

# Morphological diversity of pistil stigmas and its taxonomic significance of representatives of holoparasitic Orobanchaceae from Central Europe

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## Abstract

The stigma is the terminal part of the carpel which receives pollen during the pollination process. Although the role of the stigmas in pollination is the same for all angiosperms, stigmas structures are very diverse. This study aimed to evaluate intraspecific, interspecific and intergeneric stigmas variability and then find differences of the stigma morphology amongst 24 holoparasitic *Orobanche* and *Phelipanche* species and provide new insights into its potential taxonomic value. This paper was also focused on selecting the best diagnostic features that would be used for future stigma analysis in other species of Orobanchaceae. These analyses were conducted with fresh, dry and fixed material using stereomicroscopy from different locations from Central Europe. Twenty-one quantitative or qualitative morphological features were analysed. This study highlights the variation of stigma morphology and characters which are useful to improve the taxonomic understanding of problematic taxa. Thus, two main types of stigmas were established, based on tested features: 1—oval, rarely hemispherical in shape, most often one-coloured with lobes separated in *Phelipanche* stigmas; 2—spherical to hemispherical, rarely oval, multi-coloured with partially fused or separated lobes in *Orobanche* stigmas. The best diagnostic features of the stigmas for distinguishing the Orobanchaceae are the type and subtype of stigma, the length and area of the stigma, the width of single lobes, the width in the middle part of the stigma, the length of upper and lower separation in the middle part between lobes and the angle between lobes in the upper and lower part. The morphological features of the stigmas are important criteria for distinguishing genera, sections and subsections, as well as related species. In this study, we present the first stigma morphological studies for the most numerous genera from the tribe Orobancheae and this paper may determine features possible to use in solving certain taxonomic problems and evolutionary relationships of the species.

**Keywords**

flower morphology, gynoecium, *Orobanche*, *Phelipanche*, taxonomy

**Introduction**

Orobanchaceae is the largest parasitic plant family with 102 genera and over 2,100 species (Nickrent 2020). In this family, the tribe Orobancheae is the oldest and most species-rich lineage of holoparasitic Orobanchaceae (McNeal et al. 2013; Schneider and Moore 2017; Piwowarczyk et al. 2021). Due to the strongly reduced vegetative organs of obligate parasitic plants, they belong to the most critical genera of world flora and cause many difficulties and mistakes in species identification. They do not form vegetative organs such as leaves (reduced to simple scales) and their appearance is limited to generative stems with flowers, highly variable in colour and morphology, so any additional characteristics of systematic value would be helpful.

Recently, studies on the molecular phylogeny and taxonomy of Orobanchaceae have clarified many controversial issues (Piwowarczyk et al. 2017, 2018, 2019, 2021 and references therein). The relationships within several important lineages can also be explained by additional morphological analysis. Previous studies on holoparasitic Orobanchaceae seeds and pollen morphology (e.g. Abu Sbaih and Jury 1994; Abu Sbaih et al. 1994; Plaza et al. 2004; Domina and Colombo 2005; Zare et al. 2013; Piwowarczyk 2015; Piwowarczyk et al. 2015, 2016; Zare and Dönmez 2016; Piwowarczyk et al. 2020 and references therein) and petal micromorphology (Piwowarczyk and Kasińska 2017), as well as floral volatile organic compounds (VOC) (Tóth et al. 2016) have proved to be useful as significant complementary sources of taxonomic data.

The stigma is a part of the gynoecium, the female reproductive system of a flower, with an ephemeral and receptive surface of the style that collects the pollen and creates appropriate conditions for its hydration and germination. These female tissues also promote outcrossing or self-fertilisation by the coordinated timing of their maturation with pollen release (Heslop-Harrison 1981; Edlund et al. 2004). Understanding floral morphology is fundamental to evaluating the interactions between pollen grains and the stigmas, as well as for understanding the relationship between flowers and pollinators. Moreover, many species can only be identified by their floral characteristics (Heslop-Harrison 1992). Angiosperm stigmas are structurally very diverse and some families have stigmas of more than one type. However, stigma morphology can be used, similarly to pollen morphology, to assist taxonomic classifications (Heslop-Harrison and Shivanna 1977). The taxonomic importance and the variability of stigmas have been described in both monocotyledonous and dicotyledonous plants at the inter- and intrageneric level in for example, Bromeliaceae (Brown and Gilmartin 1989), Boragineae (Bigazzi and Selvi 2000), Vochysiaceae (Carmo-Oliveira and Morretes 2009), Convolvulaceae (Wright et al. 2011) and Cactaceae (Mosti et al. 2013).

Amongst the Orobanchaceae studied by Heslop-Harrison and Shivanna (1977), *Orobanche* s.l. presented a dry stigma with unicellular papillae. This group with dry



stigmas was characterised by lack of any surface secretion and the subgroups separate into species, based on the presence of trichomes or papillae. A review of available literature showed a scarcity of data describing the morphological variation of Orobanchae stigmas. Teryokhin et al. (1993) studied the morphological differences of the stigmas for about 50 taxa of the *Orobanche* L. and *Phelipanche* Pomel genera. The stigmas of the tribe Orobancheae show considerable variation, i.e. often subglobose in *Cistanche* Hoffmannsegg & Link and usually discoid to subglobose in *Phelypaea* L. In the *Orobanche* and *Phelipanche* genera, the stigmas are 2–4-lobed with varied shape lobes, for example, elongated, spherical, ovate and/or flattened (Kreutz 1995; Teryokhin 1997; Piwowarczyk et al. 2019). Papers on the morphological descriptions of Orobancheae species have focused mainly on the colour and the degree of fusion of the stigma lobes (e.g. Kreutz 1995; Piwowarczyk et al. 2019; Thorogood and Rumsey 2021). Therefore, studies are necessary on the morphological variation and taxonomic significance of stigmas, especially in the case of systematic division or problematic groups of species. In Orobanchaceae, *Phelipanche* and *Orobanche* s. str. are the largest holoparasitic genera that comprise ca. 50–62 and 150 species, respectively (Piwowarczyk et al. 2019), which are parasitic on the roots of other vascular plants. These genera are characterised by worldwide distribution, especially in the Mediterranean Basin, western and central Asia, north Africa, while less represented in America and Oceania (alien) (Piwowarczyk et al. 2019, 2021). In this paper, we focused on the Central European area, with only five species in *Phelipanche*, in contrast to western and central Asia and the Mediterranean, where the genus *Orobanche* represents the largest genus with about 20–23 representatives (Piwowarczyk et al. 2018).

The present study aimed to evaluate intraspecific, interspecific and intergeneric stigmas variability and then find differences of stigma morphology representatives of the *Orobanche* and *Phelipanche* genera from Central Europe using stereomicroscopy and to provide new insights into its potential taxonomic value. One of the primary objectives was to describe the stigma morphology and identify stigma characters, based on qualitative and quantitative data and to evaluate how useful these characteristics are in systematics and diagnostics for the investigated taxa. This paper also focused on selecting the best diagnostic features that could be used for future stigma analysis in the remaining genera and species of Orobanchaceae.

## Materials and methods

### Plant material

Specimens and samples of stigmas for the present study were observed, collected and photographed by the authors (primarily by Piwowarczyk), mainly during several field trips between 2006 and 2022 in Central Europe (especially Poland, Czech Republic, Slovakia and Austria) and some complementary specimens were also observed from other parts of Europe. A total of 40 samples representing 24 species were analysed (5 *Phelipanche* and 19 *Orobanche*), usually from two different localities per species (Table 1). The study was based on fresh and mature specimens collected in the natural

**Table 1.** Species used in the present study and voucher information.

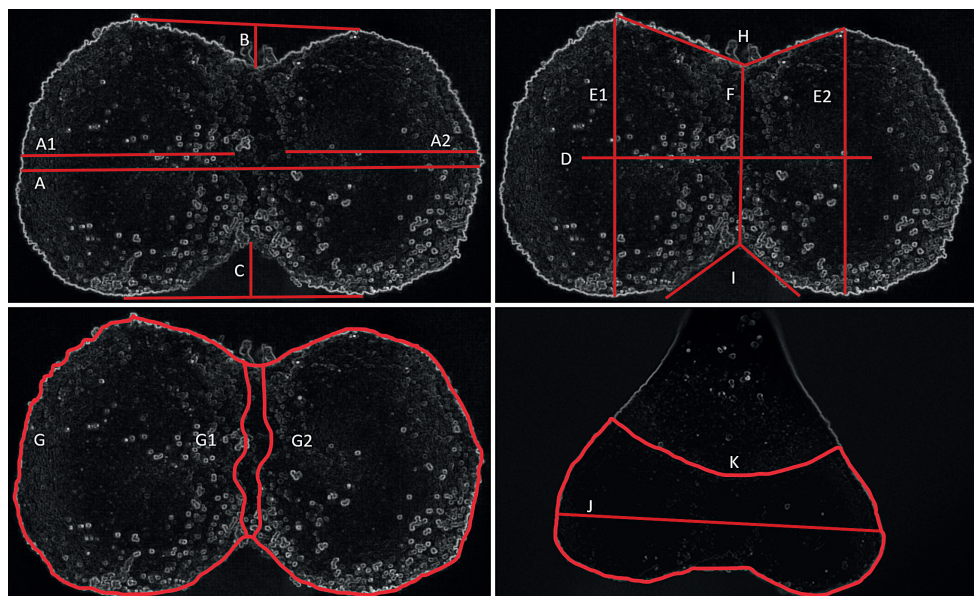
No	Species	Voucher	Host
1a	<i>Orobanchē alba</i> Stephan ex Willd.	Poland, Kąty II, 15 July 2006, <i>R. Piwowarczyk</i> (KTC)	<i>Salvia verticillata</i> L.
1b	<i>O. alba</i>	Poland, Lasocin, 27 July 2006, <i>R. Piwowarczyk</i> (KTC)	<i>S. verticillata</i>
2a	<i>O. alsatica</i> Kirschl.	Poland, Kielce, Grabina Mt., 19 June 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Peucedanum cervaria</i> (L.) Lapeyr.
2b	<i>O. alsatica</i>	Poland, Kąty near Zamość, 15 July 2006, <i>R. Piwowarczyk</i> (KTC)	<i>P. cervaria</i>
3	<i>O. artemisiae-campēstris</i> Gaudin	Czech Republic, Mikulov, 21 June 2014, <i>R. Piwowarczyk</i> (KTC)	<i>Artemisia campestris</i> L.
4a	<i>O. bartlingii</i> Griseb.	Poland, Podzamcze, 22 June 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Seseli libanotis</i> W.D.J.Koch
4b	<i>O. bartlingii</i>	Poland, Częstaków, 30 June 2006, <i>R. Piwowarczyk</i> (KTC)	<i>S. libanotis</i>
5a	<i>O. caryophyllacea</i> Sm.	Poland, Kików, 28 May 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Galium verum</i> L.
5b	<i>O. caryophyllacea</i>	Poland, Łagiewniki, 13 June 2022, <i>R. Piwowarczyk</i> (KTC)	<i>G. mollugo</i> L.
6a	<i>O. coerulescens</i> Stephan in Willd.	Poland, Pasturka, 28 June 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>A. campestris</i>
6b	<i>O. coerulescens</i>	Poland, Dobrowoda, 19 June 2010, <i>R. Piwowarczyk</i> (KTC)	<i>A. campestris</i>
7	<i>O. cumana</i> Wallr.	Ukraine, Kherson, 31 May 2019, <i>R. Piwowarczyk</i> (KTC)	<i>Artemisia</i> sp.
8a	<i>O. elatior</i> Sutton	Poland, Dzierżysław near Kietrz, 11 July 2010, <i>R. Piwowarczyk</i> (KTC)	<i>Centaurea scabiosa</i> L.
8b	<i>O. elatior</i>	Poland, Baldram, 10 July 2010, <i>R. Piwowarczyk</i> (KTC)	<i>C. scabiosa</i>
9a	<i>O. flava</i> Mart. ex F.W. Schultz	Poland, Tatra Mts, Mała Łąka Valley, 25 July 2014, <i>R. Piwowarczyk</i> (KTC)	<i>Petasites kablíkianus</i> Tausch ex Bercht.
9b	<i>O. flava</i>	Slovakia, Nizkie Tatry Mts, Ohniste, 4 August 2011, <i>R. Piwowarczyk</i> (KTC)	<i>P. kablíkianus</i>
10	<i>O. gracilis</i> Sm.	Austria, Hundsheim, 21 June 2014, <i>R. Piwowarczyk</i> (KTC)	<i>Anthyllis vulneraria</i> L., <i>Doryenium pentaphyllum</i> subsp. <i>germanicum</i> (Gremli) Gams
11	<i>O. hederæ</i> Duby	Spain, Elx, palm garden, 28 April 2009, <i>R. Piwowarczyk</i> (KTC)	<i>Hedera helix</i> L.
12a	<i>O. kochii</i> F.W. Schultz (= <i>O. centaureina</i> Bertol.)	Poland, Boria, 4 July 2021, <i>R. Piwowarczyk</i> (KTC)	<i>C. scabiosa</i>
12b	<i>O. kochii</i>	Poland, Pęczelice, Ostra Mt., 7 July 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>C. scabiosa</i>
13	<i>O. lucorum</i> A. Braun ex F.W. Schultz	Poland, Warsaw, Botanical Garden, 10 July 2009, <i>R. Piwowarczyk</i> (KTC)	<i>Berberis vulgaris</i> L.
14a	<i>O. lutea</i> Baumg.	Poland, Pęczelice, 28 May 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Medicago falcata</i> L.
14b	<i>O. lutea</i>	Poland, Żąbkowice, 29 May 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>M. sativa</i> L.
15a	<i>O. mayeri</i> (Suess. & Ronniger) Bertsch & F. Bertsch	Poland, Pieniny Mts, Trzy Korony, 30 July 2009, <i>R. Piwowarczyk</i> (KTC)	<i>Laserpitium latifolium</i> L.
15b	<i>O. mayeri</i>	Poland, Pieniny Mts, Białe Skalki, 29 July 2009, <i>R. Piwowarczyk</i> (KTC)	<i>L. latifolium</i>
16	<i>O. minor</i> Sm.	Poland, Żywiec, 19 July 2009, <i>R. Piwowarczyk</i> (KTC)	<i>Trifolium repens</i> L.
17a	<i>O. picridis</i> F. W. Schultz	Poland, Pińczów, 28 June 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Picris hieracioides</i> L.
17b	<i>O. picridis</i>	Poland, Pęczelice, Ostra Mt., 7 July 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>P. hieracioides</i>
18a	<i>O. reticulata</i> Wallr.	Lubiatowo, June 2014, <i>R. Piwowarczyk</i> (KTC)	<i>Cirsium arvense</i> (L.) Scop.
18b	<i>O. reticulata</i>	Slovakia, Nizkie Tatry Mts, Ohiste, 4 August 2011, <i>R. Piwowarczyk</i> (KTC)	<i>Carduus defloratus</i> L.
19	<i>O. teucris</i> Holandre	Austria, Hundsheim, 20 June 2014, <i>R. Piwowarczyk</i> (KTC)	<i>Teucrium montanum</i> L.
20a	<i>Phelipanche arenaria</i> (Borkh.) Pomel	Czech Republic, Mikulov, 21 June 2014, <i>R. Piwowarczyk</i> (KTC)	<i>A. campestris</i>
20b	<i>P. arenaria</i>	Poland, Młyny, 28 June 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>A. campestris</i>
20c	<i>P. arenaria</i>	Poland, Pasturka, 28 June 2022, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>A. campestris</i>
20d	<i>P. arenaria</i>	Poland, Zwierzyniec, 29 June 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>A. campestris</i>
21	<i>P. bohémica</i> (Čelak.) Holub	Poland, Zawiercie, 11 July 2010, <i>R. Piwowarczyk</i> (KTC)	<i>A. campestris</i>
22	<i>P. caesia</i> (Rchb.) Soják	Ukraine, Askania Nova, 16 June 2011, <i>R. Piwowarczyk</i> (KTC)	<i>A. austriaca</i> Jacq.
23	<i>P. purpurea</i> (Jacq.) Soják	Poland, Chrzanów, 18 June 2009, <i>R. Piwowarczyk</i> (KTC)	<i>Achillea millefolium</i> L.
24a	<i>P. ramosa</i> (L.) Pomel	Poland, Brzeziny, 4 September 2021, <i>R. Piwowarczyk &amp; K. Ruraż</i> (KTC)	<i>Nicotiana tabacum</i> L.
24b	<i>P. ramosa</i>	Poland, Szewce, 15 September 2013, <i>R. Piwowarczyk</i> (KTC)	<i>Solanum lycopersicum</i> L.

habitats and on dry herbarium specimens where mature stigmas were selected from herbarium collections, as well as on plant material which was fixed in FAA (Formalin-Aceto-Alcohol) solution. The fresh specimens were kept in the refrigerator, observed and recorded quickly to avoid dehydration within tens of minutes. Dried flowers, removed from herbarium specimens, were heated to boiling point and left to observe after several minutes. For the purpose of comparison and to eliminate variation that might be caused by sampling from different flower areas, we took mature stigmas close to the middle portion of an inflorescence. In addition, our observations and measurements of stigmas were also compared with numerous photographs of analysed species, as well as with problematic sections, subsections and/or series of species from other parts of Europe and Asia. Our samples have also been presented in recent molecular phylogenetic studies (Piwowarczyk et al. 2018, 2021). The plant names were updated, based on the World Flora Online (WFO) (2022), as well as the Index of Orobanchaceae (Sánchez Pedraja et al. 2016). Vouchers of plant material were deposited in the Herbarium (KTC) of the Institute of Biology, Jan Kochanowski University in Kielce (KTC acronym, according to Thiers 2017). Voucher information and geographic origin are included in Table 1. The terminology of stigma morphology was given according to Heslop-Harrison and Shivanna (1977), Heslop-Harrison (1992), Teryokhin et al. (1993), Kreutz (1995), Teryokhin (1997), Williams (2009), Wright et al. (2011) and Konarska and Chmielewski (2020). Systematic division was adopted according to Beck (1930) and Teryokhin et al. (1993), the scheme followed, explicitly or implicitly, by most researchers and some recent taxonomic changes (McNeill et al. 2012; Piwowarczyk et al. 2017, 2018, 2021).

### Morphometric analysis

Twenty-one quantitative and qualitative morphological features were measured. Sixteen quantitative features were analysed in the bottom view, i.e. the length of 2-lobed stigma (typical) (A) ( $\mu\text{m}$ ), the length of single lobes (A1, A2) ( $\mu\text{m}$ ), the length of upper separation in the middle part between lobes (B) ( $\mu\text{m}$ ), the length of lower separation in the middle part between lobes (C) ( $\mu\text{m}$ ), the length of the mouth of the stylar canal (slit) (D) ( $\mu\text{m}$ ), the width of single lobes (E1, E2) ( $\mu\text{m}$ ), the width in the middle part of the stigma (F) ( $\mu\text{m}$ ), the area of 2-lobed stigma (G) ( $\mu\text{m}^2$ ), the area of single lobes (G1, G2) ( $\mu\text{m}^2$ ), the angle between 2-lobed stigma in the upper part (H) ( $^\circ$ ) and the angle between 2-lobed stigma in the lower part (I) ( $^\circ$ ). In the front view, two morphological features were examined, i.e. the length of 2-lobed stigma (J) ( $\mu\text{m}$ ) and the area of 2-lobed stigma ( $\mu\text{m}^2$ ) (K) (Fig. 1). Additionally, five qualitative features, namely type, subtype, shape (in bottom view), colour and the degree of stigma lobes separation were taken into account for the morphological analysis of the stigmas.

Morphological observations of the stigmas were carried out using a Nikon SMZ-800 stereoscopic microscope coupled with a NIKON DSFi3 camera (Tokyo, Japan). Measurements were made using AxioVision SE64 Rel. 4.9.1 software (Carl



**Figure 1.** Measurement scheme of a 2-lobed stigma of *Orobanchaceae*. **A–I** bottom view **J, K** front view **A** the length of 2-lobed stigma ( $\mu\text{m}$ ) **A1, A2** the length of single lobes ( $\mu\text{m}$ ) **B** the length of upper separation in the middle part between lobes ( $\mu\text{m}$ ) **C** the length of lower separation in the middle part between lobes ( $\mu\text{m}$ ) **D** the length of the mouth of the stylar canal (slit) ( $\mu\text{m}$ ) **E1, E2** the width of single lobes ( $\mu\text{m}$ ) **F** the width in the middle part of the stigma ( $\mu\text{m}$ ) **G** the area of 2-lobed stigma ( $\mu\text{m}^2$ ) **G1, G2** the area of single lobes ( $\mu\text{m}^2$ ) **H** the angle between 2-lobed stigma in the upper part ( $^\circ$ ) **I** the angle between 2-lobed stigma in the lower part ( $^\circ$ ) **J** the length of 2-lobed stigma ( $\mu\text{m}$ ) **K** the area of 2-lobed stigma ( $\mu\text{m}^2$ ).

Zeiss, Germany). For morphological characterisation, 30–50 stigmas of mature flowers from 5–10 randomly selected individuals of each sample of species were used. Data analyses were performed using Statistica 13 (TIBCO Software Inc. 2017). Eleven quantitative and two qualitative characters of stigmas were analysed using UPGMA, i.e. the length of 2-lobed stigma (typical) (A) ( $\mu\text{m}$ ), the length of upper separation in the middle part between lobes (B) ( $\mu\text{m}$ ), the length of lower separation in the middle part between lobes (C) ( $\mu\text{m}$ ), the width of single lobes (E1, E2) ( $\mu\text{m}$ ), the width in the middle part of the stigma (F) ( $\mu\text{m}$ ), the area of 2-lobed stigma (G) ( $\mu\text{m}^2$ ), the angle between 2-lobed stigma in the upper part (H) ( $^\circ$ ), the angle between 2-lobed stigma in the lower part (I) ( $^\circ$ ), the length of 2-lobed stigma (J) ( $\mu\text{m}$ ) and the area of 2-lobed stigma ( $\mu\text{m}^2$ ) (K), as well as the type and subtype of the stigma. These features were chosen because they showed the differences and similarities between species. A dendrogram was prepared, based on the similarity matrix generated using Gower's general similarity coefficient (Gower 1966). Both analyses were performed using the MVSP package version 3.1 (Kovach 1999).

## Results

### General characteristics of stigma

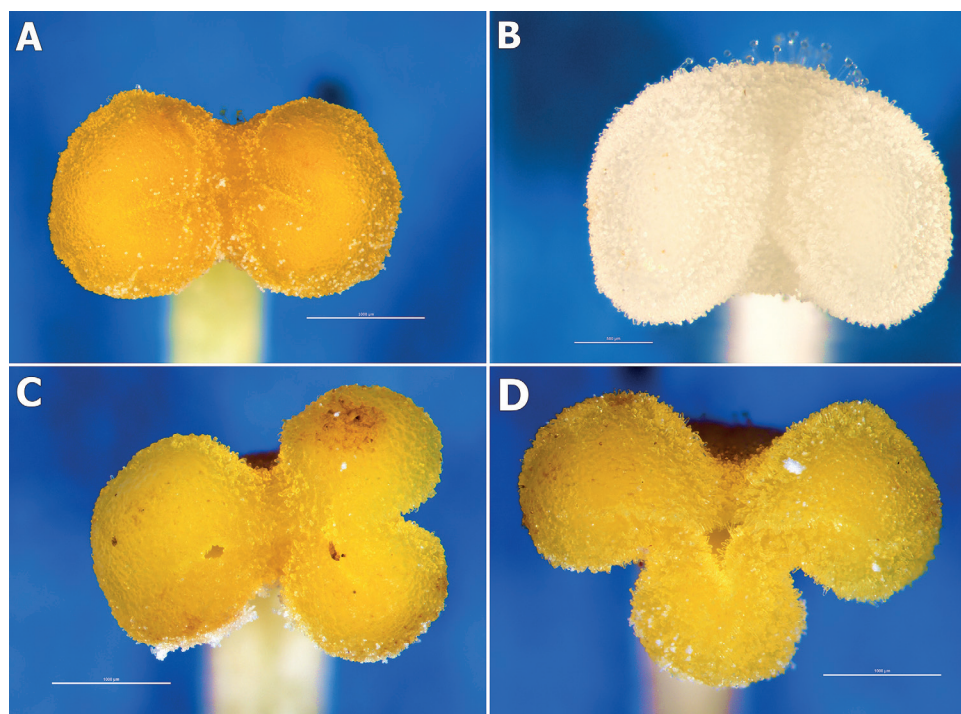
Morphological characterisation of the stigmas of *Orobanche* and *Phelipanche* has provided important data for the taxonomy of Orobanchaceae. The study showed some morphological similarities in stigma characters in both genera, for example, stigma usually 2-lobed, occasionally 3- and 4-lobed, lobes spherical to ovulate in shape (Fig. 2). The third lobe may be centrally located between the two lobes or directly under one of the lobes (Figs 2C, D). The centrally narrowed stigma was bent towards the lower lip and in the middle part was described by the presence of the mouth of the stylar canal. The stigma was covered with numerous papillae concentrated on the coloured lobes that were regularly arranged. The bow-shaped stigma had a viscous substance on the surface at the receptive stage. In buds, the length of the mouth of the stylar canal (slit) was more visible because it did not contain this substance.

### *Orobanche*

The stigmas of *Orobanche* were hemispherical, spherical, rounded, rarely oval. These stigmas were varied in colour from white, yellow, orange, pink, purple, red to dark brown with partially fused or separated lobes (Table 2, Figs 2A, 3A–P, 4A–F). In the field research, two forms were observed in *Orobanche*, euchrome (normal colour) and hypochromic (yellow), which were easy to distinguish. We observed that the hypochromic form was smaller than the euchromic in each feature tested.

The stigmas of *O. alsatica*, *O. bartlingii*, *O. elatior*, *O. flava*, *O. kochii*, *O. lucorum* and *O. mayeri* belonging to the subsect. *Curvatae* (Beck) Piwow., Ó. Sánchez & Moreno Mor. consisted of two usually yellow lobes (in *O. flava* also orange lobes) which were hemispherical, rarely oval in shape and were separated (Table 2, Figs 3C–H, 4A, B, D). The UPGMA analysis on the basis of given features distinguished three subgroups: the first one included *O. alsatica* and *O. bartlingii*, the second was represented by *O. elatior* and *O. kochii* and the third by *O. flava*, *O. lucorum* and *O. mayeri* (Fig. 5). The analysed samples of *O. alsatica* and *O. bartlingii* were in a very close relationship and represented *O. alsatica* aggr. which is a problematic complex. In *O. bartlingii*, the shape of the mouth of the stylar canal (slit) was more irregular than *O. alsatica*. A visible difference in shape between species was marked between the lobes in the lower part and there was a larger separation in *O. bartlingii*. The stigmas in the front view in *O. bartlingii* were more pronounced and marked than in *O. alsatica*, which appear more flattened (Figs 4A1, B1). The length of 2-lobed stigma (A, J) of the first subgroup fell within a range of (2770–)3281(–3525)  $\mu\text{m}$  and (2497–)3176(–3743)  $\mu\text{m}$ , with an area (G, K) varying between (2949725–)4296538(–5803293)  $\mu\text{m}^2$  and (2003320–)3118193(–3854744)  $\mu\text{m}^2$ . The width of single lobes (E1, E2) fell into a range of (1518–)1750(–1985)  $\mu\text{m}$  and (1425–)1778(–2128)  $\mu\text{m}$ , while the width in the middle part of the stigma (F) varied within a range of (625–)973(–1227)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C)





**Figure 2.** Micrographs of typical 2-lobed stigmas of *Orobanche* and *Phelipanche* and occasional 3-lobed stigmas of *Orobanche*. **A** *Orobanche* **B** *Phelipanche* **C, D** 3-lobed stigmas of *Orobanche*. Scale bars: 1000  $\mu\text{m}$  (**A, C, D**); 500  $\mu\text{m}$  (**B**). Phot. K. Ruraż, J. Pośłowska and K. Zubek.

was in a range of (150–)348(–580)  $\mu\text{m}$  and (380–)561(–935)  $\mu\text{m}$  and the angle between 2-lobed stigma in the upper and lower part (H, I), comprised (95–)120(–155)° and (36–)82(–142)° (Table 2, Fig. 6). In addition, the second and third subgroups were more similar, based on analysed features of stigmas to the previous one. The length of 2-lobed stigma (A, J) of the second subgroup (represented by *O. elatior* and *O. kochii*) comprised (2133–)2689(–3022)  $\mu\text{m}$  vs. (2289–)3021(–3389) and (2282–)2649(–3099)  $\mu\text{m}$  vs. (2958–)3389(–4025), with an area (G, K) equal to (2273587–)3147567(–3852424)  $\mu\text{m}^2$  vs. (2085214–)3183167(–3575225)  $\mu\text{m}^2$  and (1445172–)1913431(–2742252)  $\mu\text{m}^2$  vs. (2752514–)3138276(–3685224)  $\mu\text{m}^2$ . In the second subgroup, the width of single lobes (E1, E2) varied between (1370–)1508(–1650)  $\mu\text{m}$  and (1131–)1429(–1752)  $\mu\text{m}$  and in the third (*O. flava*, *O. lucorum* and *O. mayeri*) varied in a range of (887–)1283(–1482)  $\mu\text{m}$  and (884–)1302(–1487)  $\mu\text{m}$  with the width in the middle part of the stigma (F) falling into a range of (751–)1013(–1100)  $\mu\text{m}$  vs. (480–)761(–987)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) comprised (146–)248(–460)  $\mu\text{m}$  and (145–)287(–378)  $\mu\text{m}$  for *O. elatior* and *O. kochii* and (157–)218(–260)  $\mu\text{m}$  and (189–)323(–448)  $\mu\text{m}$  for *O. flava*, *O. lucorum* and *O. mayeri*. Finally, the angle between 2-lobed stigma in the upper and lower part (H, I) fell into a range of (108–)136(–162)° vs. (128–)143(–159)° and (103–)127(–153)° vs. (115–)133(–149)° (Table 2, Fig. 6).

**Table 2.** The comparison of the stigmas of selected species of *Orobanche* and *Phelipanche*.

No	Species	The length of 2-lobed stigma (A) (µm)			The length of single lobes (A1) (µm)			The length of single lobes (A2) (µm)			The length of upper separation in the middle part between lobes (B) (µm)			The length of lower separation in the middle part between lobes (C) (µm)		
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
1a	<i>Orobanche alba</i>	2406	2733	2956	1042	1267	1398	1053	1291	1420	250	400	436	175	322	390
1b	<i>O. alba</i>	2654	2769	3096	1095	1292	1423	1111	1263	1452	243	411	452	200	310	350
2a	<i>O. alsatica</i>	2770	3263	3376	1258	1389	1506	1253	1394	1524	150	320	353	380	503	563
2b	<i>O. alsatica</i>	2776	3283	3423	1280	1390	1574	1289	1468	1600	152	331	362	385	502	559
3	<i>O. artemisiae-campestris</i>	2343	2489	2635	1055	1112	1169	824	848	872	269	294	318	305	308	333
4a	<i>O. bartlingii</i>	2908	3289	3525	1324	1435	1649	1322	1440	1598	156	377	560	492	643	932
4b	<i>O. bartlingii</i>	3122	3290	3452	1352	1395	1658	1222	1352	1485	157	363	580	482	596	935
5a	<i>O. caryophyllacea</i>	2625	3234	4135	1174	1420	1814	1107	1411	1833	163	263	397	248	423	769
5b	<i>O. caryophyllacea</i>	2717	3240	3857	1193	1431	1666	1149	1423	1714	162	257	325	253	436	685
6a	<i>O. coerulescens</i>	2045	2280	2602	862	988	1047	821	939	1011	172	240	272	175	250	399
6b	<i>O. coerulescens</i>	2044	2270	2636	821	900	1100	850	990	1112	180	250	280	178	240	301
7	<i>O. cumana</i>	2191	2299	2600	650	950	1040	800	980	1200	195	243	290	190	247	325
8a	<i>O. elatior</i>	2211	2750	3022	902	1200	1425	980	1192	1422	150	254	380	150	280	360
8b	<i>O. elatior</i>	2279	2665	3002	1040	1182	1402	1055	1182	1423	182	256	460	228	293	378
9a	<i>O. flava</i>	2440	3052	3325	1161	1339	1520	1026	1313	1455	182	222	253	215	339	441
9b	<i>O. flava</i>	2480	3143	3389	1200	1400	1543	1130	1380	1487	185	227	260	220	340	448
10	<i>O. gracilis</i>	3200	4091	4522	1523	1777	1952	1452	1569	1852	290	300	315	520	555	575
11	<i>O. hederiae</i>	2316	2457	2620	1021	1114	1350	1034	1152	1250	250	300	400	270	310	410
12a	<i>O. kochii</i>	2133	2657	2957	878	1190	1361	965	1174	1355	146	241	371	145	281	352
12b	<i>O. kochii</i>	2271	2682	2986	1034	1160	1347	1024	1140	1368	174	242	455	226	292	373
13	<i>O. lucorum</i>	2289	2885	3158	1025	1205	1325	885	1210	1299	157	205	234	189	302	407
14a	<i>O. lutea</i>	2414	3213	3425	978	1251	1587	1124	1244	1509	207	261	321	313	434	555
14b	<i>O. lutea</i>	2631	3252	3489	1158	1375	1721	1159	1358	1523	229	261	440	332	432	875
15a	<i>O. mayeri</i>	2367	3024	3214	1124	1330	1501	1015	1301	1424	179	220	249	200	328	420
15b	<i>O. mayeri</i>	2380	2999	3203	1100	1289	1498	999	1299	1387	168	215	245	198	319	415
16	<i>O. minor</i>	2027	2333	2632	813	999	1113	804	987	1195	213	253	329	261	299	361
17a	<i>O. picridis</i>	2039	2341	2748	796	996	1150	864	1029	1217	233	277	318	270	308	370
17b	<i>O. picridis</i>	2035	2333	2774	824	1004	1207	814	993	1208	249	273	339	270	292	371
18a	<i>O. reticulata</i>	2200	2459	2852	1050	1280	1400	1060	1270	1452	270	442	471	154	315	382
18b	<i>O. reticulata</i>	2300	2560	2962	1105	1315	1458	1110	1305	1482	275	445	478	156	320	385
19	<i>O. teucris</i>	2617	3259	3935	1166	1339	1779	1100	1326	1756	160	230	366	250	430	770
20a	<i>Phelipanche arenaria</i>	1880	2123	2397	785	824	1086	699	712	1023	185	192	295	151	327	368
20b	<i>P. arenaria</i>	1866	2117	2360	824	947	1087	787	904	1055	191	237	309	124	279	350
20c	<i>P. arenaria</i>	1960	2158	2443	870	1048	1121	796	959	1130	202	301	345	148	326	362
20d	<i>P. arenaria</i>	1858	2181	2314	847	982	1112	711	901	1033	201	250	310	110	304	344
21	<i>P. bohemica</i>	2089	2250	2500	987	1000	1083	869	1005	1183	240	252	263	202	242	337
22	<i>P. caesia</i>	2059	2188	2400	929	961	999	948	974	986	300	305	306	286	310	389
23	<i>P. purpurea</i>	2056	2219	2312	865	966	980	859	991	1156	230	242	250	190	205	328
24a	<i>P. ramosa</i>	1159	1520	1670	481	673	717	469	565	621	163	198	226	200	236	315
24b	<i>P. ramosa</i>	1269	1568	1700	446	708	754	485	623	645	169	220	264	206	277	317

*Orobanche coerulescens* and *O. cumana* from the sect. *Inflatae* (subsect. *Inflatae* sensu Beck) consisted mostly of hemispherical, rarely rounded stigmas with two lobes which were white to yellowish-white, rarely bluish and partially separated (Table 2, Figs 3O, P). These species represented some of the smallest stigmas of the genus *Orobanche* that were analysed, i.e. (2044–)2283(–2636) µm and (1600–)2048(–2544) µm in the



Table 2. Continued.

