RESEARCH ARTICLE



# Oreocharis odontopetala, a new species of Gesneriaceae from Guizhou, China

Qiong Fu<sup>1,2,\*</sup>, Ying Xia<sup>3\*</sup>, Ying Guo<sup>4\*</sup>, Rong Huang<sup>1,2</sup>, Ying-Qiang Wang<sup>1,2</sup>

I Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou 510631, China 2 Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, School of Life Sciences, South China Normal University, Guangzhou 510631, China 3 Panzhou Bureau of Agriculture and Rural Affairs, Panzhou, Guizhou 553537, China 4 Liupanshui Niangniangshan National Wetland Park Administration Office, Panzhou, Guizhou 553522, China

Corresponding author: Ying-Qiang Wang (wangyq@scnu.edu.cn)

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#### Abstract

A new species, *Oreocharis odontopetala* Q.Fu & Y.Q. Wang from Guizhou Province in southwest China, is described and illustrated, based on morphological comparison with existing species. It is morphologically most similar to *O. elegantissima*, but can be easily distinguished by its adaxially bullate leaf blade, abaxially conspicuous reticulate veinlets, brown-purple peduncles, triangular adaxial corolla lobes and abaxial corolla lobe margins bearing 4–10 long teeth, glabrous style and shorter stamens with confluent thecae at the apex, as well as leaf epidermal characters.

#### **Keywords**

Briggsia, endemism, leaf epidermis, morphology, new taxon, taxonomy

<sup>\*</sup> Equal contribution.

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## Introduction

The genus *Briggsia* was established by Craib (1919a) and had at one point > 20 species (Wang et al. 1990, 1998; Li and Wang 2004; Wu et al. 2012; Wen et al. 2015). *Briggsia*, in its original definition, was described as having species with a large distinctly bilabiate ventricose corolla, four fertile stamens with the anthers cohered in pairs and gradually inarching filaments (Craib 1919b). However, the genus underwent various taxonomic changes and the rosette-forming species were recently subsumed in the enlarged genus *Oreocharis* that currently includes over 120 species (Möller et al. 2011, 2014, 2016, 2018). It is distributed in China, Thailand, Vietnam, Myanmar, Bhutan, NE India and Japan.

During our fieldwork on floral biology of *Oreocharis* in August 2018, we found an unrecognised species of *Oreocharis* resembling species of the former *Briggsia* on Wumeng grassland, Wumeng Town, Panzhou City, Guizhou Province, Southwest China. After carefully comparisons of diagnostic characters of *Oreocharis* specimens and consulting the relevant literature, we found it was most similar to *O. elegantissima* (H. Lév. & Vaniot) Mich.Möller & W.H.Chen (previously *Briggsia elegantissima*), but was evidently different in leaf and flower morphology. Here, we describe and illustrate the unknown species as a new species of *Oreocharis*.

# Methods

In the flowering season 2018, comparative studies on the morphology and floral ecology between the new species and Oreocharis elegantissima were carried out from two different localities. The new species grows on limestone rocks of a hill forest in Wumeng grassland, Wumeng Town, Panzhou City, Guizhou Province, southwest China (26°4.33'N, 104°37.34'E, alt. ca. 2100 m) and the studied population of O. elegantissima grows on limestone rocks in a subtropical moist forest in Heibai Village, Yuni Town, Panzhou City, Guizhou Province, southwest China (25°59.50'N, 104°51.27'E, alt. ca. 1760 m). Morphological observations and measurements were carried out on living plants, dried specimens and preserved materials under stereomicroscopes and morphological characters were described, following the terminology presented by Wang et al. (1998). Fresh experimental materials (including leaves, flowers and fruits) were obtained from the fields (at least 10 leaves and 10 flowers from 10 plants) and the micromorphological characters were further investigated with a transmission light microscope (Zeiss Axio Imager A1, Göttingen, Germany) and a scanning electron microscope (Jeol JSM-6360, Akishima, Japan). The materials for the LM study were boiled in water and then epidermal tissue was obtained from the leaves by tearing. The materials for SEM observations were macerated in 4% glutaric dialdehyde solution for about 24 hours, dehydrated in a gradient alcohol series and then critical point dried with Lecia EM CPD 300 (Vienna, Austria). Subsequently, samples were directly mounted on stubs and sputter-coated with gold-palladium. The terminology of micromorphological characters followed Dilcher (1974).

#### Taxonomy

# Oreocharis odontopetala Q.Fu & Y.Q.Wang, sp. nov.

urn:lsid:ipni.org:names:60478958-2 Figures 1, 2

**Diagnosis.** Oreocharis odontopetala is most similar to O. elegantissima, having a similar shape of leaf blade, lanceolate sepals and bracts, stellate ring-like disc, pistil and fruits. Oreocharis odontopetala differs from O. elegantissima by its adaxially bullate leaf blade (vs. not bullate), with abaxially reticulate veinlets conspicuous (vs. veinlets inconspicuous); peduncles brown-purple (vs. green); adaxial corolla lobes triangular (vs. oblong) and abaxial corolla lobe margins with 4–10 long teeth (vs. margin nearly entire); style glabrous (vs. glandular pubescent) and stamens shorter (adaxial 0.5–1.4 vs. 2.0–2.6 cm, abaxial 0.8–1.8 vs. 2.3–2.7 cm) with confluent thecae at apex (vs. not confluent).

**Type.** CHINA. Guizhou Province: Panzhou City, Wumeng Town, Wumeng grassland, growing on limestone rocks in hills, 26°4.33'N, 104°37.34'E, alt. 2100 m, 14 August 2018, *Ying-Qiang Wang*, *WYQ-2018-112* (holotype: SN!; isotypes: SN!).

Description. Perennial herbs, rosette forming. Rhizomes straight, terete, 0.8-2.1 cm long, ca. 0.8-1.1 cm in diameter. Leaves 8-18, basal; leaf blade papyraceous, usually ovate, rarely narrowly ovate and broadly ovate,  $2.4-6.4(-7.2) \times 1.3-3.5(-$ 4.0) cm, apex acute, base rounded to shallowly cordate, margin crenate-serrate, adaxially green, bullate, white pubescent except veins, abaxially pale green, rust-brown sericeous along midrib and lateral veins, white pubescent along veinlets; lateral veins 4-7 pairs per side, adaxially inconspicuous and slightly concave, abaxially prominent, reticulate veinlets conspicuous; petiole 0.4-5.4(-7.0) cm, outer leaves with long petiole, densely rust-brown sericeous. Cymes 1-6, axillary, 1-2-branched, 1-6(-11)-flowered, each plant bearing 1-18(-22) flowers; peduncle 5.0-15.3(-19.3) cm long, 0.7-1.5(-2.1) mm in diameter, brown-purple, brown villous; bracts 2, opposite, green, lanceolate, (2.5-)3.3-8.0 × 1.0-2.7 mm, outside densely brown villous, inside glabrous, apex acute, margin entire. Pedicel 1.7-3.5 cm long, ca. 0.6-0.9(-1.2) mm in diameter, brown-purple, densely white glandular pubescent. Calyx 5-parted to near base, segments lobes equal, lanceolate to narrowly lanceolate, 4.2–7.3 × 1.4–1.8 mm, outside brown villous, inside glabrous, apex acute, margin entire. Corolla purplered to purple, outside densely white glandular pubescent and sparsely villous, inside densely white glandular pubescent, 2.2-4.8 cm long; tube narrowly campanulate, gibbous abaxially, inside yellow and purple-red spotted, 1.4-2.9 cm long, 0.7-1.2 cm in diameter at middle; limb 5-lobed, zygomorphic, distinctly 2-lipped, adaxial lip 4.8–8.2 mm, nearly erect, 2-lobed to nearly middle, lobes triangular, apex acute,  $2.0-4.1 \times 2.0-3.8$  mm, abaxial lip (0.8–)1.1–1.9 cm, 3-lobed to middle, lobes elliptic to ovate, margin with 4–10 long teeth, central lobe  $5.6-9.8 \times 3.2-7.6$  mm, lateral lobes 4.0-8.6 × 3.1-6.3 mm. Stamens 4, coherent in pairs, included, adaxial stamens 0.5-1.4 cm, adnate to 4.5-8.3 mm above corolla base, abaxial stamens 0.8-1.8 cm, adnate to 3.5-7.0 mm above corolla base; *filaments* linear, slender, white glandular



**Figure 1.** *Oreocharis odontopetala.* **A** habit; **B** flower; **C** opened corolla, showing lip lobes and stamens; **D** abaxial stamens (dorsal view); **E** cohering pair of anthers (anterior view); **F** adaxial stamens (dorsal view); **G** calyx, pistil and stigma; **H** bracts (ventral and dorsal view); **I** sepals (the two on the right showing ventral view and the three on the left showing dorsal view); **J** adaxial leaf surface; **K** abaxial leaf surface. Drawn by Ms Yun-Xiao Liu based on the holotype (*WYQ-2018-112*).



**Figure 2.** Morphological comparison of (**A**) *Oreocharis odontopetala* and (**B**) *O. elegantissima.* **-1** habitat and habit. **-2** adaxial leaf surface. **-3** abaxial leaf surface. **-4** opened corolla, showering lip lobes and stamens with anthers cohering in pairs. **-5** anthers. **-6** thecae, showing confluence at apex (white arrowhead), or no confluence (black arrowhead). **-7** immature pistil and disc, showing glandular pubescence (**B**) or absence (**A**).

pubescent; *anthers* reniform, basifixed, glabrous,  $1.9-3.1 \times 1.8-3.0$  mm, thecae 2, parallel, confluent at apex; *staminode* 1, 1.1–1.8 mm long, adnate to 2.7–4.6 mm above corolla base. *Disc* stellate ring-like, yellow-green, 1.5–2.5 mm high. *Pistil* 0.7–1.2 cm long at flower opening and 1.5–2.5 cm long at maturity, glabrous; *ovary* linear, 0.4–0.9 cm long at flower opening and 1.1–1.9 cm long at maturity, 0.9–1.1 mm in diameter, 1-loculed, placentas 2, parietal, projecting inwards, 2-cleft; *style* 1.8–2.7 mm at flower opening and 3.3–5.4 mm long at maturity; *stigma* 2, equal, 2-lipped, undivided, lingulate, apex obtuse, 0.6–0.9 mm long at flower opening and 1.4–1.6 mm at maturity. *Capsule* linear, straight, glabrous, ca. 37.2×1.7 mm, dehiscing septicidally by two valves at maturity.

**Distribution and habitat.** *Oreocharis odontopetala* is only known from the type locality on Wumeng grassland, Wumeng Town, Panzhou City, Guizhou Province, China, 26°4.33'–26°8.62'N, 104°37.34'–104°36.35'E, alt. ca. 2100–2400 m.

**Ecology and phenology.** The plants grow on limestone rocks of a hillside forest. Flowering in early August to late September, fruit ripe during early-September to October.

**Conservation status.** Based on our field investigations, the new species is currently only known from the type locality Wumeng grassland. Only ca. 300 mature individuals were present and the extent of occurrence is estimated to be ca. 5000 m<sup>2</sup>. The location is not in a protected area and is accessible to casual hikers. According to the guidelines for using the IUCN Red List Categories and Criteria (IUCN 2017), the species is categorised as Endangered [EN B1abc(iv); C2a(i,ii)] due to its rarity and the threat of disturbance.

**Etymology.** The species is named after its abaxial strongly toothed corolla lobes.

**Vernacular name.** Chǐ Bàn Cū Tǒng Jù Tái (Chinese pronunciation); 齿瓣粗筒 苣苔 (Chinese name).

**Morphology (SEM) of leaf epidermis and epidermal cells of style** (Fig. 3). The leaf epidermal cells of *Oreocharis odontopetala* on both adaxial and abaxial sides were irregular, with smooth cuticular membranes and sinuate anticlinal walls (Fig. 3A1–4). The epidermal trichomes on both adaxial and abaxial leaf blades were multicellular, with rugulate membranes (Fig. 3A2). The stomata apparata were only found on the abaxial epidermis and were assigned to the anisocytic type, with rugulate membranes, stomatal length  $34.8 \pm 3.4$  (29.3–43.6) µm, stomatal width  $26.9 \pm 2.8$  (21.7–31.7) µm (Fig. 3A4). The outer stomatal rims were striate (Fig. 3A4). The epidermal cells of the style were quadrilateral or polygonal, with striate cuticular membranes and many granular derivatives (Fig. 3A5).

**Notes.** It is worth noting that *Oreocharis odontopetala* shares the narrowly campanulate, abaxial gibbose corolla tube, anthers coherent in pairs at apex, as well as similar ovary structure with all other species of the former *Briggsia*. It is most similar to *O. elegantissima*, but is distinct from its congeners by its adaxially bullate leaf blade, abaxially conspicuous reticulate veinlets, brown-purple peduncles, triangular adaxial corolla lobes and abaxial corolla lobe margins bearing 4–10 long teeth, glabrous style and shorter stamens with confluent thecae at the apex, as well as the leaf epidermal characters. The detailed morphological comparison between *O. odontopetala* and *O. elegantissima* is provided in Tables 1, 2.



**Figure 3.** Comparative morphology (LM & SEM) of leaf epidermal surface and epidermal cells of style between (**A**) *Oreocharis odontopetala* and (**B**) *O. elegantissima.* -1 adaxial leaf epidermal cells (LM). -2 adaxial leaf epidermal trichome and its cuticular membrane (SEM). -3 abaxial leaf epidermal cells (LM). -4 abaxial leaf cuticular membrane and stomata (SEM). -5 style epidermal cell shape and ornamentation (SEM).

Character	O. odontopetala	O. elegantissima	
Adaxial leaf blade	bullate	not bullate	
Abaxial leaf blade	reticulate veinlets conspicuous	veinlets inconspicuous	
Peduncle	brown-purple	green	
Corolla	purple-red to purple with white spots on the face	white, purple with purple or white striate on the face	
Corolla adaxial lip	lobes triangular, apex acute	lobes oblong, apex rounded	
Corolla abaxial lip	lobe margin with 4–10 long teeth, apex acute	lobe margin nearly entire, apex rounded	
Stamens	adaxial 0.5–1.4 cm long, abaxial 0.8–1.8 cm long	adaxial 2.0–2.6 cm long, abaxial 2.3–2.7 cm long	
Anthers	thecae confluent at apex	thecae not confluent	
Style	glabrous	glandular pubescent	

Table 1. Morphological comparison between Oreocharis odontopetala and O. elegantissima.

**Table 2.** Morphological comparisons (SEM & LM) of leaf epidermal surface and epidermal cells of style between *Oreocharis odontopetala* and *O. elegantissima*.

Characters	O. odontopetala	O. elegantissima
Leaf epidermal cell	adaxial and abaxial cuticular membranes smooth, anticlinal walls sinuate without knobs	adaxial and abaxial cuticular membranes striate, anticlinal walls sinuate with knobs
Leaf epidermal trichome	both adaxial and abaxial membranes rugulate	adaxial membranes smooth, abaxial membranes rugulate
Stomata	smaller, 34.8 $\pm$ 3.4 × 26.9 $\pm$ 2.8 µm, outer stomatal rims striate	larger, 37.7 $\pm$ 2.9 × 31.9 $\pm$ 3.0 µm, outer stomatal rim nearly smooth
Style epidermal cells	quadrilateral or polygonal, cuticular membranes many granular derivatives	long irregulate, cuticular membranes without granular derivative

Furthermore, the characteristic, abaxial corolla lobe margins with 4–10 long teeth, is easily distinguished from other species of the enlarged *Oreocharis*. The bullate leaf is quite rare in the enlarged *Oreocharis* and only occurs in the new species and other few species such as *O. bullata*, *O. curvituba*, *O. glandulosa*, *O. primuliflora*, *O. magnidens* (and to a lesser degree *O. × heterandra*), but it has a very different corolla, style and stamen amongst these species (Li and Wang 2004; Wei et al. 2016). The stellate ring-like disc is also rare in the enlarged *Oreocharis* and only found in the new species, *O. elegantissima* and *O. duyunensis* (Guo et al. 2018). Therefore, this new species has a unique morphology amongst the species of the extended *Oreocharis*.

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RESEARCH ARTICLE



# Justicia thailandica, a new species of Acanthaceae from Thailand

Yi Tong<sup>1</sup>, Yunfei Deng<sup>2,3</sup>

1 School of Chinese Materia Medica, Guangzhou University of Chinese Medicine, Guangzhou 510006, Guangdong, China 2 Key Lab of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, Guangdong, China 3 Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar

Corresponding author: Yunfei Deng (yfdeng@scbg.ac.cn)

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#### Abstract

A new species of *Justicia* (Acanthaceae), *J. thailandica*, is described and illustrated from Thailand. The new species belongs to *Justicia* sect. *Harnieria* and is similar to *J. quadrifaria* and *J. championii*, but differs on account of the obviously densely white indumentum in the inflorescence bracts and calyx, ovate leaf blades with margin usually entire, spathulate inflorescence bracts and length ratio of calyx to mature capsule. It is assessed to be "Near threatened" (NE) according to IUCN Red List Category and Criteria. Pollen and seed morphology characters are also reported. Species of *Justicia* sect. *Harnieria* in Thailand are discussed and a key to the three recognized species is presented.

#### **Keywords**

Harnieria, Calophanoides, new taxa, taxonomy

# Introduction

*Justicia* L. is the largest genus in the family Acanthaceae and consists of about 600 species distributed in tropical and temperate (to a lesser extent) regions of the world (Graham 1988; Hu et al. 2011; Mabberley 2017). It is characterized by the tubular

and bilabiate corolla with stylar furrow (rugula) in the upper lip, two stamens usually with the lower anther-theca spurred at base, "Knötchenpollen" pollen grains, and 4-(rarely 2-)seeded stalked capsules (Lindau 1894; Graham 1988; Hu et al. 2011). The recent molecular evidence (Deng et al. 2016; Kiel et al. 2017) indicated that the genus *Justicia* in the broad sense is polyphyletic and might be further separated into several independent genera. At the moment, we follow the treatment of Graham (1988) who divided the genus into sixteen sections.

Sect. *Harnieria* (Solms-Laubach) Benth. is characterized by the abbreviated axillary spikes, leaf-like inflorescence bracts, fusiform capsules and tuberculate seeds (Tong et al. 2016). It comprises approximately 76 species distributed in the tropical and subtropical regions of Africa and Asia with two species extending to Australia (Barker 1986; Hedrén 1989; Tong et al. 2016).

In the course of revising *Justicia* sect. *Harnieria* from Asia, some specimens collected from Thailand and identified in herbaria as *J. quadrifaria* (Nees) T. Anderson or *J. championii* T. Anderson appear to represent an undescribed species, which differs from the latter two species by the characters of indumentum, leaves, petiole, calyx, inflorescence bracts and length ratio of calyx to mature capsule.

#### Materials and methods

The morphological comparison with related species in *Justicia* sect. *Harnieria* was based on studies of herbarium specimens and information gathered from literature. Pollen grains and seeds were taken from dried specimens (Beusekom *et al.* 3759, MO2366671) and mounted on aluminium stubs coated with gold in a sputter coater after being cleaned in water using ultrasound, and then examined using scanning electron microcopy (SEM; JSM-6360LV). The polar (P) axis and equatorial (E) diameter were measured by imaging analyzer (Smile View 2.1; JEOL Tokyo, Japan). Pollen terminology follows Erdtman (1969) and Punt et al. (2007). Seed terminology follows Hedrén (1989) and Rueangsawang et al. (2012).

#### **Taxonomic description**

Justicia thailandica Y.F.Deng & Y.Tong, sp. nov.

urn:lsid:ipni.org:names:77197854-1 Figures 1, 2

**Type.** THAILAND. Kanchanaburi Province, Kanchanaburi District, Huay Bankau, 14°55'00"N, 98°45'00"E, mixed deciduous forest on limestone, 900 m alt., 13 Nov 1971, C. F. van Beusekom, C. Phengklai, R. Geesink & B. Wongwan 3759 (holotype: MO2366671!; isotypes: BKF!, C!, K!, L!, P!).

**Diagnosis.** The new species is similar to *Justicia quadrifaria* (Nees) T. Anderson, but differs on account of the whole plant being white villous (not pubescent), leaf



Figure 1. Justicia thailandica A habit B magnifying the portion of leaf blades showing the tomentum C adaxil surface of inflorescence bract D abaxil surface of inflorescence bract E bract F bracteoles G calyx H corolla I dorsal view of the anther J frontal view of the anthers K pistil with nectary disc L, M opened capsule N capsule with calyx O seed. (Drawn by Cui Dinghan from the holotype van Beusekom et al. 3759).



**Figure 2.** *Justicia thailandica* **A** adaxial surface of inflorescence bract **B** abaxial surface of inflorescence bract **C** bract **D** bracteoles **E** stigma **F** pistil with nectary disc **G** calyx **H** dorsal view of anther **I** frontal view of anther **J** opened capsule **K** outside of capsule **L** inside of capsule showing retinacula **M** seed.

blade ovate (not oval, oblong to rarely ovate) with margin usually entire (not slightly undulate), apex shortly caudate or acute (not acuminate), base cuneate (not decurrent), petiole 8–12 mm (not 5–7 mm) long, calyx densely white villous (not pubescent), inflorescence bracts spathulate and villous (not ovate to obovate and sparsely pubescent) and capsule longer than the calyx (not shorter than the calyx). It is also similar to *J. championii*, but differs by the whole plant being white villous (not pubescent), and inflorescence bracts spathulate and villous (not obovate-spatulate with apex emarginate and sparsely pubescent).

Perennial herbs, 20–35 cm tall. Stems cylindrical or sometimes quadrangular, base decumbent and usually rooting at nodes then erect, densely white villous. Leaves opposite; petiole 0.8-1.2 cm long, villous; blades ovate, oval to sometimes lanceolate,  $3.5-7.5 \times 1.5-4$  cm, papery, apex shortly caudate or acute, margin usually entire or slightly undulate, base cuneate to shortly attenuate, both surfaces densely white villous, mid-vein and secondary veins prominent on both surfaces, secondary veins (5)

or) 6 on each side of mid-vein, covered with grayish white strip-like cystoliths. Spikes axillary, ca. 1 cm, usually several flowers in a cluster. Inflorescence bracts leaf-like, usually spathulate, rarely obovate,  $7-8.5 \times 4-5$  mm, with a petiole 3-5 mm long, villous, pinnately veined with white strip-like cystoliths, apex round or sometimes obtuse, base decurrent onto petiole. Bracts and bracteoles linear, 1-1.2 mm long, white villous relatively sparsely below the middle. Calyx 7-9 mm, 5-lobed almost to base; lobes linearlanceolate with conspicuous membranous margins, white villous, especially above the middle, apex acuminate. Corolla ca. 9 mm long, outside white villous, tube and upper lip white, lower lip white with purple spots; tube slightly longer than limb; upper lip triangular, minutely 2-lobed; lower lip 3-lobed, lobes imbricate, suborbicular. Stamens 2, attached to corolla tube, exserted; filaments ca. 2.2 cm long, basally villous; anther bithecous, thecae superposed, upper one smaller and muticous, lower one larger and with a white spur at base, anther connective pubescent. Ovary glabrous, green, fusiform with nectary flower disc bowl-shaped at base; style ca. 5 mm long, sparsely villous at base, stigma slightly 2-lobed. Capsule fusiform, 6-7 mm, glabrous, sometimes pilose at the tip. Seeds 4, compressed, ca. 1.1 × 1 mm, somewhat heart-shaped or obovate, brown or vellowish-brown, testa tuberculate.

**Etymology.** The specific epithet "*thailandica*" is derived from Thailand, where the new species is found.

**Phenology.** The new species was recorded in flowering from August to November and fruiting from October to December.

**Distribution and habitat.** *Justicia thailandica* is only known from Thailand (Fig. 4). It grows in the thickets on the limestone hills or evergreen forest at elevations of 400–900 m.

**Conservation status.** Currently, *Justicia thailandica* is only known from seven locations of Thailand with eight collections and the estimated extent of occurrence more than 20000 km<sup>2</sup>. We speculate that it may be widespread in Thailand, but is likely to qualify for a threatened category in the near future due to its vulnerable karst habitat and human activities leading to a decline in such habitats. It is therefore assessed as "Near threatened" (NE) according to the IUCN Red List categories and criteria (IUCN 2017).

Additional specimens examined. THAILAND. Chiang Mai: Chiang Dao District, Doi Chiang Dao, SE foothills near Ban Yang Pong Luang, 575 m alt., 30 Sep 1989, J. F. Maxwell 89-1169 (A, CAS, L). Kanchanaburi: Thong Pha Phum District, Krieng Kwia, 420 m alt., 27 Nov 1982, H. Koyama, H. Terao & T. Wongprasert 30402 (BKF, C, K). Khon Kaen: Phu Khieo Game Reserve, ca. 80 km east of Phetchabun, 16°50'00"N, 101°58'00"E, 850 m alt., 8 Nov 1984, G. Murata, C. Phengklai, S. Mitsuta, T. Yahara, H. Nagumasu & N. Nantasan T-41809 (A, BKF, TI). Loei: Nam Nao National Park, 101°23'00"–28'00"N, 16°48'00"–49'00"E, near check point of road to National Park, 280–350 m alt., 28 Oct 1984, Gen Murata, C. Phengklai, S. Mitsuta, T. Yaahara, G. Nagamasu & N. Nantasan T-51534 (TI); Pha Som Dej-Phataalern, Phu Luang NP, 1000 m alt., 14 Oct 2000, M. Norsaengsri 1075 (QBG). Nakhon Ratchasima: Pak Thong Chai District, Salika Forest, 40 km SE from Pak Thong Chai, 14°40'00"N, 102°2'00"E, 400 m alt., 25 Oct 1971, C. F. van Beusekom, Chan Wid

& R. Geesink 3362 (BKF, C, K, L, MO, P). **Phetchabun**: Nam Nao District, Nam Nao National Park, 25 Dec 1982, H. Koyama, H. Terao & T. Wongprasert T-31662 (BKF); Nam Nao, 24 Aug 2006, P. Chantaranothai et al. s.n. (BKF, QBG); Loam Gow District, Nahaw Now, 900 m alt., 17 Nov 1973, J. F. Maxwell 73-614 (AAU).

# Pollen and seed morphology

Pollen grains of *Justicia thailandica* are 2-colporate, bilaterally symmetrical, elliptic in both polar and equatorial view, polar axis  $[P]=33.0\pm1.55 \mu m$ , equatorial diameter  $[E]=21.6\pm0.85 \mu m$ , P/E=1.52±0.06, aperture area with 2 rows of 6–7 unequal-sized insulae, ornamentation of the insulae microreticulate with few scattered granules between muri (Fig. 3: A–B).

Seeds of *Justicia thailandica* are compressed, somewhat heart-shaped or obovate, brown or yellowish-brown,  $1.1-1.2 \times 1-1.1$  mm, testa densely tuberculate with conspicuous rounded or oblong tubercles, ornamentation of tubercles irregular polygonal (Fig. 3: C–D).



**Figure 3.** Pollen and seed morphology of *Justicia thailandica* under SEM **A** pollen grain in equatorial view **B** exine ornamentation of pollen grain **C** seed **D** seed testa.

## Discussion

Characters of pollen grains and seed testa have proved valuable in sectional delimitation in the genus *Justicia* (Graham 1988). The pollen grains of *Justicia* sect. *Harnieria* are 2-colporate, reticulate exine ornamentation and traversed by 2 rows of unequal-sized insulae (Graham 1988; Hedrén 1989; Hu et al. 2005; Rueangsawang et al. 2013; Tong et al. 2016). Seed testa of *Justicia* sect. *Harnieria* is characterized by rugulose-tuberculate with the apices of the projections pointed (Graham 1988; Hedrén 1989; Rueangsawang et al. 2012; Tong et al. 2016), i.e. "Rugulose-tuberculate" type of Graham (1988). Our observation of the pollen and seed morphology (Fig. 3: A–D) is consistent with that of *Justicia* sect. *Harnieria* (Graham 1988; Hu et al. 2005; Hedrén 1989; Rueangsawang et al. 2012, 2013; Tong et al. 2016) and due to the characters of the abbreviated axillary spikes, leaf-like inflorescence bracts, fusiform capsules, we place the new species into that section.

Only three species of *Justicia* sect. *Harnieria* have been reported from Thailand. Hosseus (1908) reported *J. quadrifaria* from Thailand based on specimen "Hosseus et al. 228". Subsequently, Imlay (1938) added *J. quardrifaria* var. *salicifolia* (T. Anderson) Imlay based on collections "Marcan 1528 and Lakshnakara 772". Rueangsawang (2012) and Rueangsawang et al. (2012) recorded three species based on several specimens quoted in text, viz. *J. quardrifaria, J. neesiana* (Nees) T. Anderson and *J. championii*.

Justicia championii was first recorded in Thailand by Rueangsawang (2012) based on specimens (e.g. Wongprasert et al. 30402, Maxwell 73-614). However, J. championii, is currently known only from China and N Vietnam according to our worldwide specimen examination and is very similar to *J. quadrifaria*, but differs by the leaf shape and may be merged with the latter. After detailed comparison between the type specimen of *J. championii* and some Thai specimens (e.g. van Beusekom et al. 3759, Koyama et al. 30402, van Beusekom et al. 3362) identified in herbaria as I. championii or J. quadrifaria, we found they are obviously different and represent the new species described above. The clearest difference between *J. thailandica* and *I. championii* is that the calyx is densely white villous in *J. thailandica* (not pubescent) and inflorescence bracts are spathulate and villous in J. thailandica (not obovate with apex emarginate and sparsely pubescent). The leaves also tend to be different with the leaf blade ovate and white villous in *J. thailandica* (not lanceolate, oblong to oval and pubescent) with margin usually entire (not slightly undulate), apex shortly caudate or acute (not obtuse), base cuneate (not decurrent), (Fig. 5; Tab. 1). However, these leaf differences represent trends in a spectrum of variation rather than clear discontinuities.

Morphologically, *Justicia thailandica* is also similar to *J. quadrifaria*, but it can be easily distinguished from *J. quadrifaria* by the leaf blade being ovate and white villous (not oval, oblong to rarely ovate and pubescent), margin entire (not slightly undulate), petiole 8–12 mm (not 5–7 mm) long, apex shortly caudate or acute (not acuminate), base cuneate (not decurrent onto the petiole), calyx densely white villous (not pubescent), inflorescence bract spathulate and villous (not ovate to obovate and sparsely pubescent) and mature capsule longer than the calyx (not shorter than the calyx).

*Justicia quadrifaria* was recognized to be widely distributed in the tropical region of S to SE Asia from India, Indochina to Malay Archipelago and South China (Ridley



Figure 4. Distribution map of Justicia thailandica (black circle).

(1923; Hu et al. 2011). However, it is restricted to NE India and Bangladesh based on our worldwide specimen examination. *J. zollingeriana* (Nees) C. B. Clarke was reduced to *J. quadrifaria* or *Calophnodes quadrifaria* by some authors (Clarke 1907; Ridley 1923, Hu et al. 2011). However, it can be easily distinguished from the latter by the plant being glabrous (not pubescent in *J. quadrifaria*), calyx 4–5 mm (not 7–7.3 mm), capsule obviously more than 1.5 times longer than the calyx (not shorter than or subequal to the calyx) and leaves in each pair obviously unequal in size (not equal or subequal). *J. zollingeriana* is distributed in SE Asia from Thai Peninsular to Malay Archipelago. In Thailand, the species was first recorded as a synonym of *J. quadrifaria* by Hosseus (1908) based on specimen "Hosseus et al. 228", and then some specimen (e.g. Kerr 7375, Garrett 316) of the species were reported under *J. quadrifaria* by Imlay (1938), while some specimen (Marcan 1528 and Lakshnakara 772) were under *J. quadrifaria* var. *salicifolia* by Imlay (1938).

*Justicia neesiana* recorded by Rueangsawang (2012) and Rueangsawang et al. (2012) is very similar to *J. multinodis* in the lanceolate leaves, however, it differs from the latter



**Figure 5.** Comparison between *Justicia thailandica, J. quadrifaria, J. championii* and *J. salicifolia* **A** holotype of *Justicia thailandica* (Beusekom *et al.* 3759, MO2366671) **B** isotype of *J. quadrifaria* (Wallich 2479a, GZU000251567) **C** holotype of *J. championii* (Champion 210, K000884038) **D** lectotype of *Justicia barapaniensis* P. Soumya & Sunojk. (a new name (Soumya 2017) for *J. salicifolia* T. Anderson). Hooker & Thomson s.n., K000884122.

by the plant being pubescent (not nearly glabrous in later), leaf  $39-47 \times 5-7.5$  mm (not  $22-37 \times 2.2-4$  mm), base decurrent onto the petiole (not cuneate), lateral leaf vein 5 with veinlet not reticulate (not usually 7 with veinlet obviously reticulate), petiole 5-6 mm (not nearly sessile), axillary spikes usually with 3-5 flowers (not 2-3 flowers) and inflorescence bracts subrotund to oval, persistent (not lanceolate, caducous).

