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Swertia hongquanii, a new species of Gentianaceae from Mt. Wuling, southern China

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Abstract

Swertia hongquanii Jia X. Li, a new species of Gentianaceae, is described and illustrated from Mt. Wuling, southern China. It grows on the tops of steep limestone mountains surrounded by cliffs above an altitude of ca. 1400 m. This species resembles *Swertia bimaculata*, but differs from the latter by the stem leaf blades 2.0–4.5 × 1.0–2.5 cm, ovate to ovate-cordate, base cordate and subamplexicaul, the seeds irregularly polyhedral and the seed coats minutely thorny and reticulate. Based on morphological traits, i.e. the inflorescence structure and the number and structure of the nectaries, the new species may be a member of series *Maculatae*. A key to the species of series *Maculatae* of section *Ophelia* is provided.

Keywords

Swertia, new taxon, limestone cliffs, Mt. Tianmen

Introduction

Mt. Wuling is located at the eastern edge of the Yunnan-Guizhou Plateau in southern China. This plateau has an ancient geological history and complex terrain and geomorphology (Zhu et al. 2015). Due to its long history and changing paleogeography, this area presents a great variety of physiographical characteristics and abundant diverse ecosystems within a short extent of space. Additionally, it has become an important channel for the concentration, diffusion and migration of east Asiatic angiosperms (Chen et al. 2002; Li et al. 2008; Li and Yu 2014; Zhu et al. 2015). Mt. Wuling is rich in flowering plants (4083 species: Chen et al. 2002) and several new taxa have been described from this area recently (e.g. Peng et al. 2007; Li and Yu 2014; Zhang et al. 2015; Liu et al. 2016). Here we propose another new species of *Swertia* L. (Gentianaceae), collected from Tianmenshan National Forest Park in this area.

Swertia consists of 3 subgenera, 11 sections and over 160 species (Joshi 2011; Rybczynski et al. 2014; Ho and Liu 2015). The genus is easily recognisable by the rotate corolla and by the presence of coralline nectariferous glands (Suksathan 2001; Ho and Liu 2015). The genus shows a North Temperate and South Temperate disjunctive (Pan-temperate) distribution pattern (Ho and Liu 2015). The majority of species are centred in Asia. South-western China and adjacent regions are diversity centres and initial diversification centres for this genus (Ho et al. 1994; Ho and Liu 2015). In China, approximately 75 species occur, mainly in mountains at an elevation above 1000 m (Ho and Pringle 1995). Moreover, several new species have been described from China since the account of the genus for the Flora of China was published (e.g. Chen et al. 2008; Ho and Liu 2010, 2015; Chen et al. 2016).

The new species from Mt. Wuling first came to our attention in September 2007 during our plant investigation in Tianmenshan National Forest Park. It was initially misidentified as *Swertia bimaculata* (Siebold & Zucc.) Hook.f. & Thomson ex C.B.Clarke due to its lax panicles of cymes and two naked nectaries per corolla lobe. In 2016, in the course of digitising the specimens in CSFI, we found a unique specimen, collected from Mt. Tianmen by Mr. Hongquan Huang (13050406) on 20 September 2013. It was identified as *Swertia bimaculata* but differs by its petite shape and cordate leaf base. Subsequently, we again visited Tianmenshan National Forest Park to observe this plant in September and November 2017 and collected more flowering and fruiting material. After a morphological comparison, the specimens from Tianmenshan National Forest Park are confirmed to be an undescribed species of *Swertia*, probably belonging to series *Maculatae* T.N.Ho & S.W.Liu in section *Ophelia* (Griseb.) Gilg in subgenus *Ophelia* (Griseb.) C.B.Clarke.

Materials and methods

During three field expeditions in September 2007, September and November 2017, a total of fifteen flowering and five fruiting individuals from the type locality were collected from Tianmenshan National Forest Park, Zhangjiajie City, Hunan Province, Southwest China. The information and measurements of the new species were taken from live and dried herbarium specimens and from field data. Seeds were examined and imaged with a Leica M205C stereomicroscope attached to a video camera (Leica DFC495). The morphological comparisons with related species, viz., *Swertia bimaculata*, *S. tashiroi* Makino, *S. oculata* Hemsl., *S. tozanensis* Hayata, *S. cordata* (Wall. ex G.Don) C.B.Clarke and *S. shintenensis* Hayata, are based on herbarium specimens (about 2300 specimens) and relevant literature (Ho et al. 1988; Ho and Pringle 1995; Rybczynski et al. 2014; Ho and Liu 2015). Specimens deposited in the following herbaria were examined: CSFI, CSH, CZH, JIU, HTC, IBK, IBSC, LBG, KUN, PE, SYS and WUK (Thiers 2015).

The number of mature individuals was recorded in the field in twenty 1 m^2 sampling plots. We assessed the preliminary conservation status of the new species using our field knowledge and applying the IUCN (2017) criteria. The taxonomic treatment of the genus *Swertia* follows Ho and Liu (2015).

Taxonomic treatment

***Swertia hongquanii* Jia X. Li, sp. nov.**
urn:lsid:ipni.org:names:60479372-2
Figs 1–3

Diagnosis. The new species is similar to *Swertia bimaculata*, but differs from the latter by its leaf blades ovate to ovate-cordate, 2.0–4.5 × 1.0–2.5 cm with base cordate and subamplexicaul (vs. broadly elliptic to ovate-lanceolate, 3.5–9 × 1.0–4 cm with base tapered to obtuse), and its seeds irregularly polyhedral with minutely thorny and reticulate seed coat (vs. globose with seed coat finely warty) (Table 1).

Type. CHINA. Hunan province: Zhangjiajie City, Mt. Tianmen, 29°3'N, 110°28'E, elev. 1400 m, 23 September 2016, *J. X. Li 092502* (holotype CSFI, bar code: CSFI063656; isotypes CSFI, IBSC, PE).

Description. Biennial herbs, 2–40 cm tall. Roots yellow, fibrous. Stems erect, sometimes branched from the lower part, subquadrangular, 1–3 mm in diam., with narrow wings on angles. Basal leaves quickly withering, blades elliptic to obovate, 1–3.5 × 0.8–3 cm, apex obtuse, base cuneate and decurrent, veins yellow-white, distinct, pinnate; petioles flattened, winged, ca. 0.5–2.5 cm long. Stem leaves sessile or

Table 1. Morphological comparison of *Swertia hongquanii*, *S. bimaculata*, and *S. cordata* (adapted from Ho and Liu 2015).

	S. hongquanii	*S. bimaculata*	*S. cordata*
Habit	biennial	annual or biennial	annual
Height	2–40 cm tall	30–140(–200) cm tall	15–40(–80) cm tall
Stem	1–3 mm in diam., simple or branched from the lower part	2–6 mm in diam., branched from the middle	1.5–2 mm in diam., branched at base or from the middle
Stem leaves	ovate to ovate-cordate, base cordate and subamplexicaul, 2.0–4.5 × 1.0–2.5 cm	broadly elliptic to ovate-lanceolate, base narrowly tapered to obtuse, 3.5–9 × 1–4(–5) cm	ovate to ovate-cordate, base cordate and subamplexicaul, 0.8–2.3 × 0.5–1.2 cm
Inflorescence	lax, axes and pedicels spreading, few or many-flowered, 5–20(–25) × 4–20 cm	lax, axes and pedicels spreading, many-flowered, 10–50 × 10–30 cm	compact, axes and pedicels not spreading, dense, many-flowered, 3–30(–40) × 2–10 cm
Pedicel length	0.6–4 cm	0.6–4 cm	0.3–1 cm
Corolla	white with purple spots, ca.20 mm in diam.	yellow or white, with purple spots, to 25 mm in diam.	pale purple, with dark purple veins, 10–15 mm in diam.
Nectaries	2 per corolla lobe, yellow-green, semi-orbicular, in the middle of corolla lobes	2 per corolla lobe, yellow-green, orbicular, in the middle of corolla lobes	1 per corolla lobe, yellow, rhomboid to orbicular, at the base of corolla lobes
Seeds	polyhedral, ca. 1.0 mm × 0.5 mm, seed coat minutely thorny and reticulate	globose, 1–1.5 mm in diam., seed coat finely warty	ellipsoid to globose, 0.8-1 mm in diam., seed coat longitudinally and thinly corrugate

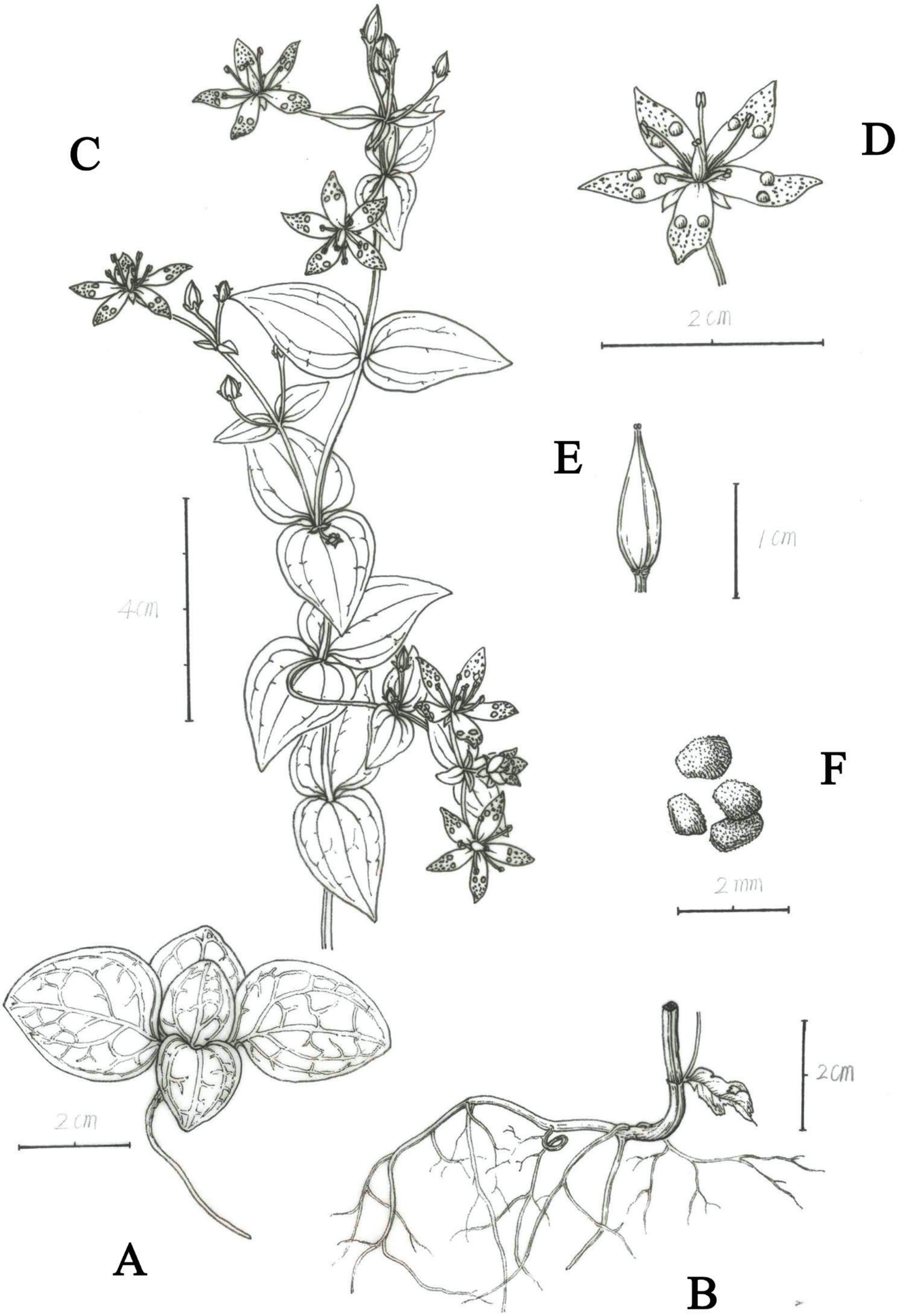

Figure 1. *Swertia hongquanii* **A** seedlings showing basal leaves **B** root **C** flowering plant **D** flower showing corolla, nectaries, stamens and pistil **E** capsule **F** seeds. Drawn by Jing Tian.

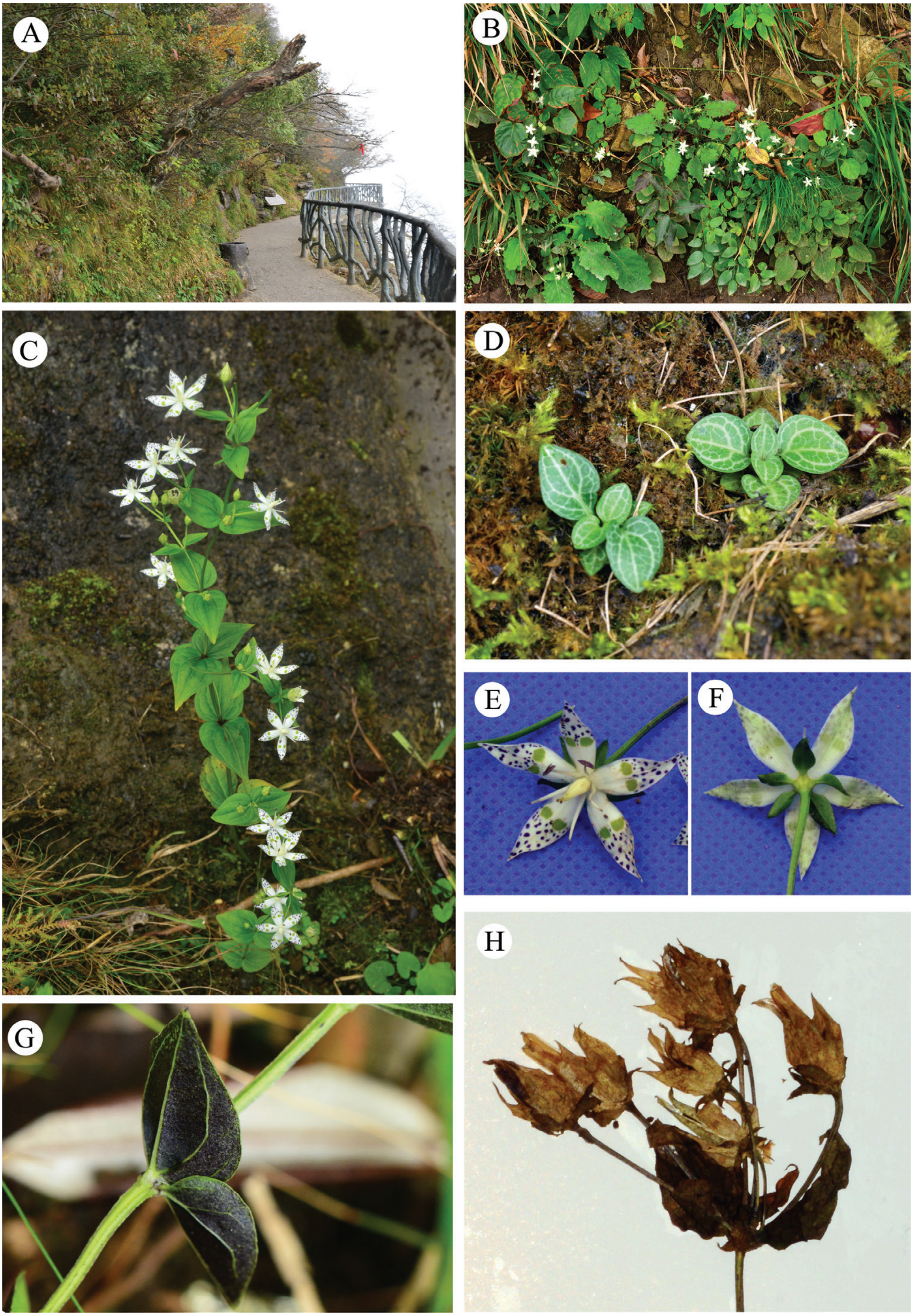

Figure 2. Habitat and morphology of *Swertia hongquanii*. **A–B** Habitat **C** flowering plant **D** seedlings showing basal leaves **E** adaxial view of flower showing calyx, corolla, nectaries, stamens and pistil **F** abaxial view of flower showing calyx and corolla **G** stem with a pair of leaves **H** flowering and fruiting plant; centrally, a capsule with persistent corolla can be seen. Photos: Jiaxiang Li.

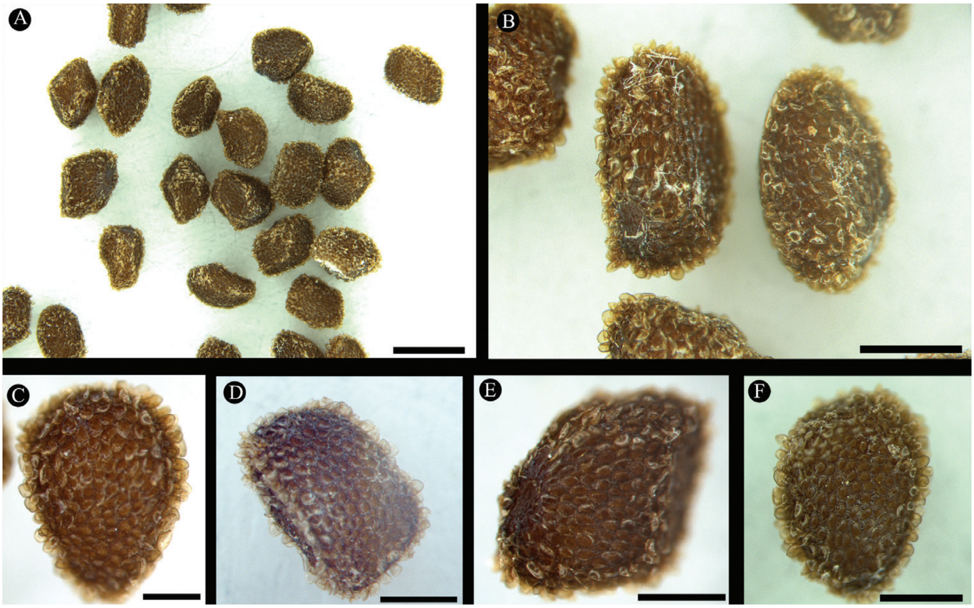

Figure 3. Seeds of *Swertia hongquanii*. Scale bars: 1 mm (**A**); 0.5 mm (**B**); 0.2 mm (**C**); 0.3 mm (**D–F**). Photos: Jiaxiang Li.

shortly petiolate, leaf blades ovate to ovate-cordate, apex acute, base cordate and subamplexicaul, 2.0–4.5 × 1.0–2.5 cm, smaller towards stem apex, veins 3–5, arcuate, distinct, green or yellow-green.

Inflorescence a panicle of cymes, lax, 5–20(–25) × 4–20 cm, few- or many-flowered; axes spreading. Flowers (4-)5-merous. Pedicels spreading to erect, slender, subquadrangular, 0.6–4 cm. Calyx 1/2 to 2/3 as long as corolla, tube 1–2 mm long, lobes narrowly elliptic, 3–6 mm long, with 3 slender and distinct veins, apex acute. Flowers to 2.0 cm in diam.; corolla white with purple spots on the upper half of the lobes adaxially but less visible abaxially, tube 1–2 mm long; lobes elliptic-lanceolate, 0.5–1.0 × 0.2–0.4 cm, widest at the middle, apex acuminate to acute. Nectaries 2 per corolla lobe, situated in the middle of corolla lobe, semi-circular, reduced to a naked gland patch without raised margin, yellow-green. Stamens with filaments ca. 4 mm long, white; anthers ellipsoid, ca. 1.5 mm long, purple. Style short, ca. 0.5 mm long; stigma lobes capitate. Capsules narrowly ovoid, to 1.3 cm long. Seeds polyhedral, compressed irregularly, with distinct angles, ca. 1.0 × 0.5 mm, dark brown; seed coat minutely thorny and reticulate.

Phenology. Flowering was observed in September and October. Fruiting was only observed in November, but probably extends till December.

Distribution and habitat. *Swertia hongquanii* is known only from the type location in Tianmenshan National Forest Park. The climate here is cool, foggy and humid (annual rainfall ca. 1700 mm) and belongs to the subtropical monsoon climate (Li et al. 2008; Zhang et al. 2015). The new species grows on the tops of steep limestone mountains surrounded by cliffs above an altitude of ca. 1400 m. The clifftops are covered by mixed evergreen-deciduous forest dominated by species of Fagaceae, Lauraceae, Betulaceae, Aceraceae and Ulmaceae (Li et al. 2008). One population occurs in the crevices of limestone cliffs; it is covered by shrubs and herbs with little soil but is rich in humus. It is accompanied by *Viola davidii* Franch., *Youngia* Cass. sp., *Thalictrum ichangense* Lecoy. ex Oliv., *Aster ageratoides* Turcz., *Calamagrostis arundinacea* (L.) Roth, *Carex filicina* Nees, *Carex* L. sp., *Leptopus chinensis* (Bunge) Pojark., *Zanthoxylum bungeanum* Maxim., *Rubus innominatus* S.Moore var. *kuntzeanus* (Hemsl.) L.H.Bailey, *Rubus henryi* Hemsl. & Kuntze, *Salix mictotricha* C.K.Schneid., *Carpinus dayongiana* K.W.Liu & Q.Z.Lin and *Betula chinensis* Maxim. Another population grows in the herb layer of forest dominated by *Quercus multinervis* (W.C.Cheng & T.Hong) Govaerts and *Hovenia dulcis* Thunb. with deep and fertile soil. Other herbaceous species are *Ophiopogon japonicus* (Thunb.) Ker Gawl., *Carex gibba* Wahlenb., *Sanicula orthacantha* S.Moore, *Pimpinella diversifolia* DC. and *Viola diffusa* Ging.

Etymology. The species is named after Mr. Huang Hongquan for his help during our field investigation. He was also the first to collect this new species.

Local name. Tianmenshan Zhang Ya Cai

Conservation status. Despite several investigations in Mt. Tianmen and the surrounding areas (96 km^2), two populations with nearly 500 individuals each (total < 1000) of *Swertia hongquanii* were found only at the type locality (towering summit terrace with an area of 2 km^2). Presently, a tourist plank walkway passes through this location and its habitat could be easily disturbed or destroyed. According to the IUCN (2017) criterion D thresholds (mature individuals < 1000, AOO < 20 km^2), the new species could be assessed as VU. However, it grows in the upper part of steep limestone mountains surrounded by cliffs, which previously made it impossible to be encountered until a plank walkway was built across the cliff face for tourists. With limited fieldwork executed at present, it is possible that more populations could be found in similar habitats of the Wuling mountain areas. Therefore, we consider the species DD (Data Deficient).

Additional specimens examined. CHINA: Hunan, Zhangjiajie City, Mt. Tianmen, 20 September 2013, 29°3'N, 110°28'E, limestone, 1400 m alt., *Hongquan Huang* 13050406 (CSFI); the same locality, 20 November 2017, *Hongquan Huang* HHQ02 (CSFI); the same locality, 25 September 2017, J.X. Li 092503 (CSFI).

Discussion

Morphologically, *Swertia hongquanii* resembles *S. bimaculata* in possessing two naked gland patches in the middle of each corolla lobe, whereas it is clearly distinguished by stem leaf and seed characteristics (Table 1). During field investigations, we observed that most *S. bimaculata* flowers were withered while the new species was just beginning to blossom in populations in the same locality (separated by a distance of ca. 300 m). We therefore infer that the morphological differences with *S. bimaculata* are interspecific rather than intraspecific variations driven by ecological conditions.

From its overall vegetative appearance, *Swertia hongquanii* also resembles *S. cordata*, with both species having slender and subquadrangular stems with wings on the angles, ovate to ovate-cordate stem leaves with cordate to subamplexicaul bases. *Swertia hongquanii* is clearly distinguished from *S. cordata*, however, by the two nectaries in the middle of the corolla lobes; *S. cordata*, in contrast, has a single nectary on the base of the corolla lobe (Table 1) (Ho and Liu 2015).

According to the classification of Ho and Pringle (1995) and Ho and Liu (1980; 2015), the species of series *Maculatae* of section *Ophelia* of subgenus *Ophelia* are distinguished by their stems being strongly branched, their inflorescences being panicles of cymes and by the presence of one or two nectaries on each corolla lobe reduced to a naked gland patch without raised margin. Six species, viz., *S. bimaculata*, *S. oculata*, *S. cordata*, *S. tozanensis*, *S. shintenensis* and *S. tashiroi* were recognised as members of series. *Maculatae*, distributed in Asia (Ho and Liu 2015). Based on morphological traits, especially those of inflorescences and nectaries, the new species may also be a member of series *Maculatae* (Figures 1–3).

The seeds of *S. hongquanii* are easily distinguished from other species of series *Maculatae*, as they are irregular polyhedrons with minutely thorny and reticulate seed coats (Figure 3), whereas those of the other species of series *Maculatae* are ellipsoid to globose with finely warty seed coats (*S. tashiroi*, *S. shintensis*, *S. bimaculata*, *S. tozanensis* and *S. oculata*) or with longitudinally and thinly corrugate seed coats (*S. cordata*) (Ho and Liu 2015).

Key to species of series Maculatae (adapted from Ho and Liu 2015)

1 Nectaries one per corolla lobe **2**

– Nectaries two per corolla lobe **3**

2 Basal leaves quickly withering; inflorescences usually narrow and dense; corolla pale purple; nectaries on base of corolla lobe; seed coat longitudinally and thinly corrugate .... ***S. cordata***

– Basal leaves persistent; inflorescences rounded and lax; corolla yellow or yellow-green; nectaries in the middle of corolla lobe; seed coat finely warty **4**

3 Leaf blades broadly elliptic to ovate-lanceolate or ovate to ovate-cordate, more than 1 cm wide; corolla with purple spots **5**

– Leaf blades linear, linear-lanceolate or lanceolate, 0.2–0.7 cm wide; corolla with yellow-green or dark spots **6**

4 Upper stem leaves lanceolate to linear; corolla unspotted; seeds 0.5 mm in diam .... ***S. tashiroi***

– Upper stem leaves ovate; corolla with purple-brown spots on upper portion; seeds 0.7–1 mm in diam. .... ***S. shintenensis***

5 Plants 30–140 (–200) cm tall; leaf blades broadly elliptic to ovate-lanceolate, base narrowly tapered to obtuse; seeds globose; seed coat warty .... ***S. bimaculata***

– Plants 2–30 cm tall; leaf blades ovate to ovate-cordate, base cordate and subamplexicaul; seeds polyhedral; seed coat minutely thorny and reticulate .... ***S. hongquanii***

6 Calyx lobes linear to linear-oblong; corolla lobes elliptic-lanceolate, white, with yellow-green spots, apex acuminate and apiculate .... ***S. oculata***

– Calyx lobes ovate-lanceolate to spathulate; corolla lobes oblong, pale-yellow, with dark spots, apex obtuse to acute .... ***S. tozanensis***

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Primulina serrulata (Gesneriaceae), a new species from southeastern Guizhou, China

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Abstract

Primulina serrulata R.B.Zhang & F. Wen, a new species from a limestone area in southeastern Guizhou, China, is described and illustrated here. The new species is morphologically related to *P. fimbrisepala* (Hand.-Mazz.) Y.Z.Wang. We examined the morphological differences between these congeners and provide illustrations and photographs of this new species in this paper.

Keywords

Flora of Guizhou, Karst region, New taxa, *Primulina fimbrisepala*

Introduction

The formerly monotypic genus, *Primulina* Hance, has become the largest genus in the subfamily *Didymocarpoideae* of Gesneriaceae in China (Weber et al. 2013, Xu et al. 2017, Wen et al. 2019). The number of *Primulina* has been growing explosively since 2005 (Möller et al. 2016, Möller 2019). For the moment, there are a total of 199 species (including infraspecies) in this genus in China (Wen et al. 2019). *Primulina* shows high levels of endemism and ecological (edaphic) specialization, especially in Karst areas.

Based on extensive literature surveys and field observations, we discovered that most species of *Primulina* occur in the karst area of southern and southwestern China and northern Vietnam. They are often limited to a single or a few caves or in specialized and narrow microhabitats of karst limestone hill systems, called island distributions (Wang et al. 1998, Li and Wang 2004, Wei et al. 2010, Hao et al. 2015). The current research result indicated that geographical isolation had been demonstrated to be one kind of reliable driver of *Primulina* diversification and speciation, for example, *P. eburnea* and *P. hochiensis* complexes (Gao et al. 2015, Wang et al. 2017, Yang et al. 2019).

In 2018, during field explorations, a local herbalist found an unknown species of *Primulina* near the Guizhou-Guangxi border at Rongjiang County, southeastern Guizhou, China (Figure 1). Several living individuals from the population found in the field were brought to the nursery of Gesneriad Conservation Center of China (GCCC) and cultivated there. The leaf blade margin characteristics of these plants look appealing because of the prominent and irregular serrations. At the same time, the beautiful silvery veins on the leaf blades and purplish-blue flowers soon caught our attention. However, this species can easily be distinguished from its congener by its morphology. After a morphological comparison between this new species and its related species and literature studies, we consider it is new to science, which is being described and illustrated here.

Methods

Morphological observations of the new species were carried out based on living plants as well as dry specimens. All morphological characters were measured with dissecting microscopes and were described using the terminology presented by Wang et al. (1998). Literature studies included all relevant monographs, i.e., Wang et al. (1998), Li and Wang (2004) and Wei et al. (2010), and also some recently published literature. Specimen checking was done at IBK, IBSC, PE and also some web databases, such as Chinese Virtual Herbarium (http://www.cvh.ac.cn/) and Global Plants (http://plants.jstor.org/).

Taxonomy

Primulina serrulata **R.B.Zhang & F.Wen, sp. nov.**

urn:lsid:ipni.org:names:77201948-1
Figures 1–3

Diagnosis. *Primulina serrulata* mainly differs from its congener, *P. fimbrisepala*, by its purplish-blue flowers which lack the dark purple spots inside the corolla (*vs*. purple to purplish-pink, with bright dark purple spots inside the corolla), cuneate leaf blade base (*vs*. obliquely cuneate, broadly cuneate or cordate), anthers glabrescent (*vs*. sparsely bearded) and smaller stigma ca. 1 mm long (*vs*. 2–3 mm long).

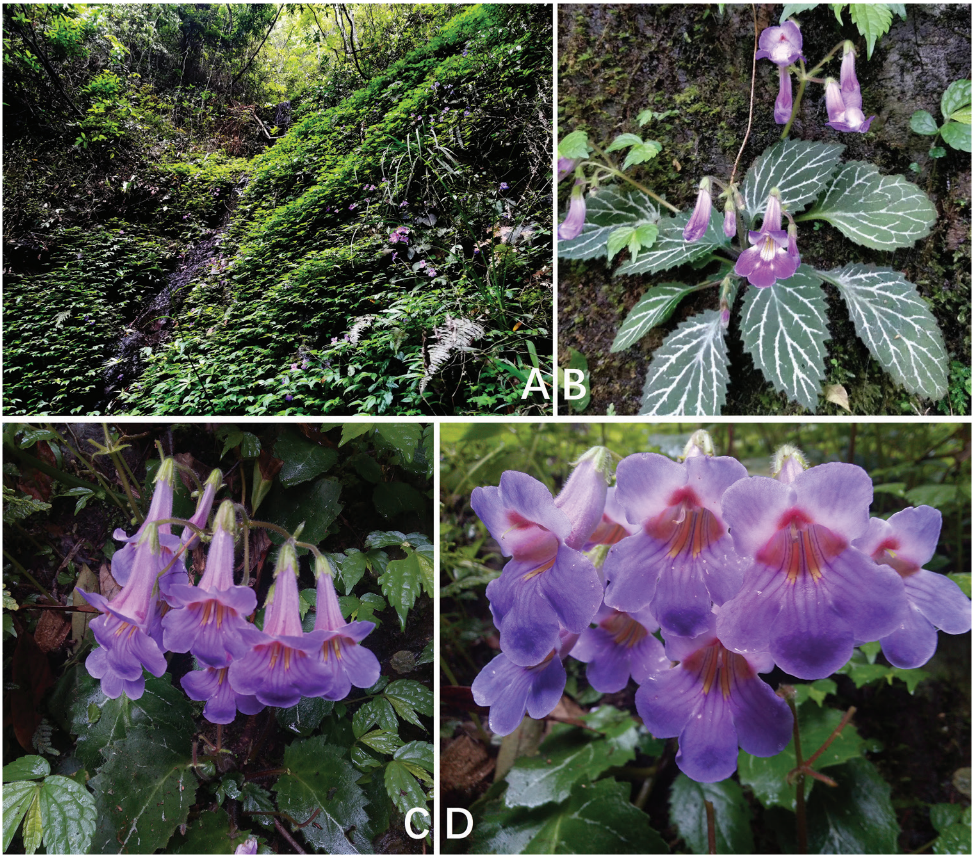

Figure 1. *Primulina serrulata*. **A** natural habitat **B** habit in flowering **C** cyme and flower in top view **D** flowers in front view. Charted by Wen-Hua Xu.

Type. CHINA. Guizhou Province, Rongjiang County, Langdong town, growing on moist limestone rocks surfaces near waterfall, alt. 780 m, 26°07'N, 108°42'E, 17 April 2018 (flowering), *Ren-Bo Zhang et al.*, *ZRB1478* (holotype: ZY!; isotype: IBK!).

Description. ***Rhizomatous*** stem subterete, ca. 6 cm long, ca. 1.5 cm in diameter. **Leaves** 4–6, all basal, opposite decussate. ***Petioles*** flatted, fragile and easy to be broken, 2–7 cm long, 4–5 mm wide, densely erect white multicellular hispid, hairs ca. 7 mm long. ***Leaf blade*** slightly fleshy and fragile when fresh, chartaceous when dried, obliquely ovate, oval to nearly rounded, 5–13 × 3–10 cm, densely erect white multicellular hispid and short hispid on both surfaces, base obliquely broadly cuneate, cordate to nearly rounded, margin conspicuously big and irregular serrate and biserrate; ***lateral veins*** 3–5 on each side, adaxially inconspicuously sunk but clearly slivery, occasionally green, abaxially conspicuously raised. ***Cymes*** 1–6, axillary, 1–2 branched, 2–5(8)-flowered; ***peduncles*** 11–16 cm long, ca. 2 mm in diameter, densely erect white multicellular hispid and short hispid; ***bracts*** 2, green, opposite, narrowly rhombic, 5–12 × 2–5 mm, margin inconspicuously serrate, apex acute, outside densely pubes-

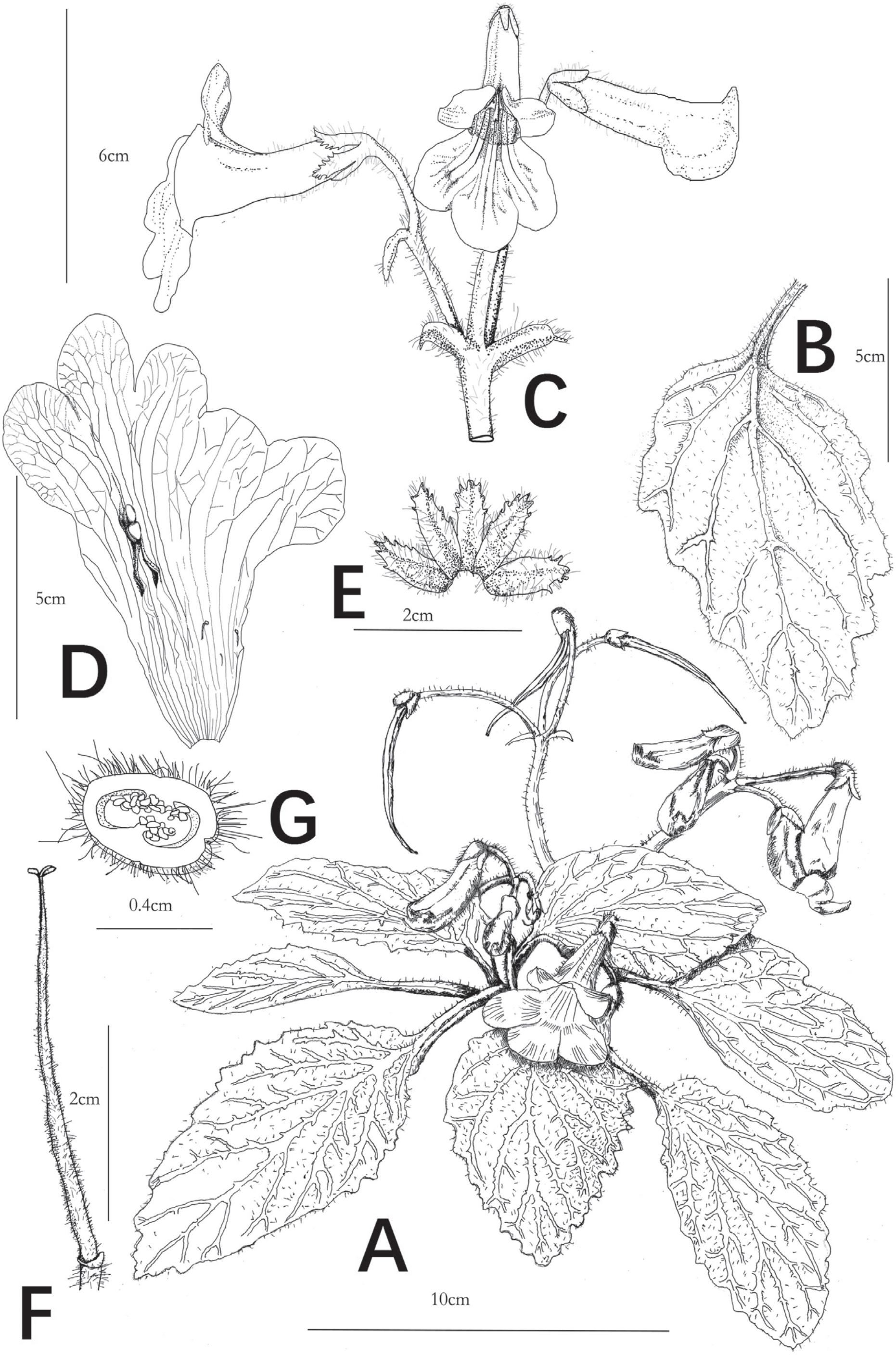

Figure 2. *Primulina serrulata*. **A** habit **B** adaxial leaf blade **C** cyme, bracts, flower in frontal view and flower in lateral view **D** opened corolla for showing stamens and staminodes **E** adaxial calyx lobes **F** pistil **G** cross section of ovary. Drawn by Tan Deng.