No	Species	The length of the mouth of the stylar canal (slit) (D) (µm)			The width of single lobes (E1) (µm)			The width of single lobes (E2) (µm)			The width in the middle part of the stigma (F) (µm)			The area of 2-lobed stigma (G) (µm <sup>2</sup> )		
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
1a	<i>Orobanchе alba</i>	850	1230	1420	1123	1334	1520	950	1183	1326	556	600	635	2757642	2850613	3000677
1b	<i>O. alba</i>	876	1249	1390	1002	1367	1457	1136	1156	1383	503	547	620	2651752	2977262	3100111
2a	<i>O. alsatica</i>	1255	1513	2024	1518	1725	1882	1534	1763	2044	713	1029	1217	2949725	4223848	5073149
2b	<i>O. alsatica</i>	1302	1513	2083	1552	1726	1889	1542	1765	2022	723	1168	1227	2952681	4234639	5045267
3	<i>O. artemisiae-campestris</i>	952	1200	1425	1315	1435	1655	1296	1436	1481	736	862	988	2632733	2961322	3289911
4a	<i>O. bartlingii</i>	1415	1759	2136	1639	1754	1974	1453	1802	2128	625	850	976	3179984	4461832	5803293
4b	<i>O. bartlingii</i>	1423	1668	2098	1652	1796	1985	1425	1782	2119	636	846	998	3175235	4265832	5795423
5a	<i>O. caryophyllacea</i>	1307	1735	2150	1321	1644	1805	1356	1689	1925	546	899	1122	2952876	4194741	5952683
5b	<i>O. caryophyllacea</i>	1322	1753	2221	1504	1612	1871	1197	1607	1920	702	887	1047	2235708	4133380	5403435
6a	<i>O. coerulescens</i>	729	888	1143	849	1257	1588	823	1307	1652	637	979	1200	1545257	2107260	2763040
6b	<i>O. coerulescens</i>	740	890	1200	870	1200	1600	840	1310	1700	650	999	1210	1586422	2108452	2794224
7	<i>O. cumana</i>	790	952	1212	888	1215	1650	860	1325	1725	690	999	1242	1725555	2249511	2800502
8a	<i>O. elatior</i>	1100	1302	1380	1400	1500	1630	1182	1450	1690	782	1020	1098	2452252	3182522	3852424
8b	<i>O. elatior</i>	1120	1305	1450	1405	1520	1650	1252	1420	1752	800	1025	1100	2575255	3125252	3825551
9a	<i>O. flava</i>	1133	1441	1567	987	1308	1441	1045	1383	1414	564	787	958	2177820	3201648	3542524
9b	<i>O. flava</i>	1150	1482	1579	995	1358	1482	1096	1399	1487	568	790	987	2198525	3235255	3575225
10	<i>O. gracilis</i>	1950	2130	2300	950	1031	1100	1000	1083	1120	590	650	720	3352552	3836796	4525252
11	<i>O. hedenae</i>	1100	1200	1420	1242	1473	1652	1288	1499	1700	818	881	1002	2722202	3049217	3952542
12a	<i>O. kochii</i>	1071	1227	1334	1370	1503	1626	1131	1402	1669	751	1005	1063	2273587	3119816	3638828
12b	<i>O. kochii</i>	1098	1238	1421	1376	1507	1646	1211	1445	1731	794	1002	1094	2397616	3135407	3657502
13	<i>O. lucorum</i>	995	1301	1358	887	1184	1275	884	1170	1247	480	687	862	2085214	3098547	3352524
14a	<i>O. lutea</i>	1057	1406	1745	1368	1608	2031	1342	1624	1939	816	999	1232	3079257	4167377	5275009
14b	<i>O. lutea</i>	1091	1461	1869	1512	1675	2125	1616	1654	2010	820	989	1337	3134725	4177720	5565740
15a	<i>O. mayeri</i>	1086	1375	1499	979	1289	1399	999	1299	1400	524	775	942	2154424	3195242	3495521
15b	<i>O. mayeri</i>	1079	1365	1487	968	1276	1384	990	1257	1397	517	768	935	2150254	3185143	3472514
16	<i>O. minor</i>	1023	1204	1423	1121	1315	1505	1176	1303	1596	577	737	923	1875234	2524575	3278563
17a	<i>O. picridis</i>	1175	1244	1480	1187	1372	1541	1200	1349	1470	732	800	952	2020061	2562134	3416204
17b	<i>O. picridis</i>	1056	1201	1448	1157	1335	1542	1181	1339	1608	543	746	935	1890329	2539792	3313251
18a	<i>O. reticulata</i>	866	1249	1423	1245	1408	1555	995	1185	1324	570	603	645	2452534	2635921	2885422
18b	<i>O. reticulata</i>	876	1286	1487	1287	1458	1599	999	1198	1357	585	624	657	2475254	2668541	2895252
19	<i>O. tueurii</i>	1250	1600	1950	1258	1671	1752	1295	1679	1885	560	885	1085	2923211	4188569	5325321
20a	<i>Phelipanche arenaria</i>	402	432	616	1210	1480	1802	1250	1458	1704	621	851	977	2489552	2669838	3547202
20b	<i>P. arenaria</i>	514	623	804	1178	1448	1750	1258	1484	1714	764	864	999	2143661	2590398	3836144
20c	<i>P. arenaria</i>	436	552	617	1238	1667	1962	1414	1673	1779	851	1010	1237	2752426	3521283	4052127
20d	<i>P. arenaria</i>	485	570	669	1338	1661	1803	1288	1617	1737	830	950	1098	2652524	2951856	3524525
21	<i>P. bohémica</i>	558	622	640	1452	1570	1700	1476	1558	1602	974	1020	1128	2825152	3129718	3352525
22	<i>P. caesia</i>	500	515	635	1500	1702	1800	1519	1610	1700	890	900	918	3118762	3156066	3222055
23	<i>P. purpurea</i>	552	602	620	1435	1462	1684	1452	1512	1598	950	1015	1114	2602565	2818684	3030555
24a	<i>P. ramosa</i>	312	367	477	811	1023	1113	814	979	1099	413	555	696	991449	1400737	1679621
24b	<i>P. ramosa</i>	327	423	486	947	1206	1274	823	1165	1274	510	661	766	996252	1647702	1893740

length of 2-lobed stigma (A, J) with an area (G, K) of (1545257–)2155074(–2800502) µm<sup>2</sup> and (785340–)1153516(–1352420) µm<sup>2</sup>. The width of single lobes (E1, E2) comprised (849–)1224(–1650) µm and (823–)1314(–1725) µm, as well as the width in the middle part of the stigma (F) varied between (637–)992(–1242) µm. The length of upper and lower separation in the middle part between lobes (B, C) fell into a range of (172–)244(–290) µm and (175–)246(–399) µm and the angle between 2-lobed

**Table 2.** Continued.

No	Species	The area of single lobes (G1) (μm <sup>2</sup> )			The area of single lobes (G2) (μm <sup>2</sup> )			The angle between 2-lobed stigma in the upper part (H) (°)			The angle between 2-lobed stigma in the lower part (I) (°)			The length of 2-lobed stigma (J) (μm)		
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
1a	<i>Orobanche alba</i>	1245823	1324428	1445236	1123574	1249113	1445834	100	159	168	100	125	138	2553	2725	2953
1b	<i>O. alba</i>	1235426	1383402	1445783	1124735	1242331	1445238	120	154	160	112	124	135	2389	2560	2756
2a	<i>O. alsatica</i>	1350001	1895292	2049205	1351501	1825268	2211640	106	121	149	90	99	142	2497	3155	3409
2b	<i>O. alsatica</i>	1345856	1864142	2057257	1325423	1948092	2354521	110	119	152	95	98	140	2500	3119	3375
3	<i>O. artemisiae-campestris</i>	1346029	1494916	1643804	1163778	1289765	1415751	110	117	125	115	127	140	2458	2559	2715
4a	<i>O. bartlingii</i>	1549428	2254127	2458532	1581602	2208461	2859009	97	118	152	36	62	96	3070	3222	3743
4b	<i>O. bartlingii</i>	1484526	2095321	2394258	1535275	2092462	2852954	95	123	155	40	68	90	3100	3207	3658
5a	<i>O. caryophyllacea</i>	1495078	2133317	2856202	1067414	2118599	2998225	108	134	148	93	116	148	2542	3173	4003
5b	<i>O. caryophyllacea</i>	1788448	2094521	2647715	1463544	2091128	2558874	123	139	146	102	116	146	3085	3160	3576
6a	<i>O. coerulescens</i>	536064	633490	733232	453078	688153	753521	133	141	151	112	122	143	1667	2019	2455
6b	<i>O. coerulescens</i>	552222	654222	745252	475525	702541	765421	138	150	160	125	120	152	1600	2000	2389
7	<i>O. cumana</i>	652224	802121	1052555	681242	855521	104855	140	158	171	130	122	160	1600	2124	2544
8a	<i>O. elatior</i>	1252522	1620222	1882522	1258522	1605525	1952522	125	138	150	110	130	140	2345	2685	3089
8b	<i>O. elatior</i>	1425254	1622252	1982522	1182525	1625444	1982202	118	140	162	115	128	153	2300	2688	3099
9a	<i>O. flava</i>	1125475	1510172	1791079	1104524	1474925	1785244	136	147	158	122	135	149	3113	3431	4002
9b	<i>O. flava</i>	1142524	1545722	1800241	1132522	1502142	1802141	139	149	159	125	137	149	3131	3458	4025
10	<i>O. gracilis</i>	1524586	1891079	2152354	1535752	1839782	2165242	115	120	129	120	142	150	3522	4145	4522
11	<i>O. hederae</i>	1259925	1418678	1602020	1252522	1421152	1625521	105	125	130	98	114	125	2225	2418	2701
12a	<i>O. kochii</i>	1173520	1569933	1787545	1190538	1526655	1847662	116	134	149	103	126	138	2300	2616	3013
12b	<i>O. kochii</i>	1324292	1501738	1917114	1043585	1500763	1906603	108	130	155	110	124	152	2282	2605	3058
13	<i>O. lucorum</i>	1052442	1395241	1675252	1042252	1395241	1685221	128	140	148	115	129	141	2958	3258	3842
14a	<i>O. lutea</i>	1413468	2076596	2643138	1599790	2080971	2590285	116	133	144	78	119	135	2603	3075	3198
14b	<i>O. lutea</i>	1540919	2167075	3080438	1607626	2150782	2816204	121	134	152	103	119	137	2690	3043	3363
15a	<i>O. mayeri</i>	1115471	1492201	1720214	1094254	1452142	1755288	129	138	147	120	130	145	3107	3401	3952
15b	<i>O. mayeri</i>	1117524	1485214	1745241	1092514	1442252	1739541	130	142	149	119	132	144	3095	3398	3925
16	<i>O. minor</i>	956424	1240557	1575224	974255	1234452	1654224	109	124	139	95	114	119	2324	2499	2799
17a	<i>O. picridis</i>	904198	1264524	1627401	968520	1284620	1677785	107	123	132	98	114	121	2157	2525	2760
17b	<i>O. picridis</i>	980526	1261885	1653207	992278	1250536	1731908	114	123	135	100	116	121	2392	2515	2890
18a	<i>O. reticulata</i>	1305424	1422813	1495242	1132531	1258456	1432574	123	150	161	92	122	140	2123	2582	2952
18b	<i>O. reticulata</i>	1312524	1432421	1502241	1272221	1475214	1562221	125	155	165	97	125	144	2157	2593	2999
19	<i>O. teucrii</i>	1402542	1922681	2795212	1402674	1954136	2752642	111	140	150	95	117	152	2354	3075	3885
20a	<i>Phelipanche arenaria</i>	1220672	1311531	1807843	1039657	1287672	1523739	120	125	134	107	127	154	2000	2183	2288
20b	<i>P. arenaria</i>	1057719	1226497	1520612	1052145	1291165	1589813	117	125	133	120	139	154	1954	2000	2235
20c	<i>P. arenaria</i>	1224027	1712603	2042492	1126734	1535574	1649937	106	126	120	103	119	152	1888	1940	2135
20d	<i>P. arenaria</i>	1096897	1303751	1679465	894185	1194634	1460135	112	120	128	120	120	156	1856	1936	2099
21	<i>P. bohémica</i>	1152525	1385251	1539616	1152555	1395225	1532525	119	125	130	111	108	121	1958	1999	2102
22	<i>P. caesia</i>	1322021	1481583	1746409	1382380	1400571	1565378	110	129	140	107	116	120	1920	2065	2255
23	<i>P. purpurea</i>	1001678	1293136	1495412	1004524	1277624	1357524	100	119	125	101	108	120	1923	1983	2025
24a	<i>P. ramosa</i>	332470	591160	759460	329541	586839	765428	82	101	116	99	108	118	1052	1288	1412
24b	<i>P. ramosa</i>	369303	755309	845729	365225	708890	849552	78	94	106	90	101	112	1110	1323	1500

stigma in the upper and lower part (H, I) fell into a range of (133–)150(–171)° and (112–)121(–160)° (Table 2, Fig. 6).

*Orobanche gracilis* from the subsect. *Cruentae* Teryokhin had hemispherical to rounded and distinctly separated stigmas with two yellow lobes and with a reddish base of the stigmas to the style tip (Table 2, Fig. 3L). The length of 2-lobed stigma (A, J) was

Table 2. Continued.

No	Species	The area of 2-lobed stigma (µm <sup>2</sup> ) (K)			Type	Subtype	Shape	Colour	Degree of stigma lobes separation
		Min	Mean	Max					
1a	<i>Orobanchе alba</i>	1645762	1751163	1863425	2	4	5	dark red or purple (rarely yellow or orange)	2
1b	<i>O. alba</i>	1594525	1725078	1852642	2	4	5	dark red or purple (rarely yellow or orange)	2
2a	<i>O. alsatica</i>	2003320	3070327	3438793	2	7	8	yellow	4
2b	<i>O. alsatica</i>	2052425	3072867	3524578	2	7	8	yellow	4
3	<i>O. artemisiae-campestris</i>	1405555	1625555	2025555	2	5	6	pink, purple-brownish, reddish	1
4a	<i>O. bartlingii</i>	2789644	3172420	3854744	2	7	8	yellow	4
4b	<i>O. bartlingii</i>	2758452	3157156	3792542	2	7	8	yellow	4
5a	<i>O. caryophyllacea</i>	2214881	3103345	3929173	2	6	7	dark brown, purple or rarely yellow or orange	5
5b	<i>O. caryophyllacea</i>	2902541	3125328	3968729	2	6	7	dark brown, purple or rarely yellow or orange	5
6a	<i>O. coerulescens</i>	785340	1105242	1235242	2	2	3	white to yellowish-white, rarely bluish	3
6b	<i>O. coerulescens</i>	796655	1100055	1245522	2	2	3	white to yellowish-white, rarely bluish	3
7	<i>O. cumana</i>	799222	1255250	1352420	2	2	3	white to yellowish-white, rarely bluish	3
8a	<i>O. elatior</i>	1555252	1922525	2742252	2	7	8	yellow	4
8b	<i>O. elatior</i>	1505557	1922442	2654444	2	7	8	yellow	4
9a	<i>O. flava</i>	2954225	3182604	3525452	2	7	8	yellow, rarely orange	4
9b	<i>O. flava</i>	2982201	3325214	3685224	2	7	8	yellow, rarely orange	4
10	<i>O. gracilis</i>	3952435	4722574	5054242	2	3	4	yellow lobes with a reddish base of the stigma to the style tip	5
11	<i>O. hederae</i>	1425256	1686525	1915252	2	5	6	yellow	1
12a	<i>O. kochii</i>	1548757	1903199	2612049	2	7	8	yellow	4
12b	<i>O. kochii</i>	1445172	1905557	2580093	2	7	8	yellow	4
13	<i>O. lucorum</i>	2752514	2958541	3295255	2	7	8	yellow	4
14a	<i>O. lutea</i>	2506590	3062143	3355113	2	6	7	yellow, rarely orange	5
14b	<i>O. lutea</i>	2800458	3083820	3481223	2	6	7	yellow, rarely orange	5
15a	<i>O. mayeri</i>	2884252	3125415	3452542	2	7	8	yellow	4
15b	<i>O. mayeri</i>	2895310	3099604	3385145	2	7	8	yellow	4
16	<i>O. minor</i>	1425775	1635554	1835447	2	5	6	pinkish, reddish or purplish rarely white	1
17a	<i>O. picridis</i>	1420156	1687767	1853354	2	5	6	purple, dark red and pink	1
17b	<i>O. picridis</i>	1485242	1674790	1925422	2	5	6	purple, dark red and pink	1
18a	<i>O. reticulata</i>	1782243	2023996	2174252	2	4	5	brownish or purplish as well as usually lighter in the upper part of the stigma	2
18b	<i>O. reticulata</i>	1824224	2162437	2302552	2	4	5	brownish or purplish as well as usually lighter in the upper part of the stigma	2
19	<i>O. teucrii</i>	2256413	3188246	3954254	2	6	7	dark brown, purple	5
20a	<i>Phelipanche arenaria</i>	1415852	1679817	1865272	1	1	1	white	4
20b	<i>P. arenaria</i>	1405527	1657854	1852727	1	1	1	white	4
20c	<i>P. arenaria</i>	1385424	1635680	1814525	1	1	1	white	4
20d	<i>P. arenaria</i>	1395472	1636061	1825577	1	1	1	white	4
21	<i>P. bohemica</i>	1502252	1702555	1842522	1	1	1	yellow-white	4
22	<i>P. caesia</i>	1385242	1654332	1834555	1	1	1	white	4
23	<i>P. purpurea</i>	1496874	1739657	1836524	1	1	1	white or pale blue or violet	4
24a	<i>P. ramosa</i>	365241	449118	589117	1	1	2	white or bright bluish, rarely yellowish	4
24b	<i>P. ramosa</i>	394527	487236	575272	1	1	2	white or bright bluish, rarely yellowish	4

Explanations: stigma type: 1–oval, rarely hemispherical, most often white, rare light blue or violet and yellow and lobes separated; 2–spherical, hemispherical, rounded, rarely oval, multi-coloured: white, yellow, orange, pink, purple, red to dark brown with partially fused or separated lobes; stigma subtype (in bottom view): 1–oval, rarely hemispherical, most often white, rare light blue or violet and yellow, lobes separated; 2–hemispherical, rarely rounded, white to yellowish-white, rarely bluish, lobes partially separated; 3–hemispherical to rounded, lobes distinctly separated; 4–spherical to rounded, multi-coloured, lobes in closer proximity; 5–hemispherical, multi-coloured, lobes in closer proximity or even partially united; 6–hemispherical to oval, multi-coloured, lobes distinctly separated; 7–hemispherical rarely oval, yellow, lobes separated; stigma shape (in bottom view): 1–oval, rarely hemispherical; 2–oval to hemispherical; 3–hemispherical, rarely rounded; 4–hemispherical to rounded; 5–spherical to rounded; 6–hemispherical; 7–hemispherical to oval; 8–hemispherical, rarely oval; degree of stigma lobes separation (in front view): 1–closer proximity or even partially united; 2–closer proximity; 3–partially separated; 4–separated; 5–distinctly separated.



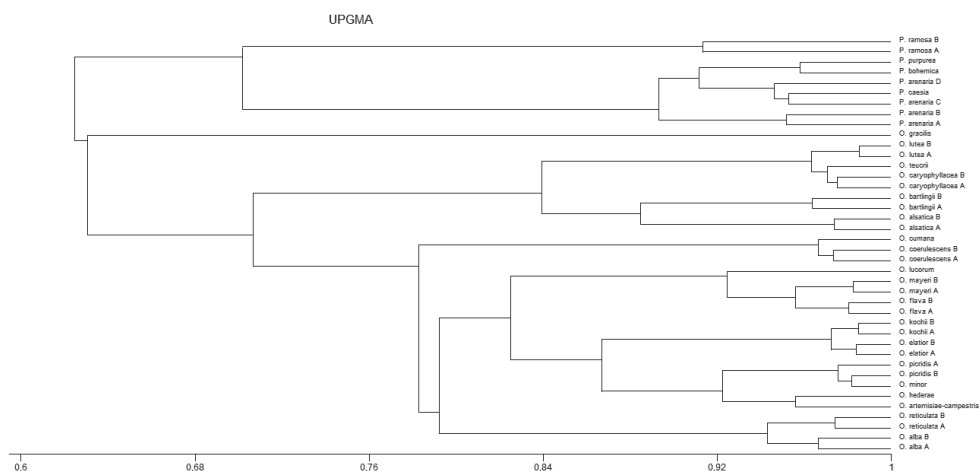
**Figure 3.** General habit of selecting flowers of Orobanchaceae species with a stigma in the front view.

**A** *Orobanche alba* **B** *O. reticulata* **C** *O. alsatica* **D** *O. bartlingii* **E** *O. kochii* **F** *O. elatior* **G** *O. flava* **H** *O. mayeri* **I** *O. caryophyllacea* **J** *O. teucritii* **K** *O. lutea* **L** *O. gracilis* **M** *O. minor* **N** *O. picridis* **O** *O. coerulescens* **P** *O. cumana* **R** *Phelipanche arenaria* **S** *P. caesia* **T** *P. purpurea* **U** *P. ramosa*. Phot. R. Piwowarczyk.





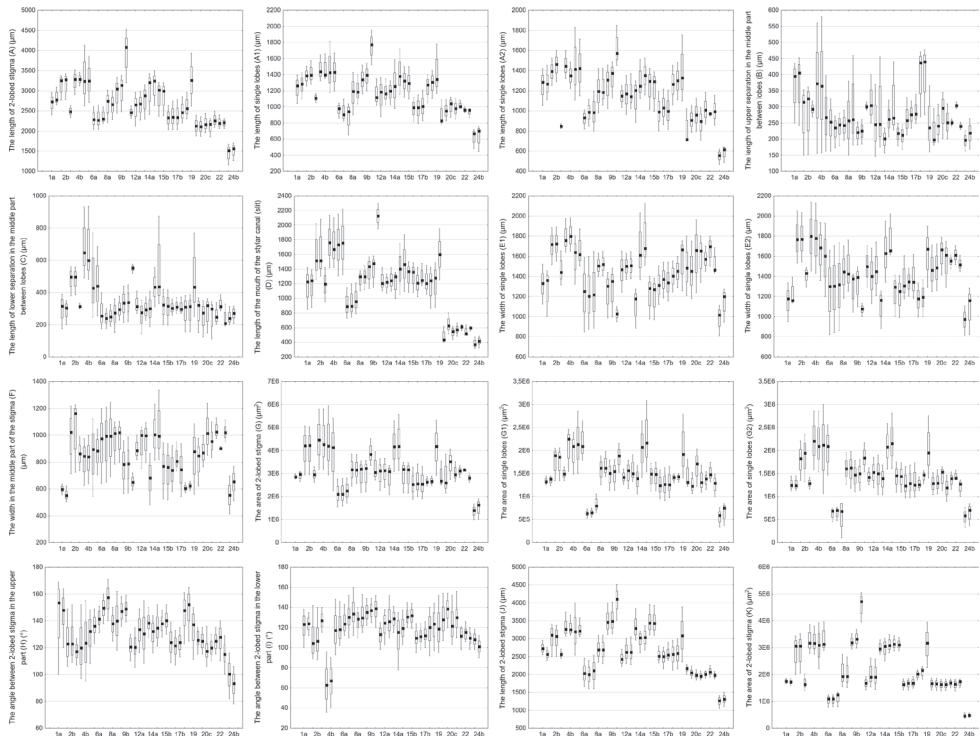
**Figure 4.** Micrographs of selected stigmas of the studied species. **A** *Orobanche alsatica* **B** *O. bartlingii* **C** *O. caryophyllacea* **D** *O. kochii* **E** *O. lutea* **F** *O. picridis* **G** *Phelipanche arenaria* **H** *P. ramosa*. **1** front view **2** bottom view **3** lateral view **4** back view. Scale bars: 1000 µm (**A1–A3, B1–C4, D3, E1–E4, G1, G3**); 500 µm (**A4, D1, D2, D4, F1–F4, G2, G4, H3**); 100 µm (**H1, H2, H4**). Phot. K. Ruraż, J. Posłowska and K. Zubek.



**Figure 5.** UPGMA dendrogram of morphological differentiation of Orobanchaceae stigmas.

the largest of all Orobanchaceae stigmas tested, comprising (3200–)4091(–4522)  $\mu\text{m}$  and (3522–)4145(–4522)  $\mu\text{m}$  with an area (G, K) varying between (3352752–)3836796(–4525252)  $\mu\text{m}^2$  and (3952435–)4722574(–5054242)  $\mu\text{m}^2$ . The width of single lobes (E1, E2) fell into a range of (950–)1031(–1100)  $\mu\text{m}$  and (1000–)1083(–1120)  $\mu\text{m}$  and the width in the middle part of the stigma (F) was equal to (590–)650(–720)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) fell within a range of (290–)300(–315)  $\mu\text{m}$  and (520–)555(–575)  $\mu\text{m}$  and the angle between 2-lobed stigma in the upper and lower part (H, I) fell into a range of (115–)120(–129)° and (120–)142(–150)° (Table 2, Fig. 6).

*Orobanche caryophyllacea*, *O. lutea* and *O. teucris*, which are represented in the *O.* subsect. *Orobanche* (subsect. *Galeatae* sensu Teryokhin) had two hemispherical to oval and distinctly separated stigma lobes. The stigmas in *O. caryophyllacea* and *O. teucris* had similar colours, namely dark brown, purple, rarely yellow or orange (in *O. caryophyllacea*), in contrast to *O. lutea* which were yellowish, rarely orange (Table 2, Figs 3I–K, 4C, E). *Orobanche caryophyllacea* and *O. teucris* stigmas were similar in terms of the analysis of qualitative and quantitative features, in contrast to *O. lutea* (Fig. 5). The length of 2-lobed stigma (A, J) of *O. caryophyllacea* and *O. teucris* fell within a range of (2617–)3244(–4135)  $\mu\text{m}$  vs. (2414–)3233(–3489) and (2354–)3136(–4003)  $\mu\text{m}$  vs. (2603–)3059(–3363), with an area (G, K) varying between (2235708–)4172230(–5952683)  $\mu\text{m}^2$  vs. (3079257–)4172549(–5565740)  $\mu\text{m}^2$  and (2214881–)3138973(–3968729)  $\mu\text{m}^2$  vs. (2506590–)3072982(–3481223)  $\mu\text{m}^2$ . In *O. caryophyllacea* and *O. teucris*, the width of single lobes (E1, E2) comprised (1258–)1642(–1871)  $\mu\text{m}$  and (1197–)1658(–1925)  $\mu\text{m}$ , while in *O. lutea* displayed a range of (1368–)1642(–2125)  $\mu\text{m}$  and (1342–)1639(–2010)  $\mu\text{m}$ , with the width in the middle part of the stigma (F) in a range of (546–)890(–1122)  $\mu\text{m}$  vs. (816–)994(–1337)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) was (160–)250(–397)  $\mu\text{m}$  and (248–)430(–770)  $\mu\text{m}$  for *O. caryophyllacea* and *O. teucris* in comparison to (207–)261(–440)  $\mu\text{m}$  and (313–)433(–875)  $\mu\text{m}$  recorded in *O. lutea*.



**Figure 6.** Box and whisker plots of quantitative morphological characters of Orobanchaceae stigmas. Points indicate the mean values (open square), boxes represent 25 and 75% percentiles and range (whiskers) represent 1 and 99% percentiles. Numbers indicate examined species (Table 2).

The angle between 2-lobed stigma in the upper and lower part (H, I) was equal to  $(108\text{--}138\text{--}150)^\circ$  vs.  $(116\text{--}134\text{--}152)^\circ$  and  $(93\text{--}116\text{--}152)^\circ$  vs.  $(78\text{--}119\text{--}137)^\circ$  (Table 2, Fig. 6).

The stigmas of species belonging to the subsect. *Glandulosae* (Beck) Teryokhin i.e. *O. alba* and *O. reticulata* consisted of two spherical to rounded lobes with different colours in closer proximity (Table 2, Figs 3A, B). *Orobancha reticulata* had more elongated and flattened stigmas with more separated lobes than *O. alba*, whose stigmas were dark red or purple (rarely yellow or orange), unlike *O. reticulata* which were brownish or purplish (mostly lighter in the upper part). The length of 2-lobed stigma (A, J) varied within limits of  $(2200\text{--}2630\text{--}3096) \mu\text{m}$  and  $(2123\text{--}2615\text{--}2999) \mu\text{m}$  with an area (G, K) falling within a range of  $(2452534\text{--}2783084\text{--}3100111) \mu\text{m}^2$  and  $(1594525\text{--}1915669\text{--}2302552) \mu\text{m}^2$ . The width of single lobes (E1, E2) comprised  $(1002\text{--}1392\text{--}1599) \mu\text{m}$  and  $(950\text{--}1181\text{--}1383) \mu\text{m}$ , as well as the width in the middle part of the stigma (F) was equal to  $(503\text{--}594\text{--}657) \mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) fell within a range of  $(243\text{--}425\text{--}478) \mu\text{m}$  and  $(154\text{--}317\text{--}390) \mu\text{m}$  and the angle between 2-lobed stigma in the upper and lower part (H, I) fell into a range of  $(100\text{--}155\text{--}168)^\circ$  and  $(92\text{--}124\text{--}144)^\circ$  (Table 2, Fig. 6).



Species from the subsect. *Minores* Teryokhin (incl. *O. hederæ* from the subsect. *Hederæ* Teryokhin) (*O. artemisiae-campestris*, *O. hederæ*, *O. minor* and *O. picridis*) had two hemispherical lobes of the stigmas in closer proximity or even partially united. *Orobanche minor* stigmas varied in colour from pinkish, reddish or purplish to rarely white, similar to *O. artemisiae-campestris* which were pink, purple-brownish, reddish and *O. picridis* with purple, dark red and pink lobes and unlike *O. hederæ* which were usually yellow. The distinguishing feature of the stigmas of this group was the presence of a well-developed and convex surface in relation to the base of the stigmas (Table 2, Figs 3M, N, 4F). The UPGMA analysis on the basis of given features distinguished two subgroups, i.e. the first one included *O. artemisiae-campestris* and *O. hederæ* and the second was represented by *O. minor* and *O. picridis* (Fig. 5). The length of 2-lobed stigma (A, J) of the first subgroup was equal to (2316–)2473(–2635)  $\mu\text{m}$  vs. (2027–)2336(–2774) and (2225–)2489(–2715)  $\mu\text{m}$  vs. (2157–)2513(–2890), with an area (G, K) varying within limits of (2632733–)3005270(–3952542)  $\mu\text{m}^2$  vs. (1875234–)2542167(–3416204)  $\mu\text{m}^2$  and (1405555–)1656040(–2025555)  $\mu\text{m}^2$  vs. (1420156–)1666037(–1925422)  $\mu\text{m}^2$ . In the first subgroup, the width of single lobes (E1, E2) varied between (1242–)1454(–1655)  $\mu\text{m}$  and (1288–)1468(–1700)  $\mu\text{m}$  and, in the second, varied in a range of (1121–)1341(–1542)  $\mu\text{m}$  and (1176–)1330(–1608)  $\mu\text{m}$ , with the width in the middle part of the stigma (F) in a range of (736–)872(–1002)  $\mu\text{m}$  vs. (543–)761(–952)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) was (250–)297(–400)  $\mu\text{m}$  and (270–)309(–410)  $\mu\text{m}$  for *O. artemisiae-campestris* and *O. hederæ* in comparison to (213–)268(–339)  $\mu\text{m}$  and (261–)300(–371)  $\mu\text{m}$  recorded in *O. minor* and *O. picridis*. The angle between 2-lobed stigma in the upper and lower part (H, I) was in a range of (105–)121(–130)° vs. (107–)123(–139)° and (98–)121(–140)° vs. (95–)115(–121)° (Table 2, Fig. 6).

### *Phelipanche*

The stigmas were oval, rarely hemispherical, with separated lobes and were most often white, rarely light blue, violet and yellow (Table 2, Figs 2B, 3R–U, 4G, H). Stigmas are less varied and smaller in size than in *Orobanche*.

The stigmas of *Phelipanche arenaria*, *P. bohémica*, *P. caesia* and *P. purpurea* belonging to the sect. *Trionychon* (Wallr.) Piwow. & Ó. Sánchez (sect. *Arenariae* Teryokhin) consisted of two white lobes which were oval, rarely hemispherical with clearly separated lobes. Most often they were white, less often light blue or violet and yellow (Table 2, Figs 3R–T, 4G). Measurable data and UPGMA analysis showed the presence of two subgroups *P. arenaria* and *P. caesia* in the first, with the second represented by *P. bohémica* and *P. purpurea* (Fig. 5). The length of 2-lobed stigma (A, J) of the first subgroup comprised (1858–)2153(–2443)  $\mu\text{m}$  vs. (2056–)2235(–2500) and (1856–)2025(–2288)  $\mu\text{m}$  vs. (1923–)1991(–2102), with an area (G, K) of (2143661–)2977888(–4052127)  $\mu\text{m}^2$  vs. (2602565–)2974201(–3352525)  $\mu\text{m}^2$  and (1385242–)1652749(–1865272)  $\mu\text{m}^2$  vs. (1496874–)1721106(–1842522)  $\mu\text{m}^2$ . In the first subgroup, the width of single lobes (E1, E2) fell within a range of (1178–)1592(–1962)  $\mu\text{m}$  and

(1250–)1568(–1779)  $\mu\text{m}$  and, in the second, was equal to (1435–)1516(–1700)  $\mu\text{m}$  and (1452–)1535(–1602)  $\mu\text{m}$ , with the width in the middle part of the stigma (F) in a range of (621–)915(–1237)  $\mu\text{m}$  vs. (950–)1018(–1128)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) was (185–)257(–345)  $\mu\text{m}$  and (110–)309(–389)  $\mu\text{m}$  for *P. arenaria* and *P. caesia*, compared to (230–)247(–263)  $\mu\text{m}$  and (190–)224(–337)  $\mu\text{m}$  recorded in *P. bohémica* and *P. purpurea*. The angle between 2-lobed stigma in the upper and lower part (H, I) fell into a range of (106–)125(–140)° vs. (100–)122(–130)° and (103–)124(–156)° vs. (101–)108(–121)° (Table 2, Fig. 6).

The stigmas of species belonging to the sect. *Phelipanche* i.e. *P. ramosa* was oval to hemispherical with separated lobes which were white or bright bluish, rarely yellowish (Table 2, Figs 3U, 4H). The length of 2-lobed stigma (A, J) was the smallest of all Orobanchaceae stigmas tested, comprising (1159–)1544(–1700)  $\mu\text{m}$  and (1052–)1306(–1500)  $\mu\text{m}$  with an area (G, K) of (991449–)1524220(–1893740)  $\mu\text{m}^2$  and (365241–)468177(–589117)  $\mu\text{m}^2$ . The width of single lobes (E1, E2) fell into a range of (811–)1115(–1274)  $\mu\text{m}$  and (814–)1072(–1274)  $\mu\text{m}$ , as well as the width in the middle part of the stigma (F) was equal to (413–)608(–766)  $\mu\text{m}$ . The length of upper and lower separation in the middle part between lobes (B, C) fell within a range of (163–)209(–264)  $\mu\text{m}$  and (200–)257(–317)  $\mu\text{m}$  and the angle between 2-lobed stigma in the upper and lower part (H, I) fell into a range of (78–)98(–116)° and (90–)110(–118)° (Table 2, Fig. 6).

## Morphometric analysis

The analysis of data from the observation of stigmas structures identified twenty-one morphological characters of stigmas in Orobanchaceae (Table 2). UPGMA analysis based on characteristics suggested two types of stigma morphology, the first one included 2-lobed, occasionally 3- and 4-lobed stigmas which were oval, rarely hemispherical in shape and most often white, rarely light blue and yellowish with separated lobes. This cluster was represented by all *Phelipanche* species studied (Fig. 5). A separate subgroup consisted of *P. ramosa* stigmas characterised by being the smallest of all stigmas tested, as well as by their more hemispherical shaped stigmas in relation to other *Phelipanche*. The second type comprised the largest group of *Orobanchaceae* species studied with 2-lobed, occasionally 3- and 4-lobed stigmas, mostly hemispherical to rounded or rarely oval and varied in colour from white, yellow, orange, pink, purple, red to dark brown with partially fused or separated lobes. This group was highly varied in terms of most useful identifying features, such as subtype, the length of 2-lobed stigma (A, J), the area of 2-lobed stigma (G, K), the width of single lobes (E1, E2), the width in the middle part of the stigma (F), the length of upper and lower separation in the middle part between lobes (B, C) and the angle between 2-lobed stigma in the upper and lower part (H, I). These features allowed several subgroups of stigmas to be distinguished corresponding to systematic and phylogenetic groups (Fig. 5). The first subgroup consisted of *O. gracilis* stigmas, which were the largest stigmas of all Orobanchaceae tested. *Orobanchaceae caryophyllacea*, *O. lutea* and *O. teucris* stigmas

created a subgroup that stands out from the rest, with hemispherical to oval stigmas and two distinctly separated stigma lobes. *O. alsatica* and *O. bartlingii* stigmas reached one of the largest sizes after *O. gracilis* (Table 2, Fig. 6). The most diverse group on the UPGMA was represented by stigmas of the species from the *Curvatae* subsection, where three subgroups were distinguished (Fig. 5). The first of them, consisting of the stigmas of *O. alsatica* and *O. bartlingii*, was further away from the other representatives of this subsection due to quantitative features, for example, these stigmas were larger in relation to the other representatives such as: *O. elatior*, *O. flava*, *O. kochii*, *O. lucorum* and *O. mayeri*. The next subgroup, corresponding to *O. coerulescens* and *O. cumana*, had the smallest stigmas of the genus *Orobanche* that were analysed with a characteristic colour from white to yellowish-white, rarely bluish. *Orobanche artemisiae-campestris*, *O. hederæ*, *O. minor* and *O. picridis* stigmas formed a separate subgroup of small stigmas (on average up to 2500 µm in length) with lobes in closer proximity or even partially united. A distinctive feature of the stigmas of this subgroup was also the presence of a well-developed and convex surface in relation to the base of the stigmas. The last subgroup, *O. alba* and *O. reticulata*, had stigmas which consist of a specific shape from spherical to rounded with two lobes in closer proximity and reaching similar sizes (Fig. 5).