In the course of revising Thailand species of sect. *Harnieria*, we have confirmed there are three species in Thailand, viz. *J. zollingeriana* (Nees) C. B. Clarke, *J. multinodis* R. Benoist and a new species, *J. thailandica*, described here.

A comparison of characters between *Justicia thailandica*, *J. quadrifaria*, *J. championii* and *J. zollingeriana* is provided in Table 1. An identification key to Thai species in sect. *Harnieria* is provided below.

	Justicia thailandica	J. quadrifaria	J. championii	J. zollingeriana
Opposite leaf	equal or subequal	equal or subequal	equal or subequal	obviously unequal
Leaf size	3.5-7.5 × 1.5-4 cm	1-2 × 5.5-6.5 cm	$1-7(-10.5) \times 0.5-$	5–10 × 2–3.5 cm
			2(-3.5) cm	
Leaf shape	ovate, oval to sometimes	oval, oblong to rarely	lanceolate, oblong to	oblong to lanceolate with
	lanceolate with margin	ovate with margin slightly	oval and pubescent with	margin slightly undulate
	entire, slightly undulate	undulate	margin slightly undulate	
Leaf apex	shortly caudate or acute	acuminate	obtuse	acuminate
Leaf base	cuneate or shortly at-	decurrent onto the petiole	decurrent onto the petiole	cuneate
	tenuate			
Petiole length	8-12 mm	5-7 mm	5–15 mm	7-15 mm
Lateral leaf vein	(5)6	7(8)	6(7)	5(6)
Indumentum	densely white villous	pubescent	densely pubescent	glabrous
Inflorescence	spathulate and densely	ovate to obovate and	obovate-spatulate with	spathulate to round and
bracts	villous sparsely pubescent ap		apex emarginate and	glabrous
			sparsely pubescent	
Calyx	7–9 mm, densely white	7–7.3 mm, pubescent	7–9.5 mm, pubescent	4–5 mm, glabrous
	long villous			
Calyx-capsule	0.7-0.82	1.07-1.32	0.89-1.06	0.59-0.67
length ratio				
Flowering	Aug–Nov.	Unknown	Aug–Oct.	Jul-Sep.
Fruiting	Oct–Dec.	Unknown	Aug–Oct.	Jul-Sep.
Distribution	Thailand	India and Bangladesh	South China and north	From Thai Peninsular to
			Vietnam	Malay Archipelago

Table I. Comparison of Justicia thailandica, J. quadrifaria, J. championii and J. zollingeriana.

#### Identification key to Thai species in Justicia sect. Harnieria

1	Mature capsule more than 1.5 times longer than the calyx in length, leaves in
	each pair obviously unequal in size Justicia zollingeriana
_	Mature capsule short than or sub-equal to the calyx, leaves in each pair sub-
	equal in size
2	Inflorescence bract and calyx lobes obviously with dense long villous hairs;
	leaf large, ovate, 3.5–7.5 × 1.5–4 cm
_	Inflorescence bract and calyx lobes nearly glabrous or with sparsely pubescent
	hairs; leaf small, narrowly lanceolate, 3-7 × 0.6-0.9 cmJ. multinodis

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RESEARCH ARTICLE



# Zeylanidium manasiae, a new species of Podostemaceae based on molecular and morphological data from Kerala, India

Remya Krishnan<sup>1</sup>, Priyanka Khanduri<sup>2</sup>, Rajesh Tandon<sup>1</sup>

**1** University of Delhi, Department of Botany, Delhi-110007, India **2** University of Calcutta, Vidyasagar Metropolitan College, Department of Botany, Kolkata-700006, India

Corresponding author: Rajesh Tandon (tandon.raj@gmail.com)

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#### Abstract

We present the description of *Zeylanidium manasiae* (Podostemaceae), a new species from Kerala, India, which is proposed based on molecular, macro- and micromorphological data. This species is characterised by its ribbon-like dichotomous thallus, floriferous shoots produced along the margins and dichotomy of the thallus, inflorescence with two bracts, unequal stigmatic lobes, ellipsoid fruits and large seeds.

#### **Keywords**

Internal transcribed spacer, Malpighiales, Podostemoideae, rheophyte, taxonomy

# Introduction

Podostemaceae represents a very distinct family of fresh water aquatic angiosperms, with unique evolutionary, ecological, morphological, developmental and embryological attributes (Cook and Rutishauser 2007, Katayama et al. 2016, Khanduri et al. 2014). It is the most diverse family of fresh water aquatic flowering plants, comprising ca. 54 genera and ca. 300 species (Koi et al. 2012, Cheek et al. 2017) distributed worldwide, but with most species presenting restricted distribution and a high degree of endemism (Philbrick et al. 2010). Podostemaceae is subdivided into three monophyletic subfamilies: Podostemoideae, Tristichoideae and Weddellinoideae (monogeneric and monospecific) (Koi et al. 2012). Southern Asia is one of the main centres of diversity for the podostemads, accounting for 17 genera and 80 species from the region (Kato 2016). India harbours 28 species of Podostemaceae, in which 23 are endemics (Khanduri et al. 2014).

The genus, *Zeylanidium* (Tul.) Engl. (subfamily Podostemoideae), is characterised by plants with crustose or ribbon-shaped thalli and caducous leaves. The flowering shoots in these species may be located either in the sinuses of the thallus lobes or borne randomly on the dorsal surface of the thallus (Mathew and Satheesh 1997). Each shoot bears a solitary, terminal and bracteate flower. The other key features include persistent spathella, anisolobous ovarian locules and many seeded capsules (Kato and Koi 2018).

*Zeylanidium* is currently represented by seven species: *Z. olivaceum* Engl., *Z. johnsonii* Engl., *Z. lichenoides* Engl., *Z. maheshwarii* C.J.Mathew & V.K.Satheesh, *Z. sessile* (Willis) C.D.K.Cook & Rutish, *Z. crustaceum* M.Kato and the recently described *Z. tailichenoide* M.Kato & Koi (Mathew and Satheesh 1996, Cook and Rutishauser 2001, Kato et al. 2015, Kato and Koi 2018). Out of these seven species, the status of *Z. johnsonii* Engl. is doubtful as it has never been reported by any study after Engler's (1930) description. All species are confined to peninsular India, Sri Lanka, Myanmar and Thailand (Mathew and Satheesh 1997, Kato et al. 2015, Kato and Koi 2018). The taxonomic delimitation of the genus is still under dispute. Two species, originally described under the New World genus *Podostemum*, were transferred to *Zeylanidium* by Cusset (1992) as *Z. barberi* (Willis) C. Cusset and *Z. subulatum* (Gardn.) C. Cusset. However, a recent combined morphological and molecular phylogenetic analysis does not support these new combinations (Khanduri et al. 2014). Therefore, these species are here excluded from *Zeylanidium* and it is our opinion that their generic placement requires further studies (pers. observ.).

During field studies in the riverine areas of Kerala, India, between 2014 and 2016, a new morphological variant of *Zeylanidium* was found. The ribbon-shaped specimens with solitary flowering shoots appeared morphologically similar to *Z. lichenoides*, but the fruits were remarkably distinct in size and shape. Detailed morphological and molecular studies revealed that the specimens were different from the remaining species on several other characters. Hence, the specimens are documented and described here as a new species, *Zeylanidium manasiae*. A detailed description with photographic documentation, illustrations, phylogenetic placement within Podostemaceae and an identification key are provided.

## Material and methods

#### Morphology

Plant specimens were collected from Thommenkuthu waterfalls, Thodupuzha, Idukki, Kerala, India (9°57'21.59"N 76°50'01.87"E, Fig. 1). Collections were made from various spots at the rapids for three consecutive years (2014–2016). Voucher specimens have been deposited at the Delhi University Herbarium (DUH), Department of Botany, University of Delhi and Calicut University Herbarium (CALI). The morphological details



**Figure 1.** Distribution map of *Zeylandium manasiae* (red dot). Thommenkuthu Waterfalls, Idukki, Kerala (Map constructed using ArcGIS 9.2 software).

of the plants were recorded in the field and documented photographically. Morphometric details of randomly collected plants (N=30) were measured by using a digital Vernier calliper and calibrated ocular micrometre. The specimens were compared with other members of *Zeylanidium* either by using preserved materials from our spirit collections [i.e. *Z. olivaceum*, *Z. lichenoides*, *Z. sessile* and *Z. maheshwarii* (Suppl. material 1)] or information available in literature (i.e. *Z. johnsonii*, *Z. crustaceum* and *Z. tailichenoides*; Mathew and Satheesh 1997, Suzuki et al. 2002, Kato et al. 2015, Kato and Koi 2018).

The differences between the taxa were compiled and are presented below (Table 1, Suppl. material 2). The terminology for vegetative and floral characters follows Mathew and Satheesh (1997), Marinho et al. (2014) and Jäger-Zürn (2003). The distribution map was constructed using ArcGIS 9.2 version (Zhan and Huang 2004).

# Anatomy

For anatomical details, flower buds of desired specimens were fixed in Karnowsky's fixative (Karnowsky 1965) and then processed to prepare resin blocks for sectioning (Feder and O'Brien 1968). Semi-thin sections (4 and 5  $\mu$ m) were obtained with the help of a rotary microtome, stained with 0.1% toluidine blue O' (pH 4.4) and mounted in DPX (O'Brien and McCully 1981). The observations were recorded with the help of a photo microscope (Carl Zeiss, Axio scope A1) with an attached digital camera (Axiocam).

# Scanning electron microscopy

For palynological and seed micromorphological studies, anthers and seeds, respectively, were fixed in Karnowsky's fixative, dehydrated in a graded series of cold acetone (10–100%, 30 min interval each), critical point dried (CPD), mounted on aluminium stubs and coated with gold-palladium alloy before making observations. The samples were examined by using a scanning electron microscope (SEM, JEOL, JSM-6610LV) at the Department of Botany, University of Delhi, India.

# DNA extraction, amplification and sequencing

Genomic DNA was extracted using DNeasy plant mini kit (Qiagen, Amsterdam, Netherlands). DNA amplification and sequencing of the entire ITS region (ITS1, 5.8S and ITS2) were performed using the primers ITS 1 and ITS 2 (White et al. 1990). The polymerase chain reaction (PCR) was executed using standard protocol with one unit of *itaq* (Taq Intron, Intron Biotechnology Inc.), 2.5  $\mu$ l of 10 X buffer, 2.5  $\mu$ l dNTPs, 1  $\mu$ l of 10 pM solution of each primer, 1  $\mu$ l of genomic DNA and 16.7  $\mu$ l distilled water. PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN) and the purified product was ligated into a pGEM-T vector (Promega, USA). The ligated mix was transformed using competent *E. coli* DH5 $\alpha$  strain. The blue-white selection method was employed for transformation (Sambrook and Russell 2001). Three clones per PCR product were sequenced at SciGenome Labs Pvt. Ltd. (Cochin, India). Contigs were assembled using DNA star Laser gene version 5.07 software (Burland 2000). Nucleotide BLAST was performed to estimate sequence similarity by using the acquired nucleotide sequence as the query. The sequences have been submitted in the GenBank (Suppl. material 3).

# Taxon sampling

ITS sequences of *Z. manasiae* and *Z. maheshwarii* were added to a dataset consisting of 39 species of Podostemaceae, produced by Khanduri et al. (2014). *Hypericum perforatum* L. and *Hypericum kouytchense* H.Lév. were included as outgroups based on the results of Ruhfel et al. (2011). The final data matrix comprised of a total of 43 accessions, representing 41 species of Podostemaceae and the two outgroups. Out of the seven species of *Zeylanidium*, five were included in the present phylogenetic analysis.

# Phylogenetic analysis

ITS sequences of all the taxa were aligned using ClustalX ver. 2.0.11 (Thompson et al. 1997) and checked manually using ClustalW (Thompson et al. 1994). Phylogenetic reconstruction was carried out using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) with the best sequence evolution model i.e. JC model under Model Test version 0.1.1 (Guindon and Gascuel 2003). Analyses were run for 1,300,000 generations until stationarity (standard deviation < 0.01). In each run, trees were sampled after every 100 generations with a sample frequency of 10. All the parameters were summarised after excluding 25% of the samples (burn-in fraction), based on the inspection of log-likelihoods of sampled trees. The summary table provides mean and mode with 95% credibility interval. The potential scale reduction factor approached 1.0 for all the parameters. Branch length information was recorded and averaged across all the retained trees and a majority rule consensus tree was computed to obtain the posterior probabilities (PP). Trees were summarised by the sump burn-in command yielding a cladogram showing PP, clade credibility for each split and a phylogram with mean branch lengths. The values between 0.95 and 1.0 were only taken into consideration for Bayesian analysis.

# Results

# Molecular analyses

DNA sequencing of the ITS region of *Z. manasiae* generated a sequence with 907 bp. This sequence aligned in the genus *Zeylanidium*, confirming its generic identity (Fig. 5). Sequence alignment of different species of *Zeylanidium* showed *Z. manasiae* to be significantly different from the other species, thereby confirms its distinction as a new species. The species was well-nested in *Zeylanidium*. The *Zeylanidium* clade was found to be sister to *Polypleurum*, which is in congruence with earlier molecular studies (Koi et al. 2012, Khanduri et al. 2014). Phylogenetic analysis revealed that all the studied species of *Zeylanidium* grouped together with the exception of *Z. sessile*, which is more closely associated with species of *Polypleurum* (Tul.) Warm. Within the major group,

there were three subgroups; one comprising Z. lichenoides and Z. maheshwarii as sister species (0.63 PP) and the other two consisting of Z. manasiae and Z. olivaceum which is supported by Bayesian posterior probability of 1.00. Zeylanidium sessile resides alone outside the Zeylanidium clade (1.00 PP). We feel that analysis with the addition of Z. johnsonii, Z. crustaceum and Z. tailichenoides might resolve the clade further. All the other major clades including Polypleurum were well-supported and are consistent with the earlier phylogenetic analysis (Khanduri et al. 2014).

#### Taxonomy

## Zeylanidium manasiae R.Krishnan, P.Khanduri & R.Tandon, sp. nov.

urn:lsid:ipni.org:names:60478968-2 Figs 2, 3, 4

**Diagnosis.** It can be distinguished from the closely related *Z. lichenoides* by the position of floriferous shoots along the margins of thallus, two bracts per floriferous shoot, unequal stigmatic lobes, larger fruits, ellipsoidal capsule and larger seeds.

**Type.** INDIA. Kerala: Idduki district, Thommenkuthu Waterfalls, River Kaliyar, 9°57'21.59"N 76°50'01.87"E, 64 m alt., 31 Dec 2015, *R. Krishnan & P. Khanduri* 8010 (holotype: DUH accession no. 14378!; isotypes: CALI accession no. 7000!, DUH accession no. 14379!)

**Description.** Herbs rheophtytic, annual. Thallus  $3.79 \pm 0.44$  mm wide, green to yellow, ribbon-shaped, dorsiventrally flattened, dichotomously branched, attached to the substrate by disc-shaped haptera,  $1.14 \pm 0.92$  mm diam. Leaves produced at the margins and sinuses/branch points of the thallus, in pairs, caducous; blades 2.5–6.75  $\times$  0.26  $\pm$ 0.03 mm, subulate, flattened, lacking a midrib. Floriferous shoots produced both marginally and at the branch points of the thallus, solitary, horizontally appressed to the thallus, composed of 2 subulate bracts subtending a single flower, successive shoots  $3.56 \pm 0.87$ mm apart; bracts 2.25-8.20 mm long, caducous, with long caducous apices. Spathella  $1.98 \pm 0.30$  mm long, obovoid, membranous, non-vascularised, persistent, enveloping the flower at pre-anthesis, rupturing longitudinally or irregularly at anthesis. Flowers green, bisexual, zygomorphic, achlamydeous, erect; pedicel measuring  $8.32 \pm 2.32$  mm long in a mature flower; tepals 2, one on either side of the andropodium,  $0.83 \pm 0.21$  mm long, filiform; stamens 2, borne on an andropodium,  $0.74 \pm 0.15$  mm long at anthesis, elongating to  $3.67 \pm 0.74$  mm long at post-anthesis, branched approximately <sup>1</sup>/<sub>4</sub> from the apex, each branch measuring  $0.13 \pm 0.04$  mm long at anthesis, elongating to  $0.80 \pm$ 0.10 mm long at post-anthesis, anthers  $0.50 \pm 0.06 \times 0.37 \pm 0.12$  mm, quadrangular, base bilobed, lobes subequal, dehiscence introrsely rimose;  $4273 \pm 941$  pollen dyads per flower,  $30.25 \pm 2.42 \times 19.62 \pm 1.99 \,\mu\text{m}$ , tricolpate, microechinate; gynoecium bicarpellate, syncarpic, ovary 2.07 ± 0.28 mm long, ellipsoidal, anisolobous, membranous septum separating two unequal locules, ovules  $78 \pm 14$ , anatropous, borne on a swollen axile placenta, style absent, stigma bifid, stigmatic lobes unequal, subconical, the longer  $0.48\pm$  $0.06 \times 0.10 \pm 0.01$  mm, the shorter  $0.42 \pm 0.06 \times 0.08 \pm 0.01$  mm. Capsule dehiscent,



**Figure 2.** *Zeylandium manasiae* **A** habit showing mature fruits **B** floral bud enclosed in spathella and subtended by two bracts **C** flower showing andropodium, tepals and unequal stigmatic lobes **D** longitudinal section of the ovary, showing unequal locules **E** capsule. Illustration by Rajesh Tandon.

loculicidal capsule measuring  $1.96 \pm 0.25 \times 0.84 \pm 0.10$  mm and pedicel elongates to  $15.55 \pm 2.21$  mm, bivalved, brown, ellipsoidal, longitudinally ridged, ridges 6, 3 on each valve, one valve persistent, the other deciduous. Seeds  $60 \pm 15.50$  per capsule,  $248.75 \pm 12.70 \times 136 \pm 8.90$  µm, spermoderm reticulate, cells rectangular with wavy striations.

**Anatomy.** Floral parts of *Z. manasiae* were anatomically investigated. The spathella is non-vascularised and consists of thick-walled polygonal cells. The anthers are of bithecous type, have secretory tapetum and each locule contains pollen dyads. The ovary is plurio-vulate and divided into two unequal locules by an apical septum. The ovules are anatropous, bitegmic and tenuinucellate that are borne on a bulbous axile placenta (Fig. 2H).

**Palynology.** The dyads of *Z. manasiae* are of the acalymmate type and measure  $30.25 \pm 2.41 \,\mu\text{m}$  in length and  $19.62 \pm 1.99 \,\mu\text{m}$  in width. Individual pollen grains



**Figure 3.** Zeylandium manasiae A habitat and habit showing plants on exposed rock surface (arrows) **B** habit of the plant showing solitary horizontally appressed flowering shoots **C** ventral surface of the thalli with haptera (arrows) **D** thalli bearing floriferous shoots on margins and point of branching **E** a young flower bud covered by bracts **F** floriferous shoot with flower subtended by two bracts. A pair of leaves can also be seen (arrows) **G** flower with spathella removed showing an anther and two tepals (arrows) **H** longitudinal section of floral bud enclosed in a spathella (sp). The ovary is bilocular and divided into two unequal halves by an apical septum (arrows). Numerous anatropous ovules (ov) are borne on a swollen placenta (p). One of the anthers in section shows a copious amount of dyad pollen **I** forked andropodium with two anthers **J** a mature capsule **K** a dehisced capsule showing persistent valve **L** comparative fruit morphology of congenerics. (Left to right) *Z. maheshwarii, Z. lichenoides, Z. olivaceum* and *Z. manasiae* **M** Comparative morphology of stigma. (Left to right) *Z. maheshwarii, Z. lichenoides, J. (D)* a flower (D) and (D) an



**Figure 4.** Scanning electron micrographs **A** seed with reticulate spermoderm pattern **B** dyad pollen with micro-echinate ornamentation over the apertural (ap) and non apertural regions (np). Scale bars: 50  $\mu$ m (**A**); 5  $\mu$ m (**B**).

are sub-prolate in shape with tricolpate aperture. The exine wall has microechinate ornamentation. The echinations on the apertural surface are larger than those on the non-apertural surface (Fig. 3B).

Additional specimens seen (paratypes). INDIA. Kerala: Idduki district, Thommenkuthu Waterfalls, River Kaliyar, 9°54'00"N 76°46'00"E, 64 m alt., 23 Dec 2016, *R. Krishnan* 8080, (DUH accession no. 14380!). The same locality, 25 Dec 2016, *R. Krishnan* 8081 (DUH accession no.14381!).

**Etymology.** The specific epithet '*manasiae*' honours the late Dr. Manasi Ram née Ghosh for her contributions to the study of embryology and systematics of Santalaceae (Ghosh 1956) and *Trapa* L. (Lythraceae; Ghosh 1954).

**Distribution and ecology.** Zeylanidium manasiae is highly endemic and is known from only one location so far, i.e. Thommenkuthu waterfalls (Figs 1 and 3A). According to a previous report, rocks of this waterfall are hornblende biotite gneiss type (Girija 2008). According to Mathew and Satheesh (1996), water in Kaliyar River is well-oxygenated hard water with low chloride content. Zeylanidium manasiae grows along with Z. lichenoides and Z. sessile. Diatoms (Cymbella C.Agardh species) were also observed forming colonies on the spathella of some of the plants.

**Conservation status.** This species is currently known to occur from a single location in Kerala and, hence, we suggest its placement in the Data Deficient category of IUCN (2017).

**Phenology.** Flowering and fruiting occurs from December to January when the water level recedes to partly expose the rocks.

# Discussion

Zeylanidium manasiae is a ribbon-shaped, dichotomously branched species, which produces leaves and flowering shoots at the margins and sinuses of the thallus. Based on morphological studies, its closest relative is *Z. lichenoides*, which is also a ribbon-shaped species. However, *Z. manasiae* can be easily distinguished from it on the basis of a number of characters (Table 1): (i) In *Z. manasiae*, leaves and floriferous shoots are present along the margins and sinuses of the thallus (Figs 2A, 3B and D), while in *Z. lichenoides*, they are borne only in the sinuses of the thallus lobes, never along the margins; (ii) There are only 2 bracts per floriferous shoot in *Z. manasiae* (Figs 2C and 3F), while *Z. lichenoides* has 4–6 bracts per floriferous shoot; (iii) *Z. manasiae* has two unequal stigmatic lobes (Figs 2E and 3M), in contrast to *Z. lichenoides*, which has equal lobes; (iv) *Z. manasiae* has comparatively larger fruits and seeds (*Z. manasiae*, capsules  $1.96 \pm 0.25 \times 0.84 \pm 0.10$  mm, seeds  $248.75 \pm 12.70 \times 136 \pm 8.90 \ \mu m$  vs. *Z. lichenoides*, capsules  $1.20 \pm 0.20 \times 0.80 \pm 0.13$ mm, seeds  $207 \pm 1.70 \times 108 \pm 1.20 \ \mu m$ ) (Figs 2B, 3J and L; Fig 4A); and (v) Capsules are ellipsoidal in *Z. manasiae*, whereas they are globose in *Z. lichenoides* (Figs 2B, 3J and L).

Zeylanidium tailichenoides and Z. sessile, the other two ribbon-shaped species in the genus, can be easily distinguished from Z. manasiae on the basis of (i) unilocular ovary in Z. tailichenoides vs. bilocular ovary in Z. manasiae; and (ii) sessile flowers and smooth capsules in Z. sessile vs. pedunculate flowers and ribbed capsules in Z. manasiae. The remaining congenerics (i.e. Z. olivaceum, Z. maheshwarii, Z. johnsonii and Z. crustaceum) have crustose thallus with leaves and flowering shoots scattered on the dorsal surface and, hence, are distinct from Z. manasiae.

Palynological studies also revealed the presence of tricolpate apertures with microechinate exine ornamentation. These characters are similar to the other *Zeylanidium* species, which confirms its generic placement. The structure of the pistil further supports generic identity of the species, since the anisolobous ovary is a characteristic feature of *Zeylanidium*. This characteristic feature separates the genus from *Polypleurum*.

Characters	Z. manasiae	Z. lichenoides	
Flower length excluding pedicel (mm)	$2.38 \pm 0.47$	$1.84 \pm 0.10$	
Anther length (mm)	$0.50 \pm 0.06 \times 0.37 \pm 0.05$	$0.4 \pm 0.16 \times 0.47 \pm 0.02$	
Pollen production per flower	4273 ± 941	$4363 \pm 92$	
Pollen size			
Polar diameter of the dyad (µm)	30.25 ± 2.41	32.08 ± 1.42	
Equatorial diameter of the dyad (µm)	$19.62 \pm 1.99$	$21.01 \pm 1.54$	
Shape of the pollen	Sub-prolate	Sub-prolate	
Ovule production per flower	78 ± 13.70	59 ± 9.27	
Pollen: Ovule ratio	55:1	74:1	
Capsule size (mm)	$1.96 \pm 0.25 \times 0.84 \pm 0.10$	$1.2 \pm 0.20 \times 0.8 \pm 0.13$	
Seed number per fruit	$60.1 \pm 15.63$	49 ± 15	
Seed size (µm)	248.75 ± 12.70 × 136 ± 8.90	$207 \pm 1.70 \times 108 \pm 1.20$	
Ovule:seed ratio	1.30	1.20	

Table 1. Morphometric differences between Zeylanidium lichenoides and Z. manasiae.



**Figure 5.** Phylogram of the consensus tree obtained by the Bayesian inference in Mr Bayes. *Zeylanidium* clade has been highlighted. Numbers above the branches indicate values of posterior probabilities.

Molecular phylogenetic analysis places *Z. manasiae* in a clade of *Zeylanidium* members which includes *Z. olivaceum*, *Z. maheshwarii* and *Z. lichenoides*. This corroborates the morphological studies done in the present work. The *Zeylanidium* clade is sister to *Polypleurum* within the subfamily of Podostemoideae.

# Key to the species of Zeylanidium s.l.

1	Thallus ribbon-like; shoots at the sinuses of the thallus or along the margins of the
	thallus
_	Thallus crustose; shoots scattered on the dorsal surface of the thallus
2	Flowers sessile; spathella apex round; capsule smooth
_	Flowers pedicellate; spathella apex acute or obtuse; capsules ribbed
3	Shoots present along the margins and sinuses of the thallus; bracts 2 per floriferous
	shoot; spathella apex obtuse; stigmatic lobes unequal; capsules ellipsoidalZ. manasiae
_	Shoots restricted to the sinuses of thallus; bracts 4-6 per floriferous shoot; spathel-
	la apex acute; stigmatic lobes equal; capsules globose
4	Spathella apex papillate; ovary 2-locular
_	Spathella apex smooth; ovary 1-locularZ. tailichenoides
5	Shoots dimorphic; primary shoot with a tuft of over 20 leaves; secondary shoots
	with 4–6 leaves
_	Shoots monomorphic with 4–6 leaves
6	Leaves up to 10 cm long; bracts 3-4; gynophore absent
_	Leaves 3–5 mm long; bracts more than 4; gynophore present7
7	Bracts 4-6; stigma bilobed; capsules 8-ribbedZ. crustaceum
_	Bracts 6-8; stigma multilobed; capsules 6-ribbed

# Conclusion

Based on the evidence drawn from the present work, it is clear that *Z. manasiae* should be recognised as a new species of *Zeylanidium*. The recognition of *Z. manasiae* brings the total number of *Zeylanidium* species to eight. The finding of new species of *Zeylanidium* indicates that the region is splendidly diverse but remains poorly explored.

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#### Supplementary material I

#### Other examined material

Authors: Remya Krishnan, Priyanka Khanduri, Rajesh Tandon

Data type: species data

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#### Supplementary material 2

#### Comparative morphology of species of Zeylanidium s.l.

Authors: Remya Krishnan, Priyanka Khanduri, Rajesh Tandon Data type: measurement

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#### Supplementary material 3

## Source locality, Herbarium Vouchers and GenBank accession numbers of newly incorporated species in the analysis

Authors: Remya Krishnan, Priyanka Khanduri, Rajesh Tandon

Data type: species data

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RESEARCH ARTICLE



### Solanum plastisexum, an enigmatic new bush tomato from the Australian Monsoon Tropics exhibiting breeding system fluidity

Angela J. McDonnell<sup>1</sup>, Heather B. Wetreich<sup>1</sup>, Jason T. Cantley<sup>2</sup>, Peter Jobson<sup>3</sup>, Christopher T. Martine<sup>1</sup>

I Department of Biology, Bucknell University, 1 Dent Drive, Lewisburg, PA, USA 2 Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA, USA 3 Northern Territory Herbarium, Alice Springs, Department of Environment and Natural Resources, Alice Springs, Northern Territory, 0870, Australia

Corresponding author: Christopher T. Martine (chris.martine@bucknell.edu)

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#### Abstract

A bush tomato that has evaded classification by solanologists for decades has been identified and is described as a new species belonging to the Australian "Solanum dioicum group" of the Ord Victoria Plain biogeographic region in the monsoon tropics of the Northern Territory. Although now recognised to be andromonoecious, S. plastisexum Martine & McDonnell, **sp. nov.** exhibits multiple reproductive phenotypes, with solitary perfect flowers, a few staminate flowers or with cymes composed of a basal hermaphrodite and an extended rachis of several to many staminate flowers. When in fruit, the distal rachis may abcise and drop. A member of Solanum subgenus Leptostemonum, Solanum plastisexum is allied to the S. eburneum Symon species group. Morphometric analyses presented here reveal that S. plastisexum differs statistically from all of its closest relatives including S. eburneum, S. diversiflorum F. Meull., S. jobsonii Martine, J.Cantley & L.M.Lacey, S. succosum A.R.Bean & Albr. and S. watneyi Martine & Frawley in both reproductive and vegetative characters. We present evidence supporting the recognition of S. plastisexum as a distinctive entity, a description of the species, representative photographs, a map showing the distribution of members of the S. eburneum species group and a key to the andromonoecious Solanum species of the Northern Territory of Australia. This new species is apparently labile in its reproductive expression, lending to its epithet, and is a model for the sort of sexual fluidity that is present throughout the plant kingdom.

#### **Keywords**

New species, andromonoecy, Solanaceae, Leptostemonum

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#### Introduction

As one of the most species-rich angiosperm genera (Frodin 2004), *Solanum* L. is also fast growing, with more than 90 species described within the last decade alone (e.g. Gouvêa et al. 2019; Gouvêa et al. 2018; Agra 2008; Stehmann and Moreira 2016). Members of the genus occupy all continents except Antarctica and inhabit diverse niches. The "spiny solanums," *Solanum* subgenus *Leptostemonum* (Dunal) Bitter, make up the largest lineage (ca. 450 spp.) within the genus and have been the subject of much recent work related to their evolution, diversity and natural history (e.g. Bohs 2005; Levin et al. 2006; Anderson et al. 2007; Stern et al. 2011; Särkinen et al. 2013; Vorontsova et al. 2013; Vorontsova and Knapp 2016; Knapp et al. 2017; Aubriot et al. 2016; Martine et al. 2019).

The spiny solanums appear to have arrived in Australia some time in the last 5-10 million years (Särkinen et al. 2013) and have continued to radiate throughout the continent with a large portion of the currently known species diversity occurring in the upper third of the continent known as the Australian Monsoon Tropics (AMT) (Bean 2004). While this radiation is reflected in diverse plant morphologies (e.g. foliage, armature, trichomes/indument, growth form, seasonal habit), the most interesting variety in form may be related to reproductive biology (Symon 1970; Symon 1979a; Symon 1979b; Symon, 1981; Anderson and Symon 1988; Anderson and Symon 1989; Martine and Anderson 2007). In particular, the spiny solanums of the AMT exhibit three primary breeding systems (breeding system used here sensu Neal and Anderson 2005): 1) hermaphroditic species with perfect flowers (i.e. S. quadriloculatum F.Muell. and approximately 10 other species), 2) cryptically dioecious species that bear functional pollen and functional gynoecia on separate individuals (i.e. S. dioicum W.Fitzg. and approximately 12 other species) and 3) andromonoecious species with inflorescences bearing one perfect flower at the base of each inflorescence and several to many staminate flowers above it (i.e. S. chippendalei Symon and about 13 other species). Our research team has recently been interested in the evolution of the andromonoecious taxa and, through field and populationlevel genomic study (in prep), has recognised a new species that is evolutionarily and morphologically distinctive.

