

Corybas papillatus (Orchidaceae), a new orchid species from peninsular Thailand

Janejaree Inuthai¹, Sahut Chantanaorrapint², Manop Poopath³,
Naiyana Tetsana³, Wittawat Kiewbang⁴, Somran Suddee³

1 Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Lampang Campus, Hang Chat, Lampang, 52190, Thailand **2** PSU Herbarium, Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand **3** Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, 61 Phahonyothin Road, Chatuchak, Bangkok 10900, Thailand **4** Forest Economics Bureau, Royal Forest Department, Bangkok 10900, Thailand

Corresponding author: Sahut Chantanaorrapint (sahut.c@psu.ac.th)

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Abstract

A new species, *Corybas papillatus*, is described and illustrated from peninsular Thailand. The new species is easily recognized through a combination of the following characters: the purplish flower, the rounded apex of the dorsal sepal, the outer surface of dorsal sepal covered with irregular papillae in the upper half, the lateral sepals adnate laterally at the base to the connate petals, the V-shaped throat, the labellum bearing short hairs, dentate to erose labellum margins, and well-developed conical spurs. A key to the species of *Corybas* in Thailand is presented.

Keywords

Helmet orchid, Khao Luang National Park, montane forest, Thai-Malay Peninsula

Introduction

Corybas Salisb. is a genus of terrestrial orchids comprising about 120 species, and is widely distributed from India, South China, peninsular Thailand, the Malesian region, to New Zealand and the Western Pacific Islands (Dransfield et al. 1986; Pridgeon et

al. 2001; Pedersen 2011; Tandang et al. 2020). The members of the genus are easily recognized by being small in size with a single cordate leaf and 1–2 underground tubers, dorsal sepal and labellum which together form a tube with expanded mouth, lateral sepals and petals often antenna-like, and labellum usually bearing two spurs (Dransfield et al. 1986). Only two *Corybas* species are currently known from Thailand, namely *Corybas ecarinatus* Anker & Seidenf. (Anker and Seidenfaden 2001; Pedersen 2011), and *C. geminigibbus* J.J. Sm. (Chantanaorrapint and Chantanaorrapint 2016).

During a recent visit to Khao Luang Mountain, Khao Luang National Park, by staff of BKF herbarium, an interesting taxon of the genus *Corybas* was collected with a unique combination of characters that did not match any of the known species. It is therefore described here as a species new to science.

Materials and methods

This study is based on material collected during July 2018 from Khao Luang National Park, Nakhon Si Thammarat province, southern Thailand. Specimens were preserved in alcohol (70% ethanol) and deposited in BKF herbarium. Morphological characters were studied using a stereo microscope Olympus SZX7 and the distinctive characters of the species were illustrated with the aid of an Olympus drawing tube. Measurements were taken from spirit material. The specimen details were compared in detail with original drawings and descriptions given in the protologues of *Corybas* species in the Malaysian region (e.g. Dransfield et al. 1986; Anker and Seidenfaden 2001; Tandang et al. 2020).

Taxonomy

Corybas papillatus Inuthai, Chantanaorr. & Suddee, sp. nov.

urn:lsid:ipni.org:names:77220208-1

Figs 1, 2

Diagnosis. Similar to *Corybas villosus* J. Dransf. & Gord. Sm., but differs in the absence of dorsal sepal keel and the lateral sepals adnate laterally at the base to the connate petals.

Type. THAILAND. Nakhon Si Thammarat province, Khao Luang National Park, near summit of Khao Luang Mt., 08°29'36.8"N, 099°43'38.9"E, ca. 1,700 m alt., 4 July 2018, *M. Poopath*, *N. Tetsana*, *W. Kiewbang*, *C. Hemrat* & *S. Jirakorn* 2201 (holotype BKF!, spirit material).

Small terrestrial herb with underground tubers. **Tubers** globose or ovoid, fleshy, 3–4 mm diam. **Stem** erect, whitish-green, 5–12 mm long, 1–1.5 mm diam., with a basal sheath; stolon whitish, hairy, up to 2 cm long, 1–1.2 mm diam. **Foliage leaf** sessile, cordate, long acuminate at apex, glabrous, 6–10 mm long, 6–8 mm wide at the widest point, flat, only slightly undulate along margin, pale green with paler veins, the veins scarcely conspicuous. **Inflorescence** one-flowered, terminal; bract pale green, lanceolate-triangular,

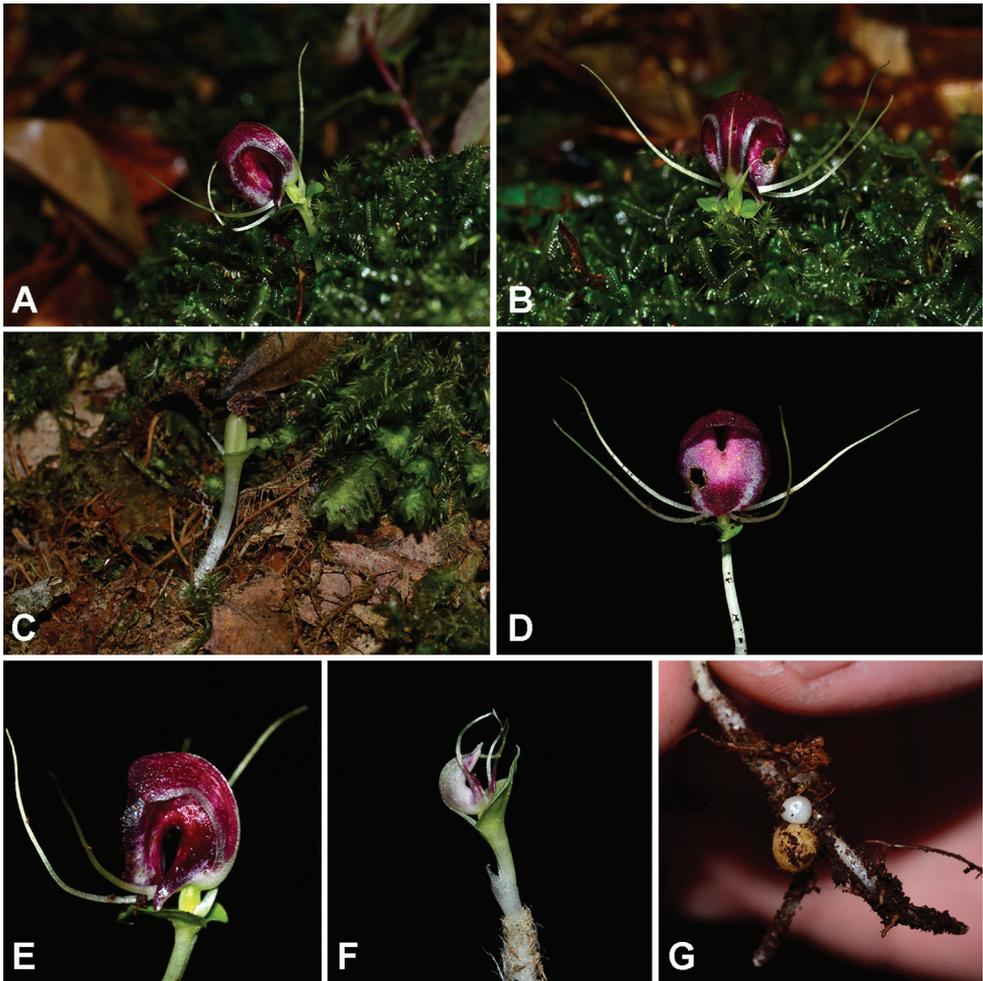


Figure 1. *Corybas papillatus* Inuthai, Chantanaorr. & Suddee **A, B** plants in natural habitat, on humus associated with *Bazzania* sp. and *Acroporium* sp. **C** plant with immature fruit **D, E** flowers **D** front view **E** lateral view **F** immature flower **G** underground parts with tubers. Photographs by W. Kiewbang.

long acuminate, 5–6 mm long, recurved. **Flower** dark purple. **Dorsal sepal** purplish, erect below, then strongly curved above, hooded and clasping labellum throughout its length, spatulate, ca. 15 mm long, 8–10 mm wide, rounded at apex, apical margins denticulate, abaxial (dorsal) surface bearing irregular papillae in the upper half. **Lateral sepals** greenish-white, linear-triangular to antenna-like, ca. 25 mm long, laterally adnate to the petals in the basal ca. 1 mm. **Petals** greenish-white, connate at the base for ca. 1 mm, free above, similar to lateral sepals but shorter, ca. 22 mm long. **Labellum** differentiated into 2 parts, erect in basal half and strongly reflexed above, ca. 17 mm long in total length; the basal half with margins strongly inrolled throughout, and forming a tube with the dorsal sepal, ca. 7 mm long; the upper half reflexed and expanded, orbicular, ca. 10 mm diam., margin erose, reddish-purple excepted for a white zone just below the margins; mouth of throat

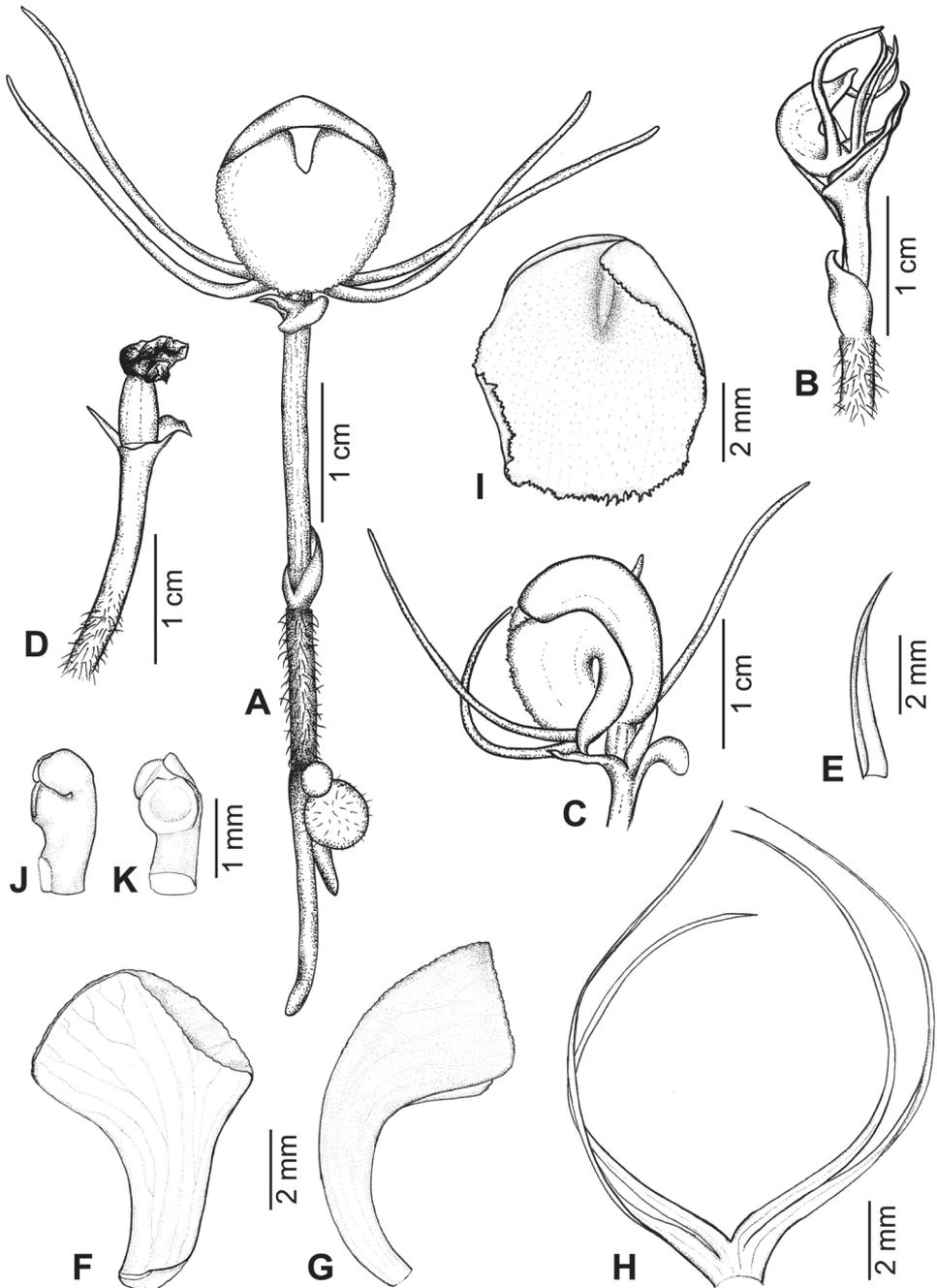


Figure 2. *Corybas papillatus* Inuthai, Chantanaorr. & Suddee **A** whole plant with flower and tubers **B** plant with immature flower **C** mature flower, lateral view **D** plant with immature fruit **E** floral bract, **F, G** dorsal sepal **F** ventral view **G** lateral view **H** lateral sepals and petals **I** labellum **J, K** column, **J** lateral view, **K** front view. Drawn by J. Inuthai.

very deeply V-shaped; adaxial surface bearing short hairs; spurs 2, divergent, conical, ca. 3 mm long. **Column** very short, ca. 1.5 mm long; stigma rounded, ca. 0.5 mm diam.; anther erect, smooth; pollinia not seen. **Ovary** green, glabrous, ca. 2 mm long. **Capsule** (immature) erect, ellipsoid or fusiform, 5–7 mm long, 2.5–3 mm diam. **Seed** not seen.

Phenology. Flowering and fruiting observed in July.

Habitat and ecology. The new species was found growing amongst bryophytes (Fig. 1A–C), such as *Acroporium* sp., *Bazzania* sp., and *Schistochila aligera* (Nees & Blume) J.B. Jack & Steph., on humus in shade in montane forest, ca. 1,700 m above sea level.

Distribution. *Corybas papillatus* is only known from the type locality (Fig. 3), however, it may also occur in other areas in peninsular Thailand with similar vegetation type.

Etymology. The specific epithet ‘*papillatus*’ alludes to occurrence of irregular papillae in upper portion of abaxial (dorsal) surface of dorsal sepal.

Conservation status. We consider it likely that if a formal assessment were performed, this species would be categorized as Critically Endangered (CR (D)) based on a preliminary risk of extinction assessment using the IUCN red list categories and criteria (IUCN 2019). This species is known from only four individuals from the type locality which attracts high numbers of camping tourists. Although we returned to the same locality and tried to find more specimens in 2020 it could not be found again. The species is, however, easily be overlooked in the field because of its small size.

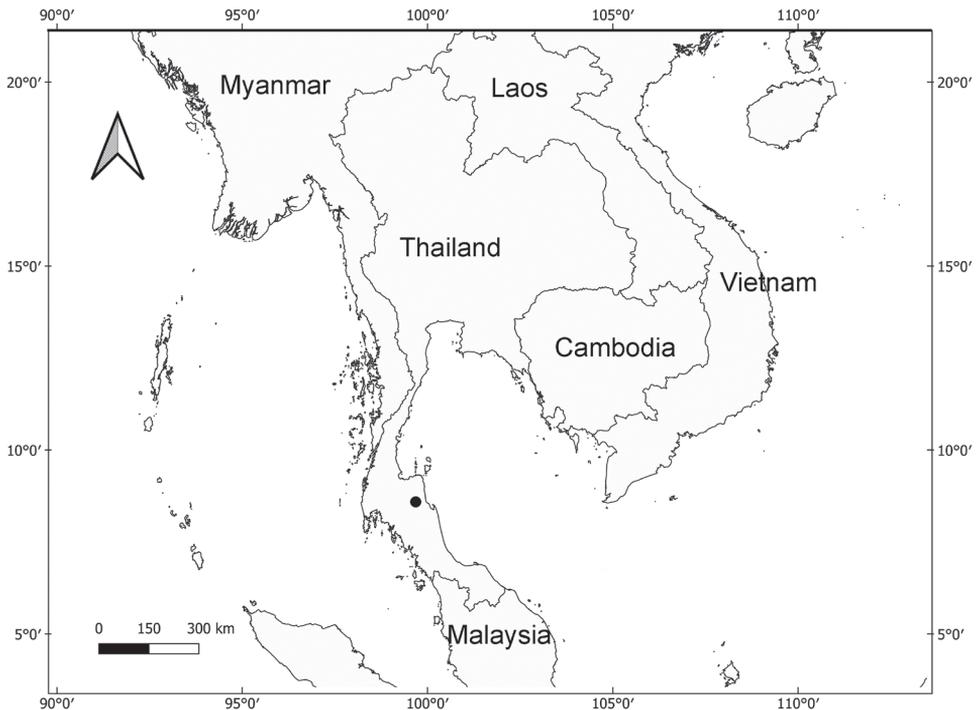


Figure 3. Type locality of *Corybas papillatus* Inuthai, Chantanaorr. & Suddee (●).

Discussion

Corybas papillatus is most similar to *C. villosus*, which is endemic to Peninsular Malaysia (Dransfield et al. 1986; Go et al. 2015). These two species share several common features, viz. flower coloration being purplish, the dorsal surface of dorsal sepal covered with irregular papillae in the upper half, the hairiness of the labellum, the V-shaped throat, irregularly dentate to erose labellum margins, and well-developed conical spurs. *Corybas papillatus* is distinguished from *C. villosus* by lacking dorsal sepal keel (strongly keeled in *C. villosus*) and the adnate lateral sepals and connate petals (all free in *C. villosus*).

Corybas papillatus might be confused with *C. ridleyanus* Schltr., another endemic to Peninsular Malaysia (Dransfield et al. 1986; Go et al. 2015), which also has purplish flowers and reddish-purple labellum excepted for a zone below the margin, and a V-shaped throat. *Corybas ridleyanus*, however, differs from *C. papillatus* by the truncate apex of the dorsal sepal and the free lateral sepals and petals.

Together with the recent discovery of a new species and new records of orchids from peninsular Thailand, especially in the Nakorn Si Thammarat mountain range (e.g. Ormerod et al. 2012; Tetsana et al. 2014; Chantanaorrapint and Chantanaorrapint 2016; Chantanaorrapint et al. 2017) it is clear that peninsular Thailand is an important region for orchid diversity and that further new species records can be expected to be found from many unexplored areas in this part of the country.

There are now three species of *Corybas* known from Thailand. A key to distinguish these is given below.

Key to the species of *Corybas* in Thailand

- 1 Lateral sepals and petals free at base; spurs inconspicuous, broad and poorly developed..... *C. geminigibbus*
- Lateral sepals adnate laterally to the connate petals at base; spurs well-developed, conical, slightly oblique to divergent **2**
- 2 Flowers pink, dorsal sepal as long as the lateral sepals, acute at apex; central portion of labellum bearing a callus *C. ecarinatus*
- Flowers purplish, dorsal sepal shorter than the lateral sepals, rounded at apex; central portion of labellum hairy without a callus *C. papillatus*

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Haplodontium altunense (Bryaceae, Bryopsida), a new moss species from Northwest China

Xiao-Rui Wang^{1*}, Min Li^{2*}, John R. Spence³, Jian-Cheng Zhao², Sulayman Mamtimin⁴

1 College of Resources and Environmental Science, Shijiazhuang University, Shijiazhuang 050035, China

2 College of Life Science, Hebei Normal University, Shijiazhuang 050024, China **3** California Academy of Sciences, Department of Botany, 55 Music Concourse Drive, Golden Gate Park, CA 94118–4503, San Francisco, USA **4** College of Life Science and Technology, Xinjiang University, Urumqi 830046, China

Corresponding author: Sulayman Mamtimin (mamtimin@xju.edu.cn)

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Abstract

Haplodontium altunense X.R.Wang & S.Mamtimin, a new moss species of the family Bryaceae from Xinjiang Uygur Autonomous Region, China is described and illustrated. Genetic analysis based on ITS sequences shows that this species is a member of the Bryaceae and in the same clade as *Anomobryum*. Particularly distinctive features of the new species include: double peristome; the exostome has raised and membranous chomata with united lamellae between two teeth proximally; the endostome is poorly developed and all the endostomial material tightly adherent to the exostome.

Keywords

Altun Mountain, *Bryum*, ITS, *Plagiobryoides*, *Ptychostomum*

Introduction

Haplodontium Hampe, classified in the family Bryaceae, has been confused with the genus *Mielichhoferia* Hornsch. Shaw and Crum (1984) transferred all species in the genus to *Mielichhoferia* because of the similar peristome. Genetic research (Cox et al. 2000; Goffinet et al. 2001; Pedersen et al. 2003, 2007; Holyoak and Pedersen 2007) has clearly shown that some species originally in *Haplodontium* are nested within Bryaceae.

* The authors contributed equally to this study.

Spence (2005) resurrected the genus *Haplodontium* and transferred two North American species of *Mielichhoferia* to *Haplodontium*. In addition, the genus *Mielichhoferia* has been accommodated in the family Mielichhoferiaceae (Shaw 2009). Pedersen et al. (2007) showed that *Haplodontium*, *Acidodontium* Schwägr. and *Anomobryum* Schimp. are sister taxa and *Haplodontium* should be included in the family Bryaceae.

Materials and methods

Morphological observations

Microscopic examination was carried out using traditional methods. The collections of *Haplodontium* and relevant species of Bryaceae in the herbarium of Hebei Normal University (HBNU), Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Institute of Botany, the Chinese Academy of Sciences (PE) and Xinjiang University (XJU) were examined.

Authors observed the plants under the dissecting microscope and examined the leaves, capsules and peristome under the compound light microscope and scanning electron microscope. Light micrographs were photographed using a Nikon E-800 microscope with a Nikon DXM1200F digital camera. The peristome and spores were mounted on double sided sticky tape on aluminium stubs, gold-coated and viewed using a Hitachi S-4800 field emission SEM. All line drawings were made using the drawing tube attachments of these optical microscopes.

Phylogenetic analyses

Twenty-one samples were used for the analyses (Table 1). To evaluate the systematic position of *Haplodontium altunense*, 20 representatives of allied genera in the family Bryaceae, including *Anomobryum*, *Bryum*, *Gemmabryum*, *Plagiobryum* and *Ptychostomum*, were also sampled as part of the ingroup (Cox et al. 2000; Goffinet et al. 2001; Pedersen et al. 2003, 2007; Kato et al. 2013). *Bryum argenteum* was selected as an outgroup. In addition to 10 sequences from GenBank, 11 sequences newly produced for the present study were included.

Genomic DNA was extracted from freshly collected and silica gel-dried plants using a Plant Genomic DNA Kit (TIANGEN Biotech (Beijing) Co., Ltd.) according to the manufacturer's protocol. One nuclear marker ITS was chosen. The following primers were used to amplify the marker: '18SF' and '26SR' for the ITS region, or sometimes '18SF' and '5.8SR' for ITS1, and '5.8SF' and '26SR' for ITS2 (Hartmann et al. 2006). PCR cycles used an initial denaturation step of 3 minutes at 95 °C, followed by 35 cycles of 30 seconds at 95 °C, 30 seconds at 50 °C, 90 seconds at 72 °C, and a final elongation of 5 minutes at 72 °C. PCR products were purified with a Gel Extraction Kit (Cwbio, Shanghai, China) following the instruction manual. These purified PCR products were sequenced by Life Technologies Inc., China (<http://www.lifetechnologies.com>).

Table 1. Voucher information and GenBank accession numbers of taxa used in the phylogenetic analyses.

Taxon	Voucher (Herbarium)	Origin	GenBank No.	Source
<i>Anomobryum auratum 1</i>	L.B. Li 20073626 (HBNU)	China	MZ470251	This study
<i>Anomobryum auratum 2</i>	L.B. Li 20073628 (HBNU)	China	MZ470252	This study
<i>Anomobryum julaceum</i>	L.B. Li 20072925 (HBNU)	China	FJ796895	Wang et al. 2011
<i>Bryum argenteum</i>	X.R. Wang 20150512031 (HBNU)	China	MZ470253	This study
<i>Bryum paradoxum</i>	J.C. Zhao 0610067 (HBNU)	China	EU878207	Wang et al. 2011
<i>Bryum recurvulum</i>	W.Q. Li 040900 (HBNU)	China	EU878217	Wang et al. 2011
<i>Gemmabryum caespiticium</i>	X.R. Wang 20156001 (HBNU)	China	MZ470254	This study
<i>Haplodontium altunense</i>	S. Mamtimin 16752 (XJU)	China	MZ470255	This study
<i>Plagiobryum zierii</i>	W.Q. Li 000514 (HBNU)	China	EU878219	Wang et al. 2011
<i>Psychostomum arcticum</i>	S. Mamtimin 15457 (HBNU)	China	MZ470256	This study
<i>Psychostomum bimum</i>	L. Hedenäs B90015 (S)	Sweden	DQ381780	Holyoak and Hedenäs 2006
<i>Psychostomum cernuum</i>	Y.L. Niu 110002 (HBNU)	China	MZ470257	This study
<i>Psychostomum inclinatum</i>	N. Cao 20050085 (HBNU)	China	EU878227	Wang et al. 2011
<i>Psychostomum lonchocaulon</i>	N. Cao 20050187 (HBNU)	China	FJ796878	Wang et al. 2011
<i>Psychostomum neodamense</i>	L. Hedenäs B65900 (S)	Sweden	DQ381772	Holyoak and Hedenäs 2006
<i>Psychostomum pallens</i>	M.X. Xiao 20091246 (HBNU)	China	MZ470258	This study
<i>Psychostomum pallescens</i>	S. Mamtimin 15265 (HBNU)	China	MZ470259	This study
<i>Psychostomum pendulum</i>	J.C. Zhao 20060463 (HBNU)	China	FJ796811	Wang et al. 2011
<i>Psychostomum pseudotriquetrum</i>	D.T. Holyoak B90021 (S)	Ireland	DQ381774	Holyoak and Hedenäs 2006
<i>Psychostomum purpurascens</i>	Y.L. Niu 110045 (HBNU)	China	MZ470260	This study
<i>Psychostomum turbinatum</i>	S. Mamtimin 15095 (HBNU)	China	MZ470261	This study

Sequence chromatograms were compiled using SeqMan II (DNASTAR Inc., Madison, WI, USA) and then aligned manually in PhyDE 0.9971 (Müller et al. 2010). Regions of partially incomplete data at the beginnings and ends of sequences were identified and excluded from subsequent analyses. Gaps were treated as missing data. The aligned ITS dataset was composed of 1213 bp.

The maximum likelihood (ML) method was performed using RAxML v.8.2.12 on the CIPRES Science Gateway (<http://www.phylo.org/>), and inferred under the default settings (Stamatakis 2014). The fast bootstrap option was used with 1000 replicates. TreeGraph 2 (Stöver and Müller 2010) was used to summarize the topologies and support values from the analyses.

Results

Taxonomic treatment

Haplodontium altunense X.R.Wang & S.Mamtimin, sp. nov.

urn:lsid:ipni.org:names:

Figs 1–3

Type. CHINA. Xinjiang, Ruoqiang County, Altun Mountain National Nature Reserve, 37°0.42'N, 88°36.35'E, 4290 m a.s.l., 22 July 2011, *S Mamtimin 16752* (**holotype**: HBNU!; **isotype**: XJU!).

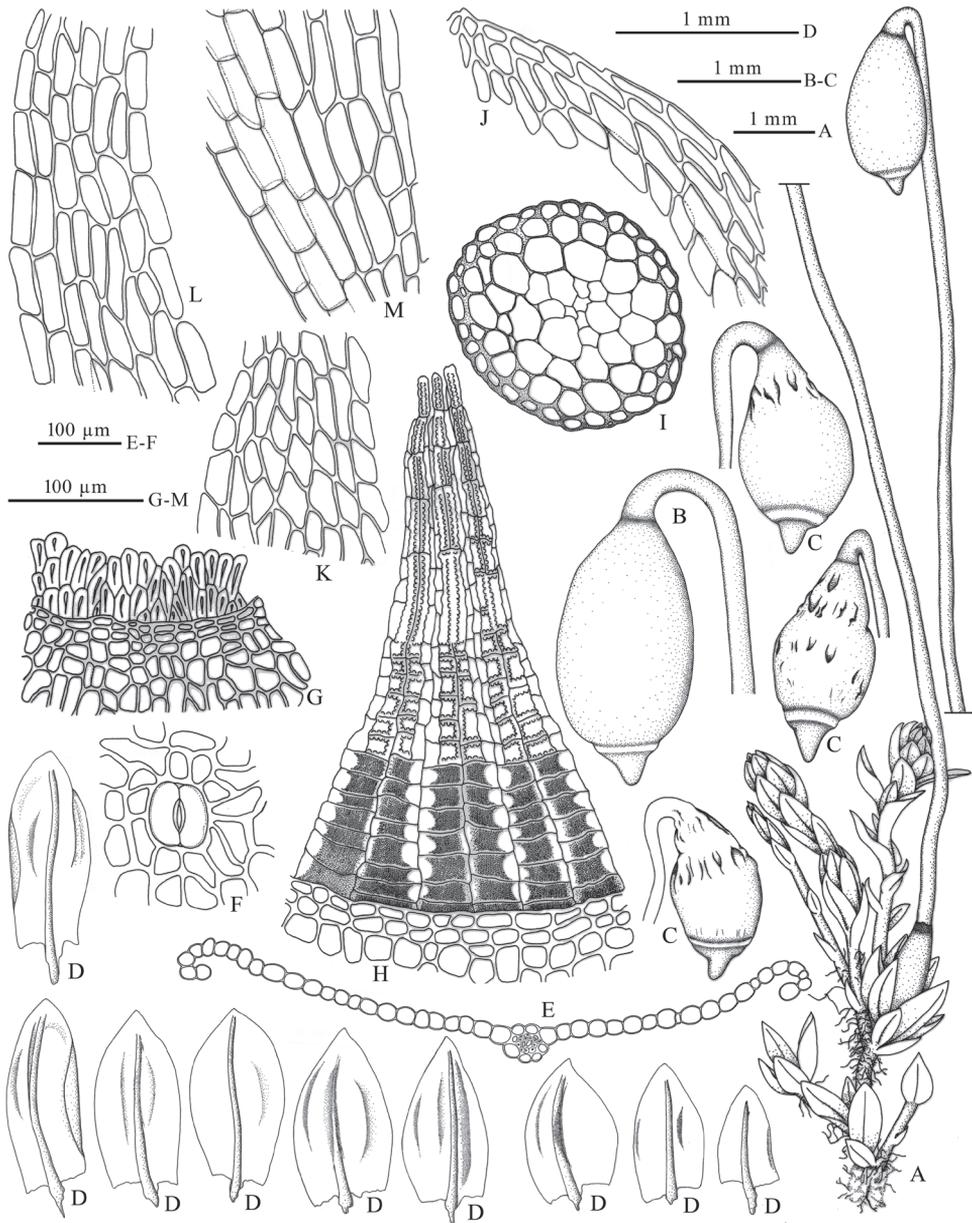


Figure 1. *Haplodontium alturnense* **A** plant (wet) **B** capsule (wet) **C** capsules (dry) **D** leaves **E** transverse section of leaf **F** superficial stoma **G** annulus growing on the capsule mouth **H** dorsal views of peristome **I** transverse section of stem **J** apical laminal cells and margin **K** apical laminal cells **L** median laminal cells **M** basal laminal cells. Drawn by Xiaorui Wang from the holotype (HBNU!).

Diagnosis. Particularly distinctive features of the new species including: double peristome; the exostome has raised and membranous chomata with united lamellae between two teeth proximally; the endostome is poorly developed and all the endostomial material tightly adherent to the exostome.

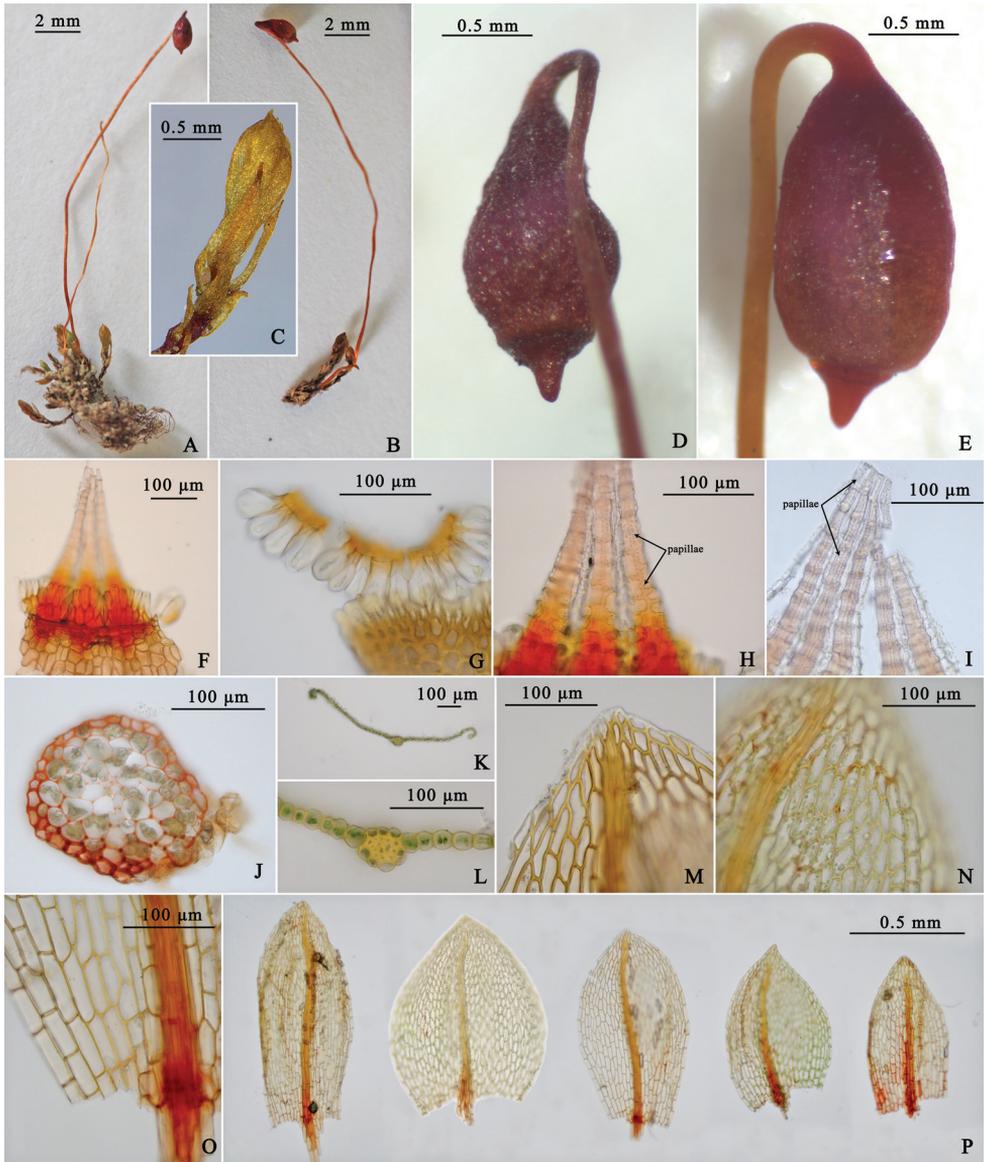


Figure 2. Light micrographs of *Haplodontium altunense* **A–C** plants (dry) **D** Capsule (dry) **E** capsule (wet) **F** annulus growing on the capsule mouth **G** annulus falling off the capsule mouth **H** dorsal views of median peristome showing the large papillae along the horizontal and median vertical lines **I** dorsal views of distal peristome showing adherent endostomial material and exostome teeth **J** transverse section of stem **K** transverse section of midleaf **L** transverse section of costa **M** leaf apex **N** median laminal cells **O** basal laminal cells **P** leaves. Photographed by Xiaorui Wang from the holotype (HBNU!).

Description. Plants small, soft and dull, brown-green. Stems short, 2.5–6 mm high, weakly julaceous, branched, circular or pentagonal circular in transverse section with small and thick-walled peripheral cells surrounding 2–3 layers gradually larger and thin-walled cortical cells, central strand weakly developed. Leaves imbricate when

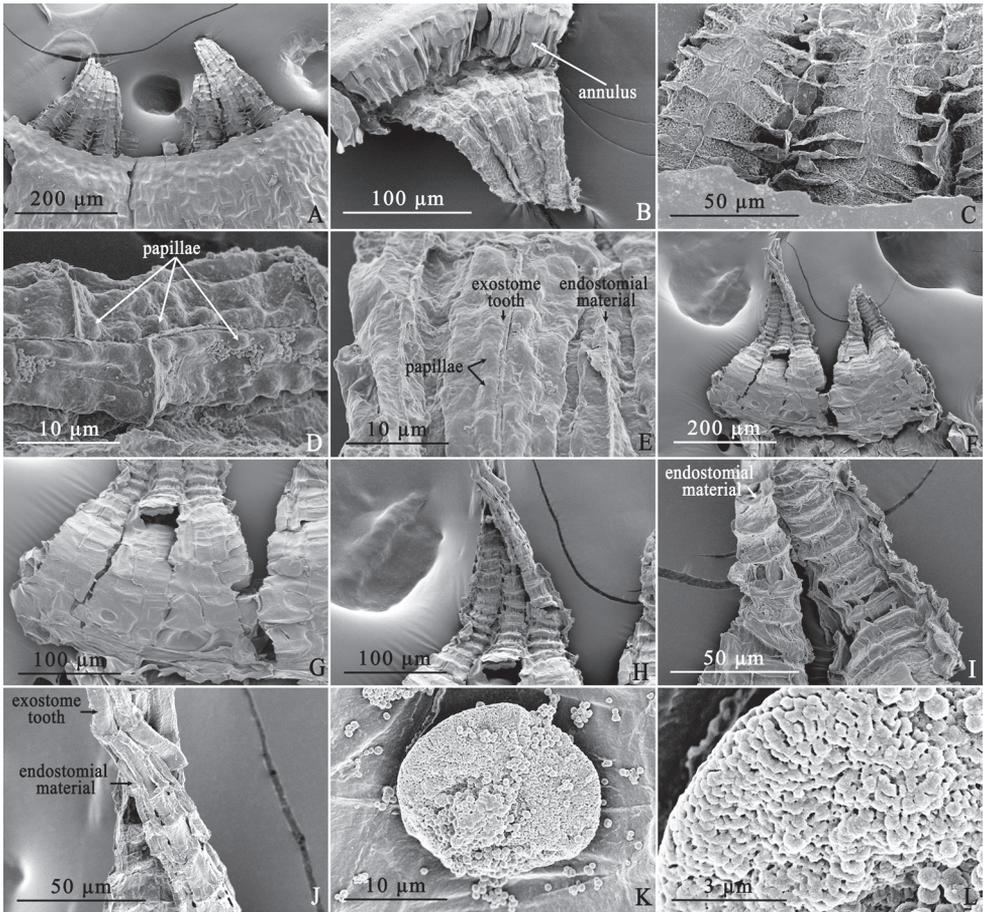


Figure 3. Scanning electron micrographs of *Haplodontium altunense* **A** dorsal views of peristome **B** dorsal views of peristome with annulus **C** dorsal surface of exostome teeth proximally showing the pores and the raised and membranous chomata horizontal lines with united lamellae between two teeth **D** dorsal surface of exostome teeth distally showing the large papillae along the median vertical lines **E** dorsal surface of exostome teeth distally showing the endostomial material adherent to the teeth **F** ventral views of peristome **G** ventral surface of peristome proximally showing the smooth endostome basal membrane **H** ventral views of peristome distally **I, J** ventral surface of peristome medially and distally showing the endostomial material adherent to the teeth **K** spore **L** exine ornamentation of spore. Photographed by Xiaorui Wang from the holotype (HBNU!).

dry, erect when moist, enlarged towards stem apex, ovate to broadly ovate, concave, 0.5–1.1×0.3–0.7 mm; base not decurrent; margins plane or recurved medially, 1-stratose, limbidium absent, smooth or finely serrulate distally; apex broadly acute; costae not reaching apex, guide cells weakly developed, 2–4 in one layer in costal transverse section, ventral and dorsal stereid bands present; alar cells not differentiated from juxtacostal cells; laminal cells lax; distal laminal cells rhomboidal, 30–44×11–21 μm, with slightly thickened walls; medial laminal cells long rhomboidal to rectangular,

37–69×12–20 μm, somewhat narrower in 2 or 3 rows toward the margins but not forming a distinct border; proximal laminal cells long rectangular, 37–56×20–28 μm. Dioicous(?). Perigonia not seen. Perichaetia at the end of short, inconspicuous stems, appearing laterally because of well-developed innovations; perichaetial leaves larger than vegetative leaves. Setae single, light brown, 15–19 mm long. Capsules nutant and symmetric, reddish brown, obovoid, 1.5–2 mm, neck short and indistinct, mouth small, stomata abundant in the neck, superficial; opercula long-conic with short rostrate; annulus present, consisting of two rows of cells, revoluble and cells with slit-like lumen; peristome double, exostome inserted below the mouth, teeth lanceolate, red-brown and pored, raised and membranous chomata with united lamellae between two teeth proximally, pale yellow to hyaline and largely papillose along horizontal and median vertical lines distally; endostome poorly developed, basal membrane smooth, segments and cilia rudimentary, all the endostomial material strongly adhere to the exostome. Spores spherical, 20–22 μm in diameter, minutely papillose.

Etymology. The specific epithet *altunense* refers to the type locality in Altun Mountain National Nature Reserve in the Northwestern China.

Distribution and habitat. China (Xinjiang). Only known from the type locality, on soil substrates at 4290 m in the Altun Mountain National Nature Reserve. The population grows in a dry, cold, and windy habitat with intense evaporation. The companion species include some xerophytic mosses of the family Pottiaceae.

Chinese name. 阿尔金拟缺齿藓 (ā ěr jīn nǐ quē chǐ xiǎn)

Phylogenetic analyses

The present phylogenetic analysis, based on the nuclear ribosomal internal transcribed spacer region ITS1–5.8S–ITS2 (hereafter, ITS) region, included 20 species from six genera, as well as *Bryum argenteum* Hedw. as outgroup (Fig. 4). The only sample of *Haplodontium altunense* is sister to the *Anomobryum* clade (81 MLBS), which is monophyletic with three members. Twelve samples of *Ptychostomum* Hornsch. formed a monophyletic clade (91 MLBS), in which *Gemmabryum caespiticium* (Hedw.) J.R. Spence was nested. *Plagiobryum zierii* (Hedw.) Lindb. is closely related to the *Ptychostomum* clade. The *Bryum* Hedw. clade (100 MLBS) with three species were basal to the main clades.

Discussion

Pending a careful examination of Bryaceae in the Altun Mountains, we discovered an unusual collection with distinctive morphological features of the peristome: endostome poorly developed and strongly adherent to the exostome. We thought this collection could belong to the genus *Synthetodontium* Cardot at first sight based on its fused peristome. Phylogenetic analysis showed that this collection was nested into Bryaceae clade. It is genetically distinct from the closely related *Anomobryum* group (Fig. 4). Further examination revealed that it has morphological characters of *Haplodontium*

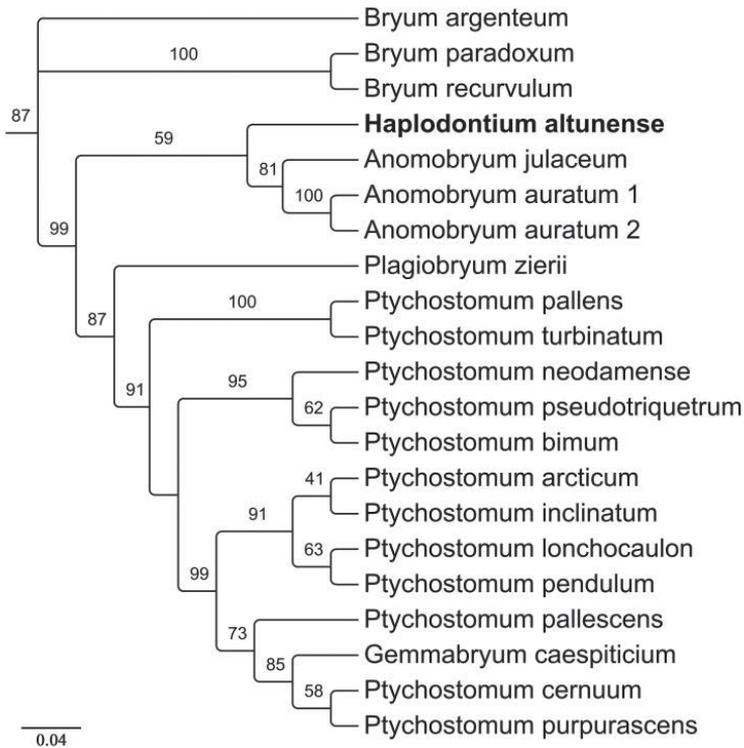


Figure 4. Phylogenetic relationships of *Haplodontium altunense* sp. nov. from ITS rDNA with related genera in Bryaceae based on Maximum Likelihood analysis. Support values are given above branches. *Bryum argenteum* was served as an outgroup.

such as stems nearly julaceous, leaf margins entire, distal and medial laminal cells laxly rhomboidal to rectangular. However, the collection is distinguished from the other previously recognized species in the region by its double peristome, and raised and membranous chomata with united lamellae between two exostome teeth proximally. We thus describe it as a new species of the genus *Haplodontium*.

The gametophytes of *Haplodontium* species are similar to those of *Plagiobryum* and *Plagiobryoides* J.R.Spence (Spence 2015). Genetic research has shown that the type species of *Haplodontium* is closely related to *Acidodontium* and *Anomobryum* (Cox and Hedderson 2003; Pedersen et al. 2007). Peristome reduction is common and complex in *Haplodontium*, from double to single to absent (Spence 2005).

The gametophyte characters of *Haplodontium altunense* are similar to *Plagiobryoides brachyneura* (Kindberg) J.R.Spence (Spence 2015). At the same time, both species have double, reduced and fused peristome. In *P. brachyneura*, setae are red-brown and 5–15 mm long, capsules are inclined to nutant, elongate-pyriform and 2–4 mm long with elongate-neck, opercula are weakly convex, exostome teeth are short and irregular in shape. However, those characters of *H. altunense* are as follows: setae are light brown and 15–19 mm long, capsules are nutant, pyriform to obovoid and 1.5–2 mm long with indistinct short-neck, opercula are long-conic with short rostrate, exostome teeth are regular long lanceolate.