## Discussion

In order to provide diagnostic information and to evaluate the utility of stigma morphological characters considered in a taxonomic and phylogenetic context, a more thorough study on stigma morphology of representatives of Orobanchaceae was performed. For this purpose, we selected species representing various localities from Central Europe and applied stereomicroscopy to provide additional evidence for distinguishing genera, sections or subsections, as well as some related species of Orobanchaceae.

*Orobanche* and *Phelipanche* genera can be divided into two groups on the basis of the analysis of features carried out in this study. It was found that some quantitative features (e.g. the length of 2-lobed stigma (A, J), the area of 2-lobed stigma (G, K), the width of single lobes (E1, E2), the width in the middle part of the stigma (F), the length of upper and lower separation in the middle part between lobes (B, C), the angle between 2-lobed stigma in the upper and lower part (H, I) and the type and subtype of the stigma) are the best diagnostic characteristics for distinguishing these genera (Fig. 5). The stigmas in *Phelipanche* were the smallest of the species studied, usually not more than 2.5 mm in length of 2-lobed stigma (A) (Table 2, Fig. 6). There was a clear difference in the size of the stigmas between the analysed sections in *Phelipanche*. The stigmas of species belonging to the sect. *Phelipanche* i.e. *P. ramosa* were smaller (up to 1700 µm) than the species representing the sect. *Trionychon* (*Arenariae*) (the minimum length of 2-lobed stigma (A) was 1858 to 2089 µm) (Table 2, Fig. 6). Morphological, ecological and molecular differences suggest that *P. bohémica* have been a separate species (Piwowarczyk 2012, 2015; Piwowarczyk et al. 2015, 2018). Morphological

studies of stigmas also showed differences between *P. purpurea* and *P. bohémica*, which had yellow-white stigmas in contrast to *P. purpurea* which was whitish (Table 2). Furthermore, the analysis of characteristics of stigmas confirmed the separation of two species of the problematic complex *O. alsatica* aggr. *Orobanché alsatica* and *O. bartlingii* were different in the shape and in the length of the mouth of the stylar canal (slit), which was more regular in *O. alsatica*, as well as there being a larger separation in the lower part between lobes in *O. bartlingii* (Figs 4A2, B2, 6). However, the similarity was evident in size, for example, both species were larger than the rest of the species in subsect. *Curvatae*. *Orobanché coerulescens* and *O. cumana* (sect. *Inflatae*) have been a transitional position between *Orobanché* and *Phelipanche*. They were placed on phylogenetic trees outside the rest of *Orobanché* (Piwowarczyk et al. 2018, 2021), which correlated with some phenotypic features (such as the violet colour of the flowers and white stigmas) or tricolpate pollen (in *O. coerulescens*) (Piwowarczyk et al. 2015) that made them similar to *Phelipanche* species. Our studies confirmed that the stigma characteristics corresponded to *Orobanché*. However, based only on the colour of the stigmas, they were close to *Phelipanche* (Fig. 5). Species belonging to the section *Minores* (incl. subsect. *Hederae*) (*O. artemisiae-campestris*, *O. hederae*, *O. minor* and *O. picridis*) and the section *Inflatae* (*O. coerulescens* and *O. cumana*) were clearly distinguished from others on the basis of their length of 2-lobed stigma (A) ( $\mu\text{m}$ ), usually not more than 3 mm in *Orobanché* (Table 2, Fig. 6). *O. hederae* (which is surprisingly regarded as a member of the *Inflatae* section, following Teryokhin et al. 1993) was clustered on phylogenetic trees with species belonging to the subsect. *Minores* (Piwowarczyk et al. 2018) and our stigma morphology (Fig. 5) supports the supposition that these species are relatives.

In addition, phylogenetic studies support seed and pollen micromorphological analysis and it is noteworthy that this study showed the separation of the subgroups into separate species, based on the morphological analysis of stigmas which corresponded to systematic and phylogenetic groups (Piwowarczyk et al. 2018, 2021). Consequently, ITS dendrograms and cluster analysis (UPGMA) were similar, for example, showing a clear difference between the morphology of the stigmas of *Orobanché* and *Phelipanche* (Fig. 5). In conclusion, the often well-defined features of the stigmas had value both as taxonomic characters and as phylogenetic data for systematic studies. Additionally, the stigmas of the pistil of other members of the tribe Orobancheae, i.e. *Cistanche* and *Phelypaea* genera, have not been thoroughly investigated morphologically. However, there are some papers describing their shape and colour. *Cistanche* species are often subglobose in shape and usually white, yellowish or bluish, while stigmas in *Phelypaea* are usually discoid to subglobose with red, pink, rarely yellow colours (e.g. Teryokhin 1997; Piwowarczyk et al. 2019).

Morphological analysis of the stigmas of Central European broomrapes showed that they were characterised by high variability at the intergeneric and interspecific level. The morphology of the stigmas has consistently provided additional data to the other characters of flower morphology used to separate species, i.e. the type and subtype of stigma, the length of 2-lobed stigma (A, J), the area of 2-lobed stigma (G, K), the width of single lobes (E1, E2), the width in the middle part of the stigma (F), the length of up-

per and lower separation in the middle part between lobes (B, C) and the angle between 2-lobed stigma in the upper and lower part (H, I) (Table 2). Interestingly, the available publications of observations of the shape of stigmas in the Orobanchaceae concerned mainly the analysis of this feature in the front view. However, as our research showed, the most useful features can be seen when observing the stigmas from the bottom side, which has not been studied before. The features studied allow us to distinguish between species taking into account both dry, as well as FAA solution material. Additionally, the length of single lobes (A1, A2) and the area of single lobes (G1, G2) could be helpful when using larger samples. Features such as shape, colour and the degree of stigma lobes separation also have diagnostic value and allow us to distinguish species and genera in Orobanchaceae (e.g. Kreutz 1995; Piwowarczyk et al. 2019; Thorogood and Rumsey 2021), based on these characters. Moreover, the colour of the stigmas and petals of the same individual were often contrasting and differ from each other. Therefore, it is important to observe fresh material because the colours of the flowers of holoparasitic species in the field turn to different shades of brown after drying.

Although the taxonomy of some Orobanchaceae is still controversial, the morphology of stigmas could provide the next important characters used to define species. In this study, we used fresh plant material, dried and fixed in FAA solution. According to Heslop-Harrison (1992), the most informative images for morphological analysis of stigmas are obtained using fresh, unfixed and uncoated material, because methods where the material is processed are unnecessary and leave some artefacts. Furthermore, it is extremely important that the morphological features of the mature stigmas are observed and collected at the stage when the stigmas are receptive for pollination. Additionally, the use of a stereoscopic microscope in research on the morphology of stigmas of Orobanchaceae are another possibility for differentiating taxonomically problematic species. Teryokhin et al. (1993) analysed the morphological differences of the stigmas of about 50 taxa of *Orobanche* and *Phelipanche* genera. In this paper, several types of stigmas were mentioned, i.e. discoid, discoid-bilobed, bilobed, two-columned-discoid, as well as two-columned stigma. This division proposed by Teryokhin et al. (1993) concerned the analysis only of the shape of the stigmas and the degree of separation of the lobes. Unfortunately, taking only these features into account, we cannot come to a conclusion about the usefulness of stigmas' features for the taxonomy of Orobanchaceae (Teryokhin et al. 1993), except on a general level. Our observations showed that the degree of stigma separation of a particular species varies depending on the stage of stigma development and was difficult to observe and measure and, therefore, this feature should not be taken into account in separate systematic considerations. In conclusion, the paper of Teryokhin et al. (1993) is the only work known to us that draws wider attention to the morphology of the stigmas of holoparasites from Orobanchaceae. According to Yang et al. (2002), a study of stigma features and other parts of flowers when considered together can allow a better understanding of the process of floral evolution of hemiparasitic *Pedicularis*, especially their significance in pollination adaptation.

Heslop-Harrison and Shivanna (1977) classified *Orobanche* s.l. stigmas as dry stigmas with unicellular papillae, but sometimes the secretion may appear under a detached

surface cuticle when it has been damaged by pollinating insects. Interestingly, the morphological adaptations of the stigmas were critical for optimum capture of pollen grains. According to Heslop-Harrison and Shivanna (1977), trinucleate pollen tends to be associated with dry stigmas. However, binucleate pollen occurs with both wet and dry stigmas and, in the case of *Orobanche*, the pollen is binucleate and the stigmas are dry. However, the basic subdivision in some families (e.g. Onagraceae) has been problematic and, in some cases, no clear separation can be made (Heslop-Harrison 1981). Amongst angiosperms families, Orobanchaceae is distinguished by the diversity in stigma type, in contrast to most families, in which stigmas are homogeneous (Heslop-Harrison 1981).

## Conclusions

Comparative studies will be required to test further findings about the morphological determinants in stigmas of such variation in the Orobanchaceae family. The possibility of using the morphology of the stigmas may help explain taxonomic relationships in the identification of specimens of problematic taxa. Flowers, including stigmas of holoparasitic plants, have evolved several adaptations for pollination as a process of their parasitic strategies. In addition, floral characters have a special significance in the investigation of parasitic plants whose life cycle has led to a reduction of vegetative structures. A study of the floral stigmas may be useful to systematics and to obtaining knowledge of ecological and co-evolutionary adaptations between the parasites and their pollinators, as well as habitats. Stigma morphology is a highly informative taxonomic criterion that helps to resolve ambiguities in plant taxonomy and evolution and has proved to be a valuable complementary tool for Orobanchaceae species identification. It is noteworthy that this study supports the division between *Orobanche* and *Phelipanche*, as well as subgroups of stigma morphology corresponding to systematic and phylogenetic groups. Our research shows that the most useful features can be seen when observing the stigmas from the bottom side (previous research were related only to observations mainly from the front view), an aspect which has not been studied before. A comprehensive survey of the general and species of holoparasitic Orobanchaceae may lead to a better understanding of the floral morphology of the family.

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# Inflorescences of *Fargesia angustissima* T.P. Yi and *Yushania pauciramificans* T.P. Yi (Poaceae, Bambusoideae) shed light on the taxonomy of the Sino-Himalayan alpine bamboos

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## Abstract

The taxonomy of the Sino-Himalayan alpine bamboos is controversial due to their complex evolutionary history and further complicated by the scarcity of inflorescence. Here, we supplement the description of the inflorescence of *Fargesia angustissima* T.P. Yi and *Yushania pauciramificans* T.P. Yi, which shed light on the taxonomy of *Fargesia* Franchet, *Borinda* Stapleton and *Yushania* Keng. *F. angustissima* has compressed inflorescence unilateral stretching out from reduced spathe, showing a transitional state between species with condensed inflorescence embraced by spathe-like bracts and species with open inflorescence without bracts. Considering that extensive gene flow existed between several clades of *Fargesia* found in recent studies, a broadly-defined *Fargesia* s. l. should be adopted. Meanwhile, the inflorescence of *Y. pauciramificans* has typical characteristics of *Yushania*, such as axilla with tuberculate glands, rachilla internodes ciliate and cylindrical florets, supporting the delimitation of *Yushania*.

## Keywords

*Borinda*, *Fargesia*, inflorescence, reproductive characters, *Yushania*

## Introduction

Although the taxonomy of bamboos has entered a new stage since the proliferation of molecular phylogenetic and phylogenomic studies (e.g., Bamboo Phylogeny Group 2012; Attigala et al. 2016; Zhang et al. 2020; Ye et al. 2021b), morphological characters play an important role in the naming and identification of the species. Reproductive characters are traditionally assumed to be critical in bamboo evolution and taxonomy, especially at the generic level (Li et al. 2006). However, due to variable blooming intervals, ranging from a couple of decades up to 120 years (Janzen 1976), many bamboo species were described without inflorescence information (Yi 1986, 1988a; Stapleton 1994; Keng and Wang 1996; Ohrnberger 1999; Li et al. 2006). This has caused the confusion in the definition of some genera, including some Sino-Himalayan alpine bamboos, e.g., *Fargesia* Franchet, *Yushania* Keng f. and *Borinda* Stapleton (Guo et al. 2002; Stapleton 2021).

*Fargesia* was delimited as having short-necked pachymorph rhizome with uni-caespitose culms and compressed inflorescence subtended by several small or large spathes, while *Yushania* has long-necked rhizome with diffuse culms and open inflorescence without bracts (Li et al. 2006; Yi et al. 2008; Shi et al. 2022). *Borinda* was described as clumping temperate bamboos, similar to *Yushania* for its inflorescence with reduced bracts, and *Fargesia* for its short-necked pachymorph rhizome (Stapleton 1994). Moreover, most species of *Borinda* were transferred from *Fargesia* (Stapleton 1998, 2006, 2021). Although morphological differentiation of rhizome and inflorescence has been used to distinguish these genera, the intermediate state of rhizome and inflorescence between them makes the genus delimitation very ambiguous. As a result, the bamboo accounts of the “Flora of China” recognize two genera, i.e., *Fargesia* and *Yushania* (Li et al. 2006).

In our recent molecular analyses of this taxonomically difficult group based on the double digest-restriction-site associated DNA sequencing (ddRAD) analyses (Ye et al. 2019), *Yushania* was resolved as a well-supported monophyletic lineage, demonstrating the phylogenetic importance of the rhizome type. If considering the rhizome type alone, *Fargesia yunnanensis* Hsueh & T.P. Yi needs to be transferred into *Yushania* for its generally long rhizome neck (12–35 cm). Actually, this species was nested in the ‘Fargesia1’ clade in the analysis of Ye et al. (2019) with high support. Therefore, more information on inflorescence knowledge should be provided for the delimitation of *Yushania*.

*Fargesia* was resolved as a polyphyly (Wang et al. 2017; Zhang et al. 2019; Zhou et al. 2019; Zhou et al. 2020; Ye et al. 2021a) and divided into several clades with high support in the recently ddRAD analyses (Ye et al. 2019). Stapleton (2021) transferred species in the ‘Fargesia3’ + ‘Fargesia4’ + *F. angustissima* clade of Ye et al. (2019) and several species sampled by Zhang et al. (2019) into *Borinda* based on the molecular phylogenetics and some floral and vegetative characteristics. Concurrently, Stapleton (2021) considered that *Fargesia* s. s. possesses tightly unilateral racemes and only distributed along the Qinling Mountains. In this case, most of the species

originally described in *Fargesia* could not be retained in this genus. Nevertheless, several species which were transferred into *Borinda* shared the floral characteristics of *Fargesia* s. s., with raceme enclosing by spathe-like sheaths and protruding from unilateral side, such as *F. edulis* Hsueh & T.P. Yi and *F. adpressa* T.P. Yi (Li et al. 2006; Shi et al. 2022). This indicates that limited reproductive characters cannot distinguish *Borinda* from *Fargesia* appropriately. Therefore, more knowledge of reproductive features should be provided to improve our understanding of the relationship of *Borinda*, *Fargesia* and *Yushania*.

In recent field surveys, we collected the floral and vegetative specimens for two bamboo species. A supplementary description of the inflorescence of these two species is presented here, providing new information on the delimitation of alpine bamboos.

## Materials and methods

We collected two specimens with both floral and vegetative organs during our field work in Yunnan (YXY2020023) and Sichuan (WL2021001), China. Morphological studies were based on the living individuals in the field, specimens, and literature (Yi 1985, 1988a, b; Keng and Wang 1996; Li et al. 2006; Shi et al. 2022). Flowering and fruit materials were dissected under an OLYMPUS DP80 digital microscope at Germplasm Bank of Wild Species of the Kunming Institute of Botany. The morphological terminology follows McClure (McClure 1966).

## Results

According to our observation and comparison of the type specimens and original literature (Yi 1985, 1988b) and bamboo accounts of “Flora Reipublicae Popularis Sinicae” (Keng and Wang 1996), “Flora of China” (Li et al. 2006) and “Illustrated Flora of Bambusoideae in China” (Shi et al. 2022), we identified specimen XYXY2020023 to be *Yushania pauciramificans* T.P. Yi based on paniculate inflorescence on terminating leafy branches without spathes subtending, pachymorph rhizomes with long neck (20–50 cm in length), culms 2–3.2 m, internodes terete, branches 1–3 at lower nodes, 5–6 at upper, culm sheaths cartilaginous, with erect gray setae and absent auricles. WL2021001 was identified to be *Fargesia angustissima* T.P. Yi according to panicles on terminating leafy branches subtended by slightly expanded bracts, pachymorph rhizomes with short neck (2–5 cm in length), uniaespitose culms with fine ridged internodes, culm node less prominent than sheath scar, culm sheaths persistent, which were longer than internodes, narrowly triangular, apically papery, linear, and narrowed for distal 1/3–1/2 of length, sparsely brown setulose, leaf blade abaxially proximally white-gray pubescent. All voucher specimens were deposited at Kunming Institute of Botany, Chinese Academy of Sciences (KUN), and epitype of these two species are also designated here (Turland et al. 2018).



## Taxonomic treatment

### *Fargesia angustissima* T.P. Yi

“油竹子” (You Zhu Zi)

Fig. 1

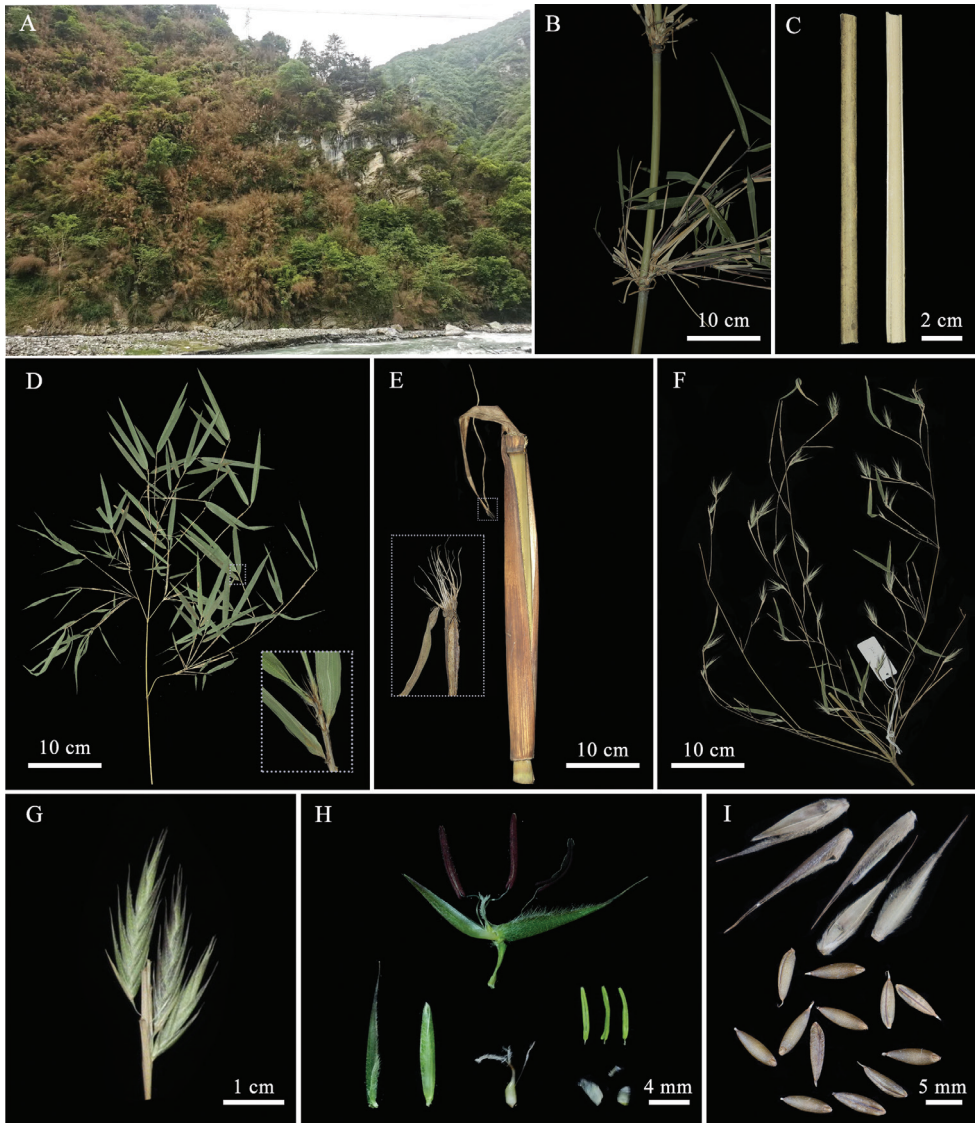
*Fargesia angustissima* T.P. Yi in J. Bamboo Res. 4(2): 21–22. pl. 4. 1985; Keng f. & Z. P. Wang, Fl. Reipubl. Poppularis. Sin. 9(1): 437. pl. 50, 1–8. 1996; D. Z. Li and Stapleton in Z. Y. Wu, P. H. Raven & D. Y. Hong, Fl. China 22: 85–86. 2006. L. B. Zhang in C. Y. Wu, P. H. Raven & H. Y. Hong, Fl. China Illustr. 22: 110. pl. 110: 1–7. 2007. ‘Type’: CHINA. Sichuan: Wenchuan County, Genda Township, 1550 m alt., live on limestone slope, 22 Sept. 1974, *T.P. Yi 74450* (holotype, SCFI!); *ibid.*, 31°4.27'N, 103°19.64'E, 1434 m alt., 23 Dec. 2021, *WL2021001* (epitype designated here, KUN, 1546903!).

≡ *Yushania ferax* subsp. *angustissima* (T.P. Yi) Demoly in Bambou. Bull. A. E. B., Sect. France. 46: 8. 2005.

≡ *Borinda angustissima* (T.P. Yi) Stapleton in Sida. 22(1): 332, 2006.

**Description.** Culms densely unicaespitose, 4–7 m tall, 1–2 cm in diameter; internodes terete, 28–35 cm long, glabrous, initially white powdery, longitudinal ribs very prominent; culm walls 1.5–2.5 mm thick; sheath scars prominent. Buds oblong. Branches 5–10 per node, slender. Culm sheaths persistent or gradually deciduous, much longer than internodes, distantly papery and narrowly banded, abaxially sparsely brown setulose, margins initially densely ciliate; auricles absent; oral setae 3–5, 5–7 mm long; ligules ca. 1 mm tall; blade reflexed, linear, narrower than apex of sheath, margins usually serrulate, readily deciduous. Foliage leaves 3–5 per branchlet; auricles absent; oral setae 5–8, 2–3 mm long; ligules convex, ca. 0.5 mm tall, external ligule pubescent; blades (1.7) 3.4–9.5 × 0.3–0.7 cm, narrowly lanceolate, abaxially proximally pubescent, second veins 2 (3) pairs, transverse veins distinct.

Flowering branches 18–60 cm long, with secondary branches; raceme composed of 1–3 spikelets, open to dense, flowering branchlet with terminal leaves 1–2, gradually deciduous, subtended by slightly inflated foliage-leaf-like sheaths, initially stretching out from one side of the sheaths; axes terete, glabrous, usually with a bract at the base of pedicels, bract lanceolate, 5 mm long. Spikelet purple-green to dark purple, 2–4 cm long, 7–10 mm wide, clustered closely; pedicels slender, curved or undulate, glabrous, 7–15 mm long; florets 2–8, 1.5–2 cm long; rachilla internode 2 mm long, expanded and pubescent at apex. Glumes 2, papery, apex acuminate, the first one narrowly lanceolate, 9–12 mm long, 2 mm wide, pubescent, apically awned, ca. 3 mm long; the second one ovoid-lanceolate, 11–15 mm long, ca. 2 mm wide, pubescent, apically awned, ca. 5 mm long. Lemma papery, ovoid-lanceolate, 15–19 mm long, 4–5 mm wide, abaxially scabrous, densely white setose, apically awned, 5–6 mm long; palea shorter than lemma, thinly papery, 5–10 mm long, 2-keeled, sulcate between



**Figure 1.** *Fargesia angustissima* T.P. Yi **A** habitat, showing flowering population **B** branches and internode **C** clum **D** branchlet, showing foliage leaves **E** culm sheath **F** flowering branches **G** inflorescence **H** anatomy of florets **I** fruit.

keels, upper part of keels ciliate, apex obtuse. Lodicules 3, membranous, transparent, elliptical-triangular, margins ciliate. Stamens 3, filaments free, anthers yellow, gradually dark purple, ovary long-ovoid, glabrous. Stigmas 3, plumose. Caryopses oblong, dark brown, ventrally grooved, 6–9 mm long, ca. 1–2 mm in diameter, glabrous, apex with persistent style.

**Phenology.** New shoots May to August. Flowering December to April; fruiting May to June.

**Distribution and habitat.** *Fargesia angustissima* is known from Dujiangyan, Wenchuan and Chongzhou of western Sichuan, and Beichuan and Pingwu of northwestern Sichuan, and mainly occurs on the steep limestone slope or along the stream at an elevation of 800–1800 m.

**Additional specimens examined.** CHINA. Sichuan: Beichuan County, Caijiaping, near Xiaozhaizigou Nature Reserve, 09 Nov. 2017, Y. X. Zhang 17142 (KUN!).

***Yushania pauciramificans* T.P. Yi**

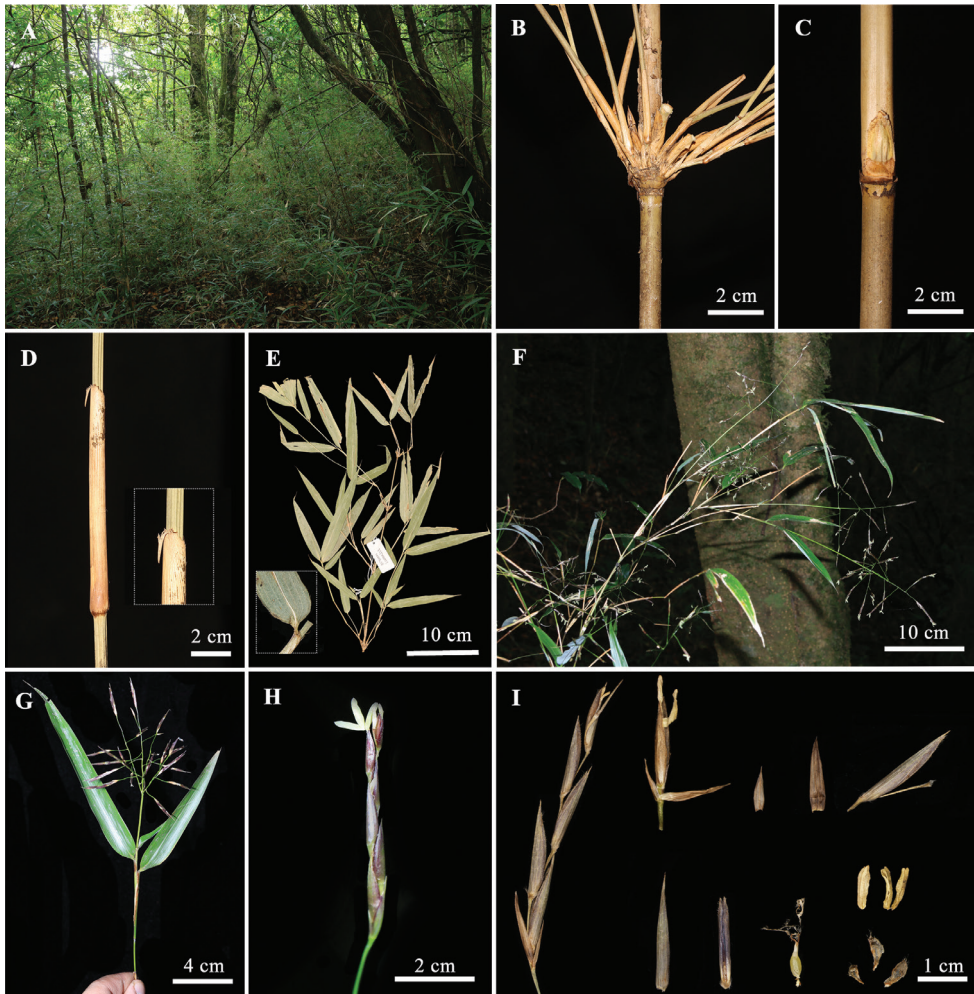
“少枝玉山竹” (Shao Zhi Yu Shan Zhu)

Fig. 2

*Yushania pauciramificans* T.P. Yi in Bull. Bot. Res. 8(4): 71–73. pl. 5. 1988; Keng f. & Z. P. Wang, Fl. Reipubl. Poppularis. Sin. 9(1): 547. pl. 164, 6–8. 1996; D. Z. Li and Stapleton in Z. Y. Wu, P. H. Raven & D. Y. Hong, Fl. China 22: 72. 2006. L. B. Zhang in C. Y. Wu, P. H. Raven & H. Y. Hong, Fl. China Illustr. 22: 96. pl. 96: 6–8. 2007. ‘Type’: CHINA. Yunnan: Xinping County, Ailao Mountain, Liangshan, 2510 m alt., under forest, 1 Sept. 1986, *T.P. Yi* 86237 (holotype, SCFI!); *ibid.*, Gasa Town, 23°57'N, 101°33.90'E, 2257 m alt., 28 May 2020, *YXY2020023* (epitype designated here, KUN, 1546904!).

**Description.** Culms diffuse, 1.5–3.5 m tall, 0.6–1.2 cm in diameter; internodes terete, 15–27 cm long, initially with a white powdery ring below nodes, glabrous; culm walls 2.5–3.5 mm thick, cavity small; nodes weakly prominent; sheath scar obviously prominent, woody. Branches 1–3 at lower nodes, ca. 5 at upper. Culm sheaths persistent, triangularly oblong,  $2/5$ – $1/2$  as long as internodes, cartilaginous, gray setose abaxially, margins densely setose; auricles absent; oral setae 2–4, erect, deciduous; ligule 1–1.5 mm tall, glabrous; blades linear lanceolate, glabrous, reflexed. Foliage leaves 2–5 per branchlet; sheath margins glabrous; auricles absent; oral setae 5–7, slightly curved; ligule 0.5–1 mm tall; blades  $5.2$ – $16 \times 1.1$ – $2.8$  cm, lanceolate or elliptic-lanceolate, base broadly cuneate or rounded, glabrous, secondary veins 4–6 pairs, transverse veins distinct.

Flowering branches 6–22 cm long, lower nodes with secondary flowering branchlets; inflorescence open, paniculate, terminal on leafy branches, composed of 5–15 spikelets, axilla with tuberculate glands, subtended by a small bract; axes terete, 2–10 cm long, glabrous. Spikelet dark purple, 3–6 cm long; pedicels slender, 1.3–3 cm long, usually slightly sinuous, glabrous; florets 2–5, 1.2–3 cm long, cylindrical, apical floret sterile and tubulose; rachilla internodes slightly flattened, ca. 5 mm long, gray white pubescent, apex more densely, margins gray white ciliate. Glumes 2, apically awned, ca. 1 mm, the first one narrowly lanceolate, 4–7 mm long, ca. 1 mm wide, distally white pubescent; the second one ovoid-lanceolate, 6–10 mm long, 1.5–2 mm wide,



**Figure 2.** *Yushania pauciramificans* T.P. Yi **A** habitat **B** branches **C** bud **D** culm sheath **E** branchlet, showing foliage leaves **F** flowering branches **G** inflorescence **H** spikelet **I** anatomy of florets.

distally white pubescent. Lemma mucronate, papery, 7–10 mm long, 3–4 mm wide, densely white setose, apically awned, ca. 1 mm; palea slightly shorter than lemma, thinly papery, 6–9 mm long, 2-keeled, densely pubescent, apex obtuse, 2-cleft. Lodicules 3, membranous, transparent, elliptical-triangular, margins ciliate. Stamens 3, filaments free, anthers yellow, ovary long-ovoid, glabrous. Pistil short, stigmas 2, plumose. Fruits unknown.

**Phenology.** New shoots August. Flowering April to June.

**Distribution and habitat.** *Yushania pauciramificans* is known from Xinping, south-central Yunnan, mainly distributed in the evergreen broadleaved forest at an elevation of 2250–2500 m.



## Discussion

Reproductive features play an important role in the delimitation of the genera of alpine bamboos, and can improve our understanding of the relationship of *Borinda*, *Fargesia* and *Yushania*. Although *Fargesia angustissima* was recombined into *Borinda* in the analysis of Stapleton (2006), its inflorescence characters provide new insight in its delimitation. According to the description, the leaf sheath underneath the inflorescence of *F. angustissima* is inflated but smaller than the spathe of some species of *Fargesia* s. s., such as *F. funiushanensis* T.P. Yi, *F. qinlingensis* T.P. Yi & J. X. Shao, while more similar to those with spikelets stretching out from one side of sheaths and arranging relatively loosely (Zhang and Ren 2016). These characteristics indicate that the inflorescence of *F. angustissima* is in a transitional state between compressed and open ones (Fig. 3). Moreover, *F. angustissima* possesses an independent position on the molecular phylogenetic trees and has a special habitat (Ye et al. 2019; Shi et al. 2022). The florets, phylogenetic position, and habitat of *F. angustissima* imply that it is different from other species of *Fargesia* and could be treated as a new genus, albeit following a narrower genus concept. Additionally, as currently circumscribed, *Borinda* is a paraphyletic group without distinct synapomorphies despite some hair-like vegetative morphology and gene flow occurring frequently between it and *Fargesia*1 clade (Ye et al. 2019; Stapleton 2021; Ye et al. 2021a). Considering the variable vegetative characters and insufficient reproductive features, any new combination of these alpine bamboos should be made cautiously, especially when extensive gene flow exists. Thus, we support the “Flora of China” in adopting a broadly-defined *Fargesia* s. l., rather than *Borinda* to minimize nomenclatural change.

The flowering branches and flowers of *Yushania pauciramificans* are similar to those of species of *Yushania*, both have similar paniculate inflorescence, axilla with tuberculate glands, ciliate rachilla internodes and florets. Combined with the reduced gene flow between *Yushania* and *Fargesia* revealed by D-statistic tests, the inflorescence state of *Y. pauciramificans* supports the monophyly of *Yushania* further (Ye et al. 2019).



**Figure 3.** Comparison of inflorescence **A** *Fargesia funiushanensis* T.P. Yi **B** *Fargesia angustissima* T.P. Yi **C** *Yushania pauciramificans* T.P. Yi.



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## Three new species of *Elatostema* (Urticaceae) from Thailand

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### Abstract

Three new species of *Elatostema* (Urticaceae) from Thailand, *E. kaweesakii* Triyutth. & L.F.Fu, **sp. nov.**, *E. rubricaula* Triyutth. & L.F.Fu, **sp. nov.** and *E. saxatile* Triyutth. & L.F.Fu, **sp. nov.**, are newly described and illustrated. These new species can be distinguished by the presence of rhizome. *Elatostema kaweesakii* is similar to *E. atroviride*. *Elatostema kaweesakii* is a lithophyte growing in limestone crevices. It differs from *E. atroviride* by its large swollen rhizome, glabrous stem, glabrous receptacle, number of tepal in staminate flower, absence of tepal in pistillate flower, presence of staminodes in pistillate flower and smooth achene. *Elatostema rubricaula* and *E. saxatile* are found on sandstone habitats. They have distinct flattened and disk-like rhizome. *Elatostema rubricaula* is distinguished by its distinct sulcate and reddish stem with flattened and disc-like rhizome and chartaceous leaves with entire margin. *Elatostema saxatile* resembles *E. bulbiferum* but differs by its flattened and disc-like rhizome, acute leaf apex, glabrous receptacle in pistillate inflorescences, presence of staminodes in pistillate flower, and its sandstone habitat. Descriptions, distribution, ecological and phenological data are provided.

