RESEARCH ARTICLE



Begonia catbensis (sect. Coelocentrum, Begoniaceae), a new species from northern Vietnam

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Abstract

Begonia cathensis, a new species in *Begonia* sect. *Coelocentrum* is described and illustrated. The new species was discovered in lowland limestone hills at Cat Ba National Park and can be easily distinguished from all its congeners by having dendritic hairs on the petiole, adaxial veins and stipules, fimbriate bracts and bracteoles, dense conical bullae on the upper surface of the leaf blade, two tepals in the pistillate flowers and a glabrescent ovary with verrucose wings. Based on IUCN Criteria, the species is currently assessed as "Endangered" (D).

Keywords

Begonia, Cat Ba, endangered plant, new species, Vietnam

Introduction

Begonia L. (Linnaeus 1753) is a highly diverse and widely distributed genus with 2001 currently accepted species (Hughes et al. 2015 onwards). Within the genus, the section Coelocentrum Irmsch. (Irmscher 1939) is mainly distributed in the karst regions and can be easily identified by having 1-locular capsules with parietal placentation (rarely 3-locular with axile placentation) (Ku Chung et al. 2014; Shui et al. 2019). The number of known species in Begonia sect. Coelocentrum has grown from 18 species (Shui et al. 2002) to 73 species (Shui et al. 2019) during the past two decades. The growth of the section has been stimulated by a series of flora diversity surveys conducted by many botanists (Shui and Chen 2005; Fang et al. 2006; Ku et al. 2006; Peng et al. 2007, 2015; Averyanov and Nguyen 2012; Qin et al. 2017; Chen et al. 2018; Radbouchoom et al. 2019; Tong et al. 2019; Liu et al. 2020; Tu et al. 2020). Some species have been merged into sect. Coelocentrum from other sections, for example, Begonia cavaleriei H.Lév (Léveillé 1909), B. pulvinifera C.-I Peng & Yan Liu (Peng et al. 2006), B. wangii Yu (Yu 1948), B. cylindrica Liang & Chen (Liang and Chen 1993), B. leprosa Hance (Hance 1883) and B. sinofloribunda Dorr (Dorr 1999) (Chung et al. 2014; Moonlight et al. 2018; Shui et al. 2019).

In Vietnam, there were only a few publications on *Begonia* before 2000. After describing eight new begonias from Vietnam (Gagnepain 1919), Gagnepain went on to record a total of 18 taxa of the genus for Vietnam in 1921 (Gagnepain 1921). In the late 20th Century, Pham (1991, 1999) made short descriptions in Vietnamese including sketches of 35 species and varieties of native *Begonia* in Vietnam. However, this publication has raised questions for taxonomists because the account does not cite specimens and has some misidentifications. For example, Kiew (2007) excluded four species of *Begonia* out of Pham's account, renamed one and described three new species for sciences which were misidentified by Gagnepain (1921) and Pham (1991, 1999). The number of species in *Begonia* sect. *Coelocentrum* recorded for Vietnam has rapidly increased in recent years, from four species in 2007 (Gagnepain 1921; Nguyen 2004; Shui and Chen 2005; Kiew 2007; Peng et al. 2007) to 21 up to now (Averyanov and Nguyen 2012; Chung et al. 2014; Peng et al. 2014, 2015; Chen et al. 2018; Radbouchoom et al. 2019).

During our field surveys of northern Vietnam in 2019, we found an interesting species of *Begonia* in lowland limestone hills at Cat Ba National Park. This begonia represents characteristics of *B. sect. Coelocentrum* (Shui et al. 2002; Chung et al. 2014), such as perennial habit, rhizomatous stems, staminate flower with 4 tepals, ovary 1-locular with parietal placentation and 3 unequally winged capsules. In having conical bullae on the upper leaf surface, it is similar to *B. ferox* C.I.Peng & Yan Liu (Peng et al. 2013), *B. fimbribracteata* Y.M.Shui & W.H.Chen (Shui and Chen 2005), *B. masoniana* Irmsch. ex Ziesenhenne (Ziesenhenne 1971), *B. melanobullata* C.I.Peng & C.W.Lin (Peng et al. 2015), *B. montaniformis* C.I.Peng, C.W.Lin & H.Q.Nguyen (Peng et al. 2015), *B. nahangensis* Aver. & H.Q.Nguyen (Averyanov and Nguyen 2012) and *B. variegata* Y.M.Shui & W.H.Chen (Shui and Chen 2005). This plant, however, is obviously differentiated from them by having dendritic trichomes on the

petioles, abaxial veins and on the keeled mid-rib of the stipules, 2-tepalled pistillate flowers and glabrescent ovaries with verrucose wings. It is clear that our plant represents a new taxon, therefore we describe and illustrate it here. Furthermore, a key to identify species of *B.* sect. *Coelocentrum* with conical bullae on the upper surface of the leaves is provided.

Materials and methods

Fresh flowers and parts of inflorescences of the new species were fixed and preserved in 50% ethanol for morphological studies. These fixed materials and dried herbarium specimens of the new species are kept at HN and IBK. Herbarium acronyms follow Thiers (2020). Conservation status assessment follows the guidelines in the IUCN Red List Categories and Criteria version 14 (IUCN 2019).

Taxonomy

Begonia cathensis L.N.Dong, K.S.Nguyen & Y.M.Shui, sp. nov.

urn:lsid:ipni.org:names:77217739-1 Figs 1–3, Table 1

Diagnosis. Morphologically similar to several *Begonia* having conically bullate leaves and others with a rugulose leaf surface with white maculation and a ciliolate tepal margin, but can be easily distinguished from them by the dendritic hairs on the petioles, abaxial veins and the keeled mid-rib of the stipules, glabrous peduncles, pistillate flowers with 2 tepals and glabrescent ovaries with verrucose wings.

Type. VIETNAM. Hai Phong City, Cat Hai District, Cat Ba National Park, remnants of primary broad-leaved evergreen forest in lowland of limestone hills, around point 20.803333°N, 106.999167°E, 50–70 m a.s.l., flowers white to greenish, fruits green, rare, 24 August 2019, *W.B. Xu, K.S. Nguyen, C.R. Lin, L.N. Dong, H.Q. Nguyen* & X.K. Nguyen 14002 (Holotype: HN!; Isotypes: IBK00421271!, HN!).

Description. Herb perennial, monoecious, epipetric, rhizomatous, rooting at nodes, about 25–35 cm tall. Rhizome succulent, elongate, 10–30 cm long, 4–7 mm in diameter, creeping and slightly suberect apically, pale greenish to purplish-green or brownish-red, internodes ca. 1.5 cm long, covered by sparse dendritic hairs. Stipules often persistent, ovate-triangular, herbaceous, brownish-red or purplish when young, later turning pale greenish speckled with purplish patches, $12-16 \times 6-12$ mm, adaxially glabrous, abaxially keeled, mid-rib with sparse dendritic hairs; margin entire or somewhat undulate and recurved; apex aristate, arista 2–3 mm long. Petiole cylindrical, succulent, 7–16 cm long, 3–4 mm in diameter, densely covered with reddish long-stalked dendritic hairs. Leaves 9–27, basal, alternate, asymmetric, unlobed, broadly ovate, $10-13 \times 5-7$ cm, papery, adaxially glossy, dark green or rarely brown, with slightly silvery green patches along the veins, surface densely bullate, bullae conical and

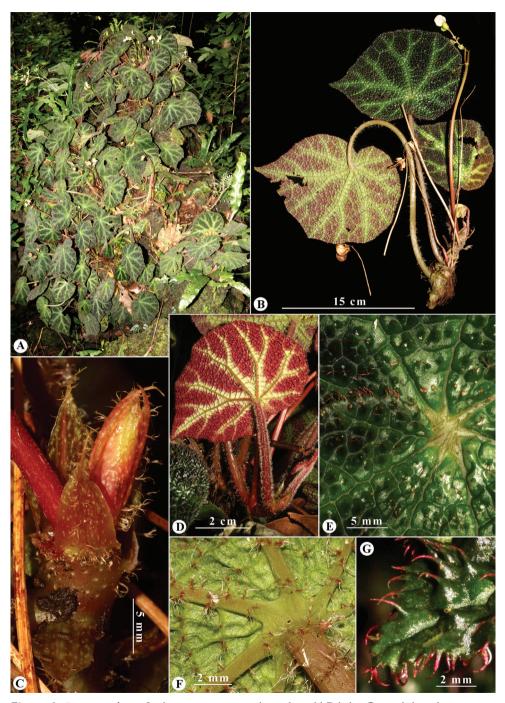


Figure 1. *Begonia cathensis* A plants growing on rocks in the wild B habit C apical shoot bearing stipules D young leaf, abaxial view E part of adaxial leaf surface showing bullae, setae and palmate veins F dendritic hairs on petiole and abaxial veins G portion of leaf showing reddish setae on bullae and along margin. Photos and layout by K.S. Nguyen & L.N. Dong.

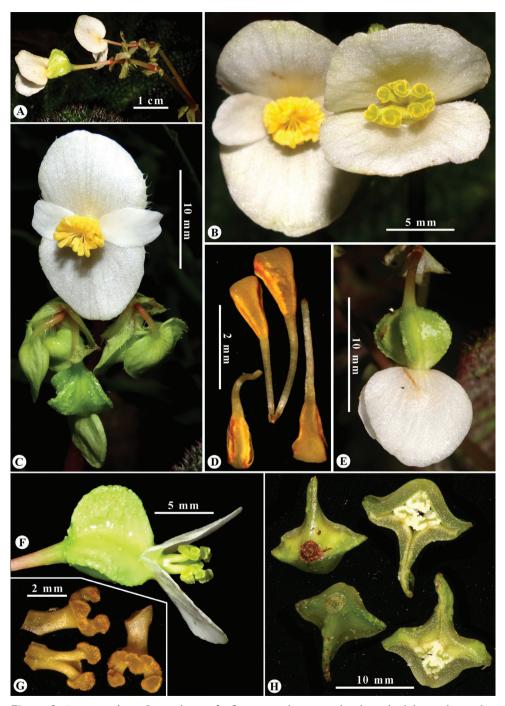


Figure 2. *Begonia cathensis* **A** apical part of inflorescence showing peduncle, pedicel, bracts, bracteoles, staminate and pistillate flowers **B** staminate and pistillate flowers **C** staminate flower (upper) in front view **D** stamens **E** pistillate flower-top view **F** pistillate flower, lateral view **G** dissected styles and stigmas **H** fruits and their cross-cut. Photos and layout by K.S. Nguyen & L.N. Dong.

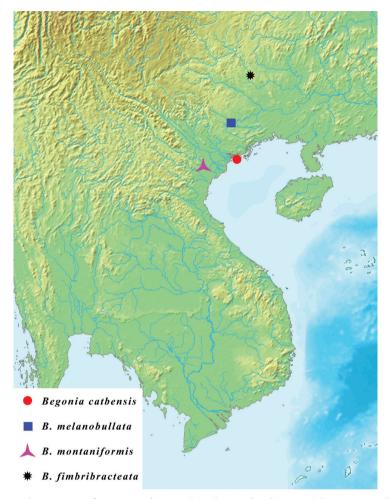


Figure 3. Distribution map of *Begonia cathensis* and its closest related species (The map is modified from https://en.wikipedia.org/wiki/Mainland_Southeast_Asia).

tipped with a red seta 1.5-2 mm long, abaxially brownish-red to red-brown (maroon), with pale green along major veins, long-stalked reddish dendritic hairs along the veins, base strongly oblique-cordate, margin repand and serrulate with red setae 1.5-2 mm long, apex acuminate; venation palmate with 5-7 primary veins, mid-rib distinct, with 2-4 secondary veins on each side, tertiary veins reticulate or percurrent, minor veins reticulate. Inflorescences axillary, dichasial cymes branched 2-3 times, arising directly from rhizome, pedunculate; peduncle terete, 13-22 cm long, 2.5-3.5 mm thick, glabrous, pale greenish-red to red; bracts and bracteoles not caducous, oblong or oval to ovate, slightly concave at the base and distally bent outwards during flowering, pale green with several longitudinal reddish veins, margin serrate-fimbriate with cilia 1.5-3 mm long, bracts $8-10 \times 4-6 \text{ mm}$, slightly larger than bracteoles ($6-8 \times 2.5-3.5 \text{ mm}$). Staminate flower: pedicel glabrous, 12-19 mm; tepals 4, pure white, outer 2 broadly

Characters	B. catbensis	B. melanobullata	B. montaniformis	B. fimbribracteata
Stipule	glabrous, except for the keeled midrib with sparse dendritic hairs	glabrous, except for the keeled midrib with unbranched hairs	glabrous, except for the keeled midrib with unbranched hairs	subglabrous
Petiole	densely covered by reddish dendritic trichomes	densely white villous when young, brownish tomentose or subglabrous later	densely white villous	sparse strigae 1–2 mm long
Leaf color	adaxially dark green or rarely brown, with slightly silvery green patches along veins abaxially brownish red to red-brown (maroon)	adaxially emerald green to yellowish green; abaxially pale green, reddish on veins and bullae	adaxially blackish-malachite green, purplish- olive or dark bluish-brown, with silvery green zone along main veins, abaxially pale green, reddish	adaxially green or brown, with white dots along major veins; abaxially reddish
Leaf bulla	tipped by a reddish seta 1.5–2 mm long	tipped by a velutinous hair 6–10 mm long	tipped by 2-6 peak-like hispidulous protrusions	tipped by a seta 1.5–2 mm long
Leaf margin	repand, serrulate and ciliolate	repand villous when juvenile	repand to shallowly denticulate and ciliate	serrulate and ciliate
Leaf apex	acuminate	caudate	acute to acuminate	rounded
Venation	5–7-veined palmate	7–9-veined palmate	7–9-veined palmate	6–7-veined palmate
Vein on abaxial surface	covered by reddish dendritic hairs	brownish tomentose	densely brownish-white tomentose	covered by strigae 1–1.5 mm long
Inflorescence	branched 2–3 times, 4–10 flowers	branched 4–6 times, numerous flowers (>20)	branched 3–8 times, numerous flowers, up to above 30	branched 2–3, ca. 5 flowers
Peduncle	glabrous	tomentose	tomentose to subglabrous	sparsely hairy
Bract	glabrous, margin serrate-fimbriate with cilia 1.5–3 mm long	glabrous, margin tomentose	abaxially velutinous along midrib, margin tomentose	glabrous, margin serrulate-fimbriate with cilia 1–2 mm long
Staminate flower tepal	abaxially glabrous	abaxially sparsely setulose	abaxially sparsely velutinous	abaxially sparsely pilose
Pistillate flower tepal number	2	<i>c</i> 0	33	6
Tepal margin	sparsely ciliolate below middle	entire, not hairy	entire, not hair	entire, not hairy
Tepal color	greenish white to white on both surface when opened	adaxially yellowish-pinkinsh, abaxially reddish	adaxially yellowish-greenish, abaxially reddish- green	pink or white
Ovary	glabrescent	sparsely dotted with sessile glands	snarselv sessile-ølandular	snarcely hairy

Table 1. Morpholocical commarison of *Beanua cathensis*. B. melanohullata. B. montantiormis (Peno et al. 2015) and B. fimbribracteata (Shui and Chen 2005).

Begonia catbensis from Vietnam

ovate to suborbicular, $9-14 \times 8-12$ mm, sparsely puberulent at the proximal margin, inner 2 glabrous, elliptic to oblanceolate, $6-8 \times 2.8-3.8$ mm; androecium actinomorphic, spherical, 5–6 mm in diameter; stamens 27–32; filaments glabrous, 1.2–1.9 mm long, fused at base, yellowish dull white; anthers somewhat greenish-yellow, narrowly obdeltoid, 1.2–1.5 mm long, widest at apex, 0.8–1 mm wide, apex obtuse, base cuneate, opened by two longitudinal slits with orange margins. Pistillate flowers: pedicel glabrous, 7–14 mm long; tepals 2, suborbicular, 8–10 mm in diameter, greenish-white when young, later turning to dull white or pure white, glabrous, margin entire or slightly undulate with sparsely puberulent at base; ovary green, glabrescent, with verrucose wings, trigonous-ellipsoid, 1-loculed; placentation parietal, with 3 placentae, each 2 branched; styles 3, fused at base or nearly free, glabrous, glossy, yellow, 3–5 mm long, apically C-shaped, stigmatic band twisted. Capsule nodding on a stipe 9–16 mm long, trigonous-ellipsoid, 13–18 mm long, 6–8 mm thick (wings excluded), fleshy, greenish when fresh, 3-winged; wings densely verrucose, unequal, abaxial crescent shaped, 7–9 mm wide, lateral 2.5–3.5 mm wide. Seeds numerous, ellipsoid, brown.

Etymology. The species is named after the type locality, Cat Ba National Park. **Phenology.** Flowering in August; fruiting in August – reported earlier.

Distribution and habitat. Cat Ba National Park is composed by many islets. All islets here are limestone mountains. We have had several days to survey in Cat Ba National Park and asked guides working in this National Park for the existence of this species, but we only found and recorded a single population here. Perhaps more populations will be found if more fieldwork is done in the future in this National Park. So far, *Begonia cathensis* is only known from the Cat Ba National Park, Cat Hai District, Hai Phong City, in northern Vietnam. Within its distribution area, the new species grows on semi-shady tops of small rocks and on steep slopes under the broad-leaved evergreen forest in lowlands of limestone hills.

Conservation status. *Begonia cathensis* is considered as a rare species because a single population with less than 200 mature individuals clustered into 10 clumps within an area of ca. 1 km² has been recorded in Cat Ba National Park. Field observation shows that the single population is strictly managed and protected by the staff of the Cat Ba National Park and the number of individuals has been stable for at least two decades. Following the guidelines of the IUCN Red List Categories and Criteria version 14 (IUCN 2019), its conservation status is currently assessed as "Endangered" (D).

Taxonomic notes. Within *Begonia* sect. *Coelocentrum, B. catbensis* is apparently closest to *B. melanobullata* and *B. montaniformis* from Vietnam (Peng et al. 2015) considering the traits of the stipules and leaves, but strikingly different from them by having dendritic (vs. unbranched) hairs on the keeled mid-rib of the stipules, petioles and abaxial veins of the lamina, 5–7 (vs. 7–9) palmate veins, 2–3-branched inflorescence with 4–10 flowers (vs. 4–6 or 3–8 branched times, with above 20 flowers), glabrous (vs. tomentose or subglabrous) peduncles, glabrous (vs. setulose or velutilous) staminate flower tepals, 2 (vs. 3)-tepalled pistilate flowers and a glabrescent (vs. sessile glandular) ovary. It resembles *B. fimbribracteata* (Shui and Chen 2005) from China in having broadly ovate leaves with adaxial conical bullae on the lamina and serrulate-ciliolate lamina margin, 2–3-branched inflorescence and glabrous bracts with fimbriate mar-

gins: however, B. cathensis is clearly distinguished by its larger leaves, $10-13 \times 5-7$ cm (vs. $6-7 \times 5-6$ cm), acuminate (vs. rotundate) leaf apex and glabrescent (vs. hairy) peduncle, staminate flower tepals and ovary. Begonia cathensis is easily distinguishable from *B. nahangensis* (Averyanov and Nguyen 2012) by its papery, asymmetric, broadly ovate leaves (vs. leathery, round to slightly asymmetric broadly oblique-ovate or oblique-reniform leaves) with an acuminate apex (vs. round to rarely broadly obtuse apex) and red setae 1.5-2 mm long along the margin (vs. white soft hairs, 3-5 mm long) and dendritic hairs (vs. villous or woolly villous hairs) on the petiole and abaxial veins and from B. variegata (Shui and Chen 2005) in having dark green or rarely brown leaves, with slightly silvery green patches along the veins (vs. dark-purple stripe near the margin of the leaves and dark brown wide bands along main veins), dendritic hairy petioles (vs. hirsute-villous), glabrous (vs. glandular hairy) peduncles, outer tepals and ovary and greenish-white to white (vs. greenish or greenish-yellow) flowers. A detailed comparison of the new species with its most morphologically similar species is listed in Table 1. To help quickly identify Begonia cathensis from its congers, a key to Begonia sect. Coelocentrum with conical bullae on their leaves is provided.

Amongst *Begonia* sect. *Coelocentrum* with a rugulose leaf surface, *Begonia catbensis* somewhat resembles *B. ningmingensis* D.Fang, Y.G.Wei & C.I.Peng and *B. retinervia* D.Fang, D.H.Qin & C.I.Peng from China (Fang et al. 2006) in leaf shape, with white maculation of the adaxial leaf surface and tepals with ciliolate margins, but it is strikingly differentiated from them in having a conically bullate (vs. rugulose) leaf surface, adaxially glabrous (vs. subsessile glandular) stipules with dendritic hairs on the mid-rib (vs. villous or glabrous), dendritic (vs. villous) petioles and abaxial veins, glabrous (vs. sparsely minute subsessile glandular) abaxial surface of staminate flower tepals and ovary and 2 (vs. 3) tepals of pistillate flower.

Identification key to *Begonia* species with conical bullae on leaf surface within sect. *Coelocentrum*

1	Dendritic hairs on petiole, along adaxial palmate veins and keeled mid-rib of
	stipule
_	Hairless or with unbranched hairs on petiole, along adaxial palmate veins and
	keeled mid-rib of stipule
2	Leaf blade with brown or dark purple maculation on adaxial surface; exterior
	surface of tepal with glandular hairs
_	Leaf blade without obvious maculation on adaxial surface; exterior surface of
	tepal without glandular hairs4
3	Leaf abaxially densely villous and tomentose, adaxially having a dark purple
	ring near the margin; peduncles and pedicels with dense glandular strigae
	B. variegata
-	Leaf abaxially sparsely long strigose, without a dark purple ring near the mar-
	gin; peduncles and pedicels subglabrous
4	Bullae 2–4 tipped
_	Bullae with a single tip

5	Leaf apex obtuse or rounded
_	Leaf apex acute to acuminate or shortly caudate7
6	Abaxial veins densely white woolly-villous; peduncle glabrous; bract margin
	entire
_	Abaxial veins laxly strigose; peduncle hairy; bract margin fimbriate
	B. fimbribracteata
7	Dense conical bullae present on all leaves; hairs on bullae tip persistent; in-
	florescence branched 4-6 times; male flowers greenish; ovary sparsely dotted
	with sessile glands
-	Conical bullae sparsely present or absent on immature leaves; hairs on bullae
	tip deciduous; inflorescence branched 3-4 times; male flowers pale pinkish-
	yellow; ovary glabrous

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RESEARCH ARTICLE



Taxonomic revision of Sageretia (Rhamnaceae) from China I: identities of S. lucida, S. thea var. cordiformis and S. yunlongensis, with the description of a new species S. ellipsoidea

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Abstract

A taxonomic revision of *Sageretia lucida, S. thea* var. *cordiformis* and *S. yunlongensis* in China is presented. *Sageretia lucida* is revised in terms of morphological characters (habit, branchlet color, phyllotaxis and rachis length), distribution, habitat, and phenology; *S. thea* var. *cordiformis* is raised to *S. cordiformis*; and *S. yunlongensis* is excluded from the genus *Sageretia* and reduced to the synonym of *Rhamnus nigricans*. Furthermore, a new species, *S. ellipsoidea*, is erected based on the paratype collections of *S. lucida*. The new species morphologically differs from *S. lucida* in having reddish brown branchlets, opposite or subopposite phyllotaxis, shorter rachises, and flowering in spring or early summer. *S. ellipsoidea* is factually closest to *S. hamosa* as they share similar woody-vine habit and larger fruit size, and fruiting in winter, whereas the former can be easily recognized based on its smaller leaf blades, fewer lateral veins, shorter rachises, and ellipsoidal or elliptic-ovoid fruits.

Keywords

Granite mountain, limestone mountain, Rhamnus, woody vine

Introduction

Sageretia Brongn., the mock buckthorn genus of Rhamnaceae, contains ca. 35 species (Chen and Schirarend 2007). The genus shows a pan-tropical distribution with most members inhabiting subtropical and tropical Asia, and a few in northeastern Africa and tropical America (Mabberley 2008; Yang et al. 2019). Generally, *Sageretia* species are shrubs or woody vines, and usually thrive in disturbed habitats which have poorly developed soils. Many members of the genus have branchlets terminating in woody thorns as a defense against herbivores, and some of them such as *S. gracilis* Drumm. & Sprague and *S. thea* (Osbeck) Johnst. are popular in bonsai gardening. Besides, the drupes of several species are edible, and the leaves are flavonoid-rich and potential substitutes for tea (Chen and Schirarend 2007; Chung et al. 2009; Hyun et al. 2015).

According to Flora of China (FOC; Chen and Schirarend 2007), a total of 19 species and 3 varieties of *Sageretia* are found in the regions south of the Qinling Mountains and the Huai River. Only one species, *S. paucicostata* Maxim., is extensively distributed northward to the Yinshan Mountains. However, two species and one variety are included in a provincial flora but absent in FOC, including *S. cordifolia* Tardieu in Flora Yunnanica (Fan 2006), *S. filiformis* (Roth) G.Don in Flora Xizangica (Chen and Zhou 1986) and Flora Yunnanica, and *S. thea* var. *taiwaniana* (Masam.) Y.C.Liu & C.M.Wang in Flora of Taiwan (Liu et al. 1993). Moreover, three species (*S. gongshanensis* G.S.Fan & L.L.Deng, *S. latifolia* Hand.-Mazz. and *S. yunlongensis* G.S.Fan & L.L.Deng) published earlier than FOC (Handel-Mazzetti 1933; Fan and Deng 1995, 1997), have not been included in FOC. Hence, together with the recently published *S. liuzhouensis* Yi Yang & H.Sun (Yang et al. 2017), 25 species and 4 varieties (19 endemic species and 3 varieties) have been recorded in China to date.

We have been studying the taxonomy, molecular phylogeny and biogeography of *Sageretia* since 2014, especially the members in China. A new species, the tropical Asian origin, and three strongly supported clades matching morphological and distributional divergences, had been reported in *Sageretia* in our previous studies (Yang et al. 2017, 2019). In this paper, we present several taxonomic problems in the genus and conduct corresponding revisions. In the process of protologue collation and specimen examination, *S. lucida* Merr., *S. thea* var. *cordiformis* Y.L.Chen & P.K.Chou and *S. yunlongensis* were found taxonomically problematic. Specifically, the paratypes of *S. lucida* distinctly differ from the type and actually represent a different species; *S. thea* var. *cordiformis* obviously diverges from *S. thea* var. *thea* in morphology and molecular phylogeny, and should be raised to a species; *S. yunlongensis* should be categorized in the genus *Rhamnus* L. rather than *Sageretia*. Thus, we here clarify the identities of these and related species.

Materials and methods

The protologues of all published names and molecularly phylogenetic studies of *Sageretia*, were carefully reviewed and collated. Specimens or digital specimen images from 26 herbaria, including A, CDBI, CSH, CZH, CSFI, E, FJSI, GXMG, HHBG, HITBC, IBK, IBSC, IMDY, K, KUN, LBG, LD, P, PE, SWFC, S, SYS, SZG, UC, US and WU (abbreviations follow Thiers 2020), were examined. In *Sageretia*, the flowers are generally too small (1–2 mm in diam.) and invariable to be used as diagnostic characters, and habit, branch, leaf and rachis characters are primarily applied in classification by contrast (Chen and Schirarend 2007; Yang et al. 2017). Despite morphological characters, habitat and phenology also perform important roles in diagnosis of *Sageretia* species. Thus, the information regarding photos in Plant Photo Back of China (PPBC, http://pbc.iplant.cn/) and Chinese Field Herbarium (CFH, http://www.cfh.ac.cn/) was also incorporated in the statistics on distribution and phenology of target species. Furthermore, field investigations were conducted at type locations of *S. lucida* and *S. thea* var. *cordiformis* to acquire real knowledge about their habit, habitat and phenology.

Results and discussion

Taxonomic treatment

Rhamnus nigricans Hand.-Mazz., Anz. Akad. Wiss. Wien. Math.-Naturwiss. Kl. 62: 234–235. 1925.

Fig. 1

Sageretia yunlongensis G.S.Fan & L.L.Deng, Sida 16(3): 477, f. 1. 1995. syn. nov. Type: CHINA. Yunnan: Yunlong County, 1300 m, 26 Oct 1987, *Expedition Team 161* (holotype SWFC!)

Type material. CHINA. Yunnan: "Beyendjing medium inter Tschuhsiung (Tsuyung) et Yungbei", 1800 m, 15 May 1915, *Hand.-Mazz. 6311* (holotype WU!; isotypes A [00051422], K [K000729152]).

Description. Evergreen vines, shrubs or small trees up to 6 m tall, dioecious. Young branches yellowish-brown pubescent; old branches scattered with tuberculate lenticels. Leaves alternate; stipules caducous; petioles 1.2-2.5 cm; leaf blades papery or thickly papery, ovate, oblong to broadly elliptic, $5-16 \times 3-7$ cm, abaxially puberulent or only on veins, adaxially usually glabrous, lateral veins 5-7 pairs, prominent abaxially, impressed adaxially, base rounded to subcordate, margin densely cartilaginous serrulate, apex acuminate to shortly caudate. Inflorescences axillary, spicate or paniculate, rachises up to 10 cm, puberulent. Flowers unisexual, 5-merous; pedicels 1-2 mm; sepals triangular; petals clawed. Drupes subglobose or globose, ca. 6 mm in diam., turning purple-black at maturity; pyrenes 2-3, asymmetrical, abaxially with a margined furrow extending over 3/4 of length.

Phenology. Flowering from May to July; ripe fruits from October to December.

Distribution and habitat. The species is distributed in southwestern China (Yunnan; Fig. 2). It grows in thickets on dry slope at elevation from 1300 to 2000 m.

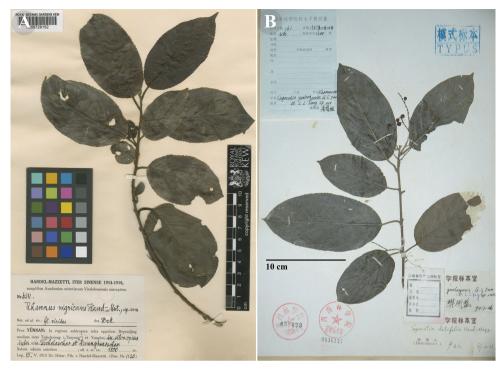


Figure 1. Type specimens of *Rhamnus nigricans* and its synonym **A** isotype of *R. nigricans* (K [K000729152]) **B** holotype of *S. yunlongensis* (SWFC). A obtained from GBIF (https://www.gbif.org/), B photographed by Y. Yang.

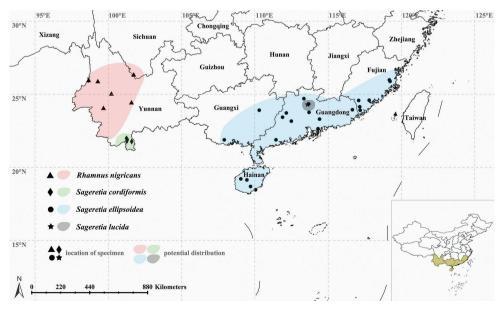


Figure 2. Distribution of *Rhamnus nigricans, Sageretia cordiformis, S. ellipsoidea*, and *S. lucida*. The sites were obtained from the specimen records, our field investigations and photo information in CFH and PPBC.

Note. Although the genera *Rhamnus* and *Sageretia* are similar in morphology, they distinctly differ in characters of inflorescence (fascicled, cymose racemes, or cymose panicles in *Rhamnus* vs. spikes or spicate panicles in *Sageretia*) and fruits (basally persistent discoid calyx tube in *Rhamnus* vs. persistent reflexed calyx or remaining inconspicuous disk in *Sageretia*). The type collection of *S. yunlongensis, Expedition Team 161*, has branched cymose panicles and fruits basally covered with discoid calyx tube, suggesting it belongs to *Rhamnus* rather than *Sageretia*. In fact, *S. yunlongensis* (Fig. 1B) extremely resembles *R. nigricans* (Fig. 1A), they share similar habit, indumentum, leaf blade shape and size, inflorescence, and fruit, and highly overlapped distribution. Thus *S. yunlongensis* is herein reduced to a synonym of *R. nigricans*.

Additional specimens examined. CHINA. Yunnan: Weishan Yi and Hui Autonomous County, 1500 m, 2012, *Weishan Expedition Team 5329271259* (IMDY); Yongde County, 1830 m, 9 Jul 2006, *E.D. Liu 170* (KUN); Shuangbai County, 1670 m, 15 Apr 1957, *W.Q. Yin 747* (KUN, LBG, PE); Lushui County, 1544 m, 12 May 2005, *Gaoligong Shan Biodiversity Survey 23964* (E).

Sageretia cordiformis (Y.L.Chen & P.K.Chou) Yi Yang, H.Sun & H.Peng, comb. & stat. nov.

urn:lsid:ipni.org:names:77217740-1 **Chinese name:** xin ye que mei teng (心叶雀梅藤) Figs 3C, D, 4A, B

S. thea var. cordiformis Y.L.Chen & P.K.Chou in Bull. Bot. Lab. North-East. Forest. Inst. 5: 74. 1979. Basionym.

Type material. CHINA. Yunnan: Xishuangbanna, Mengla County, 730 m, 28 Dec 1958, W.T. Wang 10496 (holotype KUN [1207932]; isotype KUN [1207933]).

Description. Evergreen shrubs. Branches usually alternate, armed, glabrous to puberulent; second- to fourth-year branches brown. Leaves alternate or subopposite; petioles 1–3 mm, leaf blades leathery, shiny, glabrous, cordate to ovate-orbicular, $1-3 \times 0.8-2$ cm, lateral veins 2–3 pairs, flat abaxially, impressed adaxially, base cordate or subcordate, margin crenate, apex obtuse or rounded. Inflorescences spicate or spicate-paniculate; rachises 1.5–5 (–10) cm. Flowers sessile; sepals triangular-ovate; petals clawed; stamens as long as petals. Drupes subglobose, ca. 5–6 mm in diam., from green to red and finally turn black, base with persistently reflexed calyx; pyrenes 2–3, emarginate at both ends, asymmetrical.

Phenology. Flowering in September; ripe fruits from December to January of the following year.

Distribution and habitat. The species is distributed in China (southwestern Yunnan; Fig. 2). It grows in thickets on tropical limestone mountains at elevation from 700 to 1100 m.

Note. In Flora Yunnanica, Fan (2006) reduced *Sageretia thea* var. *cordiformis* to the synonym of *S. cordifolia* (Fig. 3A, B) with no justification given. *S. cordifolia* occurs in



Figure 3. Type specimens of *Sageretia cordifolia*, *S. cordiformis* and *S. thea* **A**, **B** isotypes of *S. cordifolia* (**A**: P [P01818867]; **B**: P [06765093]) **C** holotype of *S. cordiformis* (KUN [1207932]) **D** isotype of *S. cordiformis* (KUN [1207933]) **E**, **F** isotypes of *S. thea* (E: S [S11-12914]; F: LD [1749752]). **A**, **B** and **E**, **F** obtained from GBIF, **C**, **D** from NOI (https://noi.link/).

Laos (Pakson) and factually resembles *S. thea* var *thea*. However, *S. thea* var. *cordiformis* and *S. cordifolia* differ in petiole length (1–3 mm in former vs. 4–8 mm in latter), leaf blade shape (cordate to ovate-orbicular vs. ovate-oblong to ovate-lanceolate) and size $(1-3 \times 0.8-2 \text{ cm vs. } 3.5-6 \times 1.5-3 \text{ cm})$, and number of lateral veins (2–3 pairs vs. 3–5 pairs) (Table 1). Hence, we disagree with Fan's treatment.

Based on specimen examination and our field observations, *S. thea* var. *cordiformis* and the type variety (Fig. 3E, F, Fig. 4C, D) differ in petiole length (1–3 mm in *S. thea* var. *cordiformis* vs. 2–7 mm in type variety), leaf blade texture (leathery vs. papery), and number of lateral veins (2–3 pairs vs. 3–5 (–7) pairs). In fact, Chen and Chou (1979)

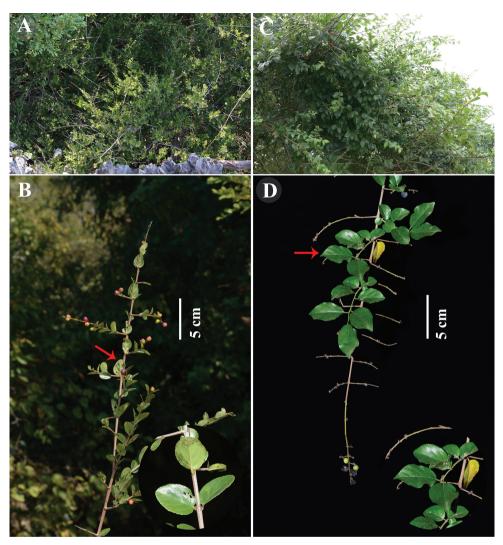


Figure 4. Field photos of *Sageretia cordiformis* (**A**, **B**) and *S. thea* (**C**, **D**) **A** and **C** habit **B** and **D** fruiting branch and leaf blades **A–C** photographed by Y. Yang, **D** by X.X. Zhu.

had clearly mentioned the sharp morphological distinctions between *S. thea* var. *cordiformis* and the type variety in the protologue. Furthermore, the results of molecular phylogenetic analyses based on five loci (ITS, ETS, *psbA-trnH*, *petA-psbJ* and *trnLtrnF*) in Yang et al. (2019) indicated the independent species status of *S. thea* var. *cordiformis* splitting from the type variety. In their study, *S. thea* var. *cordiformis*, *S. pycnophylla* C.K.Schneid. and *S. yilinii* G.S.Fan & S.K.Chen form a clade, while *S. thea* var. *thea* is in a highly supported clade with *S. subcaudata* C.K.Schneid. and *S. rugosa*. Considering the broad discrepancies between *S. thea* var. *cordiformis* and *S. thea* var. *thea* in morphology and molecular phylogeny, we raise the former to *S. cordiformis*.