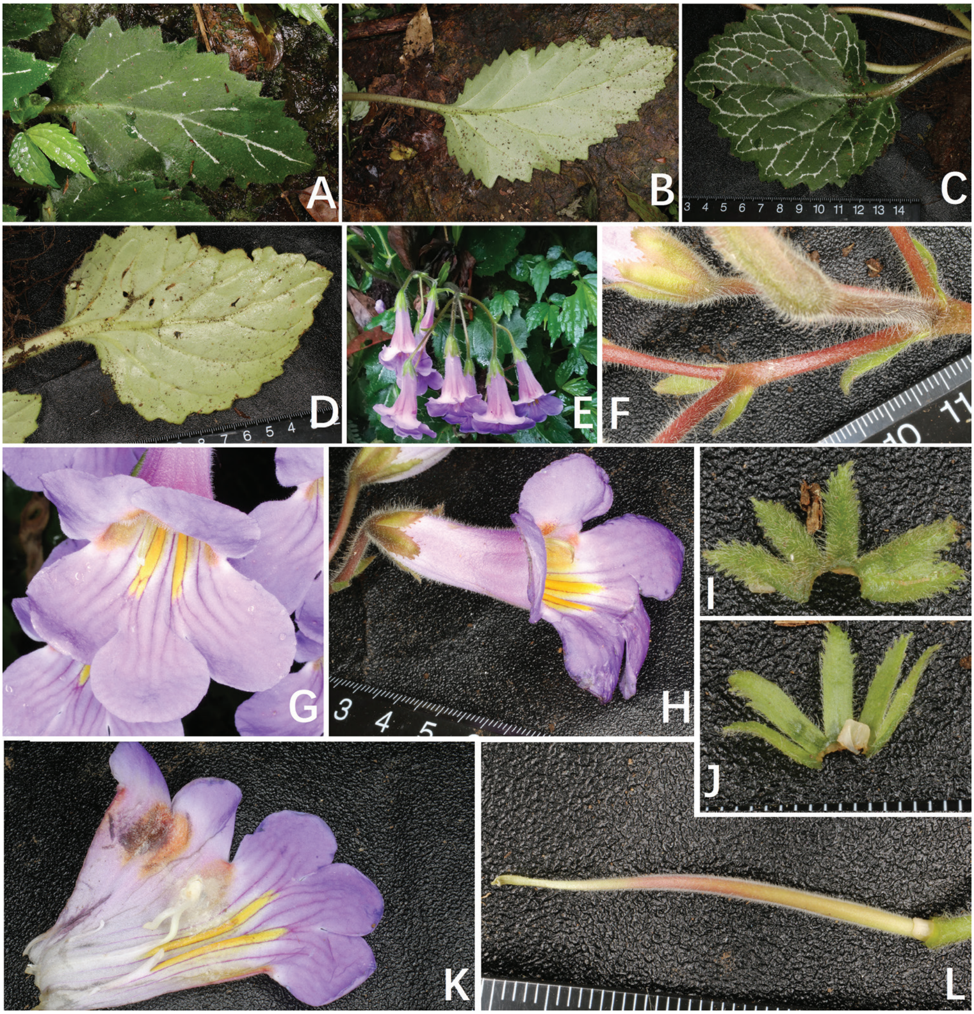

Figure 3. *Primulina serrulata*. **A, C** different shapes of adaxial leaf blades **B, D** different shapes of abaxial leaf blades **E** cyme, bracts, flower in top view **F** bracts and bracteoles **G** corolla in frontal view **H** corolla in lateral view **I** adaxial calyx lobes **J** abaxial calyx lobes **K** opened corolla **L** pistil. Charted by Wen-Hua Xu.

cent, inside pubescent; sometimes with bracteoles, opposite, 2, narrowly triangle, ca. 5 × 1 mm. ***Pedicel*** 2.5–5.5 cm long, ca. 1 mm in diameter, densely pubescent. ***Calyx*** 5-parted to near base, lobes oblong to lanceolate, green, 8–15 × 3–5 mm, outside densely pubescent, inside glabrous, apex subacute, margin entire about 2/3 of calyx lobe from the base but denticulate 3–4(5) about 1/3 of calyx lobe from the apex. ***Corolla*** pale purple to purple, the color of the throat same as corolla with two longitudinal yellow stripes along corolla tube but without dark purple spots, 4.5–6.0 cm long, outside glandular-pubescent and puberulent from the base to middle of corolla tube, inside glabrous; ***tube*** infundibuliform-tubular, 3.0–3.5 cm long, 1.0–1.5 cm in diameter at mouth, ca. 4 mm in diameter at base; ***limb*** distinctly 2-lipped, adaxial lip

2-parted, the upper part of the interior of two adaxial lip lobes with two lines of glandular hairs on the brown patch, lobes broadly ovate, 8–12 × 5–9 mm, apex rounded, abaxial lip 3-lobed, lobes oblong, 2.0–2.8 × 1.0–1.4 cm, apex rounded. ***Stamens*** 2, adnate to 10–13 mm above the corolla tube base; ***filaments*** linear, ca. 15 mm long, white, geniculate near the base, sparely puberulent from the middle to the top, the rest glabrous; ***anthers*** fused by the entire adaxial surfaces, ca. 4 mm long, glabrous. ***Staminodes*** 3, lateral ones 6–7 mm long, adnate to 12–15 mm above the corolla tube base, middle one ca. 1.5 mm long, adnate to 8–10 mm above the corolla tube base. ***Disc*** annular, the higher side ca. 1.5 mm in height but the lower side ca.0.8 mm in height. ***Pistil*** 3.5–4.5 cm long; ***ovary*** cylindrical, 2.5–3.2 cm long, densely glandular pubescent and puberulent; ***style*** 1–1.3 cm long, densely glandular-pubescent and puberulent; ***stigma*** obtrapezoid, apex 2-lobed, ca. 1 mm long, ca. 0.8 mm wide. ***Capsule*** linear, ca. 6 cm long, sparsely pubescent.

Distribution and habitat. At this time, *Primulina serrulata* is only known from the type locality in Langdong village, Langdong town, Rongjiang County, Guizhou Province, based on our field investigations. It grows on moist, shady, limestone rocks near a waterfall, at ca. 780 m altitude, with no more than 150 mature individuals. The population is close to a road, which makes it vulnerable and subject to destruction from human activities.

Phenology. This new species was observed flowering in April and fruiting from May to June.

Etymology. The specific epithet is derived from its particular leaf blade margin, having obvious serrations and bi-serrations.

Provisional IUCN conservation assessment. Because of *Primulina serrulata*'s beautiful leaves and flowers, it is being over-harvested by local people for sale. This unpublished species is therefore on the brink of extinction as a result. Before more surveys are completed to clarify its conservation status, the provisional conservation status is Critically Endangered CR B2ab (iii, v) according to the IUCN red list criteria (IUCN 2012).

Note. *Primulina serrulata* is related to its congener, *P. fimbrisepala*, by some characteristics, for example, the similar calyx lobes and infundibuliform corolla tube, but they can easily be distinguished from each other by the characters summarized in the description below. Numerous dark purple spots were covered at the throat of the corolla, and this is one stably distinctive feature of *P. fimbrisepala*. It is noticeable in different populations of *P. fimbrisepala* from South China when they are flowering (Figure 4). Table 1 below has more detailed information on how to distinguish the two species. They also grow in different substrates. *P. serrulata* only grows in limestone areas, while *P. fimbrisepala* commonly appears in weakly acidic mediums and soils of sandstone or granite mountainous regions. The different growing habitats of the two congeners indicate that geographical isolation should be one of the reliable drivers pushing this genus, *Primulina*, to generate diversification and speciation (Gao et al. 2015, Kong et al. 2017, Wang et al. 2017, Yang et al. 2019).

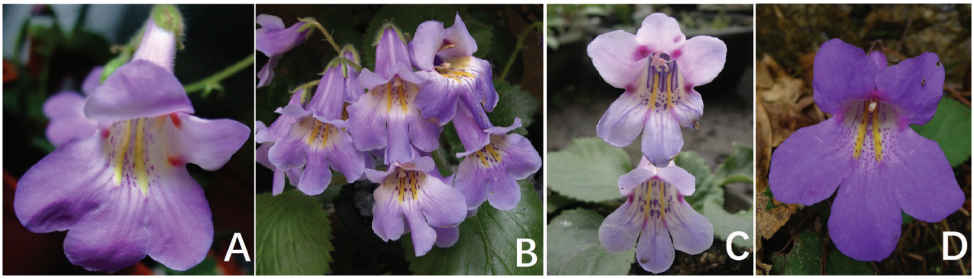

Figure 4. The corolla throat character of *Primulina serrulata*'s congener, *P. fimbrisepala*. **A** from Wenzhou city, Zhejiang **B** from Ziyuan County, Guangxi **C** from Jinxiu County, Guangxi **D** from Ruyuan County, Guangdong. Photoed by Fang Wen, Charted by Wen-Hua Xu.

Table 1. Morphological comparison of *Primulina serrulata* sp. nov. and *P. fimbrisepala*.

Characters	*P. serrulata*	*P. fimbrisepala*
Indumentum of leaf blade	densely erect white multicellular hispid and short hispid on both surfaces	adaxially puberulent and appressed pilose, abaxially sparsely puberulent to velutinous
Indumentum of peduncles	densely erect white multicellular hispid and short hispid	pubescent to appressed pilose
Calyx lobes size	8–15 × 3–5 mm wide	7–11 × 1.5–3.0 mm wide
Corolla color	pale purple to purple, throat without dark purple spots	blue, purple, to pinkish green, throat with numerous dark purple spots
Filaments length	ca. 1.5 cm long	ca. 1.3 cm long
Indumentum of anthers	Glabrescent	sparsely bearded
Stigma size	ca. 1 mm long, ca. 0.8 mm wide	2–3 mm long, ca. 1 mm wide
Flowering time	late April to early May	March to early April

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RESEARCH ARTICLE

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Rhamnella brachycarpa (Rhamnaceae), a new species from Hainan Island, China

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Abstract

Rhamnella brachycarpa Z. Qiang Lu & Y. Shuai Sun, a new evergreen woody species from Hainan Island, is described and illustrated. The specimens of this new species have previously been identified and placed under *R. rubrinervis* (H. Lév.) Rehder, with which it shares evergreen leaves, erect and climbing habits and axillary flowering branches with bracteole leaves. However, the specimens from three distinct Hainan populations significantly differ from those of *R. rubrinervis* from other regions with smaller length to width ratios of leaves, fruit and seeds, smaller sizes of fruit and seeds and mucronate seed apices. Principal Component Analysis of the closely related taxa, based on multiple morphological characters, further recognised two separated groups. One of them comprises *R. tonkinensis* and *R. rubrinervis*, the other merely includes all individuals from these distinct Hainan populations. Therefore, *R. brachycarpa*, based on these distinct Hainan populations, is here erected as a new species, distinctly different from its published relatives.

Keywords

Rhamnella brachycarpa, new species, Hainan Island

Introduction

The buckthorn family (Rhamnaceae) comprises 11 tribes and approximately 61 genera (Hauenschild et al. 2016). In this family, *Rhamnella* (Miquel, 1867) in the tribe Rhamneae is a small genus of shrubs, small trees and climbers (Chen and Carsten 2007; Hauenschild et al. 2016). In "Flora of China", *Rhamnella*, with eight described species, is recognised by the distinctly pedicellate flowers and fleshy fruits, 1-stoned drupes, pinnately veined leaves, serrate leaf margins, semi-inferior ovaries, stipules without thorns and flowers in axillary cymes (Chen and Carsten 2007). Most of them are deciduous broad-leaved woody species with erect habit. According to Flora of China, *R. rubrinervis* (H. Lév.) Rehder is the only evergreen species with both erect and climbing habits within this genus in China and is distributed in S and SE Yunnan, SW and S Guizhou, Guangxi and Hainan Island (Chen and Carsten 2007). However, four other closely related species, *R. tonkinensis* (Pit.) T. Yamaz., *R. hainanensis* Merr., *R. crenulata* (Hand.-Mazz.) T. Yamaz. and *R. longifolia* Tsai & K.M. Feng, have also been published (http://www.theplantlist.org). Only *R. rubrinervis* and *R. tonkinensis* have been accepted as valid species, while all others are treated as synonyms of *R. rubrinervis* (Chen and Carsten 2007). Interestingly, these evergreen species have been treated as an independent genus because of the unique morphology and *R. rubrinervis* has been proposed to be conspecific to *R. tonkinensis* (Fan and Yang 1997; Chen and Carsten 2007). After examining all specimens of *R. rubrinervis* preserved in the Chinese Virtual Herbarium (http://www.cvh.org.cn) and Global Biodiversity Information Facility (https://www.gbif.org/) in 2017, we found that one distinct specimen from Hainan Island placed under *R. rubrinervis* might represent a new species because it was clearly different from a representative specimen of this species from Guangxi with the characters of shorter fruit and smaller leaf length to width ratio (Figure 1). In order to further test this hypothesis, we re-examined specimens (including type specimens) in herbaria to illustrate the distinct differences of this special Hainan specimen. We also conducted a field survey across the total distribution range of *R. rubrinervis*, including this Hainan population, to collect enough specimens for the examination of their morphological variations at the population level.

Material and methods

Field surveys

We examined the specimens of *R. rubrinervis* preserved in the following herbaria: PE, KUN, GXMG, GXMI, GZAC, IBK, IMDY and MNHN (Table 1). In addition, we collected 164 specimens from 18 populations across its total distribution range for morphological comparison and clustering analysis. Voucher specimens were deposited as *Zhiqiang Lu 2018XTBG001–Zhiqiang Lu 2018XTBG019* (HITBC), *Zhiqiang Lu 2018191001–Zhiqiang Lu 2018191011* (HITBC), *Zhiqiang Lu 2018XSBN001–Zhiqiang Lu 2018XSBN003* (HITBC), *Zhiqiang Lu 2018115001–Zhiqiang Lu 2018115013* (HITBC), *Zhiqiang Lu 2018034001–Zhiqiang Lu 2018034007* (HITBC), *Zhiqiang Lu 2018027001–Zhiqiang Lu 2018027011* (HITBC), *Zhiqiang Lu 2018017001–Zhiqiang Lu 2018017008* (HITBC), *Zhiqiang Lu 2018128001–Zhiqiang Lu 2018128012* (HITBC), *Zhiqiang Lu 2018041001–Zhiqiang Lu 2018041007* (HITBC), *Zhiqiang Lu 2018033001–Zhiqiang Lu 2018033005* (HITBC), *Zhiqiang Lu 2018187001–Zhiqiang Lu 2018187003* (HITBC), *Zhiqiang Lu 2018141001–Zhiqiang Lu 2018141012* (HITBC), *Zhiqiang Lu 2018138001–Zhiqiang Lu 2018138015* (HITBC), *Zhiqiang Lu 2018035001–Zhiqiang Lu 2018035006* (HITBC), *Zhiqiang Lu 2018106001–Zhiqiang Lu 2018106009* (HITBC), *Zhiqiang Lu 2018017001–*

Figure 1. The gross morphology of two specimens identified as *Rhamnella rubrinervis*. **A** *R. rubrinervis* from Hainan Island (Qionghai, Tayang, 27 Nov 1936, *X.Q. Liu 28256*, PE) **B** *R. rubrinervis* from Guangxi (Baise, Napo, 15 Oct 2014, *B.Y. Huang et al. 074LY*, GXMG). The leaf length to width ratio and fruit size are totally different between two specimens.

Zhiqiang Lu 2018017008 (HITBC), *Zhiqiang Lu 2018HN3001–Zhiqiang Lu 2018HN3012* (HITBC) and *Zhiqiang Lu 2018HN3013–Zhiqiang Lu 2018HN3015* (GXMI). In addition, we specifically visited the site of this suspected new species and conducted specific field surveys on its total distribution range and a population census on Hainan Island, from 2018 to 2019.

Morphological analysis

The newly collected specimens of *R. rubrinervis* were used to conduct morphological comparisons, based on the characters of leaves, flowers, fruit and seeds. We examined their morphological variations within and between populations through the measurement of the typical leaf and fruit for each of 164 newly collected specimens. Seventy five specimens preserved in herbaria were also used to conduct the morphological measurement and comparison (Table 1). However, the measurement of seed characters for these specimens was abandoned, because sizes of seeds could also be reflected by those of fruit (Table 2), in addition that, we could not avoid the damage to them during this process. Finally, a total of 239 specimens and 10 morphological characters were used to carry out the Principal Component Analysis (PCA) (Table 3).

Table 1. Specimens preserved in herbarium used for Principal Component Analysis (PCA) of morphological variations. Those collection sites marked in bold indicate where the additional specimens were collected in this study.

Species name	Collector	Collection number	Collection site	Herbarium	No. of specimen
R. brachycarpa	*Z.Q. Lu*	*2018HN3001–2018HN3012*	**Baoting, Hainan**	XTBG	12
	Z.Q. Lu	*2018HN3013–2018HN3015*	**Baoting, Hainan**	GXMI	3
	X.Q. Liu	*28256*	Qionghai, Hainan	PE	1
R. hainanensis	*F.A. McClure*	*8358* (*three copies*)	Wuzhishan, Hainan	CAS	3 (isotypes)
R. rubrinervis	*Z. Huang*	*34582*	Sanya, Hainan	PE	1
	Tsang and Fung	*18207*	Danzhou, Hainan	PE	1
R. tonkinensis	*H.F. Bon*	*2246* (*three copies*)	Vietnam	MNHN	3 (isolectotypes)
R. longifolia	*G.M. Feng*	11638	Jinghong, Yunnan	KUN	1 (isotype)
R. crenulata	*Handel-Mazzetti*	*10758*	Badschai, Guizhou	HT	1 (isotype)
R. rubrinervis	*A. Rehder*	*729*	China	HUH	1 (holotype)
	China-Vietnam team	*1477* (*two copies*)	N Vietnam	PE, KUN	2
	Q.W. Wang	*76350, 75597, 75473, 441*	**Jinghong, Yunnan**	PE	4
	G.M. Feng	*11638, 14381, 159*	Menghai, Yunnan	PE	3
	Y.H. Li	*3574, 34298, 3574, 2505*	**Menglun, Yunnan**	HITBC	4
	G.D. Tao	*16289*	**Jinuoshan, Yunnan**	HITBC	1
	X.W. Li	*13103, 13015*	Yiwu, Yunnan	KUN	2
	Z.H. Yang	*1424*	**Fadou, Yunnan**	KUN	1
	Maguan team	*076, 323*	Maguan, Yunnan	IMDY	2
	Y.Z. Wang et al.	*4388*	**Malipo, Yunnan**	PE	1
	Q.W. Wang	*84943, 84942*	Yanshan, Yunnan	PE	2
	S.W. Yu	*86011, 860117*	Yanshan, Yunnan	KUN	2
	Beijing team	*893045, 896924*	**Tian'e, Guangxi**	PE	2
	S.X. Yu	*337*	**Napo, Guangxi**	GXMG	1
	Anonymous	*63, 402*	Location unknown	KUN	2
	China-Japan team	*100828*	**Xingyi, Guizhou**	KUN	1
	Guizhou team	*8180, 8427*	Xingren, Guizhou	KUN	2
	J.C. Yang	*LH0072*	Longtan, Guangxi	IBK	1
	Anonymous	*683*	**Liuzhou, Guangxi**	IBK	1
	Lingyun team	*19LY, 08LY, 48LY, 49LY, 58LY, 74LY, 51LY, 04LY, 05*	**Lingyun, Guangxi**	GXMG	9
	Tianlin team	*031* (*two copies*), *005*	**Tianlin, Guangxi**	GXMG	3
	Yongfu team	*03LY*	Yongfu, Guangxi	GXMG	1
	Anonymous	*00747, 99LY*	**Tian'e, Guangxi**	IBK, GXMG	2
	B.Y. Huang	*23672*	Shangsi, Guangxi	GXMG	1
	C.C. Huang	*16994, 00938*	Luocheng, Guangxi	GXMI	2
	X.X. Chen	*02793, 02323, 2967*	**Chongzuo, Guangxi**	GXMI	3
	Y.S. Huang et al.	*LYJX0657, LYJX0458*	**Jingxi, Guangxi**	IBK	2
	K.M. Lan	*870007, 870060*	Daozhen, Guizhou	GZAC	2
	R.C. Peng	*ML0367*	Huanjiang, Guangxi	IBK	1
	Z.Y. Cao	*1135, 597*	**Ceheng, Guizhou**	PE	2
	Y. Jiang	*7045*	**Dushan, Guizhou**	PE	1

Table 2. Morphological characters of *Rhamnella rubrinervis* and *R. brachycarpa* at the population level. Traits that differ between species are marked in bold.

Characters	*Rhamnella rubrinervis*	*Rhamnella brachycarpa*
LEAF		
Shape and size	Leaf blade oblong or ovate-oblong, 5.4–14.4 × 1.7–5.1 cm, **length to width ratio (2.5)2.8–3.9**; base commonly rounded, rarely cuneate, margin inconspicuously remotely serrate or subentire; **apex acuminate to long acuminate**; bracteole leaf similar to leaves in vegetative branches, but smaller	Leaf blade elliptic-ovate, 5.8–10.3 × 3.1–4.8 cm, **length to width ratio 1.9–2.4**; base cuneate or nearly rounded, margin inconspicuously remotely serrate or subentire; **apex short acuminate or acute**; bracteole leaf similar to leaves in vegetative branches, but smaller
Length of petiole	**3–9 mm**	**7–12 mm**
Number of lateral veins on each side of midvein	5–8	5–7
Average distance between lateral veins located in the middle of leaf	3–8 mm	3–6 mm
FLOWER		
Number of flowers for each axillary cyme	2–10	2–9
Length of pedicel	2–5	3–5
Shape and size	Flower diameter ca. 4 mm; sepals triangular, ca. 2 mm; stamens involute by petals, ca. 2 mm in length	Flower diameter ca. 4 mm; sepals triangular, ca. 2 mm; stamens involute by petals, ca. 2 mm in length
FRUIT		
Size of fleshy fruit	**10.2–12.1 × 10.1–12.5 mm**	**8.7–10.9 × 7.5–10.6 mm**
Size of dried fruit	**8.2–11.1 × 4.2–5.8 mm**	**6.5–7.5 × 4.7–6.0 mm**
Length to width ratio of dried fruit	**1.6–2.2**	**1.3–1.5**
Length of fruiting pedicel	3–6 mm	4–6 mm
SEED		
Size of seed	**7.1–9.9 × 4.0–5.5 mm**	**5.0–7.0 × 4.5–5.5 mm**
Length to width ratio of seed	**1.6–2.1**	**0.9–1.5**
Seed apex	**Rarely mucronate**	**Mucronate**

Table 3. Morphological characters measured for Principal Component Analysis (PCA) based on 239 specimens.

Character number	State	Unit	Coding (if qualitative)
LEAF			
1	Length	cm	
2	Width	cm	
3	Length to width ratio	Ratio	
4	Length of petiole	cm	
5	Apex	Qualitative	2 = Short acuminate or acute 1 = Long acuminate or acuminate
6	Base rounded or cuneate	Qualitative	2 = Cuneate 1 = Rounded
7	Number of lateral veins on each side of midvein	Count	
DRIED FRUIT			
8	Length	mm	
9	Width	mm	
10	Length to width ratio	Ratio	

Results

Our specific field survey on the special record on Hainan Island showed that no extant tree similar to *R. rubrinervis* existed in Tayang Township, Qionghai City (Figure 1A) and that the habitat was badly destroyed by human activity. However, we finally explored two new *R. rubrinervis* populations with smaller leaf length to width ratio and shorter fruit in Baoting County during our field surveys in 2018 (Table 1; Figures 2–4). Morphological comparison at the population level was conducted, showing three Hainan populations distinctly differed from those of *R. rubrinervis* from other regions with the smaller leaf length to width ratio (1.9–2.4 vs. (2.5) 2.8–3.9), shorter fruit (6.5–7.5 mm vs. 8.2–11.1 mm), smaller fruit length to width ratio (1.3–1.5 vs. 1.6–2.2), dumpier seeds (5.0–7.0 × 4.5–5.5 mm vs. 7.1–9.9 × 4.0–5.5 mm), smaller seed length to width ratio (0.9–1.5 vs. 1.6–2.1) and mucronate seed apices (Figures 3–4; Table 2). Differences were also found in leaf base shape, petiole length (3–9 mm vs. 7–12 mm) and fleshy fruit size (8.7–10.9 × 7.5–10.6 mm vs. 10.2–12.1 × 10.1–12.5 mm). No significant difference in flowers was found between the distinct Hainan populations and those of *R. rubrinervis* from other regions. A further PCA of all closely related specimens (including historical type specimens of four published synonyms), based on 10 morphological characters, was carried out (Table 3), distinguishing these three Hainan populations (in Baoting and Qionghai) and those of *R. tonkinensis* and *R. rubrinervis* from other regions into two major groups (Figure 5). One of the two groups merely represented these distinct Hainan populations; the remaining populations of *R. tonkinensis* and *R. rubrinervis* formed the other group. The first principal component axis (PC1; accounting for 38.99% of the variation) significantly separated these distinct Hainan populations from the other two species, while the second principal component axis (PC2; accounting for 20.87% of the variation) failed in separating both groups (Figure 5).

Discussion

In this study, we demonstrated that three distinct Hainan populations previously placed under *R. rubrinervis* should be described as a new species, based on the distinct morphology and clustering analysis at the population level. Morphological comparison showed that they differ from those of *R. rubrinervis* from other regions in mucronate seed apices, shorter fruit, dumpier seeds and smaller length to width ratios of leaves, fruit and seeds. PCA analysis further clustered these special Hainan populations into a separated group distinctly different from those of *R. rubrinervis* and *R. tonkinensis* from other regions (including historical type specimens of those published synonyms). Our results also showed that *R. rubrinervis* and *R. tonkinensis* had a similar morphology, indicating the obscure species boundary between them. This finding is consistent with a previous study (Fan and Yang 1997). Obviously, these special Hainan populations are distinctly different from all extant relatives from other regions (Chen and Carsten 2007). Given this, we describe them in the following as a new species.

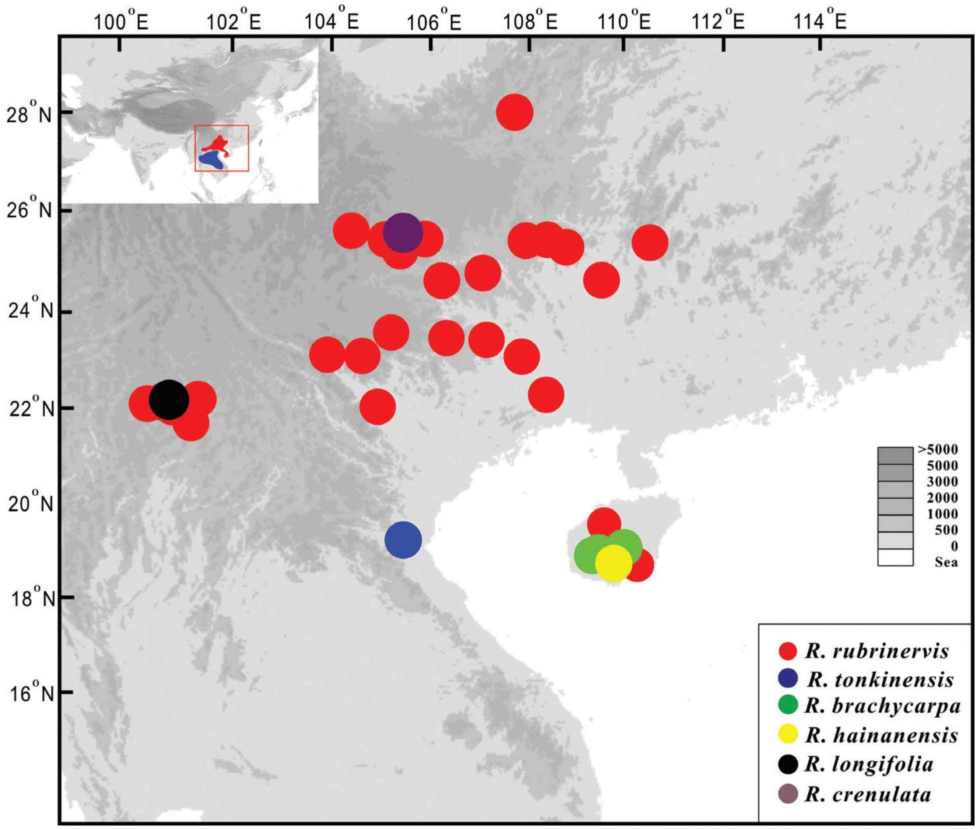

Figure 2. Distribution and locations of *R. brachycarpa*, *R. rubrinervis* and *R. tonkinensis*, based on field surveys, Chinese Virtual Herbarium (http://www.cvh.org.cn/) and Global Biodiversity Information Facility (https://www.gbif.org/).

Taxonomic treatment

***Rhamnella brachycarpa* Z. Qiang Lu & Y. Shuai Sun, sp. nov.**
urn:lsid:ipni.org:names:60479375-2
Figures 3, 4

Diagnosis. *Rhamnella brachycarpa* differs from *R. rubrinervis* and *R. tonkinensis* by leaf length to width ratio of 1.9–2.4 (compared to 2.5–3.9 in the related species) and dried fruit 6.5–7.5 × 4.7–6.0 mm in size (compared to 8.2–11.1 × 4.2–5.8 mm in *R. rubrinervis* and *R. tonkinensis*) with length to width ratio of 1.3–1.5 (compared to 1.6–2.2).

Type. CHINA. Hainan: Baoting County, Xian’an, 109°25'34"E, 18°35'37"N, 650 m alt., forest edge, 16 Oct 2018, *Z.Q. Lu 2018HN3001* (holotype, HITBC; isotypes, HITBC and GXMG).

Description. Small trees or climbing vines, evergreen. Young branches sparsely pilose or glabrous; older branches reddish-brown, grey-brown or grey, glabrous. Leaves alternate; stipules lanceolate, persistent; petiole 0.7–1.2 cm long, glabrous, narrowly

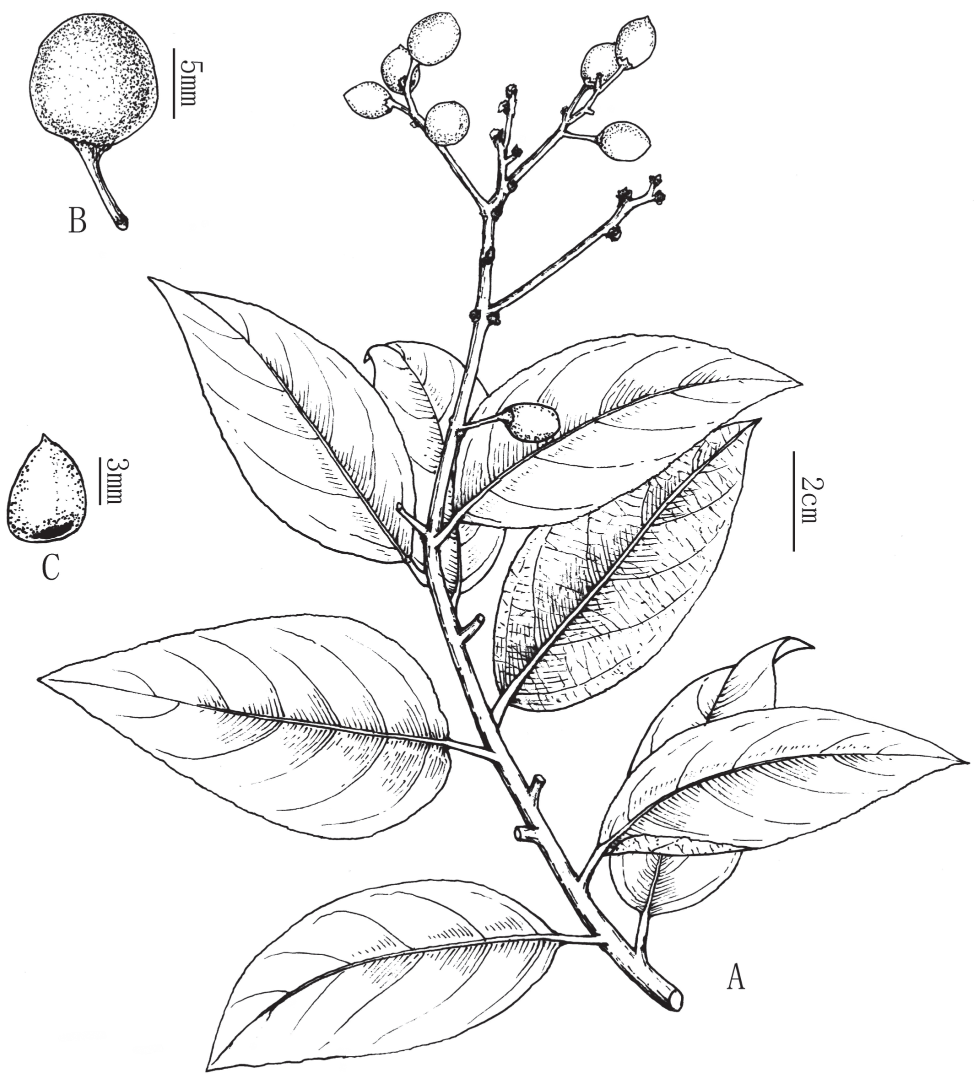

Figure 3. *Rhamnella brachycarpa* Z. Qiang Lu & Y. Shuai Sun, sp. nov., drawn from the tree of *Z.Q. Lu 2018HN3001.*

grooved on the upper surface; leaf blade abaxially dark green, shiny, adaxially pale green, elliptic-ovate, 5.8–10.3 × 3.1–4.8 cm, leathery, abaxially glabrous, adaxially glabrous, lateral veins 5–7 pairs, slightly impressed abaxially, prominent adaxially, base cuneate or nearly rounded, margin subentire, conspicuously serrate when seedlings, apex shortly acuminate or acute. Flowering branches axillary, rarely not, 7–18 cm long, glabrous. Flowers bisexual, ca. 4 mm diam., 4– or 6–merous, few to 2 or 9 in axillary cymes, subsessile or shortly pedunculate at bracteole leaf of flowering branches; bracteole leaf similar to leaves in vegetative branches, but smaller, 1.5–5.0 × 0.9–2.3 cm. Pedicel 3.3–5.2 mm long, glabrous. Sepals triangular, ca. 2 mm, adaxially mid-

Figure 4. *Rhamnella brachycarpa* Z. Qiang Lu & Y. Shuai Sun. **A** The habitat **B** Branches with leaves and fruit **C** Fruit **D** Seeds.

vein raised, rostellate at lower middle. Petals obovate, shortly clawed. Stamens involute by petals, ca. 2 mm long. Disc rounded, thick. Ovary globose, not immersed in disc. Drupe purple-red or orange at maturity, ovoid-cylindrical or globose, 8.7–10.9 × 7.5–10.6 mm, 6.5–7.5 × 4.7–6.0 mm when dried, base with persistent calyx tube; fruiting pedicel 4.3–6.2 mm, glabrous, 1-loculed, 1-seeded; seed dumpy, apex mucronate, smooth on the surface, 5.0–7.0 × 4.5–5.5 mm.

Etymology. In contrast with relatives, all individuals from these distinct Hainan populations have shorter fruit; we therefore give the epithet *Rhamnella brachycarpa*.

Phenology. Flowering from May to September and fruiting from July to October.

Habitat, distribution and conservation. According to our field surveys and records in Chinese Virtual Herbarium (CVH), *R. brachycarpa* has been found at three sites on Hainan Island (Baoting County: Xian'an and Shijia; Qionghai City: Tayang). Only two small populations with 45 individuals (including only 22 mature trees) have been found in Baoting, while no extant tree has been found in the Qionghai population due to the destruction of the habitat. Of the two small populations in Baoting, with a separation distance of about 900 m, one consists of only three

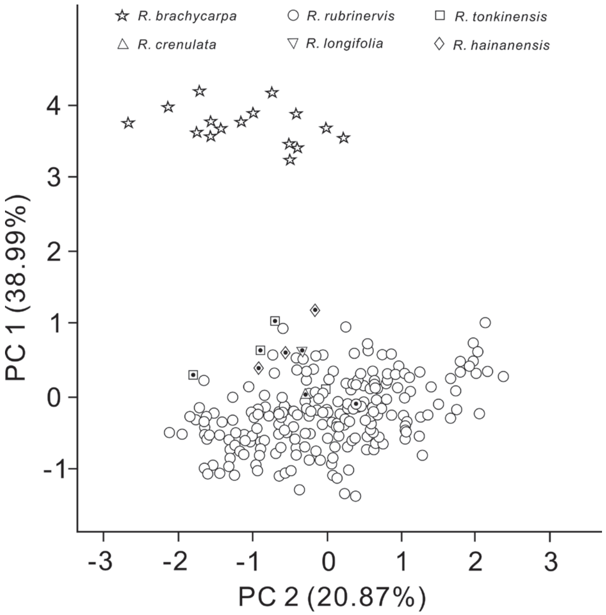

Figure 5. Morphological clustering based on Principal Component Analysis. *R. crenulata*, *R. longifolia* and *R. hainanensis* are synonyms of *R. rubrinervis*. The black dots indicate the designated type specimens.

mature trees without seedlings; the second population has 19 mature trees and 23 immature trees. All mature trees are more than 3.1 m in height and less than 1.8 m is observed for all immature trees. Forty five individuals of this new species sparsely grow along the roadsides and in forest edges or thick forest. Mature trees with climbing habit only grow in thick forest or forest edges with high canopies, but those with erect habit prefer to grow along roadsides or in forest edges without high canopies. Nevertheless, all these immature trees, sparsely growing along the roadsides and in forest edges or thick forest, present the erect habit. The rarity of this new species may be partially due to human activity because the habitat is also suitable for cultivating rubber trees and other economic plants. Comparing to the number of mature trees, the number of immature trees further indicates a decreasing population trend. In addition, all these trees are distributed in a total area of approximately 0.7 km^2. No population was found during repeated field surveys of the surrounding areas. According to the IUCN Categories and Criteria (IUCN 2016), the species is classified as “Critically Endangered” (CR). Therefore, it will be necessary to pay close attention to the conservation of this new species.

Additional specimens examined. CHINA. Hainan: Qionghai City, Tayang, open forest, 27 Nov 1936, *X.Q. Liu 28256* (PE); Baoting County, Xian’an, 109°25'34"E, 18°35'37"N, 650 m alt., forest edge, roadside and thick forest, 16 Oct 2018, *Z.Q. Lu 2018HN3002–Z.Q. Lu 2018HN3012* (HITBC); Baoting County, Shijia, 109°25'42"E, 18°36'02"N, 680 m alt., forest edge, 17 Oct 2018, *Z.Q. Lu 2018HN3013–Z.Q. Lu 2018HN3015* (GXMI).

Acknowledgements

We thank Dr. Huizhe Feng for the help in collecting samples and Mr. Xincheng Qu for drawing the picture. This work is supported by grants from "1000 Youth Talents Plan" of Yunnan Province, CAS "Light of West China" Program and start-up research fund of XTBG (No. B18114BN).

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RESEARCH ARTICLE

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An updated synopsis of *Tanaecium* (Bignonieae, Bignoniaceae)

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Abstract

Tanaecium Sw. emend L.G. Lohmann (Bignonieae, Bignoniaceae) is a genus of Neotropical lianas that is morphologically variable, especially in floral features. The genus is distributed from Mexico and the Antilles to Argentina, and centered in Amazonia. Here, we present an updated overview for *Tanaecium* that recognizes 21 species within the genus. Species delimitation was based on a detailed analysis of protologues and herbarium specimens, including type collections of all taxa. We present a detailed description for the genus and a key for the identification of all species. For each of the 21 species recognized, we present information on the nomenclature, phenology, habitat, distribution, and taxonomic notes. Furthermore, *Spathicalyx kuhlmannii* J.C. Gomes is transferred into *Tanaecium kuhlmannii* (J.C. Gomes) Frazão & L.G. Lohmann. A lectotype is proposed for *Tanaecium crucigerum* Seem.

Keywords

Tanaecium, lianas, Lamiales, lectotype, Neotropical flora, nomenclature, taxonomy

Introduction

Tanaecium Sw. emend L.G.Lohmann is a monophyletic genus, well supported by molecular characters (Frazão and Lohmann 2018), as well as by subulate and/or bromeliad-like prophylls of the axillary buds, a putative morphological synapomorphy (Lohmann and Taylor 2014). Species of the genus are lianas or shrubs distributed from Mexico and the Antilles to Argentina (Lohmann and Taylor 2014; Pace et al. 2016; Frazão and Lohmann 2018; Kaehler et al. 2019). The genus is centered in Amazonia, where 11 species occur (Lohmann and Taylor 2014; Frazão and Lohmann 2018; Kaehler et al. 2019). While some species show disjunct distributions (e.g., *Tan-*

aecium duckei A.Samp.), others are broadly distributed (e.g., *Tanaecium pyramidatum* (Rich.) L.G.Lohmann), or endemic to small geographic areas (e.g., *Tanaecium affine* (A.H.Gentry) L.G.Lohmann, *T. apiculatum* A.H.Gentry) (Lohmann and Taylor 2014; Frazão and Lohmann in prep.).

The genus was described by Swartz (1788) and originally characterized by the tubular flowers and truncate calyces. The original circumscription of *Tanaecium* included six species (see Gentry 1973; Gentry 1976), five of which remain in *Tanaecium* (i.e., *T. apiculatum*, *T. crucigerum* Seem., *T. cyrtanthum* (Mart. ex DC.) Bureau & K.Schum., *T. exitiosum* Dugand, and *T. jaroba* Sw.), while *T. nocturnum* (Barb. Rodr.) Bureau & K. Schum. was transferred to *Bignonia* L. (Lohmann and Taylor 2014). In addition to the five species originally classified as *Tanaecium*, twelve species from six previously recognized genera (i.e., *Arrabidaea* DC., *Ceratophytum* Pittier, *Pseudocatalpa* A.H.Gentry, *Paragonia* Bureau, *Periarrabidaea* A.Samp, and *Spathicalyx* J.C.Gomes) were transferred to *Tanaecium* in a revised generic classification for the whole tribe Bignonieae (Lohmann and Taylor 2014). As a result, 17 species of *Tanaecium* were recognized in the most recent synopsis of the genus (Lohmann and Taylor 2014).

Additional molecular phylogenetic studies combined with novel morphological observations indicated that *Sphingiphila tetramera* A.H.Gentry is best placed in *Tanaecium*, leading to the new combination *Tanaecium tetramerum* (A.H.Gentry) Zuntini & L.G.Lohmann (Pace et al. 2016). A new species of *Tanaecium* was subsequently described (i.e., *T. decorticans* Frazão & L.G.Lohmann) (Frazão and Lohmann 2018), while new morphological and molecular data indicates that *Tanaecium mutabile* (Bureau & K. Schum.) L.G. Lohmann is best placed within *Fridericia* Mart. emend L.G. Lohmann (Frazão and Lohmann, in prep.). More recently, Kaehler et al. (2019) transferred three species of *Fridericia* into *Tanaecium*, i.e., *Tanaecium dichotomum* (Jacq.) Kaehler & L.G.Lohmann, *T. paradoxum* (Sandwith) Kaehler & L.G.Lohmann, and *T. parviflorum* (Mart. ex DC) Kaehler & L.G.Lohmann.