Haplodontium altunense is also similar to *Ptychostomum pendulum* Hornschuch (Spence 2015) (\equiv *Bryum algovicum* Sendtner ex Müller Hal. (Li 2006; Zhang et al. 2007) in that the endostome adheres to the exostome teeth. While the former species differ from the latter one in length of stems (2.5–6 mm vs. 5–20 mm), leaf apex (broadly acute or obtuse vs. acuminate), leaf margin (indistinct bordered vs. strong limbidium), costae (ending at or near the apex vs. long-excurrent in denticulate awn), exostome teeth (united at the base, large papillose above vs. separate, smooth above), endostome (segments reduced vs. segments with ovate perforations).

Wang et al. (2017) reported one new species, *Haplodontium zangii* X.R.Wang & J.C.Zhao, from Tibet, China and transferred two Chinese species in *Mielichhoferia* to *Haplodontium* as new combinations: *H. himalayanum* (Mitt.) X.R.Wang & J.C.Zhao and *H. sinensis* (Dix.) X.R.Wang & J.C.Zhao. The most significant difference among the four species of *Haplodontium* in China is that the first three species all have single peristome, while *H. altunense* has double and fused peristome.

Key to the *Haplodontium* species in China

- 1 Peristome double, exostome teeth lanceolate, raised and membranous chomata with united lamellae between two teeth proximally, endostome reduced, all the endostomial material strongly adhere to the exostome..... ***H. altunense***
- Peristome single, exostomial..... **2**
- 2 Leaves lanceolate; costae excurrent, ending in long denticulate awns, awns 140–310 µm long..... ***H. himalayanum***
- Leaves ovate to oblong-ovate; costae subpercurrent to ending in short awns, awns 0–130 µm long **3**
- 3 Leaf apex cucullate; capsules pyriform; exostome teeth regularly lanceolate, not perforate, vertically striped on dorsal surface, smooth on ventral surface. ***H. sinensis***
- Leaf apex plane; capsules subglobose to short pyriform; exostome teeth irregularly linear-lanceolate, sometimes perforate, smooth or finely papillose ***H. zangii***

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Euphorbia mbuinzaensis, a new succulent species in Kenya from the *Synadenium* group in *Euphorbia* sect. *Monadenium* (Euphorbiaceae)

Neng Wei^{1,2,3}, Fredrick Munyao Mutie^{1,3}, Geoffrey Mwachala⁴,
Olwen M. Grace², Guang-Wan Hu^{1,5,6}, Qing-Feng Wang^{1,5,6}

1 Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, CN-430074, China **2** Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK **3** University of Chinese Academy of Sciences, Beijing, CN-100049, China **4** East African Herbarium, National Museums of Kenya, P.O. Box 451660-0100, Nairobi, Kenya **5** Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Wuhan, CN-430074, China **6** Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan, CN-430074, China

Corresponding authors: Guang-Wan Hu (guangwanhu@wbgcas.cn), Qing-Feng Wang (qfwang@wbgcas.cn)

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Abstract

Euphorbia mbuinzaensis, a succulent new species of the *Synadenium* group in Euphorbiaceae from Makueni County, Kenya, is described and illustrated. Morphologically, it is most similar to *E. pseudomollis*, but differs mainly by its shrubby habit (up to 4 m), abaxial leaves surfaces with densely stellate hairs, 2–4-forked cymes, smaller bracts (ca. 2.5 × 3.0 mm), smaller cyathia (6 mm wide), crimson glands without narrow smooth margin, smaller fruits (ca. 8 × 7 mm) and ovoid seeds (ca. 1.8 × 2.2 mm). Furthermore, we performed a molecular phylogenetic analysis of the *Synadenium* group in *Euphorbia* sect. *Monadenium*, based on complete nuclear ribosomal DNA (nrDNA) datasets. This phylogenetic inference also supports it to be a distinct species. The new species is assessed as Endangered using the IUCN criteria.

Keywords

East Africa, endangered, molecular phylogeny, succulents, *Synadenium*, taxonomy

Introduction

Synadenium Boiss. (in de Candolle (1862: 187)), was a previously recognized genus of Euphorbiaceae, confined to east and southern tropical Africa, with 14 closely related species (Carter and Leach 2001). *Synadenium* is readily distinguishable from other genera in Euphorbiaceae subtribe Euphorbiinae by the unique ring-shaped fused glands in the cyathium (Jones and Smith 1969; Carter 1988; Carter and Leach 2001). It has been regarded as a taxonomically difficult genus (Govaerts et al. 2000), since the differences between the species appear to be slight, especially when observing dried herbarium specimens only. Moreover, Brown et al. (1909) assumed that *Synadenium* species are locally endemic and probably more numerous than previously known (13 spp. at that time), given their great resemblance to one another, which may have resulted in some of them being overlooked.

Besides emphasizing leaf and cyme-branching characters, Carter and Leach (2001) suggested that emphasis should also be placed upon the habit, the shape and size of the cyathium, the color and furrowing of the involucre glands, and features of the capsules and seeds. Unfortunately, relatively few specimens have been prepared and deposited in herbaria, especially fruiting ones (Carter 1988). Although regional taxonomic treatments have been done, *Synadenium* has never been comprehensively monographed, and was regarded as a poorly known group.

Webster (1967) and Croizat (1972) questioned the delimitation of *Synadenium* and *Monadenium* as distinct genera and suggested, based on morphological similarities, that they were closely related to *Euphorbia* sect. *Tirucalli*. Analysis of molecular data over the past two decades (Steinmann and Porter 2002; Bruyns et al. 2006, 2011; Zimmermann et al. 2010; Horn et al. 2012; Dorsey et al. 2013; Wei et al. 2021), have all shown that the genera *Endadenium*, *Monadenium*, and *Synadenium* were deeply nested in *Euphorbia*. Consequently, they were merged under *Euphorbia* and treated as subgenus *Euphorbia* sect. *Monadenium*, to maintain the monophyly of *Euphorbia* (Bruyns et al. 2006). However, the *Synadenium* group has been poorly sampled to date (e.g., three species in Dorsey et al. 2013).

In a field investigation targeting Kenyan *Euphorbia* in 2018, a shrubby *Euphorbia* (*Synadenium*) species attracted the authors' attention. At first sight, it was roughly identified as *E. pseudomollis* Bruyns in Bruyns et al. (2006: 414), due to its densely pubescent leaves. More specimens of this *Euphorbia* were collected during the following field trip at the same area, and then detailed morphological studies were conducted. Based on the floral records and the examination of *Synadenium* specimens deposited in herbaria worldwide, we found that this *Euphorbia* differs from *E. pseudomollis* by a combination of several morphological characters. Furthermore, the molecular phylogeny of the *Synadenium* group based on complete sequences of the nuclear ribosomal DNA (nrDNA) revealed that our *Euphorbia* collection is sister to *E. bicompecta* Bruyns var. *bicompecta* Bruyns in Bruyns et al. (2006: 412). Hence, both morphological studies and phylogenetic analyses indicate that our *Euphorbia* collections represent a hitherto undescribed species, which we now proceed to describe and name as *Euphorbia mbuinzaensis*.

Materials and methods

Morphological observations

The morphological description of the new species is based on measurements on living specimens collected during the field trips, except for the seeds which were obtained by observations of specimens in herbaria. Comparisons with other relevant *Euphorbia* species were based on previous regional floristic accounts (Brown et al. 1909; Carter 1988; Carter and Leach 2001) as well as the examination of herbarium specimens and digitized images which are mainly from AMD, BR, BM, EA, HIB, K, P, S, and WAG (herbarium acronyms following Thiers (2021)).

Sampling, genomic DNA extraction, and sequencing

To delineate the phylogenetic placement of our *Euphorbia* collection, a total of 17 sequences, which were all newly generated in this study, were used to infer the phylogenetic tree for the *Synadenium* group in *Euphorbia*. Amongst these sequences, 14 accessions representing nine accepted species of the *Synadenium*-group were included. The remaining three accessions from traditionally recognized *Euphorbia*, *Monadenium* and *Endadenium*, were treated as outgroups, according to Dorsey et al. (2013) and Wei et al. (2021).

Sources of DNA were silica-dried leaves collected from field trips, or from dried voucher specimens in herbaria. Total genomic DNA was extracted with the Magic-Mag Genomic DNA Micro Kit (Sangon Biotech Co., Shanghai, China) following the instructions of the manufacturer's protocol. DNA quality was assessed by 1% agarose gel electrophoresis. Short inserts (350 bp) were used to construct paired-end 150-bp sequencing libraries using the NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, United States). Libraries were sequenced at Beijing Genomics Institute (Shenzhen, China) using the Illumina HiSeq 2000 Platform (Illumina, San Diego, CA, United States).

Nuclear ribosomal DNA (nrDNA) assembly and annotation

Raw sequences were quality filtered using software Trimmomatic v.0.33 (Bolger et al. 2014), to avoid any potential sequencing artefacts, improve uniformity in the read length (> 50 bp) and warrant quality (Phred score > 30) in the following assemblies. FastQC 0.11.8 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to assess the trimming quality. The remaining high-quality trimmed sequences were then *de novo* assembled in GetOrganelle (Jin et al. 2020). The produced scaffolds were viewed and then exported as the complete nrDNA in Bandage v.0.7.1 (Wick et al. 2015). The derived nrDNA sequences were annotated in Geneious v.8.0.2 (Kearse et al. 2012) against the annotated nrDNA from other members of Malpighiales in GenBank (*Linum usitatissimum* EU307117; *Hirtella physophora* KJ414478) as references.

The annotated accessions were prepared with GB2sequin (Lehwark and Greiner 2019) for GenBank submission. The complete nrDNA repeat sequence, including its seven constituent loci, i.e., external transcribed spacer (ETS), 18S, internal transcribed spacer 1 (ITS1), 5.8S, internal transcribed spacer 2 (ITS2), 26S, and intergenic spacer (IGS), was used to perform phylogenetic analyses. The sampled species, voucher information, and GenBank accession numbers are provided in Suppl. material 1.

Molecular phylogenetic analyses

The complete nrDNA sequences were aligned by MAFFT v. 7 (Katoh and Standley 2013) with the default setting. TrimAl v.1.2 (Capella-Gutierrez et al. 2009) was used to trim the alignment sequence with *automatd1* mode to reduce potentially poorly aligned regions. Besides, the trimmed alignments were also visually inspected in Geneious 8.0.2 (Kearse et al. 2012) and manually adjusted if necessary. Partition-Finder 2 (Lanfear et al. 2012, 2016) was used for best-fit substitution model selection for each region under the Akaike Information Criterion: the general time reversible model with a gamma distribution of substitution rates (GTR+G) was chosen for the ETS region; the GTR+I+G model with a proportion of invariant sites was selected for the 5.8S and 28S regions; the Hasegawa-Kishino-Yano model with a proportion of invariant sites (HKY+I) was selected for the ITS1, ITS2, and IGS regions; and the HKY+I+G model with a gamma distribution of substitution rates was selected for the 18S region. The ML tree was inferred by IQ-TREE v.1.6.8 (Nguyen et al. 2015) with 10,000 bootstrap replicates. The BI phylogenetic analysis was performed with MrBayes v.3.2.7 (Ronquist et al. 2012). Two independent Markov Chain Monte Carlo analyses (MCMC) were run with four simultaneous chains of 10 million generations, sampling one tree every 100 generations, and setting the burnin fraction as 0.25. The remaining trees were then used to construct a majority-rule consensus tree. The average deviation of split frequencies was verified by reaching a value below 0.01 at the end of the MCMC analyses. The effective sample sizes (ESS values > 200) for all parameters and statistics were also assessed using Tracer v.1.7.1 (Rambaut et al. 2018). The final phylogenetic tree was shown using the online tool iTOL (Letunic and Bork 2007).

Results

Phylogenetic relationships

The 17 complete nrDNA repeat sequences have average coverage ranging from 430.5 to 524.9 (Suppl. material 1). The aligned length of the seven concatenated nrDNA constituent loci dataset prior to trimming is 11,671 bp, whereas the trimmed alignment dataset consisted of 10,605 bp (Suppl. material 2) with 293

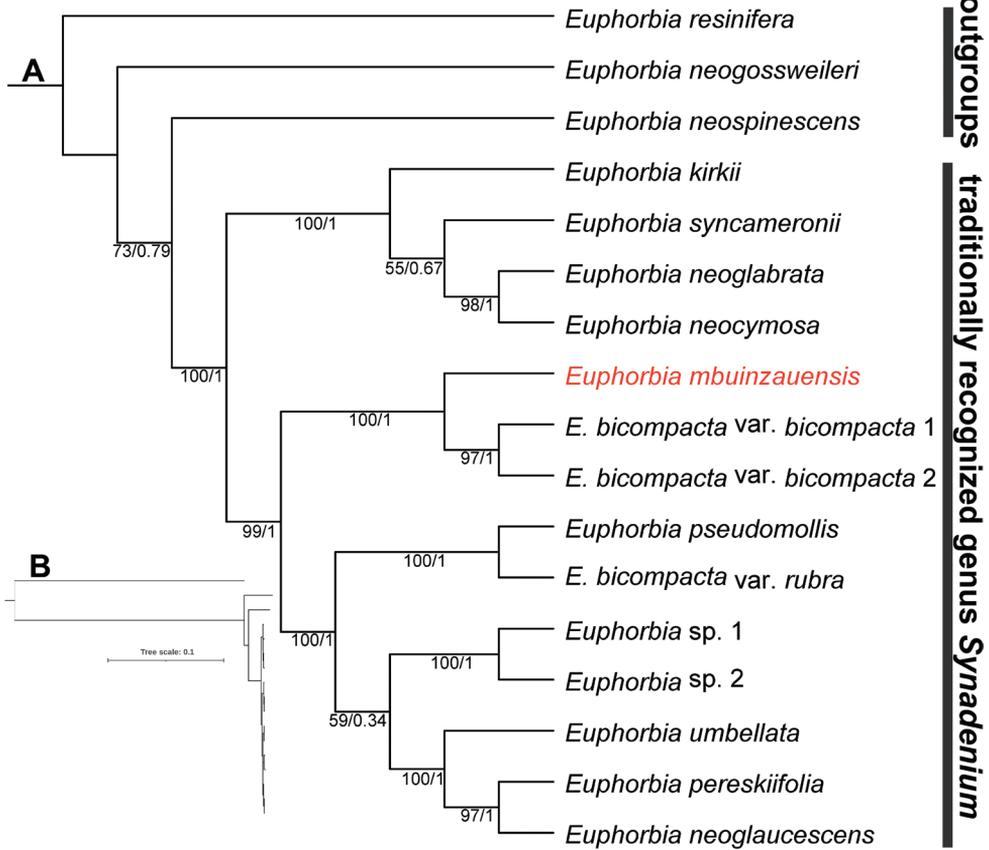


Figure 1. A The maximum likelihood tree inferred from the complete nuclear ribosomal DNA sequences to elucidate the phylogenetic position of *Euphorbia mbuinzaensis*. Bootstrap and Bayesian posterior probability values are shown below the branches (BS/PP). The new species is highlighted in red **B** same tree as A showing branch lengths proportional to nucleotide substitutions per site.

parsimony-informative sites. The ML and BI trees are identical, and the ML tree with both posterior probabilities and ML bootstrap values for each clade is shown as Figure 1. For the ML analysis of the combined seven loci of nrDNA, likelihood score ($-\ln L$) is 23609.7. Overall, the 14 accessions of the *Synadenium* group clustered together and formed a strongly supported monophyletic group (BS = 100%, PP = 1). The new species, *Euphorbia mbuinzaensis*, is sister to the clade that consists of two accessions of *E. bicompecta* var. *bicompecta* with robust support (BS = 100%, PP = 1). Despite the new species being morphologically closest to *E. pseudomollis*, they were not sister taxa in our phylogenetic tree. In addition, the accession of *E. bicompecta* var. *rubra* did not form a clade with *E. bicompecta* var. *bicompecta* as expected. Instead, it is sister to another species, *E. pseudomollis*, with robust support (BS = 100%, PP = 1).

Table 1. Characters distinguishing *Euphorbia mbuinzauensis* from *E. pseudomollis* and *E. bicompecta* var. *bicompecta*.

Character	<i>E. mbuinzauensis</i>	<i>E. pseudomollis</i>	<i>E. bicompecta</i> var. <i>bicompecta</i>
Habit	Shrub to 4 m	Shrubby tree to 9 m	Shrubby tree to 7 m
Leaf apex and margin	Apex rounded to obtuse, margin slightly undulate, crenate	Apex rounded to obtuse, margin serrate	Apex abruptly acute, margin entire, or minutely toothed
Lamina surface	Densely pubescent on adaxial surface, abaxial surfaces with densely stellate hairs, occasionally tinged purplish	Densely pubescent on both sides, abaxial surfaces without flecks	Glabrous, abaxial surfaces flecked or tinged purplish
Inflorescence	In pseudo-umbels of 2–5 cymes; cymes 2–4-forked	In pseudo-umbels of 3–5 cymes; cymes 1–2-forked	In pseudo-umbels of up to 6 cymes; cymes 2–3-forked
Bract	Ca. 2.5 × 3.0 mm, dentate	Ca. 3.5 × 3.5 mm, entire or with a few teeth	Ca. 3.0 × 3.5 mm, obscurely dentate
Cyathium	Ca. 6 mm wide	Ca. 8 mm wide	Ca. 7 mm wide
Gland	Deeply furrowed and wrinkled, crimson	Distinctly grooved, with a very narrow smooth margin, crimson to light red	Shallowly and minutely grooved, yellow to greenish red
Female flower	Styles ca. 2.0 mm long, connate at the base	Styles ca. 1.5 mm. long, connate at the base	Styles ca. 1.8 mm. long, connate to ± halfway
Capsule	To 8 × 7 mm	To 10 × 10 mm	To 8 × 7 mm
Seed	Ovoid, ca. 1.8 × 2.2 mm	Subglobose, ca. 2.5 × 2.5 mm	Ovoid, ca. 2.8 × 2.5 mm

Morphological comparisons

Morphologically, *Euphorbia mbuinzauensis* is closest to *E. pseudomollis*, a species that occurs in Kenya and Tanzania, but differs by a shorter habit (up to 4 m), abaxial surfaces of leaves densely covered with stellate hairs, 2–4-forked cymes, smaller bracts (ca. 2.5 × 3.0 mm) with dentate margin, smaller cyathia (6 mm wide), crimson gland without narrow smooth margin, smaller fruits (ca. 8 × 7 mm) and smaller ovoid seeds (ca. 1.8 × 2.2 mm). Based on the results of our phylogenetic analyses, the sister taxon of *Euphorbia mbuinzauensis* is *E. bicompecta* var. *bicompecta*. However, *E. bicompecta* var. *bicompecta* can be easily distinguished from *E. mbuinzauensis* by its glabrous leaves, shallowly and minutely grooved yellow to greenish red gland, half fused style, and larger seeds (ca. 2.8 × 2.5 mm). Further detailed morphological differences among the three species are presented in Table 1.

Discussion

Euphorbia mbuinzauensis belongs to the *Synadenium* group of subg. *Euphorbia*, which is characterized by tree-like or shrubby habit, fleshy leaves with prominent midrib, pseudo-umbels of 2–5 cymes on peduncles, cymes that are forked several times, involucre [cyathial] glands that fused in a ring-like structure, funnel-shaped involucres, and rudimentary caruncles (Brown et al. 1909; Carter 1988; Carter and Leach 2001). *Euphorbia mbuinzauensis* is distinct, however, from other species in *Synadenium* group with strong morphological and phylogenetic support.

We show the monophyly of the previously segregated genus *Synadenium*, using phylogenetic inference based on a nrDNA dataset. Nevertheless, a more representative

sampling of sect. *Monadenium*, especially of those species that belonged traditionally to *Monadenium*, is needed to draw this conclusion with greater confidence. It is worth mentioning that the phylogenetic relationships among the species in *Synadenium* group exhibited extremely short branches (Figure 1B), indicating that this lineage is likely to have radiated very recently. Interestingly, *E. bicompecta* var. *rubra* did not form a clade with *E. bicompecta* var. *bicompecta* as expected, suggesting a problem in its taxonomic placement. A comprehensive monograph based on an extensive study of specimens and a broad phylogenetic sampling is needed before the *Synadenium* group can be fully understood.

Taxonomic treatment

Euphorbia mbuinzaensis N. Wei, Mwachala, G.W. Hu & Q.F. Wang, sp. nov.

urn:lsid:ipni.org:names:77220553-1

Figures 2, 3

Type. KENYA. Makueni County, Mbuinza, 2°23'25.56"S, 37°54'42"E, 970 m, 29 Sep. 2018, *Sino-Africa Joint Investigation Team (SAJIT) 007200* (holotype HIB!; isotypes EA!, HIB!, K!)

Diagnosis. *Euphorbia mbuinzaensis* is most similar to *E. pseudomollis*, from which it differs by its shorter habit, up to 4 m (vs. to 9 m), abaxial leaves surfaces with densely stellate hairs (vs. simple hairs), 2- to 4-forked cymes (vs. 1- to 2-forked), bracts ca. 2.5 × 3.0 mm (vs. ca. 3.5 × 3.5 mm), dentate margin on bract (vs. entire or with a few teeth), cyathia 6 mm wide (vs. 8 mm wide), gland without narrow smooth margin (vs. with a narrow smooth margin), fruits ca. 8 × 7 mm (vs. ca. 10 × 10 mm), and ovoid seeds ca. 1.8 × 2.2 mm (vs. subglobose, ca. 2.5 × 2.5 mm).

Description. Many-branched shrub to 4 m high. Branches cylindrical, fleshy, and marked with prominent elliptic leaf scars. **Leaves** fleshy, subsessile, deciduous at fruiting stage, with a pair of reduced stipules; lamina subrotund to obovate, to ca. 14 × 6 cm, apex rounded to obtuse with a recurved tip, margin slightly undulate, crenate, midrib distinctly keeled beneath, blade inflated, incurved, adaxial surfaces densely pubescent, green, turning to purplish grey green during dry season, abaxial surfaces densely stellate hairy, occasionally tinged purplish, with stellate hairs along the midrib. **Inflorescences** monoecious, in dense pseudo-umbels of 2–5 cymes on pubescent peduncles to 4 cm long; cymes 2–4-forked, with pubescent branches to 1.8 cm long; bracts subquadrate, ca. 2.5 × 3.0 mm, dentate, densely pubescent. **Cyathia** ca. 2.5 × 6.0 mm, with broadly funnel-shaped involucre, pubescent below; glandular rim ca. 1.2 mm wide, deeply furrowed and wrinkled, purplish red, mostly shallowly notched on the one side, but with a deep notch when young; lobes subquadrate, ca. 2.0 × 2.5 mm, purplish red, pubescent. **Male flowers:** staminate flowers 0.8 mm long, enclosed by involucre lobes and bracteoles; bracteoles fan-shaped, lacinate, plumose, 3 mm long, with pedicels minutely pubescent; pedicels 3 mm long. **Female flowers:** styles ca. 2 mm long, connate at the base, pubescent, with distinctly bifid thickened apices, deciduous in fruit. **Capsules** obovoid, deeply acutely lobed, apex depressed, to

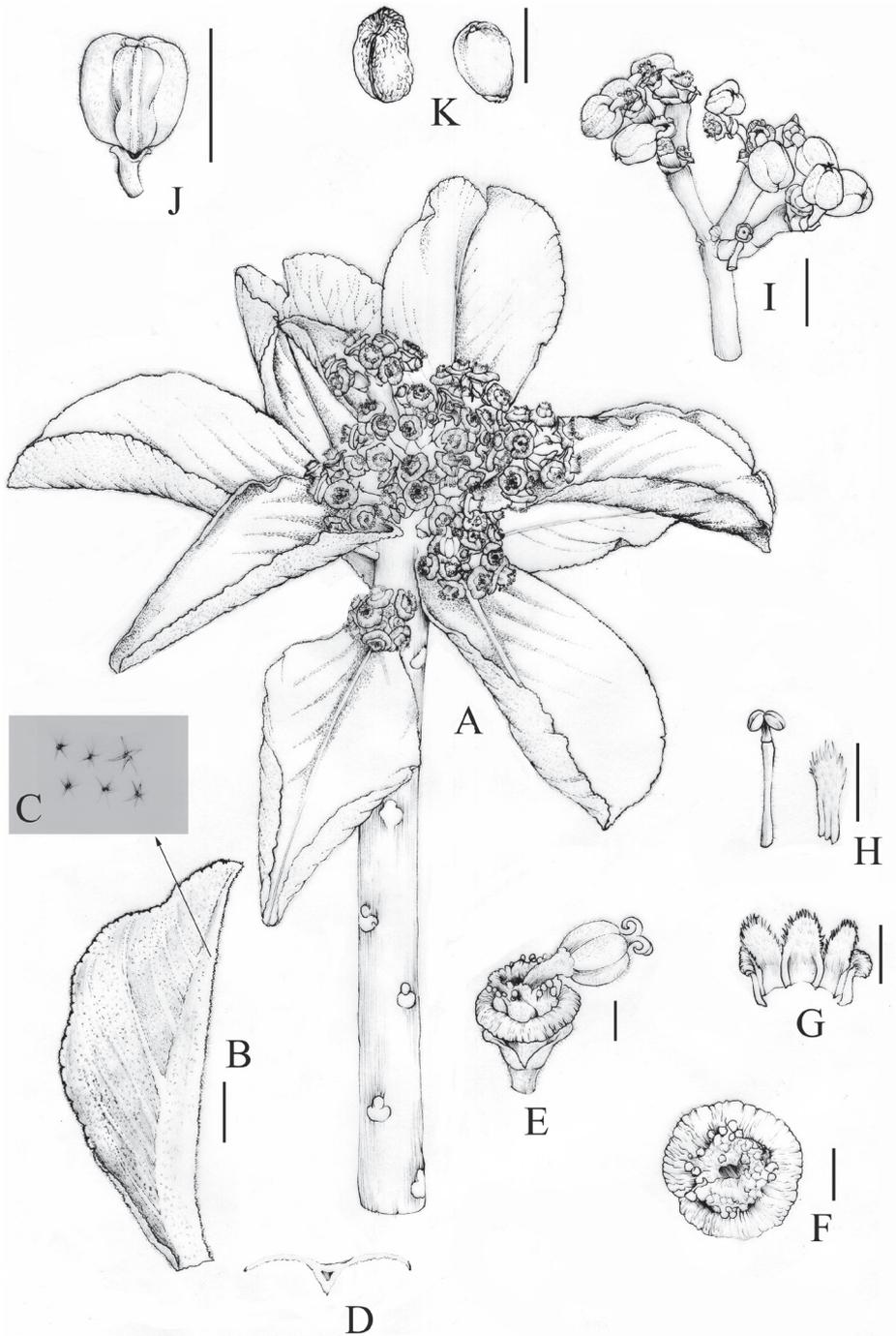


Figure 2. *Euphorbia mbuinzauensis* **A** flowering branch **B** leaf **C** the close-up of abaxial leaf to show densely stellate hairs **D** section through leaf to show the distinct keel on midrib beneath **E** cyathium, lateral view **F** cyathium, top view **G** dissected involucre to show lobes **H** male flower and bracteole **I** infructescence **J** fruit. **K.** Seeds. Scale bars: 2 mm (**C–H, K**); 1 cm (**B, I, J**). Voucher specimen: *SAJIT 007200*. Drawn by Nan Jia.

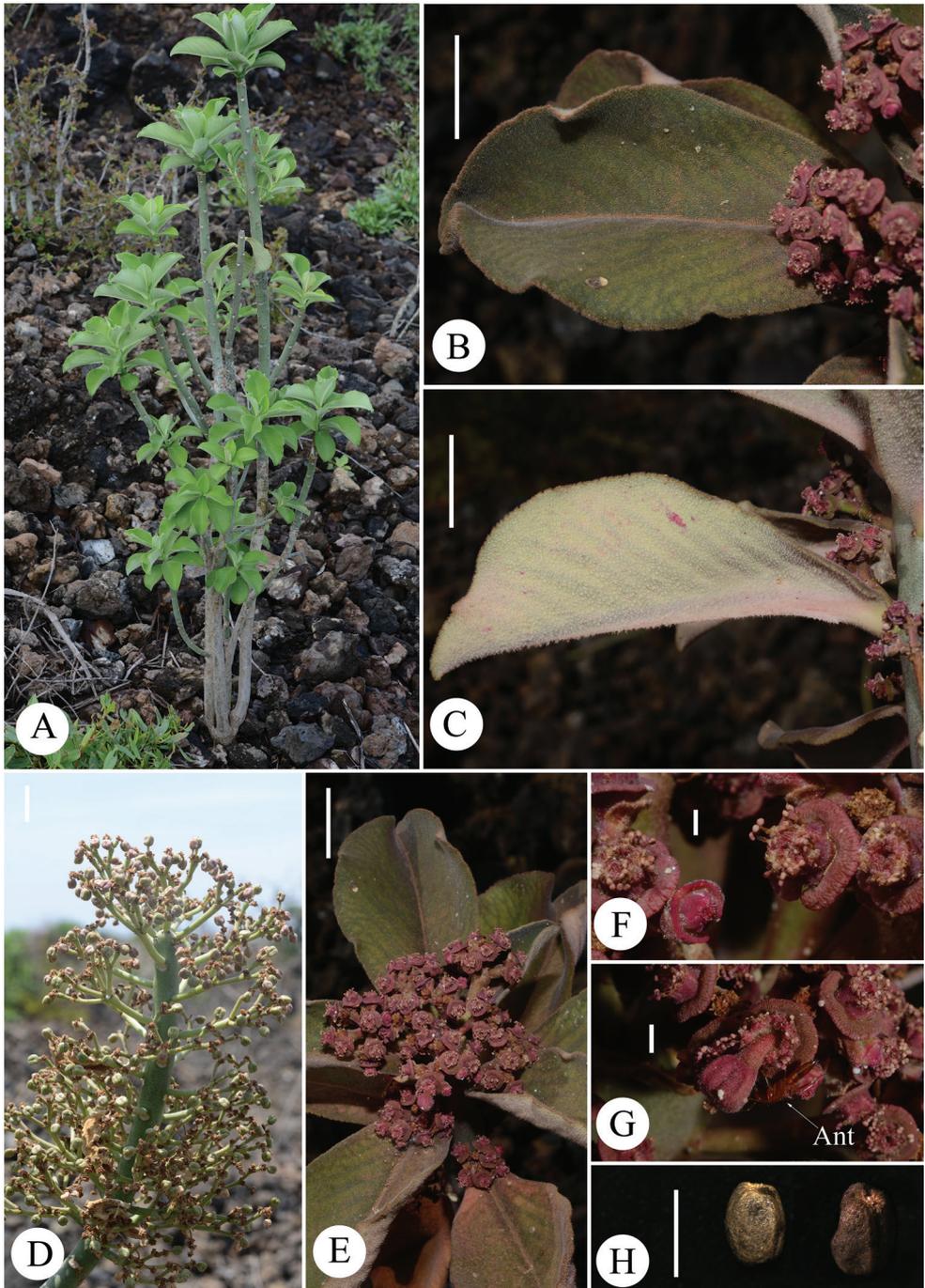


Figure 3. Morphological features of *Euphorbia mbuinzaensis* **A** habit **B** adaxial surface of leaf **C** abaxial surface of leaf **D** a branch with numerous infructescences **E** apical portion of flowering branch **F, G** Cyathium **H** seeds. Scale bars: 2 mm (**F–H**); 2 cm (**B–E**). Voucher specimens: *SAJIT 007411* (**A**), *SAJIT 007200* (**B–H**). Photo credit: Neng Wei.

8 × 7 mm, from purplish red to yellowish green, pubescent, explosively dehiscent septicidally and loculicidally into 3 2-valved cocci; pedicel recurved, pubescent, to 8 mm long; columella persistent, 6–7 mm long. **Seeds** ovoid, obscurely 4-angled, ca. 1.8 × 2.2 mm, pale brown to dark brown, shallowly tuberculate; caruncle rudimentary.

Distribution and ecology. Only one population of the new species was found at the foot of Mbuinzau hill, Makueni County, Kenya (Figure 4). Here it grows in open deciduous woodlands covered by lava outcrops at an elevation of ca. 970 m.

Conservation status. Only one population of the new species was found in an isolated woodland covered by lava outcrops (Figure 3A) in Mbuinzau area. We assess the species as Endangered (criteria D1) according to the IUCN Red List Categories and Criteria (IUCN 2001), due to the number of mature individuals (< 250) in a very small and restricted population. Unfortunately, the habitat is threatened by human activities. For instance, its habitat has been fragmented by the Mombasa–Nairobi Railway and Mombasa–Road, which are the busiest traffic routes in Kenya. Moreover, the population is under threat from habitat loss, due to the exploitation of lava rocks in its habitat for construction.

Phenology. *Euphorbia mbuinzauensis* was found with flowers in September and with fruits from September to December.

Etymology. The epithet “*mbuinzauensis*” refers to its type locality, Mbuinzau in Makueni County, Kenya.

Notes. The conspicuous latex is extremely poisonous to cattle as well as human beings, according to the comments from local farmers. We observed ants visiting the flowers and they are a possible pollinator to this species (Figure 3G).

Additional specimens examined (Paratypes). KENYA. Makueni County, Mbuinzau, 2°23'25.56"S, 37°54'42"E, elev. 970 m, 5 Dec. 2018, *SAJIT 007411* (EA!, HIB!).

Specimens examined for *Euphorbia pseudomollis*. KENYA. Makueni, Kibwezi, c. 1000 m, 2°26'S, 38°1'E, 22 Mar. 1906, *G. Scheffler 137* (isotypes AMD [AMD.66883] image!, BM [BM000911307]!, K [K000238424]!, S [S13-12929] image!, WAG [WAG0004308] image!); Taita-Taveta, Mwatate, 3°31'S, 38°24'E, 29 Apr. 1963, *P.R.O. Bally B12725* (BR [BR0000016225411] image!, K [K000238420]!); Taita-Taveta, Mwatate West of Voi, 3°30'S, 38°23'E, 15 Jul. 1960, *L.C. Leach & R. Bayliss 10258* (K [K000238421]! & [K000238422]!); Kitui, Mutomo Hill Plant Sanctuary, 900–1000 m, 1°51'S, 38°13'E, 2 May 1970, *J.B. Gillett 19141* (EA!, K [K000238423]!); Taita-Taveta, Taita, Sisal Estate, Senbi Hill, 1050 m, 3°31'S, 38°24'E, 17 Aug. 2000, *P.A. Luke & W.R.Q. Luke 6432* (EA!). TANZANIA. Kilimanjaro, above Mwembe, 1005 m, 4°8'S, 37°51'E, 9 Apr. 1972, *B.J. Harris BJH6342* (K [K000238425]!); Kilimanjaro, Mwembe, 4°10'S, 37°51'E, unknown date, *P.R.O. Bally B11499 (E54)* (K [K000238426]! & [K000238427]!); Dodoma, Mpwapwa, 1128 m, 6°21'S, 36°29'E, 30 Jun. 1938, *Hornby 911* (K [K000238428]!); Tanga, Handeni, Kideleko, 609 m, 5°29'S, 38°1'E, 1 Jul. 1965, *M.E. Archbold 471* (K [K000238430]!); Tanga, Handeni, Chanika Village, 700 m, 5°25'S, 38°1'E, 23 Sep. 1979, *O. Hedberg et al. TMP194* (K [K000238431]!); Morogoro, Kilombero, Lugoda, 1800 m, 8°42'S, 35°49'E, Aug. 1988, *E. Adibeysen 224* (K [K000238432]!); Iringa, a little north of Morogoro road, 1554 m, 7°30'S, 36°10'E, 27 Feb. 1962, *R.M. Polhill & S. Paulo 1618* (BR

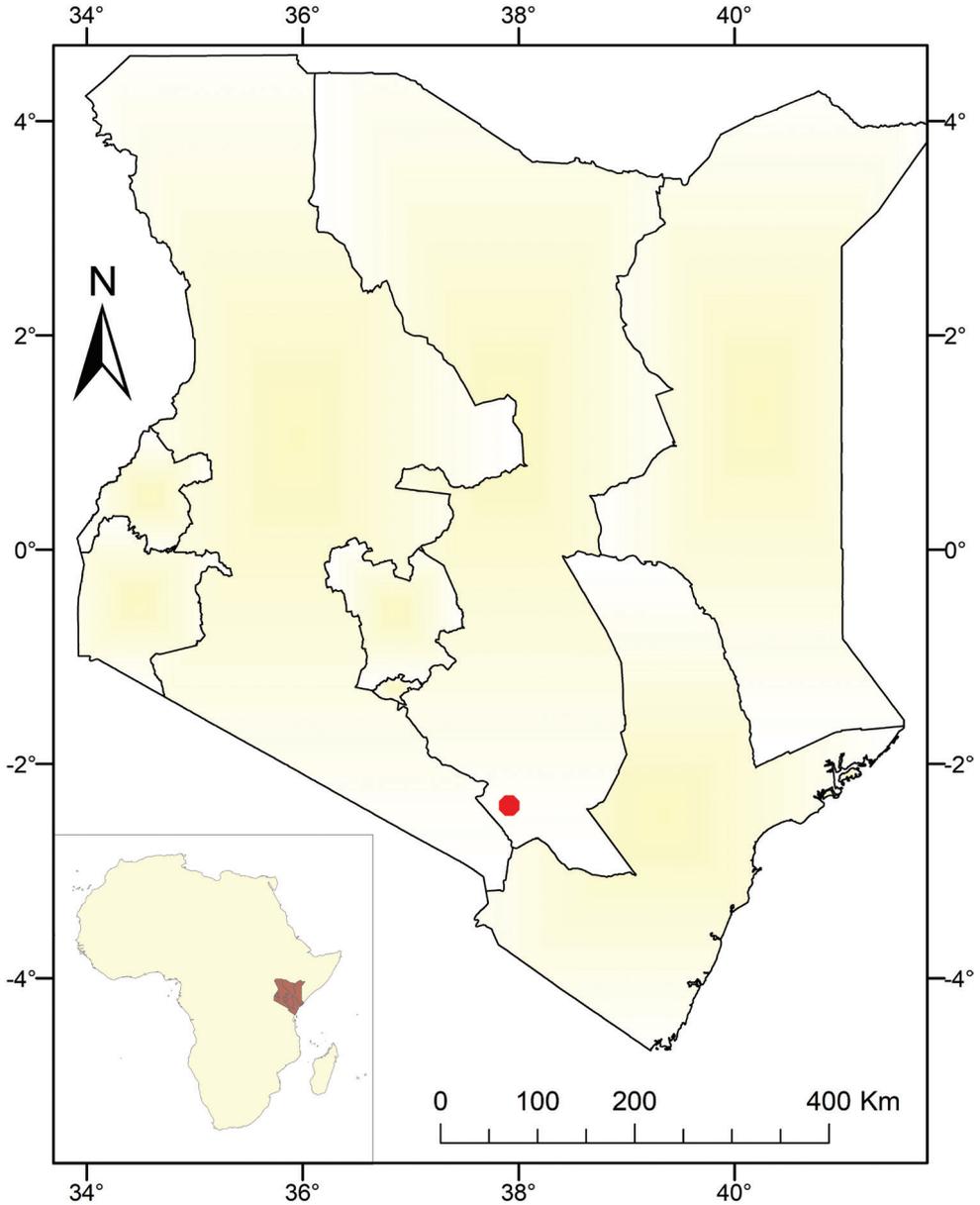


Figure 4. Known distribution of *Euphorbia mbuinzauensis* in Kenya. The red dot indicates its only known locality.

[BR0000016225435] image!, EA!, K [K000238433]!, P [P00581481] image!); Lindi, 22 Apr. 1933, *H.J.Schlieben n6383* (BR [BR0000016225428] image!).

Specimens examined for *Euphorbia bicompecta* var. *bicompecta*: KENYA. Machakos, 1°31'S, 37°16'E, 7 Jun. 1902, *T. Kassner 956* (Holotype K [K000237846]!); Isotype BM [BM000911306]!); Taita-Taveta, Wusi, 1371 m, 3°27'S, 38°21'E, May 1931, *ERN*

1322 (K [K000237843]!); Machakos, 1°31'S, 37°16'E, 27 Mar. 1940, *P.R.O. Bally E144* (K [K000237845]!); Makueni, Kibwezi, Sisal Estate, 914 m, 2°26'S, 38°1'E, Jun. 1943, *P.R.O. Bally B2573* (K [K000237847]!); Kitui, Migwani, 10 miles N of Migwani on Tharaka road, 0°57'18"S, 38°1'9"E, 3 May 1960, *D.M. Napper 1596* (BR [BR0000016224810] image!, EA!, K [K000237848]!); Nairobi, Nairobi arboretum, 1768 m, 1°17'S, 36°49'E, Aug. 1932, *I.R. Dale 2887* (K [K000237849]!); Nairobi, Langata, 1°20'S, 36°46'E, 20 March 1963, *P.R.O. Bally B12659* (K [K000237850]!); Kiambu, Muguga, 1°11'S, 36°38'E, 8 Jun. 1962, *J. Gichuru 14* (K [K000237851]!); Nyeri, Karatina, 0°29'S, 37°8'E, 24 Apr. 1943, *P.R.O. Bally B2541* (EA!, K [K000237852]!); Embu, Thuchi, crossing on Embu-Meru road, 760 m, 0°25'S, 37°52'E, 4 Apr. 1970, *J.B. Gillett & B. Mathew 19063* (BR [BR0000016224803] image!, K [K000237853]!); Laikipia, Kisima farm, 1700 m, 0°30'S, 36°30'E, 14 Jun. 1972, *P.R.O. Bally B15106* (K [K000237854]!); Taita-Taveta, Msau River Valley, 800–950 m, 3°24'S, 38°24'E, 18 May 1985, *C.H.S. Kabuye et al. 743* (K [K000237855]!); Taita-Taveta, Kasigau Mountain, 1000 m, 3°50'S, 38°40'E, 31 May 1998, *W.R.Q. Luke et al. 5344* (K [K000237856]!); Elgeyo Marakwet, Arror lower, Pt 203, 1050 m, 1°0'55"S, 35°37'27"E, 30 Jul. 2017, *Mwadime N 1861* (EA!).

Acknowledgements

We thank Leonard E. Newton and Susan Carter for their valuable comments and suggestions. We also thank Paul Rees, Imalka Kahandawala, and Zhengwei Wang for their help in collecting material for DNA extraction. Gratitude is extended to Mrs. Nan Jia for her beautiful line drawings of the new species; Mrs. Lunlun Gao from Huazhong Agricultural University for preparing the distribution map; and the Kenya Forest Service (KFS) for issuing fieldwork permits (permit number: RESEA/1/KFS 98 and RESEA/1/KFS 22) to conduct the field investigations. We would also like to thank the following herbaria AMD, BR, BM, EA, HIB, K, P, S, and WAG for hosting our visits or providing images of digitized specimens in this study. We are grateful to Dr. Dmitry Geltman for his significant editorial work, Dr. Kenneth J. Wurdack and an anonymous reviewer for their constructive comments, which helped us to improve the manuscript. This work was supported by grants from Sino-Africa Joint Research Center, CAS, China [grant numbers SAJC202101], International Partnership Program of Chinese Academy of Sciences [grant numbers 151853KYSB20190027], the National Science Foundation of China [grant number 31970211 and 32070231] and the University of Chinese Academy of Sciences Joint PhD Training Program.

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Supplementary material 1

Table S1

Authors: Neng Wei, Fredrick Munyao Mutie, Geoffrey Mwachala, Olwen M. Grace, Guang-Wan Hu, Qing-Feng Wang

Data type: docx. file

Explanation note: Accessions of nuclear ribosomal DNA (nrDNA) in *Euphorbia* used in this study. (SCBG indicates Shanghai Chenshan Botanical Garden, Chinese Academy of Sciences).

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

Link: <https://doi.org/10.3897/phytokeys.183.70285.suppl1>

Supplementary material 2

File 2

Authors: Neng Wei, Fredrick Munyao Mutie, Geoffrey Mwachala, Olwen M. Grace, Guang-Wan Hu, Qing-Feng Wang

Data type: docx. file

Explanation note: The trimmed alignment of the 17 complete nuclear ribosomal DNA (nrDNA) sequence dataset in nexus format.

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Link: <https://doi.org/10.3897/phytokeys.183.70285.suppl2>

Cirsium taiwanense (Compositae, *Cirsium* sect. *Onotrophe*, subsect. *Australicirsium*), a new species from Taiwan

Chih-Yi Chang¹, Hsy-Yu Tzeng¹, Yen-Hsueh Tseng^{1,2}

1 Department of Forestry, National Chung-Hsing University, No. 145, Hsing-Ta Rd., Taichung 402, Taiwan

2 Taiwan Forestry Research Institute, No. 53, Nanhai Rd., Zhongzheng Dist., Taipei City, 10066, Taiwan

Corresponding author: Yen-Hsueh Tseng (tseng2005@nchu.edu.tw)

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Abstract

A new species of *Cirsium*, *C. taiwanense* Y.H.Tseng & Chih Y.Chang from central-northern Taiwan is reported in this article. This species is similar to *C. hosokawae* Kitam. in having a densely cobwebby abaxial leaf surface, but differs in its yellow (vs. vivid purplish red) corolla and the angle between the midrib and the lateral veins of the leaf, which is acute as opposed to nearly at a right angle in *C. hosokawae*. *Cirsium taiwanense* has $2n = 32$ chromosomes, which is different from the other species in the Taiwanese subsect. *Australicirsium* Kitam. ($2n = 34$). An identification key to the *Cirsium* taxa of Taiwan is presented.

Keywords

Australicirsium Kitam., central-northern Taiwan, *Cirsium*, karyotype analysis, pollen morphology, subsect

Introduction

The genus *Cirsium* Mill. (Compositae) contains ca. 250 extant species, with its center of diversity in southern Europe and Caucasia (Werner 1976; Garcia-Jacas et al. 2002). This genus contains perennial, biennial, and annual spiny herbs, and has capitula with only disc florets, involucre of 5 to 20 series, setiferous receptacles, anther bases with caudate extensions, an achene apex with a short beak, and plumose pappus (Keil 2006; Funk et al. 2009). In East Asia, *Cirsium* has been reported in China (46 species, Shih and Greuter 2011), Japan (64 species, Iwatsuki et al. 1995), and Korea (8 species, Song and Kim 2007). According to Peng et al. (1998), nine species are recorded in Taiwan,

one of which is represented by two varieties. Recently, Chang et al. (2019) described a new species endemic to Taiwan, namely *C. tatakaense* Y.H.Tseng & Chih Y.Chang, and Chang and Tseng (2019) reported a newly recorded variety, *C. japonicum* DC. var. *fukienense* Kitam. In addition, Chang and Tseng (2020) reported a newly naturalized species, *C. vulgare* (Savi) Tenore. The Taiwanese species are distributed from seashores to high altitude areas.