The new taxon is the latest in a series of novelties from a set of taxa and forms within the "andromonoecious bush tomato" clade (Martine et al. 2006; Martine et al. 2009) recently described from the region (e.g. Bean and Albrecht 2008; Martine et al. 2016; Lacey et al. 2017) and part of a larger lineage of andromonoecious, hermaphrodite and functionally dioecious species in the "*S. dioicum* + *S. echinatum* Group" (*sensu* Martine et al. 2019). Across the southern margin of the AMT, where many of these taxa are distributed, mosaic habitats and corresponding environmental pressures, coupled with climatic fluctuations over the last two million years (Bowman et al. 2010), appear to have driven speciation within *Solanum* and other plant lineages of the region (Edwards et al. 2017; Edwards et al. 2018; Jobson et al. in prep; Martine et al. unpublished data).

In this paper, we describe *Solanum plastisexum* Martine & McDonnell, sp. nov., a new species restricted to a small area in the central region of the Northern Territory of Australia that has confounded field botanists since at least the early 1970s. The few historical collections made of this taxon were especially confusing to *Solanum* experts (Fig. 1) because plants may lack staminate flowers and/or the upper staminate rachis (typical for andromonoecious species) and it is often deciduous at fruit maturity. Recognition of this new species is supported by a suite of morphological characters including the lack of lobing on the leaves, a small apical leaf size and long-triangular calyx lobes on the staminate flowers. We include a morphometric comparison amongst closely-allied taxa, representative photographs, a distribution map and a key to the andromonoecious taxa of the Northern Territory along with the description of the new species.

#### Materials and methods

Fieldwork in Northern Territory during 2016 facilitated collection of specimens with male flowers and tissue for population genomic study (in prep), which has revealed that this new entity is an independently evolving lineage (unpublished data). The same population was visited again in 2018 and facilitated the collection of specimens with complete andromonoecious inflorescences (including both male and hermaphrodite flowers) and mature fruits with viable seeds, as well as information about population size, extent and local ecology. Specimens were examined from BUPL, DNA and NT (herbarium acronyms follow Index Herbariorum; Thiers 2019). We consulted herbarium records with images via the Australasian Virtual Herbarium website (https://avh.chah.org.au/) and physically consulted specimens at the Northern Territory Herbarium in Darwin (DNA).

Field-collected seeds from two subpopulations were cultivated ex situ. First, seeds were soaked for 24 hours in 1000 ppm gibberellic acid solution in the dark at room temperature. Seeds were then sown in a growth chamber that was programmed to mimic an AMT climate and light regime at Bucknell University (Pennsylvania, USA) for approximately one month. Following successful growth, plants were cultivated in an IPM-managed greenhouse. Twenty-four vegetative and reproductive characters were measured from herbarium specimens and living plants. Characters were compared amongst six species that form a monophyletic group based on a recent phylogenetic and phylogenomic study (Martine et al. 2019; Martine et al. unpublished data): S. diversiflorum F.Meull., S. eburneum Symon, S. jobsonii Martine, J.Cantley & L.M.Lacey, S. succosum A.R.Bean & Albr., S. watneyi Martine & Frawley and this new species (Fig. 2). Some of the data for S. eburneum, S. watneyi and S. jobsonii have been published previously (Martine et al. 2016; Lacey et al. 2017). Those data were supplemented and measurements from the new species and S. succosum were newly collected as a part of this study. All characters were measured on mature plants and/or specimens collected from mature plants that included fully expanded apical and basal leaves; apical refers to expanded leaves near tips of growing stems while basal refers to expanded leaves on lower parts of stems.

Comparison of characters was conducted using JMP Pro 12 (SAS Institute, Inc., Cary, North Carolina, USA). Analyses included one-way ANOVA with Student's t-test mean comparison at P < 0.05 and all the pairs by Tukey HSD to compare means and discern which species are different and in what way. A Connecting Letters Report was also generated to summarise mean values of each character across the six taxa included and to determine and assign significantly different sets when applicable. Multivariate morphometric analysis for all six taxa was also conducted using a principal components analysis (PCA) to place morphological variation in a spatial context.

#### Results

ANOVA comparisons of each character along with Student's t-tests and the Tukey HSD post-hoc comparisons reveal that species of this complex are, in large part, morphologically distinct (Table 1). Each comparison was significant and the Connecting Letters Reports reveal which sets exist for each character. Three statistically significant characters distinguish *S. plastisexum* from its closest relatives: depth of lobing on the margins of the basal leaves, surface area of the apical leaves and calyx lobe length on staminate flowers. *Solanum plastisexum* leaves are essentially unlobed, it has apical leaves that have a surface area of less than 7 cm<sup>2</sup> and the calyx lobes on male flowers are greater than 1.5 cm long. Other traits that distinguish *S. plastisexum*, although not statistically significant for all comparisons, are plants that are relatively tall (70 cm) due to the erect nature of the stems, inflorescences and leaves and the essentially unlobed apical leaves.

The PCA score plot includes all measured characters and supports the relative distinctive nature of *S. plastisexum* when compared to the other taxa sampled. The analysis identified six eigenvalues above 1.0, which reveals that our dataset is roughly six-dimensional with principal components 1 and 2 contributing most of the variation amongst the points (47.8%). Figure 3 shows the score plot with each data point plotted along with the loading plot and shows which characters had the greatest weight in placing each of the points on the PCA.

#### Taxonomic treatment

#### Solanum plastisexum Martine & McDonnell, sp. nov.

urn:lsid:ipni.org:names:77198273-1 Figs 1, 2D, 5

**Diagnosis.** Like *Solanum eburneum*, *Solanum watneyi* and *Solanum succosum*, but differing by having elliptic, unlobed (or rarely very shallowly lobed) leaves, small apical leaves, long calyx lobes on the staminate flowers and fully erect staminate inflorescence branches.

Type. AUSTRALIA. Northern Territory: ~42 km E of Top Springs, on and around the Buchanan Highway, 16°42.274'S, 132°07.446'E, elev. 286 m, 23 May

Table 1. Vegetative and reproductive characters measured for species included in this study along with associated means (M), standard deviations (SD), sample sizes
(n) and connecting letters reports (CL). Different letters in the CL for each character indicates distinctions between species; species not sharing the same letter in a row
are significantly different for that character (p < 0.5). All measurements in cm, except for seeds per fruit (n), seed length (mm), fruit wall width (mm), surface areas
(cm <sup>2</sup> ) and trichome densities (per 0.5 cm <sup>2</sup> ). The term apical refers to expanded leaves near tips of growing stems, while the term basal refers to expanded leaves on
lower parts of the stems. Connecting letters values in bold text for S. plastisexum indicate characters that statistically differentiate the species from its closest relatives.
S. diversiflorum S. eburneum S. jobsonii S. succosum S. watneyi S. sp. nov.

	S. di	iversifl	orum		S. e.	numeun	u		S. jobs	onii		,	S. succos	um		- 3	S. watn	zyi			S. sp. n	.vo	
Character	Μ	SD	a	С	Μ	SD	0	LM	SD	u	CL	Μ	SD	u	Б	Μ	SD	q	CL	Μ	SD	u	cL
Stem prickle length	0.22	0.09	30	D	0.4	0.1 1	6 A	A 0.29	) 0.12	54	BC	0.37	0.07	20	AB	0.26	0.1	24	CD	0.38	0.12	25	А
Internode length	2.23	0.91	30	В	2.16	0.67	6 B	3 1.48	3 0.51	54	υ	3.6	0.73	25	A	4.01	0.98	24	Α	1.48	0.43	25	C
Petiole length	1.03	0.53	30	D	2.68	0.8	6 E	3 0.82	2 0.39	54	Ω	1.78	0.44	25	U	3.36	0.78	24	Α	0.79	0.32	25	D
Apical leaf length	2.66	0.77	25	C	1.32	1.83	.6 A	1 5.39	9 1.33	25	В	5.13	1.09	25	В	12.39	2.51	24	Α	4.97	0.89	25	В
Apical leaf width	1.61	0.41	25	В	1.41	1.41	6 B(	C 1.6	9 1.13	25	В	1.84	0.53	25	В	2.47	0.65	24	Α	1.06	0.22	25	U
Basal leaf length	5.80	1.98	25	D	13.66	2.66 ]	6 B	9.4	2 1.84	25	υ	9.23	2.27	25	U	16.8	3.86	24	Α	8.58	1.37	25	C
Basal leaf width	3.24	0.91	25	U	2.03	0.88	6 L	(4.9	7 1.54	25	Α	4.56	1.17	25	AB	3.97	1.16	24	BC	1.59	0.37	25	D
Trichome density, adaxial, apical leaves	81.6	48.28	5	В	419 1	25.27	8	v 121.	0 61.4	2	В	345.0	43.36	Ś	A	138.33	20.26	3	В	482.2	79.82	Ś	Α
Trichome density, abaxial, apical leaves	162.4	82.84	2	B	153.2	03.03	8	A 156.4	40 47.6	5	В	412.6	63.31	Ś	A	206.0	20.78	3	В	491.6	79.01	2	Α
Depth of lobing, apical leaves	0.79	0.24	15	A	0.61	0.36 2	0 A	B 0.9	0.62	15	Α	0.91	0.4	25	А	0.27	0.36	10	BC	0.02	0.05	14	C
Depth of lobing, basal leaves	1.48	0.38	15	AB	1.31	0.67	2 E	3 2.0	1 0.92	15	A	1.22	0.49	18	В	0.87	0.59	9	В	0.02	0.06	14	c
Surface area, apical leaves	2.54	1.17	25	U	3.49	2.54 2	5	3.00	2 1.93	25	υ	9.82	3.08	20	A	10.67	6.54	25	Α	6.52	2.59	20	в
Surface area, basal leaves	9.99	4.12	25	В	7.71	1.84	F	3 19.8	9.2 39	25	Α	23.91	9.74	20	A	16.83	7.51	4	AB	11.63	6.58	11	В
Corolla diameter, male flowers	2.24	0.45	16	D	3.5	0.31	5 E	3 3.00	2 0.44	23	υ	3.11	0.34	25	BC	3.97	0.62	25	Α	3.07	0.62	6	BC
Corolla diameter, hermaphrodite flowers	2.96	0.37	16	D	4.12	0.35	.3 E	3.55	8 0.5	17	υ	3.77	0.45	17	BC	4.69	0.58	22	Α	3.54	0.33	4	BCD
Calyx lobe length, male flowers	0.35	0.09	15	щ	0.69	90.0	с С	0 1.10	5 0.14	10	Ο	0.17	0.02	25	ц	1.13	0.14	$\sim$	В	1.63	0.03	3	Α
Calyx lobe length, hermaphrodite flowers	0.35	0.09	9	D	0.9	.15	5	. 1.6	5 0.14	14	Ο	2.41	0.33	19	A	1.78	0.52	4	В	1.72	0.05	4	В
Pedicel length, in fruit	2.7	0.18	13	U	3.64	0.93	4 A	B 1.65	5 0.34	12	Ω	3.59	0.7	~	AB	4.16	0.72	17	Α	2.94	0.47	6	BC
Fruit length	3.11	0.29	14	А	1.8	1.29	3	0.1.6	5 0.13	ŝ	BC	2.88	0.26	21	A	2.15	0.34	29	В	2.0	0.37	17	BC
Fruit width	2.92	0.35	14	A	2.2	1.41	.3 E	3 1.6	8 0.28	3	В	2.69	0.28	21	A	1.96	0.36	29	В	2.2	0.35	17	В
Seeds per fruit	433	I	-	A	78.69 3	6.67	3	101.0	57 58.6	3	υ	262.81	48.26	20	В	53.11	28.45	28	U	70.08	49.87	13	C
Seed length	4.11	0.25	15	A	2.84	0.21	0	3.0	9 0.21	15	υ	3.6	0.35	11	В	3.05	0.18	20	U	3.45	0.38	14	В
Fruit wall width	4.4	I	-	AB	3.1	Ι	1 V	B 2.2(	- (	1	В	4.22	0.51	9	AB	5.5	I	1	A	3.35	0.49	2	AB
Plant height	33.8	5.35	3	B	13.62	0.86	6 B	34.0	8 7.3	9	В	69.2	27.03	Ś	A	45.85	6.91	24	в	77.33	2.52	3	A



**Figure 1.** Morphology and the earliest-known herbarium specimen of *Solanum plastisexum*. **A** Flowering stem with a single staminate flower in 2016 **B** Mature fruit **C** Erect inflorescences bearing staminate flowers in 2018 and **D** Specimen collected by P. Latz in 1974, held at DNA and annotated by D. Symon with an annotation indicating his confusion about the reproductive morphology of the specimen (male rachis visible above fruit on far left).



**Figure 2.** Closely related species of andromonoecious bush tomatoes included in this study. A *Solanum jobsonii* **B** *S. watneyi* **C** *S. succosum* **D** *S. plastisexum* **E** *S. diversiflorum* and **F** *S. eburneum*. Colours associated with each taxon also used in Figs 3, 4.

2016 (fl, fr), *C.T. Martine 4258, J.T. Cantley, L.M. Lacey, & P.C. Jobson* (holotype: DNA; isotypes to be distributed to BM, BUPL, MEL, NY, PERTH)

**Description.** Erect perennial herb 50–80 cm tall. Stems slender, woody at base, upright even when weighted by fruits; single stemmed, with some lateral branching on mature stems. Foliage and stems grey to grey-green, becoming slightly more yellow-green with age; indumentum of stems, leaves and inflorescences composed of stellate trichomes with the stalk, these short, appressed and very dense throughout (of Type 1 *sensu* Bean 2004 and Seithe 1979); stalk 0.05–0.1 mm long, with 4–6 rays 0.2–0.4 mm long, the midpoint elongate, to 0.4 mm long. Prickles scattered throughout, tan, straight, slightly widened at base, fine, 1–2 mm long. Sympodial units difoliate, the leaves solitary or geminate. Mature leaves 3–12 cm × 0.7–2.4 cm, lanceolate (elliptic), with 3–7 pairs of primary veins, with few prickles along base of abaxial midvein; both sides closely and very densely stellate-pubescent; base tapering; margins entire,



**Figure 3.** Principal components analysis score plot with eigenvalues and the contribution of each PC displayed (left) and loading plot (right) of characters and species in Table 1. Left, red triangles = *S. jobsonii*, blue circles = *S. diversiflorum*, black crosses = *S. eburneum*, pink triangles = *S. watneyi*, yellow squares = *S. succosum* and purple asterisks = *S. plastisexum*. Right, weighted characters labelled and indicated with red arrows. A Seed length **B** Fruit width **C** Number of seeds per fruit **D** Fruit length **E** Depth of lobing on apical leaves **F** Depth of lobing on basal leaves **G** Fruit wall width **H** Width of basal leaves **I** Surface area of basal leaves **N** Petiole length **O** Pedicel length in fruit **P** Corolla diameter, hermaphrodite flowers **Q** Length of basal leaves **R** Corolla diameter, male flowers **S** Length of apical leaves **T** Plant height **U** Stem prickle length **V** Trichome density, abaxial surface of apical leaves **X** Calyx lobe length, male flowers. Colours associated with each taxon also used in Figs 2, 3.

occasionally sparsely shallowly lobed; apex acute; petiole 0.3-1.8 cm long with 0-4prickles along base of adaxial midvein. Inflorescence a supra-axillary cyme 1-15 cm long, complete inflorescence consisting of a basal hermaphrodite flower and a distal group of 2-many staminate flowers with 2-3 staminate flowers typically open at the same time; peduncle typically 1.0-10.0 mm long. Flowers 5-merous, heterostylous and the plants and romonoecious. Hermaphrodite flower ca. 1.5-3 cm below the oldest staminate flower, opening first; pedicel ca. 1 cm long at anthesis, elongating in fruit, armed with 20-50 prickles, each 1-3 mm long; calyx lobes 16-18 mm long, fused for first 2-3 mm, some occasionally fused along most of their length with sepals arranged 2+2+1, armed with 40 -100 long, straight prickles and dense stellate trichomes; corolla 3.1–4.0 cm in diameter, lavender to medium purple, rotate, glabrous; stamens equal; filaments ca. 1.0 mm long; anthers 5 mm long, oblong to lanceolate, poricidal, in a tight anther cone; ovary glabrous, ca. 1.5 mm diameter at anthesis; style (including capitate stigma) dimorphic, 2.5-5 mm or 7-10.5 mm long, curved. Staminate flowers with pedicels 4–7 mm long, unarmed; calyx lobes 16–17 mm long, fused at the base, occasionally 2+2+1 as in hermaphroditic flowers, prickles absent; corolla 1.7-3.5 cm in diameter, lavender to medium purple, rotate; stamens of same proportions as in hermaphrodite flowers; ovary, style and stigma vestigial and diminutive; rachis bearing staminate flowers often deciduous in fruit. Fruit a globose berry 1.4–2.7 cm long,



**Figure 4.** Map showing geographic distribution of all taxa compared in this study. red points = *S. jobsonii*, blue points = *S. diversiflorum*, black points = *S. eburneum*, pink points = *S. watneyi*, yellow points = *S. succosum* and purple asterisk = *S. plastisexum*. All points are based on specimens databased in the Australasian Virtual Herbarium (https://avh.chah.org.au/) and specimens held at BUPL.

1.7–2.8 cm in diameter, light green with darker green striations when young, maturing to creamy yellow; flesh firm; locules 2, with little liquid; fruit wall ca. 3–4 mm thick; fruits retained on plant after maturation; fruiting pedicels 2.4–4.1 cm long; fruiting calyx covering 1/4 to 1/3 of developed fruit, the lobes narrowly deltoid, long-acuminate, tips acute, turning brown and weakly reflexing at maturity, very densely short stellate-pubescent and armed with sharp spines 2–5 mm long, these single or paired along the calyx sutures. Seeds up to ~150 per fruit, 3.0–4.5 mm long, dark brown to black, flat, reniform, the surfaces finely reticulate.

**Distribution and ecology.** Solanum plastisexum is currently known from a restricted range on and around the Buchanan Highway in the sub-arid, monsoon-influenced zone of the northern region of the Australian Northern Territory (Figs 4, 6). The species is locally abundant in two to three sites along and off of the road in *Corymbia dichromophloia* (F.Muell.) K.D.Hill & L.A.S.Johnson and *Petalostigma pubescens* Domin savanna woodland alongside *Aristida* L., *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. and other herbaceous and shrubby associates. The distribution of plants along annually-graded roadsides suggests that the species is, like many of its congeners, disturbance-adapted.



Figure 5. Scan of holotype of *Solanum plastisexum*, held at DNA.



Figure 6. Typical habitat of Solanum plastisexum, taken from the Buchanan Highway.

Nothing is known about the biotic interactions local fauna have with this species, although the floral morphology suggests the typical *Solanum* buzz pollination syndrome (Anderson and Symon 1988). Plants encountered in 2018 bore many mature fruits not taken nor eaten by frugivores, but the exposed, fleshy berries may indicate biotic seed dispersal via ingestion (Martine et al. 2019).

**Phenology.** The handful of collections that have been made of *S. plastisexum* that include flowers are all from the end of the wet season through the early months of the dry season, from January to June. Mature fruiting specimens have been collected in June.

**Etymology.** The name is based on the Latin "plastus" ("deceptive," but derived from the Greek "plastikos/plasticos/plasticus" for "able to be molded, changeable") and the Latin "sexus" for sex. We suggest the use of Dungowan Bush Tomato for the common name of this species, which refers to the cattle station on which the majority of the collections have been made.

**Preliminary assessment of conservation status.** *Solanum plastisexum* is known from only two to three extant populations, each consisting of a few dozen individuals (with some likelihood of clonality) and two historical (pre-2000) collections (Fig. 1D). The currently-known distribution of the species is not under conservation protection, but one of the populations appears to have been stable since at least the 1970s. When evaluated using the IUCN Red List Categories and Criteria for extinction risk (IUCN 2012), *S. plastisexum* falls into the Vulnerable (VU) category under Criterion B (B1ab(iii)+2ab(iii)). The VU designation is the lowest of three threatened categories, but indicates the taxon still faces a high risk of extinction in the wild. It has an Area of Occupancy that is likely much less than < 20 km<sup>2</sup> and an Extent of Occurrence < 5 km<sup>2</sup>, as calculated using the geocat.kew.

org online tool. There are fewer than 10 known locations that are possibly fragmented and a decline in overall habitat quality is likely, given population localities along a road.

**Specimens examined. AUSTRALIA. Northern Territory:** 92 km W. of Dunmarra, 16°44'S, 132°10'E, 20 June 1974 (fr), *P.K. Latz* 5482 (DNA [DNA A0041776]); Dungowan Station, 16°44'S, 132°17'E, 10 January 1978 (fl), *T.S. Henshall 1914* (DNA [DNA A0054101]); Buchanan Highway, 46.1 km E. of Top Springs, 16°43.140'S, 132°09.511'E, 22 May 2016 (fl), *C.T. Martine, J.T. Cantley, L.M. Lacey & P. Jobson 4260* (DNA, BUPL); Buchanan Highway, 42.1 km E. of Top Springs, 16°42.274'S, 132°07.446'E, 30 May 2018 (fl, fr), *C.T. Martine, A.J. McDonnell, J.T. Cantley, & P. Jobson 4743* (NT, DNA, BUPL); Buchanan Highway, 46.1 km E. of Top Springs, 16°43.140'S, 132°09.511'E, 30 May 2018 (fl, fr), *C.T. Martine, A.J. McDonnell, J.T. Cantley, & P. Jobson 4745* (DNA, BUPL).

#### Field key to andromonoecious Solanum species of Northern Territory, Australia

Couplets 3 and 4 adapted from Bean and Albrecht (2008).

1	Mature plants typically with stems 1 metre or more in height; plants pos-
	End region
_	Mature plants typically with stems 1 metre or less in height (or rarely $\geq$ 1 m);
	plants rhizomatous; foliage grey to blue green to deep green; primarily occur- ring south of Mataranka
2	Foliage yellow-green to rusty-green; leaves with several shallow or deep rounded lobes; plants perennial; typically along riverbanks around southern
	Gulf of Carpentaria
_	Foliage bright or deep green; leaves ovate to oval with none or few pointed
	lobes; plants biennial; mostly restricted to northern/western escarpments of
	the Arnhem Plateau (and Wessel Islands)
3	Leaves sessile; stellate hairs on upper leaf surface with lateral rays more or less
	porrect (held horizontally)
_	Leaves petiolate; stellate hairs on upper leaf surface mostly with ascending
	lateral rays4
4	Fruiting calyx with 2300–2700 prickles; male flowers with pedicels 3–11 mm
	long S. ultraspinosum A.R.Bean
_	Fruiting calyx with 190-310 prickles; male flowers with pedicels 11-16 mm
	long
5	Erect herbs or shrubs (though branches may become lax and the plants sprawl
	slightly in fruiting stage)6
_	Compact to weakly erect or sprawling herbs or shrubs9
6	Plants greater than 0.5 metre in height at maturity
_	Plants 0.5 metre or less in height at maturity7

7	Leaf blades lanceolate; lobes, if any, with sinuses less than 0.2 cm in depth
_	Leaf blades elliptic, ovate or rarely lanceolate: lobes frequently with sinuses $\geq$
	0.5 cm in depth
8	Leaves dissected; leaf blades ovate to oblong, 2-4 cm long, sparsely pubes-
	centS. diversiflorum F. Muell.
_	Leaves deeply lobed; leaf blades ovate to elliptic, 2.5–8 cm long, densely pu-
	bescent
9	Leaves deep green; leaf blades linear, dissected, lanceolate or elliptic10
_	Leaves grey-green; leaf blades ovate11
10	Leaf blades linear to dissected with narrow lobes or ovate to elliptic and lobed;
	berries globose; restricted to eastern Northern Territory, Limmen National
	Park region
_	Leaf blades lanceolate to elliptic and unlobed to shallowly lobed; berries
	ovoid; restricted to western Northern Territory, Bullita Homestead, Judbarra
	National Park and vicinity
11	Leaf margins shallowly lobed to entire, sinuate; fruit a dry berry
_	Leaf margins shallowly to deeply lobed, crenate to irregularly parted; fruit a
	juicy berry12
12	Plants compact, typically much less than 0.5 m tall; restricted to north-
	western Northern Territory, west of Timber Creek, in the East Baines
	River corridor S. eburneum Symon
_	Plants weakly erect to sprawling, typically reaching 1 m tall; widespread in
	Northern Territory and western Queensland S. succosum A.R. Bean & Albr.

#### Discussion

For at least five decades, the species described here has evaded easy classification by field botanists. The earliest known collections by Latz (*Latz 5482*, DNA) and Henshall (*Henshall 1914*, DNA) in the 1970s were each identified initially as *S*. aff. *eburneum*, with *Solanum* expert David Symon also suggesting *S*. aff. *chippendalei* for the former in an annotation (Fig. 1). Some of the confusion surrounding this taxon relates to the botanists' inability to clearly identify its breeding system due to the species' non-conformity to any one floral form and/or inflorescence type. Any given floral unit encountered in nature might consist of a fully andromonoecious inflorescence (basal bisexual flower with several distal staminate flowers), a solitary bisexual flower, a solitary short-styled (possibly functionally staminate) flower or an extended rachis of staminate flowers – with observers left to wonder whether any individual plant exhibits one of the three breeding systems found amongst its closest relatives; andromonoecy, hermaphroditism or functional dioecy. Labile sex expression has been observed in other *Solanum* lineages (e.g. Diggle 1991, 1994; Miller and Diggle 2003), but rarely to the same degree – and no other species amongst the ca. 30 taxa in the "*S. dioicum* +

*S. echinatum* Group" (*sensu* Martine et al. 2019) shows this same plasticity relative to reproductive expression.

Given this apparent ability to exhibit elements of all three possible breeding systems, we have chosen the name *S. plastisexum*. This name is not just a reflection of the diversity of sexual forms seen in this species, but is also a recognition that this species could prove to be a model for the sort of sexual fluidity that is present throughout the plant kingdom – where just about any sort of reproductive form one can imagine (within the constraints of genetics and development) is present (Darwin 1877).

*Solanum plastisexum* is a new species that serves as an example of for the diversity of sexual/reproductive form that has been increasingly recognised amongst plants – it is also evidence that attempts to recognise a "normative" sexual condition amongst the planet's living creatures is problematic. When considering the scope of life on Earth, the notion of a constant sexual binary consisting of distinct and disconnected forms is, fundamentally, a fallacy.

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RESEARCH ARTICLE



# Origin of the Diplazium hachijoense complex (Athyriaceae)

Kiyotaka Hori<sup>1</sup>, Noriaki Murakami<sup>2</sup>

I The Kochi Prefectural Makino Botanical Garden 4200-6 Godaisan, Kochi 781-8125, Japan **2** Makino Herbarium, Tokyo Metropolitan University, 1-1 Minami-osawa, Hachioji, Tokyo 192-0397, Japan

Corresponding author: Kiyotaka Hori (khori@makino.or.jp)

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#### Abstract

We analyzed the phylogeny of the *Diplazium hachijoense* complex using plastid *trnL-F* and low-copy nuclear marker *AKI* DNA sequences. Based on allele constitution, triploid apogamous species of the *D. hachijoense* complex appeared to have originated from the hybridization of triploid apogamous species and diploid sexual species by recurrent hybridization events. These results suggested that triploid apogamous ferns can achieve hybridization with diploid sexual species by producing diploid spores with irregular meiosis in sporogenesis. Furthermore, the present study predicted the involvement of several unknown species associated with hybridization. More sampling of *Callipteris* species from China and adjacent areas is required to determine the relationships among unknown species and the *D. hachijoense* complex.

#### Keywords

apogamous, Athyriaceae, Ferns, Diplazium, hybridization, phylogeny

#### Introduction

In sexually reproductive fern species, meiosis produces 64 haploid spores per sporangium following mitotic divisions of spore mother cells four times, and each mother cell contains half of the parental chromosome number (Manton 1950). By contrast, apogamous ferns produce 32 spores per sporangium by one of two pathways to yield chromosomally unreduced diplospores (Grusz 2016): premeiotic endomitosis (Döpp 1939, Manton 1950) or meiotic first division restitution (Braithwaite 1964). Most apogamous ferns produce their spores by the former pathway (Manton 1950). In addition, a few apogamous species possess a third mechanism of reproduction that yields either 16 or 64 relatively regular shaped spores per sporangium (Park and Kato 2003).

Apogamous reproduction is not an unusual feature in ferns. Approximately 3% of all fern species (Liu et al. 2012) and 13% of Japanese fern species, for which information regarding their reproductive modes is available, reportedly exhibit apogamous reproduction (Takamiya 1996). Although several apogamous fern species do not require sexual reproduction throughout their life cycles, they exhibit extensive morphological and genetic variation and often form species complexes with continuous morphological variation. Numerous studies have reported reticulate relationships between apogamous and sexual fern species (e.g., Watano and Iwatsuki 1988, Suzuki and Iwatsuki 1990, Lin et al. 1995, Grusz et al. 2009, Chao et al. 2012, Dyer et al. 2012, Ebihara et al. 2012, Hori et al. 2014), which are exhibited in four patterns (Figure 1): (1) tetraploid hybrids are formed between triploid apogamous species and diploid sexual species, (2) triploid hybrids are formed between diploid apogamous species and diploid sexual species, (3) triploid hybrids are formed between triploid apogamous species and diploid sexual species, and (4) tetraploid hybrids are formed between triploid apogamous species and tetraploid sexual species. In patterns (1) and (2), diploid or triploid apogamous species generate unreduced sperm (Walker 1962, Watano and Iwatsuki 1988, Grusz et al. 2009, Chao et al. 2012, Jaruwattanaphan et al. 2013, Dyer et al. 2012) or eggs (Dyer et al. 2012, Hori et al. 2014, Hori et al. 2018a) that are united with a reduced gamete from a sexual species. Alternatively, in patterns (3) and (4), a reduced gamete from a sexual species is united with a reduced diploid sperm or egg generated by an apogamous species (Ebihara et al. 2012, Hori et al. 2014, Hori et al. 2018b, Hori 2018c).

Lin et al. (1992) proposed the hybridization cycle hypothesis as the mechanism of recurrent hybridization that occurs in triploid apogamous species after the discovery of triploid apogamous *Dryopteris pacifica*, which produces diploid spores through irregular meiosis. If prothallia of such diploid spores can produce eggs or sperm, an apogamous hybrid species can repeatedly originate from the hybridization of apogamous and sexual species without an increase in ploidy. This hypothesis is supported by numerous reports of irregular meiosis in spore mother cells of triploid Polypodiales apogamous species: *Athyrium* (Athyriaceae, Kurita 1964; Hirabayashi 1970, Park and Kato 2003), *Deparia* (Athyriaceae, Hirabayashi 1970), *Diplazium* (Athyriaceae, Takamiya et al. 1999), *Cyrtomium* (Dryopteridaceae, Hirabayashi 1970), and *Dryopteris* (Dryopteridaceae, Hirabayashi 1967, Lin et al. 1992).

Diplazium hachijoense Nakai (Athyriaceae) is one of the most common triploid apogamous ferns in Japan (Takamiya et al. 1999, Ebihara 2017). It is difficult to identify because of the continuous morphological variation between other closely related triploid apogamous species (*D. conterminum*, *D. dilatatum*, *D. doederleinii*, *D. okinawaense*, *D. taiwanense*, *D. takii*, and *D. virescens*), diploid sexual species (*D. amamianum*), and tetraploid sexual species (*D. nipponicum*). In addition, *D. dilatatum* has a sexual diploid cytotype (Takamiya et al. 1999), and *D. doederleinii* has an apogamous



**Figure 1.** Hybridization patterns with germination from which apogamous species are derived. (1) Tetraploid hybrid between a triploid apogamous species and a diploid sexual species, (2) triploid hybrid between a diploid apogamous species and a diploid sexual species, (3) triploid hybrid between a triploid apogamous species and a diploid sexual species, (4) tetraploid hybrid between a triploid apogamous species and a tetraploid sexual species. Circle, sporophyte of sexual species; heart, gametophyte; square, sporophyte of apogamous species.

Table 1. The ploidy level an	reproductive mode of the D. hachi	<i>ijoense</i> complex in previous studies
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Species	Reproducive mode	ploidy level	Refereces
D. conterminum	apogamous	3×	Takamiya et al. 1999
D. dilatatum	apogamous	3×	Takamiya et al. 1999
D. doederleinii	apogamous	3×	Takamiya et al. 1999
D. doederleinii	apogamous	4×	Takamiya et al. 2001
D. okinawaense	apogamous	3×	Takamiya et al. 1999
D. taiwanense	apogamous	3×	Takamiya et al. 1999
D. takii	apogamous	3×	Takamiya et al. 1999
D. virescens	apogamous	3×	Takamiya et al. 1999
D. amamianum	sexual	2×	Takamiya et al. 1999
D. nipponicum	sexual	4×	Takamiya et al. 1999

tetraploid cytotype (Takamiya et al. 2001). The ploidy level and reproductive mode of the *D. hachijoense* complex in previous studies are both shown in Table 1. The present study aimed to determine the hybridization patterns between apogamous and sexual species within the *D. hachijoense* complex.

