

Description of four new terrestrial diatom species from *Luticola* and *Microcostatus* genera from South Africa

Mateusz Rybak¹, Natalia Kochman-Kędziora², Łukasz Peszek¹

1 University of Rzeszów, Department of Agroecology and Forest Utilization, Źwiklińskiej 1a, 35-601, Rzeszów, Poland **2** University of Rzeszów, Department of Ecology and Environmental Protection, Żelwerowicza 4, 35-601, Rzeszów, Poland

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

Academic editor: Kalina Manoylov | Received 1 March 2021 | Accepted 21 July 2021 | Published 14 September 2021

Citation: Rybak M, Kochman-Kędziora N, Peszek Ł (2021) Description of four new terrestrial diatom species from *Luticola* and *Microcostatus* genera from South Africa. *PhytoKeys* 182: 1–26. <https://doi.org/10.3897/phytokeys.181.65326>

Abstract

The knowledge about terrestrial diatom assemblages in southern Africa is rather limited, despite a long history of diatom research in this area. Terrestrial habitats are places of characteristic diatom floras, dominated by species resistant to desiccation which are able to thrive in conditions of limited water availability. The presented work expands the knowledge about these unique habitats. During the study on terrestrial moss-inhabiting diatoms from Western Cape Province (South Africa), four taxa with a unique set of valve features have been found and described herein as new species, based on light and scanning electron microscopy. These new species are: *Luticola microcephala* M. Rybak, Peszek & Kochman-Kędziora, **sp. nov.**, *Luticola asymmetrica* M. Rybak, Kochman-Kędziora & Peszek, **sp. nov.**, *Luticola terrestris* Kochman-Kędziora, M. Rybak & Peszek, **sp. nov.** and *Microcostatus meridionalis* Peszek, M. Rybak & Kochman-Kędziora, **sp. nov.** Algal community composition had low species richness (9–15 taxa per sample) and samples were dominated by: *Humidophila contenta*, *Nitzschia brevissima* and *Eunotia* aff. *pseudominor*. The three new *Luticola* species formed numerous populations, exceeding 10% of the share, whereas *Microcostatus meridionalis* reached 5.4%. Basic morphological data of associated diatom flora together with detailed micrographs are also provided.

Keywords

Bacillariophyceae, bryophytes, *Luticola*, *Microcostatus*, new species, South Africa

Introduction

Research on African diatoms has a long tradition, with the first works dating back to the 19th century (i.e. Ehrenberg 1845; Cleve 1881). The dynamic development of diatomological research took place mainly in the second half of the 20th century. Research from diverse habitats was carried out by four scientists: B.J. Cholnoky, M.H. Giffen, F.R. Schoeman and R.E.M. Archibald, whose efforts are the foundation of more recent studies (i.e. Cocquyt et al. 2017; Cocquyt and Taylor 2019; Ridder and Taylor 2020). The general diatom diversity of inland waters from southern Africa was studied by Cholnoky (e.g. Cholnoky 1960, 1962, 1966), who also described many new species (Cholnoky 1954, 1955, 1956, 1957, 1966). On the other hand, Giffen, in his works from the mid-1960s, dealt mainly with marine and estuarine diatoms (i.e. Giffen 1963, 1967, 1970, 1973, 1976), as well as some freshwater species from the Eastern Cape Region (Giffen 1966). Undoubtedly, the greatest contribution to understanding both the taxonomy and ecology of diatoms was made by Schoeman and Archibald, who, for over 15 years, published together over 20 works on diatom flora in southern Africa, supplemented by hand-drawings (i.e. Schoeman 1969, 1970; Archibald 1966) and, in subsequent years, also with microphotographs of the observed species (i.e. Schoeman and Archibald 1986a, 1986b, 1986c, 1986d; Archibald and Schoeman 1984, 1985). More recent researchers continued to work on taxonomy (i.e. Cocquyt 2006; Cocquyt et al. 2013, 2014, 2016; Taylor et al. 2014a, b), but also concentrated on ecological monitoring (i.e. Bellinger et al. 2006; Taylor et al. 2007b, c). There are two elaborate reports of the most common diatom species from the Congo and Zambezi Basins (Taylor and Cocquyt 2016) and South Africa (Taylor et al. 2007a). In recent years, many new species were described from different parts of Africa. Various studies confirm the presence of a unique diatom diversity, composed of rare and endemic diatoms species (Cocquyt et al. 2013, 2014, 2017; Cocquyt and Ryken 2017; Taylor et al. 2014b).

Despite a long history of diatom research in southern Africa, the knowledge about terrestrial diatom assemblages is rather limited (Taylor et al. 2010, 2014a; Van de Vijver et al. 2010). Although most of the studies focused on marine, freshwater and brackish diatoms, there are still some unexplored aerial habitats. A study of terrestrial moss-inhabiting diatom communities, conducted by Van de Vijver et al. (2010), resulted in the description of one species, *Muelleria taylorii* Van de Vijver & Cocquyt from Drakensburg, Freestate Province, South Africa.

Two genera are mainly considered to occur in terrestrial habitats, like mosses, rocks or soils, the genus *Luticola* and genus *Microcostatus* (Round et al. 1990; Johansen and Sray 1998). The genus *Luticola* D.G. Mann (Round et al. 1990) was distinguished from *Navicula* to accommodate species included in *Naviculae* sect. *Punctatae* with *Luticola mutica* (Kütz.) D.G. Mann as generitype. So far, over 200 species have been documented worldwide (Guiry and Guiry 2021). A monograph, summarising the entire genus (Levkov et al. 2013), presented a detailed and extensive revision of the *Luticola* genus, including more than 20 species observed from Africa. Still, more recent studies show that the diversity of the genus *Luticola* is underestimated and many new

species have been described from Europe (Levkov et al. 2017), Asia (Liu et al. 2017; Glushchenko et al. 2017; Lokhande et al. 2020), South America (Bağ et al. 2017; Straube et al. 2017; Simonato et al. 2020; Peszek et al. 2021), Madagascar (Bağ et al. 2019) and Antarctic Region (Zidarova et al. 2014; Kohler et al. 2015; Chattová et al. 2017; Kochman-Kędziora et al. 2020). The genus *Luticola* is highly diverse in valve morphology. Species of this genus are also widespread in brackish, freshwater and terrestrial ecosystems. However, the genus is ecologically characterised as being aerophilous, often noted from sites in splash zones, soil and amongst mosses (Round et al. 1990; Kociolek et al. 2017). It also shows a high degree of endemism (Kociolek et al. 2017; Lokhande et al. 2020).

The genus *Microcostatus*, described by Johansen and Sray (1998), encompasses small naviculoid species with two longitudinal depressions next to the central sternum, a simple raphe system and external microcostae. Until now, this small genus has only 24 species documented worldwide (Guiry and Guiry 2021). Taylor et al. (2010) listed 23 taxa, including three described from South Africa. More recently, papers described additional six species: *Microcostatus edaphicus* Stanek-Tarkowska, Noga, Wetzel & Ector and *M. aerophilus* Stanek-Tarkowska, Noga, Wetzel & Ector from Europe (Stanek-Tarkowska et al. 2016), *M. salinus* Li & Suzuki (Li et al. 2016) and *M. werumii* Metzeltin, Lange-Bertalot & Soninkhishig (Metzeltin et al. 2009) from Asia and two other species: *M. australoshetlandicus* Van de Vijver, Kopalová, Zidarova & E.J. Cox and *M. elisabethianus* Van de Vijver & Ector from Antarctic and Sub-Antarctic Region (Van de Vijver et al. 2013; Van de Vijver and Ector 2019).

The present paper aims to contribute information on the distribution and environmental preferences of terrestrial diatoms in South Africa. This is the first paper providing information about moss-inhabiting diatom assemblages from Western Cape Province, South Africa. Three taxa from the genus *Luticola* and one from *Microcostatus*, which cannot be identified using currently available literature, were observed during the study. Additional analysis, based on detailed light and scanning electron microscopy, showed a set of unique features allowing us to describe them as new species. Additional comparison with the most similar taxa is also provided.

Methods

The study area is situated in the Western Cape Province, on the south-western coast of South Africa. The climatological conditions in the Western Cape Province are influenced by both the Indian and Atlantic oceans. Most of the Province is considered to have a Mediterranean climate characterised by cool and wet winters (June–August), whereas summers (December–February) are warm and dry (Ndlovu et al. 2019). The Western Cape is widely-known for its high level of endemism in terrestrial plants, especially fynbos vegetation and naturally-occurring acidic water (Brown and Magoba 2009).

For this study, the moss samples were collected in September 2018 from three different study sites in Western Cape Province, South Africa. Two sites were located

Table 1. Sampling site characteristics.

Sample number	Locality	Coordinates	Altitude (m a.s.l.)	Substrate	Sampling date
2018/424	Prince Alfred's Pass, Knysna, Western Cape, South Africa	33°58.458'S, 23°08.811'E	428	terrestrial moss collected from soil	20 September 2018
2018/425	Prince Alfred's Pass, Knysna, Western Cape, South Africa	33°58.475'S, 23°08.797'E	428	terrestrial moss collected from rock	20 September 2018
2018/426	Jonkershoek Nature Reserve, Western Cape, South Africa	33°59.695'S, 18°58.726'E	397	terrestrial moss collected from rock	02 September 2018

on the edge of the forest by the Prince Alfred's Pass. The third site was situated in the Jonkershoek Nature Reserve, where the sample was collected from moss growing on the rock (Table 1). The samples were collected under permit No: CN35-285316 issued by CapeNature, OP 3570/2018.

In the laboratory, the moss samples were digested in sulphochromic mixture – a mixture of concentrated sulphuric acid and potassium dichromate in order to obtain clean diatom frustules. To remove the sulphochromic mixture, the material was centrifuged at 2500 rpm with distilled water. The cleaned diatom suspension was dried on microscope cover-slips and mounted in the synthetic Naphrax Brunel Microscopes Ltd., Chippenham, UK (refractive index 1.73). Diatoms were identified and counted under a light microscope (LM) Carl Zeiss Axio Imager A2, equipped with a 100× Plan Apochromatic objective with differential interference contrast (DIC) for oil immersion. Diatom images were captured with a Zeiss AxioCam ICc5 camera. For the observations in a scanning electron microscope (SEM), the samples were applied to a polycarbonate membrane filter with a 3 µm mesh, attached to aluminium stubs and sputter-coated with 20 nm of gold, using a turbo-pumped Quorum Q 150T ES coater. Diatoms were analysed using a Hitachi SU8010 SEM. The storage locations of holotype and isotype slides (Diatom Collections) for each of the newly-described species are indicated in the descriptions. Diatom terminology and identification was based on the following references: Round et al. (1990); Taylor et al. (2007a, 2010); Metzeltin and Lange-Bertalot (2002); Levkov et al. (2013); Taylor and Cocquyt (2016); Houk et al. (2017); Lange-Bertalot et al. (2017). Species composition and relative abundance of taxa in diatom assemblages were determined by counting 300 valves in each sample.

Results

Descriptions of new species

Division: Bacillariophyta Haeckel

Class: Bacillariophyceae Haeckel

Subclass: Bacillariophycidae D.G. Mann

Order: Naviculales Bessey

Family: Diadesmidaceae D.G. Mann

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

***Luticola microcephala* M. Rybak, Peszek & Kochman-Kędziora, sp. nov.**

Fig. 1

Holotype. Slide no. 20-093 stored at the South African National Diatom Collection (SANDC) at North-West University, Potchefstroom, South Africa.

Isotype 1. Slide no. 27525 and unmounted material with the same number at the Szczecin Diatom Collection (SZCZ) hosted by the University of Szczecin.

Isotype 2. Slide no. 2018/426 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Jonkershoek Nature Reserve, Western Cape, South Africa, 33°59.695' S, 18°58.726' E, *leg.* W. Morek and B. Surmacz, 20.09.2018.

Etymology. The specific epithet refers to the size and shape of valve apices.

Description. LM (Fig. 1A–V). Valves linear-lanceolate to lanceolate with convex margins and clearly protracted, capitate, small apices, rectangular in girdle view. The width of apices is approximately one third of the valve width. Valve dimensions ($n = 25$): length 14.0–24.0 μm , width 4.5–6.6 μm . Axial area linear, narrow. An isolated pore present in the central area, located halfway between valve margin and proximal raphe endings. Central area rectangular to slightly bow-tie-shaped and asymmetric, bordered on both sides with 3–4 areolae. Irregularly-scattered areolae and shallow depressions present in the central area. Raphe branches straight, proximal raphe endings deflecting away from isolated pore. Transapical striae radiate throughout, 19–22 in 10 μm .

Description. SEM (Fig. 1W–AD). Externally, striae composed of 1–4 areolae, decreasing from 3–4 in striae next to the central area to only one next to the apices. Areolae elongated, becoming larger towards the valve margin (Fig. 1W, X, AA, AB). On both sides, the central area bordered by 3 round, isolated areolae. Several ghost areolae present in the central area (Fig. 1AA, AC). Raphe branches positioned on the slightly raised sternum (Fig. 1Z). Proximal raphe endings shortly bent away from the small, round isolated pore (Fig. 1 AA, AC). Distal raphe fissures hooked, first deflected towards the same side as the proximal raphe endings, then hooked towards the opposite side, continuing on to the mantle (Fig. 1W, X, AB). Single row of large, usually elongated areolae present on the mantle (Fig. 1Y). Only close to the apices and in the central part of the valve, areolae becoming smaller and rounded (Fig. 1Y, Z). Copulae numerous with 1 to 3 rows of areolae (Fig. 1Z). Internally, areolae occluded by hymenes forming continuous strip (Fig. 1AD). Isolated pore opening rounded, covered by a lipped slit (Fig. 1AD). Longitudinal channel visible internally along valve edges.

***Luticola asymmetrica* M. Rybak, Kochman-Kędziora & Peszek, sp. nov.**

Fig. 2

Holotype. Slide no. 20-091 stored at the South African National Diatom Collection (SANDC) at North-West University, Potchefstroom, South Africa.

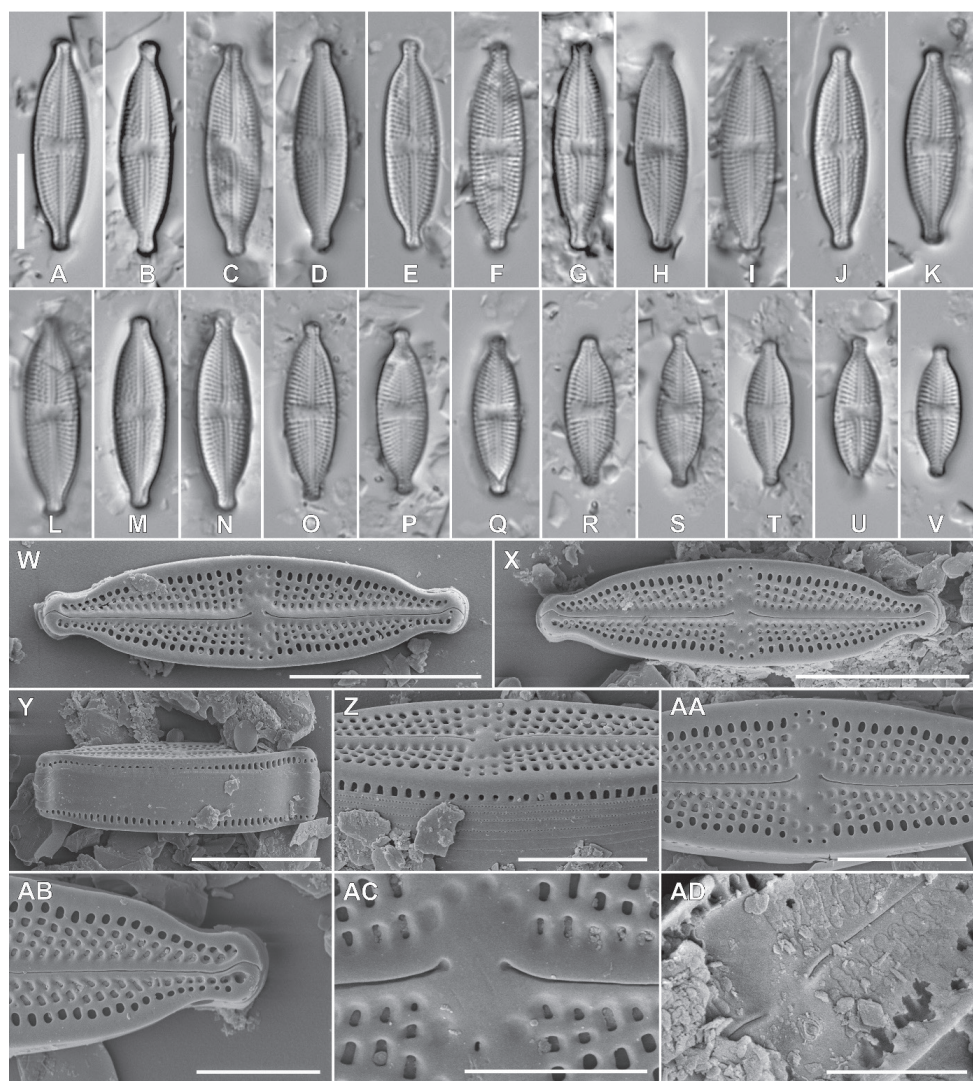


Figure 1. A–AD Holotype population of *Luticola microcephala* M. Rybak, Peszek & Kochman-Kędziora, sp. nov. A–V LM images of valve views W–AD SEM images W–X external view of valves Y external view of frustule girdle view Z partial valve view and girdle view of middle frustule section AA central area with several shallow depressions AB raphe structure with detailed view on distal raphe endings AC proximal raphe endings AD internal view of valve. Scale bars: 10 μm (A–Y), 5 μm (Z–AA), 4 μm (AB), 3 μm (AC, AD).

Isotype 1. Slide no. 27523 and unmounted material with same number stored at the Szczecin Diatom Collection (SZCZ) hosted by the University of Szczecin.

Isotype 2. Slide no. 2018/425 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Prince Alfred's Pass, Knysna, Western Cape, South Africa, 33°58.475'S, 23°08.797'E, leg. W. Morek and B. Surmacz, 20.09.2018.

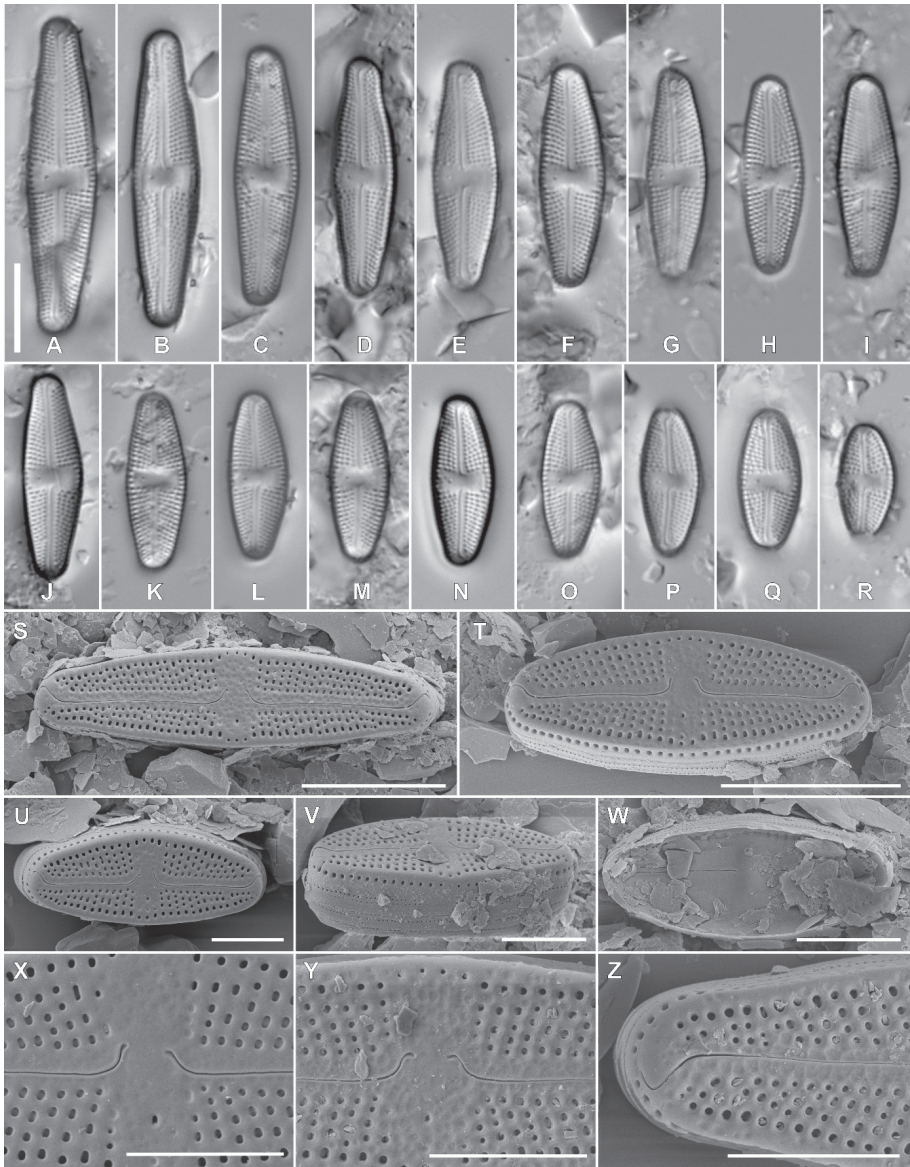


Figure 2. Holotype population of *Luticola asymmetrica* M. Rybak, Kochman-Kędziora & Peszek, sp. nov. **A–R** LM images of valve views **S–Z** SEM images **S–V** external view of an entire valve **W** internal valve face view of an entire valve **X–Y** different shape of proximal raphe endings **Z** distal raphe endings. Scale bars: 10 µm (**A–T**), 5 µm (**U–Z**).

Etymology. The specific epithet refers to the species asymmetry in valve outline and proximal raphe endings.

Description. **LM** (Fig. 2A–R). Valves weakly asymmetric with convex margins, rectangular in girdle view. Larger valves lanceolate with protracted, subrostrate apices; smaller valves elliptic–lanceolate to rhombic lanceolate with broadly-rounded apices. Valve dimen-

sions ($n = 25$): length 12.5–36.0 μm , width 6.0–8.0 μm . Axial area linear, slightly widening towards both the central area and the apices. Central area bow-tie-shaped, bordered by 3–5 shortened striae. One isolated pore present in the central area. Raphe branches straight. Proximal raphe endings unilaterally deflected away from the isolated pore. Terminal raphe fissures elongated, hooked. Striae radiate throughout, 17–20 in 10 μm .

Description. SEM (Fig. 2S–Z). Striae composed of 2–5 elongated areolae. Areolae close to the valve margin larger (Fig. 2S, T, Z). Usually 3–5 isolated areolae positioned on both sites in central area, close to the valve margin. Small, round isolated pore located in the central area, halfway between the valve margin and proximal raphe endings (Fig. 2S–U, X, Y). In some specimens, small, irregular depressions present on the valve face producing uneven appearance of the valve face (Fig. 2S, X–Z). Raphe branches straight. Proximal raphe fissures long, unilaterally deflected to the side opposite to stigma and expanded into small pores. In some specimens, proximal raphe endings asymmetrical (Fig. 2T, U, X, Y). Distal raphe fissures hooked towards opposite side, terminating shortly before valve edge (Fig. 2S–V, Z). Distal raphe fissures interrupting row of areolae on the valve mantle (Fig. 2Z). One row of round areolae present on the valve mantle (Fig. 2T–V). Copulae with 2 rows of areolae (Fig. 2V). Internally, areolae occluded by hymenes forming irregular strip. Isolated pore opening rounded, covered by a lipped slit (Fig. 2W).

***Luticola terrestris* Kochman-Kędziora, M. Rybak & Peszek, sp. nov.**

Fig. 3

Holotype. Slide no. 20-092 stored at the South African National Diatom Collection (SANDC) at North-West University, Potchefstroom South Africa.

Isotype 1. Slide no. 27524 and unmounted material with same number at the Szczecin Diatom Collection (SZCZ) hosted by the University of Szczecin.

Isotype 2. Slide no. 2018/424 and unmounted material with the same number at the University of Rzeszów, Poland.

Type locality. Prince Alfreds's Pass, Knysna, Western Cape, South Africa, 33°58.458'S, 23°08.811'E, *leg.* W. Morek and B. Surmacz, 20.09.2018.

Etymology. The specific epithet refers to the terrestrial habitat from where the new species is described.

Description. LM (Fig. 3A–W). Larger valves lanceolate with weakly-protracted apices; smaller valves rhombic-lanceolate, rectangular in girdle view. Apices usually rounded, in larger valves, slightly subcapitate. Valve dimensions ($n = 25$): length 8.0–28.5 μm , width 4.4–6.1 μm . Axial area linear, slightly widening towards both the central area and the apices. Central area bow-tie-shaped, often asymmetrical, bordered by shortened striae. One isolated pore present in the central area. Raphe branches straight. Proximal raphe endings unilaterally deflected away from the isolated pore; terminal raphe fissures elongated and hooked. Striae radiate throughout, 20–23 in 10 μm .

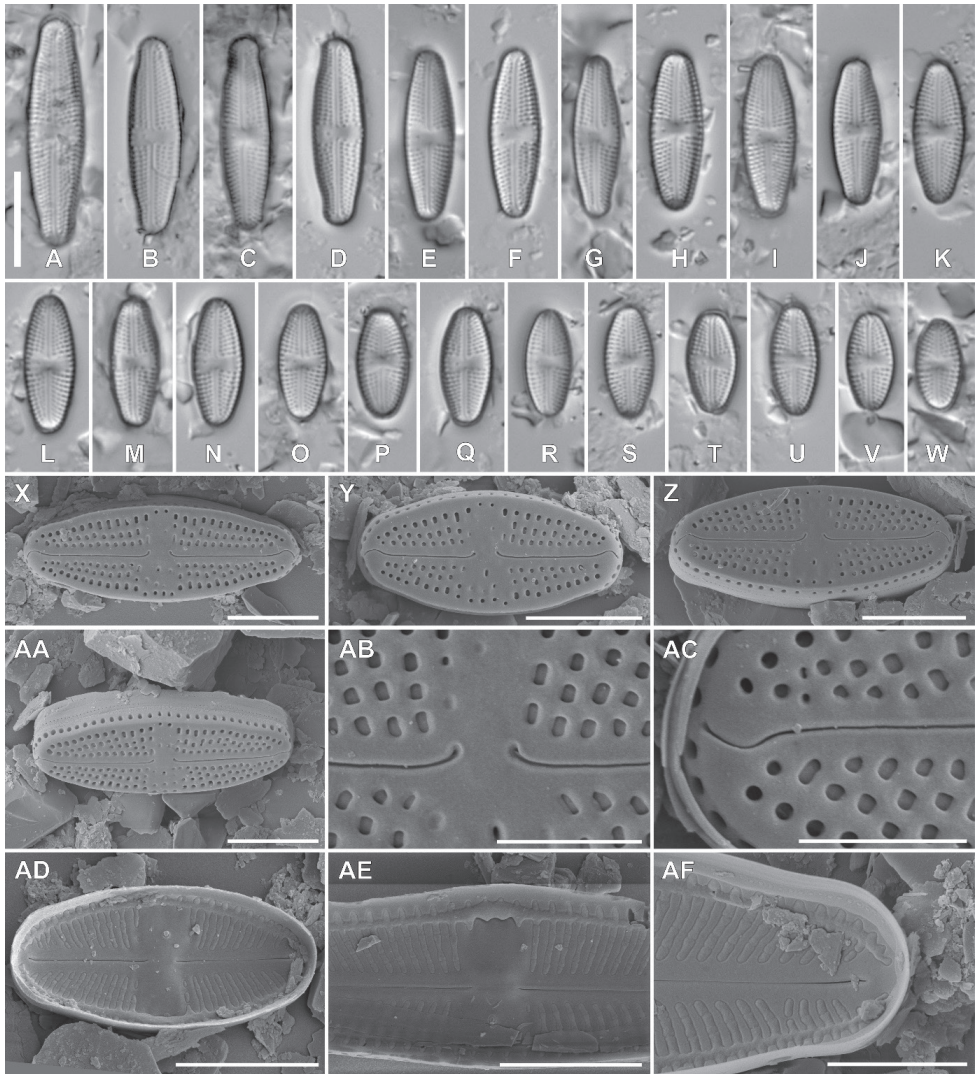


Figure 3. Holotype population of *Luticola terrestris* Kochman-Kędziora, M. Rybak & Peszek, sp. nov. **A–W** LM images of valve views **X–AF** SEM images **X–AA** external view of an entire valve with several ghost areolae in the central area **AB** proximal raphe endings **AC** distal raphe endings **AD** internal view of an entire valve **AE** central area, internal view **AF** valve apex, internal view. Scale bars: 10 μ m (**A–W**), 5 μ m (**X–AA, AD, AE**), 4 μ m (**AF**), 2 μ m (**AB, AC**).

Description. **SEM** (Fig. 3X–AF). Striae composed of 2–4 transapically elongated areolae becoming larger towards the valve margins (Fig. 3X–AA). One elongated stigma present, positioned in between the proximal raphe endings and the valve face margin (Fig. 3X–Z). Ghost areolae often present, mainly on a stigma-bearing site (Fig. 3X, AA). Raphe branches straight. Externally, proximal raphe endings deflected away from the isolated pore-bearing side with small drop-like endings (Fig. 3AB). Terminal raphe fis-

tures clearly elongated, first curved to the same side as the proximal raphe fissures, then slightly bent to the isolated pore-bearing side. Terminal raphe fissures are towards the valve apices, terminating on the valve face/mantle junction, well after the final row of areolae (Fig. 3AC). Valve mantle bearing a single row of rounded areolae (Fig. 3Z, AA). Copulae with single row of areolae (Fig. 3AA). Internally, areolae occluded by hymenes, forming a continuous strip across each stria (Fig. 3AD–AF). Internal isolated pore opening rounded, covered by a lipped slit. Proximal and terminal raphe endings weakly deflected towards the pore (Fig. 3AE), the latter terminating on to small helictoglossae (Fig. 3AF). Longitudinal channel visible along valve edges (Fig. 3AE, AF).

Division: Bacillariophyta Haeckel

Class: Bacillariophyceae Haeckel

Subclass: Bacillariophycidae D.G. Mann

Order: Naviculales Bessey

Family: Naviculaceae Kützinger

Genus: *Microcostatus* Johansen & Sray, 1998

Microcostatus meridionalis Peszek, M. Rybak & Kochman-Kędziora, sp. nov.

Fig. 4

Holotype. Slide no. 20-093 stored at the South African National Diatom Collection (SANDC) at North-West University, Potchefstroom, South Africa.

Isotype 1. Slide no. 27525 and unmounted material with same number at the Szczecin Diatom Collection (SZCZ) hosted by the University of Szczecin.

Isotype 2. Slide no. 2018/426 and unmounted material with the same number at the University of Rzeszow, Poland.

Type locality. Jonkershoek Nature Reserve, Western Cape, South Africa, 33°59.695'S, 18°58.726'E, *leg.* W. Morek and B. Surmacz, 20.09.2018.

Etymology. The name refers to the area from where the new species is described (lat. *meridional* – southern).

Description. LM (Fig. 4A–O). Valves lanceolate to elliptical-lanceolate with convex valve margins. Valve dimensions ($n = 25$): length 7.5–14.0 μm , width 3.5–4.5 μm . Striae in light microscopy invisible. Raphe branches straight. Raphe located in elevated sternum, asymmetrically concave at the centre. Proximal raphe endings clearly visible, drop-like in shape and widely spaced. Distal raphe endings barely visible, curved in the same direction.

Description. SEM (Fig. 4P–AA). Longitudinal depressions present next to the sternum (Fig. 4P–U). Striae strongly radiating (36–42 in 10 μm), composed of one row of square to rounded areolae, externally not occluded, except central area (Fig. 4P–X). Central area large, lanceolate in shape, composed of fully externally silicified areolae composing striae (Fig. 4P–T, X). Raphe straight, filiform, located in raised and asymmetrically-constricted sternum (Fig. 4P–V, Y, Z). Proximal raphe fissures slightly asymmetrical drop-shape (Fig. 4U, Y, Z). Distal raphe fissures hooked and deflect-

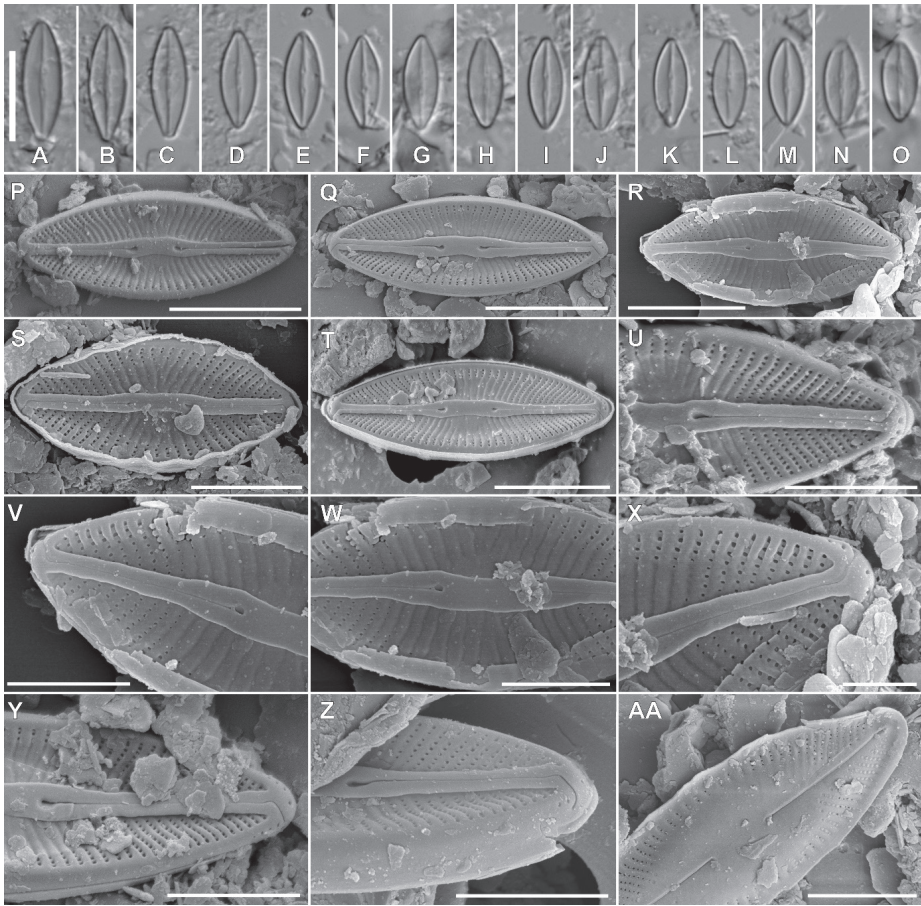


Figure 4. Holotype population of *Microcostatus meridionalis* Peszek, M.Rybak & Kochman-Kędziora, sp. nov. **A–O** LM images of valve views **P–AA** SEM images **P–T** images of entire valve external views **U–Z** view on valve central area and valve apices **AA** internal view of valve. Scale bars: 10 μm (**A–O**), 5 μm (**P, Q**), 4 μm (**R**), 3 μm (**S–U, Y–AA**), 2 μm (**V, W**), 1 μm (**X**).

ed to the same direction (Fig. 4V, X–Z). Transverse microcostae most prominent at the area near to valve margin (area of striae not covered by silica) (Fig. 4U–Y). A row of rounded to slightly elongated pores (3–5) present on the valve mantle, at the valve apices (Fig. 4U, Y, Z). Internally areolae occluded by hymenes. Raphe branches straight, forming small and elongated helictoglossae on distal endings, proximal endings drop-shaped. The central area is broad, corresponds in shape to external depression (Fig. 4AA).

Species composition and diversity of moss-inhabiting diatom communities

A total of 20 diatom taxa were observed in all samples. Amongst them, four were described as a new species (Table 2, Figs 5–9).

Table 2. List of identified taxa together with dimensions and the percentage share in the assemblage. N – number of measured specimens.

Taxa	N	Dimensions and stria density			Share in the assemblage [%]		
		Length [µm]	Width [µm]	Striae [in 10 µm]	Sample 2018/424	Sample 2018/425	Sample 2018/426
<i>Eumotia</i> aff. <i>pseudominor</i> Pavlov & Levkov	25	9.9–33.4	3.2–4.1	13–16	1.8	–	18.3
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	25	29.6–43.1	4.6–5.7	26–30	2.4	< 0.1	8.4
<i>Humidophila contenta</i> (Grunow) Lowe, Kociolek, J.R. Johansen, Van de Vijver, Lange-Bertalot & Kopalová	15	6.2–11.1	2.1–2.8	ca. 40	57.7	62.1	1.5
<i>Humidophila</i> sp. 1	5	11.6–17.7	2.5–3.1	38–39	< 0.1	< 0.1	–
<i>Humidophila</i> sp. 2	5	9.6–11.7	2.4–2.7	–	–	–	< 0.1
<i>Luticola asymmetrica</i> sp. nov.	25	12.5–36.0	6.0–8.0	17–20	–	11.6	–
<i>Luticola distinguenda</i> (Hustedt) Levkov, Metzeltin & Pavlov	10	25.7–32.3	9.5–10.6	16–19	–	< 0.1	–
<i>Luticola intermedia</i> (Hustedt) Levkov, Metzeltin & Pavlov	25	9.4–25.6	4.6–6.6	20–24	9.4	ca. 1	–
<i>Luticola microcephala</i> sp. nov.	25	14–24.0	4.5–6.6	19–22	6.6	–	12.6
<i>Luticola permuticoides</i> Metzeltin & Lange-Bertalot	25	6.3–20.4	5.1–7.4	19–23	–	12.5	–
<i>Luticola terrestris</i> sp. nov.	25	8.0–28.5	4.4–6.1	20–23	13.2	–	1.4
<i>Luticola</i> cf. <i>cristinae</i> Levkov, Metzeltin & Pavlov	2	17–21.4	5.3–6.2	23–24	–	–	< 0.1
<i>Microcostatus meridionalis</i> sp. nov.	25	7.6–13.8	3.5–4.5	42–48	–	–	5.4
<i>Muelleria</i> sp.	5	23.8–36.1	4.9–5.1	20–22	–	–	1.9
<i>Nitzschia brevissima</i> Grunow	15	15.6–41.1	3.7–4	40–43	7	< 0.1	21.8
<i>Nupela lesothensis</i> (Schoeman) Lange-Bertalot	3	8.5–12.5	2.8–3.5	42	–	–	< 0.1
<i>Orthoseira circularis</i> (Ehrenberg) R.M. Crawford	10	Ø: 11.7–24.3		22–24	3.3	11.6	< 0.1
<i>Pinnularia borealis</i> Ehrenberg <i>sensu lato</i>	20	23–49.6	6.2–9.4	4.5–5	2.3	–	13.8
<i>Pinnularia</i> sp.	10	15.7–24.8	2.8–3.3	5–6.5	–	–	3.8
<i>Stauroneis</i> cf. <i>pygmaea</i> f. <i>undulata</i> Hustedt	15	19.7–34	3.6–5.1	21–25	–	–	7.6

Discussion

The vast majority of research on diatoms in Africa was conducted in the second half of the twentieth century and many species descriptions are based on sketches and the classification of species is based on broad approaches to genera (Kociolek and Williams 2015). Many of them still wait for their verification using modern-day taxonomy focusing on detailed microscopic analysis.

Originally, many species of the genus *Luticola* were described as *Navicula* of section Punctatea. The genus verification made by Levkov et al. (2013) transferred most of them to *Luticola*. However, some taxa are still waiting for verification and potential transfer. Probably, based on sketches, the following species potentially are belonging to genus *Luticola*: *Navicula lagerheimii* f. *rotundata* Cholnoky (1954, p. 218, pl. 3, fig. 81),

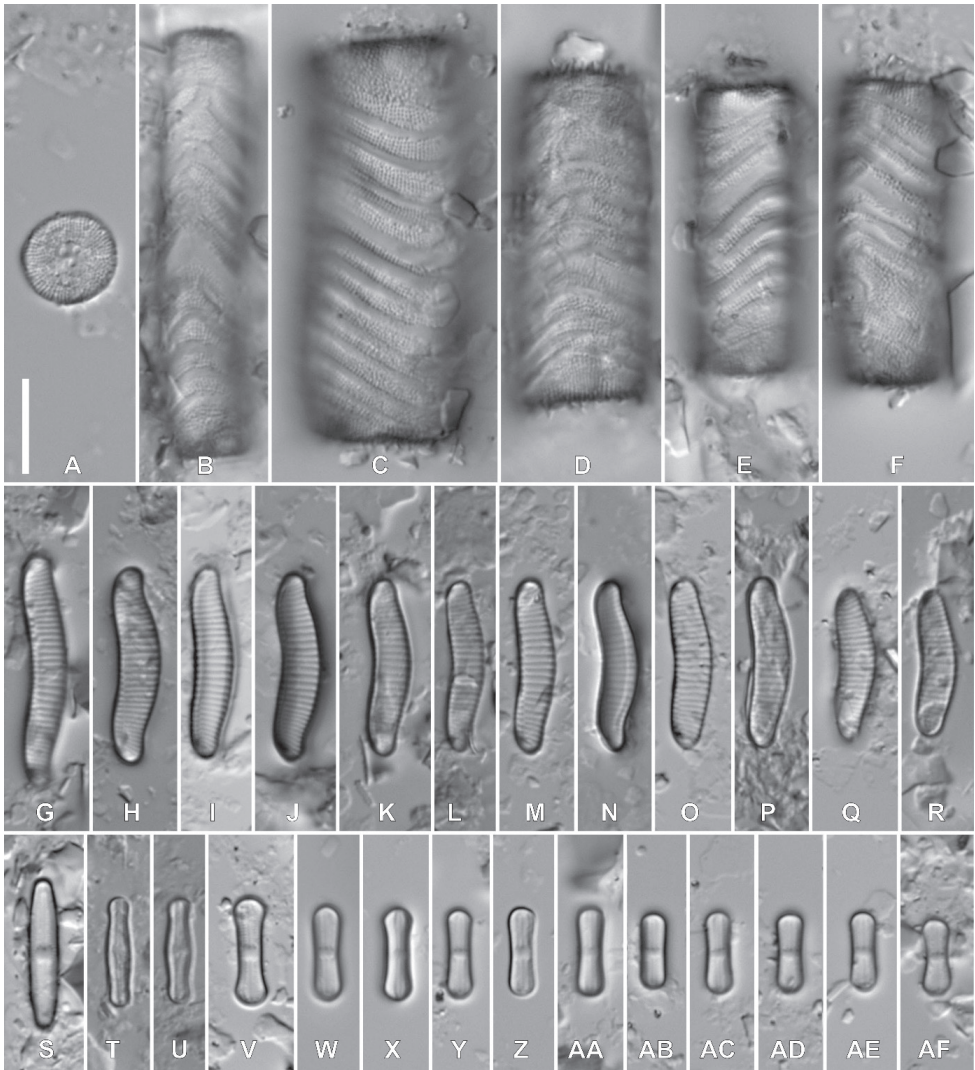


Figure 5. LM images of associated diatom flora **A–F** *Orthoseira circularis* (Ehrenberg) R.M. Crawford **G–R** *Eunotia* cf. *pseudominor* Pavlov & Levkov **S** *Humidophila* sp. 1 **T, U** *Humidophila* sp. 2 **V–A, F** *Humidophila contenta* (Grunow) Lowe et al. Scale bar: 10 μ m.

Navicula inserata var. *elliptica* Cholnoky (1960, p. 65, pl. 5, figs. 204–205), *Navicula submutica* Fusey (1964, p. 27, pl. 3, fig. 38), *N. submutica* var. *capitata* Fusey (1964, p. 27, pl. 3, fig. 41), *N. submutica* var. *elliptica* Fusey (1964, p. 27, pl. 3, fig. 40), *N. submutica* var. *rectangularis* Fusey (1964, p. 27, pl. 3, fig. 39), *Navicula guluensis* Giffen (1966, p. 238, fig. 70). However, their transfer require detailed study of type materials.

The three new *Luticola* species have comparable valve outline with protracted apices and striae density. They also share the presence of several shallow depressions in the central area visible in SEM. Despite their unique set of features, the taxa show some

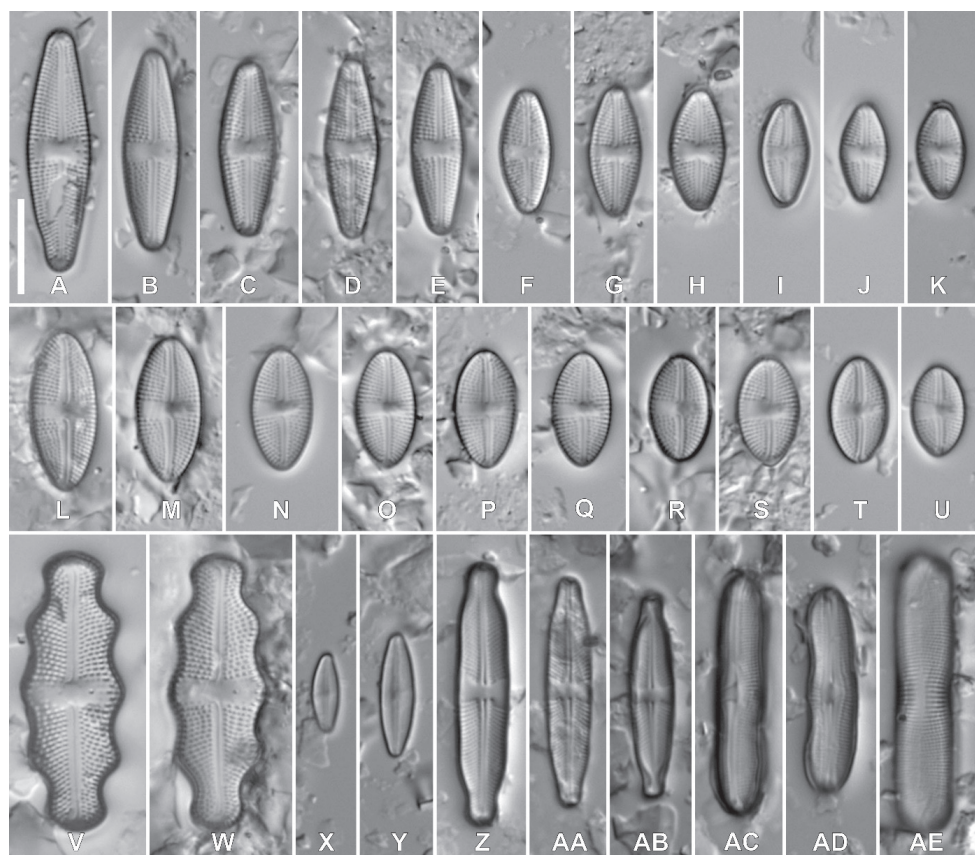


Figure 6. LM images of associated diatom flora **A–K** *Luticola intermedia* (Hustedt) Levkov, Metzeltin & Pavlov **L–U** *Luticola permuticoides* Metzeltin & Lange-Bertalot **V, W** *Luticola distinguenda* (Hustedt) Levkov, Metzeltin & Pavlov **X, Y** *Nupela lesothensis* (Schoeman) Lange-Bertalot **Z–AB** *Stauroneis* cf. *pygmaea* f. *undulata* Hustedt **AC–AE** *Muellieria* sp. Scale bar: 10 μ m.

similarities to other species of this genus, especially under the light microscope. *Luticola microcephala* sp. nov. shows the highest degree of similarity to two aerophytic taxa described from India: *Luticola jogensis* (H.P. Gandhi) Kale, Levkov & Karthick (Kale et al. 2017, p. 30, figs 2–26) and *L. gandhii* Kale, Levkov & Karthick (Kale et al. 2017, p. 33, figs 27–51). However, *Luticola microcephala* sp. nov. can be easily separated from both Indian taxa. *Luticola jogensis* has much larger valves (28.5–36.0 μ m length and 7.0–9.0 μ m width versus 14.0–24.0 μ m length and 4.5–6.6 μ m width in *L. microcephala* sp. nov.). Additionally, *L. jogensis* has 5–6 areolae per striae, whereas striae in *L. microcephala* sp. nov. are composed of 3 to 4 areolae. The second of the Indian species *L. gandhii* differs from *L. microcephala* sp. nov. in having rostrate, not capitate apices. The most pronounced difference amongst all three species is the number of rows of areolae on the valve mantle. *Luticola microcephala* sp. nov. has only one row of elongated areolae, becoming more rounded in the central part of the valve and near the

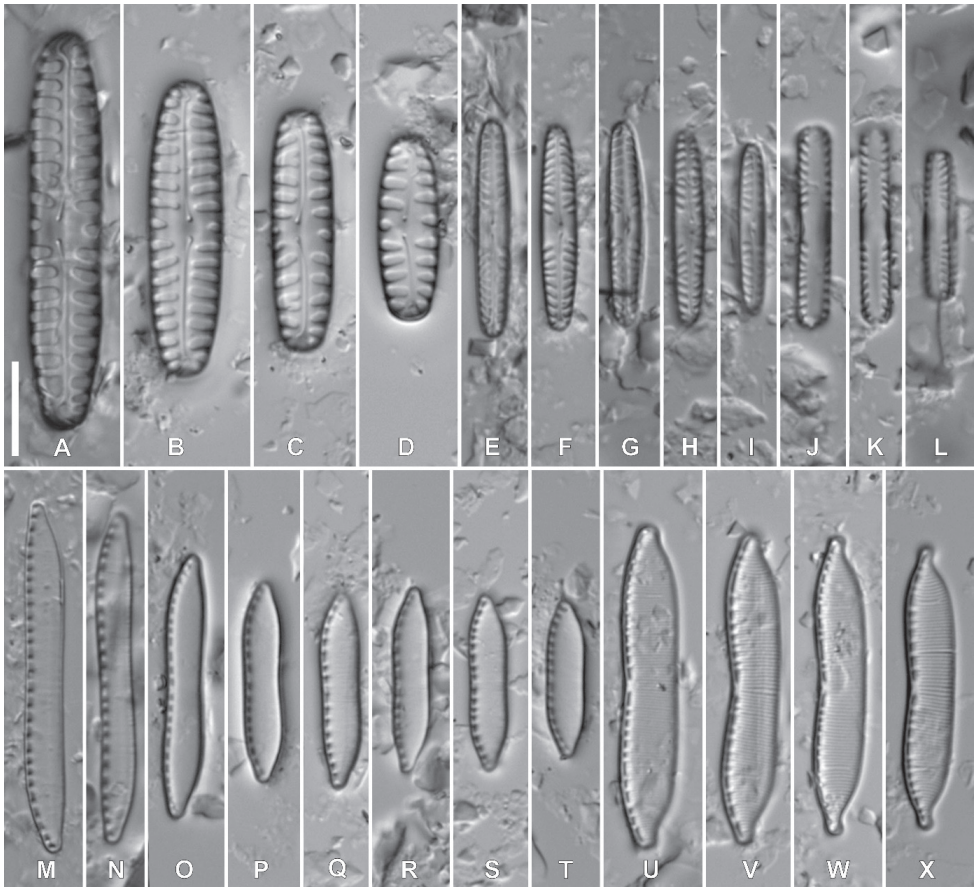


Figure 7. LM images of associated diatom flora **A–D** *Pinnularia borealis* Ehrenberg *sensu lato* **E–L** *Pinnularia* sp. **M–T** *Nitzschia brevissima* Grunow **U–X** *Hantzschia amphioxys* (Ehrenberg) Grunow. Scale bar: 10 μ m.

apices. On the contrary, *L. jogensis* has 2 or 3 rows (Kale et al. 2017, p. 34, fig. 22) and *L. gandhii* has always three rows of areolae (Kale et al. 2017, p. 37, fig. 48).

Amongst other species described from South Africa, *Navicula submutica* var. *capitata* also shows some degree of similarity to *L. microcephala* sp. nov., but there is a lack of detailed microscopic pictures of this species. However, based on the description and the line drawing of this taxon (Fusey 1964, p. 27, pl. 3, fig. 41), *Navicula submutica* var. *capitata* differs from *L. microcephala* sp. nov. in having more lanceolate valve outline, wider central area and lower striae density (16 in 10 μ m versus 19–22 in *L. microcephala* sp. nov.).

Luticola asymmetrica sp. nov. shows morphological similarity to five *Luticola* species reported from the African continent: *L. imbricatiformis* Levkov, Metzeltin & Pavlov (2013, p. 134, pl. 28, figs 1–11), *L. falknerorum* Metzeltin & Lange-Bertalot (Metzeltin and Lange-Bertalot 2007, p. 156, figs 1–9), *L. fuhrmannii* Metzeltin & Levkov in Lev-

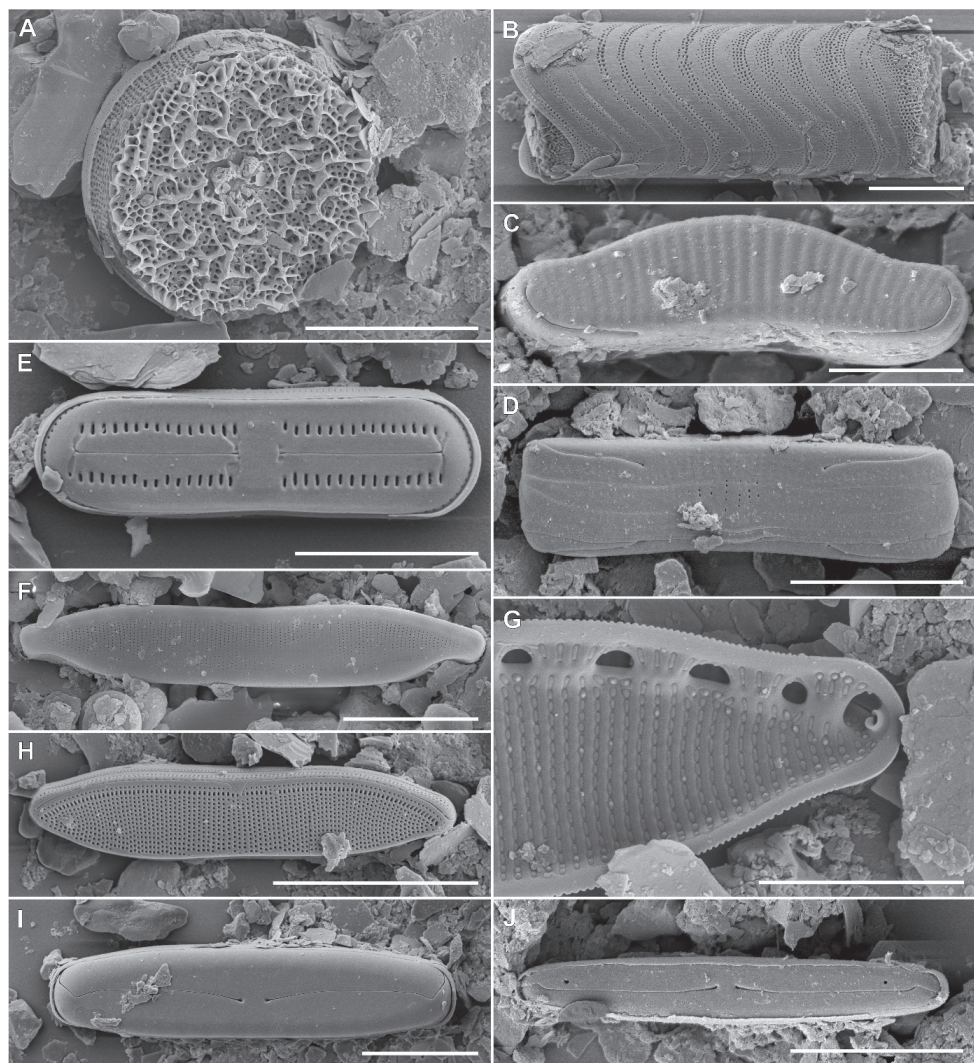


Figure 8. SEM images of associated diatom flora **A, B** *Orthoseira circularis* (Ehrenberg) R.M. Crawford **C, D** *Eunotia* cf. *pseudominor* Pavlov & Levkov **E** *Humidophila contenta* (Grunow) Lowe et al. **F** *Hantzschia amphioxys* (Ehrenberg) Grunow **G, H** *Nitzschia brevisissima* Grunow **I** *Pinnularia borealis* Ehrenberg *sensu lato* **J** *Pinnularia* sp. Scale bars: 10 µm (**A, B, D, F, H, I, J**), 5 µm (**C**), 4 µm (**E**), 3 µm (**G**).

kov et al. (2013, p. 116, pl. 23, figs 1–20), *L. gesierichiae* Levkov, Metzeltin & Pavlov in Levkov et al. (2013, p. 119, pl. 48, figs 17–28 and pl. 49, figs 1–5) and *L. frickei* Levkov, Metzeltin & Pavlov in Levkov et al. (2013, p. 114, pl. 48, figs 29–42, pl. 49, figs 6–8 and pl. 52, fig. 1). However, *Luticola asymmetrica* sp. nov. can be distinguished, based on its asymmetrical, weakly dorsiventral valve margin. Additionally, *Luticola imbricati-formis* has slightly elongated stigma positioned close to the centre of the valve (Levkov et al. 2013, p. 337, fig. 11), whereas *L. asymmetrica* sp. nov. has a round isolated pore located in the central area, halfway between the valve margin and central raphe. *Luti-*

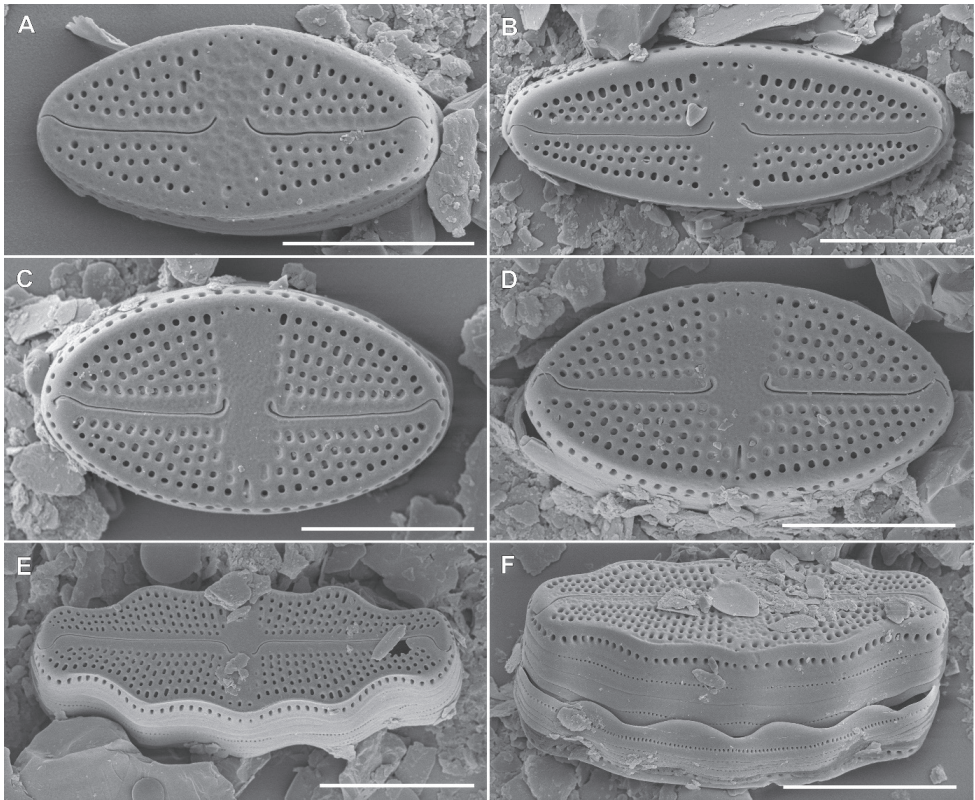


Figure 9. SEM images of associated diatom flora **A, B** *Luticola intermedia* (Hustedt) Levkov, Metzeltin & Pavlov **C, D** *Luticola permuticoides* Metzeltin & Lange-Bertalot **E, F** *Luticola distinguenda* (Hustedt) Levkov, Metzeltin & Pavlov. Scale bars: 15 μm (**A–D**), 10 μm (**E, F**).

cola falknerorum has a higher striae density (21–24 in 10 μm in comparison to 17–20 in 10 μm in *Luticola asymmetrica* sp. nov.) and more areolae per striae (5–6 versus 3–5 in *Luticola asymmetrica* sp. nov.). Both species can also be separated, based on external raphe structure. Raphe branches of *L. falknerorum* are bordered by silica ridges and have short proximal raphe endings (Levkov et al. 2013, p. 341 figs 2, 3), whereas *L. asymmetrica* sp. nov. does not have silica ridges and has asymmetrical, long proximal raphe endings, almost reaching the first row of areolae. Similar silica ridges are also present in *L. gesierichiae* (Levkov et al. 2013, p. 379, figs 2, 3). The characteristic asymmetrical valve outline with broadly-rounded apices is the main feature distinguishing *L. asymmetrica* sp. nov. from the mentioned *L. gesierichiae*, as well as from *L. frickei*. Both species have a regular linear-lanceolate valve shape with narrowly-rounded apices (Levkov et al. 2013). The last of the similar species – *Luticola fuhrmannii* is widely distributed in tropical areas of South America and Africa. Nonetheless, it can be separated, based on its elongated stigma positioned almost on the valve mantle (Levkov et. al. 2013, p. 385, fig. 4), whereas stigma of *Luticola asymmetrica* sp. nov. are round and located on the central area in the middle between the valve margin and proximal raphe endings.

