

Nomenclatural notes and typification of three synonyms of *Camellia* (Theaceae)

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Abstract

All the known generic synonyms of *Camellia* are summarized with their types clarified. *Camelliastrum* and *Desmitus* are lectotypified and *Salceda* is neotypified. “*Kailosocarpus*” and “*Parapiquetia*” were not validly published, *Theaphylla* and *Tsia* are illegitimate replacement names for *Thea*, and *Kemelia* and *Tsubaki* are illegitimate replacement names for *Camellia*. Nomenclatural notes on *Theopsis* and its type are also provided.

Keywords

Camellia cuspidata, *Camellia reticulata*, *Camelliastrum*, *Desmitus*, lectotypification, neotypification, *Salceda*, *Theopsis*

Introduction

Linnaeus (1753: 698) established the monotypic genus *Camellia* L. for the ornamental species, *Camellia japonica* L. He referenced Kaempfer’s (1712: 850, 852) description and a single illustration (Kaempfer 1712: t. 851) under *C. japonica* without citing any specimens in the protologue (Arts. 38.1, 38.5, 38.6 & 38.13 of the Shenzhen Code, Turland et al. 2018; hereafter ICN). Subsequently, Bartholomew (Jarvis et al. 1993: 29) designated the illustration (Kaempfer 1712: t. 851) as the lectotype of *C. japonica* (Art. 9 Ex. 2 of the ICN). Accordingly, this illustration serves as the type of *Camellia*.

The boundaries of *Camellia* have been gradually enlarged by several taxonomists (e.g., Sweet 1818; Seemann 1859; Sealy 1958; Chang 1981; Ming 2000). Zhang et al.

(2014) argued that *Camellia* might be a paraphyletic group in their analyses using four plastid DNA regions and the paralogous nuclear *LEAFY* marker. However, the monophyly of the genus was supported by investigations using complete plastid genome data (Yu et al. 2017) and other nuclear sequences (Vijayan et al. 2009; Li et al. 2011; Zhao et al. 2022). Nevertheless, nomenclatural problems should be resolved before a comprehensive phylogenetic study of the boundaries of *Camellia*. Although the genus, including tea, camellias and oil camellias, has been revised several times (e.g., Sealy 1958; Chang 1981; Ming 2000), the types of many taxa in *Camellia* have only recently been clarified (Zhao et al. 2017a, b, 2018, 2019; Zhao 2021). However, the types of some synonyms at generic rank remain unclear and are discussed here.

Materials and methods

Relevant collections or their images from herbaria at A, BM, E, G, IBK, IBSC, K, KUN, L, LINN, P, PE, SBT, SYS, TCD, UPS and US (acronyms following Thiers 2022), and the taxonomic literature were examined. Types were chosen based on Arts. 9 and 10 of the ICN.

Types of the synonyms of *Camellia*

Since the latest monograph of *Camellia* (Ming 2000), two further genera, *Bembiciopsis* H. Perrier (Judd 1997) and *Dankia* Gagnep. (Hò 1991; Zhao et al. 2017b), have been placed into the synonymy of *Camellia*. All 18 synonyms of *Camellia* (Linnaeus 1753; Adanson 1763; Blume 1825; Siebold 1832; Rafinesque 1830, 1838; Blanco 1845; Hallier 1921; Gagnepain 1939; Nakai 1940; Perrier 1940; Hu 1956, 1965) and their types (Jarvis et al. 1993; Lin et al. 2008; Zhao et al. 2016, 2017b, 2018, 2019; Zhao 2021) are listed in Table 1. “*Kailosocarpus*” (Hu 1957: 170) and “*Parapiquetia*” (Hu 1957: 170) were cited by Chang (1981: 12) in the synonymy of *Camellia*; however, both “*Kailosocarpus*” and “*Parapiquetia*” were not validly published since a Latin description or diagnosis was neither provided nor cited in each of the protologues (Art. 39.1 of the ICN). “*Kailosocarpus*” and “*Parapiquetia*” have no status under Art. 12.1 of the ICN and are, therefore, excluded from Table 1. The typification and nomenclatural notes of some synonyms of *Camellia* are elaborated below.

1. *Camelliastrum* Nakai, J. Jap. Bot. 16: 699. (1940)

Table 1

Type (“lectotype”, Art. 10 Note 1 of the ICN; designated here): *Camelliastrum caudatum* (Wall.) Nakai.

Nomenclatural notes. Nakai (1940) established the genus *Camelliastrum* to categorize six species in China and Japan, including *Camelliastrum assimile* (Champ. ex Benth.) Nakai, *Camelliastrum buisanense* (Sasaki) Nakai, *Camelliastrum caudatum*, *Camelliastrum gracile* (Hemsl.) Nakai, *Camelliastrum mairei* (H.Lév.) Nakai and *Camelliastrum salicifolium* (Champ.) Nakai, but did not designate a type. Based on the description in the protologue, *Camelliastrum caudatum* is selected as the type of the genus. The basionym of *Camelliastrum caudatum*, *Camellia caudata* Wall., was lectotypified by Zhao et al. (2017b: 172) as the specimen *H. Bruce s.n.* in *Wallich 978* (right-hand specimen of K001110475. An image is available at <http://www.kew.org/herbcatimg/680707.jpg>). Therefore, this specimen becomes the type of *Camelliastrum* (Art. 10.1 of the ICN).

2. *Desmitus* Raf., *Sylva Tellur.* 139. (1838)

Table 1

Type. *Desmitus reticulata* (Lindl.) Raf. ≡ *Camellia reticulata* Lindl., *Bot. Reg.* 13: t. 1078 (1827).

Lectotype (designated here): Lindl., *Bot. Reg.* 13: t. 1078 (1827).

Nomenclatural notes. Rafinesque (1838) established the monotypic genus *Desmitus* for *D. reticulata*. This was a new combination based on *C. reticulata* because he referenced the basionym by the words “Camel. do bot. reg. 1978...”. However, the taxon number cited by Rafinesque (1838: 140) is incorrect, but should be recognized as a correctable typographical error of “1078” and so does not prohibit the valid publication of the new combination (Art. 41.3 of the ICN). Nevertheless, Lindley (1827: t. 1078) described *C. reticulata* based on the living plants that bore semidouble flowers and introduced from China. No specimen was cited in the protologue of *C. reticulata*. The coloured drawing, t. 1078, was accompanied by the protologue and therefore designated as the lectotype of *C. reticulata*. Accordingly, the drawing serves as the type of *Desmitus* (Art. 10.1 of the ICN).

3. *Salceda* Blanco, *Fl. Filip.* ed. 2: 374. (1845)

Table 1

Type. *Salceda montana* Blanco

Neotype (designated here): Philippines. Luzon: Bulacan, Angat, February 1919, *Ramos & Edaña 34071* (K!; isoneotypes: BM!, P04511451 [the image is available at <https://science.mnhn.fr/institution/mnhn/collection/p/item/p04511451>]!, and US 00113902 [the image is available at <http://n2t.net/ark:/65665/3e093c26d-7aa1-4494-8723-989167baf8ba>]!).

Nomenclatural notes. Blanco (1845: 374) established the monotypic genus *Salceda* for *S. montana*. Merrill (1905: 21) transferred the species to *Thea* as *T. montana* (Blanco) Merr. and stated that “Blanco’s specimens were from Angat, Province of Bulacan”. However, the types of Blanco’s species were suggested to be either all destroyed (Merrill 1905: 6) or no longer extant (Merrill 1918: 5). I also failed to find the original material of *S. montana*. Based on the protologue (Blanco 1845: 374), a specimen collected from the same locality, *Ramos & Edaño 34071* (K), is designated as the neotype of *S. montana* because it bears flower fragments and seeds on the sheet.

Table 1. A summary of synonyms of *Camellia* L.

Synonym	Type, as species (basionym)	Type, as specimen or illustration	Notes
<i>Bembiciopsis</i> H. Perrier, Mém. Mus. Nat. Hist. Nat. 13: 300. (1940)	<i>Bembiciopsis uniflora</i> H. Perrier	<i>Le Myre de Vilers s.n.</i> , P00389083, holotype	
<i>Calpandria</i> Blume, Bijdr. fl. Ned. Ind. 178. (1825)	<i>Calpandria lanceolata</i> Blume	L 0064294, lectotype (designated by Zhao et al. 2019: 298)	
<i>Camelliastrum</i> Nakai, J. Jap. Bot. 16: 699. (1940)	<i>Camellia caudata</i> Wall.	<i>H. Bruce s.n.</i> in <i>Wallich 978</i> (K001110475, right-hand specimen), lectotype (designated by Zhao et al. 2017b: 172)	
<i>Dankia</i> Gagnep. in Humbert, Fl. Indo-Chine, Suppl. 1: 198. (1939)	<i>Dankia langbianensis</i> Gagnep.	<i>Poilane 18648</i> (P00754831), lectotype (designated by Zhao et al. 2017b: 173)	
<i>Desmitus</i> Raf., Sylva Tellur. 139. (1838)	<i>Camellia reticulata</i> Lindl.	Lindl., Bot. Reg. 13: t. 1078 (1827), lectotype, designated in this paper	
<i>Drupifera</i> Raf., Sylva Tellur. 140. (1838)	<i>Thea oleosa</i> Lour.	<i>Loureiro s.n.</i> (P00150891), holotype	
<i>Glyptocarpa</i> Hu, Acta Phytotax. Sin. 10: 25. (1965)	<i>Pyrenaria camellioides</i> Hu	<i>Wang 72468</i> (PE 00024548), lectotype (designated by Lin et al. 2008: 1701)	
<i>Kemelia</i> Raf., Sylva Tellur. 139. (1838)	<i>Camellia japonica</i> L.	Kaempfer, Amoen. Exot. Fasc. t. 851. (1712), lectotype	Illegitimate replacement name for <i>Camellia</i>
<i>Piquetia</i> (Pierre) Hallier f., Beih. Bot. Centralbl. 39(2): 162. (1921)	<i>Thea piquetiana</i> Pierre	<i>Pierre 1708</i> (P01903371), lectotype (designated by Zhao et al. 2018: 94)	
<i>Salceda</i> Blanco, Fl. Filip. ed. 2: 374. (1845)	<i>Salceda montana</i> Blanco	<i>Ramos & Edaño 34071</i> (K), neotype, designated in this paper	
<i>Sasanqua</i> T. Ness in Siebold, Nippon 2(6): 13. (1832)	<i>Camellia sasanqua</i> Thunb.	<i>Thunberg s.n.</i> (UPS No. 16143, left-hand specimen), lectotype (designated by Zhao 2021: 297)	
<i>Stereocarpus</i> (Pierre) Hallier f., Beih. Bot. Centralbl. 39(2): 162. (1921)	<i>Thea dormoyana</i>	The author and typification of the species is dependent on a binding decision to be made, see Zhao et al. (2016: 1183)	
<i>Thea</i> L., Sp. Pl. 515. (1753)	<i>Thea sinensis</i> L.	Kaempfer, Amoen. Exot. Fasc. 606, f. 1–2. (1712), lectotype (designated by Bartholomew in Jarvis et al. 1993: 93)	
<i>Theaphylla</i> Raf., Med. Fl. 2: 267. (1830)	<i>Thea sinensis</i> L.	Kaempfer, Amoen. Exot. Fasc. 606, f. 1–2. (1712), lectotype	Illegitimate replacement name for <i>Thea</i>
<i>Theopsis</i> (Cohen-Stuart) Nakai, J. Jap. Bot. 16: 704. (1940)	<i>Thea cuspidata</i> Kochs	<i>Henry 7026</i> (K000380525), lectotype (designated by Sealy 1958: 57)	
<i>Tsia</i> Adans., Fam. Pl. 2: 450. (1763)	<i>Thea sinensis</i> L.	Kaempfer, Amoen. Exot. Fasc. 606, f. 1–2. 1712, lectotype	Illegitimate replacement name for <i>Thea</i>
<i>Tsubaki</i> Adans., Fam. Pl. 2: 399. (1763)	<i>Camellia japonica</i> L.	Kaempfer, Amoen. Exot. Fasc. t. 851. 1712, lectotype	Illegitimate replacement name for <i>Camellia</i>
<i>Yunnanea</i> Hu, Acta Phytotax. Sin. 5: 282. (1956)	<i>Yunnanea xylocarpa</i> Hu	<i>Yu 16021</i> (PE 00133872), lectotype (designated by Lin et al. 2008: 1702)	

Illegitimate replacement names for *Camellia* and *Thea*

Thea L., a genus established by Linnaeus (1753: 515) for tea (*T. sinensis* L., currently *C. sinensis* [L.] Kuntze), was treated as a synonym of *Camellia* by Sweet (1818: 157). *Theaphylla* Raf. and *Tsia* Adans. are illegitimate replacement names for *Thea* because *Thea* was cited in the synonymies of them (Adanson 1763: 613; Rafinesque 1830: 267), which makes *Tsia* and *Theaphylla* nomenclaturally superfluous (Arts. 6.11, 52.1 & 52.2[e] of the ICN). Similarly, *Kemelia* Raf. and *Tsubaki* Adans. are illegitimate replacement names for *Camellia* (Adanson 1763: 399; Rafinesque 1838: 139) because *Camellia* was cited as a synonym of them (Arts. 6.11, 52.1 & 52.2[e] of the ICN). Therefore, the four names, *Kemelia*, *Theaphylla*, *Tsia* and *Tsubaki*, are rejected under Art. 52.1 of the ICN.

Nomenclatural notes on *Theopsis*

Cohen-Stuart (1916: 70) established *Camellia* sect. *Theopsis* Cohen-Stuart in his Ph.D. thesis, which constituted an effective publication under Art. 30.9 of the ICN. Subsequently, he translated the first two chapters of his original thesis in Dutch into English and published it (Cohen-Stuart 1919). Cohen-Stuart (1916, 1919) listed nine species, viz. *C. costei* H. Lév., *C. cuspidata* (Kochs) hort., *C. euryoides* Lindl., *C. forrestii* (Diels) Cohen-Stuart, *C. henryana* Cohen-Stuart, *C. lutchuensis* T. Itô ex T. Itô & Matsum., *C. parvifolia* (Hayata) Cohen-Stuart, *C. punctata* (Kochs) Cohen-Stuart and *C. rosiflora* Hook., in the key under sect. *Theopsis*. These nine species are presumably treated as the members of sect. *Theopsis* based on the structure of the key, the description of the section and the discussion. However, Cohen-Stuart (1916, 1919) did not designate a type for his sect. *Theopsis*. Nakai (1940) raised this section to generic rank to include 14 species and cited Cohen-Stuart's (1919) English article (Arts. 41.1 & 41.3 of the ICN), but without selecting a type for the genus. Remarkably, four of nine species of Cohen-Stuart's (1916, 1919) sect. *Theopsis* were excluded and five of them, including *C. euryoides*, *C. forrestii*, *C. lutchuensis*, *C. parvifolia*, and *C. rosiflora* (= *C. maliflora*, according to Cohen-Stuart 1916: 69, 1919: 241), were retained in the genus *Theopsis* (Cohen-Stuart) Nakai. Later, Sealy (1958: 14) treated *Theopsis* as a synonym of *Camellia* and resumed sect. *Theopsis* (Sealy 1958: 48). Chang (1981: 128) followed Sealy's (1958) treatment and designated *C. cuspidata*, a species excluded from Nakai's genus *Theopsis*, as the type of sect. *Theopsis* and this typification must be followed under Art. 10.5 of the ICN. However, when Nakai (1940) adopted *Theopsis* as a generic name, he did not exclude the type of Cohen-Stuart's (1916: 70) sect. *Theopsis* because the section had not yet been typified, so Arts. 48.1 & 48.2 of the ICN do not apply. According to Art. 48.1 Note 1 of the ICN, this situation should be dealt with under Art. 7.3 of the ICN. Therefore, the genus *Theopsis* is typified by the type of its basionym, sect. *Theopsis*, that is, *C. cuspidata*, based on Chang's (1981: 128) typification (Art. 10.5 of the ICN) even though the species was excluded from this genus (Art. 7.3 of the ICN).

However, *C. cuspidata* has nomenclatural problems. Kochs (1900: 586) described *T. cuspidata* Kochs and cited the single gathering *Henry 7026*. Subsequently, a name,

C. cuspidata, was provided in the list of “Awards of Merit” in *The Gardeners’ Chronicle* (Anonymous 1912: 228). The plant was described as having “small, single, white flowers” with “pale-yellow stamens” and “narrow leaves” that were about 2–2.5 inches long. The brief description could make *C. cuspidata* validly published as a new species because the requirements of Arts. 32.1 & 38.1 of the ICN are likely fulfilled (also see Art. 38 Note 2 of the ICN). However, although Kochs’s *T. cuspidata* was neither directly nor indirectly referenced in the protologue of *C. cuspidata* (Anonymous 1912: 228; Arts. 41.1–41.3 of the ICN), the latter is, nevertheless, treated as a new combination based on *T. cuspidata* under Art. 41.4 (see Ex. 12) of the ICN.

Furthermore, two duplicates of *Henry 7026* were found at K and US, viz. K000380525 (the image is available at <http://www.kew.org/herbcatimg/165067.jpg>) and US 00504123 (the image is available at <http://n2t.net/ark:/65665/308ec5722-d414-4a4d-b733-f34c3778997b>). Since Kochs (1900: 586) did not indicate a single specimen of the entire gathering as the holotype, the two duplicates at different herbaria are syntypes of *T. cuspidata* based on Art. 40 Note 1. When Sealy (1958: 57) cited “*A. Henry 7026* (K, type-number)” under *C. cuspidata*, the citation could be treated as the lectotypification of the species following Arts. 7.11, 9.10 & 9.19 of the ICN. Therefore, the lectotype of *C. cuspidata* is that of *T. cuspidata*, viz. *Henry 7026* (K000380525), which, in turn, serves as the type of *Theopsis* (Table 1).

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Taxonomic revision of the southern African species of the genus *Cynoglossum* L. (Boraginaceae)

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Abstract

The aim of the study is to provide a revision of the genus *Cynoglossum* in southern Africa. The genus is taxonomically problematic within the family Boraginaceae, due to the morphological similarities it shares with other closely related genera in the family. *Cynoglossum* plants are low-growing biennial, perennial, or rarely annual herbs which are recognizable by their hairy stems and leaves, the latter are usually basal and long petiolate. Based on the latest checklist, a total of eight species of this genus are listed for the study region: *C. alticola*, *C. amabile*, *C. austroafricanum*, *C. geometricum*, *C. hispidum*, *C. lanceolatum*, *C. obtusicalyx* (endemic to South Africa), and *C. spelaicum*. The occurrence of *C. amabile* in the region, however, requires further investigation since the only existing specimen was collected within a protected area in the KwaZulu-Natal province. Two specimens collected in the Doornpoort area in Pretoria, Gauteng province, assigned to this species appear to have been misidentified. Diagnostic characters are described, correct nomenclature, synonyms, typification, distribution maps, as well as the key for identifying the studied species, are provided.

Keywords

Boraginaceae, *Cynoglossum*, Southern Africa, taxonomic revision

Introduction

The type genus *Cynoglossum* L. of the tribe Cynoglosseae belongs to the angiosperm family Boraginaceae (forget-me-not or borage family). Members of this genus have a worldwide distribution with many species occupying the temperate as well as

the tropical regions of the Old and New Worlds. This genus comprises about 80–90 (Johnston 1924), to ca. 100 (Weigend et al. 2013), or as many as 200 species (Chacón et al. 2016). The highest diversity of species occurs in the Mediterranean region, harbouring about 20 species (Selvi and Sutory 2012), and only a few species are introduced in Australia and one in North America (i.e., *C. creticum*) (Selvi et al. 2011). The name *Cynoglossum* is derived from the Greek words ‘*cynos*’ (of a dog) and ‘*glossa*’ (tongue), depicting the texture and shape of the leaves in species of the genus (Retief 2005), hence the common name ‘hound’s tongue’. This genus was established by Linnaeus (1753) to accommodate six species that he described at the time, namely, *Cynoglossum officinale* L., *C. virginianum* L., *C. cheirifolium* L., *C. apenninum* L., *C. linifolium* L., and *C. omphaloides* L. The present state of knowledge has renamed most of these Linnaeus names to the following accepted names, i.e., *C. virginianum* L. (= *Andersonglossum virginianum* (L.) J.I. Cohen), *C. cheirifolium* L. (= *Pardoglossum cheirifolium* (L.) Barbier & Mathez), *C. apenninum* L. (= *Solenanthus apenninum* (L.) Fisch. & C.A. Mey.), *C. linifolium* L. (= *Iberodes linifolium* (L.) Serrano, R. Carbajal & S.Ortiz), and *C. omphaloides* L. (= *Omphalodes verna* Moench).

The genus is complex and has been noted by several botanists such as Miller (2005), Sutory (2010), Selvi and Sutory (2012), to be taxonomically challenging within the family due to the limited morphological variation between the species, which has rendered identification difficult. Another unresolved problem concerns the species relationships in *Cynoglossum*. Morphological characters that were traditionally used to delineate genera proved to be insufficient, especially in defining monophyletic groups within *Cynoglossum* as stated by Hilger et al. (2015). Recent phylogenetic studies by Cohen (2011, 2013, 2015), Selvi and Sutory (2012), and Weigend et al. (2013), revealed that *Cynoglossum* is paraphyletic with respect to *Cynoglossopsis* Brand, *Lindelofia* Lehm., *Paracaryum* Boiss., *Pardoglossum* Barbier & Mathez, *Rindera* Pall., *Solenanthus* Ledeb., and *Trachelanthus* Kunze, suggesting the need for generic realignment. As a contribution to the systematics of the family, Johnston (1924) grouped half a dozen of genera under a broad concept of *Cynoglossum* based on the character of corolla shape and degree of anther exertion. From this grouping, the genus *Rindera* (= *Mattia*) and *Paracaryum* (= *Mattiastrum*) were still recognised as distinct with the only diagnostic feature being the fruit ornamentation. *Cynoglossum* portraying zoochory adapted glochidiate dispersal units, *Rindera* with the broadly winged diaspores for wind dispersal, and *Paracaryum* exhibiting the combinatory features (Greuter 1981). This unresolved relationship of taxa based on morphological traits led to authors such as Greuter and Burdert’s (Greuter 1981) request a broader conception of this genus, where *Cynoglossum sensu lato* forms a single, large genus.

An article by Von Staden et al. (2013), mentioned that taxonomically problematic genera are most likely to result in poor collection and misidentification. The latter case has become a reality for the southern African species of *Cynoglossum*, whereby members of this genus are also confused with species from other closely related genera such as *Lithospermum* L. and *Myosotis* L., and members of the tribe Eritrichieae Gürke. *Lithospermum* and *Myosotis* members are characterized by ovoid nutlets with broad basal attachment scars and flat gynobases, while in *Cynoglossum* the nutlets are

depressed-ovoid or subcircular with scars restricted to the apical half of the ventral surface and have narrowly conical gynobases (Weigend et al. 2013; Otero et al. 2014). Eritrichieae are differentiated from *Cynoglossum* based on the shape of the gynobase, i.e., narrow pyramidal to subulate gynobase and mostly small nutlets, as opposed to broadly pyramidal gynobase and mostly larger nutlets for *Cynoglossum* (Hasenstab-Lehman and Simpson 2012; Weigend et al. 2013). According to Al-Shehbaz (1991), *Cynoglossum* can also be distinguished from the closely related genera *Solenanthus* Ledeb. and *Trachelanthus* Kunze in having included, instead of exerted, stamens and lastly, from *Pardoglossum* Barbier & Mathez in having slim, glabrous glochids on the nutlets, instead of swollen glochids that are densely packed with minute papillae. The fruits in members of this genus and the floral morphological characters, are normally considered to be of diagnostic importance. The nutlets of tropical *Cynoglossum* are generally smaller than those of species found in the temperate regions (Al-Shehbaz 1991).

In the southern African region, this genus has not yet been taxonomically revised since the last treatment by Wright (1904), where he recognised two species, *C. enerve* Turcz. (now *C. hispidum* Thunb.) and *C. micranthum* Desf. He cited two species, namely, *C. leptostachyum* DC. and *C. hispidum* Thunb. as being imperfectly known. Hilliard and Burtt (1986) recorded the occurrence of *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii* (Baker and C.H. Wright) Verdc. [recognised in southern Africa as *C. geometricum* Baker & C.H. Wright] for the first time in this region, while Retief and Van Wyk (1996) recorded *C. obtusicalyx* Retief & A.E. van Wyk, the only species endemic to South Africa. While a comprehensive revision is still lacking for southern African species, revisionary work has been undertaken mainly at regional or country level, for example East Africa (Verdcourt 1991), China (Shu 1995), Comoro Islands and Madagascar (Miller 2005), Italy (Selvi and Sutorý 2012), Nepal (König et al. 2015), and Taiwan (Hsiao and Liu 1998). According to the recent checklist by Germishuizen and Meyer (2003), there are eight species of *Cynoglossum* occurring in southern Africa, namely *C. alticola* Hilliard & B.L. Burtt, *C. amabile* Stapf. & J.R. Drumm, *C. austroafricanum* Hilliard & B.L. Burtt, *C. geometricum* Baker & C.H. Wright, *C. hispidum* Thunb., *C. lanceolatum* Forssk., *C. obtusicalyx* Retief & A.E. van Wyk (endemic to South Africa), and *C. spelaicum* Hilliard & B.L. Burtt. This paper aims to provide a taxonomic revision of the southern African species, to provide a diagnostic key, as well as to lay out their distribution maps in the region.

Materials and methods

A total of 316 specimens loaned from the following herbaria: PRE, GRA, NH, NU, NBG, and SAM (herbarium acronyms following Thiers 2019), were examined for distribution and morphological data. The type specimens of relevant species were studied online from the JSTOR website (<https://plants.jstor.org/>). Voucher specimens and plant material were collected during several field trips conducted throughout South Africa and Lesotho between October 2018 and December 2019. Specimens collected

were deposited in JRAU. The system by Edwards and Leistner (1971) was used for specimen citation under the section ‘additional specimen examined’.

Data on vegetative morphology was obtained by analysing all the specimens provided per species. Inflorescence structures were studied from the freshly collected samples, herbarium samples, as well as from the original author’s descriptions (in a case where all the specimens did not contain any inflorescence to study). Hand drawings representing both the vegetative and reproductive characters were made for all the species (except for *C. amabile*). The trichomes from the leaf samples and mature nutlets, from as many specimens as possible, were examined per species, except for *C. amabile*, where there was only one specimen available, and *C. obtusicalyx*, where three specimens were available. Original descriptions from JSTOR.org were also included in developing the diagnostic key. The specimens were studied using either the TESCAN VEGA3 scanning electron microscope (SEM) or the Phenom Desktop SEM. For TESCAN VEGA3 SEM analysis, samples were immersed in a mixture of 95% ethanol and isoamyl acetate (1:1) for 10 minutes and in pure isoamyl acetate for 15 minutes. After removing isoamyl acetate, the samples were placed on a holder for critical point drying for an hour. Then the dried samples were directly mounted on aluminium stubs and sputter-coated with a thin layer of gold before viewing under microscope. This was done to prevent charging of specimens due to accumulation of static electric field, and to increase the number of secondary electrons that can be detected from the surface of the specimen. For the Phenom Desktop SEM analysis, samples were only air-dried for an hour and were directly mounted on the aluminium stubs and viewed under microscope. Maps were plotted using the program CorelDraw Graphics Suite X7 (<http://coreldraw.com>).

Morphological characters of *Cynoglossum* species in southern Africa

Vegetative morphology

Members of this genus are either perennial, biennial, or rarely annual herbs which are recognizable by their hairy stems and leaves. Roots are thickened cream white taproots with small lateral roots. The stems are erect, hollow, simple at the base, and usually branched above. The basal leaves are deciduous, long petiolate, lanceolate-obtuse shaped, cross-venulate, with smooth margins, and are clustered at the lower parts of the stem forming a rosette, covered with simple trichomes on both the adaxial and abaxial surface. The stem leaves are alternate, sessile, or petiolate, lanceolate-obtuse shaped, with smooth margins. Trichomes on the leaves sometimes have a pustulate base typical of Boraginaceae members (*C. austroafricanum*, *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii* and *C. lanceolatum*). Vegetative morphology is of limited diagnostic value in distinguishing between southern African species, however a closer look at the trichomes has shown that they may be used to distinguish between similar species. For example, both *C. alticola* and *C. obtusicalyx* have a cluster of soft, woolly trichomes which differ in shape (cylindrical with a pointed tip in *C. alticola*, vs. flat surface and spherical with a blunt tip in *C. obtusicalyx*) and density (denser in *C. alticola* than in *C. obtusicalyx*) as observed in Figure 1.

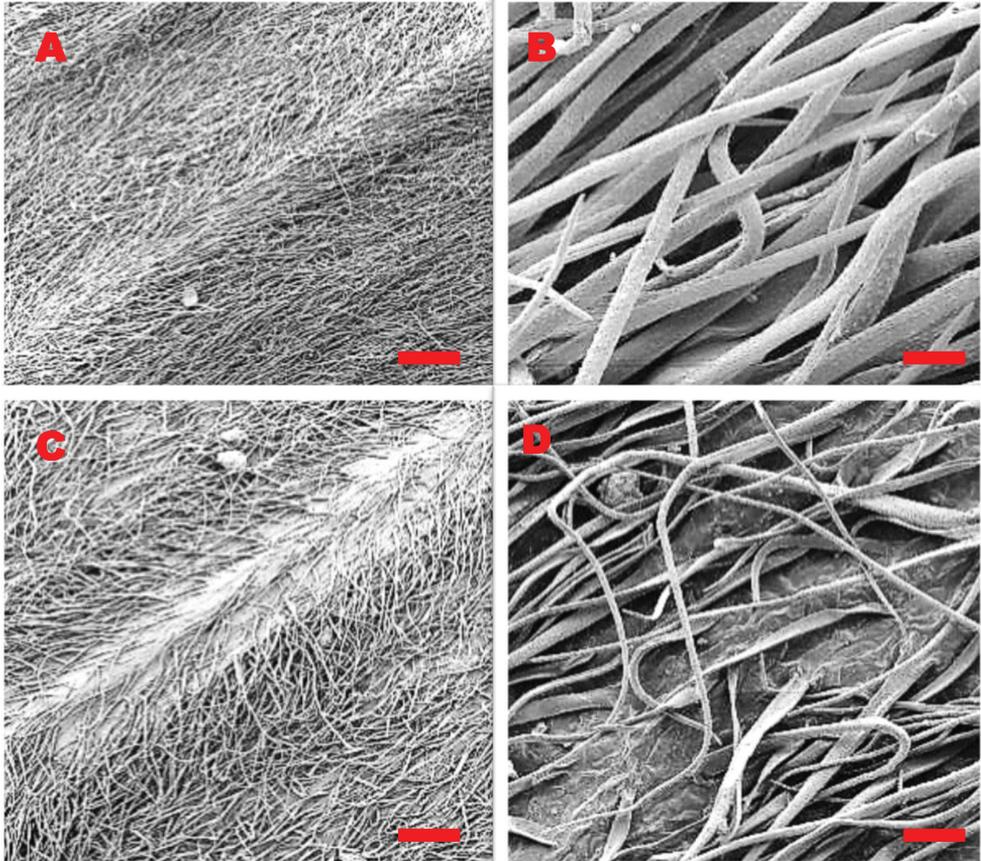


Figure 1. SEM micrographs of the adaxial leaf surfaces and the midrib of (A, B) *C. alticola* (C, D) *C. obtusicalyx* B close-up of *C. alticola* displaying cylindrical trichomes with pointed end D close-up of *C. obtusicalyx* displaying flat trichomes with blunt tip. Vouchers: A, B from L.C.C. Liebenberg 5789 (PRE) C, D from J.P.H. Acocks 8509 (PRE). SEM images scale bars: 10 mm (A); 50 µm (B); 1 mm (C); 100 µm (D).