#### Materials and methods

#### **Plant** materials

In this study, all 10 species of the *Diplazium hachijoense* complex and an additional four species, which have not yet been assigned scientific names and termed as *Diplazium* sp. 1–4, were investigated. *Diplazium chinense*, *D. esculentum*, *D. fauriei*, *D. mettenianum*, *Deparia japonica*, *De. viridifrons*, *De. unifurcata*, *Athyrium crenulatoserrulatum*, and *A. decurrentialatum* were used as outgroups. Voucher information for all samples is listed in Appendix 1. All voucher specimens have been deposited in the Makino Herbarium of Tokyo Metropolitan University, Aichi University of Education, and/or the Kochi Prefectural Makino Botanical Garden.

#### Chromosome count and assessment of reproductive modes

We counted mitotic chromosomes from *D. amamianum*, *D. dilatatum*, *D. hachijoense*, *D. nipponicum*, *D. takii*, and *Diplazium* sp. 2–4 (localities are listed in Appendix 1). Chromosome counting methodologies were performed as outlined in Takamiya et al. (1999). To assess the reproductive modes of each sample or herbarium specimen, the number of spores/sporangium were counted. Specimens were considered sexual if the number of spores/sporangium was 64; they were considered apogamous if the number of spores/sporangium was 32 (Manton 1950).

#### **DNA** extraction

For molecular analyses, total DNA was extracted from silica-dried leaves using cetyltrimethylammonium bromide solution, according to Doyle and Doyle (1990).

#### Plastid and nuclear DNA sequencing

*trnL-F* was used as the maternally-inherited plastid DNA marker (F: 5'-ATTT-GAACTGGTGACACGAG-3' and FernL 1 Ir1: 5'-GGYAATCCTGAGCAAATC-3'; Taberlet et al. 1991, Li et al. 2009). *AK1* (AK4F: 5'-GATGAAGCCATCAAGAAAC-CA-3' and AKR2: 5'-ATGGATCCAGCGACCAGTAA-3'; Hori et al. 2018b) was used as a biparentally-inherited nuclear marker for polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis, which was used to determine allelic variation in each individual (Ebihara et al. 2012, Jaruwattanaphan et al. 2013).

PCR amplification was performed using PrimeSTAR Max DNA Polymerase (Takara, Kyoto, Japan). PCR entailed an initial denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation, annealing, and elongation steps at 98 °C for 10 s, 55 °C for 5 s, and 72 °C for 5 s, respectively, using a Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Gel electrophoresis of *AK1* PCR products was performed using gels containing 2% glycerol at 15 °C for 16 h at 300 V, followed by silver staining. For sequencing of the bands separated on the SSCP gels, the polyacrylamide gel was dried after silver staining by sandwiching the gel between Kent paper and a cellophane sheet on an acrylic back plate at 55 °C for 3 h. To extract the DNA, a piece of the DNA band was peeled from the dried gel using a cutter knife and incubated in 50  $\mu$ L of Tris-EDTA buffer (10-mM Tris-HCl and 1-mM EDTA, pH 8.0) at 25 °C overnight. The supernatant solution was used as a template for further PCR amplification with the same primer set employed for initial PCR amplification.

PCR products were purified using ExoSAP-IT (USB, Ohio, USA) or Illustra ExoStar 1-Step (GE Healthcare, Wisconsin, USA) and used as templates for direct sequencing. Reaction mixtures for sequencing were prepared using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). The reaction mixtures were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems). All plant samples were classified based on their PCR-SSCP banding patterns, and each band was DNA sequenced.

#### Molecular analysis

For phylogenetic analyses, the sequences were typified and made non-redundant by removing duplicate sequences. Only one sequence representing each allele for AK1 and for each haplotype for *trnL-F* were used in the datasets (Appendices 1, 2). The sequences were aligned using MUSCLE (Edgar 2004) and assessed with Bayesian inference (BI) analysis using MrBayes 3.2.6 (Ronquist et al. 2012) and maximum parsimony (MP) analysis using the MEGA X software (Kumar et al. 2018). In the BI analysis, the best-fit model of sequence evolution for each DNA region was selected using jModelTest 2.1.10 (Darriba et al. 2012; trnL-F: HKY+G model; AK1: HKY model). In addition, we assessed BI (trnL-F: HKY+I+G model; AKI: HKY model) and MP analysis with full-data sets. Four Markov chain Monte Carlo chains were run simultaneously and sampled every 100 generations for 1 million generations in total. Tracer 1.7.1 (Rambaut et al. 2018) was used to examine the posterior distribution of all parameters and their associated statistics, including estimated sample sizes. The first 2,500 sample trees from each run were discarded as burn-in periods. The MP tree was obtained using the subtree pruning-regrafting algorithm (Swofford et al. 1996) at search level 1, at which the initial trees were obtained by the random addition of sequences (10 replicates). Indels were treated as missing characters in the MP and BI analyses. The confidence level of the monophyletic groups was estimated with 1,000 MP bootstrap pseudo-replicates.

#### Results

#### Chromosome count and estimation of reproductive mode

The ploidy level and reproductive mode of *D. hachijoense* complex species was consistent with previous reports (Takamiya et al. 1999): *D. amamianum* and *D. dilatatum*, 2n = 82, diploid sexual; *D. hachijoense* and *D. takii*, 2n = 123, triploid apogamous; and *D. nipponicum*, 2n = 164, tetraploid sexual (Appendix 1).

#### Plastid and nuclear DNA phylogenetic trees

We sequenced 719–748 bp of the *trnL-F* intergenic spacer from different specimens. The aligned *trnL-F* matrix was 748 bp, of which 114 characters (15%) were parsimony-informative. For the *AK1* intron, we sequenced 280–520 bp of the intron for each specimen, yielding a 574 bp aligned matrix, of which 74 characters (13%) were parsimony-informative. The MP trees derived from our *trnL-F* and *AK1* sequence analyses with BI posterior-probabilities (PP) and MP bootstrap percentages (BP) are shown in Figures 2a, 3a, respectively. In the phylogenies with full-data set of *trnL-F* and *AK1* (Figures 2b, 3b, respectively), 118 characters (15%) and 84 characters (14%) were parsimony-informative, respectively.

To define allelic types of the *D. hachijoense* complex, we investigated which diploid sexual or autotriploid apogamous species had each allele supported by PP and BP (Figures 2a–3b and Table 2). Alleles of nuclear genes from samples whose sequences formed a clade with particular lineages were considered to originate from the parental species. Therefore, when two alleles from one triploid apogamous species formed clades with those of parental species A and B, the allele composition of the apogamous species was AB. Unfortunately, PCR-SSCP analysis could not distinguish among the genotypes A1A1B1, A1B1B1, and A1B1 as this method cannot determine the quantity of each allele in PCR products. Therefore, the present study only showed the alleles obtained from each material, not their proportions, in Table 2 and Appendix 1.

To divide each allele number with the alphabet, we used clades supported by BP, PP, and similarity in the sequences. Furthermore, we investigated which diploid sexual or autotriploid apogamous species had each allele (Table 2). Based on allelic relationships shown in Figures 2a–3b and Table 2, *D. dilatatum* seemed to have only type A, *D. takii* had only B, and *D. doederleinii* had only C for both *trnL-F* and *AK1* sequences. Type D containing *D. amamianum* was more clearly monophyletic because the BP and PP values were higher than those for *D. dilatatum*, *D. takii*, and *D. doederleinii*. Regarding other alleles of undetected (or missing) species, we could not conclude which alleles came from the same species. Therefore, we tentatively treated these alleles as individually distinct species, outlining them as E, F, G, H, J and K.

In total, five types of plastid *trnL-F* haplotypes (Type  $\alpha - \varepsilon$ ) and 10 types of nuclear *AK1* alleles (Type A–H, J, and K) were recovered from the *D. hachijoense* complex

**Table 2.** Reproductive mode, ploidy level, plastid haplpotype (*trnL-F* intergenetic spacer), and nuclear allele (*AK1*) of the *D. hachijoense* complex in this study. Rep, reproductive mode; sex., sexual; apo., apogamous; ploi., ploidy level. Any allelic types of nuclear gene *AK1* that were identified by sequencing are in boldface.

Voucher	Locality	Species	rep.	ploi.	trnL-F	AK1
S. Serizawa 91648-1	Okinawa pref.: Kunigami village, Mt. Yonahadake	D. dilatatum	apo.		α1	A3 and A4
S. Serizawa 91648-2	Okinawa pref.: Nago city, Genka	D. dilatatum			α1	A1A4
K. Hatake 705	Kagoshima pref.: Amami city, Sumiyou village,	D. dilatatum	sex.	2×	α2	A1
	Santaro-touge, 350m alt.					
K. Hatake 974	Kagoshima pref.: Amami city, Naze	D. dilatatum	sex.	2×	α1	A4
K. Hori 3082	Kagoshima pref.: Yakushima Is, Koseda, 70m alt.	D. dilatatum			α1	A6
K. Hori 3083	Kagoshima pref.: Yakushima Is, Koseda, 70m alt.	D. dilatatum			α1	A1A2
K. Hori 3125	Kagoshima pref.: Yakushima Is, Hara, 80m alt.	D. dilatatum			α1	A6
M. Takamiya 866	Mie pref.: Minamimuro county, Kiho-cho	D. takii	apo.	3×	β1	B2
K. Hori 2924	Fukuoka pref.: Kasuya county, Hisayama-machi, 140m alt	D. takii			β1	B2
K. Hori 2958	Fukuoka pref.: Kasuya county, Hisayama-machi, 140m alt.	D. takii			β1	B2
K. Hori 2343	Mie pref.: Minamimuro county, Kiho-cho, 70m alt.	D. takii			β1	B2B3
K. Hori 3173	Kagoshima pref.: Yakushima Is, Isso-river, 390m alt.	D. doederleinii			γ2	С
K. Hatake 615	Kasgoshima pref.: Amami city, Naze, Honchya- touge, 250m alt.	D. amamianum	sex.	2×	δ1	D1
K. Hatake 985	Kasgoshima pref.: Amami city, Sumiyou village, Santaro-touge, 350m alt.	D. amamianum	sex.	2×	δ1	D1
K. Hatake 609	Kasgoshima pref.: Amami city, Naze, Ooaza-asato	D. amamianum	sex.	2×	δ4	D1
K. Hori 3084	Kagoshima pref.: Yakushima Is, Koseda, 70m alt.	D. taiwanense			β2	A2B1
K. Hori 3080	Kagoshima pref.: Yakushima Is, Koseda, 71m alt.	D. taiwanense	apo.		α1	A1A6B1
K. Hori 3087	Kagoshima pref.: Yakushima Is, Isso-river, 200m alt.	D. okinawaense	apo.		δ2	A1B2
S. Serizawa 91663-1	Okinawa pref.: Nago city, Genka	D. sp. 1	apo.		α1	A1A5G
K. Hori 3158	Kagoshima pref: Yakushima Is, Tabugawa, 200m alt.	D. conterminum			β1	B2F
K. Hori 2341	Mie pref.: Minamimuro county, Kiho-cho	D. virescens			β1	B2E
K. Hori 2342	Mie pref.: Minamimuro county, Kiho-cho	D. virescens			β1	B2E
K. Hori 3086	Kagoshima pref.: Yakushima Is, Miyanoura river, 20m alt.	D. virescens			β1	B1B2E
K. Hatake 773	Kagoshima pref.: Tokunoshima Is, Mt. Inokawadake, 200m alt.	D. hachijoense	apo.	3×	δ1	B2D1
K. Hatake 776	Kagoshima pref.: Tokunoshima Is, Mt. Inokawadake, 200m alt.	D. hachijoense	apo.	3×	δ2	B2D1
K. Hori 1681	Chiba pref.: Katori county, Tako-machi, Hayashi	D. hachijoense			δ3	B2D2
S. Serizawa 91664-1	Okinawa pref.: Nago city, Genka	D. hachijoense	apo.		δ1	B2D2
K. Hori 2957	Fukuoka pref.: Kasuya county, Hisayama-machi, 140m alt	D. hachijoense			δ3	B2D2
M Takamiya 528	Yamaguchi pref · Nagato city. Ichinoo	D hachijoense			83	B2D2
M Takamiya 919	Mie pref : Minamimuro county, Kiho-cho	D hachijoense	apo	3×	δ5	B2D3
K. Hatake 1010	Shizuoka pref.: Shimoda city, Renndaiji-onsenn,	D. hachijoense	apo.	3×	δ5	B2D3
K. Hatake 395	Kagoshima pref.: Tokunoshima Is, Mt. Inokawadake, 200m alt.	D. hachijoense	apo.	3×	δ1	B2D1
M. Takamiya 883	Mie pref.: Minamimuro county, Kiho-cho	D. hachijoense	apo.	3×	δ3	B2D2
M. Takamiya 893	Mie pref.: Minamimuro county, Kiho-cho	D. hachijoense	apo.	3×	δ3	B2D2
M. Takamiya 1883	Kagoshima pref.: Kagoshima city, Chuzann-cho, Takinoshita-river	D. sp. 3	apo.	3×	δ1	CD2
S. Serizawa 91654-1	Okinawa pref.: Kunigami village, Mt. Yonahadake	D. sp. 2			γ1	CK
S. Serizawa 91654-2	Okinawa pref.: Kunigami village, Mt. Yonahadake	D. sp. 2			γ1	CK
K. Hori 2338	Mie pref.: Minamimuro county, Kiho-cho	D. sp. 4			δ3	D1H1
M. Takamiya 929	Mie pref.: Minamimuro county, Kiho-cho	D. sp. 4	apo.	3×	δ1	D2H2
K. Hatake 1030	Kumamoto pref.: Amakusa city	D. sp. 4	apo.	3×	δ1	D2H2
K. Hori 2339	Mie pref.: Minamimuro county, Kiho-cho	D. nipponicum			ε	D2J
K. Hatake 1004	Kanagawa pref.: Minamiashigara city, Kano	D. nipponicum	sex.	4×	ε	D2J





**Figure 2a.** A 50% majority consensus tree resulting from Bayesian Markov chain Monte Carlo Bayesian (B/MCMC) analysis of plastid intergenic spacer *trnL-F* with BI PP (>0.95) and MP BP (>70) node support values. The sequences were typified and made non-redundant by removing duplicate sequences.





Figure 2b. Full-data set of a 50% majority consensus tree resulting from Bayesian Markov chain Monte Carlo Baysean (B/MCMC) analysis of plastid intergenic spacer trnL-F with BI PP (>0.95) and MP BP (>70) node support values. Blue, diploid sexual; red, triploid apogamous; green, apogamous but ploidy was not estimated in this study.



0.005

**Figure 3a.** A 50% majority consensus tree resulting from Bayesian Markov chain Monte Carlo Bayesian (B/MCMC) analysis of the nuclear gene *AK1* with BI PP (>0.95) and MP BP (>70) node support values. The sequences were typified and made non-redundant by removing duplicate sequences.

b



**Figure 3b.** Full-data set of a 50% majority consensus tree resulting from Bayesian Markov chain Monte Carlo Baysean (B/MCMC) analysis of the nuclear gene *AK1* with BI PP (>0.95) and MP BP (>70) node support values. Blue, diploid sexual; red, triploid apogamous; green, apogamous but ploidy was not estimated in this study.

(Table 2). Plastid haplotypes in the *D. hachijoense* complex were as follows (Fig. 2a, Table 2): sexual or apogamous, type  $\alpha$ - *D. dilatatum*, *D. taiwanense*, and *Diplazium* sp. 1; type  $\beta$ - *D. conterminum*, *D. taiwanense*, *D. takii*, and *D. virescens*; type  $\gamma$ - *D. doed-erleinii* and *Diplazium* sp. 2; type  $\delta$ - *D. amamianum*, *D. hachijoense*, *D. okinawaense*, *Diplazium* sp. 3, and *Diplazium* sp. 4; and type  $\varepsilon$ - *D. nipponicum*. Types  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  were well supported by PP (>0.95) and BP (>90) values. In the phylogeny with full-data set, Type  $\varepsilon$  was also supported, but Type  $\gamma$  was not supported by PP.

Allelic constitution of *AK1* in the *D. hachijoense* complex were as follows (Fig. 3a, Table 2): sexual or apogamous, type A- *D. dilatatum*; type B- *D. takii*; type C- *D. doed-erleinii*; type D- *D. amamianum*; *D. taiwanense*, one or two allele A and one allele B; *D. hachijoense*, one allele B and D; *D. okinawaense*, one allele A and B; *D. nipponicum*, one allele D and J; *D.* sp. 1, two allele A and one allele G; *D.* sp. 2, C and K; *D.* sp. 3, C and D; and D. sp. 4, D and H. Types A, B, D, and H were rather supported by PP (>0.95) and BP (>70) values. In the phylogeny with full-data set, Type E, J, and K were also supported.

#### Discussion

Figure 4 represents the reticulogram of the *D. hachijoense* complex. The ploidy levels and reproductive modes of undetected species are unknown; thus, we assigned them as either diploid sexual species (E, F, G, J, and K) or triploid apogamous species (H). If undetected species H was a diploid sexual species, we could not explain the origin of triploid apogamous *Diplazium* sp. 4 (nuclear *AK1*=D and H) because allele D belongs to diploid sexual *D. amamianum*. Thus, it must be a diploid hybrid.

The allelic constitution in D. hachijoense suggested that it resulted from hybridization between the diploid sexual species D. amamianum and the triploid apogamous species D. takii. Because plastid genomes are reported to be maternally-inherited in ferns (Gastony and Yatskievych 1992), D. amamianum is likely the maternal parent of D. hachijoense. trnL-F sequences of D. amamianum and D. hachijoense are united in Clade  $\delta$  (Figure 2). The two *AK1* alleles present in *D. hachijoense* are united in Clades B and D, with alleles from D. takii and D. amamianum, respectively. Furthermore, D. taiwanense and Diplazium sp. 3 exhibited allelic constitutions to similar to that of D. hachijoense, suggesting closely allied progenitors. Diplazium taiwanense comprises both A and B AKI biparentally-inherited alleles. However, one specimen of D. taiwanense possessed an  $\alpha$  maternally-inherited plastid haplotype derived from D. dilatatum, whereas the other specimen of *D. taiwanense* possessed a  $\beta$  plastid haplotype. The former species had one A and B allele, while the latter had two A alleles and one B allele in AK1. This suggested recurrent, reciprocal origins of D. taiwanense. In the first hybridization event, apogamous D. takii is the maternal parent of D. taiwanense, whereas sexual D. dilatatum is the maternal parent of D. taiwanense in the second hybridization event. Diplazium sp. 3 has C and D alleles of AK1 derived from D. doederleinii and D. *amamianum*, respectively, and the  $\delta$  plastid haplotype derived from *D. amamianum*.



**Figure 4.** The reticulogram of the *D. hachijoense* complex. Thin-solid arrow, autopolyploidization; Solid arrows, maternal inheritance; dashed arrows, paternal inheritance; square, triploid apogamous or diploid sexual species; dashed square, hypothesized diploid sexual or triploid apogamous species. \**D. ok-inawaense* had only nuclear *AKI* allele A of *D. dilatatum* and B of *D. takii*, although plastid haplotype was D of *D. amaminanum*.

However, *Diplazium* sp. 3 was difficult to distinguish from *D. hachijoense* because of similar morphological characteristics. Further investigations are required to determine whether *Diplazium* sp. 3 is a novel species.

The allelic constitution of *D. okinawaense* also suggested recurrent hybridization. The *trnL-F* phylogeny suggested that *D. amamianum* is the maternal progenitor of *D. okinawaense*. However, the *AK1* allelic constitution of *D. okinawaense* is A and B, neither of which is found in *D. amamianum*. The inconsistency between the plastid haplotype and the nuclear allelic constitution of *D. okinawaense* may reflect recurrent hybridization events between the triploid apogamous species *D. hachijoense* as the maternal parent and the sexual diploid *D. dilatatum*. Such a scenario may have resulted in the loss of *D. amamianum* nuclear alleles through genetic segregation with recombination (Figure 4).

Allelic constitution in other species suggested that there were six undetected parental species which have only one allele E, F, G, J, K, or H. This study could not resolve ploidy and reproductive mode of these species. Tentatively, in the reticulogram (Figure 4), we proposed *Diplazium* sp. E, F, G, J, and K as hypothesized diploid sexual species and *Diplazium* sp. H as triploid apogamous species. In the reticulogram, we interpreted the origin of six apogamous and one tetraploid sexual species as follows: *D. conterminum* originated from hybridization between apogamous *D. takii* and sexual *Diplazium* sp. E; *D. virescens* originated from *D. takii* and sexual *Diplazium* sp. F; *Diplazium* sp. 1 originated from apogamous *D. doederleinii* and sexual *D.* sp. K; hypothesized diploid sexual *D.* sp. 4 originated from sexual *D. amamianum* and apogamous

*D*. sp. H; and tetraploid sexual *D. nipponicum* originated from hybridization between *D. amamianum* and diploid sexual *D.* sp. J. In addition, *Diplazium* sp. 1, *Diplazium* sp. 2, and *Diplazium* sp. 4 were very similar to *D. dilatatum*, *D. doederleinii*, and *D. hachijoense*. More *Callipteris* species (Wei et al. 2013), including members of the *D. hachijoense* complex, need to be collected from China and adjacent areas to further dissect such dynamics.

According to the relationships of diploid sexual species and triploid apogamous species, allelic inheritance patterns in the *D. hachijoense* complex were found to be consistent with the hybridization cycle hypothesis by Lin et al. (1992, 1995) and are similar to examples from other fern taxa. For example, Hori (2018c) reported that *Deparia okuboana* (Athyriaceae) potentially had a hybrid origin from the sexual diploid species *D. viridifrons* and the apogamous triploid species *D. unifurcata*, with the latter producing unreduced diploid sperm. In *Dryopteris* (Dryopteridaceae); several studies have reported that numerous triploid apogamous species share alleles with other triploid apogamous species and with diploid sexual species (Darnaedi et al. 1990, Lin et al. 1992, Hori et al. 2014, Hori et al. 2018b, Hori et al. 2018d). Ebihara et al. (2012) reported that tetraploid apogamous *Dryopteris shibipedis* had alleles in common with apogamous *D. pacifica* and the sexual tetraploid species *D. kinkiensis*.

Morita et al. (1990) reported a similar phenomenon in the angiosperm genus *Taraxacum (Asteraceae*), revealing that diploid sexual species endemic to Japan hybridize with triploid apogamous species introduced from Europe. The resulting *Taraxacum* are tetraploid or triploid apomicts. Therefore, the hybridization cycle is considered to be an important process that facilitates the production of triploid apogamous hybrids in plant reticulation complexes.

#### Conclusions

Continuous morphological variation in the *D. hachijoense* complex reflects a history of recurrent hybridization events among sexual and apomictic taxa, an observation in line with the hybridization cycle hypothesis suggested by Lin et al. (1992, 1995). The resulting hybrid apomict species is comprised of genomes derived from *D. amamianum*, *D. dilatatum*, *D. doederleinii*, and *D. takii*. More analysis of species distributed across China and adjacent areas is required in order to further comprehensively dissect the relationships between unknown lineages, undescribed species, and all members of the *D. hachijoense* complex.

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# Appendix I

**Voucher specimens examined in this study.** Any allelic types of nuclear gene AK1 that were identified by sequencing are in boldface. Otherwise, the allelic types of nuclear gene AK1 were deduced from comparisons of band positions in SSCP gels. Data are in the order: Species name – locality, voucher (Herbarium), reproductive mode (sex: sexual, apo: apogamous), chromosome number, ploidy, haplotype of plastid *trnL*-*F*, allele of nuclear AK1. The haplotype of outgroups was only shown in Appendix 2 with accession numbers.

#### Members of the D. hachijoense complex

- Diplazium amamianum Tagawa JAPAN. Kasgoshima pref.: Amami city, Naze, Honchya-touge, 250m alt., May 7 2017, *K. Hatake 615* (KUMA), sex, 2n = 82, 2x, δ1, D1; ibid., Sumiyou village, Santaro-touge, 350m alt., May 26, 2018, *K. Hatake 985* (KUMA), sex, 2n = 82, 2x, δ1, D1; ibid., Naze, Ooaza-asato, May 6, 2017, *K. Hatake 609* (KUMA), sex, 2n = 82, 2x, δ4, D1.
- Diplazium conterminum Christ JAPAN. Kagoshima pref: Yakushima Is, Tabugawa, 200m alt., 130°36'55.92"N, 30°23'19.23"E, January 23 2019, K. Hori 3158 (MBK), β1, B2F.
- Diplazium dilatatum Blume JAPAN. Kagoshima pref.: Amami city, Sumiyou village, Santaro-touge, 350m alt., September 7, 2017, *K. Hatake 705* (KUMA), sex, 2n = 82, 2x, α2, A1; ibid., Naze, May 24, 2018, *K. Hatake 974* (KUMA), sex, 2n = 82, 2x, α1, A4; ibid., Yakushima Is, Koseda, 70m alt., 130°39'24.54"N, 30°22'44.24"E, January 20 2019, *K. Hori 3082* (MBK), α1, A6; ibid.; *K. Hori 3083* (MBK), α1, A1A2; ibid., Hara, 80m alt., 130°34'19.72"N; 30°14'41.56"E, January 22 2019, *K. Hori 3125* (MBK), α1, A6. Okinawa pref.: Kunigami village, Mt. Yonahadake, S. Serizawa 91648-1 (AICH), apo, α1, A3A4; Nago city, Genka, S. Serizawa 91658-2 (AICH), α1, A1A4.
- *Diplazium doederleinii* (Luerss.) Makino JAPAN. Kagoshima pref.: Yakushima Is, Isso-river, 390m alt., 130°29'22.32"N; 30°25'13.09"E, January 23 2019, *K. Hori3173* (MBK), apo, γ2, C.
- Diplazium hachijoense Nakai JAPAN. Chiba pref.: Katori county, Tako-machi, Hayashi, October 25 2014, *K. Hori 1681* (MAK), δ3, B2D2. Kagoshima pref.: Tokunoshima Is, Mt. Inokawadake, 200m alt., September 12 2016, *K. Hatake 395* (KUMA), apo, 2n = 123, 3x, δ1, B2D1; ibid., January 13 2018, *K. Hatake 773* (KUMA), apo, 2n = 123, 3x, δ1, B2D1; ibid., January 13 2018, *K. Hatake 773* (KUMA), apo, 2n = 123, 3x, δ2, B2D1. Mie pref.: Minamimuro county, Kihoucho, March 18 2018, *M. Takamiya 883, 893* (KUMA), apo, 2n = 123, 3x, δ3, B2D2; ibid., *M. Takamiya 919* (KUMA), apo, 2n = 123, 3x, δ5, B2D3. Okinawa pref.: Nago city, Genka, *S. Serizawa 91664-1* (AICH), δ1, B2D1. Shizuoka pref.: Shimoda city, Renndaiji-onsenn, 100m alt., 138°55'16.85"N; 34°41'55.23"E, May 22 2018, *K. Hatake 1010* (KUMA), apo, 2n = 123, 3x, δ5, B2D3. Yamagu-

**chi pref.:** Nagato city, Ichinoo, January 4 1996, *M. Takamiya 528* (KUMA), apo, 2*n* = 123, 3*x*, δ3, B2D2.

- *Diplazium nipponicum* Tagawa JAPAN. Kanagawa pref.: Minamiashigara city, Kano, March 18 2018, *M. Takamiya 1004* (KUMA), sex, 2*n* = 164, 4*x*, ε, **D2J. Mie** pref.: Minamimuro county, Kiho-cho, 70m alt., 135°59'29.5"N; 33°45'55.2"E, July 6 2016, *K. Hori 2339* (MAK), ε, **D2J.**
- *Diplazium okinawaense* Tagawa JAPAN. Kagoshima pref.: Yakushima Is, Issoriver, 200m alt., 130°28'21.08"N; 30°26'23.30"E, Jan 21 2019, *K. Hori3087* (MBK), apo, δ2, A1B2.
- *Diplazium taiwanense* Tagawa JAPAN. Kagoshima pref.: Yakushima Is, Koseda, 70m alt., 130°39'24.54"N; 30°22'44.24"E, January 20 2019, *K. Hori 3080* (MBK), apo, α1, A1A6B1; ibid., 70m alt., 130°39'24.54"N; 30°22'44.24"E, January 20 2019, *K. Hori 3084* (MBK), β2, A2B1.
- *Diplazium takii* Sa.Kurata JAPAN. Mie pref.: Minamimuro county, Kiho-cho, 70m alt., 135°59'29.5"N; 33°45'55.2"E, July 6 2016, *K. Hori 2343* (MAK), β1, B2B3; ibid.; *M. Takamiya 866* (KUMA), apo, 2*n* = 123, 3*x*, β1, B2. Fukuoka pref.: Kasuya county, Hisayama-machi, 140m alt., 130°32'2.27"N; 33°40'44.18"E, July 6 2016, *K. Hori 2924, 2958* (MBK), β1, B2.
- *Diplazium virescens* Kunze JAPAN. Mie pref.: Minamimuro county, Kihocho, 70m alt., 135°59'29.5"N; 33°45'55.2"E, July 6 2016, *K. Hori 2341, 2342* (MAK), β1, **B2E. Kagoshima pref.:** Yakushima Is, Miyanoura river, 20m alt., 130°32'31.91"N; 30°24'48.36"E, Jan 21 2019, *K. Hori 3086* (MBK), β1, **B1B2E**.
- *Diplazium* sp. 1 JAPAN. Okinawa pref.: Nago city, Genka, *S. Serizawa 91663-1* (AICH), apo, α1, A1A5G.
- **Diplazium sp. 2** JAPAN. **Okinawa pref.:** Kunigami village, Mt. Yonahadake, S. Serizawa 91654-1, S. Serizawa 91654-2 (AICH), γ1, **CK.**
- *Diplazium* sp. 3 JAPAN. Kagoshima pref.: Kagoshima city, Chuzann-cho, Takinoshita-river, *M. Takamiya 1883* (KUMA), apo, 2*n* = 123, 3*x*, δ1, CD2.
- Diplazium sp. 4 JAPAN. Mie pref.: Minamimuro county, Kiho-cho, 70m alt., 135°59'29.5"N; 33°45'55.2"E, July 6 2016, *K. Hori 2338* (MAK), δ3, D1H1; ibid., March 18 2018, *M. Takamiya 929* (KUMA), apo, 2n = 123, 3x, δ1, D2H2.
  Kumamoto pref.: Amakusa city, June 14 2018, *K. Hatake 1030* (KUMA), apo, 2n = 123, 3x, δ1, D2H2.

# Outgroups

- *Diplazium chinense* (Baker) C.Chr. JAPAN. Kochi pref.: Agawa county, Niyodogawa-cho, Iwayagawa, 250m alt., 133°03'43.15"N; 33°32'22.21"E, June 16 2018, *K. Hori 3023* (MBK).
- *Diplazium esculentum* (Retz.) Sw. JAPAN. Kagoshima pref.: Isa city, Oguchisogi, *M. Takamiya 1109* (MBK).
- *Diplazium fauriei* Christ JAPAN. Okinawa pref.: Kunigami village, Mt. Yonahadake, *S. Serizawa 91656-1* (AICH).

- *Diplazium mettenianum* (Miq.) C.Chr. JAPAN. Mie pref.: Minamimuro county, Kiho-cho70m alt., 135°59'29.5"N; 33°45'55.2"E, July 6 2016, *K. Hori 2336* (MAK).
- *Diplazium wichurae* (Mett.) Diels JAPAN. Kanagawa pref.: Zushi city, Jinnmuji, 60m alt., 139°36'18.19"N, 35°18'14.71"E, July 6 2016, *K. Hori 1763* (MAK).
- *Deparia japonica* (Thunb.) M.Kato JAPAN. Kyoto pref.: Sakyo-ku, Kibune, 300m alt., 135°45'50.79"N; 35°7'30.85"E, July 14 2018, *K. Hori 3031*(MBK).
- Deparia unifurcata (Baker) M.Kato JAPAN. Kochi pref.: Agawa county, Niyodogawa-cho, Iwayagawa, 250m alt., 133°03'43.15"N; 33°32'22.21"E, June 16 2018, K. Hori 3021 (MBK).
- *Deparia viridifrons* (Makino) M.Kato JAPAN. Kochi pref.: Takaoka county, Ochi-cho, May 30 2018, *K. Hori 2971* (MBK).
- *Athyrium crenulato-serrulatum* Makino JAPAN. Tokyo pref.: Ome city, Mt. Mitake, 880m alt., 139°08'32.09"N; 35°48'12.62"E, June 4 2018, *K. Hori 2979* (MBK).
- Athyrium decurrenti-alatum (Hook.) Copel. JAPAN. Tokyo pref.: Hachioji city, Komagino, June 5 2018, K. Hori 2986 (MBK).

