

Two new species of *Oreocharis* (Gesneriaceae) from karst regions in Yunnan and notes on *O. tetraptera* and *O. brachypoda* from China

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Abstract

Two new species of Gesneriaceae, *Oreocharis aimodisca* and *O. longipedicellata*, from the limestone area of Yunnan Province, China, are described and illustrated. Their morphological relationship with similar species is discussed and colour photographs, detailed descriptions, distribution and habitat, as well as the IUCN endangered status are provided. We also discuss the accuracy of the scientific names of the described species *O. tetrapterus* from Guangxi, China in 2019 and *O. brachypodus* from Guizhou, China, in 2015, and put forward corrections related to name form.

Keywords

flora of Yunnan, limestone area, morphology, new taxon, *Oreocharis*

Introduction

At least 30 new taxa of *Oreocharis* Benth. (Gesneriaceae) have been described and officially published (e.g., Cai et al. 2017, 2019; Do et al. 2017; Chen et al. 2018; Guo et al. 2018; Möller et al. 2018; Pan et al. 2019; Yang et al. 2019) after the generic

redefinition based on new evidence following the development of molecular phylogenetics in 2011 (Möller et al. 2011) and several later adjustments of the species (Middleton et al. 2013; Chen et al. 2014; Möller et al. 2014; Möller 2015). *Oreocharis s.l.* hitherto comprises more than 140 taxa, mainly distributed in South and Southwest China (Wen et al. 2014, 2019) and a few species extending into North Vietnam (nine species), Myanmar (two species), Bhutan (one species), India (one species), Japan (one species) and Thailand (one species) (Xuyen et al. 2016; Do et al. 2017; Möller et al. 2017, 2018; Chen et al. 2018).

Li and Li (2015) and Pan et al. (2019) each described new species of *Oreocharis*. One of the taxa with four corolla lobes is from Guangxi, China, which they named *O. tetrapterus* F.Wen, B.Pan & T.V.Do (Pan et al. 2019). The other has sessile or shorter petiole leaves and four stamens with anthers coherent in pairs from Guizhou, China, which was named as *O. brachypodus* J.M. Li & Z.M. Li (Li and Li 2015). These scientific names are improperly formed because the Latin forms ‘*tetrapterous*’ and ‘*brachypodus*’ are masculine and the Latin word of this genus, ‘*Oreocharis*’, is feminine. We revise the Latin name to ‘*tetraptera*’ and ‘*brachypoda*’ here and provide appropriate notes.

In 2018, during field investigations in the limestone area in Southeast Yunnan, China, an unknown species of Gesneriaceae without flowers was collected, then was introduced to, and cultivated in, Guilin Botanical Garden (GBG). We first observed flowering plants which were cultivated in GBG in August 2019. Thereafter, in September 2019, another unknown species of Gesneriaceae with flowers was collected from Shizong County, eastern Yunnan. We confirmed that they are both members of *Oreocharis*, based on the characteristics of leaves in a basal rosette, four separated fertile stamens and capsules dehiscing predominantly on one side. After a careful examination of the related specimens and taxonomic publications of *Oreocharis* from the adjacent regions (Wang et al. 1990, 1998; Li and Wang 2005), we concluded that these two species are both new to science. Here, *Oreocharis aimodisca* and *O. longipedicellata* are described and illustrated and their morphological characters are compared to closely-related species.

Material and methods

Extensive fieldwork has been undertaken in the east and southeast of Yunnan, China, in recent years. Samples of the two new species were respectively collected from the fields of Shizong County and living plants cultivated in Guilin Botanical Garden (GBG) which initially introduced from Malipo County, Yunnan, China. All available specimens of *Oreocharis s.l.*, stored in herbaria (E, HITBC, IBK, HN, K, KUN, P, PE and VMN), Chinese Virtual Herbarium (<http://www.cvh.ac.cn/>) in China and Global Plants on JSTOR (<https://plants.jstor.org/>) were examined. We studied all morphological characters with dissecting microscopes and described the morphological characters by using the terminology presented by Wang et al. (1990, 1998). The photographs and the specimens were taken in the field and GBG by the first and correspondence authors. All specimens seen are indicated by ‘!’.

Taxonomic treatment

Oreocharis tetraptera F.Wen, B.Pan & T.V.Do

Orthographic variant. *Oreocharis tetrapterus* F.Wen, B.Pan & T.V.Do in Pan et al. 2019: 83.

Type. CHINA. Guangxi: Hezhou City, Lisong Town, Gupo Mountain, 24°39'N, 111°36'E, elev. ca. 950 m, on moist surface of granite rocks, in flower, 25 August 2018, Wen Fang WF160825-01 (holotype: IBK!, isotype: IBK!).

Oreocharis brachypoda J.M. Li & Z.M. Li

Orthographic variant. *Oreocharis brachypodus* J.M. Li & Z.M. Li in Li and Li 2015: 296.

Type. CHINA. Guizhou: in the vicinity of Tongren city, on rather cool rocks and very steep banks of cool, clammy soil that grows a fine film of moss, elev. 1300 m, 9 April 2014, *Jia-Mei Li 2304* (holotype: HEAC!); *ibid. Jia-Mei Li 2305* (paratype: HEAC!).

Notes. The gender of the genus name, *Oreocharis*, is feminine, but the suffix of the scientific name, “-us,” is typically masculine. For *Oreocharis tetrapterus* (Pan et al. 2019), the correct orthography of the name of the new species is *O. tetraptera*, is written by using an inaccurate gender, namely “*tetrapterus*”, in the citation of the type of the new species (p. 85), in the discussion of the Etymology (p. 86) and in the notes of the illustration (pp. 86, 87 and 88). In the other new taxon, *Oreocharis brachypodus* (Li and Li 2015), the correct orthography of the epithet “*brachypoda*” should be used to replace “*brachypodus*”. The inaccurately-used name gender appeared in the citation of the type of the new species (p. 296) and in the notes of the illustration (pp. 297 and 298). Thus, here we correct and revise two new species’ names as *Oreocharis tetraptera* and *O. brachypoda*.

Oreocharis aimodisca Lei Cai, Z.L.Dao & F.Wen, sp. nov.

urn:lsid:ipni.org:names:77211926-1

Figures 1–3

Diagnosis. *Oreocharis aimodisca* is morphologically similar to *O. longifolia* (Craib) Mich.Möller & A.Weber and *O. muscicola* (Diels) Mich.Möller & A.Weber in the appearance and colour of its flowers, but differs from the latter two species in its leaf blade oval to ovate, base cordate or auriculate, margin crenate, peduncle densely brown villous and pubescent, corolla outside densely pubescent and four separated fertile stamens, pistil densely pubescent and disc blood red.

Type. China. Yunnan: Shizong County, Wulong Town, Dachang Village, Xiaofakuai, 24°39'N, 104°10'E, elev. ca. 2122 m, on the surface of wet rocks, in flower, 10 September 2019, *Lei Cai & Pin Zhang CL275* (holotype: KUN!, isotypes: KUN!, IBK!).

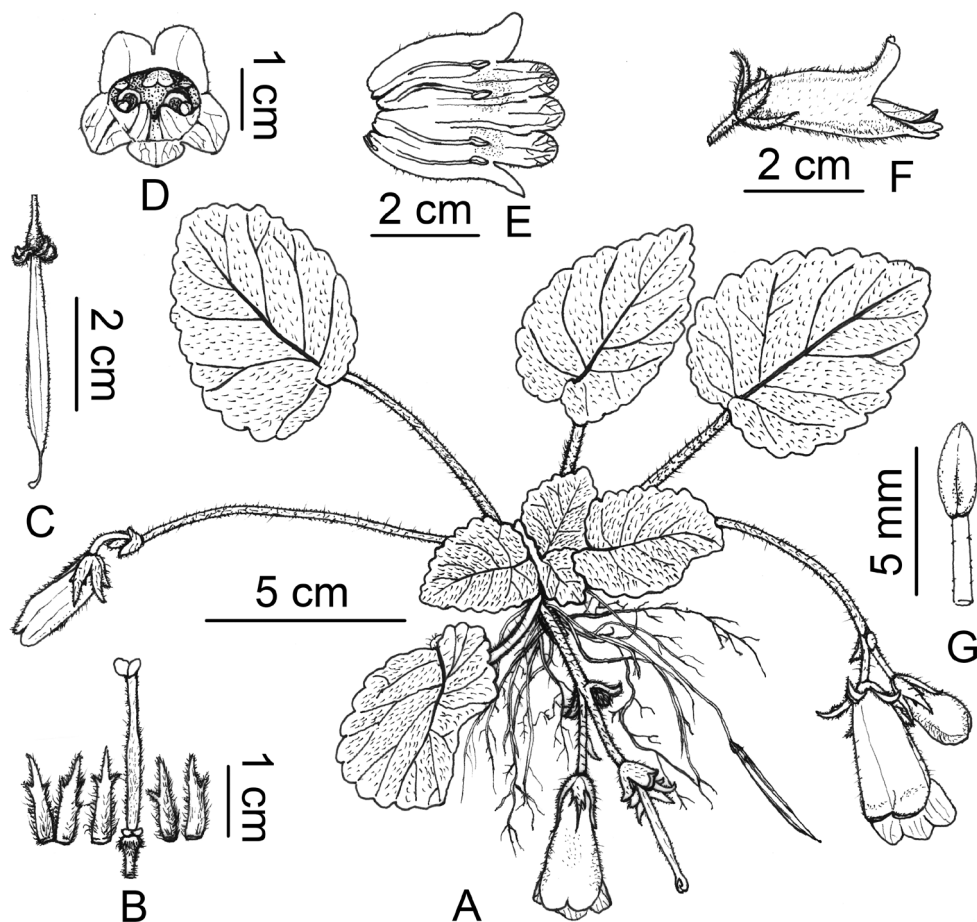


Figure 1. *Oreocharis aimodisca* Lei Cai, Z.L.Dao & F.Wen, sp. nov. **A** habit **B** pistil with disc and calyx **C** old capsule **D** front view of flower **E** opened corolla showing stamens and staminode **F** side view of a flower **G** adaxial view of the anther. Drawn by Xuan-Lin Zhu.

Description. Perennial herb, rhizome 5–18 mm long, 3–5 mm in diameter. Leaves 6–18, basal, petiole 2.5–10.5 cm long, brown villous and pubescent, leaf blade oval to ovate, 2.5–7.0 × 1.8–5.5 cm, adaxially densely appressed pubescent, abaxially puberulent, densely brown pubescent along veins, lateral veins 3–6 on each side of midrib, adaxially inconspicuous, abaxially conspicuous, apex acute, base cordate or auriculate, slightly oblique sometimes, margin crenate. Cymes axillary 2–5, inflorescence 1–5-flowered; peduncle 5.5–16 cm long, brown villous and pubescent; bracts 2, lanceolate to narrowly triangle, 5–8 × 1.5–2.8 mm, outside brown pubescent, inside glabrous, margin nearly entire to denticulate; pedicel 1.2–3.5 cm long, densely pubescent. Calyx 8–12 mm long, 5-lobed to the base, lobes unequal, linear-lanceolate or narrowly triangular, 8–12 × 1.5–2.2 mm, both sides densely pubescent, margin denticulate. Corolla yellow, 2.8–3.6 cm long, out-



Figure 2. *Oreocharis aimodisca* Lei Cai, Z.L.Dao & F.Wen, sp. nov. in natural habitat **A, B** plants with flowers in the wild **C** habitat. Photographed by Lei Cai.

side densely pubescent, inside puberulent in the throat and on adaxial lobes, the lower part forms red stripes on the throat and lobes, tube coarsely tubular, gradually expanded from base to the throat, 2.0–2.6 cm long, 6–10 mm in diameter; limb 2-lipped; adaxial lip 2-lobed to middle, semicircular, lobes 4–5 × 4–5 mm, abaxial lip 3-lobed to middle, semicircular, 5–6 × 5–7 mm. Stamens 4, 1.5–1.8 cm long, adnate to corolla 4–7 mm from the base; filaments linear, sparsely pubescent; anthers oblong, 2-loculed, dehiscing longitudinally, connective glabrous; staminode 1, 0.6–0.8 mm long, inserted ca. 3 mm from the base. Disc ca. 1.2 mm high, blood red, margin undulate. Pistil 1.6–2.4 cm long; ovary long cylindrical, densely pubescent, 1.0–1.4 cm long; style 6–10 mm long, densely pubescent; stigma bilobed, flabellate. Capsule linear, 3.5–4.8 cm long.

Phenology. Flowering from August to September; fruiting from September to December.

Distribution and habitat. *Oreocharis aimodisca* is currently known from two adjacent populations at the type locality, Shizong County, East Yunnan, China. The new species commonly growing with other plants in shady and wet places on the middle part of mountain slopes under primary evergreen broad-leaf forest and shrubbery on karstic limestone at an elevation of over 2000 m.

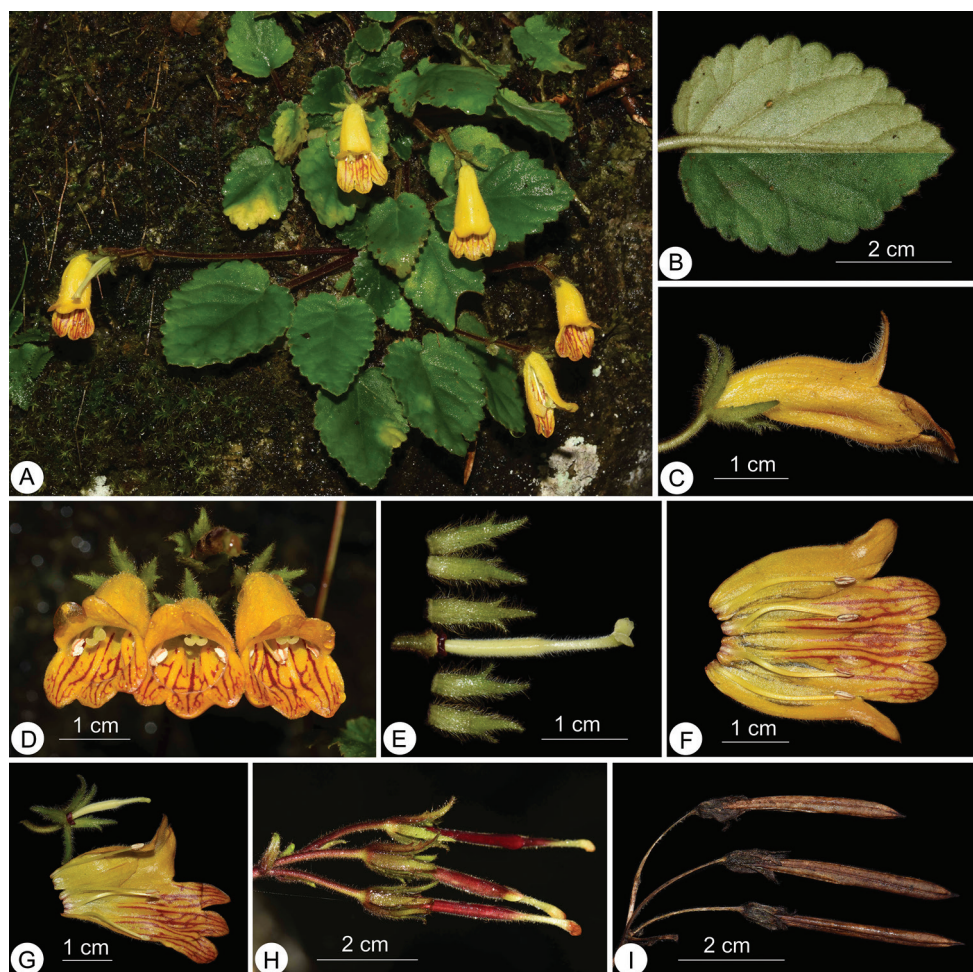


Figure 3. *Oreocharis aimodisca* Lei Cai, Z.L.Dao & F.Wen, sp. nov. **A** habit **B** adaxial (lower half) and abaxial leaf surface (upper half) **C** side view of a flower **D** front view of flowers **E** pistil with disc and calyx **F** opened corolla showing stamens and staminode **G** opened corolla with pistil and calyx **H** fresh fruits **I** old capsules. Photographed by Lei Cai.

Etymology. The original specific epithet ‘*aimodisca*’ derives from the Greek ‘αἶμα’ meaning blood red and ‘δίσκος’ meaning discus.

Vernacular name. The Chinese name of the new species is “Diān Dōng Mǎ Líng Jù Tǎi” (滇东马铃薯苔). The first two words, “Diān Dōng,” mean east of Yunnan, the next four words, “Mǎ Líng Jù Tǎi,” mean *Oreocharis* in Mandarin.

Conservation status. Based on our field investigations, the new species is currently only known from the type locality with two contiguous subpopulations, in total ca. one thousand mature individuals were present within 5000 m² (AOO). Since no special surveys were carried out for delimiting its distribution and information about threats is not very clear, this species was provisionally considered to be Critically Endangered [CR B2(a)] in terms of the IUCN Red List Categories and Criteria (IUCN 2019).

Table 1. Morphological comparison of *Oreocharis aimodisca* sp. nov., *O. longifolia* and *O. muscicola*.

Characters	<i>O. aimodisca</i>	<i>O. longifolia</i>	<i>O. muscicola</i>
Leaf-blade	oval to ovate, base cordate or auriculate, margin crenate	narrowly elliptic to oblanceolate, base attenuate, margin serrulate	narrowly elliptic to lanceolate, base often slightly oblique, narrowly to broadly cuneate, margin serrate to serrate-crenate, sometimes doubly so
Petiole	brown villous and pubescent	grey to brownish pubescent	densely rust-brown villous
Peduncle	densely brown villous and pubescent	sparsely brownish villous to pubescent	rust-brown villous and glandular-pubescent
Bract	lanceolate to narrowly triangle, outside brown pubescent margin nearly entire to denticulate	oblanceolate, outside pubescent, margin entire	lanceolate, outside rust-brown villous, margin entire
Calyx	lobes linear-lanceolate or narrowly triangular, both sides densely pubescent, margin denticulate	narrowly ovate, outside sparsely brownish pubescent, inside glabrous, margin entire	lanceolate, outside sparsely white pubescent and rust-brown villous, margin entire
Corolla	outside densely pubescent, inside puberulent in the throat and on adaxial lobes	outside sparsely glandular puberulent, inside sparsely glandular puberulent	outside sparsely puberulent, inside glandular pubescent
Filaments	sparsely pubescent	glabrous	sparsely puberulent
Anthers	oblong, separated	reniform, connected in pairs	reniform, connected in pairs
Pistil	densely pubescent	glabrous	glabrous
Disc	blood red	yellow	yellow-green

Taxonomic affinities. *Oreocharis aimodisca* is morphologically similar to *O. longifolia* and *O. muscicola* in the corolla yellow and coarsely tubular; however, it is different from the latter two species by the shape of the leaf blade, indumentum characters of the peduncle, pedicel, calyx, corolla and pistil and separated stamens. The comparison of morphological characters on related species is provided in Table 1.

***Oreocharis longipedicellata* Lei Cai & F.Wen, sp. nov.**

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Figures 4, 5

Diagnosis. *Oreocharis longipedicellata* morphologically resembles *O. panzhouensis* Lei Cai, Y.Guo & F.Wen in its ovate leaf blade, yellow flower, four separated fertile stamens, oblong anthers and bilobed, flabellate stigma, but can be easily distinguished from this species in the peduncle 20–28 cm long, bract lanceolate to elliptic, margin denticulate, the calyx 5-lobed to the base, stamens adnate to corolla 3–4 mm from base and the pistil 1.5–2 cm long.

Type. China. Yunnan: Malipo County, Mengdong, on the surface of moist rocks (Cultivated in GCCC nursery, Guilin Botanical Garden, Chinese Academy of Sciences) in flower, 24 August 2019, *Fang Wen WF190824-01* (holotype: KUN!, isotype: IBK!).

Description. Perennial herb, rhizome 0.8–2 cm long, 3–5 mm in diameter. Leaves 8–15, basal, petiole 3.5–8.0 cm long, densely brown villous, leaf blade elliptic to ovate, 3.0–5.5 × 2.4–4.5 cm, adaxially densely pubescence, abaxially pubescent, densely brown villous along veins, lateral veins 3–6 on each side of midrib, apex rounded, base slightly oblique sometimes, cordate to auricula-cordate, margin cre-

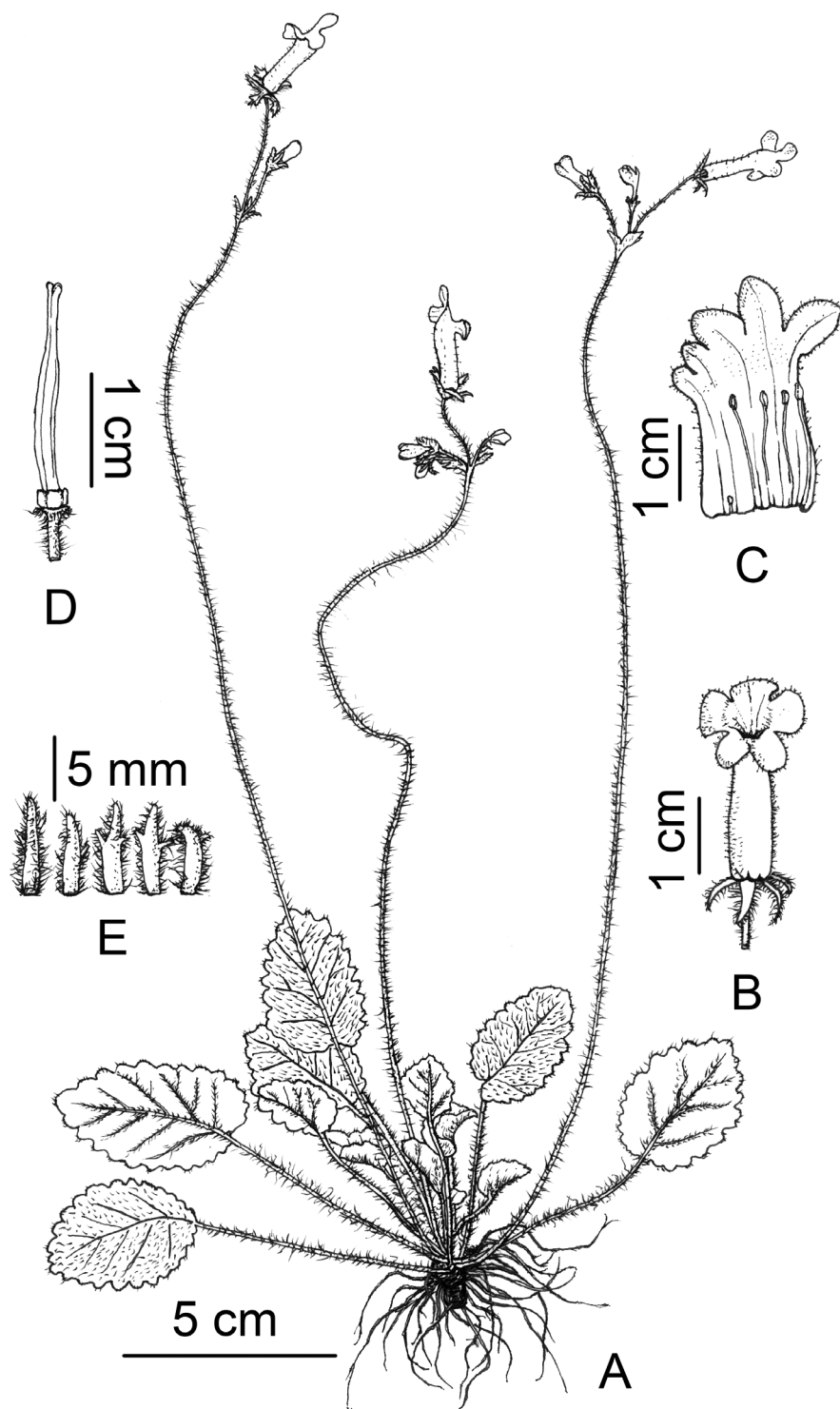


Figure 4. *Oreocharis longipedicellata* Lei Cai & F.Wen, sp. nov. **A** habit **B** front view of flower **C** opened corolla showing stamens and staminode **D** pistil with disc **E** calyx lobes. Drawn by Xuan-Lin Zhu.

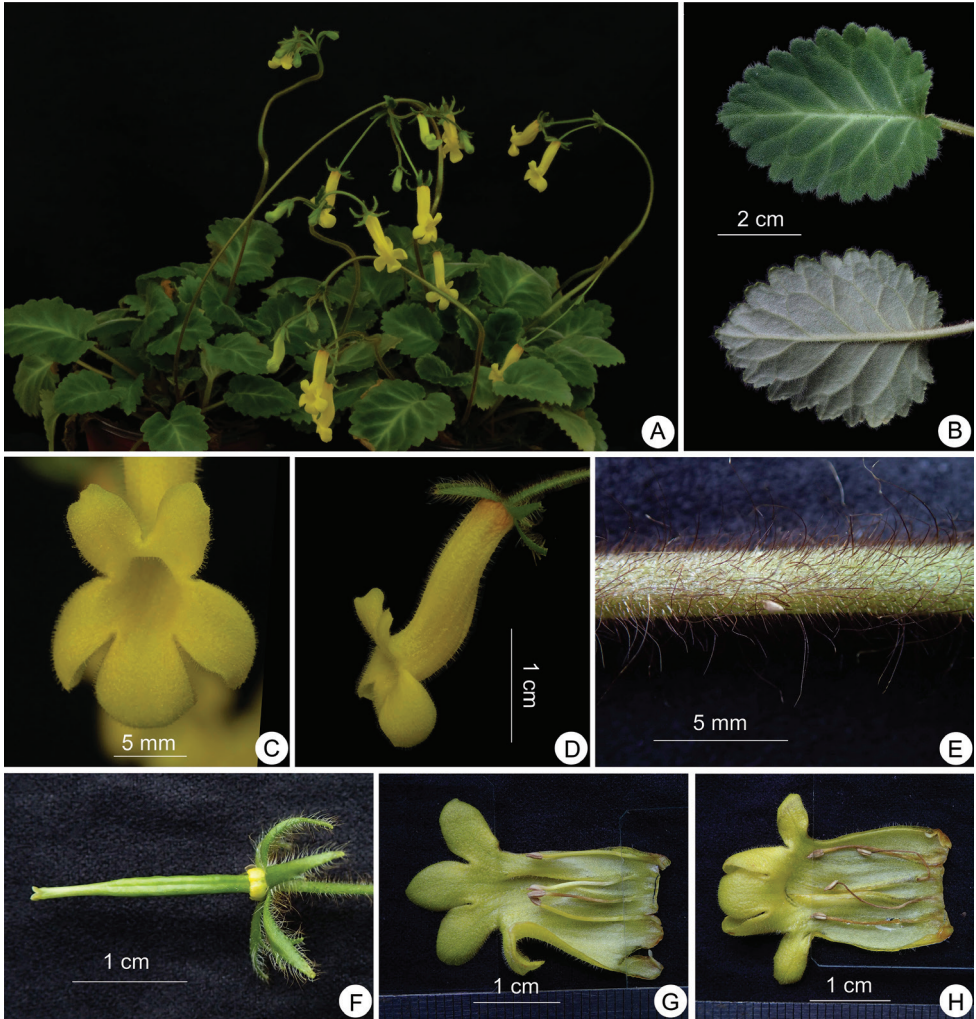


Figure 5. *Oreocharis longipedicellata* Lei Cai & F.Wen, sp. nov. **A** plants cultivated in GBG **B** adaxial and abaxial leaf surface **C** front view of flowers **D** side view of a flower **E** petiole **F** pistil with disc and calyx **G, H** opened corolla showing stamens and staminode. Photographed by Fang Wen.

nate, with brown villous. Cymes axillary 2–5, inflorescence 4–8-flowered; peduncle 20–28 cm long, brown villous; bracts 2, lanceolate to elliptic, 10–12 × 2.5–5.0 mm, adaxially densely villous, abaxially glabrous, sometimes upper part pubescent, margin denticulate; pedicel 2.0–3.5 cm long, densely villous. Calyx 6–9 mm long, 5-lobed to the base, lobes triangular lanceolate to narrowly triangular, 6–9 mm long, 1.5–2 mm wide, outside brown villous, inside glabrous, margin denticulate. Corolla sigmoid, yellow, 2.2–2.8 cm long, outside pubescent and glandular-pubescent, inside glandular-pubescent in the throat and on adaxial lobes, tube cylindrical, slightly bent near the mouth, 1.8–2.2 cm long, 5–7 mm in diameter; limb 2-lipped; adaxial lip 2-lobed to near base, semicircular, lobes 4–5 × 3.8–4.2 mm, abaxial lip 3-lobed to

Table 2. Morphological comparison between *Oreocharis longipedicellata* sp. nov. and *O. panzhouensis*.

Characters	<i>O. longipedicellata</i>	<i>O. panzhouensis</i>
Peduncle	20–28 cm long	4.5–8 cm long
Bract	lanceolate to elliptic, margin denticulate	linear to subulate, margin entire
Calyx	5-lobed to the base, lobes lanceolate to narrowly triangular, outside brown villous	5-lobed to the middle, lobes equal, broadly triangular, outside pubescent and sparsely brown villous
Corolla	sigmoid, tube cylindrical, lobes reflexed outwards slightly	not sigmoid, tube campanulate, lobes not reflexed outwards
Stamens	10–13 mm long, adnate to corolla 3–4 mm from base	5–10 mm long, adnate to corolla 5–6 mm from base
Pistil	15–20 mm long, ovary long cylindrical, 10–12 mm long; style 4–6 mm long	8–14 mm long, ovary cylindrical, 5–8 mm long; style 2–4 mm long

base, semicircular to oval, 6–8 × 5–7 mm. Stamens 4, 1.0–1.3 cm long, adnate to corolla 3–4 mm from the base; filaments linear, glabrous; anthers oblong, 2-loculed, dehiscent longitudinally, connective glabrous; staminode 1, 0.6–1.0 mm long, inserted ca. 1 mm from the base. Disc ca. 1.5 mm high, yellow, margin undulate. Pistil 1.5–2.0 cm long, glabrous; ovary long cylindrical, 10–12 mm long; style 4–6 mm long; stigma bilobed, flabellate. Fruit unknown.

Phenology. Flowering from August to October; fruiting unknown.

Distribution and habitat. *Oreocharis longipedicellata* is currently known by only one population at the type locality, Mengdong, Malipo County, southeastern Yunnan, in the China and Vietnam border area. The species was observed to grow on the surface of moist rocks in the karst region.

Etymology. The specific epithet ‘*longipedicellata*’ refers to the relatively-long peduncle of the new species. This species has almost the longest pedicels in the genus *Oreocharis*.

Vernacular name. The Chinese name of the new species is “Cháng Gěng Mǎ Líng Jù Tái” (长梗马铃薯苔). The first two words, “Cháng Gěng,” mean the long peduncle. The next four words mean *Oreocharis* in mandarin.

Conservation status. The new species could be endangered, but more data is needed to evaluate as the field distribution information is not sufficiently detailed.

Taxonomic affinities. *Oreocharis longipedicellata* most resembles recently published *O. panzhouensis* in the yellow flower, four separated stamens, calyx 5-lobed to the middle and stigma bilobed, flabellate. Nevertheless, it differs from the latter species in several other characteristics (see Table 2).

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Four new species from the diatom (Bacillariophyceae) genus *Adlafia* Moser, Lange-Bertalot & Metzeltin from waterbodies of Vietnam

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Abstract

Four species of the diatom genus *Adlafia* were found from waterbodies of Vietnam and described as new to science. Their formal descriptions are presented herein and they are illustrated by light and scanning electron micrographs. These new species are: *A. lamdongiensis* Glushch., Kulik. & Kociolek, **sp. nov.**, *A. babeiensis* Glushch., Kulik. & Kociolek, **sp. nov.**, *A. vietnamensis* Glushch., Kulik. & Kociolek, **sp. nov.** and *A. dauiensis* Glushch., Kulik. & Kociolek, **sp. nov.** These species are then compared to other similar taxa. Our new findings add to the number of species in this interesting genus and contribute to our understanding of the unique diatom flora found in Vietnam.

Keywords

Adlafia, diatoms, morphology, new species, Southeast Asia, Vietnam

Introduction

The genus *Adlafia* was proposed by Moser et al. (Moser et al. 1998). According to the original description, the genus is overwhelmingly represented by small-cell species (less than 25 µm in length). The raphe is naviculoid; external distal ends are smoothly

bent and slightly extend to the mantle externally while the external proximal ones are drop-shaped, slightly bent to the opposite side from the distal ends (Kulikovskiy et al. 2016). On the inside, the raphe is located on a raised sternum, the distal ends with small helictoglossae, the proximal ends are straight and bent to one side (Morales and Le 2005). A distinctive feature of the genus is the presence of large, often square areolae, closed externally with a hymen and a continuous silica layer (Moser et al. 1998; Lange-Bertalot 2001). Species of the genus are distinguished from those in the genus *Kobayasiella* Lange-Bertalot in Lange-Bertalot and Genkal (1999) by lacking an “umbilicus”, a deflection or nick in the raphe system on the exterior. Currently, the genus belongs to taxa with an unclear taxonomic position (*incertae sedis*). Molecular studies of the genus require the involvement of more strains (Kulikovskiy et al. 2016).

The genus includes 27 species and infraspecific taxa (Guiry and Guiry 2020). Species of the genus are distributed around the world. Most species are aerophilous, being found mainly on mosses, but others prefer oligotrophic streams and lakes with a slightly higher or lower pH value, but are rare in large rivers (Spaulding and Edlund 2009; Kulikovskiy et al. 2016; Cantonati et al. 2017). Species are also known from fossil sediments (Lange-Bertalot and Metzeltin 1996; Benson and Kociolek 2012).

Southeast Asia is a floristically interesting region, from which many new genera and species of centric and pennate diatoms have been described recently (see Gusev and Kulikovskiy 2014; Glushchenko et al. 2016, 2017, 2018, 2019; Kapustin et al. 2017, 2019; Liu et al. 2018; Kulikovskiy et al. 2019, 2020; Rybak et al. 2019). Several *Adlafia* species have been recorded previously from Southeast Asia. In Indonesia, for example, *Adlafia bryophila* (J. Petersen) Lange-Bertalot in Moser et al. 1998 and *Adlafia minuscula* (Grunow) Lange-Bertalot in Lange-Bertalot and Genkal 1999 have been reported (Bramburger et al. 2004). *Adlafia sinensis* Liu & Williams in Liu et al. 2017 was described from south-central China. They also provide a comparison of many *Adlafia* species. In Vietnam, *Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot in Lange-Bertalot and Genkal 1999 was reported from reservoirs, but without an image to document the determination (Duong et al. 2006).

The aim of our work was to identify the species diversity of the genus *Adlafia* in freshwater ecosystems of Vietnam.

Materials and methods

A list of all samples examined in this study with their geographic positions is presented in Table 1. The samples were treated with 10% hydrochloric acid to remove carbonates and washed several times with deionized water for 12 h. The samples were subsequently boiled in concentrated hydrogen peroxide (~37%) to dissolve organic matter. They were then washed four times with deionized water at 12 h intervals. After decanting and refilling with up to 100 ml deionized water, the suspension was spread onto coverslips and left to dry at room temperature. Permanent diatom preparations were mounted in Naphrax. Light microscopic (LM) observations were performed

Table 1. List of samples examined in this study. Geographic locality of samples and measured parameters indicated.

Sample/ Slide	Locality	Habitat	Coordinates	Altitude, m	Temperature, °C	pH	Conductivity, μS cm ⁻¹	Coll. date
00269	Lâm Đồng Province, Da Tien Reservoir	benthos	11°58.816'N, 108°26.987'E	1503	21.5	6.4	81	21.06.2012
00321	Khánh Hòa Province, Hòn Bà Nature Reserve, Dầu River	wet moss	12°06.768'N, 108°59.888'E	275	24	6.7	92	28.05.2012
00325	Khánh Hòa Province, Suối Tiên River	benthos and periphyton	12°12.199'N, 109°01.694'E	68	26	6.9	101	02.07.2012
02168	Bắc Kạn Province, Ba Bể Lake	benthos	22°23.605'N, 105°36.856'E	163	26	8.5	174	19.04.2015
03593	Khánh Hòa Province, Khe River	periphyton	12°16.735'N, 108°54.677'E	34	26.8	6.9	84	08.07.2010
04633	Khánh Hòa Province, Hồ Cầu Đồi Reservoir	periphyton	12°15.750'N, 109°04.012'E	9	29	6.8	110	14.09.2010

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 a JSM-6510LV scanning electron microscope (IBIW, Institute for Biology of Inland Waters RAS, Borok, Russia). For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminum stubs after air-drying. The stubs were sputter-coated with 50 nm Au in an Eiko IB 3 sputter coater. Samples and slides are deposited in the public collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia. The number of examined valves is indicated in each description of the species. The average value of the valve length, width and striae density, as well as standard deviation were calculated using Microsoft Excel 2020. Terminology of the valve follows Moser et al. 1998; Lange-Bertalot 2001; Morales and Le 2005; Kulikovskiy et al. 2016; Tusset et al. 2017 and Ciugulea et al. 2019.

Results

Division: Bacillariophyta Haeckel

Class: Bacillariophyceae Haeckel

Naviculaceae *incertae sedis*

Genus: *Adlafia* Moser, Lange-Bertalot & Metzeltin in Kulikovskiy et al. (2016)

Adlafia lamdongiensis Glushch., Kulik. & Kociolek, sp. nov.

Figs 1, 2

Holotype. Slide no. 00269 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 1A.

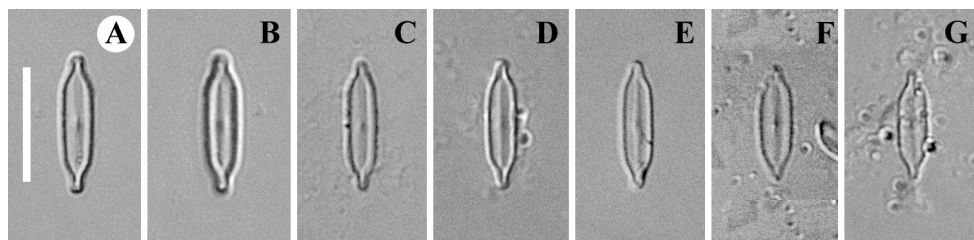


Figure 1. A–G *Adlafia lamdongiensis* Glushch., Kulik. & Kociolek sp. nov. LM, DIC, size diminution series. Slides no 00269 (**A–C, E–G**) and 03593 (**D**). Holotype (**A**). Scale bar: 10 μ m.

Type locality. Vietnam. Lâm Đồng Province, Da Tien Reservoir, benthos, 11°58.816'N, 108°26.987'E, 1503 m elev., *leg.* E.S. Gusev, 21.06.2012.

Description. LM (Fig. 1A–G). Valves linear with weakly convex margins. Ends are distinctly narrowly-rostrate. Length 9.7–13 μ m (11.4 ± 0.9 ; $n = 16$), breadth 2.5–2.8 (2.7 ± 0.1 ; $n = 16$) μ m. Striae and areolae not resolved in LM.

SEM, external view (Fig. 2A–C). Valve face flat. Axial area linear. Central area absent. Raphe filiform, weakly lateral. Proximal raphe endings slightly expanded. Distal raphe endings positioned on valve mantle, hooked and curved in same direction, terminating at valve face-mantle junction. Striae uniseriate, radiate, becoming abruptly convergent approaching apices, Striae 45–50 in 10 μ m (47.5 ± 1 ; $n = 16$). Areolae rounded or rectangular, hymenes not preserved during specimen preparation. Slit-like opening of apical areolae arranged in one row onto valve apex. Areolae 40–50 in 10 μ m (45 ± 1.8 ; $n = 16$).

SEM, internal view (Fig. 2D–F). Raphe slightly lateral, lies in a prominent and raised raphe-sternum. Proximal raphe endings deflected towards primary side of valve. Distal raphe endings terminating in small helictoglossae. Striae continuing onto valve mantle. Short striae alternate with longer striae at valve center. Areolae rounded or rectangular. Openings of apical areolae apically elongated.

Etymology. Epithet refers to the province of Vietnam (Lâm Đồng Province) where the specimens were found.

Distribution. Vietnam. Type locality (slide no. 00269) and slide no. 03593.

Adlafia babeiensis Glushch., Kulik. & Kociolek, sp. nov.

Figs 3, 4

Holotype. Slide no. 02168 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 3A.

Type locality. Vietnam. Bắc Kạn Province, Ba Bể Lake, benthos, 22°23.605'N, 105°36.856'E, 163 m elev., *leg.* E.S. Gusev, 29.04.2015.

Description. LM (Fig. 3A–L). Valves lanceolate with rostrate ends. Length 11.5–14.0 μ m (12.8 ± 0.6 ; $n = 21$), breadth 4.0–4.5 μ m (4.3 ± 0.1 ; $n = 21$). Axial area

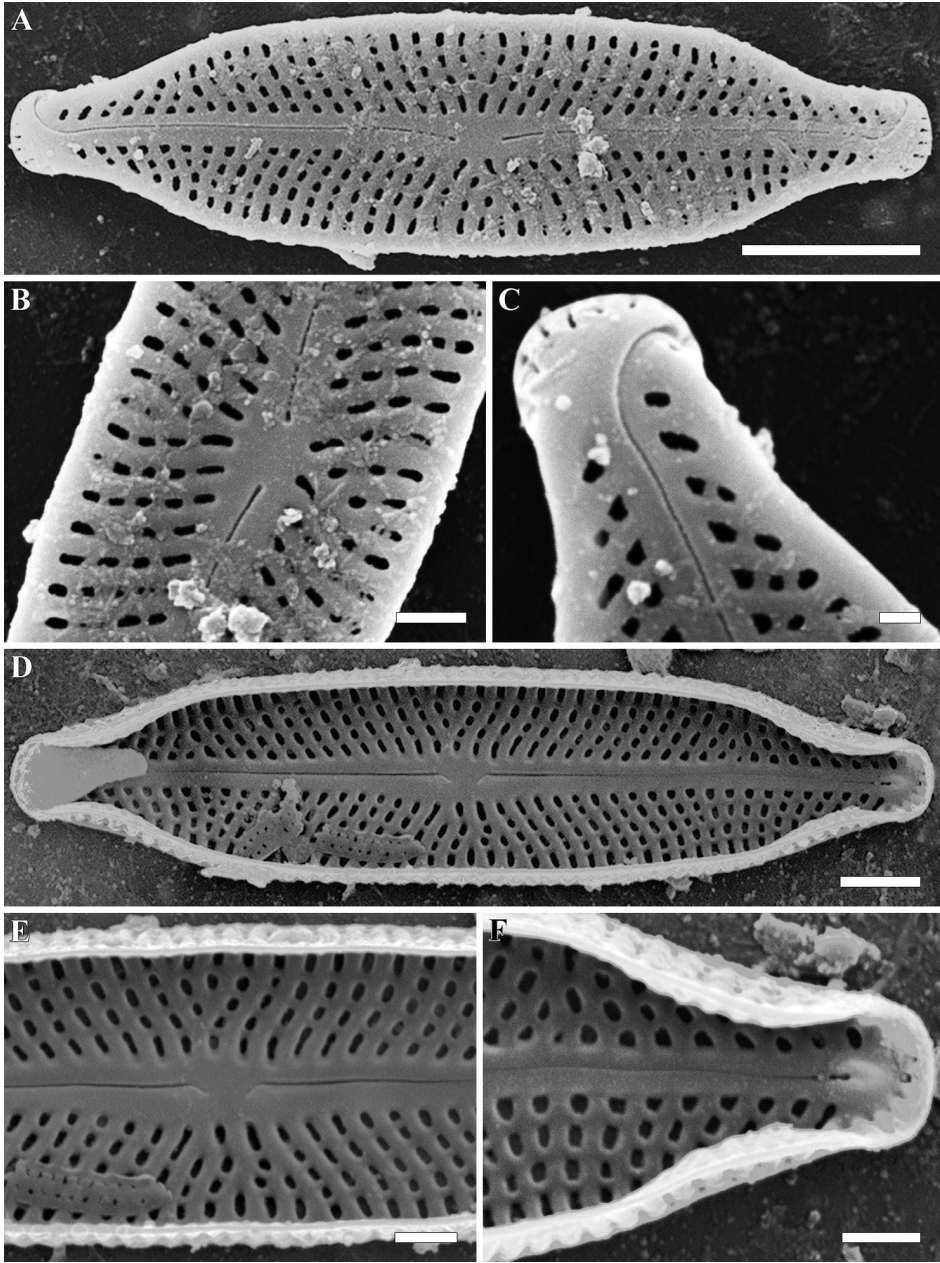


Figure 2. A–F *Adlafia lamdongiensis* Glushch., Kulik. & Kociolek sp. nov. SEM. Sample no 00269 A–C external views D–F internal views A whole valve. The valve face is flat B central area C valve end D whole valve E central area F valve end. Scale bars: 2 μm (A), 1 μm (D), 0.5 μm (B, C, E, F).

narrow, almost linear. Central area weakly expressed or absent. Raphe filiform. Striae indistinct in LM, weakly radial at the central area, convergent towards to the ends. Areolae not resolved in LM.

