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Study of terrestrial diatoms in corticolous assemblages from deciduous trees in Central Europe with descriptions of two new *Luticola* D.G.Mann taxa

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Abstract

Although many studies have examined the algae associated with various habitats in tree trunks, the diatoms in these environments are still poorly studied. Studies of corticolous algae mainly focus on green algae and cyanobacteria, which are usually immediately visible, while diatoms are mostly overlooked or not reported. During the research, 143 species of diatoms were identified, including two new representatives of the genus *Luticola: L. bryophila* **sp. nov.** with relatively large central area and short distal raphe endings and *L. confusa* **sp. nov.** characterized by the presence of small depressions on central raphe endings. Both are described herein based on light and scanning electron microscopy observations and compared to similar taxa based on literature data. Basic morphological data for almost all the diatom taxa are noted, and their habitat requirements, and photographic documentation are also presented. The present research showed that the occurrence of diatom assemblages on tree trunks is influenced by various factors like host tree species, the area where the host tree grows, and the availability of suitable microhabitats within the trunk. However, the species composition of this assemblages depends mainly on the tree species.

Keywords

Bacillariophyceae, bark chemistry, bryosphere, corticolous habitats, diversity, taxonomy

Introduction

Despite over a century of study on terrestrial algae (West and West 1894; Fritsch 1907; Petersen 1915, 1928, 1935; Beyens 1989), less is still known about these ecological groups than is known about algae from aquatic environments. Most research on terrestrial algae is floristic (Dodd and Stoermer 1962; Schlichting 1975; Camburn 1983; McMillan and Rushforth 1985; Hoffmann 1989), while few works focus on their ecology (Ress 2012; Foets et al. 2021).

Many different terms are used to name algal assemblage in their terrestrial environments depending on environmental conditions and available water sources (Ress 2012; Furey et al. 2020). Bark-associated assemblages that inhabit raised objects that receive moisture exclusively from the atmosphere are categorized as euaerial. The terms epiphylophyte and epiphloeophyte are used to refer to bark-associated algae. The former describes algae growing on leaf surfaces, while the latter describes algae inhabiting tree bark often in association with bryophytes and lichens (Chapman and Chapman 1973; Awasthi 2015; Sivakumar 2016). The term corticophiles is also sometimes used in reference to algal communities growing directly on tree barks (Kawecka and Eloranta 1994; Bhakta et al. 2014; Narasimha Rao 2017).

Bark surfaces create special microclimatic niches for algae. Thanks to cracks, they retain moisture, protect against wind, and provide shade and nutrients that are compounds from accumulated dust which dissolve in rainwater (Kawecka and Eloranta 1994). Algae inhabiting tree trunks have been the subject of a few studies, most of which focused on green algae and cyanobacteria (Foerester 1971; Wylie and Schlichting 1973; Mrozińska 1990; Thompson and Wujek 1997; Salleh and Milow 1999; Czerwik and Mrozińska 2000; Neustupa 2003, 2005; Soni and Shukla 2006; Neustupa and Škaloud 2008, 2010; Lemes-Da-Silva et al. 2010; Bhakta et al. 2014; Kharkongor and Ramanujam 2014; Štifterová and Neustupa 2015; Narasimha Rao 2017; Ambika and Krishnamurthy 2018). In research on bark-associated algae, diatoms have been reported rarely to date and are usually only listed as single taxa in species' lists (Neustupa and Škaloud 2010; Neustupa and Štifterová 2013; Kharkongor and Ramanujam 2014; Štifterová and Neustupa 2015; Nirmala et al. 2016; Eldrin 2019). Lakatos and co-authors (Lakatos et al. 2004) studied diatoms inhabiting the thallus of the arboreal lichen *Coenogonium linkii* Ehrenb., and during this research they noted 18 taxa. Research conducted by Qin et al. (2016) was focused on diatoms associated with arboreal bryophytes growing on trees in Wuhan, and they collected samples mainly from *Cinnamomum camphora* Ness & Eberm and they noted 76 taxa represented 13 genera. Studies on diatoms inhabiting arboreal mosses from the Indo-Burma biodiversity hot spot showed the presence of 56 species from 21 genera (Cheran et al. 2022). The only work from Europe is based on preliminary results from samples collected from a single tree Despite the small number of samples, 47 species have been identified (Rybak et al. 2018). In all these works, each studied sample contained only few species (usually fewer than 20) and were dominated by species from genera like: *Luticola*, *Humidophila*, *Pinnularia* and *Orthoseira*.

The aim of the study was to investigate the taxonomic diversity and ecological requirements of diatoms inhabiting various microhabitats on trunks of deciduous trees in Central Europe in areas confronting various degrees of human impact. Additionally, preferences of diatoms for host tree species and microhabitats within trunks were determined.

Methods

Samples were collected in 2017 and 2018 four times each year in the first half of April and June, and the second half of August and October from heights of 20 cm (referred to as trunk base) and 150 cm above ground level from several tree trunk microhabitats, i.e., bare bark, moss clumps, bark covered with lichens, bark with visible mats of algae (Fig. 1), at eight sampling sites and from the same trees of the following taxa: sycamore maple (*Acer pseudoplatanus* L.), linden (*Tilia* spp. L.), and poplar (*Populus* spp. L.). The designated sites included city centers (sites 1 and 4), small peripheral estates (sites 2 and 5), park complexes in suburban areas (sites 3 and 6), and the buffer zones of two national parks: Magurski National Park (site 7) and Gorczański National Park (site 8) (Table 1). Samples in form of bark pieces, together with overgrowing epiphytes, were chipped off using a hammer and chisel and placed in paper envelopes to avoid mold. During the collection of materials, no visible signs of the presence of diatoms (like slime or different color stains) were noted on the surface.

The samples were also used to prepare filtrates for pH and conductivity analyses. The filtrates were obtained by soaking bare bark pieces in deionized water (1:10 by weight) for 24 hours. The intact pieces of bark were used to obtain a solution similar to that forming on the bark surface that is a source of water and nutrients for corticolous organisms; in the case of trees completely covered by epiphytic mosses, the material together with them was used to obtain filtrates. Electrolytic conductivity and pH were measured with a MARTINI pH56 pH meter and a MARTINI EC59 conductometer (Milwaukee Electronics Kft.). The ions' content was determined using a Thermo scientific DIONEX ICS–5000+DC device in the Departmental Laboratory of Analysis of Environmental Health and Materials of Agricultural Origin at the University of Rzeszów.

A modified method by Qin et al. (2016) was used to obtain clean diatom material. For the purpose of separating bryophytes and diatoms from the bark surface, a part of the material collected was placed in beakers to which 50 ml 30% hydrogen peroxide (H_2O_2) was added, and these were left at room temperature for 48 hours. In the next step, bark fragments were rinsed with deionized water, and the resulting solution was collected in the same beaker in which the bark was digested. To obtain clean diatom valves, the solution was centrifuged to remove excess hydrogen peroxide and again digested in a mixture of sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$) until the organic matter was completely dissolved. In the last step, the burning mixture was removed by centrifugation with distilled water (at 2500 rpm).

Light microscope slides were prepared by applying the cleaned diatom suspension to cover-slips that were left to dry. The dried material was mounted in synthetic

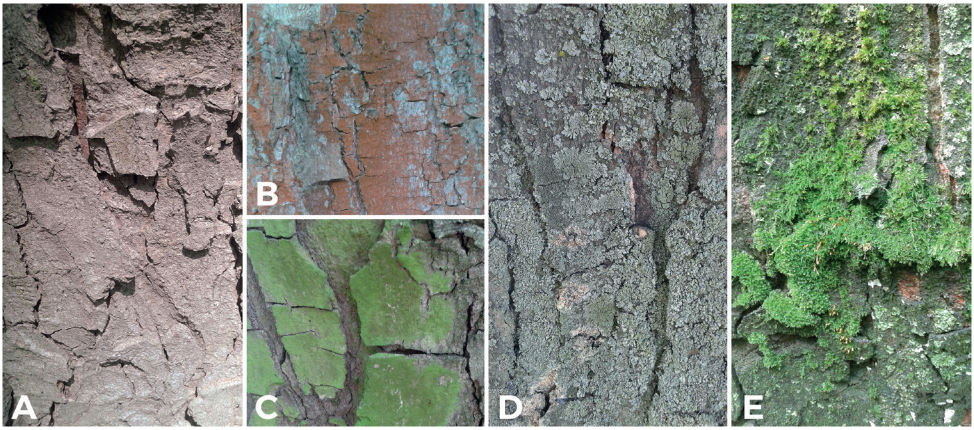

Figure 1. Types of studied microhabitats on the example of sycamore maple. Bare bark (**A**), bark covered by green algae (**B, C**), bark with lichen covering (**D**) and bark covered by bryophytes (**E**).

Table 1. List of studied sites with the sampled tree taxa and microhabitat type over their trunk.

Site	Tree species	Coordinates	Studied microhabitat
1	*Acer pseudoplatanus* L.	50°34'09.1"N, 22°03'57.5"E	bare bark, lichens
	Tilia platyphyllos Scop.	50°34'04.1"N, 22°04'01.9"E	bare bark, lichens
	Populus nigra L. 'Italica'	50°34'08.5"N, 22°03'56.8"E	bare bark, mosses, lichens
2	*Acer pseudoplatanus* L.	50°34'12.1"N, 22°04'20.4"E	lichens
	Tilia cordata Mill.	50°34'12.2"N, 22°04'20.8"E	lichens
	Populus nigra L.	50°34'16.4"N, 22°04'18.6"E	bare bark, mosses, lichens
3	*Acer pseudoplatanus* L.	50°36'13.2"N, 22°02'01.7"E	bare bark, mosses, green algae mats
	Tilia cordata Mill.	50°35'58.2"N, 22°01'49.7"E	bare bark, mosses
	Populus nigra L.	50°35'59.0"N, 22°01'49.6"E	bare bark, mosses
4	*Acer pseudoplatanus* L.	50°01'06.7"N, 22°01'01.5"E	bare bark, lichens
	Tilia platyphyllos Scop.	50°01'18.4"N, 22°00'56.7"E	bare bark, lichens
	Populus nigra L. 'Italica'	50°01'18.8"N, 22°01'00.6"E	bare bark, mosses, lichens
5	*Acer pseudoplatanus* L.	50°00'08.9"N, 22°01'54.1"E	bare bark, mosses
	Tilia cordata Mill.	50°00'11.0"N, 22°01'54.1"E	bare bark, mosses
	Populus nigra L.	50°00'09.5"N, 22°01'48.3"E	bare bark, mosses, lichens, green algae mats
6	*Acer pseudoplatanus* L.	50°00'36.3"N, 21°59'16.0"E	bare bark, mosses
	Tilia cordata Mill.	50°00'39.1"N, 21°59'20.5"E	bare bark, mosses
	Populus alba L.	50°00'42.6"N, 21°59'23.6"E	bare bark, mosses, green algae mats
7	*Acer pseudoplatanus* L.	49°30'10.3"N, 21°29'31.1"E	bare bark, mosses, green algae mats
	Tilia cordata Mill.	49°30'42.8"N, 21°29'57.0"E	bare bark, mosses, lichens
	Populus tremula L.	49°30'58.1"N, 21°29'38.3"E	bare bark, mosses
8	*Acer pseudoplatanus* L.	49°37'01.8"N, 20°04'07.0"E	bare bark, mosses, lichens
	Tilia cordata Mill.	49°37'01.4"N, 20°04'06.7"E	bare bark, mosses, lichens
	Populus nigra L.	49°37'02.8"N, 20°04'14.3"E	bare bark, mosses, lichens

Pleurax resin, Brunel Microscopes ltd. (refractive index 1.75). To better define the species composition of the assemblages analyzed, two microscope samples on coverslips were mounted on a single slide. In total, 647 samples were collected and analyzed. The diatoms were identified under a Carl Zeiss Axio Imager.A2 light microscope (LM) with a Zeiss AxioCam ICc 5 camera at 1000× magnification. For Scanning Electron Microscope (SEM) observations, the samples were coated in a Turbo-Pumped Sputter

Coater Quorum Q 150OT ES with a 20 nm layer of gold and viewed under a Hitachi SU 8010 microscope.

Two microscope slides were made from each collected sample. Diatoms were identified in both slides by observations in all possible adjacent transects. During species identification, all identified valves were counted until a number of 400 was reached. Identification of species was continued for species composition also after reaching the assumed limit of 400 valves. The dominance structure and similarity analysis were determined only for samples for which a minimum of 200 valves were counted. Species with a minimum share of 10% in the assemblages were considered dominants. The remaining samples were considered unrepresentative because of the insufficient development of assemblages or their complete absence. To present the morphological variability of the observed taxa, valves dimensions were measured under light microscope using AxioVision SE62 Rel. 4.9.1 software. For range dimension of commonly occurred taxa ca. 50 specimens were measured including the biggest and the smallest observed specimens. In the case of rare taxa (observed in <10 samples) each observed specimen was measured.

Diatom diversity was analyzed using the Shannon diversity index (H') and the Evenness index (J'). Principal Component Analysis (PCA) was performed to determine the similarity of diatom assemblages. Prior to analysis, diatom data were square root transformed. Redundancy analysis (RDA, gradient length = 1.9 SD) was performed to determine the effect of bark chemistry on diatom assemblages, but none of the parameters showed statistically significant effects on the assemblages studied ($p > 0.05$). All analyses (PCA, RDA) were performed using Canoco 5 (Ter Braak and Šmilauer 2012).

Student's t-test was used to analyze the significance of differences in the chemical parameters of the samples, and values at $p<0.05$ were considered statistically significant. All calculations were performed using Statistica 13.3 software.

Diatom terminology and identification were based on Round et al. (1990), Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Krammer (2000), Lange-Bertalot (1993), Lange-Bertalot et al. (2003, 2011, 2017), Levkov et al. (2013, 2017, 2019) and Houk et al. (2017).

Results

Chemical analyses

The pH of the analyzed filtrates indicated slightly acid to neutral condition of the barks of the tree species examined. The electrolytic conductivity values measured in the filtrates of all the trees analyzed indicated a very wide range (from 49 µm cm^{-1} to 5 846 µS cm^{-1}), regardless of sampling height (Table 2). Statistically significant differences in the parameters measured for samples taken from heights of 20 and 150 cm were noted for pH in linden and poplar and for electrolytic conductivity in sycamore maple. On the other hand, the analysis of chemical parameters of permeates depending on tree species was not statistically significantly different. The parameters measured did not differ significantly among the tree species studied (Table 2).

Table 2. Chemical parameters measured in filtrates obtained from bark of studied trees, given range (minimum and maximum), and median (brackets), bold indicates value for samples from trunk bases. * – indicates a parameter in which differences between the studied heights were statistically significant ($p > 0.05$).

Tree taxon	*Acer*	*Tilia*	*Populus*
pH	5.3–7.3 (6.3)	4.7–7.3 (6.0)*	4.8–7.5 (6.3)*
	4.8–7.5 (6.4)	**4.8–7.5 (6.4)***	**5.1–7.4 (6.5)***
Cond. [µS cm^{-1}]	49–2305 (167)*	49–462 (202)	52–2305 (323.1)
	64–604 (228)*	**69–5846 (338)**	**39–1199 (332.1)**
Cl^- [mg/l]	0.5–29.9 (3.3)	1.1–20.7 (6.3)	0.442–103.7 (9.4)
	0.5–14.5 (3.3)	**0.2–71.7 (9.0)**	**0.232–98.6 (8.8)**
NO_3^- [mg/l]	<0.001–4.6 (1.5)	<0.001–4.6 (2.5)	<0.001–159.3 (3.4)
	<0.001–16.5 (1.2)	**<0.001–89.9 (5.2)**	**<0.001–42.2 (2.3)**
PO_4^{3-} [mg/l]	<0.001–44.8 (11.1)	<0.001–34.8 (10.1)*	<0.001–36.7 (8.8)*
	2.7–42.5 (14.1)	**<0.001–39.2 (16.3)***	**<0.001–64.0 (14.3)***
SO_4^{2-} [mg/l]	0.7–55.5 (7.2)	<0.001–61.5 (18.6)	<0.001–400.9 (20.9)
	0.9–14.9 (6.5)	**0.6–3 278.2 (66.7)**	**0.2–10.7 (20.8)**
Na^+ [mg/l]	0.3–12.4 (2.5)	0.4–12.2 (3.1)*	0.273–59.2 (6.8)
	0.3–18.5 (3.4)	**0.3–21.0 (5.7)***	**0.3–55.3 (7.4)**
K^+ [mg/l]	10.2–137.9 (33.7)	9.509–68.1 (35.9)	7.8–752.2 (86.3)
	11.2–161.4 (38.4)	**<0.001–1510.2 (65.8)**	**<0.001–386.3 (75.2)**
Ca^{2+} [mg/l]	0.6–48.2 (6.7)	0.1–26.4 (10.6)	0.3–56.5 (8.5)
	0.2–34.7 (8.0)	**0.3–317.7 (16.4)**	**0.3–28.9 (8.7)**
Mg^{2+} [mg/l]	0.2–18.5 (2.4)	0.3–10.6 (2.7)	0.1–37.1 (3.5)
	0.1–14.0 (2.6)	**0.6–62.8 (3.8)**	**0.2–10.7 (3.0)**
NH_4^+ [mg/l]	<0.001–28.5 (3.8)*	0.1–23.7 (5.6)	<0.001–12.3 (2.3)*
	0.7–32.8 (7.3)*	**<0.001–228.9 (12.7)**	**<0.001–103.0 (5.8)***

Taxonomic results

Description of new species

Luticola bryophila M.Rybak, Czarnota & Noga, sp. nov.

Fig. 2

Description. Rectangular in girdle view. Valves linear to linear lanceolate with weakly protracted and moderately rounded apices, smaller specimens without protracted apices. Valve length 10–25 µm, and valve width 4–6 µm. Axial area narrow linear, slightly expanded near central area. Central area wide and rounded, bordered by 3–4 areolae. Ghost areolae commonly present in central area. Round isolated pore located half way between valve center and margin. Raphe branch straight with both endings clearly curved to site opposite to isolated pore. Distal raphe endings short, not continuing onto mantle. Internal raphe slit simple and straight, distal endings form slightly developed helictoglossae. Striae number 18–20 in 10 µm composed of 2–3 same sized, rounded to slightly elongated areolae. Single row of areolae present also on valve mantle. Internally areolae covered by hymen forming continuous strip, separated by not thickened virgae. Internally small lipped opening of isolated pore visible. Marginal channel located on valve face/mantle junction occluded by hymenes and visible internally.

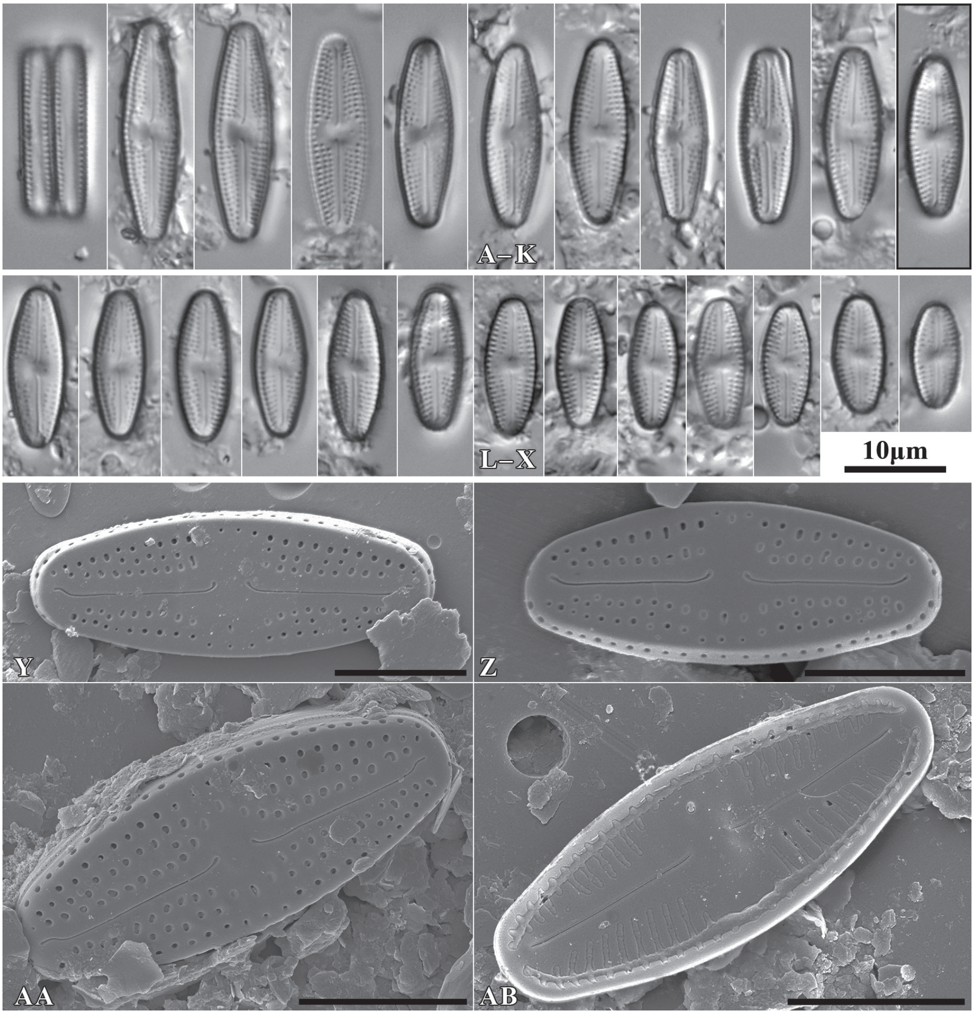

Figure 2. *Luticola bryophila* sp. nov., LM microphotographs of girdle view (**A**) and size diminution series (**B–X**), black frame indicate the holotype specimen. SEM microphotographs (**Y–AB**) of valve in external (**Y–AA**) and internal view (**AB**). Scale bars: 10 µm (**A–X**); 5 µm (**Y–AB**).

Type material. ***Holotype*:** Slide SZCZ 28844 and unmounted material with the same number stored in A. Witkowski Diatom Collection of the Institute of Marine and Environmental Sciences, University of Szczecin, holotype specimen designated here in Fig. 3K

***Isotype*:** Slide no. 2017/18 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Stalowa Wola, Podkarpacie Province, Poland, 50°34'16.4"N, 22°04'18.6"E, leg. M. Rybak.

Etymology. The name refers to the occurrence of the species mainly in terrestrial bryophytes.

Distribution. Species observed at most of the sites studied, always in single specimens, mainly in samples of bryophytes from trunk bases.

Similar species. *Luticola sparsipunctata* Levkov, Metzeltin & Pavlov (Levkov et al. 2013, p. 222); *Luticola tenuis* Levkov, Metzeltin & Pavlov (Levkov et al. 2013, p. 236).

Luticola confusa M.Rybak & Czarnota, sp. nov.

Fig. 3

Description. Valves elliptic to elliptic-lanceolate with rounded apices, rectangular in girdle view. Valve length 9–22 µm, and valve width 4.5–5.5 µm. Axial area narrow and linear, central area elliptic bordered by 3–4 areolae. Round isolated pore located halfway between valve center and margin. Raphe branch straight. Proximal raphe endings deflected to site opposite to stigma with small rounded depressions. Distal raphe endings hooked continuing onto valve mantle. Internally raphe slit simple and straight, distal endings form slightly developed helictoglossae. Striae number 20–22 in 10 µm composed mainly of 4 areolae, single row of areolae also present on valve mantle. On apices row of mantle areolae interrupted by distal raphe endings. Internally areolae covered by hymen forming continuous strip, separated by not thickened virgae. Internally small lipped opening of isolated pore visible. Marginal channel located on valve face/mantle junction occluded by hymenes and visible internally.

Type material. ***Holotype*:** Slide SZCZ28845 and unmounted material with the same number stored in A. Witkowski Diatom Collection of the Institute of Marine and Environmental Sciences, University of Szczecin, holotype specimen designated here in Fig. 4O.

***Isotype*:** Slide no. 2018/454 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Stalowa Wola, Podkarpacie Province, Poland, 50°36'13.2"N, 22°02'01.7"E, leg. M. Rybak.

Etymology. The name refers to possible past confusions in identification of the species described with other small taxa with elliptic-lanceolate valves.

Distribution. Species observed at all sites studied, always in the form of individual specimens. It mainly occurred in samples taken from the base of the trunks of the trees studied.

Similar species. *Luticola imbricata* (W.Bock) Levkov, Metzeltin and Pavlov (Levkov et al. 2013, p. 134); *L. pseudoimbricata* Levkov, Metzeltin & Pavlov (Levkov et al. 2013, p. 134); *L. obscura* Levkov, Tofilovska, C.E.Wetzel, Mitić-Kopanja & Ector (Levkov et al. 2017).

Diversity and composition of diatom assemblages

During the study 143 diatom taxa representing 39 genera were identified (Table 3, Figs 2, 3, 5–22). Most of them belonged to the genera *Luticola* (27), *Pinnularia* (13),

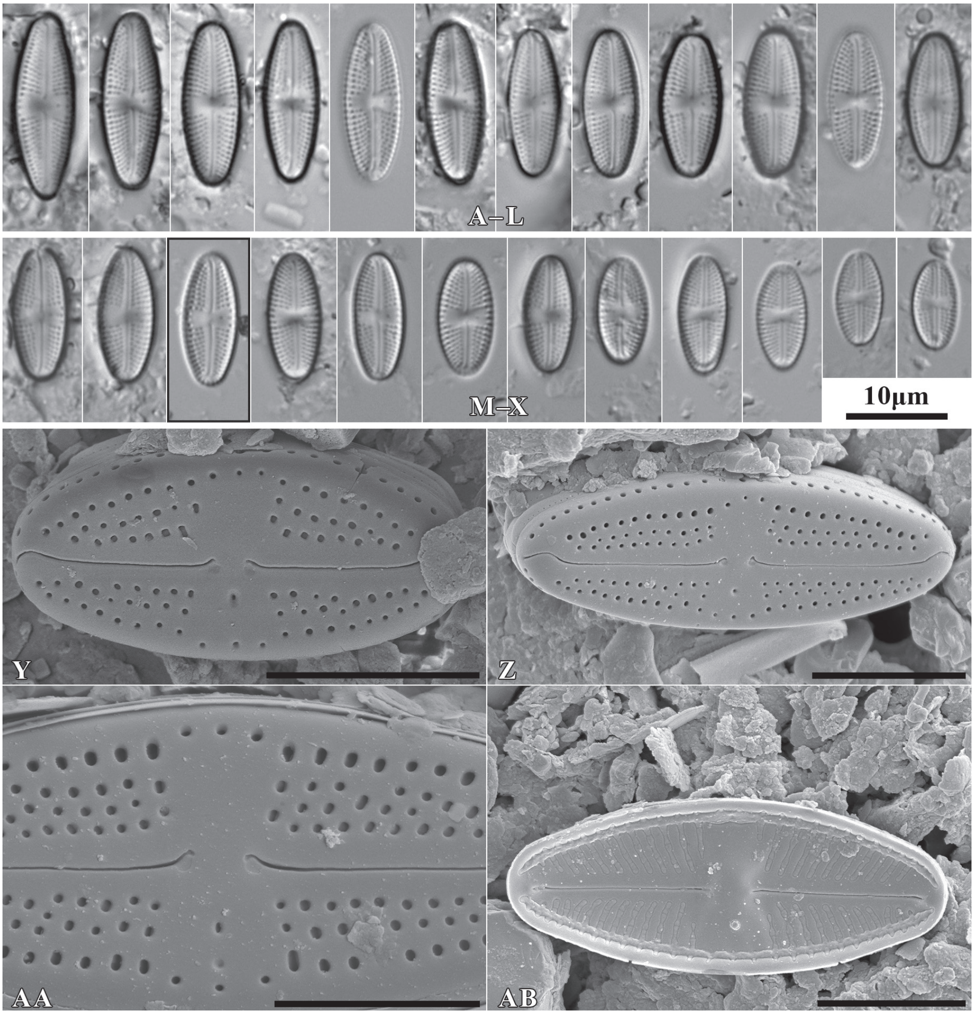

Figure 3. *Luticola confusa* sp. nov., LM microphotographs of size diminution series (**A–X**), black frame indicate the holotype specimen. SEM microphotographs (**Y–AB**), valve in external (**Y–AA**) and internal view (**AB**). Scale bars: 10 µm (**A–X**); 5 µm (**Y, Z, AB**),

and *Stauroneis* (10) (Table 3). Among the taxa identified, only a few species were found commonly in the materials studied and had noticeable shares in the communities. In most of the trees studied significantly more species were recorded in samples taken from the trunk bases (Fig. 4).

Of the 647 samples collected, only in 197 were numerous occurrences of diatoms observed. Diatoms did not occur, or occurred only, as individual valves in all samples from barks covered with lichens or algal mats. Numerous diatom assemblages were observed in 74 of 283 samples from bare bark (27 from 20 cm above ground level and

Table 3. Complete list of documented diatom taxa with measured dimension ranges (**Length / Width; striae/10µm**), frequency of occurrence in samples (**Freq.** [%]), minimum and maximum relative abundance (**Min-Max**) at sycamore maples (***Acer***), lindens (***Tilia***) and poplars (***Populus***). Bold indicates data from samples collected at 20 cm above ground level. + idicates observation of species in sample with unrepresentative assemblages.

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	Min-Max	Freq.	Min-Max	Freq.	Min-Max
Achnanthes coarctata (Brébisson & W.Smith) Grunow	15–32 / 4–7; 11–13	21 **8**	0.21–0.48 **0.30–0.34**	22 **25**	0.31–0.32 **0.10–2.47**	27 **20**	0.22–1.42 **0.19–1.38**
Achnanthidium minutissimum (Kützing) Czarnecki	5–18 / 3; 21–22	11 **9**	0.24–0.48 **0.21–0.31**	4 **12**	+ **0.32–7.41**	8 **15**	– **0.19–0.49**
Amphora pediculus (Kützing) Grunow	7–10 / 2.7–3; 17–18	– **1**	– **0.32**	– **5**	– **0.41**	– **1**	– +
Aulacoseira spp.	–	14 **20**	0.25–0.31 **0.07–0.23**	9 **16**	0.20 **0.18–0.32**	5 **10**	0.12–0.34 **0.17–0.33**
Caloneis aerophila W.Bock	12–19 / 3.5–4.5; 19–23			1 **–**	+ **–**		
Caloneis lancettula (Schulz) Lange-Bertalot & Witkowski	13–25 / 4–4.5; 23–26	4 **2**	0.37–0.48 **0.17–0.21**	1 **3**	+ **+**	2 **3**	– **0.65**
Caloneis* cf. *langebertalotioides Reichardt	17–26 / 4.5–5; 26	1 **–**	0.31 **0.25**	– **1**	+ **0.41**	**1** **3**	+ **0.17–0.29**
Caloneis leptosoma (Grunow) Krammer	23.5–32.5 / 5–6; 14–16	– **1**	– **0.26**	– **1**	– **+**		
Caloneis vasileyevae Lange-Bertalot, Genkal & Vekhov	12–18 / 4–4.5; 27–30			1 **–**	+ **–**		
Cavinula cocconeiformis (W.Gregory & Greville) D.G.Mann & A.J.Stickle	21–23.5 / 10; 27	1 **–**	0.30 **–**				
Cocconeis euglypta Ehrenberg	8–15 / 7–10; 14–22			1 **6**	+ **+**	1 **–**	+ –
Cocconeis lineata Ehrenberg	8–15 / 8–11; 14–26	5 **1**	0.26–0.37 **0.17**	4 **9**	+ **0.31–2.47**	2 **3**	0.32 **0.23**
Cocconeis pediculus Ehrenberg	17–26 / 8–11; 18			2 **4**	+ **0.32**	– **1**	– **0.47**
Cocconeis pseudolineata (Geitler) Lange-Bertalot	7–20 / 7–11; 15	– **1**	– **0.29**	2 **1**	+ **+**	– **1**	– **0.23**
Cosmioneis pusilla (W.Smith) D.G.Mann & A.J.Stickle	22.5–26 / 13–15; 15–16					1 –	+ **–**
Cyclostephanos dubius (Hustedt) Round	–	22 **17**	0.16–0.50 **0.22–0.48**	19 **26**	+ **0.31–2.47**	14 **8**	0.22–0.37 **0.17–0.37**
Epithemia adnata (Kützing) Brébisson	17–22.5 / 7–9; 14	3 **2**	0.21–0.33 **0.17–0.23**	– **1**	– **0.41**	1 **2**	+ **0.49**
Eunotia botuliformis F.Wild, Nörpel & Lange-Bertalot	6–26 / 3–3.5; 16–19	– **8**	– **0.21–0.37**	– **1**	– **+**		
Eunotia minor (Kützing) Grunow	12.5–31.5 / 4–6; 10–12			1 **–**	+ **–**	– **1**	– **0.33**
Fallacia enigmatica (H.Germain) Lange-Bertalot & Werum	8.8 / 2–2.2; not visible in LM			– **1**	– **+**	2 **–**	– +
Fallacia insociabilis (Krasske) D.G.Mann	6.5–21 / 4.5–6.5; 22–25	2 **11**	0.31–0.66 **0.17–1.30**	1 **6**	– **0.41**	2 **3**	0.47 **0.22–0.49**
Geissleria ignota (Krasske) Lange-Bertalot & Metzeltin	22.5–24 / 5; 13–14					– **1**	– **0.16**
Gomphonella olivacea (Hornemann) Rabenhorst	12–23 / 5–6; 10–11	1 **–**	0.32 **–**	– **1**	– **+**	– **1**	– **+**
Gomphonema acuminatum Ehrenberg	22 / 7; 12	1 **–**	0.24 **–**	– **1**	– **+**		
Gomphonema amoenum Lange-Bertalot	30–35 / 9–10; 10–11	1 **1**	0.31 **0.32**			– **1**	– **0.24**

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	**Min-Max**	**Freq.**	**Min-Max**	**Freq.**	**Min-Max**
Gomphonema drutelingense Reichardt	20–24.5 / 6.5–7;					–	–
	11					**1**	+
Gomphonema micropus Kützing	20–26 / 7–7.5;	2	0.13	1	+	1	+
	10–12	**4**	**0.26–0.29**	**7**	**0.20–1.23**	**4**	**0.32–0.93**
Gomphonema parvulum (Kützing) Kützing	10–15 / 6–7;	1	0.24–	–	–	2	–
	11–13	**–**		**2**	**+**	**1**	**0.31**
Halamphora montana (Krasske) Levkov	12–20 / 3.5–5.5;	18	0.20–0.34	13	0.31–0.32	16	0.17–3.79
	not visible in LM	**21**	**0.17–2.08**	**16**	**0.10–0.41**	**16**	**0.17–0.65**
Hantzschia abundans Lange-Bertalot	35–74.5 / 6–9.5;	47	0.21–2.81	51	0.30–0.61	51	0.12–6.16
	19–20	**43**	**0.17–9.95**	**77**	**0.20–2.40**	**65**	**0.16–6.92**
Hantzschia amphioxys (Ehrenberg) Grunow	12–45 / 4.5–7;	88	0.20–23.13	84	0.30–2.76	87	0.12–93.90
	21–27	**94**	**0.14–58.82**	**99**	**0.20–29.94**	**91**	**0.16–88.10**
Hantzschia calcifuga Reichardt & Lange-Bertalot	28–85 / 6.5–7.7;	18	0.21–23.18	20	+	18	0.17–0.47
	16–19	**37**	**0.18–4.02**	**25**	**0.18–4.12**	**22**	**0.22–21.80**
Hantzschia dorgaliensis Lange-Bertalot, Cavacini, Tagliaventi & Alfinito	60 / 6.7;					–	–
	18–19					**1**	**+**
Hantzschia **aff.** ***stepposa*** Maltsev & Kulikovskiy	33 / 5;					–	–
	25					**1**	**+**
Hantzschia subrupestris Lange-Bertalot	26.7–81 / 6.7–9;	9	0.25–0.66	10	+	13	0.35–0.95
	14–17	**15**	**0.18–2.99**	**16**	**0.20–0.65**	**20**	**0.16–1.01**
Humidophila brekkaensis (Petersen) Lowe et al.	8–19.5 / 6–6.5;	1	–	1	+	2	–
	not visible in LM	**5**	**0.24–12.5**	**5**	+	**3**	**0.34**
Humidophila contenta (Grunow) Lowe et al.	5–13 / 2.5–3.2;	53	0.20–23.13	32	7.50–58.51	40	0.56–59.25
	not visible in LM	**68**	**0.02–34.05**	**52**	**0.30–79.22**	**72**	**0.23–81.77**
Humidophila gallica (W.Smith) Lowe et al.	4–10 / 2–3;	2	0.31	9	0.20	10	0.35–10.90
	not visible in LM	**3**	**0.22–0.34**	**7**	**+**	**15**	**0.22–4.12**
Humidophila irata (Krasske) Lowe et al.	15–21 / 6.2–6.5;	6	0.24–0.31	2	+	2	0.24
	not visible in LM	**25**	**0.17–1.01**	**17**	**0.10–0.41**	**6**	**0.19–0.22**
Humidophila perpusilla (Grunow) Lowe et al.	6–13.5 / 4–5;			1	+	5	0.84
	not visible in LM			**2**	+	**10**	**2.21–19.06**
Lemnicola hungarica (Grunow) Round & Basson	8–13 / 4–6;	–	–	1	+	–	–
	18–21	**1**	**0.25–0.34**	**2**	+	**1**	**0.49**
Luticola acidoclinata Lange-Bertalot	10–32 / 4.8–8.7;	81	0.31–98.77	50	2.50–38.21	55	1.02–97.33
	18–23	**87**	**0.26–99.91**	**69**	**1.62–94.12**	**68**	**7.73–91.22**
Luticola binodeformis Levkov, Metzeltin & Pavlov	12–15.5 / 4–4.5;					–	–
	21–22					**1**	**0.33**
Luticola blancoi Levkov, Tofilovska, C.E.Wetzel, Mitić-Kopanja & Ector	18–21 / 6.5;			–	–	–	–
	20–21			**1**	**+**	**1**	**+**
Luticola bryophila M.Rybak, Czarnota & Noga, sp. nov.	10–25 / 4–6;	5	0.26	8	+	5	0.24
	18–20	**14**	**0.17–0.66**	**11**	**0.25–0.65**	**17**	**0.22–7.73**
Luticola cholnokyi Levkov, Metzeltin & Pavlov	15–18 / 6.5;	–	**–**				
	20–21	**1**	0.16				
Luticola cohnii (Hilse) D.G.Mann	17.5–28.5 / 8.5–10;	–	**–**	–	–		
	20–21	**1**	0.31	**1**	**+**		
Luticola confusa M.Rybak & Czarnota, sp. nov.	9–22 / 4.5–6;	20	0.24–0.32	13	–	20	0.35–3.32
	20–22	**25**	**0.18–10.45**		**0.18-2.00**	**19**	**0.17–0.53**
Luticola imbricata (W.Bock) Levkov, Metzeltin & Pavlov	15–17 / 5.5–7;	–	–	1	+	–	–
	20	**2**	**0.30–0.66**	**1**	**+**	**1**	**+**
Luticola kemalii Solak & Levkov	12–16 / 5.6–7;	2		–	–	2	+
	20–22	**–**		**1**	**+**	**2**	**0.28**
Luticola lecohui Levkov, Tofilovska, C.E.Wetzel, Mitic-Kopanja & Ector	15–23.5 / 7–8;	1	+	1	+		
	20–21	–	–	**1**	**+**		
Luticola micra Levkov, Metzeltin & Pavlov	7.5–13 / 3.8–4.5;	6	0.24–0.32	5	0.30–0.31	7	0.24–0.28
	22–24	**9**	**0.20–0.52**	**5**	**0.28–0.30**	**2**	**0.19**
Luticola nivalis (Ehrenberg) D.G.Mann	10–21 / 5.8–7.5;	16	0.28–0.36	15	0.32	21	0.17–0.95
	19–21	**17**	**0.25–0.68**	**9**	**0.10**	**33**	**0.22–0.69**

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	**Min-Max**	**Freq.**	**Min-Max**	**Freq.**	**Min-Max**
Luticola nivaloides (W.Bock) Denys & De Smet	15–23.5 / 6.5–8; 18–19	1 –	0.48 –	1 **4**	+ **0.32**	6 **8**	0.47 **0.17–0.29**
Luticola obscura Levkov, Tofilovska, C.E.Wetzel, Mitić-Kopanja & Ector	10–24.5 / 5–7; 19–22	20 **26**	0.48–0.48 **0.17–2.24**	28 **36**	0.30–0.31 **0.18–1.53**	15 **29**	0.34 **0.19–7.54**
Luticola pitranensis Levkov, Metzeltin & Pavlov	15–22.5 / 5.5–6.5; 20–22	– **2**	– **0.17–0.20**				
Luticola poulickovae Levkov, Metzeltin & Pavlov	22 / 6; 21	– **1**	– **+**				
Luticola pulchra (McCall) Levkov, Metzeltin & Pavlov	10–20 / 5.9–6.4; 20–24	1 **6**	0.32 **0.22–0.34**	7 **9**	0.31 **0.25–0.30**	15 **13**	1.90 **0.19–0.33**
Luticola pseudonivalis (W.Bock) Levkov, Metzeltin & Pavlov	13.2–16.5 / 5–6; 24	4 **1**	+ **0.28**	2 –	0.31 –	1 –	0.47 –
Luticola rotunda Solak & Levkov	12–15.5 / 6–6.5; 19–20	2 **8**	0.20–0.31 **0.31–0.68**	1 **1**	+ **+**	2 **1**	– **0.22–0.29**
Luticola sparsipunctata Levkov, Metzeltin & Pavlov	9.5–27 / 4.5–7; 18–20	14 **24**	0.28–0.40 **0.17–3.67**	19 **27**	0.31 **0.18–0.34**	**25** **32**	0.12–1.42 **0.19–7.80**
Luticola spinifera (W.Bock) L.Denys & W.H.De Smet	12–14 / 7–8; 16					1 –	+ –
Luticola undulata (Hilse) D.G.Mann	19–22.5 / 7.5–7.8; 24–25			– **1**	– **+**	– **1**	– **0.49**
Luticola vanheurckii Van de Vijver & Levkov	14.5–19 / 5.5–7.3; 18–21			10 **16**	+ **0.25–0.65**	6 **3**	+ **0.22–0.29**
Luticola ventricosa (Kützing) D.G.Mann **MT1**	10–23 / 5.5–7; 19–22	20 **11**	0.23–0.40 **0.22–10.95**	16 **17**	0.30–0.31 **0.25–0.32**	15 **27**	0.24–3.79 **0.17–2.07**
Luticola ventricosa (Kützing) D.G.Mann **MT2**	9.2–22 / 5–7; 19–22	10 **13**	0.26–0.61 **0.02–0.82**	23 **43**	0.30–0.31 **0.19–62.21**	35 **31**	0.12–1.42 **0.19–0.69**
Luticola cf. ***vesnae*** Levkov, Metzeltin & Pavlov	10–23.7 / 5–7.2; 20–24	8 **24**	0.16–0.25 **0.18–7.12**	7 **18**	+ **0.18–0.32**	9 **13**	0.12–0.34 **0.28–0.97**
Luticola sp. 1	14–18 / 5.5–7; 18–23	– **9**	– **0.34–0.37**	1 **9**	0.31 **0.32–0.41**	2 **5**	0.25 **0.16–0.19**
Luticola sp. 2	-	– **1**	+ **0.28**	– **1**	– **+**	**–1** **1**	+ **0.28**
Mayamaea asellus (Weinhold & Hustedt) Lange-Bertalot	12–16.5 / 5.5–6; 16–18	1 **4**	0.32 **+**	2 **7**	+ **0.25–0.32**	4 **2**	+ **0.28–0.29**
Mayamaea atomus (Kützing) Lange-Bertalot	7–13 / 4–5; 18–22	5 **23**	+ **0.17–6.86**	9 **37**	0.31 **0.25–0.41**	7 **15**	0.22–0.95 **0.17–0.49**
Mayamaea excelsa (Krasske) Lange-Bertalot	11–14.5 / 5–7; 16–17	2 **5**	0.22 **0.26–0.31**	– **7**	– **+**	1 **3**	+ **0.26–0.33**
Mayamaea fossalis* var. *fossalis (Krasske) Lange-Bertalot	9–11.5 / 3–4; 17–19	1 **3**	+ **0.17–0.41**	1 **4**	+ **+**	1 **1**	+ **0.33**
Mayamaea fossalis* var. *obsidialis (Hustedt) Lange-Bertalot	9–11 / 4.5; 18			– **3**	– **0.18**		
Mayamaea permitis (Hustedt) K.Bruder & Medlin	7–12 / 3.5–4.5; ca. 35	1 **7**	+ **0.17–2.69**	1**11** **11**	1 **0.20**	2 **2**	+ **0.19**
Meridion circulare (Greville) C.Agardh	13–24 / 5–6	3 **2**	0.20–0.31 **+**	7 **9**	+ **0.41**	2 **1**	0.24 **+**
Microcostatus aerophilus Stanek-Tarkowska, Noga, C.E.Wetzel & Ector	6–9.5 / 2.5–3.5; not visible in LM	1 –	+ –	– **5**	– **+**		
Muelleria islandica (Østrup) Lange-Bertalot	19.5–30 / 6.7–7.7; 22–25	2 **5**	+ **0.17–0.35**	2 **3**	+ **+**	3 **2**	+ +
Muelleria sasaensis Levkov, Vidaković, Cvetkoska, Mitić-Kopanja, Krstić, Van de Vijver & Hamilton	22.5–29.5 / 5.8–6.2; 23–24	– **3**	– **0.24–1.49**	3 **1**	+ **+**	1 **3**	– **0.25**
Muelleria terrestris (J.B.Petersen) Spaulding & Stoermer	23.5–26 / 5.2–6; 18–19	3 **3**	0.24 **0.23–0.64**	2 **3**	+ **0.50**	3 **3**	+ v
Muelleria undulata (Krasske) Levkov, Hamilton & Van de Vijver	12–22 / 4.3–5; 26–28	3 **3**	+ **0.17–3.98**	1 –	+ –	– **2**	– +

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	Min-Max	Freq.	Min-Max	Freq.	Min-Max
Navicula bjoernoeyaensis Metzeltin, Witkowski & Lange-Bertalot	17–18 / 3;	1	+	–	–	1	0.34
	18	**–**	–	**1**	**+**	**3**	**0.23**
Navicula gregaria Donkin	15.5–22 / 6–7;	–	–	1	+	1	0.12
	16	1	**0.31**	**1**	**0.41**	**3**	**0.29–0.43**
Navicula lundii Reichardt	14–25 / 4.2–6;	–	–			11	0.47
	15	**2**	**0.24–0.50**				**0.24**
Navicula neowiesneri Chaudev & Kulikovskyi	12–32 / 4.5–6;	38	0.16–0.80	36	0.20–0.32	43	0.12–5.69
	11–13	**35**	**0.18–2.03**	40	**0.18–1.75**	38	**0.17–4.14**
Navicula pseudowiesneri Chaudev & Kulikovskyi	11–24 / 4–5;	30	0.16–0.62	21	0.18–0.24	22	0.12–1.12
	11–13	**35**	**0.18–2.03**	**23**	**0.18–1.02**	**12**	**0.17–0.35**
Navicula tenelloides Hustedt	14–18 / 3–3.5;	–	–	1	+	1	+
	16	**1**	**0.25**	**1**	**0.41**	**–**	–
Navicula veneta Kützing	12–25.5 / 4.5–5.5;	1	+	–	–	1	0.12
	13–16	**2**	**0.32–0.41**	**2**	**0.32**	**4**	**0.22–0.49**
Neidium alpinum Hustedt	15–29 / 4.5–5;			–	–		
	ca. 37			**1**	+		
Neidium perforatum Schimanski	15.5–26 / 4.5–5.5;	2	0.31–0.33				
	21–23	**6**	**0.18–0.37**				
Nitzschia amphibia Grunow	9–28 / 4–5.5;	9	0.21–0.48	6	0.31	8	0.34–0.47
	15–18	**7**	**0.25–0.41**	**7**	**0.32**	**8**	**0.24–0.49**
Nitzschia amphibia* f. *rostrata Hustedt	12–15 / 3.5–4;	1		–	–	–	–
	18–20	**–**		**1**	**0.32**	**1**	+
Nitzschia communis Rabenhorst	15–20 / 4;	1	0.24			1	+
	not visible in LM	**–**	–			**3**	**0.22–0.29**
Nitzschia harderi Hustedt	19–36 / 3–4;	1	0.25				
	not visible in LM	**1**	**1.48**				
Nitzschia pusilla Grunow	7–22 / 2.5–3.5;	5	0.21–0.36	5	0.20	2	+
	not visible in LM	**11**	**0.18–1.59**	**5**	**0.10**	7	**0.22–0.33**
Nitzschia* cf. *frustulum (Kützing) Grunow	4–16.5 / 3;	15	0.25–0.36	9	–	4	0.12
	25–27	**6**	**0.30**	**16**	**0.32–0.34**	**14**	**0.22–6.45**
Nitzschia solgensis Cleve-Euler	11.5–24 / 3–4;	1	0.24	–	–	3	0.33
	21–23	**2**	**0.30**	**1**	**+**	**3**	**0.19–0.29**
Nitzschia* cf. *supralitorea Lange-Bertalot	10–20 / 2.5–3.5;			–	–	2	1.42
	27–30			**1**	**+**	**–**	–
Nitzschia linearis W.Smith	50–55 / 6;					–	–
	28–29					2	**0.22–1.74**
Orthoseira dendroteres (Ehrenberg) Genkal & Kulikovskiy	Ø – 7–26;	36	0.24–95.69	7	+	9	0.17–3.59
	20–22	**39**	**0.23–95.36**	**17**	**0.82**	16	**0.17–72.02**
Orthoseira roeseana (Rabenhorst) Pfitzer	Ø – 8–24;	4	0.24–0.71	2	+	1	0.17
	8–12	**8**	**0.17–3.55**	**–**	**–**	**4**	**0.26–2.85**
Pantocsekiella* cf. *ocellata (Pantocsek) K.T.Kiss & E.Ács	–	6	0.24–0.34	2	+	6	0.12–0.33
		10	**0.02–0.20**	**6**	**+**	**11**	**0.19–0.33**
Pinnularia borealis* var. *borealisEhrenberg	22–42 / 8–9.5;	68	0.21–58.21	52	0.30–1.81	57	1.63–97.80
	ca. 5	**80**	**0.03–52.24**	**79**	**0.30–86.57**	**67**	**0.24–66.32**
Pinnularia borealis* var. *subislandica Krammer	35–40 / 8.5–9;	–	–	1	+	1	0.47
	ca. 5	**3**	**0.26–0.32**	**2**	**0.30**	**3**	**0.29–0.65**
Pinnularia brebissonii (Kützing) Rabenhorst	10–34 / 9–12;	1	+	1	+	2	+
	10–13	**3**	**0.22–0.34**	**1**	**+**	**2**	**0.22–0.29**
Pinnularia cuneola Reichardt	24.5–30 / 7–8;	9	0.28–0.34	3	+	9	0.34
	10–11	**7**	**0.23–0.32**	**11**	**0.30–0.41**	**9**	**0.17–0.49**
Pinnularia dubitabilis Hustedt	40 / 9;	–	–				
	ca. 5	**1**	**+**				
Pinnularia* aff. *frauenbergiana* var. *caloneiopsis Lange-Bertalot & M.Werum	13–22 / 4–4.5;	3	+	1	+	2	+
	17–18	**2**	**0.33–1.35**	**2**	**+**	**1**	**0.29**
Pinnularia isselana Krammer	30–45 / 7–9;	2	0.28–0.36	2	+	1	0.24–0.47
	10–12	**–**	**–**	**10**	**0.32–0.41**	**5**	**0.24–0.49**

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	**Min-Max**	**Freq.**	**Min-Max**	**Freq.**	**Min-Max**
Pinnularia microstauron* var. *angusta Krammer	42–44 / 6.5–7; 12–13			1 –	+ –		
Pinnularia microstauron* var. *rostrata Krammer	35 / 6.5; 11			1 –	+ –		
Pinnularia obscura Krasske	9–35 / 3–5; 10–13	55**59**	0.21–1.95 **0.02–26.48**	29 **73**	0.20–0.31 **0.18–9.48**	3 **38**	0.24–1.90 **0.16–2.03**
Pinnularia perirrorata Krammer	12–25 / 4; 16–18	– **5**	– **0.25–0.27**	1 7	+ **0.30**		
Pinnularia schoenfelderi Krammer	19–34 / 5–6.5; 14–16	10 **11**	0.22–0.50 **0.22–0.37**	11 **20**	+ **0.32–2.47**	6 7	0.33 **0.23–0.32**
Pinnularia sinistra Krammer	17–35 / 4.5–6; 11–13	3 **6**	0.21–0.36 **0.27–0.37**	1 **8**	+ **0.18**	2 **2**	+ **+**
Placoneis hambergii (Hustedt) K.Bruder	14–22 / 5–7; 13–16	1 **4**	+ **0.26–0.30**	– **3**	– **0.41**		
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	4–25 / 3.5–6; 14–17	6 **5**	0.16–0.36 **0.21–0.30**	5 **22**	0.31 **0.30–2.47**	8 **11**	0.22–2.37 **0.19–1.69**
Planothidium lanceolatum (Brébisson & Kützing) Lange-Bertalot	7–25 / 4–6.5; 13–15	5 **3**	0.21–0.30 **0.29–0.30**	5 **8**	0.30 **0.30–3.29**	2 7	0.12 **0.22–3.72**
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	–			– **1**	– **+**	– **1**	– **+**
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	9–14 / 3.5–5.5; 9–13	– **1**	– **0.29**	– **1**	– **+**		
Sellaphora atomoides (Grunow) Wetzel & Van de Vijver	6–12 / 3–4; 22–25	5 **8**	0.31–0.36 **0.17–0.41**	7 **14**	+ **0.41**	4 8	0.12–1.42 **0.12–0.33**
Sellaphora harderi (Hustedt) J.Foets & C.E.Wetzel	5.5–12 / 3–4; 32–35	– **1**	– **0.28**	– **2**	– **+**	1 –	+ –
Sellaphora nana (Hustedt) Lange-Bertalot, Cavacini, Tagliaventi & Alfinito	15–18.5 / 4–4.5; 38	2 **1**	0.30 **0.41**	– **3**	– **+**	1 –	0.47 –
Sellaphora nigri (De Notaris) C.E.Wetzel & Ector	5–10 / 4; 28–30					1 **1**	0.47 **+**
Sellaphora subseminulum (Hustedt) Wetzel	7–14 / 3.2–3.8; 23–25	1 **8**	+ **0.19–1.23**	2 **15**	0.31 **0.41–3.29**	1 **5**	+ **0.22–0.34**
Stauroneis borrichii (J.B.Petersen) J.W.G.Lund	10–26 / 3.8–5; 22–26	19 **40**	0.22–0.33 **0.20–9.97**	22 **43**	0.20 **0.10–1.19**	18 **26**	0.17–0.95 **0.19–0.49**
Stauroneis laterostrata Hustedt	19–26 / 6.5–8.5; 17–20	– **8**	– **0.17–2.01**			5 **5**	+ **+**
Stauroneis leguminopsis Lange-Bertalot & Krammer	20–22 / 4.5; 24	– **1**	– **0.31**				
Stauroneis aff. ***lundii*** Hustedt	14–19 / 4–5; 23–24	2 **16**	0.20 **0.22–0.59**	4 **12**	+ **0.32–1.03**	4 **4**	0.17–0.22 **0.22–0.29**
Stauroneis muriellaJ.W.G.Lund	16–20 / 3–4; 22–26	1 **2**	+ **0.26**	2 **1**		– **1**	– +
Stauroneis obtusa Lagerstedt	15–28 / 6–8; 19–23	1 **4**	+ **0.30–6.25**	3 **5**	+ **0.41**	2 **2**	+ +
Stauroneis parathermicola Lange-Bertalot	8–18 / 3–4.5; 20–23	2 **10**	0.32–0.34 **0.17–1.01**	1 **12**	+ **0.82**	2 **2**	+ **0.22–0.24**
Stauroneis saprophila M.Rybak, Noga & Ector	30–35/ 8.5–9.5; 15–17	3 2	0.48 **0.48**	1 **2**	+ +	3 **4**	+ **0.22–0.49**
Stauroneis separanda Lange-Bertalot & Werum	13–14 / 3.8–4.2; ca. 28	1 **1**	0.30 **0.30**	1 **1**	+ +		
Stauroneis thermicola (J.B.Petersen) J.W.G.Lund	8–18 / 3–4.5; 20–23	18 **28**	0.21–0.37 **0.18–4.39**	7 **31**	+ **0.10–0.32**	2	0.12 **0.22–0.33**

Taxa	Dimensions	*Acer*		*Tilia*		*Populus*	
		Freq.	Min-Max	Freq.	Min-Max	Freq.	Min-Max
Surirella angusta Kützing	16.5–42.5 / 6–9; 24–27	3	+	1	+	2	0.12–0.47
		–	–	**2**	**0.30**	7	**0.16–1.16**
Surirella minuta Brébisson & Kützing	9–34.5 / 8–10; 26–28	–	–	2	+	2	+
		1	**0.25**	**6**	**0.41**	**3**	**0.29–0.23**
Surirella terricola Lange-Bertalot & E.Alles	11–18.1 / 6.4; 18–23	10	0.21–0.36	97	+	2	+
		10	**0.23–0.34**		**0.41**	**3**	**0.28–0.32**
Tryblionella apiculata W.Gregory	20–24 / 4.5–5.5; 17					–	–
						2	**0.49–1.08**
Tryblionella debilis Arnott & O'Meara	12–23.5 / 7–8.5; not visible in LM	–	–	11	+	**–**	–
		1	+		+	**3**	**0.22–0.49**
Tryblionella hungarica (Grunow) Frenguelli	35–42 / 5.2–6; 8–10			–	–	–	–
				1	**0.25**	**2**	**0.22–0.29**

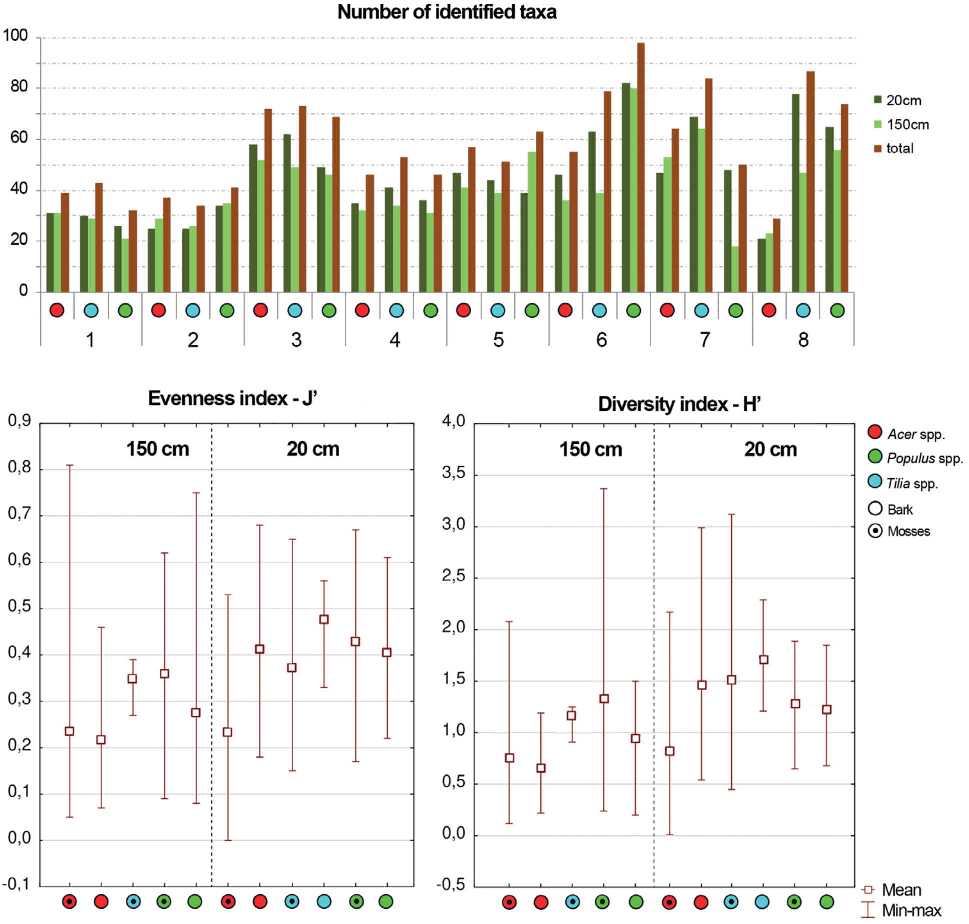

Figure 4. Number of diatom taxa identified during the research on each of the examined trees on each site and value of Eveness index (J') and Shannon index (H'). 1–8 number of sampling site.