Given all the recent taxonomic changes in *Tanaecium*, a new evaluation of the overall circumscription of the genus and its species is needed. Here, we present an overview for *Tanaecium*. We recognize 21 species for which we provide information on the nomenclature, synonymy, phenology, habitat, distribution, and taxonomic notes. Because *T. paradoxa* appeared within *Fridericia* in a recent phylogenetic study (Frazão and Lohmann, in prep.), we follow Lohmann and Taylor (2014) in treating this taxon as *Fridericia paradoxa* (Sandwith) L.G.Lohmann. A lectotype is proposed for *Tanaecium crucigerum* Seem., and the new combination *Tanaecium kuhlmannii* (J.C.Gomes) Frazão & L.G.Lohmann is proposed to accommodate novel morphological observations and recent phylogenetic findings (Frazão and Lohmann, in prep.).

Material and methods

Materials from the following herbaria were studied using standard taxonomic methods (Acronyms following Thiers 2019): INPA, IAN, MG, UFACPZ, EAC, CEN, IBGE, UB, HERBAM, ESA, RBR, RB, R, SPF, SP, UEC, HRCB, CESJ, BHCB, MBM, PY, FCQ, QCNE, QCA, NY, US, MO, A, and F. Furthermore, images of specimens from AAU, B, BR, COL, G, K, L, M, and P were accessed online through Jstor Global Plants (2019) or the online database of individual herbaria. All protologues were consulted in the Peter Raven Library (Missouri Botanical Garden) or using the online database of BHL (2019). Morphological terminology used here follows Hickey (1974) for leaf venation, Radford (1986) for leaf morphology, Weberling (1989) for inflorescence morphology, Gomes-Silva (2009) for leaflet mite-domatia, Nogueira et al. (2013) for trichomes, and Lohmann and Taylor (2014) for prophyll morphology and other morphological traits. Phenology is based on data gathered from herbarium specimens. Distributions are based in data gathered from herbarium specimens and information provided in Lohmann and Taylor (2014).

Taxonomic treatment

Tanaecium Sw., Prodr. Veg. Ind. Occ. 6: 91. 1788, emend L.G. Lohmann, Ann. Missouri Bot. Gard. 2014: 463. Type: *Tanaecium jaroba* Sw.

Paragonia Bureau, Bull. Soc. Bot. France 19: 17. 1872. Type: *Bignonia lenta* Mart. ex DC. [= *Tanaecium pyramidatum* (Rich.) L.G.Lohmann].

Sanhilaria Baill., Hist. Pl. 10: 27. 1888. *Hilariophyton* Pichon, Bull. Soc. Bot. France 92: 228. 1946. Type: *Sanhilaria brasiliensis* Baill. [= *Tanaecium brasiliensis* (Baill.) L.G.Lohmann].

Ceratophytum Pittier, J. Wash. Acad. Sci. 18: 62. 1928. Type: *Ceratophytum capricorne* Pittier [= *Tanaecium tetragonolobum* (Jacq.) L.G.Lohmann].

Periarrabidaea A. Samp., Ann. Acad. Brasil. Sci. 6: 175. 1934. Type: *Periarrabidaea truncata* A. Samp. [= *Tanaecium truncatum* (A. Samp) L.G.Lohmann].

Spathicalyx J.C.Gomes, Notul. Syst. (Paris) 15: 220. 1956. Type: *Spathicalyx kuhlmannii* J. C. Gomes [= *Tanaecium kuhlmannii* (J.C. Gomes) Frazão & L.G. Lohmann].

Pseudocatalpa A.H.Gentry, Brittonia 25(3): 241. 1973. Type: *Pseudocatalpa caudiculata* (Standl.) A. H. Gentry [= *Tanaecium caudiculatum* (Standl.) L.G.Lohmann].

Lianas or shrubs, without dimorphic juvenile growth; stems with four phloem wedges in cross section (without in *T. tetramerum*), solid (hollow in *T. apiculatum*); branchlets terete or tetragonal, without ridges, with or without striation, without peeling epidermis (present in *T. decorticans*), sparse or dense lenticels, with or without simple non-glandular trichomes (dendritic non-glandular trichomes in *T. xanthophyllum*); interpetiolar region with or without fields of patelliform glandular trichomes, and discontinuous interpetiolar ridges (sometimes continuous); prophylls of the axillary buds bromeliad-like and/or subulate (minute and triangular or foliaceous), without patelliform glandular trichomes (present in *T. selloi*). *Leaves* 2–3–foliolate (sometimes simple in *T. tetramerum*) with the terminal leaflet modified into a simple or trifid tendril (sometimes bifid in *T. pyramidatum*); leaflets without cartilaginous margin (present in *T. apiculatum*), secondary venation brochidodromous (craspedodromous in *T. parviflorum*). *Inflorescence* in a fascicule, raceme, thyrse or compound thyrse, terminal (sometimes axillary); calyx campanulate, cupular or tubular, bilabiate or truncate (sometimes spathaceous); corolla magenta, pink, yellow, pale yellow or white, infundibular or wide infundibular (campanulate or hypocrateriform), zygomorphic (actinomorphic in *T. tetramerum*), pentamerous (tetramerous in *T. tetramerum*), aestivation imbricate; androecium didynamous, pollen in monads, 3-colpate, psilate and microperforate (inaperturate and coarse-reticulate in *T. apiculatum*); nectar disk well-developed; gynoecium with ovary without stipe at the base, with one, two, or many series of ovules in each placenta, stigma papilose. *Capsule* elliptic or linear (linear-oblong), with or without lenticels, calyx caducous (persistent); seeds winged or wingless, with body smooth and glabrous, winged hyaline or opaque, linear, wingless corky or woody and rounded.

Key to species of *Tanaecium*

1 Branchlets thorn-tipped; terminal leaflets never replaced by a tendril; corollas hypocrateriform, 4-lobed .... **19. *T. tetramerum***

– Branchlets not thorn-tipped; terminal leaflets generally replaced by a tendril; corollas campanulate, infundibular or wide infundibular, 5-lobed............... **2**

2 Leaflets with caudate apices; corollas campanulate; androecium with two fertile stamens .... **4. *T. caudiculatum***

– Leaflets without caudate apices; corollas infundibular or wide infundibular; androecium with four fertile stamens **3**

3 Leaflets with dentate margins; calyces aristate (rarely mucronate); fruit apices caudate ....**14. *T. parviflorum***

– Leaflets without dentate margins; calyces not aristate; fruit apices not caudate.. **4**

4 Leaflets with apiculate apices, with cartilagenous margins; calyces with stellate simple trichomes; pollen grains inaperturated..............**2. *T. apiculatum***

– Leaflets without apiculate apices, without cartilagenous margins; calyces without stellate simple trichomes; pollen grains colpate **5**

5 Leaflets with emarginated membrane-like domatia; inflorescence nodes with patelliform trichome fields; corollas ≤ 2.6 cm long.......................**1. *T. affine***

– Leaflets without emarginated membrane-like domatia; inflorescence nodes without patelliform trichome fields; corollas > 2.6 cm long......................... **6**

6 Stems with peeling epidermis; petiolules with arrow-shaped apices; fruits with patelliform and peltate trichomes along the margins .... **7. *T. decorticans***

– Stems without peeling epidermis; petiolules without arrow-shaped apices; fruits without patelliform and peltate trichomes along the margins............ **7**

7 Leaflets 8–15 times larger than the petioles; calyces costate; corollas with cuspidate lobes..**13. *T. neobrasiliense***

– Leaflets < 8 times larger than the petioles; calyces costate; corollas without cuspidate lobes........ **8**

8 Leaflets with yellow dendritic simple trichomes; bracteoles ≥ 4:5 the flower pedicels; corollas with peltate trichomes in the ventral portion internally........ **21. *T. xanthophyllum***

– Leaflets without yellow dendritic simple trichomes; bracteoles < 4:5 the flower pedicels; corollas without peltate trichomes in the ventral portion internally.... **9**

9 Leaflets with pit domatia abaxially; calyces with constriction on the basal or medial portions; corollas pale-yellow........ **20. *T. truncatum***

– Leaflets without foveolate domatia abaxially; calyces without constriction on the basal or medial portions; corollas white, pink or magenta **10**

10 Corollas white........ **11**

– Corollas pink or magenta........ **18**

11 Leaflets with pocket and tuft domatia; petioles pulvinate (rarely absent); calyces 1:3 to 2:3 the corolla tubes; ovaries with one ovule series on each placenta........ **3. *T. bilabiatum***

– Leaflets without domatia; petioles not-pulvinate; calyces ≤ 1:3 the corolla tubes; ovaries with two or many ovule series on each placenta........ **12**

12 Stems with interpetiolular patelliform trichomes < 0.3 mm; inflorescences in corymbiform thyrse; corollas infundibular; fruits 4-lobed at base........ **18. *T. tetragonolobum***

– Stems with or without interpetiolular patelliform trichomes, > 0.3 mm when present; inflorescences not in corymbiform thyrse; corollas wide infundibular; fruits not 4-lobed at base........ **13**

13 Leaflets with basal and suprabasal venation actinodromous; tendrils trifid; calyces spathaceous; anthers curved backwards........ **14**

– Leaflets without basal and suprabasal venation actinodromous; tendrils simple; calyces not spathaceous; anthers not curved backwards........ **15**

14 Abaxial side of leaflets with patelliform trichomes ≥ 0.45 mm diam., with protrusion at the patelliform insertion; anthers ≥ 7 mm long..... **12. *T. kuhlmannii***

– Abaxial side of leaflets with patelliform trichomes < 0.45 mm diam., without protrusion at the patelliform insertion; anthers < 7 mm long..... **9. *T. duckei***

15 Adaxial side of leaflets bullate; calyces bilabiate; anthers exserted........ **10. *T. exitiosum***

– Adaxial side of leaflets not bullate; calyces truncate; anthers sub-exserted **16**

16 Caducuous when flowering; abaxial surface of leaflets with patelliform trichomes concentrated at base; calyces campanulate or cupular; fruits linear; seeds linear, with lateral seed body........ **6. *T. cyrtanthum***

– Not caducuous when flowering; abaxial surface of leaflets without patelliform trichomes concentrated at base; calyces cupular; fruits elliptic; seeds circular, with central seed body........ **17**

17 Abaxial side of leaflets whitish-tomentose .... **5. *T. crucigerum***

– Abaxial side of leaflets glabrous or pubescent .... **11. *T. jaroba***

18 Prophylls of the axillary buds foliaceous or minute and triangular; fruits with raised margins, without central ridges .... **17. *T. selloi***

– Prophylls of the axillary buds subulate or bromeliad-like; fruits with or without raised margins, with central ridges **19**

19 Leaflets with margin curvature revolute; fruits linear-oblong; seeds with vestigial wings; distributed along riparian areas in the Amazon .... **16. *T. revillae***

– Leaflets with margin curvature flat; fruits linear; seeds with well-developed wings; distributed in all habitat types throughout the Neotropics **20**

20 Petioles with patelliform trichomes at apices; tendrils bifid or trifid; fruits inflated and lenticellated .... **15. *T. pyramidatum***

– Petioles without patelliform trichomes at apices; tendrils simple; fruits flattened and not lenticellated .... **8. *T. dichotomum***

1. *Tanaecium affine* (A.H.Gentry) L.G.Lohmann. Ann. Missouri Bot. Gard. 99: 464. 2014.

Arrabidaea affinis A.H.Gentry, Novon 2(2): 159. 1992. Type: Ecuador. Sucumbios: Lake Agrio, banks of lake, 250 m, 0°6'45.28"N, 76°54'42.81"W, 1 Apr. 1980, J. Brandbyge and E. Asanza 30393 (holotype, MO [MO-083145]!; isotypes, AAU image!, AAU photo at MO!, NY [NY00000106]!).

Habitat and distribution. *Tanaecium affine* is known from humid forests with rich soils, although it has been collected in primary and secondary forests with lateritic soil in Peru (Loreto, Mayanas). It is native from Bolivia (La Paz), Colombia (Antioquia, Boyaca), Ecuador (Napo, Pastaza, Sucumbíos), and Peru (Amazonas, Junín, Loreto, Pasco, Puno).

Phenology. Flowering: February to April, September and November; fruiting: February to December.

Notes. This species is morphologically similar to *Fridericia florida* but differs by the bilabiate calyces, stems with conspicuous patelliform trichomes in the interpetiolar region, and occurrence in rich soils (Gentry 1992). In addition, *T. affine* can also be recognized by the numerous peltate trichomes distributed throughout the leaflets, emarginated membrane-like domatia, and fields of patelliform trichomes that cover the inflorescence nodes. *Tanaecium affine* shares vegetative traits with *Tanaecium tetragonolobum*, a sympatric species (Tab. 1). However, *T. tetragonolobum* can be differentiated by the glabrous leaflets (vs. leaflets covered with peltate trichomes in *T. affine*), petioles longer than petiolules (vs. petioles shorter than petiolules in *T. affine*), and subulate prophylls of the axillary buds (vs. bromeliad-like prophylls of the axillary buds in *T. affine*).

Table 1. Characters useful to recognize *Tanaecium* species.

	Tanaecium species	Branchlet section	Interpetiolar glandular field	Prophylls of the axillary buds	Tendril type	Inflores-cence type	Calyx shape	Calyx aperture	Corolla color	Corolla mouth color	Corolla shape	Ovules series	Fruit shape	Seeds wings
1.	*Tanaecium affine*	terete or tetragonal	present	subulate or bromeliad-like	simple	compound thyrse	campanulate	bilabiate	white	white	infundibular	one	linear	well-developed
2.	*Tanaecium apiculatum*	terete	absent	subulate or bromeliad-like	absent	raceme	tubular	truncate	white	white	wide infundibular	many	-	-
3.	*Tanaecium bilabiatum*	terete	absent	subulate or bromeliad-like	simple	thyrse	campanulate or tubular	bilabiate	white	yellow	infundibular	one	linear	vestigial
4.	*Tanaecium caudiculatum*	tetragonal	absent	subulate or bromeliad-like	simple	thyrse	campanulate	truncate	pale yel-low	white	campanulate	one	linear	well-developed
5.	*Tanaecium crucigerum*	terete	present	minute and triangular or bromeliad-like	simple	thyrse	cupular	truncate	white	white	wide infundibular	many	elliptic	absent
6.	*Tanaecium cyrtanthum*	terete	present	minute and triangular or bromeliad-like	simple	thyrse	cupular	truncate	white	white	wide infundibular	many	linear	well-developed
7.	*Tanaecium decorticans*	terete	present	subulate	trifid	thyrse	campanulate or cupular	truncate	pink	white	infundibular	one	linear	well-developed
8.	*Tanaecium dichotomum*	terete	present or absent	subulate or bromeliad-like	simple	thyrse	campanulate	bilabiate	pink	white	campanulate or infundibular	one	linear	well-developed
9.	*Tanaecium duckei*	terete	absent	subulate	trifid	thyrse	tubular	oblique	white	white	wide infundibular	many	linear	well-developed
10.	*Tanaecium exitiosum*	terete	absent	subulate	simple	thyrse	campanulate	bilabiate	white	white	wide infundibular	-	-	-
11.	*Tanaecium jaroba*	terete	present	minute and triangular or bromeliad-like	simple	thyrse	campanulate	truncate	white	white	wide infundibular	many	elliptic	absent
12.	*Tanaecium kuhlmannii*	terete	absent	subulate	trifid	thyrse	tubular	oblique	white	white	wide infundibular	many	linear	well-developed
13.	*Tanaecium neobrasiliense*	terete	absent	subulate or bromeliad-like	trifid	compound thyrse	campanulate	truncate	magenta	-	infundibular	two	linear	well-developed
14.	*Tanaecium parviflorum*	terete or tetragonal	absent	subulate or bromeliad-like	simple	thyrse	campanulate	truncate	white	yellow	infundibular	two	linear	well-developed
15.	*Tanaecium pyramidatum*	terete	absent	subulate or bromeliad-like	bifid or trifid	compound thyrse	campanulate	bilabiate or truncate	pink or magenta	white	infundibular	one	linear	well-developed
16.	*Tanaecium revillae*	terete	absent	subulate or bromeliad-like	simple	thyrse	campanulate	bilabiate	pink	white	infundibular	one	linear-oblong	vestigial
17.	*Tanaecium selloi*	terete	absent	minute and triangular or foliaceous	simple	thyrse	campanulate	bilabiate	pink	white	infundibular	one	linear	well-developed
18.	*Tanaecium tetragonolobum*	terete or tetragonal	present	subulate	simple	thyrse	cupular	truncate	white	yellow	infundibular	many	linear	well-developed
19.	*Tanaecium tetramerum*	terete	absent	subulate or bromeliad-like	absent	fascicule	tubular	truncate	white	white	hypocrateriform	two	elliptic	well-developed
20.	*Tanaecium truncatum*	terete	present	subulate or bromeliad-like	trifid	thyrse	campanulate	oblique or truncate	pale yel-low	yellow	infundibular	two	linear	well-developed
21.	*Tanaecium xanthophyllum*	terete	present	minute and triangular or bromeliad-like	trifid	compound thyrse	campanulate	bilabiate	yellow	yellow	infundibular	two	linear	well-developed

2. *Tanaecium apiculatum* A.H.Gentry, Ann. Missouri Bot. Gard. 63(1): 58, fig. 4. 1976.

Type. Venezuela. Monagas: Caicara, 15 May 1952, F. D. Smith 226 (holotype, US [US-2121468]!; isotype, US!, US photo at MO [MO-067514]!, [MO-067514]!)

Habitat and distribution. *Tanaecium apiculatum* is known only from the type location, Caicara, Venezuela.

Phenology. Flowering: May; fruiting (immature): May.

Notes. This species shares wide infundibular corollas with *T. crucigerum*, *T. cyrtanthum*, *T. duckei*, *T. exitiosum*, *T. kuhlmannii*, and *T. jaroba*, but can be differentiated from these taxa by the leaflets with apiculate apices and cartilaginous margins, and tubular calyces with stellate simple trichomes (Tab. 1). Out of these species, *Tanaecium crucigerum* and *T. jaroba* are the only ones that also occur in Venezuela. These species can be differentiated from *T. apiculatum* by the abaxial surface whitish-tomentose in *T. crucigerum* and abaxial surface glabrous or pubescent in *T. jaroba* (vs. abaxial surface glabrous in *T. apiculatum*).

3. *Tanaecium bilabiatum* (Sprague) L.G.Lohmann, Nuevo Cat. Fl. Vasc.Venezuela 274. 2008.

Fig. 1A, L

Memora bilabiata Sprague, Bull. Herb. Boissier (ser. 2) 6: 375. 1906.

Adenocalymma bilabiatum (Sprague) Sandwith, Recueil Trav. Bot. Néerl. 34: 213. 1937. Type: Brazil. Amazonas: Manaus, s.d., R. Spruce 1783 (holotype, K [K000492969] image!).

Habitat and distribution. *Tanaecium bilabiatum* grows in wet, flooded, riparian vegetation, or Amazonian lowlands. It occurs in Bolivia (Beni, Pando), Brazil (Acre, Amapá, Amazonas, Pará, Roraima), Colombia (Amazonas, Arauca, Guainía), French Guyana, Guyana, Peru (Madre de Dios, Loreto), Suriname (Sipaliwini, Nickerie), and Venezuela (Amazonas, Apure, Bolívar, Delta Amacuro, Guárico, Monagas, Sucre).

Phenology. Flowering: February to November; fruiting: December to October.

Notes. *Tanaecium bilabiatum* is easily differentiated from other *Tanaecium* species by the pulvinated petioles (typical of *Adenocalymma* but usually lacking in *Tanaecium* and other Bignonieae; Lohmann and Taylor 2014), large bilabiate calyces, covering 1:3 to 2:3 of the corolla tube, white corollas with yellow mouths, oblong and flattened fruits, and seeds with vestigial wings (rarely well-developed) (Tab. 1).

4. *Tanaecium caudiculatum* (Standl.) L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 464.

Petastoma caudiculatum Standl. Publ. Field Mus., Bot. 11(4): 141. 1932.

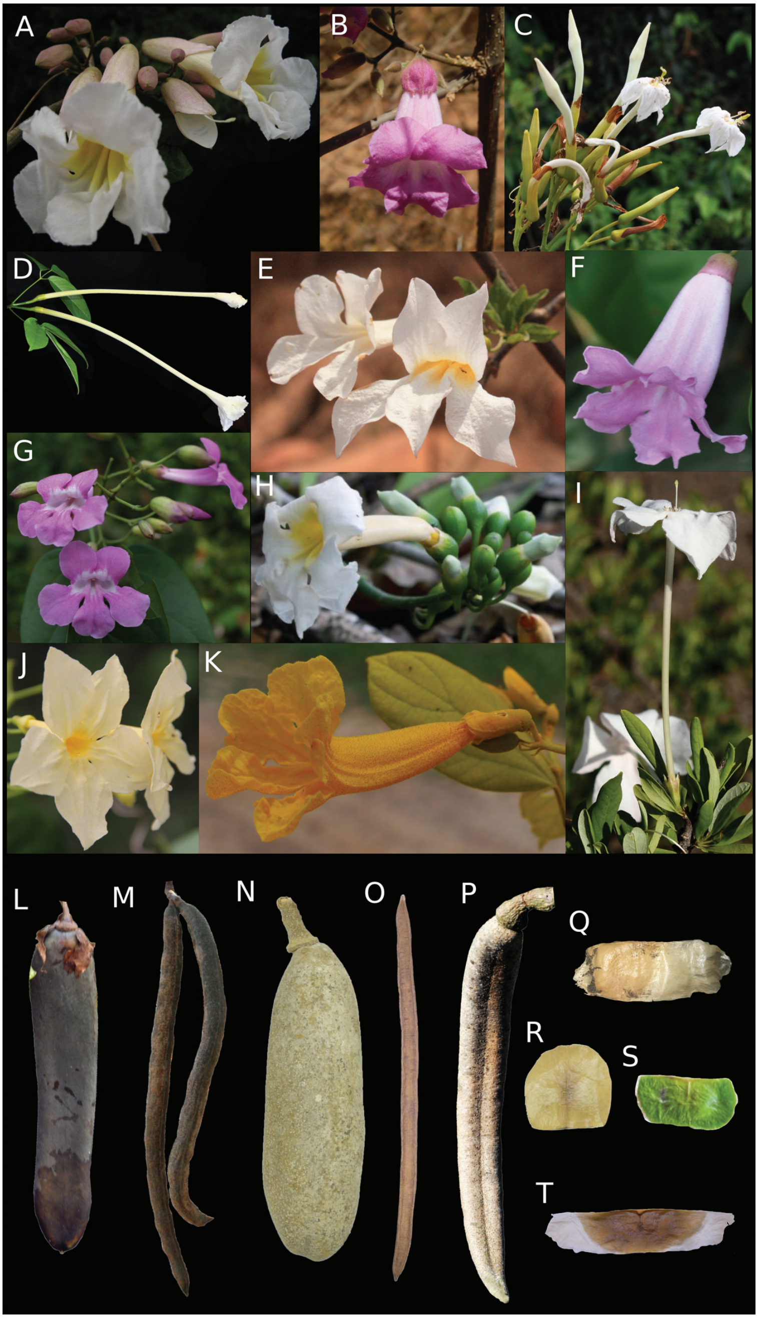

Figure 1. Morphological diversity of *Tanaecium*. **A–K** Flowers **A** *T. bilabiatum* **B** *T. dichotomum* **C** *T. duckei* **D** *T. jaroba* **E** *T. parviflorum* **F** *T. pyramidatum* **G** *T. revillae* **H** *T. tetragonolobum* **I** *T. tetramerum* **J** *T. truncatum* **K** *T. xanthophyllum*. **L–P** Fruits **L** *T. bilabiatum* **M** *T. cyrtanthum* **N** *T. jaroba* **O** *T. selloi* **P** *T. tetragonolobum*. **Q–T** Seeds **Q** *T. cyrtanthum* **R** *T. jaroba* **S** *T. revillae* **T** *T. selloi*. Photos by A. Frazão, except **A** by B. Gomes **B** by R. Lopes **E, M** by C. Siniscalchi **G** by E. Kataoka **H** by Stevens **I** by Parada-Gutierrez **J** by L.H.M. Fonseca.

Pseudocatalpa caudiculata (Standl.) A.H.Gentry, Brittonia 25(3): 241. 1973. Type: Belize. Nine Mile, Stann Creek Railway, 30 m, 22 Mar. 1932, W. A. Schipp S–297 (holotype, F!).

Habitat and distribution. *Tanaecium caudiculatum* is restricted to Central America. It is known from wet forests that grow in the mountains and sea level in Belize (Belize, Cayo, Stann Creek, Toledo), Guatemala (Alta Vera Cruz), and Mexico (Chiapas, Oaxaca).

Phenology. Flowering: March to May, July to September; fruiting: April, June, and August.

Notes. *Tanaecium caudiculatum* differs from other species in the genus by the caudate leaflets, simple tendrils that bear hooks (otherwise only found in the trifid tendrilled *Dolichandra*; Lohmann and Taylor 2014; Fonseca et al. 2017), foliaceous inflorescence bracts, stipitate glandular trichomes in the internal ventral surface of the corolla tubes, androecium with only two fertile stamens, and fruits with sinuous margins (Tab. 1).

5. *Tanaecium crucigerum* Seem., Bonplandia (Hannover) 4: 127. 1856.

Type. Lesser Antilles. Dominica, sin. loc., s. d., J. Imray 94 (lectotype, designated here, K [K000449535] image!).

Habitat and distribution. *Tanaecium crucigerum* occurs in wet forests in the Lesser Antilles (Dominica, Martinique), Trinidad and Tobago, Costa Rica (Limón), and Venezuela (Anzoátegui, Apure, Cojedes, Delta Amacuro, Guárico, Portuguesa).

Phenology. Flowering: April to July, and October; fruiting: February, April to July, and October to November.

Notes. Like Lohmann and Taylor (2014), we were also unable to locate the lectotype of *T. crucigerum* selected by Howard (1989: 334), the collection *J. Imray 95* supposedly deposited at K. This collection is thus presumed lost. As such, we select another Imray collection from Dominica studied by Seemmann (1856) deposited at K as lectotype. We selected the material *J. Imray 94* as lectotype due to high quality of this material.

This species is morphologically most similar to *T. jaroba*, sharing many characters such as the simple tendrils, wide infundibular corollas, and wingless seeds (Tab. 1). *Tanaecium crucigerum* differs from *T. jaroba* by the whitish-tomentose leaflets on the abaxial surface (vs. glabrous or pubescent leaflets on the abaxial surface in *T. jaroba*).

6. *Tanaecium cyrtanthum* (Mart. ex DC.) Bureau & K.Schum, Fl. Bras. 8(2): 186. 1896.

Fig. 1M, Q.

Tecoma cyrtantha Mart. ex DC., in A. DC., Prodr. 9: 218. 1845. Type: Brazil. Bahia: Pão d'Espinho, caatinga, Oct., C.F.P. von Martius 1860 (holotype, M [M0088980]!; isotype, G-DC!).

Habitat and distribution. *Tanaecium cyrtanthum* is distributed in dry forests, caatinga, cerrado and chaco in Bolívia (Santa Cruz, Tarija), Brazil (Bahia, Ceará, Goiás, Mato Grosso do Sul, Pernambuco, Rio Grande do Norte), and Paraguay (Alto Paraguay, Amambay, Concepción, San Pedro).

Phenology. Flowering: September to January and April; fruiting: April to August and October.

Notes. This species is generally caducous when flowering, and produces new leaves when fruiting. The tendril is simple and the leaflets have patelliform trichomes concentrated at the base abaxially. The calyces are campanulate or cupular, while the fruits are linear and inflated, bearing linear seeds, with a lateral seed body (Tab. 1).

7. *Tanaecium decorticans* Frazão & L.G.Lohmann, Pl. Syst. Evol. 304: 1248. fig. 2. 2018.

Type. Brazil. Pará: Belterra, Entrada da estrada de Aramanaí para Pindobal, próximo a Fazenda São Sebastião, 41 m a. s. l., 2°38'24.7"S, 54°59'06.6"W, 20 Sep 2015, A. Frazão 210 (holotype: SPF!; isotype: RB!, MO!).

Habitat and distribution. *Tanaecium decorticans* is known from the Brazilian Amazon (Pará, Maranhão).

Phenology. Flowering: February and September; fruiting: September and December.

Notes. This species is morphologically most similar to *T. pyramidatum*, sharing characters such as the subulate prophylls, infundibular corolla with white mouth, and linear fruits (Tab.1). However, *T. decorticans* can be differentiated from *T. pyramidatum* by the stems with peeling epidermis (vs. stems without peeling epidermis in *T. pyramidatum*), petiolules with arrow-shaped apices (vs. lacking in *T. pyramidatum*), and fruits flattened with glandular patelliform and peltate trichomes along the margins (vs. fruits inflated without glandular patelliform and peltate trichomes along the margins in *T. pyramidatum*) (Frazão and Lohmann 2018) (Tab. 1).

8. *Tanaecium dichotomum* (Jacq.) Kaehler & L.G.Lohmann, in press*

Fig. 1B

Bignonia dichotoma Jacq. Enum. Syst. Pl. 25. 1760 [also in Select. stirp. amer. hist. 183, 1763].

Fridericia dichotoma (Jacq.) L.G. Lohmann, Ann. Missouri Bot. Gard. 99: 436. 2014. Type: Colombia. Magdalena: Cartagena, not located.

Habitat and distribution. *Tanaecium dichotomum* is commonly found in dry to humid forests in Argentina (Chaco, Corrientes, Formosa, Jujuy, Misiones, Salta), Belize (Cayo),

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* TAXON in press. 2019. We do not intend to publish this combination here, but in Kaehler et al. (2019, in press.)

Bolivia (Beni, Chuquisaca, La Paz, Pando, Santa Cruz, Tarija), Brazil (Acre, Alagoas, Amapá, Amazonas, Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Sul, Rondônia, Roraima, Santa Catarina, São Paulo, Tocantins), Colombia (Amazonas, Atlántico, Bolívar, César, Chocó, Huila, La Guajira, Magdalena, Meta, Sucre, Tolima), Costa Rica (Guanacaste, Puntarenas), Ecuador (Guayas, Napo), French Guiana, Guyana (Essequibo, Rupununi), Mexico (Chiapas, Colima, Guerrero, Jalisco, Mexico, Oaxaca, Veracruz), Nicaragua (Boaco, Chontales, Granada, Matagalpa, Nueva Segovia, Río San Juan), Panama (Canal Area, Panama, Los Santos), Paraguay (Alto Paraguay, Amambay, Boquerón, Central, Chaco, Concepción, Cordillera, Ñeembucú, Nueva Asuncion, Paraguarí, Presidente Hayes, San Ramon), Peru (Cusco, Loreto, Madre de Dios, Piura, San Martín, Tumbes, Ucayali), and Venezuela (Amazonas, Anzoátegui, Apure, Aragua, Barinas, Bolívar, Carabobo, Cojedes, Distrito Federal, Falcón, Guárico, Lara, Mérida, Miranda, Monagas, Nueva Esparta, Portuguesa, Sucre, Táchira, Trujillo, Yaracuy, Zulia).

Phenology. Flowering: January to December; fruiting: January to December.

Notes. This species is widespread through the Neotropics, where it is found in many vegetation types. The species encompasses an enormous degree of morphological variation, representing a species complex. Detailed morphological and molecular studies are necessary to sort out the patterns of variation and identify putative cryptic species.

Tanaecium dichotomum shares many morphological traits with *T. selloi* and *T. revillae* (e.g., tuft domatia in the abaxial side of leaflets, bilabiate calyces), and *T. pyramidatum* (e.g., thyrsoid inflorescences, pink corollas with white mouths). However, *T. dichotomum* differs from these species by the bilabiate and cuspidate calyces, stems with patelliform glandular trichomes between the petioles, and flattened fruits without raised margins or a conspicuous central ridge (Tab. 1).

9. *Tanaecium duckei* A.Samp., Ann. Acad. Brasil. Sci. 7: 125. 1935.

Fig. 1C

Spathicalyx duckei (A.Samp.) A.H.Gentry, Phytologia 35(3): 194. 1977. Type: Brazil. Pará: Óbidos, 21 July 1918, A. Ducke s.n. (holotype, MG!; isotypes, MO [MO-077163]!, R!, RB [RB00536923]!, US [US00125782]!).

Habitat and distribution. *Tanaecium duckei* grows in Amazonian forests with sandy soils and canga vegetation. It occurs in Brazil (Acre, Amazonas, Pará, Mato Grosso), Colombia (Amazonas), and Peru (Loreto, Pasco).

Phenology. Flowering: July and September to October; fruiting: September.

Notes. This species differs from other species of *Tanaecium* by the spathaceous calyces, reflexed anthers, and vegetative structures covered by stipitate glandular trichomes. It is morphologically most similar to *T. kuhlmannii*, with which it shares trifid tendrils and spathaceous calyces (Tab. 1). It is possible to separate *T. duckei* from *T. kuhlmannii* by the lack of patelliform glandular trichomes along the tertiary veins (vs. present in *T. kuhlmannii*), green fruits with sparse stipitate glandular trichomes (vs. yellow fruits covered by stipitate glandular trichomes in *T. kuhlmannii*), smaller anthers with 4.17–4.34 mm long (vs. larger anthers with 7.0–10.0 mm long in *T. kuhlmannii*), and stamens inserted at the same height (vs. stamens inserted at two different heights in *T. kuhlmannii*) (Tab. 1).

10. *Tanaecium exitiosum* Dugand, Caldasia 1(5): 31, fig. 1. 1942.

Type. Colombia. Santander: Barrancabermeja, 50 m, 5 Apr. 1942, R. Mora s.n. (holotype, COL [COL000004390]!; isotype, COL [COL000004389]!).

Habitat and distribution. *Tanaecium exitiosum* is endemic to wet forest vegetation from Colombia (Caldas, Santander).

Phenology. Flowering: March to April and December; fruiting: unknown.

Notes. This species shares wide infundibular white flowers with *T. apiculatum*, *T. crucigerum*, *T. cyrtanthum*, *T. duckei*, *T. kuhlmannii*, and *T. jaroba*, from which it differs by the leaflets bullate adaxially, calyces campanulate and bilabiate, and anthers exserted (Tab. 1). Among the most similar species, *Tanaecium exitiosum* is only sympatric with *T. jaroba*, from which it can be distinguished by the lack of interpetiolar glandular fields (vs. present in *T. jaroba*), bilabiate calyces (vs. truncate calyces in *T. jaroba*), and exserted anthers (vs. sub-exserted anthers in *T. jaroba*) (Tab. 1).

11. *Tanaecium jaroba* Sw., Prodr. 92: 1788.

Fig 1D, N, R

Type. Jamaica, s. loc., s.d., O. Swartz s.n. (holotype, S not seen).

Habitat and distribution. *Tanaecium jaroba* grows in flooded and swampy forests (Gentry 1997) in Bolivia (Beni, La Paz), Brazil (Acre, Amazonas, Mato Grosso, Mato Grosso do Sul, Pará, Rondônia, Roraima), Colombia (Amazonas, Antioquia, Atlántico, Bolívar, Caquetá, La Guajira, Magdalena, Sucre), Costa Rica (Limón), Ecuador (Napo, Orellana), French Guiana (Cayenne), Guyana, Lesser Antilles (Jamaica, St. Vincent), Panamá (Panamá), Peru (Loreto, Madre de Dios, Ucayali), Trinidad and Tobago, and Venezuela (Amazonas, Apure, Bolívar, Carabobo, Delta Amacuro, Guárico, Zulia).

Phenology. Flowering: April to August and November to December; fruiting: March to August and December.

Notes. This species has the longest wide infundibular white flowers in the whole tribe Bignonieae, with corollas up to 35 cm long (Gentry 1997, Howard 1989). It is most morphologically similar to *T. crucigerum*, with which it shares ellipsoid fruits that bear wingless woody seeds (Tab. 1). *Tanaecium jaroba* differs from *T. crucigerum* by the glabrous or pubescent leaflets abaxially (vs. whitish-tomentose leaflets abaxially in *T. crucigerum*).

12. *Tanaecium kuhlmannii* (J.C.Gomes) Frazão & L.G.Lohmann, comb. nov.

Basionym: *Spathicalyx kuhlmannii* J.C. Gomes, Arq. Srv. Fl., Rio de Janeiro 10: 200. 1956. Type: Brazil. Rio de Janeiro: Sumaré, 5 Dec. 1932, J.G. Kuhlmann s.n. (holotype, RB!; isotype, SPF!, K image!, MO!).

Habitat and distribution. *Tanaecium kuhlmannii* is known from only a few localities within humid formations of the Atlantic Forest of Brazil (Minas Gerais, Rio de Janeiro).

Phenology. Flowering: December; fruiting: January.

Notes. Gomes (1956) originally described this species as *Spathicalyx kuhlmannii* J.C.Gomes, but Gentry (1977) synonymized it with *Spathicalyx duckei* (A.Samp.) A.H.Gentry. More recently, Lohmann and Taylor (2014) synonymized *Spathicalyx* with *Tanaecium* and recognized a single species, *Tanaecium duckei* (A. Samp.) L.G.Lohmann, following Gentry (1977). A detailed study of these taxa showed that apart from the allopatric distribution (*T. duckei* is restricted to the Amazon, while *T. kuhlmannii* is restricted to the Atlantic Forest of Brazil), *T. kuhlmannii* can be distinguished by the patelliform glandular trichomes along the tertiary veins of leaflets (vs. absent in *T. duckei*), and the ferrugineous stipitate glandular trichomes that cover the fruit surface (vs. ferrugineous stipitate glandular trichomes lacking in *T. duckei*). Furthermore, *T. kuhlmannii* has leaflets with patelliform trichomes ≥ 0.45 mm in diameter abaxially (vs. leaflets with patelliform trichomes < 0.45 mm in diameter abaxially in *T. duckei*), that also show a protrusion at the patelliform insertion (vs. without protrusion at the patelliform insertion in *T. duckei*), and anthers ≥ 7 mm long. (vs. anthers < 7 mm long in *T. duckei*). Based on these morphological features and distribution data, we here recognize both taxa as separate and propose the new combination *Tanaecium kuhlmannii* (J.C.Gomes) Frazão & L.G.Lohmann (Tab. 1).

13. *Tanaecium neobrasiliense* L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 465. 2014.

Sanhilaria brasiliensis Baill., Hist. Pl. 10: 27. 1888.

Paragonia brasiliensis (Baill.) A. H. Gentry, Ann. Missouri Bot. Gard. 63(1): 70. 1976. Type: Brazil. Minas Gerais: Itabira, 1816–1821, A.St. Hilaire 745 (holotype, P [P00458597]!; isotypes, P [P00468598]!, F [F0092570]!).

Habitat and distribution. *Tanaecium neobrasiliense* is found in caatinga and cerrado in eastern Brazil (Bahia, Ceará, Distrito Federal, Minas Gerais).

Phenology. Flowering: November to January; fruiting: January to April and June.

Notes. This species is generally confused with *T. pyramidatum* due to its pink corollas. However, it can be differentiated from *T. pyramidatum* by the leaflets 8–15 times longer than the petiole, costate calyces, and corollas with cuspidate lobes. The prophylls of the axillary buds are subulate or bromeliad-like, positioned in an acute angle in relation to the stems (vs. straight angle in *T. pyramidatum*) (Tab. 1).

14. ***Tanaecium parviflorum*** **(Mart. ex DC.) Kaehler & L.G.Lohmann, in press**[**]

Fig. 1E

Pithecoctenium parviflorum Mart. ex DC. in A.DC. Prodr 9: 197. 1845.
Arrabidaea parviflora (Mart. ex DC.) Bureau & K.Schum. in Fl. Bras. 8(2): 53. 1896.
Fridericia parviflora (Mart. ex DC.) L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 441. 2014. Type: Brazil. Bahia, Vale do Rio das Contas, October 1818, C.F.P. von Martius s.n. (lectotype, selected by Lohmann and Taylor 2014, M [M0086353]!).

Habitat and distribution. *Tanaecium parviflorum* occurs in caatinga vegetation from eastern Brazil (Bahia, Ceará, Minas Gerais, Paraíba, Pernambuco), and is also found disjunctly in Mato Grosso do Sul, in an area with drained soil.

Phenology. Flowering: December to February and April; fruiting: February to March and November to December.

Notes. *Tanaecium parviflorum* can be distinguished from all other species of the genus by the dentate leaflet margins, calyces aristate (rarely mucronate), and fruit apices caudate. Like *T. cyrtanthum* and *T. tetramerum*, this species is also caducous when flowering. However, *T. parviflorum* differs from these two species by the strongly compressed corollas (Tab. 1).

15. ***Tanaecium pyramidatum*** **(Rich.) L.G.Lohmann, Nuevo Cat. Fl. Vasc. Venezuela 274. 2008.**

Fig. 1F

Bignonia pyramidata Rich., Actes Soc. Hist. Nat. Paris 1: 110. 1792.
Tabebuia pyramidata (Rich.) DC., in A. DC., Prodr. 9: 214. 1845.
Paragonia pyramidata (Rich.) Bureau, Konigl. Danske Vidansk. Selsk. Skr., Naturivdensk. Math. Afd., ser. 6, 6: 422. 1892. Type: French Guiana. Cayenne, s. d., J. B. Leblond 292 (holotype, P-LA [P00358235]!; isotype, P-LA [P00358236]!).

