RESEARCH ARTICLE



# The genus *Eunotia* Ehrenb. (Bacillariophyta) in the Cheremsky Nature Reserve, Ukrainian Polissya, and refined terminology relevant to the raphe system morphology

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Academic editor: Kalina Manoylov | Received 25 April 2019 | Accepted 20 June 2019 | Published 23 July 2019

**Citation:** Bukhtiyarova LN (2019) The genus *Eunotia* Ehrenb. (Bacillariophyta) in the Cheremsky Nature Reserve, Ukrainian Polissya, and refined terminology relevant to the raphe system morphology. PhytoKeys 128: 1–31. https://doi. org/10.3897/phytokeys.128.35566

### Abstract

Numerous species of *Eunotia* Ehrenb., widely distributed in the world flora, prefer acidic, dystrophic or oligotrophic freshwater habitats with low conductivity and usually occur in epiphytic or epilithic hydrotopes. In Ukraine, only 32 species and eight varieties of *Eunotia* were known until this study. For the first time, 9 more species have been recorded mainly from the Cheremsky Nature Reserve, located in Ukrainian Polissya. New findings include 2 species widely distributed in the world flora on most continents and 7 rare species known from several locations, among them E. genuflexa, E. jarensis and E. ruzickae, which are probably European endemics as they have not been reported from other continents. For the present time in the Cheremsky Nature Reserve, the 20 species recorded here, the highest species richness of Eunotia in Ukraine, bring the total number of Eunotia in Ukraine to 41 species, which comprises only 7% of Eunotia species in the world flora. This is indirect evidence of insufficient investigation of the wetlands in Ukraine where *Eunotia* usually is represented with high species richness. Several definitions are suggested to describe morphological features that are peculiar to the diatom frustule particular to the Eunotia species. The genus Eunotia possesses a mirror-symmetric, mantle-offset, brevisslit raphe system, which may or may not have terminal raphe fissures. Morphological analysis provided in this study revealed the absence of terminal raphe fissures for many species of *Eunotia*. Instead, the distal ends of the raphe slits finish on the outer valve surface by funnel holes, sometimes pore-like ones, connected with the helictoglossae. However, in the literature those distal ends of the raphe slits were described erroneously as terminal raphe fissures. For the first time different types of raphe system are grounded. Two species Eunotia implicata Nörpel-Schempp et al. in Alles et al. and Eunotia incisa W. Smith ex Gregory were lectotypified.

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#### **Keywords**

*Eunotia*, functional morphology; mirror-symmetric, mantle-offset, brevisslit raphe system; taxonomy, rare species, distribution

#### Introduction

The Cheremsky Nature Reserve, located in Ukrainian Polissya, Volyn region, in the interfluve of Stokhid and Veselukha rivers, occupies about 3 thousand ha. The reserve includes large areas of untouched forests and unique wetlands which take up about 34% of the territory (Figs 1, 2). An eumesotrophic Cheremske bog (total area of about one thousand ha, peat deposition up to 10 m), relates to peripheral-oligotrophic type of development characteristic of bogs formed in lake-like basins. Two lakes within the Cheremsky Nature Reserve Cheremske (7,7 ha, max. depth 7 m) and Redychi (14,0 ha, max. depth 4,5 m) originate from glacial-karst (Konischuk and Didukh 2004, Konishchuk 2005).

Previous studies of the Bacillariophyta from the Cheremsky Nature Reserve revealed high species richness with 84 species reported by Bukhtiyarova (2008) and 57 taxa by Malakhov et al. (2017). The mesotrophic bogs contain a moderate quantity of nutrients that facilitate the development of a specific and rich diatom flora. The most species-rich genera in such habitats typically include *Eunotia* Ehrenb. and *Pinnularia* Ehrenb. Indeed, many of the diatom taxa recorded for the first time in Ukraine were from the Cheremsky Nature Reserve and included: Eunotia silvahercynia Nörpel et al. in Alles et al. (1991), Pinnularia complexa Krammer (2000), P. lokana Krammer (2000), P. nobilis var. regularis Krammer (2000), P. parvulissima Krammer (2000), P. polyonka (Bréb.) W. Smith (1856), P. rhombarea Krammer in Metzeltin and Lange-Bertalot (1998), P. stomatophora var. irregularis Krammer (2000), P. stidolphii Krammer (2000), P. subanglica Krammer (2000), P. subrupestris Krammer (1992) and its variety P. subrupestris var. cruciata Krammer (2000) (Bukhtiyarova 2008, 2009b); Eunotia myrmica Lange-Bert. in Lange-Bertalot et al. (2011) (Malakhov et al. 2017). In the Cheremsky Nature Reserve only 9 species of *Eunotia* were known from the Cheremske and Redychi lakes prior to this study.

As a part of documenting the *Eunotia* taxa it was necessary to describe the raphe system's particular properties which have taxonomical value on species rank of taxonomy. In recently published terminological glossary the following definition for the raphe was proposed: "Raphe (Lat.) – an elongated slit or pair of slits through the valve wall. When a pair of slits is present each individual slit is a branch of the raphe" (Gogorev et al. 2018: p. 299), which does not include the position of the raphe system in the diatom frustule hierarchic structure and any of its functions. In another glossary about the same definition appears: "The raphe system is composed of one or two slits, or fissures, that penetrate the valve of some diatoms. If two slits are present, each is called a branch of the raphe. Branches may be separated by a silica thickening called the central nodule" and it was indicated that raphe allows diatom cells to move (Diatoms of North America 2019). In the latest one the possible raphe position on the valve is

defined as axial (along the apical axis), eccentric (along one margin) or circumferential (around the whole margin of the valve) none of which does not specify raphe position on the valves of the *Eunotia* species. In both definitions several structural elements of the raphe system are omitted. Moreover, in the second, no distinction is made between the raphe slits and fissures, as both terms look like synonyms but they define different elements of the raphe system. The *Eunotia* taxa possess unique raphe system the morphology of which has not been studied yet in detail.

This study provides detailed information on the species of *Eunotia* Ehrenb. found in the Cheremsky Nature Reserve, including rare species recorded in Ukraine for the first time. Revised terminology to highlight morphological features of the raphe system relevant to the *Eunotia* species is also suggested.

## Materials and methods

In 2003–2004 O. Petlyovany collected epiphytic samples of algae from mosses in the Volyn region, Manevichsky district, the Cheremsky Nature Reserve mainly from the lakes Cheremske and Redychi, both from open waters and marshy locations. Sample numbers correspond to those from the Algoteca of M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine – the largest phycological collection in Ukraine.

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In accordance with Malakhov et al. (2017) hydrochemical parameters of these lakes are very similar. Lake Cheremske: conductivity – 70  $\mu$ S/cm, pH – 6.16–6.6, dissolved O<sub>2</sub> – 9.7 mg/L, NH<sub>4</sub><sup>+</sup> – 0.22 mg/L, NO<sub>2</sub><sup>-</sup> < 0.01 mg/L, NO<sub>3</sub><sup>-</sup> < 0.01 mg/L, PO<sub>4</sub><sup>3-</sup> < DL<sup>\*</sup> mg/L, Fe<sup>3+</sup> – 1.2 mg/L.

Lake Redychi: conductivity – 63  $\mu$ S/cm, pH – 6.46, dissolved O<sub>2</sub> – 9.5 mg/L, NH<sub>4</sub><sup>+</sup> – 0.20 mg/L, NO<sub>2</sub><sup>-</sup> < 0.01 mg/L, NO<sub>3</sub><sup>-</sup> < 0.01 mg/L, PO<sub>4</sub><sup>3-</sup> < DL<sup>\*</sup> mg/L, Fe<sup>3+</sup> – 1.1 mg/L.

<sup>\*</sup> detection limit

Two samples from other locations in Ukrainian Polissya collected by O.V. Kovalenko have been also studied.

- 27835 Zhytomyr region, Chervonoarmeisky district, swamp, dark films among mosses. 07/15/1983.
- **27895** Volyn region, Vladimir-Volyn district, near village Fedorovka, Western Bug River, floodplain basin, benthos. 07/21/1983.

Organic matter was removed by cold burning with concentrated sulfuric acid and cleaned materials were rinsed several times with distilled water (Wasser et al. 1989). Permanent slides with cleaned materials were mounted in Naphrax (R.1 = 1.7). Diatom species were examined with an Olympus BX 51 light microscope (LM) using a  $100 \times$  oil immersion PlanAchromat objective. The fine structure of the diatom frustules was examined with a scanning electron microscope JEOL 6060LA. The micrographs were obtained with Canon EOS 600 D digital camera using program Helicon Remote (version 3.6.2 w).

Size ranges were based on measurements typically several, sometimes single valves as all species were found in very limited numbers. Therefore the size ranges from the relevant literature were included in the species description.

In many diatom species with bipolar symmetry including *Eunotia* different morphometric data present at different valve parts, e.g. width, striae density in 10 µm etc., their dimension can be helpful in the species correct identification.

Central valve part – valve part on both sides from the transapical axis where the measuring parameter has different value comparing with other valve parts (Fig. 3a, between the arrows).

Middle valve part – valve part between central part and the beginning of valve pole (Fig. 3a, between the upper and double arrows).

Valve pole – distal valve part from its narrowest part or from the beginning of a valve narrowing to the distal end (Fig. 3a, from the double arrow to distal end of a valve).

For the algae flora of Ukraine a 'very rare species' is defined as one recorded in 1-5 localities and 'rare species' – in < 10 localities within the country territory (Palamar'-Mordvintseva et al. 1998). A 'rare species in the world flora' is defined here as one recorded in fewer than 10 localities and restricted to 1-2 continents.

**Abbreviations.** Morphometric data example: length 45–97 μm, width c6–7, m9– 12, p3–5 μm; striae density c12–16, p18–20 in 10 μm.

<b>c5</b> –7	the data for the central valve part.
m9–12	the data for the middle valve part.
p18–20	the data for the valve pole.
*	species recorded for the first time in Ukraine
٨	rare species in the world flora.



**Figures 1, 2.** Location of the studied area **I** The Cheremsky Nature Reserve on the map of Ukraine, indicated by red dot **2** map-scheme of the Cheremsky Nature Reserve.

### Results

#### Morphology and refined terminology

The revised definitions suggested here for some widely used terms and new ones are grounded on the concept of functional morphology of the diatom frustule (Bukhtiyarova 2009a, 2019) which includes a number of theses, in particular the division of all diatom frustule structures on the basic elements and functional units. Thus, this concept allows us to describe any morphological structure on a common universal basis.

Because physical-chemical properties of any material depend on the size of the particles it is compounded from, it was suggested to introduce a size scale in all the definitions of the diatom frustule structures (Bukhtiyarova 2009a).

The basic element of the diatom frustule (db-element) is a morphologically detached, homogeneous part of the frustule that possesses special physical-chemical features and provides primary basis for the frustule hierarchical construction. They belong to db-elemets of the diatom frustule as different apertures and cavities in its thickness, regularly repeated and unique silica microelemets (Bukhtiyarova 2009a: figs 1–5).

Morph of the diatom frustule (df-morph) is compound structural unit of the diatom frustule that is constructed of db-elements and/or structural units of lower orders, realizes particular functions in the diatom organism and has its own evolution (Bukhtiyarova 2009a).

The refined definitions capture raphe system as a functional unit of the diatom frustule, its different db-elements and peculiar properties relevant to *Eunotia* species. For the first time different raphe system types are grounded.

**Raphe system** – a unique compound micro df-morph of second (first) order in the diatom frustule with bipolar symmetry that consists of one or two slits, which penetrate the valve thickness, and may include additional df-morphs (central nodule, helictoglossae, tube) and/or db-elements (terminal fissures, central pores and others). One of the functions of the raphe system is active moving of the diatom unicellular organism. For the species that have an attached mode of life other functions can be performed, e.g. an attachment to substrate or between neighboring cells in a colony.

**Raphe slit** – a unique micro db-element in the shape of uniformly narrowed through opening of different profile into the valve thickness and noticeable length relative to the valve length.

**Central raphe pore** – a unique micro db-element, through hole with usually a different shape on the inner and outer valve surfaces at the proximal end of the raphe slit (Fig. 3b, arrow).

**Terminal raphe fissure (tr-fissure)** – a unique micro db-element in the shape of uniformly narrowed non-through notch continuing distal end of the raphe slit on external valve surface only (Figs 3e, f).

**Terminal raphe fissure with lacuna** – kind of fissure that finishes on distal end by lacuna (Figs 3f, 4a, 5a, 6a, 8a).

**Lacuna** – a unique micro or nano- db-element, non-through hollow of different shape and location on outer or inner valve surface.

In the genus *Eunotia* the lacunae of the raphe terminal fissures (rtf-lacuna) usually have a dish or funnel-like shape of about 100 nm in diameter (Figs 3f, 4a, 5a, 6a, 8a, double arrows).

**Helictoglossa** – a unique siliceous hyaline micro df-morph of first order on the internal valve surface usually in the shape of truncated cylinder or compressed (relative to the raphe slit) asymmetric frustum with smoothly roused up side bearing fissure at the distal end of the raphe slit and abruptly roused opposite side (Figs 13a, 29, 30a, arrows). Any functions of helictoglossae are unstudied, yet their morphology and position allow to suggest that they work like a stopper and may regulate a mucilage mass length of uniform shape that enters into the raphe slit from inside and goes out from the diatom frustule outside.

**Helictoglossa fissure (h-fissure)** – a unique micro db-element in the shape of a uniformly narrowed short non-through notch on helictoglossa continuing distal end of the raphe slit on internal valve surface only.

**Symmetric raphe system** – type of raphe system with straight equal length of the slits and similar accompanied db-elements that are located symmetrically relatively both to the longitudinal and transapical axes of bipolar frustule. Examples of this type of raphe system can be found among species of *Cavinula* D.G. Mann & Stickle in Round et al. (1990), *Hippodonta* Lange-Bertalot et al. (1996), *Navicula* Bory (1822), *Psammothidium* Bukht. & Round (1996) and other diatom genera.

**Mirror-symmetric raphe system** – type of raphe system with equal length and same shaped raphe slits, similar accompanied db-elements that all together are located mirror symmetrically relative to the transapical axis or/and in girdle view of the diatom frustule. Examples of this type of raphe system can be found in species of *Amphora* Ehrenb. ex Kütz. (1844), *Cymbella* Agardh (1830), *Eunotia* and other genera.

**Mantle-offset raphe system** (Lat.) – type of raphe system which partially or completely disposes on the valve mantle. This type of raphe system characterizes the genus *Eunotia*.

**Brevisslit raphe system** (Lat.) – type of raphe system with the slits which disposes only along part of valve length and absent on the rest of it. The examples of this raphe system type can be found in the genera *Actinella* F.W. Lewis (1864), *Eunotia, Rhoicospenia* Grunow (1860) and in others.

Thus, species of the genus *Eunotia* possess of mirror-symmetric, mantle-offset, brevisslit raphe system.

**Basal striae** – type of striae in which the areolae and all additional db-elements accompanying them occupy interstria height in whole (Bukhtiyarova 2015: figs 2, 10–15), or by other words, the valve thickness completely.

**Distant striae** – kind of striae which occupy two or more times less area than interstria area (Bukhtiyarova 2015: figs 4, 6, 8, 9–13, 17).

The terms **proportional**, **packed**, **distant kinds of striae** have been defined on the ratio between stria and interstria areas (Bukhtiyarova 2015).



**Figure 3.** Scheme of the mirror-symmetric, mantle-offset, brevisslit raphe system in frustule of the genus *Eunotia* Ehrenb. **A** valve view, different parts of valve. TA – transapical axis. Central part – between the arrows, middle part – between upper and double arrows, the beginning of valve pole – double arrow **B** girdle view, arrow – central raphe pore **C**, **D** raphe system without terminal fissures **C** arrow – a pore outer at distal end of slit, that finishes at the middle of valve pole **D** arrow – a funnel-like outer at distal end of slit, that finishes at the vental corner of valve pole **E**, **F** raphe system with terminal fissures – dotted lines between arrows, arrow – distal end of a slit **E** double arrow – the end of terminal fissure **F** double arrow – lacuna at the end of terminal fissure. (Original by L. Bukhtiyarova)

#### Taxonomy

In the hydrotopes of the Cheremsky Nature Reserve the following species of the genus *Eunotia* were recorded.

*Eunotia dorofeyukiae* Lange-Bert. & Kulikovskiy in Kulikovskiy et al. 2010b: p. 29, 65, pl. 20/figs 1–6.\*<sup>A†</sup> Figs 28, 28a, 29 (SEM)

**Illustrations.** Krammer and Lange-Bertalot 1991: pl. 143/figs 22–23 (as *Eunotia circumborealis* Nörpel & Lange-Bert.); Rivera-Rondón and Catalan 2017: pl. 25/figs 7–9.

<sup>†</sup> Species epithet 'dorofeyukiae' is corrected by M. Gury (in Guiry and Guiry 2019), the author's epithet was 'dorofeyukae'

**Diagnosis.** Morphometric data: length  $33-35 \mu$ m, width cm 7, p5–8  $\mu$ m; striae density c12–14, p18–22 in 10  $\mu$ m. Lange-Bertalot et al. 2010: length 37–58  $\mu$ m, width 7.3–8.7  $\mu$ m, striae density 10–13 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, with undulate dorsal margin and weak depression in its central part, slightly concave ventral side and subcapitate broad rounded poles. Striae basal, uniserial, distant, denser at the poles. Areolae small with round outer foramina (Fig. 28a). Raphe system consists of two short filiform slits on ventral valve mantle, distal ends of slits finish on external valve surface on ventral pole corners by small round pore outers (Fig. 28a) connected with small helictoglossae (Fig. 29); tr-fissures absent.

**Ecology.** Freshwater epiphytic species, often collected on different *Sphagnum* species, occurs in acidic (pH 5.5–5.6), oligotrophic waters with low electric conductivity and buffered by humic acids. The specimens from Type population were collected at 11–13 °C (Kulikovskiy et al. 2010b).

**Distribution.** ASIA: **Type location:** northern Mongolia, Nur bog (Kulikovskiy et al. 2010b); Russia (Kulikovskiy et al. 2016). EUROPE: Scandinavia (Krammer and Lange-Bertalot 1991 (as *E. circumborealis*); Germany (Kulikovskiy et al. 2010b); Ukraine (present paper); France, Pyrenees, Lake Mariola (Rivera-Rondón and Catalan 2017). AUSTRALIA: Tasmania (M. Guiry in Guiry and Guiry 2019). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Comments.** Illustrations of this species in Kulikovskiy et al. (2010b: pl. 20/figs 1–6) are not uniform in valve outline.

This species was described from a mountainous region with a harsh climate and was recorded later in a mountain lake in the Pyrenees. In Ukraine it inhabits in a flatland bog.

# *Eunotia formicina* Lange-Bert. in Lange-Bertalot et al. 2011: p. 105–107, pl. 222/ figs 1–7, pl. 223/figs 1–7.\*^

Figs 10, 11 (SEM)

*Eunotia formica* var. *elongta* Hustedt, 1909 *Eunotia formica* f. *elongata* (Hustedt) Ant. Mayer, 1918 *Eunotia formica* sensu Germain, 1981

Holotype. Lange-Bertalot et al. 2011: pl. 222/fig. 1.

**Illustrations.** Bąk et al. 2012: p. 131, pl. 11/7 exemplars; Ector et al. 2012: p. 249–250, 3 exemplars; Costa 2015: p. 50, pl. 100/figs 1–5; Marra et al. 2016: fig. 53.

**Diagnosis.** Morphometric data: length  $83-125 \mu$ m; width m8, cp9  $\mu$ m; striae density m9–11, p12–15 in 10  $\mu$ m. Lange-Bertalot et al. 2011: length 20–170  $\mu$ m; width c7–10, m6–8  $\mu$ m; striae density 8–12, p15–16 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves weakly dorsiventral, with gentle gibbosity in central valve part on ventral side and subcapitate broad rounded poles. Valve mantle high, of about 0.5 of valve width, perpendicular to the valve surface; valve/mantle junction

narrow hyaline (see Lange-Bertalot et al. 2011: pl. 223/fig. 4). Striae basal, uniserial, distant, irregularly spaced along the valve; on the mantle additional short intercalar striae (see Lange-Bertalot et al. 2011: pl. 223/fig. 4). Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle; central raphe pores round; tr-fissures long, widely round, follow the pole outline and finish on dorsal valve margin (see Lange-Bertalot et al. 2011: pl. 223/figs 2, 3, 5–7).

**Ecology.** Freshwater benthic species, occurs in moderately acidic, dystrophic or oligosaprobic waters (Lange-Bertalot et al. 2011).

**Distribution.** EUROPE: France, Germany, Netherlands, Poland (M. Gury in Guiry and Guiry 2019); Ukraine (present paper). S. AMERICA: Brasil (Costa 2015, Costa et al. 2017; Marra et al. 2016). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.; Lake Redychi, wetland area, epiphyton on *Sphagnum* sp.

# *Eunotia genuflexa* Nörpel-Schempp in Lange-Bertalot and Metzeltin 1996: p. 50, pl. 9/figs 14–17.\*^

Figs 4-6b (SEM)

*Eunotia flexuosa* f. *beta* A. Berg, 1939 *Eunotioforma genuflexa* (Nörpel-Schemp) Kociolek & Burliga in Burliga et al. 2013

**Illustrations.** Bąk et al. 2012: p. 132, pl. 17/1, 2, 5 exemplars from left to right; Kulikovskiy et al. 2016: p. 121, pl. 20/figs 10–14.

**Diagnosis.** Morphometric data: length 70–120  $\mu$ m; width cm2–3, p1.5–2  $\mu$ m; striae density c20, p23 in 10  $\mu$ m. Lange-Bertalot and Metzeltin 1996: length 70–160  $\mu$ m, width 1.5–2.6  $\mu$ m, striae density 19–23 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves slightly dorsiventral, with narrowed rounded poles. Striae basal, uniserial, distant, uniformly spaced along the valve (Figs 4a, 5a, 6a, b). Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle; tr-fissures long, broadly curved, sited on 0.5 of valve width along 4–5 striae, end up by round lacunae (Figs 4a, 5a, 6a).

**Ecology.** Freshwater benthic species occurs in moderately acidic, dystrophic or oligosaprobic waters. Type location had extremely low concentration of inorganic nutrient and pH = 6.5 (Lange-Bertalot and Metzeltin 1996).

**Distribution.** EUROPE: **Type location:** Finland, Lake Julma Olkky (Lange-Bertalot and Metzeltin 1996); Poland (Bąk et al. 2012); Russia (Kulikovskiy et al. 2016); Ukraine (present paper). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Fontinalis* sp.

**Comments.** Specimens with straight valve outline and subcapitate poles presented in Costa (2015: p. 55, pl. 14/figs 1–11) differ from type population significantly, therefore the author has presented other species under this name. In Bąk et al. (2012) morphology of only three exemplars corresponds to type population.



**Figures 4–6b.** New in Ukraine species *Eunotia genuflexa* Nörpel-Schempp in Lange-Bertalot and Metzeltin, outside valve surface, SEM. Arrows indicate distal ends of raphe slits, double arrows – funnel-like lacunae, terminal fissures are between the arrows. Scale bars: 10 µm (**4–6**); 2 µm (**4a, 5a, 6a**); 1 µm (**6b**).

Type species of the genus *Eunotioforma* Kociolek & Burliga is *Eunotioforma mat-togrossiana* Kociolek, Burliga & Salomoni (Burliga et al. 2013) has several characters that differ from the genus *Eunotia*: axial area on valve surface (= sternum), large heli-

ctoglossae, different number of rimoportulae (2–8 per valve), small granules along all striae which are not present in *E. genuflexa*. Strongly curved long tr-fissures are the only common character with a new genus and it can be found in some other species of *Eunotia*, for instance, *E. flexuosa* (Bréb.) Kütz. (Pavlov and Levkov 2013: pl. 7/fig. 2), *E. macedonica* Pavlov & Levkov (Pavlov and Levkov 2013: pl. 10/fig. 2). The only one character is not enough for transferring of *E. genuflexa* to the genus *Eunotioforma*.

# *Eunotia implicata* Nörpel-Schempp et al. in Alles et al. 1991: p. 206, pl. 7/figs 19-32.\*

Figs 15, 16

Eunotia impressa var. angusta Grunow in Van Heurck, 1881: pl. 33/fig. 22 [Basionym]

**Lectotype.** *Eunotia impressa* var. *angusta* Grunow in Van Heurck 1881: pl. 33/fig. 22 (= Fig. 14 here), designated here.

**Illustrations.** Krammer and Lange-Bertalot 1991: p. 197, pl. 143/figs 1–7; Ortiz-Lerín and Cambra 2007: p. 424, pl. 3/fig. C, pl. 4/figs B, I (SEM); Furey et al. 2011: p. 50, pl. 28/figs 1–8; Lange-Bertalot et al. 2011: p. 119, pl. 97/figs 1–39, pl. 225/figs 16–19; Bąk et al. 2012: p. 133, pl. 16/1, 5 exemplars from left to right; Levkov and Pavlov 2013: p. 25, pl. 56/figs 1–26, pl. 57/figs 4–7 (SEM), pl. 65/figs 1–4 (SEM); Costa 2015: p. 54, pl. 27/figs 1–7, pl. 28/figs 1–5 (SEM); Ector et al. 2015: p. 251, 30 exemplars; Bahls et al. 2018: pl. 111/fig. 15.

**Diagnosis.** Morphometric data: length 26  $\mu$ m, width 3.5  $\mu$ m, striae density c18, p20 in 10  $\mu$ m. Alles et al. 1991: length 18–30  $\mu$ m, width 3–5  $\mu$ m, striae density 14–20 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves slightly dorsiventral, linear with weakly convex dorsal margin, concave ventral margin and protracted rounded poles. The mantle's height is equal to about 0.5 of valve width, abruptly perpendicular to the valve surface (see Costa 2015: pl. 28/figs 2, 4). Striae basal, uniserial, distant, uniformly spaced along the valve and compacted at the poles, uninterrupted on dorsal mantle/valve junction and interrupted by sternum on ventral mantle; on dorsal mantle short intercalar striae present (see Costa 2015: pl. 28/figs 2, 4). Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral hyaline part of mantle, distal ends of the slits turned to the valve centre under right angle and finish on external valve surface in ventral corner of the poles by small round pores (see Costa 2015: pl. 28/figs 1, 2) connected with helictoglossae; tr-fissures absent.

**Ecology.** Freshwater, acidophilus, epiphytic species, inhabits moss vegetation, green filamentous algae. In Spain the species was collected in habitats with pH 4.3–7.9, conductivity  $4.17-720 \ \mu$ S/cm, the altitude 76–1356 m asl, SPI 12.3–20. Optimum conditions with pH 5.3–6.8, conductivity 28.7–51  $\mu$ S/cm, the altitude 472–624 m asl, SPI 19.3–19.7 (Ortiz-Lerín and Cambra 2007).

**Distribution.** EUROPE: Britain, France, Germany, Netherlands (M. Gury in Guiry and Guiry 2019); Macedonia (Pavlov and Levkov 2013), Poland, Romania, Slovakia, Spain (M. Gury in Guiry and Guiry 2019); Ukraine (present paper). AFRICA: Ghana. ASIA: Russia. AUSTRALIA: Australia, New Zealand. N. AMERICA: Canada, USA. S. AMERICA: Argentina, Brazil, Colombia (M. Gury in Guiry and Guiry 2019). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Fontinalis* sp.

**Comments.** Illustration of *E. impressa* var. *angusta* in Van Heurck (1881: pl. 35/ fig. 1) is not conspecific to *E. implicata* sensu Nörpel-Schempp et al. (in Alles et al. 1991) as it has depression on dorsal margin and the poles turned to dorsal valve side. In many literature sources the illustrations of this species are not uniform in valve outline and often do not correspond to the species lectotype.

## Eunotia incisa W. Smith ex Gregory, 1854: p. 96, pl. 4/fig. 4.\*

Figs 21-23

Lectotype. *Eunotia incisa* W. Smith ex Gregory, 1854: pl. 4/fig. 4. (= Fig. 22 here), designated here.

**Illustrations.** Krammer and Lange-Bertalot 1991: p. 221, pl. 161/figs 8–19, pl. 162/figs 1–2 (SEM), pl. 163/figs 1–7; Ohtsuka 2002: fig. 62; Ortiz-Lerín and Cambra 2007: p. 424, figs 5/O-R, 6/C, K; Taylor et al. 2007: pl. 20/5 exemplars; Furey 2011: 7 exemplars and 2 (SEM); Bak et al. 2012: p. 134, pl. 16/8 exemplars; Ector et al. 2015: p. 254–256, 25 exemplars; Costa 2015: p. 55, pl. 22/figs 1–21, pl. 23/figs 1–5 (SEM); Bahls et al. 2018: pl. 23/figs 19–28, pl. 40/figs 2,3; pl. 87/figs 11, 12; pl. 112/figs 17–19.

**Diagnosis.** Morphometric data: length 17–27  $\mu$ m, width 3.5–4.0  $\mu$ m, striae density 19–22 in 10  $\mu$ m. Costa 2015: length 18–43  $\mu$ m, width 3.0–4.5  $\mu$ m, striae density 18–21 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe, in girdle view rectangular. Valves dorsiventral with convex dorsal, straight ventral margins and gradually contracted acutely rounded poles turned to ventral valve side. Dorsal mantle arcuate with uninterrupted striae; ventral mantle abruptly perpendicular to the valve surface, hyaline, its height is about 0,5 of valve width (see Costa 2015: pl. 23/figs 1, 3, 5). Striae basal, uniserial, distant, gradually compacted from valve center to the poles (see Costa 2015: pl. 23/fig. 1). Areolae small with round outer foramina. Raphe system consists of two short filiform arcuate slits on hyaline area of ventral valve mantle, on external valve surface distal ends of the slits finish by distant from the poles round funnels on valve/mantle junction; central raphe pores are funnel-like; tr-fissures absent. One apical rimoportula has round external opening (see Costa 2015: pl. 23/figs 3, 5).

**Ecology.** Freshwater benthic species occurs in upland streams in acidic, xenooligosaprobic waters with poor electrolytes content (Ortiz-Lerín and Cambra 2007, Taylor et al. 2007). In rivers and streams of Northern Spain has been recorded highest abundance between 7–10% in conditions with pH 5.3–6, conductivity 38–51  $\mu$ S/ cm, altitude 472–484 m asl, SPI 19.3–19.7 (Ortiz-Lerín and Cambra 2007). The species was found both in oligo- and eutrophic waters: total phosphorus < 71.4 mg/cm<sup>3</sup>, conductivity 13–142  $\mu$ S/cm and pH 5.3–9.3. High abundances of *E. incisa* reported from eutrophic conditions are in disagreement with other literature data (Costa 2015).

**Distribution.** EUROPE: Baltic Sea, Belgium, Britain, Czech Republic, Finland, France, Germany, Ireland, Italy, Macedonia, Netherlands, Poland, Romania, Russia, Spain (M. Gury in Guiry and Guiry 2019); Ukraine (present paper). N. AMERICA: USA, Canada (M. Gury in Guiry and Guiry 2019). S. AMERICA: Brazil, Colombia. AFRICA: South Africa (Taylor et al. 2007); Ghana, Sudan (M. Gury in Guiry and Guiry 2019). ASIA: India, Israel; Bering Island, Korea, Russia, Singapore (M. Gury in Guiry and Guiry and Guiry 2019); Japan (Ohtsuka 2002). AUSTRALIA: New Zealand, Australia (M. Gury in Guiry and Guiry 2019). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Fontinalis* sp.

**Comments.** Distal ends of the raphe slits are clearly visible on the valve/mantle ridge in LM photos, which is a valuable character in species identification.

## *Eunotia jarensis* Lange-Bert. et al., 2003: p. 41, pl. 124/figs 7–11.\*^ Figs 12, 12a

Illustrations. Pavlov and Levkov 2013: p. 13, pl. 13/figs 1-5.

**Diagnosis.** Morphometric data: length 63  $\mu$ m, width 9  $\mu$ m, striae density c12, p18 in 10  $\mu$ m. Lange-Bertalot et al. 2003: length 36–76  $\mu$ m, width 8–10  $\mu$ m, striae density 10–12 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, uniform in width, with two very weak undulations on dorsal margin, weakly concave ventral margin and protracted broadly rounded poles slightly deflected to dorsal side. Striae basal, uniserial, distant, evenly spaced. Areolae small with round foramina (Fig. 12a). Raphe system consists of two short filiform slits on ventral valve mantle; helictoglossae average in size; one rimoportula present at the middle of pole (Fig. 12a).

**Ecology.** Freshwater species, epiphytic on the moss, at an altitude of about 2300 m a.s. (Pavlov and Levkov 2013).

**Distribution.** EUROPE: **Type locality:** ITALY, Pauli Murdegu, Insula Sardinia (Lange-Bertalot et al. 2003); North Macedonia, Shara Mountain, stream above glacial Lake Crno (Pavlov and Levkov 2013); Ukraine (present paper). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Type information.** 25.05.1994, leg. A. Bardi, (Praep. No. Eu-I-159 in Coll. Lange-Bertalot, Botan. Institut Universität Frankfurt a.M.).

**Comments.** Raphe system has not been studied from outer valve surface in SEM but this species certainly does not have tr-fissures which are always situated on a hya-



**Figures 7–12a.** New and rare in Ukraine species of *Eunotia* Ehrenb. from the Cheremsky Nature Reserve. **7–8a** *Eunotia julma* Lange-Bert. in Lange-Bertalot et al., arrow indicates distal end of raphe slit, double arrow – funnel-like lacuna, terminal fissure is between the arrows **9** *Eunotia* sp. 1 (cf *E. formica* Ehrenb.) **10, 11** *Eunotia formicina* Lange-Bert. in Lange-Bertalot et al. **12, 12a** *Eunotia jarensis* Lange-Bert. et al., arrow indicates rimoportula. Figs **7, 9–11** LM **8, 8a** outside valve surface **12, 12a** inside valve surface, SEM. Scale bars: 10 µm (**7–17**); 3 µm (**8a**); 2 µm (**12a**).

line field (Fig. 12a) and distal ends of the raphe slits finish on ventral valve margin at the poles, similar to *E. dorofeyukiae* (Fig. 27a).

Most specimens in the population from North Macedonia differ through having much narrower poles (Pavlov and Levkov 2013: pl. 13/figs 3–6) than in the type population.

Very rare species occurs only in Europe in three localities in low abundance.

*Eunotia julma* Lange-Bert. in Lange-Bertalot et al. 2011: pl. 7/figs 1–7, 8–10.\*^ Figs 7, 8, 8a (SEM)

Holotype. Lange-Bertalot et al. 2011: pl. 7/fig. 1, designated by Lange-Bertalot in Lange-Bertalot et al. 2011.

**Illustrations.** Potapova et al. 2014: fig. 1; Kulikovskiy et al. 2016: p. 122, pl. 27/ figs 14–17; Bouchard et al. 2018: pl. 1/fig. 1.

