# PhytoKeys

Research Article

# The Vitex trifolia complex (Lamiaceae) in the Philippines

Renerio P. Gentallan Jr.<sup>10</sup>, Seda Sengun<sup>10</sup>, Michael Cedric B. Bartolome<sup>10</sup>, Kristine Joyce O. Quiñones<sup>10</sup>, Nadine B. Coronado<sup>20</sup>, Teresita H. Borromeo<sup>10</sup>, Emmanuel Bonifacio S. Timog<sup>30</sup>

1 Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños, Los Baños, Laguna, Philippines

2 Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Los Baños, Laguna, Philippines

3 Department of Forest Biological Sciences, College of Forestry and Natural Resources, University of the Philippines Los Baños, Los Baños, Laguna, Philippines Corresponding author: Renerio P. Gentallan Jr. (rpgentallan@up.edu.ph)

#### Abstract

The Vitex trifolia complex in the Philippines comprises economically important medicinal species, but its taxonomic status has become ambiguous due to numerous historical nomenclatural revisions. We assembled the complete chloroplast genomes of five species belonging to this species complex to provide additional evidence for their species delimitation. Based on a detailed analysis of specimens which combined molecular and morphological data, we propose reinstating *V. elmeri* Moldenke and delineating *V. arvensis* Gentallan, Sengun & M.B.Bartolome as a new endemic species belonging to this complex. The new species is a putative hybrid between *V. bicolor* Willd. and *V. elmeri* Moldenke. The specific epithet arvensis reflects its predominantly cultivated nature, both on a commercial scale and in home gardens, as a valued medicinal plant. We also provided a key to identify the five species belonging to the *V. trifolia* complex in the Philippines.

Key words: Labiatae, lagundi, Malesia, Subfamily Viticoideae, taxonomy

#### 

Academic editor: Eberhard Fischer Received: 9 February 2024 Accepted: 14 April 2024 Published: 22 October 2024

**Citation:** Gentallan Jr. RP, Sengun S, Bartolome MCB, Quiñones KJO, Coronado NB, Borromeo TH, Timog EBS (2024) The *Vitex trifolia* complex (Lamiaceae) in the Philippines. PhytoKeys 248: 1–40. https://doi. org/10.3897/phytokeys.248.120387

**Copyright:** © Renerio P. Gentallan Jr. et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

#### Introduction

The genus *Vitex* was identified to be the most problematic group among the 'troublesome' mints (Bramley et al. 2009). Within this group, the *V. trifolia* complex was first proposed by R.P.J de Kok in 2004 at the Flora Malesiana Symposium (Sengun 2017) which consisted of six taxa, namely *V. agnus-castus* L., *V. benthamiana* Domin, *V. negundo* L., *V. pseudonegundo* (Hausskn. ex Bornm.) Hand.-Mazz., *V. trifolia* L. subsp. *trifolia* and *V. trifolia* subsp. *littoralis* Steenis. It was defined by de Kok (2007) as a group with white waxy undersurface of the leaves, attributed to their synapomorphic character of possessing conical apical cells of the hairs covered with wax plates. Its species are distributed from Greece to China and Japan, through Malesiana to Australia and the Pacific. The species complex was studied by Sengun (2017) for her doctorate, and the resulting treatment reinstated *V. bicolor* Willd., elevated *V. trifolia* subsp. *trifolia* and *littoralis* to species level as *V. trifolia* and *V. rotundifolia*, respectively, and split *V. negundo* s.l. into *V. negundo* s.s., *V. hybrida* Moldenke, and *V. collium* Sengun (Sengun et al. 2024).

Historically, taxa within the Vitex trifolia complex have often been confused with each other, resulting in numerous taxonomic treatments and nomenclatural revisions (Lamarck 1786; Lam 1919; Moldenke 1957; Munir 1987; de Kok 2007, 2008; de Kok and Sengun 2020; Sengun et al. 2024). For example, Lam (1919) placed V. bicolor as a variety under V. negundo and placed V. rotundifolia under the synonymy of V. trifolia. Moldenke (1957) later revised this and accepted V. bicolor and V. rotundifolia as varieties of V. trifolia. However, van Steenis (1957) elevated V. rotundifolia to subspecies level under the name V. trifolia subsp. littoralis. De Kok (2008) placed V. bicolor under V. trifolia subsp. trifolia and recognized V. trifolia subsp. littoralis due to differences in growth habit and distribution. Also, adding to the confusion, some of the taxa have been used as medicinal plants since ancient times and have been carried around and cultivated in several parts of the world, particularly in Asia (Sengun 2017).

In the Philippines, there are nine recorded *Vitex* species. Out of which, four species that belong to the *V. trifolia* complex have been documented: *V. bicolor*, *V. negundo*, *V. rotundifolia*, and *V. trifolia* (CDFP 2023), with only *V. negundo* recognized as introduced (POWO 2023). Throughout the taxonomic revisions of this species complex, several taxa originating from the Philippines, such as *V. elmeri* Moldenke, *V. leucoxylon* Blanco, and *V. negundo* var. *philippinensis* Moldenke, have been considered heterotypic synonyms (de Kok 2007, 2008; Sengun 2017; Sengun et al. 2024). The *Vitex trifolia* complex holds economic significance in the Philippines. Three of the four species, which occur in the country, are collectively referred to as "lagundi" (Madulid 2001). These are cultivated not only by people but also by the commercial pharmaceutical companies. The production of lagundi-based products in the Philippines was estimated to reach ₱430 million in gross sales and revenues (PCHRD 2010, as cited in Olivar et al. 2016).

Due to numerous taxonomic revisions within the complex and the same common name being applied to the different taxa, some published research data on "lagundi" in the Philippines has become ambiguous, particularly those lacking an associated type specimen and/or description (Manalo 1982; Dayrit et al. 1987; Cantoria 1989; Tadeo 2011). Moreover, upon revisiting the types of V. negundo in the Philippine Pharmacopeia 1 (PP1 2004), we observed that the herbarium specimens considered as types can be attributed to two species in the most recent taxonomic treatment. Upon further examination of all specimens within the complex, certain morphological characteristics of the revisited specimens exhibited notable and significant differences from the descriptions of the currently accepted taxa. Specifically, the leaf undersurface of some V. bicolor morphotypes from the Philippines do not dry white, resulting in the absence of a distinct color disparity ("bicolor" effect) between the abaxial and adaxial leaf surfaces in herbarium specimens. Additionally, V. negundo specimens from the Philippines have entire leaflets at maturity, contrary to the amended description of this species, which have some or all dentate leaflets throughout its life cycle (Sengun et al. 2024).

Given these observations, we aimed to elucidate the phylogenetic position and species delimitation of the taxa within the *Vitex trifolia* complex in the Philippines.

#### Methods

#### **Operational Taxonomic Units (OTUs)**

For the analysis of variance and molecular phylogeny, all specimens were assigned to eight a priori groups according to the overall pattern of morphology based on the initial observed differences. These Operational Taxonomic Units (OTUs) include:

- 1. V. agnus-castus
- 2. *V. bicolor* A: *V. bicolor* morphotype A; *V. bicolor* sensu Sengun et al. (2024); entire leaflets with densely pubescent undersurface drying white
- 3. *V. bicolor* B: *V. bicolor* morphotype B; leaflets mostly dentate when young, occasionally entire, undersurface sparsely pubescent not drying white; inflorescence not regularly dichasially branching (compared to *V. bicolor* A)
- 4. V. bicolor C: V. bicolor morphotype C; same with V. bicolor B but with petiolulate terminal and lateral leaflets
- 5. V. negundo A: V. negundo morphotype A; sensu Sengun et al. (2024); always with some dentate leaflets at maturity; Chinese taxon
- 6. *V. negundo* B: *V. negundo* morphotype B; leaflet margins entire at maturity; morphotypes from the Philippines
- 7. V. trifolia
- 8. V. rotundifolia

These OTUs were selected to represent all the clades of the currently accepted species under the *Vitex trifolia* complex (POWO 2023; WFO 2023).

#### Chloroplast genome and nrDNA assembly

The DNA samples were extracted using a slightly modified CTAB protocol of Doyle and Doyle (1987), and were sent to NovogeneAIT Genomics Singapore PTE LTD, Singapore, for sequencing using the HiSeq-PE150 platform (Illumina Inc., San Diego, CA, USA). NEBNext® Ultra<sup>™</sup> II DNA Library Prep Kit (Cat No. E7645) was used for library preparation. Fastp version 0.20.0 (Chen et al. 2018) was used for read-quality control. This generated cleaned reads of approximately 2 Gbp. We sequenced and assembled 16 accessions comprising of 9 operational taxonomic units of Vitex (Table 1). The chloroplast genomes and embryophyta plant nuclear ribosomal DNA (18S-ITS1-5.8S-ITS2-26S) sequences were assembled using GetOrganelle v1.7.5+ (Jin et al. 2020). The circularized genomes were then annotated and mapped using GeSeq (Tillich et al. 2017) and CPGAVAS2 (Shi et al. 2019). The annotated plastomes were visualized using OGDRAW (Greiner et al. 2019). The assembled chloroplast genomes and the ITS sequences were submitted to GenBank while other data sources were uploaded at Dryad (https://doi. org/10.5061/dryad.jg2bvg8h1). IRScope (Amiryousefi et al. 2018) was used to visualize the differences in the plastomes, particularly the expansion of inverted repeat (IR) regions at the junction sites among the assembled Vitex chloroplast genomes.

ΟΤυ	Plastome GenBank Accession No.*	ITS GenBank Accession No.	Voucher Information	Accession Number	Country
V. agnus-castus	PP584505	PP583618	Chase 22221	Kew DNA I.D. 44903	Unknown
V. bicolor A	NC_65871.1	PP583617	Gentallan & Bartolome 674	PBN 2018-674	Philippines
V. bicolor B	-	PP583616	Gentallan & Bartolome 879	PBN 2019-138	Philippines
		PP583605	Gentallan & Bartolome 1360	PBN 2019-619	Philippines
V. bicolor C	-	PP583612	Gentallan & Bartolome 874	PBN 2019-133	Philippines
		PP583607	Gentallan & Bartolome 1545	PBN 2023-007	Philippines
V. negundo A	PP584504	PP577658	Sengun 31	ICROPS 1443	China
V. negundo B	-	PP583606	Gentallan & Bartolome 918	PBN 2019-177	Philippines
		PP583609	Gentallan & Bartolome 132	PBN 2018-132	Philippines
		PP583610	Gentallan & Bartolome 146	PBN 2018-146	Philippines
		PP583608	Gentallan & Bartolome 116	PBN 2018-116	Philippines
		PP583613	Gentallan & Bartolome 1560	PBN 2023-022	Philippines
		PP583611	Gentallan & Bartolome 1036	PBN 2019-295	Philippines
V. rotundifolia	OQ942922.1	PP583604	Gentallan & Bartolome 1537	PBN 2021-033	Philippines
V. trifolia	NC_65871.1	PP583615	Gentallan & Bartolome 1073	PBN 2019-332	Philippines
V. parviflora (sister group)	ON597620.1	PP583619	Gentallan & Bartolome 1538	ICROPS 1373	Philippines

Table 1. Accessions sequenced for chloroplast genome assembly.

\* other source data not uploaded in GenBank are provided at Dryad.

#### **Phylogenetic analysis**

The ingroup, sister group, and outgroup were pre-identified using the updated tribal classification of Lamiaceae based on plastome phylogenomics (Zhao et al. 2021). Based on sequence availability, chloroplast genome sequences of five other Vitex species (assembled and utilized by Zhao et al. 2021) as part of the ingroup and sister group, one Congea species and one Sphenodesme species of the closely related subfamily Symphorematoideae Brig. (assembled and utilized by Zhao et al. 2021), and three Salvia species of the subfamily Nepetoideae as part of the outgroup were downloaded from the NCBI database. The whole chloroplast genome of downloaded sequences, together with the assembled chloroplast genome sequences of the nine Vitex species, were aligned using MAFFT (Katoh and Standley 2013). Using MEGA-X software (Kumar et al. 2018), the best model for phylogenetic analysis using Bayesian information criterion (BIC) was identified, and subsequently a Maximum Likelihood (ML) tree was generated (Nei and Kumar 2000) with 1,000 bootstraps. BI analyses using Markov chain Monte Carlo (MCMC) methods (Yang and Rannala 1997) were performed with MrBayes v3.2.6 (Huelsenbeck and Ronguist 2001) and implemented using Geneious Prime 2022.2.2. For each Bayesian analysis, four MCMC chains were run simultaneously for 2 million generations. Each run began with one random tree and sampled one tree every 1,000 generations. At the end of the run, chain convergence and estimated sample size (ESS) parameters were assessed. In the resulting summary tree, posterior probability values  $(PP) \ge 0.95$  were considered to be strongly supported (Suzuki et al. 2002).

To achieve the same polarity and congruence with the plastome phylogenetic analysis, the 16 assembled nuclear DNA (nrDNA) sequences were aligned to the ITS sequences available at NCBI representing the phylogenetic groupings. After alignment trimming, the same approach to phylogenetic analysis was performed using MEGA-X and Geneious Prime 2022.2.2.

# Morphological characterization and examination of herbarium specimens

Extensive fieldwork was conducted to examine living materials across the Philippines. Over 2,000 specimens of the Vitex trifolia complex, including types, were examined from various herbaria, namely B, BKF, BM, BO, BR, CAHUP, F, FOF, ICROPS, K, L, LINN, MO, NY, P, PNH, PUH, TAI, TFRI, S, SING, U, UC, and US. Following the recent taxonomic revision of the Vitex trifolia complex by Sengun et al. (2024), morphological observations and measurements were collected. Selected portions of the accessions were photographed using the Nikon COOLPIX S9500 Digital Camera and Olympus SZX7 stereomicroscope. Morphological analyses were then conducted using herbarium specimen measurements, following the methodology employed by Sengun et al. (2024) for species delineation within the V. trifolia complex. For the morphometric analysis of quantitative data measured from the specimens, our herbarium measurements were augmented with those obtained by Sengun et al. (2024) to provide a more comprehensive representation for the Operational Taxonomic Units (OTUs) involved. We examined 42 quantitative traits (Table 2). The sixth edition of the Royal Horticultural Society color chart was used to characterize color (RHS Media 2015).

#### Principal component analysis (PCA), cluster analysis and visualization

From the 42 quantitative traits examined, principal component analysis was performed using a correlation matrix to effectively summarize the variations observed in the morphological characters using the built-in R function princomp(), and the packages "FactoMineR" (Le et al. 2008) and "factoextra" (Kassambara and Mundt 2017) in R version 4.3.1. All missing data for the principal component and cluster analyses were estimated using the nearest-neighbor approach. A biplot was constructed to visually represent the contributions of original morphological variables to the principal components and the scores of individual specimens on these components to visualize distributions and identify potential patterns or clusters. Additionally, a mean point and confidence ellipse were elucidated per OTU in the biplot. For cluster analysis, the dataset underwent standardization to ensure equal weighting of variables and mitigate measurement error. A dendrogram was created using Ward's method for cluster analysis using Euclidean distance, and this was cut into clusters based on the number of clusters that minimizes entropy while maintaining meaningful separation between clusters through XLSTAT 2016.

#### Linear discriminant analysis (LDA)

To validate further the clustering of the accessions, the untransformed data of the resultant clusters were evaluated using LDA. All observations with missing data were removed. Percent correct classification rates between clusters were used to gauge the accuracy of discriminating morphology based on the clusters. Pairwise comparisons using Fisher distance and P-values were calculated at 5% level of significance. LDA was implemented using XLSTAT 2016 (Addinsoft, Inc.).

TRAIT ACRONYM	TRAIT DESCRIPTION	UNIT
txl	longest fully unfolded terminal leaflet length	cm
txw	widest fully unfolded terminal leaflet width	cm
txlwr	ratio of txl to txw	_
tml	shortest fully unfolded terminal leaflet length	cm
tmw	narrowest fully unfolded terminal leaflet width	cm
tmlwr	ratio of tml to tmw	-
tpx	terminal petiolule maximum length	cm
tpm	terminal petiolule minimum length	cm
sxl	longest fully unfolded lateral leaflet length	cm
SXW	widest fully unfolded lateral leaflet width	cm
sxlwr	ratio of sxl to sxw	-
sml	shortest fully unfolded lateral leaflet length	cm
smw	narrowest fully unfolded lateral leaflet width	cm
smlwr	ratio of sml to smw	-
spx	lateral petiolule maximum length	cm
spm	lateral petiolule minimum length	cm
bxl	longest fully unfolded basal leaflet length	cm
bxw	widest fully unfolded basal leaflet width	cm
bxlwr	ratio of bxl to bxw	-
bml	shortest fully unfolded basal leaflet length	cm
bmw	narrowest fully unfolded basal leaflet width	cm
bmlwr	ratio of bml to bmw	-
bpx	basal petiolule maximum length	cm
bpm	basal petiolule minimum length	cm
рх	petiole length (maximum)	cm
pm	petiole length (minimum)	cm
cal	calyx length	mm
tl	calyx tooth length	mm
tw	calyx tooth width	mm
tlwr	calyx tooth length-to-width ratio	_
cor	corolla length	mm
lipl	lower lip length	mm
lipw	lower lip width	mm
llwr	lower lip length-to-width ratio	_
sty	style length	mm
stig	stigma length	mm
fil	filament length	mm
ах	inflorescence axis length	cm
frl	fruit length	mm
frw	fruit width	mm
flwr	fruit length-to-width ratio	_
calcov	percentage calyx coverage	_

Table 2	Quantitative	characters	examined
Table 2.	Quantitative	characters	examineu.

#### Univariate parametric and non-parametric statistical analyses

To reveal significant differences between the means of distinct morpho-clusters across all examined taxa, univariate statistical analyses were conducted. All records with missing data were omitted from the analysis. Prior to parametric statistical testing using analysis of variance (ANOVA), the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance were applied. Once the assumptions were satisfied, ANOVA was performed, followed by a pairwise mean comparison test using the Honest Significant Difference (HSD) method at a 5% level of significance. For variables that did not meet the assumptions of ANOVA, the non-parametric Kruskal-Wallis test was employed, followed by a multiple pairwise comparison test using Dunn's test with Bonferroni correction at the same level of significance. Calculations were carried out using XLSTAT 2016 (Addinsoft, Inc.). Quantitative measurements were represented as the mean ± standard error of the mean. Violin graphs were presented as visual support for the identified traits that exhibited significant differences among the morpho-clusters tested.

#### **Results and discussion**

#### Chloroplast genome assembly and comparison

We had successfully assembled plastomes of the 16 accessions of our eight OTUs and one taxon from the sister group, *Vitex parviflora* (Table 3). These are the first de novo assembled and taxonomically verified chloroplast genomes for *V. agnus-castus*, *V. bicolor*, *V. negundo*, *V. rotundifolia*, and *V. trifolia*. The elucidated chloroplast genomes served as taxonomic references to clarify the molecular phylogenetic position of the *V. trifolia* complex accessions from the Philippines.

The lengths of the plastomes ranged from 154,024 bp (*V. parviflora*) to 154,496 bp (*V. negundo* A). The organization of the four distinct junction sites, including the number of genes and the GC% of the IR and LSC regions, of the chloroplast genome was conserved across the plastomes assembled (Fig. 1). However, we observed significant differences in the length of the IR region between *V. negundo* B and *V. negundo* A. The IR regions of *V. negundo* B, *V. rotundifolia, V. trifolia,* and *V. bicolor* were all of equal length, while *V. negundo* A exhibited the same length in this region as *V. agnus-castus* (Table 3; Fig. 1). Although the difference is merely three base pairs, this finding indicated evolutionary divergence of *V. negundo* B and *V. negundo* A, as the region displayed a more conserved nature among the regions of the plastomes examined. A similar pattern of differences persisted in the LSC and SSC regions, although intra-OTU differences

OTU	IDENTIFICATION	PLAST	OME	LS	SC	SS	C	IF	२	0			
010	NO.	bp	GC%	bp	GC%	bp	GC%	bp	GC%	Genes	MRNA	rna	IKINA
Vitex bicolor A	PBN 2018-674	154460	38.3	85158	36.4	17928	32.7	25687	43.3	135	91	8	36
Vitex bicolor B (1)	PBN 2019-138	154460	38.3	85158	36.4	17928	32.7	25687	43.3	135	91	8	36
Vitex bicolor B (2)	PBN 2019-619	154460	38.3	85158	36.4	17928	32.7	25687	43.3	135	91	8	36
Vitex bicolor C (1)	PBN 2023-007	154460	38.3	85158	36.4	17928	32.7	25687	43.3	135	91	8	36
Vitex bicolor C (2)	PBN 2019-133	154460	38.3	85158	36.4	17928	32.7	25687	43.3	135	91	8	36
Vitex trifolia	PBN 2019-332	154444	38.3	85148	36.4	17922	32.7	25687	43.3	135	91	8	36
Vitex rotundifolia	PBN 2021-033	154446	38.3	85134	36.4	17938	32.7	25687	43.3	135	91	8	36
Vitex negundo A	ICROPS 1443	154496	38.3	85196	36.4	17920	32.8	25690	43.3	135	91	8	36
Vitex negundo B (1)	PBN 2018-132	154491	38.2	85176	36.4	17941	32.7	25687	43.3	135	91	8	36
Vitex negundo B (2)	PBN 2018-116	154486	38.2	85170	36.4	17942	32.7	25687	43.3	135	91	8	36
Vitex negundo B (3)	PBN 2018-146	154492	38.2	85177	36.4	17941	32.7	25687	43.3	135	91	8	36
Vitex negundo B (4)	PBN 2018-295	154490	38.2	85176	36.4	17940	32.7	25687	43.3	135	91	8	36
Vitex negundo B (5)	PBN 2023-022	154479	38.2	85165	36.4	17940	32.7	25687	43.3	135	91	8	36
Vitex negundo B (6)	PBN 2019-177	154475	38.3	85161	36.4	17940	32.7	25687	43.3	135	91	8	36
Vitex agnus-castus	Kew DNA ID 44903	154495	38.3	85200	36.4	17915	32.7	25690	43.3	135	91	8	36
Vitex parviflora	ICROPS 1373	154024	38.3	84848	36.5	17852	32.9	25662	43.3	137	91	10	36

Table 3. Structural differences in chloroplast genome of the OTUs sequenced.



**Figure 1.** Chloroplast genome comparative analysis of assembled *Vitex* genomes using IRscope. JLB/JLA means the junction between LSC and IRb/IRa, and JSB/JSA means the junction between SSC and IRb/IRa.

existed. On the other hand, unlike *V. negundo* B which showed plastomic differences, *V. bicolor* A, B and C were observed to have the same plastome structure despite being derived from different type localities (Table 3; Fig. 1).

#### Chloroplast genome phylogenetic analysis

In the plastome-based cladogram, two major clades, the *Vitex trifolia* complex and other *Vitex* species, were elucidated with strong maximum likelihood bootstrap support (MLBS) of 100% and Bayesian information posterior probability (BIPP) of 1.00, further validating the monophyletic nature of the *V. trifolia* complex. Within the OTUs examined, *V. agnus-castus* was the earliest diverging lineage, followed by *V. negundo* A. Most of the OTUs, except for *V. bicolor* B and C, received maximal support values (MLBS > 94% and BIPP = 1.00), indicating significant differentiation among the six a priori groups.

With 100% bootstrap support, the *V. negundo* B accessions from the Philippines exhibited a closer phylogenetic relationship to *V. rotundifolia*, *V. trifolia*, and *V. bicolor*. The phylogenetic tree suggested that the collective *V. negundo* B specimens examined were evolutionarily divergent from the *V. negundo* A (Fig. 2), supporting the initial structural differences observed during plastome comparison (Fig. 1). This provided compelling evidence that the *V. negundo* B from the Philippines belongs to a different taxonomic group. At the very least, they have maternal progenitors that are evolutionarily divergent from the *V. negundo* A type from China. Our de novo assembled *V. negundo* A (*Sengun* 31) showed a close relationship to a GenBank accession of *V. negundo* var. *cannabifolia* (MT473783.1), now a heterotypic synonym of *V. negundo*, which was





utilized in updating the tribal classification of Lamiaceae based on plastome phylogenomics (Zhao et al. 2021). Hence, the plastome phylogenetic analysis, along with the plastome structural variations, supported a separate species delineation for the Philippine *V. negundo* B populations from *V. negundo* A.

On the other hand, the representative morphotypes of *V. bicolor* (A, B and C) formed a single clade, indicating that each of the *V. bicolor* accessions was closely related. However, we observed that *V. bicolor* A showed complete divergence from *V. bicolor* B and *V. bicolor* C with 1.00 BIPP and 100% MLBS (Fig. 2). Within the clades of *V. bicolor* B and *V. bicolor* C types, there was low bootstrap support (<64%), suggesting that these two types may belong to a single taxonomic unit. However, based on the plastome phylogenetic analysis, we cannot rule out the possibility that *V. bicolor* B and *V. bicolor* C are simply infraspecies genotypic variants of *V. bicolor*; thus, nrDNA-based phylogenetic analysis was done.



**Figure 3.** Tanglegram showing discordance between plastome and nrDNA cladograms. Numbers above branches indicate posterior probability from the BI analysis, below by bootstrap values for the ML analysis; accessions assembled by authors in asterisk (\*).

#### nrDNA phylogenetic analysis

The total length of the ITS alignments was 604 bp, comprising 205 variable sites (33.94%) and 120 parsimony-informative characters (PICs; 19.87%). Remarkably, a similar topology was elucidated when compared to the plastome-based phylogram, albeit with lower support values (Fig. 3). Consistent topology and comparably low support were observed with earlier phylogenies within this complex using the same marker (Bramley et al. 2009; Sengun 2017). A notable difference was the polyphyletic nature of V. bicolor B and C, resulting in a non-congruent topology in the nrDNA-based cladogram. We suspect this incongruence to be a consequence of interspecific hybridization between V. bicolor A and V. negundo B, with individuals of V. bicolor B and C carrying nuclear homologous sequences from both progenitors. Consequently, this led to the dispersed grouping of V. bicolor B and C types within the V. negundo B and V. bicolor A clades (Fig. 3). Furthermore, the plastome and nrDNA phylogenies suggest a one-way hybridization event between the maternal (V. bicolor A) and paternal (V. negundo B) progenitors. Plastid and nuclear phylogenomic discordance from hybridization events were similarly observed in Lachemilla (Morales-Briones et al. 2018), Magnolia s.l. (Dong et al. 2022), Piper (Simmonds et al. 2021), and Isodon (Chen et al. 2022); however, genome-scale data are often needed to further validate this phenomenon. Nonetheless, this corroborated the consistent morphological differences among the V. bicolor OTUs established in the a priori groups. Thus, further morphometric analysis was conducted to investigate whether these observed genetic differences can be mutually elucidated using morphological markers.

#### Multivariate morphometric analyses

The insubstantial evidence provided by the plastome phylogenetic trees on the species delimitation of *V. bicolor* OTUs, particularly the putative existence of interspecific hybrids (*V. bicolor* B and C), in the Philippines warranted an Table 4. Number of specimens per OTU sampled for the morphological analyses.

OTUs	NUMBER OF SPECIMENS
V. bicolor A	12 (Sengun 2017 measurements) + 5 (this study)
V. bicolor B	2 (Sengun 2017) + 33 (this study)
V. bicolor C	9 (this study)
V. negundo B	2 (Sengun 2017 measurements) + 18 (this study)



**Figure 4.** PCA biplot of *Vitex bicolor* (A–C) and *V. negundo* B accessions based on 42 quantitative characters, where the arrows represent contributions of the trait along the two principal components. Blue circles, *V. bicolor* A (N = 17); red circles, *V. bicolor* B (N = 35); green circles, *V. bicolor* C (N = 9); purple circles, *V. negundo* B (N = 20).

in-depth morphometric approach as additional evidence for their species delimitation. To achieve this, we conducted morphometric analyses using herbarium specimen measurements previously employed by Sengun (2017) for species delineation within the *V. trifolia* complex, particularly the *V. bicolor* OTUs with *V. negundo* B. This was augmented by our herbarium measurements to provide more comprehensive representation for the OTUs involved (Table 4).

The first two principal components accounted for 51.8% of the total variation (Fig. 4), while 73% of the total variance could be explained by the first five components. It was observed that the first principal component primarily

explained the variations in length and width measurements of the leaflets, floral parts, and fruit. Conversely, the second principal component predominantly reflects variations in the length-to-width measurements of these parts. The third principal component, contributing an additional 9.9% to the total variations, was found to be significantly influenced by variables related to petiolule lengths. These suggest that a substantial portion of the variations can be effectively explained by the first three principal components.

The biplot analysis provided clear delineation among *V. bicolor* A, *V. bicolor* (B and C), and *V. negundo* B. This further supports the hypothesis that *V. bicolor* B and C belong to a distinct single taxonomic unit, separate from *V. bicolor* A and *V. negundo* B. Furthermore, these accessions can potentially be distinguished by near-orthogonal variables, particularly length-width measurements, and their ratios, including calyx coverage. The cluster analysis yielded similar insights, as the entropy-based truncation resulted in three distinct clusters. Cluster 1 comprised *V. bicolor* (B and C) accessions, cluster 2 predominantly consisted of *V. bicolor* A accessions, and cluster 3 included *V. negundo* B accessions (Fig. 5). Hence, based on the elucidated morphometric and phylogenetic data, we propose the treatment of *V. bicolor* B and C as single distinct taxonomic unit which is a potential interspecific hybrid between *V. bicolor* A (maternal) and *V. negundo* B (paternal).

To further confirm this, a linear discriminant analysis using the revised OTU classification, wherein *V. bicolor* B and C were treated as a single taxonomic unit, was performed. Remarkably, significant Fisher's p-values were observed between the revised OTUs, and 100% correct classification was achieved in the subsequent confusion matrix using data from accessions with complete measurements (Table 5). Furthermore, analysis of Fisher's distance from the resultant OTUs revealed insightful patterns in the morphological characteristics of the examined accessions. These suggested that *V. bicolor* B and C bore closer morphological resemblance to both *V. bicolor* A and *V. negundo* B, compared to the similarity between the latter two taxa. These findings reciprocally illuminate the hypothesis derived from the earlier phylogenetic analysis, indicating that *V. bicolor* B and C are putative interspecific hybrids and should be treated as a distinct taxonomic unit.

 
 Table 5. Differences between clusters based on 42 morphological traits and their percent correct classification from discrimination analysis of 66 herbarium specimens.

Compared in ALC	JIAI 2010)						
	V. bicolor A		V. I	bicolor B & C	V. negundo B		
V. bicolor A	0			48.662		09.035	
V. bicolor B & C	<0.0001	<0.0001		0	44.461		
V. negundo B	<0.0001			<0.0001		0	
(B) Discriminant a	analysis (compute	d in XLST	AT 2016)				
FROM\T0	V. bicolor A	V. bicolo	or B & C	V. negundo B	TOTAL	% CORRECT	
V. bicolor A	9	0		0	9	100.00%	
V. bicolor B & C	0	3	8	0	38	100.00%	
V. negundo B	0	0		19	19	100.00%	
TOTAL	9	3	8	19	66	100.00%	

(A) Pairwise comparisons. Fisher's p values in lower triangle, Fisher distances in upper triangle



**Figure 5.** Dendrogram of the hierarchical cluster analysis (HCA) applied on the morphological traits of 81 herbarium samples. The scale on the bottom corresponds to the Euclidian distance between each class. The dendrogram is truncated to form three clusters based on entropy values.

#### Univariate morphometric analyses

To further identify potential characters that could delineate the resultant OTUs, univariate parametric and non-parametric tests of difference were performed. Out of the 42 characteristics examined, 38 traits exhibited significant variations among *V. bicolor* A, *V. bicolor* B and C, and *V. negundo* B accessions (Table 6). These results indicated that *V. bicolor* A had smaller, rounder leaflets, shorter petioles and inflorescence axis, larger globose fruits, and a different overall floral structure than that of *V. bicolor* B and C. Then, out of the 38 characters that were significantly varying, 12 were highly informative and exhibited significant pairwise differences across the three OTUs (Fig. 6). Most of these traits were used to construct the dichotomous key provided in the revised taxonomic treatment of the *V. trifolia* complex in the Philippines.

#### V. negundo B as Vitex elmeri Moldenke

Integrating plastome, nrDNA and morphological data, enough taxonomic evidence was collected to establish V. negundo B as a separate species, distinct from *V. negundo* A. Grouping the two OTUs will result in a paraphyletic taxon as inferred from the phylogenetic tree generated from chloroplast genome and nrDNA sequences. Throughout the multiple revisions in the history of the Vitex trifolia complex, specimens associated with V. negundo B (Ramos 8292, Merril 3627, Darling 16562, McGregor 5259) were cited Lam (1919) as V. negundo during the revision of Verbenaceae of the Malayan Archipelago. However, it was not until the publication of Moldenke in 1978 that this morphotype was recognized as a separate species, V. elmeri Moldenke. Notably, he published V. elmeri as a new species after he had already reviewed and revised most of the Vitex species in his series of publications on the "Materials toward a monograph of the genus Vitex" (Moldenke 1957). In the same publication, he had already examined Elmer 5611, the later designated holotype of V. elmeri, and identified it to be V. negundo. This clearly indicated that Moldenke, as an experienced observer and upon careful examination, was convinced that there were enough morphological differences to establish the taxon as a separate species. Moldenke (1978) identified that this species was often confused with V. negundo; however, he indicated that, "the aspect of its inflorescences is somewhat reminiscent of a depauperate V. agnus-castus L., but the pubescence is not at all mealy-canescent or albidous as in that species". In the same publication, he also indicated that the species has only entire leaflets, contrasting with the current amended description of V. negundo which always has dentate leaflets (Sengun et al. 2024). Examining herbarium specimens under V. negundo, we observed that only specimens from the Philippines (V. negundo B) bear completely entire leaflets at maturity. Despite the similarity, V. elmeri can also be delineated by its villous indumentum observed at the veins of the abaxial leaf surface (drying with prominent veins in the leaf undersurface), brown-colored fruit at maturity, shorter style, and sparsely pubescent leaf undersurface. Thus, we propose to reinstate V. elmeri Moldenke as a separate species from V. negundo.

\_

Table 6. Differences between clusters based on 42 morphological traits and their pe	r
cent correct classification from discrimination analysis of 66 herbarium specimens.	

TRAIT	Vitex bicolor A	Vitex bicolor B & C	Vitex negundo B
txl	8.9±0.44 <sup>b</sup>	12.4±0.25ª	8.9±0.34 <sup>b</sup>
txw	3.0±0.15ª	3.1±0.06ª	2.1±0.13 <sup>b</sup>
txlwr	3.0±0.09×	4.1±0.06 <sup>y</sup>	4.6±0.27 <sup>y</sup>
tml	6.0±0.37 <sup>b</sup>	10.0±0.26ª	6.4±0.34 <sup>b</sup>
tmw	2.1±0.12ª	2.4±0.08ª	1.5±0.10 <sup>b</sup>
tmlwr	3.0±0.16 <sup>×</sup>	4.2±0.09 <sup>y</sup>	4.5±0.27 <sup>y</sup>
sxl	7.0±0.3 <sup>b</sup>	10.5±0.25ª	7.3±0.28 <sup>b</sup>
sxw	2.5±0.11ª	2.7±0.07ª	1.7±0.11 <sup>b</sup>
sxlwr	2.8±0.06 <sup>x</sup>	3.9±0.09 <sup>y</sup>	4.6±0.29 <sup>y</sup>
sml	4.5±0.30 <sup>b</sup>	7.8±0.24ª	4.9±0.33 <sup>b</sup>
smw	1.6±0.96 <sup>b</sup>	2.0±0.07ª	1.0±0.08°
smlwr	2.7±0.10 <sup>×</sup>	4.0±0.08 <sup>y</sup>	4.9±0.31 <sup>z</sup>
bxl	3.3±0.31 <sup>b</sup>	5.6±0.19ª	3.9±0.24 <sup>b</sup>
bxw	1.4±0.15 <sup>b</sup>	1.9±0.07ª	1.1±0.08 <sup>b</sup>
bxlwr	2.2±0.16°	3.0±0.08ª	3.5±0.13 <sup>b</sup>
bml	2.0±0.25 <sup>b</sup>	3.2±0.16ª	1.9±0.17 <sup>b</sup>
bmw	0.9±0.12°	1.0±0.05°	0.6±0.07 <sup>b</sup>
bmlwr	2.0±0.16 <sup>×</sup>	3.1±0.12 <sup>y</sup>	3.6±0.26 <sup>y</sup>
tpx	1.5±0.11 <sup>y</sup>	1.0±0.08×	0.8±0.08×
tpm	0.7±0.09 <sup>y</sup>	0.5±0.08 <sup>xy</sup>	0.3±0.05×
spx	0.9±0.10	0.8±0.09	0.6±0.08
spm	0.2±0.05	0.3±0.06	0.2±0.04
bpx	0.0	0.0	0.0
bpm	0.0	0.0	0.0
рх	4.7±0.36 <sup>×</sup>	7.0±0.22 <sup>y</sup>	4.1±0.16 <sup>×</sup>
pm	2.4±0.19×	4.7±0.17 <sup>y</sup>	2.6±0.17 <sup>×</sup>
ax	10.8±0.98 <sup>b</sup>	15.1±0.58ª	14.0±0.58ª
cal	1.9±0.08 <sup>xy</sup>	2.0±0.05 <sup>y</sup>	1.6±0.07 <sup>×</sup>
tl	0.4±0.03°	0.7±0.02 <sup>b</sup>	0.9±0.05°
tw	0.8±0.07	0.9±0.03	0.8±0.03
tlwr	0.5±0.04×	0.7±0.03 <sup>y</sup>	1.1±0.06 <sup>z</sup>
cor	4.6±0.21 <sup>y</sup>	4.4±0.04 <sup>y</sup>	3.4±0.13 <sup>×</sup>
lipl	3.0±0.20 <sup>ab</sup>	3.2±0.08ª	2.6±0.11 <sup>b</sup>
lipw	2.8±0.17×	3.3±0.06 <sup>y</sup>	3.0±0.15 <sup>xy</sup>
llwr	1.1±0.04×	1.0±0.03 <sup>xy</sup>	0.9±0.03 <sup>y</sup>
sty	6.3±0.15 <sup>z</sup>	5.3±0.08 <sup>y</sup>	2.7±0.09×
stig	0.3±0.05 <sup>×</sup>	0.7±0.02 <sup>z</sup>	0.6±0.03 <sup>y</sup>
fil	3.7±0.17ª	3.2±0.06 <sup>b</sup>	2.9±0.12°
frl	4.3±0.17 <sup>×</sup>	4.3±0.06 <sup>×</sup>	2.5±0.09 <sup>y</sup>
frw	4.3±0.18 <sup>z</sup>	3.7±0.05 <sup>y</sup>	2.0±0.07 <sup>×</sup>
flwr	1.0±0.03°	1.2±0.01 <sup>b</sup>	1.3±0.03°
cov	0.2±0.03 <sup>z</sup>	0.6±0.01 <sup>y</sup>	1.0±0.00×

 $^{\rm a,\,b,\,c} significant$  differences observed using ANOVA and HSD tests;

x.y.zsignificant differences observed using Kruskal-Wallis and Dunn's non-parametric tests.



Figure 6. Dispersion of values for the 12 discriminating variables in the three Vitex OTUs.

#### V. bicolor B and C as new species

The pieces of taxonomic evidence support the introgression hypothesis, suggesting that *V. bicolor* B and C is a potential interspecific hybrid between proto-*V. bicolor* A and proto-*V. elmeri* (*V. negundo* B) individuals. Consequently, it should be treated as a separate new taxon, *V. arvensis* Gentallan, Sengun & M.B.Bartolome. Similar to *V. elmeri*, *V. arvensis* is often confused with *V. negundo*. Attempts to formally treat this species in the Philippines had been recorded. The earliest publication with concurring characteristics of this species dates to the publication of Blanco in 1845, under the species name *V. leucoxylon* Blanco (Blanco 1845). Although *Vitex leucoxylon* is an illegitimate name, the neotypified specimen of this species, *Merrill Sp. Blanc*. 440 (neo L [L.2768327]\*), should be correctly interpreted as *V. arvensis*. Moldenke (1978) published an infraspecies taxon associated with this morphotype, *V. negundo* var. *philippinensis* Moldenke, more than 20 years after his attempt to publish a monograph of the genus *Vitex* (Moldenke 1957).

Over time, specimens from this new taxon have been usually placed in either *V. negundo* L. (Merrill 1912; Merrill 1918; Merrill 1923; Moldenke 1957; Moldenke 1978) or *V. bicolor* Willd. (Lam 1919; De Kok and Sengun 2020; Sengun et al. 2024). This is likely due to the fact that it shares characteristics, often in intermediate form, with its putative parents, *V. elmeri* (*V. negundo* B) and *V. bicolor*, resulting from interspecific hybridization.

With *V. leucoxylon* as an illegitimate name and no other species-level names published in the literature, we propose identifying *V. bicolor* B and C types as *V. arvensis*, a new endemic putative interspecific hybrid of *Vitex* in the Philippines.

#### Taxonomic treatment of V. trifolia complex from the Philippines

Key 1. Key to species belonging to Vitex trifolia complex in the Philippines

- 1 Leaves 1-foliolate, prostrate shrubs, rooting at the nodes ......V. rotundifolia
- Leaves (1–)3–5-foliolate; small trees or shrubs, never rooting at the nodes....2

- 3 Leaflets always entire when young, undersurface densely pubescent drying white; cyme inflorescence unit regularly dichasially branching; fruit globose, drying black at maturity, covered by the calyx by 1/3 of its length ...... *V. bicolor*

#### 1. Vitex arvensis Gentallan, Sengun & M.B.Bartolome, sp. nov.

urn:lsid:ipni.org:names:77350665-1 Figs 7-11

- *Vitex leucoxylon* Blanco, Fl. Filip. 516. 1837. nom. illeg., not *Vitex leucoxylon* L.f. Type: Philippines, Rizal, Antipolo, 13 Jan. 1914, Merrill Sp. Blanc. 440 (neo-type: L [L.2768327, designated by Sengun et al. (2024)]).
- Vitex negundo var. philippinensis Moldenke, Phytologia 38: 308. 1978. Type: Philippines, Laguna, Los Baños, Apr., 1906, A.D.E. Elmer 8125 (holotype: PNH, destroyed; isotypes: K, NY [NY00138511]). syn. nov.

**Diagnosis.** This new species differs from *Vitex elmeri* Moldenke by having terminal leaflet which is narrowly elliptic to lanceolate, 4.3–16.0 by 1.0–3.7 cm, 2.4–5.7 times as long as wide, and with abaxial surface moderately pubescent (vs *V. elmeri* terminal leaflet narrowly elliptic, 4.0–12.0 by 0.7–3.5 cm, 2.5–8.4 times as long as wide); inflorescence with caducous flowers, leaving a clear set of scars on the axis (vs *V. elmeri* inflorescence with persistent flowers); fruit 3.0–4.9 by 3.0–4.3 mm (vs *V. elmeri* bears 1.8–3.1 by 0.4–0.8 mm fruits fully covered by the calyx at maturity).

**Type.** PHILIPPINES. Laguna: Los Baños, in the field genebank of the Institute of Crop Science, University of the Philippines Los Baños, 14°09'35"N, 121°14'43"E, 8 Feb. 2019, *R.P. Gentallan & M.B. Bartolome 743* (holotype: ICROPS).

Description. Shrub to small tree, 2-4 m high. Leaves (1-)3-5-foliolate, reflexed to drooping; terminal leaflet elliptic to lanceolate, 4.3-16.0 by 1.0-3.7 cm, 3.0-5.0 times as long as wide, terminal petiolules 0-2.3 cm long, moderate olive green (137B) above, grayish yellow green (191A to 191B) below; secondary veins 10-17 pairs; lateral leaflets 3.9-14.5 by 0.9-3.4 cm, 2.4-5.7 times as long as wide, lateral petiolules 0-2.5 cm long, secondary veins 8-11 pairs; basal leaflets when present, 1.9-8.4 by 0.4-3.2 cm, 2.3-4.0 times as long as wide, basal petiolules 0.4-3.2 cm; apex short to long acuminate, base attenuate, acute to short acuminate, or rounded, margin entire, but occasionally dentate when young; abaxial leaf surface sparsely to moderately pubescent, hairs whitish; petiole 2.0-10.3 cm long, round to tetragonal in cross-section, puberulent. Inflorescence lax paniculate, axis 8.8-23.5 cm long, with primary branch at panicle base and along panicle axis, consisting of lateral cymes in lax clusters attached to the panicle axis or primary branch, with the oldest terminal flower often unfertilized at maturity, angular in cross-section, pubescent; bracteole triangular, up to 2 mm long, apex acute, tomentose. Calyx 5-lobed, 5-ribbed; lobes 0.2-1.3 by 0.6-1.25 mm, acute, velutinous; flowering calyx 1.0-2.49 mm long; fruiting calyx cup-shaped, 3.0-4.3 mm diameter, covering 1/2 to 3/4 of the mature fruit. Corolla 5-lobed, strong violet to light violet (N88B to N88C), outside covered with appressed hairs; lower lip broadly ovate to orbicular, 2.0-4.5 by 2.4-4.5 mm, apex acute to obtuse, margin entire, often patent, strong violet (N89B), yellowish at base, with white and yellowish hairs at corolla mouth; corolla throat inner diameter 2.05-2.53 mm; lateral lobes 1.6-2.1 by 1.6-2.0 mm, apex round to acute, often patent to reflexed, light violet (N88C); upper lip 2-lobed, lobes 1.3–1.7 by 1.4–1.9 mm, apex acute, often patent, light violet (N88D); tube infundibular 3.5-4.7 mm long, very pale purple (85D) to very light purple (85C). Stamens strongly didynamous, exserted up to



Figure 7. Vitex arvensis A habit B part of inflorescence C side view of flower D calyx open, adaxial E Corolla open F style fruit in G calyx H fruit in cross-section I calyx. Illustration by Daryl Ceribo. Scale bars: 2.5 cm (A, B); 1 cm (C), 1 mm (D, E, F, G, H, I).

1/2 of its length, filaments 2.3–4.3 mm long, inserted 1/2 of the way on the corolla tube. Ovary 0.7–1.2 by 0.6–1.2 mm, globose, glabrous; style 3.6–6.6 mm long; stigma lobes 0.4–0.9 mm long. Fruit brown when mature; dried ellipsoid to ovoid, 3.0–4.9 by 3.0–4.3 mm, apex notched to truncated, glabrous with sparse glands.

Phenology. Produces fruits and flowers all year round.

**Habitat and ecology.** Growing primarily in farmlands, home gardens under cultivation as medicinal and/or hedge article, but recorded in secondary forests, mixed thickets, along rivers, trails and ridges, open places and in wastelands. Altitude: 15–500 m.



**Figure 8.** Morphological characteristics of *V. arvensis* **A** branch with inflorescence **B** sixth fully unfolded leaf **C** abaxial leaf lamina surface **D**–**F** flower **G** inflorescence unit **H**–**I** mature fruit with and without calyx.



**Figure 9.** Leaf undersurface pubescence of *V. arvensis* (40× magnification). Photos: L. E. Endonela.



Figure 10. Natural distribution of Vitex arvensis in the Philippines.

**Distribution.** Endemic in the Philippines, distributed primarily from Central to South Luzon but widespread throughout in cultivation.

**Vernacular names.** lagundi (Tagalog, Bisaya), dangla (Ilokano, Zambales) **Conservation status.** IUCN assessment gives the extent of occurrence (EOO) and area of occurrence (AOO), as greater than the threshold for the vulnerable category, thus the status would be least concern (LC).



Figure 11. Holotype specimen of Vitex arvensis.

**Notes.** 1. This taxon has never been recognized at the species level before. The specific epithet *arvensis* provides insight into its usually cultivated nature since it is primarily cultivated on a commercial scale, and in home gardens as a medicinal plant.

2. This is a putative natural interspecific hybrid between *V. bicolor* and *V. elmeri*. Although now distributed across the Philippines, it was primarily distributed in areas where *V. elmeri* and *V. bicolor* overlap, from South to Central Luzon. This species bears resemblance to both parents: *V. elmeri* in terms of the yellow corolla ridge color, presence of villous abaxial secondary veins, sparse pubescence on the lamina undersurface, and having dentate leaves when young; and *V. bicolor* in terms of lax inflorescence units, flower color, and leaf size.

3. Aside from bearing caducous flowers, this new species further differs from *Vitex bicolor* Willd. by having the mature ovoid fruits covered by the calyx by 1/2-3/4 along its length which dries brown, while *V. bicolor* has larger (5.2–5.8 by 4.3–5.6 mm) globose fruits covered by the calyx to only up to 1/5 along its length which dries black.

4. Unlike V. *bicolor*, V. *arvensis* was observed to have a lower germination rate of seeds, with virtually non-existent seedlings under the canopy. However, with interventions, these can be germinated ex-situ, albeit with a low success rate.

**Documented uses (in the Philippines).** Leaves heated and applied as external patch or as liniment for fever, fruits and leaves used as an insecticide; juice extracted from leaves by grinding ingested for cough relief; decoction or boiling of leaves for fever and cough.

Specimens examined. PHILIPPINES. Cagayan: Peñablanca, San Roque, May 1917, Adduru 173 (US03805856); Vendivil & Fernando 125366 (PNH); Peñablanca, Callao, Apr 1915, Castillo 22747 (US03805843); Tuao, May 1979, Sr. M. Rocero 165079 (PNH); Zambales: Botolan, May 1903, Merrill 2917 (US03805807); Botolan, Villar, Nov 1947, R. B. Fox 4928 (PNH); Bataan: Dinalupihan, Feb 1903, Merrill 1503 (US03805792); Pampanga: Arayat, Vidal 1648 (L.2768325); Bulacan: San Miguel, 15-20 m alt., B. Fegan 113564 (PNH); Doña Remedios Trinidad, Kabayanan, Dec 1994, H. Garcia et al. 15334 (L.4212458); Norzagaray, San Lorenzo, Mar 1996, E. Barbon & H. Garcia 22403 (L.4216246); Manila: Manila, Oct 1903, Merrill 3429 (US03805814); Sep 1910, M. Ramos (E01121257); near Manila, Capt. Wilkes s.n. (US 00830006); Sampaloc, Balic-balic, Aug 1890, Loher 4433 (US03805840); Mandaluyong, Malamig, G. Edaño 3783 (PNH); Cavite: Mendez, Mangubat 1343 (US03805791); Laguna: Los Baños, Dec 1903, Hallier 4055b (L.2775187); Apr 1906, Elmer 8125 (E01121256); Sep 1909, Rosenbluth & Tamesis (L.2768326); 250 m alt., Jan 1911, Holman 99 (US03805805, US03805808); Jul 1917, Elmer 18119 (US03805788, L.2768218); Makiling National Park, 100 m alt., Oct 1945, M. D. Sulit 8311 (PNH); Mar 1963, D. R. Mendoza 92452 (PNH); San Antonio, Mar 1948, E. Canicosa 9707 (PNH); College, 50 m alt., Sep 1954. J. V. Pancho 32919 (PNH); Batangas: Mataas na Kahoy, Arañez 14 (L.2768323); Rizal: Marikina, Barangka, G. Edaño 36210 (PNH); Antipolo, 100 m alt., Mar 1903, Merrill 1636 (US03805812); Jan 1913 Merr. Sp. Blanc. 440 (L.2768327); Antipolo, Boso-boso, Loher 4432 (US03805810); San Mateo, May 1904, Ahern's collector 102 (US03805809); Taytay, Feb 1953, Lorena 18 (L.0248373); Tanay, May 1903, Merrill 2320 (US03805790); Quezon: Tayabas, May 1916, Cailipan 25637 (US03805789); Infanta, Sep 1904, Whitford 853 (US03805784); Camarines Sur: Pasacao, Dalupaon, 1901, Ahern 255 (B01616593, US03805779), Naga City, Mt. Isarog, Oct 1992, Barbon et al. 8377 (L.3930320); Agusan Norte: Butuan City, Tungao, May 1991, Barbon et al. 1917 (L.4212463).

## 2. Vitex bicolor Willd., Enum. Pl. 2:660. 1809

Figs 12, 13

**Type.** 'Habitat in India Orientale', cult. Hort. Berlin from seeds sent by Klein ('Ind. 1797'), Herb. Willdenow 11709 (holotype: B-W [B11709-010])

**Description.** Shrub to small tree, 1-5 m high. Leaves (1-)-3-(5)-foliolate; terminal leaflet elliptic to narrowly elliptic, 8.9-11.6 by 3.0-3.5 cm, 2.7-3.5 times as long as wide, petiolules 0.8-1.4 cm long, secondary veins 14-18 pairs, moderate olive green above (N137A), light gray (N191C) to gravish yellow green (N191) below; lateral leaflets 5.4-9.6 by 2.5-3.7 cm, 2.1-3.3 times as wide, petiolules 0.5-0.9 cm long, secondary veins 10-14 pairs; basal leaflets when present, 2.4-4.2 by 1.5-2.2 cm, 1.1-2.5 times as long as wide; basal petiolules absent; apex acute to acuminate, base acute to cuneate, margin entire; abaxial leaf surface highly pubescent, with tomentose white hairs; petiole 3.2-4.8 cm long, round in cross-section, covered with minute hairs. Inflorescence paniculate, axis c. 17.5 cm long, with primary branch at panicle base, consisting of lateral dichasial cymes in lax clusters attached to the panicle axis or basal primary branch, with the oldest central flower often fertilized at maturity, angular in cross-section, pubescent; bracteole triangular, c. 0.75 mm long. Calyx 5-lobed, 5-ribbed; lobes 0.2-0.6 by 0.6-1.0 mm, acute, velutinous; flowering calyx 1.5-1.7 mm diameter, fruiting calyx 2.5-3.6 mm diameter, covering up to 1/5 of the mature fruit. Corolla 5-lobed, strong violet to light violet (N88B to N88C), outside covered with appressed hairs; lower lip broadly ovate to deltoid, 2.7-4.3 by 2.5-3.5 mm, apex acute, margin entire, often reflexed to patent, strong violet (N89B), purple and white at base, with white and purple hairs at corolla mouth; corolla throat inner diameter 1.7-2.4 mm; lateral lobes 1.2-1.7 by 1.2-1.9 mm, apex round, often reflexed to patent; upper lip 2-lobed, lobes 1.0-1.8 by 1.0-1.6 mm, apex rounded, often reflexed to patent, light violet (N88C); tube infundibular 5.0-6.0 mm long, light violet (N88C to N88D). Stamens strongly didynamous, shorter filaments 2.9-3.8 mm long, longer filaments 3.4-4.4 mm long, inserted c. 1/3 of the way on the corolla tube; anther 0.7-0.9 mm long. Ovary globose, glabrous; style 6.2-7.8 mm long; stigma lobes 0.3–0.6 mm long. Fruit brown to black when mature; dried ellipsoid to spherical, 5.2-5.8 by 4.3-5.6 mm, apex rounded, glabrous.

**Habitat and ecology.** Along the seashore in beach, mangrove and littoral forests to hillsides along mountain slopes; also recorded in cultivation as hedge/ fence and medicinal plant. Altitude: usually at sea level but extends up to 300 m.

**Distribution.** Sri Lanka, South China: Hainan, Japan: Okinawa; Malesia: throughout, Australia: Queensland, Pacific: Tonga, Samoa and Marianas Islands. Indigenous throughout the Philippines.

**Vernacular names.** lagundi (Tagalog, Bikolano, Bisaya), tulasi (Tagbanua, Palawan), pirabon (Palawan)

**Notes.** This species is seldom utilized for its medicinal use in the Philippines. **Documented uses (in the Philippines).** Leaves for treating dengue, cough, fever; leaves applied as poultice against the forehead and/or temples as a febrifuge.

**Specimens examined. PHILIPPINES. Cagayan**: Calayan, May 1961, E. Quisumbing & R. Del Rosario 79790 (PNH); **Bataan**: Limay, Lamao river, Nov 1903, R. S. Williams 185 (US03805765); Sep 1904, T. Borden 2035 (US03805781); Sep-Dec 1904, R. Meyer 2276 (US03805783, BO); **Aurora**: Baler, Sep 1902, Merrill 1106 (US03805785); **Quezon**: Burdeos, Karlagan, R. B. Fox 8991 (PNH); Botonan, Bar-



**Figure 12.** Morphological characteristics of *V. bicolor* **A** branch with inflorescence **B** sixth fully unfolded leaf **C** abaxial leaf lamina surface **D**–**F** flower **G** inflorescence unit **H**–**I** immature fruit with and without calyx.



Figure 13. Natural distribution of Vitex bicolor in the Philippines.

bon et al. 2047 (L.4216303); Oriental Mindoro: Puerto Galera, Minolo, Apr 1952, J. V. Santos 5258 (US03805782, L.2768221); Rombion: Magdiwang, Silum, May 1994, Reynoso 14138 (L.4216134); Palawan: Taytay, 30 May 1913, Merrill Sp. Blanc. 302 (US00699536, L.2768328); Quezon, Ampaplot, Apr 1964, R. A. Espiritu 91477 (L.2768220); Tabon, Nov 1963, E. J. Reynoso 87701 (PNH); Camarines Norte: Lagonoy, Commerson s.n. (P00657557); Camarines Sur: Pasacao, Dalupaon, Apr 1901, Ahern 166 (BO); 1902, Ahern 814 (US03805804); May 1902, Ahern 223 (US03805780, BO); Masbate: 1904, Clark 2527 (US01962875, BO); Guimaras: Nueva Valencia, Igdarapdap, June 1955, A. T. Taleon 34030 (PNH); Antique: Caluya, Kabilo, 300 m alt., Feb 1997, Romero 29662 (L.4216249); Negros Occidental: Cauayan, Bulata, 50 m alt., Aug 1995, Madulid & Majaducon, 36097; Siquijor: Larena, Sandugan, 5-10 m alt., Oct 1998, Christenhusz 86 (U.1762597); Samar: San Vicente, Medio Island, Mar 1957, Y. Kondo & G. Edaño 38739 (L.2768219, PNH); Lanao del Norte: Aug 1926, F. Guerrero 30388 (E01121263); Misamis Oriental: Jan-Feb 1913, D. P. Miranda 17976 (US03805770); Davao del Sur: Davao City, Santa Cruz, Feb 1904, Copeland 691 (US03805774); Sep 1909, Elmer 11999 (US03805774, US03805769, L.2768222, BO, PNH); Padala, Jun 1905, R. S. Williams 2978 (US03805777); Basilan: Jun 1911, Tarrosa 19553 (L.2768223); Tawi-tawi: Mapun, Pamelican, Feb 1957, Y. Kondo & G. Edaño 39032 (PNH); Languyan, Birad-dali, May 1992, F. J. M. Gaerlan & E. C. Sagcal 10053 (L.4216299).

## 3. Vitex elmeri Moldenke, Phytologia 38: 307 (1978)

Figs 14, 15

**Type.** PHILIPPINES, Union Province, Bauang, Feb. 1904, Elmer 5611 (holotype: NY [NY00138505]).

Description. Shrub to small tree, sometimes bushy, 1-4 m high. Leaves (3-)5-7-foliolate; terminal leaflet narrowly elliptic to elliptic, 4.0-12.0 by 0.7-3.5 cm, 2.5-8.4 times as long as wide; lateral leaflets 2.9-10.0 by 0.5-2.7 cm, 2.5-9.0 times as long as wide; basal leaflets 0.6-6.7 by 0.2-1.8 cm, 1.1-4.5 times as long as wide; apex acuminate, base acute, margin entire at maturity (with dentation in the middle only when young), glabrous above to very sparsely pubescent below, with the midrib and veins covered with erect villous hairs; petiole 1.4-5.5 cm long, angular in cross-section, covered with villous hairs; petiolules 0-1.4 cm long. Inflorescences paniculate, terminal axis 3-15 cm long, angular in cross-section; flowers in dense lateral clusters on peduncles up to 5 mm long; tomentose, hairs erect; bracteoles linear up to 3 mm long. Calyx 5-lobed, clearly 5-ribbed; lobes 0.5-1.3 by 0.6-1.2 mm, clearly developed, acute to acuminate, persistent, villous; glands few; flowering calyx, excluding the lobes, 1.4-2.2 mm long; fruiting calyx 1.6-2.5 mm diameter, fully enclosing the mature fruit. Corolla 5-lobed, pink; covered with appressed hairs, glands few; lower lip 2.0-3.5 by 2.0-4.0 mm, usually reflexed, apex cuneate to acute, margin entire, two well-developed ridges at corolla mouth, yellow; lateral lobes 1.2-2.4 by 1.0-2.5 mm, apex rounded to subacute, patent. Stamens: filaments 2.1-4.2 mm long, slightly to strongly didynamous, inserted halfway on the corolla tube. Ovary globose, 0.6-1.0 by 0.6-0.9 mm, glabrous; style 2.0-3.6 mm long; stigma lobes 0.4-0.8 mm long. Fruit light to dark brown when mature; dried obovoid to cylindrical, 1.8–3.1 by 0.4–0.8 mm, apex truncate, glabrous, smooth.

Phenology. Produces flowers and fruits all year round.

**Habitat and ecology.** Growing primarily in disturbed habitats (i.e. farmlands, cemeteries, wastelands) and in cultivation, but also recorded along thickets in secondary forests, and roadsides. Altitude: usually on hills and slopes, 50–500 m.

**Distribution.** Endemic in the Philippines, throughout North- to South-western Luzon, with cultivation records in Agusan del Norte, Iloilo, Antique.

Vernacular names. lagundi (Tagalog, Bisaya), dangla (Ilokano)

**Conservation status.** IUCN assessment gives the extent of occurrence (EOO) and area of occurrence (AOO), as greater than the threshold for the vulnerable category, thus the status would be least concern (LC).

**Notes.** 1. *V. elmeri* is currently synonymized under *V. negundo*, however, based on our integrative evidence, we propose to reinstate this taxon.

2. This species is predominantly used as a medicinal plant in the Northwestern part of Luzon. It pervades in disturbed areas, and the remaining populations may be remnants of its natural habitat which had now been developed. Their seeds also germinate in situ.

3. The species differs from *V. negundo* by having completely entire leaflets, with veins at the undersurface covered by villous indumentum, at maturity; flowers with pink lower lip; and fruits always drying brown at maturity.

4. There is a diversity of chlorotypes present for this taxon.

**Documented uses (in the Philippines).** Decoction of leaves prepared for flu, cough, and malaria; extracted juice from leaves by grinding for cough (internal use); utilized in preference in some parts of the llocos region.

Specimens examined. PHILIPPINES. Ilocos Norte: Banna, Balioeg, 100 m alt., Nov 1975, Iwatsuki 11 (L.2775183); Currimao, Bimmanga, 1984, Vendivil & Fernando 160201 (PNH); Carasi, Sinaligan, 1984, Vendivil & Fernando 160467 (PNH); Nueva Era, Sto. Niño, Nov 1986, Vendivil & Fernando 165304



**Figure 14**. Morphological characteristics of *V. elmeri* **A** branch with inflorescence **B** sixth fully unfolded leaf **C** abaxial leaf lamina surface **D**–**F** flower **G** inflorescence unit **H**–**I** mature fruit with and without calyx.



Figure 15. Natural distribution of Vitex elmeri in the Philippines.

(PNH); Bangui, Mt. Napidad, 500 m alt., Feb 1997, H. Garcia & L. Fernando 25321 (L.4216269); Ilocos Sur: Sta. Maria, Dec 1958, M. L. Steiner 39796 (PNH); La Union: Tubao, Anduyan, <500 m alt., Feb 1912, Vanoverbergh & Skent s.n. (BR0000026253473V); Pangasinan: Bautista, Jul 1903, Merrill 2876 (US03805806); Balungao, Mt. Balungao, 1908, Darling & Merritt 14051 (US03805859); Darling & Merritt 14063 (BR0000034903858); San Juan, San Carlos, May 1909, M. Ramos 8292 (L.2775179, L.2775180); Umingan, Apr 1914, Otanes 17995 (US03805811); Mt. Province: Ambuklao, Agno River, Nov 1953, Quisumbing 18826 (US03805838, L.2775182); Ifugao, Luta, 500 m alt., Klock 91 (US02782038); Abra: Feb 1909, Darling 16562 (L.27751881); M. Ramos 7215 (BR0000034903865); Cagayan: Sta. Praxedes, San Juan, 520 m alt., Aug 1995, H. Garcia et al. 18454 (L.4216254); Peñablanca, San Roque, 1975, Vendivil & Fernando 125368 (PNH); Isabela: Cauayan, Jun 1902, Merrill 147 (US03805855); Nueva Vizcaya: Dupax, Mar 1912, R. C. McGregor 11471 (E01121242); Tarlac: Concepcion, Nov 1903, Merrill 3627 (L.2775186); Nueva Ecija: General Mamerto Natividad, Pulong Singkamas, Sep 1908, R. C. McGregor 5259 (L.2775185); San Jose, Camanacsacan, Feb 1955, R. Martin 35234 (PNH); Batangas: Calatagan, 50 m alt., Jan 1949, E. Quisumbing 6578 (PNH).

#### **4.** *Vitex rotundifolia* **L.f., Suppl. Pl.: 294. 1782** Figs 16, 17

Vitex trifolia var. simplicifolia Cham., Linnaea 7: 107. 1832). Type: Philippines, Luzon, Cavite, Dec. 1817 – Jan. 1818, Chamisso s.n. (holotype: LE n. v.).

Vitex repens Blanco, Fl. Filip. 513. 1837. Type: Philippines, Luzon, Batangas, 11 Feb. 1915, Merr. Sp. Blanc. 814 (neotype: K [K000182650], designated by Sengun et al. (2024)).



**Figure 16.** Morphological characteristics of *V. rotundifolia* **A** branch with inflorescence **B** sixth fully unfolded leaf **C** abaxial leaf lamina surface **D**–**F** flower **G** inflorescence unit **H**–**I** mature fruit with and without calyx.

**Type.** JAPAN, Thunb. s.n. (Hb. Thunb. 14619) (holotype: LINN-SM; isotype: UPS-THUNB [V-125606]).

Description. Prostrate, decumbent, or scandent shrub, usually below 1 m tall, sometimes creeping and spreading on the ground up to 4-5 m, rooting at the nodes. Leaves 1-foliolate, round to obovate-spathulate, 0.9-5.1 by 0.5-3.7 cm, 1.3-2.8 as long as wide, base cuneate, apex rounded to subacute, margin entire; upper surface moderate yellow green (147B), pubescent, especially at the midrib; lower surface grayish yellow green (191B), velutinous, covered with whitish hairs obscuring the venation and areolation; secondary veins 4-7 pairs, slightly prominent on both surfaces; petioles, when present, up to 13 mm long. Inflorescence terminal, paniculate, 5.9-11.4 cm long axis, consisting of short dichasial cymes, often unbranched, in dense clusters, with the oldest central floret usually fertilized at maturity. Calyx 5-lobed, velutinous, with appressed hairs, lobes 0.35-0.70 by 0.9-1.9 mm; flowering calyx 1.8-4.0 mm long, 2.2-3.6 mm diameter; fruiting calyx 4.1-5.4 mm diameter, covering up to 90% of the fruit at maturity. Corolla 5-lobed, pale purple, outside covered with appressed hairs; lower lip orbicular to broadly ovate, 3.0-6.10 by 2.9-6.5 mm, apex subacute, margin entire, two clear ridges at corolla mouth, often reflexed, deep violet (N89D) to strong violet (N90A), covered with white hairs at corolla mouth; tube 4.8-9.7 mm long, infundibular, light violet (N91A to N1B). Stamens with filaments 5.2–7.5 mm long, slightly didynamous, inserted 1/3 of the way on the corolla tube, exceeding the corolla mouth from 1/3 to 1/2 of its length, anthers c. 2 mm long. Ovary 1-1.5 mm diameter, globose, glabrous, the upper half covered with glands; styles 6.0-12.4 mm long, stigma 2-lobed, lobes 0.3-1.5 mm long. Fruit when dried, black at maturity, globose, 3.7–5.5 by 4.1–5.4 mm.

Habitat and ecology. Usually along coastal strands and seashores, on sandy beaches along with Ipomoea pes-caprae and forming dense mats below the



Figure 17. Natural distribution of Vitex rotundifolia in the Philippines.

thickets of *Vitex trifolia* in the upper beach, also on sand dunes, rocky beaches, and beach forest.

Phenology. Flowering and fruiting all year round.

**Distribution.** Widespread from Japan, Korea, and eastern coast of China to north and northeast coast of Australia and extending to the Pacific until Samoa and Fiji, absent from the central Pacific with only an isolated population in Hawaii. Indigenous all throughout the Philippines, particularly toward the island borders.

**Vernacular names.** lagunding-dagat (Tagalog), lagunding-gapang (Tagalog), dungla (Tagalog), daldalaki (Ilokano), dangla-ti-baybai (Ilokano), bantige (Ilokano), agubarau (Bisaya).

**Notes.** In Sengun at al. (2024), p. 115, *Merrill Species Blancoanae* 814 has been mistakenly identified as *V. trifolia*. It is indeed *V. rotundifolia*.

**Documented uses (in the Philippines).** This species is rarely used for its medicinal properties, with the leaves being boiled or used in decoctions to treat cough.

Specimens examined. PHILIPPINES. Batanes: Sabtang, 26 Mar 1961, E. Quisumbing et al. 79299 (PNH); Batan, Mt. Iraya, 20 Mar 1991, Barbon et al. 1581 (L.4212461); Ilocos Norte: Laoaq, Sta. Maria, Apr 1955, J. V. Santos 6267 (US03805794); Laoag, 3 Jan 1965, Br. Alfred 93787 (PNH); Ilocos Sur: Tagudin, Aug 1909, R. C. McGregor 10072 (BR0000034904954); Cagayan: Aparri, June 1902, E. D. Merrill 323 (US03805761); Peñablanca, Baguio cove, 14 Apr 1981, M. S. Allen 150236 (L.2768427); Isabela: Palanan, Palanan bay, June 1913, L. Escritor 21171 (US03805797); Bataan: Limay, Lamao river, Sep 1904, T. E. Borden 1940 (US03805793, E01121261); Lamao, 1907, H. M. Curran s.n. (B01621593); Zambales: Subic, Sep 1903, H. Hallier 4230 (L.2768419, L.2768425); Sta. Cruz, Hermana Mayor Island, Dec 1954, J. V. Santos 6210 (L.2768426, US03805794); Sta. Cruz, Acoje Mining, Aug 1954, J. Yench 18928 (PNH); Central Luzon: Loher 4434 (US03805799); Manila: 1914, Perrottet s.n. (L.2768423); Batangas: Batangas City, San Pedro, Feb 1915, Merrill Sp. Blanc. 814 (US00623757, BO1359175); San Juan, Hugom, Oct 1946, M. D. Sulit 7440 (BR0000034904947); Aurora: Dipaculao, Mijares (Gupa beach), May 1992, Stone et al. 5475 (L.4212443); Quezon: Infanta, Sep 1904, H. N. Whitford 755 (US03805755); Oriental Mindoro: Calapan, Apr 1903, E. D. Merrill 898 (US03805798); June 1906, L. Mangubat 926 (US03805759); Rombion: Magallanes, Mt. Giting-giting, Apr 1910, A. D. E. Elmer 12135 (L.2768429, U.1762519, US03805796, E01121262, B01621591); Camarines Norte: Daet, Marcedes, 1903, H. Hallier 4230a (L.2768421, L.2768420); Sorsogon: Bulusan, near town, June 1958, J. Sinclair & G. Edaño 9673 (L.2768422, US03805795; E01121264); Panay: Antique, Culasi, May-Aug 1918, R. C. McGregor 32477 (L.2768418, B01359176); Samar: Capul, 27 Mar 1957, Y. Kondo & G. Edaño 36800 (PNH).

#### 5. Vitex trifolia L., Sp. Pl.: 638, 938. 1753 Figs 18, 19

#### Type. INDIA, Herb. Linn. 811/7 (holotype: LINN)

**Description.** Shrub, prostrate to erect, to small tree, up to 3 m high. Leaves (1–)3-foliolate; elliptic to obovate or obovate-spatulate, grayish olive green (NN137A) to moderate olive green (N137A) above, light greenish gray (N191C) to light gray (N191D) below, terminal leaflet 3.0–8.6 by 2.1–4.5 cm, 1.9–2.9 times as long as wide, petioles 0.4–4.7 cm, base attenuate in compound leaves, cuneate



**Figure 18**. Morphological characteristics of *V. trifolia* **A** branch with inflorescence **B** sixth fully unfolded leaf **C** abaxial leaf lamina surface **D**–**F** flower **G** inflorescence unit **H**–**I** mature fruit with and without calyx.

to round in unifoliate leaves, terminal petiolules absent, secondary veins 9-12 pairs; lateral leaflets 1.5-6.4 by 0.6-2.5 cm, 1.3-2.7 times as long as wide, secondary veins 6-8 pairs, sessile. Inflorescence terminal, paniculate, 4.9-15.1 cm long axis, seldom with primary branch at panicle base, consisting of lateral dichasial cymes in lax clusters attached to the panicle axis or primary branch, with the oldest central flower often fertilized at maturity; bracteole linear, c. 1 mm. Calyx 5-lobed, accrescent, persistent, with numerous glands; lobes 0.2-0.7 by 0.3-2.4 mm, acute, velutinous; flowering calyx 1.8-2.4 mm long, 1.5-3.2 mm diameter; fruiting calyx 2.5-4.9 mm diameter, covering up to 75% of the mature fruit. Corolla 5-lobed, pale purple, outside covered with appressed hairs; corolla throat inner diameter 2.8-4.0 mm; lower lip orbicular to broadly ovate, 1.9-5.4 by 2.2-5.6 mm, apex acute, margin entire, two clear ridges at corolla mouth, often reflexed, deep violet (N89D) to strong violet (N90A), white at base, with white hairs at corolla mouth; lateral lobes 1.9-2.8 by 2.4-3.1 mm, reflexed, apex rounded, brilliant violet (N92A to N92B); upper lip 2-lobed, lobes 1.8-2.8 by 2.0-2.9 mm, apex acute to rounded; tube infundibular 3.9-6.8 mm long, light violet (N91A to N91B). Stamens slightly didynamous, shorter filaments 3.0-5.8 mm long, longer filaments 4.1–6.1 mm long, inserted c. 1/3 of the way on the corolla tube, exceeding 1.8-3.9 mm from the corolla tube, very light purple (85C); anthers 0.6-1.1 mm long, light violet. Ovary globose, glabrous; style 7.1-9.6 mm long, light violet; stigma 2-lobed, lobes 0.3-0.8 mm long. Fruit when young green with purple tinge, turning brown to black when mature; dried obovoid, 3.4–6.4 by 3.5-6.2 mm, apex truncated, glabrous with numerous glands.

Phenology. Flowering and fruiting all year round.

**Habitat and ecology.** Beaches, but sometimes occurring in thickets and waste places, can also be found in cultivation, particularly in home gardens as both ornamental and medicinal article. Altitude: Usually at sea level but rarely up to 685(-1500) m.



Figure 19. Natural distribution of Vitex trifolia in the Philippines.

**Distribution.** Widespread from India and Sri Lanka to Southern Japan and north and east coast of Australia and the Pacific Islands. Indigenous throughout the Philippines.

**Vernacular names.** lagundi (Tagalog, Bikolano, Bisaya), lagunding-dagat (Tagalog).

Notes. This species is seldom utilized for its medicinal properties.

**Documented uses (in the Philippines).** Boiling/decoction of leaves for cough and fever; treating chickens; root decoction taken internally for malaria; seeds and fruit for poisonous and venomous animal bites such as snakes.

Specimens examined. PHILIPPINES. Ilocos Norte: Burgos, Feb-Mar 1917, M. Ramos 27118 (B01616592); Ilocos Sur: Sugpon, Nov 1908, M. L. Merritt & F. W. Darling 14079 (L.2768535); Mt. Province: Barlig, Kaleo, Mar 1914, Vanoverbergh 321 (L.2768321); Bauko, 1000-1500 m alt., Vanoberbergh & Skent s.n. (BR0000026253497V); Bataan: Limay, Lamao, Oct 1905, H. M. Curran 5295 (BR0000034904909); Manila: Tondo, Gagalangin, Loher 4436 (US03805772); Batangas: San Juan, Laiya, Oct 1946, M. D. Sulit 7441 (PNH); Quezon: Guinayangan, Vidal 850 (L.2768538); Atimonan, Aug 1904, H. N. Whitford 674 (03805776); Lucban, Mt. Banahaw, H. N. Whitford 988 (US03805773); May 1907, Elmer 7877 (US03805766, L.2768533); Calauag, Kinamaligan, E. Canicosa 9784 (PNH); Agdangan, Ibabang Kinagunan, May 1976, R. T. Francisco 170836 (PNH); Occidental Mindoro: Lubang, Apr 1903, Merrill 957 (US03805767); Lubang, Tilik, 685 m alt., 5 Jul 1996, Romero & Fuentes 37610 (L.4212394); Camarines Norte: Daet, Marcedes, H. Hallier 4230b (L.2768536); Camarines Sur: Pasacao, Dalupaon, Ahern 255 (US03805779); Pili, Carambola, May 1947, P. Convocar 2962 (PNH); Sorsogon: Irosin, Mt. Bulusan, Dec 1915, Elmer 15236 (L.2768324, U.1762516, US03805771); Mar 1958, M. L. Steiner 1327(PNH); Capiz: Jamindan, May 1918, G. Edaño 31523 (L.2768539); Leyte: Tacloban, Utap, G. Frohne 39320 (PNH).

#### Acknowledgements

We would like to thank Ronnil Beliber and Arvin Medrano for their assistance in cultivating the plant specimens; to Eddelaine Joyce Bautista, Edna Mercado and Kimberly Bejo-Locsin for the project implementation and monitoring; to Dr. Alan Paton of Kew for his help in providing access to DNA samples and the Jodrell Laboratories, Kew for sending the samples. We are grateful to the following institutions for letting us examine and collect tissues from their samples: the Philippine National Herbarium (PNH), Taiwan Forestry Research Institute (TFRI), Jose Vera Santos Memorial Herbarium (PUH), College of Agriculture Herbarium UP (CAHUP), The Singapore Herbarium (SING), Herbarium of the National University of Laos (FOF), NTU Herbarium (TAI), Forest Herbarium (BKF), and Kew (K). In addition, the first author would like to thank the Philippine Biorepository Network (of medicinal plants) team, particularly Prof. Nestor C. Altoveros, Prof. Ma. Anita Bautista, and Dr. Lourdes B. Cardenas, who started this medicinal plant journey with him; and, to his PhD dissertation adviser Dr. Antonio G. Lalusin; the committee members, Dr. Leah E. Endonela, Dr. Melguiades Emmanuel C. Reves, and Dr. Eureka Teresa M. Ocampo; to the reviewers and examiners Mr. Ulysses Ferreras, Dr. Ma. Carmina Manuel, Prof. Annalee S. Hadsall, and Dr. Rogier De Kok. The authors would also like to thank Reneliza Cejalvo, Roselle Madayag, Bartimeus Buiene

Alvaran, Angeleigh Rose Cirunay, Ison Calimpang, Josel Mansueto, Irish Alysa Herlao, and June Kristine Ando, who had been instrumental to the completion of this research project. Finally, our thanks to the Department of Science and Technology-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), Department of Science and Technology-Philippine Council for Health Research and Development (DOST-PCHRD), and UPLB (through the Academic Development Fund) for various grants which made the study possible..

#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This work was supported by the DOST-PCAARRD, DOST-PCHRD and OVCAA ADF dissertation grants.

#### **Author contributions**

Renerio P. Gentallan: Conceptualization (lead), Data curation (lead), Funding acquisition (equal), Project administration (lead), Resources (lead), Supervision (lead), Formal analysis (equal), Investigation (equal), Writing - original draft (equal), Writing - review and editing (lead). Seda Sengun: Conceptualization (equal), Data curation (equal), Resources (supporting), Supervision (supporting), Formal analysis (supporting), Investigation (equal), Writing - original draft (supporting), Writing - review and editing (supporting). Michael Cedric B. Bartolome: Conceptualization (equal), Data curation (equal), Resources (supporting), Supervision (supporting), Formal analysis (supporting), Investigation (equal), Writing - original draft (supporting), Writing - review and editing (supporting). Emmanuel Bonifacio S. Timog: Conceptualization (supporting), Data curation (equal), Resources (supporting), Supervision (supporting), Formal analysis (supporting), Investigation (supporting), Writing - original draft (supporting), Writing - review and editing (supporting). Kristine Joyce O. Quiñones and Nadine B. Coronado: Methodology (supporting), Formal analysis (supporting), Investigation (supporting), Writing - original draft (supporting), Validation (supporting), Writing - review and editing (supporting). Teresita H. Borromeo: Conceptualization (supporting), Resources (supporting), Formal analysis (supporting), Investigation (supporting), Validation (supporting), Writing - review and editing (supporting).

#### Author ORCIDs

Renerio P. Gentallan Jr. <sup>®</sup> https://orcid.org/0000-0002-6436-7878 Seda Sengun <sup>®</sup> https://orcid.org/0000-0003-3126-8707 Michael Cedric B. Bartolome <sup>®</sup> https://orcid.org/0000-0002-3579-1194 Kristine Joyce O. Quiñones <sup>®</sup> https://orcid.org/0000-0002-1709-3317 Nadine B. Coronado <sup>®</sup> https://orcid.org/0000-0002-4951-3830 Teresita H. Borromeo <sup>®</sup> https://orcid.org/0000-0002-8625-4110 Emmanuel Bonifacio S. Timog <sup>®</sup> https://orcid.org/0000-0002-0302-6519
#### **Data availability**

All of the data that support the findings of this study are available in the main text or Supplementary Information.

# References

- Amiryousefi A, Hyvönen J, Poczai P (2018) IRscope: An online program to visualize the junction sites of chloroplast genomes. Bioinformatics (Oxford, England) 34(17): 3030–3031. https://doi.org/10.1093/bioinformatics/bty220
- Blanco M (1845) Flora de Filipinas: segun el sistema sexual de Linneo. Candido Lopez, Manila. https://doi.org/10.5962/bhl.title.120031
- Bramley GL, Forest F, De Kok RP (2009) Troublesome tropical mints: Re-examining generic limits of Vitex and relations (Lamiaceae) in South East Asia. Taxon 58(2): 500– 510. https://doi.org/10.1002/tax.582014
- Cantoria M (1989) Pharmacognostical studies on *Vitex negundo* L. Lagundi (*Vitex negundo* L.). Lagundi (*Vitex negundo* L.) Monograph. Philippine Council for Health and Research Development, Department of Science and Technology, 82–83.
- CDFP (2023) In: Pelser PB, Barcelona JF, Nickrent DL (Eds) Co's Digital Flora of the Philippines. www.philippineplants.org
- Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics (Oxford, England) 34(17): i884–i890. https://doi.org/10.1093/bioinformatics/bty560
- Chen YP, Zhao F, Paton AJ, Sunojkumar P, Gao LM, Xiang CL (2022) Plastome sequences fail to resolve shallow level relationships within the rapidly radiated genus *Isodon* (Lamiaceae). Frontiers in Plant Science 13: 985488. https://doi.org/10.3389/fpls.2022.985488
- Dayrit FM, Lapid MRJ, Cagampang JV, Lagurin LG (1987) Phytochemical studies on the leaves of Vitex negundo, L. (" Lagundi"), 1: Investigations of the bronchial relaxing constituents. Philippine Journal of Science 116(4): 403–410. [Philippines]
- de Kok RP (2007) The genus *Vitex* L. (Lamiaceae) in New Guinea and South Pacific Islands. Kew Bulletin 62: 587–603.
- de Kok RP (2008) The genus *Vitex* (Labiatae) in the flora Malesiana region, excluding New Guinea. Kew Bulletin 63(1): 17–40. https://doi.org/10.1007/s12225-007-9013-7
- de Kok RP, Sengun S (2020) *Vitex*. In: Bramley GLC (Ed.) Flora Malesiana Series I, Volume 23 (Lamiaceae). Singapore Botanic Gardens, Singapore, 397–421.
- Dong SS, Wang YL, Xia NH, Liu Y, Liu M, Lian L, Li N, Li LF, Lang XA, Gong YQ, Chen L, Wu E, Zhang S-Z (2022) Plastid and nuclear phylogenomic incongruences and biogeographic implications of *Magnolia* sl (Magnoliaceae). Journal of Systematics and Evolution 60(1): 1–15. https://doi.org/10.1111/jse.12727
- Doyle J, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Greiner S, Lehwark P, Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.
  1: An expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Research 47(W1): 59–64. https://doi.org/10.1093/nar/gkz238
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754
- Jin JJ, Yu WB, Yang JB, Song Y, Depamphilis CW, Yi TS, Li DZ (2020) GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biology 21(1): 1–31. https://doi.org/10.1186/s13059-020-02154-5

- Kassambara A, Mundt F (2017) factoextra: Extract and visualize the results of multivariate data analyses (R package version 1.0.7).
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
- Lam HJ (1919) The Verbenaceae of the Malayan Archipelago. de Waal, Groningen.
- Lamarck JBAP (1786) Encyclopedie methodique: Botanique, Volume 2: Supplementary. Chez Agasse, Paris.
- Le S, Josse J, Husson F (2008) FactoMineR: An R package for multivariate analysis. Journal of Statistical Software 25(1): 1–18. https://doi.org/10.18637/jss.v025.i01
- Madulid DA (2001) A Dictionary of Philippine plant names, Volume I: local name-scientific name. The Bookmark Inc., 401 pp.
- Manalo J (1982) A study of lagundi oil: The essential oil from *Vitex negundo* Linn. Growing in the Philippines. Philippine Journal of Science 111(3): 79–97.
- Media RHS (2015) Royal Horticultural Society Colour Chart. Royal Horticultural Society, London, 2015.
- Merrill ED (1912) A flora of Manila. Bureau of printing, Manila. https://doi.org/10.5962/ bhl.title.54449
- Merrill ED (1918) Species Blancoanae: a critical revision of the Philippine species of plants described by Blanco and by Llanos (No. 12). Bureau of Printing, Manila. https://doi.org/10.5962/bhl.title.2116
- Merrill ED (1923) An Enumeration of Philippine Flowering Plants. Volume 2. Bureau of Printing, Manila. https://doi.org/10.5962/bhl.title.49412
- Moldenke HN (1957) Materials toward a monograph of the genus *Vitex* VIII. Phytologia 6: 13–64. https://doi.org/10.5962/bhl.part.9770
- Moldenke HN (1978) Notes on new and noteworthy plants CVII. Phytologia 38: 307– 308. https://doi.org/10.5962/bhl.part.23396
- Morales-Briones DF, Liston A, Tank DC (2018) Phylogenomic analyses reveal a deep history of hybridization and polyploidy in the Neotropical genus *Lachemilla* (Rosaceae). The New Phytologist 218(4): 1668–1684. https://doi.org/10.1111/nph.15099
- Munir AA (1987) A taxonomic revision of the genus *Vitex* L. (Verbenaceae) in Australia. Journal of the Adelaide Botanic Gardens 10: 31–79.
- Nei M, Kumar S (2000) Molecular Evolutionary Phylogenetics. Oxford University Press. https://doi.org/10.1093/oso/9780195135848.001.0001
- Olivar JEC, Alaba JPE, Atienza JFM, Tan JJS, Umali MT IV, Alejandro GJD (2016) Establishment of a standard reference material (SRM) herbal DNA barcode library of *Vitex negundo* L.(lagundi) for quality control measures. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment 33(5): 741–748. https://doi.org/10.1080/19440049.2016.1166525
- PCHRD (2010) Have cough? Go natural with Lagundi. Facilitated by the Philippine Council for Health Research and Development. [Published on the Internet] http://www. pchrd.dost.gov.ph/index.php/2uncategorised/3285-have-cough-go-natural-with-Lagundi [Retrieved 07 March 2015]
- POWO (2023) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. [Published on the Internet] http://www.plantsoftheworldonline.org/ [Retrieved 21 July 2023]

- PP1 (2004) Philippine Pharmacopeia, 1. Bureau of Food and Drugs, Department of Health and Japan International Cooperation Agency. Himiko Arts and Concepts, Manila.
- Sengun S (2017) Taxonomy, phylogenetics and chemosystematics of the *Vitex trifolia* complex (Lamiaceae) (Doctoral dissertation, University of London). The Royal Botanic Gardens, Kew Research Repository. https://kew.iro.bl.uk/catalog?utf8=%E2%9C%93&search\_field=all\_fields&q=Seda+Sengun+
- Sengun S, Ngrouille M, Paton A, De Kok RP (2024) Taxonomic Revision of the *Vitex trifolia* Complex (Lamiaceae). Blumea 69(2): 93–121. https://doi.org/10.3767/ blumea.2024.69.02.01
- Shi L, Chen H, Jiang M, Wang L, Wu X, Huang L, Liu C (2019) CPGAVAS2, an integrated plastome sequence annotator and analyzer. Nucleic Acids Research 47(W1): 65–73. https://doi.org/10.1093/nar/gkz345
- Simmonds SE, Smith JF, Davidson C, Buerki S (2021) Phylogenetics and comparative plastome genomics of two of the largest genera of angiosperms, *Piper* and *Peperomia* (Piperaceae). Molecular Phylogenetics and Evolution 163: 107229. https://doi. org/10.1016/j.ympev.2021.107229
- Suzuki Y, Glazko GV, Nei M (2002) Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proceedings of the National Academy of Sciences of the United States of America 99(25): 16138–16143. https://doi.org/10.1073/ pnas.212646199
- Tadeo NN (2011) Anthelmintic Efficacy of Lagundi (*Vitex negundo* Linn) and Banaba (*Lagerstroemia* Linn) Extracts on the Gastro-Intestinal Parasites of Goat. ISU-Cabagan Journal of Research 20(1): 11–23.
- Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S (2017) GeSeq-versatile and accurate annotation of organelle genomes. Nucleic Acids Research 45(W1): 6–11. https://doi.org/10.1093/nar/gkx391
- van Steenis CGGJ (1957) Miscellaneous botanical notes VIII. Blumea 8: 514-517.
- WFO (2023) World Flora Online. http://www.worldfloraonline.org/ [21 July 2023]
- Yang Z, Rannala B (1997) Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. Molecular Biology and Evolution 4(7): 717–724. https://doi.org/10.1093/oxfordjournals.molbev.a025811
- Zhao F, Chen YP, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, Min DZ, Peng H, Olmstead RG, Li B, Xiang C-L (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biology 19(1): 2. https://doi.org/10.1186/s12915-020-00931-z

# Supplementary material 1

#### Additional information

- Authors: Renerio P. Gentallan Jr., Seda Sengun, Michael Cedric B. Bartolome, Kristine Joyce O. Quiñones, Nadine B. Coronado, Teresita H. Borromeo, Emmanuel Bonifacio S. Timog Data type: xlsx
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.120387.suppl1

# **Supplementary material 2**

#### **Chloroplast genome information**

Authors: Renerio P. Gentallan Jr., Seda Sengun, Michael Cedric B. Bartolome, Kristine Joyce

O. Quiñones, Nadine B. Coronado, Teresita H. Borromeo, Emmanuel Bonifacio S. Timog Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.120387.suppl2

# **Supplementary material 3**

#### Additional specimens examined

Authors: Renerio P. Gentallan Jr., Seda Sengun, Michael Cedric B. Bartolome, Kristine Joyce O. Quiñones, Nadine B. Coronado, Teresita H. Borromeo, Emmanuel Bonifacio S. Timog Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.120387.suppl3

# PhytoKeys

Research Article

# Speciation and diversification of the *Bupleurum* (Apiaceae) in East Asia

Yong-xiu Song<sup>1</sup>, Ceng-yue Yang<sup>1</sup>, Yu-Yang Zhou<sup>1</sup>, Yan Yu<sup>1</sup>

1 Key Laboratory of Bio-Resources and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610064, China Corresponding author: Yan Yu (yyu@scu.edu.cn)

#### Abstract

Bupleurum, belonging to the Apiaceae, is widely distributed across the Eurasian continent. The origin and species diversification of Bupleurum in East Asia, remain incompletely resolved due to the limited samples in previous studies. To address these issues, we have reconstructed a robust phylogenetic framework for Bupleurum in East Asia based on the ITS and three plastid genes. Our phylogenetic analysis confirms the monophyly of Bupleurum with strong support. Both ITS and chloroplast dataset divided the Bupleurum in East Asia into East Asia Group I and East Asia Group II in this study. The divergence time and ancestral area reconstruction of ITS dataset indicated that the Bupleurum originated in the Mediterranean basin and its adjacent areas around 50.33 Ma. subg. Penninervia and subg. Bupleurum diverged at about 44.35 Ma, which may be related to the collision of India with the Eurasian continent. Both East Asia Group I and East Asia Group II originated from a common ancestor in the Mediterranean, East Asia Group I divergence around 12.95 Ma; East Asia Group II divergence around 13.32 Ma. The character reconstruction showed that the morphological characters and altitude distribution analyzed in this study exhibit a scattered distribution in East Asian Group I and East Asian Group II. Additionally, diversification rate analysis shows that the East Asian Group I and East Asian Group II exhibited no significant shifts in diversification rates in the evolutionary history according to ITS and combined dataset. Both molecular and morphological data supports that East Asian Bupleurum is a museum taxon, meaning that the species diversity of East Asian Bupleurum has gradually accumulated over time.



Academic editor: Alexander Sennikov Received: 22 July 2024 Accepted: 31 August 2024 Published: 22 October 2024

**Citation:** Song Y-x, Yang C-y, Zhou Y-Y, Yu Y (2024) Speciation and diversification of the *Bupleurum* (Apiaceae) in East Asia. PhytoKeys 248: 41–57. https://doi.org/10.3897/ phytokeys.248.132707

**Copyright:** <sup>©</sup> Yong-xiu Song et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Key words: Bupleurum, museum model, phylogeny, species diversity

# Introduction

Understanding the spatiotemporal distribution patterns of species, along with their diversification mechanisms (i.e., the historical process by which the same ancestor evolved successively through time and space to produce existing species) has long been an important issue in evolutionary biology and ecology (Shrestha et al. 2018). *Bupleurum* L. stands out as a uniquely distinct group within the Apiaceae characterized by its simple, entire leaves. It comprises about 180–200 species, of which about 50 are found in East Asia (Linczevski 1950; Sheh and Watson 2005). This genus is widely distributed in the North

temperate regions of Eurasia, with two exceptions: *B. americanum* J. M. Coult. and Rose in North America and *B. mundii* Cham. and Schltdl. in South Africa (Neves and Watson 2004). *Bupleurum* has two recognized hotspots of diversity: the Mediterranean Basin and the Himalaya-Hengduan Mountains region (Neves and Watson 2004; Huang et al. 2021a). *Bupleurum* possesses a rich morphological and ecological diversity, ranging from annual to perennial herbs, and even includes subshrubs, shrubs, such as *B. gibraltaricum* and *B. fruticosum* native to the Mediterranean and adjacent areas, and *B. dracaenoides* endemic to the Hengduan Mountains (Wang et al. 2011; Huang et al. 2021b).

Molecular phylogenetics evidence supports Bupleurum as a monophyletic group (Neves and Watson 2004; Wang et al. 2008, 2011; Banasiak et al. 2013; Wen et al. 2021). Combining morphological and phylogenetic data, existing studies have divided the Bupleurum genus into two subgenera: subg. Penninervia and subg. Bupleurum (Neves and Watson 2004). Subg. Penninervia includes the Mediterranean woody and perennial species characterized by pinnate reticulate veins; while subg. Bupleurum comprises the majority of species, distinguished by their parallel veins. Furthermore, Neves and Watson (2004) based on ITS or a few plastid markers have hypothesized that the Bupleurum originated in the western Mediterranean. Bupleurum in East Asia have been divided into two groups: East Asia Group I and East Asia Group II (Wang et al. 2008, 2011; Ma 2011; Ma et al. 2013, 2014). The two East Asia groups of the Bupleurum are believed to have originated from species near the Mediterranean, subsequently migrating eastward through the Middle East and the Caucasus to East Asia (Neves and Watson 2004; Wang et al. 2008, 2011). Moreover, a genealogical-geographical study of *B. longiradiatum* by Zhao et al. (2013). showed that B. longiradiatum is a genetically diverse species, with two corresponding refugia found throughout its distribution range.

Thus, as a monophyletic group broadly distributed across the Eurasian continent, the *Bupleurum*, with its rich species diversity, serves as an excellent subject for studying patterns in species richness distribution. Previous research on East Asian *Bupleurum* has primarily focused on taxonomy, phylogenetics, and phylogeography (Wang et al. 2011; Huang et al. 2021b). There is a lack of research on the spatiotemporal distribution patterns and species diversification within East Asian *Bupleurum*.

Here, we collected ITS and three plastid genes (matK, psbA - trnH, and rbcL) for 89 species of *Bupleurum* as a source of phylogenetic information; The purpose of our study was to (1) reconstruct a phylogenetic framework of the East Asian *Bupleurum*, and reconstruct the ancestral distribution of the East Asian *Bupleurum* to explore its origins and dispersal processes; and (2) perform divergence time estimation and diversification rate analysis, revealing the species formation patterns of East Asian *Bupleurum*.

#### Materials and methods

#### Materials collection and sequencing

89 *Bupleurum* representing all major branches within the *Bupleurum* were used for the phylogenetic analysis in this study. Of these, 25 species were newly collected from the wild. The fresh leaves were collected and preserved in silica gel.

Voucher specimens were collected and deposited in Sichuan University Herbarium (SZ). (Suppl. material 1: table S1). 61 ITS sequences and three plastid genes (56 matK, 49 psbA - trnH, and 49 rbcL) of *Bupleurum* were downloaded from NCBI. The species names and GenBank accession numbers are listed in Suppl. material 1: table S2. The downloaded sequences were used as a reference to extract the ITS sequences and chloroplast genes of the corresponding species from the next-generation sequencing data of 25 newly sequenced species and the raw sequencing da-ta of 9 *Bupleurum* species (5 from the SSR library and 4 from the study by Huang et al. (2021a) using GeneMiner (Xie et al. 2024). ITS and three chloroplast genes from 34 *Bupleurum* species were extracted using GeneMiner. A total of 86 ITS, 66 matK, 64 psbA-trnH, and 64 rbcL were used for phylogenetic analysis.

# **Phylogeny reconstruction**

Species from the Apioideae: Chamaesium novem jugum, C. mallaeanum, C. wolffianum, C. thalictrifolium, C. spatuliferum, C. delavayi and species from the Saniculoideae: Sanicula astrantiifolia, S. canadensis were used as outgroup. ITS dataset and plastid genes dataset were aligned using MAFFT (Katoh and Standley 2013) and then trimmed using TrimAl v1.2 (Capella-Gutierrez et al. 2009). Due to the limited number of parsimony-informative sites in plastid genes, which increases the potential for gene tree errors, we inferred the plastid species trees using the concatenation method, applying both Maximum Likelihood (ML) and Bayesian Inference (BI) techniques. ITS, matK, psbA-trnH, and rbcL sequences were concatenated using PhyloSuite (Zhang et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used to construct and determine the best-fitting nucleotide substitution models for each dataset. The maximum likelihood (ML) analyses of the above datasets were using FastTree 2.1 (Price et al. 2010) performing 10000 bootstrap replicates with the GTR + G model. The MrBayes v3.2.7 (Ronquist et al. 2012) was selected for Bayesian analysis under GTR + I + G model. Two independent Markov chain Monte Carlo (MCMC) runs were performed, each with two chains of 10000000 generations, of which every 1000 generation was sampled. After discarding the first 25% trees as the burn-in, a consensus tree of the remaining trees was produced.

# Estimation of divergence time

Divergence time of *Bupleurum* was estimated with a lognormal relaxed molecular clock model in BEAST v1.10.4 (Drummond and Rambaut 2007). The pollen fossil adopted by (Banasiak et al. 2013) was used to determine species node priors. The calibration point was placed at the stem node of Bupleureae (Gruascavagnetto and Cerceaularrival 1984) with a lower bound of 33.90 Mya (the end of the Priabonian) and the upper bound of 58.7 Mya (the beginning of the Thanetian). The BEAST analysis was run for 100 million generations sampling every 10000 generations. The GTR + G substitution model was selected and a Yule tree prior was used for the analysis. The stationarity of the chains and convergence of two runs was monitored by Trace v1.7 (Rambaut et al. 2018), with the effective sample size of all parameters > 200.

#### **Reconstruction of ancestral area**

The following seven regions were defined for biogeographic analyses based on the natural geography and climatic history and also according to the distribution of *Bupleurum*: (A) the Mediterranean Basin, North Africa, and Europe; (B) Central Asia and West Asia; (C) the Eastern Himalayas - Hengduan Mountains region and South Asia; (D) East Asia; (E) North Asia; (F) North America; (G) Southern Africa. The distribution areas of the study species were determined based on GBIF (2023), POWO (2024) and the WFO (2024), and field observations. Reconstructions of the ancestral area of *Bupleurum* were conducted using the Bayesian Binary MCMC as implemented in the RASP 4 (Yu et al. 2020). We used the divergence times tree based on ITS for Bayesian Binary MCMC analysis. We removed outgroups before ancestral-state reconstruction to avoid biased inferences for the crown node of the ingroups, which could arise from uncertainty in the root areas of an outgroup.

# Morphological character evolution

We collected 7 characters (key taxonomic traits for identifying species of the *Bupleurum* in the WFO (2024)) and altitude distribution for 55 species of the *Bupleurum*. The above ITS divergence time tree was used for character evolution analysis after removing the outgroups and the species with extensive missing morphological data. We conducted the reconstruction of an ancestral trait of East Asian *Bupleurum* using MultiState Reconstruction with the Bayes Traits method implemented in RASP 4 (Yu et al. 2020). All the characters were treated as unordered and equally weighted. These morphological characters and altitudes were mapped and coded in Table 1. The matrix for the East Asian *Bupleurum* was compiled based on specimens, available literature, and databases (such as GBIF 2023; WFO 2024), and is presented in Suppl. material 1: table S3.

# **Diversification rate analysis**

Time-calibrated tree based on the ITS and the combined ITS and chloroplast dataset of the *Bupleurum* were used for diversification rate analysis in BAMM (Shi and Rabosky 2015). After removing the outgroups, we ran 10000000 generations of MCMC, discarding the first 10% as burn-in and conducted analysis and plotting

Character	Character states
Plant height	A < 50 cm; B ≥ 50 cm
Stem base with fibrous remnant sheaths	A no; B yes
Rays	A < 3; B ≥ 3 < 6; C ≥ 6
Number of bracteoles	A < 5; B ≥ 5
Shape of bracteoles	A ovate (broadly ovate, obovate); B elliptic; C lanceolate (narrowly lanceolate, ovate-lanceolate); D linear; E suborbicular
Petals color	A yellow; B purple; C green;
Vittae in each furrow	A < 3; B ≥ 3
Altitudes	A < 1000 m; B 1000–3000 m; C > 3000 m

#### Table 1. Coding of morphological characters.

using the BAMMtools (Rabosky et al. 2014). Additionally, we used MEDUSA (Alfaro et al. 2009) to estimate shifts in diversification rates. After removing the outgroups, the tree was imported into the R package MEDUSA for computation.

# Results

# Gene extraction and phylogenetic analyses

The downloaded ITS and chloroplast sequence data were used as reference se-quences to evaluate the assembly results based on sequence similarity. The median se-quence similarity for all samples, except *Bupleurum fruticosum*, was above 90%, indicating a high level of reliability (Fig. 1, Suppl. material 1: table S4).

The ITS and the three plastid genes recognized the *Bupleurum* as monophyletic with robust support (PP = 1.00, BS = 100%) (Fig. 2, Suppl. material 2: fig. S1). Two major groups were recovered within East Asian *Bupleurum*, EA Group I and EA Group II. According to ITS dataset, EA Group I is sister to *Bupleurum odontites*, *Bupleurum praealtum*, *Bupleurum gerardii*; EA Group II is sister to clade of species in Mediterranean.

# **Estimation of divergence times**

Divergence time analyses based on the combined dataset are basically consistent with the ITS dataset of the *Bupleurum* (Fig. 3a, c). According to the ITS dataset, the stem age of *Bupleurum* was estimated as ca. 50.33 Ma (95% HPD: 39.16–65.36 Ma). The crown group of this genus was dated to the middle







**Figure 2.** Phylogenetic relationships of *Bupleurum* inferred from ITS based on BI methods and ML methods. PP (posterior probability) values and BS (bootstrap) values are shown above the branches (only PP < 1.0 are shown) **A** tree topology inferred by BI methods **B** tree topology inferred by ML methods.

Eocene (ca. 44.35 Ma; 95% HPD: 37.38–52.77 Ma) and then diverged into two major clades (subgen. *Penninervia* and subgen. *Bupleurum*, Fig. 3a). The crown of the subgen. *Bupleurum* was dated to the middle Oligocene (ca. 27.92 Ma; 95% HPD: 19.23–37.07 Ma); and the crown of EA Group I is predicted to have originated at ca.12.95 Ma (95% HPD: 7.61–18.66 Ma), and EA Group II originated at ~13.32 Ma (95% HPD: 7.52–19.77 Ma).

## **Reconstruction of ancestral area**

The reconstructions of the ancestral area based on the ITS dataset (Fig. 4) supported the most likely ancestral distribution of *Bupleurum* as being in the Mediterranean Basin, which served as a site of diversification. Subgen. *Penninervia* 



**Figure 3.** The estimation of divergence time and diversification rate analysis of *Bupleurum* **A**, **B** ITS **C**, **D** ITS **+** matK + psbA - trnH + rbcL **A**, **C** estimation of divergence time of Bupleurum, the 95% highest posterior density (HPD) estimates for each well-supported clade are represented by bars, historical carbon dioxide levels data from TimeTree **B**, **D** diversification rate analysis of *Bupleurum*.

and subgen. *Bupleurum* diverged in the mid-Eocene, approximately 44.35 Ma. Subg. *Bupleurum* experienced its first divergence at around 27.92 Ma, where one of its clades, diverging at 12.95 Ma, spread to the Qinghai-Tibet Plateau -Hengduan Mountains region, forming the East Asia Group I. The other branch diverged in the Mediterranean basin around 22.15 Ma, with one clade remaining in the Mediterranean. Meanwhile, another clade dispersal occurred from Central Asia to East Asia, forming the East Asia Group II.

#### **Character evolution**

The 8 characters including macroscopic characters and altitude distribution were mapped on the phylogenetic tree to reconstruct ancestral states and analyze evolution trends. The traits examined in this study exhibit a scattered distribution across



**Figure 4.** Reconstructions of the ancestral area based on ITS-based phylogeny of *Bupleurum*. node pie show the inferred ancestral ranges, pie colors to regions defined in the caption and world map.

the phylogenetic tree (Fig. 5), with every observed state of the traits occurring within both the East Asian Group I and East Asian Group II. And none of the traits' state were unique to a particular evolutionary clade. Morphological characters and altitude distribution are highly variable. The shape of the bracteoles varies widely from ovate, elliptic, lanceolate, linear, to suborbicular, and the rays varies from 2 to 18. In addition, *Bupleurum* has a wide altitudinal distribution, ranging from 100 to 4300 meters above sea level, as illustrated in Suppl. material 2: figs S2, S3, S4, S5.

#### **Diversification rate analysis**

Diversification rate analysis of the ITS dataset and the combined dataset using BAMM yielded similar results (Fig. 3b, d). Utilizing the ITS dataset, *Bupleurum* evolved at a relatively constant rate (average speciation rate  $\lambda = 0.1888$  Myr<sup>1</sup>) in the evolutionary history (Fig. 3b). The net diversification rate of all *Bupleurum* species estimated by BAMM increased slowly over time with the speciation rate ranging from a minimum of  $\lambda = 0.1048$  to a maximum of  $\lambda = 0.3587$  Myr<sup>1</sup>. The speciation rate for East Asian group I was 0.1926 Myr<sup>1</sup>, and that for East Asian group II was 0.1930 Myr<sup>1</sup>. The results from the MEDUSA analysis indicate that no significant shifts in diversification rate were detected in either the ITS dataset or the combined dataset.



Figure 5. Reconstruction of the ancestral trait of the morphological character for *Bupleurum* **a**–**h** represent different traits and different colors represent different trait states **a** plant height **b** stem base with fibrous remnant sheaths **c** rays **d** number of bracteoles **e** shape of bracteoles **f** petals color **g** vittae in each furrow **h** altitudes.

# Discussion

# The origin and spread of the Bupleurum in East Asia

As a widely distributed genus within the Apiaceae, phylogenetic relationships among the main branches of *Bupleurum* have consistently attracted significant interest in previous studies (Neves and Watson 2004; Sheh and Watson 2005; Wang et al. 2008, 2011; Huang et al. 2021a). Our phylogenetic analyses of both the ITS genes' dataset and the plastid genes dataset presented here robustly support earlier studies that *Bupleurum* is monophyletic (Wang et al. 2008, 2011). *Bupleurum* in East Asia was partitioned into two groups, namely, East Asia Group I and East Asia Group II. Species in East Asian Group I are exclusively found in the Tibetan Plateau and the Hengduan Mountains, while the species of East Asian Group II are widely distributed in the central and eastern of Asia and Europe, as well as in the northern of North America (*B. americanum*, endemic to North America).

This study conducted extensive sampling in the two distribution centers of the *Bupleurum*: the Mediterranean Basin and the Qinghai-Tibet Plateau-Hengduan Mountains region. Based on the ITS dataset, divergence time and an-

cestral area reconstructions for the Bupleurum suggest that it diverged in the Eocene (50.33 Ma) in the Mediterranean Basin (a center of diversification for many seed plants) and nearby areas, which is consistent with previous studies (Neves and Watson 2004; Banasiak et al. 2013; Wen et al. 2021; Calvino et al. 2016). In this study, the subg. Penninervia and subg. Bupleurum began to diverge at 44.35 Ma. Then, subg. Penninervia remained in the Mediterranean and nearby areas, while subg. Bupleurum spread to East Asia, forming the East Asian group. During this period, carbon dioxide levels were high, leading to a relatively warm global climate. The Indian plate and the Eurasian plate were in the midst of their collision (approximately 50-25 Ma), resulting in the rapid uplift of the Himalayas and the Tibetan Plateau. These geological events profoundly impacted the evolution of biota on the Eurasian continent. The collision between India and Eurasia, combined with the high-carbon environment, likely created new ecological niches that promoted species differentiation. This may have contributed to the divergence of the Bupleurum. (Aitchison et al. 2007; van Hinsbergen et al. 2012; Yang et al. 2021). The first diversification within subg. Bupleurum occurred at 27.92 Ma. One clade, diversifying at 19.8 Ma, spread to the Qinghai-Tibet Plateau - Hengduan Mountains region, forming the East Asian group I. Another clade diverged at 22.15 Ma in the Mediterranean Basin, with one subclade remaining there and another spreading from Central Asia to East Asia, forming the East Asian group II. During this period of divergence in the Oligocene, the global climate gradually cooled, and carbon dioxide levels decreased. These climatic changes likely exerted evolutionary pressure on the species, possibly leading to the divergence of subgenera of Bupleurum.