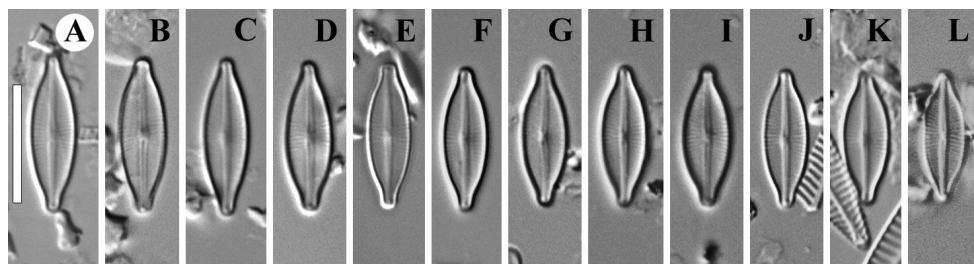


Figure 3. A–L *Adlafia babeiensis* Glushch., Kulik. & Kociolek, sp. nov. LM, DIC, size diminution series. Slide no 02168. Holotype (**A**). Scale bar: 10 µm.

SEM, external view (Fig. 4A–C). Valve face flat. Axial area linear. Central area absent. Raphe filiform, weakly lateral. Proximal raphe endings slightly expanded, deflected. Distal raphe endings positioned on the valve mantle, hooked and curved in the same direction, and terminating at the junction valve face-mantle. Striae uniseriate, radiate, becoming abruptly convergent approaching apices, Striae 36–40 in 10 µm (38 ± 0.1 ; $n = 21$). Areolae rounded or rectangular, occluded by hymenes. Slit-like opening of apical areolae invisible. Areolae 65–70 in 10 µm (67.5 ± 0.8 ; $n = 21$).

SEM, internal view (Fig. 4D). The raphe straight, lying in a prominent and raised raphe-sternum. Proximal raphe endings deflected towards primary side of valve. Distal raphe endings terminating in small helictoglossae. Striae continuing on to valve mantle. Short striae alternate with longer striae at the center of the valve. Areolae rounded. Openings of apical areolae apically elongated.

Etymology. Epithet refers to the lake of Vietnam where the new species was found.

Distribution. Vietnam. Known only from the type locality.

***Adlafia vietnamensis* Glushch., Kulik. & Kociolek, sp. nov.**

Figs 5, 6

Holotype. Slide no. 00325 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 5G.

Type locality. Vietnam. Khánh Hòa Province, Suối Tiên River, benthos and periphyton, 12°12.199'N, 109°01.694'E, 68 m elev., leg. E.S. Gusev, 02.07.2012.

Description. LM (Fig. 5A–K). Valves linear-elliptical with capitate to subcapitate ends. Length 15–22 µm (18.5 ± 1.6 ; $n = 20$), breadth 3.5–5.0 µm (4.3 ± 0.4 ; $n = 20$). Axial area narrow, almost linear. Central area weakly expressed. Raphe filiform. Striae radiate, becoming abruptly convergent approaching apices, 32–34 in 10 µm (33 ± 0.4 ; $n = 20$). Areolae not resolved in LM.

SEM, external view (Fig. 6A–C). Valve face flat. Axial area linear. Central area weakly expressed. Raphe filiform. Proximal raphe endings slightly expanded, deflected.

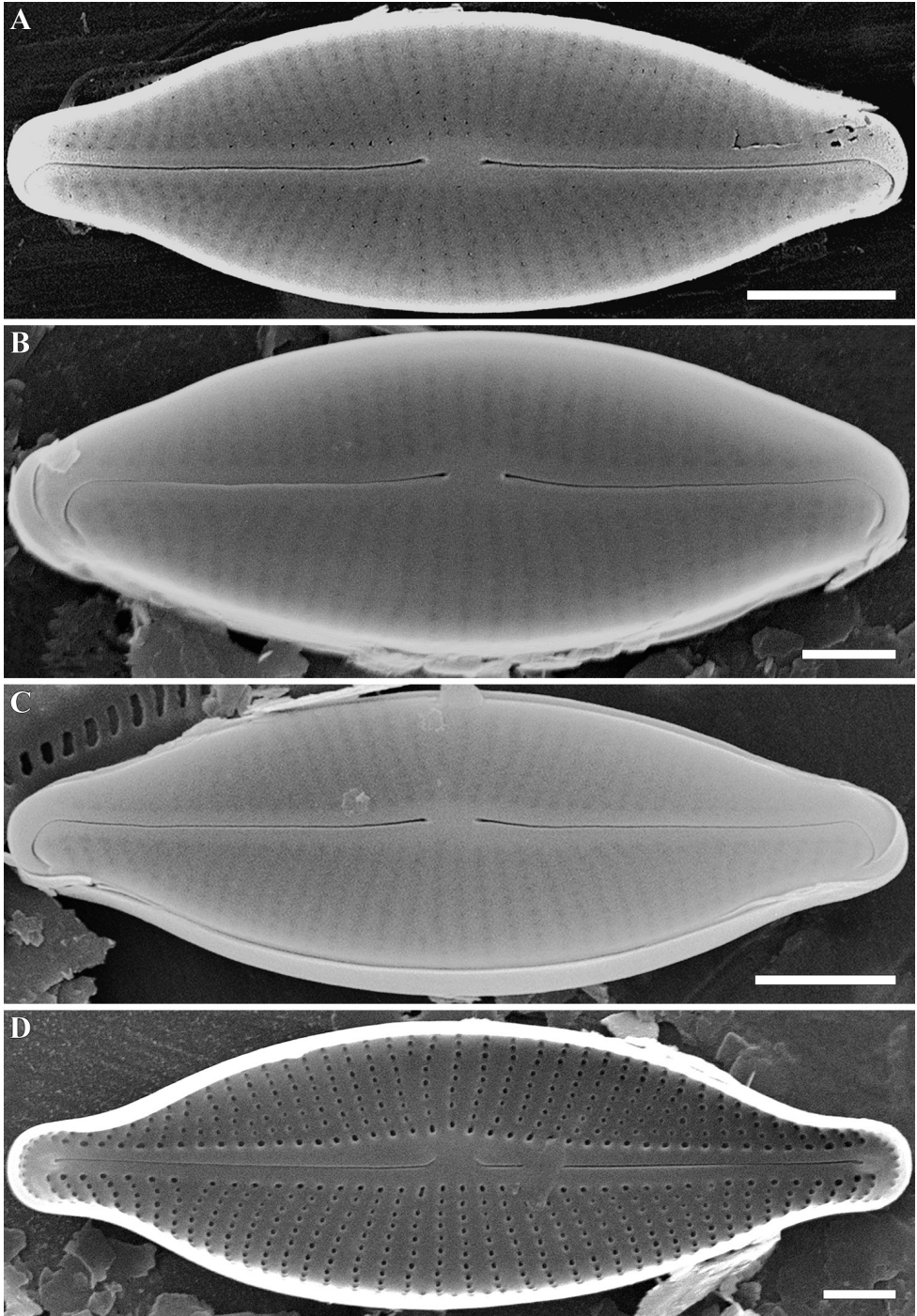


Figure 4. **A–D** *Adlafia babeiensis* Glushch., Kulik. & Kociolek, sp. nov. **A–C** SEM, external views **D** internal views. Sample no 02168. Scale bars: 2 μm (**A**, **C**), 1 μm (**B**, **D**).

Distal raphe endings positioned on the valve mantle, hooked and curved in the same direction, and terminating at the junction valve face. Striae uniseriate. Areolae rounded or rectangular, occluded by hymenes. Slit-like opening of apical areolae invisible. Areolae 50–55 in 10 μm (52.5 ± 1.0 ; $n = 20$).

SEM, internal view (Fig. 6D–F). Raphe straight, lying in a prominent and raised raphe-sternum. Proximal raphe endings deflected towards primary side of valve. Distal raphe endings terminating in small helictoglossae. Striae continuing onto valve mantle. Short striae alternate with longer striae at the center of the valve. Areolae rounded or rectangular. The openings of apical areolae apically elongated.

Etymology. Epithet refers to the country where the new species was found.

Distribution. Vietnam. Slides no. 00325 (type locality) and no. 04633.

***Adlafia dauiensis* Glushch., Kulik. & Kociolek, sp. nov.**

Figs 7, 8

Holotype. Slide no. 00321 in collection of Maxim Kulikovskiy at the Herbarium of the Institute of Plant Physiology Russian Academy of Science, Moscow, Russia, represented here by Fig. 7C.

Type locality. Vietnam. Khánh Hòa Province, Hòn Bà Nature Reserve, Dầu River, wet moss, 12°06.768'N, 108°59.888'E, 275 m elev., *leg.* E.S. Gusev, 2.07.2012.

Description. LM (Fig. 7A–K). Valves linear to linear-elliptical with subcapitate ends. Length 19.0–26.5 μm (22.8 ± 2.3 ; $n = 20$), breadth 4.5–5.5 μm (5.0 ± 0.2 ; $n = 20$). Axial area narrow, almost linear. Central area weakly expressed. Raphe filiform. Striae radiate, becoming abruptly convergent approaching apices, 32–34 in 10 μm . Areolae not resolved in LM.

SEM, external view (Fig. 8A–C). Valve face flat. Axial area linear. Central area weakly expressed. Raphe filiform. Proximal raphe endings slightly expanded, deflected. Distal raphe endings positioned on the valve mantle, hooked and curved in the same direction, and terminating at the junction valve face-mantle. Striae uniseriate. Areolae

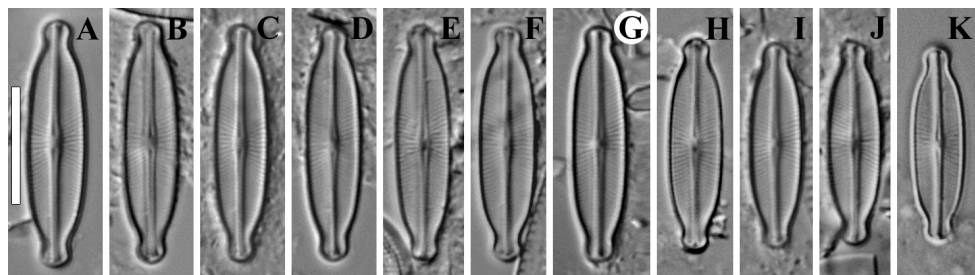


Figure 5. A–K *Adlafia vietnamensis* Glushch., Kulik. & Kociolek, sp. nov. LM, DIC, size diminution series. Slides no 00325 (B–K) and 04633 (A). Holotype (G). Scale bar: 10 μm .

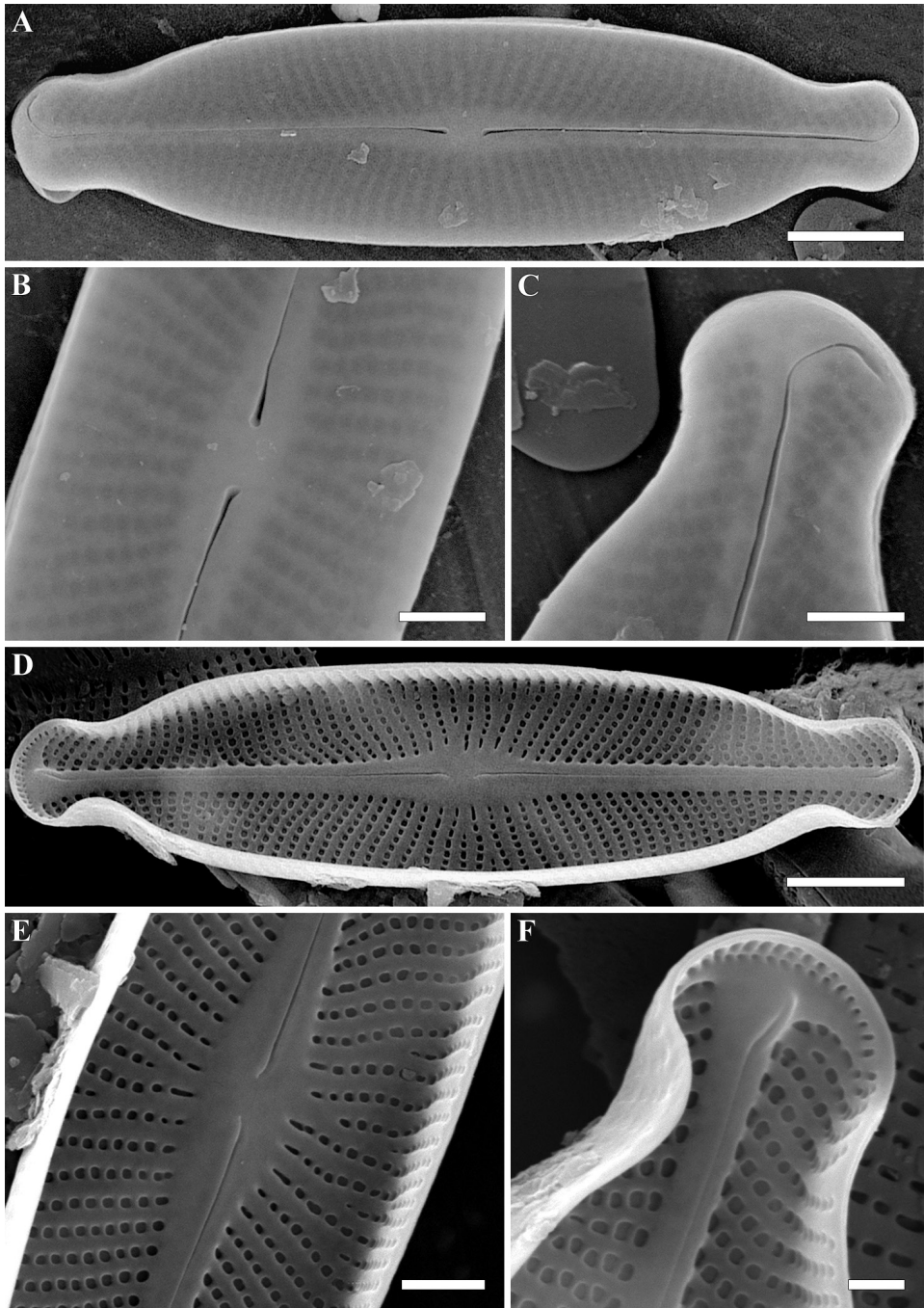


Figure 6. A–F *Adlafia vietnamensis* Glushch., Kulik. & Kociolek, sp. nov. SEM, sample no 00325 A–C external views. D, E internal views A whole valve B central area C valve end D whole valve E central area F valve end. Scale bars: 2.5 μm (A, D), 1 μm (B, C, E), 0.5 μm (F).

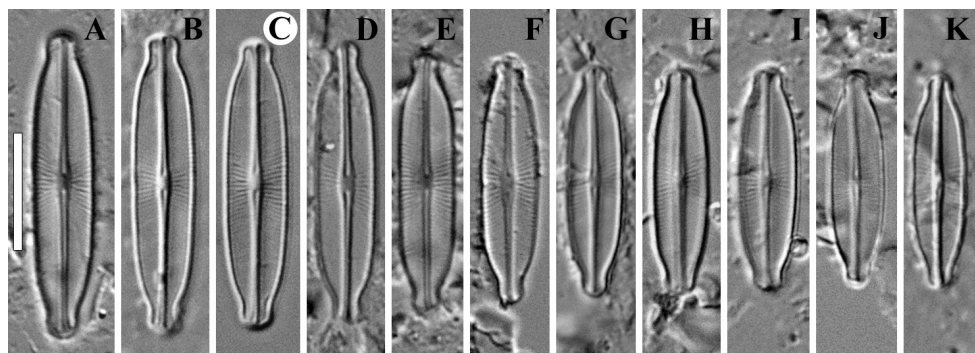


Figure 7. A–K *Adlafia dauiensis* Glushch., Kulik. & Kocielek, sp. nov. LM, DIC, size diminution series. Slide no 00321. Holotype (**C**). Scale bar: 10 μ m.

rounded or rectangular, occluded by hymenes. Slit-like opening of apical areolae invisible. Areolae 55–60 in 10 μ m (57.5 ± 1.1 ; $n = 20$).

SEM, internal view (Fig. 8D–F). Raphe straight, lying in a prominent and raised raphe-sternum. Proximal raphe endings deflected towards primary side of valve. Distal raphe endings terminating in small helictoglossae. Striae continuing onto valve mantle. Short striae alternate with longer striae at the center of the valve. Areolae rounded or rectangular. The openings of apical areolae apically elongated.

Etymology. Epithet refers to the river of Vietnam where the new species was found.

Distribution. Vietnam. Known only from the type locality.

Discussion

The four new species described here from Southeast Asia are morphologically similar to each other, but can be differentiated on the basis of valve shape, valve ends and striae density. All species share the morphological features typical for the genus *Adlafia*. A comparison of species to each other and with previously-described taxa shows that the new species from Southeast Asia are easily distinguished, unique taxa (Table 2).

Adlafia lamdongiensis sp. nov. resembles specimens identified by Lee as *Kobayasiella venezuelensis* Metzeltin & Lange-Bertalot (2007, p. 155, pl. 141, figs 10–23) specimens as illustrated with light micrographs (Lee 2012, fig. 15, K–M) on the basis of valve outline. Moreover, the valve identified by Lee in the SEM (Lee 2012, fig. 15, N) would appear to belong to the genus *Kobayasiella*, since there is a characteristic break of the raphe (the “umbilicus”) inherent to representatives of this genus. The valve has noticeably convex edges (Lee 2012, fig. 15, N), while in our material, and the light micrographs of Lee, valves are slightly convex. In our opinion, the light micrographs and a scanning image of Lee (2012) belong to species from different genera.

Adlafia babeiensis sp. nov. resembles *Adlafia multnomahii* Morales & Le (2005, p. 151, figs 1–38), differing from it mainly by having valves that are more lanceolate in shape and rostrate valve ends (Table 2). In *A. multnomahii*, on the other hand, the valve ends

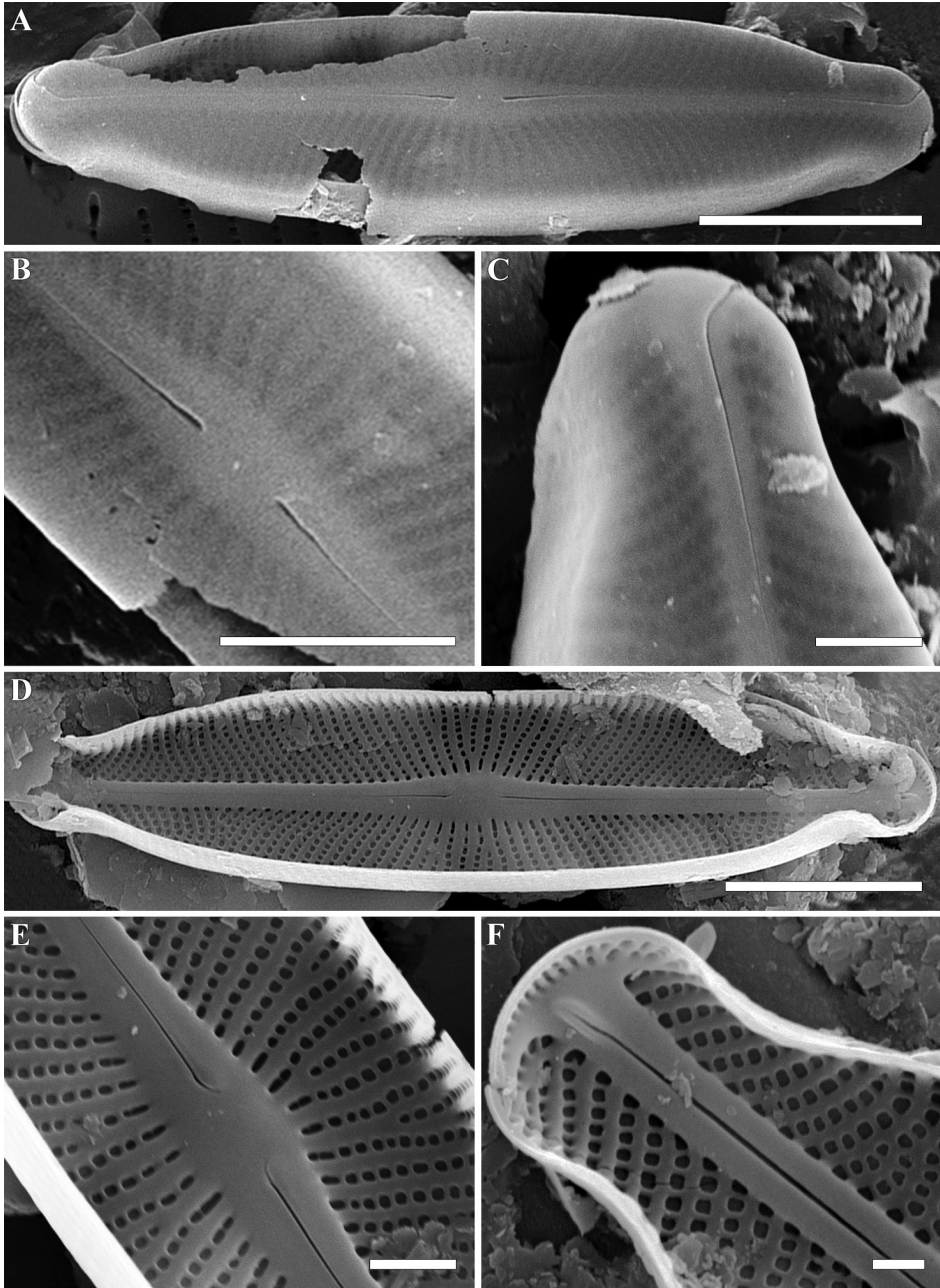


Figure 8. A–F *Adlafia dauiensis* Glushch., Kulik. & Kociolek, sp. nov. SEM, sample no 00321. **A–C** external views **D, E** internal views **A** whole valve **B** central area **C** valve end **D** whole valve **E** central area **F** valve end. Scale bars: 5 μm (**A, D**), 2.5 μm (**B**), 1 μm (**C, F**), 0.5 μm (**F**).

are capitate to rostrate. The density of striae in both species is similar (35–40 at 10 μm in *Adlafia babeiensis* sp. nov. in comparison with 37–45 at 10 μm in *A. multnomahii*). Our species also resembles *Adlafia detenta* (Hustedt) Heudre, Wetzel & Ector in Heudre et

Table 2. Morphometric features of new *Adlafia* species and comparison with similar taxa.

Taxon	Outline	Valve ends	Valve length, μm	Valve width, μm	Striae in 10 μm	Areolae in 10 μm	References
<i>A. lamdongiensis</i> sp. nov.	linear with weakly convex margins	distinctly narrowly-rostrate	9.7–13.0	2.5–2.8	45–50	40–50	This study
<i>A. babeiensis</i> sp. nov.	lanceolate	rostrate	11.5–14.0	4.0–4.5	36–40	65–70	This study
<i>A. vietnamensis</i> sp. nov.	linear-elliptical	capitate to subcapitate	15–22	3.5–5.0	32–34	50–55	This study
<i>A. dauiensis</i> sp. nov.	linear to linear-elliptical	subcapitate	19.0–26.5	4.5–5.5	32–34	55–60	This study
<i>Adlafia multinotata</i> Morales & Le	lanceolate	capitate to rostrate	9–16	4–5	37–45	65–70*	Morales and Le 2005
<i>A. detenta</i> Heudre, Wetzel & Ector in Ector et al.	elliptic to linear-elliptic	capitate	15–18	4.5–6.0	28–33	30–35	Heudre et al. 2018
<i>A. neoniana</i> Cantonati in Ciugulea et al.	elliptic-lanceolate	rostrate to subcapitate	9.4–18.5	3.7–5.1	30–32	45–50	Ciugulea et al. 2019
<i>A. decora</i> Tusset, Tremarin & Ludwig	linear-lanceolate	rostrate	18.2–26.2	4.6–5.7	24–32	50–54	Tusset et al. 2017
<i>Kobayasiella venezuelensis</i> Metzeltin & Lange-Bertalot <i>sensu</i> Lee	Linear with weakly convex margins*	subcapitate*	12.7–13.8*	2.7–2.8*	no data	no data	Lee 2012

*Data obtained from illustrations.

awl. (2018, p. 273), differing from it by the rostrate, rather than bluntly capitate, ends of the valves, striae that are more radiate in their orientation, higher density of striae (36–40 in our species versus 28–33 in 10 μm in *A. detenta*), and higher density of areolae (65–70 at 10 μm in our species versus 30–33 at 10 μm in *A. detenta*) (Table 2).

Adlafia vietnamensis sp. nov. resembles *Adlafia neoniana* Cantonati in Ciugulea et al. (2019, p. 381, figs 1, 2), by having more pronounced capitate ends, as well as less convex valves, in general, with a higher striae density (32–34 at 10 μm for our material compared to 30–32 at 10 μm for *Adlafia neoniana*) (Table 2). The density of the areolae of our species is also slightly higher (50–55 at 10 μm in *Adlafia vietnamensis* sp. nov. versus 45–50 at 10 μm in *A. neoniana*). *Adlafia vietnamensis* sp. nov. has a linearly elliptical shape of valves and valve ends from rostrate to subcapitate; *A. neoniana* is characterized by elliptical-lanceolate valves and rostrate to subcapitate ends. Our species is also similar to *Adlafia dauiensis* sp. nov. (see below) from which it differs mainly by a lower density of striae (50–55 in 10 μm in *A. vietnamensis* sp. nov. versus 55–60 in 10 μm in *A. dauiensis* sp. nov.). *A. vietnamensis* sp. nov. is slightly narrower than *A. dauiensis* sp. nov. (3.5–5.0 μm versus 4.5–5.5 μm). Valve ends of *A. vietnamensis* sp. nov. are capitate to subcapitate in shape while in *A. dauiensis* sp. nov. the ends are subcapitate. The outline of *Adlafia vietnamensis* sp. nov. is linear-elliptical, while *Adlafia dauiensis* sp. nov. has a linear to linear-elliptical outline (Table 2).

Adlafia dauiensis sp. nov. resembles *Adlafia decora* Tusset, Tremarin & Ludwig (2017, p. 261, figs 1–18), differing from it in having capitate, but not rostrate ends, as well as having less convex valves, with a slightly higher striae density (32–34 in 10 μm in our material in comparison with 24–32 to 10 μm in *A. decora*). The areola density is also different between the two species (50–54 at 10 μm in *A. decora* versus 55–60 at 10 μm in *A. dauiensis* sp. nov.) (Table 2).

These new species were found in different water ecosystems of Vietnam that show this genus is widespread in this country, especially in acidic ecosystems.

Morales and Le (2005) suggested *Adlafia* is a monophyletic group but they did not perform any formal analysis or present data to support their conclusion. Based only on a single species, Thomas et al. (2016) suggested *Adlafia* is part of a monophyletic group that could be considered the Cymbellales. No other analysis was forthcoming on this taxon, so this work did not address whether *Adlafia* is a monophyletic genus. Several authors, including in the original description of *Adlafia*, have made comparisons with *Kobayasiella* Lange-Bertalot in Lange-Bertalot and Genkal 1999 (as *Kobayasia* Lange-Bertalot, 1996, non *Kobayasia* S. Imai & A. Kawamura, 1958; see also Morales and Le 2005; Monnier et al. 2012; Van de Vijver et al. 2017). The two genera have fine striae, external distal raphe ends that are distinctly curved and external hymenate occlusions on the areolae. The difference between the two genera is usually suggested to be the absence (in *Adlafia*) or presence (in *Kobayasiella*) of a deflection (umbilicus) in the raphe system. However, this distinction has not always been applied consistently. For example, Le Cohu and Azémar (2010, figs 12, 13) showed specimens of *K. jaagi* (Meister) Lange-Bertalot, 1999 without the umbilicus. Liu et al. (2017) highlighted areas of the girdle that might help diagnose *Adlafia* as a monophyletic group, but these observations await formal analysis.

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Begonia guangdongensis, a new species of *Begonia* (Begoniaceae) from Guangdong, China

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Abstract

A new species of *Begonia* section *Coelocentrum*, *B. guangdongensis* W.H. Tu, B.M. Wang & Y.L. Li from Guangdong Province, China, is described and illustrated here. Morphologically, the new species is most similar to *B. biflora* T. C. Ku and *B. longistyla* Y. M. Shui & W. H. Chen, but differs from *B. biflora* by its rugose leaves and glabrous capsules and from *B. longistyla* by its glabrous stipules without ciliate margin, densely hirsute-pilose leaves and obtuse apex of bracts. Additionally, it is also somewhat similar to *B. chongzuoensis* Yan Liu, S. M. Ku & C.-I Peng, but there are significant distinctions in their stipules, leaves and bracts. The conservation status of *B. guangdongensis* is assessed as Critically Endangered (CR), according to the IUCN Red List Categories and Criteria.

Keywords

Begonia sect. *Coelocentrum*, Guangdong, limestone karsts, new taxon

Introduction

The genus *Begonia* L. (Begoniaceae), consisting of ca. 1900 species, is one of the ten most species-rich flowering plant genera and is widely distributed in the tropical and subtropical areas of the world (Frodin 2004; Hughes et al. 2015). Most *Begonia* species are narrowly distributed, especially those in limestone karsts (Tebbutt et al. 2006;

Ku et al. 2007; Hughes and Hollingsworth 2008). According to the recent taxonomic revision of the genus *Begonia* in Flora of China, nearly 200 species, with 141 local endemics, are reported and represent seven sections (Ku 2007; Ku et al. 2007). The *Begonia* sect. *Coelocentrum*, comprising of more than 70 species, is a typical limestone group confined to the Sino-Vietnamese karst areas and most species circumscribed within the section are rare and known only from a single collection or population (Chung et al. 2014; Peng et al. 2014). Although the section has been shown to be paraphyletic, based on phylogenetic analyses, this section is morphologically well delimited by its parietal placentation and rhizomatous perennation (Chung et al. 2014). Species within this section differ from one another by leaf texture, pubescence and stipule, inflorescence and fruit morphology (Ku et al. 2007).

During a plant diversity survey around Yangchun City in Guangdong Province in October 2019, we discovered a species of *Begonia* with parietal placentation and rhizomatous perennation on the slope of a limestone hill, which was identified as a member of *Begonia* sect. *Coelocentrum*. After critical reviewing the type specimens and protologues of relevant species of this section described from the Sino-Vietnamese karst regions, it was concluded that the species is new to science. Herein, we describe and illustrate it, as well as assess its conservation status.

Taxonomy

***Begonia guangdongensis* W.H.Tu, B.M.Wang & Y.L.Li, sp. nov.**

urn:lsid:ipni.org:names:77211928-1

Figs 1, 2

Diagnosis. *Begonia guangdongensis* is morphologically similar to *B. biflora* T. C. Ku (Wu and Ku 1997), *B. longistyla* Y. M. Shui & W. H. Chen (Shui and Chen 2005) and *B. chongzuoensis* Yan Liu, S. M. Ku & C.-I Peng (Peng et al. 2012) by sharing obliquely ovate asymmetric leaves, hairy petioles, a glabrous peduncle, 2–3 times branched dichasial cyme and glabrous trigonous-ellipsoid capsules. However, it can be easily distinguished from *B. biflora* by its stipules with aristate apex and without ciliate margin (vs. aristate and ciliate apex and ciliate margin), rugose leaves (vs. flat) and glabrous capsules (vs. pubescent); it differs from *B. longistyla* by its abaxially glabrous stipules without ciliate margin (vs. abaxially hairy stipules with ciliate margin), leaves densely hirsute-pilose (vs. densely tuberculate-based pilose-setula) and inflorescence equal or slightly shorter than petioles (vs. much longer) and it can be distinguished from *B. chongzuoensis* by its persistent stipules and bracts (vs. caducous), rugose leaves (vs. nearly flat), leaves densely white hirsute-pilose (vs. moderately to sparsely whitish-hyaline or reddish setulose) and an unequally 3-winged capsule (vs. equal or subequal). (Table 1).

Type. CHINA. Guangdong Province, Yangchun City, Chunwan Town, on a slope of a limestone hill in an evergreen forest, 22°21'44.04"N, 111°57'26.28"E, alt. 88 m, 6 October 2019, *Li et al.* 263 (holotype, CANT!; isotype, IBSC!).

Table 1. Difference between *Begonia guangdongensis*, *B. biflora*, *B. longistyla* and *B. chongzuoensis*.

Character	<i>B. guangdongensis</i>	<i>B. biflora</i>	<i>B. longistyla</i>	<i>B. chongzuoensis</i>
Stipules	persistent, ovate-triangular, apex aristate, margin eciliate, abaxially glabrous	persistent, ovate-triangular, apex aristate and ciliate, margin ciliate, abaxially glabrous or with few hairs on midrib	persistent, triangular, apex aristate, margin ciliate, abaxially with hairs	caducous, ovate or triangular-ovate, apex aristate, margin eciliate or sparsely ciliate, abaxially glabrous or with few hairs along midrib
Petioles	ca. 15–30 cm long, densely white villous	4–22 cm long, hirsute-villous	3–5 cm long, densely covered with strigae	4.5–15 cm long, sparsely hirsute-villous
Leaf blades	10–18 × 7–13 cm, apex acuminate or caudate, margin irregularly repand serrate, adaxial surface rugose, densely white hirsute-pilose, veins depressed	8–25 × 7–23 cm, apex obtuse, sometimes rounded or acute, margin crenulate and irregularly denticulate, adaxial surface flat, sparsely setulose or hispidulous, veins not depressed	6–10 × 4–6 cm, apex rotundate or with an obtuse tip, margin serrulate, adaxial surface rugose, densely tuberculate-based pilose-setulose, veins depressed	6–13 × 5–10 cm, apex acuminate or shortly acuminate, margin crenate-denticulate, adaxial surface nearly flat, moderately to sparsely whitish-hyaline or reddish setulose, veins slightly depressed
Bracts	persistent, oblong, apex obtuse	persistent, oblong or long ovate, apex undescribed	persistent, ovate, apex with a tip	caducous, ovate or rounded, apex obtuse or rounded
Inflorescence	6–15 flowers, peduncle glabrous, 15–20 cm, equal or slightly shorter than petioles	4–13 flowers, peduncle glabrous or sparsely pilose, 5–7.5 cm, shorter than petioles	20–40 flowers, peduncle glabrous, 4–8 cm long, much longer than petioles	4–8 flowers, peduncle glabrous, 5–12 cm long, shorter than petioles
Staminate flower	outer tepals 9–14 × 8–13 mm, inner tepals oblong or narrowly elliptic, 7–9 × 3–4 mm	outer tepals 4–11 × 5–9 mm, inner tepals obovate or elliptic, 6–9 × 3–5 mm	outer tepals 4–5 mm in diam., inner tepals obovate, 4.5–5 × 3–3.5 mm	outer tepals 11–14.5 × 11–15 mm, inner tepals obovate or narrowly obovate, 9–11 × 3.5–5 mm
Pistillate flower	outer tepals 6–9 × 8–12 mm, inner tepals oblong or ovate-lanceolate, styles yellow	outer tepals 6–9.5 × 6–8 mm, inner tepals oblanceolate, styles yellowish-green	outer tepals 4–5 mm in diam., inner tepals obovate, styles yellow	outer tepals 9.5–11.5 × 10–11.5 mm, inner tepals elliptic or broadly lanceolate, styles yellow
Capsules	trigonus-ellipsoid, unequally 3-winged, glabrous, with a few small red spots	oblong, unequally or subequally 3-winged, pubescent	ovate, subequally 3-winged, glabrous	trigonus-ellipsoid, somewhat compressed, equally or subequally 3-winged, glabrous
Flowering time	September to October	May	April to June	May to September

Description. Perennial herbs, rhizomatous. Rhizomes creeping, red, stout, 7–12 mm in diam., internodes 4–7 mm long, sparsely hairy. Leaves simple and alternate; stipules generally persistent, ovate-triangular, 5–8 × 3–5 mm, apex aristate, arista ca. 1.5 mm long, abaxially glabrous; petioles red, ca. 15–30 cm long, with densely white villose, \pm reflexed trichomes; blades basifixed, asymmetric, obliquely ovate, 10–18 × 7–13 cm, papery, rugose, adaxially densely white hirsute-pilose, veins depressed, abaxially hirsute-pilose, denser on primary veins, veins convex, base obliquely deeply cordate, apex acuminate or caudate, margin irregularly repand serrate and ciliate; basal palmate veins 6–7. Inflorescences axillary, arising directly from rhizome, flowers 6–15 in a 2–3 times branched dichasial cyme; peduncles 15–20 cm long, equal or slightly shorter than petioles, glabrous; bracts oblong, 2–3 × 1–1.5 mm, apex obtuse, margin serrulate and ciliate. Staminate flowers: pedicel 1–1.5 cm long, glabrous; tepals 4, outer 2 ovate to suborbicular, 9–14 × 8–13 mm, upper side pinkish-white, lower side pink with red nerves, glabrous on both sides, inner 2 white, oblong or narrowly

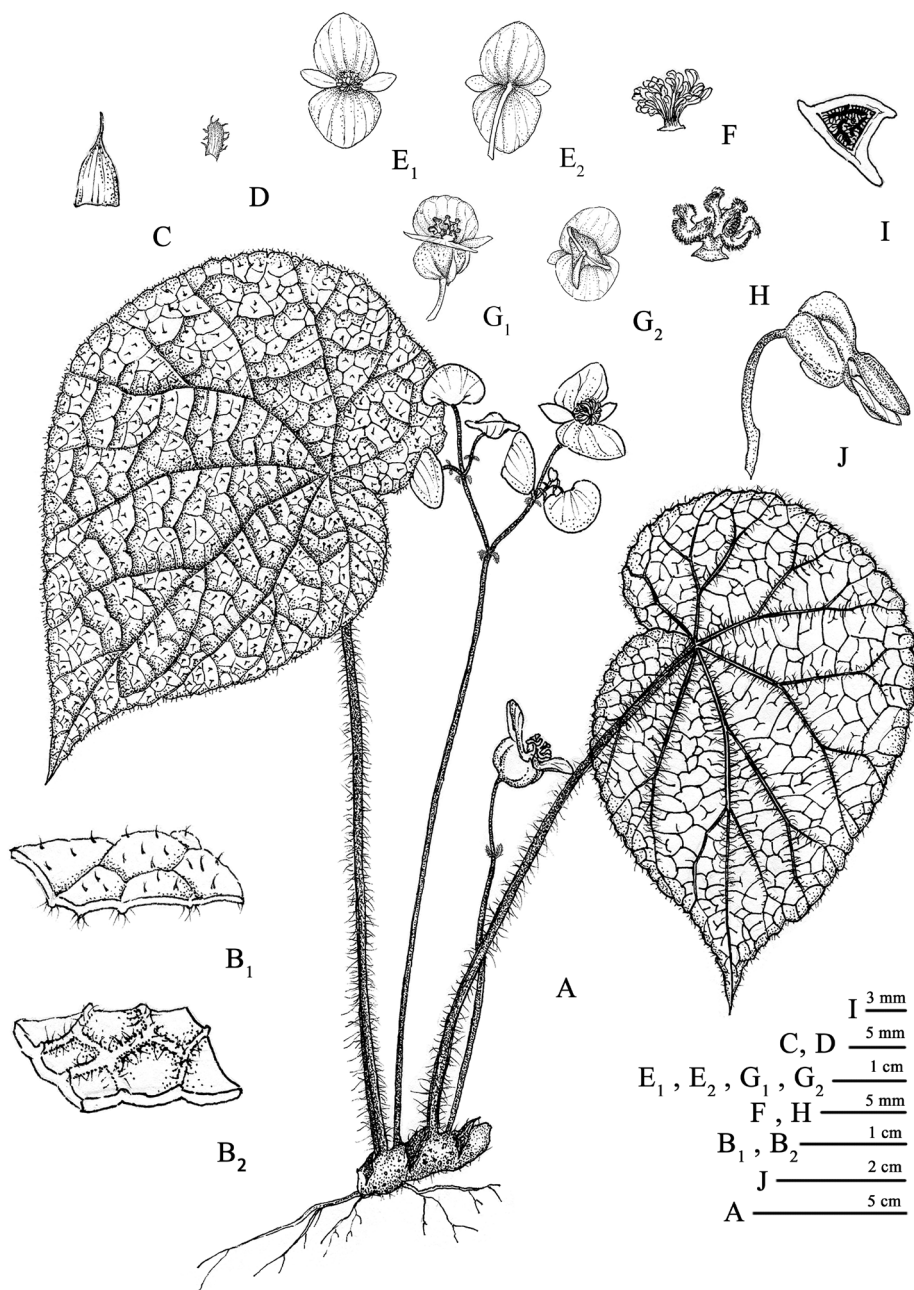


Figure 1. *Begonia guangdongensis* **A** plant **B₁** close up of adaxial surface of leaf **B₂** close up of abaxial surface of leaf **C** stipule **D** bract **E₁** and **E₂** staminate flower **F** androecium **G₁** and **G₂** pistillate flower **H** style and stigma **I** cross section of ovary in the middle part **J** immature capsule. Drawn by Zheng-meng Yang.

elliptic, 7–9 × 3–4 mm, glabrous; androecium actinomorphic, nearly spherical, yellow, stamens numerous, filaments nearly free, 1–1.5 mm long, anthers obovate, ca. 1.2 × 0.7 mm, apex emarginated. Pistillate flower: pedicel 1–1.5 cm; tepals 3, out-

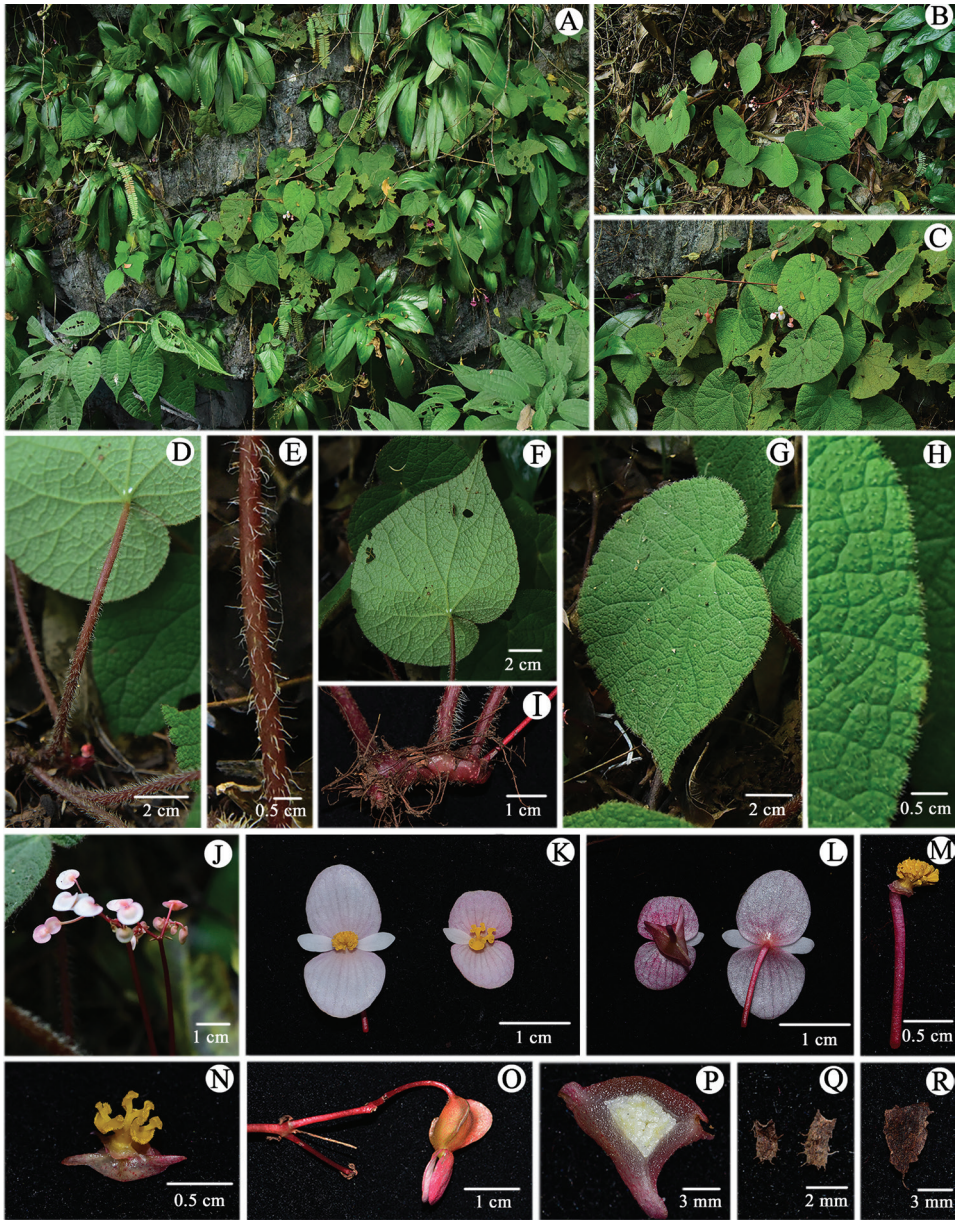


Figure 2. Habitat and morphology of *Begonia guangdongensis* **A, B** habitat **C** habit **D** petiole **E** close up of petiole **F** view of abaxial surface of leaf **G** view of adaxial surface of leaf **H** close up of adaxial surface of leaf **I** rhizome **J** inflorescences **K** view of adaxial surfaces of staminate and pistillate flower **L** view of abaxial surfaces of staminate and pistillate flower **M** androecium **N** styles and stigmas **O** immature capsule **P** cross section of ovary in the middle part **Q** dry bract **R** dry stipule.

er 2 broadly ovate to suborbicular, 6–9 × 8–12 mm, pink with red nerves, glabrous on both sides, inner 1 of left side white, oblong or ovate-lanceolate, 5–6 × 2–3 mm; styles 3, fused at base, yellow, ca. 1.5–2 mm long, the upper 2-cleft; stigmas spirally

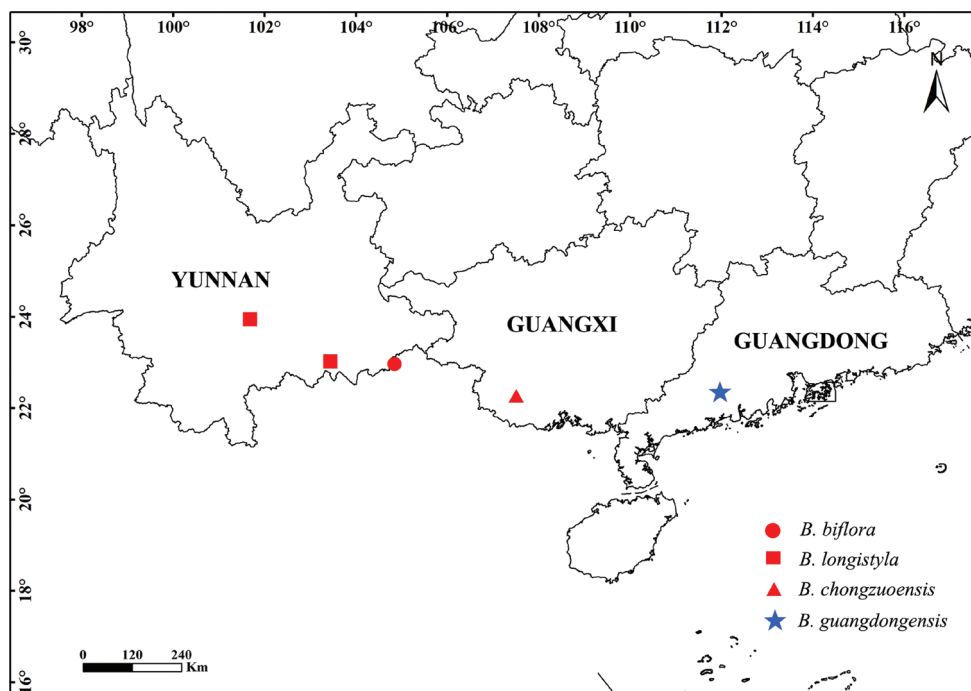


Figure 3. Distribution map of *B. biflora*, *B. longistyla*, *B. chongzuoensis* and *B. guangdongensis*.

twisted; ovary trigonous-ellipsoid, dark pink, 1-locular with parietal placentation, glabrous, 3-winged. Capsule nodding, trigonous-ellipsoid, apex obtuse, 8–10 mm long, 5–7 mm in diam. (wings excluded), surface with a few small red spots, unequally 3-winged, abaxial wing lunate, 2.5–5 mm wide, lateral wings 2–3 mm wide, glabrous.