47 from 150 cm above ground level). Numerous assemblages were also observed in 123 of 231 moss samples collected (43 from 20 cm above ground level and 80 from 150 cm above ground level).

Higher values of both indices studied (H' and J') were usually recorded for bare bark samples, and higher values of both of these indices were also recorded for samples collected from trunk bases (Fig. 4). When more than one microhabitat from a single tree was analyzed in the same season, higher values of both indexes were noted for bare bark (Fig. 4).

Principal component analysis (PCA) revealed considerable variability in the diatom assemblages. The gradient length in analysis was 2.7. The first ordination axis explained 33.48% of the variation, the second 25.00%, the third 14.02%, and the fourth 10.14% (Fig. 23). The diatom communities from sites located in city centers and small peripheral estates (sites 1, 2, 4, 5) where the trees were exposed are grouped on the left, bottom corner of the graph. Assemblages from park complexes and the buffer zones of national parks (site 3 and 6–8) form two distinct groups. Samples from sycamore

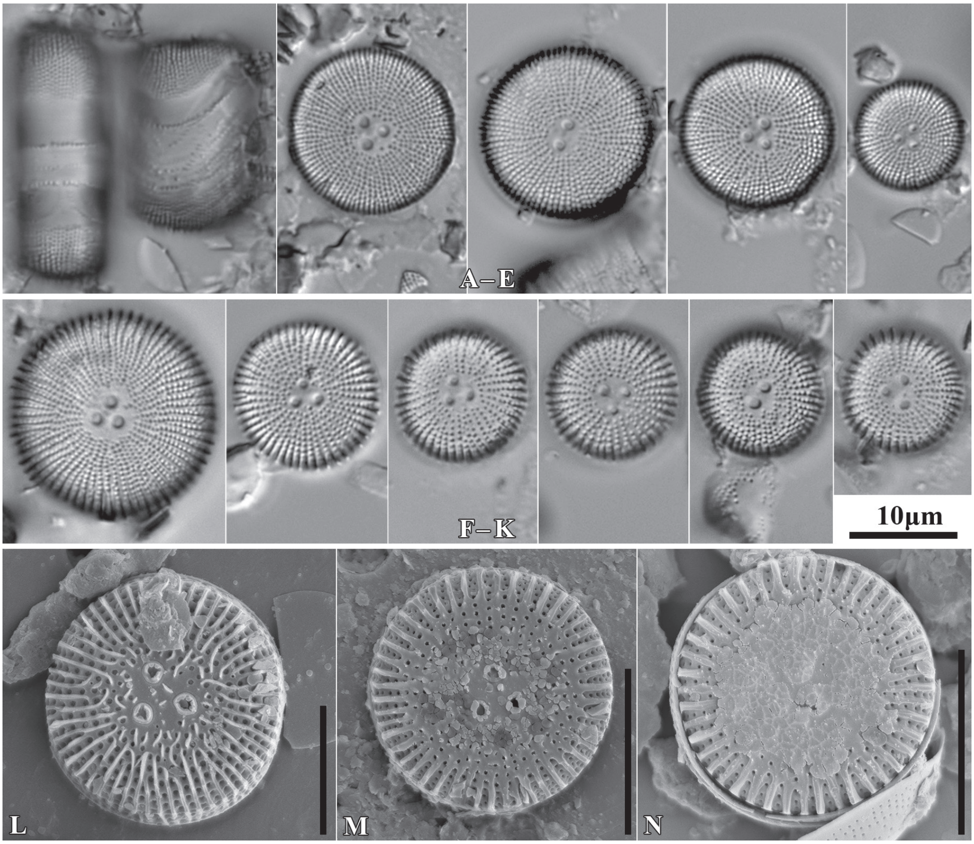

Figure 5. LM microphotographs of *Orthoseira dendroteres* in girdle view (**A**) and in size diminution series (**B–E**) and *O. roeseana* (**F–K**). SEM microphotographs of *Orthoseira dendroteres* (**L**) and *O. roeseana* (**M, N**). Scale bars: 10 µm.

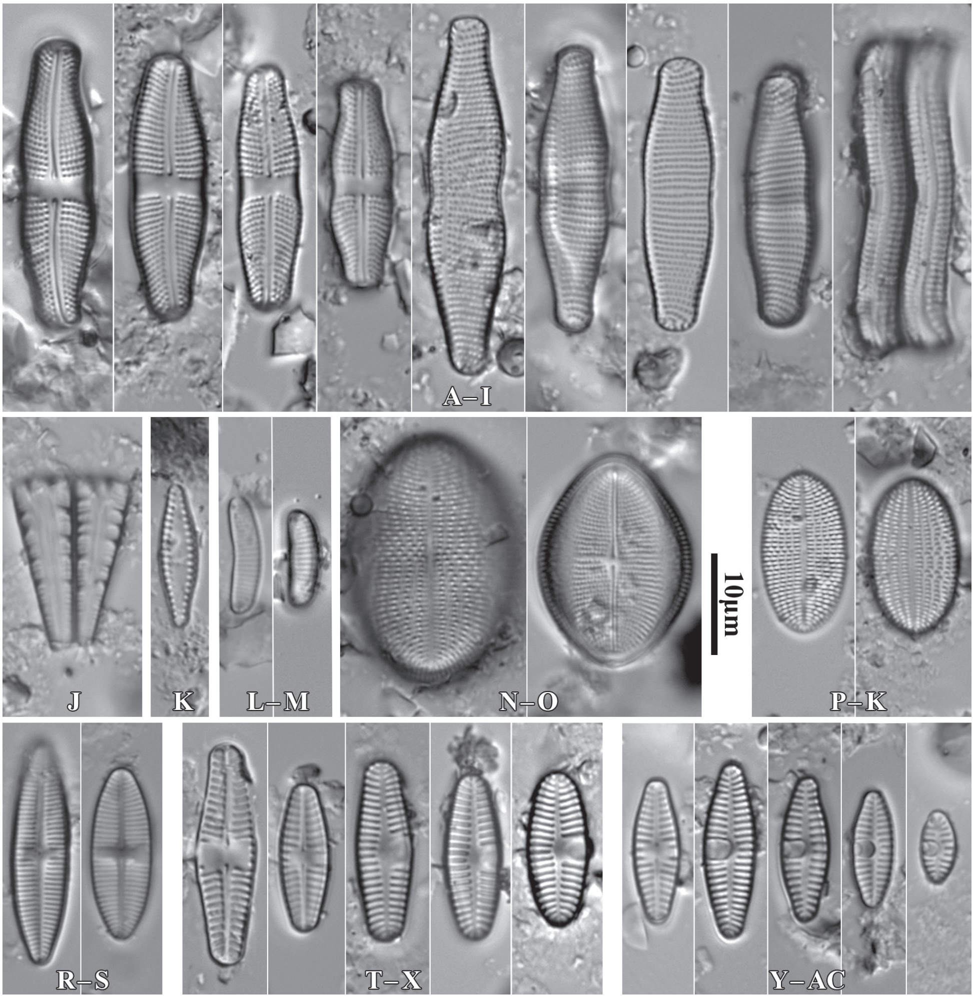

Figure 6. LM microphotographs of *Achnanthes coarctata* (**A–I**), *Meridion circulare* (**J**), *Pseudostaurosira brevistriata* (**K**), *Eunotia botuliformis* (**L, M**), *Cocconeis pediculus* (**N, O**), *C. lineata* (**P, K**), *Lemnicola hungarica* (**R, S**), *Planothidium lanceolatum* (**T–X**) and *P. frequentissimum* (**Y–AC**). Scale bar: 10 µm.

maples are grouped on the bottom right, while assemblages from lindens and poplars are grouped on the left top corner. The seasons in which the research was conducted did not affect the grouping of the tested samples.

Discussion

Characteristics of the habitat

Tree barks, thanks to their porosity, can absorb rainwater; therefore the solution on its surface is usually slightly acidic. On the other hand, pH reactions often depend on

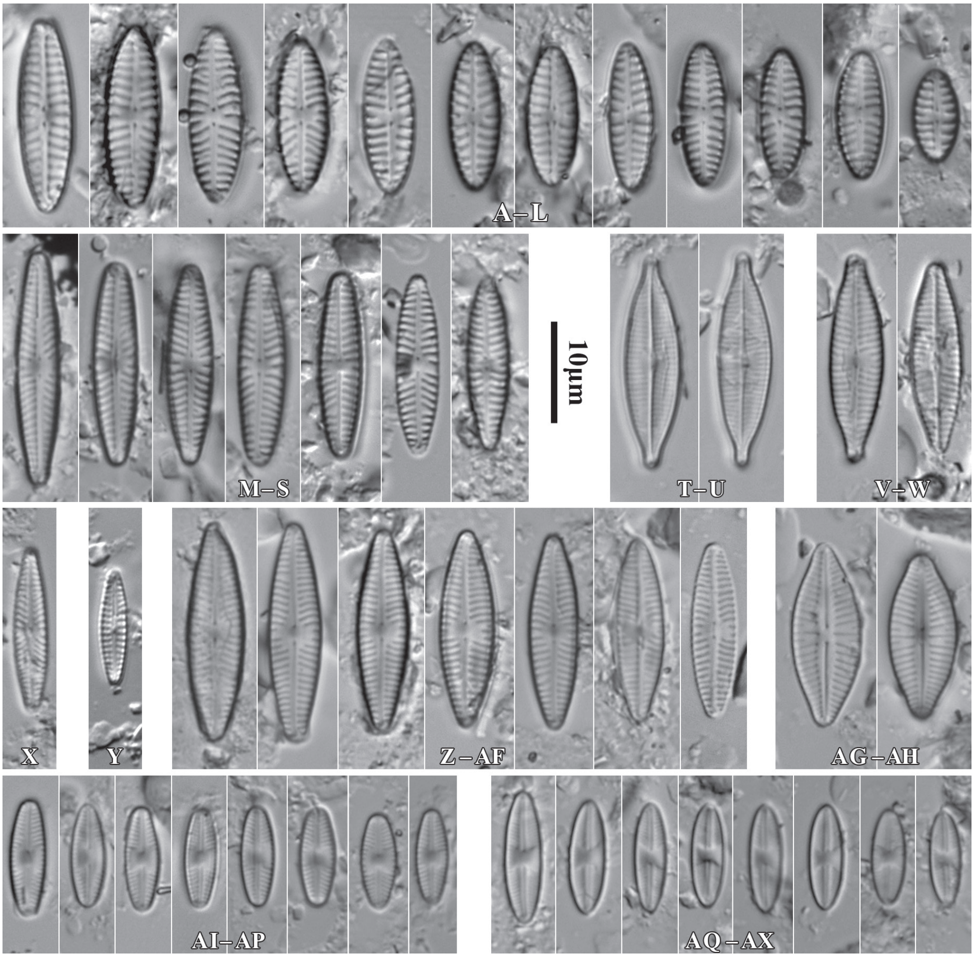

Figure 7. LM microphotographs of *Navicula neowiesneri* (**A–L**), *N. pseudowiesneri* (**M–S**), *N. gregaria* (**T, U**), *N. veneta* (**V,W**), *N. tenelloides* (**X**), *N. bjoernoeyaensis* (**Y**), *N. lundii* (**Z–AF**), *Placoneis hambergii* (**AG, AH**), *Sellaphora subseminulum* (**AI–AP**) and *S. harderi* (**AQ–AX**). Scale bar: 10 µm.

bark structures, which differ depending on tree species (Zimny 2006). A slightly acidic or close to neutral reaction is typical of the barks of most deciduous tree species, in contrast to conifers, which usually have more acidic barks (Barkman 1958; Steindor et al. 2011; Štifterová and Neustupa 2015). Additionally, the reaction of barks also can decrease with tree age (Grodzińska 1979).

Of the 647 samples collected, only 197 had developed diatom communities, and only single valves were found in the remaining samples. The almost complete or complete absence of diatoms was observed in bark samples covered with visible mats of green algae. Other studies focusing on corticolous algae assemblages, in which only a few species of diatoms have been found (Neustupa and Škaloud 2010; Neustupa and Štifterová 2013; Štifterová and Neustupa 2015), confirm that this microhabitat is not

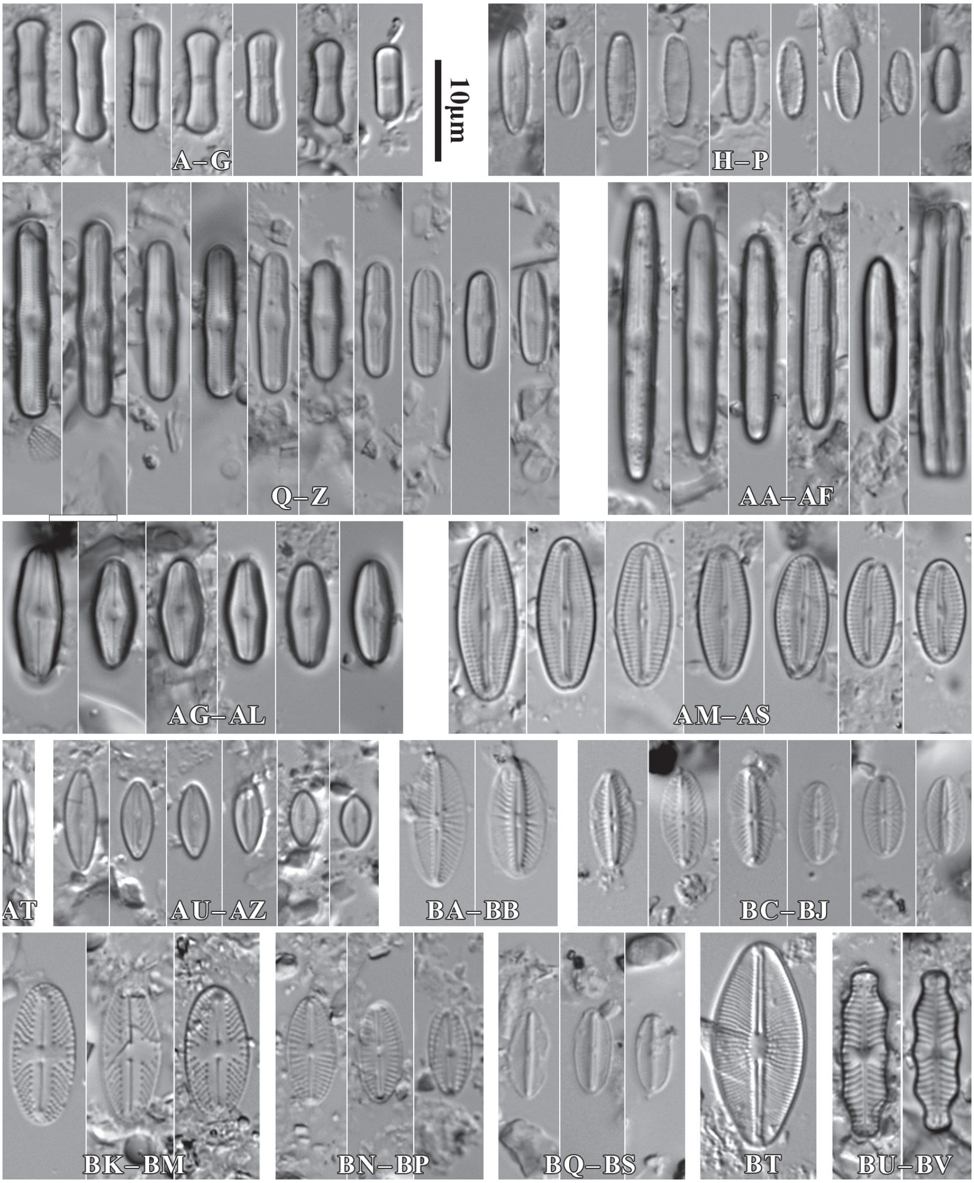

Figure 8. LM microphotographs of *Humidophila contenta* (**A–G**), *H. gallica* (**H–P**), *H. brekkaensis* (**Q–Z**), *H. irata* (**AA–AF**), *H. perpusilla* (**AG–AL**), *Fallacia insociabilis* (**AM–AS**), *F. enigmatica* (**AT**), *Microcostatus aerophilus* (**AU–AZ**), *Mayamaea excelsa* (**BA, BB**), *M. atomus* (**BC–BJ**), *M. asellus* (**BK–BM**), *M. fossalis* (**BN–BP**), *M. permitis* (**BQ–BS**), *Cavinula cocconeiformis* (**BT**) and *Geissleria ignota* (**BU, BV**). Scale bar: 10 μm.

conducive to their development. A similar situation occurred in bark samples covered with lichens. The low number of diatoms in these two microhabitats could be the result of competition for resources with arboreal cyanobacteria and green algae, or it could

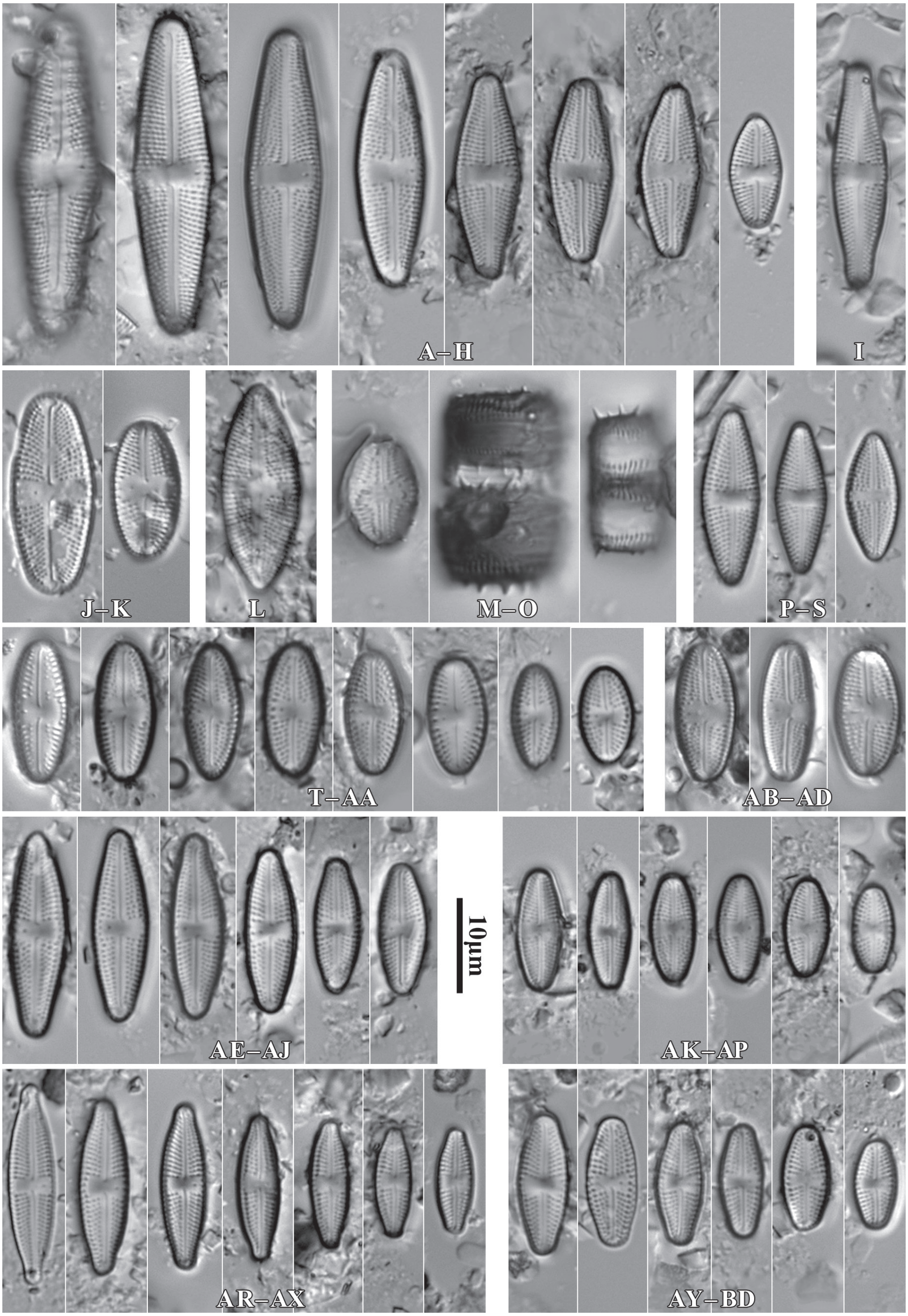

Figure 9. LM microphotographs of *Luticola acidoclinata* (**A–H**), *L. poulickovae* (**I**), *L. cohnii* (**J, K**), *L. pseudokotschyi* (**L**), *L. spinifera* (**M–O**), *L. pitranensis* (**P–S**), *L. rotunda* (**T–AA**), *Luticola* sp. (**AB–AD**), *L. imbricata* (**AE–AJ**), *L. micra* (**AK–AP**), *L.* cf. *vesnae* (**AR–AX**) and *L. obscura* (**AY–BD**). Scale bar: 10 µm.

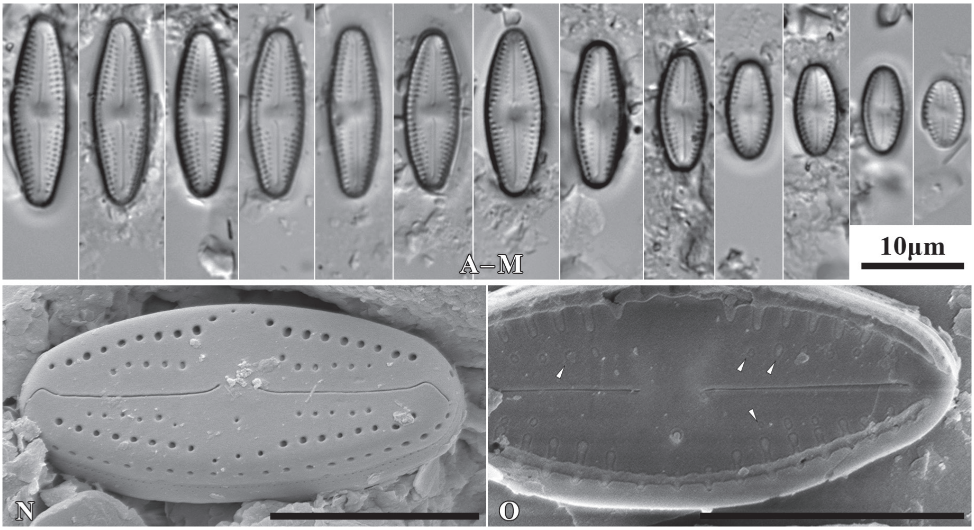

Figure 10. LM microphotographs of *Luticola sparsipunctata* in size diminution series (**A–M**). SEM microphotographs of external (**N**) and internal view (**O**). White arrowheads indicate a discontinuity in hymenes, Scale bars: 10 µm (**A–M**), 5 µm (**N, O**).

result from the allelopathic effects of algae and/or lichens on diatoms (Bhattacharyya et al. 2016; Goga et al. 2018).

Although trees growing as close as possible to each other were selected at the sites and materials from analogous microhabitats were collected from them, differences in the frequency of occurrence of diatom assemblages were clearly notable. On bare bark, diatom assemblages were found mainly in materials collected from maples and poplars at locations closest to natural sites (parks and national park buffer zones). However, diatoms were not found or only single specimens were observed in bare bark samples from site 7 (the buffer zone of Magura National Park). This was the only site where samples were collected from aspen poplar (*Populus tremula* L.), and it is possible that the bark of this host tree is an unfavorable habitat for diatoms. In urban conditions the trunks of poplars and maples were poorly inhabited by diatoms, and their diversity was concentrated in microhabitats created by bryophytes. Regardless of site, lindens seemed to be unsuitable for diatoms; they were abundant on this host tree only in single samples. The tree species-dependent bark water-holding capacity, which is related to bark features such as stability, texture, thickness, and hardness, directly influenced the intensity of desiccation and were important determinants of the distribution of various organisms overgrowing bark including algae (Valová and Bieleszová 2008; Büdel 2011; Ellis 2012).

Materials from moss microhabitats were more than half of the samples in which large numbers of diatoms were found (123 out of 197 samples). This result is similar to the study by Lindo and Gonzalez (2010) in which the bryophytes overgrowing various substrates (bryosphere), thanks to their structure that permits the accumulation of mineral particles and rainwater, create conditions for the development of different

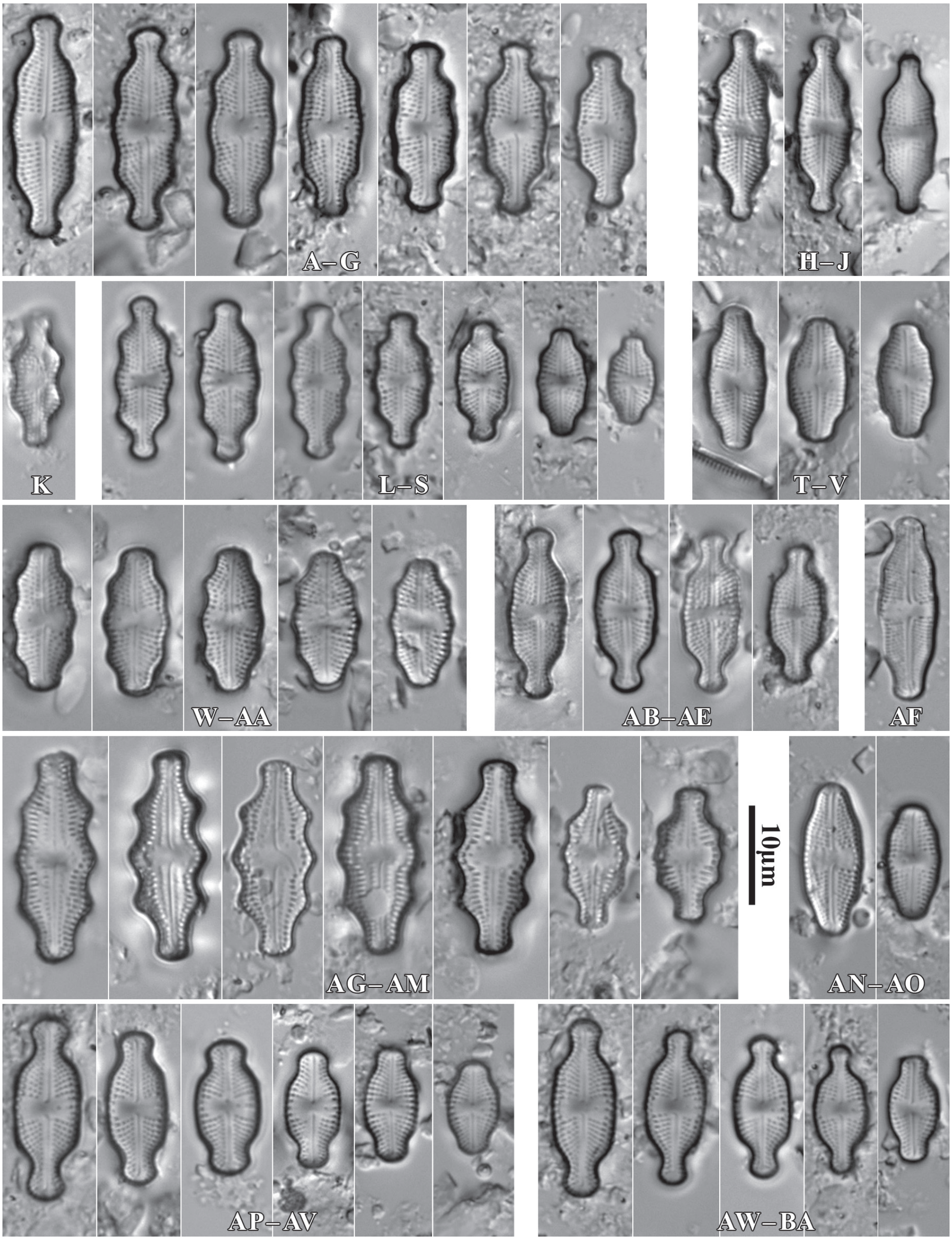

Figure 11. LM microphotographs of *Luticola nivalis* (**A–G**), *L. pseudonivalis* (**H–J**), *L. binodeformis* (**K**), *L. pulchra* (**L–S**), *L. kemalii* (**T–V**), *L. lecohui* (**W–AA**), *L. vanheurckii* (**AB–AE**), *L. undulata* (**AF**), *L. nivaloides* (**AG–AM**), *L. cholnokyi* (**AN, AO**), *L. ventricosa* MT1 (**AP–AV**) and *L. ventricosa* MT2 (**AW–BA**). Scale bar: 10 μm.

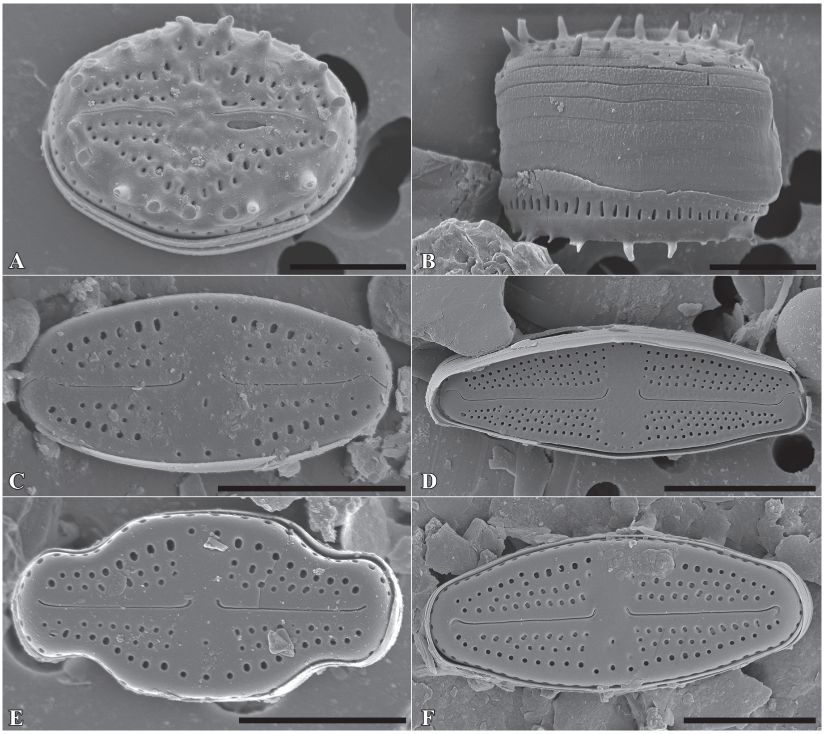

Figure 12. SEM microphotographs of *Luticola spinifera* (**A, B**), *Luticola* sp. (**C**), *L. acidoclinata* (**D**), *L. ventricosa* MT2 (**E**) and *L. obscura* (**F**). Scale bars: 10 µm (**D**); 5 µm (**A–C, E, F**).

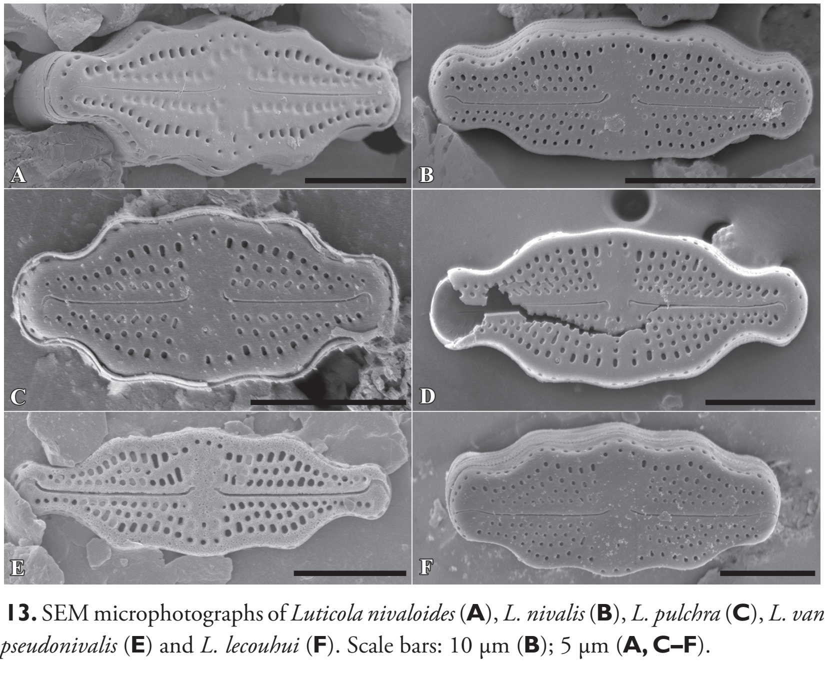

Figure 13. SEM microphotographs of *Luticola nivaloides* (**A**), *L. nivalis* (**B**), *L. pulchra* (**C**), *L. vanheurckii* (**D**), *L. pseudonivalis* (**E**) and *L. lecouhui* (**F**). Scale bars: 10 µm (**B**); 5 µm (**A, C–F**).

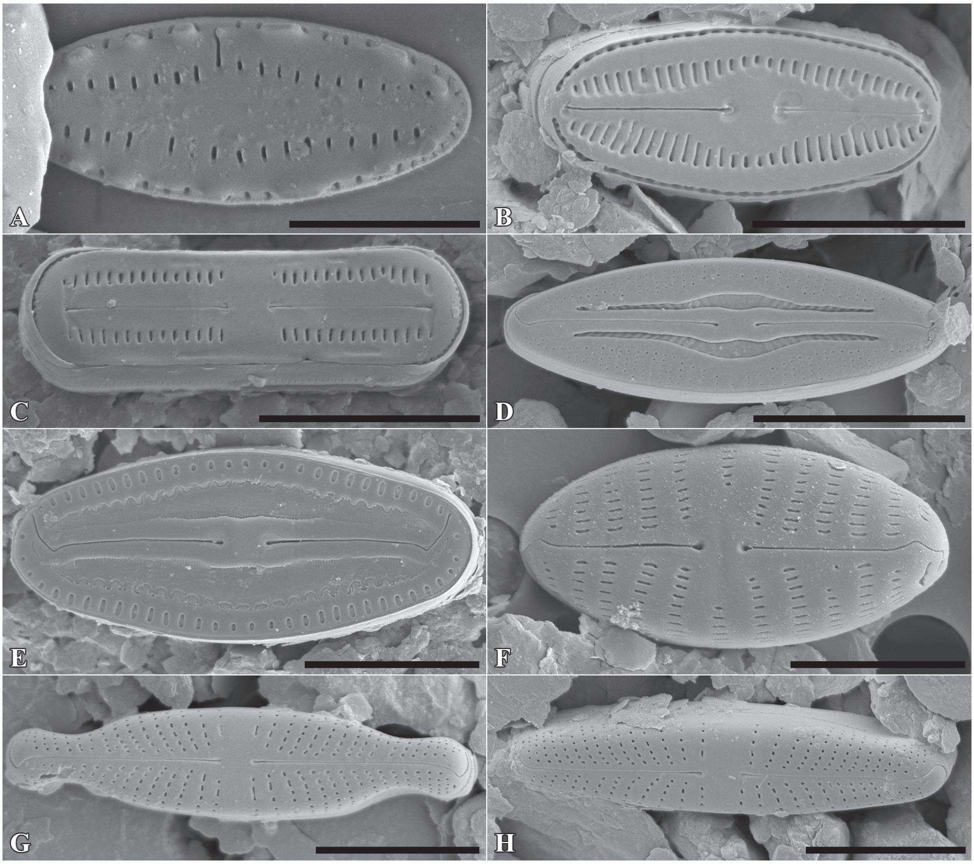

Figure 14. SEM microphotographs of *Humidophila gallica* (**A**), *H. perpusilla* (**B**), *H. contenta* (**C**), *Microcostatus aerophilus* (**D**), *Fallacia insociabilis* (**E**), *Navicula neowiesneri* (**F**), *Stauroneis thermicola* (**G**) and *S. parathermicola* (**H**). Scale bars: 5 µm (**B–H**); 3 µm (**A**).

groups of organisms. Only samples of bryophytes from linden and poplar trunks in urban sites, and again from aspen at site 7, contained a few diatoms.

The developed diatom assemblages in the microhabitats analyzed were more often recorded in samples taken from trunk bases than from a height of 150 cm above ground level, and more species were also noted in samples collected from trunk bases (Table 2). This could have been because of the greater availability of moisture from condensation, or it could have been related to the higher share of soil diatoms from the vicinity of the trees. However, the height above ground level did not influence the differences in taxonomic composition between samples from different heights within the tree trunk (Fig. 23).

Comparison of novel taxa to similar species

Both of the newly described *Luticola* represent a group of small taxa with elliptic-lanceolate to linear elliptic valves. Many taxa representing this morphological group have

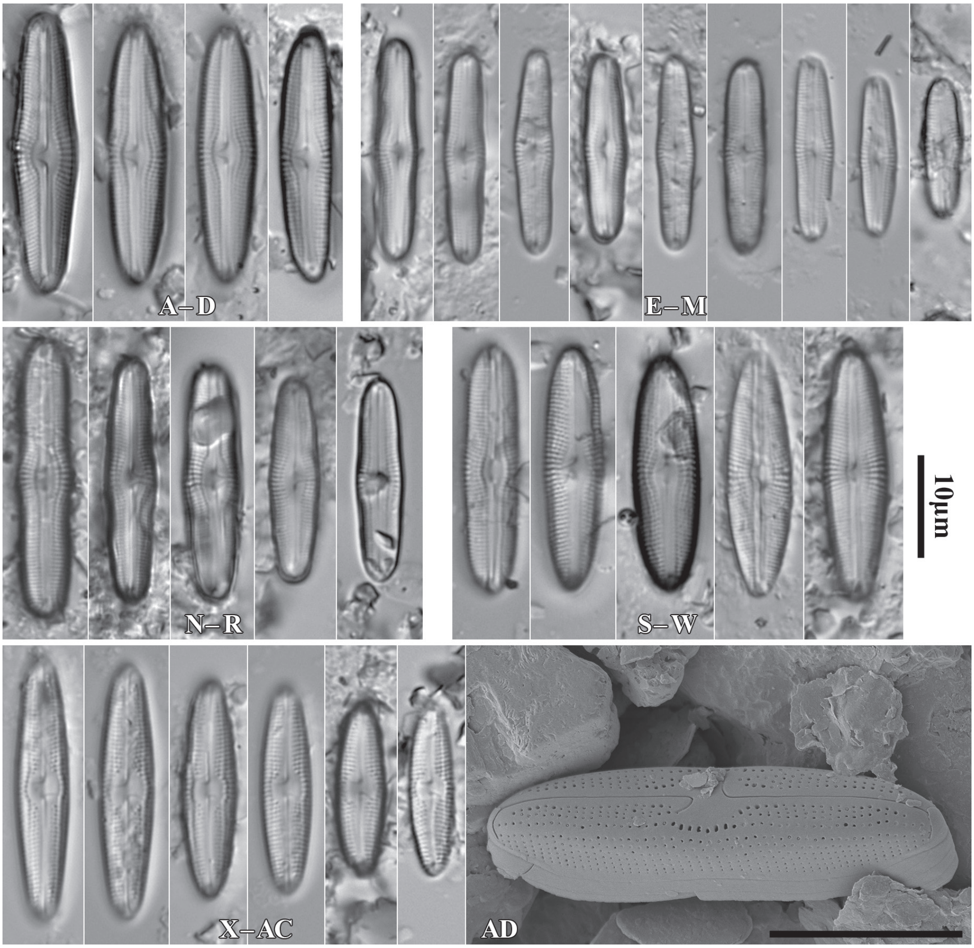

Figure 15. LM microphotographs of *Muelleria islandica* (**A–D**), *M. undulata* (**E–M**), *M. terrestris* (**N–R**), *M. sasaensis* (**S–W**) and *Neidium perforatum* (**X–AC**). SEM microphotography of *N. perforatum* (**AD**). Scale bar: 10 µm (**A–AC**); 5 µm (**AD**).

been identified previously as *L. mutica*, which is a brackish species (Levkov et al. 2013). Consequently, many modern reports of *L. mutica*, especially those from terrestrial and freshwater habitats, require systematic verification.

Luticola bryophila sp. nov. is most similar to the two European species *L. sparsipunctata* and *L. tenuis*. All three taxa share similar sizes and striae densities. The newly described species of the genus *Luticola* commonly shows ghost areolae in the central area, which are absent in both of the other taxa. Additionally, *L. bryophila* can be easily separated from *L. tenuis* based on distal raphe endings, which are short and deflected and not hooked and do not continue onto the valve mantle. *Luticola sparsipunctata* shows two morphotypes with different distal raphe ending morphology. The first of them has a hooked end that continues onto the valve mantle raphe endings, while the second has short and only deflected raphe endings (Levkov et al. 2013, pl. 33). However, *L. sparsipunctata* can

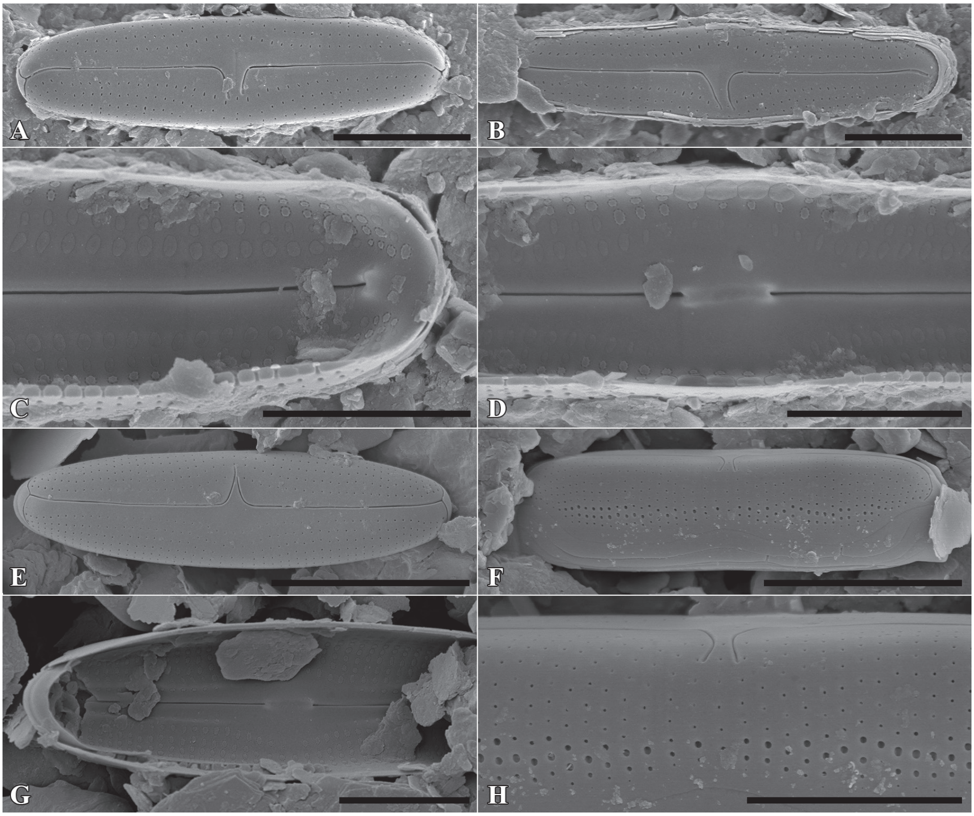

Figure 16. SEM microphotographs of *Muelleria undulata* (**A–D**) and *M. terrestris* (**E–H**). Scale bars: 10 µm (**E, F**), 5 µm (**A, B, G, H**); 3 µm (**C, D**).

also be easily distinguished from *L. bryophila* by the more lanceolate valve shape and the different striae morphology that manifests in the small areolae near the raphe slit and the large areolae near the valve edge (Levkov et al. 2013, p. 222; Fig. 11N), while in *L. bryophila* the areolae in striae are of the same size. Moreover, the internal view of the valve differs in both species. In *L. bryophila* the hymenes form a continuous strip on the striae, while in *L. sparsipunctata* the hymenes are interrupted and cover only the areolae, not the whole striae (Levkov et al. 2013, pl. 33, fig. 7; Fig. 11O).

Luticola confusa sp. nov. is highly similar in valve and central area shape and striae pattern to *L. imbricata*, *L. pseudoimbricata*, and *L. obscura*. The new species can be distinguished from *L. imbricata* based on its less lanceolate, narrower valves (5–9 µm width in *L. imbricata* vs. 4–6 µm in *L. confusa*) (Levkov et al. 2013, p. 134) and by small depressions on the proximal raphe endings, while they are absent in *L. imbricata* (Levkov et al. 2017, figs 191, 192). Compared to *L. obscura*, which has hooked distal raphe endings terminating on the valve face, *L. confusa* forms hooked distal raphe endings that continue onto the valve mantle (Levkov et al. 2017, fig 169, 170). *L. obscura* also lacks small depressions on the proximal raphe endings, while they are

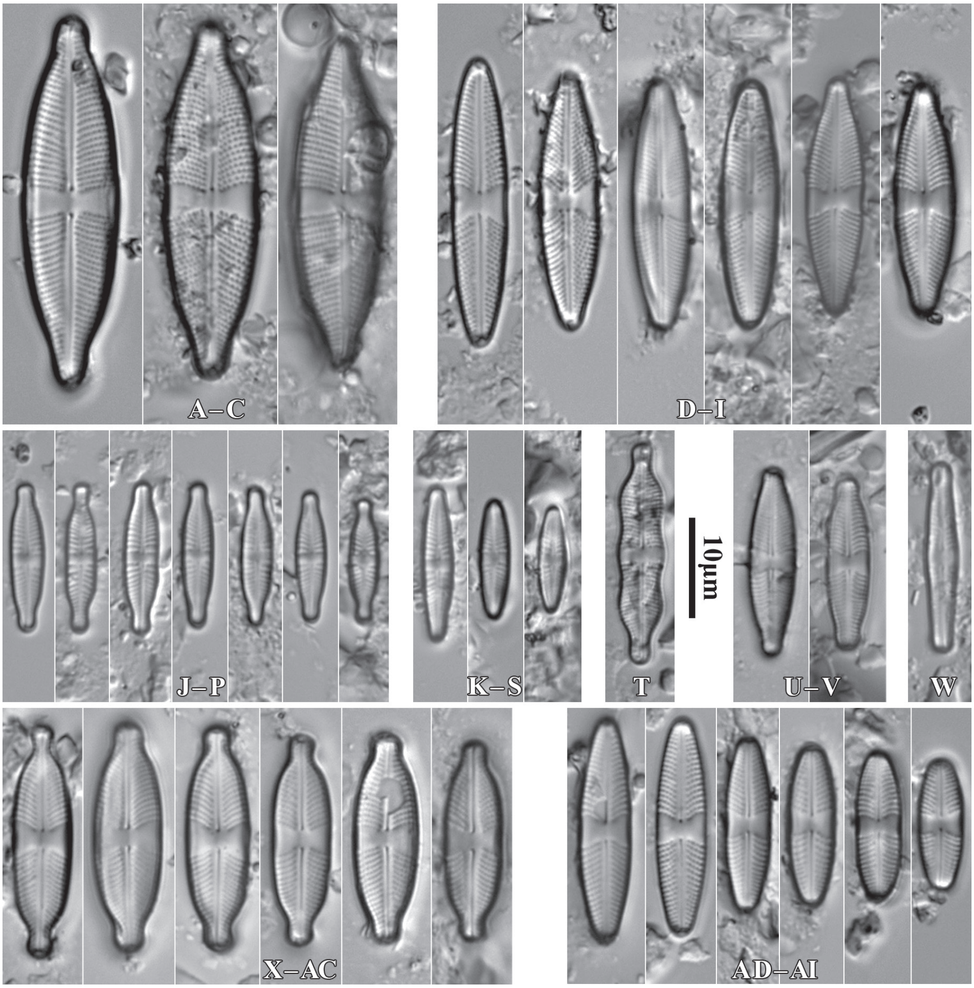

Figure 17. LM microphotographs of *Stauroneis saprophila* (**A–C**), *S. obtusa* (**D–I**), *S. thermicola* (**J–P**), *S. parathermicola* (**K–S**), *S. leguminopsis* (**T**), *S.* aff. *lundii* (**U,V**), *S. muriella* (**W**), *S. laterostrata* (**X–AC**), and *S. borichii* (**AD–AI**). Scale bar: 10 µm.

present in *L. confusa*. The structure of raphe endings also distinguishes *L. confusa* from *L. pseudoimbricata*. Both taxa share the same valve dimensions, but *L. pseudoimbricata* has a raphe with slightly deflected proximal endings and distal raphe endings terminate on the valve face (Levkov et al. 2013, pls 16, 1–5).

Diversity and composition of the assemblages analyzed

During the study 143 diatom taxa were identified, but most of them were found in single samples and often their share did not exceed 1% of communities. Only 16 species were common in the samples studied (in over 20% of the samples), of which 13

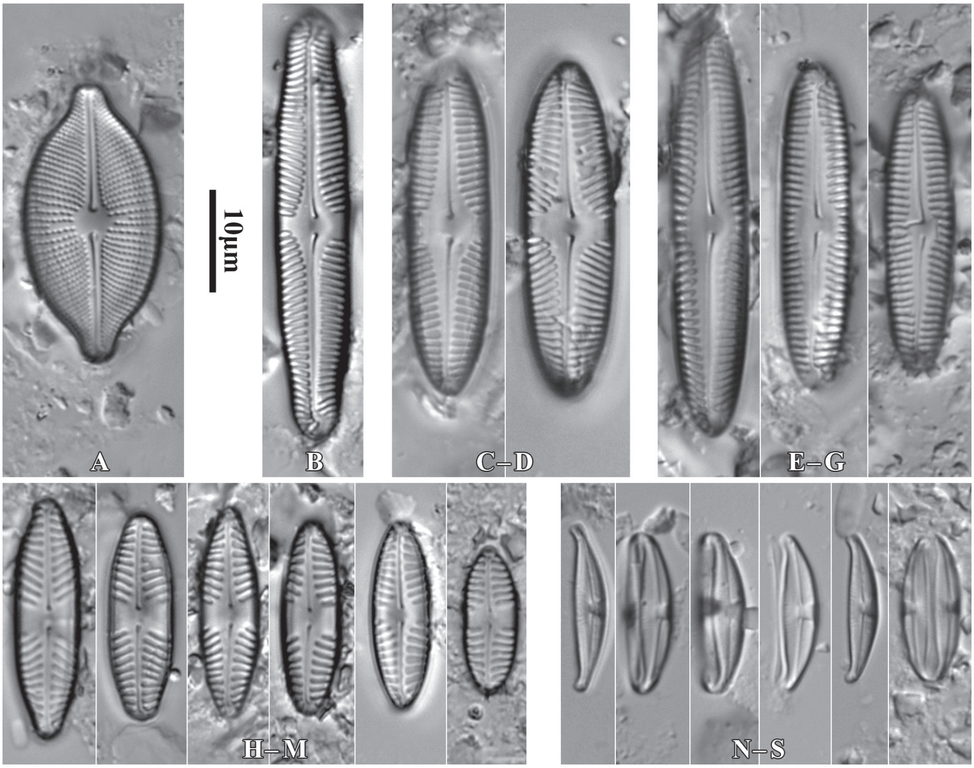

Figure 18. LM microphotographs of *Cosmioneis pusilla* (**A**), *Pinnularia microstauron* var. *angusta* (**B**), *P. brebissonii* (**C, D**), *P. isselana* (**E–G**), *P. cuneola* (**H–M**) and *Halamphora montana* (**N–S**). Scale bar: 10 µm.

species formed numerous populations (from 10% in assemblages to practically monocultures). The vast majority of the species recorded were taxa commonly identified in various terrestrial environments, mainly in soils (Lund 1946; Ettl and Gärtner 1995; Kerckvoorde et al. 2000; Stanek-Tarkowska et al. 2013; Noga et al. 2014; Barragán et al. 2018; Foets et al. 2021), but also on raised objects like cave walls (Claus 1955, 1964; Krammer and Lange-Bertalot 1991b; Poulíčková and Hašler 2007; Falasco et al. 2014), and on cliffs and walls overgrown by mosses (Round 1957; Casamatta et al. 2002; Lowe et al. 2007; Ress and Lowe 2014).

Assemblages noted on the trunks of all the trees studied growing in city centers and small peripheral estates were dominated by species able to develop in low moisture habitats with high osmotic stress (*Hantzschia abundans*, *H. amphioxys*, *Humidophila contenta*, *Pinnularia borealis*, *P. obscura*) (Lange-Bertalot et al. 2017; Foets et al. 2021). Several co-dominant species were present at these sites, which resulted in higher values of the H' and J' indexes.

Similar assemblage structures were also noted on linden and poplar at sites located in suburban park complexes and national park buffer zones. It seems that corticolous assemblages consisting mainly of drought-resistant diatom taxa are typical of these tree species regardless of the degree of tree cover in the area in which they grow.