Fluctuations in carbon dioxide levels and significant climate changes imposed various pressures on species evolution during the Miocene epoch (23-10 Ma). Both the Himalayas and the Tianshan Mountains experienced a significant uplift, and a drying event in Central Asia led to drastic reductions in rainfall, greatly impacting many taxons (Yu et al. 2014; Yang et al. 2017; Zheng et al. 2021). Thus, the diversification of the two East Asian groups of Bupleurum might be related to these events. The crown of East Asian groups I and East Asian groups II are estimated to be 12.95 Ma and 13.32 Ma, respectively. After the late Miocene (10 Ma - present), the Tibetan Plateau underwent further uplift and expansion, while the Hengduan Mountains in its southeastern margin experienced intense orogenic activity. Warm, moist air from the Indian Ocean, blocked by the Himalayas and Kailash Range, entered the East Asian region through the Hengduan Mountains, bringing significant rainfall. These continuous geological uplift events further intensified the monsoon climate in East Asia, significantly impacting the regional and global climate patterns. These events collectively shaped the complex geological and geomorphological features of the Hengduan Mountains, providing diverse habitats for plants and making it one of the global biodiversity hotspots (López-Pujol et al. 2011; He and Jiang 2014; Sklenar et al. 2014; Yang et al. 2017; Xie et al. 2019). The diversification within East Asian groups I and II may have been influenced by these geographical processes. It is speculated that the species diversification of East Asian group I was mainly influenced by the dramatic uplift of the Hengduan Mountains, while the diversification of East Asian group II, originating from Central Asia, was more influenced by the monsoon climate. Field observations and herbarium records indicate that species from East Asian group II prefer

drier, sunnier environments (Wang et al. 2008, 2011; Ma 2011; Ma et al. 2013, 2014), which may be related to the arid conditions of Central and West Asia. Additionally, ancestral area reconstructions based on ITS also suggest that the common ancestor of East Asian group II likely originated from Central Asia.

Interestingly, *B. americanum* (endemic to North America) is placed in East Asian group II. Phylogenetic indicate that it is closely related to *B. euphorbioides*, which is located at the eastern edge of the Eurasian continent. We speculate that the ancestor of *B. americanum* spread from the eastern edge of Eurasia to North America via the Bering Land Bridge. Meanwhile, *B. mundii*, an endemic species in South Africa, is placed in basal clades of East Asian group I. This suggests that the ancestor of *B. mundii* likely originated from the Mediterranean.

#### The Bupleurum in East Asia is a museum taxon

Two classical models have been used to explain patterns of species diversity from an evolutionary perspective. The first model is the "evolutionary cradle" model, which emphasizes certain events in history that created ecological opportunities for ancestral species to undergo adaptive radiation. Its main characteristic is the temporal and spatial variation in diversification rates, accumulating diversity rapidly through high species formation rates (Fischer 1960). For instance, Madagascar is often considered an "evolutionary cradle" because it hosts many unique and recently evolved species (Pastorini et al. 2009; Florio et al. 2012). The second model is the "museum" model. It highlights the comparatively stable diversification rates and lower extinction rates of species over time and space, allowing species diversity to accumulate gradually. This model posits that older evolutionary branches have more species because they have had more time to accumulate diversity, and this is unrelated to changes in diversification rates (Haffer 1969; Gentry 1982; Schley et al. 2018; Loiseau et al. 2020; Vargas and Dick 2020). An example includes the Troidini butterflies of the Neotropics (Condamine et al. 2012). Overall, the former focuses more on the recent origin and rapid evolution of species, while the latter values the longterm persistence and stability of species. Ultimately, the patterns of species diversity are generated by the processes of speciation, extinction and dispersal that occur over evolutionary time-scales (Wiens and Donoghue 2004).

In our diversification rate analysis based on ITS dataset and the combined dataset for the *Bupleurum*, we evaluated whether there were differences in evolutionary rates among different clades of the East Asian *Bupleurum*. Neither BAMM nor MEDUSA analyses based on ITS detected any diversification rate shifts within the East Asian group. Given that both distribution centers of *Bupleurum* are extensively sampled and species richness is lower in other regions, it is less likely to detect changes in species diversification rates, suggesting a low probability of significant diversification rate shifts within *Bupleurum*. The character reconstruction showed that every state of traits and altitude distribution were observed within both the East Asian Group I and East Asian Group II, showing a high degree of variability and that none of the trait states were unique to a particular evolutionary clade.

Combining the results of molecular data and character evolution, it can be hypothesized that the East Asian *Bupleurum* support the "museum" model, i.e., their rate of diversification has not undergone any abrupt shifts during their long evolutionary history. More extensive research on the diversification rates of the Apioideae supports this conclusion (Baczyński et al. 2022). Although the specific estimates of diversification rates vary, unlike rapid diversification rate shifts within groups like the Scandicineae and Tordyliinae Drude, there is no shift in diversification rates within the East Asian *Bupleurum*.

# Conclusions

This paper reconstructs the phylogenetic relationships of the Bupleurum in East Asia based on ITS dataset and the combined dataset. The results suggest that the Bupleurum is a monophyletic group, and that East Asian Bupleurum is further divided into East Asian Group I and East Asian Group II. The study on the divergence time and ancestral area reconstruction of the Bupleurum indicates that it differentiated in the Mediterranean basin and nearby areas around 50.33 Ma, with two subgenera (subg. Penninervia and subg. Bupleurum) diverging around 44.35 Ma, possibly related to the collision of India with the Eurasian continent. The speciation of East Asian Group I species might have been influenced by the dramatic uplift of the Hengduan Mountains, while the diversification of East Asian Group II could be more affected by the monsoon climate, possibly related to the arid environment of Central Asia. Additionally, the results of the diversification rate analyses based on ITS and the combined dataset, conducted using both BAMM and MEDUSA methods consistently indicated that there were no significant changes in diversification rates in the evolutionary history of the East Asian Bupleurum. The character reconstruction showed that every state of traits and altitude distribution were observed within both the East Asian Group I and East Asian Group II. Both molecular and morphological evidence support the East Asian Bupleurum as a 'museum' taxon.

# Acknowledgements

We thank the Shanghai Personal Biotechnology company for sequencing.

# Additional information

# **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

# Funding

This work was funded by the National Natural Science Foundation of China (Grant No. 32071666, 32271552) and the Science & Technology Fundamental Resources Investigation Program (Grant No. 2022FY101000).

# **Author contributions**

Methodology, conceptualization, formal analysis, and original draft preparation, Yongxiu Song; software and validation, Ceng-yue Yang; data curation, investigation, and resources, Yu-yang Zhou. and Ceng-yue Yang; funding acquisition, manuscript review and editing, visualization, and supervision, Yan Yu. All authors have read and agreed to the published version of the manuscript.

#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

# References

- Aitchison JC, Ali JR, Davis AM (2007) When and where did India and Asia collide? Journal of Geophysical Research 112(B5): 2006JB004706. https://doi.org/10.1029/ 2006JB004706
- Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ (2009) Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proceedings of the National Academy of Sciences of the United States of America 106(32): 13410–13414. https://doi.org/10.1073/pnas.0811087106
- Baczyński J, Sauquet H, Spalik K (2022) Exceptional evolutionary lability of flower-like inflorescences (pseudanthia) in Apiaceae subfamily Apioideae. American Journal of Botany 109(3): 437–455. https://doi.org/10.1002/ajb2.1819
- Banasiak A, Piwczyński M, Uliński T, Downie SR, Watson MF, Shakya B, Spalik K (2013) Dispersal patterns in space and time: A case study of Apiaceae subfamily Apioideae. Journal of Biogeography 40(7): 1324–1335. https://doi.org/10.1111/jbi.12071
- Calvino CI, Teruel FE, Downie SR (2016) The role of the Southern Hemisphere in the evolutionary history of Apiaceae, a mostly north temperate plant family. Journal of Biogeography 43(2): 398–409. https://doi.org/10.1111/jbi.12651
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348
- Condamine FL, Silva-Brandão KL, Kergoat GJ, Sperling FA (2012) Biogeographic and diversification patterns of Neotropical Troidini butterflies (Papilionidae) support a museum model of diversity dynamics for Amazonia. BMC Evolutionary Biology 12(1): 82. https://doi.org/10.1186/1471-2148-12-82

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): 214. https://doi.org/10.1186/1471-2148-7-214

- Fischer AG (1960) Latitudinal Variations In Organic Diversity. Evolution 14(1): 64–81. https://doi.org/10.2307/2405923
- Florio AM, Ingram CM, Rakotondravony Jr HA, Louis EE, Raxworthy CJ (2012) Detecting cryptic speciation in the widespread and morphologically conservative carpet chameleon (Furcifer lateralis) of Madagascar. Journal of Evolutionary Biology 25(7): 1399–1414. https://doi.org/10.1111/j.1420-9101.2012.02528.x
- GBIF (2023) Global Biodiversity Information Facility. [Published on the internet:] https:// www.gbif.org [Accessed 30.9.2023]
- Gentry AH (1982) Neotropical Floristic Diversity: Phytogeographical Connections Between Central and South America, Pleistocene Climatic Fluctuations, or an Accident of the Andean Orogeny? Annals of the Missouri Botanical Garden 69(3): 557. https:// doi.org/10.2307/2399084
- Gruascavagnetto C, Cerceaularrival M (1984) Contribution of Fossil Umbelliferous Pollen To The Paleoecological And Paleoclimatological Knowledge Of The French Eocene. Review of Palaeobotany and Palynology 40(4): 317–345. https://doi. org/10.1016/0034-6667(84)90014-9
- Haffer J (1969) Speciation in Amazonian Forest Birds. Science 165(3889): 131–137. https://doi.org/10.1126/science.165.3889.131

- He K, Jiang X (2014) Sky islands of southwest China. I: An overview of phylogeographic patterns. Chinese Science Bulletin 59(7): 585–597. https://doi.org/10.1007/s11434-013-0089-1
- Huang R, Xie X, Li F, Tian E, Chao Z (2021a) Chloroplast genomes of two Mediterranean *Bupleurum* species and the phylogenetic relationship inferred from combined analysis with East Asian species. Planta 253(4): 81. https://doi.org/10.1007/s00425-021-03602-7
- Huang R, Xie X, Chen A, Li F, Tian E, Chao Z (2021b) The chloroplast genomes of four *Bupleurum* (Apiaceae) species endemic to Southwestern China, a diversity center of the genus, as well as their evolutionary implications and phylogenetic inferences. BMC Genomics 22(1): 714. https://doi.org/10.1186/s12864-021-08008-z
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Linczevski IA (1950) *Bupleurum* L. In: Komarov VL (Ed.) Flora of USSR. (Vol. 16). Akademii Nauk SSSR, Leningrad.
- Loiseau O, Weigand A, Noben S, Rolland J, Silvestro D, Kessler M, Lehnert M, Salamin N (2020) Slowly but surely: Gradual diversification and phenotypic evolution in the hyper-diverse tree fern family Cyatheaceae. Annals of Botany 125(1): 93–103. https:// doi.org/10.1093/aob/mcz145
- López-Pujol J, Zhang F, Sun H, Ying T, Ge S (2011) Centres of plant endemism in China: Places for survival or for speciation? Journal of Biogeography 38(7): 1267–1280. https://doi.org/10.1111/j.1365-2699.2011.02504.x
- Ma XG (2011) Morphological classification and primary exploration on dna bacoding of *Bupleurum* I. from China.
- Ma X, Zhao C, Liang Q, He X (2013) *Bupleurum baimaense* (Apiaceae), a New Species from Hengduan Mountains, China. Annales Botanici Fennici 50(6): 379–385. https://doi.org/10.5735/085.050.0601
- Ma X, Zhao C, Wang C, Liang Q, He X (2014) Phylogenetic analyses and chromosome counts reveal multiple cryptic species in *Bupleurum commelynoideum* (Apiaceae). Journal of Systematics and Evolution 53(1): 104–116. https://doi.org/10.1111/jse.12122
- Neves SS, Watson MF (2004) Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. Annals of Botany 93(4): 379–398. https://doi.org/10.1093/aob/mch052
- Pastorini J, Zaramody A, Curtis DJ, Nievergelt CM, Mundy NI (2009) Genetic analysis of hybridization and introgression between wild mongoose and brown lemurs. BMC Evolutionary Biology 9(1): 32. https://doi.org/10.1186/1471-2148-9-32
- POWO (2024) Plants of the World Online. [Published on the Internet:] https://powo.science.kew.org/ [Accessed 06.06.2024]
- Price MN, Dehal PS, Arkin AP (2010) FastTree 2-Approximately Maximum-Likelihood Trees for Large Alignments. PLoS ONE 5(3): e9490. https://doi.org/10.1371/journal. pone.0009490
- Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG (2014) BAMMtools: An R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods in Ecology and Evolution 5(7): 701–707. https://doi. org/10.1111/2041-210X.12199

- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Schley RJ, de la Estrella M, Pérez-Escobar OA, Bruneau A, Barraclough T, Forest F, Klitgård B (2018) Is Amazonia a 'museum' for Neotropical trees? The evolution of the Brownea clade (Detarioideae, Leguminosae). Molecular Phylogenetics and Evolution 126: 279–292. https://doi.org/10.1016/j.ympev.2018.04.029
- Sheh ML, Watson MF (2005) Bupleurum L.[M]. Flora of China (English version). Science Press, Beijing.
- Shi JJ, Rabosky DL (2015) Speciation dynamics during the global radiation of extant bats. Evolution; International Journal of Organic Evolution 69(6): 1528–1545. https://doi.org/10.1111/evo.12681
- Shrestha N, Wang ZH, Su XY, Xu XT, Lyu LS, Liu YP, Dimitrov D, Kennedy JD, Wang QG, Tang ZY, Feng XJ (2018) Global patterns of *Rhododendron* diversity: The role of evolutionary time and diversification rates. Global Ecology and Biogeography 27(8): 913– 924. https://doi.org/10.1111/geb.12750
- Sklenar P, Hedberg I, Cleef AM (2014) Island biogeography of tropical alpine floras. Journal of Biogeography 41(2): 287–297. https://doi.org/10.1111/jbi.12212
- van Hinsbergen DJJ, Lippert PC, Dupont-Nivet G, McQuarrie N, Doubrovine PV, Spakman W, Torsvik TH (2012) Greater India Basin hypothesis and a two-stage Cenozoic collision between India and Asia. Proceedings of the National Academy of Sciences of the United States of America 109(20): 7659–7664. https://doi.org/10.1073/ pnas.1117262109
- Vargas OM, Dick CW (2020) Diversification History of Neotropical Lecythidaceae, an Ecologically Dominant Tree Family of Amazon Rain Forest. Neotropical Diversification: Patterns and Processes. Springer International Publishing, 791–809. [Reprinted] https://doi.org/10.1007/978-3-030-31167-4\_29
- Wang Q, He X, Zhou S, Wu Y, Yu Y, Pang Y (2008) Phylogenetic inference of the genus Bupleurum (Apiaceae) in Hengduan Mountains based on chromosome counts and nuclear ribosomal DNA ITS sequences. Journal of Systematics and Evolution 46(2): 142–154. https://doi.org/10.3724/SP.J.1002.2008.07107
- Wang C, Ma X, He X (2011) A taxonomic re-assessment in the Chinese Bupleurum (Apiaceae): Insights from morphology, nuclear ribosomal internal transcribed spacer, and chloroplast (trnH-psbA, matK) sequences. Journal of Systematics and Evolution 49(6): 558–589. https://doi.org/10.1111/j.1759-6831.2011.00157.x
- Wen J, Xie D, Price M, Ren T, Deng Y, Gui L, Guo X, He X (2021) Backbone phylogeny and evolution of Apioideae (Apiaceae): New insights from phylogenomic analyses of plastome data. Molecular Phylogenetics and Evolution 161: 107183. https://doi. org/10.1016/j.ympev.2021.107183
- WFO (2024) World Flora Online. [Published on the Internet:] http://www.worldfloraonline.org [Accessed 06.05.2024]
- Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. Trends in Ecology & Evolution 19(12): 639–644. https://doi.org/10.1016/j. tree.2004.09.011
- Xie C, Xie D, Zhong Y, Guo X, Liu Q, Zhou S, He X (2019) The effect of Hengduan Mountains Region (HMR) uplift to environmental changes in the HMR and its eastern adjacent area:

Tracing the evolutionary history of Allium section Sikkimensia (Amaryllidaceae). Molecular Phylogenetics and Evolution 130: 380–396. https://doi.org/10.1016/j.ympev.2018.09.011

- Xie P, Guo Y, Teng Y, Zhou W, Yu Y (2024) GeneMiner: A tool for extracting phylogenetic markers from next-generation sequencing data. Molecular Ecology Resources 24(3): e13924. https://doi.org/10.1111/1755-0998.13924
- Yang L, Hu H, Xie C, Lai S, Yang M, He X, Zhou S (2017) Molecular phylogeny, biogeography and ecological niche modelling of *Cardiocrinum* (Liliaceae): Insights into the evolutionary history of endemic genera distributed across the Sino-Japanese floristic region. Annals of Botany 119(1): 59–72. https://doi.org/10.1093/aob/mcw210
- Yang T, Yan Z, Xue C, Xin D, Dong M (2021) India Indenting Eurasia: A Brief Review and New Data from the Yongping Basin on the SE Tibetan Plateau. Geosciences 11(12): 518. https://doi.org/10.3390/geosciences11120518
- Yu X, Maki M, Drew BT, Paton AJ, Li H, Zhao J, Conran JG, Li J (2014) Phylogeny and historical biogeography of Isodon (Lamiaceae): Rapid radiation in south-west China and Miocene overland dispersal into Africa. Molecular Phylogenetics and Evolution 77: 183–194. https://doi.org/10.1016/j.ympev.2014.04.017
- Yu Y, Blair C, He X (2020) RASP 4: Ancestral State Reconstruction Tool for Multiple Genes and Characters. Molecular Biology and Evolution 37(2): 604–606. https://doi. org/10.1093/molbev/msz257
- Zhang D, Gao F, Jakovlic I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhao C, Wang C, Ma X, Liang Q, He X (2013) Phylogeographic analysis of a temperate-deciduous forest restricted plant (*Bupleurum longiradiatum* Turcz.) reveals two refuge areas in China with subsequent refugial isolation promoting speciation. Molecular Phylogenetics and Evolution 68(3): 628–643. https://doi.org/10.1016/j. ympev.2013.04.007
- Zheng H, Guo X, Price M, He X, Zhou S (2021) Effects of Mountain Uplift and Climatic Oscillations on Phylogeography and Species Divergence of Chamaesium (Apiaceae). Frontiers in Plant Science 12: 673200. https://doi.org/10.3389/fpls.2021.673200

# **Supplementary material 1**

#### **Supplementary tables**

Authors: Yong-xiu Song, Ceng-yue Yang, Yu-Yang Zhou, Yan Yu Data type: xlsx

- Explanation note: **table S1.** Voucher specimens in this study. **table S2**. GenBank accession numbers for *Bupleurum*. **table S3.** Character matrix in this study. **table S4.** median similarity percentage in this study.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/phytokeys.248.132707.suppl1

# **Supplementary material 2**

#### **Supplementary figures**

Authors: Yong-xiu Song, Ceng-yue Yang, Yu-Yang Zhou, Yan Yu Data type: zip

- Explanation note: figure S1. Phylogenetic relationships of Bupleurum inferred from matK + psbA trnH + rbcL based on BI methods and ML methods. figure S2. Reconstruction of the ancestral trait of the morphological character for *Bupleurum*. figure S3. Reconstruction of the ancestral trait of the morphological character for *Bupleurum*. figure S4. Reconstruction of the ancestral trait of the morphological character for acter for *Bupleurum*. figure S5. Reconstruction of the ancestral trait of the morphological character for *Bupleurum*. figure S5. Reconstruction of the ancestral trait of the morphological character for *Bupleurum*. figure S5. Reconstruction of the ancestral trait of the morphological character for *Bupleurum*.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.132707.suppl2



**Research Article** 

# Halamphora hampyeongensis sp. nov. (Amphipleuraceae, Bacillariophyceae), a new marine benthic diatom from a tidal mudflat in Hampyeong Bay, South Korea

Sung Min An<sup>10</sup>, Jihoon Kim<sup>20</sup>, Kichul Cho<sup>10</sup>, Hyun-Ju Hwang<sup>10</sup>

1 Department of Biological Application & Technology, National Marine Biodiversity Institute of Korea, Seocheon 33662, Republic of Korea

2 LMO Research Team, Bureau of Ecological Research, National Institute of Ecology, Seocheon 33657, Republic of Korea

Corresponding author: Sung Min An (sman@mabik.re.kr)

#### Abstract

The abundance and variety of benthic diatoms inhabiting tidal flats is widely acknowledged, although it has received relatively less attention than other research areas. In this investigation, we provide a formal description of a benthic diatom found in the tidal mudflat of South Korea, based on morphological and molecular characteristics and the similarities and differences between *Halamphora hampyeongensis* **sp. nov.**, with morphologically similar *Halamphora* species are also discussed. Morphological characteristics are described from light and electron microscopy images. *H. hampyeongensis* is distinguished by its wide ventral sides of the valve, small and rounded areolae present across the whole valve face, and dense dorsal striae biseriate (34-38 in 10 µm). Phylogenetic analysis based on 18S rDNA and *rbcL* sequence data revealed that *H. hampyeongensis* is related to *H. montana*, *H. mosensis*, and *H. specensa*. The results (morphometric and molecular) provide sufficient elements to support and propose this taxon as a new species.

Key words: 18S, morphology, new species, phylogeny, rbcL gene, taxonomy

# Introduction

Amphoroid diatoms, represented among other genus by *Amphora* Ehrenberg ex Kützing 1844 and *Halamphora* (Cleve) Mereschkowsky, 1903, are strongly dorsiventral raphid diatoms (Vyverman et al. 1998). The classification of the genus *Amphora* has been a subject of extensive study due to its non-monophyly (Cleve 1895; Mereschkowsky 1903; Krammer 1980; Mann 1994; Ed-lund et al. 2009) and prompting numerous attempts to accurately categorize this large genus (Cleve 1895; Cleve-Euler 1953; Levkov 2009; Stepanek and Kociolek 2016). Particularly, Cleve (1895), based on frustule morphology, erected nine subgenera included *Halamphora*. His proposal emerged as the most widely recognized classification system for *Amphora*. Moreover, Mereschkowsky (1903) proposed a new division of the genus *Amphora* into four



Academic editor: Bing Liu Received: 21 August 2024 Accepted: 3 October 2024 Published: 22 October 2024

**Citation:** An SM, Kim J, Cho K, Hwang H-J (2024) *Halamphora hampyeongensis* sp. nov. (Amphipleuraceae, Bacillariophyceae), a new marine benthic diatom from a tidal mudflat in Hampyeong Bay, South Korea. PhytoKeys 248: 59–71. https://doi.org/10.3897/ phytokeys.248.135034

**Copyright:** © Sung Min An et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

genera (Clevamphora, Cymbamphora, Halamphora, and Tetramphora) based on the number and morphology of the plastids in the cells. Among them, Halamphora contains no libroplasts, which are highly refractile, approximately spherical volutin granules (Mann 1989); however, this classification has not been widely accepted (Stepanek and Kociolek 2014). Levkov (2009) proposed moving Halamphora from the subgenus level to the genus level by transferring 59 existing species of the genus Amphora and adding 16 new species to it. The genus was established on the basis of the distinctive H-shaped single plastids, which are appressed ventrally and exhibit longitudinal constriction in the central region of the H-shape (Levkov 2009). This morphological feature aligns with the second type of plastid arrangement as proposed by Mereschkowsky (1903). The most obvious morphological feature that distinguishes the genus Halamphora from the genus Amphora is the absence of raphe ledge on the ventral side of the valves in the genus Halamphora (Ács et al. 2011). In addition, the genus Halamphora has the following morphological characteristics: striae composed of areolae with recessed foramina; internal central raphe endings terminating onto a fused central helictoglossae (Levkov 2009; Ács et al. 2011). Although a comprehensive and systematic revision of Amphora is currently underway, numerous molecular phylogenetic studies provide strong evidence supporting the monophyletic status of Halamphora as a distinct group separate from Amphora sensu stricto (Ruck and Theriot 2011; Sato et al. 2013; Stepanek and Kociolek 2014; Stepanek and Kociolek 2019; An et al. 2022).

Since its recognition as a genus by Levkov (2009), there has been a growing understanding of the diversity within the genus *Halamphora*. Furthermore, there has been a significant increase in the determination of new species belonging to this genus (Álvarez-Blanco and Blanco 2014; Jiang et al. 2015; Olivares-Rubio et al. 2017; López-Fuerte and Siqueiros-Beltrones 2018; Stepanek and Kociolek 2018; Zhang et al. 2019; López-Fuerte et al. 2020; An et al. 2022). According to AlgaeBase, the genus *Halamphora* constitute 155 accepted species names, including eight varieties, and is currently classified in the family Amphipleuraceae (Guiry and Guiry 2024). However, Torres-Ariño et al. (2019) deemed it appropriate to classify the species in the genus *Halamphora* under the family Catenulaceae instead of Amphipleuraceae due to the presence of dorsal marginal thickening and the absence of dorsal fascia. This is also confirmed by the phylogenetic tree of Olivares-Rubio et al. (2017).

Ecologically, *Halamphora* species are known to prefer mostly inland conductive waters or coastal waters (Levkov 2009; Stepanek and Kociolek 2018; Sala et al. 2021; Spaulding et al. 2021). Tidal flats have also been recognized as a significant habitat for the genus *Halamphora* (Desianti et al. 2017; Plante et al. 2021). However, the genus *Halamphora*, including the broader *Amphora sensu lato* group, is fairly understudied in tidal flats (An et al. 2020).

In the present study, we used light microscopy and scanning electron microscopy (SEM) to conduct morphological examinations on a novel *Halamphora* species that was isolated from a tidal mudflat in Hampyeong Bay, South Korea. We additionally performed molecular analysis of this species using 18S rDNA and *rbcL* gene, and a brief discussion has been included regarding these findings.

# Materials and methods

#### Study site, isolation and cultivation

The Sediment sample was obtained from an intertidal mudflat located in Hampyeong Bay (35°01.89'N, 126°24.31'E) on the west coast of South Korea on July 19, 2018 (Fig. 1). To obtain sediment sample containing diatoms, the surface of the tidal flat was scratched to a depth of ca. 2 mm by spatula and the sediment collected in a 50 mL conical tube. Hampyeong Bay is characterized as a semi-closed bay, encompassing a vast tidal flat spanning approximately 4,700 hectares. The bay does not receive any significant inflow from large rivers, and the contribution of fresh water from small streams is negligible. The annual mean values of temperature and salinity of pore waters in sediments varied between 12.7 and 28.6 °C and between 9.5 and 25 psu, respectively (Hwang and Koh 2012). The sediment temperature and salinity of pore water were measured using a thermometer equipped with a stainless-steel probe (Daihan Scientific Co., Wonju, South Korea) and a YSI Pro 1030 multi-parameter instrument (YSI, Yellow Springs, OH, USA), respectively. At the time of sampling, the sediment temperature was recorded at 27.8 °C, while the salinity of the pore water was measured at 24.9 psu.

A single cell was isolated using a capillary tube under an Eclipse Ti-U inverted microscope (Nikon, Tokyo, Japan) and transferred to a cell culture flask (SPL Life Sciences, Pocheon, South Korea) containing F/2 medium supplemented with silicate (Sigma Aldrich, St. Louis, MO, USA). The strains were periodically sub-cultured every 3–4 weeks and subjected to incubation at a temperature of 25 °C. The incubation was carried out under a light:dark cycle of 14:10 hours, with an irradiance of 40  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>.



**Figure 1.** Location of the sampling site in Hampyeong Bay at the west coast of Korea. The satellite image included in this figure was sourced from Google Earth.

#### Morphological observations

For the purpose of conducting morphological analysis, a sample of the culture was obtained from the initial subculture and treated with a 5% Lugol's solution for fixation. The cultured strain was treated acid treatment using sulfuric and hydrochloric acids to remove organic matter, following the modified Hendey (1974). Subsequently, the strain was affixed onto permanent slides using Pleurax (Wako Pure Chemical Industries, Osaka, Japan). Three slides were analyzed utilizing an AX10 light microscope (LM) that was equipped with an Imager A2 digital camera system (Zeiss, Göttingen, Germany). The examination was conducted under a 100× Plan-Apochromat oil-immersion objective lens (N.A. 1.30). SEM analysis was conducted following the protocol outlined by An et al. (2022). The cleaned specimens were subjected to a filtration process using a polycarbonate membrane (25 mm in diameter with a pore size of 2 µm). This was rinsed with distilled water. The membrane underwent dehydration through a series of graded ethanol concentrations ranging from 10 to 100%. Subsequently, it was dried using tetramethylsilane (Sigma Aldrich). The membrane was affixed to an aluminium stub and subjected to gold sputter-coating using an MC1000 ion sputter (Hitachi, Tokyo, Japan). The stub was used for examination utilizing a Sigma 500-VP high-resolution field-emission scanning electron microscope (Zeiss).

# **Phylogenetic analysis**

The 1.5 mL of culture strain was collected through centrifugation at a speed of 2,500 rpm for a duration of 5 minutes, and the supernatant was discarded. Genomic DNA extraction was performed using the DNeasy PowerSoil Pro Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was conducted using specific primer pairs. The primer pair Diatom9F (Lynch et al. 1997) and EukBR (Medlin et al. 1988) were used to amplify the 18S rDNA region. Additionally, the primer pair DPrbcL1 / DPrbcL7 (Daugbjerg and Andersen 1997) was employed to amplify the *rbcL* gene.

PCR conditions and reactions were implemented as per the protocols of (An et al. 2017). The PCR product was purified using the ExoSAP-IT Express PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and was subsequently sequenced by Macrogen Inc. (Seoul, South Korea). Sequence was trimmed, assembled and aligned using Geneious Prime v.2023.0.1 (Biomatters Ltd., Auckland, New Zealand). Phylogenetic trees were constructed using the Randomized Axelerated Maximum Likelihood (RAxML) v.8.2.10 (Stamatakis 2014) and MrBayes version 3.2.7 (Ronquist and Huelsenbeck 2003), based on maximum likelihood (ML) and Bayesian phylogenetic inference (BI) methods. ML analysis was conducted utilizing the GTRGAMMAIX model selected by ModelTest-NG v.0.1.7 (Darriba et al. 2020), with the number of bootstrap replicates set to 1,000 and all other settings kept at their default values. BI was conducted using the established methods as outlined in the study by López-Fuerte et al. (2020). A dataset of concatenated 18S rDNA and rbcL sequence data was constructed, including 80 Amphoroid diatom sequences from GenBank (Suppl. material 1). The dataset exclusively includes information on strains for which both 18S and rbcL sequences are available. The outgroup for this analysis was *Tetramphora chilensis* (Hustedt) Stepanek & Kociolek, 2016 strain 8531-Amph132. Trees were visualized using Figtree v. 1.4.4 and Adobe Illustrator v. 27.1.1 (Adobe Systems, San José, CA, USA).

# Results

# Halamphora hampyeongensis S.M.An & J.Kim, sp. nov. Fig. 2

**Description.** In LM, valves semi-elliptical with smoothly convex dorsal margin, nearly straight ventral margin and the valve ends narrowly rounded and slightly ventrally curved (Fig. 2A–H). Raphe slightly arched and positioned centrally to slightly ventrally on the valve face. Both dorsal and ventral striae not discernible. Valve length  $13.8-15.0 \mu m$ ; valve breadth  $2.8-3.0 \mu m$  (n = 31).

In SEM, externally, the central area slightly expanded on the ventral side only (Fig. 2I, K, asterisks). Central raphe endings straight, slightly expanded and positioned very close together (Fig. 2K, arrowhead); distal raphe endings bent towards the dorsal side (Fig. 2L, arrow). Raphe ledge well-developed on the dorsal side of the valve (Fig. 2I, K) and narrows sharply towards the dorsal valve margin. Siliceous outgrowths of various sizes arranged irregularly on the raphe ledge margin (Fig. 2K, arrows). The axial area narrow, and difficult to distinguish because of the fine striae (Fig. 2I).

Dorsal striae slightly radiate. Ventral striae are slightly radiate and more distantly spaced at the valve middle, becoming parallel to slightly convergent and denser near the valve apices (Fig. 2I, K, M). Dorsal striae biseriate under raphe ledge with two rows of small, round areolae under SEM (Fig. 2M, arrow); becoming uniseriate, composed of very small round areolae over the rest of the dorsal side (Fig. 2K, M), 34–38 in 10  $\mu$ m (n = 13). Ventral striae uniseriate, almost identical in size and shape to dorsal striae, 46–48 in 10  $\mu$ m (n = 13, Table 1). Axial longitudinal line absent on dorsal side. Internally, longitudinal rib absent. Areolae occluded by hymenes (Fig. 2N, arrows). Proximal raphe endings finish onto small and tongue-shaped fused helictoglossae (Fig. 2N, double asterisk). Poorly developed helictoglossae at distal raphe endings (Fig. 2O, arrow). Round or ovoid poroids in girdle bands, arranged in two rows of 65–67 in 10  $\mu$ m (Fig. 2P, arrows).

**Holotype.** Slide no. MABIK DI00043482 (represented by the valve shown in Fig. 2A) was deposited at the National Marine Biodiversity Institute of Korea (MABIK), located in Seocheon-gun, Chungcheongnam-do, South Korea.

**Isotype.** SEM stub no. MABIK DI00043483 and cleaned material no. MABIK DI00043484 (preserved in 99% ethanol).

**Type locality.** The intertidal mudflat located in Hampyeong Bay (35°01.89'N, 126°24.31'E), Muan-gun, Jeollanam-do, South Korea (site: HP1-3), July 19, 2018.

**Etymology.** The specific epithet *"hampyeongensis"* refers to the type locality, Hampyeong Bay, Muan-gun, Jeollanam-do, South Korea.

**Distribution and ecology.** *Halamphora hampyeongensis* is a benthic species currently known only from the type locality. The sediment temperature and salinity of pore water in the sediment at the time of sampling were measured to be 27.8 °C and 24.9 psu, respectively.

**Gene sequences.** The nucleotide sequences were deposited in GenBank under accession numbers OQ642108 (18S rDNA) and ON137728 (*rbcL* gene).



**Figure 2. A**–**H** Light microscopy photomicrographs of *H. hampyeongensis* **I**–**P** scanning electron microscopy photomicrographs of *H. hampyeongensis* **I** external valve view, with central area (asterisk) and dorsal raphe ledge (arrow) **J** internal view of a valve **K** detail of a valve externally showing siliceous outgrowths (arrows) on the margin of the raphe ledge, central area (asterisk), and proximal raphe endings (arrowheads) **L** detail of external valve apex showing the dorsally bent distal raphe ending (arrow) **M** biseriate striae (arrows) in several rows under the raphe ledge **N** detail of areolae on the dorsal side internally occluded by hymenes (arrows) and tongue-like proximal helictoglossae (double asterisk) **O** detail of internal valve apex showing poorly developed distal helictoglossae (arrow) **P** girdle bands with two rows of poroids (arrows). Scale bars: 10 μm (**A**–**H**); 5 μm (**I**, **J**); 1 μm (**K**–**M**, **O**, **P**); 0.5 μm (**N**).

#### Phylogenetic analysis

The lengths of the 18S rDNA and *rbcL* gene sequences were determined in this study for *Halamphora hampyeongensis* were 1,649 bp and 1,424 bp, respectively. Phylogenetic analysis was conducted to determine the relationship between *H. hampyeongensis* and the amphoroid diatom species retrieved from GenBank using ML and BI methods (Fig. 3). The topologies of the phylogenetic trees were similar regardless of the phylogenetic analysis methods and molecular regions utilized. Phylogenetic analysis provided robust support for the monophyletic grouping of *Halamphora* species including *H. hampyeongensis*, as indicated by a maximum likelihood bootstrap support of 98% and a Bayesian posterior probability of 0.99. *H. hampyeongensis* was located in *Halamphora* Clade Hal\_H as described by Stepanek and Kociolek (2019) with a low bootstrap value (bootstrap value = 25). This species also showed weak support (bootstrap value = 63) as the sister taxon to *H. montana* (Krasske) Levkov.

#### Discussion

Halamphora hampyeongensis sp. nov. displayed typical morphological characteristics associated with the Amphoroid as observed through microscopic examination (Fig. 2). Furthermore, phylogenetic analysis indicates that *H. hampyeongensis* belongs to the clade that includes *Halamphora* strains (Fig. 3). Therefore, considering its morphological features and molecular data, the classification of *H. hampyeongensis* in the genus *Halamphora* is deemed appropriate.

As a result of phylogenetic analysis, this species was found to be related to Halamphora Clade Hal\_H, which consists of species like H. exilis J.G.Stepanek & Kociolek, H. mosensis J.G.Stepanek & Kociolek, H. specensa J.G.Stepanek & Kociolek, H. maritima J.G.Stepanek & Kociolek and H. nipponensis J.G.Stepanek, S.Mayama & Kociolek as described by Stepanek and Kociolek (2019). This clade is characterized by specific morphological traits, including the absence of a dorsal marginal ridge, the presence of biseriate internal areolae occlusions, and hymen internal areolae occlusions. Notably, H. maritima and H. nipponensis exhibit distinct differences from the other species within the clade, specifically in their narrow ventral valve and ventral striae, which are composed of elongated areolae. In contrast, the other species typically possess a relatively wide ventral valve and fine uniseriate ventral striae. These characteristics are also observed in the *H. hampyeongensis* sp. nov. *H.* hampyeongensis shares similarities with H. caribaea (Wachnicka & E.E.Gaiser) Rimet & R.Jahn, H. exilis, H. mosensis, and H. specensa in overall valve outline, relatively broad ventral side of the valves, and specific striae characteristics, including fine uniseriate ventral striae and biseriate dorsal striae near the axial area. H. hampyeongensis has smaller areolae and denser striae (34–38 in 10  $\mu$ m) compared to *H. caribaea* (11–20 in 10  $\mu$ m) and *H. exilis* (24–26 in 10  $\mu$ m) (Table 1) (Wachnicka and Gaiser 2007; Stepanek and Kociolek 2018). Unlike H. hampyeongensis, H. mosensis has multi-seriate dorsal striae, and H. specensa has a convex ventral margin and irregularly bi-seriate dorsal striae (Stepanek and Kociolek 2018). In addition, H. hampyeongensis shares fine areolae with H. atacamana (Patrick) Levkov, and H. montana. However, H. atacamana can



**Figure 3.** A molecular phylogenetic tree obtained from ML and BI analysis based on the concatenated dataset (18S rDNA and *rbcL* gene) showing the phylogenetic positions of *H. hampyeongensis*. A phylogenetic tree was generated through a ML analysis. The values on each node indicate ML bootstrap and Bayesian posterior probabilities, respectively. The asterisk indicates 100 in ML and 1 in BI, and – indicates the topological incongruence between ML and BI trees. The *H. hampyeongensis* is highlighted in bold blue.

be differentiated from *H. hampyeongensis* based on its valve outline and lower stria density (25–28 in 10  $\mu$ m) (Levkov 2009), and *H. montana* possesses semi-stauros, making it easily distinguishable from *H. hampyeongensis*, even

		1					
	Halamphora hampyeongensis	H. atacamana	H. caribaea	H. exilis	H. montana	H. mosensis	H. specensa
Valve shape	semi-elliptical, convex dorsal and nearly straight ventral margins	semi-lanceolate, arched dorsal and straight to weakly tumid ventral margins	semi-lanceolate, convex dorsal and straight ventral margins	narrowly semi- elliptical, shallowly arched dorsal and straight ventral margins	semi-lanceolate, smoothly arched dorsal and straight to slightly convex ventral margins	narrowly semi- elliptical, smoothly arched dorsal and straight ventral margins	semi-elliptical to nearly elliptical, arched to flattened dorsal and convex ventral margins
Apices	narrowly rounded	slightly subprotracted	rostrate	narrowly rounded	broadly rounded	narrowly rounded	weakly protracted, narrowly rounded
Length (µm)	13.8-15.0	29-45	34-39	14-19	12-20	21-37	13–17
Width (µm)	2.8-3.0	4.5-8.0	6.0-8.0	2.5-3.0	3.0-4.6	4.0-4.5	3.0-3.5
Raphe	arched	arched	straight with	slightly arched	arched	arched	straight
Proximal raphe ends	slightly expanded, straight	slightly dorsally deflected	dorsally deflected	slightly dorsally deflected	slightly expanded, dorsally deflected	dorsally deflected	dorsally deflected
Dorsal striae	34-38	25-28	11-20	24-26	40-45	26-28	23-26
(in 10 µm)	bi- and uniseriate	uniseriate	ND	bi- and uniseriate	uniseriate	bi- and multiseriate	biseriate
Ventral striae (in 10 µm)	46-48	24-30	19-29	44-45	40-45	28	44-46
Habitate	brackish	brackish	marine	marine	freshwater	brackish	freshwater
References	This study (n = 31)	Levkov (2009)	Wachnicka and Gaiser (2007)	Stepanek and Kociolek (2018)	Watanabe et al. (2005); Levkov (2009)	Stepanek and Kociolek (2018)	Stepanek and Kociolek (2018)

	Table 1. Mor	phometric com	paration of Hala	amphora hami	oveonaensis	with related s	pecies, ND :	= not documente
--	--------------	---------------	------------------	--------------	-------------	----------------	--------------	-----------------

when observed under a light microscope (Watanabe et al. 2005; Levkov 2009). Furthermore, this species exhibits distinctive ornamentation on its raphe ledge, which differentiates it from other *Halamphora* species (Fig. 2K, arrows). Nevertheless, as this observation has not been substantiated through natural samples, additional verification is necessary.

Based on the aforementioned information, we propose a novel taxon found in the mudflats as a new species belonging to the genus *Halamphora*, named *H. hampyeongensis*. While benthic diatoms are the predominant organisms in benthic ecosystems of tidal flats and are recognized for their high diversity (Underwood and Barnett 2006), their overall understanding is still limited. In the future, further research is required to elucidate the species richness of diatoms in tidal flats. Consequently, it is anticipated that new and previously undocumented species will continue to be identified and reported.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This research was supported by the development of useful materials derived from marine microorganisms and microalgae (2024M00600) funded by the National Marine Biodiversity Institute of Korea (MABIK).

#### Author contributions

Conceptualization: SMA. Methodology, SMA, KC. Investigation: SMA, JK. Resources: JK. Writing – original draft: SMA. Writing – review and editing, KC, HJH. Project administration: HJH. Funding acquisition: HJH.

#### Author ORCIDs

Sung Min An <sup>©</sup> https://orcid.org/0000-0002-9180-309X Jihoon Kim <sup>©</sup> https://orcid.org/0000-0001-6363-6514 Kichul Cho <sup>©</sup> https://orcid.org/0000-0002-0685-0490 Hyun-Ju Hwang <sup>©</sup> https://orcid.org/0009-0008-4608-7021

#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

# References

- Ács É, Ector L, Kiss KT, Cserháti C, Morales EA, Levkov Z (2011) Morphological observations and emended description of *Amphora micrometra* from the Bolivian Altiplano, South America. Diatom Research 26(2): 199–212. https://doi.org/10.1080/026924 9X.2011.597987
- Álvarez-Blanco I, Blanco S (2014) Benthic diatoms from Mediterranean coasts. Bibliotheca Diatomologica band 60. J Cramer, Stuttgart, 1–409.
- An SM, Choi DH, Lee JH, Lee H, Noh JH (2017) Identification of benthic diatoms isolated from the eastern tidal flats of the Yellow Sea: Comparison between morphological and molecular approaches. PLoS One 12(6): e0179422. https://doi.org/10.1371/journal.pone.0179422
- An SM, Choi DH, Noh JH (2020) High-throughput sequencing analysis reveals dynamic seasonal succession of diatom assemblages in a temperate tidal flat. Estuarine, Coastal and Shelf Science 237: 106686. https://doi.org/10.1016/j.ecss.2020.106686
- An SM, Kim J, Kang NS, Cho K, Lee JA, Kim ES (2022) Halamphora minima (Catenulaceae, Bacillariophyta), new brackish diatom species from the mudflat in Hampyeong Bay, Korea. Phytotaxa 572(1): 115–122. https://doi.org/10.11646/phytotaxa.572.1.9
- Cleve PT (1895) Synopsis of the Naviculoid Diatoms, Part II. Kongliga Svenska-Vetenskaps Akademiens Handlingar 27(3): 1–219.
- Cleve-Euler A (1953) Die Diatomeen von Schweden und Finnland. Part III, Euraphideae. Kongliga Svenska Vetenskaps-Akademiens Handligar 4: 1–240.
- Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Molecular biology and evolution 37(1): 291–294. https://doi.org/10.1093/molbev/msz189
- Daugbjerg N, Andersen RA (1997) A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbcL* sequence data. Journal of Phycology 33(6): 1031–1041. https://doi.org/10.1111/j.0022-3646.1997.01031.x
- Desianti N, Potapova M, Enache M, Belton TJ, Velinsky DJ, Thomas R, Mead J (2017) Sediment diatoms as environmental indicators in New Jersey coastal lagoons. Journal of Coastal Research 78(10078): 127–140. https://doi.org/10.2112/SI78-011.1
- Edlund MB, Shinneman AL, Levkov Z (2009) Diatom biodiversity in Mongolia: A new amphoroid diatom from saline lakes in western Mongolia, *Amphora soninkhishigae* sp. nov. Acta Botanica Croatica 68(2): 251–262. https://hrcak.srce.hr/41428

- Guiry MD, Guiry GM (2024) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org/ [accessed 15 March 2024]
- Hendey N (1974) The permanganate method for cleaning freshly gathered diatoms. Microscopy 32: 423–426.
- Hwang DW, Koh BS (2012) Sedimentary and benthic environment characteristics in macroalgal habitats of the intertidal zone in Hampyeong Bay. Hangug Susan Haghoi Ji 45(6): 694–703. https://doi.org/10.5657/KFAS.2012.0694
- Jiang HY, Hu CQ, Yang HP, Zhang LP, Peng PF, Luo P, Zhao Z, Xia JJ (2015) Morphology and phylogeny of *Halamphora yongxingensis* sp. nov. (Bacillariophyta), a new marine benthic diatom isolated from Yongxing Island, South China Sea. Phytotaxa 195(1): 53–64. https://doi.org/10.11646/phytotaxa.195.1.3
- Krammer K (1980) Morphologic and taxonomic investigation of some freshwater species of the diatom genus *Amphora* Ehr. Bacillaria 3: 197–225.
- Levkov Z (2009) Diatoms of Europe: Diatoms of the European inland waters and comparable habitats. vol. 5. *Amphora sensu lato*. A.R.G. Gantner Verlag, Ruggel, 1–918.
- López-Fuerte FO, Siqueiros-Beltrones DA (2018) *Halamphora primus* (Bacillariophyta): A new diatom species from the Gulf of California, Mexico. Phytotaxa 369(4): 278–286. https://doi.org/10.11646/phytotaxa.369.4.6
- López-Fuerte FO, Sala SE, Lora-Vilchis MC, Murugan G (2020) *Halamphora siqueirosii* (Bacillariophyta), a new diatom species isolated from a hypersaline evaporation pond in Baja California Peninsula, Mexico. Phytotaxa 451(2): 132–144. https://doi. org/10.11646/phytotaxa.451.2.3
- Lynch ED, Lee MK, Morrow JE, Welcsh PL, León PE, King MC (1997) Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the *Drosophila* gene diaphanous. Science 278(5341): 1315–1318. https://doi.org/10.1126/science.278.5341.1315
- Mann DG (1989) The diatom genus *Sellaphora*: Separation from *Navicula*. British Phycological Journal 24(1): 1–20. https://doi.org/10.1080/00071618900650011
- Mann DG (1994) The systematics of amphoroid diatoms: The life history of *Amphora arcus*. Nova Hedwigia 58(3): 335–352.
- Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71(2): 491–499. https://doi. org/10.1016/0378-1119(88)90066-2
- Mereschkowsky C (1903) Le types de l'endochrom chez les Diatomées. Scripta Botanica 21: 1–193. [Botanisheskia Zapiski]
- Olivares-Rubio HF, Cabrera LI, Godínez-Ortega JL, Salazar-Coria L, Vega-López A (2017) Halamphora oceanica (Catenulaceae, Bacillariophyta), a new species from the epipelagic region of the southwestern Gulf of Mexico. Phytotaxa 317(3): 188–198. https:// doi.org/10.11646/phytotaxa.317.3.3
- Plante CJ, Hill-Spanik K, Cook M, Graham C (2021) Environmental and spatial influences on biogeography and community structure of saltmarsh benthic diatoms. Estuaries and Coasts 44(1): 147–161. https://doi.org/10.1007/s12237-020-00779-0
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi. org/10.1093/bioinformatics/btg180
- Ruck EC, Theriot EC (2011) Origin and evolution of the canal raphe system in diatoms. Protist 162(5): 723–737. https://doi.org/10.1016/j.protis.2011.02.003
- Sala SE, Vouilloud AA, Popovich CA, Sanchez-Puerta M, Almandoz GO, Coy BM, Montoya NG, Leonardi P (2021) Molecular, morphological, and toxinological characterizations

of an Argentinean strain of *Halamphora coffeaeformis* with potential biotechnological applications. Journal of Applied Phycology 33(2): 799–806. https://doi.org/10.1007/s10811-020-02353-4

- Sato S, Tamotsu N, Mann DG (2013) Morphology and life history of *Amphora commutata* (Bacillariophyta) I: The vegetative cell and phylogenetic position. Phycologia 52(3): 225–238. https://doi.org/10.2216/12-072.1
- Spaulding SA, Potapova MG, Bishop IW, Lee SS, Gasperak TS, Jovanoska E, Furey PC, Edlund MB (2021) Diatoms. org: Supporting taxonomists, connecting communities. Diatom Research 36(4): 291–304. https://doi.org/10.1080/0269249X.2021.2006790
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https:// doi.org/10.1093/bioinformatics/btu033
- Stepanek JG, Kociolek JP (2014) Molecular phylogeny of Amphora sensu lato (Bacillariophyta): An investigation into the monophyly and classification of the amphoroid diatoms. Protist 165(2): 177–195. https://doi.org/10.1016/j.protis.2014.02.002
- Stepanek JG, Kociolek JP (2016) Re-examination of Mereschkowsky's genus *Tetramphora* (Bacillariophyta) and its separation from *Amphora*. Diatom Research 31(2): 123–148. https://doi.org/10.1080/0269249X.2016.1183344
- Stepanek JG, Kociolek JP (2018) *Amphora* and *Halamphora* from coastal and inland waters of the United States and Japan, with the description of 33 new species. Bibliotheca Diatomologica 66. J. Cramer, Stuttgart, 1–260.
- Stepanek JG, Kociolek JP (2019) Molecular phylogeny of the diatom genera *Amphora* and *Halamphora* (Bacillariophyta) with a focus on morphological and ecological evolution. Journal of Phycology 55(2): 442–456. https://doi.org/10.1111/jpy.12836
- Torres-Ariño A, Okolodkov YB, Herrera-Herrera NV, Hernández-Barrera BL, González-Resendiz L, León-Tejera H, Gárate-Lizárraga I (2019) Un listado del fitoplancton y microfitobentos del sureste del Pacífico mexicano. Cymbella: Revista de Investigacion y Difusion Sobre Algas 5(1): 1–97.
- Underwood GJ, Barnett M (2006) What determines species composition in microphytobenthic biofilms. In: Kromkamp J (Ed.) Functioning of microphytobenthos in estuaries. Microphytobenthos symposium. The Netherlands: Royal Netherlands Academy of Arts and Sciences, Amsterdam, 121–138.
- Vyverman W, Sabbe K, Mann D, Kilroy C, Vyverman R, Vanhoutte K, Hodgson D (1998) *Eunophora* gen. nov. (Bacillariophyta) from Tasmania and New Zealand: Description and comparison with *Eunotia* and amphoroid diatoms. European Journal of Phycology 33(2): 95–111. https://doi.org/10.1080/09670269810001736593
- Wachnicka AH, Gaiser EE (2007) Characterization of *Amphora* and *Seminavis* from south Florida, USA. Diatom Research 22(2): 387–455. https://doi.org/10.1080/026 9249X.2007.9705722
- Watanabe T, Ohtsuka T, Tuji A, Houki A (2005) Picture book and ecology of the freshwater diatoms. Uchida-rokakuho, Tokyo, 1–666.
- Zhang W, Wang T, Levkov Z, Jüttner I, Ector L, Zhou QC (2019) Halamphora daochengensis sp. nov., a new freshwater diatom species (Bacillariophyceae) from a small mountain lake, Sichuan Province, China. Phytotaxa 404(1): 12–22. https://doi. org/10.11646/phytotaxa.404.1.2

# **Supplementary material 1**

# GenBank accession numbers for the amphoroid species included in the phylogenetic analyses

Authors: Sung Min An, Jihoon Kim, Kichul Cho, Hyun-Ju Hwang Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.135034.suppl1

# **Supplementary material 2**

#### Supplementary data

Authors: Sung Min An, Jihoon Kim, Kichul Cho, Hyun-Ju Hwang Data type: fas

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.135034.suppl2


**Research Article** 

# *Primula meishanensis* (Primulaceae), a new species from Sichuan, China

Tingyu Li<sup>1,2©</sup>, Xinyu Chen<sup>2®</sup>, Bo Li<sup>3©</sup>, Donglai Hua<sup>4®</sup>, Can Luo<sup>2®</sup>, Huixian Luo<sup>2®</sup>, Yun Liang<sup>5®</sup>, Jieli Yue<sup>5®</sup>, Xiaodan Xi<sup>5®</sup>, Ke Huang<sup>2®</sup>, Zhixi Fu<sup>1,2,6®</sup>

1 Ministry of Education, Key Laboratory of Land Resources Evaluation and Monitoring in Southwest (Sichuan Normal University), Chengdu 610101, China

- 2 College of Life Sciences, Sichuan Normal University, Chengdu 610101, China
- 3 Sichuan Environmental Monitoring Center, Chengdu 610091, China
- 4 College of Life Sciences, Mianyang Teachers' College, Mianyang 621000, China
- 5 Meishan Eco-environmental Monitoring Center Station of Sichuan Province, Meishan, 620010, China
- 6 Sustainable Development Research Center of Resources and Environment of Western Sichuan, Sichuan Normal University, Chengdu 610101, China

Corresponding authors: Ke Huang (542176689@qq.com); Zhixi Fu (fuzx2017@sicnu.edu.cn)

#### Abstract

*Primula meishanensis* K.Huang & Z.X.Fu, **sp. nov**., a new species of Primulaceae from Meishan City, Sichuan Province, China, is described and illustrated. The morphological data and phylogenetic analysis, based on the complete chloroplast genome, suggest that *Primula meishanensis* is a separate species closely related to *Primula dejuniana*. The complete chloroplast genome of *Primula meishanensis* was 152,175 bp and the complete chloroplast genome of *Primula dejuniana* was 151,988 bp. The new species differs from the latter by the solitary scape, the length of petiole, acute leaf blade apex and pin flower. The distribution map, morphological comparison of related species and conservation status of the new species are also provided.

Key words: Morphological characters, new species, Primula sect. Petiolares, taxonomy

#### Introduction

The genus *Primula* L. is one of the most diverse member within the Primulaceae. It consists of 38 sections and more than 500 species worldwide (Hu and Kelso 1996; Richards 2003). It is widely distributed throughout temperate and cold regions of Asia and Europe, alongside the tropical mountains of the Northern Hemisphere. The genus is composed of herbaceous plants with a basal rosette of leaves, flowers on top of a naked scape, gathered in lateral or perpendicular to the axis umbels. China hosts over 340 species, with particular biodiversity hotspots of *Primula* found in south-western China (Hu 1990; Hu 1998; Richards 2003; Ju et al. 2023; Li et al. 2023). In recent years, several new species of *Primula* from China were reported, for example, *Primula lihengiana* C. M. Hu & R. Li (Li and Hu 2009), *Primula wawushanica* G. Hao, C.M. Hu & Y. Xu, *Primula undulifolia* G. Hao, C. M. Hu & Y. Xu, *Primula pengzhouensis* C.M. Hu, G. Hao & Y. Xu., *Primula surculosa* Y. Xu & G. Hao (Xu et al. 2016a, b, 2017,



Academic editor: Bing Liu Received: 9 May 2024 Accepted: 20 September 2024 Published: 23 October 2024

Citation: Li T, Chen X, Li B, Hua D, Luo C, Luo H, Liang Y, Yue J, Xi X, Huang K, Fu Z (2024) *Primula meishanensis* (Primulaceae), a new species from Sichuan, China. PhytoKeys 248: 73–90. https://doi.org/10.3897/ phytokeys.248.127117

**Copyright:** © Tingyu Li et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). 2022), Primula dongchuanensis Z.K. Wu & Yuan Huang (Wu et al. 2019), Primula dujiangyanensis W. B. Ju, Bo Xu & X. F. Gao (Ju et al. 2021), Primula longipilosa Ze H. Wang & H. Peng (Wang et al. 2022), Primula wolongensis W.B.Ju, Bo Xu & X.F.Gao (Li et al. 2023), Primula sugongii J.D.Ya, Bin Yang & Y.H. Tan (Yang et al. 2023) and Primula xilingensis K. Huang & Z.X. Fu (Luo et al. 2023).

The *Primula* sect. *Petiolares* Pax comprises more than 60 currently recognised species (Hu 1994; Hu and Kelso 1996; Hu and Geng 2003; Rankin 2010; Wei et al. 2022; Zhang et al. 2023) and are well represented in the region of Himalaya-Hengduan Mountains, with only a few members extending into Kashmir, central China and some other regions (Hu and Kelso 1996). One of the most important diagnostic characters of this section is a globose capsule which does not open by valves, but by crumbling at the membrane apex (Hu 1994).

During the botanical expedition of Zhongyan temple in Qingshen County, Meishan City, Sichuan Province from 2022 to 2024, a population of *Primula* was discovered, photographed and collected. Based on the photographs of the flowering taxon, it appears to be closely related to *Primula dejuniana* G. Hao, C.M. Hu & Y. Xu (Xu et al. 2014) at first sight. After consultation with relevant literature and conducting morphological examination of closely-related taxa, it was determined that it represents an unreported taxon from *P.* sect. *Petiolares*. The new species could be differentiated from other members of the section by the following combination: scape solitary, the length of petiole 2.5–5 cm, a terminal umbel of (1) 2–3 (4) flowers, leaf blade smooth, oblanceolate, 19.5–25 × 2.5–5 cm, apex acute, corolla pastel violet, 5–6 lobes spreading and stamens inserted at middle of corolla tube, corolla tubes 11.0–12.0 mm long, ca. 2 mm in diam., style 4.0–7.0 mm above base of corolla tube, stamens reaching the corolla tube mouth, 7.0–12.0 mm above base of corolla tube.

In addition, in this study, the molecular data of complete chloroplast genomic data were collected and used to identify its relationship with *Primula dejuniana*. In recent years, many new species have been jointly supported by genetic and morphological data, including *Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun (Sun et al. 2020), *Aster quanzhouensis* M.Tang, G.J.Yan & W.P.Li (Xiao et al. 2022), *Primula undulifolia* G. Hao, C. M. Hu & Y. Xu (Xu et al. 2016), *Aster yaoshanensis* K. Qin, Z.X. Fu and P. Li. (Zheng et al. 2024) and *Saussurea talungensis* S.K.Ghimire & H.K.Rana (Rana et al. 2021).

In this study, we provide a detailed description of this new species, based on observations of living plants in the field and specimens in the herbarium.

#### Material and methods

#### Morphological analysis

The observation and collection of both herbarium and living materials of the new species from Qingshen County, Meishan City, Sichuan Province, were conducted in December 2022, January 2023, January 2024 and March 2024. We conducted a morphological comparison using taxonomic literature of close-ly-related species, i.e. *Primula dejuniana* (description of reference Xu et al. (2014)) and *Primula davidii* Franch. (holotype: *David*, *A.1870* (P, image!), as well as the images of specimens from the Global Plants JSTOR database (https://plants.jstor.org), i.e. *Primula epilosa* Craib (holotype: *Sun231* (KUN, image!) were

consulted. Morphological description and measurements of *P. meishanensis* were based on living plants. The taxonomic description followed the terminology used by Beentje (2016). The holotype voucher specimens were stored at the Herbarium of Sichuan Normal University (SCNU). The conservation status of the new species was assessed following the guidelines of the IUCN Red List Categories and Criteria (IUCN 2024).

#### **DNA extraction and sequencing**

Total genomic DNA was obtained using the CTAB method (Doyle and Doyle 1987). We follow the Illumina DNA Library Construction Guide to complete paired-end DNA library construction (Allen et al. 2006). The complete chloroplast genome was sequenced on the Illumina HiSeq XTen platform (San Diego, CA, USA). In this study, SPAdes v.3.10.1 software was used to assemble high-quality data by default parameters (Bankevich et al. 2012). We further used CPGView (http://47.96.249.172:16085/cpgview/view) to improve annotation, visualise the structure of the cp genome and identify gene structures including cis-splicing and trans-splicing (Liu et al. 2023). The genome sequence of *Primula dejuniana and P. meishanensis* have been deposited in GenBank (accession numbers: PQ213817 and PQ213816).

#### **Phylogenetic analysis**

The phylogenetic analysis of the complete chloroplast genomic dataset of 18 species was performed using the Maximum Likelihood (ML) method implemented in RAxML. The species *Maesa montana* A. DC. (KU569490) and *Ardisia polysticta* Migo (KC465962) were selected as outgroups. These chloroplast genome sequences were imported into MAFFT v.7.520 software (Katoh and Standley 2013) for multiple comparisons and the phylogenetic tree was constructed using CIPRES (https://www.phylo.org/), with the ML method, based on the GTRGAMMA model and the bootstrap was set to 1, 000 (Stamatakis et al. 2008) (Table 2) (Fig. 5).

#### **Result and discussion**

#### **Taxonomic treatment**

*Primula meishanensis* K.Huang & Z.X.Fu, sp. nov. urn:lsid:ipni.org:names:77350705-1

**Type.** CHINA, Sichuan Province, Meishan City, Qingshen County, Zhongyan temple, grows on moist rock surfaces amidst moss under the forest, at elevations of approximately 417 m; 29°45'47.39"N, 103°50'44.017"E; 26 December 2023 (fl.), *Ke Huang & Zhixi Fu 8200* (holotype SCNU!) (Figs 1–3).

**Diagnosis.** The new species is morphologically similar to *Primula dejuniana* in having ciliate, sharply and remotely dentate leaf blade margin, while it can be easily recognised by the following combination of characters: scape solitary, the length of petiole 2.5-5 cm, a terminal umbel of (1) 2-3 (4) flowers, leaf blade smooth, oblanceolate,  $19.5-25 \times 2.5-5$  cm, apex acute, corolla pastel



Figure 1. *Primula meishanensis* sp. nov. A plant and roots B roots C petioles D, E leaves F pin flower G thrum flower H scape and bract (Photos by XC).

violet, 5-6 lobes spreading and stamens inserted at middle of corolla tube, corolla tubes 11.0-12.0 mm long, ca. 2 mm in diam., style 4.0-7.0 mm above base of corolla tube, stamens reaching the corolla tube mouth, 7.0-12.0 mm above base of corolla tube. (Figs 1-3).

Description. Perennial herbs, 12.0-20.0 cm tall. Roots numerous, fibrous, without hairs. Leaves pilose, forming a spreading rosette, each rosette with only 2-4 leaves of previous year at flowering time; resting bud of rosette clothed by a few small paleaceous scales, basal bud scales ovate to ovate-oblong, ciliolate, rose red, apex acute; petiole 2.5-5 cm long, narrowly winged and densely covered with multicellular hairs; leaf blade oblanceolate, smooth, 19.5-25 × 2.5-5 cm, cuneate at base, abaxial surface densely along mid-vein, sparser on lateral veins covered with multicellular hairs; margin ciliate, sharply and remotely dentate; apex acute; mid-vein dull yellow in fresh state, turning brownish when dry; veins impressed adaxially, prominently raised and subalveolate abaxially. Scape solitary, 7.2–11.8 cm long, pilose, carrying a terminal umbel of (1) 2-3 (4) flowers, dull yellow in fresh state, turning brownish when dry at base. Bracts lanceolate, 4-5.5 mm long, minutely ciliate. Pedicel 0.4-1.4 cm long, shorter than leaf blade, pilose. Flowers distylous. Calyx campanulate, 8-9 mm long, parted to 1/2 of its length or slightly below; lobes linear lanceolate to lanceolate, apex acute. Corolla pastel violet, annulate, 5-6 lobes spreading, lobes broadly elliptic, 8.0-10.0 mm long, emarginate, densely yellow farinose abaxially, smooth adaxially. Pin flower: corolla tubes 9.0-10.0 mm long, stamens inserted at middle of corolla tube, 9.5-10.5 mm long, style slightly exceeding the corolla tube mouth. Thrum flower: corolla tubes 11.0-12.0 mm long, ca. 2 mm in diam., 2 times as long as the calyx, style 4.0-7.0 mm above base of corolla tube, stamens reaching the corolla tube mouth, 7.0-12.0 mm above base of corolla tube (Fig.1G).

**Phenology.** The flowering period is from December to February and the fruiting period is March to May.

**Etymology.** The epithet "meishanensis" is derived from Meishan City, located in Sichuan Province, China.

**Distribution and habitat.** *P. meishanensis* is currently known from its type locality in Zhongyan temple, Ruifeng Town, Qingshen County and roadside of Panjiaozui, forest Hongya Forest Farm, Hongya County, Meishan City, Sichuan Province, China (Fig. 4). This new species probably exists in other localities. It grows on moist rock surfaces amidst moss under the forest, at elevations of approximately ca. 400–1000 m (Fig. 2).

Additional specimens examined. CHINA, Sichuan, Meishan City, Qingshen County, 29°45'47.39"N, 103°50'44.017"E, 26 December 2023 (fl.), Ke Huang & Zhixi Fu 8201 (SCNU!); Hongya County, 29°49'57.23"N, 103°09'50.23"E, 28 May 2015 (fl.), Ya J.D. & Hu X.J. 15CS11038 (KUN!)

**Conservation status.** Data Deficient (DD). Currently, two populations with more than 100 individuals have been found in the Qingshen and Hongya Counties. The population of *P. meishanensis* inhabits moist rocks. Given its currently limited occurrence near the temple, there is a significant likelihood that the taxon may also be found in other localities. Until we have fully investigated the situation, it would be suggested to assess the conservation status of the species as Data Deficient (DD) following the IUCN Red List Criteria (IUCN 2024).



Figure 2. Primula meishanensis sp. nov. A–C habitat D–H flowers I, J fruit (Photos A, B, D, E by ZF and C, F–J by KH).



Figure 3. Holotype image of Primula meishanensis K.Huang & Z.X.Fu, sp. nov.



Figure 4. Location of the population of *Primula meishanensis* in Qingshen County and Hongya County, Meishan City and Sichuan Province (red star).

Relationship with related species. Critical examination of collected specimens, comparison with type material of allied taxa and relevant taxonomic literature revealed that P. meishanensis is a new member of the P. sect. Petiolares. Morphologically, P. meishanensis shares certain similarities with P. dejuniana. However, P. meishanensis differs from P. dejuniana in featuring the leaf apex acute (vs. the leaf apex acute, but with a small point at tip), corolla pastel violet (vs. the corolla pale rose-purple), scape solitary, 7.2-11.8 cm long, carrying a terminal umbel of (1) 2-3 (4) flowers (vs. the scape usually one per rosette, 8-12 (18) cm long, carrying a terminal umbel of 2-6 flowers), the length of petiole 2.5-5 cm (vs. 1-3 cm), basal bud scales rose red (vs. flesh pink) and flowering period is from December to February (vs. from early February to early March). The number of chloroplast genes etc. is different in the two species (Table 2, Fig. 6, Suppl. material 1). To some extent, P. meishanensis also resembles to P. davidii as a perennial herb with calyx and corolla. However, it differs from P. davidii in leaf blade margin (sharply vs. erose-dentate) and apex (acute vs. rounded). The species of P. meishanensis bears similarities to P. epilosa, yet the former is readily distinguished by its leaf apex (acute vs. rounded), corolla (pastel violet vs. rose-purple with a yellow eye) and altitude (400-420 m vs. 2000-2900 m). Further morphological comparisons amongst the species of P. meishanensis, P. davidii, P. dejuniana and P. epilosa are shown in Table 1.









Features	P. meishanensis	P. dejuniana	P. epilosa	P. davidii
Roots	numerous, without hairs	numerous, without hairs	few, without hairs	numerous, without hairs
Leaf blade	<b>smooth</b> , oblanceolate, 19.5−25 × 2.5−5 cm	rough, oblanceolate, 8−13(22) × 2−3(5.5) cm	rough, oblong-obovate to oblong-oblanceolate, 5–10 × 2–4 cm	rough, oblong to obovate- oblong, (5–)8–18 × 1.5–4 cm
Petiole	2.5-5 cm long	1-3 cm long	0.5-2.5 cm long	indistinct to nearly obsolete
Leaf apex	acute	Acute, but with a small point at tip	rounded	rounded
Leaf margin	ciliate, sharply and remotely dentate	ciliate, sharply and remotely dentate	hydathode-dentate	erose-dentate
Scape	solitary, $7.2-11.8$ cm long, pilose, carrying a terminal umbel of (1)2-3(4) flowers	usually 1 per rosette, 8–12(–18) cm long, pilose, carrying a terminal umbel of 2–6 flowers	3.5–14 cm, sparsely glandular; umbel solitary, 2–5 flowered	8–20 cm, rust-coloured pilose, umbels 2–10-flowered
Corolla	pastel violet	pale rose-purple	rose-purple with a yellow eye	pale rose-purple
Pin flowers	corolla tube 9.0–10.0 mm long, stamens inserted at middle of corolla tube, style slightly exceeding the corolla tube mouth	corolla tube ca. 1.8 cm long, stamens inserted at middle of corolla tube, style reaching annulus	corolla tube ca. 1 cm; stamens ca. 4 mm above base of corolla tube; style slightly exserted	stamens ca. 3.5 mm above base of corolla tube; style ca. as long as tube
Thrum flowers	corolla tubes 11.0– 12.0 mm long, ca. 2 mm in diam., style 4.0–7.0 mm above base of corolla tube	corolla tube ca. 2 cm long, style ca. 9 mm long reaching to middle of corolla	corolla tube 1.4–1.7 cm; style 4–5.5 mm	unknown
Altitude	400–1000 m	618-979 m	2000-2900 m	ca. 1000 m
Flowering	December to February	early February to early March	April to May	April

**Table 1.** Morphological characters comparison amongst *P. meishanensis* and closely-related species of *P. dejuniana*, *P. breviscapa* and *P. epilosa*.

#### Molecular phylogeny

In this study, 35 chloroplast genome sequences and measured chloroplast genome sequence of P. meishanensis were used to construct the ML evolutionary tree (Table 2) (Fig. 5). Primula meishanensis is sister to Primula dejuniana, forming a highly supported clade. However, the branch lengths of the two species are different, so they should be two species. The number of chloroplast genes etc. is not the same in the two species (Table 3). The complete chloroplast genome of Primula dejuniana is 151,988 bp, with the GC content of 37.01% (Fig. 7). The LSC length of Primula dejuniana is 83,888 bp, SSC length is 17,730 bp and IR length is 25,185 bp. In the nucleotide sequence of the complete chloroplast gene of the Primula dejuniana, the number of A is 47,313, the number of T is 48,431, the number of C is 28,628 and the number of G is 27,616. The complete chloroplast genome of Primula meishanensis is 152,175 bp, with the GC content of 36.93% (Fig. 8). The LSC length of Primula meishanensis is 84,052 bp, SSC length is 17,773 bp and IR length is 25,175 bp. In the nucleotide sequence of the complete chloroplast gene of the Primula meishanensis, the number of A is 47,469, the number of T is 48,508, the number of C is 28,613 and the number of G is 27,585. These complete cp genomes sequences are aligned by MAFFT (Fig. 6). We obtained a fasta file for the comparison of the two species and put it in the additional file (see Suppl. material 1). The cis-splicing genes are the same, but their positions in the sequence are different (Figs 9, 11). The rps12 is a trans-splicing gene (Figs 10, 12).

Species	Family	Genus	GenBank number
Ardisia polysticta Miq.	Myrsinaceae	Ardisia Sw.	KC465962
Maesa montana A.DC.	Myrsinaceae	Maesa Forssk.	KU569490
Primula bracteata Franch.	Primulaceae	Primula L.	NC053592
Primula bulleyana Forrest	Primulaceae	Primula L.	NC046947
Primula calliantha Franch.	Primulaceae	Primula L.	ON804895
Primula chrysochlora Balf.f. & Kingdon-Ward	Primulaceae	Primula L.	KX668178
Primula chungensis Balf.f. & Kingdon-Ward	Primulaceae	Primula L.	NC050245
Primula denticulata Wight	Primulaceae	Primula L.	NC050247
Primula dryadifolia Franch.	Primulaceae	Primula L.	NC053596
Primula filchnerae R.Knuth	Primulaceae	Primula L.	NC051972
Primula forrestii Balf.f.	Primulaceae	Primula L.	NC053602
Primula handeliana W.W.Sm. & Forrest	Primulaceae	Primula L.	MG181221
Primula jiugongshanensis J.W.Shao	Primulaceae	Primula L.	NC056335
Primula knuthiana Pax	Primulaceae	Primula L.	MG181223
Primula kwangtungensis W.W.Sm.	Primulaceae	Primula L.	KX774737
Primula matthioli (L.) V.A.Richt.	Primulaceae	Primula L.	KY235373
Primula moupinensis Franch.	Primulaceae	Primula L.	NC050244
Primula obconica Hance, J. Bot.	Primulaceae	Primula L.	NC046415
Primula odontocalyx Pax	Primulaceae	Primula L.	NC065386
Primula oreodoxa Franch.	Primulaceae	Primula L.	NC050848
Primula pellucida Franch.	Primulaceae	Primula L.	NC050248
Primula persimilis G.Hao, C.M.Hu & Y.Xu	Primulaceae	Primula L.	KX641757
Primula poissonii Franch.	Primulaceae	Primula L.	KF753634
Primula pulchella Franch.	Primulaceae	Primula L.	NC050246
Primula ranunculoides F.H.Chen	Primulaceae	Primula L.	NC056361
Primula sikkimensis Hook.	Primulaceae	Primula L.	NC050243
Primula sinensis Lour.	Primulaceae	Primula L.	KU321892
Primula stenodonta Balf.f. ex W.W.Sm. & H.R.Fletcher	Primulaceae	Primula L.	KX668176
Primula szechuanica Pax	Primulaceae	Primula L.	NC080275
Primula tsiangii W.W.Sm.	Primulaceae	Primula L.	NC046755
Primula veris L.	Primulaceae	Primula L.	KX639823
Primula wilsonii Dunn	Primulaceae	Primula L.	MW442886
Primula woodwardii Balf.f.	Primulaceae	Primula L.	MG181222
Primula dejuniana G.Hao, C.M.Hu & Yuan Xu	Primulaceae	Primula L.	PQ213817
Primula meishanensis	Primulaceae	Primula L.	PQ213816

 Table 2. Molecular analysis for the species.

 Table 3. Comparative analyses of cp genomes between the two species.

Species	Genome Size (bp)	LSC (bp)	IR (bp)	SSC (bp)	А	т	С	G	GC Content (%)
Primula dejuniana	151,988	83,888	25,185	17,730	47,313	48,431	28,628	27,616	37.01
Primula meishanensis	152,175	84,052	25,175	17,773	47,469	48,508	28,613	27,585	36.93



**Figure 7.** Circular map of *Primula dejuniana*. The map of complete chloroplast genome was generated using CPGView (http://www.1kmpg.cn/cpgview).



**Figure 8.** Circular map of *Primula meishanensis*. The map of complete chloroplast genome was generated using CP-GView (http://www.1kmpg.cn/cpgview).



**Figure 9**. Schematic map of the cis-splicing genes in the chloroplast genome of *Primula dejuniana*.













#### Acknowledgements

We are grateful to the staff of KUN and P for providing online access to specimens.

#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This study was financially supported by the National Natural Science Foundation of China (No. 32000158), the National Science & Technology Fundamental Resources Investigation Program of China (No. 2021XJKK0702), the Foundation of Sustainable Development Research Center of Resources and Environment of Western Sichuan, Sichuan Normal University (No. 2020CXZYHJZX03), Laboratory equipment research projects, Sichuan Normal University (No. SYJS20220014), Mianyang Normal University Doctoral Start-up Fund Project (QD2023A33) and Meishan Ecological Quality Sample Site Monitoring Project (No. MSJCZ20230007).

#### Author contributions

ZF and KH collected this species. BL, XC, DH, YL, JY and XX performed the data analysis. TL wrote the manuscript. TL and XC revised the manuscript. All authors have read and approved the final manuscript.

#### Author ORCIDs

Tingyu Li <sup>©</sup> https://orcid.org/0009-0005-6873-2114 Xinyu Chen <sup>©</sup> https://orcid.org/0009-0008-6347-2490 Bo Li <sup>©</sup> https://orcid.org/0000-0002-5131-8639 Donglai Hua <sup>©</sup> https://orcid.org/0009-0002-7778-2608 Can Luo <sup>©</sup> https://orcid.org/0009-0007-1815-6926 Huixian Luo <sup>©</sup> https://orcid.org/0009-0002-8332-5494 Yun Liang <sup>©</sup> https://orcid.org/0009-0002-8332-5494 Yun Liang <sup>©</sup> https://orcid.org/0009-0002-8786-2473 Xiaodan Xi <sup>©</sup> https://orcid.org/0009-0008-4917-6345 Ke Huang <sup>©</sup> https://orcid.org/0009-0006-2057-5452 Zhixi Fu <sup>©</sup> https://orcid.org/0000-0002-2789-6287

#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

#### References

Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF (2006) A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nature Protocols 1(5): 2320–2325. https://doi.org/10.1038/nprot.2006.384

- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477. https://doi.org/10.1089/cmb.2012.0021
- Beentje H (2016) The Kew Plant Glossary, an Illustrated Dictionary of Plant Terms. Kew Publishing, 184.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Hu CM (1990) *Primula*. In: Chen FH, CM Hu (Eds) FI. Reipubl. Popularis Sin., Vol. 59. Science Press, Beijing, 1–277.
- Hu CM (1994) On the geographical distribution of the Primulaceae. Redai Yaredai Zhiwu Xuebao 2(4): 1–14.
- Hu CM (1998) Diversity and distribution of *Primula* in China. In: Qiu ST, CI Peng (Eds) Plant diversity and conservation by the Cross-strait. National Museum of Natural Science, 24–27.
- Hu CM, Geng YY (2003) Two New Species of *Primula* (Primulaceae) from China. Novon 13(2): 196–199. https://doi.org/10.2307/3393518
- Hu CM, Kelso S (1996) Primulaceae. In: Wu Z-Y, Raven PH (Eds) Flora of China, Vol. 15. Science Press, Beijing and Miss. Bot. Gard. Press, St. Louis, 39–78.
- IUCN (2024) Guidelines for using the IUCN Red List categories and criteria. Version 15.1. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/resources/redlistguidelines [Accessed March 1, 2024]
- Ju WB, Deng HN, Zhu DH, Gao YD, Gao XF, Xu B (2021) *Primula dujiangyanensis* (Primulaceae) discovered from Sichuan, Southwest China. Phytotaxa 510(3): 275–280. https://doi.org/10.11646/phytotaxa.510.3.7
- Ju WB, Deng HN, Liu F, He XJ, Gao XF, Xu B (2023) *Primula medogensis*, a new species of Primulaceae from Tibet of China. PhytoKeys 230: 107–114. https://doi.org/10.3897/phytokeys.230.107008
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Li R, Hu CM (2009) *Primula lihengiana* (Primulaceae), a new species from Yunnan, China. Annales Botanici Fennici 46(2): 130–132. https://doi.org/10.5735/085.046.0208
- Li X, Cheng YH, Lin HQ, Chen C, Gao XF, Deng HN, Yu F, Anđelka PM, Ju WB, Xu B (2023) *Primula wolongensis* (Primulaceae), a new species of the primrose from Sichuan, China. PhytoKeys 218: 47–57. https://doi.org/10.3897/phytokeys.218.91161
- Liu XF, Luo JJ, Zhang MK, Wang Q, Liu J, Wu D, Fu ZX (2023) Phylogenomic analysis of two species of *Parasenecio* and comparative analysis within tribe Senecioneae (Asteraceae). Diversity 15(4): 563. https://doi.org/10.3390/d15040563
- Luo JJ, Zhang MK, Liu XF, Chen H, Li TY, Ma XD, Huang K, Fu ZX (2023) *Primula xilingensis* (Primulaceae), a new species from Sichuan, China. PhytoKeys 234: 135–143. https://doi.org/10.3897/phytokeys.234.108411
- Rana HK, Rana SK, Sun H, Fujikawa K, Luo D, Joshi LR, Ghimire SK (2021) Saussurea talungensis (Asteraceae), a new species from Humla, Nepal Himalayas. PhytoKeys 176: 55–66. https://doi.org/10.3897/phytokeys.176.61996
- Rankin DWH (2010) *Primula nghialoensis*. Curtis's Botanical Magazine 27(2): 132–139. https://doi.org/10.1111/j.1467-8748.2010.01689.x

Richards J (2003) Primula. 2<sup>nd</sup> ed. Timber Press, London, 1-346.

- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57(5): 758–771. https://doi. org/10.1080/10635150802429642
- Sun J, Zhang DG, Huang XH, Tojibaev K, Yang JY, Wang HC, Deng T (2020) *Primula sun-hangii* (Primulaceae): A new species from Hubei, Central China. PhytoKeys 156: 103–112. https://doi.org/10.3897/phytokeys.156.49137
- Wang ZH, Wang Y, Chen L, Peng H, Wu ZK, Guo G (2022) *Primula longipilosa* (Primulaceae), a new species from Yunnan, China. PhytoKeys 194: 15–22. https://doi.org/10.3897/phytokeys.194.81335
- Wei D, Wang W, Xu Y, Hao G (2022) *Primula tsaiana* (Primulaceae), a New Species from Yunnan, China, and a New Synonym of *P. wenshanensis*. Annales Botanici Fennici 59(1): 111–116. https://doi.org/10.5735/085.059.0117
- Wu ZK, Zhao FW, Chen JH, Huang Y (2019) Primula dongchuanensis (Primulaceae), a new species from northern Yunnan, China. PhytoKeys 130: 171–181. https://doi. org/10.3897/phytokeys.130.35047
- Xiao JW, Yan GJ, Li WP, Tang M (2022) *Aster quanzhouensis* (Asteraceae), a new riparian species from eastern China. PhytoKeys 195: 93–106. https://doi.org/10.3897/ phytokeys.195.82411
- Xu Y, Yuan S, Hu CM, Hao G (2014) Primula dejuniana (Primulaceae), A New Species from Sichuan, China. Annales Botanici Fennici 51(6): 372–374. https://doi. org/10.5735/085.051.0602
- Xu Y, Yu XL, Hu CM, Hao G (2016) Morphological and molecular phylogenetic data reveal a new species of *Primula* (Primulaceae) from Hunan, China. PLoS One 11(8): e0161172. https://doi.org/10.1371/journal.pone.0161172
- Xu Y, Li CH, Hu CM, Hao G (2016a) *Primula wawushanica* sp. nov. (Primulaceae) from Sichuan, southwestern China. Nordic Journal of Botany 34(2): 156–158. https://doi. org/10.1111/njb.00894
- Xu Y, Yu XL, Hu CM, Hao G (2016b) Morphological and molecular phylogenetic data reveal a new species of *Primula* (Primulaceae) from Hunan, China. PLoS One 11(10): e0165355. https://doi.org/10.1371/journal.pone.0165355
- Xu Y, Huang G, Hu C, Hao G (2017) Primula pengzhouensis (Primulaceae), a new species from Sichuan, southwestern China. Plant Diversity 39(4): 229–231. https://doi. org/10.1016/j.pld.2017.08.003
- Xu Y, He DM, Yang LZ, Hao G (2022) Primula surculosa (Primulaceae), a new species from Yunnan, China. PhytoKeys 212: 29–35. https://doi.org/10.3897/phytokeys.212.91133
- Yang B, Ya JD, Zhang W, Song Y, Wang W, Zhu ZM, He JH, Zuo YJ, Tan YH (2023) Two new species of *Primula* (Primulaceae) from Yunnan, China. Taiwania 68(2): 230–240. https://doi.org/10.6165/tai.2023.68.230
- Zhang N, Jiang XQ, Wu ZK (2023) *Primula pingbaensis* (Primulaceae), a new species from Guizhou, China. PhytoKeys 221: 85–93. https://doi.org/10.3897/phytok-eys.221.97948
- Zheng X, Qin K, Li T, Qu T, Luo J, Zhang G, Li B, Li P, Fu Z (2024) A new species, Aster yaoshanensis (Asteracae, Astereae), from Guangxi (China), based on morphology and molecular phylogenetic data. Frontiers in Plant Science 15: 1367917. https://doi. org/10.3389/fpls.2024.1367917

#### **Supplementary material 1**

#### Supplementary data

Authors: Tingyu Li, Xinyu Chen, Bo Li, Donglai Hua, Can Luo, Huixian Luo, Yun Liang, Jieli Yue, Xiaodan Xi, Ke Huang, Zhixi Fu

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.127117.suppl1



**Research Article** 

### Description and phylogenetic position of a new species, *Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil (Saxifragaceae), from the southern islands of South Korea

Ju Eun Jang<sup>1</sup>, Beom Kyun Park<sup>1</sup>, Kang-Hyup Lee<sup>1</sup>, Hyuk-Jin Kim<sup>1</sup>, Hee-Young Gil<sup>1</sup>

1 Division of Forest Biodiversity, Korea National Arboretum, Pocheon 11186, Republic of Korea Corresponding author: Hee-Young Gil (warmishe@korea.kr)

#### Abstract

We describe a new species, *Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil, belonging to the family Saxifragaceae, from the southern islands of the Republic of Korea. *Chrysosplenium insularis* is morphologically similar to *C. japonicum* (Maxim.) Makino but can be distinguished by fairly persistent bulbils, green to yellowish-green sepals, four stamens, and cylindrical papillose seeds. *Chrysosplenium insularis* is also distinguished from *C. alternifolium* L., which is distributed in Europe, northern Russia, and the Caucasus, by the absence of stolons and green bracts. Phylogenetic analyses, based on one nuclear ribosomal (ITS) and two chloroplast (*rbcL*, *matK*) regions, confirmed that the new species was monophyletic and that *C. insularis* and *C. alternifolium* formed a sister relationship with robust support. Herein, we provide a detailed morphological description of *C. insularis* with its corresponding geographical distribution and comparison table and figures of related species.

Key words: Chrysosplenium, morphology, new species, phylogeny, taxonomy

#### Introduction

*Chrysosplenium* L. is a perennial herbaceous genus of the family Saxifragaceae, consisting of more than 70 species (Kim et al. 2019; Fu et al. 2020). Species of this genus are mainly distributed in temperate regions of the Northern Hemisphere, and their habitats are shady and humid areas in the mountains (Kim and Kim 2015; Kim et al. 2018; Zhao et al. 2022). Biogeographically, *Chrysosplenium* is known to have originated in East Asia, and several independent lineages have migrated from East Asia to the New World (Soltis et al. 2001; Deng et al. 2015; Liu et al. 2016).

The genus *Chrysosplenium* is distinguished from other genera in Saxifragaceae by its tetramerous flowers with petaloid sepals and four or eight stamens (Kim et al. 2018). However, species delimitation is often difficult in this genus because of extensive morphological variations owing to differences in growth periods and habitats (Qin et al. 2018; Kim et al. 2019; Choi et al. 2020). The genus is divided into two sections, *Chrysosplenium* sect. *Alternifolia* Franch. and *C.* sect. *Oppositifolia* Franch., based on the arrangement of the leaves (Franchet 1890). However,



Academic editor: Hugo de Boer Received: 5 July 2024 Accepted: 7 October 2024 Published: 23 October 2024

**Citation:** Jang JE, Park BK, Lee K-H, Kim H-J, Gil H-Y (2024) Description and phylogenetic position of a new species, *Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil (Saxifragaceae), from the southern islands of South Korea. PhytoKeys 248: 91–104. https://doi.org/10.3897/ phytokeys.248.131291

**Copyright:** <sup>©</sup> Ju Eun Jang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Hara (1957) proposed 17 series because of the high variability in the flower, capsule, and seed traits within each section. The infrageneric classification of *Chrysosplenium* species is based on several criteria, including leaf arrangement, seed surface, pedicel length, sterile branch position, capsule shape, stem surface, ovary position, stamen length, leaf surface, sepal length, and basal leaf size (Hara 1957; Fu et al. 2020). Several phylogenetic studies on Saxifragaceae genera, including *Chrysosplenium*, have been performed based on the chloroplast *mat*K region, and their results have shown that *C*. sect. *Oppositifolia* and *C*. sect. *Alternifolia* are monophyletic (Nakazawa et al. 1997; Soltis et al. 2001; Deng et al. 2015). Recently, several new species have been described based on detailed and comprehensive morphological, molecular, and cytological studies (Liu et al. 2016; Kim et al. 2018; 2019; Wakabayashi et al. 2018; Fu et al. 2020, 2021).

Thirteen *Chrysosplenium* species belonging to seven series have been recognized in the Korean Peninsula to date (Nakazawa et al. 1997; Kim et al. 2019; Choi et al. 2020; Korea National Arboretum 2021). The following are these 13 species [*Chrysosplenium* ser. *Pilosa* Maxim.: *C. flaviflorum* Ohwi, *C. epigealum* J.W.Han & S.H.Kang, *C. ramosissimum* Y.I.Kim & Y.D.Kim, *C. valdepilosum* (Ohwi) S.H.Kang & J.W.Han, *C. aureobracteatum* Y.I.Kim & Y.D.Kim, *C. barbatum* Nakai; *C. ser. Oppositifolia* Maxim.: *C. ramosum* Maxim.; *C. ser. Nepalensia* Maxim.: *C. grayanum* Maxim.; *C. ser. Sinica* Maxim.: *C. sinicum* Maxim.; *C. ser. Macrostemon* H. Hara: *C. macrostemon* Maxim. ex Franch. & Sav.; *C. ser. Alternifolia* Maxim.: *C. japonicum* (Maxim.) Makino, *C. serreanum* Hand.-Mazz.; *C. ser. Flagellifera* Maxim.: *C. flagelliferum* F.Schmidt], and among them, *C. aureobracteatum*, *C. barbatum*, *C. epigealum*, *C. flaviflorum*, and *C. ramosissimum* are endemic to Korea (Chung and Kim 1988; Korea National Arboretum 2021; Chung et al. 2023).

During a floristic survey in the southern part of Korea in March 2020, we found a new *Chrysosplenium* species that is restricted to the southern islands of Korea (Jeju-do and Gageo-do Islands). This species is readily distinguished from previously known *Chrysosplenium* species in Korea by its greenish-yellow to green bracteal leaves at flowering and a cylindrical papillose seed surface. This species is most similar to *C. japonicum* (Maxim.) Makino, which belongs to the *C.* ser. *Alternifolia*, and is distributed throughout Northeast Asia, including Southeast China, Japan, Korea, Russia (Manchuria), and Taiwan (Nakazawa et al. 1997; Pan and Ohba 2001; Hsu et al. 2011). The new species, however, is clearly distinguishable from *C. japonicum* by the form of bulbils, color of sepals, number of stamens, and surface of seeds. Based on thorough literature surveys, extensive field observations, detailed analysis of floral morphology and seed coat characteristics, we designated this new species as *C. insularis* J.E. Jang, K.H.Lee & H.Y.Gil. Here, we provide a detailed morphological description and phylogenetic position of *C. insularis* and its geographical distribution.

#### Materials and methods

#### Material collection

Field surveys were conducted from March 2020 to March 2023. Voucher specimens were deposited at the herbarium of the Korea National Arboretum (KH, http://www.nature.go.kr/kbi/plant/smpl/KBI\_2001\_030100.do). Materials preserved in 70% ethanol were used to observe and measure the floral parts. Morphological observations and measurements of the new species were conducted on live and dried specimens, including the materials preserved at KH. Quantitative characteristics were measured based on at least 30 samples. The terminology used for description and comparison was referenced from Choi et al. (2020), Pan and Ohba (2001), Lozina (1939), Wakabayashi (2001), Kim et al. (2018), Kim et al. (2019), Fu et al. (2020), Fu et al. (2021).

#### **Microscopic observation**

The seed morphology was observed under a stereomicroscope and a scanning electron microscope (SEM). The seeds were measured using a stereomicroscope (Carl Zeiss Microscopy GmbH, Stemi 508, Zeiss, Göttingen, Germany) with an Axiocam ERc 5s. Before SEM imaging, the seeds were dehydrated using 100% ethanol and sputter-coated with gold in a KIC-IA COXEM ion coater (COXEM Co., Ltd., Daejeon, Korea). SEM imaging was performed using a COX-EM EM-30 PLUS+ table scanning electron microscope (COXEM) at 20 kV at the Seed Testing Laboratory of KH.

#### **Phylogenetic analysis**

Molecular phylogenetic analyses were conducted to confirm the phylogenetic position of the new putative species of Chrysosplenium. Sixteen accessions of four taxa, including the new and related species, were collected from seven localities in South Korea. Total DNA was extracted from silica gel-dried leaves using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) in accordance with the manufacturer's instructions. The nrDNA region (ITS) and two cpDNA regions (matK, rbcL) were subjected to polymerase chain reaction (PCR) (Choi et al. 2020) on a ProFlex 96-Well PCR System (Applied Biosystems, Foster City, CA, USA). The primers used and their sequences are listed in Table 1. Each reaction mixture contained AccuPower® PCR PreMix (Bioneer, Daejeon, South Korea), ca. 10 ng (1 µL) of genomic DNA, and 100 pM of primers in a total volume of 20 µL. The PCR conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of amplification at 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR products were visualized on 1% agarose gels and sequenced on an ABI 3730xl DNA analyzer using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences obtained were manually determined and aligned using MAFFT with Geneious Prime® 2022.1.1. (Biomatters Ltd., Auckland, NZ). The DNA sequences generated in this study have been deposited in GenBank and are indicated with an asterisk (\*) in the voucher information in Table 2.

Table 1. Primers used for phylogenetic analysis.

Fragment	Primer	Sequence 5' $\rightarrow$ 3'	Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	
rbcL	rbcL_1F	ATGTCACCACAAACAGAAAC	Fay et al. (1998)
	rbcL_724R	TCGCATGTACCTGCAGTAGC	
matK	3F_Kim_F	CGTACAGTACTTTTGTGTTTA	K.J.Kim, pers. comm.
	1R_Kim_R	ACCCAGTCCATCTGGAAATCT	

			G	enRank numh	her
Taxon	Locality	Voucher information	ITS	rbcL	matK
C. alternifolium	JAPAN: Shimane-ken	DG2019032310003	OK315466	OK315387	OK315343
C. aureobracteatum	KOREA: Gangwon-do. Mt. Gwangdeog	LeeJD et al. 17127-1	MK989508	MK989534	MK989559
	KOREA: Gangwon-do, Mt. Gwangdeog	LeeJD et al. 17127-2	MK989509	MK989533	MK989562
C. barbatum	KOREA: Jeollanam-do, Woldeung-myeon	LeeJD et al. 17008-1	MK989505	MK989538	MK989560
	KOREA: Gyeongsangbuk-do, Mt. Danseok	LeeJD et al. 17020-1	MK989506	MK989536	MK989564
	KOREA: Gangwon-do, Mt. Gwangdeog	LeeJD et al. 17066	MK989507	MK989537	MK989561
C. flagelliferum	KOREA: Gyeongsangbuk-do, Ulleung-gun, Gwanmobong	ESK21-267*	OR809214	PP133187	PP170153
	KOREA: Gyeongsangbuk-do, Ulleung-gun, Gwanmobong	ESK21-268*	OR809215	PP133188	PP170154
	KOREA: Gyeongsangbuk-do, Ulleung-gun, Seonginbong	AP22-025*	OR809213	PP133186	PP170152
	KOREA: Gyeoggi-do, Mt. Cheonma	LeeJD et al. 17014	MK989499	MK989530	MK989585
	KOREA: Gangwon-do, Mt. Cheongtae	LeeJD et al. 17052-1	MK989500	MK989529	MK989583
	KOREA: Gyeongsangbuk-do, Ulleung-do	LeeJD et al. 17122	MK989501	MK989531	MK989584
C. flaviflorum	KOREA: Gangwon-do, Mt. Pokkye	ESK21-182*	OR809216	PP133189	PP170155
	KOREA: Gangwon-do, Mt. Pokkye	ESK21-183*	OR809217	PP133190	PP170156
	KOREA: Gangwon-do, Mt. Pokkye	ESK21-184-1*	OR809218	PP133191	PP170157
	KOREA: Gangwon-do, Mt. Pokkye	ESK21-184-2*	OR809219	PP133192	PP170158
	KOREA: Chungcheongbuk-do, Mt. Gyemyeong	LeeJD et al. 17030	MK989513	MK989542	MK989569
	KOREA: Gyeongsangbuk-do, Mt. Cheonglyang	LeeJD et al. 17039	MK989514	MK989540	MK989567
	KOREA: Gangwon-do, Mt. Chiak	LeeJD et al. 17048	MK989515	MK989541	MK989568
C. grayanum	JAPAN: Hokkaido, Sapporo, Mt. Maruyama	Nakamura 16401	MK989524	MK989554	MK989574
	JAPAN: Hokkaido, Sapporo, Mt. Maruyama	Nakamura 16402	MK989523	MK989553	MK989575
	JAPAN: Hyogo prefecture, Sasayama	Lee JH & JS Shin s. n.	MK989525	MK989551	MK989576
	KOREA: Jeollanam-do, Mt. Cheongtae	LeeJD et al. 17090-1	MK989522	MK989550	MK989579
	KOREA: Jeollanam-do, Mt. Cheongtae	LeeJD et al. 17090-2	MK989520	MK989555	MK989578
	KOREA: Jeollanam-do, Mt. Cheongtae	LeeJD et al. 17090-3	MK989521	MK989552	MK989577
C. griffithii	CHINA	13PXD035	MH809138	MN185317	MN451058
C.insularis	KOREA: Jeju-do, Seogwipo-si, Hogeun-dong	SOK-2022-175*	OR809225	PP133198	PP170164
	KOREA: Jeju-do, Seogwipo-si, Hogeun-dong	J.E.Jang et al. 230322*	OR809226	PP133199	PP170165
	KOREA: Jeollanam-do, Gageodo	K.H.Lee 230514-1*	OR809227	PP133200	PP170166
	KOREA: Jeollanam-do, Gageodo	K.H.Lee 230514-2*	OR809228	PP133201	PP170167
C. japonicum	KOREA: Gyeonggi-do, Mt. Cheonma	J.E.Jang 230325-1*	OR809220	PP133193	PP170159
	KOREA: Gyeonggi-do, Mt. Cheonma	J.E.Jang 230325-2*	OR809221	PP133194	PP170160
	KOREA: Gangwon-do, Wonju-si	S.R.Lee et al. 230420-1*	OR809222	PP133195	PP170161
	KOREA: Gangwon-do, Wonju-si	S.R.Lee et al. 230420-2*	OR809223	PP133196	PP170162
	KOREA: Gangwon-do, Wonju-si	S.R.Lee et al. 230420-3*	OR809224	PP133197	PP170163
	KOREA: Jeollabuk-do, Mt. Chaegye	LeeJD et al. 17022	MK989502	MK989548	MK989586
	KOREA: Chungcheongnam-do, Palbong-myeon	LeeJD et al. 17025-1	MK989504	MK989549	MK989587
C. kamtschaticum	JAPAN: Hokkaido, Sapporo, Mt.Maruyama	Nakamura 16403	MK989516	MK989539	MK989566
C. ramosum	KOREA: Chungcheongbuk-do, Daegang-myeon Goseong	LeeJD et al. 17097-1	MK989517	MK989543	MK989571
	KOREA: Gangwon-do, Mt. Taegi	LeeJD et al. 17147	MK989518	MK989545	MK989573
	KOREA: Gyeongsangbuk-do, Mt. Irwol	LeeJD et al. 17205-1	MK989519	MK989544	MK989572
C. sinicum	KOREA: Jeju-do, Haean-dong	LeeJD et al. 17043	MK989528	MK989557	MK989582
	KOREA: Gangwon-do, Mt. Cheongtae	LeeJD et al. 17051-1	MK989526	MK989556	MK989580
	KOREA: Chungcheongbuk-do, Gagok-myeon	LeeJD et al. 17086	MK989527	MK989558	MK989581
C. valdepilosum	KOREA: Gangwon-do, Mt. Taegi	LeeJD et al. 17053-1	MK989512	MK989535	MK989563
	KOREA: Jeollabuk-do, Mt. Jiri	LeeJD et al. 17057	MK989510	MK989532	MK989565
Peltoboykinia	JAPAN: Nagano, Kiso-Fukushima,	Okuyama 035251	AB248847		
tellimoides	CHINA: Zhejiang, Suichang County	XXL170002-1		MZ7	79205

Table 2. Voucher information and GenBank number of accessions used in this study (\*newly generated sequences).

We also included 32 accessions of 13 *Chrysosplenium* species deposited in GenBank and selected *Peltoboykinia tellimoides* (Maxim.) Hara as the outgroup (Soltis et al. 1996). A total of 47 accessions from 14 taxa were used for the phylogenetic analysis. Details of the voucher information and GenBank accession numbers of the species used in this study are provided in Table 2. Phylogenetic analyses were performed using the maximum likelihood (ML) method. For the ML analysis, the best-fit model was identified using ModelFinder in Phylosuite (Kalyaanamoorthy et al. 2017; Zhang et al. 2020). ML phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under the GTR+ F + R3 model in the ITS regions and the TIM+F+R2 model in the combined chloroplast regions (Minh et al. 2013).

#### **Results and discussion**

#### **Taxonomic treatment**

*Chrysosplenium insularis* J.E.Jang, K.H.Lee & H.Y.Gil, sp. nov. urn:lsid:ipni.org:names:77350706-1 Figs 1, 4A-F

**Diagnosis.** *Chrysosplenium insularis* differs from *C. japonicum* in having fairly persistent bulbils, green to yellowish-green sepals, four stamens, and cylindrical papillose seeds.

**Type.** KOREA • Jeju, Seogwipo-si, Hogeun-dong; 33.25084, 126.54434; elev. 58 m; 25 Mar 2020 [fl]; *Kang-Hyup Lee JJ-200325-001* [holotype KH (Fig. 2); isotypes, 3 sheets, KH].

Description. Perennial herbs, hermaphroditic, 5-15 cm tall. Bulbils present near stem base, fairly persistent, pink, turning darkish brown, pilose. Roots fibrous, white. Stems erect, cespitose, light green to green, sparsely hairy, without stolons. Basal leaves of flowering stems 1-6, opposite, simple, estipulate; petiole 3-9 cm long, glabrescent or sparsely hairy; blade reniform, 13-20 mm × 15-25 mm, apex rounded and often retuse, margins dentate to crenate, 13–17 teeth, base cordate, adaxially green, pilose, abaxially pale green, subglabrous. Cauline leaves of flowering stems 1-4, alternate, simple, estipulate; petiole 5-22 mm long, glabrescent or sparsely hairy; blade flabellate to reniform, 7-12 mm × 11-18 mm, apex retuse and often rounded or obtuse, margins dentate to crenate, 9-13 teeth, base cordate to broadly cuneate, adaxially green, pilose, abaxially pale green, subglabrous. Inflorescences terminal, 6-14 flowered cyme, surrounded by leaf-like bracts; peduncles 4.59-18.54 mm long; pedicels 0.5-1.5 mm long, sparsely pilose; bracteal leaves by inflorescence 3, petiole 0.2-4.7 mm long, glabrescent or sparsely hairy; blade subflabellate to orbicular, 2-18 × 2-14 mm, apex truncate and often retuse, margins dentate to crenate, 5-9 teeth, base broadly cuneate to subcordate, adaxially green, sparsely pilose to glabrescent, abaxially pale green, subglabrous. Flowers 4-merous, actinomorphic; sepals petaloid 4, free, erect to subspreading, ovate to broadly ovate, 1.2-2.1 × 1.5-3.1 mm, apex obtuse or rounded, yellowish green to green, glabrous; stamens 4; filaments narrow conical, 0.3-0.4 mm long; anther 0.2 mm long, yellow; pistil 2-carpellate, semi-inferior; ovary 1-locular; styles 2, free, erect, 0.2-0.3 mm long; stigma round; disc present. Capsules 2-lobed, horn shaped, lobes subequal, 2.8-3.7 × 3.8-5.2 mm long, green, glabrous, dehiscent



Figure 1. *Chrysosplenium insularis* **A** habit **B** bulbils **C** stem **D** basal leaf **E** inflorescence, **F** bracteal leaves **G** flower **H**, **I** capsule **J** seed. Photographs by Ju Eun Jang and Kang-Hyup Lee.

along the adaxial suture. Seeds numerous, ovoid-ellipsoid,  $0.7-0.9 \times 0.5-0.6$  mm, brown to dark brown, cylindrical papillose on smooth surfaces.

**Phenology.** Flowering and fruiting from March to May.

**Distribution and habitat.** Southern coastal regions of Korea (Jeju-do and Gageo-do Islands). Forests, wet places in forests, shaded places on the riverside (Fig. 3).

**Etymology.** The specific epithet *"insularis"* refers to its distribution on islands. **Vernacular name.** Island golden saxifrage: Seom-gwaeng-i-nun (섬괭이눈).

**Morphological assessment.** Among the species distributed in Korea, *Chrysosplenium insularis* is morphologically similar to *C. japonicum* in terms of leaf arrangement, leaf margin, and bracteal leaf color. Despite these similarities, it is clearly differentiated by the form of bulbils [present, fairly persistent (Fig. 1B) vs. present], surface of bracteal leaves [adaxially sparsely pilose to glabres-





cent, abaxially subglabrous (Fig. 4D) vs. mainly glabrous (Fig. 4J)], color of sepals [green to yellowish green vs. yellowish green to yellow], number of stamens [4 vs. usually 8], and surface of seeds [cylindrical papillose (Fig. 4E, F) vs. papillose (Fig. 4K, L)]. Additionally, this new species is morphologically similar to *C. alternifolium*,



Figure 3. Distribution map of Chrysosplenium insularis and C. japonicum in Korea (revised from Oh et al. 2016).

which is distributed in northern Eurasia, but is distinguished by the following characteristics: stolon (absent vs. present), color of bracts (green vs. yellow), color of sepals (green to yellowish green vs. golden yellow), number of stamens (4 vs. 8), and surface of seeds [cylindrical papillose (Fig. 4E, K) vs. smooth (Fig. 4K, L)]. A comparison of the major characteristics of the new species with those of two closely related species, *C. japonicum* and *C. alternifolium*, is shown in Table 3.

**Phylogenetic analysis.** In total, 48 sequences of three regions (ITS, *matK*, and *rbcL*) were newly obtained from the 16 accessions of *Chrysosplenium insularis* and the three related taxa. We also used 93 sequences from 32 accessions obtained from GenBank (12 species of *Chrysosplenium* and one *Peltoboykinia tellimoides* as an outgroup) for the phylogenetic analysis. The aligned matrix of the ITS region and combined chloroplast regions (*matK* and *rbcL*) contained 635 and 1407 characters, respectively. We found 242 variable sites and 193 parsimony-informative sites in the ITS regions, whereas 172 variable sites and 104 parsimony-informative sites were found in the combined chloroplast regions. The GC ratios were 46.2% and 37.4% for the ITS and combined chloroplast regions, respectively. The phylogenetic tree (Fig. 5) revealed a topology similar to that obtained in a previ-

Table 3. Major characteristics of *Chrysosplenium insulalis* and two closely related taxa (\*: data from Lozina 1939; -: none known).

Charac	ter	C. insulalis	C. japonicum	C. alternifolia*	
Bulbils		present, fairly persistent	present	-	
	color	pink, turning to darkish brown	pink	-	
Stolon		absent	absent	present	
Bracteal leaves	color	green	yellowish green	yellow	
	surfaces	adaxially sparsely pilose to glabrescent, abaxially subglabrous	mainly glabrous	mainly glabrous	
Sepals	color	green to yellowish green	yellowish green to yellow	golden yellow	
Stamens	number	4	usually 8	8	
Seeds	surfaces	cylindrical papillose	papillose	smooth	
Fl. and fr.		Mar. to May	Apr. to Jun.	Apr. to Jul.	



Figure 4. Comparative photographs of the habit (A, G), inflorescence (B, H), bracteal leaves (C, I), surface of bracteal leaves (D, J), and seed (E, F, K, L) of *Chrysosplenium insularis* (A–F) and *C. japonicum* (G–L). Photographs by Ju Eun Jang and Kang-Hyup Lee.



**Figure 5.** Phylogenetic tree of *Chrysosplenium insularis* and related taxa based on ITS regions and combined CP regions (*mat*K and *rbc*L) **A** ITS region **B** combined CP regions (*mat*K and *rbc*L). The numbers above the branches are bootstrap values (BS > 50%) by the maximum likelyhood method. Newly generated sequences in this study are shown with an asterisk, and the new species are marked with a red box. The voucher information of all samples used in the analysis is indicated after the scientific names.

ous study (Choi et al. 2020). The phylogenetic results showed some topological incongruence between the ITS and combined CP trees. In the ITS tree, the most basal clade (BS = 100%) included the monophyletic *C. grayanum* and *C. sinicum* and showed a sister relationship with other *Chrysosplenium* species. However, the CP tree was divided into two clades, with *C. grayanum* and *C. sinicum* sharing the most common ancestors with the *C.* ser. *Pilosa*, *C. kamtschaticum*, and *C. ramosum* (BS = 94%). The phylogenetic relationships among the three subclades were not fully resolved, and the *C.* ser. *Pilosa*. was not monophyletic, embedding *C. kamtschaticum*. Furthermore, the series *Alternifolia* was monophyletic in the CP tree but not in the ITS tree. Both trees strongly supported the monophyly of *C. insularis* (BS = 95% in ITS, BS = 96% in CP), and it shared the most common ancestor with *C. alternifolium* distributed in Japan (BS = 98% in ITS, BS = 77% in CP). The phylogenetic trees revealed that *C. insularis* formed an independent monophyletic clade from closely related taxa (i.e., *C. japonicum* and *C. alternifolium*), suggesting the newly recognized species of *Chrysosplenium* (Fig. 5).

Additional specimens examined. Chrysosplenium insularis (Paratypes):
KOREA • Jeonnam, Sinan-gun, Heuksan-myeon, Gageodo-ri; 14 May 2023; K.H.Lee 230514-1 (KH). • Jeju, Seogwipo-si, Hogeun-dong; 28 Apr. 2020; PBK0118-001 (KH). • Jeju, Seogwipo-si, Hogeun-dong; 22 Mar. 2022; Hanon-220322-011 (KH)
• Jeju, Seogwipo-si, Hogeun-dong; 22 Mar. 2023; J.E.Jang et al. 230322-1 (KH). Chrysosplenium japonicum: KOREA • Gyeonggi, Gwangju-si, Chowol-eup,

Mugap-ri, Mugapsan; 24 Apr. 2007; *HNHM-A-158* • Gwangju-si, Toechon-myeon, Cheonjinam; 7 Apr. 2000; *KNAH014041* • Gwangju-si, Toechon-myeon, Usan-ri,

Aengjabong; 11 Apr. 2004; kjs040141 (KH) · Incheon-si, Ongjin-gun, Jawoldo Isl.; 8 Apr. 2009; NAPI-2009-1214 (KH) · Incheon-si, Ongjin-gun, Deokjeok-myeon, Mungap-ri, Gitdaebong; 9 Apr. 2014; Park140230 (KH) • Incheon-si, Ganghwa-gun, Ganghwado Isl.; 20 Apr. 2006; LeeGH6-35 (KH) • Gyeonggi, Namyangju-si, Onameup, Cheonmasan; 17 Apr. 2009; ParkSH90273 (KH) · Gyeonggi, Namyangjusi, Onam-eup, Palhyeon-ri; 25 May 2023; J.E.Jang 230325-1 (KH) · Gyeonggi, Namyangju-si, Joan-myeon, Ungilsan; 11 Apr. 2009; Y.M.Kang s.n. (KH) · Gyeonggi, Gwacheon-si, Makgye-dong, Cheonggyesan; 8 Apr. 2006; KHUS20110475 (KH) • Gangwon, Pyeongchang-gun, Yongpyeong-myeon, Jaesan-ri, Geumdangsan; 17 Apr. 2012; JSY120434 (KH) · Jeongseon-gun, Imgye-myeon; 23 Apr. 2011; 0307013 (KH) · Gangwon, Taebaek-si, Hasami-dong, Deokhangsan; 23 Apr. 2005; kjs050052 (KH) · Gangwon, Wonju-si, Panbu-myeon, Geumdae-ri; 20 Apr. 2023; S.R.Lee et al. 230420-1 (KH) · Chungbuk, Danyang-gun, Danyang-eup, Suchon-ri, Sobaeksan; 17 Apr. 2005; Sobaeksan-050417-070 (KH) · Chungbuk, Chungju-si, Sotae-myeon, Boktan-ri; 12 Apr. 2012; Namhan-548 (KH) · Gyeongbuk, Gunui-gun, Bugye-myeon, Dongsan-ri, Palgongsan; 22 Apr. 2006; CBU-070308 (KH) · Gyeongbuk, Bonghwa-gun, Myeongho-myeon, Bugok-ri, Cheongnyangsan; 27 Mar. 2006; CBU-070519 (KH) · Chungbuk, Cheongsong-gun, Hyeonseo-myeon, Bohyeonsan; 22 Apr. 2006; K.O. Yoo s.n. (KH) · Jeonbuk, Namwon-si, Ayeong-myeon, Gusang-ri, Bonghwasan; 1 May 2007; HNHM-A-283 (KH).