Reproductive morphology

The inflorescence is a cyme which is often dichotomously branched with spreading panicles. Flowers are either pedicelled or sessile, with five parted corollas; corolla white with a blue throat, blue, violet, or magenta (*C. hispidum*), or rarely white (*C. spelaum*). The stamens are included and arise from the base of the tube, they have short filaments and elliptic to oblong shaped anthers. The style is short and relatively thick, with a capitate stigma. The fruit is a schizocarp of four nutlets attached apically to a narrowly conical gynobase. The nutlets are ovoid with a convex dorsal surface. At maturity, the nutlets produce glochidia, which are sharp hair-like spines or bristles tipped with barbs. The glochidia are either swollen at the base or not bulbous-based, they either cover the whole surface or are well spaced and vary in number. The structure and shape of glochidia display an important distinguishing character amongst the southern African species (Table 1), with each species portraying a unique character as can be seen in Figure 2.

Table 1. Distinguishing characters between the southern African *Cynoglossum* species.

Characters	<i>C. alticola</i>	<i>C. amabile</i>	<i>C. austroafricanum</i>	<i>C. coeruleum</i> subsp. <i>johnstonii</i> var. <i>mannii</i>	<i>C. hispidum</i>	<i>C. lanceolatum</i>	<i>C. obtusicalyx</i>	<i>C. spelaicum</i>
Floral colour	Deep blue	Bluish purple	Pale blue	White with pale blue throat	Magenta	White with pale blue throat	Pale blue	White
Nutlet shape and size	Convex, 6–9×5–6 mm	Ovoid to convex, 2–4×3–4 mm	Ovoid, 3.0–3.5×2.5–3.0 mm	Ovoid, 3–4×2.5–3.5 mm	Convex, 5–6×3–5 mm	Ovoid-convex, 3–4×2.5–3.5 mm	Ovoid-convex, 2–4×3–4 mm	Ovoid, ca. 4×5 mm
Nutlet ornamentation	Glochidia densely packed on nutlet	Marginal glochidia are more distinct than the central glochidia	Glochidia more spread towards the margins	Glochidia more marginal and on the median line	Glochidia covering the whole nutlet	Glochidia sparsely arranged on nutlet	densely echinulate with glochidia	glochidia more marginal and acentric, marginal glochidia are longer compared to the acentric glochidia

Taxonomic treatment

Cynoglossum L., Sp. Pl. 1: 134 (1753) & Gen. Pl.: 5 (1754); Benth. and Hook. f., Gen. Pl. 2: 848 (1876); C.H. Wright in Fl. Cap. 4: 13 (1904); Baker and C.H. Wright in Fl. Trop. Afr. 4 (2): 51 (1905); Brand in Engl., Pflanzenr. 4: 252 (1921); Al-Shehbaz in J. Arnold Arbor.: 112 (1991); Selvi and Sutorý in Pl. Biosyst. 146 (2): 461–479 (2012); Hilger *et al.* in Biodivers. Data J. 3: e4831 (2015). Type species: *C. officinale* L.

Paracynoglossum Popov. Fl. URSS xix.: 717 (1953). Type species: *P. denticulatum* (DC.) Popov.

Perennial, biennial, or rarely annual herbs, often tall, up to 1,2 m in height, and slightly branched. Stems and leaves canescent. Indumentum white, simple or tubercled. Leaves alternate, lanceolate, obtuse or spatulate, entire; first year basal leaves form a rosette, lanceolate or obtuse, often long petiolate. Inflorescences usually elongate, rarely bracteate, sparingly branched or loosely paniculate. Flowers pedicelled or sessile; blue or violet with distinct veins, rarely white. Calyx five-partite, scarcely enlarged in fruit, patent or reflexed. Corolla tube short, throat closed with obtuse or arched scales; five-lobed, imbricate, obtuse, patent. Stamens five, included in the corolla tube, included, with short filaments, anthers ovoid or shortly oblong, obtuse. Ovary with four distinct lobes from an almost flat receptacle; style short or rather long; stigma small, flat or subcapitate; ovules horizontal, fixed to the central angle of the cell. Nutlets four, depressed, scarcely produced at the apex, convex or flat on the dorsal side or surrounded by an elevated margin, glochidiate (hair-like spines or short prickles). Seeds straight or slightly curve

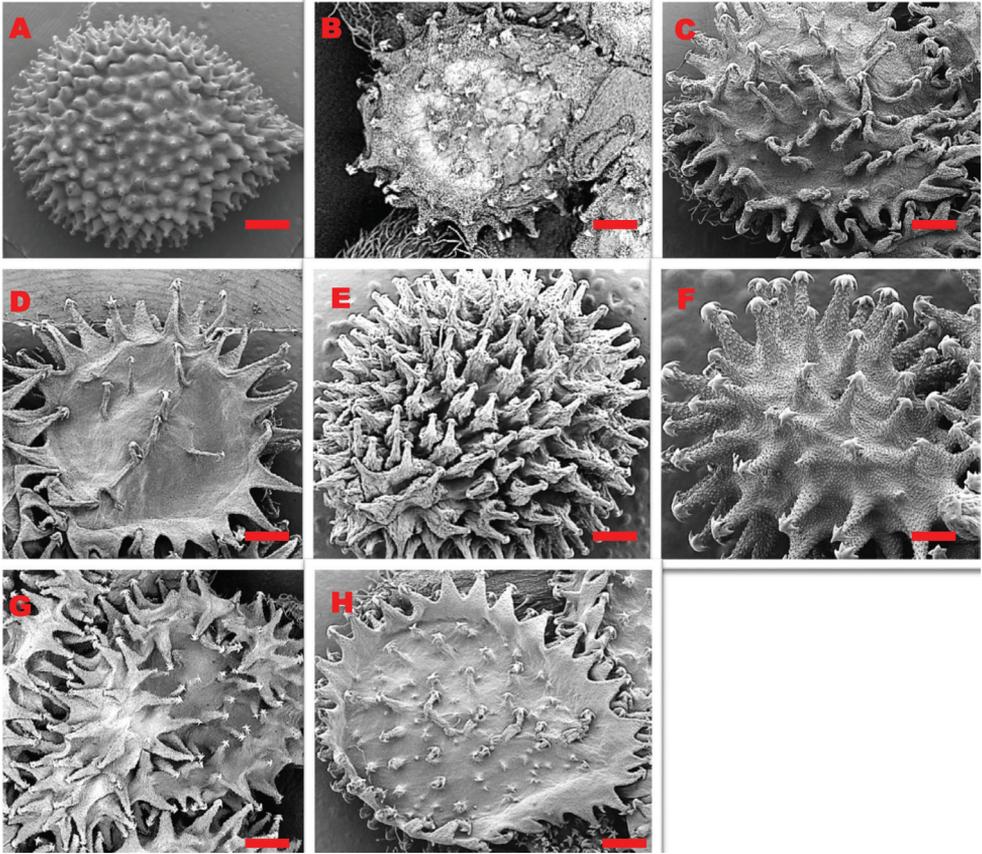


Figure 2. SEM micrographs of the adaxial surface of the nutlets of eight listed species of *Cynoglossum* **A** *C. alticola* **B** *C. amabile* **C** *C. austroafricanum* **D** *C. coeruleum* var *mannii* **E** *C. hispidum* **F** *C. lanceolatum* **G** *C. obtusicalyx* **H** *C. spelaeum*. Voucher specimens: A from L.C.C. Liebenberg 5789 (PRE) **B** from J. Stewart 2021 (NU) **C** from O.M. Hilliard and B.L. Burt 11803 (PRE) **D** from T.B. Sikhakhane 440 (NH) **E** from S.P. Bester 12958 (PRE) **F** from S.P. Bester 4653 (PRE) **G** from J.P.H. Acocks 8509 (PRE) **H** from A. Nicholas and B. Isaacs 1965 (PRE). SEM images scale bars: 1 mm (**A, C-E, G-H**); 2 mm (**B**); 500 µm (**F**).

Diagnostic key to the species

- 1 Soft woolly hairs covering the entire plant; nutlets thickened; glochidia densely arranged on the nutlet **1. *C. alticola***
- Stiff bristle hairs covering the entire plant; nutlets slightly swollen; glochidia sparsely spaced on the nutlet..... **2**
- 2 Nutlets 5 mm wide; glochidia thick at the base, fruit stalk up to 2 cm long ..
..... **5. *C. hispidum***
- Nutlets less than 5 mm wide; glochidia uniformly shaped, fruit stalk up to 1 cm long **3**

- 3 Inflorescences clustered at the apex; corolla bluish purple; glochidia dense at the margins and centre of the nutlet..... **2. *C. amabile***
- Inflorescences not clustered at the apex; corolla blue to white; glochidia dense at the margins and few at the centre of the nutlet..... **4**
- 4 Spreading long trichomes covering the whole plant; corolla longer than 7 mm long **7. *C. obtusicalyx***
- Sparsely shorter trichomes covering the whole plant; corolla shorter than 4 mm long..... **5**
- 5a Corolla white with blue throat:
- 6 Trichomes not thickened on both leaf surfaces; glochidia evenly distributed across the nutlet **6. *C. lanceolatum***
- Trichomes with pustulate base on the abaxial leaf surface; glochidia on the median line and centre of the nutlet **4. *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii***
- 5b Corolla uniformly coloured:
- 7 Leaves brightly green coloured on both surfaces, lanceolate-obtuse shaped; corolla pale blue; length of glochidia uniform throughout the nutlet **3. *C. austroafricanum***
- Leaves grey green on the abaxial surface, dark green on the adaxial surface, spatulate-obtuse shaped; corolla white; marginal glochidia longer than acentric glochidia..... **8. *C. spelaenum***

1. *Cynoglossum alticola* Hilliard & B.L.Burt, Notes Roy. Bot. Gard. Edinburgh 43(3): 343 (1986).

Type. South Africa ♀♂ Eastern Cape, Barkly East District (3027): Ben McDhui (-DB), 5 Feb 1983, *O.M. Hilliard and B.L. Burt 16468* (E-image!, holotype; NU-image!, isotype).

Perennial herbs, 0.2–0.6 m in height. Basal leaves 76–270×8–18 mm, lanceolate, densely pubescent, persistent; margins entire. Stem leaves 35–120×5–10 mm, lanceolate, apex acute, base cuneate, margins entire, soft woolly hairs. Trichomes spread equally on both the adaxial and abaxial leaf surfaces, unicellular hair base, not bulbous on both leaf surfaces. Inflorescence racemose, clustered at the apex; pedicel 4–10 mm long, lengthening considerably in fruit. Calyx ca. 4 mm long, lobes elliptic-oblong, densely hairy on inner surface, apices obtuse. Corolla deep blue; lobe 4×3 mm diameter, oblong, round apex. Nutlets convex, 6–9×5–6 mm; glochidia short and thick at the base, densely packed on nutlet, tips multi-angular (Figure 3).

Phenology. February to May.

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. *Cynoglossum alticola* can be distinguished by its thick, convex nutlets. Among the southern African species, it has a unique appearance due to

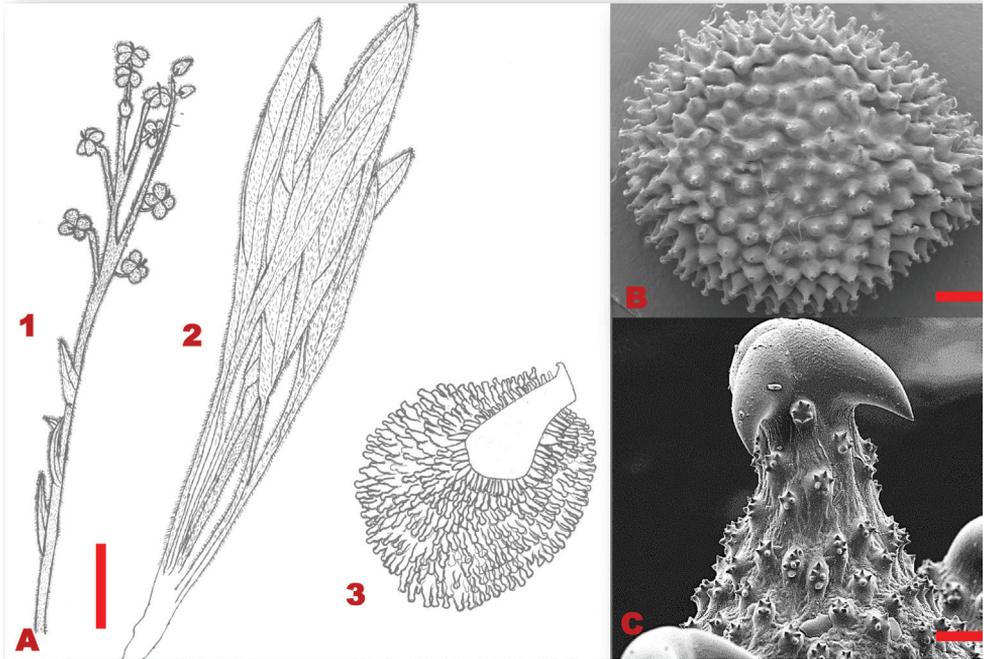


Figure 3. Vegetative and reproductive morphology of *Cynoglossum alticola* **A** line drawings of: 1. a branch showing fruiting arrangement at the apex of the stem and stem leaves; 2. long petiolate basal leaves; 3. adaxial surface of nutlet **B** SEM micrograph showing trichomes on the leaves **C** SEM micrograph showing thick and shorter glochidia on the nutlet. Voucher: *L.C.C. Liebenberg 5789* (PRE). Drawing scale bar: 8 mm. SEM images scale bars: 50 μ m (**B**); 50 μ m (**C**).

the presence of woolly trichomes that cover the whole plant. Furthermore, it has larger nutlets (6–9×5–6 mm) than other species (less than 6×5 mm). According to Hilliard and Burtt (1986), this species is related to *C. alpinum* (Brand) B.L. Burtt from the highlands of Ethiopia, with which it shares nutlet shape and size, as well as the leaf texture and colour. The difference is observed on the fornications (small crests in the corolla tube of a plant), as in *C. alticola* they are broad and short while in *C. alpinum* they are long and narrow.

Distribution and habitat. The species is restricted to the Eastern Cape Province in South Africa and Lesotho (Figure 4), where it is found growing on mountainous terrain and on damp slopes near streams.

Additional specimens examined. South Africa. Eastern Cape: 3028 (Mata-tiele): Drakensberg, near Barkly East (-CA), 19 Dec 1982, *P.B. Phillipson 705* (PRE); between Malpas and Nek (-CA), 13 Dec 1995, *T. Dold and M. Cocks 2058* (GRA).

Lesotho. 2929 (Underberg): Mokhotlong District (-AC), Jan 1953, *L.C.C. Liebenberg 5789* (PRE); 28 Feb 1947, *A. Jacot Guillarmod 997* (PRE).

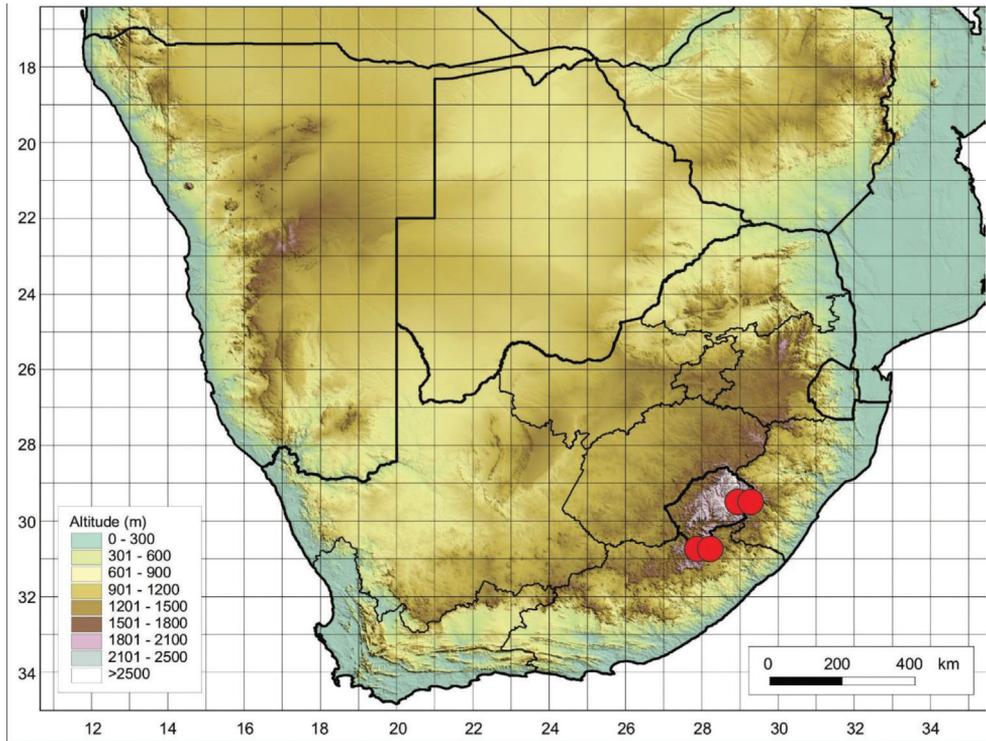


Figure 4. Distribution of *Cynoglossum alticola* based on the specimens examined.

2. *Cynoglossum amabile* Stapf & J.R.Drumm. in Bull. Misc. Inform., Roy. Bot. Gard., Kew 6:202 (1906).

Type. China ♀♂ Yunnan, Mengtze, 1894, *W. Hancock 133* (K-image! [3 sheets], lectotype, designated by Verdcourt 1991).

Perennial herb, 0.6 m in height. Basal leaves 50–100×20–35 mm, lanceolate-elliptic shaped, softly hairy, deciduous, margins entire. Stem leaves 40–100×9–20 mm, lanceolate shaped, apex acute, base cuneate, entire margins, densely covered with white brittle hairs. Trichomes soft, upright, bulbous based. Inflorescence clustered at the apex, pedicels 5–8 mm long, lengthens considerably in fruit. Calyx ca. 3 mm long, lobes ovate, grey pubescent, apex subacute. Corolla bluish purple; lobe 7×9 mm diameter, segments round. Nutlets ovoid, 2–4×3–4 mm, convex shaped; glochidia short, thick, marginal glochidia are more distinct than the central glochidia (Figure 5).

Phenology. October to November.

Conservation status. Not evaluated (Raimondo et al. 2009).

Diagnostic characters. Amongst the southern African species, *C. amabile* can be confused with *C. lanceolatum* due to their small-sized nutlets (between 2–4×2.5–4) and flowers. However, the two species are easily distinguished by their flower colour (*C.*



Figure 5. *Cynoglossum amabile*. SEM micrograph of the adaxial surface of **A** fruit nutlets **B** Glochidia. Voucher specimen: *J. Stewart 2021* (NU). SEM images scale bar: 2 mm (**A**); 100 µm (**B**).

lanceolatum has white corolla with blue throat, whereas *C. amabile* has bluish purple corolla). This species was also reported by Stapf and Drummond (1906) and König *et al.* (2015) to be like *C. furcatum* Wallich (from Nepal, China, Bhutan, Vietnam, Thailand, Philippines, and India), based on flower and fruit size. The difference can be observed in the inflorescences, whereby *C. furcatum* is a much larger plant with inflorescences up to 1 m tall, and *C. amabile* is up to 0.6 m tall.

Distribution and habitat. *Cynoglossum amabile* is widely distributed in southern China where it is usually grown for ornamental purposes and naturalised in many parts of the world (Xu *et al.* 2009). According to Germishuizen and Meyer (2003), this species is only found in KwaZulu-Natal Province (Figure 6), where it grows in open, disturbed sites, on gravel slopes and sandy, dry riverbanks.

Additional specimens examined. South Africa. KwaZulu-Natal: 2930 (Pietermaritzburg): Richmond District, Byrne Village (-CD), 23 Nov 1977, *J. Stewart 2021* (NU).

Taxonomic notes. *Cynoglossum amabile* has been described as a widespread species which grows in disturbed habitat and can be grown as an ornamental (König *et al.* 2015).

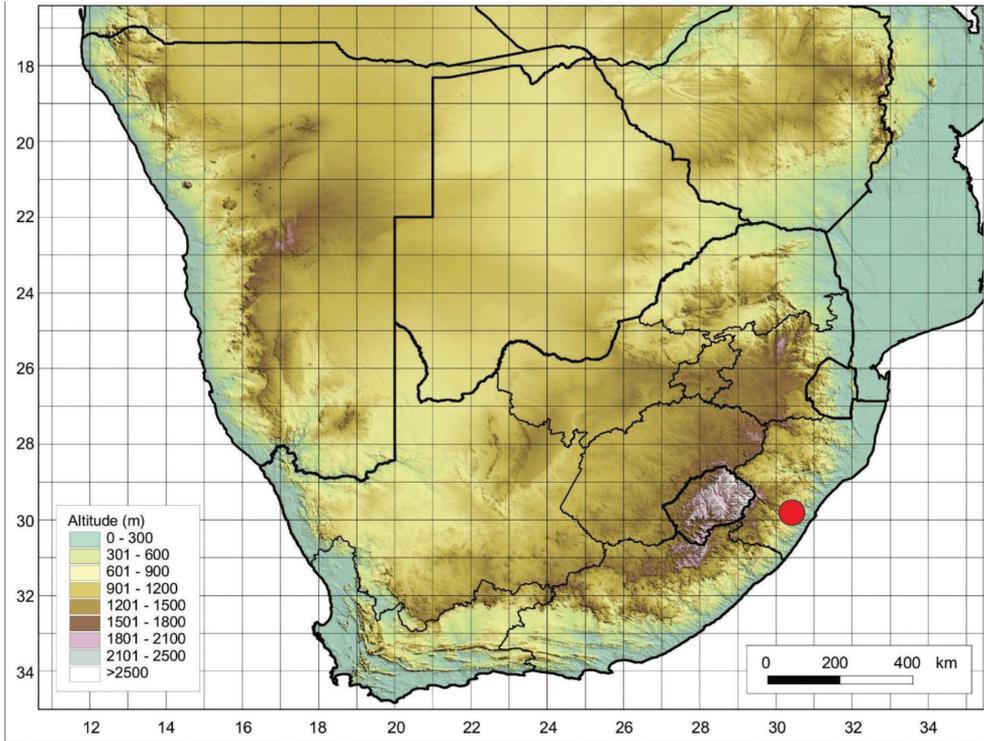


Figure 6. Recorded distribution of *Cynoglossum amabile* in southern Africa based on the specimens examined.

This species has only been collected once in South Africa by J. Stewart in 1977, since then there have been no later records or observations of this species in this region. Attempts to locate this species in the wild were futile. It is questionable whether this species occurs naturally in the southern African region since its single known locality is within a protected area in KwaZulu-Natal.

3. *Cynoglossum austroafricanum* Weim. ex Hilliard & B.L. Burtt in Notes Roy. Bot. Gard., Edinburgh 43(3):347 (1986).

Cynoglossum austroafricanum Weim., Jacot Guillarmod, Fl. Lesotho: 233 (1971); Gibbs Russell *et al.*, in Mem. Bot. Surv. S. Afr. 48:109 (1984), nom. nud.

Type. South Africa ♀♂, KwaZulu-Natal, Underberg (2929): Cobham Forest Reserve, Sipongweni, c.6500ft (-CB), 21 Feb 1981, *O.M. Hilliard and B.L. Burtt 14072* (E-image! [2 sheets], holotype; K-image!; NU-image! [3 sheets], isotype).

Perennial or biennial herbs, 0.3–0.5 m in height. Basal leaves 100–190×15–30 mm, lanceolate-obtuse, softly hairy, persistent margins entire. Stem leaves 45–100×10–21 mm, narrowly lanceolate to linear lanceolate shaped, acute apex,

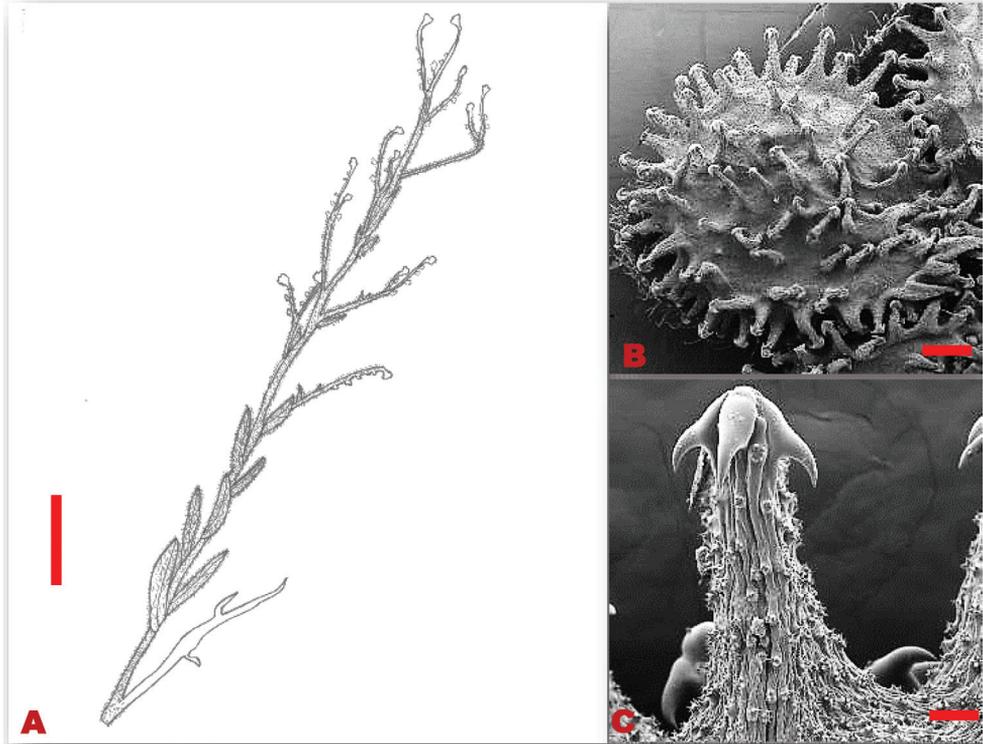


Figure 7. Vegetative and reproductive morphological features of *Cynoglossum austroafricanum* **A** line drawing of the branching pattern of the fruit stalk, and the alternating stem leaves **B** SEM micrograph of the adaxial surface of a fruit nutlet, with the arrangement of glochidia around the nutlet **C** SEM micrograph of the glochidia. Voucher specimen: *O.M. Hilliard* and *B.L. Burt* 11803 (PRE). Drawing scale bar: 7.5 mm. SEM scale bar: 1 mm (**B**); 100 μ m (**C**).

cuneate base, margins undulate, covered with stiff hairs. Trichomes unicellular, with thick round base on the adaxial surface, simple on the abaxial surface. Inflorescences dichotomously branched, loose cymes at the apex, pedicels 4–9 mm long, and lengthens considerably in fruit. Calyx ca. 2–3 mm long, lobes obtuse, pubescent on the outer surface, glabrous on the inner surface, apex acute. Corolla pale blue; lobes 2.75–4.25 mm in diameter, cruciform, apex obtuse. Nutlets ovoid, 3.0–3.5 \times 2.5–3.0 mm; glochidia more spread towards the margins, thin, tip multi-angular (Figure 7).

Phenology. December to April.

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. Amongst the southern African species, *Cynoglossum austroafricanum* can be confused with either *C. lanceolatum* or *C. coeruleum* var. *mannii*. This species differs from the two by the colour of the corolla (white corolla with pale blue throat vs pale blue corolla throughout in *C. austroafricanum*). This latter observation was also noted by Hilliard and Burt (1986).

Distribution and habitat. The species is distributed in South Africa (North-West, Gauteng, Mpumalanga, Free-State, KwaZulu-Natal, and Eastern Cape Provinces), eSwatini and Lesotho (Figure 8), where it occurs in shady, disturbed areas, and sandy, dry riverbanks.