# **Appendix 2**

**DNA** data accession numbers of the obtained nucleotide sequences used for construction of molecular phylogenetic trees in this study. Data quoted from Hori (2018c) are marked by asterisks.

## AK1

A1, LC468160; A2, LC468161; A3, LC468162; A4, LC468163; A5, LC468164; A6, LC468165; B1, LC468167; B2, LC468168; B3, LC468169; C, LC468166; D1, LC468172; D2, LC468173; D3, LC468174; E, LC468170; F, LC468171; G, LC468175; H1, LC468180; H2, LC468181; J, LC468176; K, LC468177; *Diplazium chinense*, LC468179, LC468182; *D. esculentum*, LC468186; *D. fauriei*, LC468183; LC468185; *D. mettenianum*, LC468178; *D. wichurae*, LC468187, LC468188; *Deparia japonica*, LC431726\*; *D. unifurcata*, LC421961\*; *D. viridifrons*, LC421960\*; *Athyrium crenulatoserrulatum*, LC421516\*; *A. decurrentialatum*, LC421512\*.

#### trnL-F

α1, LC468195; α2, LC468196; β1, LC468199; β2, LC468200; γ1, LC468197; γ2, LC468198; δ1, LC468201; δ2, LC468202; δ3, LC468203; δ4, LC468204; δ5, LC468205; ε, LC468208; *Diplazium chinense*, LC468193; *D. esculentum*, LC468189; *D. fauriei*, LC468206; *D. mettenianum*, LC468207; *D. wichurae*, LC468190; *Deparia japonica*, LC468194; *D. viridifrons*, LC468191; *D. unifurcata*, LC468192.

RESEARCH ARTICLE



# Dysosma villosa (Berberidaceae), a new species from Guizhou, Southwestern China

Zhiwei Wang<sup>1</sup>, Wenguang Sun<sup>2</sup>, Houcheng Xi<sup>3</sup>, Shuai Chang<sup>4</sup>

I College of Pharmacy, Guizhou University of Traditional Chinese Medicine, Guiyang 550002, Guizhou, China **2** Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China **3** Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Xishuangbanna 666303, Yunnan, China **4** Yunnan Key Laboratory for Integrative Conservation of Plant Species with Extremely Small Populations, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

Corresponding author: Houcheng Xi (xihoucheng@xtbg.ac.cn); Shuai Chang (changshuai@mail.kib.ac.cn)

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#### Abstract

A new species, *Dysosma villosa* Z.W.Wang & H.C.Xi, is described and illustrated based on collections from the Yueliang Mountains in Congjiang County of Guizhou, Southwestern China. It is morphologically similar to *D. difformis* (Hemsl. & E.H.Wilson) T.H.Wang *ex* T.S.Ying, but can be easily distinguished from *D. difformis* by its inflorescences bearing a greater number of flowers (5–13 *vs.* 2–5), white-villous stems, petioles, and abaxial leaf blade, and stigma dark purple-red. In addition, we also compare this new species to the three species of *Podophyllum* (i.e., *P. glaucescens* J.M.H.Shaw, *P. hemsleyi* J.M.H.Shaw & Stearn, and *P. trilobulum* J.M.H.Shaw) which are insufficiently known and listed as putative members of *Dysosma* in *Flora of China. Dysosma villosa* can also be easily distinguished from *P. glaucescens* (7-flowered; to 40 cm tall) and *P. hemsleyi* (4-flowered; to 40 cm tall) by its inflorescences bearing a greater number of flowers, relatively smaller stature (9–23 cm tall), stems, petioles and abaxial leaf blade densely whitevillose. Although the stems and petioles of *P. trilobulum* also possess fine short hairs, it can be easily distinguished from *D. villosa* by its trilobulate leaves, inflorescence with fewer flowers (2–5), and the position of inflorescence (inserted at or above midpoint on petiole of upper leaf).

#### Keywords

Asia, Berberidaceae, Dysosma, Podophylloideae, Podophyllum, Ranunculales

#### Introduction

Dysosma Woodson, a small genus of Berberidaceae, has long been used in traditional herbal medicine in East Asia due to the presence of podophyllotoxin, which has important biological activities, such as treating external genital warts (Beutner and Von 1990, Wang 1991, Petersen et al. 1995, Ying et al. 2011, Mao et al. 2014). It occurs at the Subtropical Evergreen Broadleaved Forest belt of China, being morphologically close to Sinopodophyllum (Royle) T.S.Ying, Podophyllum L., and Diphylleia Michx. Nonetheless, it is differentiated by comprising perennial herbs with creeping rhizomes, numerous fibrous roots, 3-9-parted or lobed-peltate leaves, umbellate inflorescences, and berries with numerous seeds (Stähelin and Von 1991, Ying et al. 2011, Mao et al. 2014). Recently, a large number of phylogenetic analyses revealed the monophyly of *Dysosma* and its sister relationship to the *Sinopodophyllum* and Podophyllum (Loconte and Estes 1989, Nickol 1995, Kim and Jansen 1998, Liu et al. 2002, Wang et al. 2007, Mao et al. 2014). In the "Flora of China" (FOC) treatment, seven species are recognised: D. delavayi (Franch.) Hu, D. pleiantha (Hance) Woodson, D. tsayuensis T.S. Ying, D. aurantiocaulis (Hand.-Mazz.) Hu, D. majoensis (Gagnep.) M.Hiroe, D. versipellis (Hance) M.Cheng ex T.S.Ying and D. difformis (Hemsl. & E.H.Wilson) T.H.Wang ex T.S.Ying (Ying et al. 2011). In addition, due to inadequate material, FOC also treats three insufficiently known species described under Podophyllum (i.e., P. glaucescens J.M.H.Shaw, P. hemsleyi J.M.H.Shaw & Stearn, and P. trilobulum J.M.H.Shaw), but that probably belong to Dysosma (Ying et al. 2011).

During a field trip to the Yueliang Mountains, Congjiang County, Guizhou Province, Southwestern China, in May 2015, an unknown species with densely white-villous stems, petioles and abaxial leaf blade, and dark purple-red stigma was found. After a detailed examination of the characters of our material and possible closely similar species in *Dysosma* (including the three species of *Podophyllum* which are putative members of *Dysosma*), we concluded that these specimens actually represent an undescribed species. Thus, due to its uniqueness in characters, it is formally described by us, below.

#### Materials and methods

Field investigations were conducted in the locality of the type specimens and other adjacent areas of Guizhou. The morphological description of the new species was based on an examination of the dried specimens in herbaria and living plants in the field. The comparison with morphologically similar species was based on an extensive check of specimens deposited in A, E, GH, PE, KUN, IBSC, HIB, HGAS, IBK and NAS, as well as the protologues and descriptions in related literature (Woodson 1928, Kim and Jansen 1996, 1998, Wang et al. 2007, Ying et al. 2011).



Figure 1. Holotype of Dysosma villosa Z.W.Wang & H.C.Xi.

### **Taxonomic treatment**

# *Dysosma villosa* Z.W.Wang & H.C.Xi, sp. nov. urn:lsid:ipni.org:names:77198709-1

Figures 1-3

**Diagnosis.** *Dysosma villosa* is most similar to *D. difformis* (Hemsl. & E.H.Wilson) T.H.Wang *ex* T.S.Ying, but differs from the latter by its inflorescences generally with more flowers (5–13 vs. 2–5), white-villous petioles, stems and abaxial leaf blade and dark purple-red stigma.

**Type.** CHINA. Guizhou: Congjiang County, Guanghui Town, Jiaya Village, Yueliang Mountains, alt. 1105 m, 25.636N, 108.293E, 09 May 2015, *Z.W. Wang & H.C. Xi WAZW15016* (holotype: CSH barcode CSH0160399!; isotype: KUN!).



**Figure 2.** Images of living plants of *Dysosma villosa* Z.W.Wang & H.C.Xi. **A** Plant **B** petiole and stem **C** abaxial leaf blade **D** inflorescence **E** flower **F** anatomy of flower.



Figure 3. *Dysosma villosa* Z.W.Wang & H.C.Xi. **A** Plant (aerial part) **B** root **C** flower **D** sepal **E** stamen **F** pistil.

**Description.** Herbs 9–23 cm tall. Rhizomes usually terete, slender, with numerous fibrous roots. Stems erect, branched, pale green, white-villous. Leaves alternate, obliquely peltate; petioles 6–12 cm long, white-villous; blades 9–17 × 13–23 cm, papery, abaxially pale green, densely white-villous, adaxially deep green, glabrous, base not deeply divided or undivided, margin sparsely denticulate. Umbels 5–13-flowered, sessile, emerging near the base of the leaf blade. Flowers pendulous, pedicels 1.6–2.2 cm long, apically gibbous, sparsely white-villous; sepals 1.4–2.2 cm × 1–4 mm, oblong-lanceolate, pale green, abaxially pubescent, adaxially glabrous, apex acuminate; petals 4–5 × 1.2–1.6 cm, oblong-loriform, dark purple-red, glabrous, apex round; stamens 6, filaments flat, ca. 0.9 cm long, dark purple-red, anthers ca. 1.4 cm long, falcate, connectives exceeding the anther sacs in measurements, anther sacs, ca. 0.9 cm, dark purple, pollen yellow; ovary obpyriform, green, ca. 0.9 cm, style ca. 2 mm long, green, densely speckled with red or purple, stigma multilobate (crown-shaped), dark purple-red. Berry globose, 1.5–2.4 cm long. Seeds numerous, lacking an aril.

**Distribution and habitat.** This new species is currently known from Yueliang mountains of Congjiang County, Guizhou Province, Southwestern China. It grows under forests, at an elevation between 800 and 1500 m.

**Phenology.** This new species has been observed flowering from April to June and fruiting from June to September.

**Etymology.** The specific epithet is derived from the character (white-villous petioles, stems and abaxial leaf blade) of this species.

Additional specimen examined. CHINA. Guizhou: Congjiang County, Guanghui Town, Baiji Village, alt. 823 m, 25.633N, 108.291E, 20 April 2016, Z. W. Wang & H.C. Xi WAZW16029 (KUN); Congjiang County, Guanghui Town, Changniu Village, alt. 936 m, 25.596N, 108.271E, 20 April 2016, Z. W. Wang & H.C. Xi WAZW16032 (CSH); Ronjiang County, Jihua Town, Baiwang Village, alt. 806 m, 25.657N, 108.269E, 18 May 2017, Z. W. Wang & H.C. Xi WAZW17024 (KUN).

#### Discussion

*Dysosma villosa* shares certain characteristics with *D. difformis* in having alternate leaves, leaf blades not deeply divided and/or undivided, inflorescence attached near the base of leaf blade, and oblong-loriform petals. However, it can be promptly recognised by its inflorescences generally having more flowers, and densely white-villose stems, petioles and abaxial leaf blade. Particularly, its densely white-villose stems, petioles and abaxial leaf blade and dark purple-red stigma are significantly different from *D. difformis*, as well as other species of *Dysosma*. In addition, it is worth mentioning that there are still three uncertain species described under *Podophyllum (P. glaucescens, P. hemsleyi*, and *P. trilobulum)* that probably belong to *Dysosma* (Shaw 2002, Ying et al. 2011). However, they are also found to be significantly different from *D. villosa* after we checked their morphological description (Ying et al. 2011) and images of type specimens from JSTOR Global Plants (http://plants.jstor.org). For instance, though

Characters	D. villosa	D. difformis	P. glaucescens	P. hemsleyi	P. trilobulum
Plant height	9–23 cm tall	15–30 cm tall	up to 40 cm tall	up to 40 cm tall	20–25(–40) cm tall
Leaf blade	abaxially densely white-villous,	glabrous; not deeply divided or	glabrous; lower leaf 4-lobed;	glabrous; lobes spatulate-	glabrous; lobes trilobulate,
	adaxially glabrous; not deeply	undivided	upper leaf with 4 obvious and 2	oblong, lobed to $4/5$ of radius	lower leaf lobes 7, upper leaf
	divided or undivided		obscure lobes	1	lobes 5
Stem and Petiole	densely white-villous	glabrous	glabrous	glabrous	with fine short hairs
Inflorescence	attached near base of blade,	attached near base of blade,	inserted on petiole of upper	inserted on petiole ca. 2 cm	inserted at or above midpoint
	5–13-flowered	2–5-flowered	leaf ca. 2 cm below blade,	below blade, 4-flowered	on petiole of upper leaf,
			7-flowered		2–5-flowered
Pedicel	1.6–2.2 cm long, sparsely	1-2 cm long, sparsely white-	4-6 cm long, densely hairy	3-4 cm long, glabrous	2.2-2.5 cm long, brown pilose
	white-villous	pubescent			
Petal	oblong-loriform, $4-5 \times 1.2-1.6$	oblong-loriform, $4-5 \times 0.8-1$	ovate-lanceolate, $10-12 \times 5-6$	oblong-spatulate, 3–3.5 cm×	ovate-lanceolate, $4-5 \times 8-10$
	cm	cm	mm	5–8 mm	mm
Pistil	ovary obpyriform, ca. 1.2 cm	ovary obpyriform, ca. 0.9 cm	ovary ovoid, ca. 5 mm long,	ovary globose to pyriform, $6-7$	unknown
	long, style ca. 2 mm long,	long, style ca. 2 mm long,	style 2–3 mm long, stigma	mm long, style 2–3 mm long,	
	stigma crown-shaped, dark	stigma crown-shaped, yellowish	peltate, corrugated, coloration	stigma globose, corrugated,	
	purple-red		unknown	coloration unknown	

Table 1. Morphological comparison of key characters amongst Dysosma villosa, D. difformis and the three insufficiently known species of Podophyllum.

the stem and petioles of *P. trilobulum* were also found with hairs, it was significantly different from *D. villosa* due to its trilobulate lobed leaves, inflorescence with fewer flowers (2–5) and the position of inflorescence (inserted at or above the midpoint on the petiole of the upper leaf). Comparisons of the key characters amongst *D. villosa*, *D. difformis* and the three insufficiently recognised species are listed in Table 1.

## Key to the species of Dysosma

1	Leaves opposite, inflorescence emerging at the petiole base
_	Leaves alternate, inflorescences emerging at or near the apex of the petiole4
2	Lobes of leaf apically trifid; petals oblong, up to 6 cm long D. delavayi
_	Lobes of leaf apically undivided; petals obovate-elliptic, ca. 3 cm long3
3	Leaf blade glabrous, palmately-lobed, lobes triangular-ovate D. pleiantha
_	Leaf blade pubescent on both surfaces, palmately parted, lobes cuneate-ob-
	long
4	Inflorescences emerging far from the leaf blade; petals obovate, 1.4-1.6 cm
	longD. aurantiocaulis
-	Inflorescences emerging near the leaf blade; petals oblong, lanceolate or obo-
	vate, 2.4–10 cm long5
5	Leaf lobes apically trifid; petals elliptic-lanceolate D. majoensis
-	Leaf lobes apically undivided; petals spatulate-obovate or oblong-loriform6
6	Leaves 4-9-lobed or deeply divided; petals spatulate-obovate; berries ca. 4 cm
	long, ellipsoid or ovoidD. versipellis
-	Leaves not deeply divided or undivided; petals oblong-loriform; berries 1.5-
	2.7 cm diam., globose7
7	Stems, petioles and leaf blades glabrous; inflorescences 2-5-flowered; stigma
	yellow
-	Stems, petioles and leaf blades white-villous; inflorescences 5-13-flowered;

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RESEARCH ARTICLE



# Insights on the systematics and morphology of Humiriaceae (Malpighiales): androecial and extrafloral nectary variation, two new combinations, and a new Sacoglottis from Guyana

Kenneth J. Wurdack<sup>1</sup>, Charles E. Zartman<sup>2</sup>

I Department of Botany, MRC-166, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA 2 Department of Biodiversity, National Institute for Amazonian Research (INPA), Av. André, Araújo 2936, Aleixo, Manaus, Amazonas 69060-001, Brazil

Corresponding author: Kenneth J. Wurdack (wurdackk@si.edu)

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#### Abstract

Humiriaceae have had little recent comparative morphological study except for their distinctive fruits. We surveyed the diversity of stamen structures in the family with consideration of dehiscence patterns and the evolutionary transitions between tetra- and disporangiate anthers. Novel interpretations of floral morphology support new combinations (*Duckesia liesneri* K.Wurdack & C.E.Zartman, **comb. nov.** and *Vantanea spiritu-sancti* K.Wurdack & C.E.Zartman, **comb. nov.**) for two species formerly in *Humirias-trum*. We investigated all eleven species of *Sacoglottis* for diagnostic features that may contribute to better species delimitations, and describe *Sacoglottis perryi* K.Wurdack & C.E.Zartman, **sp. nov.** as an endemic of the Pakaraima Mountains in western Guyana. Finally, our survey across Humiriaceae for extrafloral nectaries (EFNs) revealed their presence on leaves of all extant species as adaxial basilaminar and/or abaxial embedded glands, in addition to the frequent occurrence of marginal glandular setae. The significance of inter-generic variation in gland position and anther morphology within the family are discussed.

#### **Keywords**

anthers, Duckesia, extrafloral nectaries, floral morphology, glands, Humiriastrum, Vantanea

#### Introduction

Humiriaceae contains approximately 65 species of extant trees and shrubs characterized by small white to greenish-cream (rarely red to pink) flowers and with a center of taxonomic richness in the Rio Negro Basin, the largest tributary of the Amazon River. The family is notable for its rich fossil record of distinctive, woody endocarps, and for its unusual biogeography as a nearly wholly neotropical family except for one species in tropical West Africa. The most comprehensive synoptic revision of the family or any of its eight constituent genera was published in 1961 by noted authority José Cuatrecasas. In that visionary work, Cuatrecasas radically restructured Humiriaceae with 24 new taxa, 33 new combinations, and the segregation of four new genera of which three (Duckesia [Ducke] Cuatr., Hylocarpa [Ducke] Cuatr., and Endopleura [Huber] Cuatr.) were monotypic Amazonian endemics. The genera as defined by Cuatrecasas (1961) are well delimited by clear discontinuities in floral bauplan, anther structure, endocarp structure, and/or placentation. In the subsequent half century advances in Humiriaceae systematics and morphology have been modest, including descriptions of 15 new species in four genera, floral anatomy (Narayana and Rao 1969–1977), palynology (Bove and Melhem 2000), and reconsideration of endocarp variation (Herrera et al. 2010, 2014). Due to the highly plastic leaf morphologies and complex infraspecific taxonomy (Cuatrecasas 1961, Cuatrecasas and Huber 1999), most species are poorly understood and research remains hampered by incomplete specimens that lack critical characters from both flowers and fruits.

Although Humiriaceae is clearly monophyletic and placed in Malpighiales, its relationships within the order (Wurdack and Davis 2009, Xi et al. 2012) and among constituent genera (Herrera et al. 2010, Bardon 2015) remain equivocal. The most robust, albeit still weak, ordinal placement to date by Xi et al. (2012) indicates Humiriaceae as sister to a parietal placentation clade (i.e., six families including Passifloraceae, Salicaceae, and Violaceae); however, this affiliation is not yet corroborated by compelling morphological evidence (see Endress et al. 2013). Divergence dating indicates it is among the oldest families of Malpighiales with a mean stem-group age of 105.7 Ma (110.0–101.6, 95% HPD) but with a relatively young crown-group age of 20.7 Ma (32.1–10.4, 95% HPD). Recent reevaluation of the fossil record provides evidence for an older crown-group diversification with at least three genera (*Duckesia, Sacoglottis* Mart., *Vantanea* Aubl.) by the early Oligocene (~30–28 Ma; Herrera et al. 2010, 2014).

Glandular tissues on leaves, sepals, floral disc, and/or anthers have been reported for Humiriaceae (Cuatrecasas 1961). However, foliar glands that may function as extrafloral nectaries (EFNs), in particular, have been rarely noted (i.e., Metcalfe and Chalk 1950, Belin-Depoux 1993). Cuatrecasas (1961: 88) observed that *Humiria* J. St.-Hil. leaves are "dotted with glands near the margin on the lower side" but he provided few comments for the other taxa. This lack of basic knowledge is illustrated, for example, in the online World Checklist of Extrafloral Nectaries that only lists two genera (*Sacoglottis, Vantanea*) and three species with EFNs for the family (Weber et al. 2015). EFNs are abundant in other families of Malpighiales, especially Euphorbiaceae and Passifloraceae, although their distribution is likely also underreported across the order despite accounting for 26% of all EFN records in a recent tally (Weber and Keeler 2013). The adaptive value of EFNs for promoting mutualistic interactions with insects, especially ants, is well established (see Rico-Gray and Oliveira 2007). EFN ecology usually involves nectar secretion to attract ants that act as defenders to provide plant protection from herbivores.

*Sacoglottis*, the systematics focus of this study, is distinguished among the eight genera in having the lowest stamen number (10). Morphological and molecular evidence indicates a nested phylogenetic placement for *Sacoglottis* within the family (Herrera et al. 2010, Xi et al. 2012, Bardon 2015, Zartman et al., unpublished data), and consequently its androecium likely evolved via stamen reduction from higher numbers (mostly 20–30, but 100–200+ in some *Vantanea*). In its current circumscription (sensu Cuatrecasas 1961) the genus contains 10 species of trees distributed from Nicaragua to Brazil and Bolivia, as well as West African *S. gabonensis* (Baill.) Urb. A heretofore undescribed species of *Sacoglottis* with subglobose fruits has been known for many years among variously misidentified specimens from the northern Pakaraima Mountains of Guyana. While the earliest of the 20 known collections was made in 1951 by Bassett Maguire of The New York Botanical Garden, most are from later fieldwork sponsored by the Smithsonian Institution's Biodiversity of the Guiana Shield Program, including the 2012 Kamakusa Expedition (K. Wurdack, participant; Wurdack et al. 2013) which explicitly searched for and found the species.

We herein describe this new species of *Sacoglottis* and present comparisons of its vegetative and reproductive characters in relation to the often poorly known other species in the genus. We surveyed stamen structures from exemplars of all genera to provide more detailed information on features of systematics and evolutionary interest, especially relating to anther morphology and interpretation of sporangial reductions. As a result of this objective we also present new combinations based on novel interpretations of previously overlooked floral structure for two taxa. Finally, on noting the frequent occurrence of EFNs on Humiriaceae but little mention of them in the literature, we conducted a comprehensive EFN survey of all species to document their occurrence and any qualitative generic-level distinctions.

#### Materials and methods

Scanning electron microscopy (SEM) of untreated or rehydrated-ethanol transitioned then critical point dried (CPD) herbarium fragments was conducted with a Zeiss EVO MA15 (Carl Zeiss SMT, Inc., Peabody, Massachusetts) SEM at 5–10 kV after sputter coating with 10 nm of C + Au/Pd using a Leica EM ACE600 (Leica Microsystems GmbH, Wetzlar, Germany). Light microscopy (LM) was with an Olympus DSX100 (Olympus Corp., Tokyo, Japan) and extended focus imaging (EFI) for higher magnifications, or with a Zeiss Universal Compound Microscope. Androecial diversity of the family was broadly surveyed at US and then examined in detail with SEM and/or LM for representatives for each genus, including: Duckesia liesneri comb. nov., Henderson 933 (US); D. verrucosa (Ducke) Cuatrec., Ducke 2108 (US); Ducke [MG-16325] (US). Endopleura uchi (Huber) Cuatrec., Baker 58 (US), Assunção 605 (US). Humiria balsamifera var. imbaimadaiensis Cuatrec., Wurdack 4814 (US); H. crassifolia Mart., Cowan & Soderstrom 2145 (US). Humiriastrum cuspidatum (Benth.) Cuatrec., Cid et al. 4264 (US), Ducke 243 (US); H. dentatum (Casar.) Cuatrec., Hatschbach 56145 (US); H. diguense (Cuatrec.) Cuatrec., Quizhpe et al. 612 (US); H. glaziovii (Urb.) Cuatrec., Landrum 4262 (US). Hylocarpa heterocarpa (Ducke) Cuatrec., Ducke [JBR]-30137] (US). Sacoglottis gabonensis, Gentry & Pilz 32860 (US); S. guianensis Benth., Carvalho et al. 4346 (US); S. maguirei Cuatrec., Maguire et al. 30693 (NY, US); S. perryi sp. nov., Redden 7264 (US), Tripp 2984 (US). Schistostemon macrophyllus (Benth.) Cuatrec., Maas et al. 6577 (NY); S. oblongifolius (Benth.) Cuatrec., Maas et al. 6804 (US). Vantanea bahiensis Cuatrec., Belém & Magalhães 748 (US); V. compacta (Schnizl.) Cuatrec., Hatschbach 21265 (NY); V. depleta McPherson, Hammel & Trainer 12954 (MO), McPherson & Stockwell 10892 (US); V. micrantha Ducke, Ducke [JBRJ-30135] (US); V. peruviana J.F. Macbr., Rimachi 4577 (US); V. spiritu-sancti comb. nov., Silva et al. 1436 (US). Stamen vasculature was examined after clearing with aqueous 5% (w/v) sodium hydroxide, followed by saturated chloral hydrate. For floral anatomy of Vantanea spiritu-sancti, buds were cleared of tannins with Stockwell's bleach, paraffinembedded, sectioned at 10 µm, and stained with toluidine blue O. Stamen structure descriptions follow the work of Endress (e.g., Endress and Stumpf 1990, 1991).

Character states in Tables 1, 2 were based, except where noted, on new primary observations. Fruits vary in size and shape among *Sacoglottis* species, but distinguishing true size differences from developmental differences in their slow maturing fruits is to some degree unclear given that most fruits are collected immature. Length-to-width ratios (l/w) were used as a simple shape proxy to reflect degree of elongation in their basic ellipsoid shape, and a key character in distinguishing fruiting collections of the newly described species. Based on the many available fruits of the new species at various developmental stages (although we lack truly ripe fruit), this ratio is apparently stable across a range of sub-maturity. Similar large sample sizes were not available for most other *Sacoglottis* species. A full understanding of inflorescence structure in *Sacoglottis* with its multiple orders of often dichotomous branching is beyond the scope of our study and would be difficult to carefully assess with incomplete herbarium collections. However, we did quantify peduncle and pedicel lengths; the latter usually vary several-fold within an inflorescence.

Representative leaves from nearly all Humiriaceae species and varieties were surveyed with LM for the presence of foliar glands and leaf margin features. Type collections were utilized where possible to avoid identification uncertainty, and multiple specimens were studied, especially for taxa with glands characterized as scarce or potentially absent. In some cases, distinguishing between plant structures and leaf damage, which creates circular depressions with marginal scarring, was difficult. We employed additional search criteria of expected location and positional patterns, although the laminar glands of some species are clearly sparse and erratically distributed.

#### Results

#### Androecial morphology

The flowers of Duckesia verrucosa have (22-)25 stamens of two types, including five fertile antepetalous that each alternate with a group of 4-5 sterile on less stout filaments of varying lengths. One stamen per sterile group is usually as long or longer (clearly the medial antesepalous stamen when five per group) than the fertile stamens and the rest shorter. The filaments are subulate, complanate, basally very short-connate, proximally short-papillose, and distally smoother (especially so on the fertile stamens). Abnormally short filaments (1/3-1/2 of the normal length) lacking anthers rarely substitute for sterile stamens; these are likely the occasional staminodes reported by Cuatrecasas (1961). The filaments each contain a vascular bundle that continues into the connective protrusion for nearly its entire length, and is distally branched in the fertile but unbranched in the sterile anthers. Anthers are dorsifixed below midlength, glabrous, lack stomata, caducous, and have elongate connective protrusions, which are especially thickened and fleshy in the fertile anthers. The fertile anthers are tetrasporangiate, with a pair of larger sporangia (pollen sacs) positioned dorsally at the level of the filament insertion, and a pair at the base that are slightly smaller and nearly perpendicular to the dorsal pair (Fig. 1A, B). Dehiscence of the four thecae is by flap-like valves that open from the ventral side for each lateral sporangium and move dorsally (outward), or for a basal sporangium move from distal to proximal (drop downward; Fig. 1B). The sterile anthers consist of connectives with protrusions that are thinner than those of the fertile anthers and have little trace of sporangia (Fig. 1F); those connectives can be prolonged and misshapen due to packing in bud and form filling around the fertile anthers. The 20 stamens of Duckesia liesneri comb. nov. are of two types with five long antesepalous and 15 short. The filaments are subulate, complanate, connate for up to 1/3 of their length, smooth, and contain a vascular bundle that continues into the connective protrusion and is usually unbranched (rarely with short stubby branches distally). The anthers are all fertile, tetrasporangiate and similar to D. verrucosa; however, the pairs of sporangia lack size differentiation and are close together rather than well-separated (Fig. 1C). In bud, both species have stamens with straight filaments that are longer than the anthers.

The flowers of *Endopleura* have 20–22 stamens (to 30, fide Cuatrecasas 1961) of two types. Five antepetalous and five antesepalous long stamens are tetrasporangiate with separated lateral (dorsal) and basal pairs of sporangia (Fig. 1E, I). The 10+ short anthers alternating with the long type are usually disporangiate by missing the lateral pair, or rarely with no (sterile) or three sporangia (Fig. 1D). Filaments are subulate, complanate, basally very short-connate, and short-papillose nearly their entire length; each contains a vascular bundle that continues into the connective protrusion where it is unbranched or distally branched (branching does not appear correlated with stamen type). In bud the filaments are straight with slightly sinuous tips for the longer type (likely due to packing), and as long or longer than the anthers. Anthers are dorsifixed below midlength, glabrous, versatile, lack stomata, caducous, and variously shaped due



Figure I. Stamen structure of Humiriaceae. A *Duckesia verrucosa* tetrasporangiate anther, dorsal B *Duckesia verrucosa* tetrasporangiate anther, lateral C *Duckesia liesneri* tetrasporangiate anther, lateral D *Endopleura uchi* disporangiate anther, lateral E *Endopleura uchi* tetrasporangiate anther, lateral F *Duckesia verrucosa* sterile anther, lateral G *Humiriastrum cuspidatum* disporangiate anther, lateral H *Humiriastrum cuspidatum* disporangiate anther, lateral H *Humiriastrum dentatum* disporangiate anther, lateral K *Humiriastrum dentatum* disporangiate anther, lateral K *Humiriastrum dentatum* disporangiate anther, ventral L *Humiriastrum dentatum* disporangiate anther, lateral K *Humiria balsamifera* var. *imbaimadaiensis* stamen cluster with 2 of 3 types, dorsal. f = filament attachment location. Sources: A, B, F *Ducke 2108* C *Henderson 933* D, E, I *Assunção 605* G, H *Cid et al. 4264* J, K Hatschbach 56145 L *Quizhpe et al. 612* M *Wurdack 4814* (all US.)

to distortions of the usually thickened, elongate connective protrusions from packing in bud (e.g., Fig. 1D with impressions of adjacent sporangia indenting the connective protrusion). The four thecae and dehiscence lines resemble those of *Duckesia*.

The flowers of *Humiria* have 20 stamens of three types, including five long antesepalous, 10 short and adjacent to antesepalous, and five antepetalous of intermediate length. Two (Cuatrecasas 1961) or three (Narayana and Rao 1973b) anther types have been previously reported, and we observed three types across multiple species and varieties examined. The filaments are subulate, complanate, and connate up to half their length to form a smooth androecial tube. The free tips are smooth dorsally but otherwise papillose along their margins where the projections interdigitate, and more sparingly short-papillose ventrally. The filaments of *H. balsamifera* var. *imbaimadaiensis* each contain a vascular bundle that continues into connective protrusion for nearly its entire length and is unbranched. Anthers are disporangiate, dorsifixed at or near their base, versatile, lack stomata, and caducous (Fig. 1M). The thickened, elongate apical protrusions are glabrous and variously shaped according to their type and packing in bud; the protrusions of the long stamens wrap over and conform to the stigmas. The two sporangia are coarsely hirsute, slightly divergent, and each dehiscent from the ventral side via a dorsal hinge (opening outward).