Phenology. Flowering in September to October, fruiting in October to November.

Etymology. The new species is named after the type locality, Guangdong Province, China.

Habitat. This new species grows on the slope of a limestone hill in evergreen forests at an elevation of 80–100 m (Fig. 3).

Distribution. Only one population of this new species was discovered in Guangdong Province in China.

Conservation Status. **Critically Endangered (CR).** Limestone areas in Chunwan Town have been searched for this new species, but *Begonia guangdongensis* is known only from one population consisting of ca. 100 mature individuals. The area of occupancy (AOO) of the species is estimated to be less than 4 km², which indicates the species belongs in the Critically Endangered category under criterion B2, according to the IUCN Red List Categories and Criteria (IUCN 2019). Since the species grows on a limestone hill near two cement factories, the species is threatened by the limestone quarrying. Its habitat will likely be destroyed since the area is undergoing a continuing decline. Based on the current information (one location with area in continuing decline and AOO less than 10 km²), the new species can be assessed as Critically Endangered [B2ab(iii)] (IUCN 2019).

Discussion. *Begonia guangdongensis*, belonging to *Begonia* sect. *Coelocentrum*, is a very distinctive species in having leaf features, such as rugose and densely hirsute-pilose leaves and an obtuse apex of the capsules. Although it is more or less similar to *B. biflora*, *B. longistyla* and *B. chongzuoensis* in their obliquely ovate asymmetric leaves and glabrous trigonous-ellipsoid capsules, it differs from *B. biflora* by its sparsely hairy and smooth rhizomes (vs. rough rhizomes with many membranous scales), leaves with densely hirsute-pilose and depressed veins on adaxial surface (vs. with sparsely setula and veins not depressed) and stipule and capsule features discussed in the above diagnostic description. However, *B. guangdongensis* is quite different from *B. longistyla*, being distinguished by its oblong bracts with obtuse apex (vs. ovate bracts with a tip apex), stipule and leaf pubescence, length of inflorescence and capsules features. *B. guangdongensis* is also markedly distinct from *B. chongzuoensis* by its stipules, leaf and bract and capsule features. In addition, their distribution range is different (Fig. 3). Both *B. biflora* and *B. longistyla* are distributed in Yunnan Province and *B. chongzuoensis* in Guangxi Province, whereas *B. guangdongensis* occurs in Guangdong Province. Additionally, *B. guangdongensis* flowers in September to October, while *B. biflora* flowers in May, *B. longistyla* in April to June and *B. chongzuoensis* in May to September. Thus, even if they were growing together, they would be genetically isolated in time.

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Primulina jiuyishanica (Gesneriaceae), a new species from Hunan, China

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Abstract

Primulina jiuyishanica K. Liu, D.C. Meng & Z.B. Xin, a new species of Gesneriaceae from Hunan, China, is described and illustrated. The new species is morphologically similar to *Primulina fimbrisepala* (Hand.-Mazz.) Yin Z. Wang, but differs in its elliptic to broadly elliptic leaf blade with broadly cuneate base, peduncle densely pubescent with sparse glandular hairs, corolla throat with no purple spots inside, the yellow patch in the throat densely glandular-pubescent and pistil densely glandular-pubescent. Photographs and descriptions of the new species are provided below.

Keywords

Didymocarpoideae, flora of Hunan, *Primulina fimbrisepala*, taxonomy

Introduction

Since the original monotypic genus *Primulina* was redefined in 2011 (Wang et al. 2011; Weber et al. 2011), many taxa new to science have been published by taxonomists and researchers, making it the largest genus of the Chinese Gesneriaceae (Wen

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et al. 2019, 2020; Möller 2019). For instance, a total of 9 new species and one variety of *Primulina* were published in 2019, including *P. purpureokylin* F. Wen, Yi Huang & W.C. Chou, *P. persica* F. Wen, Yi Huang & W.C. Chou, *P. cerina* F. Wen, Yi Huang & W.C. Chou, *P. niveolanosa* F. Wen, S. Li & W.C. Chou, *P. leiyyi* F. Wen, Z.B. Xin & W.C. Chou (Li et al. 2019), *P. serrulata* R.B. Zhang & F. Wen (Jiang et al. 2019), *P. anisocymosa* F. Wen, Xin Hong & Z.J. Qiu (Hong et al. 2019), *P. chingipengii* W.B. Xu & K.F. Chung (Xu et al. 2019), *P. lianchengensis* B.J. Ye & S.P. Chen (Ye et al. 2019) and *P. sichuanensis* (W.T. Wang) Mich. Möller & A. Weber var. *pinnatipartita* H.H. Kong & L.H. Yang (Kong et al. 2019). As this trend persists, more new species will likely be discovered in the near future (Möller 2019). *Primulina*, which mainly grows in limestone areas, are found only in southern and southwestern China and northern Vietnam (Möller et al. 2016). In all, 197 species and 27 varieties of *Primulina* exist at present, including 183 species and 27 varieties recorded from China and 21 species recorded from Vietnam (Hô 2000; Burt 2002; Weber et al. 2011; Möller et al. 2016; IPNI 2020; Wen et al. 2020).

In 2016, one of the authors (LK) discovered some plants in the Jiuyishan National Nature Reserve, Hunan Province, China, which possibly represented an undescribed species. Some living plants were mailed to the Gesneriad Conservation Center of China (GCCC) for observation and conservation. Those living individuals were introduced and cultivated in the gardens of the GCCC, and the lead author continuously monitored the population in the wild for several years. A detailed comparison of these specimens and living plant materials with the type specimens and protologues of known *Primulina* species revealed that these specimens neither fit the existing protologues nor conform to the type specimens of these species. Nevertheless, the inflorescence, shape and color of the corolla, stamens and staminodes are most similar to those of *P. fimbrisepala* (Hand.-Mazz.) Yin Z. Wang. It can be distinguished from the latter by a combination of several morphological characters of the leaf blade, peduncle, corolla throat and pistil. Thus, we confirmed that it represents a new species of *Primulina*, and described and illustrated it here. The description, illustration, information on ecology, phenology, and provisional conservation assessment by using IUCN categories and criteria (2019) of the proposed new species are also provided.

Methods

The plant material for description was collected in the field at its type locality in 2017. Morphological observations and dissections of plant material of this new species were made under a stereoscopic microscope and measured and described using the terminology used by Wang et al. (1998). The literature examined included related monographs and papers (e.g., Wood 1974; Wang et al. 1998; Li and Wang 2004; Weber 2004; Haston and De Craene 2007; Chen et al. 2008; Wei et al. 2010). Specimens stored in herbaria in China, Vietnam, the United States and the United Kingdom (E, GH, HN, IBK, K, KUN, MO, PE, PH, US and VNMN) were examined.

Taxonomic treatment

***Primulina jiuyishanica* K. Liu, D.C. Meng & Z.B. Xin, sp. nov.**

urn:lsid:ipni.org:names:77211929-1

Figure 1

Diagnosis. *Primulina jiuyishanica* resembles *P. fimbrispala* (Fig. 2) in having similar inflorescence and corolla color, but can be distinguished by its leaf blade being elliptic to broadly elliptic, base broadly cuneate (*vs.* blade ovate, broadly ovate to suborbicular, base cordate), peduncle densely pubescent with sparse glandular hairs (*vs.* eglandular-pubescent to appressed pilose), corolla throat with no purple spots inside (*vs.* corolla throat with few to many purple spots inside), the yellow patch in the throat densely glandular-pubescent (*vs.* densely eglandular-pubescent) and pistil densely glandular-pubescent (*vs.* eglandular-pubescent).

Type. CHINA. Hunan Province, Yongzhou City, Ningyuan County, Jiuyishan National Nature Reserve, growing on a granite rock in the forest in a damp and moist valley, alt. 900–1300 m, 14 April 2017, *Kun Liu 20170414-01* (holotype: IBK!; isotypes: IBK!, KUN!).

Description. *Herbs* perennial, rhizomatous. *Stem* subterete, ca. 6 cm long, ca. 2 cm in diameter. *Leaves* 4–6, basal, opposite; leaf blade thickly chartaceous, elliptic to broadly elliptic, 6–9 × 6–7 cm, margin conspicuously serrate, lateral veins 4–5 on each side, abaxially conspicuous, apex obtuse, base broadly cuneate, oblique or slightly asymmetric, adaxially densely pubescent with sparse glandular hairs, abaxially densely pubescent. *Petiole* flattened, 4–5 cm long, ca. 0.5 cm wide, densely villous. *Cymes* 1–5 or more, axillary, 1–2-branched, 2–14-flowers per cyme; *peduncles* 6–8 cm long, 2.5–3 mm in diameter, densely pubescent with sparse glandular hairs; *pedicel* 1–2.3 cm long, ca. 2 mm in diameter, densely pubescent with sparse glandular hairs; *bracts* 2, opposite, narrowly lanceolate, 1.1–1.2 cm long, 2–3.5 mm wide, sparse hydathodes, both surfaces sparsely pubescent; *bracteole* 1, lanceolate, 3–5 mm long, 1–2 mm wide, sparse hydathodes, both surfaces sparsely pubescent. *Calyx* 5-parted to near base, lobes narrowly lanceolate, 5–7 × 2–3 mm, sparse hydathodes on each side; apex acute, outside densely pubescent, inside subglabrous. *Corolla* pink to bluish violet, 4–4.5 cm long, 2.6–3 cm wide; *corolla tube* funnelform, 2.5–3 cm long, 1.2–1.5 cm in diameter, outside glandular-pubescent, inside glabrous; with two distinct longitudinal ridges on the corolla tube floor; a yellow patch at corolla throat extends to the middle of the corolla tube, densely glandular-pubescent; limb distinctly 2-lipped, adaxial lip 2-lobed, lobes broadly ovate, abaxial lip 3-lobed, middle lobe narrowly orbiculate or broadly ovate, lateral lobes oval or oblong. *Stamens* 2, adnate to ca. 1.4 cm above the base of the corolla tube; 9–10 mm long, terete, geniculate near middle, knee greenish yellow, the rest white, sparsely glandular-pubescent; anthers fused by the entire adaxial surfaces, abaxially densely whitish pubescent; *staminodes* 3, lateral ones ca. 4 mm long, adnate to ca. 9 mm above the base of the corolla tube, terete, apically capitate, the middle one ca. 0.9 mm long, adnate to ca. 1.5 mm above the base of the corolla tube.

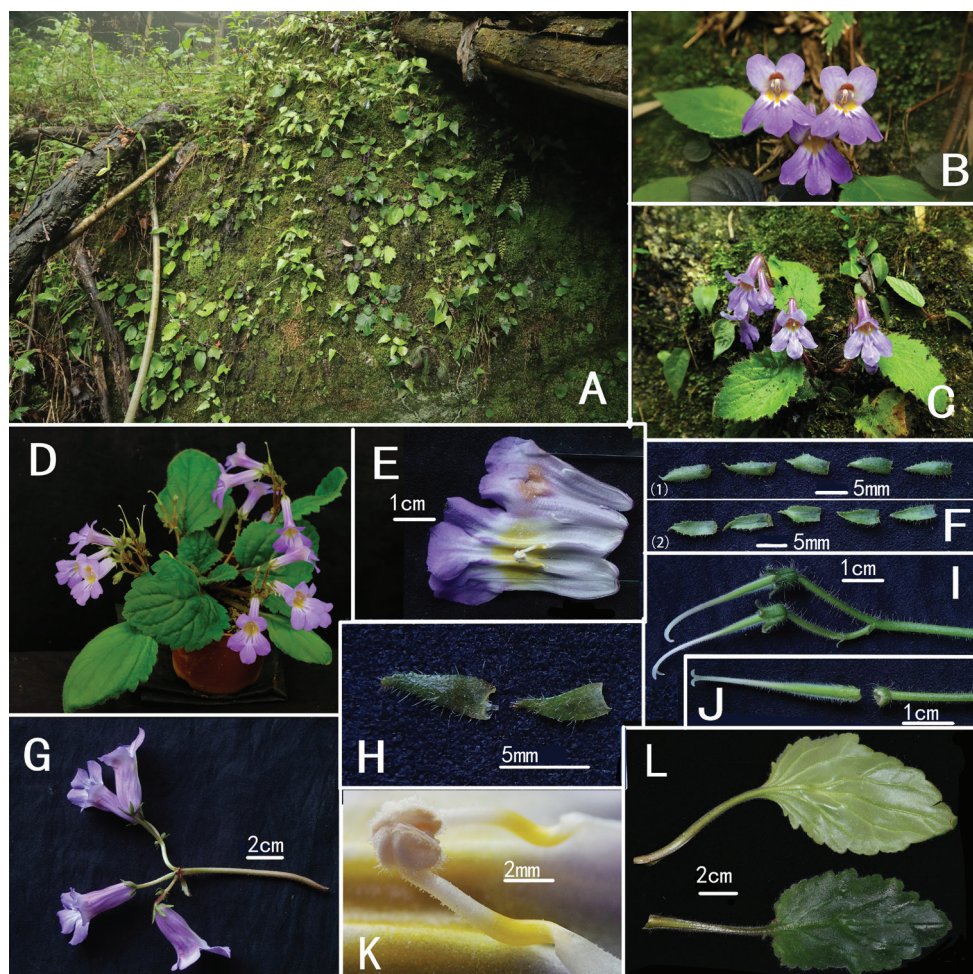


Figure 1. *Primulina jiuyishanica* **A** habitat **B–C** habit with flowers **D** cultivated plant **E** opened corolla **F** (1) adaxial surfaces of calyx lobes (2) abaxial surfaces of calyx lobes **G** inflorescence **H** bracts **I** inflorescence after the corolla shedding **J** dissected ovary, disc and pedicel **K** stamens **L** abaxial and adaxial surfaces of leaf blades (Photographed by Fang Wen).

Disc annular, ca. 1 mm high, margin repand. Pistil 3.1–3.6 cm long; ovary cylindrical, 1.2–1.6 cm long, ca. 4.5 mm in diameter, densely glandular-pubescent to glandular-puberulent; **style** 1.5–1.6 cm long, 1–1.5 mm in diameter, densely glandular-pubescent to glandular-puberulent; **stigma** chiritoid, lower lobe ca. 1 mm wide, divided, lobes ca. 4 mm long. **Capsule** linear, 5–5.5 cm long, densely glandular-pubescent to glandular-puberulent.

Distribution and habitat. We found eight populations in different places of Jiuyishan National Nature Reserve for the new species through several field investigations. It grows on granite rocks, in association with *Pileostegia viburnoides* Hook. f. & Thoms., *Hedera sinensis* (Tobl.) Hand.-Mazz, *Euonymus actinocarpus* Loes., *Viola kosanensis*

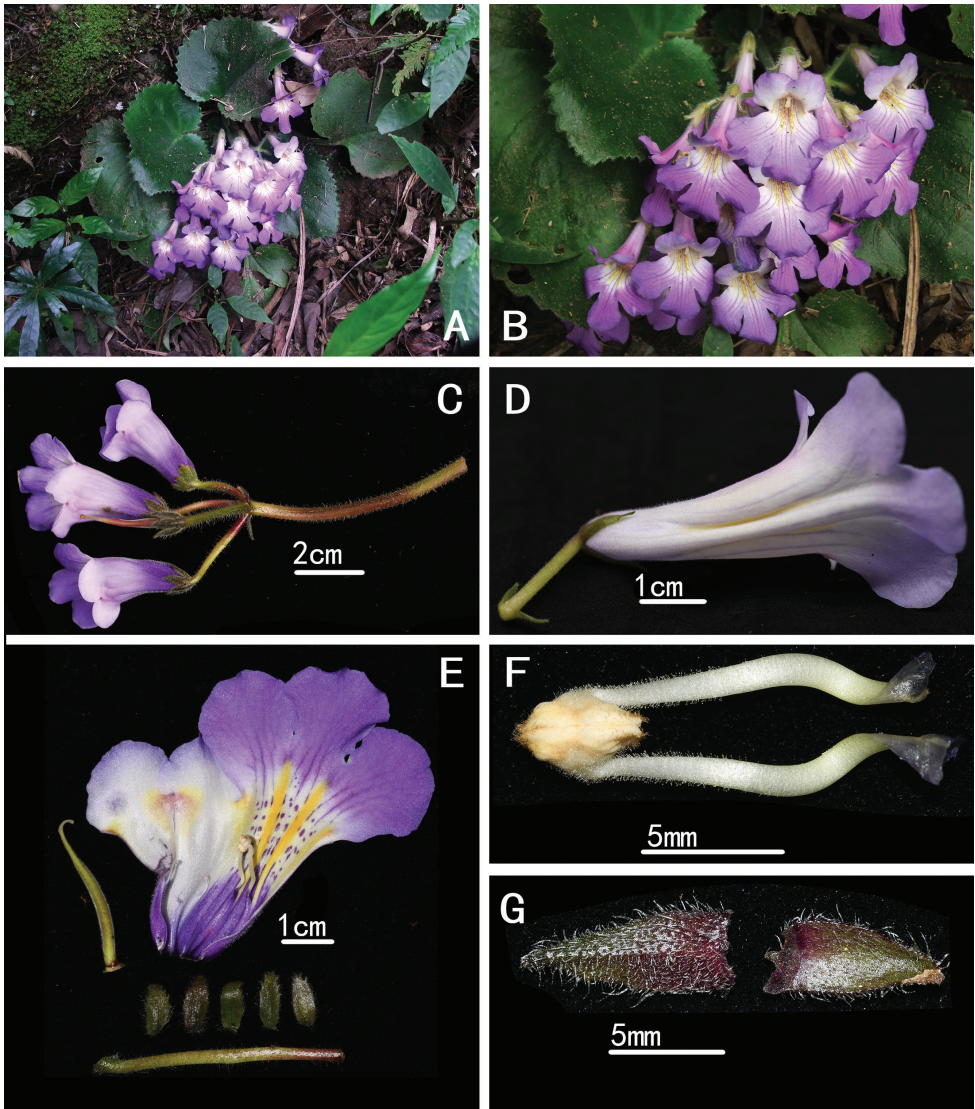


Figure 2. *Primulina fimbrisepala* (A–G) A habitat B habit in wild with flowers C inflorescence D two distinct long longitudinal ridges on the corolla tube floor E opened corolla F stamens G bracts. (A–B: Photographed by Xiao-Ning You; C–G: Photographed by De-Chang Meng).

Hayata, *Lysimachia congestiflora* Hemsl., *Goodyera biflora* (Lindl.) Hook. f., *Phyllagathis cavaleriei* Guillaum. and *Dryopteris* Adans. spp. in the forest in a damp and moist valley.

Phenology. Flowering from April to May; fruiting from June to August.

Etymology. The specific epithet is derived from the type locality, Jiuyishan National Nature Reserve, Hunan Province, China.

Vernacular name. 九嶷山报春苣苔 (Chinese name); jiǔ yí shān bào chūn jù tái (Chinese pronunciation).

Conservation status. The EOO and AOO of *Primulina jiuyishanica* are 54.28 km² and 5.2 km² respectively. So far, only eight populations of this species were found located in a nature reserve. However, we believe that more populations will be found in the future, and the EOO and AOO might increase. The eight populations have in total more than 3000 mature plants in the type locality, and additionally many seedlings were found. The plants are well protected in the nature reserve. According to the guidelines for using the IUCN Red List Categories and Criteria (IUCN 2019), the new species should be assessed as of Least Concern (LC).

Note. In figure 2, A and B were published in Wei et al. 2010, page 374, 375, under the name of *Chirita juliae* Hance, now *Primulina juliae* (Hance) Mich. Möller & A. Weber. In fact, A and B are *P. fimbrispala* (Hand.-Mazz.) Yin Z. Wang.

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Festuca drakensbergensis* (Poaceae): A common new species in the *F. caprina* complex from the Drakensberg Mountain Centre of Floristic Endemism, southern Africa, with key and notes on taxa in the complex including the overlooked *F. exaristata

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Abstract

We present taxonomic notes on the *Festuca caprina* complex from southern Africa that includes description and illustration of the new species *F. drakensbergensis* from the Drakensberg Mountain Centre of Floristic Endemism of South Africa and Lesotho. *Festuca drakensbergensis* can be differentiated from *F. caprina* s.l. by forming lax short tufts with extravaginally-branching tillers and lateral-tending cataphyllous shoots or rhizomes present, basal foliage reaching < ½ the length of the culms, with generally shorter leaves and shorter anthers, 0.8–1.6(–1.8) mm long. The species also differs from the overlooked species *F. exaristata* – currently known from two collections from Lesotho – by its fibrous basal sheaths, usually sharp, keel-like leaf blade midrib, drooping panicle with lightly to densely scabrous pendent panicle branches, longer lemmas, 4.5–5.8 mm long, with awns usually present, 0.5–3 mm long, ovary apices sparsely to densely hairy and anthers 0.8–1.6(–1.8) mm long. Taxonomic notes on the different taxa of the *F. caprina* complex in southern Africa are also provided, including images, key, and lectotypification of *F. caprina* var. *curvula*. This research adds a further two endemic species (*F. drakensbergensis* and *F. exaristata*) and two endemic varieties (*F. caprina* var. *irrasa* and *F. caprina* var. *macra*) to the Drakensberg Mountain Centre of Floristic Endemism.

Keywords

alpine grassland, Gramineae, *Festuca caprina*, Flora of Southern Africa, Lesotho, Maloti-Drakensberg, South Africa

Introduction

Carbutt's (2019) Drakensberg Mountain Centre of Floristic Diversity and Endemism (DMC) includes the only alpine region in mainland Africa south of Mount Kilimanjaro (Killick 1978), with a 2900 km disjunction. The DMC, covering some 40,000 km², comprises a montane sub-centre, dominated by C₄ grass species and an alpine sub-centre [the former Drakensberg Alpine Centre of van Wyk and Smith (2001) and Carbutt and Edwards (2004, 2006)] dominated by C₃ grass species (Brand et al. 2019). The DMC is renowned for its high levels of plant diversity and endemism, hosting 227 endemic angiosperm species that account for ca. 9% of the angiosperm flora; the DMC hosts 267 grass species in 86 genera (Carbutt and Edwards 2004), of which eight species and one genus are endemic (Carbutt 2019). Despite being the dominant ecosystem-forming component of these high elevation grasslands, grasses of the DMC are still relatively poorly studied, with only a few genera receiving attention, for example, *Anthoxanthum* L. (Mashau 2016); *Catabrosa* P. Beauv. (Soreng and Fish 2011); *Poa* L. (Soreng et al. in prep.); *Trisetopsis* Röser & A. Wölk (e.g. Mashau et al. 2010); *Pentameris* P. Beauv. (Linder and Ellis 1990).

The genus *Festuca* L. s.l. is a monophyletic lineage with ca. 650 perennials and ca. 30 annuals (beyond those in *Lolium* L.), totalling ca. 680 species (Plants of the World Online 2020 accepted species belonging to the lineage). The genus s.l. is divided into two major clades (Minaya et al. 2017): the Narrow Leaf Clade (NLC) of *Festuca* s.s., ca. 600 species (syn. [following Soreng et al. 2017 including the annuals] *Ctenopsis* De Not., *Loliolum* V.I. Krecz. & Bobrov, *Micropyrum* (Gaudin) Link, *Narduroides* Rouy, *Vulpia* C.C.Gmel. and *Wangenheimia* Moench), and the Broad Leaf Clade (BLC), ca. 82 species (perennials, and some annuals in *Lolium*), including *Drymochloa* Holub, *Leucopoa* Griseb., *Lojaconoa* Gand., *Lolium* (syn. *Micropyropsis* Romero Zarco & Cabezudo, *Schedonorus* P. Beauv.), *Patzkea* G.H. Loos, *Pseudobromus* K. Schum. and *Xanthochloa* (Krivot.) Tzvelev.

Fish and Moeaha (2015) accepted nine species of *Festuca* s.l. (but excluding *Vulpia* and *Lolium* in the narrow traditional sense) as present in the Flora of Southern Africa (FSA) region (comprising Botswana, Lesotho, Namibia, South Africa and Eswatini a.k.a. Swaziland). Generic limits of *Festuca* s.l. are still being resolved, particularly in the BLC (Soreng et al. 2017). Of the FSA species with DNA examined (Minaya et al. 2017), *F. caprina* Nees and *F. vulpioides* Steud. belong to the NLC, whereas *F. arundinacea* Schreb. (= *Lolium arundinaceum* (Schreb.) Darbysh.), *F. africana* (Hack.) Clayton (= *Pseudobromus silvaticus* K. Schum.), *F. costata* Nees, *F. killickii* Kenn.-O'Byrne, *F. longipes* Stapf and *F. scabra* Vahl belong to the BLC. Although it generally holds true,

not all NLC and BLC taxa have narrow and broad leaves, respectively, for example, *F. vulpioides* being placed in the NLC (Minaya et al. 2017; identity of voucher specimen not verified by us). *Festuca dracomontana* H.P. Linder (predicted to be BLC), *F. exaristata* E.B. Alexeev (not accounted for by Fish and Moeaha 2015, predicted to be NLC) and our new species (predicted to be NLC) have not been tested.

Festuca s.l. is one of the prominent genera present in the montane-alpine ecotone (ca. 2500–2800 m alt.) and alpine sub-centre (> 2800 m alt.) of the DMC (Irwin and Irwin 1992) and often dominates, especially in less disturbed areas (Sylvester et al. unpubl. data). One species, *F. killickii*, is currently considered to be endemic to the DMC (Carbutt 2019: table 2), although the poorly-known *F. dracomontana* and *F. vulpioides* may also be DMC endemics (Fish and Moeaha 2015). Of the species of *Festuca* recorded by Fish and Moeaha (2015), *F. caprina* is perhaps the most widespread in the Afro-montane/Afro-alpine region of White (1981), stretching from the coastal Southern Cape of South Africa to Tanzania (Fish and Moeaha 2015). *Festuca caprina* s.l. has had three varieties described from the FSA region (var. *curvula* Nees, var. *irrasa* Stapf, var. *macra* Stapf) and was considered to be a complex of species by Alexeev (1986), who recognised two new species for the complex in sub-Saharan Africa, *F. claytonii* E.B. Alexeev from Kenya and *F. exaristata* E.B. Alexeev from the DMC, and raised *F. caprina* var. *macra* to species rank. Fish and Moeaha (2015: 349) stated that the different varieties of *F. caprina* accepted in previous treatments were not upheld in their treatment because of “the variability in the species and leaf anatomy, which are constant throughout”. Although Alexeev’s (1986) taxonomy and new species were accepted by agrostologists at Kew (Phillips 1995a, b; Clayton et al. 2006 onwards), there is no mention of it in Fish and Moeaha (2015) or the older treatment of *Festuca* for the FSA region (Gibbs Russell et al. 1990) and the checklist of Lesotho grasses (Kobisi and Kose 2003), with this error also being replicated in floristic surveys of the DMC (Carbutt and Edwards 2004, 2006; Carbutt 2019).

Taxa in the *F. caprina* complex differ from other *Festuca* s.l. taxa in the FSA region by having: basal sheaths entire or splitting into narrow parallel threads (vs. coarsely fibrous in *F. costata*), glabrous or scabrous (vs. basal ones velvety in *F. scabra*); ligules < 1 mm long (vs. > 1 mm long in most, apart from *F. dracomontana* and *F. vulpioides*); collars non-auriculate (vs. auriculate in *F. arundinacea*, *F. dracomontana* and *F. vulpioides*); blades narrow, 0.2–1.5 mm wide in diameter, involute (vs. flat or relatively broad, [2–]3–15 mm wide in diameter, rarely narrower in *F. scabra*); panicles loose or contracted (vs. very open, candelabrum-shaped, in *F. longipes*, open in *F. africana*, *F. arundinacea* and *F. dracomontana*); spikelets 2 to several flowered (vs. 1-flowered in *F. africana*), awns 0–5.5 mm long (vs. 10–20 mm long in *F. africana*).

During extensive field collecting and ecological research by the authors in the DMC area (222 2 m × 2 m plots studied for all vascular plants, of which 145 plots contained *Festuca* species, with 50 collections of *Festuca* made), followed by herbarium research at PRE, clear differences were noted between specimens that were treated under *F. caprina* by Fish and Moeaha (2015). These differences included branching patterns in tillers, presence of cataphylls, abaxial leaf blade indumentum and anther

size, which are known to be taxonomically informative for distinguishing *Festuca* taxa in other parts of the World (e.g. Stančík and Peterson 2007; Ospina et al. 2015). These clear differences allowed us to distinguish the new species, *F. drakensbergensis*, and to recognise the varieties *F. caprina* var. *irrasa* and *F. caprina* var. *macra*. This new species, coupled with the overlooked species, *F. exaristata* and distinct varieties, *F. caprina* var. *irrasa* and var. *macra*, add a further two endemic species and two endemic varieties to Carbutt's (2019) checklist of DMC endemics.

The aim of this paper is therefore to:

- (i) Describe and illustrate the new DMC endemic, *F. drakensbergensis*.
- (ii) Provide taxonomic notes on the distinct varieties of *F. caprina* present in the DMC and the overlooked species, *F. exaristata*.
- (iii) Provide a revised key for the *F. caprina* complex in the FSA region.

Materials and methods

Extensive field collecting was conducted by SPS, RJS and MDPVS in the DMC between 1 Feb and 9 Mar 2020, with 42 specimens belonging to the *F. caprina* complex collected, which are deposited in the PRE, NU and US (pending export permits) herbaria [Herbarium acronyms follow Thiers (2020, continuously updated)]. Herbarium study was also conducted at PRE between 13 and 20 Mar 2020. While focus was placed on the 42 new field collections of *Festuca* and notes on variations present in our 142 plots containing taxa belonging to the *F. caprina* complex, many other older PRE herbarium specimens were studied than mentioned in the 'Selected specimens examined' sections herein, but, due to unforeseen obstructions caused by the COVID-19 pandemic, information regarding these specimens was not adequately recorded. Type images on JSTOR Global Plants (<https://plants.jstor.org>) were also assessed. We delimit taxa based on distinct discontinuities in morphological characteristics which are deemed to be phylogenetically conserved and taxonomically informative based on previous research (e.g. Stančík and Peterson 2007; Ospina et al. 2015), as well as distinct discontinuities in ecological and morphological characteristics of taxa observed during extensive fieldwork in the DMC area. Distinctive characteristics of habit, colouration and ecological preferences, notable between individual plants within and amongst populations in the field, are often difficult to sort out when dealing only with herbarium specimens. In this treatment, glabrous means without pubescence (in the sense of slender, relatively soft hairs, unless otherwise stated). Smooth indicates no prickly-hairs with broad bases and/or hooked or pointed apices (i.e. pubescence can occur on a smooth surface and a rough or scabrous surface can be glabrous). Leaf-blade anatomical characteristics were observed in cross-sections from the middle area of selected tiller blades. We collected many silica-dried leaf samples of *Festuca* s.l. for future DNA examinations.

Taxonomic treatment

Key to species of the *Festuca caprina* complex in southern Africa

Key characters separating species of the *F. caprina* complex in southern Africa are also found in Table 1.

- 1 Tillers intravaginal (cataphylls absent, elongated prophylls present at juncture of lateral shoots), lateral tending rhizomes absent; densely tufted and usually forming large tussocks with basal foliage reaching (10–)20–80+ cm tall and often $> \frac{1}{2}$ the length of the culms; sheaths of tillers and basal culm (3–)12–24 cm long; leaf blades of tillers and basal culm (4–)12–66.5+ cm long; lowermost lemmas (4.5–)5–7(–9) mm long; fertile anthers (1.8–)2–4 mm long (as short as 1.6 mm in var. *macra*, according to Alexeev 1986) (*F. caprina* s.l.).....**2**
- Tillers extravaginal (rarely some intravaginal shoots also present), lateral-tending or ascending cataphyllous shoots or lateral-tending rhizomes present; plants forming lax short tufts with basal foliage reaching (2–)4–20(–27) cm tall and $< \frac{1}{2}$ the length of the culms; sheaths of tillers and basal culm (0.5–)2–7(–10) cm long; leaf blades of tillers and basal culm (2–)5–15(–26) cm long; lowermost lemmas 4–5.8 mm long; fertile anthers 0.8–1.8 mm long.....**4**
- 2 Sheaths of old leaves falling apart (shredded) into parallel thin threads; basal foliage ca. 14–30 cm tall, often $< \frac{1}{2}$ the length of the culms; panicle branches and pedicels short-hispid or long-scabrous with hair-like prickles; lemmas, paleas and rachillas short-hispid or long-scabrous with hair-like prickles; lemma apices usually notably bifid, with awn emerging from between the lobes...
.....***F. caprina* var. *irrasa* Stapf**
- Sheaths of old leaves entire, not or rarely only very slightly disintegrating into fibres; basal foliage (10–)30–80+ cm tall, generally ($> \frac{1}{2}$) $> \frac{3}{4}$ to surpassing the length of the culms; panicle branches and pedicels short scabrous; lemmas, paleas and rachillas glabrous, scabrous, but prickles hooked, short hooked, slender or stout, not hair-like, rarely smooth; lemma apices not usually notably bifid, commonly merging into the awn**3**
- 3 Leaf blade abaxial surface antrorsely scabrous throughout
.....***F. caprina* var. *macra* Stapf**
- Leaf blade abaxial surface smooth or rarely antrorsely scaberulous towards the apex ***F. caprina* var. *caprina* Nees**
- 4 Sheaths of old leaves falling apart (shredded) into parallel thin threads; leaf blade midrib (middle vein) usually sharp, keel-like, sometimes blunt and rounded; panicles drooping; panicle branches usually pendant, lightly to densely scabrous; lowermost lemma (not including awn) 4.5–5.8 mm long; awn usually present, very rarely muticous, awn 0.5–3 mm long; ovary apex sparsely to densely hairy; fertile anthers 0.8–1.6(–1.8) mm long; basal foliage

reaching (2–)4–20(–27) cm tall

- *F. drakensbergensis* Sylvester, Soreng & M.D.P.V. Sylvester
 Sheaths of old leaves entire, not disintegrating into fibres, lustrous; leaf blade
 midrib (middle vein) blunt, rounded; panicles erect; panicle branches smooth;
 lowermost lemma (not including awn) 4–4.2 mm long; awn absent; ovary apex
 glabrous; fertile anthers 1.5–1.8 mm long; basal foliage reaching to 12 cm tall...
 *F. exaristata* E.B. Alexeev

***Festuca drakensbergensis* Sylvester, Soreng & M.D.P.V. Sylvester, sp. nov.**

urn:lsid:ipni.org:names:77211930-1

Figs 1, 2, Table 1

Type. LESOTHO. AfriSki Ski Resort, in valley just west of the resort centre with an east-southeast aspect, 28.824908S, 28.723208E, 3065 m alt., heavily grazed damp Afro-alpine grassland, 28 Feb 2020, S.P. Sylvester, R.J. Soreng & M.D.P.V. Sylvester 3660 (holotype: PRE!; isotypes: NU!, US!).

Diagnosis. Differs from *Festuca caprina* s.l. by forming lax short tufts with extravaginally branching tillers and lateral-tending or ascending cataphyllous shoots or lateral-tending rhizomes present, basal foliage reaching < ½ the length of the culms, sheaths of tillers and basal culm (0.5–)2–7(–10) cm long, leaf blades of tillers and basal culm (2–)5–15(–26) cm long, and anthers 0.8–1.6(–1.8) mm long. Differs from *Festuca exaristata* by its basal sheaths fibrous, leaf blade midrib usually sharp, keel-like, sometimes blunt and rounded, panicle branches pendent, lightly to densely scabrous, lowermost lemma (not including awn) 4.5–5.8 mm long, awn usually present, 0.5–3 mm long, ovary apex sparsely to densely hairy and anthers 0.8–1.6(–1.8) mm long.