	S. cordifolia	S. cordiformis	S. thea
Habitat	unknown	limestone mountains	hills or mountains
Branch color	gray	gray-brown to brownish	brownish
Petiole length	4–8 mm	1–3 mm	2–7 mm
Leaf blade	ovate-oblong to ovate-lanceolate,	cordate or ovate-orbicular, base	elliptic, oblong, or ovate-elliptic,
shape	base cordate, apex acute to caudate-	cordate or subcordate, apex obtuse	rarely ovate or nearly orbicular, base
	acuminate	or rounded	rounded or subcordate, apex acute,
			obtuse, or rounded
Leaf blade size	3.5–6 × 1.5–3 cm	$1-3 \times 0.8-2$ cm	2–4.5 × 0.7–2.5 cm
Leaf texture	leathery	leathery	paper
Lateral veins	3–5 pairs	2-3 pairs	3–5 (–7) pairs
Rachis	12–13 cm	1.5–5 (–10) cm	2–12 cm

Table 1. Comparison of habitat and morphology among *Sageretia cordifolia*, *S. cordiformis*, and *S. thea* based on field observation, herbarium collections, and photo information obtained from CFH and PPBC.

Specimens of *Sageretia cordifolia* examined. LAOS. Pakson, 1200 m, Nov 1938, *E. Poilane* 28562 (holotype P [01818865]; isotypes P [P01818866, P01818867, P06765093]).

Additional specimens of *Sageretia cordiformis* examined. CHINA. Yunnan: Mengla County, 25 Sept 1961, *Y.H. Li 3588* (KUN); 1200 m, 24 Nov 1975, *Y.H. Li 20033* (HITBC); 1000 m, 9 Sept 1959, *S.C. Pei 10046* (HITBC); 10 Sept 2004, *S.S. Zhou 2084* (PE); ca. 1000 m, 21 Dec 2015, *Y. Yang & Z. Chen xsbn03* (KUN); ca. 1000 m, 24 Dec 2016, *Y. Yang & L.S. Qian OYY001* (KUN).

Sageretia ellipsoidea Yi Yang, H.Sun & H.Peng, sp. nov.

urn:lsid:ipni.org:names:77217741-1

Chinese name: tuo guo que mei teng (椭果雀梅藤) Figs 5-7

Type material. CHINA. **Guangdong:** "Ying Tak, Taai Tsan, Wan Tong Shan" (Yingde City, Taizhen Town, Wentang Mountain), 17 Oct 1926, *W.T. Tsang & K.C. Wong 2718* (holotype IBSC [0404901]; isotype SYS [SYS00086833]); 18 Oct 1926, *W.T. Tsang & K.C. Wong 2723* (paratype IBSC [0404896]); 6 Oct 1926, *W.T. Tsang & K.C. Wong 2479* (paratype SYS [SYS00086832]).

Diagnosis. Similar to *S. hamosa* (Wall.) Brongn., but differs in having smaller leaves $(5-12 \times 2.5-4 \text{ cm in } S. ellipsoidea \text{ vs. } 8-15 (-25) \times 3.5-6 (-7) \text{ cm in } S. hamosa$), less lateral veins (5-7 pairs vs. 7-11 pairs), shorter rachises (1-3 (-10) cm vs. 6-20 (-25) cm), and ellipsoidal or elliptic-ovoid fruits (vs. subglobose or globose in *S. hamosa*).

Description. Woody vines. Branches opposite or subopposite, glabrous; first-year branches green, sometimes with hard-straight spine opposite to leaf, second- to fourth-year branches reddish brown. Leaves opposite or subopposite; petioles 8–15 mm, leaf blades leathery, ovate-oblong, oblong to elliptic, $5-12 \times 2.5-4$ cm, lateral veins 5–7 pairs, prominent abaxially, impressed adaxially, base rounded, margin crenate, apex

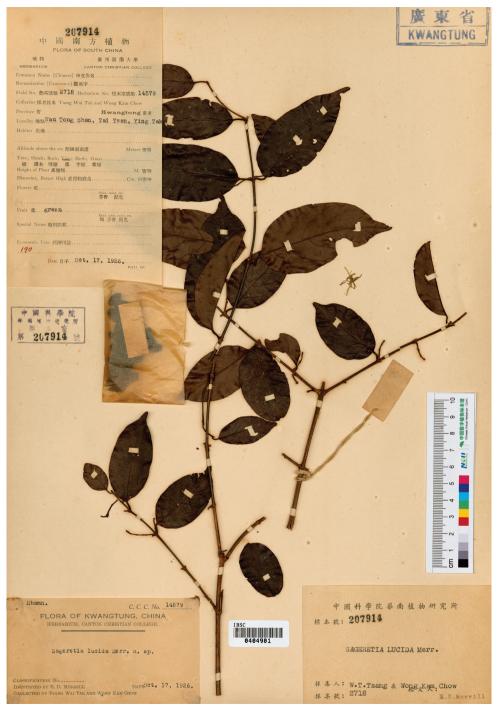


Figure 5. Holotype of *Sageretia ellipsoidea* (IBSC [0404901]). Image obtained from CVH (https://www. cvh.ac.cn/).

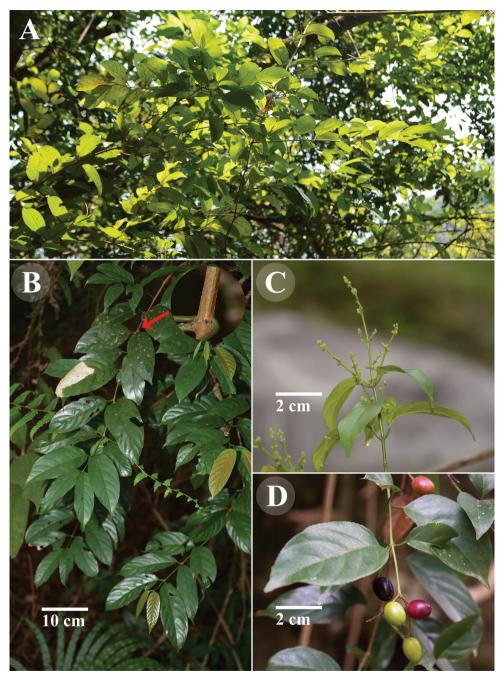


Figure 6. Field photos of *Sageretia ellipsoidea* **A** habit **B** branch **C** flowering branch **D** fruiting branch. **A–C** photographed by Y. Yang and **D** by J. Lin.

obtuse to shortly acuminate. Inflorescences usually axillary spicate, rarely spicate-paniculate; rachises 1-3 (-10) cm. Flowers subsessile, white to yellowish white; sepals triangular-ovate, ca. 1.5 mm, apex acute; petals clawed; stamens as long as petals. Drupes

Table 2. Comparison of habitat, morphology and phenology (florescence) among *Sageretia ellipsoidea*, *S. hamosa* and *S. lucida* based on field observation, herbarium collections, and photo information obtained from CFH and PPBC.

	S. ellipsoidea	S. hamosa	S. lucida
Habitat	granite mountains	non-limestone hills or mountains	limestone mountains
Habit	woody vines	wood vines	shrubs
Phyllotaxis	opposite or subopposite	alternate or subopposite	alternate
Branch color	reddish brown	reddish brown to brown	gray to dark gray
Petiole length	8–15 mm	8–15 (–20) mm	6–10 mm
Leaf blade	ovate-oblong, oblong or elliptic,	usually oblong or narrowly elliptic,	ovate-oblong to oblong, base
shape	base rounded, apex obtuse to shortly	base usually rounded, sometimes	subrounded to rounded, apex
	acuminate	cordate, apex caudate-acuminate to	acuminate
		shortly acuminate	
Leaf blade size	5–12 × 2.5–4 cm	8–15 (–25) × 3.5–7 cm	5–10 × 2.5–4 cm
Lateral veins	5–7 pairs	7–11 pairs	4–6 pairs
Rachis	1–3 (–10) cm	6–20 (–25) cm	5–10 cm
Fruits	ellipsoidal or elliptic-ovoid	globose or subglobose	unknown
Florescence	April to July	July to August	November

ellipsoidal or elliptic-ovoid, $10-12 \times 5-7$ mm, green, turning to orange-red, claret and finally purple-black, base with inconspicuous disk remains; pyrenes 1–2, emarginate at both ends, asymmetrical.

Phenology. Flowering from April to July, ripe fruits from November to January of next-year.

Etymology. This species is named for its ellipsoidal or elliptic-ovoid drupes which are different from other *Sageretia* species (subglobose or globose).

Distribution and habitat. The species is currently found in southern China (Fujian, Guangdong, Guangxi, Hainan; Fig. 2), and probably in northeastern Vietnam. It grows in moist forests along streams on granite mountains below 1200 m.

Note. When he erected the species Sageretia lucida, Merrill (1931) cited four collections, including W.T. Tsang & K.C. Wong 14340, 14579, 14584 and 15121, of which 15121 was selected as type and the other three collections were automatically treated as paratypes. However, the four numbers above belong to herbarium numbers which are ineffective nowadays, and the corresponding field numbers are W.T. Tsang & K.C. Wong 2479, 2718, 2723 and 3260, respectively. Moreover, another problem is that the paratype collections (2479, 2718 and 2723) factually represent an undescribed Sageretia species distinctly differing from S. lucida (3260) based on geological and morphological evidences. Among the four collections of S. lucida, three paratype collections were all collected from "Wan Tong Shan" (Wentang Shan) and type collection from "Chung Tung" (Zhongdong Village, about 10 km apart to the Wentang Shan). Based on field investigations, we find that Wentang Shan has granite landform while Zhongdong Village limestone landform. Furthermore, the three paratype collections are morphologically identical, but noticeably different from the type collection in terms of branchlet color (reddish brown in paratype collections vs. gray to dark gray in type collection), phyllotaxis (opposite or subopposite vs. alternate), rachis length (1-3 (-10) cm vs. 5-10 cm), and phenology (blooming in spring or early summer vs. in autumn) (seen in Table 2). Consequently, the species represented by

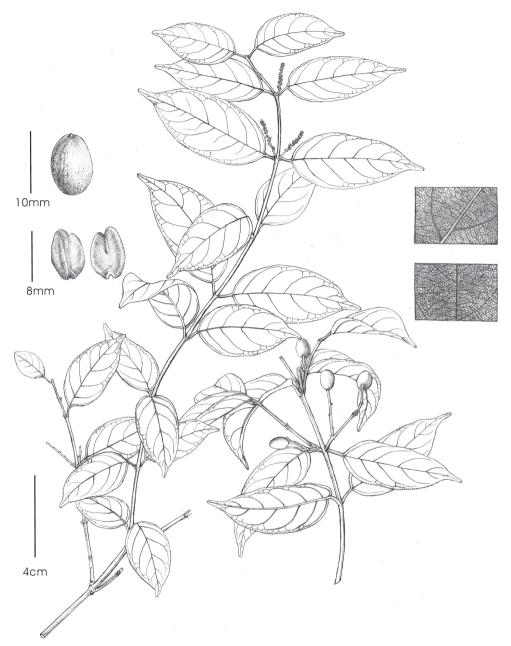


Figure 7. *Sageretia ellipsoidea* Yi Yang, H.Sun & H.Peng **A** flowering branch **B** fruiting branch **C** bottom surface of leaf blade **D** upper surface of leaf blade **E** drupe **F** pyrene (left) and seed (right).

the paratype collections of *S. lucida* is erected as a new species, namely *S. ellipsoidea* Yi Yang, H.Sun & H.Peng.

Besides, the samples of "*Sageretia lucida*" in Yang et al. (2019) factually also belong to *S. ellipsoidea*. According to Yang et al. (2019), the new species is sister to *S. hamosa* and they form an early diverging clade. In morphology and phenology, the new species

also most resembles *S. hamosa* through sharing similar habit (woody vine), larger fruit size (ca. 1 cm long or in diam.) and fruiting season (winter). Nonetheless, *S. ellipsoidea* can be easily distinguished based on its smaller leaves, fewer lateral veins, shorter rachises, and ellipsoidal or elliptic-ovoid drupes (Table 2).

Additional specimens examined. CHINA. Fujian: Minhou County, 4 Oct 2014, B. Chen & D.M. Jin CSH12700 (CSH); Nanjing County, 400 m, 19 Nov 1963, Xiamen Univ. Coll. Team 805 (PE); Pinghe County, 600 m, 23 Feb 1980, G.S. He 0475 (FJSI); Zhao'an County, 950 m, 16 Mar 2015, X.F. Zeng ZXF19893 (CZH). Guangxi: Jinxiu Yao Autonomous County, 200 m, 6 Apr 1982, Dayao Shan Expedition Team 13973 (IBSC); Shangsi County, Shiwandashan, 370-390 m, 14 Nov 2011, D.X. Nong et al. FC2011061 (GXMG). Guangdong: Dinghu District, 8 Nov 1963, G.Q. Ding & G.L. Shi 1132 (IBSC); Fengkai County, 15 Nov 1980, G.Q. Ding & G.L. Shi 6652 (CDBI); Ruyuan Yao Autonomous County, 17 Aug 1935, S.K. Lau 23948 (IBK); Huaiji County, 500 m, 26 Oct 1958, Y.G. Liu 2707 (HHBG); Yangchun City, 6 Nov 1935, C. Wang 38672 (IBK, PE); Chao'an District, 900 m, 18 Oct 2009, X.F. Zeng ZXF8404 (CZH); Conghua District, 600 m, 4 Dec 1958, L. Deng 8836 (IBK); Shenzhen City, 300-350 m, 20 Sept 2006, G.D. Wang et al. 6474 (SZG); Boluo County, 444.6 m, 1 Apr 2019, Y. Yang OYY00121 (KUN). Hainan: Baoting Li and Miao Autonomous County, 6 May 1935, F.C. How 72211 (IBK); Changjiang Li Autonomous County, 7 Jun 1934, H.Y. Liang 64162 (IBK); Baisha Li Autonomous County, 29 Apr 1936, S.K. Lau 26548 (IBK); Lingshui Li Autonomous County, 21 Oct 1956, L. Deng 2785 (KUN).

Sageretia lucida Merr. in Lingn. Sci. Journ. 7: 314. 1931. Fig. 8

Type material. CHINA. Guangdong: "Ying Tak, Taai Tsan, Chung Tung" (Yingde City, Taizhen Town, Zhongdong Village), 24 Nov 1926, *W.T. Tsang & K.C. Wong 3260* (holotype UC [319815]; isotypes A [00051501], SYS [SYS00095840], US [00094394]).

Description. Shrubs up to 3 m. Branches alternate, glabrous; second- to fourthyear branches gray to dark gray. Leaves alternate; petioles 6-10 mm, leaf blades leathery, ovate-oblong to oblong, $5-10 \times 2.5-4$ cm, lateral veins 4-6 pairs, prominent abaxially, impressed adaxially, base sub-rounded to rounded, margin serrulate, apex acuminate. Inflorescences axillary spicate; rachis 5-10 cm. Flowers sessile; sepals triangular-ovate, 1.2-1.5 mm long; petals clawed; stamens as long as petals. Fruits unknown.

Phenology. Flowering in November; fruits unknown, probably ripening from April to May of the next year.

Distribution and habitat. The species is endemic to Yingde, Guangdong, China (Fig. 2). It probably grows in thickets on limestone mountains at elevation ca. 700 m.

Note. Sageretia lucida is closest to S. henryi Drumm. & Sprague in morphology and sharing similar limestone habitats and flowering in autumn. Thus, Merrill and Chun (1940) synonymized S. lucida to S. henryi. Chen and Schirarend (2007) disagreed with the synonymization of S. lucida because S. henryi was factually compared



Figure 8. Isotype of Sageretia lucida (US [00094394]). Image obtained from GBIF.

with *S. ellipsoidea* rather than the true *S. lucida* in their study. Nonetheless, we have limited knowledge on *S. lucida* so far because of the lack of field collections, and so know nothing about the fruits. In order to get more information on the species, we

conducted investigations at the type location of *S. lucida* (Zhongdong Village) during early summer in 2016 and autumn in 2020, respectively, but failed to find any individuals. Consequently, we suggest to suspend the synonymization of *S. lucida* to *S. henryi* until more evidence has been obtained.

Additional specimens examined. CHINA. Guangdong: Yingde City, Zhongdong Village, 13 Nov 1926, W.T. Tsang & K.C. Wong 3114 (SYS).

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RESEARCH ARTICLE



On the recognition of the long neglected Vitis adenoclada Hand.-Mazz. (Vitaceae) from southern China

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from southern China. PhytoKeys 179: 29-33. https://doi.org/10.3897/phytokeys.179.65519

Abstract

This study reports the recognition of *Vitis adenoclada* Hand.-Mazz. from southern China. The species was not recognized in the *Flora Reipublicae Ropularis Sinicicae* and *Flora of China* treatments. Recent field studies and examination of herbarium collections including the type material suggest that *Vitis adenoclada* is morphologically similar to *V. heyneana*, in their densely arachnoid tomentose abaxial leaves, yet it can be easily distinguished from the latter by its red-purple glandular hairs on the young branches (vs. glandular hairs absent in *V. heyneana*) and inflorescences usually subtended by a tendril at the base (vs. only occasionally with a tendril in *V. heyneana*). *Vitis adenoclada* may be a species of hybrid origin, with the highly tomentose *Vitis heyneana* as one of the parental species, and likely the glandular-hair bearing *V. davidii* as the other parental species. *Vitis adenoclada* is recorded from southern China in Guangdong, Guangxi, Guizhou, Hunan and Zhejiang provinces.

Keywords

Grapes, species delimitation, Vitaceae, Vitis, Vitis adenoclada, Vitis heyneana

Introduction

Vitis L. (the grape genus) consists of about 75 species widely distributed in the Northern Hemisphere, with eastern Asia and North America as its current centers of diversity (Wen 2007; Wen et al. 2018a, b; Ma et al. 2020). Li (1998) and Ren and Wen (2007) did not treat *Vitis adenoclada* Hand.-Mazz., which was described from Hunan by Han-

del-Mazzetti (1925), in the *Flora Reipublicae Ropularis Sinicicae* and *Flora of China* treatments. Species delimitation of eastern Asian *Vitis* remains highly problematic (Li et al. 1996; Wan et al. 2008; Ma et al. 2018, 2020). Based on our recent field work and the examination of the type material and additional specimens, we herein propose to recognize the long-neglected *Vitis adenoclada* from southern China.

Systematics

Vitis adenoclada Hand.-Mazz., Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 62: 145. 1925.

Figures 1, 2

Vitis adenoclada Hand.-Mazz., Anz. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 62: 145. 1925. Type: China. Hunan: Xinhua Xian, near mine at Hsikwangschan (Xikuangshan), Hsinhwa (Xinhua), 600 m, 13 May 1918, in fl., *H. Handel-Mazzetti 11819* (holotype: WU, WU0029734; isotype: K, K000574937).

Description. Robust high climber 3–12 m, polygamo-dioecious. Young branches with arachnoid tomentum and red purple glandular hairs, glandular hairs hardened at maturity; tendrils bifurcate to sometimes trifurcate, stout. Leaves simple; stipules brown, narrowly triangular to ovate-triangular, $3.5-5 \times 2-2.5$ mm, membranaceous, entire; petiole 3–7 cm, arachnoid tomentose; blade oval, 7–16 × 5–12 cm, abaxially densely tomentose, adaxially pubescent, veins 7–10 pairs, base cordate to subcordate, notch obtuse, margin serrate with 20–35 teeth on each side, notch area entire, teeth fine and short, apex acute to acuminate. Inflorescence a thyrse, leaf-opposed, 7–18 cm, paniculate in shape, usually subtended by an unbranched tendril at the base; peduncle 2–7 cm, arachnoid tomentose. Pedicel 1–3 mm, pubescent. Calyx 0.1–0.2 × 0.2–0.3 mm. Petals calyptrate, 1.5–1.8 × 0.4–0.5 mm. Stamens 2–3 mm; anthers 0.6–0.7 mm. Ovary oval; style short. Fruits purple-black, globose, 9–12 mm in diam. Seeds obovoid, chalaza rounded, ventral infolds furrowed upward 1/3–1/4 from base.

Phenology. Fl. May-Jul; fr. Jul-Oct.

Distribution and ecology. In Guangdong, Guangxi, Guizhou, Hunan and Zhejiang provinces of China. 500–1015 m. Edge of forests, forests, often in limestone areas.

Additional specimens examined. CHINA. Guangdong: Huaiji Xian, 31 Oct 1958, *Y.G. Liu 02809* (IBSC). Guangxi: Jingxiu Xian, Dayaoshan, 16 km, 1015 m, 8 May 2010, floral buds, *J. Wen 11560* (US), *J. Wen 11577* (US). Guizhou: Jun 6, 1987, *BJFC Graduate Students 434* (BJFC00010633, BJFC00010634). Hunan: Hongjiang Xian, Xuefengshan, 500 m, 12 May 2014, *X.Q. Liu 114* (CCAU), *X.Q. Liu 115* (CCAU); Xinning Xian, Zhiyunshan, 13 Sep 1984, 900 m, *Zhiyunshan Exp. Team 476* (PE). Zhejiang: Nishui, 28 Jul 1959, fr, *S.Y. Zhang 6061* (KUN, PE).

Notes. *Vitis adenoclada* is morphologically similar to *V. heyneana* Roem. & Schult, with both having arachnoid tomentose abaxial leaves. It can be easily distinguished



Figure 1. General morphology of *Vitis adenoclada*, especially showing the highly tomentose leaves and the inflorescences subtended by an unbranched tendril (*J. Wen 11560*, US).

from the latter by its red-purple glandular hairs on the young branches (vs. glandular hairs absent on young branches in *V. heyneana*) and inflorescences usually subtended by a tendril at the base (vs. only occasionally with a tendril in *V. heyneana*) (Figures 1, 2). *Vitis adenoclada* may be a species of hybrid origin, with the highly tomentose *Vitis heyneana* as one of the parental species, and a glandular-hair bearing *Vitis* species, e.g., *V. romanetii* Rom.Caill. or *V. davidii* (Rom.Caill.) Foëx as the other potential parental species. Both *V. davidi* and *V. romanetii* share the unique glandular trichomes with *V. adenoclada* (see Ma et al. 2016), and *V. davidii* occurs in southern China in the same geographic area as *V. adenoclada*. We hence regard *Vitis davidii* as a more likely parental species than *V. romanetii* due to their geographic distribution and the highly

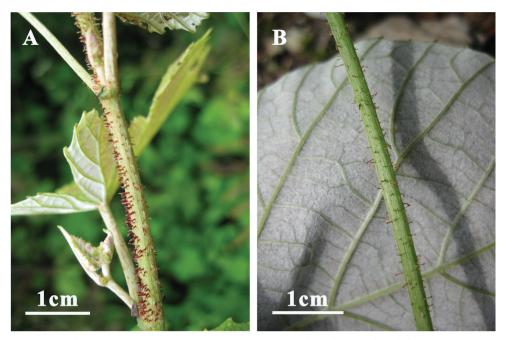


Figure 2. Characteristic glandular trichomes on young branches of *Vitis adenoclada* **A** *X.Q. Liu 114* (CCAU) **B** *X.-Q. Liu 115* (CCAU) (photo credit: Z.Y. Ma).

similar glandular trichome morphology. Ma et al. (2018, 2020) also showed that *Vitis adenoclada* and *V. davidii* grouped in one clade in the nuclear phylogeny, consistent with our hypothesis.

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RESEARCH ARTICLE



Taxonomy of *Vincetoxicum* s.str. (Asclepiadoideae, Apocynaceae) from southern Asia including three new species and resurrected names

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Abstract

This paper presents a taxonomic study of genus *Vincetoxicum* s.str. from southern Asia. Eleven regional endemic species are recognized on the basis of herbarium studies and fieldwork. Three new species are described: *V. lenifolium* **sp. nov.** (endemic to Pakistan), *V. stewartianum* **sp. nov.** (endemic to India), and *V. subcanescens* **sp. nov.** (endemic to Pakistan, Kashmir and Tibet). Three species names, *V. cabulicum*, *V. glaucum* and *V. kenouriense*, previously treated as synonyms of *V. glaucum*, *V. canescens* and *V. hirundinaria*, respectively, are resurrected. A neotype is designated for the Afghani endemic *V. cabulicum*. A lectotype is chosen from the syntypes of *V. glaucum*. We resolve the long-standing taxonomic problems in three species complexes: *V. arnottianum*, *V. luridum*, *V. sakesarense*, and *V. stocksii*; *V. glaucum*, *V. canescens* and *V. cabulicum*; and *V. hirundinaria* and *V. kenouriense*. Geo-taxonomic distinctions of southern Asian taxa are highlighted by excluding from henceforth the long misrecognized western Eurasian taxa *V. canescens* and *V. hirundinaria*. Furthermore, a detailed account of the genus including illustrations of whole plants, leaves and corona, distribution maps, a taxonomic key, morphological descriptions, synonymy, notes, and information on phenology, distribution and habitats is provided. Finally, provisional conservation assessments are provided, which indicate that *V. cardiostephanum* and *V. sakesarense* are critically endangered.

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Keywords

Afghanistan, asclepiads, endemic species, India, Pakistan, taxonomic revision, Tylophorinae, typification

Introduction

The subtribe Tylophorinae K. Schum. of Asclepiadoideae, Apocynaceae, comprises the genera *Pentatropis* R.Br. ex Wight & Arnott with five species, and *Vincetoxicum* Wolf with approximately 200 species. Liede-Schumann et al. (2012) expanded *Vincetoxicum* to include *Biondia* Schlechter, *Blyttia* Arnott, *Diplostigma* K. Schumann, *Goydera* Liede, *Pleurostelma* Baillon, *Rhyncharrhena* Mueller, and *Tylophora* R. Brown. The resulting new combinations, new names and typifications were recently published in Liede-Schumann and Meve (2018). Members of *Vincetoxicum* are distributed in Africa, Arabia, Australia and Eurasia. The plants are either erect undershrubs or twiners, often with transparent latex, leaves are simple and opposite, and flowers are mostly small with staminal corona lobes and round pollinia. The infra-generic taxa are often difficult to identify. The most important characters for identification are usually confined to the small (\pm 5 mm long) flowers. The key floral characters include color, dimensions, and internal indumentum of the corolla lobes, and shape, orientation and size of the corona lobes. The vegetative morphological features are often strikingly homomorphic among closely related species.

From the taxonomic viewpoint, Vincetoxicum is commonly considered a difficult genus, and has been confused by several authors with Cynanchum (e.g. Wight 1834; Hooker 1883; Parker 1956; Kitamura 1960; Li et al. 1995), a view opposed by others (e.g. Decaisne 1844; Boissier 1879; Bullock 1958; Rechinger 1970; Markgraf et al.1972; Browicz 1978; Liede 1996). The genus has gained attention during the past two decades along with its allies in several independent studies (e.g., Liede and Kunze 2002; Yamashiro et al. 2004a; Liede-Schumann et al. 2016; Liede-Schumann et al. 2012), leading to a new generic circumscription within Tylophorinae. Infra-generic taxonomy, however, has earned meager attention. New species discoveries from different parts of Eurasia over the past four decades (e.g. Ali and Khatoon 1982; Zaeifi 1999; Yamashiro et al. 2004b; Kidyoo 2016; Jiang et al. 2018; Shah et al. 2018) indicate that Vincetoxicum is a complex genus and that regional taxonomic revisions are important to uncover the patterns of species diversity. The need for regional taxonomic revisions is also emphasized by Liede-Schumann et al. (2012, 2016). Molecular phylogenetic studies are also fundamentally important to clarify the boundaries between cryptic species and different geographic groups.

Southern Asian members of *Vincetoxicum* s.str. have been treated periodically in different floristic and taxonomic accounts (e.g. Wight 1834; Decaisne 1844; Wight 1850; Boissier 1879; Hooker 1883; Rechinger 1970). Wight (1834) was the first on *Vincetoxicum* to describe fifteen *Cynanchum* species from British India including *Cynanchum arnottianum* Wight, *C. glaucum* Wight and *C. kenouriense* Wight.

A decade later, Decaisne (1844) moved these three species to *Vincetoxicum* whereas *C. glaucum* was synonymized with *V. canescens* (Willd.) Decaisne, a treatment which Boissier (1879) also adopted. Hooker (1883) reinstated *C. glaucum* but synonymized *V. kenouriense* (Wight) Wight with *C. vincetoxicum* (L.) Pers., a synonym of *V. hirundinaria* Medikus. Rechinger (1970) described the new species *V. cardiostephanum* (Rech. f.) Rech. f., from Afghanistan and recognized *V. glaucum* in a broader sense, merging another Afghani species, *V. cabulicum* Bornm., with the former. Hara et al. (1982) recognized *V. hirundinaria* in a much broader sense, including one additional subspecies *V. hirundinaria* subsp. *glaucum* (Wall. ex Wight) H. Hara with which Rechinger's (1970) *V. glaucum* was also synonymized.

The revision of Pakistani Vincetoxicum (Ali and Khatoon 1982) was the first detailed study on this group from southern Asia. The authors recognized six species from Pakistan including V. hirundinaria and V. canescens. These two species were recognized in a broader sense, merging Rechinger's V. glaucum into the latter. According to Ali and Khatoon (1982), these two species have a wide range from southern Asia westwards to the Mediterranean region (V. canescens) and Europe (V. hirundinaria). In a phylogenetic analysis, Liede-Schumann et al. (2016) recently recognized the Pakistani specimens as V. glaucum and distinguished them from V. canescens. The former came out as a member of the Western Himalayan subclade while V. canescens was grouped in a small Mediterranean subclade with V. creticum Browicz and V. tmoleum Boissier. The phylogenetic analysis of Liede-Schumann et al. (2016) disintegrated the V. canescens complex into the Mediterranean (V. canescens) and western Himalayan (V. glaucum) entities. Despite these advancements, to solve the V. glaucum complex, which includes plants of the so-called V. cabulicum from Afghanistan and V. glaucum from Pakistan, Kashmir, Tibet (China), India and Nepal, an inclusive taxonomic analysis is needed. Similarly, V. hirundinaria has traditionally been regarded as a variable species and spans a wide range in European Russia, Western Siberia, Turkey, the Caucasus, Kashmir, Pakistan (Hazara, Waziristan) and the Himalayas up to Sikkim (Ali 1983). Southern Asian accessions of V. hirundinaria were lacking in Liede-Schumann et al. (2016). Therefore, a comparison of accessions of V. hirundinaria throughout its range is necessary. More recently, Shah et al. (2018) elucidated the long-misunderstood complex of closely related, purple-flowered species V. arnottianum, V. sakesarense Ali & Khatoon and V. stocksii Ali & Khatoon, which resulted in the addition of a new species V. luridum Stocks ex S.A. Shah that is endemic to Balochistan (Pakistan).

This research revises *Vincetoxicum* s.str. from southern Asia. Prior to this study, a total of seven *Vincetoxicum* s.str. species were recognized in southern Asia viz., *V. arnot-tianum* (Pakistan, Kashmir and India), *V. cardiostephanum* (Afghanistan and Kurram valley of Pakistan), *V. glaucum* (Nepal westwards to Afghanistan), *V. hirundinaria* (Nepal westwards to Pakistan), *V. luridum* (endemic to Pakistan), *V. sakesarense* (endemic to Pakistan) and *V. stocksii* (endemic to Pakistan). In comparison to previous studies, we recognize 11 *Vincetoxicum* s.str. species in southern Asia (Table 1). We describe

three new species and modify previously existing descriptions. *Vincetoxicum luridum* was published just recently by Shah et al. (2018), so its description is not repeated in this paper. All species recognized in this treatment are endemic to southern Asia. The long misapplied species names *V. canescens* and *V. hirundinaria* are hereby excluded from southern Asia which breaks the connections between the southern Asian and the rest of the Eurasian, especially the western Eurasian, *Vincetoxicum* species.

Materials and methods

We morphologically examined approximately 800 herbarium specimens, including our recent collections (2015-17) from Pakistan. We conducted a total of 65 field visits to cover Himalaya, Hindukush, and Sulaiman, representing the major mountain ranges of Pakistan (Fig. 1) and extending into Afghanistan and India. We collected specimens in flowering and fruiting stages for improved characterization. We conserved live plants of V. arnottianum, V. cardiostephanum, V. kenouriense, V. lenifolium, V. luridum, V. sakesarense, V. stocksii and V. subcanescens at the Botanical Conservatory, National Agriculture Research Centre (NARC); Islamabad, Pakistan, for morphological observations and future research. We deposited our fresh collections including the type specimens in RAW and US. We used the live plants for determination of shape and dimensions of smaller structures including corona, gynostegium and calycine colleters. Colors of corollas, fruits, etc., are described from live material or herbarium labels. We carried out morphological examinations at HUP, ISL, KUH, PPFI, PMNH, RAW, and US and received herbarium loans from B, BM, GH, GOET, K, MO, and NY at US. We also retrieved digitized herbarium specimens from the databases JS-TOR Global Plants (https://plants.jstor.org/), E, and P and used either the website's measurement system or IMAGEJ for the downloaded images. Herbarium acronyms follow Thiers (2020). We studied the type specimens of the previously known names V. arnottianum, V. canescens, V. hirundinaria, V. kenouriense, V. sakesarense, V. stocksii, and V. glaucum. The type specimen of V. cabulicum was destroyed in B during World War II, therefore, we studied other specimens from the same area and designated a neotype. We followed the terminology of Ali and Khatoon (1982) for floral characters, and Harris and Harris (1994) for general morphological characters. For illustrations of the whole plant, we mostly used fresh plants and sometimes herbarium specimens. We also illustrated the leaf outlines mainly from fresh plants. During illustrations of corona types, we ensured the best angles that help determine the accurate shape of corona lobes and their orientation. We assessed the conservation status following the IUCN Red List Categories and Criteria (IUCN 2019) using the GIS-based method of Moat (2007) as implemented in the online assessment tool GeoCat (http://geocat.kew.org). The Extent of Occurrence (EOO) measures the range of the species, and the Area of Occupancy (AOO) represents the number of occupied points within that range based on the default grid size of 2 km².



Figure I. Habitats of *Vincetoxicum* species **A–D** *V. arnottianum* **E–G** *V. cardiostephanum* **H–J** *V. kenouriense* **K** *V. lenifolium* **L** *V. luridum* **M, N** *V. stocksii* **O, P** *V. subcanescens.*

General description of southern Asian Vincetoxicum s.str.

Description of vegetative parts

South Asian *Vincetoxicum* s.str. species are mostly found in mountains predominantly at higher elevations ranging from 750 to 3650 m. The plants are perennial, growing one to several stems from the rhizomatous base in spring and becoming dormant in winter. They lack milky latex and have clear latex instead. Stems are mostly longitudinally striate and pubescent all around or along one to two lines. Multiple patterns of pubescence are often found in the same species. Pubescence may be dense or sparse depending on the species. Trichomes are exclusively uniseriate, 3–5 celled, falcate or straight, with the terminal cell sometimes inflated. The phyllotaxis is opposite and decussate. However, some plants exhibit leaves in whorls or pseudo-whorls of 3–6 leaves. Laminae are discolorous and of different shapes ranging from linear to broadly ovate. Margins are always smooth. The shape of the apex and base vary depending upon the species. Laminae of the purple-flowered *Vincetoxicum* species are conduplicate. The adaxial veins, especially the midrib, are pubescent in most taxa. A cluster of small glandular structures, called colleters, are found at the bases of the laminae on the adaxial surface. Depending on the species, the remainder of foliar veins and surfaces are either glabrous or variously hairy. Foliar trichomes resemble those on the stems. Flowers are typically clustered in dense pseudo-umbels either sessile or sub-sessile or both. The pubescence on peduncles is identical to that of the stem in density, but that of the pedicels is often different.

Description of reproductive parts

The sepals are fused basally into a short calyx tube and the calyx lobes are usually adpressed to the corolla. The corolla forms a short, usually glabrous tube. The corolla lobes are either green, whitish green, purple or purple in the lower half and green up to the apex. They are either free from each other to the apex or twisted clockwise up to the apices that enclose the internal parts (in V. arnottianum and V. sakesarense only). Inside, the corolla lobes are either variously pubescent or glabrous; rarely, the trichomes are caducous. The corona is composed of five lobes basally attached to the stamens, hence called staminal corona. The corona is mostly prominent and separate from the stamens except at the base. It is the most important structure from a taxonomic point of view. However, it is difficult to study because of its small size, usually less than one millimeter in dimension. Mostly, it is fleshy and loses water content during herbarium pressing, which somewhat alters the shape and size of the lobes. Studying dried as well as fresh and/or rehydrated flowers is the most precise way of determining the shape and size of corona lobes. Another important character is the length ratio of gynostegium and corona lobes. The gynostegium possesses pollinaria in the anther locules. Each locule possesses one pollinium. The orientation of pollinaria should be noted during dissection of flowers.

The fruit is a typical asclepiad follicle. The two ovaries, if successfully pollinated, develop into paired follicles. The follicles are fused at the base, but diverge at various degrees. Each follicle contains 10–25 seeds attached to a central axis. Seeds are dispersed by wind with the help of a coma, i.e. a tuft of approximately 2–3 cm long white hairs attached to the seed apex. Margins of seeds (wings) are thin all around except at the apex.

Taxonomic treatment

Southern Asian Vincetoxicum s.str.