**Diagnosis.** Morphometric data: length 115–175  $\mu$ m, width 6  $\mu$ m, striae density c12, p16 in 10  $\mu$ m. Lange-Bertalot et al. 2011: length 70–150  $\mu$ m, 4,5–5  $\mu$ m, striae density c14, p16 in10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, uniform in width, arcuate, with rounded poles. Striae basal, uniserial, distant, evenly spaced (Figs 7, 8, 8a). Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle; tr-fissures curved on the valve surface, pass along four striae on the middle of valve and end up by round lacunae (Fig. 8a).

Ecology. Freshwater epiphytic species.

**Distribution.** EUROPE: **Type locality:** Finland, Lake Julma Olkky near Kuusamo (Lange-Bertalot et al. 2011); Netherlands (M. Gury in Guiry and Guiry 2019); Ukraine (present paper). ?ASIA: Russia, Eastern Siberia (Potapova et al. 2014); Russia (Kulikovskiy et al. 2016). N. AMERICA: Canada (Bouchard et al. 2018). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Comments.** In primary description it is indicated that " ... all specimens are consistently curved" (Lange-Bertalot et al. 2011). Our exemplars correspond to the species Holotype in valve outline and morphometry except our specimens are longer and wider. The illustrations of *E. julma* in Potapova et al. (2014: fig. 1), Kulikovskiy et al. (2016: pl. 27/figs 14–17), Bouchard et al. (2018: pl. 1/fig. 1) differ from the Holotype by almost straight valves.

# *Eunotia neocompacta* S. Mayama in Mayama and Kawashima 1998: p. 69. Figs 30, 30a (SEM)

*Eunotia exigua* var. *compacta* Hustedt, 1930: p. 176, fig. 225 [Basionym] *Eunotia compacta* (Hustedt) S. Mayama, 1997 *Eunotia neocompacta* var. *vixcompacta* Lange-Bert. in Lange-Bertalot et al. 2011 **Illustrations.** Grunow in Van Heurck 1881: pl. 34/fig. 8 – second exemplar; Krammer, Lange-Bertalot 1991: pl. 134/figs 32, 35–38 (both citations as *Eunotia nymanniana* Grunow). Mayama 1997: p. 35, figs 22–25, 26–31 (SEM) (as *E. compacta*). Lange-Bertalot et al. 2011: p. 173–174, pl. 123/figs 1, 2–16, 25–28, 32–34 (SEM); Bąk et al. 2012: pl. 19/1, 2 exemplars from left to right; Genkal and Komulaynen 2015: fig. 2π; Rivera-Rondón and Catalan 2017: pl. 33/figs 15–19 (all citations as *E. neocompacta* var. *viscompacta*). Kulikovskiy et al. 2016: p. 126, pl. 25/figs 7–11; Mimura and Ohtsuka 2016: fig. 34; Bahls et al. 2018: pl. 83/figs 8, 9.

**Diagnosis.** Morphometric data: length 27  $\mu$ m, width 3.5  $\mu$ m, striae density 20 in 10  $\mu$ m.

Mayama 1997: length 18–57 µm, width 3.5–5 µm, striae density 20–22 in 10 µm.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, weakly arcuate, uniform in width, with truncated poles strongly deflected to dorsal side. Striae basal, uniserial, distant, evenly spaced (Fig. 30a). Areolae small with round outer foramina. Raphe system consists of two short filiform slits which are straight on ventral valve mantle and widely rounded at valve poles, distal ends of the slits finish on external valve surface on the middle of the poles by small round pores connected with helictoglossae of average size (Fig. 30a; see Mayama 1997: fig. 31); tr-fissures absent (see Mayama 1997: fig. 31).

Ecology. Freshwater epiphytic species.

**Distribution.** EUROPE: Georgia, Ireland, Netherlands, Poland, Romania, Ukraine (M. Gury in Guiry and Guiry 2019); France, Pyrenees, Lake Monges (Rivera-Rondón and Catalan 2017); Russia (Genkal and Komulaynen 2015: as *E. neocompacta* var. *vixcompacta*). N. AMERICA: USA, Alaska, Atlantic Islands (M. Gury in Guiry and Guiry 2019); Canada (Bahls et al. 2018). ASIA: Japan (Mimura and Ohtsuka 2016); Russia (Kulikovskiy et al. 2016); Russia, Bering Island (M. Gury in Guiry and Guiry 2019). **In Ukraine.** First record in the Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Sphagnum* sp.

**Comments.** In some publications the illustrations of this species are not uniform in valve outline, therefore only those microphotos which correspond to the species concept in Mayama (1997) are cited in the present paper. For instance, the specimen in Bouchard et al. (2018: pl. 1/fig. 6) has arcuate valve and longer poles therefore does not match to *E. neocompacta* sensu stricto.

Based on its morphology, *Eunotia neocompacta* var. *vixcompacta* (Lange-Bertalot et al. 2011) is conspecific with *E. neocompacta*, which has also been confirmed by other authors (Kulikovskiy et al. 2016, Mimura and Ohtsuka 2016, Bahls et al. 2018).

#### Eunotia praerupta Ehrenb., 1843: p. 414.

Fig. 31 (SEM)

*Eunotia praerupta* f. *curta* (Grunow) Mayer, 1917 *Eunotia bidens* var. *praerupta* (Ehrenb.) Aysel, 2005 **Illustrations.** Krammer and Lange-Bertalot 1991: pl. 148/figs 1–3; Pavlov and Levkov 2013: p. 35, pl. 18/fig. 7 (SEM), pl. 19/figs 1–10.

**Diagnosis.** Morphometric data: length 73  $\mu$ m, width 20  $\mu$ m; striae density c5, p8 in 10  $\mu$ m. Pavlov and Levkov 2013: length 37–73  $\mu$ m, width 13–17  $\mu$ m; striae density c5–9, p8–12 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, with strongly convex dorsal and weakly concave ventral margins, gradually narrowed to rostrate poles that are about perpendicular to the valve margins. Striae basal, uniserial, distant, irregularly spaced. Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle, distal ends of the slits finish on external valve surface on about 0.3 of pole width by small round pore-like outer connected with helictoglossae (see Pavlov and Levkov 2013: pl. 18/fig. 7); tr-fissures absent.

**Ecology.** Freshwater epiphytic species.

**Distribution.** Species was recorded in most European countries and on all continents except Antarctica (M. Gury in Guiry and Guiry 2019). **In Ukraine.** First record in the Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Comments.** No illustrations were published by the author of this species, which has led to a very wide species concept and uncertain taxonomy. In this paper the concept of *Eunotia praerupta* sensu stricto proposed in Krammer and Lange-Bertalot (1991: pl. 148/figs 1–3) has been followed.

# Eunotia pseudoflexuosa Hustedt, 1949: p. 71, pl. 2/figs 16-18.\*^

Figs 13, 13a

**Illustrations.** Simonsen 1987: p. 340, pl. 522/figs 1–6; Kulikovskiy et al. 2010b: pl. 26/figs 1, 2, 4, 5 (LM), 3, 6, 7 (SEM); Kulikovskiy et al. 2016: pl. 26/figs 5–8; Bahls et al. 2018: pl. 83/fig. 1, pl. 112/fig. 6.

**Diagnosis.** Morphometric data: length 112  $\mu$ m, width 4  $\mu$ m, striae density 11 in 10  $\mu$ m.

Kulikovskiy et al. 2016: length 80–180  $\mu m$ , width 4–6  $\mu m$ , striae density c11–14, p15–20 in 10  $\mu m$ .

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, uniform in width, with subcapitate poles deflected to dorsal side. Striae basal, uniserial, distant, evenly spaced (Fig. 13a). Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle; tr-fissures long, occupy 7–8 striae in the middle of valve width; helictoglossa average in size (Fig. 13a).

**Ecology.** Freshwater epiphytic species.

**Distribution.** AFRICA: **Type location:** [Democratic Republic of the Congo, Virunga National Park]<sup>‡</sup>, vulkan region, Lake Karisimbi. EUROPE: Russia (Kulikovs-

<sup>‡</sup> current geographic names are added to this paper

kiy et al. 2016); Ukraine (present paper). ASIA: Mongolia (Kulikovskiy et al. 2010b); Russia (Kulikovskiy et al. 2016). N. AMERICA: Canada (Bahls et al. 2018); USA, Alaska (M. Gury in Guiry and Guiry 2019). **In Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

Type information. "Albert-National park in Belgisch-Kongo."

## *Eunotia ruzickae* Bílý & Marvan, 1962: p. 293, figs 1–5.\*^ Fig. 14

Illustrations. Pavlov and Levkov 2013: pl. 58/fig. 7.

**Diagnosis.** Morphometric data: length 44  $\mu$ m, width cp6, m7  $\mu$ m; striae density c16, p22 in 10  $\mu$ m. Bílý and Marvan 1962: length 40–90  $\mu$ m, width 4.5–6  $\mu$ m, striae density 13–14 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, uniform in width, with slightly undulate dorsal margin and weak depression in its central part, usually straight ventral side, sometimes with weak central convexity (see Bílý and Marvan 1962: fig. 1), and broad rounded poles deflected to dorsal side. Striae basal, uniserial, distant, denser at the poles, irregularly spaced. Raphe system consists of two short filiform slits on ventral valve mantle, distal ends of the slits terminate at the poles about 0.3 of valve width from ventral margin.

Ecology. Freshwater epiphytic species.

**Distribution.** EUROPE: **Type location:** Czech Republic (Bílý and Marvan 1962); North Macedonia, (Pavlov and Levkov 2013); Germany, Scandinavia (M. Guiry in Guiry and Guiry 2019); Ukraine (present paper). **In Ukraine.** Zhytomyr region, Chervonoarmeisky district, swamp, dark films among mosses.

**Type information.** "In bentho piscinae Řežabinec prope vicum Ražice in Bohemia meridionali atque in nonnullis locis Moraviae merdionalis", (Typus in herbario Inst. bot. Univ. Brunensis, Brno).

**Comments.** The found exemplar has denser striae than in type population. This species has typical raphe system without terminal raphe fissures, however SEM illustrations of the raphe to confirm this were not found.

### Eunotia tetraodon Ehrenberg, 1838: p. 192, pl. 21/fig. 25.

Figs 34, 35

Himantidium tetraodon (Ehrenb.) Bréb. ex Kützing, 1849 Eunotia robusta var. tetraodon (Ehrenb.) Ralfs, 1861 Eunotia diadema var. tetraodon (Ehrenb.) A. Cleve, 1953 Eunotia serra var. tetraodon (Ehrenb.) Nörpel in Krammer and Lange-Bertalot 1991

**Illustrations.** Topachevsky and Oksiyuk 1960: p. 323, pl. 119/fig. 1 (as *Eunotia robusta* Ralfs); Bahls 2012: 6 exemplars; Bąk et al. 2012: pl. 15/4 exemplars; Ector et



Figures 13–27. New and rare in Ukraine species of *Eunotia* Ehrenb. from Ukrainian Polissya and the Lectotypes of two *Eunotia* species. 13, 13a *Eunotia pseudoflexuosa* Hust., arrow indicates rimoportula at the middle of pole where raphe slit distal end finishes on the outer valve surface, double arrow – position of terminal fissure distal end on the outer valve surface, between the arrows – hyaline field from inside valve surface where the terminal fissure is located on the outer valve surface 14 *Eunotia ruzickae* Bílý & Marvan 15, 16 *Eunotia implicata* Nörpel-Schempp et al. Lectotype: 15 = *Eunotia impressa* var. *angusta* Grunow in Van Heurck 1881: pl. 33/fig. 22, designated here 17, 17a *Eunotia* sp. 2 (cf. *mongolica* Kulikovskiy et al.) 18–20 *Eunotia incisa* W. Smith ex Gregory 1854: pl. 4/fig. 4, designated here 24 *Eunotia* sp. 4 (cf. *Eunotia intermedia* (Krasske ex Hustedt) Nörpel & Lange-Bert.) 25–27 *Eunotia* sp. 5 (cf. *Eunotia meridiana* Metzeltin & Lange-Bert.). Figs 13, 17, 18 SEM: 13, 13a inside valve surface 17, 17a, 18 outside valve surface; 19–28 LM. Scale bars: 10 µm (13–17, 19–27); 5 µm (18); 1 µm (13a, 17a).

al. 2012: p. 270–271, 1 exemplar; Pavlov and Levkov 2013: pl. 16/figs 1–9, 10, 11 (SEM); Kulikovskiy et al. 2016: p. 133, pl. 29/figs 6–10; Malakhov et al. 2017: p. 67, fig. 10 (SEM); Bahls et al. 2018: pl. 21/fig. 2.

**Diagnosis.** Morphometric data: length 40  $\mu$ m, width c8–10, m10–13, p4–8  $\mu$ m; striae density c11–15, p16 in 10  $\mu$ m. Pavlov and Levkov 2013: length 25–62  $\mu$ m, width 9.5–16  $\mu$ m, striae density 6–10, m12–16 in 10  $\mu$ m.

Frustule bi-symmetric, bipolar, biraphid with mirror-symmetric, mantle-offset, brevisslit type of raphe. Valves dorsiventral, with strongly convex, four-times strongly undulate dorsal and weakly concave ventral margins, gradually narrowed to the protracted poles that continue the dorsal arc of valve margin. Striae basal, uniserial, distant, irregularly spaced, on dorsal side shortened intermediate striae present. Areolae small with round outer foramina. Raphe system consists of two short filiform slits on ventral valve mantle that follow pole margin and finish on about 0.5 of pole width by small round pore (see Pavlov and Levkov 2013: pl. 26/fig. 11) connected with helictoglossae; tr-fissures absent.

Ecology. Freshwater epiphytic species.

**Distribution.** Species was recorded in most European countries and on all continents except Antarctica (M. Gury in Guiry and Guiry 2019). **In Ukraine.** Volyn region, Manevychi district, Lake Bile; Rivnenska region, Bog Gala (Topachevsky and Oksiyuk 1960 – as *Eunotia robusta* Ralfs); Volyn region, the Cheremsky Nature Reserve, Lake Redychi (Malakhov et al. 2017); Lake Redychi, epiphyton on *Fontinalis* sp.

**Comments.** In Ukraine this species is quite rare. Only five reliable records exist that were accompanied by illustrations, including this paper, and all are from Ukrainian Polissya and Carpaty. Initially this species was reported by Topachevsky and Oksiyuk (1960) as *E. robusta* however their illustration corresponds to *E. tetraodon*. The next reports came almost 60 years later (Malakhov et al. 2017, present paper).

## *Eunotia* sp. 1 (cf. *Eunotia formica* Ehrenberg, 1843: p. 414) Fig. 9

**Morphometric data.** Length 117 µm, width c8, m6, p10 µm; striae density c9, p11 in 10 µm.

**Distribution in Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Sphagnum* sp.

**Comments.** This specimen is most similar to *E. formica* which is widely distributed and has been found on all continents except Antarctica (Gury M in Guiry and Guiry 2019). The original illustrations of *E. formica* by Ehrenberg were not introduced in primary description and the species concept in literature is different from our exemplar, which has narrower valves and rhombic poles (see Krammer and Lange-Bertalot 1991: p. 209, pl. 152/8–12a; Lange-Bertalot and Metzeltin 1996: p. 144, pl. 13/1, 2; Ortiz-Lerín and Cambra 2007: p. 424, fig.3/T; Taylor et al. 2007: pl. 22/figs 1, 3).



Figures 28–35. Species of *Eunotia* Ehrenb. from the Cheremsky Nature Reserve. 28, 28a, 29 *Eunotia* dorofeyukiae Lange-Bert. & Kulikovskiy 28a arrow indicates pore outer on the raphe slit distal end 29 arrow indicates rimoportula at the venral corner of pole where raphe slit distal end finishes on the outer valve surface 30, 30a *Eunotia neocompacta* S. Mayama 30a arrow indicates rimoportula at the venral corner of pole, absence of a hyaline field indicates absence of terminal fissure 31 *Eunotia praerupta* Ehrenb. 32 *Eunotia* sp. 6.
33 *Eunotia* sp. 7. 34, 35 *Eunotia tetraodon* Ehrenb. Figs 28–31 SEM: 28, 28a outside valve surface; 29–31 inside valve surface; 32–35 LM. Scale bars: 5 μm (28, 29, 30); 2 μm (28a); 1 μm (30a); 10 μm (31–34).

*Eunotia* sp. 2 (cf. *Eunotia mongolica* Kulikovskiy et al. 2010b: p. 124, pl. 40/figs 1–5) Figs 17, 17a (SEM)

Illustrations. Kulikovskiy et al. 2016: p. 124, pl. 28/figs 26-30.

**Morphometric data.** Length 73 μm, width 3.5 μm, striae density c16, p20 in 10 μm. Kulikovskiy et al. 2016: length 35–55, width 2.3–2.7, striae density c19, p23.

**Distribution in Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, Lake Redychi, epiphyton on *Sphagnum* sp.

**Comments.** This specimen is similar to *E. mongolica* in valve outline, however it differs in metric parameters and fine morphology, having shorter tr-fissures (Fig. 17a).

## *Eunotia* sp. 3 (cf. *Eunotia paludosa* Grunow, 1862: p. 336, pl. 3/fig. 10a–d) Figs 18–20

**Morphometric data.** Length 19–24 μm, width 3.5–4 μm; striae density c14–16, p22 in 10 μm.

**Distribution in Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Comments.** These specimens correspond to the current literature concept of *E. paludosa* in valve outline of small specimens (see Furey 2012: figs 4–6 from left; Pavlov and Levkov 2013: pl. 42/figs 23–38; Costa 2015: pl. 17/figs 1–10; Kulikovskiy et al. 2016: pl. 19/figs 8, 9). However, the illustrations by Grunow (1862: p. 336, pl. 3/ figs 10a–c) show a much more arcuate valve outline that differs significantly from the literature concept.

The other species whose small specimens have similar valve outline to the discovered specimens was reported as *Eunothia fennica* (Hustedt) Lange-Bert. in Werum and Lange-Bertalot (2004) by Noga (2019: only figs 2e–g, 3c–e). However, the detail study of *E. fennica* (Hamilton and Siver 2010: figs 1–11, 16–29) shows a clear difference from the specimens on Figs 17–19 and the ones, cited from Noga (2019): in valve outline, presence of spines, morphonetric data. Hamilton and Siver (2010) also have underlined that *E. fennica* can be confused with *E. paludosa*, more over, the authors found both species in the same sample.

In Topachevsky and Oksiyuk (1960) *E. paludosa* was considered a synonym of *Eunotia exigua* (Bréb. ex Kütz.) Rabenh. but both species are valid taxa at present.

# *Eunotia* sp. 4 (cf. *Eunotia intermedia* (Krasske ex Hustedt) Nörpel et al. 1993: p. 32) Fig. 24

**Morphometric data.** Length 23 µm, width 4 µm, striae density 11 in 10 µm.

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tral mantle,	VM+V ventral	mantle +	valve surface, VI	P valve	: pole, – absent, U	<b>JS</b> unstudied			unengunen (n	a hora	101341 314C)	
Species		Valve outlir	ne	Kind	Striae arrangement			Raphe system	on outer valve surfa	ICe		
	Dorsal margin	Ventral margin	Shape of the poles	of striae	1	Shape of slits	Dispo- sition of slits	Shape of distal ends of the slits	Disposition of distal ends of the slits	Terminal fissures shape	Disposition of terminal fissures	Shape of central pores
E. dorofevukiae	undulate	slightly concave	subcapitate, broad rounded	dis- tant	denser at the poles	N	V+MV	small round funnels	ventral VP corners	•	I	SU
E. implicata	weakly convex	concave	protracted, rounded	dis- tant	irregularly spaced, denser at the poles	NS	V+MV	turned to the valve centre under right corner, finish by small round pores	ventral VP corners	I	I	SU
E. incisa	weakly convex	Straight	gradually contracted acutely rounded	dis- tant	gradually compacted to the poles	arcuate	MV	round funnels	ventral margin	I	I	funnel-like
E. jarensis	uniform width, weakly convex	weakly concave	protracted, broadly rounded	dis- tant	evenly spaced	SU	V+MV	SU	ventral VP corners	SU	SU	SU
E. neocompacta	uniform width, weakly convex	weakly concave	truncated, strongly deflected to DS	dis- tant	evenly spaced	straight on VM, rounded at VP	V+MV	small round pores	middle of VP	I	I	funnel-like
E. praerupta	strongly convex	weakly concave	rostrate	dis- tant	irregularly spaced		V+MV	small round pores	ventral VP corners	I	I	SU
E. ruzickae	slightly undulate	straight	broad round, deflected to DS	dis- tant	irregularly spaced, compacted to the poles	SU	V+MV	SU	ventral VP corners	SU	SU	N
E. tetraodon	strongly convex, four-times strongly undulate	weakly concave	protracted,	dis- tant	irregularly spaced,	SU	V+MV	small round pores	middle of VP	I	I	N
E. formicina	weakly convex	weakly concave, gentle gibbosity	subcapitate, broad rounded	dis- tant	irregularly spaced	NS	V+MV	dd-pores	ventral VP corners	long, round	follow poles outline, finish on DM	round
E. genuflexa	weakly convex	weakly concave	narrowed rounded	dis- tant	evenly spaced	SU	V+MV	dd-pores	ventral VP corners	long, round, with lacunae	mid-valve	SU
E. julma	uniform width, convex	concave	round	dis- tant	evenly spaced	SU	V+MV	dd-pores	I	long, round, with lacunae	mid-valve	SU
E. pseudo- flexuosa	uniform width, weakly convex	weakly concave	subcapitate, deflected to DS	dis- tant	evenly spaced	SU	V+MV	SU	NS	long	mid-valve	SU

**Distribution in Ukraine.** Volyn region, Vladimir-Volyn district, near village Fedorovka, Western Bug River, floodplain basin, benthos.

**Comments.** This exemplar has more curved valves and non-narrowed poles and differs in valve outline from *E. intermedia* (see Krammer and Lange-Bertalot 1991: p. 215, pl. 143/figs 11, 13; Jüttner et al. 2019: 7 exemplars).

# *Eunotia* sp. 5 (cf. *Eunotia meridiana* Metzeltin & Lange-Bertalot, 1998: p. 67, pl. 59/figs 7–10). Figs 25–27

Figs 25–27

Morphometric data. Length 17–18 μm, width 3–3.5 μm, striae density 21 in 10 μm. Distribution in Ukraine. The Cheremsky Nature Reserve, tract Obkopane, Lake

Redychi, epiphyton on Sphagnum sp

**Comments.** These exemplars differ from *E. meridiana* in valve outline, narrower valves and higher striae density.

#### Eunotia sp. 6.

Fig. 32

Morphometric data. Length 70 µm, width 12 µm, striae density c7, p11 in 10 µm.

**Distribution in Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

*Eunotia* sp. 7. Fig. 33

Morphometric data. Length 40 µm, width 12 µm, striae density 10 in 10 µm.

**Distribution in Ukraine.** The Cheremsky Nature Reserve, tract Obkopane, ditch, epiphyton on *Sphagnum* sp.

**Comments.** Both *Eunotia* sp. 6 and *Eunotia* sp. 7 have similar morphology, valve width and striae density. Moreover, both exemplars were found in the same sample, which may suggest that they belong to the same species, but insufficient data does not provide a conclusion at present.

### **Discussion and conclusions**

The genus *Eunotia* is one of the largest within the Order Bacillariophyta and totals 589 valid taxa. This can be considered as an evolutionary success of the genus, relevant to the frustule morphology that is well suited to the ecological conditions where the *Eunotia* species inhabit.

The presence of raphe system is certainly a progressive feature which has appeared in the diatom frustule evolution since the number of species bearing it exceeds significantly the ones without raphe.

The complicated morphology of *Eunotia* species has led to the numerous synonyms – more than 60% of taxonomic names (Guiry M in Guiry and Guiry 2019). Therefore it is a necessary task to find reliable morphological characters which can be useful in species identification and description.

The genus *Eunotia* possesses of mirror-symmetric, mantle-offset, brevisslit raphe system the combination of the characters in which is unique among diatom genera. At the same time different *Eunotia* species have peculiar details in the raphe system which belong to species rank of taxonomy: presence / absence of tr-fissures and their shape, shape of raphe slits and their position on the valve etc. (Table 1).

The morphological analysis carried out in this study revealed that 8 of 12 studied species of *Eunotia* do not have terminal raphe fissures (Table 1). Instead, the distal ends of the raphe slits terminate on the outer valve surface by pore-like (Figs 3c, 28a, 30) or funnel holes (Fig. 3d, see Mayama 1997: fig. 31) which are connected with helicto-glossae. However, in the literature those distal ends of the raphe slits were erroneously described as tr-fissures (e.g. Pavlov and Levkov 2013: p. 20, pl. 18/fig. 7; p. 43, pl. 26/fig. 11). *Eunotia krammeri* Kulikovskiy et al. also has a raphe system without tr-fissures that is clearly visible on SEM illustrations both from external and internal valve surfaces (Kulikovskiy et al. 2010a: p. 102, pl. 7/figs 18, 19). Nevertheless, the authors report them in their description of this species.

In morphology of *Eunotia* the characters suitable for the species identification are quite restricted. Besides, the frustule ultrastructure is poorly studied even for the species which were investigated with SEM, in particularly, raphe system. For many species there is still no data on the slits form, central pores etc. In the meantime, among key diagnostic characters for the *Eunotia* species identification more than half include the ones which refer to the peculiarity of raphe system (Table 1). For instance, central raphe pores usually have a different shape on the inner and outer valve surfaces, however their shape on inner surface is uniform within the genera and some taxa of higher rank of taxonomy while the shape of central pores on the outer valve surface has the species rank of taxonomy (Bukhtiyarova and Pomazkina 2013).

Thus, careful application of suggested terms in future is necessary when describing new *Eunotia* taxa and will be helpful in correct identification of the known species.

Species of *Eunotia* prefer acidic, dystrophic or oligotrophic freshwater habitats, mostly of low conductivity and usually inhabit in epiphytic or epilithic hydrotopes (Alles et al. 1991, Metzeltin and Lange-Bertalot 1998, Siver et al. 2006, Cantonati and Lange-Bertalot 2011, Pavlov and Levkov 2013, Bahls et al. 2018). In Ukraine 32 species and eight varieties of *Eunotia* were known until this study and now 9 more species are reported for the first time. Thus, the total number of *Eunotia* species in Ukraine is 41, which is only 7% of the species in this genus worldwide. This is indirect evidence of insufficient investigation of the wetlands in Ukraine where *Eunotia* has high species richness. The findings in the present study include five species widely distributed in the world flora on most continents and seven rare species that

are known from several locations, among the latter are *E. genuflexa, E. jarensis* and *E. ruzickae*, which are probably European endemics. At present, in the Cheremsky Nature Reserve, 20 species have been recorded, which is the largest number of *Eunotia* species in any region of Ukraine. In total 19 *Eunotia* species were recorded in this study together with the ones which were not identified to the species level. Given the large number of poorly studied oligotrophic lakes and bogs in the country, especially in Ukrainian Polissya, it is possible to predict the future discovery of many more *Eunotia* species from Ukraine.

#### Acknowledgement

This research has been carried out under the scientific project N° 0117U004321 of the National Academy of Sciences of Ukraine. The author is extremely grateful to Dr Loren Bahls for his kind help with English language corrections and for his many valuable comments, to every reviewer and the editor Dr Kalina Manoylov for their careful patient work with the manuscript and for many suggestions which significantly improved final version of this paper.

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PhytoKeys 128: 33–38 (2019) doi: 10.3897/phytokeys.128.35446 http://phytokeys.pensoft.net

RESEARCH ARTICLE



# A new variety of Didymocarpus (Gesneriaceae) from Guangdong, China

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Academic editor: Eric Roalson   Received 14 April 2019   Accepted 28 June 2019   Published 23 July 2019
Citation: Xu W-J, Qin W-H, Wang Z-Q, Li Z-L, Fu L-F, Hong X (2019) A new variety of <i>Didymocarpus</i> (Gesneriaceae)
from Guangdong, China. PhytoKeys 128: 33–38. https://doi.org/10.3897/phytokeys.128.35446

#### Abstract

A new variety of *Didymocarpus*, *D. heucherifolius* var. *gamosepalus* from Guangdong, China, is described and illustrated with photographs. It closely resembles the more widespread *D. heucherifolius* within a number of morphological characters. However, it can be easily distinguished from the latter according to the new taxon: calyx base connate, 5-lobed from middle to above middle, larger flowers (up to 5 cm long) and glabrous corolla.

#### Keywords

New variety, Didymocarpus, Gesneriaceae, China

<sup>\*</sup> These authors contributed equally to this work.

#### Introduction

*Didymocarpus* Wall. is comprised of 31 species in China at present. W.T. Wang examined the genus *Didymocarpus s.l.* and divided them into two Sections: Section *Didymocarpus* (herbs with stems) and Section *Heteroboea* (herbs without stems) W.T. Wang auct. non Benth. Section *Heteroboea* is regarded as a distinct group, characterised by a rosulate habit and having a thick rootstock (Burtt 1998; Weber and Burtt 1998). This Section has varied considerably over time, due to the difficulty in using molecular phylogenetic studies and morphological revisions. A few more species, from China, were recently transferred to *Petrocodon* Hance (Weber et al. 2011). *Didymocarpus cortusifolius* and *D. heucherifolius* were treated as species of *Chirita* Buch.-Ham. ex D. Don. (Weber et al. 2000), but *Chirita* was cancelled in 2011 (Wang et al. 2011, Weber et al. 2011). However, they still belong to the Section *Didymocarpus* before any further research is undertaken.

In March 2019, several *Didymocarpus* specimens without flowers were collected by the authors during field investigations in Guangdong province. The plant at first looked like *D. heucherifolius* because of its similar leaf shape and leaf hair morphology. Subsequently, the living plants were cultivated in the nursery of the Gesneriad Conservation Center of China (GCCC). After we observed and collected specimens with flowers, we were surprised to find that the flower structures of the two species were different. Measurements and morphological character assessments of the putative species were undertaken and described using the living material in the GCCC. All morphological characters were studied under dissecting microscopes and are described using the terminology presented by Wang et al. (1998).

#### **Taxonomic treatment**

# *Didymocarpus heucherifolius* Handel-Mazzetti var. *gamosepalus* X.Hong & F.Wen, sp. nov.

urn:lsid:ipni.org:names:77200427-1 Fig. 1

**Diagnosis.** Didymocarpus heucherifolius var. gamosepalus can be distinguished from D. heucherifolius var. heucherifolius by its calyx base connate, 5-lobed from middle to above middle, glabrous corolla ca. 5 cm long, stamens 2.3 cm from the base, staminodes 1.4–1.8 cm from the base and 3.6–4.3 cm pistil. It also can be distinguished from D. heucherifolius var. yinzhengii by its calyx base connate, 5-lobed from middle to above middle, stamens 2.3 cm from the base, staminodes 1, pistil 3.6–4.3 cm.

**Type.** CHINA. Guangxi Province, cultivated in the nursery of Gesneriad Conservation Center of China (GCCC), introduced from north of Guangdong Province: Pingyuan County, Meizhou City, growing in rocky crevices at the foot of a calcareous sedimentary rocky hill. 22 February 2019, flowering, *WF20190222-05* (holotype: IBK; isotype: AHU)



**Figure 1.** *Didymocarpus heucherifolius* var. *gamosepalus* **A** habit **B** habitat, showing Danxia landscape **C** cyme with flowers **D** corolla, showing outside glabrous **E** bracts and bracteoles **F** calyx, showing base of calyx connate **G** opened corolla, showing staminodes **H** pistil **I** stigma **J** stamens.

Description. Acaulescent perennial herb. Rhizome horizontal, 3-4 cm long, up to 1.5 cm thick, roots fibrous. Leaves 4–8 basal, clustered at the apex of the rhizome; clearly whorled, orbicular-ovate to triangular,  $3-9 \times 3.5-11$  cm, papery, base cordate, apex rounded, margin irregularly triangular denticulate, upper surface densely covered with eglandular short hairs and sparse long hairs, lower surface sparsely covered with short and long hairs confined to the veins; basal veins 4 or 5, lateral veins 3-4 on each side of midrib, palmate; petioles terete, 2-9.5 cm long, densely covered with fuscous hairs. Cymes 1-4, axillary, 4 to many flowered; peduncle 10-18 cm long, densely covered with brown villous, pedicel 1-2.5 cm long, with same indumentum as on the peduncle. Bracts 2, opposite, subulate to subulate-triangular, ca. 6 mm long, adaxially glabrous, abaxially puberulent, margin sparsely denticulate, densely ciliary villous; bracteoles 2, opposite, subulate, 2-3 mm long, indumentum same as bracts. Calyx actinomorphic, 6–6.5 mm long, shallowly 5-lobed to about two-thirds of the calyx length from the base, lobes equal, ca.  $2 \times 1.5$  mm, apices obtuse, margin sparsely denticulate, inside glabrous, outside white puberulent. Corolla zygomorphic, up to 5 cm; glabrous both inside and outside, pink to magenta, inside with two brightly yellow strips at throat. Tube funnel-shaped to tubular, 1.8-2.2 cm long, inflated in the middle, orifice 1-1.5 cm in diameter, base constricted; limb distinctly 2-lipped, adaxial lip 2-lobed to near middle, ca.  $0.6-0.8 \times 1.6$  cm, obliquely triangular, abaxial lip 3-lobed to base, lobes rounded or oblong, ca. 1.1 × 1.1 cm, more or less equal. Stamens 2, adnate to corolla ca. 2.3 cm above the corolla base; filaments 8-10 mm long, straight, swollen at middle, white, glabrous with glandules on the surface; anthers ca. 2 mm long, white bearded. Staminodes 3, adnate to 1.4–1.8 cm above base of corolla tube, 0.3–0.5 mm long, white, glabrous. Disc annular, ca. 1 mm high. Pistil 3.6-4.3 cm long, densely puberulent; ovary white, ca. 3.3-4 cm long, cylindrical, puberulent; style ca. 3 mm long; stigma 1, terminal, depressed-globose, centrally sunken, undivided, translucent. Capsule purplish-red when young, linear-cylindrical, puberulent, up to 9 cm.

**Etymology.** The specific epithet is derived from calyx 5-lobed from middle to above middle.

**Vernacular name.** Hé è Mín Gàn Cháng Shùo Jù Tái (Chinese pronunciation); 合 萼闽赣长蒴苣苔 (Chinese name).

**Distribution and habitat.** The new variety has so far been found only in the type locality, near Pingyuan County, Meizhou City, Guangdong Province. The landform of the type locality is Danxia landform, which is formed from red-coloured sandstones and conglomerates deposited by sedimentation from lakes and streams from mainly the Cretaceous age. The new variety is locally abundant and grows on moist and shaded rocky faces on the cliff in subtropical evergreen seasonal rain forest. The average temperature of Pingyuan County is about 21.7 °C and the average annual precipitation is over 1 600 mm. Flowering is from February to March.