The island of Taiwan is located about 150 km off the southeast coast of China, between 21°45'N and 25°37'N, and 119°18'E and 122°06'E. Its climate ranges from tropical to subtropical. Taiwan is at the border between the paleotropical floristic kingdom and the Holarctic floristic kingdom (Good 1964; Takhtajan 1986). Several peaks exceed 3500 m a.s.l. and the highest is Mt. Yushan at 3952 m a.s.l., making Taiwan the fourth-highest island in the world (Chen 1980; Wang et al. 2009). Taiwan contains a diverse range of ecological niches in its mountains, which might have accelerated speciation and facilitated the evolution of endemic high-altitude plant species (Hsieh 2002). For example, there are seven native *Cirsium* species distributed from mid- to high altitudes, and all of them are endemic (Peng et al. 1998; Chang and Tseng 2019).

According to the infrageneric classification of East Asian *Cirsium* (Kitamura 1937; Shih 1984; Iwatsuki et al. 1995), the ten native species of Taiwan belong to three sections, viz. sect. *Onotrophe* (Cass.) DC., sect. *Pseudoeriolepis* (Nakai) Kitam., and sect. *Spanioptilon* (Less.) Shih. Section *Onotrophe* (Cass.) DC. is further subdivided into four subsections (Kitamura 1937), viz. subsection *Arenicola* Kitam., subsection *Australicirsium* Kitam., subsection *Nipponocirsium* Kitam., and subsection *Sinocirsium* Kitam. Following this infrageneric system, subsection *Australicirsium* has only two species in Taiwan, *C. arisanense* Kitam. and *C. hosokawae* Kitam., which are characterized by erect or nodding capitula, phyllaries with a distinct midrib, and corolla lobes that are as long as the inflated part of the corolla tube (Kitamura 1937).

Recently, we discovered an unknown *Cirsium* belonging to subsection *Australicirsium* (sect. *Onotrophe*) growing in the high mountain areas of central-northern Taiwan. This taxon appeared to be similar to *C. hosokawae*, with abaxial leaf surfaces covered with dense cobwebby hairs and by having nodding mature capitula. However, it can readily be distinguished from the latter by its yellow flowers, and the smaller angle between the midrib and the lateral veins. The aim of the present study was to elucidate the taxonomic status of this taxon using morphological, palynological and cytological approaches. After detailed examinations, we concluded that the taxon represents a new species and it is here described as *Cirsium taiwanense*.

Materials and methods

Morphological comparison

We compared the two Taiwanese taxa of subsection *Australicirsium* with the unknown taxon. Morphological measurements were made using both fresh and dried specimens.

For the morphological description, the terminology used by Peng et al. (1998) and Funk et al. (2009) was applied.

Herbarium resources

Herbarium acronyms follow Index Herbariorum (Thiers 2021, continuously updated). Voucher specimens collected for the current study were deposited in PPI, TCF, and TNM. Specimens of the following herbaria were also examined: CHIA, HAST, KYO, PPI, TAI, TAIF, TCF, TI, TNM and TNU. The holotypes of both *C. arisanense* and *C. hosokawae* were also studied.

Pollen morphology

Pollen grains were collected from fresh materials, and directly mounted on a stub. After air drying for 24 h at room temperature, the samples were sputter-coated with gold at 10–15 mA for 100 s (Quorum SC7620), and observed with a scanning electron microscope (Hitachi S-3400N). The shape, size and exine ornamentation were recorded using the methods of Erdtman (1952) and Halbritter et al. (2018). Information about voucher specimens is provided in Table 1.

Karyotype analysis

Karyotype analysis was performed using the procedures of Ozcan et al. (2011) and Yüksel et al. (2013). Root tips were collected on sunny mornings and pre-treated with 2 mM 8-hydroxyquinoline below 4 °C for 8 h, then fixed with Carnoy's solution (absolute ethanol:acetic acid, 3:1, v:v) for at least 24 h at 0 °C. The fixed roots were then stained with 2% aceto-orcein for 24 h at room temperature, squashed, and the slides were examined using an optical microscope (Accu-Scope 3025) equipped with a CCD camera (ProgRes C14 plus). Information about voucher materials is presented in Table 1.

Distribution map

A distribution map was generated using QGIS ver. 3.4 from the package developed by Lin (2018). Geographical climatic regions and altitudinal vegetation zones of Taiwan were indicated following Su (1984, 1985) (Fig. 4). The geographical range of each species was determined from information on herbarium specimens.

Data analysis

The values of the quantitative morphological and palynological traits were determined and their means and standard deviations were calculated (Table 2). Differences between taxa were analyzed using a one-way ANOVA, followed by Tukey's HSD multiple-range test ($p \leq 0.05$) (Zar 1984). All analyses were performed using the PASW Statistics ver. 18 software (Sarma and Vardhan 2018).

Table 1. Voucher material for *Cirsium* Mill. subsect. *Australicirsium* Kitam. pollen morphology and karyotype analysis.

Taxa	Location	Altitude	Coordinate (WGS84)	Collecting date	Voucher number	Pollen morphology	Karyotype analysis
<i>C. arisanense</i>	TAIWAN. Hualien County, Xiulin Township, Hsiao Fengkou	2,996 m	24.16245°N, 121.28716°E	26 June 2015	<i>C. Y. Chang</i> 602 (TNM)	●	●
	TAIWAN. Taichung City, Heping District, Mt. Syue trail 8.9 km	3,399 m	24.39229°N, 121.24166°E	3 Sept. 2015	<i>C. Y. Chang</i> 756 (TCF)	●	
	TAIWAN. Nantou County, Ren'ai Township, Rueiyuan river pipes road 2 km	2,215 m	24.11398°N, 121.20746°E	27 May 2016	<i>C. Y. Chang</i> 1275 (TCF)	●	
	TAIWAN. Nantou County, Sinyi Township, Tataka	2,609 m	23.47692°N, 120.89841°E	9 Feb. 2020	<i>C. Y. Chang</i> 2733 (TCF)		●
<i>C. hosokawae</i>	TAIWAN. Taichung City Heping District, Mt. Syue trail, Kupo to Mt. Syue East Peak	3,168 m	24.38882°N, 121.27348°E	8 Nov. 2015	<i>C. Y. Chang</i> 870 (TNM)	●	
	TAIWAN. Hualien County, Xiulin Township, Shangyuankuti, Mt. Nanhutashan	3,586 m	24.36042°N, 121.43713°E	5 Sept. 2016	<i>C. Y. Chang</i> 1432 (TCF)	●	
	TAIWAN. Ilan County, Datong Township, Mt. Nanhutashan trail, near Mt. Tochiatun	2,761 m	24.36801°N, 121.37971°E	11 Aug. 2018	<i>C. Y. Chang</i> 2023 (TCF)		●
	TAIWAN. Taichung City, Heping District, Mt. Nanhutashan trail, Sungfengling	2,648 m	24.36973°N, 121.37167°E	27 July 2019	<i>C. Y. Chang</i> 2477 (TCF)	●	
	TAIWAN. Taichung City, Heping District, Mt. Nanhutashan trail 8.5 km	2,707 m	24.36850°N, 121.37350°E	31 July 2019	<i>C. Y. Chang</i> 2499 (TCF)	●	
<i>C. taiwanense</i>	TAIWAN. Taichung City, Heping District, Mt. Syue trail 0.9 km	2,398 m	24.38520°N, 121.29254°E	3 July 2015	<i>C. Y. Chang</i> 620 (TCF)	●	
	TAIWAN. Nantou County, Ren'ai Township, Provincial Rd. No. 14A 21.5 km	2,657 m	24.11381°N, 121.22401°E	2 Oct. 2015	<i>C. Y. Chang</i> 772 (TNM)	●	
	TAIWAN. Taichung City, Heping District, Mt. Syue trail 0–1 km	2,353 m	24.38486°N, 121.29519°E	24 Apr. 2016	<i>C. Y. Chang</i> 1230 (TCF)		●
	TAIWAN. Taichung City, Heping District, Mt. Tao	2,648 m	24.41766°N, 121.30693°E	22 July 2018	<i>C. Y. Chang</i> 1926, 2133 (TCF)		●

Results

Macro-morphological differences

The abaxial leaf surface of the members of subsect. *Australicirsium* in Taiwan displays two types of indumentum. Both *C. hosokawae* and *C. taiwanense* are densely covered with cobwebby hairs, whereas *C. arisanense* is without cobwebby indumentum. The angle between the midrib and the lateral veins of the leaves of *C. hosokawae* is often almost 90°, (60–)82–90°, which differs significantly ($p \leq 0.05$) from that of *C. arisanense*, (49–)57–78° and *C. taiwanense* (44–)52–73° (Fig. 1, Table 2). In addition, the mature capitula of *C. arisanense* are erect and rarely nodding, whereas those of *C. hosokawae* and *C. taiwanense* are usually nodding. *Cirsium taiwanense* has significantly ($p \leq 0.05$) more florets in a capitulum (101–135(–194)) than *C. arisanense* (78–137) and *C. hosokawae* (54–111), and a larger number of phyllaries: 90–127 vs. 66–100 for *C. arisanense* and 68–109 for *C. hosokawae*. (Fig. 1, Table 2). Further,

Table 2. Summary of diagnostic characters of *Cirsium* Mill. subsect. *Australicirsium* Kitam. in Taiwan.

Characters		<i>C. arisanense</i>	<i>C. hosokawae</i>	<i>C. taiwanense</i>
Rosette leaves	Size (cm)	31.32 ± 13.71 ^a × 5.55 ± 1.27 ^a	19.69 ± 3.79 ^b × 4.42 ± 1.02 ^a	26.05 ± 6.18 ^{ab} × 5.55 ± 1.27 ^a
	Shape	narrowly elliptic	narrowly elliptic to oblanceolate	narrowly elliptic to oblanceolate
	Angle between midrib and lateral vein (°)	68.78 ± 5.12 ^b	83.05 ± 9.88 ^a	63.04 ± 12.00 ^b
	Cobwebby hairs on abaxial leaf surface	Absent	present	present
Cauline leaves	Size (cm)	12.70 ± 5.84 ^b × 4.10 ± 2.95 ^a	17.88 ± 0.78 ^a × 5.26 ± 0.74 ^a	16.17 ± 5.01 ^{ab} × 4.85 ± 1.56 ^a
	Angle between midrib and lateral vein (°)	65.31 ± 9.06 ^b	83.73 ± 9.53 ^a	62.30 ± 10.71 ^b
	Cobwebby hairs on abaxial leaf surface	Absent	present	present
Capitula	Mature capitula	erect or sometimes nodding	nodding	nodding
	Size (cm)	3.05 ± 0.22 ^a × 1.42 ± 0.25 ^{ab}	2.60 ± 0.66 ^a × 1.18 ± 0.32 ^b	3.03 ± 0.22 ^a × 1.61 ± 0.17 ^a
Phyllaries	Length ratio (inner vs. outer)	2.05 ± 0.75 ^{ab}	1.46 ± 0.33 ^b	2.91 ± 0.73 ^a
	Length of the reflexed part of the phyllaries (mm)	4.88 ± 1.23 ^a	2.33 ± 0.80 ^b	2.30 ± 0.18 ^b
	Number	81.3 ± 11.5 ^b	86.3 ± 12.0 ^b	111.7 ± 13.3 ^a
Florets	Length (cm)	2.54 ± 0.15 ^{ab}	2.46 ± 0.21 ^b	2.63 ± 0.21 ^a
	Inflated part of corolla tube length (mm)	4.39 ± 0.63 ^b	4.41 ± 0.46 ^b	5.11 ± 0.70 ^a
	Corolla color	Yellow	vivid purplish red	yellow
	Corolla lobes	Revolute	erect	erect
	Anther length (mm)	6.02 ± 0.78 ^b	6.10 ± 0.51 ^b	6.86 ± 0.80 ^a
	Number	102.7 ± 24.1 ^{ab}	84.6 ± 19.5 ^b	129.7 ± 30.1 ^b
Achene	Size (mm)	3.77 ± 0.16 ^a × 1.49 ± 0.06 ^{ab}	3.95 ± 0.13 ^a × 1.37 ± 0.05 ^b	3.97 ± 0.18 ^a × 1.65 ± 0.07 ^a
	Pappus length (cm)	1.66 ± 0.04 ^a	1.29 ± 0.04 ^b	1.60 ± 0.05 ^a
Pollen	Pollen size (P/E, μm)	47.61 ± 0.80 ^a / 46.37 ± 1.00 ^a	41.40 ± 0.60 ^b / 41.15 ± 0.75 ^b	48.50 ± 0.80 ^a / 47.00 ± 1.00 ^a
	Pollen spine length (μm)	4.25 ± 0.18 ^a	3.91 ± 0.14 ^{ab}	3.20 ± 0.18 ^{bc}
	Pollen spine base width (μm)	4.44 ± 0.26 ^a	5.33 ± 0.19 ^a	4.74 ± 0.26 ^a
Chromosome number		2n = 34	2n = 34	2n = 32
Distribution		Endemic to Taiwan; widely distributed in open mountain areas at 1500–3800 m a.s.l. (Chang et al. 2019).	Endemic to Taiwan; open areas at 1400–3600 m a.s.l. in central-northern Taiwan.	Endemic to Taiwan; open areas at 1400–3400 m a.s.l. in central-northern Taiwan.

^{abc} Means in the same row followed by the same letter are not significantly different ($p \leq 0.05$; Tukey's HSD test).

the corolla of *C. taiwanense* and *C. arisanense* is yellow, but that of *C. hosokawae* is vivid purplish red. Although the color of the corolla of *C. hosokawae* could not be determined from its type specimen, it is described as red in the protologue (Kitamura 1932). The populations described here have yellow corollas and are therefore regarded as *C. taiwanense*. Moreover, the corolla lobes of *C. arisanense* are revolute, whereas the two other species have erect corolla lobes (Fig. 1, Table 2). Finally, the pappus of the

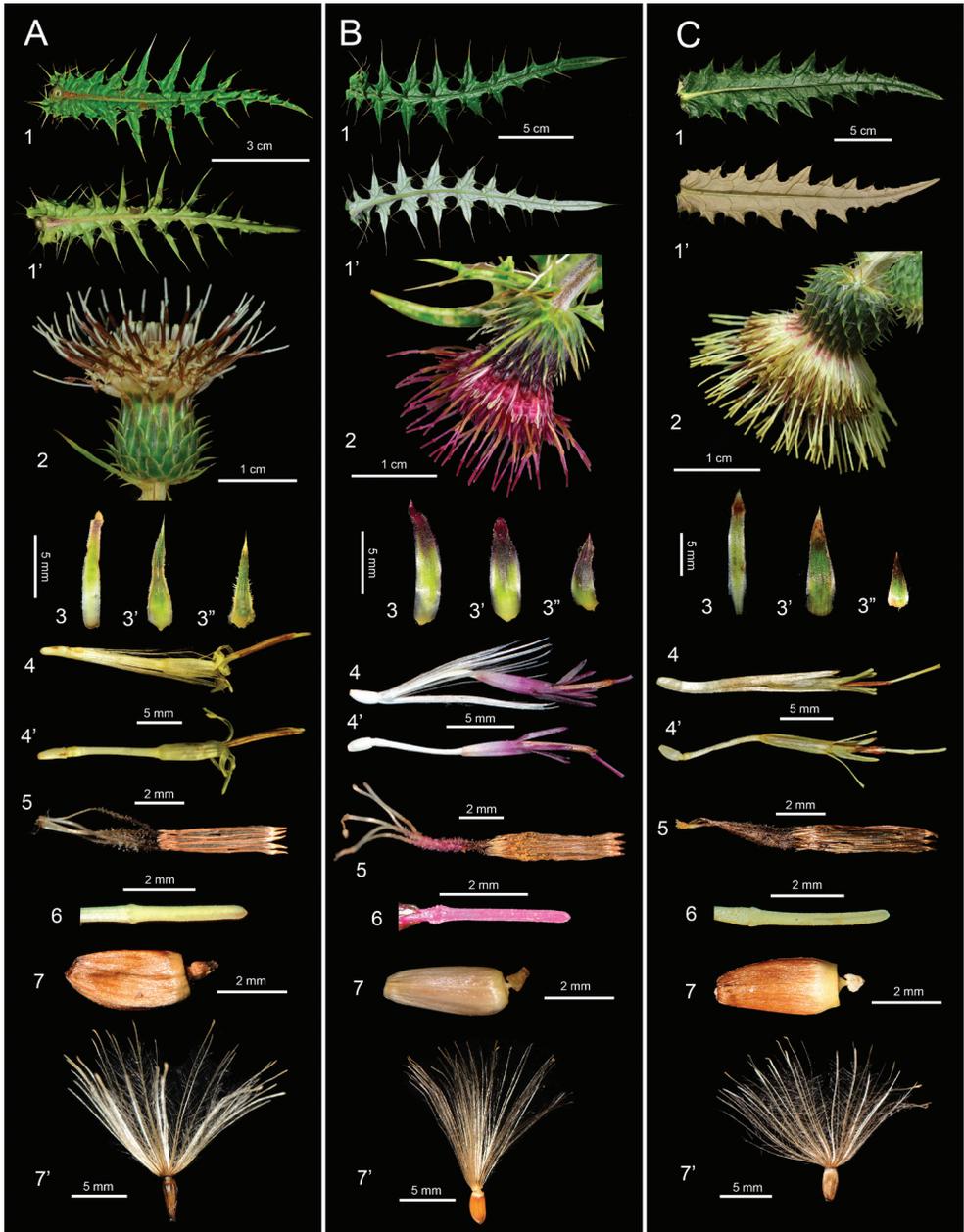


Figure 1. Comparison of the morphological characters of *Cirsium* Mill. subsect. *Australicirsium* Kitam. **A** *C. arisanense* Kitam **B** *C. hosokawae* Kitam **C** *C. taiwanense* Y.H.Tseng & Chih Y.Chang **1** cauline leaf, adaxial view **1'** cauline leaf, abaxial view **2** capitulum **3** inner phyllary **3'** middle phyllary **3''** outer phyllary **4** floret **4'** floret (pappus removed) **5** synantherous stamens **6** style branches **7** achene **7'** achene with pappus.

achene of *C. hosokawae* is significantly ($p \leq 0.05$) shorter (1.02–1.48 cm) than that of *C. arisanense* (1.44–1.73 cm) and *C. taiwanense* (1.55–1.66 cm) (Fig. 1, Table 2). In general, the leaves of *C. taiwanense* and *C. hosokawae* are similar, as the abaxial leaf

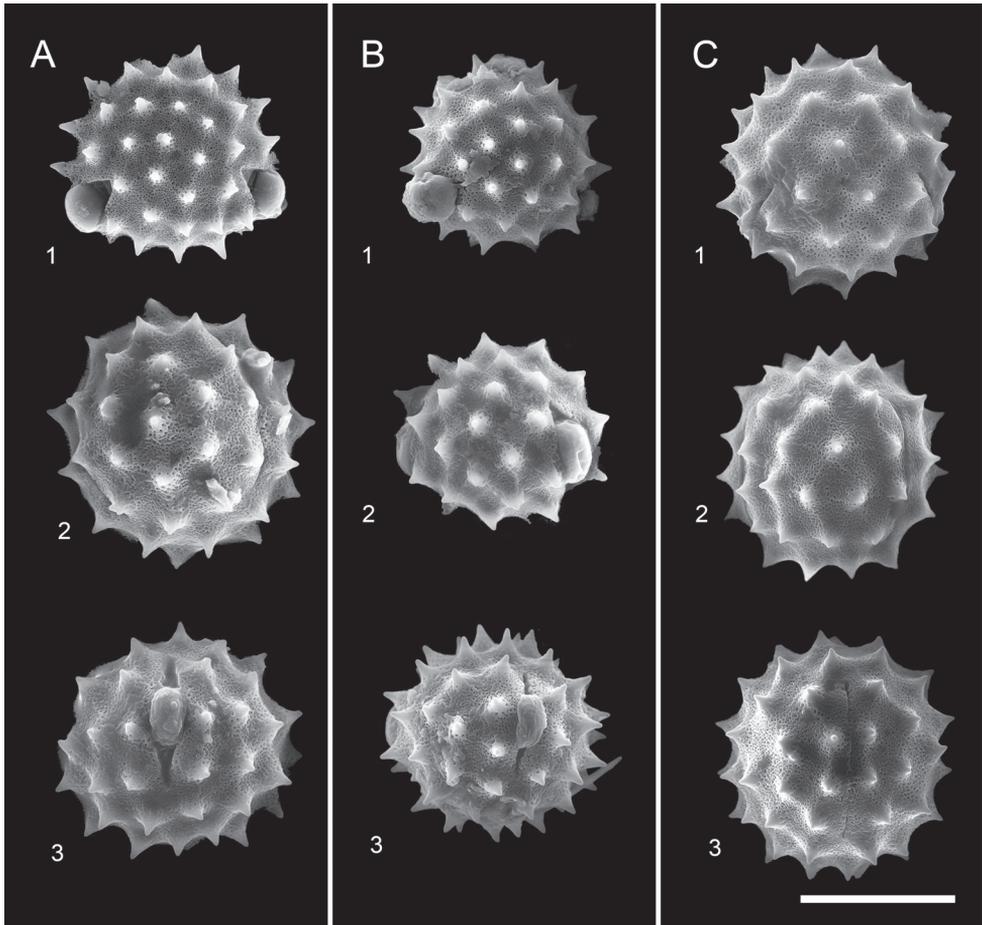


Figure 2. Comparison of the pollen morphology of *Cirsium* Mill. subsect. *Australicirsium* Kitam **A** *C. arisanense* Kitam **B** *C. hosokawae* Kitam **C** *C. taiwanense* Y.H.Tseng & Chih Y.Chang **1** polar view **2** equatorial view **3** colporate view. Scale bar: 30 μm .

surfaces of both species are covered with dense cobwebby hairs. Therefore, herbarium specimens are often misidentified. Our field observations however suggest that the color of the corolla and the angle between the midrib and the lateral veins of the leaves are reliable characters for distinguishing the two species.

Pollen morphology

The pollen grains of Taiwanese species of subsect. *Australicirsium* are tricolporate, spheroidal and of medium size. The pollen grains of *C. taiwanense* (43.5–51.8 μm) and *C. arisanense* (42.9–53.0 μm) have a significantly ($p \leq 0.05$) larger diameter than those of *C. hosokawae* (36–46 μm) (Fig. 2B). *Cirsium arisanense* has significantly ($p \leq 0.05$) longer pollen spines (2.9–5.1 μm) (Fig. 2A) than *C. taiwanense* (2.8–3.6 μm), but these are not significantly ($p \leq 0.05$) longer than those of *C. hosokawae* (3.2–4.9 μm) (Fig. 2C, Table 2).

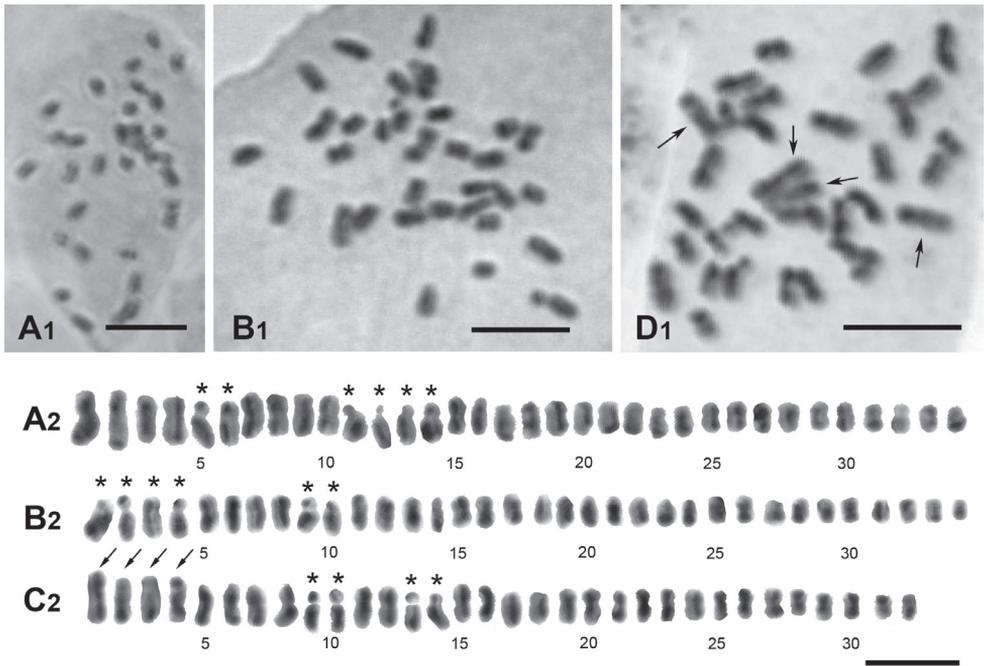


Figure 3. Karyotype of *Cirsium* Mill. subsect. *Australicirsium* Kitam **A** *C. arisanense* Kitam., $2n = 34$ **B** *C. hosokawae* Kitam., $2n = 34$ **C** *C. taiwanense* Y.H.Tseng & Chih Y.Chang, $2n = 32$ **1** cell **2** karyogram. *relatively clear satellites; arrow indicates chromosomes with secondary constriction. Scale bar: 5 μ m.

Chromosome cytology

The most common chromosome number of *Cirsium* species is $2n = 34$ (Hsu 1970; Funk et al. 2009; Chen and Yeh 2010a, 2010b). Our cytological investigation also shows that the chromosome numbers of both *C. arisanense* and *C. hosokawae* are $2n = 34$ (Fig. 3A, B). In contrast, the chromosome number of *C. taiwanense* is $2n = 32$ (Fig. 3C).

Although the chromosomes of the three taxa were too short to determine their karyotypic formula, satellites and secondary constrictions could be observed in longer chromosomes. Satellites were observed in *C. arisanense* for the 3rd, 6th and 7th pairs (Fig. 3A), in *C. hosokawae* for the 1st, 2nd and 5th pairs (Fig. 3B), and in *C. taiwanense* for the 5th and 7th pairs (Fig. 3C). In addition, only the 1st and 2nd pairs of *C. taiwanense* have secondary constrictions (arrows in Fig. 3C). Our results show that each taxon of subsect. *Australicirsium* in Taiwan has a clearly different karyotype (Fig. 3).

Distribution

Cirsium arisanense is the most common *Cirsium* species in high altitude regions in Taiwan (see distribution map in Chang et al. 2019). In comparison, *C. hosokawae* and *C. taiwanense* are less common and widespread. Both *C. hosokawae* and *C. taiwanense* occupy similar

habitats and altitudes, often occurring in open areas such as in wide roadsides and forest margins at 1400–3600 m a.s.l. However, the latitudinal distributions of the two species are different. *Cirsium hosokawae* and *C. taiwanense* are mainly found in the northwest inland region (Su 1985). However, *C. hosokawae* is absent from the central west inland region, whereas *C. taiwanense* is found less frequently near the western boundary of the north section of the east region. In general, the distribution of *C. taiwanense* is concentrated in the southwest and *C. hosokawae* is in the northeast of their combined distribution area (Fig. 4). The climate of the *C. hosokawae* habitat is usually more humid than that of *C. taiwanense*.

Discussion

The differences and the taxonomic status of the unknown *Cirsium*

Cirsium taiwanense has a unique combination of morphological characteristics: its corolla lobes are erect and yellow, and the abaxial surface of the leaves is densely covered with cobwebby hairs (Fig. 1C). Additionally, *C. taiwanense* has the largest pollen grains and shortest pollen spines of the three species of subsect. *Australicirsium* in Taiwan (Fig. 2, Table 2). The chromosome number of *C. taiwanense* is $2n = 32$ (Fig. 3C), which is different from the other known *Cirsium* species in Taiwan (Hsu 1970; Peng and Hsu 1978; Chen and Yeh 2010a, 2010b; Chang et al. 2019). Also, the 1st and 2nd pairs of chromosomes in its karyotype have secondary constrictions, which is different from other subsect. *Australicirsium* species (Fig. 3). Based on the above comparison, *C. taiwanense* is clearly different from other known congeners. We therefore here describe *C. taiwanense* as a new species.

Key to the 14 wild *Cirsium* taxa of Taiwan (modified from Chang et al. (2019), Chang and Tseng (2019), and Chang and Tseng (2020))

- 1 Biennial herb; leaves villose; involucre cylindrical or urceolate 2
- 2 Stem without wings; mature capitula nodding; involucre cylindrical.....
..... *C. ferum*
- 2* Stem with spiny wings; mature capitula erect; involucre urceolate.....
..... *C. vulgare*¹
- 1* Perennial herb; leaves glabrous, pubescent or densely cobwebby; involucre urceolate or cupuliform..... 3
- 3 All leaves cauline, basal rosette leaves absent..... 4
- 4 Leaves densely cobwebby on abaxial surface; mature capitula erect, involucre urceolate; apical parts of inner phyllaries inflated, obtuse; outer phyllaries lanceolate, apex acute without a spine; corolla lobes obviously longer than the inflated part of corolla tube..... *C. lineare*

1 naturalized species.

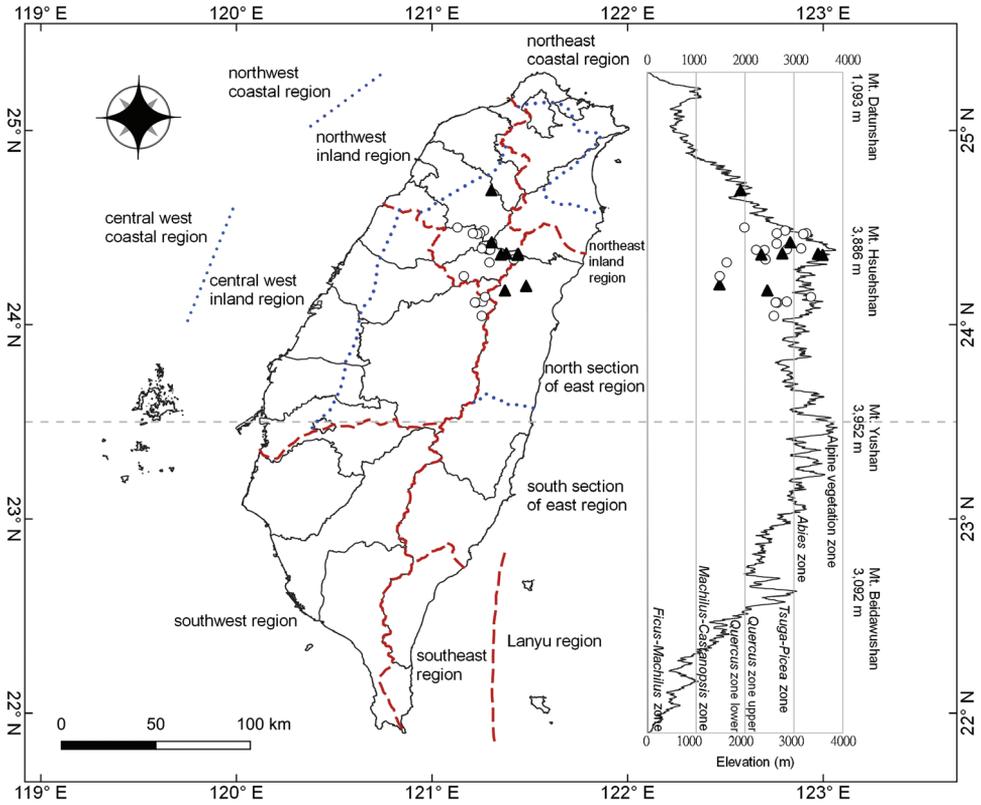


Figure 4. Distribution map of *Cirsium hosokawae* Kitam. (▲) and *C. taiwanense* Y.H.Tseng & Chih Y.Chang (○) in Taiwan. The red interrupted lines indicate major geographical climate regions as per Su (1985), and the blue dotted lines indicate sections within each climate region. The right part of the figures shows a side view of Taiwan and the vertical lines indicate altitudinal vegetation zones as per Su (1984).

- 4* Leaves glabrous on both surfaces; mature capitula nodding, involucre cupuliform; apical parts of inner phyllaries acute or acuminate; outer phyllaries elliptical with a long spine at the apex; corolla lobes as long as the inflated part of corolla tube..... 5
- 5 Corollas white; leaves pinnatisect or bipinnatisect, lobes > 1.5 cm wide.....
..... *C. kawakamii*
- 5* Corollas purple; leaves mainly pinnatisect, lobes < 1.2 cm wide.....
..... *C. tatakaense*
- 3* Leaves in both a basal rosette and cauline..... 6
- 6 Reflexed part of the phyllaries blade-like; corolla lobes as long as the inflated part of corolla tube 7
- 7 Corollas white or light purple 8
- 8 Corollas white; inner and outer phyllaries similar in length; stems cauline, without rhizome *C. brevicaule*

- 8* Corollas light purple; inner and outer phyllaries distinctly different in length; stems both cauline and rhizomatous *C. morii*
- 7* Corollas yellow or vivid purplish red..... 9
- 9 Abaxial leaf surface without cobwebby indumentum; mature capitula erect or nodding; corolla lobes revolute *C. arisanense*
- 9* Abaxial leaf surface densely cobwebby; mature capitula nodding; corolla lobes erect..... 10
- 10 Corolla vivid purplish red; angle between midvein and lateral veins of leaf (60–)82–90° *C. hosokawae*
- 10* Corolla yellow; angle between midvein and lateral veins of leaf (44–)52–73° *C. taiwanense*
- 6* Reflexed part of the phyllaries spine-like; corolla lobes shorter than the inflated part of corolla tube..... 11
- 11 Abaxial leaf surface densely cobwebby; mature capitula nodding... *C. suzukii*
- 11* Leaf surfaces pubescent, but not cobwebby; mature capitula erect..... 12
- 12 Corolla white; leaves glabrescent *C. japonicum* var. *takaoense*
- 12* Corolla purple; leaves villose, not glabrescent..... 13
- 13 Apical spines of phyllaries shorter than 2 mm; leaves pinnatifid to pinnatisect *C. japonicum* var. *australe*
- 13* Apical spines of phyllaries longer than 3 mm; leaves pinnatipartite to pinnatisect *C. japonicum* var. *fukienense*

Taxonomic treatment

Cirsium taiwanense Y.H.Tseng & Chih Y.Chang, sp. nov.

urn:lsid:ipni.org:names:77220552-1

Figures 1C, 2C, 3C, 5, 6

Diagnosis. Differs from *C. hosokawae* in having a yellow corolla (vs. vivid purplish red corolla), a narrower angle between the midrib and lateral veins of the cauline leaves ((44–)52–73° vs. 82–90°), and usually more florets (101–135(–194) vs. 54–111) and phyllaries (90–127 vs. 68–109) per capitulum. Differs from *C. arisanense* by its nodding mature capitula (vs. erect), erect corolla lobes (vs. revolute), and a densely cobwebby abaxial leaf surface (vs. without cobwebby indumentum).

Type. Taiwan. Nantou County, Ren'ai Township, Provincial Rd. No. 14A 21 km, 2605 m alt., 24.11438°N, 121.21821°E, 15 July 2020. *C. Y. Chang* 2976 (holotype: TCF; isotype: TNM, PPI).

Description. Perennial herbs, stems 0.5–1.0 m tall, internodes terete. Leaves pinnatipartite or pinnatisect, space between pinnae V-shaped, adaxial surface puberulent or cobwebby, abaxial surface densely cobwebby, margin spinose; rosette leaves narrowly elliptic to oblanceolate, base cuneate to attenuate, apex narrowly acute, 19.5–34.1 × 4.1–7.4 cm, angle between the midrib and the lateral veins (40–)55–76°; pinnae 7–11 pairs, 0.9–2.6 × 1.2–2.1 mm, space between pinnae 0.4–1.2 cm, petiole 1.5–4.0 cm; cauline

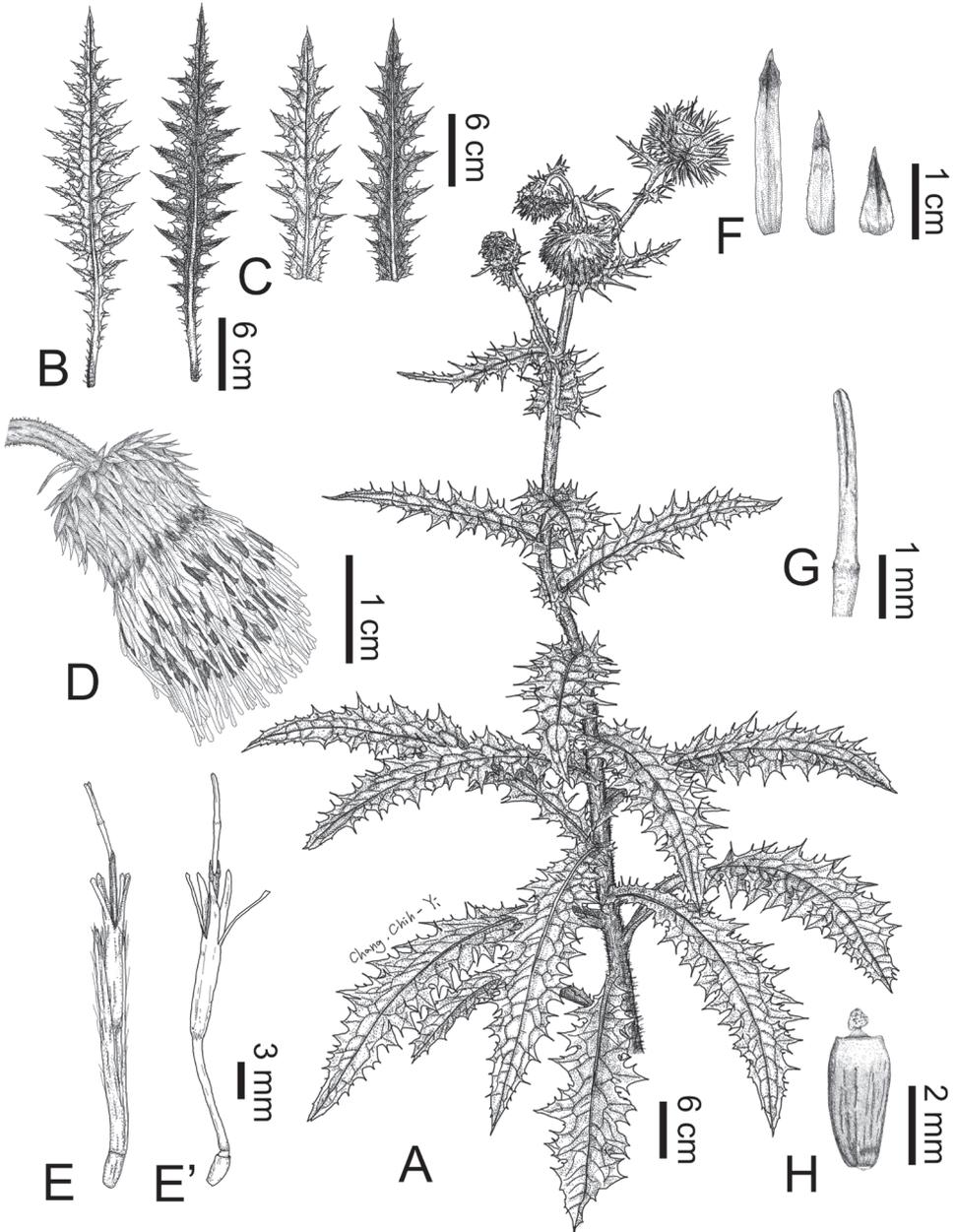


Figure 5. Line drawings of *Cirsium taiwanense* Y.H.Tseng & Chih Y.Chang **A** habit **B** rosette leaf **C** cauline leaf **D** capitulum **E** floret **E'** floret (pappus removed) **F** phyllaries **G** style branches **H** achene.

leaves narrowly elliptic to narrowly triangular, base cordate, apex narrowly acute, 9.0–25.5 × 2.2–6.9 cm, angle between the midrib and the lateral veins (44–)52–73°; pinnae 5–8 pairs, 1.3–2.1 × 0.8–1.9 cm, space between pinnae 0.6–1.5 cm, sessile. Capitula

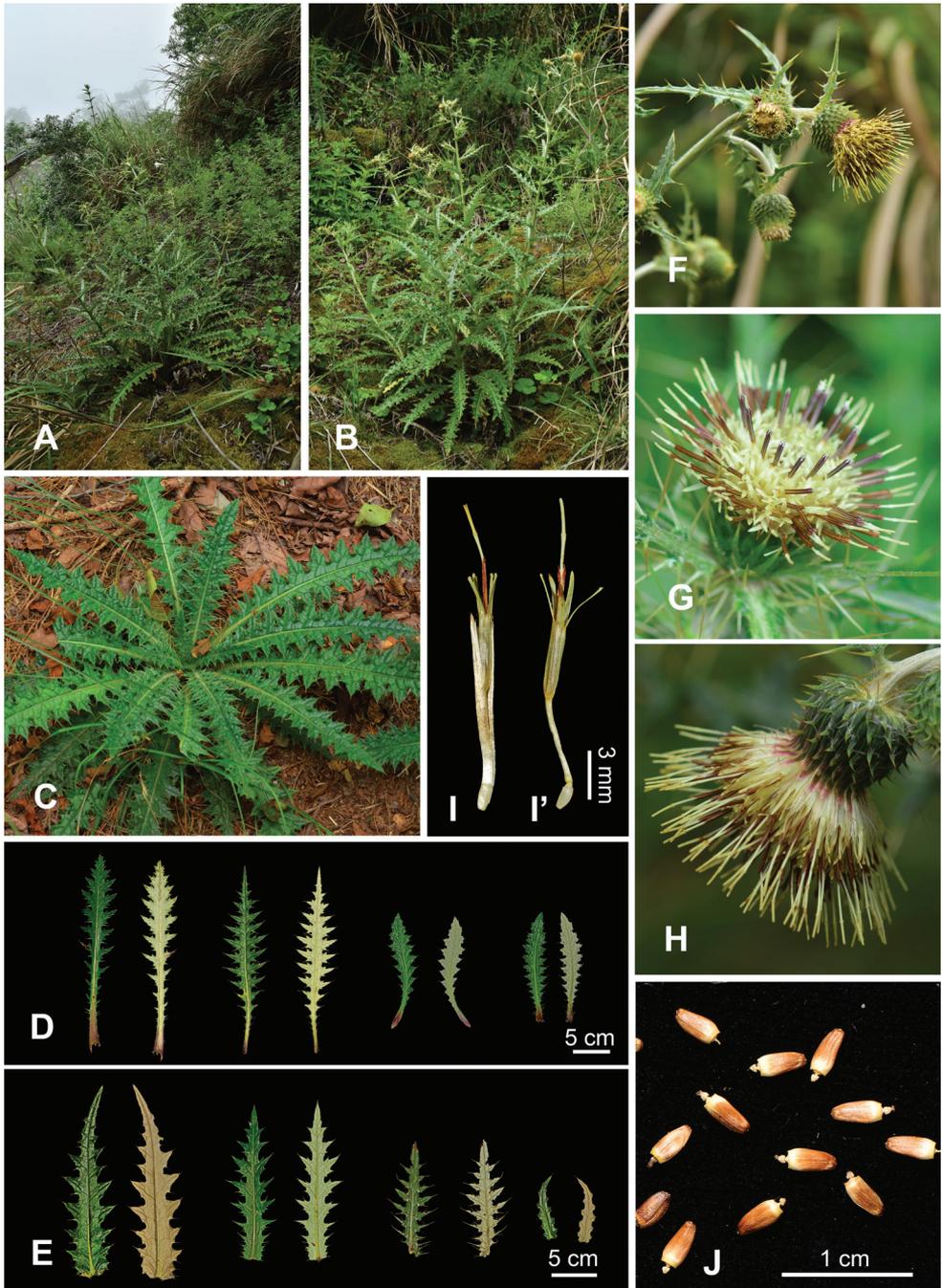


Figure 6. *Cirsium taiwanense* Y.H.Tseng & Chih Y.Chang **A** habitat **B** habit **C** basal rosette **D** variations of rosette leaves **E** variations of cauline leaves **F** inflorescences **G, H** capitulum **I, I'** floret (pappus removed) **J** achenes.

solitary or 2–6 arranged into racemes or panicles, mature capitula nodding. Involucre urceolate, more or less cobwebby, 2.8–3.4 × 1.4–1.9 cm; phyllaries 90–127, in 5–7 series, apex acute, midrib distinct, (0.3–)0.5–1.4 × 1.5–2.1 mm, length ratio of inner and outer phyllaries 2.2–3.6, the reflexed part of the phyllaries 1.5–3.1 mm long. Receptacle flat, densely bristly. Florets 101–135(–194), 2.2–3.1 cm long, with yellow corolla, corolla lobes 5, linear, erect, 3.0–5.1 × 0.4–0.7 mm, corolla tube fistulose with 2 sections, the inflated section of corolla tube 4.1–5.8 mm long; synantherous stamens 5, anthers brown, 5.9–8.3 mm long, base with caudate extensions, filaments 3.0–4.5 mm long with irregular protuberances; stigmas bifid, styles 2.0–2.5 cm long, style arm 2.6–2.7 mm long, ovaries (1.6–)3.2–4.1 mm long. Achenes oblong, base acute, apex truncate, beige, 3.5–4.5 × 1.5–1.8 mm, compressed, 4-angled, ribbed, beak heart-shaped; pappus copious, plumose, bristles in many series, 1.55–1.65 cm long, forming basal ring, easily shed.

Phenology. Flowering between June and October and fruiting between July and November.

Distribution and habitat. Endemic species of Taiwan. *Cirsium taiwanense* grows in open areas between *Quercus* forest and *Abies* forest at 1400–3400 m a.s.l. in central-northern Taiwan. *Cirsium taiwanense* usually grows at sunny sites. Common companion species are *Artemisia morrisonensis* Hayata (Compositae), *Lilium formosanum* Wallace (Liliaceae), *Salix fulvopubescens* Hayata (Salicaceae) and *Rubus pectinellus* Maxim. (Rosaceae).