### Keywords

Flora of Thailand, limestone, nettle family, rhizomatous plant, Rosales, sandstone, taxonomy

## Introduction

*Elatostema* J.R. Forst. & G. Forst. is a large genus of Urticaceae containing several hundred species distributed throughout tropical and subtropical Asia (Christenhusz et al. 2017; Fu et al. 2021). *Elatostema* is a succulent herb normally found in evergreen forest, along stream, gorges, caves and limestone mountains (Wang 2014; Fu et al. 2017; Monro et al. 2018). Based on molecular and morphological evidences, a recent systematic study (Tseng et al. 2019) demonstrated that *Elatostema* is a monophyletic genus that includes *Pellionia* Gaudich.

The first checklist of *Elatostema* (including *Pellionia*) in Thailand documented 14 species (Yahara 1984). Since then, 20 species of *Elatostema* (including *Pellionia*) were listed by Pooma and Suddee (2014). However, a recent study involving extensive investigations has resulted in 75 species of *Elatostema* in Vietnam (Fu et al. 2019), which doubles the number of species in the previous checklist (Hiep 2005). This suggests that the species diversity documentation in Thailand was out of date due to its under-sampled status (Middleton et al. 2019).

As part of ongoing research into the flora of Thailand, the authors undertook extensive field investigations and collected three unknown specimens of *Elatostema* from the northeastern region of Thailand. After a careful comparison among specimens, it was clear that these specimens belong to the clade that includes *Pellionia* and *Elatostema* in Tseng et al. (2019), but they were distinct from other species of this clade. Therefore, we confirmed them as three new species of *Elatostema*.

## Materials and methods

Between 2016 and 2022, the authors conducted several field trips throughout Thailand. The herbarium collections from AAU, BCU, BK, BKF, C, HNU, IBK, K, L, KKU, P, PSU, QBG and SING (Thiers 2022) were examined mainly by the first author. All morphological characters were measured and compared based on dried specimens from field and herbarium collections.

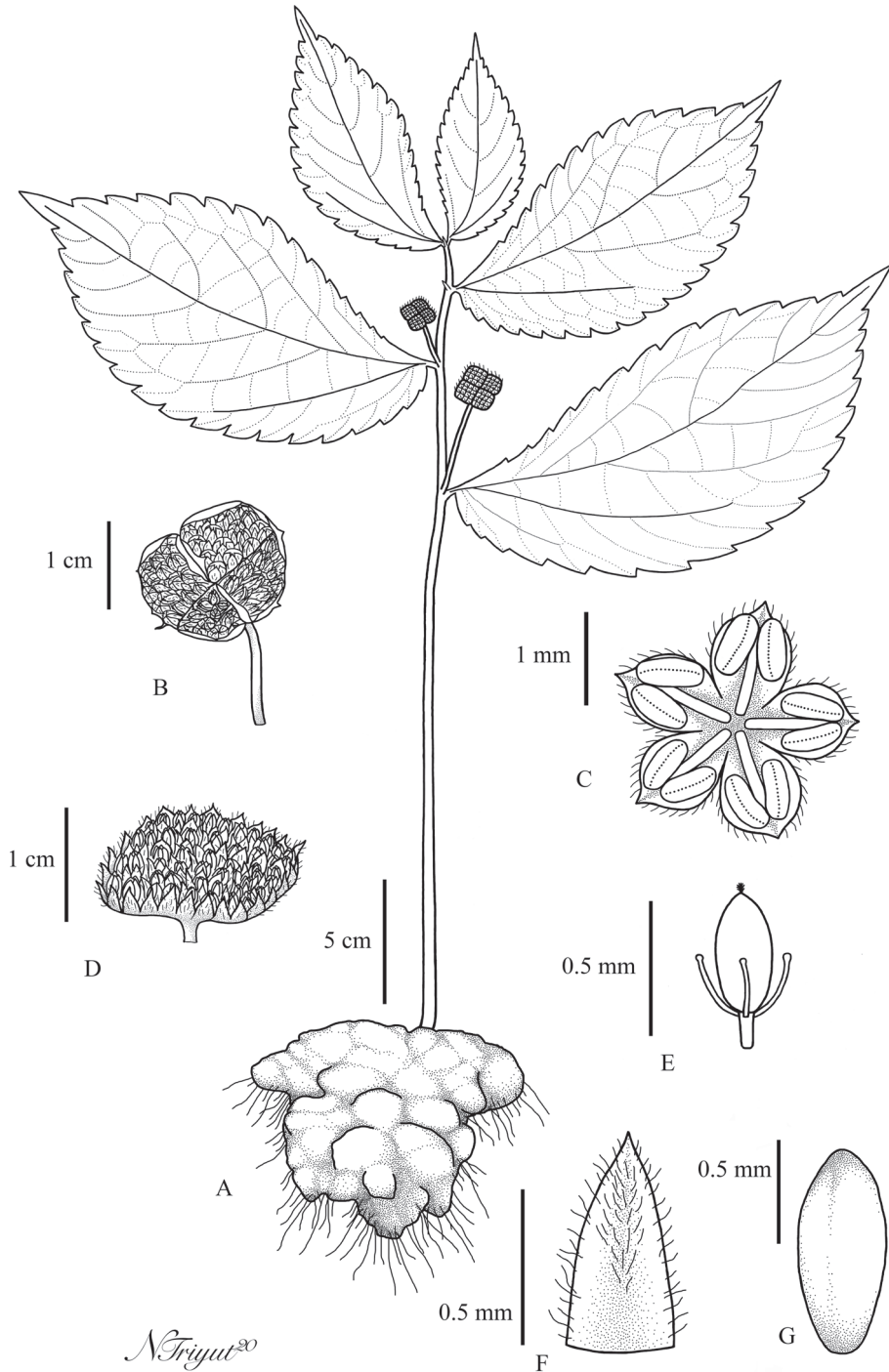
## Taxonomic treatment

### 1. *Elatostema kaweesakii* Triyutth. & L.F.Fu, sp. nov.

urn:lsid:ipni.org:names:77309834-1

Figs 1, 2

**Diagnosis.** *Elatostema kaweesakii* is similar to *E. atroviride* W.T. Wang but differed by its large swollen rhizome, staminate inflorescences with 2–7 cm long peduncle, receptacle rectangular, glabrous, staminate flower with 5 ovate tepals, pistillate inflorescences with 5–10 mm long peduncle, receptacle rectangular, glabrous, pistillate flower without tepals, staminode present and achene smooth (Table 1).



**Figure 1.** *Elatostema kaweesakii* Triyutth. & L.F.Fu, sp. nov. **A** habit **B** staminate inflorescence **C** staminate flower **D** pistillate inflorescence **E** pistillate flower **F** bracteole **G** achene (Drawn by N. Triyutthachai).



**Type.** THAILAND. Loei: Nong Hin district, 17°7.3'N, 101°56.05'E, 360 m alt., 9 August 2022, *Triyutth.* 332 (holotype KCU!; isotypes AAU!, BKF!).

**Description.** Perennial herbs, lithophytic, monoecious, rhizomatous. *Rhizome* rounded, 2–20 cm in diam., brownish. *Stems* 15–50 cm tall, simple or branched, succulent, greenish, glabrous. *Stipules* 2, persistent or sometimes caducous in reproductive stage, linear or lanceolate, 4–7 × 1–3 mm, membranous, glabrous. *Nanophylls* absent. *Leaves* distichous, alternate; petiole 0.5–1 cm long, greenish, glabrous; lamina asymmetrically ovate to elliptic, 5–20 × 3–10 cm, base oblique, margin serrate to dentate, apex acuminate, chartaceous; venation pinnate, major basal lateral veins present; upper and lower surfaces greenish, glabrous, cystoliths fusiform, 0.2–0.5 mm long. *Staminate inflorescences* axillary, solitary, capitate, 1.5–2.0 cm in diam.; peduncle 2–7 cm long, glabrous or pubescent; receptacle rectangular, 0.6–1.0 × 0.6–1.0 cm, chartaceous, glabrous; bracts ovate, connate at base, membranous, glabrous; bracteoles lanceolate, 0.6–0.8 × 0.2–0.3 mm, membranous, pubescent. *Staminate flowers* 80–100 per inflorescence, sessile to subsessile, glabrous; tepals 5, ovate, 1.0–1.5 × 1.0–1.5 mm, apex acuminate, membranous, pubescent; stamens 5, filaments 1.0–1.5 mm long, anthers 0.6–0.8 mm long. *Pistillate inflorescences* axillary, solitary, capitate 5–10 mm in diam., peduncle 0.5–1.0 cm long, glabrous; receptacle rectangular, 6–8 × 6–8 mm, chartaceous, glabrous; bracts ovate, 0.8–1.0 × 0.5–0.6 mm, membranous, pubescent; bracteoles lanceolate, 0.4–0.6 × 0.2–0.3 mm, membranous, pubescent. *Pistillate flowers* 40–100 per inflorescence, sessile to subsessile, glabrous; tepals absent; staminodes 3, oblong to linear, 0.2–0.3 mm long; ovary superior, ovoid, 0.3–0.5 mm long. *Achenes* ellipsoid, 0.8–1.2 mm long, brownish, smooth.

**Distribution.** Currently known only from Loei province in northeastern Thailand.

**Ecology.** Occur in limestone crevices.

**Phenology.** Flowering and fruiting in April–December.

**Etymology.** This species is named in honor of Mr Kaweesak Keeratikiat, who first discovered the plants.

**Conservation status.** This species was found in fewer than 5 locations at Nong Hin district, Loei province, Thailand. Moreover, it has a very small population and very restricted distribution, and the number of mature individuals is fewer than 250. According to IUCN (2022) this species should be assessed as Endangered (EN) under IUCN criteria B2a and D1.

**Additional specimens examined.** THAILAND. Loei: Nong Hin district, 16 December 2013, *S. Saengvirotjanapat* 635 (QBG!), *ibid.*, 20 April 2021, *Triyutth.* 329 (KCU!).

**Notes.** This species is similar to *E. atroviride* in their habitat as lithophytes growing in limestone crevices. Both species have rounded rhizome, absence of nanophyll and a similar shape of leaves. However, it differs from *E. atroviride* by several characters such as its large rounded rhizome (small size of rhizome in *E. atroviride*), staminate inflorescences with 2–7 cm long peduncle (vs 2–5 mm long), receptacle rectangular and glabrous (vs elliptic and pubescent), staminate flower with 5 tepals (vs 4 tepals), pistillate inflorescence with 5–10 mm long peduncle (vs 2–4 mm long), absence of tepals in pistillate flower (vs 3 tepals), 3 staminodes (vs staminode absent) and achene smooth (vs 6–8 ribbed).



**Figure 2.** *Elatostema kaweesakii* Triyutth. & L.F.Fu, sp. nov. **A** habit and habitat **B** pistillate inflorescence **C** staminate inflorescences (Photos **A**, **C** by K. Keeratikiat, **B** by Triyutth).

**Table 1.** Comparison of *Elatostema atroviride*, *E. bulbiferum*, *E. kaweesakii*, *E. rubricaula* and *E. saxatile*.

Characters	<i>E. atroviride</i>	<i>E. kaweesakii</i>	<i>E. rubricaula</i>	<i>E. saxatile</i>	<i>E. bulbiferum</i>
Habitat	lithophyte in limestone soils	lithophyte in limestone crevices	lithophyte on sandstone rocks	lithophyte on sandstone rocks	lithophyte in limestone crevices
Rhizome	slender rhizome or stoloniferous	large swollen rounded rhizome	flattened, disk-like rhizome	flattened, disk-like rhizome	swollen rounded rhizome
Stem	Simple or branched, greenish, pubescent	Simple or branched, greenish, glabrous	Simple, reddish, glabrous	Simple or branched, greenish, glabrous	Simple or branched, greenish, glabrous
Nanophyll	absent	absent	absent	present	present
Leaf margin	dentate	serrate to dentate	entire	serrate	serrate
Leaf apex	acuminate	acuminate	obtuse	acute	acuminate to caudate
Staminate inflorescences: type	Capitate	Capitate	Umbellate	Umbellate	Umbellate
Staminate inflorescences: receptacle	elliptic, chartaceous, pubescent	rectangular, chartaceous, glabrous	absent	absent	absent
Staminate inflorescences: peduncle	2–5 mm long	2–7 cm long	2–3 cm long	1–5 cm long	2–5 cm long
Staminate flower: tepal number	4	5	5	5	5
Pistillate inflorescences: type	capitate	capitate	capitate	capitate	capitate
Pistillate inflorescences: receptacle	oblong, membranous, pubescent	rectangular, chartaceous, glabrous	elliptic, membranous, glabrous	elliptic, membranous, glabrous	elliptic, membranous, pubescent
Pistillate inflorescences: peduncle	2–4 mm long, glabrous	5–10 mm long, glabrous	sessile to subsessile, glabrous	0.5–5 mm long, glabrous	0–2 mm long, glabrous
Pistillate flower: tepal number	3	absent	5	5	5
Pistillate flower: staminode	absent	3	5	5	absent
Achene	6–8 ribbed	smooth	smooth	smooth	smooth

## 2. *Elatostema rubricaula* Triyutth. & L.F.Fu, sp. nov.

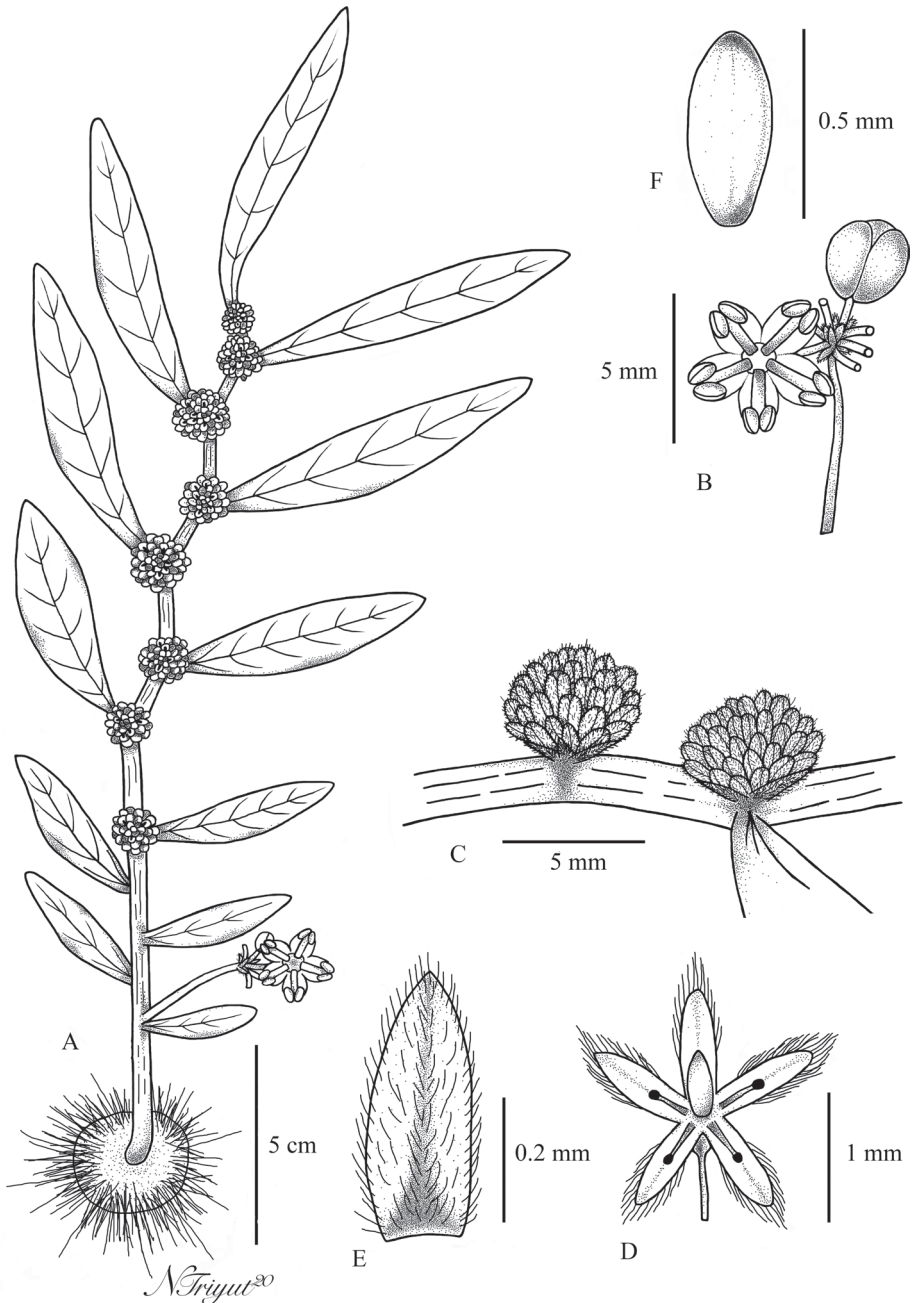
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Figs 3, 4

**Diagnosis.** *Elatostema rubricaula* differs from other Thai *Elatostema* by its distinct sulcate and reddish stem with flattened and disk-like rhizome and chartaceous leaves with entire margin. It grows on seasonally moist sandstone rock (Table 1).

**Type.** THAILAND. Buengkan: Wat A-Hong Silawas, 18°25.47'N, 103°28.2'E, 160 m alt., 13 September 2017, *Triyutth. 201* (holotype KCU!; isotypes AAU!, BKF!, IBK!, K!).

**Description.** Perennial herbs, lithophytic, monoecious, rhizomatous. **Rhizome** flattened, disk-like, 1–3 cm in diam., brownish. **Stems** 5–25 cm tall, simple, sulcate, succulent, reddish, glabrous. **Stipules** 2, persistent or sometimes caducous in reproductive stage, linear or lanceolate, 2.0–2.5 × 0.5–1.5 mm, membranous, glabrous. **Nanophylls** absent. **Leaves** distichous, alternate; petiole 0.5–2.0 mm long, reddish, glabrous; lamina asymmetrically lanceolate to elliptic, 3–5 × 0.5–1.5 cm, base asymmetrical attenuate, margin entire, apex obtuse, chartaceous; venation pinnate, major basal lateral veins



**Figure 3.** *Elatostema rubricaula* Triyutth. & L.F.Fu, sp. nov. **A** habit **B** staminate inflorescence **C** pistillate inflorescences **D** pistillate flower **E** bracteole **F** achene (Drawn by Triyutth).

absent, lateral veins 5–7 pairs; upper surface greenish, glabrous, cystolith fusiform, 0.2–0.5 mm long; lower surface cinereous, glabrous, cystoliths fusiform, 0.2–0.5 mm long. **Staminate inflorescences** axillary, solitary, umbellate; peduncle 2–3 cm long,





**Figure 4.** *Elatostema rubricaulle* Triyutth. & L.F.Fu, sp. nov. **A** habit and habitat **B** pistillate inflorescences **C** staminate inflorescence (Photos by Triyutth).



glabrous; receptacle absent; bracts lanceolate, 0.8–1.0 × 0.3–0.5 mm, membranous, pubescent; bracteoles lanceolate, 0.6–0.8 × 0.2–0.3 mm, membranous, pubescent. **Staminate flowers** 5–10 per inflorescence; pedicel 1–3 mm long, glabrous; tepals 5, ovate to oblong, 1.0–1.5 × 1.0–1.5 mm, apex obtuse, membranous, glabrous; stamens 5, filaments 1.5–2.0 mm long, anthers 0.6–1.0 mm long. **Pistillate inflorescences** axillary, solitary, capitate 5–8 mm in diam., sessile to subsessile, glabrous; receptacle elliptic, 2.0–2.5 × 1.0–1.5 mm, membranous, glabrous; bracts lanceolate, 0.4–0.6 × 0.2–0.3 mm, membranous, pubescent; bracteoles lanceolate, 0.3–0.4 × 0.2–0.3 mm, membranous, pubescent. **Pistillate flowers** 50–80 per inflorescence; pedicel 0.5–1.0 mm long, glabrous; tepals 5, lanceolate to oblong, 0.8–1.2 × 0.2–0.4 mm, membranous, pubescent; staminodes 5, linear, 0.2–0.4 × 0.1–0.2 mm; ovary superior, ovoid, 0.3–0.5 mm long. **Achenes** ellipsoid, 0.5–0.8 mm long, brownish, smooth.

**Distribution.** Currently known only from the type locality in northeastern Thailand.

**Ecology.** Occurs on seasonal moist sandstone rocks in shaded areas.

**Phenology.** Flowering and fruiting in July–October.

**Etymology.** Latin *ruber*, red, and *caulus*, stem, alluding to stem color of the new species.

**Conservation status.** *Elatostema rubricaula* is currently known only from the type locality, which is not a protected area, and the number of mature individuals is fewer than 1,000. According to IUCN (2022), this species should be assessed as Critically Endangered (CR) according to criteria B1a, B2a and D1.

**Additional specimens examined.** THAILAND. Buengkan: Wat A-Hong Silawas, 18°25.47'N, 103°28.2'E, 160 m alt., 28 April 2018, *Triyutth.* 269 (KKU!).

**Notes.** This species differs from other Thai *Elatostema* by its habitat. Most species of *Elatostema* are dwelling on limestone, stream bank, gorges and caves in evergreen forest, but *E. rubricaula* was found on moist sandstone rocks near the bank of Mekong River in Buengkan Province in the northeastern part of Thailand. The most distinguished characters of this species are the flattened and disk-like rhizome, sulcate and reddish stem, chartaceous leaves with entire margin and umbellate staminate inflorescences.

### 3. *Elatostema saxatile* Triyutth. & L.F.Fu, sp. nov.

urn:lsid:ipni.org:names:77309836-1

Figs 5, 6

**Diagnosis.** *Elatostema saxatile* is similar to *E. bulbiferum* Kurz, but differs by its flattened and disk-like rhizome, receptacle of pistillate inflorescences glabrous, staminode in pistillate flower 5, acute leaf apex and its sandstone habitat (Table 1).

**Type.** THAILAND. Nakhon Phanom: Tad Kham Waterfall, 17°57.228'N, 104°9.6'E, 160 m alt., 13 September 2017, *Triyutth.* 200 (holotype KKU!; isotypes AAU!, BKF!, IBK!).

**Description.** Perennial herbs, lithophytic, monoecious, rhizomatous. **Rhizome** flattened, disk-like, 2–7 cm in diam., brownish. **Stems** 5–35 cm tall, simple or

branched, succulent, greenish, glabrous. **Stipules** 2, persistent or sometimes caducous in reproductive stage, linear or lanceolate, membranous, glabrous. **Nanophylls** lanceolate to oblanceolate,  $0.5\text{--}1.5 \times 0.2\text{--}1.0$  cm, chartaceous, glabrous, cystoliths fusiform. **Leaves** distichous, alternate; petiole 1–3 mm long, glabrous or puberulous; lamina asymmetrically elliptic to obovate,  $1.5\text{--}7.0 \times 0.5\text{--}3.0$  cm, base oblique, margin serrate, apex acute, chartaceous; venation pinnate, major basal lateral veins absent, lateral veins 5–7 pairs; upper surface green, glabrous, cystolith fusiform, 0.2–0.5 mm long; lower surface greenish or cinereous, glabrous, cystolith fusiform, 0.2–0.5 mm long. **Staminate inflorescences** axillary, solitary, umbellate; peduncle 1–5 cm long, glabrous; receptacle absent; bracts lanceolate,  $0.6\text{--}1.0 \times 0.3\text{--}0.4$  mm, membranous, pubescent; bracteoles lanceolate,  $0.5\text{--}0.8 \times 0.2\text{--}0.3$  mm, membranous, pubescent. **Staminate flowers** 10–30 per inflorescence; pedicel 1.0–1.5 mm long, glabrous; tepals 5, ovate to oblong,  $1.0\text{--}1.5 \times 1.0\text{--}1.5$  mm, apex obtuse, membranous, glabrous; stamens 5, filaments 1.0–1.5 mm long, anthers 0.6–1.0 mm long. **Pistillate inflorescences** axillary, solitary, capitate, 2–8 mm in diam., subsessile to pedunculate; peduncle 0.5–5.0 mm long, glabrous; receptacle elliptic, 2–4 mm in diam., membranous, glabrous; bracts lanceolate,  $0.2\text{--}0.3 \times 0.8\text{--}1.0$  mm, membranous, pubescent; bracteoles lanceolate,  $0.2\text{--}0.3 \times 0.5\text{--}0.8$  mm, membranous, pubescent. **Pistillate flowers** 20–50 per inflorescence; pedicel 0.5–1.5 mm long, glabrous; tepals 5, lanceolate,  $0.8\text{--}1.2 \times 0.2\text{--}0.4$  mm, membranous, pubescent; staminodes 5, oblong,  $0.1\text{--}0.2 \times 0.1\text{--}0.2$  mm; ovary superior, ovoid, 0.4–0.8 mm long. **Achenes** ellipsoid, 0.8–1.2 mm long, brownish, smooth.

**Distribution.** Northeastern Thailand.

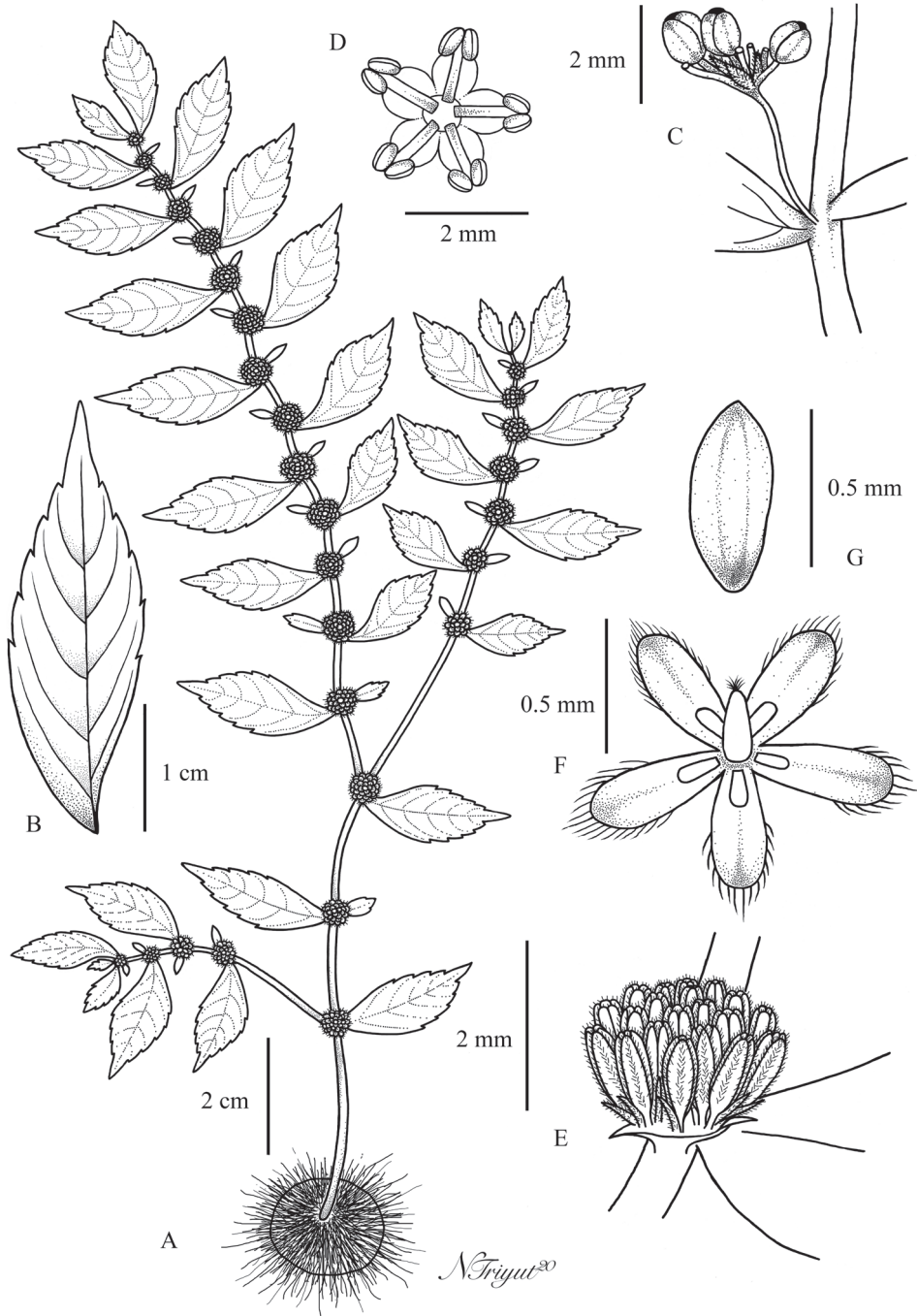
**Ecology.** Occurs on seasonal moist sandstone rocks in dry evergreen forest, at 100–200 m alt.

**Phenology.** Flowering and fruiting in May–October.

**Etymology.** Latin *saxum*, rock, and *atile*, place of growth, alluding to habitat of the new species among rocks

**Conservation status.** This species was found scattered in ca. 6 locations in the Northeastern part of Thailand and the number of mature individuals is fewer than 1,000. According to IUCN (2022), *E. saxatile* should be assessed as Vulnerable (VN) according to criteria B2a and D1.

**Additional specimens examined.** THAILAND. Buengkan: Chet Si Waterfall, 14 September 2017, *Triyutth. 202* (KKU!), *ibid.*, 27 April 2018, *Triyutth. 266* (KKU!), 267 (KKU!), Chanean Waterfall, 14 September 2017, *Triyutth. 203* (KKU!), Phu Wua, 21 May 2004, *R. Pooma et al. 4201* (BKF!), 4202 (BKF!), 4191 (BKF!), *ibid.*, 28 December 2011, *M. Norsaengsri & N. Tathana 8707* (QBG!), *ibid.*, 13 July 2016, *Triyutth. 97* (KKU!), 98 (KKU!), Phu Sing, 26 August 2001, *R. Pooma et al. 2694* (BKF!), Phu Tok, 27 April 2018, *Triyutth. 268* (KKU!), Phu Tok Noi, 22 June 1995, *C. Niyomdham 4448* (BKF!), *ibid.*, 21 June 1997, *C. Niyomdham 5052* (BKF!), Wat Tham Phra, Phu Wua, 20 May 2014, *S. Sirimongkol et al. 593* (BKF!); Nakhon Phanom: Tad Kham Waterfall, 25 August 2001, *R. Pooma et al. 2652* (BKF!), *ibid.*, 13 September 2017, *Triyutth. 199* (KKU!).



**Figure 5.** *Elatostema saxatile* Triyutth. & L.F.Fu, sp. nov. **A** habit and habitat **B** leaf **C** staminate inflorescence **D** staminate flower **E** pistillate inflorescence **F** pistillate flower **G** achene (Drawn by N. Triyutthachai).





**Figure 6.** *Elatostema saxatile* Triyutth. & L.F.Fu, sp. nov. **A** habit and habitat **B** stem and rhizome **C** pistillate inflorescences **D** staminate inflorescence (Photos by Triyutth).

**Notes.** *Elatostema saxatile* was found growing on the sandstone rocks in Buengkan and Nakhon Phanom Provinces, in the Northeastern part of Thailand. This species is similar to *E. bulbiferum* in the presence of rhizome, presence of nanophyll and umbellate staminate inflorescences, but differed by its flattened and disc-like rhizome (vs rounded rhizome in *E. bulbiferum*), receptacle of pistillate inflorescences glabrous (vs pubescent), staminode in pistillate flower 5 (vs absent), acute leaf apex (vs acuminate to caudate apex). Moreover, *E. saxatile* was found on sandstone while *E. bulbiferum* was found on limestone substrates.

## Acknowledgements

We are grateful to the Directors, Curators and staff of AAU, BCU, BK, BKF, C, HNU, IBK, K, KKU, L, P, PSU, QBG and SING for making the materials available for this study. This work was supported by the Applied Taxonomic Research Center, Khon Kaen University grant ATRC\_R6102 and the Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2016 from Graduate School and Faculty of Science, Khon Kaen University, the Carlsberg Foundation for the Flora of Thailand project, the National Natural Science Foundation of China (31860042) and the Light of West China Program of the Chinese Academic of Sciences ([2020]82).

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# A new species of *Diplostephium* (Asteraceae, Astereae) from the Atacama Desert, Chile

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## Abstract

A new species, *Diplostephium paposanum* S.T.Ibáñez & Muñoz-Schick, **sp. nov.**, is described for Chile, extending the southern distribution of the genus. Its position within the genus was confirmed by morphological and molecular data, discussed here. The new species was found in a coastal environment, new to the genus, and is geographically far removed from the other Chilean species, which are from the Andes. The formation where it occurs, known as lomas, acts as a biodiversity refuge in hyperarid environments. The presence of *D. paposanum* in this environment contributes to the evidence of a floristic connection between the Atacama Desert and the Neotropical Andes.

## Keywords

Antofagasta, coast, fog oasis, lomas, molecular analysis, Paposo, taxonomy

## Resumen

Se describe una nueva especie para Chile, *Diplostephium paposanum* S.T.Ibáñez & Muñoz-Schick, **sp. nov.**, extendiendo la distribución austral del género. Su posición dentro del género fue confirmada por datos morfológicos y moleculares, discutidos acá. La nueva especie se encontró en un ambiente costero, nuevo para el género, y está geográficamente alejada de las otras especies chilenas, que son de los Andes. La formación donde habita, conocida como lomas, actúa como refugio de la biodiversidad en ambientes hiperáridos. La presencia de *D. paposanum* en este ambiente contribuye a la evidencia de una conexión florística entre el Desierto de Atacama y los Andes Neotropicales.

**Palabras clave**

Análisis molecular, Antofagasta, costa, lomas, oasis de niebla, Paposos, taxonomía

**Introduction**

The genus *Diplostephium* Kunth, in the broad sense, is a diverse group of 111 species (Vargas 2011) distributed continuously on high altitudes of neotropical Andes, from Chile to Venezuela, with some isolated species occurring in Costa Rica and Sierra Nevada (Blake 1922; Cuatrecasas 1969; Vargas 2011). The country with the highest number of species is Colombia, with 63 species described (Vargas 2011). Although it is found in many ecosystems, species of this genus prefer highland conditions, such as open shrublands, grasslands, or upper montane forests (Weddell 1855; Cuatrecasas 1969; Vargas and Madriñán 2012; Vargas 2018).

*Diplostephium* was defined by Kunth (von Humboldt and Bonpland 1820), and separated from similar genera by vegetative and floral characters. This diagnosis was complemented by several authors as more species were described. For instance, Weddell (1855) improved the description of some morphological characters, mainly floral, and indicated the tropical Andes as the only habitat of the genus, expressing a level of uncertainty by adding a question mark to the statement. Weddell (1855) also separated the genus based on the synflorescence structure and leaf shape to help the identification, a criterion followed by Blake (1922, 1928) for his classification. The largest contribution to the genus was made by José Cuatrecasas, who described more than 90 species, 65 of them currently accepted (Vargas 2011). In his classification, Cuatrecasas (1969) used the series established by Blake (1922, 1928) and described new series, which were used by Vargas (2011) in his checklist of the genus.

Vargas et al. (2017) found that *Diplostephium* is not monophyletic, reporting two separate clades that are structured geographically. Those species present between latitudes 11°N and 3°S (60 species) were included in the genus *Linochilus* Benth (Saldivia et al. 2019), whereas the species present between latitudes 1°N and 22°S (48 species) maintained the genus name. Species included in *Linochilus* can be differentiated from *Diplostephium sensu stricto* (s. s.) by the combination of characters as habit, branching pattern, number of capitula, and the length of corolla ray florets, although no character can be used on its own to distinguish between genera (Vargas 2018).

The last molecular study of the genus *Diplostephium* (Vargas et al. 2017) corroborated previous findings (Vargas and Madriñán 2012) that indicated the lack of monophyly of the genus, and certain incongruences between phylogenies obtained with the nuclear versus chloroplast regions. This was evident in the study by Vargas et al. (2017), who used genomic technology and produced sequences for an extensive nuclear region and the whole chloroplast of most of the known species of *Diplostephium*.