The flowers of Humiriastrum (Urb.) Cuatrec. have 20 stamens of two types, including 10 long antesepalous and antepetalous, and 10 short that are adjacent to the long (Fig. 1H). The filaments are subulate, complanate, basally short- to half-connate, and smooth to sparsely short-papillose; each contains a vascular bundle that continues into the connective protrusion for half to nearly all its length, and is unbranched (H. diguense) or branched (H. dentatum). In bud, the filaments are straight and longer than the anthers. Anthers are disporangiate, dorsifixed below midlength, delicately attached, glabrous, lack stomata, caducous, and have large, thickened apical protrusions, which for the long stamens can wrap over the stigmas with tips folded inward. Cuatrecasas (1961: 49) described the "thecae of anthers basal" in Humiriastrum versus "inferolateral" in Sacoglottis and Schistostemon. However, this distinction is blurred as both orientations occur in Humiriastrum. In H. dentatum, the sole species studied by Narayana and Rao (1973a), the two sporangia are slightly divergent (V-shaped), and dehiscent from the ventral side in the manner of the other disporangiate genera (Fig. 1J-K); this configuration also characterizes H. glaziovii. Most of the remaining species in the genus have variations on (sub)parallel (not divergent) basal sporangia. For example, the sporangia of *H. cuspidatum* (Fig. 1G) are small, positioned at the anther base so as to be perpendicular to the connective long axis, and each dehiscent by a valve opening distal to proximal (drops downward). In H. diguense, the sporangia of the long stamens are oriented parallel to the connective long axis, and each open from the dorsal side via a ventral hinge (Fig. 1L); the short stamens have smaller, often asymmetrically paired sporangia. This dehiscence configuration is opposite of that found in H. dentatum, although it also differs in other details such as sporangia not divergent.

The flowers of *Hylocarpa* (examined with LM from a single, young bud and additional fragments) have 20 stamens of two types, including 12 fertile and the rest sterile (30 stamens with 5–15 fertile according to Cuatrecasas 1961), whose exact arrangement was not determined. The filaments are straight, not obviously complanate, shorter than the anthers, and smooth; the papillose filament ornamentation reported by Cuatrecasas (1961) may occur later in development. Fertile anthers are disporangiate, dorsifixed, attached in their proximal third, glabrous, and have large, carnose, clavate connectives that are unlike the tapering apical protrusions in the other genera; sterile anthers are slightly larger with connectives that are more irregularly shaped (Fig. 3G). The basal sporangia are small and divergent on the V-shaped connective base.

The flowers of Sacoglottis have 10 stamens of two types, including five long antesepalous and five short antepetalous. The filaments are subulate, complanate, basally connate to form an androecial tube, and smooth; each contains a vascular bundle that continues into the connective protrusion for nearly its entire length and distally usually bears short stubby branches. In bud, the filaments are straight and longer than the anthers. The filament width and length of connation (1/10-2/3 of total length) vary between species and can be diagnostic (see Table 2). Anthers are disporangiate, dorsifixed near their midlength or just below, versatile via a delicate attachment, glabrous, lack stomata, and caducous (Fig. 2A, B). The two sporangia are slightly divergent (Vshaped) with dehiscence lines on the ventral side that extend over both upper and lower shoulders. The stomium of each sporangium is thus eccentric with the elliptic flap-like valve longer than its dorsal hinge which is much thinner than the thickened valve lip (Fig. 2B, C). The anther connective has a thick apical protrusion, which in bud for the short stamens is compressed against and takes on the shape of the bases of the long stamens, leading to a degree of dimorphism between the two anther-types from this space filling effect. Thin staminode-like processes were rarely observed between the stamens of Sacoglottis guianensis (Fig. 2D). The anthers of S. gabonensis are the largest in the genus (to 1.5 mm long for the long stamens; 2 mm fide Cuatrecasas 1961), but structurally otherwise resemble the other species. We reexamined the limited remaining floral material on the type of S. maguirei (anthers were not seen) and could confirm it has a Sacoglottis bauplan with 10 stamens of two alternating types; its filaments resemble those of the other species and are short, wide, and connate half their length. This species otherwise vegetatively more closely resembles small-leaved species of Humiriastrum.

The flowers of *Schistostemon* (Urb.) Cuatr. have 20 stamens of three types, including five long trifurcate antesepalous, 10 short and adjacent to the antesepalous, and five of medium length antepetalous, and have 30 anthers (Fig. 2E, F). The filaments resemble those of *Sacoglottis* and are subulate, complanate, basally connate to form an androecial tube, and smooth. However, the five long stamens have a trigonous (in transverse section) free portion of the filament that then distally trifurcates, with each branch bearing an anther such that each stamen has three anthers. The lateral (dorsal) branches in the plane of the staminal tube are shorter, and the central (ventral) branch is longer and bent slightly inward (Fig. 2G). The five trifurcate stamens of *S. oblongifolius* have three co-lateral vascular bundles per filament that diverge at the branched apex with one bundle serving each anther, while the remaining 15 stamens each have a single bundle; each vascular bundle continues into the connective protrusion to nearly the tip



Figure 2. Stamen structure of Humiriaceae. A *Sacoglottis perryi* stamen cluster of 2 types, ventral B *Sacoglottis perryi* stamen cluster of 2 types, dorsal C *Sacoglottis perryi* short-stamen anther with open stomium and pollen D *Sacoglottis guianensis* androecium with interstaminal staminodes (st), dorsal E *Schistostemon macrophyllus* stamen cluster of 3 types, ventral F *Schistostemon macrophyllus* stamen cluster of 3 types, dorsal G *Schistostemon oblongifolius* trifurcate filament tip, dorsal. Sources: A–C *Tripp 2984* D *Carvalho et al. 4396* E, F *Maas et al. 6577* G *Maas et al. 6804* (all US).

and distally usually bears short, stubby branches (Fig. 3E). The disporangiate anthers of *Schistostemon* are very similar in morphology to those of *Sacoglottis*, including in details of attachment, space-filling protrusion dimorphism, branched vasculature in the apical protrusion, slight sporangia divergence (V-shaped), and dehiscence by valves that open from the ventral side and move dorsally (outward). However, the lateral anthers of each trifurcate stamen are slightly reduced in size relative to the central one.

The flowers of Vantanea vary in stamen number across the species, ranging from 15-28 in V. depleta to >200 in V. maculicarpa Sabatier & J. Engel (fide Engel and Sabatier 2018), and vary in length from 3 mm in V. spiritu-sancti to 35 mm in V. guianensis Aubl. Stamen number can also vary within collections and species, and the positions of different types (lengths) relative to sepals and petals is indeterminate with higher numbers. The filaments are much longer than the anthers, thin, subcomplanate, of multiple slightly different lengths within a flower, usually distally sinuous in bud which is retained at anthesis, and can be basally connate to form discrete whorls or less ordered. In V. peruviana, an inner cluster is differentiated by greater connation and papillose filaments, while the outer stamens are less connate and smooth. In V. micrantha, stamen whorls are poorly differentiated and all filaments are smooth. In species with relatively few, short stamens (i.e., V. depleta, V. spiritu-sancti), the filaments are clearly a single file and complanate; each contains a vascular bundle that continues into the connective protrusion for half its length and is unbranched. Anthers are tetrasporangiate with two thecae, each containing an internal septum that delineates two pollen sacs per theca. They are dorsifixed at midlength to lower third, versatile, glabrous, lack stomata, and caducous; dehiscence is by a single longitudinal slit per theca that opens widely (Fig. 3A–D). Thecae are variable in size, shape, symmetry, and orientation among the species, including elongate, extending most of the length of the anther and lateral (V. compacta), or shorter (to subovate), limited to the lower half of the anther, and slightly divergent (V-shaped in V. depleta). A theca can be (mono)symmetric, or asymmetric due to size differences between the two constituent sporangia or lateral shifts of them relative to each other. The connective protrusions are thickened and elongate, with some variation according to species such that larger protrusions appear correlated with smaller sporangia (see Fig. 3A-D). In V. spiritusancti, the 20 stamens are clearly of three alternating lengths in the manner of Schistostemon including five long antesepalous, 10 short and adjacent to the antesepalous, and five of medium length antepetalous. Its subulate filaments are the shortest in the genus at 2-3 mm, basally connate to 1 mm, distally slightly sinuous, and minutely papillose. The anthers are similar in structure to other Vantanea spp., with four sporangia, internal septa, and dehiscence by longitudinal slits (Fig. 3D). In V. depleta we found a variable stamen number with 22-28 for Hammel & Trainer 12954, and verified a lower range of 15-18 for the type collection, McPherson & Stockwell 10892, as had been reported by McPherson (1988). Its stamen structure of alternating lengths with the longest approximately antesepalous resembles that of V. spiritu-sancti, although less fixed.



**Figure 3.** Stamen structure and anatomy of Humiriaceae. **A** *Vantanea compacta* anther, lateral **B** *Vantanea compacta* anther, dorsal **C** *Vantanea depleta* anther, lateral **D** *Vantanea spiritu-sancti* anther, lateral **E** *Schistostemon oblongifolius* clearing of androecium with 3 stamen types, ventral **F** *Vantanea spiritu-sancti* longitudinal section of ovary with 2 superposed ovules per locule; lower left ovule partial **G** *Hylocarpa hetero-carpa* anthers (left to right): disporangiate dorsal, ventral; sterile, dorsal. Sources: **A**, **B** *Hatschbach 21265* **C** *Hammel & Trainer 12954* **D**, **F** *Silva et al. 1436* **E** *Maas et al. 6804* **G** *Ducke [JBR]-30137]* (all US).

#### Extrafloral nectaries and foliar glands in Humiriaceae

Foliar glands were found to be present on 64/65 accepted species and 22 infraspecific taxa of Humiriaceae (85 taxa examined, 100% presence; Appendix 1, Fig. 4). The remaining species not seen, recently described *Vantanea maculicarpa* Sabatier & J. Engel, also has scattered abaxial glands reported (Engel and Sabatier 2018). Gland distribution ranges from relatively regular within a species, to variable between collections or even leaves on the same specimen. The two basic positional types include: (1) adaxial leaf base (basilaminar) glands as 1–3(7) more or less symmetric pairs spanning the midvein (Fig. 4B, C, F), and (2) embedded laminar glands as rows (Fig. 4A, D, J) or scattered on the abaxial (rarely also adaxial) surface (Fig. 4I). Both types of glands are circular to elliptic, sunken (especially the laminar glands) or slightly raised, have



Figure 4. Extrafloral nectary and leaf margin diversity of Humiriaceae. A Vantanea depleta laminar glands, abaxial B Duckesia liesneri basilaminar glands, adaxial C Schistostemon oblongifolius basilaminar glands, adaxial D Humiriastrum ottohuberi laminar glands, abaxial E Duckesia verrucosa robust seta at margin F Sacoglottis guianensis basilaminar glands, adaxial G Humiria fruticosa shoot tip with marginal glands exposed on expanding new leaf H Humiria fruticosa marginal gland I Hylocarpa heterocarpa laminar gland, abaxial J Humiria balsamifera var. minarum dense row of marginal glands, abaxial K Duckesia verrucosa laminar gland, abaxial L Schistostemon retusus darkened scar from deciduous seta M Schistostemon retusus intact seta at margin. g = gland, s = seta scar. Sources: A Mori & Kallunki 4889 B Liesner 22589 C Maas et al. 6804 D Maguire 34912 E, K Ducke 2108 F Jansen-Jacobs et al. 1898 G, H Steyermark 103255 I Ducke [JBRJ-30137] J Mexia 5815 L Redden 3372 M Cuatrecasas 7203 (all US).

rims that are variously thickened, an outer corona free of stomata, and smooth surfaces without pores (Fig. 4H, K; 5C). In herbarium specimens the gland epidermis can be detached from the underlying gland and appear pale or whitish, which likely indicates a fluid-filled reserves pocket in the living plants (Fig. 4F, J). The extremes of gland size range from relatively large, elliptic  $(1.3-1.4 \times 0.8-0.9 \text{ mm on } Hylocarpa heterocarpa;$ Fig. 4I) to tiny, circular (<0.1 mm diameter on Sacoglottis maguirei) structures. The laminar glands are most frequently arranged in a row near or on the margin (Fig. 4D, J) but they can also be in a row at a lesser distance from the midvein (Fig. 4A), or more rarely widely scattered. The glands away from the margins are often clearly associated with higher order veins (i.e., secondaries, intersecondaries) or otherwise intercostal. The thickened lamina often obscure higher order venation that is likely associated with intercostal glands. The glands are very sparse and/or difficult to distinguish in Duckesia and some Humiriastrum (i.e., H. melanocarpum [Cuatrec] Cuatrec., H. columbianum [Cuatrec.] Cuatrec.), and many other taxa have only a subset of gland positions (i.e., either adaxial basilaminar or abaxial laminar). For example, the scattered abaxial laminar glands in Duckesia verrucosa are only a few per specimen with most leaves lacking glands. Damage to basilaminar glands, such as appearing to be chewed, was sporadically observed and may be due to insect predation.

In *Sacoglottis* the paired adaxial glands usually occur near the base of the leaf blade (Fig. 4F), and details of their position, size, and shape can differ between species. The circular basilaminar glands of *S. gabonensis*, when present (lacking on some leaves), are unusual in appearing positionally where the first proximal teeth would be along the crenate margin. However, they lack setae associated with teeth and resemble typical basilaminar glands. The other species of *Sacoglottis* have basal glands (except clearly absent in *S. maguirei*) that are more typically basilaminar and adaxial rather than marginal. Most of the other foliar glands in *Sacoglottis* are tiny, in a row near the abaxial margin, and without thickened rims (Fig. 5C), although they can be variable in size, position, and number among species. *Sacoglottis guianensis* has considerable intra- and inter-specimen variation in the number of foliar glands. The glands of *Sacoglottis maguirei* are obvious on young unexpanded leaves, and then become nearly invisible small pits on mature leaves. The deciduous stipules in *Sacoglottis* are morphologically similar to the marginal glandular setae (Fig. 5A, E).

The leaf margins are either untoothed and entire (*Humiria, Vantanea*), or have glandular setae at regular intervals associated with teeth of varying degrees of prominence (remaining taxa). The non-entire margins are variously described herein (Appendix 1) and in the literature as crenate(-serrate), crenulate(-serrate), or serrate; they are rarely subentire and nearly smooth with little evidence of setae. This terminology reflects a continuum among species in the height and curvature of their small teeth. Most species have asymmetric teeth that are shallow and rounded (crenate), and more rarely have pointed projections with strongly concave distal flanks (serrate). *Humirias-trum melanocarpum* with leaves described as having "the margin slightly crenate with small glands" (Cuatrecasas 1961: 154) also has typical non-entire margins but more prominent darkened attachments for the setae. The setae appear deciduous in almost



**Figure 5.** Micromorphology of *Sacoglottis perryi*. **A** Marginal glandular seta **B** basilaminar gland, adaxial **C** laminar gland near margin, abaxial **D** sepal tip, inner side with terminal gland **E** paired glandular stipules and petiole scar **F** pollen inside sporangium **G** stigma with ephemeral lobes intact and showing secretion **H** stigma lobes shredded showing thin walls **I** gynoecium with diagnostic hirsute ovary **J** glandular disc with erose margin. Sources: **A–C** *Gillespie 2810* **D–J** *Tripp 2984* (all US).

all species, usually rapidly so from young expanding leaves, and leave scars with sometimes darkened, glandular-appearing points of attachment to the leaf that can be used to infer their distribution in the absence of rarely collected young leaves (Fig. 4L, M). The setae have fairly uniform morphology with modest variation in size, although those of *Duckesia verrucosa* are distinctive in smooth cylindrical shape which is not collapsed on drying and greater persistence (Fig. 4E). In *Humiria* the abaxial glands are in a (sub)marginal row, 0–0.2 mm from the edge, and associated with slight sinuses so as to form a margin best described as repand or slightly undulate (Fig. 4J). This distinctive margin type clearly does not have setae or associated teeth. The few glands at the leaf base of *Humiria fruticosa* Cuatrec. are precociously exposed (Fig. 4G) prior to full unrolling of the young leaf (supervolute-involute ptyxis), and present an aspect not seen in other *Humiria* taxa which otherwise all have their usually more distal marginal glands hidden in tight involute laminar rolls. The 14 varieties of *Humiria balsamifera* Aubl. span considerable variation in leaf features including in marginal gland density and lamina size.

#### Taxonomic treatment

Duckesia liesneri (Cuatrec.) K.Wurdack & C.E.Zartman, comb. nov. urn:lsid:ipni.org:names:77198710-1

Humiriastrum liesneri Cuatrec., Phytologia 71: 165. 1991. Type: VENEZUELA. Amazonas, Depto. Rio Negro: Cerro Aracamuni, summit, Proa camp, 1400 m, 28 Oct 1987, *R. Liesner & G. Carnevali 22589* (holotype: US-3111383; isotypes: MO-3641994, NY-00329209, US-3118837, VEN-277523).

**Notes.** When Cuatrecasas (1991) originally described *Humiriastrum liesneri*, he expressed uncertainty about its anther structure and relationships due to reliance on young buds for floral details. A second collection (Venezuela. Amazonas, Sierra de Unturán, 1150 m, 3 Feb 1989, *Henderson 933*, US), correctly annotated by Cuatrecasas as *H. liesneri* in 1994, is in flower but did not cause any classification changes for the subsequent Flora of the Venezuelan Guayana (Cuatrecasas and Huber 1999). The anther morphology of this species (Fig. 1C), with its four separate sporangia, suggests an affiliation with *Duckesia* or *Endopleura*, and the transfer here to the former is supported by more similar connective morphology, absence of disporangiate anthers, and similar small leaves (Table 1). The two species of *Duckesia* are otherwise very distinct from each other; fruits remain unknown for *D. liesneri* and would likely provide additional distinguishing characters. The two collections of *D. liesneri* are ca. 60 km apart on isolated Venezuelan tepuis near the Brazilian border and north of *D. verrucosa*, which is a lowland Brazilian species.

Characters	Duckesia verrucosa	Duckesia liesneri	Endopleura uchi
Habit	Tree, 12–30 m (27–67 cm dbh)	Shrub to small tree, 2–4 m	Tree, (12)20–40 m (30–70 dbh)
Leaves	Petiole to 1 mm long; blade 5.5–7.5 ×1.7–2.3 cm, lanceolate, subcoriaceous; teeth setae robust, to 0.2 mm long, sometimes retained at maturity; abaxial glands sparse, 0–3 per leaf, 2/3 exmedial distance	Petiole to 2 mm long; blade 3.5–4.5 × 2.2–2.7 cm, elliptic to obovate, coriaceous; teeth setae delicate, to 0.3 mm long, rapidly deciduous; abaxial glands absent	Petiole 10–30 mm long; blade 17–28 × 5.5–6.5 cm, oblong to narrowly elliptic, subcoriaceous; teeth setae delicate, to 0.5 mm long, rapidly deciduous; abaxial glands in row near margin
Inflorescence	Axillary, small, to 1.5 cm long; bracts persistent	Axillary and subterminal, to 2.5 cm long; bracts deciduous	Axillary, to 6.5 cm long; bracts persistent
Flower	Calyx glabrous with minutely hispid margin; petals glabrous	Calyx hispid; petals coarsely hirsute	Calyx centrally coarsely hirsute, margin minutely hispid; petals coarsely hirsute
Androecium	Filaments papillose, very short-connate; anthers 5 tetrasporangiate and 15+ sterile	Filaments smooth, connate up to 1/3 length; anthers 20 tetrasporangiate	Filaments minutely papillose, very short-connate; anthers 10 tetrasporangiate and 10+ disporangiate
Gynoecium	Pistil longer than ovary	Pistil shorter than ovary	Pistil as long as ovary
Fruit	Ovoid; endocarp corrugated, 13+-radiate with densely packed woody ribs; resinous cavities small; valves conspicuous	Unknown	Ellipsoid; endocarp star-shaped, 10-radiate with 5 divided woody ribs; resinous cavities absent; valves inconspicuous
Distribution & ecology	Brazil (Amazonas especially near Manaus, Pará); 50–125 m elevation, terra firme	Venezuela (Amazonas on Cerro Aracamuni and Sierra de Unturán); 1150–1400 m elevation, upland cloud or semi- open tepui forest	Bolivia (Pando), Brazil (Amapá, Amazonas, Pará, Rhondônia) Guyana, Suriname, Venezuela; 50–620 m elevation, terra firme

Table 1. Comparison of select diagnostic features for Duckesia and Endopleura.

#### *Vantanea spiritu-sancti* (Cuatrec.) K.Wurdack & C.E.Zartman, comb. nov. urn:lsid:ipni.org:names:77198711-1

Humiriastrum spiritu-sancti Cuatrec., Ciencia, Mexico 23(4): 137. 1964. Type: BRA-ZIL. Espírito Santo, Mun. de Santa Tereza: Lombardia, 25 Jan 1954, *G. Dalcolmo s.n.* (holotype: RB-86212; isotype: US-2827596).

**Notes.** *Humiriastrum spiritu-sancti* was poorly known until recent ample collections allowed a fuller characterization. Giordano and Bove (2008) described its thecae as unilocular, ovary cells as uniovulate, and fruits as having five apical foramina. The transfer here to *Vantanea* is supported by anther structure that we interpret as two lateral bisporangiate thecae with internal septa (Fig. 3D; see Results), two superposed ovules per locule (Fig. 3F), apparent lack of foramina, as well as previously reported pollen details (Bove and Melham 2000), and preliminary molecular phylogenetic placement (Zartman et al., unpublished data). Upon examination of endocarps from *Amorim et al. 1391* (US-3258470, as sectioned for Herrera et al. 2010), as well as the illustration from Giordano and Bove (2008), we could not find well-developed apical foramina that otherwise characterize *Humiriastrum*. Furthermore, the androecial bauplan of *V. spiritusancti* with a low number of alternating-length stamens is similar to that of *V. depleta*.

#### Sacoglottis perryi K.Wurdack & C.E.Zartman, sp. nov.

urn:lsid:ipni.org:names:77198606-1 Figures 5–7

**Diagnosis.** Differs from *Sacoglottis guianensis* in smaller elliptic leaves, smaller shortpedunculate inflorescences with deciduous bracts, glandular sepals, hirsute ovaries, and subglobose fruits.

**Type.** GUYANA. Cuyuni-Mazaruni Region: Below 1<sup>st</sup> escarpment (of four) of Kamakusa Mt., Powis Creek (2<sup>nd</sup>) Camp, gallery forest, 5°48'26.7"N, 60°14'6.9"W, 662 m, 20 May 2012 [fl], *K. Redden 7264* (holotype: BRG, isotypes: K, NY, US-3694797). Note: type tree of 10 cm dbh was cut down (Fig. 6H) during sampling and provided a wood sample (K. Wurdack, personal observation).

Description. Habit usually small tree, 6-8 m, 10-12 cm dbh (to 14 m, 50 cm dbh), trunks lacking buttresses; bark rough, scaly, inner bark coarsely fibrous, dark brown; wood reddish-brown, diffuse porous, growth rings distinct and delimited by a fibrous zone, vessels narrow, mostly solitary, tyloses present, axial parenchyma scanty paratracheal, rays conspicuous (wood described from a 4 cm diam. trunk section); lateral leafy twigs 2-3 mm diam., puberulous, trichomes to 0.1 mm long. Stipules ca. 0.6 × 0.3 (at base) mm, narrowly triangular (rarely with smaller secondary lobes), thickened, glandular, rapidly deciduous. Leaves alternate, distichous on lateral branches, simple; petioles 6-10 (long)  $\times 1$  (wide)  $\times 0.8$  (high) mm mid-petiole, subterete (dorsiventrally slightly flattened), proximally slightly pulvinate, distally expanded to 2× wider with narrow wing extending from margins of lamina along shoulders of petiole, sparsely puberulous; blades 4.4-10.8 × 1.7-4.6 cm, length:width ratio 2.19-2.59:1 (mean = 2.37, SD = 0.256, n = 100 from 10 mature leaves × 10 collections), oblong to ovate, base angle obtuse, base obtuse to rounded, apex angle acute, apex shape acuminate with drip tip 0.5-1.5 cm long, tip ending in minute apiculate glandular tooth at distal apex of midvein; subcoriaceous, abaxially sparsely puberulous, adaxially glabrous; basilaminar glands (0-1)2, usually symmetric as pair, along adaxial margin of leaf base,  $0.2-0.5 \times 0.1-0.2$  mm, widely to narrowly elliptic; abaxial laminar glands sparse, up to 4 per side, 0.2–0.7 mm from edge, 0.1 mm wide, nearly circular, slightly sunken; margin shallowly crenate, darkened glandular spots in sinuses, 0.2-0.3 mm diam., when young these spots bearing deciduous glandular setae,  $0.3 \times 0.1$  mm; dark green above and light green below in life; venation pinnate, brochidodromous; secondaries 8-9 pairs, excurrent attachment; intersecondaries frequent. Inflorescences axillary, small (shorter than leaves), <20 flowers, 3-4 orders of branching, peduncle to 1 mm long, rachis 5–10(20) mm long, internal internodes successively shorter in higher order branches, to 2.5 mm long on secondary branches, terminal branches (pedicels) subsessile to 0.5 mm long; bracts  $1.8 \times 1-1.5$  mm, obtuse to rounded, margin entire, sparsely puberulous, rapidly deciduous leaving joint scars. Flowers bisexual, actinomorphic, mature buds  $4-4.5 \times 1.5-2$  mm; calyx cupular, sepals 5, equal, free to base of ovary (when observed from inside), connate at base of receptacle for 1-1.5 mm, imbricate, free portion widely rounded to minutely retuse, 1 × 1.5 mm, sparsely short



**Figure 6.** Macromorphology of *Sacoglottis perryi*. **A** Dried fruit with central seed embedded in woody endocarp, longitudinal split along carpel wall (dissected by Cuatrecasas) **B** dried fruit with 2 central seeds and endocarp lacunae, transverse section **C** fresh fruit with fleshy yellow-green exocarp and liquid in endocarp lacunae, transverse section **D** young inflorescence with bracts intact (b) or fallen leaving bract scars (bs) **E** mature bud with marginal sepal gland (small red dot in center) **F** partly open flower with intact anthers **G** post-anthetic flower **H** freshly cut trunk **I** type in life just before pressing. Sources: **A** *Gillespie* 2810 **B** *Tripp* 2984 **C**, **E**-**I** *Redden* 7264 **D** *Hoffman* 1600 (all US).

puberulent (trichomes shorter than elsewhere on plant) inside and out, margin finely ciliate, hyaline, usually with single gland ca. 0.1 mm diam. at minutely retuse apex, in life sepals green and gland red; petals 5, free,  $4-4.5 \times 1$  mm, oblong-lanceolate, tip acute, thick with narrow hyaline entire margin, glabrous, aestivation quincuncial, greenish-white in life and reflexed at anthesis; stamens 10, glabrous, stiffly erect in bud

and at anthesis, dimorphic, alternating in 2 lengths; 5 antesepalous longer, filaments 3.5-3.6 mm long; 5 antepetalous shorter, filaments 3 mm long; filaments of both lengths subulate, complanate, basally connate to 1.5 mm and tips free, 0.3-0.5 mm wide at start of free portion  $\times <0.1$  mm thick, in life greenish-white; anthers slightly dimorphic and differing in length of connective apex due to tight packing in bud which prevents full development of shorter anthers, antesepalous  $0.8-0.9 \times 0.5$  mm, antepetalous  $0.6-0.7 \times 0.5$  mm, dorsifixed; connective thickened, apical tip acute, base V-shaped and forcing pollen sacs to slightly diverge distally; pollen sacs 2 per anther (disporangiate), ca. 0.3 mm long, in proximal half of anther, stomium narrowly elliptic, covered by valve with a thin dorsal hinge and opening with a ventral lip, yellow-orange in life; ovary  $1-1.2 \times 1.2-1.3$  mm, subglobose, densely hirsute with long trichomes to 0.5 mm; disc 0.5-0.6 mm high, thin, cupular, margin erose with rounded irregular lobes to 0.1 mm high; style  $2-2.5 \times 0.3$  mm, single, columnar, with distinct discontinuity and slightly thinner for distal 1/3, glabrous; stigma minutely 5 lobate-capitate. Infructescence: 1-2 fruits maturing per inflorescence; fruit stalk 5-8 × 1 (diam.) mm, consisting of pedicel and 1-2 inflorescence nodes; petals and anthers caducous, sepals and filaments persistent, filaments forming protective palisade-like sheath around young developing fruit, stigmas and distal thinner part of style senescing rapidly. Fruit drupaceous, 33-35 × 23-30 mm (nearly mature, but ultimate size unknown), length:width ratio 1.07-1.44:1 (mean = 1.23, SD = 0.107, n = 19 from 9 collections, excluding *McDowell 2993*), sub-globose to ellipsoid, tip short apiculate, glabrescent but sparse trichomes remaining at apex, surface smooth when fresh, when dry sometimes slightly dimpled due to underlying endocarp, exocarp 0.5-1 mm thick; endocarp woody, obscurely bullate on surface due to underlying lacunae, with 3 slight longitudinal furrows, brown, interior with numerous lacunae; lacunae 1.5-5 × 1-4 mm, asymmetric ellipsoidal, greatly varying in size and exact shape within a fruit, in life filled with watery light brown fluid that dries to form thin dark brown, glassy, resinous layer; walls between lacunae <0.05 mm (translucent)-0.5 mm, grading thicker at vertices between multiple lacunae, distinctly thickened around seeds (locule cavity wall) and along the indistinct carpel sutures; locule snug around seed. Seeds 1-3 per fruit, 15 (long)  $\times$  5–6 (deep; parallel to embryo)  $\times$  4–5 (wide; perpendicular to embryo) mm, oblong, (sub)circular in transverse section, proximal end rounded, distal (hilar) end attenuate; coat thin, papery, brown; endosperm copious, fleshy, oily.

**Etymology.** The specific epithet commemorates Claudius Perry (1977–2011; Kelloff et al. 2011: plate 2A), a Wapishana Amerindian originally from Marunawa, who planned to be on the type-collecting trip but tragically perished on 14 Jun 2011 when a portion of his home on the Dadanawa Ranch collapsed during a storm. Although by trade a vaquero (cowboy) in the Rupununi savanna region of southern Guyana, he also served as a parataxonomist and guide on many plant and animal research expeditions (1996–2010) across Guyana. He was especially valued in the many botanical expeditions lead by H. David Clarke (Kelloff et al. 2011), Karen Redden, and the second author. He accompanied both authors herein, had a keen eye for plant diversity, and was a highly skilled collector, especially of trees that he enjoyed rapidly climbing with

spikes. He participated in expeditions that yielded the paratype collections of *Forbes* 325 and *Redden* 6582, and personally gathered the latter.

**Distribution and ecology.** Sacoglottis perryi is apparently confined to the Pakaraima Mountains of Guyana, and most localities are along mid-elevation (500-800 m) primary and secondary creeks in the headwaters of the Mazaruni River basin. It should be expected in other upland areas of the Mazaruni watershed including in adjacent Venezuela and perhaps Brazil. The tree typically grows in gallery forests at the edge of those waterways, or in forest patches in the white-sand savanna/forest mosaics of the region. Flowering occurs in May-June, with the fruits slowly developing over the course of a year. The timing of fruit ripening, likely in the fall, and features of the ripe fruit are unclear. The Kamakusa collections with the largest fruits on any specimens were hard and green when fresh (Fig. 6I; K. Wurdack, personal observation). In other species (e.g., S. guianensis; see Holanda 2013) the exocarp turns from green to deep orange, reddish, or brown, and can be fleshy and sweet. The fruits of Sacoglottis spp. are buoyant, leading to long distance transport by water (e.g., as ocean drift); they have also been documented to be dispersed by bats (Lobova et al. 2009) and monkeys (van Roosmalen 1985). The accessible position of the fruit of S. perryi on thin, pendulous, distichous-leaved branchlets over small waterway corridors appears advantageous for both bat and water dispersal.