Chinese name. Tai-wan-ji (臺灣薊).

Chromosome number. $2n = 32$ (Fig. 3B).

Palynology. Pollen grains are tricolporate, spheroidal, micro-reticulate and $46.6\text{--}51.8 \times 43.5\text{--}50.7 \mu\text{m}$ (P/E ratio: 0.9–1.1). The surface is densely covered with spines that are 2.8–3.6 μm long and 3.9–6.0 μm wide at the base. The distance between spines is 8.2–11.8 μm (Fig. 2C).

Conservation status. *Cirsium taiwanense* is common in north-central Taiwan (Fig. 4). The populations often grow in high mountain areas and experience limited disturbance by humans. Following the International Union for Conservation of Nature (IUCN) Categories and Criteria (IUCN 2019), we regard this species as of Least Concern (LC).

Additional specimens examined. TAIWAN. Hsingchu County, Jianshi Township, Mt. Itsashan, 7 Sept. 1993. C. L. Huang 78 (HAST!). Miaoli County, Taian Township, en route from 99 lodge to Mt. Tapachienshan, 11 Aug. 1985. C. I Peng 8492, 8542, 8543, 8544, 8545, 8546 (HAST!); same loc., 2 Nov. 1996. C. M. Wang 2384 (TNM!); Taian, 13 Sept. 1996. C. H. Chen 1870 (TAIE!); 99 Lodge, 2780 m alt., 2 Nov. 1996. C. M. Wang 2384 (HAST!); Mt. Hsishihshan, 27 Oct. 1976. B. P. Yang 81 (TAIF!). Taichung City, Heping District, Mt. Nanhutashan, 21 Sept. 1969. T. Yamazaki 281 (TI!); Yunleng cabin to Mt. Duojiatunshan, 26 June 1994. C. M. Wang 1026 (TNM!); en route from entrance to Yunleng cabin, 27 July 2019. C. Y. Chang 2478 (TCF); Derji, 3 July 1974. C. I Peng 15 (TAI!); en route from entrance to Chika Lodge, 1 June 2003. C. M. Wang 6908 (TNM!); same loc., 19 June 2009. C. M. Wang 13025 (TNM!); same loc., 20 Jun. 2011. C. I Huang 5273 (HAST!); same loc., 16 July 2009. Y. H. Tseng 4697

(TCF); same loc., 9 Feb. 2021. *C. Y. Chang* 3269 (TCF); en route from Chika lodge to Mt. Syue east peak, 7 Aug. 1986. *C. I Peng* 9660 (HAST!); same loc., 11 Sept. 2002. *C. I Huang* 1238 (HAST!; TNM!); same loc., 21 June 2011. *C. I Huang* 5290 (HAST!); en route from Chika lodge to 369 Lodge, 15 June 1985. *C. I Peng* 7887 (HAST!); behind 369 Lodge, margin of *Abies* forest, 9 Sept. 2009. *C. T. Chao* 922 (TCF!); en route from Wuling lodge to Mt. Tao, 24 Aug. 1988. *C. I Peng* 12012, 12020, 12100 (HAST!); same loc., 22 July 2018. *C. Y. Chang* 2133 (TCF!); en route from Wuling lodge to Taoshan waterfall, 24 Aug. 1988. *C. I Peng* 12097 (HAST!); Huanshan, 6 July 2006. *Z. H. Chen* 186 (TAIF!). Nantou County, Ren'ai Township, Guandaoxi, 22 Oct. 1932. *S. Sasao* s. n. (CHIA!); Hsinjenkang, Provincial Rd. No. 14A 21 km, 8 Sept. 1997. *S. H. Wu* 384 (HAST!); Provincial Rd. No. 14A 22 km, 3 Aug. 2011. *T. W. Hsu* 17059 (TAIE!); Mt. Hohuanshan near Yuanfeng, 13 July 1985. *C. I Peng* 8327 (HAST!); Nenggao Cross-ridge Historic Trail, 28 Feb. 2006. *M. J. Chung* x22805 (TAIF!); same loc., 31 Jan. 2018. *C. Y. Chang* 1609 (TNM); Provincial Rd. No. 14A 21.5 km, 2 Oct. 2015. *C. Y. Chang* 772 (TNM); same loc., 27 Oct. 2015. *C. Y. Chang* 839 (TNM).

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Nomenclatural notes and typification of nine names related to *Jasminum* (Oleaceae)

Bui Hong Quang^{1,2}, Ritesh Kumar Choudhary³, Joongku Lee⁴

1 Department of Botany, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, 100000, Vietnam **2** Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, 100000, Vietnam **3** Biodiversity & Palaeobiology Group, Agharkar Research Institute, G.G. Agarkar Road, Pune, Maharashtra, 411004, India **4** Department of Environment and Forest Resources, Chungnam National University, Yuseong-gu, Daejeon 34134, South Korea

Corresponding author: Joongku Lee (joongku@cnu.ac.kr)

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Abstract

Lectotypes are designated here for the following nine validly published names: *Jasminum alongense*, *J. anodontum*, *J. eberhardtii*, *J. harmandianum*, *J. lang*, *J. laxiflorum*, *J. pierreanum*, *J. rufobirtum*, and *J. sinense*. *Jasminum lang* is reinstated as a distinct species.

Keywords

Jasmine, ICN, Indochina, Nomenclature

Introduction

Jasminum L. is a genus of Oleaceae and contains about 200 species distributed widely in tropical and subtropical Asia and the Pacific Islands (Chang et al. 1996; Govaerts and Green 2021). There are 11 species and 2 subspecies of *Jasminum* in Cambodia (Cho et al. 2016), 14 species, 1 variety (1 sp. endemic) in Laos (Green 1999; Newman et al. 2007; Jin et al. 2016), and 40 species, 4 subspecies, and 1 variety (6 spp. endemic) in Vietnam (Ho 2000; Tran 2003; Bui et al. 2016, 2019; Bui 2020).

During our study on the genus *Jasminum* in Indo-China, we could not find holotypes related to nine *Jasminum* names viz. *Jasminum alongense*, *J. anodontum*, *J. eberhardtii*, *J. harmandianum*, *J. laxiflorum*, *J. pierreanum*, *J. rufohirtum*, *J. lang*, and *J. sinense*. Therefore, we have designated lectotypes for these names following Art. 9.3, and 9.11 of the Shenzhen Code (Turland et al. 2018).

Materials and methods

The following herbaria were consulted either virtually or in-person to trace the type specimens: A, BM, E, HN, IBK, IBSC, K, KUN, LE, MW, NY, P, PE, S, SING, SN, US VNM, and WU (acronyms followed Thiers 2020). Images available on JSTOR Global Plants (<https://plants.jstor.org/>), Tropicos, International Plant Names Index (IPNI), and Plants of the World Online (POWO) along with relevant monographs and Floras (Gagnepain 1933a, b; Kobuski 1939, 1959; Chang et al. 1996; Green 1993, 1995, 2000, 2003, 2004) were also scrutinized.

Typification of the names

Gagnepain in Flore Générale de L'Indochine. (Vol. 3), which was published in March 1933, documented 31 *Jasminum* species from the Indochina region and described 11 of them as new to science (1933a). In the same year, he described 13 new *Jasminum* species in Bulletin de la Societe Botanique de France [Vol. 80 (1)] including those 11 species which were already described by him (Gagnepain 1933a; Gagnepain 1933b). To confirm the publication date of the later publication, we contacted Dr. K.N. Gandhi, Sr. Nomenclature Registrar, Harvard University (*Pers. comm.*), who further communicated with the Harvard Botany librarian Ms. Gretchen Wade. The title page of Bulletin Soc. Bot. France vol. 80(1) was found, revealing “The proof for this issue was made May 23, 1933” (English translation). Therefore, we believe the priority of publication goes with Fl. Indo-Chine (Gagnepain 1933a), published in March 1933, and the *Jasminum* names are treated here accordingly.

1. *Jasminum alongense* Gagnep., Fl. Indo-Chine [P.H. Lecomte et al.] 3: 1052 (1933a); Bull. Soc. Bot. France 80: 73 (1933b)

Lectotype (here designated).—TONKIN [VIETNAM]. baie d'Along, île aux Biches, île près de Hongay, 8 November 1911, *H. Lecomte et A. Finet 840*, P00087775 (P, image!); Isolectotypes: P00087776 (P, image!); [P00087775, P00087776 (K, image!; without any K Barcode)].

Nomenclatural notes. While describing *Jasminum alongense*, Gagnepain (1933a) referred to the specimens collected by H. Lecomte and A. Finet from Tonkin (presently in North Vietnam) Hongay (Hon Gai/ present-day Ha Long Bay). Several duplicates

of the same were submitted at P. However, some of them were sent to K, evident from the label on these collections. We could locate two specimens at P (P00087775 and P00087776), and four at K. All K specimens are originally from P, pasted on new sheets and without any K barcode. Two of them, however, possess the preexisting P barcodes P00087775 and P00087776 but the remaining two do not have any). Specimens P00087775 and P00087776 at P are annotated as holotype and isotype respectively, however, they were never published effectively. A thorough survey of literature and virtual herbaria revealed that earlier workers (Ho 2000; Tran 2003) did not designate a lectotype for the name *J. alongense*. Therefore, following Art. 9.6 of the Shenzhen Code (Turland et al. 2018), P00087775 at P is being designated here as the lectotype of *J. alongense*. This specimen not only best matches with the original description but also possesses the reproductive parts and original drawing of the dissected floral parts.

Ecology and phenology. Grows on the Limestone Mountains of the islands of Ha Long Bay. Flowering in November – December, fruiting in March – April.

Distribution. VIETNAM, Endemic to Quang Ninh (Ha Long Bay, Hon Gai) Province.

2. *Jasminum anodontum* Gagnep., Fl. Indo-Chine [Lecomte et al.] 3: 1040 (Fig. 118) (1933a); Bull. Soc. Bot. France 80: 73 (1933b)

Lectotype (here designated):–VIETNAM. Cochinchine, Bien Hoa, Bruossailles [refers to scrub vegetation], Dong Nai Province, *C. Thorel 949*, P00644260 (P, image!); isolecotypes: P00644261, P00644262 (P, images!).

Syntypes. ANNAM [VIETNAM]. Ca-na, Phanrang, 500 m.a.s.l., [Ninh Thuận Province], 12 March 1923, *E. Poilane 9040*, P04046604 (P, image!); COCHINCHINE [VIETNAM]. Bien Hoa, [Dong Nai Province], *C. Thorel 943*, A00105304, A00105305 (A, images!).

Nomenclatural notes. In the protologue of *Jasminum anodontum*, Gagnepain (1933a) referred to two gatherings without citing any field number. The first referred to the collections made by E. Poilane from Ca-na, Phanrang (presently Ninh Thuận) in Annam (present-day Vietnam). Whereas the second referred to the collections made by C. Thorel at Bien-hoa, (Dong Nai Province) from Cochinchine (presently in South Vietnam). However, Gagnepain (1933b), in his other publication, only cited the specimens collected by Thorel viz. *C. Thorel 943* as the original material. We could find this specimen in duplicate at A (A00105304, A00105305), indicated as isosyntypes by P.S. Green. We found some other of Thorel's specimens P00644260, P00644261, P00644262 (all bearing the same number – *C. Thorel 949*) at P. One of them (P00644260) has the original drawings of the dissected floral parts by Gagnepain. A critical survey of literature and herbarium specimens revealed that earlier workers (Green 2000, 2003; Ho 2000; Tran 2003) did not designate lectotype for *J. anodontum*. Among all of them, we found P00644260 to represent the species best as per the protologue and, therefore, we designate this specimen here as the lectotype of *J. anodontum* following Arts. 9.3 and 9.11 of the Shenzhen Code

(Turland et al. 2018). Thorel's specimens at A are either incomplete or in fruiting, not representing all characters as per the protologue. Therefore, they are designated here as syntypes.

Ecology and phenology. Forest along the roadside, often near the water source. Flowering in January–February, fruiting in March–April.

Distribution. THAILAND, VIETNAM, (Dak Lak (Dak Mil, Dak Minh), Ninh Thuan (Thuan Nam), Binh Thuan (Phan Thiet), Dong Nai (Bien Hoa City, Vinh Cuu Dong Nai Nature-Culture Reserve). Provinces.

3. *Jasminum eberhardtii* Gagnep., Fl. Indo-Chine [Lecomte et al.] 3: 1051, 1047 (1933a), (Fig.119); Bull. Soc. Bot. France 80: 73 (1933b)

Lectotype (here designated):– VIETNAM. Hoa-Binh Province, Mai-Ha, P.A. *Eberhardt 4323*, P00644271 (P, image!); Isolectotype: P00644273 (P, image!).

Nomenclatural notes. *Jasminum eberhardtii* was described (Gagnepain 1933a,b) based on the collections made by P.A. Eberhardt (Coll. no. 4323) from Hoa-Binh Province in northern Vietnam. While searching for the original materials of *J. eberhardtii*, we could locate two specimens P00644271 and P00644273, matching the original description (Art. 9.6 of ICN, Turland et al. 2018), but neither of them was designated as a lectotype (Ho 2000; Tran 2003). Therefore, we selected P00644271, as a lectotype following Arts. 9.3 and 9.11 of ICN (Turland et al. 2018) as it best represents the species. The other specimen (P00644273) is designated as isolectotype.

Ecology and phenology. Grows in the forest between 300–1200 m.a.s.l. Flowering in April–May, fruiting in June–July.

Distribution. VIETNAM. Endemic to Hoa Binh (Mai Chau, Mai Ha), and Lao Cai Provinces.

4. *Jasminum harmandianum* Gagnep., Fl. Indo-Chine [Lecomte et al.] 3: 1045, 1047 (Fig. 119) (1933a); Bull. Soc. Bot. France 80: 74 (1933b)

Lectotype (here designated):– LAOS. *M. Massie s.n.* P00644289. (P, image!); Isolectotype: A00063064 (A, image!).

Syntypes. CAMBODIA. Expédition du Mékong, Bassac, 1866, *C. Thorel s.n.*, P00644288 (P, image!); K000901473 (K, image!). VIETNAM. delta du Mékong: Núi Cam, 01 June 1876, *F.J. Harmand 633*, A00063063 (A, image!); P00644290, P00644291, P00644292 (P, images!).

Nomenclatural notes. *Jasminum harmandianum* was described (Gagnepain 1933a,b) on the basis of three gatherings collected (*Massie s.n.*, *C. Thorel s.n.*, *F.J. Harmand 633*) from Laos, Cambodia (Expédition du Mékong, Bassac) and Vietnam. We found all these elements deposited in P and A, some of them with two duplicates. A survey of literature and virtual herbaria revealed that earlier workers (Green 2000;

Ho 2000; Tran 2003) had not designated a lectotype for this species. The specimen P00644289 at P collected by Massie represents species best and has a complete original label. Therefore, it was selected as a lectotype for *J. harmandianum* following Art. 7.11 of ICN (Turland et al. 2018). However, we found a duplicate of the same at A having barcode A00063064 which was distributed from P, as evident from its label. It is designated here as an isolectotype.

Ecology and phenology. Grows in the forest at 100–1000 m.a.s.l. Flowering in June – July, fruiting in August – September.

Distribution. CAMBODIA, LAOS, THAILAND and VIETNAM. Dak Lak (Dak Mil), Kon Tum (Chu Mon Ray National Park), and An Giang (Nui Cam) Provinces.

5. *Jasminum lang* Gagnep., Fl. Indo-Chine [Lecomte et al.]. 3: 1046 (1933a); Bull. Soc. Bot. France 80: 77 (1933b)

Lectotype (here designated):– VIETNAM. Kiên Khê, in collib. Dong Bau, (Ha Nam Province), 31 March 1885, *H.F. Bon 2869*, P00640614 (P, image!); Isolectotype: K000901460 (K, image!).

Syntypes. VIETNAM. Kiên Khê, in collib. Dong Bau, (Ha Nam Province), 19 March 1884, *H.F. Bon 2604*, P00640615 (P, image!); Presqu'île de Nui-han-heo (Hon Heo mountain, currently in Ninh Hoa Town, Khanh Hoa Province), 12 June 1933, *Poilane 6867* (K, image!), VNM00021039 (VNM, image!).

Nomenclatural notes. *Jasminum lang* was described (Gagnepain 1933a,b) based on three gatherings (*H.F. Bon 2604*, *2869* and *Poilane 6867*) collected from north, and south Vietnam. We found two duplicates matching the detailed description and specimen label information. A survey of literature and multiple herbaria revealed that a lectotype for *J. lang* had not been designated in earlier studies (Ho 2000; Tran 2003). We, therefore, selected the P specimen (P0040614) as a lectotype for *Jasminum lang* following Arts. 9.3 and 9.11 of ICN (Turland et al. 2018) as it represents the species best. The other specimen at K (K000901460) is designated here as isolectotype.

Taxonomic notes. *Jasminum lang* is known from China and Vietnam (Gagnepain 1933a,b; Chia 1952; Miao 1984; Chang et al. 1996; Ho 2000). During the course of our revisionary work, we critically compared the morphological characters of *Jasminum lang* with *Jasminum coffeinum* Hand.-Mazz. because of their morphological differences and their treatment as synonyms (Chang et al. 1996; Green 2006). Our field observation and study of the multiple specimens housed in various national and international herbaria made us realize that the species are morphologically different. Indeed, the characters used for their delimitation (Handel and Heinrich 1925; Gagnepain 1933a,b; Chia 1952; Miao 1984; Chang et al. 1996; Ho 2000), as indicated in Table 1, clearly indicate that they are different entities. Therefore, we reinstate *J. lang* Gagnep., as a distinct species.

Ecology and phenology. Grows in the forest at altitude 100–800 m.a.s.l. Flowering in February – April, fruiting May – June.

Table 1. Morphological differences between *Jasminum lang* and *J. coffeinum*.

Morphological characters	<i>Jasminum lang</i>	<i>Jasminum coffeinum</i>
Branchlets	terete or compressed	terete or 4-angled, narrowly winged
Number of secondary veins in leaf	7–11	5–9
Petiole length	0.8–2.5 cm long	1–2 cm long
Inflorescence	solitary, terminal or axillary 2–6 flowered	subopposite or fascicled in leaf axils, 3–10 flowered
Bracts	linear, 5–11 mm	ovate or spatulate, 2–5 mm
Calyx	glabrous, tube 2.8–4 mm; lobes 6–8, linear, 0.5–1.8 cm, enlarged to 2–3 cm in fruit	puberulent, tube ca. 4 mm; lobes 5, narrowly deltate, 1–2 mm, not enlarged in fruit
Corolla	white, pink outside, lobes narrowly lanceolate, 2–2.5 cm	white, lobes lanceolate, 1–1.2 cm

Distribution. VIETNAM. Endemic to Hoa Binh (Mai Chau), Ha Nam (Kien Khe), Quang Ninh (Ha Long bay), and Khanh Hoa (Hon Heo, Hon Ba Nature Reserve) Provinces.

6. *Jasminum laxiflorum* Gagnep., Fl. Indo-Chine [Lecomte et al.] 3: 1055 (1933a); Bull. Soc. Bot. France 80: 75 (1933b)

Lectotype (here designated):– VIETNAM. Austro-Cochinchine, Tanh-huyen, Thu-duc, Baria, Bien-hoa (Dong Nai Province), 01 December 1868, *L. Pierre 327*, P03868745 (P, image!); Isolectotypes: A00105308, A00105309, A00105310, A00105313 (A, images!); BM000997658 (BM, image!); P03868746, P03868747, P03868750 (P, images!).

Syntypes. VIETNAM. *C. Thorel 862*, A00105593 (A, image!); P03868756, P03868755 (P, images!); S09–37061 (S, image!); Phu-mi, 1875 *Godefroy s.n.* P03868749 (P, image!); 26 January 1903, Phu-mi, *D.G.J.M. Bois 2180*, A00105307 (A, image!); Phu-mi, P03868743 (P, image!); Bien-hoa à Xuan-loc (Dong Nai Province), 12 January 1914, *A. Chevalier 29979*, P03868744 (P, image!); Nui dinh pro de Baria (Ba Ria-Vung Tau Province), 100 m, 27 October 1919, *E. Poilane 671*, A00105311 (A, image!), P03868753, (P, image!); environs de Saigon (Ho Chi Minh city), 28 January 1926, *F. Evrard 2618*, coll. *Khai 161*, P03868742 (P, image!); km. 80.500 de la route col. N°20 pro: du Haut Donai délégation de Djyrinh (Dinh Linh, Lam Dong Province), 19 October 1931, *E. Poilane 19787*, P03868752 (P, image!); A00105312, (A, image!).

Nomenclatural notes. *Jasminum laxiflorum* was described (Gagnepain 1933a,b) based on seven gatherings (*E. Poilane 19787*, *F. Evrard 2618*, coll. *Khai 161*, *C. Thorel 862*, *L. Pierre 327*, *A. Chevalier 29979*, and *Godefroy s.n.*) collected from South Vietnam. None of them was designated as a holotype. We found nine specimens matching the original description and information given on the labels which are included in the protologue, and several duplicates. A survey of the literature (Ho 2000; Tran 2003) and multiple herbaria revealed that a lectotype for *J. laxiflorum* has not yet been designated. Therefore, *L. Pierre 327*, the specimen P03868745 at P, which best represents the species, is designated here as a lectotype following Arts. 9.3 and 9.11 of ICN (Turland et

al. 2018). Other specimens at A, P, and BM (A: A00105308, A00105309, A00105310, A00105313; BM: BM000997658; P: P03868746, P03868747, P03868750) are designated here as isolectotypes.

Ecology and phenology. Grows in the forest at 100–500 m.a.s.l. Flowering in February –March, fruiting April – May.

Distribution. VIETNAM. Endemic to Dak Lak (Dak Mil), Kon Tum (Chu Mon Ray National Park), Lam Dong (Di Linh), Dong Nai (Bien Hoa, Trang Bom) Ba Ria-Vung Tau (Ba Ria nui dinh), and Ho Chi Minh City (Thu Duc) Provinces.

7. *Jasminum pierreanum* Gagnep., Fl. Indo-Chine [Lecomte et al.] 3: 1042 (1933a); Bull. Soc. Bot. France 80: 76 (1933b)

≡ *Jasminum rarum* Kerr, Bull. Misc. Inform. Kew 28 (1938). Type: Thailand. South-western, Kanchanaburi, Bau Ke, Thailand, Kanchanburi Bau Ke, 21 July 1926, *Put. N.211* (holotype: K000901476 (K, image!); isotypes: E00284807 (E, image!), L0005365 (L, image!), P00644299 (P, image!).

≡ *Jasminum cordatulum* (Merr. & Chun ex L.C.Chia) L.C.Chia, Fl. Hainan. 3: 576 (1974). Type: China. Hainan Province, 8 September 1933, *Liang Xiangri 62960* (holotype: IBK IBK00191326 (IBK, image!); isotypes: IBK00191326 (IBK, image!), PE00027990 (PE, image!).

≡ *Jasminum seguinii* var. *cordatulum* Merr. & Chun ex L.C.Chia, Acta Phytotax. Sin. 2: 43 (1952). Type: China. Hainan, 26 August 1933, *Liang Xiangri 62823* (holotype: IBK00093762 (IBK, image!); isotypes: IBSC0459241 (IBSC, image!), PE01489419 (PE, image!), SN005501 (SN, image!).

Lectotype (here designated):– VIETNAM (Cochinchine). Prope Chiao Khan ar originum fluvii Dongnai septentrione Prov. Bien hoa “Bois Hua”, March 1877, *L. Pierre 2828*, P00640601 (P, image!); Isolectotypes: P00640600 (P, image!), VNM00021049 (VNM!).

Syntype. CAMBODIA. monts Cherreer, June 1870, *L. Pierre s.n.*, P00644300 (P, image!).

Nomenclatural notes. *Jasminum pierreanum* was described (Gagnepain 1933a,b) based on two gatherings (*L. Pierre 2828* and *L. Pierre s.n.*) collected from Cambodia and Vietnam. We found three duplicates matching the detailed description and specimen label information included in the protologue. A survey of literature and virtual herbaria revealed that a lectotype for *J. laxiflorum* has not been designated in earlier studies (Green 2000, 2003; Ho 2000; Tran 2003). We, therefore, selected the P specimen (P00640601) as a lectotype which best matched the description, following Arts. 9.3 and 9.11 of ICN (Turland et al. 2018). Other specimens at P and VNM (P00640600 and VNM00021049) are designated as isolectotypes.

Ecology and phenology. Grows in the forest at high altitude 300–1000 m.a.s.l. Flowering in August – September, fruiting in January – February.

Distribution. CAMBODIA, CHINA, THAILAND and VIETNAM. Hoa Binh (Mai Chau), Thua Thien-Hue (Phu Loc), Gia Lai (Kbang), Dak Lak (Dak Mil), and Dong Nai (Bien Hoa) Provinces.

8. *Jasminum rufobirtum* Gagnep., Fl. Indo-Chine [Lecomte et al.]. 3: 1057 (1933a); Bull. Soc. Bot. France 80: 77 (1933b)

≡ *Jasminum yunnanense* Z.P.Jien ex P.Y.Pai, Acta Bot. Yunnan. 5: 66 (1983). Type: China. Yunnan, 18 April 1956, *Sino-Soviet Yunnan Joint Investigation Group* 955 (holotype: KUN0548902 (KUN, image!; isotypes: PE00027944, PE01501575, PE01501578 (PE, images!, IBSC0460998 (IBSC, image!)).

Lectotype (here designated):– VIETNAM (Tonkin). Province de Sonla, Canton de Muong Mua Chan De Mai Son, 540 m, 26 May 1927, *Pételot* 5032, P00644294 (P, image!); Isolectotypes: P00644295 (P, image!), NY00297220 (NY, image!).

Syntype. LAOS. s.loc. *H. d'Orléans s.n.*, P00644296 (P, image!).

Nomenclatural notes. *Jasminum rufobirtum* was described (Gagnepain (1933a,b) based on two gatherings (*Pételot* 5032 and *H. d'Orléans s.n.*) collected by Pételot and H. d'Orléans from north Vietnam and Laos respectively. While tracing the holotype, we found two duplicates matching the detailed description and specimen label information which are included in the protologue. A survey of literature and virtual herbaria revealed that a lectotype for *J. rufobirtum* has not been designated by the earlier workers (Miao 1992; Chang et al. 1996; Ho 2000; Tran 2003; Prachaya et al. 2004). We selected the P specimen P00644294 as a lectotype following Arts. 9.3 and 9.11 of ICN (Turland et al. 2018) as it represents the species best. The other specimens at P, and NY, are designated here as isolectotypes.

Ecology and phenology. Grows in the forest at altitude 200–1000 m.a.s.l. Flowering in April – May, fruiting May – July.

Distribution. CHINA, LAOS, THAILAND and VIETNAM. Son La (Muong Ma), Dien Bien (Muong Cha, Ta Chua), and Lao Cai (Bat Xat Nature Reserve) Provinces.

9. *Jasminum sinense* Hemsl., J. Linn. Soc., Bot. 26: 80 (1889)

≡ *Jasminum bodinieri* H.Lév.Repert. Spec. Nov. Regni Veg. 13: 151 (1914). Type: China. Environs de Tsin-Gay, Gan-Pin./Item envir. de Tou-Chan. Juillet 97 J. Cavalerie, 15 July 1897, *E.M.Bodinier & J.P.Laborde* 1890, Type: (syntype: E00284832 (E, image!). Environs de Kouy-yang, Mont du College, 11 September 1898, *Bodinier E.M., 1890* (syntype: E00284833 (E, image!).

≡ *Jasminum sinense* var. *septentrionale* Hand.-Mazz., Symb. Sin. 7: 1012 (1936). Type: China. Yunnan: Prov. Yunnan bor.-occid.: In silva frondosa subtropica inter vicos Tjiontson et Pipiti ad fluvium Lu-djiang (Salween) infra Tschamutong. 1700 m.

asl., 17 August 1916, *Handel-Mazzetti, H.R.E. von, Handel-Mazzetti, Iter sinense 9848* (holotype: WU0060941 (WU, image!)).

≡ *Lonicera cavaleriei* H. Lév. Repert. Spec. Nov. Regni Veg. 11(271–273): 31 (1912). Type: China. Pan-choui, route de Pin-Fa Tou-Yun., 9 April 1907, *Cavalerie J., 3038* (holotype: E00284831 (E, image!)).

Lectotype (here designated):— CHINA. Hupeh [Hubei] Nan-T'o and mountains to Northward, 1887, *A. Henry 4464*, K000901325 (K, image!); Isolectotype: K000901323 (K, image!).

Syntypes. CHINA. Hupeh [Hubei] Nan-T'o and mountains to Northward, 1887, *A. Henry 2106*, K000901324 (K, image!), US00112856 (US, image!); Kwantung [Guangdong], North river, August 1887, *Ford 114*, K000901326 (K, image!), P00640606, P00640607 (P, images!), IBSC0002797 (IBSC, image!).

Nomenclatural notes. Hemsley (1889) described *Jasminum sinense* based on three gatherings (*A. Henry 2106, 4464* and *Ford 114*) from China (Hubei and Kwangtung Province). Green (1993) in one of his publications, cited these gatherings as syntypes. A survey of literature and multiple herbaria revealed that a lectotype for *J. sinense* has not been designated in earlier studies (Green 1993, Chang et al. 1996, and Bui et al. 2013). While looking for the original materials, we found eight specimens of these collections deposited in IBSC, K, P and US (IBSC0002797, K000901323, K000901324, K000901325, K000901326, P00640606, P00640607 and US00112856). One of the sheets at K holds Henry's two gatherings 4464 and 2106, together with barcodes K000901323 and K000901324, respectively. We, therefore, selected Henry's collection (No. 4464; K000901325) at K as lectotype because it represents the species best and has a complete original label, following Arts. 9.3 and 9.11 of ICN (Turland et al. 2018). Although K000901323 is also a complete and flowering specimen, it was selected as an isolectotype to avoid any confusion which the two gatherings on the same sheet (K000901323 and K000901324) may create. The remaining specimens are designated here as syntypes.

Ecology and phenology. Grows in the forest at a high altitude of 800–2000 m.a.s.l. Flowering in June – August, fruiting in September – November.

Distribution. CHINA and VIETNAM. Ha Giang Province (Pho Bang).

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Perrottetia taronensis* B.M.Barthol. & K.Armstr., sp. nov. (Dipentodontaceae), a new species from northwestern Yunnan Province, China and northern Kachin State, Myanmar and a re-examination of the Asian and Australasian taxa of *Perrottetia

Bruce Bartholomew¹, Kate E. Armstrong², Rong Li³, Peter W. Fritsch⁴

1 Dept. of Botany, California Academy of Sciences, 55 Music Concourse Dr., Golden Gate Park, San Francisco, California 94118-4503, USA **2** Institute of Systematic Botany, New York Botanical Garden, 2900 Southern Blvd., Bronx, New York 10458-5126, USA **3** Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanbei Rd., Heilongtan, Kunming 650201, Yunnan, China **4** Botanical Research Institute of Texas, 1700 University Drive, Fort Worth, Texas 76107-3400, USA

Corresponding author: Bruce Bartholomew (bbartholomew@calacademy.org)

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Abstract

Perrottetia taronensis from the Dulong Jiang valley in northwestern Yunnan Province, China and the Babulongtan mountain range in northern Kachin State, Myanmar is here described as a new species of the Dipentodontaceae. It is the third species of the genus to be recognized for China and the first to be reported for Myanmar. It is similar to *P. alpestris* s.s. but differs by characters of its leaf margins, inflorescences, and fruit. The three subspecies of *P. alpestris* recognized by Hou in “Flora Malesiana” are here recognized as three distinct species, i.e., *P. alpestris*, *P. moluccana*, and *P. philippinensis* on the basis of differences in diagnostic characters and distribution. The report in the “Flora of China” of the Taiwan species *P. arisanensis* from Yunnan is determined to be incorrect due to misidentification of two specimens at KUN.

Keywords

Huerteales, IUCN Red List, *Perrottetia alpestris*, *Perrottetia arisanensis*

Introduction

Perrottetia Kunth is a genus of about twenty species occurring mainly in tropical America and tropical Asia. In addition, two species are endemic to the Hawaiian Islands (Lorence and Wagner 2019). The genus was formerly included in Celastraceae, but evidence from nuclear and plastid genes supports its placement in Dipentodontaceae (Zhang and Simmons 2006), now treated as a separate family in the order Huerteales (Chase et al. 2016). Dipentodontaceae has two genera, *Perrottetia* and the monospecific genus *Dipentodon* Dunn (Ma and Bartholomew 2008). In the Old World *Perrottetia* occurs from mainland Asia south to New Guinea and northeastern Australia, east to the southwest Pacific islands, and north to China and Myanmar. In the “Flora of China” two species were reported (Ma and Bartholomew 2008). One of these, *P. racemosa* (Oliv.) Loes., is widely distributed in southern and southwestern China (Ma and Bartholomew 2008), occurring in the provinces of Guangxi, Guizhou, SW Hubei, NW Hunan, Sichuan, and S–SE Yunnan as well as the Chongqing Municipality which was formerly the eastern part of Sichuan Province. *Perrottetia racemosa* was first described as an *Ilex* L. by Oliver (1889) on the basis of collections made by A. Henry in Hubei Province, China, but was later transferred to *Perrottetia* by Loesner (1892). The other species in China, *P. arisanensis* Hayata (1915), was described from Taiwan Province. Recently, a third species has been found in China and Myanmar and is reported here in China from northwestern Yunnan Province in the Dulong Jiang valley of Gongshan Xian and in Myanmar from Kachin State in Putao District. This new species is similar to *P. alpestris* (Blume) Loesn. *s.s.* but differs by several characters given below in the discussion and the key to the Asian and Australasian species of *Perrottetia*. It is also disjunct from *P. alpestris s.s.* by over 2500 km.

The “Flora of China” treatment of *Perrottetia* reported that *P. arisanensis* not only occurs in Taiwan but also in central Yunnan in Eshan Xian 峨山县 and southeastern Yunnan in Xichou Xian 西畴县 (Ma and Bartholomew 2008). This Yunnan distribution is, however, incorrect as it was based on the misidentification of two specimens at KUN. The specimen from Eshan Xian (*Yuxi Team* 玉溪队 89-256 collected 7 July 1989) has been redetermined to be a *Rhamnus* L., and the specimen from Xichou Xian (*Wu Quanan* 武全安 7981 collected 7 May 1959) has been redetermined to be *P. racemosa*. With this correction, *P. arisanensis* is endemic to Taiwan.

Taxonomy

Perrottetia taronensis B.M.Barthol. & K.Armstr., sp. nov.

urn:lsid:ipni.org:names:77220797-1

Figures 1, 2, 3

Type. CHINA. Yunnan Province: Gongshan Xian 贡山县, Dulongjiang Xiang 独龙江乡, Maku Cun 马库村 [Taron River], NW facing 30–60° slope, vicinity of Nangza (Pinyin: Laza) 腊咱, W side of the Dulong Jiang valley, ca. 1.3 direct km S of Maku and ca.

3.8 direct km NE of the Myanmar border, 1970 m, 27.6747°N, 98.3015°E, 18 August 2006, *Gaoligong Shan Biodiversity Survey 32394* (holotype: KUN! accession 0856752 barcode 1418097; isotypes: BRIT! barcode BRIT478072, CAS! accession 1090250 barcode 346898, E! barcode E01016879, GH! barcode 00288213).

Diagnosis. *Perrottetia taronensis* is similar morphologically to *P. alpestris* s.s. (see Discussion) from which it differs by having much more compact and shorter inflorescences which are sparsely golden tan-tomentose rather than sparsely reddish brown-tomentose, a shorter stipe, leaf margins that are sharply serrate rather than bluntly serrate, and larger fruit when mature.

Description. Shrubs or small trees 1.5–6.0 m tall, often sprawling, likely dioecious, deciduous. Stems reddish brown, pale brown-tomentose when young, glabrescent. Stipules reddish brown-tomentose, triangular, ca. 1.5×0.5 mm, often cauducous, apex long-acuminate. Petioles 0.5–1.0 cm. Leaves alternate, mostly glabrescent; leaf buds and young leaves dactylose (Figs 1B, 3), reddish brown-tomentose (Fig. 3), persistent as small naked buds over winter; mature leaf blades chartaceous, mostly glabrescent, sometimes with domatia abaxially in axils of main lateral veins, when fresh abaxially pale pinkish greenish (Fig. 2A) and adaxially dark green (Fig. 2B), when dry abaxially pale brownish green and adaxially dark green, narrowly ovate to elliptic, $10\text{--}15 \times 3.0\text{--}7.5$ cm, abaxially aveolate, sometimes sparsely tomentose on veins, midvein prominent, secondary and tertiary veins prominent, adaxially rugose, glabrous, midvein slightly prominent, secondary and tertiary veins slightly depressed, base rounded to broadly cuneate and slightly asymmetric with margin narrowly recurrent onto petiole, margins sharply serrate with 0.5–0.8 mm forward-facing sharply pointed corniculate teeth 0.2–0.3 mm wide at their base, apex narrowly acuminate and often slightly curved. Inflorescences axillary, paniculate thyrses, 1–2 cm, ca. 25–40-flowered but much reduced in number in fruit, sparsely golden tan-tomentose, with ca. 1.2×0.5 mm narrowly triangular acuminate bracts, basal portion of inflorescences before the first branch 1–2 mm. Flowers and fruit with a basally articulate 0.5–0.6 mm stipe (Fig. 1C), pedicel 1.0–1.5 mm. Flowers 5-merous; sepals and petals only slightly differentiated, persistent in fruit (Fig. 1C). Calyx tube broadly obconical, ca. 0.5×1 mm, lobes narrowly triangular, $1.0\text{--}1.2 \times 0.5\text{--}0.6$ mm, basally overlapping corolla lobes, margins minutely denticulate, apex acuminate and often distally reflexed. Corolla lobes broadly triangular, $1.0\text{--}1.2 \times 0.5\text{--}0.7$ mm, margins minutely denticulate, apices broadly acute. Stamens 5, at the edge of the floral disc and alternating with corolla lobes. (Only one male plant of *Perrottetia taronensis* has been seen. It has two remnant undeveloped flower buds with all other flowers already fallen. In *Perrottetia* the filaments elongate after the male flowers open, so the filament characters in the observed unopened flower buds of *P. taronensis* are likely not typical of what they would be during anthesis.) Anthers globular, ca. 0.3×0.4 mm. Ovary superior, turbinate, ca. 1.2×1.0 mm. Fruit a berry, young fruit green but starting to turn red by July and turning purple and becoming fleshy when mature by August, ca. 5 mm in diam. when mature, usually 4-seeded although occasionally with only 2 or 3 seeds developing, apex emarginate; style ca. 0.2 mm, often deciduous, apically

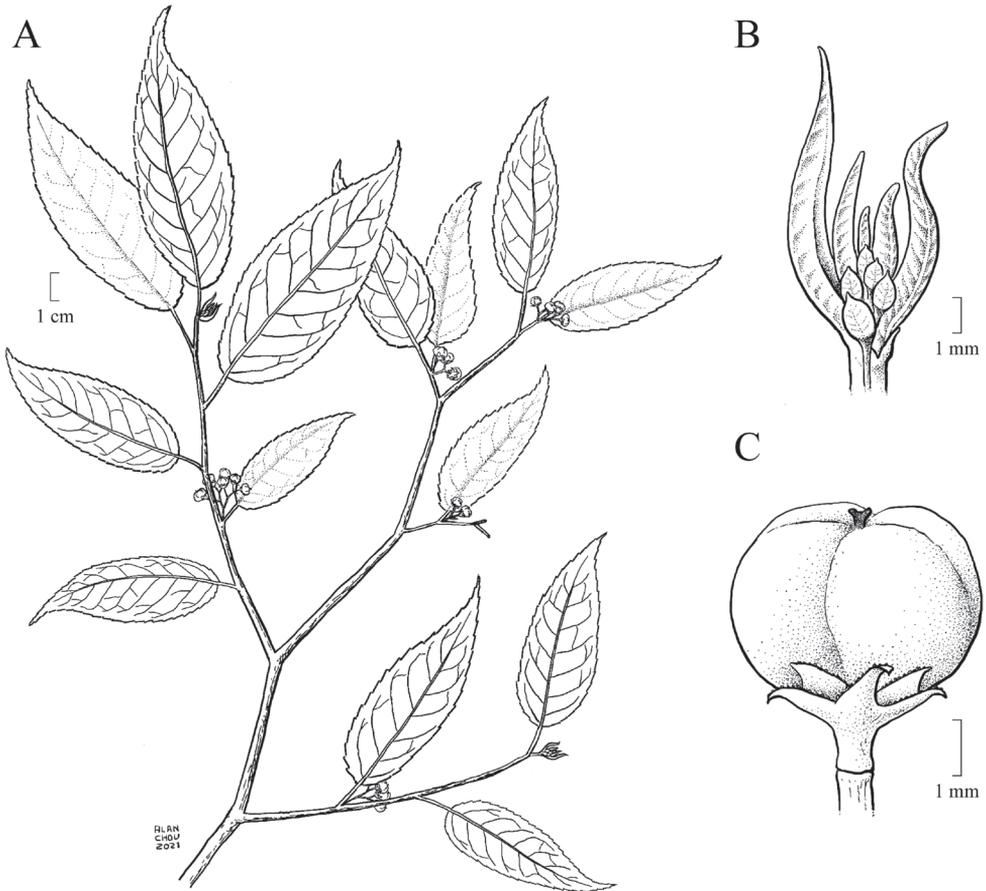


Figure 1. *Perrottetia taronensis* **A** habit, branch with fruit **B** dactylose leaf bud with young leaves and stipules **C** fruit with style, mature berry, persistent sepals and petals, and stipe. Illustrated by Alan Chou.

2-parted. Seeds brown, 1.0–1.5 mm in diam., surface with numerous shallow vertical rugose ridges when dry.

Etymology. The specific epithet “taronensis” refers to the Taron River valley in Myanmar. In China this river is named the Dulong Jiang (Dulong River) (Fig. 4). The Taron River flows into the N’Mai Hka (N’Mai River) which joins the Mali Hka (Mali River) forming one of the main northern tributaries of the Ayeyarwady River (Irrawaddy River).

Habitat and distribution. The seven collections of *Perrottetia taronensis* that have so far been made occur in the Ayeyarwady River drainage in both Yunnan Province, China and Kachin State, Myanmar at an elevation range of 1350–1970 m (Fig. 4). In China *P. taronensis* occurs on slopes in subtropical broadleaved evergreen forests, in disturbed secondary forests of *Alnus nepalensis* D. Don (an early successional tree species of secondary forests in this region), and among shrubs near river banks. In Myanmar, the habitat of the single collection of a male plant is classified within the Kachin Hills subtropical rain-forest ecosystem, a closed-canopy humid lower montane forest type occurring between

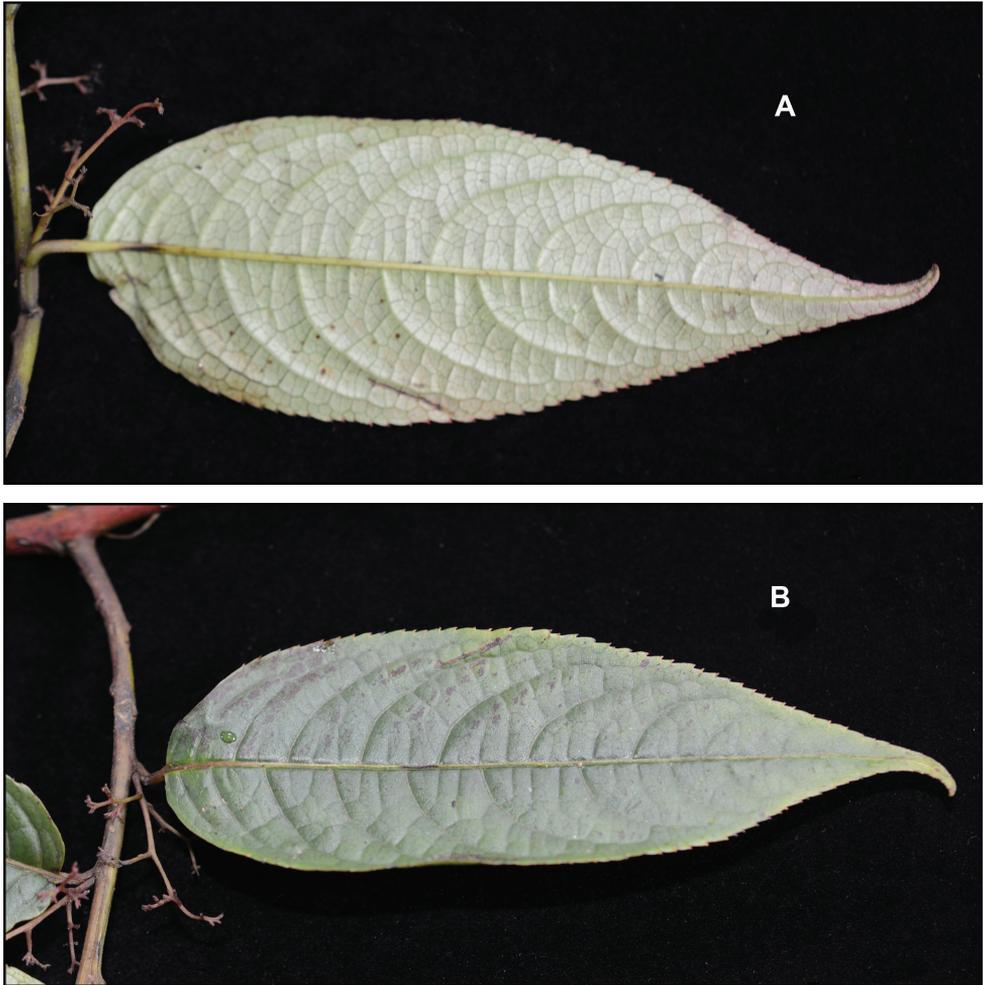


Figure 2. Leaves of *Perrottetia taronensis* showing also the old male inflorescences, from which all the flowers have dropped **A** abaxial surface **B** adaxial surface. Photos in the field of *Armstrong et al. 2983*.