## Key to the species of *Chrysosplenium* in South Korea modified from Choi et al. (2020)

1a	Cauline leaves alternate2
2a	Leaves heterophyllous; sterile branches developed; caluline and bracteal
	leaves 2-5 lobed C. flagelliferum
2b	Leaves isophyllous; sterile branch absent; cauline and bracteal leaves not
	lobed with 8–12 teeth
3a	Sepals green; stamens 4 C. insularis
3b	Sepals yellowish green or golden yellow; stamens 84
4a	Stolons present; sepals golden yellow; seed surface smooth
	C. alternifolium
4b	Stolons absent; sepals yellowish green; seed surface papillose
	C. japonicum
1b	Cauline leaves opposite5
5a	Sepals green, spreading; capsules cup-shapedC. ramosum
5b	Sepals yellow, erect; capsules horn-shaped6
6а	Plants glabrous7
7a	Stamens 4 (-6); cylindrical papillae with roundish head at the tip on smooth
	seedC. grayanum
7b	Stamens 8; cylindrical papillae with truncate tip on scabrous seed surfac-
	es8
8a	Sterile branches present; plant glabrous except petiole of sterile branches;
	stamens shorter than the sepals C. sinicum
8b	Sterile branches absent; plant glabrous; stamens longer than the sepals
	C. macrostemon
6b	Plants pubescent
9a	Seeds without tubercules10

eaves of sterile branches congested at the distal end, with white variegat- ed veins on the upper surface <b>C. flaviflorum</b>	10a
eaves of sterile branches distantly arranged, with silvery dotted upper sur-	10b
ace <b>C. epigealum</b>	
Seeds with tubercules11	9b
Seed tubercles arranged on inconspicuous longitudinal ridges <b>12</b>	11a
Sterile branches highly branched, ca. 30 cm long after fruiting; leaves of	12a
sterile branches with silvery dots, upper surface glabrous; bracteal leaves	
/ellowish-green <b>C. ramosissimum</b>	
Sterile branches unbranched, less than 15 cm long after fruiting; leaves of	12b
sterile branches without silvery dots, upper surface pilose; bracteal leaves	
pright yellow <b>C. valdepilosum</b>	
Seed tubercles arranged on prominent longitudinal ridges <b>13</b>	11b
eaves of sterile branches distantly arranged after fruiting; bracteal leaves	13a
golden yellow, greenish yellow at flowering <b>C. aureobracteatum</b>	
eaves of sterile branches congested at the distal end after fruiting; brac-	13b
eal leaves green at flowering <b>C. barbatum</b>	

#### Acknowledgements

We greatly thank Seokmin Yoon and Jin Suk Kim for providing information about this new species and respect their passion for exploring nature.

#### **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This study was financially supported by the research projects of the Korea National Arboretum [KNA 1-1-13, 14-1].

#### Author contributions

Conceptualization: KL. Resources: BKP. Visualization: KL, BKP, and JEJ. Supervision: HG. Writing the original draft: JEJ. Writing, review, and editing: JEJ, KL, HK, and HG.

#### **Author ORCIDs**

Ju Eun Jang <sup>®</sup> https://orcid.org/0000-0002-6229-8477 Beom Kyun Park <sup>®</sup> https://orcid.org/0000-0003-1894-3296 Kang-Hyup Lee <sup>®</sup> https://orcid.org/0000-0002-7189-3235 Hyuk-Jin Kim <sup>®</sup> https://orcid.org/0000-0002-3177-2914 Hee-Young Gil <sup>®</sup> https://orcid.org/0000-0003-3714-0827

#### **Data availability**

All of the data that support the findings of this study are available in the main text.

#### References

- Choi JE, In KH, Kim BS, Kim K, Kim JS, Kim YI, Lee BY, Lim CE (2020) A new distribution record of *Chrysosplenium grayanum* Maxim.(Saxifragaceae) in Korea: Evidence from morphological and molecular data. Journal of Species Research 9(1): 46–55. https://doi.org/10.12651/JSR.2020.9.1.046
- Chung YH, Kim YD (1988) Monographic Study of Endemic Plants in Korea X. Taxonomy and Interspecific Relationship of the Genus *Chrysosplenium*. Korean Journal of Environmental Biology 6(2): 33–63.
- Chung GY, Jang H, Chang KS, Choi HJ, Son DC (2023) A checklist of endemic plants on the Korean Peninsula II. Korean Journal of Plant Taxonomy 53(2): 79–101. https:// doi.org/10.11110/kjpt.2023.53.2.79
- Deng JB, Drew BT, Mavrodiev EV, Gitzendanner MA, Soltis PS, Soltis DE (2015) Phylogeny, divergence times, and historical biogeography of the angiosperm family Saxifragaceae. Molecular Phylogenetics and Evolution 83: 86–98. https://doi.org/10.1016/j. ympev.2014.11.011
- Fay MF, Bayer C, Alverson WS, de Bruijn AY, Chase MW (1998) Plastid *rbcL* sequence data indicate a close affinity between *Diegodendron* and *Bixa*. Taxon 47(1): 43–50. https://doi.org/10.2307/1224017
- Franchet AR (1890) Monographie du genere *Chrysosplenium* Tourn. Nouvelles Archives du Muséum d'Histoire Naturelle, Série 3 2: 87–114.
- Fu LF, Liao R, Lan DQ, Wen F, Liu H (2020) A new species of *Chrysosplenium* (Saxifragaceae) from Shaanxi, north-western China. PhytoKeys 159: 127–135. https://doi. org/10.3897/phytokeys.159.56109
- Fu LF, Yang TG, Lan DQ, Wen F, Liu H (2021) *Chrysosplenium sangzhiense* (Saxifragaceae), a new species from Hunan, China. PhytoKeys 176: 21–32. https://doi.org/10.3897/phytokeys.176.62802
- Hara H (1957) Synopsis of genus *Chrysosplenium* L. Journal of the Faculty of Science, University of Tokyo, Section III. Botany 7: 1–90.
- Hsu TC, Chung SW, Cheng YC (2011) *Chrysosplenium japonicum* (Saxifragaceae), Newly Recorded from Taiwan. Taiwania 56(4): 337–340.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Kim YI, Kim YD (2015) Chrysosplenium aureobracteatum (Saxifragaceae), a new species from South Korea. Novon: A Journal for Botanical Nomenclature 23(4): 432–436. https://doi.org/10.3417/2013018
- Kim YI, Cho SH, Lee JH, Kang DH, Park JH, Kim YD (2018) Chrysosplenium ramosissimum Y.I.Kim & Y.D.Kim (Saxifragaceae), a new species from Korea. PhytoKeys 111: 1–10. https://doi.org/10.3897/phytokeys.111.27182
- Kim YI, Shin JS, Lee S, Chen JH, Choi S, Park JH, Kim YD (2019) A new species of *Chrysosplenium* (Saxifragaceae) from Northeastern China. PhytoKeys 135: 39–47. https://doi.org/10.3897/phytokeys.135.39036
- Korea National Arboretum (2021) Checklist of Vascular Plants in Korea (Native Plants). Korea National Arboretum, 1006 pp.
- Liu H, Luo J, Liu Q, Lan D, Qin R, Yu X (2016) A new species of *Chrysosplenium* (Saxifragaceae) from Zhangjiajie, Hunan, central China. Phytotaxa 277(3): 287–292. https://doi. org/10.11646/phytotaxa.277.3.7

- Lozina AS (1939) *Chrysosplenium* L. In: Komarov VL, Yuzepchuk SV (Eds) Flora of the U.S.S.R, Vol. 9. Botanical Institute of Academy of Science, Leningrad, 156–158.
- Minh BQ, Nguyen MA, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi. org/10.1093/molbev/mst024
- Nakazawa M, Wakabayashi M, Ono M, Murata J (1997) Molecular phylogenetic analysis of *Chrysosplenium* (Saxifragaceae) in Japan. Journal of Plant Research 110(2): 265–274. https://doi.org/10.1007/BF02509315
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Oh BU, Ko SC, Kang SH, Paik WK, Yoo KO, Im HT, Jang CG, Chung GY, Choi BH, Choi HJ, Lee YM, Shin CH, Choi K, Han JS, Park SH, Kim HJ, Chang KS, Yang JC, Jung SY, Lee CH, Oh SH, Jo DG (2016) Distribution Maps of Vascular Plants in Korea. Korea National Arboretum, Pocheon, 216 pp.
- Pan JT, Ohba H (2001) *Chrysosplenium*. In: Wu ZY, Raven PH (Eds) Flora of China, Vol.
  8. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 346–358.
- Qin R, Lan DQ, Huang W, Liu H (2018) Research progress on chemical constituents and pharmacological activities of *Chrysosplenium* spp.(Saxifragaceae). Journal of South-Central University for Nationalities 37: 54–59. [Natural Science Edition]
- Soltis DE, Kuzoff RK, Conti E, Gornall R, Ferguson K (1996) matK and rbcL gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. American Journal of Botany 83(3): 371–382. https://doi.org/10.1002/j.1537-2197.1996.tb12717.x
- Soltis DE, Nakazawa MT, Xiang QY, Kawano S, Murata J, Wakabayashi M, Jetter CH (2001) Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on matK sequence data. American Journal of Botany 88(5): 883–893. https://doi.org/10.2307/2657040
- Wakabayashi M (2001) *Chrysosplenium* L. In: Iwatsuki K, Boufford DE, Ohba H (Eds) Flora of Japan, Vol. IIb. Kodansha Ltd., Tokyo, 58–70.
- Wakabayashi M, Takahashi H, Tomita S (2018) *Chrysosplenium suzukaense* (Saxifragaceae), a New Species from Yoro and Suzuka Mts., Central Honshu, Japan. Acta Phytotaxonomica et Geobotanica 69(1): 41–51.
- White TJ, Bruns TD, Lee SB, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, California, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular ecology resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhao J, Qiu X, Zhao Y, Wu R, Wei P, Tao C, Wan L (2022) A review of the genus *Chrysospleni-um* as a traditional Tibetan medicine and its preparations. Journal of Ethnopharmacology 290: 115042. https://doi.org/10.1016/j.jep.2022.115042



**Research Article** 

# *Papaver* recircumscribed: A review of neighbouring Papaveraceae genera, including *Afropapaver* nom. et stat. nov. and *Oreomecon*, a large, Arctic-Alpine genus

Arve Elvebakk<sup>10</sup>, Jarle W. Bjerke<sup>1,20</sup>

1 Arctic University Museum of Norway, UiT The Arctic University, PO Box 6050 Langnes, NO-9037 Tromsø, Norway

2 Norwegian Institute for Nature Research, FRAM – High North Research Centre for Climate and the Environment, PO Box 6606 Langnes, NO-9296 Tromsø, Norway Corresponding author: Arve Elvebakk (arve.elvebakk@uit.no)

#### Abstract

Papaveraceae tribus Papavereae includes an American and a mainly Eurasian group of genera. The latter is proposed here to include eight genera. Amongst these, the recently described genus Oreomecon is phylogenetically a sister group to Meconopsis, a genus from Himalaya and central China, which is reviewed here as including 95 species and 21 subspecies. By contrast, Oreomecon has a circumpolar northern alpine and Arctic distribution, including incompletely understood taxa, many threatened by climatic warming. Based on a review of literature and phylogenies, it is proposed here that Oreomecon includes 68 species and 29 subspecies. Oreomecon aurantiaca, O. cornwallisensis, O. keelei, O. ochotensis and O. uschakovii, 29 subspecies and four varieties are placed in Oreomecon here, 29 of these as recombinations, the remaining ones as nomenclatural novelties. A total of 21 existing Oreomecon names are placed into synonymy. The taxonomically challenging O. alpina group from Central Europe is treated as comprising three species, with the remaining entities positioned at the subspecies level pending further studies. The much-studied Nordic species O. radicata is treated with eight subspecies here, based on morphometric studies, whereas four accepted entities are provisionally recombined at the variety level. The name Papaver tenellum and the basionyms of Oreomecon alborosea, O. alpina subsp. corona-sancti-stephani, O. alpina subsp. degenii, O. anomala, O. lapeyrouseana subsp. endressii, O. lapponica subsp. laestadiana and O. nivalis are lectotypified here. Two replacement names, Oreomecon alpina subsp. markgrafiana and O. radicata subsp. knabeniana, are introduced.

Papaver, as currently understood, is recircumscribed here to represent four genera. The isolated section *Horrida*, from southern Africa, is raised to genus level with the new name *Afropapaver* and its only species is recombined as *Afropapaver aculeatum*. *Papaver* sect. *Californica* from California and adjacent Mexico is treated as the genus *Stylomecon*. The name has been applied to one of the two species of this group and we now recombine the other one as *S. crassifolia*, based on an older basionym replacing *Papaver californicum*. *Papaver cambricum* is accepted in its alternative position as the monotypic genus *Parameconopsis*. As reviewed here, *Papaver* comprises 59 species and 14 subspecies and is only the third-largest genus in the group. Based on the distribution of its closest relatives and oldest sections, it is hypothesised here that *Papaver* arose in the western Mediterranean. Its poricidal capsule dehiscence serves as an excellent adaptation to seed dispersal in open, arid environments, possibly explaining its later success in the Türkiye-Caucasus-Middle East area, where its diversity both at species and section level is highest.



Academic editor: Marco Pellegrini Received: 18 February 2024 Accepted: 18 September 2024 Published: 29 October 2024

**Citation:** Elvebakk A, Bjerke JW (2024) *Papaver* recircumscribed: A review of neighbouring Papaveraceae genera, including *Afropapaver* nom. et stat. nov. and *Oreomecon*, a large, Arctic-Alpine genus. PhytoKeys 248: 105–188. https://doi.org/10.3897/ phytokeys.248.121011

**Copyright:** © Arve Elvebakk & Jarle W. Bjerke. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). **Key words:** Distribution, *Meconopsis, Parameconopsis*, phylogeny, poppies, species diversity, *Stylomecon*, taxonomy

#### Introduction

*Papaver* L. and *Meconopsis* Vig. are two large plant genera with striking flowers and both are very popular in gardens, the latter restricted to areas with cool climates (Grey-Wilson 2014, 2017; Stevens 2015). In addition, *Papaver* is also very important in medicinal research (Sariyar 2002; Catania et al. 2022), while *Meconopsis* has been extensively used in regional ethno-medicine (Guo et al. 2016). These genera belong to the tribus Papavereae Dumontier of the subfamily Papaveroideae Eaton of the large family Papaveraceae with its long evolutionary history as shown by its estimated crown age of 120 Ma (Stevens 2001 onwards; Peng et al. 2023). Papavereae, with its embedded tribe Platystemoneae Spach, was split into a North American and a Eurasian clade in sister group positions after a dispersal event from Asia to North America at 81.5 Ma (Peng et al. 2023).

Papaver and Meconopsis are the largest genera within the latter group. However, the differentiation between these genera has been phylogenetically problematic. Consequently, Kadereit et al. (2011) proposed five actions to obtain monophyly. The first required action was a reclassification of Meconopsis cambrica (L.) Vig. to its former basionym Papaver cambricum L. accompanied by a necessary lectotypification, the latter made by Ferrer-Gallego (2015). The second was defining a new generitype for Meconopsis replacing M. cambrica after the recircumscription of the genus, which was done by Grey-Wilson (2012).

The long evolutionary history of *Papaver cambricum* shown by Valtueña et al. (2012), combined with its distinct morphology, calls for a status as a separate genus. Thus, the new genus *Parameconopsis* Grey-Wilson was proposed (Grey-Wilson 2014), albeit without any thorough phylogenetic discussion. Valtueña et al. (2012) showed that *Papaver cambricum* represents a phylogenetic sister group to most sections of the remaining parts of *Papaver*, except for two clades with a strongly deviating distribution. One clade consists of the southern African species *P. aculeatum* Thunb. and the second clade comprises the species pair *P. californicum* A.Gray and *P. heterophyllum* (Benth.) Greene from California and northwesternmost Mexico. Some authors have positioned the latter species within the genus *Stylomecon* G.Taylor.

A narrow concept of *Papaver* s.str. had been argued for by Kadereit et al. (1997:93, 2011:83), but Kadereit and Baldwin (2011) compared the alternatives of maintaining several lineages within *Papaver* s.lat. vs. their segregation as new genera. In the latter alternative, the lineage with *Stylomecon* had an existing name alternative. However, three of the other lineages did not and Kadereit and Baldwin (2011) favoured a more widely defined interpretation of the genus *Papaver*, which avoided splitting and nomenclatural changes. Their arguments also stated that the deviating stylar capsules of *Papaver cambricum* and *P. heterophyllum* had evolved independently.

The third action proposed by Kadereit et al. (2011) to obtain monophyly within Papavereae was to define *Meconopsis* species from basal parts of phylograms as the genus *Cathcartia* Hook.f. This proposal was strongly supported by later phylogenies (Liu et al. 2014; Xie et al. 2014; Xiao and Simpson 2015), which focused on *Meconopsis*, but included other groups. The additional recombinations needed to complete the recircumscription of *Cathcartia* were made in the extensive *Meconopsis* monograph by Grey-Wilson (2014). The fourth proposal by Kadereit et al. (2011) was to expand *Roemeria* Medik. by also transferring species of *Papaver* sect. *Argemonidium* Spach (Kadereit 1986a) into *Roemeria*. Based on the most recent revision of this section by Aghababyan (2011a), Banfi et al. (2022) completed this process by transferring nine species to *Roemeria*.

Another phylogenetically challenging clade within *Papaver* s.lat. is *P.* sect. *Meconella* Spach. Its species are perennial, scapose, mostly with bristly capsules, with deep incisions between the stigmatic rays and white or yellow to orange and pink flowers (Carolan et al. 2006). In her monograph of the section, Rändel (1974) accepted 24 species and 15 additional subspecies and presented a distribution map and a map of ploidy levels indicating possible migration routes. As an East German researcher, she profitted from close contact with botanists in the former Soviet Union, where most of the species of this group had been described. She later added three additional species and two subspecies from North America (Rändel 1977). The section included 30 species, according to Carolan et al. (2006), whereas Solstad et al. (2009), in a thesis, monographed the group, included a determination key and accepted 54 species and 15 subspecies, based on taxonomy concepts which are largely identical in the Pan-Arctic Flora review by Elven et al. (2011).

Phylogenetic analyses now clearly identify this section as a sister clade to *Meconopsis* and not as a subgroup within *Papaver* (Carolan et al. 2006; Kadereit et al. 2011; Liu et al. 2014; Xie et al. 2014). Consequently, the fifth recommendation by Kadereit et al. (2011) was that this section should be described as a new separate genus, with a new name as the genus name *Meconella* Nutt. is already in use for three unrelated species from North America (Hannan 1997).

Carolan et al. (2006) also discussed two other alternatives for obtaining monophyly within this part of Papaveraceae: *Papaver* Sect. *Meconella* could be merged into *Meconopsis* or *Meconopsis* could be merged into *Papaver*. Christenhusz et al. (2018) argued for the latter alternative, proposing it as an operating taxonomy by providing numerous new recombinations of *Meconopsis* names to be positioned within *Papaver*, also including some recombinations of *Cathcartia* and *Roemeria* names.

To our knowledge, the latter classification alternative has not been adopted by any other major study or database. However, until recently, this was still the only alternative showing monophyly within a significant part of the presently polyphyletic classification of *Papaver*. Then, Banfi et al. (2022) described the new genus *Oreomecon* Banfi et al., with *Papaver* sect. *Meconella* as basionym. A total of six species and one subspecies were recombined into the new genus. This included the well-known species *Papaver alpinum* L., which they interpreted in a broad sense following the classification by Schönswetter et al. (2009). The remaining five recombined species were referred to as being "Arctic", but their distributions are not within the Arctic as defined by Walker et al. (2005).

Recently, Galasso et al. (2023) transferred another 12 species and one subspecies to *Oreomecon* with the intention "to provide names for all the taxa now included in *Oreomecon*". In a coordinated paper, Grey-Wilson (2023) recombined another two species and one subspecies into this genus. Altogether, these additions cover only a small number of the taxa previously included in *Papaver* sect. *Meconella*, with Krivenko (2023) recombining another 61 taxa into *Oreo-mecon*. This apparently finalised the replacement of *Papaver* sect. *Meconella* names into the new genus, now totalling 81 species and three subspecies, but Krivenko (2023) did not accept any taxa at the subspecific level. He instead raised several previous names at variety and subspecies level to the rank of species without any discussions related to their original descriptions, whereas Xue et al. (2020) argued against using such a narrow species concept in treatments on a global scale. The only *Oreomecon* taxon which has been thoroughly dealt with is the subspecies level recently by Ferrer-Gallego (2024).

In our opinion, a critical review of studies dealing with *Papaver* sect. *Meconella* is needed as a basis for an evaluation of which taxa to accept and treat within *Oreomecon*. This will be done for separate geographical areas below. A state-of-the-art phylogram of existing ITS sequences will also be presented, even if ITS has not, so far, been found to be a handy phylogenetic marker for this group (Carolan et al. 2006; Solstad et al. 2009). Solstad et al. (2009) also included an extensive genetic AFLP analysis and concluded that this method is useful in comparing related taxa and populations, like in the amphi-Atlantic area, which was most densely sampled. However, it does not reflect the group's evolutionary history on a broader scope.

Examples of threatened species from all parts of the distribution area of *Oreomecon* are also presented. This is because their High Arctic and high alpine habitats are warming faster than most other biomes in the world and these species are, therefore, threatened by faster-growing forbs and woody species (Myers-Smith et al. 2020).

The present study aims to present a revised and monophyletic, generic classification of the Eurasian Papavereae group and to include an updated survey of accepted taxa within each genus. Based on existing phylogenies, evolutionary old and distinct lineages with morphological characteristics and distribution patterns are proposed as separate genera. A review of the literature on the genera and sections in the case of *Meconopsis* and *Papaver* is provided. For each of these groups, a key reference is provided for more extensive information. The global distributions of all genera are mapped with indications of their total numbers of accepted species.

#### Material and methods

The present study relies primarily on critical surveys of taxonomic and phylogenetic literature, although supported by experiences from field studies and comparative cultivation of ca. 60 species of this group in Tromsø Arctic-Alpine Botanic Garden in Tromsø, northern Norway. The plants were grown in mineral-dominated soil in rock landscapes exposed to the local climate at almost 70°N latitude and cited specimens have been in cultivation for several years.

DNA sequences were retrieved from GenBank (National Center for Biotechnology Information (NCBI), USA) via the software Geneious Prime (ver. 2023.0.1, Biomatters, Auckland, New Zealand). The same software was used for tree alignment. All sequences available for species belonging to the genus *Oreomecon* (see Results section for species) were downloaded and checked
for origins and sizes. Most sequences were from published sources, while a few sequences had not previously been used in published trees. Phylogeny was inferred from ITS-1, 5.8 S rRNA and ITS-2 sequences. Genetic distance was calculated using the Tamura-Nei genetic distance model (Tamura and Nei 1993).

For this study, species delineation within each treated genus or a major section of a genus relies on core monograph studies, as defined in Table 1. These are referred to for supplementary information, including lists of synonyms. In cases where our taxonomic apprehension deviates from or supplements these reference studies, the rationale behind our revisions is provided. The general delimitation of the Arctic follows Walker et al. (2005).

POWO (2023) and "World Plants" (Hassler 2023b) were used extensively in search of names and interpretations, although their opinions were not automatically accepted. Nevertheless, they remain a significant source of synonyms and publication information. "The International Plant Names Index" (IPNI 2023) has also been used, as has the GBIF (Global Biodiversity Information Facility) Secretariat (2023). JSTOR Global Plants (2023) was also used as a supplementary source for information on type specimens. The cited sources above are referred to for information on heterotypic synonyms of *Oreomecon* names, in addition to those referred to in the text. Selected texts in Russian were interpreted using Google Translator.

Genus	Subgroup	References	Species no.
Cathcartia		Grey-Wilson (2014)	4
Roemeria		Hassler (2023b)	16 (+1)
Oreomecon	Non-Arctic Siberia and Central Asia	Peschkova (1994)	24
	The Asian Far East	Bezdeleva (1987), Bezdeleva et al. (2006)	9 (+1)
	Arctic Asia	Tolmachev (1975), Petrovsky (1983, 1985)	14(+6)
	Arctic Alaska and Yukon and adjacent Cordilleras	Elven et al. (2011)	8 (+1)
	The major part of the North American Cordilleras	Björk (2019)	5
	Central and Eastern Canada, Greenland, Arctic Europe	Elven et al. (2011)	4 (+2)
	Non-Arctic northern Europe	Nilsson (2001)	1 (+8)
	Central Europe	Schönswetter et al. (2009)	3 (+11)
	Total		68 (+29)
Meconopsis		Grey-Wilson (2014)	95 (+21)
Afropapaver		Kadereit (1988b)	1
Stylomecon		Kadereit and Baldwin (2011)	2
Papaver	sect. Papaver	Kadereit (1986b)	4
	sect. Carinatae	Kadereit (1987)	1
	sect. Macrantha	Lack (2019a; b)	3
	sect. Rhoeadium	Kadereit (1989)	34 (+3)
	sect. Meconidium	Kadereit (1993)	7 (+5)
	sect. Pilosa	Kadereit (1996)	1
	sect. Pseudopilosa	Kadereit (1996)	8 (+2)
	Total		59 (+10)
Parameconopsis		Grey-Wilson (2014)	1
Grand Total			246 (+61)

Table 1. List of reference studies on genera and subgroups of genera referred to for further information. Number of species and subspecies (in parentheses) of each group and subgroup are indicated following the supplementary revisions and additions provided in the studies referred to in the running text.

Many of the names newly introduced here have basionyms that previously have been included in *Oreomecon* or previously treated at the same rank. They are, therefore, neither "stat. nov." nor "comb. nov." and are here referred to as nomenclatural novelties ("nomencl. nov."), following the Code (Art. 6.10; Turland et al. (2018)).

The eight genera treated here are dealt with according to their sequences shown in Fig. 1. The review of *Papaver* is organised by the section concept introduced by Kiger (1985) and later adapted by Kadereit (1988a). *Meconopsis* is also reviewed section-wise, following Grey-Wilson (2014), although sections with no or minor later changes have been lumped. In the case of *Oreomecon*, all accepted species and subspecies names are listed and they are organised by their distributions within eight geographic areas. As this northern genus is particularly vulnerable to climatic change effects, literature sources summarising endangered populations and Red List statuses are also included. The treatments below do not include hybrids, varieties and named forms, except for taxa within the much-studied Nordic species *Oreomecon radicata* (Rottb.) Banfi et al.

### Results

A simplified summary of the ITS-based phylogram by Kadereit et al. (2011) is shown here as Fig. 1 with our name conclusions for the major clades.



adopted in the present study.

#### Taxonomy

#### 1. Cathcartia Hook.f. ex Hook., Curtis's Bot. Mag. 77: t. 4596 (1851)

#### Type species. Cathcartia villosa Hook.f. ex Hook.

The genus includes four species as described by Grey-Wilson (2014), all with separate distribution areas in the East Himalayas and central parts of China, reproduced in Fig. 2. These species are accepted here.

### 2. Roemeria Medik., Ann. Bot. (Usteri) 1(3): 15. 1792

**Type species.** *Roemeria violacea* Medik., nom. illeg. [≡ *R. hybrida* (L.) DC.]

**Notes.** The genus is summarised here as including 16 species and one subspecies according to the treatment by Hassler (2023b). As shown by Fig. 2, the genus has a similar distribution to *Papaver*, but is absent from most parts of the Macaronesian Islands. It is present in Great Britain and southernmost Scandinavia, while it is absent from large parts of Central European Russia, the Arabian Peninsula and the Himalayan foothills. *Roemeria* may have had a long presence in Central Asia prior to the uplift of the Qinghai-Tibetan Plateau, based on its phylogenetic history illustrated in Fig. 1. Today, it extends as far east as Mongolia, represented by the species *R. refracta* DC. (Baasanmunkh et al. 2022).

# 3. Oreomecon Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 68. 2021

#### Type species. Papaver alpinum L., Sp. Pl. 507. 1753.

**Notes.** When this genus was introduced by Banfi et al. (2022), the relationship between the basionym and the new name was not designated by adding the expected information "nom. et stat. nov.". However, according to the International Code of Nomenclature for algae, fungi and plants (Shenzhen Code), this is a recommendation (Rec. 32A; Turland et al. (2018)) and not a requirement and the lack of this information does not affect the validity of the new



**Figure 2.** World distribution of the genera *Cathcartia* (grey), *Meconopsis* (sky blue), *Oreomecon* (yellow) and *Roemeria* (red). The total distribution of *Oreomecon* is divided into eight geographical areas (A–H) both on the map and in the textual treatment. Area E consists of two almost equally large subareas, while area H consists of one main subarea and four much smaller subareas (see arrows). Note also that the total distribution of *Cathcartia* consists of four different subareas. See Table 1 for the numbers of species and subspecies per genus and for each of the subareas of *Oreomecon*.

genus. The species belonging to the recently described genus *Oreomecon* are treated here according to their distributions within eight geographical areas (see groups A–H below). Within each area, in cases where our interpretations deviate from the cited reference studies, they are discussed and used as a basis for the conclusive list of accepted species.

Overall, this treatment shows that *Oreomecon* contains 68 species and 29 subspecies. Only *O. lapponica* and *O. nudicaulis* are listed from more than one of the geographical areas defined above, however, with different subspecies. In the geography-based enumeration of species, only areas where the nominate subspecies occur are included. A total of 38 *Oreomecon* names are newly introduced below, 29 as recombinations and nine as nomenclatural novelties, whereas 21 existing *Oreomecon* names are put into synonymy.

**Phylogeny of Oreomecon.** All ITS sequences found in the GenBank of taxa belonging to *Oreomecon* and some unpublished data were used to construct a state-of-the-art phylogram shown in the supporting document (Suppl. material 1). It is already known that this marker does not discriminate between close-ly-related taxa such as those of the *Oreomecon alpina* complex (Schönswetter et al. 2009). However, with this analysis, we wanted to check whether any major clades appeared within the genus. We found that the sequences included several misidentifications and that it was impossible to trace information on the origin of many of the samples. Our own alternative interpretations of the identities of most of the samples are, therefore, shown as a right-hand column in the figure.

Four clades were identified, although they should be interpreted with care. In branch I, three samples of *O. alpina* s.lat. are widely different from other samples. Branch IV probably includes cultivated material of "Iceland Poppy", most commonly interpreted as *O. crocea* (Elven et al. 2011). In contrast, the single sample in Branch II might represent true *O. nudicaulis*, as it was labelled *P. nu-dicaule* subsp. *nudicaule* by Carolan et al. (2006). The most exciting result from the present phylogram was the concentration of taxa from Far East Asia in Branch III, although several samples remain uninterpreted.

#### 3.1. Non-Arctic Siberia and Central Asia

#### Notes

In her monograph on Siberian species, Peschkova (1994) accepted 30 species and two subspecies and provided distribution maps of all taxa. Her study is used as a reference for treating the geographical area that is dealt with here. However, as her geographical area also included Arctic parts of Siberia, five species and two subspecies from the Arctic are instead treated in group C below, together with other Asian Arctic species. Peschkova (1994) did not treat species from the Russian Far East, which is also in a geographical area different from the one dealt with here. Mongolia, the Central Asian republics and northern China, which belong to the present geographical area, were also outside the scope of her treatment. A recent flora checklist from Mongolia (Baasanmunkh et al. 2022) follows the species concepts of Peschkova (1994), except for two accepted species not present in Siberia: *Papaver baitagense* Kamelin & Gubanov and *P. pseudotenellum* Grubov. However, Baasanmunkh et al. (2021) considered these species as synonyms of or very closely related to *P. croceum* Ledeb. and they are therefore synonymised below.

For a long time, the only species recognised from this area were *P. nudicaule* L. and *P. croceum* Ledeb. In addition, there were several taxa at infraspecific rank, two of which have intricate histories, which will be dealt with below. *Papaver nudicaule* var. *rubro-aurantiacum* Fisch. ex DC., was introduced by De Candolle (1821), although it has been treated as if it were a *nomen nudum* by several later authors. De Candolle (1821) had referred to a collection from Dahuria sent by Fischer, who apparently had suggested the name "in litt.". However, later in his treatment, below a comparison of his three varieties of *P. nudicaule*, De Candolle (1821) presented a short diagnosis for var. *rubro-aurantiacum*, for which he wrote that it might represent a true new species. His name for this taxon is, therefore, accepted as a basionym here.

Peschkova (1994) cited the taxon as P. rubro-aurantiacum Fisch. ex R. Sweet. However, Sweet (1830) only listed the name at the species level in a horticultural magazine without any attempt at a taxonomic treatment, without citing the treatment by De Candolle (1821) and Sweet's citation to the year "1822" instead refers to its introduction into British gardens. However, Hassler (2023b) cited the taxon as P. rubro-aurantiacum Fisch. ex Steud. Steudel (1841) only included the name in an enumeration where it was interpreted as a synonym of P. croceum. Fedde (1909) recombined the taxon as a subspecies. Lundström (1923) studied the holotype material collected in Dahuria by Fischer and sent to De Candolle, recombined the taxon at the species level and provided an extended description. His author citation deviates from the citation format followed by us, namely P. rubroaurantiacum (Fisch. ex DC.) C.E.Lundstr. The hyphen inserted by Lundström is deleted according to the Code (Article 60.11; Turland et al. (2018)). The name citation applied here is also in accordance with the treatment by POWO (2023). The type material was not explicitly cited by Lundström (1923), is not deposited at BG as indicated by Popov (1937) and Rändel (1974) and its herbarium affiliation is unknown to us.

Peschkova (1994) considered *P. rubroaurantiacum* to be heterogeneous, also including *P. ledebourianum* C.E.Lundstr., but mapped it as very common in southern Siberia, largely overlapping with the distribution area of *P. nudicaule*, but stated that they differ in flower colour and pubescence of sepals and capsules. Zhang and Grey-Wilson (2008), however, treated *P. rubroaurantiacum* as a synonym of *P. nudicaule*. Kamelin and Gubanov (1990) described *P. changaicum* Kamelin from Mongolia, differing from *P. rubroaurantiacum* by white flowers and it is treated as a synonym of the latter here. They also described *P. rubroaurantiacum* subsp. *chalchorum* Kamelin from calcareous steppes in Mongolia, but this taxon was not accepted by Baasanmunkh et al. (2022).

Another early described taxon is *Papaver leiocarpum* (Turcz.) Popov, with its basionym *Papaver nudicaule* var. *leiocarpum* Turcz., published in 1838. IPNI (2023) cites the basionym as *P. leiocarpum* Turcz., referring to the same publication source, which agrees with the citation in Popov (1937). However, the original publication only lists "*P. lejocarpon* m." which is a *nomen nudum* as it lacks an accompanying description. The significance of the added "m." is unknown and possibly refers to a manuscript. Therefore, it appears that the publication source of the basionym cited by Peschkova (1994) as "1842–1845, Fl. Baic.-Dahur. 1: 98" instead represents its description. Krivenko (2023) cited

the *nomen nudum* from 1838 instead of the basionym published by Peschkova (1994). However, the Code (Art. 41.6; Turland et al. (2018)) allows for erroneous basionym citations. Thus, the name *Oreomecon leiocarpa* (Turcz.) Krivenko is considered validly published, with the correct basionym citation supplied here.

In his treatment of *Papaver* for Flora SSSR, Popov (1937) included two Central Asian species of sect. *Meconella* described by Tolmachev (1931), as well as his own descriptions of five new species. Two of these, *Papaver pseudostubendorfii* Popov and *P. ajanense* Popov, were both synonymised with *P. stubendorfii* Tolm. by Peschkova (1994), whereas *P. pseudocanescens* Popov was accepted, while *P. involucratum* Popov was outside her study area. *Papaver tianschanicum* Pavlov was introduced as a *nomen nudum* in 1933. When described by Popov a year later, the name *P. tianschanicum* Popov would have been more appropriately cited as *"Papaver tianschanicum* Pavlov ex Popov", as done by Hassler (2023b). However, as this is only an alternative in the Code (Art. 46.5; Turland et al. (2018)), this correction is not followed here. The species was re-described by Popov (1937) as an isonym and was accepted by Peschkova (1994). It was synonymised with *P. canescens* Tolm. by Zhang and Grey-Wilson (2008), but accepted by Galasso et al. (2023).

Papaver amurense (N.Busch) N.Busch ex Tolm. was accepted by Peschkova (1994). In contrast, Zhang and Grey-Wilson (2008) treated it as *P. nudicaule* f. *amurense* (N.Busch) H.Chuang. POWO (2023) and Hassler (2023b) cited the species as *P. amurense* (N.Busch) Karrer, based on Karrer (1935). However, the latter is a short and non-taxonomical notice in a horticultural magazine, without even an indirect reference to a basionym as required prior to 1953 by the Code (Art. 41.3; Turland et al. (2018)) and this is not a valid recombination. Tolmachev (1971) dealt specifically with this species, but did not cite its type.

A taxon known as *Papaver tenellum* Tolm. was accepted by both Popov (1937) and Czerepanov (1995) and also by Peschkova (1994), although her statement that it "evidently represents a shade form of *P. pseudocanescens*" leads us to reject this species. Tolmachev (1930) treated it as *P. tenellum* (Korsh.) Tolm., based on a name "in sched." by Korshinsky at LE. Its author citation is, therefore, given as 'Korsh. ex Tolm.' and the Korshinsky specimen illustrated by Tolmachev (1930) is designated as lectotype here. *Papaver tenellum* was considered a synonym of *P. nudicaule* by Zhang & Grey-Wilson (2008) but accepted by Solstad et al. (2009) and POWO (2023). Following the argument by Peschkova (1994), *Oreomecon tenella* (Tolm.) Krivenko is listed below as a synonym of *Papaver pseudocanescens* Popov.

Papaver rubroaurantiacum subsp. longiscapum Rändel was recombined into species level by Krivenko (2023). However, the taxon was considered "simply the full-grown forms of *P. rubro-aurantiacum*" by Peschkova (1994) and her opinion is followed here. With the exceptions noticed above, we accept all species treated by Peschkova (1994), i.e. 23 species in total, of which six were described as new to science by her. The list also includes four species recombined by Peschkova (1994).

The diploid species *Papaver kuvajevii* Schaulo & Sonnikova (Shaulo and Sonnikova 2003) was described from a single locality in Krasnoyarsk Krai in Khakassia, Russia and is included in the list below. Only two more species have been described from the area south of Peschkova's study area. The endemic *Papaver involucratum* Popov from 2800–3300 m alt. in the western Pamir-Alay mountains in Tajikistan has been accepted by most later authorities and is also included in the list below. It has also been collected from the Afghan side of the border as shown by GBIF (Global Biodiversity Information Facility) Secretariat (2023). *Papaver angrenicum* Pazij from the western Tian Shan mountains in Uzbekistan was accepted by Rändel (1974), based on several morphological characters. It has been treated as a synonym of *P. croceum* in flora lists by Czerepanov (1995) and Sennikov and Tojibaev (2021). The latter interpretation is followed here, although a re-study vs. neighbouring species is needed to finally reject the conclusion by Rändel (1974). *Papaver involucratum* subsp. *nigrescentihirsutum* Tolm. was accepted by Solstad et al. (2009), but not by POWO (2023) nor by us. The report by Jafri and Qaiser (2011) of *Oreomecon nudicaulis* s.lat. from Pakistan probably refers to *O. crocea* in the sense of Peschkova (1994) and Sennikov and Tojibaev (2021). Notably, *Oreomecon* has not migrated westwards into the high mountains of Iran, Caucasus and Türkiye.

### Distribution

Fig. 2 shows the distribution of the genus *Oreomecon* in this geographical area, which is considered to include 24 species. *Papaver croceum* was shown by Peschkova (1994) to have a south-western Siberian distribution pattern that does not overlap with the south-eastern one of *P. nudicaule*. *Papaver amurense* overlaps with *P. nudicaule* in south-eastern Siberia, but extends into the Russian Far East and southeastwards into China (Peschkova 1994). It also occurs in North Korea (Chang et al. 2014). *Papaver pseudocanescens* Popov and *P. rubroaurantiacum* were mapped as particularly widespread and abundant by Peschkova (1994).

### Rare and red-listed species

Papaver turczaninovii is an endemic with a limited distribution south-east of Lake Baikal, while P. kuvajevii is only known from the two localities in East Sajan, as reported by Shaulo and Sonnikova (2003). An'kova et al. (2018) also briefly listed it from China, but no localities or samples were reported. In GBIF (Global Biodiversity Information Facility) Secretariat (2023), only the two localities by Shaulo and Sonnikova (2003) are available. The endemic Papaver olchonense and P. popovii were only mapped from the Lake Baikal area by Peschkova (1994), although with quite many localities and with a single locality of P. olchonense in the Republic of Sakha ca. 1,300 km north of the northern edge of Lake Baikal. Papaver popovii was cited as rare and endangered along Lake Baikal shorelines by Bukharova et al. (2021). A new locality of this species was recently recorded from Agara River, ca. 500 km west of the northern edge of Lake Baikal as shown by GBIF (Global Biodiversity Information Facility) Secretariat (2023). Papaver involucratum was described as endemic and occurring in several areas of the western Pamir-Alai mountains in Tadzhikistan (Popov 1937). Papaver turczaninovii was treated as a stenotopic endemic by Peschkova (1994), being restricted to outcrops of marble limestones at the southeastern shore of Lake Baikal. Eight of the species were listed as Nearly Threatened (NT) in Russia by Xue et al. (2023), with only P. tenellum (included in P. rubroaurantiacum above) listed as Vulnerable (VU).

#### Accepted taxa

## 3.1.1. Oreomecon ammophila (Turcz.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:1. 2023

 Papaver nudicaule var. ammophilum Turcz., Bull. Soc. Imp. Naturalistes Moscou 15: 98. 1842. Type: [Russia] Copiosissime crescit ad littus arenosum Baicalis prope monasterium Posdsolskoy, N. Turczaninov (not found)
 Papaver ledebourianum var. ammophilum (Turcz.) Peschkova, Fl. Tsentral'noi Sibiri 1: 378. 1979 = Papaver ammophilum (Turcz.) Peschkova, Fl. Sibir. 7: 16. 1994.

## 3.1.2. Oreomecon amurensis (N.Busch) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

= Papaver nudicaule subsp. amurense N.Busch, Fl. Sibir. Orient. Extremi 1: 21.
 1913 = Papaver amurense (N.Busch) N.Busch ex Tolm., Fl. Transbaikal 4:
 410. 1941 = Papaver nudicaule f. amurense (N.Busch) H.Chuang, Fl. Reipubl.
 Popularis Sin. 32: 58. 1999.

### 3.1.3. Oreomecon canescens (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver canescens Tolm., Zhurn. Russk. Bot. Obshch. 16: 77. 1931. Type: Sklony i vershiny khredta Saur [Kazakhstan, in jugo montium Saur], *Reznichenko* (LE: holotype).

## 3.1.4. Oreomecon chakassica (Peschkova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver chakassicum Peschkova, Fl. Sibir. 7: 18. 1994. Type: [Russia] Regio autonoma Chakassia distr. Askiz, in vicinitate vici Kamyschta, stepa lapidosa, 8 June 1970, E. Erschova & T. Volkova s.n. (holotype: NS)

# 3.1.5. Oreomecon crocea (Ledeb.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 71. 2022

- Papaver croceum Ledeb., Fl. Altaic. 2: 271. 1830. Type: [Russia] Altai, Ledebour (lectotype: LE) = Papaver nudicaule var. croceum (Ledeb.) Elkan, Tent. Monogr. Papaver 17. 1839 = Papaver alpinum var. croceum (Ledeb.) Ledeb., Fl. Ross. 1: 87. 1841.
- = Papaver baitagense Kamelin & Gubanov, Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 95(2): 86. 1990. Type: Jugo-zapadnaya Mongoliya, Dzhungariya, severnyi makrosklon khr. Baytag-Bogdo dolina r. Nariyn-Khargaityn-gol v 8 km

na vostok ot zastavy Baitag-Bog-do Kobposkogo aimaka, 31 July 1988, *I.A.Gubanov & E.Gaubold 2562* (holotype: MW no. 0592489)  $\equiv$  *Oreomecon baitagensis* (Kamelin & Gubanov) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

- = Papaver angrenicum Pazij, Bot. Mater. Gerb. Bot. Inst. Uzbekistansk. Fil. Akad. Nauk S.S.S.R. 3: 31. 1941. Type: [Uzbekistan] Westlicher Tien-schan, Bassin des Flusses Angren, 15 Aug 1937, Zakirov 173843 (TAK: holotypus).
- = Papaver pseudotenellum Grubov, Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R 17: 14. 1955. Type: [Mongolia] Altai Gobicus, jugum Gurban-Bogdo, mons Iche-Bogdo, fauces Narin-Churumt, latus orientale, ca. 2900 m, in fissuris rupium, 28 Aug 1948, V. Grubov 6197 (holotype: LE).

# 3.1.6. Oreomecon involucrata (Popov) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

■ Papaver involucratum Popov; Fl. URSS 7: 748. 1937. Type: [Tadzhikistan] Asia Media, Pamir-Alai, ad fl. Zeravschan superior (holotype: LE).

# 3.1.7. Oreomecon jacutica (Peschkova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver nudicaule subsp. gracile Tolm., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 20: 166. 1960. Type: Russia, E Siberia, Yakutskaya ASSR, okrestnosti g. Yakutska, Urochishche Chuchur-Muran....na peschanoy pochve, 28 Jun 1956, A.I. Tolmachev s.n. (holotype: LE; typified by Elven et al. [2009], p. 989) = Papaver jacuticum Peschkova, Fl. Sibir. 7: 19. 1994.

# 3.1.8. *Oreomecon kuvajevii* (Schaulo & Sonnikova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver kuvajevii Schaulo & Sonnikova, Turczaninowia 6(4): 5. 2003. Type: [Russia: Sajanum Occidentale. Jugum Chemtschikskij, declive generale septentrionis. Vallis fluminis Kolbak-Mis, circa ostium. Clivo montano saxoso. Schistosa]. 28 May 1982, A.E. Sonnikova s.n. (holotype: NS; isotype: SSB).

# 3.1.9. Oreomecon leiocarpa (Turcz.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

- Papaver nudicaule var. leiocarpum Turcz., Fl. Baic.-Dahur.1: 98. 1842–1845.
  Type: Russia, ad torri Bugussony, *Kuznetzoff s.n.*, 1834, (holotype: LE; isotypes: P [barcodes P00744601, P00744603], K [barcode K00065319]) = Papaver leiocarpum (Turcz.) Popov., in V.L. Komarov (ed.) Fl. SSSR 7: 604. 1937.
- Papaver nudicaule var. leiocarpum Turcz., Bull. Imp. Naturalistes Moscou 11: 86. 1838, nom. nud.

### 3.1.10. Oreomecon leucotricha (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver leucotrichum Tolm., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR. 20: 176. 1960. Type: [Russia], Siberia: Yakutia, [in jugo montium Tuora-Siss, ad ripam dextram fluminis Lenae inferioris, in cucumine montis Sokujdach], 11 Aug 1957, B. Yurtsev and B. Norin (holotype: LE).

#### 3.1.11. Oreomecon nivalis (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

 Papaver nivale Tolm., Svensk Bot. Tidskr. 24: 42. 1930. Type: [Russia] Werchojanski-Gebirge, im Tal des Tukulan, 914–977 m, 24 Jul 1935, S. Nedrigailow (lectotype: S, corresponding to major part of illustration in Tolmachev [1930: 41]; isolectotype: LE, designated here).

## 3.1.12. Oreomecon nudicaulis (L.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 71. 2022

= Papaver nudicaule L., Sp. Pl.: 507. 1753. Type: J. Dillenius, Hortus Elthamiensis 1732, t. 224, fig. 291 (lectotype).

# 3.3.13. Oreomecon nudicaulis (L.) Banfi, Bartolucci, J.-M.Tison & Galasso subsp. *nudicaulis*, Nat. Hist. Sci. 9(1): 71. 2022

■ Papaver nudicaule L., Sp. Pl.: 507. 1753. Type: J. Dillenius, Hortus Elthamiensis 1732, t. 224, fig. 291 (lectotype).

# 3.1.14. Oreomecon olchonensis (Peschkova) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

= Papaver olchonense Peschkova, Fl. Sibir. 7: 23. 1994. Type: [Russia: Ora Maris Minoris ("Malomorskoe") lacus Baical, ins. Olchon, prope vicum Chonchoi, declive boreali-occidentale], 17 Jun 1957, G. Peschkova s.n. (holotype: NSK).

# 3.1.15. Oreomecon popovii (Sipliv.) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

= Papaver popovii Sipliv., Novosti Sist. Vyssh. Rast. 10: 360. 1973. Type: [Russia] Baikal, Chivyrkuisky zaliv, ostrov Lokhmatyi Kaltygei, skaly severnogo berega, 8 Sep 1971, V.N. Siplivinskiy s.n. (holotype LE; isotype TK).

## 3.1.16. Oreomecon pseudocanescens (Popov) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

- = Papaver pseudocanescens Popov, in V.L. Komarov (ed.) Fl. SSSR 7: 749. 1937. Type: [Russia] Altai, in alpinis fluvii Topczugan, 1913, Kusnetzov and Tripolitova 2670 (holotype: LE).
- = Papaver tenellum Korsh. ex Tolm., Sv. Bot. Tidskr. 24: 40. 1930, syn. nov.; Type: [Kasakhstan] Karkaraly-Gebirge, in schattigen Schluchten, 18–20 Jun 1890, Korshinsky (lectotype: LE, illustrated by Tolmachev [1930: 40], designated here) ≡ Oreomecon tenella (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023.

# 3.1.17. Oreomecon rubroaurantiaca (Fisch. ex DC.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

- ≡ Papaver nudicaule var. rubroaurantiacum Fisch. ex DC., Syst. Nat. 2: 70. 1821; Type: Baikalien (Dahurien), Fischer (holotype) ≡ Papaver rubroaurantiacum (Fisch. ex DC.) C.E.Lundstr., Acta Horti Berg. 7: 417. 1923
- Papaver nudicaule subsp. rubroaurantiacum (Fisch. ex DC.) Fedde, in Engler, H.G.A. (ed.) Pflanzenr. IV, 104: 381. 1909.
- = Papaver rubroaurantiacum subsp. longiscapum Rändel, Feddes Repert. 84, 9-10: 683. 1974. Type: [Russia] Im Tal des Flusses Amur, am Berghang, Nähe der Siedlung Dshilinda, 17 Jun 1913, Kazanskij (holotype ['lectotype]') ≡ Oreomecon longiscapa (Rändel) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.
- = Papaver changaicum Kamelin, Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 95(2): 87. 1990. Type: Mongolia centralis, regio Uber-Changai, ad oriente ab urb. Charcharin (Karakorum), in valle fl. Tarany-gol prope montem Cecerleg-ula, 24 Jul 1983, *I.A.Gubanov 7496* (holotype: MW).
- = Papaver rubroaurantiacum subsp. chalchorum Kamelin, Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 95(2): 88. 1990. Type: Mongolia centralis, steppa chalchorum, mons Saan-Schire, ca. 80 km in via Under-Chan-Manchan, 19 Jun 1987, A.L.Budantzev et al. 20 (holotype: LE).

# 3.1.18. Oreomecon saichanensis (Grubov) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

Papaver saichanense Grubov, Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk. SSSR 17: 15. 1955. Type: [Mongolia] Altai Gobicus, jugum Gurban-Saichan, mons Dzun-Saichan, in faucibus Jalo-Ama, ad fl. Tzagan-Gol, sub rupibus, 20 Aug 1931, N. Ikonnikov-Galitzky 4192 (holotype: LE) = Papaver rubroaurantiacum subsp. saichanense (Grubov) Kamelin & Gubanov, in Gubanov I.A. Konsp. Fl. Vneshnei Mongolii: 52. 1996.

### 3.1.19. Oreomecon setosa (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

- Papaver rubroaurantiacum subsp. setosum Tolm., Svensk Bot. Tidskr. 24: 39.
  1930 = Papaver setosum (Tolm.) Peschkova, Stepnaya Fl. Baikal'skoi Sibiri: 59. 1972.
- = Papaver alpinum var. hispidissimum Ledeb., Fl. Ross. 1: 87. 1842 ≡ Papaver anomalum var. hispidatissimum (Ledeb.) Tolm., Novosti Sist. Vyssh. Rast. 7: 157. 1971;

## 3.1.20. Oreomecon smirnovii (Peschkova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver smirnovii Peschkova, Novosti Sist. Vyssh. Rast. 14: 239. 1977. Type: [Russia] Systema fl. Onon, in viciniis pag. Czindant-2, locus «Zavodskaya», in declivi schistoso-stepposo, 30 May 1911, V. Smirnov 270 (holotype and four isotypes: LE).

## 3.1.21. Oreomecon stanovensis (Petroch.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

Papaver croceum subsp. stanovense Petroch., in L.I. Malyschev (ed.). Vysokogornaya FI. Stanovogo Nagor'ya 96. 1972. Type: Russia, Buryatiya, Stanovoe Nagorye, Yuzhno-Muyskiy mountain range, the origins of the Barguzin River, in the alpine zone,1900 m alt., on the convex matted gravelly calcareous slope, 55°N, 111°E., 19 Aug 1968, Yu. Petrochenko 513 (NSK: holotype) = Papaver stanovense (Petroch.) Peschkova, FI. Sibir. 7: 28. 1994.

# 3.1.22. Oreomecon stubendorfii (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver stubendorfii Tolm., Zhurn. Russk. Bot. Obshch. 16: 80. 1931. Type: [Russia] Allakh-jun, na puti iz Jakutska v Okhotsk, Shtubendorf (LE: holotype).

# 3.1.23. Oreomecon tianschanica (Popov) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58: 2023

- Papaver tianschanicum Popov, Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 17: 84. 1934. Type: [Kyrgyzstan] Asia Media, Tian-Schan, Jugum Kungei-Alatau, ad fl. Kebin, Abolin 3264 (LE); Papaver tianschanicum Popov, in V.L. Komarov (ed.) Fl. SSSR 7: 748. 1937, isonym. = Papaver croceum subsp. tianschanicum (Popov) Kamelin, Fl. Ushchel. Reki Varzob: 140. 2021.
- Papaver tianschanicum Pavlov, Byull. Moskovs. Obshch. Isp. Prir., Otd. Biol. 1933. n.s. xiii: 126, nom. nud.

### 3.1.24. Oreomecon turczaninovii (Peschkova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver turczaninovii Peschkova, Fl. Sibir. 7: 30. 1994, nom. nov. Papaver nudicaule var. calcareum Peschkova, Fl. Tsentr. Sib. 1: 379. 1979. Type: Russia, Irkutskaya Oblast', Hamar-Daban mountain range, River Slyudyanka, talus near the marble quarry, 51°N, 105°E, 15 Jul 1964, *M. Ivanova* (holotype: NSK).

## 3.1.25. Oreomecon udocanica (Peschkova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

Papaver pseudocanescens subsp. udocanicum Peschkova, Novosti Sist. Vyssh. Rast. 14: 238. 1977. Type: Russia, Chitinskaya Oblast', Stanovoye Nagorje, Udokan Ridge, the upper reaches of Naminga River, at the upper border of the forest, 1350 m alt., on the river gravel, 56°34'41" N, 118°29'58" E, 26 Jun 1964, L. Malyschev & Yu. Petrochenko 350 (holotype: LE; isotype: NSK) = Papaver udocanicum (Peschkova) Peschkova, Fl. Sibir. 7: 30. 1994.

### 3.2. The Asian Far East

#### Notes

This area is defined as comprising north-eastern China (Liaoning, Jilin, Heilongjiang), the Korean Peninsula, Japan and non-Arctic areas of the Russian Far East, the latter defined as the eight easternmost administrative units in Russia. This area does not overlap geographically with the administrative units comprising Siberia, as dealt with by Peschkova (1994). The *Papaver* flora of the former Soviet Far East was presented by Bezdeleva (1987) and later updated for the Russian Far East by Bezdeleva et al. (2006). These two studies are used here as a combined reference study for this geographical area.

Altogether, these studies included 34 species, 30 of them presented with distribution maps, although the majority of the species are from the Arctic parts. In the present treatment, the latter are included in the next geographic group, except for three species with most of their occurrences on the American side. Therefore, they are treated in the group of Arctic Alaskan and Yukon species. Only a few of the Siberian species treated by Peschkova (1994) have marginal occurrences within the presently-defined area. To conclude, seven species dealt with by Bezdeleva (1987) and Bezdeleva et al. (2006) are exclusive to the presently-defined geographical area and are commented on below.

The earliest name from the area is *Papaver microcarpum* DC. described from Kamchatka by De Candolle (1821), based on a P.S. Pallas collection, although the true collector was possibly C.H. Merk in 1788, who passed his plant collections over to Pallas (Yakubov et al. 2001). Tolmachev (1931) described *Papaver ochotense* Tolm., but later, Tolmachev (1975) recombined it as one of four subspecies of *P. microcarpum*, another one being *P. microcarpum* subsp. *alaskanum* (Hultén) Tolm. In his distribution map, *Papaver microcarpum* subsp. *ochotense* (Tolm.) Tolm. was shown to have a distinct southern distribution

compared to *P. microcarpum* subsp. *microcarpum* with an Arctic distribution in Chukotka and *P. microcarpum* subsp. *czekanowskii* (Tolm.) Tolm., both occurring from Chukotka to much further to the west in Arctic Yakutia. These distribution patterns were confirmed by Petrovsky (1999).

These studies dealt with the Arctic and, for the Far East, Bezdeleva (1987) mapped *Papaver microcarpum* southwards to the Magadan area and southernmost parts of Kamchatka as a widely defined species without accepted subspecies, a concept also followed by Bezdeleva et al. (2006). Grey-Wilson (2023) did not even accept *P. macrocarpum* as a separate species, but recombined it as a subspecies of *Oreomecon nudicaulis*. Czerepanov (1995) and Pavlova (1999) accepted *Papaver ochotense* Tolm. as a separate species, whereas Krivenko (2023) did not recognise this taxon, but recombined *Papaver czekanowskii* Tolm. at the species level in *Oreomecon*.

Elven et al. (2011) treated the complex as three subspecies, but added that "it may consist of three (or more) separate species" and cited unpublished studies where *P. czekanowskii* and *P. microcarpum* s.str. were surprisingly different genetically. They also mentioned *Papaver omolonense* Khokr., supposedly described from the Magadan area, a name we could not find in any of the cited sources. Solstad et al. (2009) also reported on a genetically distinct taxon from Karaginsky Island off northern Kamchatka referred to as "*P.* sp. aff. *microcarpum*". It is tetraploid, whereas *microcarpum* is diploid. Following the hypothesis by Elven et al. (2011) and the evolutionary pattern within the genus in the Asian Far East, the three taxa are treated as separate species within *Oreomecon* here, with *O. microcarpu* (DC.) Krivenko as primarily a non-Arctic species. Chukotkan specimens of *P. microcarpum* subsp. *microcarpum* are left for interpretation by future studies.

Another early name from the area is Papaver anomalum Fedde (Fedde 1909). It was accepted by Popov (1937), who lumped it with P. nudicaule subsp. amurense N.Busch., a conclusion which V. Komarov (1937), the Editor of "Flora SSSR", opposed. Rändel (1974) agreed that P. anomalum sensu Fedde is different from P. amurense. However, she treated it as a subspecies of P. croceum. Bezdeleva (1987) provided a key separating this species from its most closely-related species, P. amurense and mapped the latter as very common in the southern part of the Far East, particularly near the Chinese border, whereas P. anomalum is much rarer. Peschkova (1994) and Zhang & Grey-Wilson (2008) omitted P. anomalum from their studies, but Peschkova (1994) treated P. anomalum var. hispidissimum (Ledeb.) Tolm. as a synonym of the Siberian P. setosum (Tolm.) Peschkova. Papaver anomalum was recombined into Oreomecon by Banfi et al. (2022) and POWO (2023) and Hassler (2023b) accepted this name. As emphasised by Fedde (1909), the species is very distinct by its almost globose, mostly glabrous capsules and the name is lectotypified here, based on a specimen in B where both capsule and flowers are developed. This sheet would have been readily available to Fedde and apparently carries his handwriting.

Papaver alboroseum Hultén was not typified by Hultén (1928) and, as shown by Björk (2019), two duplicates of the type exist. Here, we designate the collection at S as lectotype, as capsules are much better developed than on the alternative sheet at GB. The latter is defined as isolectotype here, related to its previous and unpublished annotation as "isotype". According to Yakubov et al. (2001), *P. alboroseum* and *P. microcarpum* co-occur on the Avachinsky Volcano in Kamchatka, where both species are frequent. Pavlova (1999) described *P. tolmatschevianum* N.S.Pavlova from Sakhalin. This species had already been described and illustrated in Flora of Sakhalin by Sugawara (1937–1940) under the name *Papaver ochotense* Miyabe & Tatew., which is an illegitimate homonym of *P. ochotense* Tolm., described by Tolmachev (1931).

Krivenko (2023) recombined *Papaver anomalum* var. *hirsutum* Tolm. as *Oreomecon hirsuta* (Tolm.) Krivenko without making any reference to the synonym *Papaver sokolovskajae* Prob., which apparently has priority at the species level. Probatova in Bezdeleva et al. (2006) described *P. sokolovskajae* as a white-flowered species from supralittoral habitats along the coast near Vladivostok. It has conspicuously glabrous and subglobose capsules, a character also noted by Krivenko (2023). However, *P. sokolovskajae* was not described as a new species, but as a nom. nov. and stat. nov. with *Papaver anomalum* var. *hirsutum* Tolm. described by Tolmachev (1971) as the basionym. Probatova did not give any arguments for introducing a replacement name and we cannot find any existing and competing *"Papaver hirsutum"* name justifying the choice. Based on the Code (Art. 6.10–11; Turland et al. (2018)), we therefore consider *P. sokolovskajae* as an illegitimate name and instead follow the recombination made by Krivenko (2023).

For the "Flora of China", Zhang and Grey-Wilson (2008) listed *Papaver radicatum* var. *pseudoradicatum* (Kitag.) Kitag. from above 1,600 m alt. on Changbai Shan in the Province of Jilin close to North Korea, also listing the taxon from Korea. Bezdeleva et al. (2006) mapped a single locality of *P. pseudoradicatum* Kitag. from the Russian side and POWO (2023) indicated this taxon to occur in Korea, "Manchuria" and the "administrative region of Khabarovsk" in the Russian Far East. Chang et al. (2014) also included *P. pseudoradicatum* from North Korea in their list of species from Korea.

Lee et al. (2011) reported *P. coreanum* Nakai to be "widely distributed in an alpine belt of Baekdu/Changbaek", a mountain chain shared by North Korea and China, also referred to as Paektu-san in Korea and Changbai Shan in China. They also cited the species to be protected in China, although the species name applied was not indicated. Zhang and Grey-Wilson (2008) and Chang et al. (2014) did not mention *P. coreanum*, whereas POWO (2023) listed both *P. coreanum* and *P. pseudoradicatum*. The Flora of Korea (Kim 2017) accepted *P. coreanum*, but did not mention *P. pseudoradicatum*. Due to their identical distributions in North Korea and the adjacent parts of China and their highly similar morphological descriptions, it is possible that these reports refer to the same species. In that case, *P. coreanum*, described in 1928, would have priority over *P. pseudoradicatum*, described in 1942. Krivenko (2023) accepted both these species and recombined them in *Oreomecon*. We accept *P. coreanum* and provisionally place *P. pseudoradicatum* in synonymy pending future studies, but have not been able to cite their types.

A species from the Kurile Islands known as *P. miyabeanum* Tatew. is closely related to *P. fauriei* (Fedde) Fedde ex Miyabe & Tatew., a local endemic from Rishiri, a volcanic island just west of the northernmost tip of Hokkaido in Japan (Takahashi and Yamagishi 2020). The former was recombined into *Oreomecon* by Banfi et al. (2022), citing a basionym originating from Miyabe and Tatewaki (1936). However, the year before, Miyabe and Tatewaki (1935) had described *Papaver nudicaule* subsp. *xanthopetalum* var. *shimshirense* Miyabe & Tatewaki listing the new name *P. miyabeanum* Tatewaki as a synonym "in sched.". Taka-

hashi and Yamagishi (2020) restudied the complex and reduced the former to a subspecies of *P. fauriei* under the name *P. fauriei* subsp. *shimshirense* (Miyabe & Tatew.) Hideki Takah. They concluded that the simultaneous publication of *P. miyabeanum* as a synonym of *Papaver nudicaule* subsp. *xanthopetalum* var. *shimshirense* by Miyabe and Tatewaki (1935) made the former name illegitimate, also when Miyabe and Tatewaki (1936) intended to name the taxon at the species level and when the taxon was recombined as *Oreomecon miyabeana* (Art. 6.4 and 58.1 in the Code; Turland et al. (2018)). *Papaver fauriei* was originally published as *Papaver nudicaule* subsp. *xanthopetalum* var. *fauriei* Fedde by Fedde (1909), who defined the specimen *Faurie 3015* at B as the holotype, which, according to Takahashi and Yamagishi (2020), has not been relocated and only isotypes are therefore listed below.

### Distribution

We conclude that this area includes nine species and one subspecies. The distribution of the genus *Oreomecon* in this area is shown in Fig. 2.

### Rare or red-listed taxa

Yamagishi et al. (2010, 2018) reported a small population of *P. fauriei* subsp. *fauriei* to be Endangered (EN) due to the threat of hybridisation with a cultivated and undetermined *Papaver* sp., which is determined here as *P. fauriei* subsp. *shimshirense* based on the data shown by Takahashi and Yamagishi (2020) and the results in our phylogram. This would then be a case of infraspecific hybridisation. Xue et al. (2023) listed *P. anomalum* as Vulnerable (VU) in Russia under the name *P. nudicaule* var. *aquilegifolium* Fedde, which is considered a synonym of *P. ammophilum* by POWO (2023). *Papaver anadyrense* V.V.Petrovsky and *P. tolmatschevianum* were listed as Nearly Threatened (NT) by Xue et al. (2023).

#### Accepted taxa

### 3.2.1. Oreomecon alborosea (Hultén) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

= Papaver alboroseum Hultén, Kongl. Svenska Vetensk. Akad. Handl., Ser. 3, 5(2): 141. 1928. Type: [Russia] Kamtchatka australis, Avatcha Volcano, 675 m alt., 30 Jul 1920, E. Hultén 508b (lectotype: S [no. S-G-4522] lectotype designated here; isolectotype: GB [barcode GB0048356]).

### 3.2.2. Oreomecon anadyrensis (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:1. 2023

Papaver anadyrense V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 68: 229. 1983. Type: [Russia] Terra Tschuktschorum australis, districtus Anadyrensis, prope pagum Otrozhnyj, in valle fl. Mavrina, in summitate monticuli, tundra schistosa, in Dryadeta, 14 Aug 1977, P. Zhukova 77-379 (holotype: LE).

### 3.2.3. Oreomecon anomala (Fedde) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 71. 2022

 Papaver anomalum Fedde, Pflanzenr. (Engler) IV.104(40).1909. Type: Central China, West Hupeh, Jun 1901, E.H. Wilson 2421 (lectotype: B [barcode B 10 0279403], lectotype designated here; isolectotypes: P [barcode P00738904], US [barcode US00099714] and LE).

# 3.2.4. Oreomecon coreana (Nakai) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

- = Papaver coreanum Nakai, Sci. Knowl. 8: 42. 1928.
- = Papaver pseudoradicatum Kitag., Rep. Inst. Sci. Res. Manchoukuo, 6, 4: 122. 1942, "pseudo-radicatum", syn. nov. ≡ Papaver radicatum var. pseudoradicatum (Kitag.) Kitag., Neolin. Fl. Manshur.: 325 (1979) ≡ Oreomecon pseudoradicatum (Kitag.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

# 3.2.5. Oreomecon fauriei (Fedde) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

*■ Papaver nudicaule* subsp. *xanthopetalum* var. *fauriei* Fedde, Repert. Spec. Nov. Regni Veg. 7: 257. 1909. Type: Japan, Hokkaido, Isl. Rishiri, *Faurie 3015*, 25 Jul 1899 (isotypes KYO; P [barcode P00744502] and P. [barcode P00744599]
 *■ Papaver fauriei* (Fedde) Fedde ex Miyabe & Tatew., Trans. Sapporo Nat. Hist. Soc. 14: 258.1936.

# 3.2.6. *Oreomecon fauriei* (Fedde) Galasso, Banfi & Bertolucci subsp. *fauriei*, Pl. Rev. 5(4): 58. 2023

= Papaver nudicaule subsp. xanthopetalum var. fauriei Fedde, Repert. Spec. Nov. Regni Veg. 7: 257. 1909. Type: Japan, Hokkaido, Isl. Rishiri, Faurie 3015, 25 Jul 1899 (isotypes KYO; P [barcode P00744502] and P [barcode P00744599].

# 3.2.7. Oreomecon fauriei subsp. shimshirensis (Miyabe & Tatew.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77351066-1

- Papaver nudicaule L. subsp. xanthopetalum Fedde var. shimshirense Miyabe & Tatew., Trans. Sapporo Nat. Hist. Soc. 14: 5. 1935. Type: Middle Kurils, Isl. Shimshir, Broughtonzaki, M. Tatewaki & Y. Tokunaga 11569, 13 Aug 1928 (SAPS no. 036731: holotype) = Papaver fauriei subsp. shimshirense (Miyabe & Tatew.) Hideki Takah, Acta Phytotax. Geobot. 71: 154. 2020.
- Papaver miyabeanum Tatew., Trans. Sapporo Nat. Hist. Soc. 14: 259. 1936, nom. illeg.; – Oreomecon miyabeana (Tatew.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 71, 2022, nom. illeg.

#### 3.2.8. Oreomecon hirsuta (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

*P. anomalum* var. *hirsutum* Tolm., Novosti Sist. Vyssh. Rast. 7: 157. 1971. Type: [Russia] Primorskiy Krai, Pos'etskiy r.-n., p[aluostr]ov Peschanyi, Kosa, 31 Jul 1931, *V. Petrov* (holotype: LE) = *Papaver sokolovskajae* Prob., Fl. Ross. Dal'nego Vostoka: 63. 2006, nom. illeg.

## 3.2.9. Oreomecon microcarpa (DC.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver microcarpum DC., Syst. Nat. 2: 71. 1821. Type: Kamchatka, P.S. Pallas (holotype: G-DC-166725/1) = Papaver nudicaule subsp. microcarpum (DC.) Elkan, Monogr. Papav.: 17. 1839 = Papaver alpinum var. microcarpum (DC.) Ledeb., Fl. Ross. 1: 87. 1841 = Oreomecon nudicaulis subsp. microcarpa (DC.) Grey-Wilson, Pl. Rev. 5 (4): 57. 2023.

## **3.2.10.** Oreomecon ochotensis (Tolm.) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350924-1

 Papaver ochotense Tolm., Zhurn. Russk. Bot. Obshch. 16: 82. 1931. Type: [Russia] Bassein r. Penshiny. Na krutom kamenistom sklone k r. Pal'matkinoy v 22 km ot ust'ya. 7 Aug 1930, V.B. Sochava (holotype: LE) = Papaver microcarpon subsp. ochotense (Tolm.) Tolm., in V.L. Komarov (ed.) Fl. SSSR 7: 31. 1975.

# 3.2.11. Oreomecon tolmatscheviana (N.S.Pavlova) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

- Papaver tolmatschevianum N.S. Pavlova, Bot. Zhurn. (Moscow & Leningrad) 84
  (2): 112. 1999. Type: [Russia: Insula Sachalin, districtus Poronajskij, brachia orientalia montium Sachalinensium Orientalium, cacumen montis Slannikovi (343 m s. m.), 2–3 km ad boreali-occidentem a promontorio Sheltingi, regio subalpina, in locis schistosis in pineto raro (*Pinus pumila*)], 16 Aug.1991, *N. S. Pavlova s.n.* (holotype: VLA; isotype: LE).
- Papaver ochotense Miyabe & Tatew., in Sugawara, S. III. Fl. Saghal. 3: 985.
  1940, homonym.

### 3.3. Arctic Asia

#### Notes

The primary treatment of this group is Tolmachev (1975) in *Arkticheskaya Flora SSSR*. He included 16 species and seven additional subspecies and he was the author or co-author of no less than 19 of these taxa, the first ones described 52 years earlier, in 1923. Two species were described together with his pupil V.V.Petrovsky (Tolmachev and Petrovsky 1973), who contin-

ued with *Papaver* studies until his early 90s, also representing an almost 50-year-long career. After the passing-away of Tolmachev in 1979, another nine species and one subspecies were described by Petrovsky (1983, 1985). The study by Tolmachev (1975), which includes an identification key and distribution maps and the two studies by Petrovsky are here considered as a combined monograph, covering 24 species and eight subspecies. The treatment below only includes new or deviating information on the cited reference studies.

The species Papaver anadyrense, P. leucotrichum, P. microcarpum, P. nivale and P. ochotense listed by the cited monograph source have been removed from this part, as they do not or scarcely reach the Arctic (Elven et al. 2011) and P. microcarpum was discussed above as a primarily non-Arctic species. They are here, instead, treated in other geographical sections. Despite being accepted by POWO (2023), Papaver minutiflorum Tolm. had been synonymised with P. lapponicum subsp. orientale Tolm. by Petrovsky (1999) and Elven et al. (2011). These sources are followed here, whereas the recently-recombined name Oreomecon minutiflora (Tolm.) Krivenko and the recently-changed status of O. orientalis (Tolm.) Krivenko are both placed in synonymy. Papaver indigirkense Jurtzev was synonymised with P. minutiflorum by Peschkova (1994). Papaver radicatum subsp. occidentale C.E.Lundstr., mapped from Wrangell Island and the Chukotka Peninsula by Tolmachev (1975), was subsumed under P. radicatum Rottb. by POWO (2023). However, Petrovsky (1999) had already explained a broad interpretation of P. radicatum as a confusion with several morphologically similar Siberian species instead. This was in agreement with Solstad et al. (1999), who considered P. radicatum to be a Nordic species.

The remaining taxa from the monograph source used here were accepted by Elven et al. (2011), although four of them only provisionally. However, the need to better understand most of the taxa was underlined. Petrovsky et al. (2019) described three subspecies of the *P. pulvinatum* Tolm. complex. The previously misinterpreted name *P. pulvinatum* subsp. *lenaense* Tolm. was shown by Petrovsky et al. (2019) to be a synonym of *P. nudicaule* var. *riparium* V.V.Petrovsky. Chepinoga et al. (2023) lifted these subspecies to the species level with later recombinations into *Oreomecon* by Krivenko (2023). Similar recombinations and status changes were done for *P. lapponicum* subsp. *jugoricum* Tolm. and *P. microcarpum* subsp. *czekanowskii* Tolm. by Krivenko (2023), whereas a status change for *P. nudicaule* subsp. *insulare* V.V.Petrovsky was undertaken by Chepinoga et al. (2023). These species-level changes were done, based on the authors' general non-acceptance of subspecific taxa and not by evaluating existing classifications in the *Papaver* literature, which is the preferred alternative here.

The Wrangel Island endemic *Papaver uschakovii* Tolm. & V.V.Petrovsky was accepted by Petrovsky (1999), Solstad et al. (2009), with molecular support and Elven et al. (2011) and is recombined into *Oreomecon* below. It was included in *Papaver polare* Tolm. by Xue et al. (2023) and POWO (2023) and was not treated by Krivenko (2023). According to Elven et al. (2011), *Papaver uschakovii* subsp. *tichomirovii* Kozhevn. from Chukotka, does not belong in *P. uschakovii*, nor in *P. dahlianum* s.lat. and may have affiliation with a still undescribed species.

A particular case concerns the species group with amphi-Beringian distributions, which comprise four species treated in the monograph sources. *Papaver detritophilum* Petrovsky has most of its distribution area on the Russian side and is treated within this group of Asian-Arctic species. *Papaver keelei* A.E.Pors., *P. gorodkovii* Tolm. & Petrovsky and *P. walpolei* A.E.Pors. have their major distribution ranges on the American side and are treated in the section on taxa from Arctic Alaska and Yukon; see below. We conclude that *Oreomecon* is represented by 14 species and six subspecies in Arctic Asia. *Oreomecon lapponica* and *O. nudicaulis* occur with separate subspecies in this area and their nominate subspecies are treated as taxa 3.6.6 and 3.1.13, respectively, below the areas where they occur.

#### Distribution

The distribution map in Fig. 3 shows a gap in Central Siberia between groups A and C. The distribution maps by Peschkova (1994) only show a connection in easternmost Siberia. However, some occurrences are shown by GBIF (Global Biodiversity Information Facility) Secretariat (2023) along the Verkhoyansk Mountain Range and we map the connection here.

### Rare and threatened species

The most exclusive ones in this group are the endemic taxa from Wrangel Island, which were treated by Petrovsky (1997, 1999) and mapped by Petrovsky in Talbot et al. (1999), each shown from 4 - 8 localities and protected in the Wrangel Island State Reserve. *Papaver gorodkovii* Tolm. & Petrovsky was included by Petrovsky (1997), but, according to Petrovsky (1999), the disjunct and large Wrangel Island population of this mostly American species is polymorphic and insufficiently understood. The remaining five species and one subspecies mapped by Talbot et al. (1999) are endemic to Wrangel Island, except *Papaver atrovirens* V.V.Petrovsky also occurring on the adjacent mainland and *P. calcareum* V.V.Petrovsky also from north-eastern Chukchi Peninsula (Elven et al. 2011).

Xue et al. (2023) did not list any Endangered (EN) or Vulnerable (VU) species from the Asian Arctic, but treated seven taxa as Near Threatened (NT). These did not include the rare species from Wrangel Island, but instead widespread taxa such as *P. czekanowskii* and *P. lapponicum* subsp. *jugoricum* (Tolm.) S.V.I. Gudoshn. They also accepted *P. indigirkense*, which we, however, consider a synonym of *P. lapponicum* subsp. *orientale*.

### Accepted taxa

## 3.3.1. Oreomecon angustifolia (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:1. 2023

Papaver angustifolium Tolm., Trudy Bot. Muz. 22: 369. 1930. Type: [Russia] Siberia: Gydan Tundra, Obvalivayushchiesya beregovye sklony u NO vhodnogo mysa Yurackoy Guby, 15 Aug 1926, A.I. Tolmachev 589 (lectotype: LE, lectotypified by Elven et al. [2009], p. 988).

#### 3.3.2. Oreomecon anjuica (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver anjuicum Tolm., in V.L. Komarov (ed.) Arkt. Fl. SSSR 7: 25. 1975. Type: Russian Far East: West Chukotka, [in parte septentrionali montium Anjuicum, ad fontes fluminis Erguveem], 11 Jul 1967, E. Zimarskaja, A. Korobkov & B. Yurtsev s.n., (holotype: LE).

## 3.3.3. Oreomecon atrovirens (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver atrovirens V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 68: 231. 1983. Type: [Russia: Insula Wrangelii, ad litus meridionale, ad sinum Somnitelnaja], 16 Jul 1971, fl. et fr. immat., V. Petrovsky 71-33 (holotype and isotypes: LE).

## 3.3.4. Oreomecon calcarea (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver calcareum V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 68: 232.
 1983. Type: [Russia: Insula Wrangelii, ad fl. Gussinaja, in declivibus glareosis calcareis], 14 Jul 1969, fl. et fr. immat., V. Petrovsky s.n. (holotype: LE).

## 3.3.5. *Oreomecon chionophila* (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver chionophilum V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 68: 233. 1983. Type: [Russia: Insula Wrangelii, ad sinum Somnitelnaya, ad fl. Somnitelnaja, alluvium], 5 Aug 1971, fl., V. Petrovsky 71-357 (holotype and isotypes: LE).

# 3.3.6. Oreomecon czekanowskii (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

 Papaver czekanowskii Tolm., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R 20: 172. 1960. Type: [Russia: Siberia, Yakutia, ad brachium delta Lenae fluminis Olenekskaja protoka dictum, ad pagum Czaj-Tumus], 19 Jul 1956, A. Tolmatchev (holotype: LE) = Papaver microcarpum subsp. czekanowskii (Tolm.) Tolm., Fl. Arct. URSS 7: 31. 1975.

# 3.3.7. *Oreomecon detritophila* (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver detritophilum V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 70: 114. 1985. Type: Russian Far East: West Chukotka, [jugum Anjujensis, in systemate fl. Anjuj Magnus, in valle fl. Bystrjanka], 25 Jul 1980, V.V.Petrovsky 80-59 (holotype: LE).

## 3.3.8. Oreomecon hypsipetes (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver hypsipetes V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 70: 113. 1985. Type: Russian Far East: West Chukotka, [districtus Bilibinskij, montes Anjujensis, jugum Ilirnejensis, ad Iacus Ilirnej Superior], 18 Jul 1973, V.V. Petrovsky 73-26 (holotype: LE).

# 3.3.9. *Oreomecon lapponica* subsp. *jugorica* (Tolm.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350925-1

= Papaver radicatum subsp. jugoricum Tolm., Bot. Mater. Gerb. Inst. Komarova Akad. Nauk S.S.S.R. 4:86. 1923. Type: [northern European Russia] Ostrov Vaigach, sev. podereshchie, bukhta Varneka, na otmeli, 2 Sep 1921, A. Tolmatchev 352 (lectotype: LE, designated by Egorova [1998] p. 99) = Papaver lapponicum subsp. jugoricum (Tolm.) Gudoschn., Fl. Krasnoy. Kraya 5 (4):
 6. 1975 = Oreomecon jugorica (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

# 3.3.10. Oreomecon lapponica subsp. orientalis (Tolm.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350926-1

- Papaver lapponicum subsp. orientale Tolm., Trudy Polyarn. Komiss. 13: 131.
  1932. Type: Siberia: Vostochniy Taimyr. Nizoviya r. Yamu-Neri (bassein Taimyrskogo ozera), raion letoviya ekspeditsii (74°50'N, 106°E), 5 Aug 1928, A.I. Tolmachev 582 (lectotype: LE, lectotype designated by Elven et al. [2009: 987]) = Oreomecon orientalis (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2.
  2023 = Papaver pospelovae Barkalov & Chepinoga, Botanica Pacifica 12, 2: 124. 2023, nom. nov., non P. orientale L. 1753.
- = Papaver minutiflorum Tolm., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 20: 180. 1960; Type: Russia, E Siberia, Yakutskaya ASSR, Tomponskii rayon. Bassein r. Tompo r. Seyule, 5 Aug. 1956, *I.D.Kildjushevskii 30/1*, (LE, lectotype, selected by Elven et al. [2009: 987]) ≡ Oreomecon minutiflora (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.
- = Papaver indigirkense Jurtzev, Novosti Sist. Vyssh. Rast. 2: 310. 1965. Type: [Russia] Jacutia orientalis, in parte superiore fl. Indigirka, ad fl. Chugutjan, in declivi substepposo, 1 Jul 1958, B.A. Jurtzev s.n. (LE, holotype).

### 3.3.11. Oreomecon multiradiata (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver multiradiatum V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 68:
 284. 1983. Type: Russian Far East: Wrangel Island, [ad sinum Somnitelnaja],
 23 Jul 1965, V.V.Petrovsky (holotype: LE).

## 3.3.12. Oreomecon nudicaulis subsp. insularis (V.V.Petrovsky) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350927-1

■ Papaver nudicaule subsp. insulare V.V.Petrovsky, Bot. Zhurn. 68: 236. 1983. Type: Russian Far East: Wrangel Island, [ad sinum Somnitelnaja], 10 Aug 1979, V.V.Petrovsky 79-96 (holotype: LE) = Papaver insulare (V.V.Petrovsky) Barkalov & Chepinoga, Botanica Pacifica 12, 2: 124. 2023.

## 3.3.13. Oreomecon paucistamina (Tolm. & V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver paucistaminum Tolm. & V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 58: 1129. 1973. Type: Russian Far East: West Chukotka, [in montibus partis centralis Terrae Tschuktschorum, in ditione fluminis Quëkvun], 26 Jul 1966, V.V.Petrovsky s.n. (holotype: LE).

# 3.3.14. Oreomecon pulvinata (Tolm.) Krivenko subsp. pulvinata, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver pulvinatum Tolm., Trudy Bot. Muz. 24: 269. 1932. Type: [Russia]: Siberia, Taimyr, Nizovya r. Yamu-Tarida (bassein Taimyrskogo ozera), raion vesnovki ekspeditsii (74°27'N, 102°50'E), 5 Aug 1928, A.I. Tolmachev 135 (lectotype: LE, lectotypified by Elven et al. [2009] p. 988).

# 3.3.15. Oreomecon pulvinata subsp. alexandri (V.V.Petrovsky) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350928-1

= Papaver pulvinatum subsp. alexandri V.V.Petrovsky, Ann. Bot. Fenn. 56: 371. 2019. Type: Russia. [NE Siberia] Yakut ASSR, N end of Kharaulakhskii Range, right bank of Bykovskaya branch (delta of the Lena River), environs of Sokol settlement (72°20'N, 125°40'E), Kiries-Khamo Bay, sandy terrace, 19 Aug 1956 T.G. Polozova, B.A. Yurtsev s.n (holotype: LE [barcode LE 01026076],

fig. 2, illustrated by Petrovsky et al. [2019]: 372; isotypes: LE [barcode LE 01026077], LE [barcode LE 01026078])  $\equiv$  *Papaver alexandri* (V.V.Petrovsky) Barkalov & Chepinoga, Botanica Pacifica 12, 2: 124. 2023  $\equiv$  *Oreomecon alexandri* (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:1. 2023  $\equiv$  *Papaver pulvinatum* subsp. *lenaense* Tolm., Arkt. Fl. SSSR 7: 24. 1975, nom. illeg.

# 3.3.16. Oreomecon pulvinata subsp. interior (V.V.Petrovsky) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350929-1

Papaver pulvinatum subsp. interius V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 65: 657. 1980) Type: Russia. West Chukotka, Anyui Upland, northern part, middle reaches of the Kytep-Guiten'ryveem River, Baraniy brook ravine, alluvial fan of a tributary, 17 Aug 1977 V. Petrovsky 77-44P (holotype: LE [barcode LE 01035172]; isotype: LE [barcode LE 01035173]) = Papaver interius (V.V.Petrovsky) Barkalov & Chepinoga, Botanica Pacifica 12, 2: 124. 2023 = Oreomecon interior (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023, "interius".

# 3.3.17. Oreomecon pulvinata subsp. tschuktschorum (Tolm.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350930-1

Papaver pulvinatum subsp. tschuktschorum Tolm., Arkt. Fl. SSSR 7: 24.
 1975. Type: N. Russian Far East, Ostrov Vrangelya, buchta Somnitelnaya, r. Somnitelnaya, 20 Jul 1964, V.V.Petrovsky s.n. (lectotype: LE [barcode LE 01042299], designated by Elven et al. [2009: 988]; illustrated by Petrovsky et al. [2019] p. 374) = Oreomecon tschuktschorum (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023.

# 3.3.18. Oreomecon schamurinii (V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver schamurinii V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad) 70: 116. 1985. Type: Russian Far East: Wrangel Island, [sinus Somnitelnaja, ad litus lacunae Bazovaja], 12 Jul 1971, V.V.Petrovsky 71-200 (holotype: LE).

### 3.3.19. Oreomecon uschakovii (Tolm. & V.V.Petrovsky) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350931-1

= Papaver uschakovii Tolm. & V.V.Petrovsky, Bot. Zhurn. 58: 1128. 1973. Type: Russian Far East: Wrangel Island, [ad sinum Rogersii], 27 Jun 1969, V.V.Petrovsky s.n. (holotype: LE).

### 3.3.20. Oreomecon variegata (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver variegatum Tolm., in V.L. Komarov (ed.) Arkt. Fl. SSSR 7: 20. 1975. Type: Siberia: Putorana, [ad litus meridionalis lacu Khantaika, prope ostium fluminis Mogaddy], 7 Aug 1970, A. Tolmachev (holotype: LE).

#### 3.4. Arctic Alaska and Yukon and adjacent Cordilleras

#### Notes

Most of the taxa of *Papaver* sect. *Meconella* in this area are shared between the Arctic parts of Alaska and Yukon as defined by Walker et al. (2005) and the northernmost part of the North American Cordilleras, whereas other species predominate further south (Kiger and Murray 1997; Björk 2019; GBIF (Global Biodiversity Information Facility) Secretariat 2023). Distribution area D is, therefore, defined as Arctic Alaska and Yukon and adjacent parts of the northern North American Cordilleras delimited southwards by the border with British Columbia and eastwards by the Mackenzie River. The primary source for area D is Elven et al. (2011), where seven species centred in Arctic Alaska were included. Only three of these species were accepted under the same names by "Flora of North America" (Kiger and Murray 1997).

The list below includes amphi-Beringian species, including *P. walpolei* A.E.Porsild, which occurs in eastern Chukotka and *P. gorodkovii* Tolm. & V.V. Petrovsky, which also extends across the Bering Strait to Wrangel Island. According to Elven et al. (2011), *P. keelei* A.E.Porsild is by far the most common species in the Arctic part of the area, ranging from Chukotka to the western mainland of the Northwest Territories. It was not treated by Galasso et al. (2023), Grey-Wilson (2023) or Krivenko (2023) and is recombined into *Oreomecon* below. *Papaver hultenii* Knaben is common in Alaska and also occurs eastwards to Nunavut, but its presence in Chukotka is uncertain, according to Elven et al. (2011). None of these species occurs south of area D, except *P. hultenii*, known from the Pink Mt. area in British Columbia (Björk 2019). Björk (2019) reported that *P. roseoalbum* Björk is mainly from south-central Alaska, with a few collections outside area D in the far north-western part of British Columbia.

Neither Björk (2019) nor Krivenko (2023) referred to *P. nudicaule* subsp. *americanum* Rändel ex D.F.Murray, which was described by Rändel (1977) and validated by Murray (1995). The taxon was studied by morphology and AFLP by Solstad et al. (2009). They concluded that it was hexaploid as opposed to the typical forms of *P. nudicaule* and that it was the only American representative from southern and eastern Alaska and Yukon of a large complex including this species. It was also accepted by Elven et al. (2011), who did not recognise it from the Arctic parts of North America. In contrast, Kiger and Murray (1997) did not discriminate between this taxon and introduced "Iceland poppies" (*P. croce-um*). The nominate subspecies of *O. nudicaulis* is treated here as taxon 3.1.13.

Krivenko (2023) omitted the author name in the citation of the basionym in his recombination of *Oreomecon alaskanum* (Hultén) Krivenko. Following the

Code (Art 41.6; Turland et al. (2018)), this is considered to be within the range of allowed erroneous basionym citations. Krivenko (2023) also transferred *Papaver denalii* Gjærevoll, a species previously accepted by Björk (2019), to *Oreomecon*. However, Solstad et al. (2009) and Elven et al. (2011) considered it a synonym of *P. mcconnellii* Hultén. Galasso et al. (2023) treated *P. macounii* var. *discolor* Hultén as a subspecies of *Oreomecon*, while Krivenko (2023) raised it to species level. In contrast, Solstad et al. (2009) and Elven et al. (2011) considered it a synonym of *P. keelei*. The latter authors are followed here in both cases.

Papaver macounii Greene is probably the rarest species in this area, known from only a few scattered sites (Elven et al. 2011). Papaver alaskanum Hultén is also a rare species, but was considered heterogeneous by Elven et al. (2011). This heterogeneity has not yet been resolved. Cortés-Burns et al. (2009) included Papaver gorodkovii and mapped its distribution in their treatment of the rare plants of the Alaskan North Slope. However, there is no coordinated evaluation of the conservation status of Papaver sect. Meconella in North America. A total of eight species and one subspecies are listed below. Papaver mcconnellii was listed as VU and P. walpolei as NT in Russia by Xue et al. (2023).

### Accepted taxa

# 3.4.1. Oreomecon alaskana (Hultén) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:1. 2023

 Papaver alaskanum Hultén, Fl. Aleutian Isl.: 190. 1937. Type: [USA] Alaska: the Aleutian Islands, Unalaska, 2 Aug 1932, E. Hultén 7197 (holotype: S [no. S-G-4519] = Papaver radicatum subsp. alaskanum (Hultén) J.P. Anderson, Fl. Alaska: 244. 1959 = Papaver microcarpum subsp. alaskanum (Hultén) Tolm., Fl. Arct. URSS 7: 30. 1975.

# 3.4.2. *Oreomecon gorodkovii* (Tolm. & V.V.Petrovsky) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

 Papaver gorodkovii Tolm. & V.V.Petrovsky, Bot. Zhurn. (Moscow & Leningrad)
 58: 1128. 1973. Type: Russian Far East: Wrangel Island, [ad sinum Somnitelnaja], 24 Jul 1971, V.V.Petrovsky & N. Taraskina s.n. (holotype: LE).

# 3.4.3. Oreomecon hultenii (Knaben) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver hultenii Knaben, Opera Bot. 3(3): 49. 1959. Type: Canada: Nunavut, Copper Mine River, M. Hammer, plants cultivated in Oslo from seeds collected in 1948 (holotype: O [barcode O-V-2014581]).

#### 3.4.4. Oreomecon keelei (A.E.Porsild) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350932-1

- = Papaver keelei A.E.Porsild, Bull. Natl. Mus. Canada 101: 20. 1945. Type: Canada: the Yukon Territory, Canal Road, Mackenzie Range, small tributary to Little Keele River, Mile 51, 8 Sep 1944, A.E.Porsild and A.J. Breitung 11,782 (holotype: CAN; isotype S [no. S-G-4528]).
- Papaver macounii var. discolor Hultén, Acta Univ. Lund., n. s., sect. 2, 41, 1: 803. 1945. Type: Alaska: Seward Peninsula, Nome, hillside, 11 Jul 1938, J.P. Anderson 3250 (holotype S) ≡ Papaver macounii subsp. discolor (Hultén) Rändel ex D.F.Murray, Novon 5: 294. 1995
- Oreomecon macounii subsp. discolor (Hultén) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023 = Oreomecon discolor (Hultén) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

# 3.4.5. Oreomecon macounii (Greene) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

= Papaver macounii Greene, Pittonia 3: 247. 1897. Type: Alaska: the Pribilof Islands, St. Paul Island, Jun-Jul 1897, J.M. Macoun s.n. (CAN, C [barcode C10016380]; K [barcode K 000653200]; NDG [barcode NDG20296].

# 3.4.6. Oreomecon mcconnellii (Hultén) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

- Papaver mcconnellii Hultén, Acta Univ. Lund. 2, 41(1): 803. 1945. Type: Canada: Northwest Territories, mountains between Peel River and La Pierre House, 1 Jul 1888, McConnell s.n. (holotype in unknown herbarium; photograph and fragment of holotype at S [no. S-G-4530]).
- = Papaver denalii Gjærev., Kongel. Norske Vidensk. Selsk. Skr. (Trondheim) 1963: 4:42. 1964. Type: Alaska, the Alaska Range, McKinley Park, Cathedral Mountain, 20 Jul 1959, O. Gjærevoll (holotype: TRH) ≡ Oreomecon denalii (Gjærev.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

### 3.4.7. Oreomecon nudicaulis subsp. americana (Rändel ex D.F.Murray) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350933-1

Papaver nudicaule subsp. americanum Rändel ex D.F.Murray, Novon 5: 295. 1995. Type: Canada: the Yukon Territory, the Klotassin area, southwest of Yukon River, between Selkirk and White River, Aug 1916, D.D. Cairnes 91890 (holotype: CAN).

### 3.4.8. Oreomecon roseoalba (Björk) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver roseoalbum Björk, Phytoneuron 2019-6: 10. 2019. Type: USA: Alaska. Populus-Alnus thicket, at mouth of small canyon, W of Portage Glacier, 12 Jul 1968, Welsh 8146 (holotype: ALA [barcode ALA274045]).

## 3.4.9. Oreomecon walpolei (A.E.Porsild) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023

= Papaver walpolei A.E.Porsild; Rhodora 41: 231 (1939). Type: Alaska: Seward Peninsula, Anvil Hill, A.E.Porsild 1352 (holotype: CAN).

### 3.5. North American Cordilleras

#### Notes

The delimitation northwards of area E is the northern boundary of British Columbia. The reference study for this area is Björk (2019), primarily focusing on British Columbia, but also dealing with material from other parts and making significant changes to the taxonomy of the group. *Papaver columbianum* Fedde ex Björk and *P. kluanense* D.Löve only extend into adjacent areas of southern Yukon, according to Björk (2019), who also showed that most of the plants from the US part of the Rocky Mountains represent *Papaver coloradense* (Fedde) Fedde ex Wooton & Standley. The central part of the North American Cordilleras now includes five species.

### Distribution

The distribution map in Fig. 2 also integrated distribution maps presented by Kiger and Murray (1997).

### Rare and protected species

Björk (2019) stated that both *P. columbianum* Fedde ex Björk and *P. luculentum* Björk may be rare. The former is only known from four collections. However, they are so far apart that the occurrence of undiscovered localities was cited to be likely.

### Accepted taxa

## 3.5.1. Oreomecon coloradensis (Fedde) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

■ Papaver nudicaule var. coloradense Fedde, Repert. Spec. Nov. Regni Veg. 7: 256. 1909. Type: USA: Colorado. East of Middle Park, 1867, Parry 147 (lectotype: BM [barcode BM574948], designated by Björk [2019], p. 17) = Papaver *coloradense* (Fedde) Fedde ex Wooton & Standl., Contr. U.S. Natl. Herb. 19: 262. 1915.

## 3.5.2. Oreomecon columbiana (Fedde ex Björk) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

■ Papaver columbianum Fedde ex Björk, Phytoneuron 2019-6: 5. 2019. Type: Canada: British Columbia, mountains at Kicking [Horse], [possibly Yoho National Park], 8000 ft, 14 Aug 1890, Macoun s.n. (holotype: US, no. 99717) = Papaver nudicaule var. columbianum Fedde; Repert. Spec. Nov. Regni Veg. 7: 255 (1909), nom. illeg.

# 3.5.3. Oreomecon kluanensis (D.Löve) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

Papaver kluanense D.Löve, Bot. Not. 109: 178. 1956. Type: Canada: Yukon Territory. North of Quill Creek Camp, alt. ca. 5000 ft, 20 mi W of Burwash Landing, 15 Jun 1953, Freedman s.n. (holotype: MAN; fragment and photograph of holotype at S [no. S S08-261]) = Papaver radicatum subsp. kluanense (D.Löve) D.F.Murray, Novon 5: 294. 1995.

# 3.5.4. Oreomecon luculenta (Björk) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

= Papaver luculentum Björk, Phytoneuron 2019-6: 7. 2019. Type: Canada: British Columbia. Boundary Ranges, ridge N of North Treaty Creek, near Bowser Lake, W of Bell Irving River, 56°38'6.88"N, 129°52'13.18"W, on fine argillite gravel scree, windblown alpine ridge, 4 Jul 2013, *Björk 32373* (holotype: UBC).

# 3.5.5. Oreomecon pygmaea (Rydb.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

Papaver pygmaeum Rydb., Bull. Torrey Bot. Club 29: 159. 1902. Type: USA: Montana. Mountain above Stanton Lake, 1 Aug 1894, Williams 992 (lectotype: NY [NY99719], designated by Björk [2019], p. 2).

### 3.6. Central and eastern Arctic Canada, Greenland and Arctic Europe

### Notes

The taxa present in the Canadian Arctic Archipelago were treated by Solstad (2007). However, the reference study for this area, where two widely distributed High-Arctic taxa are characteristic, is Elven et al. (2011). One of these

taxa is *Papaver cornwallisense* D.Löve, while the other is *Papaver dahlianum* subsp. *polare* (Tolm.) Elven & Ö.Nilsson. Solstad et al. (2014) reported both to be very common in Svalbard and equally widespread in Arctic North America. Elven et al. (2011) had left the basionym of *Papaver dahlianum* subsp. *polare* unassigned, as its type housed at LE is from Svalbard and could potentially represent *P. cornwallisense*, a more recently described taxon. However, Solstad et al. (2014) confirmed that the type represents *P. dahlianum* s.lat. Hence, *P. cornwallisense* remains the name of this distinct taxon. It was not treated in any recent *Oreomecon* studies and is, therefore, transferred to *Oreomecon* here.

Russian authors have traditionally treated *P. polare* Tolm. as distinct at the species level and this view was recently maintained by Xue et al. (2023), POWO (2023) and Krivenko (2023), the latter recombining it into *Oreomecon*. However, all recent studies comparing this taxon with *P. dahlianum* Nordh., partly with molecular support (Nilsson 2001; Solstad et al. 2003; 2009; 2014; Elven et al. 2011), conclude that these taxa are conspecific and that the older name *P. dahlianum* holds priority. Nilsson (2001) divided *Papaver dahlianum* into a wide-spread High-Arctic subsp. *polare* and a Low-Arctic subsp. *dahlianum* Nordh. *Papaver lapponicum* subsp. *dasycarpum* Tolm., recorded from Novaya Zemlya by Tolmachev (1975), was tentatively treated within *P. dahlianum* by Elven et al. (2011) and an interpretation within subsp. *polare* is followed here.

The latter subspecies occurs in the southernmost part of the Arctic in Finnmark, Norway and adjacent mountains in the Kola Peninsula, where it was described as P. lujaurense N.Semenova (Semenova-Tian-Shanskaya 1956). Based on an AFLP-based molecular analysis, Solstad et al. (2009) rejected the segregation into two distinct subspecies and this view on Papaver dahlianum was shared by Elven et al. (2011). However, Elven et al. (2022) indicated this might not be a final conclusion given the existing morphological differences and the genetic markers used. They also indicated that P. dahlianum ssp. polare has recently been discovered in the southeastern part of the Municipality of Porsanger/Porsánggu/Porsangin in Finnmark, ca. 100 km S of the polar treeline. Recently, J.O. Olsen and others posted on Artsdatabanken (2024) a number of additional localities of Papaver dahlianum from a small area in the Municipality of Gáivuotna/Kåfjord/Kaivuonu another 200 km further to the southwest. As further studies of this complex continue, we maintain Nilsson's interpretation (2001), which involves the acceptance of two separate subspecies of P. dahlianum.

Papaver lapponicum subsp. occidentale (C.E.Lundstr.) Knaben occurs in Canada and Greenland, whereas *P. lapponicum* subsp. *lapponicum* occurs from east Greenland eastwards to the westernmost parts of Siberia (Elven et al. 2011). The latter taxon was described from mountains just south of the Arctic border in the Kola Peninsula and two species described by Semenova-Tian-Shanskaya (1956) are considered synonyms. *Papaver lapponicum* subsp. *lapponicum* also occurs in the Municipalities Alta and Kvænangen in North Norway, ca. 80 km S of the Arctic tree line as defined by Elvebakk and Karlsen (2022). These populations have been treated as separate subspecies, but are included within subsp. *lapponicum* by recent authors, including Elven et al. (2022). Here, they are considered peripheral populations of an Arctic taxon and are not included amongst taxa listed from non-Arctic northern Europe. Papaver labradoricum (Fedde) Solstad & Elven from Canada and Greenland was recombined at the species level by Elven and Murray (2008). The present treatment thus includes four species and two subspecies from this area and the only taxon restricted to the vast Arctic European area is *P. dahlianum* subsp. *dahlianum*.

#### Distribution

The distribution shown in Fig. 2 includes all of Arctic Canada, Greenland and the European Arctic.

### Rare and red-listed species

Papaver dahlianum s.lat. and P. lapponicum are both endangered (EN) in mainland Norway (Artsdatabanken 2021). Papaver lapponicum subsp. lapponicum is Red-listed at the regional and national level in Russia and its populations on the Khibiny and Lovozerskie Mountains in the Kola Peninsula are protected within nature monuments as shown by Andreeva and Uotila (1998), where P. lujaurense was included within P. lapponicum.

### **3.6.1.** Oreomecon cornwallisensis (D.Löve) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350934-1

= Papaver cornwallisense D.Löve, Bot. Not. 109: 176. 1956. Type: Canada: Nunavut, ex Insula Cornwallis, in Archipelago Arctico Americae, 31 Jul 1954, J. Ritchie 663 (holotype: WIN).

# 3.6.2. Oreomecon dahliana (Nordh.) Galasso, Banfi & Bertolucci, Pl. Rev. 5(4): 58. 2023

- Papaver dahlianum Nordh., Bergens Mus. Årb. 1931, Naturvidensk. Rekke 2: 46. 1932. Type: Norway: Båtsfjord, Syltefjorden, Østerelven, på grus, 4 Jul 1930, R. Nordhagen s.n. (lectotype: O [barcode O-V-2014577], lectotype designated by Elven & Nilsson in Jonsell [2001] p. 521) = Papaver radicatum subsp. dahlianum (Nordh.) Rändel; Feddes Repert. 84: 694 (1974).
- = Papaver lujaurense N.Semenova, Fl. Murmansk. Obl. 3: 369. 1956. Type: [Russia] Peninsula Kola, in montibus Lovoserskye-Tundry, prope pag. Revda, 25 Aug 1955, N. Semenova-Tian-Shanskaya 185 (holotype: LE).

## 3.6.3. Oreomecon dahliana (Nordh.) Galasso, Banfi & Bertolucci subsp. dahliana, Pl. Rev. 5(4): 58. 2023

Papaver dahlianum Nordh., Bergens Mus. Årb. 1931, Naturvidensk. Rekke 2:
 46. 1932. Type: Norway: Båtsfjord, Syltefjorden, Østerelven, på grus, 4 Jul
 1930, R. Nordhagen s.n. (lectotype: O [barcode O-V-2014577], lectotype designated by Elven & Nilsson in Jonsell [2001] p. 521).

## 3.6.4. Oreomecon dahliana subsp. polaris (Tolm.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350935-1

- *Papaver radicatum* subsp. *polare* Tolm., Bot. Mater, Gerb. Glavn. Bot. Sada RSFSR 4: 87. 1923. Type: Norway: Svalbard, Advent Bay, 5–30 Jul 1898, Semenkevich (lectotype: LE, lectotype designated by Egorova [1998], p. 101) = Papaver polare (Tolm.) Perfil., in S.S. Stankov & V.I. Taliev, Syst. Classif. Vasc. Pl. Eur. Russ. 133. 1949 = Papaver dahlianum subsp. polare (Tolm.) Elven & Ö.Nilsson, Nordic J. Bot. 20: 522. 2001 = Oreomecon polaris (Tolm.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023.
- Papaver lapponicum subsp. dasycarpum Tolm., Trudy Bot. Muz. 25: 101.
  1932. Not lectotypified, syntypes were listed from Matotschkin Schar in Novaya Zemlya by Egorova (1998).

# 3.6.5. Oreomecon labradorica (Fedde) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023

■ Papaver nudicaule var. labradoricum Fedde, H.G.A. Engler (ed.) Pflanzenr., IV, 104: 377. 1909. Type: Greenland: Flora Groenlandiae boreali-occidentalis. Gebiet des Umanakfjords (70-71 N. Br.), 9 Aug 1893, *E. Vanhöffen 35(94)* (lectotype: B [barcode B10 0267999], lectotype designated by Elven et al. (2009) p. 986) = Papaver radicatum subsp. labradoricum (Fedde) Fedde, in Engl. & Prantl, Nat. Pflanzenfam., ed. 2, 17b: 120. 1936 = Papaver labradoricum (Fedde) Solstad & Elven, J. Bot. Res. Inst. Texas 2: 438. 2008 = Papaver lapponicum subsp. labradoricum (Fedde) Knaben, Blyttia 16: 78. 1958.

# 3.6.6. Oreomecon lapponica (Tolm.) Galasso, Banfi & Bertolucci subsp. lapponica, Pl. Rev. 5(4): 58. 2023

- Papaver radicatum subsp. lapponicum Tolm., Bot. Mater. Gerb. Glavn. Bot. Sada R.S.F.S.R., 4: 86. 1923. Type: Russia, Kola Peninsula, Oz. Imandra, 1 Aug 1911, Pohle s.n. (lectotype: LE, designated by Egorova [1998] p. 98) = Papaver lapponicum (Tolm.) Nordh., Bergens Mus. Årbog (Årbok), Naturvidensk. Rekke 2: 45. 1931.
- = Papaver nudicaule subsp. kvaenangense C.E.Lundstr., Acta Horti Berg. 7: 416. 1923; Type: Norway, Troms, Kvænangen, Burfjorddalen, Jul-Aug 1901, A.Notø (lectotype: TROM, lectotype designated by Löve [1962a] p. 133 ≡ Papaver lapponicum subsp. kvaenangense (C.E.Lundstr.) Ö.Nilsson, Nordic J. Bot. 20: 522. 2001;
- Papaver lapponicum subsp. scandinavicum Knaben, Opera Bot. 2; 3: 56.
  1959. Type: Norway: Finnmark, Alta, Talvik, Vassbotndalen, S.E. Olsen (holotype: O).
- = Papaver chibinense Semenova, Fl. Murmansk. Obl. 3: 368. 1956. Type: [Russia, Kola Peninsula] Khibinskii gornyi massiv, dolina ozera M. Vud'javr, kamenistyi otkos za morenoi meshchdy gorami Poachvumchorr i Takhtavumchorr, 8 Aug 1954, N. Semenova-Tjan-Schanskaja 127 (holotype: LE).

- = Papaver norvegicum Semenova, Fl. Murmansk. Obl. 3: 369. 1956. Type: Norway: Finnmark, Alta, Talvik, Vassbotndalen, 1930, R. Nordhagen (holotype: S).
- = Papaver tolmatchevii Semenova, Fl. Murmansk. Obl. 3: 369. 1956. Type: [Russia, Kola Peninsula] In montibus Chibinensibus ad declivitatem austro-orientalem montis Rasvumchorr, 4 Jul 1955, N. Semenova-Tjan-Schanskaja 88 (holotype: LE).

## 3.6.7. Oreomecon lapponica subsp. occidentalis (C.E.Lundstr.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350936-1

- Papaver radicatum subsp. occidentale C.E.Lundstr., Acta Horti Berg. 7, 5: 413.
  1923. Type: [Greenland]: Groenlandia orientalis, Sabine Island, 10 Jul 1899, P. Dusén 325 (lectotype: S [barcode S07-10363], lectotype designated by Elven et al. [2009] p. 987) = Papaver lapponicum subsp. occidentale (C.E.Lundstr.) Knaben; Opera Bot. 2, 3: 413 (1959).
- = Papaver lapponica subsp. porsildii Knaben, Blyttia 16: 79. 1958. Type: Canada: Nunavut, "Middle Territories, Foxe Basin, Prince Charles Island", A.E.Porsild (holotype: CAN).