**Notes.** *Didymocarpus heucherifolius* var. *gamosepalus* and the type variety, *D. heucherifolius* var. *heucherifolius*, share a number of similar vegetative characters, but the new variety differs from the latter in several morphological features, such as larger flowers and glabrous corolla, calyx base connate, 5-lobed from middle to above middle. A
detailed comparison of the diagnostic characters between *Didymocarpus heucherifolius* var. *gamosepalus* and other variety of *D. heucherifolius* is shown in Table 1.

There are nine species and two varieties in *Didymocarpus* Section *Heteroboea*, including an unpublished new species: *D. lobulatus* sp. nov. These species are mostly distributed in Eastern China, of which, more than 50% are distributed in the Zhejiang province (shown in Figure 2). The northernmost species is the *D. heucherifolius* in Linan County, Hangzhou City, Zhejiang Province, while *D. heucherifolius* var. *gamosepalus* is the southernmost species. *D. heucherifolius* is the most widespread species, which can be found in Danxia, Karst limestone and Granite landscapes. As shown in Figure 2, nine localities are Karst landscape (the green points) and nine localities are Danxia landscape (the red points). The majority of Section *Heteroboea* species (four species and two varieties) were reported on Danxia landscape.



**Figure 2.** The distribution areas of Section *Heteroboea* and their Parent material. The different colours represent the different Parent material: red – danxia landscape; yellow – granite landscape; green – karst limestone landscape; nlack – volcanic landscape).

Characters	D. h. var. heucherifolius	D. h. var. yinzhengii	D. h. var. gamosepalus
Shape of calyx	shallowly 5-lobed to the base,	shallowly 5-lobed to the	base connate, 5-lobed from middle
	lobes unequal	base, lobes unequal	to above middle, lobes equal
Size of corolla	2.5–3.2 cm long	up to 4 cm	ca. 5 cm long
Indumentum of corolla	puberulent	glabrous	glabrous
Stamens	1.0–1.2 cm from the base	1.0-1.2 cm from the base	2.3 cm from the base
Staminodes	0.6–0.8 cm from the base	absent	1.4-1.8 cm from the base
Pistil size	1.8–2.9 cm	up to 3 cm	3.6–4.3 cm

**Table 1.** Comparison of the diagnostic characters of *Didymocarpus heucherifolius* var. *gamosepalus* and other variety of *D. heucherifolius*.

#### **Acknowledgements**

This study was financially supported by the Anhui Provincial Natural Science Foundation (1908085QC1), Key University Science Research Project of Anhui Province (No. KJ2017A022), Anhui University Doctor Startup Fund, Fund of Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain (No.17-259-23).

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RESEARCH ARTICLE



# A new species of *Rhaptopetalum* (Lecythidaceae) from south-western Gabon

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Academic editor: Thomas L.P. Couvreur | Received 18 March 2019 | Accepted 24 May 2019 | Published 23 July 2019

Citation: Kenfack D, Nguema DE (2019) A new species of *Rhaptopetalum* (Lecythidaceae) from south-western Gabon. PhytoKeys 128: 39–46. https://doi.org/10.3897/phytokeys.128.34640

#### Abstract

*Rhaptopetalum rabiense* Kenfack & Nguema, **sp. nov.** from the Rabi forest in south-western Gabon is described, illustrated and assigned a provisional conservation status of "Critically Endangered". An identification key to the five Gabonese species of *Rhaptopetalum* is also provided.

#### Keywords

ForestGEO, Gabon, IUCN Red List, new species, permanent plot, Rabi, rainforest, taxonomy

# Introduction

*Rhaptopetalum* Oliv. is a genus of 12 species of trees, mostly confined in the Gulf of Guinea, with only one species occurring in west Africa (Cheek et al. 2002; Prance and Jongkind 2015). The genus was first described in 1865 by the English botanist Daniel Oliver (1865), as a member of the family Scytopetalaceae. Recent molecular phylogenetic analyses (Morton et al. 1997; Mori et al. 2007) showed that Scytopetalaceae

form a monophyletic group with Lecythidaceae. Yet, opinions still diverge about either keeping the Scytopetalaceae as a separate family (Takhtajan 2009; Reveal 2011) or as a subfamily within Lecythidaceae (Mabberley 2008; APG 2016). Here, we consider *Rhaptopetalum* as a member of the Lecythidaceae s.l. Eight species of *Rhaptopetalum* are cited in the *Flore du Gabon* account of Scytopetalaceae (Letouzey and White 1978). However, because no specimen citation was provided for four of them (*R. breteleri* R. Letouzey, *R. depressum* R. Letouzey, *R. roseum* (Gürke) Engler and *R. sessilifolium* Engler), their presence in Gabon remains doubtful. Hence, only four species are currently recognised in Gabon (Prance and Jongkind 2015).

In 2010, we established a 25-ha permanent plot in the rainforest of south-western Gabon to study the long-term dynamics of this forest (Memiaghe et al. 2016). The methods included the challenging task of identifying to species level hundreds of thousands of sterile trees and saplings with diameter at breast height  $(dbh) \ge 1$  cm. Amongst the 175,830 trees recorded in the plot, 299 were assigned to Rhaptopetalum Oliv., based on the short petiolate leaves with punctate lamina, the flowers with articulated pedicel and cupuliform calyx, the stamens attached to the base of the pseudocorolla, the poricidal anthers, the short filaments and the pubescent seeds. The identification of the Rabi material, using the key in the recent revision of the African Lecythidaceae (Prance and Jongkind 2015), was problematic from the second couplet. The species does not fit either of the leads 3 or 4, because the ovary has few (generally 1) ovules per locule, is dome-shaped, while the fruit surface is not angled. Hence, following the lead 4, the Rabi species is close to the Gabonese Rhaptopetalum belingense Letouzey with its dome-shaped ovary, its entire calyx margin, its apex placentation and smooth fruits. Following lead 3, it also resembles the Democratic Republic of the Congo R. evrardii R. Germain with its puberulous midrib, red petals and cupuliform calyx and the pedicel not articulated immediately below the calyx. However, the Rabi material also presents a suite of unique characters outlined below (Table 1) and that allows us to describe it as new to science.

	R. rabiense	R. evrardii	R. belingense
Indumentum of young branches	Puberulous	Puberulous	Glabrous
Shape of young branches	Not angular	Angular	Not angular
Lamina length (cm)	7–20	18–28	15-18
Lamina width (cm)	3–9	8-12	8-10
Pedicel length (mm)	5–7	4–5	3
Pedicel articulation	1 mm below the calyx	about 1 mm below the calyx	Directly below the calyx
Calyx margin	Entire	6–10 lobed	Entire
Calyx shape	Cupuliform	Cupuliform	Flattened patelliform
Pseudocorolla length (mm)	3–5	7–8	8
Number of ovules per locule	1	2 or 3	Many
Fruit surface	Smooth	Ridged	Smooth
Fruit diameter (mm)	15-20	10–12	15

Table I. Comparison of discriminant characters amongst Rhaptopetalum rabiense, R. evrardii and R. belingense.

#### **Taxonomic treatment**

#### Key to the Gabonese species of Rhaptopetalum

1	Leaf lamina exceeding 18 cm long and 10 cm wide2
_	Leaf lamina up to 18 cm long and 10 cm wide
2	Ovary conical, pedicel 3—5 mm long, placentation axile R. sindarense
_	Ovary dome-shaped, pedicel 8–10 mm long, placentation apical
	R. pachyphyllum
3	Leaf base cuneate, slightly decurrent onto petiole
_	Leaf base rounded
4	Young branches glabrous, petiole 5—7 mm long, ovary loci multi-ovulate
_	Young branches puberulous, petiole 2—4 mm long, ovary loci uni-ovulate

#### Rhaptopetalum rabiense Kenfack & Nguema, sp. nov.

urn:lsid:ipni.org:names:77200426-1 Figs 1, 2

**Type.** GABON. Ogooué Maritime, 25-ha Rabi Forest plot, 1°55'37.57"S, 9°52'50.66"E, 23 m alt., 27 Aug 2014 (fl), *Nguema et al. 2825a* (holotype: LBV, iso-types: BR, MO, US, K, P)

**Diagnosis.** *Rhaptopetalum rabiense* is similar to *R. belingense* by its dome-shaped ovary, its entire calyx margin, its apex placentation and smooth fruits, but differs by its pubescent (vs. glabrous) young branches, its longer pedicel (5–7 mm vs. 3 mm) articulated 1 mm below the calyx (vs. articulated directly below the calyx), and its uni-ovulated loci (vs. multi-ovulated) (Table1).

**Description.** *Tree* 4–6 m tall, bole cylindrical, to 20 cm diameter at 1.3 m aboveground; bark pale brown, slash fibrous, pink in the outer part, yellowish in the inner part; young branches slender, rusty brown, densely puberulous, conspicuously lenticellate. *Leaves* distichous; petiole 2–4(6) × 2 mm, densely puberulous; lamina obovate to elliptic, coriaceous, abundantly punctate, 8–18(21) × (2.5)5–9 cm, acuminate to broadly acute at apex, slightly unequal and rounded at base; margin entire; midrib conspicuous and flattened above, prominent and minutely puberulous beneath; secondary veins 8–11 pairs, plane above, prominent beneath, arching and joined towards the margin of the lamina. *Inflorescence* ramiflorous, axillary and supra-axillary, fasciculate, with 1–8 flowers. *Bracts* ovate, about 1 mm long. *Flower bud* ovoid to globose-oblong, rounded at apex, (2.5)3–4 mm long, pink to red directly above the calyx rim, light pink to whitish towards the apex. Pedicel in flower buds (2.5)3–5 mm, but generally 5–7 mm long in opened flowers, yellow-green, minutely puberulous to glabrescent, articulate directly beneath calyx. Calyx cupuliform, yellow-green, glabrescent to pu-



**Figure 1.** *Rhaptopetalum rabiense* **A** flowering twig **B** close-up of the lower surface showing the indumentum and the punctate lamina **C** detail of inflorescence and stem showing lenticels **D** flower bud **E** opened flower **F** flower with pseudocorolla and stamens removed showing superior ovary **G** longitudinal section of **F** showing pendulous ovules **H** fruiting branch.



**Figure 2.** *Rhaptopetalum rabiense* **A** flowering branch **B** lateral view of the flower **C** flower view from above showing the poricidal anthers and the gynoecium **D** fruiting branch. Photographs by Diosdado Nguema.

berulous, the margin entire, 2–2.5 mm in diameter on the rim, receptacle about 1 mm long. Pseudocorolla fleshy, splitting into (3)4 lobes 2.5–4 mm long oblong to ovate lobes. *Stamens* 35(37), the filament light pink to whitish, about 0.3 mm, the poricidal anthers bright yellow, slightly arched towards the centre of the flower, 2–2.5 mm long;



Figure 3. Type locations of *Rhaptopetalum rabiense* and the closely related species *R. belingense* in Gabon.

ovary superior, globose, about 1 mm high and 1.5 mm diameter, 3–4-locular, each locule with 1 or 2 axile pendulous ovules. Style 3–4.5 mm long. *Fruit* a globose capsule, green, smooth, 15–20 mm diam., fruiting pedicel 5–7 mm long, seeds  $8-12 \times 5-8$  mm.

Phenology. Flowering August–October, Fruiting September–December.

**Geographic distribution.** *Rhaptopetalum rabiense* is only known from the type locality, the Rabi forest (Figure 3).

**Habitat.** *Rhaptopetalum rabiense* grows in old growth forest, on both terra firme dry and wet depressions, with elevation 20–50 m.

Additional specimens examined. GABON. Ogooué Maritime: Rabi, 25-ha permanent plot, 24 m elev., 1°55'28.1"S, 9°52'48.26"E, 21 August 2013 (st), *Nguema et al. 1743*; Rabi, 25-ha permanent plot, 50 m elev., 1°54'51.36"S, 9°52'41.56"E, 28 October 2013 (st), *Nguema et al.* 1922; Rabi, 25-ha permanent plot, 50 m elev., 1°55'27.09"S, 9°52'41.56"E, 5 November 2013 (st), *Nguema et al. 2057*; Rabi, 25-ha permanent plot, 32 m elev., 1°55'37.57"S, 9°52'50.66"E, 2 September 2014

(fl), *Nguema et al. 2825B*; Rabi, 25-ha permanent plot, 32 m elev., 1°55'37.57"S, 9°52'50.66"E, 2 September 2014 (fr), *Nguema et al. 2825C*; Rabi, 25-ha permanent plot, 32 m elev., 1°55'30.22"S, 9°52'42.18"E, 23 September 2014 (fl), *Nguema et al. 2832*; Rabi, 25-ha permanent plot, 32 m elev., 1°55'30.35"S, 9°52'41.79"E, 2 October 2014 (fl), *Nguema et al. 2833*; Rabi, 25-ha permanent plot, 61 m elev., 1°55'36.44"S, 9°52'46.61"E, 2 December 2014 (fr), *Nguema et al. 2926*.

Preliminary conservation status. The conservation status of Rhaptopetalum rabiense was evaluated using the IUCN Red List Categories and Criteria Version3.1 (IUCN 2012). The extent of occurrence (EOO) and the area of occupancy (AOO), estimated using the web Geospatial Conservation Assessment Tool or GeoCAT (Bachman et al. 2011) and the auto-value cell size length of 2 km, were 0.214 km<sup>2</sup> and 8.00 km<sup>2</sup>, respectively. These two values meet the criteria B1 (EOO < 100 km<sup>2</sup>) and B2 (AOO < 10 km<sup>2</sup>) for Critically Endangered, following the IUCN Red List Categories and Criteria Version 3.1 (IUCN 2012). The species is not under legal protection. The type locality is an oil and gas production field and, at the same time, a logging concession. These activities are likely to result in its population reduction and/or in a fragmentation of its range. R. rabiense in the studied plot has 299 individuals with dbh  $\ge 1$  cm (12) individuals per hectare) and seems to be regenerating, based on diameter size class distribution. It is known only from its type locality and has not been recorded in the adjacent national parks of Loango National Park on the west and Moukalaba Doudou National Park on the east. We therefore assess *R. rabiense* with the preliminary IUCN Red List status of Critically Endangered CR B12ab(iii).

#### Acknowledgements

We are grateful to the Government of Gabon through the Centre National de la Recherche Scientifique et Technologique (CENAREST) for granting us permission to conduct the study and the Compagnie des bois du Gabon (CBG) for permission to establish the plot in their forest concession. The bulk of the financial and logistical support that led to the discovery of this new species was provided by Shell Gabon and Assala Energy, through the Center for Conservation and Sustainability of the Smithsonian Conservation Biology Institute. Additional funding and technical support was provided by the Smithsonian Tropical Research Institute through the Forest Global Earth Observatory. We thank the personnel of the Institut de la Recherche en Écologie Tropicale (IRET) and the Herbier National du Gabon for their great support and contribution to the success of this work. We greatly appreciate the contribution of Gauthier Moussavou, Etienne Moumoulossi, Landry Tchignoumba, Prince Biessemou, Wilfried Mbading-Mbading, Gorky Villa and all other technicians who were involved in fieldwork. Special thanks to Alfonso Alonso, Lisa Korte, Hervé Memiaghe and Pulchérie Bissengou for their leading role in this project, to Alice Tangerini of the Smithsonian NMNH for the marvellous illustration of the new species and to Dr Duncan Kimuyu for the location map. This is contribution #190 of the Gabon Biodiversity Program.

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RESEARCH ARTICLE



# Clematis guniuensis (Ranunculaceae), a new species from Eastern China

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Academic editor: M. Pellegrini | Received 15 February 2019 | Accepted 2 May 2019 | Published 23 July 2019

**Citation:** Wang R-B, Ni W-Y, Xu W-J, Gui Z-W, Zhou S-B (2019) *Clematis guniuensis* (Ranunculaceae), a new species from Eastern China. PhytoKeys 128: 47–55. https://doi.org/10.3897/phytokeys.128.33891

#### Abstract

*Clematis guniuensis* **sp. nov.**, a new narrowly endemic species of *Clematis*, is described and illustrated from the Huangshan Mountains of Eastern China. A description of *C. guniuensis* is presented along with illustrations, photographs and diagnostic differences between the new species and its putative close allies.

#### Keywords

Anhui, Early diverging eudicots, Ranunculales, Taxonomy

# Introduction

*Clematis* L. (Ranunculaceae) is a large genus of early diverging eudicots, comprising approximately 280–350 species (Tamura 1987, 1995; Johnson 1997; Grey-Wilson 2000; Wang and Li 2005), out of which 147 species are reported in China, 93 of them being

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endemic to the country (Wang and Bartholomew 2001). The species of *Clematis* are economically important for their chemical properties relating to traditional medicine and also as ornamentals due to their attractive flowers (Grey-Wilson 2000; Wang and Bartholomew 2001). The genus is distributed worldwide, showing a high degree of speciation, with adaptations to a variety of habitats, especially in eastern Asia (Tamura 1993). *Clematis* species also show considerable morphological diversity and plasticity, making the taxonomy and classifications of the genus notoriously difficult (Brandenburg 2000), with different classifications emphasising different morphological characters (e.g. Prantl 1888; Tamura 1995; Johnson 1997; Grey-Wilson 2000; Wang and Li 2005).

During floristic surveys in experimental forestry plots of this Guniujiang National Nature Reserve between 2016 and 2018, a conspicuous species bearing 1-flowered cymes was collected. After thorough comparisons of diagnostic morphological and anatomical features of similar taxa (Lin and Wei 2009; Wang 2004a, 2004b, 2006a, 2006b, 2015a, 2015b; Wang and Bartholomew 2001; Wang and Huang 2014; Wang and Li 2016; Wang and Xie 2007), we have concluded that this specimen belongs to a hitherto undescribed species. We describe this specimen as a new species, presenting a morphological description, illustrations and comments on morphologically related species.

#### Material and methods

Measurements and morphological character assessments of the putative new species were undertaken using herborised and living specimens observed in the field or cultivated at the Botanical Garden of Anhui College of Traditional Chinese Medicine. All available specimens of *Clematis*, stored in the following herbaria (acronyms according to Thiers 2017+): IBK, IBSC, N, MO, P, PE, SYS, US and some local herbaria were examined. Images of type specimens were obtained from Tropicos.org (http://www.tropicos.org) and JSTOR Global Plants (http://plants.jstor.org). All morphological characters were studied using a dissecting microscope (SZX16, Olympus, Tokyo, Japan). Characters were described, using the terminology presented by Wang and Bartholomew (2001).

#### Taxonomy treatments

*Clematis guniuensis* W.Y.Ni, R.B.Wang & S.B.Zhou, sp. nov. urn:lsid:ipni.org:names:77200428-1 Figs 1, 2, 3

**Diagnosis.** Resembles *C. florida* Thunb. and *C. huchouensis* Tamura but can be distinguished from the former one by puberulous leaflet blades, longer petiole, larger flowers with light green sepals, longer stamens and white filaments and from the latter by its longer petioles, 3-lobed leaflet blades, shorter pedicel, larger flowers, 4 sepals, filaments about 3–5 times the length of the anther, persistent style 1.5–2 cm long, and yellow plumose.



**Figure 1.** *Clematis guniuensis* W.Y.Ni, R.BWang & S.B.Zhou. **A** Habitat in flowering period **B** Inflorescences with budding flower, showing the bracts **C** Stamen **D** Pistil **E** Achene **F** Stem cross-section.

**Type.** CHINA. Anhui Province: Qimeng County, Guniujiang National Nature Reserve, Huangshan City, 30°0'57.02"N, 117°29'37.17"E, 550 m a.s.l., 15 May 2018, flowering, Rong-Bin Wang, WRB201805068 (holotype: ANUB!; isotypes: AHU!, PE!, WUH!).



Figure 2. Holotype of Clematis guniuensis W.Y.Ni, R.B.Wang & S.B.Zhou.

**Description.** Vines herbaceous, perennial; branches inconspicuously longitudinally 6-sulcate to sub-terete, densely primrose yellow puberulous covering when young, becoming glabrescent with age. Root fusiform. Leaves opposite, ternate; peti-



**Figure 3.** *Clematis guniuensis* W.Y.Ni, R.B.Wang & S.B.Zhou. **A** Habitat **B** Young branches, showing stems 6–grooved, puberulous **C** Inflorescences, showing style and abaxial surface view of leaf blade **D** Dorsal view of cymes, showing peduncles and bracts **E** Frontal view of flower, showing stamens **F** Fruit **G** Achenes, showing persistent style.

ole 7–10 cm long; leaflets 3-lobed, ovate to narrowly-ovate; central lobe  $6-7.5 \times 3.5-4$  cm, lateral lobes  $4-5 \times 2.8-3.5$  cm, margin coarsely dentate to entire, apex acuminate or sometimes caudate, base broadly cuneate to rounded, papery, adaxially dark green, densely appressed white pilose, abaxially light green, sparsely puberulous to sub-gla-



Figure 4. Distribution map of *Clematis guniuensis* (pentagon, red) and its congeners *C. huchouensis* (round, violet) and *C. florida* (square, green).

brous, basal veins abaxially slightly prominent; and with petiolule 1–2 cm long. Cymes axillary, 1-flowered; peduncles 3–6 cm long, densely puberulous; bracts opposite, subsessile, ovate,  $1.2-1.7 \times 5-7$  mm, margin entire, both surfaces puberulous. Flowers 6–8 cm diam.; pedicels ca. 2 cm long, conical, sulcate, green, densely puberulous; sepals 4, spreading, light green, ovate, ovate-lanceolate or broad-lanceolate,  $3.5-4.5 \times 1.8-2.3$  cm, apex acute, adaxially glabrous, abaxially sparsely white pubescent, trinerved; stamens numerous, 1-3 cm long, filaments linear, glabrous, about 3–5 times the length of the anthers, anthers narrowly oblong, ca. 6 mm long, white, glabrous, apex shortly apiculate; ovaries ellipsoidal, pubescent, style densely yellow-villose. Achenes dark-brown, strongly compressed, ovate to broadly ellipsoidal, ca.  $3 \times 1$  mm wide, pubescent; persistent style 1.5-2 cm long, yellow-plumose.

Phenology. Flowering from April to May; fruiting from October to November.

**Etymology.** The specific epithet is derived from the type locality, Guniujiang National Nature Reserve.

**Vernacular name.** Gū Niǘ Tiě Xián Lián (Chinese pronunciation); 牯牛铁线莲 (Chinese name).

Characters	Clematis guniuensis	Clematis huchouensis	Clematis florida
Petioles	7–10 cm long	1.7–3 cm long	2–4 cm long
Shape of leaflet blades	3-lobed	2- or 3-lobed or undivided	undivided
Indumentum of leaflet blades	puberulous	puberulous	glabrous
Flower per cyme	1-flowered	1-3-flowered	1-flowered
Size of bracts	1.2-1.7 cm long	2-3 cm long	1.4–3 cm long
Size of pedicels	ca. 2 cm long	1.2-3 cm long	3.7-8.5 cm long
Size of flowers	6–8 cm wide	2–3 cm wide	3.6–5 cm wide
Number of sepals	4	4	6
Colour of sepals	light green	white	white
Size of sepals	3.5–4.5 ×1.8–2.3 cm	1.4–2.2 × 0.3–0.6 cm	$2-3 \times 1-1.5$ cm
Size of anthers	ca. 6 mm long	2.5-3.2 mm long	2.5-3.5 mm long
Filaments	about 3-5 times the length of the anther, white	equal to the length of the anther, white	shorter than anthers, purple
Persistent styles	1.5–2 cm long, yellow plumose	0.8–1.3 cm long, appressed yellowish pubescent	ca. 8 mm long, basally spreading puberulous, apically glabrous

Table I. Diagnostic character differences amongst Clematis guniuensis, C. huchouensis and C. florida.

**Distribution and habitat.** To date, *C. guniuensis* is only known from the type locality, Guniujiang National Nature Reserve, Huangshan City, Anhui Province (Fig. 4). Currently the species is known from a few collections and there is only one known population with ca. 20 individuals at the type locality. The species is mostly found in tea plantations or forest edges along valleys of evergreen broad-leaved forests, at an elevation of 1,500 m a.s.l.

**Conservation assessment.** Based on the present field investigations, *C. guniuensis* is currently only known from the type locality and with a very small population size (ca. 20 individuals). The species should be given the IUCN status of Critically Endangered (CR) based on criteria D: "Population size estimated to number fewer than 50 mature individuals" (IUCN 2016).

**Notes.** A morphological comparison between *C. guniuensis* and morphologically related species, *C. florida* and *C. huchouensis*, is provided in Table 1. A total of 17 species of this genus was found in the Anhui province, with this new species being easily distinguished from the other species in this region by its 3-lobed leaflets, 1-flowered cymes, flowers 6–8 cm diam., sepals 4 and light green and glabrous filaments.

#### Acknowledgements

The authors are grateful to Yu-Han Xiao from Nanjing Normal University of Special Education for the beautiful hand-drawings. This study was financially supported by Long-term Plot of Forest Ecosystem Project in Guniujiang National Nature Reserve, the Anhui University Doctor Start-up Fund, Key University Science Research Project of Anhui Province (No. KJ2017A022), Anhui Natural Science Fund Project (1908085QC126), Key Projects for Academic Support of Top-level Talents in Colleges and Universities in Anhui Province (No. gxbjZD2016106), National traditional Chinese medicine resources survey project (caishe [2017] no. 66).

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# Appendix I

List of all specimens analyzed for all three species for this study, with voucher number and deposition and origin information. Species are listed in alphabetical order.

# Clematis florida Thunb.

CHINA. Anhui Province: Zhu P.Z., 64-0067 (JSPC); Ho Y.Y., Y.Y.Ho-02243 (SYS); Chongqing City: Liu Z.Y., Liu Z.Y.-972413 (IMC); Guangdong Province: Huang C., Huang-162703 (IBSC); Chen W.Y., Chun Woon Young 8416 (PE); Chun Y.F., Y.F.Chun-30488 (SYS); Shi G.L., Shi G.L. 13249 (WUK); Guangxi Zhuang Autonomous Region: Mashan Expedition Team, 450124140722047LY (GXMG); Liu Y. et al., Liu & Yu H0094 (IBK); Guangxi Investigation Team, Guangxi-3984 (PE); Guizhou Province: Huang W.L. & Tu Y.L., Huang & Tu-0459 (GNUG); Wang Z.R., 522628140720152LY (GZTM); Gao J., Gao2013233024 (QNUN); Li K., DPS2013233024 (QNUN); Henan Province: Ye Y.X., 20071024B8 (BJFC); Li G.Q., Li4257 (HEAC); Yang W.G., Yang W.G.58 (HEAC); Xu C.X., Xu 78055 (HENU); Yang X.F., Yang 28 (HENU); Wang X.F., Wang 181 (HENU); Fu J.Q., Fu 2201 (IBK); Hubei Province: Wu J.Q. & Ma X.Y., Wu & Ma 8061 (HIB); Sichuan Province: Li J.Y., ss201208040024 (BJFC); Chien Y., Y.Chien 5993 (N); Yan J.X., Yan 87029 (PEM); Chien S.S., Chien Sung Shu 5342 (SZ); Zhejiang Province: Cheng R.C., R.C. Cheng 1976 (IBSC); Rwan Chia Tsuin, Rwan Chia Tsuin 37 (N); Zhejiang Investigation Team, Shen Jia-Yu 8065 (NAS).

# Clematis huchouensis Tamura

CHINA. Guizhou Province: Huo P.Z., Zhi M.G. & Yuan D.X., 520222150126001LY (GZTM); Jiangsu Province: Wu W.X., Wu W.X.4205 (NAS); Ding Z.Z. & Wang Y.C., Ding & Wang 0939 (NAS); Wu W.X., W.X. Wu 6087 (NAS); Wu W.X., Wu W.X.4351 (WUK); Jiangxi Province: Shen S.J., Shen S.J.381 (PE); Shen S.J., Shen S.J.00565 (PE); Sichuan Province: Ju W.B. & Deng H.N., HGX13374 (CDBI); Zhejiang Province: Jiang Y.P., HZ008256 (HHBG); Tu Z.B., Tu0104710 (HIB); Chen S., S. Chen 1931 (NAS); Chen M., M.Chen 868 (NAS); Zhang S.R., Zhang S.R.1094 (PE).

RESEARCH ARTICLE



# Mosses of Gunung Senyum Recreational Forest, a tropical limestone forest in Pahang, Peninsular Malaysia

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Academic editor: Matt von Konrat | Received 14 February 2019 | Accepted 21 June 2019 | Published 25 July 2019

**Citation:** Norhazrina N, Syazwana N, Aisyah M, Aznani H, Maideen H, Nizam MS (2019) Mosses of Gunung Senyum Recreational Forest, a tropical limestone forest in Pahang, Peninsular Malaysia. PhytoKeys 128: 57–72. https://doi.org/10.3897/phytokeys.128.33860

#### Abstract

Gunung Senyum Recreational Forest harbours 59 species, two subspecies and five varieties of mosses in 32 genera and 16 families that had been identified from a total of 589 specimens collected from the area. These figures represent 11.8% out of the 558 taxa, 20.2% out of the 158 genera and 34.7% out of the 46 families of mosses reported for Peninsular Malaysia. The total also represents 14.9% of the 442 taxa, 24.0% of the 133 genera and 40.0% of the 40 families of mosses recorded in Pahang. The largest family of mosses found in this limestone forest is Calymperaceae followed by Fissidentaceae. There are two new records for Pahang, *Calymperes pallidum* Mitt. and *Taxithelium binsteadii* Broth. & Dixon. The analysis of species similarities of mosses found in the study area with some other selected areas showed that Gunung Senyum Recreational Forest had a high percentage of species similarity with Perlis State Park at Wang Kelian, another limestone forest, at 38%. Corticol is the main habitat utilised by mosses in Gunung Senyum Recreational Forest with 47 taxa, followed by the lignicol and calcicol each with 35 and 26 taxa, respectively.

#### Keywords

Mosses, limestone hill, Gunung Senyum Recreational Forest, Peninsular Malaysia

# Introduction

Forested limestone areas in Peninsular Malaysia are estimated at about 26,000 ha, mostly concentrated in the northern states and 50,000 ha in Sabah and Sarawak according to the World Wildlife Fund Malaysia (2018). More than 300 scattered limestone outcrops have been found in the Peninsular Malaysia which consist of limestone

islands in the Langkawi archipelago, with major outcrops in Kelantan, Perlis, Kedah, Perak and northern Pahang.

Gunung Senyum Recreational Forest is one of the limestone forests in Peninsular Malaysia and is located in Jengka, Pahang (latitude 3°43.0683'N and longitude 102°26.0043'E). This recreational forest consists of several series of limestone hills, including Gunung Senyum and Gunung Jebak Puyuh. They are hills located northeast of Temerloh and north of Kampung Awah (Fontaine et al. 1988). In addition to limestone hills, this area also comprises lowland forest, especially in the area between Gunung Senyum and Gunung Jebak Puyuh. Gunung Senyum has 18 caves, while seven caves have been recorded at Gunung Jebak Puyuh. These caves originated from weathering activities where some of them are archaeological sites. Several studies in geological, human civilisation and rock life have been conducted in the areas of Jengka including Gunung Senyum and Gunung Jebak Puyuh by Jasin et al. (1995) and Fontaine et al. (1988).

The collection of plant specimens from limestone hills in Peninsular Malaysia started in August 1880 by Kunstler, a collector for Sir George King, who collected specimens in the limestone area of Gopeng, Perak. In addition, there are other collectors, namely Fox, Ridley, Kelsall and Wooldridge. As a result, about 4,500 plant specimens have been collected (Chin 1977).

The study of the limestone flora in most parts of Peninsular Malaysia was initiated by Henderson from 1923 to 1935, including Gunung Senyum and other limestone areas where about 745 plant taxa were recorded here. Also, Carr had collected plant specimens at Gunung Senyum from 1928 to 1930 but most specimens collected by him are orchids and ferns (Chin 1977). Then, Chin (1977) listed about 1216 plant taxa in a comprehensive study of limestone hills in Peninsular Malaysia consisting of pteridophytes, angiosperms and gymnosperms. He also listed Gunung Senyum as one of the largest distribution area of limestone hills in Peninsular Malaysia.

The first comprehensive study on limestone moss flora in Peninsular Malaysia was conducted by Mohamed (1987), in which about 21 limestone outcrops mainly in the northern half of the country were surveyed. He listed about 73 taxa in 40 genera and 18 families of mosses. After that, Damanhuri and Maideen (2001) recorded a total of 71 taxa in 34 genera and 18 families of mosses in Perlis State Park, Wang Kelian, Perlis. Yong et al. (2002) collected about 57 taxa in 28 genera and 13 families of mosses in Wang Mu Forest Reserve, Perlis State Park, Perlis. Later, Damanhuri et al. (2007) reported about 112 taxa in 45 genera and 19 families of mosses in Kenong Forest Park, Pahang. Lastly, Kiew et al. (2014) listed about 25 taxa in 14 genera and 11 families of mosses found in Gunung Kanthan, Perak.

Some studies in Gunung Senyum had been reported before by Chin (1977), but their collections did not cover mosses. Until now, the Gunung Senyum Recreation Forest had not been explored in terms of its moss flora. Therefore, this is the first study of moss conducted there. Also, from this study, a new record for Peninsular Malaysia has been made in this area, *Calymperes pallidum* Mitt. (Ellis et al. 2018).

# Methods

This study is based on samples collected at Gunung Senyum Recreational Forest located in the Jengka Reserved Forest, Pahang (Figure 1). Collections were made along the trails in the Gunung Senyum Recreational Forest. All the specimens are curated and deposited in the Herbarium of Universiti Kebangsaan Malaysia, Bangi (UKMB).

The information regarding collection numbers, altitudes of each sample collected, date and locality of each specimen collected in Gunung Senyum Recreational Forest are shown in Table 1. Various microhabitats of mosses such as tree trunks, buttresses, rotten logs, surfaces and crevices of rocks, soil and soil banks were carefully surveyed in order to obtain as many samples and species as possible.



Figure 1. Map of Gunung Senyum Recreational Forest, Jengka Forest Reserve, Pahang.

 Table 1. Collection information of moss specimens collected in Gunung Senyum Recereational Forest.

Date	Altitude (m)	Specimen No.	Locality
16/08/2009	85-170	1-90	The foot of Gunung Senyum
17/08/2009	75–485	91-264	Trails from the foot to the summit of Gunung Senyum
18/08/2009	95–l60	265-589	Trails to Gunung Jebak Puyuh and the surrounding areas

#### **Results and discussion**

A total of 59 species, two subspecies and five varieties of mosses in 32 genera and 16 families was found in Gunung Senyum Recreational Forest, Pahang (Table 2 and Appendix 1). These numbers represent 11.8% of the 558 taxa, 20.2% of the 158 genera and 34.7% of the 46 families of mosses reported in Peninsular Malaysia. Based on the records of mosses found in Pahang, these figures represent 14.9% of the 442 taxa, 24.0% of the 133 genera and 40.0% of the 40 families of mosses in the state. Two species are new additions to the bryoflora of Pahang namely Calymperes pallidum Mitt. and Taxithelium binsteadii Broth. & Dixon, where C. pallidum had been published as a new record for Peninsular Malaysia (Ellis et al. 2018). Meanwhile, a Fissidens species remains unidentified and requires further study to ascertain its true identity. This species will contribute to new findings for the genus in Peninsular Malaysia as for the time being there are about 28 taxa recorded in Peninsular Malaysia (Syazwana et al. 2018). The new discoveries of moss species in this area can also increase the bryoflora of Pahang in which the current record is about 442 taxa of mosses in 133 genera and 40 families. The total is higher compared to neighbour states such as Kelantan (299 taxa in 105 genera and 37 families) and Terengganu (253 taxa in 88 genera and 31 families). This shows that Pahang has the highest record of moss species compared to other states in the east coast region of Peninsular Malaysia.