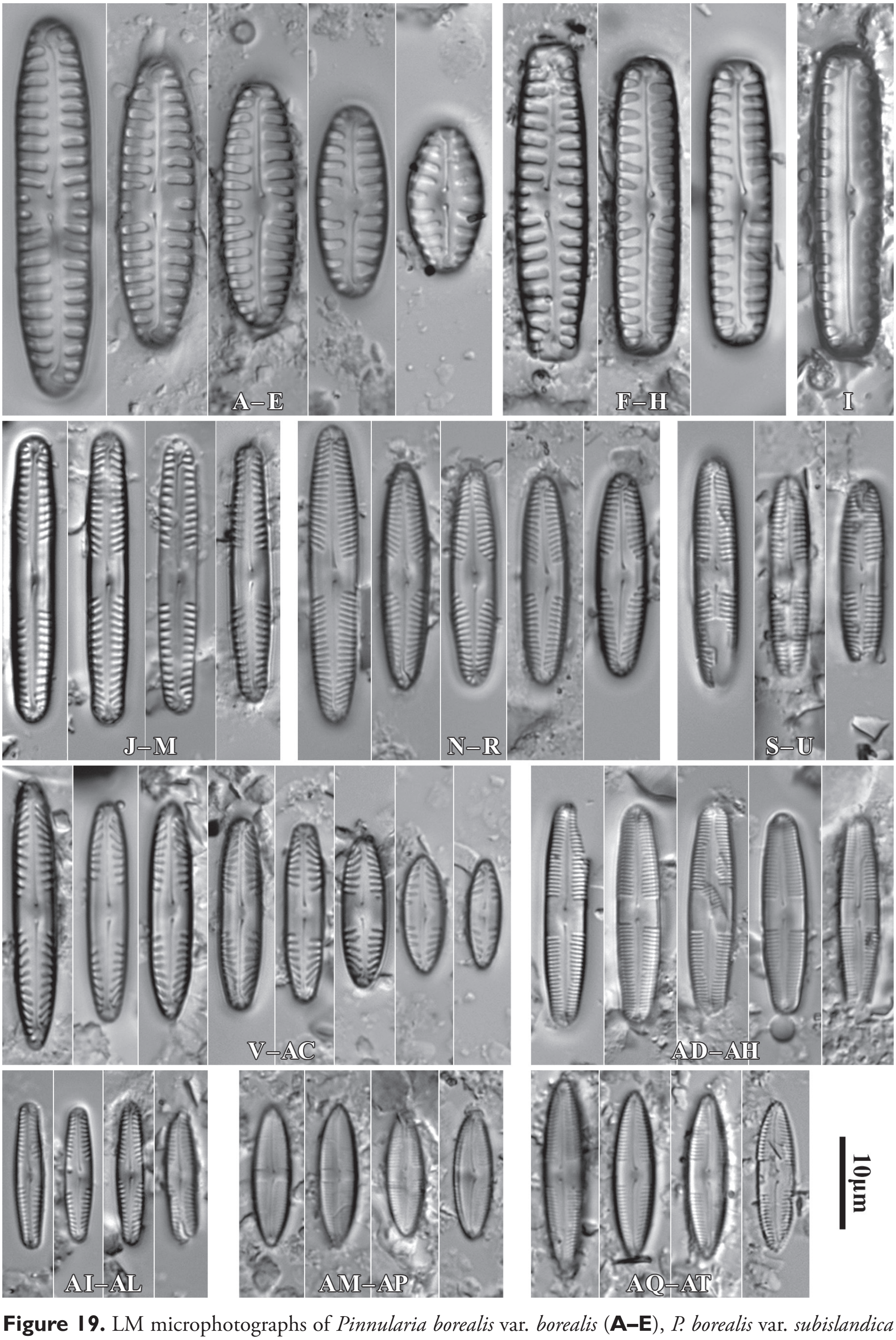

Figure 19. LM microphotographs of *Pinnularia borealis* var. *borealis* (**A–E**), *P. borealis* var. *subislandica* (**F–H**), *P. dubitabilis* (**I**), *P. sinistra* (**J–M**), *P. schoenfelderi* (**N–R**), *P. frauenbergiana* var. *caloneiopsis* (**S–U**), *P. obscura* (**V–AC**), *Caloneis* cf. *langebertalotioides* (**AD–AH**), *P. perirrorata* (**AI–AL**), *C. vasileyevae* (**AM–AP**) and *C. lancettula* (**AQ–AT**). Scale bar: 10 µm.

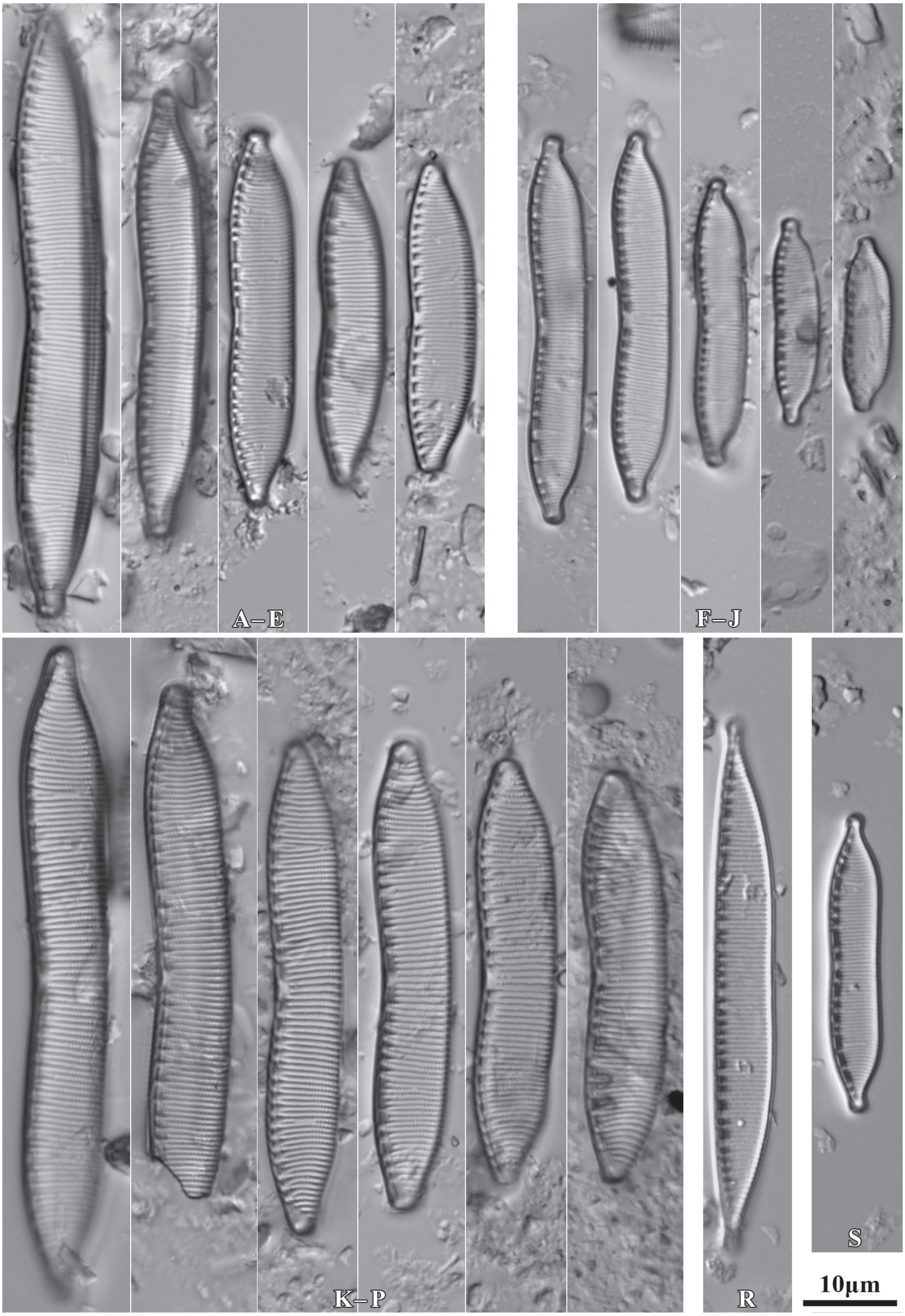

Figure 20. LM microphotographs of *Hantzschia abundans* (**A–E**), *H. amphioxys* (**F–J**), *H. subrupestris* (**K–P**), *H. dorgaliensis* (**R**) and *H. stepposa* (**S**). Scale bar: 10 µm.

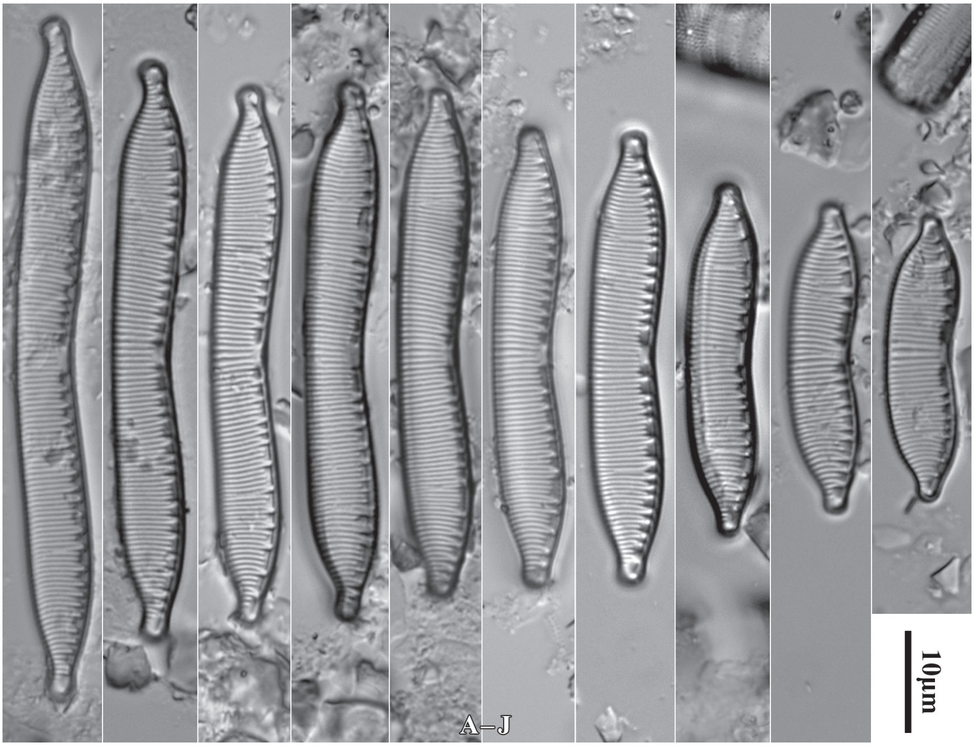

Figure 21. LM microphotographs of *Hantzschia calcifuga* in size diminution series (**A–J**). Scale bar: 10 μm.

Qin et al. (2016), who studied diatoms inhabiting arboreal mosses (collected from *Cinnamomum camphora* Ness et Eberm., *Quercus aliena* Blume and *Pterocarya stenoptera* D.CD.) in Wuhan, also found numerous specimens of *P. borealis*, *H. amphioxys*, *O. dendroteres*, and *H. contenta*. These species were also reported in works concerning mainly corticophilic green algae and cyanobacteria, (Neustupa and Škaloud 2010; Neustupa and Štifterová 2013; Štifterová and Neustupa 2015), which indicates that they are common elements of microalgal corticolous biofilms.

Assemblages from sycamore maple (except those from city centers) were distinctly different from those inhabiting linden and poplars because of the strong domination of just one species, which often formed near monocultures (Fig. 23). In parks, assemblages on sycamore were dominated (especially in the case of bryophyte samples) by *Luticola acidoclinata*, a taxon commonly inhabiting moss clumps even in conditions of high osmotic stress, that are able to survive on highly saline soils (Levkov et al. 2013, 2017). Conversely, on the same host tree species in national park buffer zones with huge tree density, the main dominant species was *Orthoseira dendroteres*, which is typical of more shaded and wet habitats like caves and riparian zones of streams (Poulíčková and Hašler 2007; Škaloud 2009; Czerwik-Marcinkowska and Mrozińska

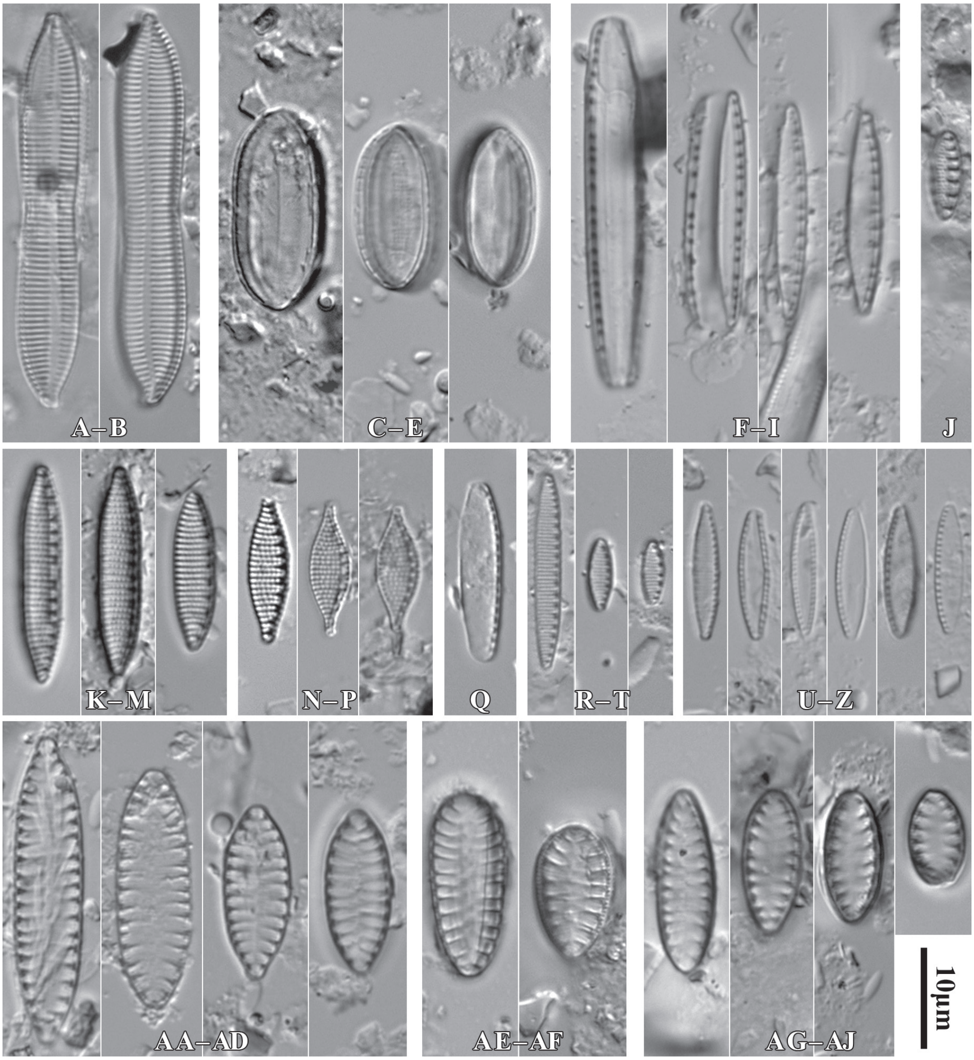

Figure 22. LM microphotographs of *Tryblionella apiculata* (**A, B**), *T. debilis* (**C–E**), *Nitzschia harderi* (**F–I**), *N. solgensis* (**J**), *N. amphibia* (**K–M**), *N. amphibia* f. *apiculata* (**N–P**), *N. communis* (**Q**), *N.* cf. *frustulum* (**R–T**), *N. pusilla* (**U–Z**), *Surirella angusta* (**AA–AD**), *S. minuta* (**AE, AF**) and *S. terricola* (**AG–AJ**). Scale bar: 10 µm.

2011; Czerwik-Marcinkowska et al. 2015; Barragán et al. 2018). The mass development of both taxa resulted in the tremendous homogenization of assemblages reflected in the very low (even 0) values of the H' and J' indexes (Fig. 4).

Additionally, many diatom species often common in terrestrial and aerophytic habitats (numerous representatives of the genera *Luticola*, *Mayamaea*, and *Muelleria*; *Microcostatus aerophilus*; *Stauroneis borrichii*; *S. parathermicola*; *S. termicola*; *Sellaphora harderi*; *S. nana*, *S. subseminulum*) rarely developed in the environments studied. They

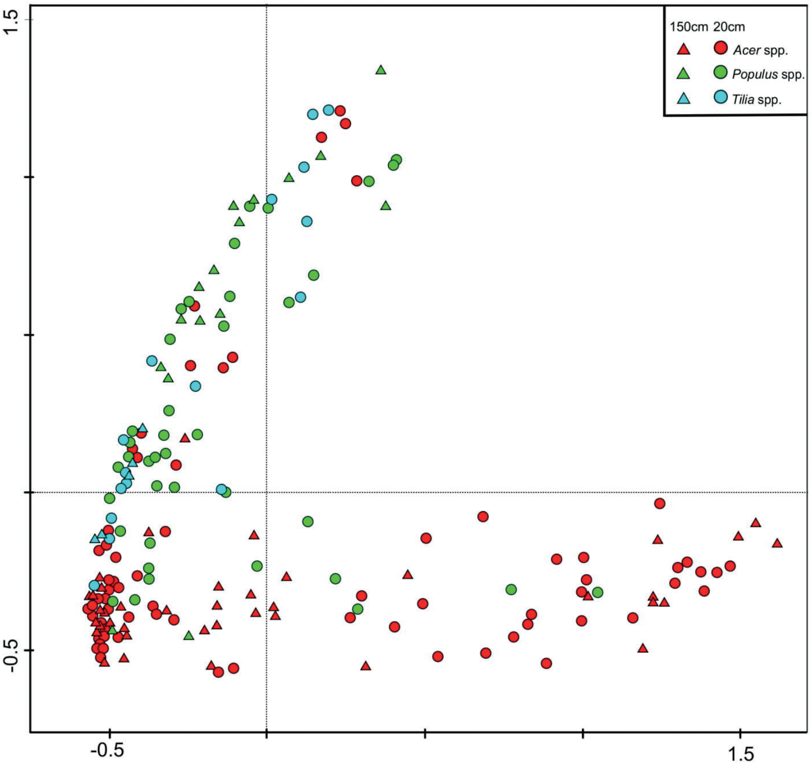

Figure 23. PCA ordination of analyzed samples determined by relative diatom assemblage composition on studied tree taxa.

were either observed in single samples or their share in assemblages did not exceed a few percent (Levkov et al. 2013; Stanek-Tarkowska et al. 2013; Noga et al. 2014; Barragán et al. 2018; Foets et al. 2021). The habitat conditions seemed to be too harsh for the development of these species in larger numbers, or the species could have found suitable conditions on different host trees that were not examined in the current study.

Except for taxa commonly reported from terrestrial habitats, diatoms that usually occur in aquatic environments were noted. The most common were centric taxa such as *Aulacoseira* spp., *Pantocsekiella* sp. and *Cyclostephanos dubius* that are recorded in freshwater planktonic assemblages. They were observed mainly as single, often damaged, frustules, but were observed in 25% of the samples analyzed. Freshwater species associated with epiphytic and epilithic communities (*Achnanthidium minutissimum*, *Cocconeis* spp., *Gomphonema* spp., *Pseudostaurosira brevistriata*) were also observed in the materials examined; however, they were noted significantly less frequently than planktonic taxa. Vacht et al. (2014), Martínez-Carreras et al. (2015) and Barragán et al. (2018) also observed typical aquatic diatoms (especially centric ones) in terrestrial environments. Their presence in the environments studied could have resulted from aerosol transmission, which is thought to be the most effective means of dispersing aerosolized microalgal cells, colonies, or whole filaments (Sharma et al. 2007; Harper and McKay 2010; Van Eaton et al. 2013; Sahu and Tangutur 2015; Tesson et al. 2016). Similar natural wind-borne "contamination" in terrestrial habitats was observed by Gottschling et al. (2020), who reported the presence of dinophyte DNA in soil samples. Based on the methods used in the present research, it is not possible to state unequivocally which of the identified species actually live in the habitats examined, only thrive there, or were only empty valves deposited in the bark cracks that were examined.

Conclusions

The present research showed that the occurrence of diatom assemblages on tree trunks is influenced by many factors, such as host tree species and the area in which these trees grow, high above soil as well as the presence of suitable microhabitats within trunks. Additionally, diatom assemblage composition was mainly influenced by the tree species.

The current research focused on communities developing on only a few tree species occurring naturally in Europe. Further research involving other tree taxa is necessary for developing a better understanding of corticolous diatom assemblages.

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Taxonomy and nomenclature of *Abutilon albidum* (Malvaceae, Malvoideae), a cryptic Saharo-Canarian species recently rediscovered in Tenerife

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Abstract

Abutilon albidum, a cryptic Saharo-Canarian species, was thought to have been last collected in 1945 in Tenerife by E.R. Sventenius. In 2019, it was rediscovered in the same area. The characteristic features of the Canarian plants are discussed, especially in relation to the morphologically similar-looking and probably closely-related species *Abutilon indicum* and *A. bidentatum*. It is concluded that the plants from Tenerife and north-western Africa indeed represent a distinct species. The species is illustrated and a key for the identification of this and related species is presented.

Keywords

Abutilon, Canary Islands, Malvaceae, nomenclature, Saharo-Canarian, taxonomy, Tenerife

Introduction

Abutilon Mill. (Malvaceae, Malvoideae) is a large genus which mostly occurs in tropical and subtropical areas around the world, with a few species extending to warm-temperate regions. The genus is most diverse in the Neotropics (Areces Berazaín and Fryxell

2007). It is one of the largest Malveae genera, although the number of its recognised species considerably varies, depending on the species delimitation. Most authors nowadays accept ca. 150–160 species (e.g. Bayer and Kubitzki (2003); Mabberley (2008); Verdcourt and Mwachala (2009)), but the boundaries of the genus remain unclear.

Abutilon lacks a solid, modern revisionary worldwide treatment, as well as an extended molecular phylogeny (a partial molecular phylogeny was presented by Donnell et al. (2012)). However, local accounts are available that cover large parts of the main areas of occurrence of the genus. For the New World, keys were published for the North and South American species (Kearney 1955, 1958; Fryxell and Hill 2015), as well as for the Mexican species (Fryxell 1988). Several accounts are available for the Caribbean as well (e.g. Adams (1972); Fryxell (1989); Areces Berazaín and Fryxell (2007)). In the Old World, taxonomic information is more fragmented and revisions covering multiple countries are scarce. In Africa, useful treatments are available for East Africa (Vollesen 1995; Thulin 1999; Verdcourt and Mwachala 2009), West Africa (Hutchinson and Dalziel 1958), southern Africa (Meeuse 1961; Roessler 1969; Exell and Gonçalves 1979), Central Africa (Hauman 1963) and North Africa (several accounts, for example, Quézel and Santa (1963); Ali and Jafri (1977); Fennane et al. (1999); Boulos (2000); El Hadidi (2000)). In Asia, the revisions for Malesia (Borssum Waalkes 1966), Pakistan (Abedin 1979), China (Tang et al. 2007), Oman (Ghazanfar 2003), Iraq (Townsend 1980), Iran (Riedl 1976), Yemen (Wood 1997) and India (Naqshi et al. 1988; Paul and Nayar 1988; Paul 1993) are worth mentioning. Some useful accounts were also published for islands in the Indian Ocean (e.g. Hochreutiner (1955), Marais (1987)). Finally, in Australia revisions are available for some of the States, such as New South Wales (Mitchell and Norris 1990). In Europe, not a single species is native, although *A. theophrasti* Medik. is sometimes mistakenly considered as such (e.g. Webb (1968)).

In their monumental “Histoire Naturelle des îles Canaries”, Webb and Berthelot (1836) provided a detailed description of “*Abutilon albidum* Nob.”, a species they explicitly based on Willdenow’s *Sida albida* Willd. It was reported from the Santa Cruz area in the south-eastern corner of the island of Tenerife. Subsequent authors (e.g. Bornmüller (1904); Pitard and Proust (1908); Lindinger (1926); Burchard (1929)) regularly confirmed its presence in a small area roughly located between Santa Cruz and Igueste de San Andrés. However, after the 1940s, it was no longer collected nor mentioned in the regional literature. Surprisingly and apparently without any explanation, Canarian records of this species were later considered to be referrable to the invasive weed *A. grandifolium* G. Don (e.g. Eriksson et al. (1974); Santos (1983); Hohenester and Welss (1993); Valdés (2011); Dobignard and Chatelain (2012); BIOTA (2023)).

In December 2019, an unknown species of *Abutilon* was observed by one of us (F.V.) in several localities in the village of Igueste de San Andrés (Municipality of Santa Cruz de Tenerife), on the verge of the natural protected area ‘Anaga Rural Park’ in the south-easternmost part of Tenerife. The observed plants clearly differed from another species of *Abutilon* that is widely naturalised and invasive in the Canary Islands, *A. grandifolium*. Although identification attempts were at first unsatisfactory, the plants eventually were determined to belong to the species that had been known from Tenerife since the end of the 18th century, but which had not been collected since 1945 and apparently completely forgotten by Canarian authors.

Materials and methods

Fieldwork was undertaken by the first author in December 2019 and March 2022 and, for revision of other localities in Tenerife, by the second author in May and June 2021. Herbarium specimens were collected and deposited at the herbarium of the Meise Botanic Garden [**BR**; for herbarium acronyms Thiers (2023) is followed]. In addition, numerous photographs were made that showed essential diagnostic features, which are less easily observed in pressed specimens. In March 2022, a detailed inventory was made of all remaining populations and all were georeferenced and registered in the nature observations platform Observation.org (https://observation.org/soort/view/1029740).

Herbarium specimens relevant for this study (including type material) were studied in the herbaria of the Meise Botanic Garden (**BR**), the Jardín Botánico Canario Viera y Clavijo (**LPA**) and the Jardín de Aclimatación de la Orotava (Instituto Canario de Investigaciones Agrarias) (**ORT**). In addition, images from several herbaria available online were also consulted (**B**, **COI**, **FI**, **G**, **K**, **L**, **MO**, **MPU**, **NYBG**, **P**, **RAB**, **UM** and **W**).

Countless literature sources (including protologues), deemed useful for this study, were checked to better understand the identity and taxonomy of the species and the characters that differentiate it from closely-related taxa.

Results

The nomenclature of *Abutilon albidum*

Willdenow (1809) validly published the name *Sida albida* Willd. for a species that was later transferred to *Abutilon* by Sweet (1826) as *A. albidum* (Willd.) Sweet. The only original extant specimen for *S. albida* is seemingly the holotype (Fryxell 2002) and is preserved at B. From this specimen and the protologue, it can be deduced that the plant described as *S. albida* definitely differs from the Saharo-Canarian one.

However, since there is no other binomial for the Saharo-Canarian plant, conservation of the name *Sida albida* with a conserved type was proposed (Verloove and Sennikov (2022) and discussion therein). If the proposal is not accepted, a new name will have to be given to this species.

The quest for the identity of an unknown *Abutilon* from Tenerife

From Tenerife, only a single, yellow-flowered species of *Abutilon* is currently known, *A. grandifolium*. This species, native to South America, is widely naturalised and invasive in the Canary Islands. Plants found in 2019 in the south-eastern part of Tenerife are strikingly different. In comparison to *A. grandifolium*, their corollas are much smaller, fruit (schizocarp) has more numerous mericarps (usually around 15), calyx is much shorter than the fruit, stem indumentum is quite different (a mixture of short stellate hairs and long patent hairs) and inflorescences consist of both solitary flowers and axillary (pseudo-)panicles. Yet, since the second half of the 20th century, Canarian authors considered historical records of *A. albidum* to be erroneous and referred them to *A. grandifolium* instead (Eriksson et al. 1974; Santos 1983; Hohenester and Welss 1993; Valdés 2011; Dobignard and Chatelain 2012; BIOTA 2023).

If the Tenerife plant is not *Abutilon grandifolium*, then what is its correct name? Identification attempts initially led – with not too much difficulty – to *A. indicum*, a common weed from the Old World tropics (e.g. Borssum Waalkes (1966)) that is also widely naturalised in the Caribbean (Fryxell 1989). However, the Tenerife plant clearly differed from that species as well, for instance, in having much shorter petals and mericarps. Its stem indumentum is also quite different: it is composed of a mixture of very short, dense stellate hairs, sparser and slightly longer multicellular hairs and rather numerous long, patent simple hairs, whereas in *A. indicum* the stem is densely covered with stellate hairs, rarely with some additional slender, simple hairs.

Identification keys from Pakistan and India key out *Abutilon indicum* together with a similar species, *A. bidentatum* (Hochst.) A. Rich., described from Ethiopia, but widely distributed from Central Africa to India. In areas where these two species occur sympatrically, they are considered to be very similar. Husain and Baquar (1974) separated *A. bidentatum* from *A. indicum*, based on its smaller, paler corollas. Bhandari (1995) distinguished *A. bidentatum* and *A. indicum*, based on carpel length (6–8 mm vs. 9–12 mm, respectively). According to Paul and Nayar (1988), both species are differentiated as follows: *A. bidentatum* has a staminal column 2–3 mm long, a schizocarp 10 mm across, mericarps 10 × 5 mm and gradually acuminate dorsally, whereas *A. indicum* has a staminal column 5–7 mm long, a schizocarp 1.5–2.5 cm across, mericarps 10–15 × 7–10 mm and acute to acuminate dorsally. The diagnostic characters put forward by these authors for *A. bidentatum* are evidently more in line with those observed in the plants found in Tenerife. However, while *A. bidentatum* has mericarps with two distinct dorsal cusps (hence the species epithet), the mericarps in the Tenerife plants are merely shortly acute-triangular dorsally at maturity, without clear protuberances.

Eventually, floristic accounts from north-western Africa, more precisely from Algeria and Morocco (Quézel and Santa 1963; Fennane et al. 1999), which are geographically close to Tenerife, threw new light on the possible identity of the Tenerife plant. Based on mericarp number and stem indumentum, the plants were easily keyed out as *Abutilon albidum* subsp. *albidum*. A subsequent study of herbarium specimens (including type material) and protologues of *Sida albida* (Willdenow 1809), *S. bidentata* (Hochstetter 1842) and *S. indica* (Linnaeus 1756) demonstrated that these three species are very similar in many characters. Yet, the plant material from Tenerife and north-western Africa is considered to be sufficiently distinct, as correctly assumed by Webb and Berthelot (1836) and other Canarian workers up to the first half of the 20th century (e.g. Bornmüller (1904); Pitard and Proust (1908); Lindinger (1926); Burchard (1929)) and by contemporary North African authors (Fennane et al. 1999). Preliminary results from a molecular analysis also showed these three species to be closely related, but distinct (Verloove, unpubl. data).

Thus, *Abutilon albidum*, *A. bidentatum* and *A. indicum* are very similar in general appearance and have often been confused. Claims of the last from Africa (e.g. Masters (1868); Ulbrich (1913)), for instance, are probably erroneous (e.g. Hauman (1963); Roessler (1969); Verdcourt and Mwachala (2009); Valdés (2011)). Most records belong, in fact, to *A. mauritianum* (Jacq.) Medik. and related or similar species like *A. bidentatum*. *A. albidum* has also been combined under *A. indicum*, as var. *albidum* (Willd.) Baker f. (Baker 1893).

Diagnostic characters

Morphological features used for the separation of *Abutilon albidum*, *A. bidentatum* and *A. indicum* are discussed below.

The **stem indumentum** of these species is different. *Abutilon indicum* has an indumentum that almost entirely or even exclusively consists of very short and dense stellate hairs. Simple, long patent hairs are always absent or very sparse (e.g. Hochreutiner (1902); Adams (1972); Townsend (1980); Fryxell (1989)). *Abutilon albidum* and *A. bidentatum*, on the contrary, have a mixture of short stellate hairs and long patent hairs; both hair types are equally abundant. In addition, rather numerous multicellular hairs are observed (some of them gland-tipped). In length, these hairs are slightly or much longer than the stellate ones. This feature is rarely mentioned in floristic accounts, although exactly the same pubescence is observable in the syntype of *A. bidentatum* kept at BR. Notably, Hochreutiner annotated a syntype at K in 1899: “Le A. bidentatum de Hochst. est une forme villeuse et légèrement glanduleuse de *A. indicum* Sw. avec lequel nous l’assimilons” [translation F.V.: *A. bidentatum* of Hochst. is a villous and slightly glandular form of *A. indicum* Sw. with which we equate it.]. Apparently, Hochreutiner also noticed the presence of the third hair type in *A. bidentatum*. Wood (1997) described *A. bidentatum* as a glandular perennial and this likely also refers to the presence of multicellular hairs. The long description of Webb and Berthelot (1836) did not refer to the presence of multicellular hairs. However, such hairs are observable in historical, as well as recent, collections from Tenerife.

These three species also differ in **flower size** and **colour**. The populations found in Tenerife are small-flowered: flowers are ca. 15 mm in diameter with petals 7–10 mm long (i.e. only slightly longer than the calyx). *Abutilon indicum*, in contrast, is a large-flowered species with corollas 25–35 cm in diameter and with petals 12–15 mm long (e.g. Borssum Waalkes (1966); Abedin (1979); Naqshi et al. (1988); Philcox (1997); Areces Berazaín and Fryxell (2007); Bano and Deora (2018)). Petals are, in fact, about twice as long as the calyx or even longer (Riedl 1976). In the syntype of *A. bidentatum* at BR, flowers are about 18 mm in diameter. In addition, the staminal column is much shorter in *A. albidum* and *A. bidentatum* than in *A. indicum* (e.g. Abedin (1979); Naqshi et al. (1988); Paul and Nayar (1988)). The plants from Tenerife and a syntype of *A. bidentatum* at BR have a staminal column 2–3 mm long, whereas the staminal column in *A. indicum* is 5–7 mm long. Finally, petals of *A. albidum* and *A. bidentatum* tend to be slightly paler; i.e. pale yellow to yellow ('pallidè luteola' for *A. albidum*, according to Webb and Berthelot (1836)), whereas those of *A. indicum* are usually said to be yellow to orange yellow (e.g. Husain and Baquar (1974); Abedin (1979); Naqshi et al. (1988)).

Fruit characters are also different in these species. Mericarps are invariably longer and wider in *Abutilon indicum*. In areas where this species and *A. bidentatum* occur sympatrically they are distinguished (often even exclusively) based on this character (e.g. Jafri (1966); Paul and Nayar (1988); Bhandari (1995)). In the plants from Tenerife, mericarps are up to 9 mm long and 5 mm wide (often slightly smaller). This is roughly in line with measurements taken on the syntype of *A. bidentatum* at BR (8 mm long and 5 mm wide). In *A. indicum*, in contrast, mericarps are often twice as long and broader. The number of mericarps per fruit tends to be higher in *A. indicum* than in *A. albidum* and *A. bidentatum*. In *A. albidum*, the number of mericarps ranges between 10 and 15 (Webb and Berthelot 1836), although most fruits have around 15 mericarps. Similar numbers are known for *A. bidentatum*, whereas Philcox (1997) gives 20–30 for *A. indicum*. We observed some overlap in these measurements, but the mericarps are, indeed, more numerous in *A. indicum*. In all these species, mericarps turn black at maturity; they are hairy outside and shiny inside. Mericarp ornamentation is considered to be an important diagnostic feature for the separation of species in *Abutilon*. The outer apical (dorsal) margin can be either rounded (without protuberances) or angled. In the latter case, the outgrowth can be merely acute, gradually acuminate to aristate. *Abutilon bidentatum* was initially described as having mericarps that are bidentate at apex ("carpellis compressis apice truncatis, bidentatis"). In the syntype at BR, a cusp ca. 1 mm long is discernible. Most authors describe the cusp length as 1–2 mm long (e.g. Thulin (1999); Verdcourt and Mwachala (2009)). At this point, the plants observed in Tenerife definitely differ from *A. bidentatum*: mericarps are angled at the dorsal margins, but a cusp or protuberance is missing. In *A. indicum*, mericarps can vary from rounded, obtuse to long acuminate (Borssum Waalkes (1966); see also Bano and Deora (2018)). The schizocarp also tends to be less wide in *A. albidum* and *A. bidentatum* (10–15 mm across) than in *A. indicum* (rather 15–25 mm across). Finally, according to Jafri (1966), mericarps in *A. bidentatum* already dehisce before breaking away from the central axis – a feature also observed in the plants from Tenerife – whereas in *A. indicum* mericarps dehisce after breaking away from the central axis.

Finally, *Abutilon albidum*, *A. bidentatum* and *A. indicum* also differ in **inflorescence shape**. All have flowers that are inserted in the leaf axils. However, whereas in *A. indicum* flowers are invariably solitary, they often merge into distinct panicles in *A. bidentatum* (e.g. Verdcourt and Mwachala (2009)). This feature was also observed in the plants that are found in Tenerife.

On the other hand, some diagnostic characteristics mentioned in literature proved to be of no value or merely erroneous. For instance, pedicels are usually said to be shorter than petioles in *A. bidentatum* and vice versa in *A. indicum* (Abedin 1979; Borssum Waalkes 1966; Ghazanfar 2003; Verdcourt and Mwachala 2009). In reality, pedicel and petiole length are variable in these three species, although pedicels often are at least as long as or longer than petioles, also in *A. bidentatum* (as can be seen in a syntype at BR, as well as in the material from Tenerife).

The features that appear to be most reliable for the separation of these three species are summarised in Table 1.

From the above, it can be concluded that *Abutilon albidum* is most closely similar to *A. bidentatum* morphologically. These two species share all characters, except that their mericarps are ornamented in different ways. Since mericarp ornamentation is considered to be an important diagnostic trait in the genus (Alzahrani et al. 2021), both species are apparently distinct. Besides these morphological differences, the species also seem to differ ecologically and occur in non-overlapping areas (see below).

Finally, another species that has often been associated with *Abutilon albidum* is *A. fruticosum* Guill. & Perr. In fact, Maire (1933) reduced the latter to subspecies rank, as *A. albidum* subsp. *fruticosum* (Guill. & Perr.) Maire (see also Quézel and Santa (1963)). A specimen of *A. albidum* at FI, annotated with 'type', is also stored under *A. fruticosum*. More or less similar criteria are also followed by POWO (2023) that considers *A. albidum* sensu Webb & Berthel. as a synonym of *A. fruticosum*. Hochreutiner (1902) already emphasised that these two species are, in fact, not related. *Abutilon fruticosum* has smaller and paler (green turning brown, never blackish), almost turbinate fruits that always have fewer mericarps (usually 9–10), smaller rounded leaves, often with almost entire margins and a stem indumentum that entirely consists of very short stellate hairs.

Table 1. Features considered most reliable for differentiating *Abutilon albidum*, *A. bidentatum* and *A. indicum* (based on our study of type material, Borssum Waalkes (1966); Abedin (1979); Vollesen (1995); Ghazanfar (2003); Verdcourt and Mwachala (2009); Randall (2017) and many other sources).

Character / species	*Abutilon albidum*	*A. bidentatum*	*A. indicum*
Stem pubescence	Stellate, tomentose, intermixed with long simple patent hairs and multicellular hairs	Stellate, tomentose, intermixed with long simple patent hairs and multicellular hairs	Dense stellate hairs, rarely with some slender, simple hairs
Staminal column length	2–3 mm long	2–3 mm long	5–7 mm long
Mericarps	10–15 in number, 9 × 5 mm, the outer apical (dorsal) margin acute-triangular, without cusps or protuberances	13–16 in number, 8–10 × 3–5 mm, the outer apical (dorsal) margin bidentate, i.e. with two cusps 1–2 mm long	15–22 in number, 10–18 × 7–9 mm, the outer apical (dorsal) margin either rounded, acute or long-acuminate
Corolla	10–15 mm across, petals ca. 8 mm long, pale yellow to yellow	10–15 mm across, petals ca. 8 mm long, pale yellow to yellow	25–35 mm across, petals 12–15 mm long, yellow to orange yellow
Inflorescence	Flowers solitary in the leaf axils or merging into distinct panicles	Flowers solitary in the leaf axils or merging into distinct panicles	Flowers solitary, in the leaf axils
Distribution	Canary Islands (Tenerife), north-western Africa	Native to eastern Africa	Native to the Indian Subcontinent and neighbouring territories; introduced elsewhere
Ecology	Basaltic rocks in the desert or semi-desert, sandy river beds	Riverine forest, river banks, alluvial Acacia wooded grassland and bushland; weedy in Egypt, Saudi Arabia and India	Waste places, roadsides, along the beach, as a weed in plantations and gardens

The species of *Abutilon* relevant to this study can be identified using the following key

See also Figs 1–5.

1 Perennial shrub; corolla large, 25–35 mm in diameter; calyx more or less urceolate, strongly keeled and cordate-saccate basally; schizocarp with ca. 10 mericarps, each mericarp with 3–5 seeds; naturalised garden escape.......... ***A. grandifolium***
– Annual herb or perennial (sub-)shrub; corolla often smaller, 10–35 mm across; calyx neither urceolate, keeled nor cordate at base; schizocarp with ca. 10 to numerous mericarps, each with 1–3 seeds; weedy and/or non-ornamental species **2**
2 Annual herb with stem indumentum of almost exclusively short hairs; corolla orange yellow; mericarp with dorsal cusps up to 5 mm long ...... ***A. theophrasti***
– Perennial subshrub with stem indumentum of short stellate hairs, often also with either multicellular hairs or long simple hairs; corolla pale to orange yellow; mericarps with dorsal margin variable, more or less rounded, acute or cuspidate (cusps, if present, up to 2 mm long)... **3**
3 Flowers 25–35 mm in diameter, with staminal column 5–7 mm long; mericarps 15–22 in number, 10–18 × 7–9 mm.. ***A. indicum***
– Flowers always smaller, 15–18 mm in diameter or less, with staminal column 2–3 mm long; mericarps up to 16 in number, (5–) 8–10 × 3–5 mm.............. **4**
4 Stem indumentum exclusively of short, dense stellate hairs; leaves small, with almost entire margins; fruit almost turbinate; mericarps 9–10, almost rounded dorsally .... ***A. fruticosum***
– Stem indumentum of short stellate hairs, multicellular hairs and long simple patent hairs; leaves with irregularly crenate-toothed margins; fruit not turbinate; mericarps more numerous, usually ca. 15, dorsally acute-triangular or with two cusps up to 2 mm long.. **5**
5 Mericarps with (apical) dorsal margin distinctly cuspidate, each cusp 1–2 mm long .... ***A. bidentatum***
– Mericarps merely acute at (apical) dorsal margin, without cusps ..... ***A. albidum***

Taxonomic treatment

Abutilon albidum **(Willd.) Sweet, Hort. Brit.: 54. 1826.**

≡ *Abutilon albidum* (Willd.) Webb & Berthel., Hist. Nat. Iles Canaries (Phytogr. Canar.) Tome troisième, Deuxième partie, Sectio 1: 39. 1836, isonym.
≡ *Abutilon indicum* var. *albidum* (Willd.) Baker f., J. Bot. 31: 213. 1893.
≡ *Sida albida* Willd., Enum. Pl.: 722.1809, nom. cons. prop.
Non *Abutilon albidum* Hooker & Arn., Bot. Beechey's Voy. 278. 1841, nom. illegit.

Type. Spain. Teneriffa, Barranco Santo, *Webb* (FI barcode FI006084), typ. cons. prop. (Verloove and Sennikov 2022).

Figure 1. *Abutilon albidum* in Igueste de San Andrés, Tenerife. General habit of plants growing below rocks along the side of the road. December 2019, F. Verloove.

Description. Erect, short-lived perennial herb or shrub up to 60(–100) cm tall. Branches densely covered by minute stellate hairs, mixed with numerous long, simple spreading hairs (especially, but not exclusively on new growth) and sparser multicellular hairs. Leaves (median cauline) up to 13 cm long and 7 cm wide, broadly ovate to almost rotund, deeply cordate at base, acute to acuminate at apex, irregularly crenate-toothed to double-toothed, very shortly stellate-pubescent on both sides; petiole up to 6 cm long, slightly shorter than blade, very densely stellate hairy mixed with rather numerous short, glandular, capitate hairs and scattered weak, simple, spreading hairs; longest stipules to 9 mm long (mostly shorter), filiform. Flowers axillary, mostly solitary, but sometimes merging to more or less distinct panicles, pedicel 2–4 cm long, elongating in fruit up to 1–8 cm, articulate and geniculate ca. 5 mm below the apex. Calyx 5-lobed for ca. ½ its length, sepals up to 8 mm long, densely long-pubescent on both sides, slightly accrescent, erect, ultimately reflexed; lobes lanceolate-acuminate. Corolla pale yellow to yellow, ca. 1.5 cm across; petals 7–10 mm long, 4–5 mm wide, obovate. Staminal column 2–3 mm long, stellate-pubescent. Fruit 8–10 mm long, ± 13 mm across; mericarps 10–15, black and rather star-like spreading at maturity, up to 9 mm long, 5 mm wide, the outer apical (dorsal) angle acute-triangular, without protuberances, stellate-pubescent towards the margin. Seeds 2(–3), brown, initially with scattered stellate hairs, pilose near margins, ca. 2 mm across.

Figure 2. *Abutilon albidum* in Igueste de San Andrés, Tenerife. Details of flower and immature fruit. December 2019, F. Verloove.

Illustrations. Webb and Berthelot (1836) presented the first superb line drawing of *Abutilon albidum*. Figs 1–4 show the species in nature in the Canary Islands (Tenerife) and Morocco (Tiglit), respectively.

Distribution. Macaronesia (Spain: Canary Islands), north-western Africa (Algeria: Hoggar Mountains; Morocco: Anti-Atlas) (Fig. 6).

Figure 3. *Abutilon albidum* in Igueste de San Andrés, Tenerife. Flowering and fruiting individual. December 2019, F. Verloove. In Tenerife, this species is now mostly found in anthropogenic habitats.

Webb and Berthelot (1836) reported *Abutilon albidum* from 'Barranco Santo', near the Zurita Bridge, in Santa Cruz de Tenerife, in the south-eastern part of the Island. It had previously been collected from Tenerife in 1796 by A. de Jussieu (P!; see specimens examined). It was considered a Canarian endemic (Masferrer y Arquimbau 1880). Nearly

Figure 4. *Abutilon albidum* near Tiglit, Morocco, December 2016, A. Garcin. In Morocco, this species is usually found in more natural habitats, often in crevices of basaltic rocks in (semi-) desert areas.

all collections that have been made since then originated from the Barranco de Santos Ravine (present-day name) in Santa Cruz (Fig. 7). Historical literature sources mostly also referred to that locality (e.g. Masferrer y Arquimbau (1880); Bornmüller (1904)). Some further historical literature sources reported it from additional localities in Tenerife. According to Lindinger (1926), it was also observed in La Orotava, ca. 35 km further

Figure 5. *Abutilon grandifolium* in Telde, Gran Canaria. April 2017, F. Verloove. For many decades, *A. albidum* was erroneously considered to be a synonym of this invasive species from South America. *Abutilon grandifolium* has much larger, more orange-yellow corollas and sepals forming longitudinal keels in bud and saccate at base.

SW. Pitard and Proust (1908) also mentioned it from another ravine in Santa Cruz, the Barranco del Bufadero. The species was said to be very rare in Tenerife. Burchard (1929) probably presented the most extensive (and most recent) update of its occurrence on the Island. It was mostly seen between San Andrés and Igueste [F.V.: i.e. Igueste de San Andrés], including below the Los Órganos cliffs [F.V.: i.e. north of the Las Teresitas Beach]. It was also said to have isolated occurrences in Santa Cruz, where it had probably become extinct. All documented occurrences, except for a single record from La Orotava, were from the extreme south-eastern part of the Island where it occupied an area of hardly more than 12 km^2. In Tenerife, it was probably last collected in 1945 by E.R. Sventenius. According to some sources, it was formerly also collected in Gran Canaria (Barranco de la Angostura) (Pitard and Proust 1908); unfortunately, no specimens are present in the LPA herbarium.

In December 2019, *Abutilon albidum* was rediscovered in Tenerife in Igueste de San Andrés, 74 years after Sventenius' collection from 1945. In the same village, several small populations of identical plants were observed in December 2019. Some plants were seen along the side of the road Carretera de Igueste de San Andrés adjacent to the Barranco de Igueste (these plants are also visible on Google Streetview images from June 2012: https://www.google.com/maps/@28.5291262,-16.1568142,3a,75y,15.61h,60.37t/

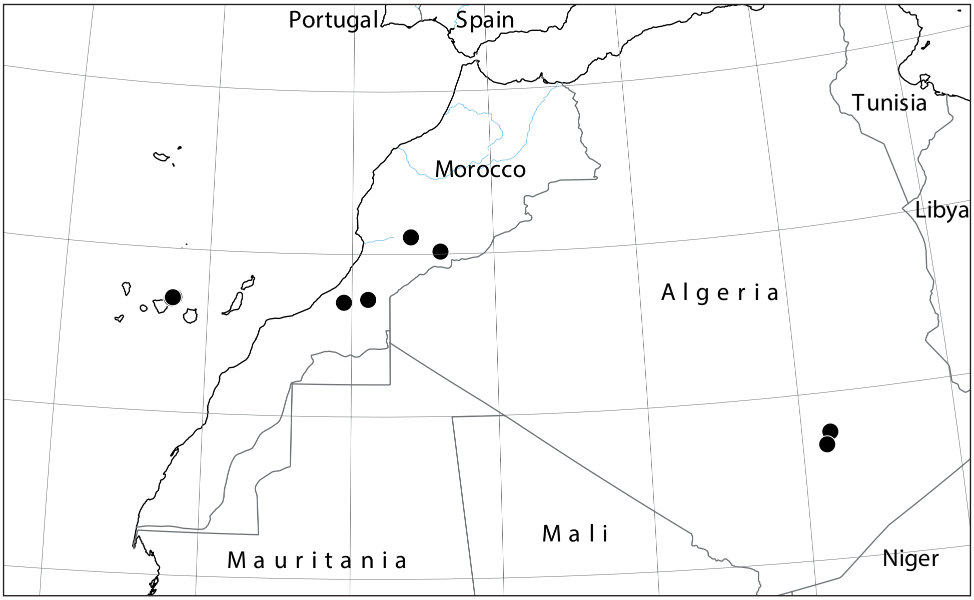

Figure 6. Worldwide distribution of *Abutilon albidum* (historical and current records).

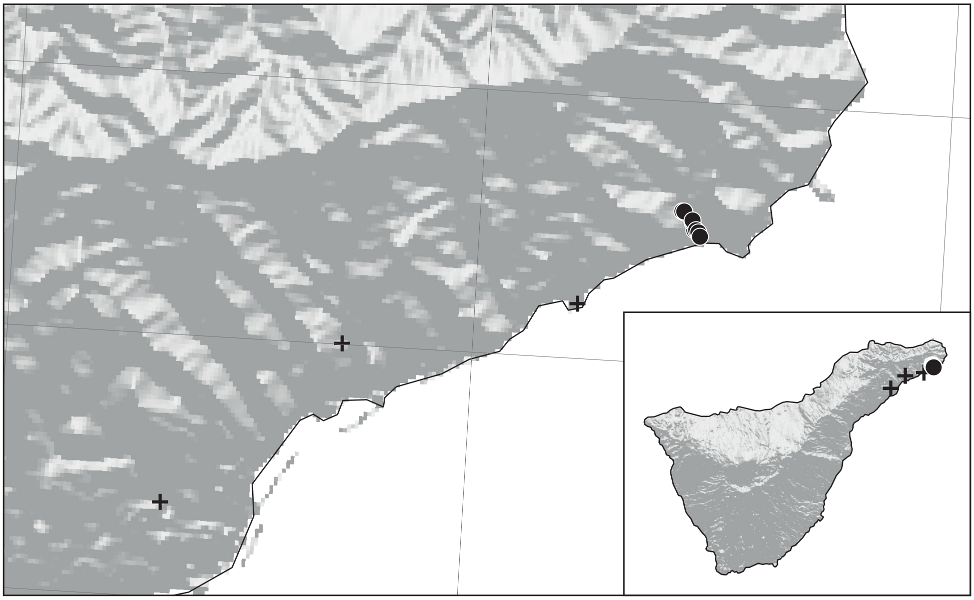

Figure 7. Old (black cross) and recent (black bullet) distribution of *Abutilon albidum* in Tenerife, Canary Islands, Spain.

data=!3m6!1e1!3m4!1s6-0-oKg77q5kAJSCf1_wCQ!2e0!7i13312!8i6656). Further plants were seen as weeds in a plantation near the San Pedro Apóstol church. Finally, the species was also observed on both sides of the Paseo el Cementerio, a track that leads to the cemetery. In March 2022, a more detailed survey was carried out (Fig. 8): the species was found in several additional localities, but less than 100 individuals in total and in an area of at most 35,000 m^2 (for details see: https://observation.org/soort/view/1029740). Interestingly, in the very same area, the Belgian amateur botanist Leon Delvosalle (1915–2012) collected this species in May 1962, but erroneously identified it as *Sida rhombifolia*, a subtropical Malvaceae weed that is also naturalised there. After the Meise Botanic Garden (BR) acquired his herbarium, this collection (consisting of four small fragments only) was re-identified by the first author as *Abutilon* cf. *indicum* in March 2013. All these localities fall within the distribution area as described by Burchard (1929). From this, it can be deduced that *A. albidum* has survived at least in Igueste de San Andrés in Tenerife. Meanwhile, Santa Cruz de Tenerife became the capital of the Island and has dramatically changed in the past century. Repeated botanical explorations over the years in the ravines where *A. albidum* formerly occurred, especially Barranco de Santos and Barranco del Bufadero, were fruitless. Additional fieldworks in May and June 2021 in potentially suitable areas in Barranco Tahodio, Barranco Valleseco, between Igueste de San Andrés and San Andrés, Playa de las Teresitas (Bajo Los Órganos) and in Playa de Las Gaviotas (Bajo Los Órganos) were also unsuccessful. The coastal area between Santa Cruz and San Andrés was mostly lost due to the expansion of the commercial port and the urbanisation of the capital. Moreover, the highly invasive grass species *Cenchrus setaceus* (Forssk.) Morrone has now colonised the entire area, including the habitats where *A. albidum* once occurred. In Los Órganos, the species probably grew near the old path between Las Teresitas and Las Gaviotas that goes through the base of the basaltic rocks of Los Órganos.

After its discovery in Tenerife, *Abutilon albidum* also was found in north-western Africa. Maire (1933) first reported it from Ideles and Imarera in the Hoggar Mountains, a highland region in the central Sahara in southern Algeria, along the Tropic of Cancer, where it was said to be rare (Quézel and Santa 1963). Its presence there has not been confirmed lately and it is likely extinct (pers. comm., Prof. Said Amrani, January 2022). From Morocco, it was first reported from Assa in the southwest of the country

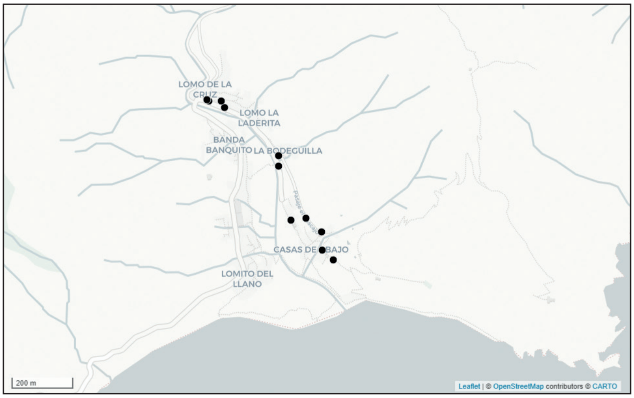

Figure 8. Distribution of recently discovered populations of *Abutilon albidum* in Igueste de San Andrés in Tenerife, Canary Islands, Spain.

(Maire 1936; Emberger and Maire 1941). Later, it was also reported from Foum Zguid in south-eastern Morocco (Fennane et al. 1999) and Maire collected it in 1937 in "Tiliouine" (Taliouine), slightly further north (UM-MPU-MPU084348!). There are apparently no recent herbarium collections or more recent reports of the species; however, it is still present at least in the surroundings of Tiglit in Morocco (https://www.teline.fr/en/photos/malvaceae/abutilon-albidum), where it had been confused with the introduced weed *A. theophrasti*. *Abutilon albidum* is probably rare throughout its entire distribution range and probably poorly known as well. It was assessed as endangered in a preliminary Red List of the Moroccon flora (Fennane 2018).

Habitat and ecology. In Algeria and Morocco, *Abutilon albidum* usually occurs on basaltic rocks in the desert or semi-desert, from subtropical to warm (Mediterranean) climate areas. It is most often found at higher elevations (up to 2,000 m above sea level), but may also grow at lower elevations. From herbarium labels, it can be deduced that the species also occurs in sandy, dried-out riverbeds. Habitats are usually natural and little disturbed although it was also collected (likely as a weed) in a palm grove (Maire 1936). It is nowhere in the world known as a weed (Randall 2017).

In Tenerife, *Abutilon albidum* is best known from its *locus classicus*, i.e. the dried-out riverbed of Barranco de Santos in Santa Cruz. It was said to grow in dry rock crevices and warm ruderal places (Webb and Berthelot 1836; Bornmüller 1904; Pitard and Proust 1908). It formerly also was reported from basaltic rocks below the Los Órganos sea cliff (Burchard 1929). However, the most recent observations are almost exclusively from more degraded, anthropogenic habitats: roadsides, plantations and ruderal places, where it is found along with, for example, *Bituminaria bituminosa* (L.) C.H. Stirt., *Cynodon dactylon* (L.) Pers., *Fagonia cretica* L., *Forsskaolea angustifolia* Retz., *Patellifolia* sp., *Setaria adhaerens* (Forssk.) Chiov. etc. In one locality, a few plants were also found on the basaltic rocks bordering the road; there, the species was accompanied by, amongst others, *Aristida adscensionis* L., *Cenchrus ciliaris* L. and *Kleinia neriifolia* Haw. In Tenerife, all populations, including the historical ones, are at elevations well below 100 m, often near to sea level.