The third of described *Luticola* species – *Luticola terrestris* sp. nov shows a high degree of similarity with several species from two informal morphological groups proposed by Levkov et al. (2013). *Luticola terrestris* sp. nov resembles *L. tenuis* Levkov, Metzeltin & Pavlov (2013, p. 236, pl. 35, figs 1–18) and *L. micra* Levkov, Metzeltin & Pavlov (2013, p. 156, pl. 35, figs 19–37) from the group A and *L. incana* Levkov, Metzeltin & Pavlov from the group B. *Luticola tenuis* and *Luticola terrestris* sp. nov. are observed from terrestrial habitats. Both species overlap dimensions; however, *Luticola terrestris* sp. nov. has higher striae density (20–23 in 10 µm versus 18–20 in *L. tenuis*). Moreover, *L. tenuis* has more linear valve outline and slightly deflected proximal raphe endings (Levkov et al. 2013, p. 35, figs 17, 18) in contrast to more curved proximal raphe fissures in *L. terrestris* sp. nov. *Luticola micra* differs from *Luticola terrestris* sp. nov. in range of valve dimensions (8–18 µm length and 4–5 µm width versus 8.1–28.3 µm length and 4.4–6.1 µm width) and the lack of ghost areolae in the central area. Additionally, *L. micra* has elongated stigma (Levkov et al. 2013, p. 35, fig. 19), whereas stigma in *L. terrestris* sp. nov. are regularly rounded. In addition, *Luticola incana* shows similarity with *Luticola terrestris* sp. nov., especially in the valve outline; however, it has a smaller range of valve dimensions (12.5–20.5 µm length and 5–6 width vs. 8.1–28.3 µm length and 4.4–6.1 µm width). Moreover, *L. incana* does not show a ghost areolae in the central area and has short, slightly deflected distal raphe endings (Levkov et al. 2013, p. 135) in contrast to *L. terrestris* sp. nov., where distal raphe endings are distinctly hooked and expanded on to the valve mantle. Finally, all three compared species are described from other continents: both *L. micra* and *L. tenuis* from Europe and *L. incana* from South America. Amongst species described from Africa, two *Navicula* taxa (which probably should be placed in the genus *Luticola*) show some degree of similarity to *L. terrestris* sp. nov. *Navicula guluensis* can be easily distinguished, based on striae density (15 in 10 µm versus 20–23 in 10 µm) and shape of raphe endings. *Navicula guluensis* has shortly deflected distal raphe endings (Giffen 1963, fig. 60; Giffen 1966, pl 3, fig. 59), whereas distal raphe endings of *L. terrestris* sp. nov are elongated and reach the valve mantle. The second of the African species *Navicula submutica* var. *rectangularis* shares with *L. terrestris* sp. nov. the valve outline, but has less dense striae with only 15–20 in 10 µm (Fusey 1964, p. 27, pl. 3, fig. 39).

Based on light microscopy observations, the *Microcostatus meridionalis* sp. nov. is the most similar to *Microcostatus egregius* (Hustedt) Lange-Bertalot (Stanek-Tarkowska et al. 2016), *Microcostatus werumii* Metzeltin, Lange-Bertalot & Soninkhishig (Metzeltin et al. 2009), *Microcostatus edaphicus* C.E. Wetzel, Noga, Ector & Stanek-Tarkowska (Stanek-Tarkowska et al. 2016), *Microcostatus aerophilus* Stanek-Tarkowska, Noga, C.E. Wetzel & Ector (Stanek-Tarkowska et al. 2016) and *Microcostatus krasskei* (Hustedt) J.R. Johansen & Sray (Johansen and Sray 1998). All species share similar valve outline and invisible or very difficult to observed striae in LM. Only in two species – *M. meridionalis* sp. nov. and *M. werumii*, apart from a raphe and valve outline, no other morphological features are discernible in LM. In other species, some additional features (for example, depressions on both sides of the sternum, visible as arch-shaped shadows or striation pattern) can be observed in LM. *Microcostatus meridionalis* sp. nov can be separated, based on its cuneate valve ends, whereas *M. werumii* has slightly rostrate valve

ends. Additionally, proximal raphe endings are different in both species – in *M. werumii*, they are poorly separated and located close to each other (Metzeltin et al. 2009, p. 225, figs 1–16). The drop-like proximal raphe endings of *M. meridionalis* are clearly visible, located on the asymmetrically constructed sternum. Under SEM, *M. meridionalis* sp. nov. poses a set of unique features that allows it to be easily distinguished from other representatives of the genus *Microcostatus*. *Microcostatus meridionalis* sp. nov. has areolae occluded only in the central area, not on the entire valve face. Moreover, it has no pseudoconopeum/conopeum in contrast to *M. aerophilus* and *M. egregius* (Stanek-Tarkowska et al. 2016, p. 166, figs 13–15). Internally, in the aspect of striae pattern and the shape of the central area, the described species is similar to *Microcostatus schoemanii* Taylor, Levanets, S. Blanco & Ector (Taylor et al. 2010, p. 180, fig. 8) and *M. cholnokyi* Taylor, Levanets, S. Blanco & Ector (Taylor et al. 2010, p. 182, fig. 25), but, based on the different shape of raphe endings and valve outline, are easy to distinguish. Regarding valve dimensions and striae density, *Microcostatus meridionalis* sp. nov. resembles *M. krasskei*. Both taxa overlap their dimensions (5–14 µm length, 3–4 µm width and 35–45 striae in 10 µm in *M. krasskei* versus 7.5–14 µm length, 3.5–4.5 µm width and 36–42 striae in 10 µm in *M. meridionalis*), but can be separated, based on the striae pattern and present of central area in *M. meridionalis* (Johansen and Sray 1998, p. 99, figs 17, 19). The others of the aforementioned most similar species, although they have a similar shape, differ in striae density. *Microcostatus egregius* has 33–36 striae in 10 µm, whereas *Microcostatus aerophilus* up to 40–50 in 10 µm (Metzeltin et al. 2009; Stanek-Tarkowska et al. 2016), unlike to *Microcostatus meridionalis* which has 36–42 striae in 10 µm. *Microcostatus aerophilus* (Stanek-Tarkowska et al. 2016) fully matches in the aspect of valve dimensions, but usually has higher striae density (40–50 in 10 µm).

The studied assemblages consisted of diatoms with various geographic distributions. Cosmopolitan species, such as: *Humidophila contenta*, *Hantzschia amphioxys*, *Nitzschia brevissima*, *Pinnularia borealis* (Krammer and Lange-Bertalot 1986; Krammer 2000; Lange-Bertalot et al. 2017) occurred together with species with a wide distribution in the tropics – *Luticola distinguenda* and *L. intermedia* (Levkov et al. 2013; Glushchenko et al. 2017; Straube et al. 2017; Da Silva-Lehmkuhl et al. 2019). On the other hand, several of the noted species have not been reported from Africa so far or their occurrence in Africa has not been confirmed. *Luticola permuticoides* was originally described by Metzeltin and Lange-Bertalot (2007) from South America. So far, the occurrence of this species in Africa has not been confirmed. However, in the original description, the authors indicated the presence of a similar taxon (Metzeltin and Lange-Bertalot 2002, plate 27, fig. 17) in Madagascar, which probably is, indeed, *Luticola permuticoides*. Another species that has not been recorded in Africa so far is *Orthoseira circularis*, a taxon originally described from South America, but observed also in materials from Java (Houk et al. 2017). Together with other species identified in our study, *O. circularis* is probably a typical inhabitant of the terrestrial environment. Based on the presented results and literature data, both mentioned taxa can be considered as having pantropical distribution in terrestrial habitats.

In the studied material, some species were scarce and their exact identification was not entirely possible. In the literature data, there is a lack of information about

valve dimensions of *Stauroneis pygmaea* f. *undulata* – the species originally described from Asia (Hustedt 1942). For this reason, despite the high level of morphological similarity in the valve outline and striae pattern, the observed species were noted as *Stauroneis* cf. *pygmaea* f. *undulata*. Two populations of *Eunotia*, observed in samples 2018/424 and 2018/426, show a highly similar pattern to *Eunotia pseudominor* Pavlov & Levkov. Both of our populations have similar dimensions: 12–35.6 µm length, 3.6–5.3 µm width and 11–15 striae in 10 µm in *E. pseudominor* (Pavlov and Levkov 2013) and 9.9–33.4 µm length, 3.2–4.1 µm width and 13–16 striae in 10 µm in our populations of *Eunotia* aff. *Pseudominor*; however, smaller valves were observed in our material. Moreover, the taxa observed in this study have less straight valve outline and more bent apices. Both taxa are also associated with moss vegetation. All of the observed differences in morphology can result from both the possible presence of two distinct species or only morphological differences within a single widely-distributed species. Therefore, we decided to identify it as similar to *E. pseudominor* (*Eunotia* aff. *pseudominor*).

Many diatom taxa develop various adaptations to changes in humidity of aerial habitats, such as: production of thickened valves, reduction of areolae number and occlusion of areolae with silica (Lowe 2011; Lowe et al. 2007; Round et al. 1990; Dodd and Stoermer 1962; Main 2003). Newly-described species represent genera commonly noted in various terrestrial habitats (Johansen and Sray 1998; Levkov et al. 2013; Lange-Bertalot et al. 2017). Amongst them, the highest level of adaptation is shown in *M. meridionalis*, which has fully covered areolae in the central part of the valve. Accompanying *Pinnularia* and *Hantzschia* taxa also show the presence of external areolae occlusions. This type of structure is also present on the surface of *Eunotia* cf. *pseudominor*, whereas the genus *Eunotia* does not create hymen or other occlusions (Round et. al 1990). The development of diatoms as epiphytes on mosses, which are able to accumulate water to provide a favourable environment for many organisms, can be also considered as form of adaptation to local environmental conditions (Round 1957; Lindo and Gonzales 2010; Glime 2017).

The investigated moss samples were characterised by a small diversity of diatom species (from 9 to 15 species per sample). This low species richness is quite typical for terrestrial environments, such as soils, rock crevices or clumps of terrestrial bryophytes. Especially in the Southern Hemisphere, terrestrial diatom assemblages are still poorly known, both in taxonomic and ecological aspects. The present study showed that these environments are often “hot spots” for the occurrence of potentially new and rare taxa.

Acknowledgements

The authors would like to thank the tardigradologist team from the Jagiellonian University (Kraków, Poland), especially Witold Morek and Bartłomiej Surmacz for collecting and sharing the materials used in the present research. We also thank Professor Jeffrey R. Johansen for consultation on *Microcostatus* species. We would like to thank the three reviewers and editorial board for their valuable and profound comments

and efforts towards improving our manuscript. The authors thank especially Jonathan Taylor – Curator of the South African National Diatom Collection at North-West University and Prof. Andrzej Witkowski from University of Szczecin (Poland). The work was supported by the program of the Minister of Science and Higher Education named "Regional Initiative of Excellence" in the years 2019–2022, project number 026/RID/2018/19.

References

- Archibald REM (1966) Some new and rare diatoms from South Africa 2. Diatoms from Lake Sibaya and Lake Nhlanga in Togoland (Natal). *Nova Hedwigia* 7: 477–496. [https://doi.org/10.1016/S0022-4618\(16\)30061-4](https://doi.org/10.1016/S0022-4618(16)30061-4)
- Archibald REM, Schoeman FR (1984) *Amphora coffeaeformis* (C.A. Agardh) Kützing: A revision of the species under light and electron microscopy. *South African Journal of Botany* 3(2): 83–102.
- Archibald REM, Schoeman FR (1985) *Amphora hybrida* Grunow (Bacillariophyceae) its identity and taxonomy. *Nova Hedwigia* 41: 159–166.
- Bąk M, Kociolek JP, Lange-Bertalot H, Łopato D, Witkowski A, Zglobicka I, Seddon AWR (2017) Novel diatom species (Bacillariophyta) from the freshwater discharge site of Laguna Diablas (Island Isabella = Albemarle) from the Galapagos. *Phytotaxa* 311(3): 201–224. <https://doi.org/10.11646/phytotaxa.311.3.1>
- Bąk M, Kryk A, Peszek Ł, Kociolek JP, Bemiasa J, Bemanaja E (2019) New and interesting *Luticola* species (Bacillariophyta) from the mangroves of Nosy Be Island, NW Madagascar. *International Journal of Oceanography and Hydrobiology* 48(1): 13–22. <https://doi.org/10.1515/ohs-2019-0002>
- Bellinger BJ, Cocquyt C, O'Reilly CM (2006) Benthic diatoms as indicators of eutrophication in tropical streams. *Hydrobiologia* 573(1): 75–87. <https://doi.org/10.1007/s10750-006-0262-5>
- Brown C, Magoba R (2009) Rivers and Wetlands of Cape Town: Caring for our Rich Aquatic Heritage. Water Research Commission Report TT 376(08): 1–377.
- Chattoová B, Lebouvier M, De Haan M, Van de Vijver B (2017) The genus *Luticola* (Bacillariophyta) on Ile Amsterdam and Ile Saint-Paul (Southern Indian Ocean) with the description of two new species. *European Journal of Taxonomy* 387. <https://doi.org/10.5852/ejt.2017.387>
- Cholnoky BJ (1954) Diatomeen und einige andere Algen aus dem “de Hoek” – Reservat in Nord-Transvaal. *Botaniska Notiser* 3: 269–296.
- Cholnoky BJ (1955) Diatomeen aus salzhaltigen Binnengewässern der westlichen Kaap-Provinz in Südafrika. *Berichte der Deutschen Botanischen Gesellschaft* 68: 11–23.
- Cholnoky BJ (1956) Neue und seltene Diatomeen aus Afrika. II. Diatomeen aus dem Tugela-Gebiete in Natal. *Österreichische Botanischeskii Zhurnal* 103: 53–97. <https://doi.org/10.1007/BF01287833>

- Cholnoky BJ (1957) Neue und seltene Diatomeen aus Afrika. III. Diatomeen aus dem Tugela-Flusssystem, hauptsächlich aus den Drakensbergen in Natal. Österreichische. Botanicheskii Zhurnal 104: 25–99. <https://doi.org/10.1007/BF01289120>
- Cholnoky BJ (1960) Beiträge zur Kenntnis der Diatomeenflora von Natal (Südafrika). Nova Hedwigia 2(1/2): 1–128.
- Cholnoky BJ (1962) Ein Beiträge zu der Ökologie der Diatomeen in dem englischen Protektorat Swasiland. Hydrobiologia 20(4): 309–355. <https://doi.org/10.1007/BF00033355>
- Cholnoky BJ (1966) Diatomeen associationen aus einigen Quellen in Südwest-Afrika und Bechuanaland. Beih. Nova Hedwigia 21: 163–244.
- Cleve PT (1881) On some new and little known diatoms. Kungliga Svenska Vetenskapsakademiens Handlingar 18(5): 1–28.
- Cocquyt C (2006) Lacustrine and Riverine Algal Biodiversity in the African Great Rift Area. In: De Dapper M, de Lange D (Eds) Africa's Great Rift: Diversity and Unity, Proceedings International Conference, Brussels, 29–30 September 2005, Royal Academy for Overseas Sciences & Royal Museum for Central Africa, 59–71.
- Cocquyt C, Ryken E (2017) Two new needle-shaped *Nitzschia* taxa from a deep East African crater lake. Diatom Research 32(4): 465–475. <https://doi.org/10.1080/0269249X.2017.1401009>
- Cocquyt C, Taylor JC (2019) Transfer of *Navicula pseudolagerstedtii* Cholnoky to *Geissleria* Lange-Bertalot & Metzeltin (Gomphonemataceae, Bacillariophyta). Notulae Algarum 101: 1–2.
- Cocquyt C, De Haan M, Taylor JC (2013) *Cavinula lilandae* (Bacillariophyta), a new diatom species from the Congo Basin. Diatom Research 28(2): 157–163. <https://doi.org/10.1080/0269249X.2012.753952>
- Cocquyt C, Taylor CJ, Wetzel CE (2014) *Stenopterobia cataractarum* spec. nov. (Bacillariophyta), a new benthic diatom from a waterfall in Zambia, Africa. Phytotaxa 158(1): 76–84. <https://doi.org/10.11646/phytotaxa.158.1.5>
- Cocquyt C, De Haan M, Lokele Ndjombo E (2016) *Eunotia rudis* sp. nov., a new diatom (Bacillariophyta) from the Man and Biosphere Reserve at Yangambi, Democratic Republic of the Congo. Phytotaxa 272(1): 073–081. <https://doi.org/10.11646/phytotaxa.272.1.4>
- Cocquyt C, Taylor JC, Kusber WH (2017) Reinvestigation of African *Surirella* taxa (Bacillariophyta) described by B.J. Cholnoky with some remarks on digitization of diatom types. Fottea 17(1): 34–56. <https://doi.org/10.5507/fot.2016.012>
- Da Silva Lehmkuhl AM, Ludwig TAV, Tremarin PI, Bicudo D (2019) On *Luticola* D.G.Mann (Bacillariophyceae) in southeastern Brazil: Taxonomy, ecology and description of two new species. Phytotaxa 402(4): 165–186. <https://doi.org/10.11646/phytotaxa.402.4.1>
- Dodd JD, Stoermer EF (1962) Notes on Iowa diatoms I. An interesting collection from a moss-like habitat. Iowa Academy of Science, Proceedings 69: 83–87.
- Ehrenberg CG (1845) Mitteilungen über die mikroskopischen Lebensformen von Portugal und Spanien, Süd-Afrika, Hinter-Indien, Japan und Kurdistan. Noverum generum et specierum brevis definitio. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich Preussischen Akademie der Wissenschaften zu Berlin, 357–381.
- Fusey P (1964) Florule algologique de la République Centrafricaine. Diatomées de quelques collections d'eau de la sous-préfecture de M'Baiki et du parc St.-Floris. Cahiers Mabokey 2(1): 20–36.

- Giffen MH (1963) Contributions to the diatom flora of South Africa. 1. Diatoms of the estuaries of the Eastern Cape Province. *Hydrobiologia* 21(3/4): 201–265. <https://doi.org/10.1007/BF01025943>
- Giffen MH (1966) Contributions to the diatom flora of South Africa IF. Diatoms of the marine littoral regions at Kidd's Beach near East London, Cape Province, South Africa. *Nova Hedwigia* 13(1/2): 244–303.
- Giffen MH (1967) Contributions to the diatom flora of South Africa III. Diatoms of the marine littoral regions at Kidd's Beach near East London, Cape Province, South Africa. *Nova Hedwigia* 13(1/2): 245–292.
- Giffen MH (1970) Contribution to the diatom flora of South Africa, IV. The marine littoral diatoms of the estuary of the Kowie River, Port- Alfred, Cape Province. *Beihefte zur Nova Hedwigia* 31: 259–312.
- Giffen MH (1973) Diatoms of the marine littoral of Steenberg's Cove in St. Helena Bay, Cape Province, South Africa. *Botanica Marina* 16(1): 32–48. <https://doi.org/10.1515/botm.1973.16.1.32>
- Giffen MH (1976) A further account of the marine littoral diatoms of the Saldanha Bay Lagoon, Cape Province, South Africa. *Botanica Marina* 19(6): 379–394. <https://doi.org/10.1515/botm.1976.19.6.379>
- Glime JM (2017) Chapter 1 – The Fauna: A Place to Call Home. *Bryophyte Ecology Volume 2: Bryological Interaction* 1: 1–16.
- Glushchenko AM, Kulikovskiy MS, Kociolek JP (2017) New and interesting species from the genus *Luticola* (Bacillariophyceae) in waterbodies of Southeastern Asia. *Nova Hedwigia. Beiheft* 146: 157–173. <https://doi.org/10.1127/1438-9134/2017/157>
- Guiry MD, Guiry GM (2021) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. [accessed 01 January 2021]
- Houk V, Klee R, Tanaka H (2017) Atlas of freshwater centric diatoms with a brief key and descriptions. Second emended edition of Part I and II. Melosiraceae, Liparogryaceae, Paraliaceae and Aulacoseiraceae. *Fottea* 17(Supplement): 1–616.
- Hustedt F (1942) Süßwasser-Diatomeen des indomalayischen Archipels und der Hawaii-Inseln. Nach dem Material der Wallacea-Expedition. *Internationale Revue der Gesamten Hydrobiologie und Hydrographie* 42(1/3): 1–252. <https://doi.org/10.1002/iroh.19420420102>
- Johansen JR, Sray JC (1998) *Microcostatus* gen. nov., a new aerophilic diatom genus based on *Navicula krasskei* Hustedt. *Diatom Research* 13(1): 93–101. <https://doi.org/10.1080/0269249X.1998.9705436>
- Kale A, Levkov Z, Karthick B (2017) Typification of two species of *Luticola* (Bacillariophyta) from aerophilic habitats of the Western Ghats, India. *Phytotaxa* 298(1): 29–42. <https://doi.org/10.11646/phytotaxa.298.1.3>
- Kochman-Kędziora N, Zidarova R, Noga T, Olech M, Van de Vijver B (2020) *Luticola puchalskiana*, a new small terrestrial *Luticola* species (Bacillariophyceae) from the Maritime Antarctic Region. *Phytotaxa* 450(1): 85–94. <https://doi.org/10.11646/phytotaxa.450.1.6>
- Kociolek JP, Williams DM (2015) How to define a diatom genus? Notes on the creation and recognition of taxa, and a call for revisionary studies of diatoms. *Acta Botanica Croatica* 74(2): 195–210. <https://doi.org/10.1515/botcro-2015-0018>

- Kociolek JP, Kopalová K, Hamsher SE, Kohler TJ, Van de Vijver B, Convey P, McKnight DM (2017) Freshwater diatom biogeography and the genus *Luticola*: An extreme case of endemism in Antarctica. *Polar Biology* 40(6): 1185–1196. <https://doi.org/10.1007/s00300-017-2090-7>
- Kohler TJ, Kopalová K, Van de Vijver B, Kociolek JP (2015) The genus *Luticola* D.G. Mann (Bacillariophyta) from the McMurdo Sound Región, Antarctica, with the description of four new species. *Phytotaxa* 208(2): 103–134. <https://doi.org/10.11646/phytotaxa.208.2.1>
- Krammer K (2000) The genus *Pinnularia*. In: Lange-Bertalot H (Ed.) *Diatoms of Europe* (Vol. 1). A.R.G. Gantner Verlag K.G, 703 pp.
- Krammer K, Lange-Bertalot H (1986) Bacillariophyceae. 1. Teil: Naviculaceae In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) *Süßwasserflora von Mitteleuropa*, Band 2/1. Gustav Fisher Verlag, Jena, 876 pp.
- Lange-Bertalot H, Hofmann G, Werum M, Cantonati M (2017) Freshwater benthic diatoms of Central Europe: Over 800 common species used in ecological assessment. English edition with updated taxonomy and added species. In: Cantonati M, Kelly MG, Lange-Bertalot H (Eds) *Koeltz Botanical Books*, Schmitt, Germany, 942 pp.
- Levkov Z, Metzeltin P, Pavlov A (2013) *Luticola* and *Luticolopsis*. In: Lange-Bertalot H (Ed.) *Diatoms of Europe, diatoms of the European inland waters and comparable habitats*. Koeltz Scientific Books, Königstein, 698 pp.
- Levkov Z, Tofilovska S, Wetzel CE, Mitic-Kopanja D, Ector L (2017) Diversity of *Luticola* D.G. Mann (Bacillariophyceae) species on halomorphic soils from Gladno Pole, Central Macedonia. *Nova Hedwigia. Beiheft* 146: 175–196. <https://doi.org/10.1127/1438-9134/2017/175>
- Li Y, Suzuki H, Nagumo T, Tanaka J, Sun Z, Xu K (2016) *Microcostatus salinus* sp. nov., a new benthic diatom (Bacillariophyceae) from estuarine intertidal sediments, Japan. *Phytotaxa* 245(1): 51–58. <https://doi.org/10.11646/phytotaxa.245.1.5>
- Lindo Z, Gonzales A (2010) The Bryosphere: An integral and influential component of the Earth's Biosphere. *Ecosystems* (New York, N.Y.) 13(4): 612–627. <https://doi.org/10.1007/s10021-010-9336-3>
- Liu B, Williams DM, Blanco S, Jiang XY (2017) Two new species of *Luticola* (Bacillariophyta) from the Wuling Mountains Area, China. *Nova Hedwigia. Beiheft* 146: 197–208. <https://doi.org/10.1127/1438-9134/2017/197>
- Lokhande V, Radhakrishnan Ch, Kociolek JP, Lowe R, Karthick B (2020) The diatom genus *Luticola* D.G. Mann (Bacillariophyceae) in the Western Ghats of India and its biogeography. *European Journal of Phycology*. <https://doi.org/10.1080/09670262.2020.1783460>
- Lowe RL (2011) The importance of scale in understanding the natural history of diatom communities. In: Seckbach, J, Kociolek, P (Eds) *The Diatom World*, Springer, New York, New York, 295–311. https://doi.org/10.1007/978-94-007-1327-7_13
- Lowe RL, Furey PC, Ress JA, Johansen JR (2007) Diatom biodiversity and distribution on wetwalls in Great Smoky Mountains National Park. *Southeastern Naturalist* (Steuben, ME) 1(Special Issue): 135–152. [https://doi.org/10.1656/1528-7092\(2007\)6\[135:DBADOW\]2.0.CO;2](https://doi.org/10.1656/1528-7092(2007)6[135:DBADOW]2.0.CO;2)
- Main SP (2003) *Diprora haenaesis* gen. et sp. nov., a filamentous, pseudoaerial, araphid diatom from Kaua'i (Hawaiian Islands). *Diatom Research* 18(2): 259–272. <https://doi.org/10.1080/0269249X.2003.9705591>

- Metzeltin D, Lange-Bertalot H (2002) Diatoms from the Island Continent Madagascar. *Iconographia Diatomologica* 11: 1–286.
- Metzeltin D, Lange-Bertalot H (2007) Tropical Diatoms of South America II. Special remarks on biogeography disjunction. *Iconographia Diatomologica* 18: 1–877.
- Metzeltin D, Lange-Bertalot H, Soninkhishig N (2009) Diatoms in Mongolia. *Iconographia Diatomologica* 20: 1–686.
- Ndlovu N, Frontasyeva M, Newman R, Maleka P (2019) Moss and lichen biomonitoring of atmospheric pollution in the Western Cape Province (South Africa). *American Journal of Analytical Chemistry* 10(03): 86–102. <https://doi.org/10.4236/ajac.2019.103008>
- Pavlov A, Levkov Z (2013) Diversity and distribution of taxa in the genus *Eunotia* Ehrenberg (Bacillariophyta) in Macedonia. *Phytotaxa* 86(1): 1–117. <https://doi.org/10.11646/phytotaxa.86.1.1>
- Peszek Ł, Rybak M, Lange-Bertalot H, Kociolek JP, Witkowski A (2021) Three new *Luticola* D.G.Mann (Bacillariophyta) species from Rapa Nui (Easter Island) found in terrestrial diatom assemblages dominated by widely distributed taxa. *PeerJ* 9: e11142. <https://doi.org/10.7717/peerj.11142>
- Ridder F, Taylor JC (2020) Diatom types of R.E.M. Archibald from Lake Sibaya and Lake Nhlanga, South Africa. *Diatom Research* 35(10): 1–18. <https://doi.org/10.1080/0269249X.2020.1733099>
- Round FE (1957) The diatom community of some bryophyta growing on sandstone. *The Journal of the Linnean Society. Botany* 362: 657–661. <https://doi.org/10.1111/j.1095-8339.1957.tb00028.x>
- Round FE, Crawford RM, Mann DG (1990) *The Diatoms. Biology and Morphology of the Genera*; Cambridge University Press, Cambridge, 747 pp.
- Schoeman FR (1969) Diatoms from the Orange Free State (South Africa) and Lesotho. No. 2. *Revista de Biologia* 7(1/2): 35–74.
- Schoeman FR (1970) Diatoms from the Orange Free State, South Africa, and Lesotho I. In: Gerlof J, Cholnoky JB (Eds) *Diatomaceae II. Beihefte zur Nova Hedwigia* 31: 331–382.
- Schoeman FR, Archibald REM (1986a) *Gyrosigma rautenbachiae* Cholnoky (Bacillariophyceae): Its morphology and taxonomy. *Nova Hedwigia* 43: 129–157.
- Schoeman FR, Archibald REM (1986b) Observations on *Amphora* species (Bacillariophyceae) in the British Museum (Natural History). I. Some species from the subgenus *Oxyamphora* Cleve. *Nova Hedwigia* 43(1/2): 113–127.
- Schoeman FR, Archibald REM (1986c) Observations on *Amphora* species (Bacillariophyceae) in the British Museum (Natural History). II. Some species from the subgenus *Psammamphora* Cleve. *Nova Hedwigia* 43(3/4): 473–484.
- Schoeman FR, Archibald REM (1986d) Observations on *Amphora* species (Bacillariophyceae) in the British Museum (Natural History). V. Some species from the subgenus *Amphora*. *South African Journal of Botany* 52(5): 425–437. [https://doi.org/10.1016/S0254-6299\(16\)31507-1](https://doi.org/10.1016/S0254-6299(16)31507-1)
- Simonato J, Kociolek JP, Sala SE, Díaz YP, Núñez-Avellaneda M (2020) Three new *Luticola* species from the Andean-Amazonian transition in Colombia: Taxonomy, morphology and preliminary considerations of the biogeography of the genus. *Diatom Research* 35(4): 1–17. <https://doi.org/10.1080/0269249X.2020.1813205>

- Stanek-Tarkowska J, Wetzel CE, Noga T, Ector L (2016) Study of the type material of *Navicula egregia* Hustedt and descriptions of two new aerial *Microcostatus* (Bacillariophyta) species from Central Europe. *Phytotaxa* 280(2): 163–172. <https://doi.org/10.11646/phytotaxa.280.2.6>
- Straube A, Tremarin PI, Ludwig TAV (2017) Species of *Luticola* D.G. Mann (Bacillariophyceae) in the Atlantic Forest rivers from southern Brazil. *Diatom Research* 32(4): 417–437. <https://doi.org/10.1080/0269249X.2017.1389771>
- Taylor JC, Cocquyt C (2016) Diatoms from the Congo and Zambezi basins-Methodologies and identification of the genera. *ABC Taxa*. 16: 1–355.
- Taylor JC, Harding WR, Archibald CGM (2007a) An illustrated guide to some common diatom species from South Africa. North-West University. Water Research Commission. Report No. TT282/07, 178 pp.
- Taylor JC, Janse Van Vuuren MS, Pieterse AJH (2007b) The application and testing of diatom-based indices in the Vaal and Wilge rivers, South Africa. *Water S.A.* 33: 51–60. <https://doi.org/10.4314/wsa.v33i1.47871>
- Taylor JC, Prygiel J, Vosloo A, De la Rey PA, Van Rensburg L (2007c) Can diatom-based pollution indices be used for bio-monitoring in South Africa? A case study of the Crocodile West and Marico water management area. *Hydrobiologia* 592(1): 455–464. <https://doi.org/10.1007/s10750-007-0788-1>
- Taylor JC, Levanets A, Blanco S, Ector L (2010) *Microcostatus schoemaniai* sp. nov., *M. chohnokyi* sp. nov. and *M. angloensis* sp. nov. three new terrestrial diatoms (Bacillariophyceae) from South Africa. *Phycological Research* 58(3): 177–187. <https://doi.org/10.1111/j.1440-1835.2010.00576.x>
- Taylor JC, Karthick B, Cocquyt C, Lang P (2014a) *Diploneis fenestrata* spec. nov. (Bacillariophyta), a new aerophilic diatom species from Zambia, Africa. *Phytotaxa* 167(1): 79–88. <https://doi.org/10.11646/phytotaxa.167.1.4>
- Taylor JC, Karthick B, Kociolek JP, Wetzel CE, Cocquyt C (2014b) *Actinellopsis murphyi* gen. et spec. nov.: A new small-celled freshwater diatom (Bacillariophyta, Eunotiales) from Zambia. *Phytotaxa* 178(2): 128–137. <https://doi.org/10.11646/phytotaxa.178.2.4>
- Van de Vijver B, Ector L (2019) *Microcostatus elisabethianus*, a new limnoterrestrial diatom species (Bacillariophyta) from the sub-Antarctic region. *Plant Ecology and Evolution* 152(3): 539–545. <https://doi.org/10.5091/plecevo.2019.1600>
- Van de Vijver B, Mataloni G, Stanish L, Spaulding SA (2010) New and interesting species of the genus *Muelleria* (Bacillariophyta) from the Antarctic region and South Africa. *Phycologia* 49(1): 22–41. <https://doi.org/10.2216/09-27.1>
- Van de Vijver B, Kopalová K, Zidarova R, Cox EJ (2013) New and interesting small-celled naviculoid diatoms (Bacillariophyta) from the Maritime Antarctic Region. *Nova Hedwigia* 97(1/2): 189–208. <https://doi.org/10.1127/0029-5035/2013/0101>
- Zidarova R, Levkov Z, Van de Vijver B (2014) Four new *Luticola* taxa (Bacillariophyta) from Maritime Antarctica. *Phytotaxa* 170(3): 155–168. <https://doi.org/10.11646/phytotaxa.170.3.2>

Youngia hangii (Asteraceae, Crepidinae), a new species from Hubei, China

Qun Liu^{1*}, Gui-Yun Huang^{2*}, Dai-Gui Zhang³,
Jian-Wen Zhang⁴, Tao Deng⁴, Zhi-Min Li¹

1 School of Life Sciences, Yunnan Normal University, Kunming 650092, Yunnan, China **2** Rare Plants Research Institute of Yangtze River, Three Gorges Corporation, Yichang 443133, Hubei, China **3** College of Biology and Environmental Sciences, Jishou University, Jishou 416000, Hunan, China **4** CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

Corresponding authors: Tao Deng (dengtao@mail.kib.ac.cn), Zhi-Min Li (lizhimin_vip@163.com)

Academic editor: Alexander Sennikov | Received 5 July 2021 | Accepted 23 August 2021 | Published 14 September 2021

Citation: Liu Q, Huang G-Y, Zhang D-G, Zhang J-W, Deng T, Li Z-M (2021) *Youngia hangii* (Asteraceae, Crepidinae), a new species from Hubei, China. PhytoKeys 182: 27–38. <https://doi.org/10.3897/phytokeys.182.71063>

Abstract

Youngia hangii T.Deng, D.G.Zhang, Qun Liu & Z.M.Li, **sp. nov.**, a new species of Asteraceae, is described and illustrated. It was collected in Wufeng County, Hubei Province, Eastern Central China. *Youngia hangii* is morphologically most similar to *Y. rubida*, but can be easily distinguished from the latter by capitula with 8–10 florets and the hairy leaf surface. Phylogenetic analyses, based on the internal transcribed spacers (ITS) and one chloroplast marker (*rps16*), showed that *Y. hangii* and *Y. rubida* were sister species with good support. The results of both phylogenetic analysis and the morphological data support the specific rank of *Y. hangii*.

Keywords

Crepidinae, Hubei, molecular phylogeny, morphology, new species, *Youngia*

* The authors contributed equally to this study.

Introduction

Youngia Cass. (Cassini 1831) (Asteraceae; Cichorieae) is an East Asian genus with about 32 species (Babcock and Stebbins 1937; Shih 1997; Shih and Kilian 2011; Urbatsch et al. 2013; Deng et al. 2014; Peng et al. 2014, 2015; Ke and Chen 2016; Chen 2018). Most of the diversity of the genus is confined to China, and their diversity is especially high in the Hengduan Mountains (Peng et al. 2017). Most species of *Youngia* in China have a narrow distribution, especially several new species of *Youngia* which have been described in recent years (Deng et al. 2014; Peng et al. 2015; Ke and Chen 2016; Chen 2018). At the same time, there are two species of *Youngia* with incorrect taxonomic status. *Youngia japonica* (L.) DC. subsp. *longiflora* Koh Nakam. & C.I. Peng is distinct from *Y. japonica* subsp. *japonica*, which is supported by micromorphological and cytological evidence (Choi et al. 2020). *Youngia nansiensis* Y.Z. Zhao & L. Ma was nested in the *Crepidiastrum* clade and, therefore, should be considered as *Crepidiastrum akagii* (Kitagawa) J.W. Zhang & N. Kilian (Shih and Kilian 2011; Peng et al. 2014).

A lack of conspicuous distinguishing morphological features makes the Cichorieae, especially the Crepidinae, taxonomically difficult (Babcock and Stebbins 1937; Shih 1997; Peng et al. 2013). The related genera, *Youngia* and *Crepidiastrum* Nakai (1920: 147) are particularly difficult to distinguish through morphological and palynological features like pollen morphology, the epidermis morphology of leaf and achenes (Babcock and Stebbins 1937; Shih 1993, 1997; Gao 2007; Sennikov and Illarionova 2008; Wang et al. 2009). First, the molecular phylogenetic studies supported *Youngia* to be part of subtribe Crepidinae Cass. ex Dumort. with the inclusion of subtribe Ixeridinae Sennikov (Kilian et al. 2009; Zhang et al. 2011; Tremetsberger et al. 2012). Then, the results of some investigations (Urbatsch et al. 2013; Liu et al. 2013; Peng et al. 2014) and the most comprehensive Crepidinae phylogeny (Wang et al. 2020) supported close relationships between *Youngia* and *Crepidiastrum* being sister groups to each other, but they are phylogenetically distant to *Ixeris*, *Ixeridium* and *Askellia*, and *Youngia* is polyphyletic. However, the circumscription of sections in *Youngia*, so far, still lacks molecular evidence (Babcock and Stebbins 1937; Shih 1997; Peng et al. 2014). Sennikov and Illarionova (2008) proposed to divide *Youngia* into four sections (*Youngia*, *Cineripappae* Sennikov, *Paleaceae* Sennikov [= *Mesomeris* Babcock & Stebbins, nom. inval.] and *Pinnatifidae* Sennikov), based on the sculpturing of the fruit surface, which was supported by the morphological structure of the achenes in the Cichorieae (Zhu et al. 2006; Zhang et al. 2013). These two studies currently lack the support of molecular results.

Due to the many floristic surveys dedicated to the flora of Hubei, a centre of Metasequoia Flora (Chen et al. 2018), many new species have been recently described from Hubei Province and the adjacent area (Lin et al. 2019; Zhang et al. 2019; Chen et al. 2020; Lv et al. 2020; Sun et al. 2020; Liu et al. 2021; Zhang et al. 2021). When conducting plant surveys in Houhe, we collected some interesting

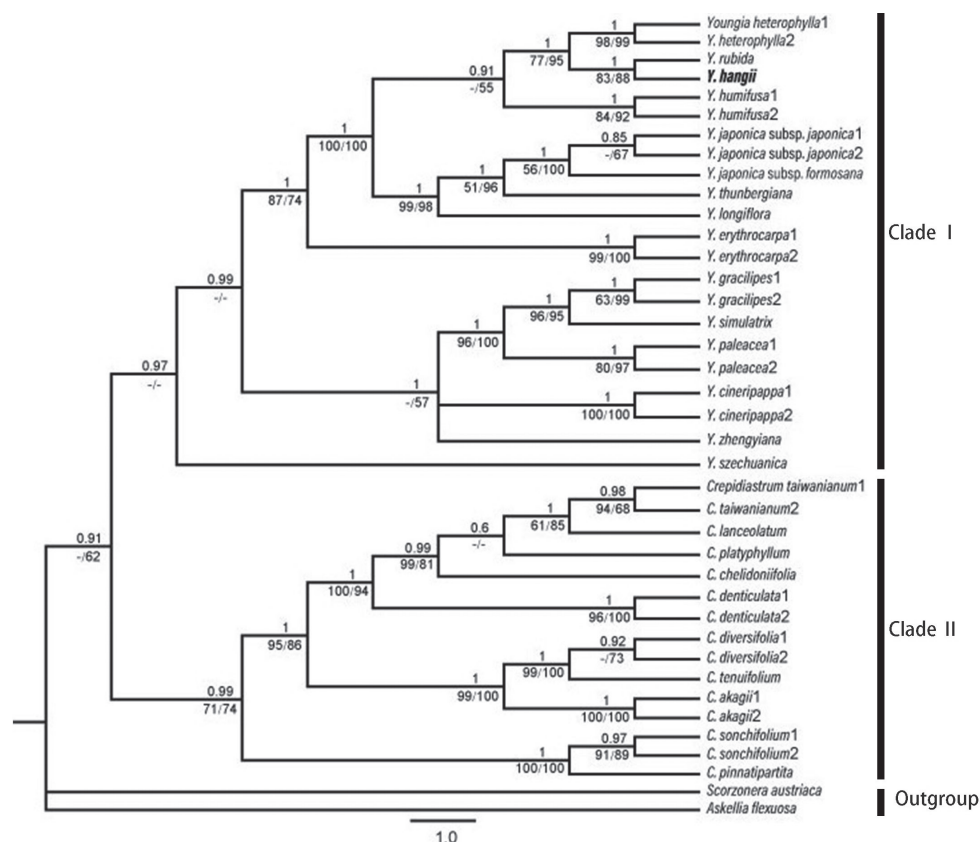


Figure 1. Bayesian consensus tree of *Youngia hangii* and related species. The BP tree is constructed, based on the combined matrix of ITS and *rps16* sequences. Numbers below branches are ML bootstraps and MP bootstraps and numbers above branches indicate Bayesian posterior probability. *Youngia hangii* is shown in bold.

plants of *Youngia* from a cave in Wufeng County, Hubei (Fig. 4). After comparing them with the known species, we determined that they represent a new taxon, which we hereby describe as *Y. hangii*.

Material and methods

Morphological assessment

We compared the shape, lobes and size of the leaves, leaf surface, phyllaries, number of florets, achenes and pappus of the new collections with *Y. rosthornii*, *Y. rubida* and *Y. heterophylla* and with descriptions in literature, in the Herbarium of the Kunming Institute of Botany (KUN). Eight individuals of the new species were examined.

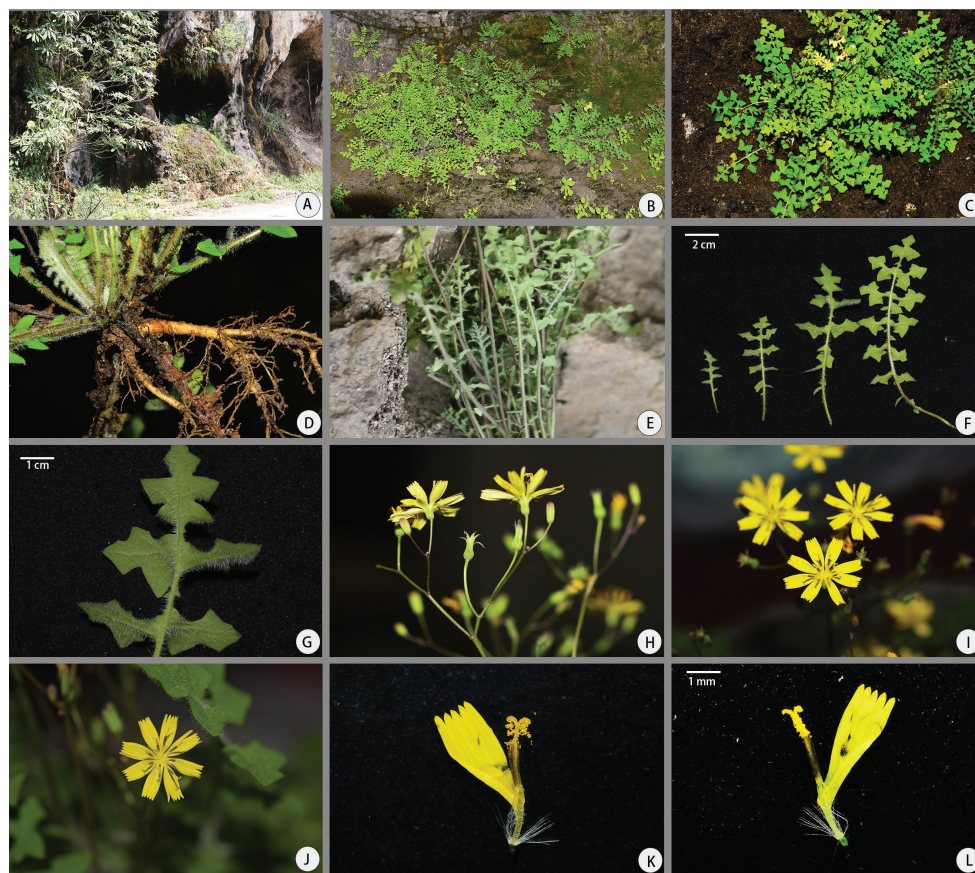


Figure 2. *Youngia hangii* T. Deng, D.G. Zhang, Qun Liu & Z.M. Li **A** habitat **B** population **C** habitat **D** root **E** stems **F, G** leaves **H–J** capitula **K, L** floret (**A–E, H–J** Photos by D.F. Zhang **F, G, K, L** Photos by Qun Liu **D, E, J–M** HAC001 (KUN)).

DNA Sequencing and Molecular Analyses

For molecular analysis, we sampled a sample from one population of the unknown species and obtained 38 samples from 26 related species from GenBank (Appendix 1). Voucher information and GenBank accession numbers are presented in Appendix 1. Total genomic DNA was extracted from dried leaves using a Plant Genomic DNA Kit DP305 (Beijing, China) and used as the template in the polymerase chain reaction (PCR). Two sequences (ITS and *rps16*) were combined by Sequence Matrix v.1.7.8 for later analysis (Vaidya et al. 2011). Multiple-sequence was aligned using the programme CLUSTAL_W v.1.4 (Rédei 2008), followed by manual adjustment in BioEdit v.7.0.5.3 (Hall 1999). Gaps were treated as missing data.

Phylogenetic trees were constructed using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). MP analyses were conducted us-

ing PAUP v.4.0a (Swofford 2004) by using a heuristic search, with random addition of 1000 replicates and tree bisection-reconnection (TBR). BI and ML analyses were conducted using MrBayes version 3.2 (Ronquist et al. 2012) and RAxML v.8.2.10 at the CIPRES Portal (<https://www.phylo.org/portal2>). The best-fit models of nucleotide substitution for individual data partitions were explored with Modeltest v.3.7 by Akaike Information Criterion (AIC). Using this procedure, GTR+I was identified as the optimal model. Bayesian tree topology was started from random trees and four Markov chain Monte Carlo (MCMC) simulations were run simultaneously. Runs were performed for 100 generations for a total of 10 million generations. The average standard deviation of split frequencies (< 0.01) was used to assess the convergence of the two runs. After the first ca. 15% of trees were discarded as burn-in, the remaining trees were imported into PAUP* and a 50% majority-rule consensus tree was then produced to obtain posterior probabilities (PP) of the clades.

Results

Taxonomic treatment

Youngia hangii T. Deng, D.G. Zhang, Qun Liu & Z.M. Li, sp. nov.

urn:lsid:ipni.org:names:77219670-1

Type. CHINA. Hubei: Wufeng County, Renheping, 30°06'27"N, 110°16'31"E, karst cave of karst topography, 500–800 m alt., 5 August 2018, *Daigui Zhang & Qun Liu HAC 001* (holotype KUN (KUN1511675); isotypes KUN (KUN1511676), JSU (HHE 3256)).

Description. Herbs, perennial, 20–35 cm tall. Taproot straight or slightly oblique, fleshy, with lateral roots (Fig. 2D). Stems erect, branched from the base, with sparse white simple hairs; stem base ribbed, with 1 or 2 leaves similar to basal leaves. Basal leaves crowded at the caudex base; petiole 2–3 cm long; blade oblanceolate, 6–18 × 2–4 cm, both surfaces short pubescent with white hairs 0.1–0.3 mm long (pubescence more evident on veins), bipinnate to pinnatifid, apical lobe halberd-shaped, apex acute-acuminate, margin deeply lobed; lateral lobes 5–10 pairs, opposite or slightly skewed, irregularly halberd-shaped (lateral lobes tapering to the base, serrate, lowermost lobes narrowly triangular), often with 1–3 pairs of triangular or oblique-ovate lobes between lateral lobes. Synflorescence corymbiform; capitula 7–10. Involucre ampullate, 3–4 mm long, 3 mm in diameter. Phyllaries in 4 rows, greyish-green; outer phyllaries 5–7, ovoid-triangular, ca. 1 × 1 mm, apex acute; inner phyllaries 7–9, lanceolate, 2–4 × 0.5–1 mm, margin white-membranous, apex acute; florets 8–10, ligules 4–6 × 1–2 mm, teeth 0.2–0.4 mm long, tube 3–4 mm; anther tube ca. 2.5 mm long; style branches ca. 0.5 mm long. Outer achenes black, fusiform, 2 mm long, apex attenuate to shortly beaked and expanded again into the pappus disc; ribs 12–14; pappus white, bristles rough, 3 mm long; inner achenes similar to the outer ones, 2.5 mm long.

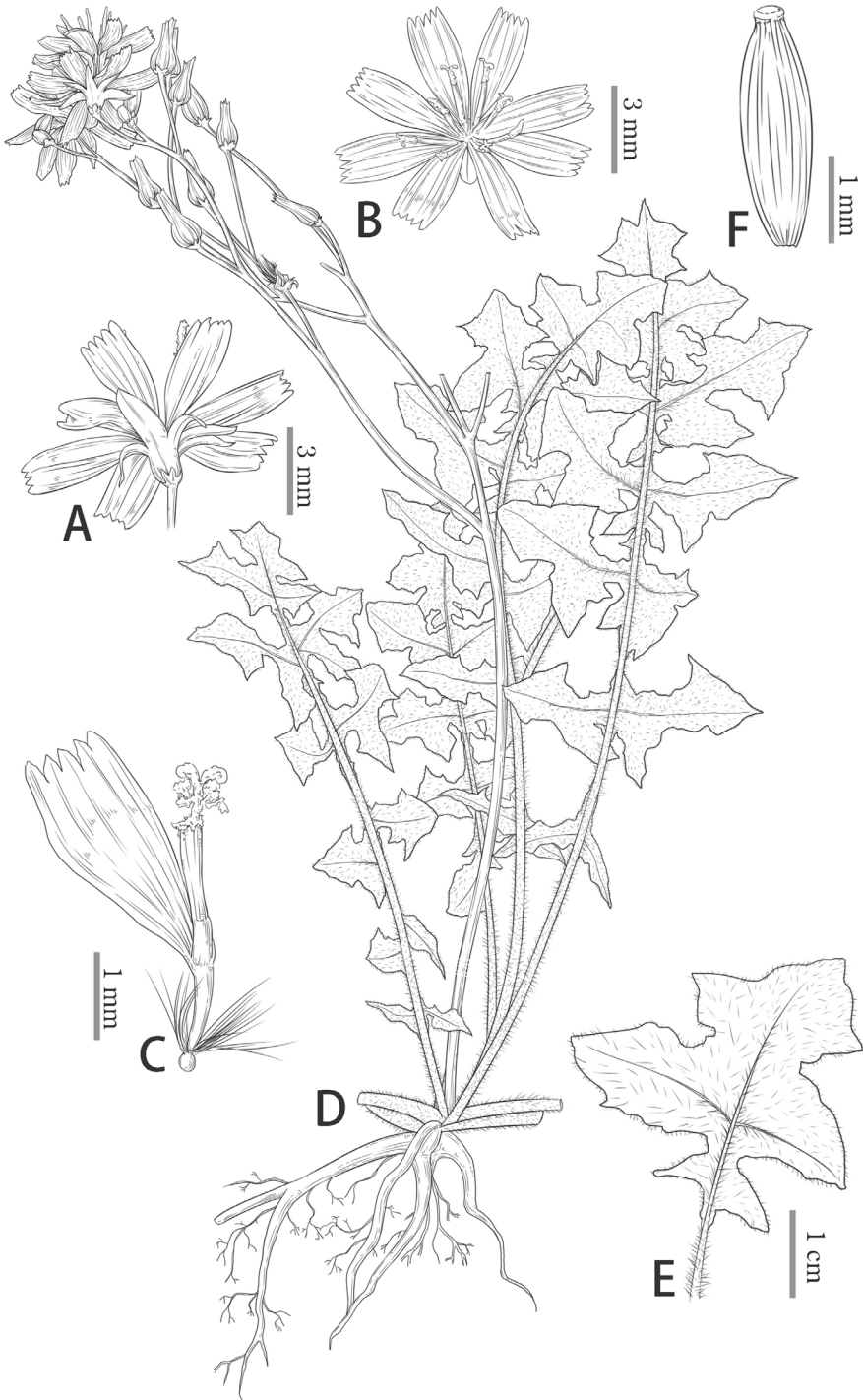


Figure 3. *Youngia bangii* T. Deng, D.G. Zhang, Qun Liu & Z.M. Li **A, B** capitula **C** floret **D** habit **E** leaf with hairs **F** achene (drawing by Jianing Yang).

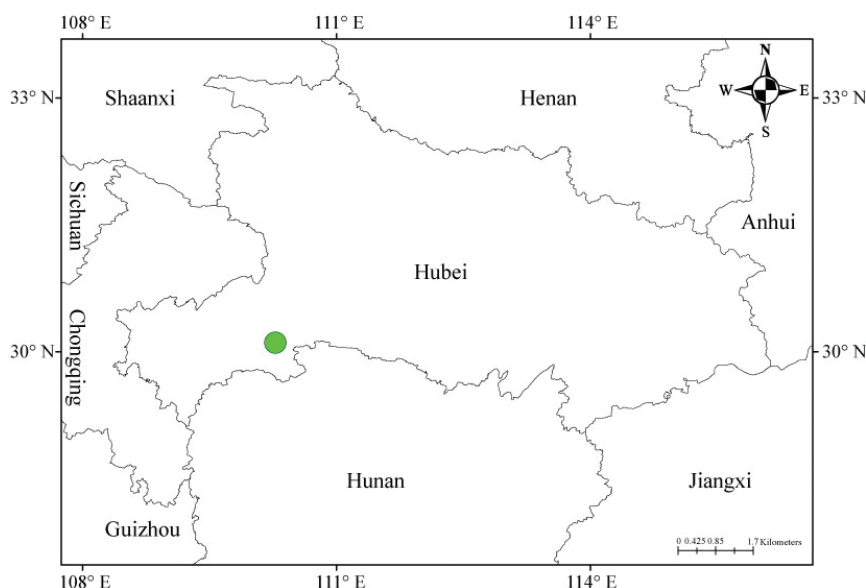


Figure 4. Distribution of *Youngia hangii* in Hubei Province, China.