Additional specimens examined. South Africa. LIMPOPO: 2430 (Tzaneen): Lekgalameetse Nature Reserve (-AA), 7 Oct 1986, *N. Stalmans 1404* (PRE). NORTH-WEST: 2626 (Lichtenburg): Lichtenburg (-AA), Feb 1918, *L. Kretzshmar 17065* (PRE). GAUTENG: 2528 (Pretoria): Brooklyn (-CA), 1 Feb 1931, *A.O.D. Mogg 16451* (PRE). MPUMALANGA: 2430 (Pilgrim's rest): Pilgrim's rest, next to the old railway line (DD), 31 Jan 2019, *A.N. Moteetee* and *L.K. Madika AL06* (JRAU). 2530 (Mashishing): Mashishing (-AB), Apr 1910, *M. Crosby 1989* (PRE); Mashishing District (-CA), 8 Feb 1904, *J. Burt-Davy 1472* (PRE). 2630 (Carolina): Between Oshoek border post and Carolina (-BA), Jan 1906, *H. Bolus 12161* (PRE). FREE STATE: 2828 (Bethlehem): Golden Gate (-AB), 22 Jan 1951, *A. Wiezer 22485* (NBG). (Fouriesburg): Bethlehem (-CA), 8 Jan 1918, *G. Potts 3246* (PRE); Witsieshoek (-DB), 28 Feb 1975, *O.M. Hilliard* and *B.L. Burt 8665* (NU). KWAZULU-NATAL: 2730 (Vryheid): Oshoek District, Wakkerstroom (-AC), 18 Jan 1961, *N.J. Devenish 480* (PRE); Amajoba District Municipality area, Luiperdkloof farm, Natural Heritage site no.47 (-AD), 25

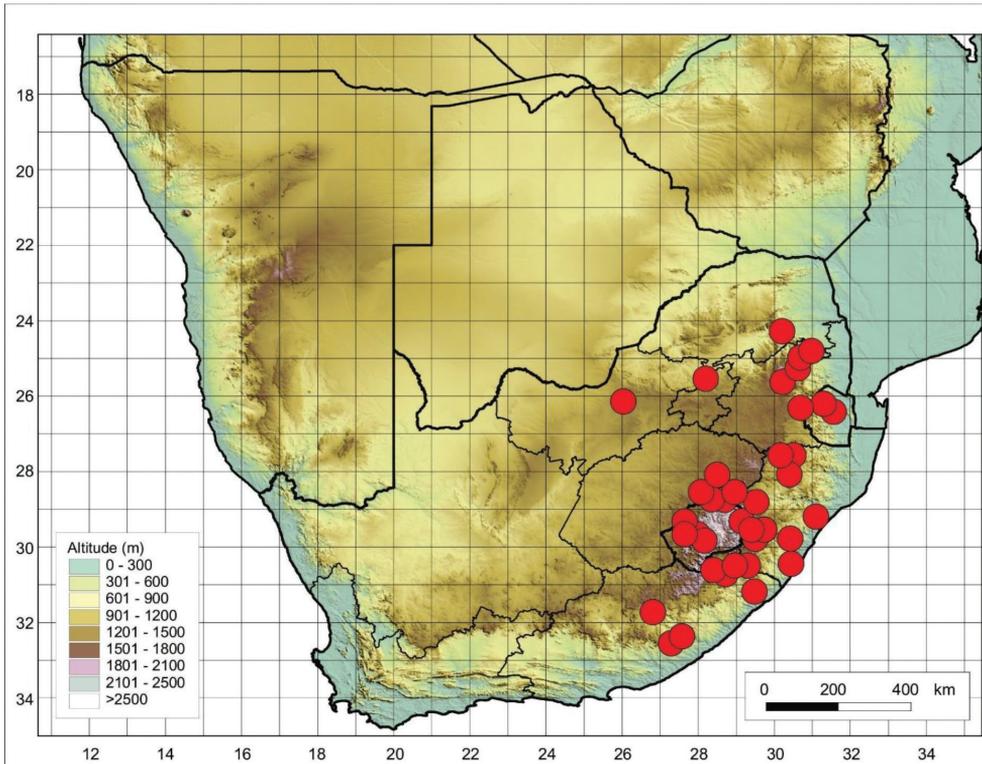


Figure 8. Known distribution of *Cynoglossum austroafricanum* in southern Africa based on the specimens examined.

Jan 2011, *A.M. Ngwenya 3601* (NH). 2829 (Harrismith): Cathedral Peak (-CC), 18 Feb 1983, *O.M. Hilliard and B.L. Burtt 16298* (NU); 12 Jan 1984, *J. Scott 76* (NH). 2830 (Dundee): Hattingspruit Station (-AA), Dec 1929, *D. Johnston 268* (NU). 2929 (Underberg): Mpendhle District, upper Loteni Valley (-AD), 5 Feb 1985, *O.M. Hilliard and B.L. Burtt 18103* (NU, PRE); Loteni Nature Reserve (-BC), 24 Dec 1978, *O.M. Hilliard and B.L. Burtt 11803* (PRE); Polela River (-CB), 21 Apr 1973, *M.A. Rennie 379* (NU); 23 Mar 1977, *O.M. Hilliard and B.L. Burtt 9793* (NU); 17 Feb 1982, *O.M. Hilliard and B.L. Burtt 15520* (NU). 2930 (Pietermaritzburg): Gate farm Keerom-Cottingham (-CC), 23 Mar 1969, *R.G. Strey 8420* (NH). 2931 (Stanger): Tugela valley (-AA), 14 Feb 1926, *Bayer 47* (NU). 3029 (Kokstad): Hillside (-CA), Jan 1956, *P. Thompson 2* (NU); on banks of streams near Kokstad (-CB), Dec 1883, *W. Tyson 1839* (NBG), 17 Jan 1957, *L.E. Taylor 5473* (NBG). 3030 (Dumisa): Port Shepstone (-AD), 22 Oct 1997, *J. Arkell 353* (NH). EASTERN CAPE: 3128 (Umtata): Transkei, on the summit of Baziya Mt. (-AD), 23 February 1988, *T. Strever 917* (PRE); Walter Sisulu University, Area 3: East of In-Service centre (-DB), 14 Feb 2001, *N. Nombekela 102* (NH). 3126 (Queenstown): Hangklip (-DD), Feb 1960, *H. Koepowitz 13147* (GRA). 3129 (Port St. John's): Ntabankulu Mountain, Gome Forest Station, Tabankulu (-AB), 11 Nov 1996, *T. Dold, E. Cloete and R. White 2940* (GRA). 3227 (Stutterheim): Fort Cunynghame Station (-AD), Nov 1894, *T.R. Sim 1860* (NU); no date 1897, *T.R. Sim 20420* (NU).

eSwatini. 2631 (Mbabane): Forbes Reef (-AA), 14 Apr 1960, *R.H. Compton 30035* (NBG); Mbabane (-AC), 17 Jan 1951, *A. Wuze 22393* (NBG).

Lesotho. 2828 (Bethlehem): Leribe District, LHDA Phase 1A (-AD), 11 Jan 1996, *P.B. Phillipson, C. Mokuku, R. Judd, and C. Hobson 4473* (GRA); Leribe (-AD), no date, *M. Dieterlen 70* (NBG; NH). 2927 (Maseru): Thaba Bosiu (-BC), 1 Mar 1978, *M. Schmitz 8205* (PRE); Mahlatsa (-BB), 18 Jan 1941, *A. JacotGuillarmod 51* (GRA); Sefikeng Ha Fako (-BD), Oct 2018, *A.N. Moteetee 56* (JRAU); 30 Dec 2018, *A.N. Moteetee 59* (JRAU). 2929 (Underberg): Mokhotlong (-AC), Mar 1949, *A. Jacot Guillarmod 1072* (PRE), 25 Feb 1949, *W.J. Barker 21515* (NBG).

No locality details: 27 Nov 1888, *H. Medley 4576* (NH); Dec 1946, *E. Mes-ton 50* (NU).

Taxonomic notes. According to Hilliard and Burtt (1986), this species was described by Dr. H. Weimarck who did not select a type specimen; therefore, in typifying it they retained the specific epithet.

4. *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii* (Baker & C.H. Wright) Verdc. in Fl. Trop. E. Africa, Boraginac.: 110 (1991).

Cynoglossum mannii Baker & C.H. Wright, in Oliver et al., Fl. Trop. Afr., 4(2.1): 52 (1905) ♀♂, Type as above.

Cynoglossum geometricum Baker & C.H. Wright, in Oliver et al., Fl. Trop. Afr. 4(2.1): 52 (1905). Type: Nyasaland [Malawi] ♀♂ Mount Chiradzulu, no date, *A. Whyte*

s.n. (K-image! lectotype, here designated). Notes: The specimen was selected as a lectotype by B.L. Burtt on the sheet but was never designated formally.

Paracynoglossum geometricum (Baker & C.H. Wright) R.R. Mill. in Notes Royal. Bot. Gard., Edinb. 41 (3): 478 (1984). ♀♂, Type: Same as above.

Cynoglossum coeruleum subsp. *geometricum* (Baker & C.H. Wright) S. Edwards in Fl. Ethiopia & Eritrea 5: 93 (2006), nom. inval.

Type. Cameroon ♀♂, Mount Cameroon, Dec 1862, *Mann 2005* (K-image, lectotype! here designated; K-image [2 sheets] isolectotype!).

Perennial, biennial, or annual herbs, 1.2 m in height. Basal leaves 90–190×28–56 mm, lanceolate-obtuse, softly hairy, deciduous, margins entire. Stem leaves 35–90×7–25 mm, lanceolate, apex acute, base acute to obtuse, covered with moderately stiff hairs margins entire. Trichomes bulbous based on the upper surface of the leaf, sometimes simple on the lower surface. Inflorescences terminal axillary cymes, few branches spreading dichotomously; pedicel 4–8 mm long, lengthens considerably in fruit. Calyx ca. 21 mm long, lobes ovate-oblong, adpressed-hairy outside, smooth inside, apex acute. Corolla white with pale blue throat; lobes ca. 2.1 mm in diameter, campanulate. Nutlets ovoid, 3–4×2.5–3.5 mm; glochidia more marginal and on the median line (Figure 9).

Phenology. December to April.

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. This variety can be easily confused with *C. lanceolatum* due to the dichotomous branching of the inflorescence but can be distinguished from it by the distribution and density of the glochidia in the nutlets. The glochidia on the nutlets of *Cynoglossum coeruleum* var. *mannii* are more marginal and on the median line, whereas they are equally distributed around the whole nutlet in *C. lanceolatum*.

Distribution and habitat. This variety is endemic to South Africa where it is known only from KwaZulu-Natal and Eastern Cape Provinces (Figure 10). It is also reported from Malawi, Mozambique, Zambia, and Zimbabwe (Mill and Miller 1984). It is found in disturbed grassland and sandy areas.

Additional specimens examined. South Africa. KWAZULU-NATAL: 2731 (Louwsburg): Itala Nature Reserve (-AD), 10 Dec 1987, *M. Jordaan 7064* (NH); 9 Dec 1987, *A.G. Hutchings 2530* (NU); Zululand District Municipality Area, Abaqulusi Municipality Area, Tygerskloof Farm (-CD), 24 Jan 2012, *A.M. Ngwenya* and *D.G.A. Styles 4034* (NH); 7 Mar 2019, *A.N. Moteetee* and *L.K. Madika AL010* (JRAU). 2828 (Bethlehem): Royal Natal National Park (-DB), 17 February 1984, *O.M. Hilliard* and *B.L. Burtt 17658* (NBG; NU). 2831 (Nkandla): Nhlazatshe farm, (-AA), 4 Mar 1994, *T.B. Sikhakhane 440* (NH). 2929 (Underberg): Mpendhle District, Loteni Nature Reserve (-AD), 1 Feb 2001, *A.M. Ngwenya 1940* (NH), Loteni, upper reaches of river (-BC), 31 Mar 1984, *O.M. Hilliard 8218* (NU, PRE), Sipongweni Mountain (-CD), 20 Mar 1987, *O.M. Hilliard* and *B.L. Burtt 8249* (PRE). 2930 (Pietermaritzburg): On the Phezulu Game Estate, Botha's Hill (-DC), 22 Jan 2005, *D. Styles 2280* (NH). 3030 (Port Shepstone): M. Stainbank's farm, mid Illovo (-BB), 23 Dec 2008, *A. Young 942* (NU). EASTERN CAPE: 3023 (Britstown): Kamberg (-CC), 21 Mar 1983, *O.M. Hilliard*

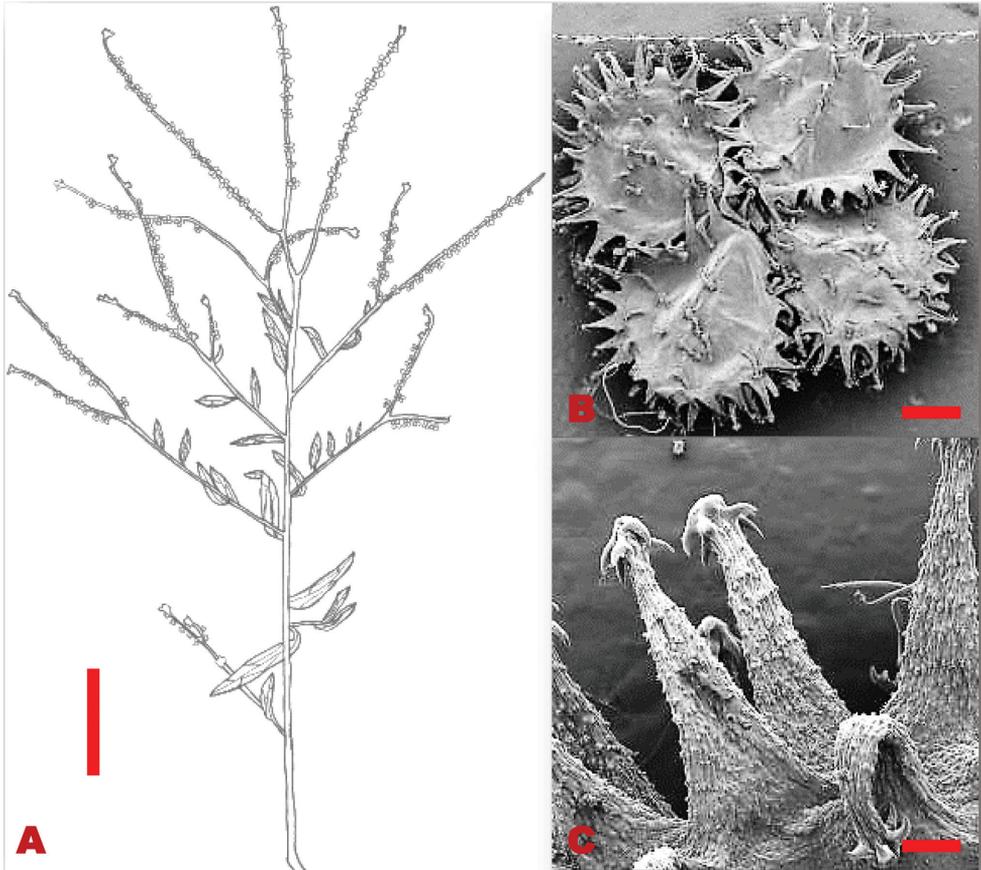


Figure 9. Vegetative and reproductive morphological features of *Cynoglossum coeruleum* var. *mannii* **A** line drawing of dichotomous branching of the fruit stalk **B** marginal and median line glochidia on the adaxial surface of a nutlet **C** Glochidia evenly sized. Voucher specimen: *T.B. Sikhakhane 440* (NH). Drawing scale bar: 7.5 mm. SEM scale bar: 2 mm (**B**); 200 µm (**C**).

8208 (NU). 3128 (Umtata): Baziya Mission (-CB), 12 Feb 1981, *O.M. Hilliard* and *B.L. Burt* 13952 (NU); Nenga River, (-DB), 26 Oct 2001, *E. Cloete* 6342 (GRA). 3226 (Fort Beaufort): Hogsback (-DB), Apr 1956, *R. Collett* 9775 (GRA); Apr 1955, *A.R.H. Martin* 9678 (GRA); Apr 1962, *A. Jacot Guillarmod* 5544 (GRA); 12 Apr 1955, *L.M. Johnson* 1152 (GRA); 4 Mar 1973, *M. Bradley* 55 (GRA).

Taxonomic notes. (i) *Cynoglossum geometricum* was recorded in the southern African region (FSA) for the first time by Hilliard and Burt (1986). Although the name seems to be accepted in the Flora of southern African region (FSA), for example, Germishuizen and Meyer (2003) and Burrows and Willis (2005), it is not the correct name for this taxon. The correct name is *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii*, as synonymized by Verdcourt (1991). It is worth noting that the latter name is erroneously listed as *C. coeruleum* var. *mannii* in websites such as Plants of the World Online (<http://powo.sci->

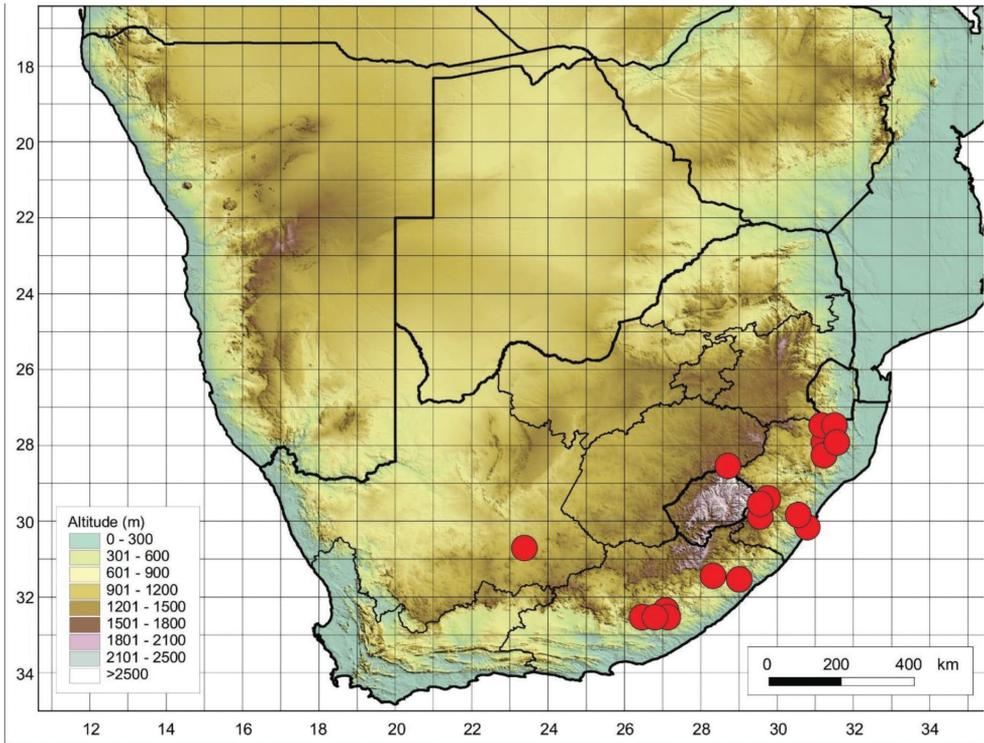


Figure 10. Known distribution of *Cynoglossum coeruleum* var. *mannii* in southern Africa based on the specimens examined.

ence.kew.org/taxon/966730-1) and the World Flora Online (<http://www.worldfloraonline.org/>). Verdcourt (1991) relegated *C. johnstonii* to subspecies level under *C. coeruleum* and then transferred *C. mannii* to *C. coeruleum* as a variety of *Cynoglossum coeruleum* subsp. *johnstonii*. (ii) There are three sheets of *Mann 2005* in Royal Botanic Gardens, Kew (K), the one with the barcode number K000418935 is chosen as a lectotype because it displays most of the important characters of the species which can be used to distinguish this species from the rest, such as the inflorescence character and the branching pattern.

5. *Cynoglossum hispidum* Thunb., Prodr. Plant. Cap. 1:34 (1794); Roemer and Schultes in Syst. Veg., ed. 15 bis 4: 79,761 (1819); C.H. Wright in F.C. 4(2):14(1904); Brand in Engl. Pflanzenr. 78 [4,252]:146 (1921).

Cynoglossum glomeratum Pursh in Fl. Amer. Sept. 2:729 (1813).

Type. United States of America ♀♂, Louisiana, no date, *Bradbury* s.n. (PH-image! holotype). *Cynoglossum enerve* Turcz. Bull. Soc. Imp. Naturalistes Moscou 1:259 (1840), E.Mey. ex DC., Prodr.10:154 (1846).

Type. South Africa. ♀♂, Eastern Cape, between Omcamwubo and Omcamcaba, no date, *Drège d* (HAL-image! lectotype, here designated; GDC-image! [3 sheets],

isolectotype). [Note: The HAL specimen is chosen as a lectotype because the specimen displays the diagnostic characters of the species as described in the protologue]. *Echinosperrum enerve* E.Mey. ex DC. Prodr. 10:154 (1846), nom. nud.

Type. South Africa ♀♂, Precise locality unknown, Lange Kloof, *Thunberg 168* sub THUNB-UPS 3996 (UPS, microfiche! holotype).

Perennial or biennial herbs, 0.5–0.76 m in height. Basal leaves 80–250×15–25 mm, lanceolate-obtuse, densely pubescent, deciduous, margins entire. Stem leaves 60–80×5–12 mm, oblong-lanceolate, apex acute, base cuneate, covered with brittle hairs, margins entire. Trichomes bulbous based on the upper surface of the leaf, sometimes simple on the lower surface. Inflorescence terminal axillary cyme, branches spreading dichotomously; pedicel up to 20 mm long, lengthens considerably in fruit. Calyx ca. 5–10 mm long, lobes obtuse, outer surface packed with bulbous-based trichomes, apex acute. Corolla magenta; lobes ca. 5 mm in diameter, cruciform. Nutlets convex, 5–6×3–5 mm, highly pubescent; glochidia short and thick at the base, tips multangular (Figure 11).

Phenology. October to March.

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. The species can be confused with *C. lanceolatum* with which it shares a similar branching pattern of the inflorescences and upright brittle hairs covering the whole plant. However, the two differ in the colour of the corolla (magenta-purplish vs. white with blue throat in *C. lanceolatum*) and pedicel length (2 cm long opposed to less than 2 cm long in *C. lanceolatum*).

Distribution and habitat. This species is widely distributed across all provinces of South Africa. It can also be found in eSwatini and Lesotho (Figure 12). It mostly occurs in open grasslands, grassy slopes, woodland marshes, and disturbed areas like abandoned lands.

Additional specimens examined. South Africa. LIMPOPO: 2229 (Waterpoort): Evelyn valley (-BD), Feb 1944, *RUC Biology Expedition 417* (GRA). 2430 (Tzaneen): Lekgalameetse Nature Reserve (-AA), 4 Dec 1985, *M. Stalmans 790* (PRE); Sekhukhune District, Leolo Mountains (-CA), 14 Mar 2007, *B. Sachse 471* (PRE). NORTH-WEST: 2523 (Pomfret): Forbes (-DA), 29 Oct 1959, *B. Dlamini s.n.* (NH). 2627 (Potchefstroom): Goedgedacht (-AA), 1 May 1932, *J.D. Sutton 676* (PRE); Losberg, Elandsfontein (-BC), 11 Dec 1934, *J.J. Theron 725* (NH); Potchefstroom (-CA), 23 Nov 1946, *W.J. Louw 1525* (PRE). GAUTENG: 2528 (Pretoria): Brooklyn (-CA), 16 Sep 1928, *A.O.D. Mogg 15247* (PRE); 6 Apr 2019, *A.N. Moteetee* and *L.K. Madika AL014* (JRAU); 1931, *A.O.D. Mogg 16601* (PRE); 30 Sep 1943, *A.O.D. Mogg 17001* (PRE); Doornpoort/ Hartbeesfontein (-CB), 24 Jan 2004, *S.P. Bester 4656* (PRE); Irene District (-CC), Oct 1929, *A.A. Obermeyer 27651* (PRE). 2628 (Johannesburg): Vereeniging District (-AC), 23 Nov 2007, *S.P. Bester 8268* (PRE); Suikerbosrand Nature Reserve (-AD), 7 Apr 1970, *A. Lambrechts 265* (PRE). MPUMALANGA: 2530 (Mashishing): Boschhoek (-AA), 16 Nov 1933, *R.S.N. Young A375* (PRE); Verlorenkloof Reserve, Welgedacht (-AD), 28 Nov 2008, *S.P. Bester 8679A* (PRE); Machado-dorp, Farm Grootvlei (-CB), 21 Oct 1988, *P. Burgoyne 457* (PRE); Songimvelo Game

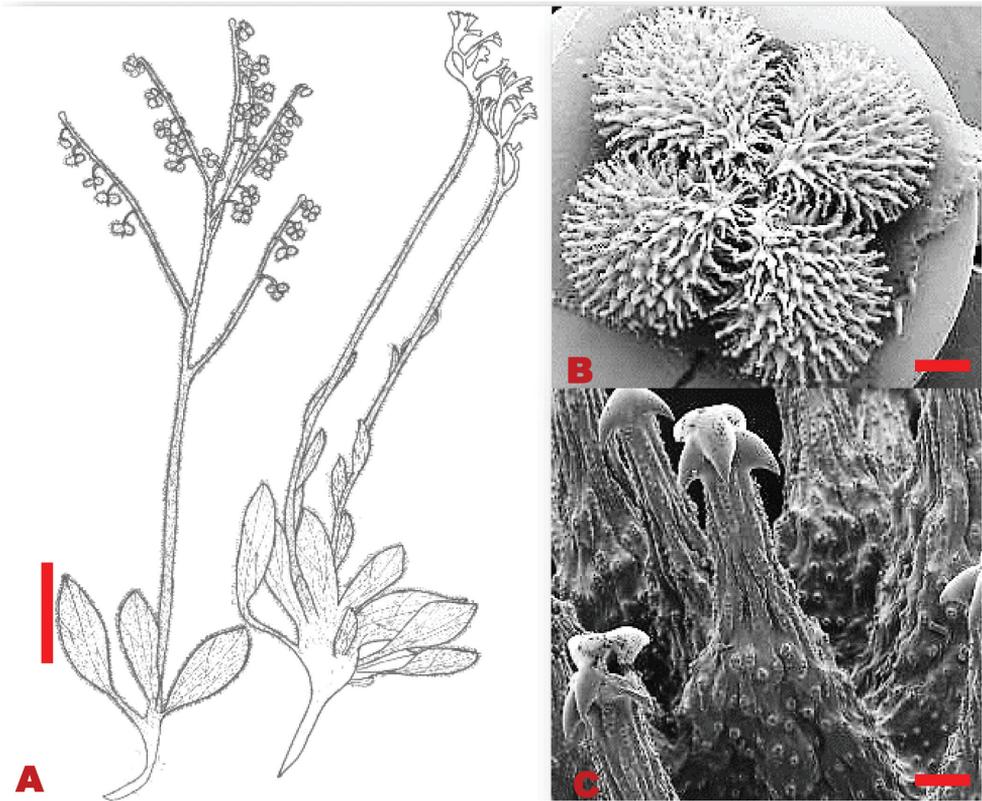


Figure 11. Vegetative and reproductive morphological features of *Cynoglossum hispidum* **A** line drawing of obtuse shaped, rosette base leaves, terminal branched fruits, terminal flowers **B** densely packed with glochidia nutlets **C** Glochidia wide at the base, with multiangular tip. Voucher specimen: *S.P. Bester 12958* (PRE). Drawing scale bar: 7.5 mm. SEM scale bar: 5 mm; 100 μ m (**C**).

Reserve (-DD), 10 Dec 1992, *M. Jordaan 2490* (PRE). 2629 (Bethal): Bethal (-AD), 14 Dec 2010, *R. Leendertz 9386* (PRE). 2630 (Carolina): Ermelo District, Spitskop (-CA), Dec 1915, *R. Potts 5007* (PRE). NORTHERN CAPE: 2820 (Kakamas): Krantzkop (-DA), Nov 1911, *J. Thode 4768* (NBG). FREE STATE. 2728 (Frankfort): Farm Reitfontein (-BC), 28 Jan 1983, *E. Retief 1071* (PRE). 2828 (Bethlehem): Qwaqwa National Park (-BC), 22 Nov 1994, *P.C. Zietsman 2558* (NH); Golden Gate National Park (-DA), 12 Dec 1988, *Gertenbach and Groenewald 8929* (PRE); Witsieshoek (-DB), 10 Mar 2015, *S. Parbhoo 81* (NH). 2829 (Harrismth): Harrismith (-AC), 17 Mar 1981, *M.L. Jacobsz 3092* (PRE); 18 Nov 1978, *M.L. Jacobsz 1299* (PRE); Rensburgskop (-AD), 10 Dec 1962, *M.L. Jacobsz 199* (NBG; PRE). KWAZULU-NATAL: 2730 (Vryheid): Oshoek District, Wakkerstroom (-AD), 19 Nov 1962, *N.J. Devenish 954* (PRE); Klipspruit Dam (-DD), 10 Feb 2005, *S.P. Bester 6540* (NH, PRE). 2731 (Louwsburg): Zululand District municipality (-CD), 29 Oct 2000, *T. Edwards and C. Potgieter s.n.* (NU); 26 Jan 2012, *A.M. Ngwenya and D.G.A. Styles 4085* (NH). 2732 (Ubombo): Ingwavuma

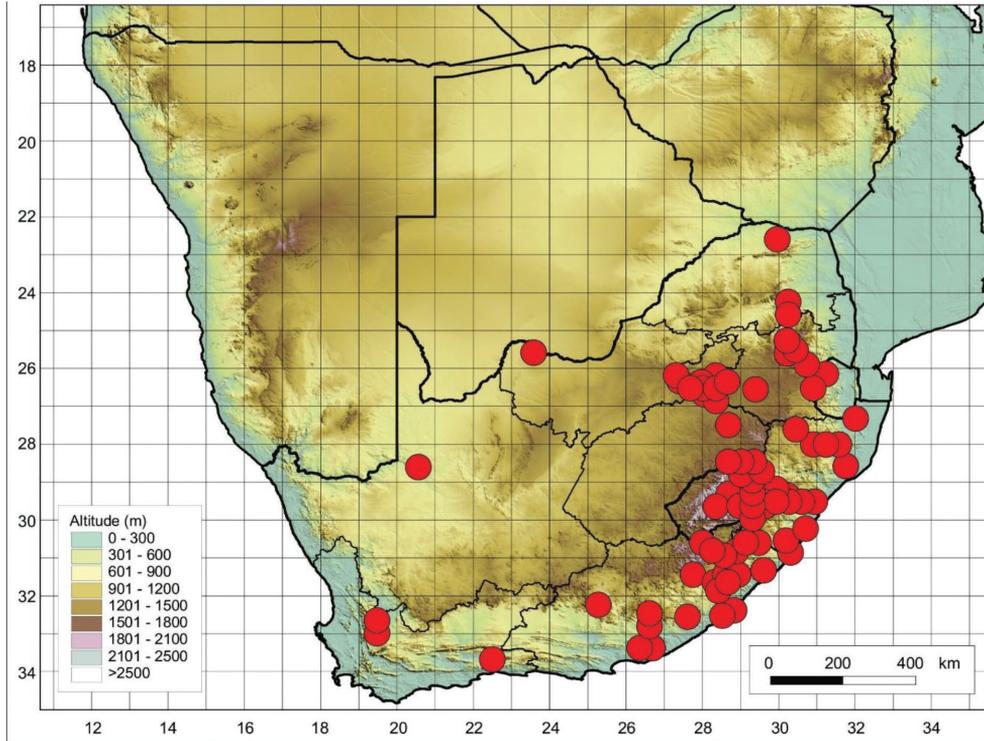


Figure 12. Known distribution of *Cynoglossum hispidum* in southern Africa based on the specimens examined.