Habitat and distribution. *Tanaecium pyramidatum* is widespread throughout the Neotropics, where it is found in dry and wet vegetation in Belize (Cayo, Toledo, Stann Creek, Belize, Orange Walk, Corozal), Bolivia (Beni, Cochabamba, La Paz, Pando, Santa Cruz), Brazil (Acre, Amapá, Amazonas, Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí,

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[**] TAXON in press. 2019. We do not intend to publish this combination here, but in Kaehler et al. (2019, in press.)

Rio de Janeiro, Rio Grande do Sul, Rondônia, Roraima, Santa Catarina, São Paulo, Tocantins), Colombia (Amazonas, Antioquia, Atlántico, Boyacá, Caquetá, Chocó, Córdoba, Cundinamarca, Guaviare, Magdalena, Meta, Nariño, Putumayo, Santander, Valle del Cauca, Vaupés), Costa Rica (Alajuela, Guanacaste, Heredia, Limón, Puntarenas, San José), Ecuador (El Oro, Esmeraldas, Guayas, Loja, Los Ríos, Manabí, Napo, Pastaza, Pichincha, Sucumbíos, Zamora-Chinchipe), El Salvador (Ahuachapán, La Libertad, Usulután), Guatemala (Alta Verapaz, Izabal, Petén), French Guiana (Cayenne, Saint-Laurent-du-Maroni), Guyana (East Berbice, Rupununi, West Demerara), Honduras (Colón, El Paraíso, Gracias a Dios, Islas de la Bahía, Olancho, Yoro), Mexico (Campeche, Chiapas, Colima, Oaxaca, Quintana Roo, Tabasco, Veracruz), Nicaragua (Atlántico Norte, Atlántico Sur, Chontales, Jinotega, Matagalpa, Río San Juan, Rivas), Panama (Bocas del Toro, Canal Area, Chiriquí, Coclé, Colón, Darién, Herrera, Los Santos, Panamá, San Blas, Veraguas), Peru (Amazonas, Cusco, Huánuco, Junín, Loreto, Madre de Dios, Pasco, Puno, San Martín, Ucayali), Suriname (Nickerie, Saramacca, Sipaliwini), Trinidad and Tobago, and. Venezuela (Amazonas, Anzoátegui, Apure, Barinas, Bolívar, Delta Amacuro, Distrito Federal, Falcón, Lara, Miranda, Monagas, Portuguesa, Sucre, Yaracuy, Zulia),

Phenology. Flowering: January to December; fruiting: January to December.

Notes. This species can be distinguished from other *Tanaecium* species by the petioles with patelliform trichomes at the apices, subulate prophylls of the axillary buds, fruits lenticellated, linear, and inflated. Despite that, *T. pyramidatum* is extremely variable morphologically. For example, populations from the Brazilian dry forests and cerrados have pubescent leaflets abaxially, a feature not found in any other population of this species. On the other hand, populations from Mexico are strongly covered by lenticels. Both of these features are found exclusively in these populations. Additional studies of *T. pyramidatum*, including phylogeographic studies based on a broad sampling of individuals collected throughout the range of this species, are necessary to identify putative cryptic species (Tab. 1).

16. *Tanaecium revillae* (A.H.Gentry) L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 466.

Fig. 1G, S

Arrabidaea revillae A.H.Gentry, Ann. Missouri Bot. Gard. 65(2): 726, fig. 1. 1978 [1979]. Type: Peru. Loreto: Maynas, distr. Pebas, Río Yahuasyacu, afluente del Río Ampiyacu, 18 Jul. 1976, J. Revilla 718 (holotype, MO [MO-086234]!; isotypes, COL [COL000004271]!, F–1797223!, NY [00313111]!, AMAZ not seen, USM not seen)

Habitat and distribution. *Tanaecium revillae* occurs in riparian vegetation and permanently flooded forest of the Amazon region. It occurs in Brazil (Amazonas, Pará, Roraima), Colombia (Caquetá), Guyana (Upper Takutu-Upper Essequibo), Peru (Loreto), and Suriname (Sipaliwini).

Phenology. Flowering: January, April, June to September and November; fruiting: July to August.

Notes. This species is well characterized morphologically and can be separated from other species of *Tanaecium* by the elliptic to ovate leaflets with cuspidate apices, tuft domatia in the abaxial surface of leaflets, fruits linear-oblong covered with peltate and patelliform glandular trichomes, and flat seeds with vestigial wings (Tab. 1).

17. *Tanaecium selloi* (Spreng.) L.G.Lohmann, Nuevo Cat. Fl. Vasc. Venezuela 274. 2008.

Fig. 1O, T

Bignonia selloi Spreng., Syst. Veg. 2: 831. 1825.

Arrabidaea selloi (Spreng.) Sandwith, Kew Bull. 8(4): 461. 1953 [1954]. Type: Brazil. Sin. loc., 1840, F. Sellow s. n. (holotype, B destroyed; lectotype, selected by Arbo 2017 in K [K000402778] image!; isolectotypes, BR [BR0000008764805] image!, G [G00133280] image!, K [K000402780] image!, L [L0412987] image!).

Bignonia coriacea Sellow ex Steud. Nomencl. Bot., ed. 2, 1: 204. 1840.

Habitat and distribution. This species is found in semi-deciduous dry or wet vegetation in Argentina (Chaco, Corrientes, Jujuy, Misiones, Salta), Bolivia (Chuquisaca, La Paz, Santa Cruz, Tarija), Brazil (Bahia, Ceará, Distrito Federal, Espírito Santo, Goiás, Mato Grosso do Sul, Minas Gerais, Paraíba, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Sul, Roraima, Santa Catarina, São Paulo), Colombia (Cesar), Paraguay (Alto Paraná, Caaguazú, Caazapá, Canindeyú, Central, Cordillera, Guairá, Paraguarí), Peru (Cusco, Junín, Tumbes), and Venezuela (Falcón, Zulia).

Phenology. Flowering: September to May and July; fruiting: January to December.

Notes. *Tanaecium selloi* differs from other *Tanaecium* species by the foliaceous or minute and triangular prophylls of the axillary buds, and fruits without a central ridge but with margins raised. Populations from semi-deciduous and dry areas of Argentina, Southern Brazil, Bolivia, and Paraguay show leaflets that are pubescent abaxially; these features are restricted to those populations (Tab. 1).

18. *Tanaecium tetragonolobum* (Jacq.) L.G.Lohmann, Nuevo Cat. Fl. Vasc. Venezuela 274. 2008.

Fig. 1K, P

Bignonia tetragonoloba Jacq., Fragm. Bot. 36. 1809 [1810].

Ceratophytum tetragonolobum (Jacq.) Sprague & Sandwith, Bull. Misc. Inform. Kew 1934: 222. 1934. Type: N. J. Jacquin, Fragm. Bot. 36, tab. 40, fig. 2 1809 [1810]–illustration! (lectotype, selected by Lohmann and Taylor 2014).

Habitat and distribution. *Tanaecium tetragonolobum* is found in dry to evergreen lowland forest vegetation (Gentry 1997) in Belize (Cayo, Orange Walk, Toledo), Bolivia (Beni, Chuquisaca, Cochabamba, La Paz, Pando, Santa Cruz), Brazil (Acre, Mato Grosso, Pará, Rondônia), Colombia (Atlántico, Bolívar, Chocó, La Guajira, Magdalena, Meta, Santander, Sucre), Costa Rica (Alajuela, Guanacaste, Guanaste, Puntarenas, San José), Ecuador (Napo, Pastaza), Guatemala (Petén), Guyana, Lesser Antilles (Grenada), Mexico (Campeche, Chiapas, Quintana Roo, Tabasco, Yucatán), Nicaragua (Atlántico Sur, Carazo, Chinandega, Chontales, Granada, León, Managua, Masaya, Río San Juan, Rivas), Panama (Canal Area, Darién, Herrera, Panama, Panamá, San Blas), Peru (Loreto, Madre de Dios, San Martín, Ucayali), Trinidad and Tobago, and Venezuela (Anzoátegui, Aragua, Barinas, Bolívar, Carabobo, Distrito Federal, Falcón, Guárico, Lara, Mérida, Miranda, Monagas, Portuguesa, Táchira, Yaracuy, Zulia).

Phenology. Flowering: February to November; fruiting: January to December.

Notes. *Tanaecium tetragonolobum* can be confused with two sympatric species, *T. jaroba* and *T. dichotomum* due to the stems with interpetiolular glandular fields (sometimes lacking in *T. dichotomum*) and subulate or bromeliad-like prophylls of the axillary buds (Tab. 1). However, *T. tetragonolobum* can be separated from *T. jaroba* by the membrane-like domatia (lacking in *T. jaroba*), lack of glandular peltate trichomes abaxially (present in *T. jaroba*), and interpetiolular patelliform trichomes < 0.3 mm (vs. interpetiolular patelliform trichomes > 0.3 mm in *T. jaroba*). On the other hand, *T. tetragonolobum* can be separated from *T. dichotomum* by the trifid tendrils (vs. simple tendrils in *T. dichotomum*) (Tab. 1).

19. *Tanaecium tetramerum* (A.H.Gentry) Zuntini & L.G.Lohmann, TAXON 65(5): 1059. 2016.

Fig. 1I

Sphingiphila tetramera A.H.Gentry, Syst. Bot. 15: 277–279, fig. 1. 1990. Type: Paraguay. Alto Paraguay: Chovoreca, moist sandy soil along pond in open cerrado vegetation, 19°20'S 59°05'W, 12 Aug 1983, W. Hahn 1600 (holotype, MO [MO–077156]!; isotypes, G [G00094221] image!, MBM–117809 not seen, MO [MO–077155]!, NY [00328929]!, PY–3783!, US [00432848]!).

Habitat and distribution. *Tanaecium tetramerum* is known from Central South America, where it occurs in Bolivia (Cochabamba, Santa Cruz), and Paraguay (Alto Paraguay, Chaco). This species occurs in xerophytic vegetation along the Chaco, in transition areas between the Chaco and Bolivian Chiquitano, Interandian, and Andean valleys. *Tanaecium tetramerum* generally grows on sandy soils or rocky outcrops.

Phenology. Flowering: January to February, August and November; fruiting: January to February, April, and July.

Notes. *Tanaecium tetramerum* is characterized by a series of unique morphological features that allow this species to be easily separated from other species of *Tanaecium* such as the thorn-tipped branchlets, terminal leaflets never replaced by tendrils, corollas actinomorphic, hypocrateriform, and 4-lobed (Gentry 1990; Pace et al. 2016) (Tab. 1).

20. *Tanaecium truncatum* (A.Samp.) L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 467. 2014.

Fig. 1J

Periarrabidaea truncata A.Samp., Bol. Mus. Nac. Rio de Janeiro 12: 86. 1936. Type: Brazil, Amazonas, Manaus, capoeira além da Villa Municipal, lugar alto, 27 July 1931, A. Ducke s.n. (holotype, RB–24093!; isotype, R–28731!).

Habitat and distribution. This species occurs in humid forest vegetation in Bolivia (Pando), Brazil (Amazonas, Mato Grosso, Rondônia), and Peru (Cusco, Loreto, Madre de Dios, Ucayali).

Phenology. Flowering: November to March, and May to October; fruiting: February, July to August, and October to December.

Notes. This species differs from other *Tanaecium* species by the foveolate domatia, calyces basally constricted, and pale-yellow corollas (Tab. 1).

21. *Tanaecium xanthophyllum* (DC.) L.G.Lohmann, Ann. Missouri Bot. Gard. 99(3): 467. 2014.

Fig. 1K

Tabebuia xanthophylla DC., in A.DC., Prodr. 9: 214. 1845.

Arrabidaea xanthophylla (DC.) Bureau & K.Schum., Fl. Bras. 8(2): 70. 1896.

Xylophragma xanthophylla (DC.) J.F.Macbr., Publ. Field Mus. Nat. Hist., Bot. Ser., 13 (pt. 5c, no. 1): 65. 1961.

Pithecoctenium xanthophyllum (DC.) Miers, Proc. Roy. Hort. Soc. London 3: 199. 1963.

Spathicalyx xanthophylla (DC.) A.H.Gentry, Phytologia 35(3): 195. 1977. Type: Brazil, Amazonas, Alto Amazonas, Rio Negro, Maribi, towards River Japurá, Dec. 1819, C.F.P. von Martius 2967 (holotype, G-DC [G00133960]!; isotypes, M [M0088929]!, M [M0088930]!, M [M0088931]!, M [M0088932]!, M [M0088933]!, M [M0088934]!, M [M0088935]!).

Habitat and distribution. This species occurs in wet forest vegetation in Bolivia (Beni, Chuquisaca, La Paz, Santa Cruz), Brazil (Acre, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia), Colombia (Amazonas, Putumayo), Ecuador (Napo, Pastaza), and Peru (Amazonas, Cusco, Junín, Loreto, Madre de Dios, San Martín, Ucayali).

Phenology. Flowering: October to July; fruiting: February to July and December.

Notes. *Tanaecium xanthophyllum* differs from other species of *Tanaecium* by the leaflets with yellow dendritic simple trichomes, bracteoles with a proportion ≥ 4:5 to the flower pedicel, corollas with peltate trichomes in the ventral portion internally. The species epithet refers to the yellow stems, leaves, inflorescences, and fruits (Tab. 1).

Incertae Sedis

Tanaecium mutabile (Bureau & K. Schum.) L.G.Lohmann. Ann. Missouri Bot. Gard. 99(3): 465.

Arrabidaea mutabilis Bureau & K.Schum., Fl. Bras. 8(2): 38. 1896. Type: Brazil. São Paulo, Campinas [“Brésil méridional” on sheet], 16 Sep 1868, J. Correia de Méllo 44 (lectotype designated by Lohmann and Taylor 2014 P [P00468542]!; isolectotypes, P [P00468543]!, P [P00468544]!, P [P00468545]!, P [P00568546]!, S [S09-21566] image!, S as photocopy at MO–2909990!, F–999017!; F–784134!).

Notes. New morphological and molecular data indicates that *T. mutabile* is nested within *Fridericia*, instead of *Tanaecium* (Frazão & Lohmann, in prep.).

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RESEARCH ARTICLE

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New morphological and DNA evidence supports the existence of *Calligonum jeminaicum* Z. M. Mao (Calligoneae, Polygonaceae) in China

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Abstract

Calligonum jeminaicum Z. M. Mao, a species regarded as endemic to China, was thought to be nonexistent owing to a lack of scientific records. The similarity of *C. jeminaicum* to *C. mongolicum* Turcz. warranted an investigation into the taxonomical relationship between these species. In this study, a naturally occurring population of *C. jeminaicum* was discovered and the taxonomical relationships of this species with *C. mongolicum* were resolved. Morphological traits, including fruit and flower characteristics, as well as nuclear (ETS, ITS) and chloroplast (*psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *rbc*L, and *trnL-F*) DNA sequence data were studied to confirm the taxonomic status of *C. jeminaicum*. The nrDNA data (ITS1-2 and ETS) from *C. jeminaicum* reflected variability from the whole *C. mongolicum* complex, showing distinctive haplotypes in the *Calligonum* sect. *Medusa* Sosk. & Alexandr. The cpDNA data supplied similar evidence, showing unique branching in Bayesian and ML tree analyses. The specific status of *C. jeminaicum* is confirmed based on both morphological and molecular analyses. Here we present a revised description of *C. jeminaicum* along with its DNA barcode and discuss suggestions for the conservation of this species. Based on current evidence, this species was evaluated as Critically Endangered (CR) according to the IUCN criteria.

Keywords

Calligonum mongolicum complex, Central Asia, desert plant, IUCN, molecular phylogenetics, morphological traits

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* These authors contributed equally to this work.

Introduction

Calligonum L. species are as ecologically important as some of the dominant shrubs and semi-shrubs in both active and inactive sand dunes in the African Sahara (Dhief et al. 2011, 2012) and the deserts of Central Asia (Losinskaya 1927; Bao and Grabovskaya-Borodina 2003; Amirabadi-zadeh et al. 2012). They are natural resources of tannins, food, medications, nectar, and antidotes (Liu et al. 2001; Badria et al. 2007; Askari-yahromi et al. 2013; Essam et al. 2014). *Calligonum* is considered to be the only genus within Polygonaceae that contains C_4 species (Pyankov et al. 2000) and displays rapid rates of evolution and diversification (Mabberly 1990). This accelerated differentiation process causes physiological (Su et al. 2005, 2013) and morphological (Mao and Pan 1986; Taia and Moussa 2011; Tao and Ren 2004) changes within these species that facilitate their tolerance of various extreme xeric conditions (Pyankov et al. 2000; Su and Zhao 2002). Thus, *Calligonum* species have been used as the major sand conservation species in northwestern China (Wang et al. 2014; Xie et al. 2014).

Calligonum jeminaicum Z. M. Mao was first described by Mao (1984) to be a local endemic species which only proliferated in the countryside near Jeminay in the northwest of the Gurbantunggut Desert (Mao 1984, 1992). It has been difficult to differentiate *C. jeminaicum* from *C. mongolicum* Turcz. owing to their similar morphological characteristics (Mao 1992; Bao and Grabovskaya-Borodina 2003). In addition, there has been no further record of this species to demonstrate its existence, leading to the question: does this endemic species actually exist? This question was resolved by specific field work in 2013 when a naturally occurring population with eight individuals of *C. jeminaicum* was found.

The rapid and complex evolutionary processes of *Calligonum* species have been reflected in their fruit morphology (Bao and Grabovskaya-Borodina 2003; Shi et al. 2009, 2016; Feng et al. 2010a; Soskov 2011). Fruit phenotype has been used as the key character to separate the whole *Calligonum* genus into four sections, namely sect. *Calliphysa* (Fish. & C. A. Mey.) Borszcz. (Fig. 1A), sect. *Pterococcus* (Pall.) Borszcz. (Fig. 1B), sect. *Calligonum* (Fig. 1C), and sect. *Medusa* Sosk. & Alexandr. (Fig. 1D). Members of sect. *Calliphysa* have membranous-saccate fruits, those of sect. *Pterococcus* have winged fruits, the fruits of sect. *Medusa* only show bristles without wings and membranes, and the fruits of sect. *Calligonum* show both wings and bristles but no membranes (Bao and Grabovskaya-Borodina 2003; Fig. 1). The most widely distributed species in Central Asia, *C. mongolicum* (sect. *Medusa*), has shown two karyotypes with different chromosome numbers ($2n = 18$ and $2n = 27$) within the same population (Shi and Pan 2015); this species has heterogeneous phenotypes and forms a *C. mongolicum* complex with inter-crossed taxonomic relationships with other species in sect. *Medusa* (Soskov 2011; Shi et al. 2011, 2012; Shi and Pan 2015). *Calligonum mongolicum* has a large distribution area bordered by Xilinhot (Inner Mongolia) in the east, Kumul and Tutotu Basin (Xinjiang) in the west, Milan (Xinjiang) in the south, and Baitashan, Qitai, and Karamay (Xinjiang) in the north. The longitudinal range of *C. mongolicum* is about 30° (Pavlov 1936;

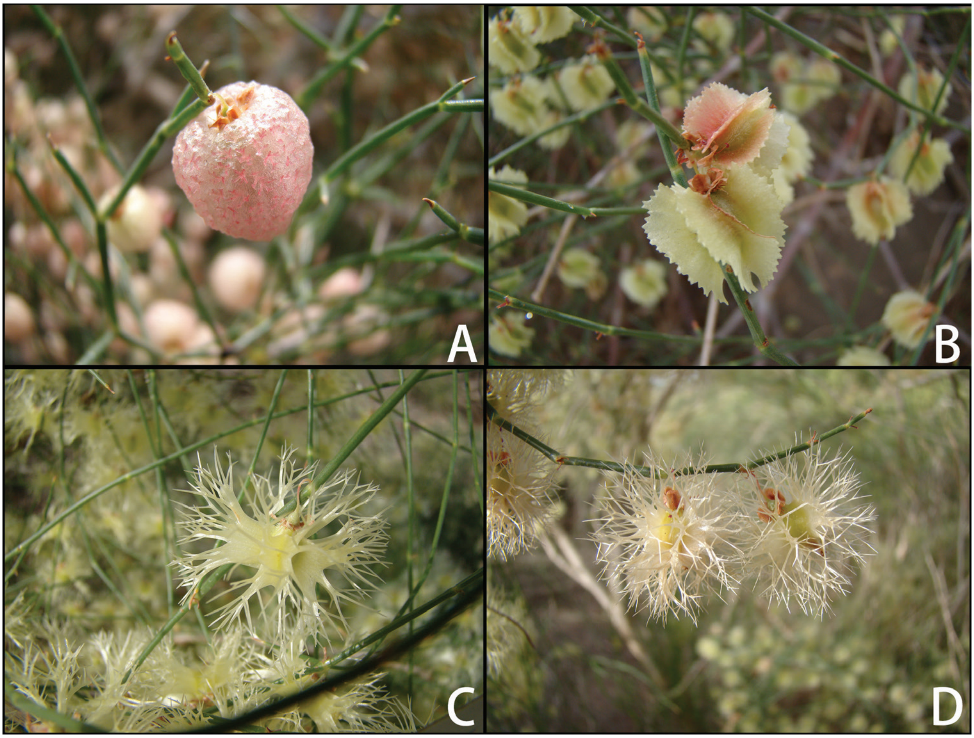

Figure 1. Fruit characters in the members of the four sections in *Calligonum* (A, sect. *Calliphysa* (Fisch. & C. A. Mey.) Borszcz.; B, sect. *Pterococcus* (Pall.) Borszcz.; C, sect. *Calligonum*; D, sect. *Medusa* Sosk. & Alexandr.).

Drobov 1953; Baitenov and Pavlov 1960; Sergievskaya 1961; Kovalevskaya 1971; Borodina 1989). The distribution range of *C. jeminaicum* lies within that of *C. mongolicum* (Mao 1992; Bao and Grabovskaya-Borodina 2003). The *C. mongolicum* complex has been the subject of several taxonomic studies, particularly those focused on species delimitation and identification (Feng et al. 2010b; Gulnur et al. 2010; Li et al. 2014; Shi et al. 2011, 2009, 2016). Both fruit and flower characteristics are used for distinguishing *C. jeminaicum* from *C. mongolicum* (Bao and Grabovskaya-Borodina 2003).

DNA analysis is regarded as one of the most important techniques to elucidate taxonomy (Kress et al. 2005; Hollingsworth et al. 2009). Previous studies have used *Calligonum* DNA data to resolve several conflicting taxonomic relationships, such as the use of RAPD markers to clarify the relationships of species in China (Ren et al. 2002), and the use of three chloroplast DNA markers (*rbcL*, *matK*, and *trnL-F*) to distinguish the Chinese species of *Calligonum*, although these conserved markers were not effective (Li et al. 2014). Additionally, cpDNA data have revealed the phylogeographic variation in different sections (Wen et al. 2015, 2016a, b), which was shown to be potentially valuable for DNA barcoding. ITS data have been used to effectively resolve taxonomical problems within the *C. mongolicum* complex (Shi et al. 2016, 2017). However, combined sequencing data from cpDNA and nrDNA have not been employed for clarifying the status of puzzling species in *Calligonum*. There is a need to further explore rapidly evolving DNA sequences that may be effective in resolving the taxonomic uncertainties in *Calligonum*.

In this study, nuclear ribosomal ITS and ETS sequences, together with five sets of cpDNA data (*psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *rbcL*, and *trnL-F*) and the morphological characters, were used to confirm the existence of *C. jeminaicum* and clarify its relationship with *C. mongolicum*. We also suggest and discuss strategies for conserving *C. jeminaicum*.

Methods

Sample selection and species identification

All samples were collected from shoots of *Calligonum* individuals from Xinjiang, Qinghai, Inner Mongolia, Gansu, and Ningxia across the northwest of China during summer from 2006 to 2015 (Table 1).

The classical identification key was used to differentiate these species mainly based on fruit characteristics and geographic locations, and the *C. mongolicum* complex has been identified by its fruit characteristics (Mao 1992; Bao and Grabovskaya-Borodina 2003), primarily based on quantifiable differences in fruit and bristle size, such as fruit length (LF), fruit width (WF), bristle length (BS), bristle distance (BD), rib distance (RD), achene length (AL), achene width (AW), and fruit shape (FF) (Shi et al. 2012, 2016; Fig. 2A). The same fruit indices have been used to compare *C. jeminaicum* with *C. mongolicum*. The flower traits for differentiating between the two species were selected based on the identification key in "Flora of China" (Bao and Grabovskaya-Borodina 2003), including the shape of perianth segments (PS, broadly elliptic or ovate Fig. 2B), pedicel length (1–2 cm in *C. jeminaicum* and 2–4 cm in *C. mongolicum*: Fig. 2C), spreading or reflexed in fruit (PSF, Fig. 2D), and pedicel joint position (below or middle). The shape of perianth segments (Fig. 2B) and pedicel length (Fig. 2C) were used to make quantitative distinctions between *C. jeminaicum* and *C. mongolicum*.

Some species with distinctive fruit characters were used as references in the DNA data analysis: *Calligonum calliphysa* Bunge, which was previously named *Calligonum junceum* (Fisch. & C. A. Mey.) Litv. (Bao and Grabovskaya-Borodina 2003), is the only species in sect. *Calliphysa*, was selected as a representative species; *Calligonum arborescens* Litv. and *Calligonum ebinuricum* Ivanova ex Y. D. Soskov (sect. *Medusa*) were used for comparison because they are regarded as distinct from the *C. mongolicum* complex. The number of individuals used for morphological analysis and DNA extraction in each population and the accession numbers of some ITS and plastid marker sequences obtained from GenBank are given in Table 1.

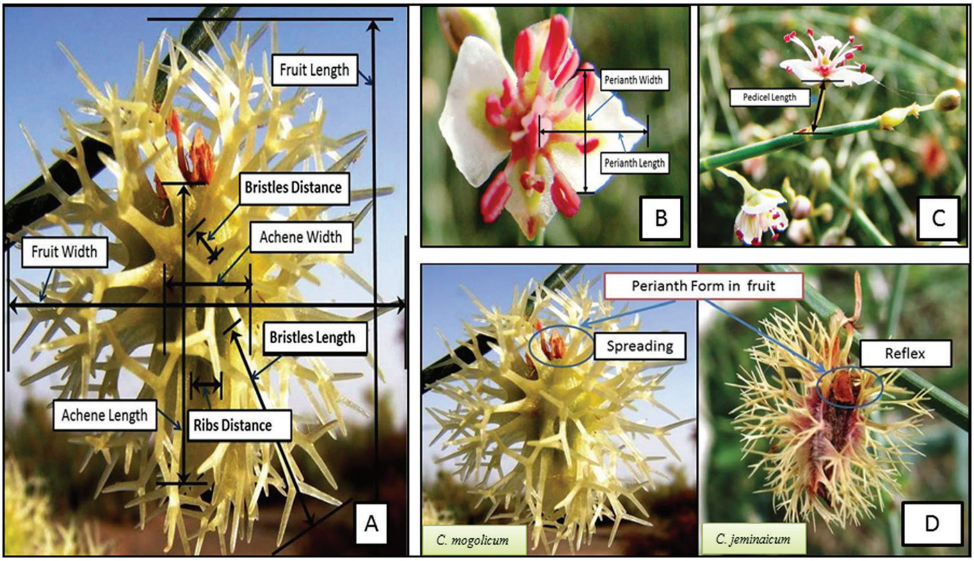

Figure 2. Measurements of fruit characters (**A**) and flower traits (**B** form of perianth segments **C** pedicel), and the distinction of the form of perianth segments in *Calligonum jeminaicum* and *C. mongolicum* fruits (**D**).

Molecular protocols

For all the newly collected samples, total genomic DNA was extracted from fresh or silica gel dried leaves according to the protocol of Doyle and Doyle (1990) or the CTAB method of Doyle and Doyle (1990). The ribosomal DNA regions are known to be potentially problematic when inferring phylogeny (Alvarez and Wendel 2003). In this study, we followed the guidelines for obtaining reliable ITS sequences in plants proposed by Feliner and Rossello (2007). The ITS regions were amplified and sequenced using the previously described primers "ITS5a" and "ITS4" (Stanford et al. 2000). The ETS primers were designed by Shi et al. (2016): the forward primer ETS-calli1: 5'-GTTACTTACACTCCCCACAACCCC-3' and the reverse primer 18SIGS: 5'-GAGACAAGCATATGACTACTGGCAGGATCAACCAG-3'. Primers and polymerase chain reaction (PCR) protocols used for the amplification of chloroplast *psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *trn*L-F, and *rbc*L (the first part of the entire *rbc*L gene) were described in previous studies (Demesure et al. 1995; Small et al. 1998; Shaw et al. 2005, 2007; Falchi et al. 2009).

The specific Sanger sequencing studies of the *Calligonum mongolicum* complex and other species were divided into two parts, with most experiments completed at the Smithsonian Institution in 2014, and additional data, particularly those concerning *C. jeminaicum*, being supplied by the Key Laboratory of Biogeography and Bioresource in Arid Land (KLBB), Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences. At the Smithsonian Institution, PCR amplification of DNA was performed using 10 ng of genomic DNA, 4 pmol of each primer, 0.5 U Taq polymerase (Bioline,

Table 1. Population information for *C. mongolicum* Turcz., *C. jeminaicum* Z. M. Mao and related species, and GenBank accession numbers of DNA sequences used in this study.

Species	Pop. No. (#, &)[1]	Location	Latitude	Longitude	Elevation	Gen-Bank accession number							Voucher Number
						ITS	ETS	*psbA-trnH*	*trnL-trnF*	*ycf6-psbM*	*rpl32-trnL*	*rbcL*	
C. mongolicum	1(10, 4)	Erjinaqi, Inner Mongolia	41°27.2'N	100°26.3'E	1002m	KU050846	KY316971	MN449309	MN449258	MN449070	MN449121	MN449172	C1101-C1110
						KU050848	KY316973	MN449310	MN449259	MN449071	MN449122	MN449173	
						–	–	MN449311	MN449260	MN449072	MN449123	MN449174	
						–	–	MN449312	MN449261	MN449073	MN449124	MN449175	
	2 (10, 2)	Hulishan, Inner Mongolia	41°58.3'N	100°35.4'E	899m	MN449220	MN449032	MN449313	MN449262	MN449074	MN449125	MN449176	C1111-C1120
						MN449221	MN449033	MN449314	MN449263	MN449075	MN449126	MN449177	
	3 (10, 2)	Qingtongxia, Ningxia	38°01.0'N	105°55.9'E	1134m	KU050847	KY316966	MN449315	MN449264	MN449076	MN449127	MN449178	C1121-C1130
						KU050853	KY316970	MN449316	MN449265	MN449077	MN449128	MN449179	
	4 (10, 3)	Mazongshan, Gansu	41°48.7'N	098°42.4'E	12364m	MN449222	MN449034	MN449317	MN449266	MN449078	MN449129	MN449180	C1145-C1154
						MN449223	MN449035	MN449318	MN449267	MN449079	MN449130	MN449181	
						–	–	MN449319	MN449268	MN449080	MN449131	MN449182	
	5 (10, 2)	Liuyuan, Gansu	43°20.5'N	091°23.6'E	1273m	KU050844	KY316963	MN449320	MN449269	–	MN449132	MN449183	C1166-C1175
						KU050845	KY316975	MN449321	MN449270	MN449081	MN449133	MN449184	
	6 (10, 3)	Kelamayi, Xinjiang	47°19.6'N	086°46.4'E	574m	MN449224	MN449036	MN449322	MN449271	MN449082	MN449134	MN449185	C2101-C2110
						MN449225	MN449037	MN449323	MN449272	MN449083	MN449135	MN449186	
						–	MN449038	–	–	–	–	–	
	7 (10, 2)	Wuerhe, Xinjiang	46°08.2'N	086°12.9'E	415m	KU050849	KY316969	MN449324	MN449273	MN449084	MN449136	MN449187	C2133-C2142
						KU050850	KY316972	MN449325	MN449274	MN449085	MN449137	MN449188	
	8 (10, 4)	Xinxinxia, Xinjiang	42°45.2'N	095°28.7'E	1744m	MN449226	MN449039	MN449326	MN449275	MN449086	MN449138	MN449189	C2165-C2174
						MN449227	MN449040	MN449327	MN449276	MN449087	MN449139	MN449190	
						MN449228	MN449041	MN449328	MN449277	MN449088	MN449140	MN449191	
						MN449229	MN449042	MN449329	MN449278	MN449089	MN449141	MN449192	
	9 (10, 2)	Qijiaojing, Xinjiang	43°35.3'N	091°25.4'E	1142m	KU050852	KY316960	MN449330	MN449279	MN449090	MN449142	MN449193	C2175-C2184
						KU050841	–	MN449331	MN449280	MN449091	MN449143	MN449194	
	10 (10, 3)	Hami1, Xinjiang	43°23.7'N	091°32.5'E	1038m	–	–	MN449290	MN449239	MN449051	MN449102	MN449153	C2011-C2020
						KU050843	KY316962	MN449291	MN449240	MN449052	MN449103	MN449154	
						–		MN449292	MN449241	MN449053	MN449104	MN449155	

Species	Pop. No. (#, &)[1]	Location	Latitude	Longitude	Elevation	Gen-Bank accession number							Voucher Number
						ITS	ETS	*psbA-trnH*	*trnL-trnF*	*ycf6-psbM*	*rpl32-trnL*	*rbcL*	
C. mongolicum	11 (10, 2)	Hami2, Xinjiang	42°44.5'N	093°55.5'E	812m	MN449205	MN449019	MN449293	MN449242	MN449054	MN449105	MN449156	C2178-C2186
						MN449206	MN449020	MN449294	MN449243	MN449055	MN449106	MN449157	
	12 (10, 3)	Tashan, Xinjiang	45°01.7'N	090°03.2'E	1018m	MN449207	MN449021	MN449295	MN449244	MN449056	MN449107	MN449158	C2274-C2283
						MN449208	–	MN449296	MN449245	MN449057	MN449108	MN449159	
						MN449209	–	MN449297	MN449246	MN449058	MN449109	MN449160	
	13 (10, 2)	Chaidamu, Qinghai	39°09.7'N	089°47.4'E	1680m	MN449210	MN449022	MN449298	MN449247	MN449059	MN449110	MN449161	C0121-C0130
						–	–	MN449299	MN449248	MN449060	MN449111	MN449162	
	14 (10, 3)	Kumishi, Xinjiang	42°14.5'N	088°13.4'E	919m	MN449211	MN449023	MN449300	MN449249	MN449061	MN449112	MN449163	C0152-C0161
						MN449212	MN449024	MN449301	MN449250	MN449062	MN449113	MN449164	
						MN449213	MN449025	MN449302	MN449251	MN449063	MN449114	MN449165	
	15 (10, 1)	Heshuo, Xinjiang	42°16.9'N	082°59.2'E	1105m	MN449214	MN449026	MN449303	MN449252	MN449064	MN449115	MN449166	C0122-C0131
	16 (10, 3)	Mingfeng, Xinjiang	36°45.1'N	082°59.3'E	1600m	MN449215	MN449027	MN449304	MN449253	MN449065	MN449116	MN449167	C0174-C0184
						MN449216	MN449028	MN449305	MN449254	MN449066	MN449117	MN449168	
						MN449217	MN449029	MN449306	MN449255	MN449067	MN449118	MN449169	
	17 (10, 2)	Yutian, Xinjiang	36°45.2'N	082°02.1'E	1648m	MN449218	MN449030	MN449307	MN449256	MN449068	MN449119	MN449170	C0147-C0158
						MN449219	MN449031	MN449308	MN449257	MN449069	MN449120	MN449171	
C. jeminaicum	18 (8, 3)	Jeminay, Xinjiang	47°19.3'N	086°45.9'E	780m	MN449232	MN449048	MN449334	MN449283	MN449094	MN449146	MN449197	C3225-C3233
						MN449233	MN449049	MN449335	MN449284	MN449095	MN449147	MN449198	
						MN449234	MN449050	–	–	–	–	–	
C. calliphysa	19 (0, 1)	Mulei, Xinjiang	44°35.8'N	090°39.7'E	574m	KX186585	KY316976	MN449338	MN449287	MN449099	MN449150	MN449202	C0112-C0121
	20 (0, 1)	Qitai, Xinjiang	44°59.4'N	089°57.5'E	540m	KX186585	KY316976	MN449339	MN449288	MN449100	MN449151	MN449203	C2301-C2310
C. ebinuricum	21 (0, 3)	Jinhe, Xinjiang	44°37.8'N	083°11.1'E	370m	MN449236	MN449045	MN449336	–	MN449096	MN449148	MN449199	C1158-C1167
						MN449237	MN449046	MN449337	MN449285	MN449097	MN449149	MN449200	
						MN449238	MN449047	–	MN449286	MN449098	–	MN449201	
C. arborescens	22(0, 2)	Huocheng, Xinjiang	44°4.58'N	080°29.2'E	639m	MN449230	MN449043	MN449332	MN449281	MN449092	MN449144	MN449195	C1168-C1177
						MN449231	MN449044	MN449333	MN449282	MN449093	MN449145	MN449196	
Pteroxygonum giraldii	23(0,1)	Ningshan, Shaanxi	33°48.5'N	108°39.7'E	1501m	MN449235	–	MN449340	MN449289	MN449101	MN449152	MN449204	P. L. Liu 431

[1] #, the number of samples used for morphological analysis; &, the number of samples used for DNA analysis

Randolph, MA, USA), and 2.5 mM $MgCl_2$ in a volume of 25 μL using a PTC-225 Peltier thermal cycler. The PCR cycling parameters were as follows: a 95 °C initial hot start for 5 min, 32 cycles of 94 °C for 30 s, primer-specific annealing (ITS and ETS: 55 °C for 60 s; the five cpDNA primers: 53 °C for 40 s), and 72 °C for 60 s, and a final extension of 72 °C for 10 min. At the Smithsonian Institute, the PCR products were isolated and purified using ExoSAP-IT (US Biological, Swampscott, MA, USA) and sequenced in both directions using the PCR primers. Cycle sequencing was carried out using an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) with 5 ng of each primer, 1.5 μL of sequencing dilution buffer, and 1 μL of cycle sequencing mix in a 10 μL reaction volume. Cycle sequencing conditions comprised 30 cycles of 30 s denaturation (96 °C), 30 s annealing (50 °C), and 4 min elongation (60 °C). The sequencing products were separated on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). At KLBB, the amplified products were purified using a PCR Product Purification Kit (Shanghai SBS, Biotech Ltd., China). Sequencing reactions were conducted with the forward and reverse PCR primers using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, U.K.) with an ABI PRISM 3730 automatic DNA sequencer (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China). Both strands of the DNA were sequenced with overlapping regions to ensure that each base was unambiguous. Electropherograms were assembled and consensus sequences were generated with Sequencher 4.5 (Gene Codes, Ann Arbor, MI, USA).

Phylogenetic and network analyses

Multiple sequence alignments were performed using MUSCLE in the Geneious v.10.0.6 platform (Kearse et al. 2012) using the default settings and manual adjustments. The phylogenetic tree reconstruction of the nrITS and ETS sequence alignment included 44 accessions: 35 newly generated nrITS sequences, 24 new ETS sequences, and nine ITS and 20 ETS sequences from GenBank (Table 1).

Phylogenetic analyses were conducted on both the nuclear and combined plastid datasets. The best-fit nucleotide substitution models for the ITS1, 5.8S, ITS2, ETS, *psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *trnL-F*, and *rbcL* regions were determined separately using jModelTest (Darriba et al. 2012) and the Akaike information criterion (AIC) were used to rank the best-fit model for the Bayesian analyses.

Phylogenetic relationships were inferred using Bayesian inference (BI) as implemented in MrBayes v.3.2.5 (Ronquist and Huelsenbeck 2003) and the maximum likelihood (ML) analyses were accomplished with RAxML v.8.2 (Stamatakis 2014). Partitioned analyses of both the nuclear and plastid datasets were implemented by applying the previously determined models to each data partition (Brown and Lemmon 2007). The nuclear ITS dataset was partitioned into ITS1, 5.8S, and ITS2 partitions. For the concatenated plastid dataset, separate partitions were used for the *psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *trnL-F*, and *rbcL* regions. 51 samples in *Calligonum* were selected as the ingroup and *Pteroxygonum giraldii* Dammer & Diels was selected as the outgroup. Two independent BI analyses with one cold and three incrementally heated Markov chain Monte Carlo (MCMC) chains were run for 10,000,000 generations, with trees sampled every 1,000 generations. All Bayesian analyses produced split frequencies of less than 0.01, indicating convergence between the paired runs. The first 2,500 trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree and posterior probabilities (PP). In the ML analyses, rapid bootstrap analysis was performed with a random seed, 1,000 alternative runs, and the same partition scheme as was used in the Bayesian analysis. The model parameters for each partition of the dataset were optimized by RAxML with the GTRCAT command. Trees were visualized in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The ML bootstrap support values (BS) were labeled on the corresponding branches of the BI trees.