Until now, three species of *Diplostephium* s. s. have been recorded in Chile; *D. cinereum* Cuatrec., *D. tacorense* Hieron. and *D. meyenii* Wedd. (Vargas 2011). The

last species has been collected at latitude 22°S (Termas de Puritama, Jul 1969, *O. Zöellner 3014* [PUCV]), the southernmost record of the genus observed by us (Moreira-Muñoz et al. 2016). This study reports a new species of *Diplostephium s. s.* found more than 200 km south of the southern limit of the genus distribution (latitude 24°30'S), herein including a newly associated habitat for the genus, which are commonly referred to as lomas. Such formations are located within the Atacama (Chile) and Sechura (Peru) deserts, and in which vegetation depends on coastal humidity for survival (Rundel et al. 1991, 2007; Cereceda et al. 2008). These formations are adapted to satisfy hydric requirements through humidity in the fog contributed by stratocumulus coming from the Pacific Ocean at low elevations (below 1,000 m), vernacularly called *camanchaca*. The effect of the stratocumulus is especially notorious in the southern Antofagasta Region, around the locality of Paposo, where vegetation thrives in a similar way as it does in the lomas formations in Perú (Johnston 1929; Ricardi 1957; Rundel et al. 1991).

## Materials and methods

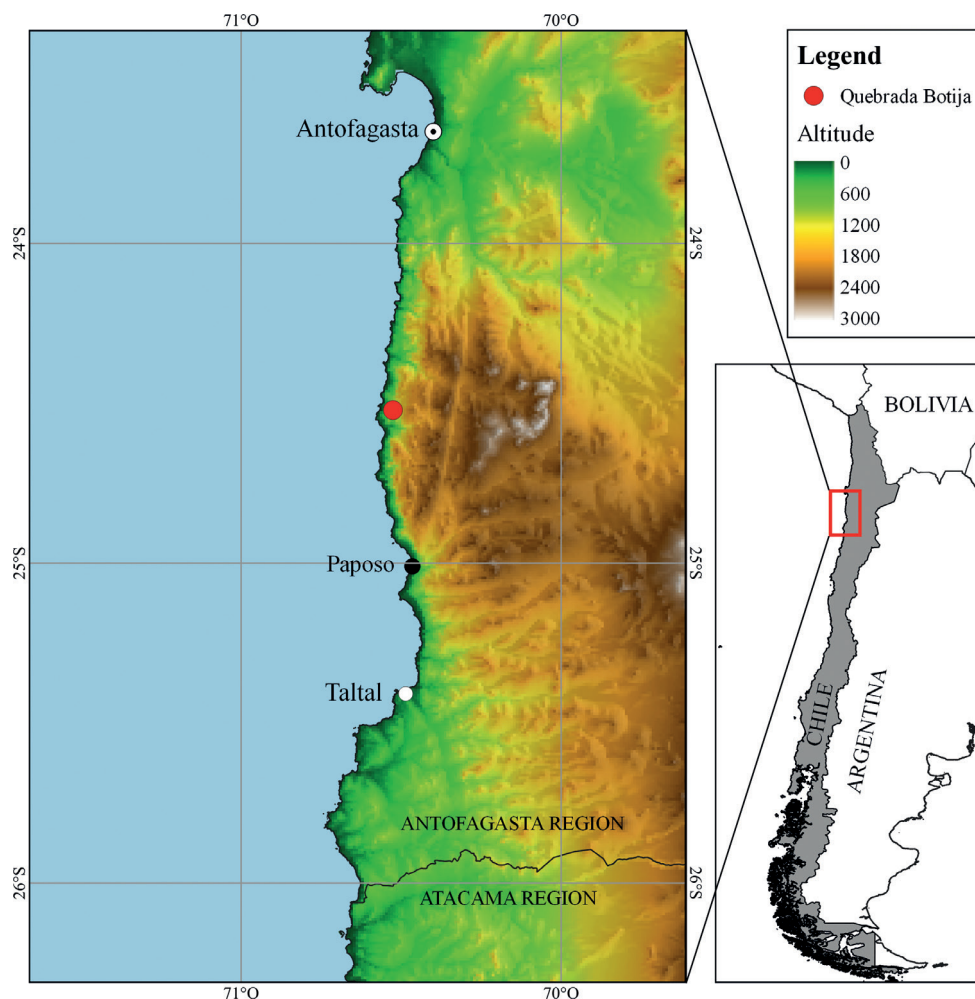
### Fieldwork

During a field campaign carried out in December 2020 on the coast of the Atacama Desert, this species was found and collected in Quebrada Botija, an area located in northern Paposo, at an altitude of 170 m (Fig. 1). Afterwards, the location was continuously visited in order to document the full extent of the population including the number of individuals, details of its natural habitat, and to collect seeds for *ex situ* conservation. Photographs in habitat were taken using a camera (Nikon D7500) with macro lens (Sigma 105 mm f2.8G).

### Study of specimens

Local floras, regional checklists (e.g. Johnston 1929, 1930, 1932; Rundel et al. 1996; Luebert et al. 2007) and herbarium specimens from SGO were revised for the coast of the Antofagasta Region, with emphasis on the family Asteraceae. Chilean *Diplostephium* species were studied, as well as species morphologically similar in leaves size and capitula arrangement (e.g. *D. sagastegui* Cuatrec., *D. foliosissimum* S.F. Blake), for which original descriptions and photographs of taxonomical types observed online (<https://plants.jstor.org/>) were compared with the new species.

For morphological study, herbarium specimens were dissected and the different morphological components of the plant were studied and recorded for the taxonomic description. Dissected parts were measured under the stereoscopic lens with a scale, and also photographed combining the lens and a camera (Canon G16), and measured afterwards using the software ImageJ (Rasband 1997–2008). Collected and studied specimens were deposited at the herbarium of the National Museum of Natural History (SGO) in Chile. Species nomenclature follows Zuloaga et al.(2008).



**Figure 1.** Location of *Diplostephium paposanum*. The red dot indicates the location of “Quebrada Botija”, the type locality of the new species.

## Molecular analysis

Because of the complexities of the phylogeny previously explained, and the fact that it was not the goal of this study to generate a genomic study, we decided to perform a Barcoding analysis using two commonly used markers for the Asteraceae, comparing the sequences for the new species to the vast number of sequences for the group available in GenBank.

Genomic DNA were isolated from silica gel-dried leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s recommendations. Two regions were amplified: The internal external transcribed spacer (ETS) region of nuclear rDNA, using primers 18S-ETS (Baldwin and Markos 1998) and Ast8 (Markos and Baldwin 2001), and the chloroplast DNA spacer trnL-trnF using primers



“c” and “f” (Taberlet et al. 1991). This combination of primers allowed amplification of a section of approximately 800 bp of this chloroplast region.

For all regions, a polymerase chain reaction (PCR) amplification were performed with 12.5 µL GoTaq Colorless Master Mix (Promega), 2.5 µL of each 10 µM primer, 2.5 µL of 1 mg/ml BSA, 2 µL of DNA template, and distilled nuclease free water for a 25 µL reaction. The PCR reaction followed the protocols of Bonifacino and Funk (2012) for ETS and Taberlet et al. (1991) for trnL-trnF. All PCR reactions consisted of 35 cycles.

Products were purified and sequenced using the Applied Biosystems sequencer ABI3500XL at the Pontificia Universidad Católica de Chile sequencing facility, using the primers described previously at a 5 µM concentration. Both forward and reverse strands were sequenced. Electropherograms of the sequenced products were edited and assembled into contigs using the DNA Baser v4 sequence assembly software (Heracle BioSoft SRL, Pitesti, Romania).

Contigs were used to perform an analysis of sequence similarity using the BLASTn (nucleotide) tool implemented in the National Centre for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). We used the option of highly similar sequences (megablast), which retrieves all sequences available in GenBank that are highly similar (95% or more) to the target sequence.

## Conservation assessment

With the information collected through fieldwork and analysed using the software QGIS (QGIS Development Team 2020), the Red List assessment of this species was completed following the guidelines of IUCN (2019).

## Results

### Taxonomic treatment

#### *Diplostephium paposanum* S.T.Ibáñez & Muñoz-Schick, sp. nov.

urn:lsid:ipni.org:names:77309837-1

Figs 2–4

**Diagnosis.** *D. paposanum* is distinctive from most species of the genus because of its lack of tomentose or lanate hairs on its vegetative parts, including the adaxial side of the leaves. Additionally, *D. paposanum* has glandular succulent leaves, and short branches with leaves that are glabrate or scarcely puberulous in the apical section of long shoots.

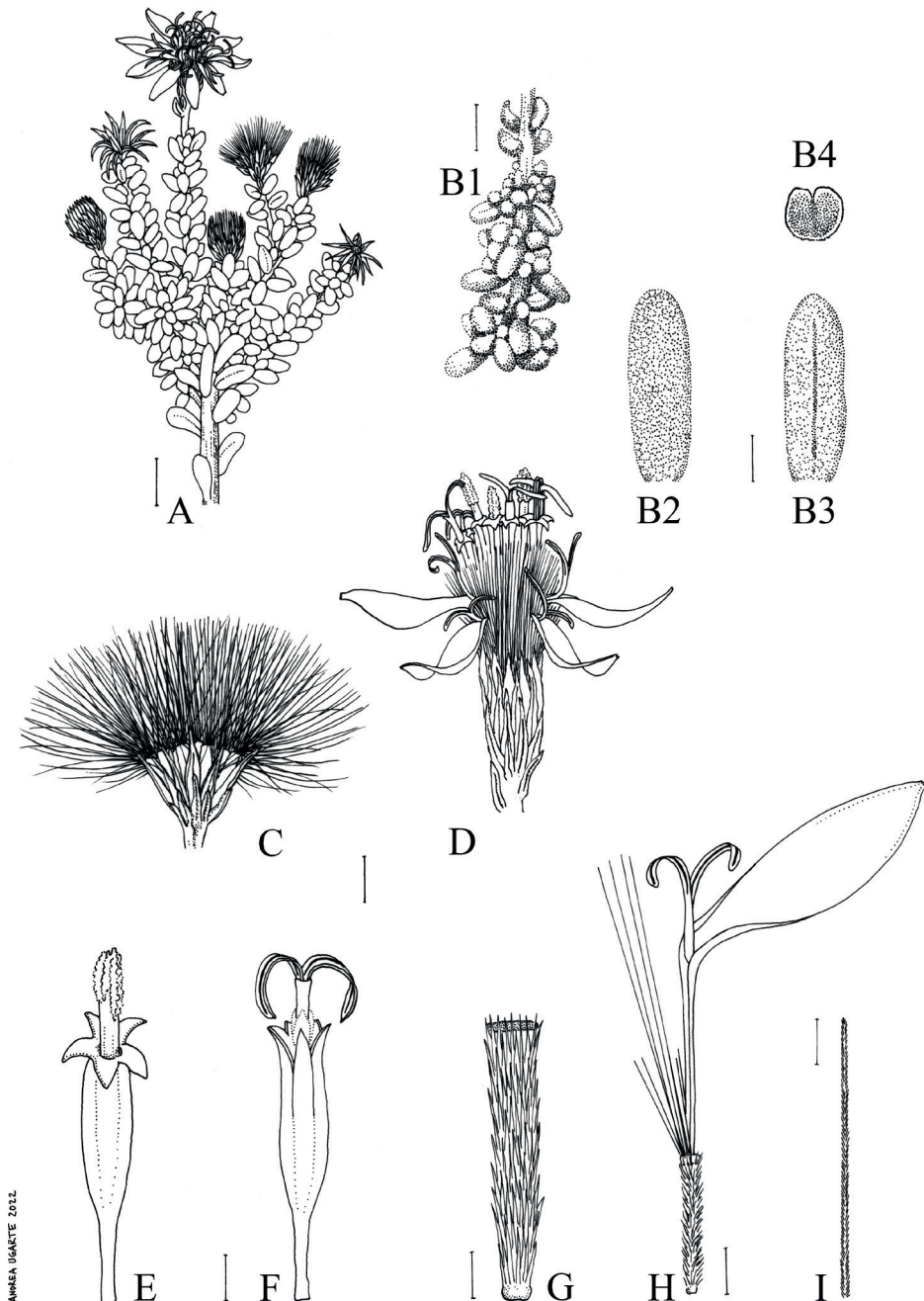
**Type.** CHILE. Región de Antofagasta: Norte de Paposo, Quebrada Botija, 24°30.334'S, 70°32.834'W, alt. 170 m, 14 Oct 2021. A. Moreira-Muñoz 3355 (holotype: SGO). Norte de Paposo, Quebrada Botija, 24°30.334'S, 70°32.834'W, alt. 170 m, 15 Dec 2020. A. Moreira-Muñoz 3204 (paratype: SGO).

**Description.** Shrub up to 70 cm tall, subglobose, resinous, generally glabrate but puberulous with hairs mixed with stipitated glands in younger twigs, with ramified erect, indeterminate, ascending, long branches, and shorter, rarely ramified, ascending lateral branches of up to 10 cm borning mostly near the apex of long branches, base of long branches naked, covered with leaves scars. Leaves alternate or fasciculate, densely covering the upper part of the branches and decreasing downward, (1-)2–5 (-8)  $\times$  0.3–0.8 mm, succulent, lamina strongly revolute, hence clavate to terete, sessile, covered with sunken glands, younger leaves in plantlets laminar, linear-oblong, with 1–2 teeth at each side of the lamina. Capitula solitary or rarely in pairs, terminal in short lateral branches, heterogamous, radiate. Peduncles (1.5-)2.8–3.9 (-5.5) mm long, often with peduncular bracts 1.0–2.3  $\times$  0.3–0.8 mm, similar to leaves but subulate and base swollen. Involucre 4.1–6.1  $\times$  3.0–4.5 mm, cylindrical; phyllaries arranged in 3–4 series, acute to obtuse, margin hyaline, central rib visible on both sides; outer phyllaries 1.3–2.6  $\times$  0.5–0.8 mm, subulate to deltoid; middle phyllaries 2.4–3.8  $\times$  0.6–0.9 mm, subulate to lanceolate, with or without distal purple spot; inner phyllaries 3.7–4.2  $\times$  0.8–1 mm wide, linear-lanceolate with a distal purple spot; receptacle 1.0–1.5 mm diameter, convex, alveolate, epaleate. Ray florets 7–10, pistillate; corolla white, tube 2.3–4.2  $\times$  0.3–0.4 mm, limb 4.2–7.1 mm  $\times$  1.6–2.2 mm, elliptic, minutely 3-lobed; style glabrous, 2.8–4.4  $\times$  0.1–0.2 mm, linear, style branches 0.8–1.7  $\times$  0.1–0.2 mm, linear, flat, with a purple marked line on the middle. Pappus composed of 17–36 bristles of two lengths, short bristles 1.2–2.1 mm, long bristles 3–7.3 mm, scabrid becoming barbellate towards apex. Cypselae of ray florets 1.4–2.9  $\times$  0.4–0.8 mm, shape as in disk florets; carpopodium 1.4–1.9  $\times$  0.4–0.8 mm. Disk florets 10–20, bisexual; corolla yellow, tube 4.3–6.0  $\times$  (0.5-)1.0–1.2 mm, narrowly infundibuliform, linear at the base, limb of 5 lobules, each 0.6–1.1  $\times$  0.3–0.6 mm, deltoid; anthers 2.4–3.5  $\times$  0.2–0.3 mm, ecaudate, cuneate, apical appendage deltoid, filaments 1.2–1.8  $\times$  0.05–0.1 mm; style 6.3–7.1  $\times$  0.2 mm, linear, style branches 1.7–2.3  $\times$  0.2 mm, lanceolate, apex acute, distal end straight. Pappus as in disk florets. Cypselae of disk florets 1.7–3.4  $\times$  0.4–0.8 mm, fusiform, slightly compressed, ribbed, villous; carpopodium present, 0.1  $\times$  0.2 mm.

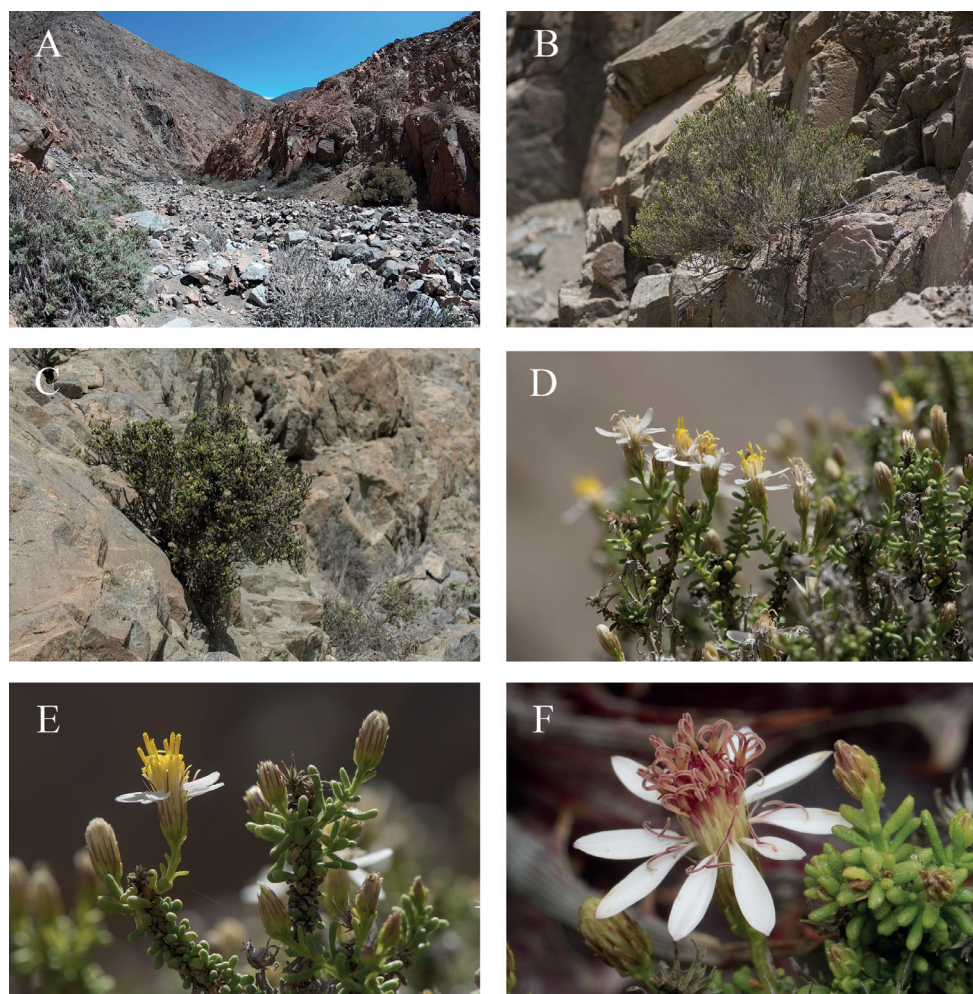
**Other material examined.** CHILE. Región de Antofagasta. Quebrada Botija, 24°31.072'S, 70°31.835'W, alt. 525 m, 4 Dec 2021. S.T. Ibáñez, C. Pañitrur & M. Acosta 771 (SGO).

**Distribution.** The species has been observed only in Quebrada Botija (24°30'S, 70°32'W), a locality 55 km north of Paposos (Fig. 1), on the coast of the Atacama Desert in the Antofagasta Region of Chile.

**Habitat.** The only population found was observed in a gorge system, growing from fissures of the ravine walls, or at the bottom of the gorge, often rooting in weathered rocks. The altitudinal range of this species is between alt. 170 and 700 m which suggests that species occurrence is influenced by the presence of the fog from the ocean. It can be found together with species such as *Spergularia arbuscula* (Gay) I.M. Johnst., *Copiapoa boliviana* (Pfeiff.) F.Ritter, *Proustia pungens* subsp. *tipia* (Phil.) Luebert, *Cristaria integerrima* Phil., *Eremocharis fruticosa* Phil., and *Jarava tortuosa* (E. Desv.) Peñail. Above the gorges, the vegetation is dominated by *Copiapoa solaris* (F. Ritter) F. Ritter.



**Figure 2.** Illustration of *Diplostephium paposanum* **A** distal end of the branch with capitula **B** details of leaves **B1** upper part of the branch covered by leaves **B2** adaxial view **B3** abaxial view **B4** transversal view **C** capitulum in dispersion of fruits **D** capitulum in bloom **E** disk florets with emerging style branches **F** disk florets with emerged and open style branches **G** detail of a cypsela of a ray floret **H** ray floret, including cypsela. Note the differences in length of pappus bristles **I** detail of a single pappus bristle. All images drawn from *Moreira-Muñoz 3355*. Illustration by Andrea Ugarte. Scale bars: 1 cm (**A**); 5 mm (**B1**); 1 mm (**B2–B4, C–F, H, I**); 0.5 mm (**G**).

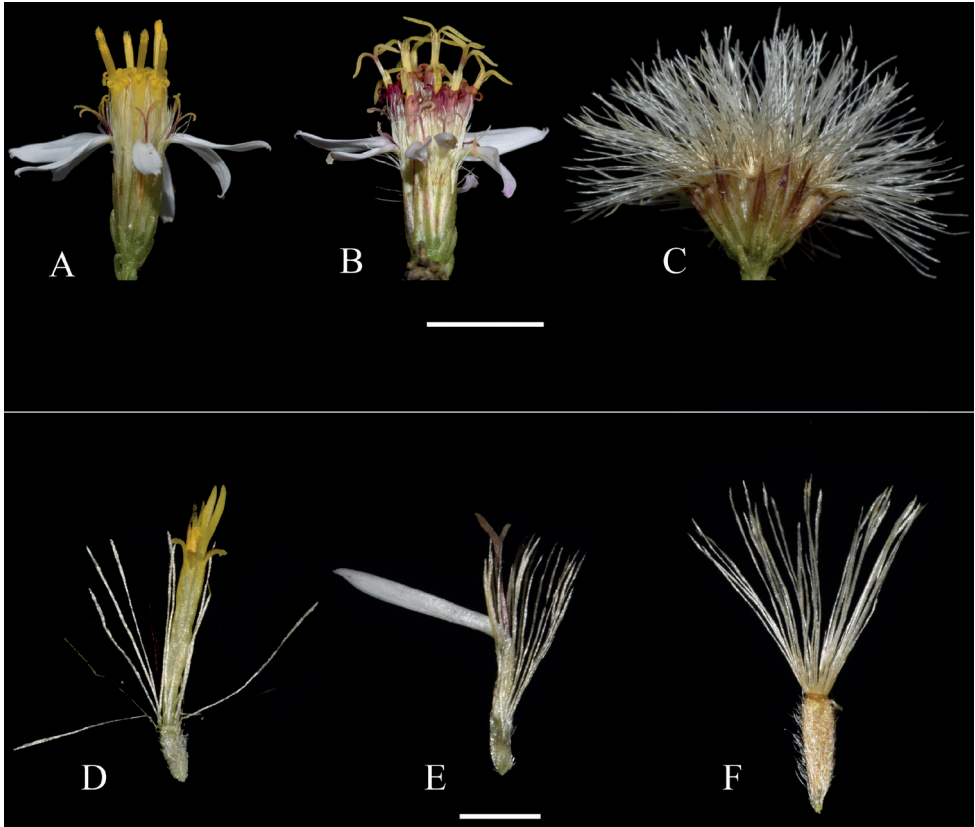


**Figure 3.** *Diplostephium paposanum* in habitat **A** general view of the type location “Quebrada Botija” **B, C** habit of individuals, growing on rocky ledges rooting on rock crevices **D** inflorescences on distal branches **E** lateral branchlets with terminal inflorescences **F** capitulum with fully unfolded style branches **E, F** note the colour differences on yellow disk florets with closed style branches and the mature purple disk florets with unfolded style branches. Photographs by S. Ibáñez (**A–E**), A. Moreira (**F**).

**Phenology.** Flowers of this species have been observed between November and January. Fruits are dispersed between December and February.

**Conservation status.** The values obtained using the Red List assessment criteria (IUCN 2019) classify this species as “Critically Endangered”, based on the criteria B1ab(iii), C2a(ii) and D. After an extensive search of the area, the only population of this species was found in Quebrada Botija, with an extent of occurrence of 4.6 km<sup>2</sup> and composed of no more than 20 individuals. Within the area delimited as the extent of occupation, there is a small mining exploitation currently





**Figure 4.** Detail of reproductive organs **A–C** capitula **A** capitulum showing early stages of development, with only anthers of disk florets and style branches of ray florets not unfolded **B** capitulum showing style branches of disk florets unfolded **C** capitulum with mature cypselae and their respective pappus fully developed **D–F** individual florets and fruits **D** disk florets with style branches barely unfolding **E** ray florets with developed style branches **F** mature cypselus of a disk floret. All images from Ibáñez, Pañitrur & Acosta 771. Scale bars: 5 mm (**A–C**); 2 mm (**D–F**).

active, in which extracted material is transported using heavy weight trucks moving along the bottom of the gorge in which the species grows. Furthermore, lomas formations are currently threatened by the decreasing amount of humidity reaching the coast in hyper arid Chile, which has led to a notorious declination of vegetation at that latitude (Schulz et al. 2012). Literature and field observation has confirmed vegetation dieback, especially between Botija and Tocopilla (Schulz et al. 2011), where high percentages of individuals of species such as *Copiapoa solaris* are currently dead.

**Etymology.** The epithet *paposanum* means “from Paposo”. Paposo is a small village located next to the foothills of the coastal cliffs which is the nearest urban centre to the species described.



## Molecular analysis

After contig assembly and trimming of the primer regions, we obtained 577 basepairs of the ETS sequence, and 824 basepairs of the trnL-F sequence. Both BLAST analyses retrieved highly similar sequences, and both markers obtained species of the genus *Diplostephium* as the most similar. For ETS, the first 40 sequences retrieved were from this genus, with 95% similarity. For trnL-F, sequences retrieved matched species of the genus *Diplostephium* with 98.9% similarity. Within the first 40 sequences retrieved, there were only two that did not correspond to this genus, but were still Asteraceae. The new sequences were uploaded to GenBank, with accession numbers OP038910 for *D. paposanum* trnL-trnF sequence and ON936842 for *D. paposanum* ETS sequence.

## Discussion

### Systematic position

The position of *D. paposanum* in *Diplostephium sensu lato* is supported by morphological characters, such as the shrubby and candelabrum-like habit, heterogamous heads with three to four series of phyllaries and alveolate receptacle, disk florets with ecaudate anthers and lanceolate style branches, ribbed and somewhat compressed cypselae with two different sizes of pappus bristles (Nesom and Robinson 2007; Vargas 2018). Additionally, this species can be placed morphologically in the group of *Diplostephium sensu stricto* because it is a small bush with up to ten capitula per main branch, all of them placed at the tip of short lateral branches near the apex, and ray flowers often longer than 10 mm. (Vargas 2018). Distribution is also consistent with *Diplostephium s. s.*, since the new species follows the southern distribution of the group, enhancing it southward to latitude 24°S.

Because of the phylogenetic complexity of the genus, we did not infer a phylogenetic position for *D. paposanum*. Nevertheless, we can assume with a high degree of confidence that genetically this new species is most similar to other species of the genus *Diplostephium s.s.* A genomic analysis would be very useful to confirm these results.

### Biogeographical implications

This species is remarkable because it is the first species of *Diplostephium* collected at low altitude, with an altitudinal range of between 170 and 700 m, whereas all species previously known were collected between 2500 and 4500 m. The new species is similar to some congeneric species, but has distinctive characters which are similar to other coastal species from the Atacama Desert. For instance, *D. paposanum* is a rather glabrate plant with leaves which become succulent and terete to clavate with age. These characters are odd in the genus, but are common in sympatric plants, such as in some shrubby *Nolana* spp. (Solanaceae), *Spergularia arbuscula* (Gay) I.M. Johnst. (Caryophyllaceae), *Heliotropium pycnophyllum* Phil. (Heliotropiaceae), or *Suaeda foliosa* Moq.

(Chenopodiaceae). The resinous glands present in *D. paposanum* are a common character found in other *Diplostephium* species, which could have led this genus to colonize dry habitats, as has been observed in other xerophytic Asteraceae species.

Despite the fact that it is not common to find high altitude Andean taxa in coastal conditions, some exceptions can be found in the particular conditions of the coastal Atacama Desert, where the coastal fog allows abundant vegetation. This can be seen in *Dalea* species which, when present in South America, are found mostly in the Andes (Piñeros-U and González 2020) with one of the exceptions being *D. azurea* (Phil.) Reiche, which is narrowly distributed around the Pajapo coastal area. Also, several phylogenetic analyses show close relationships of some plant groups in the Atacama Desert to Andean and non-Andean neotropical lineages (Luebert 2011; Ruhm et al. 2022). Moreover, numerous species have disjunct distributions, being present in the Andes at high altitudes as well as around Pajapo; for instance, species like *Bidens triplinervia* Kunth, *Stevia philippiana* Hieron., *Solanum paposanum* Phil., *Krameria lappacea* (Dombey) Burdet & B.B. Simpson, or *Euphorbia amandi* Oudejans (Johnston 1929), share this pattern. *Diplostephium paposanum*, is the newest component of a remarkable flora recognized by its level of endemism at the Pajapo-Taltal coastal fringe, especially in the Asteraceae family (Luebert et al. 2009), a coastal section also recognized as a micro-hotspot of biodiversity integrating new entomological knowledge (Pizarro-Araya et al. 2021).

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# *Microchirita minor* (Gesneriaceae), a new species from north-western Vietnam

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## Abstract

*Microchirita minor* (Gesneriaceae), a new species from the limestone area in Son La Province, north-western Vietnam, is described here. The new species resembles *M. hamosa*, but it differs by the combination of corolla tube shape, stamens number and the length of pistil. Detailed morphological description, together with photographic plates, information on phenology, distribution, ecology and preliminary conservation status of the new species are presented.

## Keywords

Didymocarpoideae, flora of Vietnam, karst limestone, *Microchirita*

## Introduction

The genus *Microchirita* (C.B.Clarke) Yin Z.Wang was raised to genus from the former *Chirita* sect. *Microchirita* C.B.Clarke, according to the results of molecular phylogenetic studies (Clarke 1883; Möller et al. 2009; Wang et al. 2011; Weber et al. 2011). The genus

currently comprises 39 species and six varieties (POWO 2022), including some species published in recent years (e.g. Puglisi et al. 2016; Puglisi and Middleton 2017; Rafidah 2019). The genus *Microchirita* grows exclusively in limestone karst habitats of tropical Asia, of which, Thailand is considered to be the centre of diversity of *Microchirita* with 29 species recorded (Puglisi and Middleton 2017), followed by Indonesia, Cambodia, China, India, Laos, Myanmar and Vietnam (Puglisi and Middleton 2017; Rafidah 2017; Middleton 2018; Vu 2018; Wei 2018; Fu et al. 2022; Wei et al. 2022; Wen et al. 2022).

Whilst conducting botanical explorations of limestone areas in northern Vietnam, we collected some interesting *Microchirita* specimens from one population within the Xuan Nha Nature Reserve, Son La Province, in north-western Vietnam. These unknown specimens showed similarity with *M. hamosa* (R.Br.) Yin Z. Wang (Brown 1839; Wang et al. 2011) in having unbranched stem, single basal leaf, cristate inflorescence, white corolla and hairy capsule: however, it differs from *M. hamosa* by the combination of corolla tube shape, stamens number and the length of pistil. These differences allow us to confirm that it represents a new species of *Microchirita*, which we describe here.

## Taxonomic treatment

***Microchirita minor* Z.B.Xin, T.V.Do & F.Wen, sp. nov.**

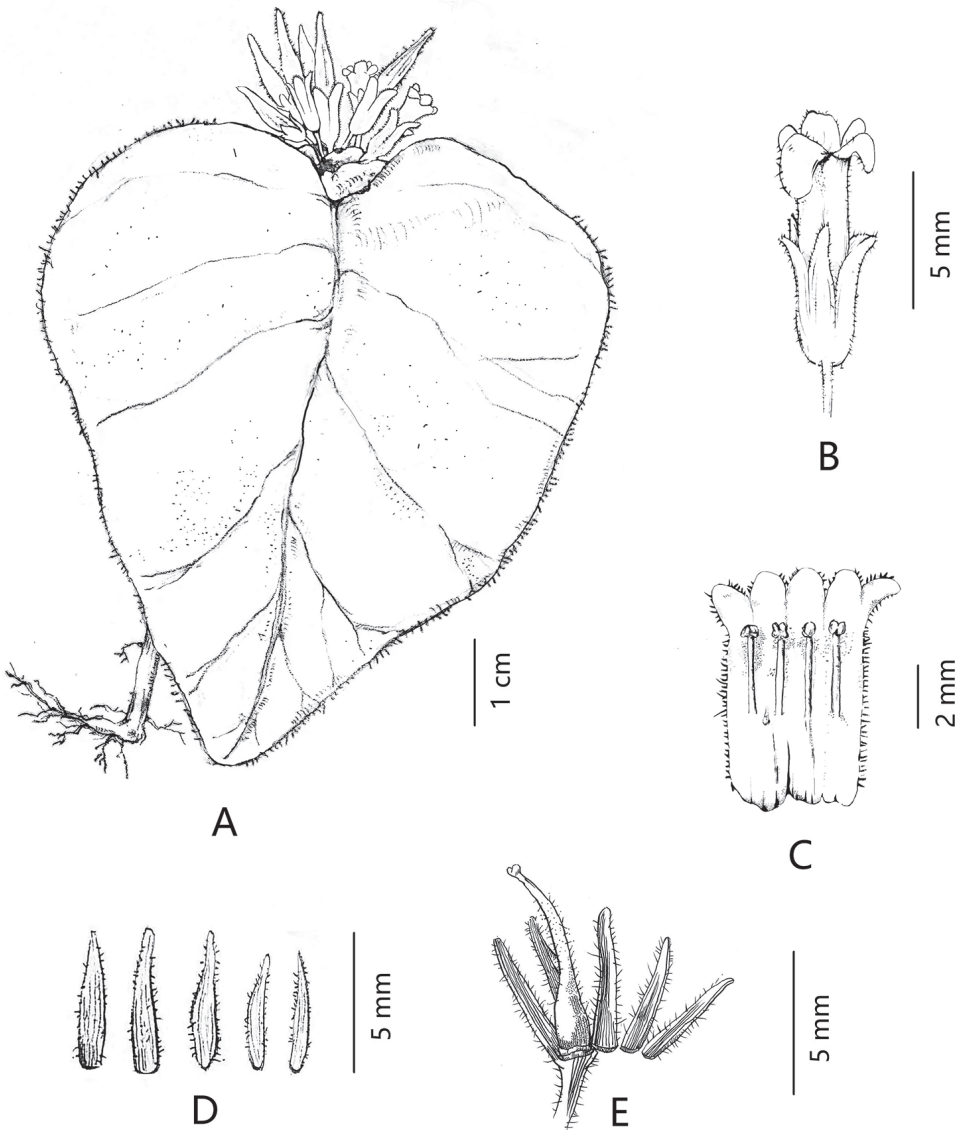
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Figs 1, 2

**Diagnosis.** The new species is morphologically similar to *Microchirita hamosa*, but it differs from the latter in its corolla tube 5–6 mm long, with four yellow patches, each one originating at the base of each filament and reaching the throat, the throat-base diameter ratio of the corolla tube 1–1.2 (vs. corolla tube 8–15 mm long, with one yellow patch ventrally, the throat-base diameter ratio of the corolla tube 2–3); stamens 4, 2–2.5 mm long, ca. 0.3 mm in diameter (vs. stamens 2, ca. 1.5 mm long, ca. 0.1 mm in diameter); pistil 6–8 mm long (vs. ca. 16 mm long); ovary 2–3 mm long (vs. ca. 14 mm long).

**Type.** VIETNAM. Son La Province: Moc Chau District, Xuan Nha Nature Reserve, in moist crevices of the limestone cliff and mouth of limestone caves in a subtropical evergreen seasonal rainforest, 20°43'32"N, 104°40'50"E, elev. ca. 539 m, 1 November 2019, *F. Wen, T.V. Do, Z.B. Xin & S. Maciejewski VMN-CN 1231* (holotype VNMN!; isotypes IBK!, VNMN!).