Phenology. Flowering and fruiting April to October.

Vernacular name. 五峰黄鹌菜, wǔ fēng huáng ān cài in Chinese Pinyin.

Etymology. The species epithet honours Prof. Hang Sun (b. 1963), a Chinese botanist who has conducted research on plant taxonomy, floristics, biogeography and evolutionary biology and inspired many people through his work. He has also given a lot of support to the plant research work in Hubei.

Distribution and habitat. *Youngia hangii* is known only from the type locality, Renheping in Wufeng Xian, Hubei, China; 500–1000 individuals are known along the edge of some small caves at the base of the karst hillside (Fig. 2A, B, C); at altitudes of 500–800 m.

Morphological assessment. Morphological characteristics suggest that *Y. hangii* is related to *Y. rubida* and *Y. heterophylla* owning 10–25 florets and resembles *Y. rosthornii* with bipinnately deeply partite leaves. The achenes of *Y. hangii* and *Y. rubida* are attenuated into a short beak, which is widened into the pappus disc. Several unique features including the shape, lobes and size of the leaves, the leaves with white simple hairs (Fig. 2E, F, G; Fig. 3F, G), phyllaries, number of florets and achenes differentiate *Y. hangii* from *Y. rubida* and *Y. heterophylla* (Table 1).

Phylogenetic analysis. The Bayesian tree showing PP support, ML bootstrap (LP) and MP bootstrap (BP) values for each clade are presented in Fig. 1. The species in clade I form a monophyletic group with PP = 0.97, but LP are with weak support and BP are in conflict with PP and they were instead with “–”, respectively in Fig. 1. *Youngia hangii* is nested within Clade I as sister to *Y. rubida* with strong support (PP = 1, LP = 83, BP = 88).

Table 1. Comparison of morphological characteristics between *Youngia hangii* and related species.

Character		<i>Y. hangii</i>	<i>Y. rubida</i>	<i>Y. heterophylla</i>	<i>Y. rosthornii</i>
Basal leaf	shape	oblanceolate, bipinnately partite	oblanceolate, pinnately deeply or completely partite	elliptic or oblong lanceolate, pinnately deeply or completely partite	long elliptic, bipinnately deeply partite with a large apical part
	lobes				
	shape	apical lobes halberd-shaped, apex acute, with a tapered tip, margin middle to deep lobed; lowest lobes narrowly triangular	apical lobes triangle, apex acute, with a tapered tip, margin serrate; lowest lobes serrate	apical lobes elliptic, irregularly elliptic, ovate or lanceolate, apex acute, with a tapered tip, margin entire, almost entire or serrate; lowest lobes narrowly halberd	apical lobes triangular, apex acute, with a tapered tip, margin entire, almost entire or serrate; lowest lobes narrowly triangular
	number of lateral lobes	5–10 pairs	2–3 pairs	1–8 pairs	5–7 pairs
	size	6–18 × 2–4 cm	3–7 × 1.5–3 cm	13–23 × 6–7 cm	20 × 8 cm
	surface	with white pubescent hairs on both surfaces, especially dense on veins	glabrous on both surfaces	sparsely pubescent on both surfaces	glabrous on both surfaces
Phyllaries		4 rows	4 rows	4 rows	4 rows
Number of florets		8–10	13–15	11–25	20
Achenes	colour	black	red	brown-purple	brown-purple
	shape	fusiform, attenuated into a narrow neck, with a conical beak	fusiform, attenuated into a narrow neck, with a conical beak	fusiform, attenuated into a narrow neck, without a beak	fusiform, attenuated into a narrow neck, without a beak
	length	2 mm	2.8 mm	3 mm	3.5 mm
	ribs	12–14 ribs with small bristles	12 ribs with small bristles	14–15 ribs with small bristles	14–15 ribs with small bristles
Pappus		white, rough, 3 mm	white, rough, 3.5 mm	white, rough, 3–4 mm	white, rough, 3.5 mm

Discussion

Owning only 8–10 florets supports a placement of *Youngia hangii* in *Y.* sect. *Youngia* and its small involucre and achenes further support that *Y. hangii* is related to *Y. rubida*. However, there are some obvious differences between *Y. hangii* and *Y. rubida* and other species in the shape, lobes and size of the leaves and in white pubescent surfaces of the leaves. Moreover, *Y. rosthornii* also has bipinnately deeply partite leaves, but its leaves with a large apical part are different from *Y. hangii*.

Based on the combined datasets of the ITS and *rps16* sequences, BI, MP and ML trees with similar topologies were constructed. *Youngia hangii* was clustered with *Y. rubida* and nested in *Y.* sect. *Youngia* with strong support (PP = 1, LP = 87, BP = 74) and was sister to the clade of *Y. rubida* with strong support (PP = 1, LP = 83, BP = 88). The results from the phylogenetic analysis are consistent with the morphological comparisons. Although only one sample of *Y. hangii* was included in the phylogenetic analysis, *Y. hangii* and *Y. rubida* have obvious differences in morphology, so the morphological data and phylogenetic results altogether support our hypothesis of *Y. hangii* being a new species.

Acknowledgements

We are grateful to Dr. David E. Boufford for revising this manuscript. This study was supported by grants from the National Natural Science Foundation of China (31960046 and 31670206), the National Natural Science Foundation of China-Yunnan joint fund to support key projects (U1802232), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA20050203), National Key R & D Program of China (2017YFC0505200), Major Program of the National Natural Science Foundation of China (31590820), Youth Innovation Promotion Association of the Chinese Academy of Sciences (2019382), Yunnan Ten-thousand Talents Plan Young & Elite Talent Project (YNWR-QNBJ–2019–154), Young Academic and Technical Leader Raising Foundation of Yunnan Province (2019HB039) and the Chinese Academy of Sciences “Light of West China” Program.

References

- Babcock EB, Stebbins GL (1937) The genus *Youngia*. Carnegie Institution of Washington, Washington DC, 106 pp.
- Chen YS (2018) *Youngia baoxingensis*, a new species of Asteraceae (tribe Cichorieae) from Sichuan, China. *Phytotaxa* 382(2): 239–242. <https://doi.org/10.11646/phytotaxa.382.2.10>
- Chen YS, Deng T, Zhou Z, Sun H (2018) Is the East Asian flora ancient or not? *National Science Review* 5(6): 920–932. <https://doi.org/10.1093/nsr/nwx156>
- Chen JT, Zhang DG, Lv ZY, Huang XH, Liu PJ, Yang JN, Yang JY, Tojibaev K, Deng T, Sun H (2020) *Oxytropis shennongjiaensis* (Fabaceae), a new species from Hubei, Central China. *PhytoKeys* 149(11): 117–128. <https://doi.org/10.3897/phytokeys.149.49533>
- Choi B, Kim SY, Jang TS (2020) Micromorphological and cytological comparisons between *Youngia japonica* and *Youngia longiflora* using light and scanning electron microscopy. *Microscopy Research and Technique* 83(12): e23538. <https://doi.org/10.1002/jemt.23538>
- Cassini H (1831) Descriptions de quelques Synanthérées de l’île Maurice. *Annales des Sciences Naturelles* 23: 84–93.
- Deng T, Zhang JW, Zhu XX, Zhang DG, Nie ZL, Sun H (2014) *Youngia zhengyiana* (Asteraceae, Crepidinae), a new species from south China, with notes on the systematics of *Youngia* inferred from morphology and nrITS phylogeny. *Phytotaxa* 170(4): 259–268. <https://doi.org/10.11646/phytotaxa.170.4.3>
- Gao ZL (2007) Study on the Subtribe Lactucinae of Compositae from Shandong. Masters degree dissertation. Shandong Normal University. [in Chinese]
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment program for Windows. *Nucleic Acids Symposium Series* 41: 95–98. <https://doi.org/10.1021/bk-1999-0734.ch008>
- Ke R, Chen YS (2016) A new species of *Youngia* (Asteraceae, tribe Cichorieae) from Yunnan, China. *Phytotaxa* 275(2): 140–148. <https://doi.org/10.11646/phytotaxa.275.2.5>

- Kilian N, Gemeinholzer B, Lack HW (2009) Tribe Cichorieae. In: Funk VA, Susanna A, Stuessy T, Bayer R (Eds) Systematics, evolution, and biogeography of the Compositae. International Association for Plant Taxonomy, Vienna, 343–383.
- Lin N, Zhang DG, Huang XH, Zhang JW, Yang JY, Tojibaev K, Wang HC, Deng T (2019) *Silene sunhangii* (Caryophyllaceae), a new species from China. PhytoKeys 135: 59–69. <https://doi.org/10.3897/phytokeys.135.36426>
- Liu Y, Chen YS, Yang QE (2013) Generic status, circumscription, and allopolyploid origin of *Faberia* (Asteraceae: Cichorieae) as revealed by ITS and chloroplast DNA sequence data. Taxon 62(6): 1235–1247. <https://doi.org/10.12705/626.14>
- Liu Q, Lin N, Zhang DG, Huang XH, Wang HC, Yang JY, Tojibaev K, Lv ZY, Deng T, Li ZM (2021) *Trichosanthes sunhangii* (Cucurbitaceae), a new species from Hubei, China. Phytotaxa 479(3): 287–294. <https://doi.org/10.11646/phytotaxa.479.3.5>
- Lv ZY, Zhang DG, Huang XH, Wang HC, Yang JY, Tojibaev K, Deng T, Li ZM (2020) *Geum sunhangii* (Rosaceae), a new species from Hubei Province, China. PhytoKeys 156(6): 113–124. <https://doi.org/10.3897/phytokeys.156.37277>
- Peng YL, Gao XF, Peng L (2013) Pollen morphology of *Youngia* and six related genera (Asteraceae: Cichorieae) and its systematic significance. Phytotaxa 139(1): 39–62. <https://doi.org/10.11646/phytotaxa.139.1.2>
- Peng YL, Zhang Y, Gao XF, Tong LJ, Li L, Li RY, Zhu ZM, Xian JR (2014) A phylogenetic analysis and new delimitation of *Crepidiastrum* (Asteraceae, tribe Cichorieae). Phytotaxa 159(4): 241–255. <https://doi.org/10.11646/phytotaxa.159.4.1>
- Peng YL, Ju WB, Gao XF, Gao YD (2015) *Youngia purpimea* (Asteraceae), a new species from Sichuan, China. Phytotaxa 236(2): 191–195. <https://doi.org/10.11646/phytotaxa.236.2.8>
- Peng YL, Gao XF, Zhang LB (2017) *Youngia jiulongensis* (Crepidinae, Cichorieae, Asteraceae), a new species from Sichuan, China. Phytotaxa 25(3): 298–301. <https://doi.org/10.3417/2015028>
- Rédei GP (2008) CLUSTAL W (improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice). Springer Netherlands 4673–4680. http://dx.doi.org/10.1007/978-1-4020-6754-9_3188
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 539–542(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sennikov AN, Illarionova ID (2008) Generic delimitation of the subtribe Ixeridinae newly segregated from Crepidiinae (Asteraceae-Lactuceae). Komarovia 5: 57–115.
- Shih C (1993) On the classification of *Ixeris* group (Compositae) from China. Acta Phytotaxonomica Sinica 31(6): 533–548.
- Shih C (1997) Compositae (10). In: Ling Y, Shih C (Eds) Flora Reipublicae Popularis Sinicae 80(1). Science Press, Beijing, 106–266.
- Shih C, Kilian N (2011) *Youngia*. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China 20–21. Science Press, Beijing & Missouri Botanical Garden, St. Louis, 252–263.

- Sun J, Zhang DG, Huang XH, Tojibaev K, Yang JY, Wang HC, Deng T (2020) *Primula sunhangii* (Primulaceae): A new species from Hubei, Central China. *PhytoKeys* 156(1): 103–111. <https://doi.org/10.3897/phytokeys.156.49137>
- Swofford DL (2004) PAUP v.4.0a: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0a. Sinauer, Sunderland.
- Tremetsberger K, Gemeinholzer B, Zetzsche H, Blackmore S, Kilian N, Talavera S (2012) Divergence time estimation in Cichorieae (Asteraceae) using a fossil-calibrated relaxed molecular clock. *Organisms, Diversity & Evolution* 13(1): 1–3. <http://www.springerlink.com/content/1439-6092>. <https://doi.org/10.1007/s13127-012-0094-2>
- Urbatsch L, Pruski JF, Neubig KM (2013) *Youngia thunbergiana* (Crepidinae, Cichorieae, Asteraceae), a species overlooked in the North American flora. *Castanea* 78(4): 330–337. <https://doi.org/10.2179/13-018>
- Vaidya G, Lohman DJ, Meier R (2011) Sequence Matrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27(2): 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Wang H, Wortley AH, Blackmore S (2009) Pollen morphology of *Crepidinae* and *Lactucinae* (Asteraceae: Cichorieae) and its systematic significance. *Grana* 48(3): 160–178. <https://doi.org/10.1080/00173130902931209>
- Wang ZH, Kilian K, Cheng YP, Peng H (2020) *Sinoseris* (Crepidinae, Cichorieae, Asteraceae), a new genus of three species endemic to China, one of them new to science. *Willdenowia* 50(1): 91–110. <https://doi.org/10.3372/wi.50.50109>
- Zhang JW, Nie ZL, Wen J, Sun H (2011) Molecular phylogeny and biogeography of three closely related genera, *Sorosseris*, *Stebbinsia*, and *Syncalathium* (Asteraceae, Cichorieae), endemic to the Tibetan Plateau, SW China. *Taxon* 60(1): 15–26. <https://doi.org/10.1002/tax.601003>
- Zhang JW, Boufford DE, Sun H (2013) Systematic significance of achene morphology in *Sorosseris*, *Syncalathium* and *Parasyncalathium* (Asteraceae: Cichorieae). *Botanical Journal of the Linnean Society* 173(3): 476–486. <https://doi.org/10.1111/boj.12046>
- Zhang XS, Jiang ZL, Yusupov Z, Zhang MH, Zhang DG, Tojibaev K, Meng Y, Deng T (2019) *Prunus sunhangii*: A new species of *Prunus* from central China. *Plant Diversity* 41(1): 19–25. <https://doi.org/10.1016/j.pld.2019.01.003>
- Zhang HJ, Zhang DG, Feng T, Liu Q, Yang JY, Chen L, Wang HC, Deng T (2021) A new species of *Potentilla* (Potentilleae, Rosaceae) from central China, with reference to molecular and morphological evidence. *Phytotaxa* 480(2): 185–194. <https://doi.org/10.11646/phytotaxa.480.2.7>
- Zhu SX, Qin HN, Shih C (2006) Achene wall anatomy and surface sculpturing of *Lactuca* L. and related genera (Compositae: Lactuceae) with notes on their systematic significance. *Journal of Integrative Plant Biology* 48(4): 390–399. <https://doi.org/10.1111/j.1744-7909.2006.00245.x>

Appendix I.

Table A1. Voucher information and GenBank accessions of species used in our study.

Species	Herbarium vouchers	GenBank accessions	
		ITS	rps16
<i>Askellia flexuosa</i>	(1)L.Peng & L.J.Tong1078(CDBI)(2)X.F.Gao,Z.M.Zhu,X.L.Zhao14954(CDBI)	KC968078 ⁽¹⁾	KR733629 ⁽²⁾
<i>Crepidiastrum akagii</i> 1 = <i>Youngia nansiensis</i> 1	(1)Y.L.Peng&L.J.Tong1151-1-3(CDBI)(2)Y. L.Peng &	KC968064 ⁽¹⁾	KC968154 ⁽²⁾
<i>Crepidiastrum akagii</i> 2 = <i>Youngia nansiensis</i> 2	L.J.Tong1151(3)Y.L.Peng & L.J.Tong1131-3(CDBI)	KC968064 ⁽¹⁾	KC968148 ⁽³⁾
<i>Crepidiastrum chelidoniifolium</i>	(1)-	AB002627 ⁽¹⁾	-
<i>Crepidiastrum denticulatum</i> 1	(1)-(2)L.Pengpl2011052701	AB002623 ⁽¹⁾	KC968107 ⁽²⁾
<i>Crepidiastrum denticulatum</i> 2		AB002622 ⁽¹⁾	KC968107 ⁽²⁾
<i>Crepidiastrum diversifolium</i> 1	(1)L.Pengpl2011072605-6(CDBI)(2)	KC968072 ⁽¹⁾	KC968150 ⁽²⁾
<i>Crepidiastrum diversifolium</i> 2	L.Pengpl2011072804(3)L.Pengpl2011072605	KC968072 ⁽¹⁾	KC968149 ⁽³⁾
<i>Crepidiastrum lanceolatum</i>	-	AB002624	AB598601
<i>Crepidiastrum pinnatipartitum</i>	(1)Y.L.Peng325-6(2)Y.L.Peng325	KC968061 ⁽¹⁾	KC968151 ⁽²⁾
<i>Crepidiastrum platyphyllum</i>	(1)K2-CR 1267(2)-	AY876264 ⁽¹⁾	AB598599 ⁽²⁾
<i>Crepidiastrum sonchifolium</i> 1	(1)SCSB-JS0086(2)Lilan245	MH808121 ⁽¹⁾	-
<i>Crepidiastrum sonchifolium</i> 2		MH808120 ⁽²⁾	-
<i>Crepidiastrum taiwanianum</i> 1	(1)-	AB002615 ⁽¹⁾	-
<i>Crepidiastrum taiwanianum</i> 2		AB002614 ⁽¹⁾	-
<i>Crepidiastrum tenuifolium</i>	(1)-	EU363645 ⁽¹⁾	-
<i>Scorzonera austriaca</i>	(1)Y.L.Peng & L.J.Tong1123-3-1(CDBI)(2)Y.L.Peng & L.J.Tong1123-2(CDBI)	KC968059 ⁽¹⁾	KC968135 ⁽²⁾
<i>Youngia cineripappa</i> 1	(1)J.W. Zhang & W.D. Zhu ZZ09041 (KUN)(2)	LT722046 ⁽¹⁾	KR733617 ⁽²⁾
<i>Youngia cineripappa</i> 2	Z.M.Zhu682(CDBI)(3)-	LT722046 ⁽¹⁾	KF732162 ⁽³⁾
<i>Youngia erythrocarpa</i> 1	-	AB598566	KF732132
<i>Youngia erythrocarpa</i> 2		AB598566	KF732169
<i>Youngia gracilipes</i> 1	(1)X.F.Gao14517-9(2)L.Pengpl2011082607-2(3)	KC968076 ⁽¹⁾	KC968155 ⁽²⁾
<i>Youngia gracilipes</i> 2	X.F.Gao14517	KC968076 ⁽¹⁾	KC968126 ⁽³⁾
<i>Youngia heterophylla</i> 1	(1)-(2)X.F.Gao,Y.L.Peng,B.Xu & X.Zheng11937-2(3)	AB598561 ⁽¹⁾	KC968123 ⁽²⁾
<i>Youngia heterophylla</i> 2	X.F.Gao,Y.L.Peng,B.Xu & X.Zheng11694	AB598561 ⁽¹⁾	KC968122 ⁽³⁾
<i>Youngia humifusa</i> 1	(1)Y.L.Peng & L.J.Tong981-3-1(2)Y.L.Peng &	KC968034 ⁽¹⁾	KC968115 ⁽³⁾
<i>Youngia humifusa</i> 2	L.J.Tong1012(3)X.F.Gao, Y.L.Peng, B.Xu & X.Zheng13147(CDBI)(4)Y.L.Peng & L.J.Tong981-1	KC968035 ⁽²⁾	KC968113 ⁽⁴⁾
<i>Youngia japonica</i> subsp. <i>formosana</i>	(1)-	AB598559 ⁽¹⁾	-
<i>Youngia japonica</i> subsp. <i>japonica</i> 1	(1)-(2)ZhangJW 388 (KUN)(3)	AB598557 ⁽¹⁾	KC968153 ⁽³⁾
<i>Youngia japonica</i> subsp. <i>japonica</i> 2	L.Pengpl2011072001-6(4)X.F.Gao,Y.L.Peng,B.Xu & X.Zheng11605	HQ436229 ⁽²⁾	KC968118 ⁽⁴⁾
<i>Youngia longiflora</i>	(1)-	AB598558	-
<i>Youngia paleacea</i> 1	(1)-(2)-(3)L.Peng pl2011082701(CDBI)(4)L.Peng	KJ502310 ⁽¹⁾	KR733620 ⁽³⁾
<i>Youngia paleacea</i> 2	pl082605-1(CDBI)	KJ502311 ⁽²⁾	KR733616 ⁽⁴⁾
<i>Youngia rubida</i>	(1)Y.L.Peng93-3(CDBI)(2)Y.L.Peng pl081201(CDBI)	KC968048 ⁽¹⁾	KR733627 ⁽²⁾
<i>Youngia bangii</i>	(1)HHE 3256(KUN)	MZ817057	MZ923644
<i>Youngia simulatrix</i>	(1)-	KJ502312 ⁽¹⁾	-
<i>Youngia szechuanica</i>	(1)-	KJ502313 ⁽¹⁾	-
<i>Youngia thunbergiana</i>	(1)-	KC539465 ⁽¹⁾	-
<i>Youngia zhengyiana</i>	(1)-	KJ502314 ⁽¹⁾	-

The history and typification of *Lilium humboldtii* J.H.Krelage (Liliaceae)

James Compton¹, Mark W. Skinner²

1 Spilsbury Farm, Tisbury Row, Salisbury SP3 6RU, UK **2** Skinner and Associates, 1275 SE River Forest Rd., Milwaukie, OR 97267 USA

Corresponding author: James A. Compton (jamiecompton@madasafish.com)

Academic editor: Lorenzo Peruzzi | Received 13 June 2021 | Accepted 20 August 2021 | Published 14 September 2021

Citation: Compton J, Skinner MW (2021) The history and typification of *Lilium humboldtii* J.H.Krelage (Liliaceae). PhytoKeys 182: 39–55. <https://doi.org/10.3897/phytokeys.182.70099>

Abstract

The history of the Californian *Lilium humboldtii* J.H.Krelage, its initial discovery and confusion in literature over its collection, distribution and naming are discussed. Neotypes are designated for the names *Lilium humboldtii* and *Lilium bloomertianum* Kellogg. Lectotypes are designated for the names *Lilium canadense* var. *puberulum* Torr. and *L. bloomertianum* var. *ocellatum* Kellogg.

Keywords

Duchartre, Krelage, Leichtlin, *Lilium humboldtii*, morphological key, nomenclature, Regel, Roetzl, typification

Introduction

The Californian lily, *Lilium humboldtii*, was first recognised to be a new and distinct taxon by the botanist John Torrey when he received material in New York sent to him from California in 1854 by the botanist John Milton Bigelow. Bigelow was employed on the government-sponsored 35th Parallel Pacific Railroad Survey of the plants found along one of the proposed Pacific Railroad routes across the USA, led by Lieutenant Amiel Weeks Whipple. Torrey published the name *Lilium canadense* var. *puberulum* Torr. in the belief that the lily was a variety of *L. canadense* L., a species native to the eastern USA (Torrey 1856: 146). Torrey added, however, “If the characters given above prove constant, this fine lily must be considered distinct from *L. canadense*” (Torrey 1856: 147).

The lily was identified as a new species sometime before 1860 by Dr Albert Kellogg (1813–1887) one of the seven founding members of the Californian Academy of Sciences. Kellogg had seen this species in cultivation in the garden of Hiram Green Bloomer (1819–1874) a botanist and another of the founders of that prestigious Academy. Kellogg was so impressed with the lily that he had it painted (possibly by Bloomer who was known to be an accomplished artist) and exhibited it in the halls of the Academy in 1860 where he proposed the name *L. bloomerianum* for it, but he did not validly publish that name for another 12 years (Kellogg 1872: 160).

This paper looks at the history of this species and follows the course of its rediscovery by a Czech plant collector in California, its arrival in Germany and its distribution to The Netherlands, France, England and Russia. We address some of the misinformation and confusion that has surrounded this magnificent Californian lily since its first recorded discovery.

Benedikt Roezl (1824–1885), the collector

The Czech traveller, botanist and plantsman, Benedikt Roezl is known largely for the immense quantity of orchids that he collected in Mexico and in various countries of South America. He started his horticultural career in 1836 in the garden of Tetschen [Děčín] Castle, the home of Leopold Graf von Thun und Hohenstein in Bohemia (Czech Republic), then later worked for Louis Van Houtte in Gentbrugge (Belgium) in 1846 where he became the new chef de culture of Van Houtte's école d'horticulture de Gand (Ghent).

Roezl collected in North America on his extended route across the USA in 1869 while travelling to California from New York, via St Louis, Chicago, Omaha, Cheyenne and Truckee (Mabberley 1985: 452). Roezl wrote a report of his Californian journey, including the discovery of *L. humboldtii* and sent it to Eduard Ortgies, Superintendent of the University of Zurich Botanic Garden and Roezl's de facto agent, who published the account in "Gartenflora" (Ortgies 1871: 108). In it, Roezl wrote (translated from the German):

"A commercial gardener in San Francisco told me about a very beautiful lily with yellow, red-dotted flowers which occurs near Nevada City. I hired two Chinese to help me and having made the acquaintance of Dr. Tiling, a doctor who is interested in the local flora, within eight days I had many bulbs of this lily. If I am permitted, I should like to give this lily the name *L. humboldtii* as it was found on the 100th birthday of von Humboldt. This beautiful lily has golden-yellow flowers with red and black spots, leaves clustered in whorls and stems five to six feet tall and bears up to 35 flowers" (Ortgies 1871: 108).

Ortgies added a footnote stating that Herr. Leichtlin handled the purchase and import of the lily whose flowers were noticeably long-lived (Ortgies 1871: 108). The Dr. Tiling mentioned by Roezl must have been the physician and botanist Heinrich Sylvester Theodor Tiling (1818–1871).

Roezl added additional information on his discovery in a short autobiography of his expeditions and collections for the "Gardeners' Chronicle" published on 18 July 1874 (Roezl 1874). In that year, he returned to Europe to settle in Smichow, Bohemia

[Smíchov, a district of Prague]. He wrote about his arrival in the USA only four years after the end of the American Civil War and having lost one of his arms while demonstrating a new sugar-cutting machine in Cuba in 1868:

“Afterwards I proceeded to New York to start on my Californian travels over the Rocky Mountains and the Sierra Nevada. I discovered here the new lilies *L. washingtonianum*, *puberulum*, *parvum* and *humboldtii*; the latter I found on the hundredth memorial day of Alexander von Humboldt and hence named one of the species after him. The lily in question does not come from the Humboldt County as some catalogues assert” (Roezl 1874: 73).

Roezl’s discovery of this new species would therefore have been on 6 May 1869 although he never published a validating description of his new lily.

Jacob Heinrich Krelage (1824–1901), the author of the name

In the world of horticulture, the great beauty and stature of this Californian lily was very quickly recognised. Roezl’s collection crossed the Atlantic to Europe in the same year that it had been collected. It was first validly named *Lilium humboldtii* in Haarlem, The Netherlands, in 1870, some five and a half thousand miles from its native habitat in the Sierra Nevada. Jacob Heinrich Krelage, son of the nurseryman Ernst Heinrich Krelage (1786–1855), was a keen and very successful commercial grower of bulbous plants. He placed two advertisements in the “Gardeners’ Chronicle and Agricultural Gazette” on 22 and 29 October 1870 for his *E.H.Krelage en zoon* nursery listing *Lilium humboldtii* as a new and interesting *Lilium*. His description in both cases was short: “from California, 5 feet high, golden flowers spotted with purple, £1 each” (J.H.Krelage 1870: 1402; 1435). There is, however, just enough descriptive matter to satisfy Art. 38.1 of the International Code of Nomenclature (ICN; Turland et al. 2018). Art. 38.1 is “one of the most difficult provisions of the ICN to apply” (J. Wiersema, pers. comm., 24 May 2021), thus the authors of this note affirmed with a number of experts involved in the current ICN, including Dr John Wiersema (US) and Dr John McNeill (E), the sufficiency of J.H.Krelage’s brief description which predates that of the English nurseryman William Bull of King’s Road, Chelsea in London. Bull, whether aware or not of Krelage’s acquisition, provided an equally short description of this recently imported Californian lily less than three months later, on 14 January 1871 (see Typification below).

Jacob Krelage waited another four years before writing a full account of *L. humboldtii* in his “De Tuinbouw-Illustratie Tijdschrift voor Tuinbouw en Plantkunde” (“The Illustrated-Horticulture Magazine for Horticulture and Botany”). He also included a plate (see Fig. 1) to accompany the comprehensive descriptive text and provided much valuable information about its history (J.H.Krelage 1874: 138, t. 31). Of further interest, the description, published by J.H.Krelage in 1870, is attributed to *E.H.Krelage en zoon*, but without specifying any individual of the business as the author of the name. Therefore, we have invoked external evidence (ICN Art. 46.9) to establish that J.H.Krelage was the only extant member of the Krelage firm (E.H.Krelage having died in 1855) and is, therefore, the author of *Lilium humboldtii*.



Figure 1. *Lilium humboldtii* subsp. *humboldtii* in “De Tuinbouw-Illustratie Tijdschrift voor Tuinbouw en Plantkunde” plate 31. (Krelage 1874).

Pierre Duchartre (1811–1894), lily writer and taxonomist

The French botanist, Pierre Etienne Simon Duchartre, was one of the founders of the Société Botanique de France in 1854. From a young age he had been fascinated by the morphology of the genus *Lilium* and, in particular, its bulb structure. He wrote extensively on the species that were cultivated in the garden of the bulb connoisseur Max Leichtlin in Karlsruhe. His articles, each entitled “Observations sur le Genre Lis”, appeared in nine separate instalments in the French “Journal de la Société Impériale et Centrale d’Horticulture de France” Ser. 2 in vols. 4 and 5 (Duchartre 1870; Duchartre 1871). In the seventh of these instalments, he wrote (translated from the French):

“*Lilium humboldtii* is one of the most beautiful discoveries made by Benedikt Roesl and one of the most precious introductions into the garden of Maximilien Leichtlin” (Duchartre 1871: 94).

Duchartre added that on 14 May 1870 Leichtlin had written a letter to him informing him that B. Roesl had found the plant at Devil’s Gate, in a ravine that ran alongside the Pacific Railroad next to a river with many rapids, before arriving at Wintah Station from where the train goes on to Mormon City. This misleading information, imparted second hand from Roesl via Leichtlin, has been the source of much confusion and is discussed below under the heading The Utah Mystery.

Duchartre also added that the description he is providing was based on two young and rather meagre specimens which he owed to the kindness of his great friend M. Leichtlin and explained that his friend Leichtlin had witnessed the species come into flower for the first time in the month of July 1870 (Duchartre 1871: 95). Finally, Duchartre provided a comprehensive Latin description and diagnosis in the firm belief that, judging from his earlier correspondence with Leichtlin, the plant known as “*Lilium humboldtii* Roezl et Leichtlin in litt.” had not yet been formally described. He added after the description:

“Hab. in Californiae montibus Sierra Nevada dictis, ubi a cl. Roezl detectum est ab eo cum cl. et amicissimo Max Leichtlin communicatum (v.v.c.)” [It grows in the Sierra Nevada mountains of California, where it was found by the famous Roezl and it was communicated to me by him along with my great and famous friend Max Leichtlin. v.v.c.] (v.v.c. = *vidi vivam cultam*: I have seen it alive in cultivation) (Duchartre 1871: 97).

Duchartre had maintained a long correspondence with Max Leichtlin (see below) in Karlsruhe [formerly Carlsruhe], also with J. H. Krelage in Haarlem and with the great nurseryman Louis Van Houtte in Gentbrugge (Duchartre 1873a: 2). Two years after his informative paper on *L. humboldtii* (Duchartre 1871), he repeated the salient extracts from that paper in a separate article that he wrote for Van Houtte’s journal “Flore des Serres”. A coloured plate (see Fig. 2) of a plant that had been cultivated in Van Houtte’s garden was included (Duchartre 1873b t. 879).

Amongst Duchartre’s correspondence, now kept in the Lenhardt Library of the Chicago Botanic Garden, is a handwritten note on *L. humboldtii* including a sketch by him of the bulb, which showed Duchartre’s particular interest in bulb diversity within the genus. The note simply says (translated from the French):

“On the 19 February 1870, I received by the post from Mr. Max Leichtlin of Carlsruhe, a beautiful lily bulb with the following letter: “*I am sending you today a bulb of the rare, new L. humboldtii of this magnificent genus. The bulbs came to me from the Sierra Nevada of North America. You can see at first glance the singular growth next to the developing bulb which sinks into the soil each year. It has orange flowers spotted with purple and carries sometimes as many as 30 flowers of a quite remarkable beauty. P.S. my plant only provisionally carries the name L. humboldtii.*”

The illustration of the bulb drawn by Duchartre in pencil also bears a short note by him (translated from the French):

“*Lilium humboldtii* (provisional name) sent from Carlsruhe by M. Max Leichtlin on 19 February 1870. The bulb is as I received it. The bulb is situated on the side of a stem fragment from under which emerge several now dried out roots. It has the general form of a martagon with large scales, thinner, almost flat, whitish, rather speckled towards the top”.

Maximilien Leichtlin (1831–1910), bulb grower extraordinaire

Max Leichtlin was one of the greatest growers of rare bulbs during the 19th century. He was the third son of Eduard Leichtlin, founder in 1823 of a successful paper-making business with his brother in Karlsruhe. Max Leichtlin briefly joined the family



Figure 2. Illustration of *Lilium humboldtii* subsp. *humboldtii*, painted from Louis Van Houtte's nursery garden and published in "Flore des Serres" 19: t. 879. (Duchartre 1873b).

business before beginning his lifelong career in horticulture, first as an apprentice in the gardens of the Schloss in Karlsruhe for the Grand Duke of Baden, then he went to the Königliche Gartenakademie [Royal Garden Academy] in Potsdam. In 1850, he travelled around Europe and South America to enhance his botanical knowledge, returning to Europe in 1856 (Hooker 1883: 1; Nelves and Cuthbertson 1931: 223). He worked for two years in the nursery of Louis Van Houtte in Gentbrugge. On the death of his eldest brother, Leichtlin rejoined the family paper business until leaving the business again in the 1860s in order to lay out his own garden near to the Stadtgarten Karlsruhe [now Zoologischer Stadtgarten] where he cultivated many rare plants. His garden included some 250 different lilies, many acquired from his ever-burgeoning list of cosmopolitan contacts, Roetzl being one of them. Leichtlin also distributed plants amongst his correspondents including to Duchartre in France and Eduard Regel (see below) in Russia.

Leichtlin had to leave Karlsruhe in 1873 owing to development in the southern part of the city. He moved to Baden-Baden 40 km south of Karlsruhe where he acquired a new garden near the Neuen Schlosses and continued to grow his rare and unusual species. His reputation by this time was huge and he was given honorary fellowship of the Royal Horticultural Society in London as well as being awarded the Veitch Memorial Medal by the Society. Leichtlin produced from this garden a series of



Figure 3. The neotype of *Lilium humboldtii* from Louis Van Houtte's nursery garden, published in "Gartenflora" 21: t. 724 (Regel 1872).

sale catalogues of the plants that he grew there. It is worth noting that, in his first list of *Lilium*, he credited his friend Duchartre with the authorship of the name "*L. humboldtii* Duchartre" (Leichtlin 1874: 16), but later, in his undated list of "Lilien", he attributed the name to Roehl as "*L. humboldtii* Roehl" without any description or additional information in either case (Leichtlin suppl. 1: 2, pre-1873).

Eduard Regel (1815–1892), the St Petersburg connection

Eduard August von Regel was a prolific German horticulturist and botanist who worked during his early career in botanic gardens in Göttingen, Bonn and Berlin. In 1842, he was appointed head of the botanic garden in Zürich. In 1852, he founded and edited the magazine "Gartenflora", in which he described several new lily species. In 1855, he became a research botanist at the Imperial Botanic Garden in St Petersburg and, from 1875, he was its director and remained so until his death.

In vol. 21 of his "Gartenflora", he described *Lilium humboldtii* with a fine coloured illustration (Regel 1872: 161–163, t. 724, see Fig. 3). This must have been sent to him courtesy of Louis van Houtte as the same image was reproduced a year later for

Duchartre (Duchartre 1873b t. 879, see typification below). Regel more or less repeats the text previously published by Duchartre (Duchartre 1871: 94–97), but adds some important information regarding the source of his own plants of the species from which the plate was prepared. His text included (translated from the German):

“We owe the depiction of the beautiful lily that our table presents here to our honoured friend Leichtlin. The bulb shown at c in the table is based on a living specimen in the garden. B. Roezl discovered this excellent and beautiful lily in California in the Sierra Nevada at Devil’s Gate, a wild mountain valley through which a river with many rapids falls, along which the Pacific Railway runs through the mountains. Mr M. Leichtlin bought from Mr Roezl all of the bulbs which he had collected, with the exception of a few specimens, which were obtained through the intermediary of Mr M. Leichtlin for the Imperial Botanic Garden in St. Petersburg”.

Regel’s comments on the provenance of the species once again have added to the confusion surrounding its origin. It seems that he is describing the Devil’s Gate in Utah, but mistakenly placing it in the Sierra Nevada in California. This error probably occurred from misinformation supplied to him by Leichtlin (see The Utah Mystery below). In addition, it is not clear in which garden the living bulb shown at c was in cultivation (but see discussion on the illustrations in Typification below).

The Utah mystery

The geographic range of *Lilium humboldtii* subsp. *humboldtii* and *Lilium humboldtii* subsp. *ocellatum* is now well established as being restricted to the Sierra Nevada, coastal ranges and Channel Islands of California (Abrams 1923; Skinner 2002). How is it, therefore, that two of Roezl’s lily collections were described by Eduard Regel (Regel 1870: 321) and as mentioned above by Duchartre (via Leichtlin) as coming from the Mormon State i. e. Utah?

The first of Roezl’s collections, originally named *Lilium roezli* Regel, but now recognised to be *L. pardalinum*, was, according to Regel:

“Die beistehen Lilie ward von Roezl im Felsengeberg in der nähe des Mormonen-Staates entdeckt und is mit *L. superbum* L. zunächst verwandt.” [The lily shown here was discovered by Roezl in the Rocky Mountains near the Mormon State and was originally thought to be related to *L. superbum* L.” (Regel 1870: 321).

The second collection, already discussed, of *L. humboldtii* includes the statement by Leichtlin that “Roezl found it at Devil’s Gate, in a ravine that ran alongside the Pacific railroad next to a river with many rapids, before arriving at Wintah Station from where the train goes on to Mormon City”. The construction of the Union Pacific Railroad linking the east with western USA in 1869 took place from two directions. The line running eastwards from San Francisco in California ran through the Sierra Nevada range, across the State of Nevada and across the north of Utah. The westwards line from Wyoming ran through the Wahsatch [Wasatch] mountains along the Weber Canyon and Devil’s Gate Pass to Uintah Station, then on to Ogden and finally up

to Promontory Junction. The opening ceremony for the joining of these two lines at Promontory was on 9 May 1869. Roezl himself stated that he had collected several lilies, including *L. humboldtii*, in the Sierra Nevada, but he clearly also travelled along the route of the Union Pacific Railroad through Devil's Gate Pass which is east of the mining town of Uintah (Leichtlin's Wintah) only three days before the joining up of the two lines. His journey down the course of the Weber River towards the Great Salt Lake must have been by horse or on foot as the line was not yet up and running.

Another curious anomaly in the two statements is that no species of the genus *Lilium* from western USA has been found as far east as the State of Utah; indeed, it is the only State in the continental US with no native *Lilium*. The localities of *Lilium humboldtii* are on the western slopes of the Sierra Nevada and along the coastal ranges in southern California some 600 miles (ca. 950 km) west of Devil's Gate in Utah. The distribution of *L. pardalinum* Kellogg is in California and southernmost Oregon. According to Roezl's report to Ortgies on his Californian travels (described above), we now know that the lily was actually collected in the Sierra Nevada in Nevada County near Nevada City (Ortgies 1871: 108).

There are, however, two species of *Fritillaria* that do occur in the Wasatch Mountains that span the States of Utah and Wyoming through which the Devil's Gate Pass runs: *F. atropurpurea* Nutt. and *F. pudica* (Pursh) Spreng. It would seem likely that, through the various communications between Roezl, Leichtlin and his associates, the provenance of the lilies and the fritillaries may have been mistakenly mixed up. In one of his undated catalogues of the plants grown in his garden, Max Leichtlin mentioned under the heading Knollen und Zwiebel-Gewächse [Tubers and Bulbs] the entry: "*Fritillaria* species from Devil's Gate" (Leichtlin undated Catalogue pre-1873: 8).

To add an extra layer of confusion, there is also a Devil's Gate in Mono County, California, but it is outside the range of both *L. pardalinum* and *L. humboldtii* and has nothing to do directly with our history.

Typification

No original material was cited in the nurseryman Jacob Krelage's catalogue advertisement and it is extremely unlikely that a specimen would have been collected. The publication, however, must serve as the protologue for the name *L. humboldtii* (J.H.Krelage 1870: 1402). To recapitulate from above, the description, albeit very short, is enough to validate the name (Art. 38.1, Turland et al. 2018; J. Wiersema, pers. comm., 24 May 2021) and predates the short advert also placed in the "Gardeners' Chronicle" the following year by another bulb enthusiast and nurseryman William Bull of London on 14 January 1871. Bull's description under the heading 'New Lilies' might have been one of the nursery entries that had later prompted Roezl to correct the misrepresentation of the locality of the species:

"*Lilium humboldtii* – a splendid species from Humboldt County, California, growing about five feet high and producing large golden-yellow flowers, which are spotted with purple. Good bulbs. Price 1 guinea each" (Bull 1871: 35).

Krelage's brief description also predates Roezl's provisional name suggested in his own report published by his agent Ortgies in "Gartenflora" (Ortgies 1871: 108), as well as the comprehensive description provided by Duchartre later that year (Duchartre 1871: 97).

Three illustrations exist of plants grown directly from Roezl's collections. The history of the two published coloured images, resulting directly from Roezl's collection in California, is rather confusing.

Upon his receipt of the bulbs from Roezl, Leichtlin must have forwarded some bulbs very soon after their arrival in Karlsruhe to Louis Van Houtte in Belgium. This information is revealed by comparing Regel's illustration (Fig. 3) in "Gartenflora" (Regel 1872: t. 724) with the illustration that was published of this species in "Flore des Serres" a year later (Fig. 2) and which is clearly a reproduction of the same image (Duchartre 1873b: t. 879). The later illustration in "Flore des Serres" actually includes the statement:

"*L. humboldtii* Roezl et Leichtl. – Regel Gartenflora, juin et août 1872, ubi tabulam nostram reperimus" [Regel Gartenflora, June and August 1872, where our plate was to be found].

This plate was initially prepared from bulbs grown in Van Houtte's garden in Gentbrugge as revealed by the legend "Off. lith. & pict. in Horto Van Houtteano" shown bottom right. In effect, Regel predated Duchartre to the publication of the same image. The two images differ from each other in the format of the lettering which was altered from capitals and with the addition of "Californie" and "plein air" [out in the open] in "Flore des Serres" (Duchartre 1873b: t. 879) to small and italicised in "Gartenflora" (Regel 1872: t. 724). The shading in the latter is also darker.

A few of Leichtlin's bulbs had also been forwarded to Regel in St. Petersburg where they were cultivated in the Imperial Botanic Garden. From these bulbs, two herbarium specimens were prepared. These can be seen in LE: LE-01072601 and LE-01072602.

The third illustration appeared another year later (Fig. 1) in "De Tuinbouw-Illustratie", the journal for the nursery of *E.H. Krelage en zoon*. Uncoloured, this was prepared from the first bulbs cultivated in Haarlem, The Netherlands that had been sent to Jacob Krelage from Leichtlin (Krelage 1874: 138–142, t. 31).

Although these illustrations are historically linked with Roezl's original collection of this species, none can be considered as original material and, therefore, a neotype must be chosen that can be representative of the species (J. McNeill, pers. comm., 7 April 2021). The illustration accompanying Regel's text in "Gartenflora" (1872: t. 724) is, therefore, chosen here as the type for the name (see Fig. 3).

Taxonomic conspectus

Lilium humboldtii J.H.Krelage, Gard. Chron. 1870: 1402 (1870)

Neotype. Designated here: [Icon], Gartenflora 21: t. 724 (1872).

Key to subspecies of *L. humboldtii*

- 1 Bulbs off-white, occasionally flecked with purple, scales always unsegmented; sepals and petals orange, spots magenta, without aureolated lighter red margins; foothills of Sierra Nevada..... ***Lilium humboldtii* subsp. *humboldtii***
- 2 Bulbs often purplish, scales segmented; sepals and petals yellow or light orange, spots red or magenta, aureolated with lighter red margins; southern California ***Lilium humboldtii* subsp. *ocellatum***

Lilium humboldtii* subsp. *humboldtii

- ≡ *Lilium canadense* var. *humboldtii* (J.H.Krelage) Baker, Gard. Chron. 1871: 1165 (1871)
= *Lilium canadense* var. *puberulum* Torr., Pacific Railr. Rep. 4 pt.5 no.4: 146 (1856)
Lectotype designated here: USA, **California**, “*K.T.Hartweg 2004*” (GH, lecto!) [GH-00106407]; syntypes: USA, **California** “border of meadows, Antelope Creek, one of the tributaries of the Upper Sacramento, 23 May 1846 *Col. Frémont 490*” (NY, syn!) [NY-0008523]; USA, **California** “near Butte Creek in the Sacramento Valley, 16 June 1848, *Hartweg 2004*” (K, syn.! × 2).
≡ *Lilium puberulum* (Torr.) Duchartre, J. Soc. Centr. Hort. France ser. 2, Vol. 4: 217 (1870).

Diagnostic description. **Bulb:** off-white, occasionally flecked with purple; scales unsegmented; stem roots absent. **Racemes:** 1–33(–40) flowered. **Flowers:** with sepals and petals orange, speckled with magenta, spots distributed distally or more proximally; pollen rust, rust-brown, rust-orange, occasionally to warm tan, becoming yellowish. **Seeds:** 114–225 per capsule.

Distribution. U.S.A., California (Amador, Butte, Calaveras, El Dorado, Fresno, Mariposa, Nevada, Placer, Tehama, Tuolumne, Yuba). *Lilium humboldtii* subsp. *humboldtii* is distributed from Tehama County south to Calaveras County; reports from further south are erroneous.

Ecology. Flowering summer (mid-June to early August), frequently in forest openings of Ponderosa pine forest (*Pinus ponderosa* Douglas ex C.Lawson) and chaparral openings; (200–1100 m).

Illustration. “Gartenflora” 21: t. 724 (1872) see Fig. 3. <https://www.biodiversitylibrary.org/item/125746#page/215/mode/1up>

***Lilium humboldtii* subsp. *ocellatum* (Kellogg) Thorne, *Aliso* 9: 195. 1978**

Basionym: *Lilium bloomerianum* var. *ocellatum* Kellogg, Proc. Calif. Acad. Sci. 5: 88 (1873). **Lectotype** designated here: [Icon] Proc. Calif. Acad. Sci. 5: 88 (1873: t. 4, see Fig. 4).

- ≡ *Lilium humboldtii* var. *ocellatum* (Kellogg) Baker, Journ. Linn. Soc. (Botany) 14(76): 245 (1874).
- ≡ *Lilium ocellatum* (Kellogg) Beane, Contr. Dudley Herb. 4: 358 (1955).
- = *Lilium bloomerianum* Kellogg, Proc. Calif. Acad. Sci. 4: 160 (1872). Type: USA, **California**, [Icon] not seen, probably destroyed; **Neotype** designated here: USA, California, *A.Kellogg & W.G.W.Harford* 978 coll. 1868–1869. (US neo!) [US-03945856].
- ≡ *Lilium humboldtii* var. *bloomerianum* (Kellogg) Purdy, J. Roy. Hort. Soc. 26: 354 (1901).
- = *Lilium humboldtii* var. *magnificum* Purdy, J. Roy. Hort. Soc. 26: 353 (1901). Type not seen
- = *Lilium fairchildii* M.E.Jones, Contr. W. Bot. 16: 39, 26 (1930) **holotype**: USA, **California**, San Diego, Mt. Palomar, west of Hot Springs, 13 July 1929, *M.E.Jones* 24762 (RSA, holo!) [RSA-0000358]

Note 1. In the absence of any type material, the description of *L. bloomerianum* by Kellogg of the bulbs as purplish and his statement “This is the most magnificent lily of the Pacific coast” indicate that he was describing *L. humboldtii* subsp. *ocellatum*. This is reinforced by Purdy’s description of *L. humboldtii* var. *bloomerianum* (with *L. bloomerianum* Kellogg also cited) as occurring in San Diego County which is within the range of subsp. *ocellatum*, but outside that of subsp. *humboldtii*. The only material indicated in the protologue as type of the name *L. bloomerianum* is the illustration donated by an unknown donor to the California Academy of Sciences which may have perished in the earthquake and fire of 1906 (Emily Magnaghi; Seth Cotterell, pers. comm.). A neotype has, therefore, been chosen.

It could be argued that, under Art. 36.1 (Turland et al. 2018), the name *L. bloomerianum* is not a validly published name as it could be seen as a “provisional name” that has been merely proposed in anticipation of its future acceptance. In his report to the California Academy of Science, Kellogg stated “Out of respect to its time-honored cultivator, Mr. H. G. Bloomer, he offered the provisional name of *Lilium bloomerianum*”. In this case, however, Kellogg added the comment “This lily is the most magnificent lily of the Pacific Coast” and the diagnostic sentence “This lily is easily discriminated from all others in any stage of its growth”. These comments are more than merely provisional. Moreover, the formal heading of “On *Lilium bloomerianum*”, as well as the full description, indicates that Kellogg is validly describing the species.

Note 2. Original herbarium material of *Lilium bloomerianum* var. *ocellatum* is stated in the protologue to have been gathered by *William George Willoughby Harford* of the U. S. Coast Survey from Santa Rosa Island. No specimen has been located. It is possible that it might also have perished in the earthquake and fire at CAS in 1906 (Emily Magnaghi, pers. comm.). We have, therefore, chosen the illustration (plate 4) that accompanies the text, as the lectotype of the name (Kellogg 1873 t. 4, see Fig. 4).

Diagnostic description. Bulb: often purplish, especially apically; scales notched, segmented with 2–5 poorly defined segments or occasionally unsegmented; stem roots frequently present. **Racemes:** 1–25-flowered. **Flowers:** with sepals and petals yellow or light



Figure 4. Illustration chosen as lectotype for the name *Lilium bloomerianum* var. *ocellatum* Kellogg, in Proceedings of the California Academy of Sciences 5: t. 4 (Kellogg 1873).

orange, speckled with large red or magenta spots aureolated with light red margins, spots larger and their margins wider and lighter towards the apex; pollen tan or peach, becoming yellow or tan-yellow, occasionally tan-orangish or rust. **Seeds:** 150–252 per capsule.



Figure 5. Representative specimen of *Lilium humboldtii* subsp. *ocellatum* in Peutz Valley near San Diego, California, showing the ocellated markings on the floral segments of the subspecies. (Mark Skinner).

Distribution. U.S.A., California (Anacapa Island, Santa Cruz Island, Santa Rosa Island, Los Angeles, Orange, Riverside, Santa Barbara, San Bernardino, Ventura). In addition to the mainland (see counties above), it occurs on the larger northern Channel Islands, where it is the only native lily.

Ecology. Flowering late spring–summer (mid-May to July). Oak canyons, chaparral; 0–1800 m elev. *Lilium humboldtii* subsp. *ocellatum* is similar to subsp. *humboldtii*, but the yellowish sepals and petals with widely margined spots, lighter-coloured pollen and purplish bulb with notched scales are distinctive.

Illustration. *Lilium humboldtii* subsp. *ocellatum* Photo. Mark Skinner (Fig. 5): Peutz Valley, east of San Diego, California

Epilogue

It took a European nurseryman to provide a formal name for a beautiful Californian lily species for the first time. It is worth noting that, despite its almost immediate appeal in Europe, there are very few records of this lily being in cultivation in its native country within the first decades of its rediscovery and distribution by Roezl. This paucity may have been due to the instability of the western part of the USA after the Mexico–American War, which was not finalised until 1848. This volatility was followed just over a decade later by the immense disruption caused by the American Civil War from 1861 to 1865. Roezl’s journey, therefore, took place only a few years after the dust had settled from the Civil War and during that period of American history when the west of the vast country was finally being conveniently connected by the railroad to the east.

One early American record of the cultivation of *L. humboldtii* is that of the nurseryman L. B. Case of Richmond, Indiana where *L. humboldtii* was listed as having flowers “yellow, with dark spots. 75c each, \$7 per doz.” (Case 1877: 23). This was, therefore, eight years after Roezl had collected and distributed his plants and six years after its first description in a Dutch nursery catalogue.

Unpublished material

The Duchartre Collection of manuscript notes, drawings and photographs on lilies (1870–1880), Chicago Botanic Garden Lenhardt Library, Illinois State Library digital archives <http://www.idaillinois.org/digital/collection/ncbglib01/id/24727>

Acknowledgements

Our thanks to Emily Magnaghi and Seth Cotterell (CAS) for searching the collections for original material of *Lilium bloomerianum* var. *ocellatum*. Many thanks to Gerard Thijssse (L) for his translations of Dutch text into English and John McNeill (E) and

John Wiersema (US) for nomenclatural advice. Thanks also to Anita Dijkstra (WUR Library) and Sara Lee (United States National Agricultural Library) for kindly searching for Krelage nursery Catalogues. Thanks to the reviewers Lorenzo Peruzzi, Michal Hroneš, Manuel B. Crespo, John McNeill and Martyn Rix for their helpful comments on the MS.

References

- Abrams L (1923) *Lilium*. In: An Illustrated Flora of the Pacific States: Washington, Oregon and California, vol. 1. Ophioglossaceae to Aristolochiaceae. Stanford University Press, 416–420. <https://doi.org/10.5962/bhl.title.62583>
- Bull W (1871) New Lilies, *Lilium washingtonianum*, *Lilium humboldtii*, Gardeners' Chronicle and Agricultural Gazette 1871(1): 35.
- Case LB (1877) *Lilium humboldtii* in L. B. Case's botanical index vol. 1: an illustrated quarterly botanical magazine. P. 23. L. B. Case, Richmond, Indiana.
- Duchartre PES (1870) Observations sur le Genre Lis pts. 1–5. Journal de la Société Impériale et Centrale d'Horticulture de France Ser. 2 vol. 4: 212–562.
- Duchartre PES (1871) Observations sur le Genre Lis pt. 7. Journal de la Société Impériale et Centrale d'Horticulture de France Ser. 2 vol. 5: 87–106.
- Duchartre PES (1873a) Observations sur les Bulbes des Lis. Bibliothèque de l'Ecole des Hautes Etudes section des Sciences Naturelles tome 7 article no. 5: 1–31. <https://doi.org/10.5962/bhl.title.15501>
- Duchartre PES (1873b) *Lilium humboldtii* Roetzl et Leichtlin (Liliaceae), Flore des Serres et des Jardins de l'Europe 19: 65–68, t. 879.
- Hooker JD (1883) To Herr Max Leichtlin, Baden Baden. Curtis's Botanical Magazine 109: 1.
- Kellogg A (1872) On *Lilium bloomerianum*. Proceedings of the California Academy of Sciences 4: 160.
- Kellogg A (1873) *Lilium bloomerianum* var. *ocellatum*. Proceedings of the California Academy of Science 5: 88, t. 4.
- Krelage JH (1870) *Lilium humboldtii* in Gardeners' Chronicle and agricultural gazette 1870: 1402.
- Krelage JH (1874) *L. humboldtii* Roetzl et Leichtlin, Lelie van Humboldt, De Tuinbouw-Illustratie tijdschrift voor tuinbouw en plantkunde: 138–142. [Pl. 31]
- Leichtlin M (1874) Pflanzen-Sammlung des Leichtlin'schen Gartens in Baden-Baden. G. Braun'schen Hofbuchdruckerei, Karlsruhe. <https://doi.org/10.5962/bhl.title.82704>
- Leichtlin M ([undated]) Catalogue and Supplement 1. Karlsruhe. [pre-1873]
- Mabberley DJ (1985) Die neuen pflanzen von Ch. Huber Frères & Co. in Hyères. Taxon 43(3): 448–456. <https://doi.org/10.2307/1221212>
- Nelmes E, Cuthbertson W (1931) Max Leichtlin (1831–1910). Curtis's Botanical Magazine Dedications 1827–1927: 222–224.
- Ortgies KE (1871) Bericht über die reisen und einföhrungen des Herrn B. Roetzl in den Jahren 1869 und 1870. Gartenflora 20: 107–117.
- Regel E (1870) *Lilium roezli* Regel. Gartenflora 19: 321–322, t. 667.
- Regel E (1872) *Lilium humboldtii* Roetzl et Leichtlin. Gartenflora 21: 161–163, t. 724.

- Roezl B (1874) Benedikt Roezl. Gardeners' Chronicle [N.S.] 2: 73.
- Skinner MW (2002) *Lilium*. In: Flora of North America Editorial Committee (Eds) Flora of North America North of Mexico, Vol. 26. Magnoliophyta: Liliidae: Liliales and Orchidales. Oxford University Press, New York; Oxford.
- Torrey J (1856) Reports of explorations and surveys to ascertain the most practicable and economical route for a railroad route from the Mississippi river to the Pacific Ocean. Volume 4, part 5: Report on the botany of the expedition, number 4, descriptions of the general botanical collections: 146. United States War Department, A. O. P. Nicholson, Washington.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF [Eds] (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2018. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>

Cyclorhiza puana (Apiaceae), a new species from Sichuan, China

Jing Zhou¹, Jun-Mei Niu¹, Xin-Yue Wang¹,
Pei Wang¹, Ming-Jia Guo¹, Zhen-Wen Liu²

1 School of Pharmaceutical Science and Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, China **2** Yunnan Academy of Forestry and Grassland, 650201, Kunming, Yunnan, China

Corresponding author: Zhen-Wen Liu (liuzw2021@163.com)

Academic editor: Alexander Sennikov | Received 6 April 2021 | Accepted 10 August 2021 | Published 20 September 2021

Citation: Zhou J, Niu J-M, Wang X-Y, Wang P, Guo M-J, Liu Z-W (2021) *Cyclorhiza puana* (Apiaceae), a new species from Sichuan, China. PhytoKeys 182: 57–66. <https://doi.org/10.3897/phytokeys.182.67009>

Abstract

A new species, *Cyclorhiza puana* J. Zhou & Z.W. Liu (Apiaceae) from Sichuan Province of China, is described and illustrated here. Morphological comparisons with congeneric species revealed that it is distinguished by its slender habit, sparse annular scars, 4-pinnatisect leaf blade with ultimate segments linear (2–4×0.5–1 mm), subequal rays, oblong fruits with slightly thickened ribs, obconic stylopodium and slightly concave seed face. A molecular analysis based on nuclear ribosomal DNA internal transcribed spacer (ITS) sequences indicated that *C. puana* is genetically distinct from the other two species of the genus. A distribution map, as well as an updated key, are provided for the species of *Cyclorhiza*.