A network analysis was carried out with SplitsTree 4.13.1 (Huson and Bryant 2006) using the uncorrected *p*-distances between the *C. mongolicum* complex and *C. jeminaicum* species from the Bayesian analyses. Branch support was estimated using bootstrapping with 1,000 replicates (Felsenstein 1985).

Results

Phenotyping

The descriptions of the shape of perianth segments in fruit (PSF) and the pedicel joint position (below or middle) used to distinguish between the two species were qualitatively compared. The shape of perianth segments in fruit differs between the two species: spreading in the fruit of *C. mongolicum*, but reflexed in that of *C. jeminaicum* (Fig. 2D).

The morphological differences between *C. mongolicum* and *C. jeminaicum* focus primarily on their fruit and flower characteristics. Compared with the ambiguous characters in *C. mongolicum*, these taxonomical characters of *C. jeminaicum* were clearer and more stable. Quantitative comparisons of the fruit traits (Fig. 2A), the perianth segment shape (broadly elliptic or ovate, identified by the value of the length of the perianth segments/width of the perianth segments: Fig. 2B), and the pedicel length (Fig. 2C) were made between the two species (Fig. 3). Although some fruit characters appeared simultaneously in the two species and led to difficulty in distinguishing *C. jeminaicum* from *C. mongolicum*, the shape of perianth segments in fruit could be regarded as an effective character for their identification (Fig. 2D).

The quantifiable morphological characters in both fruits and flowers were compared between the two species. The fruit of *C. mongolicum* (0.106–1.880 cm; 1.134 ± 0.284 cm) was significantly ($P = 0.026$) longer than that of *C. jeminaicum* (0.415–0.649 cm; 0.432 ± 0.44 mm). Additionally, the fruit width (FW) for *C. mongolicum* (0.226–1.742 cm; 0.923 ± 0.347 cm) was larger than that of *C. jeminaicum*

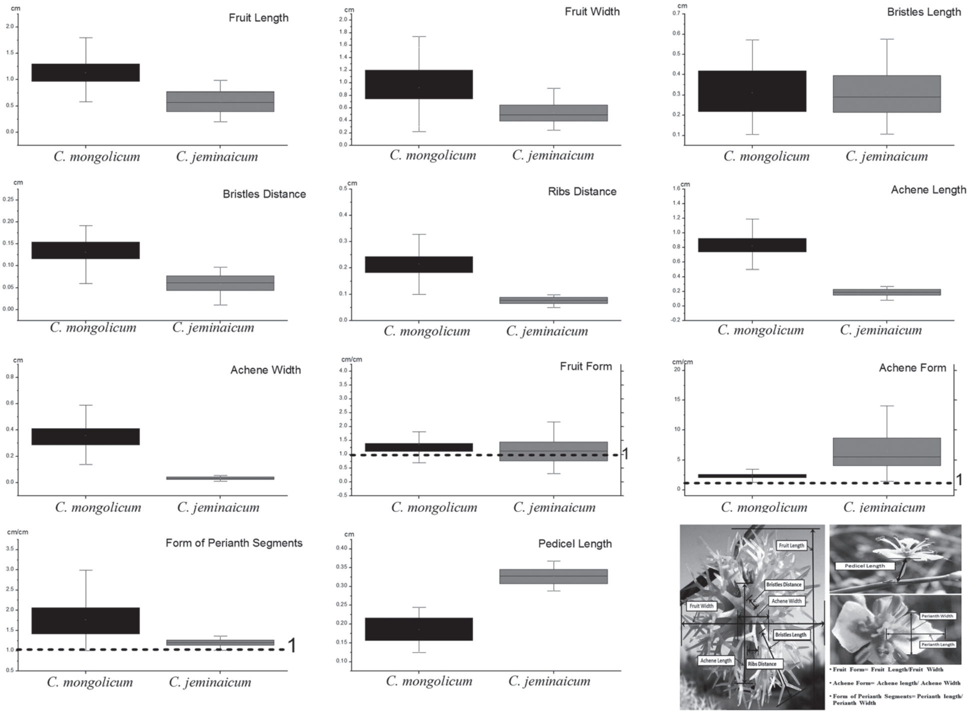

Figure 3. Quantitative comparisons of fruit and flower characters in *Calligonum mongolicum* and *C. jeminaicum*.

(0.348–0.508 cm; 0.428 ± 0.113 cm; *P* = 0.017). The bristle length of *C. jeminaicum* (0.372 ± 0.020 cm) was significantly shorter (*P* = 0.06) than that of *C. mongolicum* (0.312 ± 0.121 cm), and the bristle distance (0.077 ± 0.006 cm) and rib distance (0.087 ± 0.004 cm) of *C. jeminaicum* were significantly smaller than those of *C. mongolicum* (bristle distance 0.131 ± 0.032 cm, *P* = 0.01; rib distance 0.105 ± 0.032 cm, *P* = 0.02). Significant differences were also detected in achene length (0.823 ± 0.146 cm in *C. mongolicum* and 0.195 ± 0.105 cm in *C. jeminaicum*, *P* = 0.00) and achene width (0.359 ± 0.089 cm in *C. mongolicum* and 0.333 ± 0.004 cm in *C. jeminaicum*, *P* = 0.00) (Fig. 3), although the difference in achene width was small. The fruit shape, as the key character, was substantially different between the two species (*P* = 0.000), with the subglobose fruit of *C. jeminaicum* (1.048 ± 0.467 cm/cm) being much more rounded than the broadly ellipsoid fruit of *C. mongolicum* (1.357 ± 0.442 cm/cm). Thus, the fruit characteristics could be used to distinguish between the two species (Fig. 3). Both the pedicel length (*P* = 0.00) and the form of perianth segments (*P* = 0.01) of the two species showed significant differences. The pedicel length of *C. jeminaicum* (0.313 ± 0.004 cm) was much longer than that of *C. mongolicum* (0.219 ± 0.03 cm). The shape of perianth segments for *C. jeminaicum* (1.222 ± 0.167 cm/cm) was broader than that of *C. mongolicum* (2.544 ± 1.799 cm/cm) (Fig. 3).

Molecular phylogeny

The aligned matrix of 44 accessions of the combined nrITS and ETS sequences comprised 807 bp that did not include any abnormal SNPs or unreasonable sequences according to the Phi test (P = 0.0321). The best-fit substitution models were GTR+G for ETS (nucleotide frequencies A: 0.200803 C: 0.329510 G: 0.295074 T: 0.174613) and GTR+I+G for nrITS (nucleotide frequencies A: 0.163227 C: 0.337699 G: 0.352720 T: 0.146353) based on the jModelTest (Darriba et al. 2012) results. The GTR+G model was selected for the ML analyses of the aligned matrix of nrDNA.

The two phylogenetic tree reconstruction methods, BI and ML, produced consistent topologies. However, the nuclear and the chloroplast data were analyzed separately to reconstruct the phylogenetic relationships among *C. jeminaicum*, the *C. mongolicum* complex, and other species in *Calligonum* because obviously different topologies based on the nuclear (Fig. 4, 5) and the chloroplast (Fig. 6) data were found. In the nrDNA data, no single nucleotide polymorphism (SNP) was identified among the *C. jeminaicum* samples, but the species from the *C. mongolicum* complex showed heterogeneity and did not form a single clade (Fig. 4). The populations of the *C. mongolicum* complex, *C. arborescens*, *C. calliphysa*, and *C. jeminaicum*, were distributed within the same broad geographic region. The three individuals of *C. ebinuricum*, which had specific fruit characteristics that were different from the *C. mongolicum* complex, formed an independent clade (Fig. 4). Interestingly, the *p*-distance among the *Calligonum* taxa for the ITS and ETS regions reached 11.364% between species *C. arborescens* and *C. calliphysa*. The *p*-distance was as high as 22.54% between *C. ebinuricum* and the *C. mongolicum* complex group, which reflects their interspecific differentiation. Consistent results were obtained in the ML analysis in the same phylogenetic tree for nrDNA, conforming the *C. mongolicum* complex and *C. jeminaicum* independently (Fig. 4, PP = 1, BS = 98%).

The neighbor-net constructed for the *C. mongolicum* complex and *C. jeminaicum* using the ITS and ETS sequences (Fig. 5) also did not support a single clade for the *C. mongolicum* complex. The three *C. jeminaicum* samples formed a separate branch from other groups, which is distant from the entire *C. mongolicum* complex, with a bootstrap support value of 94.9%.

Independent phylogenetic trees were reconstructed based on the concatenated plastid dataset, including the *psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *trnL-F*, and *rbcL* regions, using the BI and ML methods. The tree topologies of the BI and ML trees were identical, and only the BI tree is shown (Fig. 6). A new haplotype (X), which occurred in all the *C. jeminaicum* individuals, was identified in the combined cpDNA dataset. The distribution of the *C. mongolicum* complex within the cpDNA tree could be separated into five to six regions that appear to reflect their geographical distribution. The first branch included sequences from six populations of the *C. mongolicum* complex (3, 4, 5, 9, 10, and 11) that were distributed in the west and

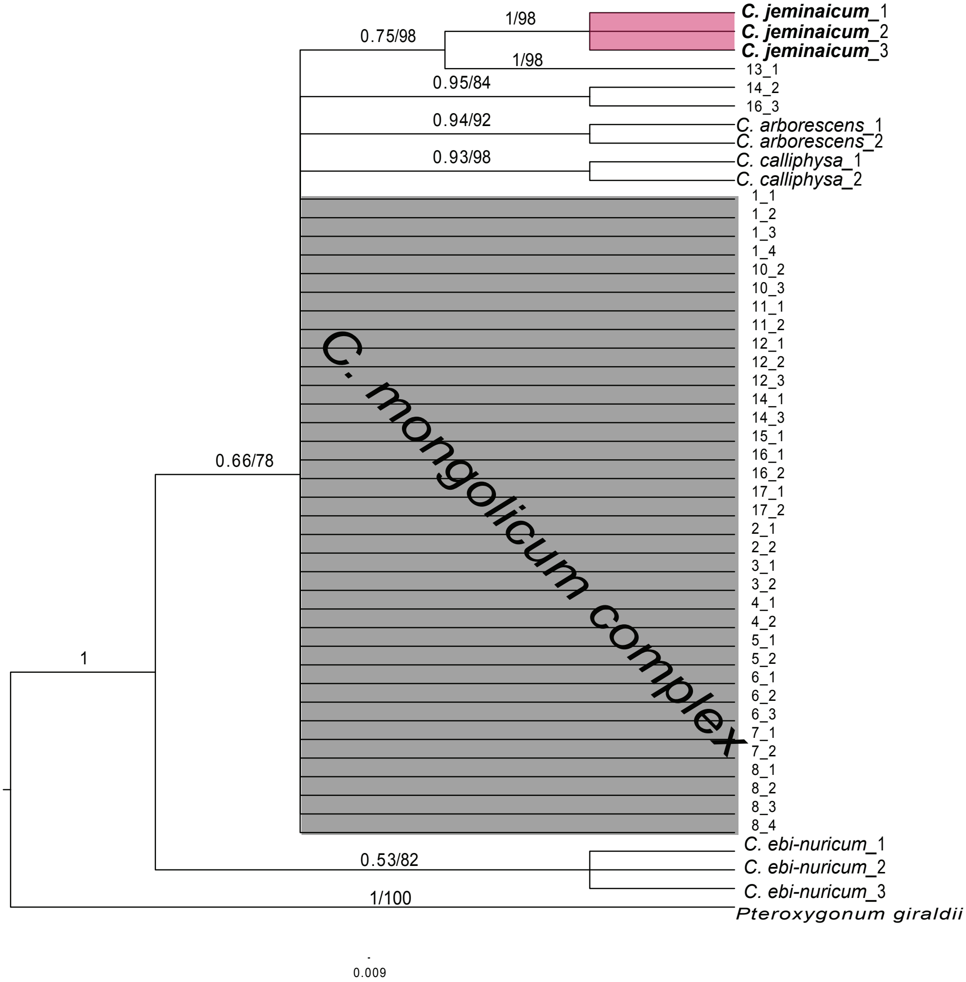

Figure 4. Bayesian inference tree of the concatenated nuclear ITS and ETS sequence data showing *Calligonum jeminaicum* and its congeners. Bayesian posterior probabilities and maximum likelihood bootstrap support values are given above the branches.

northeastern regions of the Tengger Desert, where *C. arborescens* and *C. calliphysa* occurred sympatrically with these six populations. The second independent branch included sequences from four populations (14, 15, 16 and 17) from the Taklimakan Desert. The third independent branch included sequences from three populations (6, 7, and 8) from the Gurbantunggut Desert in the east of Xinjiang. Populations 12 and 13 comprised *C. mongolicum* complex samples from the Qaidam Desert that were distributed sympatrically with *C. ebinuricum*. Population 1 was the most phylogenetically distant from other populations, perhaps owing to its geographic isolation in the extreme north of Inner Mongolia. However, the new haplotype X of *C. jeminaicum* was separated from the above-mentioned branches of the *C. mongolicum* complex with strong support (Fig. 6, PP = 1, BS = 100%). Meanwhile, the other

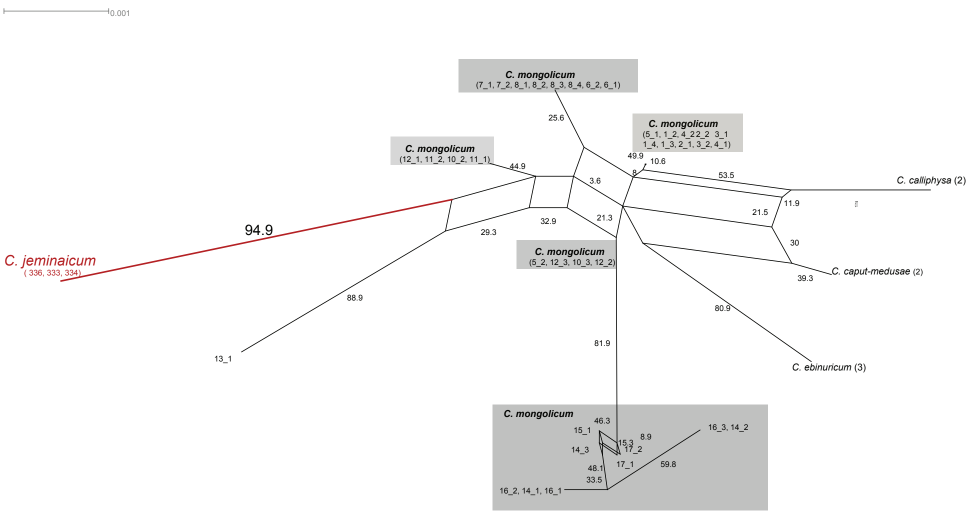

Figure 5. Neighbor-net analyses based on uncorrected p-distances of the nuclear ITS and ETS sequence data. Numbers indicate bootstrap values over 1,000 replicates.

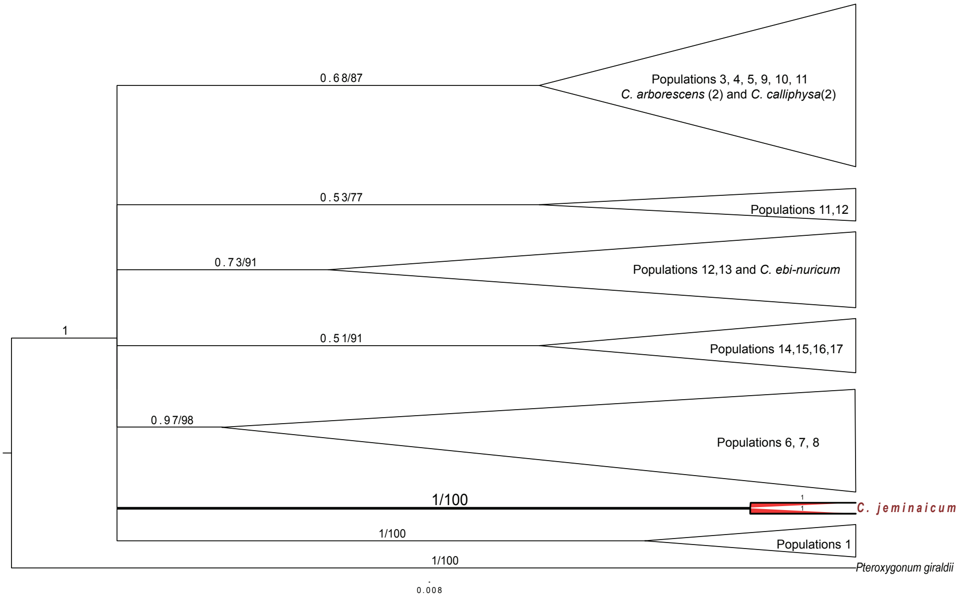

Figure 6. Bayesian inference tree of the concatenated plastid DNA sequence data (*psbA-trnH*, *ycf6-psbM*, *rpl32-trnL*, *rbcL*, and *trnL-F*) showing *Calligonum jeminaicum* and its congeners. Bayesian posterior probabilities and maximum likelihood bootstrap support values are given above the branches.

reference species of *Calligonum* (*C. ebinuricum*, *C. arborescens*, and *C. calliphysa*) did not form their own separate branches, but were interspersed within branches of the *C. mongolicum* complex (Fig. 6).

Discussion

600 species names are known in *Calligonum*, but only 90 of these were recognized (Pavlov 1936; Baitenov and Pavlov 1960; Sergievskaya 1961; Drobov 1953; Kovalevskaya 1971; Liu and Yong 1985). Most of the new names occurring in *Calligonum* were subsequently ignored or merged into existing names (Pavlov 1936; Kovalevskaya 1971; Bao and Grabovskaya-Borodina 2003). Different taxonomists have controversial opinions on species delimitations in *Calligonum* (Soskov 2011; Zhang 2007; Sabirhazi et al. 2010; Abdurahman et al. 2012; Shi et al. 2016, 2017). For example, *C. rubescens* was treated as an independent species (Soskov 2011) by merging three species, *C. pumilum*, *C. alashanicum*, and *C. jeminaicum*. The taxonomical relationships of *C. pumilum*, *C. alashanicum*, and *C. mongolicum* have been clarified, with *C. pumilum* and *C. alashanicum* being merged into *C. mongolicum* (Shi et al. 2009). Additionally, *C. rubescens* was treated as a synonym of *C. mongolicum* (Shi et al. 2016). The relationship between *C. jeminaicum* and *C. mongolicum* was analyzed in the present study.

The morphological identification system, which has been used in the *C. mongolicum* complex (Shi et al. 2009), was employed here for phenotypic discrimination. Our results demonstrated that the fruit characters, which were confusing among members of the *C. mongolicum* complex, in addition to flower characteristics, can be used to distinguish *C. jeminaicum* from the *C. mongolicum* complex by statistical analysis. *C. jeminaicum* could be identified as a good species based on its morphology (Figs 2, 3).

DNA data are used as key evidence for taxonomical conclusions, and can also reveal the systematics among species or genera (Alvarez and Wendel 2003; Feliner and Rossello 2007). Molecular analyses of both nrDNA ITS and cpDNA sequence data (*trn*L-F, *mat*K, *atp*B-*rbc*L, *psb*A-*trn*H, *psb*K-*psb*L, and *rbc*L) fail to fully elucidate the taxonomical relationships within *Calligonum* (Tavakkoli et al. 2010; Sanchez et al. 2011; Sun and Zhang 2012; Li et al. 2014; Gouja et al. 2014), but some minor and reasonable taxonomical discrepancies among the controversial species group were resolved by combining the morphological and DNA data, for example, within the *C. mongolicum* complex (Shi et al. 2009, 2016, 2017) and between *C. trifarium* and *C. ebinuricum* (Abdurahman et al. 2012). The nrDNA tree, which combined nrITS and ETS data, suggested a lack of phylogenetic structure within the *C. mongolicum* complex, but it can be used to distinguish uncontested species in sect. *Medusa*, such as *C. arich*, *C. ebinuricum*, and *C. taklimakanense* (Shi et al. 2016). In the present study, *C. jeminaicum* formed a separate branch based on the nrITS and ETS data (Figs 4, 5), which is not consistent with the past or present occurrence of hybridization or interbreeding of *C. jeminaicum* with the *C. mongolicum*. Meanwhile, the cpDNA data were employed to confirm the taxonomic relationship of the *C. mongolicum* complex with *C. jeminaicum*. A new cpDNA haplotype (X) was identified in *C. jeminaicum*, and its separation from other haplotypes of the *C. mongolicum* complex and other species in sect. *Medusa* was well supported (Fig. 6). A high level of genetic diversity was also found in previous studies based on polymorphic cpDNA markers in the sect. *Medusa*

(Wen et al. 2016b), especially in the *C. mongolicum* complex. The cpDNA information also revealed that the distributional ranges of some species in the *C. mongolicum* complex were geographically close or adjacent to each other (Figs 6). The distribution of genetic variation of the *C. mongolicum* complex in the Gurbantunggut Desert was consistent with its geographical signal, and the network analysis illustrated that genetic relationships in *Calligonum* formed a mesh pattern (Fig. 5). Compared to *C. mongolicum*, *C. jeminaicum* has a very narrow distribution with only one known population in the northwest of the Gurbantunggut Desert, which is also within the main distribution region of *C. mongolicum* (Mao and Pan 1986). It has been proposed that *C. jeminaicum* may contain only a small fraction of the total genetic variation present in its progenitor species in ancient Middle Asia (Sergievskaya 1961; Badria et al. 2007). This may have expanded the range of these xerophytes and allowed them to spread to other suitable habitats in the Jeminay area.

As an accepted name, *C. jeminaicum* has been confirmed as an endemic species which is found only within a relict area in the northwest of the Gurbantunggut Desert. *C. jeminaicum* has been on the brink of extinction over the past 40 years owing to the habitat of the only population being near the roads and the small number of individuals. Although the plants observed appeared to be healthy, the conservation of this plant species with an extremely small population (PSESP) (Wade et al. 2016) should receive appropriate attention in the future. As a result of a new policy framework, several national- and regional-level conservation strategies for China's PSESPs are being implemented (Yang et al. 2015). For many of these species (Ren et al. 2012; Wang et al. 2017), the extinction of a population is irreversible; therefore, recognizing the immediate importance of these risk factors and understanding their interactions are crucial for developing future conservation plans (Volis 2016). The *in situ* conservation of the genetic diversity of *C. jeminaicum* for the long-term survival of this species requires a new management strategy that considers its reproductive biology and the future potential of hybridization/interbreeding. In the *ex situ* conservation of *C. jeminaicum*, special efforts are needed to ensure the isolation of genetic resources.

Since *Calligonum jeminaicum* is accepted as an independent species based on our new evidence; the threatened status of this species can be evaluated according to the International Union for Conservation of Nature (IUCN) Red List categories and criteria (IUCN 2012). This species was first collected by Zumei Mao together with Borong Pan from a single site near Jeminay, Xinjiang, China in the year 1979. It was described as a new species to science in 1984 (Mao 1984). Pan searched for this species in the original site and the surrounding area in 2008 but failed to find it. The first author (Wei Shi) searched for it again in 2013 in the Jeminay area and only a population with 8 mature (fruiting) individuals was found. No seeding or young individual was found in this population. No other collection or report of this species is available. Thus we evaluated *Calligonum jeminaicum* as Critically Endangered (CR) according to criteria D "Population size estimated to number fewer than 50 mature individuals" (IUCN 2012).

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Plastome of *Quercus xanthoclada* and comparison of genomic diversity amongst selected *Quercus* species using genome skimming

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Abstract

The genus *Quercus* L. contains several of the most economically important species for timber production in the Northern Hemisphere. It was one of the first genera described, but genetic diversity at a global scale within and amongst oak species remains unclear, despite numerous regional or species-specific assessments. To evaluate global plastid diversity in oaks, we sequenced the complete chloroplast of *Quercus xanthoclada* and compared its sequence with those available from other main taxonomic groups in *Quercus*. We quantify genomic divergence amongst oaks and performed a sliding window analysis to detect the most variable regions amongst members of the various clades, as well as divergent regions occurring in specific pairs of species. We identified private and shared SNPs amongst oaks species and sections and stress the need for a large global assessment of genetic diversity in this economically and ecologically important genus.

Keywords

Phylogenomics, Quercus, Plastid genome, Genomic Diversity, Diversification, Sections, Taxonomy, Fagaceae, SNPs, Genomic Resources

Introduction

The genus *Quercus* in Fagaceae, a large and locally dominant family of trees in temperate forests of the Northern Hemisphere and in major regions of the tropics and subtropics, is one of the first plant genera described in history (Linnaeus 1753). Fagaceae comprise

8 well-recognised genera, holding ±900–1100 economically and ecologically important species (Manos et al. 2001). In the family, *Quercus* is the largest genus, with ±500–550 accepted species of broad-leaved, deciduous or evergreen trees and shrubs (Denk et al. 2017; Nixon 2006). Although best known from Europe and North America, it extends well into Central and South America, as well as the Asian tropical regions (Indo-China, Malesia) with large numbers of species (Soepadmo and Steenis 1972; Pulido et al. 2006; Phengklai 2008). South America appears to one of the most recent areas to be colonized by the genus, with *Q. humboldtii* occurring as far south as Colombia (Pulido et al. 2006). Until recently, the genus was divided in five subgenera and sections, based on molecular (Oh and Manos 2008), pollen morphology (Denk and Grimm 2009), morphological (Luo and Zhou 2002) and historical treatments (Camus 1936–1954). This was changed with a major update of the infrageneric classification (Denk et al. 2017). The genus is now divided into two subgenera holding a total of 8 sections (subg. Quercus: *Protobalanus*, *Ponticae*, *Virentes*, *Quercus*, *Lobatae*; and subg. Cerris: *Ilex*, *Cyclobalanopsis*, *Cerris*) (Denk et al. 2017). China, where oaks are among the main components of southern broad-leaved evergreen forests, holds the highest number of Asian species (Huang et al. 2009; Strijk 2019). *Quercus* and other Fagaceae are often the dominant element in tropical and subtropical evergreen forests (Huang et al. 1998) and, like Dipterocarpaceae in Asia, represent an exceptional ecological and economical resource (Cvetković et al. 2017; 2019).

Chloroplast DNA loci have been widely used in plant studies, both for evolutionary studies and for identification purposes, due to their natural abundance in plant cells (≈3–5% of the cell DNA content), when compared to nuclear DNA. In angiosperms, the chloroplast genome is a circular molecule (76–217 kilobases), with a conserved structure of two inverted repeats (IR) separated by small (SSC) and large (LSC) single-copy regions (Jansen and Ruhlman 2012). However, until recently, the number of regions in the chloroplast genome, used to address evolutionary, taxonomic and biodiversity questions, remained limited. With the rapid development of Next Generation Sequencing (NGS) approaches, analysing the entire sequence of the chloroplast using a genome skimming approach (Straub et al. 2012) has become common practice and efficient, allowing for high resolution phylogenies (Ripma et al. 2014), resolved problematic taxonomic placements (Bock et al. 2014; Zedane et al. 2016), and estimates of genomic biodiversity (Särkinen et al. 2012; Staats et al. 2013; Bakker et al. 2016). Although geography can be a major factor in shaping plastome diversity at small scales (Pham et al. 2017), dominant patterns of genomic diversity are shaped by evolutionary processes. In oaks, plastome sequences of about 20 species have been studied and are currently available online (sometimes with numerous conspecific accessions, of which many lack essential voucher information), including *Q. rubra* (Alexander and Woeste 2014), *Q. spinosa* (Du et al. 2015), *Q. aquifolioides* and *Q. aliena* (Lu et al. 2016). These sequences can thus be used in reference-guided assemblies (Ripma et al. 2014) and applied to assess the chloroplast diversity in the genus. These species – in addition to the *Q. xanthoclada* chloroplast sequence we generated – belong to the four most diverse lineages (representing more than 97% of species-level diversity). The main aim of our study was to assess levels of genetic diversity contained within the chloroplast, using a subset of oaks from key clades and using a genome skimming approach to reconstruct the complete sequence of the chloroplast for *Q. xanthoclada.* Using *xanthoclada*, we performed phylogenetic and sliding window analyses, single-nucleotide polymorphisms (SNPs) detection and genomic comparisons of diversity.

Materials and methods

Chloroplast reconstruction

Genomic DNA was extracted from 0.1 g of silica gel-dehydrated leaves using a protocol modified from Healey et al. (2014). Modifications were as follows: genomic DNA was extracted in 15 ml tubes, using 6 ml of extraction buffer, incubated at 65 °C for 60 min and two volumes of temperate absolute ethanol were added for precipitation, without incubation. Library construction and sequencing were performed by Novogene (Beijing, China), using NEBNext Ultra II DNA Library Prep Kit (Ipswich, Massachusetts, USA) and an Illumina HiSeq2500 platform (San Diego, California, USA), respectively, following specifications from the manufacturer. One Gb of raw data was generated and was imported in Geneious R11 (Biomatters Ltd, Auckland, New Zealand). Raw reads were trimmed according to their 5' and 3'-end quality and then mapped against the available chloroplast of *Q. rubra* (NC020152). Annotations were made using cpGAVAS (Liu et al. 2012) and boundary validation was performed, using ORF Finder (NCBI), with manual adjustments. The complete plastid genome was submitted to GenBank under accession number KU382355. The GenBank flatfile was used to generate a circular plastid genome map using the OrganellarGenomeDraw (OGDRAW) (Lohse et al. 2007).

Phylogenetic reconstruction

Following the recommended best practices for complete organellar sequencing (Botero-Castro et al. 2016), we performed a phylogenetic analysis to confirm the accuracy of our reconstructed plastid and our sample identification. We retrieved all the complete chloroplasts available in GenBank for the Fagales order (accessed 25 January 2019) and built both maximum likelihood and Bayesian trees using the PHYML (Guindon et al. 2009) implementation in Geneious R11 and BEAST 2.3.1 (Bouckaert et al. 2014), respectively. The ML tree was built using all positions, aligned using MAFFT (Katoh and Standley 2013) with default parameters and bootstrap values were calculated using 1000 replicates. We conducted BEAST analyses using separately aligned coding regions with MAFFT (Katoh and Standley 2013), *--auto* setting and then concatenated sections. Using only genes with annotations in all species, we excluded all those with ambiguous annotations. Analyses were performed using a GTR+I+G substitution model (4 gamma categories), a strict molecular clock model, 200 millions generations (burn-in 10%) and *Trigonobalanus doichangensis* as outgroup. We checked chain convergence and Effective Sample Size (ESS) values > 200 using Tracer 1.6 (Rambaut et al. 2014).

Comparison amongst oak species

We used the retrieved oak chloroplasts to estimate the genetic divergence amongst chloroplasts using MEGA6 (Tamura et al. 2013). We used an in-house script (available upon request) to evaluate the variability of aligned sequences amongst these five species, with a window length of 500 bp and a step size of 250 bp. Pair-to-pair comparisons were visualised using mVISTA (Frazer et al. 2004). We also assessed the number of private and shared SNPs amongst all the combination of species using a specifically developed script (available upon request). We applied Tajima's relative rate test (Tajima 1993) on each species pair, using *Lithocarpus balansae* as outgroup to test the relative rate of evolution of *Q. xanthoclada* relative to other oak species.

Results

Chloroplast reconstruction

62,060 trimmed reads were mapped on the *Q. rubra* chloroplast sequence, for a total linear length of 162,328 bp. The main sequencing depth was 47.7 (min: 2; max: 90; S.D.: 12.5), covering 100% of the reference sequence (161,304 bp). The mean mapping confidence was 35.3, with 94.5% of the bases with mapping quality > Q20, and 89.0% with quality > Q30. The frequencies of each nucleotide were 31.1% (A), 18.7% (C), 18.0% (G) and 32.2% (T), with 439 positions unresolved (N). The properties of the *Quercus xanthoclada* plastid are shown in Table 1 and in Fig. 1. The chloroplast molecule was 160,988 bp long and was 163 bp, 67 bp and 573 bp longer than those of *Q. spinosa*, *Q. aliena* and *Q. aquifolioides*, respectively. In contrast, it was 316 bp shorter than the chloroplast sequence of *Q. rubra*. The overall GC content was 36.7%. The chloroplast structure conforms to the standard found in most angiosperms, with two IRs (25,840 bp) separated by a LSC (90,353 bp) and a SSC (18,955 bp). The plastid genome contains 129 genes, including 90 coding proteins, 31 tRNA and 8 rRNA (Fig. 1). Amongst them, six (*rpl2*, *ycf15* and *ndhB* duplicated in the IRs) and two included one or two introns, respectively.

Table 1. Characteristics of the complete chloroplasts used in this study, showing the length, the GC content of each regions and the number of coding, tRNA and rRNA loci. LSC: Large Single Copy region; SSC: Small Single Copy region; IR: Inverted Repeats. Data from Du et al. (2015) (*Q. spinosa*), Lu et al. (2016) (*Q. aliena*), Yin et al. (2018) (*Q. aquifolioides*, GenBank data), Alexander and Woeste (2014) (*Q. rubra*).

Species	length	LSC	SSC	IR	GC total	GC LSC	GC SSC	GC IR	coding	tRNA	rRNA
Q. xanthoclada	160,988	90,353	18,955	25,840	36.9	34.8	31.1	42.8	90	31	8
Q. spinosa	160,825	90,371	18,732	25,861	36.87	34.7	31.2	42.6	87	29	8
Q. aliena	160,921	90,258	18,980	25,841	36.9	34.8	31.3	42.7	89	39	8
Q. aquifolioides	160,415	89,856	18,935	25,812	37.0	36.6	31.2	42.8	78	29	8
Q. rubra	161,304	90,542	19,025	25,869	36.8	34.6	30.9	42.7	89	41	8

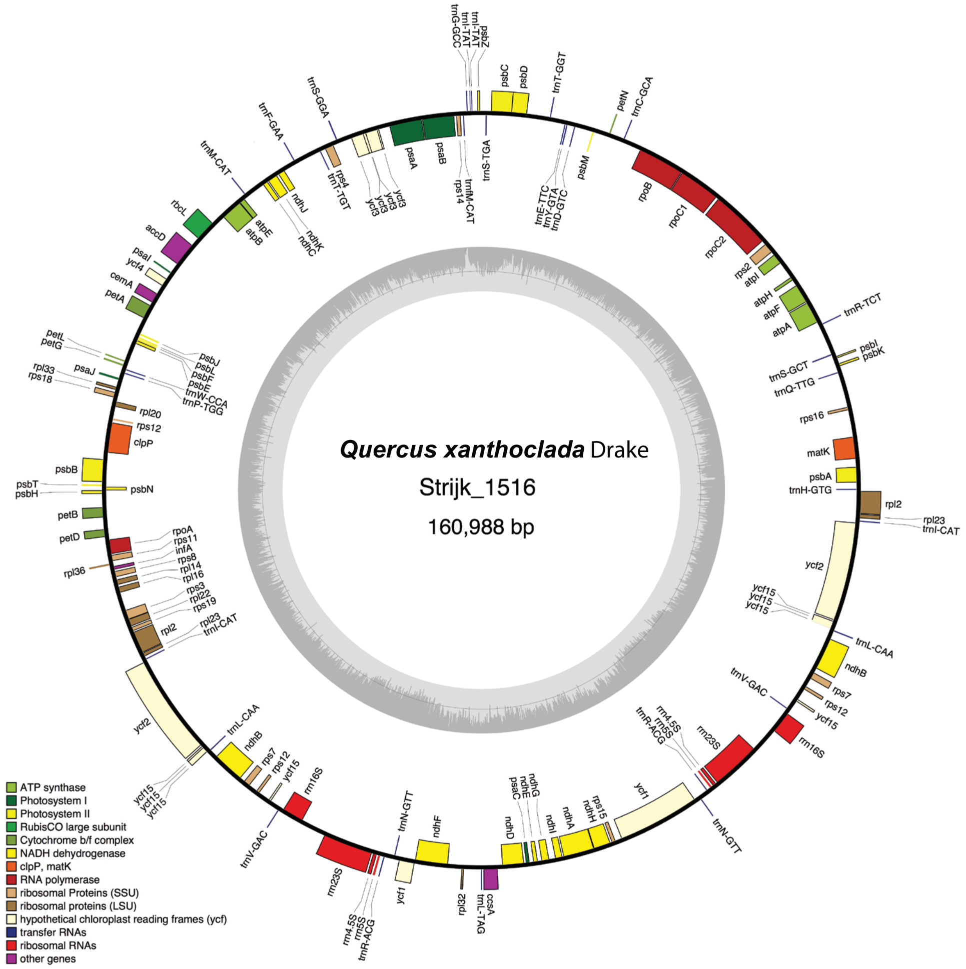

Figure 1. Circular gene map of the plastid genome of *Quercus xanthoclada*. Genes drawn within the circle are transcribed clockwise, while those drawn outside are transcribed counter clockwise. Genes are colour-coded according to their functional groups.

Phylogenetic analysis and comparison amongst oak species and sections

The maximum likelihood (ML) tree of ten *Quercus* chloroplasts available in GenBank shows that *Q. xanthoclada* is closely related to *Q. spinosa* and to the clade formed by *Lithocarpus*, *Castanea* and *Castanopsis* (Fig. 2, left). In the Bayesian analysis, *Quercus* splits into two clades, one grouping *Q. xanthoclada* and *Q. spinosa*, the other containing the remaining species (*Q. aliena*, *Q. rubra*, *Q. aquifolioides*) (Fig. 2, right). The latter is the sister group of the clade comprising *Lithocarpus*, *Castanea* and *Castanopsis* species. Both trees are in general agreement, except for the placement of the clade containing *Q. xanthoclada*, that diverged after the other *Quercus* in the ML tree and before

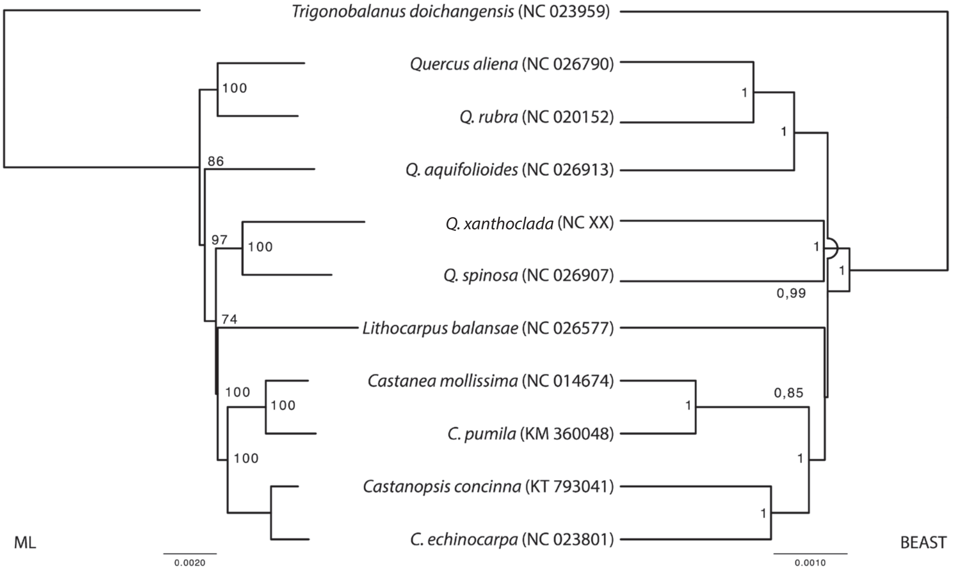

Figure 2. ML phylogenetic tree of the nine selected plastomes in GenBank, plus the plastome of *Quercus xanthoclada*. The tree is rooted with *Trigonobalanus doichangensis*. Bootstraps (1000 replicates) are shown at the nodes, values below 50% not shown. Scale in substitution per site.

this split in the Bayesian tree. Nodal support was high in both trees for all groups, except the *Q. xanthoclada* – *Q. spinosa* clade (ML, 74% bootstrap support) and the *Lithocarpus* divergence (BEAST, 0.85 PP). In both trees, the internal branches are very short, indicating more mutational events occurred in each species than amongst genera or clades (with the notable exception of *Castanea*). Furthermore, considering only the *Quercus* species, *Q. xanthoclada* appears to have evolved significantly faster than the other oak species (Table 2).

All five oak species exhibited high overall similarities (99.4–99.6%) (Table 3), but the 3766 SNPs detected were distributed unevenly across and within the plastid alignment (Fig. 3). Unsurprisingly, most of the variability amongst species was concentrated in the intergenic spacers and the gene introns. Four regions were especially SNPs-rich: the 5'end of the *trnS-GCT-trnR-TCT* spacer (49 SNPs, 750 bp), the *psbM-trnD-GTC* spacer (50 SNPs, 750 bp), the *petA-psbJ* spacer (55 SNPs, 750 bp) and the 3'end of *ndhA* (41 SNPs, 750 bp). Two regions exhibited a dramatic increase in the number of indel positions, namely the *psbZ-rps14* interval (comprising *trnI-TAT*, *trnfM-CAT* and *trnG-GCC*, 348 indel positions per kb) and the 3' end of *ndhA* (188 indel position per kb). However, the high number of indel positions in the first of these intervals actually represents regions where more than 70% of the positions were deleted in at least one species (results not shown). The less variable regions were both the IRs, with almost no SNPs or indels.