Specimens examined. Spain. Canary Islands, Tenerife: Teneriffa, Barranco Santo, *Webb* (FI006084, proposed conserved type); Canaries, s.d., herb. *Webb* (P06730933); Barranco Santo propè opp. Sta. Cruz, s.d., *Webb* (P06730936; BR); Ténérif, s.d., sine coll. (MNHN-P-P06731032); Barranco Sancto propè Sta. Cruz, s.d., sine coll. (BR 0000013462017); Teneriffe, 1796, *A. de Jussieu* (P06730938); Ténérife, Sa. Crux, 1816, *C. Smith* (G00219753, G00219752, G00219721); Teneriffa, in convalle aridiforme a Barranco Santo propè urbem Sanctam Cruceum, June 1834 (K000240407); Teneriffe, 1848, *S. Berthelot* (P06731022, K000240405, K000240406); Tenériffe, 1854, *C. Bolle* s.n. (COI00057130); Teneriffa, Barranco Santo près Santa Cruz, 12 April 1855, *E. Bourgeau* (P06730935, RAB078278); ibid. (P06641194); Barranco del Hierro, Sud-Est de Tenerife, 15 April 1855, *H. de la Perraudière* (P04694330); Reg. infer., Sud-Est de Tenerife, 15 April 1855, *H. de la Perraudière* (P06731031); Prope Sta. Crux, 15 April 1855, *H. de la Perraudière* (P06731023); ibid. (P06731030); ibid. (MPU748052); Barranco Santo, 1866, *T. Husnot* (Pl. Canarienses 632) (P04642214); ibid. (P04694328); ibid. (P06641193); ibid. (MPU748054); Teneriffa, Santa Cruz, Bco. Santo (loc. class.), 15 June 1901, *J. Bornmüller* 2132 (P06731034; BR; MPU748050); ibid. (P06731033);

Inter Sanctum Andream et Igueste oppidula Teneriffae, in rup aridis, 3 May 1907, *O. Burchard* (ORT 00001); San Andrés, Los Órganos, sitios rocosos y secos, +/- escasa, 3 April 1944, *E.R. Sventenius* (ORT 12332); San Andrés, Roque de Los Órganos, escasa, 11 January 1945, *E.R. Sventenius* (ORT 12331); Igueste [de San Andrés], bord du chemin, 5 May 1962, *L. Delvosalle* 5235 (BR); Santa Cruz de Tenerife, Igueste de San Andrés, Carretera de Igueste de San Andrés N of the Barranco de Igueste, roadside, few plants, 23 December 2019, *F. Verloove* 13743 (BR); Santa Cruz de Tenerife, Igueste de San Andrés, Paseo el Cementerio, alongside track, on both sides, ca. 15–20 individuals, 23 December 2019, *F. Verloove* 13744 (BR); Santa Cruz de Tenerife, Igueste de San Andrés, Pasaje El Cascajo, roadside, scattered individuals, 26 March 2022, *F. Verloove* 14278 (BR).

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RESEARCH ARTICLE

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Thismia kenyirensis (Thismiaceae), a new species from Taman Negeri Kenyir, Terengganu, Peninsular Malaysia

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Abstract

A new mycoheterotrophic species, *Thismia kenyirensis* Siti-Munirah & Dome from Peninsular Malaysia, is described and illustrated. *Thismia kenyirensis* differs from other previously described species in the following characteristics: the flower tube is completely orange, with alternating darker and paler-coloured longitudinal lines on the outer and inner surfaces, the outer tepals are ovate (petaloid), the inner tepals are narrowly lanceolate, each ending with a long appendage. According to the IUCN Red List categories and criteria, *T. kenyirensis* is provisionally classified as Least Concern.

Keywords

Brunonithismia, lowland dipterocarp forest, rare species

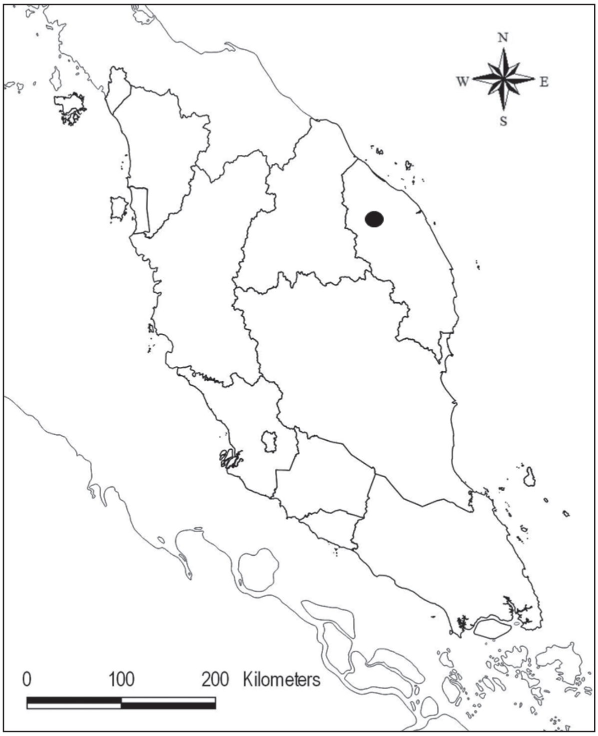
Introduction

Thismia Griff. is a genus of non-photosynthetic flowering plants belonging to the family Thismiaceae. *Thismia* species are small herbs, with scale-like leaves and actinomorphic or zygomorphic, urceolate to campanulate flowers. *Thismia* includes about 100 species worldwide (from Imhof 2010 onwards, Siti-Munirah et al. 2022) and it is the most widespread and species-rich genus in the family Thismiaceae with high endemism. Their range extends from tropical and subtropical Asia to northern and eastern Australia and New Zealand and from tropical South America to Costa Rica, with isolated occurrence in North America (POWO 2022). Many species are believed to be extremely rare and have a scattered range. They are easily overlooked in the wild due to their small size and the short-lived nature of their aboveground parts (Larsen and Averyanov 2007). In Malaysia, Terengganu is currently the state with the most *Thismia* records, with 11 reported species: *T. alba* Holttum ex Jonker, *T. arachnites* Ridl, *T. aseroe* Becc, *T. brunneomitroides* Suetsugu & Tsukaya, *T. domei* Siti-Munirah, *T. clavigeroides* Chantanaorr. & Seelanan, *T. javanica* J.J.Sm., *T. latiffiana* Siti-Munirah & Dome, *T. ornata* Dančák, Hroneš & Sochor, *T. sitimeriamiae* Siti-Munirah, Dome & Thorogood, *T. terengganuensis* Siti-Munirah (Siti-Munirah and Dome 2019, 2022a, b; Siti-Munirah et al. 2021).

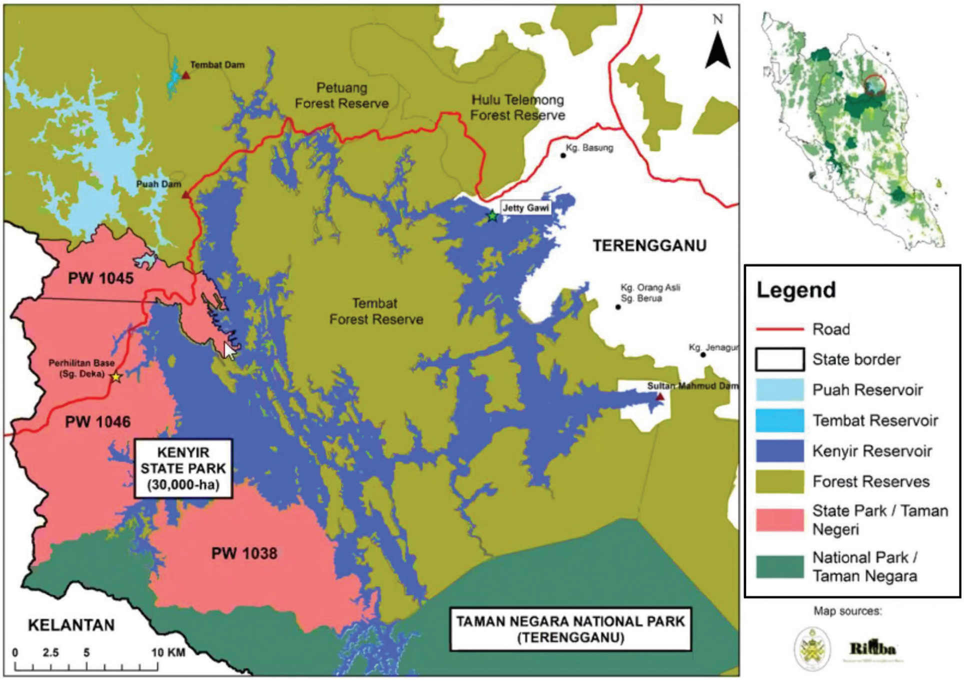
Currently, five of the species reported in Terengganu are known from the Kenyir State Park (Taman Negeri Kenyir, TNK) (Siti-Munirah and Dome 2022b). The TNK is located in the eastern part of Peninsular Malaysia in the state of Terengganu (Map 1). It used to be part of the Tembat Forest Reserve (FR), but in 2018 and 2019, parts of Tembat FR were designated as TNK. The forest area includes part of the forest around Tasik Kenyir (Kenyir Lake), which is adjacent to Terengganu National Park (Taman Negara Terengganu) and also connected to Tembat FR forest and Kelantan State (Map 2). During the scientific expedition to the TNK on 8 September 2020, the second author and his team visited the Sungai Cendana area in the southern part of the TNK and discovered a population of an unknown *Thismia* plant, which he forwarded to the first author. Together, we revisited the populations in June 2022. After further investigation and comparison with known species of *Thismia*, we concluded that this plant is distinct from previously described species of the genus. We therefore report and describe it here as a new species for science.

Materials and methods

Our assessment is based on material collected in Taman Negeri Kenyir, Hulu Terengganu, Terengganu (Map 1). The specimens were preserved in 70% ethanol. All the specimens have been deposited in Kepong Herbarium (**KEP**) and have been examined for taxonomic treatment. Morphological characters and measurements were examined using an Olympus SZ61 stereo microscope and high-resolution macrophotography. Additionally, measurements were taken from spirit material. The specimen features were compared in detail with the original drawings and descriptions of morphologically similar species of *Thismia* provided by Jonker (1938), Larsen and Averyanov (2007), Mar and Saunders (2015), Chantanaorrapint (2018), Tanaka et al. (2018), Suetsugu et al. (2018), Siti-Munirah and Dome (2019) and Nuraliev et al. (2020).



Map 1. Taman Negeri Kenyir, Terengganu (black circle), the type locality of *Thismia kenyirensis*.



Map 2. Map of Tasik Kenyir and the forest boundaries. (Map sourced from NBS).

Taxonomic account

Thismia kenyirensis Siti-Munirah & Dome, sp. nov.

urn:lsid:ipni.org:names:77315245-1
Figs 1–3

Diagnosis. Distantly similar to *Thismia hongkongensis* Mar & Saunders but differs by petaloid, ovate outer tepals which are ca. 8 mm long, narrowly lanceolate-linear, ca. 8 mm long inner tepals which are not forming a lose-dome, up to 28 mm long appendages of inner tepals, orange floral tube, which lacks reticulate pattern on its inner surface and connective apex with 3 long appendages.

Type. Malaysia. Peninsular Malaysia: Terengganu, Hulu Terengganu District, Taman Negeri Kenyir, Sungai Cendana, elev. ca 204 m, 8 Sept 2020, Dome Nikong, FRI 91122a (holotype KEP!, spirit collection, barcode no. SC12015).

Description. Achlorophyllous herb, up to 130 mm tall. ***Roots*** vermiform, unbranched, ca. 1 mm in diameter, light brown. ***Stem*** erect, up to 90 mm long, 1.5–2 mm in diameter, pale brownish-orange, bearing 1–2 flowers. ***Leaves*** 2–4, spirally arranged, triangular to narrowly triangular, scale-like, acute, margin almost entire but slightly irregularly serrate, 2–6 mm long, ca. 1 mm wide at base, pale brown. ***Involucral bracts*** 3, similar to leaves but slightly larger, spirally arranged, triangular to narrowly triangular, scale-like, acute, entire, 8 mm long, ca. 1.5–2.5 mm wide at base, pale brown or pale orange. ***Pedicel*** up to 6 mm long at anthesis, elongating to ca. 10 mm long after anthesis, pale brown. ***Flowers*** terminal, actinomorphic, ca. 52 mm long (including ovary, floral tube and inner tepal with appendage); ***floral tube*** urceolate, 12–15 mm long, ca. 5 mm wide at base, ca. 6 mm wide at middle, ca. 7 mm wide distally; ***outer surface*** glabrous, orange to brownish-orange, with 12 darker orange longitudinal ribs alternating with 12 paler longitudinal lines, ***inner surface*** smooth or rough, almost similar to outer surface, without transverse bars or reticulate ornamentation; ***outer tepals*** 3, free, petaloid, ovate, apex acute, 8 mm long, ca. 4–5.5 mm wide (ca. 4 mm at base, ca. 5.5 mm above middle), glabrous, bright orange; ***inner tepals*** 3, free, narrowly lanceolate-linear, 8 mm long, ca. 1 mm wide, glabrous, dark orange, apically bearing a tentacle-like appendage; appendage narrowing towards apex, ca. 27–28 mm long, 0.5 mm wide, dark orange at base and bright orange at apex; ***annulus*** dark orange, glabrous. ***Stamens*** 6, pendent from the annulus, outer side greenish-orange or greenish-brown, inner side dark-brown to blackish and paler, ***filaments*** free, ca. 1 mm long, curved downwards; ***connectives*** flattened at inner surface, laterally connate to form a tube, ca. 4–5 mm long, narrow at base (ca. 0.8 mm wide) and broad at apex (ca. 1.7 mm wide), connective apex with 3 appendages, slightly curved inside, each appendage ca. 1.5 mm long; outer side of connective bearing a skirt-like lateral appendage protruding towards floral tube; lateral appendage not exceeding the tip of the connective appendages, with short translucent trichomes on margin, ***interstaminal glands*** greenish translucent, inserted on the line of fusion between connectives at the level of attachment of lateral appendages. ***Ovary*** inferior, unilocular; ***placentas*** 3, free, forming columns and arising from the bottom

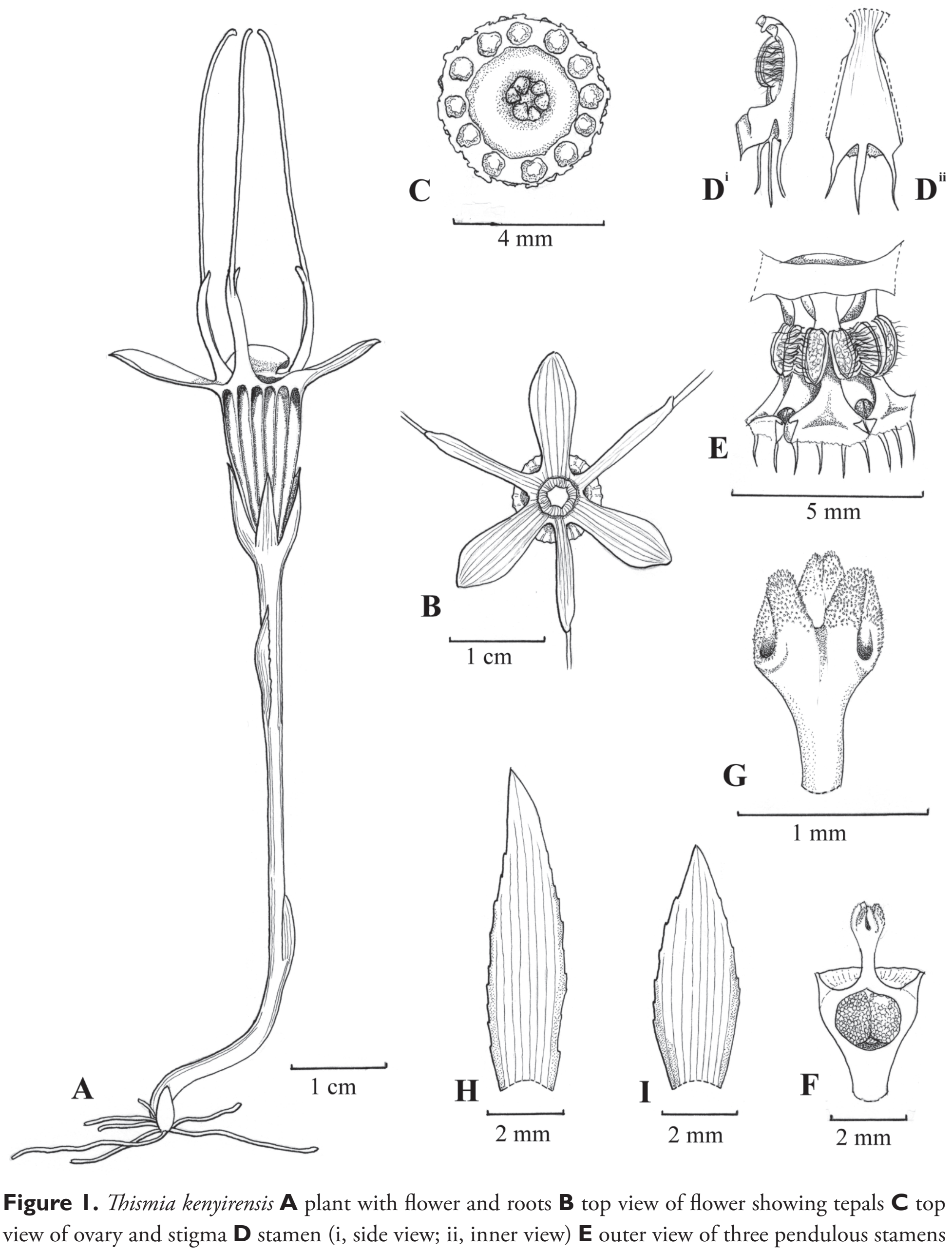


Figure 1. *Thismia kenyirensis* **A** plant with flower and roots **B** top view of flower showing tepals **C** top view of ovary and stigma **D** stamen (i, side view; ii, inner view) **E** outer view of three pendulous stamens **F** longitudinal section of ovary **G** style and stigma **H** bract **I** leaf. All from FRI91122a (spirit material). Drawings by Mohamad Aidil Noordin.

of the ovary; ovules numerous. ***Style*** dark brown-orangish, ca. 1 mm long; ***stigma*** ca. 1 mm long, papillose, 3-lobed, with lobes ± rectangular, bifurcate at apex, pale brown or orangish to whitish. ***Fruit*** cup-shaped, 3–5 mm in height, 4–6 in diameter, pale orange (or white to creamy orange), darker at upper part. ***Seeds*** unknown.

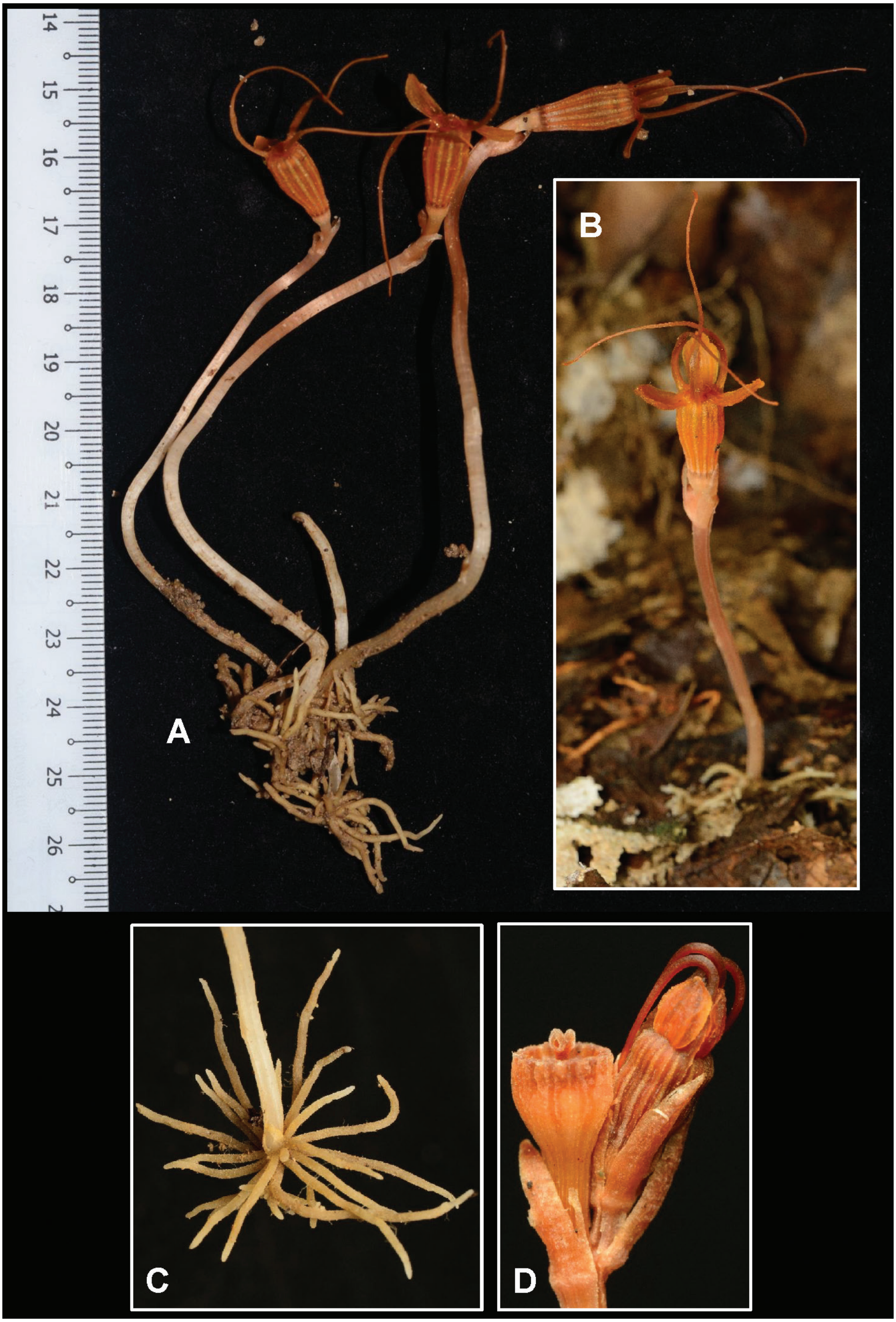


Figure 2. *Thismia kenyirensis* **A** mature plant with flowers and roots, scale in centimetres (cm) (FRI91122b) **B** mature plant in natural habitat (FRI91122a) **C** vermiform roots (FRI91122c) **D** young flower with mature ovary and stigma (FRI91122c). Photos by Siti-Munirah MY (**A**); Dome Nikong (**B–D**).

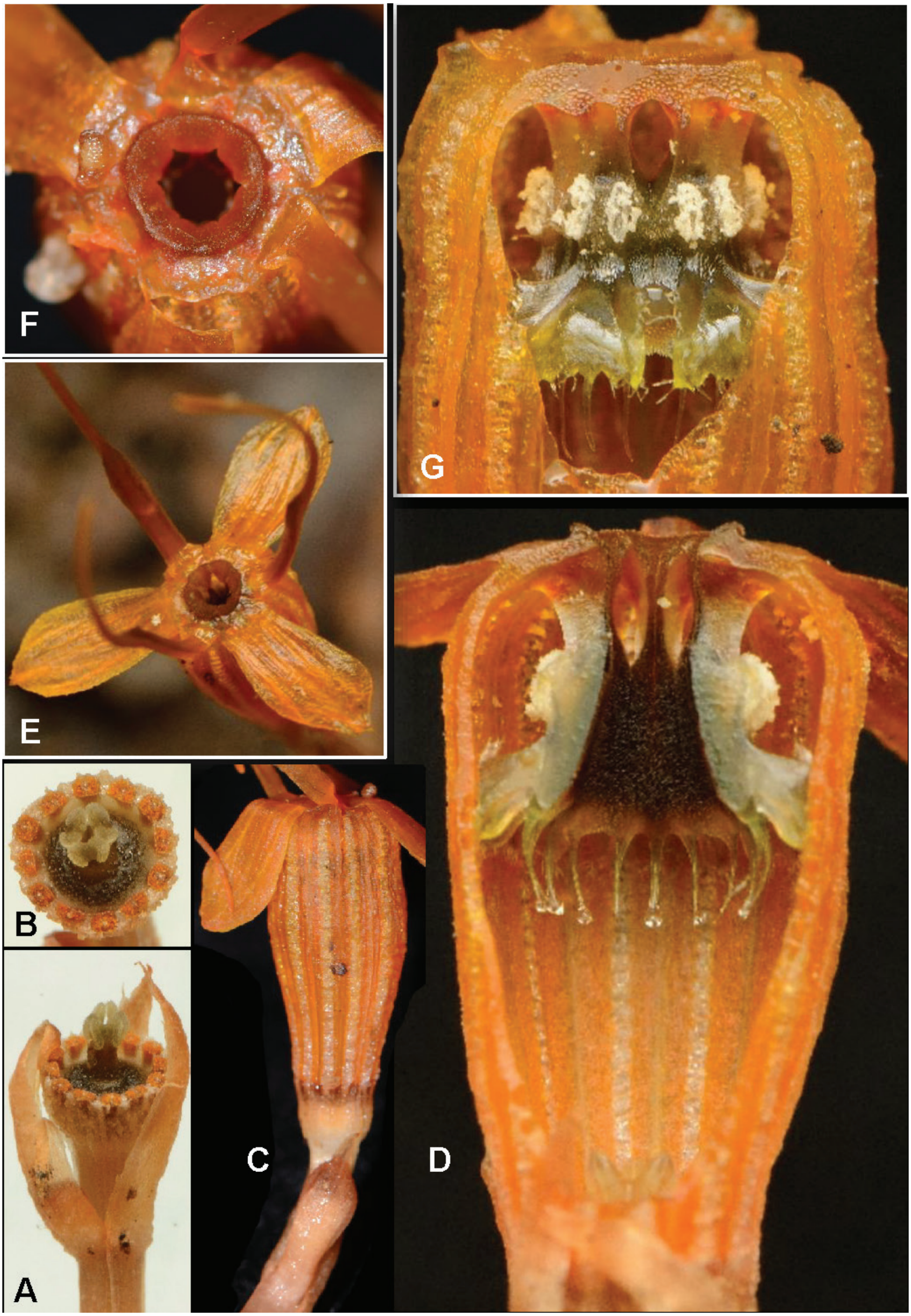


Figure 3. *Thismia kenyirensis* **A** bracts with ovary and pistil, lateral view **B** top view of stigma **C** flower, side view **D** longitudinal section of floral tube and stamens **E** flower, top view **F** annulus **G** stamens, outer view. Photos by Siti-Munirah MY (**A–C, E, F**; All from FRI 91122b); Dome Nikong (**D, G**; No specimens).

Additional specimen examined (*paratype*). Malaysia. Peninsular Malaysia: Terengganu, Hulu Terengganu District, Taman Negeri Kenyir, Sungai Cendana, elev. ca 204 m, 8 Sept 2020, Dome Nikong, FRI 91122b (KEP, spirit collection, barcode no. SC12016), FRI 91122c (KEP, spirit collection, barcode no. SC12017); Taman Negeri Kenyir, Sungai Cendana, elev. ca. 156 m, 16 June 2022, Siti-Munirah MY, FRI98678 (KEP, spirit collection, barcode no. SC12018).

Distribution. Endemic to Terengganu, Peninsular Malaysia. Currently, the occurrence is only from the type locality, Sungai Cendana area (Map 1). In addition, *T. kenyirensis* was observed in two other populations in the vicinity of Tasik Kenyir. It was sighted near Sungai Saok and on the way to Gunung Gagau (Wong Pui May personal communication), both in the Tasik Kenyir region. However, there are still no specimens and GPS information to verify these findings. This means that only the occurrence of the population in Sungai Cendana has been confirmed.

Ecology. In moist shady areas of lowland dipterocarp forest on moist soil at elevations of 150–220 m a.s.l. Flowering and fruiting mostly from September to April, but also recorded to flower in June. Historically, the type locality was botanized as early as 2007. Based on the results of Lim et al. (2008), *T. alba* was also recorded during the survey in the Sungai Cendana area. This is in contrast to *T. kenyirensis*, which was not mentioned, implying that it was not seen or collected at that time. During our recent visit, we were also able to find *T. alba* not far from the original population of *T. kenyirensis*.

Etymology. The epithet refers to Lake Kenyir, Kenyir State Park (Taman Negeri Kenyir), where the species was found.

Conservation status. According to the IUCN standards (IUCN 2022), we propose to classify the preliminary conservation status of the species as Least Concern (LC). This is because the species is likely to be widespread in TNK, where it has been sighted a few times at the type locality (Sungai Cendana) and also by other observers at two other sites, and its entire habitat is in a fully protected forest area. In addition, nearby forests in Terengganu National Park (Taman Negara Terengganu) are also fully protected. As mentioned earlier, it is very likely that there are other populations in the vicinity of Tasik Kenyir. According to the second author's observations, four individuals were sighted near Sungai Saok and one on the way to Gunung Gagau on 22 November 2022. However, no specimens are available for confirmation. *Thismia kenyirensis* occurs in intact habitats in forested areas in TNK. However, the species is very common only during the flowering season and its populations are mostly scattered, as few individuals or solitary individuals were observed during the flowering season. During the first check of the type locality, the population in Sungai Cendana in 2020, about 20 adult individuals were found. However, during a revisit in 2022, only a single individual was observed.

Notes. *Thismia kenyirensis* is easily recognised by the following combination of characteristics: vermiform roots, almost uniform light-to-dark orange flower coloration, petaloid ovate outer tepals, narrowly lanceolate-linear inner tepals with long appendages and each stamen with 3 appendages at its apex. Within the infrageneric classification by Kumar et al. (2017), *T. kenyirensis* resembles species of *Thismia* subgenus *Thismia* section *Thismia* subsection *Brunonithismia* Jonker, especially by its free and unequal tepals. Eight species are placed in subsection *Brunonithismia* by Kumar et al. (2017): *T. arachnites*, *T. brunonis*

Griff, *T. gardneriana* Hook.f. ex Thwaites, *T. hongkongensis*, *T. javanica*, *T. labiata* J.J.Sm., *T. neptunis* Becc. (proved to be only distantly related to the other species of the subsection by Shepeleva et al. 2020) and *T. tentaculata* K.Larsen & Aver. Of them, *T. arachnites* and *T. javanica* occur in Peninsular Malaysia, including the state of Terengganu. They both are readily distinguished from *T. kenyirensis* (Fig. 4, Table 1). In addition, *T. breviappendiculata* Nob. Tanaka (Tanaka et al. 2018) from Myanmar, *T. bokorensis* Suetsugu & Tsukaya (Suetsugu et al. 2018) from Cambodia, *T. gardneriana* from Sri Lanka and *T. tentaculata* from Hong Kong and Vietnam are similar to *T. kenyirensis*. In a molecular phylogenetic

Table 1. Morphological comparison of *T. kenyirensis* with similar species *T. arachnites* (Chantanaorrapint 2018), *T. bokorensis* (Suetsugu et al. 2018), *T. breviappendiculata* (Tanaka et al. 2018), *T. brunonis* (Jonker 1938), *T. gardneriana* (Jonker 1938; Bandara et al. 2020), *T. hongkongensis* (Mar et al. 2015), *T. javanica* (Siti-Munirah and Dome 2019; Nuraliev et al. 2020) and *T. tentaculata* (Larsen and Averyanov 2007; Nuraliev et al. 2020).

Characters	***T. kenyirensis***	***T. arachnites***	***T. bokorensis***	***T. breviappendiculata***	***T. brunonis***	***T. gardneriana***	***T. hongkongensis***	***T. javanica***	***T. tentaculata***
Colour of floral tube	Orange to brownish-orange with 12 darker orange longitudinal ribs	White to orange with 6 or 12 reddish vertical streaks in the upper part	Pure white	White, with 12 translucent longitudinal ridges	Yellowish	Yellowish-orange	Pinkish-white with 12 dark red vertical ribs	White to pale orange or light orangish-red	Pure white, sometimes with antetepalous veins indistinctly tinged with red
Presence of transverse bars/ornamentation on inner side of the floral tube	Absent/absent	Present/absent	Absent/absent	Present/absent	Present/absent	Absent/Not known	Absent/present	Present/absent	Absent/present
Outer tepal Shape	Ovate, petaloid	Broadly ovate	Broadly triangular, apex broadly obtuse or rounded	Ovate, obtuse	Broadly ovate, obtuse	Broad, rotundate	Triangular	Ovate-triangular	Isosceles triangular, broadly obtuse or rounded
Length (mm)	8	5–6.5	1.2	2	Not known	1.5	1.8	1–3.5	1.8–2.4
Colour	Bright orange	Yellow to orange.	Light yellow	Yellow	Not known	Yellow	Dark red	Orange to red or yellow, translucent	Light yellow or tinged with red, translucent
Inner tepal (without appendage) Shape	Narrowly lanceolate-linear	Triangular	Narrowly triangular	Isosceles triangular	Triangular at the base, caudate in long, thick, filiform tails	Subulate	Spatulate, adaxially concave	Narrowly triangular	Triangular with broad base
Length (mm)	8	6.5–9	2.6	2	Not known	Not known	3.3	1.5–3.3	
Colour	Bright orange	Orange to reddish-orange at the base	Light yellow	Yellow	Not known	Yellowish-orange	Dark red	Orange to red or yellow and usually dark red towards apex	Light yellow
Stamen Apex	3 long, slightly curved appendages	3-toothed, each tooth bearing a distinct stiff hair	3-toothed, each tooth narrowly triangular, with ca. 1 mm long	Lobed	With numerous teeth	2-toothed, each tapering into a stiff hair	2-toothed, adorned with trichomes	3-toothed, each tooth bearing hair	2-toothed, each tooth tapering into hair, sometimes with additional hairs between teeth

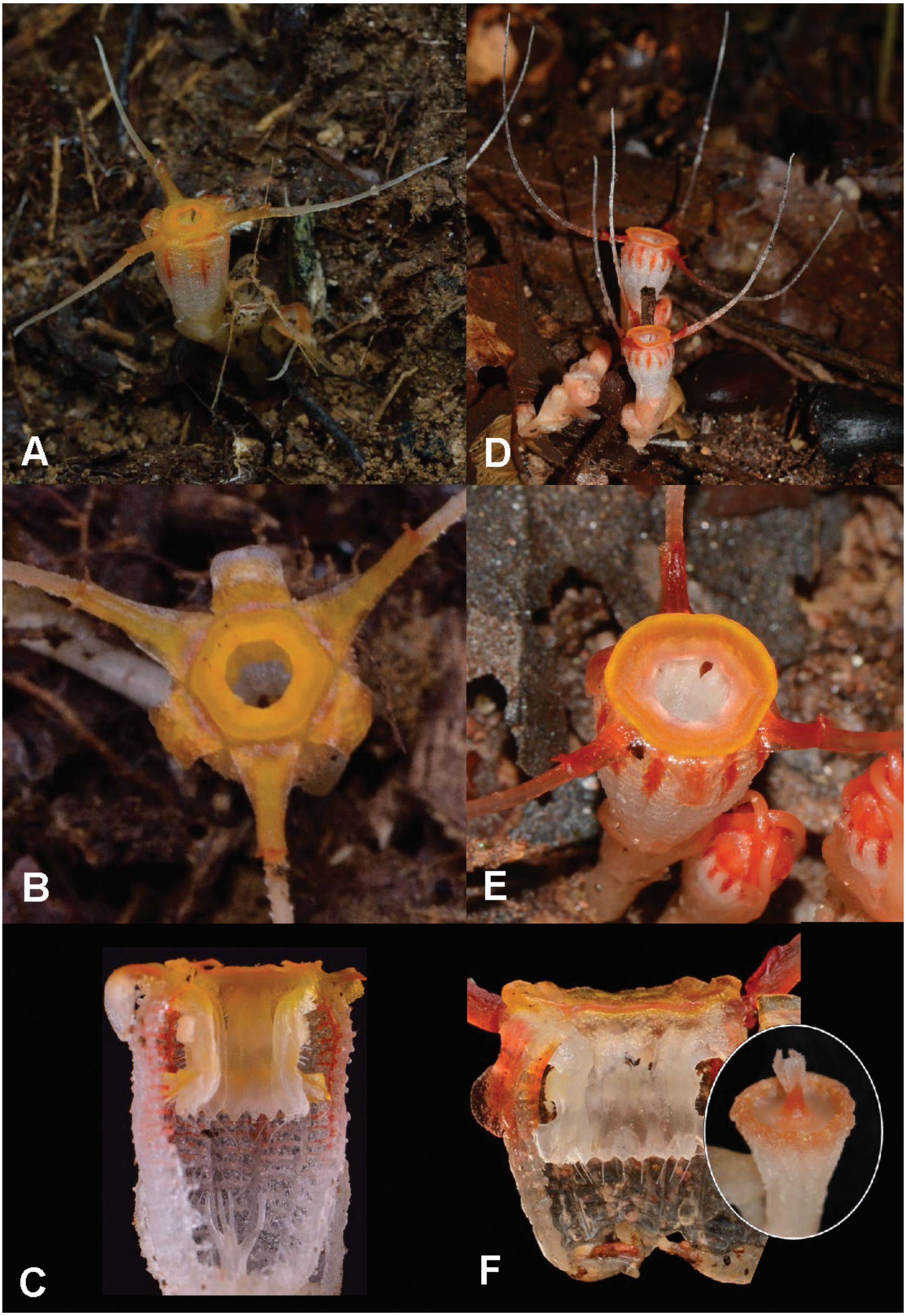


Figure 4. Morphologically close species to *T. kenyirensis* in Peninsular Malaysia **A–C** *T. arachnites* (Taman Negeri Kenyir) **D–F** *T. javanica* (Telemong Forest Reserve). Photos by Dome Nikong (**A–C, F**) and Siti-Munirah MY (**D, E, G**).

reconstruction provided by Shepeleva et al. (2020), *T. javanica*, *T. hongkongensis*, *T. gardneriana* and *T. tentaculata* occupied a relatively isolated and unstable position. All these species (and also *T. kenyirensis*) are characterised by free appendaged inner tepals and outer tepals without appendages. A more detailed phylogenetic analysis is required to test the monophyly of this morphological group, and the relationships of each of these species including *T. kenyirensis*.

Acknowledgements

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RESEARCH ARTICLE

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Hedyotis konhanungensis (Rubiaceae): A new species from the central highlands of Vietnam

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Abstract

A new species of *Hedyotis* L. (Rubiaceae), *Hedyotis konhanungensis* B.H. Quang, T.A. Le, K.S. Nguyen & Neupane, is described and illustrated from the central highlands of Vietnam based on morphological and phylogenetic evidence. The new species belongs to the morphologically diverse tribe Spermacoceae (ca. 1000 species) of the family Rubiaceae, which is represented by 70–80 species in Vietnam. The phylogenetic analysis, based on four DNA regions (ITS, ETS, petD, rps 16), confirms the new species' placement within the genus *Hedyotis* – one of the largest genera in the tribe, comprising ca. 180 species across Asia and the Pacific. *Hedyotis konhanungensis* is morphologically distinct from all southeastern Asian *Hedyotis* L. in its set of traits such as leaf type (shape and thickness), growth habit, and floral parts (color of inflorescence axis and the shape of calyx lobes). The new species shows similarities with *Hedyotis shenzhenensis*, *H. shiuyingiae*, and *H. yangchunensis* from China in its herbaceous habit, fleshy ovate leaf blades, and dark purple floral parts, but it is phylogenetically distinct and can be distinguished from them by the following combination of morphological traits: habit with slightly smaller stature (<25 cm), broadly ovate or deltoid stipules with cuspidate apex and entire margin, and ovate or nearly ovate calyx lobes.

Keywords
Gia Lai Province, *Hedyotis-Oldenlandia* complex, Indochina, phylogenetics, Spermacoceae, taxonomy

Introduction

The Asian-Pacific genus *Hedyotis* L. (ca. 180 species) lies within a polymorphic, mainly herbaceous tribe Spermacoceae (ca. 1000 species) of the family Rubiaceae. In Vietnam, this tribe is represented by 70–80 species belonging to the genera *Dimetia* (Wight & Arn.) Meisn., *Debia* Neupane & N.Wikstr., *Exallage* Bremek., *Leptopetalum* Hook. & Arn., *Hedyotis* L., *Involucrella* (Benth. & Hook.f.) Neupane & N.Wikstr., *Neanotis* W.H.Lewis, *Oldenlandia* L., and *Spermacoce* L. (Govaerts et al. 2022). The genus *Hedyotis* and its related genera that form the *Hedyotis-Oldenlandia* complex have a long history of taxonomic confusion and disagreement due to the use of inconsistent and overlapping characters in the generic delimitations. The genus *Hedyotis* is currently treated in a narrow sense based on the molecular phylogenies (Guo et al. 2013; Wikström et al. 2013; Neupane et al. 2015) where members of it are united by their predominantly shrubby to tree-like habit, septicidal capsules, flattened seeds, and tropical upland distributions in Asia and the Pacific. The species of this genus are mostly found in the mid or high-elevation slopes (to 4000 m a.s.l.) of Asia (Sri Lanka, southern India, SE China, Indochina, Malesia), Papuasia, and NW Pacific (Micronesian islands). In Vietnam there are about 20 species reported in the flora of this country (Pitard 1922; Pham 2003; Tran 2005; Do et al. 2013; Govaerts et al. 2022) but the actual number could be as high as 40 species since many species listed under *Oldenlandia* share the diplophragmous capsule characteristic of *Hedyotis* (pers. obs.).

During a botanical field survey in Kon Ha Nung Biosphere Reserve (UNESCO 2021) of the highlands of Central Vietnam, we came across a population of Spermacoceae (collection number *LTA 531*) with the following morphological features: perennial herbs, opposite obovate to nearly oval leaf blades, interpetiolar entire stipules with cuspidate apex, 4-merous flowers, bilobed stigmas, inferior ovary, and many-seeded, diplophragmous capsules with persistent ovate or nearly oval calyx lobes. The morphological features, specifically the presence of diplophragmous capsules, observed in the new population of Spermacoceae align with the diagnostic characteristics of the genus *Hedyotis* as outlined in Wikström et al. (2013) and Neupane et al. (2015). Following a thorough review of the taxonomic literature on the genus *Hedyotis* in Vietnam and surrounding regions, including Pitard (1922), Fukuoka (1970), Pham (2003), Tran (2005), Chen and Taylor (2011), Wikström et al. (2013), and Neupane et al. (2015), as well as a comprehensive morphological comparison with closely related species such as *Hedyotis shenzhenensis* T.Chen, *H. shiuyingiae* T.Chen, and *H. yangchunensis* Ko & Zhang, and utilizing phylogenetic analysis to determine its placement within the Spermacoceae, we have determined that our specimens represent a previously undescribed species within the genus *Hedyotis*. This species is hereby named *Hedyotis konhanungensis*.

Materials and methods

Morphological study

Our collected specimens were compared with all described species from southeast Asia and southern China by studying relevant literature and examining digital herbarium images. Morphological characters were recorded using Nikon SMZ745/SMZ745T stereoscopic microscope and photographs of vegetative and floral parts were taken both in the field and from the samples preserved in 70% ethanol using Canon EOS 7D. The type specimens have been stored in the following three herbaria (acronyms follow Thiers 2022, continuously updated): Vietnam National Museum of Nature (**VNMN**), Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology (**HN**), and Kon Ka Kinh National Park.

Phylogenetic analysis

To establish its phylogenetic position within the tribe Spermacoceae, total genomic DNA was extracted from silica-dried material with the DNeasy Plant Kit (Qiagen, Valencia, California, U.S.A.). Four DNA regions (nuclear genome: ITS, ETS; plastid genome: *petD*, *rps16*) that were used in our earlier studies (Neupane et al. 2015, Neupane et al. 2017), were selected for amplification from this sample. Amplifications were performed in a 25 µl reaction mixture composed of 1 µl of each primer (10 µM), 1 µl of DNA template, 12.5 µl of GoTaq Green Master Mix (Promega, Madison, Wisconsin, U.S.A.), 9.5 µl of water. The amplification protocol for nuclear and chloroplast regions follows Kårehed et al. (2008) and Groeninckx et al. (2009) respectively. Amplified PCR products were purified using ExoSAP-IT PCR Product Cleanup (Thermo Fisher Scientific) following the manufacturer's protocols and sequenced at Apical Scientific Company for sequencing (Selangor, Malaysia).

We added the DNA sequences of our sample to our existing DNA data matrix (Suppl. material 1). The GenBank accession numbers for our sample were OQ401065, OQ401066, OQ458733, and OQ401067, which correspond to the rps16, petD, ITS, and ETS regions, respectively. We also included additional sequences representing the ITS, petD, and rps16 regions for *Hedyotis yangchunensis*, *Hedyotis shenzhenensis*, and *Hedyotis shiuyingiae*, obtained from Guo et al. (2013) and available in GenBank. Each of these DNA regions was aligned using MAFFT v.7 (Katoh and Standley 2013) and concatenated into a single matrix Suppl. material 2) to infer the phylogeny. Phylogenetic analysis was conducted on the combined matrix under the maximum likelihood (ML) framework with four partition schemes (ITS, ETS, petD, rps16) for their own substitution model (GTR+GAMMA) parameters using RAxML v.8.2.12 (Stamatakis 2014). The RAxML tree search and bootstrap analysis (1000 rapid bootstrap replicates for clade support) were conducted on CIPRES (Miller et al. 2010).

Results

Molecular phylogeny

The Maximum likelihood tree obtained from RAxML analysis on the concatenated data confirms the position of our sample from Vietnam within *Hedyotis* and distinct from *H. yangchunensis*, *H. shenzhenensis*, and *H. shiuyingiae* (Fig. 1, Suppl. material 3) The new species is nested within the clade (BS = 85%) representing *Hedyotis* from Sri-Lanka, southeast Asia (primarily Borneo), and New Guinea. The new species appears sister to the Sri Lankan clade, however, this phylogenetic position is considered unresolved due to weak bootstrap support (BS = 50%).

Taxonomic treatment

***Hedyotis konhanungensis* B.H.Quang, T.A.Le, K.S.Nguyen & Neupane, sp. nov.**
urn:lsid:ipni.org:names:77315486-1
Figs 2, 3, Table 1

Type. Vietnam. Central Highlands of Vietnam, Kon Ha Nung Biosphere Reserve, Gia Lai province: K'Bang District, Kon Pne Commune, 14°20'53"N, 108°20'48"E, primary evergreen forest slopes at elevation 1150 m a.s.l., 28 November 2021, Nguyen Quoc Luan, Ngo Duy Hoang Vu & Le Tuan Anh LTA 531 (holotype: VNMN!; isotypes: HN!).

Diagnosis. *Hedyotis konhanungensis* is similar to *H. shenzhenensis*, *H. shiuyingiae* and *H. yangchunensis* from southeastern China (Guangdong and Hongkong) in the morphology of the leaf blades, floral bracts, dichasial cymes, and fruits, but differs from them by its broadly ovate or deltoid (vs. triangular or broadly triangular) stipules with entire (vs. hairy or lacerated) margins and cuspidate (vs. acute) apex, suborbicular or broadly oval (vs. subovate or ovate to lanceolate) lowest floral bracts, ovate or nearly oval (vs. triangular or subulate to lanceolate) persistent calyx lobes on fruits, and stamens in long-styled flowers inserted in lower ¼ or near the base (vs. at the middle or near the mouth) of the corolla tube (Table 1).

Description. Perennial herbs, erect, 15–25 cm tall, completely glabrous. ***Stem*** simple, rarely branched, terete, 8–18 cm long, 5–7 mm in diam.; internodes 1–1.5 cm long. ***Leaves*** 5–7 pairs per stem, decussate, evenly spaced along the stem, abaxial side dark green, adaxial side glossy, dark purple to purplish black; petioles 4–6 mm long; blade flattened, thick, fleshy (subcoriaceous when dried), obovate-lanceolate to nearly oval, 6–10 × 2–4 cm; base decurrent or cuneate; apex broadly acute or obtuse; midrib depressed adaxially and prominent abaxially; 4–5 secondary veins on each side of the midrib, inconspicuous on adaxial side. ***Stipule*** interpetiolar, fused to leaf bases or very shortly around stem, broadly ovate or deltoid, 2.5–3 mm long, 5–6 mm wide at base, flattened, dark purple outside; apex cuspidate or aristate, with aristae 3–4 mm long and 0.7–0.9 mm wide; margins entire. ***Inflorescence*** terminal, a compound dichasial cyme, 5–7 cm long, with 3–4 orders of branching, purplish, 30–50-flowered.

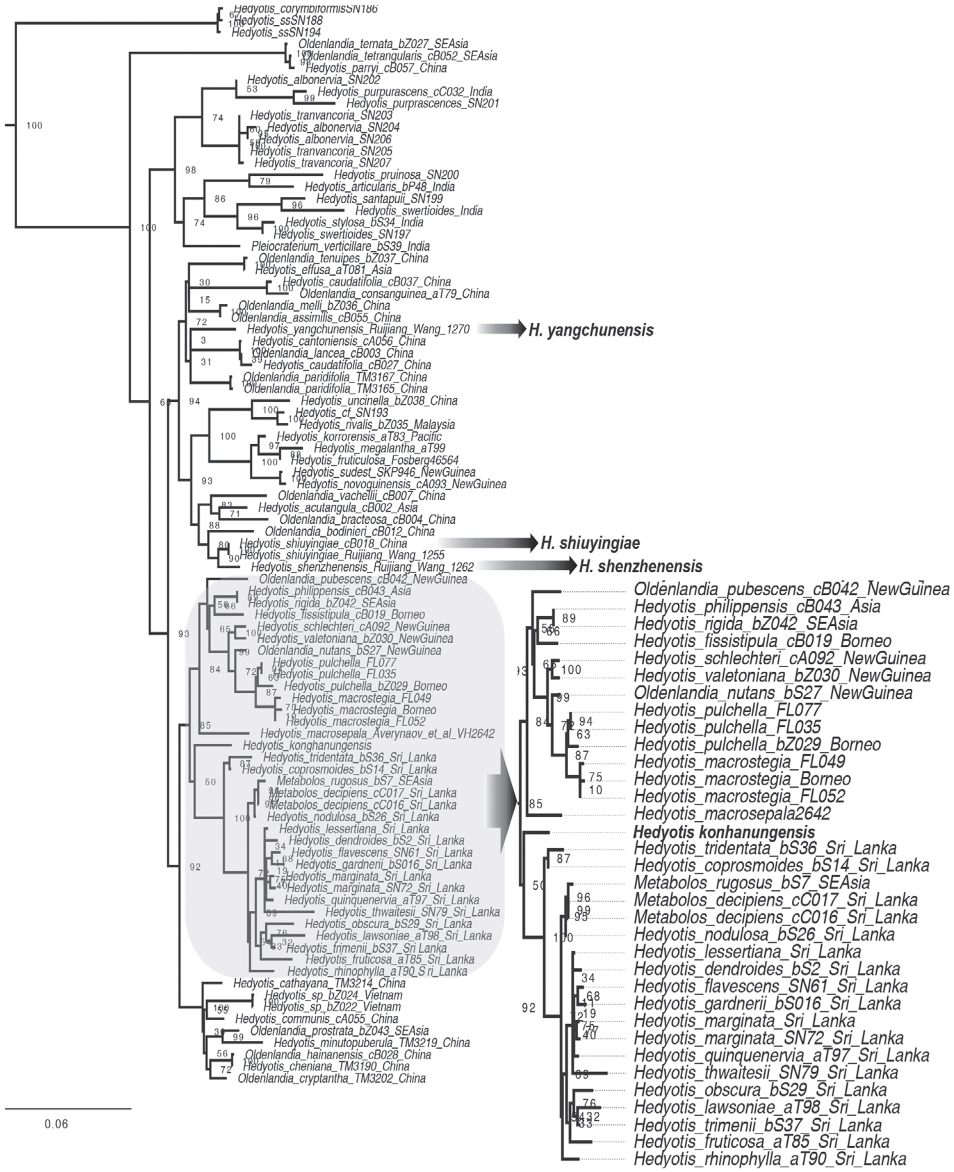

Figure 1. Maximum likelihood phylogenetic tree of *Hedyotis* based on the combined nuclear (ITS, ETS) and plastid (*petD*, *rps16*) data. The taxa discussed in the paper and the new species are in bold. Values at the nodes represent bootstrap support (BS).

Peduncle terete, 2–4 cm long; bracts subtending the basal branches of the inflorescence leaf-like, suborbicular or broadly oval, slightly concave, 2–2.5 × 1.5–2 cm, with apex rounded to broadly acute or obtuse, abaxially dark purple to dark bluish purple, pale green adaxially; bracts subtending the upper inflorescence branches smaller, ovate or lanceolate, 0.8–1.5 × 0.5–1 cm, with acute apex. Pedicels terete, 3–6 mm long, usually

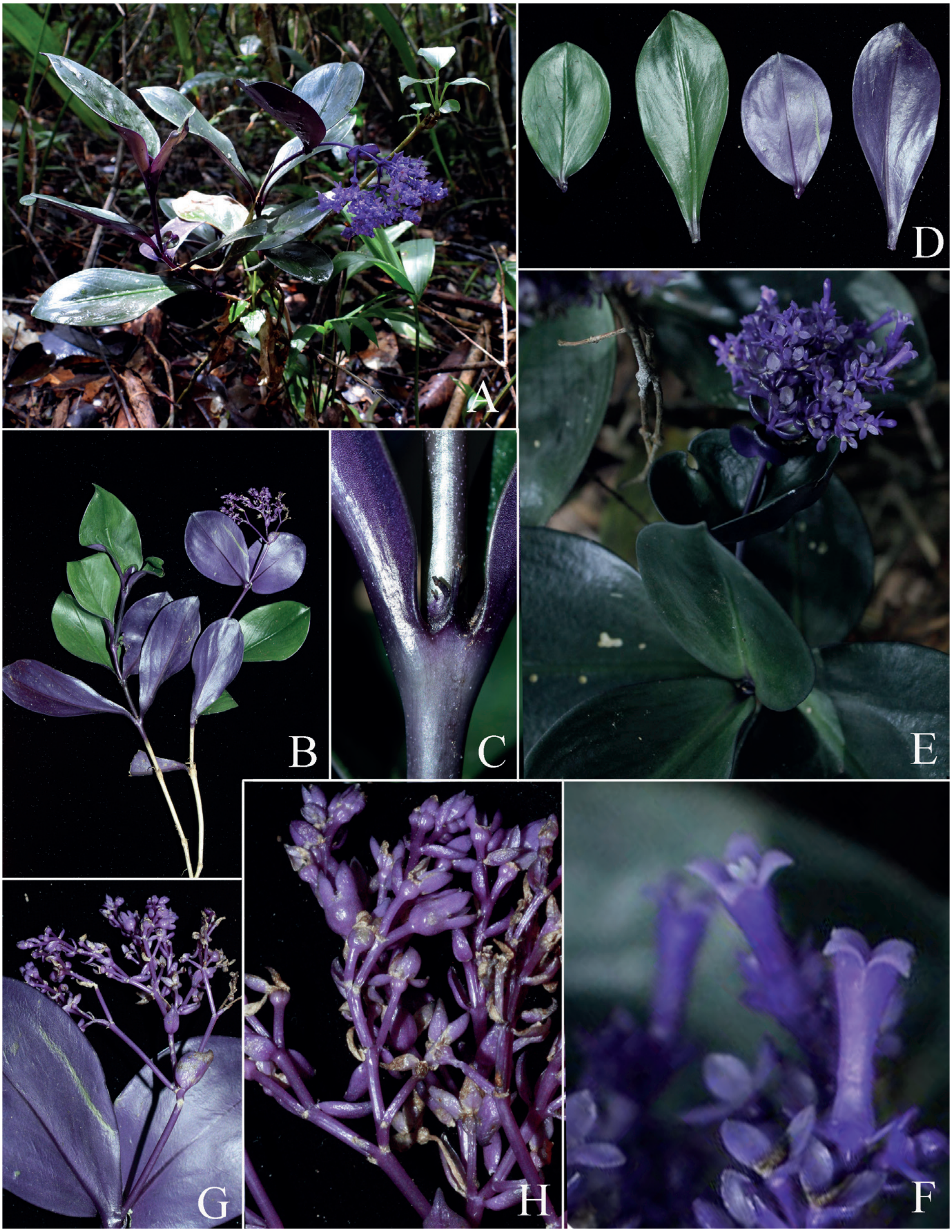

Figure 2. *Hedyotis konhanungensis* **A** habitat **B** habit **C** stipule **D** adaxial and abaxial leaf surfaces **E** inflorescences top view **F** long-styled and short-styled flower **G** infructescence **H** fruiting branch. Photos by Q.L. Nguyen and B.H. Photos by Quang, from *Luan et al. LTA 531*.

bluish purple or purplish, bracteolate or ebracteolate; bracteoles narrowly ovate or lanceolate, somewhat concave, ca. 2.5 mm × 1.5 mm, with apex broadly acute or obtuse. ***Flowers*** 4-merous, distylous. ***Calyx*** hypanthium, bluish purple, cupular, 0.8–1.2 mm

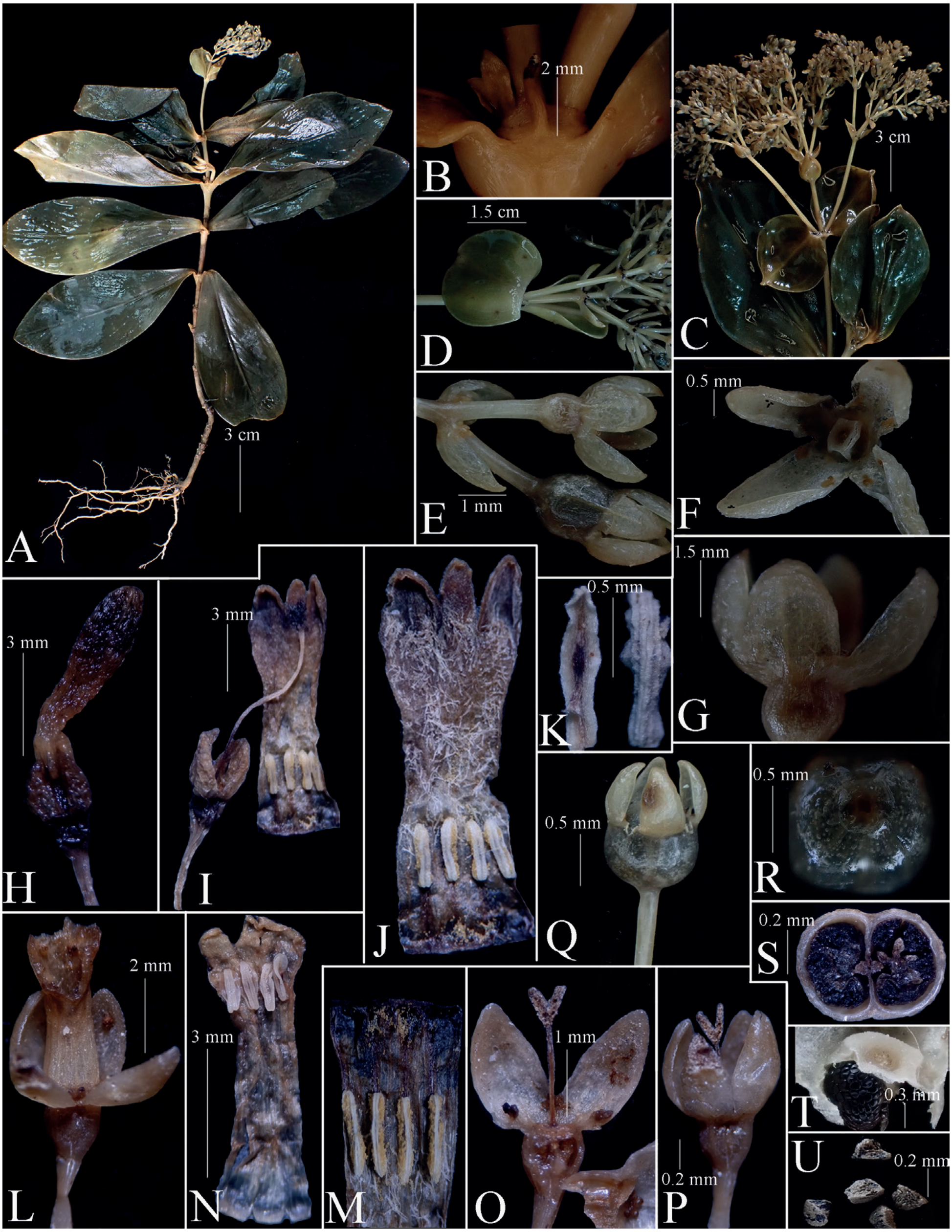

Figure 3. *Hedyotis konhanungensis* **A** habit **B** stipule **C** inflorescence **D** floral bracts **E** bracteoles and fruits **F, G** calyx top and side views **H–K** long-styled flower, entire and open showing pistil, corolla and anthers **L–P** short-styled flower, dissection showing calyx, corolla, anthers and pistil **Q–T** fruit, entire and dissection **U** seeds. Photos and design by B.H. Quang from *Luan et al. LTA 531*.

long and wide, glossy and glabrous; lobes 4, sub-equal, ovate or nearly oval, somewhat longitudinally concave, 1.7–2.2 mm long, 0.8–1.4 mm wide, broadly acute to obtuse at apex, adaxially white or purplish white, abaxially bluish purple. ***Corolla*** narrowly in-

Table 1. Morphological comparison of *Hedyotis konhanungensis* with its putative closest allies. The characters of *H. shenzhenensis* T.Chen, *H. shiuyingiae* T.Chen and *H. yangchunensis* W.C.Ko & Zhang are taken from Chen (2007, 2008), and Gao (1995) and their type specimens respectively.