700–1500 m (*Armstrong et al. 2020*; *Murray et al. 2020*). The six collections in China are from the valley of the Dulong Jiang which drains into the Taron River/N'Mai Hka in Myanmar, an upper tributary of the Ayeyarwady River. In Myanmar, the single collection is from the Babulongtan mountain range, in the drainage of the Nam Tisang/Mali Hka, which is in the adjacent river system to the west of the N'Mai Hka.

Proposed IUCN Conservation Status. The proposed Conservation Status of *Perrottetia taronensis* is Endangered (EN), B1ab(i,ii,iii,v)+2ab(I,ii,iii,v), according to the IUCN Standards and Petitions Committee (2019). This status is based on the criteria of EOO 802.498 km² and AOO 319.308 km² calculated by using GEOCAT (*Bachman et al. 2011*) and due to the occurrence in a small area, a decline in the quality of its habitat, and the low number of mature individuals observed.



Figure 3. Young leaves of *Perrottetia taronensis* showing the distinctive dactylose shape and reddish brown indumentum and also an old male inflorescence, from which all the flowers have dropped. Photo in the field of *Armstrong et al.* 2983.

Paratype specimens of *Perrottetia taronensis* examined. CHINA. Yunnan Province: Gongshan Xian 贡山县, Dulongjiang Xiang 独龙江乡, Bapo Cun 巴坡村, in the vicinity of Bapo, on the E side of the Dulong Jiang, 1350 m, 27.74°N, 98.35°E (estimated coordinates), 19 November 1990, *Dulong Jiang Investigation Team* 独龙江考察队 579 (CAS, KUN); Bapo Cun, W side of Gaoligong Shan, W of Gongshan, on the trail from

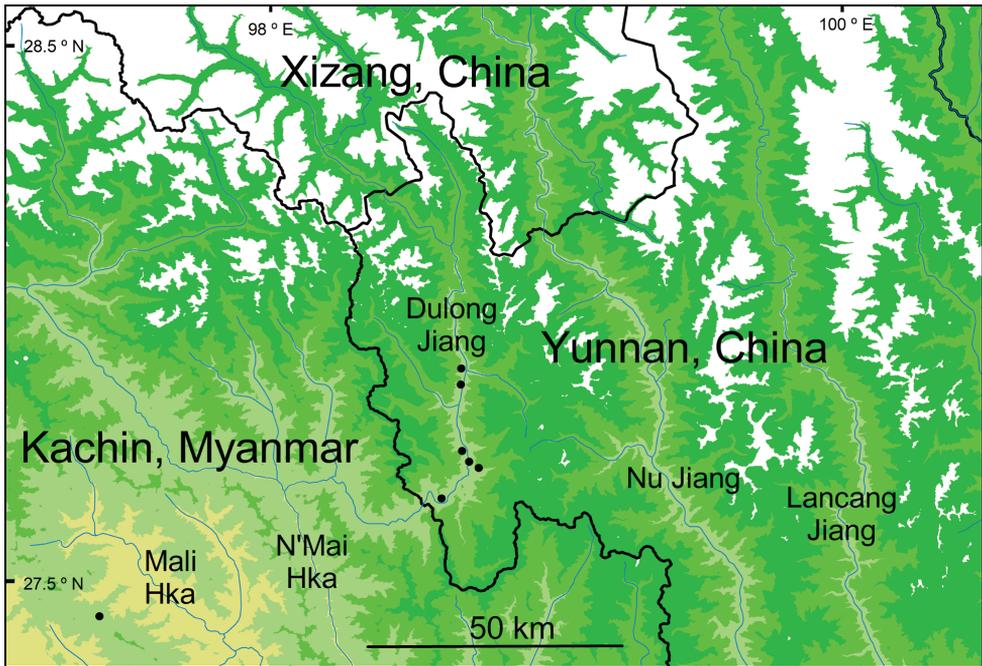


Figure 4. Distribution showing the known locations of *Perrottetia taronensis* in China and Myanmar. The map uses Shuttle Radar Topography Mission (SRTM) 90 m raster elevation data from Jarvis A, Reuter HI, Nelson E, and Guevara E, 2008, Hole-filled seamless SRTM data V4, International Centre for Tropical Agriculture (CIAT) available from <https://srtm.csi.cgiar.org> (Reuter et al. 2007). The elevation intervals for the region shown in this map have been set to 0–999 m, 1000–1999 m, 2000–2999 m, 3000–3999 m, and 4000 m and above. All seven collections are from within the 1000–1999 elevation interval shown on the map. Within China to the east and separated by the Gaoligong Shan range is the Nu Jiang (Salween River) and farther still to the east is the Lancang Jiang (Mekong River).

Qiqi 其期 to Bapo in the Dulong Jiang valley, 1900 m, 27.7290°N, 98.3670°E, 17 July 2000, Li Heng 李恒 12931 with Bruce Bartholomew, Philip Thomas, Peter Fritsch, Dao Zhiling 刀志灵, Wang Zhong-lang 王仲朗 & Li Rong 李嵘 (CAS, E, MO); Bapo Cun, W side of the Gaoligong Shan, along Gamalai He (Gamalai River) 嘎莫赖河 and Dulong Valley on the trail from Xishaofang 西哨房 to Bapo, 1350 m, 27.7592°N, 98.3377°E, 18 July 2002, Li Heng 李恒 15072 with Li Rong 李嵘 & Dao Zhiling 刀志灵 (CAS, KUN); Kongdang Cun 孔当村, W side of Gaoligong Shan, along Dulongjiang Valley on the trail from Bapo to Dizhengdang 迪政当, 1550 m, 27.8773°N, 98.3355°E, 21 Jul 2002, Li Heng 李恒 15156 with Li Rong 李嵘 & Dao Zhiling 刀志灵 (CAS, KUN); Xianjiudang Cun 献九当村, W side of Gaoligong Shan along Dulong Jiang Valley on the trail from Kongdang to Dizhengdang, 1560 m, 27.9061°N, 98.3363°E, 23 July 2002, Li Heng 李恒 15230 with Li Rong 李嵘 & Dao Zhiling 刀志灵 (CAS, KUN). MYANMAR. Kachin State, Putao District, Naungmung Township, buffer zone of Hkakaborazi National Park, along trail between Maza and Namti, 1763 m, 27.4650°N, 97.6967°E, 18 June 2017, Kate Armstrong 2983 with Thet Yu Nwe, Moe Myint Thu, San Naing Dee, Zaw Naing Tun, Hla Naing Htay & Pa Rang Gang Ken Sar (CAS, E, NY, RAF).

Discussion

As treated here *Perrottetia* is now known in Asia and Australasia as having six species of which three occur in China and one in Myanmar. The genus is herein recorded for the first time from Myanmar as it was not mentioned in either Merrill's (1941) list of "Upper Burma Plants" or in Kress et al.'s (2003) country-wide checklist. On the basis of the single Myanmar collection being a male plant and all six Chinese collections made so far being female plants in fruit, it is likely that *P. taronensis* is dioecious. This condition is common in the genus, although additional field studies in the spring during flowering are needed for confirmation.

Perrottetia taronensis is most similar morphologically to *P. alpestris* s.s. (see below) which occurs in Peninsular Malaysia as well as in Indonesia on Sumatra and Java plus nearby small islands. It differs from *P. alpestris* s.s. by several characters enumerated in the diagnosis above and in the key to the Asian and Australasian species of *Perrottetia* below. Moreover, *P. taronensis* and *P. alpestris* exhibit a north-south disjunction of about 2500 km, with *Perrottetia* not recorded from intervening continental Southeast Asia (Hou 1962; Hou et al. 2010).

In addition to *Perrottetia alpestris* s.s., two other *Perrottetia* species (*P. moluccana* (Blume) Loes. and *P. philippinensis* (S. Vidal) Loes.) occur in Southeast Asia and Australasia. Although treated as species by Loesner (1892), they were treated respectively as *P. alpestris* subsp. *moluccana* (Blume) Ding Hou and *P. alpestris* subsp. *philippinensis* (S. Vidal) Ding Hou by Hou in the treatment in "Flora Malesiana" (1962). We follow Loesner in treating all three Southeast Asian and Australasian *Perrottetia* as separate species. Not only do they differ by several diagnostic characters as can be seen in the key to the Asian and Australasian species of *Perrottetia* below, but also *P. alpestris* s.s., *P. moluccana*, and *P. philippinensis* are disjunct, as was pointed out by Hou (1962). *Perrottetia moluccana* occurs on the island of New Guinea and the Cape York Peninsula of Queensland Australia, extending into some of the adjacent islands in the southwest Pacific. *Perrottetia philippinensis* occurs in Indonesia on the islands of Borneo and Sulawesi as well as in the Philippines and on some small adjacent islands. On the basis of diagnostic morphological characters, *P. alpestris* s.s., *P. moluccana*, *P. philippinensis*, and *P. taronensis* are more similar to each other than this group of four species is to either of the other two Asian species, *P. arisanensis* and *P. racemosa*, and these similarities and differences are expressed in the key below. It is hoped that future molecular studies will confirm our interpretation based on diagnostic morphological characters.

We only know of seven collections of *Perrottetia taronensis* all of which were previously misidentified as *Celastrus* L. (Celastraceae), *Gaultheria* L. (Ericaceae), *Ilex* (Aquifoliaceae), *Maesa* Forssk. (Primulaceae), or *Rhamnus* (Rhamnaceae), so it is possible that other specimens are already in herbaria and may come to light, although a search in likely families and genera at KUN as well in the online Chinese Virtual Herbarium (<https://www.cvh.ac.cn/>) has so far not found additional specimens of this species.

Key to the Asian and Australasian species of *Perrottetia*

- 1 Inflorescences thyrses in a raceme; fruit apex rounded..... *P. racemosa*
- Inflorescences thyrses in a panicle; fruit apex emarginate..... 2

- 2 Corolla and calyx lobes ligulate, undifferentiated, not overlapping, ca. 1.0 × 0.2–0.4 mm, glabrous, margins entire; style in fruit 1.0–1.4 mm, usually persistent; inflorescences glabrous *P. arisanensis*
- Corolla and calyx lobes triangular, slightly differentiated, calyx lobes ca. 1.0 × 0.5–0.6 mm and overlapping corolla lobes, corolla lobes ca. 0.5–1.2 × 0.3–1 mm, margins of both calyx and corolla lobes minutely denticulate; style in fruit ca. 0.2 mm, often deciduous; inflorescences sparsely tomentose **3**
- 3 Leaf blades thinly coriaceous, margins entire; stems conspicuously lenticellate *P. moluccana*
- Leaf blades chartaceous, margins serrate; stems not conspicuously lenticellate **4**
- 4 Flowers 4-merous; inflorescences 5–10 cm with basal portion before first branching 2–3(–4) cm; serrations of leaf blade margins blunt with serrations 0.3–0.4 mm wide at their base; stipules apically acute *P. philippinensis*
- Flowers 5-merous; inflorescences 1–4 cm with basal portion before first branching 0.1–1.0 cm; serrations of leaf blade margins blunt or sharp with serrations 0.2–0.3 mm wide at their base; stipules apically acuminate **5**
- 5 Inflorescences 2–4 cm, sparsely reddish brown-tomentose, basal portion before first branching 0.5–1.0 cm; stipe ca. 1.5 mm; leaf blade serrations apically blunt; fruit when mature and fleshy 3–3.5 mm in diam. *P. alpestris*
- Inflorescences 1–2 cm, sparsely golden tan-tomentose, basal portion before first branching 0.1–0.2 cm; stipe 0.5–0.6 mm; leaf blade serrations apically sharply pointed; fruit when mature and fleshy ca. 5 mm in diam. *P. taronensis*

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Allium negianum (Amaryllidaceae): a new species under subg. *Rhizirideum* from Uttarakhand Himalaya, India

Anjula Pandey¹, K. Madhav Rai², Pavan Kumar Malav¹, S. Rajkumar³

1 Division of Plant Exploration and Germplasm Collection, National Bureau of Plant Genetic Resources, New Delhi 110012, India **2** ICAR-National Bureau of Plant Genetic Resources, Regional Station Bhowali, Niglat 263132, Nainital, Uttarakhand, India **3** Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India

Corresponding authors: Anjula Pandey (anjuravinder@yahoo.com), Pavan Kumar Malav (pavan.malav@icar.gov.in)

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Abstract

A new species, *Allium negianum* (Amaryllidaceae), belongs to the genus *Allium* subg. *Rhizirideum*, sect. *Eduardia* is described here from the Uttarakhand Himalayan region of India. This taxon grows in Malari region of Niti valley in Chamoli district and Dharma valley of Pithoragarh district, Uttarakhand, India. It is a narrowly distributed species and morphologically more closer to *A. przewalskianum* Regel but differentiated by its tunic color of bulb, umbel with lax flowers, peduncle length, perigone colour, size and shape and leaf anatomy. Taxonomic delineation and relationship analysis based on nuclear ribosomal Internal Transcribed Spacers (ITS) region indicated that *A. negianum* is distinct and related to *A. przewalskianum*. This study provided a comprehensive description and comparison with *A. przewalskianum*, an identification key and notes on the distribution of the species.

Keywords

Allium negianum, India, *Rhizirideum*, Seasoning spice, Uttarakhand

Introduction

Allium L., one of the largest genera in the family Amaryllidaceae, has about 1,100 species distributed world-wide (Li et al. 2010; Govaerts et al. 2021). The genus *Allium* naturally occurs in dry seasons in the northern hemisphere and South Africa (Friesen et al. 2006; Nguyen et al. 2008; Neshati and Fritsch 2009). The primary centre of evolution for the ge-

nus extends across the Irano-Turanian bio-geographical region, and the Mediterranean basin and western North America are considered as the secondary centres of diversity (Friesen et al. 2006). The genus is characterized by bulbs that are enclosed within the membranous or fibrous tunics, free tepals, often a subgynobasic style and well-known characteristic plant odour and taste due to the presence of cysteine sulphoxides (Friesen et al. 2006). The classification of global species in the genus *Allium* is based on molecular phylogenetic analyses, which includes 15 subgenera and 56 sections (Friesen et al. 2006). The Indian *Allium* includes over 10 subgenera, 22 sections and 35–40 taxa excluding cultivated species distributed in different eco-geographical areas of the temperate and alpine regions of Himalayas sharing many taxa of Chinese origin (Pandey et al. 2008, 2017; Li et al. 2010). Indian Himalayan region has two distinct centres of diversity, the western Himalaya (over 85 per cent of total diversity) and the eastern Himalaya (6 per cent), covering the alpine-sub temperate region (2500–4500 m a.s.l.) (Gohil 1992; Pandey et al. 2008).

Globally *Allium* subg. *Rhizirideum* (G. Don ex Koch) Wendelbo s.str. has ca. 37 taxa that are included in four sections distributed mainly in Europe-East Asia, in China (Friesen et al. 2006; Choi et al. 2012; Jang et al. 2021) and also in Russia, Mongolia and Kazakstan (Sinityna et al. 2016; Friesen et al. 2020). *Allium senescens* L. of sect. *Rhizirideum* a species native to northern Europe and Asia from Siberia-Korea and also naturalized in parts of Europe, is an exception (Xu and Kamelin 2000; Li et al. 2010).

Taxa of the subg. *Rhizirideum* belong to the third and the most advanced evolutionary line, which is phylogenetically sister to taxa of the subg. *Allium* L., *Cepa* L., *Reticulatabulbosa* (Kamelin) N. Friesen and *Polyprason* Radic. (Friesen et al. 2006; Memariani et al. 2007; Li et al. 2010; Choi et al. 2012). The sect. *Eduardia* N. Friesen of the subg. *Rhizirideum* is mainly distributed in the western Himalaya with Pakistan on the west and Nepal and Tibet in the centre, and southwest China on the eastern side. Its habitat mainly comprises of mountainous, snow peak grassland, dry or rocky places in forests, subalpine meadows, steppes, sunny, saline areas, sandy deserts, stony and gravelly slopes, rocky crevices along the stream banks and damp places (Fritsch and Friesen 2002; Choi and Oh 2011; Choi et al. 2012).

Despite the importance of the genus *Allium* for the Indian region, meagre comprehensive studies have been attempted pertaining to molecular and taxonomic evaluation that led to gaps in the status of interspecific and infraspecific relationships among the taxa. Meagre taxonomic studies on the native taxa, unavailability of material for research, sporadic collections from under-explored/unexplored areas and lack of the published literature have led to the possibility of finding new taxonomic records from the Indian region (Pandey et al. 2008, 2017, 2021).

The subg. *Rhizirideum* is the smallest subgenus of *Allium* as per the flora of India, and it is represented only by the sect. *Eduardia* containing only one species, *A. przewalskianum* Regel. This taxon occurs in the scrub, drier slopes, ravines and rocky crevices (2000–4500 m a.s.l.) in Leh, Jammu and Kashmir and Spiti in Himachal Pradesh. The taxa under subg. *Rhizirideum* are characterized by the presence of several narrowly ovoid-cylindric bulbs, which borne on creeping rhizome usually covered with a common reticulate membrane, leaves shorter than scape, adaxially channeled and stamens slightly longer than perigone segments, spathe with a long beak, nearly 2 to 3 times longer than the base and hemispherical umbel. Most species share a basic chromosome

number of $x = 8$ and $2n = 16$ or 32 . Occurrence of a polyploid complex in different sections of the subgenus *Rhizirideum* indicated recent origin of taxa as supported by phylogenetic and biogeographical evidences (Li et al. 2010). Areas with geographical isolation are the driving force of underestimated speciation (Seregin et al. 2015).

A new taxon, *Allium negianum*, was collected from the Indo-Tibetan border area of Malari village, Niti valley of Chamoli district in Uttarakhand (India) in 2019 and identity was confirmed by the authors. It is distinct from its closest relative, *A. przewalskianum* Regel (Table 2), the only taxon of subg. *Rhizirideum*, sect. *Eduardia* in India. It is characterized by finely reticulated red-brown outer tunics, hemispherical umbel having lax flowers, spathe with a very long beak, deep purple tepals, asynchronous flowering and inner stamen filaments having longer and sharp teeth. In the present work, *A. negianum*, is described and illustrated here. Authors have examined the evidences from morphology, eco-geography, leaf anatomy, molecular study, and taxonomic delineation from other related species.

Materials and methods

Taxon sampling and morphological descriptor

A total of 110 plants representing 7 accessions of the new species were collected from the type locality and farmers' fields in the Niti region of Uttarakhand, India. For delimitation of the taxon with other related species, plants were grown in the Field Gene Bank (FGB) at the ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), Regional Station Bhowali (Nainital), Uttarakhand for comparative study of morphological characters. Data were recorded using the *Allium* descriptor with modifications from the published literature. The floral characters were measured with separate parts to the nearest ten points of the decimal. The seeds having uniform size and maturity were recorded for ultra-features of the characters using the Stereozoom Microscope (LMI, England, model no. SZM167), and the images were captured as JPEG. Ten replicate voucher herbarium specimens of the new species were prepared as per standard procedure and deposited in the National Herbarium of Cultivated Plants (code-NHCP) (Holotype) and CAL (Isotype).

The new species was compared with its closest relative using data derived from the study of specimens preserved in the herbaria of CAL, DD, E, K and NHCP and available literature. Due to its closer affinity with *A. przewalskianum*, all the specimens from diverse sources were critically examined. Taxonomic description and identification key were provided for *Allium negianum* and affined species.

Leaf anatomy

For leaf anatomy live plants were grown in the FGB at Regional Station Bhowali (Nainital), Uttarakhand. Leaf-blades were taken from a point 3–4 cm above the sheaths and fixed in 70% alcohol. Cross-sections were made at three different lengths of leaf and stained with Sartur solution (a mix of sudan III, aniline, chloral hydrate, lactic acid, iodine), the structure was studied, and analyzed with the help of a light

microscope (Olympus BH-2) and line diagrammes drawn. The outlines of cells were diagrammatically depicted (Fig. 1F).

Taxonomic delineation and relationship analysis

DNA extraction, amplification and sequencing

Genomic DNA of nine known species and one new taxon (Table 1) which was collected from western Himalayan region and maintained as live material at Field Gene Bank (FGB), ICAR- National Bureau of Plant Genetic Resources, Regional Station, Bhowali, was isolated from fresh leaves using spin column-based Qiamp DNA kit according to the suppliers' protocol. Selection of taxa for this study was mainly based on the fact that all taxa belong the third evolutionary line representing same eco-geographical areas, and were known by similar local names. This has resulted in confusion of their identity in Indian literature. The quantity and purity of the isolated genomic DNA was tested using the spectrophotometric method. The universal primers ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS regions. The PCR protocol was run at 94 °C for 5 minutes; 30 cycles of 94 °C for 45 seconds, 55 °C for 45 seconds and 74 °C for 45 seconds and 74 °C for 5 minutes. PCR products were purified using Zymo DNA concentrator kit following the supplier's protocol. The purified PCR product was used in ABI 3730 DNA sequencer (Applied Biosystems) for generating sequences using PCR primers as sequencing primers. For remaining species from subgenus *Rhizirideum* and other related sub-genera, the ITS sequence were used from NCBI database.

Phylogenetic analysis based on the comparison of sequences

The generated DNA sequences from both the primers were checked for alignment using the BioEdit software. Multiple pairwise alignments of generated sequences and from NCBI database were made using ClustalW. The aligned sequences were used to generate the genetic distance between taxa and the evolutionary history, which was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model using MEGA7.0 (Kumar et al. 2016).

Result and discussion

Taxonomic treatment

Allium negianum A.Pandey, K.M.Rai, Malav & S.Rajkumar, sp. nov.

urn:lsid:ipni.org:names:77220799-1

Figs 1, 2

Type. India, Uttarakhand: Chamoli, rocky areas (altitude 3000–4800 m), 22 Aug. 2019, KMR/AS/02/19 (Holotype: NHCP; Isotype: CAL; Seeds conserved in the National Genebank, New Delhi: IC258493).

Table 1. List of *Allium* taxa used to generate nuclear ITS sequence in the study.

S. no.	Taxon name	Subgenus	Section	NGB accession number	District; state
1	<i>Allium tuberosum</i> Rottdler ex Spreng.	<i>Butomissa</i>	<i>Butomissa</i>	IC353524	Almora; Uttarakhand
2	<i>Allium stracheyi</i> Baker	<i>Polyprason</i>	<i>Orioprasum</i>	IC567645	Pithoragarh; Uttarakhand
3	<i>Allium przewalskianum</i> Regel	<i>Rhizirideum</i>	<i>Eduardia</i>	IC632207	Leh; Jammu and Kashmir
4	<i>Allium negianum</i> sp. nov.	<i>Rhizirideum</i>	<i>Eduardia</i>	IC258493	Chamoli; Uttarakhand
5	<i>Allium sativum</i> L.	<i>Allium</i>	<i>Allium</i>	IC278243	Chamoli; Uttarakhand
6	<i>Allium ampeloprasum</i> var. <i>ampeloprasum</i>	<i>Allium</i>	<i>Allium</i>	IC353526	Pithoragarh; Uttarakhand
7	<i>Allium cepa</i> var. <i>cepa</i> L.	<i>Cepa</i>	<i>Cepa</i>	IC410711	Uttarkashi; Uttarakhand
8	<i>Allium cepa</i> L. var. <i>aggregatum</i> G.Don	<i>Cepa</i>	<i>Cepa</i>	AP/RP/2014	Chamoli; Uttarakhand
9	<i>Allium oschaninii</i> O.Fedtsch.	<i>Cepa</i>	<i>Cepa</i>	AP/2014	Voucher; Uttarakhand
10	<i>Allium schoenoprasum</i> L.	<i>Cepa</i>	<i>Schoenoprasum</i>	IC632213	Kargil; Jammu and Kashmir

Description. Herbs, hermaphrodite, 27–50 cm tall. Rhizome condensed, 6.5–8.5 mm long, oblique. Bulb clustered, cylindrical to narrowly ovoid, 0.8–1.2 cm in diameter, 6.8–12 cm long, outer tunic finely reticulate, reddish-dark brown, inner membranous, light-brick red. Leaves 4–6, slightly shorter than scape, 12–40 cm × 1.0–3.2 mm, erect, to semi-terete to terete, dark green; base slightly bulbous. Scape terete, semi-erect, covered with leaf sheaths at base only, stout, solid in cross-section (hollow in mature), 15–30 cm × 3.5–5.5 mm. Spathe 1-valved, persistent, beak very narrow-long, 2.5–4 mm. Inflorescence umbellate, hemispheric, 30–40 lax flowered. Peduncle subequal, 16–18 × 2–3 mm, without bulbils. Flowers bisexual, perigone campanulate, tepals dark purple with distinct green mid-line; inner tepals slightly longer than outer ones, oblong-lanceolate, apex acute, 6–8 × 3–4 mm; outer segments ovate to narrowly so, 5.5–6 × 2.5–3 mm. Stamens anthers oblong, yellow-purplish (on maturity), 2.3–2.6 mm long; filaments subequal, 6.8–8.5 mm, purple, slightly exerted, connate at base and adnate to perigone segments; outer ones subulate; inner ones broadened for 1/2–1/4 to their length, one sharp toothed on each side. Ovary sub-globose, purple-tinged, 3.6–4.8 × 1.8–3.5 mm. Style terete, exerted, stigma smooth, acute-acuminate, ovules 2 per locule. Capsules trigonous, 5–5.5 × 5.8–7.2 mm; seeds obovate with a prominent notch on one side, 3.2–4.0 × 1.9–1.9 mm, testa deep black. Plant has strong onion-garlic type aroma.

Habitat. Slopes, sandy soils along rivers and streams along the alpine meadows (altitude 3000–4800 m asl) in Sumna valley (villages Gamsali, Niti, Tolma, Kailashpur and Farkya) in Chamoli district near Malari glacier of India.

Etymology. The specific epithet, “*negianum*”, is named in honour of Late Dr. Kuldeep Singh Negi, an eminent explorer who has dedicated his life in collection of indigenous *Allium* species germplasm along with associated indigenous knowledge across the country. He was also instrumental in establishing the *Allium* Field Gene Bank (FGB) at the Regional Station, Bhowali, Uttarakhand. The entire germplasm of indigenous *Allium* species collected by him from remote areas of the country are characterized and successfully conserved at *Allium* FGB, Bhowali, Uttarakhand.

Vernacular/local name. Pharan, phran, jambu, sakua, sungdung, kacho, etc. (Pandey et al. 2021).

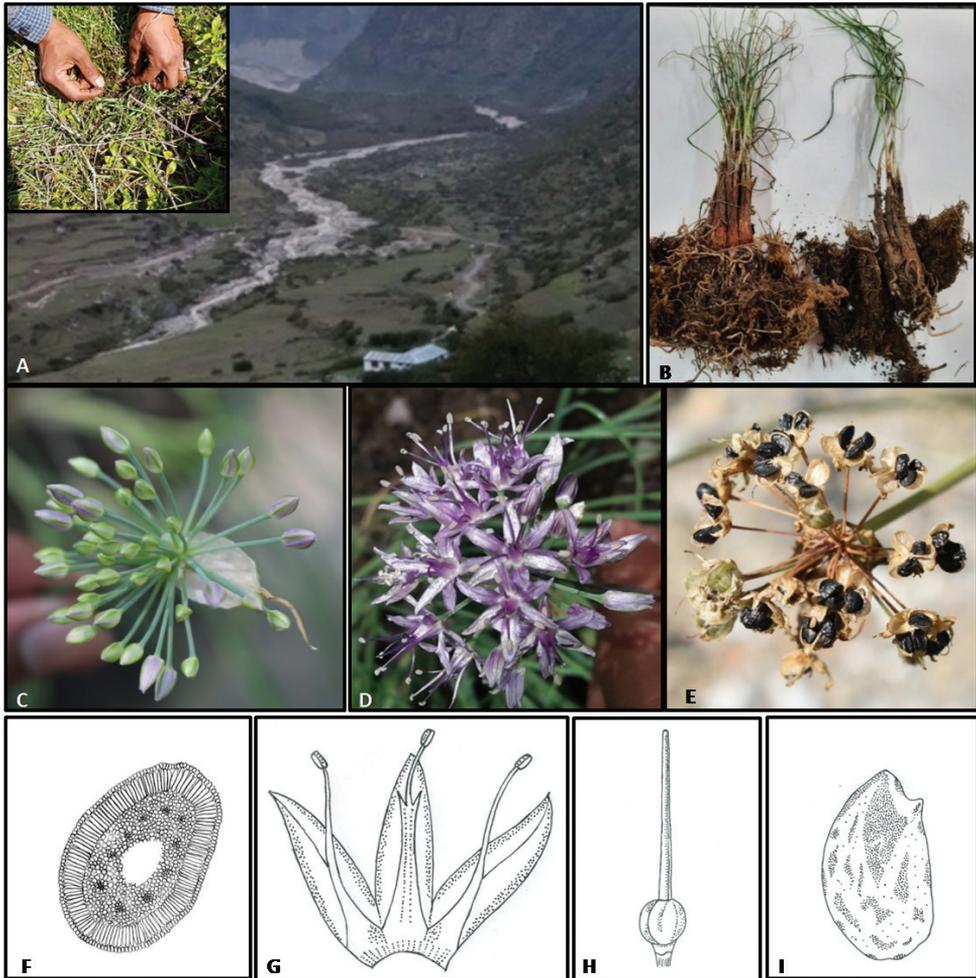


Figure 1. *Allium negianum* **A** general habitat **B** bulb covered with reticulate fiber on bulbs of *A. przewalskianum* (orange-red) and *A. negianum* (red-brown) **C** inflorescence and spathe with a very long beak, persistent **D** inflorescence **E** capsule with mature seeds **F** line-illustrations of transverse section of leaf showing hollow channel **G** longitudinal section of flower with stamen with two sharp teeth **H** ovary **I** seed with prominent beak (**C–I** magnification $\times 30$ – 40).

Phenology. Flowering and fruiting is from June to middle September (altitude 3000–4800 m a.s.l.).

Leaf anatomy. The transverse section of the leaf of *A. negianum* showed an elliptical outline. The epidermis has small cells covered with a thin cuticle layer, and stomata are narrowly distributed along the surface area. Single layered compactly arranged palisade tissue comprised of long cylindrical cells. The mesophyll cells are spongy tissue and compact in young leaf as well in the proximal ends of mature leaf while in the centre part of mature leaf, broken mesophyll cells are confused with fistulous leaf ap-

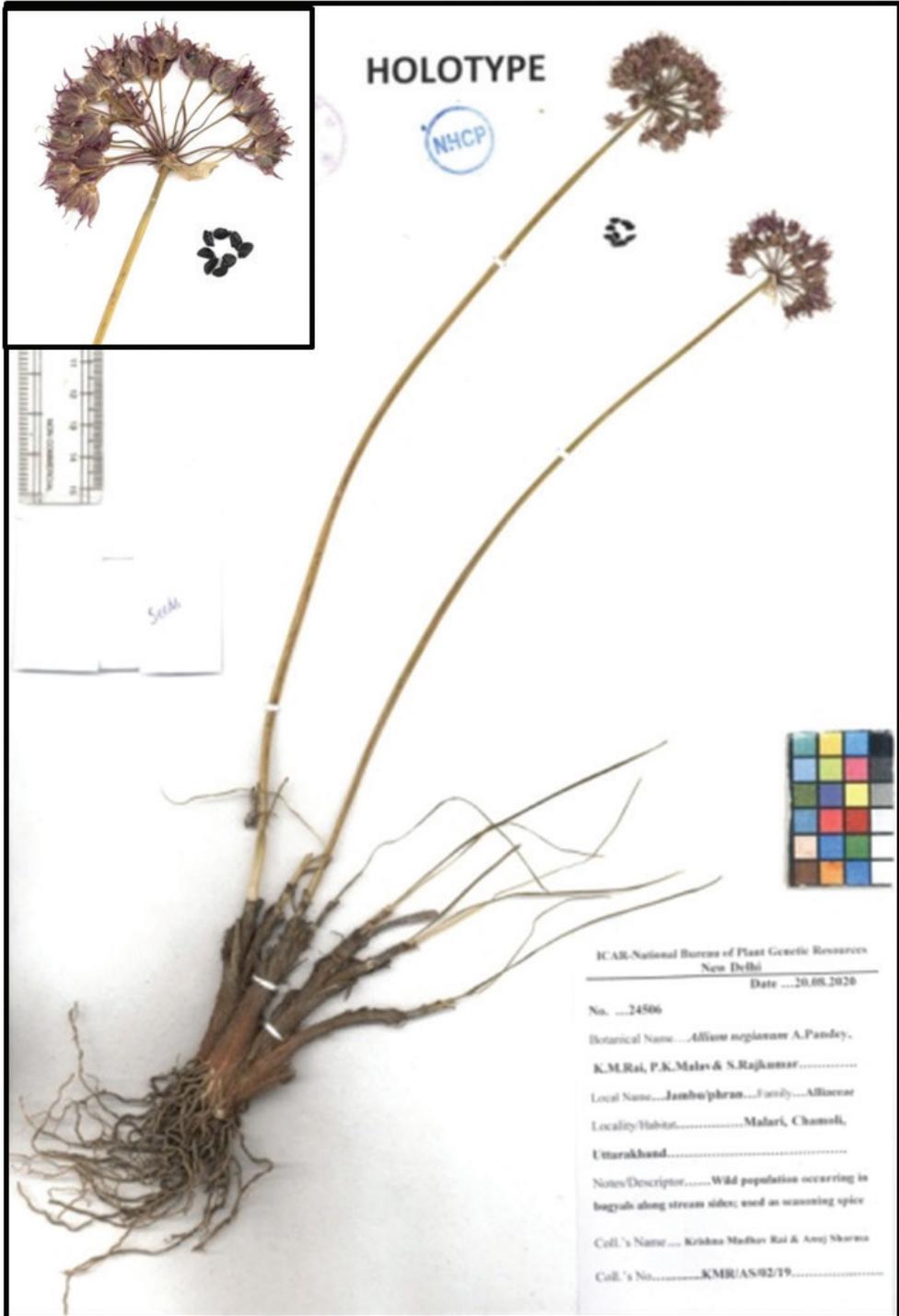


Figure 2. Holotype specimen of *Allium negianum* deposited in NHCP.

Table 2. Major morphological characters* (discriminating characters in **bold**) of *Allium negianum* in comparison with *A. przewalskianum*.

Character	<i>A. przewalskianum</i>	<i>A. negianum</i>
Habitat	Carbonaceous slates-gravel; 3300–5200 m	Grassy meadows, open sandy slopes, along rivers/ streams; 3000–4800 m
Plant habit	Erect	Semi-erect
Plant growth (under experimental condition)	Robust, shorter	Taller, plants and leaves
Plant height (cm)	20–45	27–50
Bulbs no. in cluster	2–4	2–7
Bulb no., shape	Cluster 3–4; cylindrical-narrowly ovoid	Cluster 4–8; cylindrical-narrowly ovoid
Bulb length (cm)	10.2–12.5	6.8–12
Bulb diameter (cm)	0.6–0.7	0.8–1.2
Tunic outer**	Finely reticulate; reddish-orange-brown	Finely reticulate; reddish-dark brown
Tunic inner	Membranous, brown-red	Membranous, orange-red
Rhizome type; size (mm)	Vertical, short; 3–5	Oblique; 7–12
Leaf no., colour	3–5, lighter brown-green	4–6, dark green
Leaf vs. scape	Much shorter than scape	Slightly shorter than scape
Leaf blade shape; apex	Linear, not fistular; obtuse to subrounded	Linear, filiform; acute
Leaf length (cm)	15–30	12–40
Leaf width (mm)	2.0–2.5	1–3.2
Leaf erectness	Erect	Erect-semierect
Leaf waxiness	Non-waxy	Waxy
Leaf cross section	Circular	Circular
Spathe valve if persistent	1(2)-valved, persistent	1-valved, persistent
Spathe valve shape, size	Ovate	Ovate-oblong
Spathe size (cm)	2–3 (two times the base; short, blunt)	4–6 (long narrow beak; 3 times the base)
Scape type	Solid, terete, erect, central	Solid, terete, erect to semi-erect, lateral-central
Scape size (cm)	30–40 × 0.2–0.35	20–50 × 0.36–0.48; 1/3-of the base
Pedicle vs. perigone	Subequal	2–3 times longer
Umbel flower opening pattern	Synchronous (80 per cent)	Asynchronous (30–40 per cent)
Umbel shape	Spherical-hemispherical, densely flowered, compact	Hemispherical, lax, loosely flowered
Umbel diameter (mm)	28.5–30.2	25.1–42.0
Umbel flower (no.)	25–40	30–40
Peduncle size (cm)	0.5–1.0	0.8–2.5
Flower size (cm)	0.4–0.5 × 0.3	0.5–0.5 × 0.4
Flower color	Pale red-purple pink (variable)	Dark purple (as recorded now)
Perigonium shape and color	Campanulate, pink-dark purple, tepal wide open	Campanulate, lilac, light to dark purple, tepal partly opened
Tepal shape	Ovate-lanceolate , apex obtuse	Elliptic, ovate-lanceolate ; apex-acuminate-mucronate
Tepal inner size length × width (cm)	0.3–0.4 × 0.2–0.3	0.5–0.6 × 0.3–0.4
Tepal outer size length × width (cm)	0.5–0.7 × 0.2–0.3	0.6–0.7 × 0.3–0.5
Tepal apex shape	Acute-acuminate	Acute, mucronate
Tepal maturity	Curved outwards	Slightly inwardly curved/rolled
Tepal mid-vein	Non-conspicuous; purple green-dark purple	Very conspicuous; green-light green
Anther length (mm)	6.1–9.3	6.8–8.5
Anther lobe length (mm)	Oblong-ovate, 1–2	Oblong, 1–2
Anther lobe color	Yellow-purple	Yellow-purple
Filament color	Yellowish-purple	Greenish yellow-purplish green
Filament length, position	Double the size of tepal; exerted,	Half the size of tepal; slightly exerted

Character	<i>A. przewalskianum</i>	<i>A. negianum</i>
Filament inner and outer anther	Inner – two sharp teeth up to 1/2 to 1/4 length of filament with broader base; outer narrower base	Inner – two shallower-sharper teeth up to 1/2 to 2/3 length of filament with base as wide as tepal; outer narrower base
Ovary shape	Ovoid – globose, wrinkled	Obovoid – subglobose
Ovary color	Purple green, tinged with purple	Dark-pale purple
Ovary style <i>vs.</i> anther (after pollination)	Much exerted , longer than the ovary	Slightly exerted or equal
Ovary stigma tip	Acuminate-acute	Acuminate
Stigma <i>vs.</i> stamen	Sub-equal	Slightly longer
Capsule shape	Ovoid	Sub-globose
Seed length (mm)	2.75–2.96	3.21–4.05
Seed width (mm)	1.55–1.59	1.92–1.97
Seed color	Dull black	Shiny black
Seed no./locule	2	2
1000 seed wt (g)	2.12	2.73
Odour when crushed#	Strong onion-light garlic	Strong onion-garlic

*data recorded from a minimum of 30 specimens of each taxon; additional 43 e-images; **: recorded immediately after uprooting; #: data on feedback; also refer Pandey et al. 2021.

pearance; 10–12 vascular bundles are arranged along with the palisade tissue across the entire circumference (Fig. 1F).

Seed morphology. Seed characters and testa sculptures represents a good taxonomic character in *Allium* (Neshati and Fritsch 2009; Celep et al. 2012; Lin and Tan 2017). Apparently, the seeds of the newly described species were marginally bigger than the related taxon, *A. przewalskianum*. Baasanmunkh et al. (2020) have discussed on the seed testa structure and its taxonomic implication for taxa of the subg. *Rhizirideum*. The seed size in *A. negianum* (Fig. 1I) measured 3.2–4.0 × 1.9–1.9 mm in contrast to 2.7–2.9 × 1.5–1.5 mm in the later taxon (Fig. 1I). The seeds of *A. negianum* are obovate in shape with a prominent notch on one side, gradually concave from edge to centre, with deep black and wrinkled testa.

The testa cell shape was irregularly hexagonal-pentagonal, loose with clear meshes of reticulated tissue. The anticlinal walls are usually raised, prominently small to intermediate granulate verrucae. The periclinal cells wall has several verrucae with irregular depressions. Study indicated that in subg. *Rhizirideum* testa cell shape varied from oval to irregular or oval to hemispherical; and seed length 1.30–2.35 mm, anticlinal wall were distinguished by nearly S type to straight and periclinal wall was flat to nearly convex with densely granulated verrucae (Baasanmunkh et al. 2020). *A. przewalskianum* was distinguished by irregular testa cells in a loose arrangement with reticulated tissue, straight to arched anticlinal walls, and concave periclinal walls with small to intermediate verrucae and granules (Lin and Tan 2017).

Distribution and ecology. The sect. *Eduardia* of the subg. *Rhizirideum* is distributed in the southern most range of the Himalayan region of India extending to China which is the centre of diversification. *Allium negianum* is a species recorded from the southernmost transitional zone between India and China. The distribution of *A. negianum* is restricted to the phytogeographical region of western Himalaya from Sumna

valley, Malari, Chamoli district of Uttarakhand, in western Himalaya, India where it commonly occurs along the open grassy meadows, sandy soils along rivers and streams occurring in the snow pasture lands along the alpine meadows (locally known as ‘bugyal’ or ‘bugial’) between 3000–4800 m a.s.l. (Fig. 1A; Fig. 3) in synanthropic habitats. It was reported growing as wild population in Darma valley of Pithoragarh, along Gori Ganga (also Gori Gad) river in the Munsiyari, Pithoragarh district, in Milam Glacier, in northeast of Nanda Devi, Uttarakhand, India. The seeds flowing with the melting snow led to its broader spread in the areas with good regeneration reported by the authors (Fig. 1A). Hence the taxon may be considered endemic in the area of study. Indiscriminate harvest of leaves and bulbs used for ‘seasoning’ purposes has threatened its wild population.

The first report on large scale cultivation of this taxon in Niti valley, Uttarakhand, as ‘seasoning allium spice’ called ‘jambu’ and ‘phran’ has been published (Pandey et al. 2021). Though the taxon was reported commonly under cultivation, the authors have observed the wild populations primarily from the above ‘type’ locality. The authors could not trace large scale cultivation of another taxon, *A. stracheyi* (used for same purpose and known by same local name) in the described locality in Uttarakhand (Pandey et al. 2021). Considering that *A. stracheyi* was a rare species reported from wild habitats in Uttarakhand Himalaya, the authors assume that the reports by Kuniyal and Negi (2018) on large scale cultivation may be referring to this newly described taxon which is also known by the same local name. Unfortunately, earlier studies on *A. stracheyi* did not provide any locality details, nor were the voucher specimens deposited in any herbaria of the material used in their study. Therefore, validation of the taxonomic identity could not be ascertained. Also, there is no occurrence record of the taxa belonging to subg. *Rhizirideum* from Uttarakhand, India.

Specimens examined (Paratypes). *Allium przewalskianum*: INDIA. Himachal Pradesh. Spiti, Takcha 25 Jul. 1972 U.C. Bhattacharya 48815(BSD); Tobo, Kinnaur, Lahul & Spity, 15 Sept. 2007, V.D. Verma & Ramchander (NHCP); Jammu & Kashmir. Ladakh, 25 July 1941, Ludlow & Sheriff8529 (BM); 8 Sep.1941, Ludlow & Sheriff8571 (BM); Ganglas, 1 Aug. 1988, H.J.Chowdhury & B.P.Uniyal 86043 (BSD); 1880, Aitchinson376(CAL); Kashmir. Nubra, 24 July 1980, A.R. Naqshi & G.N. Dhar7370 under *A. stracheyi*; Leh (J&K), 8 Sept. 2014, K. Pradheep & P.S. Mehta1733 (NHCP); Leh (J&K), Nov. 2014, K.Pradheep HS21817(NHCP); Pangu lake, Luthum village, Leh (4500 m), s.s. Malik & D. Gautam15298 (NHCP); Uttarakhand. Malari, Chamoli, 10 Sept. 2019, Badal Singh & K.Madhav RaiHS24013 (NHCP); *Allium auriculatum*: Uttarakhand: Brahmmathya, district Chamoli, August 1988, K.S.Negi & M.N.Kopper 9387 (NHCP).

Online herbaria. *A. stoliczki*: Ladakh, Khaedubgla, 18 Aug. 1982, P.K.Hazra98623(K), 1985, Jacquemont V. Type (K); T. Thomson, Type (K); China, 1 Jan. 1872, Przewalski N.M., #s.n., Type (P); 01 Jan. 1884, Przewalski N.M., Type (P, K); 1872–1873, Przewalski N.M., #s.n., Type (G).

There are no records on the availability of this new taxon from Uttarakhand (Dasgupta 2006). Shah (2014) has raised doubts on reported cultivation of *A. przewalskianum*

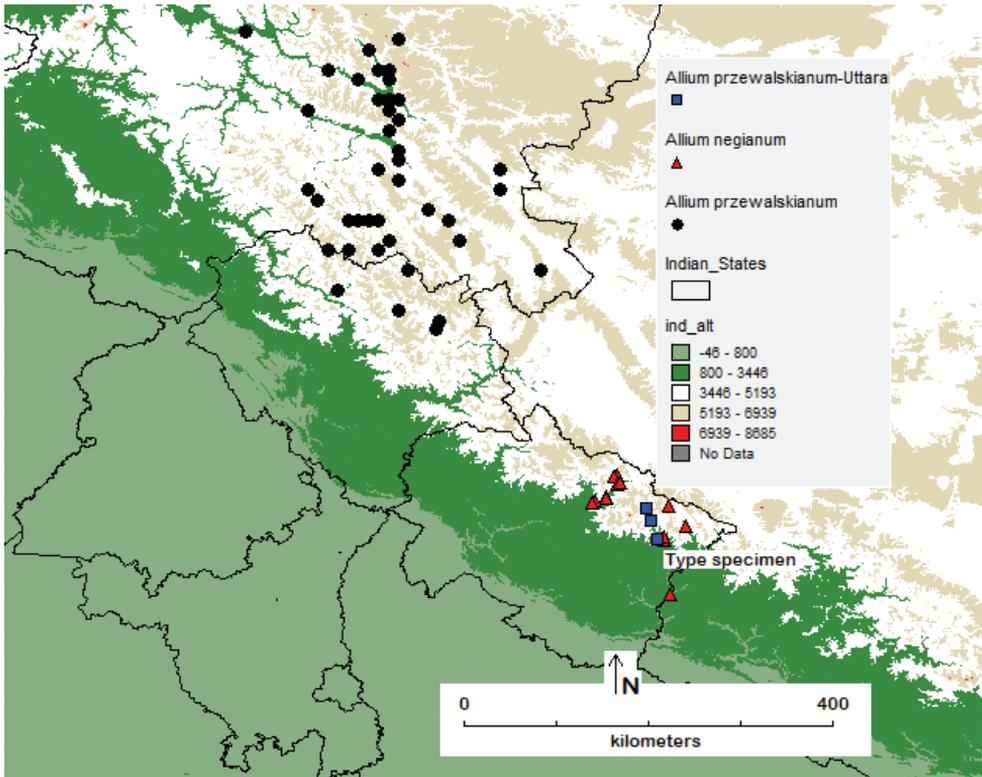


Figure 3. Distribution of taxa of *Allium* subg. *Rhizirideum* sect. *Eduardia* in India: *Allium negianum* and *A. przewalskianum* shown by the red triangle and black circled dots respectively; blue rectangle showed the occurrence of *A. przewalskianum* as per the data from GBIF (records of occurrence from Uttarakhand).

in Uttarakhand by Negi (2006). Also recorded data on the occurrence of allied taxon under *A. przewalskianum* from Gori, Kumaon, Uttarakhand (dated 16 June 2005) and Gori, Martoli, Uttarakhand (7 Oct. 2004) during the study of a total of 413 specimens in the GBIF database need critical study.