However, this overall pattern varied when considering each species pair (Fig. 4). Despite most of the overall divergent regions being equally divergent in all species,

Table 2. Results of the Tajima relative rate test. X^2 test statistic value indicated, p-value in parenthesis. p-values < 0.01 in bold. For significant rate heterogeneity comparisons, the species with slower evolutionary rate is indicated: Qsp: *Q. spinosa*, Qal: *Q. aliena*, Qaq: *Q. aquifolioides*, Qru: *Q. rubra*. For clarity, p-values lower than 0.005 are not indicated.

Species	*Q. xanthoclada*	*Q. spinosa*	*Q. aliena*	*Q. aquifolioides*	*Q. rubra*
Q. xanthoclada	/	17.37 – Qsp	30.35 – Qal	10.37 – Qaq	32.64 – Qru
Q. spinosa		/	4.17 (0.04) – Qal	0.02 (0.89)	3.47 (0.06)
Q. aliena			/	4.99 (0.03)-Qal	0.06 (0.81)
Q. aquifolioides				/	3.30 (0.07)
Q. rubra					/

Table 3. Estimates of p-distance amongst oak species. The number of base differences per site is shown. All positions containing gaps and missing data were eliminated. There were a total of 158480 positions in the final dataset.

Species	*Q. xanthoclada*	*Q. spinosa*	*Q. aliena*	*Q. aquifolioides*	*Q. rubra*
Q. xanthoclada	/	0.005	0.006	0.006	0.006
Q. spinosa		/	0.005	0.005	0.005
Q. aliena			/	0.005	0.004
Q. aquifolioides				/	0.005
Q. rubra					/

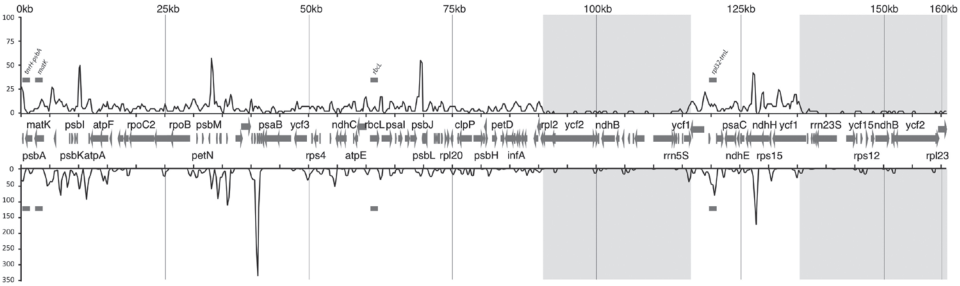

Figure 3. Sliding window analysis of the whole plastomes of five oak species. (window length: 500 bp, step size: 250 bp). X-axis: position of the mid-point of the window, Y-axis: number of SNPs (solid line) and indels (dashed line) positions of each window in bp. Coding regions and directions of transcription are indicated by arrows, inverted repeats by grey areas. Putative barcode loci are highlighted for SNPs and indels. For readability, only a few major genes are indicated.

several variable regions appeared to be more species specific: *atpF*/*atpH*, the 5'end of *trnT-GGT* and the 5'end of the *ndhA* intron in *Q. spinosa*, the 3'end of *trnS-GCT*/*trnR-TCT*, the intron of *rpoC1* and the *trnD-GTC*/*trnY-GTA* spacer in *Q. rubra*. In addition, both the *psbZ*/*trnI-TAT* (comprising *trnG-GCC*) and the *rps15*/*ycf1* spacers were more divergent from *Q. xanthoclada* in *Q. rubra* and *Q. aliena* than in *Q. spinosa* and *Q. aquifolioides*. Interestingly, the divergent portions of the *rpl32*/*trnL* spacer were different for each species. No notable divergent regions were found in the two IRs in all species pairs.

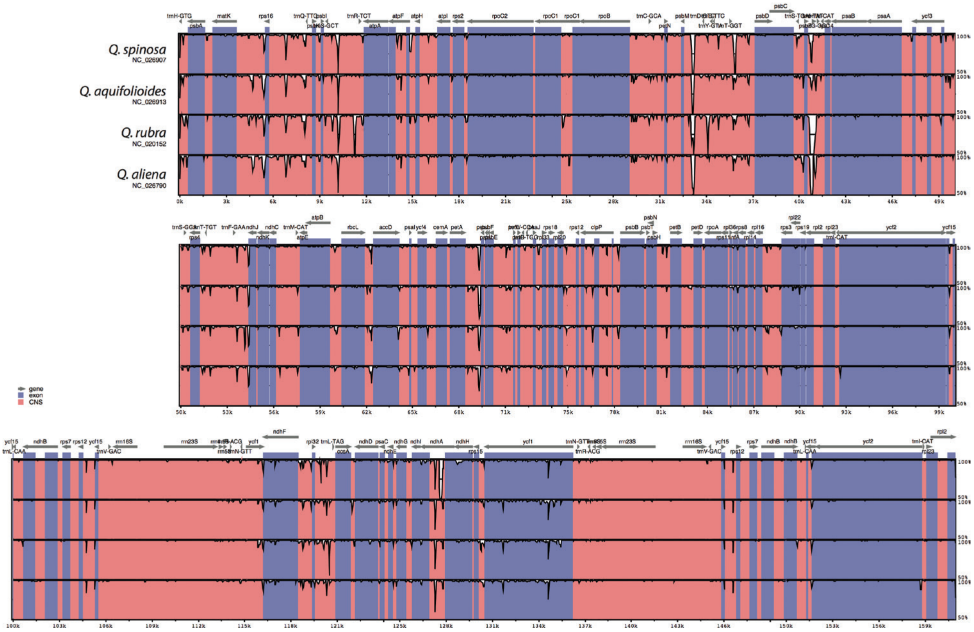

Figure 4. mVISTA percent identity plot comparing the four *Quercus* chloroplast genomes with *Q. xanthoclada* as a reference. Vertical scale indicates the percentage of identity ranging from 50% to 100%. Coding regions are in blue and non-coding regions are in pink.

Discussion

The genome-skimming approach is now widely used to reconstruct chloroplasts in angiosperms (Straub et al. 2012; Bock et al. 2014; Malé et al. 2014; Weitemier et al. 2014; Mandel et al. 2015) and an increasing number of Fagales are available (Alexander and Woeste 2014; El Mujtar et al. 2014; Hinsinger and Strijk 2015). Since the first Fagaceae (*C. concinna*; Hinsinger and Strijk 2015), the genome skimming approach has been proven to be useful in many non-model organisms (Kane et al. 2012; Nikiforova et al. 2013; Curci et al. 2016; Yu et al. 2018), despite a few disadvantages (Straub et al. 2012; Du et al. 2015), as genome skimming is a PCR-free method and a higher amount of starting material is required. Moreover, best results require high quality DNA (i.e high molecular weight DNA, usually > 10 kb, with little degradation), which is incompatible with large-scale (i.e. phylogenetic or population genomic) sampling. Nonetheless, the protocols can easily be adapted (e.g. using specific library construction kits (Zedane et al. 2016) or skipping the shearing step in degraded samples like herbarium or archaeological specimens (Särkinen et al. 2012; Staats et al. 2013). Several examples showcasing the strength of these approaches have been published. For example, Zedane et al. (2016) explored the evolutionary relationships of *Hesperelaea*, an extinct Oleaceae, by reconstructing the plastome of a century-old herbarium specimen and Renner et al. (2019) identified 3,500 years old archaeological remains as watermelon, based on their plastome sequence. With the constant progress in sequencing technology and library construction and the development of genomic resources for oaks (Kremer et al. 2012; Hipp et al. 2014), we can expect that these limitations will soon be overcome.

In addition to the complete chloroplast sequence, the genome skimming can also be use to retrieve nuclear regions found in high copy number in the genome, such as the nuclear ribosomal cistrons (NRC) (Straub et al. 2012; Ripma et al. 2014; Weitemier et al. 2014). To evaluate the relative variability of the complete chloroplast sequence versus the nuclear ribosomal cistrons, we attempted to retrieve the available raw data for the considered oak species from the Short Reads Archive (SRA). Despite the stringent chloroplast purification protocol described in Du et al. (2015), *Q. spinosa* was the only species where we were able to retrieve the NRC, following the same protocol as described above, mapping the trimmed reads against the nuclear internal transcribed spacer of *Q. robur* (FM244246) (results not shown).

In our study, the plastome of *Q. rubra* was the largest in size in oaks (161,304 bp), but in other studies, it was described as the second smallest in oaks (Alexander and Woeste 2014). Given the species diversity in oaks and with an increasing number of plastomes available, this view is likely to change in the near future.

Quercus is widespread throughout the whole of the northern hemisphere, but in our study, three of the four available species came from Asia. To fully capture the chloroplast sequence diversity on a global scale, future inclusion of and genomic comparison with American and European *Quercus* is needed. Although species in this study are members of different recognised sections and subgenera, it is likely that they represent only a fraction of the total diversity within each of these groups and inclusion of additional members will reveal more about the extent and distribution of plastome diversity on various taxonomic and global scales.

Interestingly, one of the regions showing a relatively high level of variation amongst oak species is the *rpl32-trnL* spacer. As the observed variations are located in different portions of the region, it is likely that these SNPs and indels represent section specific diagnostic regions. Indeed, *rpl32-trnL* has been hypothesised as a DNA-barcode locus in several groups (Shaw et al. 2007; Arca et al. 2012). Other proposed DNA-barcode loci showed different levels of variation, *rbcL* being the least variable, with no indels and very few SNPs, as previously demonstrated in several woody plants (Chase et al. 2005; Roy et al. 2010; Arca et al. 2012; Clement and Donoghue 2012). In contrast, *trnH-psbA* has been shown to be highly variable (Piredda et al. 2010; Arca et al. 2012; Clement and Donoghue 2012; Hinsinger et al. 2013), mostly for SNPs, while *rpl32-trnL* was more variable for indels. Briefly, the proposed standard DNA-barcode loci (namely *rbcL* and *matK* (Hollingsworth et al. 2009)) would likely fail in oaks, as demonstrated for certain subclades or specific geographic areas (Piredda et al. 2010; Simeone et al. 2013). However, not only the divergence of putative barcode loci amongst distantly related species has to be assessed, but also between closely related species, as well as amongst different populations. We highlight that *rpl32-trnL*, as well as trnH-*psbA*, should be assessed for DNA-barcoding purpose in oaks, in addition to a thorough re-assessment and estimation of the usefulness of the proposed standard loci *rbcL* and *matK*.

Most of the SNPs were either specific to one species or shared by four species and only a few shared by only two species (Fig. 5). *Q. xanthoclada* exhibited the highest number of species specific SNPs (429), whereas *Q. aliena* and *Q. rubra* segregated the lowest number of SNPs (277). Accordingly, *Q. xanthoclada* was excluded from the four species group that shared the highest number of SNPs (418), followed by *Q. aquifolioides* (348).

Although these results, in combination with those obtained in Tajima's relative rate test, seem to suggest a relative distinction of the *Cyclobalanopsis* species and their previous separation as a separate subgenus in the genus *Quercus*, this is not corroborated by any other data in our study. Neither the complete chloroplast phylogeny, nor previous studies based on nuclear and chloroplast loci (Manos et al. 2001; Oh and Manos 2008), justify this separation of *Q. xanthoclada* (and other taxa under the generic or subgeneric header of "*Cyclobalanopsis*") from other oaks.

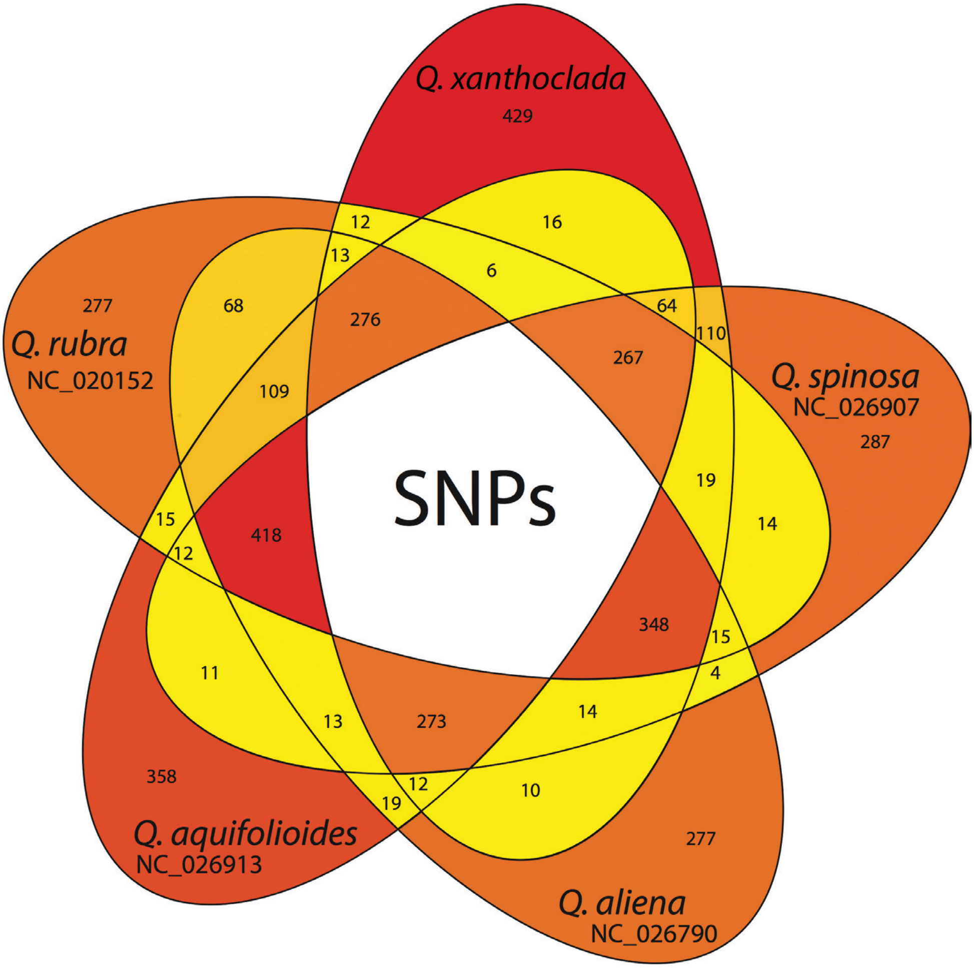

Figure 5. Venn diagram showing the private and shared SNPs amongst the five oak species. Each area is coloured according to the relative number of shared SNPs in this area.

Our work will allow for the development of new loci to be used in comparative phylogeography of the different section and subgenera (i.e. primers that are easily transferable amongst the different sections that can occur in sympatry), as well as open up new perspectives for conservation, management and the use of DNA fingerprinting to aid tracking of wood products from subtropical and tropical Asian oak species.

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RESEARCH ARTICLE

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Sedum ichangensis, a new species of Crassulaceae from Hubei, China

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Abstract

Sedum ichangensis **sp. nov.**, from Yichang, Hubei province, central China, is described and illustrated. The new species is similar to *S. elatinoides* and *S. rosthornianum* in its leaf and carpel morphology and differs in its creeping stems and solitary flowers. The conservation status of *S. ichangensis* was assessed as Endangered according to the IUCN Red List criteria.

Keywords

Crassulaceae, Flora of China, Flora of Hubei, new species, *Sedum* sect. *Filipes*

Introduction

Sedum Linnaeus (1753: 430), the largest genus in the family Crassulaceae with about 430 species, is particularly diverse in East Asia, the Mediterranean and North America (Linnaeus 1753; ’t Hart and Bleij 2003; Thiede and Eggli 2007). *Sedum* can easily be distinguished by its usually alternate leaves, sessile carpels with slightly connate at the base, separate, mostly yellow or white petals and stamens with two whorls (Thiede and Eggli 2007); however, molecular studies have revealed that *Sedum* is a highly polyphyletic group (Nikulin et al. 2016) which may be due to the high morphological plasticity and variability within the genus (Carrillo-Reyes et al. 2009). In China, 121 species were recorded in the "Flora of China" (Fu and Ohba 2001). Since 2001, six new species have been described, namely *S. hoi* X. F. Jin & B. Y. Ding (2005: 381), *S. spiralifolium* D.Q. Wang, D.M. Xie & L Q. Huang (2014: 117), *S. plumbizincicola* X.H. Guo & S.B. Zhou ex L.H. Wu (2013: 492), *S. fanjingshanensis* C. D. Yang & X. Y. Wang (2012: 389), *S. kuntsunianum* X. F. Jin, S. H. Jin & B. Y. Ding (2013: 34) and *S. peltatum* M. L. Chen & X. H. Cao (2017: 847).

According to the recent taxonomic treatment of Fu and Ohba (2001), the species of *Sedum* in China are divided into three sections, viz. *Sedum* sect. *Sedum*, sect. *Oreades* (Fröderström) K.T. Fu and sect. *Filipes* (Fröderström) K.T. Fu. The section *Sedum* is distinct from both sections *Oreades* and *Filipes* in its adaxially gibbous carpels and follicles (vs. carpels and follicles not gibbous); while the sect. *Oreades* differs from the sect. *Filipes* in its spurred (vs. spurless) leaf base and petals that are mainly yellow (vs. mainly white or reddish-purple) (Fu and Ohba 2001).

An unknown *Sedum* species, belonging to the sect. *Filipes,* was discovered in Hubei Province, Central China. The species is described as new to science in this study.

Material and methods

Three scattered populations of an unknown *Sedum* species were discovered in Yichang city of Hubei Province, Central China in 2014. These populations were continuously observed over 2 years. Fresh specimens collected from these populations were morphologically studied and illustrated. The distribution map was constructed with Arcgis 10.2, using data provided on the specimen labels.

Specimens of the morphologically similar species *Sedum elatinoides* Franchet (1883: 11) and *S. rosthornianum* Diels (1900: 361) were collected from Hubei province for comparison. Specimens of *Sedum* sect. *Filipes* deposited at PE, HIB, WH and CCAU were largely checked, based on the relevant literature (Fu and Ohba 2001, Fu 2001). Furthermore, digital images of type specimens archived at the JSTOR Global Plants website (http://plants.jstor.org) were examined.

Taxonomy

Sedum ichangensis Y. B. Wang, sp. nov.

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Figures 1, 2

Diagnosis. *Sedum ichangensis* has papillate carpels and appears to be morphologically similar to *S. elatinoides* and *S. rosthornianum*. It can be distinguished from *S. elatinoides* by its perennial habit (vs. annual) and solitary flower (vs. flowers in cymes) and from *S. rosthornianum* in its entire leaf margins (vs. leaf margins dentate), its branched stems (vs. stems simple) and its solitary flowers (vs. flowers in paniculiform cymes). (Table 1, Fig. 1, 2).

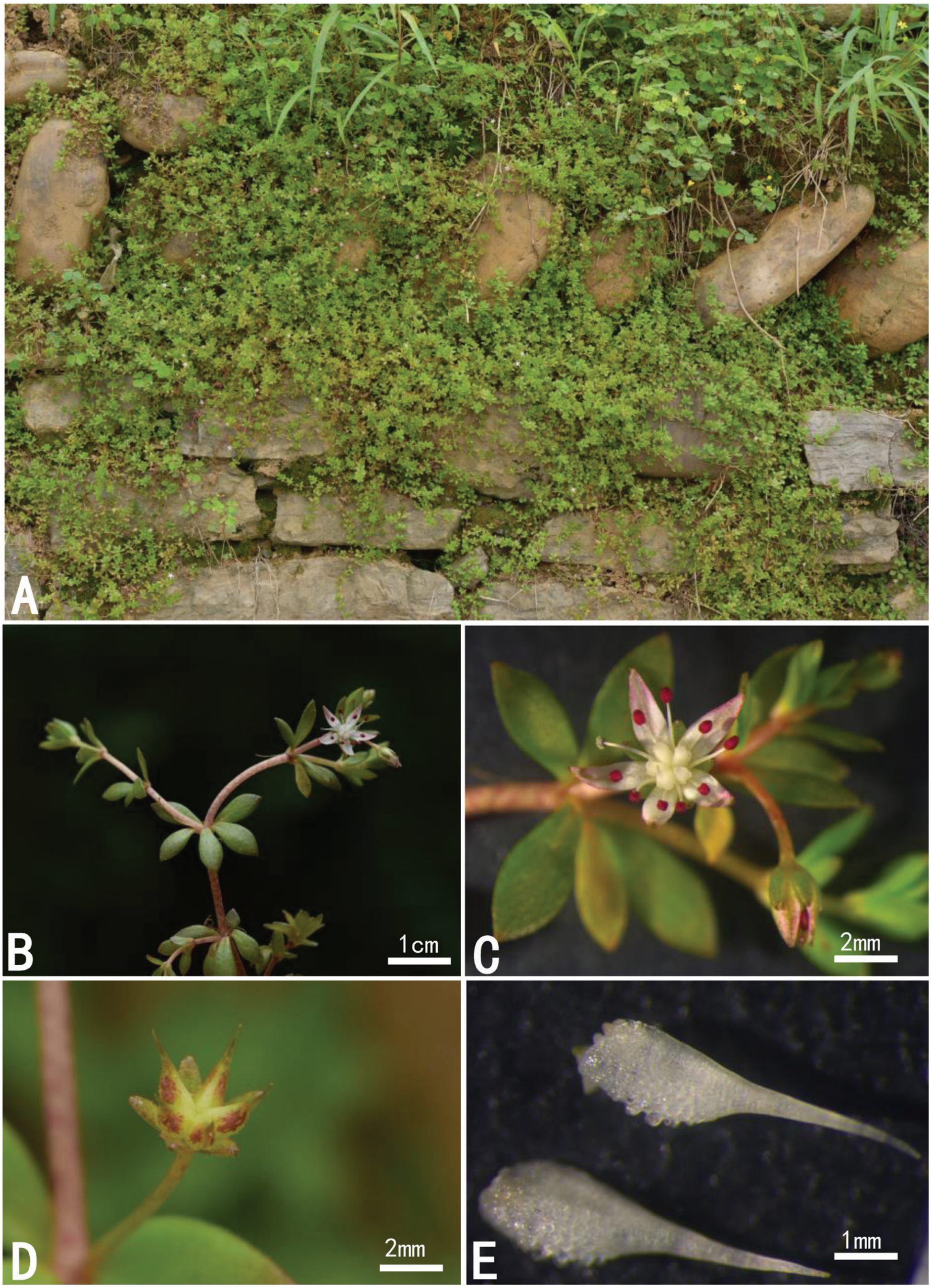

Figure 1. *Sedum ichangensis* Y. B. Wang from type locality **A** habitat **B** flowering stems **C** flower **D** unripe follicles **E** carpels with style.

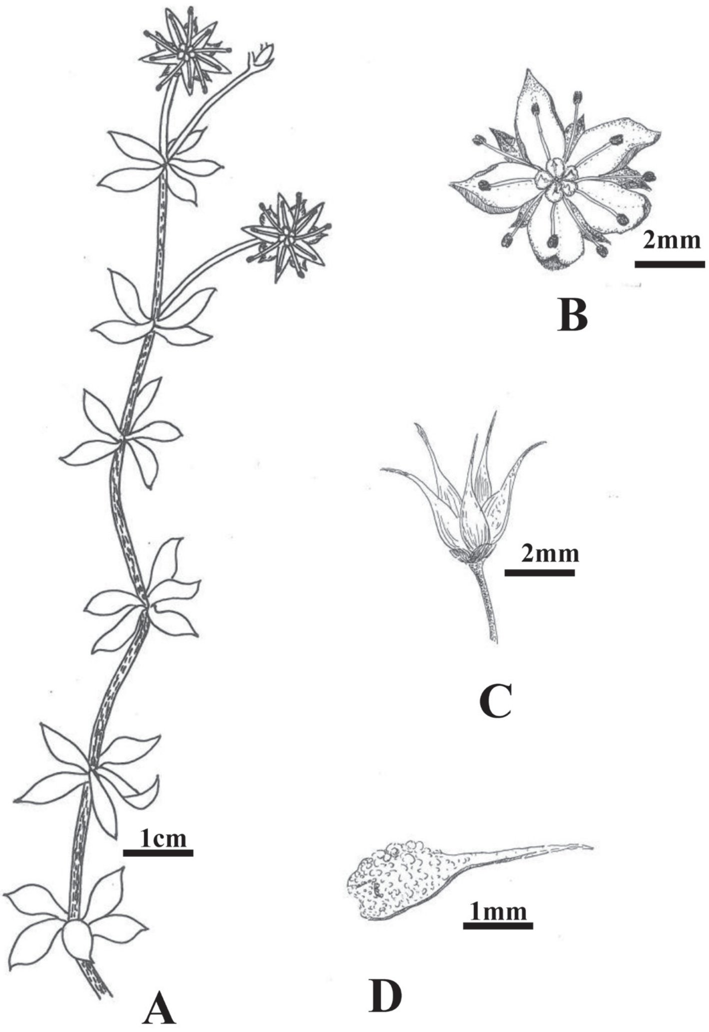

Figure 2. *Sedum ichangensis* Y. B. Wang, sp. nov. **A** habit **B** flower **C** carpels **D** single carpel.

Type. CHINA: Hubei Province, Yichang city, Changyang County, Longzhouping Town, on rocks, alt. 130 m. 30°28'N, 111°11'E, 19 Jul 2017, ycmy032 (holotype, CTGU!; isotypes HIB!, and PE!).

Description. Perennial herbs. Roots fibrous. Stems procumbent, divaricately branched, 1–2 mm in diameter, up to 35 cm long, with scattered reddish dots. Leaves 4–6-verticillate, entire, sessile, narrowly ellipsoid, 5–12 × 1.5–2.5 mm, base attenuate, apex acute. Flowers 5-merous, solitary in the axils of upper leaves, 5–8 mm in diameter. Pedicel 1.5–2.5 cm long. Sepals 5, lanceolate, 1.5–2 mm long, apex acute. Petals 5, white, pinkish towards the apex, lanceolate, 4–5 × 1–2 mm, apex acute. Stamens 10, in 2 whorls, slightly shorter than the petals, antesepalous ones ca. 4 mm long, antepetalous ones ca. 3 mm long, inserted ca. 1 mm above the petal base, filaments white, 1.6–2.4 mm long, anthers ca. 0.4 mm long, reddish. Nectar scales spatulate, ca. 0.4 mm long. Carpels 5, white, suberect, adaxially minutely papillate, broadly ovoid, ca. 2 mm long, base united for ca. 0.2 mm, styles ca. 1.5 mm long. Follicles divergent, 0.8–1.1 mm long, with scattered reddish dots, seeds numerous, brown, ca. 0.5–1 mm long, papillate.

Phenology. Flowering from early May to July, fruiting from August to October.

Distribution and habitat. *Sedum ichangensis* is known from Longzhouping town of Changyang County, Gufu town of Xingshan County and Muyang River of Yiling County in Yichang City of western Hubei Province, central China (Fig. 3). It grows on rocks of roadsides, especially in fissures filled with soil, at an elevation of ca. 100–280 m.

Chinese name. Yi-chang-jing-tian (宜昌景天).

Etymology. The specific epithet of this new species is dedicated to the Yichang city.

Taxonomic notes. *Sedum ichangensis* belongs to *Sedum* sect. *Filipes* on account of its carpels adaxially not gibbous, its spurless leaf base and its white flowers (Fu and Ohba 2001). *Sedum ichangensis* is a species easily identifiable by its floral, stem and leaf features. The new species resembles *S. elatinoides* in the leaf characters, as well as the structure of the flowers. However, *S. ichangensis* differs from *S. elatinoides* in its perennial habit with branched stems and its solitary flowers. *Sedum ichangensis* differs from *S. rosthornianum* in its much branched, decumbent stems, entire leaf margins and its solitary flowers. Here, we provide photographs (Fig. 1), line drawings (Fig. 2) and a detailed morphological comparison (Table 1), as well as a key to the species of *Sedum* sect. *Filipes* in China to facilitate its identification.

Additional specimens examined (paratypes). CHINA. Hubei Province: Xingshan County, Gufu town, 200 m alt., 31°20'N, 110°45'E, 15 May 2017, YB Wang ycmy022 (CTGU), same loc. XJ Xiong XXJ024 (CTGU); Yiling County, Muyang River, 280 m alt., 30°44'N, 111°02'E, 3 August 2017, YB Wang ycmy139 (CTGU).

Conservation status. Based on field investigations, *S. ichangensis* occurs only in three scattered areas. The total area of occupancy is less than 500 km^2; each population possesses no more than 300 mature individuals. It prefers habitats on rocks along roads. Human activities are impairing its populations severely. The type population, which grew close to a road, was seriously impacted in its survival due to herbicide spraying in 2018. Based on currently available information, the conservation status of this species is categorised as Endangered [EN] following the IUCN Categories and Criteria (IUCN 2017).

Table 1. Morphological comparison between *Sedum ichangensis* and related species.

Item	*Sedum ichangensis*	*Sedum elatinoides*	*Sedum rosthornianum*
Habit	perennial	annual	perennial
Stem	prostrate	erect	erect
Phyllotaxis	4–6-verticillate	3–6-verticillate	opposite or 3- or 4-verticillate
Leaf blade	narrowly ellipsoid, entire	narrowly oblanceolate, entire	rhombic-oblong, dentate
Inflorescence	solitary flower	paniculiform or corymbiform cyme	paniculiform cyme
Petal	white, pinkish toward the apex	white	white

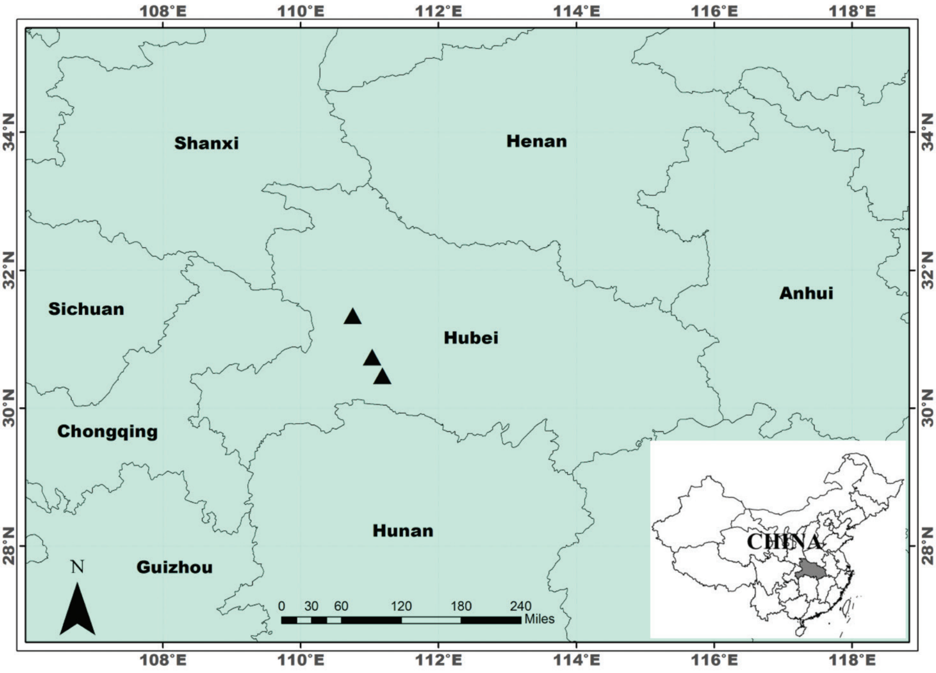

Figure 3. Distribution of *Sedum ichangensis* in Hubei province, central China. The three known localities are indicated with triangles.

Key to the species of *Sedum* sect. *Filipes* in China (adapted from Fu and Ohba 2001):

1 Plants perennial, fasciculate; stamens in 1 series .... ***S. correptum***
– Plants annual or biennial, rarely perennial, solitary or tufted; stamens in 2 series ... **2**
2 Plants glandular hairy **3**
– Plants glabrous. **4**
3 Plants annual; stems soft; leaves 2–4 × 1.4–2.5 cm .... ***S. drymarioides***
– Plants biennial; stems ± woody at base; leaves 0.7–1.5 × 0.7–0.9 cm .... ***S. stellariifolium***
4 Carpels minutely papillate **5**
– Carpels smooth 7
5 Stems simple, erect; leaf margin dentate .... ***S. rosthornianum***
– Stems many branched, decumbent; leaf margin entire **6**
6 Plants annual; stems erect, flowers in cymes .... ***S. elatinoides***
– Plants perennial, stems creeping, flowers solitary .... ***S. ichangensis***
7 Carpels 3 .... ***S. bonnieri***
– Carpels 5 .... **8**
8 Petals reddish-purple; flowering stems branched, ca. 20 cm .... ***S. filipes***
– Petals white; flowering stems simple, ca. 10 cm .... ***S. majus***

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Novelties in the genus *Viridantha* Espejo (Tillandsioideae, Bromeliaceae)

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Abstract

Based on morphological evidence, we propose to raise *Tillandsia mauryana* forma *secundifolia* to species level with the name *Viridantha secundifolia* (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr. *Viridantha secundifolia* can be readily distinguished by the falciform rosettes, the broadly oblong to square, 1–1.2 × 0.8–1.1 cm leaf sheaths and by the 1.8–2 × 0.7–1.2 cm floral bracts. Additionally, we describe and illustrate *Viridantha uniflora* Hern.-Cárdenas, Espejo & López-Ferr., from the state of Oaxaca, Mexico. The new species is morphologically similar to *Viridantha boqueronensis*, but differs by the nearly square leaf sheaths, 1.3–1.5 × 0.4–0.5 cm spikes and by the presence of only one flower per spike. A key to the taxa, morphological descriptions, list of specimens examined, illustrations and a distribution map of the described taxa are included.

Resumen

Con base en evidencia morfológica, proponemos elevar a nivel de especie a *Tillandsia mauryana* forma *secundifolia* con el nombre *Viridantha secundifolia* (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr. *Viridantha secundifolia* puede distinguirse fácilmente por las rosetas falciformes, las vainas foliares ampliamente oblongas a cuadradas, de 1–1.2 × 0.8–1.1 cm y por las brácteas florales de 1.8–2 × 0.7–1.2 cm. Además, se describe e ilustra *Viridantha uniflora* Hern.-Cárdenas, Espejo & López-Ferr., del estado de Oaxaca, México. La nueva especie es morfológicamente similar a *Viridantha boqueronensis* pero difiere por las vainas foliares cuadradas, la espigas de 1.3–1.5 × 0.4–0.5 cm y por presentar una sola flor por espiga. Se proporciona una clave de identificación, descripciones morfológicas, lista de ejemplares examinados, ilustraciones y un mapa de distribución de los taxa descritos.

Keywords
Hidalgo, México, Oaxaca, *Tillandsia*, *Viridantha*

Introduction

The genus *Viridantha* Espejo (Tillandsioideae, Bromeliaceae) is endemic to Mexico and includes 14 species (Hernández-Cárdenas et al. 2018). Due to its morphological characteristics, Smith and Downs (1977) classified some species, now placed in *Viridantha*, in *Tillandsia* subgenus *Allardtia* [*V. atroviridipetala* (Matuda) Espejo, *V. ignesiae* (Mez) Espejo, *V. mauryana* (L.B. Sm.) Espejo and *V. plumosa* (Baker) Espejo] and others in *Tillandsia* subgenus *Tillandsia* [*V. ehrenbergii* (= *V. tortilis* (Klotzsch ex Baker) Espejo) and *V. lepidosepala* (L.B. Sm.) Espejo]. Gardner (1986) included *Viridantha* in *Tillandsia* subgenus *Tillandsia* and Till (2000) included it in *Tillandsia* subgenus *Allardtia*, but as a separate group from the rest of the species in that subgenus. Espejo-Serna (2002), based on morphological characteristics, considered that this group of species constituted a distinct genus from *Tillandsia* L. and called it *Viridantha*.

Viridantha species are herbaceous plants with leaves arranged in acaulescent rosettes; protandrous flowers with petals dark green towards the apex and white towards the base; stamens equal in length, included, with filiform filaments and sub-basifixed anthers and simple-erect type style branches (Espejo-Serna 2002). The last taxonomic revision for Tillandsioideae subfamily, based on multi-loci DNA sequences phylogeny, proposed to circumscribe *Viridantha* and the *Tillandsia tectorum* E. Morren complex as *Tillandsia* subgenus *Viridantha* (Espejo) W. Till & Barfuss (Barfuss et al. 2016). However, some of the systematic changes proposed by Barfuss et al. (2016) were not supported by molecular and morphological data (Gomes-da-Silva and Souza-Chies 2017).

It should be mentioned that, in all the phylogenies reconstructed so far, *Tillandsia* emerged as polyphyletic (Gardner 1986; Terry et al. 1997; Horres et al. 2000; Barfuss et al. 2004, 2005; Barfuss 2012; Barfuss et al. 2016; Gomes-da-Silva and Souza-Chies 2017) or paraphyletic (Terry and Brown 1996; Benzing et al. 2000; Givnish et al. 2007; Donadío et al. 2015) and, consequently, its validity as a formal taxonomic entity (genus) is unacceptable. On the other hand, *Viridantha* has always been monophyletic and related to the *Tillandsia tectorum* complex (Barfuss et al. 2004, 2005, 2016; Barfuss 2012). The members of *Viridantha* have morphological, ecological and geographical coherence, all the species are easily recognised by their vegetative and reproductive characteristics and can be distinguished from the rest of the species of *Tillandsia s. l.* Furthermore, *Viridantha* species are mostly saxicolous and all are endemic to Mexico. Moreover, the species of the *Tillandsia tectorum* complex are clearly distinct from *Viridantha* by the presence of caulescent rosettes and petals purple towards the apex and white towards the base, besides the taxa of *T. tectorum* clade being endemic to northern Peru and southern Ecuador (Hromadnik 2005). By the above mentioned reasons, we maintain *Viridantha* as a genus. It becomes necessary to carry out more studies using a larger number of species and/or characteristics to propose a more precise and objective classification of *Tillandsia s.l.* and their relatives.

As a result of botanical explorations for the project Phylogeny of the genus *Viridantha* Espejo (Tillandsioideae; Bromeliaceae), we collected specimens of two different populations of *Viridantha*: the first one in the vicinity of Tolantongo, in the municipality of Metztitlán, state of Hidalgo, corresponds to *Tillandsia mauryana* L. B. Sm. forma *secundifolia* Ehlers. This form can be readily distinguished from the typical form by the falciform rosettes, the broadly oblong to square, 1–1.2 × 0.8–1.1 cm leaf sheaths, by the 1.8–2 × 0.7–1.2 cm floral bracts and other morphological characters, so we propose to raise it to species level. The second one comes from the municipality of Santos Reyes Tepejillo, in the state of Oaxaca. Initially, we thought that these specimens could correspond to *Viridantha boqueronensis* (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr.; however, after a careful and detailed revision of living and herbarium specimens, including types, we conclude that these populations correspond to an undescribed taxon.

Materials and methods

Plants were collected in Hidalgo and Oaxaca, Mexico. The material was dried and measurements and descriptions were prepared from herbarium specimens. The vouchers were deposited in UAMIZ. The morphological terms used in the descriptions were based on Radford et al. (1974) and Scharf and Gouda (2008). We revised herbarium material deposited at CHAPA, FCME, GH, IBUG, IEB, MEXU, UAMIZ and WU and all specimens are cited in the text or in Appendix 1. Comparison of the new species with *Viridantha boqueronensis*, *V. mauryana* (L.B. Sm.) Espejo and *V. penascoensis* (Ehlers & Lautner) Espejo & López-Ferr. and other morphologically related taxa was based on the protologues, living specimens collected at the type localities, as well on herbarium specimens (Appendix 1). The herbarium acronyms followed Thiers (cont. updated).

Results

Viridantha secundifolia (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr., comb. et stat. nov.

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Basionym. *Tillandsia mauryana* L.B. Sm. forma *secundifolia* Ehlers, Die Bromelie. Sonderheft 6: 56–60. Figs pp. 56, 57, 60. 2009.

Type. MEXICO. Hidalgo: Metztitlán, 1300 m a.s.l., 12 February 1992, *J. Lautner L92/3* (holotype: MEXU not found); Hidalgo, prope Tolantongo "Tolontogo", 1900 m a.s.l., 22 February 2006, *R. Ehlers & M. Kretz EM061802* (paratype: WU not found). Lectotype (here designated): figure page 56, Die Bromelie. Sonderheft 6: 56-60. 2009.