Amongst the 15 families recorded, Calymperaceae has the highest number of members (20 taxa), followed by Fissidentaceae with nine taxa. Hypnaceae, Neckeraceae and Pylaisiadelphaceae are the third largest families, each represented by six taxa. This is followed by Sematophyllaceae with five taxa, Pottiaceae and Thuidiaceae with three and two taxa, respectively. The rest, Bartramiaceae, Brachytheciaceae, Bryaceae, Leucomiaceae, Meteoriaceae, Orthotrichaceae, Plagiotheciaceae and Pilotrichaceae have one taxon each (Table 2).

The largest genus found in this study is *Calymperes* with 11 taxa which belongs to the largest family (Calymperaceae) recorded here. *Fissidens* is the second largest with nine taxa. *Taxithelium* is the third largest with five taxa followed by *Vesicularia* and *Mitthyridium* with four and three taxa respectively. Genera represented by two taxa each are the *Ectropothecium*, *Leucophanes*, *Neckeropsis*, *Pelekium*, *Pinnatella*, and *Syrrhopodon*. The remainder, *Acanthorrhynchium*, *Acroporium*, *Arthrocormus*, *Barbula*, *Bryum*, *Caduciella*, *Callicostella*, *Circulifolium*, *Exostratum*, *Floribundaria*, *Hyophila*, *Isopterygium*, *Leucomium*, *Macromitrium*, *Meiothecium*, *Papillidiopsis*, *Philonotis*, *Pseudosymblepharis*, *Pseudotaxiphyllum*, *Rhynchostegium*, and *Trichosteleum* have one taxon each.

Calymperaceae is indeed a major family in lowland forest areas in Peninsular Malaysia (Damanhuri and Maideen 2001; Damanhuri et al. 2007). In this study area, Fissidentaceae, Hypnaceae, and Neckeraceae are represented by a fairly high number of taxa since the limestone rocks are largely a habitat of choice for the members of these families (Mohamed 1987). The moss species found in Gunung Senyum Recreational Forest is compared to three other limestone forests in Peninsular Malaysia using Jaccard Coefficient of Similarity. Other limestone forest selected for comparison are Taman Rimba Kenong in Pahang (Damanhuri et al. 2007); Taman Negeri Perlis in Wang Kelian, Perlis (Damanhuri and Maideen 2001) and Gunung Kanthan in Perak (Kiew et al. 2014). Taman Rimba Kenong has the highest number of taxa, 114 taxa, followed by Taman Negeri Perlis in Wang Kelian and Gunung Senyum Recreational Forest with 72 and 66 taxa respectively. Gunung Kanthan recorded the lowest number of taxa, just 25 (Table 3).

Taman Negeri Perlis in Wang Kelian exhibited the highest degree of species similarity with Gunung Senyum Recreational Forest, which is 38.8% (Table 4). This is due to both areas consisting of limestone forests. Topographic factors also play an important role in shaping the vegetative patterns that are present in certain areas. Taman

No.	Families	Genera	Species & infraspecific taxa
1	Bartramiaceae	1	1
2	Brachytheciaceae	1	1
3	Bryaceae	1	1
4	Calymperaceae	6	18 spp. + 2 subsp.
5	Fissidentaceae	1	8 spp. + 2 var.
6	Hypnaceae	2	6
7	Leucomiaceae	1	1
8	Meteoriaceae	1	1
9	Neckeraceae	4	6
10	Orthotrichaceae	1	1
11	Pilotrichaceae	1	1 var.
12	Plagiotheciaceae	1	1
13	Pottiaceae	3	3
14	Pylaisiadelphaceae	2	5 spp. + 1 var.
15	Sematophyllaceae	5	4 spp. + 1 var.
16	Thuidiaceae	1	2
	Total	32	59 spp., 2 subsp., 5 var.

Table 2. Summary of mosses found in Gunung Senyum Recreational Forest and its vicinity.

Table 3. Summary comparing the number of moss taxa in the four areas.

Locality	Families	Genera	Taxa
Gunung Senyum	16	32	59 spp. + 2 subsp. + 5 var.
Wang Kelian	18	34	67 spp. + 1 subsp. + 3 var.
Taman Rimba Kenong	19	45	94 spp. + 4 subsp. + 16 var.
Gunung Kanthan	9	14	23 spp. + 2 var.

**Table 4.** Summary of the level of similarity of moss flora in selected areas.

	Gunung Senyum	Taman Rimba Kenong	Wang Kelian
Taman Rimba Kenong	33.6%		
Wang Kelian	38.8%	36.6%	
Gunung Kanthan	19.7%	15.0%	15.9%

Rimba Kenong shows the second highest similarity with Gunung Senyum Recreational Forest, which is 33.6%, meanwhile, Gunung Kanthan show the lowest degree of similarity with this forest which is 19.7%. This may be due to the fact that the number of mosses found in Gunung Kanthan is distinctly low compared to other comparable areas because the plant biodiversity in this location has been under threat from quarrying activity conducted there (Kiew et al. 2014).

The limestone habitats found in Gunung Senyum Recreational Forest can be divided into five groups based on classification by Chin (1977) and Mohamed (1987). The subdivisions are:

- 1. Base of hills. Species that live in this area include the foothills and the surrounding area. Examples are: *Acanthorrhynchium papillatum*, *Acroporium lamprophyllum*, *Calymperes afzelii*, *C. graeffeanum*, *Fissidens hollianus* and *Vesicularia reticulata*.
- 2. Talus slopes. Species that live in areas which cover the caves at the foot of Gunung Senyum, comprising piles of debris including limestone debris resulted from the weathering process of the rocks, Examples are: *Bryum coronatum*, *Caduciella mariei*, *Calymperes boulayi* and *C. erosum*.
- 3. Gullies and valleys. This area has plenty of sheltered places and can trap enough water. Examples are: *Fissidens ceylonensis, Macromitrium miquelii*, and *Neckeropsis lepineana*.
- 4. Cliffs and near-vertical slopes. This area provides a unique habitat for mosses because it supports very different vegetation depending on the degree of cliff gradient, presence and absence of soil and humidity levels. Examples are: *Calymperes moluccense, C. taitense, Ectropothecium dealbatum, Fissidens oblongifolius, Isopterygium pohliaecarpum* and *Pseudosymblepharis bombayensis.*
- 5. Summits. The summit of Gunung Senyum is an area composed of exposed rocks with only a small land cover. Examples are: *Floribundaria floribunda*, *Hyophila involuta*, *Isopterygium albescens* var. *albescens* and *Neckeropsis lepineana*.

Species found in this study can also be divided into four categories on the basis of their affinity to the limestone habitat (Mohamed 1987)

- 1. Exclusives. Only for species which are solely retricted to the limestone. Examples are: *Pseudosymblepharis bombayensis, Fissidens* cf. *hillianus* and *F. oblongifolius*.
- 2. Preferents. Occur mainly on limestone (50 to 75% of the time) but also found in non-limestone habitats. Examples are: *Barbula consanguinea, Bryum coronatum, Calymperes taitense, Hyophila involuta, Neckeropsis lepineana, Pelekium velatum* and *Pinnatella ambigua*.
- 3. Indifferents. Species with no particular preference for either limestone or nonlimestone habitats. Examples are: *Calymperes afzelii*, *C. boulayi*, *C. taitense*, *Ectropothecium perminutum*, *Fissidens ceylonensis*, *Homaliodendron exiguum* and *Leucophanes octoblepharioides*.
- **4. Casuals.** Non-limestone mosses which are collected on limestone. Example is: *Ectropothecium dealbatum.*

In this study, corticol is the most dominant way of life for mosses collected in this area with 47 taxa. Lignicol is second with 35 taxa, followed by calcicol or live on limestone with 26 taxa, then the terricol, with about 16 taxa. Rupicol and ramicol recorded the lowest number of taxa with eight and one taxa only. No moss species is found growing on leaves.

The mosses in Gunung Senyum Recreational Forest mostly live as corticol because this habitat provides adequate nutrients, water and exposure to sunlight. In contrast to the limestone rock which has few resources, the growth of mosses here is limited. The land surface is often limited to interstitials and limestone depressions. This factor makes the limestone environment vulnerable and hotter, even the absorption capacity of the soil is limited (Crowther 1987). In addition, the hot environment also accelerates decomposition of humus, thereby reducing the growth of mosses on decomposed materials.

# List of mosses taxa found in Gunung Senyum Recreational Forest, Taman Rimba Kenong, Wang Kelian State Park and Gunung Kanthan

The arrangement of moss taxa are arranged alphabetically. The accepted taxa for Peninsular Malaysia follow Yong et al. (2013) except for *Pelekium bifarium* (Norhazrina et al. 2017).

Taxa	G. Senyum	T. R. Kenong	Wang Kelian	G. Kanthan
Acanthorrhynchium papillatum (Harv.) M. Fleisch.	/	/	/	-
Acroporium adspersum (Hampe) Broth.	-	/	_	-
Acroporium johannis-winkleri Broth.	-	/	_	-
Acroporium lamprophyllum Mitt.	/	_	_	-
Acroporium rufum (Reinw. & Hornsch.) M. Fleisch.	-	/	_	-
Aerobryidium aureonitens (Hook. ex Schwägr.) Broth.	_	/	/	_
Aerobryidium crispifolium (Broth. & Geh.) M. Fleisch.	-	/	/	-
Aerobryopsis longissima (Dozy & Molk.) M. Fleisch. var. longissima	_	/	_	/
Arthrocormus schimperi (Dozy & Molk.) Dozy & Molk.	/	/	/	-
Barbula consanguinea (Thwaites & Mitt.) A. Jaeger	/	_	/	-
Bryum apiculatum Schwägr.	-	/	_	/
Bryum coronatum Schwägr.	/	_	/	/
Caduciella mariei (Besch.) Enroth	/	/	/	-
Callicostella papillata (Mont.) Mitt. var. papillata	/	/	_	-
Callicostella papillata (Mont.) Mitt. var. prabaktiana (Müll. Hal.) Streimann	-	/	_	-
Calymperes aeruginosum Hampe ex Sande Lac.	-	/	/	-
Calymperes afzelii Sw.	/	/	/	_
Calymperes boulayi Besch.	/	_	/	/
Calymperes erosum Müll. Hal.	/	/	/	/
Calymperes graeffeanum Müll. Hal.	/	/	/	-
Calymperes lonchophyllum Schwägr. subsp. beccarii (Hampe) M. Menzel	/	/	_	-
Calymperes lonchophyllum Schwägr. subsp. lonchophyllum	/	/	/	-
Calymperes moluccense Schwägr.	/	/	/	_
Calymperes porrectum Mitt. var. elatissimum (M. Fleisch.) A. Eddy	-	/	_	-
Calymperes porrectum Mitt. var. porrectum	_	/	_	_
Calymperes robinsonii B.C. Tan & W.D. Reese	-	/	_	-
Calymperes schmidtii Broth.	_	_	/	_
Calymperes serratum A. Braun ex Müll. Hal.	/	/	/	-
Calymperes pallidum Mitt.	/	_	_	-

Taxa	G. Senvum	T. R. Kenong	Wang Kelian	G. Kanthan
Calymperes strictifolium (Mitt.) G. Roth		/		
Calvmperes taitense (Sull.) Mitt.	/	/	/	/
Calymperes tenerum Müll. Hal.	/	_	/	_
Chaetomitrium borneense Mitt.	_	/	_	_
Chaetomitrium orthorrhynchum (Dozy & Molk.) Bosch & Sande Lac.	_	/	_	_
Chaetomitrium papillifolium Bosch & Sande Lac.	_	/	/	_
Circulifolium exiguum (Bosch & Sande Lac.) S. Olsson, Enroth & D. Ouandt	/	/	/	_
Circulifolium microdendron (Mont.) S. Olsson, Enroth & D. Quandt	_	/	/	_
Cryptogonium phyllogonioides (Sull.) Isov.	_	_	/	_
Desmotheca apiculata (Dozy & Molk.) Lindb.	_	/	_	_
Dimorphocladon borneense Mitt.	_	/	_	_
Diphyscium mucronifolium Mitt.	_	/	_	_
Ectropotheciella distichophylla (Hampe ex Dozy & Molk.) M. Fleisch.	_	/	_	_
Ectropothecium buitenzorgii (Bél.) Mitt.	_	/	/	_
Ectropothecium dealbatum (Reinw. & Hornsch.) A. Jaeger	/	_	_	_
Ectropothecium eleganti-pinnatum (Müll, Hal.) A. Jaeger	_	/	_	_
Ectropothecium ichnotocladum (Müll, Hal.) A. Jaeger	_	/	_	_
Ectropothecium monumentorum (Duby) A. Jaeger	_	1	_	/
Ectropothecium perminutum Broth, ex E.B. Bartram	/	,	/	_
Ectropothecium zollingeri (Müll, Hal.) A. Jaeger	_	_	_	/
Ethemerapsis tilbadensis K I. Goebel	_	/	_	_
Erythrodontium iulaceum (Hook, ex Schwägr.) Paris	_	,	_	_
Exostratum asperum (Mitt.) L.T. Ellis	_	,	_	_
Exostratum hlumii (Nees ex Hampe) L T Ellis	/	,	/	_
Fissidens bogoriensis M Fleisch	_	_	,	_
Fiscidens bryoides Hedw var ramossissimus Thér	1	_	, _	_
Fissidens cevlanensis Dary & Malk	,	/	/	/
Fissidens crassinervis Sande Lac var crassinervis	,	,	, _	,
Fiscidens crenulatus Mitt var elmeri (Broth ) 7 Junts & Tad Suzuki	,	,	_	_
Fisidone evictulus Brid var evictulus	/	,		/
Fissidens cristrulus Brid, var. trisputus	_	,	,	/
Fisidow flacoidus Mitt	_	,	,	_
Fiscidens guardengensis 7 Junte & 7 H Li	-	/	,	-
Fissidens hallianus Dozu & Molk	_	,		/
Fisidens invarious Dozy & Molk.	/	,	,	7
Fissidens ablangifalius Hook f. & Wilson	/	,	,	/
Fiscidens ballwidge Hornsch	,	,	,	/
Fiscidens op	/	/	,	/
Fiscidant sp.	_	_	_	/
Einidens of hilligence	1	_	_	-
Fissidens ci. mutanus	1	-		-
Flouiburd ani a flouiburd a (Dorry & Molly) M. Floiogh	1		/	_ /
Custilla tamata (Llamath ) Will & Marad	/	/		/
Groutieua tomentosa (Hornsch.) Wijk & Margad.	-		/	_
Himaniocuatum cycoprytum (With, Flain, W. Flaisch,	_	/		-
Himaniociaaium piumua (Nees) M. Fleisch	-	_	/	_
Hymenostylium recurvirostrum (Hedw.) Dixon		/		-
<i>Hyopnua mvoiua</i> (Hook.) A. Jaeger	/	/	/	_
Hyophila javanica (Nees & Blume) Brid.	-	-	/	-
Hyophila rosea R.S. Williams	_		/	-
Isopterygium albescens (Hook.) A. Jaeger var. albescens	/	/	/	-
Leucobryum aduncum Dozy & Molk. var. aduncum	-	/	/	-
Leucobryum candidum (Brid. ex P. Beauv.) Wilson	-	/	-	-
Leucopryum javense (Brid.) Mitt.	-	/	-	-
Leucobryum sanctum (Nees ex Schwägr.) Hampe var. sanctum	-	/	-	-
Leucoloma walkeri Broth.	-	-	/	-
Leucomium strumosum (Hornsch.) Mitt.	/	-	-	-
Leucophanes angustifolium Renault & Cardot	/	/	/	-
Leucophanes candidum (Schwägr.) Lindb. var. candidum	_	/	-	-

	0.0	TDV	W/ X/ I:	C K 1
Iaxa	G. Senyum	I. K. Kenong	Wang Kelian	G. Kanthan
Leucophanes glaucum (Schwagr.) Mitt.	_	/	/	-
Leucophanes octoblephariolaes Brid.	/	/	/	-
Macromurium angustijouum Dozy & Mioik.	-	,	/	_
Lac.) S.L. Guo, B.C. Tan & Virtanen	_	/	-	_
Macromitrium falcatulum Müll. Hal.	-	/	/	-
Macromitrium fuscescens Schwägr.	/	/	-	-
Meiothecium microcarpum (Harv.) Mitt. var. microcarpum	/	_	_	-
Meteorium polytrichum Dozy & Molk.	_	/	/	-
Mitthyridium constrictum (Sull.) H. Rob.	_	/	-	-
Mitthyridium fasciculatum (Hook. & Grev.) H. Rob. subsp. cardotii (M. Fleisch.) B.C. Tan & L.T. Ellis	-	/	-	-
Mitthyridium fasciculatum (Hook. & Grev.) H. Rob. subsp. fasciculatum	_	/	_	_
Mitthyridium flavum (Müll. Hal.) H. Rob.	/	/	/	_
Mitthyridium jungquilianum (Mitt.) H. Rob.	_	/	_	_
Mitthyridium repens (Harv.) H. Rob.	/	/	_	_
Mitthvridium undulatum (Dozy & Molk.) H. Rob.	/	/	/	_
Mitthvridium wallisii (Müll. Hal.) H. Rob. var. wallisii	_	/	_	_
Neckeropsis andamana (Miill, Hal.) M. Fleisch	_	_	/	_
Neckeropsis unuununu (Willi, Flat,) W. Ficisch.			,	
Neckeropsis greatlenta (Bosch & Sande Lac.) M. Eleisch	/	,	_	/
Networks grathenia (Dosen & Sante Lac.) M. Fleisch	,	,		,
Neckeropsis lepineana (Mont.) M. Fleisch.	/	/	/	/
Octoblepharum albidum Hedw.	-	_	/	-
Oedicladium pseudorufescens (Hampe) B.C. Ian & Mohamed	-	/	/	-
Orthodontium infractum Dozy & Molk.	_	_	/	-
Papillidiopsis complanata (Dixon) W.R. Buck & B.C. Tan	/	/	-	-
Papillidiopsis malesiana W.R. Buck & B.C. Tan	-	/	-	-
Pelekium bifarium (Bosch & Sande Lac.) M. Fleisch.	/	/	/	-
Pelekium gratum (P. Beauv.) A. Touw	-	-	-	/
Pelekium velatum Mitt.	/	/	/	/
Philonotis hastata (Duby) Wijk & Marg.	/	-	-	-
Pinnatella alopecuroides (Hook.) M. Fleisch.	-	-	/	-
Pinnatella ambigua (Bosch & Sande Lac.) M. Fleisch.	/	/	/	-
Pinnatella calcutensis M. Fleisch.	_	-	/	_
Pinnatella kuehliana (Bosch & Sande Lac.) M. Fleisch.	_	/	-	/
Pinnatella mucronata (Bosch & Sande Lac.) M. Fleisch.	/	/	/	/
Pogonatum piliferum (Dozy & Molk.) Lindb.	_	/	_	_
Pseudosymblepharis bombayensis (Müll. Hal.) P. Sollman	/	/	/	/
Pseudotaxiphyllum pohliaecarpum (Sull, & Lesg.) Z. Iwats,	/	_	_	_
Rhynchostegium celebicum (Sande Lac.) A. Jaeger	_	/	_	_
Rhynchostegium iavanicum (Bél.) Besch.	/	_	_	_
Stereodontopsis excavata (Broth ) Ando	, _	/	_	_
Stereophyllum tanovense (Hook) A Jagger	_	,	/	_
Surveyriguum uubyerise (1100K.) A. Jacger			,	
Synnopouon uuo-vuginuus Schwagi.	/	,	,	-
Symmopouon unsujouus with.	_	,	_	-
Syrrhopodon conjertus Sande Lac.	_	/	_	_
Syrrhopodon croceus Mitt.	-	/	-	-
Syrrhopodon involutus Schwägr.	-	/	-	-
Syrrhopodon loreus (Sande Lac.) W.D. Reese	_	/	-	-
Syrrhopodon muelleri (Dozy & Molk.) Sande Lac.	/	/	/	-
Syrrhopodon prolifer Schwägr. var. prolifer	-	/	-	-
Syrrhopodon spiculosus Hook. & Grev. var. spiculosus	-	/	/	_
Syrrhopodon trachyphyllus Mont.	-	/	_	-
Syrrhopodon tristichus Nees ex Schwägr.	-	/	-	_
Taxiphyllum taxirameum (Mitt.) M. Fleisch.	-	/	-	/
Taxithelium binsteadii Broth. & Dixon	/	-	-	-
Taxithelium instratum (Brid.) Broth.	/	/	/	_
Taxithelium isocladum (Bosch & Sande Lac.) Renauld & Cardot	/	/	_	_

Таха	G. Senyum	T. R. Kenong	Wang Kelian	G. Kanthan
Taxithelium nepalense (Schwägr.) Broth.	/	-	/	/
Taxithelium kerianum (Broth.) Broth.	/	_	_	_
Thuidium plumulosum (Dozy & Molk.) Dozy & Molk.	-	/	/	-
Thuidium pristocalyx (Müll. Hal.) A. Jaeger var. pristocalyx	-	/	_	_
Trichosteleum boschii (Dozy & Molk.) A. Jaeger	/	_	_	-
Trichostomum brachydontium Bruch	-	_	_	/
Trismegistia lancifolia (Harv.) Broth. var. lancifolia	-	/	_	-
Vesicularia dubyana (Müll. Hal.) Broth.	/	_	_	_
Vesicularia miquelii (Sande Lac.) M. Fleisch.	/	/	_	_
Vesicularia montagnei (Schimp.) Broth.	/	/	_	/
Vesicularia reticulata (Dozy & Molk.) Broth.	/	/	-	-

# Conclusion

Gunung Senyum Recreational Forest with its two unique and pristine limestone outcrops is suitable for exploration and study as this forested limestone area is inhabited by interesting flora and fauna. It is hoped that in future, the composition of moss flora in these limestone outcrops, Gunung Senyum and Gunung Jebak Puyuh, will be studied more deeply. Specimens from the steep cliffs in this area should be collected regularly to assess the true diversity of mosses in this unique limestone hill.

#### Acknowledgements

The authors would like to thank the Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme (FRGS/1/2017/STG03/UKM/02/2), Universiti Kebangsaan Malaysia for Geran Galakan Penyelidik Muda (GGPM-2017-090) and Geran Universiti Penyelidikan (GUP-2018-016) which were used to support this research. The findings have been presented in a Joint International Seminar of Universiti Kebangsaan Malaysia-Gifu University 2018 at Gifu University, Osaka, Japan on 28th of March 2018.

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# Appendix I. Moss checklist of Gunung Senyum Recreational Forest, Pahang

The arrangement of moss taxa follows Goffinet and Buck (2018), and families as well as the genera under each family and species under each genus are arranged alphabetically. The accepted taxa for Peninsular Malaysia follow Yong et al. (2013) except for *Pelekium bifarium* (Norhazrina et al. 2017). New additions to the bryoflora of Peninsular Malaysia and the state of Pahang are indicated by '\*\*' and '\*' respectively.

#### BARTRAMIACEAE

#### Philonotis Brid.

*P. hastata* (Duby) Wijk & Margad. – H. Aznani & A. Damanhuri 20, 149 & 160. On exposed roots and on limestone.

#### BRACHYTHECIACEAE

# Rhynchostegium Bruch & Schimp.

*R. javanicum* (Bél.) Besch. – H. Aznani & A. Damanhuri 339, 347 & 422. On base of trees and on limestone.

# BRYACEAE

#### Bryum Hedw.

B. coronatum Schwägr. – H. Aznani & A. Damanhuri 24, 136, 154, 153, 157, 158, 163, 167, 168, 176, 178, 179, 188, 205, 206, 208, 210, 212, 219, 220, 221, 225, 228, 234, 236, 237, 238, 241, 243, 248, 263 & 264. On rotten logs, on soil, on tree trunks and on limestone.

#### CALYMPERACEAE

#### Arthrocormus Dozy & Molk.

*A. schimperi* (Dozy & Molk.) Dozy & Molk. – H. Aznani & A. Damanhuri 458, 514 dan 522. On buttresses and base of trees.

# Calymperes Sw. ex F. Weber

- *C. afzelii* Sw. H. Aznani & A. Damanhuri 271, 278, 280, 284, 315, 326, 348, 361, 397, 398, 399, 415, 436, 477, 481 & 502. On base of trees and on buttresses.
- *C. boulayi* Besch. H. Aznani & A. Damanhuri 11, 12, 204, 218, 229 & 251. On limestone.
- C. erosum Müll. Hal. H. Aznani & A. Damanhuri 56a. On rotten log.
- C. graeffeanum Müll. Hal. H. Aznani & A. Damanhuri 405. On rotten log.
- *C. lonchophyllum* subsp. *beccarii* (Hampe) M. Menzel H. Aznani & A. Damanhuri 350. On exposed roots.
- *C. lonchophyllum* subsp. *lonchophyllum* Schwägr. H. Aznani & A. Damanhuri 287, 302, 311, 312, 330, 368 & 429. On exposed roots and on base of trees.
- C. moluccense Schwägr. H. Aznani & A. Damanhuri 116a. On tree trunk.
- \*\**C. pallidum* Mitt. H. Aznani & A. Damanhuri 56a. On burnt wood.
- C. serratum A. Braun ex Müll. Hal. H. Aznani & A. Damanhuri 294. On rotten log.

*C. taitense* (Sull.) Mitt. – H. Aznani & A. Damanhuri 91, 109, 117, 123, 133, 169, 286, 346, 370, 438 & 451. On exposed roots, on limestone and on bark of liana.

C. tenerum Müll. Hal. – H. Aznani & A. Damanhuri 387. On rotten log.

# Exostratum L.T. Ellis

*E. blumii* (Nees *ex* Hampe) L.T. Ellis – H. Aznani & A. Damanhuri 324, 393, 394, 400 & 424. On rotten log.

# Leucophanes Brid.

- *L. angustifolium* Renauld & Cardot H. Aznani & A. Damanhuri 316, 337, 376, 369, 400, 457, 490 & 498. On buttresses, on base of tree and on rotten log.
- *L. octoblepharioides* Brid. H. Aznani & A. Damanhuri 270, 284, 323, 347, 378, 395, 404, 443, 470, 487, 495, 512, 535 & 537. On base of trees, on roots of fern, on limestone and on rotten log.

#### Mitthyridium H. Rob.

- *M. flavum* (Müll. Hal.) H. Rob. H. Aznani & A. Damanhuri 274, 309, 345, 390, 410, 439, 448, 475, 499, 505, 534 & 558. On base of trees and on rotten log.
- M. repens (Harv. in Hook.) H. Rob. H. Aznani & A. Damanhuri 585. On tree trunk.
- *M. undulatum* (Dozy & Molk.) H. Rob. H. Aznani & A. Damanhuri 478. On base of tree.

# Syrrhopodon Schwägr.

- *S. albo-vaginatus* Schwägr. H. Aznani & A. Damanhuri 214, 294, 298, 314, 329, 372, 403, 507, 534, 545, 546, 578 & 579. On buttresses, on tree trunks and on rotten log.
- S. muelleri (Dozy & Molk.) Sande Lac. H. Aznani & A. Damanhuri 513 dan 526. On base of trees.

## FISSIDENTACEAE

# Fissidens Hedw.

F. bryoides var. ramosissimus Thér. – H. Aznani & A. Damanhuri 396. On tree trunk.

*F. ceylonensis* Dozy & Molk. – H. Aznani & A. Damanhuri 1, 18, 55, 64a, 65, 80, 108, 115, 124, 125, 130, 140, 142, 143, 146, 148, 151, 152, 156, 158, 161, 166, 170,

201 & 560. On limestone, on soil, on rock in the crevice of trees and on buttress.

*E. crassinervis* Sande Lac. – H. Aznani & A. Damanhuri 81, 289, 442, 426, 570, 577, 580, 583 & 584. On soil, on rocks and on soil between exposed roots.

- *F. crenulatus* var. *elmeri* (Broth.) Z. Iwats. & Tad. Suzuki H. Aznani & A. Damanhuri 519. On exposed roots.
- *F.* cf. *hillianus* H.A. Mill. & D.R. Sm. H. Aznani & A. Damanhuri 35a, 88, 98, 99, 103 & 147. On limestone.
- F. hollianus Dozy & Molk. H. Aznani & A. Damanhuri 31, 48, 49, 61, 78, 93, 97, 121, 141, 126, 265, 284, 287, 290, 318, 375, 382, 383, 388, 396, 406, 414, 416, 454, 464, 491, 499, 518, 557, 571 & 575. On roots, on base and tree trunk, on rotten log and on burnt wood.
- *F. oblongifolius* Hook.*f.* & Wilson H. Aznani & A. Damanhuri 92, 107, 110, 112, 114, 122, 127, 131, 134, 139, 155, 180, 231 & 239. On soil, on tree trunks and on limestone.

- F. pellucidus Hornsch. H. Aznani & A. Damanhuri 425 & 510. On soil and at base of tree.
- *F. zollingeri* Mont. H. Aznani & A. Damanhuri 6, 9, 13a, 14, 42, 360 & 504. On rocks, on termite hill and on soil.

#### HYPNACEAE

#### Ectropothecium Mitt.

- *E. dealbatum* (Reinw. & Hornsch.) A. Jaeger H. Aznani & A. Damanhuri 152. On limestone.
- *E. perminutum* Broth. *ex* E.B. Bartram H. Aznani & A. Damanhuri 8 & 56b. On rotten log.

# Vesicularia (Müll. Hal.) Müll. Hal.

- *V. dubyana* (Müll. Hal.) Broth. H. Aznani & A. Damanhuri 4, 19, 64, 295, 351, 384, 392a, 402, 431, 445, 456 & 459. On roots and base of tree, on soil and on rotten logs.
- *V. miquelii* (Sande Lac.) M. Fleisch. H. Aznani & A. Damanhuri 298, 444, 458 & 545. On buttresses and on rotten logs.
- *V. montagnei* (Schimp.) Broth. H. Aznani & A. Damanhuri 5, 44, 127, 317, 342, 366, 426, 430, 449, 461, 533 & 566. On soil, on tree trunks and on rotten logs.
- *V. reticulata* (Dozy & Molk.) Broth. H. Aznani & A. Damanhuri 335, 343, 362, 472, 497a & 509. On rotten log and on base of trees.

#### LEUCOMIACEAE

#### Leucomium Mitt.

L. strumosum (Hornsch.) Mitt. - H. Aznani & A. Damanhuri 423. On rotten log.

#### METEORIACEAE

# Floribundaria M. Fleisch.

*F. floribunda* (Dozy & Molk.) M. Fleisch. – H. Aznani & A. Damanhuri 245. On bark of liana.

# NECKERACEAE

# Caduciella Enroth

*C. mariei* (Besch.) Enroth – H. Aznani & A. Damanhuri 40, 353, 355, 356, 373, 418, 446, 452, 471, 473, 480, 486, 492, 520, 528a, 548 & 555. On exposed roots and on base of trees.

#### Circulifolium S. Olsson, Enroth & D. Quandt

C. exiguum (Bosch & Sande Lac.) S. Olsson, Enroth & D. Quandt – H. Aznani & A. Damanhuri 17, 29, 36, 39, 45, 50, 72, 89, 94, 100, 102, 241, 279, 281, 291, 296, 313, 332, 352, 355a, 380, 392, 455, 462, 469, 511, 528 & 551. On limestone, on exposed roots, on soil, on rotten logs and on base of trees.

#### Neckeropsis Reichardt

*N. gracilenta* (Bosch & Sande Lac.) M. Fleisch. – H. Aznani & A. Damanhuri 250, 253, 255, 258, 306, 340, 401, 440, 503, 509, 525, 529, 530, 543 & 556. On base of trees.

*N. lepineana* (Mont.) M. Fleisch. – H. Aznani & A. Damanhuri 116, 137, 164, 169, 177, 196, 215, 247 & 242. On limestone and on the edge of boulders.

#### Pinnatella M. Fleisch.

- *P. ambigua* (Bosch & Sande Lac.) M. Fleisch. H. Aznani & A. Damanhuri 291a, 340 & 358a. On limestone and on base of trees.
- *P. mucronata* (Bosch & Sande Lac.) M. Fleisch. H. Aznani & A. Damanhuri 377, 402, 539, 540a & 552. On base of trees.

# ORTHOTRICHACEAE

# Macromitrium Brid.

M. fuscescens Schwägr. - H. Aznani & A. Damanhuri 209. On rotten log.

## PILOTRICHACEAE

# Callicostella (Müll. Hal.) Mitt.

*C. papillata* (Mont.) Mitt. var. *papillata* – H. Aznani & A. Damanhuri 283, 329, 393 & 566a. On rotten log, on buttresses and on tree trunks.

# PLAGIOTHECIACEAE

## Pseudotaxiphyllum Z. Iwats.

P. pohliaecarpum (Sull. & Lesq.) Z. Iwats. – H. Aznani & A. Damanhuri 34, 67, 90 103 138, 195, 241a, 254, 324, 357, 406 & 497a (as *Isopterygium minutirameum*). On exposed roots, on soil, on limestone, on tree trunks and on rotten logs.

#### POTTIACEAE

# Barbula Hedw.

B. consanguinea (Thwaites & Mitt.) A. Jaeger – H. Aznani & A. Damanhuri 190. On limestone.

# Hyophila Brid.

*H. involuta* (Hook.) A. Jaeger – H. Aznani & A. Damanhuri 21, 41, 106, 244 & 252. On rocks, on roots of fern and on limestone.

# Pseudosymblepharis Broth

P. bombayensis (Müll. Hal.) P. Sollman – H. Aznani & A. Damanhuri 101, 105, 171, 118, 142, 145, 153a, 155, 159, 162, 172, 173, 174, 175, 181, 182, 183, 185, 187, 189, 193, 199, 203, 207, 211, 213, 214, 222, 224, 225, 227, 235, 240 & 249. On limestone.

# PYLAISIADELPHACEAE

#### Isopterygium Mitt.

*I. albescens* (Hook.) A. Jaeger var. *albescens* – H. Aznani & A. Damanhuri 8, 10, 15, 22, 35, 119, 121a, 129, 275, 292, 300, 305, 450, 506, 508, 516, 523, 527, 532 & 539. On rotten log, on soil, on exposed roots, on base of trees, on bark of lianas, on buttresses and on limestone.