Note. *Allium negianum* was previously mistaken for identity as *A. stracheyi* as noted in the published records from India. Despite no morphological similarity with the latter taxon, Kuniyal and Negi (2018) referred 'phran' as *A. stracheyi*. In literature, it was also referred as *A. auriculatum* and *A. przewalskianum* due to morphological similarity of the outer tunics (Pandey et al. 2021). However, the present study demonstrated that *A. negianum* is clearly distinguished from *A. przewalskianum* and *A. stracheyi*, particularly characters of the bulb tunic color when fresh, umbel, teeth in filament and perigone size and color (Fig. 1B–H; Table 1). *Allium negianum* is diploid ($2n = 2x = 16$) (data not produced), whereas *A. przewalskianum* is reported to be tetraploid ($2n = 4x = 32$) as well as diploid with no stated morphological variation except the stout habit. Authors noted that *A. negianum* has robust plant habit, stronger plant aroma in wild habitat as compared to plants growing under cultivation. In contrast, the related taxon of the subg. *Rhizirideum*

is currently distributed in Jammu and Kashmir, Himachal Pradesh and adjoining parts in Nepal. *A. negianum* is reported from areas of Uttarakhand and only known from the type locality (altitude 3200–4800 m a.s.l.) and has never been collected from elsewhere in India and other parts of the world. Therefore *A. negianum* is said to be localized in distribution.

Upon critical examination of specimen of *A. auriculatum* deposited in the NHCP, all plant characters were found to be closer to *A. negianum*. Four specimens of this taxon were noted in label data as frequently growing on flat rocks in Brahmmathya, district Chamoli (3800 m asl.), Uttarakhand, used as leaves cooked as a vegetable.

Allium negianum is morphologically allied to a Chinese species *A. eduardi* Stearn that occurs on the dry slopes and plains in the adjoining regions of Mongolia and Russia and shares characters of spathe beak size, hemispherical umbel and perigone shape, but differs in having yellowish-brown bulb tunic color, tepal apex with a reflexed point and shorter stamen teeth length.

Taxonomic treatment

Two species, *A. przewalskianum* and *A. negianum*, of the subg. *Rhizirideum*, sect. *Eduardia* can be distinguished from *A. stracheyi* of the subg. *Polyprason* by using the following key.

Key to *Allium negianum* and related species

- 1 Bulbs cylindrical-narrowly oblong-ovoid, outer tunic fibrous, with finely reticulate texture, reddish-dark brown, leaves semiterete-terete **2**
- Bulbs cylindrical-narrowly ovoid, outer tunic fibrous scarious, brown-darkest brown, leaves narrow, fistulous..... ***A. stracheyi***
- 2 Bulbs outer tunic reticulate, reddish, inner tunic membranous, red-orange, rarely light brown; umbel compact globose, tepal pale-red to dark purple; filaments longer than perigone segments, inner ones broadened for 1/3–1/2 their length with shallow teeth; style very much exerted after anthesis ***A. przewalskianum***
- Bulbs outer tunic reticulate, reddish-brown, inner tunic membranous red; umbel hemi-spherical, lax; tepals dark purple-pink purple; filaments equal to perigone segments, inner ones broadened at the base for 2/3–1/3 of length, sharply marked teeth; style slightly exerted after anthesis ***A. negianum***

Taxonomic delineation and relationship analysis using nuclear ITS sequence

For taxonomic delineation and relationship analysis data set comprising 18 representative taxa from diverse subgenera were selected (Table 3; Fig. 4). The DNA sequence data set of nuclear Internal Transcribed Spacers (ITS) region used for phylogenetic analysis was generated for *Allium negianum* and other taxa used in the study. The generated ITS sequences and obtained ITS sequences from NCBI (Table 3) were used to construct the maximum likelihood tree. The tree with the highest log-likelihood is shown (Fig. 4). The percentage

of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and selecting the topology with superior log likelihood value. The branch lengths measured in the number of substitutions per site.

Two major clades were found within *Allium*, comprising subgen. *Rhizirideum*, on one side and second cluster had four subg. *Butomissa*, *Allium*, *Polyprason* and *Cepa*. on the other side. This former group was divided in two sister clades, with first clade having *Allium przewalskianum*, *Allium negianum* sp. nov. *A. eduardii* (all from section *Eduardia*); *Allium subangulatum*, *A. polyrhizum* from sect. *Caespitosoprasum*; and *A. nutans*, *A. prostratum*, *A. spurium* and *A. spirale* in sect. *Rhizirideum*. One of the taxon *A. tenuissimum* from sect. *Tenuissima* grouped separately. Second clade was divided into subgenera, namely *Butomissa* with one taxon, *Allium tuberosum*; subg. *Allium*, with *Allium sativum* and *Allium ampeloprasum* var. *ampeloprasum*; subg. *Polyprason* having *Allium stracheyi*; subg. *Cepa* that was the largest having four taxa, *Allium cepa* var. *cepa*, *A. cepa* var. *aggregatum*, *A. oschaninii* and *A. schoenoprasum* from distinct sections.

Based on the likelihood tree, the new *Allium* taxon was observed to be closely related to *A. przewalskianum*, both of Indian Himalayan origin along with a Chinese taxon, *A. eduardii* to form distinct cluster supporting the morphological resemblance of this taxa with section *Eduardii* under subg. *Rhizirideum*. The species from other sections under same genus were distantly placed in the phylogenetic tree. The species which are found in same geographical area belong to different subgenera viz. *Allium*, *Cepa*, *Butomissa* and *Polyprason* were distantly placed and used as outgroup in determining the integrity of newly described species *Allium negianum*.

The above findings indicated that the new taxon is a distinct species and is closely related to *A. przewalskianum* and belongs to sect. *Eduardia* under subg. *Rhizirideum*. These findings supported the observations recorded using plant morphology, particularly the floral characters that were very distinct in both the taxa.

Recent advances in molecular phylogenetics have revolutionized our understanding of *Allium* taxonomy and evolution. However, the phylogenetic relationships in some *Allium* sections (such as the *Allium* sect. *Eduardia*) and the genetic bases of adaptative evolution remain poorly understood for the Indian taxa (Pandey et al. 2021). Molecular phylogeny study of the wild *Allium* in different centers of diversity (Nguyen et al. 2008; Xie et al. 2019; Jang et al. 2021) has helped in unlocking many aspects of the taxon relationships. The present study uncovered a new species relationship with its closest allied species and suggested that the selective habitat pressure has played an important role in the adaptation and evolution of *Allium* in this habitat which will facilitate uncover more taxa in the genus.

Conclusions

Allium negianum, a new species under the subg. *Rhizirideum*, is described using live and herbarium specimens. With the inclusion of this taxon, in the subg. *Rhizirideum* of the sect. *Eduardia* there are two taxa in India, and the latter one *A. negianum* was report-

Table 3. Details of nuclear ITS sequence used in present study.

Sl. No.	Species	Genbank accession number
1	<i>Allium tuberosum</i> Rottler ex Spreng.	MZ567234 (present study)
2	<i>Allium stracheyi</i> Baker	MZ567226 (present study)
3	<i>Allium przewalskianum</i> Regel	MZ567224 (present study)
4	<i>Allium negianum</i> sp. nov.	MZ567225 (present study)
5	<i>Allium sativum</i> L.	MZ567230 (present study)
6	<i>Allium ampeloprasum</i> L. var. <i>ampeloprasum</i>	MZ567231 (present study)
7	<i>Allium cepa</i> L. var. <i>cepa</i>	MZ567228 (present study)
8	<i>Allium cepa</i> L. var. <i>aggregatum</i> G.Don	MZ567232 (present study)
9	<i>Allium oschaninii</i> O.Fedtsch.	MZ567229 (present study)
10	<i>Allium schoenoprasum</i> L.	MZ567227(present study)
11	<i>Allium eduardii</i> Stearn ex Airy Shaw	MK917745
12	<i>Allium subangulatum</i> Regel.	AJ411870
13	<i>Allium tenuissimum</i> L.	AJ411846
14	<i>Allium nutans</i> L.	JN864787
15	<i>Allium prostratum</i> Trevi.	LN867014
16	<i>Allium spurium</i> G.Don.	LN867017
17	<i>Allium spirale</i> Willd.	JN864784
18	<i>Allium polyrhizum</i> Turcz. ex Regel	MK917742

Source: S. no. 1–10 refer table 1; 11–18: NCBI

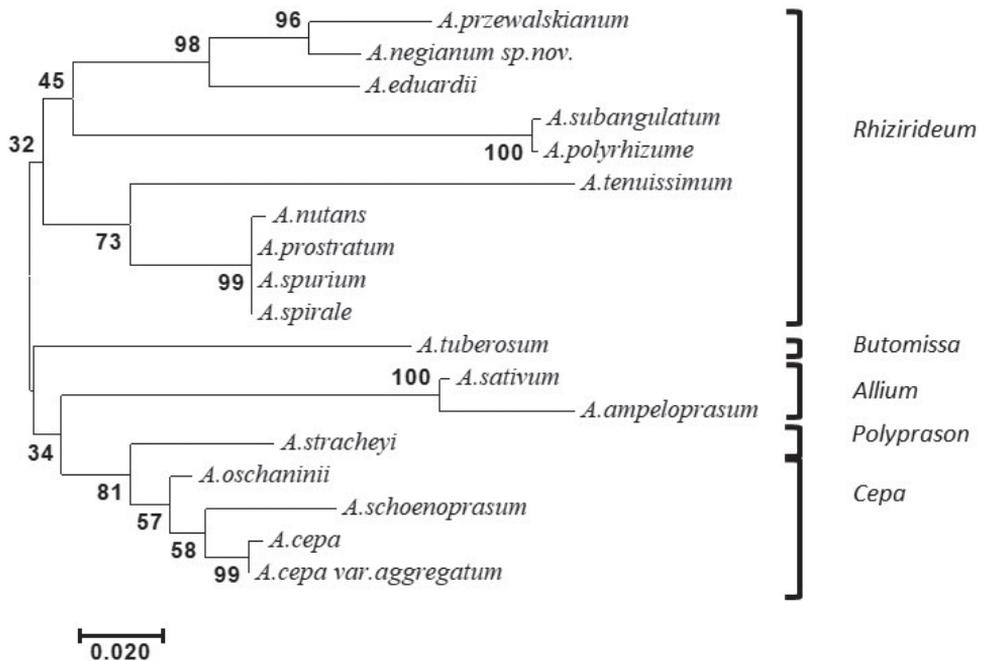


Figure 4. Maximum likelihood tree from nuclear ribosomal ITS sequence from *Allium* taxa showing distinctness of *Allium negianum* sp. nov.

edly restricted to the Uttarakhand flora. Samples of this taxon collected during earlier explorations that remained unidentified will be designated with this new name and conserved as seed in the National Gene Bank (NGB), New Delhi and vegetative material will be maintained in the Field Gene Bank (FGB) at Bhowali, Uttarakhand, India.

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Cymbopleura natellia – a new species from Transbaikal area (Russia, Siberia) described on the basis of molecular and morphological investigation

Anton Glushchenko¹, Evgeniy Gusev¹, Yevhen Maltsev¹,
John Patrick Kociolek^{2,3}, Irina Kuznetsova¹, Maxim Kulikovskiy¹

1 K.A. Timiryazev Institute of Plant Physiology RAS, IPP RAS, 35 Botanicheskaya St., Moscow, 127276, Russia **2** University of Colorado, Boulder; University of Colorado Museum of Natural History, Boulder, Colorado, 80309, USA **3** Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, 80309, USA

Corresponding author: Anton Glushchenko (gluschenkoam@studklg.ru)

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Abstract

A new cymbelloid diatom species from the genus *Cymbopleura* (Krammer) Krammer is described on the basis of molecular and morphological investigations. *Cymbopleura natellia* Glushchenko, Kulikovskiy & Kociolek, **sp. nov.** is, on the basis of results with molecular data, close to *C. naviculiformis* (Auerswald ex Heiberg) Krammer. The two species differ both by molecular distance and morphological features. Morphologically, *C. natellia* **sp. nov.** is compared with several other species in the genus. This work is a pioneer investigation of cymbelloid taxa using molecular tool from Transbaikal area.

Keywords

Bacillariophyceae, *Cymbopleura*, morphology, molecular investigation, new species, Russia

Introduction

Cymbelloid diatoms have unusually high diversity in ancient Lake Baikal but we almost have no information about them in waterbodies surrounding this lake. Historically, cymbelloid diatoms have been referred to taxa with dorsiventral symmetry (Round et al. 1990; Kulikovskiy et al. 2016). However, results from molecular investigations and a better un-

derstanding of morphological features have shown that genera with naviculoid symmetry also belong to this monophyletic group. New diatom genera from Lake Baikal, such as *Ochigma* Kulikovskiy, Lange-Bertalot & Metzeltin (Kulikovskiy et al. 2012), *Paraplaconeis* Kulikovskiy, Lange-Bertalot & Metzeltin (Kulikovskiy et al. 2012) and *Khursevichia* Kulikovskiy, Lange-Bertalot & Metzeltin (Kulikovskiy et al. 2012) are good examples of naviculoid diatoms that are more closely related to cymbelloid taxa (Kulikovskiy et al. 2012, 2016). Lake Baikal includes many “typical” dorsiventral genera and species that were comprehensively investigated by Kulikovskiy et al. (2012). In that work we described many new species from the genera *Cymbella* C.A. Agardh, *Encyonema* Kützing and *Cymbopleura* (Krammer) Krammer (Kulikovskiy et al. 2012) based on valve morphology. However, these new taxa were described without molecular investigation. Our understanding of the diversity of algae in ancient lake systems will also be enhanced by using molecular methods.

Members of the genus *Cymbopleura* were previously considered to be members of the genus *Cymbella*. *Cymbopleura* was separated from *Cymbella* based on the features of absence of apical pore fields and central raphe ends turned towards the ventral side of valve (Krammer 1982; Kulikovskiy et al. 2012). The new genus *Karthickia* Kociolek, Glushchenko & Kulikovskiy is very similar to *Cymbopleura* except that it differs by the presence of a single external stigma opening that has two elongated slit-like openings internally, residing within two longer central striae (Glushchenko et al. 2019). This new genus was described from Southeast Asia. Another new genus that is similar to *Cymbopleura* is *Yasnitskya* Rodionova & Pomazkina (Pomazkina and Rodionova 2014). This genus is characterized by interesting internal areolar morphology. *Yasnitskya* was also described on the basis morphological investigation only.

The aim of the present report is to begin a series of studies using molecular methods to study the identity and systematic placement of cymbelloid diatoms from Lake Baikal and surrounding watrebodies, with the description of new species from the genus *Cymbopleura*. We plan that this work as our pioneer investigation of dorsiventral species from this area and that this work will be continued in future with new molecular data for other known taxa in this lineage.

Materials and methods

Sample collection

The sample used in the present report was collected from Eastern Siberia, Buryatia by E.S. Gusev and M.S. Kulikovskiy on 15.07.2011. It was designated as No. 11.2 and was collected from the Zagza River, periphyton, scraping from macrophytes, $t = 14\text{ }^{\circ}\text{C}$, $\text{pH} = 8.5$, conductivity = $40\text{ }\mu\text{S cm}^{-1}$, $52^{\circ}31.656'\text{N}$, $107^{\circ}05.114'\text{E}$.

Culturing

A subsample of each collection was added to WC liquid medium (Guillard and Lorenzen 1972). A monoclonal strain was established by micropipetting a single cell under

an inverted microscope. Non-axenic unialgal cultures were maintained in WC liquid medium at 22–25 °C in a growth chamber with a 12:12 h light:dark photoperiod. The strain investigated here was designated B209.

Preparation of slides and microscope investigation

The culture was treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 h. Afterwards, the sample was boiled in concentrated hydrogen peroxide (\approx 37%) to remove organic matter. It was washed again with deionized water four times at 12 h intervals. After decanting and filling with deionized water up to 100 ml, the suspension was pipetted onto coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax. Light microscopic (LM) observations were performed with a Zeiss Axio Scope A1 microscope equipped with an oil immersion objective (\times 100, n.a. 1.4, differential interference contrast [DIC]) and Axiocam ERc 5s camera (Zeiss). Valve ultrastructure was examined by means of scanning electron microscopes JEOL JSM-6510LV (JEOL Ltd., Japan) operating at 15 kV and 8 mm of working distance (IBIW, Institute for Biology of Inland Waters RAS, Borok, Russia). For scanning electron microscopy (SEM), part of the suspensions was fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm of Au by means of a Eiko IB 3 (Eiko Engineering, Japan).

The cleaned material, sample and slide are deposited in the collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia. The type slide was designated B209.

Molecular investigation

Total DNA from the studied strain was extracted using Chelex 100 Chelating Resin, molecular biology grade (Bio-Rad Laboratories, USA), according to the manufacturer's protocol 2.2. Partial 18S rDNA (435 bp, including the highly variable V4 region) gene was amplified using primers D512for and D978rev from Zimmermann et al. (2011).

Amplification was carried out using premade polymerase chain reaction (PCR) mastermixes (ScreenMix by Evrogen, Russia). Amplification conditions for 18S rDNA gene were as follows: initial denaturation for 5 min at 95 °C followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 52 °C, and 50 s extension at 72 °C, with the final extension for 10 min at 72 °C. PCR products were visualized by horizontal electrophoresis in 1.0% agarose gel stained with SYBRTM Safe (Life Technologies, USA). The products were purified with a mixture of FastAP, 10 \times FastAP Buffer, Exonuclease I (Thermo Fisher Scientific, USA), and water. The sequencing was performed using a Genetic Analyzer 3500 instrument (Applied Biosystems, USA).

Editing and assembling of the consensus sequences were carried out by processing the direct and reverse chromatograms in Ridom TraceEdit (ver. 1.1.0) and Mega7 software (Kumar et al. 2016). The reads were included in the alignments along with corresponding sequences of 74 diatom species downloaded from GenBank (taxa names

and Accession Numbers are given in Fig. 5). Five diatom species from Rhopalodiaceae were chosen as the outgroups.

The nucleotide sequences of the 18S rDNA gene were aligned separately using the Mafft v7 software and the E-INS-i model (Katoh and Toh 2010). The final alignments were then carried out: unpaired sites were visually determined and removed from the beginning and the end of the resulting matrices. The resulting alignments had lengths of 439 characters.

The data set was analyzed using the Bayesian inference (BI) method implemented in Beast ver. 1.10.1 software (Drummond and Rambaut 2007) to construct phylogeny. For the alignment partition the most appropriate substitution model, shape parameter α and a proportion of invariable sites (pinvar) were estimated using the Bayesian information criterion (BIC) as implemented in jModelTest 2.1.10 (Darriba et al. 2012). This BIC-based model selection procedure TrN+I+G model, $\alpha = 0.5130$ and pinvar = 0.4620 for 18S rDNA gene. We used the HKY model of nucleotide substitution instead of TrN given that it was the best matching model available for BI. A Yule process tree prior was used as a speciation model. The analysis ran for 5 million generations with chain sampling every 1000 generations. The parameters-estimated convergence, effective sample size (ESS) and burn-in period were checked using the Tracer ver. 1.7.1 software (Drummond and Rambaut 2007). The initial 25% of the trees were removed, the rest were retained to reconstruct a final phylogeny. The phylogenetic tree and posterior probabilities of its branching were obtained on the basis of the remaining trees, having stable estimates of the parameter models of nucleotide substitutions and likelihood. The Maximum Likelihood (ML) analysis was performed using RAxML program (Stamatakis et al. 2008). The nonparametric bootstrap analysis with 1000 replicas was used. The programs FigTree ver. 1.4.4 and Adobe Photoshop CC (19.0) were used for viewing and editing of the trees.

Results

Cymbopleura natellia Glushchenko, Kulikovskiy & Kociolek, sp. nov.

Figs 1–4

Holotype. Slide no B209 in collection of MHA, Main Botanical Garden Russian Academy of Science, Moscow, Russia, represented here by Fig. 2J.

Reference strain. Strain B209, isolated in sample No. 11.2.

Type locality. Russia, Eastern Siberia, Buryatia, Zagza River, 52°31.656'N, 107°05.114'E.

Description. *LM* (Figs 1 and 2). Cells solitary. A single chloroplast is present per cell. The chloroplast has two lobes, that underlie the valve face, and they are connected by a wide isthmus (Fig. 1). Valves subelliptical, dorsiventral with moderately convex dorsal margin and slightly convex ventral margin, often almost straight near the valve centre. Ends are bluntly rostrate. Length 17.6–23.5 μm (20.5 ± 1.6 ; $n = 30$), width 8.7–9.5 μm (9.1 ± 0.2 ;

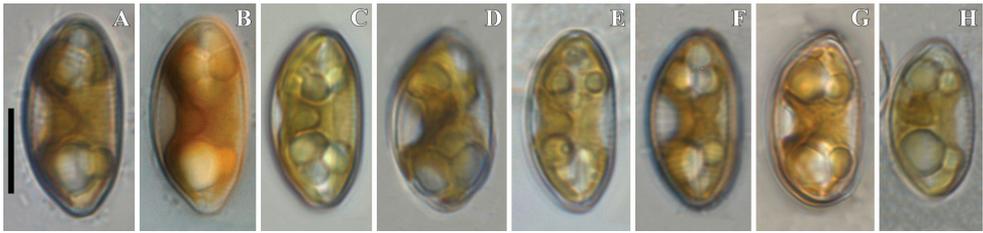


Figure 1. A–H *Cymbopleura natellia* Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Strain B209. Size diminution series. Live cells with chloroplast structure. Scale bar: 10 μ m.

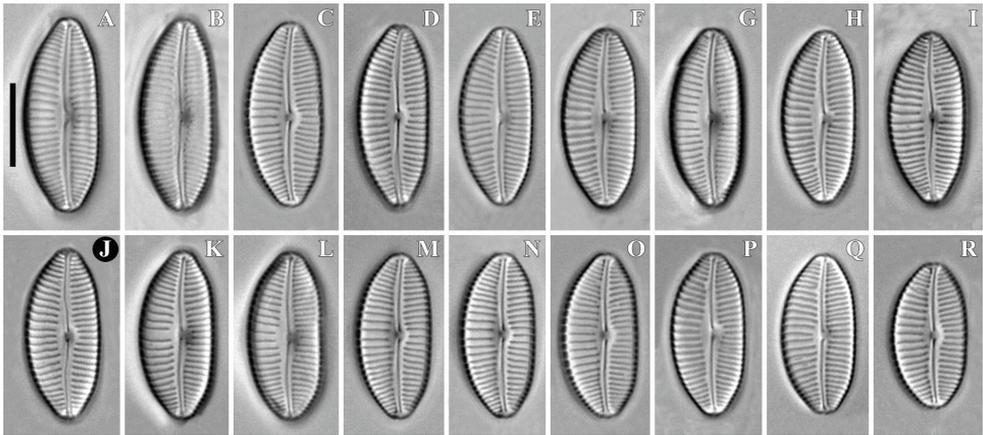


Figure 2. A–R *Cymbopleura natellia* Glushchenko, Kulikovskiy & Kociolek, sp. nov. LM, DIC. Strain B209, slide no B209. Size diminution series. Holotype (J). Scale bar: 10 μ m.

$n = 30$). Length-to-width ratio 1.97–2.52. Central area more or less pronounced, rounded, 1/3 to the valve breadth. Axial area narrow, more often weakly expands to central area, less often – almost not expanded beyond the median line of the valve. Raphe filiform near the center, lateral towards the apices, with proximal raphe ends deflected ventrally, tipped with weakly inflated pores. Striae finely punctate, radiate, becoming subparallel and condensing towards to the ends, 13–15 in 10 μ m (14 ± 0.7 ; $n = 30$) at the central part, 18–20 in 10 μ m (19 ± 0.7 ; $n = 30$) near the ends. Areolae difficult to resolve in LM (Fig. 2).

SEM, external view (Fig. 3). Valve face is flat. Central area formed by shortened striae. Striae uniseriate, extending to valve face towards mantle on both the dorsal and ventral margins, composed of very small, elongate, lineolate areolae, 30–35 in 10 μ m (32.5 ± 1.3 ; $n = 30$). Proximal raphe endings are slightly expanded, unilaterally curved. Distal raphe fissures unilaterally curved opposite the proximal raphe ends, extending onto the valve mantle.

SEM, internal view (Fig. 4). The raphe slits close to proximal endings are arcuate. Proximal raphe endings weakly deflected to the dorsal margin. Distal raphe ends terminated small helictoglossae. Areolae arranged in a series with narrow vimines, compared to the wide interstriae (virgae), occlusions absent but tectullae present.

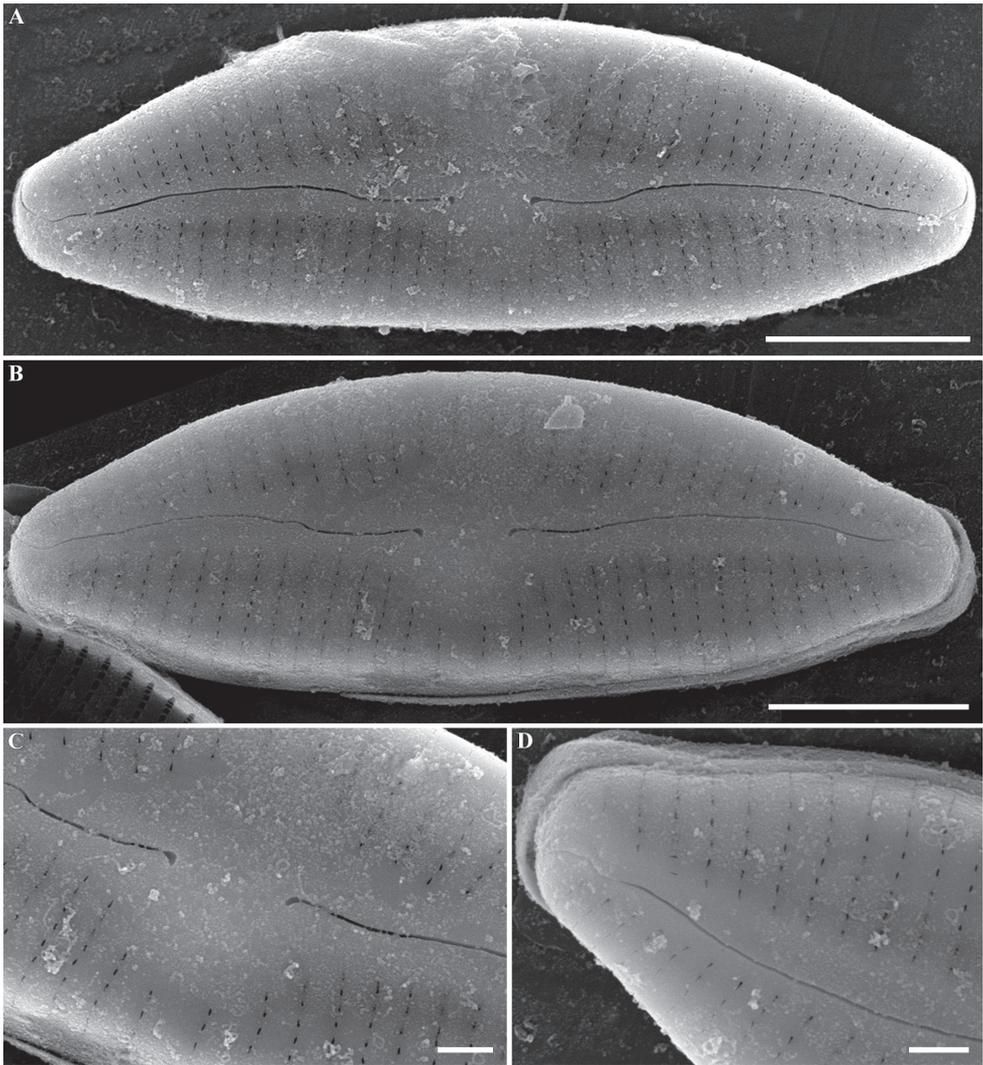


Figure 3. A–D *Cymbopleura natellia* Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Strain B209. External view **A, B** the whole valve **C** central area **D** valve end. Scale bars: 5 μm (**A, B**); 1 μm (**C, D**).

Etymology. New species is dedicated to our friend Natella Otarovna Gabuadze.

Distribution. As yet known only from the type locality.

Sequence data. Partial 18S rDNA gene sequence comprising V4 domain sequence (GenBank accession number MZ503642).

Molecular investigation

The phylogenetic analyses were conducted using a single gene dataset (Fig. 5). Sequences of *Cymbopleura* species as well as *Cymbella*, *Gomphonema*, *Placoneis* and another pennate diatom species were included in the phylogenetic analyses. According

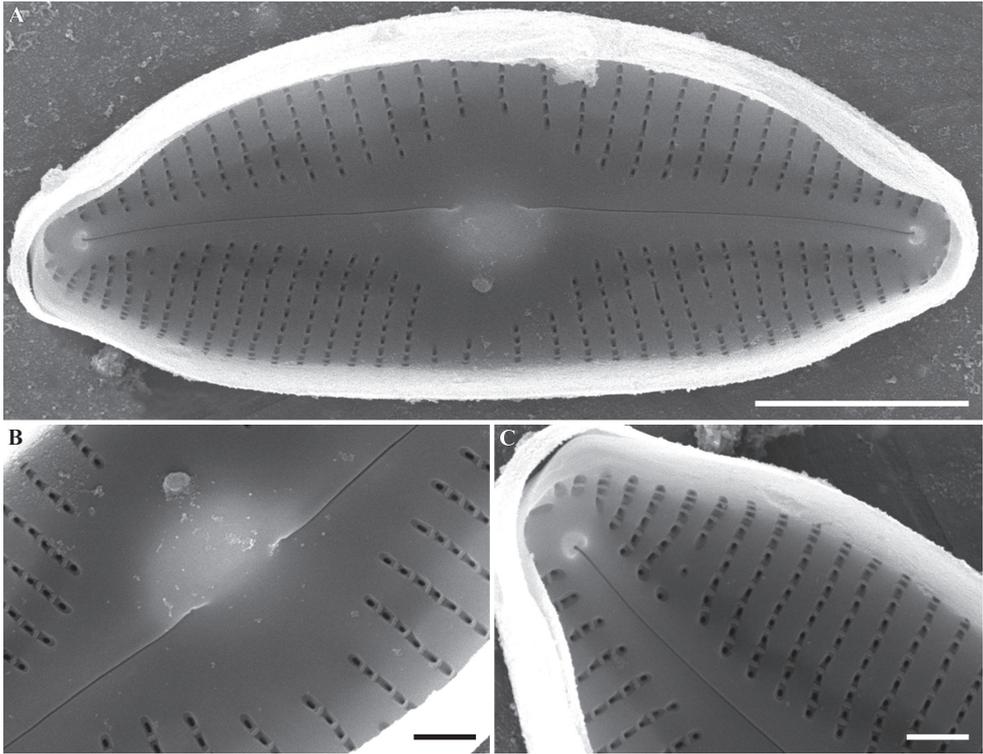


Figure 4. A–C *Cymbopleura natellia* Glushchenko, Kulikovskiy & Kociolek, sp. nov. SEM. Strain B209. Internal view **A** the whole valve **B** central area **C** valve end. Scale bars: 5 μm (**A**); 1 μm (**B, C**).

to the maximum likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses, *Cymbopleura natellia* sp. nov. (the strain B209) appeared most closely related to the strain 22 vi092D of *Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer 2003 with high statistical support (ML 94; BI 0.99) and other *Cymbopleura* strains (Fig. 5). We should point out, however, that *Cymbopleura* is non-monophyletic in these analyses, with two strains of *Cymbopleura inaequalis* and an unidentified *Cymbopleura* strain (D213 001 MK300893) having a closer relationship with some *Cymbella* species than *Cymbopleura* strains.

Discussion

According to the data of molecular analysis, the species *Cymbopleura natellia* sp. nov. is most closely related to *C. naviculiformis*. According to morphological data, the new species is distinguished by bluntly rostrate, not cuneate valve ends, whereas *C. naviculiformis* has rostrate to subcapitate, narrow-protracted valve ends. The central area of the new species is generally smaller ($1/3$ of the valve width) than the central area of *C. naviculiformis* ($1/2$ – $2/3$ of the valve width). The new species differs from *C. naviculiformis* in a shorter valve length (17.6–23.5 μm in the new species versus

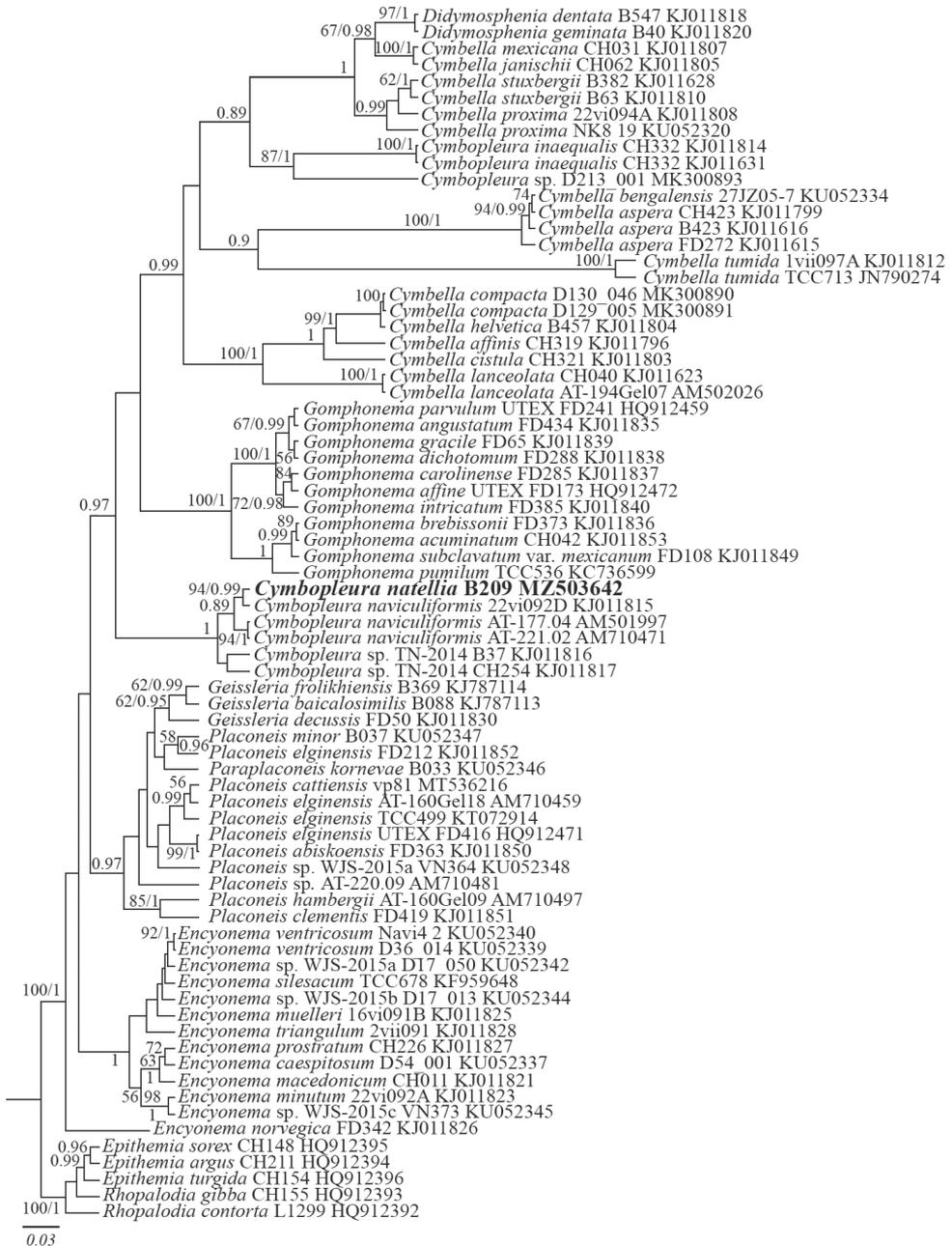


Figure 5. Phylogenetic position of *Cymbopleura natellia* B209 (indicated in bold) based on Bayesian inference for the partial 18S rDNA gene. Total length of the alignment is 439 characters. Bootstrap supports from ML (constructed by RAxML) and posterior probabilities from BI (constructed by Beast) are presented on the nodes in order. Only BS and PP above 50 and 0.85 are shown. Strain numbers (if available) and GenBank numbers are indicated for all sequences.

Table 1. Differences between species of the genus *Cymbopleura*.

	Outline	Ends	Axial area	Central area	Valve length (µm)	Valve width (µm)	Striae in 10 µm	Areolae in 10 µm	Distribution	Reference
<i>C. natellia</i> sp. nov.	subelliptical, dorsiventral with moderately convex dorsal margin and slightly convex ventral margin, which more often almost straight near the valve centre	bluntly rostrate, not protracted	narrow, more often weakly expands to central area, less often – almost not expands, almost the median line of the valve	more or less pronounced, rounded, 1/3 the valve breadth	17.6–23.5	8.7–9.5	13–15 at the central part, 18–20 near the ends	30–35	Russia: Baikal Lake	This study
<i>C. naviculiformis</i>	elliptic-lanceolate, moderately dorsiventral, dorsal margin strongly arched, ventral margin slightly convex to nearly flat	rostrate to subcapitate, narrow-protracted	narrow, linear or slightly broader towards the relatively large central area, almost in the median line of the valve	rounded, one-half to two-thirds valve width	26–50	9.0–13.0	10–15 at the central part, 16–19 near the ends	27–35	Widely distributed	Krammer 2003; Bahls 2019
<i>C. designata</i>	broadly lanceolate, moderately dorsiventral with strongly arched dorsal and ventral margins	apiculate, extending laterally from valve ends	area about 1/4 th to 1/5 th valve width, slightly curved and narrower near apices	rounded, scarcely wider than the axial area	26–37	9.0–11.6	12–14 at the central part, up to 18 near the ends	26–30	Sub-Arctic and Arctic	Lange-Bertalot and Genkal 1999; Bahls 2019
<i>C. frequens</i>	not or very slightly dorsiventral, subelliptical-lanceolate, dorsal and ventral margins moderately arched	protracted, apiculate to rostrate	narrow, linear, narrowing slightly towards to the ends, almost median line of the valve	irregular, asymmetric space of different extent	14–38	6–9	11–14 at the central part, 16 near the ends	30–36	Holarctic	Krammer 2003; Kulikovskiy et al. 2016; Bahls 2019
<i>C. hercynica</i>	slightly dorsiventral, broadly subelliptical to elliptical-lanceolate, dorsal margins strongly, ventral margin slightly arched	apiculate to subrostrate protracted	very narrow, almost the median line of the valve	1/3–1/2 the valve breadth, distinctly set off, rounded, sometimes asymmetrical and then more well-developed dorsally	16–40	7–10	12–15 at the central part, up to 20 near the ends	32–36	Holarctic, saline habitats or with higher electrolyte content	Krammer 2003; Li et al. 2007

26–50 µm in *C. naviculiformis*), as well as, in general, a smaller valve width (8.7–9.5 µm in the new species versus 9.0–13.0 µm in *C. naviculiformis*). The density of striae overlaps in species (13–15 in 10 µm in the central part, 18–20 towards the ends in the new species versus 10–15 in 10 µm in the central part, 16–19 in 10 µm towards the ends in *C. naviculiformis*) and areolae (30–35 in 10 µm in the new species versus 27–35 in *C. naviculiformis*) (Table 1).

Cymbopleura natellia sp. nov. is morphologically similar to *Cymbopleura designata* (Krammer) Bahls (in Bahls 2019). The two species are close in terms of density of striae (13–15 in the central part, 18–20 at the ends in the new species versus 13–15 in the central part, 18–20 at the ends in *C. designata*). The main difference between these two species is the shape of valves. The new species has subelliptical valve outlines while *C. designata* has a wide lanceolate shape of the valves. The central area of the new species is more differentiated than that of *C. designata*, the central area of which is slightly wider than the axial area. The ends of *C. designata* are apiculate, extending laterally from valve ends. There is also a difference in areolae density (30–35 in 10 µm in the new species versus 26–30 in 10 µm in *C. designata*).

Cymbopleura natellia sp. nov. is also morphologically similar to *C. frequens* Krammer 2003. These two species have a similar number of areolae (30–35 in 10 µm in the new species and 30–36 in 10 µm in *C. frequens*). At the same time, the species differ in the outline of the valves, with the valves of *C. frequens* being almost naviculoid or slightly dorsiventral, while in *C. natellia* sp. nov. the valves are subelliptic and evidently dorsiventral. The ends of *C. frequens* are protracted, apiculate to rostrate.

Cymbopleura hercynica (A.W.F. Schmidt) Krammer 2003 is another species that is morphologically similar to the new species, in terms of stria density (12–15 in the central part, up to 20 in 10 µm toward to the ends in *C. hercynica* versus 13–15 in the central part, 18–20 in 10 µm to the ends in the new species) and density of areolae (32–36 in 10 µm in *C. hercynica* versus 30–35 in 10 µm in the new species). Also, the two species are generally similar in valve shape. The species differ in the shape of the valve ends, in the new species the ends are wide, in *C. hercynica* they are apiculate to subrostrate protracted. The width of the central area of *C. hercynica* reaches 1/2 of the total valve width; in the new species, this ratio does not exceed 1/3. It should be noted that the new species was isolated from the Zagza River, where the water conductivity is low, while *C. hercynica* prefers brackish-water habitats or reservoirs with increased salinity (Krammer 2003; Li et al. 2007).

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Hemiboea chanii (Gesneriaceae), a new species from limestone areas of northern Vietnam

Cuong Huu Nguyen^{1,2}, Ly Van Nguyen¹, Khang Sinh Nguyen³,
Alexander A. Egorov⁴, Leonid V. Averyanov⁵

1 Vietnam National University of Forestry, Xuan Mai, Chuong My, Ha Noi, Vietnam **2** Saint Petersburg State Forestry University, Institute str., 5, St. Petersburg, 194021, Russia **3** Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Nghia Do, Cau Giay, Hanoi, Vietnam **4** St. Petersburg State University, 7-9 Universitetskaya Emb., St. Petersburg, 199034, Russia **5** Komarov Botanical Institute Russian Academy of Sciences, Prof. Popov str., 2, St. Petersburg, 197376, Russia

Corresponding author: Cuong Huu Nguyen (nguyenuucong.tvr@gmail.com)

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Abstract

Hemiboea chanii, a new species of Gesneriaceae from Ha Giang Province, northern Vietnam, is here described and illustrated. It has many branched stems, diamond-shaped involucre with two cirrose opposite apices, a pink corolla, red spotted inside, and a flowering time in January–February. Among congeners with an externally hairy corolla, this new species is morphologically close to *H. crystallina* and *H. sinovietnamica*. Diagnostic discriminative characters in all mentioned species are discussed. The conservation status of this species is considered to be “Critically endangered” (CR) according to the IUCN Red List Categories and Criteria.

Keywords

Endemism, flora of Vietnam, limestone flora, New taxon, plant diversity, plant taxonomy

Introduction

Prior to 2011, *Hemiboea* Clarke (1798) (Gesneriaceae) contained about 23 species and 5 varieties, distributed mainly from northern Vietnam to southern and eastern China to southern Japan (Weber 2004; Li and Wang 2004). In 2011 the formerly recognized genus, *Metabriggsia* W.T.Wang (1983), with two species, *Metabriggsia ovalifolia* W.T.Wang and *Metabriggsia purpureotincta* W.T.Wang, was revised and merged with *Hemiboea* (Weber et al. 2011). During last two decades, 14 new taxa were found and described in China and Vietnam: 13 new species and one new variety from Guangxi, Guizhou and Yunnan provinces of southern and southwestern China (Huang et al. 2020) and one from Thanh Hoa Province of northern Vietnam (Nguyen et al. 2019a). In addition, based on extensive morphological, phenological, and molecular studies, *H. subcapitata* var. *pteroaulis* Z.Y.Li (Li 2004) was accepted as a distinct species *H. pterocaulis* (Z.Y.Li) J.Huang, X.G.Xiang & Q.Zhang in Huang et al. (2017). Similarly, *H. subcapitata* var. *guangdongensis* (Z.Y.Li) Z.Y.Li became *H. guangdongensis* (Z.Y.Li) X.Q.Li & X.G.Xiang in Li et al. (2019). In total, before our study, the genus *Hemiboea* comprises at least 39 species and 5 varieties.

During the botanical fieldwork in limestone areas of Ha Giang Province in northern Vietnam in spring 2021, we collected an unusual species of *Hemiboea*. The genus characteristics are calyx 5-parted to the base, stamens 2, staminodes 3, stigma 1 (undivided), and capsule oblique in relation to pedicel, narrowly lanceolate, somewhat curved, dehiscent adaxially; valves 2, straight, not twisted. After consulting relevant literature of *Hemiboea* (Wang et al. 1998; Pham 2000; Li and Wang 2004; Wei et al. 2010; Do et al. 2016; Luu et al. 2018; Wei 2018; Nguyen et al. 2019b) including recently published papers for new taxa of Gesneriaceae from Vietnam (e.g. *Michaelmoelleria* F.Wen, Y.G.Wei & T.V.Do in Wen et al. 2020) as well as the examining of herbarium specimens, we identified our plants as a new species, well segregated from all known species of *Hemiboea* by its morphological characters, namely a diamond-shaped involucre with a cirrose apex; a 1-veined calyx with flat, not revolute margin; a pink corolla with red spots, and a slightly swollen stigma. This new species is described and illustrated here as *H. chanii* C.H.Nguyen & Aver.