Description. Perennial herbs, generally forming lax, short, isolated tufts, with lateral-tending or ascending cataphyllous shoots or lateral-tending rhizomes present, basal foliage (2–)4–20(–27) cm tall and generally < ½ the length of the culms, with inflorescences largely exserted. **Tillers** extravaginal, with cataphylls present, intravaginal tillers rarely also present (i.e. Sylvester et al. 3637). **Culms** (12.5–)20–46(–65) cm tall, 0.3–0.5(–1) mm diam., erect, delicate, cylindrical to slightly compressed, longitudinally striated, glabrous, smooth, with (0) 1 or 2 visible nodes, uppermost node at (1.3–)3–10(–16) cm from the base, ca. (1/10–)1/8–1/3(–½) culm height, distance between uppermost node and panicle (3–)14–33(–40) cm long, distance between uppermost node and second node down (0.9–)2.3–6.5(–9.5) cm long, nodes at the base covered by imbricate leaf sheaths. **Leaves** mostly basal, with 1 or 2 (3) cauline leaves, culm leaves similar to those of the base and tillers; **sheaths** of tillers and basal culm (0.5–)2–7(–10) cm long, proximally fused ca. ½ their length, implicate above, usually slightly obliquely truncated at the apex, herbaceous, persistent, becoming sparingly fine fibrous – decaying into longitudinal fibres – in the lower portion with age, brownish or yellowish, glabrous, usually smooth, rarely retrorsely scabrous, with 5–7 veins; flag-leaf sheaths 3.4–9.5(–12.5) cm long, fused ca. ½ their length; **auricles** 0.01–0.2 mm

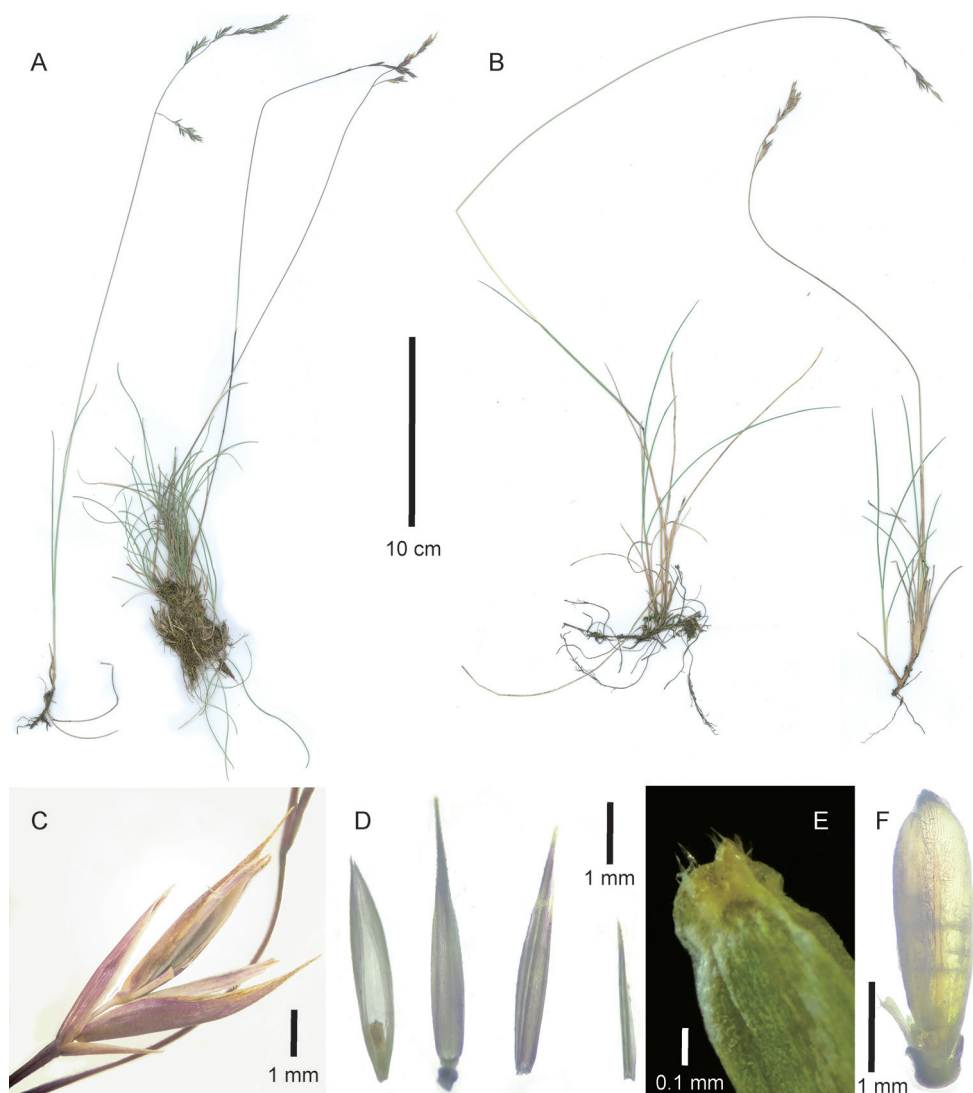


Figure 1. *Festuca drakensbergensis*, habit and inflorescence characteristics. **A, B** Whole plant **C** spikelet, lateral view **D** [from left to right] palea ventral view showing ovary, lemma dorsal view, upper glume dorsal view, lower glume lateral view **E** ovary apex, ventral view **F** caryopsis, dorsal view, with parts of torn palea and lemma at base. **A, C, D, F** of isotype S.P. Sylvester et al. 3660 (US) **B** of S.P. Sylvester et al. 3578 (US) **E** of S.P. Sylvester et al. 3687a (PRE).

long, inconspicuous, obtuse; **ligules** 0.1–0.5 mm long, membranous, moderately to strongly decurrent with the sheath margins, truncate, briefly ciliate; flag-leaf ligules 0.2–0.5 mm long; **leaf blades** of tillers and basal culm (2–)5–15(–26) cm long, 0.3–0.8(–1) mm wide as rolled or folded, setaceous, erect-curved to recurved, firm to \pm rigid, conduplicate, convolute or involute, rarely flat in upper leaves, elliptical or obo-

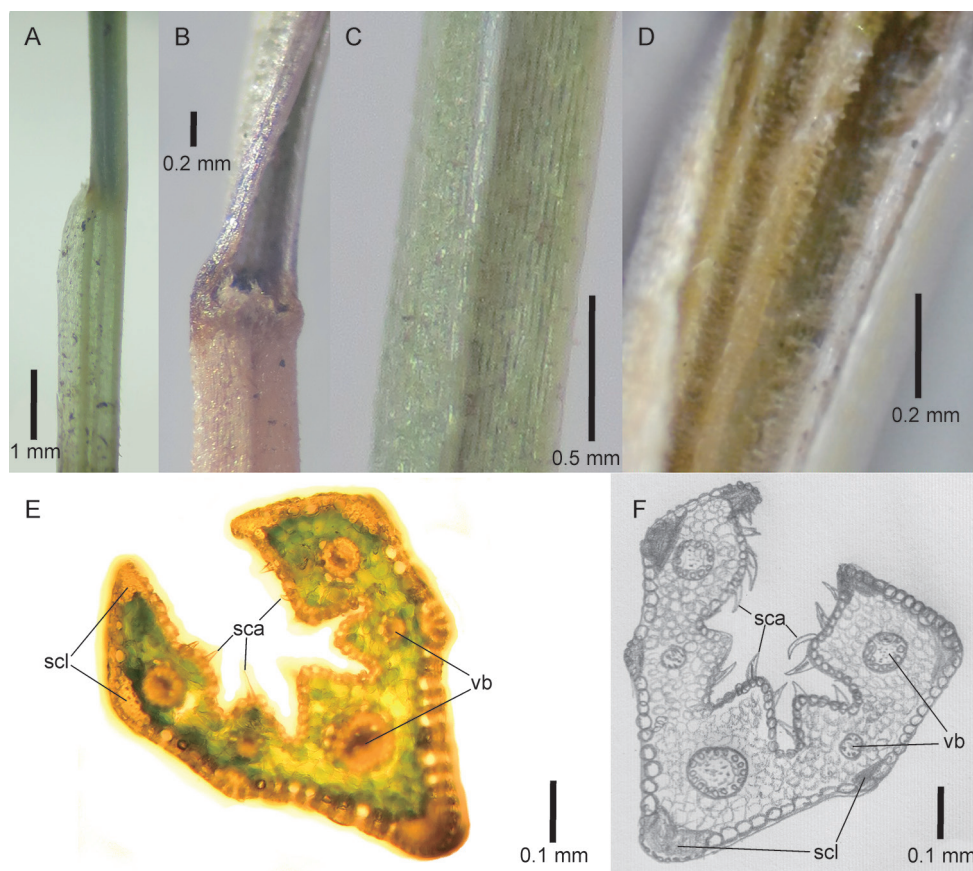


Figure 2. *Festuca drakensbergensis*, leaf morphological and anatomical characteristics. **A** Junction of tiller sheath and blade, lateral view **B** ligule of tiller, ventral view **C** abaxial tiller blade surface, showing keel **D** adaxial tiller blade surface **E, F** tiller blade cross sections, showing position of the sclerenchyma block (scl), vascular bundles (vb) and scabers (sca) on the adaxial surface. **A, B, C, E** of isotype S.P. Sylvester et al. 3660 (US) **D** of S.P. Sylvester et al. 3689 (PRE) **F** of isotype S.P. Sylvester et al. 3660 (US) drawn by M.D.P.V. Sylvester.

vate to carinate outline in cross-section, midrib (middle vein) usually sharp, keel-like, sometimes blunt and rounded, abaxial surface glabrous, usually smooth throughout or lightly antrorse-scabridulous towards the apex, adaxial surface scabrous on veins or prickles elongating to become hair-like and appearing shortly hairy, light- to dark-green, apex obtuse (to acute); upper culm leaf-blades similar to those of lower culm and tillers, but shorter and sometimes expanded; flag-leaf blades (0.2–)1.5–4(–12.5) cm long, (2–)15–40(–50)% the length of their flag-leaf sheaths, rarely longer. **Panicles** 2.5–9(–13) cm long, open to moderately congested, drooping, with (7–)8–20(–50) spikelets often held unilaterally on lower side of axis; central panicle axis smooth to lightly antrorsely scabrid, with 4–10 nodes, usually 1 branch (rarely 2 branches) per node, lowest internode (0.8–)2–4.5(–5.5) cm long, ca. 20–70% length of whole pani-

cle, lowest internode and sometimes upper internodes and panicle branches often sinuous-wavy; **panicle branches** capillaceous, generally pendent and drooping, lowermost patent to pendent, upper \pm appressed to central axis, glabrous, antrorsely scabrous to scaberulous on angles or rarely smooth; lowermost primary panicle branch (1–)1.5–6 cm long, with (1–)3–10(–17) spikelets; **pedicels** 0.5–3(–6) mm long, shorter than their spikelets, slightly thickened at their apices, glabrous, antrorsely scabrous to scaberulous on angles or rarely smooth. **Spikelets** (not including awns) (5.5–)6–9(–11.5) mm long, laterally compressed, elliptic, green or usually purplish; **florets** 2 to 5(6) fertile and usually 1 apical and \pm rudimentary, sterile, lowermost fertile floret largest, with upper fertile florets gradually reducing in size; **glumes** unequal, lower ca. $\frac{1}{2}$ – $\frac{3}{4}$ (– $\frac{5}{6}$) length and ca. $\frac{1}{3}$ – $\frac{1}{2}$ width of upper glume, narrowly scarious on the margins, usually darker purple compared to the lemmas, glabrous, keels distally scaberulous for $\frac{1}{4}$ – $\frac{1}{2}$ their length or smooth throughout, surfaces smooth throughout or sometimes sparsely scaberulous towards apex, margins usually with scattered hooks on edges in distal $\frac{1}{2}$ (– $\frac{3}{4}$), (acute or) acuminate; **lower glumes** 2.1–3(–3.8) mm long, 0.3–0.4 mm wide at base in cross section, reaching to 50–70% length of proximal lemma, linear-lanceolate, 1-veined; **upper glumes** 3.2–4(–4.9) mm long, 0.5–0.8 mm wide at base in cross section, reaching to 70–95% length of proximal lemma, ovate-lanceolate, 3-veined; **rachillas** up to ca. 0.8–1.6 mm long, slightly dorsally compressed, glabrous, smooth, lightly scabrous towards apex or densely scabrous throughout; **calluses** somewhat thick, annulated, angled downward, rugose or smooth, sometimes lightly scabrous; **lemmas** (lowermost lemma not including awn) 4.5–5.8 mm long, 0.7–1.2 mm wide at broadest point in cross section, ovate-lanceolate, herbaceous with narrowly scarious margins, glabrous, proximally smooth or sparsely to densely scabrous, especially towards the margins, distally sparsely to densely scabrous, especially towards the apex and margins, moderately to densely granulose with clear bead-like raised silica cells appearing like ‘granules’ throughout or these absent towards apex and margins, margins scabrous throughout or in the distal $\frac{1}{2}$ – $\frac{3}{4}$, green or usually greenish-purple at the margins and towards the apex, 5-veined, apices acute and tapering into a short awn, sometimes slightly bilobate with awn emerging from between the minute lobes or very rarely muticous, awn 0.5–3 mm long, straight, scabrous; **paleas** (lowermost) 4.5–5.8 mm long, subequalling to usually equalling the lemma or slightly surpassing the lemma apex by up to 0.4 mm, herbaceous with scarious margins, slightly to deeply bidentate, keels scabrous in distal ($\frac{1}{4}$ –) $\frac{1}{2}$ – $\frac{5}{6}$ or rarely throughout, between keels smooth, moderately to densely granulose with clear bead-like raised silica cells appearing like ‘granules’, margins scabrous in distal $\frac{1}{4}$ – $\frac{1}{2}$. **Flowers** proximally perfect with uppermost usually sterile; **anthers** 3 in number, 0.8–1.6(–1.8) mm long, linear, dull yellow; **ovaries** ca. 0.5–1 mm long, apex sparsely to densely pubescent; **lodicules** 0.7–0.85 mm long, bilobed with lobes ca. 2–4 mm long, both lobes \pm same size or lateral lobes to 0.2 mm shorter, glabrous, margins entire and smooth or sometimes fimbriate, acute. **Caryopses** ca. 2.6–3.5 mm long, ca. 1–1.6 mm shorter than lemma and palea, adhering to palea and lemma, narrowly elliptic to slightly narrow-obovate, deeply sulcate, hilum linear, 75–93% length of caryopsis, endosperm hard.

Table 1. Differences in key morphological characters between the species of the *Festuca caprina* complex in southern Africa.

Character	<i>F. caprina</i> var. <i>caprina</i>	<i>F. caprina</i> var. <i>irrasa</i>	<i>F. caprina</i> var. <i>macra</i>	<i>F. exaristata</i>	<i>F. drakensbergensis</i>
Tillers	intravaginal			Extravaginal, rarely also intravaginal	
Culm height (cm)	35–100	ca. 30–65	(28–)60–110(–120+)	ca. 19–35	(12.5–)20–46(–65)
Height of basal foliage	(20–)30–60+ cm tall, generally (> ½) > ¾ to surpassing the length of the culms	ca. 14–30 cm tall, often < ½ the length of the culms	(10–)30–80+ cm tall, generally > ½ the length of the culms	ca. 12–25 cm tall, generally < ½ the length of the culms	(2–)4–20(–27) cm tall, generally < ½ the length of the culms
Sheaths of old leaves	Not falling apart into parallel thin threads	falling apart (shredded) into parallel thin threads	Not falling apart into parallel thin threads		Falling apart (shredded) into parallel thin threads
Sheaths of tillers and basal culm length (cm)	ca. 6–16(–24)	ca. 2–10	(3–)12–24+	ca. 1–4(–10?)	(0.5–)2–7(–10)
Basal culm and tiller leaf blade length (cm)	(8.5–)12–60	ca. 1.8–20	(4–)13–66.5+	ca. 2–25	(2–)5–15(–26)
Leaf blade in middle vein (midrib)	Sharp, keel-like			Blunt, rounded	Usually sharp, keel-like, sometimes blunt, rounded
Abaxial leaf surface	Smooth or rarely scaberulous towards apices	Smooth or rarely scaberulous towards apices	Scabrous throughout	Smooth	Smooth or only scaberulous at apex
Panicle branches	Scabrous	Usually long-scabrous, prickles hair-like	Scabrous	Smooth	Scabrous or rarely smooth
Lowermost lemma length (mm)	(4.5–)6–7(–9)	ca. (5.2–)5.5–6.5	(4.5–)5–7.2(–9?)	4–4.2	4.5–5.8
Spikelet pubescence	glabrous	Usually hispid on lemmas, paleas and rachillas	glabrous		
Awn length (mm)	(0–)1–4.5	ca. 1.5–2.8	(0–)1.5–5.5	0	(0–)0.5–3
Anthers length (mm)	(2.1–)2.4–4	ca. 2.6–2.8	(1.6 mm?; Alexeev 1986) (1.8–)2–3.5(–4)	1.5–1.8	0.8–1.6(–1.8)
Ovary apex	Hairy, hairs sparse (sometimes just 1 or 2) or dense			Glabrous	Hairy, hairs sparse or dense

Anatomy–Outline elliptical or obovate to carinate with angled arms, ca. 5 vascular bundles all positioned in the centre of the blade and at the same level, ca. 4 grooves, ca. 5 ribs; the central rib is located in the central area of the blade. **Abaxial surface** with straight edges forming angles associated with the vascular bundles, ribs angular, composed of sclerenchyma block and found opposite all vascular bundles, smooth, macro-hairs absent, margins composed of sclerenchyma block. **Adaxial surface** markedly irregular, with rounded ribs situated opposite all vascular bundles, lacking scleren-

chyma block, prickles present and densely covering the entire surface, sometimes more prevalent on the ribs, usually extending and appearing hispid (Fig. 2E, F).

Distribution and habitat. Endemic to the high-elevation DMC of South Africa and Lesotho (Carbutt 2019). In South Africa, the species is known from the Eastern Cape and KwaZulu-Natal Provinces, with it also possibly occurring in the Free State Province, although no specimens have as yet been verified. *Festuca drakensbergensis* is a common constituent of both moderately grazed and little disturbed Afro-alpine vegetation (viz. Carbutt's 2015 'austro-alpine region'), and less often in Afro-montane vegetation, of the DMC, ca. 2150–3270+ m alt. The species is found in grassland, wetland and short Afro-alpine shrubland dominated by species in the genera *Chrysocoma* L., *Erica* Tourn. ex L., *Eumorphia* DC. and *Helichrysum* Mill. These habitats correspondent with Mucina and Rutherford's (2006) uKhahlamba Basalt Grassland (Gd 7), Lesotho Highland Basalt Grassland (Gd 8), Drakensberg Afro-alpine Heathland (Gd 10) and Lesotho Mires (AZf 5). *Festuca drakensbergensis* is rarely dominant and generally occurs in low abundance amongst the larger *F. caprina* var. *macra* or amongst other forbs or low shrubs. Of the 222 2 m × 2 m plots studied for all vascular plants across the Afro-alpine DMC (Sylvester et al. unpubl. data), *F. drakensbergensis* was encountered in usually low abundance (0.5–8[–70]% of overall plot cover) in 35 plots, highlighting its high frequency and ubiquity in these landscapes.

Preliminary conservation status. The overall extent of occurrence of *F. drakensbergensis* is relatively large compared to many DMC endemics, perhaps 30% (or 13,000 km² i.e. above 2150 m) of the total DMC area of ca. 40,000 km². Given that it is a common species without any specific habitat niche, the total population is likely well above 10,000 mature individuals. However, given the tremendous pressure that the DMC is under from communal rangeland activities – especially in Lesotho (Global Mechanism of the UNCCD 2018, 2019) – it is possibly at medium- to long-term risk from land degradation through overgrazing. Initial observations suggest that the species does have resilience, being recorded in areas disturbed by grazing and burning as well as in areas of limited disturbance. There might, however, be competition from shrubland following overgrazing (e.g. *Chrysocoma ciliata* L., *Selago melliiodora* Hilliard, *Eumorphia* spp. and *Helichrysum* spp.). Future projections of global climate change are also of concern for high-elevation species in southern Africa (Bentley et al. 2019). Accordingly, we propose the IUCN conservation status of Near Threatened (NT) until further population studies can be undertaken.

Etymology. The species epithet refers to the Drakensberg Mountain Centre (DMC) of South Africa and Lesotho (Carbutt 2019), where this species forms a common component of the Afro-alpine vegetation.

Notes. The character of extravaginal branching is not always easy to distinguish and certain specimens of *F. caprina* s.l. found growing in moss may have what appear to be rhizomes although these are, in fact, pseudostolons. However, *F. caprina* var. *caprina* and var. *macra* plants are usually much larger, with culms (28–)35–120+ cm tall, basal foliage (10–)30–80+ cm tall, generally ($> \frac{1}{2}$) $> \frac{3}{4}$ to surpassing the length of the culms, with leaf-blades of tillers and basal culm (4–)12–66.5+ cm long, often > 26 cm

long, basal sheaths entire, erect panicles with greenish or purplish spikelets on ascending branches, lower lemma often larger, (4.5–)5–7(–9) mm long, and anthers > 2 mm long (vs. culms (12.5–)20–46(–65) cm tall, basal foliage (2–)4–20(–27) cm tall, leaf-blades of tillers and basal culm (2–)5–15(–26) cm long, basal sheaths fibrous, drooping panicles with purplish spikelets on pendent branches, lower lemma 4.5–5.8, anthers 0.8–1.6(–1.8) mm long in *F. drakensbergensis*) (Table 1). *Festuca caprina* var. *irrasa* specimens can sometimes superficially resemble *F. drakensbergensis* by having shorter basal foliage reaching < ½ length of the culms, with smooth blades and fibrous basal sheaths (Table 1). However, in these cases, *F. caprina* var. *irrasa* can be distinguished by its intravaginally branched tillers which lack cataphylls, erect panicles with ascending branches, short-hispid or long-scabrous lemmas and paleas that often measure > 6 mm long, and anthers > 2 mm long (vs. extravaginally branched tillers with cataphylls present, drooping panicles with pendent branches, lemmas and paleas glabrous, scabrous, 4.5–5.8 mm long, and anthers < 1.8 mm long in *F. drakensbergensis*).

Festuca exaristata also bears extravaginally branched cataphyllous tillers or lateral-tending rhizomes, with plants forming short isolated tufts. The holotype of *F. exaristata* is very short, with basal foliage not reaching past 12 cm tall, and bears superficial resemblance to certain shorter specimens of *F. drakensbergensis*, for example, Sylvester et al. 3637. The protologue of *F. exaristata* mentions culms to 35 cm tall and leaf blades to 25 cm long, which must refer to the one paratype, du Toit 2713 (K), which has not been seen by us, showing that the species would also superficially match larger versions of *F. drakensbergensis*. However, *F. exaristata* differs by its entire, lustrous basal sheaths, blunt, rounded leaf-blade midribs, erect sub-spike-like panicles, smooth panicle branches, shorter lemmas 4–4.2 mm long which lack awns, glabrous ovary apex and anthers 1.5–1.8 mm long (vs. basal sheaths smooth or rarely retrorsely scabrous, fibrous, leaf blade midrib usually sharp, keel-like, sometimes blunt and rounded, panicles drooping, panicle branches lightly to densely scabrous, lowermost lemma (not including awn) 4.6–6 mm long, awn rarely absent, usually 0.5–3 mm long, ovary apex sparsely to densely hairy, anthers 0.8–1.6(–1.8) mm long in *F. drakensbergensis*). Although rarely some characters overlap between *F. drakensbergensis* and *F. exaristata*, the combination of characters found in *F. exaristata* is never found in specimens of *F. drakensbergensis*.

Some specimens (e.g. Sylvester et al. 3442) growing in wetlands with limited grazing were substantially larger than normal, with culms to 65 cm tall and inflorescences to 13 cm long.

Selected specimens examined. **LESOTHO.** Bokong Nature Reserve, ca. 350 m north from the information centre, 29.067203S, 28.421496E, 2972 m alt., Afro-alpine grassland dominated by *Lachnagrostis barbuligera* var. *barbuligera* with moderately controlled grazing and burning, 2 Mar 2020, S.P. Sylvester et al. 3687a (US); Bokong Nature Reserve, ca. 400 m north from the information centre, 29.065893S, 28.420137E, 2979 m alt., rocky Afro-alpine grassland dominated by *Lachnagrostis barbuligera* var. *barbuligera* with moderately-controlled grazing and burning, 2 Mar 2020, S.P. Sylvester et al. 3689 (PRE, US); Matebeng Pass, below highest summit close to the pass, 29.870708S, 28.976534E, 3094 m alt., “Lesotho Highland Basalt

Grassland” with clear elements of “Drakensberg Afro-alpine Heathland” with *Erica* and *Helichrysum* shrubs dominating the landscape, 22 Feb 2020, S.P. Sylvester et al. 3578 (PRE, US); Menoaneng Pass, on road between Rafolatsane and Thaba-Tseka, 29.427423S, 28.951273E, 3040 m alt., Afro-alpine grassland, windy ridge, grazed down to low turf, 24 Feb 2020, S.P. Sylvester et al. 3595 (NU, PRE, US); Menoaneng Pass, on road between Rafolatsane and Thaba-Tseka, 29.427403S, 28.951124E, 3039 m alt., Afro-alpine grassland, windy ridge, grazed down to low turf, 24 Feb 2020, S.P. Sylvester et al. 3605 (PRE, US); Sani Pass area, close to the top of the Pass northwest of Sani Mountain Lodge, 29.521251S, 29.200602E, 3242 m alt., short Afro-alpine grassland, close to a pool of water, frequently to heavily grazed, 26 Feb 2020, S.P. Sylvester et al. 3636 (PRE, US); Sehlabathebe National Park, lower end of the Park on the border, 29.860061S, 29.095497E, 2719 m alt., damp Afro-alpine tussock grassland, soil damp, under dripping crag, heavily grazed, close to livestock paths, 19 Feb 2020, S.P. Sylvester et al. 3531 (NU, PRE, US). **SOUTH AFRICA.** Eastern Cape: Bastervoeypad Pass area, ca. 12 km east of Mountain Shadow Hotel on Barclay Pass, 31.176139S, 27.964197E, 2176 m alt., Afro-montane transitioning to Afro-alpine grassland, 14 Feb 2020, S.P. Sylvester et al. 3505 (NU, PRE, US); Eastern Cape: between Carlisle-shoekspruit Pass and Tiffindell Ski Area, 30.677202S, 27.956643E, 2526 m alt., riparian wetland, 10 Feb 2020, S.P. Sylvester et al. 3442 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, Ben Macdhui summit, 30.647683S, 27.934042E, 2995 m alt., Afro-alpine grassland, 11 Feb 2020, S.P. Sylvester et al. 3459 (NU, PRE, US); KwaZulu-Natal: Drakensberg, top of Sani Pass, grassy slopes on bank of gully, steep east facing slope, between rocks in brown clayey soil, 9400 ft [2865 m alt.], 24 Mar 1975, P.C.V. du Toit 698 (PRE0240733); KwaZulu-Natal: Sentinel Trail, ca. 1.2 km from the chain ladders, 28.740834S, 28.886806E, 2867 m alt., Afro-montane grassland grading into Afro-alpine grassland, damp soil, infrequently grazed, 6 Mar 2020, S.P. Sylvester et al. 3714 (NU, PRE, US); KwaZulu-Natal: Sani Pass area, below southwest facing cliffs to the southeast of Sani Mountain Lodge, 29.585365S, 29.290839E, 2866 m alt., short Afro-alpine grassland, frequently to heavily grazed, 26 Feb 2020, S.P. Sylvester et al. 3637 (PRE, US); [KwaZulu-Natal?:] Probably from Mont-aux-Sources [Sentinel Peak?], E.A.C.L.E. Schelpe 1394A (PRE0024522).

Taxonomic notes on other taxa in the *Festuca caprina* complex of southern Africa

***Festuca caprina* var. *caprina* Nees, Fl. Afr. Austral. Ill. 443. 1841. *Festuca nubigena* subsp. *caprina* (Nees) St.-Yves, Rev. Bretonne Bot. Pure Appl. 2: 79. 1927.** Fig. 3, Table 1

Type. SOUTH AFRICA. [Eastern Cape:] Table mountain, Queenstown Dev., Los-Tafelberg, 5000–6000 ft [1524–1829 m alt.], [1840], [flowering in December], D.F. Drège s.n. (lectotype, designated by Alexeev 1986: 1115: K (K000345257 [image!]); isolecotypes: K (K000345258 [image!]), LE (LE00009757 [image!]); syntypes: SOUTH AF-

RICA. Plantes du Cap, Los Tafelberg, 5000–6000 ft [1524–1829 m alt.], 7 Dec 1832, D.F. Drège 8.e.3920 (P (P00434763 [image!])); [SOUTH AFRICA] Afr. Austr. D.F. Drège s.n. (L (L1262355 [image!])); SOUTH AFRICA. Los Tafelberg, Dec 1826–1834, D.F. Drège s.n. (HAL (HAL0106999 [image!])).

= *Festuca caprina* var. *curvula* Nees, Fl. Afr. Austral. Ill. 1: 443. 1841. Type: SOUTH AFRICA. [Eastern Cape:] [Monte] Los Tafelberg, an steinigen Oetern, 5000–6000 ft [1524–1829 m alt.], [flowering in December], D.F. Drège s.n. (lectotype, **designated here**: S (S-G-6704 right-hand plant annotated with ‘b’ [image!]); syntype: SOUTH AFRICA. Plantes du Cap, D.F. Drège 8.e.3920? (P (P00434764 [image!])).

= *Festuca costata* var. *longiseta* Nees, Fl. Afr. Austral. Ill. 1: 447. 1841. Type: SOUTH AFRICA. [Eastern Cape:] Stockenstrom Division, Katberg, [4000–5000 ft; 1219–1524 m alt.], 1840, D.F. Drège s.n. (lectotype, designated by Alexeev 1986: 1115: K (K000345256 [image!]); islectotype: K (K000345255 [image!])).

Notes. Alexeev (1986) separated *F. caprina* from *F. macra* (= *F. caprina* var. *macra*) based, in part, on the basal sheaths being fibrous. However, all type or original material of *F. caprina*, including the lectotype of *F. caprina* var. *caprina* designated by Alexeev (1986), had entire, often lustrous, basal sheaths apart from var. *irrasa*, which were obviously fibrous. The protologue mentions basal sheaths to be fibrous and, as such, Alexeev (1986) may have made an error in his choice of lectotype. Nevertheless, as only the type material of var. *irrasa*, which was designated by Stapf (1900), has fibrous basal sheaths, this also raises questions over the accuracy of the description in the protologue for var. *caprina*. If we treat *F. caprina* var. *caprina* based on the K lectotype and islectotype designated by Alexeev (1986) then var. *caprina* should be considered as having entire basal sheaths that do not split into fibres. Oddly, the inflorescences of all var. *caprina* specimens studied had a distinct butter-like smell upon the opening of specimen press papers, which then quickly dissipated. This odour was barely to sometimes slightly susceptible in specimens of *F. caprina* var. *macra* or var. *irrasa* or *F. drakensbergensis*. It remains to be seen whether this character is diagnostic and what phytochemical compounds are involved.

Festuca caprina var. *caprina* is more common at lower elevations in the Drakensberg Mountain Centre (Carbutt 2019) and surrounding mountainous habitats of southern Africa and extends from southern Africa to Tanzania. The species appears to prefer more mesic Afro-alpine and Afro-montane grasslands and is outcompeted by *F. caprina* var. *macra* in the drier summit area of the high escarpment in the DMC. Of the 222 2 m × 2 m plots studied for all vascular plants across the Afro-alpine DMC (Sylvester et al. unpubl. data), *F. caprina* var. *caprina* was rarely encountered, being found in only 13 plots from the Eastern Cape and Free State. The species was usually encountered in lower elevation Afro-montane transitioning to Afro-alpine grasslands at ca. 2500–2700 m alt. or exceptionally at higher elevations to 2981 m alt. in damper shaded sites, highlighting its very low frequency and commonality in the high-elevation xeric Afro-alpine zone of the DMC.

Festuca caprina var. *curvula* is also herein lectotypified. In the protologue, Nees von Esenbeck (1841: 443) only cited a single Drège s.n. collection from monte Los-Tafel-

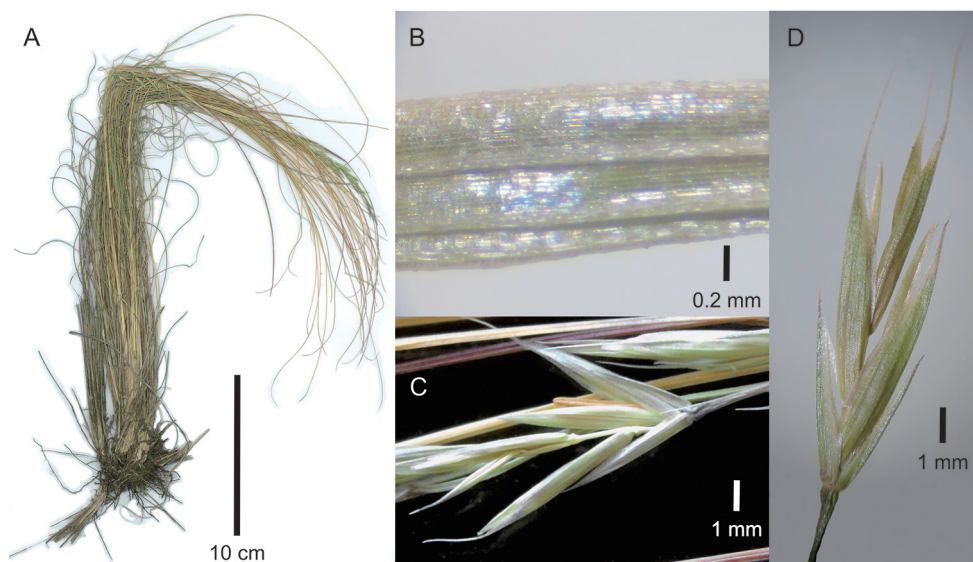


Figure 3. *Festuca caprina* var. *caprina*. **A** Whole plant **B** abaxial leaf blade surface of tiller **C, D** spikelet, lateral view. **A, B, D** of S.P. Sylvester et al. 3492 (US) **C** of S.P. Sylvester et al. 3492 (PRE).

berg, 6000 ft (1829 m alt.), which is assumed to be the same type locality as var. *caprina* that was found in Los-Tafelberg of the Eastern Cape Province, near Queenstown. Nees von Esenbeck (1841: 443) labelled var. *caprina* and var. *curvula* ‘a’ and ‘b’, respectively, with the S-G-6704 right hand plant chosen as lectotype based on this matching the protologue information and being the only specimen sheet amongst the original material to be annotated with an ‘a’ and ‘b’ in Nees von Esenbeck’s cursive handwriting. The right-hand plant annotated with ‘b’ fitted the protologue description of var. *curvula*, with Nees differentiating the variety based on its shorter height, curved blades and subsecund panicle branches with few purplish spikelets. One specimen amongst the original material, D.F. Drège 8.e.3920? (P00434764), also had ‘Curvula’ written on the label but limited locality information aside from ‘Plantes du Cap’ and is here considered a syntype of var. *curvula* as it also fits the description given in the protologue. While the differentiating characters of *F. caprina* var. *curvula* are also found in *F. drakensbergensis*, we deduce that var. *curvula* is a slight variation from the norm in *F. caprina* as neither the type specimens designated herein, nor any of the other original material from the type locality, can be attributed to *F. drakensbergensis* based on their lacking extravaginal branching and cataphyllous shoots as well as having entire lustrous basal sheaths.

Selected specimens examined. **SOUTH AFRICA.** Eastern Cape: Naudes Nek pass, near Rhodes, 30.764792S, 28.105164E, 2588 m alt., Afro-alpine tussock grassland, low rock outcrop, 13 Feb 2020, S.P. Sylvester et al. 3492 (NU, PRE, US); Free State: Witsieshoek, at beginning of Sentinel trail by parking lot, path-side, 28.733181S, 28.893296E, 2607 m alt., 5 Feb 2020, S.P. Sylvester et al. 3418 (US); Free State: Witsieshoek, Sentinel trail, along beginning of trail that leads to the chain ladders that

take you up to Amphitheatre, path-side, 28.736207S, 28.894084E, 2693 m alt., 5 Feb 2020, S.P. Sylvester et al. 3416 (US); Free State: Golden Gate National Park, summit of Wodehouse Peak, 14 Jan 1975, R.P. Ellis 2383 (PRE0464133); KwaZulu-Natal: Amphitheatre, slopes near the Tugela waterfall, Afro-alpine grassland, 28.750810S, 28.888942E, 2981 m alt., 5 Feb 2020, S.P. Sylvester et al. 3409a (US); KwaZulu-Natal: Sentinel Trail, off the main trail at the top of an east facing gully ca. 1 km from the chain ladders, 28.743162S, 28.888205E, 2953 m alt., shaded Afro-montane grassland grading into Afro-alpine grassland, damp soil, rarely grazed, 6 Mar 2020, S.P. Sylvester et al. 3713 (NU, PRE, US).

***Festuca caprina* var. *irrasa* Stapf, Fl. Cap. 7: 720. 1900.**

Fig. 4, Table 1

Type. SOUTH AFRICA. [Eastern Cape: Grahamstown], Howison Poort, Nov 1894, H.G. Flanagan s.n. (lectotype, designated by Alexeev 1986: 1115: K (K000345259 [image!])); syntype: SOUTH AFRICA. East Province of Cape Colony [Eastern Cape]: Amatole Mountains, Mar 1883, J. Buchanan 37 (K (K000345260 [image!])).

Notes. *Festuca caprina* var. *irrasa* may indeed be distinct and warrant elevating to species level. It differs from the other intravaginally branched taxa in the complex (*F. caprina* var. *caprina* and *F. caprina* var. *macra*) by the obviously fibrous basal sheaths and usually short-hispid or long-scabrous (prickles hair-like) lemmas, paleas and rachillas. The character of lemma, palea and rachilla pubescence sometimes varies with hispid hairs sometimes only found at the apex of some lemmas in the inflorescence. The panicle branches and pedicels are also usually densely short-hispid or long-scabrous with hooks elongating to become almost hair-like, a character not seen in the other members of the *F. caprina* complex, although this character also appears to vary. The variation may be due to introgressive hybridisation or lateral gene transfer between taxa, which possibly occur frequently in grasses (Kellogg 2015; Hibdige et al. 2020; Tkach et al. 2020). This could be exemplified by how one specimen (Sylvester et al. 3547) that was collected close to both var. *macra* (Sylvester et al. 3538) and var. *irrasa* (Sylvester et al. 3542) had inflorescence characteristics of var. *irrasa*, but antrorsely scabrous abaxial leaf-blade surfaces like var. *macra*. Further work is needed to clarify the circumscription and taxonomic position of var. *irrasa*. *Festuca drakensbergensis*, described herein, also usually has fibrous basal sheaths and, although not as conspicuous as *F. caprina* var. *irrasa*, can be readily distinguished based on its extravaginal tiller branching, presence of rhizomes and smaller anther size, amongst other characters.

Festuca caprina var. *irrasa* is endemic to the DMC of southern Africa, being found in Lesotho and the South African Eastern Cape and KwaZulu-Natal Provinces and possibly the Free State Province (although no specimens have been verified by us). The species appears to be more common in the KwaZulu-Natal Province. During our ecological plot-based study across the Afro-alpine DMC (Sylvester et al. unpubl. data), *F. caprina* var. *irrasa* was only encountered as locally abundant ([0.5–]5–35% of 2 m × 2 m plot

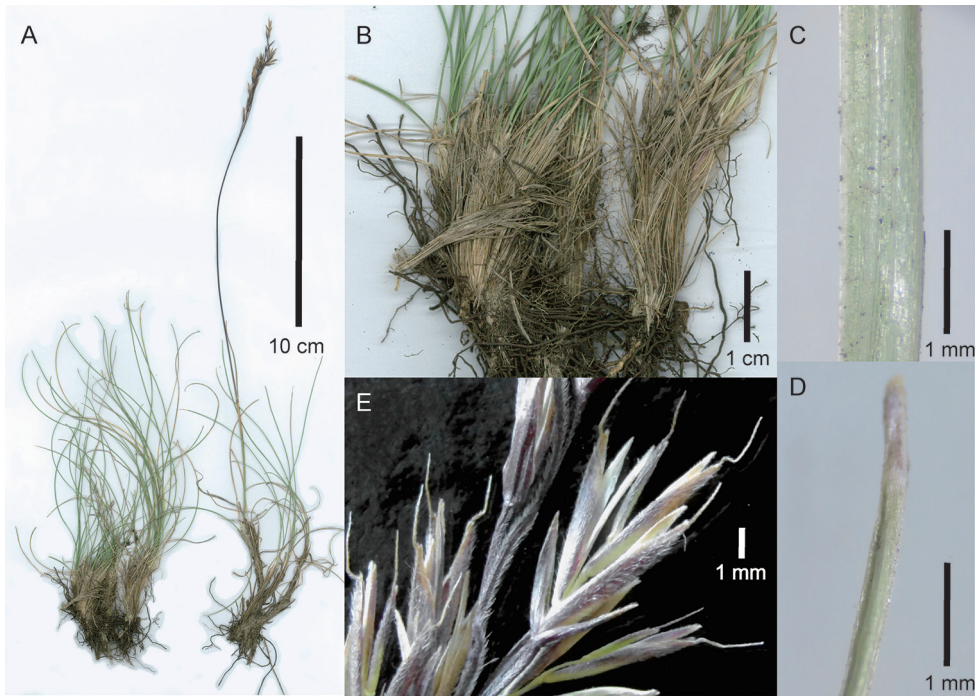


Figure 4. *Festuca caprina* var. *irrata*. **A** Whole plant **B** close-up of basal sheaths showing fibres **C** abaxial leaf blade surface of tiller **D** leaf blade apex of tiller **E** close-up of inflorescence, showing spikelets, lateral view. **A, B, E** of S.P. Sylvester et al. 3512 (US) **C, D** of S.P. Sylvester et al. 3542 (US).

cover) populations in the damper southern sites of the DMC, i.e. Sehlabathebe National Park (Lesotho) and Barclays Pass (Eastern Cape, South Africa). The species was found in only 11 plots ranging from the lower elevation Afro-montane to Afro-alpine grassland transition at ca. 2250 m alt. to wet Afro-alpine tussock grasslands at ca. 2750 m alt.

Alexeev (1986: 1115) cites "(P. Linder in Fl. South Africa. manusc.): Cape Province, Grahamstown, Howisons Poort, no. 94, H.G. Flaganan (K!)" for lectotype selection. However, upon inspection of the K lectotype, the '94' refers to the year of collection.

Selected specimens examined. **LESOTHO.** Sehlabathebe National Park, lower end of the Park on the border, 29.877593S, 29.086461E, 2606 m alt., wet Afro-alpine tussock grassland, soil damp, not grazed recently, 20 Feb 2020, S.P. Sylvester et al. 3542 (PRE, US); Sehlabathebe National Park, lower end of the Park on the border, 29.876061S, 29.086150E, 2645 m alt., gravelly slopes below basalt rock escarpment with grasses intermixed with forbs, soil damp, burned and grazed recently, 20 Feb 2020, S.P. Sylvester et al. 3547 (PRE, US). **SOUTH AFRICA.** Eastern Cape: Bastervoeetpad Pass area, ca. 12 km east of Mountain Shadow Hotel on Barclay Pass, 31.172568S, 27.964747E, 2259 m alt., Afro-montane transitioning to Afro-alpine grassland under moderately-heavy grazing, 14 Feb 2020, S.P. Sylvester et al. 3512 (US); KwaZulu-Natal: Giants Castle, 10,000 ft [3048 m alt.], 8 Jan 1915, R.E. Symons 352 (PRE0023182);

KwaZulu-Natal: Weenen county, top of Griffins Hill, ca. 5000 ft [1524 m alt.], sedgy banks of streamlet in highland sourveld, fairly frequent, 29 Oct 1944, J.P.H. Acocks 10740 (PRE0023178).

***Festuca caprina* var. *macra* Stapf, Fl. Cap. 7: 720. 1900. *Festuca macra* (Stapf) E.B. Alexeev, Bot. Zhurn. (Moscow & Leningrad) 71(8): 1116. 1986.**

Fig. 5, Table 1

Type. SOUTH AFRICA. [Kalahari Region: Orange Free State:] Wittebergen, near Harrismith, Comm. O. MacOwan, Feb 1877, Buchanan 262 (holotype: K (K000345247 [image!]); isotype: PRE! fragm. ex K).

Notes. Alexeev (1986) raised var. *macra* to species level and differentiated it from *F. caprina* based on: a) sheaths of old leaves not falling apart into parallel thin threads (vs. falling apart (shredded) into parallel thin threads in *F. caprina*, although this is now considered erroneous; see comments under *F. caprina* above); b) leaf blades more or less glaucous (vs. green in *F. caprina*); c) abaxial leaf-blade surfaces scabrous (vs. smooth or scaberulous in *F. caprina*); d) adaxial leaf-blade surfaces shortly hairy (vs. scabrous or shortly hairy in *F. caprina*); e) lemmas 4.5–5 mm long (vs. 5–7[9] mm long in *F. caprina*); f) awns 1.5–3.5 mm long (vs. [0.5]1–4 mm long in *F. caprina*); g) anthers 1.6–2.2 mm long (vs. [2–]2.5–4 mm long in *F. caprina*); h) spikelets straw-coloured-violet (vs. violet-green, rarely green in *F. caprina*). However, only the holotype of *F. caprina* var. *macra* was seen by Alexeev, as well as original material (from which a lectotype was selected) and a limited number of other specimens of *F. caprina* var. *caprina* at the K herbarium.

Upon study of numerous specimens that belong to *F. caprina* var. *caprina* and var. *macra* during extensive fieldwork in the DMC and herbarium study at PRE, it became apparent that the above-mentioned differentiating characters overlap. Both *F. caprina* var. *caprina* and *F. caprina* var. *macra* share most characteristics, such as intravaginal tillers forming dense, often large, tussocks, with entire, often lustrous, basal sheaths, narrow involute blades and similar inflorescence and spikelet morphology, with anthers usually > 2 mm long. The *F. caprina* var. *macra* holotype is on the shorter side with regards most inflorescence characters when compared with *F. caprina* var. *caprina*, with shorter spikelets, lemmas, awns and anthers according to the protologue. Nevertheless, most of these characters have also been found in specimens of *F. caprina* var. *caprina*, with variability in lengths of the spikelet parts possibly being related to ecological conditions, including seasonal variations in rainfall (C. Mashau, pers. comm.). The anther length of 1.6 mm, mentioned in the protologue for *F. macra* (Alexeev 1986: table 2), is shorter than any specimen of *F. caprina* var. *macra* studied by us, with it being plausible that the var. *macra* holotype could be somewhat intermediate between *F. drakensbergensis* and *F. caprina* var. *macra* in its broader sense, with similar plausible hybrids with a mixture of characters sometimes found in the DMC (see below). Indumentum of the adaxial leaf-blade surface was also found to vary between scabrous, long-scabrous with prickles becoming elongated and hair-like and shortly hispid in all

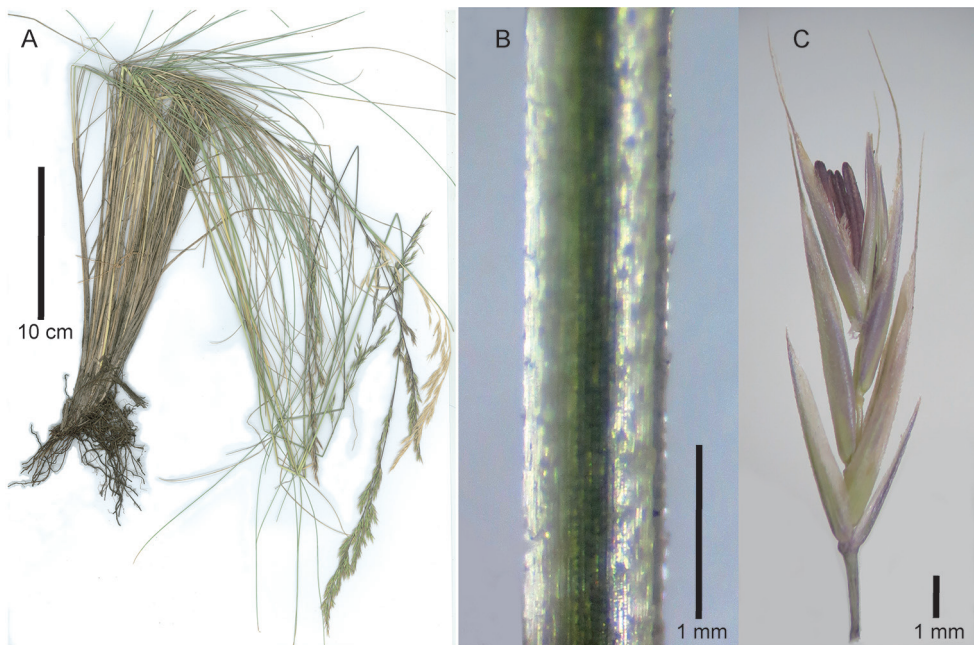


Figure 5. *Festuca caprina* var. *macra*. **A** Whole plant **B** abaxial leaf blade surface of tiller **C** spikelet, lateral view. Images of S.P. Sylvester et al. 3480 (US).

the taxa of the *F. caprina* complex from southern Africa, with this character seen to have no diagnostic value.

Festuca caprina var. *macra* was not included in the treatment of southern African grasses by Fish and Moeaha (2015), who chose not to uphold any of the varieties of *F. caprina* stating that the species was too variable. Nevertheless, we consider *F. caprina* var. *macra* to be distinct from var. *caprina* based on the character of notably antrorsely scabrous abaxial leaf-blade surfaces that is not known outside of the DMC, with all other *F. caprina* specimens across their range being smooth or exceptionally scaberulous towards their apices. Specimens with notably scabrous leaf blades were also found to be geographically and ecologically distinct during fieldwork in the DMC, these being predominantly found in drier alpine areas of the DMC, while var. *caprina* was found in more mesic environments often at lower elevations in the montane belt.

Plants of the World Online (2020), Plantlist (2020), the World Checklist of Selected Plant Families (2020) and GrassBase (Clayton et al. 2006 onwards) currently accept *F. macra* as a distinct species. While we currently disagree with this assessment, more exhaustive research may result in var. *macra* being raised to subspecies level, with certain characters still needing to be assessed such as lemma micromorphology, which has been proven to be taxonomically informative in *Festuca* (Ortúñez and Cano-Ruiz 2013).

Festuca caprina var. *macra* is often dominant in less-disturbed Afro-alpine grasslands of the DMC (Carbutt 2019), being found in Lesotho and the Eastern Cape, Free State, KwaZulu-Natal Provinces of South Africa. Of the 222 2 m × 2 m plots stud-

ied throughout the DMC, 99 were occupied and often dominated ([0.5–]20–92% of overall plot cover) by *F. caprina* var. *macra* (Sylvester et al. unpubl. data), with a total of 42 collections of the species being made. It is more palatable than *Merxmüllera* Conert species and so is less common in grazed areas (Sylvester and Soreng, pers. obs.).