Rhizomatous perennial undershrubs, sometimes herbaceous, up to 1 m tall, latex clear. Stems erect, round, longitudinally striate, glabrous or pubescent all-around with simple uniseriate trichomes or along 1–2 longitudinal lines, internodes 1–13 cm long. Leaves simple, mostly opposite, rarely both opposite and 3–4-whorled (only in *V. arnottianum*), decussate, lower and upper leaves smaller than middle ones, spreading or rarely pendent (only in *V. cardiostephanum*), petiolate or rarely sub-sessile

(only in V. hirundinaria and V. cabulicum); petioles 1-18 mm long, mostly pubescent along the adaxial channel or all around or glabrous; lamina variously shaped, mostly narrowly ovate to broadly ovate, $1.5-13 \times 1-6.5$ cm; apex acute to shortly acuminate; margins simple; secondary veins opposite or sub-opposite, 8-14 on each side of midrib, eucamptodromous, normally conspicuous, sometimes inconspicuous, rarely highly prominent (only in *V. kenouriense*); leaf surface glabrous or with different indumentum types of uniseriate simple trichomes: sub-glabrous, pubescent, densely pubescent, sub-canescent. Inflorescences lax to dense-flowered pseudoumbels, mostly sessile, sometimes pedunculate, rarely long-pedunculate; peduncles glabrous or pubescent. Flowers pentamerous except carpels, pedicellate; calyx green, persistent in fruiting, polysepalous except base, lobes 1-2 mm long, margins thin, abaxial surface pubescent or glabrous, calycine colleters single or in pairs, rarely in threes; corolla lobes ovate to oblong to rarely oblong-linear (in V. arnottianum), 2-5 × 1-2.5 mm, straight or twisted clockwise, variously coloured: mostly purple, but also green, bicolored (only in Balochistani species) or yellowish, internally glabrous or pubescent or bearded; corolla tube rarely pubescent inside (only in V. cardiostephanum); gynostegial corona lobes variously shaped: clavate, deltoid, deltoid-rhomboid, subulate, oblong, obovate, ovate, rhomboid, triangular, shorter or longer than or equal in length to the gynostegium, apices of corona lobes round or acute or fimbriate (only in V. glaucum), convergent or divergent, bases connected by soft basal tissues; pollinaria pendent, corpusculum mostly loosely embedded in the five apical corners of the gynostegium (but deeply embedded in V. glaucum), caudicles mostly ascending from corpuscular to pollinarium end, rarely slender along the entire length (only in V. luridum), whitish, pollinia yellow. Stamens with thin apical anther appendages covering the gynostegium apex and pollinia. Follicles narrowly fusiform with mostly acuminate apex, $4-9.5 \times 0.4-13$ cm, inconspicuously striate, mostly glabrous at maturation, sometimes sparsely pubescent. Seeds mostly ovate, 4-10 × 2.2–7.5 mm, brown, winged on all sides except at apex, wing up to 1 mm wide, apex comose; coma 2-3 cm long, white.

A taxonomic key to southern Asian Vincetoxicum

1	Leaves strongly discolorous, tertiary and quaternary veins prominent; flowe	rs
	green, corona lobes triangular	se
_	Leaves weakly discolorous, tertiary and quaternary veins inconspicuous; flow	7-
	ers green or purple or bicolored, corona lobes not triangular	2
2	Flowers and leaves pendent, corolla tube pubescent internally	•••
	V. cardiostephanut	n
_	Flowers and leaves not pendent, corolla tube glabrous internally	3
3	Corolla lobes glabrous within	n
_	Corolla lobes hairy within	4
4	Corona lobes exceeding gynostegium in length	5
_	Corona lobes not exceeding gynostegium in length	8

5	Apices of corona lobes convergent; leaves hairy on both sides6
_	Apices of corona lobes divergent; leaves not hairy on both sides7
6	Plants densely pubescent; corona lobes ovate; follicles up to 6 cm long
	V. luridum
_	Plants sparsely pubescent; corona lobes subulate; follicles up to 9.5 cm
	long
7	Leaves narrowly to broadly ovate; corona lobes longer than broad; toothed
	V. glaucum
_	Leaves lanceolate-ovate to elliptic-ovate; corona lobes rhomboid, broader
	than long
8	Leaves narrowly to broadly ovate, sub-canescent; corona lobes obovate9
_	Leaves narrowly to mostly lanceolate-ovate; corona lobes not obovate10
9	Leaves subsessile, corolla and inner floral parts purple
_	Leaves petiolate, corolla and inner floral parts green
10	Corona lobes rhomboid-deltoid
_	Corona lobes deltoid

1. *Vincetoxicum arnottianum* (Wight) Wight, Icones Pl. Ind. Or. t. 1614. 1850. Figs 2G–J, 3A, 4A, B, 5, 6

Type. INDIA. Kashmir: 'In itinere ad Cashmere', *Royle, 21* (*Holotype*: K! [K000872738]). Undershrubs, up to 100 cm tall. Stem striate, pubescent along one narrow line throughout the stem or alternating along two lines, sometimes both single and double lines found, internodes 0.5–10 cm long. Leaves usually opposite, rarely 3–4 whorled; petioles 2-13 mm long, pubescent along adaxial channel, abaxially glabrescent, rarely pubescent all around; lamina discolorous, narrowly ovate to lanceolate-ovate, 3-13 × 1-3.5 (-5) cm; apex acute to narrowly acute; base obtuse; veins visible on both sides, sometimes inconspicuous, secondary veins 8-10 (-14) on each side of midvein; both surfaces and abaxial veins glabrous, adaxial veins pubescent; margins sparsely pubescent. Inflorescences sessile, very rarely pedunculate; peduncles up to 1.5 cm long, pubescent; bracts linear with ciliate margins, ±1 mm long; pedicels 1-5 mm long, pubescent along single or double lines, sometimes glabrous. Flowers $4.5-5.5 \times 2-3$ mm; sepals gradually tapering to narrowly acute or acuminate apices, up to 2 mm long, margins ciliate, sometimes pubescent on abaxial surface; calycine colleters 5 or 10, unequal in length when paired; corolla dark purple, corolla tube ca. 1 mm long, lobes twisted clockwise, bearded over the whole surface within except for the lateral margin, oblong, gradually tapering to the apex, $2.5-4 \times 1-2$ mm; corona deltoid-rhomboid, broader than long, $0.6-0.8 \times 0.8-1$ mm, reaching the bases of the staminal appendages in length or rarely equalling the gynostegium, divergent. Follicles fusiform, $4-7.5 \times$ 0.8-1 cm, apex acuminate, surface glabrous, striations inconspicuous. Seeds reddish brown, $6-8 \times 3-4.5$ mm, wings up to 1 mm broad, brown dots rarely distantly present; coma up to 2 cm long.

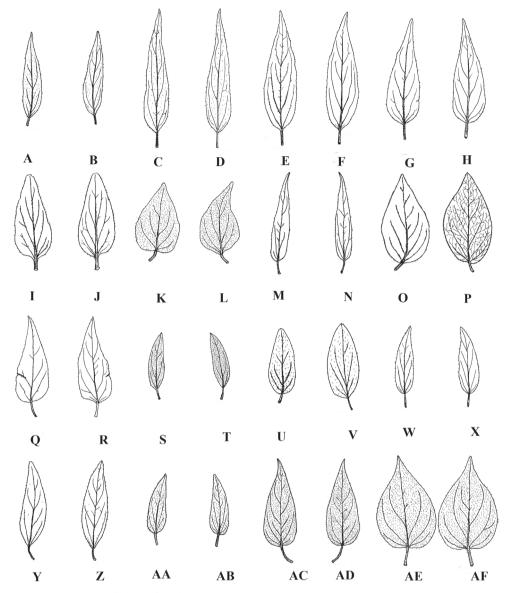


Figure 2. Outlines of leaves of southern Asian Vincetoxicum. All illustrated specimens are housed at RAW. The first image in each pair corresponds to the adaxial surface and the second to the abaxial surface. All leaves have been taken from the middle of the stem **A**, **B** Vincetoxicum sp. aff. arnottianum from S.A. Shah SAS-9 **C**, **D** Vincetoxicum sp. aff. arnottianum from S.A. Shah SAS-9 **C**, **D** Vincetoxicum sp. aff. arnottianum from S.A. Shah SAS-9 **C**, **D** Vincetoxicum sp. aff. arnottianum from S.A. Shah SAS-9 **C**, **D** Vincetoxicum sp. aff. arnottianum from S.A. Shah & F. Rahman SAS-3 **I**, **J** V. arnottianum from S.A. Shah & F. Rahman SAS-3 **I**, **J** V. arnottianum from S.A. Shah & B. Ali SAS-4 **K**, **L** V. cabulicum from D. Podlech 15814 **M**, **N** V. cardiostephanum from S.A. Shah, M. Turi et al. SAS-40 **O**, **P** V. kenouriense from S.A. Shah & L. Ahmad SAS-37 **Q**, **R** V. lenifolium from S.A. Shah SAS-44 **S**, **T** V. luridum from S.A. Shah & I. Ahmad SAS-23 **U**, **V** V. lenifolium from N. Ali 1029 **W**, **X** V. sakesarense from I. Ahmad 27821 **Y**, **Z** V. stewartianum from R.R. Stewart 16709 **AA,AB** V. stocksii from B. Gul, N. Khan et al. SAS-21 **AC,AD** V. subcanescens from A. Sultan, Z. Saqib et al. SAS-52 **AE,AF** V. subcanescens from S.A. Shah & A. Ullah SAS-8. Drawing by M. Saleem and S.A. Shah.

Species	AOO (km ²)	EOO (km ²)	Provisional conservation	Endemic to
			assessment (IUCN 2017)	
Vincetoxicum arnottianum	88	30,680	NT	Pakistan, India
V. cabulicum	20	11,583	VU	Afghanistan
V. cardiostephanum	12	53	CR	Afghanistan, Pakistan
V. glaucum	20	16,215	DD	India, Nepal
V. kenouriense	116	263,512	LC	Pakistan, India, Nepal, Bhutan
V. lenifolium	16	836	EN	Pakistan
V. luridum	52	28,013	NT	Pakistan
V. sakesarense	8	-	CR	Pakistan
V. stewartianum	4	-	DD	India
V. stocksii	36	6,123	VU	Pakistan
V. subcanescens	76	67,173	LC	Pakistan, India, Tibet (China)

Table 1. Provisional conservation assessment and areas of endemism of southern Asian Vincetoxicum.

Distribution and habitat. Based on the number of past collections and our field observations, this is the most commonly occurring species of *Vincetoxicum* in southern Asia. It is strictly western Himalayan in distribution and found in India (Himachal Pradesh), Kashmir and Pakistan (Azad Jammu, Kashmir and Hazara Division). It is a deep rooted plant with a thick root stock found on open, sunny mountain slopes in association with grasses or other herbaceous flora. Its elevation ranges from 750 to 2800 m.

Phenology. Flowering from April to September and fruiting from May to October. **Provisional conservation status.** Near threatened (Table 1). *Vincetoxicum arnottianum* is found in highly clumped and distant populations. Its populations have been mostly found in areas less than 100 m² in size and consist of less than 100 plants.

Notes. Vincetoxicum arnottianum has been confused with closely related entities for a long time. However, in recent studies (Ali and Khatoon 1982; Shah et al. 2018), those entities are regarded as separate species, namely V. lenifolium (this paper), V. luridum, V. sakesarense and V. stocksii. The inter-species relationships within the V. arnottianum complex (except V. lenifolium) were discussed in Shah et al. (2018). However, there are two associated problems that still need more clarification. Firstly, the type specimen of V. arnottianum was collected from western Himalaya, but the drawings on the herbarium sheet depict characters of a Balochistani species V. luridum, that we recently introduced as a new species in Shah et al. (2018). We hereby present our detailed observations to clarify this first problem.

Robert Wight provided two descriptions of *V. arnottianum*, notably in the protologue (Wight 1834), and a revised description (Wight 1850). In the former, he described *V. arnottianum* as a glabrous plant with oblong, obtuse or emarginate leaves, sessile umbels, 5-fid internally hairy corolla lobes, 5-fid corona lobes (shape not provided) equal to the gynostegium. The type designated was "In itinere ad Cashmere, Royle (K [K000872738])". The locality mentioned means "on the way to Kashmir". Kashmir (India) is part of the western Himalaya. For the revised description, Wight acquired specimens from Dr. Stocks collected from Balochistan and named them *V. luridum*. The name "*Vincetoxicum luridum*" was limited to those specimens and Stocks did not publish the species. Three specimens of that collection are housed in K, with

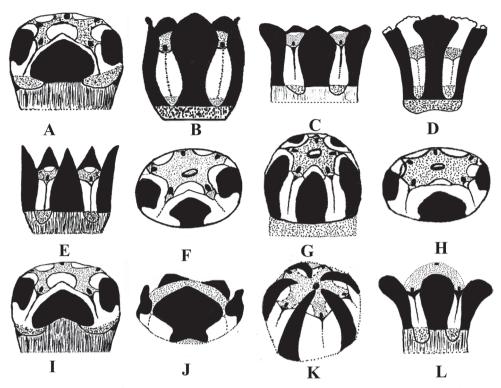


Figure 3. Illustration of corona types observed in southern Asian Vincetoxicum. The dark coloured shapes show corona lobes A Vincetoxicum arnottianum from S.A. Shah & B. Ali SAS-4 (RAW) B V. cabulicum from W. Koelz 11764 (US) C V. cardiostephanum from S.A. Shah, M. Turi et al. SAS-40 (RAW) D V. glaucum from W. Dudeeon & L. A. Kenoyer 56 (MO) E V. kenouriense from S.A. Shah & L. Ahmad SAS-37 (RAW) F V. lenifolium from S.A. Shah SAS-44 (RAW) G V. luridum from S.A. Shah & I. Ahmad SAS-23 (RAW) H A variable specimen of V. lenifolium from N. Ali 1029 (RAW) I V. sakesarense from I. Ahmad 27821 (RAW) J V. stewartianum from R.R. Stewart 16709 (RAW) K V. stocksii from J.F. Duthie 18918 (RAW) L V. subcanescens from S.A. Shah & A. Ullah SAS-8 (RAW). Drawing by M. Saleem and S.A. Shah.

K001235295 chosen as holotype of *V. luridum* (Shah et al. 2018). Wight (1850) regarded both K000872738 and Stocks' collections as one species as opposed to Stocks' view. Therefore, in the revised description, Wight (1850) added the following characters: suffruticose, climbing, branches terete, leaves succulent, umbels subsessile, manyflowered, stigma apiculate. In the geographic distribution, he mentions "Beluchistan" (correct spelling is Balochistan or Baluchistan). According to our observations, these revised characters predominantly belong to the Balochistani collections. Therefore, Wight's (1850) revised description is based on two different taxonomic entities. Balochistan is located in south-western Pakistan, not in the Himalayas, and has its own mountain ranges far away from Kashmir (see distribution map in Shah et al. (2018). Wight (1850, p. 17) acknowledged Dr. Stocks for his collections and stated: "*This species was first taken up from rather imperfect specimens, whence some alterations have here been found necessary to adapt the character to the species. I am indebted to Dr. Stocks for*

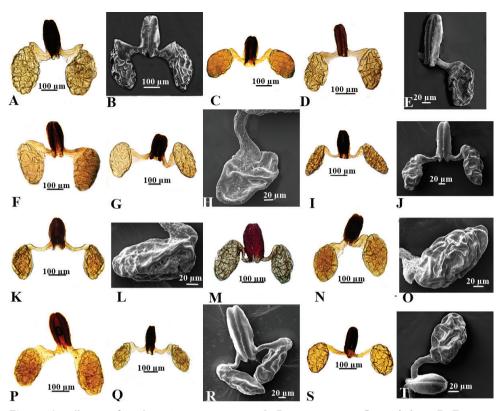


Figure 4. Pollinaria of southern Asian Vincetoxicum A, B V. arnottianum C V. cabulicum D, E V. cardiostephanum F V. glaucum G, H V. kenouriense I, J V. lenifolium K, L V. luridum M V. lenifolium N, O V. sakesarense P V. stewartianum Q, R V. stocksii S, T V. subcanescens.

the specimens from which the drawing and revised character were taken". On the other hand, one of the Stocks' specimens (K001235295) has a manuscript note in Stocks' handwriting "figured by Wight in his Icones from my specimens as his Vincetoxicum arnottianum I doubt". Wight used the term "climbing" in the revised description because he observed Stocks' specimens. He did not use this term in the 1834 description. The illustration provided in 1850 depicts a somewhat climbing habit. The type of V. arnottianum K000872738 does not show a climbing habit. The term "suffruticose" and other characters of the revised description, and some floral and pollinarium drawings were provided on a label of the type specimen K000872738. These observations further indicate that the revised characters as well as the drawings including the pollinarium pertain to Stocks' specimens (Balochistan). The illustration provided in Wight (1850) was also drawn from Stocks' specimens and perfectly resembles the Balochistani collections, not the western Himalayan specimens.

During this study, we thoroughly examined the type specimen of *V. arnottianum*, K000872738, the types of *V. luridum* (holotype K [K001235295], isotypes: K [K001235294], [K001235296]) as well as almost all *Vincetoxicum* collections



Figure 5. Field photographs of *Vincetoxicum arnottianum* **A** habit **B** leaves and inflorescences **C** flowers **D** follicles. Photos by S.A. Shah.

from western Himalaya and Balochistan. The type of *V. arnottianum* resembled western Himalayan specimens in its characters and differed from those of Balochistan. Furthermore, the specimens of western Himalaya do not match those of Balochistan in morphological characters and the two areas are geographically distant and climatically different. These findings led us to our decision in Shah et al. (2018) to introduce *V. luridum* as a new species and maintain K000872738 as the type of *V. arnottianum*. Our detailed morphological comparison is provided hereby.

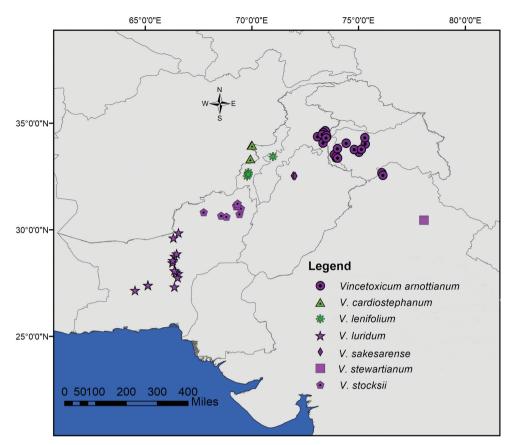


Figure 6. Geographic distribution of southern Asian *Vincetoxicum*. The colour of the symbols corresponds to the colour of the corolla.

1. The type K000872738 is glabrous, which is a character of *V. arnottianum* (western Himalayan) as also mentioned in the protologue by Wight (1834). *Vincetoxicum luridum* is densely hairy.

2. In K000872738, the inner surfaces of the corolla lobes are bearded, not pilose like in *V. luridum*.

3. In K000872738, the inflorescences are not many-flowered like those of *V. luridum*.

4. In K000872738, the corolla is dark purple as mentioned in the protologue by Wight (1834). In *V. luridum*, the corolla lobes are bicolored (lower half purple + upper half green). In this taxon, as well as in *V. stocksii*, flower color can only be correctly determined in fresh flowers.

5. In K000872738, the corolla lobes do not seem twisted because the flowers are immature and pressed. However, we have observed twisted corolla lobes in *V. arnot-tianum* while studying fresh flowers. In K000872738, the apex of the flowering bud is acute rather than obtuse as in *V. luridum*. A twisted corolla is never found in the Balochistani specimens belonging to *V. luridum*.

Secondly, we have observed significant morphological variation in certain populations of V. arnottianum. In these populations, plants are comparatively short (ca. 1 feet or rarely up to 2 feet), leaves are either pubescent (Fig. 2A–D) or glabrous on both sides (Fig. 2E-H), flower colour either purple or rarely whitish green, corolla length varies from 2 to 5 mm, corona shape is either rhomboid or deltoid-rhomboid or rarely deltoid. The geography of these populations is different from the typical V. arnottianum. They occur in Rawalpindi district (Punjab province, Pakistan) and in Malakand division (Khyber Pakhtunkhwa province, Pakistan) comprising districts Buner, Chitral, Upper Dir, Lower Dir, Malakand, Shangla and Swat. Geographically, Malakand Division represents the eastern Hindukush mountain range and borders Afghanistan in the west. The major geographic disjunction between Malakand division and the range of typical V. arnottianum (India: Himachal Pradesh, Kashmir; and Pakistan: Azad Jammu, Kashmir, and Hazara Division) is the River Indus. Therefore, in this study, we just highlight this problem and propose to identify the plants possessing the above-mentioned variations as Vincetoxicum sp. aff. arnottianum. We also recommend further detailed studies preferably including a molecular analysis using variable markers to determine whether these populations could potentially prove one or more new taxa in the purple-flowered Vince*toxicum* species complex. We cite representative specimens of these populations below.

Specimens examined. INDIA. Masrund, Chamba State, 21 June 1917, 5000 ft., *R.R. Stewart 2317* (RAW; MO); Bank of Ravi between Tiari & Siunn in dry places, Chamba State, 5000 ft., 04 July 1919, *R.N. Parker s.n.* (GH).

PAKISTAN. Azad Jammu and Kashmir (AJK): Mountain slope near Deri Nala, 2500 ft., 06 May 2015, S.A. Shah & B. Ali SAS-4 (RAW); Mandi, Kotli, 27 April 1954, A. Rashid, E. Nasir, R.R. Stewart 27007 (RAW; BM); Kotli (Azad Kashmir), ±2700 ft., 03 June 1977, Shahzad & Nisar 54346 & 54347 (ISL); Kotli (Azad Kashmir), 27 April 1954, J. Muhammad 16636 (ISL); Doongi (Kotli), ±2500 ft., 01 June 1977, Shahzad & Nisar 54341 & 54342 (ISL); Mausooh (Kotli, Azad Kashmir), 30 April 1977, Shahzad & Nisar 50160-50162 (ISL); Nawal Nadi, Poonch, ±2700 ft., 18 June 1977, Shehzad & Nisar 56415 & 56416 (ISL); Khohi Ratta (Kotli), 01 June 1977, Shahzad & Nisar 54344 & 54345 (ISL); Muzaffarabad, ±3200 ft., 12 December 1975, J. Muhammad 34527 (ISL); Zamanabad (Muzaffarabad), 25 April 1978, S. Iqbal & W. Rehman 89644, 89646 & 89647 (ISL); Datta to Rara, (Muzaffarabad), 27 April 1978, S. Iqbal & W. Rehman 89650 & 89651 (ISL); Kashmir: B-8 Pahlgam, ca. 8000 ft., s.d., R.R. Stewart 5357 (K); Kashmir, Kullogam, s.d., H. Falconer 2743 (K); Kashmir, Shapiyon, 7000 ft., s.d., C.B. Clarke 28584 A, C (K); Near Shapiyon, 6000 ft., s.d., J.R. Drummond 13948 (K); Hab River banks, Lidar Valley, Kashmir, 06 June 1939, 7000 ft., J.F. Ludlow 74 (BM); Mountains above Istahal River [Kashmir], 1915, 5000 ft., Mrs. P. Decie s.n. (BM); Tanmarg [Kashmir], s.d., A.R. Nagshi 6343 (KASH); Sonamarg, Kashmir, 9200 ft., August 1928, R.R. Stewart 13101 (MO); Kashmir, s.d., H. Falconer [?], s.n. (GOET [GOET020086]); Khyber Pakhtunkhwa: Darra, mountain slope, 3940 ft., 27 September 2016, Faizan, A. Majid & S.A. Shah SAS-47 (RAW, US); Balakot (Kaghan valley), 6 July 1954, Ch.S. Ali s.n. (RAW); Balakot-Shogran Road, 28 June 1952, I.I. Choudhri 13511 (RAW); Abbottabad, ca. 4500 ft., 01 June

1928, R.R. Stewart 295 (KUH); Parhana [Abbotabad, KPK], 11 May 1976, Shaukat & Nisar 18472 (ISL); Parhana, Hazara, 08 May 1976, M.A Siddiqi, Shahzad, Ashraf, Manzoor, Maqsood & Dilawar 22587, 22588 & 22589 (ISL); Nari, 4 miles from Abbottabad, [KPK], 29 May 1976, M.N. Chaudhri, M.A. Siddiqi, Shehzad, Ashraf, Maqsood, Lal & Akram 22597, 22598 & -22599 (ISL; GH[Col. No. 1122]); Bhonja [Mansehra], Hazara, 12 June 1976, Shaukat & Nisar 22600 & 22601 (ISL); Balakot, Hazara, 22 April 1978, S. Iqbal & N. Ahmad 89641 (ISL); Shinkiari [Mansehra], 3500 ft., 30 May 1967, E. Nasir, Siddiqi & Zaffar 4422 (KUH).

Representative specimens of the doubtful taxon Vincetoxicum sp. aff. arnottianum. PAKISTAN. Khyber Pakhtunkhwa: Buner: Elum near Kalakhela, 860 m, 23 July 2015, S.A. Shah & F. Rahman SAS-11 (RAW); Buner: On the way from Ghazi Kot to Mah Banr, 1700 m, 26 April 2015, S.A. Shah & F. Rahman SAS-3 (RAW); Swat: Karakar, road side, 1300 m, 24 July 2015, S.A. Shah SAS-12 (RAW); Mansehra: mountain slope near Bhonja village, 5740 ft., 28 September 2016, S.A. Shah SAS-46 (RAW); Swat: Sherpalam, mountain slope under Pinus trees, 3310 ft. 10 July 2016, S.A. Shah SAS-35 (RAW); Swat: Fizaghat road side near Darul Qaza, 1050 m, 10 May 2015, S.A. Shah SAS-5 (RAW); Swat: Alam Ganj village, mountain slope, 1250 m, 20 July 2015, S.A. Shah SAS-9 (RAW); Swat: Fatehpur, mountain slope, 2500 m, 28 September 2015, S.A. Shah SAS-18 (RAW); Swat: Ghalegay, road side, 2790 ft, 07 May 2016, S.A. Shah SAS-31 (RAW); Ayubia, pipeline track, 7545 ft., 04 September 2016, R. Khan SAS-41 (RAW); Malakand: mountain slope near Butkhela bazaar, 2460 ft, 07 May 2016, S.A. Shah SAS-29 (RAW); Punjab: Rawalpindi: Punjar, road side, 2250 ft., 15 May 2016, S.A. Shah SAS-25 (RAW); Rawalpindi: Murree, Masyari, [6230 ft.], 01 August 2016, Shakeel SAS-35 (RAW!).

2. Vincetoxicum cabulicum (Bornm.) Bornm. ex S.A. Shah

Figs 2K, L, 3B, 4C, 7

Cynanchum cabulicum Bornm., Bot. Jahrb. Syst. 66: 233. 1934.

Type. AFGHANISTAN. On Mt. Babour, 1800 m, 1 May 1929, *Manger s.n.* "flowers yellowish-green, inconspicuous" (*holotype* B., destroyed). *Neotype*, designated here: AFGHANISTAN. Paghman, 7500 ft., dry slope; clumps; 3 ft. high; flr. greenish, often tinged madder; 26 June 1937, *W. Koelz 12076* (US! [US03264725]).

Undershrubs, ca. 45 cm tall. Stem striate, sub-canescent all around, internodes 2.5-7 cm long. Leaves subsessile, rarely petiolate; petioles 3-8 mm long, sub-canescent all around; lamina discolorous, narrow to broadly ovate, $3-7 \times 2-4.5$ cm, both surfaces including veins and margins sub-canescent; apex acuminate; margins smooth; base sub-cordate-round; veins visible on both surfaces, secondary veins up to 8 on each side of midvein. Inflorescences sessile or pedunculate; peduncles 1-2 cm long; bracts minute, ca. 1 mm long, margins ciliate; pedicels 3-6 mm long, sub-canescent; sepals tapering to acute apices, sub-canescent, ca. 1 mm long; corolla purple, corolla tube

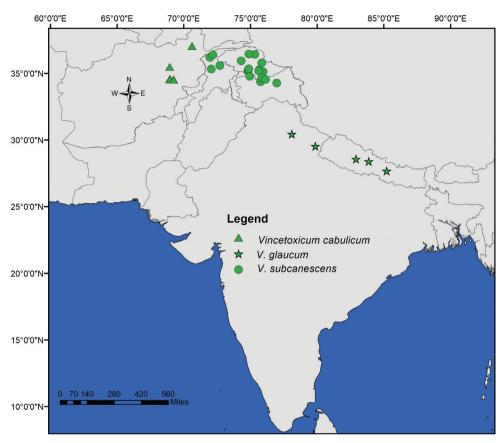


Figure 7. Geographic distribution of Vincetoxicum cabulicum, V. glaucum and V. subcanescens.

1 mm long, lobes oblong-ovate, straight, 2–2.5 mm × 1–1.5 mm, pubescent within; corona lobes obovate, ca. $0.7-1 \times 0.5$ mm, divergent, as long as gynostegium. Follicles fusiform, ca. 5.3×1.3 cm. Seeds not seen.

Distribution and habitat. Endemic to northern Afghanistan. The data on herbarium labels suggest that *V. cabulicum* grows in clumps on dry slopes of mountains. The elevation range is 1500 to 2800 m.

Phenology. Flowering from May to June and fruiting from June to October.

Provisional conservation status. Vulnerable (Table 1). *Vincetoxicum cabulicum* is collected from a few locations in Afghanistan and appears to be clumped in distribution. There are no reports on grazing. However, a possible threat appears to be habitat destruction through anthropogenic activities.

Notes. This species was originally described as *Cynanchum cabulicum* by Bornmüller (1934). In the description, Bornmüller refers to "Syn. *Vincetoxicum cabulicum* Bornm. herb.", thus creating an invalid name. Here, the name is validated as *Vincetoxicum cabulicum* (Bornm.) Bornm. ex S.A. Shah. Rechinger (1970) lumped this species with *V. glaucum* and, in turn, Ali and Khatoon (1982) lumped the latter with *V. canescens. Vincetoxicum glaucum* is hereby recognized as a separate species (see more comments on this complex below *V. subcanescens*).

Vernacular name. Rang Koh-e-Sabz (Pashto).

Specimens examined. AFGHANISTAN. **Kabul:** Paghman, near Kabul, 7000 ft., 193[0s], *F. Howland specimen B.* (US [US03264726); Westhang des Koh-i-Sher Darwasa bei Kabul, 2100 m, 10 July 1969, *D. Podlech 15814* (RAW); Chandau, 8000 ft., 07 June 1937, *W. Koelz 11764* (US); In Valle Paghman, Kabul, ca. 34°36'N, 68°56'E, 2300–2800 m, s.d., K.H. Rechinger *17141* (US, MO); Kabul, in declivibus borealibus montis Scher Darwasa, ca. 34°30'N, 69°10'E, 1800–1900 m, s.d., K.H. Rechinger *16986* (US); Paghman, 8000 ft., 22 June [19]35, *W.R. Henry 273* (K); **Badakhshan:** Faisabad district, 5000 ft., 22 May 1964, *P. Furse 6247* (K).

3. *Vincetoxicum cardiostephanum* (Rech. f.) Rech. f., Fl. Iranica 73: 14. 1970 Figs 2M, N, 3C, 4D, E, 6, 8, 9

Cynanchum cardiostephanum Rech. f., Österr. Akad. Wiss., Math.-Naturwiss. Kl. Anz. 105: 241. 1969

Type. AFGHANISTAN. Jaji, in declivibus jugi Narai Kotal versus Chakmani, in apertis quercetorum (*Qu. baloot*), substr. serpentin., 2100 m, 5 June 1967, K.H. *Rechinger* 35614 (*Holotype*: WU online [WU 1969-0013837], *Isotypes*: B online [B10 0365 118], US online [00112305]).

Small herbs to undershrubs, up to 40 cm tall. Stems striate, mostly pubescent along one or two lines, sometimes glabrous, internodes 1-4 cm long. Leaves dense, pendent; petioles 2-10 mm long, pubescent all around, sometimes only adaxial channel pubescent; lamina discolorous, narrowly lanceolate-ovate, $3-7 \times 0.6-2$ cm, both surfaces glabrous; apex acute to sometimes narrowly acute; base mostly obtuse; veins visible on both sides, sometimes inconspicuous, secondary veins up to 8 (-10) on each side of midvein, adaxial veins sparsely to densely pubescent, abaxial veins glabrous to glabrescent; margins sparsely pubescent. Inflorescences both sessile and shortly pedunculate; peduncles up to 6 mm long; bracts narrow, up to 4 mm long, margins ciliate; pedicels 2–5 mm long, pubescent along one line. Flowers $2.5-3 \times 1.5-2$ mm, pendent; sepals tapering to acute apices, up to 1 mm long, laterally sparsely ciliate, calycine colleters paired (10/flower); corolla yellowish-green, campanulate, corolla tube prominent, 1 to 1.5 mm long, lobes oblong-ovate with obtuse apices, 1-1.5 \times 1 mm; corona clavate, ca. 0.7 \times 0.6 mm, slightly exceeding the gynostegium in length, divergent. Follicles ovate-lanceolate to narrowly fusiform, up to 7 × 1 mm, apex acuminate, glabrous. Seeds dark brown, ca. 7×3 mm, wings up to 1 mm broad; coma up to 2 mm long.

Distribution and habitat. Two collections of this species are from Shalozan, Kurram valley, Pakistan. This place is located on the eastern border of Afghanistan. The type specimen is collected from Khost, Afghanistan, which is located nearby Kurram

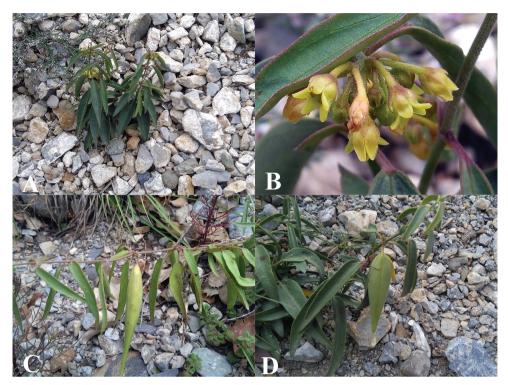


Figure 8. Field photographs of *Vincetoxicum cardiostephanum* **A** habit **B** inflorescence **C**, **D** follicle. Photos by S.A. Shah.

Valley. The habitat of the plant is open mountain slopes consisting of small stones and gravel. Elevation ranges from 2100 to 2200 m.

Phenology. *Vincetoxicum cardiostephanum* flowers from July to August and fruits from August to October.

Provisional conservation status. Critically endangered (Table 1). *Vincetoxicum cardiostephanum* is extremely rare and comprises very small populations of fewer than 50 individuals. It is known only from three localities in Pakistan and Afghanistan. It was declared critically endangered by Hussain et al. (2019). Erosion is the biggest threat for this species. We have successfully potted a plant in NARC, Islamabad. This indicates that the plant could be conserved in ex-situ conditions.

Notes. In the herbarium (RAW), only one gathering of this species from Kurram Valley, Pakistan was available, collected by Harsukh in 1894 and filed under *Cynan-chum vincetoxicum* (syn. *V. hirundinaria*). We re-discovered the species from the same area after 122 years. The population was composed of a mere 15 individuals. The type gathering of this species was collected from the adjacent area in Afghanistan.

Specimens examined. PAKISTAN. Khyber Pakhtunkhwa: Kurram Valley, 1894, Harsukh 15402 (RAW, K); Parachinar: near Khaiwas in Shalozan valley, 2200 m, 07 August 2016, S.A. Shah, W. Hussain, M. Hussain, M. Ullah SAS-40 (RAW, US).

4. *Vincetoxicum glaucum* (Wall. ex Wight) Rech. f., Fl. Iranica 73: 13. 1970 Figs 3D, 4F, 7

Cynanchum glaucum Wall. ex Wight, Contr. Bot. India: 58. 1834. Vincetoxicum hirundinaria subsp. glaucum (Wall. ex Wight) H. Hara, Enum. Fl. Pl. Nepal 3: 89. 1982.

Type. NEPAL. Chandaghir, 5 May 1821, *N. Wallich 133* [cat. #. 8229A] (*lectotype:* designated by Hara (1982), pointing generally to the set of syntypes: Asclep 133 and cat. # 8229A, specimen not chosen from syntypes); *lectotype* (designated here: K on-line [K000894587]; *isolectotypes* K online [K001129297, K000894586]; E online [E00179664, E00179665]).

Undershrubs, up to 40 cm tall. Stem striate, pubescent all around, internodes 1–6.8 cm long. Leaves petiolate; petioles 3–11 mm long, pubescent all around; lamina different shaped: narrowly ovate, oblong-ovate, elliptic ovate, lanceolate-ovate, $4-9.2 \times 1.3-3.8$ cm; margins smooth; apex acute to obtuse or sometimes mucronulate; base round or cuneate; veins visible on both surfaces, secondary veins 8–12 on each side of midvein; adaxial surface sub-glabrous, adaxial veins densely pubescent; abaxial surface glabrous to sub-glabrous, abaxial veins especially midrib pubescent; margins pubescent. Inflorescences sessile; bracts linear, ciliate; sepals tapering to acute apices, 1.5 mm long with ciliate margins; pedicels 1–3 mm long, pubescent; calycine colleters 5, exceeding the corolla tube in length; corolla green, not twisted, corolla tube 1 mm long, lobes tapering to pointed apex, 2×1 mm, bearded within; corona lobes longer than broad, 1×0.8 mm, exceeding the length of the gynostegium, base narrow, apex broad, toothed, divergent; staminal appendages obtuse; pollinaria deeply embedded in the gynostegium. Follicles and seeds not seen.

Distribution and habitat. Endemic to eastern Himalayas including India and Nepal and occurring at an elevation of over 2000 m. Herbarium label on *W. Dudgeon* & *L.A. Kenoyer 56* (MO) indicates its habitat to be open grassy places.

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

Provisional conservation status. Data Deficient (Table 1). *Vincetoxicum glaucum* is collected from a few localities in the eastern Himalayas (India and Nepal). We did not collect or observe its populations in natural habitats. Herbarium labels do not provide significant information about its populations. Therefore, it is declared as data deficient.