Additional collections examined. GUYANA. Cuyuni-Mazaruni Region: Imbaimadai, Partang River along riverbank past first rapids NE of base camp, 05°42'10.5"N, 60°16'50.1"W, 873 m, 2 Dec 2002 [fr], Forbes 325 (US); Maipuri Falls, Karowrieng River, 05°41'N, 60°14'W, 570-600 m, 20 Dec 1989 [fr], Gillespie 2810 (US); Imbaimadai Creek, 1 km W of Imbaimadai, 05°42'N, 60°18'W, 500 m, 16 May 1992 [fl, fr], Hoffman 1600 (NY, US); basecamp 8.6 km NE [of] Imbaimadai on Partang River tributary, 0.75 km E, 05°46'N, 60°15'W, 625 m, 20 May 1992 [fl, fr], Hoffman 1745 (MO, NY, US); basecamp 8.6 km NE Imbaimadai on Partang River tributary, 1.25 km E, 05°46'36"N, 60°15'49"W, 600 m, 20 May 1992 [fl], Hoffman 1755 (MO, NY, US); Mt. Aymatoi (sandstone), 05°55'N, 61°W, 1150 m, 16 Oct 1981 [fr], Maas et al. 5753 (MO, US); Imbaimadai Savannas, Upper Mazaruni River, 24 Oct 1951 [fr], Maguire & Fanshawe 32250 (MO, NY); Sagaraimadai Savanna, Upper Mazaruni River, 16 Nov. 1951 [fr], Maguire & Fanshawe 32619 (MO, NY); from Utshe River to Great Falls on Kamarang River, 4-5 km SE of Utshe camp, 05°43'N, 61°07'W, 850-975 m, 26 May 1990 [fl], McDowell 2920 (MO, NY, US); 7 km N of Paruima Village, after descent from south face of Mt. Waleliwatipu, 05°54'N, 61°02'W, 980-1060 m, 30 May 1990 [fl, fr], McDowell 2993 (MO, NY, US-2 sheets); to plateau [at] S end of Haiamatipu, 05°28'N, 60°32'W, 610–914 m, 20 Jun 1991 [fl], McDowell 4734 (NY, US); Imbaimadai Creek, W of Imbaimadai, 05°42'N, 60°18'W, 503 m, 22 Jun 1986 [fr], Pipoly 7990 (MO, NY, US); Vicinity of Chinoweing Village, 5°32'N, 60°07'W, 650-670 m, 21 Feb 1987 [fr], Pipoly 10484 (NY-2 sheets, US); Imbaimadai, Karowrieng River, towards waterfall 2.32 mi E of Base Camp 1, bordering Karowrieng Creek, 5°40'42.4"N, 60°14'30.8"W, 575 m, 22 Jan 2004 [fr], Redden 1489 (US); Mazaruni River, just above ABC Falls, trail/track 2.03 mi SW of Base Camp 6 heading



**Figure 7.** Illustration of *Sacoglottis perryi*. **A** Habit **B** bud **C** bud with petals removed **D** open flower, axial **E** open flower, lateral **F** gynoecium **G** stamen cluster of 2 types, ventral **H** floral diagram **I** post-anthetic flower **J** young fruit **K**, **L** fruit **M** fruit with 2 central seeds and endocarp lacunae, transverse section. Source: **A–M** from specimens and life photos of *Redden 7264* (US).

E, 6°4'25.2"N, 60°39'14.3"W, 605 m, 19 Feb 2004 [young fr], *Redden 2008* (US); Kako River, top of waterfall, 05°28'50.8"N, 60°50'49.3"W, 687 m, 15 May 2009 [fl, fr], *Redden 6582* (US); below 1<sup>st</sup> escarpment (of four) of Kamakusa Mt., Powis (2<sup>nd</sup>) Camp and vicinity, along creek bank at camp 5°48'34.6"N, 60°14'21.5"W, 651 m, 21 May 2012 [fl, fr], *Tripp 2984* (US); Kako River, gallery forest near rapids, 05°30'27"N, 60°50'30.3"W, 505 m, 10 May 2009 [fl], *Wurdack 4911* (US); below 1<sup>st</sup> escarpment (of four) of Kamakusa Mt., Powis (2<sup>nd</sup>) Camp and vicinity, along creek bank at camp 5°48'34.6"N, 60°14'21.5"W, 651 m, 13 Jun 2012 [young fr], *Wurdack 5898* (US).

# Discussion

#### Sacoglottis, and delimitation and morphology of newly described S. perryi

The 11 species of Sacoglottis are compared in Table 2 (gland details in Appendix 1), which highlights important diagnostic characters such as inflorescence structure (peduncle and bracts), sepal glands, ovary vestiture, and fruit shape. Especially significant is the densely hirsute ovary, a feature that only occurs in S. perryi (Fig. 51) and Central American S. trichogyna Cuatrec. All other species in the genus have glabrous ovaries. Besides ovary vestiture, no other aspect of morphology suggests a closer relationship between S. perryi and S. trichogyna, and their fruit structures are very different (Herrera et al. 2010: fig. 2N). The leaves of *S. perryi* are the second smallest in the genus after diminutive *S*. *maguirei*, and have a very pronounced drip tip and an shallowly crenate margin (Fig. 6I, 7A). Sacoglottis perryi possesses the full suite of glandular structures known for the family, including small abaxial and basilaminar glands, rapidly deciduous marginal setae, thickened stipules similar to the setae, gland-tipped sepals, a floral disc, and thickened connectives (Fig. 5A-E, J). The minute stigmas appear to secrete and consist of five hollow (presumably fluid-filled in life) thin-walled lobes that rapidly degrade atop a sturdy, persistent style (Fig. 5G, H). This unusual morphology appears typical for the genus and the family in general. The small, subspheroidal, tricolporate, microreticulate pollen of S. perryi (Fig. 5F) resembles that of other species in the genus (see Bove and Melhem 2000). Four species of Sacoglottis are now known from Guyana including S. amazonica Mart., S. cydonioides Cuatrec., S. guianensis, and S. perryi. The fruit shape of S. perryi is subglobose and intermediate between globose S. cydonioides and more elongate S. amazonica and S. guianensis. The fruits of McDowell 2993 are more elongate (length:width ratio 1.37–1.63:1, mean = 1.47, SD = 0.108, n = 4) than those typical for *S. perryi*, but in other characters the collection agrees with the species. The endocarp morphology of S. perryi does not resemble that of a putatively undescribed Sacoglottis only known from well-weathered ocean drift endocarps (van der Ham et al. 2015).

The biogeography of *Sacoglottis* includes disjunctions with clear long-distance dispersal to Africa for *S. gabonensis* and to Isla del Coco far off the Pacific coast of Costa Rica for endemic *S. holdridgei* Cuatrec., and there is direct evidence of contemporary and fossil sea drift (Herrera et al. 2012, 2014; van der Ham et al. 2015). Mostly
Characters	amazonica	ceratocarpa	cydonioides	gabonensis	guianensis	holdridgei	maguirei	mattogrossensis	ovicarpa	perryi	trichogyna
Inflorescence structure, peduncle/ pedicel lengths	Short pedunculate, 5–6 / 0.3–1 mm	Very short pedunculate, <1 / < 0.5 mm	Long pedunculate, 20–40 / 0.5–2 mm	Long pedunculate, 10–30 / <0.5 mm	Pedunculate, (2)5–35 / 0.3–3 mm	Short pedunculate, few flowered, 1–4 / 0.2–1 mm	Pedunculate, 7–15 / 1–4 mm	Long pedunculate, 15–40 / 1.5– 4.5 mm	Short pedunculate, 1–2 / < 0.5 mm	Very short pedunculate, to < 1 / < 0.5 mm	Pedunculate, clustered, 3(–6) / 0.1–0.5 mm
Bracts	Deciduous; triangular, carinate	Deciduous; shape unseen	Persistent; acute	Deciduous; triangular, carinate	Persistent; acute	Deciduous; rounded	Persistent; short, rounded	Persistent; acute	Deciduous; acute, carinate	Deciduous; obtuse to rounded	Deciduous; rounded
Sepal features	Multiple tooth-like marginal glands per sepal; margin short ciliate	No glands; sparsely short puberulent	No gland; glabrous, margin ciliate	No glands; sparsely hirsute, margin ciliate	No glands; glabrous, margin ciliate	No glands; glabrous, margin short ciliate	No glands; hispidulous	No glands; glabrous	0–1 apical gland per sepal; sparsely short puberulent, margin short ciliate	1 apical gland per sepal; sparsely short puberulent, margin ciliate	No glands; glabrous to sparsely pubescent
Petal vestiture	Glabrous	Glabrous	Hispidulous, fide Cuatrec.	Hirsute (subsericeous, fide Cuatrec)	Glabrous to puberulous	Glabrous	Hispidulous.	Glabrous	Glabrous	Glabrous	Glabrous
Filament connation (relative length)	Connate 1/3	Long connate 2/3	Connate 1/2	Short connate <1/5 (barely beyond sepals)	Connate 1/3	Connate 1/2	Connate 1/2	Connate 1/4	Connate 1/2	Connate 1/3	Very short connate ca. 1/10
Ovary vestiture	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Hirsute	Hirsute
Fruit shape; average length:width ratio	Ellipsoid; 1.4:1	Subfusiform, elongate; ca. 2.35:1	Globose; 1.02:1	Subglobose; 1.17:1	Ellipsoid- oblong; 1.6:1	Ellipsoid; 1.3:1	Subglobose; ca. 1.1:1 (very young fruit only)	Globose; 0.92:1	Ellipsoid; ca. 1.2:1 (partly dissected fruit)	Subglobose to ellipsoid; 1.23:1	Ellipsoid, large; 1.43:1
Exocarp thickness	1.5 mm	1 mm	1–2 mm	1 mm	1–2 mm	1–2.5 mm	Unknown	(0.8)1.5–2 mm	Thick, to 5 mm	0.5–1 mm	Thick, 3–6 mm
Distribution	Brazil (Amazonas, Pará), Venezuela (Delta Amacuro), Trinidad	Columbia (Vaupes), Brazil (Amazonas), Venezuela (Amazonas)	Brazil, Guyana, Surinam, Venezuela	Sierra Leone to Angola	Brazil, French Guiana, Guyana Surinam, Venezuela,	Costa Rica (Isla del Coco)	Venezuela (Amazonas: Cerro Yapacana)	Brazil (Bahia, Pará)	Colombia (Valle del Cauca)	Guyana (Cuyuni- Mazaruni: Pakaraima Mtns.)	Nicaragua, Costa Rica, Panama

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Amazonian Sacoglottis amazonica occurs in Delta Amacuro, Venezuela and reaches the nearby island of Trinidad. Otherwise the genus and indeed the entire family oddly lack a West Indian presence (Acevedo-Rodríguez and Strong 2012). Sacoglottis perryi is geographically relatively isolated from other species in the genus. It only overlaps with S. guianensis based on a single collection (Gillespie 2939, US) from the vicinity of Imbaimadai that has typical elongate fruit and a long-pedunculate inflorescence. Otherwise, S. guianensis occurs further south in the Essequibo River watershed (i.e., southern Pakaraima Mountains and Acari Mountains on the border with Brazil) and is broadly distributed elsewhere in northern South America. We reexamined the identity of the Schomburgk brothers' collections of S. guianensis ([Rob.] Schomburgk, 2nd ser. 571 but likely mistranscribed for 574, P; 574, F, G, NY, P; Rich. Schomburgk 842, F image ex B, US) from Guyana, as they collected in or near the Pakaraima Mountains, and the lectotype, Schomburgk 574 (Cuatrecasas 1961), is from "Roraima" at the drainage divide of the Mazaruni watershed. All of these specimens have long-pedunculate inflorescences and cannot be confused with S. perryi. The Schomburgk expedition routes, including the approach to Mt. Roraima, skirt around the Mazaruni headwaters and were not within the presently known range of S. perryi (Roth 1923, van Dam 2002). A sterile collection (*Guppy 308*, NY) from southern Guyana, attributed by Cuatrecasas (1961) to S. amazonica, appears based on leaf similarities to be S. guianensis. The Humiriaceae of Guyana now totals six genera and 13 species (Funk et al. 2007).

The significance of inflorescence structure variation in Sacoglottis has not been previously emphasized, although it is implicit in details of the descriptions and key of Cuatrecasas (1961). His first species-key couplet relates to deciduous versus persistent bract condition, which appears correlated with other aspects of inflorescence architecture. Taxa with persistent bracts have long-peduncled, often large inflorescences, while those with deciduous bracts have short to non-existent (giving the appearance of multiple inflorescences per leaf axil) peduncles and generally smaller inflorescences. The deciduous bracts are rapidly lost, and bract morphology can be hard to document without very young inflorescences. An exception to this inflorescence structure dichotomy is Sacoglottis gabonensis, which has deciduous bracts and a long pedunculate (1-3 cm) inflorescence. Sacoglottis maguirei, a tepui endemic known only from the type collection (Maguire et al. 30693), has been mischaracterized as deciduous (Cuatrecasas 1961), but possesses previously overlooked very short, persistent bracts that are consistent with its long-peduncled inflorescence architecture. The bract condition for the other genera of Humiriaceae is persistent (Endopleura, Humiria, Hylocarpa), deciduous (Vantanea), or mixed (Duckesia, Humiriastrum, Schistostemon).

*Schistostemon*, presently containing nine species, was formerly a subgenus or section of *Sacoglottis* and elevated to a genus principally based on differences in androecial structure with a doubling of stamen number to 20, an increase to 30 anthers, and three stamen types instead of two (Cuatrecasas 1961). Of particular note in differentiating *Schistostemon* are the antesepalous longest stamens that are trifurcate at their apex to support three anthers per stamen (Fig. 2G, 3E). An overlooked difference between the genera is in pistil morphology, which is thin and as long or longer than the stamens in

*Sacoglottis*, but in *Schistostemon* is stout and shorter than the stamens. Fruit and vegetative details are otherwise strikingly similar between the genera. The renewed suggestion by Herrera et al. (2010) of returning to a broader, pre-Cuatrecasas circumscription of the *Sacoglottis-Schistostemon* group (i.e., *Sacoglottis* s.l.) needs further study within the framework of a well-sampled molecular phylogenetic analysis that includes taxa with both types of inflorescence structure. Limited molecular phylogenetic evidence has indicated that *Schistostemon* is not monophyletic and embedded within a paraphyletic *Sacoglottis* (Bardon 2015).

#### Broader significance of androecial structure in Humiriaceae

Humiriaceae share stamens partly connate, filaments subulate and complanate, and anthers that are dorsifixed, versatile, caducous, lacking stomata, and possessing connective protrusions. Most of these shared androecial features are broadly distributed, even in combination, across the rosids (Endress and Stumpf 1991). The eight genera presently can be clearly defined based on details of androecial structure including number and morphology of both stamens and sporangia, although ovule number and endocarp structure are also important distinguishing generic characters. Our observations on gross androecial structure largely agree with and complement prior floral anatomy studies by Narayana and Rao (1969–1977). Variations in anther sporangium morphology are especially interesting from an evolutionary perspective in Humiriaceae due to transitions among the three distinct forms which include, (1) tetrasporangiatedithecal with two lateral disporangiate thecae, a typical rosid anther configuration, in Vantanea, (2) tetrasporangiate-tetrathecal with four monosporangiate thecae arranged as two superposed pairs in Duckesia and Endopleura, and (3) disporangiate-dithecal with two monosporangiate thecae arranged as one pair in the remaining five genera. Disporangiate anthers characterize some families of rosids (e.g., Cucurbitaceae, Malvaceae), but are otherwise very sparse in Malpighiales (e.g., cleistogamous flowers of Viola, Violaceae; Endress and Stumpf 1991) except for Humiriaceae.

*Vantanea* has long been considered the most ancestral genus of Humiriaceae based on androecial structure (Cuatrecasas 1961, Narayana and Rao 1969, 1977, Herrera 2010, Kubitzki 2014), although molecular phylogenetic evidence suggests a nested placement (Bardon 2015). A complex interpretation by Narayana and Rao (1976a, b, 1977) on the origin of the disporangiate anthers based on the position of the sporangia relative to the enlarged connective in bud transverse sections posits that in *Humiria* and *Schistostemon* the dorsal (outer) sporangia were lost, while in *Humiriastrum* and *Sacoglottis* the ventral (inner) sporangia were lost. We observed the anthers of *Sacoglottis* and *Schistostemon* to be nearly indistinguishable when finely comparing anthers of the same type and did not find any transverse shifts in sporangial position to suggest differing origins. Moreover, anther similarities in regard to sporangial position and dehiscence lines extend across all the disporangiate genera (in part for *Humiriastrum*), although differences exist in connective details. *Humiriastrum* is now more homogeneous with our generic transfers (i.e., Duckesia liesneri and Vantanea spiritu-sancti); however, the remaining species have two different thecal orientations, which could correspond to different origins (i.e., from dorsal or ventral sporangia). While *H. dentatum* and *H.* glaziovii in particular differ from the rest in regard to orientation, by other morphological bases they firmly belong to Humiriastrum and it is premature to reconsider generic affiliation. Whether the disporangiate bauplan has one or more origins needs further testing with floral development and phylogenetic studies. The superposed pairs of sporangia (tetrasporangiate-tetrathecal) in Duckesia and Endopleura have been thought to represent an intermediate state in the transition from tetrasporangiate-dithecal to disporangiate-dithecal anthers (Cuatrecasas 1961). Moreover, they establish homologies and clearly show that the ventral sporangia can assume a basal position with distalproximal dehiscence. Some phylogenetic evidence has indicated those genera may be embedded within a disporangiate clade and may not be sister groups (see Herrera et al. 2010, Bardon 2015), although we found no major morphological differences to contradict a single origin of the superposed-sporangia anther type. The disporangiate and superposed-sporangia anthers of Humiriaceae, sometimes described as "dehiscing by detachment" (Cuatrecasas 1961, Kubitzki 2014), have markedly eccentric stomia that fit modern definitions of valvate and have similarities in dehiscence lines to established valvate taxa such as Hamamelis L. and Grubbia P.J. Bergius (Hufford and Endress 1989, Endress and Stumpf 1990). Their fundamentally valvate nature has not been noted in prior broad comparative anther surveys and apparently does not occur in any other rosid (see Endress and Stumpf 1990, 1991).

Disporangiate-dithecal anthers are often correlated with specialized floral biology, which can be enabled by restrictive anther openings such as valves (Endress and Stumpf 1990). The pollination biology of Humiriaceae has been only carefully studied for Humiria balsamifera (Holanda et al. 2015), and indicated bee pollination of its nectariferous flowers. For the valvate taxa (i.e., all except Vantanea), further study is needed to see if the anther valves constrain pollen release. The greatest floral variation (i.e., perianth size, stamen number and length, color) occurs in Vantanea with its relatively unspecialized anthers. Vantanea guianensis in particular with its showy red flowers in large clusters is likely hummingbird pollinated and recorded as visited by hummingbirds by Gentry & Stein 46932 (US). However, it differs from typical flowers with that pollination syndrome in not being especially tubular in nature with spreading 30-40 mm long petals, which are shed early before the androecium, and stamen connation for only 5–7 mm of their 25–35 mm length. Variable morphological features that likely affect pollination biology and need further investigation in an evolutionary framework include flower color (white to greenish or red to pink), disc morphology (cupular to variously dissected), filament ornamentation (papillose to smooth), stamen length (long and thin to short and robust), stamen number (10-200+), staminodes (presence or absence), stamen connation (nearly free to half connate), anther ornamentation (hairs or connective elaborations), and mode of dehiscence (longitudinal slits or valvate). Most taxa have elaborated and likely glandular connectives and discs.

Stamen connation can affect pollinator nectar access by forming a tube around the disc that varies in height among genera and species (e.g., *Sacoglottis* spp., Table 2), although nectar robbing by piercing the tube is known for *Humiria* (Holanda et al. 2015). Anthesis is likely generally of short duration based on the morphology patterns of versatile, caducous anthers and delicate stigmas, although this has been directly little studied (Holanda et al. 2015).

#### Extrafloral nectaries of Humiriaceae

We documented EFNs in all species of Humiriaceae, and found distributional and morphological differences. Some of these differences appear informative at the generic level, including the marginal glands of Humiria, entire eglandular margins of Vantanea, and the exceptionally large laminar glands of Hylocarpa. Our EFN survey, although mostly qualitative in nature, also reveals that their species-level taxonomic value is limited due to high variation in abundance and position, and low intra-specific morphological variation. The EFN ubiquity indicates that they are phylogenetically conserved (i.e., plesiomorphic) for the family, and is a conclusion that differs greatly from character reconstructions that could be hypothesized under the prior understanding of their taxonomic distribution in only three species. This conclusion is also important when considering EFN evolution in the broader context of Malpighiales where they have been documented in 13/39 families (Weber and Keeler 2013), and when considering correlations between EFNs and higher lineage diversification rates (Marazzi and Sanderson 2010, Weber and Agrawal 2014). Humiriaceae contains relatively low extant species-richness despite EFN richness. The gross morphology and variation of Humiriaceae EFNs are consistent with reports from other families (e.g., Tilney and van Wyk 2004, Marazzi et al. 2012, Gonçalves-Souza et al. 2016), although the glands appear unremarkable with no specialized pores or unusual locations on the plants. The shallow teeth forming the crenations along the leaf margins of Sacoglottis gabonensis have been studied in detail, and on the young leaves were found to secrete nectar that attracts ants (Belin-Depoux 1993). Belin-Depoux (1993) did not comment on the basilaminar glands, which we found to be relatively easy to overlook and confuse with the marginal setae scars (see Results). The glands along the leaf margins of Humiria differ from those elsewhere in the family in their position as an often dense submarginal row. They are not modified teeth or setae, which are lacking, but appear to be typical laminar glands developmentally shifted to the margin. Humiria spp. are largely savanna dwellers unlike most of the other Humiriaceae taxa in forests, and habitat shifts may have influenced their EFN evolution. Such habitat shifts have been implicated in leaf defense adaptive evolution, including decrease in EFN abundance and increase in glandular trichomes in neotropical Bignoniaceae (Nogueira et al. 2012). In addition to the extrinsic defenses conferred with the glands, Humiriaceae leaves appear rich in intrinsic defenses due to their thickened and tannin-rich nature.

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# Appendix I

Taxon	Abaxial glands	Basilaminar (adaxial) glands	Leaf margin features	Representative voucher(s)
Duckesia		~		
<i>D. liesneri</i> (Cuatrec.) K. Wurdack & C.E. Zartman	Absent	1–2 pairs at margin	Crenate, setae present	Liesner & Carnevali 22589 (US, holotype)
<i>D. verrucosa</i> (Ducke) Cuatrec.	Sparse, 0–3 per leaf, 2/3 exmedial distance	Absent	Crenate, setae present, large	Ducke 2106 (US)
Endopleura				
<i>E. uchi</i> (Huber) Cuatrec.	Row near margin	Absent	Crenate, setae long and scars large	Assunção 605 (US)
Humiria				
<i>H. balsamifera</i> var. <i>coriacea</i> Cuatrec.	Marginal, along proximal third	Absent	Entire	Cardona 1823 (US, holotype)
<i>H. balsamifera</i> var. <i>floribunda</i> (Mart.) Cuatrec.	Marginal, along entire length	Absent	Entire	Maguire & Politi 27974 (US)
<i>H. balsamifera</i> var. <i>guaiquinimana</i> Cuatrec.	Marginal, along proximal third	Absent	Entire	Cardona 1112 (US, holotype)
<i>H. balsamifera</i> var. <i>guianensis</i> (Benth.) Cuatrec.	Marginal, along entire length	Absent	Entire	Schomburgk 270 (US, isotype)
<i>H. balsamifera</i> var. <i>iluana</i> Cuatrec.	Marginal, along proximal half	Absent	Entire	Maguire 33388 (US, holotype)
<i>H. balsamifera</i> var. <i>imbaimadaiensis</i> Cuatrec.	Marginal, along proximal half	Absent	Entire	Maguire & Fanshawe 32158 (US, holotype)

Qualitative survey of Humiriaceae for extrafloral nectaries and leaf margin features. For abaxial glands not near margins, relative exmedial distances (midvein to margin) are indicated where patterns are apparent.

Taxon	Abaxial glands	Basilaminar (adaxial) glands	Leaf margin features	Representative voucher(s)
<i>H. balsamifera</i> var. <i>laurina</i> (Urb.) Cuatrec.	Marginal, along entire length	Absent	Entire	Schomburgk 560 (US, isotype)
<i>H. balsamifera</i> var. <i>minarum</i> Cuatrec.	Marginal, along entire length	Absent	Entire	Mexia 5815 (US, holotype)
<i>H. balsamifera</i> var. <i>parvifolia</i> (A. Juss.) Cuatrec.	Marginal, along proximal half	Absent	Entire	<i>St. Hilaire</i> (US, isotype fragment)
H. balsamifera var. pilosa (Steyerm.) Cuatrec.	Marginal, along entire length	Absent	Entire	<i>Steyermark 60289</i> (NY, isotype)
<i>H. balsamifera</i> var. <i>savannarum</i> (Gleason) Cuatrec.	Marginal, along entire length or proximal half	Absent	Entire	Tate 330 (NY, type); J. Wurdack ở Monachino 41380 (US)
<i>H. balsamifera</i> var. <i>stenocarpa</i> Cuatrec.	Marginal, along entire length	Absent	Entire	Maguire
<i>H. balsamifera</i> var. <i>subsessilis</i> (Urb.) Cuatrec.	Marginal, dense along entire length	Absent	Entire	Spruce 2454 (US, isotype)
<i>H. crassifolia</i> Mart.	Marginal, sparse, denser at base and apex	Absent	Entire	Huber & Fernandez 11331 (US)
H. fruticosa Cuatrec.	Marginal, 2–4 per side near base	Absent	Entire	<i>Maguire et al. 36580</i> (US, holotype)
<i>H. wurdackii</i> Cuatrec.	Marginal, along entire length, widely spaced; absent on very narrow forms	Absent	Entire	J. Wurdack & Adderley 42760 (US, holotype); Huber 4842 (US), narrow- leaved
Humiriastrum				
<i>H. columbianum</i> (Cuatrec.) Cuatrec.	Sparse abaxial; absent on type, save for a few erratic structures that could be damage.	Absent	Serrate, setae long	Lamb 141 (US, holotype); best gland evidence is on Gentry & Stein 46898 (US); Romero Castañeda 4942 (US)
<i>H. cuspidatum</i> (Benth.) Cuatrec.	Small, sparse, near margin	1 pair, elliptic	Crenate, setae long and thin	J. Wurdack & Monachino 40881 (US)
<i>H. cuspidatum</i> var. <i>glabriflorum</i> (Ducke) Cuatrec.	Small, sparse, near margin	1 pair at margin (sometimes a single gland)	Serrate, setae scars	Ducke [JBRJ-23436] (US, isotype)
<i>H. cuspidatum</i> var. <i>subhirtellum</i> Cuatrec.	Small, sparse, near margin	0–1 pair	Serrate, setae present	<i>Fróes 25463</i> (US)
<i>H. dentatum</i> (Casar.) Cuatrec.	Small, sparse, near margin	1 pair at margin, nearly abaxial	Serrate, setae present	Hatschbach 4294 (US)
<i>H. diguense</i> (Cuatrec.) Cuatrec.	Along secondary vein loops toward margin	1–3 pairs at margin, nearly abaxial	Crenate, setae scars	<i>Cuatrecasas 14956</i> (US, isotype)
H. diguense var. anchicayanum (Cuatrec.) Cuatrec.	Along secondary vein loops toward margin	1 pair at margin, nearly abaxial	Crenate, setae scars	Cuatrecasas 14418 (US, isotype)
<i>H. diguense</i> subsp. <i>costaricense</i> Cuatrec.	Along secondary vein loops toward margin, relatively sparse	1 pair	Crenate, setae relatively stout, clearly terminating veins	<i>Allen 5812</i> (US, holotype)

Taxon	Abaxial glands	Basilaminar (adaxial) glands	Leaf margin features	Representative voucher(s)
<i>H. excelsum</i> (Ducke) Cuatrec.	Near secondary vein loops toward margin, relatively sparse	1 pair at margin	Crenate, setae present	Museu Goeldi 9672 (US)
<i>H. glaziovii</i> (Urb.) Cuatrec.	Absent	1 pair at margin, nearly abaxial	Serrate, setae present	Pabst 4726 (US)
<i>H. glaziovii</i> var. <i>angustifolium</i> Cuatrec.	Absent	1 pair at margin, nearly abaxial	Crenate, setae scars	<i>Glaziou 16724</i> (US, holotype)
H. mapiriense Cuatrec.	Sparse, toward margin	0–2 pairs	Crenate, setae scars	Buchtien 1518 (US, isotype)
<i>H. melanocarpum</i> (Cuatrec.) Cuatrec.	Very rare on few leaves, scattered, hard to distinguish from potential leaf damage	Absent	Crenate, with setae	<i>Cuatrecasas 19909</i> (US); best gland evidence is on <i>Prance</i> <i>28055</i> (US)
<i>H. mussunungense</i> Cuatrec.	Absent	0–1 pair at margin, nearly abaxial	Crenate, setae present	Folli 1393 (US, holotype)
<i>H. obovatum</i> (Benth.) Cuatrec.	Sparse, scattered	0–1 pair at margin, nearly abaxial	Subentire, setae scars sparse, hirsute	Schomburgk 1359 (US)
<i>H. ottohuberi</i> Cuatrec.	Row near margin	1–3 pairs, oval	Obscurely crenate, setae present	Steyermark
<i>H. piraparanense</i> Cuatrec.	Tiny, sparse, near margin	1–2 pairs	Crenate, setae scars	Schultes & Cabrera 15922 (US, holotype); glands better developed on García-Barriga 14287 (US)
<i>H. procerum</i> (Little) Cuatrec.	Towards margin along loops between secondary veins	1(–2) pairs at margin	Crenate, setae scars	Little & Dixon 21148 (US)
<i>H. subcrenatum</i> (Benth.) Cuatrec.	Absent	1 pair at margin	Crenate, setae scars	Martin s.n. (US, type fragment)
<i>H. villosum</i> (Fróes) Cuatrec.	Tiny, sparse, in row near margin	1 pair at margin, small	Serrate, setae scars	Humbert & Schultes 27363 (US)
Hylocarpa				
<i>H. heterocarpa</i> (Ducke) Cuatrec.	Large, erratic, near secondary or intersecondary veins, 1/2–2/3 exmedial distance	Absent	Subentire, setae scars	Ducke [JBRJ-30137] (US, isotype
Sacoglottis				
S. amazonica Mart.	Small, near margin	1 pair	Obscurely crenate, setae scars	J. Wurdack 293 (US)
<i>S. ceratocarpa</i> Ducke	Relatively large, near margin	l pair, large, narrowly elliptic, at margin of leaf base extension	Subentire, setae present	<i>Fróes 21192</i> (US)
S. cydonioides Cuatrec.	Tiny, near margin	1 pair, elliptic, sometimes large	Serrate, tooth tips with darkened spots (setae scars?)	B.W. [Surinam Forestry Dept.] 4720 (US)
S. gabonensis (Baill.) Urb.	Absent	1(–2) pairs at margin	Crenate, setae present	McPherson 13714 (US)
S. guianensis Benth.	Small, row near margin	0–1 pair at margin	Shallowly crenate; setae present, more persistent than is typical	Schomburgk 574 (NY, syntype)

Taxon	Abaxial glands	Basilaminar (adaxial) glands	Leaf margin features	Representative voucher(s)
<i>S. guianensis</i> var. <i>hispidula</i> Cuatrec.	Tiny, near margin	Absent	Serrulate, setae scars	Krukoff 6653 (US, holotype)
<i>S. guianensis</i> var. <i>maior</i> Ducke	Small, row near margin	Absent	Crenate; setae more persistent than is typical	Ducke [JBRJ- 23818 (US, isotype)
S. holdridgei Cuatrec.	Small, row near margin	1(–2) pairs	Shallowly crenate, setae scars	<i>Holdridge 5164</i> (US, isotype)
S. maguirei Cuatrec.	Sparse (0–2), tiny, near leaf apex margin, most leaves lacking	Absent	Shallowly crenate, setae scars	Maguire et al. 30693 (US, holotype)
S. <i>mattogrossensis</i> var. <i>subintegra</i> (Ducke) Cuatrec.	Small, sparse, near margin	Absent	Subentire, setae scars obscure	Ducke [JBRJ-23820] (US, isotype)
S. ovicarpa Cuatrec.	Absent	1 pair	Subentire, setae scars	Cuatrecasas 17226 (US)
<i>S. perryi</i> K. Wurdack & C.E. Zartman	Tiny, near margin	1 pair, relatively small, obscure	Shallowly crenate, setae present	McDowell 2920 (US)
S. trichogyna Cuatrec.	Absent	1 pair	Shallowly crenate, setae scars	<i>Holdridge 5216</i> (US, isotype)
Schistostemon				
<i>S. auyantepuiensis</i> Cuatrec.	Tiny, with reticulate veins near margin	1 pair, large elliptic	Very obscurely crenate, setae scars	Davidse & Huber 22804 (US)
<i>S. densiflorus</i> (Urb.) Cuatrec.	Small, row near margin	0–1 pair	Obscurely crenate, setae present	Schomburgk 543 (US isotype)
<i>S. dichotomus</i> (Urb.) Cuatrec.	Small, row near margin	1 pair	Crenate, setae scars	Sabatier 989 (US)
S. fernandezii Cuatrec.	Near margin	1 pair, oval	Obscurely crenate, small setae present	Fernandez 2276 (US, holotype)
<i>S. macrophyllus</i> (Benth.) Cuatrec.	Row near margin, widely spaced	1 pair, elliptic	Shallow teeth, setae scars	Ducke 1744 (US)
<i>S. oblongifolius</i> (Benth.) Cuatrec.	Near margin	1–2 pairs	Small, shallow teeth, setae scars	Maas et al. 6804 (US)
<i>S. reticulatus</i> (Ducke) Cuatrec.	Near margin	1–2 pairs at base, up to 3 per side near apex	Obscurely crenate	Ducke [JBRJ- 23819] (US, isotype), Klug 1564 (US)
<i>S. reticulatus</i> var. <i>froesii</i> Cuatrec.	Near margin	1 pair at base, no glands at apex	Subentire, setae scars sparse	<i>Fróes 21370</i> (US, isotype)
<i>S. retusus</i> (Ducke) Cuatrec.	Near margin, relatively regular all the way to tip	1 pair, large	Subentire, setae scars sparse	Cuatrecasas 7203 (US)
S. sylvaticus Sabatier	Row near margin	1–2 pairs at base	Very obscurely crenate, setae scars	Loubry 1074 (US)
Vantanea			1	
V. bahiaensis Cuatrec.	Tiny, near secondary or intersecondary veins	l pair	Entire	Belém & Magalhães 748 (US); Carvalho & Gatti 484 (NY, holotype)
V. barbourii Standl.	Near secondary or intersecondary veins	1 pair	Entire	Hartshorn 2139 (US)
<i>V. celativenia</i> (Standl.) Cuatrec.	Row 1/3 exmedial distance	1 pair, often others near base	Entire	Krukoff 7182 (US, isotype)
<i>V. compacta</i> (Schnizl.) Cuatrec.	Tiny, near intersecondary veins near margin	0–4 pairs per side	Entire	Hatschbach 21265 (US)