Festuca obturbans St.-Yves and its allies *F. gilbertiana* Alexeev ex S.M. Phillips and *F. macrophylla* A. Rich., described from Afro-alpine vegetation of Kenya or Ethiopia, also bear superficial similarity to *F. caprina* var. *macra* in their intravaginally branched large tussocks with entire basal sheaths and fine, involute and usually scabrid leaf blades (Alexeev 1986; Phillips 1995a,b). These also share similar inflorescence characteristics with *F. caprina* var. *macra*, such as relatively-narrow panicles with spikelets loosely arranged on short ascending branches, and spikelets with similar glume, lemma and anther sizes (Alexeev 1986; Phillips 1995a,b). These can be differentiated by their leaf blade cross sections showing sclerenchyma girders bridging both sides of the vascular bundles or, at least, the larger ones (vs. sclerenchyma only present on the abaxial blade ribs in *F. caprina* var. *macra*). *Festuca gilbertiana* can be further differentiated by its smooth leaf blades, culms 30–35 cm tall, and sparse racemose inflorescence (vs. leaf blades scabrous, culms (28–)60–110(–120+) cm tall, inflorescence usually a large loosely-contracted panicle in *F. caprina* var. *macra*). *Festuca obturbans* can be further differentiated by having sheaths open to almost their base and ovary apices glabrous (vs. sheaths closed for ca. ½ their length, ovary apices sparsely to densely pubescent in *F. caprina* var. *macra*).

Two specimens found near the Tiffindell Ski Resort of the Eastern Cape, South Africa (Sylvester et al. 3428B) and Bokong Nature Reserve, Lesotho (Sylvester et al. 3687B), bore characteristics of *F. caprina* var. *macra*, which was collected alongside them (Sylvester et al. 3428A, 3687C), such as tussock-forming habit with intravaginal branching and entire basal sheaths not splitting into fibres. However, they differed by their smooth abaxial leaf blade surfaces, placing them closer to *F. caprina* var. *caprina*, unawned lemmas, which is unusual for both var. *caprina* and var. *macra*, and short spikelets with lowermost lemmas 4.5–5.8 mm long anthers measuring ca. 1.6–1.8 mm long, placing them closer to *F. drakensbergensis*. As *F. drakensbergensis* was also collected at the same localities (e.g. Sylvester et al. 3459, 3687B), it is plausible that these could be hybrids between *F. caprina* var. *macra* and *F. drakensbergensis*. More study, including further collections, is required to ascertain the identity of these specimens.

Selected specimens examined. **LESOTHO.** AfriSki area, in valley adjoining and northwest of the valley of the AfriSki resort, on the north side of the A1 highway, 28.808394S, 28.708658E, 3104 m alt., dry upper slopes above valley, 27 Feb 2020, S.P. Sylvester et al. 3652 (NU, PRE, US); AfriSki resort, in valley just west of the resort centre, 28.822906S, 28.724602E, 3046 m alt., relatively undisturbed damp Afro-alpine grassland, 28 Feb 2020, S.P. Sylvester et al. 3663 (PRE, US); Bokong Nature Reserve, ca. 350 m north from the information centre, 29.067203S, 28.421496E, 2972 m alt., Afro-alpine grassland dominated by *Lachnagrostis barbuligera* var. *barbuligera* with moderately-controlled grazing and burning, 2 Mar 2020,

S.P. Sylvester et al. 3687b (US); S.P. Sylvester et al. 3687c (US); Matebeng Pass, below highest summit close to the pass, 29.870708S, 28.976534E, 3094 m alt., “Lesotho Highland Basalt Grassland” with clear elements of “Drakensberg Afro-alpine Heathland” with *Erica* and *Helichrysum* shrubs dominating the landscape, 22 Feb 2020, S.P. Sylvester et al. 3576 (US); Matebeng Pass, below highest summit close to the pass, 29.868524S, 28.976439E, 3125 m alt., Afro-alpine vegetation with Ericaceous shrubs dominating the landscape, heavy grazing, 22 Feb 2020, S.P. Sylvester et al. 3580 (US); Matebeng Pass, below highest summit close to the pass, 29.873765S, 28.976929E, 2947 m alt., Afro-alpine vegetation with Ericaceous shrubs dominating the landscape, heavy grazing, 22 Feb 2020, S.P. Sylvester et al. 3588 (PRE, US); Menoaneng Pass, on road between Rafolatsane and Thaba-Tseka, 29.427317S, 28.950617E, 3039 m alt., Afro-alpine grassland, windy ridge, grazed, 24 Feb 2020, S.P. Sylvester et al. 3601 (US); Sani Pass area, ca. 800 m east of Sani Mountain Lodge, 29.585198S, 29.292011E, 2896 m alt., short Afro-alpine grassland, frequently to heavily grazed, 25 Feb 2020, S.P. Sylvester et al. 3619 (PRE, US); S.P. Sylvester et al. 3620 (US); Sehlabathebe National Park, lower end of the Park on the border, 29.859882S, 29.095598E, 2779 m alt., wet Afro-alpine tussock grassland, soil damp, under dripping crag, heavily grazed, close to livestock paths, 19 Feb 2020, S.P. Sylvester et al. 3523 (NU, PRE, US); Sehlabathebe National Park, lower end of the Park on the border, 29.860180S, 29.095586E, 2733 m alt., wet Afro-alpine tussock grassland, soil damp, under dripping crag, heavily grazed, close to livestock paths, 19 Feb 2020, S.P. Sylvester et al. 3538 (PRE, US). **SOUTH AFRICA.** Eastern Cape: between Carlislehoekspruit Pass and Tiffindell Ski Area, 30.6852485S, 27.963802E, 2565 m alt., Afro-alpine grassland, 10 Feb 2020, S.P. Sylvester et al. 3428a (PRE, US); Eastern Cape: between Carlislehoekspruit Pass and Tiffindell Ski Area, 30.6852485S, 27.963802E, 2565 m alt., Afro-alpine grassland, 10 Feb 2020, S.P. Sylvester et al. 3428b (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, 30.649239S, 27.928720E, 2845 m alt., Afro-alpine grassland, 10 Feb 2020, S.P. Sylvester et al. 3446 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, 30.676006S, 27.958567E, 2527 m alt., Afro-alpine tussock grassland, 12 Feb 2020, S.P. Sylvester et al. 3480 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, Ben Macdhui summit, 30.648172S, 27.935507E, 2998 m alt., Afro-alpine grassland, 11 Feb 2020, S.P. Sylvester et al. 3462b (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, next to ski lift, 30.651034S, 27.925149E, 2778 m alt., Afro-alpine grassland, annually burnt, appears to be seeded with exotic species, 11 Feb 2020, S.P. Sylvester et al. 3463 (NU, PRE, US); KwaZulu-Natal: Amphitheatre, slopes near the Tugela waterfall, 28.754008S, 28.893853E, 2983 m alt., Afro-alpine grassland, 5 Feb 2020, S.P. Sylvester et al. 3403 (NU, PRE, US); KwaZulu-Natal: Amphitheatre, slopes near the Tugela waterfall, 28.753989S, 28.893563E, 2979 m alt., Afro-alpine grassland, 5 Feb 2020, S.P. Sylvester et al. 3406 (US); KwaZulu-Natal: Amphitheatre, slopes near the Tugela waterfall, 28.750810S, 28.888942E, 2981 m alt., Afro-alpine grassland, 5 Feb 2020, S.P. Sylvester et al. 3409b (US).

***Festuca exaristata* E.B. Alexeev, Bot. Zhurn. (Moscow & Leningrad) 71(8): 1116. 1986.**

Fig. 6, Table 1

Type. [LESOTHO] Basutoland. Above the Sani Pass, among stones, 9800 ft [2987 m alt.], 3 Feb 1959, M. McCallum Webster 483b (holotype: K (K000345250 [image!])).

Notes. This species was not included in the treatments to southern African grasses (Gibbs Russell et al. 1990; Fish and Moeaha 2015), nor in the checklist to Lesotho grasses (Kobisi and Kose 2003), but is accepted in Plants of the World Online (2020), Plantlist (2020), the World Checklist of Selected Plant Families (2020), GrassBase (Clayton et al. 2006 onwards) and Tropicos (2020). It is known from just two collections; the type from Sani Pass, bordering Lesotho and the KwaZulu-Natal Province of South Africa, and a paratype from Letsing La Letsie of the Matatiele Province of Lesotho. Exploration by the authors in the Sani Pass area failed to discover further specimens although, at the time of visiting, the authors were not searching in particular for *F. exaristata* and did not cover all the habitats present. The holotype label states ‘Above the Sani Pass’ probably referring to the mountain slopes and ridge immediately above the Sani Pass, which were not explored by us. Our exploration largely focused on the valley bottom, which experienced very heavy grazing, with it being possible that the species may have been grazed out in these areas. As the species exhibits certain characters of both *F. caprina* s.l. and *F. drakensbergensis*, as well as other characters not found on any of these (e.g. glabrous ovaries, shorter unawned lemmas), there is also the possibility that the species is a hybrid which failed to survive into subsequent gen-

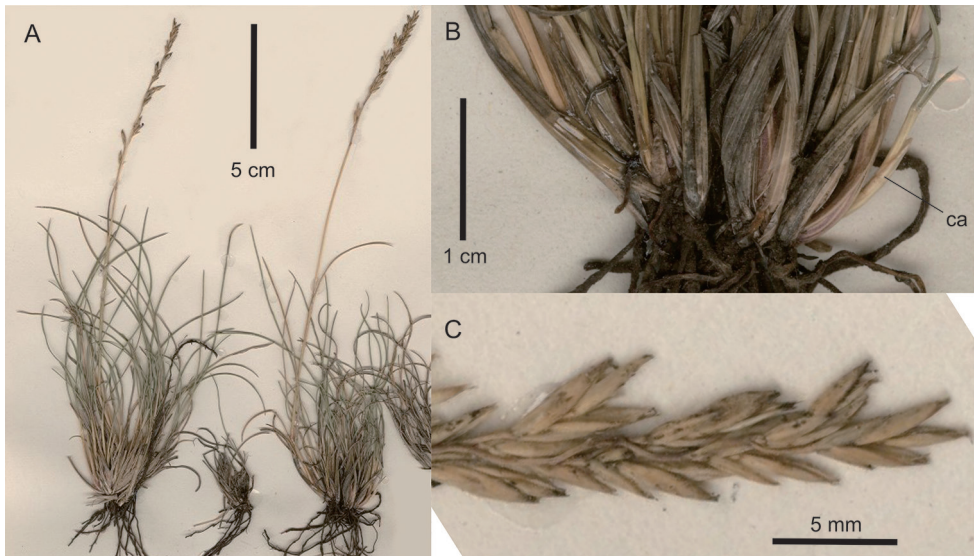


Figure 6. *Festuca exaristata*. **A** Whole plant **B** basal part of plant showing lustrous basal sheaths and extravaginally-branched tillers with cataphylls (ca) **C** close-up of inflorescence. Digitised images of holotype M. McCallum Webster 483b (K000345250), courtesy of JSTOR Global Plants (<https://plants.jstor.org>).

erations. However, the paratype, which was not seen by us, but was collected in 1977, 18 years after and ca. 130 km southwest of the type collection, raises doubt over this.

Alexeev (1986) distinguished this species from *F. macra* (= *F. caprina* var. *macra*) and *F. caprina* in part by: a) leaf blade mid-vein blunt and rounded; b) panicle branches smooth; c) lemmas 4–4.2 mm long; d) lemmas unawned; e) ovary apex glabrous; f) anthers 1.5–1.8 mm long. It can be further differentiated from *F. caprina* var. *irrasa* by the basal sheaths being entire, and from *F. caprina* var. *macra* by the leaf blade abaxial surfaces being smooth. Furthermore, although not mentioned by Alexeev (1986), the type material appears to have extravaginal branching, with cataphyllous laterally-tending shoots present, differentiating this from the intravaginally branched *F. caprina* s.l. The species does bear some resemblance to *F. drakensbergensis* (see notes under *F. drakensbergensis*).

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A new species of *Besleria* (Gesneriaceae) from the Serranía El Pinche (Cauca), southwestern Colombia

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Abstract

A new species of the genus *Besleria* (Gesneriaceae), endemic to the department of Cauca, Colombia, is described and illustrated here. The new species, *Besleria santaclarensis* Clavijo & Sánchez-Taborda, was discovered in the Regional Protective Forest Reserve “Serranía El Pinche”, Cordillera Occidental of the Colombian Andes. *B. santaclarensis* is distinguished by the epedunculate inflorescences, usually in the leafless axils near the base, with up to eight orange flowers, and by the magenta calyx that covers 2/3 of the corolla.

Resumen

Una nueva especie del género *Besleria* (Gesneriaceae), endémica del departamento del Cauca, Colombia, se describe e ilustra aquí. La nueva especie, *Besleria santaclarensis* Clavijo & Sánchez-Taborda, se descubrió en la Reserva Forestal Protectora Regional “Serranía El Pinche”, Cordillera Occidental de los Andes colombianos. *B. santaclarensis* se distingue por las inflorescencias epedunculadas, usualmente en las axilas de nudos sin hojas ubicados hacia la base, con hasta ocho flores anaranjadas por inflorescencia y cáliz magenta que cubre 2/3 de la corola.

Keywords

Andes, Cauca, Choco biogeographic, Protective Forest Reserve, San Juan de Micay, taxonomy

Introduction

Besleria Plum. ex L. includes 160 species (Clark et al. 2020) of terrestrial herbs, shrubs and small trees that grow in the rainforest understory. It is strongly supported as mono-

phyletic in the tribe Beslerieae (Smith 2000; Roalson and Clark 2006; Clark et al. 2010). Biogeographic analyses of the family suggest that it diversified in the Andes 15 Mya (Perret et al. 2013; Roalson and Roberts 2016). *Besleria* is one of the largest and least known genera among the New World Gesneriaceae; it occurs in most Neotropical rainforests with the highest diversity in the tropical Andes (>100 species), followed by Central America (20 species) (Skog and Boggan 2007; Ferreira et al. 2016). Colombia is the country with the highest diversity of *Besleria*, with more than 80 species (Cortés 2013), followed by Peru with 35 (Kvist et al. 2005), Ecuador with about 20 (Skog and Kvist 1997) and Panama with 15 (Skog 1978). In Colombia, most of the species are found in the humid forests of the Andes and the Choco Biogeographic region, whereas few species grow in the Amazon (Cortés et al. 2017). In the Andes, the largest number of species is found in the Cordillera Occidental of Colombia. For example, *Besleria* is the third largest Gesneriaceae genus with 10 species out of the 96 recorded in northern Valle del Cauca (Clavijo et al. 2014). Likewise, Pedraza and Betancur (2015) recorded five species of *Besleria* out of the 70 Gesneriaceae species collected in the National Natural Park Orquídeas (Antioquia), north of the Cordillera Occidental.

The Serranía El Pinche in Argelia (Cauca) is located in the southern portion of the Cordillera Occidental of the Colombian Andes, and is part of the Munchique-Pinche corridor, a region known for high levels of biodiversity (Paz-B et al. 2018). Biogeographically, the Serranía El Pinche is more similar to the Nudo de los Pastos and the Colombian Massif than to the northern portions of the Cordillera, mainly due to the lower elevation Paramos on the Pacific slopes (Becking 1995). For the first settlers of the Serranía, the conservation of native ecosystems and sustainable rural development were paramount in this biodiverse region. Early settlers inspired in their children the importance of preserving their natural resources by creating alliances that would allow the proper management and establishment of a formal protected area. As a result of this initiative, the Regional Protective Forest Reserve “Serranía El Pinche” was created in 2008. The Reserve is part of the San Juan de Micay river basin that ranges in elevation from 1040 to 3744 meters above sea level; it has 7,256 ha, however, after its expansion it will have in total 11,930 ha. 90.4% of the reserve corresponds to primary forests and 9.6% to perturbed forests, crops and pastures. The Reserve “Serranía El Pinche” and the buffer zone include 913 households (Paz-B et al. 2018).

The upper San Juan de Micay river basin is a unique biodiverse area in the Pacific slopes of the Andes that still has large extensions of primary tropical rainforests (Becking 1995), despite high deforestation rates caused by agriculture and the presence of illicit crops. The preservation of protected areas led by local communities in regions suffering rapid deforestation is essential to conserve species and entire ecosystems. In fact, private and community-owned protected areas have been successful in preserving natural ecosystems in the northern Andes, where biological diversity is high (Joppa et al. 2008; Armenteras et al. 2009; Rodríguez et al. 2013). Therefore, following the legacy of the first naturalists and ecologists that explored La Serranía El Pinche, we aim to contribute to the floristic knowledge of this underexplored rainforest in south-western Colombia. The plant inventory and discovery of new taxa in this region will

provide important information to support the future expansion of the Reserve toward the coast, in the municipalities of Guapi and Timbiquí, achieving a broad elevation coverage from the Pacific coast to the paramos of the Andean highlands.

Methods

During a rapid ecological evaluation carried out in September of 2017 to characterize the vegetation and establish the baseline for expanding the Regional Protective Forest Reserve “Serranía El Pinche”, we discovered a new species of the genus *Besleria* which is described and illustrated here.

The collections of the new species were processed at the herbarium of the Universidad del Cauca (CAUP) and were deposited at the Colombian National Herbarium (COL), the herbarium of the Universidad del Valle (CUVC) and the Botanical Garden of Medellin (JAUM). Specimens of *Besleria* from COL, CUVC, and JAUM were studied to confirm the identity of the species. The photographs were taken with a Nikon camera model D 5300. For the general botanical terminology we followed Beentje (2010) and Moreno (1984).

Taxonomic treatment

Besleria santaclarensis Clavijo & Sánchez-Taborda, sp. nov.

urn:lsid:ipni.org:names:77211931-1

Figs 1, 2

Type. COLOMBIA, Cauca: Municipio Argelia, corregimiento Santa Clara, vereda Santa Clara, Reserva Forestal Protectora Regional “Serranía El Pinche”, flanco oriental de la Serranía que se desprende de la vertiente pacífica de la Cordillera Occidental, camino por el borde de la quebrada La Planada, 2°23.938'N, 77°18.863'W, 1620 m. 25 Sep 2017 (fl), Jhon Alexander Sánchez-Taborda, Álex Cortés, Andrea Borrero, Fernando Joaqui, Andrés Pérez, Erminson Buitrago, Julian Uetochambo 2552 (holotype: COL!; isotype: CUVC!).

Besleria santaclarensis is distinguished by epedunculate inflorescences, usually in the leafless axils near the base of the stem, with up to 8 glabrous orange flowers, and magenta glabrous calyx that covers 2/3 of the corolla.

Terrestrial subshrub, 1–1.5 m tall. Stem erect, sometimes scandent, branched, terete in cross section, 1.9–4 mm diam., subwoody, green, surface smooth to longitudinally striated, strigose toward the apex, trichomes < 1 mm long, unbranched, white; internodes 1.7–5.8 cm long. Leaves opposite, equal in a pair, sometimes subequal, older leaves usually caducous; petiole 1.6–4.9 cm long, slightly winged in cross-section, glabrate in basal leaves, strigose in apical leaves, trichomes < 1 mm long, white; blades elliptic, coriaceous, papery when dry, 6.4–17.6 × 2.8–6.8 cm, green and glossy adaxially, olive green

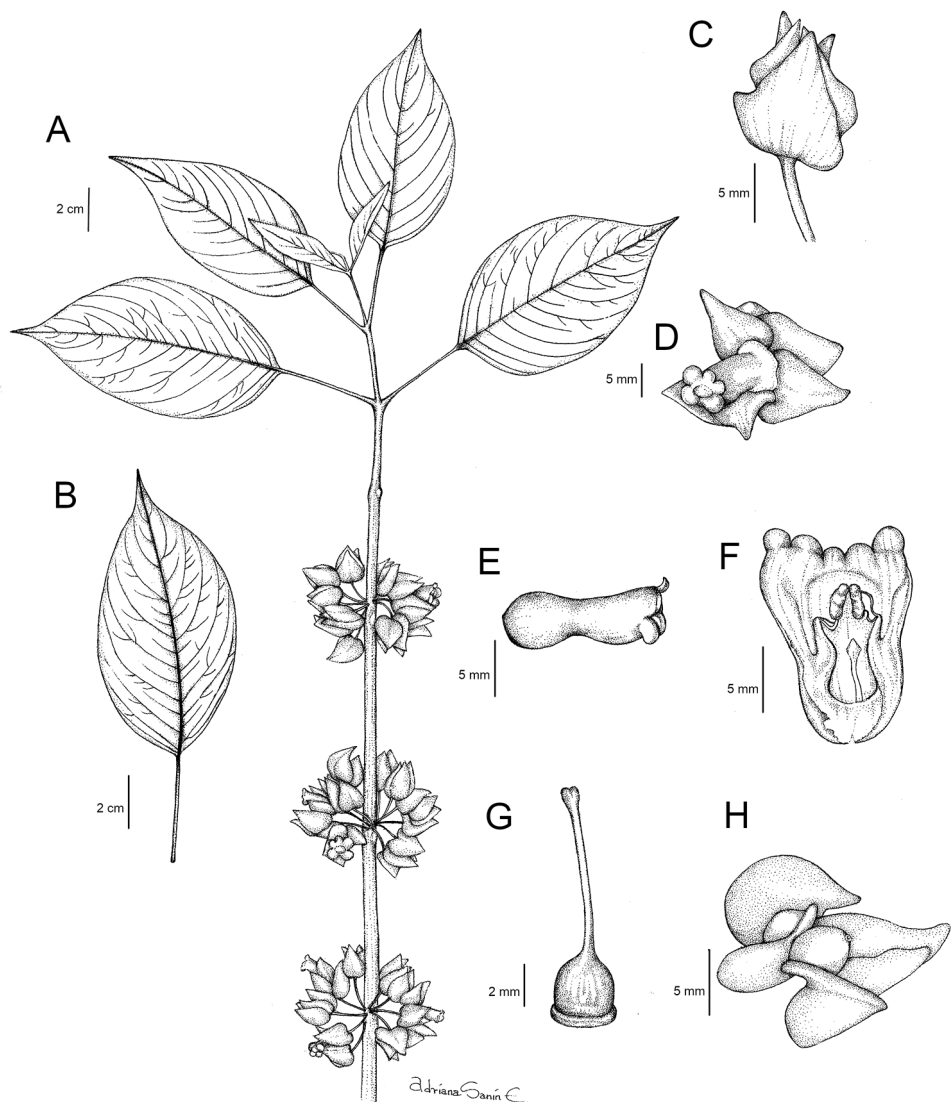


Figure 1. Drawing of *Besleria santaclarensis* Clavijo & Sánchez-Taborda, sp. nov. **A** habit **B** leaf detail, adaxial view **C** calyx, lateral view **D** flower, frontal view **E** corolla, lateral view **F** interior view of corolla showing stamens and staminode **G** gynoecium with anular nectary gland **H** fruit, lateral view. Illustration by Adriana Sanín, based on the holotype Sánchez-Taborda et al. 2552.

abaxially, apex acuminate, base cuneate, sometimes oblique, margin entire, glabrate on both surfaces, 7–10 pairs of secondary veins, obscure adaxially and raised abaxially with scarce and whitish indument, higher order of venation only evident abaxially. Inflorescence a pair-flowered cyme, axillary, usually in the leafless axils near the base of the stem, up to 8 flowers per inflorescence; peduncle and bracts absent. Pedicel oblique to perpendicular relative to the stem, 8.6–17.1 mm long, maroon, glabrous. Calyx magenta, mem-

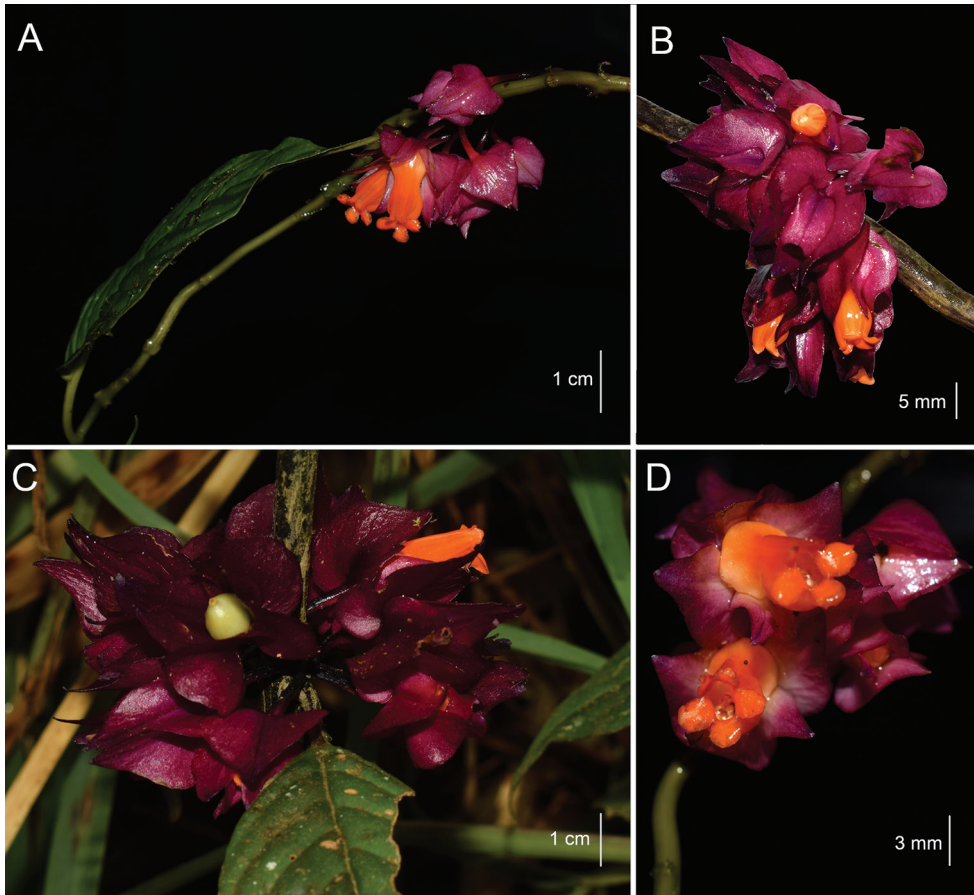


Figure 2. *Besleria santaclarensis* Clavijo & Sánchez-Taborda, sp. nov. **A** branch showing leaf and inflorescence **B** inflorescence showing flower in lateral view **C** inflorescence showing an immature fruit **D** frontal view of the corolla. (Photographs by Jhon A. Sánchez-Taborda from the paratype).

branous, persistent in fruit, venation evident, reticulated; calyx lobes 5, 4 nearly equal, free, apex acute, base truncate to cordate, margin entire, glabrous on both surfaces, ventral and lateral lobes $9\text{--}13 \times 6.2\text{--}10.6$ mm, ovate, dorsal lobe $10.1\text{--}11.9 \times 4.3\text{--}5.3$ mm, oblong; Corolla zygomorphic, protandrous, slightly gibbous, thick, $14.4\text{--}15.4$ mm long, orange, glabrous; corolla tube slightly constricted above the base and then slightly ventricose ventrally, oblique relative to calyx, $12.3\text{--}13.5$ mm long, $5.7\text{--}6.2$ mm at its widest part, constriction above the base $3.7\text{--}4$ mm diam., base $5.4\text{--}6.8$ mm wide; throat $2.3\text{--}2.8$ mm diam., inner surface with glandular trichomes; corolla lobes 5, subequal, orange, spreading, ovate, apex rounded, margin entire, glabrous on both surfaces, ventral lobe $1.4\text{--}2.6 \times 2.4\text{--}3.1$ mm, lateral lobes $2.5\text{--}3.4 \times 2.4\text{--}3.9$ mm, dorsal lobes $0.8\text{--}1.1 \times 1.8\text{--}2.3$ mm. Androecium of 4 stamens, didynamous, included; filaments $7.1\text{--}10.1$ mm long, adnate to the corolla tube for $1.9\text{--}2.6$ mm, forming a sheath, glabrous, coiling after anthesis, staminode $4.9\text{--}5.2$ mm long; anthers reniform, $1.2\text{--}1.4 \times 1.1\text{--}1.7$ mm, coher-

ent by the apex and lateral walls, dehiscence by longitudinal slits. Gynoecium with an annular nectary gland, 0.5–0.7 mm tall, glabrous; ovary superior, 3–3.1 × 2.5–2.7 mm wide, rounded, glabrous; style included, 6.4–6.8 mm long, glabrous; stigma bilobed. Fruit a berry olive green; seeds numerous.

[Measurements from flowers during the mature gynoecium phase.]

Distribution and ecology. *Besleria santaclarensis* is endemic to Colombia and known only from the type locality in the municipality of Argelia (Cauca) in the Regional Protective Forest Reserve “Serranía El Pinche” and surrounding areas (Fig. 3). This species grows in the lower montane rainforest (Holdridge 1967) between 1300 and 1600 m above sea level, on the Pacific slope of the Cordillera Occidental of the Andes. It is frequent in open areas and in forest clearings, usually near to crops, pastures and remnants of secondary and riparian forests, whose canopies reach up to 35 m and 25 m, respectively. These forests have typical Andean floristic elements, represented by the genera *Saurauia* Willd (Actinidaceae), *Schefflera* J.R. Forst. and G. Forst. (Araliaceae), *Axinaea* Ruiz and Pav. (Melastomataceae), *Ladenbergia* Klotzsch (Rubiaceae), *Wettinia* Poepp, *Socratea* H. Karst and *Iriarteia* Ruiz and Pav. (Arecaceae), *Cyathea* Sm and *Alsophila* R. Br. (Cyatheaceae).

Phenology. *Besleria santaclarensis* has been found in flower in February and September and in fruit in February.

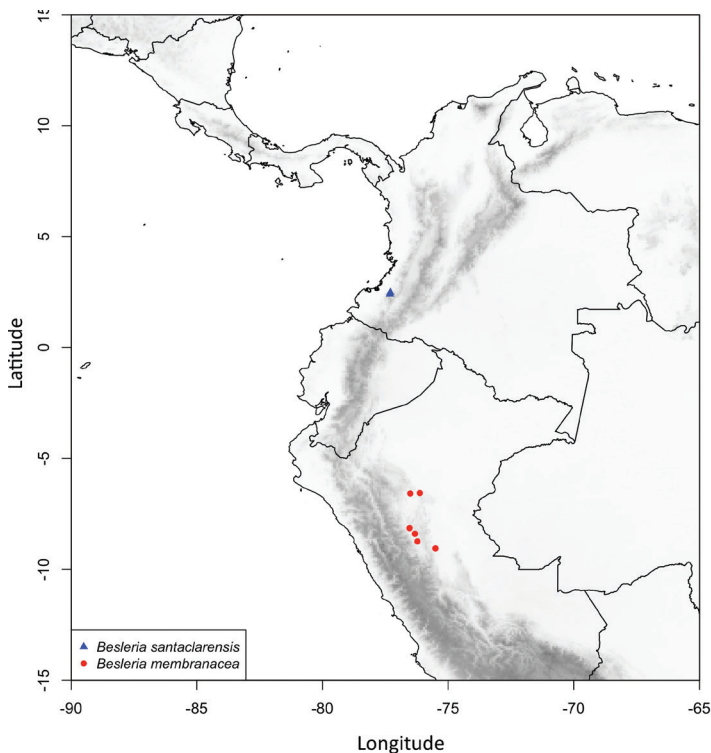


Figure 3. Distribution map of *Besleria santaclarensis* and *Besleria membranacea*.

Etymology. *Besleria santaclarensis* is named after the type locality, the Santa Clara village, in the municipality of Argelia, Cauca. The territory currently known as Argelia and areas surrounding El Pinche mountain range were initially inhabited by the *Gua-pios* indigenous people, until the arrival of the colonists who named it the Agua Clara path, due to the streams of crystalline waters present in the area. Afterwards, during evangelization, a Franciscan priest changed the name to Santa Clara because of the fertility of the land, which he called holy (Alveiro Bolaños, personal communication).

Preliminary conservation status. *Besleria santaclarensis* is only known from the Regional Protective Forest Reserve “Serranía El Pinche” and surrounding areas. Although the Reserve is a protected area, deforestation and soil degradation caused by agriculture and livestock systems, as well as the presence of illicit crops in the surrounding areas, may have a significant impact on the integrity of this narrow endemic species. *B. santaclarensis* is present in mature forests, but it is also frequent in open areas in remnants of secondary and riparian forests, suggesting it is resilient and capable of thriving in disturbed areas. A preliminary designation of Endangered (EN) category is provided, according to the criterium B2ab (IUCN 2012, 2017), based on the small population of the species, with an estimated area of occupancy of less than 20 km², and the continued habitat loss, due to the high concentration of illicit crops in the area (UNODC 2019).

Discussion

Besleria santaclarensis can be readily distinguished from its congeners by the epedunculate inflorescences, usually in the leafless axils near the base of the stem, with up to 8 glabrous orange flowers, and magenta glabrous calyx that covers 2/3 of the corolla. *B. santaclarensis* is similar to *B. membranacea* C.V. Morton in their foliage, their flowers with membranous and glabrous calyx, and their slightly gibbous and glabrous corolla tube. *B. membranacea* is endemic to Peru, particularly to the Amazon and the eastern foothills of the Andes, in the Departments of Loreto and San Martín, mainly in the basin of the Huallaga and Aguaytía rivers, between 260 and 880 m of elevation (Salinas and León 2006). *B. santaclarensis* differs from *B. membranacea* by shorter petioles (1.6–4.9 cm vs. (3.2–)6.5–11.6 cm), inflorescences with up to 8 flowers (vs. up to 4), shorter pedicels (8.6–17.1 mm vs. 13.1–20 mm), calyx base truncate to cordate (vs. never cordate), larger calyx lobes (9.0–13.0 × 4.3–10.6 mm vs. 5.8–9.3 × 1.6–4.7 mm) that cover up to 2/3 of the corolla (vs. covering up to 1/2 of the corolla), and corolla tubes shorter (14.4–15.4 mm vs. 14.9–18 mm long) and orange (vs. yellow and white). Additionally, *B. santaclarensis* is similar to an undescribed species collected in the Province of Zamora-Chinchipe, Ecuador, by Dr. John Clark (*Clark et al. 10815*). The two species share the inflorescences with several orange flowers with magenta ovate calyx lobes, but differ in that *B. santaclarensis* has glabrous calyx (vs. pilose) and slightly gibbous and glabrous corolla tube (vs. gibbous and pilose).

With the discovery and description of *B. santaclarensis* we aim to contribute to the floristic knowledge of this underexplored rainforest in southwestern Colombia, and to

provide new information to support the future expansion of the Reserve that will warrant the conservation of this and many other species.

Additional specimens examined (paratypes). COLOMBIA. Cauca: municipio Argelia, corregimiento Santa Clara, vereda El Pinche, zona aledaña a la Reserva Forestal Protectora Regional “Serranía El Pinche”, flanco oriental del Cerro Pinche, camino entre el Plateado y Guapi, en zona cercana a cultivos y pasturas con algunos relictos de bosques secundarios y riparios de la quebrada El Pinche, 2°28.809'N, 77°18.014'W, 1475 m. 11 Feb 2018 (fl, fr). *Jhon Alexander Sánchez-Taborda, Fernando Joaquín and Andrés Pérez* 2938 (JAUM).

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From chaos to order: the life history of *Hannaea inaequidentata* (Lagerstedt) Genkal and Kharitonov (Bacillariophyta), from initial cells to vegetative cells

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Abstract

This study presents observations on three species of *Hannaea* and documents and illustrates the life history of *H. inaequidentata*. We have divided the life history of *H. inaequidentata* into the following four series of successive stages: auxospore, initial cell, pre-normal vegetative cell, and normal vegetative cell. The initial cell has a cylinder-like and a frequently twisted outline, a longitudinal perizonium wholly covering the valve surface, and a disc-shaped incunabular scale, but lacks any transverse perizonium bands. The pre-normal vegetative cell cannot form ribbon-like colonies, has a wide variety of irregular outlines and is composed of two cell types: one with its epivalve composed of either the initial epivalve or the initial hypovalve, its hypovalve being newly formed, the other with both its epivalve and hypovalve newly formed. The normal vegetative cell has a regular outline and exhibits a significant length reduction so that the largest valve is at least four times longer than the smallest. From initial cell to normal vegetative cell, the developmental sequence goes from ‘chaos to order’ as happens in many phenomena in the universe. The lack of transverse perizonium bands may be the cause of the initial ‘chaos’ process during its developing period from the initial cell to the normal vegetative cell. The development of frustule/valve shape, central area, sternum, virga, vimine, rimoportula and ocellulimbus etc. during the life circle is summarised. In the genus *Hannaea*, some taxa lack the strongly buttressed central area as in *H. inaequidentata*, which also has almost parallel valve margins.

Keywords

Buttressed central area, initial cell, perizonium, pre-normal vegetative cell, uniparental initial valve

Introduction

The diatom genus *Hannaea* R.M. Patrick (in Patrick and Reimer 1966, p. 131) was first used to accommodate *Ceratoneis arcus* (Ehrenberg) Kützinger (1844, p. 104) and *Ceratoneis arcus* var. *amphioxys* (Rabenhorst) R.M. Patrick (in Patrick and Reimer 1966, p. 133), the former being the type of the genus: *Hannaea arcus* (Ehrenberg) R.M. Patrick (in Patrick and Reimer 1966). The need for the new name *Hannaea* in place of *Ceratoneis* Ehrenberg has been fully explained in Medlin and Mann (2007) and Van de Vijver and Ector (2020). *Hannaea* has recently been characterised as having valves “asymmetrical to the apical axis, usually with a small, unornamented tumid area on one side of the center of the valve” (Liu et al. 2019). Since the first use of the name *Hannaea* (as opposed to the name *Ceratoneis*), a number of species (and supra-specific taxa) have been added to the genus (ca. 13 in all, excluding varieties etc., see Liu et al. 2019) that fall into roughly four groups: (1) valves having uniseriate striae and a single rimoportula; (2) valves having biseriate striae and a rimoportula at both apices; (3) valves with poorly developed asymmetry to the apical axis; and (4) valves with (almost) parallel margins. Liu et al. (2019) note that from this diversity “two distinct groups can be identified [...]”: one with uniseriate striae and a single rimoportula, the other with biseriate striae and two rimoportulae, one at each pole. It is not yet clear if either of these “two distinct groups” are monophyletic, if they are each other’s closest relatives (hence the genus being monophyletic), or if one or another of these two sub-groups is related to species outside the genus – Liu et al. (2019) suggest that the problem is worthy of investigation.

This study offers a contribution to further that investigation. We primarily focus on specimens identified as *Hannaea inaequidentata* (Lagerstedt) Genkal and Khartitov (2008), a species with valves having almost parallel margins rather than the usual arcuate shape associated with many species of *Hannaea*. In an effort to understand the morphology and to help resolve the general relationships of *Hannaea*, this paper presents details on the entire life cycle of *H. inaequidentata*.

At present, very little is known of ‘araphid’ diatom life cycles and their ontogeny (reviews in Kaczmarska et al. 2001 and Kaczmarska et al. 2013, see Jewson and Bixby 2016). Herein, we document the morphological changes observed in the transition from what has been termed post-auxospore cells to the ‘normal’ vegetative cells, noting the changes in particular features of the valve during development. For comparative purposes we include brief descriptions of specimens identified as *Hannaea* cf. *arcus* and *Hannaea* cf. *baicalensis* (*Hannaea baicalensis* Genkal, Popovskaya & Kulikovskiy, 2008). The latter is possibly a new species from Lake Baikal, Siberia (see Williams 2019); the identity of the former remains uncertain. Both are used here simply as examples of the variation in *Hannaea*.

Material and methods

The diatom samples were collected from three different regions that are some distance from each other. The samples for *Hannaea* cf. *arcus* were collected from a tributary of

the Datong River in Qinghai province of China in August 2018. The specific sampling site is in Bazha town, Huzhu County, Qinghai province, its coordinates are 37.03684°N and 102.415849°E with an elevation of 2801 m a.s.l. Temperature, pH, and conductivity were measured in situ with a portable multimeter (HQ40D, HACH Company): pH = 8.92 ± 0.02 , conductivity = 230.6 ± 0.1 $\mu\text{S}/\text{cm}$, temperature = 15.4 ± 0.1 °C.

The samples of *Hannaea inaequidentata* were collected from Heiwan River at the foot of Fanjing Mountain in Guizhou province of China in December 2015. The specific sampling site is beside Longquan Temple which is within the Fanjing Mountain National Nature Reserve, Jiangkou County, Guizhou province. Its coordinates are 27.860093°N and 108.764229°E with an elevation of 532 m a.s.l. Temperature, pH, and conductivity were measured in situ with a portable multimeter (HQ40D, HACH Company): pH = 7.7 ± 0.1 , conductivity = 49.7 ± 0.2 $\mu\text{S}/\text{cm}$, temperature = 9.4 ± 0.1 °C.

The samples for *Hannaea* cf. *baicalensis* were collected from Lake Baikal, Siberia, as part of a Darwin Initiative (DI) project (Flower and Williams 1999; see <http://www.geog.ucl.ac.uk/ecrc/enclosed/dardata.htm>). Duplicate materials for the DI Lake Baikal collections are located in CAS (California Academy of Science), E (Royal Botanical Gardens, Edinburgh), Minsk (Laboratory of Quaternary Geology, Minsk, Belarus) and the Limnological Institute, Irkutsk, Russia.

The samples from China were scraped from stone surfaces using toothbrushes, then washed into 100 ml sampling bottles and fixed with 70% ethanol. Permanent slide preparation, light microscopy observation, and scanning electron microscopy observation follow Liu et al. (2020). A similar protocol was used for the Baikal samples.

Terminology and abbreviations

Valve morphology: We mostly follow Ross et al. (1979) and Cox and Ross (1981) for valve structure terminology and Williams (1985) for girdle band terminology. With respect to the valve central area (the “unornamented tumid area” of Liu et al. 2019, the “unilateral inflation” of Bixby et al. 2005, and other descriptions), which is of some significance for species in the genus *Hannaea*, we follow and comment upon Bixby et al. (2005).

Life cycles: We have mostly followed Kaczmarska et al. (2013, and, to a lesser extent, its precursor, Kaczmarska et al. 2001) for life cycle terminology. We introduce a few new terms that allow more precise documentation of the various stages observed in *Hannaea inaequidentata*. Below we refer to the vegetative stages during which the cells exhibit regular shapes as ‘normal’, hence ‘normal vegetative cells’. In this sense, certain ‘pre-normal cells’ can be identified.

Pre-normal vegetative period: The time between immediately after the initial cell’s first division and the presence of the first new normal vegetative cells. The cell, frustule, and valve occurring during this period can be termed ‘pre-normal vegetative cell, frustule, and valve’. Kaczmarska et al. stated that “It is often convenient to refer to the first few mitotic generations of cells produced by division of the initial cell as **post-initial cells**” (Kaczmarska et al. 2013, p. 266). Post-initial cells will include normal vegetative cells, so using the term ‘pre-normal vegetative period’ divides the life history into the

following series of successive stages: auxospore, initial cell, pre-normal vegetative cell, and normal vegetative cell.

Uniparental initial valve period: The time between the first-generation valve from the initial cell and the termination of initial valves' division. There are two types of frustule: one is composed of an initial epivalve and a non-initial hypovalve (the newly formed valve), the other is of one initial hypovalve (as epivalve in the first-generation frustule) and a non-initial hypovalve (the newly formed valve). Both the structure of the initial epivalve and initial hypovalve can be maintained for a few generations.

Standard abbreviations have been used throughout, e.g., LM = light microscopy; SEM = scanning electron microscopy, etc. Other abbreviations used in the text and figures are: Ev = epivalve; Hv = hypovalve; B1 = valvocopula; B2 = second band, copula; B3 = third band, copula; B4 = fourth band, copula; NB3 = new-born third band for hypovalve; NB4 = new-born fourth band for hypovalve.

Author names follow the International Plant Names Index (IPNI) (<https://www.ipni.org/>), herbarium names follow the Index Herbarium (<http://sweetgum.nybg.org/science/ih/>).

Results

Hannaea cf. *arcus*

Figs 1–7, 10–21

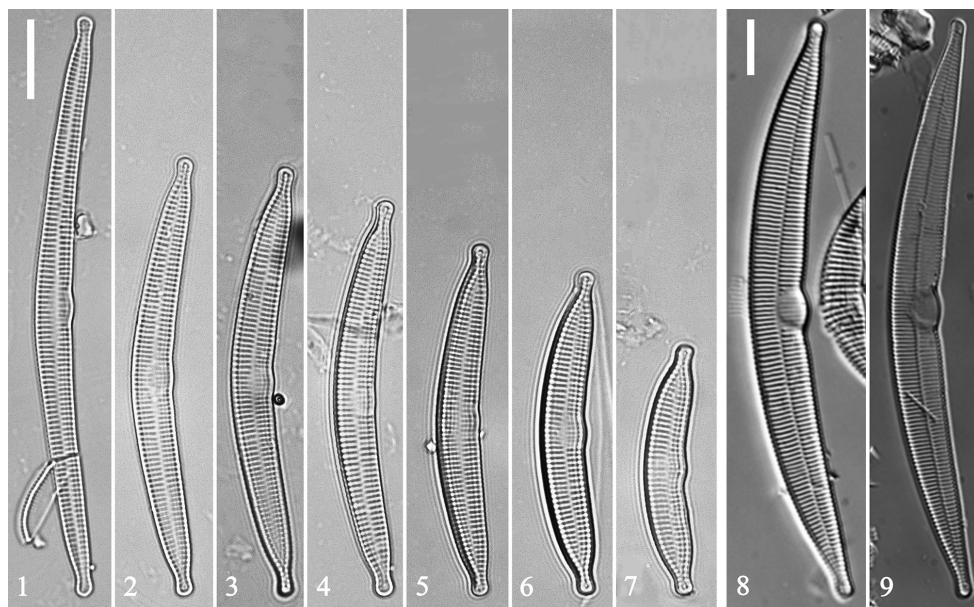
Observation: LM: Valves gently arcuate, with capitate apices (Figs 1–7). Valve dimensions ($n = 31$): length 31–85 μm , width 4.5–7.5 μm ; striae almost uniform until reaching poles. Central area as swelling on ventral side, reaching sternum, with faint ghost striae. Sternum narrow, linear. Striae mostly alternate, parallel, stria density 15–17 in 10 μm .