(-AA), 22 Nov 1969, *E.J. Moll* 4680 (NH, PRE). 2829 (Harrismith): Cathedral Peak (-CC), 10 Nov 1956, *D.J.B. Killick* 1115 (PRE); 13 Oct 1984, *J. Scott* 251 (NH). 2830 (Dundee): Dundee District (-AA), 26 Nov 1964, *N.E. Shirley s.n.* (NU); Klipriver District, Elandsplaagte (-CD), 23 Oct 1964, *N.E. Shirley s.n.* (NU). 2831 (Nkandla): Empangeni (-DB), 9 Jul 1965, *H.J.T. Venter* 1917 (PRE). 2929 (Underberg): Giants Castle Game Reserve (-AB), 8 Feb 1966, *W.R. Trausel* 575 (PRE); (-AD), 8 Nov 2001, *T.R. Green* 1217 (NU); Mpendhle District (-BC), 5 Jan 1983, *O.M. Hilliard* and *B.L. Burt* 16218 (PRE); Underberg District (-CB), 3 Feb 1975, *O.M. Hilliard* and *B.L. Burt* 7942 (NU); 3 Feb 1976, *O.M. Hilliard* and *B.L. Burt* 8905 (NU); 24 Mar 1977, *O.M. Hilliard* and *B.L. Burt* 9818 (NU); 11 Jan 1978, *M.A. Rennie* 911 (NU); 27 Jan 1982, *M.A. Rennie* 1307 (NU); 13 Feb 1983, *O.M. Hilliard* and *B.L. Burt* 17234 (NU); 6 Jan 1984, *O.M. Hilliard* and *B.L. Burt* 17290 (PRE); Underberg District (-CC), 19 Jan 1984, *O.M. Hilliard* and *B.L. Burt* 17346 (NU); 9 Jan 1986, *O.M. Hilliard* and *B.L. Burt* 18998 (NU); Hlogoma Mountain (-DC), 30 Nov 2015, *S.M. Berruti* 513 (NH); Donnybrook (-DD), 7 Nov 2013, *D.G.A. Styles* 4579 (NH); 8 Mar 2019, *A.N. Moteete* and *L.K. Madika* AL011 (JRAU). 2930 (Pietermaritzburg): Lidgetton (-AC), 23 Mar 1920, *A.O.D. Mogg* 6894 (PRE); 29 Sep 1964, *E.J. Moll* 1036 (NU, PRE); 13 Jan 1988, *B. Grove* 98 (NU); Umgeni River (-CA), 20 Oct 1984, *J. Manning* 538

(NU); 8 Mar 2019, *A.N. Moteetee* and *L.K. Madika AL013* (JRAU); Ukulinga farm (-CB), 8 Mar 1982, *J.C. Manning 212* (NU); Nagle Dam (-DA), 15 Sep 1957, *M.J. Wells 1676* (NU); Cliffdale road (-DC), 17 Aug 2002, *D.G.A. Styles 9141* (NU); 30 Jul 2003, *P. Wragg 205* (NU), 7 Nov 2014, *D.G.A. Styles 4925* (NH). 2931 (Stanger): Groutville (-AD), 14 Oct 1965, *E.J. Moll 2500* (NU, PRE). 3029 (Kokstad): Insizwa (-CA), 24 Feb 1972, *R.G. Strey 10827* (NU); Kokstad District (-DA), 1968, *C.J. Piek 53* (NH); 25 Feb 1978, *T.A. Coleman 985* (NH); no date, *F.M. Getliffe* and *T. Edwards 1266* (NU). 3030 (Port Shepstone): M. Stainbank's farm, mid Illovo (-BB), 24 Sep 2009, *A. Young 1155* (NU); Umzinto District (-BC), 3 Sep 1983, *K. Balkwill* and *J.C. Manning 828* (NU); 20 Oct 1997, *A.M. Ngwenya 1538* (NH); Port Shepstone (-CB), 3 Oct 1937, *A.O.D. Mogg 13873* (PRE); (-CD), 20 Sep 1955, *S. McNeil 142* (NU). 3030 (Umzinto): Vernon Crookes Nature Reserve (-BC), 3 Sep 1983, *K. Balkwill* and *J.C. Manning 808* (NU); 29 Sep 1984, *C.F. Kennedy 32* (NU). WESTERN CAPE: 3219 (Cape Town): Rivierground in pypsteelbosse (-CC), 18 Dec 1980, *W.J. Hanekom 2600* (PRE); Kouebokkeveld Berge, (-CC), 24 Nov 1998, *W.J. Hanekom 3120* (NBG). 3322 (Oudtshoorn): Outeniqua Mountains (-CC), 9 Nov 1986, *J.H.J Vlok 1693* (PRE). EASTERN CAPE: 3027 (Barkly East): Farm Faskally near England (-DA), 9 Nov 1995, *J.E. Victor 1586* (PRE); Ben McDui (-DC), 6 Jan 1997, *T. Dold* and *M. Cocks 3495* (GRA). 3028 (Umtata): Maclear District (-CA), 16 Jan 2016, *S.P. Bester 13207* (PRE); Maclear (-CC), 7 Nov 1993, *S.P. Bester 1535* (NH); Kloof (-DD), 28 Feb 1946, *R. Story 950* (PRE). 3128 (Umtata): Hill above Mhlanfane Forest Station (-BC), 31 Jan 1983, *O.M. Hilliard* and *B.L. Burt 16341* (NU); Lady Frere, Engcobo (-CC), 25 Nov 1990, *E. Cloete 590* (NH); (-DB), 21 Oct 1953, *G.C. Theron 1604* (PRE); Elliotdale District (-DC), 11 Jul 1966, *J.L. Gordon-Gray 529* (NH). 3129 (Umtata): Baziya, Tembuland (-CC), no date, *Baur 257* (K-image); Blesbok flats Cathcart Div. Cape, 1838, *Drège s.n.* (K-image); between St John's River and Umtsikaba River, Pondoland, 1838, *Drège s.n.* (K-image!). 3225 (Elandsfontein): Elandsfontein (-AA), 13 Dec 2005, *S.P. Bester 6347* (GRA, PRE). 3226 (Fort Beaufort): Along gravel road to Sada off the R67 (-BD), 8 Feb 1995, *J.E. Victor* and *D.B. Haare 392* (PRE); Mpopfu Game Reserve (-DA), 28 Feb 2006, *C.L. Bredenkamp 3338* (PRE); Menzieberg, Amatole Mountains (-DB), 6 Jan 1986, *P.B. Phillipson 1185* (GRA), Fort Beaufort Road, 2 miles (3.22 km) from Alice (-DD), 22 Oct 1939, *M.H. Giffen 264* (PRE). 3227 (Stutterheim): Stutterheim (-AD), 5 Dec 1942, *J.P.H. Acocks 9399* (PRE); Pirie Mission (-CC), exact date not provided 1888, *T.R. Sim 20419* (NU, PRE); 5 Aug 2014, *S. Mgcwuwa 117* (GRA); King William's Town (-DB), Nov 1891, *H.G. Flanagan 1193* (PRE). 3326 (Grahamstown): Faraway (-AD), 4 Dec 1980, *A. Jacot Guillarmod 8473* (GRA); Nov 1942, *E. Archibald 669* (GRA); Alexandria (-CB), 10 Aug 1953, *S. Johnson 691* (GRA); 11 Feb 1953, *W. Marais 185* (PRE). 3327 (Peddie): Peddie District (-AC), 5 Nov 1993, *T. Dold* and *A. Boo 481* (GRA).

eSwatini. 2631 (Mbabane): Forbes reef (-AA), 29 Oct 1959, *B. Dlamini s.n.* (NBG).

Lesotho. 2928 (Marakabei): Ntiboho valley (-AC), 2 Jan 1947, *A. Jacot Guillarmod 279* (GRA). 2929 (Underberg): Thaba Ntšo, Sehlabathebe National Park (-CC), 4–14 Jan 1973, *Jacot Guillarmod*, *Getliffe* and *Mzamane 65* (PRE); 13 Feb 1976, *A. Beverly 471* (PRE).

Unknown localities: Feb 1895, *Maurice and Evans 474* (NH); Feb 1939, *J. Wylie 30120* (NH); Apr 1943, *B. Fischer 464* (NU); 15 Dec 1943, *W.F. Barker 2796* (NBG); 18 Nov 1952, *H.B. Gilliland 26862* (PRE); 18 Dec 1966, *R.G. Strey 7047* (NH); Mar 2000, *T. Edwards 2088* (NU).

Taxonomic notes. Brand (1921) included *C. hispidum* as a synonym of *C. lanceolatum*. However, the two species have notable variations especially in the colour of the corolla (magenta-purplish vs. white with blue throat in *C. lanceolatum*) and pedicel length (2 cm long opposed to less than 2 cm long in *C. lanceolatum*).

6 *Cynoglossum lanceolatum* Forssk in Fl. Aegypt. Arab: 41 (1775).

Cynoglossum hirsutum Thunb., Prodr. Pl. Cap.: 34 (1794).

Type: South Africa ♀♂, Precise locality unknown: Roggeveld, *C.P. Thunberg 168*, sub THUNB-UPS 3995 (UPS-microfiche! holotype).

Cynoglossum micranthum Desf.: 220 (1804), nom. nud.

Cynoglossum canescens Willdenow in Enum. Pl.: 180 (1809).

Type: ♀♂, Precise locality unknown, *C.L. Willdenow, s.n.* (B-W,-image, lectotype, designated by König et al. 2015).

Cynoglossum racemosum Roxb. in Fl. Ind.: 2:6 (1824), nom. illeg.

Echinosperrum paniculatum E.Mey. ex. A.DC., Prodr. [A.P.de Candolle] 10: 149,143 (1846). ♀♂, Type: same as above.

Paracynoglossum lanceolatum (Forssk.) R.R.Mill. in Notes Roy. Bot. Gard. Edinb.: 474 (1984). ♀♂ Type: same as above.

Type. Yemen, Al Hadiyah, Mar 1763, *P. Forsskal 312* (C-image! holotype).

Annual or biennial herbs, 0.5–0.9 m in height, covered with simple hairs. Basal leaves 85–180×15–23 mm, lanceolate-obtuse, blade elliptic, softly hairy, deciduous. Stem leaves 40–65×8–18 mm, lanceolate, apex acute, base cuneate, covered with moderately stiff hairs. Trichomes brittle, simple on both leaf surfaces. Inflorescence dichotomously branched axillary cyme, pedicel 1–1.5 mm long. Calyx ca. 1–1.5 mm long, lobe ovate-obtuse, pubescent on the outer surface, inner surface glabrous, apex acute. Corolla white with pale blue throat; lobes ca. 1×1 mm, campanulate. Nutlets ovoid-convex, 3–4×2.5–3.5 mm, fully pubescent; glochidia equally thick and long (Figure 13).

Phenology. August to May.

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. Amongst the southern African species, *C. lanceolatum* is similar and possibly related to *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii* with which it shares the branching pattern of the inflorescence, flower colour, and nutlet size. The two species can be distinguished by the density of the glochidia on the nutlets and distribution. *Cynoglossum lanceolatum* nutlets are completely covered with glochidia, whereas in *Cynoglossum coeruleum* subsp. *johnstonii* var. *mannii* glochidia tend to be more marginal and acentric.

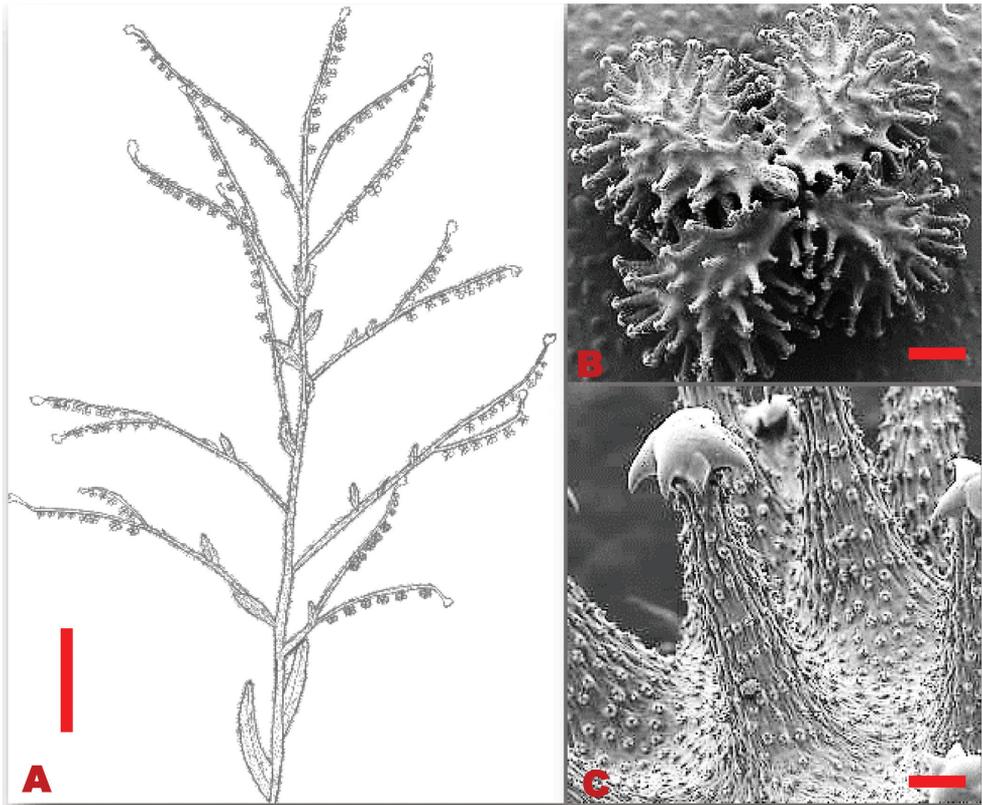


Figure 13. Morphological characters of *Cynoglossum lanceolatum* **A** line drawing of the fruiting branch **B** Glochidia evenly distributed on the adaxial surface of the nutlet **C** Glochidia uniform size, with multi-angular tip. Voucher specimen: *S.P. Bester 4653* (PRE). Drawing scale bar: 7.0 mm. SEM scale bar: 1 mm (**B**); 100 µm (**C**).

Distribution and habitat. *Cynoglossum lanceolatum* originates from Yemen (König et al. 2015) but reported from Africa (Ge-Ling et al. 1995), Pakistan, India (Joshi 2016), the Mediterranean, and throughout Asia (Verdcourt 1991) and Madagascar (König et al. 2015). In South Africa it occurs widely in all provinces, it also occurs in eSwatini and Lesotho (Figure 14). It is a widespread species that grows in disturbed habitats throughout parts of Africa.

Additional specimens examined. South Africa. LIMPOPO: 2229 (Waterpoort): Wylies Poort (-DD), 17 Oct 1938, *J.P.M. Acocks 1295* (PRE). 2230 (Messina): Sibasa District (-CD), 21 Jun 1977, *G. Hemm 163* (PRE). 2329 (Polokwane): Polokwane Nature Reserve (-CD), 12 Jan 1979, *Bredenkamp and Van Vuuren 307* (PRE). 2330 (Tzaneen): Letaba (-CA), 2 Apr 1958, *J.C. Scheepers 219* (PRE); Woodbush Forest Reserve (-CC), Jan 1923, *H. Wafen 22973* (PRE); 1 Feb 2019, *A.N. Moteetee and L.K. Madika AL07* (JRAU). 2428 (Modimolle): Waterberg, Kwaggasnek-Alma Road

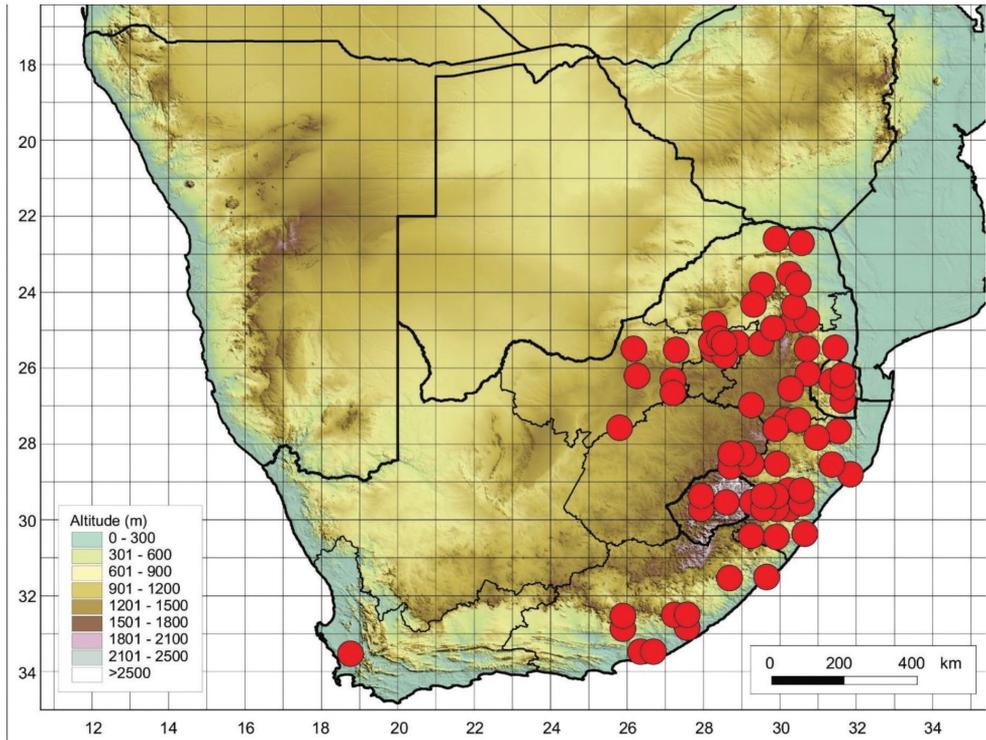


Figure 14. Known distribution of *Cynoglossum lanceolatum* in southern Africa based on the specimens examined.

(-CC), 13 Feb 1981, *K.L. Immelman 118a* (PRE). 2429 (Zebediela): Zebediela (-AA), 5 Feb 1967, *B.J. Huntley 1033* (PRE); 2430 (Pilgrim's Rest): Sekhukhune District Municipality, Leolo Mountain, SE of Tama Kgoshi (-CA), 14 Mar 2007, *B. Sachse 466* (PRE). NORTH-WEST: 2526 (Zeerust): Zeerust along road to Koster (-CA), 4 Feb 1976, *F. van der Meulen 597* (PRE). 2527 (Rustenburg): Rustenburg (-CA), 27 Dec 1949, *S.M. Johnson 854* (NBG). 2626 (Lichtenburg): Municipal Caravan Park (-AA), 19 Mar 1978, *A. Balsinhas and Harding 3303* (PRE). 2627 (Potchefstroom): Carletonville, A. Bailey Nature Reserve (-AD), Apr 1983, *S. van Wyk 180* (PRE); Mooiriver (-CA), 9 Mar 1984, *B. Ubbink 1257* (PRE). 2724 (Taung): West of Harz River near Taung, 120 km north of Kimberley (-DB), 11 Feb 1948, *R.J. Robin 3628* (PRE). GAUTENG: 2528 (Pretoria): Brooklyn (-CA), 20 May 1915, *C.A. Smith 191* (PRE); 24 May 1915, *C.A. Smith 194A* (PRE); 10 Dec 1915, *A.O.D. Mogg 11846* (PRE); Nov 1925, *C.A. Smith 1220* (PRE); Doornpoort, Airport Road (-CA), 26 Oct 2009, *S.P. Bester 9734* (PRE); 14 Nov 2018, *A.N. Moteetee and L.K. Madika AL01* (JRAU); Doornpoort/Hartbeesfontein (-CB), 24 Jan 2004, *S.P. Bester 4653* (PRE); Brooklyn (-CC), 11 Apr 1920, *K.A. Landsdell 864* (PRE). 2628 (Johannesburg): Randburg, Lanseria (-AA), 20 Nov 2018, *A.N. Moteetee and L.K. Madika AL02* (JRAU); Suikersbosrand (-CB), 27

Dec 1971, *G.J. Breidenkamp* 594 (PRE). MPUMALANGA: 2429 (Zebediela): Mashishing District (-DD), 8 January 1939, *Barnard and Mogg* 860 (PRE). 2430 (Pilgrim's Rest): Malta near Marinella (-AA), 16 Aug 1984, *M. Stalmans* 114 (PRE); Ohrigstad Dam Nature Reserve (-DC), 22 May 1973, *N. Jacobsen* 2861 (PRE). 2529 (Emalahleni): Loskop Dam (-AD), 12 Jan 1967, *G.K. Theron* 1129 (PRE). 2530 (Mashishing): Witklip (-BD), 4 Dec 1973, *J.P. Kluge* 359 (PRE); Lowveld Botanical Gardens (-BD), 18 Dec 1974, *E.J. Van Jaarsveld* 199 (NBG); Waterval Onder, Ntsinini (-CD), 26 Dec 2008, *K.W. Grieve* 250 (PRE); 23 Jan 2011, *K.W. Grieve* 342 (PRE); Barberton (-DD), 24 Jan 2000, *J.J. Meyer* 2603 (PRE). 2531 (Beersrust): White River District (-AC), Apr 1983, *W. Jacobsen* 5363 (PRE). 2630 (Carolina): Chrissiesmeer (-AC), 5 Jan 1972, *G.K. Theron* 2402 (PRE); Chrissiesmeer, on road to Lochiel (-BA), 6 Mar 1986, *M. Crosby* 273 (PRE). 2730 (Vryheid): Farm Doornhoek along Assegai river (-AA), 15 Dec 2006, *S.J. Siebert* 3246 (PRE). FREE STATE: 2729 (Volksrust): Vrede (-CB), 5 Feb 1987, *L. Smook* 6455 (PRE). 2828 (Bethlehem): Golden Gate National Park (-DA), Jan 1963, *L.C.C. Liebenberg* 6831 (PRE); Witsieshoek (-DB), Mar 1917, *H.A. Janod* 17317 (PRE). 2829 (Harrismith): Harrismith District (-AC), 8 Mar 1974, *M.L. Jacobsz* 2036 (PRE). KWAZULU-NATAL: 2729 (Utrecht): Volksrust (-BD), Jan 1928, *C.A. Smith* 5730 (PRE). 2730 (Vryheid): Oshoek District, Wakkerstroom (-AB), 26 Dec 2002, *S.J. Siebert* and *F. Siebert* 2279 (PRE); Vryheid Nature Reserve (-DC), 23 Feb 1988, *C.J. Youthed* 11 (NH); 7 March 2019, *A.N. Moteetee* and *L.K. Madika* AL09 (JRAU). 2731 (Louwsburg): Louwsburg District, Itala Nature Reserve (-CB), 15 Jan 1978, *D.J. McDonald* 443 (PRE). 2828 (Bethlehem): Crocodile River (-DB), 16 Dec 1893, *R. Schlechter* 3980 (NH); Royal Natal National Park (-DB), 17 Feb 1984, *O.M. Hilliard* and *B.L. Burt* 17662 (NU); Tugela gorge (-DB), 6 Feb 1982, *O.M. Hilliard* and *B.L. Burt* 15454 (PRE); Tugela valley (-DD), 14 Feb 1926, *A.J.W. Bayer* 47 (PRE). 2829 (Harrismith): Ladysmith (-DB), 1 Nov 1965, *N.E. Shirley s.n.* (NU). 2831 (Nkandla): Nkandla, Ferncliff Nature Reserve (-CA), 14 Feb 1994, *H. Kennedy* 533 (NU); University of KwaZulu-Natal (-DD), 27 May 2003, *S.J. Siebert* 2333 (NH). 2929 (Underberg): Loteni Nature Reserve (-AD), 13 Dec 1978, *M.L. Jacobsz* 3937 (PRE); Mpendhle District (-BC), 24 Dec 1978, *O.M. Hilliard* and *B.L. Burt* 11804 (NU, PRE); Polela District (-CB), 6 Apr 1974, *M.A. Rennie* 545 (NU); Waterfall (-DD), Mar 2002, *T. Edwards* 2731 (NU). 2930 (Pietermaritzburg): Victoria (-AA), 12 Apr 1955, *S.M. Johnson* 1152 (NH); Tweedie (-AC), 31 Dec 1927, *Forbes* 293 (NH); 28 Feb 1982, *K.L. Immanuel* 260 (PRE); Loskop District (-AD), 11 Feb 1946, *B.L. Howlett* 80 (NH); Pietermaritzburg (-CB), 29 Feb 1976, *M. Grice s.n.* (NU); 14 Oct 1987, *Renecken* 6 (GRA); Botanic Garden Estate (-CB), 8 Dec 1991, *D.G. Stielau* 135 (NH, PRE); Cottingham (-CC), 23 Mar 1969, *R.G. Strey* 8420 (NU); Durban Road (-CD), 19 Nov 1950, *J.G. Lawn* 1820 (NH); Umgeni Water Board, (-DA), 20 Oct 1984, *J. Manning* 536 (NU); Inanda (-DB), Dec, *J. Medley-Wood* 370 (NH); 25 Nov 1983, *H.H. Hilger* 35 (PRE); Pinetown District (-DD), 28 Nov 1913, *J.M. Wood* 12373 (NU). 3029 (Kokstad): Harding, Victoria East (-CA), 30 Apr 1955, *G.L. Lewis* 4914 (PRE); Harding, Alfred District (-DB), 2 Mar 1983, *O.M. Hilliard* and *B.L. Burt* 16755 (NU). 3030 (Port

Shepstone): Dumisa (-AD), 9 Dec 1992, *A.M. Ngwenya 1071* (NH). WESTERN CAPE: 3318 (Cape Town): Zondagsfontein (-DC), Dec-Mar 1930–1, *J. Thode A2838* (NH); 18 Oct 1943, *B.S. Fischer 498* (NU). EASTERN CAPE: 3128 (Mjika): Mahlahlane Forest (-BC), 6 Mar 1985, *A. Hutchings 1590* (GRA). 3129 (Port St. John's): Kloof above Port St. Johns (-DA), 31 Jan 1936, *M.C. Gillett 1257* (PRE). 3226 (Fort Beaufort): Amatole Mountain (-DB), 1 May 1986, *P.B. Phillipson 1492* (PRE); Fort Beaufort (-DD), 21 Mar 1977, *G.E. Gibbs Russell 3735* (GRA). 3326 (Grahamstown): Grahamstown Nature Reserve (-BC), Feb 1917, *J.C. Jane 17131* (PRE); 1 Apr 1952, *A.R.H. Martin 9466* (GRA); Featherstone Kloof (-BC), 18 Jul 2001, *C.J. Kayombo and A.A. Merti 3719* (GRA). 3227 (Stutterheim): Stutterheim District (-CB), 28 Jan 1979, *O.M. Hilliard and B.L. Burtt 12427* (NU); Kababu Hills (-CA), 5 Feb 1936, *M.C. Gillett 1325* (PRE); East London (-DB), 11 Dec 2001, *J.J. Meyer 4067* (PRE).

eSwatini. 2631 (Hhoho): Hhoho District, Masilela area on Maphalaleni road along Mucucene Hills (-AB), 27 Jan 1994, *G. Germishuizen 7173, 7174* (PRE); 24 Jan 1994, *S.R. Hobson 2048* (PRE); Hlambanyathi valley (-AC), 27 Nov 1954, *R.J. Compton 24864* (NBG); Mbabane (-AC), 14 Jan 1955, *R.J. Compton 24834* (PRE).

Lesotho. 2927 (Maseru): Monethi's, Berea (-BB), 1 Jan 1957, *A. Jacot Guillarmod 1910* (PRE); Mountain Road (-BD), Mar 1977, *M. Schmitz 7317* (PRE). 2928 (Marakabei): Loskop (-AB), 11 Apr 1980, *B.N. Ubbink 974* (NH); Molika-lika (-AC), 7 Jan 1954, *A. Jacot Guillarmod 1674* (PRE). 2929 (Underberg): Thaba Ntšo, Sehlabathebe National Park (-CC), 4–14 Jan 1973, *Jacot Guillarmod, Getliffe, and Mzamane 142* (PRE).

Unknown localities: No locality details, 30 Jan 1948, *B.S. Fischer 1426* (NU).

Taxonomic notes. The specimen in the National Herbarium Pretoria (PRE) collected in the Doornpoort area in Gauteng Province (Voucher number: *S.P. Bester 9734* (PRE)) belongs to *C. lanceolatum* and not to *C. amabile* since it has characters which are typical of this species, i.e., white corolla with a blue throat instead of bluish-purple corolla and divaricately branched instead of clustered at the apex.

7. *Cynoglossum obtusicalyx* Retief & A.E. van Wyk in *S. Afr. J. Bot.* 62(3): 169–172 (1996).

Type. South Africa ♀♂, Western Cape, Worcester (3319): 20 km E. of Ceres (-AD), 21 Oct 1958, *Acocks 19893* (PRE-image! holotype; NBG, isotype).

Perennial or biennial herbs, ca. 0.45 m in height. Basal leaves 175–250×5–18 mm, obtuse, densely pubescent, persistent, margins entire. Stem leaves 50–65×5–10 mm, obtuse-lanceolate, apex acute, base cuneate, soft hairs. *Trichomes* woolly, soft, non-bulbous based, unicellular, long, thin. Inflorescence dichotomously branched terminal cymes, pedicel 5–10 mm long, lengthens considerably in fruit. Calyx ca. 1.0–1.6 mm long, lobe oblong, densely pubescent, apices broadly obtuse-truncate shaped. *Corolla* pale blue; lobes 5×7 mm diameter, ovate. Nutlets ovoid-convex, 2–4×3–4 mm, densely echinulate with glochidia (Figure 15).



Figure 15. Vegetative and reproductive morphological features of *C. obtusicalyx* **A** 1. base leaves 2. stem with alternate stem leaves and terminal flowers **B** SEM micrograph of a nutlet **C** SEM micrograph of glochidia around the nutlet. Voucher: *J.P.H. Acocks 8509* (PRE). Drawing scale bar: 7.0 mm. SEM images scale bar: 1 mm (**B**); 200 μ m (**C**).

Diagnostic characters. The stems and leaves of *Cynoglossum obtusicalyx* are densely covered with soft, woolly trichomes which makes it similar to *Cynoglossum alticola*. However, the two species differ in flower colour and size (deep blue corolla, 4×3 mm vs. pale blue corolla, 5×7 mm in *C. obtusicalyx*). *Cynoglossum obtusicalyx* has pale blue flowers with corolla 5–7 mm long whereas *C. austroafricanum* has bright blue flowers with corolla 2.75–4.25 mm long. The two species also show variation in trichome texture and density, i.e., sparsely hairy with short, stiff hairs on both the stem and leaves in *C. austroafricanum* vs. densely arranged long, woolly hair on both the stem and leaves in *C. obtusicalyx*.

Phenology. October to February.

Conservation status. Least Concern (Raimondo et al. 2009).

Distribution and habitat. *Cynoglossum obtusicalyx* is endemic to South Africa where it has been recorded from Calvinia, Worcester, and Beaufort West in the Northern and Western Cape Provinces (Figure 16), it occurs in mountainous areas, often growing on screes below cliffs.