Description. Plants saxicolous, flowering 10–13 cm tall, 12–14 cm diameter; rosettes acaulescent, solitary or caespitose, falcate in outline. Leaves numerous, longer than the inflorescence; sheaths pale brown on both surfaces, broadly oblong to nearly square, 1–1.2 cm long, 0.8–1.1 cm wide, glabrous towards the base on both surfaces; blades falcate, densely white-greyish lepidote, narrowly triangular, 4.5–7 cm long, 0.5–0.8 cm wide, apical portion long attenuate. Inflorescence short pedunculated, falcate, one-branched, with 3–5 spikes; peduncle 1–1.5 cm long, 3–5 mm diameter, covered by the peduncle bracts; peduncle bracts similar to the leaves but reducing in size towards the apical portion, densely white-greyish lepidote; spikes reddish-rose, erect and appressed, flattened, elliptic, 2.5–3.5 cm long, 1–1.5 cm wide; flowers distichous, erect and appressed, 3–5 by spike; floral bracts reddish-rose at the apex, yellowish-green towards the base, ovate, 1.8–2 cm long, 0.7–1.2 cm wide, apex acute to acuminate, ecarinate to slightly carinate at the apex, lepidote abaxially; sepals pale green, lanceolate, 1.2–1.5 cm long, 0.3–0.4 cm wide, apex acute, the two adaxial ones carinate, lepidote abaxially; petals dark green, narrowly oblong, 2–2.5 cm long, 0.25–0.3 cm wide, apex rounded to obtuse; filaments white, 1.4–1.6 cm long; anthers pale to dark green, 2.5–3 mm long; ovary broadly ovoid, 3.5–4.5 mm long, 2.5–3.5 mm diameter; style white, 6–10 mm long, included; style branches green. Capsules 1.5–1.8 cm long, 5–8 mm diameter; seeds fusiform, 3–4 mm long, coma 0.8–1.2 cm long.

Habitat and ecology. *Viridantha secundifolia* is only known from the state of Hidalgo in the western and eastern regions of Metztitlán and Tolantongo municipalities, respectively, where it grows on vertical walls in xerophilous scrubs at elevations between 1100 and 1900 m a.s.l. (Figs 1, 2).

Phenology. Blooming in January and February.

Observations. *Tillandsia mauryana* was described by Lyman B. Smith (1937), based on specimens from the canyon of Metztitlán, in the state of Hidalgo, Mexico. Espejo-Serna (2002) transferred the species to *Viridantha*. Ehlers (2009) described *T. mauryana* L. B. Sm. forma *secundifolia*, differentiating it from the typical form only by the secund disposition of its leaves. Besides, Ehlers (2009) mentioned that its populations grow separated from those of *T. mauryana*. The detailed analysis of the morphological characteristics of living and dried specimens of *T. mauryana* forma *mauryana* and *T. mauryana* forma *secundifolia* allowed us to detect that, in addition to the characteristic mentioned by Ehlers (2009), there are other differences in the plants of both populations such as: the shape of the rosettes (falcate vs. spherical); the length (1–1.2 cm vs. 1.5–2.5 cm) and the shape (broadly oblong to square vs. broadly elliptic) of the leaf sheaths; the length of the floral bracts (1.8–2 cm vs. 1–1.5 cm), the presence or not of a keel on the floral bracts (absent or visible only in the apex vs. present along the bract); and the colour of the anthers (pale to dark green vs. black).

Viridantha grandispica (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr., *V. rzedowskiana* Hern.-Cárdenas, Espejo & López-Ferr. and *V. teloloapanensis* (Ehlers & Lautner) Hern.-Cárdenas, Espejo & López-Ferr., are other species morphologically similar to *V. secundifolia* (Table 1). *Viridantha secundifolia* differs from *V. grandispica* in the shape of the rosettes (falcate vs. spherical); in the shape of the leaf sheaths (broadly oblong to square vs. narrowly oblong); in the presence or not of a keel on the floral bract (absent or visible only in the apex vs. present along the bract); and in the shape of the sepals (lanceolate vs. ovate). *Viridantha secundifolia* differs from *V. rzedowskiana* in the shape of the rosettes (falcate vs. spherical); in the size of the leaf sheaths (1–1.2 cm

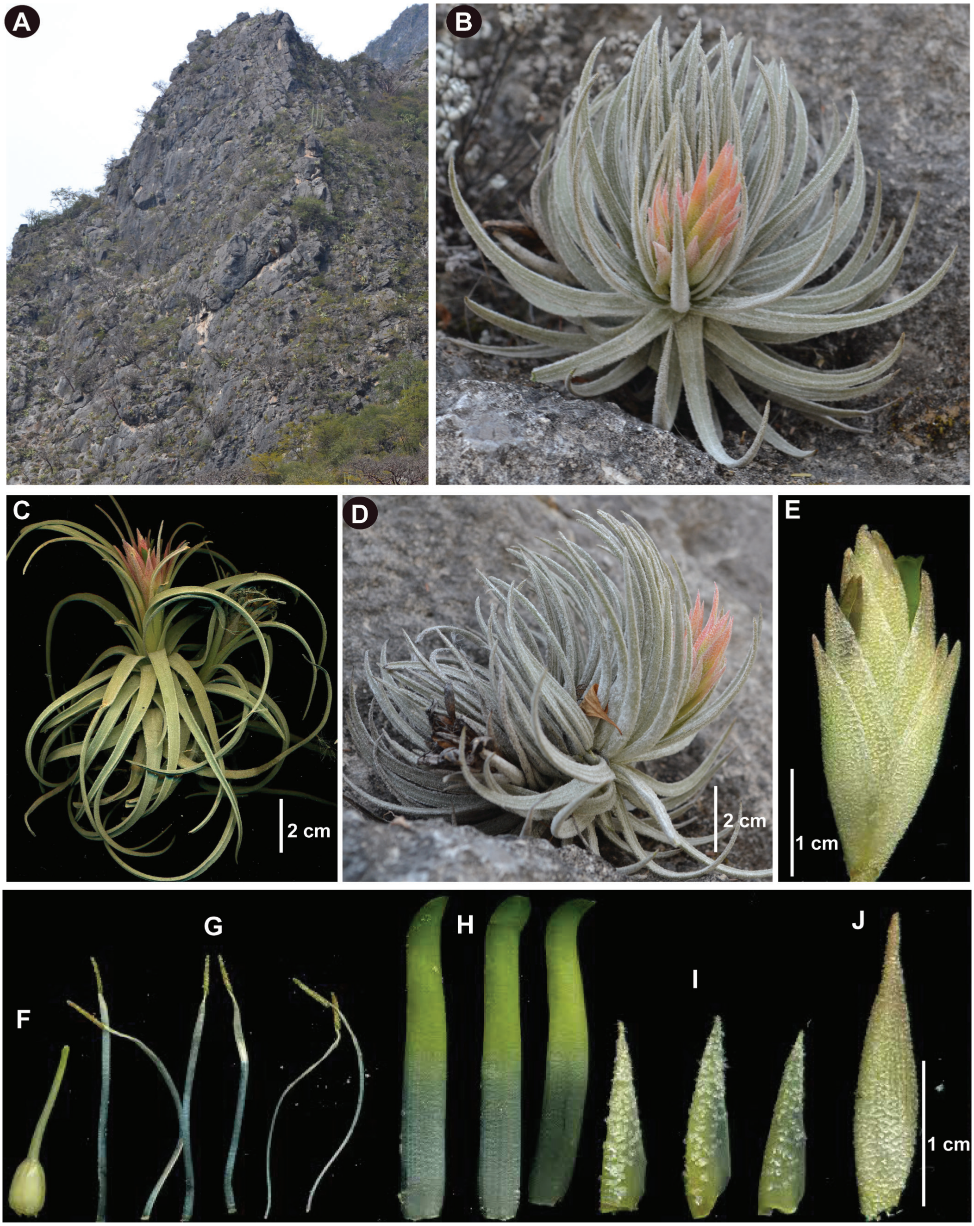

Figure 1. Morphological comparison between *Viridantha mauryana* and *V. secundifolia* (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr. *V. secundifolia* **A** habit **B, D** plant with inflorescence **E** spike **F** pistil **G** stamens **H** petals **I** sepals **J** floral bract (voucher: *Hernández-Cárdenas and Sarabia 2136*, UAMIZ). *V. mauryana* **C** plant with inflorescence (voucher: *Hernández-Cárdenas et al. 2090*, UAMIZ). Photographs by R. Hernández-Cárdenas.

× 0.8–1.1 cm vs. 1.8–2 cm × 1.5–1.7 cm); in the keel of the floral bract (absent or visible only in the apex vs. present along the bract); and in the shape of the sepals (lanceolate vs. ovate). *Viridantha secundifolia* differs from *V. teloloapanensis* in the shape of

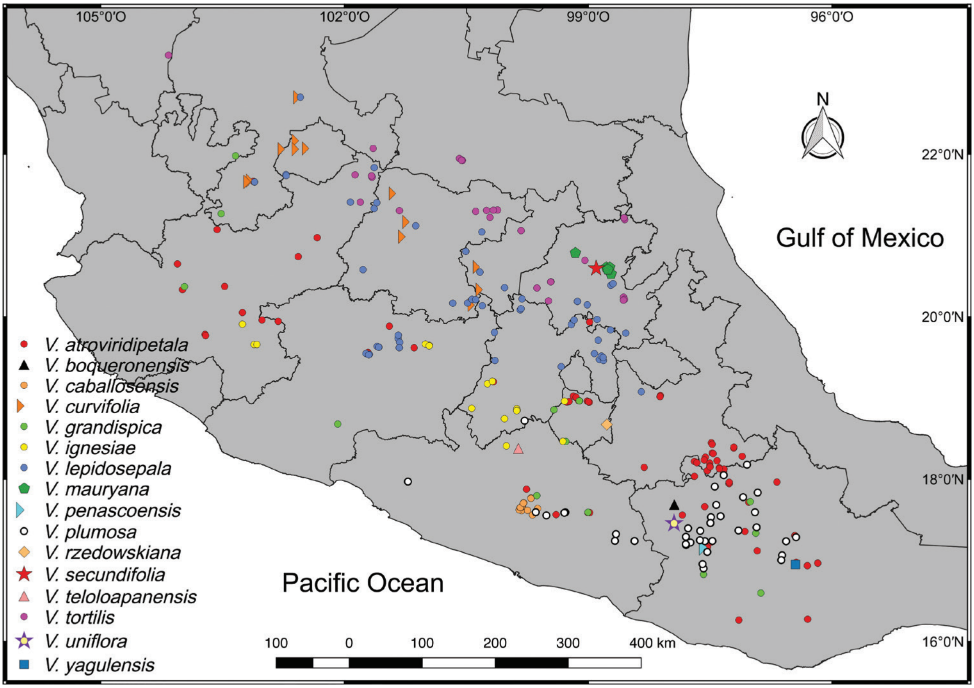

Figure 2. Geographical distribution of the genus *Viridantha*.

Table 1. Morphological differences amongst *Viridantha grandispica*, *V. mauryana*, *V. rzedowskiana*, *V. secundifolia* and *V. teloloapanensis*.

Characters	*V. grandispica*	*V. mauryana*	*V. rzedowskiana*	*V. secundifolia*	*V. teloloapanensis*
Rosettes shape in outline	Spherical	Spherical	Spherical	Falcate	Spherical
Leaf sheaths size (cm)	1–2 × 0.5–0.7	1.5–2.5 × 1–1.5	1.8–2 × 1.5–1.7	1–1.2 × 0.8–1.1	1–1.5 × 0.8–1.1
Leaf sheaths shape	Narrowly oblong	Broadly elliptic	Broadly oblong to square	Broadly oblong to square	Ovate
Leaf blades width (mm)	3–4	7–11	4.5–6	5–8	5
Floral bracts size (cm)	1.7–2.5 × 0.6–1	1–1.5 × 1–1.5	1.5–2 × 1–1.2	1.8–2 × 0.7–1.2	1–1.5 × 0.6
Floral bracts keel	Present	Present	Present	Absent or visible only in the apex	Present
Sepals width (mm)	4–6	3–5	4–6	2.5–3.5	3
Sepals shape	Ovate	Ovate	Ovate	Lanceolate	Narrowly elliptic
Anthers colour	Green	Black	Green	Pale to dark green	Green to black

the rosettes (falcate vs. spherical); in the shape of the leaf sheaths (broadly oblong to square vs. ovate); in the number of the spikes (3–5 vs. 1); and in the shape of the sepals (lanceolate vs. ovate). So we concluded that *T. mauryana* forma *secundifolia* presents different and consistent morphological characteristics to those observed on the typical form and can be considered as a distinct species.

Specimen examined. MEXICO, Hidalgo: municipio de Metztitlán. 28 km sobre el camino que va de Metztitlán a Tolantongo (20°35'43"N, 98°54'09.9"W), 1103 m a.s.l., 3 February 2018, *R. Hernández-Cárdenas y A. Sarabia 2136* (UAMIZ).

***Viridantha uniflora* Hern.-Cárdenas, Espejo & López-Ferr., sp. nov.**

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Figs 2, 3

Diagnosis. *Viridantha uniflora* is similar to *V. boqueronensis* but differs in the shape (square vs. ovate to triangular) and the width of the leaf sheath (0.7–0.8 cm vs. 1 cm); the width (0.4–0.5 cm vs. 0.7–1.3 cm) of the spikes, the number of flowers per spike (always 1 vs. 2–5); and in the shape of the floral bract (ovate vs. elliptic).

Type. MEXICO. Oaxaca: Distrito de Juxtlahuaca, municipio de Santos Reyes Tepejillo, en los alrededores del boquerón de Santos Reyes Tepejillo (17°26'58"N, 97°56'29"W), 1960 m a.s.l., 21 April 2018, *R. Hernández-Cárdenas*, *E. Negri & J. Conde 2156* (holotype: UAMIZ!; isotype: MEXU!).

Description. Plants saxicolous, flowering 7–10 cm tall, 7–9 cm diameter; rosettes acaulescent, solitary or caespitose, falcate in outline. Leaves numerous, shorter or equalling the inflorescence; sheaths pale brown on both surfaces, nearly square, 0.8–1 cm long, 0.7–1 cm wide, glabrous towards the base on both surfaces; blades falcate, densely greyish lepidote, narrowly triangular, 3–6 cm long, 0.3–0.4 cm wide, apical portion long attenuate. Inflorescence pedunculated, falcate, one-branched, with 3–5 spikes; peduncle 2.5–3.5 cm long, 0.2–0.3 cm diameter, covered by the bracts of the peduncle; peduncle bracts similar to the leaves but reducing in size towards the apical portion, densely greyish lepidote; spikes green, erect and appressed, flattened, elliptic, 1.3–1.5 cm long, 0.4–0.5 cm wide; flowers erect and appressed, only one per spike; floral bracts green to green-brownish, ovate, 1–1.5 cm long, 0.5–0.6 cm wide, apex acute to acuminate, ecarinate to slightly carinate at the apex, glabrous adaxially, lepidote abaxially; sepals green, lanceolate, 1–1.3 cm long, 0.3–0.4 cm wide, apex acute, the two adaxial ones carinate, both surfaces glabrous or lepidote abaxially mainly on the keel; petals dark green, narrowly oblong, 1.5–1.8 cm long, 0.2–0.3 cm wide, apex rounded to obtuse; filaments white, 0.8–1.2 cm long, included; anthers pale green, 1.2–1.5 mm long; ovary green, ellipsoid, 2.5–3 mm long, 2–3 mm diameter; style white, 8–10 mm long; style branches green. Capsules not seen.

Habitat and ecology. *Viridantha uniflora* is only known from the boquerón of the Santos Reyes Tepejillo municipality, located in the Sierra Madre del Sur in the northwest region of the state of Oaxaca, where it grows on vertical walls in dry oak forests and tropical deciduous forests. The plants of *V. uniflora* grow in colonies, between 1700 and 1900 m a.s.l., on the cliffs of the boquerón amongst other saxicolous herbs. (Figs 2, 3).

Phenology. The plants of *Viridantha uniflora* bloom in April and May.

Etymology. The specific epithet refers to the presence of one flower per spike, condition only known in the proposed taxon.

Observations. Plants of *Viridantha uniflora* had previously been collected by *J.I. Calzada 20057* (MEXU), but had been wrongly identified as *V. atroviridipetala* (Matuda) Espejo. However, *V. uniflora* differs from *V. atroviridipetala* in the outline shape of the rosettes (falcate vs. spherical); in the shape of the leaf sheaths (square vs.

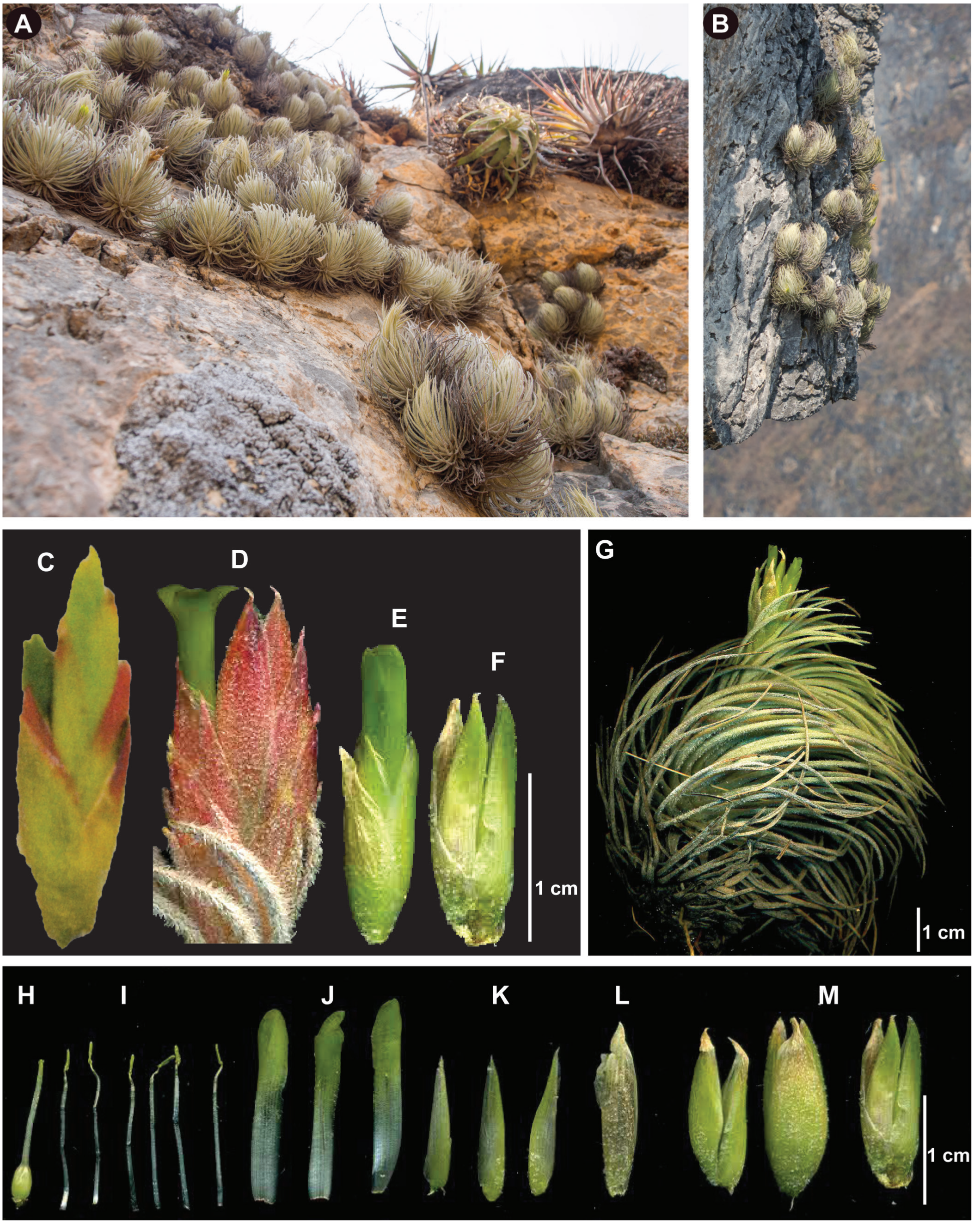

Figure 3. Morphological comparison between *Viridantha boqueronensis*, *V. penascoensis* and *V. uniflora* Hern.-Cárdenas, Espejo & López-Ferr. *V. uniflora* **A–B** habit **E–F, M** spikes **G** plant with inflorescence **H** pistil **I** stamens **J** petals **K** sepals **L** floral bract (voucher: *Hernández-Cárdenas et al. 2156*, UAMIZ). *V. boqueronensis* **C** spike (voucher: *K. and R. Ehlers EM7851*, MEXU). *V. penascoensis* **D** spike (voucher: *Hernández-Cárdenas and Sarabia 2116*, UAMIZ). Photographs A–B by E. Negri Lavín; C–M by R. Hernández-Cárdenas.

oblong to ovate); in the shape of the floral bracts (ovate vs. lanceolate to narrowly triangular) and in the number of flowers per spike (1 vs. 2–5). *Viridantha boqueronensis* and *V. penascoensis* grow in nearby locations to the type locality of *V. uniflora*, but

Table 2. Morphological differences amongst *Viridantha boqueronensis*, *V. penascoensis* and *V. uniflora*.

Characters	*V. boqueronensis*	*V. penascoensis*	*V. uniflora*
Leaf sheaths size (cm)	0.9–1.4 × 0.8–1	0.6–1 × 0.7–0.8	0.8–1 × 0.7–0.8
Leaf sheaths shape	Ovate to triangular	Broadly ovate to square	Square
Leaf blades size (cm)	4–7 × 0.3–0.4	2.5–4 × 0.2–0.3	3–6 × 0.3–0.4
Spikes colour	Green with red	Red to pink	Green
Spikes number	5–7	1	3–5
Flowers number per spike	2–5	2–3	1
Spike size (cm)	1.5–3.5 × 0.7–1.3	2–3 × 0.8–1	1.3–1.5 × 0.4–0.5
Floral bracts size (cm)	1.2–1.7 × 0.5–0.8	1.5–2 × 0.5–1	1–1.5 × 0.5–0.6
Floral bracts shape	Elliptic	Ovate	Ovate
Floral bracts keel	Present	Absent or visible only in the apex	Absent or visible only in the apex
Sepals size (cm)	1–1.3 × 0.3–0.35	1.3–1.5 × 0.3–0.5	1–1.3 × 0.3–0.4
Sepals shape	Narrowly elliptic	Ovate	Lanceolate

without overlapping its distributions. These species share the saxicolous habit and the falcate rosettes in outline. However all these species are easily distinguishable from the newly proposed taxon (Table 2). *Viridantha uniflora* differs from *V. penascoensis* in the inflorescence (branched vs. simple); in the length and in the colour (green vs. red-pink) of the spikes (1.3–1.5 cm vs. 2–3 cm). *Viridantha uniflora* differs from *V. boqueronensis* in the shape of the leaf sheaths (square vs. ovate to triangular); in the number of flowers per spike (1 vs. 2–5); in the colour of the spikes (green vs. green with red-pink); in the shape of the floral bracts (ovate vs. elliptic); and in the presence or not of a keel on the floral bracts (absent or visible only in the apex vs. present along the bract.

Additional specimens examined (paratypes). MEXICO, Oaxaca: Distrito Santiago Juxtlahuaca, municipio de Santos Reyes Tepejillo. 3 km al N de Santos Reyes Tepejillo rumbo a Corral de Piedra (17°27'N, 97°57'W), 1770 m a.s.l., 20 July 1995, *J. I. Calzada 20057* (MEXU); en los alrededores del boquerón de Santos Reyes Tepejillo (17°26'58"N, 97°56'29"W), 1960 m a.s.l., 18 March 2017, *R. Hernández-Cárdenas, F. Gómez y A. González 2120* (UAMIZ).

To facilitate the identification of the species of *Viridantha*, we include an artificial key for all representatives of the genus.

Key to the species of Viridantha

1 Rosettes irregular or falcate in outline **2**
– Rosettes spherical in outline **8**
2 Inflorescence branched **3**
– Inflorescence simple **5**
3 Flower one per spike, spikes 1.3–1.5 cm long .... ***V. uniflora***
– Flowers two or more per spike, spikes longer than 1.6 cm **4**
4 Leaf blades 3–4 mm wide; floral bract elliptic .... ***V. boqueronensis***
– Leaf blades 5–8 mm wide; floral bract ovate .... ***V. secundifolia***

5 Rosettes falcate, blades falcate **6**
– Rosettes irregular, blades squarrose **7**
6 Plants longer than 5.1 cm; spikes terete .... ***V. curvifolia***
– Plants shorter than 5 cm; spikes elliptic, flattened .... ***V. penascoensis***
7 Leaf sheaths broadly ovate to oblong; peduncle (in anthesis) longer than 4.1 cm and lesser than 2 mm diameter .... ***V. tortilis***
– Leaf sheaths broadly oblong to square; peduncle (in anthesis) shorter than 4 cm and larger than 4 mm diameter .... ***V. lepidosepala***
8 Inflorescence conspicuously pedunculate, peduncle longer than 4.1 cm **9**
– Inflorescence sessile or peduncle shorter than 3.5 cm **11**
9 Inflorescence simple, longer than 3.1 cm, flattened to terete, rarely with two small lateral spikes .... ***V. ignesiae***
– Inflorescence branched, shorter than 3 cm long, flattened **10**
10 Leaf blades wider than 2.1 mm; floral bracts triangular to ovate, carinate... ***V. plumosa***
– Leaf blades narrower than 2 mm; floral bracts elliptic to oblong, ecarinate to carinate only at the apex .... ***V. caballosensis***
11 Leaf blades wider than 7.1 mm; anthers black .... ***V. mauryana***
– Leaf blades narrower than 7 mm; anthers green **12**
12 Plants short caulescent; floral bracts elliptic .... ***V. yagulensis***
– Plants acaulescent; floral bracts variable in shape but never elliptic **13**
13 Spikes longer than 3.1 cm and wider than 1.1 cm **14**
– Spikes shorter than 3 cm and narrower than 1 cm **15**
14 Leaf sheaths broadly ovate to square, blades wider than 4.1 mm; sepals shorter than 1.5 cm .... ***V. rzedowskiana***
– Leaf sheaths narrowly oblong, blades narrower than 4 mm; sepals longer than 1.6 cm .... ***V. grandispica***
15 Spikes 3 or less; floral bracts shorter than 1.5 cm; sepals lanceolate .... ***V. teloloapanensis***
– Spikes 4 or more; floral bracts longer than 1.6 cm; sepals narrowly elliptic .... ***V. atroviridipetala***

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Appendix I

Examined specimens.

Viridantha boqueronensis (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr. Oaxaca: *Calzada 18325* (MEXU); *K. and R. Ehlers EM7851* (MEXU); *Lautner 92/57* (WU).

Viridantha grandispica (Ehlers) Hern.-Cárdenas, Espejo & López-Ferr. Guerrero: *Ehlers EM040901* (WU); *Ehlers EM991902* (WU); *R. and K. Ehlers EM911305* (WU); *Franco 8* (FCME); *Limón 6* (FCME). Jalisco: *Flores et al. 1810* (CHAPA, IBUG, IEB); *Flores 2310* (CHAPA); *Guerrero et al. 115* (IBUG). Michoacán: *Ehlers EM902503* (WU); *Steinmann 5156* (IEB). Morelos: *Ceja et al. 1049* (UAMIZ); *Flores-Palacios* and *Vergara 1048* (UAMIZ); *Hernández-Cárdenas and Moreno 2075* (UAMIZ); *Hernández-Cárdenas and Sarabia 2093* (UAMIZ); *López-Ferrari et al. 2865* (IEB, UAMIZ). Oaxaca: *Ceja et al. 1762* (IEB, UAMIZ); *Espejo et al. 6492* (UAMIZ); *Ehlers EM030203* (WU); *Ehlers EM991204* (WU); *López-Ferrari et al. 3373* (UAMIZ); *Mendoza 1399* (UAMIZ); *Téllez et al. 16039* (FCME). Zacatecas: *Espejo et al. 7065bis* (UAMIZ); *Ehlers EM001405* (WU); *Ramírez-Díaz et al. 184* (IBUG).

Viridantha mauryana (L.B. Sm.) Espejo. Hidalgo: *Ceja et al. 1967* (UAMIZ); *Ceja et al. 1768* (UAMIZ); *Gómez 533* (IEB); *Gold 2* (MEXU); *Hernández-Cárdenas et al. 2090* (UAMIZ); *López-Ferrari et al. 2133* (UAMIZ); *Maury 5747* (GH).

Viridantha penascoensis (Ehlers & Lautner) Espejo & López-Ferr. Oaxaca: *Hernández-Cárdenas* and *A. Sarabia 2116*, (UAMIZ); *Ehlers EM030202* (MEXU).

Viridantha rzedowskiana Hern.-Cárdenas, Espejo & López-Ferr. Morelos: *Hernández-Cárdenas et al. 2108* (UAMIZ).

Viridantha teloloapanensis (Ehlers & Lautner) Hern.-Cárdenas, Espejo & López-Ferr. Guerrero: *Lautner et al. EM060902* (MEXU, WU); *Schatzl 80/7* (WU).

Viridantha uniflora Hern.-Cárdenas, Espejo & López-Ferr. Oaxaca: *Calzada 20057* (MEXU); *Hernández-Cárdenas et al. 2156* (UAMIZ); *Hernández-Cárdenas et al. 2120* (UAMIZ).

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New combinations and synonyms in discoid caespitose Andean *Senecio* (Senecioneae, Compositae)

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Abstract

The names *Werneria melanandra* and *W. pygmophylla* are transferred to the genus *Senecio*. They belong to the group of the discoid caespitose Andean *Senecio*, specifically to the subgroup with blackish anthers and style branches and whitish corollas. The recognition of *S. digitatus* as a distinct species is also discussed. Within the framework of the mentioned group, the names *S. casapaltensis* and *S. macrorrhizus* are lectotypified, *S. humillimus* var. *melanolepis* is neotypified, an epitype is designated for the name *W. melanandra*, and nine new synonyms are proposed. An updated comprehensive dichotomous key including all discoid caespitose *Senecio* species from Bolivia and Peru is provided.

Keywords

Asteraceae, Bolivia, Chile, dichotomous key, Peru, taxonomy, *Werneria*

Introduction

The discoid caespitose Andean *Senecio* L. species have traditionally been placed within *S.* subser. *Caespitosi* (O. Hoffm.) Cabrera & S.E. Freire (Freire et al. 2014). This infrageneric group was conceived for embracing the strictly caespitose species but also suffrutescent plants. As circumscribed by Cabrera et al. (1999), it includes ca. 50 species from southern South America. The infrageneric classification of *Senecio* at the subserial rank has been proposed for the Argentinian species, which are reasonably well-known (Freire et al. 2014). In Bolivia and Peru, by contrast, the understanding of the genus is poorer; the infrageneric classification remains barely resolved and several species remain misplaced. This is the case for two species that were hitherto recognized as members of *Werneria* Kunth, a genus morphologically similar to *Senecio* that can be differentiated by the combination of the following characters: involucral bracts fused at the base, absence of genuine supplementary bracts (calyculus), achene trichomes not myxogenic, rosettiform habit rather than caespitose. However, some *Senecio* species sometimes also have the involucral bracts partially fused at the base or do not have supplementary bracts. Such overlapping means that historically some species have been interchangeably treated as *Senecio* or *Werneria,* depending on the authors' concepts. This is the case with *S. wernerioides* Wedd., a species that Grisebach (1874) and Kuntze (1898) transferred to *Werneria* but that is presently widely accepted as a heterotypic synonym of *S. breviscapus* DC. (Cabrera 1985; Beck and Ibáñez 2014; Freire et al. 2014). Similarly, *S. repens* var. *macbridei* (Cuatrec.) Cabrera was initially described at the specific rank as *W. macbridei* Cuatrec. In Chile, Ricardi and Marticorena (1964) described *S. pfisteri* Ricardi & Martic., a species that has been recently synonymized with *Xenophyllum esquilachense* (Cuatrec.) V.A. Funk [≡*Werneria esquilachensis* Cuatrec.] (Calvo et al. 2018). Such disparate treatments highlight the taxonomic complexity of these groups and indicate that some species are difficult to assign to one or another genus. In these cases, a detailed study based on the aforementioned set of characters is needed. In addition, the achene indumentum type appears to be a useful character for a proper identification. In arid regions *Senecio* species with myxogenic trichomes (with mucilaginous properties when soaked in water) are common (Nordenstam et al. 2009; Mukherjee and Nordenstam 2012). This character is also found in other genera within the tribe, e.g., *Dauresia* B. Nord. & Pelser, *Dolichoglottis* B. Nord., *Euryops* (Cass.) Cass. (Nordenstam et al. 2009), but it has not been reported in *Werneria*. Indeed, most species belonging to this genus have glabrous achenes or rarely scattered long trichomes near the base. Only *W. nubigena* Kunth usually displays achenes with dense, villous indumentum. It is composed of twin filiform trichomes, ca. 0.7 mm long, with acute to subacute, asymmetrical, usually forked apex, but does not exude mucilage when treated in water. On this basis, the myxogenic trichomes appear to be absent in *Werneria*, and therefore, it is another useful character to discriminate between the two genera.

Herein, we transfer *W. melanandra* Wedd. and *W. pygmophylla* S.F. Blake to the genus *Senecio*. Furthermore, and in disagreement with previous treatments (Rockhausen 1939; Cabrera 1949; Freire et al. 2014), we believe that *W. pygmophylla* and *S. digitatus* Phil. correspond to two different taxonomic entities and we justify this here accordingly. These species belong to a group of discoid caespitose Andean *Senecio* with blackish anthers and style branches and whitish corollas but differ from one another in some characters (see discussions below). Detailed illustrations and pictures are provided for each species, as well as a dichotomous key including the discoid caespitose *Senecio* species from Bolivia and Peru.

Materials and methods

This contribution is the result of an intensive review of the published bibliography and the revision of herbarium specimens kept at BOLV, CONC, HSP, LPB, MA, MOL, SGO, US, and USM. Additionally, digital herbarium specimens from LP and P were studied; herbarium acronyms follow Thiers (2018). A light microscope was used for examination of microcharacters. Field work was conducted in Bolivia, southern Peru, and northern Chile.

Results

New combinations

1. *Senecio melanandrus* (Wedd.) J.Calvo, A.Granda & V.A.Funk, comb. nov.
urn:lsid:ipni.org:names:60479386-2
Figs 1, 2, 3A, B, 4A, B

Werneria melanandra Wedd., Chlor. Andina 1: 88. 1856. Type: Bolivia. La Paz: ravin de Chuquiaguillo, 1851, H.A. Weddell s.n. (lectotype, designated by Rockhausen (1939) as "type", pg. 284: P [P04319315]). Epitype, designated here: Bolivia. La Paz: am Chacaltaya (30 km von La Paz), 4800 m, Feb 1908, O. Buchtien 1589: US [US00622639]; isoepitype: US [US00622640].

Senecio humillimus var. *melanolepis* Wedd., Chlor. Andina 1: 104. 1856. Type: Bolivia. La Paz: Larecaja, viciniis Sorata, ad lacum Yuriguana, prope Anilaya, Ancumpampa, prope Ancohuma, 3800–5000 m, Apr 1860, G. Mandon 108 (neotype, designated here: GH [GH00012144]; isoneotypes: P [P03730752, P04370980], S [S-R-10871]), syn. nov.

Senecio vegetus var. *lobatus* Cabrera, Notas Mus. La Plata, Bot. 18(89): 222. 1955. Type: Bolivia. La Paz: Ingavi, Miriquiri, 4200 m, 10 Mar 1921, E. Asplund 2866 (holotype: S [not located, Arne Anderberg in litt.]), syn. nov.

Senecio pucapampaensis H. Beltrán, Arnaldoa 15: 212. 2009. Type: Peru. Huancavelica: Pucapampa, debajo de Chonta, 4500–4600 m, 9 May 1958, O. Tovar 2959 (holotype: USM-00277274), syn. nov.

Senecio sykorae Montesinos, PhytoKeys 39: 6. 2014. Type: Peru. Moquegua: General Sánchez Cerro, Yunga, E of Yunga, on the peaks of Perusa mountain, 16°11'08"S, 70°38'14"W, 4802 m, 13 Apr 2012, D. Montesinos & F. Calisaya 3805 (holotype: USM s.n.; isotype: HUSA n.v.), syn. nov.

Senecio tassaensis Montesinos, PhytoKeys 39: 11. 2014. Type: Peru. Moquegua: General Sánchez Cerro, Ubinas, cumbre nevada del cerro Pirhuani Querala, 4650 m, 16°09'S, 70°43'W, 7 Apr 2011, D. Montesinos 3103 (holotype: HUSA n.v.; isotypes: MOL n.v. [not located, likely never sent], USM-247549), syn. nov.

Senecio canoi P. Gonzáles, Montesinos & Ed. Navarro, Anales Jard. Bot. Madrid 72(2): 1. 2015. Type: Peru. Puno: Carabaya, Corani, Minaspata, arriba de Chacaconiza, 14°01'57"S, 70°41'54"W, 4999 m, 14 Apr 2014, P. Gonzáles 2989 (holotype: USM n.v.), syn. nov.

Senecio vegetus sensu Cabrera (1955, 1985), non Weddell (1856).

Description. Caespitose perennial herb. Leaves 4–15 mm long, 1.2–2.6 mm wide, linear-oblong to spatulate, apex acute to obtuse, base narrowed, margins entire, crenate or dentate, conduplicate downwards (rarely flat), glabrous, with marginal trichomes on the narrowed base or densely pilose, somewhat fleshy, greenish or glaucous. Capitulum discoid, solitary, terminal, sessile or subsessile; involucre 5–8 mm long, 3.7–9 mm wide. Involucral bracts 11–16, oblong-lanceolate, 3.8–4.9 mm long, 0.9–1.8 mm wide, partially fused at the base, smooth, glabrous or with trichomes on the abaxial surface ca. 0.7 mm long, dark purple- or blackish-tipped. Supplementary bracts (calyculus) 2–4(–6), linear to slightly spatulate, 4.2–7.5 mm long, 0.5–1 mm wide, smooth, three-quarters to as long as the involucral bracts, with trichomes (rarely glabrous), dark purple- or blackish-tipped. Disc florets 20–45, 4.3–6.3 mm long, 0.8–1.1 mm wide, 5-lobed, conspicuously differentiated in a distinct tube and campanulate limb, whitish. Anther bases auriculate, clearly acute, dark purple to blackish; filament collar balusterform. Style branches truncate with a crown of sweeping hairs, dark purple to blackish. Achenes 2.1–2.2 mm long, ca. 0.5 mm wide, brownish, covered by dense indumentum of obtuse whitish myxogenic twin trichomes ca. 0.2 mm long; pappus 5–6 mm long, barbellate, whitish. Chromosome number: unknown.

Additional iconography. Beltrán (2008: pg. 216, fig. 2, sub *S. pucapampaensis*); Montesinos-Tubée (2014: pg. 7, fig. 2; pg. 13, fig. 4B, sub *S. sykorae*); Montesinos-Tubée (2014: pg. 12, fig. 3; pg. 13, fig. 4C, sub *S. tassaensis*); Montesinos-Tubée et al. (2015: pg. 2, fig. 1; pg. 3, fig. 2, sub *S. canoi*).

Distribution and habitat. Bolivia (Cochabamba, La Paz, Oruro, Potosí) and Peru (Apurímac, Arequipa, Ayacucho, Cusco, Huancavelica, Moquegua, Puno) (Fig. 5). It grows in exposed places mainly in the subhumid and dry puna ecoregions, at elevations of 3800–5100 m.

Phenology. Flowering mainly from January to June, although some flowering specimens have been collected in November.

Etymology. The epithet *melanandrus* means having dark or black stamens, which describes a striking character of this species.

Discussion. This species is transferred to *Senecio* on the basis that it has genuine supplementary bracts (calyculus), the involucral bracts are not clearly fused at the base, it displays a caespitose habit with short stems, and it has myxogenic achene trichomes. Furthermore, its morphologically most similar species are currently treated as *Senecio* members: i.e., *S. digitatus*, *S. madidiensis* J. Calvo & A. Fuentes, *S. pygmophyllus* (see new combination below), and *S. scorzonerifolius* Meyen & Walp. All the names included in the synonymy were also described within the genus *Senecio*.