Notes. Vincetoxicum glaucum was first described as Cynanchum glaucum Wight but soon regarded as V. canescens by Decaisne (1844), a treatment which Boissier (1879) also adopted. Hooker (1883) reinstated the original name C. glaucum. Rechinger (1970) recognized V. glaucum in a broader sense and lumped with it another Afghani species, V. cabulicum Bornm. Hara et al. (1982) regarded Rechinger's V. glaucum as a synonym of V. hirundinaria subsp. glaucum (Wall. ex Wight) H. Hara. While writing the genus Vincetoxicum for the flora of Pakistan, Ali and Khatoon (1982) lumped Rechinger's V. glaucum with V. canescens. In the present treatment, we recognize three different species in this long misunderstood species complex, namely V. cabulicum (endemic to north-

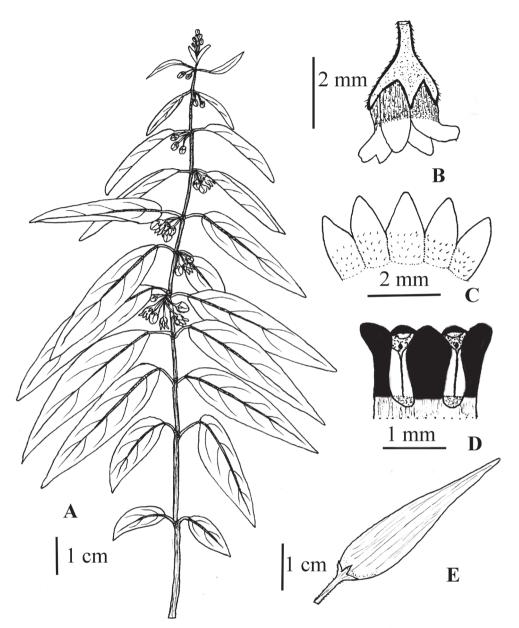


Figure 9. *Vincetoxicum cardiostephanum* illustrated by S.A. Shah & S.U. Nisa from *S.A. Shah, W. Hussain, Murtaza & M. Ullah SAS-40* (RAW!) **A** flowering plant **B** flower in natural orientation **C** corolla lobes **D** corona (black) **E** follicle.

ern Afghanistan), *V. glaucum* (endemic to India and Nepal) and *V. subcanescens* sp.nov. (endemic to Pakistan, Kashmir and Tibet), whereas *V. canescens*, a species long regarded as a member of this complex, is endemic to the eastern Mediterranean region and does not occur in southern Asia (also see notes below *V. subcanescens*).

Wight (1834) cited Wallich's collection Asclep. # 133 (Cat. # 8229A) as C. glaucum (corresponding to the typical variety *latifolium*, denoted by " α " in the protologue), Asclep. # 132 as variety *oblongifolium* (denoted by " β " in the protologue), and Asclep. # 134 and Cat. # 1554 as variety *lanceolatum* (denoted by " γ " in the protologue). He did not designate a holotype for C. glaucum, hence the specimens cited in the protologue and their duplicates were the syntypes for the respective varieties. Later on, Hara (1982) lectotypified C. glaucum by designating Wallich's Asclep. # 133 (Cat. # 8229A). This collection has at least five duplicates (syntypes) housed by K [K000894587, K001129297, K000894586] and E [E00179664, E00179665]). Possibly, Hara considered Asclep. # 133 a single specimen and did not choose a specimen as lectotype from the duplicates (syntypes). In spite of Hara's attempt, the lectotype is still unknown among the syntypes. Therefore, it is necessary to choose a specimen from the five duplicates (syntypes) of Wallich's Asclep. # 133 (Cat. # 8229A). Among them, K000894587 is chosen here as the lectotype of C. glaucum. The remaining syntypes become isolectotypes. We could not get specimen loans and examine the collections Asclep. # 132 & 134 and Cat. # 1554 that belong to the remaining two varieties of C. glaucum. Therefore, we recommend a revision for these varieties. It is also notable that there is no mention of these varieties in the postprotologue literature.

Specimens examined. INDIA. Uttarakhand: Landour, open grassy places, 7000 ft, 24 May 1920, *W. Dudgeon & L.A. Kenoyer 56* (MO [MO-2321710]); Nepal, s.d., *N. Wallich* cat. # 8229A (K [K001129296]); Kumaon/Tranquebar, India, s.d., *N. Wallich* cat. # 8229B (K [K001129299]).

5. *Vincetoxicum kenouriense* (Wight) Wight, Icon. Pl. Ind. Orient., pl. 1614. 1850 Figs 2O, P, 3E, 4G, H, 10, 11, 12

Cynanchum kenouriense Wight, Contr. Bot. India 58. 1834.

Type. INDIA. Kenour, *Royle 18* (*Holotype* K! [K000872737]).

Undershrubs, up to 1 m tall. Stem pubescent all around or along 2 dense lines, internodes 2–13 cm long. Leaves opposite; petioles 1–10 (–14) mm long, equally pubescent all around or sometimes denser along adaxial channel, lamina strongly discolorous, narrow to broadly ovate, $4-10 \times 1.8-6.5$ cm; apex narrowly acute to very shortly acuminate; base round to sub-cordate to sub-truncate; venation including tertiary and quaternary veins prominent, more prominent and raised on abaxial surface, secondary veins up to 14 on each side of midvein, trichomes absent on both surfaces, sometimes sparsely present on tertiary and quaternary veins, midrib and secondary veins densely pubescent on both surfaces, margins pubescent. Inflorescences mostly sessile, rarely both sessile and short-pedunculate inflorescences present on the same plant, peduncles up to 1.5 cm; bracts linear, up to 2 mm long, pubescent; pedicels 2–8 (–10) mm long, pubescent all around, sometimes pubescent along one or two longitudinal lines. Flowers yellowish-

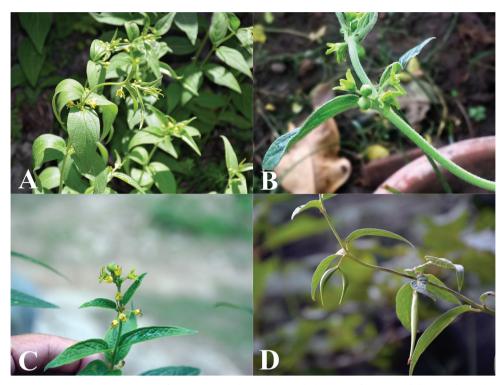


Figure 10. Field photographs of *Vincetoxicum kenouriense* **A** habit **B**, **C** flowers **D** follicles. Photos by Zahid Ullah and S.A. Shah.

green to green, $4-5 \times 2-2.5$ mm; sepals tapering to acute or narrowly acute apices, up to 2 mm long, margins sparsely ciliate, calycine colleters 5 or 10 per flower; corolla tube ca. 1 mm long, lobes oblong with obtuse apices, $3-3.5 \times 1-2$ mm, glabrous within or sparse caducous trichomes present; corona lobes long-triangular with acute apex, slightly divergent, ca. 1×0.8 mm, exceeding slightly the length of the gynostegium. Follicles fusiform, up to 7×1 cm, apex long-acuminate, surface glabrous, slightly striate. Seeds brown, $6-7 \times 3-3.5$ mm, wings less than 1 mm broad; coma 2–3 mm long.

Distribution and habitat. Endemic to a long geographic range in the Hindukush Himalayas from Bhutan in the east to Pakistan in the west. The western limit of this species is district Swat (Khyber Pakhtunkhwa province), Pakistan. It occurs on higher elevations between 1500 and 3000 m. The habitats of the species are commonly the Himalayan moist temperate forests (evergreen forests of conifers between 1500 to 3000 m elevations), rarely subalpine or open alpine lands (above 3000 m). We collected it from open stony alpine slopes and stream side slopes in a V-shaped mountain valley. The associated vegetation was either alpine herbs or conifers, and herbaceous to shrubby flora.

Phenology. Flowering from May to August and fruiting from August to October.

Provisional conservation status. Least concern (Table 1). Although having highly clumped, distant populations, *Vincetoxicum kenouriense* spans a wide range in the

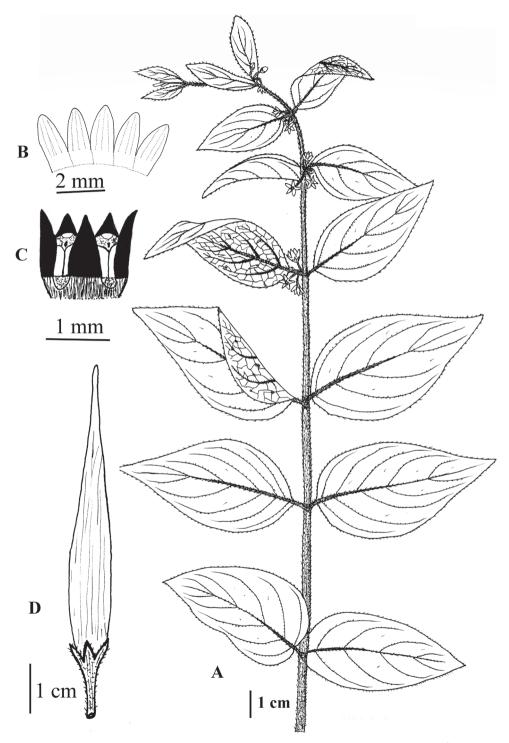


Figure 11. *Vincetoxicum kenouriense* illustrated by S.A. Shah and S.U. Nisa from *S.A. Shah & L. Ahmad SAS-37* (RAW!) **A** flowering plant **B** corolla lobes **C** corona (black) **D** follicle.

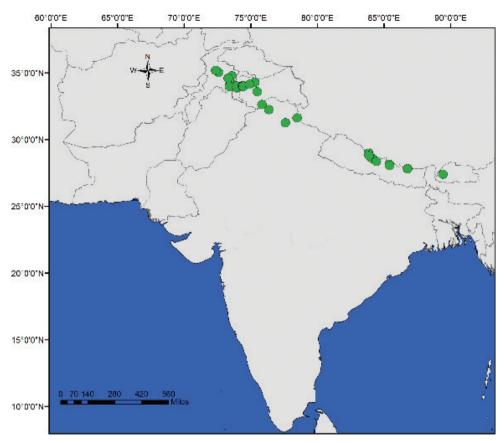


Figure 12. Geographic distribution of Vincetoxicum kenouriense.

Hindukush Himalayas. It is confined to higher altitudes and faces almost no natural threats. Anthropogenic activities, however, are deemed as a potential threat. For instance, two of its populations in Pakistan (in district Bagh, AJK, and district Swat, Pakistan) were found in recreational areas that are expected to undergo changes in the near future which might result in destruction of its habitats. It was also collected previously from Changla Gali (district Abbotabad, Pakistan). In spite of several visits in the past decade, we could not find it anymore in that area. These observations suggest that recreational activities might prove a potential threat to its existence, at least in Pakistan.

Notes. Vincetoxicum kenouriense was described by Wight (1834) from Kenour (India). Hooker (1883) synonymized it with Cynanchum vincetoxicum (syn. V. hirundinaria). Hooker's treatment has been followed in major floras (Stewart et al. 1972; Ali and Khatoon 1982) resulting in a broader circumscription of V. hirundinaria. In the present treatment, V. kenouriense is reinstated and presented as a new record for the Flora of Pakistan, Nepal and Bhutan. It replaces V. hirundinaria in southern Asia.

Specimens examined. BHUTAN. Thimpu: Paro (West Bhutan), 7800 ft., 27 June 1933, *F. Ludlow & G. Sherriff 158* (BM [BM001119300]).

INDIA. Uttarakhand: Garhwal, Mussoorie, Kidar Kantha, 13000 ft., 09 April 1952, *J.R. Drummond 22753* (K); Hab. Sikkim, 7000–10,000 ft., s.d., *J.D. H[ooker] s.n.* (GH, [GH01147527]); Daya-Balsan state, Simla hills, 7500 feet, 12 June 1937, *G.E. Parkinson 7386* (RAW); Dharmsala [India], 17 June 1929, *[R.R. Stewart] s.n.* (RAW [acc. # 1004]); s.loc., s.d., *H. Falconer s.n.* (GH [GH01147530]); Himalaya Bor. Oce., 4000–5000 ft., s.d., *W. Griffith* 3760 (GH [GH01147532]).

KASHMIR: B-8 above Gulmarg, 9000–10,000 ft., 31 July [18]92, J.F. Duthie s.n. (K); Basaoli, 8000 ft., s.d., C.B. Clarke 31523A (K); Tanmarg near Gulmarg, Kashmir, 02 September 1929, R.R. Stewart 13100 (RAW); Sonamarg, 9000 ft., s.d., R.R. Stewart 7306 (K); Below Sonamarg, Sindh Valley, 8000–9000 ft., 02 September 1940, R.R. Stewart 21328 (RAW, US); Gulmarg to Khaipur, 21 July 1890, J.F. Duthie s.n. (RAW [acc. # 1005]); Duksum-Kokernagh [Kashmir], 3000 m, s.d., G.A. Shapu 201 (KASH); Slopes above Harwan [Kashmir], 2300 m, s.d., G. Singh 833 (KASH).

NEPAL Gandaki Zone: Manang Distr. Pisang (3090 m), Humre (340 m), Manang (3360 m), 16 August 1994, *M. Mikage, N. Fujii, T. Kajita, N. Kondo, S. Noshiro & K.Yoda, 9485444* (GH); Mustang District: Gnyu Pass (4100 m), Chhengar (3700 m), Muktinath (3650 m), 14 July 2000, *Y. Lokawa, M.N. Subedi, Y. Takashi & K. Kano 20020202* (GH); On descendant and l'Imja-Khola, 3100 m, 09 April 1952, *A. Zimmermann 714* (K); Baghmati zone, before Syarpagaon, north side of Lantang River, 2600 m, 19 September 1966, *D.H. Nicolson 2445* (US); Bagmati zone: south of Gossainkunde, Blumche, 2500 m, 12 May 1967, *D.H. Nicolson 3339* (US).

PAKISTAN. Khyber Pakhtunkhwa: B-7 Hazara, Kaghan valley, 9800 ft., 09 July 1897, *Inayat 19940* (K); Nathia, July 1907, *H. Deane s.n.* (K); Mundi, UNA forest, Siran Valley, District Mansehra, 13 June 1994, *Q. Marwat 425* (RAW); C-7 Murree Hills Changlagali, 8000 ft., *M. Nath 336* (RAW); Azad Jammu and Kashmir: Bagh, 19 June 1956, *collector unknown 1507* (KUH); Bagh, Sudhan Gali to Ganga Choti, 2500–2800 m, 01 August 2016, *S.A. Shah & L. Ahmad SAS-37* (RAW, US); Bagh, Poonch, 19 June 1956, *M.A. Kazmi 1507* (RAW); C-8 Azad Kashmir, Poonch, near Trappar, below Kali, 17 September 1952, *A.R. Khan s.n.* (RAW [acc. # 998]); Raikot to Aliabad (Azad Kashmir), ±7000 [ft], 28 June 1952, *R.R Stewart & E. Nasir 23868* (KUH); Azad Kashmir, 8000 ft., 9 August 1969, *Shariq 8102* (PFI); Pir Kanthi, Uri Range, 9000 ft., 17 October 1955, *J. Mohammad s.n.* (RAW).

6. Vincetoxicum lenifolium S.A. Shah, sp. nov.

urn:lsid:ipni.org:names:77217788-1 Figs 2Q, R, U, V, 3F, 4I, J, M, 6, 13

Diagnosis. Differing from *V. stocksii* by having broader $(2.5-4.7 \times 1-4.5 \text{ cm})$, ovate leaves with lamina glabrous to inconspicuously puberulent, green flowers, internally glabrous corolla lobes, and small, ca. 0.7 mm long, usually rhomboid corona lobes. In *V. stocksii*, leaves are narrowly ovate to narrowly or broadly lanceolate, rarely elliptic-ovate $(3-6 \times 1-2 \text{ cm})$ with lamina sparsely pubescent on both sides, flowers bicolored,

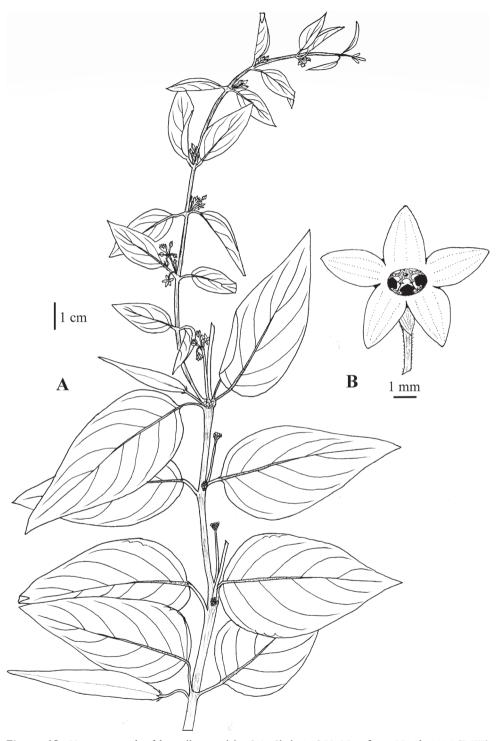


Figure 13. *Vincetoxicum lenifolium* illustrated by S.A. Shah & S.U. Nisa from *N. Ali 1035* (RAW) **A** plant with fruits and flowers **B** flower, top view.

corolla lobes pilose within, and corona lobes subulate and longer than the gynostegium (ca. 1 mm long).

Type. PAKISTAN. Razmak [North Waziristan], 193[0s], N. Ali 1035 (Holotype: RAW).

Undershrubs, ca. 50 cm tall. Stem striate, pubescent along one or two lines, rarely all around, internodes 2-7 cm long. Leaves opposite; petioles 4-15 mm long, pubescent all around or only along the adaxial channel; lamina discolorous, sometimes seemingly unifacial, lanceolate-ovate to ovate, 2.5-4.7 × 1-4.5 cm, spreading, sometimes pendent; apex acute to narrowly acute to shortly acuminate; base round or subcordate; veins prominent on both surfaces, secondary veins 8-10 (-14) on each side of midvein, both adaxial and abaxial surfaces mostly glabrous to inconspicuously puberulent, adaxial veins densely pubescent, abaxial veins and lamina margins glabrescent. Inflorescences long-pedunculate in the lower nodes to sessile in the upper nodes; peduncles up to 2.5 cm long, puberulent all around or along 1-2 lines; bracts linear, 2 mm long. Flowers green, ca. 3×2 mm; pedicels 2–5 mm long; sepals tapering to acute or narrowly acute apices, ca. 1.5 mm long, margins ciliate, abaxial surface sometimes pubescent, calycine colleters 10 per flower; corolla tube ca. 1 mm long, lobes oblong-ovate with obtuse or emarginate apex, $1.5-2.5 \times 1-1.3$ mm, glabrous within; corona lobes rhomboid, sometimes variable shapes found: conical-rhomboid, deltoidrhomboid, rarely elongated, almost equal in length and width, $0.4-0.7 \times 0.4-0.8$ mm, reaching the base of the staminal appendages, erect to slightly divergent; staminal appendages obtuse. Follicles fusiform, $5-8 \times 0.4-0.7$ cm, apex acuminate, surface glabrous to glabrescent, striate. Seeds light brown, ca. 4 × 2.2 mm, wings less than 1 mm broad; coma up to 2 cm long.

Distribution and habitat. Endemic to Khyber Pakhtunkhwa province of Pakistan. So far, it has been recorded from North & South Waziristan and Kohat districts. The elevation ranges from 1500 to 2000 m. Its habitat is open rocky slopes consisting of small stones and gravel and stream beds.

Etymology. The name is based on smooth, mostly glabrous, leaves of the species. **Phenology.** Flowering from April to June and fruiting from July to August.

Provisional conservation status. Endangered (Table 1). *Vincetoxicum lenifolium* is endemic to a small range comprising three districts of Khyber Pakhtunkhwa province, Pakistan. In 2016, we collected fresh specimens and observed its population structure in Razmak (North Waziristan). The population was clumped with less than 20 individuals. Anthropogenic activities like land degradation, settlements, roads etc. are deemed major threats to its existence.

Notes. From the general appearance, *V. lenifolium* appears as a closely related member of the purple-flowered *Vincetoxicum* including *V. arnottianum*, *V. luridum*, *V. sakesarense* and *V. stocksii*. The paratypic specimens of this species were hitherto misidentified as *V. arnottianum* or *V. hirundinaria*. However, the new species is easily distinguished by ovate leaves, green flowers and glabrous corolla from *V. arnottianum* and by inconspicuous veins, denser inflorescences and small rhomboid corona from *V. hirundinaria*. RAW houses a variable specimen, N. Ali 1029, which is silvery in appearance, with sessile inflorescences and corona lobes somewhat deltoid (Fig. 3H).

These characters and the associated geographic information do not support the recognition of the specimen as a separate taxon. The specimen is cited as *V. lenifolium* in this treatment. Long-pedunculate inflorescences are most commonly found in *V. lenifolium* and rarely in *V. stocksii*. Short-pedunculate along with sessile inflorescences are found in *V. arnottianum*, *V. cabulicum*, *V. cardiostephanum*, rarely in *V. kenouriense* and *V. sakesarense*, *V. luridum*, *V. stocksii*, and *V. subcanescens*. Sessile inflorescences are found in *V. glaucum* and *V. stewartianum*.

Specimens examined. PAKISTAN. Khyber Pakhtunkhwa: Kaniguram [South Waziristan], 13 May 1895, *J.F. Duthie 15766* (RAW); Paryat (N. Waziristan), ±5000 ft., 01 June 1979, *M. Zubair & S. Khan 114964 & 114967* (ISL); Enger (North Waziristan), 21 June 1977, *H. Ullah & Ayaz 56417 & 56418* (ISL); North Waziristan: Razmak, sandy slope on roadside near Razmak bazaar, 7545 ft., 08 September 2016, *S.A. Shah SAS-44* (RAW US); Razmak, 29 May 1979, *M. Zubair & Dilawar 112288* (ISL); Razmak, ±6500 ft., 29 May 1979, *M. Zubair & S. Khan 112298* (ISL); Razmak [North Waziristan], 193[0s], *N. Ali 1029* (RAW!); Togh Sarai, Kohat [KPK], 31 March 1979, *M. Zubair & S. Khan 113954* (ISL).

7. Vincetoxicum luridum Stocks ex S.A. Shah

Figs 2S, T, 3G, 4K, L, 6

Type. Pakistan. Balochistan: "Balochistan", 3500–5500 feet, [1849], *[Stocks] 721* (*Holotype* K! [K001235295], isotypes: K! [K001235294], [K001235296]). See more details in Shah et al. (2018).

8. *Vincetoxicum sakesarense* Ali & Khatoon, Pak. J. Bot. 14(1): 67. 1982 Figs 2W, X, 3I, 4N, O, 6

Туре. Ракізтал. Punjab: C-7 Sargodha Dist.: Sakesar hills, in protected area, 15.8. [19]72, *M. Qaiser & A. Ghafoor* 4524 (*Holotype*: KUH!).

Undershrubs, ca. 55 cm tall. Stem striate, pubescent along single or double lines, sometimes pubescent all around, internodes 1–7 cm long. Leaves opposite; petioles up to 8 mm long, pubescent along adaxial channel, sometimes sparsely pubescent abaxially; lamina narrowly ovate to lanceolate, $3-11 \times 1-3$ cm; apex acute to narrowly acute; base round to sub-cuneate, veins visible on both sides, secondary veins 6–7 (–12) on each side of midvein, both adaxial and abaxial surfaces and abaxial veins glabrous, adaxial veins pubescent, margins sparsely pubescent. Inflorescences sessile, rarely small peduncles up to 2 mm present; bracts with a few basal trichomes or completely glabrous. Flowers dark purple except sepals, 4 \times 2.5 mm; pedicels 2–5 mm long, puberulent; sepals up to 2 mm long, tapering into narrowly acute to acuminate apices, margins ciliate, abaxial surface sometimes pubescent, calycine colleters 10/flower; corolla tube up to 1 mm long, lobes 2.5 \times 1 mm, twisted clockwise, gradually tapering to the apex, inner surface of corolla lobes bearded except one lateral margin; corona lobes deltoid, longer than wide, 0.6 \times 0.5 mm, almost reaching the bases of the staminal appendages, divergent. Follicles fusiform, 5.5–6.5 \times 0.7 cm, apices acuminate, surface inconspicuously striate, glabrous. Seeds not seen.

Distribution and habitat. Endemic to a protected area in Soon Sakesar Valley, a small mountainous valley in northern Punjab, Pakistan. The maximum elevation is 1500 m.

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

Provisional conservation status (Table 1). Critically endangered. *Vincetoxicum sakesarense* is known from two localities in Soon Sakesar valley, Salt Range, Punjab province, Pakistan. The area is protected for security purposes. We and other botanists have collected the species from the type locality twice in the current decade. The species is critically endangered due to the fact that its EOO is very small (Table 1), and that it is confined to a comparatively small mountain range. Expanding human population coupled with anthropogenic activities are a serious threat. We have grown living plants in NARC, Islamabad. This indicates that this species could potentially be conserved outside its native range.

Notes. Vincetoxicum sakesarense is a member of the purple-flowered group of Vincetoxicum and most closely related to V. arnottianum. The two species can be readily distinguished by the shape of the corona lobes which is deltoid in V. sakesarense and deltoid-rhomboid in V. arnottianum. Although very similar, V. arnottianum occurs in western Himalaya while V. sakesarense occurs in the Salt Range (non-Himalayan Mountains).

Specimens examined. PAKISTAN. Punjab: Sakesar, 29 September 1951, *A. Rahman 287* (KUH); Sakesar, 27 April 1977, *M. Ajab & M. Ashraf 50159* (ISL); Sakesar, 05 May 1978, *M. Ajab & M. Ahmad 83837* (ISL); Sakesar, 27 April 1977, *M. Ajab & M. Ashraf 50158* (ISL); Ouchali, 28 April 1977, *M. Ajab & M. Ashraf 48440* (ISL); Sakesar, 01 August 1954, *I. Ahmad 27821* (RAW).

9. Vincetoxicum stewartianum S.A. Shah, sp. nov.

urn:lsid:ipni.org:names:77217789-1 Figs 2Y, Z, 3J, 4P, 6, 14

Diagnosis. Differing from *V. arnottianum* by having retrorse indumentum on the stems, ovate to elliptic-ovate leaves, light purple flowers, and large rhomboid $(1 \times 1 \text{ mm})$ corona lobes that exceed the gynostegium in length. In *V. arnottianum*, the indumentum is extrorse on the stems, leaves are mostly narrowly ovate or lanceolate-ovate, flowers dark purple, and corona lobes are deltoid-rhomboid and do not exceed the gynostegium in length.

Type. INDIA. Uttarakhand: Landour, Mussoorie, 6–7000 ft. 8 August 1938, *R.R. Stewart 16709* (*Holotype:* RAW!).

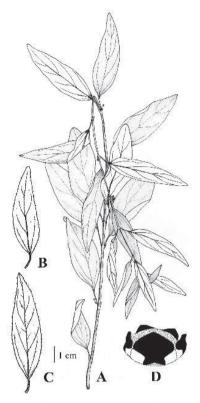


Figure 14. *Vincetoxicum stewartianum* illustrated by M. Saleem from *R.R. Stewart 16709* (RAW) **A** plant **B** adaxial surface of leaf **C** abaxial surface of leaf **D** corona.

Undershrubs, ca. 50 cm tall. Stem striate, pubescent all around, trichomes retrorse, internodes 1.5–5 cm long. Leaves opposite, petiolate; petioles 2–15 mm long, sparsely pubescent all around; lamina discolorous, ovate- or elliptic-lanceolate to narrowly ovate, 5–7.5 × 2–3 cm; apex acute to obtuse sometimes mucronulate; base obtuse to sub-obtuse; veins visible on both sides, secondary veins 8–14 on each side of midrib, both adaxial and abaxial surfaces of lamina glabrous to sometimes sub-glabrous, veins on both surfaces and margins pubescent. Inflorescences sessile; pedicels 2–3 mm long, pubescent along a narrow longitudinal line; bracts narrow tapering, pubescent. Flowers light purple, ca. 5 × 2 mm; sepals tapering to acute apices, up to 2 mm long with ciliate margins, calycine colleters single; corolla tube ca. 1 mm long, lobes oblong, narrowly tapering, ca. $3 \times 1-2$ mm, bearded within; corona lobes rhomboid, ca. 1×1 mm, exceeding the length of the gynostegium, divergent. Follicles and seeds unknown.

Distribution and habitat. Endemic to eastern Himalayan Mountains of India. The elevation range is 6000 to 7000 m. The limited herbarium materials do not provide more information.

Phenology. Vincetoxicum stewartianum flowers and fruits from August to September.

Provisional conservation status. Data deficient (Table 1). *Vincetoxicum stewartianum* is gathered from only the type locality. Besides label data on gatherings from the type locality, information is lacking about its present occurrence. A survey on its occurrence and ecology is recommended.

Etymology. Named after the collector Prof. R.R. Stewart.

Notes. The specimens of this species were hitherto misidentified as *V. glaucum* and *V. canescens.*

Specimens examined. INDIA. Himal. Bor. Occ.; Regio temp., s.d., *T. Thomson* s.n. (GH [GH01147533]; GOET [GOET020096]).

10. *Vincetoxicum stocksii* Ali & Khatoon., Pak. J. Bot. 14(1): 65. 1982 Figs 2AA, AB, 3K, 4Q, R, 6, 15

Type. PAKISTAN. Balochistan: D-5 Near Bharat Khel on way to Zhob, erect shrub, l m. tall, 15 May 1978, *S. Nazimuddin & S. Abedin 680* (*Holotype*: KUH!). Illustrated by Ali (1983: 36).

Herbs to undershrubs, up to 60 (-100) cm tall. Stem longitudinally striate, pubescent along two longitudinal lines, sometimes moderately pubescent all around, internodes 1-7 cm long. Leaves opposite, decussate, lower and upper leaves smaller than the middle ones, petiolate; petioles 2-8 mm long, glabrous to sparsely pubescent, sometimes pubescent along the adaxial channel only; lamina mostly narrowly ovate to narrowly or broadly lanceolate, rarely elliptic-ovate, sparsely pubescent on both sides, $3-6 \times 1-2$ cm; apex acute; base mostly obtuse to sometime sub-acute; margins smooth, mostly pubescent; secondary veins 7-9 on each side of midvein, visible on both surfaces, sometimes sunken, pubescent. Inflorescences axillary, mostly sessile, often both sessile and short- to long-pedunculate inflorescences present on the same plant; peduncles up to 3 cm; bracts narrow, ca. 1 mm long, pubescent; pedicels 2-4 mm long, sparsely or densely pubescent. Flowers bicolored: calvx lobes green, and lower half of corolla lobes purple, upper half green, corona and gynostegium purple; sepals ovate or tapering into acute to narrowly acute apices, ca. 1 mm long, narrowly ovate, ca. 0.5 mm long, a few marginal and surface trichomes present, calycine colleters 5 per flower; corolla tube ca. 1 mm long, lobes oblong-ovate, ca. 2 × 1 mm, pubescent within, apex obtuse or occasionally emarginate; corona lobes subulate, ca. 1 mm long, less than 0.5 mm broad, much longer than gynostegium, apices approaching those of the opposite corona lobes, convergent; staminal appendages obtuse. Follicles narrowly ovate to fusiform, 5-9.5 × 1–1.5 cm, glabrous, inconspicuously striate, apex acuminate. Seeds 6–9 × 4–6.5 mm, dorsally dotted, lateral wings less than 1 mm broad; coma white, ca. 1.5 cm long.

Distribution and habitat. Endemic to Zhob and Qila Saifullah districts in northeast Balochistan, Pakistan. It is found on dry stream beds made up of soil, stones and gravel. Its elevation ranges from 1300 to 2100 m.

Phenology. *Vincetoxicum stocksii* flowers from April to June and fruits from June to August.



Figure 15. Field and garden photographs of *Vincetoxicum stocksii* **A** habit **B** flowers. **Note:** A potted plant was photographed at the Botanical Conservatory, NARC; Islamabad. The insect is a honey bee. We did not observe successful pollination in the botanical conservatory in three years. Photos by S.A. Shah.

Provisional conservation status. Vulnerable (Table 1). *Vincetoxicum stocksii* is endemic to two districts in Balochistan province, Pakistan. The populations are partially fragmented and face the threat of habitat loss especially on the dry stream beds due to rains.

Notes. *Vincetoxicum stocksii* is a member of the purple-flowered group. Before its description as a new species by Ali and Khatoon (1982), it was identified as *V. arnottianum*. Recently, Shah et al. (2018) disintegrated *V. stocksii* into two species, separating from it *V. luridum*, an endemic of southwest Balochistan based on indumentum type on vegetative parts, corona lobes shape, pollinarium morphology and seed coat ornamentation. *Vincetoxicum stocksii* can be easily differentiated from *V. arnottianum* by the smaller pubescent leaves, the non-twisted and internally pilose corolla lobes, and the subulate corona lobes. These characters also differentiate it from *V. sakesarense*. The presence of both sessile and distinctly pedunculate inflorescences on the same plant is a common character shared between *V. stocksii* and *V. lenifolium*. However, green flowers, glabrous corolla lobes and smaller rhomboid corona lobes readily distinguish *V. lenifolium* from *V. stocksii*.

Specimens examined. PAKISTAN. Balochistan: Northeast Balochistan: ca. 30 km from Zhob on way to Quetta, 07 July 1988, *T. Ali & T. Ahmad 23361* (KUH); Janabad: ca. 33 km from Zhob on way to Qila Saifullah, 1 June 1995, *T. Ali & G.R. Sarwar 2754* (KUH); ca. 20 km from Qilla Saifullah on way to Zhob (Fort Sandeman), 19 May 1984, *S. Omer & A. Ghafoor 1640* (KUH); Murgha (Balochistan), 25 October 1950, *A.H. Khan* s.n. (RAW); Lakkaband, 21 May 1896, *J.F. Duthie* s.n. (RAW); Hindubagh, 7000 ft., 24 October 1969, *Shariq 8318 & 8322* (PFI); Quetta, in valle 12 km. N. Murgha Kibsai, 30°48'N, 69°25'E, substr. Tonschiefer, 1600 m, s.d., *K.H. Rechinger 29803* (K); Quetta: Murgha Kibsai to Fort Sandeman, ca. 30 km from Fort Sandeman, stony and sandy plain, ca. 1500 m, 19 May 1965, *J. Lamond 1440* (MO); Zhob: 15 km towards Quetta, 1390 m, 17 August 2016, *R. Khan, Z. Abedin, N. Khan, B. Gul SAS-21* (RAW).

11. Vincetoxicum subcanescens S.A. Shah & A. Sultan, sp. nov.

urn:lsid:ipni.org:names:77217790-1 Figs 2AC–AF, 3L, 4S, T, 7, 16, 17

Diagnosis. Differing from *V. cabulicm* by having distinctly petiolate leaves, green flowers, and clavate corona lobes. In *V. cabulicum*, leaves are sessile, flowers dark purple, and corona lobes obovate.

Туре. Ракізтал. Dir Upper, Patraak, mountain slope near river, 1600 m, 16 June 2015, *S.A. Shah & A. Ullah SAS-8A* (*Holotype*: RAW!).

Undershrubs, up to 75 cm tall. Stem and other vegetative parts sub-canescent, internodes 1–9 cm long, striate. Leaves opposite, rarely whorled on some nodes; petioles 1-18 mm long; lamina discolorous, narrow to broadly ovate to sometimes lanceolate-ovate, $3-9 \times 1.2-4.8$ cm; apex acute to narrowly acute to shortly acuminate; base rounded to sub-cordate to sub-truncate; veins visible on both sides, more prominent on adaxial side, secondary veins 6-12 on each side of midvein, tertiary veins sometimes visible, lamina sub-canescent on both sides. Inflorescences sessile or both sessile and pedunculate inflorescences present on the same plant; peduncles up to 2.5 cm long, sub-canescent; bracts narrow with a few marginal trichomes; pedicels 2–7 mm long, pubescent. Flowers green to yellowish-green, $3-4 \times 2-2.5$ mm; sepals tapering into acute to narrowly acute apices, pubescent along margins and abaxial surface, up to 2 mm long, calycine colleters single or in pairs; corolla tube ca. 1 mm long, lobes tapering into obtuse apex, $2-2.5 \times 1-1.5$ mm, margins appearing wavy in dry flowers, sub-bearded within; corona lobes clavate, $0.5-1 \times 0.2-0.5$ mm, reaching the base, the middle or, rarely, the apex of the staminal appendages, divergent. Follicles fusiform, $4.5-9.5 \times 0.7-1$ cm, sparsely pubescent, minutely striate, apex narrowly acuminate. Seeds $5-6 \times 2-3$ mm, wings up to 1 mm broad; coma up to 3 mm long.

Distribution and habitat. Endemic to Pakistan (Chitral, Dir, Swat and Gilgit Baltistan), Kashmir (Ladakh) and China (Tibet). The habitat of the species is open slopes. Soil type is clay to gravel to large size stones. The elevation ranges from 1600 to 2800 m.

Etymology. The name is based on the sub-canescent indumentum on the vegetative parts of the plant.

Phenology. *Vincetoxicum subcanescens* flowers from April to August and fruits from May to October.

Provisional conservation status. Least concern (Table 1). *Vincetoxicum subcanescens* is common in its range. However, the populations are extremely distant and clumped.

Vernacular name. Lovaki (in Chitrali language).

Notes. Vincetoxicum subcanescens was previously misidentified as V. canescens (Ali and Khatoon 1982; Boissier 1879). Rechinger (1970) treated it as V. glaucum in Flora Iranica and also synonymized V. cabulicum with it. Vincetoxicum subcanescens differs from V. canescens by the subcanescent indumentum, smaller flowers, clavate corona lobes, and glabrous and narrowly fusiform follicles. Vincetoxicum canescens is distributed in the eastern Mediterranean region in Turkey, Greece, Syria and Iraq. It is characterized by canescent indumentum, larger flowers, arrow-shaped corona lobes, and ovate and canescent follicles. Vincetoxicum glaucum exhibits variable leaf shapes, sub-



Figure 16. Field photographs of *Vincetoxicum subcanescens* **A** habit **B**, **C** leaves and inflorescences **D** flowers. Photos by S.A. Shah.

glabrous or glabrous leaf surfaces, sessile inflorescences and toothed corona lobes. It is distributed in the Himalayas of India and Nepal. The closest relative of *V. subcanescens* is *V. cabulicum* which differs by the sub-sessile leaves and dark purple corolla and is endemic to northern Afghanistan.