Additional specimens examined. South Africa. NORTHERN CAPE: 3119 (Calvinia): Calvinia District (-BD), 22 Sep 1955, *J.P.H. Acocks 8509* (PRE); 22 Sep 1955, *D.A Leistner 394* (PRE; NBG). WESTERN CAPE: 3222 (Beaufort West): Mountain View farm (-BD), 17 Apr 1978, *G. Gibbs Russell* and *R. Hermann 4272* (PRE).

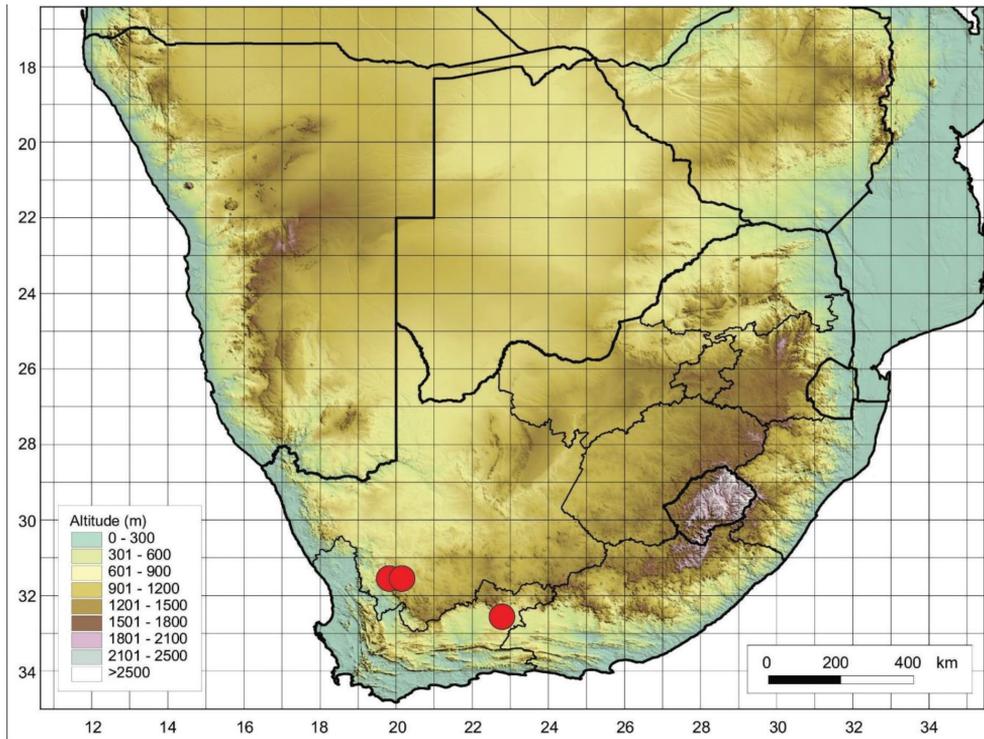


Figure 16. Known distribution map of *C. obtusicalyx* in southern Africa based on the specimens examined.

8. *Cynoglossum spelaeum* Hilliard & B.L.Burtt in Notes Roy. Bot. Gard., Edinburgh 37(2): 287 (1979).

Cynoglossum basuticum Weim.ex Guillarmod, Fl. Lesotho 233 (1973), nom. nud.

Type. South Africa ♀♂, KwaZulu-Natal, Underberg (2929): Underberg District, Cobham Forest Station, Polela valley (-CD), 20 Mar 1977, *O.M. Hilliard* and *B.L. Burtt* 9728 (E, holotype image! K-image!, NU-image!, PRE-image! [2 sheets] isotype).

Perennial herbs, ca. 0.4–0.5 m in height. Basal leaves 130–235×25–35 mm, spatulate-obtuse, soft hairs, deciduous, margins entire. Stem leaves 40–80×10–25 mm, obtuse shaped, dark green adaxial surface, grey green abaxial surface, smooth margins, soft hairs, sparsely packed. Trichomes unicellular, simple on both leaf surfaces, soft. Inflorescence corymbose panicle; threadlike pedicel 5 mm long. Calyx ca. 1.5–2.0 mm long, lobes lanceolate-oblong, softly hairy on the outer surface, inner surface smooth, apex acute. Corolla white; lobes ca. 3×3 mm diameter, obtuse. Nutlets ovoid, ca. 4×5 mm; glochidia more marginal and acentric, marginal glochidia are longer compared to the acentric glochidia (Figure 17).

Phenology. December to March.

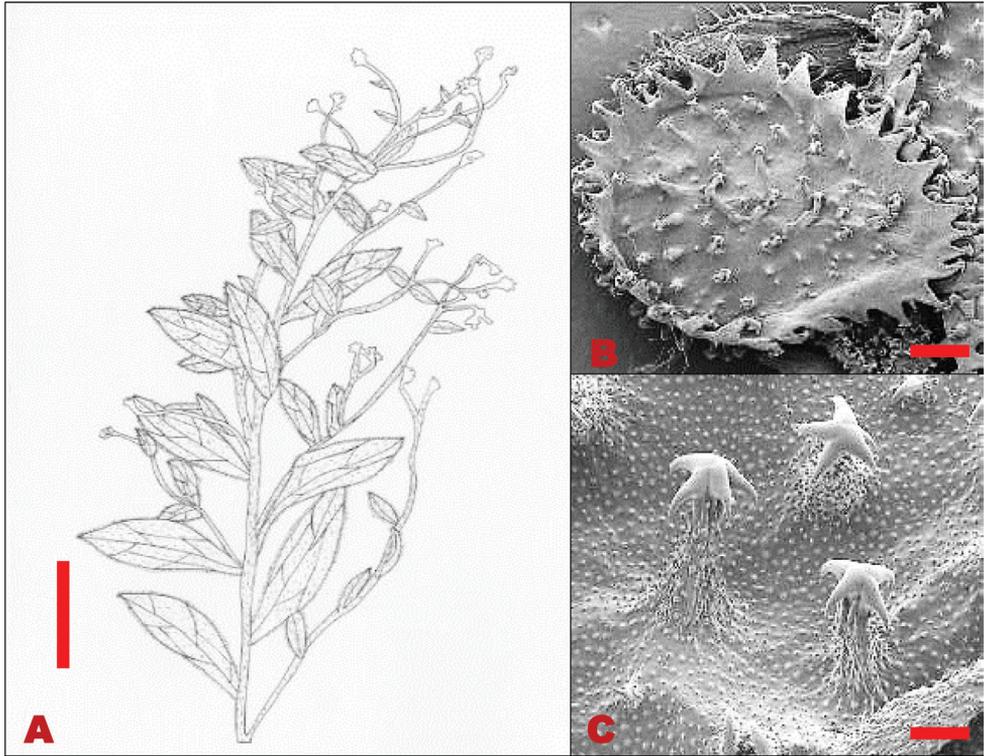


Figure 17. Morphological features of *C. spelaeum* **A** line drawing of a fruiting branch showing stem with alternate, sessile, spatulate leaves **B** SEM micrograph of nutlet showing more marginal and acentric glochidia **C** close-up SEM micrograph showing glochidia with multiangular hooks. Voucher specimen: *A. Nicholas* and *B. Isaacs* 1965 (PRE). Drawing scale 8.0 mm. SEM images scale bars: 1 mm (**B**); 200 µm (**C**).

Conservation status. Least Concern (Raimondo et al. 2009).

Diagnostic characters. This species is characterised by leaves that are sparsely covered with hairs that have a softer feel, which distinguishes it sharply from all the other southern African species which have either brittle or woolly hairs. The abaxial leaf surface has a grey-greenish appearance which is also a unique character of this species.

Distribution and habitat. The species is distributed in South Africa (Eastern Cape, Free-State, and KwaZulu-Natal Provinces) and Lesotho (Figure 18). It grows in loose sandy soil at the edge of an overhang.

Additional specimens examined. South Africa. KWAZULU-NATAL: 2729 (Utrecht): Ncandu State Forest (-DC), 8 May 1984, *A. Nicholas* and *B. Isaacs* 1965 (PRE). 2929 (Underberg): Allandale, Lion's River District (-BC), 24 Jan 1978, *O.M. Hilliard* and *B.L. Burt* 11258 (NU); Giant's Castle Nature Reserve (-BC), 07 Jan 1988, *A. Abbott* 4064 (NH); 3 Feb 1976, *O.M. Hilliard* and *B.L. Burt* 8904 (NU); 2 Jan 1978, *O.M. Hilliard* and *B.L. Burt* 11172 (NU); 20 Nov 1985, *O.M. Hilliard* and *B.L.*

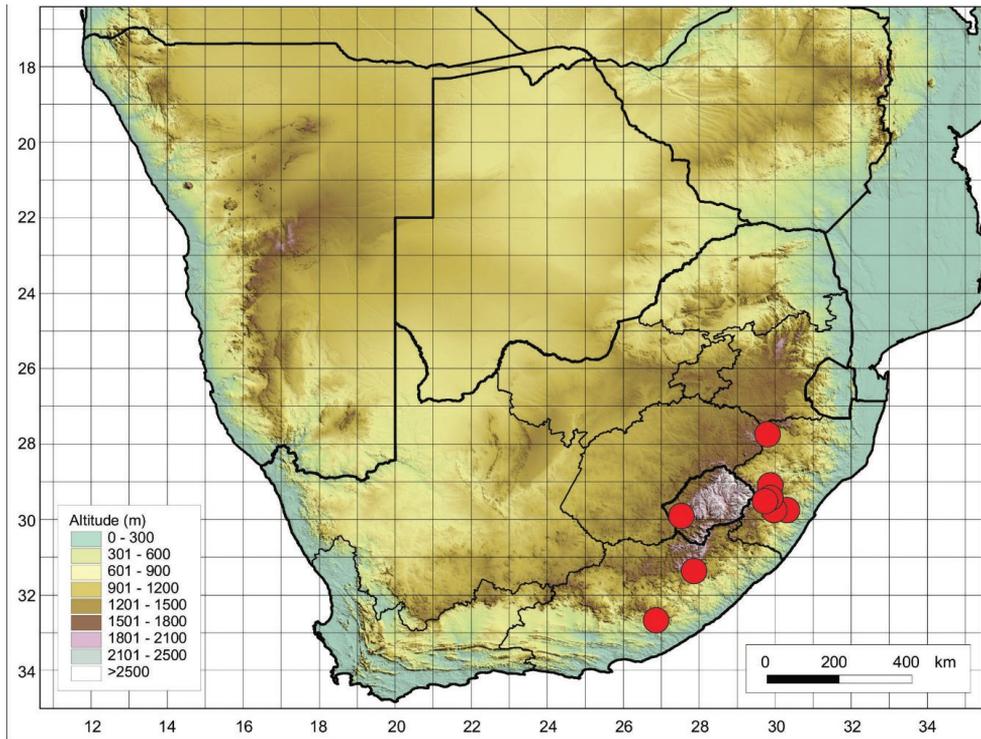


Figure 18. Known distribution of *Cynoglossum spelaeum* in southern Africa based on the specimens examined.

Burt 18242 (NU, PRE); Bulwer District (-DD), 8 Jan 1974, *M.A. Rennie 507* (NU); 8 Mar 2019, *A.N. Moteetee* and *L.K. Madika AL012* (JRAU). EASTERN CAPE: 3127 (Lady Frere); Elliot District (-BB), 22 Jan 1979, *O.M. Hilliard* and *B.L. Burt 123427* (NU). 3226 (Fort Beaufort): Amatole Mountain (-DB), 16 Feb 1986, *P.B. Phillipson 1294* (PRE).

Lesotho. 2927 (Maseru): Laikopile Mountain (-CD), Jan 1918, *A. Dieterlen 40799* (PRE).

Taxonomic notes. *Cynoglossum spelaeum* is quite distinctive among the southern African species having uniquely shaped (i.e., spatulate to obtuse) and coloured (i.e., deep green adaxial surface, grey green abaxial surface) leaves, that almost seem leathery but contain few soft trichomes. It is also the only species among the southern African species that has completely white flowers. Although Hilger et al. (2015) indicated that the species does not belong to genus *Cynoglossum*, they did not elaborate the reasons for the exclusion. Nonetheless, the species fits the generic description of four glochidiate nutlets that are adapted for zoochory. In addition, preliminary molecular data (Madika 2020), showed a close relationship between *C. coeruleum* subsp. *johnstonii* var. *mannii*, *C. lanceolatum*, and *C. spelaeum*.

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Oreocharis phuongii (Gesneriaceae), a new species from central Vietnam

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Abstract

Oreocharis phuongii, a new species of Gesneriaceae from central Vietnam, is described and illustrated here. The new species is most similar to *O. longifolia* by sharing peduncles up to 22 cm long, bracts 2, zygomorphic, yellow flowers with tubular corolla, stamens 4 with two pairs of coherent anthers and capsules up to 6 cm long. It mainly differs from the latter by the combination of some morphological characters of leaves (shape, base, apex and margin), size of calyx lobes, indumentum of corolla tube and inner surface of three lower corolla lobes. Detailed morphological description together with colour illustration, information on phenology, distribution, ecology, preliminarily conservation status of the new species and comparison with its similar species are also presented.

Keywords

Annamite Range, Didymocarpoideae, endemic to Vietnam, Flora of Vietnam, limestone flora, new taxon

* The authors contributed equally to this paper.

Introduction

The genus *Oreocharis* Bentham (Gesneriaceae DC), prior to recent phylogenetic work, comprised ca. 28 species, mainly distributed in southern China (Li and Wang 2004). Recent molecular and morphology-based analyses demonstrated that the traditionally-defined *Oreocharis* was phylogenetically intertwined with nine previously defined small genera and acaulescent, rosette forming members of *Briggsia* Craib. The re-circumscribed *Oreocharis* is a strongly supported monophyletic group and placed in the subfamily Didymocarpoideae (Möller et al. 2011; 2014; Middleton et al. 2013). Since its re-definition in 2011, *Oreocharis* s.l. comprises about 150 species making the genus one of the most morphologically diverse amongst Old World Gesneriaceae (Möller et al. 2016; Möller 2019; Jin et al. 2021). Most of the approximately 130 species are found in southern and south-western China, with a few species also in northern Vietnam, India, Bhutan, Myanmar, Thailand and Japan (e.g. Pham 2000; Vu 2005, 2018; Wei et al. 2010; Möller et al. 2011, 2016, 2018; Do et al. 2017; Chen et al. 2017, 2018; Yang et al. 2019; Cai et al. 2019; Jin et al. 2021).

Prior to this work, eight species of *Oreocharis* were known from Vietnam, of which seven new species were described from the country since 2017, i.e. *O. caobangensis* T.V.Do, Y.G.Wei & F.Wen (Do et al. 2017), *O. argyrophylla* W.H.Chen, H.Q.Nguyen & Y.M.Shui, *O. blepharophylla* W.H.Chen, H.Q.Nguyen & Y.M.Shui (Chen et al. 2017), *O. grandiflora* W.H.Chen, Q.H.Nguyen & Y.M.Shui, *O. longituba* W.H.Chen, Q.H.Nguyen & Y.M.Shui (Chen et al. 2018), *O. tribracteata* Bramley, H.J.Atkins & Mich.Möller and *O. rufescens* D.J.Middleton (Möller et al. 2018). All these species have been found in close proximity or with sympatric distributions in northern Vietnam, but none of them has been recorded from the central and southern areas of the country. Due to similarities of topography and ecological factors, the flora of northern Vietnam is similar to that of south-western and southern China, which is considered the centre of *Oreocharis* diversity (Jin et al. 2021). Additionally, there are still many parts of northern and central Vietnam (such as the Annamite Range) from which specimens of vascular plants, in general and Gesneriaceae, in particular, have not yet been well collected or for which the collecting density is very low. Hence, our understanding of the diversity and distribution of vascular plant species, in general and Gesneriaceae species, in particular, in these regions remains rudimentary.

While revising the taxonomy of Gesneriaceae for the Flora of Vietnam, we have conducted numerous field investigations throughout the country and collected some interesting Gesneriaceae specimens from two populations in some protected forest areas within the Annamite Range in central Vietnam. These specimens are characterised by leaves in a basal rosette, 2-paired stamens, ring-like disc and loculicidal capsules. Due to the above characteristics, we determined that these specimens belong to *Oreocharis* s.l. Detailed morphological comparisons with the protologues and type specimens of all previously-described species of *Oreocharis* s.l. (Pan 1987; Wang et al. 1990, 1998; Li and Wang 2004; Wei et al. 2010; Liu et al. 2012; Chen et al. 2013, 2014, 2015, 2016, 2017, 2018; Tan et al. 2013, 2015; Möller et al. 2014, 2018; Möller

2015; Li and Li 2015; Yang et al. 2015a, 2015b, 2017, 2019; Wei et al. 2016; Li et al. 2017; Do et al. 2017; Cai et al. 2019, 2020; Cai and Dao 2020) revealed that these specimens do not match with any known *Oreocharis* species. Therefore, we confirmed that these specimens represent a new species, which is here described and illustrated, namely *O. phuongii* T.V.Do.

Taxonomic treatment

Oreocharis phuongii T.V.Do, sp. nov.

urn:lsid:ipni.org:names:77296017-1

Figs 1–3

Type. VIETNAM. Thua Thien Hue Province, Nam Dong District, Thuong Lo Commune, Bach Ma National Park, on moist rocks under evergreen broad-leaved forests, 16°07'56.5"N 107°45'03.2"E, ca. 545 m alt., 21 Nov 2019, Do Van Truong ĐVT 368 (holotype: VNMN!; isotypes: IBK!, VNMN!).

Diagnosis. The new species is morphologically similar to *O. longifolia* W.H.Chen in having peduncles up to 22 cm long, bracts 2, zygomorphic, yellow flowers with tubular corolla, stamens 4 and capsules up to 6 cm long; but it differs from the latter in its elliptic to ovate lamina (vs. narrowly elliptic to oblanceolate), cuneate to nearly rounded leaf base (vs. attenuate), obtuse to almost rounded leaf apex (vs. acute), crenulate margin (vs. serrulate), 3–4.5 × 1.5–1.8 mm calyx lobe size (vs. 5–7 × 2–3 mm), glabrous inner surface of corolla tube (vs. sparsely glandular puberulent with dark-purple striations) and exclusively yellow inner surface of three lower lobes without dots and striations (vs. brown to brownish-yellow with purple dots and striations).

Description. Perennial acaulescent herbs, with conspicuous 12–14 cm long stolons, densely brown woolly, with (6–)8–14 leaves in a basal rosette. Petioles 1–3 cm long, densely brown villous. Leaf-blade elliptic to ovate, 4–7 × 2–2.8 cm, base cuneate to nearly rounded, apex obtuse to almost rounded, margin crenulate, adaxially dark-green, densely grey puberulous, abaxially pale-green, densely grey puberulous and browner appressed villous on main veins, secondary veins pinnate, 4–5 pairs, tertiary veins reticulate, lightly sunken on adaxial surface and conspicuously prominent on abaxial surface. Inflorescences cymose, subumbel-like, axillary, 2–3 cymes, each 1–3-flowered; peduncles 12–19(–22) cm long, erect, brown, sparsely villous to pubescent; bracts 2, linear-lanceolate to elliptic, 2–5 × 1.5–2 mm, outside with sparse, brown hairs, inside glabrescent, margin entire; pedicels 2–3 cm long, with sparse, brown hairs, sometimes bearing additional bracts at 1/2 to upper 1/3 of pedicel length, similar in size and morphology with bracts at branching points. Calyx equally 5-lobed, free to base, lobes triangular to lanceolate, 3–4.5 × 1.5–1.8 mm, both surfaces with a dense covering of long gland-tipped and eglandular hairs, margin entire. Corolla, zygomorphic, yellow, bilabiate, outside sparsely pubescent, inside glabrous; tube tubular, 28–30 × 12–13 mm, abruptly constricted at base, 8–9 × 2.5–3 mm; upper lip slightly 2-lobed; lobes ovate, 5–7 ×

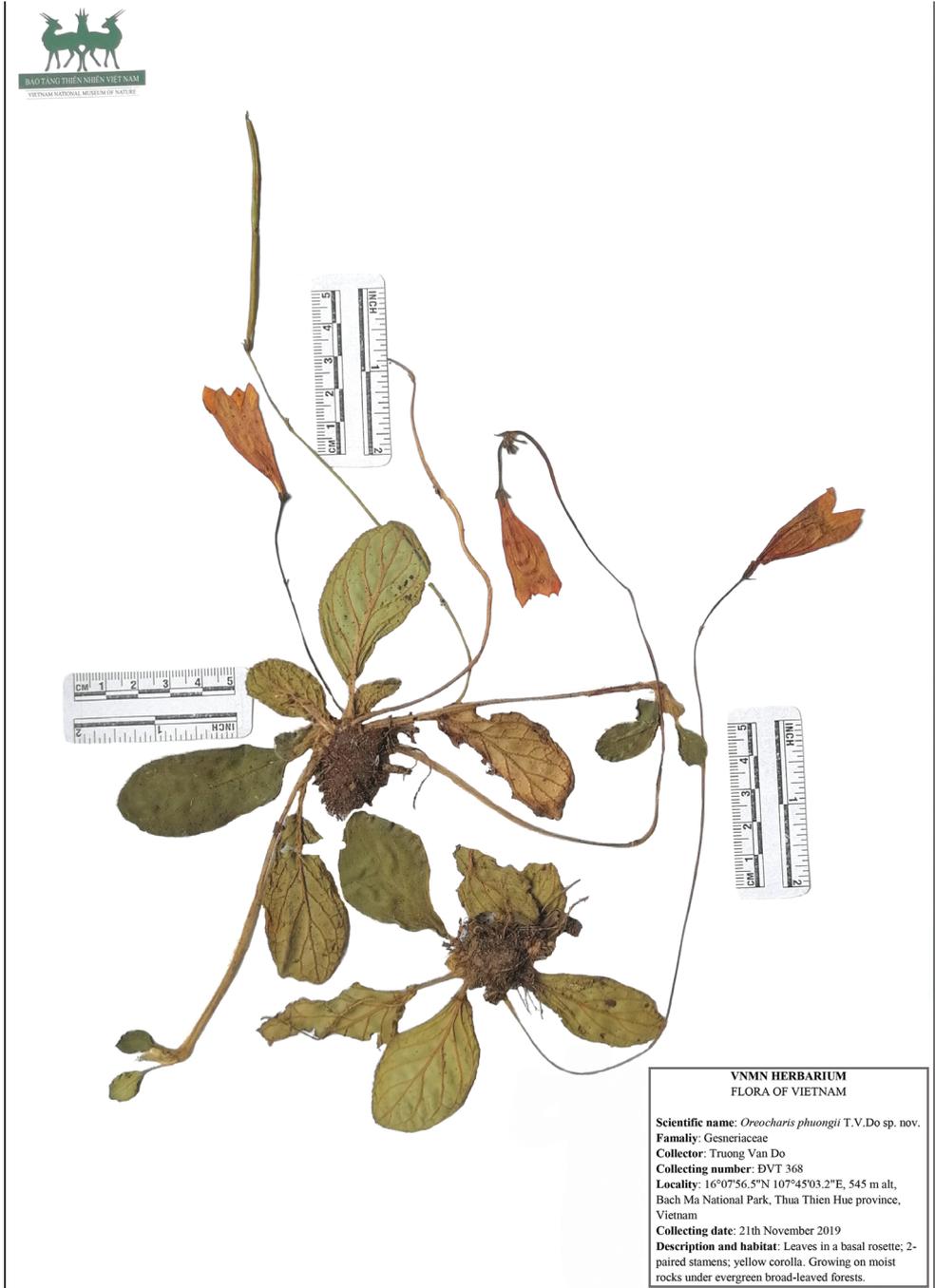


Figure 1. Holotype of *Oreocharis phuongii* sp. nov. (Truong Van Do EVT 368, deposited at VNMN).

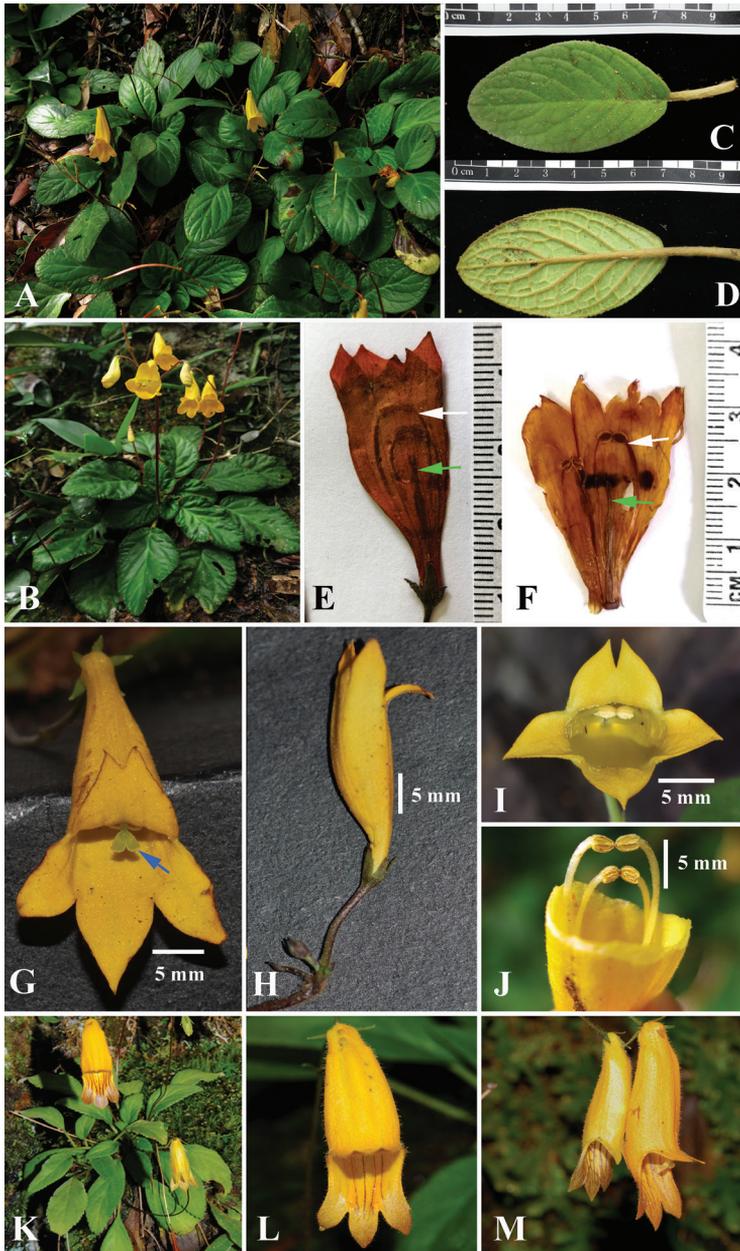


Figure 2. *Oreocharis phuongii* sp. nov. (A–J) **A** habitat **B** habit showing cymose inflorescence **C** adaxial leaf surface **D** abaxial leaf surface **E–F** structure of floral parts (two pairs of anther and filament indicated by a white arrow; shape of pistil indicated by a green arrow) **G** frontal view of opened flower showing backwards incurved upper lobes, glabrous inner surface of three lower lobes and a bilobed matured stigma longer than stamens (indicated by a blue arrow) **H** lateral view of opened flower **I** close-up of opened flower (in frontal view) **J** close-up of 2-paired stamens; *O. longifolia* (K–M) **K** habit **L** frontal view of opened flower showing brown to brownish-yellow inner surface of three lower lobes with dark-purple striations **M** lateral view of an opened flower. **A–J** photos by Do Van Truong **K–M** photos by Nicholas Turland.

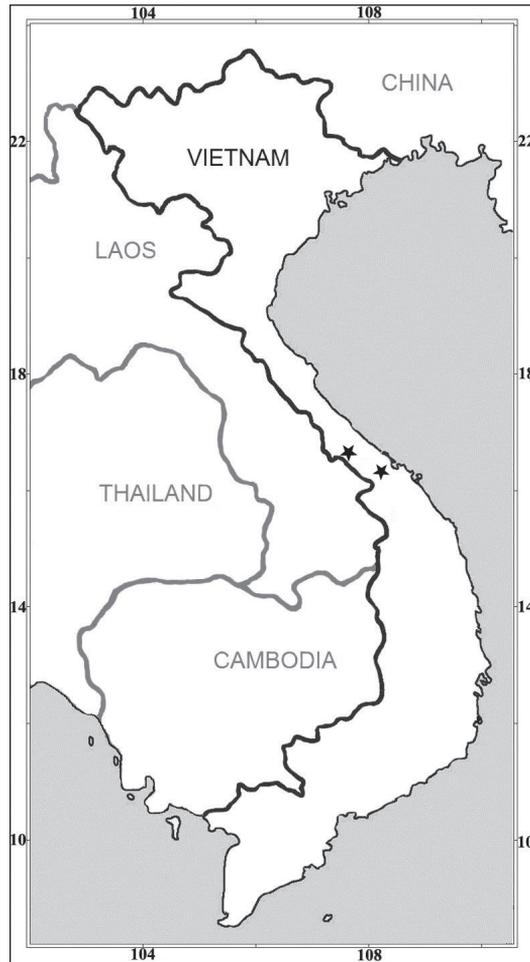


Figure 3. Distribution of *Oreocharis phuongii* sp. nov. from central Vietnam (shown by black stars).

4.5–5 mm, incurved backwards, apex obtuse to acute; lower lip 3-lobed, lobes elliptic, broadly ovate to semi-orbicular; lateral lobes 8–9 × 5–6 mm; middle lobe broader than lateral lobes, 8–10 × 6–7.5 mm, apex obtuse to acute or rounded. Stamens 4, anthers coherent in two pairs, filaments linear, glabrous; filaments of upper pair 15–17 mm long, adnate at 7–8 mm from the base of corolla tube; filaments of lower pair 1.1–1.3 cm long, adnate at 9–10 mm from the base of corolla tube; anthers reniform, 1–1.5 mm long, basifixed, glabrous; staminode absent. Disc ca. 1.3 mm in height, margin orbicular, glabrous. Pistil 21–26 mm long; ovary ca. 2.5 mm long, ca. 1 mm in diam., glabrescent; style 17–22 mm long, ca. 1 mm in diam., pubescent, longer than upper pair of filaments when mature; stigma bilobed, V-shaped, 1–1.5 mm long, glabrous. Capsules linear-oblong, straight, 5–6(–8) × ca. 0.3 cm, glabrous to glabrescent, loculicidal.

Etymology. The specific epithet honours Prof. Dr. Vu Xuan Phuong who has contributed significantly to our understanding of Gesneriaceae in Vietnam.

Phenology. Flowering was observed from October to November. Fruiting may occur from November to December.

Distribution and habitat. The new species is currently known from some protected forest areas (viz. Dakrong Nature Reserve, Quang Tri Province and Bach Ma National Park, Thua Thien Hue Province) within the Annamite Range, central Vietnam (Fig. 3). The new species grows on moist shady cliffs on the humus-rich limestone hills and moist rocks under evergreen broad-leaved forests, at elevations of 360–650 m.