#### Taxithelium Spruce ex Mitt.

- \*T. binsteadii Broth. & Dixon H. Aznani & A. Damanhuri 588. On tree trunk.
- *T. instratum* (Brid.) Broth. H. Aznani & A. Damanhuri 126, 127, 307, 312, 320, 333, 336, 359, 391, 413, 433, 437, 439, 466, 467 & 501. On rotten logs, on soil, on exposed roots, on limestone and on bark of lianas.
- *T. isocladum* (Bosch & Sande Lac.) Renauld & Cardot H. Aznani & A. Damanhuri 288 & 476. On exposed roots.
- *T. kerianum* (Broth.) Broth. H. Aznani & A. Damanhuri 275a, 283, 309, 321, 381, 474 & 573. On base of trees.
- *T. nepalense* (Schwägr.) Broth. H. Aznani & A. Damanhuri 46, 53, 73, 269, 273, 299, 304, 305, 402 & 533. On rotten log, on exposed roots, on stumps of tree inside the lake and on wood.

# SEMATOPHYLLACEAE

#### Acanthorrhynchium M. Fleisch.

A. papillatum (Harv.) M. Fleisch. – H. Aznani & A. Damanhuri 444a & 458. On buttresses.

## Acroporium Mitt.

A. lamprophyllum Mitt. – H. Aznani & A. Damanhuri 586 & 587. On rotten logs.

#### Meiothecium Mitt.

*M. microcarpum* (Harv.) Mitt. var. *microcarpum* – H. Aznani & A. Damanhuri 30, 217, 362 & 524. On limestone, on tree trunks and on rotten logs.

#### Papillidiopsis (Broth.) W.R. Buck & B.C. Tan

P. complanata (Dixon) W.R. Buck & B.C. Tan – H. Aznani & A. Damanhuri 386, 493, 531, 549, 561, 572, 574, 582 & 586. On rotten logs, on tree trunks and branches.

#### Trichosteleum Mitt.

*T. boschii* (Dozy & Molk.) A. Jaeger – H. Aznani & A. Damanhuri 303, 314a, 485, 544 & 581. On soil and rotten logs.

#### THUIDIACEAE

# Pelekium Mitt.

- P. bifarium (Bosch & Sande Lac.) M. Fleisch. H. Aznani & A. Damanhuri 3, 23, 29, 32, 38, 44, 47, 52, 55, 62, 72, 77, 87, 88, 98, 128, 300, 349 & 553 (as Aequatoriella bifaria). On limestone, on rotten log, on soil and tree trunks.
- P. velatum Mitt. H. Aznani & A. Damanhuri 2, 4, 5, 7, 8, 13, 19, 26, 33, 43, 51, 54, 57, 61, 63, 64, 66, 70, 71, 77, 104, 132, 269, 272, 296, 297, 301, 308, 322, 328, 325, 326, 334, 341, 344, 347a, 358, 359, 363, 364, 374, 385, 389, 391, 392, 402, 406, 407, 408, 411, 412, 417, 419, 428, 432, 435, 437, 441, 456, 463, 468, 472, 479, 480, 483, 488, 494, 500, 536, 550, 554, 559, 563, 564, 565, 567 & 569. On limestone, on rocks, on soil, on tree trunks, on rotten logs, on exposed roots and on base of trees.
RESEARCH ARTICLE



# A new coastal species of Pseuderanthemum (Acanthaceae) from Loyalty Islands (New Caledonia) and Vanuatu with notes on P. carruthersii

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Academic editor: Alan Paton | Received 20 May 2019 | Accepted 14 June 2019 | Published 29 July 2019

Citation: Gâteblé G, Ramon L, Butaud J-F (2019) A new coastal species of *Pseuderanthemum* (Acanthaceae) from Loyalty Islands (New Caledonia) and Vanuatu with notes on *P. carruthersii*. PhytoKeys 128: 73–84. https://doi.org/10.3897/ phytokeys.128.36325

# Abstract

When dealing with the taxonomy of Pacific coastal species within the region of New Caledonia and Vanuatu, one should examine all names published in Australasia and other Pacific islands. When the putative new species is also closely related to a highly praised ornamental species with many cultigens and with many old horticultural names, the task becomes more arduous. This is the case for the new species we describe as *Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud, which is closely related to the now pantropical cultivated species *P. carruthersii* (Seem.) Guillaumin s.l. Compared to *P. carruthersii, P. melanesicum* has carnose and shiny leaves, pedicels and sepals covered with glandular hairs, a short and enlarged corolla tube and can produce fertile capsules. The new species is a coastal taxon occurring naturally in the Melanesian archipelagos of New Caledonia and Vanuatu. This species seems uncommon in the Loyalty Islands but more common in the archipelago of Vanuatu and we propose it as Critically Endangered in New Caledonia, Vulnerable in Vanuatu and Least Concern when the IUCN evaluation is done globally.

# Keywords

Acanthaceae, New Caledonia, new species, Pseuderanthemum, taxonomy, Vanuatu

# Introduction

The Pacific and Malesian taxa of Acanthaceae Juss. are in need of a broad taxonomic revision and especially those of *Pseuderanthemum* Radlk. (Barker 1986). Pseuderanthemum is traditionally defined as a pantropical genus with about 60 species both in the Old and New World tropics; the genus belongs to the tribe Justicieae of the subfamily Acanthoideae Link (Olmstead et al. 2016). The most recent and significant taxonomic treatments of the genus in the Pacific region are the ones by Heine (1976) for New Caledonia, Barker (1986) for Australia, and Smith (1991) for Fiji. There is presently also a lack of a good molecular phylogenetic framework for the genus in the region: few Pacific and Old World members of Pseuderanthemum have been included in the most recent phylogenetic and evolutionary studies (e.g. McDade et al. 2000, Daniel et al. 2008, Kiel et al. 2018). As currently circumscribed, Pseuderanthemum is not a monophyletic genus because the sampled New World, African and Asian species are placed in different clades (McDade, pers. comm.). Because the type species of the genus Pseuderanthemum is believed to be the New World P. alatum (Nees) Radlk. (Barker 1986), the Asian and Pacific taxa will need to be transferred to another genus once the molecular phylogenies will be better resolved based on a more comprehensive taxonomic sampling.

Some recent fieldwork and specimen collection conducted by the authors in the New Caledonian Loyalty islands (Lifou and Maré) and in Vanuatu led us to think that a taxon was missing in Heine's (1976) full treatment of the genus in New Caledonia. After having looked at the many names already published in *Pseuderanthemum* and allied genera in the region to verify if a name was already available, we propose to describe it as a new taxon along with line drawings, color photos taken in the field, and an extinction risk assessment.

# Materials and methods

Most, if not all, published names of *Pseuderanthemum* and *Eranthemum* L. including some names in *Anthacanthus* Nees, *Chrestienia* Montrouz., *Graptophyllum* Nees, *Justicia* L., *Pachystachys* Nees, *Ruspolia* Lindau and *Siphoneranthemum* Kuntze said to be occurring in, or coming from, the central Indo-Pacific region were retrieved using International Plant Name Index (2012) and taxonomic publications and databases. Most of the protologues and type specimens (when scans were available) for these names were checked to know if they could be related to the new species. Careful examinations and measurements, in vivo, in the herbarium, and in alcohol, were conducted on morphological characters already used by Barker (1986) and Heine (1976) as taxonomically significant within the genus, to determine whether the species is new. Descriptions of color pertain to colors in vivo, unless otherwise noted. Descriptive terminology follows the glossary in Harris and Harris (2001). The species concept used here is based on morphological characters. Voucher specimens are

deposited in K, LOY, MPU, NOU, NY, P and PVNH [abbreviations following Index Herbariorum (2019), except for LOY, which is the future herbarium code of the new Herbier de la province des Îles Loyauté].

# Pseuderanthemum s.l. in the Australasian and Pacific regions and domestication of P. carruthersii

From our bibliographic and type specimen image searches for about 100 Pseuderanthemum s.l. names in the region, it appears that the new species could only be confused with P. carruthersii (Seem.) Guillaumin. This latter species is, however, quite variable morphologically and widespread in cultivation throughout the tropics. In the tropics and in the Southwestern Pacific, especially Vanuatu and New Caledonia, P. carruthersii s.l. is a common garden ornamental showing a great morphological variability, especially in leaf size, shape and color. Like many other plants with variegated or colored leaves [e.g. Abelmoschus manihot (L.) Medik, Acalypha wilkesiana Müll.Arg., Codiaeum variegatum (L.) Rumph. ex A.Juss., Cordyline fruticosa (L.) A.Chev., Dendrolobium umbellatum (L.) Benth., Graptophyllum pictum (L.) Griff., Hibiscus rosa-sinensis L. s.l., Pandanus tectorius Parkinson ex Du Roi, Plectranthus scutellarioides (L.) R.Br., Polyscias guilfoylei (W.Bull) L.H.Bailey and *P. scutellaria* (Burm.f.) Fosberg], we believe that *Pseuderanthemum* carruthersii s.l. was selected and moved around during the migrations and peopling of the Melanesian islands well before European arrival in the region (Gâteblé 2015). It is then difficult to assess the true area of origin of these cultigens but most of them are coming from Southeast Asia or Papua New Guinea. Like most other such cultigens, P. carruthersii is a sterile (not fruiting) plant at least in Fiji (Smith 1991), New Caledonia and Vanuatu (authors pers. obs.) and is propagated through cuttings. Future molecular studies might inform about the wild relatives of the cultivated *P. carruthersii* s.l. as well as the peopling of the west Pacific as has been done for example in bananas (Perrier et al. 2011) and sweet potatoes (Roullier et al. 2013). To our knowledge, it is not known why such a large diversity of variegated and colored leaved plant species have been selected and spread through the Melanesian archipelagos but it seems to have played an important role in Melanesian culture and "gardens" (Gâteblé 2015).

# Pseuderanthemum carruthersii s.l., a taxonomically and nomenclaturally complex taxon

*Pseuderanthemum carruthersii* was originally described by Seemann (1866) as *Eranthemum carruthersii* from specimens collected by John MacGillivray on two islands (Aneityum and Erromango) in the south of the Vanuatu (former New Hebrides) archipelago, most probably in November and December 1853 (MacGillivray 1854). According to Smith (1991) there are three syntypes in BM (one without number from Erromango and two from Aneityum under *MacGillivray 30*) while Heine (1976) stated there are several

specimens with at least the holotype in K and an isotype in BM. Both authors (Heine 1976, Smith 1991) were relying on a manuscript note from C.B. Clarke on the sheet from Erromango (BM001041151) to apply the name *E. carruthersii*. Smith (1991) chose this sheet (BM001041151) as the lectotype of the name *E. carruthersii* based on the note from Clarke describing this specimen with a short corolla tube of ca. 1 cm long. According to Clarke (in herb. and in Smith 1991), the two other syntypes *MacGillivray 30* from Aneityum in BM have a longer corolla tube (1.8–2.5 cm) and would represent a cultivated form of *E. carruthersii*. Also, Choopan et al. (2018) wrongly mentioned that Guillaumin (1948) designated the lectotype in BM.

Since *P. carruthersii* is a variable species and a highly regarded ornamental plant; it has been introduced multiple times from various sources and cultivated in European nurseries especially during the second half of the nineteen century. This has resulted in a plethora of names under *Eranthemum*, many of them being published in horticultural magazines several times with different authorships and, later on, combined or not under *Pseuderanthemum* and *Siphoneranthemum* by taxonomists. For most of the names, there is no herbarium specimen, the diagnosis is often short and/or incomplete and almost no lectotypification work has been done. It is not our intention to deal with the lectotypification and formal synonymization of all the names. Several authors have dealt, though incompletely, with the synonymization at the species or variety levels (see Fosberg 1955, Heine 1976, Fosberg and Sachet 1980, Smith 1991, Fosberg et al. 1993, Choopan et al. 2018) mainly depending on leaf shape and type of variegation.

Among all the names published so far, two are of particular interest for this work. The first one is the recently lectotypified *Pseuderanthemum maculatum* (G.Lodd.) I.M.Turner (Turner 2016) as, according to the designated lectotype (see illustration in Loddiges 1822), it is a reasonable match to *P. carruthersii* s.l. If this is the case, the name *P. maculatum* would have priority over *P. carruthersii*. The protologue does not give any indication about where the cultivated *P. maculatum* comes from but later Regel (1860) stated it comes from "*India orientalis*". The second name is *Eranthemum marmoratum* W.Bull. According to the description of the vegetative parts of *E. marmoratum* in Bull's horticultural catalogue (Bull 1874), this name might apply either to a cultigen of the new species or to a similar mottled-leaved *Graptophyllum pictum*. As there is no illustration or any herbarium specimen known to apply to the name *E. marmoratum*, it is best to consider this name as a doubtful one.

# Taxonomy of the new species

*Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud, sp. nov. urn:lsid:ipni.org:names:60479324-2 Figs 1, 2, 3

**Diagnosis.** *Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud is most similar to some cultigens of *P. carruthersii* but differs from them by its carnose leaf texture vs. chartaceous, by its pedicels and sepals with glandular hairs vs. eglandular hairs, by its



Figure 1. Distribution of *Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud, sp. nov. in some islands of New Caledonia and Vanuatu. Map done using CartoGIS Services, College of Asia and the Pacific, The Australian National University.

glabrous petals and tube vs. hairy petals and tube and by its short and enlarged corolla tube vs. longer cylindrical corolla tube.

**Type.** NEW CALEDONIA. Province des Iles Loyauté: Lifou, plant cultivated at the Agricultural Research Station Saint Louis at Mont-Dore, 4 December 2018, *G. Gâteblé 1072* (holotype P; isotypes NOU [NOU089981, NOU090339]).

**Description.** Fruticulose shrubs up to 1.5 m tall, generally decumbent to somehow erect, somewhat carnose. Branches round (living material) to angulate (dry material) in cross section, beige to brown on older stems, pale green on young stems; prominent leaf and bundles scars; lenticels few, glabrous. Leaves simple, opposite-decussate, usually ovate to broadly elliptic, rarely obovate; blade (7–)  $8-11(-12) \times (2.5-) 5-6(-6.5)$  cm, vernicose, carnose, glabrous on both surfaces; apexes obtuse to rounded, sometimes slightly retuse or acuminate, bases cuneate to attenuate, margins entire; midveins slightly impressed adaxially, prominent abaxially toward the base, glabrous; secondaries of 4-6 opposite or alternate veins, more or less brochidodromous; tertiaries in a loose reticulum; petioles (0.5–) 1–1.5 (–2.5) cm long. Inflorescences terminal, a raceme or panicle, 1–12 cm long; rachises round to quadrangulate in cross section, mostly glabrous but with some glandular hairs in its most apical part; peduncles 1-5 cm long usually glabrescent but sometimes with some glandular hairs; bracts and bracteoles lanceolate 1-4 mm × 0.5-1.5 mm, glabrescent to piloglandulose. Flowers bilaterally symmetrical; pedicels 3-11 mm long, piloglandulose. Sepals 4 or 5, lanceolate,  $3.5-4 \times 1 \text{ mm}$ , piloglandulose on the outer surface, glabrescent inside. Corollas ampliate to slightly ventricose, white with purple center, aestivation imbricate in bud; tubes ca. 1-1.3 cm long, glabrous, enlarged distally to 3-5 mm diameter before the throat, 4-5 lobed, consistently three in the lower half, and one or two in the upper half; lobes elliptic,  $5-8 \times 4-7$  mm, the lower one being the larger, glabrous. Stamens 2, slightly exserted, inserted in the tube orifice on to the upper lobe(s), filaments 4-4.5 mm long, glabrous, anthers ca.  $1.8 \times 0.8$ mm; staminodes 2, ca. 1 mm long. Ovaries conical, 4.5 × 2 mm, glabrous; styles slightly exserted, ca. 15 mm long, glabrous; stigmas bilobed, lobes ca. 0.5 × 0.15 mm. Fruits stipitate dehiscent capsules, clavate,  $1.5-3 \times 0.6-0.8$  cm, sometimes crowned with the remnant style; seed (4-?) per capsule, ovate,  $3-4 \text{ mm} \times 2-3 \text{ mm}$ .

**Distribution and ecology.** In New Caledonia and Vanuatu, *P. melanesicum* is found in coastal thickets on limestone substrate, either coastal reef, cliffs or back of the beaches, with species of *Bikkia* Reinw. ex Blume, *Dendrolobium* (Wight & Arn.) Benth., *Eugenia* P.Micheli ex L., *Heliotropium* Tourn. ex L., *Hibiscus* L., *Myoporum* Banks & Sol. ex G.Forst., *Nicotiana* L., *Pemphis* J.R.Forst. & G.Forst., *Sarcolobus* R.Br. and *Xylosma* G.Forst. at 2–60 m elevation in the Loyalty Islands. In New Caledonia, it is only known from Lifou and Maré in the Loyalty Islands and it is known from Efaté and Malakula in Vanuatu (Fig. 1). With such a distribution, the species should be more common than reflected by the available herbarium specimens. Recently (February 2019) the species was seen in relatively large populations on Erakor Island (Port Vila) and Port Resolution (Tanna). Like in other Acanthaceae, *P. melanesicum* seeds are dispersed through ballochory that could explain the many individuals found in some populations in Vanuatu. The seed seems also able to float on sea water for a few hours (observation made with only one seed).



**Figure 2.** Drawings of *Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud, sp. nov. and *P. carruthersii* **A-G** *Pseuderanthemum melanesicum* **H–I** *Pseuderanthemum carruthersii* **A, D** Structure of the inflorescence B Flower **C** Flower bud **E** Open corolla with the lower corolla lobe removed to show the arrangement of internal structures **F** Open mature and immature capsules **G** Glandular hairs on a flower bract **H** Eglandular hairs on the outer surface of the corolla tube **I** Part of inflorescence showing the long narrow tube of a flower. Voucher specimens: **A–D** *Gâteblé 1072* **E, G** *Gâteblé 722* **F** *Ramon 220* **H–I** *Gâteblé 720*. Drawings by Laurence Ramon.

**Etymology.** The new species is named after the Melanesian archipelagos of New Caledonia and Vanuatu.

**Species recognition.** With its carnose and shiny leaves (especially seen on fresh material), its short and broaden corolla tube and its many glandular hairs on pedicels and sepals, *P. melanesicum* is easily separated from the cultivated, widespread and variable taxon, *P. carruthersii*. In addition, in both countries the new species has been collected in fruit while there is, to our knowledge, no fruiting specimen of *P. carruthersii* in the region. The well-known south-western Pacific botanical specialist Peter Shaw Green (1920–2009) also thought it was a putative new species as he wrote "*Pseuderanthemum* sp.? ined" on several herbarium sheets (e.g., *Hallé 6331* and *Gillison 3539*).

**Notes.** Color figures of *P. melanesicum* have already been published twice under misapplied names of other species inhabiting Loyalty Islands and Vanuatu, once as *P. repandum* (G.Forst.) Guillaumin subsp. *loyaltyensis* (Guillaumin) Heine or «Waditcha» in Suprin (2008: 177), and the other one (fig. 119a) as *P. carruthersii* in Ramon and Sam (2015: 121). The vernacular name *Watija* in Maré can be related to *P. repandum* subsp. *loyaltyensis* but this name was not recorded recently (Lormée et al. 2011) for that species; *Watija* is clearly the local name of *Psychotria nummularioides* Baill. ex Guillaumin and has also been given to *Cleidion verticillatum* Baill., a shrub of the same size (Butaud, pers. obs.). Two local names are reported on *Gillison 3539* for Vanuatu as *Nuguvere* and *Malandi*. According to Gâteblé et al. (2018), *P. melanesicum* is only the third non-endemic species described from New Caledonia since the beginning of the 21<sup>st</sup> Century. *Pseuderanthemum melanesicum* is easily propagated by cuttings and it thrives well in cultivation. Even in cultivation, the carnose and shiny leaves are maintained (Fig. 3) and it makes a nice native ornamental plant for gardens and landscaping in open or shaded areas.

The cultivated plant from which the type specimen was prepared was originally collected by J.-F. Butaud on Lifou, north of Wé to Luecila, (2 m elevation, 20°53'34.56"S, 167°16'18.56"E) on 19 April 2014.

**Preliminary conservation status (IUCN 2017).** In New Caledonia, the species is very uncommon and was collected only recently from the east coasts of Lifou and Maré islands. Recently 15–20 shrubs were seen on Lifou and two on Maré but B. Suprin (pers. comm., 2015) states he has seen it in a few places on Maré and Lifou. On Maré, the species is threatened by feral goats that seem to graze young stems and that contribute to habitat degradation. On Lifou, in its only currently known location, the species is not clearly threatened; the only threats could come from agriculture as nearby areas are cultivated. The distribution of *P. melanesicum* in New Caledonia is similar to that of *Cyrtandra mareensis* Däniker but much rarer for the number of individuals. Its area of occupancy is 8 km<sup>2</sup> while its extent of occurrence is less than 500 km<sup>2</sup>. It is considered severely fragmented as each subpopulation could go extinct with a very reduced probability of natural recolonization from the other subpopulation. A continuing decline has been observed and/or estimated for its quality of habitat and number of mature individuals. Based on the IUCN Red List Categories and Criteria (IUCN 2017) and using criteria B, *P. melanesicum* qualifies for Critically Endangered



**Figure 3.** Field pictures of *Pseuderanthemum melanesicum* Gâteblé, Ramon & Butaud, sp. nov. and *P. carruthersii* **A–D**. *Pseuderanthemum melanesicum* **E–F** *Pseuderanthemum melanesicum* and *P. carruthersii* **A** Overview of a single shrub hanging from a coastal limestone cliff on Maré island **B** Flowering branch on Efaté island **C** Flower **D** Infructescence and ecology on Lifou island **E** Cultivated plants of *P. carruthersii* (left) and *P. melanesicum* (right) **F** Leaves and inflorescences of *P. melanesicum* (left) and two cultigens of *P. carruthersii* (center and right). Photographs by G. Gâteblé (**A**, **C**, **E–F**), L. Ramon (**B**) and J.-F. Butaud (**D**); Voucher specimens: **A** *Gâteblé et al. 1024*, **B** *Ramon 220*, **C** *Gâteblé 722*, **F** *Gâteblé 722*, *721*, *720*.

CR B2ab(iii,v) in New Caledonia. Using criteria D, with less than 50 individuals, *P. melanesicum* qualifies also for Critically Endangered (CR D). In Vanuatu, this species is commonly observed on Efaté island's seashore and the major threats are forest and coastal clearing for housing on private properties (e.g. in Havannah and Undine bays and south from Eton village). The recently seen Erakor Island (Efaté) population is also facing disturbance due to the resort while the Port Resolution (Tanna) population is not facing major threat. No threat has been identified on Malakula and the species should also be present on other southern islands (Ambrym, Epi, Erromango and Aneityum) of the archipelago of Vanuatu towards the Loyalty Islands with no major associated threat. As a putative common species in Vanuatu with between five to ten locations and with threats only in the south of Efaté, the species could qualify as VU B2ab(ii,iii,v). Globally, for both New Caledonia and Vanuatu, *P. melanesicum* could be considered as Least Concern against IUCN criteria because of there being more than ten putative locations and both the extent of occurrence and area of occupancy are above the threshold for assessment of a threatened category using criterion B.

Additional specimens examined. NEW CALEDONIA. Province des Îles Loyauté: Lifou, Luecila, 2 m, 20°53'34.56"S, 167°16'18.56"E, 15 April 2015, *J.-F. Butaud 3427* (NOU [NOU090338]); plant cultivated at the Agricultural Research Station Saint Louis at Mont-Dore, 13 May 2015, *G. Gâteblé 679* (NOU [NOU089984]); *ibid. loc.*, 7 December 2015, *G. Gâteblé 722* (NOU [NOU089982, NOU089983]); *ibid. loc.* 29 December 2015, *G. Gâteblé 740* (NOU [NOU089985, NOU090341]); *ibid. loc.* 19 February 2019, *G. Gâteblé 1073* (NOU [NOU090340]). Maré: Sentier littoral entre Eni et Shabadran, 60 m, 21°39'25.57"S, 168°0'22.45"E, 2 April 2018, *G. Gâteblé, Drouin J, Jewine A. & Wamejongo W 1024* (MPU, NOU [NOU089986], P, K, LOY). VANUATU. Efaté, Eton, plage privée (avant le blue hole), 0 m, 17°45'0.55"S, 168°33'55.46"E, 5 August 2015, *L. Ramon 220* (NY [NY03487104], P [P02434405], PVNH). Malekula, Tisbel, 29 September 1971, *N. Hallé 6331* (NOU [NOU077567], P [P04385831]); *ibid. loc.*, 28 September 1971, *A.N. Gillison 3539* (P [P04385834]).

# Acknowledgements

We are grateful to Jacqueline Tinel and the team at the NOU herbarium (IRD, Nouméa) for mounting, distributing and computerizing the specimens. Special thanks to Jérôme Munzinger who has checked the specimens at P for us. We also thank the authorities of Loyalty Islands Province (DDRA) and Vanuatu for providing collecting permits. Warm regards to Anne Marshall from the Kew Library for sending the W. Bull catalogue of 1874. Gildas Gâteblé is most thankful to Julien Drouin and Jacques Wamejongo for their enthusiastic help in the field and Giliane Karnadi-Abdelkader and Jacqueline Ounémoa, propagation and cultivation of the new species in the Saint-Louis research facilities and to L.A. McDade and B. Suprin for their personal communications. Armelle Tardivel mounted Figure 3. Jean-François Butaud thanks

Province des Îles Loyauté and Conservation International which financed botanical investigations in Loyalty Islands and more particularly Georges Kakue (PIL) and Jean-Christophe Lefeuvre (CI). Laurence Ramon thanks Chanel Sam, the Forestry Department of Vanuatu and the owner of private property around Efaté for allowing access to the seashore. George Staples is acknowledged for English proofreading and some systematic and nomenclature advices. Yunfei Deng and Ian Turner made insightful comments on a previous version of this manuscript.

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RESEARCH ARTICLE



# Isotrema sanyaense, a new species of Aristolochiaceae from Hainan, China

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Academic editor: Leandro Giacomin | Received 2 April 2019 | Accepted 24 July 2019 | Published 12 August 2019

Citation: Li R, Wang Z, Wang J, Zhu X, Xu H (2019) *Isotrema sanyaense*, a new species of Aristolochiaceae from Hainan, China. PhytoKeys 128: 85–96. https://doi.org/10.3897/phytokeys.128.35042

# Abstract

*Isotrema sanyaense* R.T.Li, X.X.Zhu & Z.W.Wang, **sp. nov.**, a new species from Hainan island, China, is described and illustrated here. It is morphologically most similar to *I. ledongense* (Han Xu, Y.D.Li & H.J.Yang) X.X.Zhu, S.Liao & J.S.Ma and *I. jianfenglingense* (Han Xu, Y.D.Li & H.Q.Chen) X.X.Zhu, S.Liao & J.S.Ma in the shape of leaf, flower, and the yellow to brown villous indumentum of the pedicel, ovary and calyx. However, *I. sanyaense* can be easily distinguished from the latter two species by its 1–5-flowered cymes, in hanging clusters of 1 to numerous branches, upper calyx tube obviously longer than basal calyx tube, calyx limb discoid, yellow inside, with purple-red stripes and spots, about 13–18 mm in diameter, glabrous, and a throat dark red without spots, 4–6 mm wide.

# Keywords

Aristolochia, Aristolochia subgenus Siphisia, Asia, morphology, taxonomy

# Introduction

*Isotrema* Raf. (Aristolochiaceae), previously treated as a subgenus of *Aristolochia* L., was recently reinstated as an independent genus based on molecular and morphological evidence (Zhu et al. 2019). It differs from other genera of Aristolochiaceae by a combination of characters: calyx strongly curved, gynostemium 3-lobed, anthers paired on the outer surface of each gynostemium segment, and capsule dehiscing basipetally

(Do et al. 2015a, Zhu et al. 2019). Currently, *Isotrema* comprises 99 species and one subspecies, mainly distributed in East and South Asia, with some species scattered in North and Central America (Zhou et al. 2019, Zhu et al. 2019). China accommodates 59 species and one subspecies, of which 47 species and one subspecies are endemic to the country (Hwang et al. 2003, Zhou et al. 2019, Zhu et al. 2019).

During our field investigations to South Hainan Province, China, in 2017 and 2018, an unknown species of Aristolochiaceae was discovered. The horseshoe-shaped calyx tube, 3-lobed calyx limb and gynostemium, anthers adnate in pairs opposite the gynostemium lobes, and capsule dehiscing basipetally indicate it to be a member of *Isotrema*. After comparing with other species of the genus, we confirmed that the unidentified species from Hainan island represents a new taxon, so here, we describe and illustrate it.

# Material and methods

Measurements and assessments of morphological data of the species described here were based on living plants obtained in the wild. Flowering and fruiting branches were pressed to specimens and deposited in the CSH and KUN herbaria (herbarium acronyms follow Thiers 2019). The comparison among similar species was based on extensive revision of specimens (including types) of *Isotrema* in A, BM, BR, CDBI, CSFI, CSH, E, EMA, GXMI, HAST, HENU, HHBG, HIB, HITBC, HNWP, IBK, IBSC, K, KUN, KYO, L, LBG, LE, NAS, NTUF, P, PE, PEM, SM, SNU, SYS, TAI, TI, W, WCU, WU, WUK, XYTC and YUKU herbaria, as well as related literature (Cheng et al. 1988, Ma 1989a, 1989b, Hwang et al. 2003, Ohi-Toma et al. 2006, Xu et al. 2011, Do et al. 2015a, 2015b, Ohi-Toma and Murata 2016, Zhu et al. 2016, 2017a, 2017b, 2018, Gong et al. 2018, Yang et al. 2018).

# Taxonomy

*Isotrema sanyaense* R.T.Li, X.X.Zhu & Z.W.Wang, sp. nov. urn:lsid:ipni.org:names:77200836-1 Figs 1–3

**Diagnosis.** *Isotrema sanyaense* is most similar to *I. ledongense* (Han Xu, Y.D.Li & H.J.Yang) X.X.Zhu, S.Liao & J.S.Ma and *I. jianfenglingense* (Han Xu, Y.D.Li & H.Q.Chen) X.X.Zhu, S.Liao & J.S.Ma (Zhu et al. 2019), but significantly differs in the following characters: cymes 1–5-flowered, in hanging clusters of 1 to numerous branches, the pedicel nearly equal in length to flower, upper calyx tube obviously longer than basal calyx tube, calyx limb discoid, yellow inside, with purple-red stripes and spots, about 13–18 mm in diameter, glabrous, the throat dark red without spots, 4–6 mm wide. A detailed morphological comparison among the three species is shown in Figure 4 and Table 1.



**Figure 1.** *Isotrema sanyaense* R.T.Li, X.X.Zhu & Z.W.Wang, sp. nov. **A** flowering branch **B** leaf **C** flower (front view) **D** flower (lateral view) **E** opened flower (showing the inside structure) **F** anthers and gynostemium **G** fruit **H** seeds. Scale bars: 6 cm (**B**); 1 mm (**C**, **D**, **E**); 5 mm (**F**); 2 cm (**G**); 3 mm (**H**).



**Figure 2.** *Isotrema sanyaense* R.T.Li, X.X.Zhu & Z.W.Wang, sp. nov. **A** habit **B** leaves (adaxially and abaxially) **C** inflorescence **D** flower (lateral view) **E** opened flower (showing the inside structure) **F** anthers and gynostemium **G** fruit **H** seeds. Scale bars: 1 cm (**B**, **C**); 2 cm (**D**, **E**, **G**); 5 mm (**F**, **H**).

**Type.** CHINA. Hainan: Sanya City, Haitang District, Haitangwan Town, 18°17'22"N, 109°39'45"E, 332m a.s.l., 28 October 2017 (fl), *X.X.Zhu & R.T.Li ZXX17105* (holotype: CSH-0146607!; isotype: CSH!, KUN!).

Description. Woody liana. Young stems terete, densely villous, with yellow to brown trichomes, old branchlets glabrous, old stems leafless. Petioles 0.8-1.7 cm long, young ones densely villous, with yellow to brown trichomes mixed with a white pubescence; blades lanceolate or elliptic-lanceolate, entire,  $9-14 \times 3-6$  cm, leathery, adaxially glabrous, abaxially villous, with sparse larger white appressed trichomes, mixed with shorter white pubescence, veins pinnate, 5 to 10 pairs, base shallowly cordate to cordate, sinus < 2-3 mm deep, apex acute. Cymes lateral on old woody stems or axillary, 1-5-flowered, in hanging clusters of 1 to numerous branches, pedicels 1.1-2.6 cm long, densely villous, with yellow to brown trichomes; bracteoles ovatelanceolate, ca.  $0.2-0.4 \times 0.4$  mm, inserted at the basis of pedicel, adaxially glabrous, abaxially densely villous, with yellow to brown trichomes. Calyx horseshoe-shaped, externally white with purple-red stripes; abaxially densely villous, with yellow to brown trichomes; basal tube ca.  $2.2 \times 0.5$  cm, inside dark purple, with white patches spaced in the middle; upper tube ca.  $2.5 \times 0.5$  cm, white inside, getting dark red in upper portion; calvx limb discoid, ca. 13–18 mm in diameter, abaxially densely villous, with yellow to brown trichomes, the inner surface yellow with purple-red stripes and spots, glabrous;



Figure 3. Holotype of Isotrema sanyaense R.T.Li, X.X.Zhu & Z.W.Wang, sp. nov. [CSH-0146607]!.



Figure 4. A, B Isotrema sanyaense R.T.Li, X.X.Zhu & Z.W.Wang, sp. nov. A habit B flowers C, D I. jianfenglingense (Han Xu, Y.D.Li & H.Q.Chen) X.X.Zhu, S.Liao & J.S.Ma C habit D flowers E, F I. ledongense (Han Xu, Y.D.Li & H.J.Yang) X.X.Zhu, S.Liao & J.S.Ma E flowering branch F flower (front view).

throat dark red, 4–6 mm wide. Anthers 6, oblong, ca. 2.2 mm long, adnate in 3 pairs to base of gynostemium, opposite to lobes, dehiscence longitudinal. Gynostemium ca. 5 mm long, 3-lobed, apex acute; ovary inferior, 6-loculed, abaxially densely villous, with yellow to brown trichomes; ovules numerous; placentation axillary. Capsule oblong-ellipsoid, ca.  $5 \times 2$  cm, dehiscing basipetally. Seeds triangular-ovate,  $4-5 \times 3-4$  mm.

**Phenology.** Flowering specimens have been collected in October and in fruiting specimens in May.

**Etymology.** The specific epithet is derived from the type locality, Sanya City, in Hainan island, China. The Chinese name is given as "三亚关木通".

**Distribution and habitat.** *Isotrema sanyaense* is currently known from Haitangwan Town, Haitang District, Sanya City, Hainan Province, China. It grows in lowland dry forests dominated by families including Euphorbiaceae, Fagaceae, Lauraceae, Myrtaceae, Arecaceae and Rubiaceae at elevations between 332–400 m.