Keywords

Apiaceae, *Cyclorhiza*, new species, nrITS, phylogeny

Introduction

Cyclorhiza M.L. Shih & R.H. Shan is a small genus of Apiaceae subfamily Apioideae, with two species currently recognised (Shih and Shan 1980; Shih and Watson 2005; Pimenov 2017). It is distributed in southwest China and characterised by carrot-like roots with prominent annular scars, bracts and bracteoles usually absent, yellow petals and fruits subpentagonal in cross section (Shih and Shan 1980; Shih and Watson 2005). Previous phylogenetic studies indicated that the two species of *Cyclorhiza* con-

stituted a highly-supported monophyletic clade in the tribe Komarovieae and showed a sister group relationship to *Calyptrosciadium* Rech. f. & Kuber from SW Asia (Zhou et al. 2009; Downie et al. 2010; Zhou et al. 2020).

During a botanical survey to examine Apiaceae in Sichuan Province of China, we discovered a small population of *Cyclorhiza*, whose morphology was clearly distinct from the other species of the genus. We checked all the collections of *Cyclorhiza* at PE and KUN, and digital resources from CVH and GBIF. We noticed a specimen identified as *C. waltonii* in CSH, whose morphology is exactly the same as in our collection. Further examination of morphological characters, coupled with molecular evidence, convinced us that this plant is a distinct new species, which is described and illustrated here.

Materials and methods

Morphological studies

The morphological characters were examined based on collected specimens. Fruits were taken from dry specimens and studied using a stereo microscope. Herbarium specimens were deposited at KUN. Morphological comparisons with the related *C. waltonii* (H. Wolff) M.L. Sheh & R.H. Shan and *C. peucedanifolia* (Franch.) Constance are provided in Table 1 and Fig. 1.

Phylogenetic analysis

For the new species, we used our own collection and the specimen deposited as CSH. *Cyclorhiza waltonii* and *C. peucedanifolia* each included two new accessions to examine the possible infraspecific molecular variation (Table 2). In addition, thirty-two ITS sequences were obtained from GenBank to determine the phylogenetic position of the new species. The taxa chosen represented a broad cross-section of sampling from the tribe Komarovieae and other clades or tribes of Apioidae, identified in previous phylogenetic studies (Downie et al. 2010; Zhou et al. 2020). Two species of Bupleureae were used to root the tree. The final data matrix comprised a total of 38 accessions. Detailed information about herbarium vouchers, GenBank accession numbers, and literature citations of previously published sequences for those taxa considered in this study are listed in Table 2.

The Plant Genomic DNA Kit (Tiangen Biotech) was used to isolate DNA from materials of silica-gel-dried and herbarium specimens, and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences were used for phylogenetic inference. Detailed information on PCR amplification and sequencing strategies was obtained from Zhou et al. (2008). Phylogenetic analyses were conducted using both Maximum Likelihood (ML) and Maximum Parsimony (MP). ML analyses were performed using MEGA7 (Kumar et al. 2016), with the GTR + G + I model and 1000 bootstrap (BS) replicates. Parsimony analysis was performed using PAUP* v. 4.0b10 (Swofford 2003). For the heuristic search, 100 random addition sequence replicates, tree bisection-reconnection (TBR), saving multiple trees and ACCTRAN optimisa-

Table 1. Morphological comparison between *Cyclorhiza puana* and congeneric species.

Character	<i>C. puana</i>	<i>C. waltonii</i>	<i>C. peucedanifolia</i>
Root	Annular scars, sparse	Annular scars, dense	Annular scars, dense
Rootstock	Slender , up to 5 mm in diameter	Stout, up to 20 mm in diameter	Stout, up to 20 mm in diameter
Stem	Up to 60 cm tall , simple or several, unbranched or upper 1–3-branched, 2–3 mm in diameter	Up to 100 cm tall, simple, branched above, 2–7 mm in diameter	Up to 150 cm tall, simple, branched above, 10–15 mm in diameter
Leaf	Triangular-ovate in outline, 4-pinnatisect, ultimate segments linear , 2–4×0.5–1 mm	Triangular-ovate in outline, 4-pinnatisect, ultimate segments linear, 4–20 × 2–6 mm	Broadly ovate-triangular in outline, 4-pinnatisect, ultimate segments ovate-oblong to linear-lanceolate, 20–60 × 3–10 mm
Bract	Absent, rarely 1	Absent	Absent, or 1–2
Ray	4–6, subequal	4–14, unequal	5–12, unequal
Bracteoles	Absent, or 1–2	Absent	Absent
Calyx	Triangular	Triangular	Triangular
Stylopodium	Obconic	Low-conic	Low-conic
Fruit	Oblong , 5×2 mm	Ellipsoid, 4×2.5 mm	Ovoid, 4–7×2–3.5 mm
Ribs	Filiform, prominent, slightly thickened	Filiform, prominent, narrowly winged	Filiform, prominent, narrowly winged
Mericaip	Subpentagonal, seed face slightly concave	Subpentagonal, seed face deeply sulcate	Subpentagonal, seed face deeply concave

tion were chosen. Gaps were treated as missing data. Bootstrap values were calculated from 1000 replicate analyses using TBR branch swapping and simple stepwise-addition of taxa.

Results

Molecular phylogenetic analysis

The final aligned data matrix contained 629 positions, in which 256 were parsimony informative. The new species yielded high sequence divergence values with the other two species of the genus, i.e. *C. waltonii* (5.21%) and *C. peucedanifolia* (4.39–4.60%). The phylogeny showed that two accessions of the new species formed a strongly-supported monophyletic group and constituted a sister branch of *Cyclorhiza* species within the tribe Komarovieae (Fig. 2). Each of the three species was resolved as monophyletic groups, with the infraspecific divergence values ranging between 0.00–0.58%.

Taxonomic treatment

Cyclorhiza puana J. Zhou & Z.W. Liu, sp. nov.

urn:lsid:ipni.org:names:77219738-1

Fig. 1G–I

Type. China. Sichuan: Luhuo County, Renda Town, 3052 m, 100°38'59.57"E, 31°24'50.76"N, 17 Aug 2016, J. Zhou, Z. W. Liu & Y. Z. Gao LZ201606120 (holotype: KUN! [KUN1519999]; isotype: KUN!).

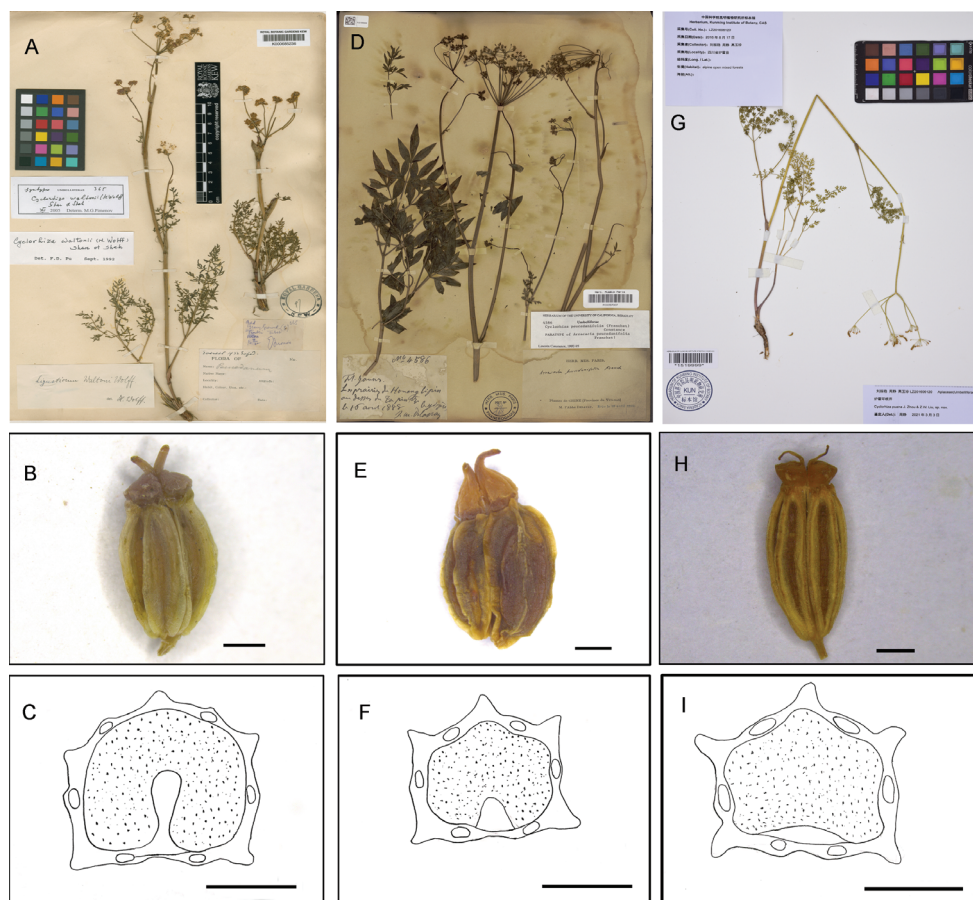


Figure 1. *Cyclorhiza waltonii* (H.Wolff) M.L. Sheh & R.H. Shan (**A–C**) **A** syntype (K000685236) **B** fruit image **C** cross-section; *C. peucedanifolia* (Franch.) Constance (**D–F**) **D** syntype (P00057007) **E** fruit image **F** cross-section; *C. puana* J. Zhou & Z.W. Liu (**G–I**) **G** holotype (KUN1519999) **H** fruit images **I** cross-section. Scale bar: 1 mm.

Diagnosis. *Cyclorhiza puana* resembles *C. waltonii* but differs from the latter in its long-cylindric roots with sparse annular scars (vs. stout, branched near stem into a cluster of several long, woody, carrot-like roots with prominent annular scars), smaller ultimate segments $2\text{--}4 \times 0.5\text{--}1$ mm (vs. $4\text{--}20 \times 2\text{--}6$ mm), rays subequal (vs. unequal), stylopodium obconic (vs. low-conic) and seed face slightly concave (vs. deeply sulcate).

Description. Herbs perennial, 40–60 cm tall, glabrous. Taproots long-cylindrical with sparse annular scars. Stem base covered in purplish-brown remnant sheaths, solitary or rarely several, ribbed, unbranched or upper 1–3-branched, 2–3 mm in diameter. Basal and lower leaves petiolate, petioles 2–6 cm long, sheaths narrow, short; blade triangular-ovate in outline, 4-pinnatisect, $2\text{--}5 \times 7\text{--}12$ cm, ultimate segments linear, $2\text{--}4 \times 0.5\text{--}1$ mm. Upper leaves smaller and reduced. Umbels loose, compound, terminal and lateral; bracts absent or sometimes 1; bracteoles absent or rarely 1–2, linear;

Table 2. Detailed information about voucher or source information and GenBank accession numbers for 38 accessions used in phylogenetic analysis.

Taxa	Source/Voucher	GenBank number
<i>Bupleurum angulosum</i> L.	Naves and Watson (2004)	AF469008
<i>B. fruticosum</i> L.	Naves and Watson (2004)	AF479298
<i>Calypotroscadium polycladum</i> Rech. f. & Kuber	Valiejo-Roman et al. (2006)	AY941266 & AY941294
<i>Chamaesium paradoxum</i> H. Wolff	Zhou et al. (2008)	EU236161
<i>C. thalictrifolium</i> H. Wolff	Zhou et al. (2008)	EU236162
<i>C. wolffianum</i> Fedde ex H. Wolff	Zhou et al. (2008)	EU236163
<i>Changium smyrnioides</i> Fedde ex H. Wolff	Hu et al. (unpubl.)	DQ517340
<i>Chuanminshen violaceum</i> M.L. Shen & R.H. Shan	Zhou et al. (2009)	FJ385040
<i>Cyclorhiza peucedanifolia</i> (Franch.) Constance	China, Yunnan, Niujaoshan, 261 (KUN)	MW807296
	Zhou et al. (2009)	FJ385042
	China, Yunnan, Lijiang, 1218 (KUN)	MW807297
<i>C. puana</i> Zhou & Liu	China, Sichuan, Luhuo, Renda Town, LZ201606120 (KUN)	MW807294
	China, Sichuan, Batang, CSH06561 (CSH)	MW807295
<i>C. waltonii</i> (H. Wolff) Sheh & Shan	China, Sichuan, Derong, 31029 (KUN)	MW807298
	China, Sichuan, Derong, 3167 (KUN)	MW807299
	Zhou et al. (2008)	EU236165
<i>Hansenia mongholica</i> Turcz.	Katz-Downie et al. (1999)	AF008643 & AF009122
<i>H. weberbaueriana</i> (Fedde ex H. Wolff) Pimenov & Kljuykov	Xin & Chen (unpubl.)	JQ936558
<i>Haplosphaera phaea</i> Hand.-Mazz.	Zhou et al. (2008)	EU236167
<i>Heptaptera anisoptera</i> Tutin	Valiejo-Roman (unpubl.)	AY941273 & AY941301
<i>Hymenidium amabile</i> (Craib & Smith) Pimenov & Kljuykov	Valiejo-Roman et al. (2012)	FJ469934 & FJ483473
<i>Hymenidium mieheanum</i> Pimenov & Kljuykov	Valiejo-Roman et al. (2012)	FJ469951 & FJ483490
<i>Komarovia anisosperma</i> Korovin	Valiejo-Roman et al. (1998)	AF077897
<i>Parasilaus afghanicus</i> (Korovin) Pimenov	Katz-Downie et al. (1999)	MK088003
<i>P. asiaticus</i> Pimenov	Katz-Downie et al. (1999)	AF008642 & AF009121
<i>Physospermopsis delavayi</i> (Franch.) H. Wolff	Zhou et al. (2009)	FJ385056
<i>Pleurospermopsis bicolor</i> (Franch.) J. Zhou & J. Wei	Zhou et al. (2014)	KF806587
<i>P. sikkimensis</i> C.B. Clarke	Spalik et al. (2010)	GQ379347
<i>Pleurospermum austriacum</i> Hoffm.	Valiejo-Roman et al. (2012)	FJ469962 & FJ483502
<i>P. cristatum</i> H. de Boissieu	Li et al. (2011)	JF977828
<i>Pseudotrachydium dichotomum</i> (Korovin) Pimenov & Kljuykov	Spalik et al. (2010)	GQ379342
<i>P. vesiculosolatum</i> (Rech. f.) Pimenov & Kljuykov	Valiejo-Roman et al. (2012)	FJ469964 & FJ483503
<i>Pterocyclis angelicoides</i> (Wall. ex DC.) Klotzsch	Valiejo-Roman et al. (2012)	FJ469967 & FJ483505
<i>P. forrestii</i> (Diels) Pimenov & Kljuykov	Valiejo-Roman et al. (2012)	FJ469965 & FJ483504
<i>P. rotundatus</i> (DC.) Pimenov & Kljuykov	China, Xizang, G18092501–1 (SZ)	MK078059
<i>Sphaerosciadium denaense</i> Pimenov & Kljuykov	Terentieva et al. (unpubl.)	FJ489358 & FJ489389
<i>Tongoloo silaifolia</i> (H.de Boissieu) H. Wolff	Zhou et al. (2008)	EU236213
<i>Trachydium roylei</i> Lindl.	Valiejo-Roman et al. (2012)	FJ469972 & FJ483510

rays 4–6, subequal; umbellules 6–14-flowered, pedicels 6–8 mm, subequal. Calyx teeth minute, triangular; petals not known; stylopodium obconic, brown; styles short. Fruit oblong, 5 × 2 mm, dark yellow; ribs 5, filiform, prominent, slightly thickened; vittae 1 in each furrow, 2 on commissure. Seed face slightly concave. Carpophore 2-cleft to base.

Etymology. The species epithet “puana” is given in honour of Prof. Pu Fading (1936–) for his outstanding contributions to the Chinese Apiaceae.

Vernacular name. The Chinese name is given as “炉霍环根芹” (lú huò huán gēn qín), referring to the locality where the type specimen was collected.

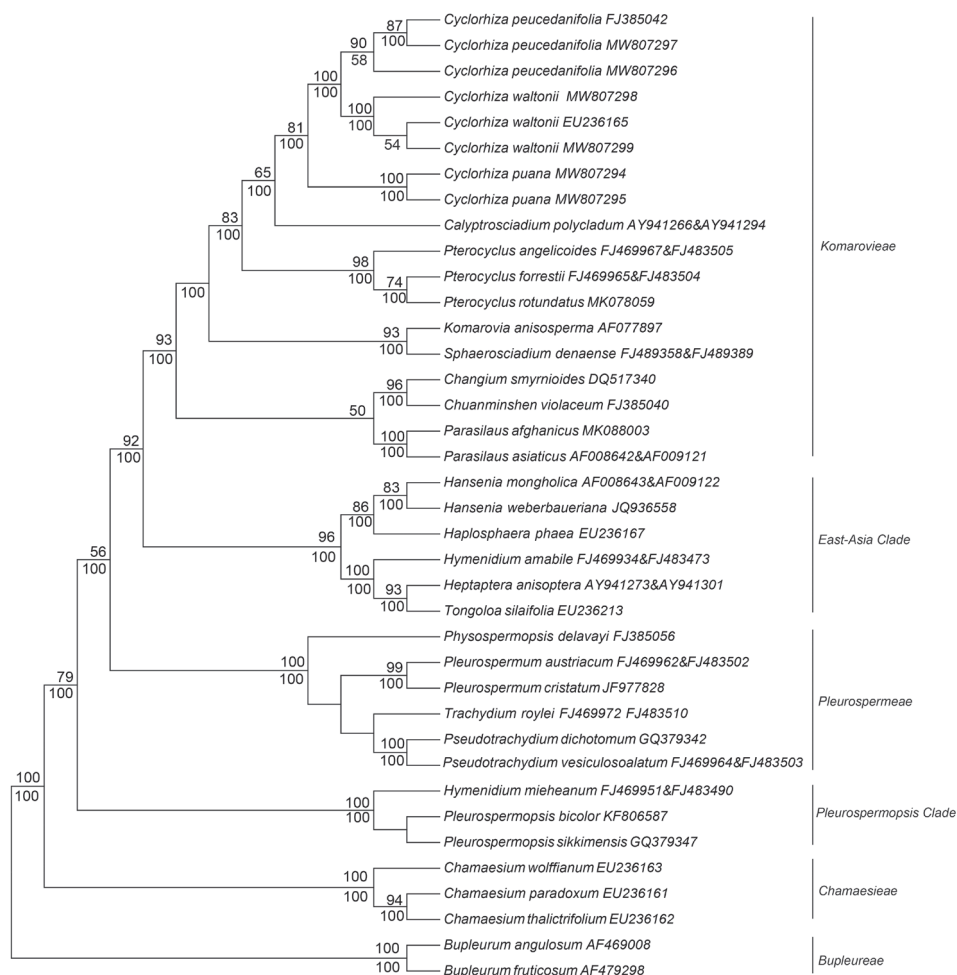


Figure 2. Phylogenetic tree derived from nrDNA ITS sequences, with support values in Maximum Likelihood and Maximum Parsimony above and below nodes, respectively.

Phenology. Flowering from June to July, and fruiting from July to September.

Distribution and habitat. The new species is distributed in Sichuan Province, China. It grows in the alpine open mixed forests at elevations of 3000–3200 m (Fig. 3).

Additional specimens examined (paratype). China. Sichuan: Batang County, Jiangbading Village, 3268 m, 99°11'51"E, 29°55'54"N, 30 Jul. 2014, X.X. *Zhu*, B. *Chen*, B. *Shen* & Y.G. *Song* CSH06561 (CSH! [CSH0037273]).

Conservation status. So far, only two populations with no more than ten individuals have been found. Through further investigations, more populations may be discovered to assess its conservation status. Based on the available data, the new species can be assessed as Data Deficient (DD) on the basis of recommendations of the International Union for Conservation of Nature (IUCN 2019).

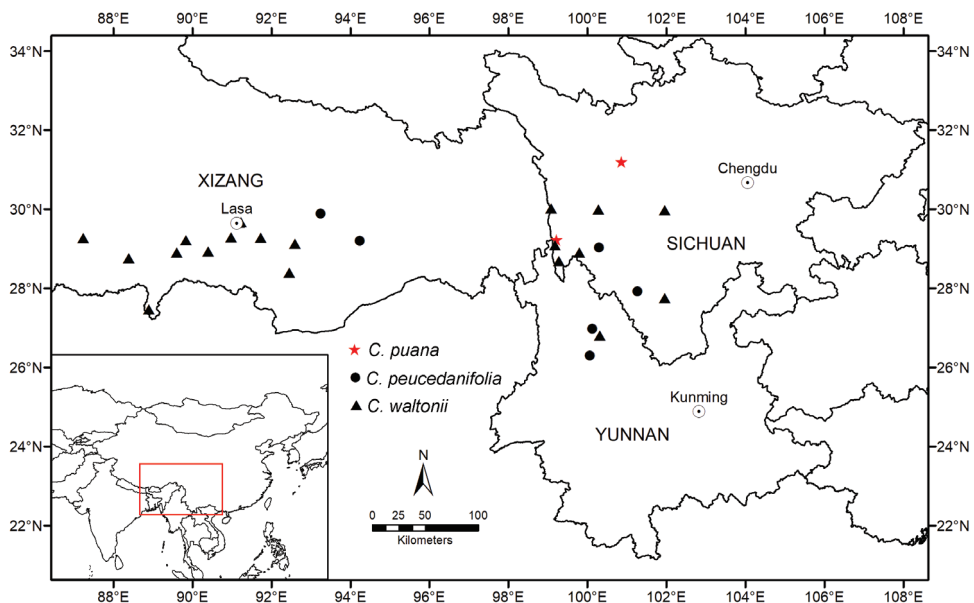


Figure 3. Distribution map of *C. waltonii*, *C. peucedanifolia* and *C. puana*.

Key to species of *Cyclorhiza*

- 1 Ultimate leaf segments ovate-oblong to linear-lanceolate, 20–60 × 3–10 mm..... *C. peucedanifolia*
- Ultimate leaf segments linear, 2–20 × 0.5–6 mm 2
- 2 Ultimate leaf segments 4–20 × 2–6 mm; rays unequal; stylopodium low-conic; seed face deeply sulcate..... *C. waltonii*
- Ultimate leaf segments 2–4 × 0.5–1 mm; rays subequal; stylopodium ob-conic; seed face slightly concave..... *C. puana*

Discussion

Due to its topographical and climatic heterogeneity, the Hengduan Mountains of the Sino-Himalayas is one of the richest regions across China in terms of biodiversity (Ying and Zhang 1984; Pu 1993). These mountains harbor an incredible number of endemic species, among which three species of *Cyclorhiza* are found. *Cyclorhiza waltonii* is found in open broad-leaved forests, scrub and alpine meadows of W Sichuan, SE Xizang and NW Yunnan Provinces at elevations of 2500–4600 m. *Cyclorhiza peucedanifolia* occurs in alpine open mixed forests, bamboo thickets and scrub of SW Sichuan (Muli), SE Xizang, NW Yunnan Provinces at elevations of 1800–3600 m (Sheh and Watson 2005). The new species seems to be relatively rare and known only from two localities in Sichuan Province, where it grows in alpine

open mixed forests at elevations of 3000–3200 m. Generally, the three species share a similar ecology and habitat.

Cyclorhiza has been regarded as a well-defined genus since it was established by Sheh and Shan in 1980. The new species possesses typical characteristics of the genus, such as taproots with prominent annular scars, bracts and bracteoles usually absent, and fruits subpentagonal in cross section, with variation in some characters (e.g. the seed face slightly concave, and the stylopodium obconic).

The specimen from CSH identified as *Cyclorhiza waltonii* actually corresponds to *C. puana*. The confusion between these two species is likely driven by the similar morphology of the leaf blades, i.e., ultimate segments. However, the ultimate leaf segments in *C. puana* are smaller, $2\text{--}4 \times 0.5\text{--}1$ mm, whereas those in *C. waltonii* are $4\text{--}20 \times 2\text{--}6$ mm (Sheh and Shan 1980). Additionally, *C. puana* can be distinguished by obconic stylopodium (vs. low-conic), ribs filiform, prominent, slightly thickened (vs. filiform, prominent, narrowly winged) and seed face slightly concave (vs. deeply sulcate). The genetic difference between these two species is relatively large (> 5%), further supporting their separate status.

Acknowledgements

We are grateful to reviewers for helpful comments on the manuscript. This work was supported by the National Natural Science Foundation of China (no. 31960048, 31460052 and 31872649), the Ten Thousand Talents Program of Yunnan (YNWR-QNBJ-2019-208) and the Hundred Talents Program of Kunming Medical University (no. 60118260127).

References

- Downie SR, Spalik K, Katz-Downie DS, Reduron JP (2010) Major clades within Apiaceae subfamily Apioideae as inferred by phylogenetic analysis of nrDNA ITS sequences. *Plant Diversity and Evolution* 128(1–2): 111–136. <https://doi.org/10.1127/1869-6155/2010/0128-0005>
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>
- Katz-Downie DS, Valiejo-Roman CM, Terentieva EI, Troitsky AV, Pimenov MG, Lee B, Downie SR (1999) Towards a molecular phylogeny of Apiaceae subfamily Apioideae: Additional information from nuclear ribosomal DNA ITS sequences. *Plant Systematics and Evolution* 216(3–4): 167–195. <https://doi.org/10.1007/BF01084397>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>

- Li DZ, Gao LM, Li HT, Wang H, Ge XJ, Liu JQ, Chen ZD, Zhou SL, Chen SL, Yang JB, Fu CX, Zeng CX, Yan HF, Zhu YJ, Sun YS, Chen SY, Zhao L, Wang K, Yang T, Duan GW (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America* 108(49): 19641–19646. <https://doi.org/10.1073/pnas.1104551108>
- Neves SS, Watson MF (2004) Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. *Annals of Botany* 93(4): 379–398. <https://doi.org/10.1093/aob/mch052>
- Pimenov MG (2017) Updated checklist of Chinese Umbelliferae: Nomenclature, synonymy, typification, distribution. *Turczaninowia* 20(2): 106–239. <https://doi.org/10.14258/turczaninowia.20.2.9>
- Pu FD (1993) Umbelliferae. In: Wang WT (Ed.) *Vascular Plants of Hengduan Mountains*, vol. 1. Science Press, Beijing, 1276–1355.
- Sheh ML, Shan RH (1980) *Cyclorhiza* and *Chuanminshen*—Two newly proposed genera in Umbelliferae (Apiaceae). *Journal of University of Chinese Academy of Sciences* 18(1): 45–49.
- Sheh ML, Watson MF (2005) *Cyclorhiza* M.L. Sheh & R.H. Shan. In: Wu ZY, Peter HR, Hong DY (Eds) *Flora of China*, vol. 14. Science Press & Missouri Botanical Garden Press, 53.
- Spalik K, Pivczynski M, Danderson CA, Kurzyrna-Mlynik R, Bone TS, Downie SR (2010) Amphitropic amphiantarctic disjunctions in Apiaceae subfamily Apioideae. *Journal of Biogeography* 37(10): 1977–1994. <https://doi.org/10.1111/j.1365-2699.2010.02334.x>
- Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), vers. 4. Sinauer, Sunderland.
- Valiejo-Roman CM, Pimenov MG, Terentieva EI, Downie SR, Katz-Downie DS, Troitsky AV (1998) Molecular systematics of Umbelliferae: Using nuclear rDNA internal transcribed spacer sequences to resolve issues of evolutionary relationships. *Botanicheskii Zhurnal* 83(7): 1–22.
- Valiejo-Roman CM, Shneyer VS, Samigullin TH, Terentieva EI, Pimenov MG (2006) An attempt to clarify taxonomic relationships in “Verwandtschaftskreis der Gattung *Ligusticum*” (Umbelliferae-Apioideae) by molecular analysis. *Plant Systematics and Evolution* 257(1–2): 25–43. <https://doi.org/10.1007/s00606-005-0383-8>
- Valiejo-Roman CM, Terentieva EI, Pimenov MG, Kljuykov EV, Samigullin TH, Tilney PM (2012) Broad Polyphyly in *Pleurospermum* s.l. (Umbelliferae-Apioideae) as inferred from nrDNA ITS and chloroplast sequences. *Systematic Botany* 37(2): 573–581. <https://doi.org/10.1600/036364412X635593>
- Ying TS, Zhang ZS (1984) Endemism in the flora of China – studies on the endemic genera. *Zhiwu Fenlei Xuebao* 22: 259–268.
- Zhou J, Peng H, Downie SR, Liu ZW, Gong X (2008) A molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nuclear ribosomal DNA internal transcribed spacer sequences. *Taxon* 57: 402–416. <https://doi.org/10.2307/25066012>
- Zhou J, Gong X, Downie SR, Peng H (2009) Towards a more robust molecular phylogeny of Chinese Apiaceae subfamily Apioideae: Additional evidence from nrDNA ITS and cp-

- DNA intron (rpl16 and rps16) sequences. *Molecular Phylogenetics and Evolution* 53(1): 56–68. <https://doi.org/10.1016/j.ympev.2009.05.029>
- Zhou J, Wang WC, Liu MQ, Liu ZW (2014) Molecular authentication of the traditional medicinal plant *Peucedanum praeruptorum* and its substitutes and adulterants by DNA-barcoding technique. *Pharmacognosy Magazine* 10(40): 385–390. <https://doi.org/10.4103/0973-1296.141754>
- Zhou J, Gao YZ, Wei J, Liu ZW, Downie SR (2020) Molecular phylogenetics of *Ligusticum* (Apiaceae) based on nrDNA ITS sequences: Rampant polyphyly, placement of the Chinese endemic species, and a much-reduced circumscription of the genus. *International Journal of Plant Sciences* 181(3): 306–323. <https://doi.org/10.1086/706851>

Three new species of *Columnea* (Gesneriaceae) from the western Andean slopes of Ecuador and Colombia

John L. Clark¹, Francisco Tobar^{2,4}, Laura Clavijo³,
Mathieu Perret⁵, Catherine Helen Graham⁶

1 Science Department, The Lawrenceville School, Lawrenceville, NJ 08648, USA **2** Área de Investigación y Monitoreo de Avifauna, Aves y Conservación – BirdLife en Ecuador, Quito, Ecuador **3** Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia **4** Instituto Nacional de Biodiversidad, Herbario Nacional del Ecuador QCNE, Quito, Ecuador **5** Conservatoire et Jardin botaniques de la Ville de Genève, Ch. de l'Impératrice 1, CH-1292 Chambésy, Switzerland **6** Biodiversity and Conservation Biology Unit, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

Corresponding author: John L. Clark (jlclark@lawrenceville.org)

Academic editor: Ricardo Kriebel | Received 22 May 2021 | Accepted 3 August 2021 | Published 20 September 2021

Citation: Clark JL, Tobar F, Clavijo L, Perret M, Graham CH (2021) Three new species of *Columnea* (Gesneriaceae) from the western Andean slopes of Ecuador and Colombia. *PhytoKeys* 182: 67–82. <https://doi.org/10.3897/phytokeys.182.69016>

Abstract

Three new species of *Columnea* (Gesneriaceae, tribe Gesnerieae) are described from the western Andean slopes of northern Ecuador and southern Colombia. *Columnea angulata* J.L. Clark & F. Tobar and *Columnea floribunda* F. Tobar & J.L. Clark are described from northern Ecuador. *Columnea tecta* J.L. Clark & Clavijo is described from southern Colombia and northern Ecuador. The three new species are facultative epiphytes with dorsiventral shoots and are readily recognized by bright red tips on the abaxial and adaxial leaf surfaces. The species described here are vegetatively similar to the sympatric species *Columnea picta* H. Karst. and are readily differentiated by floral features that are illustrated, described and featured with digital images.

Abstract

Se describen tres especies nuevas de *Columnea* (Gesneriaceae, tribu Gesnerieae) originarias de la vertiente occidental de los Andes de Ecuador y Colombia. *Columnea angulata* J.L. Clark & F. Tobar y *Columnea floribunda* F. Tobar & J.L. Clark se describen del norte de Ecuador; *Columnea tecta* J.L. Clark & Clavijo se describe del sur de Colombia y norte de Ecuador. Las tres especies nuevas son epífitas facultativas con vástagos dorsiventrales, las cuales se reconocen fácilmente por las puntas de las hojas de color rojo brillante en ambas superficies; son vegetativamente similares y simpátricas con *Columnea picta* H. Karst., pero se pueden diferenciar por las características florales que se ilustran, describen y detallan con imágenes digitales en este artículo.

Keywords

Colombia, *Columnea*, Ecuador, Gesneriaceae, taxonomy

Introduction

The flowering plant family Gesneriaceae, with over 3400 species and 150+ genera (Weber 2004; Weber et al. 2013), is in the order Lamiales. The family is divided into three subfamilies and seven tribes (Weber et al. 2013, 2020), which represent monophyletic lineages (Ogutcen et al. 2021). The majority of New World members are in the subfamily Gesnerioideae and are represented by 1200+ species and 77 genera (Clark et al. 2020). *Columnea* L. is classified in the tribe Gesnerieae and subtribe Columneinae (Weber et al. 2013, 2020).

The genus *Columnea* is primarily epiphytic. It ranges from Mexico south to Bolivia, and is most diverse in the northern Andes of Colombia and Ecuador. With over 210 species (Clark et al. 2020), *Columnea* is the largest genus in the subfamily Gesnerioideae (Weber et al. 2013, 2020). The genus is distinguished by fruits that are indehiscent berries in contrast to fleshy bivalved capsules in closely related genera. *Columnea* is strongly supported as a monophyletic genus based on molecular phylogenetic studies (Smith et al. 2013; Schulte et al. 2014). The species described here were discovered during exploratory research expeditions and ongoing taxonomic research of herbarium specimens. The three newly described species are similar to many taxa recognized in the section *Collandra* (Lem.) Benth. or previously classified as members of the genus *Dalbergaria* Tus-sac. We refrain from classifying the new species to a subgeneric rank because most are artificially defined and not supported by phylogenetic studies (Smith and Carroll 1997; Smith 2000; Clark and Zimmer 2003; Clark et al. 2012; Smith et al. 2013; Schulte et al. 2014). The three species described here are distributed on the western Andean slopes of northern Ecuador and southern Colombia (Fig. 1). Herbarium specimens representing these three species are often annotated as “*Columnea* aff. *picta*” or “*Columnea* cf. *picta*” because they share a similar vegetative feature of apical red leaf apices on the upper and lower leaf surfaces. In contrast, most species of *Columnea* have red leaf apices on the lower leaf surface, but not on the upper leaf surface. Table 1 summarizes prominent characters to differentiate the three new species from each other and from *Columnea picta*.

Taxonomic treatment***Columnea angulata* J.L. Clark & Tobar, sp. nov.**

urn:lsid:ipni.org:names:77219739-1

Figs 2, 3

Diagnosis. Differs from *Columnea picta* by a shallow bilabiate corolla limb (vs. deeply bilabiate corolla limb) and a corolla perpendicular to oblique relative to the calyx (vs. corolla straight relative to the calyx).

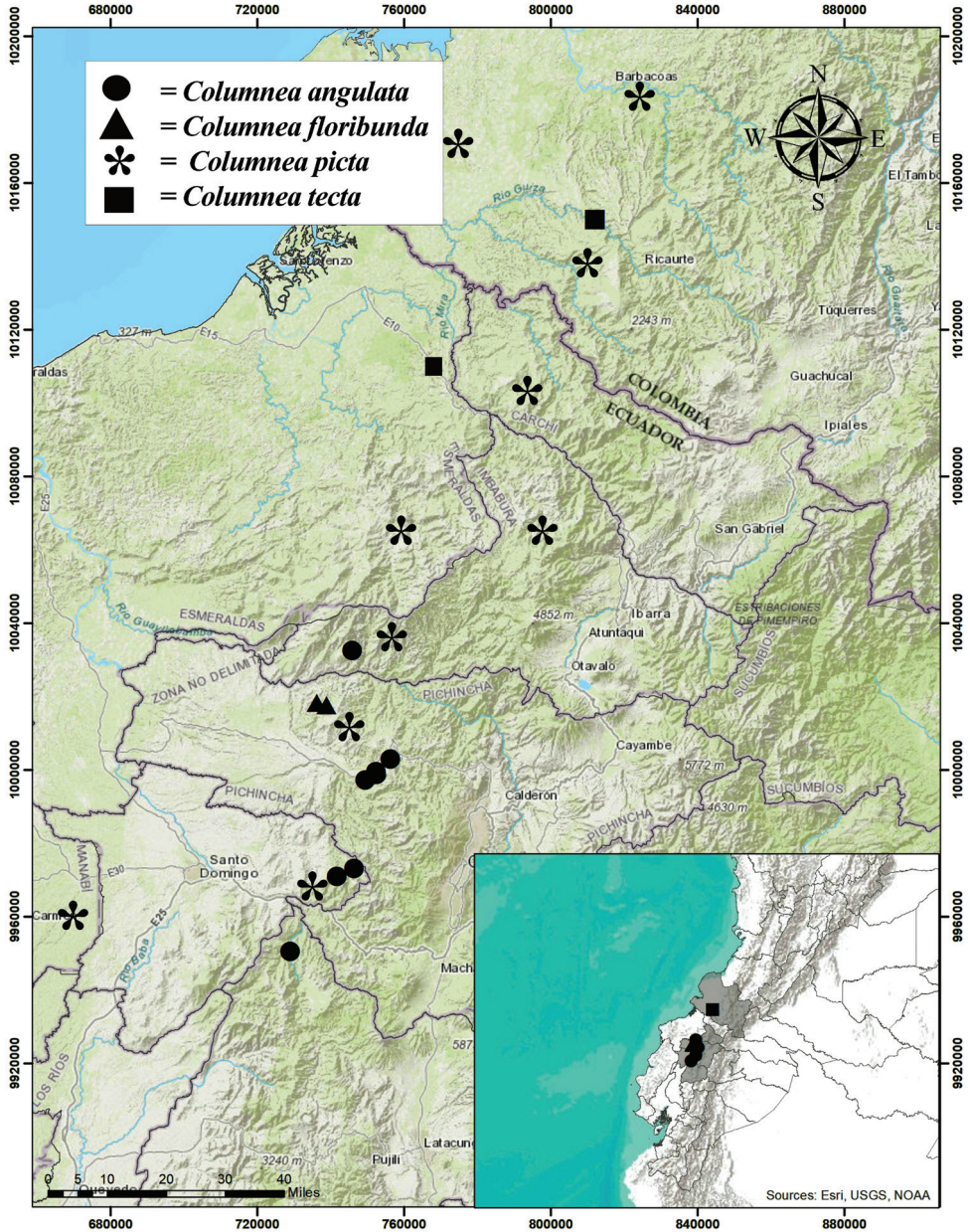


Figure 1. Distribution of *Columnnea angulata* (circles), *C. floribunda* (triangles), *C. tecta* (squares), and *C. picta* (asterisks). Note that *C. picta* ranges from southern Ecuador to northwestern Colombia (exceeding the range of the currently described species) (Map generated by Marco Monteros).

Type. ECUADOR Imbabura: cantón Cotacachi, parroquia García Moreno, Cordillera de Toisán, Cerro de la Plata, Bosque Protector Los Cedros, sendero Camino del Oso, north of lodge, 0°18'N, 78°46'W, 1500–2600 m, 19 Mar 2003, J.L. Clark, F

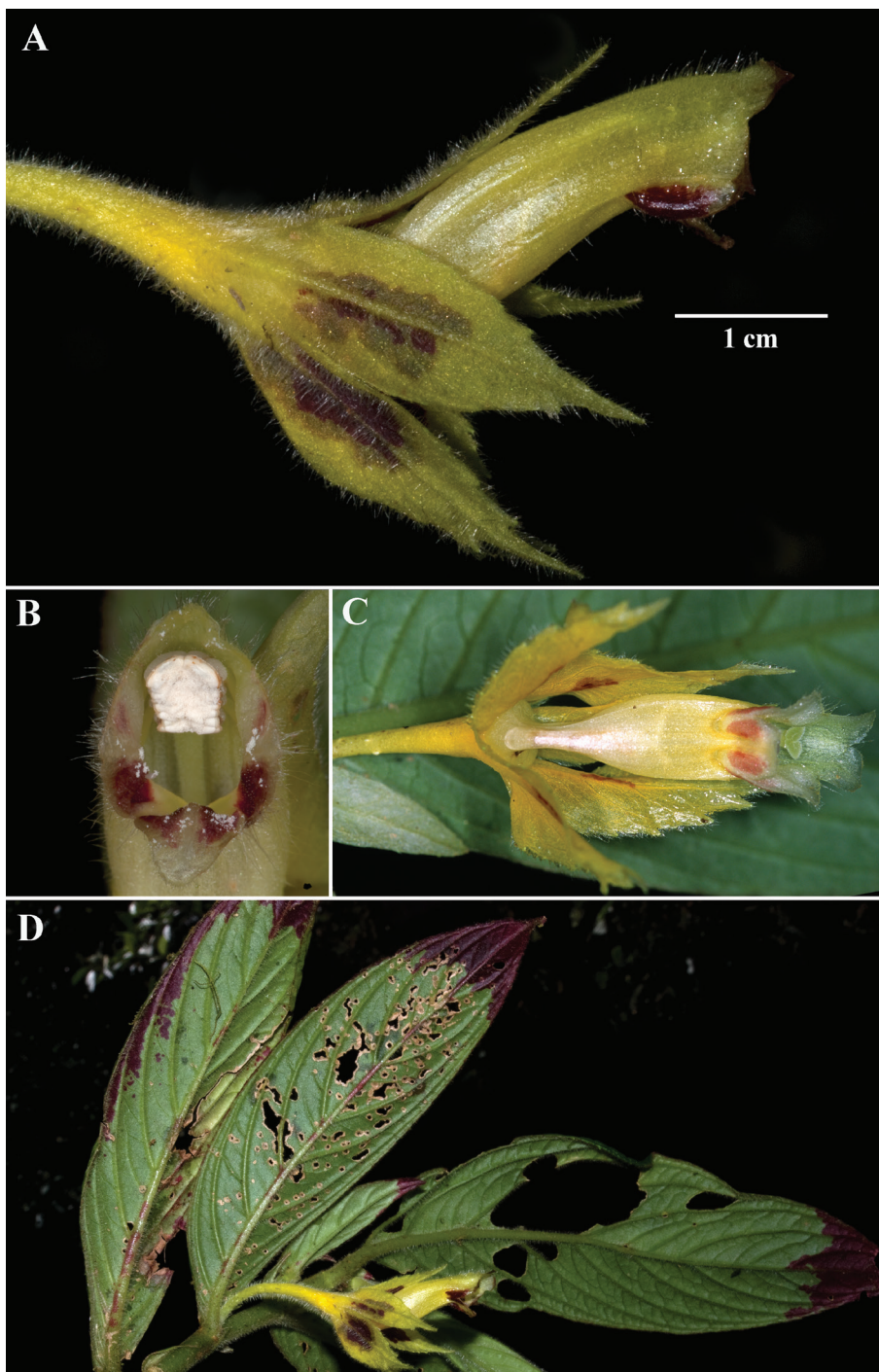


Figure 2. *Columnnea angulata* J.L. Clark & F. Tobar **A** mature flower **B** front view of flower **C** ventral view of flower showing laterally compressed corolla tube **D** dorsiventral habit (**A**, **D** from J.L. Clark et al. 12198 **B** from J.L. Clark 10968 **C** from J.L. Clark et al. 7413). Photos by J.L. Clark.

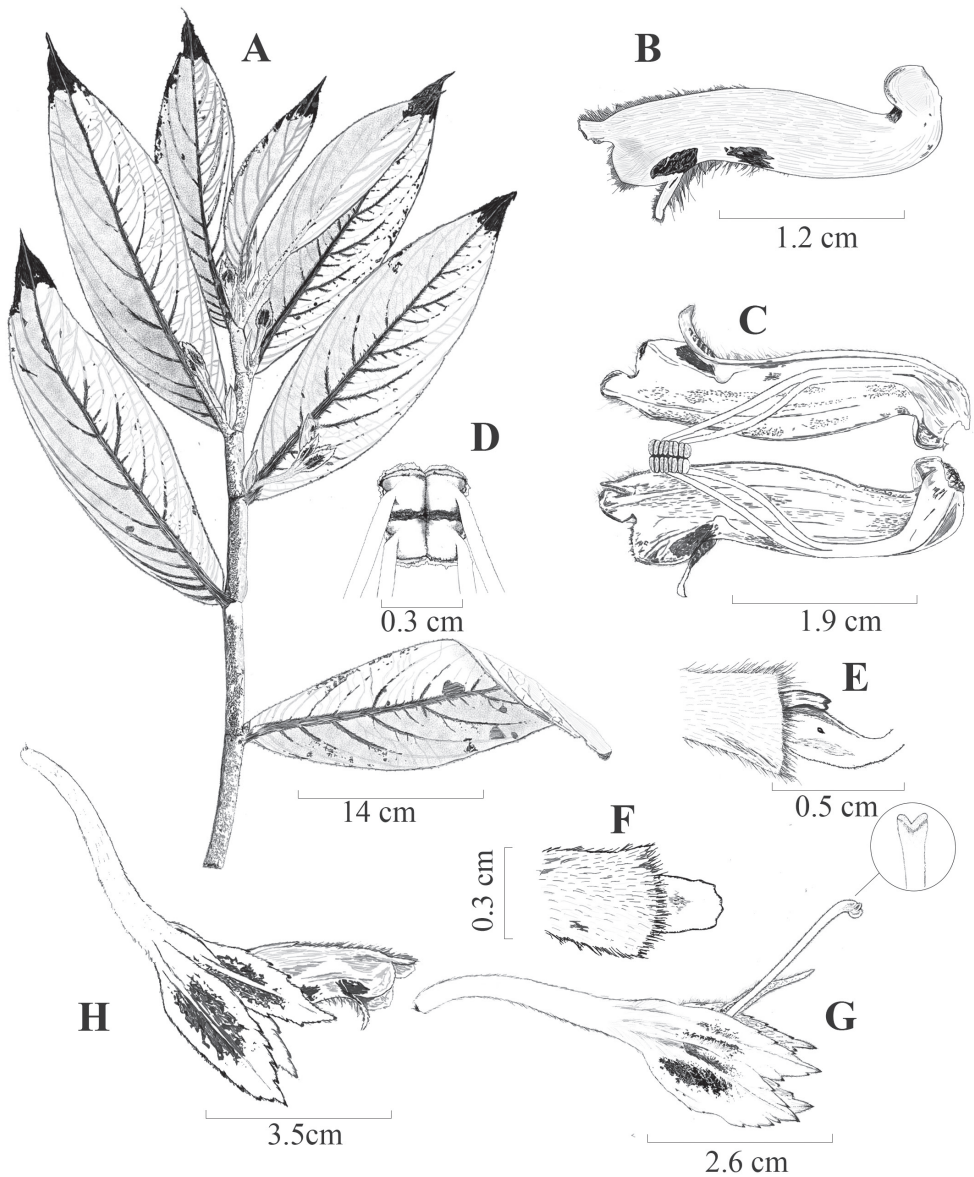


Figure 3. *Columnea angulata* J.L. Clark & F. Tobar **A** dorsiventral habit **B** lateral view of flower **C** dissected corolla showing filaments and ventral view of the anthers **D** dorsal view of the anthers **E** lateral view of ovary and nectary gland **F** dorsal view of nectary gland **G** lateral view of calyx and style, showing shallowly bifid stigma **H** lateral view of flower. Illustrated by M.J. Gavilanes, based on *F. Tobar et al 3409*.

Nicolalde & R. Hall 7413 (holotype: US [US3492386]; isotypes: AAU, COL, K, MO, QCA, QCNE, SEL, UNA).

Description. Facultative epiphyte with dorsiventral shoots to 1.5 m long, sub-woody, suffrutescent, glabrescent below, sparsely pilose above; internodes 7–10 cm

Table 1. General geographic distribution (names in parentheses indicate Ecuadorian provinces and Colombian departments) and comparison of morphological characters between *Columnnea angulata*, *C. floribunda*, *C. tecta*, and *C. picta*.

	<i>Columnnea angulata</i> J.L. Clark & F. Tobar	<i>Columnnea floribunda</i> F. Tobar & J.L. Clark	<i>Columnnea picta</i> H. Karst	<i>Columnnea tecta</i> J.L. Clark & L. Clavijo
Calyx lobe margin	serrate towards apex	serrate	entire	serrate towards apex
Calyx lobe shape	elongate to lanceolate	ovate	broadly ovate	ovate
Corolla posture relative to calyx	oblique to perpendicular	straight	straight	straight
Corolla tube angulation	angulate	not angulate	not angulate	not angulate
Corolla deeply or shallowly bilabiate	shallowly bilabiate (nearly tubular)	shallowly bilabiate (nearly tubular)	deeply bilabiate	shallowly bilabiate (nearly tubular)
Number of flowers/axil	single (rarely 2–3)	4–6	2–3	2–4
Relative length of corolla tube vs. calyx lobes	exceeds length of calyx lobes	exceeds length of calyx lobes	exceeds length of calyx lobes	equal to or less than length of calyx lobes
Distribution	western Andean slopes in northern Ecuador (Cotopaxi, Imbabura, Pichincha, and Santo Domingo de los Tsáchilas)	western Andean slopes of northern Ecuador (Pichincha)	widespread on the western Andean slopes in Colombia & Ecuador	western Andean slopes of northern Ecuador (Esmeraldas) and southern Colombia (Nariño)

near base, then clustered at branch apex. Leaves opposite, strongly anisophyllous, papyraceous when dry; larger leaf nearly sessile, petioles 0.1–0.4 cm long, pilose; blade asymmetric, oblanceolate to oblong, 7–28 × 2–9.5 cm, base oblique, apex acuminate, margin serrate, adaxially uniformly dark green to red with dark red apex, glabrous, abaxially light green, upper regions of margins, and apical third of leaf dark red, sparsely pilose to densely pilose along the venation, lateral veins 7–15, primary and secondary veins occasionally red; smaller leaf sessile, sometimes appressed to the dorsal surface of stem; blade asymmetric, lanceolate 0.5–2 × 0.4–0.6 cm, base oblique, apex acuminate, margin serrate, green with red apex on both surfaces, glabrous adaxially, sparsely pilose to densely pilose along the venation and margins abaxially. Inflorescence reduced to a single axillary flower (rarely 2–3); peduncles absent or highly reduced (< 0.2 cm); bracts 1–2, light green, lanceolate, 0.7–1.2 × 0.2–0.4 cm, glabrous on both sides. Flowers subtended by elongate pedicels, 1.5–2.5 cm long, sparsely to densely pilose, tightly appressed to the abaxial leaf surface when immature, becoming pendent during anthesis; calyx lobes 5, nearly free, mostly equal in size and shape, dorsal lobe slightly smaller, lobes appressed to flower when immature and spreading during anthesis, from uniformly yellow, to red with yellow margins, to yellow with a large reddish mid-region, 1.5–3.5 × 0.5–1 cm, ovate to broadly oblong, apex acuminate to acute, margin serrate, pilose on both surfaces; corolla tubular, appearing perpendicular to calyx via a sigmoid-shaped corolla tube, 2.1–3.5 cm long, outer and inner surfaces pilose, base appearing laterally compressed, limb shallowly bilabiate, white suffused with yellow on lower two thirds, more yellow toward apex, splotches of dark red on lower portion of lateral and ventral lobes, light yellow patch below lobes, red streaks abaxially, lobes 0.3–0.4 × 0.3–0.5 cm. Androecium of 4 stamens, filaments connate at the base and forming a filament curtain for 0.2–0.4 cm, free portion of filaments 3–3.5 cm long,

glabrous; anthers longer than broad, ca. 2×1.5 mm, dehiscing by longitudinal slits; staminode absent; nectary a bilobed dorsal gland, glabrous; ovary superior, densely pilose, $0.2\text{--}0.4 \times 0.2$ cm, style ca. 2.5 cm long, glabrous, stigma included and shallowly bifid. Fruit not observed.

Phenology. This species has been found with flowers in two periods: February to May and August to October.

Etymology. The specific epithet is in reference to the angulate or bent corolla tube. The corolla is nearly perpendicular to the calyx lobes because of the sigmoid-shaped tube.

Distribution and preliminary assessment of conservation status. *Columnnea angulata* is locally abundant in forests along the western slopes of the Ecuadorian Andes in the provinces of Cotopaxi, Imbabura, Pichincha, and Santo Domingo de los Tsáchilas (Fig. 1) where it grows in mature forests and the shaded understory of recently cleared forests, from 1500 to 2600 m in elevation. It is especially common along the old highway between Quito and Santo Domingo. It has been documented in two protected areas: Reserva Florística-Ecológica Río Guajalito and Bosque Protector Los Cedros. According to the IUCN Red List criteria (IUCN 2001) for limited geographic range (B1, less than 20,000 km²) and considering the uncertain future of habitat conservation of western Andean forests (B2b, c), *Columnnea angulata* should be listed in the category Vulnerable (VU).

Comments. *Columnnea angulata* is unique from other *Columnnea* by the posture of the pendent mature flowers where the corolla tubes are oriented oblique to perpendicular relative to the calyx (Figs 2A, 3B). Another defining character is a constriction at the base of the corolla tube that makes it appear laterally compressed (Fig. 2C). *Columnnea picta* and *C. angulata* are vegetatively similar and grow sympatrically. These two species are differentiated by the presence of deeply bilabiate corolla tubes in *Columnnea picta* (Fig. 6A) in contrast to the shallowly bilabiate corolla tubes in *C. angulata* (Fig. 2B, C). *Columnnea angulata* differs from *C. tecta* by an elongate corolla tube (vs. corolla tube that does not exceed the calyx lobes in *C. tecta*) and single axially flowers (rarely 2–3) in contrast to the abundant clusters of 3–5 axially flowers in *C. floribunda*. *Columnnea picta* and *C. angulata* are the two most commonly collected species in this complex and readily differentiated by the entire calyx margin in *C. picta* and serrate calyx margin in *C. angulata*.

Specimens examined. ECUADOR Cotopaxi: cantón Sigchos, parroquia San Francisco de las Pampas, Bosque Integral Otonga, 0°25.17'S, 79°0.19'W, 1900 m, 26 Jan 2001, J.L. Clark and Muñoz 6125 (QCA, QCNE, SEL, UNA, US); Pichincha: cantón Quito, parroquia Nono, El Pahuma Orchid Reserve, 17 km east of Nanegalito, 0°1'S, 78°37'W, 1700 m, 17 Apr 2003, J.L. Clark et al. 7648 (QCA, QCNE, SEL, UNA, US); cantón San Miguel de los Bancos, Mindo Loma Cloud Forest Reserve, km 73.5 via Calacali-La Independencia, 3 km past the entrance to the village of Mindo, 0°0'44"S, 78°44'29"W, 1800 m, 23 May 2011, J.L. Clark & C. Aulestia 12198 (QCNE, UNA, US); cantón San Miguel de los Bancos, Las Gralarias Reserve, 1.2 km east of the lodge, 0°05'N, 78°43'W, 1900 m, 15 Aug 2017, F. Tobar, A. Nieto, A. Marçayata & S. Imba 2832 (QCA); cantón San Miguel de los Bancos, Las Gralarias Reserve, Puma trail,

0°05'N, 78°43'W, 1900 m, 21 May 2018, *F. Tobar*, *F. Richter* 3280 (QCA); cantón San Miguel de los Bancos, Puyucunapi Reserve, cultivada cerca de la casa de la reserva, 0°01'N, 78°41'W, 1800 m, 13 Oct 2019, *F. Tobar* & *M. Gavilanes* 3409 (HPUCESI, QCNE); cantón San Miguel de los Bancos, a 2.6 km al este de San Tadeo en la vía a Bellavista Lodge, 0°01'N, 78°44'W, 1893 m, 11 Mar 2020, *F. Tobar* & *M. Gavilanes* 3475 (QCNE); cantón San Miguel de los Bancos, Puyucunapi Reserve, a 800 m de la entrada del transecto principal, 0°01'N, 78°41'W, 1995 m, 12 Mar 2020, *F. Tobar* & *M. Gavilanes* 3479 (QCA); **Santo Domingo de los Tsáchilas:** cantón Santo Domingo de los Colorados, Bosque Protector Río Guajalito, located on the old Quito-Santo Domingo road, between Chiriboga and La Palma, 0°18'50"S, 78°55'35"W, 1796 m, 30 May 2009, *J.L. Clark et al.* 10968 (NY, QCNE, SEL, US); Reserva Florística-Ecológica Río Guajalito, km 59 de la carretera antigua Quito-Sto. Domingo de los Colorados, a 3.5 km al NE de la carretera, 0°13'53"S, 78°48'10"W, 1800–2200 m, 3 Apr 2003, *J.L. Clark*, *N. Muchhala* & *A. Hoyos* 7618 (QCA, QCNE, SEL, UNA, US).