Proposed IUCN conservation status. Two large-sized populations of *Oreocharis phuongii* were found in the core-zones of Dakrong Nature Reserve, Quang Tri Province and Bach Ma National Park, Thua Thien Hue Province, central Vietnam, which are almost entirely covered by primary forest and are well protected. Furthermore, our field observations of these populations indicated that there are many healthy individuals and seedlings that regenerate in well-protected habitats and there is no immediate threat to the populations from human activities. Thus, the new species is probably not at risk in the near future. This species is preliminarily assessed as Least Concern (LC) according to the IUCN Categories and Criteria (IUCN Standards and Petitions Subcommittee 2019).

Notes. In the size and shape of the corolla and the structure of inflorescences, *O. phuongii* is similar to some species with the acaulescent and rosette-forming stems of the formerly circumscribed *Briggsia*, which previously comprised ca. 30 species and four varieties and was mainly distributed in Bhutan, China, India, Myanmar and Vietnam (Wang et al. 1990, 1998; Vu 2018). Nineteen species and four varieties of acaulescent, rosette forming *Briggsia* (Craib 1920; Pan 1988) were moved to *Oreocharis* s.l. in a later revision (Möller et al. 2011, 2014). Of which, *O. phuongii* is most similar to *O. longifolia* (Craib) Mich.Möller & A. Weber in having peduncles up to 22 cm long, bracts 2, zygomorphic, yellow flowers with tubular corolla, stamen 4 with two pairs of coherent anthers and capsules up to 6 cm long, but it clearly differs from the latter in the shape of leaf blade, leaf base, leaf apex, leaf margin, number of flowers, shape and size of calyx lobes, inner surface of corolla tube and inner surface of three lower corolla lobes. Detailed morphological comparisons of the new species with *O. longifolia* are shown in Table 1 and Figure 2.

This new species is the first record of the genus *Oreocharis* occurring in central Vietnam, which raised the species number of *Oreocharis* in Vietnam to nine. Amongst the nine known *Oreocharis* species from Vietnam, the new species shares the yellow to orange corolla with five other species: *O. aurea*, *O. argyrophylla*, *O. grandiflora*, *O. longituba* and *O. tribracteata* (Chen et al. 2017, 2018; Möller et al. 2018). However, it is clearly different from these five species by having a tubular corolla tube (vs. funnel to narrowly funnel corolla tube in *O. argyrophylla*, *O. grandiflora*, *O. longituba*, *O. tribracteata* and urceolate corolla tube in *O. aurea*). Jin et al. (2021) showed that *Oreocharis* sl. could be separated into two clades: Clade A was mainly distributed in SW China and predominantly showed yellow to orange corollas; Clade B was mainly distributed in S and SE China and predominantly showed purple corollas, of which, Clade A includes ca. 20 species. In

Table 1. Detailed morphological comparisons of *Oreocharis phuongii* with *O. longifolia*.

Characters	<i>O. phuongii</i> sp.nov.	<i>O. longifolia</i> *
Stems	with conspicuous stolons	without stolons
Leaves		
lamina	elliptic to ovate	narrowly elliptic to oblanceolate
base	cuneate to nearly rounded	attenuate
apex	obtuse to almost rounded	acute
margin	crenulate	serrulate
Cymes	1–3-flowered	1–10-flowered
Peduncle length (cm)	12–19(–22)	5.5–22
Calyx		
shape	equally 5-lobed, lobes triangular to lanceolate	subequally 5-lobed, lobes ovate
size (mm)	3–4.5 × 1.5–1.8	5–7 × 2–3
Corolla		
tube size (cm)	2.8–3 × 1.2–1.3	(1–)1.8–2.3 × 0.8–1.6
outer surface of tube	sparsely pubescent	densely trichomes
inner surface of tube	glabrous without striations	sparsely glandular puberulent with purple striations
inner surface of three lower lobes	exclusively yellow without dots and striations	brown to brownish-yellow with purple dots and striations
Distribution	Central Vietnam	South-western China and northern Myanmar

* Morphological characters following Wang et al. (1998) and our own observations.

order to facilitate identification, a key to five yellow to orange species of *Oreocharis* in Vietnam is provided.

Additional specimen examined. VIETNAM. Quang Tri Province, Dakrong District, Dakrong Nature Reserve, on moist shady cliffs on the humus-rich limestone hills, 16°29'50.97"N, 107°00'09.25"E, 650 m alt., 18 Oct 2019, Do Van Truong ĐVT 362 (VNMN).

A key to yellow to orange species of *Oreocharis* in Vietnam

- 1 Corolla tube urceolate, corolla less than 2.5 cm long; anthers free..... *Oreocharis aurea*
- Corolla tube funnel-shaped to tubular, corolla more than 2.5 cm long; anthers coherent in pairs..... 2
- 2 Corolla tube tubular, abruptly constricted near base *Oreocharis phuongii*
- Corolla tube funnel-shaped to narrowly funnel-shaped 3
- 3 Calyx lobes divided to about 2/3 of their length; bracts 3 *Oreocharis tribracteata*
- Calyx lobes free to base or almost so; bracts 2 4
- 4 Leaf base cordate..... *Oreocharis longituba*
- Leaf base cuneate to nearly rounded..... 5
- 5 Petiole less than 3 cm long; leaf margin crenate; corolla 3.3–3.6 cm long, deep orange *Oreocharis grandiflora*
- Petiole 4–9 cm long; leaf margin serrulate towards apex; corolla 2.5–3.1 cm long, yellow *Oreocharis argyrophylla*

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Orobanche ingens (Orobanchaceae) – a poorly known species of the Greater Caucasus: taxonomic problems, distribution, hosts and habitats

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Abstract

Orobanche ingens is an endemic species from the Caucasus, especially the Greater Caucasus, parasitising on large Apiaceae (usually *Heracleum*). This species was misclassified over the years and little was known about its range and habitats. Here, we clarify the typification, as well as provide notes about the taxonomy of this species. Additionally, we presented distribution, habit and host range of *O. ingens* and morphological features that distinguish it from similar species.

Keywords

Caucasus, distribution range, *Heracleum*, holoparasitic Orobanchaceae, host, *Orobanche*, typification clarification

Introduction

This taxon was described by Beck (1922) on the basis of a part of the materials collected by Th. Alexeenko in Kasi-Kumukh (Dagestan). These materials are preserved on three herbarium sheets in LE. These three sheets contain original labels, two of them equal and printed in blue ink by Alexeenko (LE01015385 and LE01015386) and the third handwritten, probably by Beck (LE01015387); furthermore, the three labels have two of Beck's manuscript numbers: 10524 (LE01015386) and 10544 (LE01015385 and LE01015387). Beck considered the most typical material (today with the barcode LE01015386) as a robust form (f. *ingens*) of *O. alba* Stephan ex Willd.; remaining specimens, collected at the same place and on the same date (today with the barcodes LE01015385 and LE01015387) and with the number 10544 on their main labels, were determined by him as f. *bidentata* of *O. alba*. Beck seemed to see transitional forms between the specimens of Alexeenko's gathering, while in our opinion, they are all similar and correspond to the same species (*O. ingens* (Beck) Tzvelev). Later, Beck (1930) included his f. *ingens* within the taxonomically elevated var. *bidentata* [*O. alba* var. *bidentata* f. *ingens* Beck], presumably based on the morphology of the calyx segments (deep and long bidentate). Novopokrovsky, in 1947, in his herbaria reviews ('Notae criticae'), considered this species closely related to *O. crenata* Forssk.; on a herbarium label, he even came to subordinate the form described by Beck to *O. crenata*.

Years later, Tzvelev (1957 and 1958 [in Novopokrovsky and Tzvelev 1958]), supposedly based on the material deposited in LE and collected in the Greater Caucasus (cf., for example, LE s.n. – Blyumental and Karpova 1946 n. 139), published *O. alsatica* var. *heraclei* Tzvelev. Tzvelev did not directly or indirectly mention f. *ingens* described by Beck and, furthermore, indicated that his new variety can be found in Azerbaijan extending its distribution range to the Greater Caucasus. Finally, on the basis of the same gathering of Alexeenko used by Beck (1922), Tzvelev (1990) raised the taxon described by Beck (1922) to the rank of species, indicating its correct basionym, but continued to point out its relationship with the *O. alsatica* aggregate.

Tzvelev (1990: 182; 2015: 211) indicated the sheet of Th. Alexeenko (LE s.n.; nowadays as LE01015386) as the 'Typus' of f. *ingens* with the reference to the number '10524' (today in the scan of the LE herbarium covered by a detached flower). This sheet (with a single uniform gathering) is the lectotype (Turland et al. 2018: Art. 9.3). Although Beck did not indicate a single sheet, of the three sheets collected by Alexeenko from this location and available at present in LE, only one was used by Beck to describe his new form (Turland et al. 2018: Art. 9.4, 'original material'). This sheet, on the original label of Alexeenko, has, in the manuscript by Beck, in black ink, the number '10524' and the annotation '*Heracleum parasitica*'. In addition, it contains another label manuscript by Beck, the description of his new form. This description is very similar to that published by Beck (1922, for example, 'Floribus ... 27–28 mm longi'), almost literally copied by himself in 1930. The other two sheets (LE01015385 and LE01015387), which contain Tzvelev's (1988) review label as 'Isotypus', are not really isotypes because they are not a part of the original material used by Beck (cf. the

LE virtual herbarium as ‘Isotype of *Orobanche ingens* (G. Beck) Tzvelev’, who, at the time of describing his f. *ingens*, treated them to belong to a different taxon of the same rank, f. *bidentata*.

Moreover, the new variety (var. *heraclei*), described by Tzvelev (1957) and (1958) [in Novopokrovsky and Tzvelev 1958], was not published validly according to the ICN (Turland et al. 2018). The first time (Tzvelev 1957), it was published with a Russian, but no Latin description or diagnosis, making it a not validly published name (Turland et al. 2018: Art. 39.1). For the second time of publication (Tzvelev 1958) [in Novopokrovsky and Tzvelev 1958], indicated by Tzvelev (2015) as ‘nom. nud.’, the same applies: the name, accompanied by some diagnostic characters, the habitat and the host plant are described, but everything is in Russian without a Latin description or diagnosis.

Clarification and history of the typification

***Orobanche ingens* (Beck) Tzvelev in Novosti Sist. Vyssh. Rast. 27: 182 (1990)**
Figs 1, 2

Orobanche alba f. *ingens* Beck in Repert. Spec. Nov. Regni Veg. 18: 38[470] (1922)

Type. lectotype (Tzvelev 1990: 182; Turland et al. 2018: Art. 9.3, 7.11; we do not know if additional elements were used by Beck): Russia: 1. “Flora Caucasi / 10524 [m. Beck]. / *Heracleum* parasitica [m. Beck] / Prov. Daghestan, Distr. Kasi-Kumukh [Kazi-Kumykh, Laksy distr.]. / Ad viam inter pagos Chelussun et Kumukh / 13 Jul 1897 6400’–7000’ / leg. *Th. Alexeenko*”. – 2. “*Orobanche alba* Steph. nov. forma *ingens* mihi / Luxurians... [description] ... / Cum forma *bidentata* Beck Mon. 211 formis / intermediis conjuncta / Beck [m. Beck]”. – 3. “*Orobanche crenata* Forssk.! / var. *ingens* (Beck) m.c.n. / 15.X.1947 / Determ. I. Novopokrovsky”. – 4. “? *O. crenata* Forssk. / ... / 1955.II.15 Teste: Tzvelev”. – 5. “*Orobanche ingens* (G. Beck) Tzvel / comb. et stat. nov. / Typus! / 1988.III.5 Teste Tzvelev” (LE01015386, Fig. 1).

Additional non-type material. Russia: 1. “Flora Caucasi // 10544. *Orobanche alba* Steph. / f. *bidentata* Beck [m. Beck] // Prov. Daghestan, Distr. Kasi-Kumukh [Kazi-Kumykh, Laksy distr.]. / Ad viam inter pagos Chelussun et Kumukh / 13 Jul 1897 6400’–7000’ / leg. *Th. Alexeenko*”. – 2. “Af. *O. owerini* G. Beck [m. Beck, handwritten on the sheet]”. – 3. “I. Novopokrovsky. Notae criticae / *Orobanche crenata* / Forssk.! / 1948.8.XI”. – 4. “*Orobanche ingens* (G. Beck) Tzvel / Isotypus! / 1988.III Teste Tzvelev” (LE01015385, Fig. 2).

1. “Flora Caucasi / 10544. / *Orobanche alba* Steph. / f. *bidentata* Beck / Prov. Daghestan, Distr. Kasi-Kumukh [Kazi-Kumykh, Laksy distr.]. / Ad viam inter pagos Chelussun [sic] et Kumukh / 6400’–7000’ / 13 Jul 1897 / leg. *Th. Alexeenko* [m. Beck]”. – 2. “an. *O. picridis-hieraciodes* / Holandre [handwritten on the sheet]”. – 3. “*Orobanche ingens* (G. Beck) Tzvel / Isotypus! / *O. crenata* Forsk. / 1955.II.2 [date of the first revision] Teste Tzvelev III.1988” (LE01015387, Fig. 2).

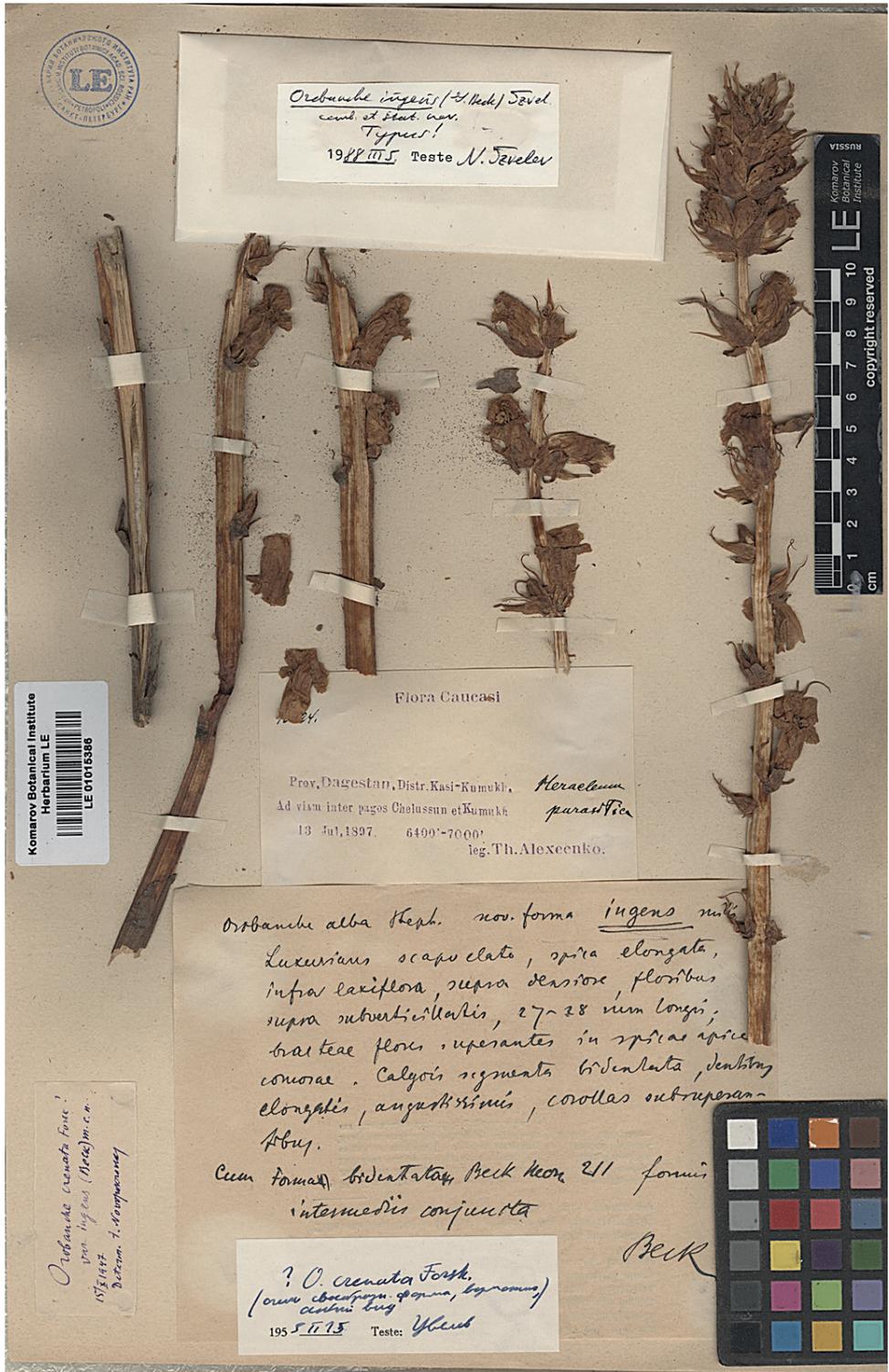


Figure 1. Lectotype of *Orobanche ingens* (LE01015386) (<http://re.herbariumle.ru/01015386>).

Synonyms. *Orobanche alsatica* var. *heraclei* Tzvelev, Fl. Azerb. 7: 591 (1957), nom. inval. (Turland et al. 2018: Art. 39.1); *Orobanche alsatica* var. *heraclei* Tzvelev in Schischk., Fl. SSSR 23: 111 (1958), nom. inval. (Tzvelev 2015: 211, nom. nud. [sic]).

Misapplied names. *Orobanche alba* var. *bidentata* sensu Beck in Engl., Pflanzenr. 96: 155 (1930) [saltem p.p.], non *O. alba* f. *bidentata* Beck in Biblioth. Bot. 19: 211 (1890).

Distribution, ecology and taxonomic problems

General distribution. Caucasus, mainly Greater Caucasus range, Russia (Krasnodar Krai, Karachay-Cherkessia, North Ossetia, Dagestan) and Georgia. Needs confirmation in Azerbaijan. Endemic to the Caucasus (Fig. 3).

Specimens examined. GEORGIA. Racha-Lechkhumi and Kvemo Svaneti prov., tall herb communities, forest edges and glade, near the trail to Udziro lake, 1.5–2 km S of Shovi Village, 42°41'07"N, 43°39'56"E, 2050–2100 m a.s.l., on *Heracleum leskovii*, but probably also on *Ligusticum alatum*, 18 July 2018, R. Piwowarczyk (KTC); RUSSIA: DAGESTAN. Distr. Kasi-Kumukh [Laksky distr.], ad viam inter pagos Chelussun et Kumukh, 6400'–7000', 13 July 1897, Th. Alexeenko (LE) [as *O. ingens* by Tzvelev in 1988, Typus]; prov. Dagestan, distr. Samur, ad riparian fl. Dulty-chaj, 7300', 12 July 1897, Th. Alexeenko (LE) [as "10526" *O. alba* f. *rubiginosa* by Beck (Beck 1930: 148, sub *O. alba*), *O. alsatica* by Novopokrovsky in 1949, *O. flava* by Tzvelev in 1955, *O. ingens* by Tzvelev in 1988]; Dagestan, Dokuzparinskiy distr., Kiler, meadow near road, parasitising on *Heracleum sosnowskyi* [root attachment verified], 41°22'50"N, 47°53'55"E, [ca. 1130 m a.s.l.], 26 June 2021, A.V. Fateryga & S.A. Svirin (KTC, YALT); KARACHAY-CHEKKESSIA. North Caucasus, upper reaches of the Bolshaya Laba river, upper part of Zagedanka river, tall grassy vegetation in subalpine meadow, on the roots of *Heracleum*, 2300 m a.s.l., 19 July 1946, Blyumental & Karpova (LE) [as *O. owerinii* by Novopokrovsky in 1946, *O. alsatica* Kirschl. var. *heraclei* Tzvel. by Tzvelev in 1955, *O. ingens* (G. Beck) Tzvel. by Tzvelev in 1988]; North Caucasus, basin of the Urup river [Kuban distr.], 9 Aug 1945, V.I. Grubov & L.I. Ivanina (LE) [as *O. ingens* by Tzvelev in 1988]; near Dombay, Alibek river valley, 29 July 2014, M. Skotnikova [phot., <https://www.plantarium.ru/page/image/id/268327.html>, <https://www.plantarium.ru/page/image/id/256514.html>, <https://www.plantarium.ru/page/image/id/256512.html>, as *O. alsatica*]; Dombay, Alibek river valley, 26 August 2013, S. Banketov [phot., <https://www.plantarium.ru/page/image/id/205049.html>, <https://www.plantarium.ru/page/image/id/205050.html>, <https://www.plantarium.ru/page/image/id/205051.html>, <https://www.plantarium.ru/page/image/id/205052.html>, as *O. alsatica*]; Karachay-Cherkessia, Dombay, Dombay-Ulgen river valley, above the Russian glade, about 2000 m a.s.l., 21 July 2010, E. Komarov [phot., <https://www.plantarium.ru/page/image/id/66557.html>, <https://www.plantarium.ru/page/image/id/66558.html>]; Karachayevsky distr., near Dombay, 11 Aug 2010, E. Suslova [phot., <https://www.inaturalist.org/observations/70214391>, as *O. alsatica*]; Karachay-Cherkessia, Malokarachaevsky distr., slope of Bolshoi Bermamyt peak, ca. 2000 m a.s.l., subalpine meadow, 5 Aug 2017, T. Gaidash [phot., <https://www.plantarium.ru/page/image/id/527245.html>, <https://www.plantarium.ru/page/image/id/527246.html>].



Figure 2. Additional non-type material of *Orobanche ingens* (LE01015385, LE01015387) (<http://re.herbariumle.ru/01015385>, <http://re.herbariumle.ru/01015387>).



Figure 2. Continued.

html, as *O. grossheimii*); Karachay-Cherkessia, Malokarachaevsky distr., Eshkakon river valley, 15 June 2013, *I. Tabunova* [phot., <https://www.plantarium.ru/page/image/id/193231.html>]; KRASNODAR KRAI. North Caucasus, Krasnodar territory, Mostovskoi distr., 6–7 km N from Shedok, vicinity of Dyatlovo, small piece of fallow land at the field margin, not far from dried-up stream, on *Heracleum mantegazzianum*, 4 June 2002, *D.V. Geltman* (LE) [as *O. alsatica*]; NORTH OSSETIA. Vladikavkaz, July 1888, *Akinfiew* (LE) [as *O. flava?* by Tzvelev in 1955, as *O. ingens* by Tzvelev in 1988]; North Ossetia, Irafsky distr., upper part of Urukht river, ca. 1700 m a.s.l., subalpine meadow, 9 July 2016, *M. Skotnikova* [phot., <https://www.plantarium.ru/page/image/id/479013.html>], as *O. alsatica*].

Habitat. In tall herbaceous habitats, mainly in subalpine meadows, pastures, edges of forests and shrubs, forest glades, near river valleys, as well as fallow lands, usually (1000) 2000–2300 (2500) m a.s.l.

In Georgia, in the locality on a subalpine glade, numerous pollinators of this species – workers of *Dolichovespula sylvestris* (Scop.) (Hymenoptera, Vespidae) – have been observed.

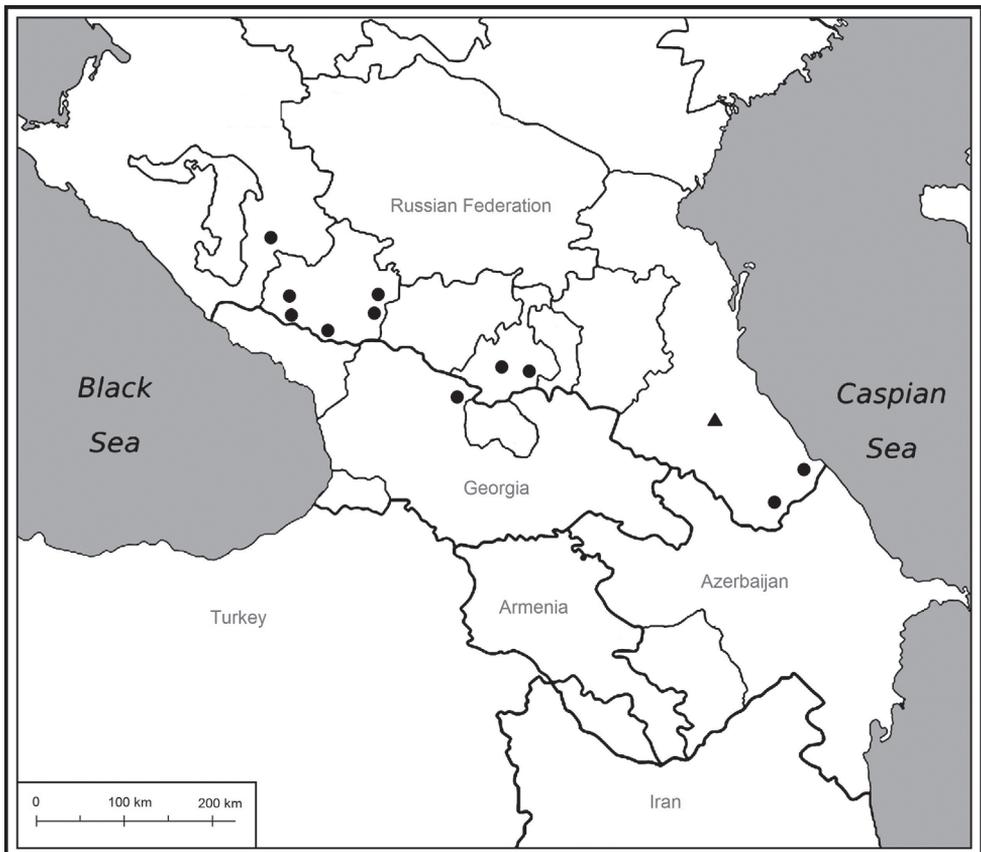


Figure 3. Distribution of *Orobanche ingens*, triangle indicates *locus classicus*.

Host. Parasitises *Heracleum* species (Apiaceae), such as *H. leskovii* Grossh., *H. sosnowskyi* Manden. and *H. mantegazzianum* Sommier & Levier. Probably also on other large Apiaceae species, such as *Ligusticum alatum* (M. Bieb.) Spreng. (= *Cnidiocarpa alata* (M. Bieb.) Pimenov & Kljuykov), but this needs confirmation (observed by R. Piwowarczyk in Georgia, but root attachment was not verified).

Apart from *O. ingens*, only one *Heracleum* parasite is known – *Phelipanche sevanensis* Piwow., Ó. Sánchez & Moreno Mor., described from the slopes of Sevan lake in Armenia, where it parasitises *H. trachyloma* Fischer & Meyer (Piwowarczyk et al. 2017, 2019).

Phenology. Flowering (June) July–August, fruiting (July) August (September).

Note. At first, the species was mistakenly described as a form of *O. alba* (subsect. *Glandulosae*); next Tzvelev (1990, 2015) continued to indicate the relationship of *O. ingens* with *O. alsatica* aggr., especially with *O. bartlingii* Griseb. (subsect. *Curvatae*). However, according to morphological features (Figs 1, 2, 4, Table 1), it is far from the *O. alsatica* group and subsect. *Curvatae*, but belongs to the *Minores/Speciosae* subsection. In the *Minores/Speciosae* subsection, the corolla is usually tubular or campanulate, almost straight in the middle part, the pubescence of the corolla is usually a mixture of longer and stiff whitish hairs, the calyx segments are long, entire or bidentate, usually subulate or filiform at the tip. In contrast, in the subsect. *Curvatae*,

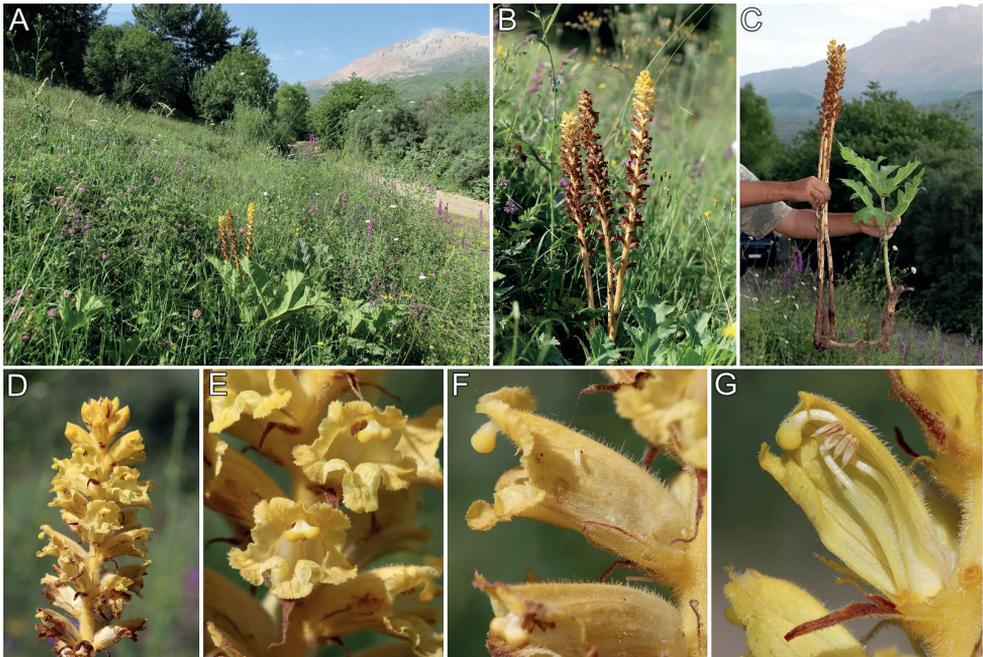


Figure 4. *Orobanche ingens* from Kiler in Dagestan **A, B** habitat and general habit **C** attachment with host root of *Heracleum sosnowskyi* **D** top of inflorescence **E** flowers in front view **F** flower in lateral view **G** flower in longitudinal-section. Phot. A. Fateryga.

Table 1. Distinctive morphological characters and hosts of the studied species and its Caucasian relatives.