Senecio melanandrus is a highly variable species that has been variously interpreted. The poor condition of the type material probably helped to maintain the uncertainty

Figure 1. *Senecio melanandrus*. Habit (drawn from *Buchtien 1589*). Illustration by Alice Tangerini.

surrounding the application of this name. Weddell (1856) described the leaves as "integerrimis vel nonnullis dente triangulari, […] glabriusculis vel inconspicue ciliolatis" [entire or with a few triangular teeth, rather glabrous or inconspicuously ciliate]. Several years later Rockhausen (1939), who published the first comprehensive taxonomic revision of the genus *Werneria*, stated that the leaves have "marginibus obsolete glanduloso-ciliolata" [margins scarcely glandular-ciliate]. On the basis of our studies, this species displays an unusually wide variability in leaf margin and indumentum of leaves and involucre, which is reflected in the number of names included in the synonymy. The leaf margin may be entire, crenate or dentate, variability that can be even found in the same individual. Likewise, the leaf indumentum varies from densely pilose (Fig. 3A, B) to almost glabrous (Fig. 4A, B). In Bolivia, the pilose forms are common although some glabrescent specimens are found near Nevado Sajama (*Liberman 821*, LPB, US) and in northern La Paz Department (*Menhofer 1901*, US). The glabrous forms also ap-

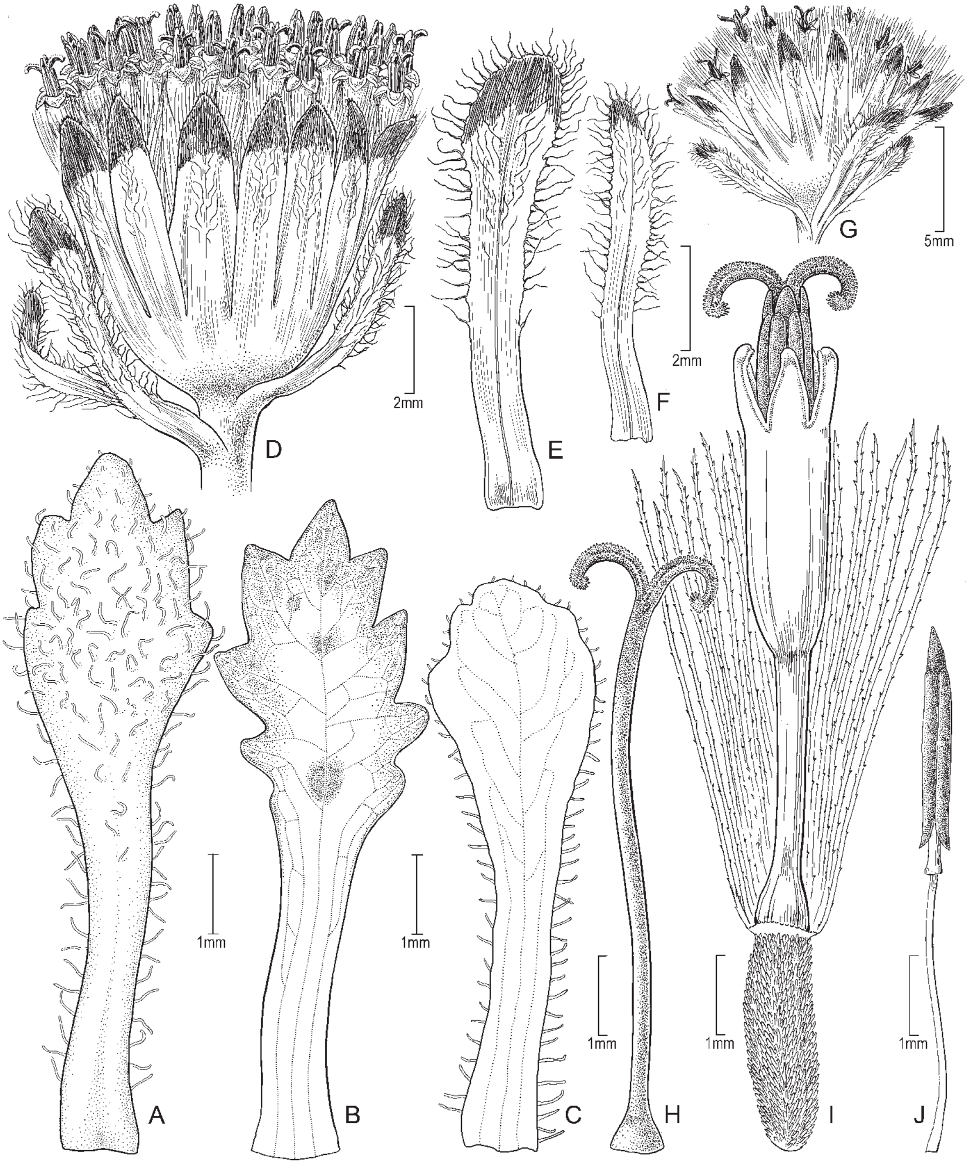

Figure 2. *Senecio melanandrus* **A–C** variability of leaves **D** capitulum **E, F** supplementary bracts **G** involucre **H** style **I** achene and floret **J** stamen. All details drawn from *Weberbauer 5446* except for **A** (drawn from *Calvo & Zárate 7872*), **C** (drawn from *Montesinos 3103*), and **G** (drawn from *Buchtien 1589*). Illustration by Alice Tangerini.

pear in the Peruvian regions of Huancavelica, northern Ayacucho, and northern Puno, and were recently treated under the names *S. pucapampaensis* H. Beltrán and *S. canoi* P. Gonzáles & al. [see Calvo and Fuentes (2018)]. These glabrous plants usually have dentate leaves, but forms exist with rather subentire leaves (*Gonzáles 3568,* USM). The

Figure 3. A, B *Senecio melanandrus* (pilose form) **C, D** *Senecio pygmophyllus* **E, F** *Senecio digitatus* **A** habit (Peru, Cusco, Sibinacocha; *Meneses et al. 6968*) **B** leaves (Bolivia, Potosí, Kari Kari; *Calvo & Zárate 7872*) **C** habit (Chile, Tarapacá, Colchane; *Moreira-Muñoz 2876*) **D** leaves (Chile, Arica-Parinacota, Las Cuevas; *Moreira-Muñoz & Luebert 2470*) **E** habit **F** leaves (Chile, Antofagasta, Pacana; *Calvo 7926*). Picture **A** by Jim Farfán **B, E, F** by Joel Calvo **C, D** by Andrés Moreira-Muñoz.

dentate, pilose forms that are frequently found in Bolivia were described as *S. tassaensis* Montesinos on the basis of a single collection from Moquegua (southern Peru). From the same region, a form with almost entire, glabrous leaves was named *S. sykorae* Montesinos. This form was also collected near the Bolivian locality of Ulla Ulla (*Menhofer 1901,* US). This puzzling distribution pattern and a continuum of intermediates sug-

Figure 4. A, B *Senecio melanandrus* (glabrous form) **C, D** *Senecio pygmophyllus* **A** habit **B** leaves (Peru, Puno, pr. Ananea; *Funk et al. 13184*) **C** habit **D** leaves (Peru, Moquegua, pr. Anillune; *Funk et al. 13153*). Pictures by Mauricio Diazgranados.

gest that these forms do not deserve taxonomic recognition. Despite this variability, *S. melanandrus* is well characterized by supplementary bracts that are almost as long as the involucral bracts, the leaf lamina narrowed at the base, the blackish anthers and style branches, the whitish corollas, and by its myxogenic achene trichomes. The apex of the involucral bracts is usually remarkably dark-colored. Indeed, the epithet *melanolepis* of Weddell's varietal name, here included in the synonymy, explicitly refers to this character, i.e., having black scales (involucral bracts). It is noteworthy that the anther bases were hitherto described as obtuse; however, they are auriculate and clearly acute.

The name *Senecio vegetus* var. *lobatus* Cabrera, here synonymized with *S. melanandrus*, was included by Cabrera (1985) in the synonymy of *S. vegetus* (Wedd.) Cabrera. Cabrera (1955, 1985) described this latter species as having silky-pubescent achenes. We had the opportunity of studying some of the specimens that he examined and they indeed correspond to *S. melanandrus* (i.e., *Beck 7952*, *Mandon 108*, *Menhofer 2013*, *Weberbauer 7491*). Cabrera's interpretation of *S. vegetus* (≡*S. humillimus* var. *vegetus* Weddell) might be explained by the fact that one of the syntypes (P [P01816588]) contains mixed material and some plants certainly correspond to *S. melanandrus* (the individual on the left hand and likely the fragment at the right hand below). The syntype P

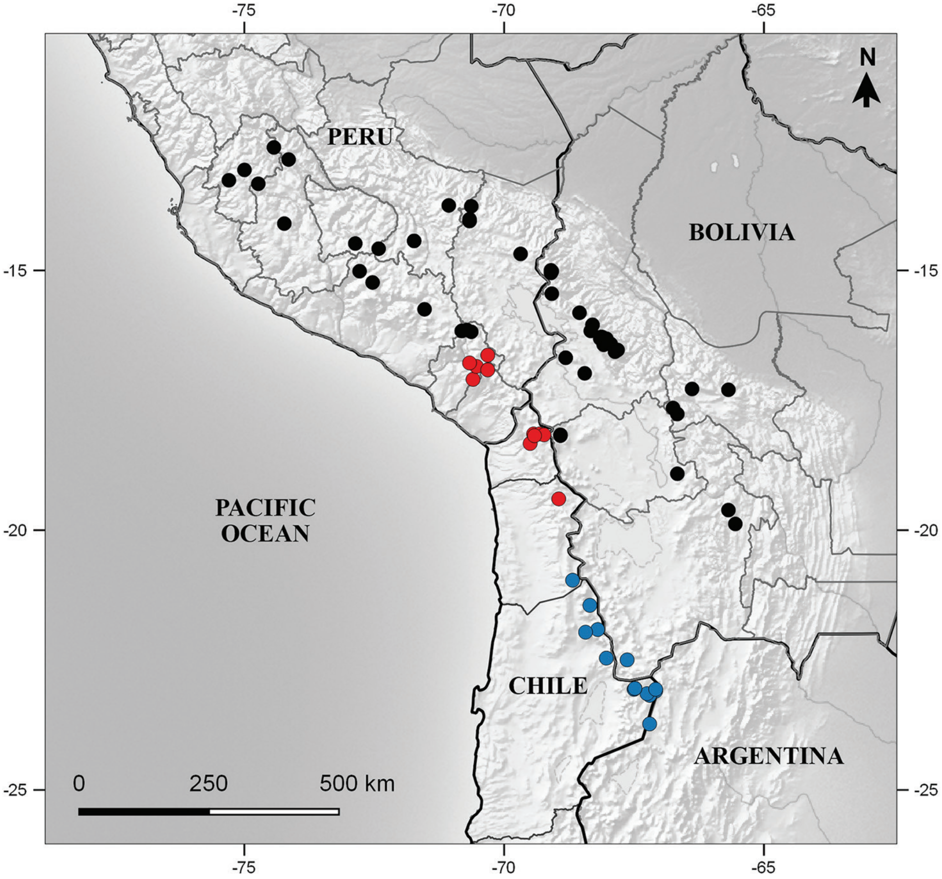

Figure 5. Distribution map of *Senecio melanandrus* (black circle), *S. pygmophyllus* (red circle), and *S. digitatus* (blue circle).

[P01816917], not containing admixtures, shows plainly glabrous plants with the leaves entire and obtuse at the apex. Therefore, and in disagreement with Cabrera, we consider that *S. vegetus* and *S. melanandrus* correspond to distinct taxonomic entities. The former belongs to the subgroup with yellowish anthers, style branches, and corollas whereas the latter is a member of the subgroup displaying blackish anthers and style branches and whitish corollas. However, it is important to point out that the accurate taxonomic position of *S. vegetus* remains uncertain. Because of the number of involucral bracts, the leaf morphology, the yellowish corollas, and the presumed glabrous achenes, we believe that this taxon is related to *S. gamolepis* Cabrera. Additional studies are needed to establish its correct taxonomic position. For the time being, we prefer not including it in the key.

Our efforts to locate the type material of *S. humillimus* var. *melanolepis* Wedd. were unsuccessful. In fact, all the collections cited in the protologue that were located correspond to the other varieties described by Weddell. For that reason, we selected as the neotype a Mandon collection that perfectly matches the diagnosis provided by Weddell. Moreover, it was identified as *S. humillimus* var. *melanolepis* by Schultz Bipontinus [see Mandon (1865)], which supports our interpretation of this taxon. The specimen P03730757 is excluded because it contains mixed material.

The holotype of the name *S. canoi* should be housed at USM (Montesinos-Tubée et al. 2015); however, it was not located. The paratype *Gonzáles 3429* (USM) was also not located at USM. As a result, we studied the collections *Gonzáles 3428* and *Gonzáles 3441* (USM), both collected around the *locus classicus* on the same day as the paratype. Likewise, the holotype of *Senecio vegetus* var. *lobatus* appears to be missing (Arne Anderberg in litt.). Cabrera indicated as paratype the collection *Mandon 108*, which is here selected as neotype for *S. humillimus* var. *melanolepis.*

Finally, in order to remove any uncertainty surrounding the application of this name, and considering that the conditions of the type material are deficient for a proper study of the diagnostic characters, we consider it appropriate to designate an epitype. The selected specimen is *Buchtien 1589* (US00622639) from Chacaltaya, a mountain not far from the *locus classicus* of *W. melanandra*. A duplicate was found at US.

Selected specimens examined. BOLIVIA. Cochabamba: Arque, Cruce Ventilla, 17°46'S, 66°40'W, 17 May 1981, O. Murgia 276 (LPB); cordillera del Tunari, cumbres del cerro Tunari, 17°17'S, 66°23'W, 25 Mar 1990, G. Navarro 653 (BOLV); Tapacarí, arriba rancho Wacakhariña, 3 km al NE de Japo K'asa (km 125 Cbba-Oruro), 17°39'S, 66°45'W, 9 Mar 1995, H.U. Pestalozzi 446 (BOLV); Tiraque, P.N. Carrasco, cordillera Juno, 17°18'S, 65°41'W, 18 Mar 2001, M. Zárate & D. Méndez 1087 (LPB); **La Paz:** Murillo, La Paz 32 km hacia Unduavi, 16°19'S, 68°2'W, 3 Apr 1983, S.G. Beck 7952 (LPB); Murillo, camino La Paz-Lambate, cerca Apacheta entrando al desvío hacia el Illimani, 2 km entrando hacia Milla Milla, 16°34'S, 67°52'W, 6 Apr 2012, S.G. Beck 32782 (LPB); Murillo, La Paz subiendo el valle Kaluyo hasta el albergue ecoturístico Pampalarama, 16°19'S, 68°4'W, 22 Mar 2009, S.G. Beck 33091 (LPB); Murillo, subiendo el valle de Irpavi hasta Palcoma, subiendo el río Hati Jahuira, 16°25'S, 67°57'W, 26 Apr 2013, S.G. Beck 34141 (LPB); Los Andes, above cumbre (pass) on rd. through Hichu-Kkota valley on rd. to mina La Fabulosa, 21 km from base of lag. Khara Kkota, 16°10'S, 68°20'W, 29 Apr 1995, V.A. Funk 11406 (US; the duplicate at LPB corresponds to *Werneria apiculata* Sch. Bip.); Murillo, Zonga valley, laguna Pata Kkota, 1.5 km S of pass, 16°18'S, 68°7'W, 11 Apr 1995, V.A. Funk & N. Bernal 11270 (LPB, US); Murillo, nev. Huayna Potosí, E slopes above rd., 16°17'S, 68°8'W, 12 Apr 1995, V.A. Funk & N. Bernal 11284A (US); Franz Tamayo, Canhuma (Ulla-Ulla), subiendo al cerro Laramani, 15°0'S, 69°6'W, 22 Jan 1983, X. Menhofer 1901 (US); Franz Tamayo, estancia Okaria (Ulla-Ulla), 15°3'S, 69°6'W, 24 Feb 1983, X. Menhofer 2013 (LPB); Murillo, 3.4 km N of Milluni on road to Zongo, 16°18'S, 68°7'W, 25 Apr 1985, J.C. Solomon & M. Moraes 13440 (LPB, US); Ingavi, cantón Jesús de Machaca, comunidad Titicani-Tacaca, a 20 km de Guaqui, 16°41'S, 68°49'W, 8 Apr 1989, X. Villavicencio 457 (LPB); **Oruro:** Eduardo Abaroa, Challapata, comunidad Churacani, cerca a la laguna Chullumpiri, 18°55'S, 66°40'W, 1 Apr 2018, M. Guzmán 125 (LPB); Sajama, nevado de Sajama, sur del cerro Jasasuni [Asa-asuni], 18°11'S, 68°55'W, 18

Mar 1984, M. Liberman 821 (LPB, US); Sajama, cantón Sajama, 18°10'S, 68°55'W, 17 Feb 1998, F. Loza de la Cruz 315 (LPB); **Potosí:** cordillera Kari Kari, aprox. 3.2 km arriba de la laguna San Sebastián, 19°37'S, 65°41'W, 13 Feb 2019, J. Calvo & M. Zárate 7872 (BOLV); José M. Linares Lizarazu, comunidad Alkatuyo, cerro Ichurata, 53 km SE de Potosí, 14 km al N de la escuela de Alkatuyo, 19°53'S, 65°33'W, 22 Jan 1994, F. Marino 309 (LPB). **PERU. Apurímac**: Antabamba, Juan Espinoza Medrano, paraje Ccanccahuane a 18 km al S de la comunidad campesina de Mollebamba, zona Minaminayoc, 14°29'S, 72°52'W, 5 Jun 2017, B. Espinoza-Prieto 534 (USM); **Arequipa:** pr. Chivay, ladera S del nevado Huarancante, 15°45'S, 71°32'W, 1 Apr 2005, C. Aedo & A. Galán 11022 (MA, USM); Castilla, Orcopampa, minas de Poracota, cerca a quebrada Faculla, 15°14'S, 72°32'W, 20 Apr 2011, H. Beltrán 7112 (USM); La Unión, Huaynacotas, Sarajorepampa, 15°1'S, 72°47'W, 18 Mar 2011, D. Montesinos 2949 (USM); **Ayacucho:** Huanca Sancos, Putajasa, 14°6'S, 74°14'W, 24 Feb 2002, A. Cano et al. 11963 (USM); Huanta, mt. Razuhuilca, 12°52'S, 74°9'W, 4–6 Feb 1926, A. Weberbauer 7491 (CONC, F); **Cusco:** Chumbivilcas, Santo Tomás, compañía minera Azuca (borde departamental Cusco-Apurímac), 14°35'S, 72°25'W, 13 Apr 2011, H. Beltrán 7032 (USM); Velille, Uchucarco, cerca a Soracocha, 14°26'S, 71°44'W, 23 Apr 2015, P. Gonzáles 3600 (USM); Velille, Uchucarco, cerca a Soracocha, 14°26'S, 71°44'W, 23 Apr 2015, P. Gonzáles 3601 (USM); cordillera de Vilcanota, cuenca de la laguna Sibinacocha, cerro Rititica, 13°45'S, 71°4'W, 5 Mar 2019, R.I. Meneses et al. 6968 (LPB); **Huancavelica:** Huaytará, Pilpichaca (abra Apacheta), 13°20'S, 74°44'W, 4 Jul 2010, A. Cano, W. Mendoza & A. Delgado 19680 (USM); Huachocolpa, alrededores de la unidad minera Caudalosa, 13°4'S, 75°0'W, 23–31 Mar 2015, P. Gonzáles 3568 (USM); Castrovirreyna, cordillera between Pisco and Ayacucho, 13°16'S, 75°18'W, May 1910, A. Weberbauer 5446 (F, GH); **Moquegua:** General Sánchez Cerro, Ubinas, S of Pillone, 16°10'S, 70°49'W, 24 Mar 2013, D. Montesinos 4023 (USM); General Sánchez Cerro, Ubinas, Matazo, 16°10'S, 70°49'W, 28 Mar 2015, D. Montesinos 4242 (USM); General Sánchez Cerro, Ubinas, Querala, 16°10'S, 70°49'W, 2 Mar 2018, D. Montesinos 5918 (USM); **Puno:** just W of abra on unpaved track, ca. 17 km from Puno-Ananea rd., 14°41'S, 69°41'W, 16 Mar 2014, V.A. Funk, M. Diazgranados & E. Cochachin 13184 (US, USM); Carabaya, Corani, Chacaconiza, 14°1'S, 70°40'W, 14 Jan 2015, P. Gonzáles 3428 (USM); Carabaya, Corani, Chacaconiza, 14°3'S, 70°40'W, 14 Jan 2015, P. Gonzáles 3441 (USM); Carabaya, Corani, Chacaconiza, 14°3'S, 70°40'W, 14 Jan 2015, P. Gonzáles 3444 (USM); Carabaya, alrededores de Condena, 13°46'S, 70°38'W, 9 Nov 2017, H. Trinidad 4192 (USM).

2. *Senecio pygmophyllus* (S.F. Blake) J.Calvo, A.Granda & V.A.Funk, comb. nov.

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Figs 3C, D, 4C, D, 6

Werneria pygmophylla S.F. Blake, J. Washington Acad. Sci. 18: 491. 1928. Type: Peru. Moquegua: cordillera East of Carumas, 4500–4600 m, 7–8 Mar 1925, A. Weberbauer 7358 (holotype: F [F-552587]; isotypes: CONC [CONC-28864], G [G00356025], US [US00622822]).

Senecio laucanus Ricardi & Martic., Gayana, Bot. 11: 17. 1964. Type: Chile. Arica-Parinacota: camino de Putre a Chucuyo, km 17, 4250 m, 12 Feb 1964, C. Marticorena, O. Matthei & M. Quezada 208 (holotype: CONC [CONC-29864]; isotype: CONC), syn. nov.

Description. Caespitose perennial herb. Leaves long pseudopetiolate; leaf lamina 2.5–5.5 mm long, 2.4–5.5 mm wide, ovate to suborbiculate, obtuse at the apex, rounded to truncate at the base, typically crenate-lobate with 3–9 rounded lobes, revolute, usually strongly conduplicate downwards, pilose on both surfaces, somewhat fleshy, glaucous; pseudopetiole 5–25 mm long, flat, slightly broadened at the base, marginally ciliate. Capitulum discoid, solitary, terminal, sessile or subsessile; involucre 6–8 mm long, 7–10 mm wide. Involucral bracts 16–21, oblong-lanceolate, 2.5–4 mm long, 0.7–1.7 mm wide, partially fused at the base, smooth, with trichomes on the abaxial surface 0.5–0.8 mm long, dark purple- or blackish-tipped. Supplementary bracts ca. 3, linear, 6–7.5 mm long, 0.5–0.8 mm wide, smooth, three-quarters to as long as the involucral bracts, with trichomes on the margins, dark purple- or blackish-tipped. Disc florets 50–82, 3.5–5.1 mm long, 0.6–1 mm wide, 5-lobed, conspicuously differentiated in a distinct tube and campanulate limb, whitish. Anther bases auriculate, clearly acute, dark purple to blackish; filament collar balusterform. Style branches truncate with a crown of sweeping hairs, dark purple to blackish. Achenes 1.7–1.8(–2.5) mm long, ca. 0.5 mm wide, brownish, covered by dense indumentum of obtuse whitish myxogenic twin trichomes ca. 0.2 mm long; pappus 3–4.5 mm long, barbellate, whitish. Chromosome number: unknown.

Additional iconography. Blake (1928: pg. 496, fig. 1F, G, sub *W. pygmophylla*); Ricardi and Marticorena (1964: pg. 19, fig. 6, sub *S. laucanus*).

Distribution and habitat. Chile (Arica-Parinacota, N Tarapacá) and Peru (Moquegua) (Fig. 5). The species is also expected in the Peruvian department of Tacna and in the Bolivian region bordering northern Chile, although no collections have been studied from there. It grows in exposed places on sandy soils, between elevations of 4100–4700 m.

Phenology. Collected in bloom from January to June, although full bloom probably takes place between March and April.

Etymology. The epithet refers to the resemblance of the leaves to a fist.

Discussion. Blake (1928) placed his new species within *Werneria* arguing that the involucral bracts were connate half way. Otherwise, he assumed a close similarity between it and a *Senecio* species collected by Pennell here identified as *S. moqueguensis* Montesinos (see protologue of *W. pygmophylla*). It is certain that the involucral bracts of *S. pygmophyllus* are usually partially fused at the base; however, this character alone cannot be used as diagnostic to place a species in one or another genus. Rather, we prefer to base a decision on a set of characters, i.e., presence or absence of genuine supplementary bracts, involucral bracts free or fused at the base, achene trichomes

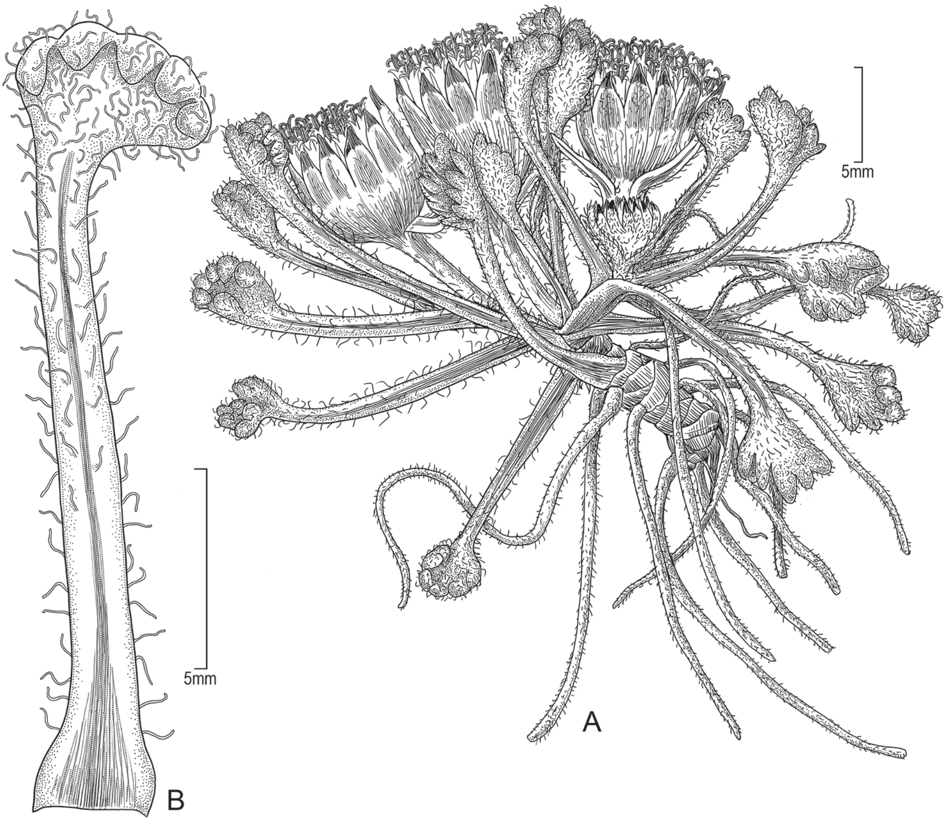

Figure 6. *Senecio pygmophyllus* **A** habit (drawn from *Funk et al. 13153*) **B** leaf (drawn from *Weberbauer 7358*). Illustration by Alice Tangerini.

myxogenic or not, and rosettiform or caespitose habit. Accordingly, we consider that this species should be placed within *Senecio* on the basis of the following characters: presence of supplementary bracts, myxogenic achene trichomes, and caespitose habit. This decision is also supported by the fact that it was inaccurately considered a synonym of *S. digitatus* for a long time.

Rockhausen (1939) was the first author who treated *W. pygmophylla* as a heterotypic synonym of *S. digitatus*. Since then, most authors followed his treatment (e.g., Cabrera 1949; Freire et al. 2014). Ricardi and Marticorena (1966), in disagreement, concluded that they correspond to two distinct taxonomic entities. We agree with Ricardi and Marticorena's treatment after studying the respective type materials, further collections from southern Peru and northern Chile, and living plants. The two species can be differentiated by their leaf shape and indumentum type. *Senecio pygmophyllus* has a lamina clearly differentiated from the pseudopetiole (petioliform base); usually the lamina is remarkably reduced when compared with the pseudopetiole length (at least in the more basal leaves). The lamina we observed were ovate to suborbicular, typically crenate-lobate with 3–9 rounded lobes and revolute margins (Fig. 4C). In contrast, *S. digitatus* has linear to slightly spatulate leaves narrowed at the base (Fig. 3E, F). This latter species is extremely variable with regard to the leaf margin, which can be dentate, pinnatipartite or distantly pinnatisect, with clearly acute teeth; however, specimens with entire leaves and even individuals displaying both entire and dentate leaves were occasionally observed. The leaf apex is acute and usually shows a whitish callus-like tip, whereas in *S. pygmophyllus* the apex is always plainly obtuse and unadorned (Fig. 3C, 4D). Both species usually have abundant indumentum on the leaves, involucre, and supplementary bracts but the type of trichomes differs and is useful to separate them from one other. The indumentum of *S. pygmophyllus* is pilose whereas in *S. digitatus* the trichomes are clearly arachnoid, longer, and intermingled. Moreover, the indumentum of *S. digitatus* is essentially concentrated on the adaxial surface, whereas in *S. pygmophyllus* the leaf lamina has trichomes on both surfaces. Their distribution areas do not overlap (Fig. 5).

Senecio pygmophyllus might be confused with those forms of *S. melanandrus* displaying pilose, dentate leaves. A useful character to discriminate them from each other is the leaf shape, although some overlap has been detected in a few specimens. In *S. pygmophyllus* the leaves are clearly pseudopetiolate and the ratio lamina/pseudopetiole length usually is very low in the more basal leaves (Fig. 4D). In contrast, *S. melanandrus* displays a lamina progressively narrowed at the base (Fig. 3B). The distinctive pseudopetiole length of *S. pygmophyllus* might be an adaptation to the sandy soils where this species thrives because the plants usually appear to be partially sunken. Additionally, the number of disc florets tends to be higher in *S. pygmophyllus* (50–82 vs. 20–45), as well as the number of involucral bracts (16–21 vs. 11–16). Since the mentioned morphology coincides with geographical separation, we consider it appropriate to recognize it as a distinct species.

The name *S. laucanus* Ricardi & Martic. was described from northern Chile (Arica-Parinacota) and it was hitherto considered endemic to this country (Moreira-Muñoz et al. 2016). It is included in the synonymy of *S. pygmophyllus* since we failed to identify any diagnostic character to differentiate them. In some specimens from Chile the more basal leaves are not so long pseudopetiolate as in the typical forms (e.g., *Moreira-Muñoz & Luebert 2470*), but it is considered as part of the variability encompassed by this species; indeed, this morphology probably responds to the fact that these plants grow on less sandy soils.

Specimens examined. ***Senecio digitatus*. ARGENTINA. Salta:** Los Andes, Huaitiquina, 23°44'S, 67°12'W, 27 Feb 1972, Cabrera et al. 22559 (LP). **BOLIVIA. Potosí:** Sud Lípez, a 1 km al W de salar Chalviri, 22°30'S, 67°38'W, 7 May 1999, N. Massi & C. Salles 726 (LPB) [first record for Bolivia]. **CHILE. Antofagasta:** El Loa, camino entre Ascotán y San Pedro de Conchi, 21°58'S, 68°26'W, 4 Apr 1985, M. Arroyo 85-606 (CONC); El Loa, cerro Losloyo, ladera SE, 23°9'S, 67°15'W, 9 Apr 1997, M. Arroyo, L. Cavieres & A. Humaña 97331 (CONC); El Loa, cerro Nevados de Poquis, ladera SO, 23°4'S, 67°5'W, 9 Apr 1997, M. Arroyo, L. Cavieres & A. Humaña 97343 (CONC); El Loa, pampa Laguna Helada, 23°6'S, 67°5'W, 9 Apr 1997, M. Arroyo, L. Cavieres & A. Humaña 97403 (CONC); El Loa, pampa Loyoques, 23°11'S, 67°12'W, 9 Apr 1997, M. Arroyo, L. Cavieres & A. Humaña 97408 (CONC); El Loa, cordón cerro de la Pacana, cuesta entre salar de Aguas Calientes y quebrada Quepiaco, 23°3'S, 67°29'W, 11 Apr 1997, M. Arroyo, L.

Cavieres & A. Humaña 97477 (CONC); El Loa, cordón cerro de la Pacana, cuesta entre salar de Aguas Calientes y quebrada Quepiaco, 23°4'S, 67°30'W, 11 Apr 1997, M. Arroyo, L. Cavieres & A. Humaña 97498 (CONC); El Loa, Toconao, camino a Tara, monjes de La Pacana, 23°3'S, 67°29'W, 6 Mar 2019, J. Calvo 7926 (SGO); cruce camino internacional Paso Jama con camino a salar de Tara, 23°3'S, 67°29'W, 19 Dec 1996, A. Moreira-Muñoz 317 (SGO); Machuca-Copacoya, 22°28'S, 68°2'W, 18 Feb 1885, F. Philippi s.n. (LP, SGO); laguna de Llaillai, 21°55'S, 68°12'W, 23 Feb 1885, F. Philippi s.n. (CONC, LP, SGO, SI); El Loa, Ascotán, 21°27'S, 68°21'W, 23 Jan 1943, E. Pisano & J. Venturelli 1753 (SGO); El Loa, entre Machuca y Tatio, 15 Feb 1943, E. Pisano & J. Venturelli 1866 (CONC, SGO); **Tarapacá:** [without locality], Feb 1885, F. Philippi s.n. (K); Iquique, Collaguasi, San Carlos, 20°58'S, 68°41'W, 22 Jan 1994, S. Teillier 3286A (CONC).

***Senecio pygmophyllus*. CHILE. Arica-Parinacota:** cerca de laguna de Cotacotani, camino a Guane Guane, 18°10'S, 69°14'W, 9 Mar 1984, M. Arroyo 84-724 (CONC); portezuelo entre cerro Guane Guane y cerro Larancagua, 18°9'S, 69°19'W, 22 Apr 1984, M. Arroyo 84-935 (CONC); Las Cuevas, antes del Chaku, 18°11'S, 69°25'W, 20 Mar 2015, A. Moreira-Muñoz & F. Luebert 2470 (SGO); camino de Putre a Portezuelo de Chapiquiña, 18°20'S, 69°30'W, 28 Mar 1961, M. Ricardi, C. Marticorena & O. Matthei 277 (CONC); **Tarapacá:** Colchane, géiser Puchultiza, 100 m antes del géiser, 19°24'S, 68°57'W, 16 Jun 2018, A. Moreira-Muñoz 2876 (SGO). **PERU. Moquegua:** minera Quellaveco, 17°6'S, 70°36'W, 8 Apr 1999, ESCO 7238 (US); area between the carretera-binacional and the interoceanica sur, on unpaved road that connects the two main roads and borders a large bofedal, 16°51'S, 70°32'W, 12 Mar 2014, V.A. Funk, M. Diazgranados & E. Cochachin 13153 (US, USM); Mariscal Nieto, Carumas, Ancolacaya, 16°38'S, 70°19'W, Mar–Apr 2018, V. Morales 140 (USM); 5 km East of lago Suche, 16°55'S, 70°19'W, 19 Jan 1952, O.P. Pearson 5 (CONC, UC).

New synonyms

1. *Senecio casapaltensis* Ball, J. Linn. Soc., Bot. 22: 47. 1885.

Senecio sanmarcosensis H. Beltrán, Arnaldoa 15: 211. 2009. Type: Peru. Ancash: Huari, San Marcos, Ccolla Chica, 09°40'28"S, 77°03'10"W, 5600 m, 4 May 2008, H. Beltrán 6476 (holotype: USM [USM-00277272]; isotypes: CUZ n.v., HUT n.v.), syn. nov.

Senecio repens var. *taraxacifolius* A. Gray [“taraxicifolius”], nom. nud. in sched. (Turland et al. 2018, ICN Art. 38.1) (US [US00829056]).

Type. Peru. Lima: supra Casapalta, 4265–4360 m, 22 Apr 1882, J. Ball s.n. (lectotype, designated here: K [K000497782]; isolectotype: E [E00417028]).

Discussion. *Senecio casapaltensis* Ball was described from central Lima near the border between Lima-Junín departments, whereas the type material of *S. sanmarcosensis* H. Beltrán comes from southeastern Ancash Department. After studying several specimens from both regions, we can conclude that the differences concerning the shape and size of the leaves are not significant. The populations from Ancash tend to have a denser indumentum composed of capitate trichomes, whereas those specimens from Lima are glabrescent or the indumentum is rather deciduous and composed of shorter glandular trichomes. Nonetheless, the existence of intermediate specimens makes their recognition as distinct species inadvisable and, therefore, *S. sanmarcosensis* is here synonymized with *S. casapaltensis*.

Among the located original material of *S. casapaltensis*, the specimen at K is designated as the lectotype due to it being more complete than the duplicate at E.

2. *Senecio expansus* Wedd., Chlor. Andina 1: 107. 1856.

Senecio macrorrhizus Wedd., Chlor. Andina 1: 108. 1856. Type: Peru. Cusco: dept. de Cuzco, Oct 1839–Feb 1840, C. Gay 1870 (lectotype, designated here: P [P01816797]), syn. nov.

Type. Bolivia. Potosí: montagnes des lagunas de Potosí, [without date], A. d'Orbigny 1418 (lectotype, designated by Cabrera (1966) as "typus", pg. 21: P [P01816805]; isolectotypes: BR [BR0000552801], G [G00356020], K [K000497783]).

Discussion. *Senecio macrorrhizus* Wedd. was described from Cusco (Peru) and distinguished from *S. expansus* Wedd. mainly by having a thicker, longer, and more sinuous rhizome, larger capitulum, and rosettes less spread out (Weddell 1856). The mentioned differences fall within the variability encompassed by *S. expansus*, and here we synonymize them.

Gay's specimen P01816797 perfectly matches the protologue information, and therefore, it is designated as the lectotype of the name *S. macrorrhizus*.

Key to the discoid caespitose *Senecio* species from Bolivia and Peru

The dwarf shrubs developing erect stems are excluded (e.g., *S. apolobambensis* Cabrera, *S. puchei* Phil., *S. trifurcifolius* Hieron.). *Senecio aquilaris* Cabrera was cited for Bolivia (Beck and Ibáñez 2014) and Peru (Gonzáles et al. 2016); it is not included in the key because its identification is doubtful and further studies are required. The rosettiform species *S. expansus* and *S. hyoseridis* (Benth.) L. Salomón & S.E. Freire were placed in *S.* ser. *Culcitium* (Bonpland) Cabrera (Freire et al. 2014; Salomón et al. 2018) but they are included in the key because they fit well within the discoid caespitose species group. The color of the anthers, style branches, and corollas has a relevant taxonomic value within the group and it is readily noticeable on living plants. However, on dried specimens a careful study is required in order to avoid misidentifications.

1 Plants in rosette form **2**
– Plants developing prostrate or decumbent stems **6**

2 Leaves pinnatilobate to lyrate-pinnatisect.......... ***S. casapaltensis***

– Leaves subentire to pinnatipartite **3**

3 Capitula sessile, solitary or several; leaf lamina longer than or similar to the pseudopetiole.......... **4**

– Capitulum shortly pedunculate, solitary; leaf lamina clearly shorter than the pseudopetiole.......... **5**

4 Leaves densely white tomentose on both faces, concolorous....... ***S. expansus***

– Leaves only densely white tomentose beneath, discolorous.......... ***S. hyoseridis* s.l.** (further research needed)

5 Leaves ovate-deltate, crenate-dentate, puberulous on both faces... ***S. genisianus***

– Leaves elliptic-suborbicular, subentire, with scattered long hispid trichomes above and nearly glabrous beneath.......... ***S. sagasteguii***

6 Anthers and style branches yellowish; corolla yellowish.......... **7**

– Anthers and style branches blackish; corolla whitish **16**

7 Leaves and involucre covered by whitish lanate indumentum.......... **8**

– Leaves and involucre glabrous or covered by arachnoid or pilose indumentum.......... **9**

8 Leaves arranged along true stems; leaves oblanceolate to oblong .... ***S. carhuanishoensis***

– Leaves arranged in rosettiform clusters arising directly from rhizome-like stems; leaves obovate-spatulate.......... ***S. evacoides***

9 Involucre 4–5 mm long; involucral bracts 8(–9) .... ***S. humillimus***

– Involucre 6–12 mm long; involucral bracts (9–)12–15(–20).......... **10**

10 Achenes with indumentum.......... **11**

– Achenes glabrous **13**

11 Leaves glabrous, entire or subentire.......... ***S. woodii***

– Leaves covered with trichomes, dentate.......... **12**

12 Involucral bracts 9–12; leaves sparsely covered with trichomes.......... ***S. moqueguensis***

– Involucral bracts 15–20; leaves densely covered with trichomes .... ***S. ticsanicus*** (further research needed)

13 Leaves dentate or lobate, rarely only shallowly crenate **14**

– Leaves entire **15**

14 Leaves dentate, rarely only shallowly crenate.......... ***S. menesesiae***

– Leaves pinnatilobate.......... ***S. pinnatilobatus***

15 Leaves (15–)20–50 mm long, arranged along the stems.......... ***S. algens***

– Leaves 5–10 mm long, arranged in rosettiform clusters.......... ***S. gamolepis***

16 Achenes papillose, with visible ribs; leaves linear .... ***S. scorzonerifolius***

– Achenes silky-pubescent, usually with invisible ribs; leaves linear, linear-oblong or spatulate **17**

17 Leaf lamina glabrous.......... **18**

– Leaf lamina with indumentum **19**

18 Supplementary bracts 4.1–5.7 mm long, a third to a half as long as the involucral bracts, glabrous; leaves linear-oblong, flat, acute at the apex .... ***S. madidiensis***

– Supplementary bracts 4.2–7.5 mm long, almost as long as the involucral bracts, usually pilose; leaves linear-oblong to spatulate, usually conduplicate downwards, rather obtuse at the apex................................. ***S. melanandrus***

19 Lamina ovate to suborbicular, differentiated from the pseudopetiole............ .... ***S. pygmophyllus***

– Lamina linear, linear-oblong or narrowly spatulate, progressively narrowed at the base..**20**

20 Leaves dentate, pinnatipartite or distantly pinnatisect (rarely entire), arachnoid, usually with a callus-like tip..***S. digitatus***

– Leaves entire, crenate or dentate, pilose, unadorned at the apex .... ***S. melanandrus***

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