#### 3.7. Non-Arctic Northern Europe

#### Notes

In his monograph on the alpine *Papaver* taxa in Scandinavia, Nordhagen (1932) described three new species. In addition, he described four new subspecies and two new varieties, including one variety of *P. radicatum* Rottb. He also briefly mentioned a 'doubtful race' ("± zweifelhaften Rasse"), which was intermediate between *P. radicatum* subsp. *dovrense* Nordh. and *P. relictum* (E.Lundstr.) Nordh., but it was not described. His suggested name, *P. radicatum* subsp. *dovrense* var. *intermedium* Nordh., for this taxon is, therefore, a *nomen nudum*, which makes later homotypic recombinations of this name illegitimate. A replacement name is, therefore, introduced below and a diagnosis is supplied; for a further description, see Nilsson (2001).

In a flora treatment, Löve (1945) described *P. radicatum* subsp. *stefanssonii* Á.Löve from Iceland, including both the white- or pink-flowered plants *P. radicatum* f. *albiflora* Stefánsson and *P. radicatum* f. *rubriflora* Stefánsson presented in the flora by Stefánsson (1901). However, like these, the taxon is illegitimate as no Latin diagnosis was provided (Art. 39.1 in the Code; Turman et al. (2018)) and the name cannot be applied to homotypic recombinations (Art. 6.4 in the Code; Turman et al. (2018)). Löve (1955, 1962b) interpreted the type material of *P. radicatum* to originate from Greenland and considered this species to be limited to Greenland and Canada. Löve (1955) considered Nordic material to belong to four species, namely *P. steindorssonianum* Á.Löve and *P. stefanssonianum* Á.Löve from Iceland and *P. relictum* and *P. nordhagenianum* Á.Löve from Scandinavia. In addition, he accepted *P. lapponicum* and *P. laestadianum* (Nordh.) Nordh. from northernmost Fennoscandia. By an epithet name change, *Papaver stefanssonianum* became the valid basionym of the taxon now often

treated as *P. radicatum* subsp. *stefanssonii*, for example, by Nilsson (2001) and Wąsowicz (2020).

Löve (1962a) revised his concepts and transferred the octoploid species *P. lapponicum* and *P. laestadianum* to the subspecies level of his concept of the North American octoploid *P. radicatum*. In contrast, all Nordic decaploid taxa were united within one species. When merging *P. nordhagenianum* and *P. relictum*, he described five subspecies of *P. nordhagenianum*, three of which also have several varieties. His selected species is the younger of the two alternatives and, as already shown by Knaben and Hylander (1970), the recombinations are not in accordance with the priority rules. Thus, eight of the names published by Löve (1962a) are here considered illegitimate. Löve later reached the same conclusion, as he stated that all these taxa instead belong within *P. relictum* (Löve 1970). However, he only provided valid recombinations of the Icelandic-Faroese taxa.

Knaben (1958) and Knaben and Hylander (1970) argued convincingly why the type of *P. radicatum* originated in Iceland. The view that *P. radicatum* is a North Atlantic taxon known primarily from alpine localities in Norway, Sweden, Iceland (with secondary localities in lowland screes and river banks) and the Faroe Islands has been maintained later (Elven et al. 2011; 2022) and subspecific taxa described from elsewhere all represent other species (Solstad et al. 2003). Knaben (1970) stated that her intention was to treat the *P. radicatum* taxa at the variety level. However, she later concluded that this might have led to confusion in light of the high number of synonyms already published (Knaben 1985).

Most of the Scandinavian taxa were studied morphometrically by Selin and Prentice (1988) and Selin (1998, 2000), who referred to these taxa as subspecies. Nilsson (2001) treated 13 Nordic taxa at the subspecies level and provided morphological descriptions and a determination key. Solstad et al. (2003), however, did not find support for such a diversification, based on an analysis of isozyme patterns and a later AFLP-based study concluded on the presence of two groups, one comprising populations from northern Scandinavia, another one comprising populations from southern Scandinavia and Iceland (Solstad et al. 2009). Elven et al. (2011, 2022) maintained that the variation in the North Atlantic area does not, with one exception, merit recognition as a subspecies. In a checklist from Iceland, Wąsowicz (2020), on the other hand, recently accepted three subspecies and the distribution of two of these subspecies have previously been mapped, showing that they reach the Arctic parts of Iceland (Nilsson 2001).

Papaver radicatum has been a key issue in Nordic discussions on whether plant life survived the Weichselian glaciation or immigrated post-glacially, for example, the review by Solstad et al. (1999, 2003) and the discussion by Selin (2000). The species has been extensively studied by all generations of Nordic botanists, still without a unified conclusion on its taxonomy. However, Elven et al. (2022) remarked that future application of other genetic markers might instigate a revised taxonomic concept for this species. To reflect the Nordic name tradition and facilitate communication, Elven et al. (2022), therefore, treated nine formerly named taxa of *P. radicatum* from mainland Norway at the variety level; this was done as an informal treatment without providing the required recombinations. The same approach has been followed by Artsdatabanken (2024). Below, we present a review of the classifications of the taxa within *P. radicatum*, with all important synonyms cited as presented in the original literature, involving a number of deviations from those listed by Nilsson (2001) and POWO (2023). In the absence of modern molecular data, we use morphological criteria and vicariant evolution as criteria for accepting subspecies, as underlined by Molinari (2023) and POWO (2023). Assessments of morphological differentiation rely on the morphometric studies by Selin and Prentice (1988), Selin (1998; 2000) and Øvstedal and Grung (2015).

In a study on five of the entities from southern Norway, Selin and Prentice (1988) concluded that *P. radicatum* subsp. *intermedium* (Nordh.) Knaben and *P. radicatum* subsp. *oeksendalense* Knaben were distinct, whereas *P. radicatum* subsp. *groevudalensis* Knaben and *P. radicatum* subsp. *gjaerevollii* Knaben were clustered quite closely with *P. radicatum* subsp. *ovatilobum* Tolm. from a neighbouring mountain area. When recombining these taxa in Oreomecon below, we therefore treat *P. radicatum* subsp. *ovatilobum*, *P. radicatum* subsp. *oeksendalense* and the new name of *P. radicatum* subsp. *intermedium* at the subspecies level. The former is the older of the three names from mountains further to the north in southern Norway and takes priority when *P. radicatum* subsp. *groevudalensis* and *P. radicatum* subsp. *gjaerevollii* are treated as synonyms, as done also by Nilsson (2001). Øvstedal and Grung (2015) concluded that the sixth southern Norwegian entity, *P. radicatum* subsp. *relictum* was morphometrically distinct from *P. radicatum* subsp. *oeksendalense* and deserved its position at the subspecies level, a conclusion followed here.

Concerning northern Scandinavia, Selin (1998) found *P. radicatum* subsp. *subglobosum* Nordh. to be morphologically distinct, whereas *P. radicatum* subsp. *hyperboreum* Nordh. and *P. radicatum* subsp. *macrostigma* (Nordh.) Nordh. were similar. The latter was originally described as *P. radicatum* subsp. *hyperboreum* var. *macrostigma* Nordh. by Nordhagen (1932). The local endemic *P. radicatum* subsp. *avkoënse* Knaben has not been studied morphometrically by the studies referred to above. Only *P. radicatum* subsp. *subglobosum* and *P. radicatum* subsp. *hyperboreum* are, therefore, recombined at the subspecies level below. Material from Iceland and the Faroe Islands has not been subject to morphometric analyses, except for unpublished data referred to by Selin (2000). Here, the taxon known as *P. radicatum* subsp. *stefanssonii* was related to south Scandinavian taxa in seed characters and to northern Scandinavian material in capsule morphology. It also deviates from all entities in its range of flower colours and is accepted here as a subspecies together with the nominate subspecies.

The conclusion below is that *Oreomecon radicata* is accepted with eight subspecies and four varieties from the Nordic area.

#### The identity of Papaver radicatum subsp. laestadianum Nordh.

The exception referred to above is *P. radicatum* subsp. *laestadianum* Nordh., a name used for a taxon limited to a small alpine area of Troms in north Norway and adjacent Sweden (Solstad et al. 2009). It shares the chromosome number (2n = 56) with *P. lapponicum*, contrasting 2n = 70 for *P. radicatum*. However, *P. radicatum* subsp. *laestadianum* was not integrated into the biosystematic study by Knaben (1959a; b) as material was not available for her extensive cul-

tivation experiments. Nannfeldt (1963) included it in *P. lapponicum* and Knaben (1983) concluded that it deserves status as a subspecies of *P. lapponicum*.

Tromsø Arctic-Alpine Botanic Garden holds material of P. radicatum subsp. laestadianum in cultivation from the locality Isdalsfjella/Njearrečazagáisi. This corresponds to the place name "Causigaisa" used in older maps and is within the area where the type material of this taxon was collected (Nordhagen 1932). Nordhagen reported it from two additional localities and provided illustrations of samples from two of them. However, he did not designate any type or mention any type of candidate and a lectotypification is therefore required. Amongst the syntypes at O and shown by Natural History Museum, University of Oslo (2023), there are annotations regarding two alternative typifications. One includes two sheets, O-V-2017486 and O-V-2017487, with at least five different individuals, as shown by the presence of tap roots, in addition to nine rosettes and 20 single leaves and references are given to illustrations from both sheets published by Nordhagen (1932, 1970). The annotations are undated and the handwriting is by T. Engelskjøn, although not formally documented. An alternative typification is presented by the annotation "Typus: individual marked 'NB' (by R. Nordhagen?) 30 Jun 1978, Gunvor Knaben". This refers to the specimen to the far left on the latter of these sheets, which is a well-defined individual, as shown by its tap root. None of these annotations fulfils the requirement of effective publication (Art. 7.10 in the Code; Turland et al. (2018)) and the taxon is, therefore, lectotypified below. The specimen with Knaben's annotation is selected as the lectotype. All additional specimens on six sheets in O are designated as isolectotypes.

Fig. 3 shows the capsule and leaf morphology of specimens from the comparative cultivation of four taxa in the Tromsø Arctic-Alpine Botanic Garden. Material from the lectotype locality of *Papaver radicatum* subsp. *laestadianum* shows clear affinity in capsule and leaf morphology with *P. lapponicum* subsp. *lapponicum* and appears very distinct from the two varieties of *P. radicatum* included. The black capsule hairs of our cultivated specimens are smooth in *P. radicatum* subsp. *laestadianum* and *P. lapponicum*, whereas they are decurrently dentate in both varieties of *P. radicatum*. It also differs in several minor characters from *P. lapponicum* subsp. *lapponicum*. We, therefore, interpret *P. radicatum* subsp. *laestadianum* as a subspecies of *P. lapponicum*, prior to its recombination in *Oreomecon* below. Its affinity to *P. lapponicum* is also supported by its chromosome number.

It should be added that a population of *P. lapponicum* subsp. *laestadianum* from the Mountain Márkos, which is situated ca. 15 km north of the type locality, was studied by Solstad et al. (2009) and by Nevermo (1997), both studies concluding on an affinity to *P. radicatum*. However, both Heggelund (1993), who presented its known distribution and Nevermo (1997) commented that plants on Márkos were morphologically heterogeneous. We, therefore, intend to bring samples from more populations of this taxon into comparative cultivation to test its possible heterogeneity pending future molecular studies.

Overall, northern non-Arctic Europe includes one species, distributed within Iceland, the Faroe Islands and the mountains of Scandinavia, in addition to one endemic subspecies of a different species (Fig. 2). The nominate subspecies of *Oreomecon Iapponica* is treated as taxon 3.6.6 here.
### Rare species and red-list treatments

Only the two fully-accepted subspecies of *P. radicatum* were treated by the Norwegian Red List (Artsdatabanken 2021), both as EN. These subspecies are subsp. *radicatum* and subsp. *laestadianum*. In Sweden, these two taxa are assessed as NT and VU, respectively (Eide et al. 2020). *Papaver radicatum* subsp. *stefanssonii* Knaben was treated as Vulnerable (VU) in Iceland (Wąsowicz and Heiđmarsson 2019) below its homonym *P. radicatum* subsp. *stefanssonii* (Á.Löve) Jonsell & Ö.Nilsson.

### Accepted taxa

# 3.7.1. Oreomecon lapponica subsp. laestadiana (Nordh.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350937-1

Papaver radicatum subsp. laestadianum Nordh. Bergens Mus. Årbog (Årbok) 1931 (2): 49. 1932. Type: Norway: Troms, Målselv, Rostadalen: Causigaisa nær grensen mot Moskovarre-Pältsa, på kalkholdig glimmerskifer, ca. 1100 moh., 28 Jul 1930, *R. Nordhagen* (lectotype: O, specimen marked "NB" to the far left on sheet O-V-2017487, lectotypification designated here, isolectotypes designated here: O [barcodes O-V-2017486; O-V-2014582; O-V-2014583; O-V-362047; O-V-362048, specimens other than the lectotype on O-V-2017487]) = Papaver laestadiananum (Nordh.) Nordh.; Bot. Not. 1939: 693. 1939 = Oreomecon laestadiana (Nordh.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:2. 2023.

# 3.7.2. Oreomecon radicata (Rottb.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 71. 2022

Papaver radicatum Rottb., Skr. Kiøbenhavnske Selsk. Laerd. Elsk. 10: 455. 1770. Type: III. in Rottbøll (1770), Tab. VIII, No. XXIV (lectotype designated by Knaben [1958], p. 62. Epitype: Iceland, Barðastrandarsýsla, Brjánslækur, 23 Jul 1962, Nannfeldt 17564, UPS [barcode UPS 207575], lower left specimen, designated by Nilsson & Elven in Jonsell [2001], p. 520) = Papaver nudicaule var. radicatum (Rottb.) DC., Syst. Nat. 2: 70. 1821 = Papaver nudicaule subsp. radicatum (Rottb.) Fedde, Beibl. Bot. Jahrb. Syst. 81: 34. 1909.

# 3.7.3. Oreomecon radicata subsp. radicata (Rottb.) Banfi, Bartolucci, J.-M. Tison & Galasso, Nat. Hist. Sci. 9(1): 71. 2022

Papaver radicatum Rottb., Skr. Kiøbenhavnske Selsk. Laerd. Elsk. 10: 455. 1770. Type: III. in Rottbøll (1770), Tab. VIII, No. XXIV (lectotype designated by Knaben [1958], p. 62. Epitype: Iceland, Barðastrandarsýsla, Brjánslækur, 23 Jul 1962, Nannfeldt 17564, UPS [barcode UPS 207575], lower left specimen, designated by Nilsson & Elven in Jonsell [2001], p. 520. = Papaver nordhagenianum subsp. islandicum Á.Löve, Nytt Mag. Bot. 4: 16. 1955. Type: Eyri in Ísafjørdur Islandiae occidentalis-septentrionalis, 1925, *I.* Óskarsson (holotype: ICEL) ≡ Papaver relictum subsp. faeroense var. islandicum (Á.Löve) Á.Löve, Taxon 19: 300. 1970 ≡ Papaver nordhagenianum subsp. faeroense var. islandicum (Á.Löve) Á.Löve, Taxon 11: 137. 1962, nom. illeg.

# 3.7.4. Oreomecon radicata var. avkoensis (Knaben) Elvebakk & Bjerke, comb. et stat. nov.

urn:lsid:ipni.org:names:77350938-1

- = Papaver radicatum subsp. avkoënse Knaben, Opera Bot. 2 (3): 39. 1959. Type: Plant grown from seed collected in Norway, Troms, Nordreisa, Avko in 1952 by O. Gjærevoll (holotype: O).
- Papaver nordhagenianum subsp. nordhagenianum var. avkoënse (Knaben) Á.Löve, Taxon 11: 136, nom. illeg.

## 3.7.5. Oreomecon radicata var. faeroensis (C.E.Lundstr.) Elvebakk & Bjerke, comb. et stat. nov.

urn:lsid:ipni.org:names:77350939-1

Papaver radicatum subsp. faeroënse C.E.Lundstr., Acta Horti Berg. 7: 412.
 1923. Type: Faroe Islands, Fugloy, Harz & Ostenfeld (lectotype: C, designated by Löve [1962a], p. 137) = Papaver nordhagenianum subsp. faeroënse (C.E.Lundstr.) Á.Löve, Nytt Mag. Bot. 4: 16. 1955 = Papaver relictum subsp. faeroense (C.E.Lundstr.) Á.Löve, Taxon 19: 300. 1970.

# 3.7.6. Oreomecon radicata subsp. hyperborea (Nordh.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350940-1

- Papaver radicatum subsp. hyperboreum Nordh., Bergens Mus. Årbok 1931, Naturv. r. 2; 48. 1932. Type: Norway, Troms, Målselv, Alappen, 30 Jul 1930, R. Nordhagen (holotype: O).
- Papaver nordhagenianum subsp. nordhagenianum Á.Löve, Nytt Mag. Bot. 4: 15. 1955. Type: Nissontjåkko, Lapponia tornensis Sueciae, *H. Smith* (holotype: UPS).

# 3.7.7. Oreomecon radicata subsp. knabeniana Elvebakk & Bjerke, comb., stat. et nom. nov.

urn:lsid:ipni.org:names:77350941-1

= Papaver radicatum subsp. dovrense var. intermedium Nordh., Bergens Mus. Årbok 1931, Naturv. r. 2; 43. 1932, nom. nud. Type: Norway, Oppland, Vågå, Besshøe, 1923, *R. Nordhagen* (holotype: BG) ≡ *Papaver radicatum* subsp. *intermedium* (Nordh.) Knaben, Opera Bot. 2(3): 34. 1959, nom. illeg. ≡ *Papaver nordhagenianum* subsp. *ovatilobum* var. *intermedium* (Nordh.) Á.Löve, Taxon 11: 136. 1962, nom. illeg.

**Diagnosis.** Differs from *O. radicata* subsp. *ovatiloba* in ovoid capsules which are wider near the top and not near the middle, capsules which are densely covered by pale brown, mostly appressed and not suberect hairs and leaf lobes often lanceolate to ovate and not ellipsoid to ovate.

**Etymology.** Oreomecon radicata subsp. knabeniana is named in honour of the substantial contributions to the knowledge of this genus made by Gunvor Snekvik Knaben (1911–1993), who was affiliated with the University of Oslo during most of her career.

## 3.7.8. Oreomecon radicata var. macrostigma (Nordh.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350942-1

 Papaver radicatum subsp. hyperborea var. macrostigma Nordh., Bergens Mus. Årbok 1931, Naturv. r. 2; 48. 1932. Type: Norway, Finnmark, Stjernøya, Hundneset, 14 Jul 1930, R. Nordhagen (lectotype: O, designated by Elven & Nilsson in Jonsell [2001], p. 521) = Papaver radicatum subsp. macrostigma (Nordh.) Nordh., Norsk flora: 225. 1940 = Papaver nordhagenianum var. macrostigma (Nordh.) Á.Löve, Nytt Mag. Bot. 4: 15. 1955.

# 3.7.9. Oreomecon radicata subsp. oeksendalensis (Nordh.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350943-1

- Papaver radicatum subsp. oeksendalense Knaben, Opera Bot. 2(3): 38. 1959. Type: Plant grown from seed collected in Norway, Møre & Romsdal, Sunndal, Øksendalen, Jønnstadnibba, 4 Aug 1948, R. Nordhagen (holotype: O); ≡ Papaver nordhagenianum subsp. ovatilobum var. oeksendalense (Knaben) Á.Löve, Taxon 11: 136. 1962, nom. illeg.
- = Papaver angusticarpum Nordh., Norsk Flora: 629. 1970, nom. nud.

# 3.7.10. Oreomecon radicata subsp. ovatiloba (Tolm.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350944-1

■ Papaver radicatum subsp. ovatilobum Tolm., Bot. Mater. Gerb. Glavn. Bot. Sada RSFSR 4: 85. 1923. Type: Norway, Sør-Trøndelag, Oppdal, Kongsvold, Aug 1889, G.H. Hagelin (lectotype: S, designated by Löve [1962a] p. 136) = Papaver nordhagenianum subsp. ovatilobum (Tolm.) Á.Löve, Nytt Mag. Bot. 4: 15. 1955.

- = Papaver radicatum subsp. gjaerevollii Knaben, Opera Bot. 2 (3): 38. 1959. Type: Plant grown from seed collected in Norway, Sør-Trøndelag, Trollheimen, Gjevilvasskammen by O. Gjærevoll (holotype O); = Papaver nordhagenianum subsp. ovatilobum var. gjaerevollii (Knaben) Á.Löve, Taxon 11: 136. 1962, nom. illeg.
- = Papaver radicatum subsp. groevudalense Knaben, Opera Bot. 2 (3): 38. 1959. Type: Plant grown from seed collected in Norway, Møre, Sunndalen, Grøvudalen by R. Nordhagen (holotype: O); = Papaver nordhagenianum subsp. ovatilobum var. groevudalense (Knaben) Á.Löve, Taxon 11: 136. 1962, nom. illeg.

## 3.7.11. Oreomecon radicata subsp. relicta (C.E.Lundstr.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350945-1

■ Papaver nudicaule subsp. relictum C.E.Lundstr., Acti Horti Berg. 7: 415. 1923. Type: Norway, Oppland, Vang, Vassendfjeld, 15 Aug 1870, Söderén & Eisen (lectotype: S, designated by Elven & Nilsson in Jonsell [2001], p. 521) = Papaver radicatum subsp. relictum (C.E.Lundstr.) Tolm., Svensk Bot. Tidskr. 21: 78. 1927 = Papaver relictum (C.E.Lundstr.) Nordh., Bergens Mus. Årbok 1931, Naturv. r. 2; 45. 1932; = Papaver nordhagenianum subsp. relictum (C.E.Lundstr.) Á.Löve, Taxon 11: 136. 1962, nom. illeg.

# 3.7.12. Oreomecon radicata subsp. stefanssoniana (Á.Löve) Elvebakk & Bjerke, comb. et stat. nov.

urn:lsid:ipni.org:names:77350946-1

- = Papaver stefanssonianum Á.Löve, Nytt Mag. Bot. 4: 14. 1955. Type: Iceland, Norðvestur-Ísland, Gufudalsháls, 8 Aug 1893, S.Stefánsson & Ó.Davíðsson (lectotype: ICEL, designated by Löve [1955] p. 14).
- Papaver radicatum f. albiflora Stefánsson, Fl. Islands: 100. 1901, nom. illeg;
  Papaver radicatum f. rubriflora Stefánsson, Fl. Islands: 100. 1901, nom. illeg;
  Papaver radicatum subsp. stefanssonii Á.Löve, Izlendsk. Jurt.: 149. 1945, nom. illeg.; Papaver radicatum subsp. stefanssonii (Á.Löve) Jonsell & Ö.Nilsson, in Jonsell (2001): 521, nom. illeg.; Papaver nordhagenianum subsp. stefanssonii Á.Löve, Taxon 11: 137. 1962, nom. illeg.; Papaver relictum subsp. faeroënsis var. stefanssonii (Á.Löve) Á.Löve, Taxon 19: 300. 1970, nom. illeg.

# 3.7.13. Oreomecon radicata var. steindorssoniana (Á.Löve) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350947-1

■ Papaver steindorssonianum Á.Löve, Nytt Mag. Bot. 4: 15. 1955. Type: Iceland, Austur-Ísland, Ós i Breiðdalur, Aug 1944, S.Steindórsson (holotype: AMNH) = Papaver relictum subsp. faeroënsis var. steindorssonianum (Á.Löve) Á.Löve, Taxon 19: 300. 1970 = *Papaver radicatum* subsp. *steindorssonianum* (Á.Löve) Knaben ex Ö. Nilsson, Nordic J. Bot. 20: 521. 2001.

Papaver nordhagenianum subsp. faeroënse var. steindorssonianum (Á.Löve)
 Á.Löve, Taxon 11: 137. 1962, nom illeg.

## 3.7.14. Oreomecon radicata subsp. subglobosa (Nordh.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350948-1

 Papaver radicatum subsp. subglobosum Nordh., Bergens Mus. Årbok 1931, Naturv. r. 2; 47. 1932. Type: Norway, Nordland, Meløy, Svartisen, Engabreen, 1 Aug 1930, R. Nordhagen (holotype: O) = Papaver nordhagenianum var. subglobosum (Nordh.) Á.Löve, Nytt Mag. Bot. 4: 16. 1955.



**Figure 3.** Specimens of Oreomecon Iapponica subsp. Iapponica, O. Iapponica subsp. Iaestadiana, O. radicata subsp. ovatiloba (1992-681) and O. radicata subsp. stefanssoniana (2001-76) under comparative cultivation in Tromsø Arctic-Alpine Botanic Garden (TAABG).

> **Notes.** The origin of the seeds for the cultivated samples shown in Fig. 3 are: *Oreomecon lapponica* subsp. *lapponica* - ex Norway, Troms, Kvænangen,

Raudfjellet, Aug 1988, A. Elvebakk, TAABG 1992-4053.

Oreomecon lapponica subsp. laestadiana - Norway, Troms, Målselv, N slope of Isdalsfjellet/Njearrečazagáisi facing Čorrováhgáisi, schistose scree, 1000 m alt., 23 Aug 2011, A. Granmo; L. Mølster, I.A. Mølster, TAABG 2014-248/TROM V-991064.

Oreomecon radicata subsp. ovatiloba - Botanic Garden of Tøyen, Oslo; TA-ABG 1992-681.

Oreomecon radicata subsp. stefanssoniana - undocumented commercial source; TAABG 2001-76.

#### 3.8. Central Europe

#### Notes

Kadereit (1990) monographed the *Papaver alpinum* L. complex, including eight subspecies with mostly non-overlapping distribution ranges. All these subspecies were later subject to an RAPD analysis, which produced five weakly-supported geographically-based clusters (Bittkau and Kadereit 2002). The correlation with subspecies was low. They also analysed four subspecies with respect to ITS1 sequences, which did not show any differentiation.

Schönswetter et al. (2009) studied 12 named entities by using DNA sequencing, AFLP fingerprinting and morphological traits. They concluded with a similar set of four weakly-supported geographically-based groups (Slovenia, Balkan, most of the Alps/Tatra, Central Italy) and two more strongly-supported groups from south-eastern France and the Pyrenees. They did not find any consistent morphological or molecular characters differentiating the taxa. They concluded that all the previously named variation was best treated within a single, widely defined species, Papaver alpinum, except for the Iberian entity, which was accepted as P. alpinum subsp. lapeyrousianum (Gutermann ex Greuter & Burdet) Kerguélen. They found most sampled population groups or populations to be genetically distinct and explained this as genetic drift within diploid and rapidly reproducing plants in often small populations. The concept of P. alpinum used by Schönswetter et al. (2009) was followed by Banfi et al. (2022) when the latter recombined P. alpinum and its subsp. lapeyrouseanum into Oreomecon, although Banfi et al. (2022) applied a different subspecies epithet for the Iberian entity.

The classification system by Schönswetter et al. (2009) has been followed by many treatments, such as Hassler (2023a, b), although the two latter studies made an exception for *Papaver tatricum* (A.Nyár.) Ehrend. ex Soó and *P. tatricum* subsp. *fatraemagnae* Bernát, which were accepted as separate entities. POWO (2023) accepted a widely defined *Oreomecon alpina*. However, they also made exceptions by maintaining four taxa in the complex as separate *Papaver* species. *Flora Gallica* (Tison and de Foucault 2014) also adopted *P. alpinum* in a broad sense. In contrast, other major floras from the area, for example, *Flora Helvetica* (Lauber et al. 2018) and *Flora d'Italia* (Pignatti et al. 2017), adopted a multi-species approach.

Fragnière et al. (2020) and Pittet et al. (2020) studied in detail *Papaver occidentale* (Markgr.) H.E.Hess et al., a white-flowered species from the western Alps. It appears similar to the other white-flowered species, *P. alpinum* s.str., *P. sendtneri* Kern. ex Hayek and *P. tatricum* (A.Nyár.) Ehrend. and might be characterised as the least distinct taxon, at least from its original description as *Papaver alpinum* subsp. *tatricum* var. *occidentale* Markgr. (Markgraf 1958a). Based on a detailed genetic study, Pittet et al. (2020) concluded that *P. occidentale* is a genetically and morphologically well-defined entity. It has apparently survived the Late Glacial Maximum both in periglacial areas and in nunatak situations. They underlined that further studies are needed to sort out the taxonomy of this complex.

The emerging pattern is that of an immigrating ancestral taxon into Central Europe, which had split into a western and a central group, with further differentiations leading to genetically distinct populations in numerous discrete areas (Schönswetter et al. 2009). As indicated by Schönswetter et al. (2009), the immigration and expansion situations are more likely to have taken place during a glacial period. In contrast, an interstadial represents a bottleneck situation favouring isolation and differentiation within this complex.

The studies by Bittkau and Kadereit (2002) and Schönswetter et al. (2009) indicate that some names do not correlate with the patterns in the molecular studies. This mismatch in the data by Bittkau and Kadereit (2002) would have been reduced if accepting *P. aurantiacum* Loisel. and *P. alpinum* subsp. *occidentale.* The southern populations of *P. alpinum* subsp. *kerneri* (Hayek) Fedde in Bosnia and Herzegovina and Montenegro may also represent separate entities, according to data from Schönswetter et al. (2009). With their present names, plants in central Italy do not match the molecular data shown in these two studies. Conversely, other names appear to be redundant, for example, *P. alpinum* subsp. *victoris* (Škornik & Wraber) Wraber from Slovenia, although the conclusion by Škornik and Wraber (1988) was based on a comparison with the neighbouring species. When treating this difficult complex within *Oreomecon*, we find that plants of the central group are better maintained at the subspecies level than left unnamed. The study by Schönswetter et al. (2009) is here used as the main source on nomenclature.

Markgraf (1958a) is the only study on the complex where type localities are indicated, although with incomplete information. The Bulgarian Papaver alpinum subsp. degenii (Urum. & Jáv.) Markgr. was cited with type specimen from "El Tepe, Pirin, 1915, Dimonie", whereas the locality which had been presented in the protologue by Urumov (1920) was "in graminosis aridis m. Pirin, legi 1915" without collector information. In a biography on Mihael Dimonie, Pachschwöll et al. (2019) presented his botanical activities, indicating that he instead only visited the Pirin Mountains in June, July and August 1909. The area was then within the Ottoman Empire and the peak of Mt. Vihren was then referred to as El Tepe or Jel-tepe. His only collections surviving two fires are those distributed commercially to several herbaria under the heading "Plantae Macedonicae". Three of his collections of this taxon from Vihren at WU, as documented by Virtual Herbaria JACQ (2024), have slightly different label texts from the one in the protologue by Urumov (1920). However, in the absence of any known collections from 1915, these are designated as lectotypes and isolectotypes below. The label texts are identical, except that the lectotype includes altitude information.

According to Schönswetter et al. (2009), *Papaver aurantiacum* Loisel. was described in Flora Gallica (Loiseleur-Deslongchamps 1807). However, it was described in a later supplement (Loiseleur-Deslongchamps 1809), where it was compared with the leaves and flowers of *Papaver alpinum* and where a type specimen was cited for having been collected at Mont Ventoux by M. Requien. We suppose the holotype specimen is at P, but we have not been able to trace it there or elsewhere.

Zapałowicz (1911) described *Papaver corona-sancta-stephani* Zapał. as common at 2000–2200 m alt. on the northwest slope of Mt. Ineu (= Vârful Ineu, Munții Rodnei) in the Romanian Carpathians and Paszko et al. (2020) indicated that the type is housed at KRAM. Zapałowicz (1911) described the type locality, but did not select a type. We have seen six vouchers of the typical form from KRAM. The article by Zapałowicz (1911) was published in October 1911

and a collection made in August 2011 by D. Herbich is referred to. Therefore, four vouchers from the period August 2010 to August 2011 are candidates for lectotypification. Below, we designate the specimen out of the five that presents the best-developed floral buds as the lectotype. Zapałowicz (1911) also described *Papaver corona-sancta-stephani* f. *hispidulum* Zapał. and *P. corona-sancta-stephani* var. *angustisectum* Zapał. from the same locality. The former is present as a single collection at KRAM and is interpreted by us as the holotype. The latter is lectotypified below, while both are listed as heterotypic synonyms of *P. corona-sancta-stephani* s.str.

Markgraf (1958a) is correct in stating that the name Papaver rhaeticum Leresche was first mentioned by Gremli (1874) in the second edition of his "Excursionsflora für die Schweiz". however, on p. 80 and not on p. 66, as repeatedly stated in basionym citations. Page 66 instead refers to the similar treatment in the sixth flora edition (Gremli 1889), as indicated by Fedde (1909). In both these floras, the name is presented as a nomen nudum and the treatment by Gremli (1889) is only 'like P. pyrenaicum, but only in Engadin'. Nyman (1889) recombined it as P. alpinum subsp. rhaeticum (Leresche) Nyman and Banfi et al. (2022) corrected its author citation to P. alpinum subsp. rhaeticum (Leresche ex Gremli) Nyman. The first valid description of the taxon was made by Markgraf (1958a: 311), who also cited its type specimen from Oberengadin in Switzerland. He did not cite its herbarium affiliation and we have not been able to trace it. Markgraf (1958a) cited his taxon as "Papaver alpinum subsp. rhaeticum (Ler.) Mkr.". The correct citation appears to be P. alpinum subsp. rhaeticum (Leresche ex Gremli) Nyman ex Markgr. for what is the basionym of a described taxon. However, the name presented by Markgraf (1958a) is illegitimate (Art. 6.4 and 58.1 in the Code, Turland et al. (2018)) and a new homotypic replacement name honouring F. Markgraf is, therefore, introduced below.

Nyárády (1942) described *Papaver alpinum* subsp. *tatricum* A.Nyár. from 'Tatri Magni' without adding a type. He described *P. alpinum* subsp. *tatricum* var. *angustisectum* A.Nyár. and *P. alpinum* subsp. *tatricum* var. *latisectum* A.Nyár. with illustrations of leaves from several collections from the Tatra Mountains. He also cited two collections of *P. alpinum* subsp. *tatricum* from Haute-Savoie in France; however, did not describe the nominate variety of this subspecies. Markgraf (1958a) described the plants from France as *P. alpinum* subsp. *tatricum* var. *occidentale* Markgr. with one of the samples mentioned by Nyárády (1942) as the holotype. He also provided a diagnosis of *P. alpinum* subsp. *tatricum* var. *tatricum* A.Nyár. and listed as type a collection from Hohe Tatra with Nyárády as collector. This is defined here as the lectotype of *P. alpinum* subsp. *tatricum*, but we have not been able to localise it. The type of *Papaver sendtneri* Kern. ex Hayek at B is possibly a holotype, but we have not been able to verify whether duplicates exist, requiring a lectotypification.

Krivenko (2023) recently recombined one of the entities of the *O. alpina* complex as *Oreomecon tatrica* (A.Nyár.) Krivenko, whereas Grey-Wilson (2023) introduced the name *Oreomecon corona-sancti-stephani* (Zapał.) Grey-Wilson. Both these authors selected single taxa from amongst those included within a broad concept of *O. alpina* as defined by Banfi et al. (2022), leaving the remaining ones embedded in *O. alpina* without explaining why these did not deserve similar treatment. As they did not cite any studies supporting these selected recombinations, the two names are not accepted below.

On the other hand, the western entities were well separated from the remaining samples in the analysis by Schönswetter et al. (2009) and the Iberian plants are treated as a separate species here. As shown by Banfi et al. (2022), the name *P. pyrenaicum* subsp. *suaveolens* P.Fourn. is heterotypic as compared with *P. suaveolens* Lapeyr. The latter is illegitimate since the earlier name *P. aurantiacum* Loisel. was cited as a synonym, which prevents the epithet "suaveolens" from being adopted at the species level for the basionym *P. pyrenaicum* subsp. *suaveolens* P.Fourn. Greuter (1981) introduced the name *Papaver lapeyrousianum* Gutermann ex Greuter & Burdet. Banfi et al. (2022) concluded that this name has priority at the species level, while Ferrer-Gallego (2024) recently recombined it into *Oreomecon*.

Kropf et al. (2006) studied the Iberian populations and concluded on vicariance in the development of populations both in the Sierra Nevada, the eastern Pyrenees and the western Alps, while long-distance dispersal, surprisingly from Sierra Nevada to Central Pyrenees, explains the latter populations. Relying on genetic structure and morphological dissimilarity, they concluded that the eastern Pyrenean populations are different and deserve a separate variety name, which had been introduced by Ascherson (1869). Both Bittkau and Kadereit (2002) and Schönswetter et al. (2009) confirm that the Iberian populations are heterogeneous. Thus, we follow Kropf et al. (2006) and conclude that the Sierra Nevada and Central Pyrenean populations represent one distinct subspecies within *Papaver Iapeyrouseanum*, namely subsp. *Iapeyrouseanum*, which is different from the east Pyrenean *P. Iapeyrouseanum* subsp. *endressii* (Asch.) Greuter & Burdet.

The AFLP analysis by Schönswetter et al. (2009) showed that both the Iberian material and samples corresponding to *P. aurantiacum* are genetically very distinct from the remaining parts of the complex. We also recombine the latter taxon at the species level below to reflect this pattern.

#### Distribution

The present list of accepted taxa includes three species with 11 additional subspecies distributed within the area presented in Fig. 2.

#### Rare populations and red-listed taxa

Fragnière et al. (2020) conclude that *P. occidentale* is doomed to extinction in the wild due to rapid global warming. Their hypothesis can probably be extended to other taxa of the group. *Papaver aurantiacum, P. occidentale* and *P. sendtneri* are Red-listed as near threatened (NT) in Switzerland (Bornand et al. 2016) and *P. alpinum* s.lat. is threatened in Germany (Metzing et al. 2018). From Sierra Nevada, Blanca et al. (2002) presented the orange-flowered *P. lapeyrousianum* as forming a single population of less than 2,500 individuals known from four very small subareas between 3,200 and 3,450 m a.sl., near the peak of Mulhacén. The Sierra Nevada population is obviously in danger, as opposed to the larger populations in the Pyrenees, which are considered to be of least concern (LC).

Papaver degenii is a local endemic of the Mountain Pirin in Bulgaria, where it is rare and occurs between 1,915 and 2,850 m alt.. It has been treated at the species level and as vulnerable (VU) both by Stoeva (2009) and by later online versions of the Bulgarian Red Data Book (Stoeva 2023), where a distribution map shows three population centres, with single minor occurrences, each consisting of 20–60 individuals over areas of only a few m<sup>2</sup>. The species is protected and listed as a glacial relict. Gorgorov et al. (2011) showed that the taxon had reduced sexual reproduction capacity and they tried *in vitro* propagation as an additional effort in *ex-situ* conservation. In Romania, the distribution and ecology of *P. alpinum* subsp. *corona-sancti-stephani* (Zapał.) Borza was presented by Bartók et al. (2016) and its Red-list status in Romania was cited as "rare", referring to Oltean et al. (1994). The rarest taxon in Central Europe is probably *P. tatricum* subsp. *fatraemagnae* Bernát., a taxon with deviating flowers with wedge-shaped petals known from limestone slopes at only 890 m altitude, at a locality in Slovakia, where it is isolated from the distribution area of *P. tatricum* s.str. (Bernátóvá 2002).

### Accepted taxa

## 3.8.1. Oreomecon alpina (L.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 69. 2021

= Papaver alpinum L., Sp. Pl. 507. 1753. Type: [Austria] Niederösterreich, Mount Schneeberg (lectotype: UPS [Herb. Burser IX:58], lectotype designated by Markgraf [1965], p. 145).

## 3.8.2. Oreomecon alpina subsp. alpina (L.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 69. 2021

Papaver alpinum L., Sp. Pl. 507. 1753. Type: [Austria] Niederösterreich, Mount Schneeberg (lectotype: UPS [Herb. Burser IX:58], lectotype designated by Markgraf [1965], p. 145).

# 3.8.3. Oreomecon alpina subsp. corona-sancti-stephani (Zapał.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350949-1

- Papaver corona-sancti-stephani Zapał., Bull. Int. Acad. Sci. Cracovie, Cl. Sci. Math., Sér. B, Sci. Nat. 1911(8B): 620. 1911. Type: [Romania: Sub culmine montis] Ineu [(2280 m) Alpium Rodnensium in valle voraginosa versus septentrionalem occidentem sita, solo mico schistoso 2000-2200 m alt.], 18 Aug 1911, H. Zapałowicz (lectotype: KRAM [barcode KRAM00026854]; isolectotype KRAM [barcode KRAM00026853], designated here) = Papaver alpinum subsp. corona-sancti-stephani (Zapał.) Markgr., Phyton (Horn) 7: 306. 1958 = Papaver pyrenaicum subsp. corona-sancti-stephani (Zapał.) Borza in Bul. Grăd. Bot. Univ. Cluj 8: 114. 1928 = Oreomecon corona-sancti-stephani (Zapał.) Grey-Wilson, Pl. Rev. 5(4): 57. 2023.
- Papaver corona-sancti-stephani f. hispidulum Zapał., Bull. Int. Acad. Sci. Cracovie, Cl. Sci. Math., Sér. B, Sci. Nat. 1911(8B): 621. 1911. Type:: [Romania: Sub culmine montis] Ineu [ (2280 m alt.) Alpium Rodnensium in valle

voraginosa versus septentrionalem occidentem sita, solo mico schistoso 2000–2200 m alt.], 18 Aug 1911, *H. Zapałowicz* (holotype: KRAM [barcode KRAM00026857]).

= Papaver corona-sancti-stephani var. angustiscum Zapał., Bull. Int. Acad. Sci. Cracovie, Cl. Sci. Math., Sér. B, Sci. Nat. 1911(8B): 621. 1911. Type:: [Romania: Sub culmine montis] Ineu [ (2280 m alt.) Alpium Rodnensium in valle voraginosa versus septentrionalem occidentem sita, solo mico schistoso 2000–2200 m alt.], 3 Aug 1910, S. Fedorowicz (lectotype: KRAM [barcode KRAM00026858], designated here).

# 3.8.4. Oreomecon alpina subsp. degenii (Urum. & Jáv.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350950-1

 Papaver pyrenaicum subsp. degenii Urum. & Jáv., Magyar Bot. Lapok 18: 33.
 1920. Type: [Bulgaria, Blagoevgrad oblast] In rupestribus alpinus mt. Jel-tepe Perin dag finis turco-bulgare, 2560 m alt., Jun 1909, *M. Dimonie* (lectotype: WU [barcode WU 0105034]; isolectotypes WU [barcodes WU 0105032; WU 01050333], all designated here) = Papaver degenii (Urum. & Jáv.) Kuzmanov, Fl. Reipubl. Popularis Bulg. 4: 282. 1970 = Papaver alpinum subsp. degenii (Urum. & Jáv.) Markgr., Phyton (Horn) 7: 312. 1958.

# 3.8.5. Oreomecon alpina subsp. ernesti-mayeri (Markgr.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350951-1

= Papaver alpinum subsp. ernesti-mayeri Markgr., Phyton (Horn) 7: 312. 1958. Type: [Slovenia] Julische Alpen, Triglav, Staničeva Koča, 1956, F. Markgraf (holotype not found) = Papaver ernesti-mayeri (Markgr.) Wraber; Proteus (Ljubljana) 44: 238 (1982).

# 3.8.6. Oreomecon alpina subsp. fatramagnae (Bernát.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350952-1

Papaver tatricum subsp. fatraemagnae Bernát., Fl. Slovenska 5(4): 765. 2002.
 Type: [Slovakia], Vel'ká Fatra (Fatra Magna) in calcareis declivitatum septentrionali-occidentalis montis Ostrá supra l.d. Konský dol incola], ca. 890 m alt., 8 Aug 1999, D. Bernátová (holotype: BBZ).

**3.8.7.** Oreomecon alpina subsp. kerneri (Hayek) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350953-1

■ Papaver kerneri Hayek, Österr. Bot. Z. 53: 170. 1903. Type: [Slovenia] Steiermark, Sanntaler Alpen, bei den Korošicahütte, 1800 m alt., 18 Jul 1900, A. von Hayek s.n. (lectotype GB [barcode GB-004 8359], lectotypified by Markgraf [1958a], p.  $41 \equiv Papaver alpinum$  subsp. kerneri (Hayek) Fedde, Engl. Pflanzenreich IV. 104(40): 375. 1909.

## 3.8.8. Oreomecon alpina subsp. markgrafiana Elvebakk & Bjerke, nom. et comb. nov.

urn:lsid:ipni.org:names:77350954-1

 Papaver alpinum subsp. rhaeticum (Leresche ex Gremli) Nyman ex Markgr., Phyton (Horn) 7: 311. 1958. Type: Switzerland, Oberengadin (not found), nom. illeg. = Papaver rhaeticum Leresche ex Gremli, Gremli, Excursionsfl. Schweiz: 80. 1874, nom. nud. = Papaver alpinum subsp. rhaeticum (Leresche) Nyman, Consp. Fl. Eur. Suppl. 2: 16. 1889, nom. nud. = Papaver pyrenaicum subsp. rhaeticum (Leresche) Fedde, in H.G.A. Engler (ed.), Pflanzenr., IV, 104: 372. 1909, nom. nud.

**Etymology.** Oreomecon alpina subsp. markgrafiana is named in honour of the very large contributions to the knowledge of the *O. alpina* group made by the German botanist Friedrich Markgraf (1897–1987).

# 3.8.9. Oreomecon alpina subsp. occidentalis (Markgr.) Elvebakk & Bjerke, comb. et stat. nov.

urn:lsid:ipni.org:names:77350955-1

Papaver alpinum subsp. tatricum var. occidentale Markgr., Phyton (Horn) 7: 313. 1958. Type: [France] Hochsavoyen, Vergy, I. Dörfler (holotype: B [Dörfler Herb. Norm. 5209] = Papaver occidentale (Markgr.) H.E.Hess & Landolt, Fl. Schweiz Gebiete 3: 778. 1973.

# 3.8.10. *Oreomecon alpina* subsp. *sendtneri* (Kern. ex Hayek) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350956-1

 Papaver sendtneri Kern. ex Hayek; Österr. Bot. Z. 53: 406. 1903. Type: Austria, Tirol, Hafelekar bei Innsbruck, Kerner s.n. (holotype?: B [barcode B\_10\_0294933]) = Papaver alpinum subsp. sendtneri (Kern. ex Hayek) Schinz & Keller; Fl. Schweiz, ed. 3, 1: 223. 1909.

## 3.8.11. Oreomecon alpina subsp. tatrica (A.Nyár.) Elvebakk & Bjerke, nomencl. nov.

urn:lsid:ipni.org:names:77350957-1

Papaver alpinum subsp. tatricum A.Nyár., Acta Geobot. Hung. 5: 19. 1942.
 Type: Karpaten, Hohe Tatra, A. Nyárády (lectotype: not found, designated by

Markgraf [1958a: 313]) = Papaver tatricum (A.Nyár.) Ehrend., Oesterr. Bot. Z. 122: 268. 1973 = Oreomecon tatrica (A.Nyár.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023.

# 3.8.12. Oreomecon alpina subsp. victoris (Škornik & Wraber) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77350958-1

 Papaver victoris Škornik & Wraber, Biol. Vestn. 36(3): 82. 1988. Type: Slovenija, Julijske Alpe, in glareosis declitivitatis septentrionalis montis Matajurski vrh, inter montes Črna prst et Rodica, solo calcareo, 1900 m alt., 20 Jul 1981, *M.Krajit & A.Podobnik 9749/3* (holotype: LJU, no. 109984) = Papaver alpinum subsp. victoris (Škornik & Wraber) Wraber, Hladnikia 10: 42. 1998.

### **3.8.13**. Oreomecon aurantiaca (Loisel.) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350959-1

Papaver aurantiacum Loisel., J. Bot. (Desvaux) 2: 340. 1809. Type: [France] Mont Ventoux, M. Requien (holotype: not found) = Papaver alpinum var. aurantiacum (Loisel.) Markgr. Phyton (Horn) 7: 311. 1958 = Papaver pyrenaicum var. aurantiacum (Loisel.) Dalla Torre, Alpenfl. 173. 1882 = Papaver pyrenaicum subsp. aurantiacum (Loisel.) Fedde, Beibl. Bot. Jahrb. Syst. 81: 38. 1905.

# 3.8.14. *Oreomecon lapeyrouseana* (Gutermann ex Greuter & Burdet) P.P. Ferrer, Taxon 73: 919. 2024

- Papaver lapeyrouseanum Gutermann ex Greuter & Burdet, Willdenowia 11: 43.
   1981. Type: France, Lapeyrouse s.n. (lectotype: B [barcode B\_10\_0294931], lectotypified by Greuter [1981], p. 43) = Papaver alpinum subsp. lapeyrousianum (Gutermann ex Greuter & Burdet) Kerguélen, Index Synonym. Fl. France (Coll. Patrim. Nat., 8): xv. 1993.
- Papaver lapeyrouseanum Gutermann, Österr. Bot. Z. 122: 268. 1973, nom. illeg.
- Papaver alpinum subsp. lapeyrousianum (Gutermann) Kadereit, Bot. Jahrb., 112(1): 84. 1990, nom. illeg.
- *Papaver pyrenaicum* subsp. *suaveolens* P.Fourn., Quatre FI. France 4: 372. 1936. Type: [France] Lin. sommets elevés, fentes des rochers. Mail du Crystal, Cambredases, Pic de Midy, Erezlidtz, Houle Marboré, *Lapeyrouse s.n* [before 1813] (lectotype, TLM, bottom-right individual, lectotype designated by Banfi et al. [2022], p. 69, illustrated by Banfi et al. [2022], p. 70) ≡ Oreomecon alpina subsp. *suaveolens* (P.Fourn.) Banfi, Bartolucci, J.-M.Tison & Galasso, Nat. Hist. Sci. 9(1): 69. 2022 ≡ Papaver alpinum subsp. *suaveolens* (P.Fourn.) Rändel, Feddes Repert. 84 (9–10): 173. 1974 ≡ Papaver alpinum subsp. *suaveolens* 0.Bolòs & Vigo, Bull. Inst. Catalana Hist. Nat., Secc. Bot. 38(1): 73. 1974, isonym ≡ Oreomecon suaveolens (P.Fourn.) Krivenko, Nov. Syst. Pl. Vasc. 54: e06:3. 2023.
- Papaver suaveolens Lapeyr., Hist. Pl. Pyrenées Suppl. 72. 1818, nom. illeg.

## 3.8.15. Oreomecon lapeyrouseana subsp. lapeyrouseana (Gutermann ex Greuter & Burdet) P.P.Ferrer, Taxon 73: 919. 2024

Papaver lapeyrouseanum Gutermann ex Greuter & Burdet, Willdenowia 11: 43.
 1981. Type: France, Lapeyrouse s.n. (lectotype: B [barcode B\_10\_0294931], lectotypified by Greuter [1981], p. 43).

## 3.8.16. Oreomecon lapeyrouseana subsp. endressii (Asch.) Elvebakk & Bjerke, comb. nov.

urn:lsid:ipni.org:names:77351065-1

Papaver suaveolens var. endressii Asch., Bot. Zeitung (Berlin) 27: col. 127. 1869. Type: [France] Ceuillade de Nourri, 8400 ft., Aug 1829, Unio itineraria, P.A.C. Endress (lectotype: W; isolectotype: JE [barcode JE00018778], designated here) = Papaver lapeyrouseanum subsp. endressii (Asch.) Greuter & Burdet, Willdenowia 11: 43. 1981 = Papaver alpinum var. endressii (Asch.)
 O. Bolòs & Vigo, Butl. Inst. Catalana Hist. Nat., Secc. Bot. 38(1): 73. 1974 = Papaver lapeyrouseanum var. endressii (Asch.) Rivas Mart., Itinera Geobot., 15: 705. 2002.

#### 4. Meconopsis Vig., Hist. Nat. Pavots: 20. 1814, nom. cons.

Type species (conserved): Meconopsis regia G.Taylor.

**Notes.** Grey-Wilson (2014) treated 76 species, 20 subspecies and three named hybrids. Since then, new species and revised concepts of existing taxa have been published, most of them within the monocarpic sections *Racemosae* and *Forrestianae*.

Overall, the genus *Meconopsis* currently includes 95 species and 21 subspecies. As shown in Fig. 2, its distribution largely follows the distribution map by Grey-Wilson (2014), but with a small extension into Xinjiang (China) in the western-most part following the distribution map of *Meconopsis aculeata* Royle in the same source. The westernmost species are all rare, with *M. aculeata* Royle as critically endangered in Pakistan (Majid et al. 2015), *M. latifolia* Prain as a local endemic and *M. neglecta* G.Taylor only known from its type collection (Jafri and Qaiser 2011).

## 4.1. *Meconopsis* sect. *Racemosae* C.Y.Wu & H.Chuang, Acta Bot. Yunnan. 2(4): 374. 1980

#### Type species. Meconopsis racemosa Maxim.

**Notes.** In their phylogenetic study, Xiao and Simpson (2015) concluded that species in the strongly bristly section *Racemosae* do not merit classification within the two series proposed by Grey-Wilson (2014), but show a complicated and partly reticulate evolutionary pattern. Therefore, Xiao and Simpson (2015) proposed that these species should be treated as the "*M. horridula* Hook. f. & Thomson species complex" pending further phylogeographic studies. This involves the following additional taxa not accepted at the species level by Grey-Wilson (2014): *M. calciphila* Kingdon-Ward, *M. castanea* H. Ohba, Tosh. Yoshida & H. Sun, *M. pseudohorrida* C.Y.Wu & H.Chuang and *M. rigidiuscula* Kingdon-Ward. Two more species were later described from Bhutan/adjacent India by Yoshida et al. (2016a, b) and one from Sichuan/Yunnan by Yoshida and Sun (2019b). The latter study did not accept all species included in the *M. horridula* species complex by Xiao and Simpson (2015). We conclude that the section comprises 18 species, significantly increasing from the 11 species monographed by Grey-Wilson (2014).

## 4.2. *Meconopsis* sect. *Forrestianae* C.Y.Wu & H.Chuang, Acta Bot. Yunnan. 2 (4) 375. 1980

#### Type species. Meconopsis forrestii Prain.

**Notes.** *Meconopsis lancifolia* subsp. *lepida* (Prain) Grey-Wilson was raised to the species level by Yoshida and Sun (2017). Yoshida and Sun (2018) described four new species in the *Forrestianae* section and another four were described by Yoshida and Sun (2019a). They also positioned the species *M. pleurogyna* W.T.Wang (Wang 2019) here, reduced *M. sinomaculata* Grey-Wilson and *M. xiangchenensis* R.Li & Z.L.Dao to variety level and described two more subspecies of *M. lancifolia* (Franch.) Franch. ex Prain. The monograph by Yoshida and Sun (2019a) concluded that the section includes 15 species.

#### 4.3. Meconopsis sect. Impeditae Grey-Wilson, Gen. Meconopsis: 46. 2014

#### Type species. Meconopsis impedita Prain.

**Notes.** Section *Impeditae* Grey-Wilson (Grey-Wilson 2014) partly overlaps with section *Forrestianae* as monographed later by Yoshida and Sun (2019a) and referred to above. Two new species of *Impeditae*, *M. angustipetala* W.T.Wang and *M. brachynema* W.T.Wang, were described from China (Wang 2019).

#### 4.4. Meconopsis sect. Grandes (Prain.) Fedde, Engl., Pflanzenr. 4, 104: 262. 1909

#### Type species. Meconopsis grandis Prain.

**Notes.** Grey-Wilson (2014) included four conspicuously yellow species in Section *Grandes* (Prain) Fedde. Subsequently, two additional species were added to the section; one is the new species, *M. wanbaensis* Tosh. Yoshida (Yoshida 2019), the other is the recombined *M. uniflora* (C.Y.Wu & H.Chuang) Tosh. Yoshida et al. (Yoshida et al. 2019). Whereas almost all *Meconopsis* species described after the publication of the monograph by Grey-Wilson (2014) are monocarpic and, in the case of the *Forrestianae* and *Impeditae*, rather small plants, one large and blue perennial poppy has also been described. This is *M. gakyidiana* Tosh. Yoshida et al., the famous blue poppy of Bhutan, which is also the country's national flower (Yoshida et al. 2016b). It has remained in cultivation since George Forrest's introduction in the 1930s, partly under various cultivar names (Grey-Wilson 2017), partly as *M. grandis* subsp. *orientalis* Grey-Wilson, until the latter was raised to species level under the name referring to Bhutan's "gakyid" concept of national happiness.

#### 5. Afropapaver (Elkan) Elvebakk & Bjerke, nom. et stat. nov.

urn:lsid:ipni.org:names:77350960-1

= Papaver sect. Horrida Elkan, Tent. Monogr. Papav. 32. 1839. Type species: Papaver aculeatum Thunb. [= Afropapaver aculeatum (Thunb.) Elvebakk & Bjerke].

**5.1.** *Afropapaver aculeatum* (Thunb.) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350961-1

- = Papaver aculeatum Thunb., Prodr. Pl. Cap. 2: 92. 1800. Type: e Cap. b. Spei ("eastern Cape of Good Hope"), C.P. Thunberg, UPS-THUNB (V-106276).
- = Papaver horridum DC., Syst. Nat. 2: 79. 1821. Type: Hab. in Nova-Hollandia, Caley (holotype: BM).
- = Papaver gariepinum Burch. ex DC., Trav. S. Afr. 1: 318. 1822. Type: Africa extratropica ad ripas fluminis Gariep seu Orange-River, *Burchell 1633* (holotype: K).

**Notes.** This is a new, monospecific genus. The basionym name "horrida" is an adjective in the plural. There are examples of adjectives used as nouns in the names of genera. However, according to the recommendations in the Code (20A.1.[f, g]; Turland et al. (2018)), one should avoid adjectives as nouns and one must not use the epithet or derived form of the epithet of one of the species of the genus in question. The present synonym, *Papaver horridum*, is based on anthropogenically induced material from Australia (Kadereit 1988c) and would have priority in case Australian material is lifted to the species level by future studies. Therefore, a replacement name is a preferred alternative. *Papaver sect. Horrida* was monographed by Kadereit (1988c), including synonyms and types.

**Etymology.** *Afropapaver* refers to its relationship to *Papaver* and its strongly isolated occurrence in southernmost Africa (Kadereit 1988c).

**Distribution.** Its distribution, as shown here in Fig. 4, is based on Kadereit (1988c), who concluded that the species is an early human introduction to Australia and is also synanthropic in Namibia.

#### 6. Stylomecon G.Taylor, J. Bot. 68: 140. 1930

Type species. Stylomecon heterophylla (Benth.) G.Taylor.

**Notes.** *Stylomecon heterophylla* (Benth.) G.Taylor and the species known as *Papaver californicum* A.Gray are endemic to California and adjacent parts of Mexico, where they are grossly disjunct as they are the only representatives of the mostly Eurasian clade of Papavereae in America. Samples from this group of species diverged from the remaining samples of *Papaver* and *Parameconopsis* at ca. 19–20 Ma according to the phylogenies by Valtueña et al. (2012) and Xie et al. (2014) and a similar phylogenetic position was shown by Liu et al. (2014). Catania et al. (2022) concluded that *Papaver californicum* was the earliest branching species from a common ancestor in the *Papaver* lineage, which had a gene fusion event basal for the further synthesis of the morphinan group of alkaloids, a divergence dated at 16.8 Ma.



**Figure 4**. World distribution of the genera *Afropapaver* (green), *Papaver* (red), *Parameconopsis* (grey) and *Stylomecon* (violet). See Table 1 for species number per genus.

According to these phylogenies, these two species definitively should be congeneric. Kadereit and Baldwin (2011) dealt with their morphology, ecology and distribution in detail and showed differences in flower and capsule morphology. They concluded that the style in *S. heterophylla* probably evolved independently from other lineages and a structure similar to the stigmatic disc of *P. californicum* and they treated both species within a broad definition of *Papaver*.

Given that these two Californian-Mexican species have an old evolutionary history, a similar phylogeny and distribution and a diverging style which probably evolved relatively recently within its lineage, the clade is best treated as a separate genus. The name *Stylomecon* is available and a new combination is needed for the species known as *P. californicum*.

Stylomecon heterophylla was briefly described as Meconopsis heterophylla Benth. by Bentham (1835), who also described Meconopsis crassifolia Benth. Both type specimens shown by JSTOR Global Plants (2023) carry the label information "Nova California, 1833, Douglas'. According to Bentham (1835), David Douglas travelled from present-day Oregon to then Mexican California and carried out botany studies in the surroundings of Monterey during the years 1831 and 1832. Then he travelled to what is now named Hawaii and dispatched his plants by ship to England before he returned to Oregon. The year of the label should, therefore, refer to the year of the plants' arrival in England and not the year of collecting. According to Brentham (1835), cultivation attempts failed and the plants preserved are, therefore, those collected by Douglas. Although classified as isotypes by JSTOR Global Plants (2023), below, we list these as holotypes in the absence of known duplicates.

Steudel (1841) included both *Meconopsis heterophylla* and *Meconopsis crassifolia* within a widely defined genus *Stylophorum* Nutt., including two from

California, two others from northern America, one from Europe and two from Nepal. In a flora of the San Francisco area, Greene (1894) accepted both names as species of *Papaver* together with *P. californicum* and *P. lemmonii* Greene. Much later, Kadereit (1988a) and Kadereit and Baldwin (2011) accepted only *Papaver californicum* and *Stylomecon heterophylla* and did not present interpretations of the name *Meconopsis crassifolia*. Grey-Wilson (2014), however, considered the latter to be a synonym of the very different *Stylophorum diphyllum* (Michx.) Nutt. distributed on the opposite side of the North American continent, an interpretation followed by POWO (2023).

Kadereit and Baldwin (2011) explained and illustrated the leaves of *Stylome-con heterophylla* to be very different from those of *Papaver californicum*, the latter being strikingly heterophyllous. The holotypes of *Meconopsis heterophylla* and *M. crassifolia* illustrated by JSTOR Global Plants (2023) differ in the same way and also match the diagnoses by Bentham (1835). We conclude that *Meconopsis crassifolia* and *Papaver californicum* are synonyms and that the former holds priority.

### Accepted taxa

## **6.1.** *Stylomecon crassifolia* (Benth.) Elvebakk & Bjerke, comb. nov. urn:lsid:ipni.org:names:77350962-1

- *■ Meconopsis crassifolia* Benth., Trans. Hort. Soc. London, Ser. 2, 1: 408. 1835: Type: USA, Nova California,1833, *D. Douglas s.n.* (holotype: BM) *■ Stylophorum crassifolium* (Benth.) Steud., Nomencl. Bot. ed. 2, 2: 650. 1841 *■ Papaver crassifolium* (Benth.) Greene, Man. Bot. San Francisco: 9. 1894 *■ Papaver heterophyllum* var. *crassifolium* (Benth.) Jeps., Fl. W. California: 209. 1901.
- = Papaver californicum A.Gray; Proc. Ameri. Acad. Arts 22: 323. 1887. Type: USA: California, Santa Inez Mountains, J. Spence s.n. (holotype: GH), syn. nov.
- Papaver lemmonii Greene, Pittonia 1: 168. Type: USA: California, San Luis Obispo County, 1887, J.G. Lemmon s.n. (holotype: NDG).

#### 6.2. Stylomecon heterophylla (Bentham) G. Taylor, J. Bot. 68: 140. 1930

■ Meconopsis heterophylla Benth., Trans. Hort. Soc. London, Ser. 2, 1: 408. 1835: Type: USA, Nova California,1833, D. Douglas s.n. (holotype: BM) = Stylophorum heterophyllum (Benth.) Steud. Nomencl. Bot. red. 2, 2: 650. 1841 = Papaver heterophyllum (Benth.) Greene, Pittonia 1: 168. 1888.

#### 7. Papaver L., Sp. Pl. 1: 506. 1753

#### Type species. Papaver somniferum L.

**Notes.** The sections of *Papaver* s.str. have been thoroughly dealt with in a series of monographs; see summary below. The sections have also been compared, for example, by Kadereit (1988b) and phylogenetically explored, for example, by Carolan et al. (2006).

As circumscribed and reviewed here, the genus *Papaver* includes 59 species and 14 subspecies and most of the changes, compared with the cited

reference studies, have taken place in the large section *Rhoeadium* Spach. The distribution of the genus is shown in Fig. 4, where anthropogenic occurrences are excluded. The genus ranges from the endemic species *P. gorgoneum* on the Cape Verde Islands (Kadereit and Lobin 1990) through Central Europe and the Mediterranean area. Latitudinally, it occurs from Central European Russia and the western Tian Shan southwards into the Arabian Peninsula. Eastwards, the genus reaches as far east in Central Asia as Kyrgyzstan with the species *P. laevigatum* M.Bieb. (Sennikov and Tojibaev 2021) and *P. macrostomum* extends eastwards to Assam along the foothills of the Himalayas (Fig. 4).

### 7.1. Papaver L. sect. Papaver, Sp. Pl. 1: 506. 1753

#### Type species. Papaver somniferum L.

Notes. This section includes four species and one subspecies, according to the monograph by Kadereit (1986b). However, the section was not monophyletic, according to Carolan et al. (2006). Based on seed morphology, Jesus et al. (2023) recently showed archaeological evidence of the cultivation of Papaver somniferum in Europe dating back about seven millennia. During the first millennia, seeds indicating the wild and weedy morphotype were mostly treated as P. somniferum subsp. setigerum (DC.) Arcang., were dominant and, after a transition period, the cultivated morphotype became dominant about 3000 years ago. This selection towards larger seeds appears from these data to be primarily a result of human domestication and not one of vicariant evolution. The latter is a major criterion applied for the subspecies category by POWO (2023). However, Hong et al. (2022) convincingly proved that the wild type is genetically clearly distinct and in a sister group position to P. somniferum, coupled with the morphological difference shown by Kadereit (1986b). As indicated by Liu et al. (2020), it is unlikely that the tetraploid wild type is the progenitor of the diploid P. somniferum. We, therefore, interpret the wild-growing taxon as the separate species P. setigerum DC.

## 7.2. *Papaver* sect. *Carinatae* Fedde, Engler (ed.) Pflanzenr. 40 (4; 104): 334. 1909

**Type species.** *Papaver macrostomum* Boiss. & Huet, Boiss. Fl. Orient. 1: 115. 1867. **Notes.** Includes a single species, *P. macrostomum* Boiss. & A.Huet, with a dehiscing capsule disc, which is an exclusive character according to Kadereit (1987), who only included four varieties. POWO (2023) also accepted *P. halophilum* (Fedde) Cullen and *P. piptostigma* Bien. ex Fedde. However, they were treated as synonyms of *P. macrostomum* by Kadereit (1987) and Tavakkoli and Assadi (2016).

#### 7.3. Papaver sect. Macrantha Elkan, Tent. Monogr. Papaver 19: 1839

Type species. Papaver orientale L., Sp. pl. 508. 1753.

**Notes.** This section includes the famous and perennial species *Papaver orientale* L. and two more species according to most studies, including the recent monographic treatments by Lack (2019a, b), who argued convincingly why the name *P. pseudo-orientale* (Fedde) Medw. should be conserved. Solomon et al. (2014) accepted *P. lasiothrix* Fedde as an additional species. However, this had been rejected in a study on Iranian species, which treated it as a synonym of *P. bracteatum* Lindl. (Tavakkoli and Assadi 2013), a conclusion also reached by Lack (2019a) and POWO (2023). The new species *Papaver yilderimlii* Ertekin was described from a single site in the province of Siirt in Türkiye as similar to *P. lasiothrix*, except for smaller and globose capsules (Yıldırımlı and Ertekin 2008). *Papaver yilderimlii* was accepted by POWO (2023), but not treated by Lack (2019 a, b) nor in other recent studies dealing with this complex. It is treated here as a synonym of *P. bracteatum*. The species concept in the section was also confirmed by a study on morphology, phylogeny and chemistry in Türkiye (Parmaksız and Özcan 2011). In contrast, another Turkish phylogenetic study which applied ISSR markers (Gürkök et al. 2013) did not clearly sort out the given sample names over the major clades.

### 7.4. Papaver sect. Rhoeadium Spach, Hist. Nat. Veg. Phan. 7: 16. 1839

**Type species.** *Papaver segetale* Schimp. & Spenn., Fl. Friburg. 3: 1829 [= *Papaver rhoeas* L.].

**Notes.** This section was monographed by Kadereit (1989), who accepted 16 species and five subspecies, whereas the Cape Verdean *P. gorgoneum* Coutinho with one additional subspecies was added by Kadereit and Lobin (1990). The following changes indicated by POWO (2023) and Hassler (2023b) are accepted here: *Papaver guerlekense* Stapf is reduced to synonymy of *P. rhoeas* L., *P. stylatum* Boiss. is reduced to synonymy of *P. umbonatum* Boiss. and three subspecies of *P. dubium* L. are raised to species level as *P. glabrum* Royle, *P. lecoqii* Lamotte and *P. laevigatum* M. Bieb., respectively.

The following taxa were not accepted by Kadereit (1989) or were described subsequently. *Papaver postii* Fedde, treated as a synonym of *P. rhoeas* L. by Kadereit (1989), was accepted as a deviating perennial member of *Rhoeadium* by Cullen (1965) and later also accepted by POWO (2023). In a study from Cyprus, Aghababyan et al. (2011) compared *P. postii* Fedde with the new species *P. paphium* M.V.Agab. et al. and *P. cyprium* (Chrtek & B.Slavik) M.V.Agab. et al., the latter being a taxon originally described as a subspecies of *P. rhoeas*. These taxa are all accepted by POWO (2023) and Hassler (2023b) and are also accepted here.

Some rare species were dealt with in a study on the Red-listed species of Caucasus in a broad sense by a joint effort of botanists from Türkiye, Georgia, Armenia, Azerbaijan, Russia and Iran (Solomon et al. 2014). The *Papaver* species agreed on by these authors are, with one exception, accepted here. Their treatment includes three endemic species from Armenia described by Aghababyan and Fragman-Sapir (2007), two within this section. *Papaver roseolum* M.V.Agab. & Fragman was listed as endangered and *P. gabrielianae* M.V.Agab. as vulnerable (Solomon et al. 2014). *Papaver schelkovnikovii* N.Busch from Azerbaijan was listed as endangered by Solomon et al. (2014) and included within the top 50 national conservation priorities of this country. It is known from two localities and cited as endemic, although POWO (2023) cited it to have a wider distribution. In addition, Aghababyan (2013) described *P. gorovanicum* M.V.Agab. as a local endemic from sandy soils near the village of Gorovan in the Ararat Region of Armenia. Papaver albiflorum (Elkan) Pacz., previously included as a variety within *P. dubium* by Kadereit (1989), was accepted by POWO (2023), who treated *P. paczoskii* Mikheev as a synonym. *Papaver confine* Jord., from the same complex in Europe, was also accepted by POWO (2023) and by Hassler (2023b). Mikheev (1993), who reviewed the Papaveraceae flora of the Russian Caucasus, reduced his *P. alberti* Mikheev to a synonym of *P. stevenianum* Mikheev, which later was treated as *P. dubium* subsp. *stevenianum* (Mikheev) Kubát & Šipošová, an alternative followed here. Solomon et al. (2014, as *P. alberti*) treated the taxon as Nearly Threatened (NT). Mikheev (1999) described the new species *P. maschukense* Mikheev from the foot of the Maschuk Mountain in the Russian part of Caucasus.

Tavakkoli and Assadi (2016) monographed *Papaver* in Iran, but did not mention the Iranian species *P. pasquieri* Dubuis & Faurel, which is accepted by POWO (2023) and Hassler (2023b). Tavakkoli and Assadi (2016) also maintained *P. bipinnatum* C.A.Mey. as a synonym of *P. arenarium* M. Bieb. and treated *P. lacerum* as a synonym of *P. commutatum* Fisch., C.A.Mey. & Trautv. instead of *P. laevigatum*. Dar et al. (2010) described two new local species from near Srinagar in north-westernmost India and *P. kachroianum* Tabinda, Dar & Naqshi was only described from its holotype. Its etymology was explained as commemorating the botanist P. Kachroo and the orthography of the epithet is corrected to *"kachrooianum"* here according to the Code, Art. 60.8; Turland et al. (2018). *Papaver pamporicum* Tabinda, Dar & Naqshi was described from cultivated specimens originating from saffron fields in Pampore in the same area. *Papaver stewartianum* Jafri & Qaiser was described from Pakistan, based on the type specimen from grain fields at Campbellpore. It was described as a possible hybrid, but has been maintained by Jafri and Qaiser (2011) and is accepted by POWO (2023) and Hassler (2023b).

Papaver maireii Batt. and P. malviflorum from North Africa, both previously included within P. dubium, were accepted by POWO (2023) and Hassler (2023b), the former referring to flora treatments. The conclusion is that section *Rhoediana* includes 34 species and three subspecies.

#### 7.5. Papaver sect. Meconidium Spach, Hist. Nat. Veg. Phan. 7: 21. 1839

**Type species.** *Papaver armeniacum* (L.) DC. Prodr. 2: 79. 1821. [≡ *Argemone armeniaca* L., Sp. pl.: 509. 1753].

**Notes.** Kadereit (1993), in his monograph for the section of orange-flowered, biennial species with valvate capsules, accepted four species in addition to five subspecies. In addition, Tavakkoli (2012) and POWO (2023) accepted *Papaver acrochaetum* Bornm. ex Fedde. The species *P. sjunicicum* M.V.Agab. was described by Aghababyan & Fragman-Sapir (2007) from altitudes above 3200 m in the Zangezura area of Armenia. It was listed as a data-deficient species from Armenia in the Red List treatment for the widely-defined Caucasus Region (Solomon et al. 2014). POWO (2023) considers this species a synonym of *P. armeniacum* subsp. *armeniacum* (L.) DC, which is a taxon with several other synonyms that are still widely in use, for example, *P. caucasicum* M. Bieb., *P. fugax* Poir. and *P. triniifolium* Boiss. Aghababyan & Fragman-Sapir (2007) treated *Papaver sjunicicum* as different from *P. zangezuricum* Mikheev, another species from 3500 m alt. in the same area of Armenia, see Mikheev (1993). The latter species is accepted by POWO (2023) and both are accepted here. Papaver shepardii Post ex Dinsm. from southern Türkiye near the border of NW Syria was considered a Critically Endangered (CR) species by Cullen (1965). It is probably identical to *P. persicum* ssp. *tauricola* (Boiss.) Kadereit. *Papaver shepardii* was not mentioned by Kadereit (1993). It was considered unresolved by Aghababyan (2011b) and is not accepted here, although it was so by POWO (2023). Our summary thus shows that this section is comprised of seven species and five subspecies.

## 7.6. *Papaver* sect. *Pilosa* Prantl, Engler & Prantl, Nat. Pflanzenfam. 3, 2: 142. 1889

Type species. Sibt. & Smith, Fl. Graeca Prodr. 1: 360. 1973.

**Notes.** This section consists of a single species, *Papaver pilosum* Sm., with four subspecies, all distributed in western parts of Türkiye (Kadereit 1996).

#### 7.7. Papaver sect. Pseudopilosa M.Popov ex Günther, Flora 164: 436. 1975

**Type species.** *Papaver rupifragum* Boiss. & Reut., Pugill. Pl. Afr. Bot. Hispan.: 6. 1852.

**Notes.** This section was monographed as including three species with two additional subspecies by Kadereit (1996), showing a wide disjunction between the species pair *P. atlanticum* (Ball) Coss. and *P. rupifragum* Boiss & Reut. in Morocco and southern Spain and *P. lateritium* K. Koch in east Türkiye and Transcaucasus. In a more recent monograph, Aghababyan (2009) also accepted *P. oreophilum* Rupr., regarded as an endemic of central parts of the main Caucasus mountain chain. He also accepted *P. monanthum* Trautv. as an endemic distributed from east Türkiye through the Trans-Caucasian mountains from southern Georgia to northern Armenia, whereas *P. lateritium* was considered a local endemic of east Türkiye.

Furthermore, Aghababyan (2009) treated *P. lisae* N.Busch, a local endemic of the Russian Republic of Kabardino-Balkaria on the northern side of the central main Caucasus. Popov (1937) considered this species problematic and could not easily assign it to any section. The same conclusion was reached by Kadereit et al. (1997), who cited that it had been proposed that a separate section be formed by an unpublished study. Aghababyan (2009), on the other hand, considered it to be surprisingly similar to the Moroccan species *P. atlanticum*. It was mapped and treated as Endangered (EN) by Solomon et al. (2014). Grey-Wilson (2023) recently transferred *P. lisae* to *Oreomecon*. However, we follow Aghababyan (2009) and accept it as a species within section *Pseudopilosa*.

Papaver talyshense Grossh. was considered to be a dubious species by Aghababyan (2009), but was mapped from a single locality in Azerbaijan and regarded as endangered by Solomon et al. (2014). They listed it amongst the country's top 50 national conservation priorities. The species is accepted by Mikheev (1993), POWO (2023) and Hassler (2023b). Overall, we conclude that section *Pseudopilosae* includes eight species and two subspecies.

#### 8. Parameconopsis Grey-Wilson, Gen. Meconopsis: 367. 2014

Type species. Parameconopsis cambrica (L.) Grey-Wilson.

**Notes.** This monospecific genus includes *Parameconopsis cambrica* (L.) Grey-Wilson, see treatment by Grey-Wilson (2014). The distribution map in Fig. 4 is based on Valtueña et al. (2012).

### 8.1. Parameconopsis cambrica (L.) Grey-Wilson, Gen. Meconopsis: 367. 2014

 = Papaver cambricum L., Sp. Pl.: 508. 1753. Type: In Pyrenaeis. Herb. Burser IX: 45 (lectotype: UPS-BURSER, designated by Ferrer-Gallego [2015], p. 208) = Meconopsis cambrica (L.) Vig., Hist. Nat. Pavots: 48. 1814.

#### Discussion

The present treatment of Papavereae is based on a review of literature on the taxonomy of what Carolan et al. (2006) referred to as the Old-World clade of Papaveroideae, including the genera *Meconopsis, Papaver, Roemeria* and *Stylomecon* and comprising approximately 130 species of the two former, three of *Roemeria* and one of *Stylomecon*. Based on a set of defined reference studies and discussed deviations from these, the present study accepts eight genera, a total of 246 species and 61 subspecies, many evidently in need of further studies. Three of the genera are large and the number of accepted species and subspecies is 95 + 21 for *Meconopsis*, 68 + 29 for *Oreomecon* and 59 + 14 for *Papaver*, the latter surprisingly being only the third largest genus of the group.

#### Generic concepts and phylogenies within Papaveraceae

The arrangements of the presently defined genera were quite similar in the large, mainly ITS-based phylogenies by Carolan et al. (2006), Kadereit et al. (2011), Valtueña et al. (2012), Liu et al. (2014) and Xie et al. (2014). Three of the studies also included chronologies and they indicated that the Eurasian group within Papavereae, including the eight genera accepted in the present study, diverged from the American clade during the early Tertiary, at ca. 52 Ma according to a calibration with the oldest Papaveraceae fossil by Kadereit et al. (2011). This divergence time was recently estimated to be 81.5 Ma (Peng et al. 2023). Four of the studies above showed *Cathcartia* to have the earliest divergence, a pattern confirmed by Peng et al. (2023). Kadereit et al. (2011) dated this divergence at ca. 38 Ma followed by a split at ca. 28 Ma of the clade including *Roemeria*, *Meconopsis* and *Oreomecon* vs. a clade including further divergences, first of *Afropapaver*, then *Stylomecon* and finally a split-off of *Parameconopsis* and *Papaver* at 12.7 (6.6–19.0) Ma, see Fig. 1.

As compared with Kadereit et al. (2011), Valtueña et al. (2012) added more samples of *Parameconopsis cambrica*, but their phylogeny is very similar, except for the oldest dichotomies where *Cathcartia* was lacking. Xie et al. (2014) estimated the divergence of *Meconopsis* and *Oreomecon* at 16.8 Ma vs. ca. 23.5 Ma by Kadereit et al. (2011). Xie et al. (2014) only included a single sample of the presently defined genus *Roemeria*. However, *Meconopsis* was their focal point compared to *Parameconopsis* by Kadereit et al. (2011) and Valtueña et al. (2012).

Afropapaver and Stylomecon are both represented by only one or two sequences each in the cited phylogenies on this group and further phylogenetic studies with more markers are evidently needed, cf. the exploration of new markers by Liu et al. (2020). However, their patterns clearly indicate two early diverging genera. The type material of the *Afropapaver* synonym *Papaver horridum* DC. (POWO 2023) was collected by George Caley in Australia in 1803, just after the very onset of British colonisation. However, we follow Kadereit (1988b) in accepting *Afropapaver aculeatum* as a remarkably early human introduction into Australia. In addition to its isolated distribution, the monotypic genus *Afropapaver* has several distinct characters, including a spiny indumentum, yellow filaments and anthers, 2n = 11 and a racemose inflorescence (Kadereit 1988b). His description does not explicitly include the dehiscence structure, but it is illustrated as short-valvate. Thus, the combination of genetics, morphology, karyology and distribution supports the status of *Afropapaver* as a separate genus, which evolved most likely after a long-distance dispersal event, possibly > 20 Ma ago.

Thompson (2005) also cited several other examples of generic pairs between the Cape and the Mediterranean floras thought to have ancient origins, modified here as *Moraea* Mill. with neighbouring genera vs. *Iris* Tourn. ex L. (Goldblatt et al. 2002), *Lobostemon* Lehm. vs. *Echium* Tourn. ex L. (Hilger and Bohle 2000) and *Passerina* L. (Bredenkamp and van Wyk 2006) vs. *Daphne* Tourn. ex L. Cowling and Holmes (1992) concluded from a study in the Cape Region that regional or local endemics over-represented in several genera had a particular biological profile. They were primarily non-sprouting dwarf shrubs with ant-dispersal of seeds over short distances in combination with soilstored seed banks or they had microsymbiont-mediated nutrient uptake. None of these traits is valid for the monotypic genus *Afropapaver*.

The two species in *Stylomecon* have been taxonomically challenging as their capsules have such contrasting morphology and *S. californica* was positioned in the monotypic section *Papaver* sect. *Californica* Kadereit by Kadereit (1988a). The section name has been corrected to plural here according to the Code (Art. 21.2; Turland et al. (2018)). The first study treating these as sister species based on molecular data was the one by Kadereit et al. (1997), later confirmed by Carolan et al. (2006) and Kadereit et al. (2011) and supported by a study on morphology and ecology by Kadereit and Baldwin (2011).

Catania et al. (2022) indicated, based on a whole-genome analysis, that the gene fusing event basal for the synthesis of morphine-types of alkaloids had a monophyletic origin in *Stylomecon californica* and it was dated at 16.8–24.1 Ma. It could have evolved in the *Stylomecon* lineage or its ancestor, but posterior to the divergence dated at 24 Ma of a branch including *Roemeria* and *Oreomecon*, where this gene fusion had not occurred. The most likely hypothesis is that it has taken place in the *Stylomecon* ancestor lineage prior to the establishment of the taxon in California/Mexico. Otherwise, one would need to postulate a re-migration into Eurasia, allowing for later diversification of this chemosyndrome. The enigmatic distribution of *Stylomecon* could be explained by an extreme long-distance dispersal event from western Eurasia, where its closest relatives occur, including the likely source area of *Afropapaver*, as indicated by Kadereit (1988b). Kadereit and Baldwin (2011) stated that there is no fossil evidence for a postulated wide distribution of the ancestral lineage in North America, which would have reduced the migration distance needed from western Eurasia. A third hy-

pothesis is a less dramatic dispersal from East Asia to California. However, this would require postulating an extensive lineage extinction in eastern and central Eurasia. Thus, the early members of this group only survived as *Afropapaver* and *Stylomecon* and not in Eurasia, where only later divergences were present.

Parameconopsis and Papaver s.str. also diverged early and the former survived the Quarternary as a Tertiary relict species in several disjunct areas in western Europe, as shown convincingly by Valtueña et al. (2012). It is also a sister group to Papaver s.str. in the phylogenies by Carolan et al. (2006), Liu et al. (2014) and Xie et al. (2014). This leaves Papaver and Parameconopsis as the most recently evolved sister group among these genera. Parameconopsis also contains yellow flower pigments known as nudicaulins. They are absent from Papaver, but different nudicaulins are present in intensely yellow or orange flowers of Oreomecon (Tatsis et al. 2013).

In the phylogram by Kadereit et al. (2011), where *Papaver* as defined in the present study is denoted as "*Papaver* s.str.", the earliest divergence amongst its numerous sections is section *Pseudopilosa*, based on analyses of the Moroccan/Spanish species pair *P. atlanticum* and *P. rupifragum*. Together with the present distribution of *Parameconopsis*, this could indicate that *Papaver* arose in the western part of the Mediterranean. Section *Pseudopilosa* has a remarkable disjunction between Morocco and Türkiye (Kadereit 1996). The remaining sections of *Papaver* all have their diversity centres in the latter country and adjacent areas and an evolutionary origin here has been a prevailing view (Kadereit 1998).

Styles are very short or obsolete in the oldest genus, *Cathcartia* (Grey-Wilson 2014) and have a different ontogeny and evolutionary history in *Parameconopsis* as compared to *Meconopsis* (Kadereit and Erbar 2011). Carolan et al. (2006) consider valvate capsules to be the most ancient character in Eurasian *Papaverae*. The valvate capsules of *Cathcartia* can be interpreted as a synapomorphy being maintained within all dichotomies involving the evolution of the genera *Meconopsis*, *Oreomecon, Afropapaver, Stylomecon* and *Parameconopsis*, but lost in *Stylomecon heterophyllum*. Alternatively, the capsule dehiscence in the latter can be interpreted as short-valved from the illustrations in Kadereit and Baldwin (2011). Outside of these six genera within Old-World *Papaverae*, valvate capsules occur only in *Papaver* sect. *Meconidium* (Kadereit 1993). As indicated by Carolan et al. (2006), this section represents the most recent divergence within *Papaver* and its acquisition of valvate capsules evidently occurred as a separate evolutionary event.

Poricidal capsule dehiscence occurs in *Roemeria* and *Papaver* and probably represents separate evolutionary events. As opposed to the long-valvate genera *Cathcartia*, *Meconopsis* and *Parameconopsis* from mesic habitats, the genera above occur in open, arid areas, sharing the evolutionary advantages of poricidal capsule dehiscence for seed dispersal in wind-exposed and open habitats. The seeds would be retained within the capsules during calm periods and dispersed predominantly during windy episodes when they travel further.

During the late Miocene, global cooling was coupled with increasing aridity and ecosystem changes (Herbert et al. 2016). Pound et al. (2011) showed that, during the Tortonian Miocene, 11.6–7.25 Ma, warm-temperate, wet forests covered central and southern parts of Europe, except for southern and western parts of the Iberian Peninsula. Türkiye, by contrast, had broadleaved temperate savannahs with large extensions further east. The success of the genus *Papaver*, as opposed to its three small and geographically isolated neighbouring genera, might be its adaptations to the expanding arid ecosystems of the east Mediterranean. However, its evolutionary origin could hypothetically have been further west.

*Oreomecon* is also of particular interest regarding capsule morphology. As shown by Kadereit (1988a), their capsules have valvate dehiscence. However, they are so short-valved that they share the evolutionary adaptations to open areas with the genera with true poricidal capsules. The scapose pedicels of *Oreomecon* species, rigid when capsules are mature, also add to an adaptation to harsh Arctic and Alpine habitats. Seeds will be dispersed over time and during windy episodes since the valvate openings are small. Growing on exposed sites, the scapes often protrude through a thin snow cover and are adapted to efficient long-distance dispersal also on the snow. A study from Svalbard by Alsos et al. (2007) showed that plant colonisation to these High Arctic islands had predominantly occurred across the sea-ice from the west or the east. This probably explains the broad distributions of only a few High-Arctic taxa of *Oreomecon*, such as *O. dahliana* subsp. *polaris* and *O. cornwallisensis*, over the northernmost land areas from Canada to Arctic Europe.

The clade combining *Meconopsis* and *Oreomecon* evolved as a response to the dramatic uplift of the Himalayan and the neighbouring Hengduan Mountain ranges and the eastern Tibetan Plateau at 25–20 Ma (Xie et al. 2014). *Meconopsis* adapted to monsoonal climates initiated at ca. 20 Ma, although the major clades within the genus had a rapid early divergence involving polyploidy. Some also invaded drier plateau habitats to the north (Xie et al. 2014) and section *Aculeatae* is concentrated in the drier areas and is centred in northernmost Pakistan and adjacent India (Grey-Wilson 2014). Wen et al. (2014) showed how the uplift of the Qinghai-Tibetan Plateau has worked as a driver of evolution, instigating spectacular radiations and species diversification also in numerous other genera, for example, *Pedicularis* L., *Primula* L., *Rhodiola* L., *Rhododendron* L., *Saxifraga* L. and *Saussurea* DC.

At about 20 Ma, the global climate was about 4 °C warmer than at present and alpine climates probably occurred only at altitudes between 5,000 and 6,500 m. However, the global climate cooled at ca. 12 Ma (Herbert et al. 2016), facilitating the evolution of *Oreomecon* (Xie et al. 2014). *Papaver* and *Meconopsis* are classified within systems of several sections, most well-defined in morphology and genetics. This is not the case with *Oreomecon*, which shares a median crown age of 16.6 Ma with *Meconopsis*, according to Xie et al. (2014). According to Kadereit et al. (2011), the diversification of extant species of *Oreomecon* started much later, at ca. 5 Ma. This may indicate that early clade representatives became extinct, whereas those surviving were well adapted to the extreme cooling during the Pleistocene.

The distribution maps of *Meconopsis* and *Oreomecon* in Fig. 2 show a rather narrowly overlapping zone between them. Concerning *Oreomecon*, Fig. 2 shows that it has its concentration of species in Asia, with 24 species in central parts, ten taxa in the Asian Far East and 20 taxa in Arctic Asia. It extends into Arctic Alaska and Yukon and adjacent Cordilleras with nine taxa, with another five species further to the south in the North American Cordillera. However, in the vast area ranging from central Arctic Canada throughout Greenland, including the entire Arctic Europe, only six species and subspecies occur. The Nordic *O. radicata* complex is apparently the most studied part of the genus, although different classification alternatives have been proposed. Here, we conclude on eight subspecies and four varieties, based on morphometric studies, which are

pending future molecular studies. In Central Europe, where the taxonomy of this genus is also controversial, we propose to accept three species and recognise the remaining entities preliminarily at the subspecies level.

Rändel (1974) argued that *Oreomecon* had its evolutionary centre in Central Asia, as indicated by the concentration of diploids there. Later migrations northwards and longitudinally involved a high degree of polyploidy and hybridisation. Exceptions are the American *O. walpolei* and *O. pygmaea* and the European *O. alpina* s.lat. as they are diploids outside the postulated area of origin. Kadereit (1988a) found this explanation hypothetical, although the much later phylogenetic conclusions that *Oreomecon* and *Meconopsis* are sister genera support the views of Rändel (1974).

Applying molecular analyses, Solstad et al. (2009) confirmed that *O. radicata* is, indeed restricted to the previously heavily glaciated Nordic area. Early Scandinavian authors like Nordhagen (1932) used the fragmented distribution pattern of *Oreomecon* taxa as evidence of glacial survival in the Nordic countries. In contrast, later authors (i.e. Nordal (1987)) argued in favour of post-glacial immigration. Westergaard et al. (2018), however, presented evidence for Weichselian survival in the case of *Carex scirpoidea* Michx., a species widely distributed in northern North America, but absent from Europe, except for a few sites in north Norway. Here, it grows in low-alpine habitats, which starkly contrasts *Oreomecon* at higher altitudes in the high-alpine zone. Hence, *Oreomecon* species therefore appear to be better adapted to glacial survival.

Oreomecon radicata probably originated from neighbouring taxa within a continuous adjacent distribution and the species forms an evolutionary group with *O. lapponica* subsp. *jugorica* as its closest taxon (Solstad et al. 2009). The relationship between *O. radicata* and the *O. dahliana* group is more distant. This group is represented in the amphi-Beringian area by a still undescribed species referred to as "Papaver aff. dahlianum" by Solstad et al. (2009). Their conclusion from molecular studies is that these species do not have connections to any known diploid ancestral species.

Our recombination of *O. lapponica* subsp. *laestadiana* is based on comparative cultivations in the rock garden exposed to the cool climate of Tromsø. This taxon was evaluated by Nordhagen (1939) to be the most exclusively high-alpine representative of the genus in Norway and very difficult to cultivate in the lowlands of south Norway, as opposed to *O. radicata*.

Oreomecon lapponica specimens are highly modified by their contrasting habitats. This is evident from herbarium specimens at TROM, partly collected by the first author from High Arctic Canada (subsp. occidentalis) and east Greenland (probably subsp. lapponica). Those from higher latitudes and altitudes are compact, in contrast to the very elongated specimens, for example, from 68°N in west Greenland (subsp. occidentalis) and only 60°N in the Ural Mts. (ssp. jugorica). The same contrast is shown for herbarium specimens of *O. lapponica* subsp. lapponica from lowland riverbanks in north Norway vs. high-alpine populations of *O. lapponica* subsp. laestadiana. Population samples can be more reliably identified and compared with other taxa by comparative cultivation, an approach strongly recommended for Papaver (Knaben 1959b; 1979) and other vascular plants (e.g. Emig and Kadereit (1993)).

The Central European complex of Oreomecon most likely originated from an extreme long-distance dispersal event. According to Popov (1937), "P. nivale is

readily distinguished from all other Soviet representatives of the section Scapiflora and is related to the European P. alpinum". This was confirmed by Rändel (1974), who only found differences in capsule hair colour. The Oreomecon alpina complex is genetically not closely related to any analysed Asian species (Solstad et al. 2009) and is also confirmed by our summary of existing ITSbased phylogenies (Suppl. material 1). No recent studies have addressed the relationship between O. alpina and O. nivalis; the latter is a species belonging to the O. pulvinata complex, according to Petrovsky (1999). The possibility of a Weichselian long-distance dispersal event cannot be excluded; compare the case of the local endemic Oxytropis deflexa subsp. norvegica Nordh., known from populations in two screes in continental northern Norway. The ancestor of Oxytropis deflexa subsp. norvegica migrated postglacially across a then vast, open and gravelly periglacial landscape of present-day Russia, then becoming extinct there, leaving a large, present-day distribution gap to the nearest occurrences of Oxytropis deflexa subsp. deflexa (Pall.) DC. in Kazakhstan (Elvebakk 1984; POWO 2023).

However, *Oreomecon nivalis* is tetraploid (Solstad et al. 2009) and the closest relative of *O. alpina* has still not been identified (Kadereit et al. 2008). The *O. alpina* complex has a distribution pattern strikingly similar to that of *Primula* sect. *Auricula* Duby (Zhang et al. 2004; Aymerich et al. 2014). The latter group comprises 26 species, which are reasonably well differentiated morphologically and genetically (Zhang et al. 2004). This suggests a considerably older origin than that of the *O. alpina* complex. Zhang et al. (2004) estimated the diversification of the former from an Asian ancestor to have taken place at 3.59 Ma.

The treatment followed here, with the formal recognition of both subspecies and varieties of *O. radicata* in northern Europe, parallels the treatment of the *Androsace vitaliana* (L.) Lapeyr. complex, previously the genus *Vitaliana* Sesl., by Dixon et al. (2016). Except for being absent from east European mountains, this group has a similar distribution pattern as the *Oreomecon alpina* complex. Dixon et al. (2016) accepted ten taxa, all with allopatric distributions. Only three of the six subspecies were considered to be morphologically distinct. The Pyrenean subspecies appears to have evolved through gene flow from entities further south in the Iberian Peninsula, where four geographically distinct varieties within one subspecies are maintained despite unconfirmed morphological and genetic characteristics.

Our chosen alternative of accepting three western taxa in addition to a complex of *O. alpina* further east was proposed already by Ascherson (1869). Claiming to follow the then-new Darwinian theory, he put forward the hypothesis that a northern Asian member of the circumpolar *P. nudicaule* s.lat. complex had dispersed to Central Europe. Here, it first evolved at the species level as the taxon now referred to as *P. aurantiacum*, with a distinct morphology, as cited from its type locality at Mt. Ventoux. Further evolution produced another distinct species in western Europe, referred to as *P. suaveolens*, which in the east Pyrenees diverged into another taxon, *P. suaveolens* var. *endressii* Asch., distinguished by its dissected leaves. In the central and eastern parts of the Alps, various distinct forms with intermediates referred to as the *P. alpinum* complex had not yet reached differentiation corresponding to species level.

Burbrink et al. (2022) proposed to abandon the use of the subspecies category in taxonomy, as the major criterion of reproductive isolation used in their field of vertebrate zoology is often difficult to adapt. Phylogeny should, therefore, decide whether populations represent a species or whether to be left untreated by formal taxonomy. In botany, many taxa have not been studied or have been insufficiently studied by phylogeny and Molinari (2023) proposed morphology as a decisive additional criterion, whereas Wood et al. (2015) and POWO (2023) emphasised vicariant evolution. These criteria have been used to define the numerous subspecies accepted by the present study. This is particularly the case for *Oreomecon* subspecies from areas with a dramatic history of glaciation, where time has been insufficient for a taxon to develop into a fully-defined species.

Solstad et al. (2009) concluded that "Papaver alpinum seems to have been isolated in Europe for a fairly long time". This is supported by the many geographically defined lineages which are genetically distinct. Many such named lineages are now threatened and Red-listed, primarily because of the effects of global warming and conservation issues are becoming imminent in many countries. Further studies need to address the patterns of genetic vs. morphological variation, including improved genetic markers and also comparative cultivation. However, we believe that maintaining a traditional, although imperfect, concept of several *Oreomecon alpina* subspecies is to be preferred over the alternative of leaving them unnamed.

### Conclusions

We propose that the evolution of the Old-World group of Papavereae is best reflected within a system of eight genera. The sister genera Meconopsis and Oreomecon are the two most speciose genera. Their evolution was instigated by the dramatic uplift of the Qinghai-Tibetan Plateau, where Meconopsis is concentrated in the southern monsoon-influenced parts. Oreomecon probably evolved in the northern rain-shadow area and eight of the Central Asian species treated by Peschkova (1994) were characterised by being from steppe or steppe-like habitats. From Central Asia, the genus radiated to high mountains and Arctic areas on a circumpolar scale (Rändel 1974), shown in Fig. 2 as a map and with numbers of accepted taxa for eight geographical areas provided in Table 1. The genus Oreomecon was recently described as a genus replacing Papaver sect. Meconella (Banfi et al. 2022) and more recent recombinations have resulted in 82 species names proposed for the new genus. Here, we argue in favour of accepting subspecies status for many taxa according to traditional treatments by Papaver experts. Oreomecon, which comprises many incompletely understood taxa, is reduced to 68 species here, with 38 Oreomecon names newly introduced, most of them as recombinations, whereas 21 existing Oreomecon names are reduced to synonymy.

The sections *Horrida* and *Californica* of *Papaver* are here treated as the genera *Afropapaver* and *Stylomecon*, respectively. We accept the generic status *Parameconopsis* for the species now most commonly treated as *Papaver cambricum* and define *Papaver* s.str. as the sister group of *Parameconopsis* after the latest major divergence in the group. The distributions of the genera are illustrated here (Figs 2, 4), with indications of the number of accepted species and subspecies based on key reference studies supplied by a review of subsequent literature.

### Acknowledgements

The authors acknowledge assistance from Eirik A. Finne, the Norwegian Institute for Nature Research, for retrieving a free map base from *R* and from Magne Rundberg and colleagues at the University Library of UiT The Arctic University of Norway for retrieving less accessible literature. We also appreciate that Alfred Granmo (1944–2024), previously at the Arctic University Museum of Norway, provided seeds from the type locality of *Oreomecon lapponica* subsp. *laestadiana* and appreciate valuable comments on the manuscript by Curtis R. Björk, University of British Columbia Herbarium, Vancouver, Canada. Dr. Agnieszka Nikel, W. Szafer Institute of Botany, Kraków, Poland, kindly provided information on vouchers housed at KRAM, and Dr. Hans-Joachim Esser, Botanische Staatssammlung München, Germany, on vouchers at M.

### Additional information

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

Parts of JWB's research time allocated to this study was supported by basic financial support to the Norwegian Institute for Nature Research, granted by The Research Council of Norway, project no. 160022/F40.

### Author contributions

Conceptualisation: AE. Funding acquisition: JWB. Investigation: AE, JWB. Methodology: AE, JWB. Writing: AE, JWB.

### **Author ORCIDs**

Arve Elvebakk <sup>(1)</sup> https://orcid.org/0000-0002-7682-3797 Jarle W. Bjerke <sup>(1)</sup> https://orcid.org/0000-0003-2721-1492

### **Data availability**

All of the data that support the findings of this study are available in the main text or Supplementary Information.

### References

Aghababyan MV (2009) Critical notes on *Papaver* sect. *Pseudopilosa* from Caucasia. Flora, rastitel'nost', rastitel'nye resursy Armenii 17: 17–20.

Aghababyan MV (2011a) A revision of *Papaver* sect. *Argemonidium* Spach (Papaveraceae). Takhtajania 1: 38–43.

Aghababyan MV (2011b) Papaveroideae. Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. http://www.europlusmed.org [Accessed 15.2.2023]

- Aghababyan MV (2013) *Papaver gorgovanicum* M.V.Agab. (Papaveraceae), a new species from Ararat Valley (Armenia). Takhtajania 2: 134–135.
- Aghababyan MV, Fragman-Sapir O (2007) Three new *Papaver* species (Papaveraceae) from Armenia. Flora, rastitel'nost', rastitel'nye resursy Armenii 16: 27–29.
- Aghababyan MV, Christodoulou CS, Hand R (2011) Papaveraceae. In: Hand R (Ed.) Supplementary notes to the flora of Cyprus VII. Willdenowia 41: 341–355. https://doi. org/10.3372/wi.41.41215
- Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY, Taberlet P, Brochmann C (2007) Frequent long-distance plant colonization in the changing Arctic. Science 316: 1606–1608. https://doi.org/10.1126/science.1139178
- An'kova TV, Shaulo DN, Aleshina TE (2018) *Papaver kuvajevii*. In: Marhold K, Kučera M (Eds) IAPT chromosome data 27. Taxon 67: 1041–1047. https://doi.org/10.12705/675.24
- Andreeva V, Uotila P (1998) Papaver Iapponicum ssp. Iapponicum (Papaveraceae). In: Kotiranta H, Uotila P, Sulkava S, Peltonen S-L (Eds) Red data book of East Fennoscandia. Ministry of the Environment, Finnish Environment Institute & Botanical Museum, Finnish Museum of Natural History. Helsinki, 95–96.
- Artsdatabanken (2021) Norsk rødliste for arter 2021. https://www.artsdatabanken.no/ lister/rodlisteforarter/2021 [Accessed 26.10.2023]
- Artsdatabanken (2024) Artskart. https://artskart.artsdatabanken.no/#map/ [Accessed 20.07.2024]
- Ascherson P (1869) Ueber Formen von *Papaver alpinum* L. Botanische Zeitung (Berlin) 27: 121–129.
- Aymerich P, López-Alvarado J, Sáez L (2014) *Primula subpyrenaica* (Primulaceae), a new species from the Pyrenean range (south-western Europe). Phytotaxa 163(2): 77–90. https://doi.org/10.11646/phytotaxa.163.2.2
- Baasanmunkh S, Oyuntsetseg B, Oyundari C, Oyundelger K, Urgamal M, Darikhand D, Soninkhishig N, Nyambayar D, Khaliunaa K, Tsegmed Z, Kechaykin AA, Shmakov AI, Erst AS, Friesen N, Ritz CM, Wesche K, Choi HJ (2021) The vascular plant diversity of Dzungarian Gobi in western Mongolia, with an annotated checklist. Phytotaxa 501(1): 1–55. https://doi.org/10.11646/phytotaxa.501.1.1
- Baasanmunkh S, Urgamal M, Oyuntsetseg B, Sukhorukov AP, Tsegmed Z, Son DC, Erst A, Oyundelger K, Kechaykin AA, Norris J, Kosachev P, Ma J-S, Chang KS, Choi HJ (2022) Flora of Mongolia: Annotated checklist of native vascular plants. PhytoKeys 192: 63–169. https://doi.org/10.3897/phytokeys.192.79702
- Banfi E, Bartolucci F, Tison J-M, Galasso G (2022) A new genus for Papaver sect. Meconella and new combinations in Roemeria (Papaveraceae) in Europe and the Mediterranean area. Natural History Sciences 9(1): 67–72. https://doi.org/10.4081/ nhs.2022.556
- Bartók A, Hurdu BI, Szatmari PM, Ronikier M, Puşcaş M, Novikoff A, Bartha L, Vonica G (2016) New records for the high-mountain flora of the Făgăraş Mts. (southern Carpathians) with discussion on ecological preferences and distribution of studied taxa in the Carpathians. Contributii Botanice 50: 77–153.
- Bentham G (1835) Report on some of the more remarkable hardy ornamental plants raised in the Horticultural Society's Garden from seeds received from Mr. David Douglas, in the years 1831, 1832, 1833. Transactions of the Horticultural Society of London, Series 2: [1; LII:] 403–414.
- Bernátová D (2002) 5. Papaver tatricum (A.Nyár.) Ehrend. Mak tatranský. In: Bernátová D, Kubát K, Mártonfi P, Somogyi J, Šípošová H (Eds) Papaveraceae Juss. Makovité. Flóra Slovenska 5(4): 47–54.

- Bezdeleva TA (1987) Sem. makov'ye Papaveraceae. In: Kharkevich SS (Ed.) Sosudis'te rasteniya sovetskogo dal'nego vostoka (Plantae vasculares orientis extremi sovietici). Tom. 2. Leningrad, Izdatel'stvo Nauka, 37–68.
- Bezdeleva T, Pavlova NS, Probatova NS (2006) Sem. 41. Makovye Papaveraceae Juss. In: Kozhevnikov AE, Probatova NS (Eds) Flora rossiiskogo Dal'nego Vostoka: Dopolneniya i izmeneniya k izdaniyu "Sosudistye rasteniya sovetskogo Dal'nego Vostoka." Tt. 1–8 (1985–1996) [Flora of the Russian Far East: Supplements and Amendments to the "Vascular Plants of the Far East", 1985–1996, Vols 1–8]. Dal'nauka, Vladivostok, 59–67.
- Bittkau C, Kadereit JW (2002) Phylogenetic and geographical relationships in *Papaver alpinum* L. (Papaveraceae) based on RAPD data. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 123: 463–479.
- Björk CR (2019) Taxonomy of *Papaver* sect. *Meconella* (Papaveraceae) in British Columbia. Phytoneuron 6: 1–18.
- Blanca G, Onieva MRL, Lorite J, Lorola MJM, Mesa JM, Quintas S, Girela MR, Varo MÁ, Vidal S (2002) Flora amenazada y endémica de Sierra Nevada. Editorial Universidad de Granada, Granada, Spain, 410 pp.
- Bornand C, Gygax A, Juillerat P, Jutzi M, Möhl A, Rometsch S, Sager L, Santiago H, Eggenberg S (2016) Liste rouge plantes vasculaires. Espèces menacées en Suisse. L'environnement pratique 1621. Office fédéral de l'environnement & Info Flora, Bern, Genève, 178 pp. https://www.bafu.admin.ch/bafu/fr/home/themes/biodiversite/publications/ publications-biodiversite/liste-rouge-plantes-vasculaires.html [Accessed 26.10.2023]
- Bredenkamp CL, van Wyk AE (2006) Phytogeography of *Passerina* (Thymelaeceae). Bothalia 36(2): 191–199. https://doi.org/10.4102/abc.v36i2.361
- Bukharova EV, Anenkhonov OA, Badmaeva NK, Burdokovskiy AI, Pykhalova TD, Luzhkova NM (2021) Flora of the Chivyrkuyiskiy Bay Islands: Main features and patterns (Zabaikalskiy National Park). Botanicheskii Zhurnal 106(5): 483–493. https://doi. org/10.31857/S0006813621050021
- Burbrink FT, Crother BI, Murray CM, Smith BT, Ruane S, Myers EA, Pyron RA (2022) Empirical and philosophical problems with the subspecies rank. Ecology and Evolution 12(7): e9069. https://doi.org/10.1002/ece3.9069
- Carolan JC, Hook ILI, Chase MW, Kadereit JW, Hodkinson TR (2006) Phylogenetics of *Papaver* and related genera based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL Intron and trnL–F intergenic spacers. Annals of Botany 98(1): 141–155. https://doi.org/10.1093/aob/mcl079
- Catania T, Li Y, Winzer T, Harvey D, Meade F, Caridi A, Leech A, Larson TR, Ning Z, Chang J, de Peer YV, Graham IA (2022) A functionally conserved STORR gene fusion in *Papaver* species that diverged 16.8 million years ago. Nature Communications 13(1): 3150. https://doi.org/10.1038/s41467-022-30856-w
- Chang C-S, Kim H, Chang KS (2014) Provisional checklist of vascular plants for the Korea Peninsula Flora (KPF). Version 1.0. 1–660. http://hosting03.snu.ac.kr/~quercus1 [Accessed 26.10.2023]
- Chepinoga VV, Seregin AP, Barkalov VY, Ebel AL, Efimov PG, Friesen NV, Gontcharov AA, Kechaykin AA, Knyazev MS, Korobkov AA, Korolyuk EA, Kosachev PA, Luferov AN, Melnikov DG, Nikiforova OD, Ovzinnikova SV, Schekhovstsova IN, Troshkina VI (2023) New combinations and new names in vascular plants of Asian Russia. Botanica Pacifica 12(2): 120–133. https://doi.org/10.17581/bp.2023.12s06
- Christenhusz MJM, Fay MF, Byng JW [Eds] (2018) Plant Gateway's The Global Flora. A practical flora to vascular plant species of the world. GLOVAP nomenclature Part 1. Plant Gateway, Bradford, 155 pp.

- Cortés-Burns H, Carlson ML, Lipkon R, Flagstad L, Yokel D (2009) Rare vascular plants of the North Slope. A review of the taxonomy, distribution and ecology of 31 rare plant taxa that occur in Alaska's North Slope Region. Technical Report 58. US Department of the Interior, Bureau of Land Management, Alaska State Office, Anchorage, 116 pp.
- Cowling RM, Holmes PM (1992) Endemism and speciation in a lowland flora from the Cape Floristic Region. Biological Journal of the Linnean Society. Linnean Society of London 47(4): 367–383. https://doi.org/10.1111/j.1095-8312.1992.tb00675.x
- Cullen J (1965) *Papaver* L. In: Davis PH (Ed.) Flora of Turkey and the East Aegean Islands, Vol. 1. Edinburgh University Press, Edinburgh, 219–296.

Czerepanov SK (1995) *Papaver* L. In: Czerepanov SK (Ed.) Vascular plants of Russia and adjacent states (the former USSR). Cambridge University Press, Cambridge, 333–334.