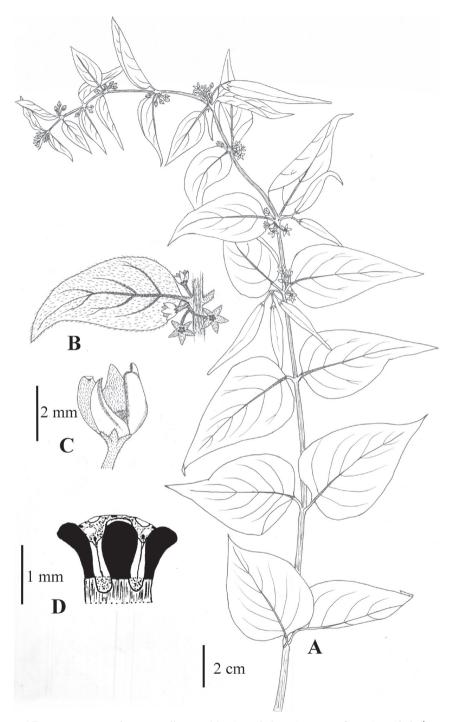


Figure 17. *Vincetoxicum subcanescens* illustrated by S.A. Shah & S.U. Nisa from *S.A. Shah & A. Ullah SAS-8* (RAW!) **A** plant with flowers and fruits **B** flowering node and leaf **C** flower **D** corona (black).

Specimens examined. CHINA. Tibet: Hab. Tibet, 10,000–12,000 ft., s.d., *J. J. s. n.* (GOET [GOET020098; GOET020099]).

KASHMIR. Nurla, Ladak, Kashmir. On sandy slope, 28 August 1931, *W. Koelz 2707* (RAW, US); North of Kamri Pass, above Shankargarh, ca. 10,000 ft., s.d., *R.R. Stewart 22773* (K, US); Kargil, Ladakh, Kashmir, 27–28 July 1933, *W. Koelz 6132* (US).

PAKISTAN. Khyber Pakhtunkhwa: Chitral, Rosh Gol Tirich Mulkhow, 9039 ft., dry hard soil, 08 October 2013, Kifayat 516 (HUP); Chitral to Pirpesh, 8 June 1958, I.I. Choudhri 7 (RAW); Dir [Upper]: Patraak, 13 July 1968, Y. Nasir 5089 (RAW); Swat: Batain above Ushu cliffs, 27 July 1953, R.R. Stewart & A. Rehman 25301 (RAW); Gilgit Baltistan: Satpura Nullah near Skardu, s.d., A. Ghafoor 567 (KUH); Near Rattu above Astor, 21 August 1939, R.R & I.D. Stewart 18834 (RAW); Bagocha to Olding, Indus valley, ca. 8500 ft., 22 August 1940, R.R. Stewart 21009 (RAW); Indus Valley: below Parkutta, 20 August 1940, R.R. Stewart 20908 (RAW); Between Arkote and Biafo base camp, 3200 m, 05 June 1962, Schussalide 1095 (KUH); Astor: ± 30 km from Pagru on way to Shimshol, ±3000 m, 25 June 2007, J. Alam et al. 3941 (KUH); Astor: Gorikot P.R.C, 26 May 2008, A. Noor & Basharat 1489 (KUH); Astor: Peer rant village, bank of cultivated field, 12 September 2004, A. Noor 52 (KUH); Astor: above Rattu, 2642 m, 23 August 2014, A. Sultan SAS-52 (RAW); Hunza, Gilgit, ±35 km from Posu on way to Shimshol, 28 June 2007, J. Alam, Karimuddin & M. Khan 3941 (KUH); Dharkot, 10,500 ft., 19 June 1976, B. Lyon 8144 (KUH); Skardo-Dras, 8800 ft., petals green, acute, s.d., C.B. Clarke 30512A (K); B-9 Baltistan, Paskyum, 9600 ft., flowers greenish, s.d., B.B. Osmaston 129 (K); Gilgit: Chamrot, 29 July 1957, M.B. Zaman & D.K. D 1876 (PFI); Gilgit: Passu between lake and glacier, 16 August 1994, S.Z. Hussain, R.A.W. Lowe, M. Shah & L.S. Springate 632 (PMNH).

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RESEARCH ARTICLE



Thismia sitimeriamiae (Thismiaceae), an extraordinary new species from Terengganu, Peninsular Malaysia

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Abstract

We describe an extraordinary new species in the genus *Thismia* from the Terengganu State of Malaysia in the Malay Peninsula. The new species, which we name *Thismia sitimeriamiae*, is distinct from all other *Thismia* species known to science, most notably in its unique mitre configuration formed by the inner tepals and its floral surface morphology characterised by conspicuous orange trichomes. We discuss our findings in the context of underestimated species diversity in the genus *Thismia* and implications for their conservation. We recommend assigning *T. sitimeriamiae* the conservation status as Critically Endangered (CR) according to IUCN criteria.

Keywords

conservation, forestry, mycoheterotrophy, species diversity, taxonomy

Introduction

The Thismiaceae is a highly unusual family of flowering plants belonging to the order Dioscoreales (APG IV 2016). There are currently 86 accepted species of *Thismia* Griff. (POWO 2019), all of which are small, diminutive herbs that lack chlorophyll. The genus is distributed across tropical and subtropical regions of Asia, Australasia and America, extending into some temperate regions (Yudina et al. 2021). Over a third of the known species of *Thismia* have been recorded to occur in Malaysia where the majority have very local distributions. These include 24 species in East Malaysia with six species in Sabah and 16 in Sarawak (Dančák et al. 2020a, b, c) and two species which occur in both states: *T. goodii* Kiew (Kiew 1999) and *T. brunneomitra* Hroneš, Kobrlová & Dančák (Hroneš et al. 2015). A further 15 species occur in Peninsular Malaysia (PM) (Jonker 1948; Siti-Munirah et al. 2021; Siti-Munirah and Dome 2021) including *T. clavigera* (Becc.) F.Muell. which occurs in both PM and Sarawak (Stone 1980; Dančák et al. 2020c).

Many species of *Thismia* have been seen or collected only once, from a single locality and their biology and ecology are poorly known. These elusive plants are visible only transiently, emerging to flower and set seed after heavy rain (Guo et al. 2019) and, like the related genus *Oxygyne* Schltr., are easily overlooked because they occur in deep shade, often under leaf litter (Thorogood 2019). Therefore, they can be difficult to relocate after their initial discovery. The extremely local distributions of mycoheterotrophs, like *Thismia*, may be restricted by host specificity for associated mycorrhizal fungi (Yamato et al. 2011; Gomes et al. 2017). The Malay Peninsula falls in the centre of diversity of *Thismia* and many new species have been described here in recent years, all of which are indeed very rare or local.

An area of exceptional diversity of the genus Thismia is the Malaysian State of Terengganu, which contains a significant reservoir of primary rainforest, although this has diminished at a rapid and alarming rate in recent decades. To date, six species of Thismia have been reported in the State of Terengganu: T. alba Holttum & Jonker, T. aseroe Becc., T. domei Siti-Munirah, T. javanica J.J.Sm., T. latiffiana Siti-Munirah & Dome, T. terengganuensis Siti-Munirah (Jonker 1948; Siti Munirah and Dome 2019, 2021). This astonishing spate of species discoveries triggers the need for a reassessment of the genus in the Flora of Peninsular Malaysia and their conservation requirements. Here, we describe an additional new species which we name Thismia sitimeriamiae Siti-Munirah, Dome & Thorogood from the vicinity of Gunung Sarut, which is located in the Hulu Nerus Forest Reserve (FR) in the State of Terengganu. The plant was discovered in two sites in close proximity growing along the summit trail of the mountain (Fig. 1) by the second author in 2019. In February 2020, the authors revisited the locality during a botanical exploration of the Hulu Nerus FR led by the Forest Research Institute Malaysia (FRIM) Flora Team. Within the only known population, we found that one of the two sites had been destroyed by wild boar activity and the other had just a single fruiting specimen (Fig. 1). In December 2020, the second author revisited and observed just a single specimen after a thorough assessment of the area.

The distinctive structure, colour and morphology of this exceptionally rare and ephemeral plant make this species amongst the most eye-catching in the genus described from Peninsular Malaysia to date.

Materials and methods

Our assessment is based on material collected by the second author in December 2019 and 2020 from Hulu Nerus FR, Setiu District, Terengganu (Map 1). The specimens were preserved in 70% ethanol. In total, two specimens have been deposited in Kepong Herbarium (KEP) and have been examined for taxonomic treatment. Morphological characters and dimensions were examined using Olympus SZ61 and LEICA M125 stereomicroscopes and high-resolution macrophotography.

Taxonomic account

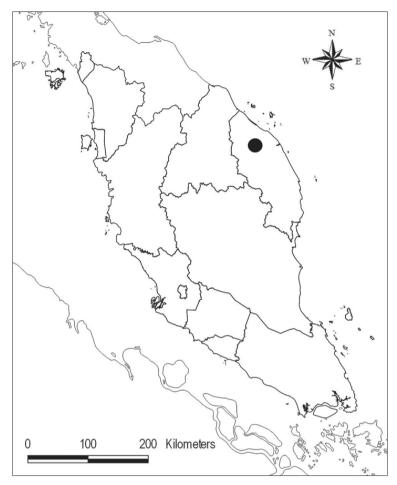
Thismia sitimeriamiae Siti-Munirah, Dome & Thorogood, sp. nov. urn:lsid:ipni.org:names:77217986-1

Figures 1-4

Diagnosis. *Thismia sitimeriamiae* has a unique combination of morphological traits, by which it differs from all its congeners including its orange colour, its surface characteristics and its floral architecture: the flower is strigose with pale orange simple and stellate trichomes covering the outer surface of the floral tube, mitre and ovary; the upper surface of the annulus is covered by very short trichomes; the outer tepals are reduced and inconspicuous and the inner tepals are curved upwards, connate, forming an umbrella/parasol-like mitre with a flattish apex.

Type. MALAYSIA. Peninsular Malaysia: Terengganu, Setiu District, Hulu Nerus Forest Reserve, ca. 209 m alt., 23 December 2019, *Dome Nikong*, *FRI 91118* (holotype KEP!, spirit collection, No. barcode 280006).

Description. Achlorophyllous herb, ca. 2.2 cm tall. *Roots* coralliform, creeping, light brown. Stem 8 mm tall, erect-ascending, unbranched, 2 mm thick, whitish. Leaves ca. 6, spirally arranged, triangular, scale-like, acute, entire, 3-4 mm long and 1 mm wide at base, pale whitish. Bracts 3, widely triangular to ovate, entire, acute to acuminate, ca. 5 mm long, 2 mm wide, pale greenish to brown. *Pedicel* 1 mm long, elongating after anthesis, greenish. Inflorescence (flower) solitary, actinomorphic, ca. 1.5 cm long; pale to dark orange; *floral tube* (hypanthium) conical in shape, ca. 1 cm in height, widest (1.2 cm) in the upper part; outer surface bright orange, sparsely covered with pale orange, simple trichomes (occasionally apically stellate); inner surface partially convex/reticulum; outer tepals 3, rather reduced and inconspicuous, divided, forming a narrow overhanging fringe around the mouth of the perianth tube; inner tepals well-developed, each pale orange, distally connate, forming a flattish, umbrella-like mitre ca. 3–4 mm tall, proximally flattened with revolute margins, arching over the floral tube; *mitre* circular in outline, ca. 8-10 mm wide, the upper surface bright orange, covered sparsely with orange trichomes, the lower surface glabrous, bright orange; *annulus* (apical part of the floral tube) pale orange,



Map I. Hulu Nerus Forest Reserve (black circle), the type locality of Thismia sitimeriamiae.

rounded, ca. 1.2 cm wide, the rim raised, connected with the inner tepals; upper surface covered with short trichomes; aperture, ca. 4–5 mm, with a blackish margin; *stamens* 6, orange, pendent from the annulus; *filaments* orange, curved downwards, *connectives* laterally connate, forming a tube, ca. 3 mm long, glabrous on the inner surface, the apex of each connective with two lobes, each pointed with transparent trichomes; *outer side of connectives* forming a lateral appendage, protruding towards the floral tube, horn-like on each side, shallowly dentate and sparsely hairy on the free margins; thecae pale, surrounded by tufts of hairs; *interstaminal glands* inserted along the line of fusion between the connectives; **ovary** inferior, obconical, flushed pale orange and greenish, covered by bracts; *placentas* 3, parietal; *pistil* slender, clavate, pale greenish-orange; *style* ca. 1.5 mm long, slender, erect, glabrous; *stigma* ca. 1 mm, papillose, 3-lobed, narrowly-rectangle, each without furrowed,



Figure 1. *Thismia sitimeriamiae* **A** the habitat in Gunung Sarut, in the State of Terengganu (PM) **B** the habit of flowering specimen **C** a fruiting specimen (in situ).

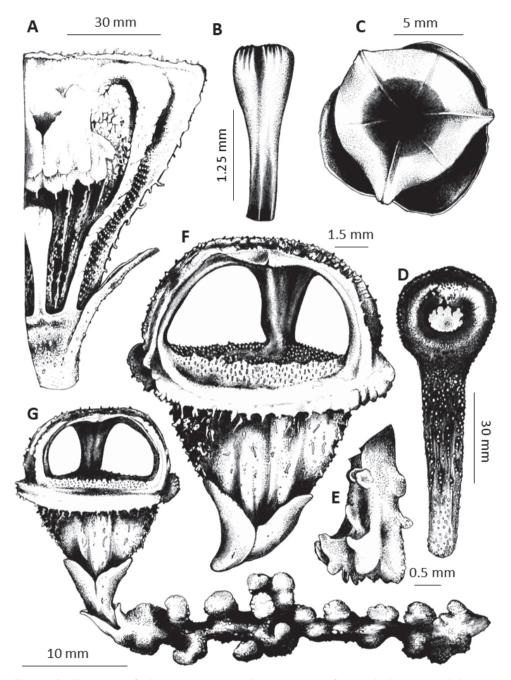


Figure 2. Illustration of *Thismia sitimeriamiae* **A** cross section of perianth showing pendulous stamens (above) and slender pistil (beneath) **B** pistil **C** aerial view of flower showing mitre and portions of apical part of floral tube and outer tepals **D** fruit **E** stamen (showing outer side of connective) **F** flower, lateral view **G** habit, showing inflorescence (flower) and roots. All illustrated from *FRI 91118* (excluding **D**, in situ).

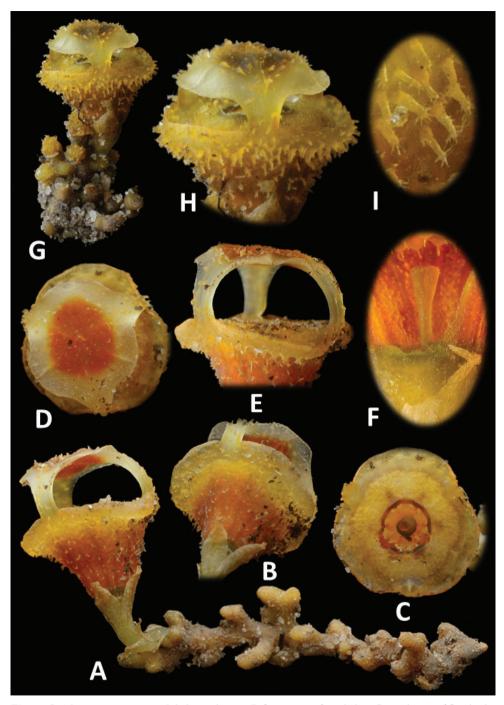


Figure 3. *Thismia sitimeriamiae* **A** habit with roots **B** flower, view from below **C** aerial view of floral tube (mitre removed) **D** flower, aerial view **E** mitre, lateral view **F** ovary and pistil, lateral view **G** flower, lateral view **H** mitre, lateral view **I** stellate trichomes on the outer surface of floral tube. All from *FRI 91118* (**A–F**) and a plant photographed in situ (**G–I**). Images not to scale (see dimensions in description and Figure 2).

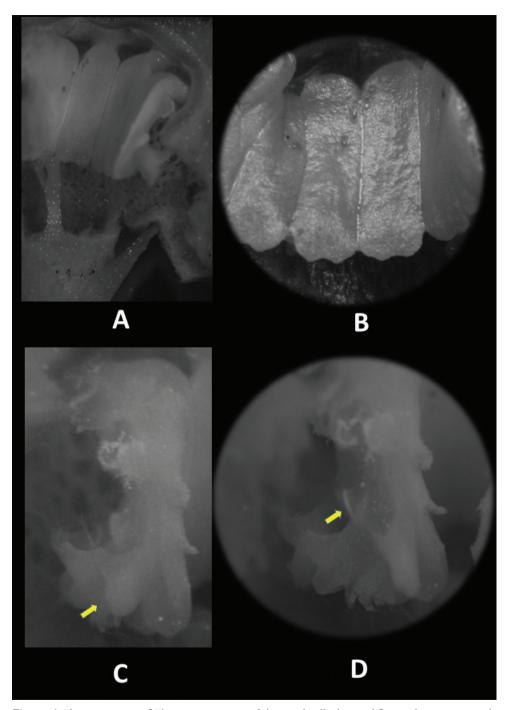


Figure 4. Flower structure of *Thismia sitimeriamiae* **A** longitudinally dissected flower, showing inner side of stamens and pistil **B** stamens, (inner view) **C** lateral view of stamens showing lateral appendage (arrow) **D** lateral view of outer stamens in which interstaminal glands (arrow) are discernible. All photos from *FRI 91123*. For dimensions, see description and Figure 2.

orange; *fruit* a cup-shaped capsule, greenish, borne on an elongated pedicel up to 6 cm long; *seeds* brown.

Additional specimen examined (paratype). MALAYSIA. Peninsular Malaysia: Terengganu, Setiu District, Hulu Nerus Forest Reserve, ca. 209 m a.s.l., 25 December 2020, *Dome Nikong FRI 91123* (KEP, spirit collection, barcode 28007).

Distribution. Endemic to Terengganu, Peninsular Malaysia. Currently known only from the type locality (Map 1).

Ecology. Lowland dipterocarp forest on moist soil in shade at an elevation of 209 m a.s.l. Flowering and fruiting from December to February.

Etymology. *Thismia sitimeriamiae* is dedicated to Siti Meriam, the mother of the second author (Dome Nikong), in honour of her unparalleled support for the conservation activities pursued by Dome Nikong and her help in maintaining his plant collections.

Conservation status. In accordance with the IUCN Red List Categories and Criteria (IUCN 2019), *Thismia sitimeriamiae* is assigned as Critically Endangered (CR), based on criterion B2 (ab (ii,iii)), because it is only known from the type locality, where fewer than five individuals have been observed (both in flower and in fruit), since survey work began.

Discussion

Taxonomy

Thismia sitimeriamiae is markedly distinct from all other species of Thismia reported to date. This species possesses unique morphological features including an outer floral surface covered with mixed simple and stellate trichomes, a connate mitre formed by the inner tepals with a flat elevated, umbrella-like portion and a pistil far more slender than those of other species described which normally possess a short and thick style. Thismia sitimeriamiae is morphologically most similar to T. coronata Hroneš, Dančák & Sochor (Dančák et al. 2020b) and T. kelabitiana Dančák, Hroneš & Sochor (Dančák et al. 2018) described from Sarawak, Malaysia. All three species possess yellow to orange flowers. However, T. sitimeriamiae differs in many traits, including its mitre structure and also the pistil shape which is more slender than those of *T. coronata* and *T. kelabitiana*; furthermore, the stigma of the latter two species is longitudinally furrowed, a pattern absent in T. sitimeriamiae. A detailed account of the morphological differences amongst the species is given in Table 1. The coralliform roots and presence of inner tepals connately fused to form a mitre indicate that T. sitimeriamiae belongs to Thismia sect. Sarcosiphon (Kumar et al. 2017). Based on recent molecular phylogenetic work carried out by Shepeleva et al. (2020), T. sitimeriamiae is likely to belong to a clade (denoted 'clade 3'), characterised by species which possess coralliform roots and inner tepals fused into a mitre without foveae and includes the morphologically similar species T. kelabitiana.

Character	T. sitimeriamiae	T. coronata	T. kelabitiana
Height (cm)	2.2	4.5-8	5–18
Stem		-	-
Length (cm)	0.8	2.4-4.2	1.5–16
Form	simple	branched	branched
Colour	whitish	reddish-brown to orange	dark pinkish to reddish-brown (to
			almost grey or orange)
Inflorescence (flower	Solitary	1–6	1–3
number)			
Leaves	1		
Number	6	4-11	3–10
Size			
Length (mm)	3–4	2–5	4.5–5.5
Width (mm)	1	1–2	1.8–2
Colour	pale, whitish	light brown to reddish	light brown to pinkish
Bracts			
Shape	broadly triangular to ovate	lanceolate triangular	broadly triangular to ovate
Margin	entire	entire to irregularly dentate	entire to irregularly dentate, often
			deeply dissected
Size			
Length (mm)	5	4-8	6–8
Width (mm)	2	2–3	2.5-4
Colour	pale greenish to brown	reddish to brown	pinkish to brown
Pedicel colour	greenish	reddish-brown to orange	dark pinkish to reddish brown (to
	0		almost grey or orange)
Flowers		l	
Size			
Length (cm)	1.5	1.8–2.3	2.6–2.8
Width (cm)	1.2	0.7–1	1.2–1.3
Colour	pale to dark orange	dark yellow to orange	white to bright yellow
Floral tube (hypanthiu		, ,	
Shape	conical	funnel-shaped towards the	funnel-shaped towards the base,
1		base, apically urceolate	apically urceolate
Colour	outer surface more or less	dark yellow to orange, with 6	white to bright yellow at the top,
	uniformly bright orange	brownish-orange, prominent	with six brownish non-prominent
	, , , ,	longitudinal ribs alternating with 6	longitudinal ribs and six yellow
		brown-orange longitudinal stripes	to brown longitudinal stripes on
		on outer surface	outer surface
Surface texture			
Outer	sparsely covered with pale orange,	glabrous (trichomes absent)	glabrous (trichomes absent)
	simple trichomes (occasionally		
	apically stellate)		
Inner	inner surface partly convex/	inner surface weakly reticulated,	inner surface reticulated
	reticulum	especially apically	
Outer tepals	3, rather reduced and	3, conspicuous, entire or slightly	3, deeply divided into 8-10 acute
	inconspicuous, somewhat divided	sinuate, often single-toothed,	lobes forming a conspicuous fringe
	along the margin to form a narrow,	forming a fringe around the floral	around the floral tube mouth
	inconspicuous fringe around the	tube mouth	
	floral tube mouth		
Inner tepals (and	pale whitish-orange, distally	orange, distally connate, forming	bright yellow to brownish-yellow,
mitre)	connate, forming an elevated, more	an elevated, more or less flat,	distally connate, forming a
	or less circular, umbrella-like mitre;	triangular mitre; lobes proximally	conspicuously elevated, more
	lobes proximally flattened with	filiform, pillar-like, arched over the	or less triangular mitre; lobes
	revolute margins (not filiform),	floral tube; trichomes absent	proximally filiform, pillar-like,
	arched over the floral tube; upper		arched over the floral tube;
	surface covered sparsely with		trichomes absent
	conspicuous orange trichomes	1	1

Table I. Morphological comparison of *Thismia sitimeriamiae*, *T. coronata* and *T. kelabitiana* (Dančák et al. 2018; Dančák et al. 2020b).

Character	T. sitimeriamiae	T. coronata	T. kelabitiana
Annulus (apical part of floral tube)	present (upper surface covered with short trichomes)	absent	absent
,	with short trichomes)		
Connectives	1	1	
Colour	orange	white	white
Length (mm)	3	5–6	7
Inner surface	glabrous	with prominent longitudinal rib extending along inner side	with prominent longitudinal rib extending along inner side
Apex of each connective	2 lobes, each pointed with a transparent trichome	1 central lobe (extension of rib) and 2 smaller lobes, each lobe with a long transparent trichome	1 central lobe (extension of the rib) and 2 smaller lobes pointing somewhat centrifugally, each with a transparent trichome
Outer surface	lateral appendage protruding towards floral tube, horn-like on each side, shallowly dentate and sparsely hairy on free margins	lateral appendage box-shaped, protruding towards floral tube, not reaching apex of connective, shallowly dentate and hairy on apical margin, with tufts of hairs on lateral margins	lateral appendage box-shaped, protruding towards perianth tube, not reaching the apex of the connective, shallowly dentate and sparsely hairy on free margins
Ovary			
Colour	flushed pale orange and greenish	dark brown	dark reddish-brown
Pistil			1
Style	slender	short	short
Stigma	lobes narrowly-rectangular, not furrowed; orange	lobes rectangular, longitudinally furrowed; dark brown	lobes rectangular, longitudinally furrowed; dark reddish-brown
Capsule			
Colour	greenish	pale brown to reddish at maturity	dark brown to blackish or reddish, maturing to become pinkish
Pedicel	elongated to 60 mm	10–25 mm	very short (dimensions not known)

Further work should augment the molecular phylogenetic reconstruction of Shepeleva et al. (2020) to resolve the phylogenetic relationships of *T. sitimeriamiae* with related taxa.

We should note that our assessment of *T. sitimeriamiae* is based on a very limited sample due to the rarity of the plant and a paucity of material. Similar challenges were encountered for other species in this taxonomically difficult genus in PM, for example, *T. chrysops*, *T. fumida*, *T. racemosa* and *T. kelantanensis* (Jonker 1948; Siti-Munirah 2018). Indeed, the majority of descriptions of *Thismia* have been based on single collections due to a lack of accessibility of their habitats, the difficulty of finding them in flower or because of habitat loss and probable extinction. Whilst the type locality of *T. sitimeriamiae* is readily accessible, attempts to recollect the species in the site and in surrounding areas in Terengganu have so far proved fruitless.

Reproductive ecology

The mating system and reproductive ecology of *T. sitimeriamiae* are unknown, as with most species of *Thismia*. Shepeleva et al. (2020) recommended a re-evaluation of the floral anatomy across the genus. The most detailed analysis of the floral architecture, anatomy and development of *Thismia* was carried out by Nuraliev et al. (2021). This work has identified an exceptional array of reproductive structures. The drivers of the exceptional floral diversity of *Thismia* are unknown, but possibly linked to their pollination biology. Mycoheterotrophs often produce inconspicuous flowers that are self-

pollinated to maximise the seed set in areas where pollinators are scarce (Waterman and Bidartondo 2008; Guo et al. 2019). However, Mar and Saunders (2015) and Guo et al. (2019) identified outcrossing in species of *Thismia* and observed fungus gnats as the pollinators. We did not observe any floral visitors to *T. sitimeriamiae*; however, efforts to understand its reproductive biology would be impeded by its exceptional rarity.

Conservation

We assess *T. sitimeriamiae* as Critically Endangered (CR B2 ab (ii,iii)) in accordance with the IUCN Red List Categories and Criteria because it is known only from the type locality, where just four individuals have been observed in total, including two flowering specimens in December 2019, one in fruit in February 2020 and a further specimen in flower in December 2020. The type locality is on a tourist trail within a forest reserve. Due to the sensitivity and human footfall of the habitat, the risk of disturbance is high. *T. sitimeriamiae* is probably unpredictable in appearance, like other species in the genus. Based on the authors' long-term observations of the type locality and associated areas, the species is exceptionally rare or absent elsewhere. Furthermore, based on recent observations by the second author (June 2021), the only known location of the plant has been destroyed by wild boar activity, meaning that the species is now at considerable risk of extinction.

Mycoheterotrophic plants, such as *Thismia*, present an interesting challenge in plant conservation. Most are probably intractable to cultivation due to their very exacting ecologies and dependency on specific fungal partners. Botanic gardens could play a part in the conservation of *Thismia* as they have for other intractable plants. For example, botanic gardens have played a role in the conservation of the mycoheterotrophic orchid *Rhizanthella gardneri* in Australia through survey work and successful transplant and propagation (Swarts and Dixon 2009; Thorogood et al. 2019). In the Philippines, conservation of parasitic *Rafflesia* has involved increasing the availability of host plants in fragmented habitats with rooted *Tetrastigma* cuttings (Pelser et al. 2017). Creative approaches may, therefore, be required for conserving rare and poorly understood species with complex ecological interdependencies. Given the rarity and inaccessibility of the vast majority of species of *Thismia* (many of which have been found only once), *in situ* conservation seems to be the only realistic approach.

Concluding remarks

Since the most comprehensive taxonomic revision of the genus *Thismia* in the 1930s and 40s (Jonker 1938, 1948), the number of species described in this genus has proliferated. Recent species discoveries and investigations of the floral anatomy and evolution of the genus have brought an astonishing diversity of floral structure to our attention (Shepeleva et al. 2020; Nuraliev et al. 2021). The extraordinary morphology and extreme scarcity of *Thismia sitimeriamiae* raise interesting questions about the ecology, evolution and conservation of this and other species in this exceptional genus. Further survey work and taxonomic assessment will provide information for the setting of

conservation priorities for these mysterious plants, many of which have been seen only once and some may never be seen again.

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RESEARCH ARTICLE



Aphyllorchis yachangensis (Orchidaceae), a new holomycotrophic orchid from China

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Abstract

Aphyllorchis yachangensis, a new holomycotrophic orchid from Guangxi, southern China is described and illustrated here. This new species is similar to *A. caudata* but differs from the latter mainly by the sepals acute at the apex, the hypochile with 2 smaller and semicircular wings, the epichile adaxially smooth, acute, the lateral lobes triangular-ovate and the column clavate.

Keywords

Aphyllorchis, Guangxi Province, new taxa, saprophytic Orchidaceae, taxonomy

Introduction

Aphyllorchis Blume (1825) includes about 30 species, and is mainly distributed in tropical Asia and the Himalayas, extending to the north of Japan and to the south of Australia (Chen and Stephan 2009; Tian et al. 2013). The species of *Aphyllorchis* are holomycotrophic herbs, with short rhizomes; fleshy roots; erect, unbranched stems, with sheaths; racemose inflorescences; petals similar to sepals, free; lip 2-partite, hypochile smaller than epichile, sometimes without hypochile; staminodes 2; pollinia 2, granular-farinaceous, caudicles absent (Chen and Stephan 2009). In China, six species and one variety are reported from the South, Southwest and Taiwan Island, including *Aphyllorchis alpina* King & Pantling, *A. caudata* Rolfe ex Downie, *A. gollanii* Duthie, *A. montana* H. G. Reichenbach var. *montana*, *A. montana* H. G. Reichenbach var.

membranacea T. C. Hsu, *A. pallida* Blume and *A. simplex* Tang & F. T. Wang (Chen and Stephan 2009; Fan et al. 2011; Hsieh et al. 2013; Huang et al. 2014; Hsu and Chung 2017; Lin 2017).

In late July 2018, during a botanic expedition in the Yachang Orchid National Nature Reserve, Guangxi Province in China, the first author collected a specimen (*Ying Qin* et al. *YC3639*) belonging to *Aphyllorchis*, with residual flowers. The narrow hypochile with 2 unobvious wings at base and purple epichile of the species drew the author's attention. However, due to the incomplete structure of sepals, petals and column, it was not accurately identified at that point. Fortunately, after two further botanic expeditions, one in July 2019 and the other in July 2020, we successfully collected two specimens (*Ying Qin* et al. *QY20190719001* and *Ying Qin* et al. *QYYC20200703009*) belonging to the same taxon, but on these occasions with complete flowers. After dissecting and examining those flowers, and consulting the relevant literature (Reichenbach 1876; Downie 1925; Fukuyama 1934; Pearce and Cribb 1999; Roy et al. 2009; Aravindhan et al. 2013; Merckx et al. 2013; Tian et al. 2013; Campbell 2014; Sun et al. 2017; Suetsugu et al. 2018), we finally confirmed that it is a new species, which is described here.

Material and methods

From July 2018 to July 2020, we examined four specimens of *Aphyllorchis* in IBK on the field, and also examined forty specimens of *Aphyllorchis* in PE, HITBC, KUN, AU, IMDY, SZG, etc. through CVH (https://www.cvh.ac.cn/index.php). Except for *Ying Qin* et al. *YC3639, Ying Qin* et al. *QY20190719001* and *Ying Qin* et al. *QYYC20200703009* collected by the first author and kept in IBK, none of the specimens belong to *Aphyllorchis yachangensis*. Photographs of plants and flowers were taken using a Canon PowerShot G16. Morphological characters of the new species were measured with a ruler on living plants in the wild. The terminologies used to describe parts of the new species, such as rhizome, ovary, sepals, petals, lip, hypochile, epichile, column, etc. come from Flora of China (Chen and Stephan 2009).

Taxonomic Treatment

Aphyllorchis yachangensis Ying Qin & Yan Liu, sp. nov. urn:lsid:ipni.org:names:77218063-1 Figures 1, 2

Diagnosis. Aphyllorchis yachangensis is similar to A. caudata but differs from the latter mainly by its hypochile with two wings of $1-1.4 \times 0.4-0.6$ mm (vs. $2-3 \times ca.4$ mm), epichile adaxially smooth (vs. densely papillose), lateral lobes triangular-ovate (vs. semicircular), sepals acute at apex (vs. long cuspidate) and column clavate (vs.

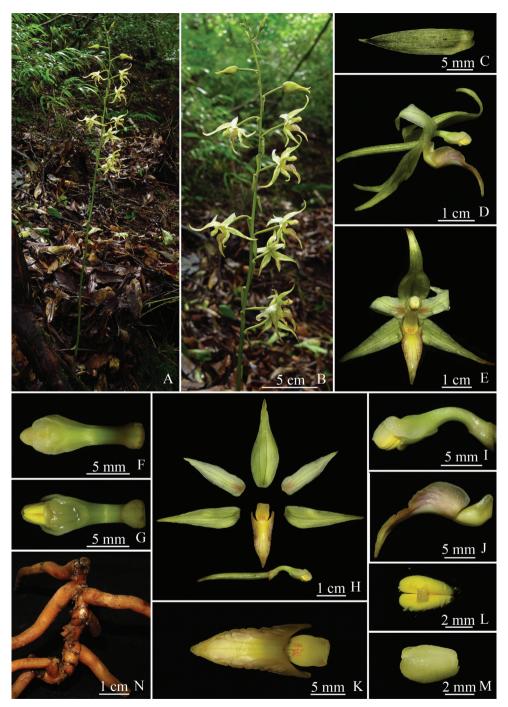


Figure 1. *Aphyllorchis yachangensis* **A** flowering habit **B** inflorescence **C** bract **D** flower in side view **E** flower in front view **F** column in top view **G** column in ventral view **H** lip, dorsal sepal, lateral sepals, petals, column and ovary **I** column in side view **J** lip in side view **K** lip in top view **L** pollinaria **M** anther cap in top view **N** rhizome and roots. Photographed by Ying Qin from *Ying Qin* et al. *QY20190719001* (holotype: IBK!).

approximately cylindrical). Detailed morphological comparisons between *A. yachan*gensis and *A. caudata* are provided in Table 1.

Type. CHINA. Guangxi, Baise city, Leye county, Yachang Orchid National Nature Reserve, 1862 m, 19 July 2019, *Ying Qin* et al. *QY20190719001* (*holotype*: IBK! isotype: IBK!).

Description. Herb holomycotrophic, leafless, 107-113 cm tall. Rhizome ca. 10.8 cm long, 4.9-6.2 mm in diameter, densely noded; internodes 2.4-12 mm long. Roots spreading, stout, 3.4-6.4 mm in diameter. Stem 61-71 cm tall, 4.5-7.3 mm in diameter, yellow-green, usually with many dark purple stripes and spots, with 1.4-4 cm long sheaths; the uppermost sheath lanceolate; other sheaths tubular. Inflorescence terminal, racemose, 42-47 cm long, with 19-23 well-spaced flowers; rachis sparsely glandular puberulent, yellow-green, usually with many dark purple stripes and spots. Bracts lanceolate, shorter than pedicel and ovary, 1.2-3 cm long, 2.8-6.8 mm wide, yellow-green, usually with many dark purple stripes and spots on abaxial surface, sparsely glandular puberulent. Ovary cylindrical, yellow-green, usually with many dark purple stripes and spots, sparsely glandular puberulent, 2.2-2.8 mm in diameter, including pedicel to 2.3-3.5 cm long. Sepals yellow-green, usually with many dark purple stripes and spots on abaxial surface, abaxially sparsely glandular puberulent; dorsal sepal lanceolate, slightly concave, 2.5-4 cm long, 7.9-10.7 mm wide, apex acute, reflexed; lateral sepals lanceolate, slightly oblique at base, 2.5-4 cm long, 6.1-8.2 mm wide, apex acute, reflexed. Petals lanceolate, reflexed, 2.2-2.7 cm long, 5.6–7.2 mm wide, yellow-green, pale purple to dark purple at base, apex acute. Lip 2-partite; hypochile narrow, concave, 6.6-7.1 mm long, 3.6-4.5 mm wide, yellow or reddish brown, with 2 unobvious wings at base; wings semicircular, 1–1.4 mm wide at base, 0.4–0.6 mm tall; epichile ca. 1.9–2.2 cm long, 1.3–1.5 cm wide when flattened, 3-lobed, yellow with pale purple, or purple to dark purple, adaxially smooth, margin slightly erose; lateral lobes triangular-ovate, 8.2–8.9 mm long, 3.6–4.3 mm wide; midlobe narrowly triangular, 1.1-1.3 cm long, 5.6-5.9 mm wide, acute at apex, margin slightly involute. Column clavate, arcuate, 1.4–1.6 cm long, 1.8–3.3 mm in diameter in lower part, 4.3-6.1 mm in diameter in upper part, yellow-green, usually purple from middle to base; stigma ovate, slightly concave; staminodes 2, on both sides of apex, yellow-white to pale yellow-green; pollinia 2, ovate, granular-farinaceous, 4 mm long, yellow; anther cap ovate, slightly laterally compressed, 4 mm long, pale yellow.

Distribution, habitat and ecology. *Aphyllorchis yachangensis* was discovered in the Yachang Orchid National Nature Reserve, Leye county, Baise city, Guangxi Province, China. The holotype subpopulation is distributed in a subtropical evergreen and deciduous broad-leaved mixed forest, and is found growing with *Chimonobambusa quadrangularis* (Franceschi) Makino (Gramineae), *Lithocarpus glaber* (Thunberg) Nakai (Fagaceae), *Manglietia fordiana* Oliver (Magnoliaceae), *Juglans mandshurica* Maximowicz (Juglandaceae), *Dysosma majorensis* (Gagnep.) Ying (Berberidaceae), *Ophiopogon bockianus* Diels (Liliaceae), *Disporopsis fuscopicta* Hance (Liliaceae), *Calanthe brevicornu* Lindley (Orchidaceae), *Goodyera biflora* (Lindley) J. D. Hooker (Orchidaceae), *G. velutina* Maximowicz ex Regel (Orchidaceae), etc.