SEM: Virgae slightly raised, wide, vimines much smaller, evenly spaced (Figs 10–21); spines present, situated at valve margin, between two adjacent virgae, interrupting striae (Figs 10–13). Striae uniseriate, with round or oblong areolae. Central area tumid, with transversely raised virgae, faint ghost striae, lacking buttressing (Figs 14, 15). Sternum central, linear. One rimoportula per valve, with paired lips, situated at apex (Figs 16, 17); ocellulimbus located on valve margin at each pole, composed of vertical rows of ca. 5–10 poroids. Valvocopula open, same shape as valve, closely attached to mantle interior, surrounding valve margin (Figs 18, 21). Each valvocopula with single row of poroids bisecting pars interior and exterior, located at mid-line (Figs 19–21). Valvocopula with sawtooth-shaped projections attached to valve, internally visible over each virga (Figs 19, 20, arrows).

Hannaea cf. *baicalensis*

Figs 8, 9, 22–34

Observation: LM: Valves arcuate, with capitate to sub-capitate apices (Figs 8, 9). Valve 40–150 μm long, 4–12 μm wide. Striae almost uniform until reaching poles. Central area as



Figures 1–9. *Hannaea cf. arcus* and *Hannaea cf. baicalensis*, LM **1–7** seven valves showing valve size diminution series for *Hannaea cf. arcus* **8, 9** two valves of *Hannaea cf. baicalensis*. Scale bar: 10 μm (**1, 8**).

definite swelling on ventral side, reaching sternum, ghost striae just beyond. Sternum narrow, linear, well-defined. Striae mostly alternate, parallel, striae density 10–15 in 10 μm .

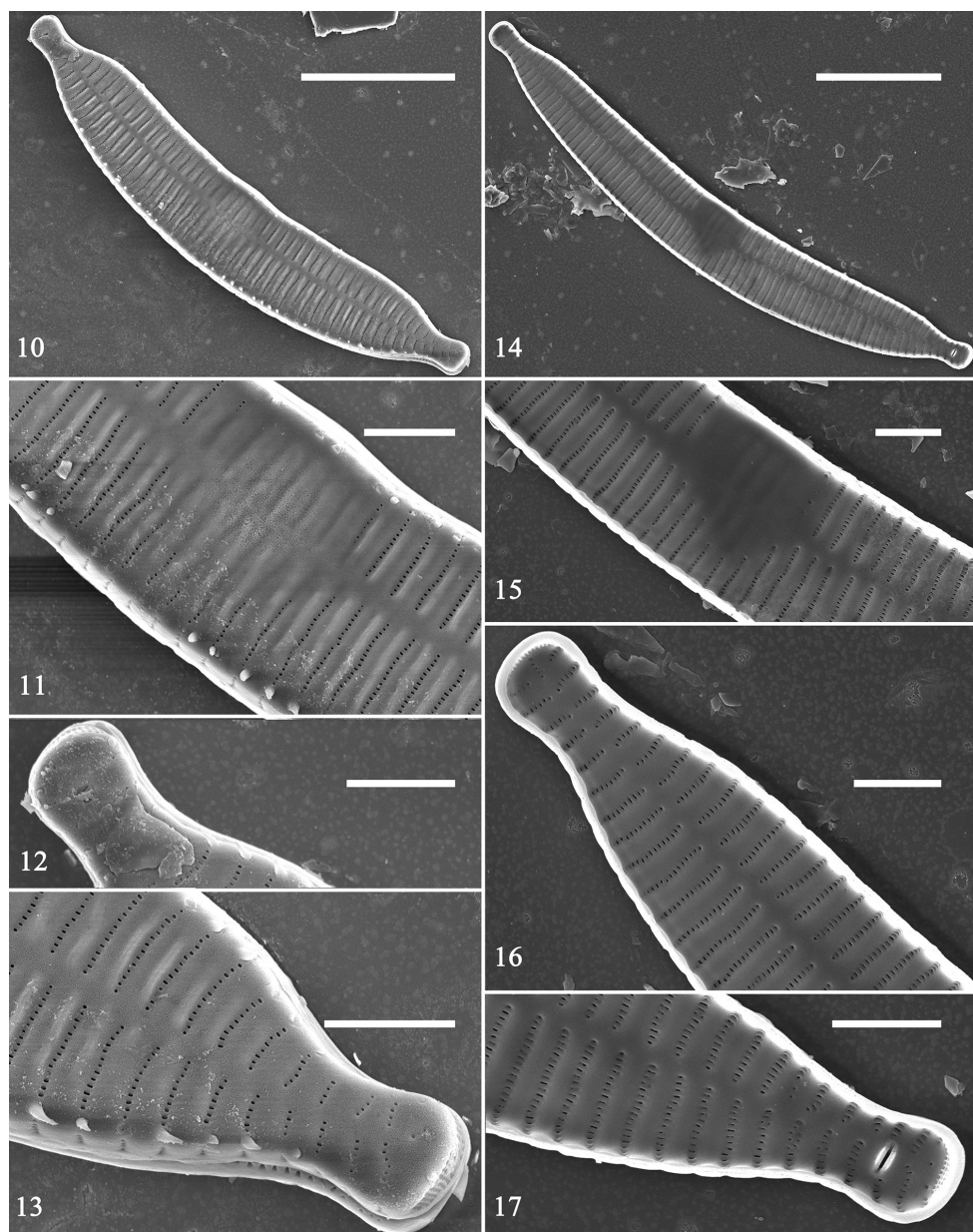
SEM: Virgae raised, relatively wide, vimines much smaller, sunken but evenly spaced (Figs 22–28); spines present as short ill-defined spurs, approaching spatulate, most emanating from vimines (Figs 24, 25, 28). Striae biseriate, with round areolae occurring opposite each other, uniseriate at poles, formed by merging vimines. Central area tumid, with transversely raised virgae, vimines filled in forming ghost striae, buttressing demarcating either side of central area, fusing with sternum (Figs 23, 33). Sternum central, linear, level with virgae. Rimoportula at each pole, two per valve, each with simple paired lips (Fig. 27); ocellulimbus well-developed, located on valve margin at each pole, composed of parallel vertical rows of ca. 15–20 poroids (Figs 29, 34). Cingulum composed of simple open bands, possibly four per valve, single row of poroids either side of pars media, only par exterior (usually) visible (Figs 29, 31, 34). Valvocopula with fringed edge to affix internal portion of valve (Figs 30, 32). All bands similar structure, lacking pleurae.

Hannaea inaequidentata

Figs 35–136

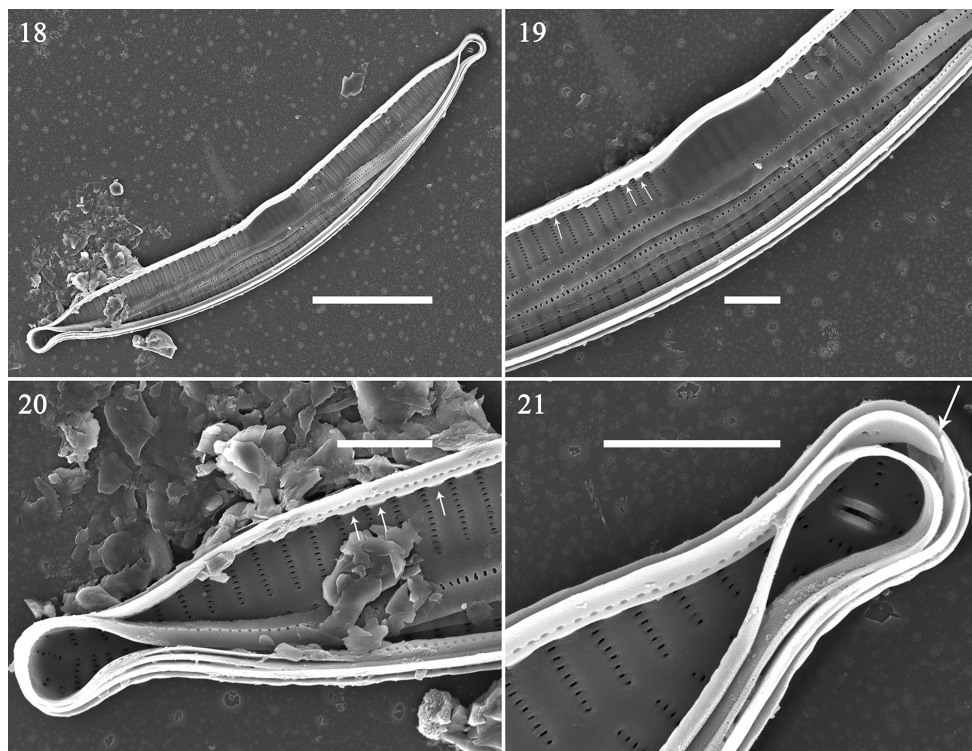
Observation: *Normal vegetative colony and frustule* Figs 35–47.

Frustules forming valve face-to-face colonies, via interlocking linking spines (Fig. 35, with three complete frustules, i.e., with epivalve, hypovalve and cingulum; two incomplete; Fig. 36, with two complete frustules). Frustules composed of epivalve,



Figures 10–17. *Hannaea* cf. *arcus*, SEM **10–13** external view of valve, note linking spines and central area **14–17** internal view of valve, note central area lacking buttressing (**15**). Scale bars: 10 μm (**10, 14**), 2 μm (**11–13, 15–17**).

hypo- and cingulum of 6–8 open bands. For each vegetative, but not dividing, frustule, a 4:2 configuration of girdle bands visible (Figs 37–39 indicated by 4 and 2), i.e., four girdle bands visible for epivalve, two for hypo- (Figs 37–39, labelled B1 to B4 and B1 to B2, respectively). For dividing frustule with newly formed hypo-



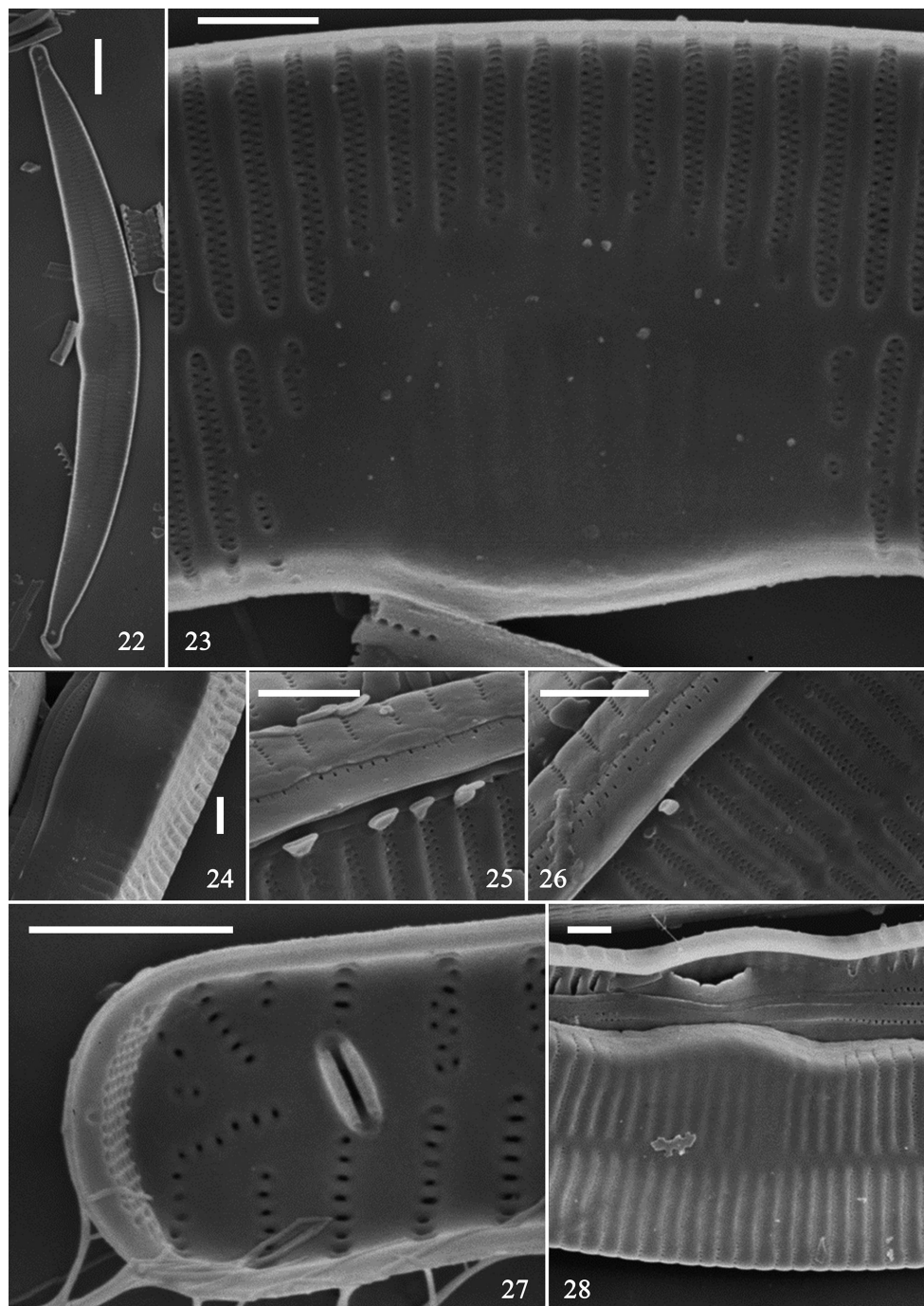
Figures 18–21. *Hannaea* cf. *arcus*, SEM **18** complete valve with valvocopula. **19–21** details of Fig. 18, note valvocopula with sawtooth-shaped projections attached to valve (**19, 20**, arrows), valvocopula open at one pole (**21**, arrow). Scale bars: 10 μ m (**18**), 2 μ m (**19–21**).

occurring (Figs 38, 39, two arrows for two new hypovalves), a 4:4 configuration of girdle bands (Figs 38, 39, indicated by 4 and 4), i.e., four girdle bands visible for epivalve and four (with two newly added bands, Figs 38, 39 labelled NB3 and NB4) for hypovalve (Figs 38, 39, upper frustule, labelled B1 to B4 and B1 to NB4). Plaques located at mantle edge, strongly developed, distinct (Fig. 37). Interlocking linking Y-shaped spines (Fig. 37, arrowheads) at valve centre, becoming more acute towards each apex, frustules separate from each other at each apex (Figs 37–39). Girdle bands open, with a row of poroids located at centre line dividing pars interior and exterior (Figs 40–47, Fig. 45, arrow), poroids interrupted at centre (Figs 42, 46, arrow). Valvocopula with crenulated pars interior attaching to valve, internally visible over each virga (Figs 41, 46, arrowheads); copulae with smooth pars interior (Figs 41–43).

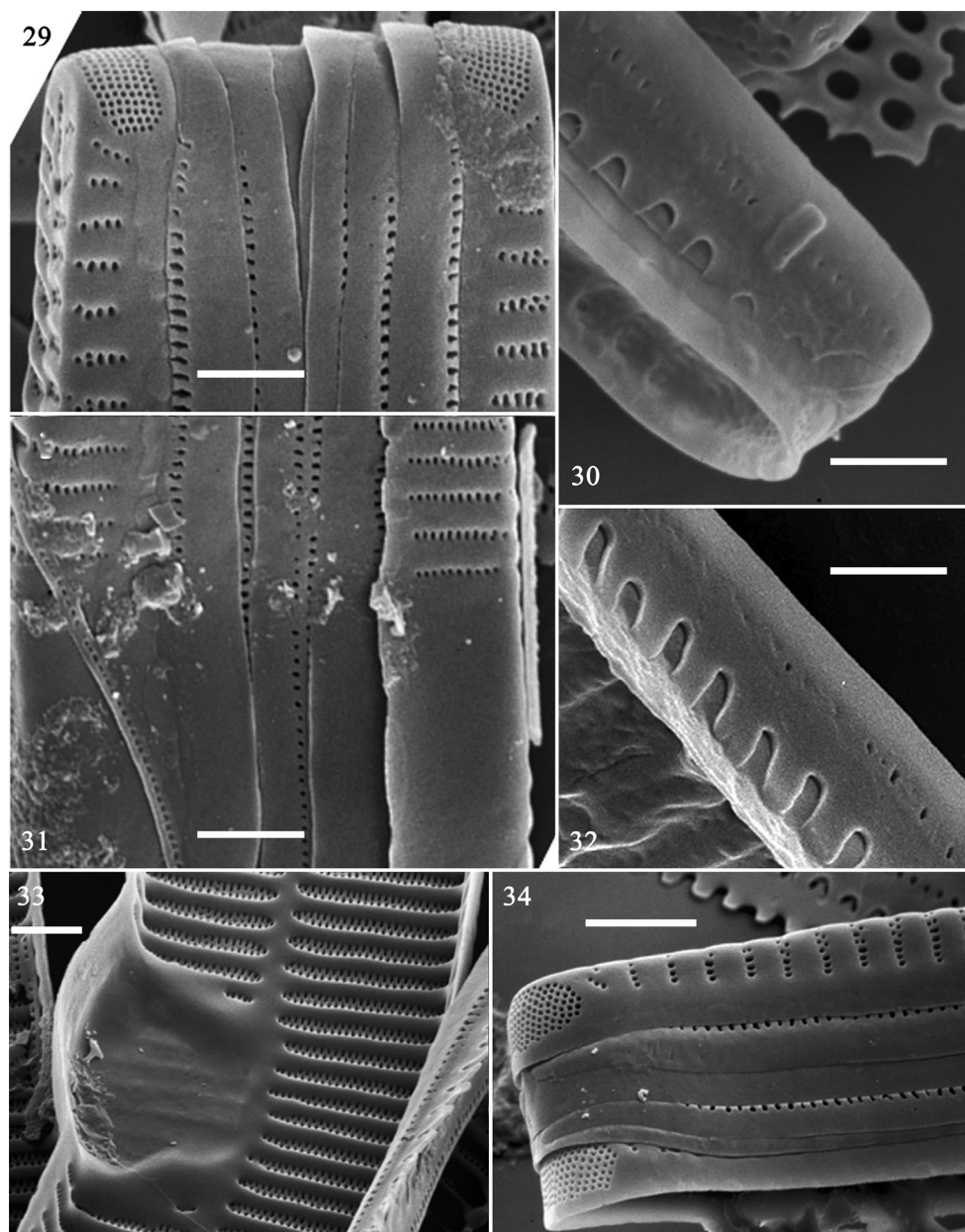
Normal vegetative valve

Figs 48–69

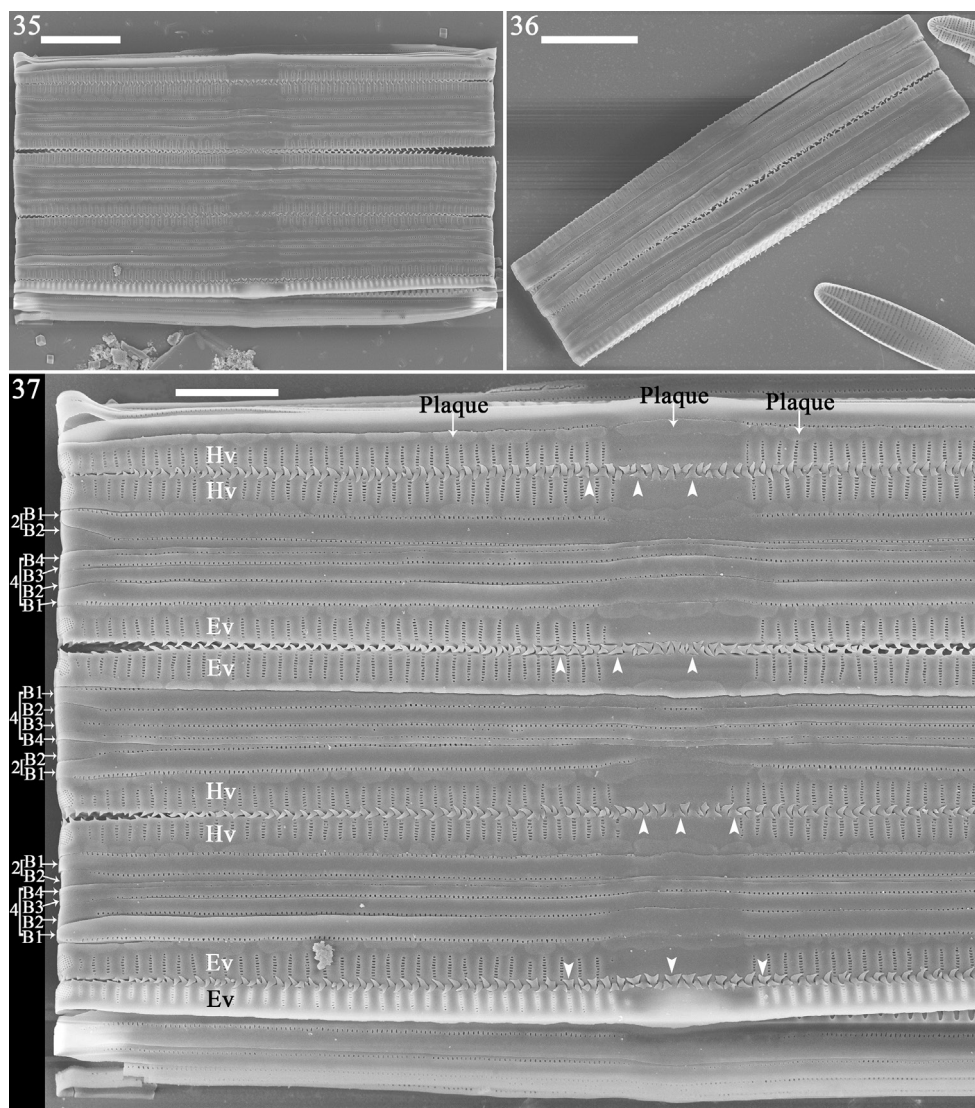
LM: Valves lanceolate, slightly arcuate in larger specimens (Figs 48–55), almost parallel in smaller specimens (Figs 56–59), with capitate to sub-capitate apices. Largest valve



Figures 22–28. *Hannaea* cf. *baicalensis*, SEM. **22** complete valve, internal view, note rimoportula at each pole **23–28** detail of valve structure. Scale bars: 10 µm (**22**), 2 µm (**23–28**).



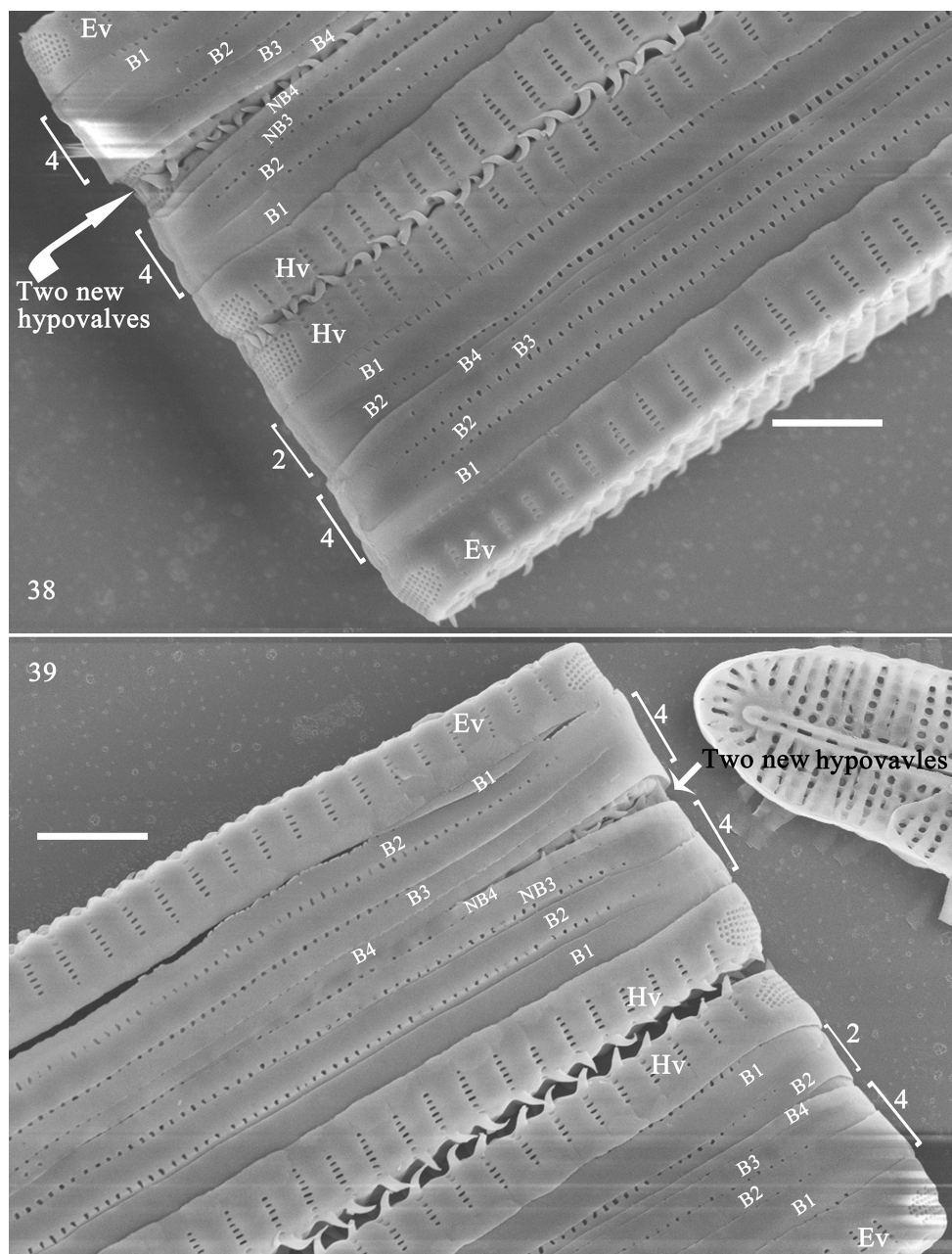
Figures 29–34. *Hannaea cf. baicalensis*, SEM. **29–32, 34** details of cingulum **33** central area of valve, internal view, note buttressed central area. Scale bar: 2 μm (**29–34**).



Figures 35–37. *Hannaea inaequidentata*, girdle view, SEM **35** colony with ca. 5 frustules **36** colony with two frustules **37** detail of Fig. 1, showing epivalves and hypovalves, distinct mantle plaques, fork-shaped interlocked linking spines at valve middle (arrowheads) and more acute spines towards each apex; note 4:2 configuration of girdle bands in three normal but not dividing vegetative frustules. Scale bars: 10 μ m (**35, 36**), 5 μ m (**37**).

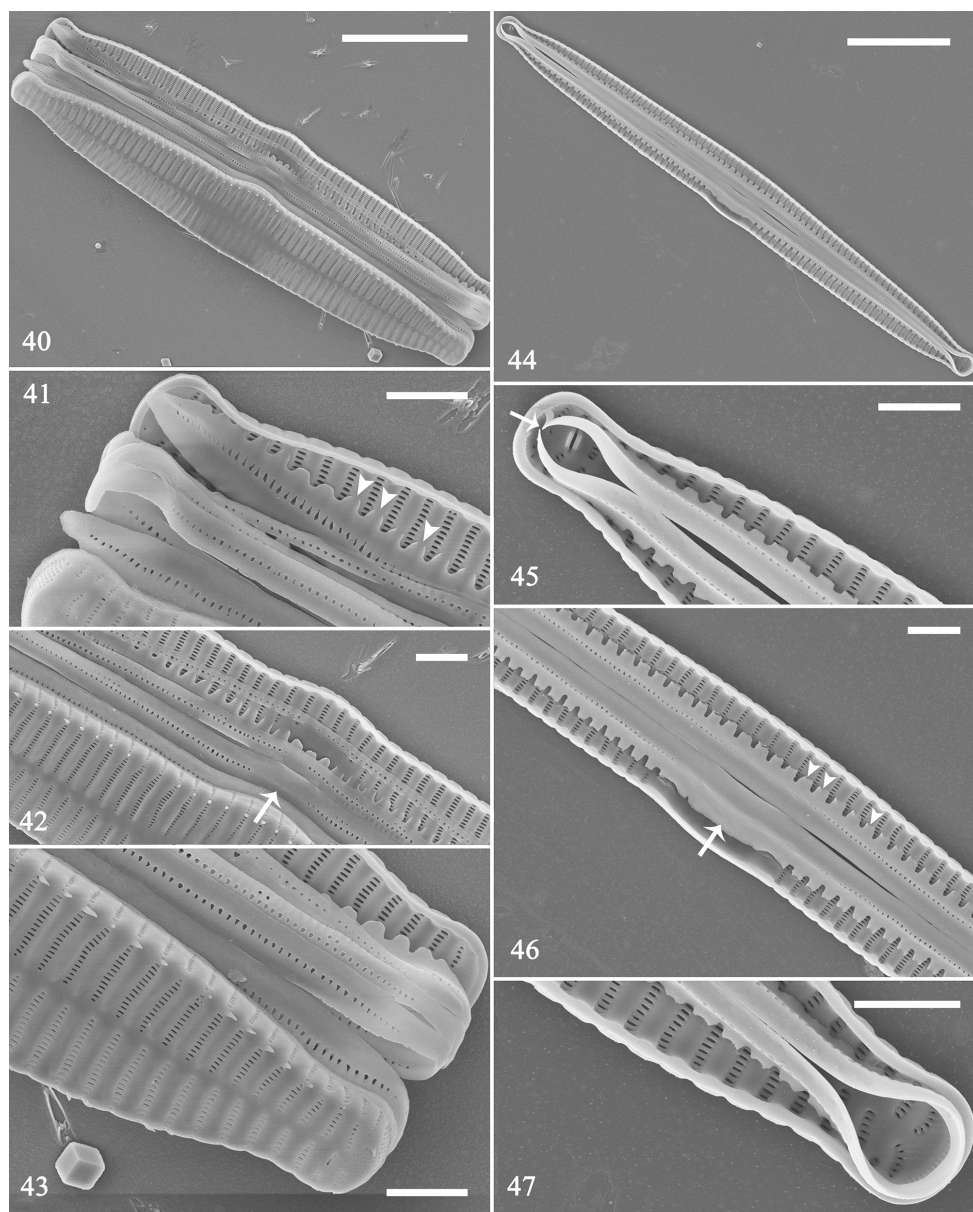
(Fig. 48) four times longer than smallest valve (Fig. 59). Valve dimensions ($n = 44$, Table 1): 24–102 μ m long, 5–7 μ m wide at the centre. Central area as swelling on ventral side with faint ghost striae. Sternum narrow, almost linear. Striae mostly alternate, parallel, except near each apex where slightly radiate, striae density 14–16 in 10 μ m.

SEM: external view: Virgae raised, vimines depressed on valve surface, spines situated along valve face/mantle junction (Figs 60–65). Spines mostly located between



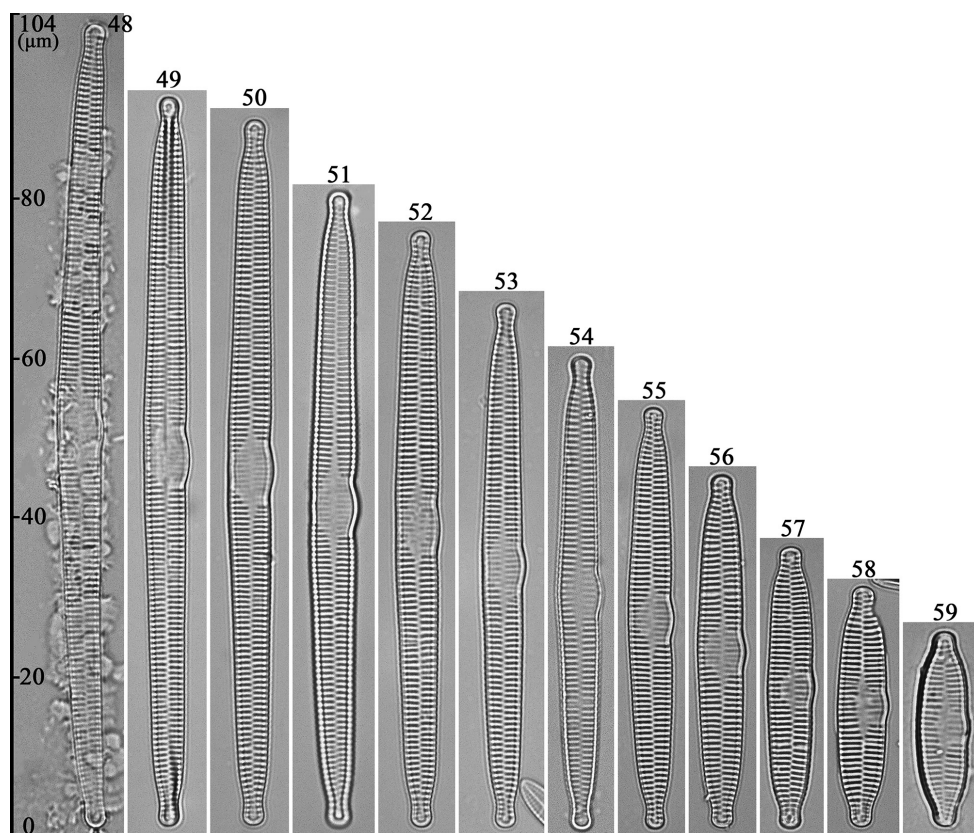
Figures 38, 39. *Hannaea inaequidentata*, girdle view, SEM. **38, 39** details of two apices from Fig. 36, showing epivalves and hypovalves, 4:2 configuration of girdle bands in normal but not dividing vegetative frustule (lower frustule), 4:4 configuration of girdle bands in dividing vegetative frustule (upper frustule); note two new hypovalves (arrows) are interlocked by linking spines. Scale bars: 2 μ m.

two adjacent virgae within vimines, occasionally on virgae (Fig. 61, arrowheads). Central area with transversely raised virgae, with faint ghost striae (Fig. 61). Each valve bearing one rimoportula at apex, in frustule each pole with one rimoportula



Figures 40–47. *Hannaea inaequidentata*, SEM **40–43** frustule details showing open girdle bands, note row of poroids interrupted at centre (**42** arrow; also see Fig. 46, arrow) **44** valve with complete valvocopula **45–47** details of Fig. 44 showing open valvocopula (**45** arrow), sawtooth-shaped projections attached to valve, internally visible over each virga (**46** arrows; also see Fig. 41, arrows). Scale bars: 10 μ m (**40, 44**), 2 μ m (**41–43, 45–47**).

(Figs 62, 63, two arrows, respectively). Striae uniseriate, areolae rounded to slit-like, internally occluded by hymens (Figs 64, 65). Ocellulimbus located under valve face, vertical row of poroids composed of ca. 3–7 poroids, and ocellulimbus surface covers



Figures 48–59. *Hannaea inaequidentata*, normal vegetative valves, LM 48–59 12 valves showing diminution series, note slightly arcuate, lanceolate valve outlines, and largest valve (48) 4× longer than smallest (59).

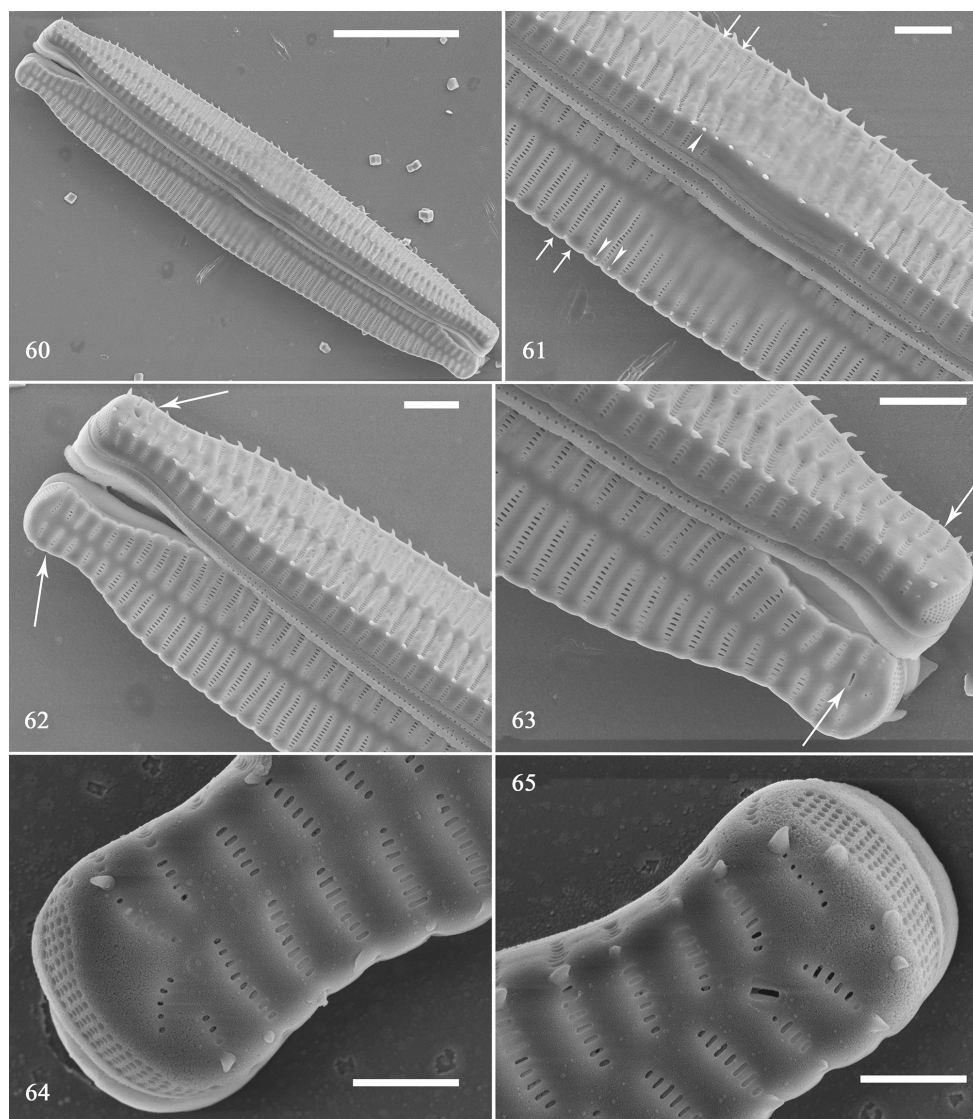
valve polar margin (Figs 64, 65). Internal view: valve slightly arched, lanceolate, sternum central, almost straight (Fig. 66). Central area with swollen valve middle margin, virgae raised, vimines depressed, with no apparent buttressing (Figs 66, 67). Rimoportula as paired lips, striae near each apex slightly radiate (Figs 68, 69).

Initial frustule

Figs 70, 71, 79–96

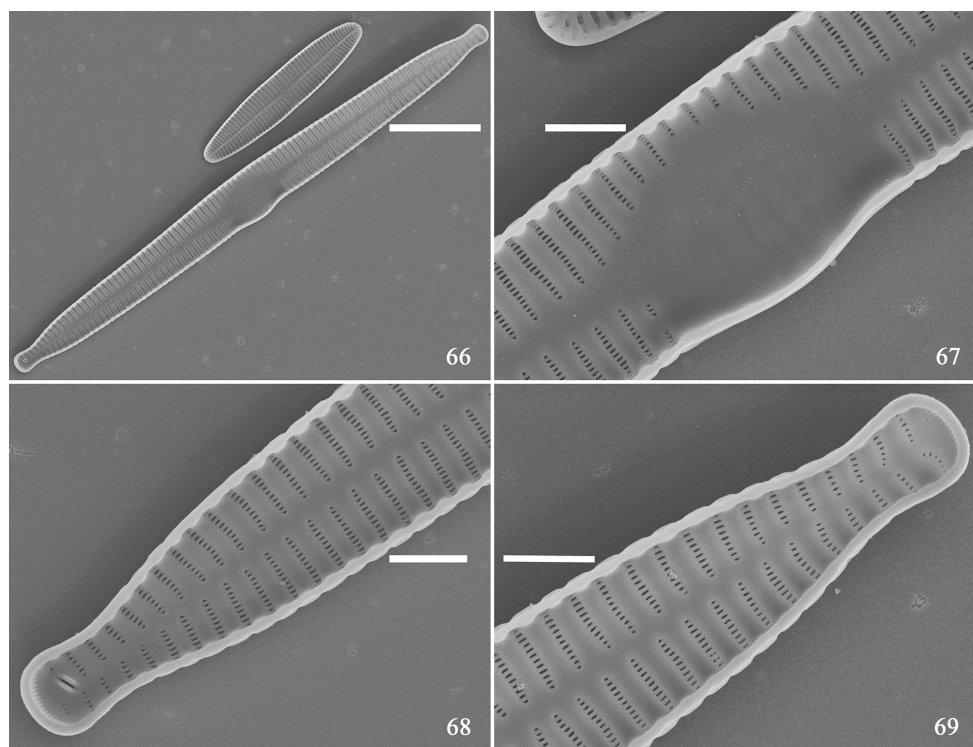
In LM, the initial frustules have either a curved (Fig. 70) or arcuate (Fig. 71) irregular outline. The sternum is present but not entirely obvious, and the valve face is ill-defined (Figs 70, 71). With SEM, three initial frustules are illustrated to document its fine structure.

The first initial frustule is illustrated in Figs 79–84. It is cylindrical and twisted from pole to pole (Fig. 79). The virgae and vimines are almost flush to each other, with the virgae relatively wide with respect to the vimines (Figs 80–84). The central area is an area completely (or almost) silicified, with no appreciable distinction between virgae and vimines; even ghost striae are not evident, nor is a sternum (Fig. 80; Fig. 81, arrow; Fig.



Figures 60–65. *Hannaea inaequidentata*, normal vegetative valves, external view, SEM **60** displaced frustule **61** detail of Fig. 60, showing well-developed virgae and vimines (arrows), spines mostly located between two adjacent virgae, sometimes situated on virgae (arrowheads) **62, 63** apex details of Fig. 62 showing rimoportula configuration in two valves forming a cell: each cell with two rimoportulae, located diagonally at both apices of each cell (two arrows, respectively) **64, 65** another two apices showing a regular ocellulimbus and areolae occluded internally by hymens. Scale bars: 10 µm (**60**), 2 µm (**61–63**), 1 µm (**64, 65**).

83, arrow). There are two girdle bands (Figs 82, 84, labelled B1 and B2); the incunabular scales are disc-shaped, slightly dendritic (cf., “dendroid scales (dendroid spine scales)”, Kaczmarzka et al. 2013, p. 283; see Fig. 83, curved arrow, Fig. 84, arrow). The perizonium plate cannot be detected because it tightly covers the valve surface.

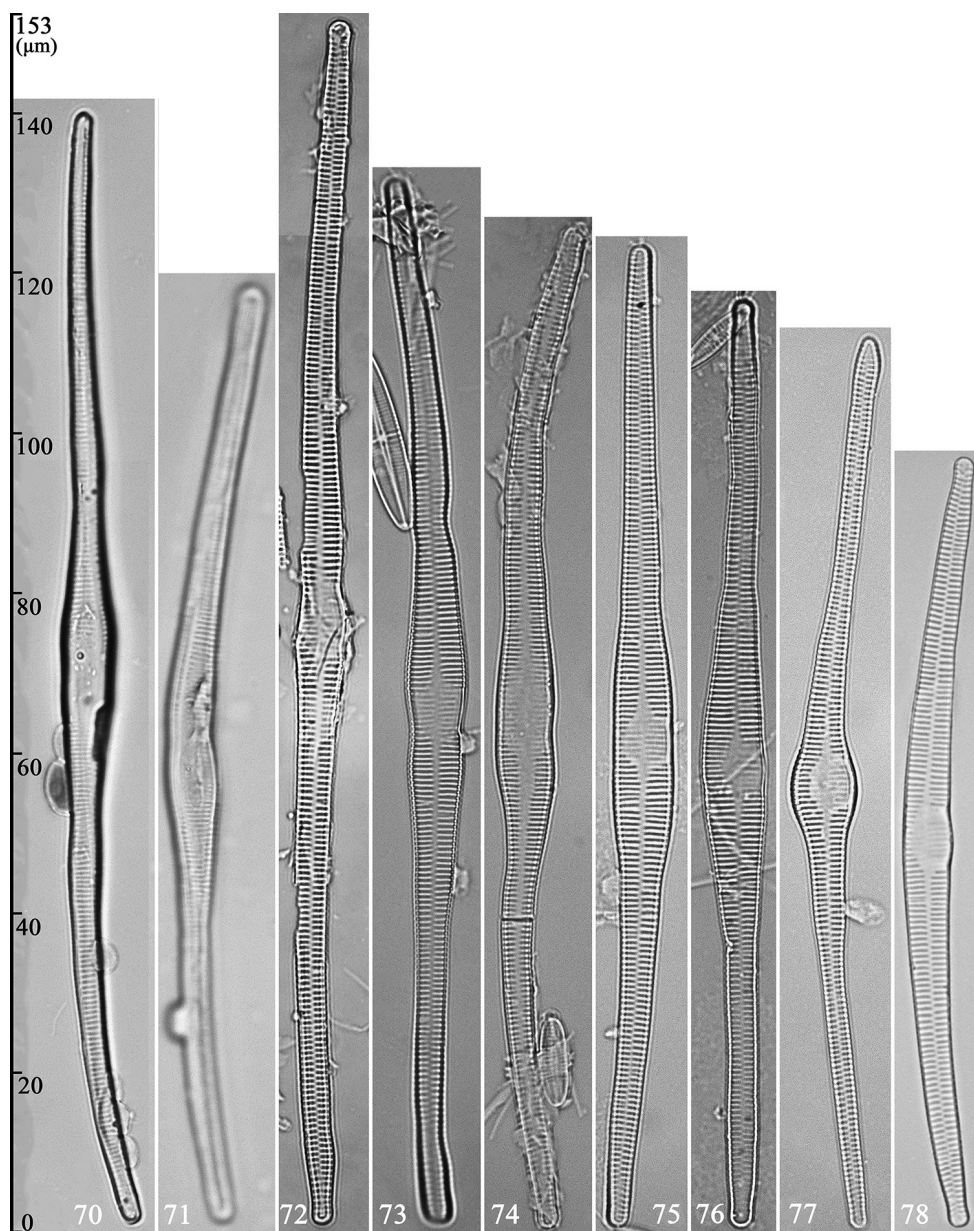


Figures 66–69. *Hannaea inaequidentata*, normal vegetative valve, internal view, SEM **66** complete valve **67** detail of Fig. 66 showing unilateral swollen middle **68, 69** details of Fig. 66 showing regular sternum and radiating striae near each apex. Scale bars: 10 µm (**66**), 2 µm (**67–69**).