- Dar GH, Rashid T, Naqshi AR, Khuroo AZ, Malik AH (2010) Two new species of *Papaver* L. (Papaveraceae) from Kashmir Himalaya, India. Pakistan Journal of Botany Special Issue 42: 57–62. [SI Ali Festschrift]
- De Candolle AP (1821) Regni vegetabilis systema naturale, sive ordines, genera et species plantarum secundum methodi naturalis normas digestarium et descriptarum. Volumen secundum. Treuttel et Würtz, Parisiis, 745 pp.
- Dixon CJ, Gutermann W, Schönswetter P, Schneeweiss GM (2016) Taxonomy and nomenclature of the polymorphic European high mountain species *Androsace vitaliana* (L.) Lapeyr. (Primulaceae). PhytoKeys 75: 93–106. https://doi.org/10.3897/phytokeys.75.10731
- Egorova TV (1998) Rod *Papaver* L. (Papaveraceae) vo flore vostochnoi Evropii. Novosti Sistimatiki Vysshikh Rastenii 31: 90–118.
- Eide W, Ahrné K, Bjelke U, Nordström S, Ottosson E, Sandström J, Sundberg S [Eds] (2020) Rödlistade arter i Sverige 2020. SLU Artdatabanken, Uppsala, 242 pp. https:// www.artdatabanken.se/var-verksamhet/rodlistning/ [Accessed 30.10.2023]
- Elvebakk A (1984) Flora og vegetasjon i utbyggingsområdet ved Alta/Kautokeino-vassdraget. I. Økologi, innvandringshistorie og bestandsstatus for masimjelt (*Oxytropis deflexa* ssp. *norvegica*). Tromura Naturvitenskap 42. Tromsø Museum, Tromsø, 69 pp.
- Elvebakk A, Karlsen SR (2022) Det arktiske Finnmark ein bioklimatisk studie av område nord for den polare skoggrensa. Blyttia 80: 147–174.
- Elven R, Murray DF (2008) New combinations in the Panarctic vascular plant flora. Journal of the Botanical Research Institute of Texas 2: 433–446. https://www.jstor.org/ stable/41971656
- Elven R, Petrovsky VV, Solstad H (2009) Typification of names in *Papaver* sect. *Meconella* (Papaveraceae). Taxon 58(3): 985–990. https://doi.org/10.1002/tax.583028
- Elven R, Murray DF, Petrovsky VV, Solstad H (2011) *Papaver*. In: Elven R (Ed.) Annotated checklist of the Panarctic Flora (PAF). Vascular plants. http://panarcticflora.org/flo-ra#paf-37 [Accessed 27.10.2023]
- Elven R, Bjorå CS, Fremstad E, Hegre H, Solstad H (2022) Norsk flora. 8<sup>th</sup> edn. Det Norske Samlaget, Oslo, 1255 pp.
- Emig W, Kadereit JW (1993) The comparative biology of the closely related Senecio nebrodensis and S. viscosus, a narrow endemic and a widespread ruderal. Nordic Journal of Botany 13(4): 369–375. https://doi.org/10.1111/j.1756-1051.1993.tb00064.x
- Fedde F (1909) Papaveraceae-Hypecoideae et Papaveraceae-Papaveroideae. In: Engler A (Ed.) Das Pflanzenreich: Regni vegetabilis conspectus 40(IV:104): 1–430.
- Ferrer-Gallego PP (2015) Lectotypification of *Papaver cambricum* L. (Papaveraceae). Candollea 70(2): 207–210. https://doi.org/10.15553/c20I5v702a5
- Ferrer-Gallego PP (2024) (3034) Proposal to reject the name Argemone pyrenaica (Papaver pyrenaicum) (Papaveraceae). Taxon 73(3): 918–919. https://doi.org/10.1002/tax.13194

- Fragnière Y, Pittet L, Clément B, Bétrisey S, Gerber E, Ronikier M, Parisod C, Kozlowski G (2020) Climate change and alpine screes: No future for glacial relict *Papaver occidentale* (Papaveraceae) in Western Prealps. Diversity 12(9): 46. https://doi.org/10.3390/ d12090346
- Galasso G, Banfi E, Bartolucci F (2023) New combinations in Oreomecon. The Plant Review 5(4): 58.
- GBIF (Global Biodiversity Information Facility) Secretariat (2023) GBIF Backbone Taxonomy. [Checklist dataset accessed via GBIF.org on 30.4.2023] https://doi. org/10.15468/39omei
- Goldblatt P, Savolainen V, Porteous O, Sostaric I, Powell M, Reeves G, Manning JC, Barraclough TG, Chase MW (2002) Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. Molecular Phylogenetics and Evolution 25(2): 341–360. https://doi.org/10.1016/S1055-7903(02)00235-X
- Gorgorov RN, Yankova EP, Baldjiev GA, Apostolova II, Yurukova-Grancharova PD, Stanilova MI (2011) Reproductive capacity and in vitro cultivation of the glacial relict *Papaver degenii* (Papaveraceae). Phytologia Balcanica 17: 333–339.
- Greene EL (1894) Manual of the botany of the region of Francisco Bay. San Francisco, Cubery & Company, 368 pp. https://doi.org/10.5962/bhl.title.57343
- Gremli A (1874) Excursionsflora für die Schweiz. Zweite gänzlich umgearbeitete Auflage. Ph. Wirz-Christen, Aarau, 471 pp. https://doi.org/10.5962/bhl.title.153315
- Gremli A (1889) Excursionsflora für die Schweiz. Sechste, vermehrte und verbesserte Auflage. J.J. Christen, Aarau, 509 pp.
- Greuter W (1981) Med-Checklist Notulae, 3. Willdenowia 11: 23-43.
- Grey-Wilson C (2012) Proposal to conserve the name *Meconopsis* (Papaveraceae) with a conserved type. Taxon 61(2): 473–474. https://doi.org/10.1002/tax.612026
- Grey-Wilson C (2014) The genus *Meconopsis*. Blue poppies and their relatives. Kew Publishing, Royal Botanic Gardens, Kew, Richmond, Surrey, 399 pp.
- Grey-Wilson C [Ed.] (2017) *Meconopsis* for gardeners. The lure of the blue poppy. Alpine Garden Society and the Meconopsis Group, Pershore, Worcestershire, 384 pp.
- Grey-Wilson C (2023) Saving Meconopsis. The Plant Review 5(4): 54-57.
- Guo Q, Ruifeng B, Baosheng Z, Feng X, Zhao Y, Tu P, Chai X (2016) An ethnopharmacological, phytochemical and pharmacological review of the genus *Meconopsis*. The American Journal of Chinese Medicine 44(3): 439–462. https://doi.org/10.1142/ S0192415X16500257
- Gürkök T, Kaymak E, Boztepe G, Koyuncy M, Parmaksız İ (2013) Molecular characterization of the genus *Papaver* section *Oxytona* using ISSR markers. Turkish Journal of Botany 37: 644–650. https://doi.org/10.3906/bot-1208-16
- Hannan GK (1997) *Meconella*. In: Flora of North America Editorial Committee (Eds) Flora of North America, Vol. 3. Oxford University Press, New York and Oxford.
- Hassler M (2023a) Flora Germanica Die Farn- und Blütenpflanzen Deutschlands. Version 8.33. https://www.flora-germanica.de/flora-von-deutschland/artenliste [Accessed 16.07.2023]
- Hassler M (2023b) World Plants. Synonymic checklist and distribution of the world flora. Version 16.1. https://www.worldplants.de/world-plants-complete-list/completeplant-list [Accessed 16.07.2023]
- Heggelund I (1993) Nye lokaliteter for læstadiusvalmue (*Papaver laestadianum*) i Signaldalen ["New localities for *Papaver laestadianum* in Signaldalen, Troms"]. Polarflokken 17: 471–484.

- Herbert TD, Lawrence KT, Tzanova A, Peterson LC, Caballero-Gill R, Kelly CS (2016) Late Miocene global cooling and the rise of modern ecosystems. Nature Geoscience 9(11): 843–847. https://doi.org/10.1038/ngeo2813
- Hilger HH, Bohle U-R (2000) *Pontechium*: A new genus distinct from *Echium* and *Lobostemon* (Boraginaceae). Taxon 49(4): 737–746. https://doi.org/10.2307/1223974
- Hong UVT, Tamiru-Oli M, Hurgobin B, Okey CR, Abreu AR, Lewsey MG (2022) Insights into opium poppy (*Papaver* spp.) genetic diversity from genotyping-by-sequencing analysis. Scientific Reports 12(1): 111. https://doi.org/10.1038/s41598-021-04056-3
- Hultén E (1928) Flora of Kamtchatka and the adjacent islands, 2. Kungliga svenska vetenskapakademiens handlingar, tredje serien 5(2): 1–218.
- IPNI (2023) International Plant Names Index. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. https://www.ipni. org [Accessed 16.07.2023]
- Jafri SMH, Qaiser M (2011) Papaveraceae Juss. Flora of Pakistan. http://www.tropicos. org/Project/Pakistan [Accessed 27.10.2023]
- Jesus A, Bonhomme V, Allowen E, Soteras RI, Jacomet S, Bouby L, Antolín F (2023) Morphometrics of waterlogged archaeological seeds give new insights into the domestication and spread of *Papaver somniferum* L. in Western Europe. PLoS ONE 18(5): e0286190. https://doi.org/10.1371/journal.pone.0286190
- Jonsell B (2001) Additional nomenclatural notes to Flora Nordica (Chenopodiaceae-Fumariaceae). Nordic Journal of Botany 20(5): 519–523. https://doi. org/10.1111/j.1756-1051.2000.tb01596.x
- JSTOR Global Plants (2023) JSTOR Global Plants. Ithaka, New York. http://plants.jstor. org/plants/browse [Accessed 27.11.2023]
- Kadereit JW (1986a) A revision of *Papaver* L. section *Argemonidium*. Notes from the Royal Botanic Garden Edinburgh 45: 25–43.
- Kadereit JW (1986b) A revision of *Papaver* sect. *Papaver* (Papaveraceae). Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 108: 1–16.
- Kadereit JW (1987) A revision of *Papaver* sect *Carinatae* (Papaveraceae). Nordic Journal of Botany 7(5): 501–504. https://doi.org/10.1111/j.1756-1051.1987.tb02016.x
- Kadereit JW (1988a) *Papaver* L. sect. *Californicum* Kadereit, a new section of the genus. Rhodora 90(861): 7–13.
- Kadereit JW (1988b) Sectional affinities and geographical distribution in the genus *Papaver* L. (Papaveraceae). Beiträge zur Biologie der Pflanzen 63: 139–156.
- Kadereit JW (1988c) The affinities of the south-hemispherical *Papaver aculeatum* Thunb. (Papaveraceae). Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 109: 335–341.
- Kadereit JW (1989) A revision of *Papaver* L. section *Rhoeadium* Spach. Notes from the Royal Botanic Garden Edinburgh 45: 225–286.
- Kadereit JW (1990) Notes on the taxonomy, distribution, phylogeny and ecology of *Papaver alpinum* L. (Papaveraceae). Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 112: 79–97.
- Kadereit JW (1993) A revision of *Papaver* sect. *Meconidium*. Edinburgh Journal of Botany 50(2): 125–148. https://doi.org/10.1017/S0960428600002523
- Kadereit JW (1996) A revision of Papaver sects. Pilosa Prantl and Pseudopilosa M. Popov ex Günther (Papaveraceae). Edinburgh Journal of Botany 53(3): 285–309. https:// doi.org/10.1017/S0960428600003747
- Kadereit JW (1998) The genus *Papaver* L. in the Mediterranean area. Lagascalia 19: 83–92.

- Kadereit JW, Baldwin B (2011) Systematics, phylogeny, and evolution of *Papaver* californicum and Stylomecon heterophylla (Papaveraceae). Madrono 58(2): 92–100. https://doi.org/10.3120/0024-9637-58.2.92
- Kadereit JW, Erbar C (2011) Evolution of gynoecium morphology in Old World Papaveroideae: A combined phylogenetic/ontogenetic approach. American Journal of Botany 98(8): 1243–1251. https://doi.org/10.3732/ajb.1100066
- Kadereit JW, Lobin W (1990) The taxonomy and affinities of *Papaver gorgoneum* from the Cape Verde Islands. Nordic Journal of Botany 9(6): 643–648. https://doi. org/10.1111/j.1756-1051.1990.tb00556.x
- Kadereit JW, Schwarzbach AE, York KB (1997) The phylogeny of *Papaver* s.lat. (Papaveraceae): Polyphyly or monophyly? Plant Systematics and Evolution 204(1–2): 75–98. https://doi.org/10.1007/BF00982533
- Kadereit JW, Licht W, Uhink CH (2008) Asian relationships of the flora of the European Alps. Plant Ecology & Diversity 1(2): 171–179. https://doi. org/10.1080/17550870802328751
- Kadereit JW, Preston CD, Valtueña FJ (2011) Is Welsh Poppy, Meconopsis cambrica (L.) Vig. (Papaveraceae), truly a Meconopsis? New Journal of Botany 1(2): 80–88. https:// doi.org/10.1179/204234811X13194453002742
- Kamelin RV, Gubanov IA (1990) Novye taksony Papaver L. iz Mongolii. Byulleten' Moskovskogo Obshchestva Ispytateley Prirody. Otdel Biologicheskiy 95: 86–88.
- Karrer S (1935) Papaver amurense (Amur-Mohn), Nudicaule-Typ. Möllers Deutsche Gärtner-Zeitung 50: 247.
- Kiger RW (1985) Revised sectional nomenclature in *Papaver* L. Taxon 34(1): 150–152. https://doi.org/10.2307/1221582
- Kiger RW, Murray DF (1997) Papaver. In: Flora of North America Editorial Committee (Eds) Flora of North America, Vol. 3. Oxford University Press, New York, Oxford, 323– 333.
- Kim M (2017) Papaveraceae. In: Flora of Korea Editorial Committee (Eds) Flora of Korea Vol. 2a Magnoliidae-Ranunculaceae. National Institute of Biological Resources, Ministry of Environment, Korea, 104–107.
- Knaben G (1958) *Papaver*-studier, med et forsvar for *P. radicatum* Rottb. som en islandsk-skandinavisk art. Blyttia 16: 61–80.
- Knaben G (1959a) On the evolution of the *Radicatum*-group of the *Scapiflora* Papavers as studied in 70 and 56 chromosome species. A. Cytotaxonomical aspects. Opera Botanica 2(3): 1–74.
- Knaben G (1959b) On the evolution of the *Radicatum*-group of the *Scapiflora* Papavers as studied in 70 and 56 chromosome species. B. Experimental studies. Opera Botanica 3(3): 1–96.

Knaben G (1970) Om artsbegrepet hos fjellvalmuer. Blyttia 28: 187-193.

- Knaben GS (1979) Additional experimental studies in the Papaver radicatum group. Botaniska Notiser 132: 483–490.
- Knaben GS (1983) Den arktiske valmuen. Polarflokken 7: 54–72.
- Knaben GS (1985) Neo-polyploids in the North Atlantic region. Botanica Helvetica 95: 177–191.
- Knaben G, Hylander N (1970) On the typification of *Papaver radicatum* Rottb. and its nomenclatural consequences. Botaniska Notiser 123: 338–348.
- Komarov VL [Ed.] (1937) Flora SSSR, Vol. VII, Ranales and Rhoeadales. Izdatel'stvo Akademii Nauk SSSR, Moskva, Leningrad. [English translation by Singh BSMP, published by Koeltz Scientific Books, Königstein, 1985].
- Krivenko DA (2023) New combinations in the genus Oreomecon (Papaveraceae). Novitates Systematicae Plantarum Vascularium 54: 97–100 [Pe06: 1–4]. https://doi. org/10.31111/novitates/2023.54.06
- Kropf M, Comes HP, Kadereit J (2006) Long-distance dispersal vs vicariance: The origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. The New Phytologist 172(1): 169–184. https://doi.org/10.1111/j.1469-8137.2006.01795.x
- Lack HW (2019a) (2680) Proposal to conserve the name *Papaver pseudo-orientale* (Fedde) Medw. against *P.×pseudo-orientale* E.G.Camus (Papaveraceae). Taxon 68(2): 407–408. https://doi.org/10.1002/tax.12042
- Lack HW (2019b) The discovery and naming of *Papaver orientale* s.lat. (Papaveraceae) with notes on its nomenclature and early cultivation. Candollea 74(1): 47–64. https://doi.org/10.15553/c2019v741a7
- Lauber K, Wagner G, Gygax A (2018) Flora Helvetica. Illustrierte Flora der Schweiz mit Artsbeschreibungen und Verbreitungskarten von 3200 wild wachsenenden Farn- und Blütenpflanzen, einschliesslich wichtiger Kulturpflanzen. Sechste, vollständig überarbeitete Auflage. Haupt Verlag, Bern, 1686 pp.
- Lee D-U, Park JH, Wessjohann L, Schmidt J (2011) Alkaloids from *Papaver* coreanum. Natural Product Communications 6(11): 1593–1594. https://doi.org/10.1177/1934578X1100601109
- Liu Y-C, Liu Y-N, Yang FS, Wang X-Q (2014) Molecular phylogeny of Asian *Meconopsis* based on nuclear ribosomal and chloroplast DNA sequence data. PLoS ONE 9(8): e104823. https://doi.org/10.1371/journal.pone.0104823
- Liu L, Du Y, Shen C, Li R, Lee J, Li P (2020) The complete chloroplast genome of *Papaver* setigerum and comparative analyses in Papaveraceae. Genetics and Molecular Biology 43(3): e20190272. https://doi.org/10.1590/1678-4685-gmb-2019-0272
- Loiseleur-Deslongchamps JLA (1807) Flora Gallica, seu enumeratio plantarum in Gallia sponte nascentum. Pars secunda. Lutetiae, Paris, Matthei Migneret, 337–742.
- Loiseleur-Deslongchamps JLA (1809) Suite de la notice sur les plantes à ajouter à la Flore de France (Flora Gallica), avec quelques corrections et observations. Journal de Botanique (Desvaux) 2: 321–370. https://doi.org/10.5962/bhl.title.6361
- Löve Á (1945) Íslenzkar jurtir með myndum eftir Dagny Tande Lid. Ejnar Munksgaard Kaupmannahöfn, 291 pp.
- Löve Á (1955) Cytotaxonomical remarks on the Icelandic *Papaver*. Nytt Magasin for Botanikk 4: 5–18.
- Löve Á (1962a) Nomenclature of North Atlantic papavers. Taxon 11(4): 132–138. https://doi.org/10.2307/1217741
- Löve Á (1962b) Typification of *Papaver radicatum* a nomenclatural detective story. Botaniska Notiser 115: 113–136.
- Löve Á (1970) Emendations in the Icelandic flora. Taxon 19(2): 298–302. https://doi. org/10.2307/1217990
- Lundström E (1923) Über *Papaver nudicaule* L. und *P. radicatum* Rottb. in Fennoskandia und Arktis, sowie über einige mit *P. nudicaule* verwandten Arten. Acta Horti Bergiani 7(5): 403–430.
- Majid A, Ahmad H, Saqib Z, Ali H, Alam J (2015) Conservation status assessment of *Meconopsis aculeata* Royle; a threatened endemic of Pakistan and Kashmir. Pakistan Journal of Botany 47: 1–5.
- Markgraf F (1958a) Eine neue Gliederung der Alpenmohne. Phyton 7: 302–314.
- Markgraf F (1958b) Papaveraceae. In: Hegi G (Ed.) Illustrierte Flora von Mitteleuropa, 2<sup>nd</sup> edn., Vol. IV, 1/2, Hanser, München, 15–49.

- Markgraf F (1965) Die Typisierung von Papaver alpinum L. Taxon 12(4): 144–146. https://doi.org/10.2307/1216181
- Metzing D, Garve E, Matzke-Hajek G, Adler J, Bleeker W, Breunig T, Caspari S, Dunkel FG, Fritsch R, Gottschlich G, Gregor T, Hand R, Hauck M, Korsch H, Meierott L, Meyer N, Renker C, Romahn K, Schulz D, Täuber T, Uhlemann I, Welk E, van de Weyer K, Wörz A, Zahlheimer W, Zehm A, Zimmermann F (2018) Rote Liste und Gesamtartenliste der Farn- und Blütenpflanzen (Trachaeophyta) Deutschlands. In: Metzing D, Hofbauer N, Ludwig G, Matzke-Hajek G (Eds) Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands. Band 7: Pflanzen. Naturschutz und Biologische Vielfalt 70. Landwirtschaftsverlag, Münster, 13–358.
- Mikheev AD (1993) Obzor vidov semeistva Papaveraceae floriy Kavkaza (The synopsis of the species of the family Papaveraceae from the Caucasian flora). Botanicheskii Zhurnal 78: 115–124.
- Mikheev AD (1999) Nov'ii vid roda *Papaver* (Papaveraceae) s severnogo Kavkaza (A new species of the genus *Papaver* (Papaveraceae) from the northern Caucasus). Botanicheskii Zhurnal 84: 105–197.
- Miyabe K, Tatewaki M (1935) Contributions to the Flora of Northern Japan V. Transactions of the Sapporo Natural History Society 14: 1–10.
- Miyabe K, Tatewaki M (1936) Contributions to the Flora of Northern Japan VIII. Transactions of the Sapporo Natural History Society [Hist. Soc.] 14: 255–270.
- Molinari J (2023) A bare-bones scheme to choose between the species, subspecies, and 'evolutionarily significant unit' categories in taxonomy and conservation. Journal for Nature Conservation 72: 126335. https://doi.org/10.1016/j.jnc.2023.126335
- Murray DM (1995) New names in *Papaver* section *Meconella* (Papaveraceae). Novon 5(3): 294–295. https://doi.org/10.2307/3392270
- Myers-Smith IH, Kerby JT, Phoenix GK, Bjerke JW, Epstein HE, Assmann JJ, John C, Andreu-Hayles L, Angers-Blondin S, Beck PSA, Berner LT, Bhatt US, Bjorkman AD, Blok D, Bryn A, Christiansen CT, Cornelissen JHC, Cunliffe AM, Elmendorf SC, Forbes BC, Goetz SJ, Hollister RD, de Jong R, Loranty MM, Macias-Fauria M, Maseyk K, Normand S, Olofsson J, Parker TC, Parmentier F-JW, Post E, Schaepman-Strub G, Stordal F, Sullivan PF, Thomas HJD, Tømmervik H, Treharne R, Tweedie CE, Walker DA, Wilmking M, Wipf S (2020) Complexity revealed in the greening of the Arctic. Nature Climate Change 10(2): 106–117. https://doi.org/10.1038/s41558-019-0688-1
- Nannfeldt JA (1963) Taxonomic differentiation as an indicator of the migratory history of the North Atlantic flora. In: Löve Á, Löve D (Eds) North Atlantic biota and their history. Pergamon Press, the Macmillan Company, New York, 87–98.
- Natural History Museum, University of Oslo (2023) Natural History Museum, University of Oslo. https://samlingsportal.nhm.uio.no/museum/nhm [Accessed 2.12.2023]
- Nevermo I (1997) Økofysiologiske studier av *Papaver laestadianum* (Nordh.) Nordhagen. Cand. Scient. Thesis, Institutt for biologi, Universitetet i Tromsø, 68 pp. [unpubl.]
- Nilsson Ö (2001) *Papaver* L. In: Jonsell B (Ed.) Flora Nordica, Vol. 2. Chenopodiaceae Fumariaceae. The Bergius Foundation, Stockholm, 345–359.

Nordal I (1987) Tabula rasa after all? Botanical evidence for ice-free refugia in Scandinavia reviewed. Journal of Biogeography 14(4): 377–388. https://doi.org/10.2307/2844945

Nordhagen R (1932) Studien über die skandinavischen Rassen des *Papaver radicatum* Rottb. sowie einige mit denselben verwechselte neue Arten. Bergens Museums Årbok 1931. Naturvitenskapelig Rekke 2: 1–50. https://doi.org/10.1002/fedr.4870292305

Nordhagen R (1939) Bidrag til fjellet Pältsas flora. Ett nytt funn av *Stellaria longipes.* Botaniska Notiser 1939: 691–700.

- Nordhagen R (1970) Norsk flora. Tegninger av Miranda Bødker. Illustrasjonsbind, fjerde hefte. H. Aschehoug, Oslo, 639–944.
- Nyárády A (1942) Magyarország vadontermő *Papaver* fajai. Acta Geobotanica Hungarica 5: 3–68 [2 tábla].
- Nyman CF (1889) Conspectus florae europaeae. Supplementum II. Pars prima. Typis oficinae bohlinianae, Örebro, 404 pp.
- Oltean M, Negrean G, Popescu A, Roman N, Dihoru G, Sanda V, Mihăilescu S (1994) Lista roșie a plantelor superioare din România. Studii, sinteze, documentații de ecologie 1. Academia Română, Institutul de Biologie, București, 52 pp.
- Øvstedal DO, Grung B (2015) Underartene hos vår fjellvalmue er de virkelig forskjellige? Årringen 2014: 81–89.
- Pachschwöll C, Reich D, Tan K (2019) The botanical legacy of Mihael Dimonie (1870– 1935), an almost forgotten plant collector in the southern Balkan Peninsula before the First World War. Willdenowia 49: 257–279. https://doi.org/10.3372/wi.49.49213
- Parmaksız İ, Özcan S (2011) Morphological, chemical, and molecular analyses of Turkish *Papaver* accessions (Sect. *Oxytona*). Turkish Journal of Botany 35: 1–16. https:// doi.org/10.3906/bot-1003-39
- Paszko B, Nikel A, Mueller-Bienek A, Paul W (2020) Zapałowicz's *Conspectus florae Galiciae criticus*: Clarification of publication dates for nomenclatural purposes and bibliographic notes. PhytoKeys 155: 53–85. https://doi.org/10.3897/phytokeys.155.51072
- Pavlova NS (1999) Noviy vid roda *Papaver* (Papaveraceae) s Ostrova Sakhalin. Botanicheskii Zhurnal 84: 112–115. [A new species of the genus *Papaver* (Papaveraceae) from Sakhalin]
- Peng H-W, Xiang K-L, Erst AS, Lian L, Ortiz RDC, Jabbour F, Chen Z-D, Wang W (2023) A complete genus-level phylogeny reveals the Cretaceous biogeographic diversification of the poppy family. Molecular Phylogenetics and Evolution 81: 107712. https:// doi.org/10.1016/j.ympev.2023.107712
- Peschkova IA (1994) Rod Papaver L. In: Malyschev LI, Peschkova GA (Eds) Flora Sibiri, Vol. 7, Berberidaceae-Grossulariaceae. Nauka, Novosibirsk, 12–31. [in Russian; Flora of Siberia, Vol. 7. Science Publishers, Inc. Enfield, USA, English translation, 2004, 4–25]
- Petrovsky VV (1983) Novye taksony roda *Papaver* (Papaveraceae) s severo-vostoka Azii (New taxa of the genus *Papaver* (Papaveraceae) from the northeast of Asia). Botanicheskii Zhurnal 68: 229–236.
- Petrovsky VV (1985) Tri novikh vida roda *Papaver* (Papaveraceae) s severo-vostoka Azii (Three new species of the genus *Papaver* (Papaveraceae) from the north-east of Asia). Botanicheskii Zhurnal 70: 113–117.
- Petrovsky VV (1997) Areas of intensive plant speciation in the Beringian Arctic Shelf. Opera Botanica 132: 19–25.
- Petrovsky VV (1999) The problem of the species-width in poppies of Russian Arctic. In: Nordal I, Razzhivin VY (Eds) The species concept in the High North – a Panarctic flora initiative. Det Norske Videnskaps-Akademi. I. Matematisk-Naturvitenskapelig Klasse. Skrifter, Ny Serie 38: 243–259.
- Petrovsky VV, Sokolova IV, Mikhailova MA (2019) Taxonomy of the subspecies of *Papa-ver pulvinatum* (Papaveraceae). Annales Botanici Fennici 56(4–6): 369–377. https://doi.org/10.5735/085.056.0423
- Pignatti S, Guarino R, La Rosa M (2017) Papaveraceae. In: Pignatti S, Guarino R, La Rosa M (Eds) Flora d'Italia, 2<sup>nd</sup> edn. Edagricole de New Business Media, Bologna, 883–900.
- Pittet L, Fragnière Y, Grünig S, Bétrisey S, Clément B, Gerber E, Ronikier M, Kozlowski G, Parisod C (2020) Genetic structure of the endemic *Papaver occidentale* indicates

survival and immigration in the Western Prealps. Alpine Botany 130(2): 129–140. https://doi.org/10.1007/s00035-020-00238-3

- Popov MG (1937) Fam. LXVIII Papaveraceae B. Juss. In: Komarov VL (Ed.) Flora SSSR, Vol. VII. Ranales and Rhoeadales. Izdatel'stvo Akademii Nauk SSSR, Moskva-Leningrad. [English translation by Singh BSMP, published by Koeltz Scientific Books, Königstein, 1985), 573–717, 748–750.]
- Pound MJ, Haywood AM, Salzmann U, Riding JB, Lunt DJ, Hunter SJ (2011) A Tortonian (Late Miocene, 11.61–7.25 Ma) global vegetation reconstruction. Palaeogeography, Palaeoclimatology, Palaeoecology 300(1–4): 29–45. https://doi.org/10.1016/j.pa-laeo.2010.11.029
- POWO (2023) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. https://powo.science.kew.org/ [Accessed 16.07.2023]
- Rändel U (1974) Beiträge zur Kenntnis der Sippenstruktur der Gattung *Papaver* L. Sectio *Scapiflora* Reichenb. (Papaveraceae). Feddes Repertorium 84(9–10): 655–732. https://doi.org/10.1002/fedr.19730840903
- Rändel U (1977) Über Sippen des subarktisch-arktischen Nordamerikas, des Beringia-Gebietes und Nordost-Asiens der Sektion *Lasiotrachyphylla* Bernh. (Papaveraceae) und deren Bezeichungen zueinander und zu Sippen anderer Arealteile der Sektion. Feddes Repertorium 88(7–8): 421–450. https://doi.org/10.1002/fedr.19770880705
- Rottbøll CF (1770) Afhandling om en Deel enten gandske nye eller vel forhen bekiendte, men dog for os rare Planter som i Island og Grønland ere fundne, tillige med en kort Indledning om Urtelærens Tilstand i Dannemark. Skrifter Kiøbenhavnske Selskab Lærde Videnskabers Elskere 10: 393–462.
- Sariyar G (2002) Biodiversity in the alkaloids of Turkish Papaver species. Pure and Applied Chemistry 74(4): 557–574. https://doi.org/10.1351/pac200274040557
- Schönswetter P, Solstad H, Garcia PE, Elven R (2009) A combined molecular and morphological approach to the taxonomically intricate European mountain plant *Papaver alpinum* s.lat. (Papaveraceae) taxa or informal phylogeographical groups? Taxon 58(4): 1326–1343. https://doi.org/10.1002/tax.584020
- Selin E (1998) Morphometric analyses of capsule and seed traits in Scandinavian *Papaver radicatum* (Papaveraceae) in relation to the bicentric distribution pattern. Nordic Journal of Botany 18(6): 689–700. https://doi.org/10.1111/j.1756-1051.1998.tb01551.x
- Selin E (2000) Morphometric differentiation between populations of Papaver radicatum (Papaveraceae) in northern Scandinavia. Botanical Journal of the Linnean Society 133(3): 263–284. https://doi.org/10.1111/j.1095-8339.2000.tb01546.x
- Selin E, Prentice HC (1988) Morphometric analysis of disjunct *Papaver radicatum* (Papaveraceae) populations in southern Norway. Plant Systematics and Evolution 159(3–4): 237–247. https://doi.org/10.1007/BF00935975
- Semenova-Tian-Shanskaya NZ (1956) Sem. XXXIV. Makovye Papaveraceae (B. Juss.) Durande. In: Polyarkova AI (Ed.) Flora Murmanskoy oblasti. Viputsk III. Izdatel'stvo Akademii Nauk SSSR, Moskva-Leningrad, 296–305.
- Sennikov AN, Tojibaev KS (Eds.) (2021) Checklist of vascular plants of the Tian-Shan Mountain System. Korea National Arboretum, Pocheon, 607 pp.
- Shaulo D, Sonnikova A (2003) A new species of the genus *Papaver* L. (Papaveraceae) from the Western Sayan. Turczaninovia 6: 5–6.
- Škornik M, Wraber T (1988) *Papaver victoris*, novi rumenocvetni alpski mak v Julijskih Alpah. Biološki Vestnik 36: 79–93.
- Solomon J, Shulkina T, Schatz E [Eds] (2014) Red list of the endemic plants of the Caucasus: Armenia, Azerbaijan, Georgia, Iran, Russia, and Turkey. Monographs in Sys-

tematic Botany from the Missouri Botanical Garden 125, Missouri Botanical Garden Press, Saint Louis, 451 pp.

- Solstad H (2007) Papaveraceae. In: Aiken SG, Dallwitz MJ, Consaul LL, McJannet CL, Boles RL, Argus GW, Gillett JM, Scott PJ, Elven R, LeBlanc MC, Gillespie LJ, Brysting AK, Solstad H, Harris JG (Eds) Flora of the Canadian Arctic Archipelago: Descriptions, Illustrations, Identification, and Information Retrieval. NRC Research Press, National Research Council of Canada, Ottawa. https://nature.ca/aaflora/data/index [Accessed 26.10.2023]
- Solstad H, Elven R, Nordal I (1999) Are there too many species and subspecies in the *Papaver radicatum* complex? In: Nordal I, Razzhivin VY (Eds) The species concept in the High North a Panarctic flora initiative. Det Norske Videnskaps-Akademi. I. Matematisk-Naturvitenskapelig Klasse. Skrifter, Ny Serie 38: 281–294.
- Solstad H, Elven R, Nordal I (2003) Isozyme variation among and within North Atlantic species of *Papaver* sect. *Meconella* (Papaveraceae) and taxonomic implications. Botanical Journal of the Linnean Society 143(3): 255–269. https://doi.org/10.1046/ j.1095-8339.2003.00211.x
- Solstad H, Ehrich D, Kvernstuen LG, Trávníček P, Elven R (2009) Taxonomic variation in a complex polyploid plant group – *Papaver* sect. *Meconella* (Papaveraceae) – inferred by Amplified Fragment Length Polymorphism, morphology and DNA ploidy. In: Solstad H (Ed.) Taxonomy and evolution of the diploid and polyploid *Papaver* sect. *Meconella* (Papaveraceae). PhD Thesis, University of Oslo, Oslo 64 pp. [+ unpaginated figures and table]
- Solstad H, Eriksen PB, Little L, Elven R (2014) To valmue-arter på Svalbard, og litt om fjell- og polarvalmuer (Two species of *Papaver* sect. *Meconella* in Svalbard, and some notes on alpine and arctic poppies). Blyttia 72: 187–196.
- Stefánsson S (1901) Flóra Íslands. Hinu Íslenzka Bókmenntafjelagi, Kaupmannahöfn/ Copenhagen.
- Steudel ET (1841) Nomenclator botanicus, seu synonymia plantarum universalis enumerans ordine alphabetico nomina atque synonyma, tum generica tum specifica, et a Linnaeo et a recentioribus de re botanica scriptoribus plantis phanerogamis imposita. Pars II Lit. L-Z. J.G. Cottiae, Stuttgartiae et Tubingae, 810 pp. https://doi. org/10.5962/bhl.title.655
- Stevens PF (2001) [onwards] Angiosperm Phylogeny Website. Version 14, July 2017. http://www.mobot.org/mobot/research/apweb/ [Accessed 10.1.2024]
- Stevens E (2015) A pictorial guide to the big blue poppies (*Meconopsis*). Dander Publishing, Nottingham, 95 pp.
- Stoeva M (2009) *Papaver degenii* (Urum. & Jav.) Kuzmanov. In: Petrova A, Vladimirov V (Eds) Red List of Bulgarian vascular plants. Phytologia Balcanica 15: 86.
- Stoeva M (2023) Papaver degenii. In: Golemansky V, Peev D, Chipev N, Beron P, Biserkov V (Eds) Red Data Book of the Republic of Bulgaria. Digital Edition. Bulgarian Academy of Sciences, Ministry of Environment and Waters of Bulgaria, Sofia. http://e-ecodb.bas.bg/rdb/en/vol1/Papdegen.html [Accessed 31.1.2023]

Sugawara S (1937–1940) Illustrated flora of Saghalien. Vol. 1–4. Tokyo, 1957 pp.

- Sweet R (1830) Sweet's Hortus Britannicus, or, a catalogue of plants, indigeneous, or, cultivated in the gardens of Great Britain, 2<sup>nd</sup>. edn., Ridgway, London, 623 pp. https:// doi.org/10.5962/bhl.title.10527
- Takahashi H, Yamagishi M (2020) A taxonomic re-investigation of *Papaver miyabeanum* and *P. fauriei* (Papaveraceae). Acta Phytotaxonomica et Geobotanica 71: 147–155. https://doi.org/10.18942/apg.201916

- Talbot SS, Yurtsev BA, Murray DF, Argus GW, Bay C, Elvebakk A (1999) Atlas of rare endemic vascular plants of the Arctic. Conservation of Arctic Flora and Fauna (CAFF) Technical Report 3. U.S. Fish and Wildlife Service, Anchorage, [iv +] 73.
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.
- Tatsis EC, Böhn H, Schneider B (2013) Occurrence of nudicauline structural variants in flowers of papaveraceous species. Phytochemistry 92: 105–112. https://doi. org/10.1016/j.phytochem.2013.04.011
- Tavakkoli Z (2012) Micromorphological and taxonomical study of the genus *Papaver* sect. *Meconidium* (Papaveraceae) in Iran. Iranian Journal of Botany 18: 270–283.
- Tavakkoli Z, Assadi M (2013) Comparison of morphological and micromorphological studies in the genus *Papaver* sect. *Oxytona* (Papaveraceae) and interspecific hybrids. Iranian Journal of Botany 19: 235–249.
- Tavakkoli Z, Assadi M (2016) Evaluation of seed and leaf epidermis characters in the taxonomy of some annual species of the genus *Papaver* (Papaveraceae). Nordic Journal of Botany 34(3): 302–321. https://doi.org/10.1111/njb.00833
- Thompson JD (2005) Plant evolution in the Mediterranean. Oxford University Press, New York, 293 pp. https://doi.org/10.1093/acprof:oso/9780198515340.001.0001
- Tison J-M, de Foucault B [Eds] (2014) Flora Gallica: Flore de France. Biotope Éditions, Mèze, 1196 pp.
- Tolmachev ['Tolmatchew'] Al (1930) *Papaver nudicaule* L. und einige verwandte asiatische *Papaver*-Formen. Svensk Botanisk Tidskrift 24: 33–43.
- Tolmachev AI (1931) Tri novykh sibirskiky vida r. Papaver. Zhurnal Russkogo Botanicheskogo Obshchestva 16: 77–82.
- Tolmachev AI (1971) O Papaver anomalum Fedde i P. amurense N.Busch. Novosti Sistematiki Vysshikh Rastenii 7: 148–164.
- Tolmachev AI (1975) Rod *Papaver* L. (Genus *Papaver* L.) In: Tolmachev AI (Ed.) Arkticheskaya Flora SSSR. VII. Papaveraceae-Cruciferae. Nauka, Moskva-Leningrad, 7–32.
- Tolmachev AI, Petrovsky VV (1973) Novye vidy *Papaver* s kraynego severo-vostoka Azii (A new species of *Papaver* from the far Northeast of Asia). Botanicheskii Zhurnal 58: 1127–1130.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeil J, Monro AM, Prado J, Price MJ, Smith GF (Eds) (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten, Koeltz Botanical Books. https://doi.org/10.12705/Code.2018
- Urumov IK (1920) Neue und seltene Pflanzen Bulgariens I. Magyar Botanikai Lapok 18: 33–40.
- Valtueña FJ, Preston CD, Kadereit JW (2012) Phylogeography of a Tertiary relict plant, *Meconopsis cambrica* (Papaveraceae), implies the existence of northern refugia for a temperate herb. Molecular Ecology 21(6): 1423–1437. https://doi.org/10.1111/ j.1365-294X.2012.05473.x
- Virtual Herbaria JACQ (2024) Database search. https://herbarium.univie.ac.at/database/search.php [Accessed 30.08.2024]
- Walker DA, Raynolds MK, Daniëls FJA, Einarsson E, Elvebakk A, Gould WA, Karenin AE, Kholod SS, Markon CJ, Melnikov ES, Moskalenko NG, Talbot SS, Yurtsev BA (2005)

The Circumpolar Arctic vegetation map. Journal of Vegetation Science 16(3): 267–282. https://doi.org/10.1111/j.1654-1103.2005.tb02365.x

- Wang WT (2019) Three new species of *Meconopsis* (Papaveraceae). Guihaia 30: 1–6. [in Chinese with English summary]
- Wąsowicz P (2020) Annotated checklist of the vascular plants of Iceland. Fjölrit Nátturufræðistofnunar 57: 1–191. https://doi.org/10.33112/1027-832X.57
- Wąsowicz P, Heiđmarsson S (2019) A vascular plant Red List for Iceland. Acta Botanica Islandica 16: 31–48.
- Wen J, Zhang J-Q, Nie Z-L, Zhong Y, Sun H (2014) Evolutionary diversification of plants on the Qinghai-Tibetan Plateau. Frontiers in Genetics 5: 1–16. https://doi.org/10.3389/ fgene.2014.00004
- Westergaard KB, Zemp N, Bruederle LP, Stenøien HK, Widmer A, Fior S (2019) Population genomic evidence for plant glacial survival in Scandinavia. Molecular Ecology 28(4): 818–832. https://doi.org/10.1111/mec.14994
- Wood JRI, Williams BRM, Mitchell TC, Carine MA, Harris DJ, Scotland RW (2015) A foundation monograph of *Convolvulus* L. (Convolvulaceae). PhytoKeys 51: 1–282. https:// doi.org/10.3897/phytokeys.51.7104
- Xiao W, Simpson BB (2015) Phylogenetic analysis of *Meconopsis* (Papaveraceae) and evaluation of two controversial taxonomic species. Lundellia 18(1): 14–27. https://doi.org/10.25224/1097-993X-18.1.14
- Xie H, Ash JE, Linde CC, Cunningham S, Nicotra A (2014) Himalayan-Tibetan Plateau uplift drives divergence of polyploid poppies: *Meconopsis* Viguier (Papaveraceae). PLoS ONE 9(6): e99177. https://doi.org/10.1371/journal.pone.0099177
- Xue J, Chepinoga VV, Liu Y, Keping M (2020) Mapping Asia plants: Historical outline and review of sources on floristic diversity in North Asia (Asian Russia). Global Ecology and Conservation 24: e01287. https://doi.org/10.1016/j.gecco.2020.e01287
- Xue J, Shcherbakov AV, Kipriyanova LM, Zhu L, Ma K (2023) Mapping Asia Plants: The threat status and influencing factors of rare and endangered vascular plant species in North Asia (Asian Russia). Plants 12(15): 2792. https://doi.org/10.3390/plants12152792
- Yakubov VV, Chernyagina OA, Berkutenko AN (2001) Flora of the Avachinsky volcano (south-east Kamchatka Peninsula). In: Berkutenko AN, Lumsden HG, Lumsden D (Eds) Flora and climatic conditions of the North Pacific: a collection of scientific papers. Institute of Biological Problems of the North, North-East Scientific Center, Federal Research Center of the Far East Branch of the Russian Academy of Sciences, Magadan, 3–30.
- Yamagishi M, Yoshida E, Aikoh T, Kondo T, Takahashi H (2010) A cultivated poppy (*Papaver* sp.) invades wild habitats of *Papaver fauriei* in the mountain area of Rishiri Island, Japan. Landscape and Ecological Engineering 6(2): 155–159. https://doi.org/10.1007/s11355-009-0089-0
- Yamagishi M, Onoma H, Kondo T, Kosugi K (2018) Ability of the endangered *Papaver fauriei* to produce hybrids with a cultivated poppy (*Papaver* sp.). Plant Species Biology 33(3): 167–173. https://doi.org/10.1111/1442-1984.12206
- Yıldırımlı Ş, Ertekin AS (2008) Two new species, *Papaver yilderimlii* Ertekin (Papaveraceae) and *Salvia ertekinii* Yıldırımlı (Lamiaceae) from Siirt, Turkey. Ot sistematik Botanik Dergisi 15: 1–8.
- Yoshida T (2019) New taxa of *Meconopsis* (Papaveraceae) from Wanba, southwestern Sichuan, China. Harvard Papers in Botany 24(1): 31–39. https://doi.org/10.3100/ hpib.v24iss1.2019.n6

- Yoshida T, Sun H (2017) Meconopsis lepida and M. psilonomma (Papaveraceae) rediscovered and revised. Harvard Papers in Botany 22(2): 157–192. https://doi. org/10.3100/hpib.v22iss2.2017.n11
- Yoshida T, Sun H (2018) Plants related to *Meconopsis psilonomma* (Papaveraceae) in northern Sichuan and southeastern Qinghai, China. Harvard Papers in Botany 23(2): 313–331. https://doi.org/10.3100/hpib.v23iss2.2018.n16
- Yoshida T, Sun H (2019a) Revision of *Meconopsis* Section *Forrestianae* (Papaveraceae). Harvard Papers in Botany 24(2): 379–421. https://doi.org/10.3100/hpib. v24iss2.2019.n20
- Yoshida T, Sun H (2019b) Revision of *Meconopsis castanea* (Papaveraceae) and its allies. Harvard Papers in Botany 24(2): 359–378. https://doi.org/10.3100/hpib. v24iss2.2019.n19
- Yoshida T, Yangzom R, Long DG (2016a) A new species of *Meconopsis*. Plantsman 15: 177–181.
- Yoshida T, Yangzom R, Long DG (2016b) Dancing butterflies of the East Himalayas new *Meconopsis* species from East Bhutan, Arunachal Pradesh and South Tibet. Sibbaldia 14: 69–96. https://doi.org/10.24823/Sibbaldia.2016.193
- Yoshida T, Xu B, Boufford DE (2019) Revision of *Meconopsis integrifolia* var. *uniflora* (Papaveraceae). Harvard Papers in Botany 24(1): 41–46. https://doi.org/10.3100/hpib. v24iss1.2019.n7
- Zapałowicz H (1911) Krytyczny przegląd roślinności Galicyi (część XXII) Conspectus florae Galiciae criticus (pars XXII). Bulletin International de l'Academie des Sciences de Cracovie, Classe des Sciences Mathématiques et Naturelles, Série B: Sciences Naturelles. Année 8B: 620–622.
- Zhang M, Grey-Wilson C (2008) 3. *Papaver* Linnaeus, Sp. Pl. 1: 506. 1753. In: Wu Z, Raven P (Eds) Flora of China, Vol. 7. Menispermaceae through Capparaceae. Missouri Botanical Garden Press, Saint Louis, 278–280.
- Zhang L-B, Comes HP, Kadereit JW (2004) The temporal course of Quaternary diversification in the European high mountain endemic *Primula* sect. *Auricula* (Primulaceae). International Journal of Plant Sciences 165(1): 191–207. https://doi. org/10.1086/380747

## **Supplementary material 1**

#### A phylogram including all ITS sequences of Oreomecon available at GenBank

Authors: Arve Elvebakk, Jarle W. Bjerke

- Data type: docx
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.121011.suppl1

## PhytoKeys

**Research Article** 

## *Maesa flabellifera* (Primulaceae), a new species from southeast Yunnan, China

Dan Wei<sup>1</sup>, Yuan Xu<sup>2,3</sup>, Gang Hao<sup>1</sup>, Timothy M. A. Utteridge<sup>4</sup>

1 College of Life Sciences, South China Agricultural University, Guangzhou 510642, China

2 State Key Laboratory of Plant Diversity and Specialty Crops, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

3 South China National Botanical Garden, Guangzhou 510650, China

4 Botanical Research, Singapore Botanic Gardens, 1 Cluny Road, Singapore 259569, Singapore

Corresponding author: Gang Hao (haogang@scau.edu.cn)

#### Abstract

*Maesa flabellifera* (Primulaceae-Maesoideae) from southeast Yunnan, China, is described and illustrated here. This new species belongs to the informal long corolla-tube species group and is morphologically similar to *M. permollis* and *M. kurzii*, but can be distinguished by lacking hairs, membranaceous leaves and long panicles with 7–16 branches. According to the IUCN criteria, *M. flabellifera* is assessed as "Least Concern".

Key words: Ericales, Maesoideae, morphology, taxonomy, Yunnan

### Introduction

The genus *Maesa* Forssk. (Primulaceae) contains approximately 185 species mainly distributed in tropical regions of the Old World from southern Africa through to the islands of the Pacific (Mez 1902; POWO 2024). It was originally placed in the monotypic subfamily Maesoideae of Myrsinaceae and later elevated to familial level as Maesaceae by Anderberg (2000). It is now included in an enlarged Primulaceae *s.l.* which includes four former families (Maesaceae, Myrsinaceae, Primulaceae and Theophrastaceae) and recognised as the only genus in the subfamily Maesoideae (APG 2016). According to the phylogenetic analysis, *Maesa* was found to form a basal branch, sister to all other Primulaceae species (Anderberg and Stähl 1995; Anderberg et al. 1998; Källersjö et al. 2000). Species of the genus *Maesa* are shrubs, trees or scramblers (Bremer et al. 2009) and can be distinguished from other genera of Primulaceae by the semi-inferior ovary, two bracteoles subtending each flower and black glandular lines scattered on the leaves, flowers and fruits (Sumanon et al. 2023).

Currently, approximately 35 species and two varieties (with 13 endemics) of *Maesa* have been recorded from China and they are mainly distributed in south-western China, especially in Yunnan Province. The first comprehensive revision of *Maesa* in China was conducted by Walker (1940). Chen (1979) recognised 29 species and one variety in China and divided these species into two sections, namely *Maesa* sect. *Maesa* and *M.* sect. *Doraena* [Thunb.] Nakai.



Academic editor: Avelinah Julius Received: 24 August 2024 Accepted: 14 October 2024 Published: 30 October 2024

Citation: Wei D, Xu Y, Hao G, Utteridge TMA (2024) *Maesa flabellifera* (Primulaceae), a new species from southeast Yunnan, China. PhytoKeys 248: 189–197. https://doi. org/10.3897/phytokeys.248.135449

**Copyright:** © Dan Wei et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Chen and Pipoly (1996) reviewed the Chinese species in the Flora of China and recognised 29 species. Recently, a few taxonomic reports of *Maesa* have been published from China (Yu et al 2016; Ding et al 2023).

In February 2021, during a field survey by the first author and colleagues in Dawei Mountain National Nature Reserve in Pingbian County, Honghe Prefecture, Yunnan, an unknown species of *Maesa* in blossom was encountered and gathered. In March 2023, the same plants were discovered again in two other counties of Honghe Prefecture, namely Yuanyang and Lüchun. After a careful comparison with similar species from China and adjacent countries, it was confirmed that this species is a distinct new one and is described here.

## Materials and methods

Three field populations from Honghe Prefecture were observed and collected in February 2021 and March 2023; examination of herbarium specimens also revealed its occurrence in some other counties of Honghe Prefecture. Morphological observations were based on living plants in field and specimens deposited at IBSC and KUN. Measurements were taken with a ruler or stereomicroscope (EZ4W). The new species was compared with type specimens of similar species of *Maesa* available at the JSTOR Global Plants, as well as the specimens in BKF, E, HN, K and PE. Relevant taxonomic literature (Utteridge 2000, 2001, 2015, 2021; Utteridge and Saunders 2001, 2004; Sumanon et al. 2021, 2023) was extensively consulted. Morphological terminology follows Beentje (2016), Hewson (2019), Hickey (1979) and the Systematics Association Committee for Descriptive Biological Terminology (1962). The conservation status of the new species was assessed following the guidelines for using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2024).

## **Taxonomic treatment**

Maesa flabellifera D.Wei, G.Hao & Utteridge, sp. nov.

urn:lsid:ipni.org:names:77351092-1 Figs 1, 2

**Type.** CHINA • Yunnan Province: Honghe Prefecture, Pingbian County, Dawei Mountain National Nature Reserve; 22.93, 103.69; 1871 m alt.; 26 February 2021 (fl.); *D. Wei et al. Xu210531* (Holotype: IBSC! barcode IBSC1025516).

**Diagnosis.** Maesa flabellifera is morphologically similar to *M. permollis*, but clearly differs from the latter in the indumentum (lacking hairs vs. rusty hirsute hairs present), inflorescence structure (panicles 4.0–6.5 cm long with 7–16 branches vs. racemes or panicles 1–3 cm long with up to 3 branches). It is also similar to *M. kurzii*, but can be distinguished by the indumentum (lacking hairs vs. presence of rusty tomentose and strigose hairs) and lamina texture (membranaceous vs. chartaceous).

**Description.** Large shrub, up to 2.5 m tall. *Indumentum* all parts lacking hairs, scales present on leaves, inflorescences and fruits, scales peltate, black, ± sessile, circular with irregular margins. *Branches* dark green with scattered lenticels, sparsely scaly. *Leaves* lamina broadly elliptic to obovate, 15–35 cm long, 6–20 cm wide, membranaceous, dark green above, pale grey-green below,



Figure 1. Holotype of Maesa flabellifera D.Wei, G.Hao & Utteridge, sp. nov. (D. Wei et al. Xu210531, IBSC1025516, IBSC).



Figure 2. *Maesa flabellifera* **A** habitat **B** habit **C** node with petiole and base of inflorescence **D** abaxial and adaxial surfaces of leaf **E** inflorescence **F** bract (borne at base of pedicel) and bracteole (borne at base of the hypanthium) **G** flower after removal of corolla **H** corolla from G, opened flat **I** Infructescences.

adaxial and abaxial surface sparsely scaly; base obtuse to cuneate; margins serrulate-serrate with 20-34 teeth on each side; apex acuminate to obtuse, sometimes emarginate; mid-rib sparsely scaly adaxially and abaxially; secondary veins 10-18 pairs, craspedodromous; densely longitudinally glandular lines; petiole 1.5-3.0 cm long, sparsely scaly. Staminate inflorescences lateral (axillary), sometimes terminal, panicles, with 7-16 branches, 4.0-6.5 cm long, axis scaly; pedicels 0.5-1.5 mm long; bracts ovate, 1.20-1.65 mm long, scaly to densely scaly, margins entire, apex acute; bracteoles ± opposite, inserted at the base of the hypanthium, triangular, 0.90-1.35 mm long, 0.4-0.6 mm wide, apex acute, margins entire, scaly. Staminate flowers pentamerous, white; calyx lobes triangular, 1.25-1.60 mm long, 0.70-1.05 mm wide, margins entire, apex acute to rounded; corolla tube 1.9-2.3 mm long, corolla lobes broadly triangular, 1.45-1.55 mm long, 1.5-1.8 mm wide, margins entire, apex rounded; stamens 5, arising 0.8-1.0 mm from the base of the corolla, filaments 1.14-1.37 mm long, anthers 0.59-0.69 mm long; hypanthium 0.75-1.20 mm long, scaly to sparsely scaly; style 1.5-2.0 mm long, stigma ± 3-lobed. Pistillate inflorescences and flowers not seen. Fruits sub-globose, ca. 3.5 mm long, ca. 3 mm in diameter, scaly to sparsely scaly; pedicels at fruiting 0.50-1.66 mm long; bracteoles remaining ± opposite at the base of the fruit; persistent calyx lobes non-overlapping.

**Distribution and habitat.** According to the specimens examined and the recent field investigations, *Maesa flabellifera* is presently found in Honghe Prefecture, Yunnan Province (Map 1). It is common in evergreen broad-leaved mixed forests at elevations of 1500–2200 m.

**Phenology.** Flowering from January to March, fruiting from April to December.

**Etymology.** The specific epithet '*flabellifera*' is derived from the Latin 'flabella' and 'fera' to refer to its inflorescence with 7–16 branches of almost equal length and spreading, looking like a branching fan.





**Vernacular name.** Chinese Mandarin: shan xing du jing shan (扇形杜茎山).

**Preliminary conservation status.** *Maesa flabellifera* is widely distributed in southeast Yunnan. In the populations in the Dawei Mountain National Nature Reserves (43993 hm<sup>2</sup>) and Huanglian Mountain National Nature Reserves (65058 hm<sup>2</sup>), the habitats are well-protected and not threatened and individuals have been found locally common in each site. Based on currently available data, *M. flabellifera* is preliminarily assessed as Least Concern (LC) according to IUCN Categories and Criteria (IUCN Standards and Petitions Committee 2024).

Additional specimens examined (paratypes). CHINA, Yunnan, Honghe Prefecture • Yuanyang County, Xinjie Town; 1891 m alt.; 22 March 2023 (fl.); Wei et al. Xu231213 (IBSC, barcode IBSC1025520) • Lüchun County, Huanglian Mountain National Nature Reserve; 1865 m alt.; 23 March 2023 (fl.); Wei et al. Xu231222 (IBSC, barcode IBSC1025523) • Jinping County; 2192 m alt.; 16 January 2010 (fl.); Southeast Yunnan expedition. GBOWS956 (KUN, barcode KUN1279679) • Pingbian County; 1520 m alt.; 23 November 2009 (fr.); Qian et al. Pbdws151 (KUN, barcode KUN1339632).

**Notes.** Based on a phylogenetic analysis of molecular data, a new infrageneric classification of *Maesa* was proposed, dividing it into five subgenera, based on distribution and morphological characters (Sumanon et al. 2023). The species-level tree shows a strong signal of geographical distribution for the new infrageneric classification. It is speculated that *Maesa flabellifera* should be placed in the *Maesa* subg. *Indicae* Sumanon, Eiserhardt & Utteridge, by far the most species-rich subgenus in China, with species of trees or shrubs mainly from the Asian Continent, since its morphology and distribution is consistent with this clade especially the leaf morphology, such as the serrulate-serrate margins.

Maesa flabellifera belongs to the group of species with a longer corolla-tube. In the Flora Reipublicae Popularis Sinicae (Chen 1979), Maesa was divided into two sections based on the ratio of corolla-tube length to lobe length, namely Maesa sect. Maesa and M. sect. Doraena [Thunb.] Nakai. There are eight species with a long corolla-tube similar to M. flabellifera in sect. Doraena [Thunb.] Nakai. This treatment was not adopted in the Flora of China (Chen and Pipoly 1996). Although the long corolla-tube is a good character for species-level identification as a 'spot character', the group is not monophyletic in the phylogenetic analysis (Sumanon et al. 2023) and is used here as a comparative tool.

Maesa flabellifera is unique within the long corolla-tube species group, differing from all others by the following characters: lacking hairs on all parts; leaves thick, membranaceous and broadly elliptic to obovate, 15–35 cm long and 8–20 cm wide; long paniculate inflorescences, up to 6.5 cm long, with 7–16 branches, each branch almost equal in length, looking like a branching fan arising from the leaf axils.

In the key to *Maesa* in the Flora of China (Chen and Pipoly 1996), *M. flabel-lifera* would key out close to *M. permollis* Kurz as they share the same leaf features and the long corolla-tube. However, *M. flabellifera* is unlikely to be confused with *M. permollis* by examination of the indumentum and inflorescence structure. Based on the herbarium and field observations, *M. permollis* is conspicuously hairy throughout with rusty hirsute hairs and the inflorescences are short, dense clustering of numerous flowers, forming compact, many-flowered inflorescence clusters. Moreover, *M. flabellifera* is found in higher elevations

around 1500–2200 m, compared to *M. permollis* which is encountered at lower elevations around 500–1600 m.

Compared to the other *Maesa* species with long corolla-tubes, *M. flabellifera* is most similar to *M. kurzii*, sharing broadly elliptic to obovate leaves and long paniculate inflorescences. However, the indumentum and lamina texture make *M. flabellifera* very distinctive and easily separated from *M. kurzii*, which has chartaceous leaves, usually rusty tomentose hairs throughout and inflorescences with rusty strigose hairs. Furthermore, the distributions of these two species are distinctly different and non-overlapping. *Maesa flabellifera* is currently only known from southeast Yunnan, situated in Honghe Prefecture. *Maesa kurzii* is located within Myanmar. A detailed comparison of these three species is shown in Table 1.

Features	M. flabellifera	M. permollis	M. kurzii
Indumentum	lacking hairs	rusty hirsute hairs	rusty tomentose and strigose hairs
Leaf texture	membranaceous	membranaceous	chartaceous
Inflorescence structure	panicles with 7-16 branches	racemes or panicles with up to 3 branches	panicles with 4-10 branches
Inflorescence length	4.0-6.5 cm	1-3 cm	3.0-4.5 cm
Elevation	1500-2200 m	500-1600 m	500-1000 m

Table 1. Morphological and ecological comparison between Maesa flabellifera and its allies.

### Acknowledgements

We greatly appreciate Herbaria of IBSC, KUN, BKF, E, HN, K and PE for access to specimen consultancy.

## Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

The study was financially supported by the National Natural Science Foundation of China (grants no. 32070220 and 32070230).

#### Author contributions

All authors have contributed equally.

#### Author ORCIDs

Timothy M. A. Utteridge D https://orcid.org/0000-0003-2823-0337

#### **Data availability**

All of the data that support the findings of this study are available in the main text.

## References

- Anderberg AA (2000) Maesaceae, a new primuloid family in the order Ericales s.l. Taxon 49(2): 183–187. https://doi.org/10.2307/1223834
- Anderberg AA, Stähl B (1995) Phylogenetic interrelationships in the order Primulales, with special emphasis on the family circumscriptions. Canadian Journal of Botany 73(11): 1699–1730. https://doi.org/10.1139/b95-184
- Anderberg AA, Stahl B, Källersjö M (1998) Phylogenetic relationships in the Primulales inferred from *rbcL* sequence data. Plant Systematics and Evolution 211: 93–102. https://doi.org/10.1007/BF00984914
- APG [Angiosperm Phylogeny Group] (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of Linnean Society 181(1): 1–20. https://doi.org/10.1111/boj.12385
- Beentje H (2016) The Kew Plant Glossary: an illustrated dictionary of plant terms, second edition. Royal Botanic Gardens Kew, London, 192.
- Bremer B, Bremer K, Chase MW, Fay MF, Reveal JL, Bailey LH, Soltis DE, Soltis PS, Stevens PF (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of Linnean Society 161(2): 105–121. https://doi.org/10.1111/j.1095-8339.2009.00996.x
- Chen J (1979) Myrsinaceae. In: Chen J (Ed.) Flora Reipublicae Popularis Sinicae 58. Science Press, Beijing, 1–38.
- Chen J, Pipoly JJ (1996) Myrsinaceae. In: Wu ZY, Raven PH (Eds) Flora of China 15. Science Press, Beijing and Missouri Botanical Garden Press, St Louis, 1–38.
- Ding HB, Wang LY, Quan DL, Yang B, Yue MM, Wang PY, Yang YJW, Gong QB, Zhou SS, Wang L, Li JW, Tan YH (2023) Additions to the seed plant flora in Yunnan, China. Biodiversity Science 31(10): 23254, 1–12. https://doi.org/10.17520/biods.2023254
- Hewson HJ (2019) Plant Indumentum: A Handbook of Terminology, Revised edition. Australian Biological Resources Study, Commonwealth Department of the Environment and Energy, 47 pp.
- Hickey LJ (1979) A revised classification of the architecture of dicotyledonous leaves. In: Metcalfe CR, Chalk L (Eds) Anatomy of the Dicotyledon. Clarendon Press, Oxford, 25–39.
- IUCN Standards and Petitions Committee (2024) Guidelines for using the IUCN Red List categories and criteria. Version 16: Prepared by the Standards and Petitions Committee in March 2024.
- Källersjö M, Bergqvist G, Anderberg AA (2000) Generic realignment in primuloid families of the Ericales *s.l.*: a phylogenetic analysis based on DNA sequences from three chloroplast genes and morphology. American Journal Botany 87(9): 1325–1341. https:// doi.org/10.2307/2656725
- Mez C (1902) Myrsinaceae. In: Engler A (Ed.) Das Pflanzenreich 9. IV.236. Wilhelm Engelmann, Berlin, 1–437.
- POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Garden, Kew. http://www.plantsoftheworldonline.org/
- Sumanon P, Eiserhardt WL, Balslev H, Utteridge TMA (2021) Six new species of *Maesa* (Primulaceae) from New Guinea. Phytotaxa 505: 245–261. https://doi.org/10.11646/ phytotaxa.505.3.1
- Sumanon P, Balslev H, Utteridge TMA, Eiserhardt WL (2023) A species-level phylogenetic framework and infrageneric classification for the genus *Maesa* (Primulaceae). Taxon 72(4): 1–20. https://doi.org/10.1002/tax.12991

- Systematics Association Committee for Descriptive Biological Terminology (1962) IIa. Terminology of Simple Symmetrical Plane Shapes (Chart 1a), Addendum. Taxon 11 (8): 245–247. https://doi.org/10.2307/1217034
- Utteridge TMA (2000) Two new species of *Maesa* (Myrsinaceae) from Puncak Jaya, New Guinea. Contributions to the Flora of Mt. Jaya, I. Kew Bulletin 55: 443–449. https://doi.org/10.2307/4115658
- Utteridge TMA (2001) Two new species of *Maesa* (Maesaceae) from New Guinea. Kew Bulletin 56: 677–683. https://doi.org/10.2307/4117694
- Utteridge TMA (2015) A new species of *Maesa* (Primulaceae-Maesoideae) from New Guinea. Blumea 59: 226–228. https://doi.org/10.3767/000651915X689505
- Utteridge TMA (2021) Flora of Singapore precursors 26: The genus *Maesa* (Primulaceae) in Singapore and clarification of *Maesa ramentacea* in Malesia. Gardens' Bulletin Singapore 73(2): 267–278. https://doi.org/10.26492/gbs73(2).2021-04
- Utteridge TMA, Saunders RMK (2001) Sexual dimorphism and functional dioecy in *Maesa perlarius* and *M. japonica* (Maesaceae/Myrsinaceae). Biotropica 33(2): 368–374. https://doi.org/10.1111/j.1744-7429.2001.tb00190.x
- Utteridge TMA, Saunders RMK (2004) The genus *Maesa* (Maesaceae) in the Philippines. Botanical Journal of the Linnean Society. 145: 17–43. https://doi.org/10.1111/j.1095-8339.2003.00261.x
- Walker EH (1940) A revision of the eastern Asiatic Myrsinaceae. Philippine Journal of Sciences 73(1/2): 1-258.
- Yu SX, Hao G, Jin XF (2016) Spermatophytes. In: Chen YY (Ed.) Species Catalogue of China 1. VII. Science Press, Beijing, 128–130.



**Research Article** 

## *Ophiorrhiza liuyanii* (Rubiaceae), a new species from south-western China and northern Vietnam

Chu-Yu Liu<sup>1</sup>, Xiao-Wen Liao<sup>2</sup>, Li-Chun Ye<sup>2</sup>, Yun-Hong Tan<sup>3</sup>, Khang Sinh Nguyen<sup>4,5</sup>, Tran Duc Thien<sup>5,6</sup>, Lei Wu<sup>2</sup>

- 1 School of Minerals Processing and Bioengineering, Central South University, Changsha 410083, China
- 2 College of Forestry, Central South University of Forestry and Technology, Changsha 410004, China
- 3 Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
- 4 Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18, Hoang Quoc Viet Road, Cau Giay, Hanoi 10072, Vietnam
- 5 Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi 10072, Vietnam
- 6 Regional Research and Development Institute, Ministry of Science and Technology, No. 70, Tran Hung Dao, Hoan Kiem District, Hanoi, Vietnam

Corresponding author: Lei Wu (wuleiibk@163.com)

#### Abstract

Ophiorrhiza liuyanii, a new species from south-western China and northern Vietnam, is described and illustrated. The new species is characterised by the glabrous surfaces on almost all plant parts, congested inflorescences and broad-ovate to ovate bracts  $9-22 \times 4-11$  mm. It morphologically differs from the closest species, *O. baviensis*, mainly in most plant parts being glabrous, bracts broad-ovate to ovate, apex acute or sometimes obtuse, corollas inside with a ring of white hairs at the middle and anthers inserted near the middle in long-styled flowers. According to IUCN Categories and Criteria, *O. liuyanii* is assessed as Least Concern (LC).

Key words: New taxon, Ophiorrhiza, Rubiaceae, taxonomy

### Introduction

The genus *Ophiorrhiza* Linnaeus is an Indo-Malesian genus of Rubiaceae with species distributed in tropical and subtropical regions of Asia, with only a few extending to Australia, New Guinea and the Pacific Ocean (Tran 2005; Chen and Taylor 2011; Hareesh et al. 2015; Li 2020; Schanzer and Nabatov 2022; Shang et al. 2024). Representatives of this genus are annual or perennial herbs or rarely sub-shrubs, easily recognised by having obcordate and compressed fruits that are dehiscent with two valves along a transverse slit at the top (Darwin 1976; Lo 1990; Wu et al. 2015) and usually growing in moist locations or stream-sides under evergreen forests (Darwin 1976; Deb and Mondal 1997; Chen and Taylor 2011; Hareesh et al. 2015). In spite of the clear monophyly of the whole genus, based on capsule shape, the total species number of the genus is unclear and is estimated to be from 200 species (Lo 1999; Li 2020) to as many as 300 species (Alfeche et al. 2020; Taher et al. 2020; Zhou et al. 2020; Idrees et al. 2023) due to the lack of a worldwide revision.



Academic editor: Petra De Block Received: 19 August 2024 Accepted: 10 October 2024 Published: 30 October 2024

Citation: Liu C-Y, Liao X-W, Ye L-C, Tan Y-H, Nguyen KS, Thien TD, Wu L (2024) *Ophiorrhiza liuyanii* (Rubiaceae), a new species from south-western China and northern Vietnam. PhytoKeys 248: 199–206. https://doi.org/10.3897/ phytokeys.248.135078

**Copyright:** © Chu-Yu Liu et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

China is a diversity centre of Ophiorrhiza with about 74 taxa recorded (Wu et al. 2017, 2018; Tu et al. 2018; Yang et al. 2018; Hu et al. 2021; Liu et al. 2023; Shang et al. 2024; Zhan et al. 2024). Most of Chinese Ophiorrhiza are distributed in southern and south-western China, particularly in Guangxi and Yunnan Provinces (Lo 1999; Chen and Taylor 2011). While examining Ophiorrhiza specimens at PE Herbarium in 2013, we found an unusual sheet with congested inflorescences, broadly ovate bracts and winged corolla outside. Due to the single specimen and lack of information inside the corolla, we tentatively treated it as O. baviensis Drake and thought that the difference in bracts might be a variable character within this species. In recent field surveys in Menghai County, south-western Yunnan, the peculiar plants of this species with fruits in 2014 and flowers in 2024 were observed and re-collected. After carefully examining fresh and dried material of the abovementioned species, we found that it is distinctly different from O. baviensis by the glabrous surfaces on most plant parts, larger bracts broad-ovate to ovate, the indumentum inside corollas and the placement of stigma and anthers (Wu et al. 2019). Further, from a comprehensive comparison between this peculiar plant with other known species of the genus, we concluded that it represents a new taxon, which is described hereafter.

## Materials and methods

Field observations were carried out in south-western China in 2014 and 2024 and northern Vietnam in 2022. The morphological characteristics of a new *Ophiorrhiza* species were observed and measured in the field and laboratory. The morphological variations of 30 individuals were measured with a ruler and a micrometer. Specimens of the new species were preserved in the Forest Plant Herbarium (CSFI) of Central South University of Forestry and Technology and other herbaria (BNU, CSFI, HITBC, HN and LE). Acronyms for all herbaria in the text follow Thiers (2024). The conservation status of the new species was evaluated, based on field observations and referred to the IUCN Red List Guidelines (IUCN 2023).

## **Taxonomic treatment**

*Ophiorrhiza liuyanii* L.Wu, Y.H.Tan & K.S.Nguyen, sp. nov. urn:lsid:ipni.org:names:77351093-1

Figs 1, 2A-K

**Type.** CHINA • Yunnan Province: Menghai County, Mengsong Village, growing along a stream or on moist slopes under densely evergreen broad-leaved forests, 21°30'37.36"N, 100°30'17.33"E, elevation 1715 m, 13 Apr 2024 (fl.), *X.W. Liao LXW0217* (holotype: CSFI!; isotypes: CSFI!).

**Diagnosis.** Morphologically similar to *O. alatiflora* and *O. baviensis*, but the new species differs from the former by its congested (vs. developing) inflorescences and infructescences, broad-ovate to ovate (vs. linear or linear-lanceolate) bracts, 4–11 (vs. 0.8–1.5) mm wide and from the latter by its glabrous (vs. densely pubescent or puberulent) peduncles, broad-ovate to ovate (vs. lanceolate) bracts, corollas tube inside with (vs. without) a ring of white hairs at the middle in long-styled flowers.





**Description.** Perennial herbs, erect or ascending at the base, up to 80 cm tall; stem, leaves, petiole, stipule, bract, outside flower and capsule glabrous. Leaves generally in equal pairs (usually isophyllous); petioles 1-3 cm, pale green; leaf blades drying papery, dark green adaxially, pale green abaxially, elliptic, oblong or ovate-elliptic,  $7-15 \times 3-6$  cm, cuneate at base, acuminate at apex, margins entire; secondary veins 9-13 at each side; stipules small, broadly triangular, ca. 1 mm long, caducous, with glands at the inner base. Inflorescences congested cymose, many-flowered, drooping at the early stage, then erect; peduncles 1-2 cm long, pale green; bracts broad-ovate to ovate,  $9-22 \times 4-11$  mm, apex acuminate, acute or sometimes obtuse. Flowers heterostylous; pedicels to 3 mm long, puberulent.



Figure 2. Ophiorrhiza liuyanii A habitat B habit C stipule D inflorescence in side view E inflorescences in different development stages F bracts from lower part to upper part of inflorescence G corollas in top view H longitudinally dissected long-styled flower I longitudinally dissected short-styled flower J infructescence in side view K infructescence in top view. Ophiorrhiza alatiflora L habit M inflorescence in lateral view N infructescence. Scale bars: 1 cm. Photos by L. Wu, X. W. Liao and K. S. Nguyen.

Calyx puberulent; hypanthium oblate,  $1.5-1.8 \times 1.8-2.2$  mm; lobes triangular to ovate triangular, 0.8-1.6 mm long, acuminate at apex. Corolla white or pink-ish-white, subtubular; tube 1.0-1.6 cm long, outside longitudinally winged from

apex to base, wings straight or undulate, ca. 0.8-2 mm wide; lobes 5, ovate-triangular,  $3.8-4.8 \times 2.8-3.5$  mm, inside pubescent, apex acute, slightly incurved. Stamens 5; anthers linear, 2.2-3.2 mm long. Stigma bilobed; ovary 2-celled. Longstyled flowers: inside with a ring of white hairs at the middle of the corolla tube and puberulent from the middle up to the throat; stamens included, positioned a little below the middle of the corolla tube; style 8-12 mm long, densely pubescent; stigma positioned at the corolla throat, lobes elliptic, ca. 1.8 mm long. Short-styled flowers: sparsely pubescent at the middle of the corolla tube; stamens reaching slightly beyond corolla throat, not exserted; style 3.8-5.5 mm long, pubescent; stigma lobes lanceolate, ca. 2.8 mm long. Capsules mitriform, ca.  $4.5 \times 10$  mm.

**Phenology.** Flowering from April to May; fruiting from May to July.

**Distribution and habitat.** *Ophiorrhiza liuyanii* is currently known from south-western China (Menghai County of southern Yunnan Province) and north-western Vietnam (Dien Bien Province). It grows along streams or moist places under evergreen broad-leaved forests at an elevation range from 1500–1850 m, in which the vegetation is dominated by the tree families Lauraceae, Fagaceae, Magnoliaceae, Theaceae and Betulaceae, shrub families Ericaceae and Symplocaceae and herbaceous families Urticaceae, Balsaminaceae and Begoniaceae.

**Preliminary conservation status.** Our field surveys revealed that three populations of *Ophiorrhiza liuyanii* have a total of matured individuals of ca. 5000 plants. The population of the holotype locality is the largest and is in good condition because their occupied area is included in the Nabanhe River Watershed National Nature Reserve and, during our 10 yearly re-visitation, we found the habitats had been well-protected. Therefore, the new species is preliminarily assessed as Least Concern (LC) according to IUCN (2023).

**Etymology.** The species epithet is named after Prof. Yan Liu, Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and the Chinese Academy of Sciences, who has made great contributions to plant taxonomy in China.

Chinese name. 宽翅蛇根草 (kuan-chi-she-gen-cao).

Additional specimens examined (*paratypes*). CHINA • Same village as holotype, elevation 1700 m, 2 Apr 2001 (fl.) H. Wang 4311 (PE 2014155!), elevation 1500 m, 7 Jun 2014 (fr.), L. Wu 3706 (BNU! CSFI!), 21°30'42.43"N, 100°30'18.73"E • elevation 1700 m, 13 Apr 2024 (fl.), X.W. Liao LXW0219 (CSFI!), 21°30'27.65"N, 100°30'27.12"E • elevation 1695 m, 13 Apr 2024 (fl.), X.W. Liao LXW0220 (CSFI!).

VIETNAM • Dien Bien Province: Muong Nhe Distr., Muong Nhe Natural Reserve, Sin Thau Municipality, Ta Mieu Village, around point 22°24'02"N, 102°08'38"E, elevation 1800–1850 m, old humid secondary evergreen broad-leaved montane forest of very steep mountain slopes composed of sandstone, terrestrial herb to 0.5 m tall, flower pinkish-white, common, 14 May 2022, L. Averyanov, H.T. Tran, K.S. Nguyen, H.C. Nguyen, T. Maisak, C.K. Bac, VR 1637 (HN!, LE!).

**Notes.** Ophiorrhiza liuyanii is morphologically most similar to *O. baviensis* on having congested inflorescences and distinct, persistent bracts. However, the former differs from the latter mainly by its glabrous (vs. pubescent or puberulent) stems, peduncles and calyx (Figs 2B, C, D, E, 3E, F), broad-ovate to ovate (vs. lanceolate) bracts with larger in size,  $9-22 \times 4-11$  (vs.  $6-15 \times 2-7$ ) mm (Figs 2D–F, 3C, D, G), corolla tubes inside with (vs. without) a ring of white hairs at the middle and anthers positioned near the middle (vs. base) in long-styled flowers (Figs 2H, I, 3E, F). The new species also resembles *O. alatiflora* by having wings longitudinally and wider than 0.8 mm outside corolla, but it clearly differs by its congested (vs. developing)



Figure 3. Ophiorrhiza baviensis A habit B inflorescence in top view C, D inflorescence in side view E longitudinally dissected long-styled flower F longitudinally dissected short-styled flower G infructescence in top view. Scale bars: 1 cm. Photos by L. Wu.

	Table 1	. Morphological	comparison of	Ophiorrhiza liu	ıvanii. O. a	alatiflora and O	baviensis.
--	---------	-----------------	---------------	-----------------	--------------	------------------	------------

	Ophiorrhiza liuyanii	O. alatiflora	O. baviensis
stem	glabrous	glabrous	glabrous to densely pubescent
peduncles	glabrous	glabrous	densely pubescent or puberulent
inflorescence	congested	congested when young, then developing when matured	congested
bracts	broad-ovate to ovate, 9–22 × 4–11 mm, apex acute or sometimes obtuse, glabrous	linear or linear-lanceolate, 5–15 × 0.8–1.5 mm, apex acute, glabrous	lanceolate, $6-15 \times 2-7$ mm, apex accumulate, puberulent or ciliate
calyx	glabrous	puberulent	pubescent, sometimes densely
corolla	subtubular	subtubular	tubular, slightly swollen at base
long-styled flowers inside	inside with a ring of white hairs at the middle	inside with a ring of white hairs at the middle	inside densely pubescent, but without a ring of white hairs at the middle
anthers and stigma	inserted near the middle and the throat of corolla tube in long-styled flowers respectively, while opposite in the short-styled flowers	inserted near the middle and the throat of corolla tube in long-styled flowers respectively, while opposite in the short-styled flowers	inserted near the base and above middle of corolla tube in long-styled flowers respectively, while opposite in the short-styled flowers

inflorescences and infructescences (Figs 2B, D, E, J, K, M, N), broad-ovate to ovate (vs. linear or linear-lanceolate) bracts, 4-11 (vs. 0.8-1.5) mm wide (Figs 2F, J, K, N). Further distinctive characteristics of the three species are shown in Table 1.

## Acknowledgements

The authors are grateful to Miss Ming-Min Chen for her excellent drawing; the staff of BNU, HITBC, HN, IBK, IBSC, KUN and PE for permission to examine their specimens.

## **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

#### Ethical statement

No ethical statement was reported.

#### Funding

This study was supported by the project of National Plant Specimen Resource Center (NPSRC) (grant. no. 0117G1001). The work of KSN was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.03-2023.21.

#### Author contributions

All authors have contributed equally.

## **Author ORCIDs**

Xiao-Wen Liao I https://orcid.org/0000-0003-2652-2862 Khang Sinh Nguyen I https://orcid.org/0000-0001-5171-4140 Lei Wu I https://orcid.org/0000-0003-1451-7855

#### Data availability

All of the data that support the findings of this study are available in the main text.

## References

Alfeche NKG, Alejandro GJD, Meve U, Liede-Shumann S (2020) Two new endemic species of *Ophiorrhiza* L. (Rubiaceae: Ophiorrhizeae) from Davao Oriental, Philippines. Nordic Journal of Botany 38(3): e02581. https://doi.org/10.1111/njb.02581

Chen T, Taylor CM (2011) *Ophiorrhiza*. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol. 19). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 258–282.

- Darwin SP (1976) The Pacific species of Ophiorrhiza L. (Rubiaceae). Lyonia 1: 48–101. https://doi.org/10.3897/phytokeys.121.30570
- Deb DB, Mondal DC (1997) Taxonomic revision of the genus *Ophiorrhiza* L. (Rubiaceae) in Indian subcontinent. Nelumbo 39: 1–148. https://doi.org/10.20324/nelumbo/ v39/1997/74298
- Hareesh VS, Sreekumar VB, Kumar KMP, Nirmesh TK, Sreejith KA (2015) *Ophiorrhiza sa-hyadriensis* (Rubiaceae), a new species from southern Western Ghats, Kerala, India. Phytotaxa 202(3): 219–224. https://doi.org/10.11646/phytotaxa.202.3.6
- Hu YH, Liu WJ, Song XF, Deng GX, Nakamura K, Wu L, Liu QR (2021) A discussion of the relationship between *Ophiorrhiza exigua* and *O. michelloides* (Rubiaceae) with the description of a new species. Nordic Journal of Botany 39(6): e03138. https://doi. org/10.1111/njb.03138

Idrees M, Li M, Zhang Z (2023) Transfer of six species of *Spiradiclis* Blume to *Ophiorrhiza* L. (Rubiaceae). Phytotaxa 579(3): 225–227. https://doi.org/10.11646/phytotaxa.579.3.8

IUCN (2023) Guidelines for using the IUCN Red List categories and criteria, version 14. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/resources/redlistguidelines [Accessed 28 July 2023]

- Li DZ (2020) The Families and Genera of Chinese Vascular Plants (Vol. III). Science Press, Beijing, 1761 pp.
- Liu Q, Chen AX, Liao XW, Liu QR, Wu L (2023) *Ophiorrhiza pseudonapoensis* (Rubiaceae), a new species from Yunnan, southwestern China. Phytotaxa 607 (4): 228–234. https://doi.org/10.11646/phytotaxa.607.4.1
- Lo HS (1990) Taxonomic revision of the Chinese species of *Ophiorrhiza* (Rubiaceae). Bulletin of Botanical Research 10(2): 1–82.
- Lo HS (1999) *Ophiorrhiza*. In: Lo HS (Ed.) Flora Reipublicae Popularis Sinicae (Vol. 71(1)). Science Press, Beijing, 110–174.
- Schanzer IA, Nabatov AA (2022) Taxonomic reassessment and lectotypification of 24 species names in *Ophiorrhiza* (Rubiaceae, Rubioideae) from Thailand. Nordic Journal of Botany 2022(2): e03280. https://doi.org/10.1111/njb.03280
- Shang C, Xue J, Yang Y, Liao X, Liu Q, Wu L (2024) *Ophiorrhiza reflexa* (Rubiaceae), a new species from a karst region in Guangxi, China. PhytoKeys 238: 231–240. https://doi. org/10.3897/phytokeys.238.116767
- Taher M, Shaari SS, Susanti D, Arbain D, Zakaria ZA (2020) Genus *Ophiorrhiza*: a review of its distribution, traditional uses, phytochemistry, biological activities and propagation. Molecules 25: 2611. https://doi.org/10.3390/molecules25112611
- Thiers B (2024) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: http://sweet-gum.nybg.org/science/ih/ [accessed 12 August 2024]
- Tu RH, Li JL, Wu L, Hareesh VS (2018) *Ophiorrhiza gaoligongensis* (Rubiaceae), a new species from southwestern China. Novon 26(4): 351–354. https://doi. org/10.3417/2018309
- Wu L, Wang JL, Liu QR (2015) Spiradiclis pauciflora (Rubiaceae), a new species from limestone areas in Guangxi, China. Annales Botanici Fennici 52: 257–261. https:// doi.org/10.5735/085.052.0318
- Wu L, Deng YF, Tan YH (2017) Notes on Ophiorrhiza hispida (Rubiaceae) from China. Journal of Tropical and Subtropical Botany 25: 597–600. https://doi.org/10.11926/ JTSB.3750
- Wu L, Tan YH, Hareesh VS, Liu QR (2018) Ophiorrhiza macrocarpa (Rubiaceae), a new viviparous species from Yunnan, south western China. Nordic Journal of Botany 36(4): 1–5. https://doi.org/10.1111/njb.01637
- Wu L, Liu WJ, Nguyen KS (2019) Revision of three taxa of *Ophiorrhiza* (Rubiaceae) from China. Phytotaxa 87: 129–139. https://doi.org/10.11646/phytotaxa.387.2.5
- Yang CD, He XZ, Gou GQ (2018) Ophiorrhiza guizhouensis (Rubiaceae), a new species from Guizhou Province, southwestern China. PhytoKeys 95: 121–126. https://doi. org/10.3897/phytokeys.95.22506
- Zhan M, Liao XW, Song F, Xue L, Liu QR, Wu L (2024) *Ophiorrhiza paralatiflora* (Rubiaceae), a new species from limestone areas in Guangxi, China. Nordic Journal of Botany 2024(7): e04391. https://doi.org/10.1111/njb.04391
- Zhou SS, Li R, Quan RC, Shine L, Duan LD (2020) Ophiorrhiza monsvictoriae (Rubiaceae, Rubioideae), a new species from Myanmar. In: Jin XH, Xia NH, Tan YH (Eds) Plant diversity of Southeast Asia-II. PhytoKeys 138: 219–223. https://doi.org/10.3897/phytokeys.138.38966

## PhytoKeys

**Research Article** 

# Resolving the *Drymonia killipii* (Gesneriaceae) complex results in a new species from the northwestern Andes of South America

Laura Clavijo<sup>1,2</sup>, John L. Clark<sup>2</sup>

1 Universidad Nacional de Colombia, Sede Bogotá, Facultad de Ciencias, Instituto de Ciencias Naturales, Bogotá, D.C., 111321, Colombia

2 Marie Selby Botanical Gardens, Sarasota, FL, USA

Corresponding author: Laura Clavijo (lvclavijor@unal.edu.co)

#### Abstract

A new species, *Drymonia quadrangulata* Clavijo & J.L.Clark, **sp. nov.** (Gesneriaceae, Columneinae), is described from the western Andean slopes of southern Colombia and northern Ecuador. The new species has been historically confused with *D. killipii*, an endemic species to Colombia, restricted to the lowlands of the Chocó biogeographic region in the departments of Cauca, Chocó, and Valle del Cauca. These two species have large foliaceous calyx lobes that cover at least half of the corolla tube, and tubular-infundibuliform corollas. The new species differs by strigose quadrangulate and sometimes winged stems, leaves reticulate abaxially with obtuse to subcordate bases, midveins green, corolla lobes white to yellow with margins incised to short laciniate, and indehiscent berry fruits. Digital photographs, detailed morphological comparisons with the similar species, and an IUCN preliminary risk extinction assessment are provided for the new species.

#### Resumen

Se describe una nueva especie, *Drymonia quadrangulata* Clavijo & J.L.Clark, **sp. nov**. (Gesneriaceae, Columneinae), de la vertiente occidental de los Andes del sur de Colombia y el norte de Ecuador. La nueva especie ha sido históricamente confundida con *D. killipii*, una especie endémica de Colombia, restringida a las tierras bajas del Chocó biogeográfico en los departamentos de Cauca, Chocó y Valle del Cauca. Estas dos especies tienen un cáliz foliáceo que cubre al menos la mitad del tubo de la corola y corolas tubulares-infundibuliformes. La nueva especie se distingue por sus tallos estrigosos, cuadrangulares y a veces alados, hojas reticuladas en el envés con bases obtusas a subcordadas, nervadura central verde, lóbulos de la corola blancos a amarillos con márgenes incisos a cortamente laciniados y frutos en bayas indehiscentes. Se proporcionan para la especie nueva fotografías digitales, comparaciones morfológicas detalladas con las especies similares y una evaluación preliminar de riesgo de extinción de acuerdo con los criterios de la UICN.

Key words: Andes, Chocó biogeographic region, Colombia, Columneinae, Ecuador

## Introduction

*Drymonia* Mart. ranks as the third-largest genus within neotropical Gesneriaceae, surpassed only by *Columnea*, with 210+ species, and *Besleria*, with 175+ species (Clark et al. 2020; GRC 2024). Based on our ongoing revision of the



Academic editor: Michael Moeller Received: 17 March 2024 Accepted: 29 July 2024 Published: 31 October 2024

**Citation:** Clavijo L, Clark JL (2024) Resolving the *Drymonia killipii* (Gesneriaceae) complex results in a new species from the northwestern Andes of South America. PhytoKeys 248: 207–222. https://doi. org/10.3897/phytokeys.248.123248

**Copyright:** © Laura Clavijo & John L. Clark. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). genus, *Drymonia* comprises 87 described species, most of them concentrated in the northern Andes and the Chocó biogeographic region, especially in Colombia (40 species) and Ecuador (38 species). Molecular sequence data strongly supports the monophyly of *Drymonia* (Clark et al. 2015; Ogutcen et al. 2021). *Drymonia* is classified in the subtribe Columneinae that represents 16% (approximately 525+ spp.) of the total species diversity in the Gesneriaceae (Weber et al. 2013, 2020).

Drymonia is a heterogenous genus characterized by a diverse array of leaf shapes, indumenta, corolla shapes and colors, and fruit types. Species within the genus range from herbs, sub-shrubs, shrubs, vines or lianas. The scandent habit is best summarized as a nomadic climber (Moffett 2000) characterized as germinating on the ground, ascending onto other plants using scandent stems or adventitious roots, and potentially shedding older stem parts in the process of ascent (Zotz 2013). Other common habits in Drymonia include epiphytes (facultative or obligate) or terrestrial. Corollas vary from campanulate to tubular, infundibuliform or hypocyrtoid (i.e., constricted apically with a ventral pouch) with a broad diversity of limb colors and margins. The corolla lobe margins range from entire or crenate, to laciniate or fimbriate. Anthers typically dehisce by basal pores, historically a defining character for Drymonia (Wiehler 1983); however, at least two clades within the genus exhibit longitudinal dehiscence, indicating that poricidal dehiscence has been likely lost twice in Drymonia (Clark et al. 2006, 2015). Fruit shapes in Drymonia are classified into the following four types (Clark and Clavijo 2022): 1) fleshy display capsules (no separate endocarp), 2) fleshy capsules with tardily dehiscent endocarps, 3) fleshy capsules with non-dehiscent endocarps, and 4) berries.

Our ongoing studies on the genus Drymonia based on extensive fieldwork and revision of herbarium collections have allowed us to identify a significant number of collections misidentified as Drymonia killipii Wiehler (Wiehler 1977) (Fig. 1). Subsequently, our observations of species concepts based on herbarium research were confirmed with our preliminary DNA sequence data from two nuclear and two plastid regions, supporting D. quadrangulata as not closely related to D. killipii (Clavijo 2016) and, therefore, recognized here as a new species. Hans Wiehler collected Drymonia killipii in the Colombian department of Valle del Cauca in 1972 (H. Wiehler 72142), brought it into cultivation, and later described it as a new species (Wiehler 1977). Living plants of Drymonia killipii were cultivated at Marie Selby Botanical Gardens in Sarasota (Florida, USA) until they were lost around 2019. Fortunately, Selby Gardens shared cuttings with Atlanta Botanical Garden where individuals of this species are currently thriving (David Ruland pers. comm.). Drymonia killipii is a distinctive species, featuring terete stems covered by a hirsute indumentum that becomes glabrescent with age, and large leaves (up to 45 cm long) with suppressed inter-secondary and tertiary venation abaxially (Fig. 1). It also has large foliaceous calyces and a tubular-infundibuliform corolla with a royal purple to maroon limb suffused with lemon-yellow toward the throat (Fig. 1B). Notably, this species is characterized by fragrant flowers described as having a lemon scent (Wiehler 1977). It is a narrow endemic, found in the lowlands of the Chocó biogeographic region of Cauca, Chocó, and Valle del Cauca (Colombia) and is only known from 29 collections, most of which were made in the past century.



Figure 1. Drymonia killipii Wiehler A holotype at SEL (H. Wiehler 72142) B dorsal view with front of corolla C cultivated collection from the Atlanta Botanical Garden (leaves are smaller due to cultivation conditions) (B from H. Wiehler live collection W-1726 C from J.L. Clark 10048).

Here, we describe a new species that has been confused with *Drymonia killipii*, clarify their morphological differences, and compare the new species with similar congeners (Table 1). The description of *D. quadrangulata* is based on the study of herbarium specimens, living plants in their natural habitats, and photographic images; the terminology used in the description follows Beentje (2016).

## **Taxonomic treatment**

## Drymonia quadrangulata Clavijo & J.L.Clark, sp. nov.

urn:lsid:ipni.org:names:77351102-1 Figs 2, 3

**Diagnosis.** Differs from *Drymonia killipii* by quadrangular to strongly angulate stems in cross-sections vs. terete; stems strigose apically vs. hirsute; blades 12.0–26.7 cm long vs. 20.5–45.0 cm long; corolla lobes white to yellow vs. royal purple to maroon; and fruits indehiscent globose berries vs. bivalved fleshy capsules.

**Type.** ECUADOR. Imbabura: Ibarra. Parroquia: Lita. Unpaved road heading south, near Rocafuerte (between San Gerónimo and La Carolina), accessed via the Lita-San Lorenzo highway (KM 98), road recently developed through the SolGold mining concession (Alpala); 0.7526389°N, 78.373889°W; 1550–1650 m; 23 Jul 2022; *J.L. Clark, Álvaro Pérez, Francisco Tobar & Russell Clark 17079* (holotype: QCA; isotypes: COL, CUVC, E, F, G, MO, NY, QCNE, SEL! [barcode: SEL086219], US).

Description. Terrestrial herb or shrub, 0.6–1.5 m tall. Stem scandent basally and then erect, usually branched, adventitious roots usually absent, quadrangular in cross-section to strongly angulate, sometimes winged, 4.2-10.5 mm in diameter, herbaceous to succulent, green to green with maroon spots, smooth, strigose apically, becoming glabrescent with age, lenticels sometimes present, internodes 2.2-10.2 cm long, reduced toward the apex. Leaves opposite, decussate, equal to subequal in a pair; petiole (2.2-)5.2-15.0 cm long, green with maroon spots, terete in cross-section, grooved, flattened at base, pairs of petiole bases fused together forming a perfoliate-like flap or wing, 1-2 mm wide, petiole enations present at base of petiole, strigose in apical leaves, glabrescent in basal leaves; blade elliptic to ovate, 12.0-26.7 × 5.2-18.5 cm, coriaceous, green adaxially, light green suffused with maroon abaxially, brown-maroon when dried, apex acute to acuminate, base obtuse, rounded or subcordate, margin serrulate to serrate, minutely strigose to glabrescent adaxially, glabrescent abaxially, 5-7 pairs of main lateral veins, minutely puberulent, reticulated, evident on both surfaces but more so abaxially. Inflorescence axillary, a reduced pair-flowered cyme with 1-6 flowers per inflorescence; bracts usually caducous, 7.0-16.9 × 2.0-3.7 mm, light green suffused with maroon to mostly maroon, lanceolate to oblong, apex acute, base obtuse, margin entire, glabrescent adaxially, strigulose abaxially; peduncle absent. Flowers non-resupinate; pedicel erect to perpendicular 9.5-42.0 mm long, green, strigulose, enations scattered along the pedicel. Calyx green, green with reddish or maroon margins, or mostly maroon, membranaceous, persistent in fruit, calyx lobes 5, 4 nearly equal, nearly free, fused at the base for 0.7-2.0 mm, with margins overlapping at least half their length, lanceolate, apex attenuate, base cordate, margins entire to minutely serrulate, glabrescent adaxially, glabrescent but strigulose at



Figure 2. Drymonia quadrangulata Clavijo & J.L.Clark A lateral view of corolla B front view of flower (yellow form) C lateral view of flower D front view of flower (white form) E quadrangular stem F abaxial surface of leaf G gynoecium H lateral view of immature fruit (A, G from L. Clavijo et al. 1879 B from J.L. Clark 13609 C, E J.L. Clark et al. 10344 D J.L. Clark 17079 F from J.L. Clark 18123 H from J.L. Clark 16320). Photos: A, G by L. Clavijo, B–F, H by J.L. Clark.



Figure 3. Isotype of Drymonia quadrangulata Clavijo & J.L.Clark (SEL).

base abaxially, ventral and lateral lobes 25.4-53.2 × 10.3-36.0 mm, dorsal lobe smaller, 25.0-37.6 × 8.4-19.0 mm. Corolla zygomorphic, protandrous, oblique to perpendicular relative to calyx, tubular, 42.7-64.6 mm long; tube gibbous at base, slightly constricted above base and wider at the middle, 30.8-49.5 mm long, 5.8-9.9 mm wide at constriction above base, 10.7-16.6 mm wide at the middle, outside white and puberulous, inside light yellow and glabrescent, with glandular trichomes toward throat on dorsal surface, nectary chamber 4.7-10.2 mm long; throat 9.1-15.1 mm in diameter, outside white and puberulous, inside yellow, ventrally darker and thickened forming a groove, dorsally with glandular trichomes; corolla lobes 5, subequal, white to yellow, orbicular, apex rounded, margin incised to short laciniate, glabrous adaxially, glabrous to strigulose abaxially, ventral lobe larger, straight to spreading, 14.2-23.9 × 12.2-33.4 mm, lateral lobes spreading, 12.1-23.1 × 12.7-25.0 mm, upper lobes, 10.3-22.3 × 10.3-21.6 mm. Androecium of 4 didynamous stamens, included, filaments 29.0-35.0 mm long, adnate to the corolla tube for 5.1-14.0 mm, white, glabrous, coiling after anthesis, staminode absent; anthers oblong, sagittate, coherent by the lateral walls, dehiscence by basal pores that develop into longitudinal slits,  $4.2-7.0 \times 0.7-2.0$  mm. Gynoecium with a single dorsal nectary gland, oval, apex irregularly acute to obtuse, 1.7-2.7 mm long, white, glabrous; ovary superior, 4.2-10.0 × 2.1-6.7 mm, ovate, yellow to yellow-green, puberulous to velutinous; style included, 23.5-35.1 mm long, puberulous to velutinous, reddish at base, white apically; stigma stomatomorphic, white. Fruit a berry, 6.8-17.0 × 5.5-16.5 mm, globose, yellow at maturity. Seeds numerous,  $0.8-1.0 \times 0.4-0.5$  mm, brown, fusiform, longitudinally ridged.

**Phenology.** Collected with flowers throughout the year. Fruits collected in May and June.

**Etymology.** The specific epithet is in reference to the quadrangular to strongly angulate stem cross-section which is occasionally winged (Figs 2E, 3).

Distribution and preliminary conservation assessment. Drymonia quadrangulata has been recorded from the western slopes of the Andes in southwestern Colombia (Cauca and Nariño) and northern Ecuador (Carchi, Esmeraldas, Imbabura, and Pichincha), between 250 and 2300 m (Fig. 4). It grows in the premontane rainforest, in the transition between the Tropical Andes and the Tumbes-Chocó-Magdalena Hotspots (Mittermeier et al. 2004), in a region characterized by high precipitation, which in some areas may reach more than 7000 mm per year (FELCA 2024). In Colombia, this species has been documented in protected areas such as the National Natural Park Munchique, and the Natural Reserves Río Ñambí and La Planada, with few collections in their surroundings. In Ecuador, it has also been found in protected areas such as the Mache-Chindul Ecological Reserve, the Dracula Natural Reserve (Fundación Ecominga), and the Bosque Protector Mashpi (Mashpi Lodge). According to GeoCAT (Bachman et al. 2011), the following values were calculated: EOO = 34,836 km<sup>2</sup> and the AOO = 132 km<sup>2</sup>. Based on the IUCN Red List Categories and Criteria (2012) and updated criteria in the IUCN Standards and Petitions Committee (2024), the AOO satisfies criterion B2 for Endangered (<500 km<sup>2</sup>), but the other criteria do not support a threatened category. Given the large EOO and the presence of several populations in protected areas, Drymonia quadrangulata is preliminarily assessed as being of Least Concern (LC).



Figure 4. Distribution map of Drymonia quadrangulata (blue circles), D. killipii (orange circles), D. chiribogana (red circles), and D. lanceolata (green circles).

Comments. Drymonia quadrangulata is morphologically similar to D. killipii (Fig. 1), D. chiribogana (Fig. 5), and D. lanceolata (Fig. 6). These taxa possess foliaceous calyx lobes, nearly free, covering at least half of the corolla tube and sometimes the entire tube. The calyx lobe apices are acute to attenuate, with margins folded longitudinally outwards, and tubular-infundibuliform corollas. Drymonia quadrangulata is often collected, but misidentified in most herbaria as D. killipii, a rarely collected narrow endemic in Colombia. However, D. guadrangulata can be distinguished from D. killipii by its strigose indumentum in vegetative and reproductive structures that becomes glabrescent with age (Fig. 2E) vs. hirsute, becoming glabrescent with age (Fig. 1C), stem guadrangular to strongly angulate (Figs 2E, 3), sometimes winged vs. terete (Fig. 1C), petiole of (2.2-)5.2-15.0 cm long vs. 2.0-6.4 cm long, blade base obtuse to subcordate (Figs 2F, 3) vs. cuneate (Fig. 1A), inter-secondary and tertiary venation reticulated (Fig. 2F) vs. obscure or suppressed (Fig. 1A), pedicel and calyx lacking glandular trichomes vs. presence of glandular trichomes, calyx lobes lanceolate (Fig. 2C) vs. ovate (Fig. 1A), corolla lobes white to yellow (Fig. 2B-D) vs. royal purple to maroon, suffused with lemon-yellow toward the throat (Fig. 1B), corolla lobes margin incised to short laciniate (Fig. 2A-D) vs. subentire (Fig. 1B), and indehiscent berry fruits (Fig. 2H) vs. bivalved fleshy capsules. Additionally, Wiehler (1977) noted the fragrant lemon scent of D. killipii, while no scent has been recorded for D. quadrangulata.



Figure 5. Drymonia chiribogana Wiehler A habit B front view of corolla C mature fleshy bivalved capsule D lateral view of flower (A, B, D from J.L. Clark et al. 10935 C from J.L. Clark et al. 7358). Photos by J.L. Clark.



**Figure 6**. *Drymonia lanceolata* (Hanst.) C.V.Morton. **A** erect habit **B** front view of corolla **C** immature fruit **D** lateral view of flower (**A** from *J.L. Clark et al.* 13257 **B** from *J.L. Clark et al.* 13325 **C** from *J.L. Clark* 13341 **D** from *J.L. Clark et al.* 13618). Photos by J.L. Clark.
*Drymonia quadrangulata* can be distinguished from *D. chiribogana* (Fig. 5) by its terrestrial habit vs. epiphytic, stem quadrangular to strongly angulate (Fig. 2E) vs. terete to subquadrangular (Fig. 5A), stem surface smooth when dried vs. papyraceous, blade green with midvein green adaxially vs. green with veins whitish or silvery, blade glabrescent abaxially vs. with glandular trichomes sunken into the epidermis, inter-secondary and tertiary venation reticulated (Figs 2F, 3) vs. obscure or suppressed, calyx lobes lanceolate (Fig. 2C) vs. broadly ovate (Fig. 5D), corolla of 4.3–6.5 cm long (Fig. 2A–D) vs. 3.2–4.3 cm long (Fig. 5D), corolla lobes white to yellow (Fig. 2B–D) vs. magenta, with red or yellow toward the throat (Fig. 5B), corolla lobes margin incised to short laciniate (Fig. 2A–D) vs. subentire to incised (Fig. 5B, D), and berry fruit (Fig. 2H) vs. bivalved fleshy capsule (Fig. 5C).

While *Drymonia quadrangulata* shares a terrestrial habit, quadrangulate to angulate stems, and berry fruits with *D. lanceolata* (Fig. 6), it can be differentiated by the stem surface smooth when dried vs. papyraceous, blade base obtuse to subcordate (Fig. 2F) vs. cuneate to attenuate (Fig. 6A), veins minutely puberulent abaxially vs. strigose to tomentose, calyx lobes nearly free with margins folded longitudinally outwards vs. free with margins flat (Fig. 6D), corolla lobes incised to short laciniate (Fig. 2A–D) vs. long-fimbriate (Fig. 6B, D), and stomatomorphic stigma vs. deeply bilobed. The morphological differences between *Drymonia quadrangulata*, *D. killipii*, *D. chiribogana*, and *D. lanceolata* are summarized in Table 1.

Specimens examined. COLOMBIA - Cauca. · El Tambo; PNN Munchique, camino a López de Micay, entre El Boquerón y La Cueva; Bosque Pluvial premontano; 2.76816667°N, 76.974361°W; 900-1050 m; 14 Jun 2017; fl; L. Clavijo, et al. 1868 (COL, CAUP, CUVC) • ibid.; 15 Jun 2017; fl, fr; L. Clavijo et al. 1879 (COL) • ibid.; PNN Munchique, camino a López de Micay, alrededores de la quebrada Aguaclara, Bosque Pluvial premontano; 2.76816667°N, 76.967417°W; 1045-1220 m; 17 Jun 2017; fr; L. Clavijo et al. 1900 (COL); • ibid.; bud; L. Clavijo et al. 1901 (COL). - Nariño. · Barbacoas; Corregimiento Altaquer; vereda El Barro, Reserva Natural Río Ñambí; 1.299103°N, 78.084362°W; 1325 m; 4 Dec 1993; J. Betancur 4518 (COL) • ibid.; 1.28333333°N, 78.066667°W; 1200-1400 m; 19 Apr 2004; N.R. Salinas 516 (COL) · ibid.; Reserva Natural Río Ñambí, sendero hacía Puente Piedra y bosque al otro lado del río Peie; 1.4523911°N, 78.259865°W; 1400 m; 25 Jul 2011; fr; L. Clavijo, M. Flores & A. Vasguez 1667 (COL) • ibid.; Reserva Natural Río Ñambí, vertiente occidental andina, bosque fluvial premontano, bosque primario poco intervenido, margen derecha del Río Ñambí; 1.3°N, 78.133333°W; 1325 m; 8 Dec 1993; J. Betancur 4738 (COL) • ibid.; Reserva Natural Río Ñambí; 1.16666667°N, 78.133333°W; 1325 m; 6 Dec 1993; P. Franco 4991 (COL) • corregimiento Junín; vereda Gualte; small patch of forest along Hwy Junín - Barbacoas (3-5 KM north of Junín), western slopes of the Cordillera Occidental;1.55987778°N, 78.2198083°W; 910 m; 17 May 2013; fl; J.L. Clark, L. Clavijo, O. Marín, & H. García 13609 (CAUP, COL, CUVC, HUA, PSO, SEL, US) · vereda Gualte; trail from north side of Hwy Junín-Barbacoas towards Río Ñambí, western slopes of the Cordillera Occidental; 1.368333°N, -78.083056°W; 775 m; 14 May 2013; bud; J.L. Clark et al. 13500 (COL, SEL) • carretera entre Altaquer y Junin, Cuyamba; 1450 m; 17 Nov 1967; L.E. Mora O. 4170 (COL) • Ricaurte; La Planada Reserve, 7 km from Chucunes; 1.083333°N, 78.01667°W; 1800 m; 22 Dec 1987; st; A. Gentry & P. Keating 59691 (MO) • La

**Table 1.** General geographic distribution and comparison of morphological characters to differentiate Drymonia quadran-gulata, D. killipii, D. chiribogana, and D. lanceolata.