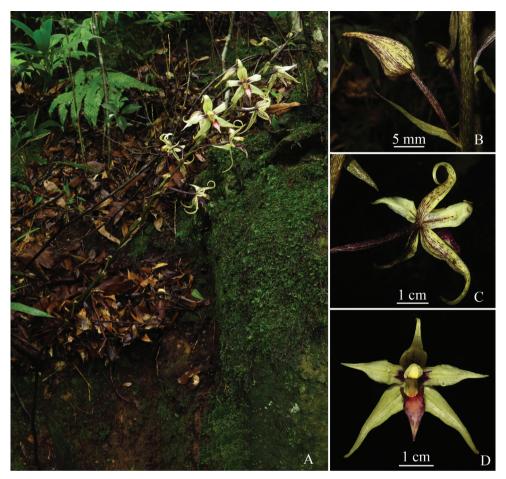


Figure 2. *Aphyllorchis yachangensis* (an individual with dark colors in the same environment with *Ying Qin* et al. *QY20190719001*) **A** flowering habit **B** bud **C** flower in back view **D** flower in front view. Photographed by Ying Qin from *Ying Qin* et al. *QYYC20200703009* (paratype: IBK!).

Table	I. Morphological	comparison	of Aphyllorchis	yachangensis	and A. caudata.
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Characters	A. yachangensis	A. caudata	
Plant size	107–113 cm	100 cm	
Inflorescence	42–47 cm	rachis to 50 cm	
Bracts	lanceolate, 12–30 × 2.8–6.8 mm	narrowly lanceolate, 40–48 × 5–6 mm	
Dorsal sepal	lanceolate, 25–40 × 7.9–10.7 mm, apex acute	linear-lanceolate or lanceolate, 30–35 × ca. 8.0 mm,	
		apex long cuspidate	
Lateral sepals	lanceolate, 6.1–8.2 mm wide, apex acute	linear-lanceolate or lanceolate, 6–7 mm wide, apex long	
		cuspidate	
Petals	lanceolate, 22–27 × 5.6–7.2 mm	lanceolate, ca. 20 × 6–7 mm	
Hypochile	6.6-7.1 mm long, with 2 wings; wings semicircular,	2-3 mm long, with 2 wings; wings ligulate, 2-3 × ca.	
	1–1.4 × 0.4–0.6 mm	4 mm	
Epichile	1.9-2.2 cm long, adaxially smooth; lateral lobes	ca. 1.2 cm long, adaxially densely papillose; lateral	
	triangular-ovate	lobes semicircular	
Column	1.4-1.6 cm long, clavate (apex obviously inflated)	1.1–1.4 cm long, approximately cylindrical	
Flowering period	late June and July	July and August	

Phenology. Flowering in late June and July, capsules not seen.

Etymology. The specific epithet is derived from the type locality, Yachang Orchid National Nature Reserve.

Chinese name. 雅长无叶兰 (Ya Chang Wu Ye Lan)

Additional specimens examined (paratypes). CHINA: Guangxi: Baise city, Leye county, Yachang Orchid National Nature Reserve, 1862 m, 28 July 2018, *Ying Qin* et al. *YC3639* (IBK!); Guangxi: Baise city, Leye county, Yachang Orchid National Nature Reserve, 1859 m, 03 July 2020, *Ying Qin* et al. *QYYC20200703009* (IBK!).

Key to Aphyllorchis taxa in China

1	Lip similar to lateral petals
_	Lip distinctly different from petals
2	Bracts longer than pedicel and ovary
_	Bracts shorter than pedicel and ovary
3	Flowers yellowish green; bracts linear to linear-lanceolate, 3-4 mm wide; lip
	contracted at middle into hypochile and epichile
_	Flowers pale purplish brown; bracts ovate to elliptic-lanceolate, 6-8 mm
	wide; lip contracted near base into hypochile and epichile
4	Sepals equal to or longer than 25 mm
_	Sepals equal to or shorter than 11 mm
5	Sepals apex long cuspidate; epichile adaxially densely papillose A. caudata
_	Sepals apex acute; epichile adaxially smooth
6	Sepals 4–5 mm long
_	Sepals 9–11 mm long
7	Lip fleshy A. montana var. montana
_	Lip membranous A. montana var. membranacea

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RESEARCH ARTICLE



New circumscriptions add two northern Andean species to Kohleria (Gesneriaceae)

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Abstract

Recent studies of type specimens and exploratory research expeditions in the northern Andes have resulted in an updated circumscription and recognition for two species of *Kohleria* (Gesneriaceae) in Ecuador and Colombia. A change in the rank from a variety to species is recognized for *Kohleria anisophylla* (Fritsch) Wiehler. The combination *Kohleria andina* (Fritsch) J.L. Clark & Jost, **comb. nov.** is provided here and a lectotype is designated. The updated circumscriptions of these two species are supported by morphology and geographic distribution. The presence of an epiphytic habit for *Kohleria* is discussed. Field images based on recent expeditions are provided to support the circumscriptions presented here.

Resumen

Los estudios recientes de las colecciones tipo y las expediciones exploratorias en el norte de los Andes han dado como resultado la actualización en la circunscripción y reconocimiento de dos especies de *Kohleria* (Gesneriaceae) en Ecuador y Colombia. Se reconoce el cambio de rango de variedad a especie para *Kohleria anisophylla* (Fritsch) Wiehler. Se presenta la nueva combinación *Kohleria andina* (Fritsch) J.L. Clark & Jost, **comb. nov.** con la designación de su lectotipo. La circunscripción actualizada de estas dos especies está soportada por caracteres morfológicos y distribución geográfica. Se discute la presencia del hábito epífito en *Kohleria.* Se presentan imágenes obtenidas en las expediciones de campo para soportar las circunscripciones propuestas aquí.

Keywords

Colombia, Ecuador, Gesneriaceae, Kohleria, taxonomy

Introduction

The flowering plant family Gesneriaceae, with over 3400 species and 150+ genera (Weber 2004; Weber et al. 2013) is in the order Lamiales. The family is divided into three subfamilies and seven tribes (Weber et al. 2013, 2020). The majority of New World members are in the subfamily Gesnerioideae and are represented by 1200+ species and 77 genera (Clark et al. 2020). The New World subfamily Sanangoideae is limited to one genus and one species (Weber et al. 2013, 2020). *Kohleria* Regel is classified in the tribe Gesnerieae Dumort. and subtribe Gloxiniinae G. Don (Weber et al. 2013, 2020).

Kohleria was monographed by Kvist and Skog (1992), who recognized 17 species. An additional two species were transferred to *Kohleria* from *Capanea* Decaisne ex Planchon (Roalson et al. 2005a) based on molecular phylogenetic analyses of the tribe Gloxinieae (Roalson et al. 2005b). Clark and Skog (2008) described *Kohleria hypertrichosa* J.L. Clark & Skog from the western Andes of northern Ecuador. The recognition of two more *Kohleria* species here brings the total number in the genus to 22 species.

Kvist and Skog (1992) broadly defined many species in their monographic revision of *Kohleria*. For example, *Kohleria hirsuta* (Kunth) Regel var. *hirsuta* (sensu Kvist and Skog 1992) includes more than 40 names representing 15 heterotypic synonyms. Kvist and Skog (1992) noted a wide range of morphological variation when circumscribing taxa, and that variation was attributed to hybridization. Molecular tools, fieldwork, and ready access to digital images are necessary for evaluating many of these broadly circumscribed species. A current doctoral dissertation project by Kimberly Hansen from Washington State University (USA) and an undergraduate thesis by Katherin Arango-Gómez from the Universidad del Valle (Colombia) are evaluating the phylogeny and taxonomy of *Kohleria* based on the use of molecular tools, herbarium specimens, and extensive field work. The updated circumscriptions provided here will hopefully play a role in facilitating these active projects.

Results

New generic placement requires new combination and lectotypification for *Kohleria andina*

Kohleria andina (Fritsch) J.L. Clark & Jost, comb. nov.

urn:lsid:ipni.org:names:77218228-1

Capanea andina Fritsch, Bot. Jahrb. Syst. 50: 431–432. 1913 ("1914"). Type: Ecuador. Andes Quitenses, Tunguragua, 1857, *R. Spruce 5178* (lectotype K000395097, designated here; isolectotypes: BM000953512, E00062367, G00370826, G00370838, K000395097).

Remarks. One of the key characters discussed by Kvist and Skog (1992) as the basis for the generic circumscription of *Kohleria* was a terrestrial (i.e., non-epiphytic) habit. In the

generic delimitation of *Kohleria* (Kvist and Skog 1992), the habit is described as herbs, subshrubs, shrubs, or rarely scandent shrubs. The terrestrial habit was considered a character by Kvist and Skog (1992) to differentiate *Kohleria* from closely related genera that are epiphytes or lianas. Phylogenetic studies by Roalson et al. (2005b) showed that *Kohleria* was paraphyletic with the exclusion of "*Capanea*", a group of epiphytic subshrubs from the Andes. Thus, many of the features that differentiated "*Capanea*" from *Kohleria*, such as an epiphytic habit and four-valved capsules, are autapomorphic. The transfer of two species from "*Capanea*" to *Kohleria* is well-supported, and combinations were made by Roalson et al. (2005b). Roalson et al. (2005b) did not make a combination for *Kohleria andina* because it was considered a heterotypic synonym of *Kohleria affinis*. Examination of material in the field and in herbaria allowed us to recognize *K. affinis* and *K. andina* as different species. Outlined here are characters to differentiate *K. andina* from *K. affinis* (see Table 1 for a summary of the characters that are discussed below).

Another feature that defines the clade previously recognized as "*Capanea*" is the presence of resupinate flowers via a twisted pedicel. The androecium and gynoecium are located in the lower region of the corolla tube (Fig. 1B, C and Fig. 2B, C). In contrast, all other *Kohleria* and closely related genera have the androecium and gynoecium in the upper region of the corolla tube.

The flowers of *Kohleria affinis* are often photographed because of their conspicuous clusters of brightly colored purple-red corolla tubes with contrasting green lobes (Fig. 1). It is common to see individuals with 50+ pendent flowers, especially in abandoned cow pastures or recently cleared forests. Herbarium specimens do not preserve floral colors and most corollas dry uniformly black. Thus, corolla colors are challenging to determine on dried herbarium specimens unless noted by collectors in the descriptions. Use of field-based images, review of taxonomic literature, and examination of type specimens provided information for re-assessing the circumscription of *Kohleria affinis* and *K. andina*.

The corolla tube of *Kohleria andina* is white, but appears bright yellow from dense tomentose yellow trichomes (Fig. 2). In contrast, the corolla tube of *Kohleria affinis* is dark red to bright purple (Fig. 1). The corolla tube in most *Kohleria affinis* is narrow, but some populations from Colombia are broad. The corolla tube of *Kohleria andina* is consistently broad. Corolla length in *Kohleria affinis* is highly variable and ranges from 3 to 6 cm. In contrast, the corolla tubes of *Kohleria andina* are usually less than 3.5 cm long. Both species have bright green corolla lobes that contrast with dark purple spots on the inner surface (Figs 1, 2).

	Kohleria affinis (Fritsch) Roalson & Boggan	Kohleria andina (Fritsch) J.L. Clark & Jost
Corolla tube shape	usually narrow, rarely broad (Colombia)	broad
Corolla tube color	dark red to bright purple	white
Corolla tube trichome color	transparent	yellow
Corolla tube length	3–6 cm	< 3.5 cm
Peduncle and pedicel trichome color	transparent	purple
Distribution	widespread in Colombia, Ecuador, and	endemic to the Ecuadorian province of
	northern Peru	Tungurahua)

Table 1. Morphological differences and general distribution of Kohleria affinis and K. andina.

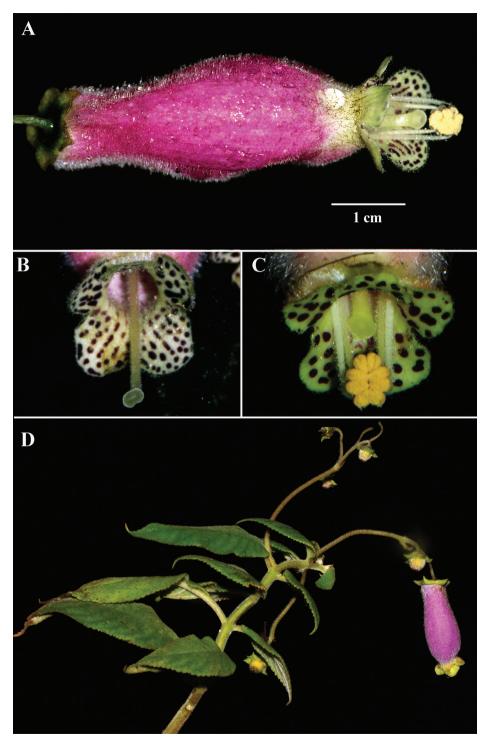


Figure I. *Kohleria affinis* (Fritsch) Roalson & Boggan **A** lateral view of flower **B** female phase of mature flower **C** male phase of mature flower **D** habit (**A** *Clark et al. 7698* **B** *Clark s.n.* **C** *Clark et al. 12979* **D** *Clark et al. 15845*). Photos by J.L. Clark.

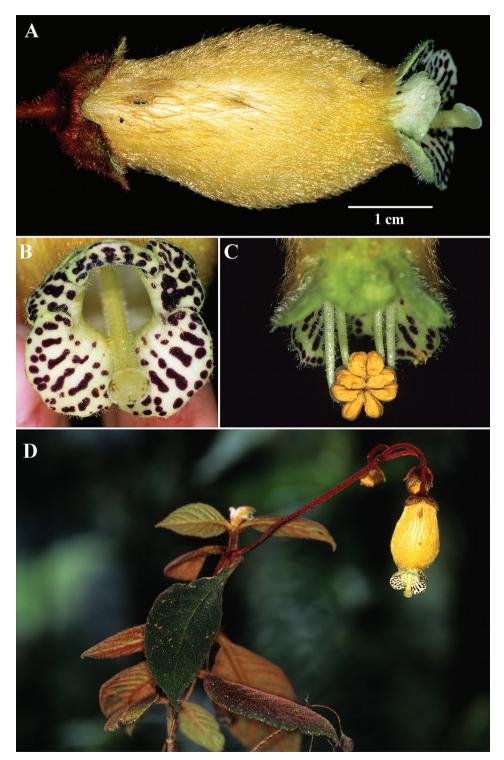


Figure 2. *Kohleria andina* (Fritsch) J.L. Clark & Jost **A** lateral view of flower **B** female phase of mature flower **C** male phase of mature flower **D** habit (**A–D** *Clark et al. 7750*). Photos by J.L. Clark.

An additional character useful for differentiating *Kohleria andina* from *K. affinis* is the presence of dark red-purple trichomes on the peduncles and pedicels (Fig. 2A, D). The red-purple trichomes on the peduncles were noted by Fritsch (1913: page 432) in the protologue, "Pedunculi axillares elongati purpureo-villosi." In contrast, the peduncles in *Kohleria affinis* are sparsely pilose and appear green due to transparent trichomes.

Kohleria andina and K. affinis are geographically separated by elevation. K. andina is endemic to elevations above 2500 meters on the western Andean slopes (Cordillera Oriental) in the Tungurahua province of Ecuador. In contrast, Kohleria affinis is widespread in the northern Andes of Colombia (Antioquia, Boyacá, Caldas, Caquetá, Cauca, Chocó, Cundimarca, Huila, Nariño, Putumayo, Quindío, Risaralda, and Valle del Cauca), Ecuador (Azuay, Bolívar, Carchi, Chimborazo, Cotopaxi, Esmeraldas, Imbabura, Loja, Napo, Pichincha, Santo Doingo, Tungurahua, and Zamora-Chinchipe), and northern Peru (Amazonas and Cajamarca). The authors' field work from the upper slopes of Tungurahua, an active volcano in the western Andean slopes of the Cordillera Oriental, revealed little overlap. Kohleria andina is locally endemic to elevations above 2500 meters and K. affinis is widespread and located in forests below 2500 meters. Intermediate forms were not found here, indicating that these two forms are geographically separated by elevation and supported as different biological species.

Lectotypification. Syntypes are from two distinct localities: *F.C. Lehmann* 4869 (F0060498) from Colombia and *R. Spruce* 5178 from K (K000395097) from Tungurahua, Ecuador. The specimen of *F.C. Lehmann* 4869 (F) is more similar to the widespread *Kohleria affinis*. The specimen of *R. Spruce* 5178 from (K) is similar to the locally endemic *Kohleria andina*, and is designated as the lectotype to stabilize this species concept. According to Fritsch (1913), Richard Spruce cites Tunguarahua as a locality and the specimens have characters that are congruent with the Tungurahua populations featured in the images here (Fig. 2). The lectotype has a corolla that is wide and more ampliate (Fig. 2) relative to the narrower corolla tube of *K. affinis* (Fig. 1). An additional character that is congruent with material from the type locality and the lectotype (*R. Spruce* 5178) is the presence of dark red trichomes on the peduncles and pedicels. In contrast, the peduncle and pedicel trichomes on *F.C. Lehmann* 4869 are transparent and more similar to *K. affinis*.

Revised species circumscription for Kohleria anisophylla

Kohleria anisophylla (Fritsch) Wiehler

Kohleria anisophylla (Fritsch) Wiehler.

Kohleria anisophylla (Fritsch) Wiehler, Selbyana 5: 62. 1978. Type: Based on Diastema anisophyllum Fritsch.

Kohleria villosa var. anisophylla (Fritsch) Kvist & Skog, Smithsonian Contr. Bot. 79: 70. 1992. Type: Based on *Diastema anisophyllum* Fritsch. Basionym.

- Diastema anisophyllum Fritsch, Bot. Jahrb. Syst. 50: 408. 1913 ("1914"). Type: Colombia. [Nariño] Piedra Ancha, West of Andes of Tuquerres, *F.C. Lehmann 5843* (B, holotype not extant, lectotype K000509983, designated by Wiehler (1978: 62), isolectotype K000509984).
- Nematanthus erianthus Bentham, Pl. Hartw: 231. 1846. Type: Ecuador. Pichincha: Quito towards Nanegal, *Hartweg s.n.* (holotype K000509985).
- Columnea eriantha (Bentham) Hanstein, Linnaea 34: 391. 1865. Type: Based on Nematanthus erianthus Fritsch.
- Diastema anisophyllum Fritsch var. quitense Fritsch. Bot. Jahrb. Syst. 50(4): 408. 1913 ("1914"). Type: Ecuador. [Pichincha] Quito, W. Jameson s.n. (holotype W).

Remarks. *Kohleria anisophylla* (Fig. 3) was previously recognized by Kvist and Skog (1992) as a variety of *Kohleria villosa* (Fig. 4). The strongly anisophyllous leaves and dorsiventral shoots (Fig. 3D) are more similar to *Kohleria hypertrichosa* (Fig. 5D) than *K. villosa* (Fig. 4D). All three species are found on the northwestern Andean slopes of Ecuador. Only *Kohleria anisophylla* is documented from Colombia (Nariño department). Wiehler (1978) made the combination *Kohleria anisophylla* and recognized it at the rank of species. Kvist and Skog (1992) recognized this taxon as *Kohleria villosa* var. *anisophylla*. Based on limited material, Wiehler (1978) cited the type (*F.C. Lehmann 5843*) and a recently collected specimen from Ecuador (*C. Luer & A. Hirtz 2672*). Kvist and Skog (1992) cited the same Ecuadorian collection and mentioned the study of eleven additional specimens. This species is common along the northwestern slopes of the Ecuadorian Andes, especially along the old road between Quito and Santo Domingo where many of the images were taken for Figure 3. Outlined here are characters to differentiate *K. anisophylla*, *K. villosa*, and *K. hypertrichosa* (see Table 2 for a comparison of characters that are discussed below).

The recent transfer (Roalson et al. 2005b) of *Kohleria affinis* and *K. tigridia* (Ohlend.) Roalson and Boggan represented an autapomorphic synapomorphy of epiphytism in traditionally recognized *Kohleria*. What is noteworthy about *Kohleria anisophylla* and *K. hypertrichosa* is their previously unreported epiphytic habits. Thus, the presence of an epiphytic habit in *K. anisophylla* and *K. hypertrichosa* could represent an additional independent origin of epiphytism in *Kohleria*. Several populations of *Kohleria anisophylla* were observed and documented with dorsiventral shoots, a

Table 2. Morphological differences and general distribution of *Kohleria anisophylla*, *K. villosa*, and *K. hypertrichosa*.

	Kohleria anisophylla (Fritsch)	Kohleria villosa (Fritsch) Wiehler	Kohleria hypertrichosa J.L. Clark
	Wiehler		& L.E. Skog
Habit	facultative epiphyte	terrestrial	facultative epiphyte
Shoots	dorsiventral	erect	dorsiventral
Relative leaf size	anisophyllous	isophyllous	anisophyllous
Corolla vestiture	villous	villous	tomentose
Distribution	Ecuador (Bolívar, Carchi, Pichincha)	Ecuador (Bolívar, Carchi, Cotopaxi,	northern Ecuador (Carchi and
	and Colombia (Nariño)	Esmeraldas, Imbabura, Pichincha)	Esmeraldas)

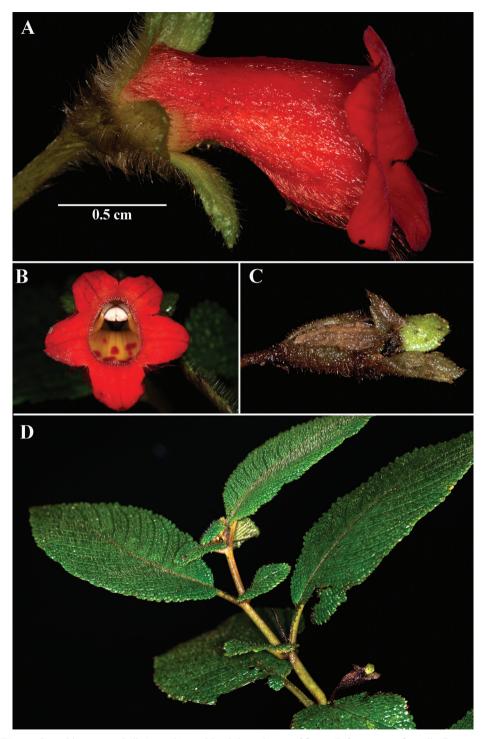


Figure 3. *Kohleria anisophylla* (Fritsch) Wiehler **A** lateral view of flower **B** front view of corolla **C** mature fruit **D** dorsiventral habit with anisophyllous leaves (**A**, **B** *Clark et al. 10981* **C** *Clark 10948* **D** *Clark et al. 14295*). Photos by J.L. Clark.

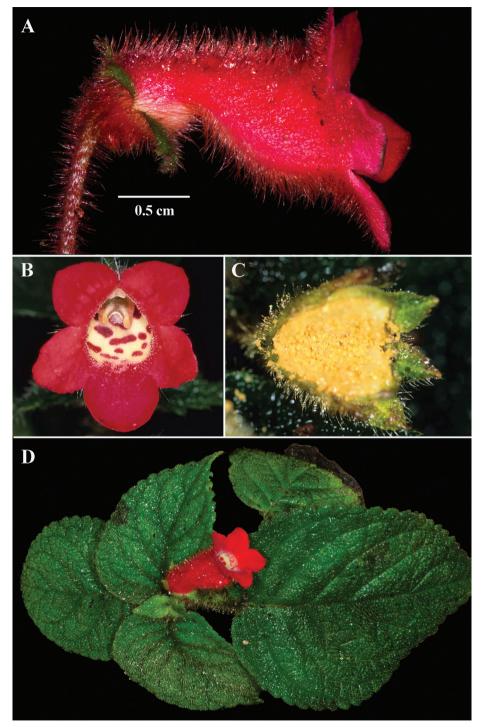


Figure 4. *Kohleria villosa* (Fritsch) Wiehler A lateral view of flower B front view of corolla C mature fruit
D erect herbaceous habit with isophyllous leaves (A *Clark et al. 14295* B *Clark 7331* C *Clark et al. 7400*D *Clark et al. 14295*). Photos by J.L. Clark.

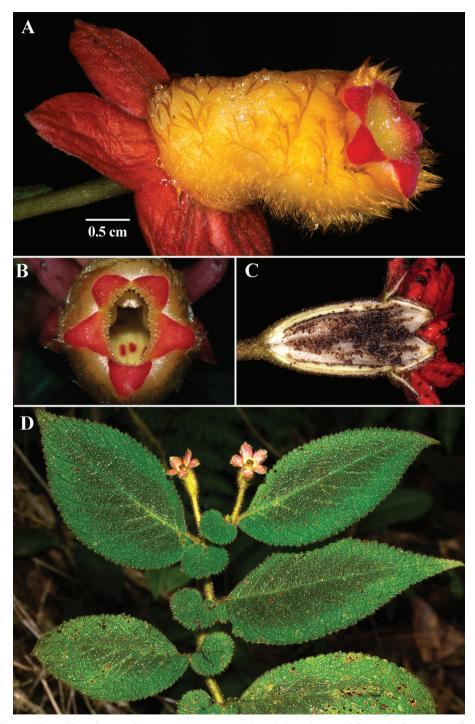


Figure 5. *Kohleria hypertrichosa* J.L. Clark & L.E. Skog **A** lateral view of flower **B** front view of corolla **C** mature fruit **D** dorsiventral habit with anisophyllous leaves (**A** *Clark et al. 15900* **B** *Clark 6539* **C** *Clark et al. 10310* **D** *Clark et al. 14942*). Photos by J.L. Clark.

feature that is common in facultative epiphytes in other Gesneriaceae genera. Many members of *Columnea* have strongly anisophyllous leaves – especially species that are facultative epiphytes with dorsiventral shoots. Other species of Gesneriaceae that are facultative epiphytes with dorsiventral shoots include *Cremosperma anisophylla* J.L. Clark & L.E. Skog, *Drymonia anisophylla* L.E. Skog & L.P. Kvist, and the majority of species in *Monopyle* Moritz ex Benth. and *Trichodrymonia* Oerst. Likewise, *Kohleria anisophylla* and *K. hypertrichosa* are facultative epiphytes with dorsiventral shoots are facultative epiphytes with dorsiventral shoots and anisophylla and *K. hypertrichosa* are facultative epiphytes with dorsiventral shoots and anisophyllous leaves. In contrast, *Kohleria villosa* is a terrestrial herb with isophyllous leaves (Fig. 4D).

The corollas of *Kohleria villosa* and *K. anisophylla* are villous (Figs 3, 4). The corollas of *Kohleria hypertrichosa* are densely tomentose (Fig. 5). The specific epiphyte, "*hypertrichosa*" refers to the abundance of trichomes, which is why it is commonly known in the horticultural community as "Chewbacca," a reference to the Wookie (fictional character) in the movie Star Wars.

Kohleria villosa and *K. anisophylla* are easily recognized when sterile. The opposite leaves of *Kohleria anisophylla* are consistently unequal in size or anisophyllous (Fig. 3D). In contrast, the opposite leaves of *Kohleria villosa* are consistently equal in size or isophyllous (Fig. 4D). In addition, the dorsiventral shoots distinguishes *K. anisophylla* from the erect shoots of *K. villosus*.

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We thank Don Hector Yela for facilitating fieldwork in the Reserva Dracula where populations of *Kohleria hypertrichosa* are currently preserved. We thank Laura Clavijo from the Instituto de Ciencias Naturales – Universidad Nacional de Colombia for the Spanish translation of the Abstract and for providing helpful comments on the manuscript. Finally, we are grateful to Christian Feuillet and an anonymous reviewer for providing valuable feedback on an earlier version of the manuscript.

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RESEARCH ARTICLE



Morphology of pollen in Ferula genus (Apiaceae)

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Abstract

In this study, the pollen morphology of all *Ferula* species distributed throughout the country of Turkey was studied with light and scanning electron microscopy for the first time. The aim is to identify the pollen morphological characteristics of 23 *Ferula* species. The pollen is radially symmetrical, isopolar and tricolporate in all examined species. Pollen grains are prolate and perprolate with the polar axis ranging from 22.28 to 40.47 μ m and the equatorial axis from 13.70 to 18.73 μ m. Their polar shapes are triangular, triangular to subtriangular and circular to subcircular. Several types of exine ornamentations have been observed on pollen through the use of scanning electron microscopy. The dendrogram constructed by using Average Linkage of the examined data revealed two main groups. It was determined that some pollen characteristics are more useful for classification than others. In particular, P, E, the ratio of P/E (pollen shape) and ornamentation in the polar and equatorial views are the most valuable variables for discrimination the *Ferula* species.

Keywords

Apiaceae, Ferula, LM, pollen morphology, SEM, Turkey

Introduction

Apiaceae is one of the largest plant families in the world. Apiaceae comprise approximately 450 genera and 3700 species, chiefly in north temperate regions (Pimenov and Leonov 2004).

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The largest centere of biodiversity for this family in Asian countries is Turkey, with about 160 endemic species included in 44 genera (Bilgili et al. 2016). These family members are of considerable economic importance as food, flavouring and ornamental plants.

The genus *Ferula* is a very important pharmaceutical plant of the Apiaceae family. There are different opinions about the taxonomy on the subfamily, tribe, genus and species of Apiaceae as the taxonomic system of Apiaceae is based on the typical umbrella anthotaxy and its fruit with specific secretory tube. *Ferula* is traditionally classified in the tribe Peucedaneae and six subgenera are recognised with in the genus (Pimenov and Leonov 1993; Korovin 1947). However, serological studies by Shneyer et al. (1995) indicated the distant position of *Ferula* from all other genera of traditionally delimited Paucedaneae. *Ferula* species are typically tall perennials or biennials with stout stems and finely divided leaves with inflated sheaths. Morphological charactereristics of the basal leaves and mature fruits are necessary for the accurate identification of the species (Kurzyna-Młynik et al. 2008).

The chemical constituents of plants in the genus *Ferula* (Umbelliferea) have been fairly thoroughly studied, with the most common compounds being sesquiterpenes and sesquiterpene coumarins. As such, many members of the genus have been used in China for the treatment of rheumatoid arthritis and stomach diseases. Modern pharmacological studies have established the anti-ulcerative, antibacterial, anti-inflammatory and immunopharmacological activities of this genus (Yang et al. 2006; Li et al. 2014).

Globally, the genus *Ferula* L. (Apiaceae) contains 180–185 species, with the most diversity found in Central and Southwest Asia (Pimenov and Leonov 2004). In Turkey, about 130 species can be found, of which approximately 100 are endemic (Korovin 1951; Chamberlain and Rechinger 1987). The first revision of *Ferula* in Turkey was prepared by Peşmen (Peşmen 1972), where18 species were recognised, one of which was incompletely known and nine of which were endemic (Sağıroğlu and Duman 2008). A comprehensive revision of Turkish *Ferula* has been undertaken by Sağıroğlu since 2005 and a large number of specimens have been collected from all over Turkey. Four new species have since been added to the Flora of Turkey and one was made a synonym (Duman and Sağıroğlu 2005; Sağıroğlu and Duman 2006; Sağıroğlu and Duman 2007; Sağıroğlu and Duman 2010; Sağıroğlu and Duman 2014). *F. divaricata* and *F. pisidica* were published by Pimenov and Akalın (Pimenov and Kljuykov 2013; Akalın et al. 2020). According to recent studies, the *Ferula* genus includes 23 species, of which 14 species are endemic (Güner et al. 2012).

Pollen morphology of various members of the family Umbelliferae has been studied over time. For example, Erdtman (1952) studied pollen morphology of the family Umbelliferae, while Ting (1961) examined pollen of some American species of family Umbelliferae. Additionally, Ting et al. (1964) examined pollen morphology of the subfamily Hydrocotyloideae: Umbelliferae. Pollen morphology of the North European Flora of the family Umbelliferae was examined by Punt (1984). However, the most comprehensive study on pollen morphology of family Umbelliferae is that of Cerceau-Larrival (1971, 1981). Van Zeist et al. (1977) described the following pollen types from Iran: *Anisosciadium, Bunium, Eryngium, Ferula, Ma*-

labaila, Pimpinella, Sium erectum, and *Turgenia* types. Pollen morphology of the family has also been studied by Erdtman et al. 1961, 1969; Visset 1972; Faegri and Iversen 1975; Nilsson et al. 1977; Moore and Webb 1978; Nigaud 1977, 1980; Punt 1984; Perveen and Qaiser 2006. Descriptions by several authors have been given in regional pollen floras (Aytuğ et al. 1971; Kuprianova and Alyshina 1972; Punt 1984; Perveen and Qaiser 2006; Punt et al. 2007; Güner et al. 2011; Pehlivan et al. 2009; Mungan et al. 2011; Baldemir et al. 2018), but only a few studies have been conducted on the genus *Ferula*.

To date, no information is available on the pollen morphology of species of *Ferula* found in Turkey. In the present study, an attempt has been made to provide complete information on pollen morphology of these genera in Turkey. For all 23 taxa belonging to the genus *Ferula*, pollen morphology was examined from samples which were collected from their natural habitat. This research is a palynological study of *Ferula*, collected from different regions in Turkey and was conducted to shed light on the properties of the pollen taxa that were examined. The present research aims to provide detailed quantitative and qualitative data on the pollen morphology of the genus, as well as to evaluate the taxonomic value of those data.

Materials and methods

Plant material

The material used for this study was collected from various locations throughout Turkey during the year 2018. The voucher specimens were deposited in the herbarium of Sakarya University and the Faculty of Science of Gazi University, Ankara, Turkey (GAZI). Plant localities, collection dates and collector numbers can all be seen in Table 1.

Palynological and morphological analysis

For Light Microscope Studies: Pollen slides were prepared using the Wodehouse (1935) technique. The pollen grains were mounted in unstained glycerine jelly, stained with safranin and studies were made using an Olympus BX-21. The measurements were based on 30 readings from each specimen. Polar axis (P), equatorial diameter (E), P/E ratio, exine (ex), intine (in), colpi long axis (clg), colpi short axis (clt), pori long axis (plg), pori short axis (plt) and costae (c) were also measured.

For SEM studies: Dried pollen grains were transferred on to aluminium stubs and coated with gold at 20 Kv for 4 min in a sputter-coater. The SEM examination was carried using a ZEISS Supra 55 Scanning Electron Microscope at the SEM Laboratory of the Central Research Laboratory (MERLAB), Yuzuncu Yil University, Van.

The pollen terminology was adopted from Faegri and Iversen (1975), Punt (1984) and Punt et al. (2007) and the shape classification followed that of Erdtman (1969), based on the P/E ratio in Tables 2, 3.

Taxa	Locality	Collector
F. szowitsiana D.C.	B6 Sivas: Zara road, 13. km, 1300 m, 14.06.2018	M.S 6859
F. drudeana Korovin	C5 Kayseri: Yahyalı, Faraşa Village, 1550 m, 10.06.2018	M.S 6854
F. coskunii H. Duman & M. Sağıroğlu	C6 Hatay: Hatay-Yayla Mountain, 1200 m, 15.07.2018	M.S 6881
F. mervynii M.Sağıroğlu & H. Duman	A9 Erzurum: Uzundere-Artvin road, Dam vicinity 27.07.2018	M.S 6885
F. communis L.	A6 Samsun: Samsun-Ankara road, Samsun output, 50 m, 03.05.2018	M.S 6829
F. tingitana L.	C1 Izmir: Efes ruins, 18.05.2018	M.S 6833
F. duranii M.Sağıroğlu & H. Duman	C3 Antalya: Alanya castle, 40 m, 30.05.2018	M.S 6844
F. lycia Boiss.	B4 Konya: Hadim-Bozkır road, 54. km, 1050 m, 29.05.2018	M.S 6842
F. hermonis Boiss.	C6 Adana: Between Gürümze-Feke, 1600 m, 12.06.2018	M.S 6857
F. anatolica Boiss.	B2 Manisa: Alaşehir, Kozluca Village, 1000 m, 25.05.2018	M.S 6836
F. orientalis L.	B7 Elazig: Elazig-Diyarbakir road, 62. km, 1250 m, 17.06.2018	M.S 6869
<i>F. brevipedicellata</i> Peşmen ex M. Sağıroğlu & H. Duman	B9 Bitlis: Hizan-Pervari road, 26. km, 1000 m, 15.06.2018	M.S 6861
F. halophila Peşmen	B4: Tuz Lake, islands, 908 m, 02.06.2018	M.S 6846
<i>F. parva</i> Freyn & Bornm.	C4 Konya: Karaman-Mut road, 5. km, 1130 m, 16.07.2018	M.S 3175
F. tenuissima HubMor. & Peşmen	C6 Osmaniye: Zorkun Plateau, 5. km, 1600 m, 15.07.2018	M.S 6880
F. haussknechtii Wolff ex Rech.	B9 Bitlis: Between Tatvan-Van, 66. km, 1950 m, 15.06.2018	M.S 6862
F. elaeochytris Korovin	C5: Nigde-Ulukışla, Alihoca-Maden villages between, 1500 m, 04.06.2018	M.S 6850
F. longipedunculata Peșmen	B6 Maraş: Maraş-Göksün, Keklikoluk Village, Işık Mountain, 1900 m, 18.06.2018	M.S 6872
F. divaricata Pimenov	B3: Eskişehir-Sivrihisar yolu, Beylikova road, 920 m, 19.06.2018	M.S 4542
F. huber-morathii Peşmen	B8 Bingöl: Elazig-Bingöl road, Yolçatı, 1300 m, 16.06.2018	M.S 6864
F. caspica Bieb.	A4Ankara: Ankara-Nallıhan, Davutoğlan, 500 m, 27.05.2018	M.S 6839
F. rigidula DC.	B5 Yozgat: Yozgat-Şefaatli output, 2. km, rocks, 920 m, 20.05.2018	M.S 6835
F. pisidica Akalın & Miski	C4 Konya: Hadim-Beyreli Village, 1570 m, 2 1.06.2018	M.S 6876

Table 1. List of taxa examined, localities and collector.