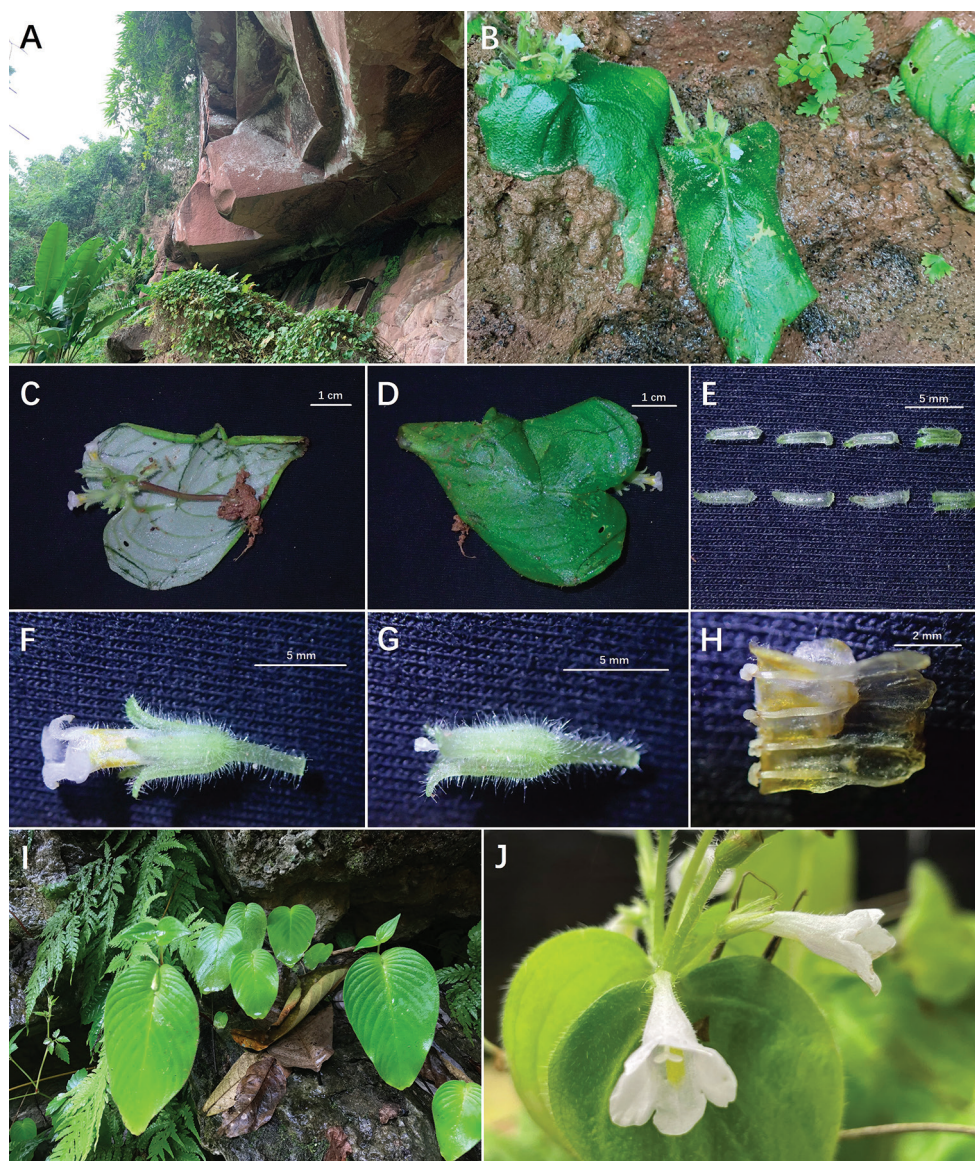
**Description.** Annual herb, up to 7 cm tall. Stems maroon green, erect or sub-erect, sparsely eglandular pubescent; unbranched. Leaves 1, rarely 3 to 5, lowermost solitary, leaves towards apex opposite; petioles 1–10 mm long, densely and shortly pubescent; blades mid-green adaxially, paler abaxially, ovate to elliptic, 1–10 cm long, 0.5–8 cm wide, base cordate broadly attenuate to obtuse, apex acute to acuminate, eglandular pubescent adaxially and abaxially, margin near entire, mid-rib impressed adaxially, prominent abaxially, lateral veins 5–7 pairs, sparsely eglandular pubescent. Inflorescences cristate, epiphyllous, 5–15-flowered; peduncles extremely short, ca. 1 mm long;



**Figure 1.** *Microchirita minor* sp. nov. **A** habit **B** flower **C** opened corolla with stamens and staminodes; **D** abaxial surface of calyx lobes **E** pistil and calyx. Drawn by Rui-Feng Li.

bracts absent; pedicels pale green, 4–6 mm long, densely glandular and eglandular pubescent. Calyx 5, segments unequal, upper lobes 3-parted to near the base, lower lobes 2-parted to the base, the central upper lobe (alternate to the upper corolla lobes) shorter and thinner than the other lobes, pale green, lobes lanceolate, larger lobes 5–7 × ca. 1 mm, smaller lobes ca. 4 × 0.6 mm, apex acuminate, margin entire, densely glandular and eglandular pubescent outside, glabrous inside. Corolla 8–10 mm long, tube white, with four yellow patches inside the tube, each one of them originating at





**Figure 2.** **A–H** *Microchirita minor* sp. nov. **I–J** *M. hamosa* **A** habitat **B** plants with flowers **C** abaxial surface of leaf blade **D** adaxial surface of leaf blades **E** calyx lobes (adaxial surface above, abaxial surface below) **F** lateral view of the flower **G** pistil and calyx **H** opened corolla with stamens and staminodes **I** habitat **J** plant with flowers. (Photos **C–H** from type material).

the base of each filament and reaching the throat, corolla tube tubular, 5–6 mm long, 1.7–2.2 mm in diameter, eglandular hairy outside, glabrous inside; lobes elliptic, upper lobes 1–1.2 × 1.2–1.5 mm, lower lobes 1.2–1.5 × 1.5–2.0 mm. Stamens 4, inserted ca. 3 mm above the corolla base; filaments straight, white, glabrous, 2–2.5 mm long, ca. 0.3 mm in diameter; anthers white, papilionaceous, ca. 0.8 × 0.6 mm; staminode 1,

adnate to ca. 2.5 mm above the corolla base, 0.3–0.5 mm long. Disc annular, margin entire. Pistil 6–8 mm long, densely glandular and eglandular pubescent from the base, more sparsely towards the stigma; ovary 2–3 mm long; style 3–4 mm long, eglandular pubescent, stigma elliptic, ca.  $1 \times 0.8$  mm. Capsule green, 1.4–1.6 cm long, 1.5–2.0 mm in diameter, eglandular pubescent, straight.

**Etymology.** Latin *minor*, smaller, alluding to size of plants and flowers.

**Phenology.** Flowering was observed from October to November. Fruiting from November to December.

**Distribution and habitat.** The new species is currently only known from one population within Xuan Nha Nature Reserve, Moc Chau District, Son La Province, north-western Vietnam. The new species grows in moist crevices of the limestone cliff and mouth of limestone caves in a subtropical evergreen seasonal rainforest, at elevations of 530–545 m.

**Proposed IUCN conservation status.** The new species is only known from a single population in Xuan Nha Nature Reserve, Moc Chau District, Son La Province, north-western Vietnam. This single population has no more than 1000 mature individuals, all growing on moist and shaded rocky surfaces on the cliff. They are easily disturbed by human activities as the known habitat is located in the buffer zone of the Nature Reserve and near the sugar-cane field. The species is provisionally assessed as data deficient (DD), following the IUCN Red List Categories and Criteria (IUCN 2022), because more surveys are needed.

**Notes.** The most striking character of *Microchirita minor* is the tiny, white corolla with four stamens. It is most easily confused with *M. hamosa*, from which it differs in the much smaller corolla, shorter corolla tube (5–6 mm long vs. 8–15 mm long), with four yellow patches inside the tube, each one of them originating at the base of each filament and reaching the throat, four larger and sturdier stamens (2–2.5 mm long, ca. 0.3 mm in diameter vs. ca. 1.5 mm long, ca. 0.1 mm in diameter) and shorter pistil (6–8 mm long vs. ca. 16 mm long). Detailed morphological comparisons of the new species with *M. hamosa* are shown in Table 1. The floral measurements of *M. hamosa* were mainly derived from Wang et al. (1990, 1998), Puglisi and Middleton (2017) and our own observations and dissection.

**Table 1.** Detailed comparison of *Microchirita minor* and its relative *M. hamosa*.

Characters	<i>M. minor</i>	<i>M. hamosa</i>
Height of the mature plant	up to 7 cm tall	up to 25 cm tall
Peduncles	extremely short, ca. 1 mm long	up to 4 mm long
Corolla tube length	5–6 mm long	8–15 mm long
Throat-base diameter ratio of the corolla tube	1–1.2	2–3
Corolla tube colour	white with four yellow patches inside the tube, each one of them originating at the base of each filament and reaching the throat	white with one yellow patch ventrally
Stamens	four, 2–2.5 mm long, ca. 0.3 mm in diameter	two, ca. 1.5 mm long, ca. 0.1 mm in diameter
Pistil	6–8 mm long	ca. 16 mm long
Ovary	2–3 mm long	14 mm long
Stigma	ca. 1 mm long	ca. 0.2 mm long

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# *Quercus mangdenensis*, a new species of *Quercus* (Fagaceae) from Kon Tum Province, Vietnam

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## Abstract

*Quercus mangdenensis* Binh & Ngoc, **sp. nov.** (Fagaceae) is newly described from Mang Den Town in the central highland of Vietnam. The new species is characterized by lanceolate to oblong-lanceolate leaves with entire margin, 1–5-fruited infructescence, larger fruit size 6–10.5 cm long, broadly bowl-shaped cupules enclosing 1/5 of the nut, bracts of cupule entire and arranged in 5–7 rings, and cylindrical-ellipsoid and basally flat nuts 4.5–7.5 cm long. *Quercus mangdenensis* is morphologically similar to *Q. bidouensis* Binh & Ngoc and *Q. kontumensis* A.Camus in having similar leaf shape, cuneate leaf base, and bracts arrangement in cupules. However, it differs from *Q. bidouensis* and *Q. kontumensis* by cupules broadly bowl-shaped, much larger fruits, cylindrical-ellipsoid nut shape, and cupule enclosing 1/5 of the nuts. A description, photographs, and preliminary species conservation status of the new species are also provided.

## Keywords

Fagales, flora, Kon Plong, Mang Den, taxonomy

## Introduction

*Quercus* L. is the biggest genus of the family Fagaceae, comprising more than 500 species widely distributed in Europe, North America, the Mediterranean, temperate deciduous forests in East Asia, and tropical montane forests in Southeast Asia (Nixon 1993;

Huang et al. 1999; Phengklai 2008; Hubert et al. 2014; Valencia-A et al. 2016). In Vietnam, a total of 52 *Quercus* species have been reported (Ho 2003; Ban 2005; Li et al. 2016; Binh et al. 2018a, b, c; Binh et al. 2021; Ngoc et al. 2022), of which, recent efforts in taxonomic works of the genus *Quercus* have resulted in the description of seven new species from Vietnam (Binh et al. 2018a, b, c; Binh et al. 2021; Ngoc et al. 2022), indicating that the species diversity of *Quercus* of the country is significant. Therefore, taxonomic studies of the Vietnamese *Quercus* are still required.

Mang Den town belongs to Kon Plong District, Kon Tum Province of Vietnam with a total area of 148.07 km<sup>2</sup> (Kon Tum Province 2022) (Fig. 1). In this area, the annual temperature range is 18.7–24.9 °C and the average annual rainfall is around 1780–2200 mm with the rainy season from August to February (Tri et al. 2022). In the region, 273 woody plants have been recorded (265 angiosperms and 8 gymnosperm species). As for Fagaceae, 20 species have been recorded from Kon Plong District (Thanh et al. 2001).

From April to July 2022, we conducted botanical surveys in Mang Den and collected several individuals of the genus *Quercus* that could not be placed with any previously described species. After careful morphological examination and taxonomic review, here we describe those individuals as *Quercus mangdenensis* Binh & Ngoc along with morphological comparisons with the most similar species, photographs, and a preliminary conservation assessment of the new species.

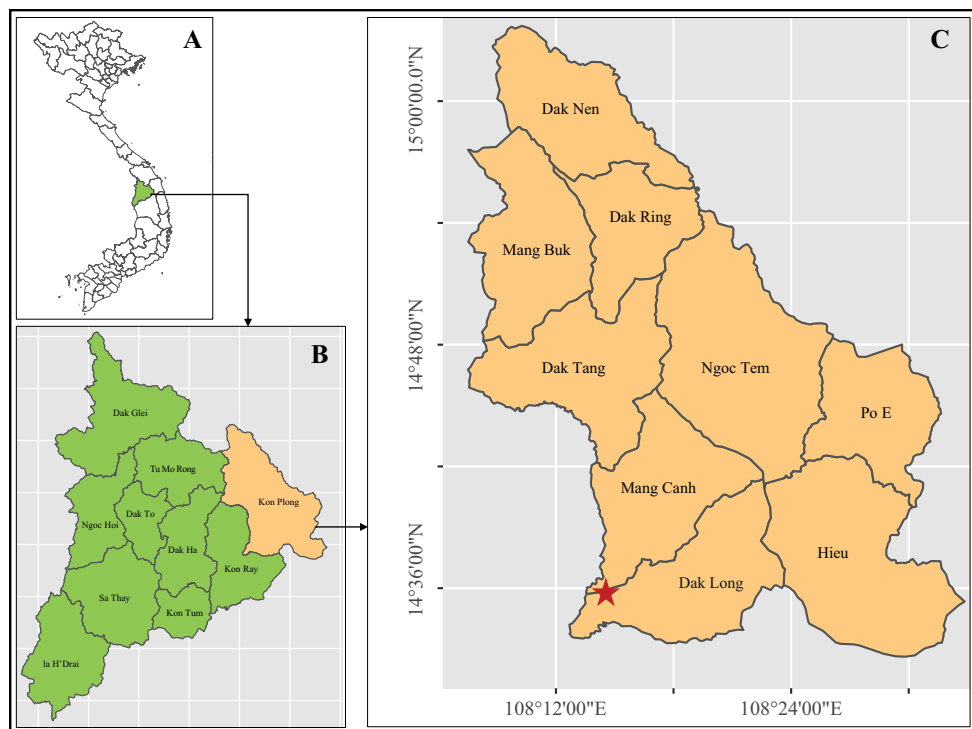
## Materials and methods

### Plant materials

In this study, six specimens of the new species (*Binh et al. QC204, 205 206, 207, 208, 209*) were collected from Mang Den, Kon Plong District, Kon Tum Province (Fig. 1). In addition, we used type specimens and specimens that were collected from the type locality of the following species: *Q. bidoupensis* (*Tagane et al. V4328* [DLU!], *Binh et al. QC27, 29, 30, 45, 57* [DLU!]) and *Q. kontumensis* (*Poilane 18381* [P00754004] and [P00754005]), which are morphologically most similar to the new species in having a lanceolate to oblong-lanceolate leaf shape, cuneate leaf base, and bracts of cupule fusing in 5–7 rings.

### Morphological observations

To confirm that the species was undescribed, the relevant literature including Camus (1934), Soepadmo (1972), Huang et al. (1999), Ho (2003), Ban (2005), and Phengklai (2008), Binh et al. 2018a, b, c; Binh et al. 2021; Ngoc et al. 2022 were consulted. Then, we examined specimens in the herbaria at DLU, K, P, and VNM as well as digital images on the websites of JSTOR Global Plants (<https://plants.jstor.org/>) and the Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>).



**Figure 1.** Type locality of *Quercus mangdenensis* Binh & Ngoc **A** map of Vietnam **B** map of Kon Tum province **C** map of Kon Plong District, the red star indicated the type locality: Mang Den Town, Dak Long Commune.

## Taxonomic treatment

### *Quercus mangdenensis* H.T.Binh & Ngoc, sp. nov.

urn:lsid:ipni.org:names:77309987-1

Fig. 2

**Diagnosis.** *Quercus mangdenensis* is morphologically most similar to *Q. bidoupensis* and *Q. kontumensis* with a lanceolate to oblong-lanceolate leaf shape, cuneate leaf base, and bracts of cupule fusing in 5–7 rings. However, *Q. mangdenensis* is distinguished from *Q. bidoupensis* by its entire leaf margin (vs. undulate, distinctly serrate in the upper 1/2), longer petioles 2.5–4 cm long (vs. 1.3–2 cm long), broadly bowl-shaped cupules (vs. obconical-shaped), larger cupule (2.6–3.5 cm high × 4–5 cm in diam. vs. 1.3–1.5 cm high × 1.3–1.7 cm in diam.), enclosure of cupule (1/5 vs. 1/3 of the nut), cylindrical-ellipsoid nut (vs. ovoid), larger nut (4.5–7.5 cm high × 3–4 cm in diam. vs. 2.2 cm high × 1.4 cm in diam.), and flat basal scar of the nut (vs. convex). It differs from *Q. kontumensis* in having larger cupule enclosing 1/5 of the nut (2.6–3.5 cm high × 4–5 cm in diam. vs. 1–1.2 cm long × 1.5–2 cm in diam., enclosing 1/2 of the nut), larger cylindrical-ellipsoid nut 4.5–7.5 cm high × 3–4 cm in diam. (vs. cylin-

drical-ovoid, 2 cm high × 1.5 cm in diam.), and scales of cupule fusing into 5–7 entire ridges (vs. undulate) (Table 1).

**Type.** VIETNAM. Kontum Province, Kon Plong District, Mang Den Town, alt. 1179 m, 14°36'39.1"N, 108°15'00.5"E, 23 July 2022, *H. T. Binh & N. V. Ngoc QC204* (holotype DLU!; isotypes HNI, VNM!)

**Description.** Tree, evergreen, 20–25 m tall, 60–80 cm girth. Bark whitish gray. Buds perulate, globose to oblate, 2.5–4 mm high, 2.5–3.5 mm in diam., bud scales imbricate, in 2–3 rows, ovate-triangular, 2.0–3.0 × 1.5–2.0 mm, apex obtuse, margin ciliate, covered with appressed white hairy outside, glabrous inside. Young twigs dark pale green, hairy, old twigs grayish-brown, glabrous, sometimes sulcate, lenticellate. Leave alternate; blades thickly coriaceous, lanceolate to oblong-lanceolate, (5.5–)11–16.5(–19.5) × (2.6–)3.5–5.5 cm, acute at apex, broadly cuneate at base, margin entire, glossy green adaxially, bright green abaxially, conspicuously pale creamy brown to yellowish brown *in sicco*, glabrous on both surfaces, midrib slightly raised on the upper surface, prominently raised on the lower surface, lateral veins (5–)7–9 pairs, prominent on the lower surface, at an angle of 45–55 degree from the midrib, straight and then curved near margin, tertiary veins scalariform-reticulate, faintly visible on both surfaces; petioles (1.3–)2.5–4 cm long, yellowish brown when dry, glabrous. Male and female inflorescences not seen. Infructescences axillary or pseudo-terminal (sometimes in upper leaf-scars), erect, rachis 1–1.5 cm long, 1–1.3 cm in diam., woody, glabrous, dark yellow *in vivo*, yellowish brown *in sicco*, lenticellate. Mature fruits 6–10.5 cm high (including cupule), solitary, sessile on woody rachis; nuts cylindrical-ellipsoid, 4.5–7.5 cm high, 3–4 cm in diam., rounded at the top, white tomentose around stylopodia, stylopodia 1.5–2.5 mm long, basal scar 2–2.5 cm in diam., flat; cupules broadly bowl-shaped, 2.6–3.5 cm high, 4–5 cm in diam., enclosing 1/5 of the nut when mature, outside tomentose with reddish hairs to glabrous, inside with appressed densely reddish hairs, wall

**Table 1.** Morphological comparison amongst *Quercus mangdenensis* Binh & Ngoc, sp. nov., *Quercus bidoupensis* Binh & Ngoc and *Quercus kontumensis* A. Camus.

Characters	<i>Q. mangdenensis</i>	<i>Q. bidoupensis</i> <sup>(1,2)</sup>	<i>Q. kontumensis</i> <sup>(3,4)</sup>
Buds shape	gobose to oblate	oblong to ellipsoid	ovoid to ellipsoid
Leaf shape	lanceolate to oblong-lanceolate	oblong-lanceolate	lanceolate to oblong-lanceolate
Leaf margin	entire	margins undulate, distinctly serrate in the upper 1/2	entire or distinctly serrate in the upper 1/5
Length of petioles	(1.3–)2.5–4 cm long	1.3–2 cm long	2.5–4.2 cm long
Number of secondary veins	(5–)7–9 pairs	10–13 pairs	5–8 pairs
Cupule shape and size	broadly bowl-shaped, 2.6–3.5 cm high, 4–5 cm in diam.	obconical-shaped, 1.3–1.5 cm high, 1.3–1.7 cm in diam.	cup-shaped, 1–1.2 cm long, 1.5–2 cm in diam.
Number of rings on cupule	5–6 rings	5–6 rings	6–7 rings
Cupule bract margin	entire	entire	undulate
Cupule coverage	enclosing 1/5 of the nut	enclosing 1/3 of the nut	enclosing 1/2 of the nut
Nut shape and size	cylindrical-ellipsoid, 4.5–7.5 cm high, 3–4 cm in diam.	ovoid, 2.2 cm high, 1.4 cm in diam.	cylindrical-ovoid, 2 cm high, 1.5 cm in diam.
Base of the nut	Flat	Convex	Flat

<sup>(1)</sup> From the protologue (Binh et al. 2018b); <sup>(2)</sup> From *Binh et al. QC27* (DLU); <sup>(3)</sup> From Camus (1934); <sup>(4)</sup> From the material *E. Poilane 18381* (P [P00754005, P00754005]).

0.5–1.3 cm thick, woody, bracts fusing and arranged in 5–7 ridges (rings), the rings' margin completely entire (without scale-like structure).

**Distribution.** Vietnam. Kon Tum Province, Kon Plong District, Mang Den Town (Fig. 1).

**Habitat.** *Quercus mangdenensis* were found in the scattered evergreen forest, from 1050 to 1200 m elevation.

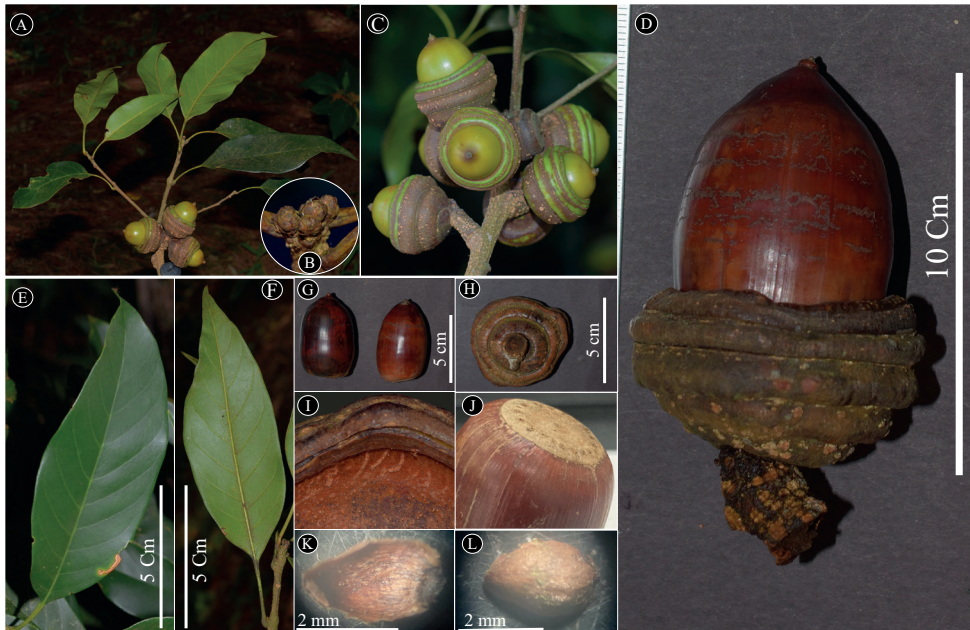
**Etymology.** The specific epithet is derived from its type locality, Mang Den Town, Kon Plong District, Kon Tum Province, Vietnam.

**Vernacular name.** Sồi Măng Đen (suggested here).

**Phenology.** Fruiting specimens and fallen mature fruits were collected in July.

**Additional specimens examined.** VIETNAM. Kontum Province, Kon Plong District, Mang Den, alt. 1168 m, 14°36'37.4"N, 108°15'11.6"E, 23 July 2022, *H. T. Binh & N. V. Ngoc QC205* [fr.] (DLU!, HN!, VNM!); *ibid.*, 1180 m elev., 14°36'32.5"N, 108°15'18.2"E, 23 July 2022, *H. T. Binh & N. V. Ngoc QC207* [fr.] (DLU!, HN!, VNM!); *ibid.*, 1130 m elev., 14°36'36.5"N, 108°15'31.3"E, 23 July 2022, *H. T. Binh & N. V. Ngoc QC208* [fr.] (DLU!, HN!, VNM!); *ibid.*, 1065 m elev., 14°36'36.5"N, 108°15'31.3"E, 23 July 2022, *H. T. Binh & N. V. Ngoc QC209* [fr.] (DLU!, HN!, VNM!).

**Preliminary conservation status.** Our botanical inventories were conducted at Mang Den and the surrounding areas from April to July 2022 and we found five sub-populations of 5–7 mature individuals of *Quercus mangdenensis* in the scattered ever-



**Figure 2.** *Quercus mangdenensis* Binh & Ngoc: **A** twigs with young fruit **B** terminal buds **C** infructescences **D** fallen mature fruit **E**, **F** adaxial and abaxial surface of the leaves **G** nuts **H** outside of cupule **I** densely reddish hairs inside of cupules **J** basal scar of the nut **K**, **L** inside and outside of bud scale. Materials from *H. T. Binh and N. V. Ngoc QC204*.



green forests. In addition, the forest in this area is severely fragmented and continuing to decline caused by human activities, such as farming, logging, and harvesting of non-timber forest products, etc., while almost all of the individuals of *Q. mangdenensis* are distributed along the boundary between the evergreen forest and local people's farms. According to criterion D of the IUCN Red List criteria (IUCN 2019), the species classifies as Critically Endangered (CR). The area of occupancy (AOO) and the extent of occurrence (EOO) were calculated are 0.415 km<sup>2</sup> and 8.0 km<sup>2</sup>, respectively. Based on criterion B of the IUCN Red List criteria (IUCN 2019), the new species is qualified as Critically Endangered [CR B1ab(i,ii,iii,iv,v) B2ab(i,ii,iii,iv,v)].

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# Nomenclature and typification of *Cathaya argyrophylla* (Pinaceae)

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## Abstract

In 1958, Chun and Kuang described *Cathaya* Chun & Kuang as a new genus of Pinaceae. They included one fossil species, *C. loehri* (Engelh. & Kink.) Chun & Kuang and two extant species, *C. argyrophylla* Chun & Kuang and *C. nanchuanensis* Chun & Kuang. Under Art. 40.1 of the Shenzhen Code, they did not validly publish *Cathaya* because they did not designate a type species for the generic name. Four years later (Chun and Kuang 1962), they again published on *Cathaya* (in Chinese) where they cited the 1958 publication and included one species *C. argyrophylla* (with *C. nanchuanensis* in synonymy) under *Cathaya*. According to Art. 40.3, they validated both the generic name *Cathaya* and *C. argyrophylla* in 1962. Further examination of the type collection and botanical history of the discovery of *C. argyrophylla* revealed that the type collection *Guang-Fu-Lin-Qu Exped. 00198* consists of 11 duplicates in the South China Institute of Botany (IBSC) and 9 duplicates in other herbaria (GAC, IBK, PE, SZ) and that the intended type specimen (IBSC0000004) consists of two gatherings: the bark, reproductive shoot and seed cones collected in 1955, whereas pollen-bearing cones were collected in 1956. We thus lectotypify the name *C. argyrophylla* with the specimen *Guang-Fu-Lin-Qu Exped. 00198* (IBSC0000004 excl. pollen-bearing cones).

## Keywords

*Cathaya argyrophylla*, gathering, nomenclature, Pinaceae, Shenzhen Code, typification

*Cathaya argyrophylla* Chun & Kuang is the sole extant species of *Cathaya* Chun & Kuang (Pinaceae). The genus was widely distributed during the Tertiary (Wang et

al. 1998; Liu and Basinger 2000), but is presently relictual and locally restricted in Guangxi, Chongqing, Hunan and Guizhou provinces, China, perhaps due in part to unfavorable conditions brought about by the Quaternary glaciations (Liu and Basinger 2000; Manchester et al. 2009). *Cathaya argyrophylla* is represented in nature by fewer than 5,000 living individuals and is considered to be Vulnerable (VU, Qin et al. 2017; Yang 2021). It is listed under the first class of protection in the recently released National Key Protected Wild Plant Species (<http://www.forestry.gov.cn/main/3954/20210908/163949170374051.html>, accessed 26 August 2022; Yang et al. 2021).

In 1955, J. X. Zhong led a group of botanists to collect an unusual conifer in Guang-Fu-Lin-Qu in Longsheng Xian, Guangxi Province, China (Fu and Cheng 1981, Fu 1998). Specimens with seed-bearing cones were collected on 16 May 1955; pollen-bearing cones were collected in the following year, 1956 (Fu and Cheng 1981; Fu 1998). Chun and Kuang (1958) determined that the collections represented both an undescribed genus and an undescribed species. They examined herbarium specimens deposited in PE and found that similar specimens had been collected in Nanchuan Co., Chongqing, China (Fu and Cheng 1981; Fu 1998). In reporting their discovery, Chun and Kuang (1958) described the new genus *Cathaya* Chun & Kuang with two extant new species, *C. argyrophylla* and *C. nanchuanensis*, and made one new combination to include a fossil species. Under Article 40.1 of the Shenzhen Code, the generic name *Cathaya*, however, was not validly published because they included three species within the genus, but did not designate a type for the generic name (Doweld 2016, 2018). The two specific names were not validly published because of the invalid status of the generic name. When Chun and Kuang (1962) introduced the species to Chinese audiences, they fully cited the Latin description from their earlier paper (Chun and Kuang 1958) for both the generic and specific names and cited only one species, *C. argyrophylla*, under the genus name *Cathaya*. Under Art. 40.3, they validly published *Cathaya* and *C. argyrophylla* at the same time. However, one year earlier, in 1961, the Russian botanist, Karavaev, published *Cathaya* Karav. as the generic name of a fossil plant (Karavaev 1961; Doweld 2016, 2018). When this was noticed, Doweld (2016) proposed conserving the name of the living *Cathaya* of Chun and Kuang against the fossil homonym *Cathaya* Karav. The proposal was approved, and *Cathaya* Chun & Kuang is now a conserved name (appendix III, Turland et al. 2018).

A further complication, however, was recently discovered. Chun and Kuang (1958, 1962) designated Guang-Fu-Lin-Qu Exped. (as ‘*Expeditionis Kwangfu Lingchu*’) 00198 (IBSC) as the type of *C. argyrophylla*. We examined the collection and found 11 duplicates of ‘*Expeditionis Kwangfu Lingchu*’) 00198 in IBSC. Under Art. 40.2 Note 1, these duplicates should be considered as syntypes (duplicates in other herbaria are considered isosyntypes). Of the 11 duplicates, IBSC0000004 (Fig. 1) bears the annotation ‘TYPUS’ and was published as Plate VIII in the paper of 1958. We consider that it was Chun and Kuang’s (1958) intention to indicate this specimen as the type. In examining the specimen further, we found that it includes reproductive shoots of seed-bearing cones, seeds in bags, bark, pollen-bearing cones in a bag and a branch bearing a pollen cone. Prof. L.K. Fu, a retired gymnosperm specialist in China, commented that the seed-bearing cones were collected in May, 1955, while the pollen-bearing cones were collected in 1956





(Fu and Cheng 1981; Fu 1998). Based on these clues, we consider that IBSC0000004 consists of two gatherings, pollen-bearing cones collected in 1956 and other parts of the plant collected in 1955, but which were later mounted on a single herbarium sheet. Here we lectotypify the name *C. argyrophylla* Chun & Kuang with IBSC0000004, excluding the branch with pollen-bearing cones and the bag containing two pollen-bearing cones.

## Typification

***Cathaya* Chun & Kuang, Acta Bot. Sin. 10: 245. Sep 1962 [Pin. / Pin.], nom. cons.**

**Type species.** *C. argyrophylla* Chun & Kuang.

***Cathaya argyrophylla* Chun & Kuang, Acta Bot. Sin. 10: 245. Sep 1962.**

**Type.** CHINA. Guangxi, Longsheng Xian., alt. 1400 m, on sunny rocky slopes, 16 May 1955, *Guang-Fu-Lin-Qu 00198* (lectotype: IBSC0000004 excl. pollen-bearing cones, photo!, here designated; isolectotypes: GAC0005145 photo!, IBK00200027 photo!, IBK00190034 photo!, IBK00190035 photo!, IBK00190036 photo!, IBSC0000005, IBSC0011250, IBSC0003243 photo!, IBSC0003246 photo!, IBSC0003247, IBSC0003248 photo!, IBSC0003249 photo!, IBSC0003250 photo!, IBSC0003251 photo!, IBSC0003252 photo!, PE00000497 photo!, PE00000498 photo!, SYS00095331 photo!, SZ00004502 photo!).

**Note.** Photos are in Lin (2014, citing PE00000497) and in the Chinese Virtual Herbarium (2022).

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# *Lysimachia coriacea* (Primulaceae, Myrsinoideae), a new species from Chongqing, China

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## Abstract

A new species, *Lysimachia coriacea*, from Chongqing, China, is described and illustrated. It is assigned to subgen. *Lysimachia* sect. *Nummularia* ser. *Paridiformes* and resembles *L. paridiformis* var. *stenophylla*, but is characterised by smaller leathery leaves with black glandular striations near the margin. It is also similar to *L. nanpingensis* in its two to three pairs of leaves sub-whorled at the stem apices, but differs by smaller leathery leaves and densely glandular stem, petiole and pedicel, and calyx lobes with sparse black glandular stripes.

## Keywords

field expedition, *Lysimachia*, morphology, Nanchuan County, taxonomy

## Introduction

*Lysimachia* L. is the largest genus in the tribe Lysimachieae (Primulaceae), which consists of approximately 200 species worldwide (Chen and Hu 1979; Chen and Hu 1989; Hu and Kelso 1996; Marr and Bohm 1997; Hao et al. 2004; Julius et al. 2016). Traditionally, the classification of Lysimachieae includes six genera (*Anagallis* L., *Asterolinon* Hoffmanns. & Link, *Glaux* Tourn. ex L., *Lysimachia*, *Pelletiera* A.St.-Hil., and *Trientalis* Ruppius ex L.) based on several characters, such as the dehiscing pattern of the capsule and the number of corolla lobes. Molecular evidence even suggested expanding the delimitation of *Lysimachia* by including all satellite genera in the tribe (e.g. Anderberg et



al. 2007). China has been considered as the diversity centre of this genus with approximately 130 species recorded in Flora of China (Hu and Kelso 1996). Subsequently, at least 20 new species have been discovered in the last two decades (Peng and Hu 1999; Shao et al. 2004; Shao et al. 2006; Yan and Hao 2012; Liu et al. 2014a, b; Estes et al. 2015; Zhou et al. 2015; Baskose et al. 2016; Julius et al. 2016; Wang et al. 2016; Yan et al. 2017; Wang et al. 2018; Huang et al. 2019; Liu et al. 2020; Mou et al. 2020; Yi 2020; Ju et al. 2021; Lu et al. 2021). In recent years, multiple field expeditions have been conducted in the Municipality of Chongqing, China. During several fieldworks, a species new to science of *Lysimachia* was discovered and is described below.

## Materials and methods

Multiple field investigations were conducted between April 2019 and July 2022 to collect the specimens of the putative new species. The morphological descriptions are based on both living and dried materials, which are deposited in the Herbaria of South China Botanical Garden, Chinese Academy of Sciences (IBSC) and Kunming Institute of Botany, Chinese Academy of Sciences (KUN). This study was based on an examination of herbarium specimens at IBSC, PE, KUN and digital images from the Chinese Virtual Herbarium (CVH: <https://www.cvh.ac.cn/index.php>), Global Plants JSTOR (<http://plants.jstor.org/>) and Plants of the World Online (POWO: <http://www.plantsoftheworldonline.org/>). In addition, the descriptions of its most similar species (*L. paridiformis* var. *stenophylla* Franch. and *L. nanpingensis* F.H. Chen & C.M. Hu) from the relevant taxonomic literature (Chen and Hu 1979; Chen and Hu 1989; Hu and Kelso 1996) were also consulted. The conservation assessment of the putative new species was undertaken using IUCN categories of threat (IUCN Standards and Petitions Committee 2022).