	D. quadrangulata	D. killipii	D. chiribogana	D. lanceolata
Habit	Terrestrial, rarely a nomadic climber	Epiphytic, rarely terrestrial	Epiphytic	Terrestrial
Stems	Branched, rarely unbranched	Branched	Sparsely branched	Unbranched, rarely branched
Indument	Strigose, glabrescent with age	Hirsute, glabrescent with age	Glabrescent	Strigose, glabrescent with age
Surface	Smooth	Smooth	Papyraceous	Papyraceous
Shape	Quadrangular to strongly angulate, sometimes winged	Terete	Terete to subquadrangular	Quadrangular to angulate
Leaf pairs	Equal to subequal	Subequal to unequal	Subequal to unequal	Equal
Petiole length	(2.2-)5.2-15.0 cm	2.0-6.4 cm	1.8-10 cm	2.5-6(-14.4) cm
Indument	Strigose	Hirsute	Glabrescent	Strigulose
Blade shape	Elliptic to ovate	Elliptic to obovate	Oblanceolate-elliptic, asymmetrical	Elliptic
Base	Obtuse, rounded or subcordate	Cuneate	Cuneate, oblique	Cuneate to attenuate
Margin	Serrulate to serrate	Subentire	Entire to subentire	Serrate
Indument abaxially	Glabrescent	Hirsute to glabrescent	Glandular trichomes sunken into the epidermis	Puberulous to strigulose
Veins abaxially	Reticulate	Suppressed	Suppressed	Reticulate
Veins indument	Minutely puberulent	Hirtellous to hirsute	Glabrescent	Strigose to tomentose
Pedicel indument	Hirsute with glandular trichomes	Glabrescent	Strigose to strigulose	Strigulose
Calyx lobes color	Green, green with reddish or maroon toward margins	Yellow green	Light green, often suffused with purple or maroon	Green
Fusion	Nearly free	Nearly free	Nearly free	Free
Shape	Lanceolate	Lanceolate	Ovate	Lanceolate to ovate
Apex	Attenuate	Acute to attenuate	Acute	Long acuminate
Base	Cordate	Cordate	Strongly cordate	Cordate
Margin	Entire to minutely serrulate, folding outwards	Subentire or ciliate, folding outwards	Subentire, folding outwards	Entire, flat
Indument abaxially	Glabrescent, strigulose at base	Sparsely hirsute with scattered glandular trichomes	Glabrescent	Puberulous to strigose
Corolla length	4.3-6.5 cm	5.5–7.5 cm	3.2-4.3 cm	(3.6-)4.5-5.1 cm
Tube indument	Outside puberulous, inside glabrescent	Outside sparsely pilose, inside glabrous	Glabrous	Outside puberulous, inside glabrescent
Throat color	Yellow	Lemon-yellow, with brown spots and dots	Yellow with red lines to red	Yellow
Limb color	White to yellow	Royal purple to maroon	Magenta	White, suffused with light yellow at base
Corolla lobes margin	Incised to short laciniate	Subentire	Subentire to incised	Long-fimbriate
Ovary indument	Puberulous to velutinous	Hirsute with glandular trichomes	Glabrous	Glabrous to strigulose
Style indument	Puberulous to velutinous	Hirsute with glandular trichomes	Glabrous	Strigillose
Stigma shape	Stomatomorphic	Stomatomorphic	Stomatomorphic	Deeply bilobed
Fruit	Berry	Bivalved fleshy capsule	Bivalved fleshy capsule	Berry
Distribution	Northwestern Ecuador and Southwestern Colombia	Endemic to Colombia (Chocó and Valle del Cauca)	Endemic to Ecuador	Widely distributed from Costa Rica to Ecuador
Elevation	250-2250 m	0-200 m	80-1800 m	140-2400 m

Planada, trail to El Hondón, 6-12 km SW of La Planada; 1.066667°N, 78.03333°W; 1750-1800 m; 5 Jan 1988; bud; A. Gentry 60390 (LPB, MO, US) • Trail from La Planada to Pielapi, wet lower montane cloud forest; 1.066667°N, 78.03333°W; 1600-1800 m; 22 July 1988; st; A. Gentry 63678 (MO) · Reserva Natural La Planada; Ricaurte, trail behind Centro Cientifico leading to mountain top, 1.1666667°N, 77.96667°W; 1830-1930 m, 3 Mar 1989 (bud) J.F. Smith & M. Galeano 1516 (COL, WIS) • Reserva Natural La Planada; 7 km above Chucunés (along road between Tuquerres and Ricaurte) along trail to El Hondón, beginning at Quebrada Tejón and for 0.5 km beyond, 1.1333333°N, 77.9°W; 780-800 m, 15 Mar 1990, T. Croat 71474 (PSO) · Reserva Natural La Planada; 7 km de Chucunés; 1.166667°N, -77.96667°W; 1800 m; 13 Dec 1987; bud; O de Benavides 9024 (MO, US) • ibid.; 27 Sep 1989; fl; O. de Benavides 10942 (MO, US) • ibid.; Camino a Pialapí; 1.166667°N, 77.96667°W; 1800 m; 21 May 1992 ; fl, fr; R. Giraldo 138 (HUA) • ibid.; Trocha al Hondón; 1.166667°N, 77.96667°W; 1800 m; Oct 1995; fl; R. Giraldo 12 (HUA) • ibid.; El Hondón; 1.166667°N, 77.96667°W; 12 Apr 1994; H. Mendoza 595 (COL) • ibid.; 1.1525°N, 77.992833°W; 1800 m, 2 Mar 1995; fl; H. Mendoza 778 (PSO) · ibid.; 22 Feb 1993, C.A. Agudelo 2967 (COL) • Reserva Natural La Planada; 1.1525°N, 77.992833°W; 16 Jan 1990; bud; O. de Benavides 11147 (MO) • ibid.; 1800 m; 1 Feb 1993; M. Amaya 221 (COL) • ibid.; 1 Sep 1993; M. Amaya 283 (COL) • Vicinity Ricaurte, along rio Imbí, ca 2-3 km above Ecopetrol Campamento Palmar, located 3 km NW of Ricaurte, along trail to Ramos (indigenous settlement); 1.133333°N, 77.93333°W; 1150 m; 16 Mar 1990; bud; T. Croat 71528 (PSO) · Camino Las Cruves-Curcuel; 1.133333°N, 77.85°W; 1700–1800 m; 4 Nov 1995; fl; M.S. González, B.R. Ramírez & A. Muñoz 1274 (PSO) • Resguardo Indígena Pialapí-Pueblo Viejo; Reserva Natural La Planada; sendero Natural El Tejón; 1.1581624°N, 77.981004°W; 1700-1850 m; 19 Jul 2011; fl; L. Clavijo & C. Caicedo 1600 (COL, CUVC, PSO). Ecuador. - Carchi. Stream by Rafael Quindi's finca flowing into Río Verde, above Untal (along road) to Chical), 0.5 km from finca; 0.8833333°N, 78.133333°W; 1730 m; 25 Nov 1987; fl; W.S. Hoover & S. Wormley 1532 (MO) • Stream by Rafael Quindi's finca flowing into Río Verde, above Untal (along road to Chical); 0.8833333°N, 78.133333°W; 1730 m; 25 Nov 1987; fl; W.S. Hoover & S. Wormley 1548 (MO) • collections from forest area along trail from Rafael Quindí's house to his mountain finca; 0.8666667°N, 78.1333333°W; 1890 m; 28 Nov 1987; W.S. Hoover & S. Wormley 1899 (MO) • embankments along Río Verde, from point at which trail from Rafael's mountain finca crosses river, 1.5 km.; 0.8666667°N, 78.133333°W; 1890 m; 29 Nov 1987; fl ; W.S. Hoover, 1922 (MO) • ibid.; W.S. Hoover, 1995 (MO) · trail from Paílon to Gualpi Chico area of Awá Reservation, 1.5 km past Río Blanco; 0.85°N, 78.266667°W; 1000–1450 m; 14 Jan 1988; fl; W.S. Hoover et al. 2434 (MO, US) • trail to Pailon encampment, Gualpi Chico area of Awá Reserve; 0.9666667°N, 78.266667°W; 1350-1400 m; 21 Jan 1988; bud, W.S. Hoover et al. 3601 (MO) • up small mountain SW of Rafael Quindi's finca along small stream and descending mountain trail; 0.86666667°N, 78.133333°W; 1930-2100 m; 28 Nov 1987; W.S. Hoover & S. Wormley 1813 (MO) • Tulcan, parroquia Chical, Reserva Drácula (Fundación EcoMinga), trail from Chical along Río Blanco and then to summit of Cerro Oscuro; 0.83322°N, 78.2335°W; 2224 m; 17 Mar 2016; fl; J.L. Clark, S. Ginzbarg & H. Yela 15000 (ECUAMZ, QCA, UNA, US) • Chical; Cerro Golondrinas, ridgeline from campsite to base of Golondrinas; 0.8621861°N, 78.1674667°W; 2200 m; 25 Jan 2024; fl; J.L. Clark et al. 18123

(QCA, SEL) • ibid.; Cerro Golondrinas, trek from main road (km 22) to campsite; 0.892463889°N, 78.19465833°W; 1650-1900 m; 22 Jan 2024; bud; J.L. Clark, J. Mia & E. Nolan 17947 (QCA, SEL) • ibid.; collection made along path from the village of Quinyal towards an area known locally as "Gualpi" (near the border of the Reserva Awa); 0.965083333°N, 78.22258333; 1200-1700 m; 6 Dec 2001; bud; J.L. Clark & O. Mejia 6297 (MO, QCNE, SEL, UNA, US). - Esmeraldas. • Eloy Alfaro; Reserva Ecológica Cotacachi - Cayapas, parroquia Luis Vargas Torres, Río Santiago, estero Angostura; 0.816666667 S, 78.75°W; 250 m; 8 Dec 1993; bud; M. Tirado et al. 772 (MO, US) · Quinindé; Bilsa Biological Station, Mache Mountains, 35 km W of Quinindé, 5 km W of Santa Isabel; 0.35°N, 79.73333333°W; 400-600 m; 15 Jul 1996; bud, J.L. Clark et al. 2860 (QCNE, SEL, US) •ibid.; Mache-Chindul Ecological Reserve, Bilsa Biological Station, 35 km W of Quinindé; 0.35°N, 79.733333333°W; 500 m; 2 Oct 1996; bud, J.L. Clark 3007 (QCA, QCNE, US) · San Lorenzo; parroquia Alto Tambo, mature forest 4-8 km west of El Cristal, 0.837778°N, 78.51778°W; 1500-1650 m, 27 May 2008; bud; J.L. Clark, J, Melton, O. Solarte 10301 (QCNE, UNA, US)• ibid.; parroquia Alto Tambo, mature forest 4-8 km west of El Cristal; 0.837778°N, 78.51778°W; 1500-1650 m, 27 May 2008, J.L. Clark, J, Melton, O. Solarte 10302 (QCNE, UNA, US) • ibid.; parroquia Alto Tambo, finca Bufalito (Empresa Golden Land), 10-15 km NW of Lita; 0.8747222°N, 78.486944°W; 900-1300 m; 27 Mar 2003; bud; J.L. Clark & R. Hall 7601 (QCNE, US). - Imbabura. · Ibarra; parroquia Lita, comunidad San Francisco, next to Río Verde (13 air-km south of Lita); 0.755833°N, 78.4525°W; 900-1100 m; 24 Mar 2003; fl; J.L. Clark, R. Hall & F. Nicolalde 7521 (MO, QCNE, SEL, UNA, US) · ibid.; parroquia Lita, unpaved road heading south, near Rocafuerte (between San Gerónimo and La Carolina), accessed via the Lita - San Lorenzo highway (KM 98), road recently developed through the SolGold mining concession (Alpala), finca de Rene Chavez, known locally as "La Esperanza" and adjacent to Río Verde; 0.7330556°N, 78.373889°W; 1550-1700 m; 25 Jul 2022; J.L. Clark et al. 17143 (MO, NY, SEL, US). - Pichincha. • Quito; parroquia Pacto, Mashpi Lodge (Reserva Mashpi), 0.158333°N, 78.8855°W; 1000 m; 16 Mar 2019; J.L. Clark & L. Jost 16320 (UNA, US) • parroquia Pacto, Mashpi Lodge (Reserva Mashpi); 0.158333°N, 78.8855°W; 788 m, 25 Mar 2024; fl; hiking trail to Río Magnolia; J.L. Clark, S. Enriquez, S.G. Clark, A. Clark & C. Correa 18587 (QCA, SEL, US).

# Acknowledgments

We thank the Ministry of the Environment of Ecuador for providing specimen collection and transportation permits (Research permit Aves y Conservación N° 007-2018-IC-FLO-FAU and mobilization permit Aves y Conservación 005-FLO-2019-DPAP-MA). The National Science Foundation provided support for JLC (DEB-0841958 & DEB-0949169) and the Nellie Sleeth Scholarship from The Gesneriad Society, Inc. provided support for LC. We thank the Resguardo Indígena Awá Pialapí Pueblo Viejo, the Fundación Ecológica los Colibríes de Altaquer (FELCA), and the Colombian National Natural Parks System and the Ecohabitats Foundation for facilitating access to the Natural Reserve Río Ñambí, to the Natural Reserve La Planada, and to the National Natural Park Munchique, respectively. We are grateful to the Department of Research and Biology–Mashpi Lodge (especially Mateo Roldan, Chiara Correo, and Santiago Enriquez) and Fundación EcoMinga (especially Sara Chingal, Jeovanni Guerra, Luis Micanquier, Patricio Chugar, Milton Cantancruz, Viviana Casanova, Nolan Exe, Mia Johnson, and Marco Monteros) for logistical support during field research in western Ecuador. We thank all the herbaria that granted access to their collections (CAUP, COL, CUVC, HUA, LPB, MO, NY, PSO, QCA, QCNE, SEL, UNA, US, and WIS), David Ruland from Atlanta Botanical Garden for sharing images of *Drymonia killipii* in cultivation, and Alejandro Tobos from Universidad Nacional de Colombia for providing the map (Fig. 4). We thank Jeanne Katzenstein, Alain Chautems, Michael Möller, and an anonymous reviewer for providing valuable feedback on an earlier version of the manuscript.

## **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

No funding was reported.

#### Author contributions

Investigation: LC. Writing - original draft: LC. Writing - review and editing: JLC.

#### **Author ORCIDs**

Laura Clavijo https://orcid.org/0000-0002-3009-9158 John L. Clark https://orcid.org/0000-0002-1414-6380

#### **Data availability**

All of the data that support the findings of this study are available in the main text.

# References

- Bachman S, Moat SJ, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109
- Beentje H (2016) The Kew Plant Glossary: An Illustrated Dictionary of Plant Identification Terms. Bibliovault OAI Repository, University of Chicago Press, 160 pp.
- Clark JL, Clavijo L (2022) *Drymonia peponifera*, a new species of Gesneriaceae from Ecuador with an overview of *Drymonia* fruit traits. Brittonia 74(1): 87–94. https://doi. org/10.1007/s12228-021-09688-3

Clark JL, Herendeen PS, Skog LE, Zimmer EA (2006) Phylogenetic relationships and generic boundaries in the Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological data. Taxon 55(2): 313–336. https://doi.org/10.2307/25065580

Clark JL, Clavijo L, Muchhala N (2015) Convergence of anti-bee pollination mechanisms in the Neotropical plant genus *Drymonia* (Gesneriaceae). Evolutionary Ecology 29(3): 355–377. https://doi.org/10.1007/s10682-014-9729-4

- Clark JL, Skog LE, Boggan JK, Ginzbarg S (2020) Index to names of New World members of the Gesneriaceae (Subfamilies Sanangoideae and Gesnerioideae). Rheedea 30: 190–256. https://doi.org/10.22244/rheedea.2020.30.01.14
- Clavijo L (2016) Systematics of the Neotropical genus *Drymonia* (Gesneriaceae). PhD Thesis, The University of Alabama, USA.
- FELCA (2024) [continuously updated] Reserva Natural Río Ñambí. https://www.felca-colombia.org/reserva-natural-rio-nambi [accessed 25.03.2024]
- GRC (2024) [continuously updated] Gesneriaceae Resource Centre. Royal Botanic Garden Edinburgh. https://padme.rbge.org.uk/GRC [accessed 25.03.2024]
- IUCN Standards and Petitions Committee (2024) Guidelines for using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Subcommittee. IUCN, Gland, Switzerland; Cambridge, United Kingdom. https://www. iucnredlist.org/resources/redlistguidelines
- Mittermeier RA, Robles Gil P, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonseca GAB (2004) Hotspots revisited: Earth's biologically richest and most endangered ecoregions. CEMEX, Mexico City, Mexico, 392 pp.
- Moffett MW (2000) What's 'up'? A critical look at the basic terms of canopy biology. Biotropica 32(4a): 569–596. https://doi.org/10.1111/j.1744-7429.2000.tb00506.x
- Ogutcen E, Christe D, Nishii K, Salamin N, Möller M, Perret M (2021) Phylogenomics of Gesneriaceae using targeted capture of nuclear genes. Molecular Phylogenetics and Evolution 157: 107068. https://doi.org/10.1016/j.ympev.2021.107068
- IUCN Red List Categories and Criteria (2012) Version 3.1. Second edition. IUCN Species Survival Commission. Gland, Switzerland; Cambridge, United Kingdom.
- Weber A, Clark JL, Möller M (2013) A new formal classification of Gesneriaceae. Selbyana 31(2): 68–94.
- Weber A, Middleton DJ, Clark JL, Möller M (2020) Keys to the infrafamilial taxa and genera of Gesneriaceae. Rheedea 30: 5–47. https://doi.org/10.22244/rheed-ea.2020.30.01.02
- Wiehler H (1977) New genera and species of Gesneriaceae from the Neotropics. Selbyana 2: 67–132.
- Wiehler H (1983) A synopsis of the Neotropical Gesneriaceae. Selbyana 6: 1-219.
- Zotz G (2013) 'Hemiepiphyte': A confusing term and its history. Annals of Botany 111(6): 1015–1020. https://doi.org/10.1093/aob/mct085



Research Article

# Molecular phylogenetic and biogeographic evidence of *Lepidagathis* Willd. (Acanthaceae, Barlerieae) focusing on Indian endemics

Suhas K. Kadam<sup>1</sup><sup>®</sup>, Rohit N. Mane<sup>2</sup><sup>®</sup>, Asif S. Tamboli<sup>1</sup><sup>®</sup>, Akshay P. Jangam<sup>3</sup><sup>®</sup>, Yeon-Sik Choo<sup>1</sup><sup>®</sup>, Jae Hong Pak<sup>1</sup><sup>®</sup>

1 Research Institute for Dok-do and Ulleung-do Island, Kyungpook National University, Daegu, Republic of Korea

2 Department of Botany, Rayat Shikshan Sansthas, Balwant College, Vita, Sangli, India

3 Department of Botany, The New College, Kolhapur, Maharashtra, India

Corresponding author: Jae Hong Pak (jhpak@knu.ac.kr)

#### Abstract

Lepidagathis Willd., a genus belonging to the Acanthaceae family, is primarily distributed in tropical and subtropical regions worldwide, encompassing approximately 153 species. While considerable morphological research has been conducted on Lepidagathis, it has not completely dispelled taxonomic ambiguities and conflicting interpretations. Molecular analysis emerges as a valuable tool for resolving these taxonomic uncertainties, but the availability of nucleotide sequence data for Lepidagathis has been limited thus far. This study delivers a phylogenetic analysis of Lepidagathis species, utilizing both chloroplast and nuclear regions. The results of Bayesian Inference and Maximum Likelihood phylogenetic analyses consistently segregate the studied Lepidagathis species into two principal clades, denoted as Clade A and Clade B. Notably, this analysis firmly positions the Indian endemic Lepidagathis within Clade A, supported by robust statistical evidence. Furthermore, our biogeographical analysis strongly suggests that the origin of Lepidagathis might be traced back to Eurasia. This research establishes a foundational molecular phylogeny of Lepidagathis, offering valuable insights for future taxonomic investigations. Additionally, it sheds light on the evolutionary history and biogeographical origins of the Lepidagathis genus.

Key words: Barlerieae, biogeography, ITS, Lepidagathis, molecular phylogeny, trnL-F, trnS-G

### Introduction

The genus *Lepidagathis* Willd. (Acanthaceae, Barlerieae) comprises a total of 153 species found globally, with a predominant presence in pantropical regions (Gnanasekaran et al. 2023; More et al. 2023; POWO 2023). The genus displays quincuncial corolla aestivation, a characteristic it shares with other genera within the same tribe. This shared trait places *Lepidagathis* within the broader context of the Barlerieae tribe within the Acanthoideae subfamily (Manzitto-Tripp et al. 2022). The genus *Lepidagathis* was originally described by Willdenow in 1800, primarily based on the species *L. cristata* Willd. The genus can be recognized by axillary or terminal heads or spikes type of inflorescence, that are often 1-sided or sometimes fascicled; usually conspicuous bracts; bracteoles smaller than



Academic editor: Alan Paton Received: 2 August 2024 Accepted: 13 October 2024 Published: 31 October 2024

**Citation:** Kadam SK, Mane RN, Tamboli AS, Jangam AP, Choo Y-S, Pak JH (2024) Molecular phylogenetic and biogeographic evidence of *Lepidagathis* Willd. (Acanthaceae, Barlerieae) focusing on Indian endemics. PhytoKeys 248: 223–236. https://doi.org/10.3897/ phytokeys.248.133776

**Copyright:** © Suhas K. Kadam et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). bracts; calyx consists of deeply 4–5 unequal sepals; bilabiate corolla with four didynamous stamens attached at the base of the throat and included within the tube; anthers all subequal 2-celled, and a recurved style with capitellate stigma, capsule 2 or 4-seeded and hairy seeds (Hooker 1892; Borude et al. 2020). In India, the genus *Lepidagathis* is represented by a total of 39 taxa, and notably, 27 of these taxa are exclusive to India (Modified after Bramhadande and Nandikar 2023). Furthermore, over the past 16 years, researchers have made a noteworthy discovery of 12 new additions to *Lepidagathis* in India (More et al. 2023).

The genus *Lepidagathis* has a long history of taxonomic exploration, but it has remained relatively understudied from a molecular perspective. Despite extensive taxonomic investigations, numerous uncertainties persist in its complex taxonomy. To address these challenges, molecular studies have become imperative (Kadam et al. 2023a, 2023b).

In this research, we have constructed the phylogeny of *Lepidagathis*, incorporating some species from the genus *Barleria* (Barlerieae). This phylogeny is based on sequences from the nuclear ITS and chloroplast intergenic spacers (*trnL*-F and *trnS*-G). The primary objectives of this study were to determine the phylogenetic placement of specific Indian endemic *Lepidagathis* species, to establish a robust and comprehensive phylogeny that can assist in resolving future taxonomic challenges, and to gain insights into the biogeography of *Lepidagathis*.

# Material and methods

#### Taxon sampling

We successfully procured eight distinct species of *Lepidagathis* (Fig. 1), with five demonstrating an exclusive endemic presence within India. To substantiate our research, voucher specimens were meticulously prepared for each collected plant sample and have been duly archived at the Department of Botany, Shivaji University, Kolhapur (Suppl. material 2: table S1). A geographical distribution of these sampled species within India can be observed in Fig. 2. The data of the remaining species were derived from our prior comprehensive research (Kadam et al. 2023b).

The fresh leaf material of *Lepidagathis cristata* Willd, *L. dalzelliana* S.More, Mane, M.Sawant & H.S.Bhosale, *L. fasciculata* (Retz.) Nees, *L. incurva* Buch.-Ham. ex D.Don, *L. mahakassapae* S.More, M.Sawant, H.S.Bhosale & Kambale, *L. purpuricaulis* Nees, *L. shrirangii* Natekar, Kambale & Chandore, and *L. ushae* Borude, Gosavi & Chandore were used to extract the total genomic DNA using the DNeasy® Plant Mini Kit (QIAGEN, Germany). The quality and integrity of the extracted DNA were rigorously assessed through gel electrophoresis on a 2% agarose gel.

#### PCR amplification and sequencing

For PCR amplification we employed the set of genetic markers, ITS, *trn*L-F, and *trn*S-G, and PCR reactions setup and conditions were adapted from Kadam et al. (2023b). The purity and quality of the resulting PCR products were assessed via electrophoresis on a 2% agarose gel. Subsequently, the amplified genes were further purified and bidirectionally sequenced at Macrogen Corporation



**Figure 1.** Sampled Indian *Lepidagathis* species a. *Lepidagathis* cristata, b. L. *dalzelliana*, c. L. *fasciculata*, d. L. *incurva*, e. L. *mahakassapae*, f. L. *purpuricaulis*, g. L. *shrirangii*, h. L. *ushae*.



Figure 2. Distribution map of all sampled Lepidagathis species.

(Seoul, South Korea). For reference, we have documented the accession numbers for our generated *Lepidagathis* sequences alongside retrieved sequences from other *Lepidagathis* species and outgroup taxa, which are accessible in Table 1. To construct comprehensive DNA sequence data matrices, we assembled data from a total of 26 taxa, encompassing 23 *Lepidagathis* species, and three *Barleria* species as an outgroup. To account for any missing sequences, we supplemented the dataset with blank sequences.

Taxa name	ITS	trnL-F	trnS-G
Lepidagathis villosa Hedrén	AF169752	AF063121	_
Lepidagathis scabra C.B.Clarke	EU528896	EU528931	EU528974
Lepidagathis incurva BuchHam. ex D.Don	KT004484	-	KP744313
Lepidagathis formosensis C.B.Clarke ex Hayata	EU528895	EU528930	EU528973
Lepidagathis falcate Nees	EU528894	EU528929	EU528972
Lepidagathis alopecuroidea (Vahl) R.Br. ex Griseb.	AF169753	AF167702	EU528971
Lepidagathis chiapensis (Acosta) Kameyama	EU528897	EU528932	EU528975
Lepidagathis uxpanapensis (Acosta) Kameyama	EU528898	EU528934	EU528977
Lepidagathis sessilifolia (Pohl) Kameyama ex Wassh. & J.R.I.Wood	_	EU528933	EU528976
Lepidagathis riedeliana Nees	EU528875	EU528913	EU528940
Lepidagathis rigida Dalzell	OM337591	OM314919	OM314924
Lepidagathis cuspidata Nees	OM337592	OM314920	OM314925
Lepidagathis lutea Dalzell	OM337593	OM314921	OM314926
Lepidagathis sabui Chandore, Borude, Madhav & S.R.Yadav	OM337594	OM314922	OM314927
Lepidagathis clavata Dalzell	OM337595	OM314923	OM314928
Lepidagathis cristata Willd.	_	OR532599*	OR532591*
Lepidagathis fasciculate (Retz.) Nees	_	OR532600*	OR532592*
Lepidagathis incurva BuchHam. ex D.Don	OR529469*	OR532601*	OR532593*
Lepidagathis mahakassapae S.More, M.Sawant, H.S.Bhosale & Kambale	_	OR532602*	OR532594*
Lepidagathis purpuricaulis Nees	OR529471*	OR532603*	OR532595*
Lepidagathis shrirangii Natekar, Kambale & Chandore	_	OR532604*	OR532596*
Lepidagathis dalzelliana S.More, Mane, M.Sawant & H.S.Bhosale	OR529470*	OR532605*	OR532597*
Lepidagathis ushae Borude, Gosavi & Chandore	_	OR532606*	OR532598*
Outgroup	·	'	'
Barleria prionitis L.	MK066159	AF063118	MK066212
Barleria lupulina Lindl.	MK066150	AF289758	MK066202
Barleria ovata E.Mey. ex Nees	KT345485	KT345418	KT345460
*Sequences generated in this study		·	

Table 1. GenBank accession numbers of nuclear and chloroplast region used for molecular analyses.

#### Phylogeny

After the sequencing run, the DNA sequences were analyzed, edited, and assembled using CodonCode Aligner version 9.0.2, developed by CodonCode Corporation. Subsequently, multiple sequence alignment was conducted using MEGA 10, as described by Kumar et al. (2016), utilizing the MUSCLE program developed by Edgar (2004). Further refinement of sequences in each aligned region was carried out using BMGE v 1.1 (Criscuolo and Gribaldo 2010). The data incongruence (ILD) test (Farris et al. 1994) was carried out using PAUP 4.0a 152 (Swofford 2002) to assess the phylogenetic congruence between the nuclear and chloroplast datasets. The test was performed with 1000 heuristic replicates, and the results showed no significant conflict between the two datasets (p-value = 0.001). Additionally, the topology of all individual phylogenies was largely consistent. As a result, all subsequent phylogenetic analyses were conducted on the combined dataset.

For a better understanding of the relationships among Lepidagathis taxa, we employed both Bayesian Inference (BI) and Maximum Likelihood (ML) methods to construct phylogenies based on nuclear, chloroplast, and combined (nuclear + chloroplast) datasets. The best-fit nucleotide substitution models for each sequence dataset were determined using the jModelTest 2 program (Darriba et al. 2012) based on the Akaike information criterion (AIC). The GTR+G model was found to be the best fit for combined datasets (ITS+trnS-G+trnL-F) and thus, it was employed for phylogeny construction. The ML analysis was executed with RaxML-HPC v.8.0 (Stamatakis 2014) via the XSEDE resource through the CIPRES science gateway (Miller et al. 2010) (https:// www.phylo.org/). We employed the rapid bootstrap algorithm, conducting 1000 bootstrap replicates to obtain support values. However, BI phylogenetic analyses were carried out using MrBayes v.3.2.7a (Ronguist and Huelsenbeck 2003) on XSEDE. In the Bayesian analysis, we conducted Markov Chain Monte Carlo (MCMC) with four separate runs, each comprising 50,000,000 generations. These runs consisted of three heated chains and one cold chain, with tree sampling occurring every 1000 generations. The initial 10% of trees were discarded as burn-in, and the remaining trees were used to generate a 50% Majority-rule consensus and All-compatible group Bayesian tree with posterior probability values for each node.

#### **Biogeographic analysis**

Biogeographic regions were delineated by considering the distribution patterns of all *Lepidagathis* species. The distribution of *Lepidagathis* was categorised as follows: (A) America, (B) Africa and Arabia, (C) India and Sri Lanka, (D) Eurasia up to Wallace's Line, and (E) the Pacific, (areas east of Wallace's Line and Australia). To analyze the historical biogeography, the S-DIVA (Statistical Dispersal-Vicariance Analysis) was conducted using an All-compatible Bayesian tree in RASP v 4.2 (Yu et al. 2015). To ensure the reliability of the biogeographic analysis, 382 binary trees were employed for running the S-DIVA analysis.

# Results

#### Molecular phylogeny

This study incorporated sequence data from three distinct genomic regions, namely nrITS, *trn*L-F, and *trn*S-G, with respective sequence lengths of 546, 387, and 666 base pairs. These sequences were combined to form a composite matrix comprising both plastid and nuclear loci, which was used to construct molecular phylogenies of the *Lepidagathis* genus using ML and BI methods. This comprehensive dataset, encompassing nuclear and chloroplast sequences, encompassed 26 different species and comprised a total of 1599 characters, as detailed in Suppl. material 1. The resulting phylogenetic analysis based on this combined dataset effectively segregated *Lepidagathis* into two major clades: Clade A (PP = 1 and BS = 100) and Clade B (PP = 1 and BS = 100) (Fig. 3). Furthermore, the combined dataset provided robust support, as indicated by higher BI PP and ML BS, for delineating the evolutionary relationships between the *Barleria* and *Lepidagathis* lineages.



**Figure 3.** Bayesian phylogenetic tree based on the combined (ITS + *trn*S-G + *trn*L-F) dataset. Bayesian posterior probability values and Maximum Likelihood bootstrap values (BI PP / MLBS) are provided above branches. The species sampled from India are highlighted in red color and \* represents bootstrap value less than 50.

#### Ancestral area reconstructions

The biogeographic analysis of *Lepidagathis*, using the S-DIVA method, was performed on a combined dataset specifically curated for this study. We categorized distribution areas into five regions, considering both the historical distribution of ancient supercontinents and the distribution patterns of *Lepi-dagathis* species. The resulting analysis revealed a maximum S-DIVA value of 1390.6670, which serves as robust evidence supporting our conclusions regarding ancestral range inference (Fig. 4). S-DIVA analysis unveiled a complex biogeographic history, marked by 20 dispersal events and 5 vicariance events that have significantly influenced the current distribution patterns observed in *Lepidagathis*. Notably, we identified two instances of global extinction events at Node 50 and Node 51, which occurred when a descendant lineage inherited a range different from that of its parent lineage. Furthermore, Node 48 indicated a dispersal event, with an 80% likelihood that Eurasia (D) may have served as the originating region for the common ancestor of *Lepidagathis* (Fig. 4). The distribution codes for *Lepidagathis* species used in the biogeographic analysis along with the output data from the RASP program for S-DIVA analysis, particularly focusing on the significant nodes are mentioned in Suppl. material 2.





#### Discussion

We have undertaken molecular phylogenetic analysis concerning *Lepidagathis*, a significant genus belonging to the Barlerieae tribe (Acanthaceae). Our research focused on delving into the phylogeny of *Lepidagathis* by examining the combined nrITS+cpDNA region. Despite its significance, *Lepidagathis* has not received substantial attention in terms of phylogenetic studies, which prompted us to expand upon our previous investigation in this area. Furthermore, our study incorporates biogeographical analyses, providing valuable insights into the relationships among members of the genus. Consequently, this study sheds light on the biogeographic analysis of *Lepidagathis* and the phylogenetic placement of eight recently described species, five of which are endemic to India.

#### Phylogeny of Lepidagathis

The molecular phylogenetic analysis, based on the combined nrITS+cpDNA region, reveals that *Lepidagathis* forms a monophyletic group with robust BS PP and ML BS values. We concluded that for future phylogenetic investigations in this plant group, researchers can enhance their sampling size and reconstruct the phylogeny based on the combined (ITS + *trn*S-G + *trn*L-F) dataset.

As discussed in previously published work (Kadam et al. 2023b), the correct identification of *Lepidagathis* remains a challenging task due to historical misinterpretations, with several new genera mistakenly derived from it. This study, like the previous one, supports systematic revisions, including the incorporation of *Lophostachys* and *Acanthura* species into *Lepidagathis*. The lack of a universal classification system for *Lepidagathis*, likely due to its confusing morphology, persists, and resolving the taxonomic issues requires a proper classification system. The phylogenetic tree presented here confirms the existence of two major clades within *Lepidagathis*: Clade A and Clade B. All the Indian endemic species, including *L. clavata*, *L. mahakassapae*, *L. dalzelliana*, *L. sabui*, *L. ushae*, *L. shrirangii*, and *L. cristata* are clustered together in Clade A.

Recently, Bramhadande and Nandikar (2023) summarized the genus Lepidagathis in India, grouping species based on morphological similarities. This artificial classification aims to improve understanding and utilizes traits such as endemism, growth habits, leaves, flowers, and ovules to categorize 38 Indian taxa. Notably, the authors synonymized L. ushae with L. prostrata Dalzell. However, King et al. (2023) later recognized L. ushae as a distinct species following detailed morphological analyses of both fresh and herbarium specimens. Based on this, we have adopted the recognition of L. ushae as a separate species. According to Bramhadande and Nandikar's (2023) classification, L. cristata, L. shrirangii, and L. lutea fall under Group A, characterized by decumbent herbs or subshrubs with glabrous to scabrid, linear to narrowly ovate leaves, sessile and acute; axillary spikes forming a congested, globose head near the roots, with mucronate-spinescent bracts. In our phylogeny, L. shrirangii, described by Natekar et al. (2019), and its closely related species L. lutea are positioned as sister taxa with robust support. Both species originate from the Konkan region of Maharashtra, India; however, L. cristata is not included in this clade (Fig. 3).

In Group C, Bramhadande and Nandikar (2023) placed *L. sabui* and *L. mahakassapae* based on their prostrate shrub habit, axillary or terminal erect spikes,

and two ovules; all these species are endemic to peninsular India. Since L. ushae also shares these traits, we have included it in Group C as well. Our phylogeny supports this grouping as these species are placed in the same clade. However, L. dalzelliana, which was placed in Group H by Bramhadande and Nandikar (2023), appears in our phylogeny alongside species from Group C. Notably, L. mahakassapae shares a close relationship with L. sabui but can be distinguished by its oblanceolate acuminate leaves, pubescent terminal elongated spikes, large floral structures, small sterile bracts, and lanceolate broader segments of the bracteole (More et al. 2022). Similarly, L. dalzelliana bears a resemblance to L. clavata in appearance but differs in having long lanceolate, hairy bracts, and spatulate, oblanceolate to ovate-lanceolate glabrous leaves (More et al. 2023). In addition, L. mahakassapae and L. dalzelliana are also closely related species, sharing a sister relationship in their evolutionary lineage (Fig. 3). Both of these species inhabit high-altitude plateaus within the Satara district (Maharashtra) and coexist in the same geographical area. They are characterized as perennial, decumbent, prostrate, sub-shrubs, hairy bracts and seeds. The primary distinguishing features between them include flower color, hairiness of bracts, nerve number of bract, nature of bracteole, color of seed hairs and flowering and fruiting time (More et al. 2022; More et al. 2023). Similarly, L. sabui and L. ushae are found in lower-altitude lateritic plateaus and share a close phylogenetic relationship according to our research. These two species are also characterized as perennial, procumbent, prostrate, rigid herb, cylindrical stem, pinkish flower color (Borude et al. 2020; Chandore et al. 2020). In addition, L. clavata which was placed in Group D by Bramhadande and Nandikar (2023), appears in our phylogeny alongside species from Group H (L. rigida and L. cuspidata). Furthermore, L. cuspidata, L. rigida, L. clavata, L. dalzelliana, L. mahakassapae, L. sabui, and L. ushae co-occur in the same region, with many of them also described from the Konkan region of Maharashtra. Consequently, our phylogenetic analysis groups these species together, indicating their close relationship.

According to Bramhadande and Nandikar (2023), *L. fasciculata* (Retz.) Nees is the lone species from Group E, and it is placed near *L. cristata* in our phylogeny, though with weak support. *L. incurva* Buch.-Ham. ex D.Don and *L. purpuricaulis* Nees are classified as Group F species based on shared characteristics, such as their erect to decumbent herb or shrub habit, linear to elliptic-ovate leaves, and axillary or terminal inflorescences. However, our phylogeny separates these species into distinct clades. Additionally, both sampled and adapted species of *L. incurva* cluster together with strong ML support.

In summary, our phylogeny aligns with certain aspects of Bramhadande and Nandikar's (2023) classification but diverges in others. A universal classification system for *Lepidagathis* is crucial, and a more comprehensive, robust molecular phylogeny is needed to resolve the remaining taxonomic issues.

#### Biogeography

The study of evolutionary history through molecular phylogeny is essential for gaining a precise understanding of biogeographical evolution (Ali et al. 2012). Surprisingly, no biogeographical investigation has been conducted on *Lepidagathis* thus far. Our S-DIVA analysis suggests that Eurasia (D) may serve as the

probable center of origin for *Lepidagathis*. Within *Lepidagathis*, we observe a further division into Clade A, which is distributed across Eurasia and subsequently diverged in India and America (Node 42). In contrast, Clade B expanded initially in Eurasia (Node 47) and later dispersed into America (Nodes 43 and 44).

The shared ancestry of the former *Lophostachys* species (*Lepidagathis chiapensis*, *L. sessilifolia*, and *L. uxpanapensis*), the previous *Acanthura mattogrossensis* (now *L. riedeliana*), and *L. alopecuroidea* are traced back to a common origin during the migration from the Old World to the New World. (McDade et al. 2008). Our phylogenetic analysis positions these species collectively, corroborating the notion of their shared ancestry, observed at nodes 45 and 42, corresponding to the mentioned dispersal event.

It's worth noting that species within *Lepidagathis* have received relatively little attention in terms of molecular phylogenetic research, and many remain unsequenced. However, it is important to emphasize that this study represents a preliminary exploration, shedding light on potential avenues for future molecular investigations into *Lepidagathis*.

# Conclusion

This study serves as phylogenetic research on *Lepidagathis* and offers insights into the phylogenetic placement of certain Indian endemic species. Moreover, our biogeographic investigations have indicated that Eurasia is a potential place of origin for this genus. The combined dataset comprising nrITS, *trn*L-F, and *trn*S-G sequences has proven effective in resolving the phylogeny of *Lepidagathis*. Therefore, this dataset holds promise for future comprehensive phylogenetic studies within the genus. It's important to note that *Lepidagathis* encompasses a diverse array of species, and only a limited subset has undergone sequencing. To resolve the remaining taxonomic issues, a universal classification system for *Lepidagathis* is crucial, alongside a more comprehensive and robust molecular phylogeny. Extensive sampling efforts will also be essential to gain a deeper understanding of the genus and to fully explore its biogeography.

# **Additional information**

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1A6A1A05011910). APJ is thankful to Principal, The New College, Kolhapur and MAHAJYOTI for financial assistance [MAHAJYOTI/Nag./Fellowship/2021-22/1042 (275)]. RNM is thankful to the Head, Department of Botany and Principal, Balwant College, Vita for providing necessary facilities.

#### Author contributions

The study was designed by Suhas K. Kadam. Material was collected in the field by Rohit N. Mane and Akshay P. Jangam; data analysis was done by Suhas K. Kadam and Asif S. Tamboli; creation of the first draft of the manuscript was performed by Suhas K. Kadam, Yeon-Sik Choo and Jae-Hong Pak. All authors read and approved the final manuscript.

#### **Author ORCIDs**

Suhas K. Kadam <sup>©</sup> https://orcid.org/0000-0002-2396-4932 Rohit N. Mane <sup>©</sup> https://orcid.org/0000-0002-3198-6816 Asif S. Tamboli <sup>©</sup> https://orcid.org/0000-0003-2146-670X Akshay P. Jangam <sup>©</sup> https://orcid.org/0000-0002-4916-4220 Yeon-Sik Choo <sup>©</sup> https://orcid.org/0000-0001-7307-325X Jae Hong Pak <sup>©</sup> https://orcid.org/0000-0001-9085-3741

#### Data availability

The data that support the findings of this study are openly available in Science Data Bank, at https://doi.org/10.57760/sciencedb.15983.

# References

- Ali SS, Yu Y, Pfosser M, Wetschnig W (2012) Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). Annals of Botany 109: 95–107. https://doi.org/10.1093/aob/mcr274
- Borude DB, Natekar PD, Gosavi KVC (2020) *Lepidagathis ushae*: a new species of Acanthaceae from the lateritic plateaus of the Konkan region, Maharashtra, India. Kew Bulletin 75: 1. https://doi.org/10.1007/s12225-020-9878-2
- Bramhadande SP, Nandikar MD (2023) A synopsis of the genus *Lepidagathis* (Acanthaceae) in India, new taxa and notes on Dalzell's species. Journal of Asia-Pacific Biodiversity 16: 4. https://doi.org/10.1016/j.japb.2023.08.004
- Chandore AN, Borude DB, Madhav NA, Yadav SR (2020) *Lepidagathis sabui* (Acanthaceae), a new species from the lateritic plateaus of Konkan region of Maharashtra, India. Phytotaxa 464(2): 159–166. https://doi.org/10.11646/phytotaxa.464.2.2
- Criscuolo A, Gribaldo S (2010) BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Ecology and Evolution 10: 210. https://doi.org/10.1186/1471-2148-10-210
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772–775. https://doi. org/10.1038/nmeth.2109
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with re-duced time and space complexity. BMC Bioinformatics 5: 113–131. https://doi.org/10.1186/1471-2105-5-113
- Farris JD, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of incongru-ence. Cladistics 10(3):315–319. https://doi.org/10.1111/j.1096-0031.1994.tb00181.x
- Gnanasekaran G, King AFJ, Kasim SM, Arisdason W (2023) *Lepidagathis gandhii* (Barlerieae: Acanthaceae), a new species from Tamil Nadu, India. Kew Bulletin 78: 203–212. https://doi.org/10.1007/s12225-023-10086-z
- Hooker JD (1892) Acanthaceae. In: Clarke CB (Ed.) The Flora of British India 4. L. Reeve & Co. London, 387–558.

- Kadam SK, Tamboli AS, Mane RN, Yadav SR, Choo Y-S, Burgos-Hernández M, Pak JH (2023a) Revised molecular phylogeny, global biogeography, and diversification of palms subfamily Coryphoideae (Arecaceae) based on low copy nuclear and plastid regions. Journal of Plant Research 136: 159–177. https://doi.org/10.1007/s10265-022-01425-5
- Kadam SK, Tamboli AS, Mane RN, Jangam AP, Yadav SR, Choo Y-S, Pak JH (2023b) Molecular phylogeny and genetic diversity of some *Lepidagathis* species (Acanthaceae) based on nuclear and chloroplast regions. Plant Biosystems 157: 455–464. https:// doi.org/10.1080/11263504.2023.2165557
- King AFJ, Prajapati SR, Patel RM, Gnanasekaran G, Darbyshire I, W. Arisdason (2023) Taxonomy and lectotypifcation of *Lepidagathis hamiltoniana* (Acanthaceae: Barlerieae), an endemic species of India. Rheedea 33(2): 74–82. https://doi.org/10.22244/ rheedea.2023.33.02.04
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054
- McDade LA, Daniel TF, Kiel CA (2008) Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). American Journal of Botany 95(9): 1136–1152. https://doi.org/10.3732/ajb.0800096
- Manzitto-Tripp EA, Darbyshire I, Daniel TF, Kiel CA, McDade LA (2022) Revised classification of Acanthaceae and worldwide dichotomous keys. Taxon 71(1): 103–153. https://doi.org/10.1002/tax.12600
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 1–8. https://doi.org/10.1109/ GCE.2010.5676129
- More S, Kambale S, Sawant M, Mane R, Bhosale H (2022) *Lepidagathis mahakassapae* sp. nov. (Acanthaceae: Barlerieae) from the high elevated Lateritic Plateau of northern Western Ghats of Maharashtra, India. Nordic Journal of Botany 7: e03345. https://doi.org/10.1111/njb.03345
- More S, Mane R, Sawant M, Bhosale H (2023) *Lepidagathis dalzelliana* (acanthaceae), a new species from the northern Western ghats and lectotypification of the name *Lepidagathis prostrata* Dalzell. International Journal of Advanced Research 11(06): 907–911. https://doi.org/10.21474/IJAR01/17143
- Natekar PD, Borude DB, Kambale SS, Chandore AN (2019) *Lepidagathis shrirangii* (Acanthaceae) a new species from Konkan region of Maharashtra, India. Phytotaxa 405(4): 215–220. https://doi.org/10.11646/phytotaxa.405.4.6
- POWO (2023) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew.
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033
- Swofford DL (2002) PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Willdenow CL (1800) Species Plantarum 4 ed. vol. 3. Berlin.
- Yu Y, Harris AJ, Blair C, He X (2015) RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. Molecular Phylogenetics and Evolution 87: 46–49. https://doi.org/10.1016/j.ympev.2015.03.008

#### **Supplementary material 1**

#### Sequence matrix used for phylogenetic analysis

Authors: Suhas K. Kadam, Rohit N. Mane, Asif S. Tamboli, Akshay P. Jangam, Yeon-Sik Choo, Jae Hong Pak

Data type: txt

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.133776.suppl1

# **Supplementary material 2**

#### Information of nodes discussed in biogeography analysis

Authors: Suhas K. Kadam, Rohit N. Mane, Asif S. Tamboli, Akshay P. Jangam, Yeon-Sik Choo, Jae Hong Pak

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.133776.suppl2

# PhytoKeys

**Research Article** 

# Newly found and rediscovered hornworts (Anthocerotophyta) in Poland: Indicators of climate change impact in Central Europe

Vítězslav Plášek<sup>1,2</sup>, Lukáš Číhal<sup>3</sup>, Frank Müller<sup>4</sup>, Martina Pöltl<sup>50</sup>, Mariusz Wierzgoń<sup>60</sup>, Ryszard Ochyra<sup>70</sup>

- 1 Department of Biology and Ecology, University of Ostrava, Chittussiho 10, CZ-710 00 Ostrava, Czech Republic
- 2 Institute of Biology, University of Opole, Oleska 48, PL-45-052 Opole, Poland
- 3 Silesian Museum, Nádražní okruh 31, CZ-746 01 Opava, Czech Republic
- 4 Institut für Botanik, Technische Universität Dresden, D-01062 Dresden, Germany
- 5 Studienzentrum Naturkunde Joanneum Graz, Weinzöttlstraße 16, AT-8045 Graz, Austria
- 6 Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Jagiellońska 28, PL-40-032 Katowice, Poland
- 7 National Collection of Biodiversity, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland Corresponding author: Vítězslav Plášek (vitezslav.plasek@osu.cz)

#### Abstract

In 2023, field research in south-western Poland led to the noteworthy discovery of two hornworts: *Notothylas orbicularis*, a species previously unrecorded in this country, and the rediscovery of *Anthoceros neesii* for the Polish bryoflora. These findings are significant as they suggest a response to climate change, which is facilitating the range expansion of hornworts within Central Europe. Detailed descriptions of the new localities for both species are provided, highlighting the specific environmental conditions and habitats where they were found. Distribution maps for *Notothylas orbicularis* and *Anthoceros neesii* in Poland are provided, as well as SEM micrographs of spores. Additionally, a key to the identification of Polish hornwort species is also included. Furthermore, a model projecting the potential future spread of these hornworts within Poland and the broader Central European region is presented. This model considers climatic variables and habitat availability, offering insights into possible range shifts. This study contributes to the growing body of evidence that climate change is a driving factor in the redistribution of bryophytes.

**Key words:** Anthoceros neesii, arable fields, bryophytes, Central European endemic, distribution modelling, diversity, expansion, key to determination, *Notothylas orbicularis*, SEM micrographs

# Introduction

Hornworts (Anthocerotophyta), as the sister group to liverworts and mosses, are critical in understanding the evolution of key land plant traits (Frangedakis et al. 2021, 2023). Approximately 250 species of hornworts exist worldwide (Villarreal et al. 2010, 2012; Garcia et al. 2012; Peng and Zhu 2013). They are characterized by their dorsiventral thalli and horn-like sporophytes. In temperate climates, hornworts grow terrestrially, preferring open, moist, or shaded places with nutrient-rich soils (Glime 2017). Especially in the northern hemisphere,



Academic editor: Matt von Konrat Received: 14 August 2024 Accepted: 8 October 2024 Published: 31 October 2024

Citation: Plášek V, Číhal L, Müller F, Pöltl M, Wierzgoń M, Ochyra R (2024) Newly found and rediscovered hornworts (Anthocerotophyta) in Poland: Indicators of climate change impact in Central Europe. PhytoKeys 248: 237–261. https://doi. org/10.3897/phytokeys.248.134729

**Copyright:** © Vítězslav Plášek et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). they are frequently found in agricultural landscapes, such as stubbled fields, where regular soil disturbance provides the exposed substrate they require (Bisang et al. 2021a, 2021b). These conditions are prevalent in areas subjected to ploughing and other forms of soil turnover, creating the bare ground necessary for hornwort colonization (Bisang 1998; Bisang, Bergamini 2020; Schuster 1992; Villarreal et al. 2010). These plants are often among the first colonizers in these environments, taking advantage of the lack of competition and the availability of light and moisture. In addition to agricultural settings, hornworts commonly inhabit other types of open soil environments, including paths, ditches, and riverbanks, where periodic disturbance or water flow maintains the open soil conditions (Glime 2017). Ecologically, hornworts contribute to soil stabilization and the early stages of soil formation (Bisang 1998). Their presence can influence soil microbial communities and nutrient cycling, as their thalli provide a substrate for microorganisms, and their decomposition contributes organic matter to the soil. The symbiotic relationship between hornworts and cyanobacteria, which fix atmospheric nitrogen, also enhances soil fertility, benefiting other plant species that follow in succession (Bisang 1998; Bisang et al. 2009).

Hornworts are often overlooked because of their small size, the seemingly uninteresting habitat in which they grow, and their short life cycle, which means that they are often only seen for a short period during the year (Bisang 2004). As a result, distributional data for these plants are often absent from relatively large areas. However, to better understand their spread and ecology, we can utilize the results of various floristic reports and ecological surveys. These studies highlight the niche preferences of hornworts and the factors influencing their distribution.

# History of findings of hornworts in Poland

The history of the study of hornworts in Poland is quite confusing, reflecting the complicated and chequered taxonomic and nomenclatural history of European species of this group of bryophytes. In older literature (Limpricht 1876; Błoński 1888; Rejment-Grochowska 1950) there are only two species reported from Poland classified in the one genus of Anthoceros L., namely A. punctatus L. and A. laevis L. Szweykowski (1958), in his fundamental work on the liverworts in Poland, based on the taxonomy of these bryophytes as taken from the opus of Müller (1906-1911, 1912-1916), reported three species of Anthoceros from Poland, A. crispulus (Mont.) Douin, A. laevis and A. punctatus. In turn, Rejment-Grochowska (1966), in the first volume of the Flora of Polish liverworts, based her treatment of the Anthocerotophyta on the study by Proskauer (1958) and provided four species from the country classified into two genera, namely Phaeoceros Prosk. (Ph. laevis (L.) Prosk.) and Anthoceros (A. punctatus, A. crispulus and A. neesii Prosk.). In addition, she provided a description and illustration of Notothylas orbicularis (Schwein.) Sull., a species and genus found in the neighbouring countries of Germany and the Czech Republic (then Czechoslovakia) close to the border with Poland, suggesting it would very likely be found in Poland. Finally, Koła and Turzańska (1995) reported five species of hornworts from Poland, namely Anthoceros punctatus, A. neesii and A. agrestis Paton (= A. punctatus auct.) and Phaeoceros laevis and Ph. carolinianus (Michx.) Prosk.

In the most recent critical lists of hornworts in Poland, Szweykowski (2006), Klama (2006a, 2006b) and Klama and Górski (2018) recognised only three spe-

cies of these bryophytes from the country, belonging to the genera Anthoceros (A. agrestis and A. neesii) and Phaeoceros (Ph. carolinianus). According to Szweykowski (2006), the occurrence of Ph. laevis in Poland is very doubtful, and a revision of all available herbarium materials so named showed that they actually belonged to Ph. carolinianus. Similarly, specimens published and deposited in herbaria under the name Anthoceros punctatus actually represented A. agrestis. This species was common throughout the country half a century ago, but now is very rare or locally absent in the central and northern lowlands and is more frequent only in the foothills of the Carpathians and the Sudetes in the south of the country.

The knowledge about the occurrence of *Anthoceros neesii* in Poland is relatively poor. It was first recognized as a separate taxon, *Anthoceros punctatus* f. *monocarpus* Nees, by Nees von Esenbeck (1838) who described it on the basis of the material he had collected from Grodna hill in the village of Staniszów [*German* Stohnsdorf am Stangenberg] in the Jelenia Góra Basin at the foothills of the Giant Mountains (*Polish* Karkonosze, *Czech* Krkonoše, *German* Riesengebirge) in Lower Silesia in SW Poland. This taxon was actually first collected in the Czech Republic by Corda (1829), who illustrated it in detail as *A. punctatus*. However, Nees von Esenbeck (1838) demonstrated that all illustrations by Corda (1829) but one showing the habit of this hornwort actually represented the form of this species described by himself from specimens collected within the present borders of Poland.

Proskauer (1958) examined the original material of *Anthoceros punctatus* f. *monocarpus* in the Nees von Esenbeck herbarium which is apparently housed in STR (Grolle 1976; Stafleu and Cowan 1981) and concluded that this form indeed deserved the status of a separate species. As a result, he raised the form described by Nees von Esenbeck (1838) to the rank of species, which he named *Anthoceros neesii* and designated the original specimen of this form examined by himself as the type, i.e. the holotype. Unfortunately, this is not a completely correct interpretation, as Nees von Esenbeck (1838) also cited Corda's (1829) illustration in the protologue, so lectotypification is necessary in this situation. According to Art. 9.10 of the current ICN (Turland et al. 2018), Proskauer's citation of Nees von Esenbeck's specimen as the holotype is an error to be corrected to lectotype.

Apart from the type specimen, Proskauer (1958) cited three additional specimens of *Anthoceros neesii* collected in Poland, unfortunately, without any details regarding collector(s), dates, and herbaria in which they were located. Two specimens were collected in the Jelenia Góra Basin in Lower Silesia in close proximity of the type locality, namely in Malinnik near Cieplice Śląskie Zdrój [*German* Herischdorf bei Warmbrunn] and by the road to the Krzyżna Góra village [*German* am Wege nach Kreuzberg], and the other specimen was collected in the village of Rusinowo [*German* Ruschendorf] in the Wałcz Lakeland in West Pomerania in NW Poland.

After its inception, *Anthoceros neesii* was recorded only once at the type locality from the farmland around the Staniszów village and not far from this place, in the hamlet of Wilcza Poręba in Karpacz in the Karkonosze (Koła and Turzańska 1993). At all sites *A. neesii* co-occurred with other ephemeral bryophyte species typical of habitats of disturbed arable fields. However, the species has not been rediscovered in Poland in the past three decades and therefore it was initially classified as rare (R) (Klama 2006b), but in the latest Red List of Polish liverworts and hornworts *A. neesii* is considered to be critically endangered (CR category) in the country (Klama and Górski 2018). Interestingly, in the European checklist of bryophytes, Hodgetts and Lockhart (2020) ignored the latter Red List and continued to treat *A. neesii* as extremely rare (R) in Poland, but not critically endangered. It is worth noting that despite intensive searches, no historical specimens of this species were found in the main Polish bryological herbaria.

The present study provides data on the rediscovery of *Anthoceros neesii* at two new localities in Lower Silesia in SW Poland. Additionally, the information about the first discovery of *Notothylas orbicularis* in Poland is provided, thus confirming the anticipation of its occurrence in the country expressed by Rejment-Grochowska (1966).

# Material and methods

# Plant material and description of the localities

On October 7 and 8, 2023, a bryophyte collection expedition was conducted on several arable and stubble fields in the southern part of the Lower Silesian Voivodeship in Poland. The primary motivation was to gather fresh material of bryophytes for a bryological course for students at Opole University. However, in addition to finding common species of ephemeral bryophytes, two very interesting species of hornworts were discovered. After a preliminary examination in the field, the material was collected and studied in detail in the laboratory. In addition to using classic optical microscopes (Olympus SZ61 and Olympus BX53F), SEM microscopy (Jeol SEM microscope) was also employed to study the surface and ornamentation of the spores in detail, which are crucial for distinguishing hornworts, especially within the genus *Anthoceros*. The distribution of the hornwort species in Poland was plotted on maps in the ATMOS grid square system (Ochyra and Szmajda 1981). The specimens are stored in the OSTR and KRAM herbaria.

A list of localities where the hornworts were recently collected (Fig. 1A-F):

- S Poland, Lower Silesian Voivodeship, Central Sudetes, Kłodzko Basin, 5 km W of Kłodzko town, between Szalejów Dolny and Szalejów Górny villages, stubble field near the national road No. 8, on open soil near a small forest, 359 m a.s.l., 50°25'53.0"N, 16°34'13.8"E, ATMOS grid square Fb-25, 7 Oct 2023, leg. V. Plášek (Fig. 1A, B).
- S Poland, Lower Silesian Voivodeship, Central Sudetes, Kłodzko Basin, 12 km WNW of Kłodzko town, 3 km SE of Wambierzyce village, small stubble field near the provincial road No. 388, on open soil, 450 m a.s.l., 50°28'06.7"N, 16°28'33.8"E, ATMOS grid square Fb-15, 7 Oct 2023, leg. V. Plášek (Fig. 1C, D).
- 3. S Poland, Lower Silesian Voivodeship, Western Sudetes, Karkonosze (Giant Mountains), 8 km S of Kłodzko town, 250 m NE of Przełęcz Mielnicka (=Mielnik pass) in the small village of Mielnik, small stubble field near the national road No. 33, on open soil near a small forest, 382 m a.s.l., 50°21'14.6"N, 16°40'08.2"E, 8 Oct 2023, leg. V. Plášek (Fig.1E, F).



**Figure 1.** A view of the localities where the hornworts were recently collected **A**, **B** loc. 1 (between the villages of Szalejów Dolny and Szalejów Górny) **C**, **D** loc. 2 (near the village of Wambierzyce), and **E**, **F** loc. 3 (near the village of Mielnik). Photographs were taken by Vítězslav Plášek (7–8 Oct 2023).

# Modelling

Maxent version 3.4.4 software (Phillips et al. 2024) was used to calculate the individual models. The R (R Core Team 2024) and QGIS (QGIS Development Team 2024) programs were used for data preparation and analysis.

#### Species occurrence data

For our analysis, we utilized a comprehensive set of incidence data sources. We incorporated field observation data from the Czech Republic, Germany, and Austria, which were compiled through the review of herbarium specimens, literature excerpts, and using data from national bryophyte distribution databases. Additionally, we integrated data for *Anthoceros neesii* and *Notothylas orbicularis* sourced from the Global Biodiversity Information Facility (GBIF) [https://www.gbif.org/]. These data were accessed via the "sp\_occurrence" function within the "geodata" package (Hijmans et al. 2023). Specifically, the data included occurrences for *Anthoceros neesii* (GBIF.org, accessed January 15, 2024, GBIF Occurrence Download: https://doi.org/10.15468/dl.hdc36q) and *Notothylas orbicularis* (GBIF.org, accessed January 15, 2024, GBIF Occurrence Download: https://doi.org/10.15468/dl.hdc36q).

To address potential sampling biases and errors in the GBIF data, we employed multiple cleaning approaches from "CoordinateCleaner" (Zizka et al. 2019). Additionally, we mitigated sampling bias using the "spThin" package (Aiello-Lammens et al. 2015) in R (R Core Team 2024). With a thinning parameter ("thin.par") set to 2 km (after testing) and restricted to the geographical extent of (5.8667, 24.1333, 46.3167, 55.05), we obtained 50 samples of *Anthoceros neesii* and 62 samples of *Notothylas orbicularis* for subsequent analysis (Table 1).

Species	Time Period	GBIF data After Cleaning	Bryol. Coll.	Overall	Data After Thinning (2 km)
Anthoceros neesii	1980-2010	1	42	43	20
	2011 =>	1	48	49	30
Notothylas orbicularis	1980-2010	1	88	89	36
	2011 =>	7	37	44	26

Table 1. Numbers of records for different datasets.

#### Environmental layers and variable selection

Nineteen environmental variables (bio1-bio19) at a resolution of 30 seconds (~1 km<sup>2</sup>) were downloaded from the CHELSA dataset (Karger et al. 2017), covering the historical period from 1980 to 2010. For the current/future period (2011-2040), the same 19 environmental variables were obtained from the CHELSA dataset using CMIP6 Global Circulation Models (GCMs), namely GDFL-ESM4 and IPSL-CM6A-LR, under two Shared Socio-economic Pathways (SSPs) 126 and 585.

This publication also utilized information from the European Union's Copernicus Land Monitoring Service, specifically the Land Cover data for the years 2000 (LC2000) and 2018 (LC2018). These datasets were selected to correspond with the two distinct time periods (LC2000 for 1980–2010 and LC2018 for 2011–2040). The Land Cover data provide detailed information categorized into 15 distinct classes based on updated Land Cover illustrated nomenclature guidelines (Kosztra et al. 2017), ensuring compatibility with Maxent modelling by limiting the number of categories.

SHORTNAME	LONGNAME	EXPLANATION
bio1	Mean annual air temperature	Mean annual daily mean air temperatures averaged over 1 year.
bio3	Isothermality	Ratio of diurnal variation to annual variation in temperatures.
bio4	Temperature seasonality	Standard deviation of the monthly mean temperatures.
bio8	Mean daily mean air temperatures of the wettest quarter	The wettest quarter of the year is determined (to the nearest month).
bio9	Mean daily mean air temperatures of the driest quarter	The driest quarter of the year is determined (to the nearest month).
bio14	Precipitation amount of the driest month	The precipitation of the driest month.
bio15	Precipitation seasonality	The coefficient of variation is the standard deviation of the monthly precipitation estimates expressed as a percentage of the mean of those estimates (i.e. The annual mean).
LC2000	CORINE land cover 2000	The pan- European CORINE land cover inventory for 44 thematic classes for the 2000 reference year.
LC2018	CORINE land cover 2018	The pan-European CORINE land cover inventory for 44 thematic classes for the 2018 reference year.

Table 2. Uncorrelated environmental variables used in Maxent modelling.

**Note:** Throughout all different time periods, scenarios, and SSPs, the same variables were identified as uncorrelated. The only variation lies in the Land Cover (LC) data utilized for the different time periods, either LC2000 or LC2018, in conjunction with environmental data.

To maintain consistency with CHELSA layers, the Land Cover data were resampled to match their dimensions using the "resample" function from the "raster" library (Hijmans 2024) with the nearest neighbor method. From the initial set of 19 environmental variables and two land cover (LC) variables, we eliminated those showing high collinearity using the "vifstep" function from the "USDM" package (Naimi et al. 2014) with a threshold of 0.9. This process yielded a set of 8 uncorrelated variables for each model (bio1, bio3, bio4, bio8, bio9, bio14, bio15, LC2000, or LC2018) (see Table 2).

LC2000 and LC2018 data were assessed in combination with bio1-19 separately, considering potential temporal differences. For each variable combination (across different time periods, scenarios, and SSPs), a distinct Maxent model was constructed. This strategy ensures tailored models for specific conditions, capturing nuances and enhancing the accuracy and interpretability of results compared to utilizing a single model and reprojecting it onto various conditions.

#### Background points and background area

The study area encompasses Central Europe, defined by the geographical coordinates (5.8667, 24.1333, 46.3167, 55.05), predominantly covering the territories of the Czech Republic, Austria, Germany, and Poland. This region was selected for its ecological significance and alignment with our research objectives.

To maintain spatial consistency throughout the modelling process, we generated 10,000 random background points confined to the entire study area, following the methodology outlined by Phillips and Dudík (2008). This approach of selecting background points from the entire study area mitigates potential issues associated with reprojection to a different territory than that used for model creation.

#### **Used algorithm**

Maxent, a machine-learning software program, was utilized for species distribution modelling. It calculates raw probability values for each pixel within the study region and is widely acknowledged for its efficacy in predicting species distributions (Elith et al. 2006).

## Model settings and evaluation

Maxent was employed with varying model complexities to strike a balance between model fit and overfitting. Subsequently, the jackknife method, employing jackknife cross-validation with n-1 folds, was utilized for model complexity estimation. This approach proves advantageous for species with limited occurrence data, facilitating a robust evaluation of model performance across different complexities (Pearson et al. 2007; Shcheglovitova and Anderson 2013).

Model assessment relied on the Area Under the Curve (AUC) metric, offering a comprehensive evaluation of the model's discriminative ability between suitable and unsuitable habitats across various threshold values. Following the tuning process and subsequent testing, default settings for Feature Class and Regularization were retained, as they exerted minimal impact on the model.

# Results

# New finds of Notothylas orbicularis and Anthoceros neesii in Poland

During the field visit on 7–8 October 2023, two notable findings of hornworts were made in Poland. Firstly, *Notothylas orbicularis* was discovered for the first time in Poland. Secondly, the occurrence of *Anthoceros neesii* in this country was reconfirmed after 36 years. *Notothylas orbicularis* was collected at localities No. 1 and No. 2, while *A. neesii* was found at localities No. 2 and No. 3 (for locality details, see the Material and methods chapter). Populations of both species were relatively abundant, each consisting of several dozen fertile plants. Along with these interesting species, other bryophytes were recorded (listed in alphabetical order): *Anthoceros agrestis, Amblystegium serpens* (Hedw.) Schimp., *Barbula unguiculata* Hedw., *Brachythecium rutabulum* (Hedw.) Schimp., *Bryum argenteum* Hedw., *B. klinggraeffii* Schimp. ex Klinggr., *B. rubens* Mitt., *Dicranella schreberiana* (Hedw.) Dixon, *D. staphylina* H.Whitehouse, *Fissidens taxifolius* Hedw., *Riccia sorocarpa* Bisch., *Tortula acaulon* (With.) R.H.Zander and *T. truncata* (Hedw.) Mitt.

To prevent potential confusion regarding hornwort species, we provide a brief summary of the most important diagnostic features of *Notothylas orbicularis* and *Anthoceros neesii* with a special reference to the shape and sculpture of the spores (Fig. 3) and a key to the determination of all four species of hornworts occurring in Poland. Moreover, all literature and herbarium records of these two species are presented and their distribution is mapped (Figs 4, 5).



Figure 2. Detail view of the hornworts A Notothylas orbicularis B Anthoceros neesii. Photographs were taken by Štěpán Koval.

#### **Notothylas orbicularis** (Fig. 2A)

The species is characterized by small, rosette-like, flat, prostrate, and round thallus, typically measuring 5–7 (up to 12) mm in diameter. They are dark green, and irregularly lobed at the edge, 6–10 cells thick medially and thinning 2–3-stratose toward margins. Small colonies of cyanobacteria of the genus *Nostoc* Vaucher *ex* Bornet & Flahault are visible in the thallus. The species is monoicous, typically with 2–6 club-shaped antheridia in each cavity. The involucres are scattered near thallus margins, often paired, and they bear capsules that are decumbent on the thallus and project over the edge. The capsules are approximately 1 mm long and oblong-ovate in shape, with a reduced or absent columella. The pseudoelaters are unicellular. The spores are golden-yellow to yellow-green, measuring 35–45 µm in diameter, delicately vermiculate with both proximal and distal faces appearing virtually smooth (Fig. 3J–L). In the size and sculpture the spores of *N. orbicularis* somewhat resemble the spores of *Phaeoceros carolinianus* in which the proximal faces usually appear nearly smooth but the distal faces are finely echinulate to verruculose (Fig. 3G–I).

Notothylas orbicularis is a rare species of hornwort in Central Europe, occurring in Germany, Austria, the Czech Republic and now recorded in Poland. According to T. Pócs and A. Sass-Gyarmati, Eger, and P. Širka, Zvolen, this species is, respectively, absent in Hungary and Slovakia. In Poland, it is currently known only from two following localities in the Kłodzko Basin in the Central Sudetes (Fig. 4), although it is very likely that careful field studies will yield additional records of this species in this region. In Europe, the species is otherwise reported from Italy, and Croatia (Rimac et al. 2019; Hodgetts and Lockhart 2020). It is



**Figure 3.** SEM photographs of the spores of hornworts occurring in Poland **A–C** Anthoceros agrestis **D–F** Anthoceros neesii **G–I** Phaeoceros carolinianus **J–L** Notothylas orbicularis. Photographs were taken by Vítězslav Plášek. Scale bar: 10 µm.

placed in the IUCN European Red List of Mosses, Liverworts and Hornworts in the category of Endangered B2ab (ii, iii, v) (Hodgetts et al. 2019) and is listed on Annex II (animal and plant species of Community interest whose conservation requires the designation of special areas of conservation) of EU Habitats Directive (European Community 1992).



Figure 4. Distribution map for Notothylas orbicularis in Poland. Inset: The location of Lower Silesia in Europe.

# List of localities of Notothylas orbicularis in Poland

- Fb-15 Central Sudetes, Kłodzko Basin: 3 km SE of the Wambierzyce village, small stubble field near the provincial road No. 388, on open soil, alt. 450 m a.s.l., 7 Oct 2023, leg. V. Plášek (OSTR #8301, KRAM B-278059).
- **Fb-25** Central Sudetes, Kłodzko Basin: between the villages of Szalejów Dolny and Szalejów Górny, on open soil on a stubble field near the national road No. 8, alt 359 m a.s.l., 7 Oct 2023, leg. V. Plášek (OSTR #8302, KRAM B-278060).

#### Anthoceros neesii

(Fig. 2B)

Unlike the more frequently occurring species *Anthoceros agrestis*, *A. neesii* is a very small plant, with rosette-like thalli up to 5 mm in diameter. They are 3–4 cells thick medially and arched on the upper surface. The species is



Figure 5. Distribution map for Anthoceros neesii in Poland. Inset: The location of Lower Silesia in Europe.

monoicous, typically with numerous club-shaped antheridia, up to 60  $\mu$ m long, in each cavity. The capsules are somewhat club-shaped, brown distally at maturity, mostly 3–7 mm long and 0.3–0.4 mm wide, with a distinct columella. The pseudoelaters are formed of two to several short cells. The mature spores are blackish-brown, 45–54  $\mu$ m in diameter, with spinulate-blunt protuberances on the proximal faces and simple spines on the distal faces (Fig. 3D–F). The sculpturing of the spores immediately differentiates *A. neesii* from *A. agrestis* which has an almost smooth proximal face with indentations and a distal face covered with forked spines (Fig. 3A–C).

Anthoceros neesii is a rare and widely scattered Central European endemic species which is placed in the IUCN European Red List of Mosses, Liverworts and Hornworts in the category of Endangered B2ab(iii) (Bisang et al. 2019; Hodgetts et al. 2019). Apart from Poland, it is known from the Czech Republic, Germany and Austria only (Hodgetts and Lockhart 2020). In Poland, the species has been recorded in seven grid squares in the ATMOS system for mapping bryophytes (Ochyra and Szmajda 1981). In three grid squares it was recorded prior to 1944, in two in the second half of the twentieth century, and in the other two in 2023 (Fig. 5). All localities but one are situated in the Sudetes in the south-western part of the country and only one historical discovery originates from the northern lowlands in West Pomerania. It is worth noting that *A. neesii* was rediscovered in the early 1990s at the type locality in the Jelenia Góra Basin in Lower Silesia and, additionally found at one locality in the Giant Mountains (Koła and Turzańska 1993). However, these discoveries were ignored by the authors of the treatment of *A. neesii* in the IUCN Red List of Threatened Species 2019 (Bisang et al. 2019), who indicated the occurrence of this species in Poland to be "uncertain".

## List of localities of Anthoceros neesii in Poland

- **Cb-14** South Baltic Lakelands, South Pomeranian Lake District, Wałcz Lakeland: Rusinowo (Proskauer 1958).
- Ea-69 Western Sudetes, Jelenia Góra Basin: District Malinnik of Jelenia Góra east of Cieplice Śląskie Zdrój District (Proskauer 1958).
- **Eb-70** Western Sudetes, Jelenia Góra Basin: Grodna Hill in Staniszów (Proskauer 1958); Staniszów (Koła and Turzańska 1993)
- **Eb-71** Western Sudetes, Krzyżna Góra in the Góry Sokole [= Falcon Mountains] massif east of Jelenia Góra (Proskauer 1958).
- **Eb-80** Western Sudetes, Karkonosze: Wilcza Poręba in Karpacz (Koła and Turzańska 1993).
- Fb-15 Central Sudetes, Kłodzko Basin: 3 km SE of the Wambierzyce village, small stable field near the provincial road No. 388, on open soil, 450 m a.s.l., 7 Oct 2023, leg. V. Plášek (OSTR #8303, KRAM B-278061).
- Fb-25 Central Sudetes, Kłodzko Basin: between the villages of Szalejów Dolny and Szalejów Górny 5 km west of Kłodzko town, stable field near the national road No. 8, on open soil near a small forest, 359 m a.s.l., 7 Oct 2023, leg. V. Plášek (OSTR #8304, KRAM B-278062).

# Key to identification of the hornworts species in Poland

- 2 Capsules 10–30 mm long, proximal spore faces almost smooth, with indentations, distal faces forming forked spines\*....... *Anthoceros agrestis*
- Capsules 3–7 mm long, proximal spore faces with spinulate-blunt protuberances, distal faces forming simple (not forked) spines.....
  - .....Anthoceros neesii (Fig. 2B)

<sup>\*</sup> For detailed views of the spore faces of each species, see SEM photographs (Fig. 3A-L).

#### Maxent modelling results

In this section, we outline the outcomes of various Maxent models conducted for each studied species across different scenarios (Figs 6, 7). Additionally, we provide average AUC results and highlight the most significant variables identified for each scenario.



**Figure 6.** This figure displays the probabilities generated by Maxent across different time periods, GCMs, and SSPs for the species *Anthoceros neesii*. The colour gradient ranges from blue (representing low probabilities, close to 0) to red (representing high probabilities, close to 1). The state borders are outlined for reference. For explanation of the maps **A–E** and detailed values see Table 3.

Madal	Average AUC		
Model	Anthoceros neesii	Notothylas orbicularis	
1980-2010	0,960 (Fig. 6A)	0,955 (Fig. 7A)	
2011-2040_gfdl-esm4_ssp126	0,929 (Fig. 6B)	0,863 (Fig. 7B)	
2011-2040_gfdl-esm4_ssp585	0,933 (Fig. 6C)	0,829 (Fig. 7C)	
2011-2040_ipsl-cm6a-lr_ssp126	0,938 (Fig. 6D)	0,853 (Fig. 7D)	
2011-2040_ipsl-cm6a-lr_ssp585	0,934 (Fig. 6E)	0,855 (Fig. 7E)	

Table 3. Average AUC values for each species and model.



**Figure 7.** This figure displays the probabilities generated by Maxent across different time periods, GCMs, and SSPs for the species *Notothylas orbicularis*. The colour gradient ranges from blue (representing low probabilities, close to 0) to red (representing high probabilities, close to 1). The state borders are outlined for reference. For explanation of the maps **A–E** and detailed values see Table 3.

The following values present estimates of the relative contributions of the variables to the final Maxent model (in %) for different time period, GCMs and SSPs -only variables with contributions to the final model >5% were left in the final models (average contributions in Table 4):

#### Anthoceros neesii:

1980–2010: bio8 – 34.5, LC – 33.6, bio14 – 20, bio1 – 6.1, bio15 – 5.9; 2011–2040\_gfdl-esm4\_ssp126: bio1 – 36.6, bio14 – 32.7, LC – 13.2, bio15 – 12.1, bio8 – 5.4; 2011–2040\_gfdl-esm4\_ssp585: bio1 – 36.7, bio14 – 32.8, LC – 13.7, bio15 – 8.6, bio8 – 8.2; 2011–2040\_ipsl-cm6a-lr\_ssp126: bio14 – 38.3, bio1 – 36.3, LC – 12.7, bio15 – 7.9, bio8 – 5.7; 2011–2040\_ipsl-cm6a-lr\_ssp585: bio1 – 40.3, bio14 – 32.3, LC – 13.9, bio15 – 8.2, bio8 – 5.3.

#### Notothylas orbicularis:

1980–2010: bio8 – 35.1, bio14 – 25.3, LC – 23.9, bio15 – 9.6, bio1 – 6; 2011– 2040\_gfdl-esm4\_ssp126: bio14 – 29, bio15 – 24.6, bio1 – 20.2, LC – 17.4, bio8 – 8.8; 2011–2040\_gfdl-esm4\_ssp585: bio14 – 30.6, bio15 – 28.3, LC – 21.4, bio1 – 13.7, bio8 – 6; 2011–2040\_ipsl-cm6a-lr\_ssp126: bio15 – 27.5, bio14 – 22.7, bio1 – 21.2, LC – 18.2, bio8 – 10.4; 2011–2040\_ipsl-cm6a-lr\_ssp585: bio15 – 26.2, bio14 – 21.7, bio1 – 20.8, LC – 18.6, bio8 – 12.6.

 Table 4. Average relative contribution of the variables calculated from all models for each species to the final models.

Variable	Anthoceros neesii	Notothylas orbicularis
bio14	25.86	31.22
bio15	23.24	8.54
LC(2000 or 2018)	19.9	17.42
bio1	16.38	31.4
bio8	14.58	11.62

\*For this purpose, LC2000 and LC2018 were calculated together.

## Discussion

Although *Notothylas orbicularis* is often designated as a cosmopolitan species (Dierßen 2001), it is actually a highly disjunct panholarctic temperate species, with the main center of its occurrence in eastern North America (Schuster 1992), and scattered localities in Central and South Europe, as well as in China and Japan in East Asia (Yamada and Iwatsuki 2006; Peng and Zhu 2013). Outside the Holarctic, it only occasionally penetrates into the tropics, with records from Colombia and Ecuador (Gradstein 2021) and eastern Brazil (Gradstein and Costa 2003) in South America, as well as Uganda and the Democratic Republic of the Congo in mainland Africa (Stieperaere and Matcham 2007; Wigginton 2018; Gradstein 2023), and Madagascar (Ellis et al. 2018). Additionally, the species was discovered in Western Australia and Queensland in Australia (Cargill 2016).

In Europe, the species has been recorded in three Central European countries (Austria, Germany, Czech Republic) and two Southern European countries (Italy, Croatia) (cf. Jack 1898; Ludwig et al. 1996; Saukel and Köckinger 1999; Weddeling 2002; Manzke 2004, 2005; Fischer et al. 2008; Koval and Zmrhalová 2010; Kučera 2011; Köckinger 2017; Rimac et al. 2019; Pöltl et al. 2020). Herein, its occurrence is also documented for Poland. In its fertile stage, *N. orbicularis* is nearly unmistakable in the field due to its distinctively formed capsules. However, the young thallus of this species can resemble *Anthoceros agrestis*. A critical distinguishing feature is that the capsules of *N. orbicularis* grow almost horizontally on the thallus or are partially embedded within it and never grow perpendicularly or obliquely as seen in species of *Anthoceros or Phaeoceros*. The capsules are ellipsoidal and cigar-shaped, enclosed in a longitudinally warty involucre throughout the ripening period. Fully mature capsules may exhibit a dark tip, and mature spores are smooth on both sides (Fig. 3J–L).
Anthoceros neesii (Fig. 2B) has a limited global distribution, being recorded only in the Czech Republic, Poland, Germany, and Austria (Proskauer 1958; Düll 1983; Grolle 1983; Düll and Meinunger 1989; Koła and Turzańska 1993; Dierßen 2001; Weddeling 2002; Manzke 2004, 2005; Meinunger and Schröder 2007; Fischer et al. 2008; Teuber and Göding 2009; Koval and Zmrhalová 2010; Kučera 2011; Schlüsslmayr 2011; Schröck et al. 2014; Köckinger 2017; Pöltl et al. 2020). Given its distribution, A. neesii is classified as a Central European endemic. In the field, A. neesii can be initially distinguished from other hornworts by its generally smaller stature. The capsules are short and grow perpendicularly or obliquely to the thallus surface, displaying a significant constriction at the transition to the upper dark part where mature spores are located. However, reliable differentiation, especially from small forms of A. agrestis, can only be achieved when mature spores are present, which exhibit distinct microscopic features (Fig. 3D-F). Additionally, there is a small difference in mean spore size (n = 50) between A. neesii and A. agrestis (Pöltl et al. 2020). According our observation, the spores of A. neesii measure 45-54 µm and are slightly smaller than those of A. agrestis, which measure 38-62 µm.

For both studied species, an increase in the areas suitable for their occurrence in the future is projected. For *Anthoceros neesii*, these areas are primarily located in the border regions of the Czech Republic, as well as in southern, northern, and central Poland, and central regions of Germany. Additionally, suitable areas are expected in the states of Oberösterreich and Steiermark in Austria, as well as certain regions of Slovakia. For *Notothylas orbicularis*, the projected suitable areas are mainly in the border and central regions of the Czech Republic, with additional suitable areas expected to emerge in Slovakia, Poland, and Austria. However, there is a projected decrease in suitable areas in Germany when comparing the period from 1980–2010 to future projections. Overall, there appears to be an expansion of suitable areas for the spread of both species.

According to the results of our study, climate change is significantly influencing the distribution and expansion of hornworts (Anthocerotophyta) in Central Europe. Rising temperatures and altered precipitation patterns are causing shifts in their traditional habitats (Bates and Preston 2011; van Zuijlen et al. 2024). Warmer temperatures can extend the growing season, potentially allowing hornworts to colonize areas previously too cold for their establishment. However, these advantages are often counterbalanced by increased risks of drought and habitat desiccation (Gignac 2001; Bisang et al. 2021a, 2021b).

Additionally, climate change can lead to alterations in land use patterns, such as changes in agricultural practices and forest management, further impacting hornwort habitats (Dale 1997; Olesen and Bindi 2002; Oliver and Morecroft 2014; van Zuijlen et al. 2024). For example, an increased frequency of intense agricultural activities may create more disturbed soils (Ewert et al. 2005), which can be beneficial for some hornwort species. Conversely, such practices may also result in habitat loss through land conversion. Additionally, the rapid ploughing of harvested fields, especially cereal fields, poses a risk to both species.

Hornworts depend heavily on moisture for their growth and reproduction. Changes in precipitation patterns, particularly reduced summer rainfall and an increased frequency of droughts, can adversely affect their populations (Spinoni et al. 2015). In Central Europe, the variability in seasonal precipitation has led to fluctuating soil moisture levels, impacting hornworts' ability to thrive in their typical moist and shaded microhabitats. Despite these challenges, some hornwort species may find new niches in disturbed areas created by climate-induced events such as flooding (Madsen et al. 2014). Floodplains and areas prone to periodic waterlogging can provide suitable environments for hornworts, potentially aiding in their expansion. Furthermore, milder winters and reduced snow cover might also facilitate hornwort survival and propagation in regions previously constrained by harsh winter conditions.

Research indicates that climate change may also affect the symbiotic relationships hornworts have with cyanobacteria, which are crucial for nitrogen fixation. Changes in temperature and moisture levels could disrupt these symbioses, impacting hornworts' growth and their role in nutrient cycling within ecosystems (Tylianakis et al. 2008).

The northward migration of certain hornwort species has been observed and is attributed to the warming climate. This shift is a response to changing temperature and precipitation regimes, allowing species to move into new territories where conditions have become more favourable (cf. Zanatta et al. 2020; van Zuijlen et al. 2024). However, this migration is not uniform across all species and depends on their specific ecological requirements (Zanatta et al. 2020). The fact that *A. neesii* is endemic to Europe underscores the significant responsibility of the countries where this species occurs to ensure its protection.

# Conclusions

The impact of climate change on these hornworts is multifaceted. While certain changes may offer new opportunities for colonization, others pose significant risks by disrupting their delicate ecological niches. Continuous research and monitoring are crucial to comprehending these dynamics and formulating strategies to mitigate adverse effects on these species.

In conclusion, hornworts represent a distinctive and ecologically significant component of Central Europe's bryophyte flora. Their distribution is influenced by a combination of climatic, edaphic, and anthropogenic factors. Continued research and conservation efforts are imperative to ensure the persistence of these unique plants amidst ongoing environmental changes.

# Acknowledgements

We would like to thank colleagues from Hungary (T. Pócs and A. Sass-Gyarmati) and Slovakia (P. Širka) for their valuable advice and information. Special thanks are due to Krzysztof Świerkosz, Zygmunt Dajdok, and Magdalena Turzańska (Wrocław, Poland) and Katarzyna Buczkowska-Chmielewska (Poznań, Poland) for checking the herbarium collections and providing all the important historical information. We also express our gratitude to Štěpán Koval (Sobotín), for generously providing the two photographs that greatly enhanced the quality of this article, and Marian Wysocki (Kraków, Poland) for assistance in generating distribution maps of species in Poland.

# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

The project is co-financed by the EU structural funding CZ.1.05/2.1.00/19.0388 and funding from the Ministry of Education, Youth, and Sports of the Czech Republic: L01208. Work of Lukáš Číhal was financially supported by the Ministry of Culture of the Czech Republic (Silesian Museum, MK000100595). Ryszard Ochyra obtained support from the statutory fund of the W. Szafer Institute of the Polish Academy of Sciences in Kraków. Work of V. Plášek was carried out at MCBR UO (International Research and Development Center of the University of Opole), which was established as part of a project co-financed by the European Union under the European Regional Development Fund, RPO WO 2014–2020, Action 1.2 Infrastructure for R&D. Agreement No. RPOP.01.02.00-16-0001/17-00 dated January 31, 2018.

#### Author contributions

VP collected the specimens, identified them, and prepared SEM micrographs and photographs of the habitats; LČ performed data analyses and prepared Maxent models; FM, MP obtained and provided data on the distribution of the hornworts concerned in Germany and Austria, which was used for the preparing the Maxent models; RO, MW analyzed historical data on the distribution of hornworts in Poland and compared them with the current distribution; RO generated the distribution maps of *Notothylas orbicularis* and *Anthoceros neesii* in Poland; VP, LČ, FM, MP, MW and RO prepared the draft version of the manuscript, which was reviewed, edited, and approved by all co-authors.

#### Author ORCIDs

Vítězslav Plášek © https://orcid.org/0000-0002-4664-2135 Lukáš Číhal © https://orcid.org/0009-0009-2740-1326 Frank Müller © https://orcid.org/0000-0001-9482-9423 Martina Pöltl © https://orcid.org/0000-0002-3001-058X Mariusz Wierzgoń © https://orcid.org/0000-0002-4809-2202 Ryszard Ochyra © https://orcid.org/0000-0002-2541-0722

#### Data availability

All of the data that support the findings of this study are available in the main text.

# References

- Aiello-Lammens ME, Boria RA, Radosavljevic A, Vilela B, Peterson RP (2015) spThin: An R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38(5): 541–545. https://doi.org/10.1111/ecog.01132
- Bates JW, Preston CD (2011) Can the effects of climate change on British bryophytes be distinguised from those resulting from other environmental changes? In: Tuba Z, Slack NG, Stark LR (Eds) Bryophyte Ecology and Climate Change. Cambridge University Press, New York, 371–407. https://doi.org/10.1017/CB09780511779701.020
   Bisang I (1998) The occurrence of hornwort populations (Anthocerotales, Anthocero-
- topsida) in the Swiss Plateau: the role of management, weather conditions and soil characteristics. Lindbergia 23: 94–104.

- Bisang I (2004) Population development, demographic structure, and life cycle aspects of two hornworts in Switzerland. Lindbergia 28: 105–112.
- Bisang I, Bergamini A (2020) Agricultural intensification, sustainable farming and the fate of arable bryophytes in Switzerland. In: Hurford C, Wilson P, Storkey J (Eds) The changing status of arable habitats in Europe, Springer Nature Switzerland, 139–156. https://doi.org/10.1007/978-3-030-59875-4
- Bisang I, Bergamini A, Lienhard L (2009) Environmental-friendly farming in Switzerland is not hornwort friendly. Biological Conservation 142: 2104–2113. https://doi. org/10.1016/j.biocon.2009.04.006
- Bisang I, Schnyder N, Caspari S, Hedenäs L, Hodgetts N, Kiebacher T, Kučera J, Ştefănuţ S, Vana [sic!] J (2019) Anthoceros neesii. The IUCN Red List of Threatened Species 2019: e.T39217A87732549. https://doi.org/10.2305/IUCN.UK.2019-2.RLTS. T39217A87732549.en
- Bisang I, Lienhard L, Bergamini A (2021a) Three decades of field surveys reveal a decline of arable bryophytes in the Swiss lowlands despite agri-environment schemes. Agriculture, Ecosystems and Environment 313: 107325. https://doi.org/10.1016/j. agee.2021.107325
- Bisang I, Schnyder N, Bergamini A (2021b) Are agri-environment schemes beneficial to arable specialist bryophytes in Switzerland? Field Bryology 125: 50–59.
- Błoński F (1888) Materyjały do flory skrytokwiatowéj krajowéj. Wątrobowce Królestwa Polskiego (Hepaticae polonicae) [Matériaux pour server à la flore cryptogame. Hépatiques de la Pologne]. Pamiętnik Fizyjograficzny, Dział 3 Botanika i Zoologija 8: 156–202 [+ Tab. ii-v].
- Cargill DC (2016) Rare and peculiar hornworts: *Notothylas orbicularis* and *N. javanica* (Notothyladaceae), new genus and species records for Australia. Phytotaxa 275(1): 1–13. https://doi.org/10.11646/phytotaxa.275.1.1
- Corda AJ (1829) Monographia Rhizospermacearum et Hepaticorum. Die Wurzelfarren und Lebermoose nach ihren Gattungen und Arten, organographisch-phytotomisch bearbeitet. 1. Heft. Prag: in der Sommerschen Buchdruckerei, i-vi + 7-16 + Taf. i-vi.
- Dale VH (1997) The relationship between land-use change and climate change. Ecological Applications 7(3): 753–769. https://doi.org/10.1890/1051-0761(1997)007[0753 :TRBLUC]2.0.CO;2
- Dierßen K (2001) Distribution, ecological amplitude and phytosociological characterization of European bryophytes. Bryophytorum Bibliotheca 56: 1–289.
- Düll R (1983) Distribution of the European and Macaronesian liverworts (*Hepaticophyti-na*). Bryologische Beiträge 2: 1–115.
- Düll R, Meinunger L (1989) Deutschlands Moose. Die Verbreitung der deutschen Moose in der BR Deutschland und in der DDR, ihre Höhenverbreitung, ihre Arealtypen, sowie Angaben zum Rückgang der Arten. 1. Teil: Anthocerotae, Marchantiatae, Bryatae: Sphagnidae, Andreaeidae, Bryidae: Tetraphidales – Pottiales. Bad Münstereifel – Ohlerath IDH-Verlag, 1–368.
- Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JM, Peterson AT, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29(2): 129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x
- Ellis LT, Afonina OM, Andriamiarisoa RL, Asthana G, Bharti R, Aymerich P, Bambe B, Boiko M, Brugués M, Ruiz E, Sáez L, Cano MJ, Ros R, Číhal L, Deme J, Csiky J, Dihoru G,

Dřevojan P, Ezer T, Fedosov VE, Ignatova EA, Seregin AP, Garcia CA, Martins A, Sérgio C, Sim-Sim M, Rodrigues ASB, Gradstein SR, Reeb C, Irmah A, Suleiman M, Koponen T, Kučera J, Lebouvier M, LiQun Y, Long DG, Maksimov AI, Maksimova TA, Muñoz J, Nobis M, Nowak A, Ochyra R, O'Leary SV, Osorio F, Pisarenko OYu, Plášek V, Skoupá Z, Schäfer-Verwimp A, Schnyder N, Shevock JR, Ştefănuţ S, Sulayman M., Sun B-Y, Park SJ, Tubanova DYa, Váňa J, Wolski J, Yao K-Y, Yoon Y-J, Yücel E. (2018) New national and regional bryophyte records, 56. Journal of Bryology 40(3): 271–296. https://doi.org/10.1080/03736687.2018.1487687

- European community (1992) Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities L 206/7, 1–44.
- Ewert F, Rounsevell M, Reginster I, Metzger MJ, Leemans R (2005) Future scenarios of European agricultural land use I. Estimating changes in crop productivity. Agriculture Ecosystems and Environment 107: 101–116. https://doi.org/10.1016/j.agee.2004.12.003
- Fischer E, Killmann D, Buchbender V (2008) Zum Status von *Notothylas orbicularis* und *Anthoceros neesii* (Anthocerotopsida) im Westerwald/Rheinland-Pfalz. Decheniana 161: 33–39. https://doi.org/10.21248/decheniana.v161.3828
- Frangedakis E, Shimamura M, Villarreal JC, Li F-W, Tomaselli M, Waller M, Sakakibara K, Renzaglia KS, Szövényi P (2021) The hornworts: morphology, evolution and development. New Phytologist 229: 735–754. https://doi.org/10.1111/nph.16874
- Frangedakis E, Marron AO, Waller M, Neubauer A, Tse SW (2023) What can hornworts teach us? Frontiers in Plant Science 14: 1108027. https://doi.org/10.3389/ fpls.2023.1108027
- Garcia C, Sérgio C, Villarreal JC, Sim-Sim M, Lara F (2012) The hornworts *Dendroceros* Nees and *Megaceros* Campb. in São Tomé e Prīncipe (Africa, Gulf of Guinea) with the description of *Dendroceros paivae* sp. nov. Cryptogamie Bryologie 33: 3–21. https:// doi.org/10.7872/cryb.v33.iss1.2012.003
- Gignac LD (2001) Bryophytes as indicators of climate change. Biological Conservation 10(2): 131–142. https://doi.org/10.1639/0007-2745(2001)104[0410:BAIOCC]2.0.CO;2
- Glime JM (2017) Bryophyte ecology. Volume 1. Physiological ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. https://digitalcommons.mtu.edu/bryophyte-ecology1/
- Gradstein SR (2021) The liverworts and hornworts of Colombia and Ecuador. Memoirs of the New York Botanical Garden 121: 1–723. https://doi.org/10.1007/978-3-030-49450-6
- Gradstein SR (2023) Bryophytes. Introduction. Division A. Anthocerotophyta. In: Sosef MSM (Ed.) Flore d'Afrique Centrale (République démocratique du Congo – Rwanda – Burundi). Jardin Botanique de Meise, 1–33.
- Gradstein SR, Costa DP (2003) The Hepaticae and Anthocerotae of Brazil. Memoirs of the New York Botanical Garden 87: 1–318.
- Grolle R (1976) Verzeichnis der Lebermoose Europas und benachbarter Gebiete. Feddes Repertorium 87: 171–279. https://doi.org/10.1002/fedr.19760870303
- Grolle R (1983) Hepatics of Europe including the Azores: an annotated list of species, with synonyms from the recent literature. Journal of Bryology 12: 403–459. https:// doi.org/10.1179/jbr.1983.12.3.403
- Hijmans RJ (2024) Raster: Geographic data analysis and modeling. R package version 3.6-27. https://cran.r-project.org/web/packages/raster/raster.pdf
- Hijmans RJ, Barbosa M, Ghosh A, Mandel A (2023) Geodata: download geographic data. https://cran.r-project.org/web/packages/geodata/geodata.pdf

- Hodgetts N, Lockhart N (2020) Checklist and country status of European bryophytes update 2020. Irish Wildlife Manuals 123: 1–214.
- Hodgetts N, Cálix M, Englefield E, Fettes N, García Criado M, Patin L, Nieto A, Bergamini A, Bisang I, Baisheva E, Campisi P, Cogoni A, Hallingbäck T, Konstantinova N, Lockhart N, Sabovljevic M, Schnyder N, Schröck C, Sérgio C, Sim Sim M, Vrba J, Ferreira CC, Afonina O, Blockeel T, Blom H, Caspari S, Gabriel R, Garcia C, Garilleti R, González Mancebo J, Goldberg I, Hedenäs L, Holyoak D, Hugonnot V, Huttunen S, Ignatov M, Ignatova E, Infante M, Juutinen R, Kiebacher T, Köckinger H, Kučera J, Lönnell N, Lüth M, Martins A, Maslovsky O, Papp B, Porley R, Rothero G, Söderström L, Ştefănuţ S, Syrjänen K, Untereiner A, Váňa Jł, Vanderpoorten A, Vellak K, Aleffi M, Bates J, Bell N, Brugués M, Cronberg N, Denyer J, Duckett J, During HJ, Enroth J, Fedosov V, Flatberg K-I, Ganeva A, Gorski P, Gunnarsson U, Hassel K, Hespanhol H, Hill M, Hodd R, Hylander K, Ingerpuu N, Laaka-Lindberg S, Lara F, Mazimpaka V, Mežaka A, Müller F, Orgaz JD, Patiño J, Pilkington S, Puche F, Ros RM, Rumsey F, Segarra-Moragues JG, Seneca A, Stebel A, Virtanen R, Weibull H, Wilbraham J, Żarnowiec J (2019) A miniature world in decline: European Red List of mosses, liverworts and hornworts. Brussels, Belgium: IUCN.
- Jack J (1898) Lebermoose Tirols. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 48: 173–191.
- Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M (2017) Climatologies at high resolution for the earth's land surface areas. Scientific Data 4: 170122. https://doi.org/10.1038/sdata.2017.122
- Klama H (2006a) Systematic catalogue of Polish liverwort and hornwort taxa. In: Szwey-kowski J (Ed.) An annotated checklist of Polish liverworts. Biodiversity of Poland, Vol.
  4. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 83–100.
- Klama H (2006b) Red list of the liverworts and hornworts in Poland. In: Mirek Z, Zarzycki K, Wojeworda W, Szeląg Z (Eds) Red list of plants and fungi in Poland. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 21–33.
- Klama H, Górski P (2018) Red List of liverworts and hornworts of Poland (4<sup>th</sup> edition, 2018). Cryptogamie, Bryologie 39(4): 415–441. https://doi.org/10.7872/cryb/v39. iss4.2018.415
- Köckinger H (2017) Die Horn- und Lebermoose Österreichs (Anthocerotophyta und Marchantiophyta). Catalogus Florae Austriae, II. Teil, Heft 2. In: Ehrendorfer F (Ed.) Biosystematics and Ecology Series No. 32. Wien: Verlag der Österreichischen Akademie der Wissenschaften, 1–382. https://doi.org/10.2307/j.ctt1v2xvg0.4
- Koła W, Turzańska M (1993) Zbiorowiska pól uprawnych Dolnego Śląska. Prace Botaniczne 53: 3–12.
- Koła W, Turzańska M (1995) Wątrobowce (Hepaticopsida) i glewiki (Anthocerophytina). Klucz do oznaczania. Część I. Wątrobowce plechowate i glewiki. Wrocław: Wydawnictwo Uniwersytetu Wrocławskiego, 1–153.
- Kosztra B, Büttner G, Hazeu G, Arnold S (2017) Updated CLC illustrated nomenclature guidelines. European Environment Agency: Wien, Austria, 1–124. https://land.copernicus.eu/user-corner/technical-library/corine-land-cover-nomenclature-guidelines/docs/ pdf/CLC2018\_Nomenclature\_illustrated\_guide\_20190510.pdf [Accessed on May 2024]
- Koval Š, Zmrhalová M (2010) Znovuobjevení hlevíků Anthoceros neesii a Notothylas orbicularis v České republice. Bryonora 46: 38–46.
- Kučera J (2011) Zajímavé bryofloristické nálezy XVIII. Bryonora 48: 59-67.
- Limpricht KG (1876) Lebermoose. In: Cohn F (Ed.) Kryptogamen-Flora von Schlesien. Breslau: J. U. Kern's Verlag (Max Müller), 225–352.

- Ludwig G, Düll R, Philippi G, Ahrens M, Caspari S, Koperski M, Lütt S, Schulz F, Schwab G (1996) Rote Liste der Moose (Anthocerophyta et Bryophyta) Deutschlands. 295. Schriftenreihe für Vegetationskunde 28: 189–306.
- Madsen H, Lawrence D, Lang M, Martinkova M, Kjeldsen TR (2014) Review of trend analysis and climate change projections of extreme precipitation and floods in Europe. Journal of Hydrology 519: 3634–3650. https://doi.org/10.1016/j.jhydrol.2014.11.003
- Manzke W (2004) Zur Verbreitung und Bestandssituation von Notothylas orbicularis, Anthoceros neesii, Anthoceros agrestis, Phaeoceros carolinianus und Riccia ciliata auf Stoppelfeldern im Vogelsberg (Hessen). Hessische Floristische Briefe 53: 53–65.
- Manzke W (2005) Zur Verbreitung und Gefährdung von Notothylas orbicularis und Anthoceros neesii im Vogelsberg (Hessen). Bryologische Rundbriefe 86: 1–3.
- Meinunger L, Schröder W (2007) Verbreitungsatlas der Moose Deutschlands. Band 1. Herausgegeben von Oliver Dürhammer für die Regensburgische Botanische Gesellschaft von 1790 e. V. Verlag der Gesellschaft, Regensburg, 1–636.
- Müller K (1906–1911) Die Lebermoose Deutschlands, Oesterreichs u. d. Schweiz mit Berücksichtigung der übrigen Länder Europas. I. Abteilung. In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Band 6. Zweite Auflage. Die Lebermoose. Leipzig: Verlag von Eduard Kummer, 1–870.
- Müller K (1912–1916) Die Lebermoose Deutschlands, Oesterreichs u. d. Schweiz mit Berücksichtigung der übrigen Länder Europas. II. Abteilung. In: Dr. L. Rabehorsts's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Band 5. Zweite Auflage. Die Lebermoose. Leipzig: Verlag von Eduard Kummer, 1–947.
- Naimi B, Hamm NAS, Groen TA, Skidmore AK, Toxopeus AG (2014) Where is positional uncertainty a problem for species distribution modelling? Ecography 37(2): 191–203. https://doi.org/10.1111/j.1600-0587.2013.00205.x
- Nees von Esenbeck ChG (1838) Naturgeschichte der Europäischen Lebermoose mit besonderer Beziehung auf Schlesien und die Oertlichkeiten des Riesengebirgs. Band 4. Breslau: bei Grass, Barth und Comp. Ixxii + 539 pp.
- Ochyra R, Szmajda P (1981) La cartographie bryologique en Pologne. In: Szweykowski J (Ed.) New perspectives in bryotaxonomy and bryogeography. Second Bryological Meeting, Poznań, June 26<sup>th</sup> 29<sup>th</sup>, 1980. Adam Mickiewicz University, Poznań, 105–110.
- Olesen JE, Bindi M (2002) Consequences of climate change for European agricultural productivity, land use and policy. European Journal of Agronomy 16(4): 239–262. https://doi.org/10.1016/S1161-0301(02)00004-7
- Oliver TH, Morecroft MD (2014) Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. Environmental Science. Wiley Interdisciplinary Reviews: Climate Change 5(3): 317–335. https://doi.org/10.1002/wcc.271
- Pearson RG, Raxworthy CJ, Nakamura M, Peterson AT (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of Biogeography 34(1): 102–117. https://doi.org/10.1111/ j.1365-2699.2006.01594.x
- Peng T, Zhu R-L (2013) A revision of the genus *Anthoceros* (Anthocerotaceae, Anthocerotophyta) in China. Phytotaxa 100(1): 21–35. https://doi.org/10.11646/phytotaxa.100.1.3
- Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. Ecography 31(2): 161–175. https://doi. org/10.1111/j.0906-7590.2008.5203.x

- Phillips SJ, Dudík M, Schapire RE (2024) Maxent software for modeling species niches and distributions (Version 3.4.4). http://biodiversityinformatics.amnh.org/open\_ source/maxent [Accessed on July 2024]
- Pöltl M, Berg C, Köckinger H, Caspari S, Ephan N, Gey S, Kiebacher T, Kropik M, Zechmeister H (2020): Neues zur Moosflora der Steiermark 3. Joannea Botanik 17: 45–59.
- Proskauer J (1958) Nachtrag zur Familie Anthocerotaceae. In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Band 6, 2. Abteilung, 3 Auflage. Leipzig: Akademische Verlagsgesellschaft Geest & Portig K.-G., 1303–1319.
- QGIS Development Team (2024) QGIS Geographic Information System. Open Source Geospatial Foundation Project. https://qgis.org
- R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Rejment-Grochowska I (1950) Wątrobowce (Hepaticae). Występowanie, budowa, rozmnażanie, systematyka oraz klucz do oznaczania 100 pospolitych gatunkow z 15 tablicami rysunkowymi. Warszawa: Państwowe Zakłady Wydawnictw Szkolnych, 1–178.
- Rejment-Grochowska I (1966) Wątrobowce (Hepaticae). Tom I. In: Czubiński Z, Kochman J, Krzemieniewska H, Motyka J, Skirgiełło A, Starmach K, Rejment-Grochowska I, Szafran B (Editorial Committee) Flora Polska. Rośliny zarodnikowe Polski i ziem ościennych. Warszawa: Państwowe Wydawnictwo Naukowe, 1–258.
- Rimac A, Šegota V, Alegro A, Koletić N, Vuković N (2019) Novelties in the hornwort flora of Croatia and Southeast Europe. Cryptogamie, Bryologie 40(22): 289–295. https:// doi.org/10.5252/cryptogamie-bryologie2019v40a22
- Saukel J, Köckinger H (1999) Rote Liste gefährdeter Lebermoose (Hepaticae) und Hornmoose (Anthocerotae) Österreichs. Grüne Reihe des Lebensministeriums 10: 172–179.
- Schlüsslmayr G (2011) Soziologische Moosflora des Mühlviertels (Oberösterreich). Stapfia 94: 1–480.
- Schröck C, Köckinger H, Schlüsslmayr G (2014) Katalog und Rote Liste der Moose Oberösterreichs. Stapfia 100: 1-247.
- Schuster RM (1992) The Hepaticae and Anthocerotae of North America east of the hundredth meridian. Volume 6. Field Museum of Natural History, Chicago, 1–937.
- Shcheglovitova M, Anderson RP (2013) Estimating optimal complexity for ecological niche models: A jackknife approach for species with small sample sizes. Ecological Modelling 269(C): 9–17. https://doi.org/10.1016/j.ecolmodel.2013.08.011
- Spinoni J, Naumann G, Vogt J, Barbosa P (2015) European drought climatologies and trends based on a multi-indicator approach. Global and Planetary Change 127: 50– 57. https://doi.org/10.1016/j.gloplacha.2015.01.012
- Stafleu FA, Cowan RS (1981) Taxonomic literature. A selective guide to botanical publications and collections with dates, commentaries and types. Volume 3: Lh–O. Ed. 2.
  [Regnum vegetabile Vol. 105]. Utrecht: Bohn, Scheltema & Holkema and The Hague: dr. W. Junk b. v., Publishers, 1–980. https://doi.org/10.5962/t.207599
- Stieperaere H, Matcham HW (2007) Notothylas orbicularis (Schwein.) Sull.in D. R. Congo and Uganda, new to Africa and N. javanica (Sande Lac.) Gottsche new to D. R. Congo (Anthocerotophyta, Notothyladaceae). Journal of Bryology 29: 3–6. https://doi. org/10.1179/174328207X160559
- Szweykowski J (1958) Prodromus Florae Hepaticarum Poloniae. Poznańskie Towarzystwo Przyjaciół Nauk, Wydział Matematyczno-Przyrodniczy, Prace Komisji Biologicznej 20 (Plantae Cryptogamae), 1–600 [+ 1 map].

- Szweykowski J (2006) An annotated checklist of Polish liverworts. Biodiversity of Poland, Vol. 4. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 1–114.
- Teuber U, Göding H (2009) Neu- und Wiederfunde einiger seltener Moosarten im östlichen Niederbayern. Hoppea 70: 175–180.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzen Code) adopted by the Nineteenth International Botanical Congress, Shenzen, China, July 2017. [Regnum Vegetabile Vol. 159]. Glashütten: Koeltz Botanical Books, 1–254. https://doi.org/10.12705/Code.2018
- Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. Ecology Letters 11(12): 1351–1363. https://doi.org/10.1111/j.1461-0248.2008.01250.x
- van Zuijlen K, Bisang I, Nobis MP, Bergamini A (2024) Extinction risk of European bryophytes predicted by bioclimate and traits. Biological Conservation 293: 110584. https://doi.org/10.1016/j.biocon.2024.110584
- Villarreal JC, Cargill DC, Hagborg A, Söderström L, Renzaglia KS (2010) A synthesis of hornwort diversity: Patterns, causes and future work. Phytotaxa 9: 150–166. https:// doi.org/10.11646/phytotaxa.9.1.8
- Villarreal AJC, Campos SLV, Uribe MJ, Goffinet B (2012) Parallel evolution of endospory within hornworts: *Nothoceros renzagliensis* (Dendrocerotaceae), sp. nov. Systematic Botany 37: 31–37. https://doi.org/10.1600/036364412X616594
- Weddeling K (2002) Bericht über die *Notothylas* Exkursion 27.–29. 09. 2002 in Hessen. Bryologische Rundbriefe 59: 1–3.
- Wigginton MJ (2018) Checklist and distribution of the liverworts and hornworts of sub-Saharan Africa, including the East African islands (edition 4, 25 June 2018). Tropical Bryology Research Reports 9: 1–138.
- Yamada K, Iwatsuki Z (2006) Catalog of the hepatics of Japan. The Journal of the Hattori Botanical Laboratory 99: 1–106.
- Zanatta F, Engler R, Collart F, Broennimann O, Mateo RG, Papp B, Muñoz J, Baurain D, Guisan A, Vanderpoorten A (2020) Bryophytes are predicted to lag behind future climate change despite their high dispersal capacities. Nature Communications 11: 5601. https://doi.org/10.1038/s41467-020-19410-8
- Zizka A, Silvestro D, Andermann T, Azevedo J, Ritte, CD, Edler D, Farooq H, Herdean A, Ariza M, Scharn R, Svanteson S, Wengström N, Zizka V, Antonelli A (2019) Co-ordinateCleaner: standardized cleaning of occurrence records from biological collection databases. Methods in Ecology and Evolution 10(5): 744–751. https://doi.org/10.1111/2041-210X.13152



**Research Article** 

# New genera and new species of Catenulaceae (Bacillariophyta) from Coral Reef habitat of two Indonesia islands—Bawean and Sulawesi—A morphological approach

Oktiyas Muzaky Luthfi<sup>1,20</sup>, Sulastri Arsad<sup>1,20</sup>, Adrian Kryk<sup>10</sup>, Yenny Risjani<sup>2,30</sup>, Yunianta<sup>40</sup>, Mateusz Rybak<sup>50</sup>, Łukasz Peszek<sup>60</sup>, Rafał J. Wróbel<sup>70</sup>, Janice L. Pappas<sup>8\*0</sup>, Małgorzata Bąk<sup>10</sup>, Andrzej Witkowski<sup>1+0</sup>

- 1 Institute of Marine and Environmental Sciences, University of Szczecin, ul. Mickiewicza 16, 70-383 Szczecin, Poland
- 2 Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Jl. Veteran, Malang, 65145, Indonesia
- 3 Center for Algae and Environment, (ALGAEN) LPPM, Universitas Brawijaya, Jl. Veteran, Malang, 65145, Indonesia
- 4 Faculty of Agricultural Technology, Universitas Brawijaya, Jl. Veteran, Malang, 65145, Indonesia
- 5 College of Natural Sciences, University of Rzeszów, ul. Zelwerowicza 4, 35-601, Rzeszów, Poland
- 6 Department of Agroecology and Forest Utilization, University of Rzeszów, ul. Ćwiklińskiej 1A, 35-601 Rzeszów, Poland
- 7 Engineering of Catalytic and Sorbent Materials Department, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Szczecin, Poland
- 8 Department of Mathematics, University of Michigan, Ann Arbor, MI, USA

Corresponding author: Oktiyas Muzaky Luthfi (omuzakyl@ub.ac.id)



Academic editor: Kalina Manoylov Received: 11 July 2024 Accepted: 11 September 2024 Published: 1 November 2024

Citation: Luthfi OM, Arsad S, Kryk A, Risjani Y, Yunianta, Rybak M, Peszek Ł, Wróbel RJ, Pappas JL, Bąk M, Witkowski A (2024) New genera and new species of Catenulaceae (Bacillariophyta) from Coral Reef habitat of two Indonesia islands—Bawean and Sulawesi—A morphological approach. PhytoKeys 248: 263–291. https://doi. org/10.3897/phytokeys.248.131839

**Copyright:** © Oktiyas Muzaky Luthfi et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

#### \* Retired.

† Deceased.

#### Abstract

Indonesia is listed as a region with high marine biodiversity, especially when considering the three large tropical ecosystems: seagrass beds, mangroves and coral reefs. It is likely that the biodiversity of diatoms in this area is also high. Samples for this research were taken from the remote area of Bawean Island and Tomini Gulf in Central Sulawesi. In this research, we explored marine benthic diatoms from coral reef areas and presented two new genera: *Paracatenula* and *Wallaceago*, and seven new species: *Paracatenula porostriata* **sp. nov.**, *Wallaceago porostriatus* **sp. nov.**, *Catenula boyanensis* **sp. nov.**, *C. komodensis* **sp. nov.**, *C. decusa* **sp. nov.**, *C. densestriata* **sp. nov.**, and *Catenulopsis baweana* **sp. nov.** The new genus *Paracatenula* is characterized by its perforated cingulum, and the genus *Wallaceago* is distinguished by its proximal and apical raphe ends bent to the ventral side and the presence of a stauros in the mantle.