Numerical analysis

Using the SPSS 21.0 statistical programme, clustering analysis was performed to determine the similarities between groups (Fig. 5). Seven palynological characters were selected to distinguish the 23 taxa of the genera (Table 3). Each of these palynological characters is included in the analysis by giving numerical values. Since Gower's formula (Gower 1971) was modified by Podani (1999), it now allows the inclusion of ordinal variables and missing scores in the data matrix. Thus, it was used to calculate the primary mixed data for dissimilarities. Additionally, Scatterplot analysis was performed by using P and E values. The graph obtained is shown in Figure 6.

Results

The pollen properties of 23 species of Turkish *Ferula* are here described for the first time. All of the morphological parameters investigated are shown in Tables 2, 3 and in Figs 1–6. The pollen grains are radially symmetrical and isopolar. The shape is prolate and perprolate. Their apertures are operculate and tricolporate with costae. In this study, the genus *Ferula* was found to have three types of polar shapes; triangular, triangular to subtriangular, and circular to subcircular (Figs 1, 2).

Taxon	Р	Е	P/E	Clg	Clt	Plg	Plt	Exine	Intine	Costa
F. szowitsiana	35.03±2.67	15.82±1.17	2.21	23.28±2.14	0.68±0.18	5.80±0.68	8.42±1.24	1.23±0.24	0.53±0.17	0.94±0.21
F. drudeana	34.27±2.13	15.18±1.42	2.26	23.23±2.47	$0.63 {\pm} 0.17$	5.73±0.64	7.07 ± 0.81	$1.38 {\pm} 0.14$	$0.48 {\pm} 0.14$	$1.03 {\pm} 0.19$
Ecoskunii	25.33±1.95	13.70±1.16	1.85	18.95±2.47	$0.62 {\pm} 0.25$	5.18±0.77	7.80 ± 1.21	$0.81 {\pm} 0.19$	0.45 ± 0.19	0.75 ± 0.21
F. mervynii	22.28 ± 2.17	$14.40 {\pm} 1.50$	1.55	16.83±1.90	$0.70 {\pm} 0.20$	3.77±0.68	$4.52{\pm}0.88$	$0.79 {\pm} 0.27$	$0.51 {\pm} 0.17$	$0.68 {\pm} 0.17$
F. communis	$32.73 {\pm} 1.89$	$18.73 {\pm} 1.63$	1.75	24.63 ± 2.28	$0.66 {\pm} 0.24$	$5.68 {\pm} 0.55$	$9.28{\pm}0.61$	$1.55 {\pm} 0.24$	$0.58 {\pm} 0.12$	$1.54 {\pm} 0.19$
F. tingitana	32.03±1.59	$15.27 {\pm} 1.08$	1.78	27.27±1.34	$0.81 {\pm} 0.22$	$5.87 {\pm} 0.64$	$7.00 {\pm} 0.71$	$0.98 {\pm} 0.16$	$0.39 {\pm} 0.13$	$1.24 {\pm} 0.20$
F. duranii	$30.73 {\pm} 2.03$	18.72 ± 1.43	1.64	24.27 ± 1.41	$0.45 {\pm} 0.18$	$5.20{\pm}0.92$	$7.08{\pm}0.79$	$0.68{\pm}0.21$	$0.37 {\pm} 0.10$	$0.71{\pm}0.12$
F. lycia	34.43 ± 1.37	16.33 ± 1.21	2.11	25.92±1.66	$0.72 {\pm} 0.17$	$5.35 {\pm} 0.45$	$6.73 {\pm} 0.67$	$1.40 {\pm} 0.27$	$0.65 {\pm} 0.26$	$1.30{\pm}0.27$
F. hermonis	$34.40 {\pm} 2.81$	$16.52 {\pm} 1.38$	2.08	23.98±1.20	$0.60 {\pm} 0.12$	5.65 ± 0.49	$8.78 {\pm} 1.25$	$1.18 {\pm} 0.25$	$0.53 {\pm} 0.14$	$0.77{\pm}0.18$
F. anatolica	27.83 ± 1.34	$16.47 {\pm} 1.28$	1.69	27.17±1.68	$0.78 {\pm} 0.19$	5.07 ± 0.76	$6.87{\pm}0.92$	$1.09 {\pm} 0.12$	$0.50 {\pm} 0.18$	$0.73 {\pm} 0.15$
F. orientalis	37.17 ± 2.70	$18.33 {\pm} 1.79$	2.03	26.03±2.38	$0.66 {\pm} 0.19$	$5.48 {\pm} 0.59$	8.17±0.95	1.34 ± 0.22	$0.33 {\pm} 0.12$	1.35 ± 0.22
F. brevipedicellata	32.77±2.17	16.05 ± 1.32	2.04	26.28±1.97	$0.61 {\pm} 0.13$	$4.67 {\pm} 0.67$	$6.10 {\pm} 0.64$	$1.30 {\pm} 0.28$	$0.48 {\pm} 0.11$	$1.16 {\pm} 0.26$
F. halophila	33.50 ± 1.43	17.16 ± 1.26	1.95	24.87±1.78	$0.70 {\pm} 0.19$	5.40 ± 0.66	7.90 ± 1.15	$1.89 {\pm} 0.24$	$0.73 {\pm} 0.20$	1.73 ± 0.22
F. parva	33.15 ± 2.19	$16.33 {\pm} 1.20$	2.03	24.03±2.65	$0.50{\pm}0.11$	$5.03{\pm}0.94$	$6.30{\pm}1.20$	$1.68 {\pm} 0.23$	$0.47 {\pm} 0.18$	$1.53{\pm}0.19$
F. tenuissima	$40.47{\pm}3.09$	17.23 ± 1.68	2.35	30.73±2.65	$0.78{\pm}0.21$	$5.70{\pm}0.48$	$8.80{\pm}0.68$	$1.40 {\pm} 0.25$	$0.39 {\pm} 0.213$	$1.37{\pm}0.16$
F. haussknechtii	$31.23 {\pm} 1.63$	16.43 ± 1.54	1.90	23.97±1.63	$0.55{\pm}0.10$	$5.63{\pm}0.57$	$6.83{\pm}0.70$	1.45 ± 0.17	$0.55 {\pm} 0.15$	$1.22{\pm}0.29$
F. elaeochytris	$30.83 {\pm} 1.70$	$16.10 {\pm} 1.18$	1.92	25.17±1.62	$0.48{\pm}0.16$	$3.91{\pm}0.62$	$5.50 {\pm} 1.03$	$1.23{\pm}0.21$	$0.42 {\pm} 0.18$	$1.52{\pm}0.22$
F. longipedunculata	$32.87 {\pm} 2.58$	17.27 ± 1.33	1.90	24.83±1.62	$0.78{\pm}0.18$	$5.82{\pm}0.50$	$8.47{\pm}0.87$	$1.51 {\pm} 0.19$	$0.34 {\pm} 0.12$	1.45 ± 0.19
F. divaricata	$34.70 {\pm} 2.60$	17.20 ± 1.42	2.02	24.77±1.77	$1.02{\pm}0.21$	5.65 ± 0.53	$8.20{\pm}0.82$	$1.52 {\pm} 0.18$	$0.58 {\pm} 0.25$	$1.50{\pm}0.19$
F. huber-morathii	35.08 ± 1.74	$16.87 {\pm} 1.50$	2.08	23.70 ± 2.22	$0.76{\pm}0.18$	5.88 ± 0.60	7.82 ± 1.13	$1.48 {\pm} 0.25$	$0.46 {\pm} 0.14$	$1.29{\pm}0.23$
F. caspica	27.03 ± 1.87	$14.37 {\pm} 1.22$	1.88	22.63±1.61	$0.50{\pm}0.14$	$4.53{\pm}0.92$	5.77 ± 0.84	$1.17 {\pm} 0.22$	$0.49 {\pm} 0.17$	$1.10{\pm}0.18$
F. rigidula	$33.90 {\pm} 1.88$	$18.20 {\pm} 1.61$	1.86	19.83±1.97	0.53 ± 0.16	6.05 ± 0.56	5.95 ± 0.58	1.55 ± 0.27	$0.48 {\pm} 0.16$	$1.67 {\pm} 0.21$
F. pisidica	32.17 ± 2.52	17.83±2.12	1.80	25.50±2.75	$0.57 {\pm} 0.18$	5.05 ± 1.03	7.62±1.39	$1.52 {\pm} 0.19$	$0.37 {\pm} 0.13$	$1.50{\pm}0.19$

Table 2. Pollen morphological parameters in the investigated taxa.

NOMENCLATURE: P: Polar axis E: Equatorial axis Clg: Length of the colpus Clt: Width of the colpus Plg: Length of the porus Plt: Width of the porus.

(Average µm± standard deviation)

The polar axis (P) ranges from 22.28 to 40.47 μ m and the equatorial axis (E) ranges from 13.70 to 18.73 μ m. The polar axis is longest in *F. tenuissima* (40.47 μ m) and shortest in *F. mervynii* (22.28 μ m); the equatorial axis is longest in *F. communis* (18.73 μ m) and shortest in *F. coskunii* (13.70 μ m). The dimensions are smaller in *F. mervynii* and larger in *F. tenuissima*. In all taxa examined, the width of the porus (plt) is greater than the width of the colpus (clt). The colpus is short to rather long (16.83–30.73 μ m), narrow (0.45–1.02 μ m) and slit-like. The highest values were observed in *F. tenuissima* and *F. tingitana*. *F. mervynii* has the smallest measures of colpus. Intine thickness ranges between 0.33 and 0.73 μ m. Intine was thickest in *F. halophila* and thinnest in *F. orientalis*. The exine is tectate and 0.68–1.89 μ m in thickness in the equatorial area. Exine was thickest in *F. halophila* and thinnest in *F. duranii*. There is a thickening around the aperture of exine (costae) with a decreasing diameter towards the poles. In addition, *F. halophila* (1.73 μ m) and *F. rigidula* (1.1.67 μ m) have the thickest costae (Table 2).

According to LM investigations; prolate pollen shape were observed in *F. coskunii*, *F. mervynii*, *F. communis*, *F. tingitana*, *F. duranii*, *F. anatolica*, *F. halophila*, *F. haussknechtii*, *F. elaeochytris*, *F. longipedunculata*, *F. caspica*, *F. rigidula* and *F. pisidica*. Other taxa are perprolate. In other words, in terms of pollen shape, about half of the studied taxa is prolate and the other half is perprolate.

Through SEM investigation, several types of ornamentations were observed in the equatorial area, polar area and around the pore on pollen surfaces. In many pollens, it

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No	Taxa	Ь	Э	P/E	Exine	Costae	Ornamentation, polar area	Ornamentation, polar area Ornamentation, equatorial area Ornamentation, pore around	Ornamentation, pore around
FI	F. szowitsiana	35.03	15.82	Perprolate	1.23	0.94	striate-reticulate	rugulate	rugulate-striate
$\mathbf{F2}$	F. drudeana	34.27	15.18	Perprolate	1.38	1.03	striate-reticulate	rugulate	rugulate-striate
$\mathbf{F3}$	F. coskunii	25.33	13.70	Prolate	0.81	0.75	striate-perforate	rugulate	rugulate-striate
F4	E mervynii	22.28	14.40	Prolate	0.79	0.68	striate-reticulate	rugulate-striate	rugulate-striate
F5	F. communis	32.73	18.73	Prolate	1.55	1.54	striate-reticulate	rugulate-perforate	striate-reticulate
F6	F. tingitana	32.03	15.27	Prolate	0.98	1.24	striate-reticulate	rugulate-striate	rugulate-striate
$\mathbf{F7}$	F. duranii	30.73	18.72	Prolate	0.68	0.71	striate-reticulate	rugulate	striate
$\mathbf{F8}$	F. lycia	34.43	16.33	Perprolate	1.40	1.30	rugulate-striate	rugulate-striate	rugulate
F9	F. hermonis	34.40	16.52	Perprolate	1.18	0.77	striate-reticulate	rugulate	striate
F10	F. anatolica	27.83	16.47	Prolate	1.09	0.73	striate-reticulate	rugulate	striate
F11	F. orientalis	37.17	18.33	Perprolate	1.34	1.35	striate-reticulate	rugulate	striate
F12	F.brevipedicellata	32.77	16.05	Perprolate	1.30	1.16	rugulate-striate	rugulate	rugulate-striate
F13	F. halophila	33.50	17.16	Prolate	1.89	1.73	striate-reticulate	rugulate	striate
F14	F. parva	33.15	16.33	Perprolate	1.68	1.53	striate-reticulate	rugulate	striate
F15	F. tenuissima	40.47	17.23	Perprolate	1.40	1.37	striate-reticulate	rugulate	striate
F16	F. haussknechtii	31.23	16.43	Prolate	1.45	1.22	rugulate-verrucate	verrucate	verrucate
F17	F. elaeochytris	30.83	16.10	Prolate	1.23	1.52	striate-reticulate	rugulate	rugulate-striate
F18	<i>Elongipedunculata</i>	32.87	17.27	Prolate	1.51	1.45	striate-reticulate	rugulate	striate
F19	F. divaricata	34.70	17.20	Perprolate	1.52	1.50	striate-reticulate	rugulate	rugulate-striate
F20	F. huber-morathii	35.08	16.87	Perprolate	1.48	1.29	striate-reticulate	rugulate	striate
F21	F. caspica	27.03	14.37	Prolate	1.17	1.10	striate-reticulate	rugulate	striate-reticulate
F22	F. rigidula	33.90	18.20	Prolate	1.55	1.67	striate-reticulate	rugulate	verrucate
F23	E nisidica	37 17	17 83	Drolota	157	1 50	eteinte retion hte	rugulate	ct rioto



Figure I. LM micrographs of pollen grains in the *Ferula* taxa examined 1–3 *F szowitsiana* 4–6 *F. drudeana* 7–9 *F.coskuni* 10–12 *F. mervynii* 13–15 *F. communis* 16–18 *F. tingitana* 19–21 *F. duranii* 22–24 *F. lycia* 25–27 *F. hermonis* 28–30 *F. anatolica* 31–33 *F. orientalis* 34–36 *F. brevipedicellata* 37–39 *F. halophila* 40–42 *F. parva.* Scale bar: 20 μm. (Equatorial view: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40. Polar view: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41. Aperture view: 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42).

has been determined that the ornamentation around the pore is different from that in both polar and equatorial areas. In the equatorial area, ornamentation was determined to be; rugulate in 18 species, rugulate-striate in three species, rugulate-perforate in one species and verrucate in one species. In the polar area, ornamentation was striate-retic-

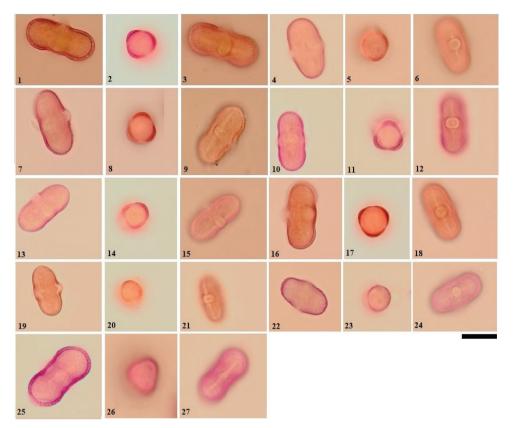


Figure 2. LM micrographs of pollen grains in the *Ferula* taxa examined 1–3 *F. tenuissima* 4–6 *F. haussknechtii* 7–9 *F. elaeochytris* 10–12 *F. longipedunculata* 13–15 *F. divaricata* 16–18 *F. huber-morathii* 19– 21 *F. caspica* 22–24 *F. rigidula* 25–27 *F. pisidica.* Scale bar: 20 μm. (Equatorial view: 1, 4, 7, 10, 13, 16, 19, 22, 25. Polar view: 2, 5, 8, 11, 14, 17, 20, 23, 26. Aperture view: 3, 6, 9, 12, 15, 18, 21, 24, 27).

ulate in 19 species, rugulate-striate in two species, striate-perforate in one species, and rugulate-verrucate in one species. Around the pore area, ornamentation was; striate in 10 species, rugulate-striate in eight species, striate-reticulate in two species, verrucate in two species and rugulate in one species (Figs 3, 4).

In the genus *Ferula*, different palynological characters, which became evident over the course of the investigation, were measured, leading to the realisation of a dendrogram. This dendrogram shows the similarities or dissimilarities which exist amongst the taxa being studied. The dendrogram obtained in this research is based on the seven palynological variables of the 23 taxa of *Ferula* genus and is presented in Figure 5. The dendrogram was constructed by using Average Linkage (Between Groups) of the examined data revealed through two main groups. From the dendrogram, it is evident that *F. coskunii*, *F. mervynii*, *F. anatolica* and *F. caspica* are quite different from the other species and were the first to separate. In scatterplot graphs, it is seen that the species with the smallest (*F. mervynii*) and largest (*F. tenuissima*) pollen differ from other species (Fig. 6).

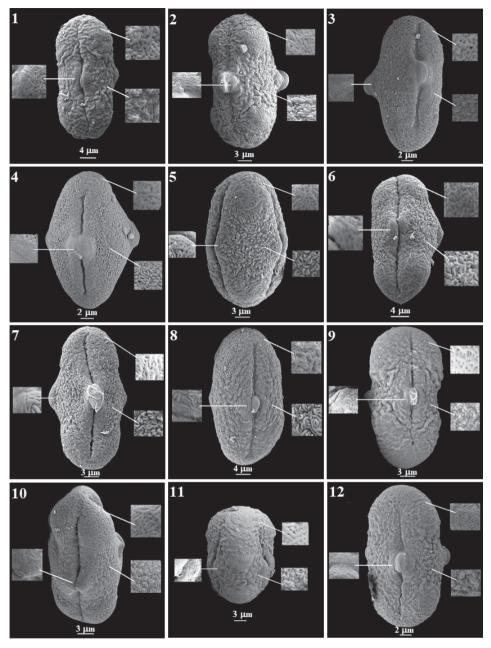


Figure 3. SEM micrographs of pollen grains in the *Ferula* taxa examined 1 *F. szowitsiana* 2 *F. drudeana* 3 *F. coskuni* 4 *F. mervynii* 5 *F. communis* 6 *F. tingitana* 7 *F. duranii* 8 *F. lycia* 9 *F. hermonis* 10 *F. anatolica* 11 *F. orientalis* 12 *F. brevipedicellata.*

Discussion

Pollen morphology of 23 taxa has been examined by light and scanning microscope. Pollen grains of *Ferula* taxa were generally tricolporate, with the shape of the grains

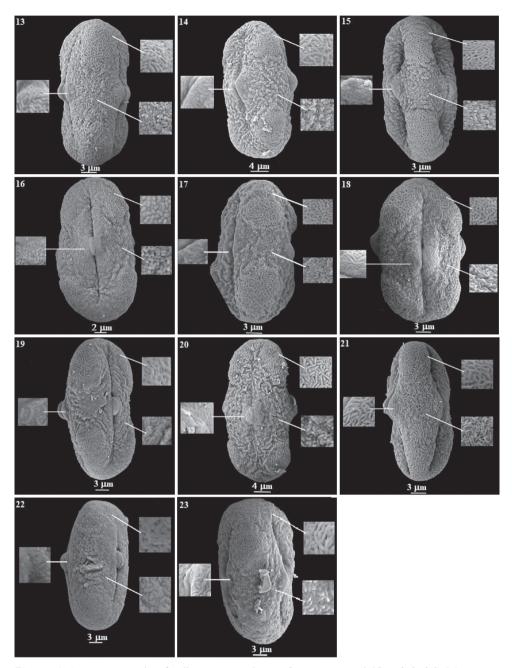


Figure 4. SEM micrographs of pollen grains in the *Ferula* taxa examined 13 *F. halophila* 14 *F. parva* 15 *F. tenuissima* 16 *F. haussknechtii* 17 *F. elaeochytris* 18 *F. longipedunculata* 19 *F. divaricata* 20 *F. hubermorathii* 21 *F. caspica* 22 *F. rigidula* 23 *F. pisidica.*

being prolate and perprolate. The P/E ratio ranged from 1.55 to 2.35. The pollen in approximately half of the species examined in this study has a perprolate shape, which is characteristic for the Apiaceae family (Baczyński et al. 2021). In the analysis, accord-

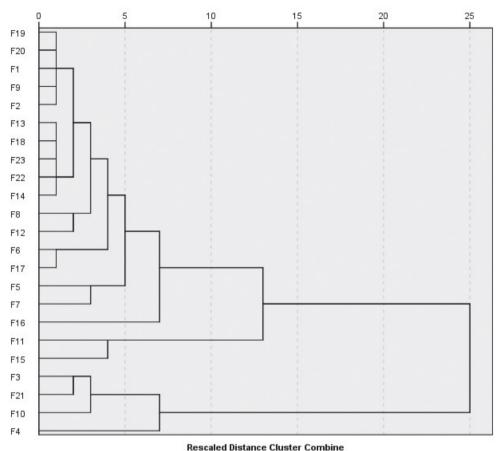


Figure 5. Dendrogram using Average Linkage (Between Groups) of the examined data (F1: *F. szowit-siana*; F2: *F. drudeana*; F3: *F.coskuni*; F4: *F. mervynii*; F5: *F. communis*; F6: *F. tingitana*; F7: *F. duranii*; F8: *F. lycia*; F9: *F. hermonis*; F10: *F. anatolica*; F11: *F. orientalis*; F12: *F. brevipedicellata*; F13: *F. halophila*; F14: *F. parva*; F15: *F. tenuissima*; F16: *F. haussknechtii*; F17: *F. elaeochytris*; F18: *F. longipedunculata*; F19: *F. divaricata*; F20: *F. huber-morathii*; F21: *F. caspica*; F22: *F. rigidula*; F23: *F. pisidica*).

ing to the ratio of P and E values, the largest pollen grains were found in *F. tenuissima* and the smallest in *F. duranii* (Table 2).

The pollen morphologies of the Turkish *Ferula* species have taxonomic significance. Variation was mainly observed in pollen shape and pollen sculpturing. The sculpturing of the pollen exine is useful for ascertaining relationships amongst species (Brochmann 1992). They differ in sculpturing from the equatorial area to the poles. In addition, different ornamentations around the pore were found (Table 3, Figs 3, 4).

Prolate, perprolate and tricolporate pollen with costae grains were seen in all species. The common aperture type of Apiaceae pollen grains is 3-colporate (rarely 4-colporate porate) and colpi with costae (Perveen and Qaiser 2006; Yousefzadi et al. 2006; Pehlivan et al. 2009; Güner et al. 2011). *Ferula* pollen grains have only a tricolporate aperture. It has been suggested that the outline of the endo-aperture is

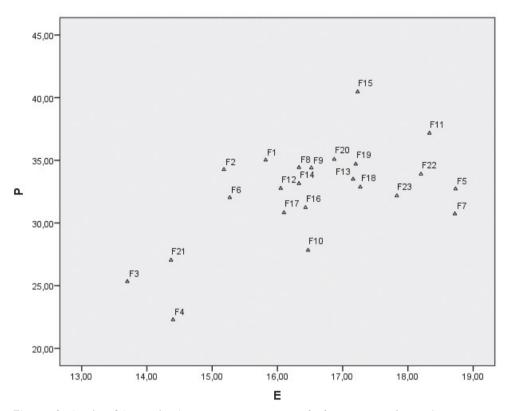


Figure 6. Graphs of Scatterplot (F1: *F szowitsiana*; F2: *F. drudeana*; F3: *F.coskuni*; F4: *F. mervynii*; F5: *F. communis*; F6: *F. tingitana*; F7: *F. duranii*; F8: *F. lycia*; F9: *F. hermonis*; F10: *F. anatolica*; F11: *F. orientalis*; F12: *F. brevipedicellata*; F13: *F. halophila*; F14: *F. parva*; F15: *F. tenuissima*; F16: *F. haussknechtii*; F17: *F. elaeochytris*; F18: *F. longipedunculata*; F19: *F. divaricata*; F20: *F. huber-morathii*; F21: *F. caspica*; F22: *F. rigidula*; F23: *F. pisidica*).

most important for the identification of Apiaceae (Cerceau-Larrival 1962; Punt 1984; Hebda 1985).

Palynological contributions on the family Umbelliferae are numerous, but often fragmentary and concern few species (Anefrod 1960; Erdtman 1971; Moore and Webb 1978; Punt 1984; Faegri and Iversen 1989). The most complete and interdisciplinary studies to date have been carried out by Cerceau-Larrival (1962, 1963, 1965, 1967, 1968, 1971, 1974, 1981), Cerceau-Larrival and Deroquet (1975) and De Leonardis et al. (2009), which not only allow correlation between the shape of fruits with the symmetry of radiosymmetric pollen, the pollen shape with the size of cotyledonous leaves and the value of the P/E ratio with the phenotype stability of the belonging tribe, but also further deepen the knowledge of the shape of the pollen grains, the variability of the sporodermic wall and the presence of columellar hypertrophy as adaptation to environmental conditions.

Cerceau-Larrival (1962) divided the pollen of Umbelliferae into five types based on P/E ratio: subrhomboidal (type1, P/E: 1–1.5), subcircular (type 2, P/E: 1–1.5), oval (type 3, P/E: 1.5–2), subrectangular (type 4, P/E: 2) and equatorially constricted (type 5, P/E: over 2). In the present study, all of the taxa examined belong to all of the pollen types as described by Cerceau-Larrival, the oval-type with a P/E ratio of 1.5–2 to the equatorially constricted type with a P/E ratio greater than 2. The diversification in the family Apiaceae of the phyletic series was subrhomboidal > subcircular > oval > subrectangular > equatorially constricted (Gruas-Cavagnetto and Cerceau-Larrival 1978), where a suboval or rectangular shape is a more advanced feature (De Leonardis et al. 2008).

Cerceau-Larrival (1959, 1962) and Punt (1984) observed that the polar view of Apiaceae was important for the differentiation of the pollen types. In this study, it was found that the genus *Ferula* had three types of polar shapes: triangular, triangular to subtriangular and circular to subcircular. Punt (1984) divided the family into 50 pollen types, based on the two outer contour shapes (outer contour of mesocolpium side straight or convex and concave or slightly concave). The outer contours of generally examined taxa are concave, slightly concave or straight, while those of *F. mervynii* and *F. communis* are convex, according to Punt's classification.

Pollen ornamentation is one of the most significant characteristics that can be used to separate taxa (Pinar et al. 2009; Mačukanović-Jocić et al. 2017; Zhang et al. 2017). It was observed in the present research that *Ferula* was striate-reticulate, rugulate-striate, striate-perforate and rugulate-verrucate in polar view; rugulate, rugulate-striate, rugulate, striate and verrucate in equatorial view; and rugulate-striate, striate-reticulate, striate and verrucate around the pore in exine ornamentation. This situation reflects the variation between the species.

Pollen morphology of 50 species representing 27 genera of the family Umbelliferae from Pakistan was examined by Perveen and Qaiser (2006). They determined that the pollen grains of Umbelliferae were generally tricolporate, the shape of the grains varied from prolate-perprolate, and the P/E ratio ranged from 1.2 to 2.6. Tectum is uniformly striate to striate-rugulate. In their study, all the taxa examined belong to all the pollen types as identified by Cerceau-Larrival; that is, subrhomboidal type to equatorially constricted type. The present study has very similar results to these palynological properties. According to the findings of this current study; pollen grains are tricolporate, the shape of the pollens is prolate-perprolate, the P/E ratio ranges from 1.55 to 2.35 and all of the examined taxa belong to the oval to subrhomboidal type. Related to the tectum, three distinct pollen types are recognized viz., in Pakistan, *Bupleurum gilessii*-type, *Pleurospermum hookeri*-type, *Trachyspermum ammi*-type. The tectal surface of the *Pleurospermum hookeri* type is rugulate-striate, which was observed particularly around the polar, equatorial and pore areas of the taxa examined in the present study.

In another study, Güner et al. (2011) observed the following ornamentation types of *Seseli*: rugulate in the equatorial area, psilate at the poles; striate-reticulate at equator, rugulate at poles; rugulate at equator, striate at poles; and rugulate-granulate at equator, striate at poles. Ornamentation types such as rugulate, striate-reticulate and striate were also observed in *Ferula* pollens.

The results of the cluster analysis show that the examined members of *Ferula* that fall into two main groups coincide with pollen sizes (Fig. 5). According to the

dendrogram using Average Linkage (Between Groups) analysis, based on pollen morphological data, each species was distinctly separated from each other. Pollen morphological characteristics, such as polar axis (P), equatorial axis (E), the ratio of P/E and ornamentation at the polar and equatorial view, are the most valuable variables for separating the *Ferula* species. In the scatterplot graphs, *F. coskunii*, *F. mervynii*, *F. anatolica* and *F. caspica* species were grouped together, just as in the dendrogram. These species were found as the external taxa separating from the other taxa at first in the dendrogram and the plot (Figs 5, 6). It was found that the P value of these four species is smaller than the other species. At the same time, three of these four species have lower E values than other species. In this case, it can be said that the P value is the primary valuable variable and E value had secondary importance for separating the species of *Ferula*.

According to Cluster analysis; F. szowitsiana, F. drudeana, F. hermonis, F. divaricata and F. huber-morathii species are in the same clade. The pollen shapes of these taxa are perprolate and ornamentation in the polar area is striate-reticulate, while in the equatorial area it is rugulate. F. halophila, F. parva, F. longipedunculata, F. rigidula and F. pisidica are found in the same clade and have P and E values that are very close to each other, with ornamentation in the polar area being striate-reticulate and rugulate in the equatorial area. F. lycia and F. brevipedicellata are more similar, the pollen shapes of these taxa are perprolate and ornamentation in the polar area is rugulate-striate. *E. tingitana* and *E. elaeochytris* species are in the same clade and their pollen shapes are prolate, with ornamentation in the polar area being striate-reticulate. F. communis and F. duranii species are similar, with perprolate pollen shapes, E values that are very close to each other and ornamentation in the polar area being striate-reticulate. *F. orientalis* and F. tenuissima species are more similar because the pollen shapes of these two taxa are perprolate, exine and costa values are very close to each other and ornamentation in the polar area is striate-reticulate, while it is rugulate in the equatorial area. The species *F. haussknechtii* differs from the species closest to it in that its ornamentation is rugulate-verrucate in the polar area and verrucate in the equatorial area.

Some *Ferula* species in the same clade are closely related to each other morphologically; for example, *F. szowitsiana* and *F. drudeana*; *F. coskunii* and *F. mervynii*; *F. halophila*, *F. parva* and *F. rigidula*. In other words, in some species, palynological data support the separation of taxa according to morphological characteristics.

Conclusion

In conclusion, analysis of pollen grains of 23 *Ferula* species in Turkey by LM and SEM revealed that palynological characteristics are reliable criteria for explaining the relationships between these species. The results of the cluster analysis showed that the most important variables in order to separate the taxa of *Ferula* in this study are the P and E values, ratio of P/E (pollen shapes) and ornamentation in the polar and equatorial area. In other words, these particular pollen characteristics seem to have the potential for evaluation of infrageneric relationships in the genus *Ferula*.

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RESEARCH ARTICLE



Big fruits with tiny tepals: An unusual new species of Lauraceae from southwestern China

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Abstract

We collected an unusual new plant of *Phoebe* (Lauraceae) from southeastern Yunnan, China, which possesses more or less oblong leaves, paniculate inflorescences with strictly opposite lateral cymes, trimerous flowers with 4-locular stamens, and large fruits with tiny, equal, persistent tepals. Our molecular phylogenetic study based on nrITS, *LEAFY* and plastid *mat*K sequences suggests that this species belongs to a clade of *Phoebe* including *P. puwenensis*, *P. megacalyx*, and *P. macrocarpa*. However, this species differs from the latter three species by subglabrous twigs, leaves and inflorescences (vs. pubescent twigs, leaves and inflorescences in the latter three species), larger fruits (5–8 cm long vs. 1–4 cm long in the latter three species), and smaller tepals (1–2.5 mm long vs. 5–15 mm long in the latter two species). As a result, *Phoebe jinpingensis* sp. nov. is described and illustrated here as new to science.

Keywords

Lauraceae, morphology, new species, *Phoebe*, phylogeny, taxonomy

^{*} These authors equally contributed to this work.

Introduction

The Lauraceae are woody plants, except for the hemiparasitic climber genus *Cassytha* L. (Linnaeus 1753), and have more than 3,000 species mainly distributed in tropical regions (Rohwer 1993). This family is notorious for its complicated taxonomy. Many Lauraceae are large trees, which makes it difficult to collect specimens in the field. Good quality specimens are rarely represented in herbaria, many species being known from only one or a few imperfect specimens lacking floral or fruiting characters. The integration of morphological and molecular evidence represents a promising way to better understand the diversity of the tropical family Lauraceae.

Phoebe Nees (Nees von Esenbeck 1836) contains ca. 100 species that are distributed in tropical and subtropical Asia (van der Werff 2001; Wei and van der Werff 2008). Morphologically, *Phoebe* is well-defined and differs markedly from other Asian genera of the *Persea* group by the persistent and appressed tepals at the base of fruits (vs. deciduous or persistent and spreading tepals), e.g. *Alseodaphne* Nees (Wallich 1831), *Alseodaphnopsis* H.W.Li & J.Li (Mo et al. 2017), *Dehaasia* Blume (Blume 1837), *Machilus* Nees (Wallich 1831), *Nothaphoebe* Blume (Blume 1851).

Recently, we collected both flower and fruit materials of one notable tree species while conducting field investigations in southeastern Yunnan, China. Further morphological and molecular phylogenetic studies indicate that this species is a new species of *Phoebe*. We thus describe it here as new to science.

Material and methods

Morphology and Anatomy

We conducted field collections and morphological observations, obtained voucher specimens, preserved flowers in FAA, and dried leaf materials with silica gel. Photographs of vegetative and reproductive characters were taken with a Nikon camera (D700). The preserved flowers were dissected and observed, and photographs were taken under a stereo microscope (Leica S8APO).

Phylogeny

To determine the systematic position of our new species, we conducted a phylogenetic study using nrITS, *LEAFY* and plastid *mat*K sequences. This phylogeny included 40 species from five genera of the *Persea* group (*Alseodaphne, Alseodaphnopsis, Dehaasia, Machilus* and *Phoebe*). *Litsea acuminata* (Blume) Sa. Kurata (Kurata 1968) and *Litsea akoensis* Hayata (Hayata 1911) were selected as the outgroup. Markers used in this phylogenetic study were either sequenced in this study or obtained from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Vouchers and accession numbers of sequences are listed in Table 1.

Species	ITS	LEAFY	matK	Voucher specimen	Locality
Alseodaphne huanglianshanensis H.W.Li &	MW826229	MW849576	MW849603	Bing Liu 2447	China, Yunnan
Y.M.Shui					
Alseodaphnopsis andersonii (King ex Hook.f.) H.W.Li & J.Li	MW826228	MW849575	MW849602	Bing Liu 1584	China, Yunnan
Alseodaphnopsis hokouensis (H.W.Li) H.W.Li & J.Li	MW826237	MW849583	MW849610	Bing Liu 2358	China, Yunnan
Alseodaphnopsis petiolaris (Meisn.) H.W.Li & J.Li	MW826234	MW849580	MW849607	Bing Liu 1266	China, Yunnan
Alseodaphnopsis sichourensis (H.W.Li) H.W.Li & J.Li	MW826235	MW849581	MW849608	Bing Liu 1980	China, Yunnan
Alseodaphnopsis ximengensis H.W.Li & J.Li	MW826236	MW849582	MW849609	Bing Liu 4726	China, Yunnan
Dehaasia incrassata (Jack) Kosterm.	MW826238	MW849585	MW849612	X.H.Jin IN004	Indonesia.
Litsea acuminata (Blume) Kurata	MW826239	MW849586	MW849613	Y.Yang, T.T.Sun & W.Y.Jin 2016110811	China, Taiwan
<i>Litsea akoensis</i> Hayata	MW826240	MW849587	MW849614	Y.Yang, T.T.Sun & W.Y.Jin 2016110810	China, Taiwan
Machilus bonii Lec.	MW826241	MW849588	MW849615	Bing Liu 2133	China, Yunnan
Machilus breviflora (Benth.) Hemsl.	MW826242	MW849589	MW849616	Bing Liu 1827	China, Guangdong
Machilus melanophylla H.W.Li	MW826243	MW849590	MW849617	Bing Liu 1950	China, Yunnan
Machilus robusta W.W.Sm.	MW826244	MW849591	MW849618	Bing Liu 1524	China, Yunnan
Phoebe angustifolia Meisn.	MW826246		MW849620	Bing Liu 1490	China, Yunnan
Phoebe cavaleriei (H.Lév.) Y.Yang & Bing Liu	MW826245		MW849619	Bing Liu 2215	China, Yunnan
Phoebe chekiangensis C.B.Shang	MW826247	MW849592	MW849621	Bing Liu 2232	China, Hubei
Phoebe formosana (Hayata) Hayata	MW826248	MW849593	MW849622	Y.Yang, T.T.Sun & W.Y.Jin 2016110836	China, Taiwan
Phoebe jinpingensis Liu B. 1477	MW826231	MW849584	MW849611	Bing Liu 1477	China, Yunnan
Phoebe jinpingensis Liu B. 2050	MW826232	MW849578	MW849605	Bing Liu 2050	China, Yunnan
Phoebe jinpingensis Liu B. 2052	MW826233	MW849579	MW849606	Bing Liu 2052	China, Yunnan
Phoebe jinpingensis Liu B. 2417	MW826230	MW849577	MW849604	Bing Liu 2417	China, Yunnan
Phoebe lanceolata (Wall. ex Nees) Nees	MW826249	MW849594	MW849623	Bing Liu 2532	China, Yunnan
Phoebe macrocarpa C.Y.Wu	MW826250	MW849595	MW849624	Bing Liu 1239	China, Yunnan
Phoebe megacalyx H.W.Li	MW826251	MW849596	MW849625	Bing Liu 1988	China, Yunnan
Phoebe nanmu (Oliv.) Gamble	MW826252	MW849597	MW849626	Bing Liu 2227	China, Yunnan
Phoebe neurantha var. brevifolia H.W.Li	MW826253	MW849598	MW849627	Bing Liu 2270	China, Yunnan
Phoebe neurantha (Hemsl.) Gamble	MW826254	MW849599	MW849628	Bing Liu 1570	China, Yunnan
Phoebe puwenensis W.C.Cheng	MW826255	MW849600	MW849629	Bing Liu 2242	China, Yunnan
Phoebe sheareri (Hemsl.) Gamble	MW826256	MW849601	MW849630	Bing Liu 2224	China, Hubei
Phoebe tavoyana (Meisn.) Hook.f.	MW826257	-	MW849631	Bing Liu 1822	China, Hainan

Table 1. Sequences obtained in this study and their vouchers. All voucher specimens are deposited in PE.