Taxon	Abaxial glands	Basilaminar (adaxial) glands	Leaf margin features	Representative voucher(s)
<i>V. deniseae</i> W.A. Rodrigues	Near secondary or intersecondary veins	Absent	Entire	Freitas et al. 860 (US)
<i>V. depleta</i> McPherson	Near secondary veins, row half exmedial distance	1 pair	Entire	McPherson & Stockwell 10892 (US, isotype)
V. guianensis Poir.	Scattered	Absent	Entire	Maguire et al. 56006 (US)
V. macrocarpa Ducke	Scattered	Absent	Entire	<i>Ducke 2230</i> (US)
<i>V. magdalenensis</i> Cuatrec.	Tiny, near secondary and intersecondary veins	1 pair	Entire	Lamb 133 (US, holotype)
V. micrantha Ducke	Scattered	Absent	Entire	<i>Ducke 751</i> (US)
V. minor Benth.	Large, sparse, proximal half near primary vein	Absent	Entire	Liesner 23974 (US)
V. morii Cuatrec.	Absent	2(4) pairs	Entire	Mori & Benton 13181 (US, holotype)
V. obovata Benth.	Near secondary or intersecondary veins	1–2 pairs	Entire	Anderson et al. 36781 (US)
<i>V. occidentalis</i> Cuatrec.	Large, near intersecondary veins, row 1/3 exmedial distance	Absent	Entire	Patiño 12 (US)
V. ovicarpa Sabatier	Tiny, close to secondary veins	0–1 pair, rarely others near base	Entire	Granville 10792 (US)
V. paraensis Ducke	Near secondary or intersecondary veins	1 pair, often others along secondary near base	Entire	Ducke [JBRJ-23430] (US)
<i>V. parviflora</i> Lam.	Along loops of secondary veins, near margin	Row of 2–4(5) pairs, usually close to mid-vein	Entire	<i>Prance et al. 17770</i> (US)
V. parviflora var. puberulifolia Cuatrec.	Along loops of secondary veins, near margin, usually surrounded by vein	2 (1–3) pairs, closer to midvein	Entire	Ducke [JBRJ-23428] (US, holotype)
<i>V. peruviana</i> J.F. Macbr.	Large, row, mostly near intersecondary veins, 1/4–1/3 exmedial distance	Absent	Entire	Rimachi 4577 (US)
V. spichigeri A.H. Gentry	Row, 1/5 way to margin, often near secondary veins	1–2 pairs	Entire	Abadie s.n. 12-9-74 (US), Spichiger et al. 1743 (NY, isotype)
<i>V. spiritu-sancti</i> (Cuatrec.) K. Wurdack & C.E. Zartman	Sparse, small along secondary or intersecondary veins, half exmedial distance	1–2 (type); 5–7 pairs along proximal edge	Entire	Dolcolmo [JBR]- 86212] (US, isotype fragment); Silva et al. 1436 (US), more abundant
<i>V. tuberculata</i> Ducke	Along secondary veins, half exmedial distance	Absent	Entire	Ducke [JBRJ-30134] (US, isotype)

RESEARCH ARTICLE



# Thismia domei and T. terengganuensis (Thismiaceae), two new species, and T. javanica, a new record from Terengganu, Peninsular Malaysia

Siti-Munirah Mat Yunoh<sup>1</sup>, Dome Nikong<sup>2</sup>

l Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia **2** DigitalDome Photography, 21500 Permaisuri, Terengganu, Malaysia

Corresponding author: Siti-Munirah Mat Yunoh (sitimunirah@frim.gov.my)

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#### Abstract

Two new species of the mycoheterotrophic genus *Thismia* Griff. (Thismiaceae), *Thismia domei* Siti-Munirah and *T. terengganuensis* Siti-Munirah from Peninsular Malaysia, are described and illustrated. *Thismia domei*, characterized by its perianth lobes that are upright and curve inward, but are imperfectly connate, falls within section *Odoardoa*. *Thismia terengganuensis* is characterized by its mitre with three appendages on its apex, so falls within section *Geomitra*. Both new species are unique and totally different from other described species, *T. domei* by the trichomes on its outer perianth tube surface and *T. terengganuensis* by its mitre with slender appendages. *Thismia javanica* J.J.Sm, also from Terengganu, is a new record for Peninsular Malaysia.

#### Keywords

Thismia, taxonomy, mycoheterotrophy, new species, Telemong Forest Reserve, Terengganu, Peninsular Malaysia

# Introduction

*Thismia*, a genus of small, mycoheterotrophic herbs, currently comprises about 70 accepted species (Hroneš et al. 2018; Suetsugu et al. 2018). In 2018, eight new species were described from East Malaysia (Sabah and Sarawak). We can expect more new species in future. *Thismia* is poorly known because the above-ground parts are ephemeral

and are often overlooked due to their small size. Well-preserved herbarium specimens are rare but spirit-preserved material and field images are informative. Currently in Malaysia, including these two new species and the new record, there are 27 species. Thirteen occur in Peninsular Malaysia, namely: *Thismia alba* Holttum *ex* Jonker, *T. arachnites* Ridl., *T. aseroe* Becc., *T. chrysops* Ridl., *T. clavigera* F. Muell., *T. crocea* (Becc.) J.J.Sm, *T. fumida* Ridl., *T. grandiflora* Ridl., *T. javanica* J.J. Sm., *T. kelantanensis* Siti-Munirah, *T. racemosa* Ridl., (Jonker 1948, Siti Munirah 2018) and the two new species described here. However, based on Jonker (1948), *T. crocea* is considered as highly dubious for Peninsular Malaysia. Its status in Peninsular Malaysia needs to be revisited.

Endemism is high, about half the Peninsular Malaysian species are endemic to Peninsular Malaysia and *T. kelantanensis*, *T. racemosa*, *T. grandiflora* and *T. chrysops* have only been collected once. *Thismia alba*, *T. arachnites*, *T. clavigera* and *T. javanica* also occur in Thailand; and *T. fumida* and *T. aseroe* in Singapore. The most common species, which is found from many places in Peninsular Malaysia, is the bright yellow species, *T. alba*.

In 2018, six new species were described from Sarawak and two from Sabah. Sochor et al. (2018a) recently rediscovered the magnificent *Thismia neptunis* in Sarawak after 150 years. In total, Sarawak has about 10 species of which 9 are endemic: *Thismia acuminata* Hroneš, Dančák & Sochor, *T. laevis* Sochor, Dančák & Hroneš, *T. nigra* Dančák, Hroneš & Sochor, *T. viridistriata* Sochor, Hroneš & Dančák, (Sochor et al. 2018b), *Thismia cornuta* Hroneš, Sochor & Dančák (Hroneš et al. 2018), *T. kelabitiana* Dančák, Hroneš & Sochor (Dančák et al. 2018), *T. bifida* M.Hotta, *T. clavigera*, *T. episcopalis* (Becc) F.Muell, *T. neptunis* Becc. (Jonker 1948). Five taxa have been described from Sabah of which three are endemic: *Thismia bryndonii* Tsukaya, Suetsugu & Suleiman (Tsukaya et al. 2017), *T. hexagona* var. *grandiflora* (Tsukaya et al. 2014), *T. goodii* Kiew (Kiew 1999), *T. kinabaluensis* Nishioka & Suetsugu (Nishioka et al. 2018) and *T. pallida* Hroneš, Dančák & Rejžek (Hroneš et al. 2018).

The two new species and *Thismia javanica* were discovered by the second author, Mr Dome Nikong during a photographic trip in the Hulu Telemong Forest Reserve (FR) area, in the state of Terengganu, Peninsular Malaysia (Map 1). Hulu Telemong FR, located to the north of Kenyir Lake, was previously logged for timber. However, some patches of pristine primary rain forest have survived.

#### Materials and methods

This study is based on material collected by the second author in November and December 2018 from Hulu Telemong FR, Kuala Berang District, Terengganu. The specimens were preserved in spirit. Morphological characters were studied using stereo microscope and high-resolution macrophotography. Measurements were taken from live and spirit material. The specimen details were compared in detail with original drawings and descriptions given in the protologues of *Thismia* species in the Malaysian region.



**Map 1.** Hulu Telemong Forest Reserve (•), the type locality of *Thismia domei* and *T. terengganuensis* and the locality of *T. javanica*. NFI III Courtesy of Forest Department Peninsular Malaysia.

# Taxonomic account

*Thismia domei* M.Y.Siti-Munirah, sp. nov. urn:lsid:ipni.org:names:77198712-1 Figures 1, 2, 3

**Diagnosis.** The whole plant is completely white translucent and the flower is strigose with white trichomes covering the outer surface of the perianth tube and ovary; the apex of the perianth tube is partially covered by the apical part of flower tube with a ring-like annulus and together with six perianth lobes are upright and curve inwards with a dorsal long appendage on each lobe.

**Type.** MALAYSIA. Peninsular Malaysia: Terengganu, Kuala Berang District, Hulu Telemong Forest Reserve, ca 207 m alt., 22 Nov 2018, *Dome Nikong, FRI 91111* (holotype KEP!).

Description. Terrestrial, achlorophyllous, whitish herb to 9 cm tall. Roots vermiform brownish with white apices. Stems erect (sometimes curved), unbranched, 0.2 to 2.5 cm long, glabrous with ridges. Leaves scale-like, appressed, 2-7 mm long, 2 mm wide, smaller at the base leaf increasing in size above, alternate, single, triangular to lanceolate, translucent white, apex acute or acuminate, base appressed. Internodes 4-10 mm. Involucral bracts 3, translucent white, ca. 1-1.2 cm long, lanceolate, apex acute to acuminate, margin entire, base appressed, with a central vein. Bud to 7 mm long, pale to dark purple. Pedicel 4-5 mm long. Flowers solitary to 7-8 cm long (including appendages); perianth lobes 6, each  $3 \times 2$  mm (excluding appendage), obovate to spathulate are upright and curve inwards over perianth chamber, apex truncate, with erect subulate appendage abaxially, ca. 3.5-5.5 cm long, ca. 0.5 mm wide, cylindric, apex rounded or falcate; perianth tube bowl-like, 5–5.5 mm long, 3–6 mm wide, narrowed just above the ovary, widest just below the lobes, white translucent, outer surface with longitudinal ribs covered with numerous white translucent thick trichomes; apical part of perianth tube with annulus and opening circular aperture ca. 2 mm in diameter, from above ring-like, whitish, glabrous, Stamens 6, pendulous attached to the inner wall of perianth tube, ca. 2.5 mm long, apex lobed with 3 apical appendages with glandular tip (visible in fresh plant); filaments short, free, white, each with two rounded oblong thecae facing the inner wall of the perianth tube; anthers oblong, 2 mm long; lateral appendage box-shaped; ovary inferior, cup-shaped, ca. 4 mm long, whitish to brownish, outer surface smooth, without longitudinal ribs, covered with numerous white translucent thick trichomes, unilocular with 3 parietal placentas; style ca. 0.5 mm long; stigmas 3, ca. 0.6 mm long, oblong, papillate, 3-lobed, lobes slightly folded, apex truncate. Fruit cup-shaped, translucent white.

**Distribution.** Endemic in Peninsular Malaysia, Terengganu. Currently known only from the type collection.

**Ecology.** In lowland dipterocarp forest on wet, moist soil in shade at an altitude of 207 m. Flowering in November-December. This new species was encountered on bamboo leaf litter near an elephant trail. When mature *T. domei* produces an unpleasant smell like rotting fish.

**Etymology.** The species is named after Mr Dome Nikong, professional photographer and freelance researcher, who first discovered this species and the other new species described below as well as *T. javanica*.

**Conservation status.** Critically Endangered (B2 ab(iii)). Following the 2012 IUCN Red List Categories and Criteria, (IUCN 2012), this species is assessed as critically endangered because it is only known from the type locality. It is very rare. Fewer than 10 individuals were observed, which included both flowering and fruiting individuals. The type locality is within a forest reserve in disturbed forest area near the river bank at an elevation of c. 207 m. The species is under threat because selective logging activities are currently on-going within the forest reserve.

**Notes.** *Thismia domei*, based on colour, is most like *T. clavarioides* K. Thiele (Thiele and Jordan 2002) and *Thismia taiwanensis* S.-Z. Yang, R. M. K. Saunders & C.-J. Hsu, (Yang et al. 2002). Both have completely white perianth lobes that are upright and



Figure 1. *Thismia domei* Siti-Munirah A habit (A1 roots A2 tip of filiform appendages), B top view of plant C perianth tube covered with trichomes (C1 trichome C2 white spots D top view of flower showing perianth lobes overlapping and appendages on the each lobes forming loose mitre E top perianth tube covered with apical part of flower tube and annulus F longitudinal section of flower (F1 base perianth tube and ovary) G perianth lobes from outside H perianth lobes from inside I pendulous stamen attached to the inner wall of perianth tube (abaxial view of stamens) J pendulous stamen attached to the inner wall of perianth tube (adaxial view of stamens) (J1 stamen from side view J2 three appendages at apical margin) K ovary showing stigma (K1 ovary). Photo credit: A, A1, B, C, C1, D, F, G, H, J, J1, J2, K Dome Nikong; A2, C2, E, F1, I, K1 Siti-Munirah MY.



**Figure 2.** *Thismia domei* Siti-Munirah **A**, **B** habit of two plants **C** leaf **D** bract **E** perianth lobes from top **F** top view of apical part of perianth tube with annulus **G** inner adaxial view of six pendulous stamens **H** stamen attached to perianth tube **I** a perianth lobe with perianth tube from side view **J** ovary with stigma and placenta (below). All from Dome Nikong FRI 91111. Drawn by M. Aidil. The drawing is based on spirit material.



**Figure 3.** *Thismia domei.* **A, B** habitat on leaf litter within a bamboo clump (plant indicated by red arrow) **C** habit of the flowering *T. domei* **D** Mr Dome Nikong showing *T. domei* at different stages of anthesis **E** habit of *T. domei*. Photo credit: Dome Nikong.

curved inwards towards the perianth tube with an appendage on each lobe. However, *T. domei* differs from these species in that its perianth tube is fully covered with thick trichomes and it has six perianth lobes each with an appendage as opposed to being completely glabrous and *T. taiwanensis* from Taiwan has only three perianth lobes with appendages and the perianth of *T. clavarioides* forms a mitre from Australia. *T. domei* is unusual in its conspicuous cell inclusion white spot which appear to be aleurone grains.

#### Thismia terengganuensis M.Y.Siti-Munirah, sp. nov.

urn:lsid:ipni.org:names:77198713-1 Figure 4, 5, 6

**Diagnosis.** *Thismia terengganuensis* is unique in its perfect mitre with the long filiform appendages not seen in any other species of *Thismia*. It has a racemose sessile inflorescence with large bracts. Its flower parts are brownish with brown striae with inner perianth lobes forming a dark brown mitre with a white slender appendage attached at apex. The outer perianth lobes at interval appear like a wing. It has creeping vermiform brown roots.

**Type.** MALAYSIA. Peninsular Malaysia: Terengganu, Kuala Berang District, Hulu Telemong Forest Reserve, ca 227 m alt., 2 Dec 2018, *Dome Nikong, FRI 91112* (holo-type KEP!).

Description. Small perennial achlorophyllous herbs. Roots creeping, vermiform, brown. Stem white, 3-3.5 cm long, ascending, glabrous. Leaves scale-like, triangular, ca. 3 mm long, apex acute, alternate. Bracts many, large, 1-1.5 cm long, crowded. Pedicel short or up to 3-4 cm long. Inflorescence racemose. Flowers sessile, perianth tube urceolate, ca. 1 cm long, 5 mm wide in the upper third, brownishwhite, with inconspicuous brown longitudinal stripes from upper part to the base. Apex of perianth tube covered with the dark brown apical part of perianth tube, circular, slightly raised annulus with opening aperture ca. 2 mm in diameter. Perianth lobes divided into 2 types; inner perianth lobes 3, erect, convergent to connate at apex, forming a mitre, dark brown, each with long and slender filiform appendages, 5–7 cm long; outer perianth lobes 3, recurved, all equal in shape and size, long triangular, boat-like, 4–5 mm long, 2 mm wide at base, white, tapering into curved apex. Stamens 6, brownish white; filaments short, free, attached to the mouth of the perianth tube, curved downwards; connectives blunt (tongue-like), without apical appendages; thecae yellow, not connected; lateral appendage skirt-like. Ovary white, cup-shaped, inferior, free central placentation; style short; stigma 3-lobed, papillose. Fruit cup-shaped, brownish.

**Distribution.** Endemic in Peninsular Malaysia, Terengganu. Currently only known from the type locality.

**Ecology.** In lowland dipterocarp forest on wet, moist soil in shade at altitude 227 m. Flowering in November-December on forest floor under canopy of dense shrubs.

Etymology. The epithet refers to the state, Terengganu, where it was found.

**Conservation status.** Critically Endangered (B2 ab(iii)). Following the 2012 IUCN Red List Categories and Criteria, (IUCN 2012), this species is assessed as critically endangered because it is only known from one locality where less than 5 individuals flowering and fruiting individuals were observed. *Thismia terengganuensis* is currently known only from the type locality and is certainly a very rare species. The locality is within the forest reserve but is threatened by selective logging activities within the forest reserve that are currently ongoing.



**Figure 4.** *Thismia terengganuensis* Siti-Munirah **A** habit of flowering and fruiting plants **B** top view of plant **C** root and stems **D** perianth tube **E** perianth lobes (inner forming a mitre, outer curved) **F** ovary with pistil (stigma) **G** fruits with seeds **H** stamens attached to the inner wall of mouth of the perianth tube (adaxial view) **I** stamens deflexed (abaxial view). Photo credit: **A–D**, **G** Dome Nikong; **E**, **F**, **H**, **I** Siti-Munirah MY.



**Figure 5.** *Thismia terengganuensis* Siti-Munirah **A** flower and fruit **B** habit of fruiting plant **C** leaf **D** bracts **E** stamens (adaxial view) **F** stamens (abaxial view) **G** ovary showing free central placentation **H** stigma. All from Dome Nikong FRI 91112. Drawn by M. Aidil.

**Notes.** *Thismia terengganuensis* is most similar to species in sect. *Sarcosiphon* and sect. *Geomitra* in the shape of the perianth tube and mitre but differs in all other morphological parts, e.g. in its slender filiform appendages on the apex of mitre and also its connectives that are blunt without any apical appendages.



**Figure 6.** *Thismia terengganuensis* **A** habitat **B** habit of a fading *T. terengganuensis* plant. Photo credit: Dome Nikong.

#### New record

# *Thismia javanica* J.J. Sm., Ann. Jard. Bot. Btzg. 23: 32. 1910 Figure 7

*Thismia javanica* J.J. Sm., Ann. Jard. Bot. Btzg. 23: 32. 1910; Jonker, Fl. Malesiana 1,4: 23. 1948; Larsen, Fl. Thailand 5,1: 125. 1987. Specimen: *Dome Nikong FRI* 91114 (KEP!)

**Note.** Stem erect simple, rarely branched, up to 12 cm tall, 1-few flowered. Leaves scale-like, lanceolate to ovate, 3 mm long. Involucral bracts 3, orange. Perianth-tube urceolate, pale orange with darker stripes, with longitudinal bars inside connected by several transverse bars; outer perianth lobes orange, ovate, inner ones triangular, terminated by a 2-3 cm long appendage. Anthers 3-toothed at the apical margin, each tooth terminated by a hair; connective broad with quadrangular appendage. Ovary obovoid; style short; stigma truncate. Fruit orange, 6 mm long.

Distribution. Indonesia, Malaysia and Thailand.

**Conservation status.** We propose a regional conservation status for *T. javanica* in Peninsular Malaysia as Critically Endangered (B2 ab(iii)). Following the 2012 IUCN Red List Categories and Criteria, (IUCN 2012), this species is assessed as critically endangered because it is only known from one locality where less than 6 individuals flowering and fruiting individuals were observed. It lies within a forest reserve that is threatened by selective logging activities that are currently on-going.

**Notes.** The specimens of *Thismia javanica* were found not far from the *T. terengganuensis* population. We believe that *T. javanica* has a wider distribution in Peninsular Malaysia based on photographs of a specimen from Langkawi, Kedah, by late Abd Ghani Hussain. Unfortunately, there are no specimen to verify this.

**Discussion.** The genus *Thismia* is divided into two subgenera, subg. *Ophiomeris* (Miers) Maas & Maas and subg. *Thismia* (Kumar et al., 2017). All Peninsular Malaysian species belong to subgenus *Thismia*. It is divided into five sections of which three occur in Peninsular Malaysia, (a) sect. *Thismia* with two subsections, subsect. *Brunonithismia* Jonker (*T. arachnites, T. javanica*) and subsect. *Odoardoa* Schlechter (*T. alba, T. aseroe, T. chrysops, T. domei, T. fumida, T. grandiflora, T. racemosa*), (b) sect. *Sarcosiphon* (Blume) Jonker (*T. crocea*), and (c) sect. *Geomitra* Kumar & S.W. Gale (*T. kelantanensis, T. clavigera* and *T. terengganuensis*). *Thismia terengganuensis* is a unique species which can hardly be assigned to any section of *Thismia.* However, we currently locate it under sect. *Geomitra* as its perianth lobes form a mitre with appendages.



**Figure 7.** *Thismia javanica* from Terengganu **A** habit **B** flower **C** opened to show the inside of the perianth tube. Photo credit: Dome Nikong.

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RESEARCH ARTICLE



# Impatiens jenjittikuliae (Balsaminaceae), a new species from Thailand

Saroj Ruchisansakun<sup>1</sup>, Piyakaset Suksathan<sup>2</sup>

I Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand **2** Queen Sirikit Botanic Garden, The Botanical Garden Organization, Chiang Mai, Thailand

Corresponding author: Saroj Ruchisansakun (s.ruchisansakun@gmail.com)

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#### Abstract

*Impatiens jenjittikuliae* Ruchis. & Suksathan, a new species from a limestone area in Thasongyang District, Tak Province, Northern Thailand, is described and illustrated. This endemic new species is distinguished from the most similar, *I. lacei* Hook.f. through having pilose lateral sepals vs glabrous, and by the absence of long hairs along the lamina margin. Its pollen and seed morphology, stem anatomy, and pollination ecology are also observed. Furthermore, its conservation status as Critically Endangered is also assessed.

#### Keywords

Uniflorae, lithophytic, endemic, critically endangered

## Introduction

Balsaminaceae consists of *Hydrocera* Blume ex Wight & Arnott (1834: 140) with only a single species, *H. triflora* (L.) Wight & Arnott (1834: 140), and *Impatiens* L. (1753: 937), comprising over 1,000 species (Ruchisansakun et al. 2018). In Southeast Asia, many new *Impatiens* species were recently described (Souvannakhoummane and Suksathan 2015; Ruchisansakun et al. 2017; Ruchisansakun et al. 2018; Suksathan and Triboun 2009).

In Thailand, sixty-one native *Impatiens* species have been enumerated in previous works (Shimizu 1970, 1977, 1991, 2000; Shimizu and Suksathan 2004; Suksathan

and Triboun 2009; Ruchisansakun et al. 2014). In 2017, the first author cited here noticed an unnamed *Impatiens* from Tak Province via Weerayuth Laohajinda's Facebook and later on traveled to examine the plant in 2018. After a detailed study, it turned out to be a species new to science. It is therefore described here.

# Material and methods

Fieldwork was conducted in October 2018. The plants were prepared for making herbarium specimens. Each part of the flower was separately glued on the hard paper and dried separately. All parts were measured and described in line with terminology in Ruchisansakun et al. (2018). For a palynological study, mature pollen grains were collected, air-dried, and fixed to aluminum stubs, then sputter-coated with gold. Micrographs were taken with a Field Emission Scanning Electron Microscope (FE-SEM) (Hitachi SU8010). The pollen grains and seeds were measured by ImageJ and described according to the terminology of pollen grains and seeds (Janssens et al. 2012). For an anatomical study, the fresh stem was dissected at the base and stained by diluted Safranin-O for 20 sec, and observed under a light microscope.

# Result

Taxonomy

Impatiens jenjittikuliae Ruchis. & Suksathan, sp. nov.

urn:lsid:ipni.org:names:77198714-1 Figs 1–5

**Diagnosis.** *Impatiens jenjittikuliae* is most similar to *I. lacei* Hook.f. It differs from *I. lacei* by its densely pilose lateral sepal (versus glabrous) and by having no long hairs along its lamina margin (versus distinct long hairs especially along the lower-half of leaf margin).

**Type.** THAILAND. Tak Province, Thasongyang District [17°30'1"N, 98°3'60"E], limestone area near waterfall in mixed deciduous forest at 540 m alt., 20 October 2018, *S. Ruchisansakun* 900 (holotype: QBG; isotypes: BK, BKF, Mahidol University Herbarium)

**Description.** Lithophytic, annual herb, up to 6–30 cm tall. Stem erect, up to 1.2 cm in diam., cylindrical, branched, green, densely pilose with short white hairs. Leaves spirally arranged. Petiole 3–7.5 cm long, ca. 2.5 mm in diam., pale green to green to pink, pilose; with 5–7 pairs of long hairs on petiole, up to 2 mm long, green, sometime with red tips. Lamina 9–20 × 3–7 cm, ovate to elliptic, apex acute, base cuneate, margin shallowly serrate, adaxial green, abaxial pale green, pilose on both sides; lateral veins 10–12 pairs. Inflorescence raceme, axillary, 8–12 florets; peduncle 7–10 mm



**Figure 1.** *Impatiens jenjittikuliae.* **A** Habit **B** Flower, front view **C** Inflorescence with flower in lateral view **D** Lateral sepals **E** Lower sepal **F** Dorsal petal **G** Lateral united petals. Drawn by Saroj Ruchisansakun.

long, 1.5–2 mm in diam., pale green, densely pilose; rachis 8–20 mm long, ca. 1.5 mm in diam., pale green, densely pilose, hairs shorter than those on peduncle. Flowers ca.  $20 \times ca. 15$  mm, ca 3 mm deep, pinkish white with reddish purple lip. Bracts ca.  $1 \times 0.5$  mm, linear to narrowly lanceolate, apex acute, base cuneate, green, caducous, abaxial densely pilose with white hairs. Pedicel 12–15 mm long, less than 1 mm in diam.,



**Figure 2.** *Impatiens jenjittikuliae* **A** flower, front view **B** flower, lateral view **C** habit *in situ*. Photographs by Saroj Ruchisansakun.

pink, densely pilose with white hairs. Lateral sepals 2,  $5-6 \times 6-7$  mm, free, broadly ovate, the apex mucronate, the base truncate, pale pink, abaxially densely pilose with white hairs. Lower sepal  $11-13 \times 8-10$  mm, ca. 14–17 mm deep, broadly navicular to subsaccate, apex acuminate and mucronate, white with green tip, densely pilose to

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Figure 3. Pollen of Impatiens jenjittikuliae (FE SEM) A entire pollens B, C sexine ornamentation.

strigose outside with long white hairs, distal part gradually constricted into a curved spur, 14–15 mm long, white to pale pink. Dorsal petal  $11-12 \times 14-15$  mm, broadly ovate to obcordate, cucullate, apex emarginate and mucronate, base cordate, white to pale pink, densely pilose with white hairs, abaxial midvein with a white crescent-shaped crest, 1–1.5 mm high. Lateral united petals 20–24 mm long, free: the upper petals 9–10 × 10–11 mm, broadly oblong, apex truncate, base cuneate, upper outer part white to pale pink, lower inner part dark purple; the lower petals 17–19 × 8–10 mm, oblong, apex truncate, pink to reddish-purple; with a pink auricle at the base, ca 1 mm high. Stamens 5; filaments 4–5 mm long, white; anthers white. Ovary ca 4 mm



**Figure 4.** Seed of *Impatiens jenjittikuliae* (FE SEM). **A, B** Entire seeds **C** Inflated cells with granulate walls **D** Thick finger-like cells.

long, 1.5–2 mm in diam., short fusiform, 5-carpellate, green, glabrous. Fruits, short fusiform capsule, 11–12 mm long, 6–8 mm in diam., subglobose, 5–lobed, green, glabrous. Seeds ca. 20 per fruit, ca. 1.34 mm long, ovoid, brown.

**Pollen morphology:** Pollen grains 4-colpate (Fig. 3A). Equatorial view oblong, ca  $35 \times 18-19 \mu m$  (length/width = 1.89); Polar view nearly elliptic, ca. 17  $\mu m$  thick, colpi four, linear, ca  $9-10 \mu m$  (Fig. 3A); surface entirely covered with numerous irregular lumens, 1.2–2  $\mu m$  diam, lumens deep, sparsely granulate (Fig. 3B); muri slightly straight, joint of muri slightly corniculate (Fig. 3C).

**Seed morphology:** Brown ovoid, ca  $1.34 \times 0.93$  mm, ca  $0.51 \mu$ m thick (length/ width = 1.44) (Fig. 4). Seed coat a composite of two types, thick finger-like cells, and inflated cells with granulate walls (Figs. 4C, D).

*Stem anatomy:* Stem herbaceous, without lignification (Figs 5A–C). Only angular collenchyma for stem-strengthen were found (Fig. 5D).

Phenology. Flowering from Oct. to Nov.; fruiting Oct. from Nov.

**Distribution.** The new species is only known from the type locality in Tak Province, Thailand.


Figure 5. Stem of *Impatiens jenjittikuliae*, transverse sections.



Figure 6. Floral visitation by bee in the locality of *Impatiens jenjittikuliae*.

**Ecology.** *Impatiens jenjittikuliae* grows on limestone close to waterfall in a mixed deciduous forest, 520–600 m elevation (pers. obs.).

**Proposed IUCN conservation assessment.** Critically Endangered B1ab (i, ii, iii) + 2ab (i, ii, iii). This species is only known from the type locality; the extent of occurrence is estimated to be less than 5 km, where it occurs as a small population (IUCN 2012).

**Etymology.** The new species is named in honor of Dr. Thaya Jenjittikul who encouraged the first author to step in and study this lovely plant family.

**Pollination ecology.** The author observed five visitations by bees from the family Apidae (identified by an entomologist, Pornpimon Tangtorwongsakul) during the expeditions. The size of bee body fit well with the floral entrance (Fig. 6). Moreover, the floral structure of this new species is similar to other bee-pollinated species, e.g. *I. psittacina* (Ruchisansakun et al. 2016). Hence, we concluded that it is a bee-pollinated species.

## Discussion

*Impatiens jenjittikuliae* is similar to *I. lacei* and the other species closely related to *I. pul-chra* Hook.f (= *I. mengtszeana* Hook.f. in Ruchisansakun et al. 2015) in its raceme inflorescence, shape of flower, and short fusiform capsule. The short fusiform capsule and the 4-colpate pollen grains of the new species support its placement in the subgenus *Impatiens* (Yu et al. 2015). In addition, *I. jenjittikuliae* has seeds coated with inflated cells with granulate walls similar to those described in species, such as *I. napoensis* Y. L. Chen, within the sect. *Uniflorae* (Janssens et al. 2012; Yu et al. 2015).

The cross sections of the stem of *I. jenjittikuliae* have shown that the new species is herbaceous, similar to the morphologically similar species in the sect. *Uniflorae*, *I. pulchra*, which also show in Lens et al. (2012) as *I. mengtszeana* Hook.f. (Lens et al. 2012; Ruchisansakun et al. 2015; Yu et al. 2015).

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