**Conservation status.** *Isotrema sanyaense* is only known from two populations in Sanya City, Hainan island, China, with fewer than 30 individuals seen at each site. Therefore, the new species is assigned a preliminary status of Vulnerable (VU D2) according to IUCN Red List Criteria (IUCN 2012), indicating a population with a very restricted area of occupancy (typically less than 20 km<sup>2</sup>) or number of locations (typically five or fewer).

Additional specimens examined (paratypes). CHINA. Hainan: Sanya City, Haitang District, Haitangwan Town, 18°17'24"N, 109°39'43"E, 400m a.s.l., 28 October 2017 (vegetative), *X.X.Zhu & R.T.Li ZXX17106* (CSH); same location, 18°17'29"N, 109°39'46"E, 376m a.s.l., 21 May 2018 (fr), *X.X.Zhu & J.Wang ZXX18075* (CSH, KUN).

Characters	I. sanyaense	I. ledongense	I. jianfenglingense
Leaf blade	adaxially glabrous, abaxially sparsely with white pubescence	adaxially mixed with yellow- brown villous and white pubescence, abaxially densely yellow-brown villous	adaxially sparsely yellow-brown villous and white pubescence, abaxially mixed with yellow-brown villous and white pubescence
Inflorescence	cyme 1–5-flowered, in hanging clusters of 1 to numerous branches	solitary	solitary
Pedicel	1.1–2.6 cm, nearly equal in length to flower	0.7–1 cm, significantly shorter than flower	2.7–3.5cm, nearly equal in length to flower
Calyx	white, with purple-red stripes; basal tube ca. 22 × 5 mm, upper tube significantly longer than basal tube	light yellow, with purple-red stripes; basal tube 15–16 × 4.5–5 mm, upper tube almost equal to basal tube	light red brown, without stripes; basal tube 23–26 × 5–6 mm, upper tube significantly longer than basal tube
Limb	discoid, yellow, 13–18 mm in diameter, with purple-red stripes and spots, lobes without papillae and pubescent	discoid, yellow, 5–7 mm in diameter, only with unobvious light red spots, lobes densely papillate	trumpet-shaped, pink, 8–9 mm in diameter, lobes densely papillae and white pubescent
Throat	dark red without spots, 4–6 mm wide, significantly smaller than the limb	dark red with light yellow spots, ca. 5 mm wide, significantly smaller than the limb	pink with red-brown spots, ca. 8–9 mm wide, approximately to the limb
Gynostemium	ca. 5 mm long, lobes nearly equal in length to anthers, apex acute	ca. 3 mm long, lobes significantly shorter than anthers, apex obtuse	ca. 4 mm long, lobes significantly shorter than anthers, apex curved

**Table 1.** Morphological comparison of key characters among *Isotrema sanyaense*, *I. ledongense* and *I. jianfenglingense*.

# Discussion

Morphologically, Isotrema sanyaense resembles I. ledongense and I. jianfenglingense in having similar leaf blade shape (lanceolate or elliptic-lanceolate, entire, base shallowly cordate) and the yellow to brown villous indumentum of the pedicel, ovary and calyx, but I. sanyaense and I. ledongense are significantly different in the inflorescence (cymes 1-5-flowered, in hanging clusters of 1 to numerous branches vs. solitary), the pedicel (nearly equal in length to flower vs. significantly shorter than flower), the calyx tube (upper tube obviously longer than basal tube vs. upper tube almost equal to basal tube), the calyx limb (about 13-18 mm in diameter, with purple-red stripes and spots vs. 5-7 mm in diameter, only with unobvious light red spots), and the throat (dark red without spots vs. dark red with light yellow spots). Isotrema sanyaense can also be easily distinguished from I. jianfenglingense by the inflorescence (cymes 1-5-flowered, in hanging clusters of 1 to numerous branches vs. solitary), the calyx (white with purple-red stripes vs. light red to brown without stripes), the calyx limb (discoid, yellow, with purple-red stripes and spots, lobes without papillae and pubescent vs. trumpetshaped, pink, without stripes and spots, lobes densely papillae and pubescent), and the throat (dark red without spots, significantly smaller than the limb width vs. pink with red-brown spots, approximately to the limb width) (summarized in Table 1). Considering the discovery of this new species from Hainan island, along with the species previously described in China, Myanmar, Peninsular Malaysia, Thailand and Vietnam in recent years (González and Poncy 1999, Phuphathanaphong 2006, Hansen and Phuphathanaphong 2009, Liu and Deng 2009, Xu et al. 2011, Yao 2012, Huang et al. 2013, 2015, Wu et al. 2013, 2015, Do et al. 2014, 2015a, 2015b, 2015c, 2015d, 2017, 2019, Huong et al. 2014, Lu and Wang 2014, Ohi-Toma et al. 2014, Ravikumar et al. 2014, Zhu et al. 2015, 2016, 2017a, 2017b, 2018, Gong et al. 2018, Yang et al. 2018, Zhou et al. 2019), we predict that more and more new species of *Isotrema* will be found after extensive investigations and studies.

# Key to *Isotrema sanyaense* and closely related species (including all species of Aristolochia and *Isotrema* in Hainan island, China)

1	Calyx tube rectilinear or slightly curved; with short stipe connected to ovary; limb
	ligulate; gynostemium 6-lobed; anthers 6, opposite to lobes of gynostemium;
	capsule deniscing acropetally
_	Calyx tube norsesnoe-snaped at middle; without short stipe connected to ovary;
	limb discoid or obliquely trumpet-snaped; gynostemium 5-lobed; anthers 6, ad-
2	Lashlada nalum ambia avata ar avata daltata ta assistata usuallu daanku 3 lahad
L	smaller 2.5.55 x 2.6 cm; seeds avoid 2.5 x 2 mm; flowering from October to
	November <b>A polymorpha</b>
_	Leaf blade ovate-cordate or oblong-ovate entire larger 8–24 x 4–22 cm: seeds
	triangular to subcordiform, ca 8 x 8 mm flowering from May to August
	A. tagala
3	Calvx limb trumpet-shaped, throat as long to the calvx limb
_	Calvx limb discoid, bell-shaped, throat significantly shorter than the calvx limb <b>5</b>
4	Throat yellow without spot; leaf blades ovate to ovate-lanceolate, lateral veins 5 to
	7 pairs
_	Throat pink with red-brown spots; leaf blades lanceolate to elliptic-lanceolate,
	lateral veins 16 to 18 pairs I. jianfenglingense
5	Calyx limb discoid; width of throat > 10 mm; leaf blades polymorphic, broad-
	ly oblong-oblanceolate, linear, or oblong, widest at upper half, often shallowly
	2–3-lobed
-	Calyx limb bell-shaped or discoid; width of throat $\leq$ 6mm; leaf blades uniform,
	widest at middle or lower half, not lobed
6	Calyx limb bell-shaped, inner surface purple black <i>I. fulvicomum</i>
_	Calyx limb discoid, inner surface yellow, sometimes with red stripes and spots7
/	Upper calyx tube almost equal to basal calyx tube; calyx limb 5–7 mm in diam-
	eter, only with unobvious light red spots; gynostemium lobes significantly shorter
	than anthers, with obtuse apices
-	Upper calyx tube significantly longer than basal calyx tube; calyx limb about
	13–10 mm m dameter, with purple-red stripes and spots; gynostemium lobes
	incarry equal in length to anthers, with acute aprees

The authors are very grateful to Ms. Shizhen Qiao for the illustration of the new species. This study was jointly supported by the National Natural Science Foundation of China (grant nos. 31760053 and 31600161), the Natural Science Foundation of Education Department of Guizhou Province (QianJiaoHe KY Zi [2018]089), the Foundation of Guizhou Administration of Traditional Chinese Medicine (QZYY–2018–097), the Doctoral Research Project of Guiyang College of Traditional Chinese Medicine "Research on Polygonati Rhizoma and its adulterants", the Education Department of Guizhou Province (KY[2017]018), the Foundation of the Science and Technology Department of Guizhou Province ([2019]5658) and the First-class Construction Disciplines Sub Project in Guizhou Province (Science of Chinese materia medica; GNYL[2017]008 Hao–7).

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RESEARCH ARTICLE



# Synopsis of the tribe Stipeae (Poaceae) in Nepal

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Academic editor: Maria Vorontsova | Received 18 March 2019 | Accepted 17 July 2019 | Published 19 August 2019

**Citation:** Nobis M, Gudkova PD, Pendry CA (2019) Synopsis of the tribe Stipeae (Poaceae) in Nepal. PhytoKeys 128: 97–119. https://doi.org/10.3897/phytokeys.128.34637

# Abstract

In Nepal the Stipeae consists of six genera: Achnatherum, Orthoraphium, Piptatherum, Ptilagrostis, Stipa, Trikeraia, and 15 species. Two new combinations, Ptilagrostis duthiei (Hook. f.) M.Nobis & P.D.Gudkova and Achnatherum staintonii (Bor) M.Nobis & P.D.Gudkova, are proposed, and new country records for Stipa klimesii, Ptilagrostis dichotoma, Ptilagrostis concinna and Achnatherum jacquemontii are reported. The records of Stipa roborowskyi, S. przewalskyi, S. capillata, S. consanguinea, S. mongholica, and S. sibirica, previously thought to occur in Nepal were based on misidentifications, and these have been excluded from the list of Nepalese Stipeae. We present keys for the identification of genera and species, and a checklist including information on nomenclatural types, regional and national distribution, and habitat. A lectotype is designated for Stipa brandisii Mez.

# **Keywords**

Checklist, Identification key, Nepal, Poaceae, Stipeae

# Introduction

The tribe Stipeae L. (feather grasses) is composed of extratropical and high-mountain grasses consisting of about 680 species distributed on all continents except Antarctica (Tzvelev 1977; Barkworth 2007; Romaschenko et al. 2008, 2010, 2011, 2012; Soreng et al. 2003, 2015, 2017, Nobis et al. 2019). It is an early divergent, highly specialized, monophyletic lineage within the subfamily Pooideae Benth. The Stipeae are characterized by their single-flowered spikelets without rachilla extensions, lemmas with apical awns where the awn is the result of fusion between the central and two lateral vascular

traces, florets with three (rarely two) lodicules, and usually the palea is concealed by the lemma (if the palea is exposed when the floret is closed, then the palea is coriaceous (Roshevitz 1934; Tzvelev 1976; Freitag1985).

Although agrostologists have maintained a broad concept of the genus *Stipa* L. since its description (Hitchcock 1935, Clayton and Renvoize 1986; Freitag 1985; Columbus et al. 2019 and others), recent molecular phylogenetic studies suggest that ca. 34 genera should be recognized within the tribe (Hamasha et al. 2012; Nobis et al. 2019; Peterson et al. 2019). However, the species composition of some genera still requires further study.

All previous treatments of the Stipeae in Nepal have followed a broad generic concept. The Annotated checklist of the flowering plants of Nepal (Press et al. 2000, http://www.efloras.org/flora\_page.aspx?flora\_id=110) and the Flora of Mustang, Nepal (Ohba et al. 2008) recognized 11 species of *Stipa: Stipa breviflora* Griseb., *S. capillata* L., *S. consanguinea* Trin. & Rupr., *S. duthiei* Hook. f., *S. koelzii* R.R.Stewart, *S. mongholica* Turcz. ex Trin., *S. przewalskyi* Roshev., *S. roborowskyi* Roshev., *S. roylei* (Nees) Mez., *S. sibirica* (L.) Lam. and *S. staintonii* Bor and three species of *Oryzopsis: O. gracilis* (Mez) Pilg., *O. lateralis* (Regel) Stapf ex Hook. f. and- *O. munroi* Stapf ex Hook.f. Two other species have recently been reported from Nepal, *Ptilagrostis milleri* (Noltie) M.Nobis & A.Nobis (=*S. milleri* Noltie), and *S. krylovii* Roshev. (Nobis et al. 2015; Gudkova et al. 2017a). Unfortunately, identification of Nepalese feather grasses is difficult due to the lack of recent, comprehensive, regional taxonomic studies. The main goal of this paper is to provide an identification key and checklist including information on types, nomenclature, distribution, and habitat for all Nepalese species of Stipeae.

# Materials and methods

Our treatment is based on herbarium specimens deposited in BM, E, GOET, K, KATH, KRA, KUN, LE, M, NY, P (Thiers 2018). Each species is listed with complete nomenclatural and type information (the type specimens examined has exclamation mark after a herbarium code) synonyms, habitat requirements, and Nepalese and general distribution. The distribution within Nepal is given by District (Fig. 1). Elevation ranges and habitat requirements have been determined from herbarium specimen labels and from the literature.

# Morphological analyses

Nineteen morphological characters scored for each taxon were included in the analysis (Table 1). Each species was treated as an Operational Taxonomic Unit (OTU) following Sokal and Sneath (1963). Cluster analysis was performed on all OTUs to estimate morphological similarities among the species. The similarities among OTUs were calculated using Gower's general similarity coefficient. A cluster analysis (UPGMA) was carried out using PAST software (Hammer et al. 2001).



Figure 1. Districts of Nepal.

Table I. Morphological characters and character stat	es.
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Characters	States
Macromorphological characters:	
Length of anthecium (lemma + callus) [mm]	mean length
Length of callus [mm]	mean length
Ratio lemma / palea	subequal (1); lemma longer than palea (2)
No. of awn geniculations	without geniculations (0); unigeniculate (1); bigeniculate (2)
Length of awn [mm]	mean length
Hairs on column [mm]	mean length
Hairs on seta [mm]	mean length
Length of glumes [mm]	mean length
Apex of glumes	twisted (1), straight (2)
Ligules of vegetative leaves [mm]	mean length
Ratio lower glume / upper glume	subequal (1); lower longer than upper (2)
Hard prickles at lemma apex	absent (1); present (2)
Micromorphological characters of the lemm	na epidermis:
Length of long cells	1–3(–5) times as long as wide (1); (4–)5–9(–11) times as long as wide (2), as wide as long (3)
Side walls of long cells	not thickened (1), thickened (2)
Presence of hooks	frequent (more than 12 on area of $0.015 \text{ mm}^2$ ) (1); sparse (less than 12 on area of $0.015 \text{ mm}^2$ ) (2), absent (3)
Presence of silica cells	frequent (more than 20 per area of 0.015 mm <sup>2</sup> ) (1); sparse (less than 20 per area of 0.015 mm <sup>2</sup> ) (2); rare (less than 5 per area of 0.015 mm <sup>2</sup> ) (3)
Constriction of silica cells	with constrictions (1), without constrictions (2)
Shape of silica cells	ovate (1); elongated to ovate (2), elliptic or reniform (3)

# Stipeae Dumort., Observ. Gramin. Belg. 83, 88, 134 (1824)

Plants perennial, usually cespitose, occasionally rhizomatous. Culms erect, unbranched. Leaf blades flat or convolute, abaxial surface smooth, scabrous or pubescent, adaxial surface prominently ribbed, with 0.05–1 mm long hairs. Ligules membranous. Inflorescence a dense or open panicle. Spikelets with one bisexual floret. Glumes clearly unequal to subequal, membranous, obtuse or acute, tapering into a long tip. Awns scabrid to plumose, straight, uni- or bi-geniculate. Lemmas narrowly lanceolate, terete, usually leathery, usually hairy. Callus rounded or acute to sharply pointed.

# **Results and discussion**

Detailed analyses of macro- and micromorphological structures of the lemma epidermis of Nepalese species of Stipeae confirmed that they form two main clusters, one with three subclusters (Fig. 2). The clusters correspond to the four lemma epidermal patterns (LEP): *Stipa*-like, *Ptilagrostis*-like, *Piptatherum*-like and *Achnatherum*-like (Fig. 3). The taxa from cluster I belonging to *Stipa* have long cells and hooks on the lemma epidermis in an ordered saw-like pattern (Romaschenko et al. 2012; Fig. 3a). Within subcluster A of cluster II, there are three genera, *Ptilagrostis, Trikeraia* and *Orthoraphium* (Fig. 2), that have LEPs dominated by elongated basal cells, frequent silica bodies and cork cells (Fig. 3b–d, h). However, the presence of deflexed, hard prickles in the case of *Orthoraphium roylei* (Fig. 3d), as well as 2–3 mm long awn-like lemma lobes



**Figure 2.** Cluster analysis (UPGMA method of classification and Gower's general similarity coefficient) performed on 16 qualitative morphological characters for all of the Nepalese members from the tribe Stipeae.



Figure 3. Lemma epidermal patterns (LEPs) of Old World Stipeae: a *Stipa breviflora* [Kyrgyzstan, near Issyk-Kul Lake, *M. Nobis* (KRA)] b *Ptilagrostis concinna* [India, Ladakh, Himalayas, *L. Klimeš* (KRA)]
c *Ptilagrostis duthiei* [India, Himalayas, *J.F. Duthie 3585* (LE) d *Orthoraphium roylei* [India, Himalayas, *J.F. Duthie 3568* (LE)] e *Trikeraia hookeri* [China, Tibet (PE 718306)] f *Piptatherum munroi* [Nepal, Solukhumbu, *M.F. Watson et al. DNEP3 AX33* (E)] g *Achnatherum brandisii* [India, Kashmir, *R.R. Stewart 18120* (NY)] h *Achnatherum staintonii* [Nepal, Mustang, *M.A. Farille 81-340* (E)]. Annotations: b – basal cells, c – cork cells, sb – silica bodies, m – macro-hairs.

in the case of *Trikeraia hookeri* (Fig. 3e) are unique characters which distinguish them from other members of the subcluster. Subcluster B comprises species from the genus *Piptatherum*. These species differ from those in subcluster A in their extremely short callus, less numerous and rounded silica bodies on the lemma surface (Fig. 3f). Taxa in subcluster C, all of which belong to the genus *Achnatherum*, have a maize-like type of LEP (Romaschenko et al. 2012), characterized by numerous silica bodies and very short basal cells (Fig. 3g–h). All of these species have lemmas distinctly longer than paleas.

# Key to genera

1	Lemma with deflexed (retrorse), apical prickles Orthoraphium
_	Lemma lacking deflexed, apical prickles2
2	Lemma lobes awn-like, 2-3 mm long, setaceous
_	Lemma without awn-like lobes, lobes (if present) flat and less than 1 mm
	long
3	Awns straight, scabrous. Anthecium usually dorsally compressed. Callus up
	to 0.3 mm long Piptatherum
_	Awns geniculate, scabrous or variously pilose. Anthecium not compressed or
	laterally compressed. Callus longer than 0.3 mm
4	Callus longer than 0.9 mm. Lemma epidermis with numerous minute hooks
	(visible under high magnification) Stipa
_	Callus up to 0.8 mm long. Lemma epidermis smooth or rarely with infre-
	quent minute hooks
5	Lower segment of awn pilose, with hairs over 0.3 mm long. Surface of lemma
	epidermis covered with elongated basal cells (4-11 times longer than wider)
	and occasional, 1-3-constricted silica bodiesPtilagrostis
_	Lower segment of awn scabrous, with hairs up to 0.1 mm long. Surface of
	lemma epidermis covered with rounded or once-constricted silica, underlying
	cells as wide as long or wider than longerAchnatherum

# Orthoraphium Nees, Proc. Linn. Soc. Lond. 1: 94 (1841)

Type. Orthoraphium roylei Nees.

# Orthoraphium roylei Nees, Proc. Linn. Soc. Lond. 1: 94 (1841).

*≡ Stipa orthoraphium* Steudel, Syn. Pl. Glumac. 1: 131 (1855) nom. superfl.;

- $\equiv$  *Stipa roylei* (Nees) Duthie, Grasses North-Western India 27 (1883);
- ≡ Stipa roylei (Nees) Mez, Repert. Spec. Nov. Regni Veg. 17: 207 (1921).

Type. (India, W Himalaya) Kadarkanal, Royle (holotype: LIV).

General distribution. Himalayas: Bhutan, S China, Ladakh, N Myanmar, N India, Nepal (Bor 1960; Freitag 1985; Wu and Phillips 2006).

**Distribution in Nepal.** Baglung, Bajura, Darchula, Dolkha, Humla, Jumla, Lalitpur, Mugu, Myagdi, Ramechhap, Rasuwa, Rukum, Sankhuwasabha, Solukhumbu, Taplejung.

Habitat. Alpine meadows, *Rhododendron* scrub, oak-laurel forests.

Altitudinal range. 2200–4000 m.

Selected Nepalese specimens studied. Baglung: Dhorpatan, Vallee de Dhorpatan du cote est, 28°29'18"N, 83°4'1"E, 2800-3350 m, 16 Aug. 1981, M. Farille 81-168 (E). Bajura: Birseni – Porakya, 2250 m, 12 Aug. 1991, K.R. Rajbhandari 14829 (KATH). Darchula: Dopakhe, Dandar – Dopakhe, rocky slope, 2200 m, 28 Aug. 1980, K.R. Rajbhandari & K.J. Malla 5684 (KATH). Dolkha: Gyalche Kharka - Thang Dingma, 3100 m, 1 Sep. 1983, K.R. Rajbhandari 9744 (KATH); Bhitte Kharka – Patlo Pokhari, shady place in forest, 3700 m, 12 Sep. 1983, K.R. Rajbhandari 10123 (KATH); Bhitte Kharka - Patlo Pokhari, 3800 m, 12 Sep. 1983, K.R. Rajbhandari 10164 (KATH). Humla: Tambe Danda, 2750 m, Q. semecarpifolia forest, 15 Aug. 1977, K.R. Rajbhandari & B. Roy 2268 (KATH). Jumla: Jumla, 29°16'31"N, 82°11'0"E, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 9327 (KATH). Lalitpur: Phulchoki, 27°34'14"N, 85°24'4"E, 2600 m, 14 Oct. 1990, K.R. Rajbhandari 4122 (KATH). Mugu: Chankheli Lagna, 29°38'21"N, 82°6'52"E, 3500 m, 9 Aug. 1979, K.R. Rajbhandari & B. Roy 4445 (KATH). Myagdi: Gurjakhani, North-West of Gurjakhani, 28°36'N, 83°13'E, 3480-3490 m, 31 Jul. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 3685 (BM, E, K). Ramechhap: Khola Kharka – Thare, 3600 m, 22 Jul. 1985, H. Ohba et al. 60583 (KATH); Bhandar – Deorali – Khasrubus – Shivalaya, 2600 m, H. Ohba et al. 62276 (KATH). Rasuwa: Rupchet Kharka - Balchagam, 3200 m, 16 Aug. 1994, K.R. Rajbhandari 17910 (KATH); Laurebina Yak, 28°5'32"N, 85°22'52"E, 3450 m, 30 Jul. 1995, T. Hoshino et al. 9537289 (KATH). Rukum: Sing Khola – Farkama 3636 m, 18 Sep. 1976, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 3720 (KATH). Sankhuwasabha: Hile Ghot, 27°24'N, 87°26'E, 3500 m, 20 Aug. 1972, J.F. Dobremez 1594 (BM, E). Solu Khumbu: Imja Khola Valley, Omoga, sandy clay, floor of north-west/south-east river valley, west facing slope, mossy slope in shade, 27°50'38"N, 86°47'10"E, 3600–2300 m, 26 Sep. 2005, DNEP3 AX131 (E, KATH); Chaurikharka, Q.semecarpifolia forest, mossy slope, 27°41'46"N, 86°43'31"E, 2729 m, 30 Sep. 2005, DNEP3 BY229 (E, KATH); Dudh Kund - Samakang, 27°41'N, 86°50'E, 3500 m, 24 Aug. 1995, F. Miyamoto et al. 9592410 (E); Samakang Kharka 27°41'N, 86°50'E, 3500 m, 24 Aug. 1995, F. Miyamoto et al. 9592410 (KATH); Beni Kharka, 3100 m, 3 Sep. 1985, H. Ohba et al. 62035 (KATH); Beni Kharka, 3600-2300 m, 2 Sep. 1985, H. Ohba et al. 61907 (KATH); Loding, 27°32'N, 86°32'E, 2700 m, 5 Sep. 1985, H. Ohba et al. 62108 (KATH); 62155; Pike Bhanjyang, 3700 m, 6 Sep. 1985, H. Ohba et al. 62155 (KATH); Pike Dongshar, Rhododendron campanulatum thicket, 27°30'N, 86°27'E, 2300-3600 m, 9 Sep. 1985, H. Ohba et al. 62192 (KATH); Rangdu Kharka, 27°8'N, 86°48'E 3550 m, 9 Aug. 1997, K.R. Rajbhandari 9740203 (KATH); Tangnag – Mosom Kharka, in forest on mossy ground, 3700 m, 21 Aug. 1997, K.R. Rajbhandari 9740472 (KATH); Beni – Tokchardingma, Basa Valley,

27°32'59"N, 86°35'13"E, 3750 m, 2 Aug. 1995, *K. Tsuchiya 40841* (KATH); Sengephuk, Beni VDC, 3580 m, 29 Aug. 1995, *K. Tsuchiya 41782* (KATH); Luminasa, Basa Valley, Beni, 3780 m, 8 Aug. 1995, *K. Tsuchiya* 42188 (KATH). **Taplejung**: forest ridge et Manedhanjang, N end of Milke Danda Ridge, Rhododendron / Bamboo forest, caespitose grass, 27°27'N, 87°28'E, 3340 m, 22 Oct. 1991, *D.G. Long et al. 966* (E, KATH); Topke Gola, Arun-Tamur watershed S of Topke Gola, on slopes, 27°38'25"N, 87°34'59"E, 3940 m, 13 Sep. 1956, *J.D.A. Stainton 1728* (BM, E).

# Stipa L., Sp. Pl. 1: 78 (1753)

Type. Stipa pennata L.

# Key to the genus Stipa

1	Upper part of awn (seta) scabrous, with hairs up to 0.4 mm long2
_	Upper part of awn (seta) pilose, with hairs over 0.4 mm long
2	Upper part of awn and tips of glumes spirally twisted
_	Upper part of awn and tips of glumes not twisted
3	Ligules of vegetative shoots up to 0.3 mm long. Awn column with 0.6-
	0.7 mm long hairs
_	Ligules of vegetative shoots over 2 mm long. Awn column with 1.5–2.5 mm
	long hairs

# Stipa breviflora Griseb., Nachr. Ges. Wiss. Göttingen, Math.-Phys. Kl. 3:82 (1868).

= S. aliciae Kanitz, Növényt. Gyujtesek Eredm. Grof Szechenyi Bela Keletazsiai Utjabol 61, t. 7 (1891).

**Type.** (China) Tibet, Gnari (Nari) Khorsum, *Schlagentweit 7105* (holotype GOET!, isotype LE!).

General distribution. China, Kyrgyzstan, N India, Mongolia, Nepal (Grubov 1982; Tzvelev 1968, 1976; Freitag 1985; Wu and Phillips 2006).

Distribution in Nepal. Mustang.

Habitat. High altitude steppes, scree.

Altitudinal range. 2750–3600 m.

Selected specimens studied. Mustang: Entre Jomsom et Kagbheni, dans la steppe aride a *Caragana gerardiana* et *C. brevispina* (limite), 28°46'51"N, 83°43'27"E, 2750 m, 17 Sep. 1981, *M.A. Farille 81-362* (E); Muktinath, on open slopes near cultivations, 28°48'58"N, 83°51'47"E, 3640 m, 8 Jun 1954, *J.D.A. Stainton, W.R. Sykes* & *L.H.J Williams 5647* (E, K).

# Stipa klimesii M.Nobis, Phytotaxa 174(3): 166–168 [174–176] (2014).

= Stipa basiplumosa Munro ex Hook. f., Fl. Brit. India 7(22): 229 (1896) var. longearistata Munro ex Hook. f., Fl. Brit. India 7(22): 229 (1896).

**Type.** India, NW India, Jammu and Kashmir State, Ladakh, Indus Vy: Zhung (Leh), Ganglas – upper part, springs with drinking water, 3880–4000 m, 30 Jul. 2001, 34°12.3'N, 77°36.8'E, *L. Klimeš 1155, 1156* (holotype KRA!, isotype PRA!).

**General distribution.** Bhutan, China (Tibet), India (Ladakh, Sikkim), Nepal, Pakistan, (Nobis et al. 2014, 2015).

Distribution in Nepal. Mustang.

Habitat. High mountain steppes and alpine mats, among subalpine shrubs and on rocky ledges.

Altitudinal range. 3500–5000 m.

**Notes.** These specimens were previously identified as *S. roborowskyi*, but this species does not occur in Nepal. This species differs from *S. klimesii* in having shorter ligules on the vegetative shoots [0.5-1.5(-2) vs. (2-)3.5-7.5(-9) mm], shorter anthecium [(6-)6.5-7.5(-7.7) vs. (7-)8.3-9.5(-10) mm] and shorter hairs on seta  $[(0.3)0.5-1.1(-1.4) \text{ vs. } (1-)1.3-2(-2.3) \text{ mm} \log n, respectively].$ 

**Selected specimens studied. Mustang:** Damodar, on dry sandy slope, 29°11'N, 83°58'E, 28 Jul. 1979, *P.R. Shakya, S.R. Adhikari & K.R. Amatya 5111* (KATH); Kunda, 28°59'9"N, 84°9'26"E, 4720–4740 m, 11 Aug. 2001, *S. Noshiro, M. Amano & T. Kurosawa 20104179* (KATH).

# Stipa krylovii Roshev., Izv. Glavn. Bot. Sada S.S.S.R. 28: 379 (1929).

- ≡ S. sareptana subsp. krylovii (Roshev.) Cui, Fl. Xinjiang. 6: 299 (1996);
- ≡ S. sareptana var. krylovii (Roshev.) Kuo & Sun, Fl. Reipubl. Popularis Sin. 9(3): 275, pl. 65, f. 37–41 (1987).
- = S. capillata var. coronata Roshev., Fl. Aziat. Ross. 1(12): 168, pl. 8, 8b (1916);
- S. densiflora P.A.Smirn., Repert. Spec. Nov. Regni Veg. 26: 265 (1929) hom. illeg. non Hughes;
- = S. densa P.A.Smirn., Del. sem. Hort. Bot. Univ. Mosquensis 15 (1930);
- = S. decipiens P.A.Smirn., Ucen. Zap. Moskovsk. Gosud. Univ. 2: 338 (1934).

**Type.** Selenginskaya Dauriya, gory mezhdu Temnikom i Dzhidoi, yugo-zapadnaya chast khr. Borgoiskogo, na sklonakh so stepnoi rastitelnostyu, 28 Jul. 1912, *V. Smirnov 524* (lectotype: LE!, designated by Tzvelev 1976).

**General distribution.** Widely distributed throughout Central Asia (Eastern Kazakhstan, Russia (Siberia: Altai, Khakasiya, Tuva, South Krasnoyarsk, Irkutsk, Buryatiya, Chita, South Yakutia); China (Gansu, Hebei, Nei Mongol, Ningxia, Qinghai, Shanxi, Xinjiang, Xizang), Mongolia, eastern Kyrgyzstan, Tajikistan (Pamir), North India, Nepal. (Tzvelev 1968, 1976; Freitag 1985; Wu and Phillips 2006; Gudkova et al. 2017a, 2017b).

**Distribution in Nepal.** Mustang (Gudkova et al. 2017a).

Habitat. High mountain semi-desert.

Altitude range. 3900–4000 m.

**Notes.** These specimens were previously identified as *S. capillata*, but this species does not occur in Nepal. *Stipa krylovii* differs from *S. capillata* mainly in having a ring of hairs at the top of the lemma.

**Selected specimens studied. Mustang**: s.loc., on dry sandy ground, 29°14'N, 83°52'E, 13000 ft, 3 Aug. 1954, *Stainton, Sykes, Williams 2161* (E, K, BM).

# Stipa capillacea Keng, Sunyatsenia 6(2): 100, pl. 15 (1941).

= S. koelzii R.R.Stewart, Brittonia 5: 441 (1945).

**Type.** Open grass land in rear of Shaowusze Agricultural Station, Taining district, Sikang province, 22 Jul. 1940, *K.L. Chü 7449* (holotype: N, isotype: PE!).

**General distribution.** Bhutan, S China, N India, Nepal, Pakistan, (Freitag 1985; Noltie 2000; Wu and Phillips 2006).

Distribution in Nepal. Mustang, Rasuwa, Solukhumbu.

Habitat. Alpine meadows.

Altitudinal range. 2800–4100 m.

**Note.** These specimens were previously identified as *Stipa consanguinea*, but this species does not occur in Nepal. *Stipa capillacea* differs from other species of the genus in awns twisted together at top of panicle.

**Selected specimens studied. Mustang:** Kali Gandaki, Thulo Bugin, ESE Lete, S- facing steep slope, 28°38'4"N, 83°36'20"E, 2820 m, 10 Oct. 1977, *G. Miehe 762b* (BM); at edge of field, 13000 ft, 3 Aug. 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 2150* (BM, K); **Rasuwa:** Langtang, 28°13'N, 85°3'E, 3900 m, 7 Aug. 1970 *J.F. Dobremez 522* (BM, E); Kyanjin Gompa, 28°12'42"N, 85°34'1"E 11500–12500 ft, 10 Aug. 1969, *A. Richard 103* (BM); Ganesh Himal, 28°20'N, 85°10'E, Apr. 1975 - May 1975, *B. Yon 252* (E). **Solukhumbu:** Orsho, east facing slope open ground, grazed area with scattered Juniperus indica, 27°52'15"N, 86°48'44"E, 4100 m, 19 Sep. 2005, *DNEP3 AX83* (E, KATH).

# Ptilagrostis Griseb., Fl. Ross. 4(13): 447 (1852)

Type. Ptilagrostis mongholica (Turcz. ex Trin.) Griseb.

#### Key to the genus Ptilagrostis

2	Glumes, lemma and palea distinctly unequal (lower glume 1.5–3.5 mm longer
	than the upper and lemma 1–2.5 mm longer than palea)
_	Glumes, lemma and palea equal or only slightly unequal
3	Panicles open, 3-5 cm wide, branches up to 6 cm long, spreading
	P. dichotoma
_	Panicles compressed, 0.7-2 cm wide, branches 0.3-2.8 cm long, suberect or
	narrowly ascending

# Ptilagrostis duthiei (Hook.f.) M.Nobis & P.D.Gudkova, comb. nov.

urn:lsid:ipni.org:names:77200949-1

Stipa duthiei Hook.f., Fl. Brit. India 7: 232, 1896. (Basionym).

*E Achnatherum duthiei* (Hook.f.) Kuo & Lu, Fl. Reipubl. Popularis Sin. 9(3): 322, pl. 80, f. 9–14 (1987).

**Type.** [India] Tehri Garwhal, Lekhus, below Srikanta, 12000–13000ft, 11 Aug. 1853, *Duthie 273* (holotype K 32097!).

General distribution. China, N India, Kashmir, Nepal (Freitag 1985; Wu and Phillips 2006).

Distribution in Nepal. Myagdi.

Habitat. Mountain shrublands.

Altitudinal range. 3400-3800 m.

Selected specimens studied. Myagdi: North of Barse, among dwarf Rhododendron, 3940 m, [28°35'N, 83°11'E], 14 Aug. 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 3844* (E 690624).