***Columnea floribunda* Tobar & J.L. Clark, sp. nov.**

urn:lsid:ipni.org:names:77219740-1

Figs 4, 5

Diagnosis. Differs from *Columnea picta* by a nearly tubular corolla (vs. deeply bilabiate corolla). Differs from *Columnea angulata* by the straight corolla relative to the calyx (vs. oblique to perpendicular corolla relative to the calyx). Differs from *Columnea tecta* by corollas that exceed the length of the calyx lobes (vs. corollas that are equal to or less than the length of the calyx lobes).

Type. ECUADOR Pichincha: cantón Pichincha, parroquia Pacto, Bosque Protector Mashpi, sendero Mashpi Capuchin, 5 km al norte de Lodge, 0°09'N, 78°52'W, 900–1200 m, 18 Jan 2020, *F. Tobar*, *C.H. Graham*, *T. Santander* & *E. Guevara* 3527 (holotype: QCA; isotypes: QCNE, US).

Description. Facultative epiphyte with dorsiventral shoots to 2–3 m long, subwoody, suffrutescent, glabrescent below, sparsely pilose above; internodes 3–16 cm near base, then clustered at branch apex. Leaves opposite, strongly anisophyllous, papyraceous when dry; larger leaf nearly sessile, petioles 0.1–0.2 cm long, pilose; blade asymmetric, oblanceolate to oblong, 1–28 × 4.8–6.2 cm, base oblique, apex acuminate, margin serrate, adaxially uniformly dark green with bright red, glabrous, abaxially light green with bright red apex, sparsely pilose, lateral veins 7–12; smaller leaf sessile, blade asymmetric, lanceolate 1.4–2.5 × 0.3–0.5 cm, base oblique, apex acuminate, margin serrate, green with red apex on both surfaces, adaxially glabrous, abaxially sparsely pilose. Inflorescence reduced to axillary clusters of 3–5 flowers; peduncles absent or highly reduced (< 0.2 cm long); bracts 1–2, light green, oblong, 0.5–1.2 × 0.2–0.3 cm, glabrous on both sides. Flowers subtended by elongate pedicels, 2.2–3.3 cm long, sparsely pilose; calyx lobes 5, nearly free, mostly equal in size and shape, dorsal lobe elongate and slender, 1.7–2.1 × 0.7–1.3 cm, ovate, apex acute, margin serrate, mostly

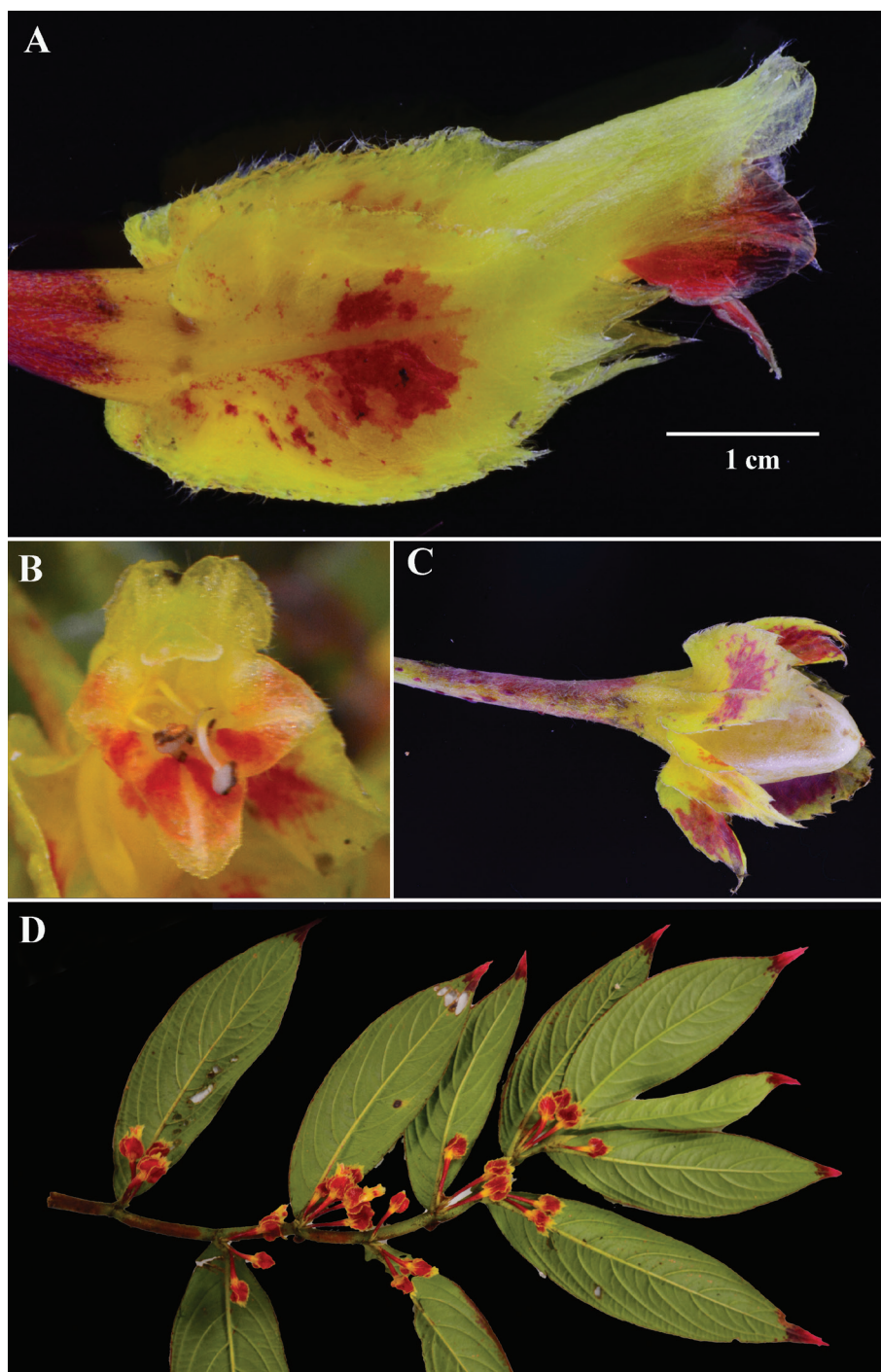


Figure 4. *Columnnea floribunda* F. Tobar & J.L. Clark **A** mature flower **B** front view of flower during anthesis **C** mature fruit **D** dorsiventral habit **A–D** from F. Tobar *et al.* 3527 (**A–D** from F. Tobar *et al.* 3527, holotype). Photos by F. Tobar.

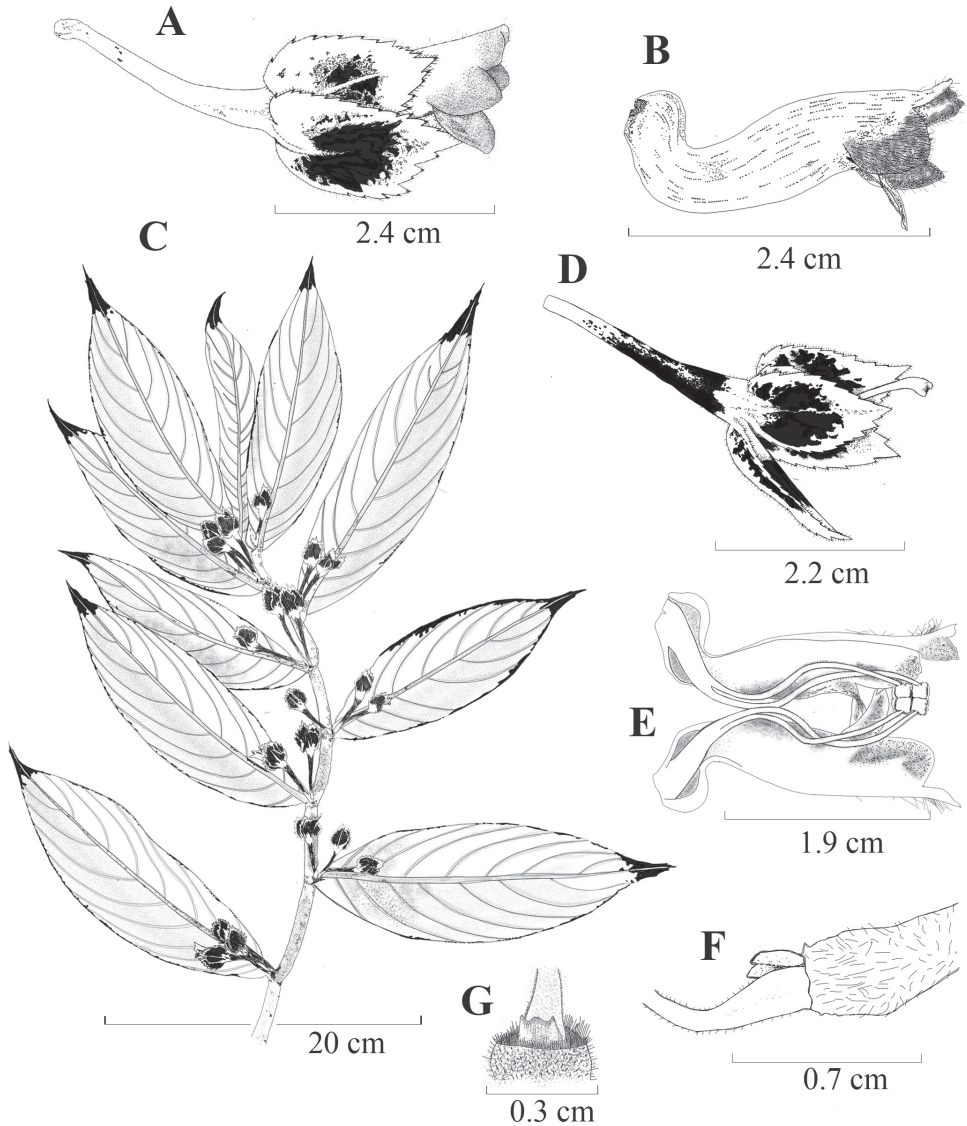


Figure 5. *Columnnea floribunda* F. Tobar & J.L. Clark **A** mature flower **B** Lateral view of corolla **C** dorsiventral habit **D** lateral view of calyx and style, showing shallowly bifid stigma **E** corolla dissected showing filaments and ventral view of anthers **F** lateral view of ovary and nectary gland **G** dorsal view of nectary gland. Illustrated by M.J. Gavilanes, based on F. Tobar *et al.* 3527, holotype.

yellow with red splotches in the center, inner and outer surfaces pilose; corolla tubular, 0.6–2.1 cm long, mostly yellow with whitish base, outer and inner surfaces pilose, limb shallowly bilabiate, corolla lobes $0.3\text{--}0.5 \times 0.2\text{--}0.4$ cm, lateral and lower lobes red, upper lobes yellow. Androecium of 4 stamens, filaments connate at the base and forming a filament curtain for 0.2–0.3 cm, free portion of filaments 1.5–1.9 cm long, minutely

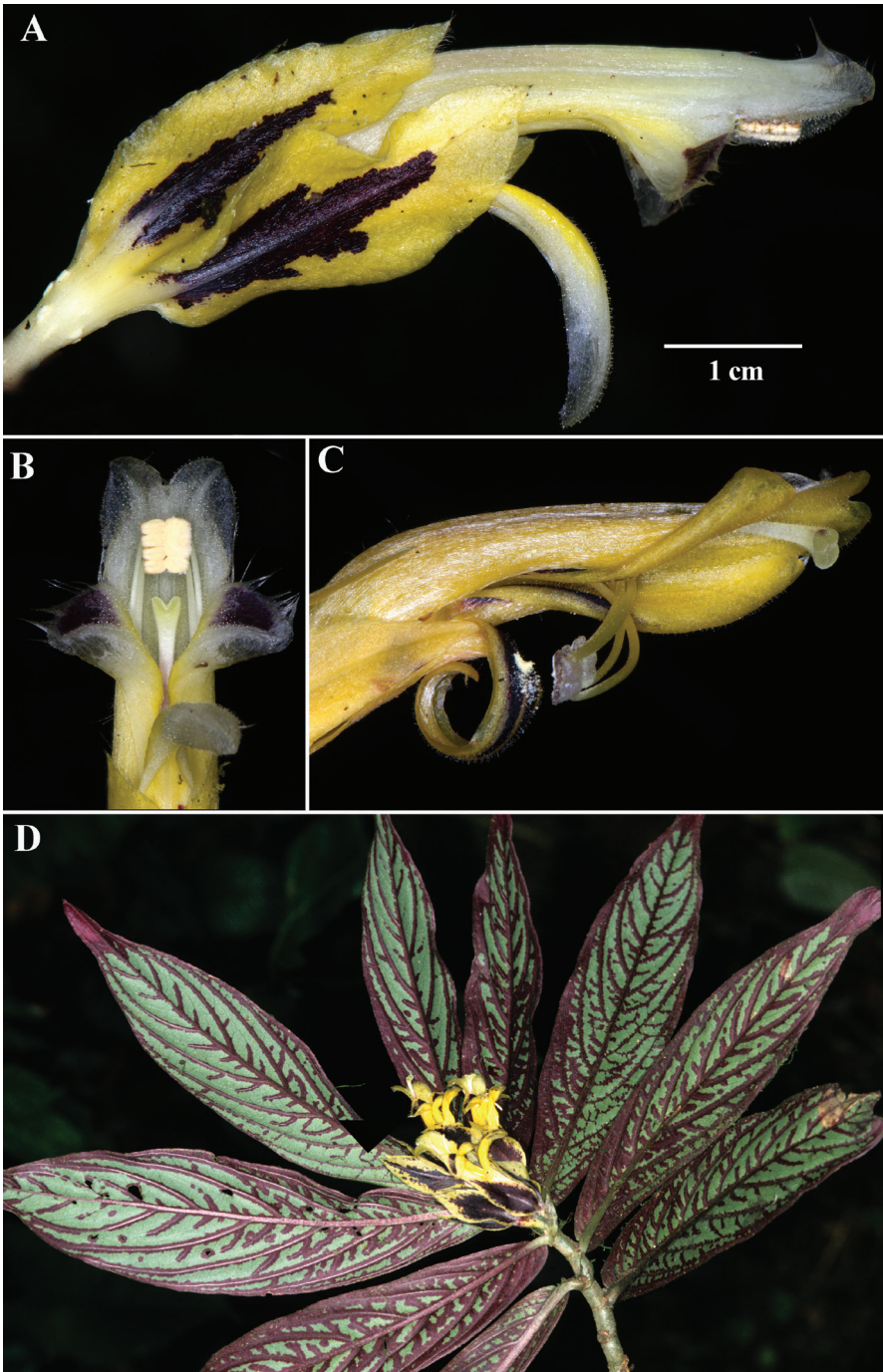


Figure 6. *Columnnea picta* H. Karst **A** lateral view of flower featuring deeply bilobed corolla **B** ventral view of flower during anthesis **C** mature flower featuring curled lower lobe **D** dorsiventral habit (**A, B** from J.L. Clark & L. Jost 16301 **C** from J.L. Clark et al. 15393 **D** from J.L. Clark, M. Mailloux & S. Seger 7942). Photos by J.L. Clark.

pubescent; anthers longer than broad, ca. 0.3×0.2 mm, dehiscent by longitudinal slits; staminode absent; nectary a trilobed dorsal gland, glabrous; ovary superior, densely pilose, $0.2\text{--}0.4 \times 0.2$ cm, style $1.5\text{--}1.8$ cm long, minutely pubescent, stigma included and shallowly bifid. Fruit an indehiscent globose white berry. Seeds not observed.

Phenology. Collections of *Columnea floribunda* are documented with flowers between January and April and between June and October. Fruits have been recorded during March.

Etymology. The specific epithet refers to axillary clusters of several flowers (3–5).

Distribution and preliminary assessment of conservation status. *Columnea floribunda* is locally abundant in the Mashpi Rainforest Biodiversity Reserve (900–1340 m) and the surrounding roads, a Chocó biogeographic forest relict in northern Ecuador. It is likely that additional populations are located in the adjacent provinces of Imbabura and Esmeraldas. Future research expeditions to unexplored areas of the Cotacachi Cayapas Ecological Reserve will hopefully result in additional documented populations of *C. floribunda*. According to the IUCN Red List criteria (IUCN 2001) for limited geographic range (B2a, less than five locations) and considering the uncertain future of habitat conservation, *Columnea floribunda* should be listed in the category Endangered (EN).

Comments. *Columnea floribunda* is readily distinguished from all other congeners by the elongate corolla tubes that exceed the length of the calyx lobes (Fig. 4A), in contrast to the corolla tubes of *C. tecta* that are equal to or less than the length of the calyx lobes (Fig. 7C); the corolla posture relative to the calyx that is straight (Fig. 4A), in contrast to the oblique to perpendicular corolla relative to the calyx of *C. angulata* (Fig. 2A); and the axillary clusters of three or more flowers (Fig. 4D).

Specimens examined. ECUADOR Pichincha: cantón Pacto, Mashpi Lodge, transecto Mashpi Laguna, a 500 m de la entrada del transecto, $0^{\circ}09'N$, $78^{\circ}52'W$, 880 m, 21 Sep 2017, *F. Tobar & A. Nieto 2903* (QCA); cantón Pacto, transecto Mashpi Capuchin, entrada del transecto, $0^{\circ}10'N$, $78^{\circ}52'W$, 800 m, 20 Mar 2018, *F. Tobar, A. Marcatayata & K. Cortez 3161* (QCA); cantón Pacto, km 20, carretero entre La Delicia y el pueblo de Mashpi, $0^{\circ}09'N$, $78^{\circ}51'W$, 1200 m, 18 Dec 2019, *F. Tobar & M. Gavilanes 3509* (QCA); cantón Pichincha, Amagusa Reserve, 1200 m dentro del sendero principal hacia el río, $0^{\circ}09'N$, $78^{\circ}51'W$, 1213 m, 18 Mar 2018, *F. Tobar, C. Poveda, S. Basantes & M. Gavilanes 3465* (HPUCESI, QCNE); cantón Pacto, Mashpi reserve, road to lodge, $0^{\circ}09'38''N$, $78^{\circ}50'58''W$, 1338 m, 7 Feb 2019, *M. Perret & F. Tobar 258* (QCNE).

Columnea tecta J.L. Clark & Clavijo, sp. nov.

urn:lsid:ipni.org:names:77219741-1

Fig. 7

Diagnosis. Differs from *Columnea picta* by a nearly tubular corolla (vs. deeply bilabiate corolla) that is equal to or shorter than the calyx lobes (vs. corolla that extends beyond the calyx lobes).

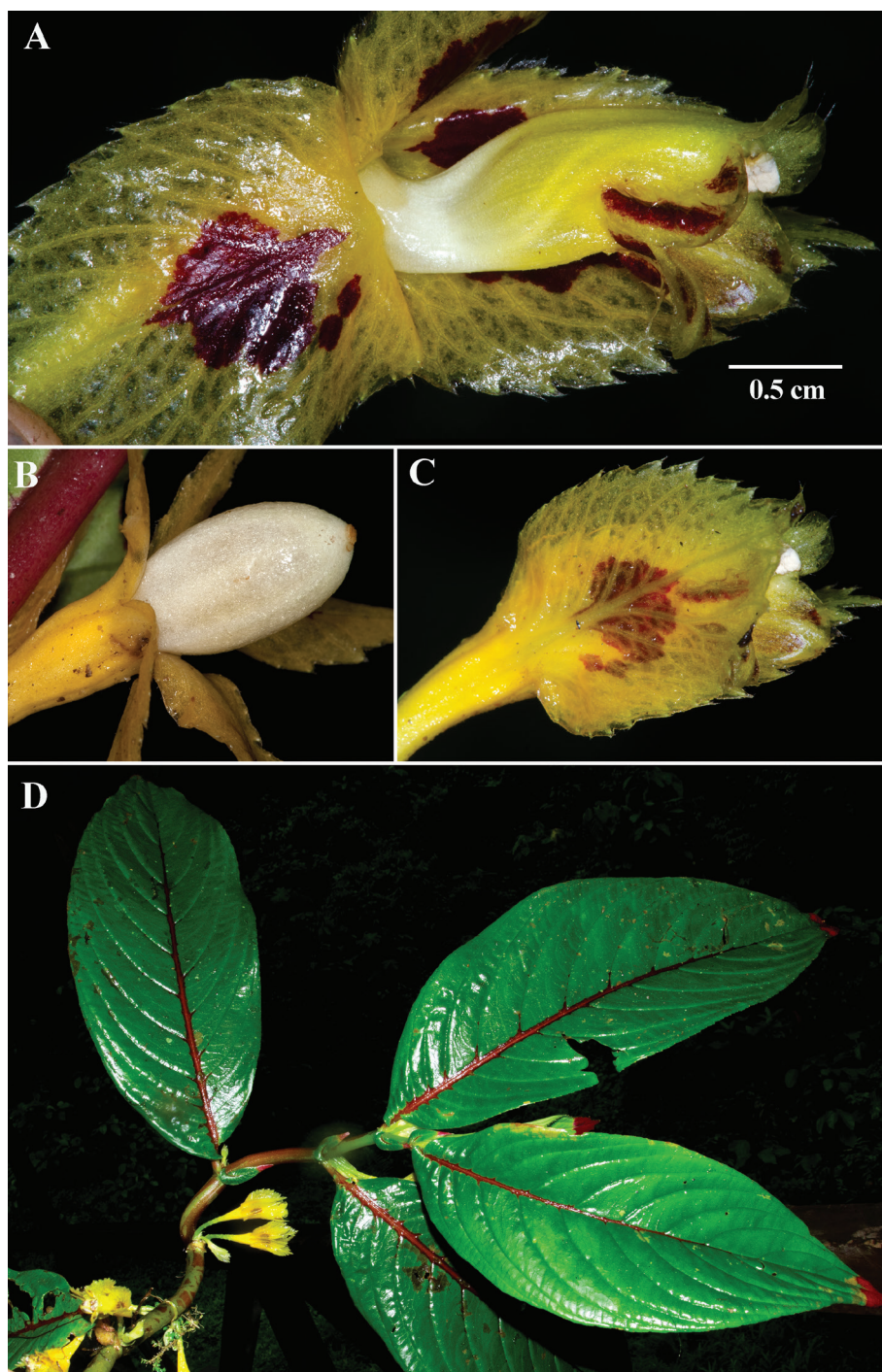


Figure 7. *Columnnea tecta* J.L. Clark & Clavijo **A** mature flower with lateral calyx lobe pulled back **B** Oblong white berry **C** mature flower **D** dorsiventral habit (**A–D** from J.L. Clark *et al.* 13433). Photos by J.L. Clark.

Type. ECUADOR Esmeraldas: cantón San Lorenzo, remnant patch of forest along highway Ibarra-San Lorenzo, between the towns of Durango and Alto Tambo, 0°57'21"N, 78°33'38"W, 664 m, 3 Jun 2009, J.L. Clark & 2009 Gesneriad Research Expedition Participants 11104 (holotype: US [3693986]; isotypes: MO, NY, QCNE, SEL).

Description. Facultative epiphyte with dorsiventral shoots to 1.5 m long, subwoody, suffrutescent, glabrescent below, sparsely pilose above; internodes 5–10 cm near base, then clustered at branch apex. Leaves opposite, strongly anisophyllous, papyraceous when dry; larger leaf nearly sessile, petioles succulent, 0.3–0.8 cm long, glabrous; blade asymmetric, broadly oblanceolate, 7–30 × 3–6.6 cm, base oblique, apex acuminate, margin serrate, adaxially uniformly green with bright red apex, glabrous, abaxially light green with bright red apex, sparsely pilose along the venation, lateral veins 7–14, primary vein bright red, secondary veins red at base and green adaxially; smaller leaf sessile and often clasping the base of the stem; blade asymmetric, lanceolate 0.5–1.5 × 0.4–0.5 cm, base oblique, apex acuminate, margin serrate, green with red apex on both sides, adaxially glabrous, abaxially sparsely pilose. Inflorescence reduced, appearing in clusters of 1–4 axially flowers; peduncles absent or highly reduced (< 0.2 cm long); bracts 1–2, light green, oblanceolate, 0.7–1 × 0.2–0.3 cm, glabrous on both sides. Flowers subtended by elongate pedicels, 1.5–2.4 cm long, sparsely pilose, with enations near the apex; calyx lobes 5, nearly free, mostly equal in size and shape, dorsal lobe slightly smaller, 1.5–2.3 × 1–2 cm, ovate, apex acute, margin serrate, yellow with red splotches in the center, inner and outer surfaces sparsely pilose; corolla tubular and erect, 1–1.9 cm long, outer and inner surfaces pilose, limb shallowly bilabiate, mostly yellow with red striations on lateral and ventral lobes, corolla lobes 0.2–0.5 × 0.2–0.4 cm. Androecium of 4 stamens, filaments connate at the base for 0.1–0.3 cm and forming a filament curtain, free portion of filaments ca. 1.5 cm long, glabrous; anthers longer than broad, ca. 2 × 1.5 mm, dehiscing by longitudinal slits; staminode absent; nectary a bilobed dorsal gland, glabrous; ovary superior, densely pilose, 0.2–0.4 × 0.2 cm, style ca. 1.4 cm long, glabrous, stigma included and shallowly bifid. Fruit an indehiscent oblong white berry, 1.2 × 0.72 cm.

Phenology. This species was documented with flowers in June and May. Fruits have been recorded in June.

Etymology. The specific epithet *tecta* refers to the “hidden” or relatively short corolla tube that does not exceed the length of the calyx lobes, an unusual character in *Columnnea*.

Distribution and preliminary assessment of conservation status. This species has not been found in any formally protected areas. According to the IUCN Red List criteria (IUCN 2001) for limited geographic range (B2a, less than five locations) and considering the uncertain future of habitat conservation of western Andean forests, *Columnnea tecta* should be listed in the category Endangered (EN).

Comments. *Columnnea tecta* is readily distinguished from all other congeners by relatively short corollas that barely exceed the length of the calyx lobes (Fig. 7). The corollas of *Columnnea tecta* have limbs that are shallowly bilabiate (Fig. 7A) in contrast to the deeply bilabiate corollas of *Columnnea picta* (Fig. 6A). *Columnnea tecta* and *C. picta*

are vegetatively similar by the presence of a dorsiventral habit with red apices on both leaf surfaces. The corolla tubes of *C. tecta* are short (less than the length of the calyx lobes and shallowly bilabiate) relative to the longer corolla tubes of *C. picta* (exceeding the length of the calyx lobes and deeply bilabiate). *Columnea tecta* differs from *C. angulata* by a straight orientation of the corolla relative to the calyx (vs. oblique to perpendicular in *C. angulata*).

Specimens examined. COLOMBIA Nariño: municipio Barbacoas, corregimiento El Diviso, western slopes of the Cordillera Occidental, trail from El Diviso towards Río Güiza, 1°21'21"N, 78°11'45"W, 404 m, 13 May 2013, J.L. Clark, L. Clavijo, O. Marín & M. Flores 13433 (COL, CUV); Altaquer to Junín, near Altaquer, 10 May 1972, H. Wiehler, R.L. Dressler, N.H. Williams & N.F. Williams 72222 (SEL).

Acknowledgements

Support for J.L. Clark was provided by the National Science Foundation (DEB-0841958 & DEB-0949169). Support for F. Tobar, M. Perret and C.H. Graham was provided by the Swiss Federal Research Institute (WSL) – National Geographic Society (Grant N° 9952-16); Swiss National Science Foundation (SNF Grant N° 173342) – European Research Council Advanced Grants (ERC Grant N° 787638) Aves y Conservación. We thank the Ministry of the Environment of Ecuador for providing permits for specimen collection and transportation (Research permit Aves y Conservación N° 007-2018-IC-FLO-FAU and mobilization permit Aves y Conservación 005-FLO-2019-DPAP-MA). Maria Jose Gavilanes is acknowledged for the illustrations (Figs 3, 5). Marco Monteros is acknowledged for producing the distribution map (Fig. 1). We are grateful to Christian Feuillet (US) for helping us select specific epithets. Oscar Marín, Mauricio Flórez and the Fundación Ecológica Los Colibríes are acknowledged for facilitating the research expedition that resulted in the discovery of *Columnea tecta*. We are grateful to the Mashpi & Amagusa Reserve, Mashpi Lodge, and the Mindo Cloud Forest for supporting our research by providing access to their reserves. We thank Fred R. Barrie and Alain Chautems for providing valuable feedback on an earlier version of the manuscript.

References

- Clark JL, Zimmer EA (2003) A preliminary phylogeny of *Alloplectus* (Gesneriaceae): Implications for the evolution of flower resupination. *Systematic Botany* 28: 365–375.
- Clark JL, Funke MM, Duffy AM, Smith JF (2012) Phylogeny of a Neotropical clade in the Gesneriaceae: More tales of convergent evolution. *International Journal of Plant Sciences* 173(8): 894–916. <https://doi.org/10.1086/667229>
- Clark JL, Skog LE, Boggan JK, Ginzburg S (2020) Index to names of New World members of the Gesneriaceae (Subfamilies Sanangoideae and Gesnerioideae). *Rhedeia* 30: 190–256. <https://doi.org/10.22244/rhedeia.2020.30.01.14>

- IUCN [Species Survival Commission] (2001) IUCN Red List Categories and Criteria: Version 3.1. IUCN, Gland and Cambridge.
- Ogutcen E, Christe D, Nishii K, Salamin N, Möller M, Perret M (2021) Phylogenomics of Gesneriaceae using targeted capture of nuclear genes. *Molecular Phylogenetics and Evolution* 157: e107068. <https://doi.org/10.1016/j.ympev.2021.107068>
- Schulte LJ, Clark JL, Novak SJ, Ooi MT, Smith JF (2014) Paraphyly of Section *Stygnanthe* (*Columnnea*, Gesneriaceae) and a revision of the species of section *Angustiflorae*, a new section inferred from ITS and chloroplast DNA Data. *Systematic Botany* 39(2): 613–636. <https://doi.org/10.1600/036364414X680861>
- Smith JF (2000) Phylogenetic resolution within the tribe Episcieae (Gesneriaceae): Congruence of ITS and *ndhF* sequences from parsimony and maximum-likelihood analyses. *American Journal of Botany* 87(6): 883–897. <https://doi.org/10.2307/2656896>
- Smith JF, Carroll CL (1997) A cladistic analysis of the tribe Episcieae (Gesneriaceae) based on *ndhF* sequences: Origin of morphological characters. *Systematic Botany* 22(4): 713–724. <https://doi.org/10.2307/2419437>
- Smith JF, Ooi MT, Schulte LJ, Amaya-Márquez M, Pritchard R, Clark JL (2013) Searching for monophyly in the subgeneric classification systems of *Columnnea* (Gesneriaceae). *Selbyana* 31: 126–142.
- Weber A (2004) Gesneriaceae. In: Kadereit J (Ed.) *The Families and Genera of Vascular Plants*. Vol. 7. Flowering Plants. Dicotyledons. Lamiales (Except Acanthaceae Including Avicenniaceae). Springer, Berlin, 63–158. https://doi.org/10.1007/978-3-642-18617-2_8
- Weber A, Clark JL, Möller M (2013) A New Formal Classification of Gesneriaceae. *Selbyana* 31(2): 68–94.
- Weber A, Middleton DJ, Clark JL, Möller M (2020) Keys to the infrafamilial taxa and genera of Gesneriaceae. *Rheedeana* 30: 5–47. <https://doi.org/10.22244/rheedeana.2020.30.01.02>

Re-appraisal of *Nertera* (Rubiaceae) in Taiwan

Wei-Chih Chen¹, Chih-Chiang Wang², Kun-Cheng Chang³

1 Graduate Institute of Bioresources, National Pingtung University of Science and Technology, Pingtung 912, Taiwan
2 Department of Forestry, National Pingtung University of Science and Technology, Pingtung 912, Taiwan **3** Department of Forestry and Nature Resources, National Chiayi University, 300 Syuefu Rd., Chiayi city 600, Taiwan

Corresponding author: Kun-Cheng Chang (kcchang@mail.ncyu.edu.tw)

Academic editor: Peter de Lange | Received 25 June 2021 | Accepted 24 August 2021 | Published 20 September 2021

Citation: Chen W-C, Wang C-C, Chang K-C (2021) Re-appraisal of *Nertera* (Rubiaceae) in Taiwan. *PhytoKeys* 182: 83–91. <https://doi.org/10.3897/phytokeys.182.70685>

Abstract

A revision of *Nertera* (Rubiaceae) in Taiwan was carried out by classical taxonomic methods and the presence of two endemic species was confirmed. Only one species, misapplied as *N. granadensis*, had been reported in the second edition of “Flora of Taiwan”, but there were two additional endemic species in this genus: *N. nigricarpa* and *N. taiwaniana* confirmed. *Nertera nigricarpa* is characterised by the entire leaf, purple-black petals, black fruits and dark-purple seeds with raised striate. *Nertera taiwaniana* has leaves with undulated margins, yellowish-green petals, red fruits and yellow-white seeds without striate. *N. granadensis* is excluded from the flora of this Island.

Keywords

Nertera depressa, *Nertera granadensis*, *Nertera nigricarpa*, *Nertera taiwaniana*, Rubiaceae

Introduction

There are six known species in the genus *Nertera* Banks ex Gaertn. in the family Rubiaceae in Australia, New Zealand, South America, Indonesia (Java), China, Taiwan and the Philippines (Chen and Taylor 2011). Moreover, Thompson (2010) suggested about 15 species in the genus. Hayata (1908) first described *N. nigricarpa* Hayata and reported that it is endemic to Taiwan and distributed at mid- to high-altitudes across the Island. Later, Hayata (1918) provided line drawings of *N. nigricarpa*. Masamune (1938) first described *N. taiwaniana* Masam., which has red fruits and was only known from the Jingshueiyang area in southern Taiwan. However, *N. taiwaniana* has recently

been discovered near Yuanyang Lake in northern Taiwan and at Lijia Industry Road in eastern Taiwan. Yamamoto (1938, 1940) believed that *N. depressa* Banks & Sol. ex Gaertn. in the Philippines and *N. taiwaniana* in Taiwan were the same species. Chao (1978) included both *N. depressa* and *N. nigricalpa* in the first edition of “Flora of Taiwan” and also treated *N. taiwaniana* as being a synonym of *N. depressa* in accordance with Yamamoto (1938, 1940), which was followed by Ko (1999). Liu and Yang (1998) only recorded one species, *N. granadensis* (Mutis ex L. f.) Druce, in the second edition of “Flora of Taiwan” and treated *N. depressa* and *N. nigricalpa* as synonymous.

Materials and methods

This study is based on field observations and detailed examinations of herbarium specimens. Specimens examined included those from the Herbaria CHIA, HAST, TAI, TAIF and TCF. The other type specimens were accessed as digital images of *Nertera depressa* and *N. granadensis* from Herbaria LINN and WELT. Morphological comparisons of fresh leaves, flowers, fruits and seeds were observed and stereomicroscopic pictures were taken of plants from Taiwan. Their sizes were measured by a digital caliper.

Results and discussion

Based on field observations and detailed examinations, we verified that the black-fruited *Nertera* and the red-fruited *Nertera*, native to Taiwan, are different species (Fig. 1). *Nertera taiwaniana* has leaves with undulating margins and secondary veins that are distinctly convex on the upper surface, yellowish-green petals, red fruits and yellow-white seeds without striate surfaces. *Nertera nigricalpa* is characterised by leaves entire without undulating margins and secondary veins which are not apparent on the upper surface or, if apparent, then only slightly convex, purple-black petals, black fruits and dark-purple seeds with raised striate.

Furthermore, after studying the lectotypes of *Nertera granadensis* (Mutis s.n., LINN) and a live individual, growing in its country of origin, Columbia, we found that the leaves of *N. granadensis* are nearly fleshy, with no apparent veins on both surfaces, without undulating margins, with an obtuse to acute apex and obtuse to shallowly truncate base and with red fruits that have no black spots. Therefore, *N. granadensis* is morphologically distinguishable from *N. nigricalpa* and *N. taiwaniana*. Although, the fruits of both *N. granadensis* and *N. taiwaniana* are red, *N. taiwaniana* possesses leaves that are membranous to papery with distinctly convex veins, an acute apex, a cordate or truncate base and fruits with black spots.

Nertera granadensis has often been referred to as *N. depressa* Banks & Sol. ex Gaertn, the type of the genus (Chen and Taylor 2011). Chao (1978) treated *Nertera taiwaniana* as a synonym of *N. depressa*. We also compared at syntype of *N. depressa* (Banks & Solander s.n., WELT SP063852). *Nertera depressa* possesses slightly fleshy leaves with no apparent

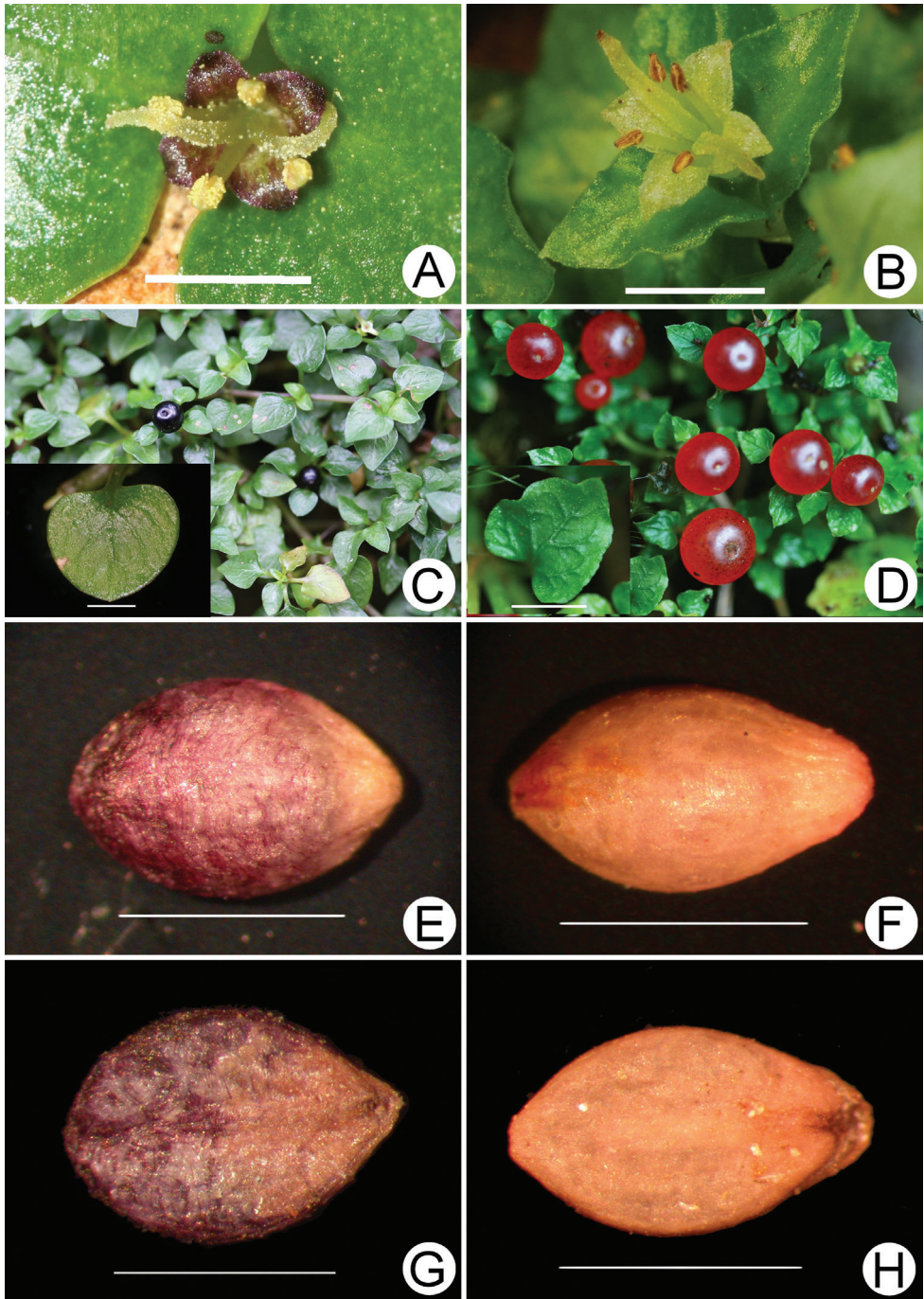


Figure 1. Photographs of *Nertera nigricarpa* Hayata and *N. taiwaniana* Masam **A** flower of *N. nigricarpa*. **B** flower of *N. taiwaniana* **C** fruits and leaf shape of *N. nigricarpa* **D** fruits and leaf shape of *N. taiwaniana* **E** top side of *N. nigricarpa* seed **F** top side of *N. taiwaniana* seed **G** underside of *N. nigricarpa* seed **H** underside of *N. taiwaniana* seed. Scale bars: 2 mm.

Notes. Creeping glabrous herbs; branches slender, 5–20 cm long. Leaves reniform to broad-ovate or deltoid, papery to thick-papery, 6–13 mm long, 4–8 mm wide; apex obtuse to acute, base cordate or truncate, margins entire without undulating; petioles 2–7 mm long; secondary veins 2–3 on each side of mid-vein, usually not apparent on upper surface but, if visible, slightly convex, slightly impressed on lower surface; stipules lanceolate to triangular, membranous, ca. 1.3 mm long and 0.9 mm wide. Flowers sessile, solitary, terminal, ca. 1.5 mm long and 1.5 mm wide; calyx truncate, glabrous;

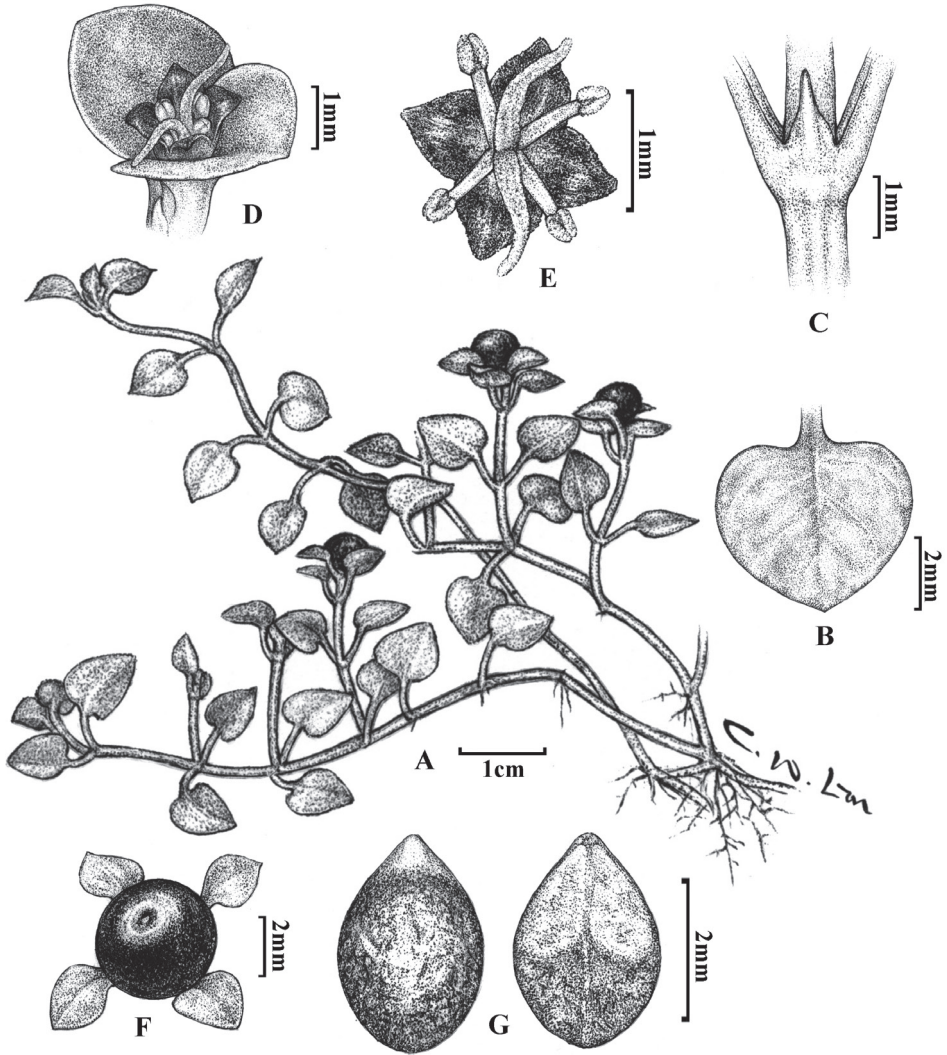


Figure 2. *Nertera nigricarpa* Hayata **A** habit **B** leaf **C** stipule **D** inflorescence **E** flower **F** fruit and **G** seeds.

corolla purple-black, lobes 4, deltoid to ovate, ca. 0.6 mm long and 0.5 mm wide, apex acute; stamens 4, anthers ovate, ca. 0.36 mm long and 0.2 mm in diameter, filaments ca. 0.4 mm long; ovary ellipsoid, two-celled, each with one ovule; two styles, free, ca. 1 mm long. Fruit globose drupe, 3–5 mm in diameter, black at maturity; two seeds, dark-purple, ovate to long-ovate, 2–3 mm long, 1.7–2 mm wide, surfaces striated.

Distribution and habitat. *Nertera nigricarpa* is endemic in Taiwan, at medium altitudes throughout the Island.

Specimens examined. TAIWAN, Taipei: Bunzangun, 6 Aug 1938, *T. Nakamura* 673 (TAI); Mt. Chiamu, 11 May 1935, *N. Fukuyama* 19242 (TAI); Hsintien, 15 Dec 2000, *Chen et al.* 3771 (TAIF); Hsinchu: Mt. Tapachienshan, 6 Sept 1993, *C. L. Huang* 71

Table 1. Comparison of Taiwanese *Nertera* native species and the excluded species *Nertera granadensis*.

	<i>Nertera granadensis</i>	<i>Nertera taiwaniana</i>	<i>Nertera nigricarpa</i>
Leaf Texture	Nearly fleshy	Membranous to papery	Papery to thick-papery
Veins	Usually not apparent or, if apparent, slightly convex on upper surface	Distinctly convex on upper surface	Usually not apparent or, if apparent, slightly convex on upper surface
Margins	Undulate	Undulate	Entire without undulating
Flower Colour	Yellowish	Yellowish-green	Purple-black
Fruit Colour	Red without black spots	Red with black spots	Black
Seed Colour	Yellow-white	Yellow-white	Dark-purple
Ornamentation	Not striate	Not striate	Striate

(HAST), 1 Nov. 1996, *C. M. Wang* 2370 (TAIF); **Taichung**: Suyuan, 13 Jan 2000, *Y. P. Cheng* 2950 (TAIF); Mt. Pahsien, 7 Aug 1959, *T. I. Chung* 2697 (HAST); Mt. Amma, 20 Oct 1957, *T. S. Liu* 197 (HAST); **Nantou**: Mayfeng to Sungkang, 21 Jan 1986, *C. I. Peng* 9079 (HAST); Yuanfeng, 5 Jul 2000, *Y. P. Cheng* 3253 (TAIF); Tunyuan to Yunhai, 20 May 1993, *C. C. Liao* 1299 (HAST); Tatchiaanpu to Lulinshanchuang, 7 Aug 1991, *W. P. Leu* 1208 (HAST); Kuankao to Patungkuan, 4 Jul 1985, *C. I. Peng* 8181 (HAST); **Chiayi**: Shimeng Valley, 6 Feb 2007, *K. C. Chang* et al. 3802 (TCF); Mt. Ali, 17 Dec 1939, *Nakamura & Yamamoto* 4133 (TAI); 10 Oct 1983, *C. I. Peng* 6018 (HAST); **Kaohsiung**: Chungtzukuan, 19 Dec 2000, *S. J. Yang* 29999 (TAIF); Chuyunshan, *H. L. Ho* 899 (HAST); **Pingtung**: Mt. Peitawu, 23 May 1918, *E. Matuda* 1710 (TAI); 7 Feb 1990, *C. I. Peng* 13276 (HAST); 5 May 2004, *K. C. Chang* 2419 (CHIA); **Ilan**: Fanfan, 27 Aug 1918, *E. Matuda* 430 (TAI); Tananao, 21 Jul 1929, *S. Suzuki* 628 (TAI); Mt. Chialoshan, 27 Sept 1930, *S. Suzuki* 6220 (TAI); Mt. Chililo, 5 Aug 1928, *S. Suzuki* s. n. (TAI); Mt. Taiping, 25 Jul 1929, *S. Suzuki* 920 (TAI); 26 Aug 1962, *Kao et al.* 4961 (TAI); 2 Mar 1966, *Kao & Chuang* 4093 (TAI); 26 Aug 1962, *M. T. Kao* 4691 (HAST); Chililo-Hsulawa, 16 Jul 1932, *S. Suzuki* 7206 (TAI); Kiyarawa, 25 Sept 1930, *S. Suzuki* 6182 (TAI); **Hualien**: Hoping Logging Trail, 15 Apr 2006, *S. W. Chung* 8489 (TAIF); Mt. Chingshui, 9 Sept 1939, *T. Nakamura* 3780 (TAI); Tailoku-Tailokutaishan, 15 Jun 1933, *A. T. Hsieh* s. n. (TAI); Mt. Mukua, 7 Aug 1940, *T. Nakamura* 4526 (TAI); 23 Sept 1984, *C. I. Peng* 7269 (HAST); Tayulin, 17 Oct 2004, *J. H. Lii* 1135 (TAI); Mt. Lintien, 12 Feb 1962, *J. M. Chao* 810 (TAI); **Taitung**: Siangyang, 18 Mar 2006, *K. C. Chang & C. C. Wang* 3069 (TCF).

***Nertera taiwaniana* Masam., Trans. Nat. Hist. Soc. Form. 28: 144. 1938.**

Figs 1b, d, f, h, 3; Table 1

Nertera granadensis auct. non Druce; Liu & Yang, Fl. Taiwan 2nd 4: 306. pro parte; Chen & Taylor, Fl. China 19: 257. 2011. syn. *N. taiwaniana*.

Nertera depressa auct. non Banks & Sol. ex Gaertn: Yamamoto, Journ. Soc. Trop. Agr. 10: 276. 1938, 12: 24. 1940; Chao, Fl. Taiwan 4: 315. pro parte; Ko, Fl. Reipubl. Popularis Sin. 71 (2): 164. syn. *N. taiwaniana*.

Notes. Creeping herbs; branches slender, 5–15 cm long, glabrous. Leaves opposite, deltoid to ovate, membranous to papery, 4–12 mm long, 2–8 mm wide, apex

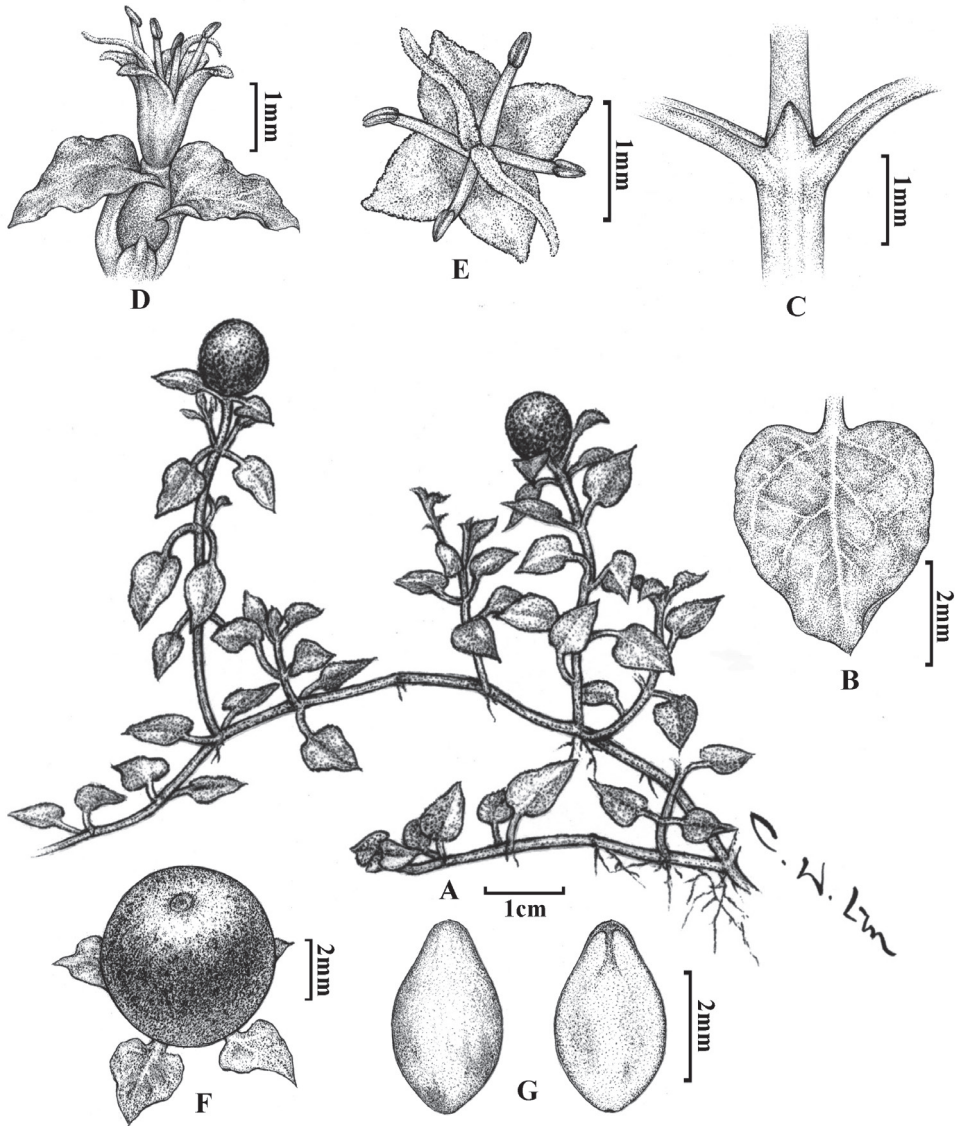


Figure 3. *Nertera taiwaniana* Masam **A** habit **B** leaf **C** stipule **D** inflorescence **E** flower **F** fruit and **G** seeds.

acute to acuminate, base cordate or truncate, margins entire, more or less undulate; petioles 1.5–6 mm long; secondary veins 2–3 on each side of mid-vein, distinctly convex on upper surface, not apparent on lower surface; stipules triangular, membranous, ca. 1.5 mm long and 1.5 mm wide. Flowers sessile, solitary, terminal, ca. 2 mm long and 1.8 mm wide; calyx truncate, glabrous; corolla yellowish-green, lobes 4, deltoid to ovate, ca. 0.7 mm long and 0.6 mm wide, apex acute; stamens 4, anthers oblong, ca. 0.29 mm long and 0.15 mm in diameter, filaments ca. 0.5 mm long; ovary ellipsoid, two-celled, each with one ovule; two styles, free, ca. 1 mm long. Fruit globose drupe, 4.5–6.5 mm in diameter, red with black spots at maturity.

ty; seeds 2, yellowish-white, long-ovate to spatulate, 2.5–3.5 mm long, 1.5–2 mm wide, surfaces smooth.

Distribution and habitat. *Nertera taiwaniana* is endemic in Taiwan. Growing on hillsides at medium altitudes in the eastern and southern parts of the Island.

Specimens examined. **TAIWAN, Pingtung:** Chunjih Hsiang, 30 Dec 1999, *C. I Peng 17902* (HAST); Tahan Forest Road, 26 Jul 2001, *Y. Y. Huang 554* (HAST); Chinshuiying, 23 Jun 1999, *K. F. Chung 1348* (HAST); 18 May 2008, *K. C. Chang & C. C. Wang 4447* (TCF); 6 Jun 2009, *K. C. Chang & C. C. Wang s. n.* (TCF); **Ilan:** Shenmihu, 28 Dec 1987, *Y. M. Hsu 554* (TAI); Chialohu, 10 May 2002, *C. I Huang 815* (HAST); Mt. Taiping, 22 Dec. 1995, *C. H. Chen 1519* (HAST); Mt. Fanpao, 17 Apr 1996, *C. C. Liao 1798* (HAST); **Taitung:** Mt. Sung, 30 Aug 1932, *S. Suzuki s. n.* (TAI); 13 May 1988, *S. Y. Lu 22900* (TAIF); Mt. Kutzulun, 20 Jul 1937, *H. Simizu 3888* (*Nertera taiwaniana*, holotype: TAI!).

Excluded species to the Flora of Taiwan

Nertera granadensis (Mutis ex L. f.) Druce, Rep. Bot. Soc. Exch. Club Brit. Isles 1916: 637. 1917; Chen & Taylor, Fl. China 19: 257. 2011. excl. *Taiwan* – *Gomozia granadensis* Mutis ex L. f., Suppl. Pl. 129. 1781.

Specimens examined. Colombia. South America: no date, *Mutis s.n.* (LINN).

Distribution and notes. *Nertera granadensis* has an unusually extensive trans-continental distribution surrounding the Pacific Ocean, occurring from New Zealand subantarctic Islands (Tristan da Cunha), South America (Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, Venezuela), Central America (Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama), North America (Mexico) and in Hawaii, eastern Australia, Indonesia, Malaysia, Papua New Guinea and the Philippines. As we observed that the morphology of *Nertera* populations differed between regions, further and more detailed research is necessary for classification. Considering the current evidence, we suggest that *N. granadensis* is excluded from the Flora of Taiwan.

Acknowledgements

We thank Mr C. W. Lin for his excellent line drawings and we thank Mr S. K. Yu, Ms P. F. Lu and Dr C. M. Wang for their assistance with the fieldwork. We thank the curators of the Herbaria CHIA, HAST, TAI, and TAIF for allowing us to study specimens. We are very grateful for the helpful suggestions and comments made by the editor and reviewers: Peter de Lange, Matt Renner and Ewen Cameron. English Language editing and review services supplied by ARPHA Proceedings, Pensoft Publishers Ltd. and Wallace Academic Editing.