Total DNA was extracted from silica-gel-dried leaves using Tiangen Plant Genomic DNA kits. To amplify the nrITS, *LEAFY* and plastid *mat*K fragments, we followed Li et al. (2004, 2011b) and Sang et al. (1997) in the primers and the PCR amplification.

Sequences were edited with Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan, USA), aligned using MAFFT 7 (Katoh and Standley 2013), and then manually adjusted using BioEdit 7.2.5 (Hall 1999). MEGA-X was used to compute variable and parsimony-informative sites (Kumar et al. 2018). Phylogenetic analyses were based on concatenated sequences in Phylosuite v1.2.2 (Zhang et al. 2020). The best-fit nucleotide substitution model for each sequence was determined by Modelf-inder (Kalyaanamoorthy et al. 2017) in Phylosuite v1.2.2 based on BIC (Schwarz and

Gideon 1978). Bayesian inferences (BI) based on the concatenated sequences were carried out with MrBayes (Ronquist et al. 2012) in Phylosuite v1.2.2 with the following designations: number of generations 2,000,000, sampling frequency 1000; a relative burnin of 25.0% for diagnostics. Maximum likelihood (ML) analyses were run with the IQtree (Nguyen et al. 2014) in Phylosuite v1.2.2 with 1000 bootstrap replicates.

Figure treatments

The line drawing was done manually with a black ink pen. Illustrations and photos showing morphological characters were edited and merged in Adobe Photoshop CS2 ver. 9.0. Phylogenetic trees were browsed and adjusted in FigTree ver. 1.4.0 (Rambaut 2012) and then improved with Adobe Illustrator CS ver. 11.0.0. A distribution map was generated with ArcGis ver. 10.0 (ESRI, Redlands, CA, USA; http://www.esri.com).

Red list assessment

Extinction risk was assessed using IUCN categories and criteria (IUCN 2012). Population data and area of occupancy were obtained/estimated according to our field investigations.

Results

Phoebe jinpingensis Bing Liu, Y.Yang, W.Y.Jin & Zhi Yang, sp. nov.

urn:lsid:ipni.org:names: Figs 1–3

Type. CHINA. Yunnan Province: Jinping County, Mengla, Tuomazhai, 22°37'N, 103°01'E, elev. 956 m, 8 Apr 2014, *B.Liu, Y.Yang, Q.W.Lin, L.Jiang & X.J.Li 2050* (*holotype:* PE!; *isotypes:* PE!).

Diagnosis. This species is similar to *P. macrocarpa* C.Y.Wu (Wu and Wang 1957) and *P. megacalyx* H.W.Li (Lee et al. 1979) in the large fruits over 3 cm in diam., but differs from the latter two species by the subglabrous leaves being more or less oblong-elliptic and the larger fruits having smaller tepals.

Description. Trees (Fig. 1A), up to 15 m tall, to 40 cm in DBH (diameter at breast height). Bark yellowish gray. Branchlets purplish, slender, longitudinally ridged, subglabrous. Leaves alternate, usually clustered at the apex of branchlets, thinly coriaceous to chartaceous, subglabrous, oblong to oblanceolate (Fig. 1B), 15–25 × 5–8 cm, apex acute to slightly acuminate, base acute, midvein impressed adaxially, and prominently elevated abaxially, lateral (secondary) veins 7–10 pairs, immersed adaxially and elevated abaxially, transverse minor (tertiary) veinlets connecting lateral veins visible; petioles 1.2–4 cm, subglabrous. Panicles slender, 4–9 cm long, subglabrous. Flowers yellowish (Fig. 1C). Tepals subequal, 2–2.4 mm long. Fertile stamens in three whorls; filaments of the first and second whorls 1–2 mm; anthers 4-locular, locules arranged in

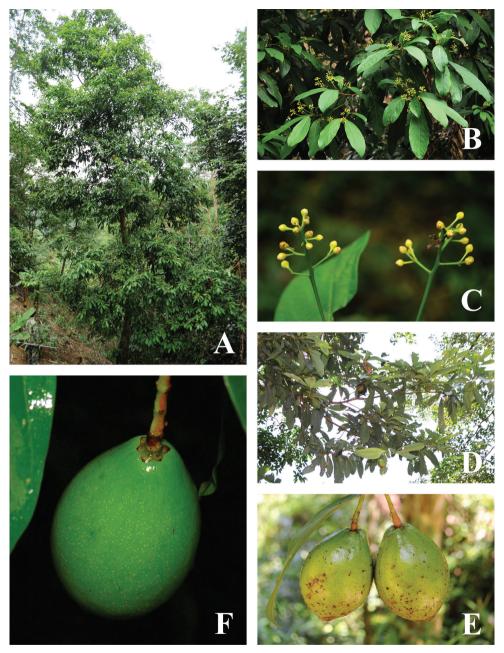


Figure 1. Morphology of *Phoebe jinpingensis* sp. nov. **A** habit of the tree **B** flowering branches **C** portion of a branch bearing inflorescences **D** fruiting branches **E** two fruits displaying swollen base and inconspicuous tepals **F** a fruit displaying the tiny tepals at the base.

trapezoid shape; each filament of the 3rd staminal whorl possessing two yellow cordate glands at its base. Staminodes sagittate. Fruits ellipsoid to obovoid, avocado-shaped, 5–8 cm long, and 3.5–5.2 cm in diam. (Fig. 1D–F); tepals persistent, equal, triangular

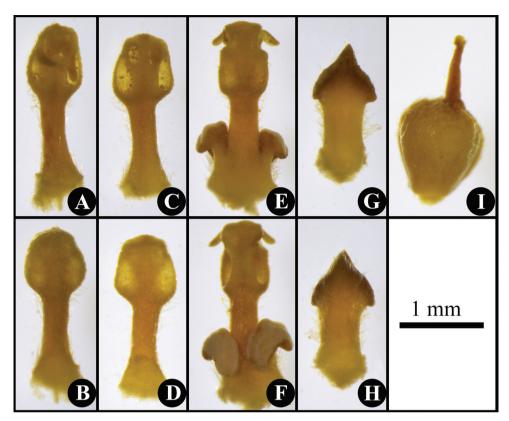


Figure 2. Anatomy of a flower of *Phoebe jinpingensis* sp. nov. showing morphology of stamens, staminodes, and pistil **A** stamens of the first staminal whorl, adaxial side **B** stamen of the first staminal whorl, abaxial side **C** stamen of the second staminal whorl, adaxial side **D** stamen of the second staminal whorl, abaxial side **E** stamen of the third staminal whorl, adaxial side **F** stamen of the third staminal whorl, abaxial side **G** staminode of the fourth staminal whorl, adaxial side **H** staminode of the fourth staminal whorl, abaxial side **I** pistil.

to ovate, tiny, 2–2.5 mm long, clasping the fruit base (Fig. 1F), concealed and inconspicuous when fruit becoming swollen (Fig. 1E). Fruit peduncles not thickened.

Distribution. So far, this species has only been found in southeastern Yunnan, China (Fig. 4).

Habitat. This species occurs in montane evergreen forests at an altitudinal range of 900–980 m. It blooms in April, and the fruits mature from September to December.

Etymology. The specific epithet '*jinpingensis*' refers to the type locality "Jinping County".

Additional specimens examined. CHINA. Yunnan Province: Jinping County, Mengla, Tuomazhai, 8 Apr 2014, fruit, *B.Liu, Y.Yang, Q.W.Lin, L.Jiang & X.J.Li 2052* (PE!); Jinping County, Mengla, Tuomazhai, 9 Oct 2011, fruit, *B.Liu 1477* (PE!); Jinping County, Mengla, Tuomazhai, 14 Sep 2014, fruit, *B.Liu, Y.Song, H.Lai & X.Yao 2417* (PE!).

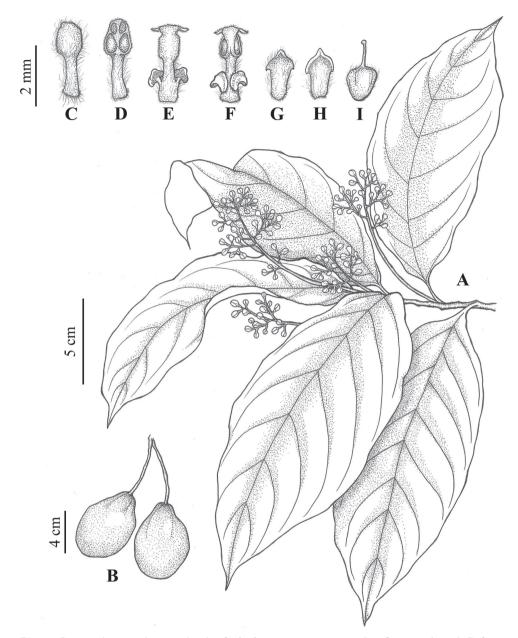


Figure 3. Line-drawing showing details of *Phoebe jinpingensis* sp. nov. **A** a flowering branch **B** fruits **C** fertile stamens of the first staminal whorl, abaxial side **D** fertile stamens of the first staminal whorl, adaxial side **E** fertile stamen of the third staminal whorl with the two glands at the base of the filaments, adaxial side **F** fertile stamen of the third staminal whorl whorl with the two glands at the base of the filaments, abaxial side **G** staminode of the fourth staminal whorl showing pubescence, adaxial side **H** staminode of the fourth staminal whorl showing pubescence, adaxial side **I** pistil, with obovoid pubescent ovary and linear style.

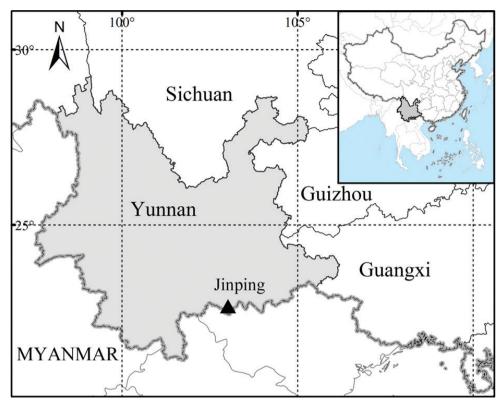


Figure 4. A map showing the distribution of Phoebe jinpingensis sp. nov.

Table 2. The best-fit nucleotide substitution model of the three markers for both BI and ML analyses.

Analysis	nrITS	LEAFY	matK
BI	HKY+F+I+G4	HKY+F+G4	HKY+F+I
ML	HKY+F+G4	HKY+F+G4	HKY+F

Conservation. There is only one population with 10 mature individuals occupying ca. 400 m². Fewer than 10 juvenile individuals were found. All the individuals have not been protected in any nature reserve, and a rubber plantation exists nearby the population. Based on IUCN Red List Categories and Criteria (IUCN 2012), the new species is categorized as "Critically Endangered" (CR Blab (v); D).

Phylogeny

We finally obtained 30 sequences of nrITS, 27 sequences of *LEAFY* and 30 sequences of *mat*K (Table 2). The aligned nrITS sequences had 745 nucleotides including 149 variable sites among which 98 sites are parsimony-informative. The aligned *LEAF* sequences consisted of 896 nucleotides including 158 variable sites and 57 parsimony-informative. The aligned *mat*K sequences contained 763 nucleotides with 22 variable

Item	nrITS	LEAFY	matK	all
Sequence length (nt)	745	896	763	2404
Variable (polymorphic) sites (nt)	149	158	22	329
Parsimony informative sites (nt)	98	57	13	168

Table 3. Information of nrITS, LEAFY, matK and concatenated sequences.

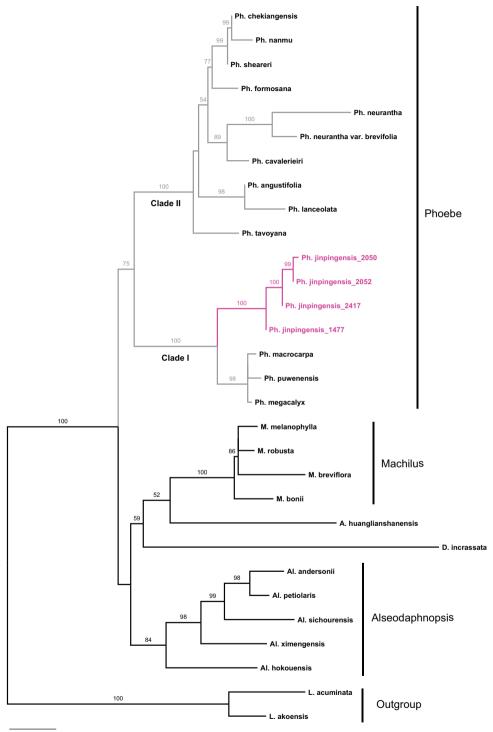
sites and 13 parsimony-informative sites. The concatenated matrix was 2404 nucleotides in length and included 329 variable sites and 168 parsimony-informative sites. The best-fit nucleotide substitution models for the three markers for both BI and ML analyses are listed in Table 3.

Our phylogenetic study gave rise to BI and ML trees showing similar topology (Figs 5, 6). The genus *Phoebe* constituted a clade with moderate to high support (BS: 75, PP: 0.91), the two clades within the genus were robustly supported (BS: 100 and PP: 1). Clade I consisted of *P. puwenensis* W.C.Cheng (Cheng et al. 1963), *P. macro-carpa, P. megacalyx,* and *P. jinpingensis.* The four samples of our new species fell within a robust clade (BS: 100, PP: 1). Clade II includes *P. angustifolia* Meisn. (Meissner 1864), *P. cavaleriei* (H.Lév.) Y.Yang & Bing Liu (Yang and Liu 2015), *P. chekiangensis* C.B.Shang (Shang 1974), *P. formosana* (Hayata) Hayata (Hayata 1915), *P. lanceolata* (Wall. ex Nees) Nees (Nees von Esenbeck 1836), *P. nanmu* (Oliv.) Gamble (Sargent 1914), *P. neurantha* (Hemsl.) Gamble (Sargent 1914), *P. sheareri* (Hemsl.) Gamble (Sargent 1914), and *P. tavoyana* (Meisn.) Hook.f. (Hooker 1886).

Machilus was monophyletic (BS: 100 and PP: 1). *Alseodaphne huanglianshanen*sis H.W.Li & Y.M.Shui (Li and Shui 2004) and *Dehaasia incrassata* (Jack) Kosterm. (Kostermans 1952) were close to *Machilus* (BS: 59%, PP: 0.91), but relationships among them were not resolved. *Alseodaphnopsis*, a recently established genus, constituted a monophyletic group which received high support (BS: 84%, PP: 1). Relationships among *Alseodaphnopsis*, *Phoebe*, and *Machilus-Dehaasia-Alseodaphne* were ambiguous.

Discussion

Modern taxonomy is based on phylogeny. There is no phylogeny with full sampling of *Phoebe*, only a few molecular phylogenetic studies including partial sampling of the genus (Rohwer et al. 2009; Li et al. 2011a, b; Song et al. 2017). Frey (2015) stated that the genus *Phoebe* is polyphyletic but provided no evidence. Li et al. (2011a, b) reconstructed a phylogeny based on nrITS and nuclear *LEAFY* indicating that *Phoebe* as a monophyletic group receives low bootstrap value (<50%) but high posterior probability (Li et al. 2011a, b). Our new phylogenetic study using three markers reaches a similar conclusion. However, Song et al. (2017) reconstructed a phylogeny of *Phoebe* based on 15 highly variable regions of the chloroplast genome and found that the genus as a clade receives high support (BS: 100%; PP: 1.00). These phylogenetic studies consistently suggested that the traditional subdivision of the genus into two sections, i.e. sect. *Phoebe* and *Caniflorae* Meisn. (Meissner 1864), is unreasonable because some species



0.006

Figure 5. Maximum Likelihood tree based on nrITS, *LEAFY* and plastid *mat*K indicating the phylogenetic position of *Phoebe jinpingensis* sp. nov. Bootstrap support values (>50%) are shown above the branches.

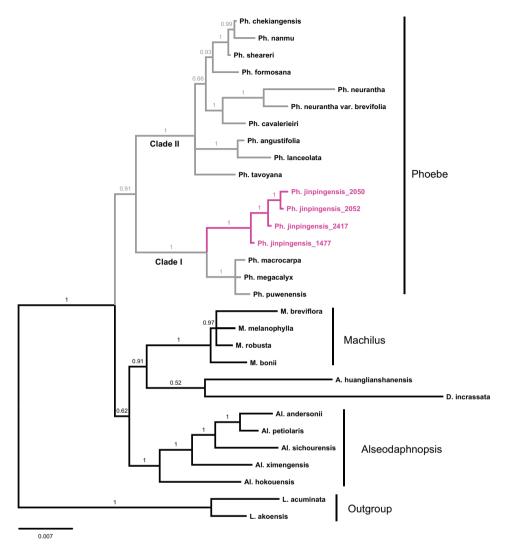


Figure 6. Bayesian tree based on nrITS, *LEAFY* and plastid *mat*K showing phylogenetic position of *Phoebe jinpingensis* sp. nov. Bayesian posterior probabilities (>0.50) are indicated above the branches.

of sect. *Caniflorae* are actually closer to species of sect. *Phoebe*. According to our phylogenetic study, the new species *Phoebe jinpingensis* belongs to the genus *Phoebe*, and falls within a robustly supported clade including *P. macrocarpa*, *P. megacalyx*, *P. puwenensis*, which were formerly ascribed to the sect. *Caniflorae*.

The genus *Phoebe* possesses persistent appressed tepals at the base of the fruits, whereas *Alseodaphne* and *Alseodaphnopsis* have fruits lacking persistent tepals (van der Werff 2001; Li et al. 2008; Mo et al. 2017). A few species formerly assigned to *Phoebe* with less indurate tepals slightly recurving at the apex and globose fruits were transferred to *Machilus* based on molecular phylogenetic studies (Li et al. 2011a). Traditional taxonomic studies have suggested that the genus *Phoebe* is characterized

by fruits with persistent appressed tepals (van der Werff 2001; Li et al. 2008). Our new species, *P. jinpingensis*, clearly belongs to the genus *Phoebe*, because of its fruits having persistent and appressed tepals in fruits (Fig. 1E). Our phylogenetic study also confirmed that the new species belongs to *Phoebe*.

Phoebe jinpingensis is unusual in the genus *Phoebe*. First, the fruit is very large and avocado-shaped. Second, the tepals of *P. jinpingensis* are quite tiny (1–2.5 mm long) and concealed and not obvious in well-developed swollen fruits (Fig. 1E). Third, the leaf shape of *Phoebe* is usually oblanceolate, while our new species possesses oblong to oblanceolate leaves which is unusual in the genus and more similar to *Alseodaphne/Alseodaphnopsis*.

The genus *Phoebe* usually possesses small fruits ca. 1 cm, the largest fruits being seen in *P. megacalyx* (ca. 3.2 cm long) and *P. macrocarpa* (3.5–4.2 cm long) (Wei and van der Werff 2008). Our new species is most similar to *P. megacalyx* and *P. macrocarpa* in its large fruits, but differs from the latter two species by the subglabrous twigs, leaves and inflorescences (vs. pubescent or tomentose twigs, leaves, and inflorescences), the bigger fruits (5–8 cm long vs. 3–4 cm long in the latter two species), and the smaller tepals (1–2.5 mm long vs. >5 mm long in the latter two species). *Phoebe puwenensis* also belongs to the same clade as *P. jinpingensis* in our phylogenetic trees. Our new species differs from *P. puwenensis* in the subglabrous twigs and leaves (vs. tomentose twigs and leaves in the latter species) and the bigger fruits (5–8 cm long vs. 1–2 cm long in the latter species). For taxonomic purposes, we provide a new key to these closely related species of clade I of the genus *Phoebe*.

Key to species of clade I of the genus Phoebe

1 Leaves, inflorescences, and twigs subglabrous; fruits lan	rge, up to 8 cm long,
having tiny tepals ca. 2–2.5 mm long	P. jinpingensis
- Leaves, inflorescences, and twigs usually tomentose	or pubescent; fruits
smaller, usually ca. 1–4 cm, possessing bigger tepals 5 n	nm or longer2
2 Fruits 3–4 cm long	
 Fruits 1–2 cm long 	
3 Leaves asymmetrical at the base; tepals woody, ca. 15 mm	n long P. <i>megacalyx</i>
- Leaves symmetrical at the base; tepals coriaceous, 5–6 r	nm long

Acknowledgements

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Supplementary material I

Figures S1–S8, Tables S1–S3

Authors: Zhi Yang, Wei-Yin Jin, Bing Liu, David Kay Ferguson, Yong Yang Data type: Phylogenetic trees and tables

- Explanation note: Figures S1–S8. Phylogenetic trees. Figures S1–S6. BI and ML trees of Asian species of the *Persea* group using single markers. Figures S7, S8. BI and ML trees of the Persea group using concatenated sequences of nrITS, *LEAFY* and *mat*K. Table S1. Sequences obtained from NCBI for phylogeny of the *Persea* group. Table S2. Substitution models of nrITS, *LEAFY* and *mat*K for phylogeny using separate markers. Table S3. A tabulated comparison of fruit size of *Phoebe* from China.
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RESEARCH ARTICLE



Vaccinium exiguum (Ericaceae, Vaccinieae), a new species from the ultramafic summit of Mt. Victoria, Palawan Island, Philippines

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Abstract

Vaccinium exiguum from the ultramafic summit of Mt. Victoria, Palawan Island, Philippines is here described as a new species of Ericaceae. It closely resembles *V. hamiguitanense* but is distinct by having much shorter petioles and leaves, longer and glabrous calyx lobes with serrate lobe margins, a larger corolla with deeper sulcations, and longer stamens with spurs oriented laterally. *Vaccinium exiguum* represents the third *Vaccinium* species found on the Island of Palawan and 36th in the Philippines.

Keywords

Ericales, endemic species, Malesia, sect. Bracteata, Vaccinioideae

Introduction

The Island of Palawan is situated on the south-western side of the Philippine Archipelago and is bordered by the West Philippine Sea in the north and the Sulu Sea in the south. Palawan is a biodiverse area, regarded both biogeographically and geologically as a portion of the Sunda Shelf, with many of its species shared with Borneo (Dickerson 1928; Heaney 1986; Voris 2000; Esselstyn et al. 2004). The long and complex geohistory of the island which started during the mid-Oligocene, as well as its highly variable elevation and climate, has promoted high rates of speciation and endemism (Anacker 2011; Galey et al. 2017). In particular, the extensive areas of ultramafic substrates have resulted in the evolution of many endemic plant species in Palawan (e.g. Robinson et al. 2009, 2016; Malabrigo 2020; Quakenbush et al. 2020; Tandang et al. 2020) and it is to be expected that more species await discovery in these ultramafic regions as they are further explored and studied.

The tropical species of *Vaccinium* L. are predominantly montane inhabitants with a high degree of endemicity (Argent 2018). The genus is the most species-rich of the Philippine genera of the family and is currently represented by 35 species, 32 of which are endemic to the country (Argent 2008; Pelser et al. 2011 onwards). The highly regarded taxonomic treatments by Copeland (1930) and Sleumer (1966–1967) are the most comprehensive, thus far, for Philippine *Vaccinium*. However, gaps in our knowledge of Philippine *Vaccinium* remain, especially in various species complexes (e.g. *V. caudatum* Warb./*V. benguetense* S.Vidal) and many character ambiguities used for the treatments require clarification and resolution. After Sleumer's work (1966–1967), three species have been added to the list, viz. *V. cebuense* Salares and Pelser, *V. hamiguitanense* P.W.Fritsch, and *V. oscarlopezianum* Co (Co et al. 2002; Salares et al. 2018; Fritsch et al. 2020).

During fieldwork on Mt. Victoria, Palawan Island in February 2021, author Bustamante documented a species of *Vaccinium* that grows at the ultramafic summit of the mountain (Fig. 1A) and closely resembles the recently described *V. hamiguitanense* from Mt. Hamiguitan, Mindanao Island. However, the inflorescence of this species differs from that of *V. hamiguitanense* in shape. After detailed morphological examination, it was confirmed that the specimen possesses distinguishing characters demonstrating its status as a species new to science, which we describe here under a biological species concept (Mayr 2000). Our discovery increases the number of species of *Vaccinium* in the Philippines to 36 and increases the number of known *Vaccinium* species from Palawan Island to three. Photographs and an illustration of the new species are also provided.

Materials and methods

The description was based on *in-situ* observations, herbarium specimens, and spirit samples preserved in Copenhagen solution. Microscopic details were described with the aid of a Swift SM100 stereo zoom microscope (30× magnification). Relevant literature and taxonomic keys (i.e. Copeland 1930; Sleumer 1966–1967; Argent 2018) were also consulted.

Taxonomy

Vaccinium exiguum M.N.Tamayo, R.Bustam. & P.W.Fritsch, sp. nov. urn:lsid:ipni.org:names:77218415-1

Figs 1B, 2, 3

Type. Philippines, Palawan Island (= Palawan Province), Municipality of Narra, Mt. Victoria, 9°21'56"N, 118°20'02"E, exposed ultramafic summit, ca. 1700 m elevation, 27 February 2021, *PTI-8 by R. Bustamante* (holotype: PNH!; isotypes: PPC!; BRIT!).

Diagnosis. *Vaccinium exiguum* closely resembles *V. hamiguitanense*, but is distinct by having shorter petioles (0.5–1.0 mm vs. 1.2–1.8 mm), shorter leaves (4.9–9.3 mm vs. 8.8–16.0 mm), longer calyx lobes (1.8–2.0 mm vs. 1.1–1.3 mm) that are glabrous (vs. merely ciliate) with serrate calyx lobe margins (vs. entire), a larger corolla (6.0–7.0 \times 4.0–5.0 mm vs. 4.6–5.0 \times ca. 2.6 mm) with deeper (vs. shallow) sulcations, and longer stamens (3.5–4.0 mm vs. 2.8–3.5 mm) with spurs oriented laterally (vs. slightly upcurved and oriented \pm apically). The calyx lobes of *V. exiguum* are unique amongst Philippine species in that they are nearly as long as or longer than the hypanthium. Unlike *V. hamiguitanense*, *V. exiguum* has a corolla that appears distinctly star-shaped in cross section because of its deep sulcations.

Description. *Habit* shrubs, terrestrial, evergreen, 0.3–1.5 m tall, densely branched. Branchlets when young with finely translucent erect, straight trichomes ca. 0.2 mm long, at maturity brown, slightly compressed and often ridged, 0.5-1.5 mm wide, not lenticellate, outer surface brownish; perennating buds compressed-ovoid, 0.5-0.8 mm long, with several obscurely overlapping scales. *Leaves* persistent on older branchlets, densely crowded, spirally and evenly arranged; petiole green with a tinge of red, 0.5- 1.0×0.4 –0.8 mm, nearly as long as wide, with translucent, erect, straight trichomes ca. 0.1 mm long, in cross section abaxially rounded, adaxially nearly flat; leaf blade elliptic or oblong, larger leaves on each branchlet $4.9-9.3 \times 3.0-6.0$ mm, coriaceous, both surfaces greenish red when young, glabrous, except occasionally puberulent at base, smooth, abaxial surface without punctae, green and glossy, light brown in sicco, adaxial surface green and nitid, dark brown in sicco, base cuneate, margin with 4 or 5 impressed \pm evenly distributed crenations per side with occasional minute translucent lanceolate glands on crenations, thinly recurved, apex obtuse to rounded, the very tip with a gland, marginal glands 3 or 4 per side, scattered along length of margin, ca. 0.1 mm diameter, midvein slightly raised abaxially, flattened adaxially or nearly so, secondary veins 2 to 4 on each side of midvein with first pair arising from base and remainder along midvein, arc-ascending, slightly raised or obscure abaxially, obscure adaxially, tertiary veins faintly evident or obscure. Inflorescences pseudo-terminal or terminal, racemose, ca. 1.5 cm long at anthesis, developing beyond confines of perennating bud, densely flowered, 3 to 8 per axil, (3 to)5 or 6-flowered; rachis green, puberulent, slightly ridged with translucent trichomes ca. 0.1 mm long; bracts sub-



Figure I. *Vaccinium exiguum* **A** ultramafic summit of Mt. Victoria **B** flowering branchlets *in situ*. Photo **A** by Alastair Robinson. Photo **B** by Rene Alfred Anton Bustamante.

tending pedicels, foliaceous, greenish, brown *in sicco*, ovate to elliptic, planar or occasionally cucullate, $3.0-8.0 \times 4.0-7.5$ mm, coriaceous, glabrous, margin crenulate or serrulate with 4 or 5 (or 6) impressed crenations per side, with minute translucent lanceolate glands on crenations, apex obtuse or rounded. *Flowers* articulated at junction with pedicel, 6.0-7.0 mm long. *Pedicel* nodding, $2.0-5.0 \times 0.8-0.9$ mm at anthesis, white-puberulent; bracteoles persistent, 2, borne at base of pedicel, margin with minute translucent lanceolate glands on crenations, linear-lanceolate to oblong or nearly so, $1.0-1.2 \times 0.3-0.5$ mm long, glabrous, margin serrate, apex sharply acute. *Hypanthium* green, glossy, cupuliform, $1.4-1.8 \times 0.9-1.0$ mm, glabrous; calyx limb

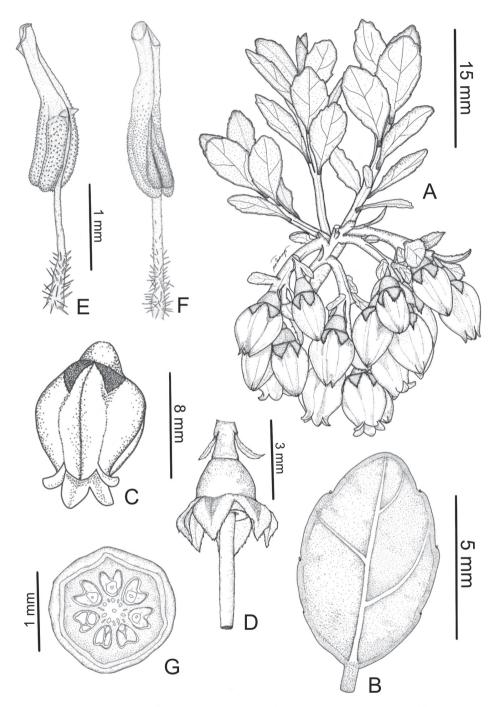


Figure 2. *Vaccinium exiguum* **A** flowering branchlet **B** leaf, abaxial view **C** lateral view of flower showing ribbed corolla **D** distal portion of pedicel, as well as hypanthium, calyx lobes and style **E** stamen in oblique-lateral view showing spurs **F** stamen in ventral view **G** cross section of ovary showing seven locules. Illustrated by Maverick N. Tamayo.

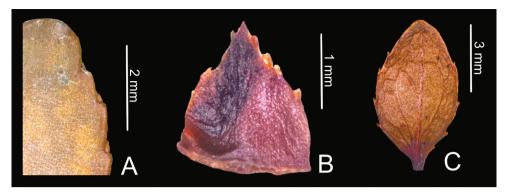


Figure 3. Translucent glands of *Vaccinium exiguum* **A** leaf margin **B** calyx lobe **C** bract. Photos by Maverick N. Tamayo.

0.7–1.0 mm long, glabrous; calyx lobes broadly triangular, 1.8–2.0 mm long, glabrous both sides, margin serrate, with minute translucent lanceolate glands on crenations, apex acute, without sessile and terminal gland. Corolla in bud closed, broadly urceolate and strongly 5- to 7-ribbed (sulcations) along the petal midveins, pale green, at anthesis strongly 5- to 7- ribbed, white with tinge of pale red or pink near and on lobes, 6.0-7.0 × 4.0-5.0 mm, glabrous inside and outside; corolla lobes 5 to 7, ca. 1.0×1.0 mm, apex acute or obtuse. *Stamens* 8 to 10, monomorphic, free from each other, 3.5–4.0 mm long; filaments straight, 1.9–2.0 mm long, white-pubescent mainly at base, trichomes ca. 0.1 mm long (shorter distally); anthers 1.5-2.1 mm long, cells 1.2–1.3 mm long, echinulate, tubules parallel, broadly cylindrical, 0.8–1.0 mm long, slightly narrower than cells, opening by oblique ventrally oriented apical pores, pore apex rounded, spurs present, minute, borne \pm midway along anther, laterally oriented, 0.15–0.20 mm long. *Ovary* 5 or 6 (or 7)-locular, but appearing pseudo-10- to 12- (to 14-) locular with false partitions extending 0.20-0.25 mm from inner wall; ovules in two columns per locule, each column separated by false partitions; disk circular with prominent ridges on margin, ca. 2.0 mm in diameter, glabrous; style not exserted from corolla, 4.0–5.0 mm long, glabrous. Fruit not observed.

Etymology. The epithet "*exiguum*" refers to the overall small stature and leaf morphology of the new species.

Distribution and habitat. *Vaccinium exiguum* is currently known from a single individual from a single location at the exposed ultramafic forest summit of Mt. Victoria, Palawan Island, Philippines at ca. 1700 m elevation.

Conservation status. Only a single flowering plant was documented from a single location at the summit of Mt. Victoria, Palawan. Other summits within the mountain range are similar to the type locality in elevation, but these have yet to be explored. As such, we propose the conservation threat status Data Deficient (DD) (IUCN Standards and Petitions Committee 2019) due to the scarcity of data with which to confidently assess the species against the IUCN guidelines.

Many species are endemic to Mt. Victoria (Robinson et al. 2009, 2016). Though harboring high biodiversity, Mt. Victoria is unfortunately not legislated as a protected area (PA). Mining activities within the area are considered a significant threat that poses the risk of habitat and forest degradation. Thus, the lack of legal protection is the greatest threat to this species and to the biodiversity of the area.

Phenology. The new species was observed flowering during the month of February.

Discussion

Vaccinium exiguum belongs to section *Bracteata* Nakai (Nakai and Koidzumi 1927) sensu Sleumer (Sleumer 1966–1967) as exhibited by its well-developed and often many-flowered racemes, corolla consisting of a single homogenous layer, and the absence of a membranaceous wing at the sinuses and anthers that open by short introrse slits or terminal pores (Co et al. 2002; Salares et al. 2018; Fritsch et al. 2020).

In the key of Sleumer (1996–1967) of the Malesian *Vaccinium* section *Bracteata*, *V. exiguum* best keys to *V. gitingense* Elmer (endemic to the Philippines). However, the new species differs from *V. gitingense* by having shorter petioles (0.5–1.0 mm vs. ca. 2.0 mm), smaller leaf blades ($4.9-9.3 \times 3.0-6.0$ mm vs. $15-35 \times 8-15$ mm), glabrous calyx lobes (vs. finely ciliate), a non-glaucous and glabrous corolla (vs. glaucous and occasionally bears few hairs), and longer filaments (1.9-2.0 mm vs. ca. 1.5 mm) (Elmer 1912). In the key to the Bornean species of *Vaccinium* (Argent 2018), the species best keys to *V. coriaceum* var. *stapfianum* (Sleumer) Argent (restricted to Mt. Kinabalu, Malaysia); however, *Vaccinium exiguum* differs by having shorter leaf blades (4.9-9.3 mm vs. 6.0-13.0 mm), a crenate leaf margin (vs. entire), longer calyx lobes (1.8-2.0 mm vs. ca. 1.3 mm) that are triangular (vs. ovate), and a corolla with deep sulcations (vs. no sulcations). Likewise, the presence of translucent lanceolate glands on the crenations along the margins of the leaves, bracts, bracteoles, and calyx lobes of *V. exiguum* (Fig. 3) is a character shared by the three species.

Although the species exhibits morphological similarities with *V. hamiguitanense*, the flowers of *V. exiguum* are unique amongst all other Philippine *Vaccinium* in having a broadly urceolate and strongly 5- to 7-ribbed corolla and calyx lobe margins that are serrate. *Vaccinium exiguum* also possesses the smallest leaves amongst the Philippine *Vaccinium* species with a size range near *V. hamiguitanense* and *V. microphyllum* Reinw. The latter species is easily distinguished from *V. exiguum* by its axillary solitary flowers (vs. terminal multi-flowered inflorescences). In addition to the characters distinguishing *V. exiguum* from *V. hamiguitanense* as specified in the diagnosis, the two species have distinct geographical ranges.

Currently, two species of *Vaccinium* are recorded in Palawan: *V. brachytrichum* Sleumer and *V. palawanense* Merr. The new species can be easily differentiated from *V. brachytrichum* by bearing fewer flowers per inflorescence [(3 to)5 or 6 vs. 5 to 8)], longer anthers (1.5–2.1 mm vs. ca. 1 mm), and a glabrous (vs. pubescent) hypanthium. It differs from *V. palawanense* in the short and densely flowered inflorescences that are much shorter than the leaves (vs. flowers in racemes about as long as the leaves), a shorter pedicel (2.0–5.0 mm vs. 5.0–7.0 mm), and a 5- or 6-(or 7-) locular ovary (vs. 5) (Merrill 1908; Sleumer 1961). Moreover, the strongly ribbed corolla and the small leaves with crenate margins are also remarkable characters of the new species that easily distinguish it from the two Palawan species. At its type locality, *V. exiguum* was observed to be sympatric with *V. palawanense*.

Due to the paucity of collections and relative lack of study, intraspecific morphological variation within the species of Philippine *Vaccinium* (Salares et al. 2018), as well as sectional boundaries and composition, are imprecisely known (Vander Kloet and Dickinson 2009; Fritsch et al. 2020). A detailed monographic study of this group is warranted.

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