# Ptilagrostis dichotoma Keng ex Tzvelev, Rast. Tsentr. Azii 4: 43 (1968).

**Type.** China, Kansu and Tsinghai border [in regione opp. Labrang], *I.C. Wu 478* (holotype N, isotype LE!).

**General distribution.** Bhutan, Birma, China (Tibet), N India, Nepal (Wu and Phillips 2006; Nobis et al. unpbl).

**Distribution in Nepal.** Bajhang, Bajura, Dolakha, Jumla, Mustang, Myagdi, Ramechhap, Rasuwa, Rukum, Solukhumbu.

Habitat. Alpine meadows, grassy mountain slopes.

Altitudinal range. 3300-5000 m.

**Note.** These specimens were previously identified as *Ptilagrostis mongholica* [=*Stipa mongholica*] (Bor 1960; Freitag 1985), but the two species are easily distinguished as *P. dichotoma* has a tuft of short hairs at the apex of anthers (glabrous in *P. mongholica*). They are disjunctly distributed with *P. dichotoma* found in the mountains of southern-central Asia while *P. mongholica* occurs mainly in the mountains of northern-central Asia; (Tzvelev 1968; Wu and Phillips 2006).

Selected specimens studied. Bajhang: Manane Lekh 29°36'45"N, 80°59'35"E, 3830 m, 14 Jul. 2009, Bajhang 09 20917078 (E, KATH); Saipal, 29°57'51"N, 81°13'6"E, 3909 m, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 1808 (KATH); Saipal, 29°57'51"N, 81°13'6"E, 30 Jul. 1976, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 4024 (KATH); Saipal, 29°57'51"N, 81°13'6"E, 31 Jul. 1976, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 4028 (KATH). Bajura: Chauki Lekh, 29°35'34"N, 81°38'5"E, 4427 m, 15 Aug. 2017, BSH C42; Chauki Lekh, 29°37'16"N, 81°34'30"E, 4427 m, 16 Aug. 2017, BSH C52. Dolakha: Dudh Kunda, 4550 m, 5 Sep. 1983, K.R. Rajbhandari 9915 (KATH). Dolpa, Nahure, 14,000 ft, 24 Jun. 1952, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 1434. Jumla: Maharigaon, 15,000 ft, 20 Jul. 1952, Polunin, O.V., Sykes, W.R. & Williams, L.H.J. 226. Mustang: Muktinath Himal, Muktinath Range, 28°44'37"N, 83°53'14"E, 480-5000 m, 18 Sep. 1981, M.A. Farille 81-415 (E); Muktinath Himal, Muktinath Range, hab dans la praire alpine, 28°44'37"N, 83°53'14"E, 4800-5000 m, 18 Sep. 1981, M.A. Farille 81-415 (E); Kaisang - Omang Kharka 3800 m, 1 Aug. 1996, K.R. Rajbhandari 9672251 (KATH); Muktinath, 28°48'58"N, 83°51'47"E, 4180 m, 12 Oct. 1976, H. Tabata, K.R. Rajbhandari, K. Tsuchiya & Y. Konno 6310 (KATH); Muktinath, alpine grassland, 28°48'58"N, 83°51'47"E, 4120 m, 12 Oct. 1976, H. Tabata, K.R. Rajbhandari, K. Tsuchiya & Y. Konno 6342 (KATH). Myagdi: 28°32'0"N, 83°13'0"E, 3360 m, 20 Sep. 1996, M. Mikage, R. Hirano, A. Takahashi & K. Yonekura 9682900 (KATH). Ramechhap: Thare Og, 27°45'N, 86°28'E 24 Jul. 1985, H. Ohba, M. Wakabayashi, M. Suzuki, N. Kurosaki, K.R. Rajbhandari & *S.K. Wu 60657* (KATH). **Rasuwa**: Gosainkund, 28°4'58"N, 85°24'51"E, 4300 m, 27 Jul. 1995, T. Hoshino, K. Dan, H. Koba, Y. Omori, C.P. Rauniyar, M. Sato, P. Shrestha & S. Takatsuki 9537190 (KATH); Gosainkund, 4350 m, 28°4'58"N, 85°24'51"E, 27 Jul. 1995, T. Hoshino, K. Dan, H. Koba, Y. Omori, C.P. Rauniyar, M. Sato, P. Shrestha & S. Takatsuki 9537218 (KATH); Gosainkund, 28°4'58"N, 85°24'51"E, 4300 m, 28 Jul. 1995, T. Hoshino, K. Dan, H. Koba, Y. Omori, C.P. Rauniyar, M. Sato, P. Shrestha & S. Takatsuki 9537221, 9537–222 (KATH). Rukum: Chalikhe Pahar, near Chalike Pahar, exposed south facing slopes, 28°40'N, 83°4'E, 4240 m, 17 Jun 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 3163 (E). Solukhumbu: Seto Pokhari (4810m) - Chhomalang Base Camp (4495), 27°47'N, 86°57'E, 4810 m, 17 Aug. 1995, F. Miyamoto, M. Amano, H. Ikeda, C.M. Joshi, K. Arai & T. Komatsu 9592313 (E); Beni, alpine meadow, 27°32'59"N, 86°35'13"E 4600 m, K. Tsuchiya 42693 (KATH).

#### Ptilagrostis yadongensis Keng & Tang, J. SouthW. Agric. Univ. 4: 44 (1985).

- ≡ *Ptilagrostis macrospicula* Cai, Acta Bot. Boreal.-Occid. Sin. 23(11): 2018 (2003). superfl. name.
- = Stipa milleri Noltie, Edinburgh J. Bot. 56(2): 288 (1999); ≡ Ptilagrostis milleri (Noltie) M.Nobis & A.Nobis, Nordic J. Bot. 31: 623 (2013).

**Type.** China. Xizang: Yadong, 14 Sep. 1974, *Qinghai-Xizang Exped. 74–2496* (lapsus calami as *74–2469*; holotype HNWP, isotype PE).
**General distribution.** Bhutan, China (Tibet), Nepal (Noltie 1999; Nobis and Nobis 2013; Nobis et al. 2015; Zhang et al. 2016).

Distribution in Nepal. Bajhang, Dolkha, Rasuwa, Solukhumbu.

Habitat. Alpine meadows, moist grassy places, under shrubs, swampy places, *Kobresia* moors.

Altitudinal range. 3600-4600 m.

**Note.** These specimens were previously identified as *P. concinna* which also occurs in Nepal and which can be distinguished by its subequal glumes, lemma and palea. It has also been confused with *Ptilagrostis bhutanica* (Noltie) M.Nobis (basionym: *Stipa bhutanica* Noltie 1999: 289; Nobis et al. 2016), from Bhutan and China. However, these two taxa differ in the upper part of the awn which is scabrous in *P. bhutanica* and shortly pilose (with hairs over 0.5 mm long) in *P. yadongensis*.

Selected specimens studied. Bajhang: Saipal Aletsoura, 29°57'51"N, 81°13'6"E, 4333 m, 31 Jul. 1976, *H. Tabata, K.R. Rajbhandari & K. Tsuchiya 1941* (KATH). Dolkha: Rolwaling Dudh Kunda, 4520 m, 15 Jul. 1975, *P.R. Shakya, K.R. Rajbhandari & H.K. Saiju 75/2978* (KATH). Rasuwa: Ya La, 3600 m, 29 July 1972, *A.Maire, AMA 250* (E); Upper Langtang, 4600 m, 30 Sep. 1986, G. Miehe 13090 (KATH). Solukhumbu, Chola Tsho, north side of lake, SE facing slope, rocks and sand near lakeside, Juniperus indica dwarf scrubland with Rhododedron setosum and Potentilla fruticosa, 27°55'18"N, 86°47'50"E, 4500 m, 21 Sep. 2005, *DNEP3 AX98* (E, KATH).

# Ptilagrostis concinna (Hook. f.) Roshev., Fl. URSS 2: 75 (1934).

Stipa concinna Hook. f., Fl. Brit. India 7(22): 230 (1897) (Basionym).

**Type.** Sikkim-Himalaya, Tibetan region, 14000–16000ft, 1861, *Hooker* (holotype K!, isotypes G, GOET!, LE 9267!).

**General distribution.** Himalayas: China (Tibet), India (Ladakh and Sikkim), Nepal (Freitag 1985; Wu and Phillips 2006).

Distribution in Nepal. Solukhumbu, Mustang.

Habitat. Alpine meadows, moist grassy places, under shrubs, swampy places, Kobresia moors.

# Altitudinal range. 4400–5300 m.

**Selected specimens studied. Solukhumbu**: Seto Pokhari, 27°47'N, 86°55'E, 4495–4810 m, 17 Aug. 1995, *F. Miyamoto, M. Amano, H. Ikeda, C.M. Joshi, K. Arai & T. Komatsu 9592313* (KATH). **Mustang**: Thorung La, 5200–5300 m, 19 Sep. 1981, *M.A. Farille 81-434* (E 189114).

# Achnatherum P.Beauv., Ess. Agrostogr.: 19, 146, pl. 6, f. 7 (1812)

Type. Achnatherum calamagrostis (L.) P.Beauv.

#### Key to the genus Achnatherum

1	Glumes distinctly unequal. Lemma apex with ring of hairs over 3 mm long.
	Callus 0.5–0.7 mm long, acute at the apex
_	Glumes equal or almost so. Lemma apex with ring of hairs up to 2 mm long.
	Callus up to 0.5 mm long, rounded at the apex2
2	Lemma and palea clearly unequal. Leaves filiform, inrolled. Culms up to 45
	cm long. Panicle with very short branches
_	Lemma and palea subequal. Leaves flat. Culms over 60 cm long. Panicle with
	widely spreading branches

#### Achnatherum brandisii (Mez) Z.L.Wu, Acta Phytotax. Sin. 34: 154 (1996).

*Stipa brandisii* Mez, Repert. Spec. Nov. Regni Veg. 17(13–18): 207 (1921) (Basionym). = *Stipa subeffusa* Ohwi, Acta Phytotax. Geobot. 17: 15 (1957).

**Type.** [India] N. W. Himalaya, Kulla, Oct. 1876, *Brandis* 1005 (lectotype K 32092! selected and labeled by H. Freitag on 15 March 1984 but **designated here**).

**General distribution.** Afghanistan, Bhutan, China, NW India, Nepal, Pakistan (Freitag 1985; Wu and Phillips 2006).

Distribution in Nepal. Dolpa, Manang, Mustang.

**Habitat.** Open dry slopes, among shrubs and in Bamboo (*Sinarundinaria* sp.) thicket. **Altitudinal range.** 2400–4000 m.

**Note.** *Stipa brandisii* was described by Mez (1921) based on a specimen housed at B but destroyed during the Second World War. In his original description of the species Mez (1921) reported that the species had been collected in 'Western-Himalaya, Kulla ([by] Brandis)', but did not provide further information about the date of specimen collection, number and place where it was housed. In the absence of the original material, the specimen at K (http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000032092) was selected as lectotype by H. Freitag in 1984 and subsequently cited as the holotype (Freitag 1985). We designate this here as the lectotype.

Selected specimens studied. Dolpa: Above Chong, near Tibrikot Growing, among shrubs on open slopes, 29°1'40"N, 82°46'22"E, 2580 m, 11 Sep. 1952, O.V. Polunin, W.R. Sykes & L.H.J. Williams 3314 (E). Manang: Humde, Bhraka, open place, 3400 m, Aug. 1983, K.R. Rajbhandari 8786 (KATH); Boraga, Entre Braga et Ghyaru, 28°39'24"N, 84°2'22"E, 3500 m, 22 Sep. 1981, M.A. Farille 81-486 (E); Boraga, Entre Braga et Ghyaru, bois ouvert xerophile, 28°39'24"N, 84°2'22"E 3500 m, 22 Sep. 1981, M.A. Farille 81-491 (E); Boraga Annapurna Himal, Manang, Annapurna III, north-slope above Braga, on pastures, 28°39'24"N, 84°2'22"E, 3850 m, 14 Oct. 1969, T. Wraber, 36404(502) (BM). Mustang: Versant de Muktinath Range, faisant face a Jomsom, 50 m au dessus du village Rochers [on

rocks], environnement rocheux, mais dans les touffes d'epineux oub d'Artemisia, 28°46'51"N, 83°43'27"E, 2750 m, 16 Sep. 1981, M.A. Farille 81-358 (E); Ommang 28°44'N, 83°45'E, 3600 m, 31 Jul. 1996, T. Hoshino, M. Amano, H. Koba, N. Miyoshi, K.R. Rajbhandari, M. Sato, P. Shrestha & S. Takatsuki 9662100 (KATH); Jomsom, 28°46'51"N, 83°43'27"E, 3350 m, 31 Jul. 1996, T. Hoshino, M. Amano, H. Koba, N. Miyoshi, K.R. Rajbhandari, M. Sato, P. Shrestha & S. Takatsuki 9670079 (KATH); Kali Gandaki, Tangdung-Khola, S-facing, wind-blown slope, 2490-2480 m, Aug. 1977, G. Miehe (BM); Cha Lungpa, NE-facing slope, alpine pastures 3940 m, 27 July 1977, G. Miehe 351b (BM); NW of Tukche, valley of Yamkin Khola, in Bamboo (Sinarundinaria sp.) thicket, 28°41'15"N, 83°37'35"E, 2840 m, 20 Sep. 1995, M. Mikage & K. Yonekura 9552331 (KATH); Tukucha (Kali Gandaki), 3180 m, [28°42'33"N, 83°38'37"E], 21 Aug. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 7363 (BM, E 619028); Tukucha, Kali Gandaki, amongst hillside shrubs, 28°42'33"N, 83°38'37"E, 3030 m, 12 Sep. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 7813 (E); Chimgaon (N of Tukucha) Kali Gandaki, on dry slopes, 28°43'38"N, 83°40'45"E, 2880 m, 14 Sep. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 9887 (BM).

# Achnatherum jacquemontii (Jaub. & Spach) P.C.Kuo & S.L.Lu, Fl. Reipubl. Popularis Sin. 9(3): 323, pl. 80, f. 15–19 (1987)

Stipa jacquemontii Jaub. & Spach, Ill. Pl. Orient. 4: 60, pl. 339 (1851) (Basionym).

- ≡ Lasiagrostis jacquemontii (Jaubert & Spach) Munro ex Boiss., Fl. Orient. 5: 506 (1884);
- ≡ Lasiagrostis jacquemontii (Jaub. & Spach) Munro ex Aitch., J. Linn. Soc., Bot. 18: 107 (1880).
- *Stipa jacquemontii* Jaub. & Spach subsp. *chuzomica* Noltie, Edinburgh J. Bot. 56(2):
   290, f. 1Q–U (1999).

**Type.** [India] ad ruped in excelsis Emodi Cashemyrianim 2750 m, 1831, *Jacquemont 994*, (holotype P, isotype K!).

**General distribution.** E Afghanistan, Bhutan, China (Tibet), NW India, Nepal, Pakistan (Freitag 1985; Noltie 2000; Wu and Phillips 2006).

Distribution in Nepal. Mustang.

Habitat. Dry mountain slopes, especially in rock crevices.

Altitudinal range. 2500–3000.

**Selected specimens studied. Mustang:** Marpha, pentes rocheues arides, 28°45'11"N, 83°41'28"E, 2650 m, 16 Sep. 1981, *M. Farille 81-340* (E); Versant de Muktinath Range, faisant face a Jamson, 100 m au dessus du Village Rochers, 2800 m, 16 Sep. 1981, *M.A. Farille 81-347* (E 188712); Barsumg Khola, on a dry cliff, 28°52'N, 83°16'E, 10000 ft, 18 Jul. 1963, *J.D.A. Stainton 4417* (E 00619022).

# Achnatherum staintonii (Bor) M.Nobis & P.D.Gudkova, comb. nov.

urn:lsid:ipni.org:names:77200950-1

*Stipa staintonii* Bor, Bull. Bot. Surv. India 7: 133 (1965) (Basionym). ≡ *Stipella staintonii* (Bor) Röser & Hamasha, Pl. Syst. Evol. 298: 365 (2012), nom. inval.; ≡ *Stipellula staintonii* (Bor) Röser & H.R. Hamasha, Schlechtendalia 24: 92 (2012).

**Type.** Nepal, near Seng Khola, 12500 ft [3810 m.], exposed cliffs, 4 Okt 1954, *Stainton, Sykes & Williams 4677* (holotype K!, isotype BM!).

General distribution. Nepal (endemic; Bor 1965; Freitag 1985).

Distribution in Nepal. Baglung, Dolpa, Manang, Mustang, Rukum.

Habitat. open rocky or stony sandy slopes and scrublands.

Altitude. 3000–4200 m.

**Note.** Although *Achnatherum staintonii* has been confused with *Stipa przewalskyi*, the latter species does not occur in Nepal. *Achnatherum staintonii* is easily distinguished from *Stipa przewalskyi* by having maize-like vs. saw-like LEPs and in having unequal glumes and distinctly longer lemma than palea vs. glumes as well as lemma and palea subequal, respectively. Röser (2012) transferred five species of *Stipa*, including *Stipa staintonii*, into their new genus *Stipellula* on the basis of his earlier molecular analysis (Hamasha et al. 2012). *Stipellula* is characterized by its maize-like lemma epidermal pattern which clearly distinguishes it from *Stipa* and confirms that these species belong to the achnatheroid group of grasses within the Stipeae. However, there are no unique, diagnostic morphological characters to separate *Stipellula* from *Achnatherum* which is itself polymorphic and highly polyphyletic (Romaschenko et al. 2012; Hamasha et al. 2012). Thus, we prefer to treat *Stipa staintonii* as a member of *Achnatherum*.

Selected specimens studied. Baglung: Sing Khola, wet rocky cliff, 18 Sep. 1976, H. Tabata, K.R. Rajbhandari & K. Tsuchiya 3711 (KATH). Dolpa: Ringmo, dry hillslope, 29°10'20"N, 82°55'50"E, 3400 m, 2 Aug. 1973, S. Einarsson, L. Skärby & B. Wetterhall 3128 (BM); Barbung Khola, 28°52'N, 83°15'E, 3030 m, 18 Jul. 1963, J.D.A. Stainton 4417 (BM); Barbung Khola, 28°52'N, 83°18'E, 3030 m, 13 Jul. 1963, J.D.A. Stainton 4417 (E); Suligad, Rhagaon, rocky slope, 29°28'N, 82°55'E, 2600 m, 25 Sep. 1982, K.R. Rajbhandari & K.J. Malla 6740 (KATH); Karnali, Ringmigaon, on dry hilslope, 3400 m, 2 Aug. 1973, S. Einarsson, L. Skärby, B. Wetterhall 3126 (UPS); Karnali, Manang: Bhraka, Humde, open rocky slope, 28°38'24"N, 84°5'36"E, 3400 m, 3 Aug. 1983, K.R. Rajbhandari 8814 (KATH); Marsyandi valley, Tangi above Manangbhot, on stony sandy places, 28°39'56"N, 84°1'33"E, 3800 m, 12 Oct. 1969, T. Wraber 36427 (E); Tangi, above Manangbhot, Tangje, on stony sandy places, 28°39'22"N, 84°2'2"E, 3800 m, 12 Oct. 1969, T. Wraber 479 (BM). Mustang: Entre Marpha et Syang; Syang, Marpha, 28°45'11"N, 83°41'28"E, 2650 m, 16 Sep. 1981, M.A. Farille 81-340 (E); Entre Larjung et Tukuche, 2550 m, 14 Sep. 1981, M.A. Farille 81-313 (E); Cha Lungpa, in E-facing Cupressus forest, 3030 m, 3 Oct. 1977, G. Miehe 80 (BM); Phalyak, dry place 28°49'24"N, 83°44'23"E, 4110 m, 9

Aug. 2002, F. Miyamoto, N. Kurosaki, S. Akiyama, H. Ikeda, Y. Iokawa, Y. Takahashi, M. Tsusaka & M.N. Subedi 20210022 (KATH); Phalyak to Pongio Kharka, 28°49'0"N, 83°45'0"E, 3800–4000 m, 9 Aug. 2002, F. Miyamoto, N. Kurosaki, S. Akiyama, H. Ikeda, Y. Iokawa, Y. Takahashi, M. Tsusaka & M.N. Subedi 20220034 (KATH); Chele – Samar, 28°57'43"N, 83°48'6"E, 3450-3670 m, 4 Aug. 2001, S. Noshiro, M. Amano, Y. Iokawa, T. Kurosawa, & M.N. Subedi 20105005 (KATH); Tukuche, open rocky slope, 28°42'33"N, 83°38'37"E, 2630 m, 19 Jul. 1983, K.R. Rajbhandari 7991 (KATH); Jomsom, open slope, 28°46'51"N, 83°43'27"E, 2760 m, 21 Jul. 1983, K.R. Rajbhandari 8072 (KATH); Jomsom to Sayang, cliffs in dry valley with scrub (Caragana, Clematis, Rosa etc.), 28°46'51"N, 83°38'37"E, 3180 m, 21 Aug. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 7352 (BM). **Rukum**: Sen Khola, 28°42'54"N, 82°57'21"E, 3790 m, 4 Oct. 1954, J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 4677 (BM).

### Piptatherum P.Beauv., Ess. Agrostogr.: 17: 173 (1812)

Type. Piptatherum coerulescens (Desf) P. Beauv.

#### Key to the genus Piptatherum

1	Panicle compressed	2
_	Panicle lax	
2	Awn terminal	P. laterale
_	Awn subterminal	P. gracile
3	Lemma almost equal to glumes, apical part gradually narrowed	l into a persis-
	tent awn	P. aequiglume
_	Lemma much shorter than glumes, apical part abruptly con	tracted into a
	slender, caducous awn	P. munroi

# *Piptatherum aequiglume* (Duthie ex Hook.f.) Roshev., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 14: 113 (1951).

*Oryzopsis aequiglumis* Duthie ex Hook. f., Fl. Brit. India 7(22): 234 (1896) (Basionym). = *Piptatherum sinense* Mez, Repert. Spec. Nov. Regni Veg. 17(486–491): 211 (1921).

Type. India: distr. Jansar, Gamble 15143 (lectotype K! designated by Bor 1970).General distribution. Afghanistan, Bhutan, S China, NW India, Nepal, Pakistan (Freitag 1975; Noltie 2000; Wu and Phillips 2006).

Distribution in Nepal. Rukum.

Habitat. Moist mesophytic forests.

Altitude range. 3500–4000 m.

**Selected specimens studied. Rukum**: near Dogadi Khola, 3660 m, 8 Aug. 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 3801* (E 814753); nr. Dogadi Khola, 3790 m, 8 Aug. 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 3794* (E 814768).

# Piptatherum gracile Mez, Repert. Spec. Nov. Regni Veg. 17(486-491): 211 (1921).

≡ Oryzopsis gracilis (Mez) Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 14: 347 (1939:).

**Type.** Tibet occ., 3900–4000 m., *Thomson* s.n. (lectotype W designated by Freitag 1975, isolectotype K!).

**General distribution.** Afghanistan, China, N India, Nepal, Pakistan, Tajikistan (Freitag 1975; Wu and Phillips 2006).

Distribution in Nepal. Mustang.

Habitat. alpine steppes and meadows.

Altitude range. 2500–4000 m.

**Selected specimens studied. Mustang:** Marpha, 28°45'11"N, 83°41'28"E, 2670 m, 16 Sep. 1981, *M.A. Farille 81-336* (E); Jomsom, 28°46'51"N, 83°43'27"E, 3200 m, Versant de Muksant Range, faisant face a Jomsom, 500 m au dessus du village, on rocks, 16 Sep. 1981, *M.A. Farille 81-352* (E); Jharkot – Kagbeni, 28°50'17"N, 83°47'3"E, 2800–3550 m, 17 Sep. 1981, *M.A. Farille 81-359* (E); 10 Jul. 2000, *Y. Iokawa, M.N. Subedi, Y. Takahashi & K. Kano 20020054* (E); Dzong Pura (Muktinath), 28°49'41"N, 83°51'19"E 3640 m, 29 July 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 2087* (E); Tange, 29°0'38"N, 83°56'45"E, 3640 m, 1 Aug. 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 2125* (E); Kagbeni, 28°50'17"N, 83°47'3"E, 3030 m, 8 Jun 1954, *J.D.A. Stainton, W.R. Sykes & L.H.J. Williams 5659* (E); Ekle Bhatti, on sunny rocky steep slope at pathside, 2270 m, 22 Sep. 1995, *M. Mikage et al. 9552384* (E 224287).

# *Piptatherum munroi* (Stapf ex Hook.f.) Mez, Repert. Spec. Nov. Regni Veg. 17: 212 (1921).

Oryzopsis munroi Stapf ex Hooker, Fl. Brit. India 7(22): 234 (1897) (Basionym).

= Oryzopsis stewartiana Bor, Kew Bull., 272 (1953);

= Oryzopsis geminiramula Ohwi, Acta Phytotax. Geobot. 17: 14 (1957).

**Type.** NW India, Chenab Himalayas, 1852, Thomson (lectotype E 360583!, designated by Freitag 1975).

General distribution. China, N India, Kashmir, Nepal (Freitag 1975).

**Distribution in Nepal.** Dolpa, Jumla, Mustang, Sankhuwasabha, Solukhumbu. **Habitat.** Among dwarf *Rhododendron* shrubland and in coniferous forest. **Altitude range.** 3490–4500.

**Selected specimens studied. Dolpa**: Sangdan, 28°55'N, 83°41'E, 4550 m, 21 Jul. 1963, *J.D.A. Stainton 4443* (E). **Jumla**: Maharigaon, grassy slope, 29°19'50"N, 82°22'15"E, 4090 m, 18 Jul. 1952, *O.V. Polunin, W.R. Sykes, & L.H.J. Williams 219* (E). **Mustang**: 2800 m, 16 Sep. 1981, *M. Farille, 81-348* (E); Lo Tsho Dhyum, Nr. Kali Grandaki River, Dhi (Dhee) area, stony river bank, scattered open vegetation, 22 Jul. 1998, *W.R. Sykes 285/98* (E). **Sankhuwasabha**: Thudam, 27°45'31"N, 87°32'59"E, 3490–3480 m, 2 Nov 1971, *L.W. Beer, C.R. Lancaster & D. Morris 10679* (E). **Solukhumbu**: Namche Bazar, along the trail to Phurte, South east facing grassy slopes, open grassy slopes with bushes of Juniperus, 27°48'24"N, 86°42'46"E, 3420 m, 13 Sep. 2005, *F.M. Watson et al. DNEP3 AX33* (E, KATH).

# *Piptatherum laterale* (Regel) Munro ex Nevski, Trudy Bot. Inst. Akad. Nauk SSSR Ser. 1, Fl. Sist. Vyssh. Rast. 4: 217 (1937).

Milium laterale Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 7: 645 (1881) (Basionym).

- ≡ Oryzopsis lateralis (Regel) Stapf ex Hook. f., Fl. Brit. India 7(22): 234 (1896);
- ≡ Piptatherum laterale (Regel) Roshev., Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Kazahsk. SSR 14: 117 (1951).
- = Oryzopsis pubiflora Hack., Denkschr. Kaiserl. Akad. Wiss., Wien Math.-Naturwiss. Kl. 50(2): 8 (1885); ≡ Piptatherum pubiflorum (Hack.) Roshev., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 14: 111 (1951).
- = Oryzopsis vavilovii Roshev., Trudy Prikl. Bot. Selekts. 19(1): 123 (1928); ≡ Piptatherum vavilovii (Roshev.) Roshev., Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Kazahsk. SSR 14: 118 (1951).

**Type.** Afghanistan, Kurram valley, Sikarm, common at 3650 m., dry localities, 1879, *Aitchison* (holotype LE!, isotype K).

General distribution. widely distributed species, occurring from Turkey up to Bhutan and S China (Freitag 1975, Tzvelev 1976).

**Distribution in Nepal.** Although we did not find any specimens of *Piptatherum laterale* during this study, it is known from nearby regions of Bhutan, China, India (Freitag 1975, Noltie 2000, Wu and Phillips 2006), so it is very likely also to be present in Nepal.

#### Trikeraia Bor, Kew Bull. 9(4): 555, f. s.n. (1954)

Type. Trikeraia hookeri (Stapf) Bor.

#### Trikeraia hookeri (Stapf) Bor, Kew Bull. 9(4): 555-556 (1954).

Stipa hookeri Stapf, J. Linn. Soc., Bot. 30: 120 (1894) (Basionym).

≡ Achnatherum hookeri (Stapf) Keng, Claves Gen. Sp. Gram. Prim. Sinic. 106, 213 (1957).

= Timouria aurita Hitchc., J. Wash. Acad. Sci. 23: 134 (1933).

**Type.** Tibet, 4500 m, sheltered nullahs near water, Jul-Sep. 1891, Thorold 124 (holo-type K!, isotype C).

General distribution. China (Tibet), India (Sikkim, Ladakh), Pakistan (Freitag 1985; Wu and Phillips 2006).

**Distribution in Nepal.** Although we did not find any specimens of *Trikeraia hook-eri* during this study we include it here because it is known from nearby regions (Freitag 1975, Wu and Phillips 2006), so it is very likely to be present in Nepal. Freitag (1985) reported this species from Nepal based on *Sufed 104* (K), Mt. Everest, Tinkye palin, 4270 m, but this specimen appears to have been collected on the Tibetan side of Mt Everest.

Habitat. Scrublands, alpine mats.

Altitude range. 4000–4300 m.

**Note.** The 2005 DNEP3 expedition to Solukhumbu collected several specimens which were identified as *Trikeraia oreophila* Cope by H. Noltie [Dingboche, on trail south to the Lobuche Khola bridge, east facing valley side, 27°52'50"N, 86°49'7"E, 4230 m, 23 Sep. 2005, *M.F. Watson et al. DNEP3 AX107* (E, KATH)]. These specimens are characterized by having 4–6 mm long lemma lobes, the awn arising below the middle of the lemma and ovary with two stigmas. Recently, *Trikeraia oreophila* was found to be conspecific with *Sinochasea trigyna* Keng (WCSP 2019). Morphological and molecular studies have shown that the genus *Sinochasea* is distinct not only from *Trikeraia*, but also from all the other genera of the tribe Stipeae, and therefore it was transferred to the tribe *Phaenospermateae* Renvoize & Clayton (Schneider et al. 2011; Romaschenko et al. 2012; Kellogg 2015).

#### Acknowledgements

We would like to express our gratitude to Paul Peterson and Mary Barkworth for their valuable comments and improvements to the manuscript of the paper as well as to the curators of BM, E, GOET, K, KATH, KRA, KUN, LE, NY, P, for their kind hospitality and assistance during visits and for making specimens of *Stipa* available on loan. We thank The Sibbald Trust, the Davis Expedition Fund and the Russian Foundation for Basic Research (RFBR) research project no. 18-34-20112). Financial support for M. Nobis research came from the National Science Center, Poland (grant no. 2013/09/B/ NZ8/03287).

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RESEARCH ARTICLE



# Systematic relevance of pollen morphology in tribe Hylocereeae (Cactaceae)

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Academic editor: <i>Alice Calvente</i>	Received 30 April 2019	Accepted 24 July 2019	Published 19 August 2019

**Citation:** Ruiz-Domínguez C, Vovides AP, Sosa V (2019) Systematic relevance of pollen morphology in tribe Hylocereeae (Cactaceae). PhytoKeys 128: 121–140. https://doi.org/10.3897/phytokeys.128.35842

#### Abstract

Hylocereeae is one of the nine tribes in the subfamily Cactoideae (Cactaceae), for which the limits and recognition of genera have been controversial. Essentially, this group comprises epiphytic and hemiepiphytic genera with stems modified as climbing structures. The aim of this paper is to examine pollen attributes in representative species of genera of Hylocereeae, focusing on *Selenicereus* whose current circumscription comprises *Hylocereus* and three *Weberocereus* species, to find whether significant potentially apomorphic and/or autapomorphic systematic characters can be discovered. Utilizing SEM and light microscopy, 25 pollen characters were observed and measured. Tribe Hylocereeae is stenopalynous, with pollen grains isopolar and radially symmetrical monads, mostly tricolpate, except in *Kimnachia, Pseudoripsalis* and *Weberocereus*, whose pollen grains are pantocolpate. Seven attributes (five qualitative and two continuous) exhibited useful variation and were coded. The character of brevicolpate pollen grains was shared by *Kimnachia ramulosa* and *Pseudorhipsalis amazonica*. Convex quadrangular outline in the polar view was shared by *Weberocereus tunilla* and *S. glaber*. The absence of spinules on the exine was shared by *S. minutiflorus* and *S. stenopterus*. The largest pollen grain, found in *Selenicereus megalanthus*, might be correlated with polyploidy. *Selenicereus* is the taxon with the highest variation in pollen attributes, including species with an exine with or without spinules and variable polar area index and shape (subprolate or oblate-spheroidal).

#### Keywords

Hylocereus, pantocolpate, Selenicereus, stenopalynous, tricolpate

### Introduction

Hylocereeae is one of the nine tribes in subfamily Cactoideae (Cactaceae), in which the limits and recognition of genera have been controversial (Britton and Rose 1920; Buxbaum 1958; Barthlott and Hunt 1993; Bauer 2003; Korotkova et al 2017). With the exception of *Acanthocereus*, this group comprises epiphytic and hemiepiphytic genera with approximately 82 species with stems modified as climbing structures (Barthlott and Hunt 1993). It includes ornamentals in genera such as *Selenicereus, Epiphyllum* and *Disocactus*, whose flowers are night-blooming and known as queen of the night. It also includes *Hylocereus* with several species producing edible fruits known as dragon fruit, pitaya or pitahaya, which are cultivated in tropical areas around the world (Anderson 2001). Species in the tribe share characters such as angled stems and branches, with ribs, furrows or rarely smooth, aerial roots and usually spiny or hairy ovary areoles (Fig. 1) (Bauer 2003; Hunt et al. 2006).

Five taxonomical studies have introduced classifications for tribe Hylocereeae in the last century. First, the group was considered a subtribe in tribe Cereeae by Britton and Rose (1920) recognizing 9 genera with 48 species (Aporocactus, Deamia, Hylocereus, Mediocactus, Selenicereus, Strophocactus, Weberocereus, Werckleocereus and Wilmattea). Later, Buxbaum (1958) raised this group to the tribe level, tribe Hylocereeae, adding three genera to the previous classification: Disocactus, Epiphyllum and Pseudorhipsalis; however, Deamia was not accepted. The subsequent classification by Barthlott and Hunt (1993) differed notably from the preceding taxonomies, with the tribe comprising only six genera: Discocactus, Epiphyllum, Hylocereus, Pseudorhipsalis, Selenicereus and Weberocereus. The most recent classification based on the monophyletic groups identified by a plastid molecular phylogeny, recognized eight genera in tribe Hylocereeae: Acanthocereus (including Peniocereus subg. Pseudoacanthocereus), Aporocactus, Disocactus, Epiphyllum, Kimnachia (a new genus for Pseudorhipsalis ramulosa), Pseudorhipsalis, Selenicereus (incorporating Hylocereus, Weberocereus alliodorus, W. glaber and W. tonduzii) and Weberocereus. In comparison with previous definitions of tribe Hylocereeae, Deamia and Strophocactus were excluded, and Acanthocereus was added (Korotkova et al. 2017).