Morphological characters	*H. konhanungensis*	*H. shenzhenensis*	*H. shiuyingiae*	*H. yangchunensis*
Plant height, including the inflorescences (cm)	15–25	20–40	15–35	30–39
Stem	terete or subterete, 8–18 cm long	terete or subterete,1–2 cm long	slightly tetragonal, 10–22 cm long	tetragonal or subterete, 15–30 cm long
Leaf arrangement	evenly spaced along the stem	fascicled on short rosette-like stem	evenly spaced along the stem	evenly spaced along the stem
petiole length (mm)	4–6	0.5	5–10	15–20
blade shape and size (cm)	obovate to oval, 6–10 × 2–4	elliptic, oblong-elliptic or obovate, 8.5–15 × 5–9	broadl, lanceolate or obovate-lanceolate, 2–19 × 1.4–8	narrowly elliptic, oblanceolate or elliptic-oblong, 3–12 × 1–4.5
Stipule shape and size (mm)	broadly ovate or deltoid, apex cuspidate, margin entire, 2.5–3 × 5–6	triangular, apex acute, margin shortly hairy, 3–5 × 5–10	triangular, apex acute, margin dense trichomes, 4–8 × 5–10	triangular, apex and margin lacerate or deeply divided into several linear lobes, ca. 16 × 8
Inflorescence	flowers lax or not congested into a head-like inflorescence	flowers lax or not congested into a head-like inflorescence	flowers congested into a head-like inflorescence	flowers congested into cymose or capitate inflorescence
Peduncle length (cm)	2–4	10–18	10	3–4
Lowest floral bracts	suborbicular or broadly oval, 2–2.5 × 1.5–2 cm	subovate, 2.5–3 × 1.8–2 cm or sometimes larger	ovate to lanceolate, 0.6–2.5 × 0.2–1 cm	ovate, ca. 1.5 × 0.5–0.6 cm
Calyx lobes	ovate or nearly oval, 1.7–2.2 × 0.8–1.4 mm	subulate, ca. 1.5 mm long	subulate, ca. 3 mm long	unknown
Corolla				
outer colour	bluish purple	white	purplish white	unknown
tube dimension and shape	6–7 × 1–1.5 mm, slightly enlarged both ends	ca.3 × 1.5–1.8 mm, slightly narrower near mouth	tube ca. 4.5 × 2–2.5 mm, enlarged at mouth	unknown
Stamens of long-styled flowers	inserted in lower ¼ (or near the base) of corolla tube	inserted in upper ¼ (or near the throat) of corolla tube	inserted in ½ (or middle) of corolla tube	unknown
Stamens of short-styled flowers	inserted in the upper ¼ (or near the throat) of corolla tube;	inserted near the throat of corolla tube;	inserted near base of corolla tube;	unknown
	filaments ca. 0.2 mm long, not exserted beyond corolla mouth	filaments ca. 0.8 mm long, exserted beyond corolla mouth	filaments ca. 0.3 mm long, not exserted beyond corolla mouth	
Persistent calyx lobes on fruits	ovate or nearly oval, 1.8–2.3 × 0.8–1.5 mm, unveined	narrowly triangular or subulate, 1.2–2 × 0.5–0.7 mm, unveined	narrowly triangular or subulate, 3–4 × ca.1 mm, unveined	lanceolate, 3–6 × 1.2–3 mm, veined

fundibuliform or tubular, bluish purple outside, white pubescent inside; tube 6–7 mm long, 1–1.5 mm wide, slightly enlarged at both ends; lobes 4, strongly reflexed, triangular-ovate, 1.5–1.8 × 1.2–1.5 mm, acute, adaxially white or purplish white, sparsely puberulent near base on adaxial surface. ***Longistylous flowers*:** stamens inserted in the lower ¼ of corolla tube, 1.5–2 mm above the base of tube; filaments very short, ca. 0.2 mm long; anthers linear, 1–1.2 × 0.2 mm, dorsifixed, introrse; style filiform, 5.5–6.5 mm long, white, glabrous, 2-parted; lobes oblong, 0.6–0.7 mm long, abaxially minutely papillose, pure white, slightly exserted beyond the corolla mouth; ovary inferior, 2-locular, with numerous ovules, placentation axile. ***Brevistylous flowers*:** stamens inserted on the upper ¼ of corolla tube, 1–1.5 mm below the corollas mouth; filaments and anthers similar to those in the long-styled flowers; style filiform, 1.5–1.8 mm long, 2-parted; lobes oblong, 0.6–0.7 mm long, densely papillose abaxially. ***Disks*** fleshy, ring-shaped, concave in the center, glabrous, white. ***Fruits*** capsular, nearly cupuliform with slightly concave top, 2.5–3 mm in diam., glabrous, purplish, crowned by the persistent, unveined, oval or nearly oval calyx lobes. Seeds many, black, irregularly angular, reticulate, minute, 0.3–0.5 mm long.

Additional specimens examined (Paratypes). Vietnam. Central Highlands of Vietnam. Kon Ha Nung Biosphere Reserve, Central Highlands of Vietnam, Gia Lai province: K'Bang District, Kon Pne Commune, 14°20'43.06"N, 108°20'38.33"E, 906 m a.s.l., 26 March 2022, *Bui Hong Quang et al. BHQ 453* (HN, and herbarium of Kon Ka Kinh National Park).

Etymology. This species is named after the "Kon Ha Nung Biosphere Reserve" where it was discovered.

Vernacular name. Vietnamese: An điền Kon Hà Nừng

Phenology. Flowering in October to November, fruiting from November to December.

Distribution and ecology. *Hedyotis konhanungensis* is recorded only from the type locality in the Kon Pne Commune of the Central Highlands of Vietnam, which is part of the Annamite Range. This range is known for its rich biodiversity and high number of endemic species (Averyanov et al. 2003). The species grows in the understorey of the evergreen forests in the valleys or on flat areas to slopes of sandstone mountains. Within its occupancy areas, the new species was associated with some shrubs or herbs such as *Pavetta bauchei* Bremek., *Lasianthus biflorus* (Blume) M.Gangop. & Chakrab., *Staurogyne* sp., *Popowia* sp., *Huperzia* sp.

Discussion

The genus *Hedyotis*, as currently circumscribed, corresponds to a primarily woody genus characterized by "diplophragmous" capsules (septicidally dehiscent capsules that separate into two distinct valves, as described by Wight and Arnott in 1834) and "fruiticosa type" seeds (dorsiventrally flattened seeds with a ventral hilar ridge topped by a punctiform apical hilum, as described by Terrell and Robinson in 2003). The members of this hyperdiverse genus (approximately 180 species) primarily occupy the mountains of Asia and the Micronesian Islands (in the northwestern Pacific). The molecular dating analysis suggests that *Hedyotis* split from its sister lineages of approximately 10 species (the African/Malagasy *Agathisanthemum* group and the African-tropical Asian-North American *Edrastima* group) nearly 27 million years ago (Neupane et al. 2017). The phylogenetic comparative analysis of evolutionary correlations shows that the evolution and diversification of the hyperdiverse *Hedyotis* along tropical mountain orogeny are strongly linked to the formation of a woody habit and many narrow endemic species (Neupane et al. 2017). The new species *Hedyotis konhanungensis*, found in the mountains of central Vietnam, is another example of a tropical montane species in this genus. This species is resolved within the clade containing other southeast Asian species and is unique among the Indochinese *Hedyotis* in its thick and fleshy oval leaves and completely dark purple floral parts (calyx and corolla) including the inflorescence stalk. Despite the geographic distance, its morphological similarity with Chinese members and non-monophyletic group of *H. shenzhenensis* and *H. shiuyingiae* with *H. yangchunensis* (Fig. 1) suggests the independent evolution of fleshy leaves and somewhat stunted growth habit among these species. Here we provide both morphological and molecular evidence to confirm the novelty of this taxon and propose it as species new to science.

Acknowledgements

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Supplementary material 1

Taxa and NCBI GenBank accessions used to estimate phylogeny of Spermacoceae for this study

Authors: Bui Hong Quang, Khang Sinh Nguyen, Tuan Anh Le, Le Thi Mai Linh, Quoc Luan Nguyen, Duy Hoang Vu Ngo, Lei Wu, Suman Neupane

Data type: Excel datasheet

Link: https://doi.org/10.3897/phytokeys.221.95895.suppl1

Supplementary material 2

A concatenated nexus alignment of four DNA regions (ITS, ETS, *petD*, *rps16*) used to estimate phylogeny of Spermacoceae for this study

Authors: Bui Hong Quang, Khang Sinh Nguyen, Tuan Anh Le, Le Thi Mai Linh, Quoc Luan Nguyen, Duy Hoang Vu Ngo, Lei Wu, Suman Neupane

Data type: Nexus alignment

Link: https://doi.org/10.3897/phytokeys.221.95895.suppl2

Supplementary material 3

Output of RAxML tree analysis with bootstrap values (1000 replicates)

Authors: Bui Hong Quang, Khang Sinh Nguyen, Tuan Anh Le, Le Thi Mai Linh, Quoc Luan Nguyen, Duy Hoang Vu Ngo, Lei Wu, Suman Neupane

Data type: Newick tree file

Link: https://doi.org/10.3897/phytokeys.221.95895.suppl3

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RESEARCH ARTICLE

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Primula pingbaensis (Primulaceae), a new species from Guizhou, China

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Abstract

Primula pingbaensis Na Zhang, X.Q.Jiang & Z.K.Wu, a new species of Primulaceae from Gaofeng Mountain of Pingba county, Guizhou, China, is described and illustrated. Morphological evidence supports *P. pingbaensis* as a member of *P.* sect. *Petiolares* on account of scape elongating, pedicels conspicuously thickening in fruit, and its capsule cracking irregularly round the top and crumbling away. Amongst the members of subsect. *Davidii*, the new species is characterized by having a uniquely smooth leaf blade due to inconspicuously raised veinlets and homostylous flowers with the style usually extending beyond the anthers. The distribution, phenology and conservation status of the new species are also provided.

Keywords

Guizhou, Karst, new species, ping ba bao chun, *Primula* sect. *Petiolares*

Introduction

Primula L., comprising 521 species mostly found in the temperate zone, is one of the largest genera in Primulaceae (Hu 1994; POWO 2022). More than 300 species of *Primula* were found in China, and distributed mainly in the southwestern part (Yunnan, Sichuan and Tibet), with the modern diversity center of the genus ranging from the Himalaya-Hengduan mountains chain (Hu 1994; Hu and Kelso 1996; Richards 2003).

In *Primula*, floral morph (i.e., heterostyly or homostyly) is one of the important characters for species identification. Heterostyly is perhaps the most distinctive feature of *Primula* (Richards 2003) because most of the species (92%) are heterostylous (Wang et al. 2021). In these species, all the flowers on a plant either have a long style ('pin morph') or a short style ('thrum morph') differing reciprocally in stigma and anther height. This reciprocal herkogamy is often accompanied by an incompatibility system that makes intermorph crosses more successful than intramorph crosses (Richards 2003). In contrast, the remaining species are almost monomorphic for style condition (i.e., homostylous), having either long styles and long-level stamens (long homostyly) or short styles and short-level stamens (short homostyly). Recently, a novel floral form in *Primula* was reported by one of the authors (Wu et al. 2023), which is characterized by bell-shaped flowers with exceptionally short tubes, stamens set just above the mouth of the tube and the style either as long as, or exserting, the corolla. In addition, some species have both heterostylous and homostylous populations, such as in *P. chungensis* Balf.f. & Kingdon-Ward and *P. oreodoxa* Franch. (Hu 1990).

The *Primula* sect. *Petiolares* Pax is well represented in the Himalaya-Hengduan mountains, with only a few members extending into Kashmir, central China, and some other regions (Hu 1990; Hu and Kelso 1996). One of the most important diagnostic characters of the section is that of the capsule being globose, included in the persistent calyx, not opening by valves but crumbling at the membrane apex. More than 60 species of this section are now recognized worldwide (Hu 1994; Hu and Kelso 1996; Hu and Geng 2003; Richards 2003; Li and Hu 2009; Rankin 2010; Hu and Hao 2011; Xu et al. 2014a, 2015, 2016a, 2022a; Ju et al 2018; Yuan et al. 2018).

Guizhou is one of the biodiversity hotspots in China, marked by its karst landscape. According to relevant literatures and Flora of China, there are more than a dozen species of *Primula* distributed in Guizhou, of which some species are endemic to Guizhou, such as *P. esquirolii* Petitm. (Wu et al. 2022), *P. fangingensis* Chen et C.M.Hu, *P. kweichouensis* W.W.Smith, *P. lithophila* F.H.Chen & C.M.Hu and *P. levicalyx* C.M.Hu et Z.R.Xu (Smith et al. 1977; Hu 1990). During the last decade, some newly published *Primula* species from the adjacent areas were also found within Guizhou, such as *P. pelargoniifolia* G.Hao, C.M.Hu & Z.Y. Liu (Xu et al. 2014b), *P. persimilis* G.Hao, C.M.Hu & Y.Xu (Xu et al. 2016b) and *P. centellifolia* G.Hao, C.M.Hu & Y.Xu (Xu et al. 2017). Recently, a newly described species, namely *Primula chishuiensis* C.M.Hu, G.Hao & Y.Xu (Xu et al. 2022b) was reported from Guizhou.

Multiple field surveys were carried out from type locality and adjacent areas from 2018 to 2022 to obtain more reliable information on the field distribution of *P. esquirolii*, species that was last recorded in the 1910s in Pingba county, Guizhou, China. During field surveys in April 2018, we found a *Primula*, which is suspected to be a member of sect. *Petiolares* with fruits from Gaofeng mountain in Pingba county. At first, we thought it was *P. esquirolii*, but different compared to the type specimen of *P. esquiroliii* in its lamina character, where *P. esquirolii* is conspicuously bullate on the upper surface and strongly honeycombed-reticulate beneath. For further clarification on the identity of the newly collected *Primula*, the Gaofeng Mountain and adjacent areas were revisited the following years (2019–2022) to observe and collect the plants in flower and fruit. The collected *Primula* is a slender dwarf perennial herb with smooth, thin papery lamina, bearing homostylous flowers. After comparing the relevant literature for related species, we concluded that this plant was unique and should be described as a new species. Therefore, we describe and illustrate the taxon as new to science here.

Materials and methods

The morphological description of the new species was based on living plants from Gaofeng Mountain. For comparison purposes, specimens of closely related species, *P. coerulea* from Yunnan and *P. esquirolii* from Guizhou were also collected from their type locality. In addition, specimens' images online of the closely related species from herbaria P, E, KUN, PE, K, IBSC, and relevant literature (Smith et al. 1977; Hu 1990; Hu and Kelso 1996) were also consulted. All morphological characters of *P. pingbaensis* and its morphologically similar species in the section *Petiolares*, including *P. esquirolii* and *P. coerulea*, were measured using a vernier calliper. The conservation assessment of the new species was evaluated using the IUCN categories of threat (see IUCN 2012 and IUCN Standards and Petitions Committee 2022).

Taxonomic treatment

Primula pingbaensis **Na Zhang, X.Q.Jiang & Z.K.Wu, sp. nov.**
urn:lsid:ipni.org:names:77315510-1
Figs 1A–I, 2

Note. The new species most resembles *Primula esquirolii* and *P. coerulea*, sharing similar floral morphology and short or almost obsolete scapes at flowering time. However, the new species differs from the homostylous *P. esquirolii* mainly in its lamina smooth on upper surface, deeper flower color, oblong flower lobes, and the style usually extending beyond the anthers. Compared to the heterostylous *P. coerulea*, the new species differs mainly in its homostylous flower, and the whole plant is usually covered with sparse glands. The main morphological distinctions between *P. pingbaensis*, *P. esquirolii* and *P. coerulea* are summarized in Table 1.

Type. China. Guizhou: Pingba county, Gaofeng Mountain, Wanhua Temple. 26°22'31"N, 106°24'24"E, 1432 m alt., 14 January 2021 (fl.), *ZKWU 2021010* (holo-type: GZTM!).

Description. A perennial slender, dwarf herbaceous, efarinose, with a short rhizome and numerous fibrous roots. ***Leaves*** forming a rosette, at flowering time 2–4 cm long including the petiole, 2–3 cm broad, spatulate or elliptic-obovate, obtuse or rounded at the apex, gradually tapering into the winged petiole; petiole up to 1/3 as long as leaf blade; lamina thin papery, upper surface smooth, lower surface midrib conspicuous but reticulate veins inconspicuous, sparsely glandular, margin with regular sparsely acute serrate. ***Scape*** at flowering time almost obsolete, to 1 cm in fruit, sparsely glandular, usually 1–3 flowered. ***Bracts*** linear-lanceolate, 3–6 mm long, sparsely glandular; pedicel 5–12 mm, glandular. ***Flower*** homostylous; calyx campanulate, 5–6 mm long, glandular-puberulous, parted nearly to 1/3 of its length, lobes triangular-lanceolate, apex acute; corolla funnel-shaped, violet, tube 13–15 mm long, usually three times the length of the calyx, limb 15–20 mm wide, lobes oblong, 4–6 mm long, apex emarginate; stamens with anthers 1.5 mm long, inserted at apex of corolla tube, style

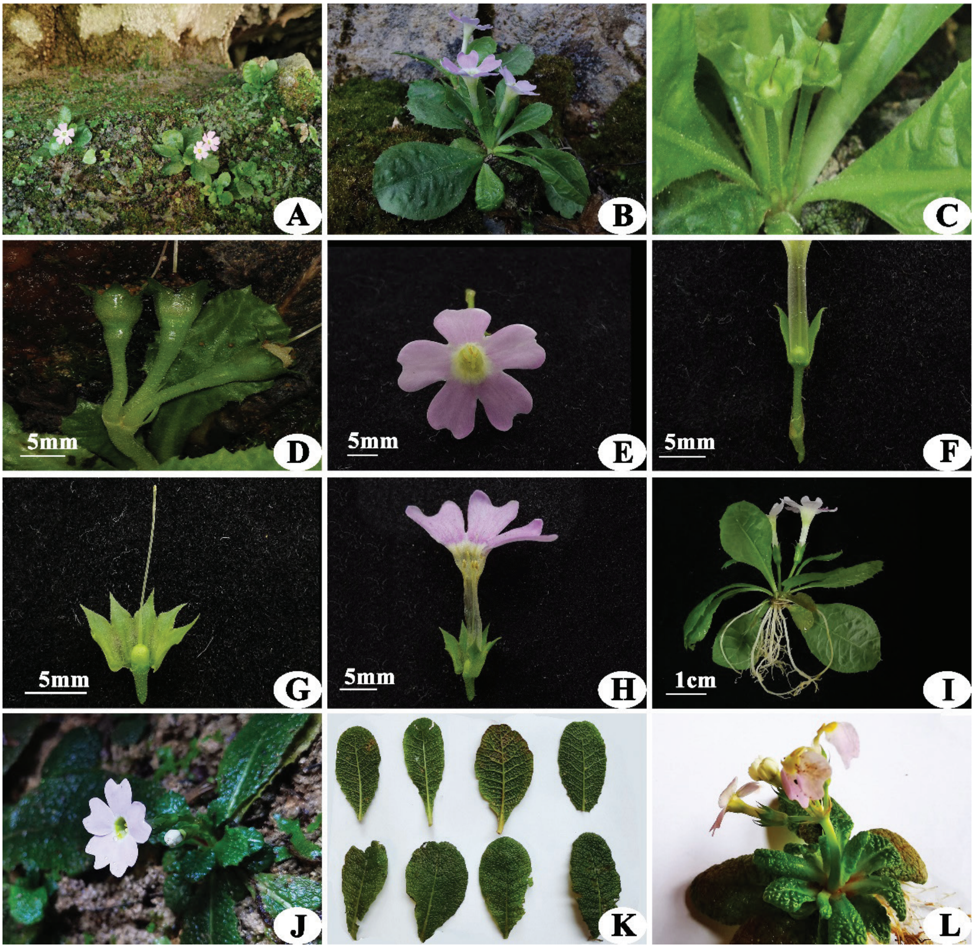

Figure 1. A–I *Primula pingbaensis* sp. nov.: **A** habitat **B** habit in flowering **C** capsule in early-fruiting **D** capsule in later-fruiting **E** flower, front view **F** bracts and calyx **G** calyx and stigma **H** dissected corolla showing the anthers and stigma **I** specimen in flowering; **J–L** *Primula esquirolii*: **J** habitat **K** leaves, both surfaces **L** habit. Photographed by Z.K Wu.

14–15 mm long, usually beyond the anthers. ***Capsule*** subglobose, 4–6 mm in diameter, nearly equal to calyx.

Distribution and ecology. *Primula pingbaensis* is only known from the type locality on Gaofeng mountain in Pingba county, Guizhou, China. The plant grows on moist walls of karst cliffs. (Fig. 1, Map 1).

Phenology. Flowering occurs from January to March; fruiting from April to June.

Etymology. The epithet of the new species is derived from the name of Pingba county, Guizhou, where the new species was discovered and collected (Map 1).

Vernacular name. Chinese mandarin: ping ba bao chun (平坝报春).

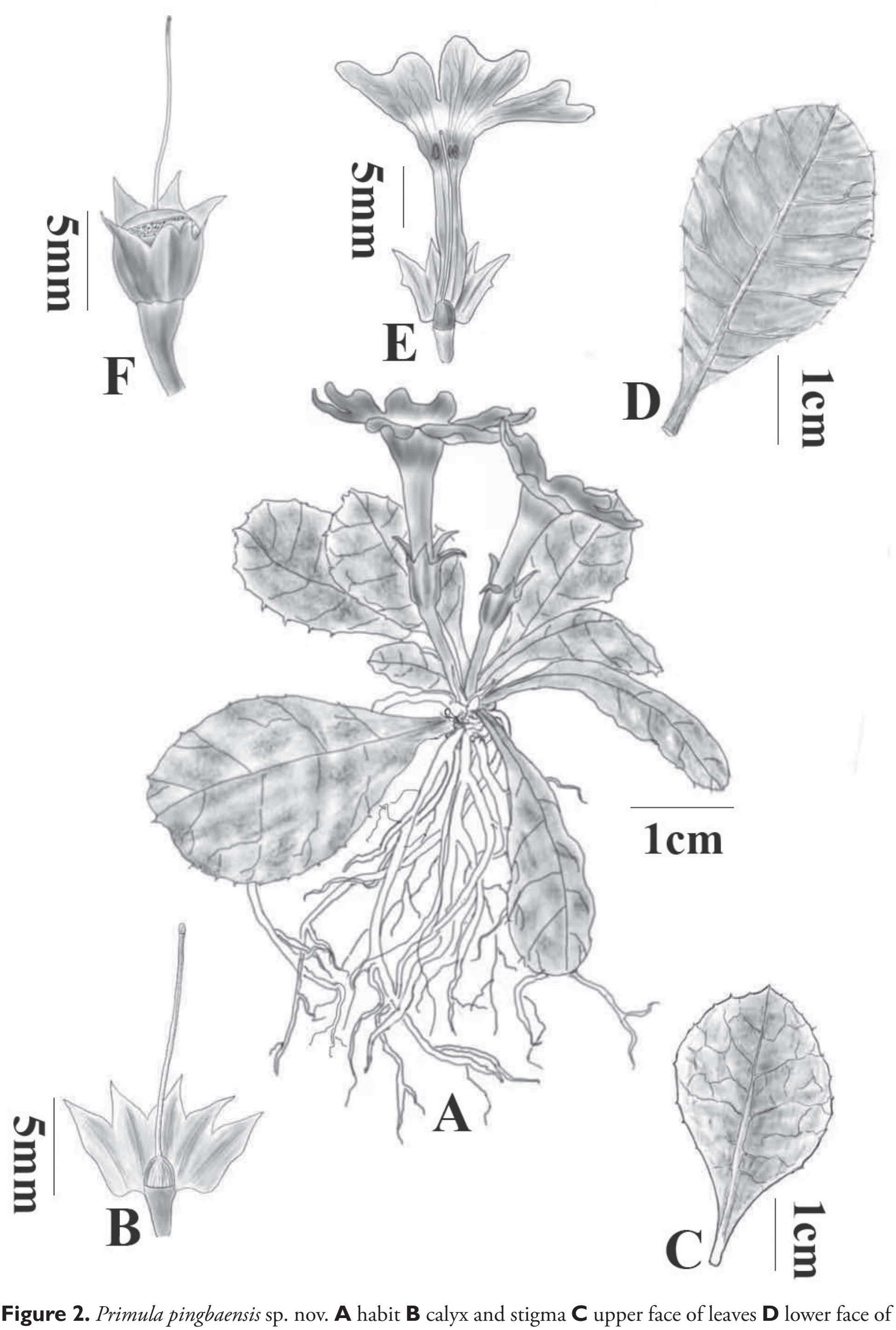

Figure 2. *Primula pingbaensis* sp. nov. **A** habit **B** calyx and stigma **C** upper face of leaves **D** lower face of leaves **E** dissected corolla **F** capsule. Drawn by Ms. Xiang-Li Wu.

Map 1. Location of the population of *Primula pingbaensis* in Pingba county.

Provisional conservation status. Critically Endangered (CR B2ab(iii)). Field surveys were conducted several times in the type locality and adjacent districts for this new species, and only one population of *Primula pingbaensis* was discovered, with ca. 40–60 adult individuals, distributed over about 100 m^2 in the type locality. This site is on the grounds of a temple, and some individuals grow close to the path for visitors and face a strong threat from human activities. Its status should therefore be of concern and addressed in further investigations.

We estimated the extent of occurrence of the species to be less than 100 km^2. Over the last four years, we have observed a steady decline in the territory area of the habitat due to the temple building maintenance and road construction. Considering the present field information and IUCN categories of threat, this species should be included in the category Critically Endangered (CR B2ab(iii)).

Discussion

Most species in the sect *Petiolares* of *Primula* have heterostylous flowers. Only *P. hookeri* G.Watt, *P. chamaethauma* W.W.Smith, *P. esquirolii* and our newly discovered species *P. pingbaensis* have homostylous flowers. Species with homostylous morphs are usually accompanied by a compatibility system that makes intramorph

Table 1. Morphological comparisons of *Primula pingbaensis* with *P. esquirolii* and *P. coerulea*.

Characters	*P. pingbaensis*	*P. esquirolii*	*P. coerulea*
Scape	almost obsolete in flowering time	almost obsolete or to 1 cm in flowering time	apparent scape in flowering time
Leaf blade	2–4 × 2–3 cm, spatulate or elliptic–obovate, thin papery, abaxially sparsely glandular, adaxially smooth	5–13 × 1.5–5 cm, elliptic–obovate to obovate–oblanceolate, subcoriaceous, abaxially densely short glandular pubescent along veins, adaxially bullate	3.5–10 × 2–4.5 cm, elliptic to oblong–oblanceolate, firm papery, abaxially with multicellular hairs along veins, adaxially bullate
Petioles	1/3 as long as leaf blade, sparsely glandular	Short or almost obsolete, sparsely glandular	1–7 cm, with long dense multicellular hairs
Calyx	5–6 mm long	5–7 mm long	8–13 mm long
Calyx lobes	glandular-puberulous, parted nearly to 1/3 of its length	sparsely glandular, parted nearly to middle	pubescent, cut to slightly below middle
Corolla	usually 3 times the length of the calyx, limb 15–20 mm wide, lobes oblong	usually 2–3 times the length of calyx, limb 15–20 mm wide. lobes obovate	nearly one time as the length of the calyx, limb 25–35 mm wide, lobes broadly obovate
Flower color	violet	pale blue or rose or violet	purplish blue
Flower	Homostylous	Homostylous	Heterostylous
Style	14–15 mm long, usually beyond the anthers	style barely reaching base of anthers	Pin flowers: stamens ca. 5 mm above base of corolla tube; style as long as tube. Thrum flowers: stamens 12–13 mm above base of corolla tube; style ca. 6 mm

(and thus self-) crosses more successful (Richards 2003). The selfing homostylous lineages often have a high genetic load, which makes them sensitive to environmental changes and renders population expansion over short periods difficult (Wu et al. 2022). Our observation on *Primula* species distribution indicated that homostylous species usually have fewer populations or smaller population sizes than closely related heterostylous species. *Primula hookeri* and *P. chamaethuma* are distributed in the alpine areas of Yunnan and Tibet (usually over 4000 m a.s.l.) while *P. esquirolii* and *P. pingbaensis* occur in the karst areas of Guizhou (usually lower than 1500 m a.s.l.). Field surveys on *P. esquirolii* showed that it is a species with extremely small populations and was evaluated as‘Critically Endangered’ (Wu et al. 2022), like our new finding of *P. pingbaensis.*

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RESEARCH ARTICLE

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Solved at last: The Philippine endemic *Psychotria philippensis* is a synonym of *Scyphiphora hydrophylacea* (Rubiaceae, Scyphiphoreae)

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Abstract

Psychotria philippensis (Rubiaceae) was described by Chamisso and Schlechtendal in 1829, was the first *Psychotria* name published for the Philippines and is currently considered a Philippine endemic. The name remained in a taxonomic limbo for almost two centuries as it was variously accepted, synonymized or considered obscure, probably because the type specimen in the Berlin herbarium was destroyed and no original material has survived or is currently known. A combined analysis of the information on morphology, type locality and ecology contained in the protologue and a review of relevant literature on the study of the name by various authors over the last two centuries finally clarified the identity of *P. philippensis*. The name is confirmed here as a synonym of the rubiaceous mangrove *Scyphiphora hydrophylacea*, as first proposed by Schumann, one of the authorities of the family in the late 19th century, and the application of *P. philippensis* is fixed by neotypification. This reduces the number of Philippine species of *Psychotria* by one, but fortunately, this is not happening through extinction, as has been the case with too many species of the highly endangered Philippine flora. In addition, the history of the discovery and study of *S. hydrophylacea* and its synonyms are described in detail, and one lectotype and one neotype are designated.

Keywords

Chamisso, herbarium history, Paleotropical Rubiaceae, The Philippines

Introduction

The Philippines is one of the world's top 25 biodiversity hotspots and one of the eight "hottest hotspots" in terms of endemic species and habitat loss (Myers et al. 2000; Brooks et al. 2006; Posa et al. 2008; Webb et al. 2010). One of the largest groups in the Philippine flora and the tropics is the coffee family (Rubiaceae), with more than 13,000 described species. Rubiaceae is represented in the Philippines by more than 455 native species in 74 genera and 25 tribes (Alejandro 2007). Merrill (1923–1925, 1923a, b, 1926) published the first complete account and enumeration of Philippine flowering plants and formed the basis for subsequent research on the Philippine Rubiaceae. Later, Alejandro and Liede (2003) and Alejandro (2007) updated Merrill's list with species described since then and changes in generic concepts, but noted that some groups are still poorly known due to the size and taxonomic complexity of the family and the need for modern revision. Fortunately, several recent monographic studies have greatly improved our knowledge of the family in the region (e.g., Alejandro et al. 2010, 2011, 2016; Wong and Low 2011; Obico and Alejandro 2012; Pereira and Ridsdale 2012; Chavez et al. 2020).

Among the genera revised recently in the Philippines, is *Psychotria* (Psychotrieae), the largest and most complex genus of Rubiaceae in the Philippines and elsewhere. Sohmer and Davis (2007) recognized 112 *Psychotria* species and 17 infraspecific taxa, of which 106 are endemic and 25 are only known from the type. The broad circumscription of *Psychotria* was subsequently narrowed following morphological and phylogenetic studies, which led to the exclusion of numerous segregates in the tribe Palicoureeae (Nepokroeff et al. 1999; Robbrecht and Manen 2006; Razafimandimbison et al. 2014). On the other hand, long-recognized segregates such as the myrmecophytic Hydnophytinae C.R.Huxley & Jebb (or the genera *Cremocarpon* Boivin & Baill. and *Pyragra* Bremek. not present in the Philippines) were found to be nested in *Psychotria*. In the current delimitation of *Psychotria*, its center of diversity lies in the Palaeotropics, and it includes at least 1,600 species of trees, treelets, shrubs, subshrubs, herbs, climbers and epiphytes (Razafimandimbison et al. 2014). Following these authors, the two species comprising the Membranifolia Species Group in Sohmer and Davis (2007) were excluded from *Psychotria* and transferred to an expanded *Eumachia* within Palicoureeae (Taylor et al. 2017).

With few exceptions, all of the 110 remaining *Psychotria* species are probably endemic to the Philippines, but a full assessment would require a better knowledge of the Southeast Asian and especially the Bornean species. They are predominantly found in primary forests and have narrow distributions making them prone to extinction. Following a drastic decline in primary forest cover over the last century, many species have not been seen for decades, leading Koopowitz et al. (1998) and Sohmer (2001) to assume that more than half of the Philippine species of *Psychotria* are extinct. In contrast, Sohmer and Davis (2007) provided a more optimistic estimate of extinction rates of up to 28%, but a few of these presumably extinct species have since been rediscovered by local botanists (Ordas et al. 2019; Biag and Alejandro 2020, 2022; Batuyong et al.

2021). Apparently, several primary forest species can tolerate some disturbance and escape extinction in relatively more widespread secondary forests (Sohmer and Davis 2007; Biag and Alejandro 2022).

Taxonomic history of *Psychotria philippensis* Cham. & Schltdl.

Five *Psychotria* names could not be assigned in the revision of Sohmer and Davis (2007). These include the first *Psychotria* name published for the Philippines and named after the archipelago: *Psychotria philippensis* Cham. & Schltdl. (Chamisso and Schlechtendal 1829a). Sohmer and Davis (2007: 238) excluded the name from *Psychotria* but could not assign it to a genus due to the missing type material.

A year after its publication in 1829, *Psychotria philippensis* was already in use and was included in the *Prodromus* of de Candolle (1830: 505–506) in an attempt to summarize all known seed plants. However, Candolle did not examine any specimens, and his description is essentially an abbreviated version of that published in the original protologue, a limitation that many subsequent authors had in understanding the identity of the name. Miquel (1857: 282) was the first to conclude that *P. philippensis* was misplaced in *Psychotria* but incorrectly suggested an affinity with *Chassalia*, a palaeotropical genus of the tribe Palicoureeae. Naves and Fernández-Villar (1880: 112, "Verè distinctissima species") again accepted *P. philippensis* as a species of *Psychotria*, referring to a specimen they identified as this species and had seen in the herbarium of Sebastián Vidal (now at MA, the herbarium of the Real Jardín Botánico, Madrid, Spain). However, the identity of the material they examined seems doubtful since they gave the Caraballo Mountains in central Luzon as the locality, where the presence of the species (a mangrove species, see below) is ecologically impossible. Kuntze (1891) considered *Uragoga* Baill. the correct name for *Psychotria* and proposed the combination *U. philippensis* to accommodate the species. In his enumeration of Philippine Rubiaceae, Elmer (1906: 32) retained *P. philippensis* as an accepted species. Furthermore, he recognized that the elongated, spindle-shaped or fusiform drupes of *P. philippensis* are unusual and differentiate the species from all congeners.

Karl Moritz Schumann (1851–1904), professor of botany and curator at the Berlin herbarium (B), contributed the family Rubiaceae to Engler's "Die Natürlichen Pflanzenfamilien". He was the first to place *Psychotria philippensis* in the synonymy of the rubiaceous mangrove tree, *Scyphiphora hydrophylacea* (Schumann 1897: 80). He did not give details of his reasoning, but likely he may have seen the type specimen in B, where he worked. Years later, Merrill examined the type at B again (Merrill 1915: 129). He did not reference Schumann's earlier work but reached the same conclusion and synonymized *P. philippensis* under *S. hydrophylacea* in his "Studies on Philippine Rubiaceae" (Merrill 1915: 129) and in his "Enumeration of Philippine Flowering Plants" (Merrill 1923b: 533).

Most of the Berlin herbarium was destroyed during World War II (Hiepko 1987), the type of *Psychotria philippensis* was lost and the identity of the name remained un-studied for several decades. Sohmer and Davis (2007: 238) again excluded the name from *Psychotria* and concluded an affiliation with Rubiaceae but could not assign the name to any genus. The species was not included in the list of Philippine Rubiaceae by Alejandro (2007) but was again listed as an accepted species of *Psychotria* in the checklist of Philippine vascular plants by Pelser et al. (2011 onwards), and subsequently in the World Checklist of Rubiaceae (Govaerts et al. 2022, published online and accessed Oct. 2022, but recently discontinued) and the Plants of the World Online (http://powo.science.kew.org, accessed Feb. 2023), who probably overlooked the synonymizations by Schumann and Merrill or reached a different conclusion on the identity of the name. Wong et al. (2019) gave the most recent treatment and synonymy of *Scyphiphora*, but *P. philippensis* was not listed as a synonym.

The identity of *Psychotria philippensis* as a synonym of *Scyphiphora hydrophylacea* is finally established here by a combined analysis of morphological and ecological information in the protologue and an analysis of the historical studies of the name by various authors. Furthermore, the application of the name is fixed by neotypification. This reduces the number of Philippine species of *Psychotria* by one, but fortunately, this is not happening through extinction, as has been the case with too many species of the highly endangered Philippine flora.

Psychotria philippensis and *Scyphiphora hydrophylacea*

Chamisso and Schlechtendal (1829a) described *Psychotria philippensis* as a resinous shrub of mangroves (“in maritimis”) with paired axillary or supraxillary inflorescences and subcylindric drupes with acute ridges. Resin production, inflorescence structure and habitat immediately exclude the name from *Psychotria*, which has terminal or solitary-pseudaxillary inflorescences and does not occur in mangroves or other coastal habitats (e.g., Sohmer and Davis 2007; see also Taylor 1996, 2020 and Lachenaud 2019). Instead, the detailed and elaborate description in the protologue is an excellent and unambiguous match for *Scyphiphora hydrophylacea*, a rare and endangered mangrove species that is distributed from East India and Madagascar to the Western Pacific and Northern Australia, including the Philippines (e.g., Puff and Rohrhofer 1993; Alejandro and Liede 2003; Alejandro 2007; Almazol and Cervancia 2013). Thus, there is no reason to doubt the identifications of Schumann (1897: 80) and Merrill (1915, 1923b), who did extensive work on Philippine and other Rubiaceae and were probably the only botanists to examine any of the type specimens.

The monotypic *Scyphiphora* is unusual within Rubiaceae in a number of ecological and morphological characters and is surveyed in detail by Puff and Rohrhofer (1993): It is the only member of the family that occurs in mangrove vegetation. Thus, it is exposed to the influence of salt or brackish water, which only a few Rubiaceae, such as the coastal *Guettarda speciosa* L. (Guettardeae) and *Hydrophylax maritima* L. f., can withstand (e.g., Puff et al. 2021). *Scyphiphora* is typically associated with species of the Rhizophoraceae and other mangroves but is never a dominant component. Its phylogenetic position was long unclear until molecular phylogenetic data showed that it belongs to the Vanguerieae alliance within the subfamily Ixoroideae and is best placed in the monotypic tribe Scyphiphoreae Kainul. & B. Bremer (Mouly et al. 2009; Razafimandimbison et al. 2011; Kainulainen et al. 2013). Finally, recent phylogenetic studies have demonstrated that Scyphiphoreae is sister to the monotypic Southwest Chinese Trailliaedoxeae Kainul. & B. Bremer, and together are sister to the *Glionnetia*–Vanguerieae–Greeneeae–Ixoreae clade (Wikström et al. 2020).

The fruits of *Scyphiphora* are somewhat reminiscent of those of *Psychotria*. Both genera have bilocular drupaceous fruits, but *Psychotria* has fleshy, usually red, bird-dispersed drupes, usually with two one-seeded pyrenes embedded in the fruit pulp. When fresh, these fruits are more or less globose to ovoid or obovoid, and the longitudinally ribbed pyrene surface becomes apparent only in dried fruits (e.g., Taylor 1996, 2020; Sohmer and Davis 2007; Lachenaud 2019). The only exceptions from that fruit type are species of the former genus *Cremocarpon* and *Pyragra* with dry schizocarps nested within a broadly defined *Psychotria* (e.g., Razafimandimbison et al. 2014; Taylor 2020).

Meanwhile, the fruits of *Scyphiphora* are drift fruits adapted for sea dispersal, a rare dispersal mechanism in the family, otherwise found in few other members of the family that occur in coastal vegetation, for example, in *Guettarda speciosa*. The fruits of *Scyphiphora* are two-locular with two superposed ovules per locule, one ascending, the other descending. When the fruits are mature, they are elongated, dry, brownish and strongly longitudinally ridged. Most of the pericarp and the prominent ribs are composed of mesocarp, which consists of dead, thin-walled, lignified cells. Their lumina are connected by numerous pits and are probably air-filled to increase their buoyancy. The skin-like exocarp is thin, parenchymatic, dry and usually detaches to some extent when the fruits mature. Finally, the endocarp is thin, dry, and heavily sclerified, enclosing each of the two locules. In mature fruits, the seeds are connate by layers of parenchymatic cells, the fruits therefore do not separate into mericarps and the seeds are dispersed together. As pointed out by Puff and Rohrhofer (1993), the fruits were often referred to as “fleshy” or “drupes”, but the entire pericarp is dry when mature and these terms therefore are inaccurate.

Chamisso and his collections

Between August 1815 and August 1818, the Chancellor of the Russian Empire, Count Nikolai Romanzoff (1754–1826), commissioned an expedition around the world on the Russian brig Rurik under the command of Captain Otto von Kotzebue (1787–1846). In addition to their primary goal of finding the Northeast Passage from the Bering Strait to the Atlantic Ocean, they aimed to make scientific collections, for which they hired the famous poet and belated botanist Ludolf Karl Adelbert von Chamisso (1781–1838; see Schlechtendal 1839 and Schmid 1942 for biography and bibliography), zoologist Johann Friedrich von Eschscholtz (1793–1831) and artist Louis Choris (1795–1828). The extensive diaries and travelogues of Chamisso (1836a, 1836b), Kotzebue (1821) and Choris (1822) give rather detailed contemporary views of the Romanzoffian expedition, and Bździach (2004) and Maaß (2016) provided excellent historical contextualization.

In his first report to Romanzoff, Chamisso (1818: 206) estimated that they had collected about 2,500 plant species, one-third of which were undescribed. Upon returning from the expedition, Chamisso received permission to take his entire collection to Berlin to study and publish the taxonomic novelties, where they remained until his death (Hiepko 2004; Maaß 2016: 134; see also Chamisso 1818: 208). To this day, the total size of the collections remains unknown. Still, it is clear that the same species were often collected in several places, and each gathering consisted of numerous duplicates, which partly explains the wide dissemination of many Chamisso specimens (e.g., Maaß 2016: 171–172).

Chamisso donated a complete set of specimens from the expedition on the Rurik to the Berlin Herbarium (B). As stipulated in his will, an additional set of "1,800 plant species" was given to his successor at B, Johann Friedrich Klotzsch, who also donated them to the herbarium (Schlechtendal 1839: 104; Urban 1917: 19, 22, 336). Unfortunately, most of B was destroyed during World War II, including most of the two sets of Chamisso's collections deposited there (Hiepko 1987). Duplicates are expected in HAL (see below), where the herbarium of his close friend Schlechtendal, professor of botany and director of the Botanical Garden of the University of Halle, is kept (Werner 1988; Braun and Wittig 2003). Schlechtendal received part of Chamisso's collections during their joint work in Berlin on the publication of the plants from the expedition on the Rurik (e.g., Chamisso 1826, 1830; Chamisso and Schlechtendal 1826a, b, 1827, 1829a, b).

In 1840, two years after his death, Chamisso's private herbarium, which contained up to 12,000 species and 60,000 specimens of his and many of his contemporary collectors (Ruprecht 1864: 4), was acquired by the St. Petersburg Academy of Sciences and is now in the herbarium of the Komarov Botanical Institute (LE). This collection is said to include most of the specimens from the Romanzoffian expedition, but the specimens are generally unmounted and difficult to access and study (Imchanitzkaja 2004).

Taxonomic treatment

Scyphiphora hydrophylacea **C.F.Gaertn., Suppl. Carp. 1(2): 91–92, tab. 196, fig. 2. 1806.**

= *Epithinia malayana* Jack, Malayan Misc. 1(5): 12–13. 1820. ≡ *Scyphiphora malayana* (Jack) Bedd., Fl. Sylv. S. India Forester's Man. Bot.: cxxxiv–3, tab. 29., fig. 5. 1874, nom. inval. Type: Singapore. Singapore Island [protologue: "Found in Mangrove swamps on the Island of Singapore."], s.d., *W. Jack s.n.* (lectotype, designated by Wong et al. 2019: 283: L [L 0001344]).

= *Psychotria philippensis* Cham. & Schltdl., Linnaea 4(1): 21–22. 1829a. ≡ *Uragoga philippensis* (Cham. & Schltdl.) Kuntze, Revis. Gen. Pl. 2: 962. 1891. Type: Philippines. Luzon, Calabarzon Region, Cavite Province: Noveleta [protologue: "Legimus in maritimis circa Tierra-alta Luçoniae."], 1817–1818, *L. K. A. von Chamisso s.n.* (type, B†). Neotype: Philippines, Palawan Province: Culion Island, August 1913, *L. Escritor s.n.* in *Merrill: Species Blancoanae 635* (neotype, here designated: US [00624079]; isoneotypes: L [L.2962064], P [P03972577], W [W 0131765]).

= *Ixora manila* Blanco, Fl. Filip.: 60–61. 1837. Type: Philippines. Palawan Province: Culion Island, August 1913, *L. Escritor s.n.* in *Merrill: Species Blancoanae 635* (first-step neotype, designated by Merrill 1918: 364; second-step neotype, here designated: US [00624079]; isoneotypes: L [L.2962064], P [P03972577], W [W 0131765]).

= *Hydnophytum costatum* Drake, J. Bot. (Morot) 9: 240–241. 1895. Type: Vietnam. Quảng Ninh Province: Surroundings of Quảng Yên [protologue: "Environs de Quang-Yen, au milieu des palétuviers (685)."], August 1885, *B. Balansa 685* (lectotype, designated by Wong et al. 2019: 283: P [P00836559]).

Type. Indonesia. Java, Jawa Barat: Anyer [protologue: "*Hydrophylax*. Collect. Banks."], 2. Oct. 1770, *unknown collector* in *J. Banks s.n.* (lectotype, here designated: BM [BM000945301]).

The type of *Psychotria philippensis*

Psychotria philippensis was published in a series on the botanical results of the Romanzoffian expedition on the Rurik prepared by Chamisso and Schlechtendal (1829a). The protologue gives "in maritimis circa Tierra-alta Luçoniae" as a rather minimalistic collection locality. Fortunately, more detailed information can be derived from the diaries and travelogues of Chamisso (1836a, b) and Kotzebue (1821). According to them, the Rurik stayed in the port of Cavite from 18 December 1817 to 28 January 1818 for refurbishments, the most important Philippine fortress and an arsenal of the Spaniards at that time. Knowing that Cavite had little to offer a botanist, Chamisso quickly settled in Tierra Alta, then a small village on the high shore of Manila Bay, where the sandy headland of Cavite joins the adjacent mainland.

Chamisso noted that the lush forests around Tierra Alta extend from the mountains to the coast, where "*Rhizophora* and other trees reach into the sea" (Chamisso 1836b: 118). He spent most of his time in the Philippines here and roamed the area around Tierra Alta, where he also collected the type specimen of *Psychotria philippensis* (Chamisso and Schlechtendal 1829a). Chamisso first passed through Tierra Alta on 27 December 1817, when he travelled overland from Manila back to Cavite. The French nobleman Don San Jago de Echaparre offered him hospitality there, and he returned to work in Tierra Alta a few days later. He stayed there until 12 January 1818, when he first left for an eight-day expedition to Taal Lake and Volcano in the interior, returning to Cavite shortly thereafter.

The type collection of *Psychotria philippensis* (apparently mentioned by him as one of the "other trees" in the remark quoted above) can thus be dated and localized to the mangroves around Tierra Alta and a period of about two weeks. According to information on the neotype, US 00624079, *Scyphiphora hydrophylacea* was already extinct in the entire Manila Bay region by around 1913, where it was once widespread (Blanco 1837: 60–61, as *Ixora manila*). This is even more tragic as Blanco reported that the native Tagalog names he attributed to the species, *nilad* and *manilad* or *may-nilad*, places where *nilad* is abundant, ultimately led to the name Manila. This notion is, however, rejected by modern authors (e.g., Baumgartner 1975).

After the destruction of B, where Schumann and Merrill had seen the only known type specimen, there should have been additional original material of *Psychotria philippensis* found in HAL and LE, which hold the largest extant parts of Chamisso's herbarium (see above). However, no specimens are currently known in either of these herbaria (HAL: Braun and Wittig 2003; LE: Larisa Orlova, personal comment) or other collections (JSTOR Global Plants database, http://plants.jstor.org; accessed February 2023). Searches in the herbarium of the Natural History Museum, Vienna (W), where three Chamisso specimens of *Psychotria* recently resurfaced in the extensive private herbarium of Stephan Ladislaus Endlicher (1804–1849), professor of botany and director of the Botanical Garden and Botanical Museum of Vienna, were also unsuccessful (Berger 2018; see also Bräuchler et al. 2021).

Therefore, the name *Psychotria philippensis* is neotypified here, fixing the application of the name after nearly two centuries of uncertainty (ICN, Art. 9.8, 9.13; Turland et al. 2018). The specimen designated as neotype was collected by Leonicia Escritor on Culion Island, Palawan, and is no. 635 of the exsiccatae series "Species Blancoanae" issued by Merrill. In this series, he distributed selected specimens that he considered particularly characteristic of the species published by Francisco Manuel Blanco (1778–1845) in the first two editions of his "Flora de Filipinas" (Blanco 1837, 1845) and the third edition edited by Andrés Náves (Blanco 1877). Blanco did not preserve specimens, and the interpretation of some of his names remained problematic until Merrill (1918) published his critical revision "Species Blancoanae" after a thorough study of the publication. In 1916, he issued a corresponding set of "illustrative specimens" for each of Blanco's names as he understood them.

For example, Merrill (1918: 364) identified *Ixora manila* (Blanco 1837) as the previously published *Scyphiphora hydrophylacea* and distributed the specimens mentioned above to clarify the application of the name *Ixora manila* (see also Naves and Fernández-Villar 1880: 109, tab. 277). Thus, he anticipated the concept of the neotype, and his "illustrative specimens" can be understood today as first-step neotypifications of the respective names. In a subsequent second-step neotypification the designation may be narrowed to a single specimen (ICN, Art. 9.17, Turland et al. 2018; see also Nicolson and Arculus 2001). Indeed, many of Blanco's names were later neotypified with specimens from Merrill's "Species Blancoanae" (e.g., Nicolson and Arculus 2001).

According to a letter preserved at the United States National Herbarium (US), Merrill prepared 15 sets of his "Species Blancoanae", each with 1046 specimens, and sent the first to US. Furthermore, he stated that the set at US was the only set that included original data such as field labels or notes and typewritten drafts of the treatments in the "Species Blancoanae". According to Nicolson and Arculus (2001), specimens from these sets are now represented in many herbaria including A, B, BM, BO, CAL, F, GH, K, L, MO, NSW, NY, P, U, UC, US and W. These usually have minimal labels stenciled "Merrill: Species Blancoanae No." in black ink followed by a number stamped in blue ink (Nicolson and Arculus 2001).

Although the above-mentioned gathering "Species Blancoanae" 635 is not from the Manila Bay, it serves as an excellent neotype for *Psychotria philippensis* because the gathering has duplicates in many herbaria and agrees with both Merrill's concept of *Scyphiphora hydrophylacea* and his interpretation of the type of *P. philippensis* as its synonym. Therefore, a specimen of the gathering is here designated as the neotype of *P. philippensis* and the second-step neotype of *Ixora manila*.

The type of *Scyphiphora hydrophylacea*

With the application of *Psychotria philippensis* fixed, some further notes on *Scyphiphora* and *Scyphiphora hydrophylacea* seem useful. As to the type of genus and species, the names were published by the German botanist Carl Friedrich von Gaertner (1772–1850, original German orthography Karl Friedrich von Gärtner) in a "Supplementum" (Gaertner 1806) to his father Joseph Gaertner's (1732–1791, original German orthography Joseph Gärtner) pioneering work on fruit and seed morphology "De fructibus et seminibus plantarum" (Gaertner 1788, 1790–1792). In writing his "Supplement", C. F. Gaertner made extensive use of his father's fruit and seed collection, which was largely based on specimens received from contemporary botanists. One of the most important sources J. Gaertner consulted was Banks's herbarium in London in 1778, and large parts of his 'Carpologia' were based on material that he received on loan or as duplicates through Banks' generosity (Deleuze 1805: 23–24; Stafleu 1969). Following in his father's footsteps, C. F. Gaertner also travelled to London in 1802 with the same aims (Stafleu 1969).

J. Gaertner's collection of fruits and seeds is kept in the herbarium of the University of Tübingen (TUB), as is the herbarium of his son, who added to his father's carpological collection and kept his own herbarium (Stafleu 1969). Yet, there is no extant original material of *Scyphiphora hydrophylacea* in the Gaertner herbarium at TUB (Uta Grünert, personal comment). However, the protologue ("*Hydrophylax*. Collect. Banksian.") indeed gives reference to traveler, naturalist and patron of science, Sir Joseph Banks (1743–1820), whose extensive private herbarium later became the foundation of the herbarium at the Natural History Museum, London (BM). There are a number of specimens and drawings of *S. hydrophylacea* at BM associated with Banks, his herbarium and endeavors.

Two specimens, both mounted together on one sheet, are curated there as types of the name. The sheet is annotated in pencil as type of *Scyphiphora hydrophylacea* with the place of publication of the name. The two specimens are labelled '1' and '2' in black ink on the mounting tape at the base of the shoots. The numbers correspond to meagre annotations of locality and collector on the verso of the sheet in the upper left corner, written in black ink, as was common practice in many herbaria at that time. These annotations are here-interpreted as being in the hand of Samuel Törner, who was employed as amanuensis in the Banks herbarium from 1792 to 1797 (Marshall 1978).