References

- Chao JM (1978) *Nertera*: Rubiaceae. In: Li HL et al. (Eds) Flora of Taiwan. Vol. 4. Epoch Publishing, Taipei, 315–317.
- Chen T, Taylor CM (2011) *Nertera* Banks ex Gaertner. In: Wu ZY, et al. (Eds) Flora of China. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis 19: 257–258.
- Hayata B (1908) Flora montana formosae. The Journal of the College of Science. Imperial University of Tokyo 19: 115–116.
- Hayata B (1918) Icones plantarum formosanarum. The Bureau of Productive Industry. Government of Formosa 7: e32.
- Ko WC (1999) *Nertera*: Rubiaceae. In: Lo HS et al. (Eds) Flora Reipublicae Popularis Sinicae. Science Press, Beijing, China, Tomus 71(2): 162–165. [In Chinese]
- Liu HY, Yang TYA (1998) *Nertera*: Rubiaceae. In: Huang TC et al. (Eds) Flora of Taiwan. 2nd edn. Vol. 4. Editorial Committee of the Flora of Taiwan, Department of Botany, NTU, Taipei, 306–307.
- Masamune G (1938) Miscellaneous notes on the flora of Eastern Asia (III). Transactions of the Natural History Society of Formosa 28(176): 138–144.
- Thompson IR (2010) A new species of *Leptostigma* (Rubiaceae: Coprosminae) and notes on the Coprosminae in Australia. *Muelleria* 28(1): 29–39.
- Yamamoto Y (1938) Observationes ad Floram Formosanam XXI. Japanese Society for Tropical Agriculture 10(2): 274–280.
- Yamamoto Y (1940) Materials for a flora of the South-Eastern Asia, including Formosa and Ryu-Kyu, South-China, Malay Archipelago and others. III. Japanese Society for Tropical Agriculture 12(2): 243–250.

Lectotypification of six names in the genus *Elleanthus* (Orchidaceae) described from J. J. Linden's collection

Magdalena Dudek¹, Dariusz L. Szlachetko¹

¹ Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland

Corresponding author: Magdalena Dudek (magdalena.dudek@ug.edu.pl)

Academic editor: Lorenzo Peruzzi | Received 17 May 2021 | Accepted 25 August 2021 | Published 24 September 2021

Citation: Dudek M, Szlachetko DL (2021) Lectotypification of six names in the genus *Elleanthus* (Orchidaceae) described from J. J. Linden's collection. PhytoKeys 182: 93–106. <https://doi.org/10.3897/phytokeys.182.68782>

Abstract

The lectotypification of six names of species, originally described as *Evelyna* Lind. (Orchidaceae), based on collections of Jean Jules Linden from locations that are currently in Venezuela and Colombia, is proposed. We also provide the number and location of duplicates of the type material.

Keywords

Elleanthus, *Evelyna*, Jean Jules Linden, lectotype, nomenclature, syntype

Introduction

The Neotropical orchid genus *Elleanthus* was proposed by Czech botanist Carl Borivoj Presl in 1827 (Presl 1927). As the genus has never been comprehensively taxonomically revised, we can assume that it currently comprises over 120 species (Dodson and Luer 2010) which are widely distributed throughout the tropical and subtropical zones of the New World (Pridgeon et al. 2005). We can also assume that a significant number of taxa are still awaiting their formal description. The works by Garay (1978), Dodson (1998) and Dodson and Luer (2010) – the only partial revisions of the genus – concern solely the species growing in Ecuador, which is a comparatively minor part of the whole range of the genus. The difficulties in studying *Elleanthus* likely ensue from the

high morphological diversity of these plants, especially the structure of the flowers (e.g. shape, size and texture of floral bracts, shape and placement callus on the lip, size and shape of lip, structure of gynostemium), but also the vegetative parts (e.g. type of stem, size, shape and texture blade of leaf). The strong floral polymorphism is also the reason for many systematic ambiguities with reference to the genus.

Over the years, researchers have described a few similar genera, *Evelyna* Poepp. & Endl., *Adeneleterophora* Barb.Rodr. and *Epilyna* Schltr., which have been considered synonymous with *Elleanthus* (Reichenbach 1861; Dressler 1981, 1993; Szlachetko 1995; Pridgeon et al. 2005). The first of them was characterised by flowers densely packed in capitate inflorescence, equal sepals, a sub-rounded lip that is saccate at the base and features two calli and a semi-terete, naked gynostemium. It originally embraced five species (*Evelyna aurea*, *E. capitata*, *E. gaminifolia*, *E. oligantha* and *E. strobilifera*) which were described by Poepping and Endlicher (1835).

Many orchid species were described in the 19th century without indicating any type material, or information about the location of the reference collection. The examination of any original material cited in the protologue is a remarkable step in taxonomic work. Our studies towards revising the genus *Elleanthus* sensu lato has revealed specimens, belonging to the Jean Jules Linden's gatherings, where Lindley (1846) described seven species of *Evelyna* (*E. bractescens*, *E. columnaris*, *E. ensata*, *E. flavescens*, *E. furfuracea*, *E. kermesina* and *E. lupulina*). We also found the original material for one species (*Evelyna coriifolia*), which was described by Reichenbach (1852). Though they do not have a capitate inflorescence, they were placed in the genus proposed by Poeppig and Endlicher in 1835. However, several years later, Reichenbach (1861) came to the conclusion that they are congeneric and transferred species of this genus to *Elleanthus*.

Jean Jules Linden was a Belgian botanist and was particularly fond of orchids. His first scientific expedition to Brazil lasted less than two years (September 1835–March 1837). Nicolas Funck, Auguste Ghiesbreght and he collected and brought back to Europe a large collection of plants and animals. During the following expedition (September 1837–December 1840) to Cuba and Mexico, he focused mainly on observing the habitat of orchids and collected them for breeding purposes and for adding to European herbaria. He was the first botanist whose findings revolutionised the cultivation of orchids in Europe (Ceulemans and Braem 2006). His other trip to Venezuela and Colombia (1841–1844) resulted in gathering a variety of orchids species that Lindley (1846) described in Orchidaceae Lindenianae including *Evelyna* species. Lindley mentioned in his work that Linden had made partial contributions to some English institutions. However, the most of plants collected by Linden were cultivated in Brussels, Ghent and Paris (Ceulemans and Braem 2006), and during our studies we found Linden's collection at P and BR Herbaria.

Currently, we are carrying out a revision of *Elleanthus* sensu lato (in preparation), which is proving that some names of species are lacking type. In order to stabilise the nomenclature of these species, they require typification. Therefore, in this paper, a lectotypification of the names of species of *Elleanthus* described from Linden's gathering is proposed. For one name, we could not indicate a lectotype and this is widely discussed.

Typifications

***Elleanthus bractescens* (Lindl.) Rchb.f., Annales Botanices Systematicae 6: 479. 1861.**

Basionym. *Evelyna bractescens* Lindl., Orchidaceae Lindenianae 10,11, no 59. 1846.

Type: Venezuela, Merida, “on the stunted trees skirting the paramos of the Province Merida at the height of 8000 to 10 000 feet [2438–3480 m], July”, *Linden 2215*.

Note. In the protologue of *Evelyna bractescens*, Lindley (1846:11) included just a short note concerning the locality and collector of the reference specimen. In his *Orchids of Peru*, Schweinfurth (1958) noted that the type of *Evelyna bractescens* is stored in Lindley Herbarium at Kew. We were, however, unable to locate there any sheets labelled *Evelyna bractescens*. Interestingly, instead we found a specimen of *Maxillaria longissima* Lindl. collected by Linden in Merida Province and numbered 2215 (K-000779830). This sheet has the original



Figure 1. *Evelyna bractescens* Lindl. **a** specimen *Linden 2215* at the Naturhistorisches Museum, Vienna (W-R13950) **b** Reichenbach's drawing **c** based on Szlachetko's drawing of the Fragment 2 of original material (Baranow): A – dorsal sepal, B – lateral sepal, C – petal, D – lip, E – gynostemium.

label with Linden's handwriting. Additionally, we checked a protologue of *M. longissima* and it appeared that this species was described, based on the gathering named *Linden 2215* [coll. orig. 'Forests of Merida, at the height of 6000 feet; July (No. 2215)'] (Lindley 1846).

During our research in herbaria, we located only a single *Linden 2215* collection corresponding to *Evelyna bractescens*. It is deposited in the Naturhistorisches Museum in Vienna (W-R13950). This sheet includes five plant fragments and Reichenbach's drawing, signed as *E. bractescens* and *Lin. 2215* (Fig. 1a, b). The first fragment, including a sterile stem with leaves, but no flowers, has a label with the locality recorded as 'Tungurahura [Ecuador] in fruticetis, alt. 1000'–11000', Jan. 1859' (Fig. 1a). Therefore, it is not mentioned in the protologue. Fragments 3, 4 and 5 (Fig. 1a) have no labels; they comprise only a very small part of the stem and a few leaves, hence they are indeterminable. However, Fragment 2 (Fig. 1a) is the only one to include an inflorescence with flowers. After a detailed analysis of the flower's parts and a comparison with the original description, it seems that Fragment 2 corresponds to the description of *Evelyna bractescens*. However, it has no locality data to confirm the information contained in the protologue. Only the note on the drawing suggests Linden's gathering. We can suspect that Reichenbach prepared this drawing (Fig. 1b), based on Fragment 2. Unfortunately, we are unable to clearly indicate where the type collection is stored, so we decided not to lectotypify this name.

***Elleanthus columnaris* (Lindl.) Rchb.f., Annales Botanique Systematicae 6: 483. 1861.**

Basionym: *Evelyna columnaris* Lindl., Orchidaceae Lindenianae 11. no. 62. 1846.

Type: Venezuela, Trujillo, "Agua de Obispo and Sierra Nevada, at high of 9000 feet [2743 m], May to August", *Linden 620*; Lectotype (designated here): P (P00389742), drawing of the lectotype (K); Syntypes: Venezuela, Caracas, April 1842, *Linden 620* (W-R51649); Agua de Obispo, prov. Truxillo, 7000 feet, May, *Linden 620* (BR0000013083625); prov. Merida, *Linden 620* (W-R17081); no thorough locality from Venezuela, *Linden 620* (W-R51295).

Note. In the protologue of *Evelyna columnaris*, Lindley (1846:11) cites, as the type, the collection named *Linden 620* from 'Agua de Obispo and Sierra Nevada, at the height of 9000 feet, May to August'. We found that the collection labelled *Linden 620* actually consists of six specimens deposited at W, P, BR and K herbaria. These specimens were collected at four distinct Venezuelan localities: Caracas, Agua de Obispo prov. Truxillo (=Trujillo), prov. Merida, the high Andes of Truxillo and Merida and the last one with no precise location. Thus, these specimens should be treated as syntypes (Art. 9.4 Shenzhen Code), with the Parisian one serving as the lectotype (Fig. 2). It contains not only vegetative parts, which are very well preserved, but also the inflorescence with many flowers. It was collected in the high Andes mountains in the Provinces of Trujillo and Merida, which corresponds to the protologue. A sheet kept at Kew bears a drawing of flower segments (a dorsal sepal, a lateral sepal, a petal and a lip) and a gynostemium, which were made, based on the original material.



Figure 2. *Evelyna columnaris* Lindl. Specimen *Linden 620* at the Museum National d'Histoire Naturelle, Paris (P00389742) designated here as lectotype (CC BY 4.0; <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00389742>).

***Elleanthus coriifolius* (Rchb.f ex Linden) Rchb.f., Annales Botanices Systematicae 6: 478. 1861.**

Basionym: *Evelyna coriifolia* Rchb.f. ex Linden, Botanische Zeitung (Berlin) 10: 710. 1852. Type: Colombia, “Neu Granada”, *Linden 1272*; Lectotype (designated here): W-R (W-R51672).

Note. In the protologue of *Evelyna coriifolia* Rchb.f., the author indicates the gathering *Linden 1272* as the type. We found only one specimen of this collection deposited in the Naturhistorisches Museum in Vienna (Fig. 3a). This specimen has no type designation, but it is labelled with the same collector as in the protologue. We analysed the vegetative and floral features and compared them with diagnosis. It is noteworthy that our analytical drawing (Fig. 3c) is somewhat different from details depicted by Reichenbach (Fig. 3b).

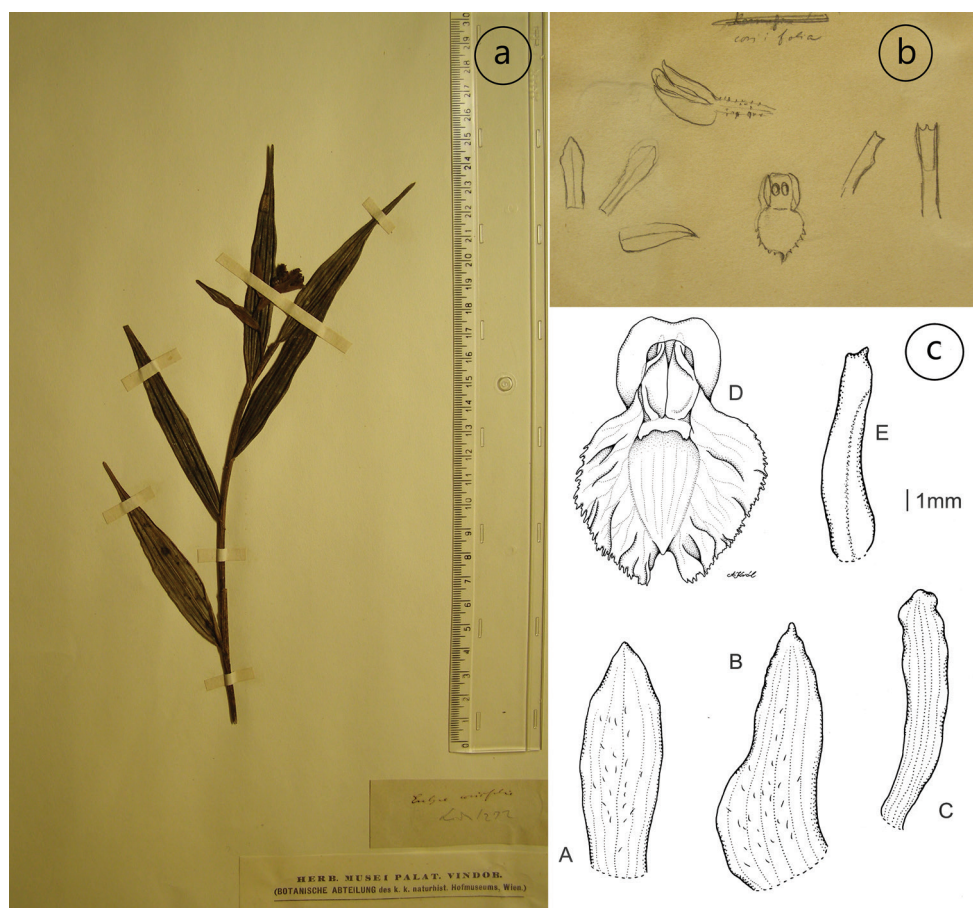


Figure 3. *Evelyna coriifolia* Rchb.f. ex Linden **a** specimen *Linden 1272* at the Naturhistorisches Museum, Vienna (W-R51672) designated here as lectotype **b** Reichenbach's drawing **c** based on Szlachetko's drawing of the original material (A. Król): A – dorsal sepal, B – lateral sepal, C – petals, D – lip, E – gynostemium.

In our opinion, the only explanation is that Reichenbach prepared his sketches, based on premature flower and this is clearly visible on his illustration of the flower (upper part of the sketch), which is narrowly tubular. His presentation of the lip clearly suggests that the apical part of the lip was not fully pressed and corpuscles are undeveloped.

In addition, in Kew, there is a sheet which bears a drawing made on the basis of a specimen from Vienna. It is marked as a type as well; however, it was not made by the author of the species. According to the Code of Nomenclature (Turland et al. 2018) this drawing cannot be treated as part of the original material.

***Elleanthus ensatus* (Lindl.) Rchb. f., *Annales Botanices Systematicae* 6: 482. 1861.**

Basionym: *Evelyna ensata* Lindl., *Orchidaceae Lindenianae* 11–12. no. 64. 1846. Type: Venezuela, Merida, “Sierra Nevada, at the height of 8000 feet [2438 m], August”, *Linden* 664; Lectotype (designated here): P (P00389702); isolectotypes: W (W-R51384), W (W-R51392), W-R-drawing (W-R30233).

Note. In the protologue of *Evelyna ensata* Lindl., the author indicates in a short note the gathering of *Linden* 664 as the type. We found that this collection actually consists of three specimens deposited in two institutions: the Naturhistorisches Museum in Vienna and the Muséum National d’Histoire Naturelle in Paris. The Vienna specimens have no annotation on their status, but they have a designation of the collection number *Linden* 664; the specimen stored in P is labelled as the type. We have analysed and compared all of them with the diagnosis. We analysed not only vegetative features, but also we compared the floral structures. These specimens have all the features of *Evelyna ensata* and one of them was selected as the lectotype (Fig. 4).

There is a Reichenbach drawing kept at W (W-R30233), labelled *E. ensata* and numbered 644. In the protologue, Lindley (1846: 12) indicates a gathering *Linden* 664. However, drawing deposits in Vienna Herbarium definitely correspond with Lindley’s species. Therefore, we can assumed that this is probably an error.

***Elleanthus flavescens* (Lindl.) Rchb.f., *Annales Botanices Systematicae* 6: 479. 1862.**

Basionym: *Evelyna flavescens* Lindl., *Orchidaceae Lindenianae* 10, no. 59. 1846. Type: Venezuela, Trujillo “between Humucoro-Bajo and the Agua de Obispo, in the Province of Truxillo, at the height of 7000 feet [2133 m], May”, *Linden* 625; Lectotype (designated here): K-L; isolectotypes: P (P00389695), W-R (W-R51662, W-R51664, W-R30242 [drawing]), BR (BR0000013083618).

Note. In the short note following the protologue, Lindley (1846) mentions a gathering which may refer to this species: *Linden* 625. Dodson and Luer (2010) selected the specimen kept in the Kew Herbarium as the holotype, but according to Arts. 9.11 and



Figure 4. *Evelynia ensata* Lindl. Specimen *Linden* 664 at the Museum National d'Histoire Naturelle, Paris (P00389702), designated here as lectotype (CC BY 4.0; <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00389702>).

9.12 of the Code of Nomenclature, their designation did not constitute a typification (Turland et al. 2018). This specimen could be treated as the lectotype.

During our studies, we found five more specimens which are labelled *Linden* 625. Three of them are deposited in the Naturhistorisches Museum in Vienna, one in the Muséum National d'Histoire Naturelle in Paris and one in Meise Botanic Garden in Belgium. The specimens from Vienna have no type annotation, but do mention the collection as *Linden* 625. We analysed the vegetative and floral characteristics and compared them with the diagnosis; two of them (W-R51662 and W-R51664) represented *Elleanthus flavescens*. The third one (W-R30242) is a drawing which is likely based on the type material as the same collector and number are recorded on it as in the protologue.

The specimen deposited in the Paris Herbarium (P00389695) was labelled by Garay as *Elleanthus furfuraceus* (Lindl.) Rchb.f. However, we are of the opinion that it is a part of the type material of *Elleanthus flavescens*. This specimen has lanceolate, acuminate leaves, an oblong, cylindrical inflorescence which is loose at the base, floral bracts that are shorter than the flowers and a pair of ovate corpuscles at the base of the lip with a strongly thickened transverse ridge, just like *E. flavescens*. It also mentions the number *Linden* 625.

Elleanthus flavescens is more similar to *E. aurantiacus* than *E. furfuraceus*. Some authors, such as Foldats (1969) and Luteyn (1999), treat this species as a synonym of *E. aurantiacus*. However, it is distinguished from the latter by smaller flowers, less cone-shaped inflorescences and a decidedly thicker transverse callus (Dunsterville and Garay 1966, 1979; Dodson and Luer 2010).

***Elleanthus furfuraceus* (Lindl.) Rchb.f., Annales Botanices Systematicae 6: 480. 1861.**

Basionym: *Evelyna furfuracea* Lindl., Orchidaceae Lindenianae 12. no. 65. 1846.

Type: Venezuela, Trujillo “Agua de Obispo, at the height of 9000 feet [2743 m], May”, *Linden* 627; Lectotype (designated here): P (P00389698); isolectotypes: P (P00389697), BR (BR0000013083588), W-R (W-R17083); Syntype: Venezuela, Merida “forest of Merida, at the height of 5500 feet [1676 m], June”, *Linden* 619 (unknown location).

Note. In describing *Evelyna furfuracea*, Lindley (1846: 12) cited two *Linden* collections: 619 and 627. Unfortunately, we were unable to locate *Linden* 619. However, collection *Linden* 627 is stored in the Muséum National d'Histoire Naturelle in Paris (two specimens), in Meise Botanic Garden (one specimen) and in the Naturhistorisches Museum in Vienna (one specimen). However, only the specimens in P and BR are marked as the type collection and only one of them is a complete specimen (P00389698) (Fig. 5). It can be characterised by lanceolate, acuminate and coriaceous leaves; terminal, laxly to subdensely flowered (with few to several) inflorescences; and subrounded-ovate, acute bracts. This specimen has also a lip obovate to suborbicular in outline, concave, unlobed and retuse in the front and saccate at the base with two large, well-separated, ellipsoid/ovoid calli. Therefore, it has been selected as the lectotype (Fig. 5).



Figure 5. *Evelynia furfuracea* Lindl. Specimen *Linden* 627 at the Museum National d'Histoire Naturelle, Paris (P00389698) designated here as lectotype (CC BY 4.0; <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00389698>).

***Elleanthus kermesinus* (Lindl.) Rchb.f., Annales Botanices Systematicae 6: 478. 1862.**

Basionym: *Evelyna kermesina* Lindl., Orchidaceae Lindenianae 11. no 61. 1846.

Type: Venezuela, Tolima, Mariquita “from the forests of Tolima in the Province of Mariquita at the height of 9000 feet [2743 m], January”, *Linden* 1276; Lectotype (designated here): P (P00419576); isolectotype: BR (BR0000013083366).

Note. In the short note following the protologue, Lindley (1846: 11) cited the collection *Linden* 1276. We have found two specimens corresponding to the original description. All of them bear *Linden* 1276 and were labelled as *Evelyna kermesina* Lindl. and designated as the type. These are deposited in the following Herbaria: the Muséum National d'Histoire Naturelle and Meise Botanic Garden. However, in the Botanic Garden in Kew, there was found a drawing based on type material, but it was not made by the author of species. This sheet embraces particular segments of flowers (a dorsal sepal, a lateral sepal, a petal and a lip), a floral bract and a gynostemium. The best preserved specimen, kept in the Paris Herbarium (P00419576), contains not only vegetative parts, but also an inflorescence with a flower (Fig. 6). The specimen has linear/lanceolate, mucronate and coriaceous leaves, a fractiflex inflorescence and two small, oval calli on the base of the lip, which displays transverse thickening. According to Arts. 9.11 and 9.12 (Turland et al. 2018), this specimen could be treated as the lectotype. The specimen from Meise Botanic Garden (BR0000013083366) is a sterile plant without flowers.

***Elleanthus lupulinus* (Lindl.) Rchb.f., Annales Botanices Systematicae 6: 483. 1861.**

Basionym: *Evelyna lupulinus* Lindl., Orchidaceae Lindenianae 11. no. 63. 1846. Type:

Venezuela, Merida “plant from the vicinity of the Paramo of the Sierra Nevada, at the height of 10 000 feet [3038 m], August”, *Linden* 642; Lectotype (designated by Garay 1978: 88): K-L; isolectotypes: P (P00389658), W-R (W-R17080, W-R51365), MO (MO1109600).

Note. Dodson and Luer (2010) selected a specimen from the Kew Herbarium as the holotype. This action is against the rules of the Code of Botanical Nomenclature. In the protologue, Lindley (1846) did not indicate where the type collection was deposited. In accordance with Arts. 9.11 and 9.12 of the ICN (Turland et al. 2018), if the plant name was published without indicating a holotype, a lectotype can be selected. We found that the gathering of *Linden* 642 actually consists of five specimens deposited at K, P, W and MO. All of these specimens are labelled as the type and a comparison of the features against the original description of the species reveals that they correspond



Figure 6. *Evelynia kermesina* Lindl. Specimen *Linden* 1276 at the Museum National d'Histoire Naturelle, Paris (P00419576) designated here as lectotype (CC BY 4.0; <http://coldb.mnhn.fr/catalognumber/mnhn/p/p00419576>).

to *Evelynia lupulina* Lindl. In such a situation, the lectotype may be designated from amongst these specimens. However, Garay (1978), in *Flora of Ecuador*, used the term type for the K specimen, while it can serve as the lectotype. According to Art. 9.10 of the Code of Nomenclature (Turland et al. 2018), Garay unknowingly designated a lectotype. A misused term may be corrected because this case meets the requirements of Art. 7.11 (Turland et al. 2018).

Acknowledgements

We would like to express our gratitude to the curators and staff of the Herbaria cited herein for their kind hospitality during our visits to their institutions. Additionally, we would like to thank Przemyslaw Baranow and Anna Król for preparing the drawings and Slawomir Nowak for his helpful comments and valuable advice on the manuscript. I am grateful to LINGUA LAB (<https://lingualab.pl/>) for English language editing. We would like also to thanks the Naturhistorisches Museum in Vienna and the Museum National d'Histoire Naturelle in Paris for the authorisation granted for the reproduction of images their specimens. The project has been supported by the Polish Ministry of Science and Higher Education (research grant nos. 5819/B/PO1/2010/39 and 8124/B/PO1/2011/40).

References

- Ceulemans N, Braem GJ (2006) Linden – Explorateur – Père des Orchidées. Fonds Mercator, Brussels, 256 pp.
- Dodson CH (1998) Native Ecuadorian Orchids, Volume II: Dresslerella–Lepanthes. Dodson Trust, Sarasota, 209–419.
- Dodson CH, Luer CA (2010) Flora of Ecuador 25(3). Botanical Institute, University of Göteborg, Riksmuseum, Stockholm.
- Dressler RL (1981) The Orchids: Natural History and Classification. Harvard University Press, Cambridge, 332 pp. <https://doi.org/10.2307/1219717>
- Dressler RL (1993) Phylogeny and Classification of the Orchid Family. Dioscorides Press, Portland, 330 pp.
- Dunsterville GCK, Garay LA (1966) Venezuelan Orchids Illustrated vol. IV. Andr'e Deutsch Ltd., London.
- Dunsterville GCK, Garay LA (1979) Venezuelan Orchids. An Illustrated Field Guide, vol. VI. Andr'e Deutsch Ltd., London.
- Foldats E (1969) Orchidaceae. In: Lasser T (Ed.) Flora de Venezuela vol. 15. Fundacion Instituto Botanico de Venezuela Press, Caracas, 153–154.
- Garay LA (1978) *Elleanthus*. In: Harling G, Sparre B (Eds) Flora of Ecuador. Department of Systematic Botany, University of Goeteborg and Section for Botany, Riksmuseum, Stockholm, 57–110.
- Lindley J (1846) Orchidaceae Lindenianae: Notes open a collection of orchids formed in Colombia and Cuba by Mr. J. Linden. Bradbury and Evans, Whitefriars, London, 28 pp. <https://doi.org/10.5962/bhl.title.66687>
- Luteyn JL (1999) Paramos – a checklist of plant diversity, geographical distribution, botanical literature. The New York Botanical Garden Press, 302 pp.
- Poepping EF, Endlicher SFL (1835) Nova Genera ac Species Plantarum, in Regno Chilensis Peruviano et in Terra Amazonica, 62 pp.
- Presl CB (1827) Orchideae Juss. In: Haenke T (Ed.) Reliquiae Haenkeanae seu Descriptiones et Icones Plantarum quas in America Meridionali et Boreali, in Insulis Phillipinis et Marianis Collegit. (Orchidaceae), fasciculus 2. Calve, Prague, 91–104.

- Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (2005) *Genera Orchidacearum*. Vol. 4: Epidendroideae (Part 1). Oxford University Press, 672 pp.
- Reichenbach HG (1852) Neue Orchideen der Expedition des Herrn J. de Warszewicz (Fortsetzung). *Botanische Zeitung* (Berlin) 10: 710.
- Reichenbach HG (1861) Orchides. *Annales Botanices Systematicae* 6: 167–933.
- Schweinfurth C (1958) Orchidaceae, Orchids of Peru. *Fieldiana. Botany* 30: 787–1005.
- Szlachetko DL (1995) *Systema Orchidarium*. *Fragmenta Floristica et Geobotanica* 3: 1–152.
- Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber WH, Li DZ, Marhold K, May TW, McNeill J, Monro AM, Prad J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Koeltz Botanical Books, Glashütten. <https://doi.org/10.12705/Code.2018>

Indigofera wenholdiae (Indigofereae, Fabaceae), a new species from the Western Cape Province, South Africa

Brian du Preez^{1,2}, Leanne L. Dreyer², Charles H. Stirton¹,
A. Muthama Muasya¹, Brian D. Schrire³

1 Bolus Herbarium, Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch, 7701, South Africa **2** Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa **3** Comparative Plant and Fungal Biology Department, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK

Corresponding author: Brian du Preez (brian.enviro@gmail.com)

Academic editor: Patrick Herendeen | Received 26 July 2021 | Accepted 20 August 2021 | Published 1 October 2021

Citation: du Preez B, Dreyer LL, Stirton CH, Muasya AM, Schrire BD (2021) *Indigofera wenholdiae* (Indigofereae, Fabaceae), a new species from the Western Cape Province, South Africa. *PhytoKeys* 182: 107–112. <https://doi.org/10.3897/phytokeys.182.72170>

Abstract

In this study, *Indigofera wenholdiae*, a new species of *Fabaceae* from the Agulhas Plain Region of the Western Cape Province, South Africa, is described. A composite photographic plate is included along with a distribution map, description of habitat and ecology and proposed IUCN conservation status. *Indigofera wenholdiae* is unique in the *I. brachystachya* group by having digitately compound (vs. pinnately compound) leaves, white and unscented flowers (vs. pink and sweetly scented flowers) and grows on sandstone hillsides (vs. coastal limestone plains and outcrops).

Keywords

Indigofera, Leguminosae, Greater Cape Floristic Region, fynbos, taxonomy

Introduction

Fabaceae represents the second largest plant family in the Cape Floristic Core Cape Region (CCR), approaching 800 species (Manning and Goldblatt 2012). The genus *Indigofera* L. with about 90 species in the region, is second only to *Aspalathus* L.

(270+ species) in size (Dahlgren 1988; Schrire in Manning and Goldblatt 2012). *Indigofera* species in the CCR are largely part of a monophyletic clade referred to as the Cape Clade by Schrire et al. (2009). Unlike the three main tropical clades that have members dispersed across multiple continents, the Cape Clade is endemic to South Africa and especially within the winter-rainfall area (Schrire et al. 2009; Schrire in Manning and Goldblatt 2012). A large group within the Cape Clade, Section *Brachypodae* Schrire, is prolific in Fynbos vegetation and has many localised species, often associated with preferences for specific microhabitats. Diverse geological formations, as seen in the Agulhas Plain region (Thwaites and Cowling 1988), have resulted in localised radiations in many plant groups (Cowling and Holmes 1992; Manning and Goldblatt 2012). For example, over 20 new legume species have been recently described from various genera, including *Aspalathus* L., *Otholobium* C.H.Stirt., *Polhillia* C.H.Stirt., *Psoralea* L. and *Rhynchosia* Lour. (Curtis et al. 2013; Moteetee et al. 2014; Stirton and Muasya 2016, 2017; Bello et al. 2017; Du Preez et al. 2021). Members of *Indigofera* Section *Brachypodae*, particularly the *I. brachystachya* group (*I. brachystachya* (DC.) E.Mey. and *I. hamulosa* Schltr.), are especially diverse in this region, with several putative undescribed taxa related to *I. brachystachya*, noted from recent field studies (Schrire and du Preez, unpublished data). While further, more detailed, analyses are required to resolve this species complex, we here describe a new, distinctive species from the *I. brachystachya* group first found in the Grootbos Nature Reserve in 2020.

Material and methods

The description of morphological characters is based on freshly collected material and herbarium voucher specimens. The conservation assessment was done using the Categories and Criteria of the IUCN (2012). The Extent of Occurrence (EOO) and Area of Occurrence (AOO) were calculated using GeoCAT (www.geocat.kew.org). The distribution map was made using QGIS 3.18 software (www.qgis.org).

Species treatment

***Indigofera wenholdiae* du Preez & Schrire, sp. nov.**

urn:lsid:ipni.org:names:77220006-1

Diagnosis. Similar to *I. brachystachya*, but differs in its digitately compound, 5–7 foliolate leaves (versus pinnately compound, 7–9 foliolate leaves), leaflets 4–6 mm long (versus leaflets 9–15 mm long), racemes up to 7 mm long, flowers $\pm$ 4–5 per raceme (versus racemes > 10 mm long, flowers > 8 per raceme), flowers creamy white, unscented (versus flowers pale pink, scented), branching divaricate (versus branching random), populations restricted to sandstone fynbos (versus populations restricted to limestone fynbos).

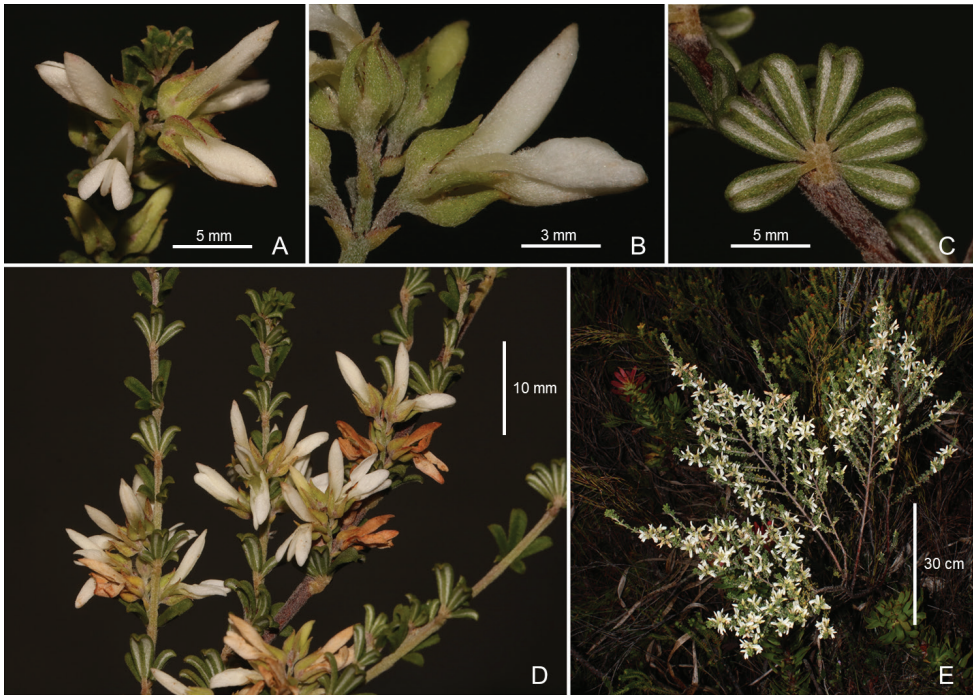


Figure 1. *Indigofera wenholdiae* du Preez & Schrire **A** single inflorescence **B** flower lateral view **C** leaf abaxial view **D** multiple inflorescences on branches **E** whole plant. Photographs by Brian du Preez. Voucher *B. du Preez* 836 (BOL).

Type. SOUTH AFRICA, Western Cape Province, track to Beacon Peak in Grootbos Nature Reserve, Gansbaai District, elevation 257 m, 34°31'32.84"S, 19°30'19.08"E, 7 May 2020, flowering, *B. du Preez* 836 (Holotype: BOL; Isotypes: K!, NBG!, PRE!).

Description. Erect perennial shrub 0.4–0.8 m tall, robust, sparsely branching, divaricate; obligate reseeder. **Branches** up to 10 mm thick, terete to ribbed on fresh growth, densely strigose with sessile whitish biramous hairs, glabrescent later, reddish-brown, woody. **Stipules** 0.8–1.2 mm long, $\pm$ 1 mm wide at base, broadly triangular, asymmetric, navicular-cucullate, attenuate, apex aristate, adpressed to branch, adnate to base of petiole, densely strigose, $\pm$ soft-textured and thickened, pale green, pearl bodies present along margin. **Leaves** alternate, digitately 5–7 foliolate, petiole $\pm$ 1 mm long, sub-terete, flattened adaxially; rachis $\pm$ 0.5 mm long, terete, stipels absent; petiolules $\pm$ 0.5 mm long; terminal leaflet 4–6 mm $\times$ 1.5–2.5 mm, narrowly oblanceolate, apex apiculate, hooked, base cuneate, upper surface sparsely strigose, bright green, paler below; lower surface densely strigose; mid-vein sunken adaxially, prominent abaxially; margins not thickened, strongly revolute; lateral leaflets similar, opposite. **Racemes** axillary, up to 7 mm long, erect, parallel with branch, roughly equalling the leaf length, including a peduncle 2–3 mm long, ribbed, soft-textured; $\pm$ 4–5 flowered; bracts $\pm$ 1.5 mm long, lanceolate, cucullate, apex apiculate, adpressed to petiole, persistent until after flowering, pearl bodies present along margin. **Bracteoles** absent. **Pedicels** 1–1.5 mm long.



Figure 2. Distribution map of *Indigofera wenholdiae*.

Flowers 8–9 mm long, unscented. **Corolla** creamy-white, petals persistent after anthesis. **Calyx** 3.5–4.2 mm long, pale green, lobes lanceolate, distinctly navicular-cucullate, 2.5–3 mm long, $\pm$ three times tube length, moderately strigose, pearl bodies present along margin. **Standard petal** 8.7–9.3 mm $\times$ 3.3–3.7 mm, broadly oblong, tapering gradually to a short claw at the base; blade concave, nectar guide plain white; apex acute-obtuse; back of standard strigose, no visible colouration patterns. **Wing petals** 7.5–8 $\times$ 1.5–2 mm, shortly clawed at base, unguiculate portion $\pm$ half total petal length, blade asymmetrically navicular, apex rounded; blade moderately strigose. **Keel petals** 7.5–8 $\times$ 1.8–2.3 mm, valvately connate distally, lateral spurs up to 1 mm long, blade asymmetrically lanceolate, dorsal margin curving slightly downwards to an acute-obtuse apex, densely strigose; claws 2.5–3 mm long. **Stamens** 5–5.5 mm long, exceeding calyx by 1.5–2 mm, staminal tube pale creamy-green; hair clusters present below anthers. **Gynoecium** 4–4.5 mm long, strigillose on distal half; style $\pm$ 2 mm long, erect to $\pm$ 1 mm high distally; stigma capitate. **Fruit** 13–16 $\times$ 2–2.2 mm, oblong, woody, reddish-brown, densely strigillose, 4–5 seeded, dehiscent, ripe fruit not seen. **Seeds** not seen.

Distribution, habitat and ecology. *Indigofera wenholdiae* is restricted to a few sandstone hills from the Grootbos Nature Reserve to Pearly Beach on the Agulhas Plain of the Western Cape Province (Figure 2). The species is occasional in Overberg Sandstone Fynbos (FFs12, Mucina and Rutherford 2006), favouring south-facing slopes and hilltops.

Phenology. Flowering takes place from April to July.

Etymology. The specific epithet “wenholdiae” is assigned in honour of Mrs Hanerrie Wenhold who has funded large-scale conservation efforts in this area, including

the funding of the botanist post at Grootbos Nature Reserve of Miss Rebecca Dames who brought this species to our attention in April 2020.

Conservation status. *Indigofera wenholdiae* is a range-restricted species, with an EOO of 30 km² and AOO of 24 km². A total of six subpopulations across four locations have been discovered, with the number of mature individuals estimated to be less than 10 000. Most subpopulations occur within protected areas or on farms earmarked for conservation. Alien vegetation is prominent in the region, although only one location is currently threatened by invasive species, while alien eradication projects at other locations have largely controlled or eradicated invasive species. The Red List category VU D2 is thus recommended, based on the IUCN Categories and Criteria (IUCN 2012).

Related species. *Indigofera wenholdiae* is part of Section *Brachypodae*, a large group ($\pm$ 30 species) of *Indigofera* species almost exclusively found in Fynbos vegetation and characterised by having five or more foliolate leaves with short petioles. The *I. brachystachya* group resolves within this section and includes *I. brachystachya*, *I. hamulosa* and several putative undescribed taxa related to the former. All of these species are characterised by a corolla morphology unique in the genus, with elongate and concave standard petals and, in general, robust rather than delicate petals. All species in this group are coastally distributed and most often occur on limestone substrate. Apart from the distinguishing features noted above, *I. wenholdiae* is an erect to less than 1 m tall, divaricately branching shrub with deep green leaves and white flowers, in contrast with the bushier growth of *I. brachystachya*, with its typically dull grey leaves and pale pink flowers.

Additional specimens examined. Grootbos Nature Reserve, (3419DA), 12 September 2020, *B. du Preez* 879 (BOL, NBG).

Acknowledgements

The permit for the collection of herbarium specimens was obtained from the Western Cape Nature Conservation Board (Permit: AAA 008-00222-0028). We wish to thank Miss Rebecca Dames for bringing this species to our attention and who, along with Mr Sean Privett, searched for and located further populations in the area. We wish to also thank Mr Michael Lutzeyer for allowing us access to Grootbos Nature Reserve to study this species. Running costs for research, including fieldwork, were covered by an NRF – FBIP grant awarded to Prof. A.M. Muasya (FBIP180529337017) and personal bursaries awarded by the NRF – FBIP (FBIP 128327) and the Science Faculty PhD Fellowship from the University of Cape Town.

References

- Bello A, Stirton CH, Chimphango SBM, Muasya AM (2017) Taxonomic revision of African *Psoralea pinnata* species complex (Psoraleaceae, Leguminosae). South African Journal of Botany 112: 128–179. <https://doi.org/10.1016/j.sajb.2017.05.003>

- Cowling RM, Holmes PM (1992) Endemism and speciation in a lowland flora from the Cape Floristic Region. *Biological Journal of the Linnean Society*. Linnean Society of London 47(4): 367–383. <https://doi.org/10.1111/j.1095-8312.1992.tb00675.x>
- Curtis OE, Stirton CH, Muasya AM (2013) A conservation and floristic assessment of poorly known species rich quartz-silcrete outcrops within Rûens Shale Renosterveld (Overberg, Western Cape), with taxonomic descriptions of five new species. *South African Journal of Botany* 87: 99–111. <https://doi.org/10.1016/j.sajb.2013.03.017>
- Dahlgren R (1988) *Aspalathus* (3,6). In: Leistner OA (Ed.) *Flora of Southern Africa*, 16, Botanical Research Institute, Pretoria.
- Du Preez B, Dreyer LL, Stirton CH, Muasya AM (2021) A monograph of the genus *Polhillia* (Genisteae: Fabaceae). *South African Journal of Botany* 138: 156–183. <https://doi.org/10.1016/j.sajb.2020.12.022>
- IUCN (2012) IUCN Red List Categories and Criteria, Second ed. IUCN, Gland, Switzerland and Cambridge, UK.
- Manning J, Goldblatt P [Eds] (2012) *Plants of the Greater Cape Floristic Region. 1: the core Cape flora*. South African National Biodiversity Institute, Pretoria, 853 pp.
- Moteetee AN, Boatwright JS, Jaca TP (2014) A review of *Rhynchosia* Section *Polytropia* (Phaseoleae, Fabaceae) and a new species from the Western Cape Province, South Africa. *Systematic Botany* 39(4): 1127–1131. <https://doi.org/10.1600/036364414X683859>
- Mucina L, Rutherford MC [Eds] (2006) *The vegetation of South Africa, Lesotho and Swaziland*. Strelitzia 19. South African National Biodiversity Institute, Pretoria, 801 pp.
- Schrire BD, Lavin M, Barker NP, Forest F (2009) Phylogeny of the tribe Indigofereae (Leguminosae-Papilionoideae): Geographically structured more in succulent-rich and temperate settings than in grass-rich environments. *American Journal of Botany* 96(4): 816–852. <https://doi.org/10.3732/ajb.0800185>
- Stirton CH, Muasya AM (2016) Seven new species and notes on the genus *Aspalathus* (Crotalariaeae, Fabaceae). *South African Journal of Botany* 104: 35–46. <https://doi.org/10.1016/j.sajb.2015.10.007>
- Stirton CH, Muasya AM (2017) Ten new species and a new record for the genus *Otholobium* (Psoraleae, Leguminosae) from South Africa. *Kew Bulletin* 72(50): 1–27. <https://doi.org/10.1007/S12225-017-9722-5>
- Thwaites RN, Cowling RM (1988) Soil-vegetation relationships on the Agulhas Plain, South Africa. *Catena* 15(3–4): 333–345. [https://doi.org/10.1016/0341-8162\(88\)90055-0](https://doi.org/10.1016/0341-8162(88)90055-0)

Begonia pseudoedulis, a new species in *Begonia* sect. *Platycentrum* (Begoniaceae) from southern Guangxi of China

Xin-Xin Feng^{1*}, Yan Xiao^{2,3,4*}, Zhi-Xian Liu¹,
Ren-Kun Li⁵, Dan Wei⁶, Dai-Ke Tian^{3,4}

1 Dongguan Botanical Garden, Dongguan 523086, China **2** College of Life Sciences, Hunan Normal University, Changsha, 410081, China **3** Shanghai Chenshan Plant Science Research Center, Chinese Academy of Sciences, 3888 Chenhua Road, Songjiang, Shanghai 201602, China **4** Shanghai Key Laboratory for Plant Functional Genomics and Resources, Shanghai Chenshan Botanical Garden, 3888 Chenhua Road, Songjiang, Shanghai 201602, China **5** Enshi Dongsheng Plant Development Co. Ltd., Enshi 445000, China **6** Guangdong Academy of Forestry, Guangzhou 510520, China

Corresponding authors: Dai-Ke Tian (dktian@cemps.ac.cn), Xin-Xin Feng (xinxin8715@163.com)

Academic editor: Hanno Schaefer | Received 24 May 2021 | Accepted 17 July 2021 | Published 1 October 2021

Citation: Feng X-X, Xiao Y, Liu Z-X, Li R-K, Wei D, Tian D-K (2021) *Begonia pseudoedulis*, a new species in *Begonia* sect. *Platycentrum* (Begoniaceae) from southern Guangxi of China. *PhytoKeys* 182: 113–124. <https://doi.org/10.3897/phytokeys.182.69074>

Abstract

Begonia pseudoedulis, a new species in *Begonia* sect. *Platycentrum* (Klotzsch) A.DC. (Begoniaceae) from southern Guangxi of China, is here described and illustrated. It morphologically resembles *B. edulis* H.Lév. and *B. dielsiana* E.Pritz. ex Diels but differs easily by its hairy petioles and inflorescences, and red hispidulous flower tepals, ovary and capsules. The molecular phylogenetic analysis based on ITS supported that the new species was a monophyletic lineage, separating from both *B. dielsiana* and *B. edulis*. Due to its isolated distribution with several small populations, which are possibly disturbed by human activities, the species is considered as “Near Threatened” (NT) according to the IUCN Red List Categories and Criteria.

Keywords

Molecular evidence, morphology, new taxon, southern China, taxonomy

* The authors contributed equally.

Introduction

As a pan-tropically distributed and the sixth largest genus in the angiosperms (Hughes et al. 2015–onwards; Moonlight et al. 2018), *Begonia* L. consists of 2007 known species belonging to 70 sections (Hughes et al. 2015–onwards; Moonlight et al. 2018). Although showing a very high diversity in the specific level, the classification and phylogeny of this mega genus remain uncertain due to inadequate field surveys, high similarity and variation in morphology, and wild hybridization (Moonlight et al. 2018; Tian et al. 2018a). *Begonia* species are commonly sensitive to the environment, particularly with a low tolerance to both high and low temperature and strong sunlight. In addition, due to their high ornamental and medicinal values, some wild begonias have been commercially over-collected (Tian et al. 2018a). The vulnerability of the restricted distribution and the fact that it is easily disturbed by human activities has resulted in many *Begonia* species becoming endangered (Shui and Chen 2018).

In the past 20 years, the recognized *Begonia* species increased from 80 to 200 in China (Tian et al. 2018b). Over 220 species belonging to 10 sections have been illustrated in China, which is one of the diversity centers of Asian begonias. China plays a more and more significant role in classical taxonomy, phylogeny, utilization, and diversity conservation of *Begonia* (Tian et al. 2018a, 2018b).

In China, the reported *Begonia* taxa are mainly distributed in southeastern Yunnan and southwestern Guangxi. Over 90 species have been described from Guangxi, most of which belong to *B.* sect. *Coelocentrum* (Gu et al. 2007; Dong and Liu 2018). We conducted field surveys in several places in southwestern Guangxi on April 1, 2015, November 13, 2016, and in October 2019, respectively and found a putative new *Begonia* species similar to *B. edulis* H.Lév. and *B. dielsiana* E.Pritz. ex Diels in morphology. In October 2020, the type specimens with flowers were collected from Shiwan-shan Mountain. Based on further detailed morphological observation and comparison with its allied species and molecular phylogenetic evidence, the species is confirmed as a new one belonging to *B.* sect. *Platycentrum* (Klotzsch) A.DC.

Materials and methods

Taxonomic observation

The morphological traits were observed and recorded both in the field and from specimens. The photographs were taken during field surveys in southern Guangxi in China. The specimens are deposited at Chenshan Herbarium (**CSH**) of Shanghai Chenshan Botanical Garden and Herbarium of Sun Yat-sen University (**SYS**), respectively.

DNA sequencing and molecular analysis

The 16 species from sect. *Platycentrum* and 9 species from other sections of *Begonia* native to mainland China were selected to reconstruct the phylogenetic relationships (Table 1).

Table 1. *Begonia* species and populations included in the phylogenetic analysis (Sectional placement follows Moonlight et al. 2018).

Taxon	Origin	Genbank accession no.	Section	Collector, voucher (Herbarium)
<i>B. acetosella</i> Craib	Sapa, Vietnam	AF485102	<i>Platycentrum</i>	Forrest, L.L.108 (E)
<i>B. biflora</i> T.C.Ku	Malipo, Yunnan, China	JF975965	<i>Coelocentrum</i>	Shui, Y.M. et al. 20484 (KUN)
<i>B. chingii</i> Irmsh.	Napo, Guangxi, China	KP710820	<i>Reichenheimia</i>	Tian, D.K., Li, C. TDK785 (CSH)
<i>B. circumlobata</i> Hance	Xinyi, Guangdong, China	KP710815	<i>Platycentrum</i>	Tian, D.K., Li, X.P. TDK866 (CSH)
<i>B. circumlobata</i> Hance	Youxi, Fujian, China	MZ145342	<i>Platycentrum</i>	Tian, D.K., et al. TDK2541(CSH)
<i>B. lipingensis</i> Irmsh.	Liping, Guizhou, China	MZ145346	<i>Platycentrum</i>	Xiao, Y., et al. XY01 (CSH)
<i>B. dielsiana</i> E.Pritz. ex Diels	Wulong, Chongqing, China	KP710805	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2356 (CSH)
<i>B. dregei</i> Otto & Dietr	South Africa	AF469126	<i>Augustia</i>	Forrest, L.L.241 (E)
<i>B. edulis</i> H.Lév.	Bama, Guangxi, China	KP710813	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK757 (CSH)
<i>B. edulis</i> H.Lév.	Debao, Guangxi, China	MZ145343	<i>Platycentrum</i>	Tian, D.K., et al. TDK3111_14(CSH)
<i>B. edulis</i> H.Lév.	Jingxi, Guangxi, China	MZ145344	<i>Platycentrum</i>	Tian, D.K., et al. TDK3101_19(CSH)
<i>B. emeiensis</i> C.M.Hu ex C.Y. Wu & T.C.Ku	Emeishan, Sichuan, China	KP710816	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2249 (CSH)
<i>B. fimbripila</i> Hance	Fangchenggang, Guangxi, China	KP710826	<i>Diplocinium</i>	Li, C. Yang, L.H. TDK2268 (CSH)
<i>B. grandis</i> Dryand.	Yongshun, Huhun, China	KP710828	<i>Diplocinium</i>	Li, X.P., Li, X.J. LXJ022 (CSH)
<i>B. handelii</i> Irmsh.	Fengshan, Guangxi, China	KP710818	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK763 (CSH)
<i>B. huangii</i> Y.M.Shui & W.H.Chen	Gejiu, Yunnan, China	JF976001	<i>Coelocentrum</i>	Shui, Y.M., et al. 40782 (KUN)
<i>B. jinyunensis</i> C.-I Peng, Ding & Q. Wang	Jinyunshan, Chongqing, China	MZ145345	<i>Platycentrum</i>	Tian, D.K., et al. TDK623 (CSH)
<i>B. laminariae</i> Irmsh.	Pingbian, Yunnan, China	KP710814	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK1338 (CSH)
<i>B. longifolia</i> Blume	Wuming, Guangxi, China	MZ145347	<i>Platycentrum</i>	Tian, D.K., et al. TDK3007_8 (CSH)
<i>B. palmata</i> D.Don.	Jinxu, Guangxi, China	MZ145348	<i>Platycentrum</i>	Li, C., Yang, L.H. TDK1848_1(CSH)
<i>B. palmata</i> D.Don.	Jinxu, Guangxi, China	MZ145349	<i>Platycentrum</i>	Li, C., Yang, L.H. TDK1848_2 (CSH)
<i>B. palmata</i> D.Don.	Jinxu, Guangxi, China	MZ145350	<i>Platycentrum</i>	Li, C., Yang, L.H. TDK1848_3 (CSH)
<i>B. pedatifida</i> H.Lév.	Tianlin, Guangxi, China	KP710809	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK1924 (CSH)
<i>B. pedatifida</i> H.Lév.	Tianlin, Guangxi, China	KP710810	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK774 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Fangchenggang, Guangxi, China	MZ145352	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2428_2 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Fangchenggang, Guangxi, China	MZ145353	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2428_3 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Fangchenggang, Guangxi, China	MZ145354	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2428_4 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Fangchenggang, Guangxi, China	MZ145355	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2423_5 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Fangchenggang, Guangxi, China	MZ145356	<i>Platycentrum</i>	Tian, D.K., Tian, L.Z. TDK2423_7 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Wuming, Guangxi, China	MZ145357	<i>Platycentrum</i>	Tian, D.K., et al. TDK3008_1 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Wuming, Guangxi, China	MZ145358	<i>Platycentrum</i>	Tian, D.K., et al. TDK3008_4 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Wuming, Guangxi, China	MZ145359	<i>Platycentrum</i>	Tian, D.K., et al. TDK3008_5 (CSH)
<i>B. pseudododulis</i> D.K.Tian, X.X.Feng & R.K.Li	Shangsi, Guangxi, China	MZ153095	<i>Platycentrum</i>	Feng, X.X., et al.FXX201001 (CSH)
<i>B. pulchrifolia</i> D.K.Tian & C.H.Li	Meinfeng, Leshan, Sichuan, China	KP710811	<i>Platycentrum</i>	Tian, D.K., et al. TDK2243 (CSH)
<i>B. ruboides</i> C.M.Hu ex C.Y.Wu & T.C.Ku	Hekou, Yunnan, China	JF976047	<i>Diplocinium</i>	Shui, Y.M. D-38 (KUN)
<i>B. scorpiuroloba</i> D.K.Tian & Q.Tian	Fangchenggang, Guangxi, China	MZ145351	<i>Platycentrum</i>	Li, C. Yang, L.H. TDK2269 (CSH)
<i>B. setifolia</i> Irmsh.	Lvchun, Yunnan, China	KP710827	<i>Diplocinium</i>	Tian, D.K., Li, C. TDK1280 (CSH)
<i>B. silletensis</i> (A.D.C.) C.Clarke	Lincang, Yunnan, China	AF048988	<i>Platycentrum</i>	X.J.Y.01012 (KUN)
<i>B. wenshanensis</i> C.M.Hu ex C.Y.Wu & T.C.Ku	Kunming Botanic Garden, China	AF048974	<i>Diplocinium</i>	X.J.Y.01010 (KUN)
<i>B. wilsonii</i> Gagnep.	Nanchuan, Chongqing, China	KP710819	<i>Diplocinium</i>	Tian, D.K., Tian, L.Z. TDK2111 (CSH)

B. dregei Otto & A.Dietr. of sect. *Augustia* (Klotzsch) A.DC. from South Africa was used as outgroup in the phylogenetic analysis. The methods for DNA extraction, amplification and data analysis were adopted from Tian et al. (2014, 2015). The nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region was amplified with primers from Chung et al. (2014). The PCR products of ITS were directly sent to Tsingke Biotechnology Co., Ltd. (Shanghai, China) to be sequenced.

The phylogenetic analysis of Bayesian inference (**BI**) was performed in MrBayes v3.2.7 (Ronquist et al. 2012). The GTR+G model was chosen as the optimal model of nucleotide substitution using the Akaike information criterion (**AIC**; Burnham and Anderson 2002) as implemented in IQ-TREE (Trifinopoulos et al. 2016). The Markov chains were run for 1,000,000 generations and sampled at each 100 generations, with the first 25% discarded as burn-in.

Taxonomy

***Begonia pseudoodulis* D.K.Tian, X.X.Feng & R.K.Li, sp. nov.**

urn:lsid:ipni.org:names:77220007-1

Figs 1–3

Chinese name: 假食用秋海棠

Type. **China** Guangxi, Shangsi County (上思县), Shiwanshan (十万山), 21°58'4.71"N, 108°16'50.05"E (Fig. 4), 163 m alt., near a stream under the broad leaf forest, at late period flowering, October 2020, X. X. Feng, Z. X. Liu, & R.K. Li, FXX201001 (**holotype**: CSH0185896, CSH!; **isotypes**: CSH! & SYS!).

Diagnosis. The new species shows high resemblance to both *B. edulis* and *B. dielsiana* from the same section (sect. *Platycentrum*) in their stout and creeping rhizome, erect stem at anthesis, asymmetric ovate and chartaceous leaf blade, palmate venation, dichasial cyme, four tepals of staminate flowers, unequally 3-winged capsules. However, it differs from the latter two mainly by its shorter (vs. taller) plants, variation (variegated more than pure green vs. green or dark-green for *B. edulis* and pure green for *B. dielsiana*) in leaf color, hairy (vs. glabrous or nearly so) petioles and red hairs (vs. glabrous or nearly so) on abaxial surface of outer tepals, stable five (vs. usually five and rarely six for *B. edulis*, and more six than five for *B. dielsiana*) tepals of pistillate flowers.