The aim of this paper is to examine pollen attributes in representative species of genera of tribe Hylocereeae focusing on the current concept of *Selenicereus* that includes *Hylocereus* and three species of *Weberocereus* to find whether potentially apomorphic and/or autapomorphic character states can be discovered. Traditionally, pollen has provided valuable and significant characters in plant taxonomy (Larson and Skvarla 1962; Nowicke and Skvarla 1979; Ferguson 1985), and in particular for Cactaceae, pollen characters continue to be useful taxonomically (e.g. Anderson and Skillman 1984; Rose and Barthlott 1994; Halbritter et al. 1997; Aguilar-García et al. 2012; Gonzaga et al. 2019; Mouga et al. 2019). Furthermore, pollen attributes have been used as a tool to clarify the taxonomy of diverse and complex angiosperm groups such as Poaceae (Dórea et al. 2017), and of difficult genera such as *Rosa* or *Psidium* (Wrońska-Pilarek and Jagodziński 2011; Tuler et al. 2017). Likewise, pollen morphology has been useful



Figure 1. Morphological variation in tribe Hylocereeae. A Aporocactus martianus (Photo S. Avendaño) B Disocacus ackermannii (Photo C. Ruiz) C Kimnachia ramulosa subsp. ramulosa (From Flora de Nicaragua, O.M. Montiel) D Selenicereus atropilosus (From Hunt 2006) E Epiphyllum hookeri (Photo C. Ruiz) F Pseudorhipsalis amazonica (From Mobot - Hammel 24524) G Acanthocereus tetragonus (Photo C. Gómez-Hinostrosa) H Selenicereus (Hylocereus) undatus (Photo C. Ruiz) I Weberocereus tunilla (From Mobot – B. Hammel 22442).

in systematic determinations at generic level in large families such as Asteraceae (Zhao et al. 2000), Liliaceae (Du et al. 2014), Ericaceae (Wrońska-Pilarek et al. 2018), and Bignoniaceae (Burelo-Ramos et al. 2009).

Leuenberger (1976) compiled the most complete description of pollen morphology of 600 cactus species, and found that aperture ratio was one of the most variable and useful characters at different taxonomic levels, from subfamily to genus. In addition, Kurtz (1963) in his study of pollen in Cactaceae – which included several genera in Hylocereeae – identified relevant variation in pollen size in *Hylocereus* and *Selenicereus*, differences in the number of furrows in a number of genera such as *Weberocereus*, and useful variation in pollen sculpture in the length of spinules and perforation of the exine in several genera. Likewise, the identification of species in Cactaceae using pollen in countries such as Brazil, Peru and Argentina found useful characters such as variation in size, shape, and exine thickness to determine taxa at tribe and genus levels (Santos and Watanabe 1996; Santos et al. 1997; Garralla and Cuadrado 2007; Cuadrado and Garralla 2009; Lattar and Cuadrado 2010; De la Cruz et al. 2013; Miesen et al. 2015; Cancelli et al. 2017).

The study of the pollen of tribe Hylocereeae is part of our current project on the evolution and systematics of the *Hylocereus* clade. We aim to incorporate pollen characters with morphological and molecular data to better understand the limits and phylogenetic position of this group, along with phylogenetic relationships of its species, the evolution of chemical and fruit characters. In this paper, the pollen attributes of 27 representative taxa of the genera comprising tribe Hylocereeae, with a main focus in the current concept of *Selenicereus* (including *Hylocereus* and three *Weberocereus* species), are examined to determine whether character states are shared or are exclusive in these taxa.

### Materials and methods

#### Sampling

Pollen grains of 27 species of tribe Hylocereeae, corresponding to 8 genera according to classifications of this group, were collected (Korotkova et al. 2017). Anthers were either collected directly in the field and preserved in envelopes or from herbarium specimens. Representative species in the following genera were sampled (No. spp. sampled/No. spp. in the genus, *sensu* Korotkova et al. 2017): *Acanthocereus* (2/13); *Aporocactus* (1/2); *Disocactus* (2/14); *Epiphyllum* (2/10); *Kimnachia* (1/1); *Pseudorhipsalis* (1/5); *Weberocereus* (1/6). *Selenicereus* (comprising *Hylocereus* and *Weberocereus pro parte*) (17/31). The species vouchers are included in the descriptions of pollen morphology

#### Pollen preparation

The acetolysis method proposed by Erdtman (1960) was used for processing the pollen grains for observation. For difficult material such as collapsed grains or delicate pollen, the suggestions of Fonnegra (1989) were implemented. Pollen grains were mounted in jelly and sealed. For observing pollen with scanning electron microscope (SEM), the material was dried at critical point and sputter coated in palladium gold (Boyde and Wood 1969). SEM observations were made and electromicrographs taken with a Jeol JSM-5600LV scanning electron microscope.

#### Qualitative pollen characters

For the species studied, fourteen qualitative characters were coded: 1) shape of pollen grain, 2) type of polar area, 3) aperture (based in polar area index = apocolpium/ equatorial diameter in polar view), 4) outline of the pollen grain polar view (amb), 5) pollen unit, 6) pollen type (according to polar axis longitude), 7) polarity, 8) aperture class (colpate or brevicolpate), 9) number of colpi, 10) symmetry, 11) tectum (perforate or imperforate), 12) exine (tectate or semitectate), 13) exine spinules (present or absent), 14) margo (present or absent) (Fig. 2). The terminology for pollen grain characters follows Punt et al. (2007), and character denomination follows Erdtman (1952) and



**Figure 2.** Quantitative and qualitative characters from pollen grains: **A**, **B** optical Microscope Photographs (800×) **A** polar view: Apocolpium (A), Mesocolpium (M), Equatorial diameter in polar view (EDP), Exine length (EL), Amb, Polarity, Symmetry, Aperture length, Polar Area Index (PAI) = A/EDP **B** equatorial view: Equatorial diameter (ED), Polar axis (PA), Pollen unit, Aperture (number, position, character), Pollen type, Shape class (PE) = PA/ED **C** scanning Electron Microscope Photograph (6000x); Exine Ornamentation, Spinule length (SL), Spinule base (SB), Perforation diameter (PD).

Salgado-Labouriau (1966). The evaluation of attributes in pollen grains was based on one specimen, following Wrońska-Pilarek et al. (2014). Their study corroborated that the number of pollen grains measured is more important than the number of analyzed specimens, indicating that a sample should contain at least 25 pollen grains (Wrońska-Pilarek et al. 2014).

#### Morphometric pollen characters

Eleven morphological continuous pollen characters of the studied species were measured, including 1) equatorial diameter in polar view, 2) apocolpium (area delimited by lines connecting the apices of the colpi at the pole of the pollen grain), 3) mesocolpium (area delimited by lines between the apices of adjacent colpi), 4) polar axis in equatorial view, and 5) equatorial diameter in equatorial view. They were measured for a maximum of 25 pollen grains from at least three preparations of a single specimen for each species, with 800× optical zoom under a Carl Zeiss Fomi III Optical Microscope, equipped with a Cannon Power Shot G9 digital camera. Additionally, under 1250× optical zoom, 10 pollen grains were observed to measure 6) exine thickness (Fig. 2). For further analysis, SEM electromicrographs on 10 pollen grains for each species with 6000× magnification SEM analysis was performed on acetolyzed and non-acetolyzed pollen material. With SEM, the following exine characters were measured: 7) spinule length, 8) spinule base, and 9) perforation diameter. In addition, the following ratios were estimated: 10) PAI (Polar Area Index) PAI= Apocolpium/ Equatorial diameter in polar view and 11) PE (Shape class) PE=Polar axis/Equatorial diameter in equatorial view. Character measurements from optical microscopy were obtained with the software Axio Vision ver. 4.7.2, and characters from SEM were acquired using ImageJ 1.45 software (Abramoff et al. 2004) (Fig. 2). To identify character states, every continuous character was coded following Almeida and Bisby (1984), ordered in boxplot diagrams by median values, and examined for dips or gaps. Gaps based on the first and third quartiles are codified as discontinuities and the corresponding character states are assigned.

#### Exploratory multivariate analyses

A principal component analysis (PCA) was performed using the packages Factoextra and FactoMine in R (R Development Core Team 2018) to reduce the dimensionality of phenotypic variation and summarize the variables that are correlated. PCA was carried out to identify the characters that explained the greatest proportion of the variability and to identify pollen grains occupying different spaces.

### Results

The taxa studied in tribe Hylocereeae are stenopalynous, i.e. there is slight variation in pollen grains. They are isopolar and radially symmetrical monads, mostly tricolpate, with the exception of *Kimnachia*, *Pseudoripsalis* and *Weberocereus*, whose pollen grains are pantocolpate, with 12 to 15 colpi.

In the following paragraphs detailed descriptions of the pollen grains are provided.

#### Acanthocereus (Engelm. ex A. Berger) Britton & Rose

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: varies from subprolate to oblate-spheroidal (P/E=0.97–1.29). **Apertures**: 3, colpate, large; polar area of medium size (PAI=0.31–0.40). **Measurements**: pollen grains of large size (49.02)56.81(68.53) × (49.89)63.62(79.4) µm in equatorial view; exine thickness (2.41)3.29(4.19) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.05)1.43(1.76) µm length × (1.08)1.37(1.83) µm diameter in base; perforations (0.17)0.25(0.33) µm in diameter (Figure 3A).

**Species examined.** *Acanthocereus tetragonus* (L.) Hummelinck (Colombia, Valle del Cauca. C. Ruiz et al. 576 CUVC); *Acanthocereus chiapensis* Bravo (México, Chiapas. C. Gómez-Hinostrosa et al. 2325 MEXU).

#### Aporocactus Lemaire

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: subprolate (P/E=1.15). **Apertures**: 3, colpate, large; polar area of medium size (PAI=0.36). **Measurements**: pollen grains large to very large, (75.98)93.62(110.47)  $\times$  (80.52)106.91(117.69) µm in equatorial view; exine thickness (3.16)3.66(4.34) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.31)1.64(1.93) µm length  $\times$  (1.18)1.53(2.10) µm diameter in base; perforations (0.14) 0.23(0.34) µm in diameter.

**Species examined.** *Aporocactus martianus* (Zucc.) Britton & Rose. (México, Veracruz. H. Narave et al. 308 XAL).



Figure 3. Pollen grains of tribe Hylocereeae (Scanning Electron Microscope photographs). Left polar view, right equatorial view A Acanthocereus tetragonus (C. Ruiz et al. 576) B Disocactus ackermanii (R. Torres et al. 309) C Epiphyllum oxypetalum (C. Ruiz et al. 640) D Selenicereus costaricensis (C. Ruiz et al. 555)
E S. escuintlensis (C. Ruiz et al. 635) F S. guatemalensis (M. Véliz et al. 20227) G S. minutiflorus (C. Ruiz et al. 627) H S. ocamponis (M. Cházaro 7334) I S. polyrhizus (C. Ruiz et al. 566) J S. sp. (C. Ruiz et al. 608).

#### Disocactus Lindley

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: varies from subprolate to prolate-spheroidal (P/E=1.13–1.18). **Apertures**: 3, colpate, large; polar area of medium size (PAI=0.33–0.34). **Measurements**: pollen grains large to very large, (80.36)97.51(116.99) × (99.25)112.18(125.04) µm in equatorial view; exine thickness (2.24)3.27(3.99) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.22)1.63(2.50) µm length × (1.07)1.45(2.06) µm diameter in base; perforations (0.30)0.61(0.98) µm in diameter (Figure 3B).

**Species examined.** *Disocactus ackermanii* (Haw.) Ralf Bauer (México, Oaxaca. R. Torres et al. 309 MEXU); *Disocactus speciosus* (Cav.) Barthlott (México, Edo de México. J. Canek Ledesma 2211 MEXU).

#### Epiphyllum Haworth

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: varies from oblate-spheroidal to prolate-spheroidal (P/E=0.90–1.06). **Apertures**: 3, colpate, large; polar area of medium size (PAI=0.32–0.46). **Measurements**: pollen grains large, (69.33)88.76(111.93) × (74.43)86.49(105.34) µm in equatorial view; exine thickness (2.24)2.73(3.67) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.22)1.69(2.02) µm length × (0.97)1.26(1.57) µm diameter in base; perforations (0.32)0.51(0.65) µm in diameter (Figure 3C).

**Species examined.** *Epiphyllum oxypetalum* (DC.) Haw. (Guatemala, Sacatepéquez. C. Ruiz et al. 640 BIGU); *Epiphyllum thomasianum* (K. Schum.) Britton & Rose (Guatemala, Sacatepéquez. C.K. Horich 572922 MEXU).

#### Kimnachia S. Arias & N. Korotkova

**Pollen.** pantocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: prolate-spheroidal (P/E=1.07). **Apertures**: 15, brevicolpate, very large; polar area small (PAI=0.05). **Measurements**: pollen grains medium-sized to large,  $(43.95)49.14(55.73) \times (48.04)52.45(58.32) \ \mu m$  in equatorial view; exine thickness  $(3.10)3.84(4.74) \ \mu m$ . **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of  $(0.19)0.33(0.43) \ \mu m$  length ×  $(0.34)0.45(0.55) \ \mu m$  diameter in base; perforations  $(0.09)0.18(0.35) \ \mu m$  in diameter (Figure 4G).

**Species examined.** *Kimnachia ramulosa* (Salm-Dyck) S. Arias & N. Korotkova (Guatemala, Suchitepéquez. L. Velásquez et al. 4884 BIGU).



Figure 4. Pollen grains of tribe Hylocereeae (Scanning Electron Microscope photographs). A–D: Left polar view, right equatorial view A S. undatus (C. Ruiz et al. 560) B Weberocereus tunilla (C.K. Horich BGA 58344) C S. setaceus (H. Bravo et al. 2755). D–J: left polar view, left details of spinules and perforations D S. megalanthus (C. Ruiz et al. 563) E S. setaopterus (C.K. Horich s.n.) F S. triangularis (C. Gómez-Hinostrosa 2110) G Kimnachia ramulosa (L. Velásquez et al. 4884) H Pseudorhipsalis amazonica (E. Gudiño 145) I S. alliodorus (A. Ruiz Velazco et al. 86) J S. glaber (C.K. Horich BGA 57239).

#### Pseudorhipsalis Britton & Rose

**Pollen.** pantocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: oblate-spheroidal (P/E=0.99). **Apertures**: 12, brevicolpate, small; polar area large (PAI=0.62). **Measurements**: pollen grains large, (73.59)81.71(93.64) × (76.00)81.23(86.72) µm in equatorial view; exine thickness (2.85)3.19(3.48) µm. **Exine**: marginated. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (0.64)0.78(0.99) µm length × (0.39)0.48(0.54) µm diameter in base; perforations (0.05)0.11(0.19) µm in diameter (Figure 4H).

**Species examined.** *Pseudorhipsalis amazonica* (K. Schum.) Ralf Bauer (Ecuador, Napo. E. Gudiño 145 MEXU).

#### Selenicereus (A. Berger) Britton & Rose

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: prolate-spheroidal (P/E=1.06–1.07). **Apertures**: 3, colpate, large; polar area medium-sized (PAI=0.36–0.37). **Measurements**: pollen grains large to very large,  $(66.74)87.12(110.39) \times (70.79)90.62(119.77) \mu m$  in equatorial view; exine thickness (2.19)2.68(3.39)  $\mu m$ . **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.09)1.58(2.07)  $\mu m$  length × (0.92)1.30(1.86)  $\mu m$  diameter in base; perforations (0.17)0.30(0.56)  $\mu m$  in diameter.

**Species examined.** Selenicereus grandiflorus (L.) Britton & Rose (México, Veracruz. Rivera-Alarcón et al. 37 XAL); Selenicereus hamatus (Scheidw.) Britton & Rose (México, Veracruz. D. Jimeno-Sevilla 1079 XAL).

#### Selenicereus (Hylocereus sect. Hylocereus clade)

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: varies from suboblate to subprolate (P/E=0.86–1.18). **Apertures**: 3, colpate (brevicolpate in *S. minutiflorus*), large (small in *S. minutiflorus*); polar area medium-sized to large (PAI=0.30–0.59). **Measurements**: pollen grains of medium to very large size,  $(54.52)78.67(97.83) \times (45.94)83.68(102.53)$  µm in equatorial view; exine thickness (1.66)2.95(4.10) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules (*S. minutiflorus* and *S. stenopterus* without spinules) of (1.06)1.47(2.13) µm length × (0.72)1.23(1.53) µm diameter in base; perforations (0.20)0.39(0.89) µm in diameter (Figures 3D, 3E, 3F, 3G, 3I, 3J, 4A, 4E, 4F).

**Species examined.** Selenicereus costaricensis (F.A.C. Weber) Britton & Rose (Colombia, Valle del Cauca. C. Ruiz et al. 555 CUVC); S. escuintlensis Kimnach (Guatemala, Escuintla. C. Ruiz et al. 635 BIGU); S. guatemalensis (Eichlam ex Weing.) Britton & Rose (Guatemala, El Progreso. M. Véliz et al. 20227 BIGU); S. minutiflorus Britton & Rose (Guatemala, Izabal. C. Ruiz et al. 627 BIGU); S. monacanthus (Lemaire) Britton & Rose (Honduras, Francisco Morazán. C. Ruiz et al. 493 TEFH); *S. polyrhizus* (F.A.C. Weber) Britton & Rose (Colombia, Valle del Cauca. C. Ruiz et al. 566 CUVC); *Selenicereus* sp. (México, Oaxaca. C. Ruiz et al. 608 XAL); *S. stenopterus* (F.A.C. Weber) Britton & Rose (Costa Rica, Alajuela. C.K. Horich s.n. MEXU); *S. triangularis* (L.) Britton & Rose (México, Yucatán. C. Gómez-Hinostrosa 2110 MEXU); *S. undatus* (Haworth) Britton & Rose (Colombia, Valle del Cauca. C. Ruiz et al. 560 CUVC).

#### Selenicereus (Hylocereus: Salmdyckia clade)

**Pollen.** trizonocolpate, radially symmetrical, isopolar with circular contour in polar view. **Shape**: Prolate-spheroidal to subprolate (P/E=1.01–1.16). **Apertures**: 3, colpate, large; polar area medium-sized (PAI=0.30–0.40). **Measurements**: pollen grains large to very large, (81.09)89.11(127.4) × (83.13)98.23(129.26) µm in equatorial view; exine thickness (1.94)2.92(4.56) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.17)1.61(2.07) µm length × (0.81)1.20(1.53) µm diameter in base; perforations (0.23)0.46(0.70) µm in diameter (Figure 3H, 4C, 4D).

**Species examined.** *S. megalanthus* (K. Schumann ex Vaupel) Ralf Bauer (Colombia, Valle del Cauca. C. Ruiz et al. 563 CUVC); *S. ocamponis* (Salm-Dyck) Britton & Rose (México, Michoacán. M. Cházaro 7334 MEXU); *S. setaceus* (Salm-Dyck ex DC) Ralf Bauer (Brazil, Rio de Janeiro. H. Bravo et al. 2755 MEXU).

#### Selenicereus (ex Weberocereus)

**Pollen.** trizonocolpate to pantocolpate, radially symmetrical, isopolar with circular or convex-quadrangular contour in polar view. **Shape**: prolate-spheroidal (P/E=1.04–1.12). **Apertures**: 3 or 12, brevicolpate to colpate; polar area medium-sized to large (PAI=0.41–0.60). **Measurements**: pollen grains large to very large, (50.9)77.50(100.2) × (66.79)82.21(100.86) µm in equatorial view; exine thickness (2.29)2.96(3.77) µm. **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of (1.12)1.48(1.82) µm length × (1.13)1.44(1.91) µm diameter in base; perforations (0.17)0.29(0.50) µm in diameter (Figure 4I, 4J).

**Species examined.** *Selenicereus alliodorus* (México, Oaxaca. Gómez-Hinostroza & H. M. Hernández) S. Arias & N. Korotkova (A. Ruiz Velazco et al. 86 MEXU); *S. glaber* (Eichlam) G.D. Rowley (Guatemala, Sacatepéquez. C.K. Horich BGA 57239 MEXU).

#### Weberocereus Britton & Rose

**Pollen.** pantocolpate, radially symmetrical, isopolar with convex-cuadrangular contour in polar view. **Shape:** prolate-spheroidal (P/E=1.07). **Apertures:** 12–15, brevi-

colpate, small; polar area large (PAI=0.56). **Measurements**: pollen grains large,  $(77.07)82.94(89.61) \times (82.67)88.73(98.94) \ \mu\text{m}$  in equatorial view; exine thickness  $(1.96)2.43(2.85) \ \mu\text{m}$ . **Ornamentation**: smooth surface with tectum perforate, ornated with spinules of  $(1.24)1.41(1.58) \ \mu\text{m}$  length  $\times (1.28)1.65(1.91) \ \mu\text{m}$  diameter in base; perforations  $(0.17)0.28(0.45) \ \mu\text{m}$  in diameter (Figure 4B).

**Species examined.** *Weberocereus tunilla* (F.A.C. Weber) Britton & Rose (Costa Rica, Cartago. C.K. Horich BGA 58344 MEXU).

**Qualitatitative pollen characters.** Of the fourteen characters examined, five were identified as variable: Amb (the outline of a pollen grain seen in polar view), colpi number, aperture type (colpate or brevicolpate pollen), marginate exine (an area of the exine around an ectocolpous that is differentiated from the remainder of the exine by difference in thickness and the presence of spinules). The other qualitative characters were not variable (Table 1, Suppl. material 1: Table S1). Pollen grains of the studied

**Table 1.** Qualitative and quantitative morphological characters of pollen for the representative species in the tribe Hylocereeae. Spinule length and perforation diameter are coded based on the simple gap method by Almeida and Bisby (1984). PE ratio =Polar axis/Equatorial Diameter; PAI ratio =Apocolpium/Equatorial diameter in polar view (Polar Area Index); Amb: Outline in polar view. Spinule length (0 = 0  $\mu$ m, 1 = 0.33–0.78  $\mu$ m, 2 = 1.27–1.86  $\mu$ m); Perforation diameter (0 = 0.11–0.18  $\mu$ m, 1 = 0.23–0.72  $\mu$ m). P=present, A=Absent.

Species	PE ratio	Shape class	PAI ratio	PA type	Amb	Pollen type	Aperture	Margo	No of colpus	Spninules	Spinule length	Perforation diameter
Acanthocereus chiapensis	1.30	Subprolate	0.32	Medium	Circular	Large	Colpate	А	3	Р	2	1
Acanthocereus tetragonus	0.97	Oblate-spheroidal	0.41	Medium	Circular	Large	Colpate	А	3	Р	2	1
Aporocactus martianus	1.15	Subprolate	0.36	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Disocactus ackermanii	1.19	Subprolate	0.34	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Disocactus speciosus	1.13	Prolate-spheroidal	0.35	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Epiphyllum oxypetalum	1.07	Prolate-spheroidal	0.32	Medium	Circular	Large	Colpate	А	3	Р	2	1
Epiphyllum thomasianum	0.90	Oblate-spheroidal	0.47	Medium	Circular	Large	Colpate	А	3	Р	2	1
Kimnachia ramulosa	1.07	Prolate-spheroidal	0.05	Small	Circular	Large	Colpate	А	15	Р	1	0
Pseudorhipsalis amazonica	0.99	Oblate-spheroidal	0.63	Large	Circular	Large	Colpate	Р	12	Р	1	0
Selenicereus alliodorus	1.12	Prolate-spheroidal	0.41	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus costaricencis	1.18	Subprolate	0.38	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus escuintlensis	0.97	Oblate-spheroidal	0.37	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus glaber	1.05	Prolate-spheroidal	0.61	Large	Convex quadrangular	Large	Colpate	А	12	Р	2	1
Selenicereus grandiflorus	1.07	Prolate-spheroidal	0.37	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Selenicereus guatemalensis	1.04	Prolate-spheroidal	0.31	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus hamatus	1.06	Prolate-spheroidal	0.37	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus megalanthus	1.01	Prolate-spheroidal	0.40	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Selenicereus minutiflorus	0.87	Suboblate	0.60	Large	Circular	Large	Colpate	А	3	А	0	1
Selenicereus monacanthus	1.11	Prolate-spheroidal	0.34	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus ocamponis	1.17	Subprolate	0.31	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Selenicereus polyrhizus	1.07	Prolate-spheroidal	0.34	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus setaceus	1.10	Prolate-spheroidal	0.34	Medium	Circular	Very Large	Colpate	А	3	Р	2	1
Selenicereus sp.	1.11	Prolate-spheroidal	0.35	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus stenopterus	1.17	Subprolate	0.38	Medium	Circular	Large	Colpate	А	3	А	0	1
Selenicereus triangularis	0.91	Oblate-spheroidal	0.45	Medium	Circular	Large	Colpate	А	3	Р	2	1
Selenicereus undatus	1.04	Prolate-spheroidal	0.40	Medium	Circular	Large	Colpate	А	3	Р	2	1
Weberocereus tunilla	1.07	Prolate-spheroidal	0.57	Large	Convex quadrangular	Large	Colpate	А	12, 15	Р	2	1

species in tribe Hylocereeae are oblate-spheroidal, tricolpate with spinules of variable size (Figs 3 and 4). *Weberocereus, Pseudorhipsalis,* and *Kimnachia* differ from the rest of the tribe by having pollen grains with a small aperture, brevicolpate, with differences in the number of colpi as well. *Aporocactus, Acanthocereus, Disocactus, Epiphyllum,* and *Selenicereus* (including *Hylocereus*) have tricolpate pollen. In addition, two autoapomorphic characters were identified: convex-quadrangular outline in *Weberocereus*, marginate exine in *Pseudorhipsalis* (Figs 3, 4).

**Continuous pollen characters.** The size of pollen grains for the 27 taxa examined varies from 55.47 to 154.42  $\mu$ m in polar axis, and this is large according to Erdtman (1952). Boxplot diagrams ordered by median values found gaps for spinule length and perforation diameter based on the first and third quartiles (Fig. 5). These were coded based on the simple gap method by Almeida and Bisby (1984). Spinule length differentiates *Kimnachia* from *Pseudorhipsalis*. For *Selenicereus* (*Hylocereus* clade), spinule length was found to be polymorphic because it includes representatives lacking spinules and representatives with spinules of larger dimensions than those of *Kimnachia* and *Pseudorhipsalis* (Table 1).

**Multivariate analyses.** The PCA graph displays projections of pollen characters in a multidimensional space in which the first two components explained 62.4% of the observed variance. PC 1 explains 44% of the variance and is associated with size (equatorial diameter, polar axis, and spinule dimensions), while PC 2, which explains 18.4% of the variance, is associated with proportions (PAI and PE ratios) (Fig. 6A). The length of the arrows in Figure 6A indicates adequate sampling for all characters, except for exine length (EL) and perforation diameter (PD). The size of pollen grains in the polar and in equatorial views had a positive correlation and, similarly, there is



**Figure 5.** Box plots of two coded pollen characters, which are discriminant for genus in tribe Hylocereeae. **A** Spinule length **B** perforation diameter. Boxes represent the first and third percentiles and black lines indicate median values. Character states are indicated (0, 1 and 2) for each character and measurement range is indicated. Measurements are in  $\mu$ m.



**Figure 6.** Principal Component Analysis graph. **A** Scatterplot displaying projections in a multidimensional space of the pollen grain characters: Apocolpium (A), Mesocolpium (M), Equatorial diameter in polar view (EDP) Exine length (EL), Polar Area Index (PAI), Equatorial diameter (ED), Polar axis (PA), Shape class (PE), Spinule length (SL), Spinule base (SB), Perforation diameter (PD). PC1 explains 44% of the variance and PC2 explains 18.4% of the variance **B** Sorting of the 27 species of tribe Hylocereeae, in relation to the morphometric variables of pollen grains.

a negative correlation between the PE and PAI ratios, as expected in spherical forms. There is a negative correlation between exine length (EL) and the other measures. Figure 5B displays the species studied along the principal components in relation to pollen characters. The association of species is defined by the size of the pollen grain and its shape from prolate to oblate spheroidal). A core association of species is formed by representative species from all genera, including pollen mainly subprolate to prolate-spheroidal. The only species with no representatives in the core group and that appear as outliers are *Pseudorhipsalis amazonica* and *Kimnachia ramulosa*, with prolate to oblate pollen. Remarkably, *Selenicereus (Hylocereus) megalanthus*, along with *S. setaceus* are two of the species with prolate-spheroidal pollen grains with the largest polar axis; *S. minutiflorus and K. ramulosa* have the smallest pollen grains (smallest polar axis). *Selenicereus minutiflorus* is the representative of *Selenicereus (Hylocereus)* with the unique characters of suboblate pollen and an exine lacking spinules (Fig. 3).

#### Discussion

Pollen grains of the representative species of the genera of tribe Hylocereeae studied here share the pollen type common to Caryophyllales: tricolpate to pantocolpate with the exine spinulose and perforate (Nowicke and Skvarla 1979). Furthermore, in particular for Cactaceae, tricolpate pollen has been described in all tribes of subfamilies Pereskioideae and Cactoideae (Lehuenberger 1976; Kurtz 1948, 1963) and is the most common pollen type in eudicots (Erdtman 1952; Furness and Rudall 2004).

From the 25 pollen characters analyzed, only seven attributes (five qualitative and two continuous) exhibited useful taxonomic variation. Four genera in Hylocereae: *Epiphyllum, Acanthocereus, Disocactus, Selenicereus* (comprising *Hylocereus* and three species of *Weberocereus*), and *Aporocactus* have pollen grains with essentially similar morphology. That being said, *Disocactus* and *Epiphyllum* form part of the Phyllocac-toid clade while *Selenicereus* and *Weberocereus* form part of the Hylocereoid clade in the molecular phylogeny constructed by Korotkova et al. (2017).

Despite the fact that the majority of taxa studied here share many pollen attributes, certain characters were common to limited groups of species. By way of example, *Kimnachia ramulosa* and *Pseudorhipsalis amazonica* are the only two species included in our study that share the attributes of brevicolpate pollen grains with small apertures. *Kimnachia* is a recently described genus whose sole species was previously included in *Pseudorhipsalis* (Korotkova et al. 2017). In addition, *Kimnachia ramulosa* and *Pseudorhipsalis amazonica* also share the character of pollen grains with 12–15 colpi with *Weberocereus*. Furthermore, *Selenicereus glaber* and *Weberocereus tunilla* share the character of convex quadrangular contour in polar view (Amb); *S. glaber* was previously included in *Weberocereus* (Barthlott and Hunt 1993).

Two species in *Selenicereus* (*S. minutiflorus* and *S. stenopterus*) stand out for lacking spinules in the exine in tribe Hylocereeae. They were retrieved in the *Hylocereus* clade in the plastid phylogeny of Korotkova et al. (2017) and transferred with all *Hylocereus* spp. to *Selenicereus*. They have remarkable morphology with miniature plants bearing pinkish flowers in contrast to the rest of the species in the current concept of *Selenicereus* whose flowers are white. Bauer (2003) transferred these two species from *Selenicereus* to *Hylocereus* and classified them in the *Salmdyckia* group of *Hylocereus*. Previously, Britton and Rose (1920) included these taxa (*S. minutiflorus and S. stenopterus*) in *Mediocactus*, a genus with intermediate morphological characteristics between *Selenicereus* and *Hylocereus*, with spines on the pericarpel.

The *Salmdyckia* group, including *S. ocamponis*, *S. setaceus* and *S. megalanthus*, possesses the largest pollen grains in the genus. Of these three species, *Selenicereus megalanthus* had the largest pollen grains, with a pollen grain size that could be correlated with polyploidy, a process that can produce large to very large pollen grains (Muller 1979). Chromosome counts for this species indicate that it is tetraploid (Tel-Zur et al. 2004; Tel-Zur et al. 2011).

Furthermore, multivariate analyses corroborated the results of discrete and qualitative characters, displaying species such as *Selenicereus megalanthus*, *S. stenopterus*, *S. multiflorus* and *Kimnachia ramulosa* as outliers in the multidimensional space. Variation in these analyses was found to be mainly associated with size (equatorial diameter, polar axis, and spinule dimensions).

Of the genera in Hylocereeae, *Selenicereus* in its current concept including the species previously considered in *Hylocereus* and three species formerly classified in *Weberocereus*, is the taxon with the greatest variation in pollen grains. For instance, it includes species with and without spinules in the exine, variable shape (subprolate to oblate-spheroidal), and polar area index is either small, medium or large. Moreover, the

generic limits of *Hylocereus* and *Selenicereus* have changed over time (Gómez-Hinostrosa and Hernández 2014; Gómez-Hinostrosa et al. 2014; Cruz et al. 2016; Korotkova et al. 2017). Pollen size, the absence of spinules and the morphological characters in species such as *S. minutiflorus* and *S. stenopterus* suggest they might belong to a genus other than *Hylocereus* or *Selenicereus*.

Pollen research that concentrates on finding crucial taxonomical characters in Cactaceae has been scarce. Nevertheless, current studies in other plant groups have demonstrated their utility and that of other data sources (e.g. Kriebel et al. 2017; Niu et al. 2018; Siniscalchi et al. 2017; Wrońska-Pilarek et al. 2018). The most complete study on the palynology of the entire Cactaceae by Leuenberger (1976b) was published in a series of dissertations and is difficult to acquire. Probably the delicate pollen in Cactaceae which is difficult to process is one of the causes of the limited number of studies on pollen.

The pollen attributes identified here and that are shared by a number of species belonging to different genera that have recently been segregated or grouped together, suggests that additional evidence should be gathered and new phylogenetic analyses performed to clarify boundaries. Circumscription of the genera in tribe Hylocereeae has only been carried out based on a set of molecular or morphological characters. Our project on the *Hylocereus* clade will include the palynological characters determined here, along with other sources of attributes such as their morphological, ecological and molecular traits.

#### Acknowledgments

The authors thank the two anonymous reviewers for comments that greatly improved this paper. We also thank the curators of the MEXU, XAL and BIGU herbaria for kindly allowing us to sample pollen. We are grateful to Diana Isabel Vergara and Sonia Galicia for their invaluable help in the anatomical lab and with optical microscopy; to Tiburcio Laez for SEM photographs; Carlos Gómez-Hinostrosa and María del Carmen Mandujano for their review of the manuscript; Mario Véliz, Germán Sandoval, Lilian Ferrufino, Guillermo Reina, Isabel Nicholls, Favio González, and Diego Angulo for help during field trips. We thank Bianca Delfosse for her editorial work on our English. This research was supported by CONACYT, Mexico to V. S. (PNDP2015/1023). C.R-D. was supported by a Doctorate in Science scholarship from CONACyT, Mexico (300231).

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# Supplementary material I

#### Table S1

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Data type: species data

- Explanation note: Qualitative and quantitative morphological characters of pollen for the representative species in the tribe Hylocereeae.
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Link: https://doi.org/10.3897/phytokeys.128.35842.suppl1