Specimen '1', BM 000945301, mounted on the upper left side of the sheet and annotated on the verso '1. Java prope Angerpoint. J. B.', is a small fragment of a fruiting branch with a single leaf. 'J. B.' refers to Joseph Banks and links the specimen to James Cook's first voyage aboard HMS Endeavour from 1768 to 1771, which is confirmed by a printed label of a later date reading ‘Java 1770–71 Banks & Solander’. The specimen was collected at ‘Angerpoint’, here identified as the present-day coastal town of Anjer or Anyer on Java, a natural harbor and important victualling station during the passage of the Sunda Strait. The diaries and travelogues of Cook and Sydney Parkinson (1745–1771), Banks’ illustrator (Hawkesworth et al. 1773: 705; Parkinson 1773: 171; Cook 1893: 349), reported that a boat was sent ashore at Anger Point on 2 October 1770, to gather supplies, and the specimen was most likely collected then. However, neither Banks nor botanist Daniel Carlsson Solander (1733–1782) went ashore that day, as can be seen from Banks’s travelogue published by Hooker (1896: 363). For that reason, the gathering must have been made by one of the sailors gathering supplies, which probably explains the scrappy condition of the specimen.

Specimen ‘2’, BM 000945302, consists of four flowering branches and is annotated ‘2. Paolo Candor. Dav. Nelson’. It was collected by David Nelson (1740–1789), who participated in Cook’s third voyage aboard HMS Resolution (1776–1780) on behalf of Banks. The modern transcription of ‘Paolo Candor’ is Pulo Condor, now Côn Sơn Island, Vietnam. The gathering can be dated to 21–28 January 1780, based on King’s (1784: 450–464) account of the Resolution’s visit to the island.

Banks and his collaborators had already collected *Scyphiphora hydrophylacea* in flower and fruit in Australia, which the Endeavour expedition visited before returning home via the Dutch East Indies and the Cape of Good Hope (Banks et al. 1901: 46). Based on these gatherings, illustrations were produced under the unpublished name ‘Ixoroides littoralis’, and the corresponding artwork is held in the “Cook First Voyage Artwork Collection” in the library of the Natural History Museum in London. These materials include a first pencil sketch with color notes by Parkinson, made in 1770 somewhere at the mouth of the Endeavour River (plate number A4/169A). After Parkinson died on the return from the voyage in 1771 (see Parkinson 1773), the watercolors were completed by James Miller (plate number A4/169B, A4/169 2) and engraved by Charles White (plate number A4/169C, also water colored A4/169 5), both under the patronage of Banks. The illustration remained unpublished for over a century until a posthumous publication on Banks’s Australian plants from the expedition, edited by James Britten (Banks et al. 1901: 46, tab. 143). Corresponding specimens that could be located are BRI-AQ0450760, P00836560 and W0131767, all received from BM, and the former two are curated as types of *S. hydrophylacea*.

Another notable holding of *Scyphiphora hydrophylacea* material at BM is a long-unpublished watercolor by Ferdinand Bauer, made during the 1801–1803 circumnavigation of Australia on HMS Investigator under Captain Matthew Flinders. The watercolor shows a complete specimen with analysis of flowers and fruits, and was based on gatherings from the Northern Territory, also in the library of the Natural History Museum in London (Botany Library no. 70, Admiralty Library no. 122; see Mabberley and Moore 1999: 122, plate 70).

The materials of the Investigator expedition (1801–1803) were definitely not available at the Banksian herbarium during C. F. Gaertner’s visit in 1802. It is also unlikely that the Australian materials of the Endeavour expedition (1768–1771) were seen by him, although they were surely accessioned in a timely manner after Banks' return. The specimens were gathered under the herbarium name 'Ixoroides littoralis', and there is no reference to that name in the protologue of *Scyphiphora hydrophylacea*, where the material seen by Gaertner in the Banks herbarium is instead referred to the genus *Hydrophylax* L. f. (Rubiaceae: Spermacoceae). The only species currently assigned to that genus, *Hydrophylax maritima*, inhabits sea shores in India, Sri Lanka, Bangladesh and the western Thai part of the Malay peninsula. It has indehiscent fruits that superficially resemble *Scyphiphora* (e.g. Groeninckx et al. 2009; Puff 1986; Puff et al. 2021), which explains the use of the generic name for the undescribed material in the Banksian Herbarium, and also lends its name to the species epithet. Hence, the two specimens curated as types of *Scyphiphora* at BM remain to be considered as original material.

C. F. Gaertner described and illustrated the fruits of *Scyphiphora hydrophylacea*, but made no mention of the flowers (Gaertner 1806: "Cor. … Stam. …") and the vegetative parts as for many other species that he described. Therefore it seems more likely that C. F. Gaertner either received isolated fruits on loan and did not study one or both of the complete specimens, or that the material had already been studied or received on loan by J. Gaertner during his visit in 1778, two years before the collection of Nelson's specimen, and that C. F. Gaertner built upon sketches, manuscripts or other materials inherited from his father. For these reasons, the flowering specimen gathered by Nelson (BM000945302) is not considered original material here, and the single fruiting specimen, BM000945301, is here designated as lectotype of the name *S. hydrophylacea*.

A few years after Gaertner's (1806) publication, the plants were again described as a monotypic genus, *Epithinia*, by Jack, based on one of his gatherings from Singapore (Jack 1820: 12–13). *Epithinia malayana* was first synonymized with *Scyphiphora hydrophylacea* by Korthals (1851: 203–204), and from Miquel (1857: 238–239) and especially Gray (1859: 307), onwards the names were consistently considered synonyms. The combination *S. malayana* (Jack) Bedd. was ascribed to Beddome and considered a valid name in various publications and databases. Although the name indeed first appears in the caption accompanying the respective figure analysis in his rare *Foresters Manual* (Beddome 1874: tab. 29, fig. 5), it is not accepted in the corresponding text (Beddome 1874: cxxxiv–3, using a combination of Roman and Arabic pagination). There, Beddome explains that the name used in the figure should be changed to *S. hydrophylacea* ("Under the name of Scyph. malayana, which should be altered as above."). Apparently, the plates were engraved with the name before the text was finished, and Beddome changed his mind on the identity of the material during the completion of the text, as with few other species in the text. *S. malayana* is therefore not accepted by Beddome and is not valid according to Art. 36.1 (ICN, Turland et al. 2018). Wong et al. (2019: 283) designated the only currently known duplicate of the respective gathering *W. Jack s.n.* at L as lectotype.

Finally, the plants were again described as *Hydnophytum costatum* Drake (Drake del Castillo 1895) and based on *B. Balansa 685* gathered in Vietnam. Again, the single known specimen was designated as lectotype by Wong et al. (2019: 283).

Bibliography of *Scyphiphora*

Selected and more or less useful historic and contemporary information and illustrations on *Scyphiphora hydrophylacea* can be found in chronological order in the following publications, some of them under synonyms added in parentheses:

Gaertner (1806: 91–92, tab. 196, fig. 2), Jack (1820: 12–13, as *Epithinia malayana*), Blume (1826–1827: 955), Chamisso and Schlechtendal (1829a: 21–22, as *Psychotria philippensis*), de Candolle (1830: 477–478, as *E. malayana*, 505–506, as *P. philippensis*, 577), Richard (1830: 79, tab. 4, fig. 1), Wight and Arnott (1834: 423–424, as *E. malayana*), Blanco (1837: 60–61, as *Ixora manila*), Endlicher (1838: 525–526, 545, on the latter page as *E. malayana*), Korthals (1851: 203–204), Griffith (1854a, b: 269–271, tab. 478, as *E. malayana*; 1854b: tab. 644A, is the same figure in different composition but named *Lumnitzera pentandra*, the corresponding text accompanying the figure – 1854a: 684 – apparently describes the actual *Lumnitzera*), Hasskarl (1855: 16–17), Miquel (1857: 238–239), Gray (1859: 307), Bentham and Mueller (1867: 417–418), Beddome (1874: 134-3, tab. 29, fig. 5), Kurz (1877: 4), Hooker (1880: 125), Naves and Fernández-Villar (1880: 109, tab. 277), Vidal (1883a, b: 29, tab. 57, fig. G, 1886: 154), Trimen (1894: 337), Schumann (1897: 80, fig. 29 B, C), Banks et al. (1901: 46, tab. 143), Koorders and Valeton (1902: 124–127), King and Gamble (1904: 227–228), Elmer (1906: 32), Koorders (1912: 258), Koorders and Valeton (1915: fig. 563), Merrill (1918: 364, 1923b: 533), Pitard (1922–1924: 280–282, tab. 23, figs 5–8), Ridley (1923: 88–89), Watson (1928: 84–87, tab. 43), White (1929), Valeton (1930: 303), Corner (1940: 559), Henderson (1950: 217–218, fig. 196), Bakhuizen van den Brink (1955: 101–102, 1975: 34–35), Backer and Bakhuizen van den Brink (1965: 316), Wong (1988: 197), Wong (1989: 408), Keng (1990: 161), Hộ (1993: 206, fig. 7607), Puff and Rohrhofer (1993, richly illustrated), Turner (1995: 446), Ridsdale (1998: 235), Mabberley and Moore (1999: 122, pl. 70), Banerjee et al. (2002: 262), Puff et al. (2005: 82, pl. 3.1.19), Tao et al. (2011: 323) and Wong et al. (2019: 282–285, fig. 71).

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RESEARCH ARTICLE

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A new species of *Sedum* (Crassulaceae) from Mount Danxia in Guangdong, China

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Abstract

Sedum jinglanii, a new species of Crassulaceae from Mount Danxia in Guangdong, China, is described and illustrated. Phylogenetic analysis based on the internal transcribed spacer (ITS) region of *nr*DNA suggests that the new species belongs to *S.* sect. *Sedum* sensu Fu and Ohba (2001) in the "Flora of China", and is sister to a clade comprising *S. alfredi* and *S. emarginatum* with high support values (SH-aLRT = 84, UFBS = 95) but is distantly related to *S. baileyi*. The new species is morphologically similar to *S. alfredi* but it can be distinguished from the latter in its opposite leaves (vs. alternate leaves), its usually wider leaves (0.4–1.2 cm vs. 0.2–0.6 cm), its usually shorter petals (3.4–4.5 mm vs. 4–6 mm), its shorter nectar scales (0.4–0.5 mm vs. 0.5–1 mm), its shorter carpels (1.5–2.6 mm vs. 4–5 mm), and its shorter styles (0.6–0.9 mm vs. 1–2 mm). The new species can be easily distinguished from *S. emarginatum* which both have opposite leaves by its short, erect or ascending rhizome (vs. long and prostrate rhizome in the latter), shorter petals (3.4–4.5 mm vs. 6–8 mm) and shorter carpels (1.5–2.6 mm vs. 4–5 mm). It can also be easily distinguished from *S. baileyi* by its short, erect or ascending rhizome (vs. long and prostrate rhizome) and its shorter style (0.6–0.9 mm vs. 1–1.5 mm).

Keywords

Danxia landscape, morphology, nrITS, *Sedum* sect. *Sedum*

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[*] These authors contributed equally to this work.

Introduction

According to Fu and Ohba (2001) in the "Flora of China", *Sedum* Linnaeus is the most species-rich genus of the family Crassulaceae, comprising about 470 species. However, as presently circumscribed, the genus is highly polyphyletic, and a monophyletic circumscribed genus *Sedum* s.l. would comprise approximately 755 species by inclusion of all 14 genera currently recognized in tribe Sedeae into it (Messerschmid et al. 2020). The genus is distributed in temperate and subtropical environments, and the diversity center is in the Mediterranean Sea, Central America, the Himalayas and East Asia (Stephenson 1994; Thiede and Eggli 2007). In China, 121 *Sedum* species are documented, amongst which 91 species are endemic (Fu and Ohba 2001).

During the past 20 years, about seventeen *Sedum* species have been newly described from China, including *S. hoi* X.F.Jin & B.Y.Ding (Wang et al. 2005), *S. plumbizincicola* X.H.Guo & S.B.Zhou (Wu et al. 2012), *S. fanjingshanense* C.D.Yang & X.Yu Wang (Yang et al. 2012), *S. kuntsunianum* X.F.Jin, S.H.Jin & B.Y.Ding (Jin et al. 2013), *S. tarokoense* H.W.Lin & J.C.Wang (Lu et al. 2013), *S. spiralifolium* D.Q.Wang, D.M.Xie & L.Q.Huang (Xie et al. 2014), *S. peltatum* M.L.Chen & X.H.Cao (Chen et al. 2017), *S. kwanwuense* H.W.Lin, J.C.Wang & C.T.Lu and *S. taiwanalpinum* H.W.Lin, J.C.Wang & C.T.Lu (Lu et al. 2019), *S. ichangense* Y.B.Wang (Wang and Xiong 2019), *S. lipingense* R.B.Zhang, D.Tan & R.X.Wei (Zhang et al. 2019), *S. nanlingense* Yan Liu & C.Y.Zou (Zou et al. 2020), *S. cirenianum* S.S.Ying, *S. shaoakouense* S.S.Ying and *S. shengkuangense* S.S.Ying (Ying 2022a), and *S. parviflorum* S.S.Ying and *S. tachingshuianum* S.S.Ying (Ying 2022b).

Molecular data unambiguously demonstrate the polyphyletic nature of *Sedum* with its species placed in four major crown clades of the crassulacean tree, for example, *Acre*, *Aeonium*, *Leucosedum*, and *Sempervivum*. There is no agreement between specialists regarding the infrageneric structure of *Sedum* (reviewed in Nikulin et al. 2016). According to Fu and Ohba (2001), Chinese *Sedum* are divided into three sections, including *S.* sect. *Sedum*, *S.* sect. *Oreades* (Fröderström) K.T. Fu, and *S.* sect. *Filipes* (Fröderström) S.H. Fu. *S.* sect. *Sedum* is distinguishable from the two latter sections by the adaxially gibbous carpels and follicles, while *S.* sect. *Oreades* differs from *S.* sect. *Filipes* in the spurred leaf base and yellow petals (vs. spurless leaf base and white or reddish-purple petals) (Fu and Ohba 2001). During our investigations in Danxiashan National Park, Guangdong Province, China, an unknown *Sedum* species with opposite leaves was collected. After several years of field observations, comprehensive literature studies and molecular analysis, we confirmed that it was a new species and it is described and illustrated here.

Materials and method

Field investigations and observations were conducted during the flowering and fruiting periods of the putative new species. We obtained morphological data of this putative species by measurements based on 6–8 living samples. Mean values of these statistical data were calculated and then were compared with six other related species (Table 2). The specimens were collected in Danxiashan National Park, Renhua County, Guangdong Province, China. Voucher specimens were deposited in the herbarium of Sun Yat-sen University (**SYS**).

Two representative individuals from different populations were selected for further molecular experiments, one from Bazhai of Mount Danxia (*Y. S. Huang 21040301*) and another one from Yanyan of Mount Danxia (*Q. Fan et al. DNPC 2873*). Fresh leaves of the two individuals were collected and stored with silica gel in zip-lock plastic bags until use. Total DNA was extracted using the modified CTAB method (Doyle and Doyle 1987). The region of the partial internal transcribed spacer 1, 5.8S ribosomal RNA gene and partial internal transcribed spacer 2 was amplified using previously reported primers ITS1 and ITS4 (White et al. 1990). PCR amplifications were performed following Huang et al. (2021).

In order to explore the phylogenetic position of the putative new species in *Sedum*, ITS sequences of 56 accessions representing 46 *Sedum* taxa and three outgroup species (*Aeonium lancerottense*, *Aeonium viscatum*, and *Greenovia aizoon*) were downloaded from the Genbank public database at the National Center for Biotechnology Information (NCBI) (Table 1). The sequences were aligned using ClustalW 1.8 (Thompson et al. 1994) and then adjusted manually. Besides, to improve the credibility, we also aligned the sequences using MAFFT v.7.402 (Katoh and Standley 2013), and the alignments generated from the two methods were consistent. The best-fit nucleotide substitution model was determined by ModelFinder (Kalyaanamoorthy et al. 2017). Based on the maximum likelihood (ML) method, the phylogenetic tree was constructed using IQ-Tree v. 2.0.3 (Nguyen et al. 2015) by executing 5,000 replicates of SH approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap (UFBS) (Hoang et al. 2018). Finally, the tree file was visualized by the online tool of Interactive Tree Of Life (iTOL) v5 (Letunic and Bork 2021).

Results and discussion

The alignment length of the ITS sequences was 624 bp, amongst which 340 were parsimony-informative. Within the new species, only one variable site was detected, but 40 variable sites were detected between the new species and *S. alfredi* and 40 variable sites were detected between the new species and *S. emarginatum*, indicating that pronounced genetic differentiation existed between the new species and *S. alfredi* as well as *S. emarginatum*. The best-fit nucleotide substitution model was estimated as SYM+I+G4 according to the Bayesian Information Criterion (BIC).

As the ML phylogenetic tree shows (Fig. 1), seven subclades were resolved with moderate to high support values. Accessions of the putative new species, *S. alfredi*, *S. emarginatum*, and *S. lungtsuanense* together formed subclade 7 with high support values (SH-aLRT = 92, UFBS = 98), all belonging to *S.* sect. *Sedum* sensu Fu and Ohba (2001).

Table 1. Taxa, voucher information, and GenBank accession numbers of the sequences used in this study.

Taxon	Voucher	Accession numbers	References
Sedum Sect. *Oreades*			
S. oreades	*Rao 090803-03*	KF113733	Zhang et al. 2014
S. trullipetalum	*Miyamoto et al. 9420132*	AB088630	Mayuzumi and Ohba 2004
S. bergeri	*Ni et al.*	AY352897	Ni et al. unpublished
S. erici-magnusii	*Ito 2077*	LC229235	Ito et al. 2017a
Sedum Sect. *Sedum*			
S. jinglanii	*Huang 21040301*	OP288035	This study
	Fan et al. DNPC 2873	OQ162326	This study
S. actinocarpum	*Ito 1749*	LC229265	Ito et al. 2017a
S. alfredi	*Kokubugata 17190*	AB930259	Ito et al. 2014a
	Kokubugata 17191	AB930260	Ito et al. 2014a
	Kokubugata 17192	AB930261	Ito et al. 2014a
	WUK415208	FJ919953	Wang and Shu unpublished
S. baileyi	*LBG0064555*	FJ919935	Wang and Shu unpublished
S. bulbiferum	*Ito 416*	LC229234	Ito et al. 2017a
	130514hs41	KM111166	Xie et al. 2014
	130524qz09	KM111165	Xie et al. 2014
S. emarginatum	*130512hs27*	KM111145	Xie et al. 2014
S. erythrospermum	*Tsutsumi 1504*	AB906473	Ito et al. 2014b
S. formosanum	*Ito 1260*	LC229279	Ito et al. 2017a
S. hakonense	*Mayuzumi C00005*	AB088625	Mayuzumi and Ohba 2004
S. hangzhouense	*Ito 2604*	LC260130	Ito et al. 2017b
S. japonicum	*Kokubugata 16749*	AB906475	Ito et al. 2014b
S. senanense	*Ito 2200*	LC229238	Ito et al. 2017a
S. oryzifolium	*Ito 2285*	LC229239	Ito et al. 2017a
S. japonicum var. *pumilum*	*Ito 2287*	LC229240	Ito et al. 2017a
S. japonicum ssp. *uniflorum*	*Ito 447*	LC229241	Ito et al. 2017a
S. boninense	*Ito 2371*	LC229242	Ito et al. 2017a
S. jiulungshanense	*Ito 76*	LC229243	Ito et al. 2017a
S. kiangnanense	*CMQ1030*	LC229244	Ito et al. 2017a
S. lineare	*Mayuzumi C00120*	AB088623	Mayuzumi and Ohba 2004
S. lungtsuanense	*Ito 3563*	LC260131	Ito et al. 2017b
S. makinoi	*Kokubugata 16730*	AB906476	Ito et al. 2014b
S. morrisonense	*Ito 2765*	LC229290	Ito et al. 2017a
S. multicaule	*Miyamoto et al. TI9596136*	AB088631	Mayuzumi and Ohba 2004
S. nagasakianum	*Ito 2064*	LC229249	Ito et al. 2017a
S. nokoense	*Kokubugata 10426*	AB906478	Ito et al. 2014b
S. oligospermum	*Ito 74*	LC229250	Ito et al. 2017a
S. yabeanum	*Ito 396*	AB906490	Ito et al. 2014b
S. polytrichoides var. *setouchiense*	*Ito 2298*	LC229253	Ito et al. 2017a
S. polytrichoides	*CMQ1057*	LC229251	Ito et al. 2017a
S. rupifragum	*Ito 2070*	LC229254	Ito et al. 2017a
S. sarmentosum	*Ito 978*	LC229255	Ito et al. 2017a
S. satumense	*Ito 2295*	LC229256	Ito et al. 2017a
S. subtile	*Shimizu 1999*	AB088622	Mayuzumi and Ohba 2004
	Ito 2259	LC229257	Ito et al. 2017a
S. subtile	*Ito 624*	AB930277	Ito et al. 2014a
S. taiwanianum	*Ito 2770*	LC229297	Ito et al. 2017a
S. tetractinum	*Ito 3623*	LC260135	Ito et al. 2017b
S. tianmushanense	*Ito 355*	LC229261	Ito et al. 2017a
S. tosaense	*Kokubugata 16726*	AB906483	Ito et al. 2014b
S. triactina	*9596091*	AB088629	Mayuzumi and Ohba 2004
S. tricarpum	*Ito 2269*	LC229259	Ito et al. 2017a
S. lipingense[*]	*ZRB1479*	MN150061	Zhang et al. 2019
S. mexicanum[*]	*Ito 647*	LC229247	Ito et al. 2017a
S. truncatistigmum[*]	*Ito 3254*	LC229306	Ito et al. 2017a

Taxon	Voucher	Accession numbers	References
*S. zentaro-tashiroi**	*Ohba 1998*	AB088619	Mayuzumi and Ohba 2004
Outgroups			
Aeonium lancerottense	*Mort 1518*	AY082143	Mort et al. 2002
Aeonium viscatum	*Mort 1432*	AY082154	Mort et al. 2002
Greenovia aizoon	*Mort 1425*	AY082112	Mort et al. 2002

*Not recorded in Fu and Ohba (2001).

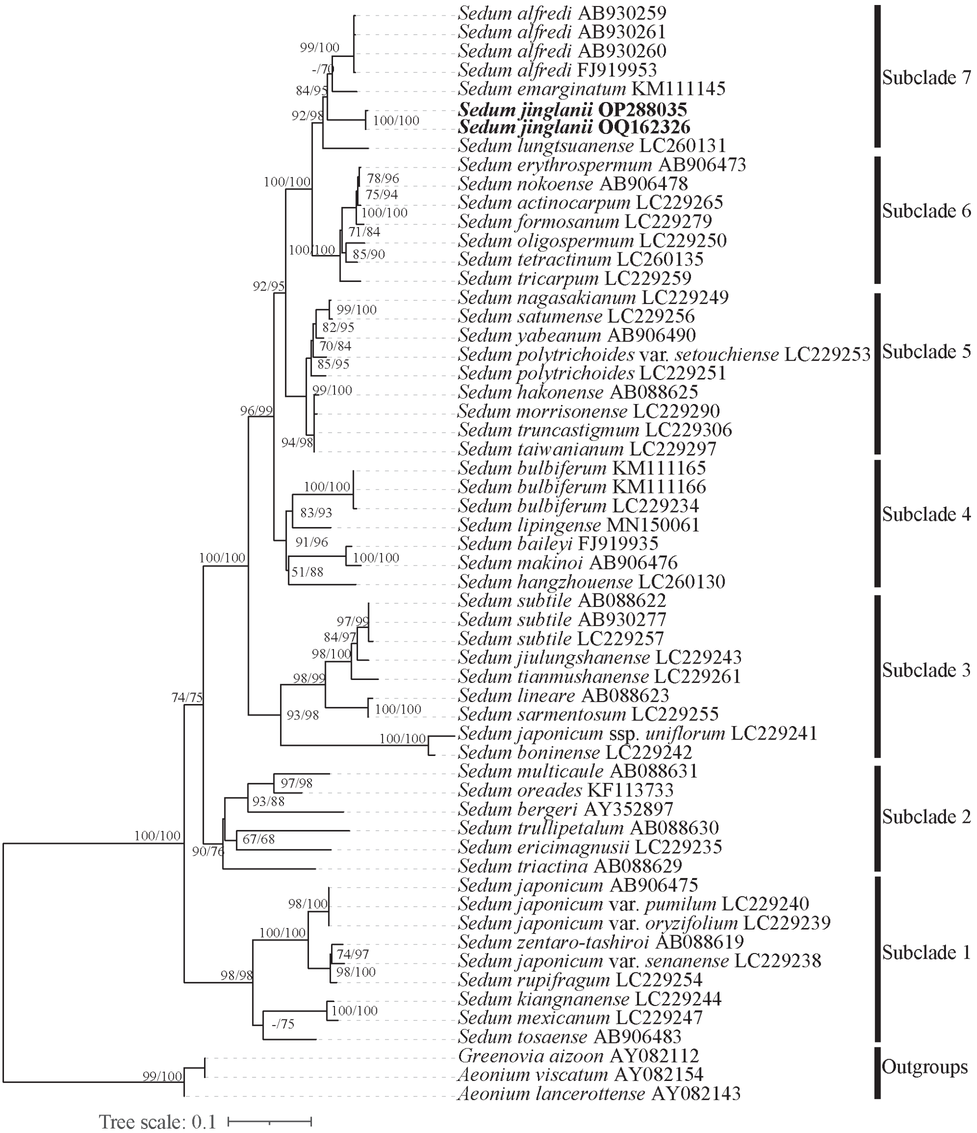

Figure 1. Maximum Likelihood tree based on ITS sequences for Eastern Asian species of *Sedum* sect. *Sedum*, four species of *S.* sect. *Oreades*, and three outgroups. Numbers near the branches are SH approximate likelihood ratio test (SH-aLRT) and ultrafast bootstrap (UFBS) support values. The new species is highlighted in bold.

Morphologically, the putative new species is similar to *S. alfredi* from which it can be easily distinguished by its opposite leaves (vs. alternate leaves in the latter). Furthermore, the leaves of the putative new species are usually wider than those of *S. alfredi* (0.4–1.2 mm vs. 0.2–0.6 mm), the petals are usually shorter (3.4–4.5 mm vs. 4–6 mm), the nectar scales shorter (0.4–0.5 mm vs. 0.5–1 mm), the carpels shorter (1.5–2.6 mm vs. 4–5 mm) and the styles shorter (0.6–0.9 mm vs. 1–2 mm) (Table 2). Phylogenetically, the putative new species is closely related to *S. emarginatum*. Although the leaves of both species are opposite, it can be easily distinguished from the latter by its short, erect or ascending rhizome (vs. long and prostrate rhizome), shorter petals (3.4–4.5 mm vs. 6–8 mm) and shorter carpels (1.5–2.6 mm vs. 4–5 mm). The putative new species was distantly related to *S. baileyi* in the phylogenetic tree although both are morphologically similar (Table 2). Also, it can be easily distinguished from the latter by its short, erect or ascending rhizome (vs. long and prostrate rhizome) and its shorter style (0.6–0.9 mm vs. 1–1.5 mm).

Additionally, four representatives of *Sedum* sect. *Oreades* sensu Fu and Ohba (2001) (*S. oreades*, *S. trullipetalum*, *S. bergeri*, and *S. erici-magnusii*) were also included in our analysis. However, these four species were nested within species belonging to *S.* sect. *Sedum* sensu Fu and Ohba (2001), thus showing that *S.* sect. *Sedum* might not be monophyletic. This result is consistent with previous studies (Nikulin et al. 2016; Zhang et al. 2019; Messerschmid et al. 2020).

Through numerous scientific investigations, more than a dozen new species were found on Mount Danxia in Guangdong in recent years, and most are endemic to it such as *Lespedeza danxiaensis* Q.Fan, W.Y.Zhao & K.W.Jiang (Zhao et al. 2021), *Asplenium danxiaense* K.W.Xu (Xu et al. 2022), *Pilea danxiaensis* L.F.Fu, A.K.Monro & Y.G.Wei (Fu et al. 2022), *Wikstroemia fragrans* W.B.Liao, Q.Fan & J.R.Chen (Chen et al. 2022), and *Commelina danxiaensis* Q.Fan, Long Y.Wang & W.Guo (Wang et al. 2023). As a World Heritage site and tourist attraction, Danxia landform possesses special and complicated habitat differences at a small scale, which might contribute to the plant endemism at Mount Danxia (Peng et al. 2018).

Taxonomic treatment

Sedum jinglanii **Yan S.Huang & Q.Fan, sp. nov.**

urn:lsid:ipni.org:names:77315511-1
景兰景天

Type. China. Guangdong Province, Renhua County, Mount Danxia, Bazhai, in the cliff of steep slopes, 25°00'N, 113°39'E, 520 m a.s.l., 3 April 2021, *Y. S. Huang 21040301* (holotype: SYS; isotype: SYS) (Figs 2, 3).

Diagnosis. This new species is similar to *S. alfredi*, but differs from the latter in its opposite leaves (vs. alternate leaves), its usually wider leaves (0.8–2.9 × 0.4–1.2 cm vs. 1.2–3.0 × 0.2–0.6 cm), usually shorter petals (3.4–4.5 mm vs. 4–6 mm), shorter nectar scales (0.4–0.5 mm vs. ca. 0.5–1 mm), and shorter carpels (1.5–2.6 mm vs.

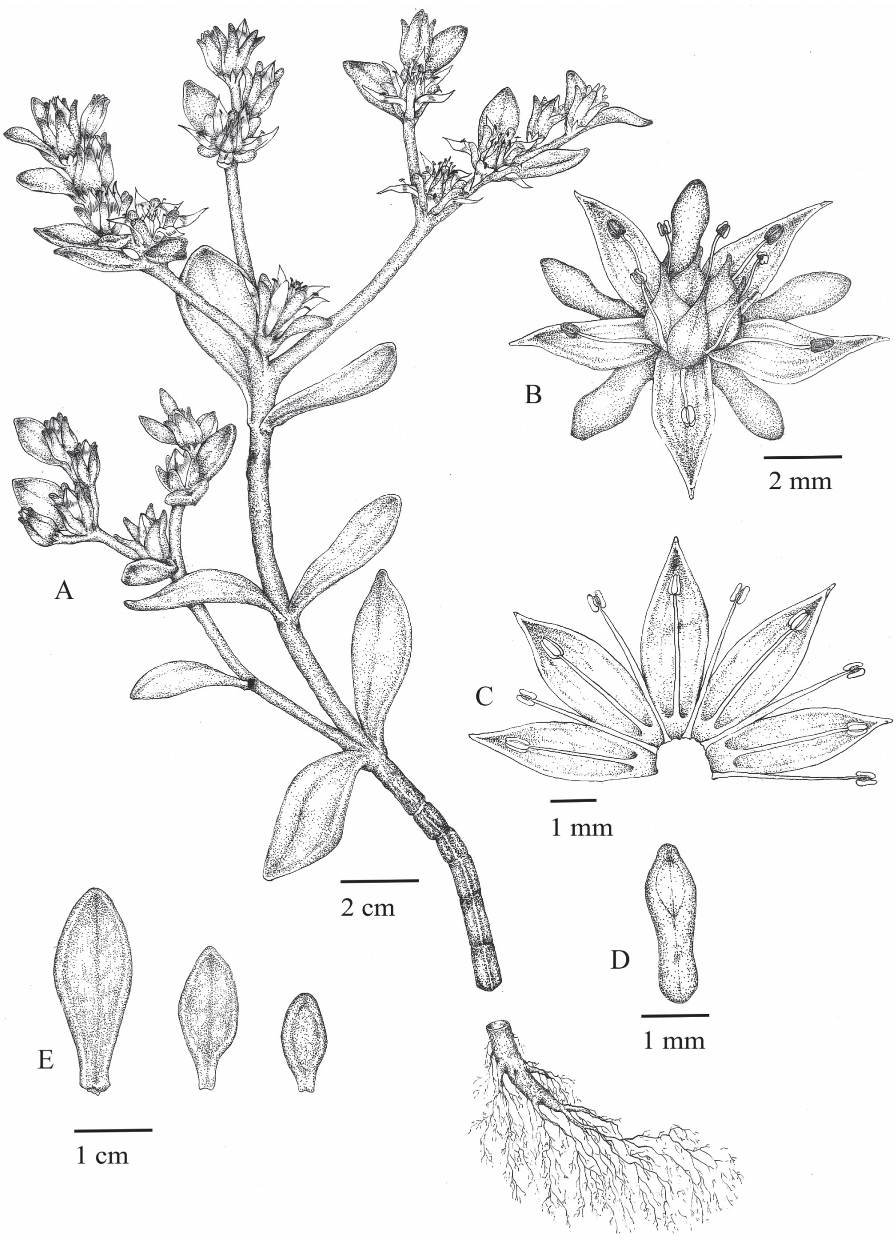

Figure 2. *Sedum jinglanii* sp. nov. **A** habit **B** flower with sepals, petals, stamens and carpels **C** petals and stamens **D** sepal **E** leaves. Illustration by Yuan-Yuan Sun based on living field-collected material (*Y. S. Huang 21040301*).

4–5 mm). Although the leaves of this new species and of *S. emarginatum* are opposite, it can be easily distinguished from the latter by its short, erect or ascending rhizomes (vs. long and prostrate rhizomes), shorter petals (3.4–4.5 mm vs. 6–8 mm) and shorter carpels (1.5–2.6 mm vs. 4–5 mm).

Table 2. Morphological comparisons between *S. jinglanii*, *S. alfredi*, *S. baileyi*, *S. emarginatum*, *S. kuntsunianum*, *S. makinoi*, and *S. satumense*.

Characters	***S. jinglanii***	***S. alfredi***[†]	***S. baileyi***[‡]	***S. emarginatum***[§]	***S. kuntsunianum***[|]	***S. makinoi***[¶]	***S. satumense***[#]
Leaf blade	Spatulate or obovate	Linear-cuneate, spatulate or obovate	Obovate-spatulate	Spatulate-obovate to broadly obovate	Widely obovate or suborbiculate, spatulate	Obovate or obovate-spatulate	Narrowly obovate or spatulate
Leaf size (cm)	0.8–2.9 × 0.4–1.2	1.2–3.0 × 0.2–0.6	1–2.5 × 0.6–0.8	1–2.5 × 0.5–1.2	1.4–2.0 × 0.9–1.5	1–2 × 0.6–0.8	1.0–2.2 × 0.6–0.9
Phyllotaxy	Opposite	Alternate	Opposite	Opposite	Opposite, or rarely alternate at base	Opposite	Opposite
Rhizome	Short, erect or ascending	Short, erect or ascending	Long, prostrate	Long, prostrate	Absent	Short, erect or ascending	Short, erect or ascending
Sepal length (mm)	2–3.1	2–5	1.5–2.5	2–5	5–9	2–3	6–7
Petal length (mm)	3.4–4.5	4–6	4–5	6–8	7–8	4–5	7–8
Stamen length (antepetalous) (mm)	2.2–2.6	2.5–3.5	2–3	3–4	ca. 5	2.5–3.2	–
Stamen length (antesepalous) (mm)	3.2–3.3	3.8–4.5	3–4	4–5	ca. 6	2.8–4.5	–
Nectar scale length (mm)	0.4–0.5	0.5–1	0.4–0.6	0.6–0.8	ca. 0.5	0.5–0.7	ca. 0.5
Carpel length (mm)	1.5–2.6	4–5	2–3	4–5	ca. 5	4–5	–
Style length (mm)	0.6–0.9	1–2	1–1.5	1.5–2	ca. 1	1–2	1.0–1.5

[†,‡,§,¶] Based on Fu and Ohba (2001) and own measurements at IBSC and SYS; [|]Jin et al. (2013); [#] Ohba (2003).

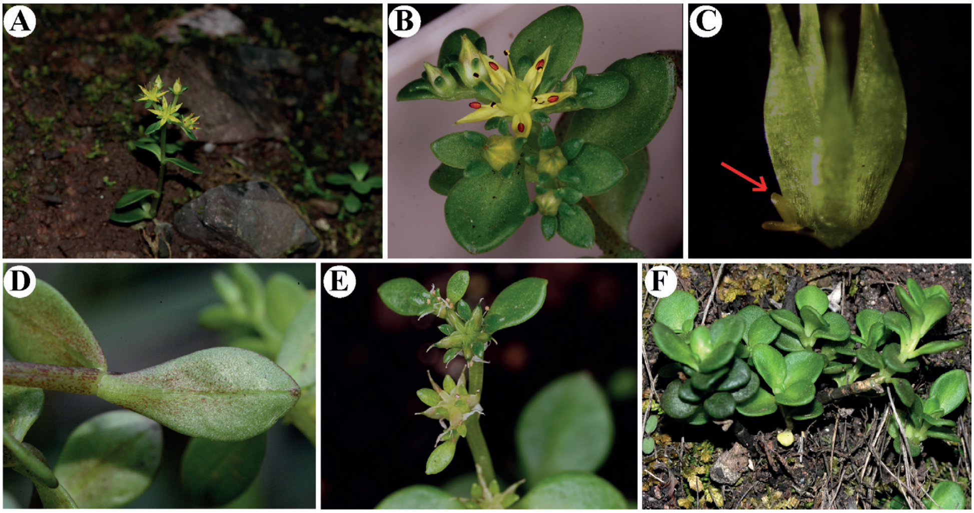

Figure 3. *Sedum jinglanii* sp. nov. **A** habit **B** flower, front view, showing brownish-red anthers **C** carpels and a nectar scale (red arrow) **D** abaxial leaf surface **E** young unripe fruits **F** young sterile individuals **A, B, D** photographed by Qiang Fan in the field (Pingtouzhai, 6 April 2022, *Q. Fan et al.*, *DNPC 1953*) **C** photographed by Min Lin in the lab (7 April 2022, *Q. Fan et al.*, *DNPC 1953*) **E, F** photographed by Yan-Shuang Huang (Bazhai, 3 April 2021, *Y. S. Huang 21040301*).

Description. Fleshy herbs, perennial; stems glabrous, greenish, often with small reddish dots thus appearing more or less reddish, ascending; leaves opposite, usually deciduous, crowded distally on the stem, succulent; leaf blade spatulate or obovate, 8–29 mm long, 4–12 mm wide, base narrowly cuneate and spurred, apex obtuse and sometimes emarginate; inflorescence in dense terminal cymes, usually two to four branched; bracts leaflike, 1.7–2.4 mm long, 0.7–1.1 mm wide; flowers usually sessile, rarely with short pedicels to 0.8 mm long, unequally 5-merous; sepals green, linear-spatulate, 2–3.1 mm × 0.7–1.4 mm, base shortly spurred; petals yellow, lanceolate to lanceolate-oblong, 3.4–4.5 mm × 0.8–1.1 mm, base connate for 0.1–0.2 mm, apex mucronate; stamens 10, yellow, filiform, arranged in 2 whorls; antesepalous ones 3.2–3.3 mm, antepetalous ones 2.2–2.6 mm; anthers brownish red, long ellipsoid. Nectar scales yellow green, spatulate-quadrangular, 0.4–0.5 × 0.2–0.3 mm, apex obtusely truncate. Carpels yellow green, erect, ovoid-lanceolate, 1.5–2.6 mm long, 0.6–0.9 mm wide, adaxially gibbous, base shortly connate; styles 0.6–0.9 mm long. Follicles yellowish, obliquely divergent. Seeds numerous, brown, oblong, 0.5–0.6 mm, papillate.

Phenology. Flowering from April to May. Fruiting from June to August.

Etymology. *Sedum jinglanii* is named after Prof. Jing-Lan Feng (1898–1976), an academician of the Chinese Academy of Sciences and one of the founders of mineralogy in China. In 1928, he discovered and named the red beds and related strata in North Guangdong as “Danxia Formation” for the first time (Peng 2020).

Distribution and habitat. Presently, this new species is only known from the type locality, Mount Danxia, Renhua County, Guangdong Province, China. It grows on the cliff of steep slopes at altitudes of 200–550 m a.s.l.

Conservation status. Only five populations were found with no more than 1,000 mature individuals. Thus, the conservation status could be considered as Vulnerable (VU; D1), according to the IUCN Red List Criteria (IUCN Standards and Petitions Subcommittee 2022).

Additional specimens examined (paratypes). China. Guangdong: Renhua County, Mount Danxia, Pingtouzhai, 25°00'N, 113°37'E, 536 m a.s.l., 6 April 2022, *Q. Fan et al.*, *DNPC 1953* (SYS); Renhua County, Mount Danxia, Yanyan, 25°02'N, 113°61'E, 263 m a.s.l., 27 December 2022, *Q. Fan et al. DNPC 2873* (SYS).

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RESEARCH ARTICLE

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Kengiochloa, a new bamboo genus to accommodate the morphologically unique species, *Pseudosasa pubiflora* (Poaceae)

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Abstract

Pseudosasa was confirmed as polyphyletic by recent phylogenetic analyses, with Chinese species of *Pseudosasa* distantly related to those from Japan. Among the Chinese species of *Pseudosasa*, *Pseudosasa pubiflora* is a morphologically unique as well as taxonomically problematic species endemic to South China, of which the generic designation is still uncertain. Molecular analyses based on both plastid and nuclear genomic data demonstrated that this species is closest to the recently published genus *Sinosasa*. Morphologically, the two are somewhat similar to each other in flowering branches developing at the nodes of every order of branches, raceme-like units of inflorescence with 3–5 short spikelets, each spikelet with few florets including a rudimentary one at the apex, and each floret with 3 stamens and 2 stigmas. However, *P. pubiflora* is very different from *Sinosasa* species in many reproductive and vegetative characters, such as the morphology of paracladia (lateral spikelet "pedicels"), the absence or existence of pulvinus at the base of paracladia, the relative length of the upper glume and the lowest lemma, the shape of lodicules and primary culm buds, the branch complement, the morphology of nodes, culm leaves and dried foliage leaf blades, and the number of foliage leaves per ultimate branchlet. The morphological and molecular evidence warrants recognition of a new genus to accommodate this unique species, which is here named *Kengiochloa*. After consulting related literature and examination of herbarium specimens or specimen photos, a taxonomic revision of *K. pubiflora* and its synonyms was made, and it was confirmed that four names, viz. *P. gracilis*, *Yushania lanshanensis*, *Arundinaria tenuivagina* and *P. parilis*, should be merged with *K. pubiflora*, while *Indocalamus pallidiflorus* and *Acidosasa paucifolia* are distinct species.

Keywords
morphology, phylogeny, plastome, synonyms, taxonomy

Introduction

Pseudosasa Makino ex Nakai, with ca. 20 species mainly distributed in East Asia and Vietnam, belongs to the subtribe Arundinariinae of the tribe Arundinarieae (Poaceae: Bambusoideae) (Zhang et al. 2020; Soreng et al. 2022; POWO 2022). Many molecular phylogenetic studies have shown that this genus is polyphyletic, as the Japanese species including the generic type, *P. japonica* (Siebold & Zucc. ex Steud.) Makino ex Nakai, and those from China are always distributed in different clades in the phylogenetic trees (Triplett and Clark 2010; Zhang et al. 2012; Guo et al. 2021; Triplett and Clark 2021). Morphologically, the two groups of species are also different: *P. japonica* has persistent culm leaf sheaths, a solitary branch at each node, or sometimes three branches at the top nodes of the culm, and three to five stamens (which also suggest that *P. japonica* has a hybrid origin between Japanese *Pleioblastus* Nakai and *Sasa* Makino & Shibata or *Sasamorpha* Nakai, as indicated by Triplett and Clark (2010, 2021)), while Chinese *Pseudosasa* species usually have deciduous culm leaf sheaths, one to three branches per node, and three stamens (Chen et al. 1996; Zhu et al. 2006; Zhang et al. 2012; Kobayashi 2017). Among those sampled *Pseudosasa* species, one species endemic to South China, viz. *P. gracilis* S. L. Chen & G. Y. Sheng, drew the attention of many researchers. This species was consistently associated with some Chinese *Sasa* species (which were recently transferred to the newly established genus, *Sinosasa* L. C. Chia ex N. H. Xia et al., see Qin et al. 2021), and far related with other *Pseudosasa* species in previous studies (Zhang et al. 2012; Guo et al. 2021) (Fig. 1). Zhang et al. (2012) argued that *P. gracilis* was not a typical member of *Pseudosasa* in terms of morphology, and suggested that more work was required to clarify the ascription of this species and its phylogenetic placement. In Flora of China, Zhu et al. (2006) considered that *P. gracilis* was possibly a synonym of *P. pubiflora* (Keng) Keng f. ex D. Z. Li & L. M. Gao, which they thought may not belong to *Pseudosasa* but *Indocalamus* Nakai.

Pseudosasa pubiflora has a relatively complicated taxonomic history. In 1936, Yi-Li Keng, the first Chinese botanist who studied the bamboo classification in China, described *Arundinaria pubiflora* Keng from northern Guangdong based on only one collection (*To & Tsang 12284*) with flowers (Keng 1936). His son, Pai-Chieh Keng then transferred it to *Pseudosasa* Makino ex Nakai without giving any reason (Keng 1957 & 1959). However, the new combination was invalid due to lack of a clear and direct reference to the basionym (Zhu et al. 2006). Yang and Chao (1994) recognized this species in *Arundinaria* Michx.., and considered several other names, including *P. gracilis*, *Indocalamus pallidiflorus* McClure, *Yushania lanshanensis* T. H. Wen and *Acidosasa paucifolia* W. T. Lin as well as several combinations based on these names, as synonyms of this species.

The *P. pubiflora* published in Flora of China (Zhu et al. 2006) was a combination largely based on Yang and Chao's (1994) treatment, except that *P. gracilis* was suggested as "possibly a synonym of *P. pubiflora*". Besides, two more names, viz.

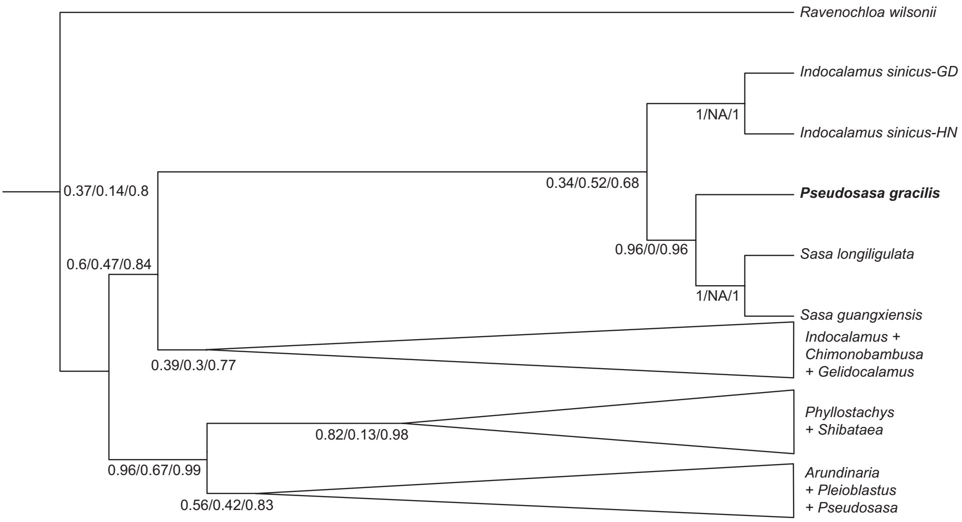

Figure 1. Phylogenetic position of *Pseudosasa gracilis*, adapted from Guo et al. 2021.

Arundinaria teuivagina W. T. Lin and *P. parilis* T. P. Yi & D. H. Hu, were further synonymized with *P. pubiflora*. Consequently, the distribution of this species was extended to include three provinces, i.e., Guangdong, Hunan and Jiangxi. Moreover, Zhu et al. (2006) argued that, for *P. pubiflora*, “the woolly internode apex is unusual in *Pseudosasa* and rather suggestive of *Indocalamus* Nakai”. Thus, the relationships between *P. pubiflora* and its synonyms and the generic designation of this species need a further study.

Materials and methods

Field trips to the type locality of *P. pubiflora* as well as its several synonyms including *I. pallidiflorus*, *Arundinaria teuivagina* and *P. parilis* were conducted from 2018 to 2022. The complete specimens including rhizomes, culms with culm leaves, branches and foliage leaves as well as fresh young foliage leaves used for molecular analyses were collected. The types and other specimens of the pertinent species housed at CANT, IBSC, N, SYS and ZJFI, as well as photographs of specimens housed at K, US and W, were examined. Herbaria acronyms follow Thiers (2022). The morphological description is based on specimens and referred to related literature (Keng 1936; Chen et al. 1983; Wen 1985, 1986; Lin and Wu 1990; Yi 1995; Chen et al. 1996; Zhu et al. 2006). General morphological terms follow Beentje (2016). The terms applied to the flowering structure in this study mainly follow the synflorescence concept applied to grasses (Vegetti and Anton 1996; Tivano et al. 2009; Cai and Xia 2021).

To study the phylogenetic position of *P. pubiflora* within the tribe Arundinarieae, the whole chloroplast genomes were used for building the phylogenetic tree. Five *Pseudosasa* species including one from Japan, also the type species of the genus, viz. *P. japonica*, and four from China were sampled. In total, there were 29 species represented by 30 samples of all the five subtribes in the tribe Arundinarieae (two samples for *P. pubiflora*) and one sample of *Bambusa bambos* (L.) Voss from the tribe Bambuseae was the outgroup. Species names, voucher information and GenBank accession numbers are provided in Table 1.

Total genomic DNA was isolated from silica gel-dried leaves using TIANGEN Genomic DNA Extraction Kit (TIANGEN, Beijing, China). The extracted genomic DNA was fragmented randomly by Covaris M220 (Covaris, Woburn, MA), and the fragments with insert size of 350 bp were selected by using AxyPrep Mag PCR Clean Up Kit. The selected fragments were enriched by PCR after undergoing end repair, the addition of polyA tail and adaptor ligation. The paired-end (2 × 150 bp) libraries were constructed on NovaSeq 6000 platform. Finally, 20 Gb genome skimming data were generated for each sample.

Table 1. Species names, voucher information and GenBank accession numbers of the 30 whole chloroplast genomes used in this study.

Taxon	Voucher information	GenBank accession
Ingroup		
Acidosasa glauca B. M. Yang	CZY56 (IBSC)	*OP850353
Ampelocalamus actinotrichus (Merr. & Chun) S. L. Chen T. H. Wen & G. Y. Sheng	MPF10003 (KUN)	MF066245
Chimonobambusa quadrangularis (Franceschi) Makino	Not provided by the author	MW928533
Ferrocalamus rimosivaginus T. H. Wen	Zhang08019 (KUN)	HQ337794
Ferrocalamus strictus Hsueh & Keng f.	NH001 (IBSC)	*OP850355
Gaoligongshania megalothyrsa (Hand.-Mazz.) D. Z. Li, Hsueh & N. H. Xia	MPF10056 (KUN)	JX513419
Gelidocalamus stellatus T. H. Wen	BH102 (IBSC)	*OP850347
Gelidocalamus tessellatus T. H. Wen & C. C. Chang	MPF10049 (KUN)	JX513420
Hsuehochloa calcarea (C. D. Chu & C. S. Chao) D. Z. Li & Y. X. Zhang	MPF10050 (KUN)	KJ496369
Indocalamus longiauritus Hand.-Mazz.	MPF10168 (KUN)	HQ337795
Indocalamus latifolius (Keng) McClure	CZY76 (IBSC)	*OP850354
Indocalamus sinicus (Hance) Nakai	ZMY037 (KUN)	MF066250
Indosasa crassiflora McClure	BH58 (IBSC)	*OK558536
Phyllostachys nidularia f. *farcta* H. R. Zhao & A. T. Liu	2020-JZ01 (NF)	LC590826
Pleioblastus chino (Franch. & Sav.) Makino	NH029 (IBSC)	*OP850356
Pseudosasa amabilis (McClure) Keng f.	NH032 (IBSC)	*OP850358
Pseudosasa cantorii (Munro) Keng f.	MPF10006 (KUN)	MF066255
Pseudosasa japonica (Siebold & Zucc. ex Steud.) Makino ex Nakai	Pjc-1 (ZJFC)	KT428377
Pseudosasa nanunica (McClure) Z. P. Wang & G. H. Ye	XNH36 (IBSC)	*OP850361
Pseudosasa pubiflora (Keng) Keng f. ex D. Z. Li & L. M. Gao	CZY146 (IBSC)	*OP850350
Pseudosasa pubiflora (Keng) Keng f. ex D. Z. Li & L. M. Gao	XNH63 (IBSC)	*OP850362
Shibataea chinensis Nakai	NH036 (IBSC)	*OP850359
Sinobambusa tootsik (Makino) Makino ex Nakai	NH031 (IBSC)	*OP850357
Sinosasa fanjingshanensis N. H. Xia, Q. M. Qin & J. B. Ni	BH124 (IBSC)	*OP850348
Sinosasa sp.	CZY173 (IBSC)	*OP850352
Sinosasa longiligulata (McClure) N. H. Xia, Q. M. Qin & J. B. Ni	CZY163 (IBSC)	*OP850351
Ravenochloa wilsonii (Rendle) D. Z. Li & Y. X. Zhang	MPF10146 (KUN)	JX513421
Oligostachyum sulcatum Z. P. Wang & G. H. Ye	CZY113 (IBSC)	*OP850349
Yushania basihirsuta (McClure) Z. P. Wang & G. H. Ye	XNH144 (IBSC)	*OP850360
Outgroup		
Bambusa bambos (L.) Voss	B1-1 (ISC)	KJ870988

Note: Asterisk (*) indicates the DNA data obtained in this study.

After quality control of 20 Gb clean data by Trimmomatic v 0.39 (Bolger et al. 2014), the whole chloroplast (cp) genomes were assembled using the software GetOrganelle v 1.7.4 pipeline (Jin et al. 2018), with the reference cp genome sequence of *Ampelocalamus sinovietnamensis* (MW525255) and K-mer sizes of 45, 65, 85, 105 and 125. The generated whole cp genome was annotated using the program Plastid Genome Annotator (Qu et al. 2019) based on the annotation of *A. sinovietnamensis*. The transferred RNAs (tRNAs) were adjusted using the software tRNAscan-SE service (Schattner et al. 2005). The initiation and termination codons of all the coding genes were manually verified in Geneious Prime v 9.1.4 (Kearse et al. 2012).

All the cp genomes were aligned using MAFFT v. 7.450 (Katoh and Standley 2013). Maximum Likelihood (ML) analysis was conducted by RAxML v 8.2.12 (Stamatakis 2014) with the rapid bootstrap algorithm. GTRGAMMAI was selected as the best-fit model recommended by jModelTest v2.1.6 (Darriba et al. 2012). The number of 12345 was specified as the random seed of parsimony tree inference with 1000 replicates performed. For Bayesian Inference (BI), the data matrix was calculated using MrBayes v3.2.2 software (Ronquist et al. 2012). The best-fit model was selected as SYM+G under the Akaike information criterion (AIC) using MrModeltest v 2.3 (Nylander 2004). Rates of variations across sites were trimmed as gamma. For each analysis, two simultaneous runs of four Monte Carlo Markov Chains (three heated and one cold) were run for 20 million generations with a random tree as the starting point and saving trees every 1000 generations. After discarding the first 25% samples as burn-ins, the optimized topology was generated. The final results were visualized with Figtree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The chloroplast genomes ranged from 139,093 bp (*Pleioblastus chino* Makino) to 140,064 bp (*Gaoligongshania megalothyrsa* (Hand.-Mazz.) D. Z. Li, Hsueh & N. H. Xia) with an alignment length of 144,771 bp. The plastid data matrix was characterized by sequence divergence with 9,751 variable sites (6.74%), including 6,477 parsimony informative sites (4.47%) and 3,274 singleton variable sites (2.27%).

The reconstructed phylogenetic tree is shown in Fig. 2. Similar to some previous analyses on plastid sequence data (Triplett and Clark 2010; Zhang et al. 2012), *Pseudosasa* was resolved as polyphyletic in this analysis. *Pseudosasa pubiflora* was sister to the clade of *Sinosasa* with strong support (maximum likelihood bootstrap value/ Bayesian inference posterior probability BS/PP = 100%/1.00). The other *Pseudosasa* taxa form a clade with five other genera of the subtribe Arundinariinae, namely *Oligostachyum* Z. P. Wang & G. H. Ye, *Acidosasa* B. M. Yang, *Sinobambusa* Makino, *Indosasa* Maclure and *Pleioblastus* (MLBS/PP = 100%/1.00).