Description. Perennial evergreen herb, monoecious, 30–50 cm tall. **Rhizome** stout and creeping, ca. 10–12 cm long and 10–15 mm in diameter, internodes obvious and crowded; erect stem only at anthesis, usually reddish-brownish, rarely green, sparsely pilose. **Stipules** membranous, triangular, reddish or green, glabrous. **Leaves** 6–8 basal and 2–3 aerial, petiole pale- to reddish-green, 18–50 cm long, 5–10 mm thick, densely red pilose, 3–5 mm long at young stage and then sparsely short reddish-brownish or gray hairs in mature; leaf blade variable in shape and coloration, usually widely ovate, 12–23 × 9–22 cm, adaxially dark-green along main veins or evenly green, rarely white spotted, puberulent, abaxially purple-red along veins or evenly green, subglabrous

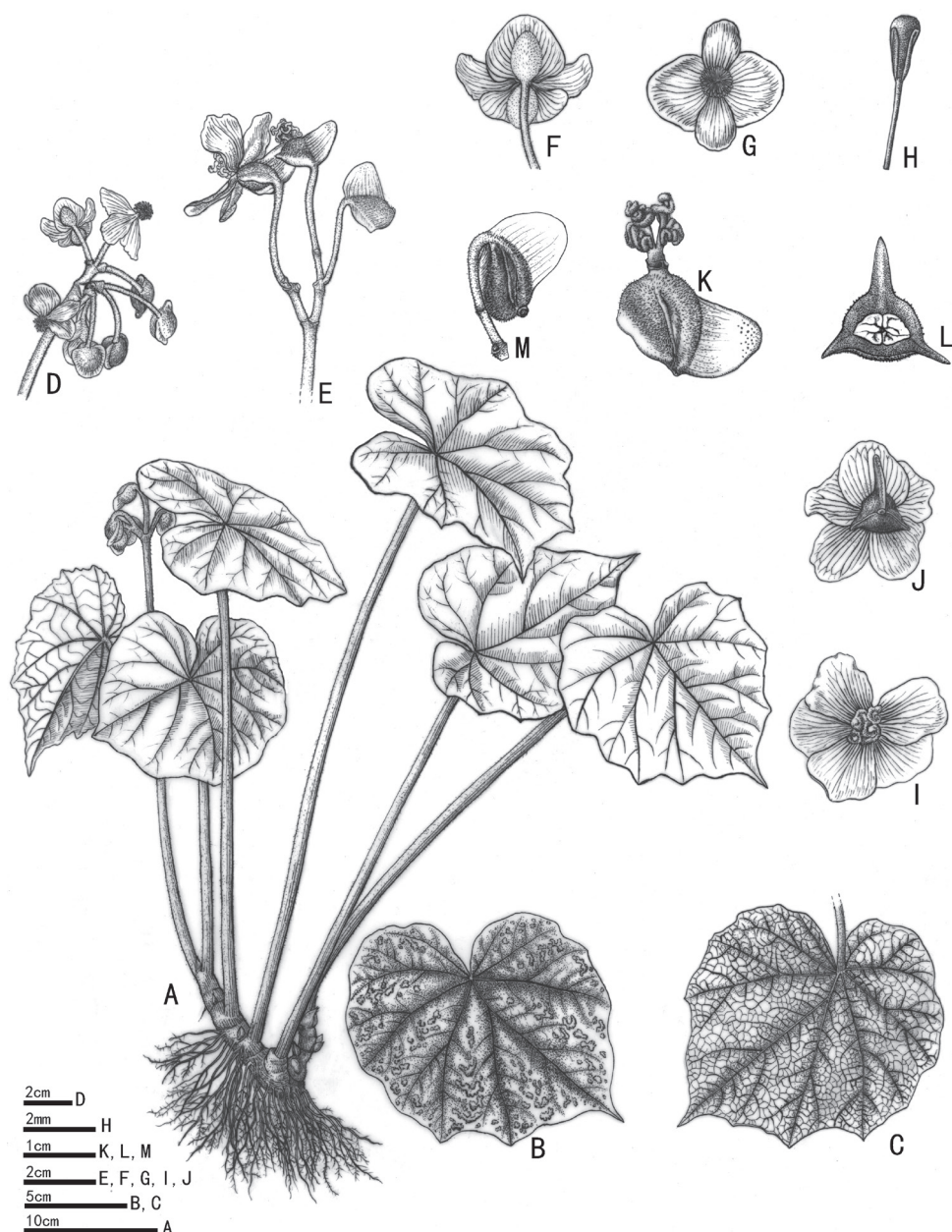


Figure 1. *Begonia pseudoedulis* **A** habitat **B** adaxial leaf blade **C** abaxial leaf blade **D, E** inflorescences **F** staminate flower (abaxial) **G** staminate flower (adaxial) **H** stamen **I** pistillate flower (adaxial) **J** pistillate flower (abaxial) **K** ovary and stigma **L** cross section of ovary **M** capsule (Illustration drawn by Yunxiao Liu).

except main veins, base strongly oblique-cordate, margin triangularly denticulate to shallowly lobed, apex caudate. Venation palmate, primary veins 7–8, adaxially slightly concave, abaxially convex. **Inflorescences** arising from erect stem at anthesis, dichasial

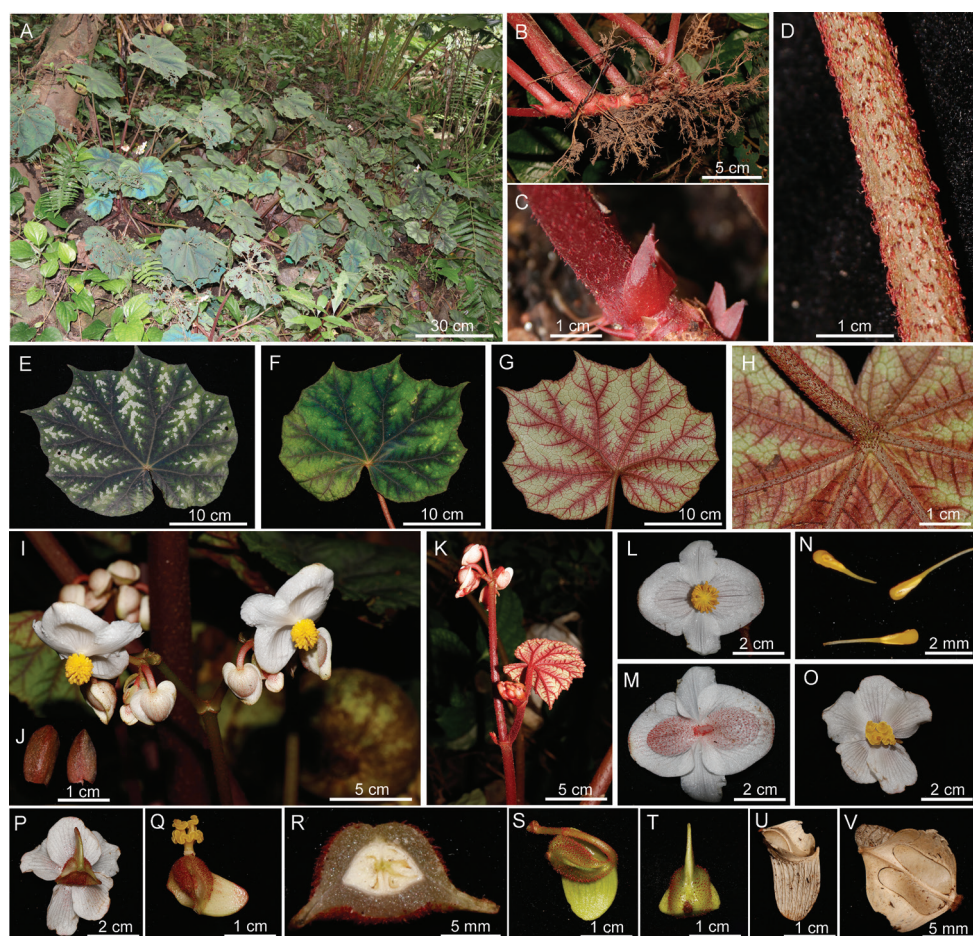


Figure 2. Habitat and morphology of *Begonia pseudoedulis* **A** habitat **B** creeping rhizome **C** stipule **D** petiole showing hairs **E** mature leaf blade (adaxial) **F** juvenile leaf blade (adaxial) **G, H** mature leaf blade (abaxial) **I** inflorescence **J** bracts **K** erect stem at anthesis **L** front view of staminate flower **M** back view of staminate flower **N** stamens **O** front view of pistillate flower **P** back view of pistillate flower **Q** ovary with styles and stigmas **R** cross section of ovary **S, T** Immature capsule (different views) **U** dry capsule showing abaxial wing **V** dry capsule showing lateral wings.

cymes branching only once, peduncle 16–23 cm long, red short strigose, flowers unisexual, 5–8 flowers per inflorescence; bracts membranous, oblong triangular to widely ovate, reddish-brownish, 8–15 × 4–8 mm, glabrous. **Staminate flower:** pedicels pink, 2.0–2.5 cm long, hairy nearly same as peduncles; tepals 4, white or nearly so, outer 2 broadly ovate, 18–23 × 17–22 mm, middle part thicker, adaxially concave, abaxially convex, red short strigose, veins distinct, apex obtuse, margin entire; inner 2 nearly obovate, ca. 18 × 14 mm, glabrous; androecium spheroid, ca. 11 mm across; stamens numerous, ca. 4–5 mm long; filaments fused at base, anthers yellow, clavate, base cuneate, ca. 2 mm long. **Pistillate flower:** pedicels pink, 2.0–2.5 cm long, hairy nearly

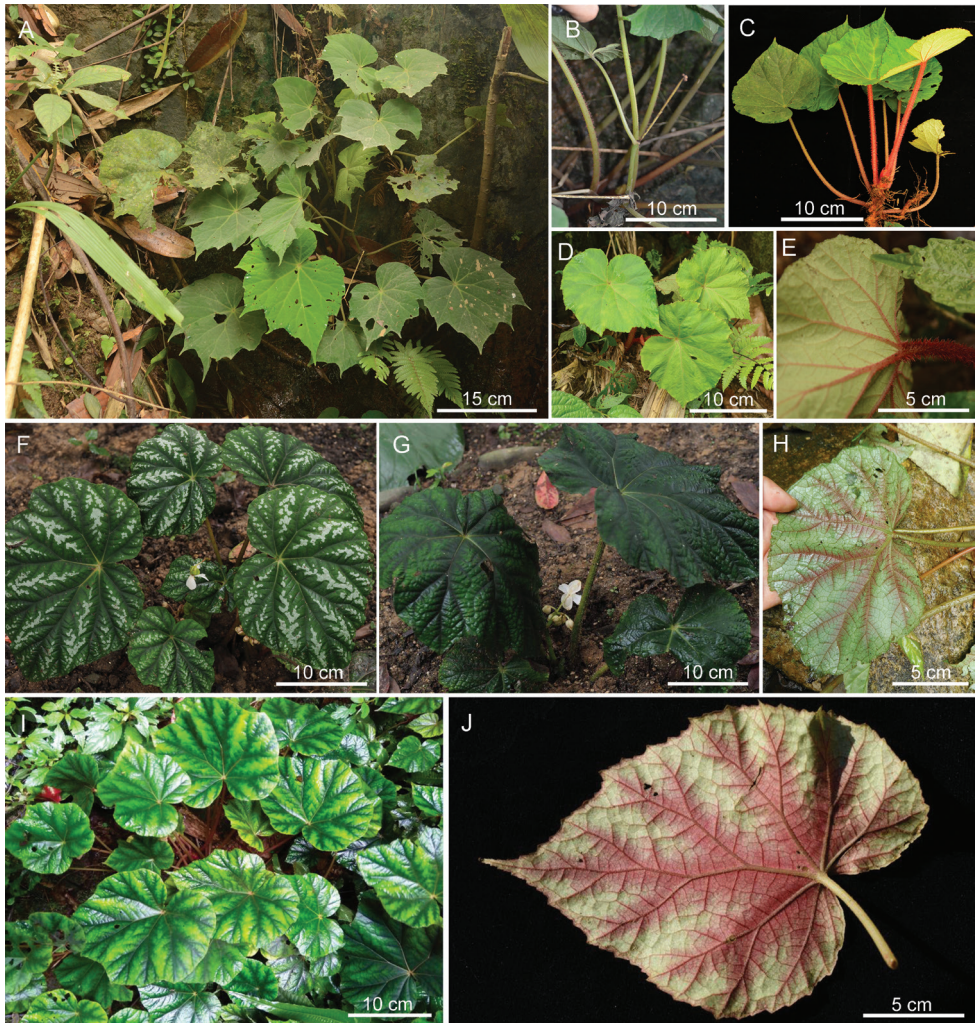


Figure 3. Variation in leaf morphology of different populations of *Begonia pseudoedulis* **A–E** the population from Fangcheng, Fangchenggang, Guangxi (**A, B** mature plants **C–E** juvenile individuals showing dense hairs on petioles) **F–H** wuming, Nanning, Guangxi **I, J** daxin, Chongzuo, Guangxi (photos **A–E, J** by Dai-Ke Tian **F–H** by Jun Liu from Zhejiang University; **I** by Chen-Yang Zhao from Daxin County of Guangxi). Note: The population from Daxin, Chongzuo (**I, J**) is only recognized by morphology without molecular evidence.

same as peduncles; tepals 5, white or nearly so, irregularly suborbicular, fan-shaped or broadly ovate, sub-equal, 16–19 × 15–17 mm, abaxially sparsely red short strigose, apex obtuse; ovary yellowish-green, trigonous-ellipsoid, 12–14 × 8–9 mm (wings excluded), red hispidulous; 2-loculed, placentation axile, placentae bifid per locule; styles 2, fused at base, yellow, ca. 7–9 mm long; stigma U-shaped, spirally twisted. **Capsules** nodding, trigonous-ellipsoid, ca. 18 × 8–10 mm (wings excluded), yellowish-green,



Figure 4. Distribution of *Begonia pseudoedulis* and the allied *B. edulis* and *B. dielsiana*.

red hispidulous; wings 3, unequal, abaxial wing semicircle-shaped or rectangular, ca. 15 mm long; lateral wings narrow, 3–4 mm long.

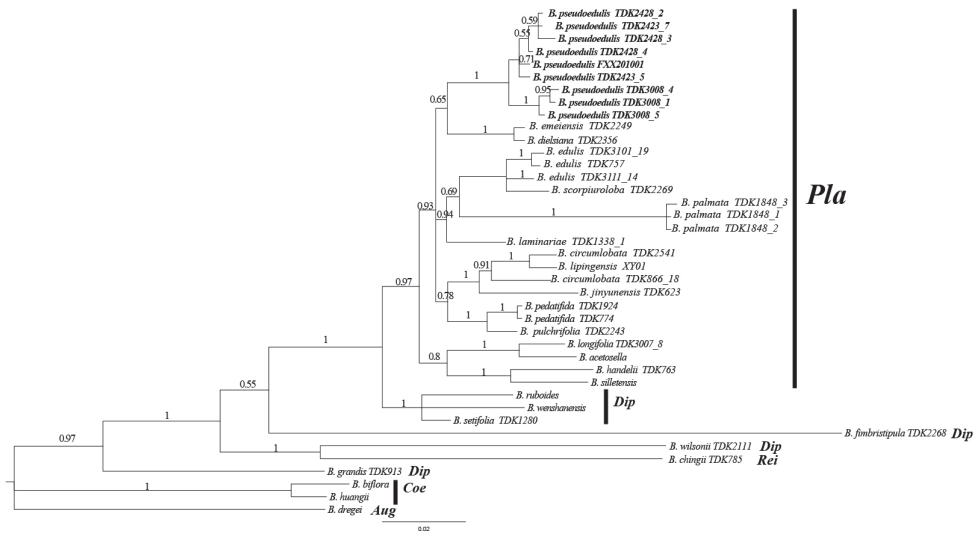
Additional specimens examined. CHINA **Guangxi:** Fangchenggang District (防城港区), Dongzhong Town (峒中镇), Nameng Village (那蒙村), roadside of S325, near stream under bamboo forest, 21°38'32.63"N, 107°35'48.91"E, elev. 380 m, 1 April 2015, *Dai-Ke Tian, Li-Zhi Tian, TDK2423* (CSH!); Dongzhong Town (峒中镇), Dakeng Village (大坑村), Maan'ao (马鞍坳), 21°38'25.32"N, 107°39'34.40"E, elev. 650 m; Wuming County (武鸣县), Liangjiang Town (两江镇), Daming Mountain Nature Reserve (大明山保护区), 23°25'38"N, 108°27'35"E, elev. 550 m, *Dai-Ke Tian, Yan Xiao, Yi Tong & Li-Zhi Tian, TDK3008* (CSH!); Wuming County, Liangjiang Town, 23°26'19.74"N, 108°24'31.92"E, *Ya-Hong Gao TDK4001* (collected at Shanghai Chenshan Botanical Garden from introduced plants)

Distribution and habitat. Currently known from four localities with elev. 160–650 m. (Fig. 4). It grows in shaded environment along the stream or near waterfall under the broad-leaved forest.

Phenology. Flowering August–October, fruiting September–November.

Etymology. The specific epithet “*pseudoedulis*” refers to its similarity to *B. edulis*, because both are easily confused based on appearance when the inflorescences and flowers are invisible.

Conservation status. Only four populations with under 1000 estimated individuals have been found so far in three counties of Guangxi. Each population consisting of approximately 20–300 individuals is distributed in no more than 200 m² area. The



population size is prone to decrease by illegal collection for medicinal and ornamental uses. Considering the disturbance of human activities and narrow distribution, *B. pseudoedulis* is currently assessed as “Near Threatened” (NT) according to the IUCN Red List Categories and Criteria (IUCN, 2019).

The ITS data set containing 40 accessions represented 26 species, four main sections of *Begonia* in China and one section from Africa (Table 1). The aligned matrix of ITS region was 664 bp. The result of Bayesian inference analysis was shown in Fig. 5. The sect. *Platycentrum* appeared monophyletic with a high Bayesian posterior probability (bpp = 0.97). The putative new species was a monophyletic lineage (bpp = 1) and sister with *B. dielsiana* and *B. emeiensis*. *Begonia edulis* with the highest morphologic resemblance of *B. pseudoedulis* formed another clade.

Discussion

Begonia pseudoedulis is assigned to *B.* sect. *Platycentrum* by its 2-loculed ovary, placentation axile, and placentae bifid per locule (Gu et al. 2007). In the same section, it is most similar to *B. edulis* and *B. dielsiana* and *B. emeiensis* in morphology (Fig. 4).

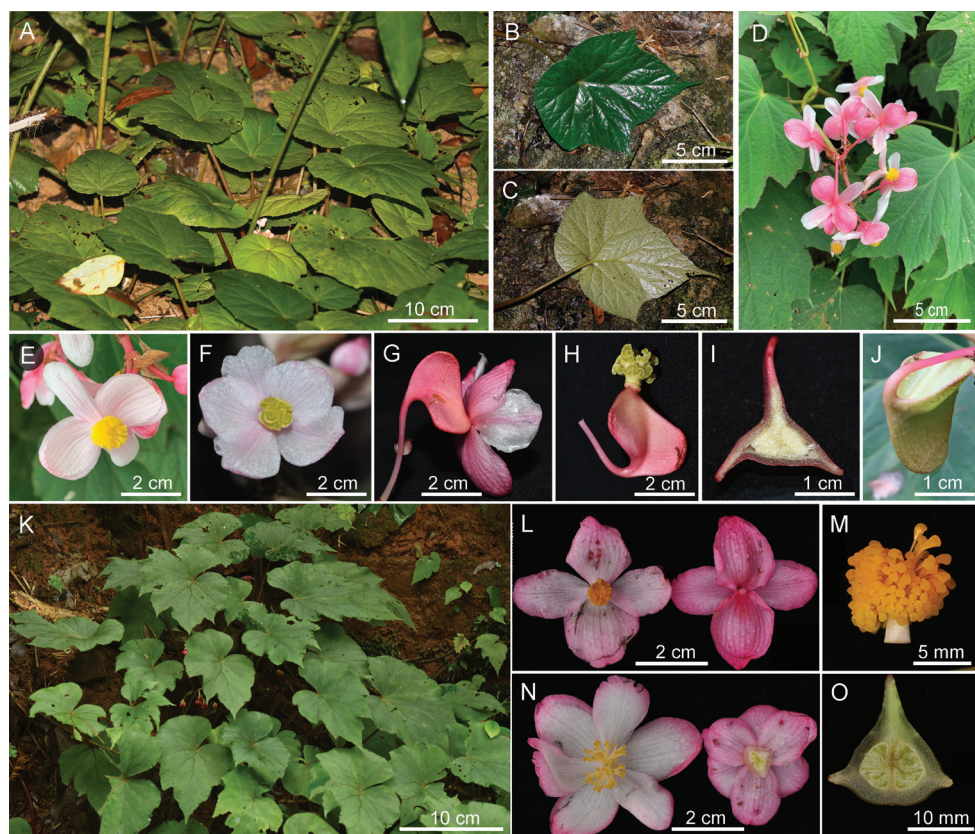


Figure 6. *B. edulis* (A–J) and *B. dielsiana* (K–O) for comparison to *B. pseudoedulis* A habitat B, C leaf blade (adaxial and abaxial) D inflorescence E staminate flower F, G pistillate flowers H ovary & stigma I cross-section of ovary J immature capsule K habitat L staminate flowers M androecium N pistillate flowers O cross-section of ovary.

The new species shows high resemblance to *B. edulis* in stout creeping rhizome, erect stem at anthesis, palmate veins, chartaceous leaf blade, dichasial cyme, unequally 3-winged capsule (Table 2, Fig. 6; Gu et al. 2007). However, it can be distinguished from *B. edulis* mainly by its densely red hirsute (gradually wide down to base) (vs. glabrous to subglabrous) petioles and red hispid (vs. glabrous or nearly so) on the abaxial surface of outer tepals of staminate flowers, ovary and fruits, and usually variegated and seldom pure green (vs. evenly green or dark green) leaf blades.

B. emeiensis shares almost the same morphologic characters and geographic distribution (Central China) with *B. dielsiana* and could be treated as a synonym or variety of *B. dielsiana* based on the unpublished data from Daike Tian's lab. They can be easily distinguished from the new species in having evenly green leaves with glabrous to subglabrous petioles, glabrous flower tepals, and usually six (less in five) tepals of the pistillate flowers (Table 2, Fig. 6).

Table 2. Morphological comparisons among *B. edulis*, *B. dielsiana* and *B. pseudoedulis*.

Character	<i>B. edulis</i>	<i>B. dielsiana</i>	<i>B. pseudoedulis</i>
Plant height	up to 1.5 m	up to 90 cm	30–50 cm
Petiole	green to red, glabrous to subglabrous	green, glabrous to subglabrous	usually reddish, seldom green, densely hairy at young stage
Leaf color	adaxially green or dark green, abaxially light green or purple red	both sides green	adaxially green but dark green along main veins, abaxially light green and purple red along main veins, seldom evenly green
Inflorescence	peduncles green or red, glabrous to subglabrous	peduncles green, glabrous to subglabrous	peduncles red or occasionally green, densely red hairy particularly at young stage
Staminate flower	pedicels and tepals glabrous	pedicels and tepals glabrous	pedicels and outer tepals red hispidulous
Pistillate flower	pedicels glabrous, tepals 5 rarely 6, glabrous, unequal, inner one smallest	pedicels glabrous, tepals 6 rarely 5, subequal, glabrous	pedicels hairy, tepals 5, subequal, outer three hairy
Ovary and capsule	glabrous	glabrous	Hairy
Flowering time	Jun.–Sep.	Jul.–Aug.	Aug.–Oct.
Distribution	100–1500 m, Hunan, Guangdong, Guangxi, Guizhou, Yunnan; Vietnam	950–1350 m, Chongqing, Hubei, Hunan, Sichuan	160–650 m, Guangxi only

Among these three species, *B. edulis* is most commonly seen in China usually with large populations, and has the widest distribution followed by *B. dielsiana*. The new species has the narrowest distribution range, which is only found in Guangxi. Although the three species share high similarity in morphology, the molecular evidence strongly supported that they are three different taxa.

Acknowledgements

This study was supported by the Dongguan Science and Technology of Social Development Program (2020507101165; 2016108101018; 2014108101046), the National Natural Science Foundation of China (31570199), the Guangdong Rural Revitalization Strategic Foundation (20211800400111), the Natural Science Foundation of Guangdong Province, and the Guangzhou Science and Technology Program (201903010076). Special thanks are given to Mr. Jun Liu from Zhejiang University and Mr. Chen-Yang Zhao from Daxin County of Guangxi, for providing photos, and to Ya-Hong Gao from Hangzhou Botanical Garden for providing living plants introduced from Wuming County of Guangxi. We are also grateful to the editor and the reviewers for reviewing our manuscript.

Reference

- Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: a practical information-theoretic approach. Springer, New York, 488 pp.
- Chung KF, Leong WC, Rubite RR, Repin R, Kiew R, Liu Y, Peng CI (2014) Phylogenetic analyses of *Begonia* sect. *Coelocentrum* and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. Botanical Studies 55(1): e1. <https://doi.org/10.1186/1999-3110-55-1>

- Dong LN, Liu Y (2018) Supplement to *Begonia* L. in Flora of Guangxi. *Guihaia* 39(1): 16–39.
- Gu CZ, Peng CI, Turland NJ (2007) Begoniaceae. In: Wu CY, Raven PH, Hong DY (Eds) *Flora of China* (Vol. 13). Science Press & Missouri Botanical Garden, Beijing & St. Louis, Missouri, 153–207.
- Hughes M, Moonlight P, Jara A, Pullan M (2015) *Begonia* Resource Centre. <http://padme.rbge.org.uk/begonia/> [updated: 16 April 2021]
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee of the IUCN Species Survival Commission. <http://cmsdocs.s3.amazonaws.com/RedListGuidelines.pdf> [accessed on 15 May 2020]
- Moonlight PW, Ardi WH, Padilla LA, Chung KF, Fuller D, Girmansyah D, Hollands R, Jara-Muñoz A, Kiew R, Leong W-C, Liu Y, Mahardika A, Marasinghe LDK, O'Connor M, Peng C-I, Pérez ÁJ, Phutthai T, Pullan M, Rajbhandary S, Reynel C, Rubite RR, Sang J, Scherberich D, Shui Y-M, Tebbitt MC, Thomas DC, Wilson HP, Zaini NH, Hughes M (2018) Dividing and conquering the fastest-growing genus: Towards a natural sectional classification of the mega-diverse genus *Begonia* (Begoniaceae). *Taxon* 67(2): 267–323. <https://doi.org/10.12705/672.3>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Shui YM, Chen WH (2018) *Begonia* of China. Yunnan Science & Technology Press, Kunming, 285 pp.
- Tian DK, Li C, Yan YH, Li XP, Meng J (2014) *Begonia intermedia*, a new species of Begoniaceae from Hainan, China. *Phytotaxa* 166(2): 114–122. <https://doi.org/10.11646/phytotaxa.166.2.2>
- Tian DK, Li C, Li CH, Li XJ (2015) *Begonia pulchrifolia* (sect. *Platycentrum*), a new species of Begoniaceae from Sichuan of China. *Phytotaxa* 207(3): 242–252. <https://doi.org/10.11646/phytotaxa.207.3.2>
- Tian DK, Shui YM, Hughes M, Kiew R, Tebbitt M, Moonlight PW (2018a) *Begonia* Special Issue 2018. *Phytotaxa* 381(1): 100–106. <https://doi.org/10.11646/phytotaxa.336.1.9>
- Tian DK, Xiao Y, Tong Y, Fu NF, Liu QQ, Li C (2018b) Diversity and conservation of Chinese wild begonias. *Plant Diversity* 40(3): 75–90. <https://doi.org/10.1016/j.pld.2018.06.002>
- Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44(W1): 232–235. <https://doi.org/10.1093/nar/gkw256>

Leaf epidermal micromorphology defining the clades in *Cinnamomum* (Lauraceae)

Zeng Gang^{2,3}, Bing Liu², Jens G. Rohwer⁴, David Kay Ferguson⁵, Yong Yang¹

1 College of Biology and Environment, Nanjing Forestry University, 159 Longpan Road, Nanjing 210037, Jiangsu, China **2** State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 10093, China **3** Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China **4** Universität Hamburg, Institute of Plant Science and Microbiology, Ohnhorststr. 18, 22609 Hamburg, Germany **5** University of Vienna, Department of Paleontology, 1090 Vienna, Austria

Corresponding author: Yong Yang (yangyong@njfu.edu.cn)

Academic editor: Elton John de Lirio | Received 13 April 2021 | Accepted 7 September 2021 | Published 4 October 2021

Citation: Gang Z, Liu B, Rohwer JG, Ferguson DK, Yang Y (2021) Leaf epidermal micromorphology defining the clades in *Cinnamomum* (Lauraceae). *PhytoKeys* 182: 125–148. <https://doi.org/10.3897/phytokeys.182.67289>

Abstract

In this study, we sampled 48 species of Asian *Cinnamomum* covering the species groups that were identified in recent phylogenetic studies and conducted leaf micromorphological observations using both light microscopy (LM) and scanning electron microscopy (SEM). Synapomorphies were determined by means of mapping micromorphological characters on a phylogenetic tree. The results indicate that *Cinnamomum* exhibits two different types of leaf upper epidermis: Type I has smooth/non-reticulate periclinal walls whereas Type II has reticulate periclinal walls and is unusual in the family Lauraceae. We found that the two types of micromorphological characters are clade-specific, sect. *Camphora* s.s. possesses Type I leaf upper epidermis, and sect. *Cinnamomum* s.l. has Type II leaf upper epidermis. Our study also reveals that *C. saxatile*, a member of sect. *Camphora* s.l. in the traditional classification, actually has Type II leaf upper epidermis, thus reinforcing the result of a recent molecular phylogeny that has this species in a clade consisting mainly of species of sect. *Cinnamomum*.

Keywords

Anatomy, *Cinnamomum*, Lauraceae, scanning electron microscope (SEM), systematics

Introduction

In the family Lauraceae, there are some named generic complexes according to molecular systematic studies, e.g. the *Beilschmiedia* group, the *Persea* group, the *Litsea* group, the *Alseodaphne* group, and the *Cinnamomum* group (e.g. Chanderbali et al. 2001; Rohwer et al. 2009, 2014; Huang et al. 2016; Trofimov et al. 2016, 2019; Mo et al. 2017; Rohde et al. 2017; Trofimov and Rohwer 2020; Xiao et al. 2020; Liu et al. 2021). A number of macromorphological characters have been used in the past to define the genera in each complex, but it now seems that these macromorphological characters were either plesiomorphic (e.g. in *Ocotea* Aubl. s.l.) or originated through parallelism, i.e. evolved several times. For instance, in the *Litsea* group, *Lindera* Thunb. differs from *Litsea* Lam. in the number of anther locules (2-locular in *Lindera* vs. 4-locular in *Litsea*). Phylogenetic studies based on DNA sequences suggest that *Lindera* is polyphyletic, and comprises many different clades (Li et al. 2004; Fijridiyanto and Murakami 2009). How these clades can be recognized using morphological characters has become an important question in the taxonomy of the group.

The *Cinnamomum* group is amphi-Pacific and distributed in tropical America and tropical to subtropical Asia with relatively few species found in Africa and Australia (Rohwer 1993; van der Werff 2001). The group belongs to the Laureae-Cinnamomeae clade of the core Lauraceae (Chanderbali et al. 2001; Rohwer and Rudolph 2005; Song et al. 2017, 2020), and consists of several closely related genera, i.e. *Cinnamomum* Schaeff., *Aiouea* Aubl. and the *Ocotea* complex (Chanderbali et al. 2001; Huang et al. 2016; Rohde et al. 2017). The group is thought to have originated ca. 55 mya and was once widely distributed in the palaeotropical Arcto-Tertiary flora of Laurasia during the Eocene, then migrated southwards and, with cooling temperatures, split, resulting in the modern amphi-Pacific disjunct distribution (Huang et al. 2016).

Cinnamomum is generally considered to consist of ca. 300 species, with the highest diversity in tropical Asia (Rohwer 1993; van der Werff 2001) and only a few species in Australia (Hyland 1989) and some Pacific Islands. Species of the genus are characterized by inflorescence Type II of van der Werff and Richter (1996), i.e. paniculate inflorescences with strictly opposite ultimate cymes, flowers with nine fertile stamens plus three staminodia with a conspicuous cordate to sagittate glandular head, and a more or less developed cupule with or without persistent (remnants of) tepals (van der Rohwer 1993; van der Werff 2001). Traditionally, the Asian species have been classified into two sections: *Camphora* Meisn. (1864: 24) and *Cinnamomum*. Species in sect. *Camphora* have alternate leaves, usually with domatia in the axils of lateral veins, pinnate to subtriplinerved venation, and often perulate buds. Species in sect. *Cinnamomum* have (sub)opposite and tripliveined leaves lacking domatia in the axils of lateral veins, and no perulate buds (Fig. 1, e.g. Li et al. 1982). Recent phylogenetic studies have consistently suggested that Asian *Cinnamomum* is not monophyletic and contains two robust clades (Huang et al. 2016; Trofimov and Rohwer 2020). *Cinnamomum* sect. *Camphora* s.s. (Clade 1) excluding three species of the traditional sect. *Camphora*, and sect. *Cinnamomum* s.l. (Clade 2) containing three species previously attributed to sect.

Camphora s.l. (*C. saxatile* H.W. Li (1975: 44), *C. longipetiolatum* (Gamble) N. Chao ex H.W. Li (1975: 47), and an unidentified species labelled *C. sp.* C684; Huang et al. 2016). The species of the Neotropical clade (Clade 3) recognized by Huang et al. (2016) have recently been transferred to *Aiouea* (Rohde et al. 2017), as the result of a study of nrITS sequences and two chloroplast spacers (*psbA-trnH* and *trnG-trnS*).

Various studies have suggested different topologies, e.g. sect. *Cinnamomum* s.l. as sister to the Neotropical clade in Huang et al. (2016), but sect. *Camphora* s.s. appears to be sister to the Neotropical clade in the ITS result of Rohde et al. (2017). According to Rohde et al. (2017), sect. *Camphora* s.s. and the Neotropical clade share alternate, penninerved to moderately triplinerved leaves. Huang et al. (2016) suggested that alternate and penninerved leaves, perulate buds and domatia are potential synapomorphies for sect. *Camphora* s.s., but these characters do not seem to be very reliable (Huang et al. 2016). Though Trofimov and Rohwer (2020) confirmed that Asian *Cinnamomum* is diphyletic, they gave different relationships of the two sections of the Asian *Cinnamomum*: sect. *Camphora* appears to be sister to *Sassafras* J. Presl, and together they constitute a clade which appears to be sister to the Neotropical *Ocotea* complex, whereas sect. *Cinnamomum* is sister to *Kuloa* Trofimov & Rohwer, an African genus recently described. Liu et al. (2021) found pervasive conflicts between plastid data and nrITS, and suggested that Asian *Cinnamomum* was paraphyletic with respect to *Sassafras*. They included only a single Neotropical sample from the Cinnamomeae, *Nectandra angustifolia* (Schrad.) Nees et Mart. (1833: 48). In any case, Asian *Cinnamomum* contains two robust clades. However, it remains unclear how the clades of *Cinnamomum* should be defined morphologically.

Leaf epidermal micromorphology has been considered to be of taxonomic importance within the Lauraceae (Christophel et al. 1996; Nishida and Christophel 1999; Nishida and van der Werff 2007, 2011, 2014; Yang et al. 2012; Zeng et al. 2014; Nishida et al. 2016; Trofimov and Rohwer 2018), but its systematic significance has rarely been discussed within a phylogenetic context. In this study, we report micromorphological observations in Asian *Cinnamomum*, and discuss their systematic significance.

Material and methods

Mature leaf materials were taken from herbarium specimens. Our sampling covered the two clades that were identified in recent molecular phylogenetic studies (Huang et al. 2016; Rohde et al. 2017), and contained 48 species of Asian *Cinnamomum*. Three *Sassafras* species were also sampled for comparison because the result based on *psbA-trnH* and *trnG-trnS* sequences in Rohde et al. (2017) suggested that *Sassafras* was closely related to at least some species of *Cinnamomum*.

Leaf samples were obtained from herbarium specimens deposited in the Herbarium of the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany (PE), the Chinese Academy of Sciences (Table 1). Earlier studies have suggested that leaf epidermal characters are stable within species (e.g. Nishida and Chris-

Table 1. Voucher information of leaf samples for this study.

Latin Name	Collection	Locality
<i>Cinnamomum appelianum</i> Schewe (1925: 20)	S.H. Chun 10175	China, Guangxi
<i>C. aromaticum</i> Nees (1831b: 74)	Anshun Exped. 049	China, Guizhou
<i>C. austrosinense</i> H.T. Chang (1959: 20)	S.Y. Chang 3133	China, prov. unknown
<i>C. bejolghota</i> (Buch.-Ham.) Sweet (1826: 344)	S.Z. Cheng & B.S. Li 03985	China, Xizang
<i>C. bodinieri</i> H. Lév. (Léveillé 1912: 369)	P.C. Tsoong 34	China, Guizhou
<i>C. burmannii</i> (Nees & T. Nees) Blume (1826: 569)	G.Z. Li 15650	China, Guangxi
<i>C. camphora</i> (L.) J. Presl (1825: 47)	S.Y. Chang 4819	China, Zhengjiang
<i>C. camphora</i>	L.D. Duan 2601	China, Hunan
<i>C. camphora</i>	C.F. Liang 33262	China, Guangxi
<i>C. chartophyllum</i> H.W. Li (1975: 49)	B. Liu 1366	China, Yunnan
<i>C. chekiangense</i> Nakai (1939: 23)	H. Zou 01435	China, Anhui
<i>C. daphnoides</i> Siebold & Zucc. (Siebold and Zuccarini 1846: 402)	T. Yahara 6641	Japan, Kyushu
<i>C. doederleinii</i> Engl. (Engler 1884: 57)	M. Furuse 43512	Japan, Kyushu
<i>C. glanduliferum</i> (Wall.) Meisn. (Meisner 1864: 25)	Y.M. Shui 2217	China, Yunnan
<i>C. ilicioides</i> A. Chev. (Chevalier 1918: 855)	F.C. How 72957	China, Hainan
<i>C. iners</i> (Reinw. ex Nees et T. Nees) Blume (1826: 570)	Y. Tsiang 12772	China, Yunnan
<i>C. insularimontanum</i> Hayata (1913: 158)	T.Y.A. Yang et al. 08378	China, Taiwan
<i>C. japonicum</i> Siebold (1830: 23)	Zhejiang Bot. Exped. 27696	China, Zhejiang
<i>C. jensenianum</i> Hand.-Mazz. (Handel-Mazzetti 1921: 63)	T.T. Yü 3125	China, Sichuan
<i>C. liangii</i> C.K. Allen (1939: 58)	S.K. Lau 26252	China, Hainan
<i>C. litseifolium</i> Thwaites (1861: 253)	M. Poilane 14784	Cambodia
<i>C. longipaniculatum</i> (Gamble) N. Chao ex H.W. Li (1975: 48)	Z.W. Yao 3567	China, Sichuan
<i>C. macrostemon</i> Hayata (1913: 160)	Y.H. Lai 27	China, Taiwan
<i>C. mairei</i> H. Lév. (Léveillé 1914: 174)	Z.W. Yao 4980	China, Yunnan
<i>C. micranthum</i> (Hayata) Hayata (1913: 160)	M. Poilane 10707	Cambodia
<i>C. migao</i> H.W. Li (1978: 90)	Beijing Team 891144	China, Guangxi
<i>C. osmophloeum</i> Kaneh. (Kanehira 1917: 428)	C.M. Wang 05395	China, Taiwan
<i>C. ovalifolium</i> Gardner ex Meisn. (Meisner 1864: 11)	T. Koyama 13513	Sri Lanka
<i>C. parthenoxylon</i> (Jack) Meisn. (Meisner 1864: 26)	Sichuan Bot. Exped. 2355	China, Sichuan
<i>C. parthenoxylon</i>	IBCAS Team. 814	China, Jiangxi
<i>C. pauciflorum</i> Nees (1831b: 75)	Mt. Ziyun Exped. 411	China, Hunan
<i>C. pedunculatum</i> (Thunb.) J. Presl (1825: 37)	Jiangxi Exped. 947	China, Jiangxi
<i>C. pingbienense</i> H.W. Li (1978: 91)	B. Liu 1363	China, Yunnan
<i>C. pittosporoides</i> Hand.-Mazz. (Handel-Mazzetti 1925: 19)	Yunnan Exped. of CAS 311	China, Yunnan
<i>C. pseudopedunculatum</i> Hayata (1913: 161)	M. Furuse 52925	Japan, Kyushu
<i>C. randaiense</i> Hayata (1911: 238)	T.Y. Liu et al. 201	China, Taiwan
<i>C. reticulatum</i> Hayata (1911: 239)	G.F. Zhong et al. 1374	China, Taiwan
<i>C. rigidissimum</i> H.T. Chang (1959: 19)	C.F. Wei 122561	China, Hainan
<i>C. saxatile</i> H.W. Li (1975: 44)	B. Liu 1327	China, Yunnan
<i>C. scortechinii</i> Gamble (1910: 219)	M. Poilane 11143	Unknown locality
<i>C. septentrionale</i> Hand.-Mazz. (Handel-Mazzetti 1936: 213)	H. Yu 177	China, Sichuan
<i>C. subavenium</i> Miq. (Miquel 1858: 902)	W.C. Cheng 3649	China, Zhejiang
<i>C. subavenium</i>	M.J. Wang 3768	China, Anhui
<i>C. tamala</i> (Buch.-Ham.) T. Nees & Eberm. (Nees 1831a: 2)	Qinghai-Xizang Veg. Exped. 4584	China, Xizang
<i>C. tenuifolium</i> (Makino) Sugim. (Sugimoto 1928: 57)	M. Furuse 8173	Japan
<i>C. tetragonum</i> A. Chev. (Chevalier 1918: 855)	M. Poilane 378	Unknown locality
<i>C. tonkinense</i> (Lecomte) A. Chev. (Chevalier 1918: 856)	Liu Bing 1326	China, Yunnan
<i>C. tsangii</i> Merr. (Merrill 1934: 26)	Jiangxi Exped. 124	China, Jiangxi
<i>C. validinerve</i> Hance (1882: 80)	X.G. Li 200631	China, Guangdong
<i>C. verum</i> J. Presl (1825: 37)	N. Wallich 2573B	Unknown locality
<i>C. wilsonii</i> Gamble (1914: 66)	Z.C. Luo 191	China, Hunan
<i>C. zollingeri</i> Lukman. (Lukmanoff 1889: 4)	S. Saito 1388	Japan, Nagato
<i>Sassafras albidum</i> (Nutt.) Nees (1836: 490)	S.C. Chen et al. 715	China, Taiwan
<i>S. randaiense</i> (Hayata) Rehder (1920: 244)	W.M. Wang 93	USA, Georgia
<i>S. tzumu</i> (Hemsl.) Hemsl. (Hemslay 1907: 55)	B. Liu 1372	China, Yunnan

*All specimens examined in this study are deposited in PE.

tophel 1999; Nishida and van der Werff 2007, 2011; Yang et al. 2012). Therefore, in most cases, one specimen was sampled to represent the species in this study. For the purpose of comparison and to eliminate variation that might be caused by sampling from different leaf areas, we took samples close to the basal portion on the left hand side of the midvein of a leaf. The leaf materials were then cut into small rectangular pieces (ca. 3 mm×10 mm).

For light microscopic observation, the samples were dipped in 40% NaClO at 60 °C until the samples began to bleach. The samples were then washed in distilled water. The epidermis of both surfaces of the leaves was peeled off under a light microscope (Zeiss Stemi 2000), then stained in 1% safranin-50% ethanol for 30 minutes, dehydrated with gradations of ethanol, and treated with gradations from ethanol to xylene, and finally the epidermis pieces were mounted in Canada balsam (Yang et al. 2012; Zeng et al. 2014). The preparations were dried at 40 °C in an incubator. Photographs were taken using a Zeiss Axio Imager A1 light microscope with a 10× eyepiece and 40× objective.

For SEM observations, leaf samples were cut into small pieces of ca. 3 × 3 mm. Leaf samples were soaked in 100% ethanol for 15 minutes, followed by ultrasonic cleaning for 10 minutes at 100 hz, after which the ethanol was replaced by isoamyl acetate, and critical-point dried using carbon dioxide for five hours (equipment: HCP-2; Yang et al. 2012; Zeng et al. 2014). The treated leaf pieces were then fixed on stubs with the inner surface of leaf epidermis exposed, coated with palladium under 15 mA for 110 s, observed and photographed under a HITACHI s-4800 scanning electron microscope (10.0KV; State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences). To clarify the ornamentation of the upper leaf epidermis, the peeled upper leaf epidermis was also observed.

Observed features of leaf epidermis included the following: (1) epidermal cell shape, (2) anticlinal walls of normal epidermal cells, i.e. non-stomatal cells, (3) periclinal walls of normal epidermal cells, and (4) the stomatal complex (including subsidiary cells). Line-drawings were made with Adobe Photoshop CS2 ver. 9.0 using the Pen Tool.

Because our aim was to understand the evolution of micromorphological characters in the context of phylogeny, we selected published sequences according to our sampling for the micromorphological studies to reconstruct a phylogeny. *Phoebe zhennan*, *P. hungmoensis*, *Alseodaphnopsis rugosa*, and *A. hainanensis* were selected as the outgroup for phylogenetic analysis of *Cinnamomum*. Micromorphology of these outgroup species was observed but has not been published yet (Zeng 2018). All sequences were downloaded from NCBI (Table 2). We used three nuclear genes to reconstruct the phylogeny of the Asian *Cinnamomum*, i.e. nrITS, *LEAFY* intron II, and *RPB2*. Sequences were aligned with MAFFT v7.304b (Katoh and Standley 2013). MrModeltest 2.3 (Nylander 2008) and Paup* v.4.0b10 (Swofford 2003) were used to select the best-fit evolutionary model using Akaike Information Criterion (AIC). Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 on XSEDE (Huelsenbeck and Ronquist 2001). The Markov Chain Monte Carlo (MCMC) algorithm was run for 3,000,000 generations, sampling one out of every 1000 generations. The first 25% trees were discarded as burn-in. The remaining trees were used to calculate the posterior probabilities (PP)

Table 2. Sequences obtained from the GenBank for phylogeny of Asian *Cinnamomum*.

Taxon	ITS	RPB2	LEAFY
<i>Alseodaphnopsis hainanensis</i> (Merr.) H.W. Li & J. Li (2016: e0186545 (9))	FJ755440	KU140409	HQ697006
<i>A. rugosa</i> (Merr. & Chun) H.W. Li & J. Li (2016: e0186545 (9))	HQ697183	KU140410	HQ697012
<i>Cinnamomum appelianum</i>	KU139817	KU140330	KU140244
<i>C. austrosinense</i>	KU139818	KU140331	KU140245
<i>C. bejolghota</i>	KU139822	KU140335	KU140249
<i>C. bodinieri</i>	KU139824	KU140336	KU140251
<i>C. burmannii</i>	KU139825	KU140337	KU140252
<i>C. camphora</i>	KU139826	KU140338	KU140253
<i>C. chartophyllum</i>	KU139832	KU140344	KU140259
<i>C. chavarrianum</i> (Hammel) Kosterm. (Kostermans 1988: 442)	AF272261	KU140345	–
<i>C. chekiangense</i>	MF110041	KU140346	KU140260
<i>C. daphnoides</i>	FM957803	KU140352	KU140266
<i>C. doederleinii</i>	KU139842	–	KU174408
<i>C. glanduliferum</i>	KU139843	KU140354	KU140269
<i>C. iners</i>	KU139849	KU140360	KU140275
<i>C. insularimontanum</i>	KY271510	KU140361	KU174418
<i>C. japonicum</i>	KU139851	KU140361	KU140277
<i>C. jensenianum</i>	KU139853	KU140363	KU140279
<i>C. liangii</i>	KU139856	KU140366	KU174422
<i>C. longipaniculatum</i>	KX546754	KT248715	KU140283
<i>C. macrostemon</i>	GU598521	–	–
<i>C. mairei</i>	KU139859	KU140368	KU174423
<i>C. micranthum</i>	KY271519	KU140369	GQ260581
<i>C. osmophloeum</i>	KY271528	KU140375	–
<i>C. parthenoxylon</i>	KU139871	KU140377	KU140295
<i>C. pauciflorum</i>	KU139872	KU140378	KU140296
<i>C. pingbienense</i>	KU139873	KU140379	KU140297
<i>C. pittosporoides</i>	KU139874	KU140380	KU140298
<i>C. reticulatum</i>	KU139879	–	KU174432
<i>C. rigidissimum</i>	KU139881	KU140386	KU140305
<i>C. saxatile</i>	KU139882	KU140387	KU140306
<i>C. septentrionale</i>	KU139883	KU140388	KU140307
<i>C. subavenium</i>	KU139888	KU140393	KU140312
<i>C. tamala</i>	KX822090	KU140396	KU174439
<i>C. tenuifolium</i>	KU139892	KU140397	KU140316
<i>C. tenuifolium</i>	–	–	KU140316
<i>C. tonkinense</i>	KU139895	KU140400	KU140319
<i>C. tsangii</i>	KU139900	KU140405	KU140324
<i>C. verum</i>	MF110061	KU140407	KU140326
<i>C. wilsonii</i>	KU139904	KU140408	KU140328
<i>Phoebe hungmoensis</i> S.K. Lee (1963: 190)	HQ697206	KU140413	HQ697138
<i>P. zhennan</i> S.K. Lee & F.N. Wei (1979: 61)	HQ697212	KT248761	HQ697161

*Note 1: these species were not included in phylogenetic studies, but belong to Clade I according to their non-reticulate periclinal walls.
*Note 2: these species lack phylogenetic information, should belong to the clade II according to their reticulate periclinal walls.
#*C. saxatile* is traditionally ascribed to sect. *Camphora*.
Invisible refers to those species possessing thick trichomes or appendages covering the stomata.

and construct the consensus tree. Maximum Likelihood (ML) analyses were performed using RAxML-HPC2 on XSEDE with the GTRCAT model to search the best-scoring ML tree and generate a tree block at the same time. 1000 bootstrap replicates were performed in each analysis to obtain the confidence support. The ML tree block was

read in FigTree v1.4.0 and saved as a nexus file which was then opened in Mesquite v3.04. Micromorphological characters were collected based on leaf anatomy in this study, and were manually input into the “Character Matrix” in Mesquite. All characters were treated as unordered and equally weighted. To reconstruct character evolution, a maximum likelihood approach using Markov k-state 1 parameter model (Mk1; Lewis 2001) was used. We selected the “Trace-Character-Over-Trees” command to calculate ancestral states at each node including probabilities in the context of likelihood reconstructions. To carry out these analyses, characters were plotted onto trees that were sampled in ML analyses. The results were finally summarized as percentage of changes of character states on a given branch among the stored trees utilizing the option of “Average-frequencies-across-trees”. Trees with reconstructed ancestral character states were then exported as pdf files which were then manually adjusted in Adobe Illustrator CS6.

Results

Leaf epidermal micromorphology is presented in Figs 2, 3. Illustrations in Fig. 4 display main characters and their variation. The main results are presented in Table 3.

Micromorphology

The upper leaf epidermal micromorphology of Asian *Cinnamomum* species falls clearly into two types according to the reticulation of the periclinal walls of leaf upper epidermis, cell shape and straightness of anticlinal walls. Type I is characterized by polygonal epidermal cells, the anticlinal walls being straight or nearly so, the periclinal walls smooth and not reticulate (Figs 2, 4a). This type of epidermal cell is rather homogeneous. Variation occurs in the thickness of the anticlinal walls, e.g. not thickened, somewhat beaded (Fig. 2A), prominently thickened (Fig. 2B), or more or less thickened (Fig. 2C). Thickening of the anticlinal walls was based on visual perception and no measurements were made. Type II possesses epidermal cells with irregular outlines, with the anticlinal walls undulate to sinuous, and the periclinal walls reticulate (Figs 3, 4b, d). The cell shape and anticlinal walls are variable in straightness. In most species, the epidermal cells have an irregular shape, the anticlinal walls being either sinuous (Fig. 4b) or extremely sinuous, almost stellate in appearance (Fig. 4c). In a few species, the epidermal cells are polygonal and the anticlinal walls are straight or curved, e.g. *C. daphnoides*, *C. doederleinii*, *C. pittosporoides*, *C. reticulatum*, *C. scortechinii*, *C. validinerve*, and *C. ovalifolium*. The reticulation of the periclinal walls of the Type II results from the uneven thickening of the periclinal walls (Fig. 5A–D). In Type I, the periclinal walls are evenly thickened (Fig. 5E, F). The leaf epidermal characters such as the anticlinal wall straightness, cell shape and periclinal wall ornamentation are stable within species and not influenced by external stimuli.

The leaf micromorphology of *Sassafras* is presented in Fig. 6. The upper leaf surface is very similar to Type I of *Cinnamomum*, the epidermal cells being rectangular or po-

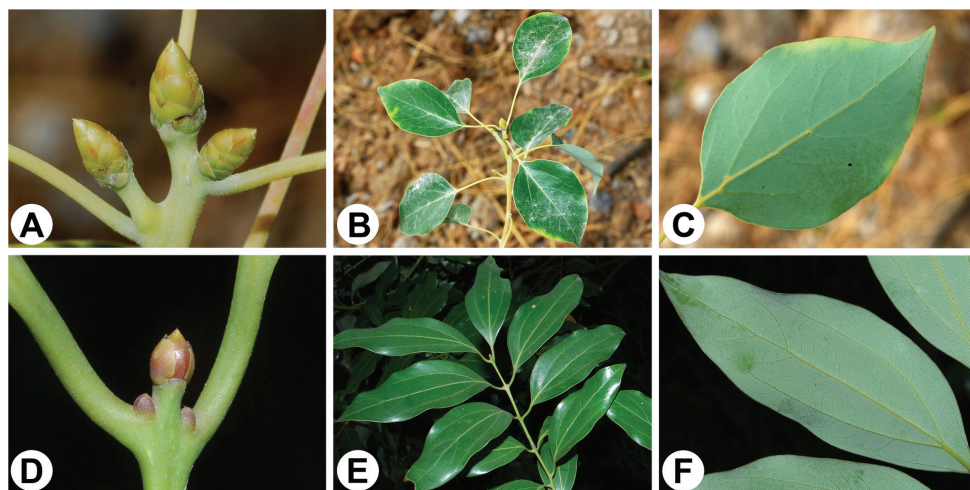


Figure 1. Morphology of the two sections of the Asian *Cinnamomum* **A–C** *Cinnamomum camphora* of sect. *Camphora* **A** perulate terminal buds **B** branch portion displaying the alternate leaf arrangement **C** a leaf showing the pinnate venation and the domatia in axils of lateral veins **D, E** *Cinnamomum japonicum* of sect. *Cinnamomum* **D** terminal buds lacking helically arranged scales **E** branch portion exhibiting the subopposite leaf arrangement **F** a leaf displaying the tripliveined venation and the absence of domatia in axils of lateral veins.

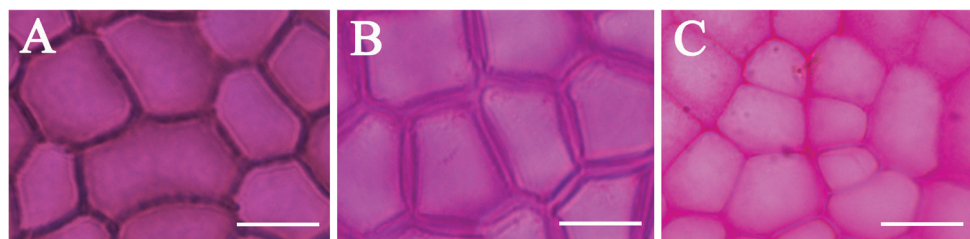


Figure 2. Leaf epidermal micromorphology of the Asian *Cinnamomum*, displaying the Type I upper leaf epidermis with polygonal cell shape, periclinal walls lacking reticulate ornamentations, and variable thickness of anticlinal walls **A** *C. parthenoxylon* **B** *C. inunctum* **C** *C. bodinieri*. Bars: 20 µm.

lygonal, the anticlinal walls straight or nearly so, and the periclinal walls not reticulate (Fig. 6A–C). The stomata are elliptic in outline, with reniform subsidiary cells, or lip-shaped with narrower subsidiary cells, the subsidiary cells usually raised. The periclinal walls of epidermal cells are slightly wrinkled and immersed in *S. albidum*, but raised in *S. tzumu* and *S. randaiense*.

Lower leaf epidermis comprises epidermal cells and stomata. Epidermal cells are polygonal (e.g. *C. camphora*, *C. daphnoides*, and *C. glanduliferum*), round (e.g. *C. bodinieri*, *C. glanduliferum*, *C. migao*, and *C. porrectum*) or irregular/amoeboid in shape (e.g. *C. burmannii*, *C. randaiense*, *C. saxatile* and *C. subavenium*). Anticlinal walls are straight and angular (e.g. *C. camphora* and *C. glanduliferum*), or round (e.g.