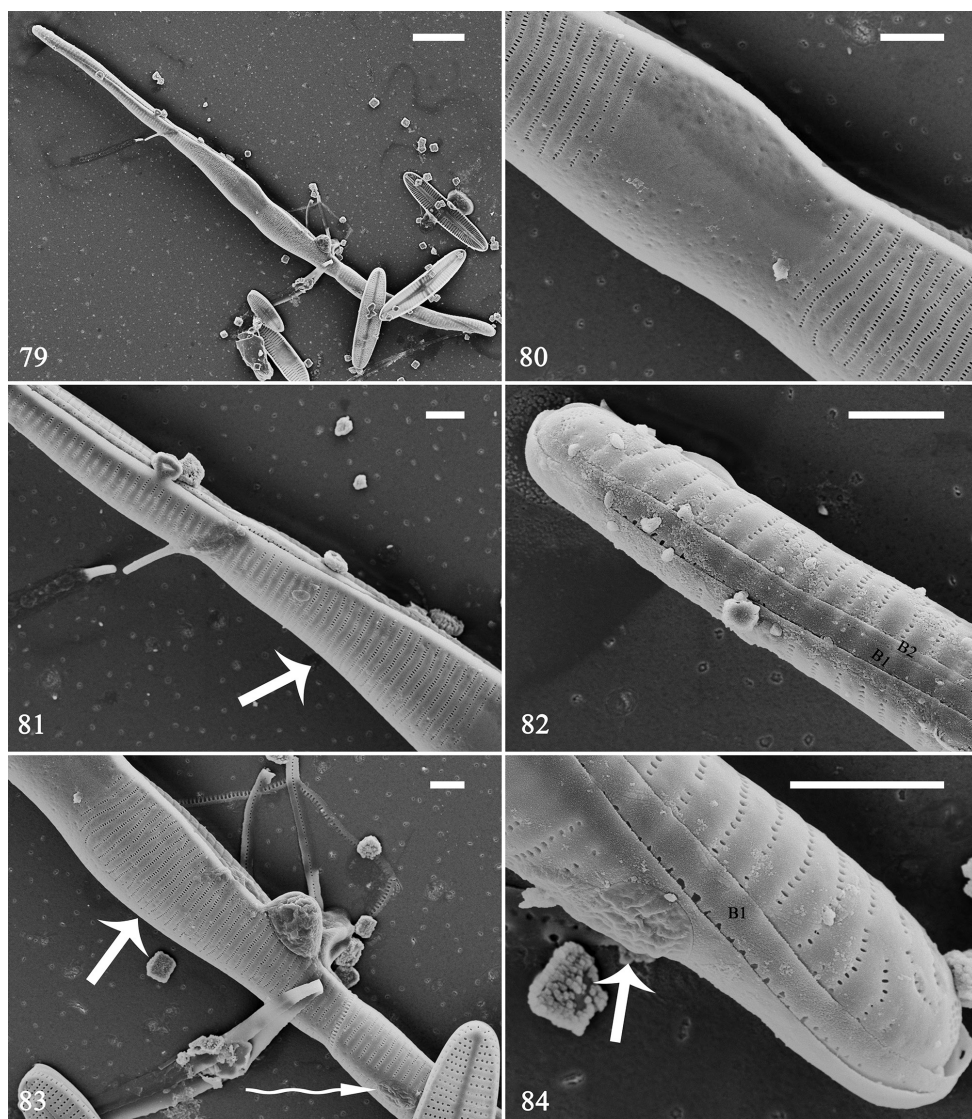
Table 1. Dimensions of initial valves, pre-normal valves and normal valves in *Hannaea inaequidentata*; values are range (mean ± SD).

	Initial valve length (µm) (n = 7)	Pre-normal valve length (µm) (n = 41)	Normal valve length (µm) (n = 44)
<i>H. inaequidentata</i>	107–139 (122 ± 13)	97–151 (119 ± 12)	24–102 (59 ± 19)

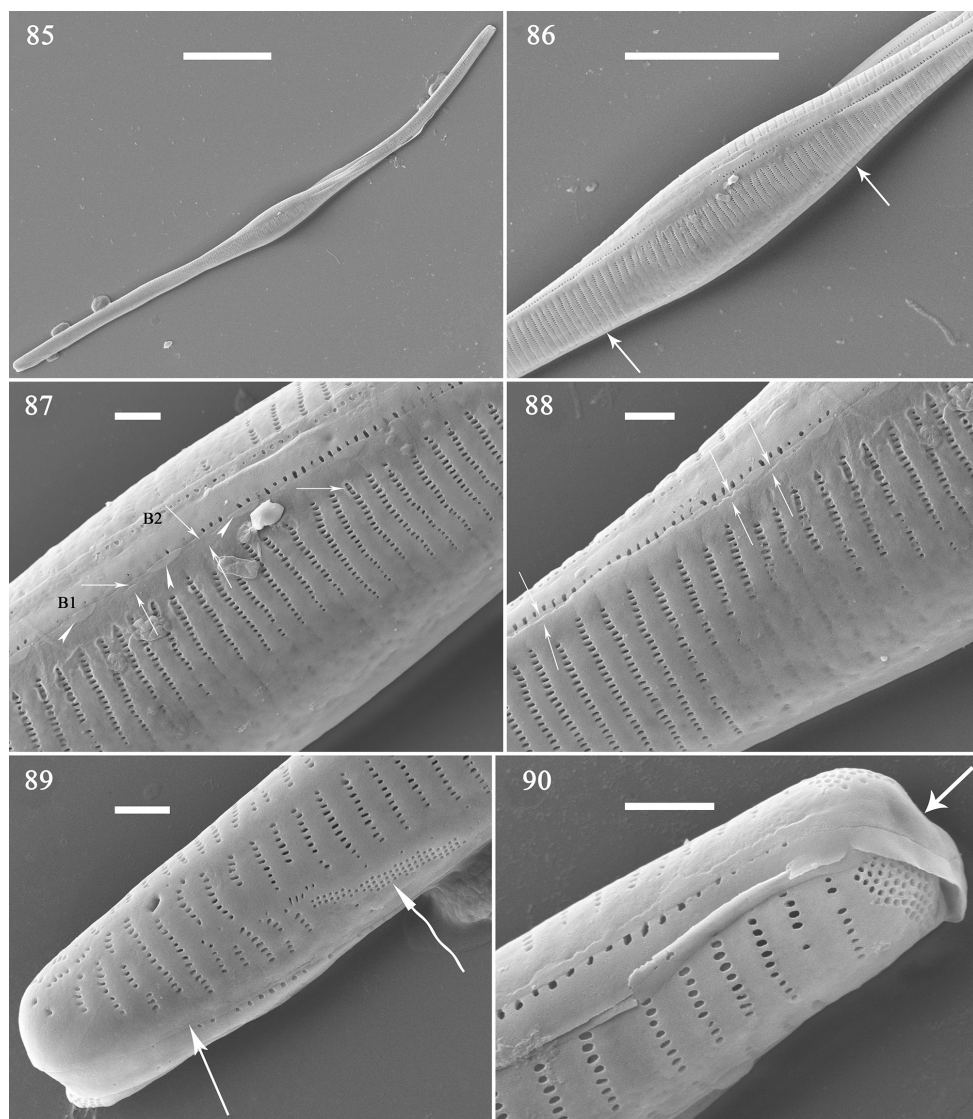
The second initial frustule is illustrated in Figs 85–90. It is cylindrical and twisted (Fig. 85). The central area is an area completely (or almost) silicified, with some noticeable distinction between virgae and vimines; ghost striae are evident, but a sternum is not (Fig. 86, two arrows). The longitudinal perizonium plate covers the valve surface, but no transverse perizonium bands were observed (Figs 87–89, arrows). Plaques are present, more spaced out than on the normal vegetative valves (Fig. 87, arrowheads). There are two girdle bands (Fig. 87, labelled B1 to B2). There is a cluster of small poroids on the valve margin giving the appearance of a pore-field or ocellulimbus (Fig. 89, curved arrow); the ocellulimbus occurs at the poles (Fig. 90). The longitudinal perizonium plate approaches a corrugated appearance at one pole (Fig. 90, arrow).



Figures 70–78. *Hannaea inaequidentata*, initial frustules and pre-normal vegetative valves, LM **70, 71** two initial frustules, note nonexistent (undeveloped) sternum and irregular valve face **72–78** seven pre-normal vegetative valves showing seven irregular valve shapes: almost straight with undulate valve margins (**72**), sigmoid with constricted two middle margins (**73**), double S-shaped with one middle margin constricted (**74**), parallel middle margins with one half of valve straight and the other deflexed (**75**), swollen middle part with almost straight valve (**76**), arcuate with globular middle part (**77**), and nearly normal but distinctly arcuate (**78**).

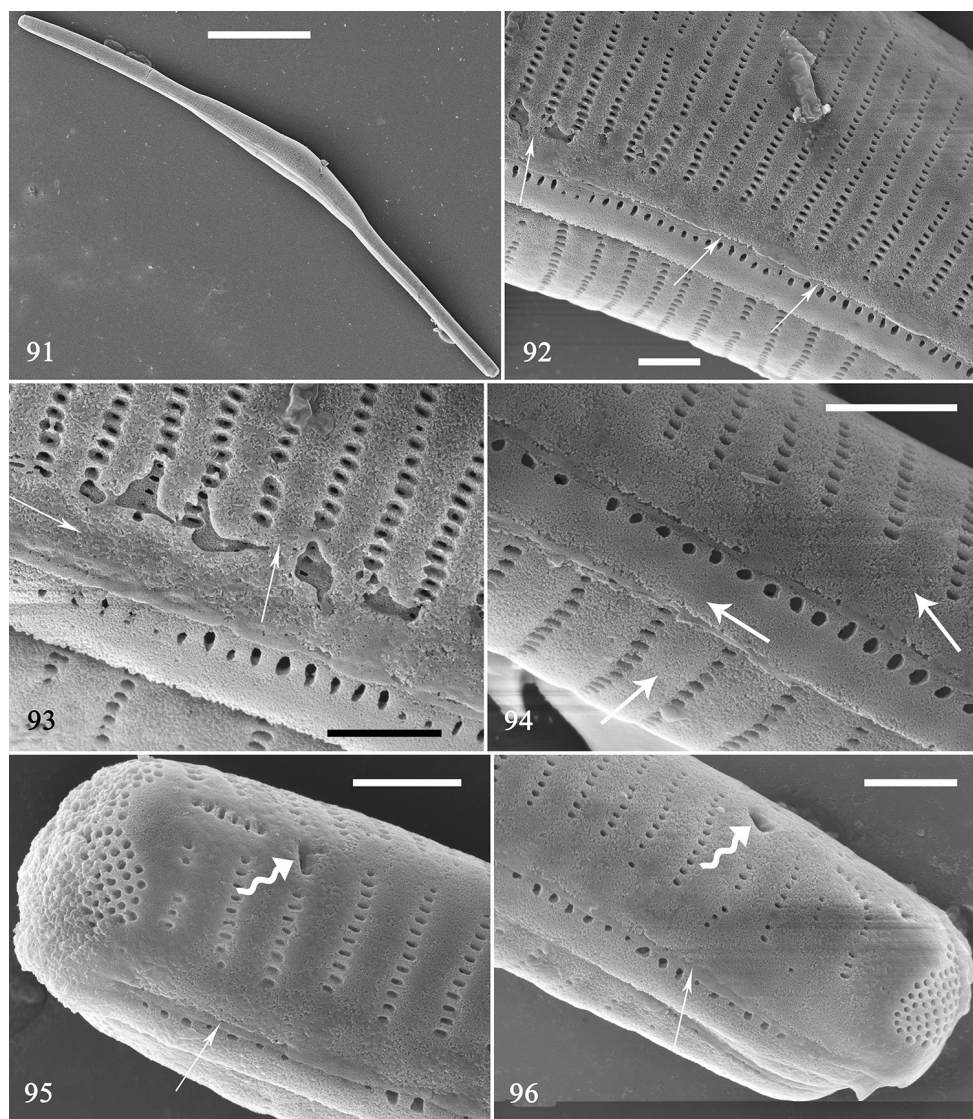


Figures 79–84. *Hannaea inaequidentata*, an initial frustule, external view, SEM **79** complete initial frustule, note its rounded, cylinder-like, twisted outline **80** middle detail of Fig. 79, showing central area, sternum not developed (i.e. striae continue across valve surface, also see **81**, **83**, arrow), longitudinal perizonium wholly covering valve surface, no transverse perizonium bands (also see Figs 85–106) **81** detail of Fig. 79 showing sternum not developed (arrow) **82** apex detail of Fig. 79 showing two girdle bands for this initial frustule, sternum not developed. **83**, **84** details of Fig. 79 showing two disc-shaped incunabular scales with cerebral-cortex-like surfaces (**83** curved arrow; **84** arrow). Scale bars: 10 μm (**79**), 2 μm (**80–84**).



Figures 85–90. *Hanaaea inaequidentata*, an initial frustule, external view, SEM **85** complete initial frustule, note its rounded, cylinder-like, twisted outline **86** middle detail of Fig. 85, showing central area, sternum not developed (two arrows), longitudinal perizonium wholly covering valve surface, no transverse perizonium bands **87** detail of Fig. 85 showing longitudinal perizonium (arrows), plaques (arrowheads), and two girdle bands **88** detail of Fig. 86 showing longitudinal perizonium (arrows) **89** apex detail of Fig. 85 showing longitudinal perizonium (arrow) and irregular ocellulimbus located in valve margin (curved arrow) **90** another apex of Fig. 85, note depressed pole (arrow). Scale bars: 20 µm (**85**), 10 µm (**52**), 1 µm (**86–90**).

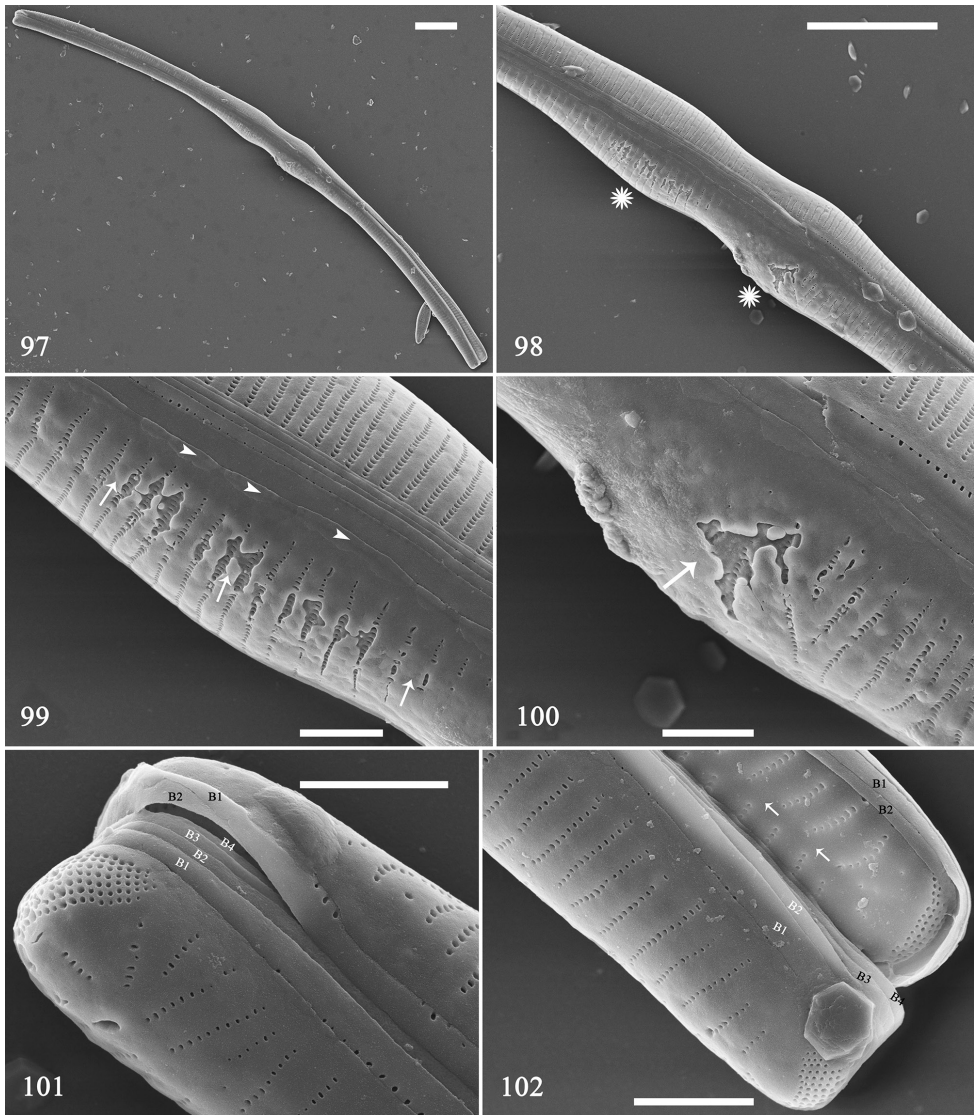
The third initial frustule is illustrated in Figs 91–96. It is cylindrical and slightly twisted (Fig. 91). The virgae and vimines are almost flush to each other and the longitudinal perizonium plate can be observed from the centre to the pole (Figs 92–96,



Figures 91–96. *Hannaea inaequidentata*, an initial frustule, external view, SEM **91** complete initial frustule, note its rounded outline **92** detail of Fig. 90, showing longitudinal perizonium wholly covering valve surface, no transverse perizonium bands (arrows) **93** detail of Fig. 90 showing longitudinal perizonium (arrows) **94** detail of Fig. 91 showing longitudinal perizonium on two valves and one girdle band (arrows) **95, 96** two apex details of Fig. 91 showing longitudinal perizonium (arrows), irregular ocellulimbus, and two rimoportulae per valve (curved arrows). Scale bars: 20 μm (**91**), 1 μm (**92–96**).

arrows): the two valves and one girdle band are all covered by the longitudinal perizonium plate and band (Fig. 92, three arrows). One initial valve has two rimoportulae, one at each pole (Figs 95, 96, two curved arrows).

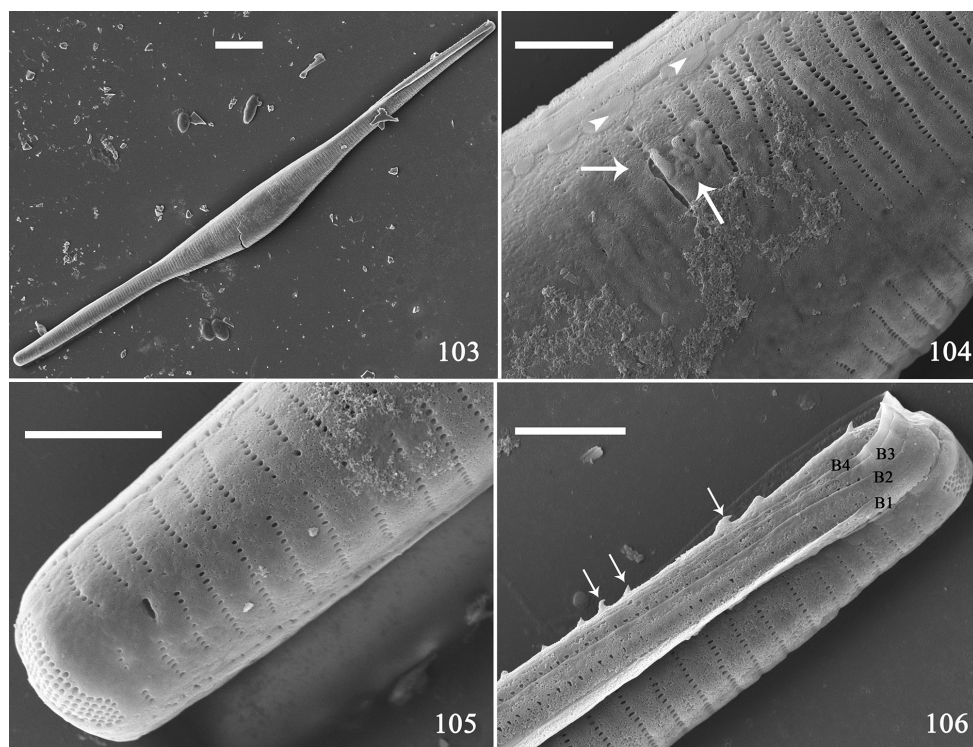
Overall, the three examples illustrate the changes exhibited from a relatively disorganised structure to a more conventional and regular vegetative valve (see Table 2).



Figures 97–102. *Hanaea inaequidentata*, dividing half mother frustule, external view, SEM **97** slightly displaced half mother frustule, note its rounded outline **98** middle detail of Fig. 97 showing broken longitudinal perizonium **99**, **100** details of Fig. 98 (two asterisks) showing broken longitudinal perizonium (arrows) and distinctive plaques (arrowheads) **101** apex detail of Fig. 97 showing irregular ocellulimbus and 4:2 configuration of girdle bands **102** another apex detail of Fig. 97 showing 4:2 configuration of girdle bands and a new-born hypovalve with regular sternum (two arrows). Scale bars: 10 μm (**97**, **98**), 2 μm (**99**–**102**).

Pre-normal frustule/valve Figs 72–78, 97–136.

LM: Seven pre-normal vegetative valves are illustrated, each an irregularly shaped valve. Some with almost with parallel margins, one half of the valve linear, the other half deflexed (e.g. Fig. 75), most tapering towards the poles (e.g. Fig. 72); some with



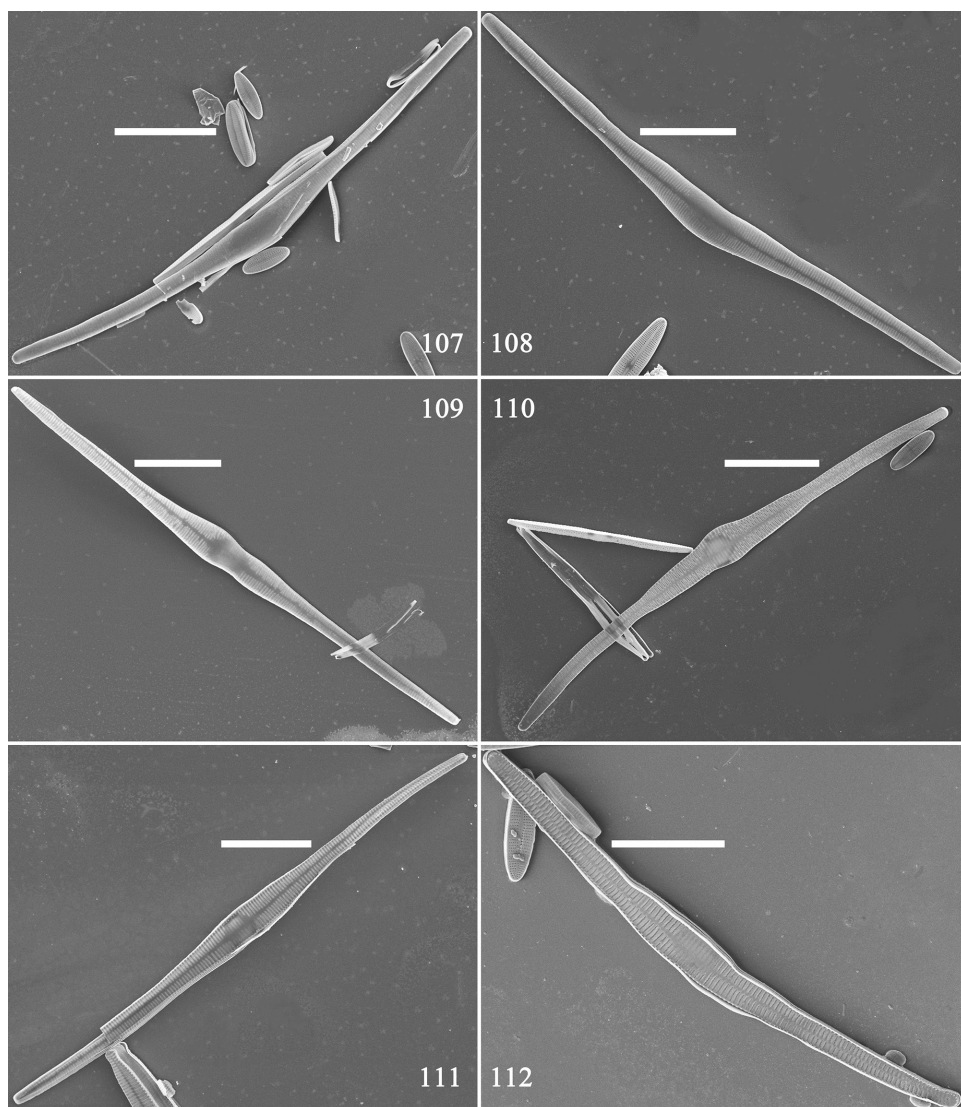
Figures 103–106. *Hannaea inaequidentata*, half mother frustule, external view, SEM **103** half mother frustule, note its rounded outline **104** middle detail of Fig. 103 showing longitudinal perizonium (arrows) and plaques (arrowheads) **105** apex detail of Fig. 103 **106** apex detail of Fig. 103 showing four girdle bands and hypovalve with spines (arrows). Scale bars: 10 μm (**103**), 2 μm (**104–106**).

an undulating appearance (Figs 74, 76), others slightly sigmoid with constrictions at the central margins (Figs 73, 74). Some have an expanded central area on one side of the valve (e.g. Figs 77, 78), others with the central area across the whole valve from margin to margin (e.g. Figs 73–76), some partially across valve surface (e.g. Fig. 77), and others with central area on one side of the valve but having a distinctly arcuate outline (e.g. Fig. 78).

Pre-normal frustule with uniparental initial epivalve

Using SEM, we illustrate two pre-normal frustules with uniparental initial epivalves. The first is illustrated in Figs 97–102. It is cylindrical with a constriction at its centre (Fig. 97). The longitudinal perizonal plate can be observed from centre to pole (Figs 98–102). There are six girdle bands (Figs 101, 102, labelled B1 to B4 and B1 to B2). One new-born hypovalve has a central sternum (Fig. 102, two arrows) and a more conventionally structured ocellulimbus.

The second is illustrated in Figs 103–106. It is cylindrical with an expanded central part (Fig. 103). The longitudinal perizonal plate can be observed (Fig. 104, two ar-

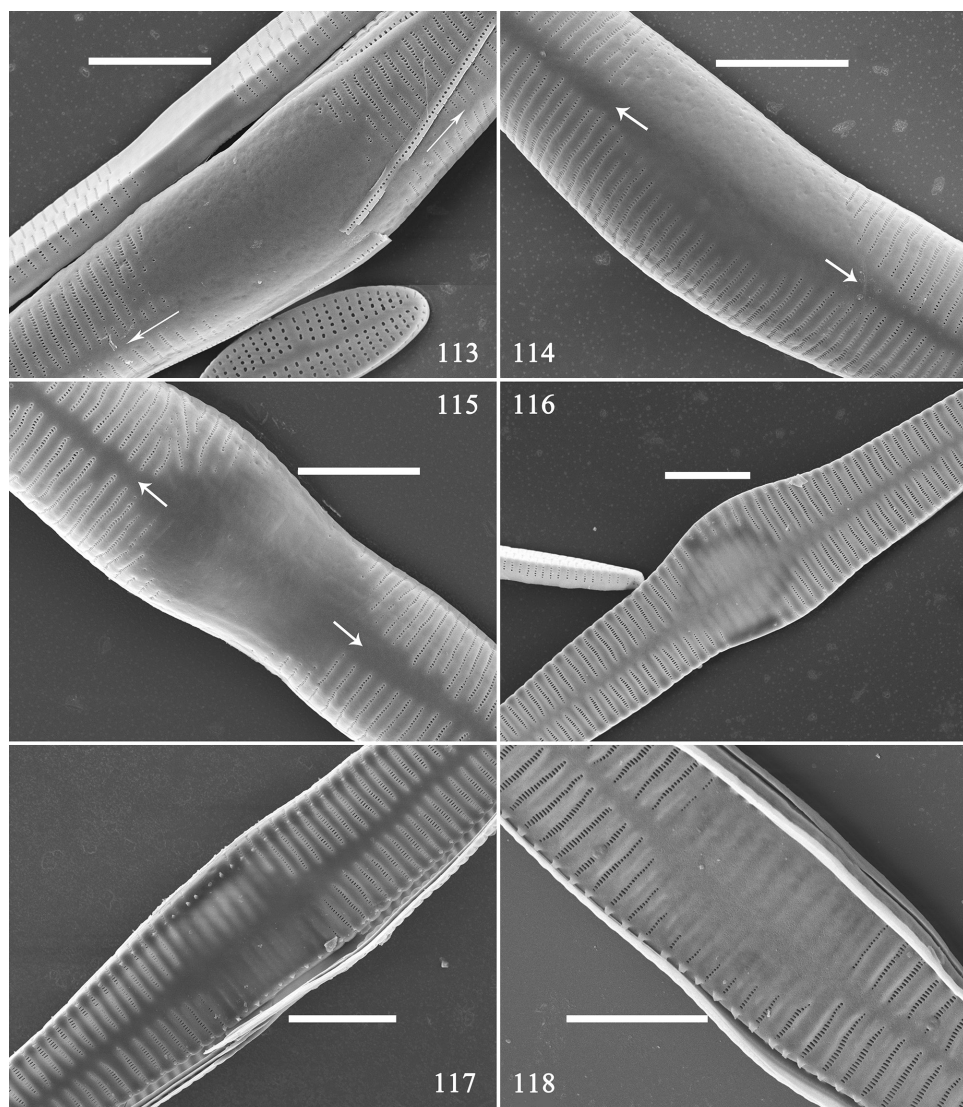


Figures 107–112. *Hannaea inaequidentata*, pre-normal frustules, external view, SEM **107** frustule with arcuate outline and swollen middle **108** frustule with developed sternum **109** frustule with bi-constricted middle and developed sternum **110** frustule with globular middle and developed sternum **111** twisted frustule with developed sternum **112** frustule with distinct virgae and developed sternum. Scale bars: 20 μm (**107–112**).

rows). The virgae and vimines are almost flush with each other (Figs 104–106). There are four girdle bands (Fig. 106, labelled B1 to B4). The hypovalve has spines (Fig. 106, arrows) indicating that the initial epivalve may have passed a few generations.

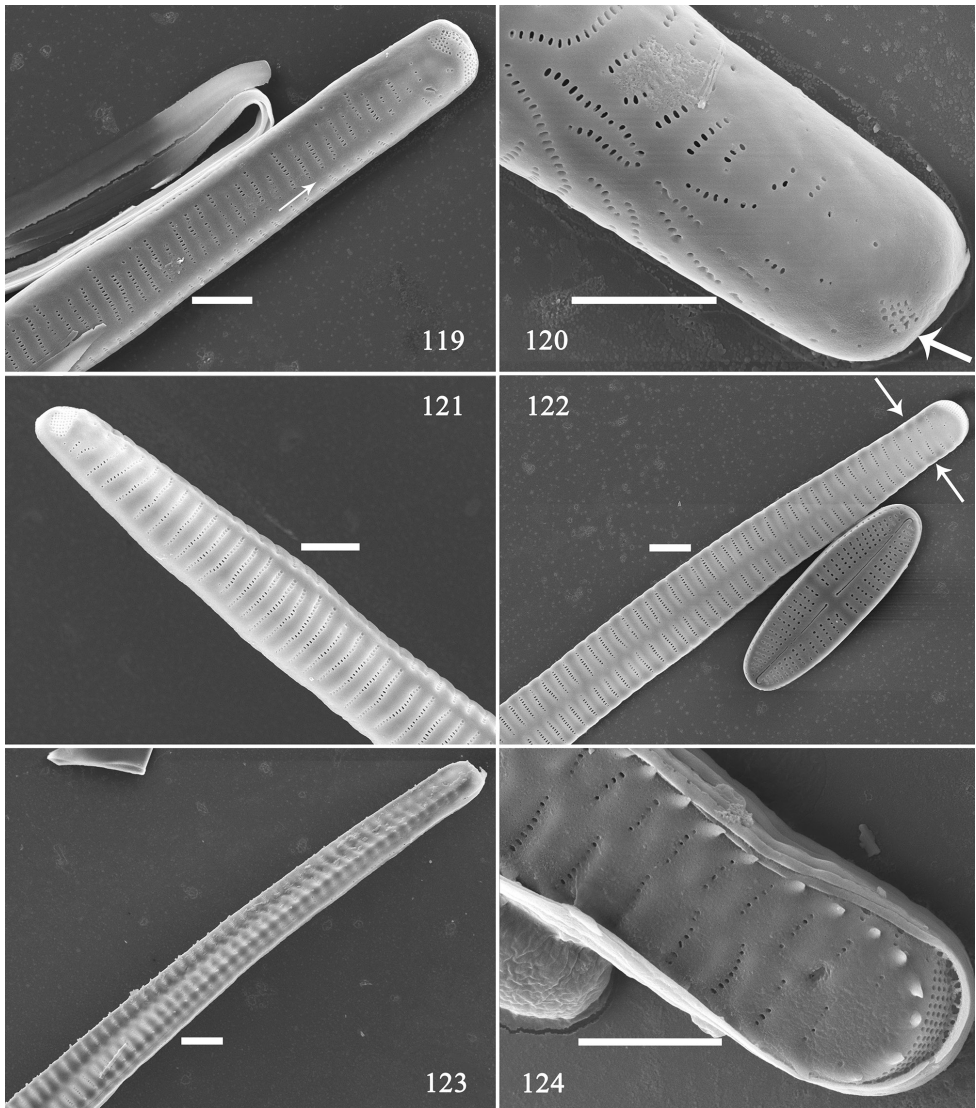
Pre-normal frustule composed of new-born epivalve and hypovalve

Using SEM, we illustrate six frustules in external view (Figs 107–112) to document how the pre-normal vegetative frustules gradually develop into normal vegetative



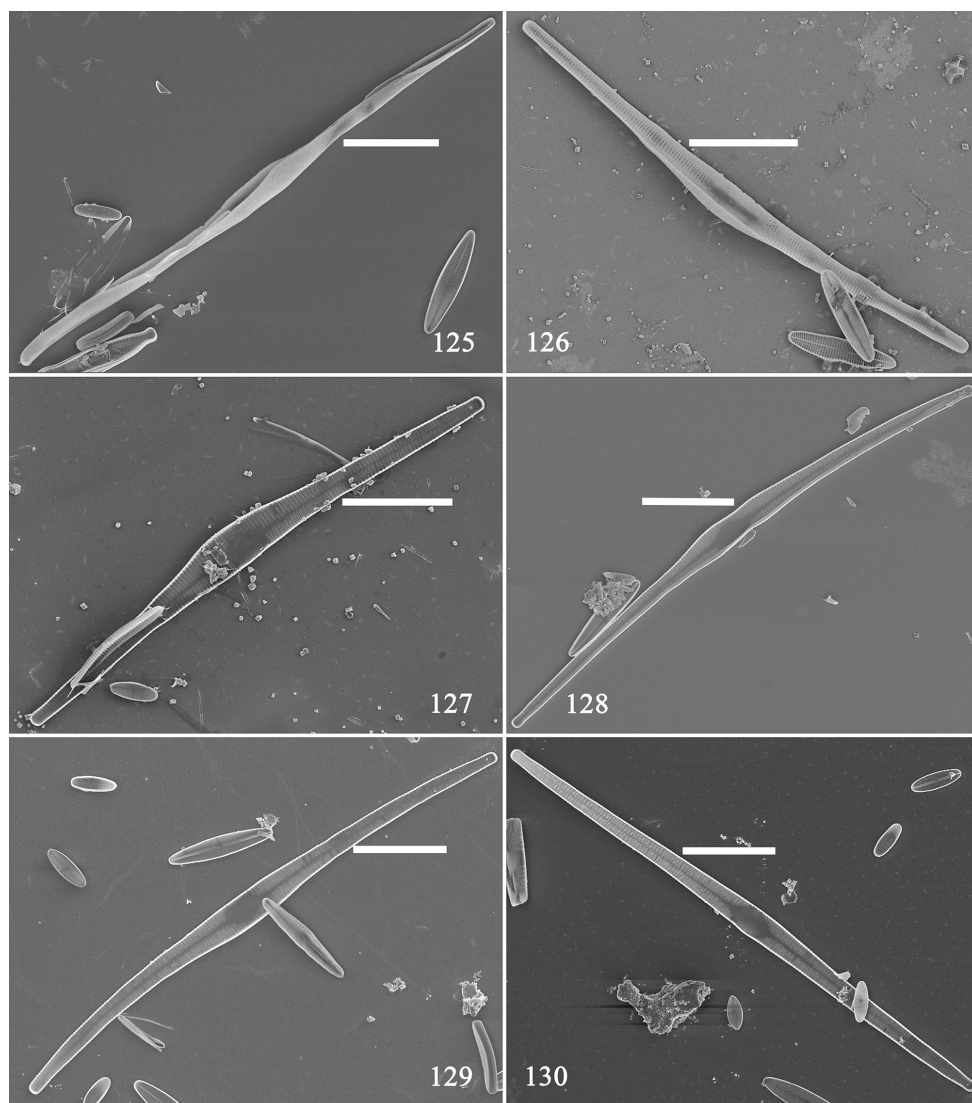
Figures 113–118. *Hannaea inaequidentata*, middle details of pre-normal cells, external view, SEM **113** middle part of Fig. 107 showing deflexed sternum (two arrows) and central area **114** detail of middle illustrated in Fig. 108 showing sternum (two arrows) and central area **115** detail of middle illustrated in Fig. 109 showing sternum (two arrows) and central area **116** detail of middle part illustrated in Fig. 110 showing developed virgae and vimines **117** detail of middle part illustrated in Fig. 111 showing developed spines **118** detail of middle part illustrated in Fig. 112 showing well-developed virgae, vimines and spines. Scale bars: 5 μ m (**113–118**).

frustules (Figs 113–124). The lateral sternum (Figs 107, 113, 119) gradually becomes central sternum (Figs 108–112, 114–118, 120–124). The central area develops from an area without ghost striae (Figs 113–115) and gradually occupies one half of the valve and ghost striae are evident (Figs 116–118). The spines appear forming as out-growths of a vimine (in most cases) (e.g., Figs 117, 118, 123, 124). At the outset, the



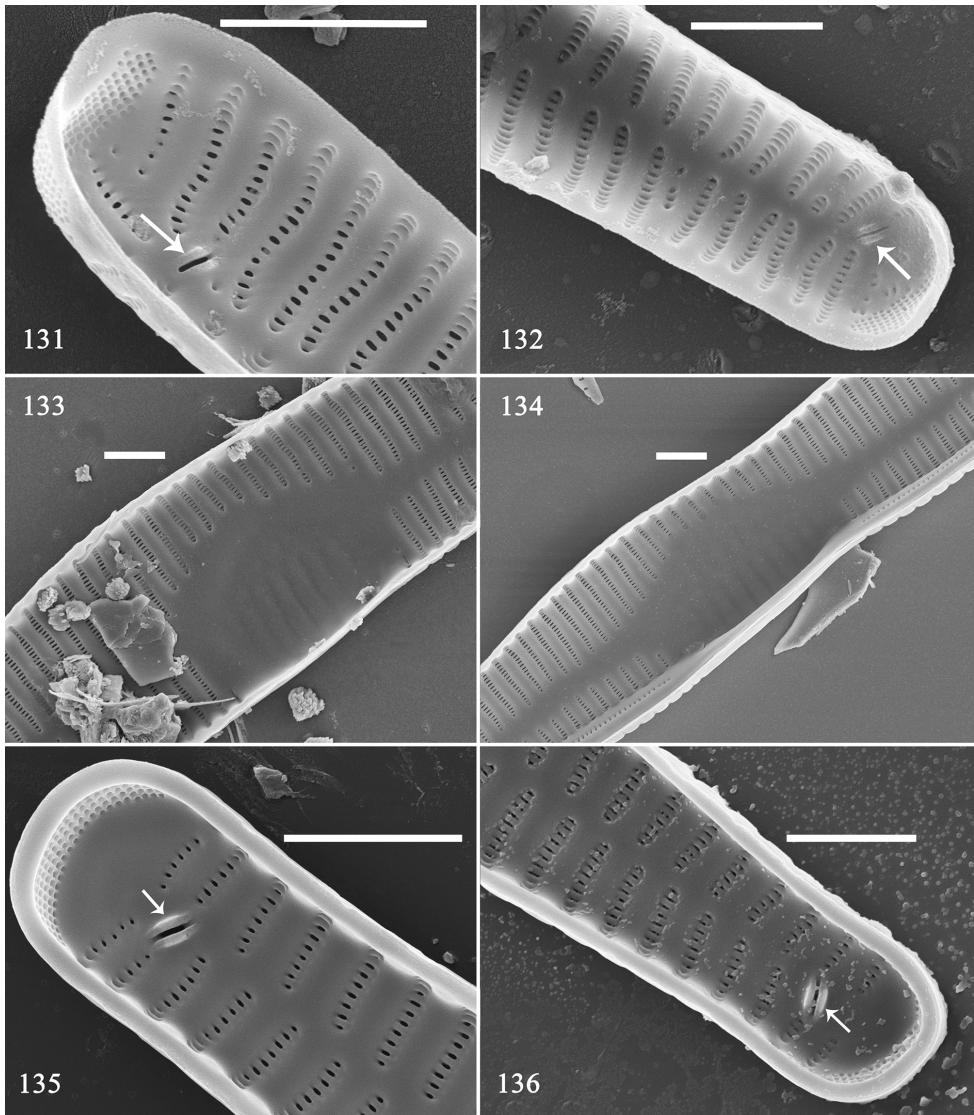
Figures 119–124. *Hannaea inaequidentata*, apex details of pre-normal cells, external view, SEM **119** apex detail of Fig. 107 showing deflexed sternum (arrow) **120** apex detail of Fig. 108 showing irregular striae and small ocellulimbus (arrow) **121** Apex detail of Fig. 109 showing twisted valve **122** apex detail of Fig. 110 showing almost normal sternum and striae (two arrows) **123** apex detail of Fig. 111 showing twisted valve and not well-developed spines **124** apex detail of Fig. 112 showing well-developed spines and almost normal ocellulimbus. Scale bars: 2 μm (**119–124**).

virgae and vimines occur on the same plane (Figs 113–115), with the virgae becoming raised away from the vimines (Figs 116–118), and the ocellulimbus gradually become more regular in its structure (Figs 119–124).



Figures 125–130. *Hannaea inaequidentata*, pre-normal vegetative valves, internal view, SEM **125** twisted and rounded valve **126** arcuate valve with swollen middle part **127** valve with sternum and swollen middle part **128** valve with bi-constricted middle part and sternum **129** slightly arcuate valve with parallel middle part and sternum **130** nearly normal valve. Scale bars: 20 μm (**125–130**).

Using SEM, we illustrate six pre-normal vegetative valves in internal view (Figs 125–130). These valves have different outlines: a twisted, rounded valve (Fig. 125); an arcuate valve with swollen centre (Fig. 126); a valve with sternum and swollen centre (Fig. 127); a valve with bi-constricted centre area and a central sternum (Fig. 128); a slightly arcuate valve with parallel centre and central sternum (Fig. 129); and a nearly



Figures 131–136. *Hannaea inaequidentata*, details of pre-normal vegetative valves, internal view, SEM **131, 132** two apices of Fig. 126 showing two rimoportulae per valve (two arrows) **133** middle part detail of Fig. 127 showing swollen central area and ghost striae **134** detail of Fig. 128 showing the bi-constricted middle part and ghost striae **135, 136** two apices of Fig. 130 showing two rimoportulae per valve. Scale bar: 2 μm (**131–136**).

normal valve (Fig. 130). Internally, as noted above for the external view, the virgae and vimines first occur on the same plane, with the virgae becoming raised away from the vimines and the lateral sternum becomes central (Figs 131–136). As with the initial valve, some pre-normal new-born valves also have two rimoportulae per valve (Figs 131, 132, 135, 136, two arrows respectively).