## Taxonomic treatment

***Lysimachia coriacea* S.R. Yi & H.F. Yan, sp. nov.**

urn:lsid:ipni.org:names:77310050-1

Figs 1, 2

**Type.** CHINA. Chongqing: Nanchuan District, Nanping Town, Shangengqiao, 29°02'N, 107°08'E, 740 m a.s.l., 28 April 2019, *Si-Rong Yi* YSR8174 (holotype IBSC!; isotypes KUN!).

**Diagnosis.** The new species belongs to subgen. *Lysimachia* sect. *Nummularia* ser. *Paridiformes* Chen & Hu (1979: 36) characterized by verticillate upper leaves, scale-like lower leaves and umbellate inflorescence. It is similar to *L. paridiformis* var. *stenophylla* in having scale-like lower leaves, upper leaves in a terminal whorl and terminal umbels, but it is easily distinguished by its smaller and leathery leaves (vs. papery) only with black

glandular stripes near the margin (vs. black glandular stripes on the whole leaf blade), pedicels with dense stalked glands (vs. glabrous), and corollas without black glandular stripes (vs. occasionally with black glandular stripes) (Hu and Kelso 1996). At first glance, it also looks similar to *L. nanpingensis*, but differs by its smaller leathery leaves (vs. papery) and densely glandular stem, petiole and pedicel (vs. densely fulvous pubescent), and glabrous calyx lobes with sparse black glandular stripes (vs. pubescent and sparsely reddish glandular punctate). A more detailed comparison of the three species is provided in Table 1.

**Description.** Herbs perennial, 5–15 cm tall. **Rootstock** with numerous fibrous roots. **Stem** terete, erect or lower part procumbent, rooting at nodes, simple or branched, with dense stalked glands when young. **Leaves** opposite, lowest 1–2 pairs scale-like, upper 2–3 pairs closely crowded; blades elliptic or ovate-elliptic, 1.8–3.5 × 1.0–1.8 cm, leathery, adaxially dark green, smooth and glabrous, lustrous; abaxially light green, glabrous, black glandular striate near entire margin, base cuneate, apex acute, mid-vein impressed above, raised below, lateral veins and veinlets inconspicuous; petiole 5–15 mm long, with dense stalked glands. **Flowers** 1–5, crowded at apex of stems, bracts oblanceolate, 6 mm long, green, sparsely glandular; pedicel 6–15 mm long, with dense stalked glands, erect in fruit; calyx lobes narrowly lanceolate, 4.5–6.8 × 0.9–1.5 mm, divided to base, apex acuminate, abaxially sparsely black glandular striate, margin glandular, membranous; corolla yellow, tube 2–3 mm high, lobes elliptic, 6–9 × 5.5–6.8 mm, apex obtuse or slightly emarginate; filaments connate basally into a tube, ca. 2 mm high, with dense granular glandular spots, free parts 2–3 mm long; anthers oblong, 0.8–1.2 mm long, dorsifixed; ovary subglobose, 0.9–1.2 mm in diam., glabrous; style 4.5–6.0 mm long, glabrous. **Capsule** subglobose, 2.5–4.5 mm in diam.

**Distribution and habitat.** *Lysimachia coriacea* is only known from the western slopes of Jinfo Mountain, Nanchuan, Chongqing, China. It grows on damp rocks or cliffs in forests, along roadsides and in mountain valleys at elevations of 740–1,300 m.

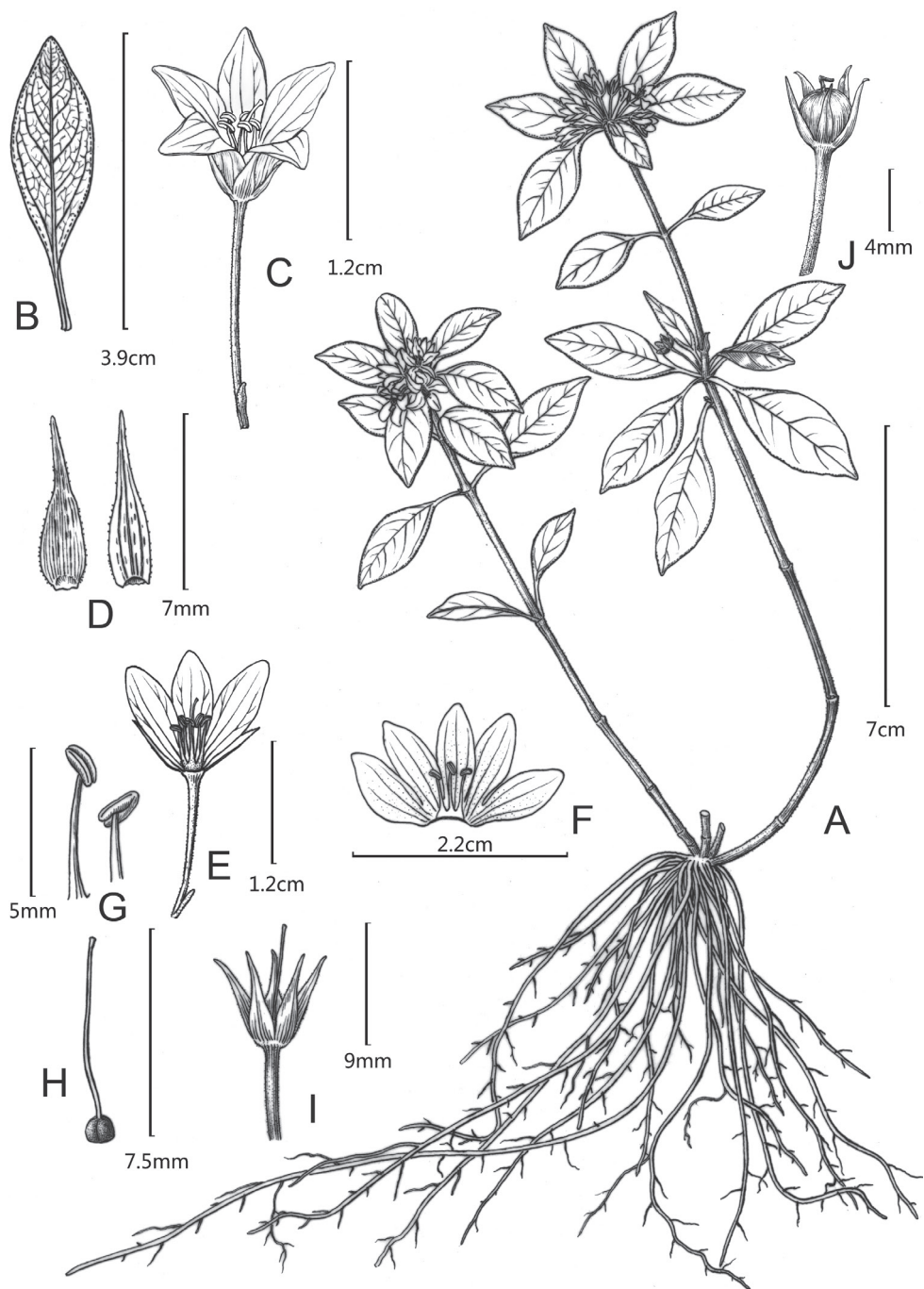
**Conservation status.** This new species has only been found on Jinfo Mountain with at least four populations, where its area of occupancy is less than 10 km<sup>2</sup>. Fortunately, the current distribution area of the species is under the protection of the Jinfo Mountain National Natural Reserve. Thus, it is assigned the status of “Least Concern” (LC) according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2022).

**Phenology.** Flowering from April to May and fruiting from May to June.

**Etymology.** Latin *coriacea*, leathery, alluding to texture of leaves.

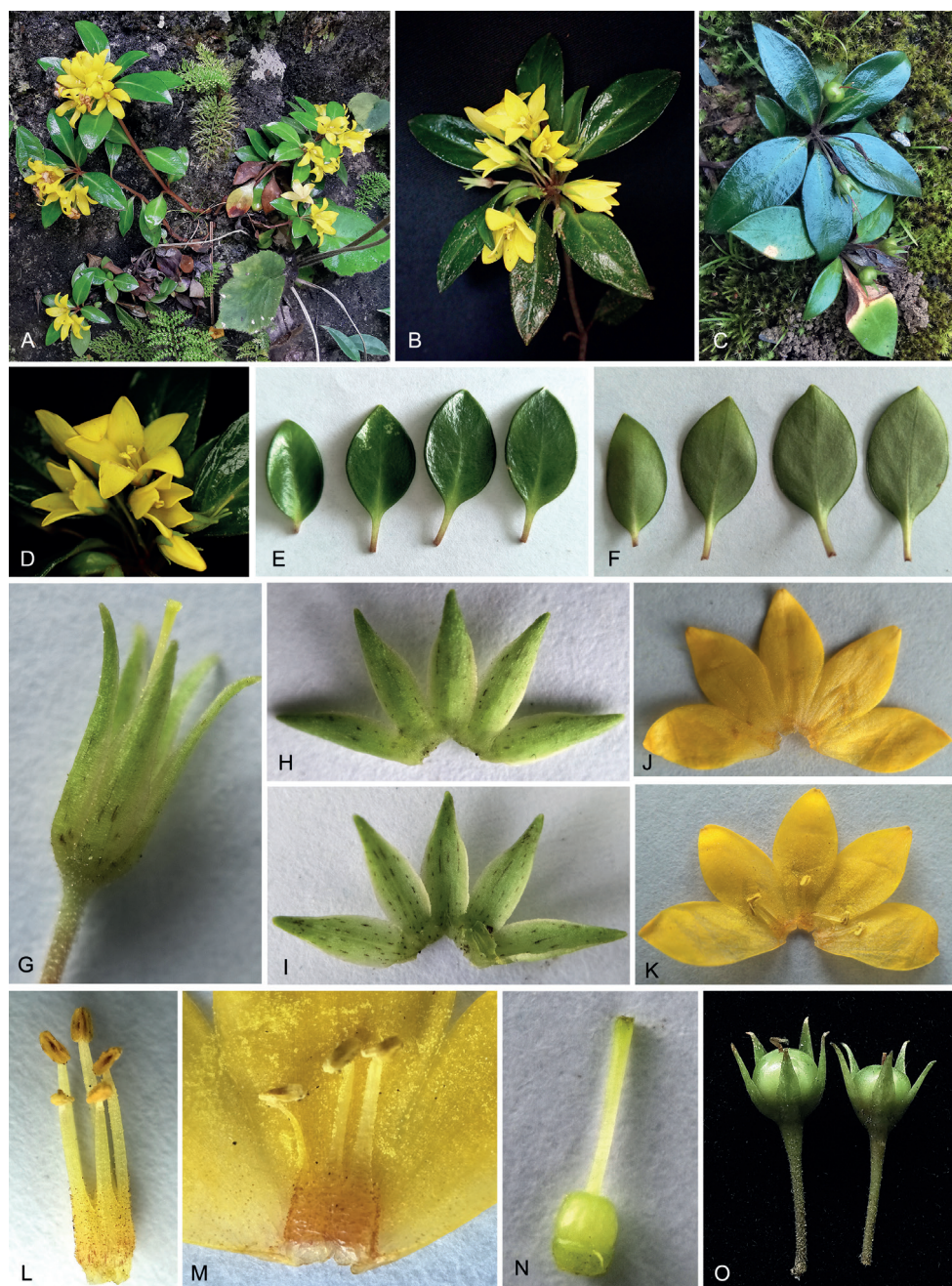
**Chinese name.** 革叶过路黄 (Gé Yè Guò Lù Huáng).

**Additional specimens examined.** CHINA. **Chongqing:** Nanchuan District, Nanping Town, Shangenqiao, 29°02'N, 107°08'E, 740 m a.s.l., in shady fissures of wet rocks, 7 June 2019, *Si-Rong Yi* YSR8004 (KUN1510469!); Nanchuan District, Nanping Town, Huangniya, on a damp rocky roadside, 1,300 m a.s.l., 28 May 1986, *Z. Y. Liu* 0663 (PE01895529!); Nanchuan District, Dutouma (from Lanba to Sanhui), on the roadside, 860 m a.s.l., 7 May 1957, *J.H. Xiong & Z.L. Zhou* 90705 (IBSC0019656!); Nanchuan District, Sanhui Dianchanggou, on a damp rock in forests, 970 m a.s.l., 5 July 1957, *J.H. Xiong & Z.L. Zhou* 91820 (IBSC0019657!).



**Figure 1.** *Lysimachia coriacea* **A** habit **B** leaf **C** flower **D** calyx lobes, abaxial view (right) and adaxial view (left) **E, F** opened corolla, showing filaments connate at base **G** anthers, front view (left) and back view (right) **H** pistil **I** flower with corolla removed **J** young fruit with persistent calyx. Drawn by Yun-Xiao Liu.





**Figure 2.** *Lysimachia coriacea* **A** flowering plants **B** inflorescence **C** fruiting plants **D** flowers **E, F** leaves **G** flower with corolla removed **H** calyx, abaxial view **I** calyx, adaxial view **J** corolla, abaxial view **K** corolla, adaxial view **L–M** stamens **N** pistil **O** young fruits. Photographed by Si-Rong Yi.

**Table 1.** Comparison of diagnostic characters of *Lysimachia coriacea*, *L. paridiformis* var. *stenophylla* and *L. nanpingensis*.

	<i>L. coriacea</i>	<i>L. paridiformis</i> var. <i>stenophylla</i>	<i>L. nanpingensis</i>
Stem	with dense stalked glands when young	glabrous	with dense fulvous multicellular hairs
Leaf blade	elliptic or ovate-elliptic, 1.8–3.5 × 1.0–1.8 cm, leathery; base cuneate, apex acute; with black glandular stripes near the margin; veins inconspicuous, except mid-vein	narrowly elliptic to broadly lanceolate or linear-lanceolate, 4–16 × 1.2–5 cm, papery; base cuneate, apex short acuminate; with or without black glandular stripes; veins 4 or 5 pairs, conspicuous	elliptic to ovate-elliptic, 3.5–5.5 × 2–4.5 cm, papery; base subrounded, apex acute; indistinctly glandular punctate; veins inconspicuous, except mid-vein
Petiole	5–15 mm long; with dense stalked glands	sessile or subsessile; glabrous	3–12 mm long; densely fulvous pubescent
Calyx lobes	4.5–6.8 × 0.9–1.5 mm; glabrous on both sides, sparsely ciliate on the margin, with sparse black glandular stripes	8–13 × 2.5–3.5 mm; glabrous on both sides, occasionally ciliate on the margin, occasionally with black glandular stripes	6–7.5 × 1.2–1.9 mm; pubescent on the abaxial surface, obscurely glandular punctate
Pedice	6–15 mm long, erect in fruit, with dense stalked glands	3–15 mm long; erect in fruit, glabrous	4–9 mm long, recurved in fruit, densely fulvous pubescent
Corolla lobes	6–9 × 5.5–6.8 mm, without glandular stripes	9–11 × 4–4.5 mm, with or without black glandular stripes	9–11 × 3.5–4 mm, sparsely reddish glandular punctate

**Notes.** Specimens of the new species were first collected by Ji-Hua Xiong and Zi-Lin Zhou in 1957 and deposited in IBSC (IBSC0019656 and IBSC0019657). The two specimens were identified as “*Lysimachia paridiformis* var. *stenophylla* Franch.” by Chi-Ming Hu. However, Hu should also notice the differences between this specific taxon and *L. paridiformis* var. *stenophylla*, because he wrote a temporary name “*Lysimachia nitida* Chen et C.M. Hu” on the annotation labels of the specimens. Later, specimens of the new species were collected by some other collectors (see above).

Acknowledgements

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# ***Gastrochilus heminii* (Orchidaceae, Epidendroideae), a new species from Sichuan, China, based on molecular and morphological data**

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## **Abstract**

*Gastrochilus heminii* (Orchidaceae), a new orchid species from Sichuan Province, Southwest China, is described and illustrated. It morphologically resembles *G. affinis* and *G. yei*, but differs markedly from the former in having a thinner and slightly rolled downwards reniform epichile and the central thickened purple-red mat with irregular folds (vs. subtriangular epichile curves upwards, with 2 thick, brown to purplish-brown median ridges from base to apex), and can be clearly distinguished from the latter by having reniform epichile with lobed apex and subconical hypochile with bilobed apex that splits into two conical protrusions (vs. semi-rounded epichile not lobed and subconical hypochile not bilobed). The results of molecular phylogenetic analysis based on nuclear ribosome internal transcribed spacer (nrITS) and four chloroplast DNA fragments (*matK*, *psbA-trnH*, *psbM-trnD*, and *trnL-F*) of 36 *Gastrochilus* species showed that *G. heminii* was closely related to *G. affinis* and *G. distichus*.

## **Keywords**

*Gastrochilus*, morphological, Orchidaceae, phylogeny, taxonomy

\* These authors contributed equally to this work as co-first authors.

## Introduction

*Gastrochilus* D.Don (Orchidaceae, Epidendroideae; Don 1825) is diagnosed by a subdivided labellum with a recurved epichile and a saccate hypochile attached to the column at the base and two subglobose pollinia attached to a slender and filiform stipe (Tsi 1999; Pridgeon et al. 2014). The genus comprises more than 70 accepted species, most of which are distributed in Southeast Asia (Kumar et al. 2014; Liu et al. 2016, 2019; Liu and Gao 2018; Rao et al. 2019; Govaerts et al. 2021; Nguyen et al. 2021; Zhou et al. 2021a, b; Zhang et al. 2022). Liu et al. (2019) first constructed the phylogenetic relationships of *Gastrochilus* and divided them into five clades. Recently, the phylogenetic studies by Liu et al. (2020), Li et al. (2022), and Zhang et al. (2022) also supported the monophyly of *Gastrochilus*.

An unusual arboreal miniature orchid was recently discovered for the first time in Wolong Nature Reserve (Wenchuan County, Sichuan Province, China) during a routine survey. It was tentatively identified as a species of *Gastrochilus* sect. *Microphyllae* Bentham & Hooker (Bentham and Hooker 1883), characterized by plants with extremely small flowers, distichous and alternate leaves compared to plants in the other sections (larger flowers or clustered leaves). However, a morphological examination revealed that this species shows an unusual combination of characters that does not match any known species of *G.* sect. *Microphyllae*. The phylogenetic analysis using five DNA markers (nrITS, *matK*, *psbA-trnH*, *psbM-trnD*, and *trnL-F*) confirmed the monophyly of this taxon. The objectives of this study are (1) to describe, (2) to examine both molecular and morphological affinities of this new *Gastrochilus* species, *Gastrochilus heminii* M.Liao, B.Xu & Yue.H.Cheng, sp. nov.

## Materials and methods

### Morphological analyses

The measurements and description of *Gastrochilus heminii* were based on two living plant individuals and two herbarium specimens (voucher information: *Min Liao & Yue-Hong Cheng ZJY143*; *Min Liao & Yue-Hong Cheng ZJY167*), respectively. The taxonomic description follows the terminology used by Beentje (2012). Voucher specimens and additional silica-gel dried leaves are deposited in CDBI Herbarium (herbarium follows Thiers 2021).

### DNA extraction, amplification and sequencing

The sequences of the two individuals of the new species newly generated in this study, and the sequences of the remaining 42 species used in the molecular phylogenetic analysis, were retrieved from GenBank. The information on the DNA fragments and four complete plastid genomes were listed in Appendix 1. Total DNA was

extracted exclusively from silica-gel dried leaves via a Plant DNA Isolation Kit (Cat. No.DE-06111). We used the same primers as Liu et al. (2019) to amplify the nuclear ribosome internal transcribed spacer (nrITS) and the four chloroplast DNA fragments (i.e., *matK*, *psbA-trnH*, *psbM-trnD*, and *trnL-F*) through polymerase chain reaction (PCR). All DNA samples were sent to TSINGKE Biotech Co. Ltd (Chengdu, China) for sequencing. The sequences were then deposited with GenBank, with the following accession numbers: *G. heminii*, nrITS (ON286752, ON286753), *matK* (ON331126, ON331127), *psbA-trnH* (ON331128, ON331129), *psbM-trnD* (ON331130, ON331131), and *trnL-F* (ON331132, ON331133).

## Phylogenetic analyses

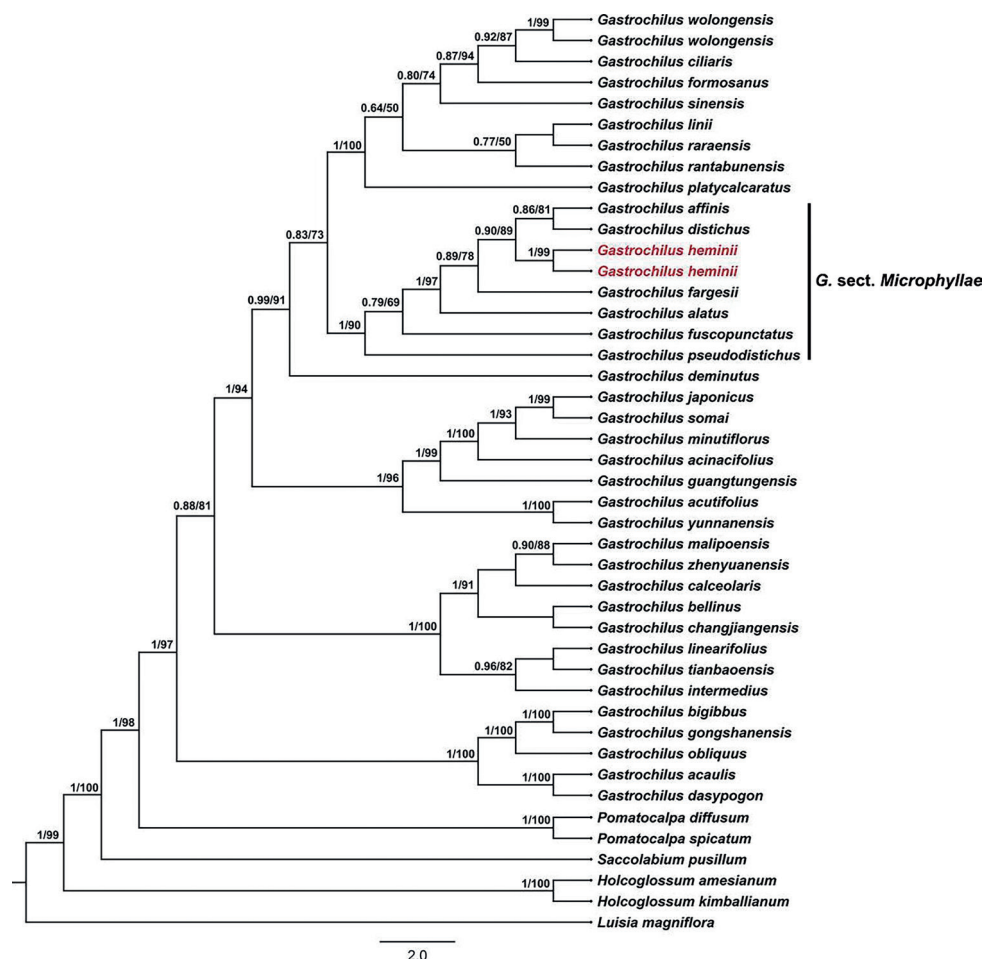
All sequences were edited via Sequencher v4.1.4 (Gene Codes, Ann Arbor, Michigan, USA) and aligned via MAFFT v7.475 (Katoh and Standley 2013) with default parameters. We performed phylogenetic analyses based on combined nuclear ribosome internal transcribed spacer (nrITS) and the four chloroplast DNA fragments. The nucleotide substitution model for the data matrix was estimated using jModeltest v2.1.6 (Posada 2008) software and the best-fit model (GTR+I+G) was selected using the corrected Akaike Information Criterion (AICc). Two different methods, Maximum likelihood (ML) and Bayesian inference (BI), were employed. The ML analysis was performed using IQ-TREE v1.4.2 (Nguyen et al. 2014) with branch support estimated by 2,000 replicates of ultrafast bootstrapping algorithm (UFboot) (Minh et al. 2013). The BI analysis was conducted using MrBayes v3.2.7a (Ronquist and Huelsenbeck 2003), with two separate Markov-chain Monte Carlo (MCMC) chains (1,000,000 generations and sampled every 1,000 generations). The first 25% of the trees were discarded as burn-in, and the remaining trees were used to generate a majority-rule consensus tree.

## Results

The molecular phylogenetic tree showed that the 36 taxa of *Gastrochilus* formed a well-supported monophyletic group (BI/ML = 1/97, Fig. 1). The two individuals of *G. heminii* were resolved as sisters to each other (BI/ML = 1/99, Fig. 1). Our data recovered a sister relationship between *G. affinis* (King & Pantl.) Schltr. (King and Pantling 1898; Schlechter 1913) and *G. distichus* (Lindl.) Kuntze (Lindley 1859; Kuntze 1891); these two species formed a monophyletic group with *G. heminii* (BI/ML = 0.90/89, Fig. 1), which formed a subclade of section *Microphyllae* together with *G. fargesii* (Kraenzl.) Schltr. (Kraenzlin 1903; Schlechter 1919), *G. alatus* X.H.Jin & S.C.Chen (Jin et al. 2007), *G. fuscopunctatus* (Hayata) Hayata (Hayata 1912, 1917), and *G. pseudodistichus* (King & Pantl.) Schltr. (King and Pantling 1895; Schlechter 1913).

Morphologically, *G. heminii* is closest to *G. affinis* and *G. yei* JianW.Li & X.H.Jin (Li et al. 2021). A thorough morphological comparison among *G. heminii*, *G. affinis*, *G. yei* and *G. distichus* is summarized in Table 1 and Fig. 2.





**Figure 1.** Maximum likelihood tree of *Gastrochilus* from phylogenetic analysis of combined nrITS and plastid DNA markers. Numbers before and after the slash indicate Bayesian posterior probabilities and ML bootstrap supports, respectively. The position of *Gastrochilus heminii* is indicated in red.

## Taxonomic treatment

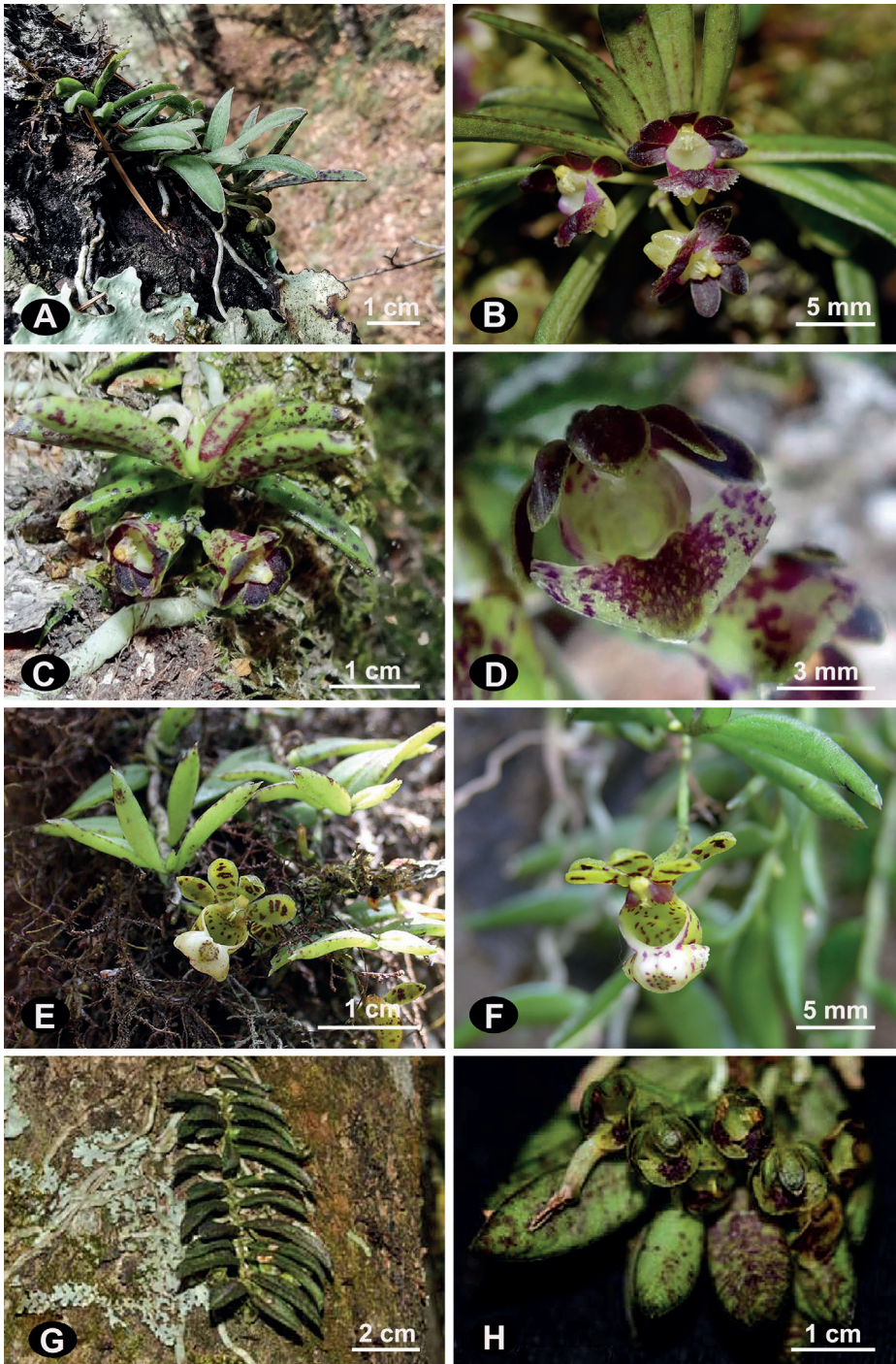
### *Gastrochilus heminii* M.Liao, B.Xu & Yue H.Cheng, sp. nov.

urn:lsid:ipni.org:names:77310051-1

Figs 2A, B, 3

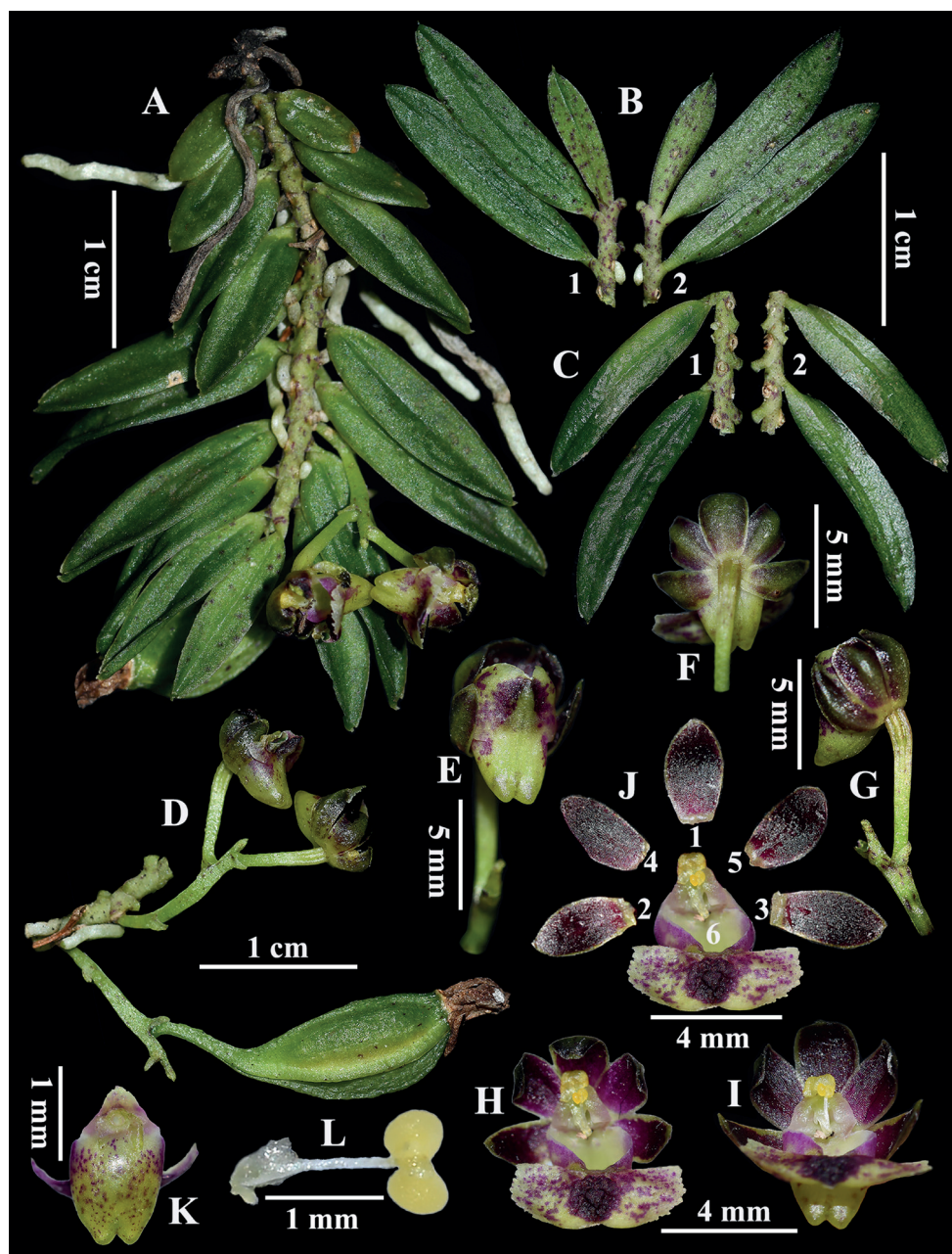
**Type.** CHINA. Sichuan: Wenchuan, coniferous and broadleaf mixed forest, on tree trunk, elev. ca. 2640 m, in flowering and fruiting, 15 March 2022, *Min Liao & Yue-Hong Cheng ZJY143* (holotype CDBI!).

**Diagnosis.** *Gastrochilus heminii* is morphologically related to *G. affinis* and *G. yei* based on vegetative and floral characteristics such as similar habit, distichous and alternate leaves, epichile surface smooth and glabrous, sepals and petals with purplish-red



**Figure 2.** Comparison of three taxa of *Gastrochilus* **A, B** *Gastrochilus heminii* **C, D** *G. affinis* **E, F** *G. distichus* **G, H** *G. yei*. [Images **C, D** cited from Jalal et al. 2020; image **E** reproduced from website (<http://www.orchidspecies.com/gastdistichus.htm>); image **F** cited from Kumar et al. 2014, which was photographed by Xiao-Hua Jin; images **G** and **H** cited from Li et al. 2021].





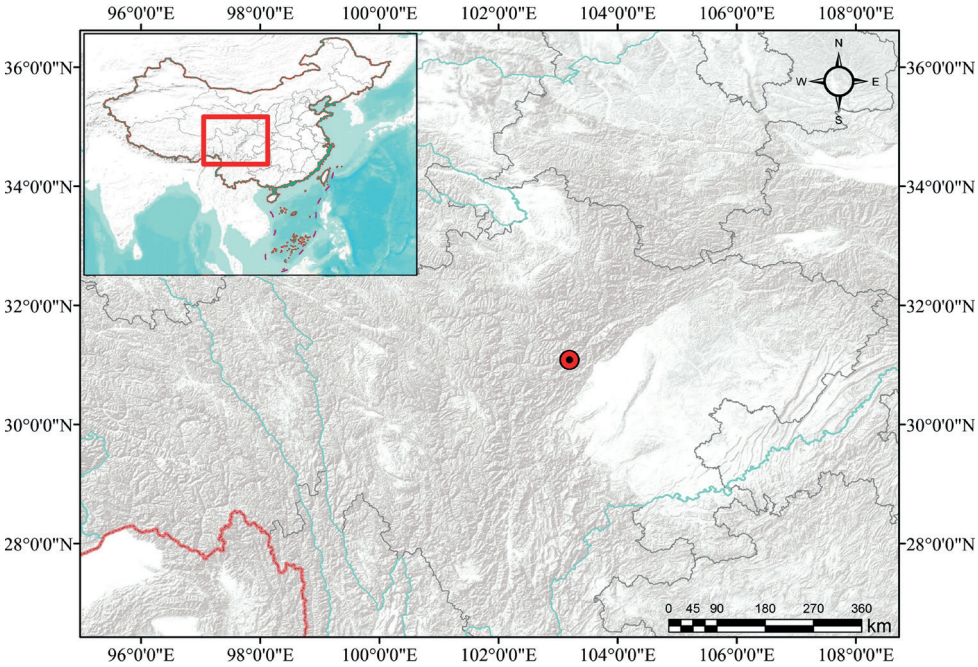
**Figure 3.** *Gastrochilus heminii* **A** flowering plant **B** young leaves (B1: front view; B2: reverse view) **C** old leaves (C1: front view; C2: reverse view) **D** raceme and capsule (side view) **E–I** flowers (different views) **J** anatomy of flower (J1: dorsal sepals; J2 and J3: lateral sepals; J4 and J5: petals; J6: labellum) **K** hypochile (dorsal view) **L** stamens.

patches. However, it can be differentiated from *G. affinis* on the basis of flower numbers (1–2 in the former vs. 1–4 in the latter), peduncle length (0.4–0.7 cm in the former vs. 1.5–2.0 cm in the latter) and an additional morphological characteristic: young leaves are densely covered with purple-red spots and old leaves have hardly any purple-red spots in the former (both have purple-red spots in the latter); the reniform epichile is rolled downwards, smooth and glabrous above, and central thickened purple-red mat with irregular folds in the former (subtriangular epichile curves upwards, with 2 thick, brown to purplish-brown median ridges from base to apex in the latter). It differs from *G. yei* by having reniform and lobed epichile (not lobed in the latter), apex of hypochile bilobed and splits into two conical protrusions (not bilobed in the latter), apex of the leaf with 1–2 lobules, lobes setaceous (apex of leaf with a tine in the latter).