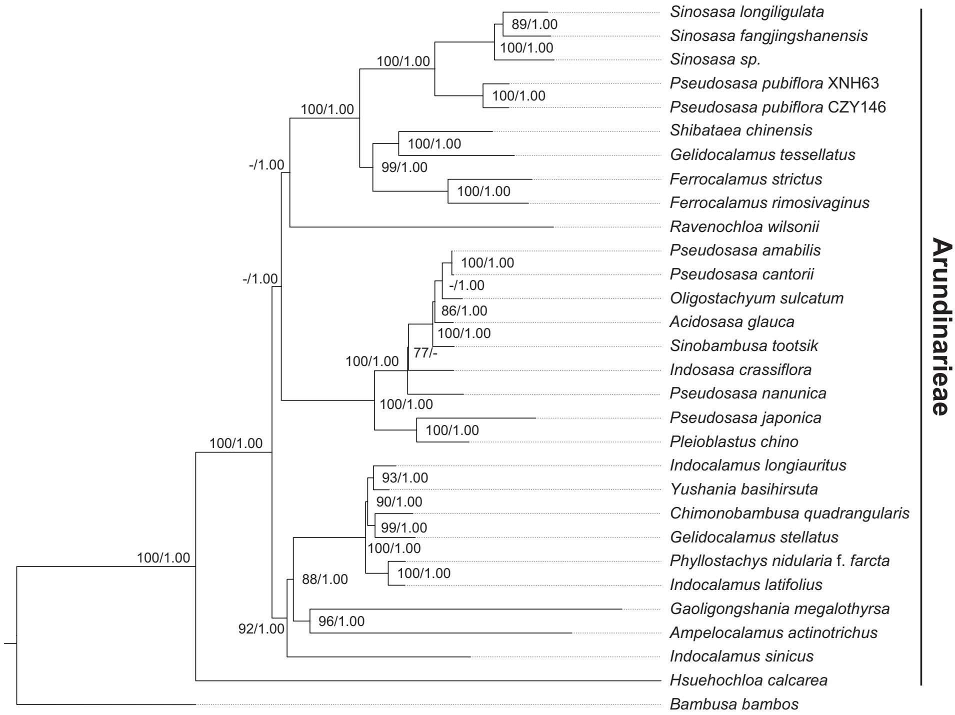

Figure 2. Phylogeny of Arundinarieae based on whole chloroplast genome derived from maximum likelihood and Bayesian analyses. Only bootstrap values (BS) ≥ 70% and posterior probabilities (PP) ≥ 0.95 are indicated at each node.

Discussion

Taxonomic revision of *Pseudosasa pubiflora* and its synonyms

After consulting related literature and examining the types and other herbarium specimens or specimen photographs, we agree with Yang and Chao (1994) that *P. gracilis*, *Yushania lanshanensis* should be merged into *P. pubiflora*, and with Zhu et al. (2006) that *Arundinaria teuivagina* and *P. parilis* are also synonyms of *P. pubiflora*, since all these species share the same key diagnostic characters, mainly including the leptomorph rhizome, the pluricaespitose, short (< 2 m) and thin (< 0.8 mm) culm (Fig. 3C), the flat node, the narrowly ovate culm buds (Fig. 4B), the branch complement with two to four branches at each mid-culm node, the persistent papery culm leaf sheath with fragile oral setae that are adnate at base (Fig. 3E), the erect and amplexicaul culm leaf blades that are usually longer than sheath (Fig. 3D), and the ultimate branchlet with one to four lanceolate or narrowly lanceolate foliage leaves (Fig. 3B). Among these characters, its oral setae and culm leaf blade are very special in Arundinarieae.

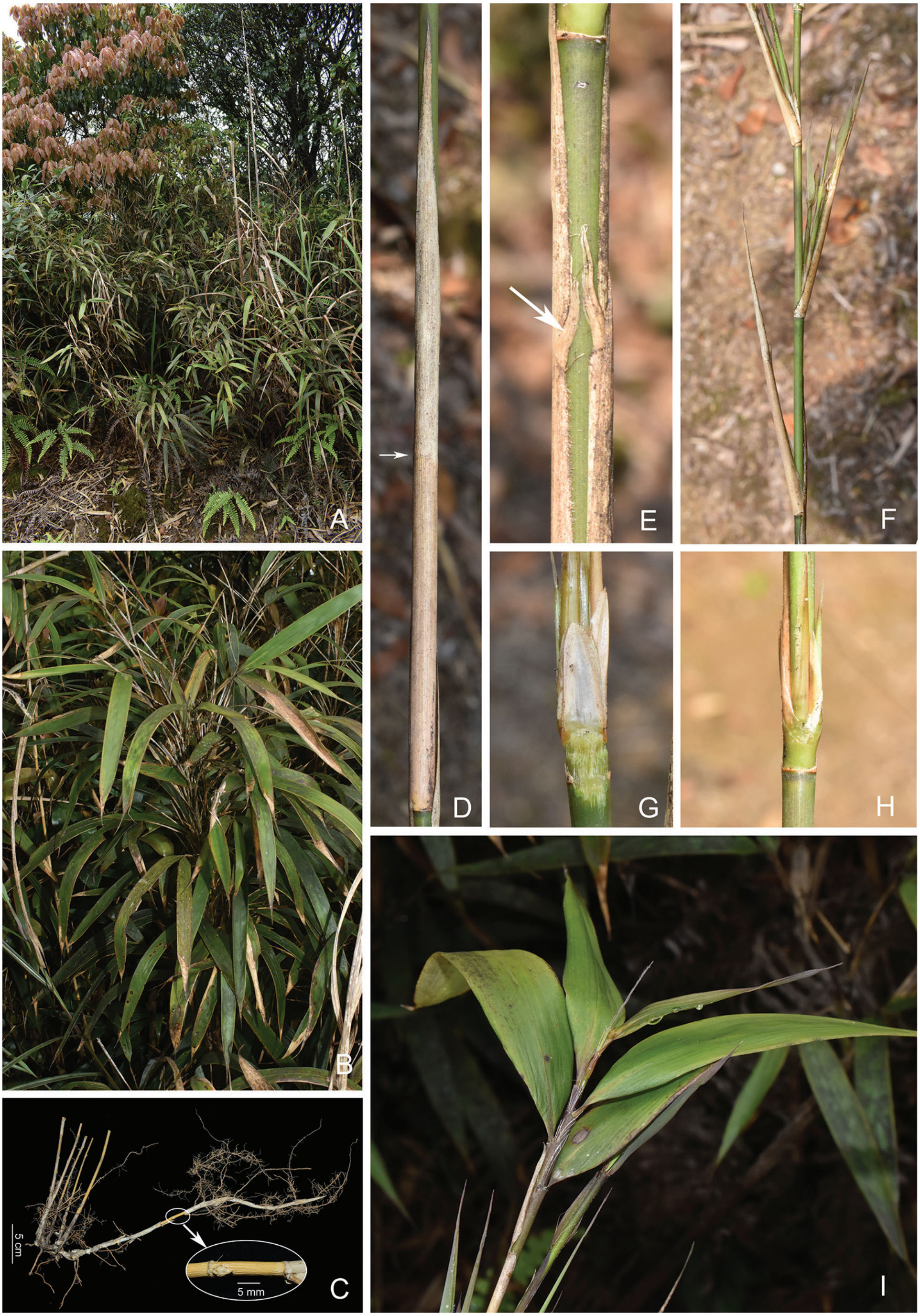

Figure 3. *Kengiochloa pubiflora* **A** habitat **B** habit **C** leptomorph rhizome and pluricaespitose culms, the arrow showing an internode with a bud **D** culm leaf, the arrow showing the junction of sheath and blade **E** culm leaf oral setae **F** intravaginal branching **G** prophyll of primary branch **H** three young branches at a node **I** foliage leaf branch. Photos by Y. H. Tong.

However, we do not agree that *I. pallidiflorus* and *Acidosasa paucifolia* are identical to *P. pubiflora*. *Indocalamus pallidiflorus* (≡ *Pseudosasa pallidiflora* (McClure) S. L. Chen & G. Y. Sheng) markedly differs from *P. pubiflora* in the woolly (vs. glabrous) infranodal region, the culm leaf sheath oral setae present (vs. absent) and the culm leaf blade much shorter (vs. usually longer) than sheath. Actually, the description of *P. pubiflora* in Flora of China is mainly based on that of *I. pallidiflorus* made by McClure (1940), which conflicts a lot with the protologue of *P. pubiflora* (Keng 1936). We cannot find the type specimens of *Acidosasa paucifolia* despite an exhaustive search in CANT where they should be preserved, as indicated by the author (Lin 1992). According to the description and illustration in the protologue (Lin 1992), this species significantly differs from *P. pubiflora* by the deciduous culm leaf with abaxially more or less hispid (vs. usually glabrous) sheaths and much longer ligules (2 mm vs less than 0.5 mm) and without (vs. with) oral setae, the absence (vs. existence) of foliage leaf oral setae and much longer foliage leaf inner ligules (3 mm vs. 0.3–1.5 mm). Thus, this species should not be synonymized with *P. pubiflora* either. The identities of these two taxa need further study.

Generic designation of *Pseudosasa pubiflora*

Recent research has proved that the formerly recognized *Arundinaria* in a broad sense should not be adopted any more, which at present is thought to be a small genus with only three species restricted to North America (Zhang et al. 2012, 2016, 2020; Guo et al. 2021; Soreng et al. 2022). Thus, *Pseudosasa pubiflora* should not be placed into *Arundinaria*. This species has many characteristics that are very unusual for *Pseudosasa*, such as the lanceolate (vs. narrowly terete or linear in other *Pseudosasa* species, the same below) and rather short (1.6–2 cm long vs. usually much longer and up to 20 cm) spikelets with robust and erect (vs. slender and porrect) pedicels and very few florets (2–4 vs. 3–30), each floret with two (vs. three) stigmas, the papery (vs. leathery) culm leaf sheath and the glabrous and nonpowdery (vs. conspicuously powdery) infranodal regions (Table 2). The phylogenetic analyses of both our study based on the chloroplast genomes and Guo et al. (2021) based on nuclear markers from ddRAD data demonstrated that this species had a distant relationship with other *Pseudosasa*. Thus, *P. pubiflora* should not be a member of *Pseudosasa*.

Interestingly, both of the two phylogenetic studies suggested a close relationship between *Pseudosasa pubiflora* and *Sinosasa*. In the analysis of Guo et al. (2021), *Indocalamus sinicus* (Hance) Nakai is closest to the *Pseudosasa pubiflora* + *Sinosasa* clade (called "*Pseudosasa gracilis* + *Sasa* subg. *Sasa*" clade (Fig. 1) in that paper) with a strong support. Morphologically, this small clade is characterized by having persistent papery culm leaf sheath, short (less than 2.5 cm) and lanceolate spikelet with few florets (2–4) including a terminate rudimentary one and each floret with two stigmas in the subtribe Arundinariinae. However, the flowering branch of *Indocalamus sinicus* is terminal on culm or foliage leaf branches with a large and panicle-like unit of inflorescence composed of many spikelets, while the flowering branches of the other two develop at the nodes of every order of branches with a raceme-like unit of inflorescence composed of only 3–5 spikelets. The culm buds of *Indocalamus sinicus* are also very special; they are triangular with lower half completely adnate to the culm (Fig. 4A).

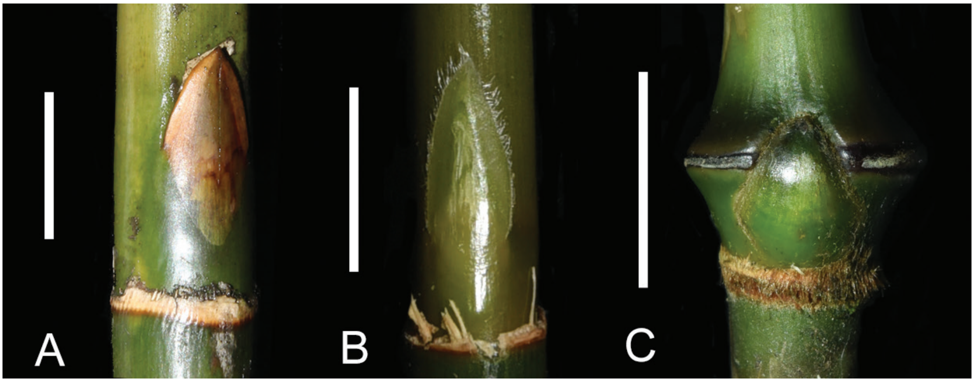

Figure 4. Culm buds of three bamboos **A** *Indocalamus sinicus* **B** *Kengiochloa pubiflora* **C** *Sinosasa* sp. Photo **A** by Xi-Rong Zheng, **B, C** by Zhuo-Yu Cai. Scale bars: 1 cm (**A, C**); 5 mm (**B**).

Last, there are so many different vegetative and productive characters between *Pseudosasa pubiflora* and *Sinosasa* that they cannot belong to the same genus. Specifically, *Pseudosasa pubiflora* differs from *Sinosasa* in having narrowly ovate culm buds (vs. trullate) (Fig. 4), branch complement with 2–4 branches at mid-culm nodes (vs. solitary), flat nodes (vs. prominent with a strongly raised supranodal ridge), infranodal region being glabrous and nonpowdery (vs. with a sericeous or villous band), culm leaf with oral setae adnate at base (vs. distinct) and amplexicaul blade being usually longer than sheath (vs. not amplexicaul and much shorter than sheath), 1–4 foliage leaves per ultimate branchlet (vs. 5–17) with blades flat when dry (vs. wavy) and much shorter inner ligule (0.3–1.5 mm vs. (8–)10–20 mm), robust and erect (vs. slender and porrect to reflexed) paracladia being appressed to the axis and without (vs. with) pulvinus at base, upper glumes typically shorter than (vs. nearly equal to) the lowest lemma and lodicules with a rounded (vs. acute) apex (Detailed comparison is provided in Table 2). In conclusion, both the morphological and molecular phylogenetic evidence strongly supports the recognition of a new genus to accommodate *Pseudosasa pubiflora*.

Taxonomic treatment

Kengiochloa Y.H.Tong & N.H.Xia, gen. nov.

urn:lsid:ipni.org:names:77315562-1

Type. *Kengiochloa pubiflora* (Keng) Y. H. Tong & N. H. Xia (≡ *Arundinaria pubiflora* Keng).

Diagnosis. This new genus is close to *Sinosasa*, but differs in having narrowly ovate culm buds, branch complement with 2–4 branches at mid-culm nodes, flat nodes, glabrous and nonpowdery infranodal region, culm leaf oral setae adnate at base, amplexicaul culm leaf blades that are usually longer than sheath, 1–4 foliage leaves per ultimate branchlet with blades flat when dry and short inner ligule, robust and erect paracladia (lateral spikelet pedicels) appressed to the axis and without pulvinus at base, upper lemma typically shorter than the lowest lemma and lodicules with a rounded apex.

Table 2. Comparison of key morphological characters of *Pseudosasa pubiflora*, *Sinosasa*, *Indocalamus sinicus* and *Pseudosasa*. The characters unique to *P. pubiflora* among these taxa are indicated in bold.

	P. pubiflora	***Sinosasa***	***Indocalamus sinicus***	***Pseudosasa***
Culm bud	**Narrowly ovate**	Trullate	Triangle, lower half adnate to culm	Trullate to narrowly trullate
Branches at mid-culm nodes	2 to 4	Solitary	Solitary	1 to 3
Nodes	Flat	Prominent, supranodal ridge strongly raised	Flat	Flat or weakly prominent
Infranodal region	Glabrous, nonpowdery	With a sericeous or villous band	Glabrous, nonpowdery	Powdery
Culm leaf sheath	Persistent, papery	Persistent, papery	Persistent, leathery-papery	Persistent, usually leathery
Culm leaf oral setae	**Adnate at base**	Distinct	Distinct or absent	Distinct or absent
Culm leaf blades	Erect, amplexicaul, **usually longer than sheath**	Erect or slightly spreading, not amplexicaul, much shorter than sheath	Erect or reflexed, not amplexicaul, narrowly triangular-lanceolate, much shorter than sheath	Erect or reflexed, amplexicaul or not, narrowly triangular to strap-shaped, shorter than or as long as sheath
Number of foliage leaves per ultimate branchlet	1 to 4	5 to 17	7 to 14	2 to 10
Inner ligule	Very short, 0.3–1.5 mm	Long, (8–)10–20 mm	Short, 2–3 mm	Short to long, 1–5 mm
Foliage leaf blades	Flat when dry	Wavy when dry	Flat when dry	Flat when dry
Flowering branch	Developing at the nodes of every order of branches	Developing at the nodes of every order of branches or culm	Terminal on culm or foliage leaf branches	Developing at the nodes of every order of branches or culm
The unit of inflorescence of the synflorescence	Raceme-like with 3–5 spikelets	Raceme-like with 3–5 spikelets	Panicle-like with many spikelets	Raceme or panicle-like with several to many spikelets
Paracladia	**Robust and erect, appressed to the axis**	Slender, porrect to reflexed	Slender, porrect	Slender, porrect
Pulvinus at the base of pracladia	Absent	Present	Present	Absent
Spikelet	Lanceolate, 1.6–2 cm long	Lanceolate, 0.5–1.5 cm long	Lanceolate, 1.5–2.5 cm long	Narrowly terete or linear, 2.5–20 cm long
Number of floret per spikelet	2–4	2–3	3–4	3–30
Terminate rudimentary floret	Exist	Exist	Exist	Exist or not
The relative length of upper glume and the lowest lemma	Upper glume shorter than the lowest lemma	Upper glume nearly equal to the lowest lemma	Upper glume shorter than the lowest lemma	Upper glume shorter than the lowest lemma
Lodicule	Oblong-obovate, spatulate or obovate, apex rounded	Ovate or narrow oblong, apex acute	Ovate to oblong, apex obtuse	Oblong, obovate-lanceolate, spathulate or lanceolate, apex acute or obtuse
Stigma number	2	2	2	3 to 5

Description. Shrubby bamboo. Rhizomes leptomorph. Culms pluricaespitose, short and thin, less than 2 m tall and 8 mm in diam.; nodes flat. Culm buds narrowly ovate. Branches intravaginal, 2–4 at each mid-culm node. Culm leaf sheath persistent, papery; auricles absent; oral setae fragile, adnate at base; blade erect and amplexicaul, usually longer than sheath; ligule convex, short. Foliage leaves 1–4 per ultimate branchlet; auricles obscure; inner ligule short. Flowering branches developing at the nodes of every order of branches; the unit of inflorescence of synflorescence raceme-like with 3–5 spikelets; paracladia robust and erect, appressed to the axis and without pulvinus at base; florets 2–4 per spikelet including a rudimentary one at the apex; glumes 2, upper one shorter than the lowest lemma; lemma longer than palea; palea 2-keeled; lodicules 3, apex rounded; stamens 3, anthers pale yellow; styles 2, base slightly connate; stigmas 2, plumose. Caryopsis unknown.

Etymology. *Kengiochloa* is named in honor of Professor Yi-Li Keng (1897–1975), a renowned botanist and the first Chinese who studied the bamboo taxonomy in China. The type species of this genus was first described by him, too. Its Chinese name is given as 以礼竹属 (*pinyin*: yǐ lǐ zhú shǔ).

***Kengiochloa pubiflora* (Keng) Y.H.Tong & N.H.Xia, comb. nov.**
urn:lsid:ipni.org:names:77315564-1

≡ *Arundinaria pubiflora* Keng, Sinensia 7: 414, fig. 4. 1936; Yang & Chao, J. Bamb. Res. 13(1): 5. 1994, excl. syn. of *Indocalamus pallidiflorus*. ≡ *Pseudosasa pubiflora* (Keng) Keng f. ex D. Z. Li & L. M. Gao in Zhu et al., Fl. China 22: 118. 2006, excl. descr. and syn. of *Indocalamus pallidiflorus* and *Acidosasa paucifolia*; Keng f. in Keng, Clav. Gen. Sp. Gram. Prim. Sin. 154. 1957 & Fl. Ill. Pl. Prim. Sin. 32 & pl. 22. 1959, *nom. inval.* Type: China, Guangdong, on top of the hill at the rear of Iu [Yao] village, Lung Tau Mt. [Longtou Mountain], 30 May 1924, *To & Tsang 12284* (holotype US00130296 (sheet 1) & US00065420 (sheet 2), image!, isotypes US0031360, image!, US00144993, image!, W19390008723, image!, SYS!). Figs 3, 4B; also see fig. 150 in Vol. 22 of Flora of China Illustrations (available at http://www.efloras.org/object_page.aspx?object_id=95963&flora_id=2).

= *Pseudosasa gracilis* S. L. Chen & G. Y. Sheng, Acta Phytotax. Sin. 21: 405. 1983; Chen et al., Fl. Reipubl. Popularis Sin. 9(1): 658. 1996; Zhu et al., Fl. China 22: 119. 2006. Type: China, Hunan, Yizhang County, Zeping, 6 May 1977, *Z. P. Wang, H. R. Zhao & G. Y. Sheng 77004* (holotype NAS, not seen, isotypes N019022078!, N019022079!, N019022080!).

= *Yushania lanshanensis* T. H. Wen, J. Bamboo Res. 4(2): 13. 1985. ≡ *Arundinaria lanshanensis* (T. H. Wen) T. H. Wen, J. Bamboo Res. 5(1): 19. 1986. Type: China, Hunan, Lanshan County, Ziliang Township, 1125 m, 17 June 1984, *S. Q. Chen CX84860* (holotype ZJFI!, isotypes ZJFI!).

= *Arundinaria tenuivagina* W. T. Lin & Z. M. Wu, J. S. China Agric. Univ. 11(3): 48. 1990. Type: China, Guangdong Province, Xinyi City, Dawuling, 1400 m, 12 April 1987, *Z. M. Wu 0167* (holotype CANT00002410!, isotypes CANT00002408!, CANT00002409!, CANT00002411!, CANT00002412!, CANT00002413!, CANT00002414!, CANT00002415!).

= *Pseudosasa parilis* T. P. Yi & D. H. Hu in Yi, J. Bamboo Res. 14(1): 20. 1995. Type: China, Jiangxi, Suichuan County, Dabali, 1400 m, 21 November 1991, *Q. Hu & D. H. Hu 032* (holotype SAUT, not seen, isotype Herbarium of Ji'an Forestry Institution, not seen).

Description. Rhizomes amphipodial, internodes terete, 2–2.5 cm long, ca. 2.5 mm in diam. Culms 1.2–2 m tall, up to 8 mm in diam.; internodes terete, 8–24 cm long, green, not powdery, glabrous; wall thick, cavity with woolly or irregularly lamellate pith; supranodal ridge flat, remains of sheath base persistent, sheath scar pubescent or glabrous. Culm buds narrowly ovate. Branches intravaginal, 2–4 at each mid-culm node, base attached to culm. Culm leaf sheaths gradually deciduous or rather persistent, 1/2 to 4/7 as long as internodes, thickly papery, glabrous or sometimes sparsely brown appressed hispidulous, margins ciliolate; auricles absent; oral setae 5–11, lateral 1–4 curved and downward, others straight forward, 6–9 mm long, fragile, base usually adnate; ligule short, less than 0.5 mm, unevenly laciniate, glabrous; blade erect, amplexicaul, striate, broadly ovate-lanceolate, usually longer than sheath, both surfaces glabrous, margins ciliolate, apex acuminate. Foliage leaves 1 to 4 per ultimate branch; sheath 2–4 cm long, upper half more or less white-pilose abaxially, margins ciliolate, glabrescent; outer ligule ca. 0.5 mm long, margin white-pilose; auricles obscure; oral setae present, to 1.4 cm long; inner ligule short, 0.3–1.5 mm; pseudopetiole 1.5–3 mm long, slightly pilose adaxially, glabrous abaxially; blade lanceolate or narrowly lanceolate, 8–24 × 0.9–2 cm, slightly pilose at base adaxially, hispidulous abaxially, glabrescent, base cuneate, margins serrulate, apex acuminate; lateral veins 4–5 pairs, veinlets conspicuous. The unit of inflorescence of the synflorescence 3–9 cm long; main axis glabrous, flattened on the branching side, basal internode 0.8–1.8 cm long. Paracladia 2–4, 0.4–1 cm long, robust, close to the axis, puberulent or appressed-pubescent at the apex, base without a pulvinus. Spikelets 1.6–2 cm long, stramineous; developed (fertile) florets 1–3, uppermost one rudimentary; rachilla segments thickened upwards, 3–4 mm long, glabrous except the upper part white-tomentose; glumes 2, the lower one lanceolate to narrowly lanceolate, 7–10 mm long, glabrous at lower part, pubescent towards the apex, apex acuminate; the upper one with the same shape and indumentum as the lower one, 7–12 mm long; callus bearded with white- or grey-tomentose hairs; lemmas ovate, the lowest one 10–12 mm long, densely adpressed-pubescent abaxially, 9-veined, apex acuminate-mucronate; palea 7–8 mm long, apex obtuse, glabrous except the ciliate and strongly curved keels; lodicules 3, ca. 2 mm long, anterior 2 oblong-obovate, posterior 1 spatulate or obovate, rounded at apex, lower part brown-nerved, upper part hyaline and minutely ciliate; anthers 3, pale yellow, 5 mm long; ripe ovary 2 mm long, brownish when dry; styles 2, persistent, ca. 1 mm long, base slightly connate, stigmas very thin, plumose, ca. 3 mm long. Fruit unknown. Description of inflorescence follows Keng (1936).

Phenology. Culm shoots produced in April to July, flowering in May.

Distribution and habitat. This species is distributed in Hunan, Jiangxi and Guangdong, China. It grows under evergreen broadleaved forests at an elevation from 1100–1600 m.

Chinese name. 以礼竹 (pinyin: yǐ lǐ zhú).

Additional specimens examined. *Kengiochloa pubiflora*: China. Guangdong: Ruyuan County, Tianjingshan, 4 May 1978, *Z. P. Wang & S. T. Liu 780047* (N019022073, N019022074, N019022075, N019022076); Shixing County, Longtou Moutain, elev. 1131 m, 25 April 2021, *Z. Y. Cai & X. H. Ye czy-146* (IBSC); Xinyi City, Mianbeiding, 20 April 2020, *N. H. Xia*, *Y. H. Tong*, *J. B. Ni*, *S. J. Zeng & B. M. Wang XNH-63* (IBSC). Hunan: Yizhang County, Mang Mountain, elev. 1600 m, 4 July 1964, *B. M. Yang 026501* (N019022077). Jiangxi: Suichuan County, Dabali, 22 April 2022, *C. Long BH-148* (IBSC); ibid. 13 November 2022, *C. Long s.n.* (IBSC). *Indocalamus pallidiflorus*: CHINA. Guangdong: Longmen County, Nankun Mountain, 12 April 1932, *W. T. Tsang 20216* (SYS00095376, US00065467 (image), US00312706 (image), US00312707 (image)); ibid., 18 April 2018, *X. R. Zheng*, *J. B. Ni & Y. Y. Zhang zxr-145* (IBSC). *Indocalamus sinicus*: CHINA. Hongkong: hill sides, *C. Wright s.n.* (K00092161 (image)); without precise locality, *Hance s.n.* (K00092162 (image), K00092163 (image)).

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RESEARCH ARTICLE

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Didymodon changbaiensis (Pottiaceae, Musci), a new species from Changbai Mountain, China and its phylogenetic position based on molecular data

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Abstract

Changbai Mountain, located in northeast China, is one of the areas with the most complete natural ecosystem preservation in China. A new species, *Didymodon changbaiensis* C.Feng, J.Kou, H.-X. Xiao & T.-T. Wu from north slope of Changbai Mountain in Jilin Province of China is described and illustrated. It is characterised by ovate or ovate-lanceolate leaves that are appressed when dry, acute leaf apex, lamina red or reddish-orange with KOH, unistratose lamina throughout, plane and unistratose leaf margins, percurrent costa with one layer of guide cells and without ventral stereids, upper and middle laminal cells with elliptical papillae over the transverse walls between two immediately adjacent cells and basal laminal cells not differentiated from the median cells. Our morphological analyses and molecular results, based on DNA sequences of ITS, *rps*4 and *trn*M-*trn*V, confirm that *D. changbaiensis* is revealed to be sister to *D. daqingii* J. Kou, R.H. Zander & C. Feng. This new species is compared with similar species and its phylogenetic position and ecology are discussed.

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* These authors contributed equally to this work.

Keywords
Asia, northeast China, phylogenetic analysis, taxonomy

Introduction

Changbai Mountain National Reserve is located at the junction of three counties: Antu County, Fusong County and Changbai County in the southeast of Jilin Province, with a total area of 196,000 ha^2. It is one of the earliest national nature reserves established in China. The vertical height difference of the Nature Reserve is nearly 2000 m and the altitude is between 720 and 2691 m (Jia et al. 2020). From bottom to top, the landform can be clearly divided into three annular zones: lava platform, lava plateau and volcanic cone. The protected area belongs to the temperate humid monsoon climate, which is generally characterised by long and cold winters, short and cool summers, strong and dry winds in spring and foggy and cool autumns. The plants in the area belong to the flora of Changbai Mountain and the vegetation changes vertically with the altitude gradient. The vegetation in this area has obvious vertical zonal distribution due to the influence of climate and it is generally divided into four vertical vegetation zones. From the foot of the mountain to the top of the mountain are generally: broad-leaved Korean pine forest belt (below 1100 m), spruce fir forest belt (1100–1800 m), Yuehua forest belt (1800–2100 m) and alpine tundra belt (greater than 2100 m) (Ping et al. 2016). Due to the diverse characteristics of topography, climate and ecosystem, the area is rich in biodiversity resources. There are nine species of amphibians, 12 species of reptiles, 24 species of fish, 56 species of mammals, 230 species of birds and 1255 species of insects in the Reserve, respectively (Tang et al. 2011). In addition, there are 430 species of fungi, 200 species of lichens, 311 species of bryophyte, 78 species of ferns, 11 species of gymnosperms and at least 1325 species of neutrophils (Zhang et al. 2016).

Although the bryophyte flora of Changbai Mountain has been well studied, most of these studies were nearly twenty years ago (e.g. Koponen et al. 1983; Cao and Guo 2000; Cao et al. 2002; Guo et al. 2002). In the background of the recent revolution of genus *Didymodon* (Zander 2013; Zander 2019) which was split into seven smaller genera: *Aithobryum* R.H. Zander, *Didymodon* s. str., *Exobryum* R.H. Zander, *Fuscobryum* R.H. Zander, *Geheebia* Schimp., *Trichostomopsis* Card. and *Vinealobryum* R.H. Zander, based on macro-evolutionary analysis and the dissilient genus concept applied (Zander 2013; Zander 2019), a re-evaluation of *Didymodon* in China is being conducted by the authors (e.g. Feng et al. 2022a; Feng et al. 2022b). During a recent excursion in Changbai Mountain, many specimens have been collected. Amongst them, two samples of *Didymodon* s. lat. from stony habitats are different from species previously reported in the area (Li et al. 2001). They mostly resemble *Didymodon tibeticus* J.Kou, X.-M.Shao & C.Feng. To clarify their taxonomic identity, we conducted phylogenetic analysis and confirm that these samples belong to the genus *Didymodon* s. str. (Zander 2013), but do not match with any species known in the genus. Here, we describe this unknown moss as a new species.

Materials and methods

Morphological observations

Over 3000 specimens of the genus *Didymodon* s. lat. were examined during our revision of Pottiaceae in China. More than 50 field investigations were conducted in past years and the specimens included in this study were housed in the Herbaria at IFP, KUN and NMAC. Microscopic examinations and measurements were taken with a ZEISS Primo Star light microscope and photomicrographs were obtained with a Canon EOS 70D camera, mounted on this microscope. Specimens were examined in 2% potassium hydroxide (KOH). Three plants were dissected from each collection and, for each shoot, every possible structure from the gametophyte had to be examined and a record kept of what was found for each individual species. Specific morphological and anatomical features of taxonomic importance were assessed mainly following Zander (1993). Leaves were always taken from the upper and middle parts of the stem and cross-sections were made in the middle part of the stem. Measurements of leaf width were taken at the base, mid-leaf and upper part. Cross-sections were made at mid-leaf.

Phylogenetic analyses

To test the phylogenetic position of the new species, one specimen (234) collected from Changbai Mountain was sampled. Another species (188) that was discovered nearby the new species was also sampled. We employed one nuclear (ITS) and two chloroplast markers (*rps*4 and *trn*M-*trn*V), which had been used successfully in previous phylogenetic studies in *Didymodon* s. lat. and enabled the re-use of earlier results and easier interpretation of new data (Zhang et al. 2022). Phylogenetic trees are created and shown separately. The complete list with sample names and GenBank accession numbers is presented in Tables 1 and 2. DNA extraction, PCR amplification and sequencing procedure followed the protocols described by Wang et al. (2010).

The sequences were aligned by using MAFFT 7.222 (Kazutaka and Daron 2013) and then edited in BioEdit 7.0.1 (Hall 1999). The concatenation of individual *rps*4 and *trn*M-*trn*V fragments was performed by our custom Perl script. The phylogenetic position of the new species was analysed within a comprehensive phylogenetic analysis of *Didymodon* s. lato. species by our previous study (Zhang et al. 2022). Phylogenetic analyses were performed by using the Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Parameter configuration and convergence estimation followed Zhang et al. (2022).

Table 1. New sequences used in this study, including taxa vouchers information and GenBank accession numbers.

Species	Voucher information	ITS	*rps*4	*trn*M-*trn*V
234 (new species)	China, Changbai Mountain, Jin Kou 20200902234	OP641837	OP641840	OP656316
188 (unknown species)	China, Changbai Mountain, Jin Kou 20200902188	OP641838	OP641839	OP656315

Table 2. Sequences from GenBank used in this study, including taxa and GenBank accession numbers.

Species	ITS	*rps4*	*trnM-trnV*
Acaulon triquetrum	MW398556		
Aloina rigida	MW398549		
Aloinella andina	MW398550		
Andinella churchilliana	MW398720		
Andinella coquimbensis	MW398711		
Andinella elata	MW398708		
Andinella granulosa	MW398714		
Andinella limensis	MW398710		
Andinella oedocostata	MW398733		
Andinella pruinosa	MW398726		
Barbula unguiculata	MW398553	HM147777	JQ890366
Bryoerythrophyllum recurvirostrum	MW398547	JQ890468	JQ890407
Bryoerythrophyllum rubrum	MW398548		
Chenia leptophylla	MW398561		
Cinclidotus riparius	MW398554		
Crossidium squamiferum	MW398558		
Didymodon acutus	AY437111	KP307551	KP307667
Didymodon alpinus	MW398606		
Didymodon andreaeoides	MW398768		
Didymodon anserinocapitatus	MW398649	KP307545	KP307640
Didymodon asperifolius	MW398594	JQ890472	KP307600
Didymodon australasiae (*Trichostomum australasiae*)	MW398737	KP307571	KP307651
Didymodon brachyphyllus (*Vinealobryum brachyphyllum*)	MW398817		
Didymodon buckii	MW398578		
Didymodon caboverdeanus	MW398607		
Didymodon californicus (*Vinealobryum californicum*)	MW398819		
Didymodon canoae	MW398584		
Didymodon cardotii	MW398729		
Didymodon challaensis (*Trichostomopsis challaensis*)	MW398748		
Didymodon constrictus	MW398613		
Didymodon cordatus	MW398664	KP307564	KP307668
Didymodon ditrichoides	MW398642		
Didymodon eckeliae (*Vinealobryum eckeliae*)	MW398826		
Didymodon edentulus	MW398685		
Didymodon epapillatus	MW398665		
Didymodon erosodenticulatus	MW398792	MF536597	MF536635
Didymodon erosus	EU835148	MF536609	MF536646
Didymodon fallax (*Geheebia fallax*)	MW398779	KP307552	KP307663
Didymodon ferrugineus (*Geheebia ferruginea*)	MW398796	MF536588	MF536625
Didymodon fragilicuspis	KP307482		
Didymodon fuscus	MW398689	KP307537	KP307601
Didymodon aff. *fuscus*		KP307546	KP307615
Didymodon gaochienii		KP307538	KP307658
Didymodon gelidus	MW398693		
Didymodon giganteus	MW398786	KP307548	KP307669
Didymodon glaucus	MW398612		
Didymodon guangdongensis (*Vinealobryum guangdongense*)	MW398657		
Didymodon hedysariformis	MW398582	KP307569	KP307629
Didymodon hengduanensis	MW398629		
Didymodon hegewaldiorum	MW398739		

Species	**ITS**	***rps4***	***trnM-trnV***
Didymodon herzogii	MW398746		
Didymodon humboldtii	MW398667		
Didymodon icmadophilus	MW398632	KP307598	KP307604
Didymodon imbricatus	MW398646		
Didymodon incrassatolimbatus	MW398572		
Didymodon incurvus	MW398680		
Didymodon insulanus (*Vinealobryum insulanum*)	MW398811		
Didymodon japonicus	MW398757		
Didymodon jimenezii	MW398622		
Didymodon johansenii	MW398589	KP307542	KP307662
Didymodon kunlunensis	MW398610		
Didymodon laevigatus	MW398618		
Didymodon lainzii	MW398575		
Didymodon leskeoides (*Geheebia leskeoides*)	MW398777	MF536604	MF536642
Didymodon luehmannii	MW398718		
Didymodon luridus	AY437098	MF536587	MF536624
Didymodon maschalogena	MW398615		
Didymodon maximus (*Geheebia maxima*)	MW398784	MF536591	MF536628
Didymodon mesopapillosus	MW398758		
Didymodon molendoides	MW398687		
Didymodon mongolicus	KU058175		
Didymodon murrayae	KP307513	KP307563	KP307650
Didymodon nevadensis	MW398730		
Didymodon nicholsonii (*Vinealobryum nicholsonii*)	MW398808		
Didymodon nigrescens	LC545516	KP307543	KP307611
Didymodon norrisii	MW398830	KP307585	KP307617
Didymodon novae-zelandiae	MW398769		
Didymodon obtusus	MW398666		
Didymodon occidentalis		KP307533	KP307599
Didymodon ochyrarum	MW398763		
Didymodon paramicola (*Trichostomopsis paramicola*)	MW398740		
Didymodon patagonicus	MW398675		
Didymodon perobtusus	KP307523	KP307539	KP307609
Didymodon revolutus (*Husnotiella revoluta*)	MW398569	JQ890471	KP307646
Didymodon revolutus var. *africanus*	MW398568		
Didymodon rigidulus	MW398602	KP307589	KP307647
Didymodon rigidulus var. *subulatus*	MW398672		
Didymodon rivicola	MW398599	KP30756	KP307607
Didymodon santessoni	MW398705		
Didymodon sicculus	MW398801	MF536606	MF536643
Didymodon sinuosus	MW398567	JQ890476	JQ890410
Didymodon spadiceus (*Geheebia spadicea*)	MW398795	MF536593	MF536631
Didymodon subandreaeoides	AY437108	KP307570	KP307630
Didymodon tectorum	MW398659		
Didymodon tibeticus	MW398638		
Didymodon tomaculosus	AY437114		
Didymodon tophaceus	MW398807	MF536607	MF536644
Didymodon tophaceus var. *anatinus*		MF536589	MF536626
Didymodon torquatus	MW398719		
Didymodon umbrosus (*Trichostomopsis umbrosa*)	MW398742		
Didymodon validus	MW398650		
Didymodon vinealis (*Vinealobryum vineale*)	MW398815	JQ890475	KP307606

Species	ITS	*rps4*	*trnM-trnV*
Didymodon vinealis var. *rubiginosus*	MW398822		
Didymodon vulcanicus	MW398636		
Didymodon waymouthii	MW398770		
Didymodon wisselii	MW398655		
Didymodon xanthocarpus	MW398696	KP307534	KP307638
Didymodon zanderi	MW398585	KP307535	KP307621
Dolotortula mniifolia	MW398555		
Erythrophyllopsis andina	MW398546		
Gertrudiella uncinicoma	MW398698		
Gertrudiella uncinicoma var. *serratopungens*	MW398701		
Guerramontesia microdonta	MW398543		
Hennediella heimii	GQ339750		
Hennediella polyseta	GQ339759		
Leptodontium excelsum	MW398545		
Microbryum curvicolle		JX679986	JX679936
Microbryum davallianum	MW398557		
Pseudocrossidium hornschuchianum	MW398551	JQ890481	JQ890420
Pseudocrossidium revolutum	MW398552		
Pterygoneurum ovatum	MW398560		
Sagenotortula quitoensis	GQ339761		
Stegonia latifolia	MW398559		
Syntrichia ruralis	MW398564	FJ546412	FJ546412
Tortula muralis	MW398562	JN581679	JQ890421
Tortula subulata	MW398563		
Triquetrella arapilensis	MW398544		
Tridontium tasmanicum	MW398750		

Results

The combined length of ITS and cpDNA (*rps*4 and *trn*M-*trn*V) is 4622 bp. The position of the new species is different between the BI and ML phylogenetic trees and, thus, both of them are reserved. The topology, based on ML analyses, shows that the new species is sister to *Didymodon daqingii* J. Kou, R.H. Zander & C. Feng and they nested within the monophyletic group comprising *Didymodon hengduanensis* J.A. Jiménez, D.G. Long, Shevock & J. Guerra, *D. icmadophilus* (Schimp. ex Müll. Hal.) K. Saito, *D. mesopapillosus* J. Kou, X.-M. Shao & C. Feng, *D. tibeticus* and *D. vulcanicus* J.A.Jiménez, Hedd. & Frank Müll (Fig. 1). The BI tree was more similar to that of the ML tree, but with weakly-supported values (Fig. 2).

Discussion

Our molecular analyses reveal that the new species belongs to *Didymodon* s.str. Morphologically, the combination of characters: concave leaves, plane leaf margins, percurrent to excurrent costa, seldom papillose laminal cells, costa with quadrate or

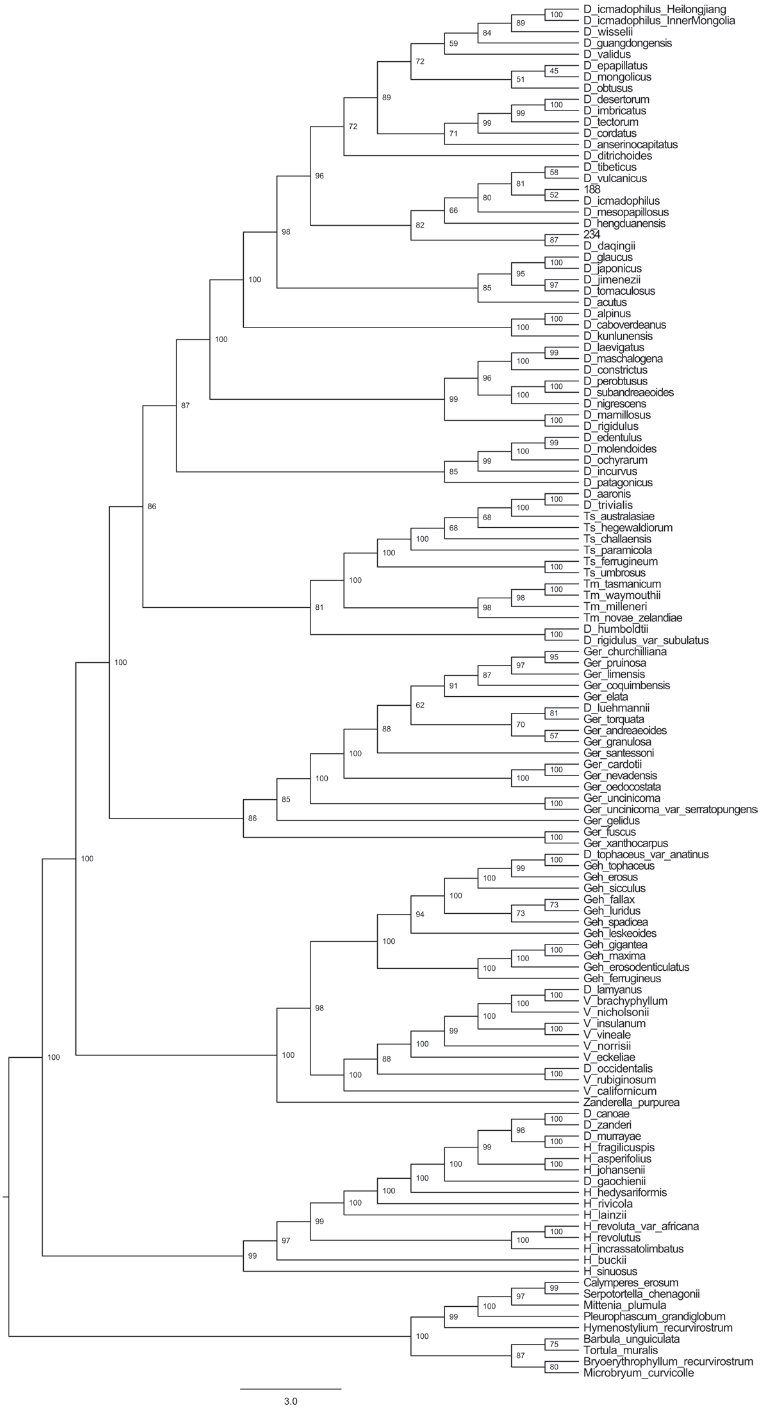

Figure 1. Maximum Likelihood tree inferred from concatenated ITS, *rps*4 and *trn*M-*trn*V datasets. Numbers indicate Maximum Likelihood bootstrap values. The numbers 234 and 188 show the sample of *D. changbaiensis* and an unknown species, respectively.

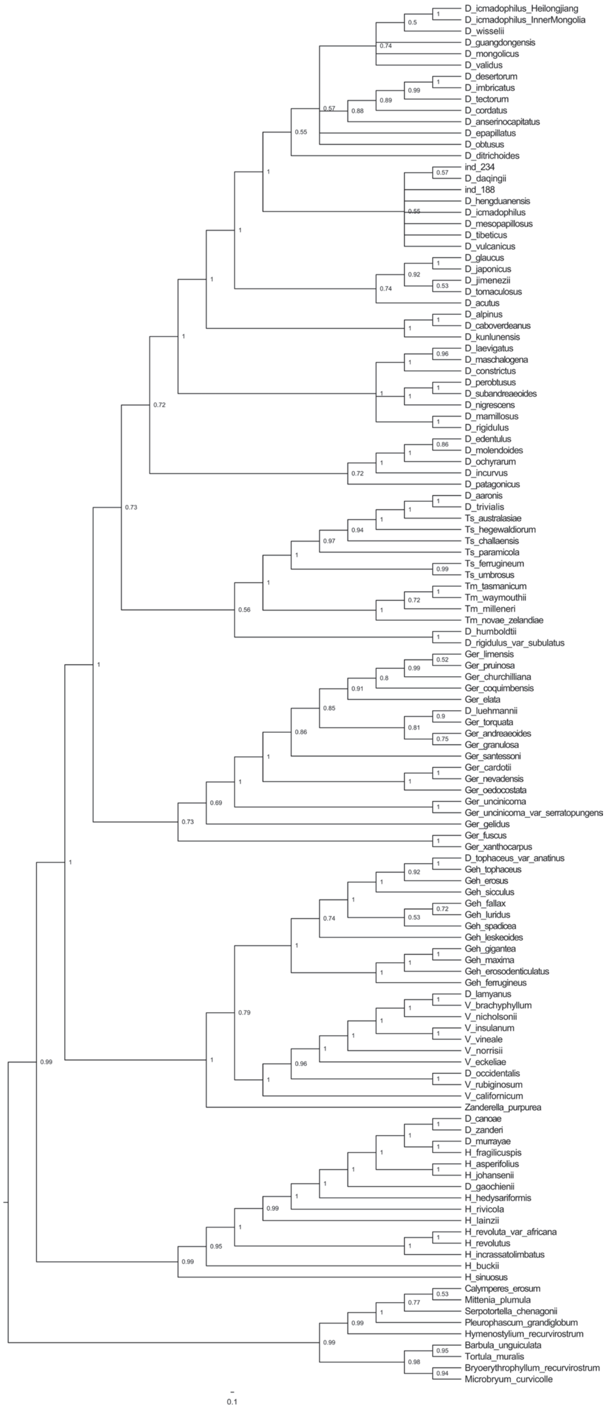

Figure 2. Phylogenetic relationships (50% majority consensus tree) from the Bayesian Inference of the concatenated ITS, *rps*4 and *trn*M-*trn*V datasets. Numbers indicate posterior probability from the BI analysis. The numbers 234 and 188 show the sample of *D. changbaiensis* and an unknown species, respectively.

occasionally short-rectangular superficial adaxial cells and absence of costal adaxial stereid band also suggests the placement of the new species in the emended genus *Didymodon* s.str. Zander (1978, 1993, 2013). The new species is distinguished from all congeners by the following combination of diagnostic features: ovate or ovate-lanceolate leaves that are appressed when dry, acute leaf apex, lamina red or reddish-orange with KOH, unistratose lamina throughout, plane and unistratose leaf margins, percurrent costa with one layer of guide cells and without ventral stereids, upper and middle laminal cells with elliptical papillae over the transverse walls between two immediately adjacent cells and basal laminal cells not differentiated from the median cells.

The phylogenetic analyses support that the new species is closely related to *D. daqingii*, a species that was recently described from Inner Mongolia, China (Kou et al. 2019). They have similarity in quadrate or subquadrate ventral cells of costa in the upper middle part of the leaf, bulging costal ventral surface cells, transverse section of costa round at mid-leaf and costa without ventral stereids. However, *D. daqingii* differs from the new species by the long-lanceolate leaves, very fragile and bistratose leaf apices, distally bistratose and recurved leaf margins, costa with guide cells in 2–3 layers, laminal cells with 1–3 simple papillae per cell and yellow-green KOH laminal colour reaction.

There are five species that are related to the new species, based on our phylogenetic analysis. Amongst them, the new species is most similar to *D. tibeticus*, based on morphological characters. They share such distinctive characteristics as the shape of the leaves, lamina red or reddish-orange with KOH, unistratose lamina throughout, plane and unistratose leaf margins and percurrent costa. Nevertheless, *D. tibeticus* can be separated from the new species by the costa with a ventral costal pad of cells and 0–1 layer of ventral stereids, transverse section of costa flattened at mid-leaf and laminal cells with 1–2 simple or bifurcate papillae per cell. Other species, *Didymodon hengduanensis* J.A. Jiménez, D.G. Long, Shevock & J. Guerra, *D. icmadophilus* (Schimp. ex Müll. Hal.) K. Saito, *D. mesopapillosus* J. Kou, X.-M. Shao & C. Feng and *D. vulcanicus* J.A.Jiménez, Hedd. & Frank Müll. are distinguished from the new species by their recurved leaf margins and costa with ventral stereids.

Taxonomic treatment

***Didymodon changbaiensis* J.Kou, C.Feng, H.-X.Xiao & T.-T.Wu, sp. nov.**

Figs 3, 4

Chinese name: 长白山对齿藓

Type. China. Jilin Province: Changbai Mountain, 42°3'35.316"N, 128°3'51.516"E, on soil over rocks, elevation 1864 m, 2 September 2020, *Jin Kou 20200902234* (holotype: NENU!; isotype: NMAC 20200902234!).

Diagnosis. It differs from the otherwise similar *D. tibeticus* in its costa without a ventral costal pad of cells and ventral stereids, transverse section of costa round at mid-leaf and laminal cells with elliptical papillae over the transverse walls between two immediately adjacent cells.

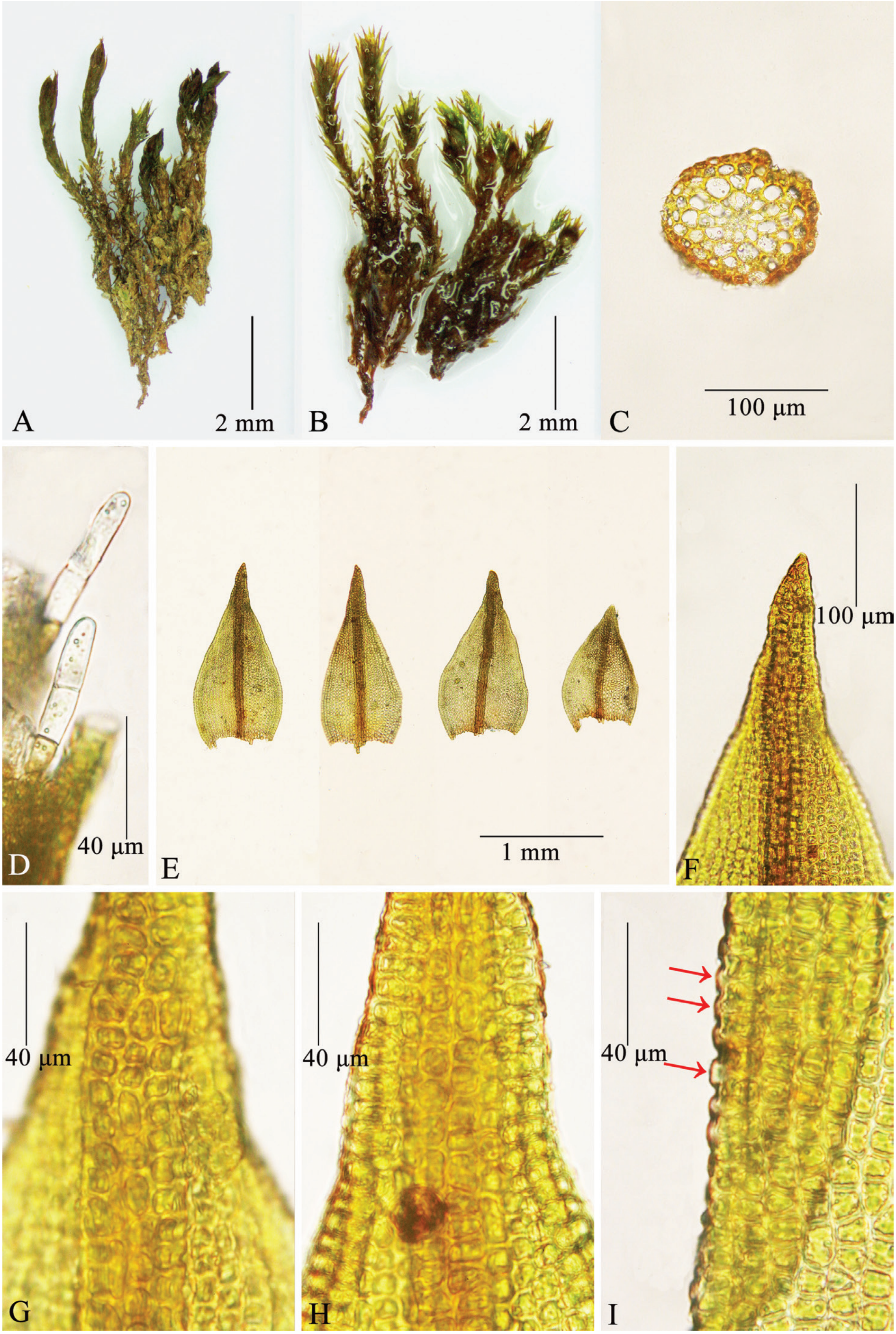

Figure 3. *Didymodon changbaiensis* **A** dry plants **B** moist plants **C** cross-section of stem **D** axillary hairs **E** leaves **F** leaf apex **G** upper part of costa (dorsal) **H** upper part of costa (ventral) **I** upper leaf margin, arrows shows the laminal cells with elliptical papillae over the transverse walls between two immediately adjacent cells. Photographed on 25 May 2022 by Chao Feng from the isotype.

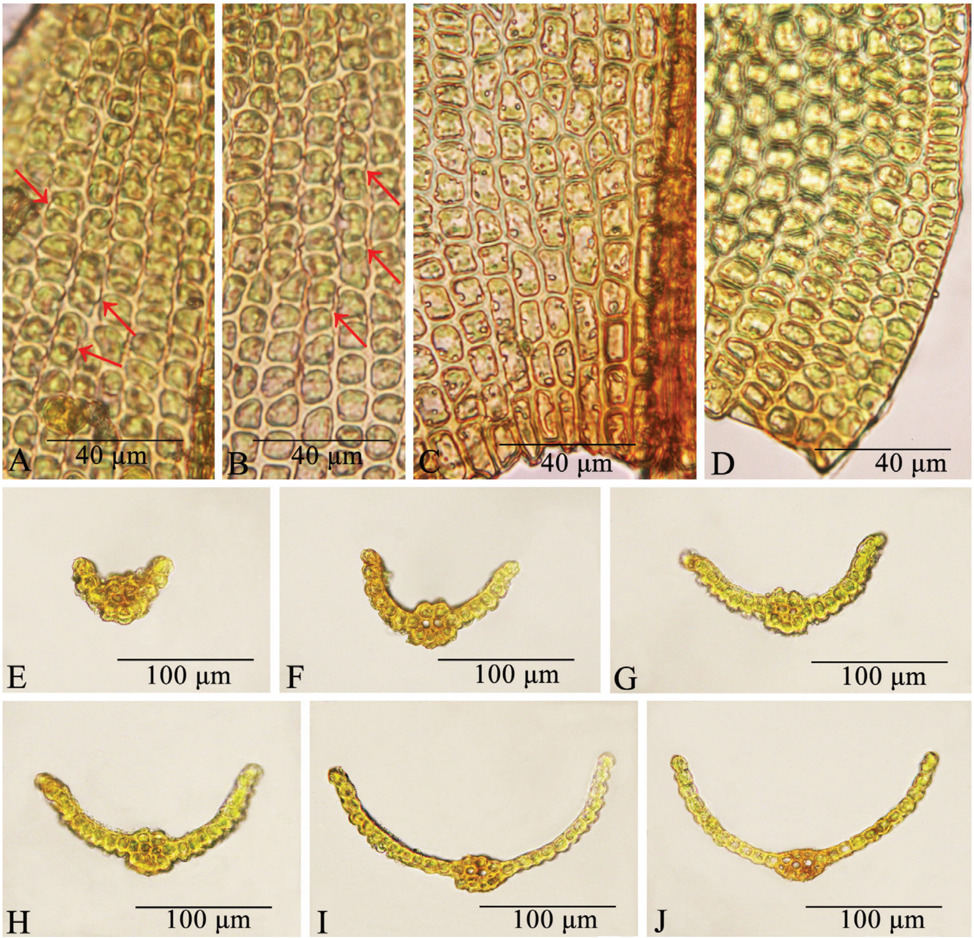

Figure 4. *Didymodon changbaiensis* **A, B** median leaf cells, arrows show the laminal cells with elliptical papillae over the transverse walls between two immediately adjacent cells **C** basal juxtacostal cells **D** basal marginal cells **E–J** cross-sections of leaves, sequentially from apex to base. Photographed on 25 May 2022 by Chao Feng from the isotype.