Material and methods

All collected and studied specimens of the newly discovered species are presently stored in the Herbaria of China, Russia, and Vietnam (IBK, IBSC, KUN, LE, PE, VNF). The photographs used for the species illustration were taken in the species natural habitats. Morphological observations and measurements were made on living plants, dry specimens, and materials preserved in alcohol. Morphological characters are described using the terminology proposed by Wang et al. (1998).

Taxonomic treatment

Hemiboea chanii C.H.Nguyen & Aver., sp. nov.

urn:lsid:ipni.org:names:77221221-1

Figs 1, 2

Type. VIETNAM. Ha Giang Province: Vi Xuyen District, Minh Tan Village, primary evergreen broad-leaved forest, around point 23°00'14.9"N 104°54'55.9"E, altitude 533 m, 19 January 2021, *N.V.Ly*, *NVL 20210119001* (holotype: VNF!; isotype: LE <http://en.herbariumle.ru/?t=occ&id=91550>).

Diagnosis. *Hemiboea chanii* is similar to *H. crystallina* Y.M.Shui & W.H.Chen in the shape of leaf blade, leaf margin and the externally pubescent corolla, but differs in having non-inflated nodes, diamond-shaped not winged involucre, flat calyx margin, corolla red-spotted inside with a ring of hairs, and stigma slightly swollen. It also morphologically resembles *H. sinovietnamica* W.B.Xu & X.Y.Zhuang but differs in having 4–8 branches on main stem, repand-crenate leaf margin, involucre diamond-shaped with a cirrose apex, and pink corolla and a slightly swollen stigma (Table 1).

Description. Perennial lithophytic herb. Stem ascending to erect, with 12–16 nodes on the main stems, not inflated, glabrous, subterete, 50–90 cm tall, 3–7 mm in diameter, with 4–8 branches. Leaves petiolate, opposite, unequal to sub-equal in a pair; petiole 1.5–4.5 cm long, about 2 mm in diameter, glabrous, green to purple; leaf blade narrowly ovate, ovate, oblong or elliptic, coriaceous when dry, 7.5–12 cm long, 2.5–5 cm wide, glabrous, adaxial surface dark green, abaxial surface pale greenish, at base cuneate, sometimes slightly oblique, apex acute or shortly acuminate, repand-crenate along the margin, median and lateral veins inconspicuous adaxially and protuberant abaxially, lateral veins in 6–10 pairs. Inflorescence subterminal, 2–3-flowered cyme; peduncle 0.8–1 cm long, about 1 mm in diameter, glabrous, green to pale green; involucre diamond-shaped, not winged, 1.6–1.8 cm in diameter, outside glabrous, with two cirrose opposite apices. Calyx white, actinomorphic, 5-lobed, dissected from the base; segments subequal, narrowly lanceolate 1.3–1.4 × 0.2–0.3 cm, glabrous, margin entire flat, 1-veined. Corolla infundibular, outside pink with numerous red spots on adaxial lip, inside red spotted, 3.5–4 cm long; tube 3.0–3.6 cm long, 1.4–1.6 cm in diameter at the orifice, 3–4 mm in diameter at the base, sparsely glandular pubescent outside; inside with a ring of hairs adnate to 3–4 mm above the corolla base; limb distinctly two-lipped; adaxial lip 3–4 mm long, 2-lobed at apex, lobes subequal, nearly semi-circular, margin recurved; abaxial lip 6–8 mm long, 3-lobed, lobes unequal, with rounded apex, median lobe larger, broadly ovate, lateral 2 smaller, slightly obliquely ovate. Stamens 2, adaxial, adnate to 1.1–1.3 cm above the corolla base; filaments filiform, coiled, 1.2–1.4 cm long, 1 mm in diameter; anthers basifixed, globular, 1.5–1.7 mm in diameter, coherent at the apex. Staminodes 3, linear, glabrous, with inflated apex, adnate to 13–15 mm above the corolla base, the middle one 2–3 mm long,



Figure 1. *Hemiboea chanii* C.H.Nguyen & Aver., sp. nov. **A** plants in natural habitat **B** leaf blade, abaxial surface **C** flowering branch **D** flower, frontal view **E–G** flower, side and half side views **H** stamens and staminodes **I** involucre **J** flower inside and outside views **K** ripening capsule, cross section **L–M** pistil and ripening capsule, side view **N** calyx segments. Photos by Nguyen Van Ly, correction and design by C.H. Nguyen.



Figure 2. *Hemiboea chanii* C.H.Nguyen & Aver., sp. nov. isotype specimen detail. N.V.Ly, NVL 20210119001 (isotype: LE 01077046 <http://en.herbariumle.ru/?t=occ&id=91550>).

lateral ones 9–10 mm long. Disc circular, lemon-yellow, 1.2–1.4 mm high, margin repand, glabrous. Pistil 24–26 mm long; ovary narrowly cylindrical, glabrous, 7–8 mm long, about 2 mm in diameter; style 17–18 mm long, terete, glabrous, about 1 mm in diameter, apex curved; stigma slightly swollen, truncate. Capsule oblique in relation to pedicel, terete to narrowly fusiform, 2.6–3 cm long, glabrous, slightly curved, dehiscent adaxially; valves 2, straight, not twisted.

Distribution and habitat. The new species is only known from Minh Tan Village, Ha Giang Province, growing in cracks of limestone rocks in moist shady places or occasionally in the upper part of slopes, and on rocky hilltops in primary evergreen broad-leaved forests on karstic limestone at elevations 500–700 m a.s.l. Some of main herbaceous species accompanying the new species have been recorded as *Aglaonema modestum* Schott ex Engl., *Amorphophallus tonkinensis* Engl. & Gehrm., *Begonia* spp., *Impatiens bonii* Hook.f., *Laportea interrupta* (L.) Chew, *Primulina balansae* (Drake) Mich.Möller & A.Weber, and *Rhaphidophora decursiva* (Roxb.) Schott

Phenology. Flowering from January to February, and fruiting from February to April.

Etymology. *Hemiboea chanii* is named in honor of the lecturer, Mr. Le Mong Chan, for his outstanding contributions to the conservation of the flora of Vietnam.

Proposed IUCN conservation status. At present, only one known population with less than 100 mature individuals is confirmed in the field assessment. Its estimated area of occupancy is less than 5 km². The population and habitat are greatly susceptible to various human activities and damage, particularly forest logging, agriculture, and grazing. There is a high risk of habitat degradation in the future because it is located close to the local village. Following the IUCN Red List Categories and Criteria (IUCN 2017), the new species may be assessed tentatively as Critically Endangered [B2ab (ii, iii), CR].

Note. *Hemiboea chanii* is morphologically similar to *H. crystallina* and *H. sinovietnamica* in its ecology. It has only been found in a limestone area and grows in the evergreen broad-leaved forest. The new species can be easily distinguished from *H. crystallina* in having a subterete stem up to 90 cm tall (vs. stem terete up to 40 cm tall), non inflated nodes (vs. nodes inflated), 1-veined calyx with flat, not revolute margin (vs. calyx 3-veined with revolute margin), corolla red spotted inside with a ring of hairs (vs. corolla inside purplish-red spotted with pale yellowish lines and absence of hair ring), and stigma slightly swollen (vs. stigma not swollen). The new species differs from *H. sinovietnamica* in having a subterete, 4–8 branched stem up to 90 cm tall (vs. stem subtetragonal, simple, up to 45 cm tall), repand-crenate leaf margin (vs. entire leaf margin), involucre diamond-shaped with a cirrose apex (vs. involucre triangular with acute apex), corolla pink (vs. corolla yellowish), stigma slightly swollen (vs. stigma distinctly capitate), and a flowering period lasting in January–February (vs. flowering period lasting during August–October). The comparison of the key morphological characters of *H. chanii*, *H. crystallina*, and *H. sinovietnamica* is presented in Table 1.

Table 1. Morphological characters of *H. chanii*, *H. crystallina* and *H. sinovietnamica*.

	<i>H. chanii</i>	<i>H. crystallina</i>	<i>H. sinovietnamica</i>
Stem height and shape of cross-section	50–90 cm, subterete	40 cm or less, terete	25–45 cm, subtrapezoidal
Number of stem branches	4–8	2–6	stem simple
Node	not inflated	inflated	not inflated
Leaf margin	repand-crenate	repand-crenate	entire
Involucre characters	diamond-shaped, not winged, apex cirrose	quadrangular, winged on costas, apex acute	triangular, not winged, apex acute
Calyx lobe characters	margin flat, not revolute, 1-veined	margin revolute, 3-veined	margin flat, not revolute, 1-veined
Corolla characters	pink, inside red spotted with a ring of hairs	white, inside purplish-red spotted with pale yellowish lines, hair ring absent	yellowish, inside purple-spotted with a ring of hairs
Stigma	slightly swollen	slightly obtuse	distinctly capitate
Flowering period	January–February	November	August–October

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Marine and brackish *Luticola* D.G.Mann (Bacillariophyta) species from the Java Sea and South China Sea coasts with the description of three new species

Mateusz Rybak¹, Andrzej Witkowski^{2,3}, Łukasz Peszek¹, John P. Kociolek⁴, Yenny Risjani⁵, Duc Hung Nguyen⁶, Jinpeng Zhang⁷, Yunianta⁸, Van Duy Nguyen⁶, Romain Gastineau^{2,3}, Thi Thuy Duong⁹, Philippe Rosa¹⁰, Vona Meleder¹⁰

1 Department of Agroecology and Forest Utilization, Institute of Agricultural Sciences, Land Management and Environmental Protection, University of Rzeszów, ul. Ćwiklińskiej 1a, 35-601 Rzeszów, Poland **2** University of Szczecin, Institute of Marine and Environmental Sciences, Mickiewicza 16A, 70-383 Szczecin, Poland **3** Natural Sciences Education and Research Centre, University of Szczecin, Mickiewicza 16a, 70-383 Szczecin, Poland **4** Museum of Natural History and Department of Ecology and Evolutionary Biology University of Colorado, Boulder, Colorado, 80309 USA **5** Faculty of Fisheries and Marine Sciences, Brawijaya University, Jl. Veteran, 65145 Malang, Indonesia **6** Faculty of Natural Sciences Pedagogy, Saigon University, Ho Chi Minh City, Vietnam **7** Guangzhou Marine Geological Survey, China Geological Survey/Key Laboratory of Marine Mineral Resources, Ministry of Natural Resources, 188 Guanghai Rd., Guangzhou 510760, China **8** Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, 65145 Malang, Indonesia **9** Institute of Environmental Technology, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam **10** Université de Nantes, EA 2160 Mer – Molécules – Santé 2, Rue de la Houssinière, 44322 Nantes, France

Corresponding author: Mateusz Rybak (matrybak91@gmail.com)

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Abstract

In this study, samples were collected from the Java Sea coasts, from the South China Sea in Hainan Island coasts and Quảng Yên region and Rú Chá mangrove near Hue in Central Vietnam. In studied samples a total of eight *Luticola* species have been observed. Three of the taxa studied are described herein as species new to science – *Luticola orientalis* sp. nov., *L. cribriareolata* sp. nov. and *L. halongiana* sp. nov. Under light microscopy (LM) *L. orientalis* sp. nov. and *L. cribriareolata* sp. nov. are similar with rhombic-lan-

ceolate to rhombic/ elliptic-lanceolate to elliptic valve shapes and narrowly rounded apices. Both species can be easily distinguished by stria density (higher density in *L. orientalis*). Under SEM *L. cribriareolata* is characterized by cribrate areola occlusions, a character thus far observed only in three established species. The remaining species of the whole genus known thus far are characterized by hymenate areola. Similar morphology *Luticola* species have been observed from tropical mangrove forests from Madagascar but they all can be easily distinguished based on the lack of grooves in the central area. The third species – *L. halongiana* **sp. nov.** has rhombic-elliptic to rhombic-lanceolate valves with broadly rounded to slightly protracted apices in larger specimens. This species has a relatively broad central area. Also unique among brackish-water *Luticola* is the small, rounded stigma positioned almost midway between the valve center and valve margin. In the habitats from which the new species are described we also identified five established *Luticola* taxa including, *L. belawanensis*, *L. celebesica*, *L. inserata*, *L. seposita* and *L. tropica*. For those species we provide detailed SEM characteristics of valve ultrastructure, as well as the range of environmental conditions and geographic distribution within the study area.

Keywords

Brackish waters, *Luticola* genus, marine tropical coasts, morphology, offshore aquaculture

Introduction

The genus *Luticola* was established in Round et al. (1990). It shows great variability in size and shape of the valve, as well as in the types of environment in which they may occur. In a monograph on the genus *Luticola*, (Levkov et al. 2013) presented about 200 species, 93 of which they described as new. The taxonomic revision and update on established taxa, and description of a wealth of new ones performed by the above authors, are considered to be starting points for further taxonomic research on *Luticola*. In the following papers descriptions or appropriate transfers of almost 50 taxa from various parts of the world have been published (Guiry and Guiry 2021). Most of the new taxa descriptions and new combinations are concerned with species found in Asia (Glushchenko and Kulikovskiy 2015; Kale et al. 2017; Glushchenko et al. 2017; Liu et al. 2017; Lokhande et al. 2020). Numerous new taxa have also been described from the Antarctic region (Zidarova et al. 2014; Kohler et al. 2015; Chattová et al. 2017; Kochman-Kędziora et al. 2020) as well as from South America (Bağ et al. 2017; Bustos et al. 2017; Straube et al. 2017; Da Silva-Lehmkuhl et al. 2019; Simonato et al. 2020), from Europe (Levkov et al. 2017; Coste et al. 2019; Hindáková and Noga 2021) and from Madagascar (Bağ et al. 2019). And, most recently, three *Luticola* species new to science were described from the cave entrance of one of the most remote islands in the World Ocean, the Rapa Nui (i.e. Easter Island, Chile) (Peszek et al. 2021).

Material for this study was collected from various microhabitats from coastal regions surrounding the Java Sea coasts (north coast of Java Island) and from the South China Sea in Hainan Island coasts (S China), Quang Yen, Quang Ninh province (NE Vietnam) and Ru Cha mangrove, Thua Thien Hue province (Central Vietnam). Collections were derived from a wide range of salinities, from a range of brackish water sites up to a fully marine site, and included various biofilms from tidal mudflats, oyster shells, rocks and hydrotechnical constructions that turned out to host abundant and

sometimes even dominant populations of *Luticola* species. Most of the species observed in our samples are well-known from the tropical ocean coasts across the globe and are known to prefer brackish water environments (Levkov et al. 2013). Brackish water and marine habitats are unusual for *Luticola* species as most of them either inhabit freshwaters or can be found in various kinds of terrestrial habitats including soils (e.g. Levkov et al. 2013, 2017; Zidarova et al. 2014; Kochman-Kędziora et al. 2020). Until now only a few species have been reported from brackish environments and, except the generic type species *L. mutica* which can be abundant in European estuaries (Levkov et al. 2013; Lange-Bertalot et al. 2017), almost all of them occur in the tropics (Levkov et al. 2013). Some, including e.g. *L. tropica* Levkov, Metzeltin & Pavlov, are widely distributed in tropical estuaries (Fernandes et al. 1990; Navarro and Lobban 2009; Levkov et al. 2013; Straube et al. 2017; Glushchenko et al. 2017) whereas the others occur in more restricted areas like in waters from SE Asia to the coast of Australia (*L. belawanensis* Levkov & Metzeltin, *L. inserata* (Hustedt) D.G.Mann, *L. lacertosa* (Hustedt) D.G.Mann, *L. novaeguineensis* (Tempère) Levkov, Metzeltin & Pavlov) (Foged 1978; Levkov et al. 2013; Glushchenko et al. 2017), to Madagascar (*L. madagascarensis* M. Bağ, Kryk & Peszek and *L. nosybeana* Kryk, M. Bağ & Peszek in Bağ et al. 2019) and to Galapagos islands (*L. galapagoensis* Witkowski, Bağ, Kociolek, Lange-Bertalot & Seddon and *L. darwinii* Witkowski, Bağ, Kociolek, Lange-Bertalot & Seddon in Bağ et al. 2017). It is worth mentioning that the morphologically similar genus *Luticolopsis* Levkov, Metzeltin & Pavlov, which is monotypic (*L. vietnamica* Levkov, Metzeltin & Pavlov), is also found inhabiting brackish water habitats (Levkov et al. 2013).

The aim of this paper is to provide a description of three new species – *Luticola orientalis* M. Rybak, Peszek, J.P. Zhang & Witkowski sp. nov., *Luticola cribriareolata* Witkowski, M. Rybak, Risjani & Yuniarta sp. nov. and *L. halongiana* M. Rybak, Witkowski, H-D. Nguyen & D-V. Nguyen sp. nov. We also provide for the first time detailed characteristics of valve ultrastructure and supplementing of knowledge of the following *Luticola* species: *L. belawanensis* Levkov & Metzeltin and *L. inserata* (Hustedt) D.G.Mann, *L. seposita* (Hustedt) D.G.Mann and *L. tropica* Levkov, Metzeltin & Pavlov. Based on published sources, the geographic distribution of the established *Luticola* species is provided. These taxa seem to comprise a group of brackish-water to fully marine species confined to tropical coasts, primarily mangroves and tidal flats, but also biofilms on rock surfaces, oyster shells and hydrotechnical constructions.

Material and methods

Sampling sites (Fig. 1)

Site 1 – The Java Sea, East Java north coast (E Java, N coast); 07°46'42"S, 113°16'34"E

The sampling area was located on the north coast of eastern part of Java Island bordered by the southern part of the Java Sea. In contrast to the south coast of East Java, the north-



Figure 1. Map showing location of sampling sites: 1–The Java Sea, East Java north coast, 2–NW South China Sea, Hainan Island, China 3–W South China Sea, Quang Ninh province, NE Vietnam, 4–Rú Chá mangrove, Central Vietnam.

ern part of Java is less bright, and has lower light levels penetrating the water column due to, in some places, turbid waters heavily loaded with sediment. Measured environmental parameters according to Risjani et al. 2021 are presented in Table 1. For this study we used samples with accession number SZCZ 27006 and SZCZ 27007, both of which originate from the Probolinggo coastal zone. The habitats sampled involved periphyton from a plastic pier and from the boulders protecting the coastal zone in Probolinggo at Pantai Bentar. Material was collected by Y. Risjani and Yunianta on March 1st 2020.

Site 2 – NW South China Sea, Hainan Island; 18°35'2"N, 110°10'31"E

The sampling area was located on the coast of Hainan Island, in the NW South China Sea (Fig. 1). This island has numerous bays (e.g. Yangpu Bay, Sanya Bay) and provides suitable habitats for rich diatom assemblages of sandy beaches, rocks, mangroves and coral reefs with numerous hydrotechnical constructions (Hainan Provincial Local History Office 2020; Hainan Provincial Bureau of Statistics 2020). The coastal sea water temperature around Hainan Island in winter, at Haikou is 18.7 °C, while in Sanya in

Table 1. Samples characteristics and physicochemical parameters of water of studied sites (– means no data available).

Sampling site	Site 1: The Java Sea, East Java north coast	Site 2: NW South China Sea, Hainan Island	Site 3: W South China Sea, Quảng Yên, NE Vietnam	Site 4: Rú Chá mangrove, Central Vietnam
Sample number	SZCZ 27006 SZCZ 27007	SZCZ27176	SZCZ26472	SZCZ26505
Sample type	periphyton from a plastic pier and boulders	microbial mat developed on a pier	periphyton from oyster shells from aquaculture	sediments from mangrove area
Water temperature [°C]	30.0–32.6	24.9	27–32	29.6
pH	7.7–8.3	8.2	5.6–6.6	6.5
Salinity [psu]	27.7–30.4	32.8	22.0–23.0	7.0
Conductivity [μ S/cm]	–	–	34 700–36 500	–
Dissolved oxygen [%]	–	–	–	5.9
Dissolved oxygen [mg/L]	3.5–6.6	–	–	0.45

the south it increases to 22 °C. The annual difference in sea water temperature oscillates between 7 and 11 °C (Hainan Provincial Local History Office 2020; Hainan Meteorological Service 2020). The average annual salinity of the surface seawater along the coast of Hainan Island is 32.64. The salinity extremes are as high as 36.0 psu and 36.2 psu in Dongfang and Yinggehai respectively (Hainan Provincial Local History Office 2020). Water transparency ranges between 0.5 and 20 meters with eastern and southern coastal areas highly transparent, while the western and northern regions are less transparent. Abundant populations of *Luticola* spp. were recorded in the sample with accession number SZCZ27176 collected by A. Witkowski and J.P. Zhang on April 20th 2015. It was a microbial mat developed on a pier at Fenjiezhou Island. Results of the measured environmental parameters are in Table 1.

Site 3 – W South China Sea, Quảng Yên, Quảng Ninh province, NE Vietnam; 20°13'20"N, 106°32'14"E

Quảng Yên is one of the coastal towns of Quảng Ninh province that is located in the northern part of Vietnam, and the biggest oyster (*Ostrea edulis* Linnaeus, 1758) aquaculture area of the Ha Long Bay. Quảng Yên has a climate characteristic of the tropical monsoon with cold winters. Total rainfall amounts to ca. 500–700 mm. Quảng Yên is considered an area sensitive to the impacts of climate change regarding mangrove forests and biodiversity. Periphyton from oyster shells from offshore aquaculture facility, by V. Méléder, P. Rosa and T.T. Duong on October 28th 2018. Accession number SZCZ26472. Water parameters measured in situ are in Table 1.

Site 4 – W South China Sea, Rú Chá mangrove, Thua Thien Hue province, Central Vietnam; 16°33'28"N, 107°36'41"E

Rú Chá mangrove functions as an ecotone between the mainland and the lagoon. With an overall area of about 50 to 100 hectares, the core species of the area of more than 5 hectares is *Excoecaria agallocha* L. The mangrove flora in Rú Chá has 27 species (10

true mangrove species and 17 mangrove associated species. In 2014, an assessment of surface water and sediment of the Rú Chá mangrove showed that the surface water had a high concentration of total nitrogen (3.4 mg L^{-1}) and total phosphorus (0.3 mg L^{-1}). The sediments were saline, strongly acidic, frequently waterlogged and rich in organic matter (Ha et al. 2015). A sample with access number SZCZ26505 was collected by V. Méléder and P. Rosa on November 11th 2018 from the mangrove area. Water parameters measured *in situ* are presented in Table 1.

Diatom analysis

Diatom samples were collected using tooth brush to detach the periphyton from solid substrate (pier, boulders) and with a plastic tube pressed into the sediment in case of soft substrate (microbial mat, sediment). Diatom samples were cleaned by boiling with 30% hydrogen peroxide (H_2O_2) for a few hours. Cleaned diatom material was pipetted on to coverslips and dried, and then mounted on glass slides using Naphrax mounting medium (Brunel Microscopes Ltd, Wiltshire, U.K.). Light microscopy (LM) observations were made with a Zeiss Axio Imager A2 (Carl Zeiss, Jena, Germany) using a $\times 100$ Plan Apochromatic oil immersion objective (NA 1.46) equipped with Differential Interference Contrast (DIC). Diatom images were captured with a Zeiss AxioCam ICc5 camera (Jena, Germany). For scanning electron microscope (SEM) examination, a few drops of cleaned material were put onto Whatman Nuclepore polycarbonate membrane filters (Fisher Scientific, Schwerte, Germany). Once dried, the membranes were mounted on to aluminum stubs and coated with 20 nm of gold using a turbo-pumped Quorum Q 150T ES coater. SEM observations were performed at the University of Rzeszów, using a Hitachi SEM SU8010. The diatom terminology follows: Round et al. (1990) and Levkov et al. (2013).

Results

Descriptions of new *Luticola* species

Phylum: Bacillariophyta Haeckel

Class: Bacillariophyceae Haeckel

Subclass: Bacillariophycidae D.G.Mann

Order: Naviculales Bessey

Family: Diadesmidaceae D.G.Mann

Genus: *Luticola* D.G.Mann in Round et al. (1990)

Luticola orientalis M.Rybak, Peszek, JP.Zhang & Witkowski, sp. nov.

Figures 2A–AH, 3

Description LM. Valves rhombic-lanceolate to rhombic in smaller specimens with narrowly rounded apices. Valves $9.5\text{--}22.1 \mu\text{m}$ in length, $5.4\text{--}8.5 \mu\text{m}$ in width ($n = 30$). Raphe filiform, axial area narrow and linear expanding into rectangular, narrow central

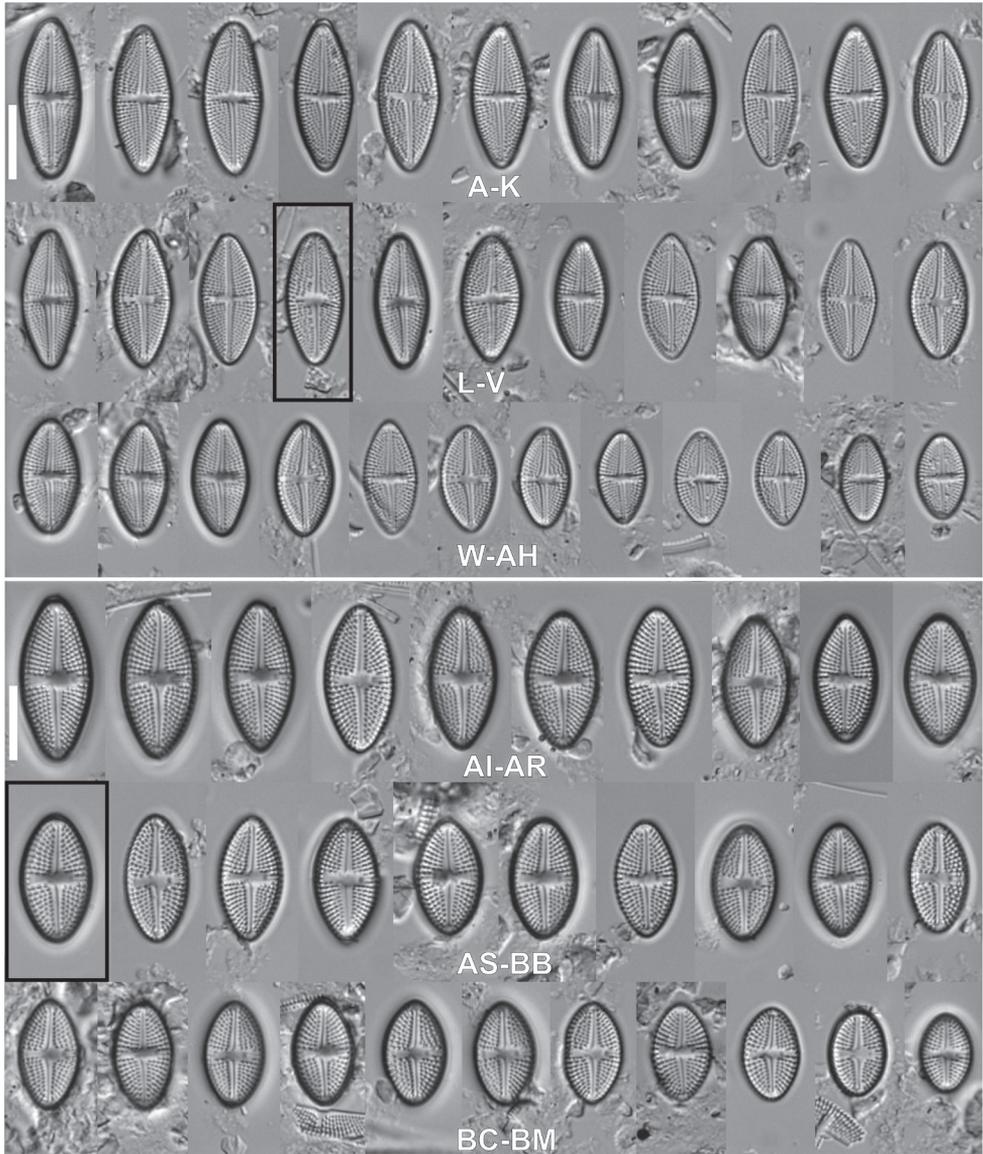


Figure 2. LM micrographs of size diminution series of *Luticola orientalis* M.Rybak, Peszek, JP.Zhang & Witkowski sp. nov. (A–AH) and *Luticola cribriareolata* M.Rybak, Witkowski, Risjani & Yuniarta sp. nov. (AI–BM). Holotype specimen of *Luticola orientalis* sp. nov. – black frame (O). Holotype specimen of *Luticola cribriareolata* sp. nov. – black frame (AS). Scale bar: 10 μ m.

area, stigma side of the central area bordered by 2–3 areolae, on side opposite stigma bordered by 1–2 areolae. Stigma located close to valve margin. Transapical striae easily distinguishable with LM, radiate throughout, 18–22 in 10 μ m.

Description SEM. Valve surface flat, the transition between valve face and the mantle abrupt marked with a stripe of hyaline silica. Axial area narrow becoming broader toward the valve middle, expanding into the rectangular central area. External-

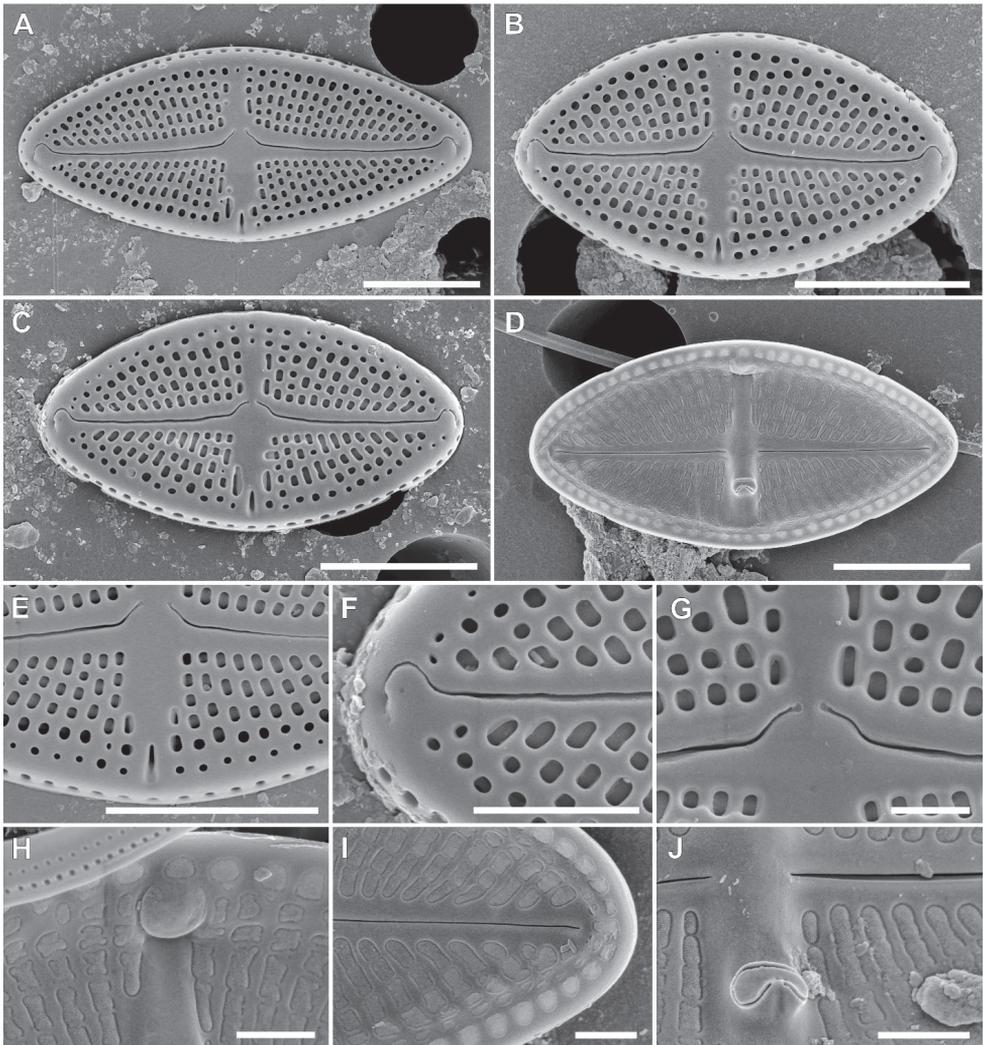


Figure 3. SEM micrographs of *Luticola orientalis* M.Rybak, Peszek, JP.Zhang & Witkowski sp. nov. External valve view (**A–C**; **E–G**), Internal valve view (**D**; **H–J**). Detailed view of showing external view of stigma opening **E** distal **F** and proximal **G** raphe endings. Detailed close-ups showing internal silica flap on longitudinal channel **H** distal raphe endings **I** Detailed view of proximal raphe endings and stigma opening (**J**). Scale bars: 5 μm (**A–D**), 1 μm (**E**, **G**, **I**, **J**), 2 μm (**F**, **H**).

ly raphe filiform and straight, distally strongly hooked in the same direction on valve apices, proximal raphe endings close to each other, simple and clearly bent towards the primary valve side (opposite the stigma). Valve mantle with a single row of elliptical areolae. Internally raphe branches straight, with proximal ends simple and relatively distant, terminating at the apices as small, indistinct helictoglossae. Transapical striae composed of 4–6 rounded or slightly transapically elongated areolae, often becom-

ing smaller close to valve margin, internally occluded by hymenes. Areola occlusions positioned at the internal valve surface. Externally elongated stigma positioned close to valve margin of the valve secondary side. Internal stigma opening with large-lipped structure. Internally longitudinal channel visible on face and mantle conjunction, with relatively large silica flap on site opposite to stigma opening. Longitudinal channel covered by hymen similar to those occluding areola.

Holotype. Slide SZCZ27007 stored in A. Witkowski Diatom Collection of the Institute of Marine and Environmental Sciences, University of Szczecin, holotype specimen is Fig. 2O.

Isotype. Slide no. 2018/425 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Indonesia. Java Island: Pantai Bentar in Probolinggo at North coast, a periphyton from a boulder, 07°46'41"S, 113°16'34"E, *leg.* Y. Risjani, Yunianta and A. Witkowski, 1st March 2020.

Etymology. The name refers to the geographical location – east (lat. *orientalis* – eastern).

Distribution. Abundant in holotype sample SZCZ27007, and in periphyton from the plastic pier at Pantai Bentar in Probolinggo, and was also present in sample SZCZ27006 very close to the holotype habitat. The new species was also observed in an epilithic sample from Fenjiezhou Island at the coast of Hainan Island, NW South China Sea in sample SZCZ27176, and from the Xuân Thúy Mangrove in NE Vietnam where it was found in the biofilm from wild oysters, sample SZCZ26472.

Taxonomic comment. Valve shape of *Luticola orientalis* sp. nov. is similar to *Luticola cribriareolata* sp. nov., however, the former species can be distinguished by stria density, which are finer than in *L. cribriareolata* sp. nov. *Luticola orientalis* sp. nov. is also similar in terms of valve outline to *L. nosybeana* and *L. madagascarensis* from Nosy Be Island, however, the former species has simple proximal raphe endings without any grooves (Table 2) which are distinct in both Madagascar species (Bağ et al. 2019).

***Luticola cribriareolata* M.Rybak, Witkowski, Risjani & Yunianta, sp. nov.**

Figures 2AI–BM, 4

Description LM. Valves elliptic-lanceolate to elliptic with rounded apices. Valves 9.8–28.3 µm in length, 6–11.6 µm in width (n = 30). Raphe filiform, axial area narrow at apices becoming broader towards valve middle part, expanding into asymmetrical central area, broader opposite the stigma and bordered by 2–3 areolae. Stigma present close to valve margin. Transapical striae easily distinguishable with LM, radiate throughout, 14–16 in 10 µm.

Description SEM. Valve surface flat with the transition to the mantle abrupt and marked with distinct hyaline stripe. Axial area narrow becoming broader toward the valve middle, expanding into the rectangular central area. Raphe filiform and straight, external proximal raphe endings close to each other, clearly bent to the valve primary

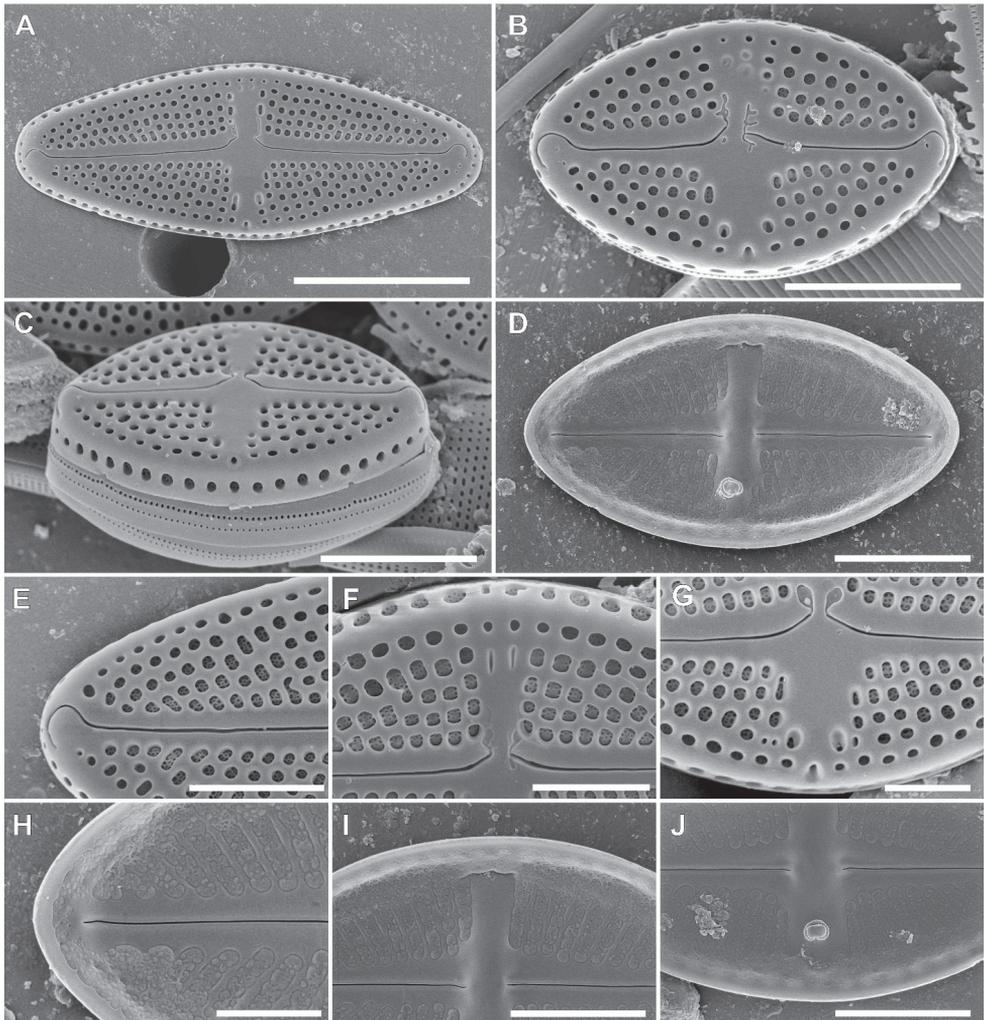


Figure 4. SEM pictures of *Luticola cribriareolata* M.Rybak, Witkowski, Risjani & Yunianta sp. nov. External valve view (**A–C**, **E–G**), External view with cribrate mantle areolae **C** internal valve view (**D**, **H–J**). External details of distal raphe ending and areolae **E** proximal raphe endings with shallow grooves, and ghost areolae **F** proximal raphe endings with shallow grooves and stigma opening **G** internal details of raphe branch with distal raphe end and irregular hymenate structure **H** proximal raphe endings and longitudinal channel **I** proximal raphe endings and stigma opening **J**. Scale bars: 10 μm (**A**, **C**, **D**), 5 μm (**B**), 4 μm (**E**, **I**, **J**), 3 μm (**F**), 2 μm (**G**, **H**).

side and associated with irregular in shape shallow grooves expanded in the direction opposite the stigma. External raphe distal ends strongly hooked on valve face and terminating in an indistinct groove in central area, at apical part of mantle. Valve mantle steep with a single row of oblong areolae. Girdle composed of a few copulae each with two rows of small circular pores. Internally, raphe branches straight, with proximal endings slightly bent. Internally, raphe terminates in a small helictoglossae. Transapi-

cal striae composed of 2–5 large areolae. Areolae on both valve face and valve mantle are deeply embedded, and occluded with reticulated cribra positioned on the inner valve surface. Within central area ghost areolae are often observed, oblong to strongly elongated in shape. Elongated stigma positioned close to margin of the valve primary side. Externally, stigma small and slightly elongated. Internal stigma opening with large-lipped structure. Internally, longitudinal channel visible, with small silica flap on site opposite to stigma. Internally, areolae and longitudinal channel occluded with irregular hymenate structure.

Holotype. Slide SZCZ27007 stored in A. Witkowski Diatom Collection of the Institute of Marine and Environmental Sciences, University of Szczecin, represented here by Fig. 2AS.

Isotype. Slide no. 2018/425 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Indonesia. Java Island: Pantai Bentar in Probolinggo on the north coast, a periphyton from a boulder, 07°46'41"S, 113°16'34"E, *leg. Y. Risjani, Yunianta and A. Witkowski, 1st March 2020.*

Etymology. The species name is derived from its areola occlusions which are in the shape of reticulated cribra, hence the stem “cribr-” of the word “cribrum” is left, the connecting vowel “-i-” and “areolata” are added = criבריareolata.

Distribution. Observed thus far from the holotype sample SZCZ27007, and in periphyton from the plastic pier at Pantai Bentar in Probolinggo and in sample SZCZ27006 very close to the holotype habitat.

Taxonomic comment. *Luticola criבריareolata* has valve shape similar to *Luticola orientalis* sp. nov., however, the two species can be easily distinguished based on the stria density, which is coarser in *L. criבריareolata*. *Luticola criבריareolata* sp. nov. is also similar in terms of valve outline to *L. nosybeana* and *L. madagascarensis* described from Nosy Be Island from NW Madagascar. The newly described species has simple proximal raphe endings, whereas both *L. madagascarensis* and *L. nosybeana* have external proximal raphe endings with distinct grooves (Bąk et al. 2019) (Table 2).

***Luticola halongiana* Witkowski, M.Rybak, H-D.Nguyen & D-V.Nguyen, sp. nov.**
Figure 5

Description LM. Valves rhombic-elliptic to rhombic-lanceolate with broadly rounded to slightly protracted apices in larger specimens, 9.9–22.1 µm in length and 5.4–7.7 µm in width (n = 30). Raphe filiform, slightly bent, axial area narrow, expanding into rectangular to bow-tie shaped central area bordered by 3–4 areolae. Stigma round, present near the valve margin. Transapical striae radiate throughout, 20–24 in 10 µm.

Description SEM. Valve face flat, the transition from valve face to the mantle abrupt, marked with distinct hyaline stripe. Axial area narrow linear, slightly broadened towards the valve middle, expanding into the bow-tie shaped central area with

Table 2. Morphological characteristics of all *Luticola* taxa listed here with comparisons to most similar brackish taxa based on literature data. Data marked with an asterisk (*) are obtained from photomicrographs.