Key words: Biodiversity hotspot, coastal habitats, diatom, epipsammic, taxonomy

# Introduction

The family Catenulaceae initially had been described by Mereschkowsky (1902) who named one species, *Catenula pelagica*, and then transferred *Navicula adhaerens* Mereschkowsky into *Catenula* (Silva 1980; Sundbäck and Medlin 1986). The Catenulaceae name came from the Latin word *catēna* and *ula* which literally has the meaning of a diminutive chain. The early described

Catenulaceae was characterized by the number, form and arrangement of chloroplasts instead of valve characteristics. Since Catenula as the genus had been described at that time as having a single chromatophore, it was placed in the Monoplacatea (Sundbäck and Medlin 1986; Cox 1987). The recent family description is based on frustule characteristics like dorsiventral valves with the presence of two raphe slits. Additionally, features that are important to determine the genus are the number of areolae per striae, number of girdle bands, and the presence/absence of pores on the valve. Based on those criteria, the members of the diatom family Catenulaceae include several genera, e.g., Amphora Ehrenberg ex Kützing, Catenula Mereschkowsky, Undatella Paddock & Sims, Halamphora (Cleve) Levkov, Oxyamphora Stepanek & Kociolek, Clevamphora Mereschkowsky and Catenulopsis Kryk, Witkowski, Kociolek & Mayama (Paddock 1980; Round et al. 1990; Levkov 2009; Cox 2015; Stepanek and Kociolek 2016; Kryk et al. 2021). Based on a morphological approach, Undatella has a central keel raphe and a stauros similar to Staurotropis so this genus falls into the Bacillariales (Medlin and Kaczmarska 2004). A revised position of Undatella indicates that it belongs in the Thalassiophysales (Ruck and Theriot 2011; Louvrou and Economou-Amilli 2012). Another study based on a single molecular gene, SSU rDNA, resulted in this genus being moved back into Bacillariales (Ruck and Theriot 2011) and was stated as incertae sedis by www.marinespecies.org and Ashworth et al. (2017) (www.algaebase. org). The most recent classification is that Undatella belongs to Catenulaceae (www.diatombase.org, www.marinespecies.org). The genus Halamphora (Levkov 2009) previously was placed in Catenulaceae (Olivares-Rubio et al. 2017), but recently has been moved into the Family Amphipleuraceae (www. marinespecies.org, www.algaebase.org, www.diatombase.org). The genus Clevamphora was placed into the Subclass Bacillariophyceae incertae sedis (www.marinespecies.org, www.algaebase.org, www.diatombase.org). Based on a recent taxonomy review, the Catenulaceae family members are Amphora, Catenula, Undatella, Oxyamphora and Catenulopsis (www.diatombase.org, Kryk et al. 2021).

The genus *Catenula* has been described by Mereschkowsky (1902) as raphid diatoms which form ribbon-like colonies. *Catenula* is distinguished by valve asymmetry to the apical axis, eccentric raphe, clear helictoglossae (terminal nodule), and short terminal fissures on valve apices (Round et al. 1990; Kryk et al. 2021). The genus *Catenula* is very rarely reported in scientific publications to date, and only 6 species have been noted from all over the world. Since 1901, the species, *C. adhaerens*, had been described by Mereschkowsky, who two years later, published *C. pelagica*. Almost 100 years later, Witkowski et al. (2000) reported a new species as *C. robusta*, then another three species were described two decades later, i.e., *C. exigua* (Robert et al. 2019), *C. brotasiae*, and *C. javanica* (Kryk et al. 2021).

The monotypic genus *Catenulopsis* with its only representative, *Catenulopsis catenulafalsa* Kryk & Witkowski, is characterized by dorsiventral valves with rectangular apices that are dull or obtusely formed. The position of the raphe is eccentric on the ventral side which has distal and proximal end bending on the ventral side. While the copulae have several pores (areolae), they have a punctate and lineolate form. Furthermore, the striation only can be found in the

central margin (Kryk et al. 2021). This study presents the description of coral reef diatoms from the family Catenulaceae and describes new species of *Paracatenula*, *Wallaceago*, *Catenula* and *Catenulopsis*.

# Materials and methods

# Samplings

Sample materials were collected on 7 January 2021 at two sites on Bawean Island, Java Sea, Indonesia with local names *Mangrove Hijau Daun* – MHD (5°50'57.5"S, 112°43'3.6"E) and Gili Iyang (5°51'11.70"S, 112°38'51.10"E) (Fig. 1A). The diatom samples were taken from reef sand and coral rubble (pieces of dead coral) within the reef flat area which is located 1 km away from the mangrove swamp area or near the reef crest in MHD and around 500 m from the coast in Gili Iyang (Fig. 1D). The diatom samples from Sulawesi were taken from the intertidal area at Tanjung Perak of Tomini Gulf (1°18'2.974"S, 120°37'37.009"E) on 29 September 2022. They were collected from rocks in the coral reef area. The sampling point is part of Tomini Gulf, the largest gulf in Indonesia, which has 1,350 km of coastline, and the distance between the indentation mouth shorelines was 44.4 km (Fig. 1B).



**Figure 1.** Map of sampling sites **A** sites Gili Iyang Harbour and MHD on Bawean Island **B** site Tanjung Perak on Central Sulawesi **C**, **D** detailed location of sampling areas on Sulawesi Island and East Java **E** location of Sulawesi and Java Island on Indonesian archipelago.

## Light and scanning electron microscopy

The samples were treated with 10% HCl, removing carbonate minerals (ca. two days), and washed with deionized water thereafter. Samples then were treated by adding 37%  $H_2O_2$  and boiling for 3–5 hours to remove organic matter (Górecka et al. 2021; Kryk et al. 2021). Finally, the boiled samples were rinsed five times with deionized water. Thereafter, the water suspension was pipetted onto coverslips, left at room temperature for 24 h until evaporation of the water, and mounted onto glass slides using Naphrax®. Light microscopy (LM) images were taken with a Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany), with a 100 × Plan Apochromatic oil immersion objective (n.a. = 1.40) equipped with a Canon EOS 500D camera (Li et al. 2015). For identification and terminology reference of diatoms, sources such as Witkowski et al. (2000) and Round et al. (1990) were used.

In addition, for scanning electron microscopy (SEM) imaging, a few drops of cleaned or raw material were placed on a Whatman (pore size 5 or 1  $\mu$ m) Nuclepore® filter and rinsed twice with distilled water. Filters were mounted onto aluminum stubs and air-dried before coating with gold-palladium alloy using a sputter coater. SEM examination involved a Hitachi SU8020 from West Pomerania University of Technology in Szczecin (Poland), at the Faculty of Chemical Technology and Engineering, and a Hitachi SU8010 at the University of Rzeszów, Poland.

## Results

Phylum: Bacillariophyta Karsten Subphyllum: Bacillariophytina Medlin & Kaczmarska Class: Bacillariophyceae Haeckel Sub-class: Bacillariophycidae D.G. Mann Order: Thalassiophysales D.G. Mann Family: Catenulaceae Mereschkowsky

#### Paracatenula Witkowski, Luthfi & M.Rybak, gen. nov.

**Etymology.** The name of the genus is derived from its resemblance to *Catenula*. "Para" in Greek means alongside, besides, near, resembling, beyond, apart from, and abnormal, referring to the superficial similarity of the new genus to *Catenula* but does not conform to the type of the genus as typified with *C. pelagica* Mereschkowsky. *Paracatenula* means resembling *Catenula*.

**Description.** Frustules strongly dorsiventral attached with valve faces to form short chains, plastid unknown. In girdle view, several bands perforated with one or two rows of small pores. Valves asymmetrical about the apical axis with dorsal margin gently arched and ventral margin straight usually with apices slightly deflected towards the ventral side. Raphe sternum positioned close to the ventral margin, raphe slits externally almost straight with simple proximal and apical ends. Striae on valve face usually absent or observed as shallow transapical grooves filled in with silica. On the dorsal mantle, solitary pore-like areolae present, whereas on the dorsal mantle, short striae usually

composed of several small poroids. Valve face internally hyaline without any areolae, and valve mantle having a row of areolae along the valve margin, which is occluded by hymens. Internally, raphe slits are bent toward the dorsal margin and proximally terminate in a simple somewhat raised end, whereas apically, they terminate in oblique helictoglossae. Present sulcus in apical area. The sulcus clearly found in the internal view of the diatom valve after the distal ending (Fig. 3B).

## Paracatenula porostriata Luthfi, Witkowski & M.Rybak, sp. nov.

**Type materials.** *Holotype*: Slide number SZCZ 27552 at repository of University of Szczecin.

Isotype: SZCZ 27553 at repository of University of Szczecin.

**Type locality.** Rubble of coral reef at Gili Iyang harbour, Bawean Island, East Java, Indonesia

**Etymology.** The species epithet 'porostriata' is a combination of the Latin words *porus* meaning pore or punctum and *striatus* meaning striated or having striations to show that this species consists of porous striations on the mantle and cingulum.

**Distribution.** The diatom species *P. porostriata* sp. nov. has been consistently found in coral rubble specimens from both Gili Iyang harbor and MHD, Bawean Island.

**Description.** *Light microscopy* (Fig. 2A–N): The valves are semi-lanceolate, apices broadly rounded, protracted and rostrate, which tend to deflect to the ventral side. The ventral margin is straight, and the dorsal side is smoothly arched or curved. Raphe slits are observed on the valve face as short lines. Valve length 10.1–25.4  $\mu$ m (n = 25) and width 1.7–4.7  $\mu$ m (n = 25). Striation is parallel in the middle and then slightly radial near the ends.

Scanning electron microscopy: (Fig. 20-U external view; Fig. 3A-E internal view): valve shape semi-circular to semi-lanceolate dorsiventral. The surface of the valve, both externally and internally, is completely flat. The transition between the valve face and valve mantle is sharp with a slightly diminished rib. Central area is distinct only on the valve face; lack of fascia on valve mantle. Axial area wide on the dorsal side and narrower on the ventral side (Fig. 20, P). Externally, the raphe is lateral, short, simple and positioned in close proximity to the edge of the ventral valve face. Proximal raphe ends straight, distance to each other is  $3.8 \,\mu m$  (n = 12). Distal raphe ends straight, distance to the apices is 2.6  $\mu$ m (n = 11). Externally, transapical striae are arranged by one row along the dorsal face about 14-20 in 10 µm. Uniseriate striae observed along the valve face (Fig. 20-R). Sometimes, scattered unoccluded pores are found on apices (Fig. 20). Girdle band open with two rows of pores (Fig. 2S-T). Frustules contain five girdle bands and have a rectangular form in girdle view (Fig. 2U). Internally, the raphe slits are short, simple, and arcuate, with proximal and distal ends bent toward the dorsal side. Raphe fissure terminating in distinct helictoglossae (Fig. 3A-E). Line-like and depressed siliceous areolae (Latin sulcus, plural sulci) are present internally at the apical areas, approximately 1.3 µm in length (Fig. 3B).



Figure 2. Paracatenula porostriata Luthfi, Witkowski & M.Rybak, sp. nov. A–N light micrographs O–U SEM images of external valve view O, P external view of valves, unoccluded pores at one or both apices are present (white arrowhead, O) Q protracted apex of valve R pores on the valve mantle shown in the white arrowhead S, T cingulum perforated by biseriate striae externally at the dorsal side (black arrowheads) and one row of elongated pores internally at the ventral side (white arrowhead, S) U whole girdle view of frustule, with depressed areolae on valve mantle. Scale bar: 10 µm (A–N).

## Wallaceago Witkowski, Arsad, Luthfi & M.Rybak, gen. nov.

**Etymology.** The name of the genus is dedicated to Alfred Russel Wallace in recognition of his contribution to the biogeography of the Indonesian Islands. The ending "-ago" is used to denote an explorer or traveller.



**Figure 3.** *Paracatenula porostriata* Luthfi, Witkowski & M.Rybak, sp. nov. SEM images of internal view **A** valve with protracted apices **B** valve with linear grooves (Latin *sulcus*, plural *sulci*) on apices area (black arrowhead, also on **A**–**E**) **C** valve view showing occluded areola on ventral and dorsal valve mantel **D**, **E** internal apical raphe end bent and ending with indistinct helictoglossa.

**Description.** Frustules strongly dorsiventral, plastid unknown, and girdle not observed. Valves asymmetrical about the apical axis. Raphe sternum close to the ventral side along the apical axis. Raphe slit straight with externally simple proximal and apical ends that bent towards the ventral margin. Transapical striae absent in the valve face, but short striae present on the ventral margin, which is composed of a series of small areolae. On the ventral side, a distinct, rhomboidal central nodule is observed. Valve face internally flat, central nodule with distinct siliceous deposition. Areolae occluded by hymenate structures. Raphe slits internal, exhibiting slight dorsal curvature; proximally elevated above central nodule deposition, terminating apically in indistinct helictoglossae.

#### Wallaceago porostriatus Arsad, Witkowski, Luthfi & M.Rybak, sp. nov.

**Holotype.** Slide number SZCZ 28814 at the repository of the University of Szczecin.

**Type locality.** Rock scrape in Tanjung Perak, Poso Pesisir Regency, Central Sulawesi/ Celebes, Tomini Gulf, Indonesia

**Etymology.** This species is dedicated to Alfred Russel Wallace in gratitude for his contribution to exploring the Indonesian archipelago. The term *porostriatus* is a Latin adjective that means having porous striations or with striations composed of pores.

**Distribution.** The new species so far is only found in Tanjung Perak, Sulawesi, Indonesia.

**Description.** *Light microscopy* (Fig. 4A–J): The valves are dorsiventral, apices dull without protracted ends. The narrower valves are weakly protracted. Valve length 7.1–14.8  $\mu$ m (n = 17) and width 1.6–3.1  $\mu$ m (n = 17). Raphe branches cannot be found. The central area is resolvable with LM, marked by a light color in the middle of the ventral margin.

**Scanning electron microscopy** (Fig. 4K-R): Valves exhibit semi-lanceolate dorsiventral morphology, transitioning to pyramidal form. The valve face presents as flat and smooth, devoid of striae ornamentation both externally and internally (Fig. 4K-R). The dorsal mantle transitions gradually from the valve face, while the ventral mantle transition is abrupt, characterized by a distinct groove resembling a rabbet at the valve face edge (Fig. 4L, M). Ventral mantle seriation is uniseriate, with 70-80 areolae per 10 µm. Areolae are rounded and separated by a pore-free, hyaline silica thickening, forming a stauros (Fig. 4M). Seriation grooves are prominently marked internally (Fig. 4P-R). The dorsal mantle lacks areolae (Fig. 4P, R). The raphe externally filiform with a simple, rabbet-like profile, positioned close to the ventral valve margin (Fig. 4L-N). External proximal raphe ends simply, slightly deflected ventrally, and somewhat distant from each other (1.1 µm). Distal raphe endings are simple, lacking fissures, and bent towards the ventral margin. Internally, raphe endings straight, terminating slightly towards the dorsal valve at the central area (Fig. 40, P). Distal raphe endings are straight and situated close to the apices (0.5 µm), with indistinct or absent helictoglossae (Fig. 4Q, R). Girdle bands were not observed in the specimens examined.

## Catenula boyanensis Luthfi, Witkowski & M.Rybak, sp. nov.

**Holotype.** Slide number SZCZ 27552 at the repository of the University of Szczecin.

**Type locality.** Sand and coral boulders of coral reef at Gili Iyang harbour Bawean Island, East Java, Indonesia.

**Etymology.** The species name is derived from local Bawean Island people called Boyan.

**Distribution.** The diatom species *C. boyanensis* has been regularly observed in samples from the harbour of Gili lyang on Bawean Island.

**Description.** *Light microscopy* (Fig. 5A–G): The valves are semi-lanceolate, dorsiventral. Raphe is clearly observed through the ventral area with proximal



Figure 4. Wallaceago porostriatus Arsad, Witkowski, Luthfi & M.Rybak, sp. nov. (A–R) A–J light microscopy images K–R SEM images K–N external valve view O-R internal valve view L, M striation in mantle consists of 2–5 tiny areolae (white arrowhead, L), proximal and distal raphe ends bent in the same direction towards the ventral side (black arrowheads, L) M a presence of stauros (black arrowhead), ornamented raphe and the edge of the valve by a rabbet (white arrowheads) O-Q raphe straight with simple ends R distinct virgae on the mantle with unoccluded pores. Scale bar: 10 µm (A–J).

raphe endings distant from each other and distal raphe endings distant to apices (Fig. 5A–F). Valve length 8.2–22.2  $\mu$ m, n = 29 and width 2.1–4.9  $\mu$ m, n = 17. Transapical striae can be observed on the dorsal face. Apices obtusely rounded with distinct helictoglossae.

**Scanning electron microscopy** (Figs 5, 6): Frustules exhibit significant dorsiventral. Several non-perforated girdle bands compose the frustule (Fig. 5H, M). Valves display strong asymmetry around the apical axis, with a



Figure 5. Catenula boyanensis Luthfi, Witkowski & M.Rybak, sp. nov. A–G light microscopy images H–O SEM images in external view H, M girdle view of frustule showing rectangular with obtuse shape on corners and a row of elongated striae on mantle (M, black arrowhead) I detail of raphe branch J detail of apical part of a frustule with girdle band M the mantle of valve K–L external valve view showing transapical irregular grooves and the central area N open unperforated cingulum O detail of frustule central part. Scale bar: 10 μm (A–G).

gently arched dorsal margin and straight ventral margin (Fig. 5K, L). The raphe sternum is positioned near the ventral margin, with nearly straight raphe slits (Fig. 5L). Raphe occupies a more central location rather than along the ventral margin, with proximal raphe endings distantly spaced ( $1.5-3.8 \mu m$ ) (Fig. 5K, L). External proximal and apical ends are filiform, short, and straight (Fig. 5I). Transapical striae appear as shallow grooves on the valve face, with a row of striae visible on the dorsal mantle (Fig. 5H, J, M). The internal valve face is flat. Small areolae on the valve surface are covered by a thin hymen layer. Internally, raphe slits bend very slightly toward the dorsal margin, maintain straight proximal ends, and terminate apically in helictoglossae (Fig. 6D).

Oktiyas Muzaky Luthfi et al.: Catenulaceae genera and species in Bawean and Sulawesi coral reefs



**Figure 6.** Internal view of *Catenula boyanensis* Luthfi, Witkowski & M.Rybak, sp. nov. SEM images **A–D** internal valve view showing arcuate raphe slit, a row of linear areolae on mantle **D** detail of raphe branch and proximal raphe endings with apical raphe ending bent and finishing with small helictoglossae (arrowhead).

## Catenula decusa Luthfi, Witkowski, Arsad & M.Rybak, sp. nov.

**Type materials.** *Holotype*: Slide number SZCZ 27552 at repository of University of Szczecin.

*Isotype*: Slide number SZCZ 28814 at repository of University of Szczecin (Fig. 7S).

**Type locality.** Sand and coral boulder of coral reef at Gili Iyang harbour, Bawean Island, East Java, Indonesia.

**Etymology.** The species name is derived from the Latin word *decus* which literally means an ornament, decoration, or embellishment. The new species exhibits a distinct central area.

**Distribution.** The diatom species *C. decusa* has a unique distribution, being found exclusively in Bawean and Sulawesi Islands, Indonesia. Interestingly, both of these locations share a similar habitat, characterized by the presence of coral reef areas.

**Description.** *Light microscopy* (Fig. 7A–Q): The valves are semi-lunate or semi-lanceolate dorsiventrally. Thickening silica in the central area is very clear under LM. A row of linear areolae can be distinguished in the dorsal margin (Fig. 7F). Valve length 8.2–16.5  $\mu$ m, n = 18 and width 1.9–2.9  $\mu$ m, n = 17. Apices broadly rounded with indistinct, dot-like helictoglossae. The frustule is rectangular in girdle view, 1.1  $\mu$ m depth. Raphe slits observed in ventral area.

**Scanning electron microscopy** (Fig. 7R-Y): Frustules semi-circular to semi-lanceolate, dorsiventral; valve face flat, smooth, abruptly transitioning



Figure 7. Catenula decusa Luthfi, Witkowski, Arsad & M.Rybak, sp. nov. A–Q light micrographs R–Y SEM images R, S, V external view T, U, W–Y internal view R external view of frustule with three cingula S external valve view with rectangular central area created by striation interruption in dorsal and ventral margin T, Y internal view of entire valve U valve with open unperforated girdle band V external view an arcuate raphe with simple proximal and apical end W straight raphe slit in internal valve view X rectangular central area with interrupted dorsal and ventral mantle striation. Scale bar: 10 μm (A–Q).

to mantle. Distinct rhomboidal central nodule observed on ventral and dorsal sides (Fig. 7S, T). Valve face devoid of transapical striae; striae present on dorsal and ventral mantle (Fig. 7R). Raphe sternum positioned near ventral margin;

raphe slits straight medially, curving towards apices. External proximal raphe ends simple, slightly expanded; distal ends simple, close to apices ( $\bar{x}$ =0.72 µm, n = 21), deflected dorsally. Central nodules and helictoglossae indistinct. Dorsal mantle striation density 35–40 in 10 µm; ventral mantle 40–50 in 10 µm. Internal raphe filiform, straight (Fig. 7T, U, Y); mantle areolae occluded, flask-shaped (Fig. 7X). Girdle open, comprising multiple unperforated plain bands (Fig. 7R, U).

## Catenula komodensis Witkowski, Risjani, Yunianta, M.Rybak & Luthfi, sp. nov.

**Type materials.** *Holotype*: Slide number SZCZ 27552 at repository of University of Szczecin.

*Isotype*: Slide number SZCZ 28814 at repository of University of Szczecin (Fig. 8N).

**Type locality.** Sand and coral boulders of coral reef at Gili Iyang harbour Bawean Island, East Java, Indonesia.

**Etymology.** The species name is derived from the Komodo dragon that is endemic to 4 islands: Komodo, Rinca, Flores, and Gili Motang, East Nusa Tenggara, Indonesia.

**Distribution.** The diatom species *C. komodensis* is distributed on Bawean and Sulawesi Islands, Indonesia.

**Description.** *Light microscopy* (Fig. 8A–K): The valves are lanceolate, dorsiventral to linear-lanceolate with straight to less convex margins. Apices are narrowly pointed. Thickened silica in the central area was observed clearly on some valves under LM. Valve length  $8.3-15.6 \mu m$ , n = 13 and width  $1.7-2.6 \mu m$ , n = 13.

**Scanning electron microscopy** (Fig. 8L–U): Valve face flat, abruptly transitioning to mantle. Raphe filiform, positioned near ventral margin externally and internally (Fig. 8L–N, T–U). External raphe slits arcuate; proximal endings simple, dorsally oriented, distantly spaced (1.1–1.9  $\mu$ m). Distal raphe endings simple, terminating 0.8–2.8  $\mu$ m from apices. Areolae absent on dorsal and ventral valve surfaces. Dorsal side exhibits shallow or indistinct parallel grooves. Internal raphe slits curved; apical endings deflected dorsally (Fig. 8T–U). Internal distal raphe endings simple, without fissures, terminating 1.7–1.8  $\mu$ m from apices. Helictoglossae indistinct (Fig. 8S). Dorsal and ventral sides striated; areolae occluded by dome-like structures. Striae density slightly lower dorsally; secondary silica deposition observed on ventral valve side (Fig. 8U). Striae density 34–35 in 10  $\mu$ m ventrally, 35 in 10  $\mu$ m dorsally (n = 13).

#### Catenula densestriata, Luthfi, Witkowski, M.Rybak & Arsad, sp. nov.

**Holotype.** Slide number SZCZ 27553 at the repository of the University of Szczecin.

**Type locality.** Sand and rubble of coral reef at Daun, Bawean Island, East Java, Indonesia.

**Etymology.** The name of the species was derived from Latin meaning "densely packed." This species has dense striation in the dorsal area.



Figure 8. *Catenula komodensis* Witkowski, Risjani, Yunianta, M.Rybak & Luthfi, sp. nov. **A–K** light micrographs **L–U** SEM images. **L–N** external view on entire frustules; note a row of slit-like ventral and dorsal mantles (arrowheads) **O** detail of proximal raphe ending, externally **P** detail of apical raphe ending, externally **Q–S** detail of internal proximal raphe ending, arcuate raphe slit and distal raphe ending, respectively; note a row of linear small protruding flaps (**R** arrowhead) **T–U** internal valve view with 2 thickenings of silica on central area and near slit ends (**U** arrowheads). Scale bar: 10 μm (**A–K**).



Figure 9. Catenula densestriata Luthfi, Witkowski, M.Rybak & Arsad, sp. nov. A–M light micrographs N–X SEM images N external view of valve is semi-lanceolate dorsiventral and semi-circular (P, S) O, Q, X internal view of valves R two frustules are attached to each other by the valve face S areolae on dorsal mantle T a close-up of the detail of the proximal raphe ending as simple and straight U detail of the apical raphe ending deflected on dorsal side V detail of internal view, indistinct helictoglossa (white arrowhead) and linear occluded areolae (black arrowhead) W detail of frustule showing linear areola on mantle valve with several cingula X unperforated girdle band is shown at white arrowhead. Scale bar: 10 μm (A–M).

**Distribution.** The diatom species *C. densestriata* is found solely on Bawean Island and Tiga Warna Beach in East Java, Indonesia.

**Description.** *Light microscopy* (Fig. 9A–M): The frustules are rectangular in girdle view, 1.8 µm deep, joining each other on the valve face (Fig. 9A). Valves exhibit asymmetry and are dorsiventral, characterized by a curved dorsal margin and a straight ventral margin. Some dorsal margins are slightly flat in the middle, as semi-lanceolate (Fig. 9C, G, J, K, M). Valve apices cuneate with dot-like helictoglossae. Raphe slits positioned on the ventral side close to the margin (Fig. 9B–E, H). The terminal raphe ends are closed. Valve length 10.7–17.5 µm, n = 15 and width 1.8–3.5 µm, n = 14. The striae on the dorsal side are indistinct.

Scanning electron microscopy (Fig. 9N–X): Valves semi-lanceolate, dorsiventral, with flat faces transitioning abruptly to dorsal and ventral mantles (Fig. 9N–Q). Short chains formed; plastid unknown. Externally, shallow fine radiate striae visible dorsally, absent ventrally (Fig. 9N). Linear pores present in rows on dorsal and ventral mantles (Fig. 9S, X). Internally, valve face lacks parallel striation; occluded pores as small protruding flaps on dorsal and ventral sides, density 40–50 in 10 µm dorsally, 40–55 in 10 µm ventrally. Raphe slits arcuate, deflected to same side. Internal raphe slightly terminated by indistinct helictoglossae toward dorsal side. Proximal raphe endings more distant internally (mean 1.5 µm, n = 14) than externally (mean 1.26 µm, n = 11). Central nodule indistinct. Girdle bands open, unperforated, comprising at least four cingula.

#### Catenulopsis baweana Luthfi, Witkowski, M.Rybak & Kryk, sp. nov.

**Holotype.** Slide number SZCZ 27552 at the repository of the University of Szczecin.

**Type locality.** Sand and rubbles of coral reef at Gili Iyang harbour, Bawean Island, East Java, Indonesia.

**Etymology.** The name of the species is derived from the geographic location of the type habitat, i.e., Bawean Island. The meaning of *Bawean* in Sanskrit is sunlight.

**Distribution.** The diatom species *Ca. baweana* has a unique distribution, being found exclusively on Bawean Island, Indonesia.

**Description.** *Light microscopy* (Fig. 10A–S): Valves are asymmetrical, dorsiventral, with the dorsal margin curved and the ventral margin straight. Valves are 10.7–16.1  $\mu$ m long (n = 19) and 1.8–3.1  $\mu$ m in width (n = 19). The apices look subcapitate when focusing on multiple planes using LM; the helictoglossae appear as a darker grey spot. A white line appearing near the ventral margin is a raphe. The frustule in girdle view is rectangular or widely rectangular because it consists of several cingula. Transapical striae are difficult to resolve with LM.

**Scanning electron microscopy** (Fig. 10T–AB): Frustules strongly dorsiventral, rectangular in girdle view around 1.9–2.9  $\mu$ m deep (Fig. 10T–V, X). The girdle is composed of several perforated bands (2–3 cingula), each with uniseriate small pores, 50–65 in 10  $\mu$ m. Valves strongly asymmetrical about the apical axis, 8.8–17.1  $\mu$ m long (n = 17) and 1.7–2.9  $\mu$ m in width (n = 9). External and internal valves are flat and end abruptly at the mantle. The dorsal margin is gently arched, and the ventral margin is straight, apices obliquely cuneate. Raphe sternum is positioned close to the ventral margin, raphe slits almost straight.



Figure 10. Catenulopsis baweana Luthfi, Witkowski, M.Rybak & Kryk, sp. nov. A–S light micrographs T–AB SEM images T girdle view of the whole frustule U perforated valvocopula of frustule V, W detail of striation in the ventral mantle X frustule of diatoms attached at valve face Y, AA external view of valves semi-lanceolate dorsiventral with raphe branches. Proximal and apical endings deflected to ventral side Z–AB internal valve view shows simple raphe slits. Scale bar: 10 μm (A–S).

External proximal and apical ends are simple, bent towards the valve ventral margin. Proximal raphe ends somewhat distant from each other,  $1.3-1.9 \mu m$ . Transapical striae present only on the valve face, dorsal and ventral mantle, and composed of long linear slits, 22-34 in  $10 \mu m$  (n = 12). The internal valve face is flat. The areolae are closed with hymenate occlusions. Internally, raphe slits are very straight and skewed towards the dorsal area, and the proximal raphe ends terminate in a slightly expanded drop-like structure, whereas apically, they terminate in small helictoglossae.

## Discussion

#### The new genera Paracatenula and Wallaceago

*Paracatenula* has unique features that make it recognisable, including its shape, striation in the mantle, girdle band, and a slit-like depression in the apical area, which we call a sulcus. The valve margin of the new genus has a dorsal convex and a ventral straight side, sharing similarities with *Catenula*. However, the valve apices in the new genus are protractedly deflected toward the ventral margin, which cannot be found in *Catenula*. The most similar genus which has the pointed valve ends that curve or bend downward is *Halamphora*, e.g., *Halamphora normanii* (Rabenh) (Levkov 2009) and *H. montana* (Krasske) (Levkov 2009), which have capitate valve apices in shape. In contrast, *Paracatenula* has rostrate valve apices (Table 1).

Striation in the valve mantle of *Paracatenula* is ornamented by single tiny uniseriate areolae. In other dorsiventral diatoms, such as Catenula and Catenulopsis, the mantle is featured by a row of elongated slits which are positioned perpendicularly to the surface of the valves (Fig. 2U). Paracatenula features an intricate unique cingulum structure with an open channelled and perforated band. Externally, on the dorsal side, the cingulum is ornamented with biseriate striae, creating a double row of fine, closely spaced lines. Internally, on the ventral side, it features a single row of elongated pores (Fig. 2S, T). The absence of such perforation patterns in Catenula highlights this distinctive morphological feature of Paracatenula. Several diatom genera with a perforated cingulum include Catenulopsis and Halamphora. Frustules of Catenulopsis are supported by several perforated girdle bands (Kryk et al. 2021). Another dorsiventral diatom which is composed of girdle bands perforated with a few rows of poroids is Halamphora catenulafalsa (Witkowski et al. 2016). However, the two unique types of cingula striation previously described are only found in Paracatenula. The perforated cingulum is common and found in centric diatoms, e.g., Thalassiosira and Stephanodiscus (Round et al. 1990).

The genus *Paracatenula* exhibits several distinctive characteristics that justify its classification within the Catenulaceae family (Table 1). The frustules of *Paracatenula* are rectangular in girdle view, have asymmetric dorsiventral valves in the apical plane, the raphe position is eccentric close to the ventral margin, valve apices are protracted and rostrate, there is striation in the mantle, and there is a plain valve face with transapical ribbing (Round et al. 1990; Levkov 2009; Pliński and Witkowski 2020). Additionally, the family Catenulaceae exhibits a variety of apical valve morphologies. For instance, *Catenula* has no protracted apices, while *Amphora* and *Halamphora* do have protracted,

	Paracatenula	Wallaceago	Catenulopsis	Catenula	Amphora	Lunella	Parlibellus
	This study	This study	Kryk et al. (2021)	Mereschkowsky (1902)	Kützing (1844)	Snoeijs (1996)	Cox (1988)
Valves	strongly asymmetric and dorsiventral	strongly asymmetric, dorsiventral, semi-rhombic	strongly asymmetric and dorsiventral	strongly asymmetric and dorsiventral	asymmetric and dorsiventral	semi-lanceolate and dorsiventral	symmetric, bluntly lanceolate
Raphe position	eccentric, near ventral mantle	eccentric, near ventral mantle	eccentric, near ventral mantle	eccentric, near ventral mantle	moderate to strong eccentric toward ventral mantle or valve margin, raphe ledge present	near the ventral margin	on valve face, in the centre
Raphe shape	biarcuate	straight biarcuate	slightly biarcuate or sinusoidal	biarcuate	straight to biarcuate (sigmoid)	slightly biarcuate	straight
Raphe distal Endings	bent to dorsal, distant to poles	curved to ventral, distant to poles	curved to ventral, distant to poles	bent to dorsal, distant to poles	curved to dorsal face	terminating to ventral	terminating to secondary side
Raphe proximal Endings	straight, distant	close and curved to ventral	close and curved to ventral	straight, distant	terminate on ventral; on valve face; close to distant	straight to deflected on ventral; close	straight and distant
Sternum	narrow	none	narrow	narrow	narrow	narrow	narrow
Valve face/ mantle transition	abrupt	abrupt	abrupt	abrupt	abrupt	gradual both ventral and dorsal	gradual
Helictoglossae	well developed	poorly developed	poorly developed	well developed	poor to well- developed	well developed	well developed
Cingulum	open, perforated small pore	unknown	several plain porous copulae	open, non– porous band	plain or perforated, one or more areolae	porous copulae	wide girdle region, perforated

Table 1	<ol> <li>Mor</li> </ol>	phologica	I comparison	of Paracatenula	and Wallaceago	with the most	t similar genera

capitate apices that terminate on the dorsal mantle. Moreover, the protracted, rostrate valve apices of *Paracatenula* are rarely reported from other genera of Catenulaceae. Only *C. pelagica* has been reported to have slightly rostrate apices (Round et al. 1990).

The newly described genus Wallaceago differs from all established catenuloid genera in terms of its valve shape, stauros, and raphe. Wallaceago exhibits a distinctive valve morphology characterized by a semi-rhombic, dorsiventral shape with broadly rounded apices. The valves display a pronounced bilateral flattening along the dorsiventral axis while maintaining a semi-rhombic outline tapering towards the apices. This combination of dorsiventral compression and rounded apical terminations distinguishes Wallaceago from other closely related genera, e.g., Amphora, Halamphora, Catenula and Catenulopsis which possess valves with a semi-lanceolate, dorsiventral form (Barber and Haworth 1981; Round et al. 1990; Robert et al. 2019; Stepanek and Patrick Kociolek 2019; Kryk et al. 2021). So far, only Wallaceago exhibits these features. Moreover, striation in the mantle of Wallaceago is characterized by a distinctive pattern on the ventral side (70-80 striae per 10 µm) which is interrupted by a stauros. The stauros has been previously reported in C. adhaerens from Zimbros, Brazil but it is located in the central area of the valve face (Garcia and Talgatti 2011, p. 103, figs 16, 17). Note, in our opinion those described specimens are similar to C. decusa sp. nov. in this paper. The stauros is a distinctive valve feature found primarily in Catenula (Garcia and Talgatti 2011) and in the freshwater diatom,

genus *Stauroneis* (Levkov et al. 2016). For distinguishing the fascia from the stauros, a key morphological feature in certain diatom taxa, refer to the comprehensive review by Cox (2012).

*Wallaceago* exhibits a distinctive biarcuate raphe system, characterised by both proximal and distal ends bent towards the ventral side of the valve. A unique feature of the raphe in this genus is the ornamentation along the slit, which resembles a framed profile. While *Wallaceago* shares some similarities in raphe morphology with the genus *Catenulopsis*, notable differences exist. In *Catenulopsis*, the raphe displays a more pronounced sinusoidal curvature, whereas in *Wallaceago*, the raphe tends to be less curved, with a straighter trajectory (Kryk et al. 2021).

The genus *Wallaceago* shows several distinctive characteristics that justify its classification within the Catenulaceae family. The frustule of *Wallaceago* has asymmetric dorsiventral valves, is rectangular in girdle view, the raphe position is eccentric close to the ventral margin, valve apices are never protracted, and there is striation in the mantle with a plain valve face with transapical ribbing (Round et al. 1990; Levkov 2009; Pliński and Witkowski 2020). The genus *Wallaceago* appears similar to the genus *Catenulopsis* in terms of the raphe position and direction of the raphe ends. Another shared characteristic between *Wallaceago* and *Catenulopsis* is the presence of striations on the valve mantle. However, a notable difference lies in the specific arrangement and nature of these striations. In *Wallaceago*, the striations are confined solely to the ventral mantle and consist of uniseriate areolae. In contrast, *Catenulopsis* exhibits striation patterns on both the dorsal and ventral mantles, with these striations appearing as single rows of puncta or pores. In addition, *Catenulopsis* exhibits areolate striations composed of columnar or lanceolate shapes similar to *Amphora*.

## Novel Paracatenula species

*Paracatenula porostriata* and *C. javanica*, exhibit intriguing similarities and differences. Both species possess a dorsiventral valve, a characteristic that is shared among catenuloid diatoms. Their transapical striae, a key feature in their structure, are barely resolvable when observed through the LM. However, a notable distinction lies in their apical forms. *P. porostriata* is characterized by protracted rostrate apices, a feature that is absent in *C. javanica*. As indicated in Table 2, there is a significant difference in the lengths of the two species. Specifically, the length of the newly described species, ranges from  $10.1-26.6 \mu m$ . On the other hand, *C. javanica*, is shorter, with a length ranging from  $9.7-15.4 \mu m$  (Table 2). Under LM observation, *P. porostriata* is easily misunderstood to be *C. javanica* (see Kryk et al. 2021; figs 53, 60) which has a dorsiventral shape with slightly protracted rostrate apices. Both species also have refractive proximal and distal raphe ends that appear as dots in a bright colour.

Ultrastructural observations using an SEM revealed distinct differences between the apices of the two species. *Paracatenula porostriata* exhibited apical pores that were conspicuously absent in *C. javanica* (Fig. 20). A notable difference was observed in the transapical striae, with *P. porostriata* having fewer (14–22/10 µm) compared to *C. javanica*, which had 26–32/10 µm (Table 2). Furthermore, the raphe slit of *P. porostriata* was significantly longer, measuring 11.8 µm, in contrast to *C. javanica*, which measured only 6.8 µm. The cingulum

	Paracatenula porostriata sp. nov.	Wallaceago porostriatus sp. nov.	Catenula boyanensis sp. nov.	Catenula decusa sp. nov.	Catenula komodensis sp. nov.	Catenula densestriata sp. nov.	Catenula brotasiae	Catenula javanica
	This study	This study	This study	This study	This study	This study	Kryk et al. (2021)	Kryk et al. (2021)
Length (µm)*	10.1-26.6	5.2-8.6	8.2-22.2	6.8-16.5	8.1-19.3	8.2-20.3	8.5-11.8	9.7-15.4
Width (µm)*	1.7-4.7	0.9-1.2	1.1-4.9	1.0-1.7	1.1-2.1	1.6-3.5	2.8-3.5	2.4-3.5
Valve shape	semi-lanceolate dorsiventral	semi-rhombic dorsiventral	semi- lanceolate dorsiventral	semi- lanceolate dorsiventral	lanceolate dorsiventral	semi- lanceolate dorsiventral	dorsiventral	dorsiventral
Girdle shape	rectangular	rectangular	rectangular	rectangular	rectangular	rectangular	rectangular	rectangular
Sternum	narrow	narrow	absent	absent	narrow	absent	absent	narrow
Raphe slits path	straight	biarcuate	straight	arcuate	biarcuate	arcuate	straight	straight
Distal raphe ending	straight (external), deflected to dorsal (internal)	deflected to ventral (external), straight (internal)	straight (external), deflected to dorsal (internal)	deflected to dorsal (external), straight (internal)	straight (external), deflected to dorsal (internal)	deflected to dorsal (external), straight (internal)	straight (external), deflected to dorsal (internal)	straight (external), deflected to dorsal (internal)
Proximal raphe ending	straight, distant (external and internal)	deflected to ventral (external), straight (internal), close	straight, distant (external and internal)	straight, close (external and internal)	deflected to ventral, close (external and internal)	straight, close (external and internal)	straight, close (external and internal)	straight (external), deflected to dorsal (internal), distant
Raphe slits length (µm)	11.8	4.7	9.1	6.8	9	9.9	8.4	6.8
Raphe position	ventral area	ventral area	ventral area	ventral area	ventral area	ventral area	ventral area	ventral area margin
Central nodule	absent	absent	absent	absent	absent	absent	present	absent
Central area	present	present	present	present	present	present	present	present
Apices	broadly rounded, protracted, rostrate	broadly rounded, not protracted	broadly rounded, not protracted	broadly rounded, not protracted	acutely rounded, not protracted	broadly rounded, not protracted	broadly rounded, not protracted	obtusely rounded, never protracted
Helictoglossa	indistinct	absent	indistinct	absent	indistinct	distinct	indistinct	indistinct
Striation	transapical and parallel	absent	transapical and parallel	absent	transapical and parallel	transapical and parallel	uniseriate	transapical and parallel
Areolae in mantel	punctate	punctate	slit-like	slit-like	slit-like	slit-like	slit-like	slit-like
Number of dorsal striae (in 10 µm)	14-22	-	10-20	35-40	30-35	40-50	40-50	26-32
Number of ventral striae (in 10 µm)	20-36	70-80	22-30	40-50	30-45	40-55	-	36-42
Cingulum	open and perforated	unknown	open and plain	open and plain	unknown	open and plain	open and plain	unknown

Table 2. Comparison of newly described species with the most similar taxa.

\*average (LM+SEM).

of *P. porostriata* was characteried by biseriate striae externally and a single row of elongated pores internally. However, both shared a similarity in biarcuate shape of the internal raphe branches.

## Novel Wallaceago species

Based on observations of ultrastructures by using an SEM, *Wallaceago porostriatus* is characterised by an isosceles (semi-rhombic dorsiventral) triangular valve shape and a rectangular girdle shape. The established genus with a similar valve shape is *Seminavis* D.G. Mann, e.g., *S. basilica*. This species has a rhombic-lanceolate structure with truncated apices (Danielidis and Mann 2003). Distinct differences in the raphe structure differentiate the species *S. basilica* from *W. porostriatus*. Specifically, *S. basilica* is characterised by a straight raphe with a raphe fissure oriented towards the dorsal side. In contrast, *W. porostriatus* lacks such a raphe fissure. The raphe slit of *W. porostriatus* is notably short, measuring only 4.7  $\mu$ m (Table 2). This is significantly shorter when compared to the similar genus *Catenulopsis*, which has a raphe slit length of 8.3  $\mu$ m (Table 3; Kryk et al. 2021). Interestingly, the raphe slit length of *W. porostriatus* is nearly identical to that of *Medlinella*, which measures approximately 4.0  $\mu$ m (Frankovich et al. 2016). The distal ends of the raphe in *S. basilica* and *Medlinella* exhibit a similarity to those of *W. porostriatus*, specifically, that they are curved towards the ventral side. However, the raphe of *W. porostriatus* is slightly arcuate in the middle, distinguishing it from the others. In general, raphe shape for *W. porostriatus* has slight curvatures whereas similar genera have raphe shapes that are more pronounced in bending or sinusoidal patterns along the ventral side. Another unique shape of the *W. porostriatus* raphe is attributed to the presence of a rabbet.

Among those species that have striation on the mantle, the type of striation differs. *W. porostriatus* has punctate striation, which means the striations appear as tiny, point-like dots. On the other hand, other species have slit-like striations, where the striations appear as small, elongated slits. The number of striations in the ventral area of the new species is higher, 70–80 per 10  $\mu$ m, compared to *M. amphoroidea* (Tables 2, 3; Frankovich et al. 2016). Under LM, *W. porostriatus* and *M. amphoroidea* are identified as small diatoms, each with distinct valve lengths. The valve length of *W. porostriatus* ranges from 5.2–8.6  $\mu$ m, while *M. amphoroidea* exhibits a slightly larger span of 7–13  $\mu$ m. In contrast, *Ca. catenulafalsa* presents a longer valve length, measuring between 8.4–14.6  $\mu$ m. Notably, the transapical groove in both *M. amphoroidea* and *Ca. catenulafalsa* is clearly visible under LM, a feature that is conspicuously absent in *W. porostriatus*.

	Ca. catenulafalsa	Ca. baweana sp. nov.
	Kryk et al. (2021)	This study
Length (in 10 µm)	8.4-14.6	8.9-17.1
Width (in 10 µm)	3-4.1	1.7-3.1
Valve shape	dorsiventral	semi-lanceolate dorsiventral
Girdle shape	rectangular	rectangular
Sternum	narrow	narrow
Raphe slits path	biarcuate	biarcuate
Distal raphe ending	deflected to ventral (external), straight (internal)	deflected to ventral (external), straight (internal)
Proximal raphe ending	deflected to ventral (external), straight (internal), close	deflected to ventral (external), straight (internal), close
Raphe slits length (µm)	8.3	9.6
Raphe position	ventral area, very close to ventral margin	ventral area
Central nodule	absent	absent
Central area	present	present
Apices	obtusely rounded, never protracted	obtusely rounded, never protracted
Helictoglossa	indistinct	distinct
Striation	transapical and parallel	transapical and parallel
Areolae in mantel	slit-like	slit-like
Number of dorsal striae (in 10 µm)	30-40	14-20
Number of ventral striae (in 10 µm)	30-40	22-34
Cingulum	open and perforated	open and perforated

Table 3. Morphological characteristics of Catenulopsis species.

## Novel Catenula species

Catenula boyanensis shows similarities and distinct morphological characteristics with C. javanica (Table 2). Its valve length and breadth are slightly less than C. javanica (Table 2). The valve shape of this species is semi-lanceolate dorsiventral, and it has a rectangular girdle shape. In C. javanica, the valve shape is strictly lanceolate with a broader dorsal side. The primary distinction lies in the external positioning of the raphe. In the case of C. javanica, it is situated nearly along the edge of the ventral valve face. Conversely, for C. boyanensis, the raphe is located on the valve face itself, significantly further from the margin. In C. boyanensis the path of the raphe slits is straight, with the distal raphe ending being straight externally but deflected to the dorsal side internally. The proximal raphe ending is straight and distant, both externally and internally. The length of the raphe slits is 9.1 µm, and they are positioned in the ventral area. On the other hand, the raphe distinctly exhibits an undulating pattern in C. javanica externally and shares a similar pattern internally. The raphe slit length is lower at 9.1 µm. The number of dorsal and ventral striae for C. boyanensis is lower compared to C. javanica (Table 2). Upon observation with LM, the valve shapes of both species appear to be similar.

The diatom species Catenula decusa shares similarities to C. adhaerens in terms of valve morphology and girdle structure. Both species exhibit a semi-lanceolate dorsiventral valve shape and a rectangular girdle form. However, notable distinctions can be observed between the two taxa. C. decusa is characterised by a shorter and slimmer frustule, with valves displaying a more pronounced lanceolate outline and a slender profile, in contrast to the broader, semi-lanceolate valves of C. adhaerens (length 6.8-12.4 µm, width 1.0-1.7 µm). Furthermore, the striation density is remarkably higher in C. decusa, with 35-40 striations per 10 µm, compared to the lower density of 25–35 striations per 10 µm observed in C. adhaerens. Additionally, the proximal ends of the raphe system in C. decusa exhibit a closer proximity 0.9 µm, while the distal ends are positioned nearer to the apices (0.8 µm). Another distinct difference is the thickening of the central area externally and internally in C. decusa with interrupted striation in the mantle, a feature absent in C. adhaerens. When observed under LM the central area seems lighter than other areas due to reflecting more light. Unlike C. adhaerens, C. decusa lacks helictogossae. In Kryk et al. (2021, p.10, figs 85, 93), the specimens that were incorrectly identified as C. adhaerens are C. decusa.

Catenula komodensis. The newly described species is distinct and does not resemble previously described catenuloids. This diminutive diatom species has some valves shaped as linear rhombic dorsiventral and others mostly semi-lanceolate dorsiventral. The second difference is, internally, the presence of a second silica deposit close to the apices on the ventral side that splits the striation. This species shares some similarities with two other species, *W. porostriatus* and *Halamphora veneta*, but *C. komodensis* is characterised by a smoothly arched dorsal margin, that distinguishes it from these related species. Valve morphometrics reveal a length ranging from  $8.1-19.3 \mu m$  and a width spanning  $1.1-2.1 \mu m$ , dimensions slightly larger than those of *W. porostriatus* (Table 2) yet approximately half the size of *H. veneta* (Levkov 2009). Notably, the proximal raphe endings are deflected towards the ventral side with a narrow gap between them, both externally and internally. The central nodule of *C. komodensis* extends towards the dorsal and ventral margins, manifesting itself as a thickening of the

valve face similar to *C. decusa*. However, *C. komodensis* has two distinct silica deposits, situated in the central and apical areas, respectively. These deposits interrupt the striation patterns on both the dorsal and ventral aspects of the valve. Under LM, the valve shape is semi-lanceolate, and in smaller sizes, it appears lanceolate, and the silica thickening in the central and apical areas is clearly visible.

Catenula densestriata. Newly described *C. densestriata* is similar to Catenula adhaerens, Catenulopsis catenulafalsa, and Catenulopsis baweana. The significant difference is the number of dorsal mantle striae density in 10  $\mu$ m which is 24–36 for *C. adhaerens* and 44–50 for the new species. Apical raphe ends are closer in proximity for Catenula densestriata at 0.3–1.2  $\mu$ m in contrast to 1.5  $\mu$ m for *C. adhaerens*. Internally, raphe slits of Catenulopsis are straight versus Catenula densestriata having raphe slits that are straight and apical raphe ends bent toward the dorsal side. One remarkable characteristic that distinguishes *C. densestriata* from other catenuloid species is its high striation density. Notably, the number of striations observed in this new species is approximately twice that of *C. adhaerens* (Sundbäck and Medlin 1986; Garcia and Talgatti 2011; Kryk et al. 2021), a significant characteristic of valve ornamentation.

Secondly, the distance between the external and internal proximal raphe endings in *C. densestriata* is nearly identical, measuring 1  $\mu$ m and 0.9  $\mu$ m, respectively. This feature is distinctly different from *C. adhaerens*, which exhibits twice the distance between its internal proximal endings and its external proximal endings (Kryk et al. 2021). Moreover, the distance between the distal raphe end and the apices is remarkably close in *C. densestriata*, 0.8  $\mu$ m, with almost no difference between the external and internal morphologies. In contrast, *C. adhaerens* exhibits a greater distance of approximately 1.5  $\mu$ m or twice that of the newly described species. Thirdly, the shape of the raphe slit's path in *C. densestriata* exhibits an arcuate form, both internally and externally. Meanwhile, in *C. adhaerens*, the raphe slit is arcuate externally but straight internally (Kryk et al. 2021). Based on several distinguishing characteristics of *C. densestriata*, we believe that the specimen described as *C. adhaerens* in Kryk et al. (2021, p. 10, figs 84, 86) is *Catenula densestriata*.

Based on the description and explanation provided, the four new species *C. boyanensis*, *C. decusa*, *C. komodensis*, and *C. densestriata* exhibit the following general characteristics: a dorsiventral valve shape, a rectangular appearance when viewed from the girdle perspective, and slit-like areolae present on both the dorsal and ventral mantles. Additionally, they possess an eccentrically positioned raphe on the ventral side. Notably, in the case of *C. boyanensis*, distinct transapical grooves are observed. Furthermore, these species exhibit an open and unperforated cingulum structure.

## Novel Cantenulopsis species

Catenulopsis baweana sp. nov. (Ca. baweana) is the second species of the genus discovered and described thus far. The type of the genus is Catenulopsis catenulafalsa (Ca. catenulafalsa) described from the tidal flat habitats in Nosy Be Island in NW Madagascar (Kryk et al. 2021). Whereas there is a distinct overlap in valve length between the two taxa, firstly, the valve width is smaller in the newly described species, with 1.7–3.1 µm versus 3–4.1 µm in the species from Nosy Be. The most distinct differences are: the girdle bands pore density, which is 30–40 in 10 µm in Ca. catenulafalsa and 50–65 in 10 µm

in Ca. baweana; the striated valve in Ca. catenulafalsa versus the plain valve face in the newly described species; and strongly dorsally bent raphe slits in the former species versus slightly bent in the latter species. Furthermore, Ca. baweana has a semi-lanceolate shape with an abrupt transition between the valve face and mantle. In contrast, Ca. catenulafalsa has a smoother transition between these structures (see Kryk et al. 2021, figs 27-32). Secondly, the raphe of the new species is situated significantly distant from the valve margin. However, the raphe of Ca. catenulafalsa is situated in a transition area. Consequently, the distal raphe endings clearly extend into the mantle (Kryk et al. 2021). Thirdly, both species possess biarcuate raphe slits; however, in the new species, the raphe is straight in the central region and exhibits curvature at the proximal and distal ends. This contrasts with the raphe in Ca. catenulafalsa, which tends to follow a sinusoidal path and is shorter in length (Table 3). Fourthly, unlike Ca. catenulafalsa, which is characterized by the presence of ribs on the ventral valve face, the new species exhibits a distinct absence of such structures. Fifthly, the dorsal and ventral mantles of Ca. catenulafalsa are striated by the irregular shape of long solitary areolae, while the newly described species has a lower number in 10 µm of dorsal striae (Table 3).

## Key to catenuloid in this study with all described Catenula taxa

# Order: Thalassiophysales Family: Catenulaceae

## Key to genera

orange.
mphora
rallel to
atenula
ther, ra-
ulopsis
ventral
mphora
rallel to
atenula
bet, dis-
laceago

## Genus: Catenula Mereschkowsky

## Key to species

1	Valve shapes semi-lanceolate dorsiventral	2
1a	Valve shapes lanceolate dorsiventral	9
2	Valve length 6-20 μm	3
2a	Valve length 8–26 µm	8
3	Ventral margin straight	4
3a	Ventral margin curved	C. brotasiae

4	Internal valve has one thickening silica	C. decusa
5	Striae density 40-50 in 10 µm	C. densestriata
6	Striae density < 40 in 10 µm	C. adhaerens
6a	Transapical striae coarse	C. robusta
6b	Raphe eccentric along the edge of valve face	C. javanica
7	Striae density ~ 90 in 10 µm	C. exigua
8	Raphe eccentric on the ventral valve face	C. boyanensis
8a	Valve long 23–38 µm, wide 6–11 µm	C. pelagica
9	Internal valve has two thickening silica	<mark>C. komodens</mark> is

#### Genus: Catenulopsis Kryk, Witowski, Kociolek & Mayama

#### Key to species

## Genus: Paracatenula Witkowski, Luthfi & M.Rybak, gen. nov.

#### Key to species

#### Genus: Wallaceago Witkowski, Arsad, Luthfi & M.Rybak, gen. nov.

#### Key to species

# Conclusion

The present study significantly expands upon the foundational work of Kryk et al. (2021) by introducing 7 new catenuloids species from Indonesian marine environments. This discovery enriches our understanding of the biodiversity within the Catenulaceae family, particularly in the understudied regions of the Indonesian archipelago. Our ongoing research further supports the notion of a diverse diatom community within this archipelago, as evidenced by the identifi-
cation of an additional amphoroid species that will soon be described as a new member of the Catenulaceae family. These findings collectively highlight the unexplored potential for discovering novel diatom taxa in the vast and ecologically complex coral reef ecosystems of Indonesia.

# Dedication

This article is dedicated to the memory of a researcher, diatomist, and our supervisor, the late Professor Andrzej Witkowski, who passed away on September 17, 2023. As a pioneering figure in the study of diatoms, his contributions to our understanding of these remarkable organisms have been invaluable.

# Acknowledgments

The authors express their sincere gratitude to Muhammad Faisal for his invaluable assistance in field sample collection. We also acknowledge M. Azurea BL for their expertise in creating the map. Our thanks extend to the two reviewers for their thorough and insightful feedback, and to the editorial board for their continuous support and guidance throughout the review process.

# **Additional information**

# **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

# Funding

This work was partly supported by the International Society for Diatom Research for the funding through Luc Ector Early Career Award 2023 and this publication is Co-financed by the Minister of Science the Republic of Poland under the Regional Excellence Initiative Program for 2024-2027 (RID/SP/0045/2024/01).

# **Author contributions**

Conceptualization: AW, OML. Data curation: OML, AK. Formal analysis: AW, OML, MB, AK. Funding acquisition: MB, AW. Investigation: SA, YR, OML. Methodology: MR, LP, RJW. Project administration: Y. Resources: AW, MR, LP, RJW. Supervision: MB, JLP. Validation: AW, MB, MR, LP. Visualization: OML. Writing - original draft: AW, OML, AK. Writing - review and editing: JLP, MB.

# **Author ORCIDs**

Oktiyas Muzaky Luthfi https://orcid.org/0000-0002-9550-9381 Sulastri Arsad https://orcid.org/0000-0002-7322-7834 Adrian Kryk https://orcid.org/0000-0003-4959-4199 Yenny Risjani https://orcid.org/0000-0002-6191-5824 Yunianta https://orcid.org/0000-0002-9684-8131 Mateusz Rybak https://orcid.org/0000-0001-8998-9537 Łukasz Peszek https://orcid.org/0000-0002-9132-2210 Rafał J. Wróbel <sup>©</sup> https://orcid.org/0000-0003-2593-0813 Janice L. Pappas <sup>©</sup> https://orcid.org/0000-0002-1207-8388 Małgorzata Bąk <sup>©</sup> https://orcid.org/0000-0001-6465-5047 Andrzej Witkowski <sup>©</sup> https://orcid.org/0000-0003-1714-218X

### Data availability

All of the data that support the findings of this study are available in the main text.

# References

- Ashworth MP, Lobban CS, Witkowski A, Theriot EC, Sabir MJ, Baeshen MN, Hajarah NH, Baeshen NA, Sabir JS, Jansen RK (2017) Molecular and morphological investigations of the stauros-bearing, raphid pennate diatoms (Bacillariophyceae): Craspedostauros EJ Cox, and Staurotropis TBB Paddock, and their relationship to the rest of the Mastogloiales. Protist 168(1): 48–70. https://doi.org/10.1016/j.protis.2016.11.001
- Barber HG, Haworth EY (1981) A guide to the morphology of the diatom frustule: with a key to the British freshwater genera.
- Cox EJ (1987) *Placoneis* Mereschkowsky: The re-evaluation of a diatom genus originally characterized by its chloroplast type. Diatom Research 2(2): 145–157. https://doi.or g/10.1080/0269249X.1987.9704994
- Cox EJ (2012) Ontogeny, homology, and terminology-wall morphogenesis as an aid to character recognition and character state definition for pennate diatom systematics 1. Journal of Phycology 48(1): 1–31. https://doi.org/10.1111/j.1529-8817.2011.01081.x
- Cox EJ (2015) Coscinodiscophyceae, Mediophyceae, Fragilariophyceae, Bacillariophyceae (Diatoms). In: Jaklitsch W, Baral HO, Lücking R (Eds) Syllabus of Plant Families, 64–103.
- Danielidis DB, Mann DG (2003) New species and new combinations in the genus *Seminavis* (Bacillariophyta). Diatom Research 18(1): 21–39. https://doi.org/10.1080/026 9249X.2003.9705570
- Frankovich TA, Ashworth MP, Sullivan MJ, Vesela J, Stacy NI (2016) *Medlinella amphoroidea* gen. et sp. nov.(Bacillariophyta) from the neck skin of Loggerhead sea turtles (Caretta caretta). Phytotaxa 272(2): 101–114. https://doi.org/10.11646/phytotaxa.272.2.1
- Garcia M, Talgatti D (2011) Morfologia e distribuição de *Catenula adhaerens* Mereschkowsky (Bacillariophyceae) no sul do Brasil. Iheringia. Série Botânica 66: 99–108.
- Górecka E, Ashworth MP, Davidovich N, Davidovich O, Dąbek P, Sabir JS, Witkowski A (2021) Multigene phylogenetic data place monoraphid diatoms Schizostauron and Astartiella along with other fistula-bearing genera in the Stauroneidaceae1. Journal of Phycology 57(5): 1472–1491. https://doi.org/10.1111/jpy.13192
- Kryk A, Witkowski A, Ribeiro L, Kociolek JP, Mayama S, Wróbel RJ, Risjani Y, Yunianta, Bemiasa J, Bemanaja E (2021) Novel Diatoms (Bacillariophyta) from tropical and temperate marine littoral habitats with the description of *Catenulopsis* gen. nov., and two *Catenula* species. Diatom Research 36(3): 265–280. https://doi.org/10.1080/02 69249X.2021.1974572
- Levkov Z (2009) Diatoms of Europe: diatoms of the European inland waters and comparable habitats. 5. *Amphora* sensu lato: Zlatko Levkov. Gantner.
- Levkov Z, Tofilovska S, Jovanovska E, Cvetkoska A, Metzeltin D (2016) Revision of the *Stauroneis smithii* Grunow (Bacillariophyceae) species complex from Macedonia. Botanica Serbica 40: 167–178.
- Li CL, Ashworth MP, Witkowski A, Dąbek P, Medlin LK, Kooistra WHCF, Sato S, Zgłobicka I, Kurzydłowski KJ, Theriot EC, Sabir JSM, Khiyami MA, Mutwakil MHZ, Sabir MJ,

Alharbi NS, Hajarah NH, Qing S, Jansen RK (2015) New insights into Plagiogrammaceae (Bacillariophyta) based on multigene phylogenies and morphological characteristics with the description of a new genus and three new species. PLoS ONE 10(10): e0139300. https://doi.org/10.1371/journal.pone.0139300

- Louvrou I, Economou-Amilli A (2012) Transfer of four taxa of genus *Nitzschia* Hassal to genus *Psammodictyon* DG Mann (Bacillariophyceae). Journal of Biological Research (Thessaloniki) 17: 148.
- Medlin LK, Kaczmarska I (2004) Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. Phycologia 43(3): 245–270. https://doi.org/10.2216/i0031-8884-43-3-245.1
- Mereschkowsky, C (1902) Sur *Catenula*, un nouveau genre de Diatomées. Scripta Botanica (Botanisheskia Zapiski). St. Petersburg, 19: 93–116 [43–66] [pl. 3].
- Olivares-Rubio HF, Cabrera LI, Godínez-Ortega JL, Salazar-Coria L, Vega-López A (2017) Halamphora oceanica (Catenulaceae, Bacillariophyta), a new species from the epipelagic region of the southwestern Gulf of Mexico. Phytotaxa 317(3): 188–198. https:// doi.org/10.11646/phytotaxa.317.3.3
- Paddock TBB (1980) Observations on the marine diatom genus *Auricula* and two new genera *Undatella* and *Proboscidea*. Bacillaria 3: 161–196.
- Pliński M, Witkowski A (2020) Diatoms from the Gulf of Gdańsk and surrounding waters (the southern Baltic Sea): a key to the identification of the species. Wydawnictwo Uniwersytetu Gdańskiego, 442 pp.
- Robert K, Bosak S, Van de Vijver B (2019) *Catenula exigua* sp. vov., a New Marine Diatom (Bacillariophyta) Species from the Adriatic Sea. Phytotaxa 414(2): 113–118. https://doi.org/10.11646/phytotaxa.414.2.3
- Round FE, Crawford RM, Mann DG (1990) Diatoms: biology and morphology of the genera. Cambridge University Press, 747 pp.
- Ruck EC, Theriot EC (2011) Origin and evolution of the canal raphe system in diatoms. Protist 162(5): 723–737. https://doi.org/10.1016/j.protis.2011.02.003
- Silva PC (1980) Names of classes and families of living algae. Regnum Vegetabile 103: 1–156.
- Stepanek JG, Kociolek JP (2016) Re-examination of Mereschkowsky's genus *Tetramphora* (Bacillariophyta) and its separation from *Amphora*. Diatom Research 31(2): 123–148. https://doi.org/10.1080/0269249X.2016.1183344
- Stepanek JG, Patrick Kociolek J (2019) Molecular phylogeny of the diatom genera *Amphora* and *Halamphora* (Bacillariophyta) with a focus on morphological and ecological evolution. Journal of Phycology 55(2): 442–456. https://doi.org/10.1111/jpy.12836
- Sundbäck K, Medlin LK (1986) A light and electron microscopic study of the epipsammic diatom *Catenula adhaerens* Mereschkowsky. Diatom Research 1(2): 283–290. https://doi.org/10.1080/0269249X.1986.9704974
- Witkowski A, Lange-Bertalot H, Metzeltin D (2000) Diatom flora of marine coasts I. Iconographia Diatomologica Annotated Diatom Micrographs, ARG Gantner, Königstein, Germany, 925 pp.
- Witkowski A, Li C, Zgłobicka I, Yu S, Ashworth MP, Dąbek P, Qin S, Tang C, Krzywda M, Ruppel M, Theriot EC, Jansen RK, Car A, Płociński T, Wang Y, Sabir JSM, Daniszewska-Kowalczyk G, Kierzek A, Hajrah NH (2016) Multigene Assessment of Biodiversity of Diatom (Bacillariophyceae) Assemblages From the Littoral Zone of the Bohai and Yellow Seas in Yantai Region of Northeast China With Some Remarks on Ubiquitous Taxa. Journal of Coastal Research 74: 166–195. https://doi.org/10.2112/SI74-016.1

# PhytoKeys

Research Article

# Cardamine zhangjiajieensis, a new species of Brassicaceae in China

Jia-Lu Li<sup>10</sup>, Cheng Zhang<sup>2,30</sup>, Yi He<sup>10</sup>, Quan-Ru Liu<sup>1</sup>

1 Key Laboratory of Biodiversity Science and Ecological Engineering, Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, China

2 Shenzhen Key Laboratory for Orchid Conservation and Utilization, and Key Laboratory of National Forestry and Grassland Administration for Orchid Conservation and Utilization, Shenzhen 518114, China

3 The National Orchid Conservation Center of China and the Orchid Conservation & Research Center of Shenzhen, Shenzhen, 518114, China Corresponding author: Quan-Ru Liu (liuquanru@bnu.edu.cn)

#### Abstract

*Cardamine zhangjiajieensis*, a new species from Hunan Province, China, is described and illustrated. *Cardamine zhangjiajieensis* is similar to *C. circaeoides*. From the latter, *C. zhang-jiajieensis* is readily distinguished by the terminal leaflet of the cauline leaf 4.5–7 cm (vs. 2.5–4.5 cm), mostly round or oblong (vs. oblong-oval to triangular-oval), margin undulating sinuses (vs. crenate or subentire), seed apically narrow wing (vs. wingless).

Key words: Flora of Hunan, morphology, new species, phylogeny, taxonomy

# Introduction

*Cardamine* L. (Brassicaceae) is a cosmopolitan genus with over 280 species (Marhold et al. 2021), mainly distributed in the temperate regions. According to the *Flora of China*, there are 48 species of *Cardamine* in China, 24 of which are endemic (Zhou et al. 2001). Since 2001, new species and distribution records of Chinese *Cardamine* have been published continuously (Marhold et al. 2007; Al-Shehbaz and Boufford 2008; Al-Shehbaz 2015a, 2015b; An et al. 2016). To date, 61 species of *Cardamine* have been reported in China, 31 of which are endemic.

We examined literature and specimens of *Cardamine*. Field surveys were conducted in Hunan, Sichuan, Jilin, Hebei, Yunnan from 2021 to 2023. During the specimens examination of the genus *Cardamine*, the author picked out one specimen which was identified as *Cardamine macrocephala* Z. M. Tan & S. C. Zhou (Tan and Zhou 1996), *Zhang D. G. 130502028* collected from Zhangjiajie Nature National Reserve, Hunan Province. This name had been recorded as a synonym of *C. circaeoides* Hook.f. & Thomson (Zhou et al. 2001). The morphological characters of the plants on the specimen *Zhang D. G. 130502028* are significantly inconsistent with those of *C. circaeoides*, whose leaves are heart-shaped, 1.5–5.5 cm long, margin entire. In contrast, the leaves of *Zhang D. G. 130502028* are oval to nearly round, 3–7 cm long, with wide undulating teeth. Therefore, in order to clarify the taxonomic status of the above two groups, this study conducted field investigations, morphological and systematic analysis. This species, *C. zhangjiajieensis*, is proposed as new to science.



Academic editor: Karol Marhold Received: 29 January 2024 Accepted: 29 September 2024 Published: 4 November 2024

**Citation:** Li J-L, Zhang C, He Y, Liu Q-R (2024) *Cardamine zhangjiajieensis*, a new species of Brassicaceae in China. PhytoKeys 248: 293–304. https://doi. org/10.3897/phytokeys.248.119678

**Copyright:** © Jia-Lu Li et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

# Method

## Sampling and morphological analyses

Based on the literature examination, more than 5000 specimens from PE, KUN, CDBI, SZ and other herbaria were examined. Field surveys were conducted in Zhangjiajie Nature National Reserve during April 2021, May 2022 and May 2023, specimens' collection sites are shown in Table 1, Fig. 1.

Morphology characters of rhizome, stem, leaf, flower and silique have been carefully revalued by Image J. A list of morphological characters and their acronyms used in analyses has been shown in Table 2. The description of character was referred to (Šlenker et al. 2018). 14 unrelated characters were selected, and PCA was performed by Past 3 after standardization by IBM SPSS Statistics (v.27).

# **Phylogenetic analyses**

Fresh plant leaves were collected in the field and quickly dried with silica gel. Plant samples were sent to Beijing Novogene Corporation for quality testing and re-sequencing. The sequencing platform, Illumina HiSeq X Ten and BGI, was used to generate approximately 2–10 GB of data for each sample. The chloroplast genome was assembled from the clean data using GetOrganelle (Jin et al. 2020). Plastid Genome Annotator (PGA) was used to annotate chloroplast genome with *Amborella trichopoda* Baill. from software as references (Qu et al. 2019). Then, 26 plastid genome sequences were downloaded from NCBI (Table 5), including 23 species of *Cardamine* and 2 species, *Rorippa sylvestris* (L.) Besser, *Rorippa indica* (L.) Hiern as outgroup.

Taxa and sample sites	No. of individuals	Morphological analyses	Flow cytometric measurements
C. zhangjiajieensis J.L. Li & Q.R. Liu			
China Hu'nan: Zhangjiajie Nature Reserve, Wulingyuan, 29.340766°N, 110.45326°E, 880 m, May 21, 2022, J.L.Li & C. Zhang BNU2022ZJJ02	3	$\checkmark$	$\checkmark$
China, Hu'nan: Zhangjiajie Nature Reserve, Wulingyuan, 29.324980°N, 110.433310°E, 600 m, May 21, 2022, <i>J.L.Li &amp; C. Zhang BNU2022ZJJ0</i> 6	5	$\checkmark$	
China, Hu'nan: Zhangjiajie Nature Reserve, Wulingyuan, 29.341153°N, 110.457903°E, 820 m, May 21, 2022, <i>J.L.Li &amp; C. Zhang BNU2022ZJJ0</i> 4	3	$\checkmark$	
China, Hu'nan: Zhangjiajie Nature Reserve, Jinbianxi, 2021, C. Zhang, zhang670	9	$\checkmark$	
C. circaeoides Hook.f. & Thomson			
China Yunnan: Ailaoshan, alt. 2523 m, 24.535°N, 101.023198°E, 26 May 2022, C. Zhang & X.J.Zhao BNU2022ALS01	12	$\checkmark$	
China Yunnan: Ailaoshan, alt. 2475 m, 24.525649°N, 101.008964°E, 26 May 2022, C. Zhang & X. J .Zhao BNU2022ALS03	5	$\checkmark$	
China Sichuan: Yinchanggou Village, alt. 1200 m, 28°30'9.17"N, 103°30'40.83"E, 30 April 2022, <i>C. Zhang &amp; J.L. Li BNU2022XN01</i>	9	$\checkmark$	
China Sichuan:Yinchanggou Village, alt. 1200 m, 28°30'0.91"N, 103°29'10.44"E, 30 April 2022, C. Zhang & J.L. Li BNU2022XN03	4	$\checkmark$	
China Hubei: Xuan'en, Shadaogou, C. Zhang, zhang660	5	$\checkmark$	
China Hu'nan: Jishou, Hangxia Spring, C. Zhang, zhang551	2	$\checkmark$	
China Hu'nan: Jishou, Hangxia Spring, C. Zhang, zhang531	6	$\checkmark$	
C. cf. scutata Thunb.			
China Sichuan: Michangshan, alt. 1256 m, 32.633917°N, 106.517870°E, 14 May 2022, C. Zhang & J.L. Li BNU2022MCS02	3		$\checkmark$
C. hupingshanensis K.M.Liu, L.B.Chen, H.F.Bai & L.H.Liu			
China Hu'nan: Hupingshan April 2023, BNU2023HPS01	2		√

Table 1. Taxa and sample sites.

The annotated sequences were imported into PhyloSuite (Zhang et al. 2020), the Mafft module was used for sequence alignment (Katoh et al. 2019), and the ModelFinder module was used to calculate the nucleotide substitution model for the aligned sequences. The maximum likelihood (ML) tree was constructed using IQ-TREE (Minh et al. 2020), with the nucleotide substitution model set to GTR+R3+F and a standard bootstrap value of 1000.



**Figure 1.** Collection site of *C. zhangjiajieensis*, plus represents *C. zhangjiajieensis* and the cross represents *C. circaeoides*.

Table 2. List of	f morphological	characters and their a	cronvms used in analvse	s
				~

Acronym	Description of character
RW	Indument in the plant (0-hairs present, 1-hairs absent)
BL	Length of the terminal leaflet of the basal leaf (mm)
BW/BL	Ratio of the length and width of the terminal leaflet of the basal leaf
BWL/BL	Ratio of the widest part length and length of the terminal leaflet of the basal leaf
BTA	Angle of the apex of the terminal leaflet of the basal leaf (mm)
BBA	Angle of the basal of the terminal leaflet of the basal leaf (mm)
No.BL	Mean number of lateral leaflets of the basal leaf (mm)
BTD	Depth of sinuses of the terminal leaflet of the basal leaf (mm)
CL	Length of the terminal leaflet of the cauline leaf (mm)
No.CL	Mean number of lateral leaflets of the cauline leaf (mm)
CTA	Angle of the apex of the terminal leaflet of the cauline leaf (mm)
CBA	Angle of the basal of the terminal leaflet of the cauline leaf (mm)
CBL/CL	Ratio of the widest part length and length of the terminal leaflet of the basal leaf
CTL	Depth of sinuses of the terminal leaflet of the cauline leaf (mm)
PL	Length of petals (mm)
PW	Width of petals (mm)
SL	Length of sepals (mm)
SW	Width of sepals (mm)
LFL	Length of longer filaments (mm)
SFL	Length of shorter filaments (mm)
PdL	Length of the pedicel of the lowermost siliqua of the main inflorescence (mm)
SqL	Length of the lowermost siligua of the main inflorescence (mm)

### Flow cytometric measurements and estimation of DNA ploidy levels

Methods referring to Marhold et al. (Marhold et al. 2010) and Kobrlová and Hroneš (Kobrlová and Hrones 2019) measured the nuclear DNA content using flow cytometry. Inferred the DNA ploidy levels within the studied populations based on Cardamine species with known ploidy (Suda and Trávníček 2006; Marhold et al. 2021). The relative nuclear DNA content was determined using PI, a DNA intercalating fluorescent dye, with arbitrary units (a.u.) as the unit of measurement. The buffer solution used was LB01. Dehydrated leaves, preserved by drying at 40 °C for 18-24 months, were used for the determination of chromosome ploidy. The sample sources and voucher specimens are presented in the Table 1. In a pre-cooled culture dish, 1-2 mL of LB01 buffer solution and 2 cm<sup>2</sup> of dry leaves were added. After rapid chopping, the mixture was filtered through a 400-mesh gauze, centrifuged at 4 °C, 3000 rpm for 10 minutes, and the supernatant was discarded. The pellet was resuspended in 600 µL of LB01 buffer solution, followed by the addition of 100  $\mu$ L of PI solution (50  $\mu$ g/mL), and stained in the dark for 15 minutes. Ploidy level of the stained cell suspension was determined by flow cytometry (ACEA NovoCyte 3130). Using 488 nm blue light excitation, 10,000 cells were collected at a time. The other samples were determined under the same voltage bar using C. scutata (2n = 4x = 32) as the reference for tetraploid.

# Result

# Morphological analyses

The ordination diagrams of PCA based on individual plants (Fig. 2) showed that, the cumulative proportion of eigenvalue of the first five principal component axes exceeds 75%, indicated success of dimension reduction. *C. circaeoides* and *C. zhangjiajieensis* were separated on principal component axis 1 without overlap. The characters that contributed to this axis are the angle of the basal of the terminal leaflet of the basal leaf (BBA), depth of sinuses of the terminal leaflet of the basal leaf (CBL/CL), indicating significant differences in cauline leaf morphology. The principal component axis 2 and axis 3 have overlapping distribution ranges.



**Figure 2**. Ordination diagrams of principal component analyses, orange plus refers to *C*. *circaeoides*, cyan dots refers to *C*. *zhangjiajieensis*.

## **Phylogenetic analyses**

The aligned plastid genome dataset included 32 species with 161195 bp characters; 6552 (4.06%) were parsimony informative. As shown in the Fig. 2, the plants used in this study were divided into three branches, *Cardamine* species formed a monophyletic group with moderate support (BS = 1). *C. zhangjiajieensis* and *C. scutata* converged into a single lineage, formed a clade with *C. amariformis* Nakai. in Fig. 3.

### Flow cytometric measurements and estimations of DNA ploidy levels

As shown in Table 3, the chromosome ploidy was determined by the materials stored for 18-24 months after being dehydrated, and the coefficient of variation was between 6.9% and 7.9%, indicating that dried specimens could be used in this study (Suda and Trávníček 2006). According to the relative DNA content and chromosome number of *C.scutata*, the relative DNA content of *C. zhangjiajieensis* and *C. hupingshanensis* was measured, as shown in the table. The chromosome ploidy of *C. zhangjiajieensis* was estimated to be  $2n \approx 6x$ .



**Figure 3.** The strict consensus tree resulted from IQ-tree analysis using plastid genome. Bootstrap (BS) are showed below branches. Blue refers to species distributed in Hunan province, rose red refers to the novelty.

**Table 3.** Relative genome sizes obtained for *C. zhangjiajieensis*, *C. scutata*, *C. hupingshanensis*, the Relative genome sizes of *C. scutata* refer to (Marhold et al. 2010).

Taxa and Voucher information	Relative genome size in a.u. (arbitrary units); mean (minimum-maximum)	(DNA) Ploidy level	Mean relative genome size per monoploid genome	Variation (%)
C. zhangjiajieensis	0.727 (0.670-0.784)	≈бх	-	7.9%
C. scutata	0.466 (0.405-0.527)	4x	0.117	6.9%
C. hupingshanensis	0.911 (0.842-0.980)	Зx	0.304	7.6%

### **Taxonomic treatment**

Cardamine zhangjiajieensis Q.R.Liu & J.L.Li, sp. nov. urn:lsid:ipni.org:names:77351286-1 Figs 4, 5 Common name: 张家界碎米荠

**Type.** CHINA • Hunan: Zhangjiajie Nature Reserve, Wulingyuan, 29.340766°N, 110.45326°E, 880 m, May 21, 2022, *Li JL, Zhang C, BNU2022ZJJ02* (holotype:B-NU0057018, isotype: BNU0057017, BNU0057016).

**Diagnosis.** Cardamine zhangjiajieensis is similar to *C. circaeoides*. From the latter, *C. zhangjiajieensis* is readily distinguished by the terminal leaflet of the cauline leaf 4.5–7 cm (vs. 2.5–4.5 cm), lateral leaflets 0–2 pairs (vs. 0–1 pairs), without auriculate petioles (vs. auriculate petioles), mostly round or oblong (vs. oblong-oval to triangular-oval), margin undulating sinuses (vs. crenate or subentire), seed apically narrow wing (vs. wingless).

**Description.** Herbs perennial, 15–40 cm tall, glabrous. Rhizomes short, sometimes with a few stolons. Stems erect, simple or branched above middle. Basal leaves not rosette, simple or 2–4 foliolate; petiole 3–6 cm, glabrous; leaf blade or terminal leaflets cordate or ovate, 3-5 cm  $\times 3-5$  cm, glabrous, base cordate, sometimes subtruncate, or subcuneate, margin often sinuous, apex obtuse; lateral leaflets (when present) petiolulate or sessile, much smaller than terminal one. Cauline leaves simple or 1 of which 2– or 3–foliolate, petiolate or rarely uppermost subsessile; petiole (0.4-)1–5 (-6) cm; leaf blade similar to that of basal leaves, cordate, sometimes ovate to ovate-lanceolate, 4.5 (-6)  $\times$  3(-4) cm. Fruiting pedicels 3–12 (-15) mm, ascending, base sometimes rooting, emitting new plants. Sepals ovate or oblong, 2.8-3.2 mm  $\times$  1.4–1.9 mm, glabrous, margin often membranous. Petals white, spatulate, 4.5-6.5 mm  $\times$  2.5–3 mm, apex notch. Median filament pairs 3.5-4.5 mm, lateral pair 1.5–2.5 mm; anthers oblong. Siliques linear, 2.5-4 cm; valves torulose, glabrous; style (0.5-)1–2 mm. Seeds brown, ovate or broadly oblong,  $1.4-1.6 \times 0.9-1.1$  mm, with a narrow wing. Fl. and fr. April–Jul. 2n  $\approx$  6x.

**Distribution.** Only found in Zhangjiajie Nature Reserve, Hunan Province, grows in wet places of roadside, forest, river sides.

**Specimens examined.** CHINA • Hunan: Zhangjiajie Nature Reserve, Wulingyuan, 29.324980°N, 110.433310°E, 600 m, May 21, 2022, *Li JL, Zhang C, BNU2022ZJJ06* (BNU, 4 duplicates) • Zhangjiajie Nature Reserve, Wulingyuan, 29.341153°N, 110.457903°E, 820 m, May 21, 2022, *Li JL, Zhang C, BNU2022ZJJ04*, (BNU, 7 duplicates); April 25, 2023, *Li JL, Song QR, BNU2023ZJJ01*, (BNU, 2 duplicates); Jinbianxi, 2021, *Zhang C zhang670*, (BNU, 7 duplicates) • Tianmenshan, May 2, 2013, *Zhang DG 130502028* (JIU33520) • Hunan, Exact location unknown, March 18, 1955, *Xia JL 20*, (PE01995438).

**Discussion.** Major centers of *Cardamine* diversity estimated by the number of taxa appear to be in the European Mediterranean and the Caucasus; Eastern Asia and the Himalayas; and North and Central America (Marhold et al. 2018). Utilizing data from this study, we selected common *Cardamine* in Hunan and Central China, such as *Cardamine lyrata*, *Cardamine anhuiensis*, *Cardamine engleriana*, *Cardamine fragariifolia*, *Cardamine circaeoides*, *Cardamine scutata*, *Cardamine fallax*, *Cardamine occulta* and determined the phylogenetic position of *C. zhangjiajieensis* within the genus. Phylogenetic analysis shows that

Jia-Lu Li et al.: Cardamine zhangjiajieensis, a new species of Brassicaceae in China



**Figure 4.** *Cardamine zhangjiajieensis* Q.R.Liu & J.L.Li **A** plants in fruit period **B** new leaves emanating from aerial roots **C** basal leaves **D** flower **E** petal and sepals **F** stamens **G** style **H** siliques and seed, All drawn by Quan-Ru Liu from voucher specimens *Li JL, Zhang C BNU2022ZJJ02* (BNU!) (**A**), *Li JL, Zhang C BNU2022ZJJ04* (BNU!) (**B**–**H**).



Figure 5. Morphology and habitat of *C. zhangjiajieensis* **A**, **B** plants in flowering and fruit period **C** flower **D** siliques and seed morphology **E** flower (lateral view) **F** leaves, indicating cauline leaves ( $\mathbf{F}_1$ ,  $\mathbf{F}_2$ ), basal leaves ( $\mathbf{F}_4$ ), and new leaves emanting from aerial roots ( $\mathbf{F}_3$ ). Photographs **A**, **B**, **D**, **E** by Zhang C, **C**, **F** by Li JL.

*C. zhangjiajieensis* falls in a clade sister to *C. scutata*. From the latter, *C. zhangjiajieensis* is readily distinguished by lateral leaflets 0–2 pairs (vs. 1–4), the terminal leaflet of the cauline leaf 4–7 cm × 3.5–6.5 cm (vs. 2–5 × 1.5–4 cm), petals 4.5–6.5 mm × 2.5–3 mm (vs. 2.5–4.5 × 1.5–2.5 mm), sepals 2.8–3.2 mm × 1.4–1.9 mm (vs. 1.5–2.5 × 0.9–1.4 mm), vegetative propagated by adventitious buds in fruit stage.

As a widespread species in East Asia, the speciation of *C. scutata* has been elucidated. Allotetraploid *C. scutata* originated by hybridization between two diploid species, *C. parviflora* and *C. amara* (Mandáková et al. 2019). Based on chloroplast genome data, our study showed that *C. parviflora*, *C. scutata*, *C. zhangjiajieensis*, *C. amariformis* formed a highly supported subclade, *C. parviflora* located at the base of clade, which supported the results of previous studies. Our analysis also suggested that *C. scutata* (2n = 4x = 32) may be involved in the speciation of *C. zhangjiajieensis* ( $2n \approx 6x$ ) as hybrid parent according to the ploidy level.

Another notable character of *C. zhangjiajieensis* is vegetative reproduction by adventitious buds in fruit stage, which is also found in *C. hupingshanensis*, an endemic species to Hupingshan Mountains (Long 2004). From the latter, *C. zhangjiajieensis* is readily distinguished by glabrous (vs. hirsute), terminal leaflet of the cauline leaf oblong, not petiole auriculate-amplexicaul at base (vs. reniform or orbicular, petiole auriculate-amplexicaul at base), seed apically narrow wing (vs. wingless), as shown in Table 4. *C. hupingshanensis* has 2n = 24. In natural conditions, the reproduction of *C. hupingshanensis* relies on adventitious roots growing on each stem node after lodging, indicating that the ploidy level of *C. hupingshanensis* is 2n = 3x. To further clarify speciation and evolution of karyotype within *Cardamine*, studies with comprehensive sampling and seeds germination of *C. zhangjiajjeensis* are needed.

	C. zhangjiajieensis	C. circaeoides	C. scutata	C. hupingshanensis
Life cycle	Perennial	perennial	annual or biennial	perennial
Trichome	Glabrous	pilose	glabrous or pilose	hirsuta
Plant height	15-40 cm	9-36 cm	(5-)15-50(-70) cm	30-100 cm
Base of petiole	Simple	auriculate	simple	auriculate
No. of lateral leaflet or lobes of the basal leaf	(0-)1-2(-3)	0(-2)	1-4	0(rare 1)
Length of terminal leaflet of the basal leaf	3–5 cm	2-4 cm	1.5-2.5 cm	4-13 cm
Terminal leaflet of the basal leaf	Orbicular	cordate or oval	reniform or cordate	reniform or orbicular
Base of the basal leaf	Truncate	cordate	cordate	cordate, petiole auriculate- amplexicaul at base
Length of terminal leaflet of the cauline leaf	4.5-7 cm	1-3.5 cm	2-5 cm	4-13 cm
Terminal leaflet of the cauline leaf	orbicular to oblong	cordate, ovate to ovate-lanceolate	oblong-oval	reniform or orbicular
Base of the cauline leaf	Ovate	cuneate	ovate	cordate, petiole auriculate- amplexicaul at base
Margin of cauline leaf	undulating sinuses	crenate or subentire	apically sinuses 3–5	crenate
Sepals (mm)	2.8-3.2 × 1.4-1.9	2-3.5 × 0.8-1.5	1.5−2.5 × 0.9−1.4	5-6 × 3-4
Petals (mm)	4.5-6.5 × 2.5-3	5-7 × 2-2.5	2.5-4.5 × 1.5-2.5	8-10 × 7-9
Stamens (mm)	1.8-3	2.5-5	2-3.5	3-6
Ovules of ovary	18-40	20-42	20-40	Not seen
Seed (mm)	1.4-1.6 × 0.9-1.1	0.8-1.1 × 0.6-0.9	0.9-1.2 × 0.6-0.9	1.2-1.8 × 0.9-1.3
Wing of seed	apically narrow wing	wingless	with wing	wingless
(DNA) ploidy level	2n ≈ 6x	2n = 2x = 16	2n = 4x = 32	2n = 3x = 24

Table 4. Comparison of characters of C. zhangjiajieensis, C. circaeoides and C. scutata.

GenBank accession numbers	Specimens	Species name
NC026446		Cardamine resedifolia L.
MT136871		Cardamine quinquefolia (M.Bieb.) Schmalh.
MK637691		Cardamine pentaphyllos (L.) Crantz
NC036964		Cardamine parviflora L.
NC036963		Cardamine oligosperma Nutt.
MZ043777		Cardamine occulta With.
MF405340		Cardamine macrophylla Willd.
MZ846206		Cardamine lyrata Bunge
MK637684		Cardamine kitaibelii Bech.
NC026445		Cardamine impatiens L.
ON322745		Cardamine hupingshanensis K.M.Liu
MK637681		Cardamine hirsuta L.
MN651504		Cardamine heptaphylla (Vill.) 0.E.Schulz
MK637680		Cardamine glanduligera O.Schwarz
MZ043778		Cardamine fallax L.
NC049605		Cardamine enneaphyllos (L.) Crantz
OL634846		Cardamine circaeoides Hook.f. & Thomson
NC049603		Cardamine bulbifera (L.) Crantz
MN651509		Cardamine bipinnata (C.A.Mey.) O.E.Schulz
MZ043776		Cardamine amariformis Maxim.
NC036962		Cardamine amara L.
NC060863		Cardamine abchasica Govaerts
KJ136821		Cardamine impatiens L.
NC069649		Rorippa sylvestris (L.) Besser
NC065833		Rorippa indica (L.) Hiern
PP114745	ZJJ02, Li JL, Zhang C	Cardamine zhangjiajieensis
PP114744	Msc02, Li JL, Zhang C	Cardamine scutata Thunb.
PP719683	Zhang549, Zhang C	Cardamine anhuiensis D.C.Zhang & C.Z.Shao
PP719686	Zhang664, Zhang C	Cardamine hygrophila T.Y.Cheo & R.C.Fang
PP719685	EM105, Li JL, Zhang C	Cardamine fragariifolia O.E.Schulz
PP719684	Msc05, Li JL, Zhang C	Cardamine engleriana O.E.Schulz

Table 5.	Таха	in	phy	logenetic	analyses.
----------	------	----	-----	-----------	-----------

# Acknowledgments

We would like to express gratitude to the staff of PE, KUN for their support for this study; thanks to researcher Dai-Gui Zhang from Jishou University for his help in this project.

# **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

# **Ethical statement**

No ethical statement was reported.

### Funding

This research was financed by the National Natural Science Foundation of China (No. 210100203 & No. 32200170).

# **Author contributions**

All authors have contributed equally.

### Author ORCIDs

Jia-Lu Li <sup>©</sup> https://orcid.org/0009-0002-8147-5157 Cheng Zhang <sup>©</sup> https://orcid.org/0000-0003-1020-0850 Yi He <sup>©</sup> https://orcid.org/0000-0002-6925-7299

### **Data availability**

All of the data that support the findings of this study are available in the main text.

# References

- Al-Shehbaz IA (2015a) Cardamine hongdeyuana (Brassicaceae), a new species from Xizang, China. Kew Bulletin 70(1): 1–4. https://doi.org/10.1007/s12225-014-9556-3
- Al-Shehbaz IA (2015b) *Cardamine xinfenii* (Brassicaceae), a New Species from Sichuan (China). Harvard Papers in Botany 20(2): 145–146. https://doi.org/10.3100/hpib. v20iss2.2015.n3
- Al-Shehbaz IA, Boufford DE (2008) *Cardamine tianqingiae* (Brassicaceae), a new species from Gansu Province, China. Harvard Papers in Botany 13(1): 89–91. https://doi.org/ 10.3100/1043-4534(2008)13[89:CTBANS]2.0.C0;2
- An M, Lin Y, Yu L, Yang Y, Cheng G, Li J (2016) *Cardamine kuankuoshuiense* (Brassicaceae), a new species from Guizhou, China. Phytotaxa 267(3): 233–236. https://doi. org/10.11646/phytotaxa.267.3.7
- Jin JJ, Yu WB, Yang JB, Song Y, DePamphilis CW, Yi TS, Li DZ (2020) GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biology 21(1): 241. https://doi.org/10.1186/s13059-020-02154-5
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kobrlová L, Hrones M (2019) First insights into the evolution of genome size in the borage family: A complete data set for Boraginaceae from the Czech Republic. Botanical Journal of the Linnean Society 189(2): 115–131. https://doi.org/10.1093/botlinnean/boy079
- Long YS (2004) Study on Karyotype Analysis of Cardamine hupingshanesis, a New Species in *Cardamine* and the Evolution of the Intel Transcibed Spacer (ITS)Sequences of the Species in the *Cardamine*. Master, Hunan: Hunan Normal University, 27–28. [in Chinese]
- Mandáková T, Zozomová-Lihová J, Kudoh H, Zhao Y, Lysak MA, Marhold K (2019) The story of promiscuous crucifers: Origin and genome evolution of an invasive species, *Cardamine occulta* (Brassicaceae), and its relatives. Annals of Botany 124(2): 209–220. https://doi.org/10.1093/aob/mcz019
- Marhold K, Lihova J, Al-Shehbaz IA, Kudoh H (2007) The correct interpretation and lectotypification of the name *Cardamine fallax* (Brassicaceae). Journal of Plant Research 120(5): 655–660. https://doi.org/10.1007/s10265-007-0107-y
- Marhold K, Kudoh H, Pak JH, Watanabe K, Spaniel S, Lihova J (2010) Cytotype diversity and genome size variation in eastern Asian polyploid *Cardamine* (Brassicaceae) species. Annals of Botany 105(2): 249–264. https://doi.org/10.1093/aob/ mcp282

- Marhold K, Šlenker M, Zozomová-Lihová J (2018) Polyploidy and hybridization in the Mediterranean and neighbouring areas towards the north: examples from the genus *Cardamine* (Brassicaceae). Biologia Serbica 40.
- Marhold K, Kempa M, Kučera J, Skokanová K, Smatanová J, Šingliarová B, Šlenker M, Zozomová-Lihová J (2021) Datababase of names, chromosome numbers, ploidy levels and genome sizes of the tribe Cardamineae. https://cardamine.sav.sk
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37(5): 1530–1534. https:// doi.org/10.1093/molbev/msaa015
- Qu XJ, Moore MJ, Li DZ, Yi TS (2019) PGA: A software package for rapid, accurate, and flexible batch annotation of plastomes. Plant Methods 15(1): 50. https://doi. org/10.1186/s13007-019-0435-7
- Šlenker M, Zozomova-Lihova J, Mandakova T, Kudoh H, Zhao Y, Soejima A, Yahara T, Skokanova K, Spaniel S, Marhold K (2018) Morphology and genome size of the widespread weed Cardamine occulta: How it differs from cleistogamic C. kokaiensis and other closely related taxa in Europe and Asia. Botanical Journal of the Linnean Society 187(3): 456–482. https://doi.org/10.1093/botlinnean/boy030
- Suda J, Trávníček P (2006) Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry–New prospects for plant research. Cytometry. Part A 69A(4): 273–280. https://doi.org/10.1002/cyto.a.20253
- Tan ZM, Zhou SC (1996) Two new species of Cruciferae from Sichuan. Journal of Sichuan University [Natural Science Edition], 139–142. [in Chinese]
- Zhang D, Gao FL, Jakovlic I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhou T, Lu L, Yang G, Al-Shehbaz IA (2001) Flora of China. 8. Brassicaceae through Saxifragaceae [Hauptbd.], Science Press.

# PhytoKeys

**Research Article** 

# *Carrierea leyensis*, a new species of Salicaceae from limestone areas of Guangxi, China

Zhao-Cen Lu<sup>1,2</sup>, Zhi-Rong Liu<sup>3</sup>, Ming-Lin Mo<sup>1,4</sup>, Shi-Li Chang<sup>1,4</sup>, Wei-Bin Xu<sup>1,2</sup>

- 1 Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin, 541006, Guangxi, China
- 2 Nonggang Karst Ecosystem Observation and Research Station of Guangxi, Chongzuo, 532499, Guangxi, China
- 3 Guangxi Forestry Inventory and Planning Institute, Nanning, 530011, Guangxi, China
- 4 College of Life Sciences, Guangxi Normal University, Guilin, 541006, Guangxi, China

Corresponding author: Wei-Bin Xu (gxibwbxu@163.com)

### Abstract

*Carrierea leyensis* Z.C.Lu & W.B.Xu, a new species of Salicaceae was discovered from limestone areas of Guangxi, China. The morphology of *C. leyensis* is similar to *C. dunniana*, but differs by its evergreen nature; shorter petioles, only 3–8 mm long, and tomentose or glabrous when old; elliptic leaf blade with cuneate base; shorter inflorescence (1.8–4.5 cm long); smaller flowers; and smaller capsules (1.7–2.7 cm long, 5–9 mm in diam.).

Key words: Carrierea calycina, Carrierea dunniana, morphology, new taxa, taxonomy

# Introduction

*Carrierea* Franchet (1896: 498) is a small genus in Salicaceae, distributed from southern China to northern Vietnam (Lai 1999; Yang and Zmarzty 2007). *Carrierea* is closely related to *Poliothyrsis* Oliv. and *Itoa* Hemsl., all of which have ellipsoidal to spindle-shaped capsules that split from both the base and the apex (Alford 2005). These genera were once placed in the Flacourtiaceae (Fan 1990, 1995; Yang and Zmarzty 2007), but molecular (Chase et al. 2002) or molecular and morphological data (Alford 2005) indicate that these genera are very closely related to *Salix* L. and *Populus* L. (Salicaceae *s. str.*). Four names have been published in *Carrierea* previously, but recent revisions have treated *C. rehderiana* Sleumer as a synonym of *C. calycina* Franch. (1896: 498), and *C. vieillardii* Gagnep. as a synonym of *Itoa orientalis* Hemsl. (Lai 1999; Yang and Zmarzty 2007). *Carrierea calycina* and *C. dunniana* Léveillé (1911: 458) are accepted taxa, both of which are distributed in China, and *C. calycina* is endemic to China (Yang and Zmarzty 2007).

During our investigation of plant diversity from July 2023 to May 2024, an unusual plant that could belong to an unknown species of Salicaceae with flowers and mature capsules was collected from limestone forests in Leye County, Baise City, Guangxi, China. Based on the smaller capsule, the previous year's zigzag dehiscence capsule and the shorter inflorescence with 2–11 flowers,



Academic editor: Irina Belyaeva Received: 16 June 2024 Accepted: 2 October 2024 Published: 5 November 2024

Citation: Lu Z-C, Liu Z-R, Mo M-L, Chang S-L, Xu W-B (2024) *Carrierea leyensis*, a new species of Salicaceae from limestone areas of Guangxi, China. PhytoKeys 248: 305–313. https://doi.org/10.3897/ phytokeys.248.129824

**Copyright:** © Zhao-Cen Lu et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). this unknown species was placed in the genus *Carrierea*. After carefully checking the morphological characters of the specimens, consulting relevant literature (Fan 1990, 1991, 1995; Lai 1999; Yang and Zmarzty 2007), studying herbarium specimens, and examining the other related specimens of *Carrierea*, we confirmed that this species is new to science, and it is described below.

# Materials and methods

Specimens of this new species were observed, photographed, and collected from Leye County, Baise City, Guangxi, China. Herbarium specimens were deposited at CSH, GXMG, GXMI, IBK, IBSC, KUN and PE (Herbarium codes follow Thiers 2022). Morphological characters of the specimens, including the size, shape, and color of bark, petioles, leaf blades, bracts, bracteoles, inflorescences, staminate and pistillate flowers, sepals, capsules and seeds were recorded. Online images of the other specimens of *Carrierea* were examined from the Chinese Virtual Herbarium (https://www.cvh.ac.cn/), JSTOR Global Plants (https://plants.jstor.org), and Kew Herbarium Catalogue (http://apps.kew.org/herbcat/gotoHomePage.do). The morphological description for the new species is based on the cited type specimens (holotype, isotypes, and paratypes).

# **Taxonomic treatment**

### Carrierea leyensis Z.C.Lu & W.B.Xu, sp. nov.

urn:lsid:ipni.org:names:77351405-1 Figs 1-3 Chinese name: 乐业山羊角树 (lè yè shān yáng jiǎo shù)

**Diagnosis.** Carrierea leyensis Z.C.Lu & W.B.Xu differs from *C. dunniana* H.Lév. in its evergreen nature; shorter petioles, only 3–8 mm long, and tomentose or glabrous when old; elliptic leaf blades with cuneate base; shorter inflorescences; smaller flowers; and smaller capsules.

**Type.** CHINA • Guangxi: Baise City, Leye County, Tongle Township, Fengdong Village, Laomudong, around the point 106.482109°E, 24.75169°N, in forests of limestone slope, elevation ca. 1330 m, 4 May 2024, *W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18270* (holotype: IBK00461833; isotypes: IBK00461831, IBK00461832).

**Description.** Trees or small trees, monoecious, evergreen, 5–12 m tall; bark gray-brown; branchlets grayish, with white lenticels and leaf marks, tomentose, glabrous when old; winter buds conical, scales hairy; stipules absent. Petiole 3–8 mm long, tomentose to glabrous when old; leaf blade greenish abaxially, deep green adaxially, glabrous, elliptic,  $(2.8-)4-9.5(-12.5) \times 1.7-4.6$  cm, leathery or thinly leathery, both surfaces glabrous or abaxially sparsely appressed-villous along midveins, weakly 3-veined at base, lateral veins 5–8 pairs, veins distinct on both sides, midvein raised below, base cuneate, margin remotely serrate, with spheroidal to torus-shaped glands at the tips of the teeth (salicoid teeth), apex acuminate to long acuminate. Inflorescence terminal or axillary, 2–11-flowered, rarely single flower axillary, 1.8–4.5 cm long including flowers, tomentose, pistillate flowers in terminal part of inflorescence, staminate ones in lower part; bracts ovate-lanceolate, 1–1.35 cm long, papery, both surfaces sparsely to densely



Figure 1. *Carrierea leyensis* sp. nov. A habit B trunk C flowering branches D fruiting branches E leaf, view from adaxial side F leaf, view from abaxial side.

tomentose. Pedicels 0.5-2.5 cm long, 2-bracteolate near middle; bracteoles similar to bracts, opposite, narrowly oblong, 3-7 mm long, papery, both surfaces sparsely to densely tomentose. Sepals (2 or 3 rare) 4-5, valvate, ovate-deltoid to oblong,  $3.5-8 \times 2-3$  mm, papery, both sides tomentose, apex acute. Petals absent. Staminate flowers: smaller than pistillate flowers, stamens with filaments unequal in length, 2-3 mm long, glabrous; anthers ca. 0.5 mm long. Pistillate flowers: staminodes like stamens but much reduced; ovary superior, elliptic, densely tomentose, 0.6-1 cm long; placentas 3 or 4; styles 3 or 4, 0.5-1 mm long, connate



Figure 2. *Carrierea leyensis* sp. nov. A flowering branches with staminate flowers and young capsules B inflorescences C staminate flowers in frontal view D pistillate flower in frontal view E pistillate flower in dorsal view F dissection of staminate flower G dissection of pistillate flower H bracts I bracteoles J long-section and cross-section of ovary K capsules L seeds.

at least at base to form a column, sparsely tomentose like the ovary; stigmas reflexed, drying black, flattened, triangular, 2-3 mm long, irregularly lobed, glabrous. Capsule fusiform, slightly curved, 1.7-2.7 cm long, 5-9 mm in diam., tomentose, valves splitting from both apex and base; fruiting pedicel stout, 1-2.8 cm long; seeds compressed, including wing 9-12 mm long; sterile seeds smaller.



Figure 3. The holotype sheet of Carrierea leyensis (IBK).

**Etymology.** The specific epithet '*leyensis*' refers to the locality where the new species was collected.

**Phenology.** Flowering April to May (spring); fruiting July to October (summer to autumn).

**Distribution and habitat.** *Carrierea leyensis* has only been collected from limestone areas of Leye County, Baise City, Guangxi, China. It grows sporadically in forests on limestone slopes, at an elevation of 1100–1350 m. Associated species include *Handeliodendron bodinieri* (H.Lév.) Rehder, *Pistacia chinensis* Bunge, *Pittosporum tonkinense* Gagnep., *Machilus cavaleriei* H.Lév., *Triadica rotundifolia* (Hemsl.) Esser, *Jasminum lanceolaria* Roxb., *Eriobotrya seguinii* (H.Lév.) Cardot ex Guillaumin, *Carex brunnea* Thunb., *Ophiorrhiza* species, and *Carpinus* species. **Conservation status.** The new species has been found in three localities in Leye County, Baise City, Guangxi, China. We did not complete additional surveys in the area, so the number of living populations is unknown. According to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022), *Carrierea leyensis* should be considered in the Data Deficient (DD) category at present.

Additional specimens examined (paratypes). CHINA · Guangxi: Baise City, Leye County, Tongle Township, Fengdong Village, around the point 106.502383°E, 24.769041°N, limestone slope, alt. 1190 m, 30 July 2023, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 16830 (IBK, GXMG, IBSC); • ibid., 30 July 2023, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 16832 (IBK, PE, KUN); • ibid., 10 October 2023, W. B. Xu, Z. C. Lu, S. L. Chang & J. Q. Huang 17742 (IBK, CSH); • Tongle Township, Longmen Village, Caojiadongtun, around the point 106.456363°E, 24.736441°N, limestone slope, alt. 1230 m, 31 December 2023, W. B. Xu, Z. C. Lu, S. L. Chang & J. Q. Huang LZC2195 (IBK, GXMI); • ibid., 24 September 2023, Z. R. Liu LKY-YC468 (IBK); • ibid., 4 May 2024, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18273 (IBK, CSH); • ibid., 4 May 2024, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18274 (IBK, GXMI); • Tongle Township, Fengdong Village, Laomudong, around the point 106.481868°E, 24.751426°N, limestone slope, alt. 1300 m, 4 May 2024, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18271 (IBK, PE, KUN); • ibid., 4 May 2024, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18264 (IBK, GXMG, IBSC); • ibid., 4 May 2024, W. B. Xu, Z. C. Lu, M. L. Mo, S. L. Chang & J. Q. Huang 18266 (IBK).

**Notes.** *Carrierea leyensis* can be easily distinguished from the other two species of *Carrierea* in its evergreen nature, shorter petioles, the shape of leaf blades, shorter inflorescences, smaller flowers, and smaller capsules. The morphological differences between *C. leyensis* and the two related species *C. dunniana* (Fig. 4A–D) and *C. calycina* (Fig. 4E–I) are summarized in Table 1.

This study also provides field documentation that individuals of *Carrierea* can be monoecious. Although some reports of *Carrierea* indicate that it is dioecious, our results affirm the observations of Yang and Zmarzty (2007), who noted that the type specimens of *C. dunniana* and *C. calycina* had both pistillate and staminate flowers.

Characters	C. leyensis	C. calycina	C. dunniana
Habit	Evergreen	Deciduous	Deciduous
Petiole	3–8 mm long, tomentose or glabrous when old	(2.5) 3–7 cm long, pubescent or glabrous	2–5 cm long, glabrous
Leaf blade	Elliptic, base cuneate, apex acuminate to long acuminate	Ovate-oblong, oblong, or slightly obovate, less often elliptic, base rounded to cordate, apex obtuse to acute	Ovate to oblong, base rounded, apex acuminate
Inflorescence	Terminal or axillary, 1.8-4.5 cm long	Terminal, 5–10 cm long	Terminal or axillary, 7–15 cm long
Sepals	(2 or 3 rare) 4–5, ovate-deltoid to oblong, 3.5–8 mm long	4–6, broadly ovate, 1.5–2 cm long	4–5, obovate to elliptic, 5–10 mm long
Bracts	Ovate-lanceolate, 1–1.35 cm long	Lanceolate to narrowly elliptic, 1–3 cm long	Ovate, 5–10 mm long
Bracteoles	Narrowly oblong, 3–7 mm long	Narrowly oblong, 4–8 mm long	Broadly oblong, ovate, or elliptic, 2.5–5 mm long
Capsule	1.7-2.7 cm long	3–8 cm long	2.5–4 cm long

Table 1. Comparison among Carrierea leyensis, C. calycina and C. dunniana.



Figure 4. Carrierea dunniana A flowering branches B inflorescence C staminate flower in frontal view D dissection of staminate flower; Carrierea calycina E flowering branches F inflorescence G pistillate flower in frontal view H pistillate flower in dorsal view I dissection of pistillate flower.

# Identification key to the species of Carrierea

# Acknowledgments

The authors are grateful to Mr. Jin-Quan Huang (IBK) for assistance during the fieldwork, and to Chun-Yu Zou (IBK), who provided the photos of *Carrierea calycina*. We also thank the reviewers and editors of the journal for their constructive comments.

# **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

### Funding

This study was funded by the project on the plants and macrofungi biodiversity investigation in the assessment area of Southwest Karst National Park (Grant no. GZZ2023-22), and also partly supported by scientific research capacity building project for Nonggang Karst Ecosystem Observation and Research Station of Guangxi (Grant no. GK23-026-273).

### Author contributions

Zhao-Cen Lu and Wei-Bin Xu conceived the study. All authors participated in specimen collection. Zhao-Cen Lu, Zhi-Rong Liu, Ming-Lin Mo and Shi-Li Chang conducted the measurements of morphological characters. Zhao-Cen Lu wrote the manuscript. All authors approved the final version of the manuscript.

# Author ORCIDs

Zhao-Cen Lu <sup>©</sup> https://orcid.org/0000-0002-1725-0247 Zhi-Rong Liu <sup>©</sup> https://orcid.org/0009-0001-5171-5693 Ming-Lin Mo <sup>©</sup> https://orcid.org/0009-0000-8895-5474 Shi-Li Chang <sup>©</sup> https://orcid.org/0009-0005-9801-4121 Wei-Bin Xu <sup>©</sup> https://orcid.org/0000-0002-5602-8753

# Data availability

All of the data that support the findings of this study are available in the main text.

# References

Alford MH (2005) Systematic studies in Flacourtiaceae. PhD Thesis, Cornell University, USA.

Chase MW, Zmartzy S, Lledó MD, Wurdack KJ, Swensen SM, Fay MF (2002) When in doubt, put it in Flacourtiaceae: A molecular phylogenetic analysis based on plastid *rbcL* DNA sequences. Kew Bulletin 57(1): 141–181. https://doi.org/10.2307/4110825

- Fan GS (1990) A preliminary study on Flacourtiaceae from China. Journal of Wuhan Botanical Research 8(2): 131–141.
- Fan GS (1991) The distribution and origin of Flacourtiaceae. Journal of Southwest Forestry University 11(2): 125–133.

- Fan GS (1995) Systematic taxonomy of Flacourtiaceae from China. Journal of Southwest Forestry University 15(3): 32–33.
- Franchet AR (1896) *Carrierea* Franch. In: Carriere EA, Andre ED (Eds) Revue Horticole. Journal D'horticulture Practique, 497–499.
- IUCN Standards and Petitions Committee (2022) Guidelines for using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee of the IUCN Species Survival Commission. https://www.iucnredlist.org/ resources/redlistguidelines [accessed on 13 June 2024]
- Lai SK (1999) *Carrierea* Franch. In: Gu CZ (Ed.) Flora Reipublicae Popularis Sinicae. Science Press, Beijing 51(1): 60–63.
- Léveillé AAH (1911) *Carrierea dunniana* H.Lév. In: Fedde F (Ed.) Repertorium Specierum Novarum Regni Vegetabilis, Berlin 9: 458. https://doi.org/10.1002/fedr.19110091603
- Thiers B (Ed.) (2022) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg. org/science/ih/
- Yang QE, Zmarzty S (2007) Carrierea Franch. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China. Science Press, Beijing & Missouri Botanical Garden Press, St Louis 13: 126–127.