	<i>O. bartlingii</i>	<i>O. ingens</i>	<i>O. owerinii</i>	<i>O. laxissima</i>	<i>O. minor</i>
Inflorescence	cylindrical to ovate, ± dense, usually shorter than the rest of the stem	usually long, rarely short (small specimens), cylindrical, somewhat lax, usually many-flowered	usually short-cylindrical to ovate, ± lax, usually few-flowered	usually long, or less frequently short (small specimens), cylindrical, very lax, usually many-flowered	usually long, rarely short (small specimens), cylindrical, dense at the first of anthesis, becoming lax later, usually many-flowered (except small specimens)
Floral bract	about two thirds of the length of the corolla	as long as the corolla tube, slightly shorter than the corolla	as long as the corolla or slightly shorter or longer than the corolla	as long as the corolla tube, slightly shorter than the corolla	as long as the corolla
Calyx segments	bidentate, teeth short, usually subulate at tip	deeply bidentate, teeth frequently filiform	segments entire or less frequently bidentate, narrowly subulate	bidentate, rarely with 4 teeth, teeth narrowly subulate to filiform	bidentate or less frequently entire, teeth subulate to narrowly subulate but not filiform
Corolla, length	12–18 mm	(20–) 23–24 (–30) mm	15–20 (–30) mm	(20) 22–24 (–25) mm	10–19 mm
Corolla, form	tubular	campanulate	campanulate-infundibuliform	tubular-infundibuliform	tubular
Corolla, dorsal line	evenly curved at the proximal and distal part, almost straight at the middle	almost straight, except evenly curved at the proximal part	curved at the proximal part and, sometimes, slightly bent forward at the distal part	almost straight, except evenly curved at the proximal part and, sometimes, slightly bent forward at the distal part	evenly curved at the proximal part, almost straight at the middle and slightly bent forward at the distal part
Corolla, colour	yellow, brownish-yellow, pinkish ± tinged with purple	yellow, rarely violet, dark pink	pale violet to purple, rarely cream	purple, dark or light pink, rarely dirty yellow, light brown, sometimes with ± dark purplish veins	whitish or yellowish-white, more or less coloured purple, with dark purplish veins, rarely entirely yellow
Filaments	inserted 1–3 mm above the corolla base; with long hairs at the basal half and sparsely glandular pubescent under the anthers	inserted 1 (–2) mm above the corolla base; with long hairs at the basal half and sparsely glandular pubescent (almost glabrous) under the anther	inserted 3–4 mm above the corolla base; hairy at the basal half (or third) and sparsely glandular pubescent under the anthers	inserted (2–)3–5 mm above the corolla base; hairy at the base and sparsely glandular pubescent under the anthers	inserted 2–3 (–5) mm above the corolla base; glabrous or sparsely hairy at the base and sparsely glandular pubescent under the anthers
Stigma	yellow	mainly yellow or orange, rarely reddish, violet, dark pink	reddish to deep purple	dark pink, purple, violet, rarely orange, pale to dark yellow	pinkish, ± deep purple, rarely yellow or whitish
Host	Apiaceae (<i>Seesli</i>)	Apiaceae (<i>Heraclenum</i>)	Fabaceae (<i>Trifolium</i> and <i>Vicia</i>) and rarely Asteraceae (<i>Leontodon</i> and <i>Lactuca</i>)	trees and shrubs, Betulaceae, Oleaceae, Fagaceae, Sapindaceae, Cornaceae, Lythraceae, Fabaceae, Anacardiaceae (e.g., <i>Fraxinus</i> , <i>Fagus</i> , <i>Carpinus</i> , <i>Rhus</i>)	Fabaceae, Asteraceae, Apiaceae, etc. (e.g., <i>Trifolium</i> , <i>Medicago</i> , <i>Hypochaeris</i> , <i>Leontodon</i> , <i>Chondrilla</i> , <i>Daucus</i>)

the corolla is usually tubular-infundibuliform or \pm broadly tubular, the dorsal line of the corolla is evenly curved over its entire length, the pubescence is rarely a mixture of whitish hairs. Molecular studies do not indicate the validity of dividing the species into the subsect. *Speciosae* because the species included here are both morphologically and genetically very similar to those of the subsect. *Minores* (Piwowarczyk et al. 2021). It is worth emphasising that individuals of *O. ingens* are usually very tall, over 60 to 100 cm, with corolla (20–) 23–24 (–30) cm long, usually with a mixture of longer and stiff whitish hairs (Fig. 4F; without dark glandular hairs which are characteristic for the subsect. *Glandulosae*) and both the corolla and stem can be very variable in colour, even within one population, from yellow to pink and purple; additionally, the colour of the stigma can be variable, from yellow through to orange, pink to purple (Fig. 4 and photos from Plantarium.ru and iNaturalist.org, links above).

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Townsendia lemhiensis (Asteraceae, Astereae): A narrowly endemic new species from Idaho, USA

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Abstract

Townsendia lemhiensis (Asteraceae) is described from the Lemhi Valley of east-central Idaho. From a genus with weak intrinsic isolating barriers, *T. lemhiensis* remains distinct apparently due to apomixis and to its isolation and habitat specialization on spatially limited occurrences of ashy white soils in the Lemhi Valley. Despite similarities to *T. spathulata*, this new species differs in its persistent pappus, fewer series of phyllaries and sericeous rather than long woolly hairs.

Keywords

Apomixis, compositae, Flora of Idaho, Lemhi Valley

Introduction

Townsendia Hook. includes about 29 recognized species distributed predominantly throughout the Rocky Mountain and Great Basin regions of western North America (Larsen 1927; Beaman 1957; Shultz and Holmgren 1980). Species of *Townsendia* are generally characterized by showy inflorescences nestled in a dense rosette of leaves or on short stems; and by a pappus of short bristles. Many species also have strong

edaphic affinities that delimit their distribution (Lowrey and Knight 1994). Six species of *Townsendia* are known to occur in Idaho (*T. condensata* Parry, 1874, *T. florifera* (Hook.) A.Gray, 1880, *T. hookeri* Beaman, 1957, *T. leptotes* (A.Gray) Osterh., 1908, *T. montana* M.E.Jones, 1895, and *T. parryi* D.C.Eaton, 1874), yet none of these species approach the combination of characteristics present in *T. lemhiensis*.

Methods

Field methods

Specimens were initially collected during cursory exploration by Curtis Björk on May 20th, 2012. Curtis Björk further sampled the area in 2013, and two additional populations were found. In 2018, Chris Lee and Jeannette Whitton used localities provided by Curtis Björk to collect additional samples. We also used satellite imagery of nearby areas to identify potential habitat for *T. lemhiensis*, and these areas were targeted for additional surveying. Several voucher specimens were taken during each site visit, and dried between newspaper and cushioned cardboard in a plant press. Characters were measured directly from herbarium specimens. Herbaria acronyms follow Index Herbariorum (Thiers 2016).

Pollen counts

We removed 2–3 florets from individual plants and placed them in a 200 µL microtube. After adding approximately 20 µL of lactophenol blue stain to each tube, we vortexed tubes for 20–30 seconds to release pollen into suspension. Slides were prepared using the resulting suspension, and viewed under a standard light microscope. This procedure was repeated multiple times on various specimens but yielded no pollen grains, thus no pollen counts or measurements could be completed. We later examined disk florets from individuals of all known populations under a dissecting microscope, and found that stamens were abortive and evidently non-functional; no anthers were observed.

Taxonomic treatment

Townsendia lemhiensis C.Lee, Björk & Whitton, sp. nov.

urn:lsid:ipni.org:names:77296133-1

Holotype. *Björk* 29248 (UBC V252324) 20 May 2012. USA, Idaho, Lemhi County, 18 Mile Wilderness Area, ca. 24 km SSE of Leadore, 44.44806°N, 113.17222°W, on dry ashy white soil on slope (7368 ft) in sagebrush steppe (Fig. 1A). **Paratypes:** *Björk* 30627 (UBC V252325) 14 May 2013. USA, Idaho, Lemhi County, 18 Mile Wilderness Area, on a ridge crest near the middle road, 44.46707°N, 113.22570°W, on ashy



Figure 1. **A** habitat of the type locality of *Townsendia lemhiensis* **B** habit of *Townsendia lemhiensis* at the type locality **C** close-up of *Townsendia lemhiensis* capitulum **D** close-up of *Townsendia lemhiensis* phyllaries. Photos A-B by Björk; Photos C-D by Whitton.

white slope, sparsely vegetated, soil derived from tuff conglomerates, in upper elevation (6968 ft) sagebrush steppe in intermontane valley; *Björk* 30772 (UBC V252326) 21 May 2013. USA, Idaho, Lemhi County, 18 Mile Wilderness Area, south of McFarland Road, 44.48000°N, 113.20333°W, on ashy white flats and slopes, sparsely vegetated, in upper elevation (6857 ft) sagebrush steppe in intermontane valley; *Whitton* 252 (UBC V252322) 13 May 2018. USA, Idaho, Lemhi County, 18 Mile Wilderness Area, 44.47293°N, 113.20670°W, on open hillside (7355 ft) with sparse sagebrush and antelope brush on grey-brown rocky substrate; *Whitton* 256 (UBC V252323) 14 May 2018. USA, Idaho, Lemhi County, 18 Mile Wilderness Area, 44.44530°N, 113.17541°W, on exposed rocky slope (7371 ft), in areas of grey-white substrate, with bunchgrass, and antelope brush.

Ab *Townsendia spathulata* pappi ad maturitatem persistens, series phyllariorum pauciores, pilis caulibus sericeus non villosus, folia non carnosa differt.

Description. Plants perennial, 25–30 mm tall, rosette forming, rosettes 8–17 mm wide, solitary or (more often) arising from a few-branched caudex; stems essentially absent, or when visible, then villosulous; leaves 4–14 × 2 mm, narrowly oblanceolate to narrowly elliptic-oblanceolate, silvery, moderately sericeous but not villous, apex acute; capitula one per fertile rosette, sessile or nearly so; involucre campanulate, 9–10 mm

wide, phyllaries appressed, narrowly elliptic-lanceolate, 6–7 × 1 mm, graduated, in 2–3 series, reddish brown, sericeous, apex acute to acuminate; ray florets 15–24, pistillate; ray corollas true ray, 7–8 mm long, surpassing pappus bristles, light brownish pink or whitish and tipped in pink, slightly darker abaxially, aglandular; disc florets 24–30, functionally pistillate; disc corollas tubular, 4 mm long, shortly surpassed by the pappus bristles, yellow, aglandular; stamens reduced to strap-like staminodes, anthers and pollen absent; cypselae 3 × 1 mm, oblanceolate and compressed, with glochidiate hairs; pappus bristles 23–26 in disc florets, 14–20 in ray florets, 6–7 mm long, white, barbelate, persistent. (Figs 1B–D, 2A–F).

Etymology. *Townsendia lemhiensis* is named after the Lemhi Valley, Idaho, where individuals of this species were first noticed by Curtis Björk.

Distribution and habitat. *Townsendia lemhiensis* is known only from the Lemhi Valley in east-central Idaho, which is situated within a region known for its numerous geographically endemic plants (Moseley and Conley 1989; Moseley et al. 1990; Ertter and Moseley 1992). The species has been found in three populations, within an area of 4.9 km².

Townsendia lemhiensis grows on ashy white slopes of eroded rhyolite tuff. These slopes of powdery soils and friable rock are sparsely vegetated, forming edaphic islands of open ground within a more densely vegetated surrounding matrix of sagebrush steppe. Numerous other plants having narrow geographical ranges occupy similar ashy slopes elsewhere in dry interior regions of western North America (Grimes 1984; Reveal and Björk 2004; Brown and Mansfield 2017). The Lemhi Valley is lined on the east and west by alpine ridges, and the valley floor sits at a high elevation, making it cooler than the sagebrush steppes both further south on the adjacent Snake River Plains and further north in the Salmon-Challis Valleys region.

Associated species growing with *Townsendia lemhiensis* on the ashy slopes include: *Artemisia frigida* Willd., 1838, *Artemisia tridentata* subsp. *wyomingensis* Beetle & A.L.Young, 1965, *Chaenactis douglasii* Hook. & Arn., 1839, *Comandra pallida* A.DC., 1857, *Cymopterus bipinnatus* S.Wats., 1885, *Elymus lanceolatus* (Scribn. and J.G.Sm.) Gould, 1949, *Eriogonum mancum* Rydb., 1917, *Eriogonum soliceps* Reveal & Björk, 2004, *Ipomopsis spicata* subsp. *orchidacea* (Brand) D.Wilken & R.L.Hartm., 1991, *Linum lewisii* var. *alpicola* Jeps., 1936, *Oreocarya humilis* Greene, 1896, *Packera cana* (Hook.) W.A.Weber & Á.Löve, 1981, *Penstemon humilis* Nutt. ex A.Gray, 1862, *Phlox muscoides* Nutt., 1831, *Stenotus acaulis* (Nutt.) Nutt., 1840, *Townsendia hookeri* Beaman, 1957, *T. leptotes* (A.Gray) Osterh., 1908, and *T. parryi* D.C.Eaton, 1874.

Phenology. We observed *T. lemhiensis* in flower in mid-May over three years (2012, 2013, 2018). At this time, some individuals had some capitula in bud, and others had mature seeds or open flowers. On this basis, we describe flowering as likely occurring throughout May, and seedset through late May or possibly into June. Further studies are needed to document the timing of bud formation, and potential variation in flowering and fruiting phenology. Another early-flowering species, *Townsendia hookeri*, co-occurs with *T. lemhiensis*, and has been observed to set buds in fall that open soon after snow melt (Lee and Whitton, personal observation). Given the early flowering

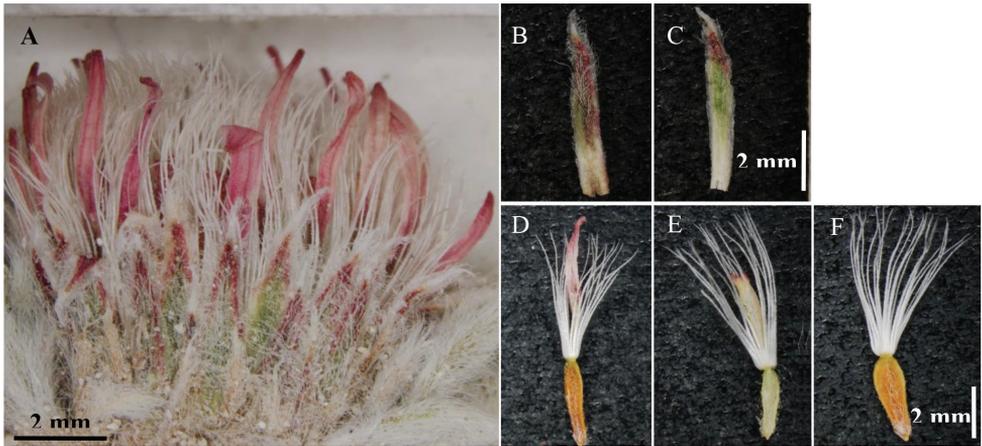


Figure 2. **A** capitulum **B** outer involucre bract **C** inner involucre bract **D** ray floret **E** disc floret **F** mature disc cypselus. Photos by Spencer Goyette (UBC Herbarium).

of *T. lemhiensis* and the presence of snow patches persisting in surrounding areas, we suspect fall bud set may also occur in this species. Co-occurring species of *Townsendia* were found on site in bud (*T. parryi*) and late-bud (*T. leptotes* and *T. hookeri*).

Discussion

Townsendia lemhiensis is most similar in morphology to *T. spathulata* Nutt., which shares a generally hairy appearance. However, *T. lemhiensis* is morphologically distinct because its leaves are narrowly oblanceolate with short dense pubescence, instead of the fleshy, spathulate leaves covered in long, tangled hairs in *T. spathulata*. Also, the cypselae of *T. lemhiensis* have persistent pappi rather than the rare characteristic (in *Townsendia*) of deciduous pappi found only in *T. spathulata*, *T. microcephala* and *T. condensata* (Beaman 1957; Dorn 1992). Its capitulum is protected by fewer phyllary series (2–3), rather than 3–4 phyllary series found in *T. spathulata*.

Townsendia lemhiensis is morphologically distinct from the seven other species of *Townsendia* known from this region. We include *T. spathulata* here, because of its similarity to *T. lemhiensis*, and because it has been documented in the Beaverhead range overlooking the Lemhi Valley, Idaho (>9000 ft) at the border with Montana (Fig. 3). The seven species of *Townsendia* in Idaho and adjacent Montana can be distinguished using the taxonomic key below. Four of these species tend to occur at higher elevations (*T. condensata*, *T. leptotes*, *T. spathulata* and *T. montana*), while *T. florifera* is often at lower elevations than *T. lemhiensis*, and is associated with basalt. We found *T. parryi*, *T. leptotes* and *T. hookeri* co-occurring with *T. lemhiensis*, but these species were in bud, while *Townsendia lemhiensis* was in full flower. The nearby locality of *T. spathulata* is

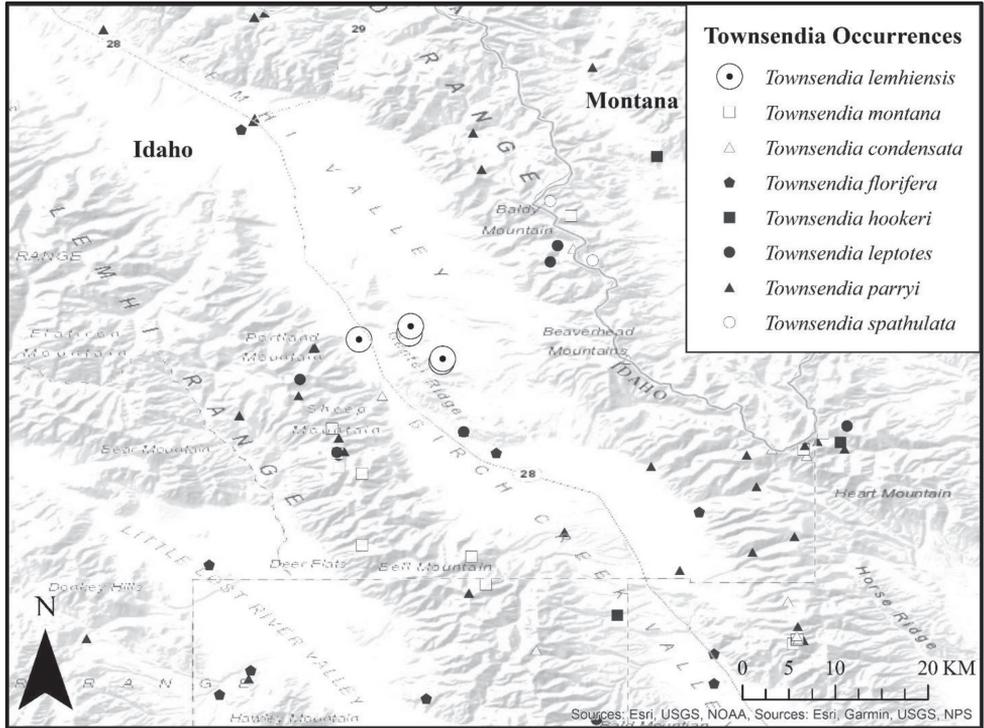


Figure 3. Occurrence records of *Townsendia lemhiensis* and other nearby *Townsendia* spp. (ArcMap v.10.3.1) (Consortium of Pacific Northwest Herbaria 2022)

somewhat unusual; more typically, *T. spathulata* inhabits the semi-arid plains of Wyoming and Montana along the eastern side of the Great Divide.

Although many *Townsendia* species occupy distinct geographical ranges and possess a multitude of unique character traits, Beaman (1957) describes them as lacking strong (intrinsic) genetic isolating barriers, based on crossing studies in the greenhouse, and on observed patterns of morphological intergradation in nature (Beaman 1954). While interbreeding is sometimes possible in cultivation, and likely occurs in nature, geographic isolation, phenological differences, and habitat differentiation, especially edaphic specialization, contribute to maintaining species boundaries in this genus. The species recognized by Beaman all include sexually reproducing populations, but apomictic populations also occur in a number of *Townsendia* species (Beaman 1954, 1957). Sexual populations are diploid and outcrossing, while known apomicts are triploid or tetraploid (Beaman 1954). Sexual and apomictic forms typically have distinct (sometimes overlapping) distributions, and provide classic examples of geographical parthenogenesis (Bierzchudek 1985). Apomixis also contributes to maintaining species boundaries, because hybridization involving apomicts is not likely. As a result, hybrids will not necessarily be produced, even where multiple *Townsendia* species co-occur.

Although direct genetic or experimental evidence of apomixis is not available for *T. lemhiensis*, no pollen was detected on florets sampled from our collections, which strongly suggests that these populations are apomictic. As a result, despite the physical proximity of *T. lemhiensis* to *T. hookeri*, *T. leptotes*, and *T. parryi*, hybridization is unlikely given that in this region, all four species are likely apomicts (Beaman 1957; Thompson and Whitton 2006). We note that while it is uncommon for species in *Townsendia* to be recognized based solely on apomictic populations, to date, apomicts have generally fit within the boundaries of morphological descriptions of known sexual populations. In this case, *T. lemhiensis* has no known sexual populations; whether these are undiscovered or extinct, or whether *T. lemhiensis* is of hybrid origin is not known.

The first author examined the majority of *Townsendia* specimens from UBC, UAC, SASK, RM, CS, and UNLV from 2008–2014, and the second author examined all Asteraceae at ID in 2002, and did not find any specimens of *Townsendia lemhiensis*. Additionally, from 2008–2014, the first author undertook targeted searches for all species of *Townsendia* throughout their range in WA, ID, MT, WY, CO, NV, NM and CA, and no similar populations were encountered.

Despite the second author's searches in apparently suitable habitats throughout the Lemhi Valley and adjacent valleys in Idaho and Montana in the years 1999–2013, only the three reported populations have been located. Thus it appears that *T. lemhiensis* is a high priority for conservation. All known occurrences of *T. lemhiensis* are situated on public land administered by the Bureau of Land Management. Hence, the landscape surrounding this species is somewhat protected from threats, but such a small, rare species may still undergo population reduction from factors such as trampling by cattle or recreationists, invasive exotic plant species, or from climate change that could cause higher frequency and severity of drought and excessive heat events.

Key to *Townsendia* of Idaho and adjacent Montana

- 1a Lax-growing plants with elongated stems, many internodes > 2 mm long, plants often lacking sterile rosettes when flowering **2**
- 2a Stems erect or sub-erect; longest phyllaries > 9 mm long..... **3**
- 3a Rays purplish; longest phyllaries mostly > 10 mm long; stems erect, unbranched..... ***T. parryi***
- 3b Rays white or pink; longest phyllaries mostly < 10 mm long; stems sub-erect, often branched..... ***T. florifera***
- 2b Stems decumbent; longest phyllaries up to 9 mm long..... **4**
- 4a Stems gray-white, obscured by dense hairs ***T. incana***
- 4b Stems lightly to moderately strigose ***T. strigosa***
- 1b Compact plants, stems scarcely elongated, internodes < 2 mm long; sterile rosettes commonly present..... **5**
- 5a Plants with abundant spreading hairs..... **6**
- 6a Pappus persistent; leaf hairs sericeous..... ***T. lemhiensis***

- 6b Pappus easily breaking away from the cypselae at maturity; leaf hairs villous7
- 7a Phyllaries > 40, the longest ones > 9 mm long, involucre > 12 mm long.....*T. condensata*
- 7b Phyllaries < 40, the longest ones < 9 mm long, involucre < 12 mm long.....*T. spathulata*
- 5b Plants with appressed hairs only, or spreading hairs few and inconspicuous .. **8**
- 8a Phyllaries 2–5 × long as wide; rays purplish, often glandular abaxially**9**
- 9a Phyllaries acute to acuminate; ray laminae 5–8 mm long.....*T. leptotes*
- 9b Phyllaries blunt; ray laminae 7–16 mm long.....*T. montana*
- 8b Phyllaries at least 6 × long as wide; rays white to pink, glabrous.....**10**
- 10a Longest phyllaries > 12 mm long and > 1.5 mm wide; disc pappi 6.5–11+ mm long; leaf midvein apparent*T. exscapa*
- 10b Longest phyllaries < 12 mm long and ca. 1 mm wide; disc pappi 4–6(–7.5) mm long; leaf midvein obscure*T. hookeri*

Acknowledgements

We are grateful to Ryan Batten for his assistance in the field. The curatorial staff of UBC, CS, ID, RM, SASK, UAC, and UNLV are thanked for their kind and generous help facilitating specimen research. In particular, we appreciate the rapid assistance from Linda Jennings and Spencer Goyette at UBC in processing accession numbers, and mounting, photographing and scanning specimens for this study.

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Supplementary material I

Townsendia lemhiensis occurrence records

Authors: Christopher Lee, Curtis R. Björk, Jeannette Whitton

Data type: Occurrence.

Explanation note: Type and paratype specimens collected of *Townsendia lemhiensis*.

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Link: <https://doi.org/10.3897/phytokeys.193.76365.suppl1>

Elsholtzia zhongyangii (Lamiaceae), a new species from Sichuan, China

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Abstract

Elsholtzia zhongyangii (Lamiaceae), a new species from Sichuan Province, China, is described and illustrated. The new species is morphologically similar to *E. feddei* f. *feddei*, but it can be easily distinguished from *E. feddei* f. *feddei* by smaller corolla (3.2–3.5 mm vs. 4.5–5.3 mm), bract indumentum (glabrous, except margin ciliate vs. villous, especially on veins abaxially, glabrous adaxially) and bract stalked (ca. 1.2 mm vs. sessile). Phylogenetic analyses, based on two nuclear ribosomal (ETS, ITS) and five plastid (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) regions, confirmed that the new species formed a monophyletic clade with robust support. The new species is currently known from western Sichuan.

Keywords

Aromatic plant, Elsholtzieae, phylogeny, traditional medicinal herbs, taxonomy

* These authors contributed equally to this work.

Introduction

Elsholtzia Willdenow (Lamiaceae) is a member of the tribe Elsholtzieae (Harley et al. 2004). The genus is characterised by verticillasters in continuous or interrupted spikes or capitula, compact spikes cylindric or secund, and two-lipped corolla (Huang 1977) (Huang 1977). *Elsholtzia* is mainly distributed in East Asia, with 35 species found in China (Huang 1977; Li and Hedge 1994; Harley et al. 2004; Pu et al. 2012; Xiang and Liu 2012). Many species of *Elsholtzia* are important plant resources used both as medicine and flavouring. More than 60% of the Chinese species have been widely used as traditional medicinal herbs, nectar source plants, vegetables and spices (Huang 1977; Li and Hedge 1994; Editorial Board of Zhong Hua Ben Cao 1999).

During our botanical expedition to Yajiang County, Sichuan Province in September 2012, we discovered an unknown species of *Elsholtzia*. It is similar to *Elsholtzia feddei* f. *feddei* in calyx (villous, with two long and three short teeth with spinescent apices) and leaf apices acute, but differs from the latter by its smaller corolla (3.2–3.5 mm vs. 4.5–5.3 mm), bract glabrous, with a ciliate margin (vs. villous, especially on veins abaxially, glabrous adaxially) and bract stalked (ca. 1.2 mm vs. sessile). After carefully checking specimens and literature, together with evidence from molecular phylogenetic analyses, based on combined nrDNA (ETS, ITS) and combined ptDNA (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) datasets, we demonstrated that it is, indeed, a new species which is described and illustrated here.

Materials and methods

Morphological study

The morphological characters were examined, based on the living plants and specimens. *Elsholtzia* specimens, collected from Sichuan, were checked in the Herbaria of **CDBI**, **CDCM**, **CQNW**, **HITBC**, **HNWP**, **HZU**, **IBK**, **IBSC**, **KUN**, **NAS**, **PE**, **SM**, **SZ**, **TIE**, **WCSBG**, **WUK** and **WZUH** (acronyms as in Thiers 2021).

Taxon sampling and molecular analyses

A total of 48 individuals of *E. zhongyangii* were collected from Yajiang County, Sichuan Province, China from September 2012 to December 2021 (Fig. 1). Voucher specimens were deposited in Wenzhou University (**WZUH**). The nuclear (ETS, ITS) and plastid (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) regions were used for reconstructing the phylogeny of the new species and its related taxa (Li et al. 2017). A total of 20 individuals, representing 16 species of *Elsholtzia*, were sampled, with *Elsholtzia densa* Benth. as the outgroup. The GenBank accession numbers are listed in Suppl. material 1. Most sequences were downloaded from GenBank, except for the new species, which was newly sequenced in the present study.

Two samples of the new species were sequenced using the genome skimming approach, then 14 sequences [each sample has two nrDNA (ETS, ITS) and five ptDNA (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) sequences as listed in Suppl. material 1] were mined for the phylogenetic analyses. Genomic DNA was extracted from approximately 20 mg of silica-gel-dried leaves using DNA Plantzol Reagent (Hangzhou Lifefeng Biotechnology Co., Ltd.) according to the manufacturer’s protocol. For each species, genomic DNA ($m > 1 \mu\text{g}$, $c > 12.5 \text{ ng}/\mu\text{l}$) was sheared (yielding $< 800 \text{ bp}$ fragments) and the quality of fragmentation was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies). Preparation of short-insert (350 bp) paired-end libraries and sequencing were performed by the Beijing Genomics Institute (Shenzhen, China). These samples were pooled with others and run in a single lane of an Illumina HiSeq XTEN with a read length of 150 bp. For the two samples of the new species, we used the GetOrganelle pipeline (Jin et al. 2020) for de novo assembly of plant plastome and nuclear ribosomal RNA (ETS-18S-ITS1-5.8S-ITS2-26S-ETS). The nuclear (ETS, ITS) and chloroplast (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) segments were extracted from these assembled genomic sequences.

Maximum Likelihood (ML) analyses were performed in IQtree v.1.6.12 (Nguyen et al. 2015). The best-fitting substitution models (GTR+F+G4 for nrDNA and TVM+F+G4 for ptDNA) were selected by ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) according to the Bayesian Information Criterion (BIC). An ultrafast bootstrap (UFB) (Bui et al. 2013) of 1000 replications and the SH-aLRT

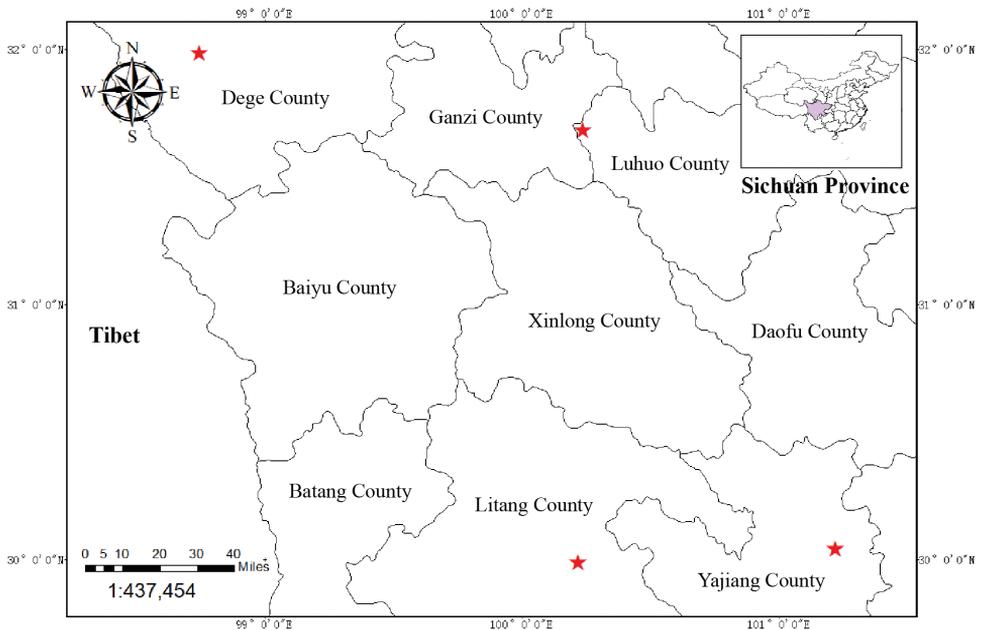


Figure 1. Distribution of *Elsholtzia zhongyangii* Pan Li & X.J. Jin. The red stars indicate the recorded localities of *E. zhongyangii*.

test were used in the analysis to assess branch supports. Bayesian Inference analyses (BI) were conducted in MrBayes 3.2.6 online interface (Miller et al. 2010). MrModeltest 2.3 (Nylander 2004) was used to determine the appropriate DNA substitution model using the Akaike Information Criterion (AIC) and the results indicated that GTR+G (both for nrDNA and ptDNA) is the best-fit model. Four (one cold and three hot) simultaneous Markov chains were run for one million generations with sampling every 1000th generation until the average deviation of split frequencies fell below 0.01. The posterior distribution of trees was summarised by the > 50% majority rule consensus tree after discarding the first 25% of samples as burn-in.