	Size [µm] Length/Width	Striae [in 10 µm]	Areolae characteristic	Proximal raphe endings	Distal raphe endings	Distribution	References
<i>L. orientalis</i> sp. nov.	9.5–22.1/5.4–8.5	18–22	4–6 per striae, round or slightly elongated	slightly deflected, close to each other	hooked	Java, Hainan Island, Vietnam	this study
<i>L. cribriareolata</i> sp. nov.	9.8–28.3/6.0–11.6	14–16	3–5 per striae, with deeply positioned cribrum	slightly deflected with long irregular thread-like grooves	hooked	Java	this study
<i>L. halongiana</i> sp. nov.	9.9–22.1/5.4–7.7	20–24	3–4(5) per striae, round or slightly elongated	strongly deflected with small rounded groove	hooked	Vietnam, Java	this study
<i>L. belawanensis</i>	8.4–19.0/6.1–9.0	18–21	3–4(5) per striae mainly slightly elongated	bent with small C-shaped or irregular grooves	hooked	Vietnam	this study
	15.5–27.0/15.5–27	18–20	3–4 per striae	Deflected	–	Sumatra	Levkov et al. 2013
	9–29/5–10	18–19	3–5 per striae*	–	–	Vietnam	Glushchenko et al. 2017
<i>L. celebesica</i>	10.6–27.1/7.3–13.1	17–19	4–6 per striae	Deflected	hooked	Vietnam	this study
	11.5–39.0/11.5– 39.0	18–21	(4)5–6 per striae	deflected	hooked	Sulawesi	Levkov et al. 2013
<i>L. nosybeana</i>	9–27/6.0–10.5	20–24	4–5 per striae, round to elliptic	with irregular “insect-antennae- like” or “butterfly- like” grooves	hooked	Madagascar	Bak et al. 2019
<i>L. madagascarensis</i>	13.0–22.5/6.0–7.5	20–24	3–4 per striae, round to elliptic or slit-like	with L-shaped grooves	hooked	Madagascar	Bak et al. 2019
<i>L. inserata</i>	12.2–33.5/8.2–14.0	15–20	4–5 per striae, round to elongated with small spines on margin	bent with irregular thread-like grooves	hooked	Vietnam	this study
	23–28/12	18	–	–	–	Sumatra	Hustedt 1955
	18–28/10.0–13.5	16–19	5–6 per striae, round to elongated	deflected	hooked	Indonesia, Australia	Levkov et al. 2013
	15–25/9–12	20	4–6 per striae, round to elongated*	–	–	Vietnam	Glushchenko et al. 2017
	16.8–24.4/9.5–12.4	14–17	4–5 per striae, round to slightly elongated areolae	bent, with small C-shaped grooves	hooked	Hainan Island	this study
<i>L. seposita</i>	23/11	16–18	–	bent	bent slightly S-shaped	Sulawesi	Hustedt 1942
	18–24/10–12	18–21	4–5 per striae, transapically elongated	Hook-shaped	hooked	–	Levkov et al. 2013
	11.8–21.2/7.5–11.1	17–20	4–5 per striae	clearly bent with long irregular thread-like grooves	hooked	Hainan Island, Vietnam	this study
	12–22/7–9	20	–	–	–	South Africa	Cholnoky 1960
<i>L. tropica</i>	15.5–24.0/8–11–5	20–24	4–5 per striae, round to transversally elongated	Bent and expanded into central pores	hooked	–	Levkov et al. 2013
	11–24/7–9	20–24	4–5 per striae*	–	–	Vietnam	Glushchenko et al. 2017
	8.8–19.8/6.3–10.3	16–18	4–5 per striae, round or slightly elongated*	slightly deflected with long irregular thread-like grooves*	hooked*	Brazil	Straube et al. 2017

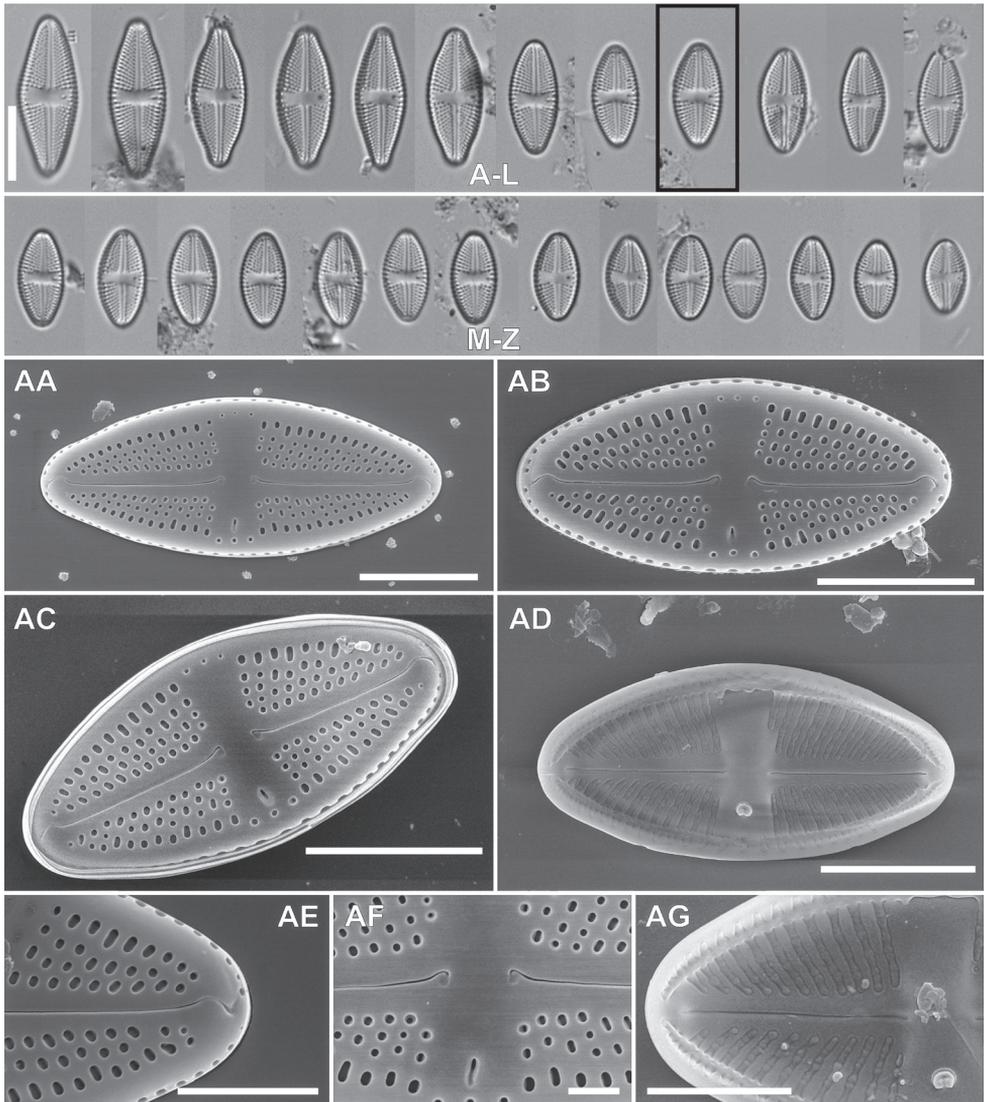


Figure 5. LM micrographs of *Luticola halongiana* Witkowski, M.Rybak, H-D.Nguyen & D-V.Nguyen sp. nov. in size diminution series (**A–Z**). Holotype specimen – black frame **I** external view of frustule (**AA–AC**). Internal view of frustule (**AD**). Detailed external close-ups showing distal raphe ending (**AE**), proximal raphe endings with small rounded grooves and opening of stigma (**AF**). Detailed internal close-up showing distal and proximal raphe endings, hymen structure and stigma opening (**AG**). Scale bars: 10 μm (**A–Z**), 5 μm (**AA,AB,AC,AD**), 3 μm (**AE,AG**), 1 μm (**AF**).

slit like opening of stigma bordered by 3–4 small, rounded areolae. Raphe filiform and straight. Raphe branches very slightly bent with external proximal raphe endings strongly deflected to the valve primary side (opposite to stigma) with small, rounded grooves on the stigma-bearing side. External distal raphe ends slightly hooked and terminate on the apex valve mantle. The valve mantle bearing one row of oblong areola. Internally, raphe straight, with proximal endings slightly bent, and distal raphe end-

ings terminating in small helictoglossae. Transapical striae composed by 3–4(5) round to slightly elongate areolae. Internally, areolae covered by hymen forming continuous strip. Internal stigma opening with circular lipped structure. Internally, longitudinal channel visible, with small silica flap on site opposite to stigma.

Holotype. slide SZCZ26472 stored in A. Witkowski Diatom Collection of the Institute of Marine and Environmental Sciences, University of Szczecin, represented here by Fig. 5I

Isotype. Slide no. 2018/425 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. NE Vietnam: W South China Sea, Quảng Yên, in Halong region, oyster offshore aquaculture, 20°54'1"N, 106°54'17"E, *leg. Vona Meleder and Philipp Rosa, 10th October 2018.*

Etymology. The specific epithet refers to the type location, Ha Long, NE Vietnam.

Distribution. Species occur rarely, observed thus far at the type locality Quảng Yên in biofilm on shells, and on the north coast of Java in Indonesia, from periphyton from the plastic pier (slide SZCZ27006).

Taxonomic comment. *Luticola halongiana* sp. nov. has a unique set of characters and it is difficult to point out any similar established species. The only exception is the position of the stigma, which is located almost in the middle between the valve center and valve margin. This makes it similar to *L. madagascarensis*, however, the latter species has external proximal raphe endings with long and distinct grooves on a side opposite the stigma; these grooves are indistinct in *L. halongiana*. Also, *Luticola mutica* (Kützing) D.G.Mann shows some similarities to *L. halongiana* sp. nov. but it can be easily distinguished based on the narrower central area. Also *L. mutica* has areolae containing cribrum which is not present in described species.

Morphological characteristic of recently established *Luticola* taxa observed during this study.

Luticola belawanensis Levkov & Metzeltin

Figure 6

Description LM. Valves elliptic to elliptic-lanceolate with rounded apices. Valve length 8.4–19 µm, breadth 6.1–9.0 µm, with easily distinguishable radiate striae (18–21 in 10 µm) (n = 15). Axial area lanceolate. Central area asymmetrical, with wider side opposite the stigma, bordered on each margin by a row of areolae. Stigma elongated, located close to valve margin.

Description SEM. Valve face flat, raphe filiform and straight, distally strongly hooked on valve face at the apices. Proximal raphe endings clearly bent to the side opposite the stigma with small C-shaped or irregular grooves evident. Internally, raphe straight, proximal endings only slightly bent, whereas distal raphe endings terminate in small helictoglossae. Transapical striae composed of 4–5 round to slightly elongate areolae. Single row of areolae occurs also on valve mantle. Internally, areolae occluded

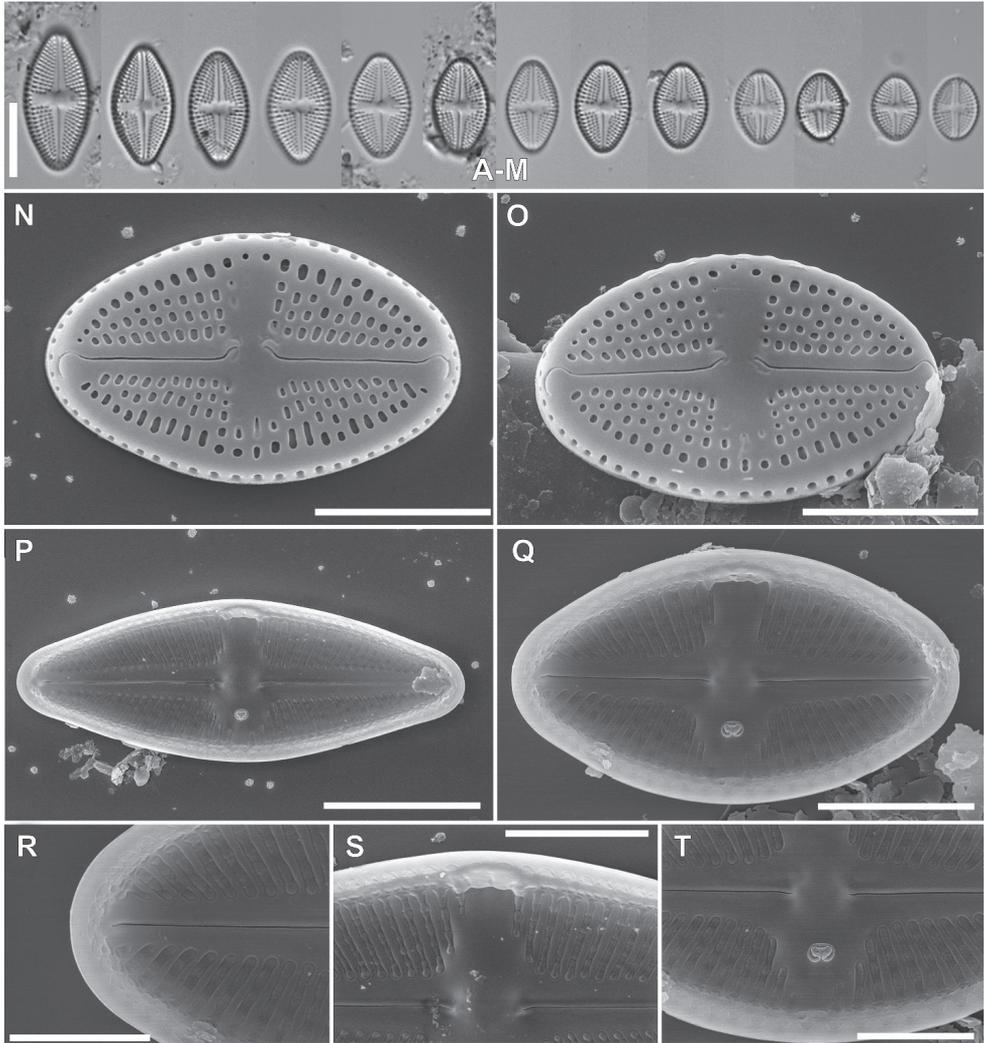


Figure 6. LM pictures of *Luticola belawanensis* Levkov & Metzeltin in size diminution series (**A–M**). External view of frustule (**N, O**). Internal view of frustule (**P, Q**). Internal details of: raphe branch with distal raphe end and hymen structure **R** proximal raphe endings and longitudinal channel **S** proximal raphe endings and stigma opening **T**. Scale bars: 10 μm (**A–M, P**), 5 μm (**N, O, Q, S**), 3 μm (**R, T**).

with hymen forming continuous strips. External opening of stigma slit-like, positioned close to valve margin but separated by a single areola. Internal stigma opening with large-lipped structure positioned mid-way between valve margin and valve center. Internally, longitudinal channel present along the valve margin, with small silica flap on side opposite the stigma.

Distribution. Occurred rarely only in sample SZCZ26472 from Western South China Sea, Quảng Yên, in Ha Long region of NE Vietnam, collected from oyster shells in offshore aquaculture area.

***Luticola celebesica* Levkov, Metzeltin & Pavlov**

Figure 7

Description LM. Valves elliptic to rhombic-elliptic with rounded apices, 10.6–27.1 μm in length, 7.3–13.1 μm in width ($n = 9$). Axial area broad, clearly expanded near central area, asymmetrical bordered by shortened striae, composed of 2–3 areolae while on opposite site of a single areola. Raphe branches straight with hooked distal raphe endings and proximal endings deflected to site opposite to stigma. Transapical striae easily distinguishable, radiate, 17–19 in 10 μm . Slit-shaped stigma located close to valve margin.

Distribution. This is a very rare species, observed only in sample SZCZ26505 from Rú Chá Mangrove mud flat in Hue, the western South China Sea coast, Central Vietnam. Due to the rare occurrence of this species, a detailed description of valve ultrastructure was impossible up until the present.

***Luticola inserata* (Hustedt) D.G.Mann**

Figures 8, 9

Description LM. Valves lanceolate-elliptical to broadly elliptic with weakly undulated margins with rounded, rostrate to capitate apices, 12.2–33.5 μm in length, 8.2–14.0 μm in width ($n = 20$). Axial area narrow, gradually broadening towards valve center, central area rectangular, asymmetrical, bordered by two or three shortened striae with slit-like stigma located close to valve margin. Transapical striae radiate, becoming strongly radiate toward apices, 15–20 in 10 μm . Copulae open.

Description SEM. Valve face flat, raphe branches filiform and straight. External proximal raphe endings clearly bent to the site opposite the stigma with irregularly-shaped grooves expanded opposite the stigma. External distal raphe endings terminate on apices, strongly hooked. Internally, raphe branches straight, only proximal endings slightly bent, distal raphe endings terminating in small helictoglossae. Transapical

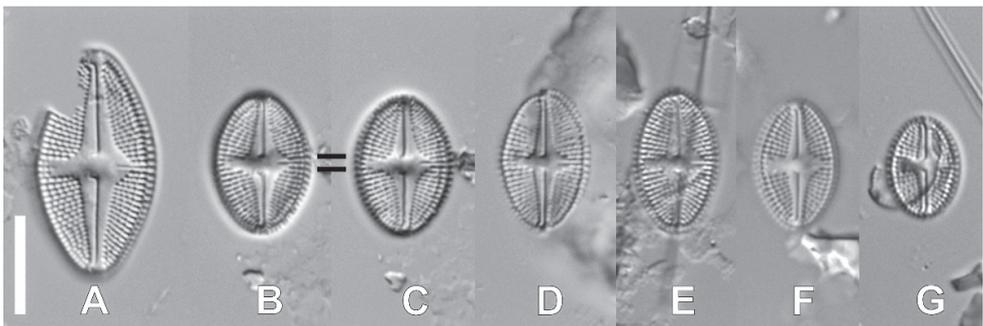


Figure 7. LM micrographs of *Luticola celebesica* Levkov, Metzeltin & Pavlov in size diminution series (A–G). Scale bar: 10 μm .

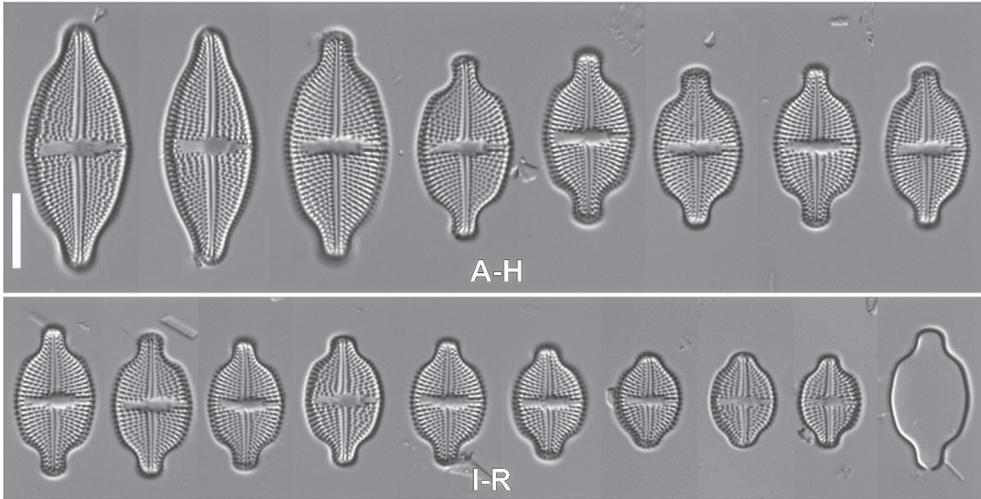


Figure 8. LM micrographs of *Luticola inserata* (Hustedt) D.G.Mann in size diminution series (A–R). Scale bar: 10 μ m.

striae composed of 4–5 round to elongate areolae. Single row of elongate areolae occurs also on valve mantle. Both areolae on mantle and valve face with small spines on edges. Internally, areolae covered with hymen forming continuous strips. Ghost areolae rarely present within central area. External opening of stigma small and rounded, positioned very close to valve margin. Internal stigma opening with large-lipped structure positioned mid-way between valve margin and valve center. Internally, longitudinal channel present along the valve margin, with small silica flap on side opposite the stigma.

Distribution. This species was observed only in sample SZCZ26472 from Western South China Sea, Quảng Yên, in Halong region of NE Vietnam, shell scrape from oysters in offshore aquaculture area.

Luticola seposita (Hustedt) D.G.Mann

Figures 10, 11

Description LM. Valves linear-elliptic to elliptic with weakly undulate margins, with rounded, rostrate to capitate apices, 16.8–24.4 μ m in length, 9.5–12.4 μ m in width ($n = 20$). Axial area narrowly-lanceolate. Central area bordered by two or three shortened striae with slit-like stigma positioned close to the valve margin. Transapical striae radiate becoming strongly radiate toward apices, 14–17 in 10 μ m.

Description SEM. Valve face flat, raphe filiform and straight, distally strongly hooked at the apices. External proximal raphe endings bent to side opposite the stigma with small C-shaped grooves. Internally, raphe straight, only proximal endings slightly bent, with distal raphe endings terminating in small helictoglossae. Transapical striae composed of 4–5 round to slightly elongated areolae. Single row of areolae present

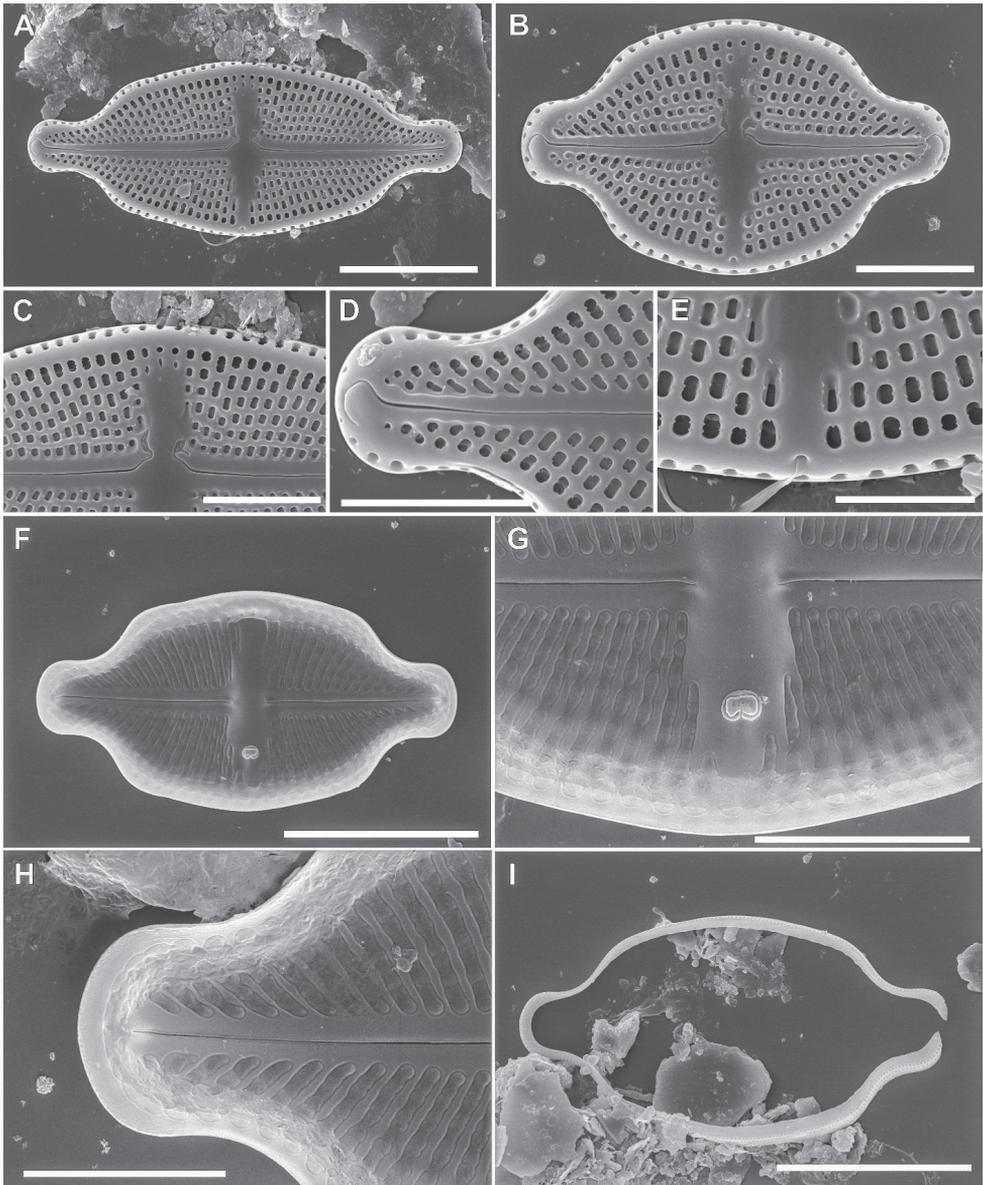


Figure 9. SEM micrographs of *Luticola inserata* (Hustedt) D.G.Mann. External view of valve (**A**, **B**). Detailed external views showing proximal raphe endings with irregular shallow grooves **C** distal raphe ending, areolae **D** stigma opening **E** internal view of valve **F** detailed internal view showing proximal raphe endings and stigma opening **G** and distal raphe ending and hyminate structure **H** open copulae **I**. Scale bars: 10 μm (**A**, **F**, **I**), 5 μm (**B**–**D**, **G**), 3 μm (**E**), 4 μm (**H**).

also on valve mantle. Internally, areolae occluded with hymen forming continuous strips. External elongate ghost areolae present within central area. External opening of stigma slightly elongate and positioned close to valve margin. Internally, as a large-lipped structure positioned mid-way between valve margin and valve center. Internally,

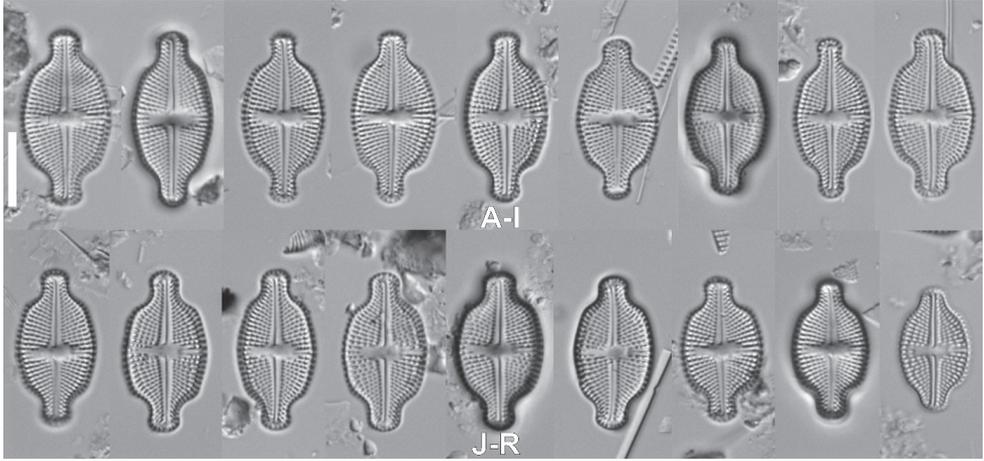


Figure 10. LM micrographs of *Luticola seposita* (Hustedt) D.G.Mann in size diminution series (A–R). Scale bar: 10 μ m.

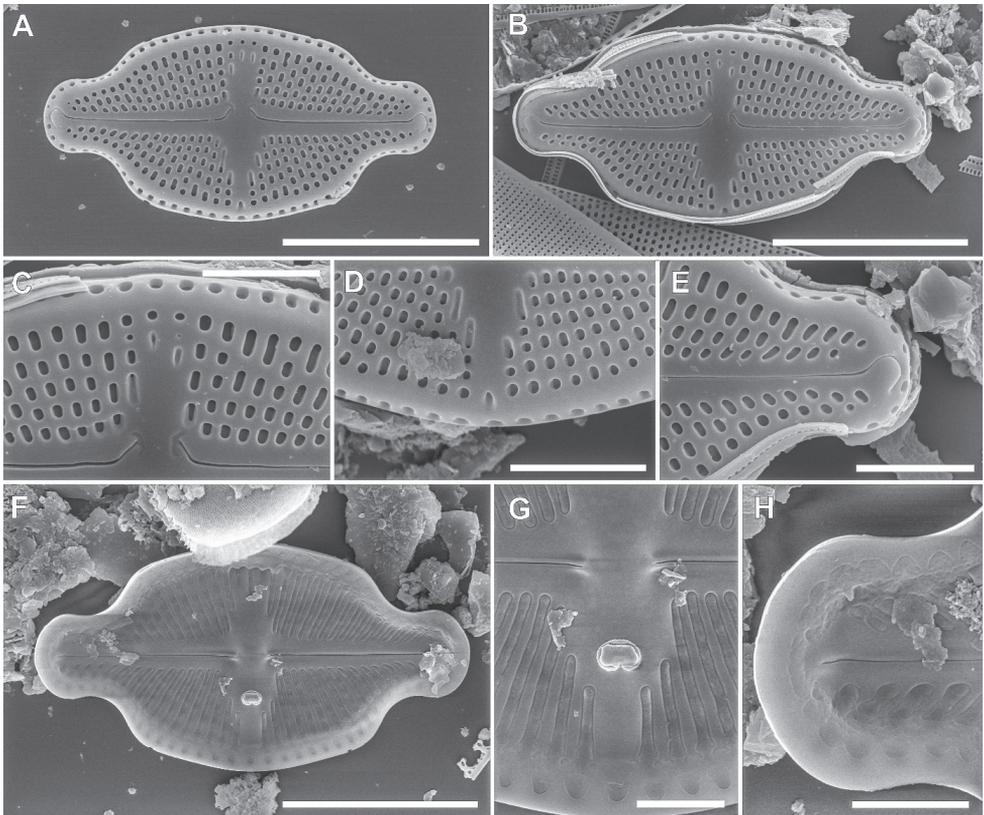


Figure 11. SEM micrographs of *Luticola seposita* (Hustedt) D.G.Mann. External view of valve (A, B). Detailed external views showing proximal raphe endings with C-shaped grooves C stigma opening D and distal raphe ending E internal view of valve F detailed internal views showing proximal raphe endings, stigma opening and hymentate structure G and distal raphe ending H. Scale bars: 10 μ m (A, B, F), 3 μ m (C, E), 4 μ m (D), 2 μ m (G, H).

longitudinal channel is present along the valve margin, with small siliceous flap on side opposite to stigma.

Distribution. This species was observed only in epilithic sample from a sampling site called Fenjiezhou Island located on the coast of Hainan Island, NW South China Sea (China) in sample SZCZ27176.

Luticola tropica Levkov, Metzeltin & Pavlov

Figure 12

Description LM. Valves elliptic-lanceolate with triundulate margins with rostrate and broadly rounded apices, 11.8–21.2 μm in length, 7.5–11.1 μm in width ($n = 25$). Axial area narrow linear, slightly broadening towards valve middle, expanding into rectangular central area bordered on each margin by 2–4 shortened striae. Transapical striae clearly punctate, radiate becoming strongly radiate toward apices, 17–20 in 10 μm . Stigma slightly elongated, close to the valve margin.

Description SEM. Valve face flat, raphe filiform and straight, distally strongly hooked at the apices. External proximal raphe endings strongly bent to the side opposite the stigma, expanding into thread-like grooves that are variable in shape. Internally, raphe straight, only proximal endings slightly bent, distal raphe endings terminate in small helictoglossae. Transapical striae composed of 4–5 round to slightly elongate areolae. Single row of areolae also occurs on the valve mantle. Internally, areolae occluded with hymen forming continuous strips. A few ghost areolae present within central area. Slightly elongate stigma positioned close to the valve margin. Internal stigma opening with large-lipped structure, located midway between raphe endings and valve margin. Internally, longitudinal channel present along the valve margin, with small siliceous flap on side opposite the stigma.

Distribution. This species was abundant in epilithic sample from a sampling site called Fenjiezhou Island located on the coast of Hainan Island, NW South China Sea (China) in sample SZCZ27176, and in Xuân Thúy Mangrove in NE Vietnam, as bio-film from wild oysters, sample SZCZ26472.

Discussion

Brackish and marine water *Luticola*

The genus *Luticola* D.G.Mann contains species with various ecological preferences. However, most of the research on this genus concerns species inhabiting terrestrial and freshwater environments, while the brackish and marine species are still poorly studied. Likewise, poorly known is their geographic distribution and autecology, except the generitype of the genus i.e. *Luticola mutica*, a species widely distributed in estuaries and brackish-water basins of the Northern Hemisphere (e.g. Hofmann et al. 2011; Levkov et al. 2013; Lange-Bertalot et al. 2017).

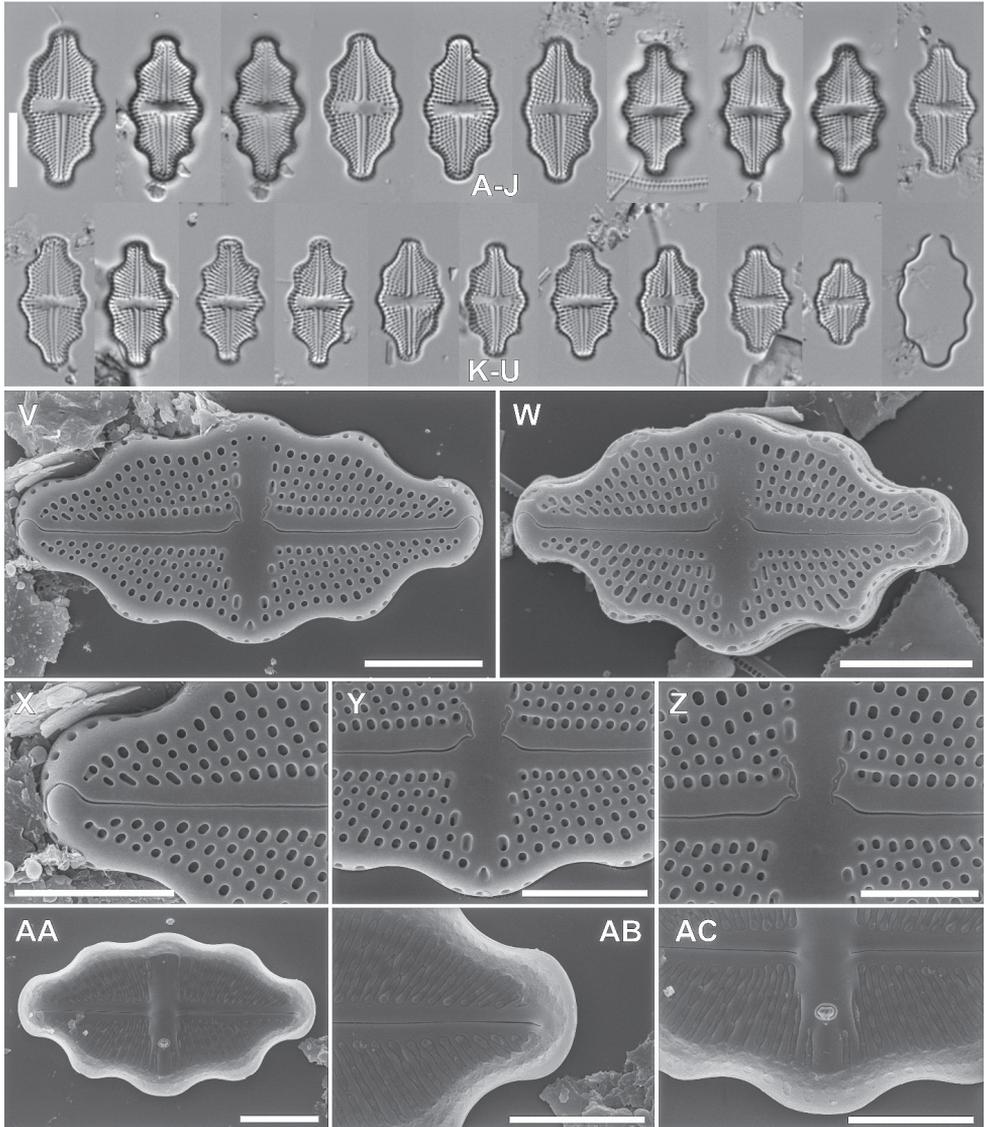


Figure 12. LM micrographs of *Luticola tropica* Levkov, Metzeltin & Pavlov in size diminution series (A–T) and isolated open copula U external view of valve (V,W) Detailed external views showing distal raphe ending X stigma opening Y proximal raphe endings with thread-like grooves (Y, Z) and areolae structure (X–Z). Internal view of valve AA detailed internal close-ups showing distal raphe ending AB proximal raphe endings and stigma opening AC. Scale bars: 10 μm (A–U), 5 μm (V,W,AA), 4 μm (X,Y,AB,AC), 3 μm (Z).

For the coasts studied to date, *Luticola* species seem to be inhabitants of mudflats and part of various kinds of biofilms related to human activity (oyster shells from off-shore aquaculture, hydrotechnical constructions). These habitats have negative impacts on environmental conditions, which seem to be interconnected at least in the north

coast of Java and Hainan Island (S China). The existing environmental data suggest that the North Java coast which abounds with *Luticola* spp. is affected by a strong human impact of densely populated coastal area. Likewise, the Vietnamese and Hainan coasts we sampled are well known to be densely populated regions. All these findings related to brackish water *Luticola* species from SE Asia are confirmed by the autecology of *L. mutica* distributed in human impacted rivers (Lange-Bertalot 1979) and estuaries in Europe (Hofmann et al. 2011; Levkov et al. 2013).

Levkov et al. (2013) selected a few *Luticola* taxa confined to tropical brackish-water and marine habitats, but the information on their morphology was mostly only on LM data and the occurrence represents only a few findings. Included in this group were e.g. *L. belawanensis*, *L. inserata*, *L. celebesica* and *L. tropica*. Interestingly, all these species were described from Indonesia and, in particular, from Sumatra Island coastal region either by Hustedt (1942, 1955) and included in *Naviculae* section *Punctatae* Cleve or by Levkov et al. (2013). Taxa described by Hustedt were later transferred in *Luticola* by D.G. Mann in Round et al. (1990). Interestingly for the taxa described by Levkov et al. (2013) the holotypes were designated from the slides studied by Hustedt (1942, 1955) although some of these taxa have the pantropical geographic distribution. An example of this pattern can be seen in *L. tropica*. Our LM and SEM observations revealed that the investigated samples from coastal waters of Hainan Island (China), Vietnam and Java coasts show a high *Luticola* diversity in terms of species and high relative abundance. In addition to the established taxa reported herein, we have also observed three taxa new to science. Compared with other regions of the world, coastal areas of SE Asia have high diversity of *Luticola* species. Indeed, several *Luticola* species have been described from brackish-waters of marine coasts of the Nosy Be Island in NW Madagascar (Bağ et al. 2019) and from Laguna Diabla in Isabela Island of Galapagos Archipelago (Bağ et al. 2017). This makes together four species (two from Nosy Be and two from Galapagos) and according to present information the number of established brackish-water and marine *Luticola* with our novel taxa slightly exceeds a dozen (Levkov et al. 2013; Bağ et al. 2017, 2019, this study). However, as shown in our recent study on tropical *Luticola* from marine coasts of China, Indonesia and Vietnam, the potential for discoveries of new species is high if the appropriate habitats are sampled. With several hundred samples from the above coasts we sampled only those enriched in organic matter e.g. tidal flat, biofilm revealed significant content (even dominance) of *Luticola* spp. (Risjani et al. 2021). The highest relative abundance of *Luticola* were observed in highly populated coasts of North Java (Probolinggo area) and in Hainan Island. Whereas in Probolinggo the dominant taxa were *L. cribriareolata* and *L. orientalis*, in Hainan Island these were *L. seposita* and *L. tropica*. Interestingly, the third novel species *L. halongiana* was observed in biofilm on oyster shells from an offshore aquaculture area. It is a well-known fact that *L. goeppertiana* and *L. mutica* are tolerant to high and moderate loads of organic contents in freshwaters and in coastal marine waters (Hofmann et al. 2011). Results of our study seem to conform such high tolerance abilities, at least in the case of *L. orientalis* and *L. cribriareolata*, which are dominant in turbid waters of North Java and Hainan Island.

Two of the newly described species – *L. orientalis* sp. nov. and *L. cribriareolata* sp. nov. show a high similarity to each other in the valve shape. However, they can be easily distinguished based on the stria density. Also valve ultrastructure as resolved with SEM allows for the easy separation of these species (Table 2). These two taxa are similar in their valve outline to two species described from NW Madagascar (Nosy Be): *L. nosybeana* and *L. madagascarensis*. *Luticola orientalis* sp. nov., despite having overlapping length and width dimensions, can be easily distinguished from both Madagascar species based on denser areolae per stria. Also *L. orientalis* sp. nov. has a much narrower central area than both above mentioned taxa. The major distinguishing characters of the new *Luticola* species versus Madagascar taxa are simple proximal raphe endings without any grooves, which are present both in *L. madagascarensis* and *L. nosybeana* (Bağ et al. 2019), (Table 2). Despite the similar valve outline and presence of grooves on proximal raphe endings, the newly described *L. cribriareolata* sp. nov. can be easily distinguished from *L. madagascarensis* and *L. nosybeana*. Both Madagascar species share the same stria density (20–24 in 10 µm), which are denser than in *L. cribriareolata* sp. nov. which has 14–16 striae in 10 µm. Also *L. cribriareolata* sp. nov. has deeply positioned cribra which are not observed in either *L. madagascarensis* or in *L. nosybeana* (Bağ et al. 2019).

The presence of areola occluded with cribra in *Luticola cribriareolata* sp. nov. is a very rarely observed character in *Luticola*. Up until now only 3 cribrum-bearing *Luticola* species are known, *L. mutica* from Europe, *L. rionegrensis* from Rio Negro in South America and *L. subcrozetensis* from Maritime Antarctica (Levkov et al. 2013; Zidarova et al. 2016). From these taxa only *L. mutica* and *L. rionegrensis* have (same as *L. cribriareolata* sp. nov.) cribra positioned deeply within areola, while cribra in *L. subcrozetensis* are located almost at the external surface of the valve (Zidarova et al. 2016: 205, fig. 19). In contrast to cribra-bearing *Luticola* species (as well as all described *Luticola*), the internal hymen of *L. cribriareolata* sp. nov. does not form a regular continuous strip but forms strips of irregularly-shaped occlusions conjoined with occlusions of a longitudinal channel.

The newly described *L. halongiana* sp. nov. possesses slightly hooked proximal raphe endings with only small depressions on the stigma-bearing side. This species also shows highly variable shape of the valve apices. In contrast to other brackish-water *Luticola* with rhombic-elliptic or rhombic-linear margins, this species has a relatively broad central area. Also unique among brackish-water *Luticola* is the small, rounded stigma positioned almost midway between the valve center and valve margin. A similar position of the stigma is found in *L. madagascarensis*. However, *L. madagascarensis* can be distinguished from species described herein, based on their external proximal raphe endings with distinctive long grooves on the side opposite the stigma.

Also *Luticola mutica* (Kützing) D.G.Mann shows some similarities to *L. halongiana* sp. nov., however, it can be easily distinguished based on its narrower central area, less dense striae (16–18 vs. 20–24 in 10 µm) and presence of cribrum in areolae which does not occur in *L. halongiana* sp. nov.

Biogeography of the *Luticola* studied

The biogeography of most of the established species has been originally observed and described from Indonesian Islands (Hustedt 1942, 1955), assigned to *Navicula* and later transferred to *Luticola* either in Round et al. (1990) or in Levkov et al. (2013). The latter species seems to have wide environmental amplitude as it can be abundant in brackish and freshwater habitats (this study; Levkov et al. 2013).

We saw great variability in the distribution and relative abundances. For example, *L. celebesica* and *L. belawanensis* were observed only rarely and found from one sample site. *L. inserata*, *L. seposita* and *L. tropica* occurred in high relative abundance and from a few sampling sites. The published data on their geographic distribution shows that some of them, e.g. *L. tropica*, are globally distributed in tropical estuaries and marine coasts (Fernandes et al. 1990; Navarro and Lobban 2009; Levkov et al. 2013; Straube et al. 2017; Glushchenko et al. 2017), whereas the others occur in more restricted areas like in waters from SE Asia to the coast of Australia (*L. belawanensis* and *L. inserata*). *Luticola celebesica* was described from Makassar on Sulawesi Island, however, the species description does not indicate the habitat in which this species was found (Levkov et al. 2013). In the analyzed materials, this species occurred very rarely, which made it impossible to make a detailed description of the observed population which would include the ultrastructure of the valves. Several specimens of *L. celebesica* have been observed only on the mud flat of Rú Chá Mangrove near Hue, the western South China Sea coast in Central Vietnam. *Luticola belawanensis* was described for the first time from the mouth of the River Belawan on Sumatra and later it was reported from mangrove forests in Vietnam by Levkov et al. (2013) and Glushchenko et al. (2017). We observed *L. belawanensis* in periphyton from oysters shell in offshore aquaculture areas, Quảng Yên, in Ha Long region of NE Vietnam from Western South China Sea. All reports of these two taxa confirm that both of them prefer brackish-water conditions.

Likewise, *Luticola inserata* was described from the Sumatran coast at the mouth of the Belawan River (Hustedt 1942, 1955; Simonsen 1987). The species has a very rich published record of occurrence from the coastal waters of SE Asia (Indonesia, Vietnam) (Hustedt 1955; Amossé 1969; Glushchenko et al. 2017) and NE Australia (Foged 1978). Levkov et al. (2013) characterize *L. inserata* as a tropical, brackish-water species. This species shows great variability of shape, from lanceolate-elliptical in early stages of life cycle (Fig. 8A, B; Glushchenko et al. 2017) to broadly elliptical in smaller specimens (Fig. 8C–R). We have observed it in high relative abundance in sample SZCZ26472 from periphyton from oysters shell in offshore aquaculture from Quảng Yên, Western South China Sea, in Halong region of NE Vietnam. Despite it being a commonly reported species, a detailed description of valve ultrastructure of this species was not published until the observations presented herein. We have observed it in high relative abundance in sample SZCZ26472 and have been able to resolve the valve ultrastructure. In our SEM observations this species shows some unique characters including areola (both on valve face and valve mantle) with short spines on margins and only partially elevated raphe sternum. Both of these features allow it to be distinguished from *L. seposita* which has an almost identical valve outline and overlapping valve size dimensions (Table 2).

Luticola seposita was described by Hustedt (1942) from Mahalon-See (Lake Danau Mahalona) on Sulawesi Island. The species was considered to prefer nutrient poor, circumneutral waters with elevated metal concentration and up until now was not reported from marine habitats. In our study *L. seposita* was only observed in an epilithic sample from Fenjiezhou island on the coast of Hainan Island, NW South China Sea in high relative abundance. Seemingly, *L. seposita* is capable of adapting to a broad array of environmental conditions (Levkov et al. 2013, this study). It's also worth mentioning that *L. seposita* was reported from Australia but the valves shown here (John 2020: fig. 141O–Q, p. 117) have a much larger central area that is bordered by a higher number of shortened striae. It is highly possible that these Australian specimens do not represent *L. seposita*, but another (possibly) undescribed species.

Luticola tropica is reported in the diatomological literature as a widely distributed species confined to tropical estuaries and marine coasts. This species is based on *Navicula inserata* var. *undulata* Hustedt (Hustedt 1955) and its type habitat is the mouth of Belawan River on the Sumatran coast. The species has been reported from marine coasts and estuaries of the Atlantic Ocean in Brazil (Fernandes et al. 1990), East African coast of Natal in South Africa (Cholnoky 1960), Pacific Ocean tropical Islands (Navarro and Lobban 2009) and tropical coasts of Ha Long Bay in NE Vietnam and Hainan Island (this study). From all reported taxa only *Luticola tropica* seems to have the widest (pantropic) distribution among the brackish-water and marine taxa treated here. Until now, it has been reported from the mouth of rivers and coastal waters of South-East Asia (Vietnam), Africa (Ghana, Gambia, KwaZulu-Natal), South America (Brazil) and the Pacific tropical island of Guam (Cholnoky 1960; Foged 1966, 1986; Navarro and Lobban 2009; Levkov et al. 2013; Straube et al. 2017). Despite its wide distribution, particular populations do not show significant morphological differences (Table 2).

From eight identified taxa (including three new to science), seven of them were found only in samples collected from marine ecosystems (salinity 22.0–32.8 psu). Based on the literature data as well as on the presented results, it seems that all of them are species that find their ecological optimum in marine habitats. Only *L. celebesica*, which was described from Sulawesi Island (Indonesia), seems to be a species that prefers waters with increased salinity (brackish environment) and does not occur in typically marine diatom assemblages.

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(WCP) with the staff members of the Brawijaya University by the Indonesian Ministry of Education and Culture. Sampling and research of Hainan material has been funded within bilateral international cooperative project ‘SECEB’ and ‘ERES’ implemented between the Guangzhou Marine Geological Survey, China (GZH201500207), and the University of Szczecin, Poland (Polish National Science Centre (NCN) allocated decision No. DEC-2011/01/N/ST1007708 and No. DEC-2016/21/B/ST10/02939).

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