**Description.** Miniature trunk epiphyte. Roots vermiform, 2.0–7.0 cm long and ca. 1.5 mm thick. Stem pendulous, slender, purplish-red spots, 3.0–6.5 cm long and ca. 1.8 mm thick. Leaves alternate, distichous, narrowly oblong or falcate oblong, 0.9–2.3 × 0.3–0.5 cm, apex acute and with 1–2 lobules, lobes setaceous, young leaves with conspicuous purplish-red spots, old leaves with hardly any purplish-red spots. Raceme with 1 or 2 flowers; inflorescence stalk curved upward and thickened, 4.0–9.0 mm long, proximally covered with two sheaths; floral bracts ovate-lanceolate, 0.7–1.0 mm long, apex acute; pedicel and ovary connate, 4.0–5.5 mm long; flowers spreading, ca. 6.0 × 5.0 mm; sepals and petals heterochromatic on both surfaces, outside yellow-green with purplish-red spots, inside purplish-red with yellow-green margin; dorsal sepals and lateral sepals similar and equal in size, elliptic-oblong, ca. 2.4 × 1.5 mm, apex obtuse; petals narrowly oblong, ca. 2.6 × 1.3 mm, apex acute, base narrowed; epichile reniform, yellow-green with purplish-red spots, 4.2–6.5 × 2.0–3.0 mm, margin erose, smooth and glabrous above, central thickened purple-red mat with irregular folds; hypochile subconical, yellow-green with purple-red spots, 2–2.4 × 1.6–2 mm, dorsally compressed, slightly bent outward, the apex splits into two conical protrusions; column cylindrical, ca. 1.0 mm; anther cap subhemispheric, with two chambers, 0.7 × 0.4 mm, hanging from both ends of the stipe; pollinia 2, 0.4 × 0.3 mm, yellow, full and nearly spherical, with a depression in the center; stigma deeply sunken, inverted V-shaped, ca. 0.6 mm long, yellow, apically forked, forked in a subtriangular outline. Capsule shuttle-shaped with six ribs, green with sparse purplish-red spots, ca. 1.1 cm long, inflated to ca. 0.6 cm in the middle, persistent and growing for one year until maturity.

**Additional specimens examined.** —CHINA. Sichuan: Wenchuan, coniferous and broadleaf mixed forest, on tree trunk, elev. ca. 2640 m, 18 April 2022, *Min Liao & Yue-Hong Cheng* ZJY167 (CDBI).

**Distribution, habitat and phenology.** The new species is currently known only from Wenchuan County, Sichuan Province, Southwest China (Fig. 4). It is found epiphytic on the trunk of *Tsuga chinensis* (Franch.) Pritz. in a subalpine mixed coniferous forest at elevation ca. 2640 m. *Gastrochilus heminii* flowers from March to April.



**Figure 4.** Distribution map of *Gastrochilus heminii*.

**Table 1.** Morphological comparison of *Gastrochilus heminii* with three related species, *G. affinis*, *G. distichus* and *G. yei*.

Character	<i>G. heminii</i>	<i>G. affinis</i>	<i>G. distichus</i>	<i>G. yei</i>
Plant length	3.0–6.5 cm	4.0–15.0 cm	1.5–20.0 cm	3.0–8.0 cm
Leaf shape	narrowly oblong or falcate oblong	oblong-lanceolate to subspathulate	lanceolate or falcate lanceolate	blade lanceolate
No. of flowers per inflorescence	1 or 2 flowers	1–4 flowers	2–4 flowers	2–6 flowers
Peduncle length	0.4–0.7 cm	1.5–2.0 cm	1.0–1.5 cm	0.7–1.0 cm
Dorsal sepal	elliptic-oblong, ca. 2.4 × 1.5 mm, concave, apex obtuse	elliptic-oblong, 3–5 × 1–1.3 mm, concave, apex obtuse	oblong-elliptic, 4.5–0.5 × 2.5–3 mm, concave, apex obtuse	oblong, 3.3 × 1.9–2.0 mm, apex rounded
Lateral sepals	similar to dorsal sepal, equal in size	elliptic-ovate, 3.5–4 × 0.7–1.3 mm, slightly oblique and incurved, apex obtuse	similar to dorsal sepal, equal in size	oblong, 3.9–4.0 × 1.8–1.9 mm, apex obtuse
Petals	narrowly oblong, ca. 2.6 × 1.3 mm, apex acute, base narrowed	ovate-elliptic to elliptic, 3–4 × 1–1.3 mm, apex obtuse	subobovate, slightly smaller than sepals, apex obtuse	oblong, 3.5 × 1.8 mm, apex rounded
Epichile	reniform, 4.2–6.5 × 2.0–3.0 mm, margin erose, smooth and glabrous above, central thickened purple-red mat with irregular folds	subtriangular, decurved, subacute at apex, margin finely erose at base, disk with 2 thick, brown to purplish-brown median ridges from base to apex.	subcircular, 5 × 3 mm, apex obtuse, margin entire, smooth and glabrous above and thickened cushion-like centrally, with 2 conical callosities near base	semi-rounded, 2.0–2.2 × 4.0–4.2 mm, glabrous, with a thickened central, rugose cushion, tint with purple, margin irregularly denticulate
Hypochile	subconical or helmet-shaped, ca. 2–2.4 × 1.6–2 mm, dorsally compressed, slightly bent outward, the end splits into two conical protrusions	obconical, 3–4 × 2–3 mm, dorsally compressed, slightly bent outward, subacute to obtuse and shortly bifid at apex	subcupular, 4 × 2–3 mm, rounded at end, dorsally compressed, slightly bent outward	subconical, 3 mm tall, 3 mm in diameter, apex rounded



**Etymology.** Named in honor of Mr. He-Min Zhang, the advocate of the panda “Release to the Forest”, one of the pioneers of panda research in China, renowned as the “father” of pandas, in recognition of his contribution to the conservation of flora and fauna in the region which was one of the earliest reserves dedicated to preserving the habitat of wild pandas. A Chinese name, *he min peng ju lan* (和民盆距兰), is suggested here.

**Conservation status.** Three populations with a total of ca. 200 individuals of *Gastrochilus heminii* have been documented during our investigation. However, similar habitat of this new species is widespread in the Wolong Natural Reserve. Therefore, we assessed the conservation status of *Gastrochilus heminii* as DD (Data Deficient) according to the IUCN (2022).

## Acknowledgements

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Appendix I

Table A1. The GenBank accession numbers for DNA sequences used in this study.

Taxa	nrITS	matK	psbA-trnH	trnL-F	psbM-trnD
<i>G. acaulis</i> (Lindl.) Kuntze	KM583455	KM583465	—	—	—
<i>G. acinacifolius</i> Z.H.Tsi	KJ733412	KJ733569	KJ733492	KJ733649	MK357216
<i>G. acutifolius</i> (Lindl.) Kuntze	MT225573	MW433889	—	—	MK357230
<i>G. affinis</i> (King & Pantl.) Schltr.	—	MK357141	—	—	MK357141
<i>G. alatus</i> X.H.Jin & S.C.Chen	—	—	—	—	MK357228
<i>G. bellinus</i> (Rchb.f.) Kuntze	KY966597	KY966884	MK357164	MK357202	MK357240
<i>G. bigibbus</i> (Rchb.f. ex Hook.f.) Kuntze	—	MN124439	MN124439	MN124439	MN124439
<i>G. calceolaris</i> (Buch.-Ham. ex Smith) D.Don	MN517123	MK357144	MK357169	MK357204	MK357232
<i>G. changjiangensis</i> Q.Liu & M.Z.Huang	MK357124	—	MK357166	—	MK357236
<i>G. ciliaris</i> F.Mackawa	—	MK357148	MK357173	—	MK357225
<i>G. dasypogon</i> (Lindl.) Kuntze	DQ091685	MK357149	MK357181	MK357197	MK357219
<i>G. deminutus</i> J.M.H.Shaw	KY966600	KY966887	—	—	—
<i>G. distichus</i> (Lindl.) Kuntze	KJ733414	KJ733571	KJ733494	KJ733651	—
<i>G. fargesii</i> (Kraenzl.) Schltr.	KJ733415	KJ733572	—	KJ733652	—
<i>G. formosanus</i> (Hayata) Hayata	KJ733416	KJ733573	KJ733495	KJ733653	MK357226
<i>G. fuscopunctatus</i> (Hayata) Hayata	MK317970	MK357150	MK357171	MK357192	MK357231
<i>G. gongshanensis</i> Z.H.Tsi	—	MN124438	MN124438	MN124438	MN124438
<i>G. guangtungensis</i> Z.H.Tsi	KJ733417	KJ733574	KJ733496	KJ733654	KJ733654
<i>G. heminii</i> M.Liao, B.Xu & Yue.H.Cheng	ON286752	ON331126	ON331128	ON331130	ON331132
<i>G. heminii</i> M.Liao, B.Xu & Yue.H.Cheng	ON286753	ON331127	ON331129	ON331131	ON331133
<i>G. intermedius</i> (Griff. ex Lindl.) Kuntze	MK357121	MK357151	MK357172	MK357190	MK357213
<i>G. japonicus</i> (Makino) Schltr.	KJ733418	KF545886	KF545866	KF545897	KX871236
<i>G. linearifolius</i> Z.H.Tsi & Garay	MK357133	MK357136	MK357187	MK357194	MK357229
<i>G. linii</i> Ormerod	—	MK357152	MK357176	MK357198	MK357224
<i>G. malipoensis</i> X.H.Jin & S.C.Chen	—	MK357147	MK357177	MK357200	MK357235
<i>G. minutiflorus</i> Aver.	—	MK357153	MK357179	—	MK357215
<i>G. obliquus</i> (Lindl.) Kuntze	MK357131	MK357137	KJ733498	KJ733656	MK357211
<i>G. platycalcaratus</i> (Rolfé) Schltr.	MK357122	—	MK357175	—	MK357222
<i>G. pseudodistichus</i> (King & Pantl.) Schltr.	MK357132	—	MK357170	—	MK357221
<i>G. rantabunensis</i> C.Chow ex T.P.Lin	—	MK357155	MK357184	MK357193	MK357223
<i>G. nanaensis</i> Fukuyama	KJ733420	KJ733577	KJ733499	KJ733657	MK357239
<i>G. sinensis</i> Z.H.Tsi	OM985813	OK042953	OK172399	OK172401	—
<i>G. somai</i> (Hayata) Hayata	MK357128	MN124436	MK357180	MN124436	MK357220
<i>G. tianbaoensis</i> Q.Liu & Y.H.Tan	MK357120	MK357157	MK357186	MK357207	MK357214
<i>G. wolongensis</i> Jun.Y.Zhang, B.Xu & Yue.H.Cheng	OM985810	OK172400	OK172402	OK172404	OK172403
<i>G. wolongensis</i> Jun.Y.Zhang, B.Xu & Yue.H.Cheng	OM985811	OM974209	OM974211	OM974210	—
<i>G. yunnanensis</i> Schltr.	MK165469	MK357158	MK357185	—	MK357212
<i>G. zhenyuanensis</i> Q.Liu & D.P.Ye	MK357127	MK357146	MK357168	MK357199	MK357237
<i>Holcoglossum amesianum</i> (Rchb.f.) Christenson	HQ404389	JF763779	HQ404439	—	—
<i>Holcoglossum kimballianum</i> (Rchb.f.) Garay	HQ452901	JF763787	HQ404452	—	—
<i>Luisia magniflora</i> Z.H.Tsi & S.C.Chen	KJ733426	KJ733583	KJ733505	—	KJ733663
<i>Pomatocalpa diffusum</i> Breda	AB217576	AB217752	—	—	EF670432
<i>Pomatocalpa spicatum</i> Breda	DQ091706	KJ733595	KJ733518	—	KJ733675
<i>Saccolabium pusillum</i> Blume	AB217580	AB217756	—	—	—

# *Aphyllorchis periactinantha* (Orchidaceae, Neottieae), a new mycoheterotrophic species from peninsular Thailand

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## Abstract

A new orchid species from southern Thailand, *Aphyllorchis periactinantha*, is described and illustrated. The novelty is characterized by the subactinomorphic flowers, the concave labellum, not divided into hypochile and epichile, the reduced staminodes, the shallowly bilobed stigma and the semicircular rostellum. A key to the species of *Aphyllorchis* in Thailand is updated.

## Keywords

achlorophyllous orchid, dipterocarp forest, Epidendroideae, peloric flower, Thai-Malay Peninsula, Ton Nga Chang Wildlife Sanctuary



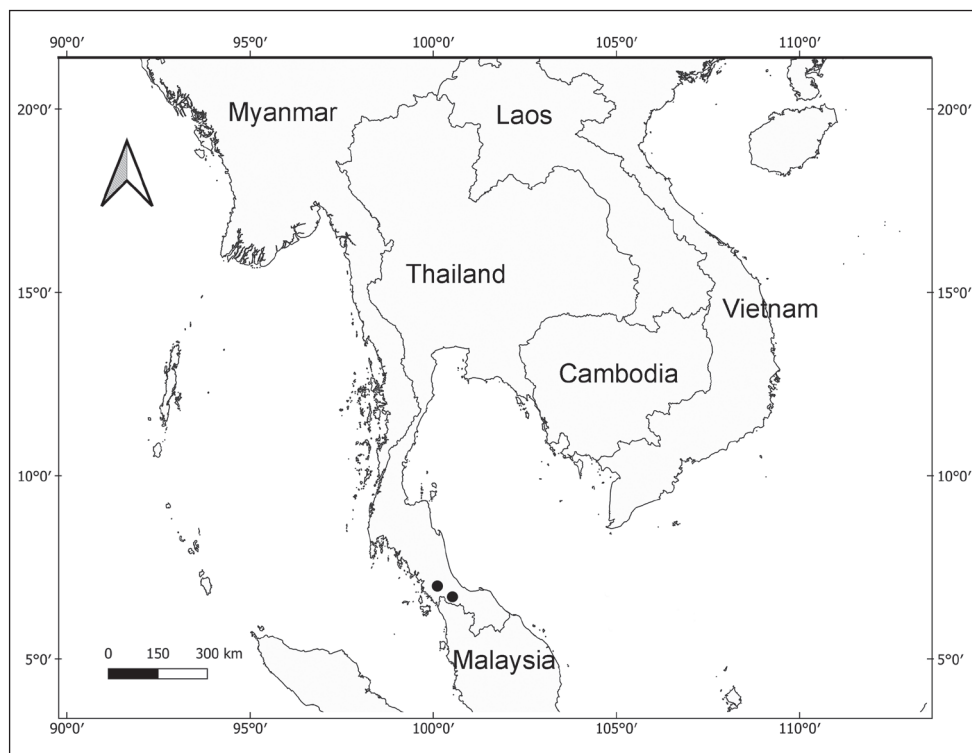
## Introduction

*Aphyllorchis* Blume is one of the largest mycoheterotrophic orchid genera with 19 currently accepted species (POWO 2022), mainly distributed in tropical Asia and the Himalayas, extending to Japan and Australia (Pridgeon et al. 2005; Suetsugu et al. 2018; Qin et al. 2021). Members of this genus are leafless and achlorophyllous herbs with erect and unbranched stems, racemose, many-flowered inflorescences, petals similar to sepals but shorter and narrower, and a labellum usually divided into hypochile and epichile (Pridgeon et al. 2005; Hsieh et al. 2013; Tian et al. 2013; Suetsugu et al. 2018; Qin et al. 2021). In Thailand, four species have been recorded: *A. caudata* Rolfe ex Downie, *A. evrardii* Gagnep., *A. montana* Rchb.f. and *A. pallida* Blume (Downie 1925; Seidenfaden 1978; Roy et al. 2009; Pedersen 2014).

In November 2015, during an orchid survey in Ton Nga Chang Wildlife Sanctuary (TNCWS), Songkhla province, southern Thailand, an immature inflorescence of an unknown achlorophyllous orchid was observed. However, due to the immature flowers, it could not be accurately determined. Later, four further botanical surveys to TNCWS and Ban Yang Ko community forest resulted in four more collections of this unknown orchid. After careful examination, these specimens were identified as belonging to *Aphyllorchis*, however, they differed from all the other known species of the genus in Thailand. These specimens resemble *A. anomala* Dockr. from Australia, *A. montana* Rchb.f. var. *rotundatipetala* (C.S.Leou, S.K.Yu & C.T.Lee) T.P.Lin from Taiwan, *A. simplex* Tang & F.T.Wang from China and Vietnam, and *A. striata* (Ridl.) Schltr. from Peninsular Malaysia and Borneo in having subactinomorphic (peloric) flowers and a labellum not divided into hypochile and epichile as in most species of *Aphyllorchis*. Following a detailed comparison with closely related taxa, we here describe and illustrate these specimens as a new species.

## Materials and methods

Field surveys were carried out in TNCW and Ban Yang Ko community forest, Songkhla province, southern Thailand (Fig. 1) in October 2017, November 2018, December 2020 and 2021. The specimens were photographed and deposited in BKF and PSU. Morphological characters were studied using an Olympus SZX12 stereomicroscope and the distinctive characters of the species were illustrated with the aid of an Olympus drawing tube (SZX-DA). The measurements and description were prepared from living plants and spirit materials. Comparisons of diagnostic characters were based on Thai specimens, as well as digital images of specimens held at BRI, BKF, K, PSU, and SING, available online, and on relevant taxonomic literature (e.g. Blume 1825; Downie 1925; Dockrill 1965; Seidenfaden 1978; Seidenfaden and Wood 1992; Hsieh et al. 2013; Tian et al. 2013; Pedersen 2014; Suetsugu et al. 2018; Qin et al. 2021). Herbarium acronyms follow Index Herbariorum (Thiers 2022). The preliminary conservation status was assessed following the International Union for Conservation of Nature (IUCN) Red List criteria (IUCN 2022) and using GeoCAT (Bachman et al. 2011) to calculate the area of occupancy (AOO) and extent of occurrence (EOO).



**Figure 1.** Distribution of *Aphyllorchis periactinantha* A.Chantanaorr. & Chantanaorr. (black circle) in Thailand.

## Taxonomic treatment

### *Aphyllorchis periactinantha* A.Chantanaorr. & Chantanaorr., sp. nov.

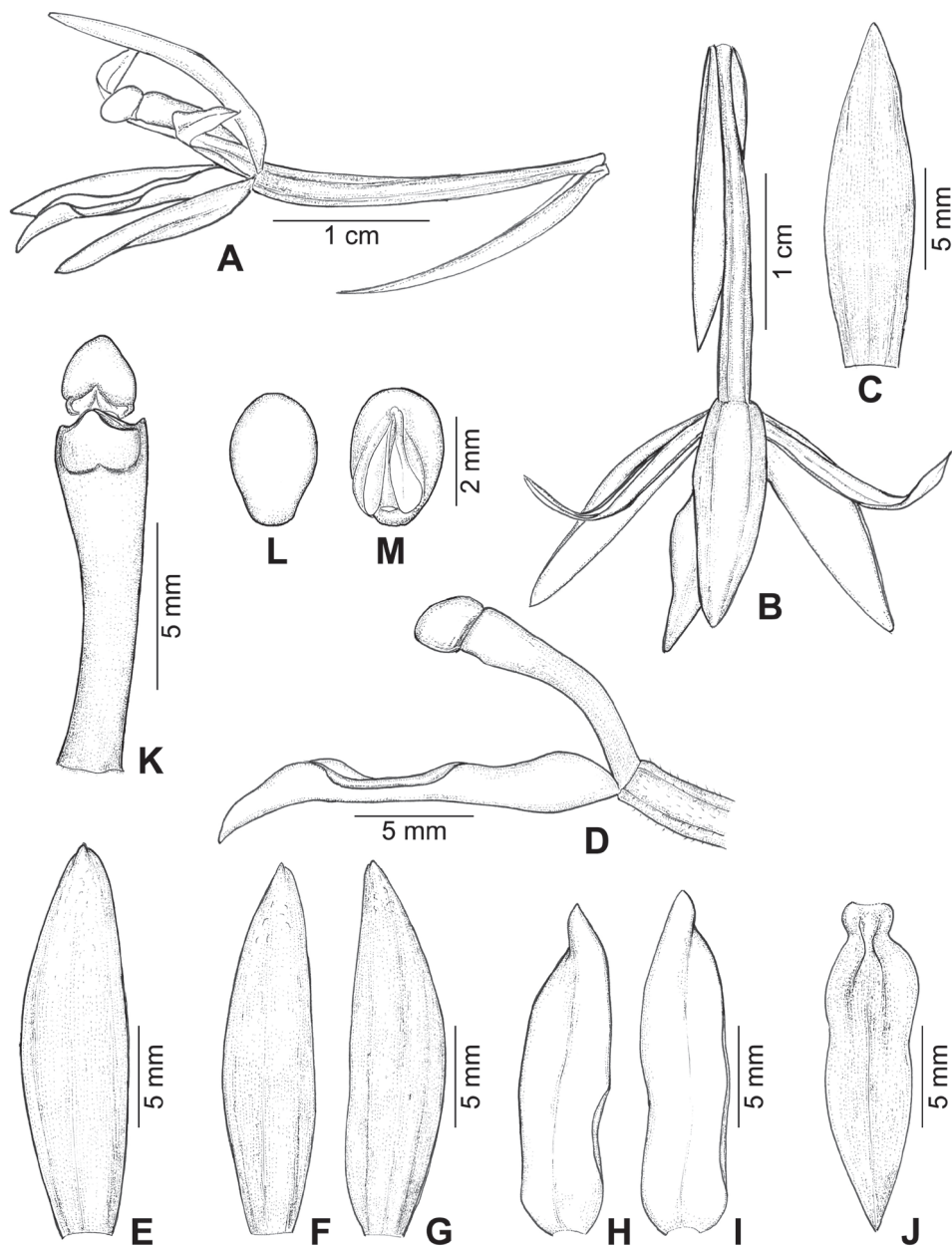
urn:lsid:ipni.org:names:77310465-1

Figs 2, 3

**Diagnosis.** Similar to *A. anomala*, but differs in having a concave labellum without purple veins, acute at the apex, and a semicircular rostellum.

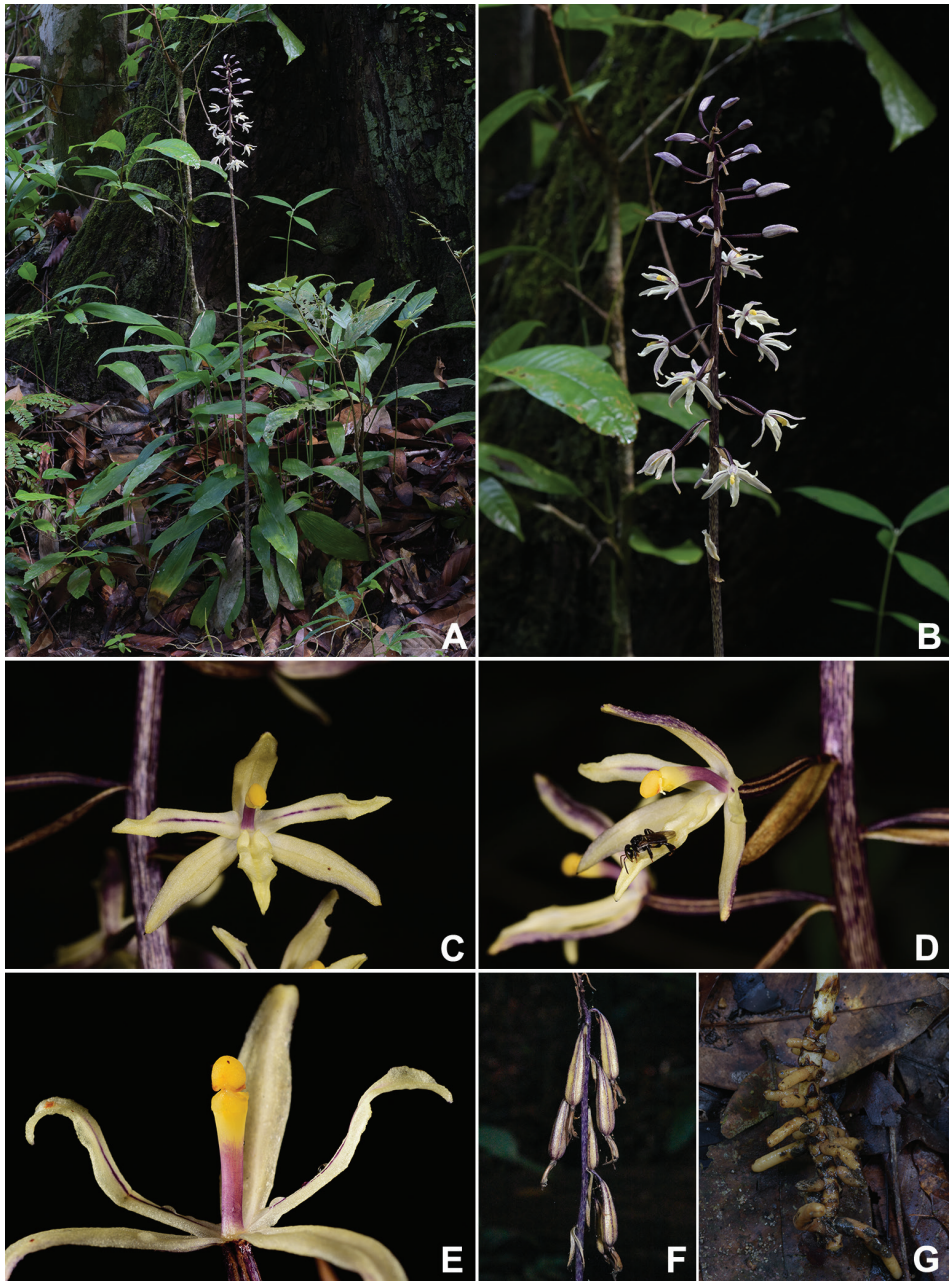
**Type.** THAILAND. Songkhla Province: Ton Nga Chang wildlife sanctuary, ca. 100 m, 06°59'43.91"N, 100°09'00.57"E, 26 December 2020, *S. Chantanaorrapint* & *A. Chantanaorrapint* 3109 (holotype: BKF!; isotype: PSU! [PSU00019495]).

**Description.** Terrestrial, achlorophyllous herbs, with a suberect rhizome and an erect flowering shoot. **Rhizomes** pale brown, producing numerous horizontal roots; roots fleshy, 3.5–6.5 mm in diameter, pale brown, glabrous. **Flowering shoots** 70–150 cm tall, up to 9 mm in diameter at base, unbranched whitish or pale yellow, marked with purple stripes or spots, with 5–9 membranous sheaths at base and 10–15 scales above. **Inflorescence** racemose, terminal, 15–30 cm long, up to 32 flowers; rachis glabrous. **Floral bracts** reflexed, lanceolate to linear-lanceolate, acute to acuminate, 16.5–21.5 × 4.5–5.5 mm, whitish or pale yellow with purple stripes.



**Figure 2.** *Aphyllorchis periactinantha* A.Chantanaorr. & Chantanaorr. **A** flower, lateral view **B** flower, top view **C** floral bract **D** column and labellum, side view **E** dorsal sepal **F, G** lateral sepals **H, I** petals **J** labellum **K** column and anther cap in ventral view **L, M** anther caps. Drawn by S. Chantanaorrapint.

**Flowers** opening widely, creamy white to pale yellow. **Sepals** creamy white to pale yellow, minutely tuberculate and sparsely pubescent on the adaxial surface, scattered with purple stripes or dots, concave, margins entire, apex acute; dorsal sepal narrowly



**Figure 3.** *Aphyllorchis periactinantha* A.Chantanaorr. & Chantanaorr. *in situ* **A** habit **B** inflorescence **C** flower in front view **D** flower in oblique view with stingless bee (*Tetragonula* sp.) **E** column in ventral view **F** immature fruits **G** rhizome and roots **A–E, G** photographed by S. Chantanaorrapiñt **F** by C. Leeratiwong.

ovate to lanceolate, 17.5–19.6 × 4.3–5.2 mm; lateral sepal obliquely narrow-ovate to obliquely lanceolate, 16.8–19.0 × 4.2–5.1 mm. **Petals** creamy white to pale yellow with purple veins, oblong-lanceolate, 16.0–16.5 × 3.9–4.3 mm, slightly falcate



at apex, base obtuse to subtruncate, apex acute, margin entire or minutely erose and slightly recurved backward in the middle, keeled adaxially along midrib. **Labellum** creamy white to pale yellow, simple and not divided into hypochile and epichile, oblong-lanceolate,  $16.2\text{--}17.0 \times 3.8\text{--}4.5$  mm, more or less folded along a midrib, concave at the basal part, apex acute, margin nearly entire or minutely erose, abaxial and adaxial surfaces nearly smooth. **Column** slender, subclavate, yellow at the apical third and purple at the proximal two thirds, gently curved throughout its entire length, 10–12 mm long, without column wing or a hook-shaped appendage on ventral side; clinandrium with a rather large central dome-like outgrowth; stigma more or less ovate in outline, shallowly bilobed at the lower margin; anther cap ovoid in outline, 2.0–2.2 mm long, apex obtuse; pollinia 2, soft and mealy, without caudicles. **Pedicel with ovary** slightly bent upwards or downwards, 18.5–23.5 mm long, ca. 2 mm in diameter, dark purple, pubescent, bearing sparse glandular hairs. **Capsules** (immature) pendulous, claviform or fusiform, 5.5–6.5 cm long, 0.7–1.1 cm diameter. **Seeds** not seen.

**Additional specimens examined (paratypes).** THAILAND. Songkla Province: Ton Nga Chang Wildlife Sanctuary, ca. 100 m,  $06^{\circ}59'43.91''\text{N}$ ,  $100^{\circ}09'00.57''\text{E}$ , 22 October 2017, *S. Chantanaorrapint & A. Chantanaorrapint 2810* (PSU), 16 November 2018, *S. Chantanaorrapint & O. Suwanmala 2732* (PSU); Ban Yang Ko Community Forest, 7 December 2021, *C. Leeratiwong 21-1752* (PSU).

**Phenology.** Flowering and fruiting observed from October to December.

**Distribution, habitat and ecology.** *Aphyllorchis periactinantha* is known only from two localities in Songkhla province (Fig. 1); however, it may also occur in other areas in southern Thailand with a similar vegetation type. The new species was found growing amongst leaf litter, in shade in lowland broad-leaf forest dominated by dipterocarps such as *Anisoptera costata* Korth., *Dipterocarpus kerrii* King, *Hopea ferrea* Lanness., *H. odorata* Roxb., and *Shorea gratissima* (Wall. ex Kurz) Dyer, ca. 100 m above sea level. During the field surveys, we found a stingless bee (*Tetragonula* sp.) visiting the flower (Fig. 3D). However, its status as a pollinator could not be confirmed.

**Etymology.** Greek prefix *peri-*, about, *actis*, ray or radiate, and *anthos*, flower, alluding to subactinomorphic flowers.

**Conservation status.** This species is currently known from two subpopulations, representing two locations, which are in protected areas (Wildlife Sanctuary and community forest). One of the subpopulations is located beside a waterfall, which is a common visiting site for tourists. Therefore, habitat quality is threatened by trampling and other destructive activities potentially caused by regular visits by tourists to the area, namely the attraction of wild boars. Together, these have the potential to cause a population reduction. The other subpopulation is also somewhat disturbed by human activities such as illegal logging. Moreover, the number of mature individuals observed is fewer than 50. The extent of occurrence cannot be estimated because the species is known only from two subpopulations and its area of occupancy is estimated to be only 8 km<sup>2</sup>. *Aphyllorchis periactinantha* is, therefore, assigned a preliminary status of Critically Endangered based on subcriterion C2a(i).



## Discussion

*Aphyllorchis periactinantha* is morphologically similar to *A. anomala*, which is endemic to Queensland, Australia (Dockrill 1965). These two species share several common features, viz. the peloric flower, the simple labellum lacking any ridges or appendages and not being divided into hypochile and epichile, the wingless column, the clinandrium with a rather large central dome-like outgrowth, the bilobed stigma, and the ovoid anther cap.

The new species can be confused with *A. striata* from Peninsular Malaysia and Borneo (Seidenfaden and Wood 1992), in general appearance. However, *A. striata* differs from *A. periactinantha* in having smaller flowers, a trilobed labellum (with indistinct side lobes), an anther cap with an acute apex, and the lingulate rostellum.

The new species also resembles *A. montana* var. *rotundatipetala*, endemic to Taiwan (Hsieh et al. 2013; Lin et al. 2016) and *A. simplex* from China and Vietnam in having peloric flowers and a simple labellum. However, *A. montana* var. *rotundatipetala* can be easily distinguished from *A. periactinantha* by the obtuse apices of petals and labellum and the presence of staminodes on the ventral side of column. *Aphyllorchis simplex* differs from *A. periactinantha* by the shape of labellum and the apex of the column. The comparison of morphological characters between *A. periactinantha* and other related species is summarized in Table 1.

**Table 1.** Morphological differences among *Aphyllorchis periactinantha*, *A. anomala*, *A. montana* var. *rotundatipetala*, *A. simplex* and *A. striata*. The characters of previously described species are taken from the protologues and from recent publications (Ridley 1893; Dockrill 1965; Seidenfaden and Wood 1992; Averyanov 2011; Hsieh et al. 2013).

Characters	<i>A. periactinantha</i>	<i>A. anomala</i>	<i>A. montana</i> var. <i>rotundatipetala</i>	<i>A. simplex</i>	<i>A. striata</i>
dorsal sepal	narrowly ovate to lanceolate, 17.5–19.6 × 4.3–5.2 mm, apex acute	oblong-cuculate, ca. 12 × 3 mm, apex acuminate	oblong-cymbiform, 10–12 × 4.0–5.5 mm, apex obtuse	narrowly elliptic, 8–10 mm long, apex acute to obtuse	lanceolate, ca. 12–14 × 2.5–3.0 mm, apex acute
petals	oblong-lanceolate, 16.0–16.5 × 3.9–4.3 mm, apex acute	oblong, ca. 12 × 2 mm, apex acuminate	oblong, 9.5–11.5 × 3.5–4.0 mm, apex rotundate or obtuse	narrowly elliptic, 8–10 mm long, apex acute to obtuse	lanceolate, ca. 11–14 × 2.0–2.5 mm, apex acute
labellum	oblong-lanceolate, 16.2–17.0 × 3.8–4.5 mm, folded along a midrib, concave at the basal part apex acute, lateral margins not decurved	oblong-acuminate, ca. 11 × 2.5 mm, flat entirely, apex acuminate and slightly twisted, lateral margins decurved	oblong, 9.5–11.5 × 3.5–4.0 mm, flat entirely, apex obtuse or rotundate, lateral margins recurved backward at middle	oblanceolate, 9–10 × 2–2.5 mm, flat entirely, apex acute and slightly decurved, lateral margins slightly decurved	3-lobed with indistinct side lobes, ca. 11–14 × 2.5–3.0 mm, almost flat, apex acute, lateral margins rolled at the basal part
staminode	reduced	reduced	Present	present	reduced
rostellum	semicircular, entire apex	triangular, entire apex	ovate-lingulate, entire apex	ovate, emarginate apex	lingulate, entire apex



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