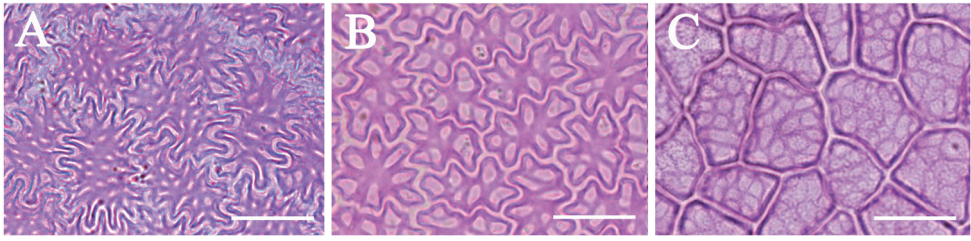


Figure 3. Leaf epidermal micromorphology of *Cinnamomum*, displaying the Type II upper leaf epidermis with irregular or polygonal cell shape, reticulate periclinal walls, and straight, sinuous to extremely sinuous anticlinal walls **A** *C. iners* **B** *C. appelianum* **C** *C. pittosporoides*. Scale bars: 20 μm .

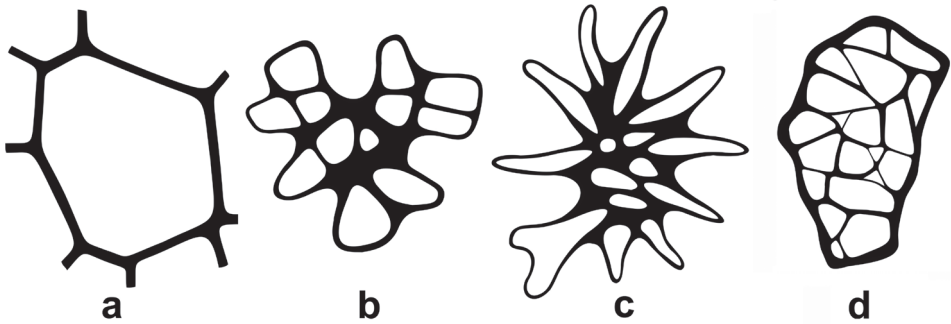


Figure 4. Line-drawing displaying variation of the leaf upper epidermis **a** type I, displaying the non-reticulate periclinal wall, the polygonal cells, and the round to polygonal cell shape **b** type II, displaying the sinuous anticlinal wall, the irregular cell shape, and the reticulate periclinal wall **c** type II, displaying an extremely sinuous anticlinal wall, the irregular cell shape, and the reticulate periclinal wall **d** type II, displaying the straight or nearly so anticlinal wall, the polygonal cell shape, and the reticulate periclinal wall.

C. bodinieri, *C. glanduliferum* and *C. porrectum*), or sinuous (e.g. *C. iners* and *C. saxatile*), thickened (e.g. *C. daphnoides*, *C. randaiense*, and *C. subavenium*) or not (e.g. *C. camphora*, *C. longepaniculatum*, *C. migao*, and *C. porrectum*). Periclinal walls of epidermal cells are either smooth (e.g. *C. camphora*, *C. glanduliferum*, *C. longepaniculatum*, *C. migao*, and *C. porrectum*) or reticulate (e.g. *C. burmannii*, *C. iners*, and *C. randaiense*). The lower stomatal ledges of *Cinnamomum* under LM include different types, e.g. wide lip-shaped (e.g. *C. randaiense*, Fig. 7E), narrow lip-shaped (e.g. *C. migao*, Fig. 7C), bat-shaped (e.g. *C. longepaniculatum*, Fig. 7D), butterfly-shaped (e.g. *C. burmannii* and *C. daphnoides*, Fig. 7A, B, Table 3). The wide lip-shaped and narrow li-shaped sometimes concur in a certain species (e.g. *C. verum*, Fig. 7F). Stomatal surfaces under SEM possess at least five different types, i.e. circular (e.g. *C. chartophyllum*, Fig. 8A), eyelid-shaped (e.g. *C. tonkinense* and *C. jensenianum*, Fig. 8G and 8H), globose (e.g. *C. septentrionale* and *C. camphora*, Fig. 8E and 8F), lip-shaped (e.g. *C. longepaniculatum* and *C. migao*, Fig. 8C and 8D), and invisible when the stomata are densely covered with wax/appendages (Table 3).

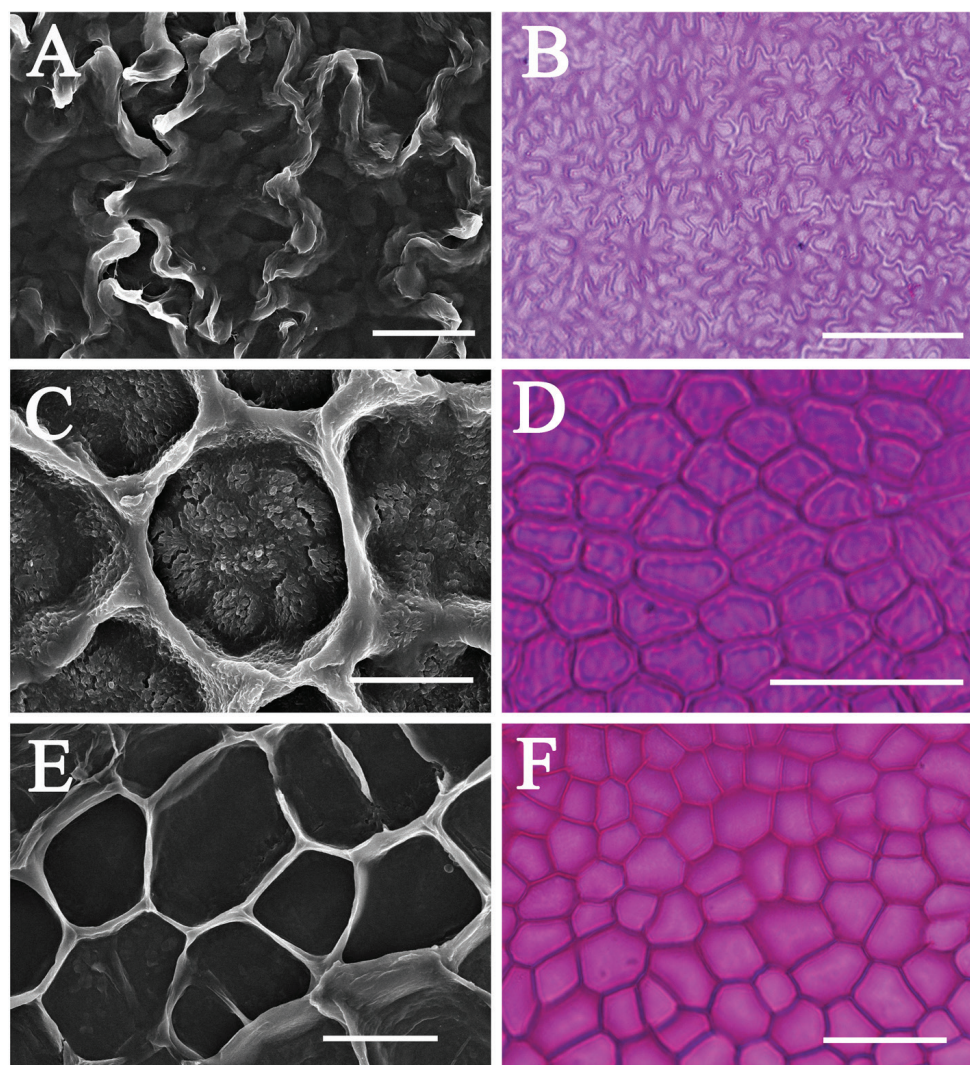


Figure 5. A comparison between the upper leaf epidermis under scanning electron microscope and light microscope, SEM images of internal surface of the upper leaf epidermis displaying the possible origin of the reticulations of the periclinal walls **A, B** *C. aromaticum* **C, D** *C. daphnoides* **E, F** *C. ilicioides*. Scale bars: 10 μm ; (**A, C**); 20 μm ; (**E**); 50 μm (**B, D, F**).

Phylogeny and character evolution

Asian *Cinnamomum* diverged into two robust clades (BS: 100; PP: 1.00, Fig. 9), one containing the species of sect. *Camphora* s.s. except *C. saxatile*, the other including the species of sect. *Cinnamomum* plus *C. saxatile*, which was previously ascribed to sect. *Camphora*. This latter clade is considered as sect. *Cinnamomum* s.l. here. However, relationships within the two clades were not completely resolved. A number of nodes

Table 3. Micromorphology of the leaf epidermis of Asian *Cinnamomum* under light microscopy (LM) and scanning electron microscope (SEM).

Clade	Latin name	Cell shape	Periclinal wall	Anticlinal wall	Lower stomatal ledge (LM)	Stomatal surface (SEM)	Section
Clade 1	<i>C. bodinieri</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped	eyelid-shaped	Sect. <i>Camphora</i>
	<i>C. camphora</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped or bat-shaped	globose	
	<i>C. chartophyllum</i>	polygonal	non-reticulate	straight/rounded	narrow lip-shaped	circular	
	<i>C. glanduliferum</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped or bat-shaped	globose	
	<i>C. longepaniculatum</i>	polygonal	non-reticulate	straight/rounded	bat-shaped	lip-shaped	
	<i>C. micranthum</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped	circular	
	<i>C. parthenoxylon</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped	globose	
	<i>C. septentrionale</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped	globose	
	<i>C. ilicioides</i>	polygonal	non-reticulate	straight/rounded	wide lip-shaped	eyelid-shaped	
	<i>C. migao</i>	polygonal	non-reticulate	straight/rounded	narrow lip-shaped	lip-shaped	
	<i>C. appelianum</i>	irregular	reticulate	Sinuuous	wide lip-shaped or bat-shaped	eyelid-shaped	
	<i>C. austrosinense</i>	irregular	reticulate	Sinuuous	wide lip-shaped or bat-shaped	eyelid-shaped	
	<i>C. bejolghota</i>	irregular	reticulate	Sinuuous	-	globose	
	<i>C. burmannii</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	
	<i>C. cassia</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	
Clade 2	<i>C. chekiangense</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	Sect. <i>Cinnamomum</i>
	<i>C. iners</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. insularimontanum</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. japonicum</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. jensenianum</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	
	<i>C. mairei</i>	irregular	reticulate	Sinuuous	wide lip-shaped	invisible	
	<i>C. osmophloem</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. pauciflorum</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	
	<i>C. pedunculatum</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. pingbienense</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. randaiense</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. rigidissimum</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	#C. saxatile	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. subavenium</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. tamala</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	lip-shaped	
	<i>C. tenuifolium</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. tonkinense</i>	irregular	reticulate	Sinuuous	wide lip-shaped or bat-shaped	eyelid-shaped	
	<i>C. tsangii</i>	irregular	reticulate	Sinuuous	wide lip-shaped	invisible	
	<i>C. verum</i>	irregular	reticulate	Sinuuous	wide lip-shaped or narrow lip-shaped	eyelid-shaped	
	<i>C. wilsonii</i>	irregular	reticulate	Sinuuous	wide lip-shaped or butterfly-shaped	eyelid-shaped	
	<i>C. zollingeri</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. daphnoides</i>	polygonal	reticulate	straight/rounded	butterfly-shaped	invisible	
	<i>C. doederleinii</i>	polygonal	reticulate	straight/rounded	wide lip-shaped	eyelid-shaped	
	<i>C. pittosporoides</i>	polygonal	reticulate	straight/rounded	wide lip-shaped	globose	
	<i>C. reticulatum</i>	polygonal	reticulate	straight/rounded	bat-shaped	eyelid-shaped	
	<i>C. scortechinii</i>	polygonal	reticulate	straight/rounded	butterfly-shaped	globose	

Clade	Latin name	Cell shape	Periclinal wall	Anticlinal wall	Lower stomatal ledge (LM)	Stomatal surface (SEM)	Section
*Note 2	<i>C. liangii</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	Sect. <i>Cinnamomum</i>
	<i>C. litseifolium</i>	irregular	reticulate	Sinuuous	narrow lip-shaped	eyelid-shaped	
	<i>C. macrostemon</i>	irregular	reticulate	Sinuuous	butterfly-shaped	eyelid-shaped	
	<i>C. pseudopedunculatum</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. tetragonum</i>	irregular	reticulate	Sinuuous	wide lip-shaped	eyelid-shaped	
	<i>C. validinerve</i>	polygonal/irregular	reticulate	undulate/rounded	wide lip-shaped	eyelid-shaped	
	<i>C. ovalifolium</i>	polygonal/irregular	reticulate	undulate/rounded	wide lip-shaped	eyelid-shaped	

*Note 1: these species were not included in phylogenetic studies, but belong to Clade I according to their non-reticulate periclinal walls. *Note 2: these species lack phylogenetic information, should belong to the clade II according to their reticulate periclinal walls. #*C. saxatile* is traditionally ascribed to sect. *Camphora*. Invisible refers to those species possessing thick trichomes or appendages covering the stomata.

were only poorly supported, bootstrap values were less than 50 and posterior probabilities were less than 0.70.

When simply mapping on the phylogenetic tree, the two character states of the periclinal wall reticulation allowed clear separation into two clades: non-reticulate for sect. *Camphora* s.s. and reticulate for sect. *Cinnamomum* s.l.; there is no overlap. However, neither epidermal cell shape nor anticlinal wall straightness are clear-cut. Sect. *Cinnamomum* s.l. usually possess sinuuous anticlinal walls and irregular cell shapes, but a few species with straight or curved anticlinal walls and polygonal cell shapes were found to belong to this clade, e.g. *C. reticulatum*, *C. doederleinii*, *C. daphnoides*, and *C. pittosporoides*. Straight or curved anticlinal walls and polygonal cell shapes were common in sect. *Camphora* s.s., and we found no exception. For evolutionary history of periclinal wall reticulation (Fig. 10), the ancestral node A of sect. *Cinnamomum* s.l. was reticulate with high probability (95.18%), and the ancestral node B of sect. *Camphora* s.s. was non-reticulate with very high likelihood (99.99%). Anticlinal wall straightness and epidermal cell shape resulted in the same reconstruction (Fig. 11), the ancestral node of sect. *Camphora* s.s. possessed straight or curved anticlinal walls (node B), and polygonal cell shapes (99.17%), while it was uncertain whether the ancestral node of sect. *Cinnamomum* s.l. had sinuuous anticlinal walls and irregular cell shapes or not, the probability being only 56.54% (node A).

Discussion

In this study, leaf epidermal micromorphology of 48 species representing the two macromorphological sections of Asian *Cinnamomum* was studied. Our sampled species largely overlapped with the species sampling of the two recent molecular phylogenetic studies (Huang et al. 2016; Rohde et al. 2017), permitting an assessment of the systematic significance of leaf epidermal micromorphology within a phylogenetic context.

The polygonal to irregular epidermal cell shape and the straight to sinuuous anticlinal walls have been described in previous reports (e.g. Christophel et al. 1996; Nishida

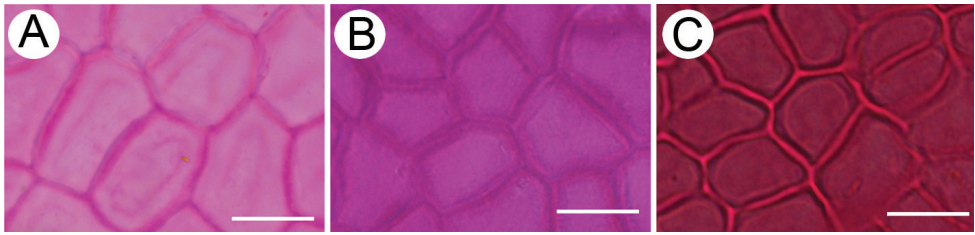


Figure 6. Leaf epidermal micromorphology of *Sassafras* displaying the non-reticulate periclinial wall in the genus **A** *S. albidum* **B** *S. randaiense* **C** *S. tzumu*. Scale bars: 20 μ m (**A–C**).

and Christophel 1999; Nishida and van der Werff 2007, 2011; Yang et al. 2012; Trofimov and Rohwer 2018), but our study suggests that sect. *Cinnamomum* s.l. possesses an unusual reticulate periclinial wall which has not been studied carefully before in Lauraceae. We studied the reticulate periclinial wall under SEM, and hypothesize that uneven thickening of the periclinial wall gives rise to the reticulation under LM (Fig. 5). The reticulations of the periclinial wall are usually coarse in sect. *Cinnamomum* s.l., the spaces dividing the periclinial wall into reticulations are narrow. The reticulations in sect. *Cinnamomum* s.l. are rarely fine and appear to be ‘punctate’, e.g. in *C. iners* and *C. japonicum*, which is similar to that of a few species of *Beilschmiedia* Nees (Nishida and van der Werff 2007), where the spaces are wide. We prefer to describe the unusual periclinial wall in sect. *Cinnamomum* as reticulate but not punctate because they appear to be coarse but not dot-like.

Asian *Cinnamomum* species are classified into two sections according to the persistence of tepals, presence of perulate buds, leaf arrangement either alternate or subopposite, and leaf venation, i.e. sect. *Camphora* s.l. and sect. *Cinnamomum* s.s. (syn.: sect. *Malabathrum* Meisn. (1864: 10)). This classification was proposed by Meisner (1864) and followed by subsequent authors (e.g. Li et al. 1982). A recent phylogeny based on three nuclear sequences (ITS, *RPB2* and *LEAFY*) suggests that a few taxa placed in sect. *Camphora* based on macromorphological characters actually belong to the clade consisting mainly of sect. *Cinnamomum* s.l., namely *C. saxatile*, *C. longipetiolatum* and an unidentified sample (Huang et al. 2016). Sect. *Camphora* s.s. is characterized by alternate, pinnately veined or weakly tripliveined leaves, mostly perulate buds and presence of domatia in the axils of lateral veins. However, these features occur also in sect. *Cinnamomum* s.l. when the clade includes *C. saxatile* and *C. longipetiolatum*. As a result, the current definition of both sect. *Cinnamomum* s.l. and *Camphora* s.s. using presence or absence of these morphological characters is problematic.

Our study suggests that the leaf epidermal micromorphology can be divided into two different types and the two types of leaf epidermal micromorphology are surprisingly congruent with the clades retrieved in the analysis of Huang et al. (2016): the taxa of sect. *Camphora* s.s. possess the smooth upper epidermis, while those of sect. *Cinnamomum* have the reticulate upper epidermis. The reticulate type of periclinial walls is derived, because this type has not been found in any other groups of the fam-

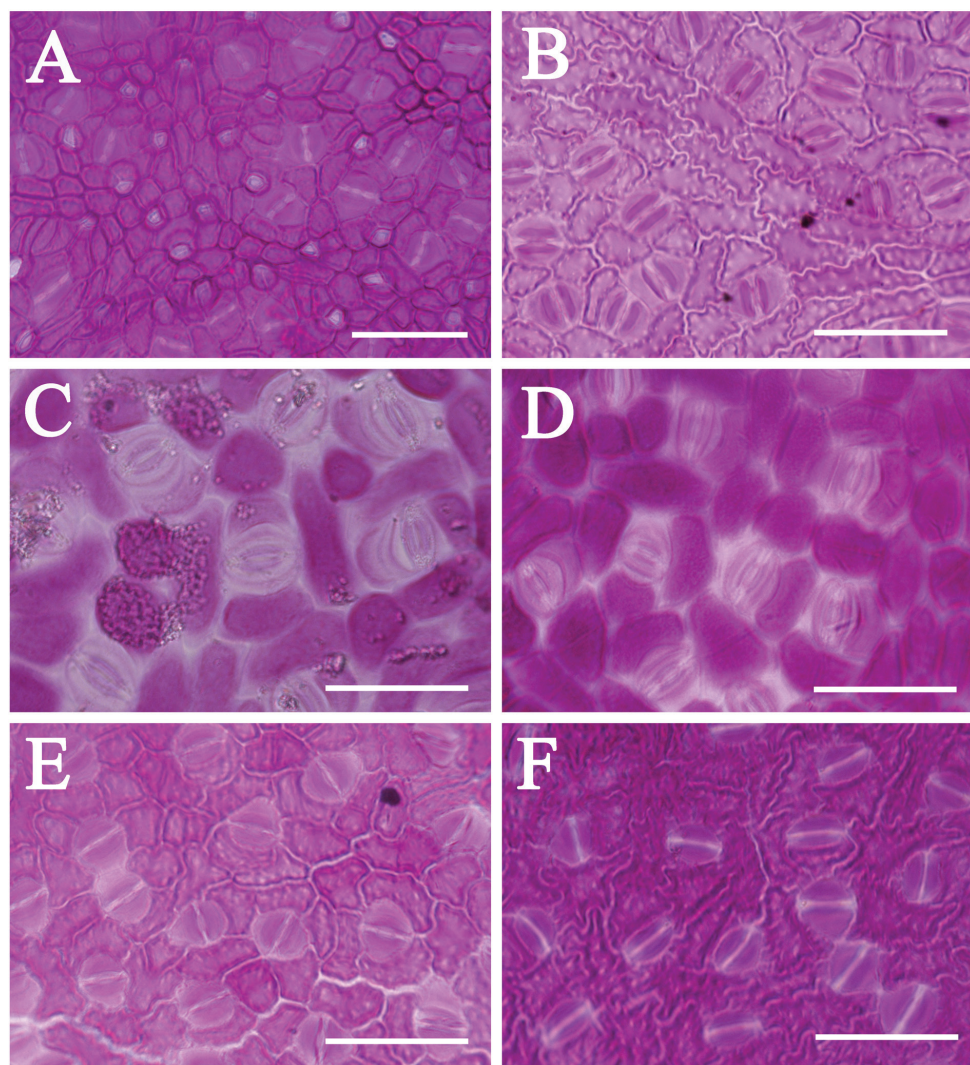


Figure 7. Lower leaf epidermis of *Cinnamomum* under light microscope (LM) **A** *C. daphnoides* displaying butterfly-shaped stomata **B** *C. burmannii* displaying butterfly-shaped stomata **C** *C. migao* displaying narrow lip-shaped stomata **D** *C. longepaniculatum* displaying bat-shaped stomata **E** *C. randaiense* displaying narrow lip-shaped stomata **F** *C. verum* displaying wide lip-shaped/wide lip-shaped stomata. Scale bars: 50 μ m.

ily (see Christophel et al. 1996; Nishida and Christophel 1999; Nishida and van der Werff 2007, 2011, 2014; Yang et al. 2012; Zeng et al. 2014; Nishida et al. 2016). In the character reconstruction, the ancestral node of the Asian *Cinnamomum* possesses a non-reticulate type of periclinal walls with high probability (95.34%). The two types of periclinal walls are clade-specific (Fig. 10), and the reticulate type appears to have originated in the ancestor of sect. *Cinnamomum* s.l. The reticulate type is shared by sect. *Cinnamomum* s.l. and its ancestor with a probability of 95.18%, and the non-

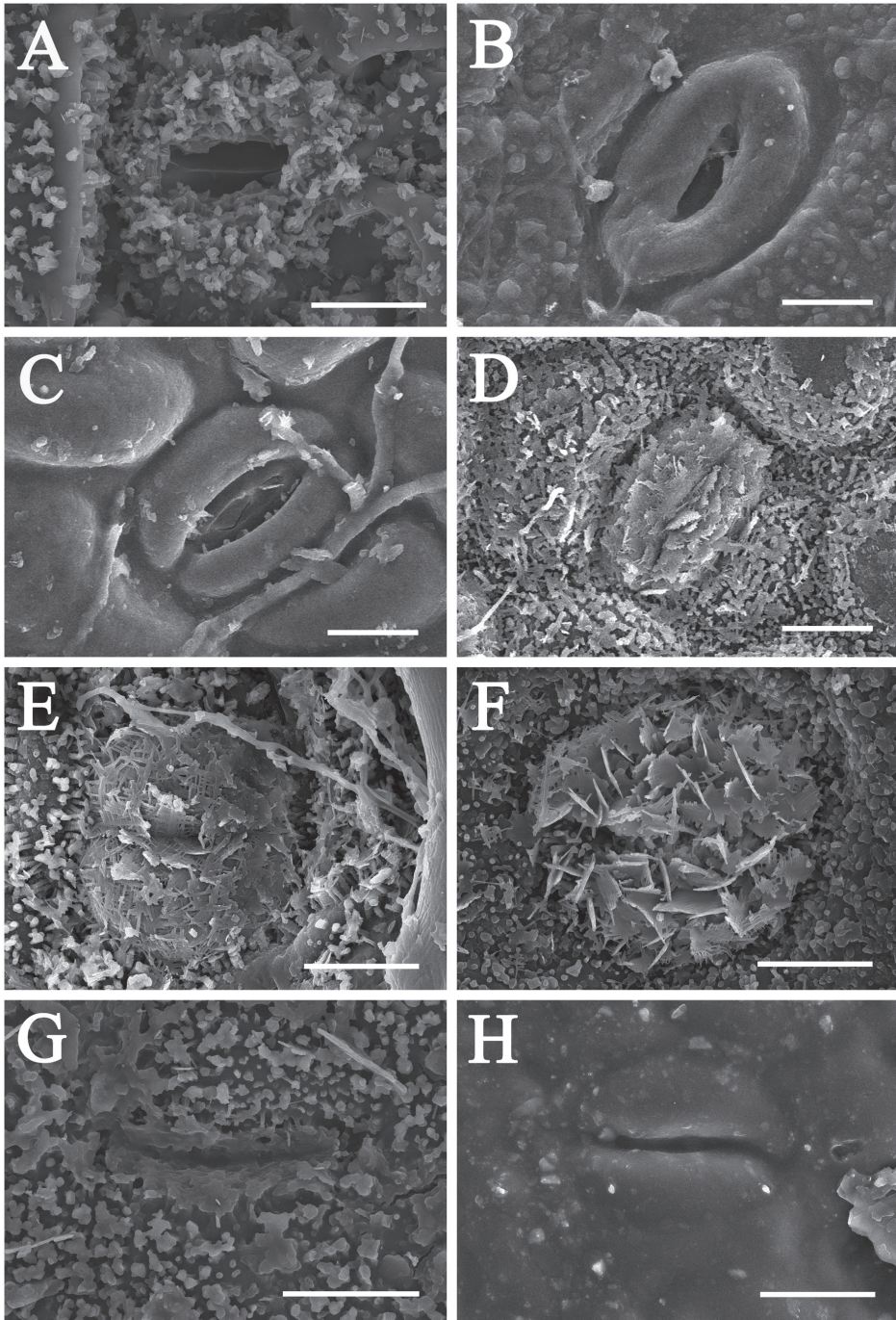


Figure 8. Lower leaf epidermis of *Cinnamomum* under scanning electron microscope displaying stomatal features **A,B** circular stomata **A** *C. chartophyllum* **B** *C. micranthum* **C,D** lip-shaped stomatal **C** *C. migao* **D** *C. longepaniculatum* **E, F** globose stomata **E** *C. septentrionale* **F** *C. camphora* **G, H** eyelid-shaped stomata **G** *C. tonkinense* **H** *C. jensenianum*. Scale bars: 10 µm.

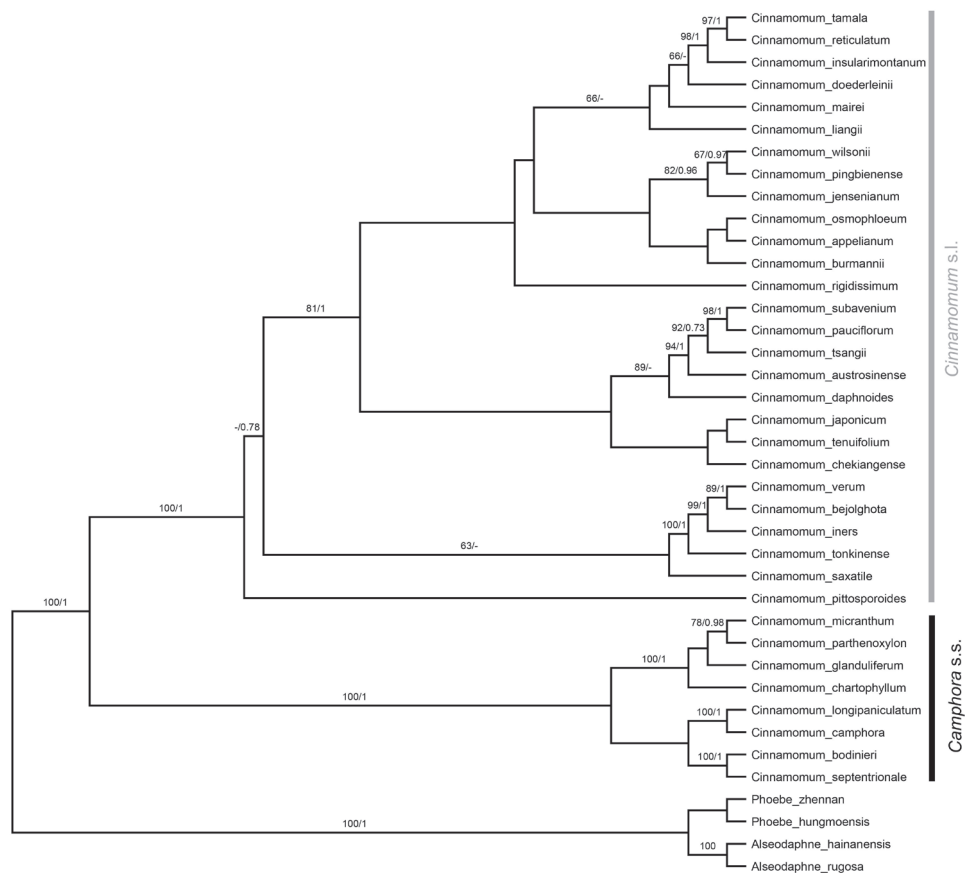


Figure 9. Phylogeny of the Asian *Cinnamomum* incorporating ML and BI trees. Upper number of the slash refers to the bootstrap value of the ML tree and the lower number of the slash refers to the posterior probabilities of the BI tree.

reticulate type is shared by sect. *Camphora* s.s. and its ancestor with a probability of 100%. We consider that the reticulate type of periclinal walls is a synapomorphy of sect. *Cinnamomum* s.l., and is useful in classification of the two clades.

Both leaf epidermal cell shape and the straightness of anticlinal walls are not clade specific and transitional between the two groups/clades of Asian *Cinnamomum* (Fig. 11). In sect. *Cinnamomum* s.l., a few species possess polygonal epidermal cell shape and straight/curved anticlinal walls, which are common in sect. *Camphora* s.s., e.g. *C. daphnoides*, *C. doederleinii*, *C. pittosporoides*, *C. reticulatum*, and *C. scortechinii*. These species possess opposite triveined/tripliveined leaves. A few other species were not examined in phylogenetic studies, but they too possess reticulate periclinal walls, and opposite/subopposite, triveined/tripliveined leaves lacking domatia, viz. *C. liangii*, *C. litseifolium*, *C. macrostemon*, *C. pseudopedunculatum*, *C. tetragonum*, *C. validinerve*, and *C. ovalifolium*. We thus expect them

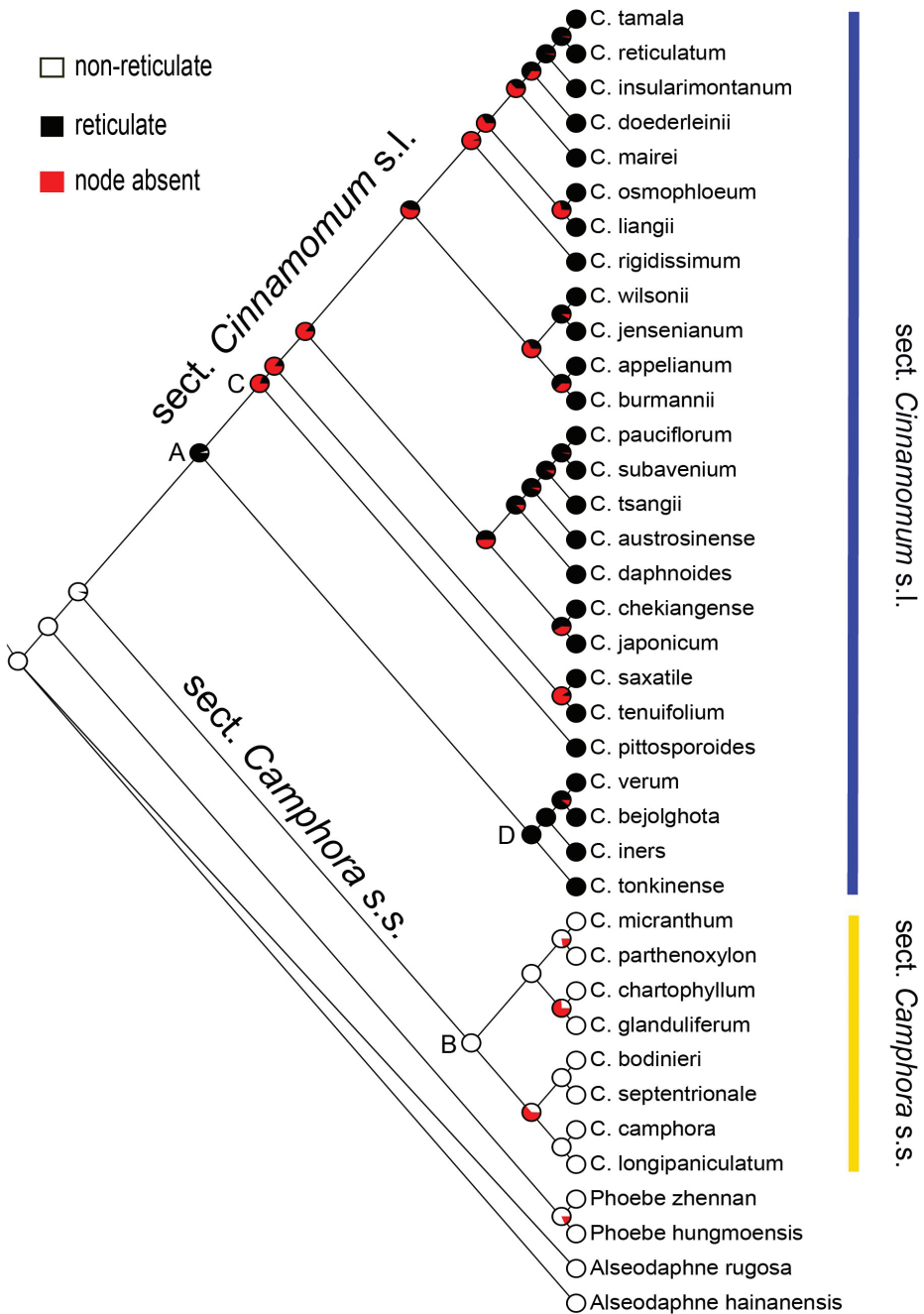


Figure 10. Ancestral character reconstruction of the periclinal wall reticulation by applying a ML tree block in Mesquite with a maximum likelihood approach and MK1 model. The common ancestor of Node A possesses reticulate periclinal wall with high likelihood (95.18%), and the ancestral Node B is reticulate with high likelihood (99.99%).

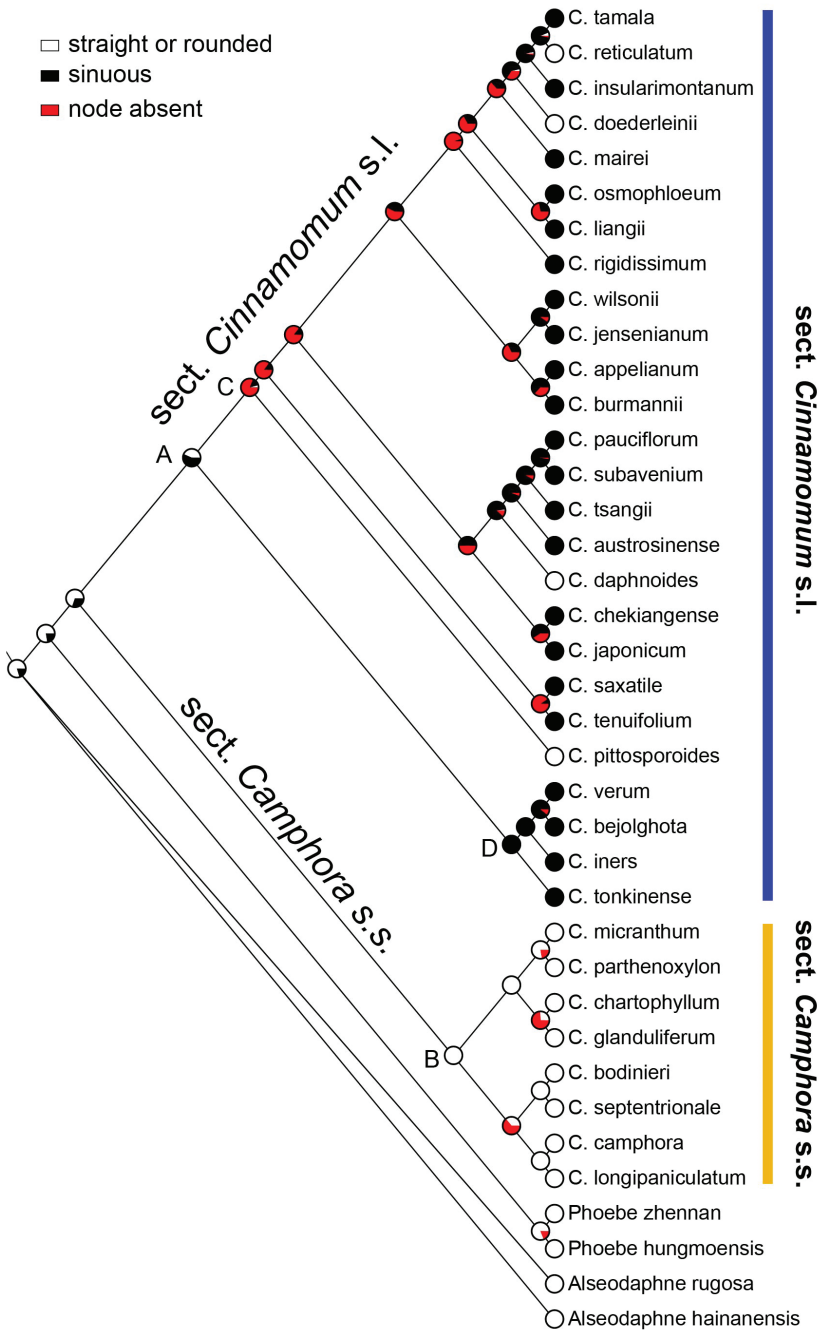


Figure 11. Ancestral character reconstruction of the epidermal cell shape and the straightness of anticlinal wall by applying a ML tree block in Mesquite with a maximum likelihood approach and MK1 model. Node A: the ancestral node of sect. *Cinnamomum* s.l. had sinuous anticlinal walls and irregular cell shapes or not, the probability being only 56.54%; Node B: the ancestral node of sect. *Camphora* s.s. possessed straight or curved anticlinal walls and polygonal cell shapes, the probability being 99.17%.

to belong to sect. *Cinnamomum* s.s. Another two species examined here, *C. ilicoides* and *C. migao* possess non-reticulate periclinal walls, polygonal cell shape, straight/rounded anticlinal walls, perulate buds, and alternate and penninerved leaves which bear inconspicuous domatia in axils of lateral veins, so they clearly belong to sect. *Camphora* s.s.

Phylogenetic relationships of *Sassafras* have not been resolved. Rohde et al. (2017) gave conflicting phylogenetic results on *Sassafras* based on nrDNA and cpDNA sequences. The phylogeny based on nrITS indicates that *Sassafras* is sister to the *Cinnamomum*+*Aiouea*+*Ocotea* complex, nevertheless, the phylogeny based on cpDNA suggests that *Sassafras* forms a clade together with two species of sect. *Camphora*, i.e. *C. bodinieri* and *C. glanduliferum*, making the sect. *Camphora* polyphyletic. Trofimov and Rohwer (2020) indicated that *Sassafras* is sister to sect. *Camphora* based on nrITS and *psbA-trnH*. Liu et al. (2021) reported conflicts between nuclear and plastid phylogenetic results. In their analysis, *Sassafras* is either sister to sect. *Camphora* (nrDNA phylogeny) or to a clade consisting of *C. caudiferum* and *C. porrectum* (plastome phylogeny), but their result on *Sassafras* is not conclusive due to poor sampling of *Cinnamomum*. Our leaf anatomy indicates that *Sassafras* does possess Type I upper leaf epidermis as in sect. *Camphora* (and most other Lauraceae), i.e. polygonal epidermal cells, straight anticlinal walls, and non-reticulate periclinal walls.

The genus *Cinnamomum* was formerly considered to be amphi-Pacific (Rohwer 1993; Lorea-Hernandez 1996; van der Werff 2001), but a recent phylogenetic study (Rohde et al. 2017) suggested that the American species are closer to the likewise predominantly American *Ocotea* complex than to Asian *Cinnamomum*; they have now been accommodated in *Aiouea* (Rohde et al. 2017). The Old World *Cinnamomum* is thus a diphyletic group, and includes two clades (Huang et al. 2016; Rohde et al. 2017, and this study). Sect. *Cinnamomum* appears to be sister to the Neotropical clade in Huang et al. (2016) but it is the sect. *Camphora* that appears to be sister to the Neotropical clade in nrITS analysis of Rohde et al. (2017). Whichever is correct, the Asian *Cinnamomum* is not a monophyletic group and should be further subdivided into two genera. Our new study clearly suggests that use of leaf epidermal micromorphological characters leads to the recognition of two distinct groups that are clade-specific and highly predictive. We thus provide micromorphological support to classify the Asian *Cinnamomum* into two genera.

Acknowledgements

The authors thank D.J. Mabberley and H. van der Werff for their instructive suggestions on the manuscript. Thanks are also due to F. Alves, S. Nishida and an anonymous reviewer for their valuable suggestions. This work was supported by the National Natural Science Foundation of China [31770211, 31470301, 31270238] and the *Metasequoia* funding of Nanjing Forestry University to YY.

References

- Allen CK (1939) Studies in the Lauraceae. II. Some critical and new species of *Cinnamomum* and *Neocinnamomum*. Journal of the Arnold Arboretum 20: e58.
- Blume CL (1826) Bijdragen tot de Flora van Nederlandsch Indie, pt.11. Ter Lands Drukkerij, Batavia, 569 pp. <https://doi.org/10.5962/bhl.title.395>
- Chanderbali AS, van der Werff H, Renner SS (2001) Phylogeny and historical biogeography of Lauraceae: Evidence from the chloroplast and nuclear genomes. Annals of the Missouri Botanical Garden 88(1): 104–134. <https://doi.org/10.2307/2666133>
- Chang HT (1959) Notulae plantarum Austro-Sincarum. Acta Scientiarum Naturalium Universitatis Sunyatseni 1959(2): 19–22.
- Chevalier AJB (1918) Premier inventaire des bois et autres produits forestiers du Tonkin. Bulletin Économique (de l'Indochine) 20: 742–884.
- Christophel DC, Kerrigan R, Rowett AI (1996) The use of cuticular features in the taxonomy of the Lauraceae. Annals of the Missouri Botanical Garden 83(3): 419–432. <https://doi.org/10.2307/2399871>
- Engler A (1884) Lauraceae. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 6: e57.
- Fijridiyanto IA, Murakami N (2009) Phylogeny of *Litsea* and related genera (Laureae-Lauraceae) based on analysis of *rpb2* gene sequences. Journal of Plant Research 122(3): 283–298. <https://doi.org/10.1007/s10265-009-0218-8>
- Gamble JS (1910) New Lauraceae from the Malayan region. II. Bulletin of Miscellaneous Information. Royal Gardens, Kew 1910(7): 218–228. <https://doi.org/10.2307/4111848>
- Gamble JS (1914) Lauraceae. In: Sargent CS (Ed.) Plantae Wilsonianae. An Enumeration of the Woody Plants Collected in Western China for the Arnold Arboretum of Harvard University, Vol. 2. The University Press, Cambridge, MA, 66–86.
- Hance HF (1882) A decade of new Hong-Kong plants. Journal of Botany. British and Foreign. London 20: 79–80.
- Handel-Mazzetti HRE (1921) *Cinnamomum jensenianum* Hand.-Mazz. Anzeiger der Akademie der Wissenschaften in Wien, Mathematische-naturwissenschaftliche Klasse, Wien 58: e63.
- Handel-Mazzetti HRE (1925) *Cinnamomum pittosporoides* Hand.-Mazz. Anzeiger der Akademie der Wissenschaften in Wien, Mathematische-naturwissenschaftliche Klasse, Wien 61: e19.
- Handel-Mazzetti HRE (1936) *Cinnamomum septentrionale* Hand.-Mazz. Oesterreichische Botanische Zeitschrift 85: e213. <https://doi.org/10.1007/BF01255464>
- Hayata B (1911) Materials for a flora of Formosa. Journal of the College of Science, Imperial University of Tokyo 30(Art. 1): 235–258.
- Hayata B (1913) Icones Plantarum Formosanarum nec non et Contributiones ad Floram Formosanam, Vol. 3. The Bureau of Productive Industries, Government of Formosa, Taihoku [Taipei], 157–167.
- Hemsley WB (1907) *Sassafras* in China. Bulletin of Miscellaneous Information. Royal Gardens, Kew 1907(2): 55–56. <https://doi.org/10.2307/4111625>

- Huang JF, Li L, van der Werff H, Li HW, Rohwer JG, Crayn DM, Meng HH, van der Merwe M, Conran JG, Li J (2016) Origins and evolution of cinnamon and camphor: A phylogenetic and historical biogeographical analysis of the *Cinnamomum* group (Lauraceae). *Molecular Phylogenetics and Evolution* 96: 33–44. <https://doi.org/10.1016/j.ympev.2015.12.007>
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* (Oxford, England) 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Hyland B (1989) A revision of Lauraceae in Australia (excluding *Cassytha*). *Australian Systematic Botany* 2(2): 135–367. <https://doi.org/10.1071/SB9890135>
- Kanehira R (1917) *Formosan Trees: an Account of Trees, Shrubs, Bamboos, Palms, and Tree Ferns Indigenous or Commonly Cultivated in Formosa*. Taihoku [Taipei], 428 pp.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kostermans AJGH (1988) Materials for a revision of Lauraceae V. *Reinwardtia* 10: 439–469.
- Lee SK (1963) Notes on Hainan Lauraceous plants. *Acta Phytotaxonomica Sinica* 8: 181–196.
- Lee SK, Wei FN, Wei YT, Li HW (1979) *Materiae ad Floram Lauracearum Sinicarum* (III). *Acta Phytotaxonomica Sinica* 17: 45–74.
- Léveillé H (1912) LXIII. Decades plantarum novarum. LXXXV–LXXXIX. *Repertorium Specierum Novarum Regni Vegetabilis* 10(24–26): 369–378. <https://doi.org/10.1002/fedr.19120102402>
- Léveillé H (1914) XLV. Decades plantarum Novarum. CXXXII–CXXXV. *Repertorium Specierum Novarum Regni Vegetabilis* 13(9–12): 174–180. <https://doi.org/10.1002/fedr.19140130912>
- Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50(6): 913–925. <https://doi.org/10.1080/106351501753462876>
- Li HW (1975) The relation between plant classification and chemistry of essential oil in *Cinnamomum glanduliferum* (Wall.) Nees and its allies. *Acta Phytotaxonomica Sinica* 13: 36–50.
- Li HW (1978) *Materiae ad Floram Lauracearum Sinicarum* (I). *Acta Phytotaxonomica Sinica* 16: 90–92.
- Li HW, Pai PY, Lee SK, Wei FN, Wei YT, Yang YC, Huang PH, Cui HB, Xia ZD, Li JL (1982) Lauraceae. In: Li HW (Ed.) *Flora Reipublicae Popularis Sinicae* (Vol. 31). Science Press, Beijing, 1–463.
- Li J, Christophel DC, Conran JG, Li HW (2004) Phylogenetic relationships within the ‘core’ Laureae (*Litsea* complex, Lauraceae) inferred from sequences of the chloroplast gene *matK* and nuclear ribosomal DNA ITS regions. *Plant Systematics and Evolution* 246(1–2): 19–36. <https://doi.org/10.1007/s00606-003-0113-z>
- Liu ZF, Ma H, Ci XQ, Li L, Song Y, Liu B, Li HW, Wang SL, Qu XJ, Hu JL, Zhang XY, Conran JG, Twyford AD, Yang JB, Hollingsworth PM, Li J (2021) Can plastid genome sequencing be used for species identification in Lauraceae? *Botanical Journal of the Linnean Society* 197(1): 1–14. <https://doi.org/10.1093/botlinnean/boab018>

- Lorea-Hernández FG (1996) A systematic revision of the Neotropical species of *Cinnamomum* Schaeffer (Lauraceae). PhD Thesis. University of Missouri, St. Louis. <https://doi.org/10.5962/bhl.title.10824>
- Lukmanoff A (1889) Nomenclature et Iconographie des Canneliers et Camphriers. F. Debons ETJ, Paris, 4. <https://doi.org/10.5962/bhl.title.540>
- Meisner CDF (1864) Lauraceae. In: de Candolle A (Ed.) Prodrromus Systematis Naturalis Regni Vegetabilis, sive, Enumeratio Contracta Ordinum, Generum, Specierumque Plantarum Hucusque Cognitarium, Juxta Methodi Naturalis, Normas Digesta, Vol. 15(1). Sumptibus Sociorum Treuttel & Würtz, Parisii, 1–260.
- Merrill ED (1934) Unrecorded plants from Kwangtung Province III. Lingnan Science Journal 13: e26.
- Miqeul FAW (1858) Flora van Nederlandsch Indie, Vol. 1. C.G. van der Post, Amsterdam, 902.
- Mo YQ, Li L, Li JW, Rohwer JG, Li HW, Li J (2017) *Alseodaphnopsis*: A new genus of Lauraceae based on molecular and morphological evidence. PLoS ONE 12(10): e0186545. <https://doi.org/10.1371/journal.pone.0186545>
- Nakai T (1939) Flora Sylvatica Koreana. The Forest Experiment Station, Government General of Chosen. Keijyo 22: e23.
- Nees TFLE (1831a) Handbuch der Medicinisch-Pharmaceutischen Botanik, Vol. 2. Düsseldorf, Arnz & Comp., 426 pp.
- Nees CGE (1831b) Laurinae Indie Orientalis. In: Wallich N (Ed.) Plantae Asiaticae Rariores: or, Descriptions and Figures of a Select Number of Unpublished East Indian Plants, Vol. 2. Treuttel and Würtz, London, 58–76.
- Nees CGE (1836) Systema Laurinarum. Sumptibus Veitii & Sociorum, Berolini, 720 pp.
- Nishida S, Christophel DC (1999) Leaf anatomy of *Beilschmiedia* (Lauraceae) in the Neotropics. Nature and Human Activities 4: 9–43.
- Nishida S, van der Werff H (2007) Are cuticular characters useful in solving generic relationships of problematic of Lauraceae. Taxon 56(4): 1229–1237. <https://doi.org/10.2307/25065914>
- Nishida S, van der Werff H (2011) An evaluation of classification by cuticular characters of the Lauraceae: A comparison to molecular phylogeny. Annals of the Missouri Botanical Garden 98(3): 348–357. <https://doi.org/10.3417/2010054>
- Nishida S, van der Werff H (2014) Do cuticle characters support the recognition of *Alseodaphne*, *Nothaphoebe*, and *Dehaasia* as distinct genera? Reinwardtia 14(1): 53–66. <https://doi.org/10.14203/reinwardtia.v14i1.395>
- Nishida S, de Kok R, Yang Y (2016) Cuticular features of *Cryptocarya* (Lauraceae) from Peninsular Malaysia, Thailand and Indo-China and its taxonomic implications. Phytotaxa 244: 026–044. <https://doi.org/10.11646/phytotaxa.244.1.2>
- Nylander JAA (2008) MrModeltest 2.3. Dept. Systematic Zoology, EBC, Uppsala University, Sweden.
- Presl JS (1825) O Pirozenosti Rostlin, Obsahugcj Gednanj o Ziwobitj Rostlin, Vol. 2. W. Praze, Jos. Kaus 37: e47.
- Rehder A (1920) American and Asiatic species of *Sassafras*. Journal of the Arnold Arboretum 1: e244.

- Rohde R, Rudolph B, Ruthe K, Lorea-Hernández FG, de Moraes PLR, Li J, Rohwer JG (2017) Neither *Phoebe* nor *Cinnamomum* – the tetrasporangiate species of *Aiouea* (Lauraceae). *Taxon* 66(5): 1085–1111. <https://doi.org/10.12705/665.6>
- Rohwer JG (1993) Lauraceae. In: Kubitzki K, Rohwer JG, Bittrich V (Eds) The families and genera of vascular plants (Vol. II). Springer-Verlag, Berlin, 366–391. https://doi.org/10.1007/978-3-662-02899-5_46
- Rohwer JG, Rudolph B (2005) Jumping genera: The phylogenetic positions of *Cassytha*, *Hypodaphnis*, and *Neocinnamomum* (Lauraceae) based on different analyses of *trnK* intron sequences. *Annals of the Missouri Botanical Garden* 92: 153–178.
- Rohwer JG, Li J, Rudolph B, Schmidt SA, van der Werff H, Li XW (2009) Is *Persea* (Lauraceae) monophyletic? – Evidence from nuclear ribosomal ITS sequences. *Taxon* 58(4): 1153–1167. <https://doi.org/10.1002/tax.584009>
- Rohwer JG, de Moraes PLR, Rudolph B, van der Werff H (2014) A phylogenetic analysis of the *Cryptocarya* group (Lauraceae), and relationships of *Dahlgrenodendron*, *Sinopora*, *Triadodaphne*, and *Yasunia*. *Phytotaxa* 158(2): 111–132. <https://doi.org/10.11646/phytotaxa.158.2.1>
- Schewe (1925) *Cinnamomum appelianum* Schewe. *Anzeiger der Akademie der Wissenschaften in Wien, Mathematische-naturwissenschaftliche Klasse*, Wien 61: 20.
- Siebold PF (1830) *Verhandelingen van het Bataviaasch Genootschap van Kunsten en Wetenschappen*, Vol. 12. Egbert Heemen, Batavia, 23.
- Siebold PF, Zuccarini JG (1846) *Florae Japonicae. Abhandlungen der Mathematisch-Physikalischen Classe der Königlich Bayerischen Akademie der Wissenschaften*, Munich 4(3): e402.
- Song Y, Yu WB, Tan YH, Liu B, Yao X, Jin JJ, Padmanaba M, Yang JB, Corlett RT (2017) Evolutionary comparisons of the chloroplast genome in Lauraceae and insights into loss events in the Magnoliids. *Genome Biology and Evolution* 9(9): 2354–2364. <https://doi.org/10.1093/gbe/evx180>
- Song Y, Yu WB, Tan YH, Jin JJ, Wang B, Yang JB, Liu B, Corlett RT (2020) Plastid phylogenomics improve phylogenetic resolution in the Lauraceae. *Journal of Systematics and Evolution* 58(4): 423–439. <https://doi.org/10.1111/jse.12536>
- Sugimoto J (1928) *Cinnamomum tenuifolium* (Makino) Sugim. *Nippon Journal of Botany* 1(2): e57.
- Sweet R (1826) *Sweet's Hortus Britannicus: or a Catalogue of Plants Cultivated in the Gardens of Great Britain, Arranged in Natural Orders with Reference to the Linnean Class and Order to Which Each Genus Belongs; the Whole Brought up to the Present Time, and Contains above 34,000 Plants*. J. Ridgway, London. <https://doi.org/10.5962/bhl.title.43792>
- Swofford DL (2003) PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland.
- Thwaites GHK (1861) *Enumeratio Plantarum Zeylanicae*. Dulau & Co., London, 253 pp.
- Trofimov D, Rohwer JG (2018) Epidermal features allowing identification of evolutionary lineages in the *Ocotea* complex (Lauraceae). *Perspectives in Plant Ecology, Evolution and Systematics* 31: 17–35. <https://doi.org/10.1016/j.ppees.2017.12.003>

- Trofimov D, Rohwer JG (2020) Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae): An analysis with emphasis on the Old World taxa and description of the new genus *Kuloa*. Botanical Journal of the Linnean Society 192(3): 510–535. <https://doi.org/10.1093/botlinnean/boz088>
- Trofimov D, Rudolph B, Rohwer JG (2016) Phylogenetic study of the genus *Nectandra* (Lauraceae), and reinstatement of *Damburneya*. Taxon 65(5): 980–996. <https://doi.org/10.12705/655.3>
- Trofimov D, Moraes P, Rohwer J (2019) Towards a phylogenetic classification of the *Ocotea* complex (Lauraceae): Classification principles and reinstatement of *Mespilodaphne*. Botanical Journal of the Linnean Society 190(1): 25–50. <https://doi.org/10.1093/botlinnean/boz010>
- van der Werff H (2001) An annotated key to the genera of Lauraceae in the Flora Malesiana region. Blumea 46: 125–140.
- van der Werff H, Richter HG (1996) Toward an improved classification of Lauraceae. Annals of the Missouri Botanical Garden 83(3): 409–418. <https://doi.org/10.2307/2399870>
- Xiao TW, Xu Y, Jin L, Liu TJ, Yan HF, Ge XJ (2020) Conflicting phylogenetic signals in plastomes of the tribe Laureae (Lauraceae). PeerJ 8: e10155. <https://doi.org/10.7717/peerj.10155>
- Yang Y, Zhang LY, Liu B, van der Werff H (2012) Leaf cuticular anatomy and taxonomy of *Syndiclis* (Lauraceae) and its allies. Systematic Botany 37(4): 861–878. <https://doi.org/10.1600/036364412X656518>
- Zeng G (2018) Floral organs and leaf epidermis of a few groups of the Lauraceae. Ph.D. thesis. Chinese Academy of Sciences, Beijing.
- Zeng G, Liu B, van der Werff H, Ferguson DK, Yang Y (2014) Origin and evolution of the unusual leaf epidermis of *Caryodaphnopsis* (Lauraceae). Perspectives in Plant Ecology, Evolution and Systematics 16(6): 296–309. <https://doi.org/10.1016/j.ppees.2014.07.003>

Supplementary material I

Phylogenetic trees of *Cinnamomum* and sequences obtained from the GenBank

Authors: Zeng Gang, Bing Liu, Jens G. Rohwer, David Kay Ferguson, Yong Yang

Data type: Docx file.

Explanation note: Phylogenetic trees.

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

Link: <https://doi.org/10.3897/phytokeys.182.67289.suppl1>