Description. Plants to 1 cm high, growing in dense turfs, brown-reddish below, green above. Stems erect, frequently branched, in transverse section rounded, central strand weakly differentiated, hyalodermis and sclerodermis absent; axillary hairs filiform, usually 3–4 cells long, with one brown basal cell and hyaline upper ones. Rhizoidal tubers absent. Leaves appressed when dry, erect when moist, ovate or ovate-triangular with a broad base, 0.85–1.3 × 0.43–0.65 mm, channelled ventrally in the upper part; lamina completely unistratose, reddish-orange in KOH; apex acuminate to acute, not cucullate; margins entire, plane, completely unistratose; costa 51.7–86.2 µm wide at base, percurrent to short-excurrent; ventral cells of costa in upper middle part of leaf quadrate or subquadrate, sparsely papillose; dorsal cells of costa in upper middle part of leaf quadrate or subquadrate, sparsely papillose; transverse section of costa round at mid-leaf; with 3–4 guide cells in one layer, absence of ventral stereids, 1–2 layers of dorsal stereids, without hydroids, ventral surface cells bulging, not forming a pad of a single layer of cells, papillose, dorsal surface cells papillose; upper and middle laminal cells subquadrate, hexagonal or shortly rectangular, 5.5–11.1 × 8.9–12.2 μm, dorsally with one low elliptical papilla over the transverse walls which reaches the two immediate cells; basal cells weakly differentiated, smooth, basal juxtacostal cells hexagonal or short-rectangular, 11.1–20 × 5.56–10 μm, evenly thick-walled; basal marginal cells oblate, 5.56–10 × 6.67–10 μm, with regular thickened transverse walls and thin longitudinal walls. Gemmae absent. Dioicous. Sporophyte unknown.

Etymology. The specific epithet refers to Changbai Mountain, the type locality.

Habitat and distribution. The new species is currently known only from the type locality at the north slope of Changbai Mountain, Jinlin Province, China, growing on thin soil over rocks.

Acknowledgements

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RESEARCH ARTICLE

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Carex quixotiana (Cyperaceae), a new Iberian endemic from Don Quixote's land (La Mancha, S Spain)

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Abstract

Despite centuries of work, the basic taxonomic knowledge of the flora of the Iberian Peninsula is still incomplete, especially for highly diverse and/or difficult genera such as *Carex*. In this study, we conducted an integrative systematic study based on molecular, morphological and cytogenetic data to elucidate the taxonomic status of several problematic *Carex* populations from La Mancha region (S Spain) belonging to *Carex* sect. *Phacocystis*. These populations have been traditionally considered of uncertain taxonomic adscription, but close to *C. reuteriana* due to their morphological appearance and ecological preferences. A detailed morphological and cytogenetic study was performed on 16 La Mancha's problematic populations (Sierra Madrona and Montes de Toledo) to compare them with the other Iberian sect. *Phacocystis* species. In addition, a phylogenetic analysis was conducted using two nuclear (ITS, ETS) and two plastid (*rpl*32-*trn*L^{UAG}, *ycf*6-*psb*M) DNA regions, including representatives from all species of sect. *Phacocystis*. We found a significant degree of molecular and morphological differentiation that supports the recognition of La Mancha's problematic populations as a new Iberian endemic species, described here as *Carex quixotiana* Ben. Benítez, Martín-Bravo, Luceño & Jim.Mejías. Our results reveal that *C. quixotiana*, unexpectedly, is more closely related to *C. nigra* than to *C. reuteriana* on the basis of phylogenetic relationships and chromosome number. These contrasting patterns reflect the taxonomic complexity in sect. *Phacocystis* and highlight the need for integrative systematic approaches to disentangle such complicated evolutionary scenarios.

Keywords

Cytogenetics, Iberian Peninsula, Mediterranean, morphometrics, new species, *Phacocystis*, phylogenetics, *Quercus* forest, taxonomy

Introduction

The Iberian Peninsula is one of the three large peninsulas of Southern Europe projecting into the Mediterranean Sea, and as such, it harbours a high diversity of plant species and endemism relative to the rest of Europe (Myers et al. 2000; Mittermeier et al. 2011; Vargas 2020). Phytogeographically, it is part of the Mediterranean Basin biodiversity hotspot (Myers et al. 2000; Mittermeier et al. 2011). *Carex* L. (Cyperaceae) is among the three most speciose angiosperm genera in the Iberian Peninsula (Aedo et al. 2017). Floras, monographs and checklists published from the second half of the 20th century have progressively increased the number of reported *Carex* native taxa in the Iberian Peninsula (Vicioso 1959: 74 taxa; Chater 1980: 95 taxa; Luceño and Aedo 1994: 98 taxa; Luceño 2008: 101 taxa; Luceño et al. unpublished data: 108 taxa). These studies, along with the finding of new Iberian records (e.g., *C. cespitosa*, Jiménez-Mejías et al. 2007), re-evaluation of neglected taxa (e.g., *C. paui*, Benítez-Benítez et al. 2017; Troia et al. 2018) or even description of new ones (e.g., *C. lucennoiberica*, Maguilla and Escudero 2016; *C. camposii* subsp. tejedensis, Sánchez-Villegas et al. 2022), demonstrate that the taxonomic and biogeographic knowledge of *Carex* in this territory is still in progress.

Section *Phacocystis* Dumort. (subg. *Carex*) is among the largest sections of *Carex* (ca. 112 spp. in Roalson et al. 2021), although the number of taxa differs considerably among treatments (see Benítez-Benítez et al. 2021). It has a sub-cosmopolitan distribution that somewhat mirrors that of the whole genus, with higher species diversity in temperate and cold areas in the Northern Hemisphere, but also with several species present in the Southern Hemisphere (Benítez-Benítez et al. 2021). The typical ecological requirements of the section include habitats with high water availability, such as wetlands, river shores, mountain bogs and wet coastal sands, which are mostly in freshwater systems, although a few high latitude species also grow in halophytic environments. In the Iberian Peninsula, it is represented by six species and eight taxa: *C. acuta* L., *C. cespitosa* L., *C. elata* All. subsp. *elata*, *C. nigra* (L.) Reichard subsp. *nigra*, *C. nigra* subsp. *intricata* (Tineo ex Guss.) Rivas Mart., *C. reuteriana* Boiss. subsp. *reuteriana*, *C. reuteriana* subsp. *mauritanica* (Boiss. & Reut.) Jim.Mejías & Luceño, and *C. trinervis* Degl. Section *Phacocystis* is one of the most controversial *Carex* groups from a taxonomic perspective, as species in the group are affected by the complex interplay of high intraspecific morphological variability, faint species boundaries, and interspecific hybridization (see references in Jiménez-Mejías et al. 2014a; Benítez-Benítez et al. 2021). In addition, the use of certain species as taxonomic hotchpotches (e.g., *C. acuta*, *C. cespitosa*), and frequent misidentifications during the taxonomic history of sect. *Phacocystis*, have greatly obscured the knowledge of the group in certain areas (e.g., Jiménez-Mejías and Martinetto 2013; Jiménez-Mejías et al. 2014b). An example of this among the Iberian taxa of sect. *Phacocystis* may be observed in *C. elata* and *C. reuteriana*, for which different taxa have been miscited or even confused with other species such as *C. acuta* or *C. nigra* (Luceño and Aedo 1994; Jiménez-Mejías et al. 2011, 2020).

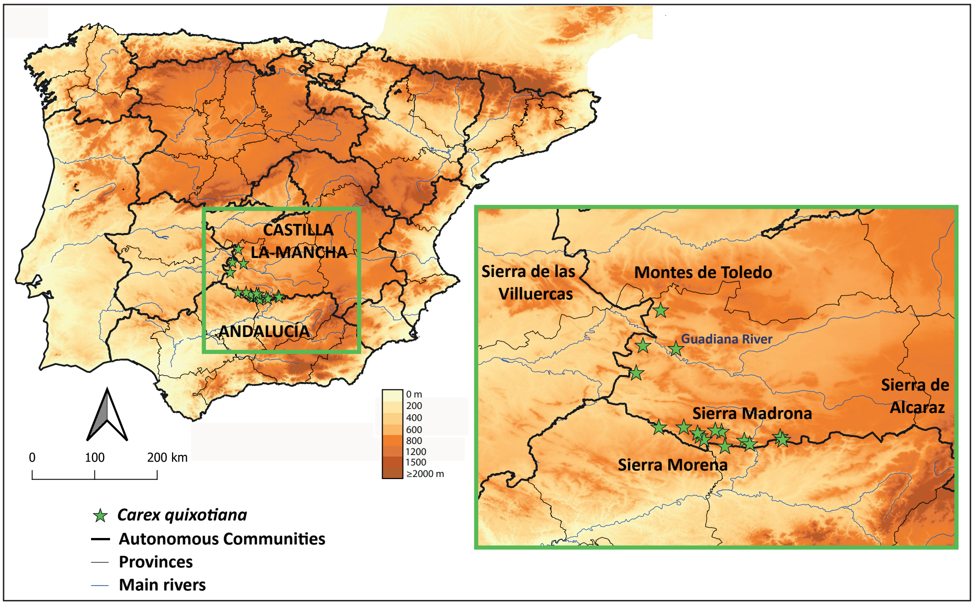

Figure 1. Distribution map representing the studied populations of *Carex quixotiana*.

La Mancha (Fig. 1) is a natural and historical region placed in the south-southeastern limit of the Iberian Central Plateau, mostly belonging to the Guadiana River basin. It is popularly known as the main setting for Miguel de Cervantes's (1547–1616) novel Don Quixote (de Cervantes 1605, 1615). At an approximate average elevation of 500–600 m, La Mancha has a relatively cold, semi-arid climate and low topographic relief. The region is delimited by Sierra Morena to the south, Montes de Toledo to the north, and Sierra de Alcaraz to the east (Fig. 1), all delimiting the Guadiana basin, and Las Villuercas to the west, across which the River Guadiana stretches towards the Atlantic Ocean. While the central part of La Mancha is mostly formed by carbonate sediments, Sierra Morena and Montes de Toledo are composed of metamorphic siliceous materials, including quartzite, schist and slate. The majority of the present La Mancha landscape is dedicated to agriculture, but potential vegetation would be primarily evergreen sclerophyllous *Quercus ilex* forest on the lower lands, and *Q. pyrenaica* and/or *Q. faginea* woodlands on the more elevated ones (Martín Herrero et al. 2003). According to Ramos-Gutiérrez et al. (2021), there are only two plant species endemic to La Mancha, both belonging to the agamospermic genus *Limonium* (*L. pinillense* Roselló & Peris and *L. squarrosum* Erben) and inhabiting inland saltmarshes. There are about 20 species of *Carex* present in La Mancha (Luceño et al. unpublished data). Most of them are limited to habitats with year-round water availability, such as springs, streams or *bonales* (relictic aquifer-fed peat bogs) (Martín-Blanco and Carrasco de Salazar 2005), although a few species also grow on relatively dry soils as part of the Mediterranean forest understory.

During the preparation of Flora Iberica (Luceño and Jiménez-Mejías 2008) a set of problematic *Carex* sect. *Phacocystis* populations from Sierra Madrona (a northern subrange of Sierra Morena belonging to La Mancha) were tentatively assigned to *C. reuteriana* (Jiménez-Mejías et al. 2011), but with ambiguous affinities regarding the subspecies (*C. reuteriana* subsp. *reuteriana* and *mauritanica*; treated under *C. elata* in Flora Iberica, Luceño and Jiménez-Mejías 2008). A preliminary AFLP assessment (Jiménez-Mejías and Martín-Bravo, unpublished) associated with a study focusing on *C. reuteriana* (Benítez-Benítez et al. 2018) revealed that, surprisingly, the problematic populations were not as closely related to *C. reuteriana* as expected. Subsequently, we found several additional of these problematic populations in La Mancha. The detailed comparison of the material with other Iberian species of the group revealed superficial morphological affinities with *C. reuteriana* or *C. nigra*. However, its ecological preferences were confounding, since the problematic populations inhabited generally small streams, rivers and springs and wet meadows in marcescent and sclerophyllous *Quercus* forests (Fig. 2), while *C. reuteriana* grows exclusively in permanent streams, and *C. nigra* thrives at much higher altitudes in wet mountain meadows and bogs. Here we conduct a detailed systematic study of these La Mancha's problematic populations, including the comparison with other Iberian species within sect. *Phacocystis*, using a combined molecular, morphological and cytogenetic approach demonstrated to be useful for disentangling the complex taxonomy of this group (e.g., Jiménez-Mejías et al. 2011). Our objective was to elucidate the systematic status of these populations and to warrant their taxonomic recognition if required.

Materials and methods

Sampling

We collected specimens from 10 problematic populations from La Mancha region (Sierra Madrona and Montes de Toledo; Fig. 1) that were deposited at UPOS herbarium, with duplicates at GDA, JAEN, K, MA, NY, P and SALA (acronyms following Thiers 2023). A special sampling effort was conducted in Sierra Madrona, resulting in a representative sampling across the entire range (Fig. 1). Additional herbarium specimens were obtained from JAEN, MA, MACB and SALA (Suppl. material 1). Some of these specimens came from sites very close to the 10 mentioned populations (e.g., in the same watercourse) and/or had imprecise coordinates, so they were considered to belong to the same population, but 6 additional populations were identified (Suppl. material 1).

Morphological study

Material from all 16 sampled populations (Suppl. material 1) was carefully compared with all the other sect. *Phacocystis* species present in the Western Palearctic using specialized literature (Schultze-Motel 1968; Nilsson 1985; Kukkonen 1998; Egorova 1999; Jermy et al. 2007; Dean and Ashton 2008; Luceño and Jiménez-Mejías 2008; Jiménez-Mejías et al. 2015), with emphasis on Iberian species and specifically the morphologically similar *C. nigra* and *C. reuteriana*. We examined the most important

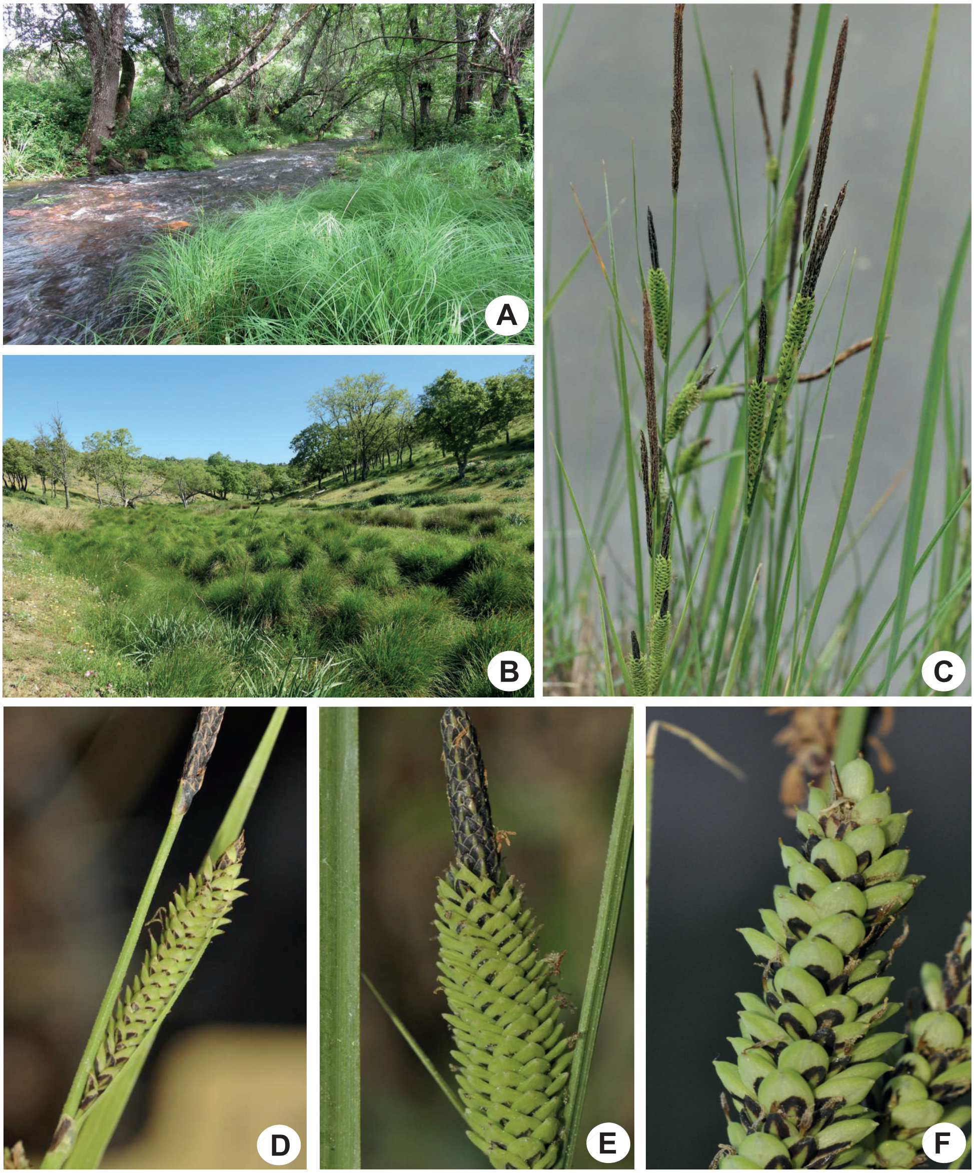

Figure 2. Representative photos of *Carex quixotiana* **A** habitat (riparian forest with *Alnus lusitanica* and *Fraxinus angustifolia*: Ciudad Real, Solana del Pino, Robledillo River; 57SMB17, UPOS-8924; *C. quixotiana* plants are in the lower right corner) **B** habitat (stream in *Quercus pyrenaica* open forest: Ciudad Real, between El Viso del Marqués and San Lorenzo de Calatrava; 8CBB18, UPOS-16897; *C. quixotiana* are the tussocks along the stream) **C** inflorescence **D, E** detail of terminal male and lateral androgynous spikes **F** detail of lateral female spikes and utricles (Ciudad Real, Fuencaliente, Minas del Horcajo, 54SMB17, UPOS-8922).

morphological characters for the taxonomy of *Carex* sect. *Phacocystis*: general habit, basal sheaths colour and form, stomata distribution on leaf faces, relative length of lowest bract/inflorescence, spike size and sex distribution, and utricle size, colour and indumentum. Measurements above 1 cm were taken with a standard 30 cm ruler, and those below 1 cm with an ocular micrometer (only for specimens from the 10 collected populations deposited at UPOS).

Molecular and phylogenetic study

To tackle the phylogenetic placement of La Mancha's problematic populations, we included these samples in the molecular phylogeny of sect. *Phacocystis* (Benítez-Benítez et al. 2021). We used the same markers as in that phylogeny: two nuclear ribosomal markers (ITS-ETS) and two plastid markers (*rpl*32-*trn*L^{UAG} and *ycf*6-*psb*M). The new sequenced material (Suppl. material 2, including Genbank accessions) was integrated with the complete singleton matrix (Benítez-Benítez et al. 2021) (Suppl. material 3), which provided the best phylogenetic resolution in that study. That matrix included 75% of the species of the section and 80% of the so-called Western Palearctic clade in particular (Benítez-Benítez et al. 2021). Inferred hybrid samples (e.g., *C. acuta*, *C. buekii*, *C. randalpina*, *C. salina*) were excluded from the complete singletons matrix to avoid deleterious topological effects as explained in Benítez-Benítez et al. (2021). The morphological and biogeographic affinities of La Mancha's problematic populations clearly point to the Western Palearctic lineage as the best candidate for them to be placed in.

DNA extraction and sequence amplification followed Benítez-Benítez et al. (2021). All PCR products were sequenced by Macrogen (Madrid, Spain). Sequence chromatograms were edited using GENEIOUS v.11.0.2 (Biomatters Ltd., Auckland, New Zealand). The sequences were aligned with MUSCLE v.5 (Edgar 2004). We carried out a scaffolding strategy following Jiménez-Mejías et al. (2016) and Benítez-Benítez et al. (2021) methodologies. First, we compiled all accessions containing both nuclear sequences (ITS and ETS) and built a nrDNA reference tree using maximum likelihood (ML) with RAXML (Stamatakis 2014; 100 bootstrap replicates). The resulting tree was used to build a query tree placing all the remaining accessions through the Evolutionary Placement Algorithm (EPA; Berger et al. 2011), also implemented in RAXML. Branch support for the query tree was calculated using the non-parametric Shimodaira-Hasegawa (SH) from approximate likelihood ratio test (SH-aLRT support; Guindon et al. 2010; Anisimova et al. 2011). All analyses were implemented in CIPRES Science Gateway (Miller et al. 2010).

To explore the systematic relationships of La Mancha's problematic populations at a finer scale and with respect to the other Iberian representatives of sect. *Phacocystis*, a statistical parsimony analysis of plastid haplotypes was conducted with TCS v. 1.2.1 (Clement et al. 2000). We included only those accessions from specimens from the Iberian Peninsula that contained both plastid *rpl*32-*trn*L^{UAG} and *ycf*6-*psb*M regions, except for *C. acuta* and *C. buekii* as explained above. To calculate the most parsimonious haplotype network we set a 95% parsimony connection limit for the minimum number of mutations differentiating the haplotypes. Gaps in sequences were coded as a fifth character.

Cytogenetic study

Chromosome counts for five La Mancha's problematic populations (Suppl. material 1) were performed. Anthers, from young male flowers, were fixed during meiosis following the method by Luceño (1988). The obtained chromosome numbers and meiotic configurations were compared with those of closely related species, since cytogenetics have been demonstrated to have a strong diagnostic potential in *Carex*, including sect. *Phacocystis* (Jiménez-Mejías et al. 2011).

Conservation Assessment

Following the taxonomic recognition of La Mancha's problematic populations (see below), we evaluated their conservation status at the global level following criteria, categories, and guidelines from IUCN (2017). Area of occupancy (AOO) and extent of occurrence (EOO) were calculated using the GeoCAT tool (Bachman et al. 2011) based on the 16 studied populations (see morphological study).

Results

Morphological study

The detailed examination of diagnostic morphological characters for La Mancha's problematic populations and its comparison with closely related species revealed qualitative and quantitative morphological differences regarding the other Iberian taxa of sect. *Phacocystis* (Table 1). In particular, all character states of La Mancha's problematic populations were distinct from at least one of the other Iberian taxon studied.

Molecular study

The query tree built (see Suppl. material 4) using the singletons matrix from Benítez-Benítez et al. (2021) recovered a topology equivalent to that found in the reference paper. The Western Palearctic clade was relatively well-supported (SH=89) and arranged in two main clades (Fig. 3): one containing *C. reuteriana* and the closely related *C. panormitana* (marginally supported, SH=64), and another with the rest of the species of the clade (SH=90). La Mancha's problematic populations were placed unresolved among the species of this latter clade.

The statistical parsimony analysis revealed 14 different haplotypes (Suppl. materials 5, 6). La Mancha's problematic populations displayed an exclusive haplotype, not shared with any of the other Iberian species. The most closely related haplotype was that displayed by the Sierra Nevada populations of *C. nigra*, separated by only one mutation.

Table 1. Comparison of main morphological diagnostic characters, chromosome numbers, and habitat (on the Iberian Peninsula) among *C. quixotiana* and all the other Iberian *Carex* sect. *Phacocystis* taxa. Measurements from the other taxa have been taken from Luceño and Jiménez-Mejías (2008).

	C. quixotiana	***C. acuta***	***C. cespitosa***	***C. elata* subsp. *elata***	***C. nigra***		***C. reuteriana***		***C. trinervis***
					subsp. *nigra*	**subsp. *intricata***	**subsp. *reuteriana***	**subsp. *mauritanica***	
Habit	From tussock-forming to rhizomes elongated	From more or less caespitose to rhizomes elongated	Tussock-forming	From tussock-forming to laxly caespitose	Rhizomes elongated		Tussock-forming		Rhizomes elongated
Basal sheaths (at the base of fertile stems)	Scale-like, sometimes elongated, creamy-yellow, rarely reddish-brown	Culm bases bearing brown old-leaf remains, without conspicuous scale-like basal sheaths	Scale-like, dark purple	Scale-like to elongated, creamy-yellow	Culm bases with or without conspicuous scale-like basal sheaths, dark brown when present		Scale-like, orange to reddish-brown		Culm bases bearing straw-colored old-leaf remains, without conspicuous scale-like basal sheaths
Leaf section	Flat to keeled	Flat	Flat to keeled	Flat to keeled	Flat to keeled		Flat to keeled		Strongly plicate to canaliculate
Leaf width (mm)	(1.8)2–3.2(4.7)	3–5(7)	2–4	3–6(7)	3–6(10)		(3)3.5–5.5(6)	(3)4–8(10)	(0.5)1.5–2.5(3)
Stomata distribution	Amphistomatic	Hypostomatic	Hypostomatic	Hypostomatic	Epistomatic	Amphistomatic	Hypostomatic		Amphistomatic
Relative lowermost bract-inflorescence length	Longer to shorter than the inflorescence	Longer than the inflorescence	Shorter than the inflorescence, rarely equalling it	Shorter than the inflorescence, rarely equalling or exceeding it	Equalling to slightly shorter than the inflorescence, rarely exceeding it		Equalling the inflorescence		Longer than the inflorescence
Terminal (male) spikes number	(1)2–3	2–4	1	1–2	1(2)		1(2)	(1)2–4	(1)2–3
Terminal (male) spike length	(15)20–60(85)	(15)20–50(60)	(12)15–30	25–80	(5)10–30		(15)20–60(70)	(10)20–70	(15)25–40
Lateral androgynous spikes	(0)1–3(4)	1–2	0	0–3(4)	(0)1		0–3(6)	(0)1–5	2–4
Lateral female spikes	0–2(3)	2–4	1–2(3)	0–2(3)	2–4		0–3(4)	0(1)	2–4
Utricle nerves	Nerved	Nerved	Nerveless	Nerved	Nerved		Nerved		Nerved

	C. quixotiana	***C. acuta***	***C. cespitosa***	***C. elata* subsp. *elata***	***C. nigra***		***C. reuteriana***		***C. trinervis***
					subsp. *nigra*	**subsp. *intricata***	**subsp. *reuteriana***	**subsp. *mauritanica***	
Utricle indumentum	With high whitish papillae on the upper half or towards the apex, sometimes aculeolate at the apex	Almost entirely covered with high papillae, somewhat inflated	With whitish high papillae towards the apex	With whitish high papillae, at least towards the apex, not inflated	Covered with high papillae at least the upper half		Smooth, very rarely with some scattered, low papillae towards the apex		Covered with low papillae
Chromosome number (2n)	82–83	84–86	78–80	74–80	(80)82–88(92?)		73–76	(72)74–76	82–85
Habitat (Iberian Peninsula)	Small streams, rivers and springs in riparian forests (*Alnus lusitanica*, *Fraxinus angustifolia*, *Salix* spp.), humid meadows, in marcescent and sclerophyllous *Quercus* forests, on siliceous bedrock substrate	River shores, usually in deciduous forests, without clear edaphic preferences regarding bedrock substrate	River shores in deciduous forest, on granitic substrates	River shores (or lakes) in diverse types of vegetation, on calcareous bedrock substrates, rarely on siliceous ones, also on coastal swamps	Montane-alpine wet meadows, bogs, and swamps, also in river and lake shores, in diverse types of vegetation and without clear edaphic preferences regarding bedrock substrate		Stream and river shores in diverse types of vegetation, usually on siliceous bedrock substrates, rarely on calcareous ones	Stream and river shores in riparian forests, in marcescent and sclerophyllous *Quercus* forests, without clear edaphic preferences regarding bedrock substrate	Sandy coastal swamps
Altitude (m)	400–800	0–1300	10–40	10–1750	1000–3300		300–1900	20–650	0–30

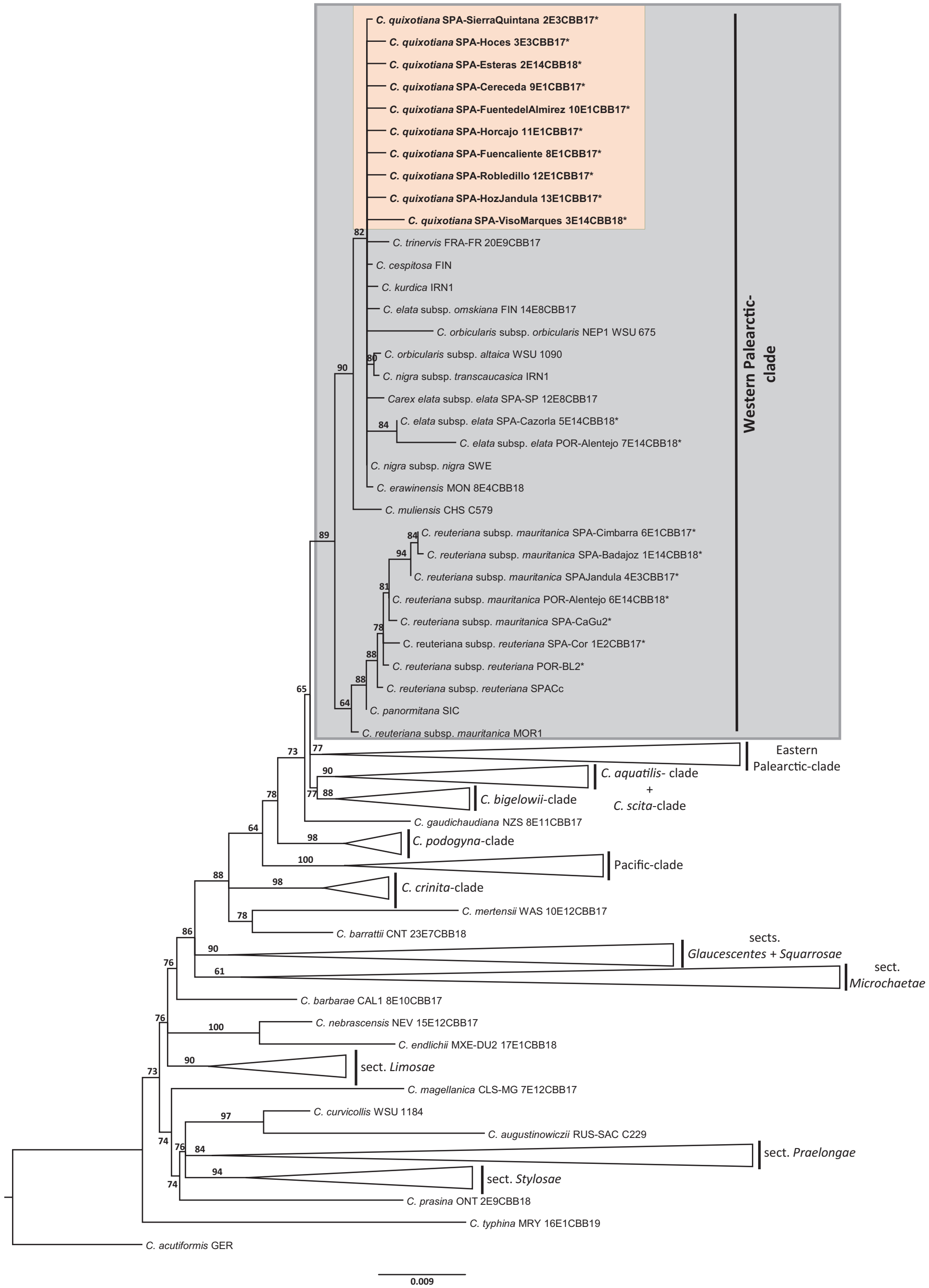

Figure 3. Phylogram of sect. *Phacocystis* s.l. based on the maximum likelihood phylogenetic reconstruction of the complete singletons tree (ITS, ETS, *rpl*32-*trn*L$^{\mathrm{UAG}}$, *ycf*6-*psb*M) by Benítez-Benítez et al. (2021). SH supports > 60 are given above branches. Tip codes are included in Suppl. material 3. The samples with asterisk (*) have been newly sequenced for this study. The orange highlighted populations represent La Mancha's problematic samples herein described as *Carex quixotiana*.

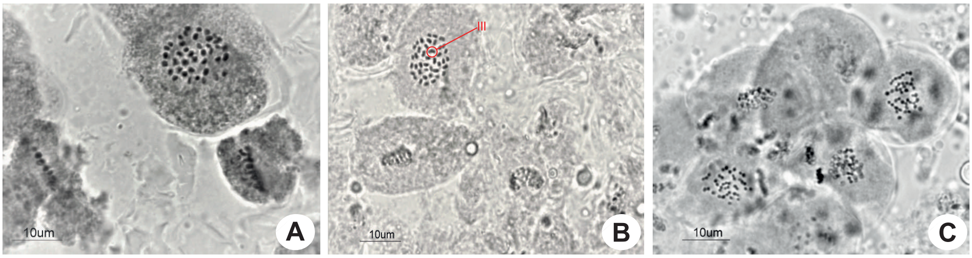

Figure 4. A regular meiotic configuration of *Carex quixotiana* in Metaphase I (2n=82) **B** meiotic configuration showing a trivalent in Metaphase I ($2n=40^{II} + 1\ ^{III}=83$) **C** pollen grain mitosis of the irregular cytotype in which two cells are visible (n=41 and n=42).

Cytogenetic study

The studied meiotic plates, representing five different La Mancha's problematic populations (Suppl. material 1), mostly displayed 82 chromosomes, with regular pairing (Fig. 4). Interestingly, the population 59SMB17 also showed $40^{II} + 1^{III}$ in Metaphase I, and n=41, 42 in pollen grain mitosis (from 30 counted plates, 16 showed n=41 and 14 n=42). Therefore, the inferred diploid chromosome numbers were 2n=82, 83 (Fig. 4).

Discussion

Different sources of evidence (morphological, cytogenetic, molecular) support the systematic distinctiveness of the studied La Mancha's problematic populations (Table 1, Fig. 3, Suppl. material 6). The greater morphological affinities of these populations are with *C. reuteriana* and *C. nigra*, however they show clear-cut morphological differences (Table 1). La Mancha's populations differ from these two species by leaf width and basal sheath colour. In addition, it differs from *C. reuteriana* by leaf stomata distribution, utricle indumentum and chromosome number (*C. reuteriana* (72)73–76 vs. *C. quixotiana* 82–83), and from *C. nigra* by the terminal male spike length. In addition to the appearance, the ecology of *C. nigra* in Sierra Nevada is fairly distinct as it is a dwarf sedge that forms dense tufts at habitats often above the timberline (from 1000 m and up to 3300 m), while La Mancha's populations are medium-size herbs that grow in low-medium elevation in riparian vegetation within *Quercus* forests.

Remarkably, the DNA markers used in the phylogenetic reconstructions showed that La Mancha's problematic populations do not group with *C. reuteriana*, to which they had been traditionally assigned (Luceño and Jiménez-Mejías 2008), while they are more related to *C. nigra* s.l. (Fig. 3). In addition, the haplotype network pointed to more genetic affinities with the populations of *C. nigra* from Sierra Nevada (Suppl. material 6), which are ca. 150 km south of La Mancha, than with *C. reuteriana.*

According to the evidence presented, La Mancha's problematic populations warrant taxonomic recognition, so we proceed to describe them as a new species.

Taxonomic treatment

Carex quixotiana Ben.Benítez, Martín-Bravo, Luceño & Jim.Mejías, sp. nov.

urn:lsid:ipni.org:names:77315699-1

Diagnosis. Similar in appearance to *C. reuteriana* Boiss. & Reut., from which it differs by the creamy-yellow, rarely reddish-brown basal sheaths (vs. orange to reddish-brown), amphistomatic leaves (vs. hypostomatic), and utricles with high papillae (vs. smooth or rarely with low papillae). It is also similar to *C. nigra* (L.) Reichard, from which it can be distinguished also by the creamy-yellow, rarely reddish-brown basal sheaths (vs. absent or dark brown when present), narrower leaves (1.8)2–3.2(4.7) mm (vs. 3–6(10) mm), and longer terminal male spike (18)20–60(85) mm (vs. (5)10–30 mm).

Type. Spain. Ciudad Real: Fuencaliente, Azor stream recreational area, stream edges in *Quercus faginea* forests, 733m, 38.44906944, -4.327163889, 10 May 2017, S. Martín-Bravo & C. Benítez-Benítez 41SMB17 (holotype!: UPOS-8925, 41SMB17(5); isotypes!: GDA, JAEN, K, MA, NY, P, SALA and UPOS).

Selected material examined (paratypes): Spain. Ciudad Real: Fuencaliente, Cereceda stream, with *Alnus lusitanica*, 695m, 38.42363889, -4.297472222, 10 May 2017, S. Martín-Bravo & C. Benítez-Benítez 47SMB17 (UPOS-8927); Fuente del Almírez, puddled meadows in *Quercus pyrenaica* forests, 800 m, 38.47152778, -4.344888889, 10 May 2017, S. Martín-Bravo & C. Benítez-Benítez 48SMB17 (UPOS-8926); Minas del Horcajo, gorge of Nacedero stream with *Salix* sp., 729 m, 38.51397222, -4.445750000, 11 May 2017, S. Martín-Bravo & C. Benítez-Benítez 54SMB17 (UPOS-8922); Solana del Pino, Robledillo River, riparian forests with *Alnus lusitanica* and *Fraxinus angustifolia*, 453 m, 38.41783333, -4.003388889, 11 May 2017, S. Martín-Bravo & C. Benítez-Benítez 57SMB17 (UPOS-8924); Solanilla del Tamaral, gorge of Jandula River (Hoz del Jándula) with *Alnus lusitanica*, 392 m, 38.39186111, -3.96333333, 11 May 2017, S. Martín-Bravo & C. Benítez-Benítez 59SMB17 (UPOS-8923); Valdemancos de Esteras, riverside of Esteras River with *Fraxinus angustifolia* and *Salix* sp., 453 m, 38.90733333, -4.794222222, 15 May 2018, C. Benítez-Benítez & S. Martín-Bravo 6CBB18 (UPOS-16896); Between Viso del Marqués and San Lorenzo de Calatrava, riverside of Ballesteros stream, in open forest of *Quercus pyrenaica* and *Salix* sp., 872 m, 38.44450000, -3.740444444, 16 May 2018, C. Benítez-Benítez & S. Martín-Bravo 8CBB18 (UPOS-16897); Puebla de Don Rodrigo, Sala del Halconcillo stream, 559 m, 39.10527778, -4.744722222, 15 June 2022, M. Sanz-Arnal, P. García-Moro & P. Jiménez-Mejías 13MSA22 (UPOS-16898); Horcajo de los Montes, Chorrera de Horcajo, 642 m, 39.36111111, -4.614999999, 15 June 2022, M. Sanz-Arnal, P. García-Moro & P. Jiménez-Mejías 16MSA22 (UPOS-16899); Viso del Marqués, Las Hoces, 780 m, 38.42303569, -3.721722958, 16 May 1991, C. Fernández García-Rojo (JAEN-914254); Puebla de Don Rodrigo, Río Frío mountain range near a birch forest, 600 m, 39.08243815, -4.503007649, 9 May 1992, Carrasco, Garrido & Martín-Blanco (MACB-68849); Solana del Pino, valley of Nacedero stream, 590 m, 38.482505309, -4.169451970, 26 April 1997, R. García

Río (MA-596319); Hinojosas, valley of Montoro River with *Alnus glutinosa*, Cervigón, 700 m, 38.491048629, -4.215454806, 5 May 1997, R. García Río (MA-596320); Almodóvar del Campo, Guadalmez River with *Alnus glutinosa*, 510 m, 38.513064049, -4.628724543, 4 July 1997, R. García Río (MA-596324). Jaén: Andújar, Sierra Quintana, Valmayor River, 570 m, 38.37460677, -4.144817294, 27 June 1985, E. Cano & C. Fernández García-Rojo (JAEN-855238).

Other material. (see Suppl. material 1).

Morphological description. ***Rhizomes*** from dense and tussock-forming to elongated. ***Stems*** (48)60–80(95) cm long, (0.8)1–1.2(1.3) mm wide below the inflorescence, sharply trigonous, smooth for most of its length, densely scabrid above. ***Basal sheaths*** scale-like, sometimes elongated, creamy-yellow, rarely reddish-brown, coriaceous. ***Leaves*** (1.8)2–3.2(4.7) mm wide, pale green to bluish-green, amphistomatic, flat to keeled, usually shorter or equalling the stems, antrorsely scabrid on the margins, especially distally, and on the mid-vein on the abaxial side to the apex; ***ligule*** (2)3–7(10) mm long, usually more than twice as long as wide, apex acute to obtuse, rarely rounded or truncate, hyaline, the margins brownish to orange-brown. ***Inflorescence*** (9)12–19(22) cm long, all spikes erect, exceptionally the lowermost slightly nodding. Lowest bract leaf-like, very rarely setaceous, longer to shorter than the inflorescence, sheathless, with hyaline, pale brownish to dark purplish-brown auricles at its base. Terminal ***male spikes*** (1)2–3; the uppermost spike (15)20–60(85) × 2–3.5 mm, subsessile to long pedunculate, oblong-cylindrical to narrowly fusiform, densely flowered; subterminal male spikes (0)1–2, (5)10–30(33) × (1)1.5–2.3(2.6) mm, similar in outline to the terminal one, basally overlapping with it or with an internode up to 20 mm. Lateral spikes female or androgynous with the flowers spirally arranged; ***female spikes*** 0–2(3), (19)25–50(79) × (2)2.8–4(5.2) mm, cylindrical, densely to more or less laxly flowered proximally; ***androgynous spikes*** (0)1–3(4), (9)20–50(62) × (1)1.7–2.7(5) mm, with the male portion (1)3–15(50) mm long and the female one (6)10–40(50) mm long. ***Male glumes*** (1.9)2.3–3.9(4.1) × (0.6)0.7–1.2(1.4) mm, oblong to obovate-oblong, apex rounded, usually dark purplish brown, with a green, 1-veined central band, with or without whitish hyaline margins, sometimes also becoming hyaline towards the base. ***Female glumes*** 1.3–2.3(2.5) × (0.6)0.7–0.9(1) mm, lanceolate to ovate, rarely elliptical, apex obtuse, acute or mucronate, usually shorter and narrower than the utricles, very rarely shortly exceeding them, dark purplish brown, rarely pale brown, with a green, 1–3 veined central band, with or without whitish hyaline margins, mainly in the distal part. ***Utricles*** (1.8)2–2.7(3) × (1.1)1.3–1.8(2) mm, plano-convex, widely elliptical to almost suborbicular, green to straw-coloured, distally whitish, sometimes purplish-dotted or purplish-tinged towards the apex, with high, whitish papillae on the upper half or towards the apex, sometimes also aculeolate at the upper margins, faintly to conspicuously nerved, shortly stipitate, more or less abruptly contracted into a short, cylindrical, truncate, more rarely emarginate beak (0)0.1–0.2(0.3) mm long, whitish, sometimes brown-tinged. ***Achenes*** 1.5–2.1 × (1.1)1.3–1.5(1.7) mm, widely elliptical to suborbicular, straw-coloured to pale brown, biconvex, more or less stipitate; style base terete to slightly conical, up to 0.3 mm. Stigmas 2.

Distribution. (Fig. 1) Endemic to South-Central Spain (Ciudad Real and marginally Jaén provinces). So far known from 16 populations (Suppl. material 1), mostly located in the Sierra Madrona range, but also extending north reaching the southern foothills of the Montes de Toledo range. Since it is a medium-size sedge rarely collected, there might be additional populations in these areas.

Habitat. Small streams, rivers and springs in riparian forests (*Alnus lusitanica*, *Fraxinus angustifolia*, *Salix* spp.), and humid meadows, in marcescent and sclerophyllous *Quercus* forests, on siliceous bedrock substrate. 400–800 m.

Phenology. (April) May-June (July).

Chromosome number. 2n=82, 83.

Iconography. Fig. 5.

Conservation status. So far known from 16 populations (subpopulations according to IUCN; Fig. 1), deduced from the studied material (Suppl. material 1). This implies a relatively restricted distribution range enclosed in an extent of occurrence (EOO) of 4.920 km^2, and an area of occupancy (AOO) of just 64 km^2. This would point to the application of criteria B1 and B2 of the Endangered (EN) category (threshold of 5.000 km^2 and 500 km^2 for EOO and AOO, respectively; IUCN 2017). The species' overall demographic tendency and number of mature individuals are unknown. In addition, the number of locations (16; n > 5) prevents the application of EN category since two conditions of criteria B must be fulfilled. Some populations are located in protected land (see below) so they should not be likely submitted to plausible threats in the short term (but see García Río 2007). With the currently available data, and taking into account the restricted EOO and AOO, we hypothesize that *C. quixotiana*'s formal IUCN conservation category at the global level would be Data Defficient (DD). As it is an Iberian endemic with a relatively small number of populations and distribution range, it would benefit from legal protection and inclusion in in-situ/ex-situ conservation programmes, at least at the regional level (Castilla-La Mancha, Andalucía).

Etymology. The species epithet, *quixotiana* (pronounced *kee·how·tee·a·na* in English) is derived from Miguel de Cervantes's (1547–1616) masterpiece Don Quixote (de Cervantes 1605, 1615), globally considered one of the best works in the history of literature, and whose number of editions and translations is only surpassed by the Bible. The setting of Don Quixote is La Mancha, the region of Spain where almost all populations of *Carex quixotiana* occur. We would like this epithet to serve as a double tribute: (1) First to Cervantes and his novel Don Quixote, flagship of Spanish culture. And (2) To Pedro Jiménez-Mejías's father, Pedro Jiménez Gilabert, an enthusiastic reader who always enjoys reading Don Quixote above all other books, and who always transmitted Pedro's curiosity and love for nature. In contrast to the first words in Don Quixote, ("En un lugar de La Mancha, de cuyo nombre no quiero acordarme (...)" [In a village of La Mancha, the name of which I have no desire to call to mind (…)]), we desire to remember and commemorate the dedication of this epithet we are coining.

Figure 5. Analytical drawing of *Carex quixotiana* (Spain, Ciudad Real, Sierra Madrona) **A** habit **B** plant **C** androgynous spike **D** female glume **E** male glume **F** achene **G** utricle **H** details of papillae in the upper part of the utricle. Drawing by M. Sánchez-Villegas.

Systematic notes. *Carex quixotiana* populations have been consistently assigned to *C. reuteriana*. Local floristic studies have predominantly identified them as *C. reuteriana* subsp. *reuteriana* (e.g., García Río 2004, 2007; Martín Blanco and Carrasco 2005; Fernández García-Rojo 2015; Fernández García-Rojo and Salazar-Mendías 2019), but also as *C. reuteriana* subsp. *mauritanica* (García Río 1999, 2004). Specialized taxonomic treatments have considered the populations as morphologically intermediate between the two *C. reuteriana* subspecies, which has been attributed to hybridisation in a putative contact zone between the two *C. reuteriana* subspecies (Luceño and Jiménez-Mejías 2008).

These problematic populations clearly illustrate the taxonomic complexity of sect. *Phacocystis*. Their overall morphological appearance and ecological preferences suggested that the populations were conspecific with *C. reuteriana* (Luceño and Jiménez-Mejías 2008). On the other hand, phylogenetic relationships (Fig. 2) and the relatively high chromosome number (see below) indicate closer genetic affinity with *C. nigra*. These misleading patterns denote the necessity of integrative approaches comprising different data sources to unmask complex systematic scenarios. Further phylogenomic studies based on High-Throughput-Sequencing techniques like GBS might provide a greater number of loci and therefore might help to shed more light on the systematic placement of *C. quixotiana* (Benítez-Benítez et al. unpublished data).

The chromosome number has been used as a biosystematic tool for sect. *Phacocystis* in the Iberian Peninsula (Luceño and Aedo 1994; Luceño and Jiménez-Mejías 2008). Our chromosome counts indicate that *C. quixotiana* (2n=82–83) has a greater cytogenetic affinity with *C. nigra* (2n=(80)82–86) than with *C. reuteriana* (2n=(72)73–76).

Biogeographic and conservation issues. Considering the new species described here, the Iberian Peninsula has 12 endemic *Carex* taxa (*C. asturica* Boiss., *C. camposii* Boiss. & Reut. subsp. *camposii*, *C. camposii* subsp. *tejedensis* R. Sánchez-Villegas, M.Escudero & Luceño, *C. caudata* (Kük.) Pereda & Laínz, *C. durieui* Steud. & Kunze, *C. furva* Webb, *C. lainzii* Luceño, E.Rico & T.Romero, *C. lucennoiberica* Maguilla & M. Escudero, *C. nevadensis* Boiss. & Reut., *C. quixotiana*, *C. reuteriana* ssp. *reuteriana* and *C. rorulenta* Porta). However, this may appear as a relatively small number of endemics in proportion to the total number of *Carex* Iberian native taxa (108; Luceño et al. unpublished data), especially when compared with other species-rich genera (e.g., *Alchemilla*, *Armeria*, *Centaurea*, *Limonium*, *Teucrium*) with much larger numbers of Iberian endemics (>40 each; Aedo et al. 2017; Buira et al. 2020). This highlights the novelty of the description of an additional Iberian endemic in *Carex*. Indeed, *C. quixotiana*, with a highly restricted distribution in southern-central Spain, would be the first Iberian endemic species belonging to sect. *Phacocystis*, although one subspecies (*C. reuteriana* ssp. *reuteriana*) is also endemic to C-NW parts of the Iberian Peninsula. Other species of sect. *Phacocystis* with limited distribution range in the Mediterranean basin are *C. reuteriana* ssp. *mauritanica* (S Iberian Peninsula-NW Africa), *C. nigra* ssp. *intricata* (Western Mediterranean mountains), and *C. panormitana* Guss. (Sicily, Sardinia and Tunisia).

As explained above, known populations of *C. quixotiana* are mostly located in La Mancha limits, in Sierra Madrona and, to a lesser extent, southern Montes de Toledo. Sierra Madrona is one of the secondary mountain ranges within the larger Sierra Morena range, which stretches for about 450 kms from W to E across the S Iberian Peninsula, separating the southern half of the Central Plateau from the Guadalquivir Valley. Sierra Madrona runs almost in parallel (NW–SE) along the northern side of the main range for about 80 kms. It includes the highest altitudes of all Sierra Morena (Bañuela peak, 1332 m). As the whole Sierra Morena, it is mainly composed by old Paleozoic siliceous rocks, especially quartzites. On the other hand, Montes de Toledo is a mountain range entirely belonging to the southern half of the Central Plateau, separating the Tajo and Guadiana River basins. It stretches for about 350 kms from E to W and is composed by quartzite ridges of relatively uniform elevation, around 1400 m, and a generally eroded relief (Muñoz Jiménez 1976). The immediate foothill landscapes are highly anthropised, with the vast majority of La Mancha dedicated to non-irrigated crops, mostly cereal (wheat, barley and oat), and vineyards.

Sierra Madrona is currently protected as a natural park established in 2011 (Valle de Alcudia and Sierra Madrona). Several restricted plant endemics from Sierra Morena are found in this range and its surroundings (i.e., *Armeria pauana* (Bernis) Nieto Fel., *Coincya longirostra* (Boiss.) Greuter and Burdet; García Río 2004). It also includes many different habitats protected at European level (Directive "Habitats" 92/43/CEE; see García Río 2004, 2007), including some considered of high conservation priority. *Carex quixotiana* habitats in Sierra Madrona, although included in a protected area, are not free of threats, so their biodiversity has been considered as highly valuable and sensitive (García Río 2007). They include populations of other endangered and protected plant species at regional (e.g., *Erica tetralix* L., *Drosera rotundifolia* L.) or even at national level (e.g., *Myrica gale* L., *Narcissus muñozii-garmendiae* Fern.Casas).

The other known populations of *C. quixotiana* occur mostly in rivers and creeks in the southern foothills of Montes de Toledo in W Ciudad Real province. These populations are located within or close to remarkable habitats, such as relictual peatbogs (e.g., Bonales de Puebla de Don Rodrigo) and birch forests (e.g., Abedular de Ríofrío), which are on protected land and considered to be of extraordinary conservation value. In addition, *C. quixotiana* marginally reaches NE Jaén province in Sierra Quintana, a small southern subrange of Sierra Madrona province (Cano-Carmona and Valle-Tendero 1996), which separates Castilla-La Mancha from Andalucía (Fig. 1). Sierra Madrona and Montes de Toledo are considered biogeographically close (García Río 2007). Interestingly, *C. reuteriana* subsp. *reuteriana* occurs in northern parts of Montes de Toledo, whereas *C. reuteriana* subsp. *mauritanica* is widespread in the main Sierra Morena range, therefore flanking C. quixotiana's distribution (Luceño and Jiménez-Mejías 2008; see maps in Benítez-Benítez et al. 2018). It would be interesting to precisely delimit these species' range and to explore potential contact zones in detail in order to investigate the possible co-occurrence and/or hybridisation processes.

Identification key to the Iberian taxa of *Carex* section *Phacocystis*

1 Leaves strongly plicate to canaliculate, rigid, up to 2.5(3) mm wide; stems obtusely trigonous; plants bluish-green .... ***C. trinervis***
– Leaves flat to carinate, soft to medium rigid, (1.8)2–8(10) mm wide; stems sharply trigonous; plants green to bluish-green **2**
2 Utricles not veined; basal sheaths dark purple .... ***C. cespitosa***
– Utricles faintly to prominently veined; basal sheaths yellowish-brown to reddish-brown **3**
3 Utricles smooth, rarely with some scattered, green, low papillae.................. **4**
– Utricles densely papillose, at least on the apex, with whitish, more or less high papillae **5**
4 Male spike usually solitary, rarely 2; lowest spikes usually female; leaves up to 6 mm wide .... ***C. reuteriana* subsp. *reuteriana***
– Male spikes (1)2–4; lowest spikes usually androgynous; leaves (3)4–8(10) mm wide.................................... ***C. reuteriana* subsp. *mauritanica***
5 Leaves epistomatic or amphistomatic **6**
– Leaves hypostomatic **8**
6 Male spikes (1)2–3, the terminal one (15)20–60(85) mm long; leaves (1.8)2–3.2(4.7) mm wide; plants usually tussock-forming; basal sheaths scale-like, sometimes elongated, creamy yellow, rarely reddish-brown .... ***C. quixotiana***
– Male spike solitary, rarely 2, the terminal one (5)10–30 mm long; leaves 3–6(10) mm wide; plants rarely tussock-forming; scale-like basal sheaths absent or dark brown when present **7**
7 Leaves densely stomatic on both sides (amphistomatic) .... ***C. nigra* subsp. *intricata***
– Leaves epistomatic or densely stomatic on the upper surface and with a few scattered stomata on the lower surface .... ***C. nigra* subsp. *nigra***
8 Lowest bract much longer than the inflorescence; utricles strongly biconvex, somewhat inflated; culm bases bearing brown old leaf remains, without conspicuous scale-like basal sheaths .... ***C. acuta***
– Lowest bract always shorter than the inflorescence; utricles plano-convex to slightly biconvex, not inflated; basal sheaths scale-like to elongated, creamy-yellow .... ***C. elata* subsp. *elata***

Conclusion

Integrative approaches based on different sources of evidence are required to unveil complex systematic scenarios. Our study of some problematic Iberian populations belonging to the taxonomically difficult sect. *Phacocystis* revealed that they display a congruent set of diagnostic morphological, molecular, ecological and cytogenetic characters that allow their distinction with respect to close relatives (e.g., *C. reuteriana*, *C. nigra*). Therefore, we here described a new species to science (*C. quixotiana*) for these populations, which is endemic to the Iberian Peninsula.

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Supplementary material 1

List of studied material of *C. quixotiana*, including the 16 known populations highlighted in red

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: occurrence data (excel document)

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl1

Supplementary material 2

List of specimens newly sequenced in the present study

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: Occurrence data (excel document)

Explanation note: Including taxon, sample code, locality, voucher and/or herbarium number, and NCBI accession numbers for each molecular marker (ITS, ETS, *rpl*32-*trn*LUAG, *ycf*6-*psb*M).

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl2

Supplementary material 3

List of studied material, with their accession numbers included, which were previously published by Benítez-Benítez et al. (2021)

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: occurrence data (excel document)

Explanation note: Tip codes with "spm" denote samples previously published by Global Carex Group works (Jiménez-Mejías et al. 2016 Martín-Bravo et al. 2019).

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl3

Supplementary material 4

Cladogram of sect. *Phacocystis*, including newly sampled sequences

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: phylogenetic tree (PDF file)

Explanation note: The cladogram is based on the maximum likelihood phylogenetic reconstruction of complete singleton's tree (ITS, ETS, *rpl32-trn*L, *ycf6-psb*M) following a scaffolding approach previously implemented by Benítez-Benítez et al. (2021). SH supports > 60 are given above branches.

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl4

Supplementary material 5

List of studied material for haplotype network analysis, displaying the corresponding haplotype number obtained for each sample

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: table (excel document)

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl5

Supplementary material 6

Haplotype network obtained from the statistical parsimony analysis of the combined plastid sequences of Iberian *Carex* sect. *Phacocystis* species, including *C. quixotiana*.

Authors: Carmen Benítez-Benítez, Pedro Jiménez-Mejías, Modesto Luceño, Santiago Martín-Bravo

Data type: image (PDF file)

Explanation note: Small circles represent inferred extinct or not sampled haplotypes and lines mutational connections.

Link: https://doi.org/10.3897/phytokeys.221.99234.suppl6