**Research Article** 

# *Leucheria peteroana* (Nassauvieae, Asteraceae), a new species of *Leucheria* endemic to the Andes of Central Chile, and insights into the systematics of Nassauviae

Nicolás Lavandero<sup>10</sup>, Fernanda Pérez<sup>10</sup>, Nicolás Pinilla<sup>10</sup>

1 Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Avenida Libertador B. O'Higgins 340, Santiago, Chile Corresponding author: Nicolás Lavandero (nglavand@uc.cl)

### Abstract

A new species, *Leucheria peteroana* **sp. nov.**, endemic to a restricted area of the Andes of Central Chile, is here described. Using newly sequenced nDNA and cpDNA data, the phylogenetic affinities of *Leucheria* and closely related taxa within Nassauvieae are revisited. This new species shows a unique set of characters that clearly distinguish it from other species of *Leucheria*. Phylogenetic analyses place this perennial species close to annual species found in the pre-Andean environments of Central Chile. A detailed description, distribution map, insights about its habitat, conservation status, and photographs are provided.

Key words: Andes, Asteraceae, Laguna Teno, Leucheria, Maule, Nasauvieae, taxonomy

# Introduction

The genus Leucheria Lag. is one of the largest genera within the Nassauvieae tribe. It occurs in the Southern Cone of South America, distributed across Peru, Bolivia, Chile, and Argentina, including the Falkland Islands (Crisci 1976; Katinas et al. 2022). Most species are found within the Patagonian-Andean and Subantarctic Phytogeographic domains (Cabrera and Willink 1973). Leucheria has a rich and complex taxonomic history. For a complete and detailed history of its taxonomy, see Crisci (1976). In the latter work, the first modern revision of the genus, 46 species were recognized. Since Crisci (1976), at least three new species (Katinas et al. 2008a; Katinas et al. 2018; Lavandero et al. 2020) and a new variety (Ratto et al. 2014), which was later elevated to species level (Jara-Arancio et al. 2019), have been described. A recent work by Apodaca et al. (2021) made a significant reclassification, synonymizing 10 annual species of Leucheria into Leucheria tomentosa (Less.) Crisci. In the most recent taxonomic synopsis by Katinas et al. (2022), the number of species of Leucheria was reduced to 29. More recently, Muñoz-Schick and Moreira (2022) included Leucheria graui Katinas, M. C. Tellería, & Crisci within the synonymy of Leucheria apiifolia Phil., further reducing the current number of accepted species of Leucheria to 28.

The diversity center of *Leucheria* overlaps with the Central and Southern Chilean biodiversity hotspot (Moreira-Muñoz et al. 2012), one of 35 world biodiversity



Academic editor: Oscar Vargas Received: 26 July 2024 Accepted: 14 October 2024 Published: 7 November 2024

**Citation:** Lavandero N, Pérez F, Pinilla N (2024) *Leucheria peteroana* (Nassauvieae, Asteraceae), a new species of *Leucheria* endemic to the Andes of Central Chile, and insights into the systematics of Nassauviae. PhytoKeys 248: 315–337. https://doi. org/10.3897/phytokeys.248.133202

**Copyright:** <sup>©</sup> Nicolás Lavandero et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). hotspots recognized by Myers et al. (2000) and later by Mittermeier et al. (2005), due to their great diversity and high levels of endemism, combined with a past and ongoing loss of habitat and biodiversity (Myers et al. 2000). Central Chile features a Mediterranean-type climate, although significant climatic heterogeneity exists due to latitudinal and altitudinal gradients, ranging from sea-level to up to 6570 m (Armesto et al. 2007; Luebert and Pliscoff 2017). The extraordinary environmental heterogeneity in this region, along with fluctuating changes due to the glacial history throughout the Quaternary, may have led to the higher species diversity and endemism observed in this area (Arroyo et al. 1995; Villagrán 1995).

Phylogenetic relationships within Nassauvieae have been the subject of several studies, from Phenetics (Crisci 1974) to Phylogenomics (Zhang et al. 2024). In particular, the relationship of Leucheria to Oxyphyllum and Marticorenia was earlier suggested by Crisci (1980). Hellwig (1985) suggested a close relationship of Leucheria to Moscharia, later guestioned by Katinas (1994). Early molecular studies, based on the ndhF gene of the chloroplast genome, suggested a close relationship to Jungia L.f. (Kim et al. 2002). Subsequently, Panero and Funk (2008), using 10 chloroplast loci, found Leucheria as sister to a clade formed by Nassauvia, Perezia, Acourtia, Dolichlasium, Trixis and Jungia. However, Panero and Funk (2008) only sampled 9 genera within Nassauvieae. A broader sampling was used by Katinas et al. (2008c) where Leucheria is placed as sister to Polyachyrus, and this clade appears as sister to Moscharia, using both nuclear ITS and the chloroplast trnL-trnF intergenic spacer. Luebert et al. (2009) examined the relationships of monotypic Oxyphyllum within Mutisieae. By using the plastid rbcL and ndhF genes, the trnL-trnF spacer and nuclear ITS region, their work supported the close relationship of Oxyphyllum to Leucheria and Polyachyrus. More recently, Jara-Arancio et al. (2017), based on a broader sampling within Nassauvieae, and using the ITS region and two chloroplast intergenic spacers, placed Leucheria as sister to a clade formed by Moscharia and Marticorenia. More recent studies, using target capture methods (Mandel et al. 2019) and phylotranscriptomics (Zhang et al. 2021; Zhang et al. 2024), have only used a limited sampling within the tribe. Until now, generic and infrageneric relationships within Nassauvieae remain unresolved, with at least two genera, Criscia and Cephalopappus, without any genetic information available.

In the context of a modern taxonomic revision of the genus, unusual specimens of *Leucheria* were collected in the vicinity of the Lagunas del Teno (35°11'15"S, 70°33'33"W), in the Andes of the Maule Region of Chile. This area is characterized by a steep topography, with elevations up to 4112 m above sea level (Volcán Azufre). This area of the Andes Mountain range is characterized by active volcanic activity, with six volcanic complexes actively monitored in the present (SERNAGEOMIN 2018). Although several botanical collections have been made around this area, most were done near roads, which are limited to a few Andean Mountain passes and international border crossings between Chile and Argentina.

This work aims to describe a new species of *Leucheria*, providing a distribution map as well as information on its habitat, ecology and phenology. A provisional assessment of its conservation status is also provided. We further investigated its phylogenetic affinities, aiming to re-evaluate the phylogeny of *Leucheria* presented by Jara-Arancio et al. (2017), and the systematics and taxonomy of the most recent taxonomic synopsis of the genus by Katinas et al. (2022).

# Methods

### Herbarium and fieldwork

In March 2019, a botanical exploration was conducted around the Lagunas de Teno (2549 m). Specifically, we collected around the Laguna El Planchón (2549 m a.s.l.), one of the two lakes forming the Lagunas del Teno (Fig. 1). This lake is of glacial origin, created by the natural damming of the glaciers due to the activity of the Volcanic complex Planchón-Peteroa (Caputo et al. 2013). Specimens of *Leucheria* that could not be assigned to any of the currently accepted species were found. Herbarium specimens were collected, together with leaf material preserved in silica gel and capitula preserved in 70% ethanol. Herbarium specimens were deposited at SGO herbarium. A systematic examination of herbarium specimens and keys were prepared after examining all available specimens.

### **Conservation status**

A tentative assessment of the conservation status of the species was made using the International Union for Conservation of Nature (IUCN 2012) categories and criteria, following the most recent guidelines (IUCN Standards and Petitions Committee 2024). The extent of occurrence (EOO) and area of occupancy (AOO) were calculated using GeoCat (Bachman et al. 2011).

### Taxon sampling and phylogenetic analysis

DNA sequences for nDNA (ITS), as well as cpDNA intergenic spacers (*rpl32-trnL* and *trnF-trnL*) were obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank) for the outgroup genera within Mutisioideae and Barnadesioideae, including 25 of the 27 currently accepted genera of Nassauvieae (Katinas et al. 2008b). Due to conflicts in the identification of the herbarium samples used by Jara-Arancio et al. (2017), we decided to redo the phylogenetic analysis. We collected field samples for most of the taxa recognized by Crisci (1976) and subsequently by Katinas et al. (2022), which can be considered a "lumper" perspective of the former. All sequences of *Leucheria*, except for *Leucheria cantillanensis* Lavandero (Lavandero et al. 2020), were generated in the present study.

Total genomic DNA was extracted from silica-dried material collected in the field using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Santiago, Chile) following the manufacturer's instructions. Genomic DNA was used to amplify by PCR one nuclear region, the internal transcribed spacer region (ITS), and the chloroplast intergenic spacers *trnL-trnF* (Taberlet et al. 1991) and *rpl32-trnL* (Shaw et al. 2007). For the ITS region, we used the newly generated primers ITS4\_leu (5' TGATATGCTTAAACTCAGCGGG 3') and ITS5\_leu (5' GGAAGGAGAAGTCGTAA-CAAGG 3'), modified from White et al. (1990). For the *trnL-trnF* region, we used the c and f primers following Taberlet et al. (2007). We amplified all regions of *Leucheria* in 25 µl PCR reactions using the following thermocycling conditions: initial denaturation of 95 °C for 5 min; 35 cycles at 95 °C for 1 min, a specific annealing temperature for 1 min (51 °C for *trnL-trnF* and *rpl32-trnL*; 55 °C for ITS), 72 °C for 1 min; and a final elongation period of 72 °C for 15 min. Sanger sequencing was performed in the Plataformas UC de Secuenciación y Tecnologías Ómicas, Pontificia Universidad Católica de Chile, using the ABI PRISM 3500 xl Genetic Analyzer (Applied Biosystems<sup>™</sup>). GenBank accession numbers for all DNA sequences used in this study are given in Suppl. material 3.



**Figure 1.** Distribution map of *Leucheria peteroana* (white triangles) in Chile, Maule Region, based on the type locality and collections.

The assembled sequences were aligned using the MAFFT v7.450 algorithm (Katoh et al. 2002; Katoh and Standley 2013) in Geneious Prime 2022.2.1 (https://www.geneious.com). Phylogenetic analyses were run for both Maximum-likelihood (ML) (Felsenstein 1981), using RAxML-AVX3 version (Stamatakis 2014) included in RAxMLGUI v.2.0 beta (Silvestro and Michalak 2012; Edler et al. 2020), and Bayesian inference (BI) using MrBayes x64 v3.2.7 (Ronquist et al. 2012), respectively. The best-supported model of nucleotide sequence evolution for each partition was determined based on the Akaike Information Criterion (AIC) using MrModeltest v2 (Nylander 2004). For both partitions, the GTR+I+G model was selected. Before analysing the combined nuclear and chloroplast regions, an Incongruence Length Difference (ILD) Test was performed in PAUP v. 4.0a (Swofford 2003). Phylogenetic reconstruction was performed for each region independently, to compare topological incongruences between the nuclear and chloroplast dataset. For the combined analysis, two partitions were used, corresponding to the nuclear and the chloroplast regions. Maximum likelihood analyses were run using the GTRGAMMA approximation, including the proportion of invariant sites (+I option). The analysis included 1000 ML slow bootstrap replicates with 100 runs. Bayesian analyses were conducted under the respective best fit models for each partition, with two independent runs of 15 million generations each, sampling every 10000 generations. Time series plots and effective sample size (ESS) were analysed using TRACER v.1.7 (Rambaut et al. 2018) to check convergence for each run. The first 3 million generations were discarded as burn-in.

# Results

### Molecular phylogenetic analyses

The total DNA alignment contained 2841 characters (718 ITS, 950 trnL-trnF, and 1173 rpl32-trnL) representing 34 ingroup and 40 outgroup accessions. The incongruence-length difference test showed a significant conflict between the nuclear and chloroplastidial partitions (P < 0.001), so the combined dataset analysis must be interpreted cautiously, considering the topological discordances in some of the clades. In both ML and BI analyses (Suppl. materials 1, 2), there is a consistent incongruence between nuclear and chloroplast data regarding the position of Mutisieae (represented by Mutisia spinosa and Adenocaulon chilense in this work). Nuclear data places this tribe as sister to a clade formed by Onoserideae and Nassauvieae, whereas chloroplast data places this tribe within Nassauvieae, and Onoserideae (represented by Plazia daphnoides and Gypothamnium pinifolium in this work) as sister to this clade. However, all analyses and datasets retrieved Macrachaenium, placed within Nassauvieae by Panero and Funk (2008), as sister to Mutisia. Within Nassauvieae, Spinoliva ilicifolia subsp. ilicifolia appears as sister to the whole clade for the nuclear dataset, whereas its placement is unresolved for the chloroplast dataset. A consistent clade is retrieved by both datasets consisting of *Berylsimpsonia*, *Trixis*, Jungia, Pleocarphus, Ameghinoa, Dolichlasium and Leunisia (PP = 1.0, BS = 100 for ITS; PP = 0.95, BS = 77 for chloroplast). Another clade consistently retrieved (PP = 1.0, BS = 87 for ITS; PP = 0.98, BS = 73 for chloroplast) is formed by Nassauvia, Triptilion, Calopappus, Pamphalea, Perezia, Calorezia, Acourtia, Burkartia, and *Holocheilus*. Within this clade, both datasets consistently retrieved a clade formed by *Acourtia*, *Burkartia* and *Holocheilus* (PP = 1.0, BS = 100 for ITS; PP = 0.96, BS = 88 for chloroplast), and another clade formed by *Nassauvia*, *Triptilion*, *Calopappus*, *Pamphalea*, *Perezia*, and *Calorezia* (PP = 1.0, BS = 99 for ITS; PP = 1.0, BS = 47 for chloroplast). The position of *Holocheilus* differs in the chloroplast ML analysis, as it appears in the latter clade.

In both datasets, a well-supported clade formed by Marticorenia foliosa, Moscharia, Oxyphyllum, Polyachyrus and Leucheria was retrieved (PP = 1.0, BS = 100 for both ITS and Chloroplast). In both datasets, Marticorenia appears as sister to Moscharia (PP = 1.0, BS = 100), and this clade is sister to Oxyphyllum, Polyachyrus and Leucheria. Oxyphyllum appears as sister to Leucheria and Polyachyrus in the nuclear dataset with moderate support (PP = 0.98, BS = 73), whereas in the chloroplast dataset, it appears unresolved. A remarkable finding is that Leucheria, as presented by Jara-Arancio et al. (2017) and Katinas et al. (2022), appears as paraphyletic, as it includes Polyachyrus in both the nuclear and chloroplast datasets. Leucheria appears to be formed by three main clades, differing in many cases to the ones shown in Jara-Arancio et al. (2017). A first clade is formed by acaulescent species of Leucheria, coinciding with the clade "A" of Jara-Arancio et al. (2017). It includes L. eriocephala, L. purpurea, L. achillaeifolia, L. hahnnii, L. leontopodioides, L. millefolium, L. candidissima, L. nutans, L. scrobiculata, L. cantillanensis, and L. salina. This clade is strongly supported on both datasets (PP = 1.0, BS = 100). A second clade is formed by several species of Leucheria, plus Polyachyrus fuscus. It is moderately to strongly supported (PP = 0.99, BS = 64 for ITS; PP = 1.0, BS = 100 for chloroplast), and it is mostly comprised of tall perennials with leaves all along the stems, such as L. bridgesii, L. lithospermifolia, L. rosea, L. garciana, L. gilliesii, L. meladensis, L. viscida, and L. polyclados. This clade also includes annual species formerly recognized by Apodaca et al. (2021) as a single species, Leucheria tomentosa. Phylogenetic analyses show that samples formerly assigned to L. tenuis, L. tomentosa, L. oligocephala, L. glandulosa, L. glabriuscula, and L. cerberoana (currently synonymized with L. tomentosa) belong to five different clades. The nuclear dataset includes in this clade the morphologically distinct L. floribunda, whereas the chloroplast dataset is ambiguous regarding its position. A third clade retrieved with strong support (PP = 1.0, BS = 97 for ITS; PP = 1.0, BS = 94 for chloroplast) is mostly formed by perennials with a basal rosette, such as L. hieracioides, L. integrifolia, L. runcinata, L. gayana, L. amoena, L. coerulescens, and L. glacialis. It also includes two annual species considered by Katinas et al. (2022) as synonyms of L. tomentosa: L. glandulosa and L. glabriuscula. The two latter species form a strongly supported clade (PP = 1.0, BS = 97 for ITS; PP = 0.99, BS = 85 for chloroplast) with the putative new species, L. peteroana. In the combined analysis (Fig. 2), the Marticorenia, Moscharia, Oxyphyllum, Polyachyrus and Leucheria clade is again retrieved, with the same topology for Marticorenia and Moscharia. The clade comprised of Oxyphyllum + Leucheria + Polyachyrus is strongly supported (PP = 1.0, BS = 100), but the clade formed by Leucheria + Polyachyrus has low support (PP = 0.63, BS = 57). The three distinct clades of Leucheria are also retrieved. Finally, the phylogenetic position of L. peteroana is again confirmed as sister to the annual species L. glandulosa and L. glabriuscula with strong support (PP = 1.0, BS = 99).



**Figure 2.** Phylogeny of Nassauvieae resulting from Maximum likelihood analysis of the combined nuclear ITS and plastid *rpl32-trnL* and *trnL-trnF* dataset. For each node, the values of bootstrap support under Maximum likelihood and Bayesian posterior probabilities are to the left and right of the slash, respectively. The new species, *Leucheria peteroana* is highlighted in bold. Ba: Barnadesioideae, On: Onoserideae, Mu: Mutisieae.

### **Taxonomic treatment**

#### Leucheria peteroana Lavandero, sp. nov.

urn:lsid:ipni.org:names:77351414-1 Figs 3, 4

**Diagnosis.** *Leucheria peteroana* is most similar to *Leucheria runcinata* but differs by its simple aboveground stems (vs. branching stems), solely glandular indumentum (vs. lanose and glandular), lack of any type of scent (vs. strongly pungent or fetid odour), completely white corollas on the adaxial side (vs. lilac to blue), pink-purplish anther apical appendages (vs. blue), pink style branches (vs. white styles), and pappus pectines of 250–520 µm long (vs. 130–160 µm) (Figs 5, 6). *Leucheria peteroana* also differs from *Leucheria apiifolia* by its larger height (vs. plants not taller than 30 cm.), two types of glandular trichomes in the vegetative part (vs. only one type of glandular trichome), lack of any type of scent (vs. soft and lemony odour), completely white corollas on the adaxial side (vs. pale gold corollas), outer lip completely extended at full anthesis (vs. outer lip revolute), and pink-purplish anther apical appendages (vs. beige to dark brown) (Fig. 7).

**Type.** CHILE • Región del Maule: Provincia de Curicó, comuna de Romeral. Lagunas de Teno, alrededores de la Laguna Planchón, sector Norte, 35°10'1.06"S, 70°32'37.79"W, 2593 m., 5 March 2019, fl. And fr., *N. Lavandero*, *L. Santilli y C. Ossa* 1873 (holotype: SGO 171859!; Isotype CONC!).

Description. Perennial herb 40-70 cm tall, forming clumps of seasonally persistent annual stems. Rhizome dark brown, round, 25-50 mm wide, bifurcating, oblique to vertical. Roots brown, ca. 1 mm wide. Stems green, erect, fistulose, 2.5-5.0 mm wide, simple, never branching, round, internodes up to 10 cm long, densely covered by two types of trichomes with clear and sticky resin, not fragrant, and without any pungent or noticeable scent when touched or pressed (same indumentum up to the corolla tube): short glandular, capitate, (60–)90–150 µm long, multicellular 8-15-celled trichomes; long glandular, (300-)500-1500(-2300) µm long, multicellular 10-30(-50)-celled trichomes. Leaves green, alternate; basal leaves attenuate, more densely arranged at the base, but not forming a conspicuous rosette; upper leaves sessile, amplexicaul, loosely arranged, gradually reduced in size towards the capitulescence. Lamina oblanceolate, pinnatipartite to pinnatisect, with 6-9 segments per side, almost tripartite towards the apex, (70-)130-180(-190) × (20-)50-60(-65) mm; base attenuate, amplexicaul, apex mucronate; margin serrate, texture coriaceous, densely glandulous on both surfaces; segments at the base 1(-2)-dentate, apex mucronate; segments in the middle 4-7-dentate; apical segments fused, 3-7-dentate; venation prominent on abaxial side, with primary vein ending in apical mucro, secondary veins ending in apical mucro of each segment, and tertiary veins ending in lateral teeth of each segment. Capitulescence a single corymbiform cyme per stem. Capitula 5-9 per stem, pedunculate, homogamous, discoid; pedicels (2-)8-10(-14) cm long. Involucres hemispheric 10.1-11.0 × 14.2-15.2 mm, two-seriate, alternate. Receptacle convex, epaleate (no flowers between bracts), glabrous. Outer involucral bracts (10-12(-14), green, lanceolate, concave on the inner face,  $8.1-9.2 \times 1.8-1.9$  mm, with 3 dark-green longitudinal veins (including the midrib), margin ciliate, apex ciliate, texture coriaceous to hyaline-membranaceous towards the margins, abaxial side densely covered by short and long glandular trichomes, adaxial side glabrous.



Figure 3. *Leucheria peteroana* Lavandero, sp. nov. (Lavandero & Pérez 1504, SGO) **A** habit **B** detail of the capitulum, apical anther appendages and styles **C** capitulum, mid-upper view **D** capitulum, lateral view **E** leaf, abaxial side **F** leaf, detail of adaxial side **G** leaf, detail of abaxial side. All photographs by Nicolás Lavandero.



**Figure 4.** Habit and habitat of *Leucheria peteroana* Lavandero, sp. nov. **A** overview of Laguna El Planchón and the volcanic complex Planchón-Peteroa **B** detail of the plant **C** another plant, growing among rocks **D** detail of plant clump at full sun exposure, but right next to an Andean spring. All photographs by Nicolás Lavandero.


**Figure 5.** Comparison between *Leucheria peteroana* (NL-1873, SGO) (**A**, **C**, **E**) and *Leucheria runcinata* (NL-1867, SGO) (**B**, **D**, **F**) **A**, **B** frontal view of capitula **C**, **D** detail of capitula and flowers **E**, **F** cypselae and detail of pappus bristles. All photographs by Nicolás Lavandero, except E and F (Nicolás Pinilla).



**Figure 6.** Leaf morphology comparison between *Leucheria peteroana* and *Leucheria runcinata*. For each leaf, both adaxial and abaxial sides are shown **A** *Leucheria peteroana*, adaxial side (NL 1873, SGO) **B** *Leucheria peteroana*, abaxial side (NL 1873, SGO) **C** *Leucheria runcinata*, adaxial side (NL-1867, SGO) **D** *Leucheria runcinata*, abaxial side (NL-1867, SGO).



**Figure 7.** Other *Leucheria* species found along the Río Teno Basin in the Maule Region, Central Chile **A** *Leucheria* amoena **B** *Leucheria* apiifolia **C** *Leucheria* candidissima **D** *Leucheria* garciana **E** *Leucheria* glacialis **F** *Leucheria* integrifolia **G** *Leucheria* lithospermifolia **H** *Leucheria* millefolium. All photographs by Nicolás Lavandero, except L. amoena (Joaquín E. Sepúlveda) and *L. candidissima* (Guillermo Debandi).

Inner involucral bracts half the number of outer involucral bracts, (5-)6(-7), green, lanceolate, concave to flat 9.0-9.2 × 1.9-2.2 mm, with one dark-green longitudinal stripe (midrib), apex acute, texture leaf-like to hyaline-membranaceous towards both lateral margins, margin ciliate, central portion of the abaxial side sparsely covered by short glandular trichomes, hyaline lamina glabrous, adaxial side glabrous. Flowers isomorphic, bisexual, (40-)43(-45) per capitulum. Corollas bilabiate, white, sometimes pinkish white on the abaxial side, tube 4.3-4.6 mm long, 1.0-1.1 wide; corolla tube sparsely covered by glandular trichomes. Outer lip oblanceolate, 6.8-7.1 × 3.2-3.5 mm at its widest, apex 3-toothed, teeth equal, 4-veined, glabrous. Inner lip bifid, lacinae linear, 3.4-3.6 × 0.20-0.29 mm at its widest, connivent, glabrous. Stamens 5, 6.2-6.7 mm long, glabrous. Anthers sagittate, 3.0-3.3 mm long; apical appendages pink-purplish, lanceolate, 1.6-1.9 mm long, apex acute; tails long, lanceolate, 1.1-1.2 mm long, apex rounded, smooth. Style pink, 6.5-7.0 mm long, cleft into two truncate branches of pink colour, branches 1.1-1.3 mm long, with stigmatic papillae on internal surface and apical crown papillose. Cypselae dark-brown, 3.5-3.6 × 1.1-1.2 mm, obovoid, strigose; covered by two types of trichomes: glandular biseriate trichomes, 100-130 µm long, and twin trichomes, 230-280 µm long. Pappus uniseriate, fused at their bases into a ring, deciduous; bristles 23-30, white, sub-plumose, 7.4-7.7 mm long; pectines long, filiform, 250–400(–520) µm long, laterally inserted.

**Distribution and habitat.** *Leucheria peteroana* is endemic to the Andes of Central Chile. It is known only from the type locality on the whereabouts of Laguna El Planchón, Maule Region (Fig. 1). It grows at full sun in margins of Andean wetlands or shaded by large boulders and rock walls near 2500 m a.s.l. with SE orientation (Fig. 4). L. peteroana occurs associated with other high Andean plants such as *Grausa lateritia* (Gillies ex Arn.) Weigend & R.H. Acuña, *Calceolaria williamsii* Phil., *Acaena ovalifolia* Ruiz & Pav., *Calceolaria filicaulis* Clos, and *Erythranthe lutea* (L.) G.L. Nesom.

Phenology. Flowering between December and March. Fruiting in March.

**Etymology.** The specific epithet refers to the active volcanic complex Planchón-Peteroa. The Andean Lake where the species occurs lies at the foot of this volcano.

Informal conservation status. Leucheria peteroana can be tentatively considered as Critically Endangered (CR) under the IUCN categories and criteria B2ab(ii,iii). Criterion B2 was selected because its Area of Occupancy is < 10 km<sup>2</sup> (4 km<sup>2</sup>). Criterion "a" was selected because it is known to exist at only a single location, with only two known subpopulations. Criterion b(ii,iii) was selected because we expect a continuing decline of suitable conditions for the species to thrive. There is evidence of a decreasing snow cover extent during the dry season of near 15% per decade in the Andes at mid-latitudes (Cordero et al. 2019). It is also likely that explosive volcanic eruptions of the Planchón-Peteroa complex, close to the only known locality of the species, may wipe out the whole population. These events are relatively common, with at least 20 eruptions documented since 1600 CE, the most recent occurring between September 2018 and April 2019 (Romero et al. 2020). Leucheria peteroana is not present in any known protected area. Although there have not been appropriate efforts to exhaustively locate more populations of L. peteroana in the area, it is likely that these would be subject to the same threats as the already known populations. The extent of occurrence (EOO) could not be calculated since only two populations are known.

Additional specimens examined. CHILE • Región del Maule: Provincia de Curicó, departamento de Curicó. A Orillas de la Laguna Teno. 2500 m. 10 March 1967. *Marticorena & Matthei 892* (CONC!); En los alrededores de la Laguna Teno. 2570 m. *Lavandero & Pérez 1504*. 8 January 2022 (SGO!).

**Notes.** Crisci (1976) and posteriorly Katinas et al. (2022) identified *Marticorena & Matthei 892* as *Leucheria apiifolia*. The differences between these species are notorious (Fig. 7), since the leaf shape and flower colour differ, but the fact that both plants lack lanate indumentum and the dark colour both species acquire once pressed, may have led to this misidentification.

#### Key for the species of Leucheria present in the Río Teno basin

This key aims to cover the diversity of species collected by the authors around this valley. The taxonomy slightly differs from that of Katinas et al. (2022), as it recognizes *Leucheria garciana* as a distinct taxonomic unit from *Leucheria gilliesii*, and *Leucheria millefolium* from *Leucheria purpurea*. These decisions are based on our own ongoing revision of the genus, based on field observations, phylogenetic analyses and herbarium work, rather than solely on the latter, as done by Katinas et al. (2022). Fresh material is often easy to identify. However, herbarium material can be challenging to identify, especially if the belowground structures are incomplete, and the colour of the flower structures are not recorded. Photographs of all species in the field, other than *L. peteroana*, are provided in Fig. 7.

1	Plants acaulescent, not taller than 20 cm, with a long creeping rhizome
	with dark long roots emerging from the internodes2
-	Plants caulescent, up to 170 cm, without a creeping rhizome with dark
	long roots emerging from the internodes, either with a basal leafy rosette
	or leaves distributed along the stem4
2	Leaves with deeply appressed and dense lanate indumentum in both fac-
	es, greyish in appearance <i>L. candidissima</i> (Fig. 7C)
-	Leaf indumentum not as above, green in appearance3
3	Leaves with glandular indumentum only, citric pungent scent when
	crushed. Flowers yellow L. apiifolia (Fig. 7B)
-	Leaves with sparsely lanate indumentum, without any scent. Flowers
	pink <i>L. millefolium</i> (Fig. 7H)
4	Plants with a conspicuous basal rosette or leaves distributed mostly at
-	
-	the basal portion of the stem
_	the basal portion of the stem
- 5	the basal portion of the stem
- 5	the basal portion of the stem
- 5 -	the basal portion of the stem
- 5 -	the basal portion of the stem
- 5 - 6	the basal portion of the stem
- 5 - 6	the basal portion of the stem
- 5 - 6	the basal portion of the stem
- 5 - 6	the basal portion of the stem
- 5 - 6	the basal portion of the stem
- 5 - 6	the basal portion of the stem

- Plants with capitula evenly arranged along the capitulescence. Involucre with glandular and lanose indumentum ......L. integrifolia (Fig. 7F)
- Plants with mostly appressed lanate indumentum in all organs, involucre lanate, flowers white to lilac.
  L lithospermifolia (Fig. 7G)

## Discussion

Chloroplast markers and the nuclear ribosomal cistron regions have been extensively used to infer phylogenetic relationships within Nassauvieae (Katinas et al. 2008c; Jara-Arancio et al. 2017; Jara-Arancio et al. 2018; Sancho et al. 2018; Nicola et al. 2019). However, not all studies documented the topological incongruences between the chloroplast and nuclear ribosomal cistron. Moreover, only a few of these studies explicitly performed tests to evaluate incongruence between nuclear and chloroplast partitions. In the present study, we have confirmed that there are several topological incongruences between these two datasets for Nassauvieae at both generic and infrageneric level. These results suggest that any systematic study and reclassification proposal within Nassauvieae using these datasets alone or combined should be taken into consideration with caution.

In the present study, *Leucheria*, as recognized by Crisci (1976) and more recently by Katinas et al. (2022), appears to be paraphyletic, including the morphologically distinct genus *Polyachyrus*. This result was not shown by Jara-Arancio et al. (2017), as it did not include any sample of *Polyachyrus*. Additionally, the position of *Oxy-phyllum* as sister to *Leucheria* + *Polyachyrus* clade was not retrieved by Jara-Arancio et al. (2017), as it also did not include *Oxyphyllum* in the sampling. Our analyses, including 25 of the 27 accepted genera of Nassauvieae, consistently retrieved a clade comprising *Leucheria*, *Moscharia*, *Marticorenia*, *Oxyphyllum* and *Polyachyrus*.

Within Leucheria, an interesting finding is that our phylogenetic results are more consistent with the taxonomy proposed by Crisci (1976) than the recent proposal by Katinas et al. (2022), which dramatically reduced the number of accepted species of Leucheria. The synonymization of Leucheria millefolium into Leucheria purpurea is not supported by our phylogenetic analyses. Likewise, the synonymization of Leucheria garciana into Leucheria gilliesii is also not supported by the present work. The proposal of lumping 10 species of Leucheria into Leucheria tomentosa by Apodaca et al. (2021), including all the former annual species recognized by Crisci (1976) is here contested. Based on morphology and the revision and comparison of our own field collections with the type specimens, we sampled at least six taxa recognized as annuals by Crisci (1976), and lumped into Leucheria tomentosa by Apodaca et al. (2021). Interestingly, our phylogenetic results indicate that Leucheria tomentosa, as recognized by Katinas et al. (2022), is not monophyletic, suggesting that an important taxonomic work on this group needs to be done, considering both field, herbarium and phylogenetic evidence available (Lavandero et al., in prep.). An unexpected finding was that the new species Leucheria peteroana, is sister to a clade comprised by two annual species found in the Mediterranean

region of Central Chile, *Leucheria glandulosa* and the poorly collected *Leucheria glabriuscula*. Although these three species have little in common morphologically, it is noteworthy that *Leucheria glandulosa* has pink anther appendages, whereas the sister clade comprises species with only blue anther appendages. This character, although rarely recorded on herbarium sheets, is taxonomically important. Likewise, *Leucheria glabriuscula* has a similar pappus, with long pectines (~300–400 µm long), an unusual character within the genus.

The discovery of a new species of *Leucheria*, restricted to a small region of the Andes of Central Chile highlights the importance of more field sampling, even for widely collected genera. Most herbarium collections, including those in Chile, have a significant collection bias (Daru et al. 2017; Hughes et al. 2021), particularly noticeable in areas with harsh topography and lack of roads, as most of the Andes Mountain range. The access to the Lagunas del Teno, and therefore, the finding of *Leucheria peteroana*, was only possible because there is an unpaved road that leads to a dam that controls the water flow from the lake, used for irrigation (Caputo et al. 2013). This area is also known to harbour several rarely collected species, such as *Calceolaria williamsii* Phil. and *Leucheria apiifolia*. As more fieldwork is done in remote areas of the Andes, it is more likely that new species will be found, particularly those with restricted distributions or with very specific requirements, as confirmed by recent additions to the Andean flora of Chile (Villarroel et al. 2021, 2022; Menegoz et al. 2024).

This work highlights the importance of taxonomic revisions that integrate herbarium studies, field collections and ecology, along with a robust phylogenetic framework, especially for diverse and complex genera, such as *Leucheria*. Revisions made solely on herbarium species may leave behind important characters that are only visible when dealing with live plants in the field, such as life form, growth habit, texture, odours and colours that eventually fade once pressed. A fully resolved phylogeny of *Leucheria* is still lacking, and its monophyly has been put to the test by our results. In future works, we will aim to resolve the systematics of Nassauvieae, particularly *Leucheria* and closely related genera, based on low-copy nuclear genes, following a target capture approach (Mandel et al. 2014).

# Acknowledgements

We are grateful to the curators and staff of CONC and SGO herbaria. We would like to thank Ludovica Santilli, for her help during fieldwork and for carefully revising the manuscript. We would like to thank Dr. Nicolás García and one anonymous reviewer for their helpful comments on the submitted manuscript. We thank Gioconda Peralta and Loreto Carrasco of the Plataformas UC de Secuenciación y Tecnologías Ómicas, Pontificia Universidad Católica de Chile for laboratory support and expert capillary electrophoresis analysis. We would like to thank Joaquín E. Sepúlveda and Guillermo Debandi for sharing photos of Leucheria amoena and Leucheria candidissima, respectively.

## Additional information

#### **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

#### Funding

This work was funded by the Agencia Nacional de Investigacion y Desarrollo (ANID) Fondecyt-Chile grant 1211765 and Subdirección de Capital Humano/Doctorado Nacional/2023-21230402.

#### Author contributions

Conceptualization: NL. Formal analysis: NL, NP. Funding acquisition: FP. Methodology: NP, FP, NL. Resources: FP. Supervision: FP. Writing - original draft: FP, NL, NP. Writing - review and editing: FP, NP.

#### Author ORCIDs

Nicolás Lavandero III https://orcid.org/0000-0002-2390-5078 Fernanda Pérez III https://orcid.org/0000-0001-5730-9760 Nicolás Pinilla III https://orcid.org/0000-0002-1494-215X

#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

### References

- Apodaca MJ, Palacio FX, Katinas L (2021) Morphology and morphometry support a single species in the *Leucheria cerberoana* species complex (Asteraceae, Nassauvieae). Phytotaxa 489(1): 27–48. https://doi.org/10.11646/phytotaxa.489.1.3
- Armesto JJ, Arroyo MTK, Hinojosa LF (2007) The Mediterranean environment of central Chile. In: Veblen TT, Young KR, Orme AR (Eds) The physical geography of South America. Oxford University Press, New York, 184–199. https://doi.org/10.1093/ oso/9780195313413.003.0019
- Arroyo MTK, Cavieres L, Marticorena C, Muñoz-Schick M (1995) Convergence in the Mediterranean Floras in Central Chile and California: Insights from Comparative Biogeography. In: Arroyo MTK, Zedler PH, Fox MD (Eds) Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia. Springer, New York, 43–88. https://doi.org/10.1007/978-1-4612-2490-7\_3
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity science. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109
- Cabrera AL, Willink A (1973) Biogeografía de América latina. Secretaría General de la Organización de los Estados Americanos, 120 pp.
- Caputo L, Alfonso G, Givovich A (2013) Limnological features of Laguna Teno (35 S, Chile): A high altitude lake impacted by volcanic activity. Fundamental and Applied Limnology 183(4): 323–335. https://doi.org/10.1127/1863-9135/2013/0551
- Cordero RR, Asencio V, Feron S, Damiani A, Llanillo PJ, Sepulveda E, Jorquera J, Carrasco J, Casassa G (2019) Dry-season snow cover losses in the Andes (18–40 S) driven by changes in large-scale climate modes. Scientific Reports 9(1): 16945. https://doi. org/10.1038/s41598-019-53486-7

- Crisci JV (1974) A numerical-taxonomic study of the subtribe Nassauviinae (Compositae, Mutisieae). Journal of the Arnold Arboretum 55(4): 568–610. https://doi.org/10.5962/p.67292
- Crisci JV (1976) Revisión del género *Leucheria* (Compositae: Mutisieae). Darwiniana 20: 9–126.
- Crisci JV (1980) Evolution in the subtribe Nassauviinae (Compositae, Mutisieae): A phylogenetic reconstruction. Taxon 29(2–3): 213–224. https://doi.org/10.2307/1220283
- Daru BH, Park DS, Primack RB, Willis CG, Barrington DS, Whitfeld TJ, Seidler TG, Sweeney PW, Foster DR, Ellison AM, Davis CC (2017) Widespread sampling biases in herbaria revealed from large-scale digitization. The New Phytologist 217(2): 939–955. https://doi.org/10.1111/nph.14855
- Edler D, Klein J, Antonelli A, Silvestro D (2020) RaxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12: 1–5. https://doi.org/10.1101/800912
- Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17(6): 368–376. https://doi.org/10.1007/ BF01734359
- Hellwig F (1985) Bau der Blütten und Köpfchen von *Moscharia* und verwandten Gattungen der Compositae-Mutisieae. Mitteilungen aus der Botanischen Staatssammlung München 21: 1–47.
- Hughes AC, Orr MC, Ma K, Costello MJ, Waller J, Provoost P, Yang Q, Zhu C, Qiao H (2021) Sampling biases shape our view of the natural world. Ecography 44(9): 1259– 1269. https://doi.org/10.1111/ecog.05926
- IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, UK, [iv +] 32 pp. https://portals.iucn.org/library/ sites/library/files/documents/RL-2001-001-2nd.pdf
- IUCN Standards and Petitions Committee (2024) Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf
- Jara-Arancio P, Vidal PM, Panero JL, Marticorena A, Arancio G, Arroyo MTK (2017) Phylogenetic reconstruction of the South American genus *Leucheria* Lag. (Asteraceae, Nassauvieae) based on nuclear and chloroplast DNA sequences. Plant Systematics and Evolution 303(2): 221–232. https://doi.org/10.1007/s00606-016-1366-7
- Jara-Arancio P, Vidal PM, Arroyo MTK (2018) Phylogenetic reconstruction of the genus *Triptilion* (Asteraceae, Nassauvieae) based on nuclear and chloroplast DNA sequences. Journal of Systematics and Evolution 56(2): 120–128. https://doi.org/10.1111/ jse.12294
- Jara-Arancio P, Ratto F, Bartoli A, Arancio G, Carmona-Ortiz MR (2019) A new species of the genus *Leucheria* (Asteraceae, Nassauvieae) from Argentina. Phytotaxa 404(1): 51–57. https://doi.org/10.11646/phytotaxa.404.1.5
- Katinas L (1994) Un nuevo género de Nassauviinae (Asteraceae, Mutisieae) y sus relaciones cladísticas con los géneros afines de la subtribu. Boletín de la Sociedad Argentina de Botánica 30(1–2): 59–70. https://botanicaargentina.org.ar/wp-content/ uploads/2018/08/59-70011.pdf
- Katinas L, Tellería MC, Crisci, JV (2008a) A new species of *Leucheria* (Asteraceae, Mutisieae) from Chile. Novon: A Journal for Botanical Nomenclature 18(3): 366–369. https://doi.org/10.3417/2006108
- Katinas L, Pruski J, Sancho G, Tellería MC (2008b) The subfamily Mutisioideae (Asteraceae). Botanical Review 74(4): 469–716. https://doi.org/10.1007/s12229-008-9016-6

- Katinas L, Crisci JV, Jabaily RS, Williams C, Walker J, Drew B, Bonifacino JM, Sytsma KJ (2008c) Evolution of secondary heads in Nassauviinae (Asteraceae, Mutisieae). American Journal of Botany 95(2): 229–240. https://doi.org/10.3732/ajb.95.2.229
- Katinas L, Crisci JV, Marticorena A (2018) Una nueva especie de *Leucheria* (Asteraceae), endémica de Chile. Boletín de la Sociedad Argentina de Botánica 53(1): 1–10. https://doi.org/10.31055/1851.2372.v53.n1.19909
- Katinas L, Apodaca MJ, Crisci JV (2022) A synopsis of *Leucheria* (Asteraceae, Nassauvieae), with notes on the morphology. Smithsonian Contributions to Botany, No. 115. Smithsonian Institution Scholarly Press, Washington, D.C. https://doi.org/10.5479/ si.19773379
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14): 3059–3066. https://doi.org/10.1093/nar/gkf436
- Kim HG, Loockerman DJ, Jansen RK (2002) Systematic implications of ndhF sequence variation in the Mutisieae (Asteraceae). Systematic Botany: 598–609. https://doi. org/10.1043/0363-6445-27.3.598
- Lavandero N, Rosende B, Pérez MF (2020) *Leucheria cantillanensis* (Nassauvieae, Asteraceae), a new species endemic to Central Chile. PhytoKeys 169: 99–117. https://doi. org/10.3897/phytokeys.169.57532
- Luebert F, Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile: segunda edición. Editorial universitaria, Santiago-Chile, 381 pp.
- Luebert F, Wen JU, Dillon MO (2009) Systematic placement and biogeographical relationships of the monotypic genera *Gypothamnium* and *Oxyphyllum* (Asteraceae: Mutisioideae) from the Atacama Desert. Botanical Journal of the Linnean Society 159(1): 32–51. https://doi.org/10.1111/j.1095-8339.2008.00926.x
- Mandel JR, Dikow RB, Funk VA, Masalia RR, Staton SE, Kozik A, Michelmore R, Rieseberg L, Burke JM (2014) A target enrichment method for gathering phylogenetic information from hundreds of loci: An example from the Compositae. Applications in Plant Sciences 2(2): 1300085. https://doi.org/10.3732/apps.1300085
- Mandel JR, Dikow RB, Siniscalchi CM, Thapa R, Watson LE, Fun VA (2019) A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. Proceedings of the National Academy of Sciences of the United States of America 116(28): 14083–14088. https://doi. org/10.1073/pnas.1903871116
- Menegoz K, Villarroel AE, Lavandero N (2024) Phylogeny of Berberidopsidales based on nuclear and chloroplast loci, with the description of a new species of *Berberidopsis* endemic to Central Chile. Taxon 73(3): 800–817. https://doi.org/10.1002/tax.13170
- Mittermeier RA, Gil PR, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Da Fonseca GAB (2005) Hotspots revisited: Earth's biologically richest and most threatened ecoregions. CEMEX, Mexico City, 391 pp.
- Moreira-Muñoz A, Morales V, Muñoz-Schick M (2012) Actualización sistemática y distribución geográfica de Mutisioideae (Asteraceae) de Chile. Gayana. Botánica 69(1): 9–29. https://doi.org/10.4067/S0717-66432012000100003
- Muñoz-Schick M, Moreira A (2022) Acerca de la identidad de *Leucheria apiifolia* Phil. (Asteraceae) y la ampliación de la distribución de *Marticorenia foliosa* (Phil.) Crisci (Asteraceae) en la cordillera de Curicó, Chile. Chloris Chilensis. Año 25(1): 96–112.

https://www.chlorischile.cl/25-1-web/Mu%C3%B1oz%20&%20Moreira-Leucheria%20&%20Marticorenia.pdf

- Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi. org/10.1038/35002501
- Nicola MV, Johnson LA, Pozner R (2019) Unraveling patterns and processes of diversification in the South Andean-Patagonian *Nassauvia* subgenus *Strongyloma* (Asteraceae, Nassauvieae). Molecular Phylogenetics and Evolution 136: 164–182. https://doi. org/10.1016/j.ympev.2019.03.004
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Panero JL, Funk VA (2008) The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Molecular Phylogenetics and Evolution 47(2): 757–782. https://doi.org/10.1016/j.ympev.2008.02.011
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032
- Ratto F, Bello M, Bartoli A (2014) Novedades en *Leucheria* (Asteraceae, Mutisieae). Boletín de la Sociedad Argentina de Botánica 49(1): 91–92. https://doi. org/10.31055/1851.2372.v49.n1.7827
- Romero JE, Aguilera F, Delgado F, Guzmán D, Van Eaton AR, Luengo N, Caro J, Bustillos J, Guevara A, Holbik S, Tormey D, Zegarra I (2020) Combining ash analyses with remote sensing to identify juvenile magma involvement and fragmentation mechanisms during the 2018/19 small eruption of Peteroa volcano (Southern Andes). Journal of Volcanology and Geothermal Research 402: 106984. https://doi.org/10.1016/j.jvolgeores.2020.106984
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Sancho G, Katinas L, Viera Barreto JN, Moreira-Muñoz A, Luebert F (2018) Phylogenetic relationships and generic reassessment of *Proustia* and allies (Compositae: Nassauvieae). Taxon 67(1): 113–129. https://doi.org/10.12705/671.7
- SERNAGEOMIN (2018) Chile: Territorio Volcánico. Servicio Nacional de Geología y Minería, Santiago-Chile, 139 pp.
- Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. American Journal of Botany 94(3): 275–288. https://doi.org/10.3732/ajb.94.3.275
- Silvestro D, Michalak I (2012) raxmlGUI: A graphical front-end for RAxML. Organisms, Diversity & Evolution 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0]
- Stamatakis A (2014) Raxml version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033
- Swofford DL (2003) PAUP\* Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4.0a. https://paup.phylosolutions.com/
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17(5): 1105– 1109. https://doi.org/10.1007/BF00037152

- Villagrán C (1995) Quaternary history of the Mediterranean vegetation of Chile. In: Arroyo MTK, Zedler PH, Fox MD (Eds) Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia. Springer, New York, 3–20. https://doi.org/10.1007/978-1-4612-2490-7\_1
- Villarroel AE, Menegoz K, Lavandero N (2021) *Rayenia malalcurensis* (Escalloniaceae), a new genus and species endemic to Central Chile. Phytotaxa 484(1): 96–112. https://doi.org/10.11646/phytotaxa.484.1.4
- Villarroel AE, Menegoz K, Le Quesne C, Moreno-Gonzalez R (2022) Valeriana praecipitis (Caprifoliaceae), a species new to science and endemic to Central Chile. PhytoKeys 189: 81–98. https://doi.org/10.3897/phytokeys.189.73959
- White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols. Academic Press, 315–322. https://doi.org/10.1016/ B978-0-12-372180-8.50042-1
- Zhang C, Huang CH, Liu M, Hu Y, Panero JL, Luebert F, Gao T, Ma H (2021) Phylotranscriptomic insights into Asteraceae diversity, polyploidy, and morphological innovation. Journal of Integrative Plant Biology 63(7): 1273–1293. https://doi.org/10.1111/jipb.13078
- Zhang G, Yang J, Zhang C, Jiao B, Panero JL, Cai J, Zhang ZR, Gao LM, Gao T, Ma H (2024) Nuclear phylogenomics of Asteraceae with increased sampling provides new insights into convergent morphological and molecular evolution. Plant Communications 5(6): 100851. https://doi.org/10.1016/j.xplc.2024.100851

# **Supplementary material 1**

# Phylogenies of Nassauvieae resulting from Maximum likelihood of nuclear ITS, and chloroplast *rpl32-trnL* and *trnL-trnF* datasets

Author: Nicolás Lavandero

Data type: tif

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.133202.suppl1

# Supplementary material 2

# Phylogenies of Nassauvieae resulting from Bayesian analyses of nuclear ITS, and chloroplast *rpl32-trnL* and *trnL-trnF* datasets

Author: Nicolás Lavandero

Data type: tif

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.133202.suppl2

# **Supplementary material 3**

# GenBank accession numbers for the ITS, *rpl32-trnF*, and *trnL-trnF* sequences used in this study

Author: Nicolás Lavandero

Data type: xlsx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/phytokeys.248.133202.suppl3

# PhytoKeys

**Research Article** 

# A taxonomic revision of *Garcinia* sections *Dicrananthera* and *Macrostigma* (Clusiaceae) in Thailand

Chatchai Ngernsaengsaruay<sup>1,20</sup>, Pichet Chanton<sup>30</sup>

1 Department of Botany, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

2 Biodiversity Center, Kasetsart University (BDCKU), Chatuchak, Bangkok 10900, Thailand

3 Suan Luang Rama IX Foundation, Nong Bon Subdistrict, Prawet District, Bangkok, 10250, Thailand

Corresponding author: Chatchai Ngernsaengsaruay (fsciccn@ku.ac.th)

#### Abstract

*Garcinia* sections *Dicrananthera* and *Macrostigma* (Clusiaceae) is revised for Thailand. Three species are enumerated, i.e., one species, *G. thorelii* belongs to the section *Dicrananthera*, and two species, *G. nuntasaenii* and *G. prainiana*, are in the section *Macrostigma*. Detailed morphological descriptions and illustrations are provided, together with notes on distribution, habitats and ecology, phenology, conservation assessments, etymology, vernacular names, uses, and specimens examined. An identification key to the species of section *Macrostigma* is presented. One name, *G. thorelii*, is lectotypified here. For *Garcinia nuntasaenii* we recommend a conservation status of Endangered [EN B2ab(v)] and for the other two species (*G. prainiana* and *G. thorelii*) a conservation status of Least concern [LC]. A number of vegetative characters and features of reproductive organs, especially the flowers, distinguish the two sections and we enumerate these herein.

**Key words:** Dioecy, *Discostigma*, edible fruits, Guttiferae, lectotypification, Malpighiales, taxonomy

# Introduction

*Garcinia* L. is a group of evergreen trees, occasionally shrubs, which are usually dioecious but sometimes polygamo-dioecious. It also has obligately and facultatively agamospermous species. The color of latex secreted from cut boles, twigs, leaves, and fruits can be yellow, pale yellow, white, cream, or clear. The genus consists of c. 400 species (Gaudeul et al. 2024; POWO 2024), and is the largest genus in the Clusiaceae Lindl. (Guttiferae Juss.). It is a pantropically distributed genus and has centers of diversity located in Africa (Madagascar), Australasia, and Southeast Asia (Sweeney and Rogers 2008; Gaudeul et al. 2024). In Asia, *Garcinia* is most diverse in the Malesian region but also spreads north into southern China, west to India, and east to the Micronesian islands (Nazre et al. 2018).

The last monograph of the genus *Garcinia* was published by Engler (1893), who recognized 34 sections. Engler's work was an elaboration of Pierre (1882, 1883), who established the first monograph of *Garcinia* and used mainly flower and inflorescence characters to classify the species into 37 sections. Vesque



Academic editor: Hanno Schaefer Received: 23 June 2024 Accepted: 17 October 2024 Published: 7 November 2024

**Citation:** Ngernsaengsaruay C, Chanton P (2024) A taxonomic revision of *Garcinia* sections *Dicrananthera* and *Macrostigma* (Clusiaceae) in Thailand. PhytoKeys 248: 339–360. https://doi. org/10.3897/phytokeys.248.130311

#### Copyright: ©

Chatchai Ngernsaengsaruay & Pichet Chanton. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). (1893) used floral morphology and leaf anatomy to classify the species into three subgenera and nine sections. A worldwide sectional treatment of *Garcinia* was presented by Jones (1980) in an unpublished Ph.D. thesis in which the genus was classified into 14 sections based largely on floral morphology, especially staminate flowers and pollen morphology. The latest is an updated infrageneric classification of the genus proposed by Gaudeul et al. (2024), who recovered nine major clades falling within two major lineages, and recognized 11 sections.

Gaudeul et al. (2024) resurrected Pierre's (1882: t. 62) *Garcinia* section *Dicrananthera* for a morphologically coherent group of species that was designated the "*G. stipulata*" group in Sweeney et al. (2022). The section contains five species: *G. nujiangensis* C. Y. Wu & Y. H. Li, *G. paucinervis* Chun & F. C. How, *G. stipulata* T. Anderson, *G. thorelii* Pierre, and *G. yaatapsap* K. Armstr. & P. W. Sweeney. Jones (1980) placed *G. stipulata* and *G. thorelii* into section *Discostigma*, creating a new subsection for them based on Pierre's (1883: 8) sectional name *Dicrananthera* [*Garcinia* section *Discostigma* subsection *Dicrananthera* (Pierre) S. W. Jones, *nom. inval.* following Art. 30.9 of the ICN (Turland et al. 2018)]. These species all share prominent stipuliform structures (rare in Clusiaceae, Stevens 2007), leaves with prominent, widely spaced, curved secondary veins and percurrent tertiaries, male flowers with numerous stamens united into an annular mass encircling and attached to the pistillode (in *G. paucinervis* and *G. nujiangensis* the stamens are described as being in four bundles [Chun and How 1956; Li 1981]), and ellipsoid fruits with a discoid stigma and one to two seeds (Sweeney et al. 2022).

Garcinia section Macrostigma includes largely species that were included in Jones (1980) sections Macrostigma, Mungotia, and Tripetalum. This is a heterogenous group and it is difficult to find distinguishing features shared by all of the species in the section. Many species, especially those that were placed into sections Macrostigma and Tripetalum, often have stamen bundles adnate to the petals (Gaudeul et al. 2024). In the phylogeny, this clade includes three species that have been variously placed into other sections by other authors (Lauterbach 1922; Jones 1980): G. hollrungii Lauterb., G. prainiana King, and G. warrenii F. Muell. In addition to molecular data, these species have morphological characters that support their placement in section Macrostigma. This section was recently updated by Gaudeul et al. (2024), who recognized 29 species (e.g., G. nuntasaenii Ngerns. & Suddee, G. phuongmaiensis V. S. Dang, H. Toyama & D. L. A. Tuan). The section is distinguished by its staminate flowers lacking pistillode (usually, but rudimentary or well-developed pistillode present in some species); stamens united into central column (sometimes lobed with lobes equalling number of petals), or into completely separate antepetalous fascicles; androecium often adnate to the petals to varying degrees; two-thecous anthers; (three-) four to eight locular ovaries; unlobed and smooth or divided and papillose stigmas; fruits with smooth walls or faintly to deeply furrowed; and axillary or terminal inflorescences with one to many flowers (Gaudeul et al. 2024).

A taxonomic revision of *Garcinia* in Thailand has recently been undertaken by the first author as part of the *Flora of Thailand* project. More recently, Ngernsaengsaruay and Suddee (2016, 2022) described additional new species: *G. nuntasaenii* from North-Eastern and *G. santisukiana* Ngerns. & Suddee from Eastern Thailand, respectively. Ngernsaengsaruay (2022) recognized three species in *Garcinia* section *Brindonia* (Thouars) Choisy in Thailand: *G. atroviridis* Griff. ex T. Anderson, *G. lanceifolia* Roxb., and *G. pedunculata* Roxb. ex Buch.-Ham. Ngernsaengsaruay et al. (2022a, 2023a) published additional new species records from Peninsular Thailand: *G. dumosa* King and *G. exigua* Nazre, respectively. Ngernsaengsaruay et al. (2022b) described *G. siripatanadilokii* Ngerns., Meeprom, Boonthasak, Chamch. & Sinbumr. as a new species from Peninsular Thailand. *Garcinia* section *Xanthochymus* (Roxb.) Pierre was revised for Thailand with four native species, i.e., *G. dulcis* (Roxb.) Kurz, *G. nervosa* (Miq.) Miq., *G. prainiana*, and *G. xanthochymus* Hook. f. ex T. Anderson (Ngernsaengsaruay et al. 2023b). *Garcinia* section *Garcinia* was treated for Thailand with three species and one variety, i.e., two native species: *G. celebica* L. and *G. exigua* Nazre, and one cultivated species: *G. mangostana* L. var. *mangostana*, including excluded and unplaced species, *G. anomala* Planch. & Triana (Ngernsaengsaruay et al. 2024a). Finally, Ngernsaengsaruay et al. (2024b) published an additional new species record from Peninsular Thailand, *G. minutiflora* Ridl.

From these publications, the genus has a total of c. 30 accepted species in Thailand. However, identifications mostly rely on the literature and the type specimens, and this is the case for *Garcinia* sections *Dicrananthera* and *Macrostigma*, which have never been revised for Thailand. Therefore, in this paper, we provide here an updated account for these two sections in Thailand in order to present a taxonomic treatment that includes lectotypification, detailed morphological descriptions, and illustrations, along with notes on distributions, habitats and ecology, phenology, conservation assessments, etymology, vernacular names, uses, and specimens examined. An identification key to the species of the section *Macrostigma* is presented.

# Materials and methods

The collected specimens were examined by consulting taxonomic literature (e.g., Pierre 1882, 1883; Vesque 1889, 1893; King 1890; Pitard 1910; Ridley 1922; Gagnepain 1943; Corner 1952; Whitmore 1973; Jones 1980; Ngernsaengsaruay and Suddee 2016; Ngernsaengsaruay et al. 2023b), and by comparing with herbarium specimens housed in the following herbaria: AAU, BK, BKF, BM, C, CMUB, K, P, PSU, QBG, and those included in the virtual herbarium databases of AAU (https://www.aubot.dk/search\_form.php), CAL (https://ivh.bsi.gov.in/phanerogams), E (https://data.rbge.org.uk/search/herbarium/), K (including K-W) (http://www.kew.org/herbcat), L (https://bioportal.naturalis.nl/), P (https:// science.mnhn.fr/institution/mnhn/collection/p/item/search/form), and US (https://collections.nmnh.si.edu/search/botany/). All herbaria acronyms follow Thiers (2024). All specimens cited have been seen by the authors unless stated otherwise. The taxonomic history of the species was compiled using the taxonomic literature and online databases (IPNI 2024; POWO 2024). The morphological characters, distribution, habitats and ecology, phenology, and uses were described from historic and newly collected herbarium specimens and the author's observations during field work. The vernacular names were compiled from the specimens examined and the literature (e.g., Pooma and Suddee 2014; Ngernsaengsaruay and Suddee 2016; Ngernsaengsaruay et al. 2023b). Thailand floristic regions follow Flora of Thailand Vol 4(3.3) (The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, 2023). The assessment of conservation status was performed following the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022)

to yield a preliminary assessment of the conservation category in combination with GeoCAT analysis (Bachman et al. 2011) and field information. The calculation of Extent of Occurrence (EOO) and Area of Occupancy (AOO) are based on GeoCAT (https://www.kew.org/science/our-science/projects/geocat-geospatial-conservation-assessment-tool).

# **Results and discussion**

# **Taxonomic treatment**

Sectional classification sensu Gaudeul et al. (2024)

*Garcinia* section *Dicrananthera* Pierre, Fl. Forest. Cochinch. 1(5): 8. 1883; M. Gaudeul et al., PhytoKeys 239: 95. 2024.

Type. Garcinia thorelii Pierre, Fl. Forest. Cochinch. 1(4): t. 62. 1882.

Garcinia section Dicrananthera is distinguished by the presence of a pair of interpetiolar stipuliform structures (rare in Clusiaceae); leaves with prominent, widely spaced secondary veins and scalariform tertiary veins; axillary or terminal cymose inflorescences with three to many flowers [i.e., *G. yaatapsap* (3–5-flowered), *G. paucinervis* (4–10-flowered), *G. stipulata* (4–6-flowered), *G. nujiangensis* (6–10-flowered), and *G. thorelii* (20–40-flowered thyrse)]; flowers with 4 sepals and 4 petals; male flowers with numerous stamens united into an annular mass encircling and attached to the pistillode (i.e., *G. stipulata* and *G. yaatapsap*) or united into 4 bundles surrounding the pistillode (i.e., *G. nujiangensis*, *G. paucinervis*, and *G. thorelii*), 2-thecous anthers; 1–2-locular ovaries; unlobed and smooth stigmas; and fruits with a smooth surface and unlobed. Distinguishing morphological characters reported here for this section were taken from Pierre 1882, Pitard 1910, Chun and How 1956, Jones 1980, Li 1981, Li et al. 2007, Stevens 2007, Gaudeul et al. 2024, and from our observations.

A section of five species worldwide (Gaudeul et al. 2024); one species in Thailand.

*Garcinia thorelii* Pierre, Fl. Forest. Cochinch. 1(4): t. 62. 1882; Vesque, Epharmosis 2: 16. t. 146. fig. 77. 1889 et in A. DC. & C. DC., Monogr. Phan. 8: 367. 1893; Engl. in Engl. & Prantl, Die Naturlichen Pflanzenfamilien 3(6): 236. 1893; Pit. in Lecomte et al., Fl. Indo-Chine 1(4): 301. 1910; Craib, Fl. Siam. 1(1): 118. 1925; Gagnep. in Gagnep., Fl. Indo-Chine Suppl.: 260. 1943; S. W. Jones, Morphology and Major Taxonomy of *Garcinia* (Guttiferae), Ph.D. Thesis (unpublished): 375. fig. 7/13. 1980. Figs 1, 2, 3

**Type.** LAOS • Paklai, fl. & fr., Expedition Mekong 1866–1868, *C. Thorel 3365* (lectotype designated here P [P04701082!]; isolectotypes [P04701076!, P04701080!, P04701081!, P04701083!], K [K000677688!].

**Description.** *Habit* evergreen tree, 8–20 m tall, 50–180 cm GBH; latex yellow, sticky; branches decussate, horizontal or nearly horizontal; branchlets green, 4-angular, glabrous. *Bark* reddish brown or brown, cracked or shallowly fissured;



Figure 1. *Garcinia thorelii* **A** branchlets, leaves, and male inflorescences **B** branchlets and male inflorescences es with male flower buds and fully open male flowers **C** male inflorescences with male flower buds and early stage of open male flower **D** early stage of open male flower **E** fully open male flower showing 4 petals and numerous stamens united into 4 bundles surrounding a pistillode **F** infructescence **G** fruit. Photo: Drawn by Wanwisa Bhuchaisri.

inner bark pale brown. **Terminal bud** concealed between the bases of the uppermost pair of petioles. **Stipuliform structures** 2, interpetiolar, caducous, triangular,  $1.2-2.7 \times 1-2.8$  mm, apex acute. **Leaves** decussate; lamina elliptic, narrowly elliptic or oblanceolate-obovate,  $7.5-18 \times 3-7.5$  cm, apex acuminate or acute, base cuneate or obtuse, margin repand, coriaceous, dark green above, paler below, glabrous on both surfaces, midrib shallowly grooved or flattened above, raised below, secondary veins 5-10 each side, curving towards the margin and connected in distinct loops and united into an intramarginal vein, flattened above, slightly raised below, intersecondary veins usually absent,



**Figure 2**. *Garcinia thorelii* **A** habit and stem **B** slashed bark with yellow latex **C**, **D** branchlets, leaves, and male inflorescences with male flower buds and open male flowers **E** interpetiolar stipuliform structure **F** branchlets and male inflorescences with male flower buds and early stage of open male flower **G** branchlets and male inflorescences with male flower bud and fully open male flowers showing 4 petals and numerous stamens united into 4 bundles surrounding a pistillode **H** branchlets, leaves, and fruit. Photos: Chatchai Ngernsaengsaruay (**A**–**G**), Yotsawate Sirichamorn (**H**).



**Figure 3.** Types of *Garcinia thorelii*, *C. Thorel 3365* from Paklai, Laos, Expedition Mekong 1866–1868 **A** lectotype designated here P [P04701082!] **B** isolectotype P [P04701076!]. Photos: Muséum National d'Histoire Naturelle, Paris, France, http://coldb.mnhn.fr/catalognumber/mnhn/p/p04701082, http://coldb.mnhn.fr/catalognumber/mnhn/p/p04701082, http://coldb.mnhn.fr/catalognumber/mnhn/p/p04701076.

tertiary veins scalariform, veinlets reticulate, visible below, interrupted long wavy lines (glandular wavy lines, also called exudate containing canals) present, of differing lengths, running across the secondary veins to the apex, faint; petiole green, 0.6-1.5 cm long, grooved above, slightly transversely rugose, glabrous, with a basal appendage clasping the branchlet. Inflorescences axillary or at leafless nodes (in axils of fallen leaves), a short thyrse of many flowers, 2-2.5 cm long; bracts caducous, triangular, 0.6-2 mm long, apex acute; peduncle 5-8 mm long, 4-angular; rachis 0.8-2 cm long, 4-angular. Flowers unisexual, plants dioecious, 4-merous, fully open flowers 0.5-1.5 cm in diam.; bracteoles caducous, triangular, 0.8-1.5 mm long, apex acute; pedicels 2-4 mm long; sepals 4 and petals 4, decussate, glabrous; sepals pale green, not concave, triangular, 1-2.5 × 1-2.5 mm, subequal, apex obtuse; petals pale yellow or creamish white, concave, suborbicular, obovate or broadly elliptic, 3-5.5 × 2-4.5 mm, subequal, apex rounded, margin irregularly dentate. Male flowers: stamens numerous united into 4 bundles surrounding a pistillode, bundles 1.3-2.8 × 0.6-2 mm; filaments very short; anthers small; pistillode fungiform (mushroom-shaped), 2-3 mm long; rudimentary ovary slender, cylindrical, 0.5-1.5 mm long; sterile stigma pale yellow, sessile, convex, unlobed, 0.8-1.5 mm in diam., papillate.

**Female flowers:** staminodes absent; pistil fungiform, 2.5–3 mm long; ovary ellipsoid, c. 1.5 mm long, glabrous, 1–2-locular; stigma convex, unlobed, 1.5–2 mm in diam., smooth. **Fruits** berries, ellipsoid or broadly ellipsoid, 1.5–2.5 × 1–2 cm, green, smooth, glabrous, with persistent sepals; persistent stigma convex, unlobed, 2.5–4 mm in diam., smooth; fruiting stalks 2–5 mm long, glabrous. **Seeds** 1, ellipsoid, 1.5–2 × 1–1.2 cm, with a thin fleshy pulp.

Distribution. Vietnam, Laos, and Thailand (Fig. 4).

Distribution in Thailand. Northern: Nan, Lamphun, Lampang, Phrae, Phitsanulok; North-Eastern: Phetchabun, Loei, Bueng Kan, Nakhon Phanom; Eastern: Chaiyaphum, Nakhon Ratchasima; South-Western: Kanchanaburi, Phetchaburi (Fig. 4).



**Figure 4**. Distribution of *Garcinia thorelii*, known from Vietnam, Laos, and Thailand. In Thailand, this species is known to be naturally distributed in the northern, the north-eastern, the eastern, and the south-western regions.

**Habitat and ecology.** It is found in dry evergreen forests, lower montane rain forests, mixed deciduous forests, often along streams, sometimes on lime-stone hills, 50–1,300 m amsl.

**Phenology.** Flowering and fruiting more than once, nearly throughout the year. **Conservation status.** *Garcinia thorelii* is distributed from Vietnam to Laos and Thailand. It is known from many localities and has a large EOO of 338,548.61 km<sup>2</sup> and a small AOO of 128 km<sup>2</sup>. In Thailand, this species is known to be naturally distributed in four floristic regions and 13 provinces and has an EOO of 145,632.54 km<sup>2</sup> and an AOO of 96 km<sup>2</sup>. Because of its number of localities and because it does not face any threat of extinction we therefore suggest the conservation status Least Concern (LC).

**Etymology.** The specific epithet of *Garcinia thorelii* honors Clovis Thorel (1833–1911), a French physician, botanist, and plant collector (Stafleu and Cowan 1986), who collected the type specimen of this species.

**Vernacular names.** Kok (ก็อก) (Phitsanulok, from the specimen A. F. G. Kerr 5816); Khrak khamin (ครากขมีน), **Ma da khi non** (มะดะขีหนอน) (Northern); Mai khwak lueang (ไม้ขวากเหลือง) [Phrae, from the specimen Unknown s.n. (BKF40333)]; Khi non (ขีหนอน) (Laos, from the specimen J. E. Vidal 5952).

**Uses.** The wood is used for house construction [from the specimen *Unknown s.n.* (BKF40333)].

**Lectotypifications.** *Garcinia thorelii* was named by Pierre (1882: t. 62), who cited the specimen *Thorel 3365* collected from Laos, Paklai. He did not choose a holotype nor did he mention the name of the herbarium where the specimen was housed. However, we located five sheets of *C. Thorel 3365* at P [P04701076, P04701080, P04701081, P04701082, P04701083] and one sheet at K [K000677688], and following Art. 9.6 of the ICN (Turland et al. 2018), these are syntypes. The P [P04701082] specimen is the best preserved and is selected here as the lectotype, following Art. 9.3 and 9.12 of the ICN (Turland et al. 2018).

**Notes.** According to Jones 1980, the shape and size of leaves of *Garcinia thorelii* are narrowly elliptic, ovate or obovate and  $18-20 \times 6-9$  cm. However, from our observations, we found the shape and size of leaves of this species can be elliptic, narrowly elliptic or oblanceolate-obovate and sometimes smaller,  $7.5-18 \times 3-7.5$  cm.

According to Jones 1980, male flowers of this species have numerous stamens united into an annular mass around and attached to a pistillode in a narrow ring halfway up. However, from our examinations, the stamens are united into 4 bundles surrounding a pistillode. In the early stage of open flowers, the stamen bundles are attached to a pistillode (not spreading), then spreading in the fully open flowers.

According to Jones 1980, the shape and size of fruits of this species is narrowly ellipsoid and c.  $1.5 \times 0.6$  cm. From our examinations, we found the shape and size of fruits of this species are ellipsoid or broadly ellipsoid and larger,  $1.5-2.5 \times 1-2$  cm.

Additional specimens examined. THAILAND. Northern • Nan [Nam Haeng, young fr., 14 Jul 1926, *Winit 1751* (BKF, K [K003964565])] • Lamphun [along the trail to Tat Moei Waterfall, Doi Khun Tan National Park, Mae Tha District, fl., 29 Jun 1993 (as *Garcinia hanburyi*), *J. F. Maxwell 93-728* (BKF, CMUB, L [L2403744, L2403745]) • Doi Khun Tan National Park, fl., 29 Apr 1994

(as G. hanburyi), J. F. Maxwell 94-562 (BKF, CMUB)] • Lampang [Mae Saloi, fl., 29 Oct 1925, Winit 1492 (BKF, K [K003964566]) • Chae Son National Park, Wang Nuea District, 3 fl., 2 Jun 1996 (as G. hanburyi), J. F. Maxwell 96-792 (BKF, CMUB) • ibid., sterile, 3 Jun 2014 (as G. hanburyi), T. Riythiwigrom 1 (CMUB)] • Phrae [Locality unspecified, fl., 1912, Luang Vanpruk 279 (BKF, K [K003964569]) • Locality unspecified, fl., s.d., Luang Vanpruk 514 (E [E00839797], K [K003964567]) • Huai Ta, Mae Song, fl., 28 May 1912, Luang Vanpruk 303 (BKF, K [K003964568]) . Long District, fl., 12 Mar 1930, Winit 1909 (BKF, K [K003964570]) • Thung Laeng Subdistrict, Long District, fr., 4 Feb 1963 (Garcinia sp.), Unknown s.n. (BKF40333) • Huai Rong Waterfall, fr., 19 Jun 1996 (as Garcinia sp.), R. Pooma & P. Puudjaa 1400-1 (BKF) • Phitsanulok [Nakhon Thai District, fl., 11 Apr 1922, A. F. G. Kerr 5816 (BM, E [E00839798], K [K003964572], P [P04701077]) • Huai Sai, fl., 18 Jun 1967 (as Garcinia sp.), S. Phusomsaeng 241 (BKF, K [K003964575], P [P05062032]) • Political and Military School, Phu Hin Rong Kla National Park, Nakhon Thai District, A fl., 30 Sep 2007, C. Ngernsaengsaruay G56-30092007 (BKF, spirit material)] • Province unspecified [Pa Hia, near Pang Pue, fl., 28 Mar 1914, A. F. G. Kerr 3174 (BM, E [E00160901], K [K003964571])]; North-Eastern • Phetchabun [Khao Paya Paw, fr., 4 Mar 1931, A. F. G. Kerr 20342 (BKF, BM, C, E [E00160902], K) • Nam Nao National Park, fr., 8 Apr 1976 (Garcinia sp.), Bunnak 3108 (BKF) • Wang Pong District, fr., 1 Feb 2001, T. Wongprasert 012-3 (BKF, L [L3811042]) • Huai Phrom Laeng, Nam Nao National Park, fl., 18 Jan 2003 (as Garcinia sp.), P. Chantaranothai et al. 512003 (AAU) • along stream, Nam Nao National Park, fl., 28 May 2013 (as Garcinia sp.), C. Maknoi 5589 (QBG)] · Loei [Phu Luang Wildlife Sanctuary, sterile, 17 May 1998 (as Garcinia sp.), K. Chayamarit et al. 1480 (BKF) • Huai Baeng Forest Protection Station, Phu Luang Wildlife Sanctuary, Wang Saphung District, fr., 22 Jun 2003, T. Wongprasert 036-47 (BKF)] • Bueng Kan [Bueng Khong Long, Kinnari Waterfall, Phu Langka National Park, fr., 20 May 2014, S. Sirimongkol et al. 596 (BKF, K [K003964580], L [L4367330])] • Nakhon Phanom [Tat Kham Waterfall, Phu Langka National Park, fr., 30 Oct 1998, T. Wongprasert s.n. (BKF120854)]; Eastern · Chaiyaphum [Nam Phrom, fr., 10 Dec 1971 (as Garcinia sp.), C. F. van Beusekom et al. 4085 (BKF, C, K [K003964578], P [P05062035]) • Ban Nam Phrom, fr., 24 May 1974 (as Garcinia sp.), R. Geesink et al. 6910 (BKF, K [K003964574], L [L2409515]) • Phu Khiao, young fr., 3 Aug 1972 (Garcinia sp.), K. Larsen et al. 31312 (BK, K [K003964576], L [L2409542]) • Phu Khiao Wildlife Sanctuary, fr., 8 Nov 1984 (as Garcinia sp.), G. Murata T-50251 (BKF) • ibid., fr., 3 May 1997 (as Garcinia sp.), R. Pooma 1539 (BKF, CMUB) • Tabo-Huai Yai Wildlife Sanctuary, fr., 22 Aug 2019 (as Garcinia sp.), N. Boonruang 0350 (QBG)] • Nakhon Ratchasima [Koa Lem, fl. & fr., 12 Jan 1925, A. F. G. Kerr 9978 (BM, E [E00839799], K [K003964573], P [P04701078]), 9979 (BM, P [P04701079]) • Khao Yai National Park, fl. & fr., 9 Apr 1974, T. Smitinand 12001 (BKF)]; South-Western • Kanchanaburi [Erawan Waterfall, fl., 25 Jan 1962 (as Garcinia sp.), K. Larsen & T. Smitinand 9271 (BKF, C, K [K003964577]) • Sai Yok, fr., 26 Nov 1971 (as Garcinia sp.), C. F. van Beusekom et al. 3987 (BK, C, K [K003964579], P [P05062045]) Vajiralongkorn Dam, Tha Khanun Subdistrict, Thong Pha Phum District, fl., 11 Jan 1985 (as Garcinia sp.), H. Koyama T-49019 (BKF)] • Phetchaburi [Mae Kradang La Waterfall, Kaeng Krachan National Park, Nong Ya Plong District, female fl. & fr., Feb 2024, Y. Sirichamorn personal observation with photos].

**VIETNAM** • Tonkin [prov. de Lauyson, fr., 19 Dec 1913 (as *Garcinia* sp.), *Unknown 29663* (P [P04788166]) • Hoa-Binh, fr., 1 Nov 1929 (as *G. bonii*), *M. Brillet unreadable number* (P [P04899809, P04899810])].

LAOS • Luang Phrabang [Locality unspecified, fl., 20 Mar 1932 (as *Garcinia* sp.), *Poilane 20452* (P [P04899801])] • Sayaboury [Mekong River, Na Konken Village, ♂ fl., 28 Apr 2012, *J. F. Maxwell 12-154* (CMUB, L [L4345648])] • Vientiane [Reservoir Nam Ngum, fr., 19 Oct 1974 (*Garcinia* sp.), *J. E. Vidal 5952* (P [P05061722, P05061726])].

# *Garcinia* section *Macrostigma* Pierre, Fl. Forest. Cochinch. 1(6): 63. 1883; M. Gaudeul et al., PhytoKeys 239: 94. 2024.

Type. Garcinia latissima Miq., Ann. Mus. Bot. Lugduno-Batavi 1: 209. 1864.

Garcinia section Macrostigma is characterized by its axillary or terminal cymose inflorescences with two to many flowers (in a simple cyme or in fascicles of several simple cymes), or a solitary flower (in female flowers); 4(-5)-merous flowers [4-merous, e.g., *G. nuntasaenii*; 5-merous, e.g., *G. phuongmaiensis*, *G. prainiana*]; male flowers with numerous stamens united into a central column, sometimes lobed with lobes equaling number of petals [a single weakly 4-lobed bundle, e.g., *G. nuntasaenii*], or into completely separate antepetalous bundles [united into 5 bundles, e.g., *G. phuongmaiensis*, *G. prainiana*]; androecium often adnate to the petals to varying degrees; 2-thecous anthers; pistillode absent in male flowers, but rudimentary or well-developed pistillode present in some species; (-3)4-8-locular ovaries; unlobed or lobed and smooth or papillate stigmas; and fruits with a smooth surface, unlobed or faintly, shallowly or deeply lobed. Distinguishing morphological characters of this section based on Gaudeul et al. 2024, which includes additional information.

A section of 29 species worldwide (Gaudeul et al. 2024); two species in Thailand (i.e., *G. nuntasaenii* and *G. prainiana*).

#### A key to the species of Garcinia section Macrostigma in Thailand

A related species from Vietnam is included in the key.

1. *Garcinia nuntasaenii* Ngerns. & Suddee, Thai Forest Bull., Bot. 44(2): 134. figs 1, 2, 3. 2016; Tagane et al., Edinburgh J. Bot. 75(1): 110. fig. 2G. 2018. Fig. 5

**Type.** THAILAND • Bueng Kan Province, Bung Khla District, Phu Wua Wildlife Sanctuary, fr., 13 Dec 2008, *N. Nuntasaen 10* (holotype BKF!; isotype BKF!).

**Description.** *Habit* shrub, 1–2 m tall; latex white, turning pale yellow, sticky; branches decussate, horizontal or nearly horizontal; branchlets green, 4-angular, glabrous. Bark green when young, turning dark brown when mature, smooth, usually lenticellate; inner bark pale yellow. Terminal bud concealed between the bases of the uppermost pair of petioles. Leaves decussate; lamina lanceolate-ovate, ovate or elliptic, 6-17 × 3-7.5 cm, apex acuminate or acute and rigid, base subcordate, margin thick, entire and slightly undulate (repand), coriaceous, slightly bullate or bullate, apical part of leaves conduplicate, twisted and recurved, glossy dark green above, paler below, glabrous on both surfaces, midrib shallowly grooved above, raised as a prominent ridge below, secondary veins 12-20 each side, curving towards the margin and connected in distinct loops and united into an intramarginal vein, flattened above, slightly raised below, conspicuous on both surfaces, intersecondary veins conspicuous, veinlets reticulate, visible on both surfaces, interrupted long wavy lines present, of differing lengths, nearly parallel to the midrib, running across the secondary veins to the apex, visible below; petiole green, 0.2-1 cm long, not grooved, transversely rugose, glabrous, with a basal appendage clasping the branchlet; young leaves red. Inflorescences terminal or at leafless nodes (in axils of fallen leaves), cymose. Flowers unisexual, plants dioecious, 4-merous, fully open flowers 0.8-1 cm in diam.; bracteoles narrowly triangular or triangular, 2-10 × 1.5-2.5 mm, apex acute or acuminate, somewhat thick; pedicels short; sepals 4 and petals 4, decussate, pale yellow or creamish white, somewhat thick; sepals obovate or elliptic, 3-6 × 2.5-3.5 mm, concave, apex rounded; petals broadly obovate, 5.5-8.5 × 3.5-7 mm, apex rounded, apical part recurved. Flower buds: sepals pink or pink-pale yellow. Male flowers in fascicles of several simple cymes; stamens numerous, united into a single weakly 4-lobed bundle; filaments very short; anthers 0.3-0.5 mm long; pistillode usually absent. Female flowers usually in a cluster of 3 flowers (a simple cyme); staminodes numerous, united into a single weakly 4-lobed bundle, surrounding the ovary; pistil fungiform; ovary depressed globose, 1.5-2 × 2-3 mm, shallowly 4-6-lobed, 4-6-locular; stigma convex, hemispherical,  $1.5-2 \times 2.5-3.5$  mm, papillate. Fruits berries, depressed globose, 0.5–0.7 × 1–2 cm, 4–6-lobed, green with white dots, turning red when ripe, smooth, glabrous, glossy, with persistent sepals; persistent stigma circular, flat, radiately lobed or unlobed; fruiting stalk 2-4 mm. long. Seeds 4-6, with fleshy pulp. The description of this species is based on Ngernsaengsaruay and Suddee 2016, which includes additional information.

**Distribution.** Central Laos and North-Eastern Thailand.

Distribution in Thailand. North-Eastern: Bueng Kan, Nakhon Phanom.

Habitat and ecology. It is found in dry evergreen forests, 150–220 m amsl.

**Phenology.** Flowering and fruiting more than once; flowering in December to July; fruiting in December to April.



**Figure 5.** *Garcinia nuntasaenii* **A** habit and habitat **B** branchlets and young leaves **C** terminal bud concealed between the bases of the uppermost pair of petioles **D** terminal inflorescences with flower buds **E** branchlets and male inflorescences in axils of fallen leaves with male flower buds and open male flowers **F** female flower (top view) **G** branchlets, leaves, and fruit. Photos: Chatchai Ngernsaengsaruay (**A–D, F**), Manop Poopath (**E, G**).

**Conservation status.** *Garcinia nuntasaenii* is known only from four localities in Bueng Kan and Nakhon Phanom Provinces, North-Eastern Thailand and Nam Kading National Protected Area, Laos. All occurrences are in small populations. It has a small EOO of 796.87 km<sup>2</sup> and a relatively small AOO of 16 km<sup>2</sup>. Because of its number of localities, and because the roots of this species are used for medicinal purpose by nearby villagers in Thailand, the population is suspected to be declining. We therefore suggest the conservation status Endangered [EN B2ab(v)].

**Etymology.** The specific epithet of *Garcinia nuntasaenii* honours Mr Narong Nuntasaen, a staff member and a plant collector of BKF, who collected the type specimen.

Vernacular names. Chang nga ek (ช้างงาเอก) (Bueng Kan, Nakhon Phanom).

**Uses.** The fruits are edible (from the specimen *M. Norsaengsri & N. Tathana 8630*). The roots are locally used for medicinal purposes (Ngernsaengsaruay personal observation).

**Note.** *Garcinia nuntasaenii* is similar to *G. phuongmaiensis* in its habit (shrubs); sticky white latex, turning pale yellow when exposed to the air; 4-angular branchlets, especially when young; coriaceous, bullate, shiny dark green, subcordate leaves with a short petiole; flower size, c. 1 cm in diam.; and the color of fruits, turning red when ripe, but differs in having 4-merous flowers [vs (4–)5-merous flowers]; pale yellow or creamish white petals (vs white petals); 4–6-lobed fruits (vs 3–4-lobed fruits); and is also distributed in Central Laos and North-Eastern Thailand (vs distributed in Central Vietnam). The morphological characteristics and distribution of *G. phuongmaiensis* were taken from Tuan et al. (2023).

Additional specimens examined. THAILAND. North-Eastern • Bueng Kan [Phu Wua Wildlife Sanctuary, Bung Khla District, fl., 8 Nov 1996 (*Garcinia* sp.), *C. Niyomdham* 4910 (BKF) • ibid., fl., 27 Aug. 2001 (*Garcinia* sp.), *R. Pooma et al.* 2791 (BKF) • ibid., ♂ fl., 1 Jan 2008 (spirit specimen), *N. Nuntasaen* 11 (BKF) • ibid., fr., 27 Dec 2011 (*Garcinia* sp.), *M. Norsaengsri & N. Tathana* 8630 (BKF, QBG) • ibid., 12 Feb 2015, *M. Poopath* 981-1, 981-2 (BKF) • ibid., ♀ fl., 24 Jul 2015, *C. Ngernsaengsaruay & N. Meeprom* 754 (BKF) • The Upper Northeast Wild Plants Conservation Center, Bung Khla District, ♂ fl., 24 Jul 2015, *C. Ngernsaengsaruay & N. Meeprom* 750, 751 (BKF) • ibid., ♀ fl., 24 Jul 2015, *C. Ngernsaengsaruay & N. Meeprom* 752, 753 (BKF)] • Nakhon Phanom [Ban Phaeng District, Phu Langka National Park (Narong Nuntasaen own observation)].

**LAOS** • Bolikhamxay [Nam Kading National Protected Area, ♂ fl., *L8* • ibid., fl. & fr., *L431* • ibid., *L1034* (Tagane et al. 2018).

2. *Garcinia prainiana* King, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 59(2): 171. 1890; Vesque in A. DC. & C. DC., Monogr. Phan. 8: 329. 1893; Ridl., Fl. Malay Penins. 1: 180. 1922; Corner, Wayside Trees Mal. 1: 320. fig. 112. ed. 2. 1952; Corner & Watan., Ill. Guide Trop. Pl.: t. 193. 1969; Whitmore in Whitmore, Tree Fl. Malaya 2: 220. 1973; I. M. Turner, Gard. Bull. Singapore 47(1): 263. 1995; Ngernsaengsaruay et al., PeerJ, DOI 10.7717/peerj.16572: 28. figs 13, 14, 15. 2023. Fig. 6

**Type.** Peninsular Malaysia • Perak, Kuala Dipang (originally "Kwala Dipang" on the label; originally published "Kwala Dynong"), fl., February 1885, *B. Scortechini* 1796

(lectotype designated by Ngernsaengsaruay et al. 2023b: 28, CAL [CAL0000005844, photo seen]; isolectotypes K [K000677678!], P [P04701324, photo seen]).

**Description.** *Habit* evergreen trees, 3-12 m tall, 15-75 cm GBH; latex white, sticky; branches decussate, horizontal or nearly horizontal; branchlets 4-ridged, glabrous. *Bark* pale brown, grayish brown or blackish brown, smooth or slightly rough; inner bark pale yellow. *Terminal bud* concealed between the bases of the uppermost pair of petioles. *Leaves* decussate; lamina elliptic, oblong or elliptic-oblong, sometimes narrowly oblong,  $12.5-27.5 \times 5.5-11.5$  cm, apex acute or obtuse, base subcordate, often subamplexicaul, margin repand and slightly revolute, coriaceous, bullate or slightly bullate, shiny dark green above, paler below, glabrous on both surfaces, midrib flattened above, raised as a prominent



**Figure 6.** *Garcinia prainiana* **A**, **B** branchlets, young leaves, and mature leaves **C** inflorescences with male flower buds and open male flower **D** branches and branchlets with mature and ripe fruits **E** branchlets, leaves, and mature and ripe fruits **F** ripe fruits (pericarp removed and in transverse section showing seeds with fleshy pulp). Photos: Chatchai Ngernsaengsaruay (**A**–**C**, **E**, **F**), G Rawit Sichaikhan (**D**).

ridge below, secondary veins 9-20 each side, curving towards the margin and connected in distinct loops and united into an intramarginal vein, flattened above, raised below, conspicuous on both surfaces, intersecondary veins conspicuous, veinlets reticulate, visible on both surfaces, interrupted long wavy lines present, of differing lengths, nearly parallel to the midrib, running across the secondary veins to the apex, visible below; petiole green, 1.5-6 mm long, 2-5 mm in diam., not grooved, transversely rugose, glabrous, with a basal appendage clasping the branchlet; young leaves shiny pale green. Inflorescences terminal, sometimes on short, leafless lateral branchlets, cymose, usually in dense fascicles of several to many flowers. Flowers unisexual, plants dioecious, 5-merous, fully open flowers with spreading petals; bracteolate; sepals 5 and petals 5, quincuncial, coriaceous, glabrous. Male flowers 2.5-3.5 cm in diam.; bracteoles pale green, triangular 2.3-4.5 × 1.8-3.7 mm, apex acute, conduplicate with a central keel; pedicel pinkish green, reddish green or greenish red, 3-6 mm long, 2.5-4 mm in diam., widened at the apical part, terete, glabrous; sepals pinkish green, reddish green or greenish red, concave, broadly ovate or suborbicular 4.8-8 × 5-7.8 mm, unequal, apex rounded; petals variable in color: pale yellow, yellowish pink, yellowish red, pinkish red, pink or red, broadly obovate or obovate,  $0.8-1.4 \times 0.6-1.1$  cm, subequal, sometimes unequal, apex rounded; stamens numerous, united into 5 bundles surrounding a pistillode, antepetalous, 1.7-4.2 mm long, each bundle 1.2-4 mm wide, pale yellow, pink or red; filaments fused throughout their entire length; anthers yellow, 0.3-0.6 mm long; pistillode fungiform, 5.5-7.5 mm long; sterile stigma pale yellow, pink or red, sessile, convex, indistinctly lobed, 5-6 mm in diam., papillate. Female flowers 2.5-4 cm in diam.; bracteoles and pedicel same as in male flowers; sepals and petals same as or slightly larger than in male flowers; staminodes absent; pistil fungiform, 6-8.5 mm long; ovary pale green, depressed globose  $4-6 \times 4.5-6.5$  mm, unlobed, glabrous, 5-8-locular; stigma pale yellow, pink or red, sessile, convex, weakly 5-8-lobed or indistinctly lobed, 5-7 mm in diam., papillate. Fruits berries, depressed globose or depressed subglobose, sometimes globose, 2-3.5 × 2-5.3 cm, sometimes oblique, asymmetrical, unlobed, slightly concave or flattened at the apex, green, turning greenish yellow, bright yellow, orangish yellow and bright orange when ripe, smooth, glabrous, glossy, then exocarp becoming dark brownish black and slightly sinuously wrinkled when dry, pericarp 3.5-8 mm thick, exocarp thin; persistent stigma dark brown or blackish brown, circular, button-like, 0.6-1.1 cm in diam., slightly concave or flattened, weakly 5-8-lobed or indistinctly lobed, papillate; persistent sepals pale green, turning yellowish green and orangish green, larger than in flowering material; fruiting stalk green, thick, 0.4-1.5 cm long, 3-6 mm in diam., Seeds 1-6, often aborted, brown, broadly ellipsoid, ellipsoid or subglobose, 0.9-1.6 × 0.7-1.4 cm, with pale orange fleshy pulp. The description of this species was taken from Ngernsaengsaruay et al. 2023b.

**Distribution.** Known only from Peninsular Thailand and Peninsular Malaysia. It is widely distributed in Peninsular Malaysia (Perlis, Kedah, Penang, Perak, Kelantan, Terengganu, Pahang, Selangor, Negeri Sembilan, Malacca, Johor) (Corner 1952; Whitmore 1973; Turner 1995; Azuan and Salma 2018). It can be found mainly in Pahang, Perak, and Negeri Sembilan (Syazwani 2020).

**Distribution in Thailand. Peninsular:** Yala (Than To), Narathiwat (Waeng, Sungai Kolok).

**Habitat and ecology.** It is found in tropical lowland evergreen rain forests, occasionally along streams, 30–200 m amsl. It is also cultivated in villages and botanical gardens.

In Peninsular Malaysia, it occurs in lowland and hill forests, on hillsides and ridges up to elevations of 1,000 m amsl. It is also cultivated in villages (Whitmore 1973; Syazwani 2020; from the specimen *T. C. Whitmore Kep. FRI4018*).

**Phenology.** Flowering and fruiting more than once; flowering nearly throughout the year, usually in February to May; fruiting April to June and September to December.

Conservation status. LC (Ngernsaengsaruay et al. 2023b).

**Etymology.** The specific epithet of *Garcinia prainiana* refers to Sir David Prain (1857–1944), a British botanist, a herbarium curator of the Royal Botanic Garden, Calcutta (1887–1898), and a director of the Royal Botanic Gardens, Kew (1905–1922) (Stafleu and Cowan 1983).

**Vernacular names. Chupu** (จูป) (Malay-Narathiwat); Cerapu, Chekau, Chepu, Cherapu, Cherupu, Chupak, Chupu, Kechupu, Kecupu, Menchepu, Menchupu (Malay); Button mangosteen (English).

**Uses.** Garcinia prainiana is locally cultivated for its fruits in peninsular Thailand. The fruits (pericarp and fleshy pulp surrounding the seeds) are edible and have a sour or sweet-sour taste. It is also grown in some botanical gardens as an ornamental plant to provide botanical education.

In Peninsular Malaysia, it is commonly cultivated in village gardens. The ripe fruits are edible and are sometimes used fresh in beverages (Allen 1965; Burkill et al. 1966). The pulp of fruits has high antioxidant content of about 91.9% and vitamin C content of about 27.3 mg per 100 g fresh weight (Azuan and Salma 2018). In a traditional Malay recipe, the raw fruits are described as being cooked with dried fish (Zawiah and Othaman 2012). The wood is used for house building (Allen 1965; Burkill et al. 1966). It is an excellent ornamental plant for use in landscape gardens in parks (National Parks Flora and Fauna Web 2023).

**Notes.** According to Ngernsaengsaruay et al. (2023b), the male flowers of *Garcinia prainiana* were reported to have a small ring-shaped disk surrounding the base of the pistillode. However, in this study, we re-examined the flowers, and a small ring-shaped disk is absent.

Garcinia prainiana is also similar to *G. phuongmaiensis* in having coriaceous, bullate, shiny dark green, subcordate, subamplexicaul leaves with a short petiole; 5-merous flowers; and numerous stamens, united into 5 antepetalous bundles surrounding a pistillode, but differs in relatively larger habit as a 3-12 m tall tree (vs smaller habit, shrubs, 1-3 m tall); larger leaves,  $12.5-27.5 \times 5.5-11.5$  cm (vs smaller leaves,  $4-11 \times 2.5-5$  cm); larger flowers, 2.5-4 cm in diam. (vs smaller flowers, c. 1 cm in diam.); variable in color of petals: pale yellow, yellowish pink, yellowish red, pinkish red, pink or red (vs white petals); staminodes absent (vs present); unlobed fruits, turning bright yellow, orangish yellow and bright orange when ripe (vs shallowly 3-4-lobed fruits, turning bright red when ripe); seeds with pale orange fleshy pulp (vs seeds with white fleshy pulp); and is distributed in Peninsular Malaysia and Peninsular Thailand (vs Central Vietnam). The morphological characteristics and distribution of *G. phuongmaiensis* were taken from Tuan et al. (2023).

Vesque (1893) placed *Garcinia prainiana* with species of *G*. section *Xantho-chymus* (subgenus *Xanthochymus*), and this placement was followed by Jones

(1980). However, the flowers of *G. prainiana* have a pistillode and lack receptacular disks and antepetalous appendages, unlike those found in other *G.* section *Xanthochymus* species (e.g., *G. dulcis, G. subelliptica* Merr.) or other species in "lineage A". These flowers, with staminal phalanges adnate to the petals, and *G. prainiana*'s branching, adaxial, exudate-containing canal pattern agree with the molecular data and support its placement within a subclade (clade 9) of "lineage B" with which it shares many features (Sweeney 2008). More recently, Gaudeul et al. (2024) reported that in addition to molecular data, *G. prainiana* has morphology that supports its placement into *G. section Macrostigma*.

Additional specimens examined. THAILAND. Central • Nakhon Nayok [Phrueksaphan Thepparat Botanicical Garden, Chulachomklao Royal Military Academy, cultivated, 31 May 2019, C. Ngernsaengsaruay & W. Boonthasak G30-31052019 (BKF); Peninsular • Trang [Khao Chong Botanical Garden, Chong Subdistrict, Na Yong District, cultivated, 16 Feb 2022, C. Ngernsaengsaruay et al. G32-16022022 (BKF)] • Yala [Chulabhorn Phatthana 7 Project, Than To District, near waterfall, 27 Nov 2019, C. Ngernsaengsaruay & G. Sichaikhan G31-27112019 (BKF)] • Narathiwat [Hala-BalaWildlife Sanctuary, Ban Bala, Lo Chut Subdistrict, Waeng District, d fl., 13 May 2005 (as Garcinia sp.), M. Poopath 274 (BKF) • Hala-Bala Wildlife Sanctuary, Waeng District, fr., 22 Sep 2005, C. Niyomdham & P. Puudjaa 7593 (BKF) • Su-ngai Kolok District, fr., 20 Apr 2002, U. Upho 556 (QBG) • Su-ngai Kolok District, fl., cultivated, 20 May 2003, U. Upho 550 (BKF)].

**PENINSULAR MALAYSIA** • Perak [Kwala Dipang,  $\mathcal{J}$  fl., Dec 1896, *C. Curtis 3273* (K [K000677679]) • Kg Kepayang near Ipoh, fr., 30 Oct 1971, *Syed Abu Bakar Kep. FRI20440* (L [L2417220])] • Pahang [Su-ngai Bertam at Kuala Mensum, fl., 2 Jun 1971, *T. C. Whitmore Kep. FRI20091* (L [L2417222]) • Path leading to Kuala Mensum from Boh Tea, Cameron Highlands, fr. 24 Sep 1971, *H. S. Loh Kep. FRI19187* (L [L2417221]) • Cameron Highlands Road, fr., 18 Jan 1982, *K. M. Kochummen Kep. FRI29377* (L [L2417225]) • Kelantan [0.5 mile east of Gua Musang, fr., 14 Jul 1967, *T. C. Whitmore Kep. FRI4018* (L [L2417226]) • Su-ngai Lebir, below Kuala Relai at Jentah, fl., 24 Apr 1976, *B. C. Stone & M. Sidek 12426* (BKF, L [L2417224], US [US02961246]) • Su-ngai Long off Su-ngai Pergau, Jeli, fr., 26 Sep 1986, *A. Latiff et al. ALM1856* (L [L3806490], PSU) • Ketam, Cicar Tinggi, Kampung Bata, Pasir Mas, Q fl. & fr., 1 Aug 1992, *A. Noorsiha et al. Kep. FRI39214* (L [L3878683]) • Pasir Putih, fl., 23 Oct 1992, *H. Husmady et al. Kep. FRI39551* (L [L3806959]) • near Brooke Camp, Gua Musang, fl., 2 Jun 1994, *H. Husmady et al. Kep. FRI41841* (L [L2417223])].

# Acknowledgements

We would like to thank the curators and staff of the following herbaria AAU, BK, BKF, BM, C, CMUB, K, P, PSU, and QBG for their assistance during visits and allowing access to the herbarium specimens, and those included in the digital herbarium databases of AAU, CAL, E, K (including K-W), L, P, and US. We are grateful to the plant collectors of *Garcinia* sections *Dicrananthera* and *Macrostigma*. We also would like to thank Wanwisa Bhuchaisri for the line drawings, Dr Nattanon Meeprom and Weereesa Boonthasak for their kind help with field work, Manop Poopath for two photos of *G. nuntasaenii*, G Rawit Sichaikhan for a photo of *G. prainiana*, and Asst. Prof. Dr Yotsawate Sirichamorn for a photo and information of *G. thorelii* from Kaeng Krachan National Park, Phetchaburi Province.

# **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

#### Ethical statement

No ethical statement was reported.

#### Funding

This research was funded by the Basic Research Fund (BRF) and the International SciKU Branding (ISB), Faculty of Science, Kasetsart University.

#### Author contributions

Conceptualization: CN. Data curation: CN, PC. Formal analysis: CN. Funding acquisition: CN. Investigation: CN. Methodology: CN, PC. Project administration: CN. Resources: CN, PC. Writing - original draft: CN, PC. Writing - review and editing: CN.

## Author ORCIDs

Chatchai Ngernsaengsaruay https://orcid.org/0000-0002-7131-976X Pichet Chanton https://orcid.org/0009-0001-7325-6109

#### Data availability

All of the data that support the findings of this study are available in the main text.

## References

- Allen BM (1965) Malayan Fruits: An Introduction to the Cultivated Species. Eastern Universities Press, Ltd., Singapore.
- Azuan A, Salma I (2018) Fruit for the future: cerapu (*Garcinia prainiana*) [Abstract] in Conference: MARDI Science and Technology Exhibition (MSTE 2008) at Serdang, Selangor.
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity Science. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109
- Burkill IH, Birtwistle W, Foxworthy FW, Scrivenor JB, Watson JG (1966) A Dictionary of the Economic Products of the Malay Peninsula Vol 1 (A–H). Government of Malaysia and Singapore, Ministry of Agriculture and Co-Operatives, Kuala Lumpur, 1063–1074.
- Chun WY, How FC (1956) Species novae arborum utilium Chinae meridionalis. Acta Phytotaxonomica Sinica 5(1): 1–25.
- Corner EJH (1952) Wayside Trees of Malaya. 2<sup>nd</sup> edn. The Government Printing Office, Singapore, 312–320.
- Engler A (1893) Guttiferae. In: Engler HGA, Prantl KAE (Eds) Die Natürlichen Pflanzenfamilien Vol 3(6). Verlag von Wilhelm Engelmann, Leipzig, 194–242.
- Gagnepain F (1943) Guttiféres. In: Humbert H, Gagnepain F (Eds) Supplément a la Flore Générale de L'Indo-Chine Vol. 1(3). Muséum National D'Histoire Naturelle, Phanérogamie, Paris, 254–277.
- Gaudeul M, Sweeney P, Munzinger J (2024) An updated infrageneric classification of the pantropical species-rich genus *Garcinia* L. (Clusiaceae) and some insights into

the systematics of New Caledonian species, based on molecular and morphological evidence. PhytoKeys 239: 73–105. https://doi.org/10.3897/phytokeys.239.112563

- IPNI (2024) International plant names index. The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium. http://www.ipni.org
- IUCN Standards and Petitions Committee (2022) Guidelines for Using the IUCN Red List Categories and Criteria Version 15.1. Standards and Petitions Committee. https:// www.iucnredlist.org/resources/redlistguidelines
- Jones SW (1980) Morphology and Major Taxonomy of *Garcinia* (Guttiferae). PhD Thesis, University of Leicester and British Museum (Natural History), London, 474 pp.
- King G (1890) Materials for a flora of Malay Peninsula. Journal Asiatic Society of Bengal Part 2. Natural History 59(2): 113–206.
- Lauterbach C (1922) Beitrage zur Flora von Paupasien, series IX. 80. Die Guttiferen Paupasiens. Botanische Jahrbücher für Systematik 58(1): 1–49.
- Li YH (1981) Some new species of the genus *Garcinia* from South China. Acta Phytotaxonomica Sinica 19: 490–499.
- Li XW, Li J, Stevens P (2007) *Garcinia* (Clusiaceae). In: Wu CY, Raven PH, Hong DY (Eds) Flora of China Vol 13 (Clusiaceae through Araliaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 40–47.
- National Parks Flora and Fauna Web (2023) *Garcinia prainiana*. National Parks Board. https://www.nparks.gov.sg/florafaunaweb
- Nazre M, Newman MF, Pennington RT, Middleton DJ (2018) Taxonomic revision of *Garcinia* section *Garcinia* (Clusiaceae). Phytotaxa 373(1): 1–52. https://doi.org/10.11646/ phytotaxa.373.1.1
- Ngernsaengsaruay C (2022) Lectotypifications of three names in *Garcinia*, synonymy of *Garcinia pedunculata* and detailed descriptions of three species in *Garcinia* section *Brindonia* (Clusiaceae). Diversity 14(7): 1–29. https://doi.org/10.3390/d14070556
- Ngernsaengsaruay C, Suddee S (2016) *Garcinia nuntasaenii* (Clusiaceae), a new species from Thailand. Thai Forest Bulletin 44(2): 134–139. https://doi.org/10.20531/ TFB.2016.44.2.09
- Ngernsaengsaruay C, Suddee S (2022) *Garcinia santisukiana* (Clusiaceae), a new species from Thailand. Kew Bulletin 77(2): 121–125. https://doi.org/10.1007/s12225-021-09979-8
- Ngernsaengsaruay C, Duangnamon D, Boonthasak W (2022a) *Garcinia dumosa* (Clusiaceae), a new record for Thailand, with associated lectotypifications. Thai Forest Bulletin 50(1): 66–74. https://doi.org/10.20531/tfb.2022.50.1.07
- Ngernsaengsaruay C, Meeprom N, Boonthasak W, Chamchumroon V, Sinbumroong A, Wessapak P, Duangjai S (2022b) *Garcinia siripatanadilokii* (Clusiaceae), a new species from Peninsular Thailand. Kew Bulletin 77(4): 905–913. https://doi.org/10.1007/s12225-022-10059-8
- Ngernsaengsaruay C, Boonthasak W, Meeprom N, Tetsana N, Suddee S (2023a) *Garcinia exigua* (Clusiaceae), a new record for Thailand. Thai Forest Bulletin 51(1): 36–44. https://doi.org/10.20531/tfb.2023.51.1.05
- Ngernsaengsaruay C, Chanton P, Chaiprasongsuk M, Leksungnoen N (2023b) A taxonomic revision of *Garcinia* section *Xanthochymus* (Clusiaceae) in Thailand. PeerJ 11(e16572): 1–54. https://doi.org/10.7717/peerj.16572
- Ngernsaengsaruay C, Chanton P, Leksungnoen N, Chaiprasongsuk M, Thunthawanich R (2024a) A taxonomic revision of *Garcinia* section *Garcinia* (Clusiaceae) in Thailand. PhytoKeys 244: 175–211. https://doi.org/10.3897/phytokeys.244.126207

- Ngernsaengsaruay C, Tetsana N, Suddee S, Boonthasak W, Meeprom N (2024b) *Garcinia minutiflora* (Clusiaceae), a new record from Peninsular Thailand, with lectotypification. Thai Forest Bulletin 52(2): 72–79. https://doi.org/10.20531/tfb.2024.52.2.10
- Pierre JBL (1882) Flore Forestiére de la Cochinchine Vol 1(4). Octave Doin, Éditeur, Paris, t. 49–64.
- Pierre JBL (1883) Flore Forestiére de la Cochinchine Vol. 1(5, 6). Octave Doin, Éditeur, Paris, t. 65–83, 84–96.
- Pitard CJ (1910) Hypéricacées, Guttiféres, Ternstræmiacées et Stachyuracées. In: Lecomte PH (Ed.) Flore Générale de l'Indo-Chine Vol. 1(4). Masson et Cie, Éditeurs, Paris, 289–448.
- Pooma R, Suddee S (Eds) (2014) Tem Smitinand's Thai Plant Names, Revised Edition 2014. Office of the Forest Herbarium, Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Bangkok, 260–261.
- POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/
- Ridley HN (1922) Guttiferae. The Flora of the Malay Peninsula Vol. 1. L. Reeve & Co. Ltd, London, 166–192. https://doi.org/10.5962/bhl.title.10921
- Stafleu FA, Cowan RS (1983) Taxonomic Literature: A Selective Guide to Botanical Publications and Collections with Dates, Commentaries and Types Vol. 4 [P– Sak]. 2<sup>nd</sup> edn., Bohn, Scheltema & Holkema, Utrecht, The Netherlands. https://doi. org/10.5962/t.206493
- Stafleu FA, Cowan RS (1986) Taxonomic Literature: A Selective Guide to Botanical Publications and Collections with Dates, Commentaries and Types Vol. 6 [Author Sti– Vuy]: 2<sup>nd</sup> edn., Bohn, Scheltema & Holkema, Utrecht, The Netherlands. https://doi. org/10.5962/t.206495
- Stevens PF (2007) Clusiaceae-Guttiferae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants Vol 9, Flowering Plants, Eudicots: Berberidopsidales, Buxales, Crossosomatales, Fabales pp, Geraniales, Gunnerales, Myrtales pp, Proteales, Saxifragales, Vitales, Zygophyllales, Clusiaceae alliance, Passifloraceae alliance, Dilleniaceae, Huaceae, Picramniaceae, Sabiaceae. Springer-Verlag, Berlin, 48–66. https:// doi.org/10.1007/978-3-540-32219-1\_10
- Sweeney PW (2008) Phylogeny and floral diversity in the genus *Garcinia* (Clusiaceae) and relatives. International Journal of Plant Sciences 169(9): 1288–1303. https://doi. org/10.1086/591990
- Sweeney PW, Rogers ZS (2008) Nomenclatural notes on Garcinia (Clusiaceae) from Madagascar and the Comoros. Novon: A Journal for Botanical Nomenclature 18(4): 524–537. https://doi.org/10.3417/2006146
- Sweeney PW, Nwe TY, Armstrong KE (2022) *Garcinia yaatapsap* (Clusiaceae), a new species from northern Myanmar. Phytotaxa 545(2): 121–127. https://doi.org/10.11646/phytotaxa.545.2.1
- Syazwani A (2020) Garcinia prainiana King (Clusiaceae). Malaysia Biodiversity Information System (MyBIS). https://www.mybis.gov.my/art/275
- Tagane S, Souladeth P, Rueangruea S, Okabe N, Zhang M, Chayer S, Yang CJ, Yahara T (2018) Flora of Nam Kading National Protected Area II: 30 new records of angiosperms for Laos. Edinburgh Journal of Botany 75(1): 107–116. https://doi. org/10.1017/S0960428617000361
- The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation (2023) Flora of Thailand Vol 4(3.3). Prachachon Co. Ltd., Bangkok.

- Thiers B (2024) [continuously updated] Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/
- Tuan DLA, Toyama H, Nguyen XMA, Phan TTN, Truong VB, Dang VS (2023) Garcinia phuongmaiensis, a new species of Garcinia sect. Xanthochymus (Clusiaceae) from central Vietnam. Kew Bulletin 78(2): 189–195. https://doi.org/10.1007/s12225-023-10077-0
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code) Adopted by the Nineteenth International Botanical Congress Shenzhen, China, 2017. Koeltz Botanical Books, Regnum Vegetabile 159, Glashütten. https:// doi.org/10.12705/Code.2018
- Turner IM (1995) A catalogue of the vascular plants of Malaya. Gardens' Bulletin (Singapore) 47(1): 1–346. https://doi.org/10.26492/gbs71(1).2019-01
- Vesque J (1889) Epharmosis sive materiae ad instruendam anatomiam systematis naturalis Vol. 2 (Gentalia foliaque Garciniearum et Calophyllearum). Vincennes: Delapierre, 1–29 [pl. 1–162]. https://doi.org/10.5962/bhl.title.828
- Vesque J (1893) Guttiferae. In: de Candolle ALPP, de Candolle ACP (Eds) Monographiae Phanerogamarum, Prodromi Nunc Continuatio, Nunc Revisio Vol. 8. Sumptibus G. Masson, Paris, 669 pp.
- Whitmore TC (1973) Guttiferae. In: Whitmore TC (Ed.) Tree Flora of Malaya Vol. 2. Wing Tai Cheung Printing Co. Ltd, Hong Kong, 62–236.
- Zawiah N, Othaman H (2012) 99 Spesies Buah di FRIM. Institut Penyelidikan Perhutanan Malaysia.