Table 2. Features of initial cell, pre-normal and normal vegetative in *Hannaea inaequidentata*.

Feature	Initial frustule/valve	Pre-normal vegetative frustule/valve	Normal vegetative frustule/valve
Colony	solitary	solitary	ribbon-like colony
Girdle band number	two	four	not dividing frustule has six, with 4:2 configuration; dividing frustule has eight, with 4:4 configuration
Plaques	present	present	present
Valve outline	cylinder-like, often twisted	irregular (see Figs 72–78)	slightly arcuate, lanceolate
Valve apex	rounded	rounded, cuneate, rostrate, or sub-capitate	capitate to sub-capitate
Sternum	non-existent or lateral sternum	lateral to central sternum	central sternum, i.e. normal, situated on the middle line of valve
Central area	present or ca. as half with short striae to one side	present or ca. as half with short striae to one side	half with short striae in one side
Virga/vimine	virgae and vimines almost flush with each other	vimines slightly lower than virgae	virgae raised, vimines sunken
Linking spines	not present	gradually developed	present and interlocking cells forming ribbon-like colony
Rimoportula number per valve	one, sometimes two	one, sometimes two	one
Ocellulimbus	extending valve face, perivalvar row of poroids not vertical	perivalvar rows of poroids gradually becoming vertical	perivalvar rows of poroids all vertical

Summary of morphological features changing

The morphological features that change during the life circle of *Hannaea inaequidentata* are summarised in Table 2. From initial frustule/valve, via pre-normal vegetative frustule/valve, to normal vegetative frustule/valve, the colony, girdle band numbers, valve outline, valve apex, sternum, central area, virga and vimine, linking spines, rimoportula number per valve, and ocellulimbus all gradually become normal (Table 2). The valve plaques are a constant feature, occurring in the initial valve, pre-normal valve, and normal valve.

Discussion

We noted above that *Hannaea* is usually characterised as having valves “asymmetrical to the apical axis, usually with a small, unornamented tumid area on one side of the center of the valve” (Liu et al. 2019, p. 42) – four groups have been recognised, based on a combination of striae structure and rimoportula number: one group has uniseriate striae and a single rimoportula, another has biseriate striae and two rimoportulae, one at each pole; these two groups are both asymmetrical about the apical axis. The additional two groups are those that have either poorly developed asymmetry to the apical axis or with parallel margins (cf. Liu et al. 2019, p. 42). These latter two groups are those possibly related to *Fragilaria* Lyngbye.

Initial cell and pre-normal vegetative cell

In ‘araphid’ diatoms there are very few reports of transverse perizonal bands. For example, in *Fragilariforma virescens* (Ralfs) D.M. Williams and Round, Williams noted “no sign of transverse perizonal bands at all” (Williams 2001) and in a species of *Ulnaria* (Kützing) Compère, Williams and Metzeltin noted that the auxospore/initial cells were rather large (in excess of 250 µm), curved along their length, with an irregular basal siliceous layer and the valve outline sometimes interrupted by undulations or a central inflation (Williams and Metzeltin 2004, see also Sato et al. 2004 for further comparisons).

Structure and ontogeny

Valve changes: This study is primarily based on *Hannaea inaequidentata*, a species with almost parallel valve margins, its overall structure similar to some species currently in *Fragilaria* (as noted first by Cleve 1898; *Fragilaria* as defined by *F. pectinalis* (O.F. Müller) Lyngbye, see Tuji and Williams 2006).

Van de Vijver and Ector have documented the changes in shape of valves in *Ceratoneis amphioxys* such that “a continuum is present from longer valves showing the typical valve morphology of *Hannaea arcus* to shorter valves with the indentations that are typical for *Hannaea arcus* var. *amphioxys*” (Van de Vijver and Ector 2020, p. 2, see also Jewson and Bixby 2016). In addition, *Hannaea arcus* and *Hannaea arcus* var. *amphioxys* were considered to be synonymous, with Van de Vijver and Ector noting that “Based on the results of the morphological analysis using light microscopy, we propose to treat *Hannaea arcus* var. *amphioxys* (Rabenhorst) R.M. Patrick as a heterotypic synonym of *Hannaea arcus* (Ehrenberg) R.M. Patrick” (Van de Vijver and Ector 2020, p. 3); Van de Vijver and Ector record others who have previously expressed the same view: Krammer and Lange-Bertalot (1991, p. 134, as a shape variant, ‘*Umrissvariation*’) and implied in Genkal and Kharitonov (2008, p. 17, pl.1, fig. 8). Many of currently valid taxon names may turn out to be simply stages in individual life-cycles, e.g. *Ceratoneis arcus* f. *trigibba* C. Zimmermann (Zimmermann 1915: 36, pl. 4, fig. 10) and the various valves illustrated in Meister (1919) (see Van de Vijver and Ector 2020 for illustration and discussion).

The 1979 terminology paper defined the central area as “an expanded or otherwise distinct portion of the axial area midway along its length” (Ross et al. 1979, p. 518). This definition related more to raphid diatoms than ‘araphid’ diatoms. Bixby et al., in their study of *Hannaea*, suggested some useful additional terms that help describe more accurately the structure of the central area. In valves of *Hannaea superiorenensis* Bixby and Edlund (in Bixby et al. 2005, p. 231), internal views shows that the central area is demarcated by a central swollen portion of the valve with an area demarcated by *buttressing* (as in: “buttressed central inflation”, Bixby et al. 2005, p. 235, p. 234, fig. 11). In *Hannaea superiorenensis*, the “buttressed central inflation” extends up to the sternum. The buttresses are effectively a pair of heavily silicified virgae situated either side of the demarcated central area enclosing a series of “ghost striae”, the latter being a more heavily silicified

set of virgae and vimines but with each visible (Bixby et al. 2005, p. 234, fig. 11). Most species of *Hannaea* have this kind of central area construction, but not all – see *Hannaea tibetiana*, for example, which has a simple plain area demarcated by the virgae and vimines being more silicified in this area (Liu et al. 2019, p. 46, fig. 3; figure 3B is of the ‘plain’ internal view). The buttressing is less obvious in species such as *Hannaea arcus* and *H. inaequidentata*. Here we noted that in the normal vegetative valves, *H. inaequidentata* has a central area on the ventral side of the valve with faint ghost striae, and transversely raised virgae are evident. Further, in the initial cells, the central area appears without any obvious distinction between virgae and vimines, hence ghost striae and the sternum are not evident. In the ‘pre-normal frustule/valve’, the central area varies in shape, from slightly sigmoid, expanded on one side of the valve, extending across the whole valve, margin to margin, often with varying shapes. Finally, the central area occupies one half of the valve and the ghost striae become evident. The implication is that the virgae in the central area being laid down later emerge from the silica basal layer rather than forming first with the vimines and then being filled in. Thus, while the structure called the ‘central area’ is obviously composed of various parts of the valve structure and is now better known, its relevance to taxon relationships remain less than obvious.

Relationships

At present, it is not clear if *Hannaea*, consisting of all the various groups of species, is monophyletic, in spite of the conclusions offered by Bixby et al. (2005). As we noted above, Bixby et al. (2005) based its monophyly on a combination of the presence of a unilateral inflation, the lack of striae in that inflation, and a valvocopula with an ad valvar crenate margin. None of these characters appear unique (synapomorphic) to *Hannaea* as currently formulated. For example, the asymmetrical valve shape can be found elsewhere in freshwater ‘araphid’ diatoms currently included in *Fragilaria* (e.g., *Fragilaria flexura* Hoff and Lange-Bertalot in Hoff et al. 2011, which is admittedly an unusual species of *Fragilaria*) and, as we noted above, the “small, unornamented tumid area” is also found in a few other species (e.g., *Synedra mazamaensis* Sovereign 1958 (as the current definition of *Synedra* refers to a marine genus, this species clearly does not belong there – it is probably not a species of *Fragilaria sensu stricto* either, but that requires further investigation, see Williams & Karthick, In Review, for comments on the name *Synedra*; other species to consider might be *Fragilaria bidens* Heiberg and its relatives). It is also not clear if the four sub-groups noted above are themselves monophyletic or just ‘convenience’ groups to aid identification.

Final comments

The diversity of species in *Hannaea* is currently recognised by the array of names available, some 30+ for *Ceratoneis arcus* alone, for example. Many of these may turn out to be definable taxa, but others will simply be stages in the individual life cycles, e.g., *Ceratoneis arcus* f. *trigibba* (see Van de Vijver and Ector 2020). Schmid (1997) suggest-

ed that species in *Hannaea* may simply be teratological forms of *Fragilaria*, in a similar fashion to the tri-radiate cells of *Centronella* M. Voigt. This is certainly a possibility but the work of Van de Vijver and Ector (2020) suggests that while there are shape changes to the valves, they should not be considered teratological forms but natural. That viewpoint is supported here. Nevertheless, it would seem essential at this stage to perform life-cycle studies where possible to ascertain not just how valves form and how exactly valve characters emerge, but to utilise this information to establish evidence for the relationships of taxa at all ranks.

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Phylogenetic relationships and status of taxa of *Pulsatilla uralensis* and *P. patens* s.str. (Ranunculaceae) in north-eastern European Russia

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Abstract

We studied the allopatric complex *Pulsatilla patens* (L.) Mill. s.lat. (Ranunculaceae) in north-eastern European Russia and the Urals. In this region, there are two kinds of *P. patens* with different perianth colours in monochrome and polychrome populations. To clarify their taxonomic boundaries, we used the sequences of chloroplast DNA (*rbcL* and *matK*) and nuclear DNA (ITS2), in addition to morphological characteristics. The combination of three markers (*rbcL*+*matK*+ITS2) was found to be the most effective for phylogenetic resolution. The samples of two morphologically-different taxa *P. uralensis* and *P. patens* s.str. were shown to form a single clade on the phylogenetic tree. Based on the molecular phylogenetic analysis, we were not able to unequivocally prove the independent existence of *P. uralensis*.

Keywords

DNA barcode, molecular phylogeny, taxonomy, Ranunculales, ITS2, *rbcL*, *matK*

Introduction

The genus *Pulsatilla* Mill., which is sometimes included in *Anemone* s.lat. (e.g. Hoot et al. 1994, 2012), comprises more than 30 taxa which, as a rule, form intricate species complexes with a high degree of morphological variability (Stepanov 2014; Li et al. 2019; Sramko et al. 2019). The general classification, boundaries and the number of species and lower taxa have been revised several times, but no consensus has yet been reached (e.g. Tamura 1995; Tzvelev 2012; Grey-Wilson 2014). Furthermore, taxa of *Pulsatilla* often hybridise with each other in common habitats (Akeroyd 1993; Bakin 2005; Stepanov 2014), which makes it difficult to define the species' boundaries. Many species and subspecies of *Pulsatilla* are rare and subject to protection (Holub and Prochazka 2000; Council of the European Union 2007; Bardunov and Novikov 2008; The IUCN Red List of Threatened Species 2019). In the Russian Federation, even widespread *Pulsatilla* species are subject to protection due to the small number of habitats, small population sizes and high anthropogenic impact. Fourteen species of *Pulsatilla* are recorded in the European part of Russia (Tzvelev 2001) and 11–15 in the Asian part (Malyshev 2012; Timokhina 1993). In addition, there are several questions regarding the identification and size of taxa within the *P. patens* s.lat. complex, despite several recent studies on molecular phylogenetic relationships in the genus (Li et al. 2019; Sramko et al. 2019).

In the Urals and the adjacent parts of the Russian Plain, four taxa of *Pulsatilla* ser. *Patentes* can be found: *P. patens* s.str., *P. uralensis* (Zamelis) Tzvelev, *P. multifida* (Pritz.) Juz. and *P. angustifolia* Turcz. (Tzvelev 2001, 2012). The main diagnostic characteristics used to recognise taxa within *P. patens* s.lat. are the colour of the perianth, the degree of dissection of the leaf blade (i.e. number of teeth), the presence and length of the petiolule of the apical segment and the width of the apical segment (Juzepczuk 1937; Tzvelev 2001; Egorova et al. 2017). All of them have extensive ranges of distribution and different centres of speciation, but only *P. patens* s.str. and *P. uralensis* are found in the Komi Republic. The European *P. patens* s.str. (= *P. patens* subsp. *patens*) only slightly expands beyond the boundaries of Europe. It is mainly distributed on the western macro-slope of the Urals (Central and Southern Urals) and also extends into Siberia and Central Asia (Tzvelev 2001; Sushentsov 2008). In Europe, this taxon is morphologically fairly uniform, but becomes extremely polymorphic in the Urals and Siberia (Juzepczuk 1937). *Pulsatilla uralensis* is distributed in the Central and Southern Urals (Kulikov 2005; Sushentsov 2008) and the adjacent part of the Russian Plain, in the basin of the Vyatka River (Egorova et al. 2017).

Due to the past separate geographical range, which suggests allopatric geographic isolation, and nomenclature confusion the circumscription of some species is a matter of much debate. For instance, *P. uralensis* (Zamelis) Tzvelev (= *P. patens* subsp. *uralensis* Zamelis) is often used synonymously with *P. flavescens* (Zucc.) Juz. [nom. illeg., non-Boros = *P. patens* subsp. *flavescens* (Zucc.) Zamelis]. However, we consider these taxa to

be conspecific and, despite the widespread use of both names in regional floristic surveys and databases (see The Plant List 2020; World Flora Online 2020; NCBI 2020), the name *P. flavescens* is illegitimate (Somlay 2000). Therefore, in this study, we will use the commonly-accepted name *P. uralensis* (Zamelis) Tzvelev (see Tzvelev 2001, 2012; The Euro+Med PlantBase 2020) in order to also avoid confusion with the homonym of the species.

To date, no particular research of *Pulsatilla* species in the allopatric zone in the northern part of European Russia (within 59°12'–68°25'N and 45°25'–66°15'E) has been carried out. The northern boundary of the *Pulsatilla* range passes through the Komi Republic, while over 100 localities of plants of different coloured flowers are included under *P. patens* in the Red Book for the region (Martynenko 2009). The location of the region in the allopatric zone of European *P. patens* s.str. and Ural-Siberian *P. uralensis*, plus the elevated degree of polymorphism of diagnostic characteristics (i.e. colour of the perianth and dissection of the leaf blade) indicate the need to supplement the morphological methods with modern molecular genetic studies. Therefore, the aims of this work were to: 1) identify the taxa of *P. patens* s.lat. in north-eastern European Russia using herbarium specimens and wild populations; and 2) describe their phylogenetic relationships using plastid markers (*matK* and *rbcL*) and a nuclear marker (ITS2) recommended for plants by the DNA Barcode consortium.

Materials and methods

The study area is located in north-eastern European Russia (Fig. 1). Thirty-one samples from ten populations were collected by the authors from the Komi Republic, Orenburg Oblast and Sverdlovsk Oblast as detailed in Table 1. Most samples were collected from the Komi Republic within 59°12'–68°25'N and 45°25'–66°15'E. In each population, generative specimens were collected during the flowering period, at least 5–15 m apart from each other. We collected: 1) nine samples from hybridogenous polychrome populations (i.e. I, II and III) with yellow or blue-violet flowers; 2) eleven samples of *P. uralensis* from monochrome populations (i.e. IV, V, VII, VIII and X) with yellow (from pale yellow to deep yellow) and occasionally with white flowers; and 3) nine samples of *P. patens* s.str. from monochrome populations (i.e. VI and IX) with blue-violet flowers (Table 1).

Furthermore, we analysed over 120 specimens of *P. patens* s.lat. from the Herbarium of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (SYKO). According to morphological characteristics, these plants were identified as *P. patens* s.str. (Fig. 2) or *P. uralensis* (Fig. 3). *Pulsatilla patens* s.str. has blue-violet flowers, while *P. uralensis* has pale yellow to yellow flowers. The main morphological differences between *P. patens* s.str. and *P. uralensis* are summarised in Table 2.

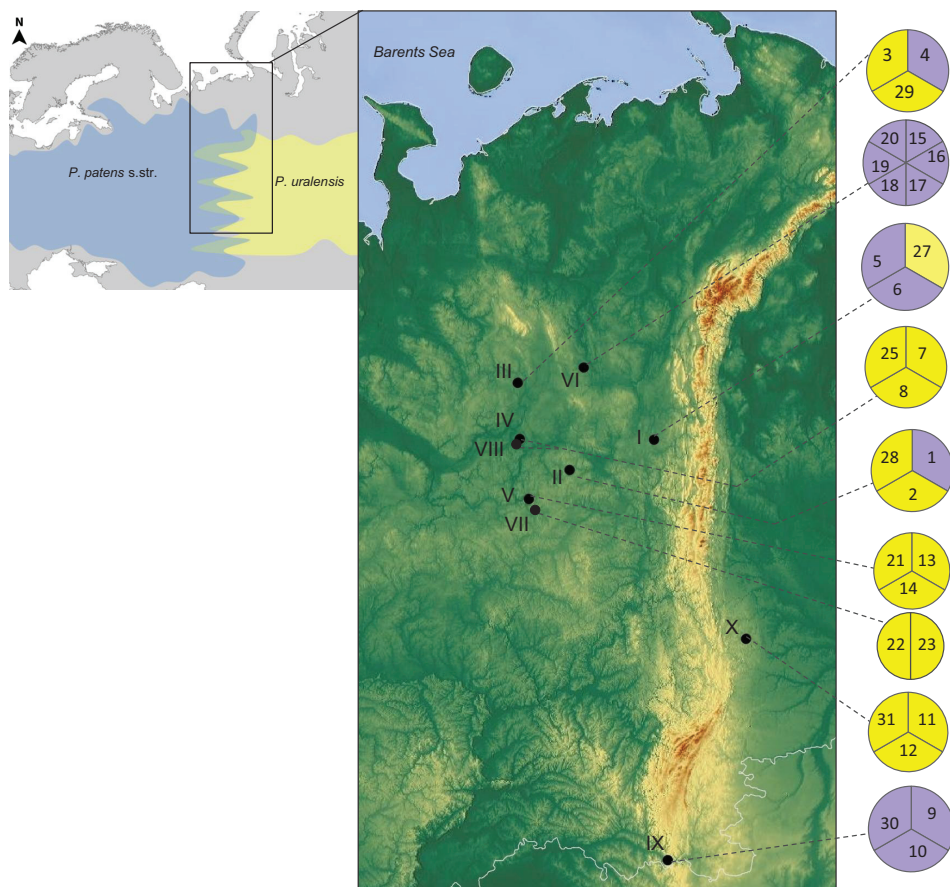


Figure 1. Distribution map of the sampling sites of *P. patens* s.str. and *P. uralensis* in north-eastern European Russia and the Urals. The colour on the diagrams indicates the colour of the perianth in different taxa: blue-violet – *P. patens* s.str., yellow – *P. uralensis*. The locations (I–X) and samples correspond to Table 2.

DNA extraction, amplification and sequencing

Sequences of plastid DNA (*rbcL* and *matK*) and nuclear DNA (ITS2) have been successfully used for plant identification and DNA barcoding in various taxonomic groups (Cai et al. 2010, Wattoo et al. 2016, Saddhe Kumar 2017), including the genus *Pulsatilla* (Li et al. 2019). ITS2 is considered to be the most effective barcode for the identification of more than half of *Pulsatilla* species (Li et al. 2019). Therefore, we tested the ability of DNA barcoding to distinguish between the taxa of *P. patens* s.lat. Thirty-one samples were analysed and three barcode regions (*rbcL*, *matK* and ITS2) were amplified, sequenced and aligned.

Total genomic DNA was isolated from dried leaves using the Sorb–GMO–B kit (Sintol, Russia) in accordance with the manufacturer’s instructions. PCR fragments

Table 1. The studied *Pulsatilla* populations, characteristics of the samples, and GenBank and BOLD Systems numbers.

Population number	Geographic location/Habitat/ Coordinates	Population characteristics*	Taxon	Perianth colour	Sample number	GenBank accession number			BOLD Systems sample ID
						<i>rbcL</i>	<i>matK</i>	ITS2	
I	Russia, Komi Republic, Troitsko-Pechorsky District, near settlement of Znamenka, 2 km to the east, right bank of the Pozhieg River / Shrub - green moss - lichen pine forest / 61.9670°N, 56.8894°E	Hybridogenous polychrome population of <i>P. patens</i> s.str. × <i>urandensis</i>	<i>P. patens</i> s.str.	blue-violet	5	MK050017	MK050048	MK424550	SYKO-PV-17508
			<i>P. urandensis</i>	yellow	6	MK050018	MK050049	MK424551	SYKO-PV-17512
			<i>P. patens</i> s.str.	blue-violet	27	MK050019	MK050050	MK424552	SYKO-PV-17524
II	Russia, Komi Republic, Ust-Kalmosky District, near settlement of Nizhny Yarashyu, valley of the Vyehgda River / Lichen pine forest / 62.0927°N, 54.2982°E	Hybridogenous polychrome population of <i>P. patens</i> s.str. × <i>urandensis</i>	<i>P. patens</i> s.str.	blue-violet	1	MK050020	MK050051	MK424553	SYKO-PV-17509
			<i>P. urandensis</i>	yellow	2	MK050021	MK050052	MK424554	SYKO-PV-17526
			<i>P. urandensis</i>	yellow	28	MK050022	MK050053	MK424555	SYKO-PV-17525
III	Russia, Komi Republic, Kuyazh Pogorsky District, near the settlement of Meshchura, valleys of the Vym and Elva Rivers / Green moss - lichen pine forest / 63.3375°N, 50.9150°E	Hybridogenous polychrome population of <i>P. patens</i> s.str. × <i>urandensis</i>	<i>P. urandensis</i>	yellow	3	MK050023	MK050054	MK424556	SYKO-PV-17510
			<i>P. patens</i> s.str.	blue-violet	4	MK050024	MK050055	MK424557	SYKO-PV-17511
			<i>P. urandensis</i>	yellow	29	MK050025	MK050056	MK424558	SYKO-PV-17532
IV	Russia, Komi Republic, Syktyvdinsky District, near settlement of Kocchoyag, 300–400 m north of station Yazel / Railway mound / 61.9588°N, 50.6117°E	Monochrome population of <i>P. urandensis</i>	<i>P. urandensis</i>	yellow	7	MK050026	MK050057	MK424559	SYKO-PV-17513
			<i>P. urandensis</i>	yellow	8	MK050027	MK050058	MK424560	SYKO-PV-17527
			<i>P. urandensis</i>	yellow	13	MK050034	MK050065	MK424567	SYKO-PV-17535
V	Russia, Komi Republic, Koigorodsky District, near settlement of Vezhye (Uzhga-2) / Lichen pine forest / 60.6284°N, 51.0303°E	Monochrome population of <i>P. urandensis</i>	<i>P. urandensis</i>	yellow	14	MK050035	MK050066	MK424568	SYKO-PV-17536
			<i>P. urandensis</i>	yellow	21	MK050036	MK050067	MK424569	SYKO-PV-17531
			<i>P. patens</i> s.str.	blue-violet	15	MK050037	MK050068	MK424570	SYKO-PV-17516
VI	Russia, Komi Republic, Ukhtinsky District, near settlement of Shudayag, the Imanian limestones / Cowberry- green-moss pine forest / 63.5199°N, 53.5949°E	Monochrome population of <i>P. patens</i> s.str.	<i>P. patens</i> s.str.	blue-violet	16	MK050038	MK050069	MK424571	SYKO-PV-17517
			<i>P. patens</i> s.str.	blue-violet	17	MK050039	MK050070	MK424572	SYKO-PV-17518
			<i>P. patens</i> s.str.	blue-violet	18	MK050040	MK050071	MK424573	SYKO-PV-17519
VII	Russia, Komi Republic, Koigorodsky District, near settlement of Vezhye (Uzhga-1) / Lichen pine forest / 60.6005°N, 50.9959°E	Monochrome population of <i>P. urandensis</i>	<i>P. patens</i> s.str.	blue-violet	19	MK050041	MK050072	MK424574	SYKO-PV-17520
			<i>P. patens</i> s.str.	blue-violet	20	MK050042	MK050073	MK424575	SYKO-PV-17521
			<i>P. urandensis</i>	yellow	22	MK050043	MK050074	MK424576	SYKO-PV-17522
VIII	Russia, Komi Republic, Syktyvdinsky District, near settlement of Kocchoyag / Lichen pine forest / 61.9428°N, 50.6281°E	Monochrome population of <i>P. urandensis</i>	<i>P. urandensis</i>	yellow	23	MK050044	MK050075	MK424577	SYKO-PV-17530
			<i>P. urandensis</i>	yellow	24	—	—	—	—
			<i>P. urandensis</i>	yellow	25	MK050046	MK050077	MK424579	SYKO-PV-17523
IX	Russia, Orenburg Oblast, Gaysky District, near village of Khmelevka, slope facing to east / Steppe meadow / 51.13°N57.54°E	Monochrome population of <i>P. patens</i> s.str.	<i>P. patens</i> s.str.	blue-violet	9	MK050028	MK050059	MK424561	SYKO-PV-17514
			<i>P. patens</i> s.str.	blue-violet	10	MK050029	MK050060	MK424562	SYKO-PV-17528
			<i>P. patens</i> s.str.	blue-violet	30	MK050030	MK050061	MK424563	SYKO-PV-17533
X	Russia, Sverdlovsk Oblast, near city of Rezh, carbonate rocks / Pine forest / 57.23°N, 61.25°E	Monochrome population of <i>P. urandensis</i>	<i>P. urandensis</i>	yellow	11	MK050031	MK050062	—	SYKO-PV-17515
			<i>P. urandensis</i>	yellow	12	MK050032	MK050063	MK424565	SYKO-PV-17529
			<i>P. urandensis</i>	yellow	31	MK050033	MK050064	MK424564	SYKO-PV-17534

Note: * polychrome populations are formed by plants with yellow and blue-violet flower colours of varying intensity; monochrome populations are formed only by plants with a yellow flower colour or only a blue-violet flower colour of varying intensity.

were amplified in 50 µl of a mixture containing 10 µl of Screen Mix (Eurogen, Russia), 10 µl of each primer (0.3 µM) (Eurogen, Russia), 18 µl of ddH₂O (Ambion, USA) and 2 µl of DNA template (1÷100 ng). The ITS2 sequences were amplified with universal primers ITS-5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'); the *rbcL* and *matK* sequences – with primers SI_For (5'-ATGTCACCACAAACAGAGACTAAAGC-3'), SI_Rev (5'-GTAAAATCAAGTCCACCRCG-3') and KIM 3F (5'-CGTACAGTACTTTTGTGTTTACGAG-3'), KIM 3R (5'-ACCCAGTCCATCTGGAAATCTTGTTTC-3'), respectively (Kress et al. 2009). Thermal cycling included heating to 95 °C for 4 min, followed by 34 cycles of 60-s melting at 95 °C, 30-s annealing at 50 °C (for *rbcL*), 55 °C (for ITS2), 61 °C (for *matK*) and 40-s extension at 72 °C, with a final extension for 5 min at 72 °C. PCR and sequencing were carried out using the equipment of the Center for Collective Usage «Molecular Biology» of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences (Syktyvkar, Russia).

Phylogenetic analysis

Multiple alignments of nucleotide sequences were obtained using ClustalW in the MegaX programme (Thompson et al. 1994, Kumar et al. 2018). There were some missing data (see Table 2) and the alignments of the *rbcL*, *matK* loci and the ITS2 region were analysed separately and in concatenation (*rbcL*+*matK*+ITS2).

Phylogenies were constructed based on the GTR+Γ+I model for all alignments using the Bayesian Inference (BI) and Maximum Likelihood (ML) analysis. The BI analysis was conducted using MrBayes-3.2.5 (Ronquist and Huelsenbeck 2003). Three “hot” and one “cold” Markov chains were run for 1×10^6 cycles in two repetitions with the selection of each 200th generated tree. The phylogenetic tree and the probabilities of its branching were obtained after discarding the first 25% of the model for estimating the parameters of nucleotide substitutions and their probabilities. The ML analysis was performed using the MegaX programme (Kumar et al. 2018) with bootstrap analysis from 1,000 replicas. Graphical viewing and editing of trees were carried out in the programmes Fig Tree (ver. 1.4.2) and Adobe Photoshop CC (19.0).

In the analysis, we used nucleotide sequences obtained by us or taken from the NCBI database (GenBank) and BOLD Systems (accessions numbers on phylogenetic trees are indicated in Table 2). Representatives of some species of *Anemone*, *Anemoclema*, *Clematis* and *Hepatica* were used as an external group. In this study, we discuss the phylogenetic hypotheses obtained from each individual dataset separately (*rbcL*, *matK* and ITS2) and analysis of the combined dataset of all three markers. All new *rbcL*, *matK* and ITS2 sequences, obtained by us and used in this study, were deposited to the GenBank (accession No. MK050017–MK050077, MK424550–MK424579) and Barcode of Life databases (BOLD Systems sample ID: SYKO-PV-17508–SYKO-PV-17536) (Table 2). Other *Pulsatilla* species from the BOLD Systems (BOLD Systems 2020) and GenBank (NCBI 2020) databases that had all three sequences (*rbcL*, *matK* and ITS2) were used in phylogenetic analysis.



Figure 2. *Pulsatilla patens* s.str. (L.) Mill. (*P. patens* subsp. *patens*) **A–C** flowers with different perianth colour **D** herbarium specimen of a flowering shoot and typical leaf blade **E** plant just after flowering with unripe fruits. The photographs show sample number 5 (**A, D**), sample number 15 (**B**) and sample number 18 (**C**).



Figure 3. *Pulsatilla uralensis* (Zamelis) Tzvelev **A–C** flowers with different perianth colour **D** herbarium specimen of a flowering plant and different leaf blades **E** fruiting plant. The photographs show sample number 6 (**B**), sample number 7 (**C**) and sample number 25 (**D**).

Table 2. Morphological comparison between *P. patens* s.str. and *P. uralensis*.

Characters	<i>P. patens</i> s.str.	<i>P. uralensis</i>
Flower colour	Blue-violet (different saturation)	Yellow (white or light yellow)
Lobes of basal leaves	≤ 26 (17–30)	≥ 26 (22–43)
Width of leaf-lobes	0.3–0.6 cm	0.2–0.5 cm
Stalk of the central lobe of the basal leaves	Absent	0.7–1.2 cm / absent
Flowering time	Early May to early June	Early May to early June

Results

The data matrix of *rbcL* sequences included 486 bp, *matK* – 775 bp and ITS2 – 214–215 bp. The concatenated data matrix of *rbcL*+*matK*+ITS2 sequences included 1,476 bp.

We reconstructed molecular phylogenetic trees using BI and ML analyses and obtained similar topologies for concatenated dataset trees (*rbcL*, *matK* and ITS2), including 37 terminals of *Pulsatilla* species and ten outgroups (Fig. 4). *Pulsatilla alpina* (subgenus *Preonanthus*) and *P. kostyczewii* (subgenus *Kostyczewianae*) were the first two splits within the genus *Pulsatilla*, respectively. All closely-related taxa from *P.* section *Pulsatilla* (*P. patens* s.str., *P. uralensis*, *P. patens* subsp. *multifida*, *P. vernalis* and *P. vulgaris*) and *P.* section *Semicampanaria* (*P. turczaninowii*, *P. chinensis*, *P. cernua* and *P. dahurica*) of the subgenus *Pulsatilla* belong to a clade with a high support (95% BS and 1 PP). *P. vulgaris* and the section *Semicampanaria* belong to a clade with an elevated bootstrap value (78% BS and 0.72 PP). *Pulsatilla patens* s.str. (samples No. 5, 15–20 and 30) and *P. vernalis* belong to a clade with a low bootstrap value (56% BS). We were not able to establish relationships for the remaining samples of *P. patens* s.str. (sample numbers 1, 4, 9, 10 and 27) and *P. uralensis* (sample No. 2, 3, 6, 7, 8, 12–14, 21–23, 25, 28, 29 and 31) from different geographic locations (Table 2), since they arose as terminals from a polytomy.

Trees that were constructed based on the sequences of single genes had a low resolution. On the phylogenetic tree of the plastid region *rbcL*, all species of the subgenus *Pulsatilla* were also united into one clade (see Suppl. material 1). The exceptions are the apical clade represented by two sequences of *P. cernua* and *P. chinensis* (63% BS and 0.91 PP) and the subclade represented by eight sequences of *P. patens* s.str. (samples No. 5, 15–20 and 30) and *P. vernalis* (FBPL086-12) (65% BS and 0.82 PP). The molecular analysis of the sequences showed that samples of *P. patens* s.str. (No. 5, 15–20 and 30) differ from the others by one variable site in the *rbcL* region (A/G₆₁) (see Suppl. material 2). Comparison of the *matK* and ITS2 sequences could not separate *P.* subgenera *Pulsatilla*, *Kostyczewianae* and *Preonanthus* (see Suppl. material 3, 4). On the *matK* phylogenetic tree, the same clade is distinguished, including eight blue-flowered samples of *P. patens* s.str. (5 and 15–20), however, with low support (0.67 PP). For such samples, one variable site (C/T₄₇₂) was identified (Suppl. material 2).



Figure 4. Combined Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic tree (*rbcl*+*matK*+ITS2) of 37 *Pulsatilla* samples and 10 outgroup samples. All new 28 samples of *P. patens* s.str. and *P. uralensis* are marked with dots. Outgroups include *Anemone*, *Anemoclema*, *Clematis* and *Hepatica* species. ML bootstrap support (left) and BI posterior probability (right) are recorded along branches. Values below 50% are not shown.

Discussion

Global climate perturbations throughout the Quaternary period caused active migrations of *Pulsatilla* within Eurasia, followed by secondary polyploidy and increased polymorphism (Sramko et al. 2019). The ancestor of the modern *P. patens* s.lat. popu-

lations inhabiting north-eastern European Russia most likely grew in the steppes of Eastern Eurasia (Sramko et al. 2019). During the Pleistocene, the ancestor of the modern *P. patens* s.lat., perhaps, together with the southern Siberian forest-steppe species, spread to the west and further north along the Ural Mountains (Knyazev et al. 2007, Kulikov et al. 2013). Climate change associated with the Quaternary period contributed significantly to the diversification of *P. patens* s.lat. The disruption of the continuous distribution of species by the Pleistocene glaciations led to geographical disjunction and formation of a number of allopatric morphological forms in the European and Asian parts of the range of the species (Bobrov 1944; Tzvelev 2001; Ronikier et al. 2008; Kricsfalussy 2015 etc.). During the post-glacial colonisation, the ranges of migrants from several refugia started to touch or overlap in contact zones.

A part of the territory of north-eastern European Russia, including the Komi Republic, was covered by the last Late Pleistocene glaciations (Andreicheva 2002; Ilchukov 2010) and many of the known *Pulsatilla* locations in this region are the result of post-glacial colonisation. On the northern limit of the range, the most typical habitats for *P. patens* s.lat. are arid pine and mixed forests, forest clearings and edges. Large populations (up to 500–1,000 and more specimens) in the region are rare and are confined to lichen forests in the southern part of the Komi Republic (60–62°N, 51–54°E). During the Pleistocene, climate changes led to repeated Meridional displacements of vegetation zones (Andreicheva 2002). Evidence of these processes is provided by the fragments of a relict petrophytic floristic complex that currently exists on limestones in the north-east of European Russia, including the Timan limestones (Yudin 1963; Teteryuk et al. 2006). Small and isolated populations of *P. patens* s.lat. (up to 150–200 specimens) survived at the outcroppings of bedrocks; they grow together with the species of the relict petrophytic floristic complex in the river valleys of the Timan limestones (63–64°N, 52–53°E). Therefore, it is likely that some populations of *P. patens* s.str. (e.g. population VI), together with some species of bushes and herbaceous plants, survived during the last glaciations and are the part of more ancient vegetation compared to the adjacent flatland ecosystems that formed after the last ice-sheet glaciations.

Molecular phylogenetic analyses showed that all samples of *P. patens* s.str. and *P. uralensis* belong to a single clade and some groups within it arose with moderate statistical support, which makes it impossible to draw more definitive conclusions. Such low resolution is generally associated with *Pulsatilla* species (Li et al. 2019) and with specific factors related to the evolution of the species (by hybridisation and polyploidisation). Therefore, our discussion will be carefully constructed, given the low resolution that we obtained.

The obtained results of the molecular phylogenetic analysis (nuclear and chloroplast loci) are not consistent with the traditional morphological approach, according to which the isolation of *P. patens* s.str. and *P. uralensis* is based on the colour of the perianth – the distinctive feature on the level of species (Juzepczuk 1937; Tamura 1995; Tzvelev 2012; Egorova et al. 2017; amongst others). Only a small part of the samples belongs to a subclade (Fig. 4) that is represented exclusively by blue-flowered samples of *P. patens* s.str. (64% BS and 0.97 PP). This small subclade includes only blue-flowered

P. patens s.str. (5, 15–20) from the Komi Republic, *P. patens* s.str. (30) from the Orenburg Oblast of monochrome populations and European plant *P. vernalis* (MK550989, FBPL086-12). Such a relationship of the complex of *P. patens* s.lat. and *P. vernalis* with erect flowers and less dissected leaf blades was found by Sramko et al. (2019), who suggested to include *P. vernalis* into Series *Patentes*. Our phylogenetic trees showed that *P. vernalis* is close to *P. patens* s.str. (Fig. 4). These species are very different in morphology (e.g. *P. vernalis* has basal overwintering leathery leaves) and the range of *P. vernalis* does not currently cover north-eastern European Russia. It is an alpine species which, during the Ice Age in Europe, was widespread in the lowlands in sparse pine forests and heathlands. It is currently preserved in the Scandinavian refugia (Ronikier et al. 2008). Hybridisation and/or introgression between the ancestor of the modern forms of *P. patens* s.str. and *P. vernalis* may have occurred a long time ago during speciation.

According to our phylogenetic results, all the populations from different locations studied (except for population VI) belong to the same species. Even though ITS2, *matK* and *rbcL* were found to be suitable regions for barcoding in species of *Pulsatilla*, in our study, they did not allow us to isolate *P. uralensis* from monochrome populations in north-eastern European Russia and the Urals into a separate group. The absence of divergence in the tree (see Fig. 4) may indicate the hybrid origin of all yellow-flowered samples and the hybrid origin of modern *P. uralensis* populations. Our results indicate recent speciation and incomplete lineage sorting, resulting in very few accumulated genetic differences. This study suggests that *P. uralensis* should not be recognised as a separate species, despite its Ural-Siberian detached range, yellow flowers, and finer leaf lobes. Despite being morphologically identified by the colour of its perianth and leaf structure, our phylogenetic results do not support this distinction. Using the ITS2, *matK* and *rbcL* markers, we found only limited phylogenetic resolution within the *P.* subgenus *Pulsatilla*. Molecular data showed that the yellow-flowered taxon should be considered a subspecies within the *P. patens* s.lat. complex. At least in north-eastern European Russia and in the Urals, where its west distribution border passes, this taxon does not separate clearly from *P. patens* s.str. It is possible that the standard DNA barcode markers, ITS2, *matK* and *rbcL*, are not applicable within the *P. patens* complex and do not allow us to assess the divergence between these closely-related taxa.

Given the high morphological variability of *P. patens* s.lat. and the widespread presence of hybridogenic populations in the Urals in the Orenburg and Sverdlovsk Oblasts (Kulikov 2005, Sushentsov 2008) and the vicinity of the Komi Republic on the Russian Plain (Egorova et al. 2017), we can expect that many plants in the zone of overlapping of their ranges are hybrids. The morphological analysis also supports the opinion that *P. uralensis* is polymorphic with high variability of leaf characteristics (Sushentsov 2008). In order to clarify the species' limits of *P. uralensis*, a more detailed study with different methodological approaches is required, involving data on closely-related taxa from a wider area from Siberia and other parts of their range. This is beyond the scope of this study.

An indication of hybrid processes in *Pulsatilla* populations is their polychromy (Bobrov 1944). In greenhouse experiments, the hybrid progeny of *P. patens* s.lat. most

often has yellow, sometimes white and blue and rarely pink perianth (at a ratio of 4:2:1) (Pavlova 1990). Taking into account the prevalence of polychrome and monochrome yellow-flowered populations of *Pulsatilla* in the Komi Republic, we believe that an intricate complex with a significant participation of hybrid forms has formed in this territory, whereas the populations of *P. patens* s.str. have limited distribution (e.g. population VI). It is possible that there is an active process of introgression and “supplanting” of the European blue-flowered *P. patens* s.str., followed by the “absorption” of this species by the Ural-Siberian yellow-flowered *P. uralensis*.

The obtained data are consistent with the results of the study of the population variability of morphological features of *Pulsatilla* in the adjacent territory (Sushentsov 2008; Egorova et al. 2017). To the south, in the basin of the Vyatka River (57–58°N), we identified populations of *P. patens* s.str., as well as hybridogenous populations of *P. patens* s.str. × *uralensis* (Egorova et al. 2017). They occupy a different ecological niche, which is preserved within the territory of the Komi Republic. Yellow-flowered populations are common on the pine terraces in lichen pine forests. Such conditions (sandy biotopes favourable for cross-pollination and seed renewal with reduced competition from other plants) contribute to the manifestation of *Pulsatilla* polymorphism in hybridization zones. In the large monochrome yellow-flowered population No. V of *P. uralensis* in the pine forests of lichen type in the south of the republic, we found specimens with highly dissected leaves (samples No. 13, 14 and 21) that were also included in the clade of phylogenetic trees, together with the hybrid forms and *P. patens* s.str. Leaf-blade lobes of *P. uralensis* are finely dissected and the ultimate lobes (up to 43 pieces) are linear-lanceolate to narrowly linear, the central leaflet has an 8–12 mm petiolule.

Thus, using the sequences of chloroplast (*matK*, *rbcl*) and nuclear DNA (ITS2), we showed that, in north-eastern European Russia and the Urals, populations with yellow and blue-violet flowers belong to the same species (*P. patens* s.lat.). Isolated monochrome populations of *P. patens* s.str. with blue-violet flowers are preserved within the territory of the Komi Republic and have a limited distribution range.

Since there are still many questions about the taxonomy of *P. patens* s.lat. complex, we believe that, in order to improve the ability to distinguish closely-related taxa in this group, it is necessary 1) to analyze a large number of plants with an accurate identification of the main diagnostic characteristics; to scrutinise additional morphological and micro-morphological characteristics; 2) to obtain information on the location of the studied samples in the species range (the centre or the border of the range); to obtain information on their possible hybridisation and introgression with other species of the genus *Pulsatilla* on this territory, taking into account the factor of overlapping and the relict nature of the communities; 3) to obtain information on the abundance, distribution range and genetic structure of populations. The use of DNA barcoding process, geographically-expanded sampling and analysis of the genetic structure of populations of closely related *Pulsatilla* species are necessary to reconstruct the phylogenetic relationships between taxa of series *Patentes*.

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Supplementary material 1

Maximum Likelihood and Bayesian Inference phylogenetic tree (*rbcL*)

Authors: Olga E. Valuyskikh, Ludmila V. Teteryuk, Yana I. Pylina¹, Oleg E. Sushentsov, Nikita A. Martynenko, Dmitry M. Shadrin

Data type: phylogenetic tree

Explanation note: Maximum Likelihood and Bayesian Inference phylogenetic tree (*rbcL*) of 13 sequences of *P. patens* s.str. and 16 sequences of *P. uralensis*, plus the nine selected sequences in GenBank and BOLD Systems for different *Pulsatilla* species. Posterior probability and bootstraps are shown at the nodes, values below 50% are not shown.

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Link: <https://doi.org/10.3897/phytokeys.162.53361.suppl1>

Supplementary material 2

Informative nucleotide sites in cpDNA (*matK*, *rbcL*) for *Pulsatilla*

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Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.162.53361.suppl2>

Supplementary material 3

Maximum Likelihood and Bayesian Inference phylogenetic tree (matK) of 13 sequences of *P. patens* s.str. and 16 sequences of *P. uralensis*

Authors: Olga E. Valuyskikh, Ludmila V. Teteryuk, Yana I. Pylina¹, Oleg E. Sushentsov, Nikita A. Martynenko, Dmitry M. Shadrin

Data type: phylogenetic tree

Explanation note: Maximum Likelihood and Bayesian Inference phylogenetic tree (matK) of 13 sequences of *P. patens* s.str. and 16 sequences of *P. uralensis*, plus the nine selected sequences in GenBank and BOLD Systems for different species. Bootstraps and posterior probabilities are shown at the nodes, values below 50% are not shown.

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Supplementary material 4

Phylogenetic tree (ITS2) constructed using the Maximum Likelihood and Bayesian Inference of the 13 sequences of *P. patens* s.str. and 15 sequences of *P. uralensis*

Authors: Olga E. Valuyskikh, Ludmila V. Teteryuk, Yana I. Pylina¹, Oleg E. Sushentsov, Nikita A. Martynenko, Dmitry M. Shadrin

Data type: phylogenetic tree

Explanation note: Phylogenetic tree (ITS2) constructed using the Maximum Likelihood and Bayesian Inference of the 13 sequences of *P. patens* s.str. and 15 sequences of *P. uralensis*, plus the nine selected sequences in GenBank and BOLD Systems for different species. Bootstraps and posterior probabilities are shown at the nodes, values below 50% are not shown.

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