Incongruences amongst different datasets (combined ptDNA dataset, combined nrDNA dataset) were explored through visual comparison of tree topologies and support values. Hard incongruence was defined as BS \geq 80% and/or PP \geq 0.95 (Pelsner et al. 2010).

Results

Morphological comparison

Detailed morphological comparisons between the new species and four other sympatric or morphologically similar taxa are summarised in Table 1. In morphology, the putative new species is most similar to *Elsholtzia feddei* f. *feddei*, sharing features, such as two long and three short calyx teeth with spinescent apices, white villous outside of the calyx, acute leaves apex, obtuse serrate leaf margin and villous leaves. However, the new species differs from the latter by smaller corolla (3.2–3.5 mm), bract stalked (ca. 1.2 mm vs. sessile) and bract glabrous, except margin ciliate (Table 1, Figs 3–4). The other four species could be easily distinguished from the new species by their larger corolla (4.5–7 mm vs. 3.2–3.5 mm) and sessile bract (sessile vs. stalked ca. 1.2 mm) (Table 1, Fig. 4).

Molecular phylogenetics

The aligned sequences of nrDNA (ETS, ITS) and ptDNA (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) for phylogenetic analyses were 1,023 bp and 7,010 bp in length, respectively. *Elsholtzia zhongyangii* was recovered as a monophyletic clade in the two resulting phylogenetic trees obtained in this study (PP: 100, BS: 1, Fig. 2). In the ptDNA tree, *E. zhongyangii* formed a sister group to the *E. argyi-E. splendens* clade consisting of *E. argyi*, *E. minima*, *E. hallasanensis*, *E. splendens* 1, *E. splendens* 2 and *E. saxatilis* (PP: 100, BS: 1, Fig. 2). *Elsholtzia zhongyangii* and the *E. argyi-E. splendens* clade were sister to the *E. feddei-E. strobilifera* clade (PP: 95, BS: 1, Fig. 2). In the nrDNA tree, based on nrDNA, *E. zhongyangii* was sister to the *E. feddei* f. *feddei-E. ciliata* clade and the *E. argyi-E. splendens* clade, with *E. strobilifera* as one of the basal clades (Fig. 2).

Table 1. Morphological comparisons among *Elsholtzia zhongyangii* and its close relatives.

Species Characters		<i>Elsholtzia zhongyangii</i>	<i>E. splendens</i>	<i>E. feddei</i> f. <i>robusta</i>	<i>E. feddei</i> f. <i>feddei</i>	<i>E. ciliata</i>	<i>E. souliei</i>
Plant	Tall	10–45 cm	30–50 cm	20–30 cm	10–20 cm	30–50 cm	< 10 cm
Inflorescence	Bract villous	Glabrous	Glabrous	Densely villous abaxially, glabrous adaxially	Villous, especially on veins abaxially, glabrous adaxially	Subglabrous abaxially, glabrous adaxially	Villous abaxially, glabrous adaxially
Flower	Corolla size	3.2–3.5 mm	6–7 mm	4.6–5.0 mm	4.5–5.3 mm	ca. 4.5 mm	ca. 6 mm
	Calyx villous	White villous outside	White hispidulous	White villous outside	White villous outside	Pilose	White villous
	Calyx apices	Apex spinescent	Caudate	Apex spinescent	Apex spinescent	Apex spinescent	Apex spinescent
	Calyx length	Two long and three short	Subequal	Two long and three short	Two long and three short	Two long and three short	Two long and three short
	Bract	Stalked, ca. 1.2 mm	Sessile	Sessile	Sessile	Sessile	Sessile
Leaves	Apices	Acute	Acuminate	Acute	Acute	Acuminate	Acuminate
	Margin	Obtuse serrate	Remotely serrate	Obtuse serrate	Obtuse serrate	Denticle	Obtuse serrate
	Blade	Villous	Sparsely fine pilose	Villous	Villous	Sparsely minutely hispid	Villous

Visual comparison of the resulting topologies, based on supporting values, revealed well-supported discrepancies for the combined nrDNA (ITS, ETS) and combined ptDNA (*rbcL*, *matK*, *trnL-F*, *ycf1*, *ycf1-rps15*) datasets. Two trees received strong support, but with conflicting tree topologies. The *E. feddei*-*E. ciliata* clade was sister to *E. strobilifera* in the ptDNA tree (PP: 99, BS: 1, Fig. 2), but was sister to *E. argyi*-*E. splendens* clade in the nrDNA phylogeny (PP: -, BS: 0.89, Fig. 2). Due to the discrepancies between the ptDNA and nrDNA datasets, we did not combine the two matrices to further reconstruct the phylogeny.

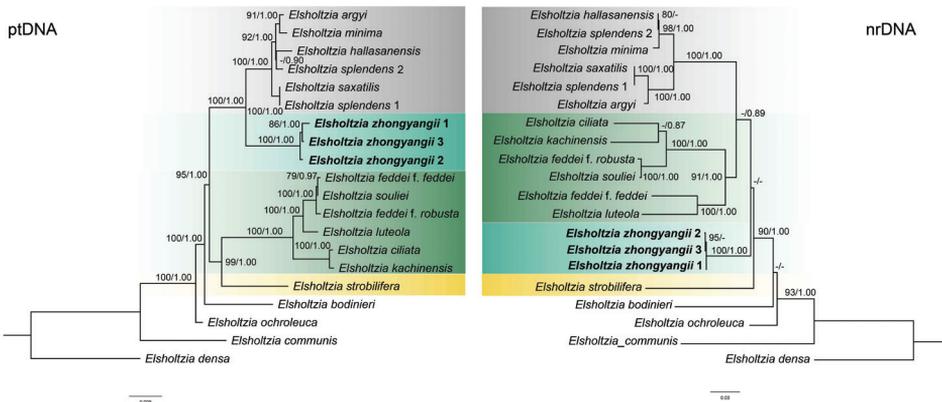


Figure 2. The Maximum Likelihood tree, based on the combined ptDNA (*ycf1*, *ycf1-rps15*, *rbcL*, *trnL-F*, *matK*) and nrDNA (ITS, ETS) dataset without gap coding. Support values displayed on the branches follow the order ML-BS/BI-PP (“-” indicates support values of less than 75% or less than 0.85).

Taxonomic treatment

Elsholtzia zhongyangii P. Li & X.J. Jin, sp. nov.

urn:lsid:ipni.org:names:77296134-1

Type. China. Sichuan: Yajiang County, Agakeyong, 30°2.54'N, 100°15.01'E, 3237 m a.s.l., 18 Sep 2018, *Pan Li* LP185940 (holotype, ZM; isotypes CSH, CDBI, HZU, KUN, PE, SZ, WZUH). (Fig. 3 and Fig. 5)

Diagnosis. *Elsholtzia zhongyangii* is most similar to *E. feddei* f. *feddei* morphologically in having calyx villous, spinescent calyx apex, two long and three short calyces and acute leaf apices, but differs from the latter by its smaller corolla (3.2–3.5 mm vs. 4.5–5.3 mm), bract stalked (ca. 1.2 mm vs. sessile) and bract glabrous, except margin ciliate (vs. villous, especially on veins abaxially, glabrous adaxially).

Description. Annual herbs, erect. Stems 10–45 cm tall, tawny purple; pilose or pilulose, multi-branched above base, branch apex with inflorescence, internode 0.5–4.8 cm long. Leaves ovate triangular, ovate oblong to oblong lanceolate or lanceolate, 0.7–3.6 cm long and 0.3–1.7 cm wide, apex acute, base broadly or narrowly cuneate, extending to petiole, with dense glandular. Leaves villous adaxially, villous especially on veins abaxially. Leaf margin serrate, obtuse, occasionally acute, serrate margin usually purple. Inflorescence a terminal spike, secund, ca. 0.9–7.9 cm, flowers 8–14 at each node of the inflorescence (a pair of cymes); bracts subcircular to broadly ovate, ca. 1.2–3.2 × 1.1–4.5 mm, caudate cuspidate, apex ca. 0.7–2.1 mm, glabrous, except margin ciliate, with dense glandular, dark magenta at maturity, bract stalked, ca. 1.2 mm. Pedicel ca. 2 mm long, enlarged at apex, glabrous. Calyx ca. 2.0–3.2 mm, teeth 5, teeth triangular, two long and three short, apex spinescent, white hirsute, margin ciliate. Corolla deep purple, 3.2–3.5 mm, slightly incurved, narrowly funnel-shaped, with hairs transparent and purple interlaced, villous outside, pilose annulate inside, the base of the corolla tube is about 0.5 mm wide, widening upwards, throat about 1.2 mm wide, upper lip emarginated; middle lobe of lower lip semicircular, lateral lobes subcircular, shorter than middle lobe. Stamens 4, protruding from corolla, the anterior longer than the posterior, filaments are glabrous, anthers purple-black. Style longer than stamens at maturity, exerted, 2-cleft, with lobe equal, linear. Ovary 4-cleft; disc persistent, 1 nectary rises on the edge of the ovary. Nutlets 4, brown, oblong, ca. 1.1 mm long, 0.7 mm in diameter, surface becoming mucilaginous when wetted.

Phenology. Flowering and fruiting from September to December.

Chinese name. Zhong-yang-xiang-ru (钟扬香薷).

Etymology. The specific epithet is named in memory of Prof. Yang Zhong, a Chinese botanist who was dedicated to botanical research and education in Xizang (Tibet), China.

Distribution, ecology and habitat. – Endemic to China, Sichuan Province, Yajiang County (Wolonsi, Weidi, Agakeyong, Jialulongba and Zhusang), Litang County (Wenquan), Ganzi County, Dege County (Ezhi), Luhuo County (Zhuwo) and Batang County. Growing in grassland on mountain slopes or even along the road, at an elevation of 3000–4000 m.

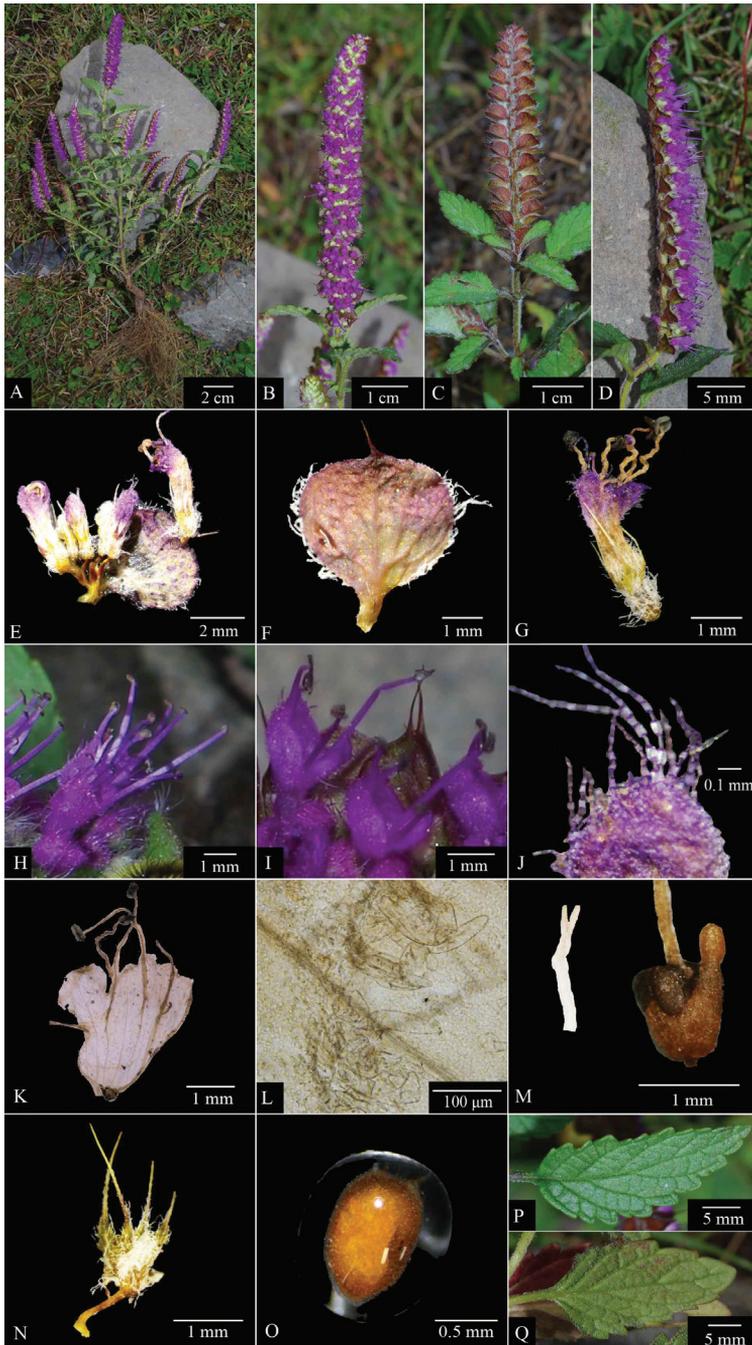


Figure 3. *Elsholtzia zhongyangii* sp. nov. **A** blooming plant **B** front view of inflorescence **C** dorsal view of inflorescence **D** side view of inflorescence **E** cyme **F** bract **G** flower **H** side view of flower **I** front part of flower **J** villous outside corolla **K** dissected corolla **L** pilose annulate inside **M** ovary 4-cleft, 1 nectary rises on the edge of the ovary **N** outside of calyx **O** nutlet (with mucilage) **P** padaxial surface of leaf **Q** abaxial surface of leaf. Photos by Xin-Jie Jin & Pan Li.

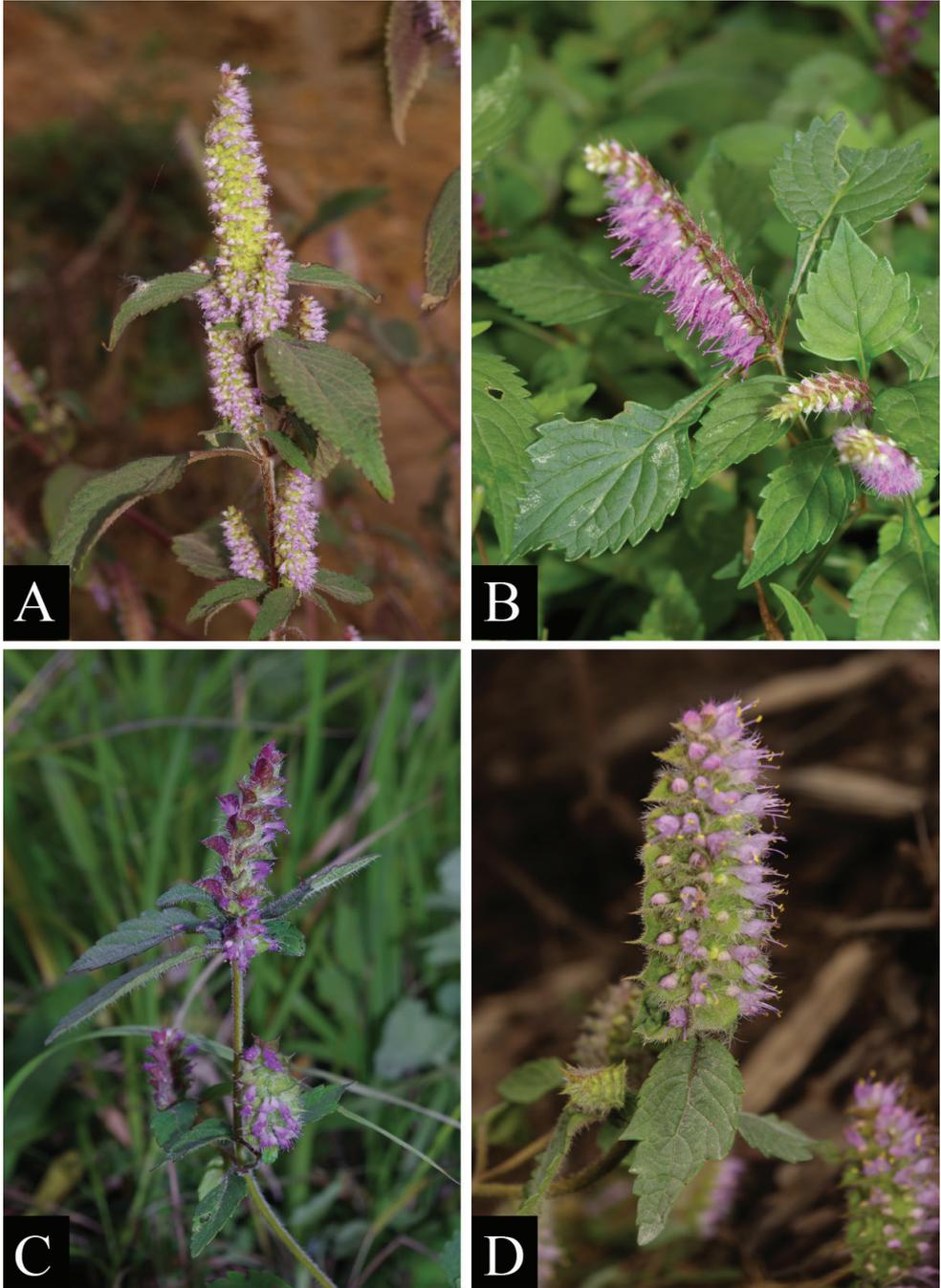


Figure 4. Four other sympatric or morphologically similar species **A** *Elsholtzia ciliata* **B** *Elsholtzia splendens* **C** *Elsholtzia feddei* f. *feddei* **D** *Elsholtzia feddei* f. *robusta*. Photos by Pan Li.

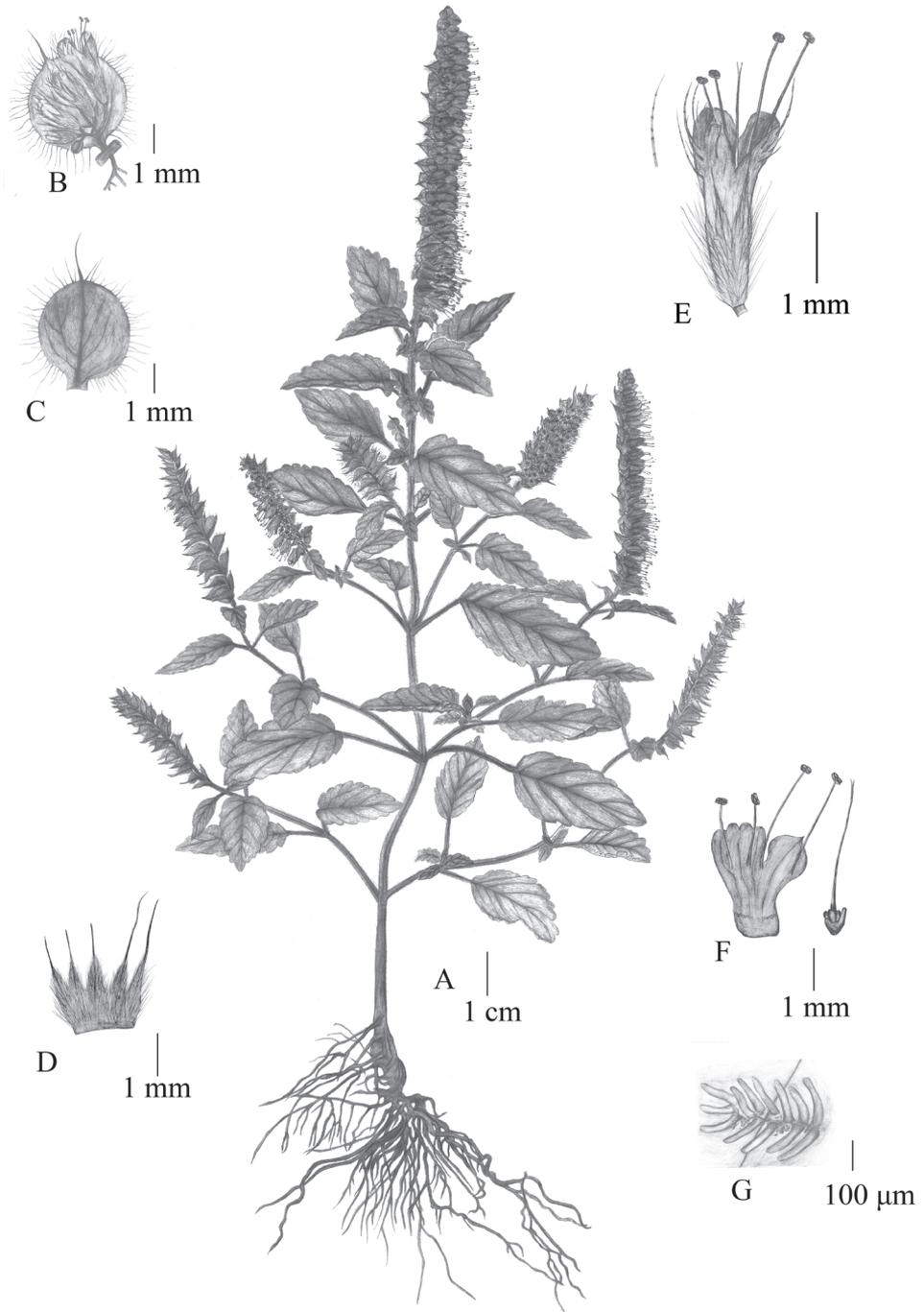


Figure 5. Illustration of *Elsoltzia zhongyangii* Pan Li & X.J. Jin **A** the whole plant **B** cyme **C** bract **D** outside of calyx **E** flowers and villous outside corolla **F** dissected corolla **G** pilose annulate inside. Drawn by Xin-Jie Jin.

Conservation status. The known localities of *Elsholtzia zhongyangii* are not in protected areas. During our field surveys between September 2012 and December 2021, populations in Wolongsi, Weidi, Agakeyong, Jialulongba and Zhusang of Yajiang County were found. Specimen records show that it also occurs in Litang County (Wenquan), Ganzi County, Dege County (Ezhi), Luhuo County (Zhuwo) and Batang County. Taking into consideration that it was distributed along the roadsides like weeds, we believe that it should have a much wider distribution than what is now known. Due to its wide distribution range and large population size, *Elsholtzia zhongyangii* is here recommended as Least Concern (LC), according to the IUCN Categories (IUCN Standards and Petitions Subcommittee 2019).

Additional specimens examined (paratypes). China. Sichuan: Yajiang County, Wolongsi, 30°2.54'N, 101°15.08'E, 3293 m a.s.l., 26 August 2015, *Pan Li & Xinglv Xie* LP150689 (PE, KUN, WZUH); Yajiang County, Weidi, 30°3.13'N, 101°12.66'E, 3028 m a.s.l., 18 September 2018, *Pan Li* LP185942 (PE, WZUH); Yajiang County, Jialulongba, 30°2.94'N, 101°18.42'E, 3760 m a.s.l., 26 August 2015, *Pan Li & Xinglv Xie* LP150686 (PE, KUN, CDBI, SZ, CSH, WZUH); Yajiang County, Zhusang, 19 September 2012, *Pan Li PNL120120191* (WZUH); Litang County, Wenquan, 4000 m a.s.l., 11 September 1974, Y.Q. He & H.J. Wang 8281 (WUK); Ganzi County, 10 September 1951, *W.G. Hu 13099* (WUK); Dege County, Ezhi, 1900 m a.s.l., 31 August 1979, *Dege team 0685* (SM); Luhuo County, Zhuwo, 3700 m a.s.l., 2 August 1974, *Sichuan vegetation team 07693* (CDBI); Batang County, 3100 m a.s.l., 2 September 1973, *Sichuan vegetation team 3971* (CDBI).

Discussion

Elsholtzia zhongyangii usually grows together with *E. ciliata* or other *Elsholtzia* species in grassland on mountainsides or even along roadsides, thus it must have been overlooked previously. We found that the plant is fragrant with dense glands. This species may be suitable as an aromatic plant, and thus it has the potential for development and application values.

There is one other *Elsholtzia* species with small corolla recorded in Sichuan, i.e. *E. souliei*. However, *E. souliei* is distantly related to the new species in the phylogenetic tree (Fig. 2) and the plant is much smaller (less than 10 cm tall).

Within the genus *Elsholtzia*, the taxonomy of the *E. splendens*-*E. ciliata* clade is the most problematic. For example, in the Flora Reipublicae Popularis Sinicae, Huang (1977) divided *E. feddei* into four different forms, which looks very different from each other. However, Li and Hedge (1994) treated all the forms as synonyms of *E. feddei* in the Flora of China. A recent molecular phylogenetic study (Li et al. 2017) included two forms of *E. feddei* and showed that they did not cluster together, with *E. feddei* f. *robusta* sister to *E. souliei*. Besides, they also found that *E. splendens* from China and South Korea did not form a clade, with the Chinese *E. splendens* sister to a sympatric *E. saxatilis*, and the Korean *E. splendens* sister to *E. hallasanensis* (endemic to Jeju

Island). Our phylogenetic result (Fig. 2) is consistent with previous study. Together with the extremely variable morphology and controversial taxonomic treatments, these findings imply that the so-called “*E. feddei*” and “*E. splendens*” may contain some hidden diversity. Future studies based on comprehensive sampling and genomic data are needed to shed light on the systematics of this problematic group.

Acknowledgements

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Supplementary material I

Appendix I

Authors: Xin-Jie Jin, Yue Huang, Yu-Kun Wei, Qing Ma, Lu-Xian Liu, Zhi-Xi Fu, Gui-Fang Wu, Yong-Hua Zhang, Pan Li

Data type: COL.

Explanation note: Taxa and Genbank accessions involved in the present study. Missing sequences are indicated by a double slash (//). Accession numbers in bold are newly generated sequences.

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Actinostephanus (Gesneriaceae), a new genus and species from Guangdong, South China

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Abstract

Actinostephanus, a new genus from southern China, is described and colorfully illustrated with a single species, *A. enpingensis*. This new genus is morphologically most similar to *Boeica* and *Leptoboaea*, nevertheless, it can be easily distinguished from the latter two by the following characteristics, such as leaves in whorls of three, all closely clustered at the top; corolla bowl-shaped, 5-lobed, actinomorphic; capsule hard, oblong-ovoid, short, 3–4 mm long, densely appressed villous, wrapped by persistent densely pubescent calyx lobes, style persistent. The new genus and related genera were sequenced using the next-generation sequencing technique. The whole plastid genome of the new genus is 154,315–154,344 bp in length. We reconstructed phylogenetic trees using the dataset of 80 encoded protein genes of the whole plastid genome from 47 accessions based on ML and BI analyses. The result revealed that the new genus was recovering in a polytomy including *Boeica*,

* These authors contributed equally to this work as co-first authors.

Rhynchocheum, and *Leptoboea* with strong support, congruent to the morphological evidence. A global conservation assessment was also performed and classifies *A. enpingensis* as Least Concern (LC). In addition, after a review of recently described species of Gesneriaceae, we propose that plant enthusiasts, especially Gesneriad fans, have been playing an increasingly important role in the process of new taxa-discoveries.

Keywords

Boeica, Didymocarpoideae, flora of China, IUCN, *Leptoboea*, phylogeny

Introduction

The family Gesneriaceae has been extensively studied since the 1970s in China. There have been 19 new genera from China discovered and published by Chinese taxonomists and botanical researchers before 2005, i.e., *Allostigma* W.T.Wang, *Boeicopsis* H.W.Li, *Briggsiopsis* K.Y.Pan, *Buxiphyllum* W.T.Wang & C.Z.Gao, *Calcareaoboea* C.Y.Wu ex H.W.Li, *Chiritopsis* W.T.Wang, *Didymostigma* W.T.Wang, *Deltocheilos* W.T.Wang, *Dolicholoma* D.Fang & W.T.Wang, *Gyrocheilos* W.T.Wang, *Gyrogyne* W.T.Wang, *Hemiboeopsis* W.T.Wang, *Metabriggsia* W.T.Wang, *Paralagarosolen* Y.G.Wei, *Pseudochirita* W.T.Wang, *Schistolobos* W.T.Wang, *Thamnocharis* W.T.Wang, *Tumidinodus* H.W.Li, *Wentsaiboea* D.Fang & D.H.Qin (Wang 1981a, b; Li 1982, 1983; Wang 1983a, b, c, 1984a, b; Pan 1985; Fang and Qin 2004; Wei 2004). However, the confirmation of all the genera mentioned-above relied only on the significances of morphological characters. Some were subsequently canceled in the process of compiling *Flora Reipublicae Popularis Sinicae* (Vol. 69) and *Flora of China* (Vol. 18) (Wang et al. 1990, Wang et al. 1998). *Boeicopsis*, *Buxiphyllum*, *Schistolobos* and *Tumidinodus* were treated as synonyms of *Boeica* Clarke, *Paraboea* (Clarke), Ridley, *Opithandra* Burt, and *Anna* Pellegr, respectively (Wang et al. 1990, Wang et al. 1998; Li and Wang 2005). In addition, *Opithandra* was merged into *Oreocharis* Benth. (Möller et al. 2011).

Apart from morphological data, recognizing and describing new taxa with molecular evidence will result in a more robust and rational taxa (Fu et al. 2021). The first Chinese paper to mention the molecular method, estimated a molecular phylogeny of the previous subfamily Cyrtandroideae using two DNA regions (Wang and Li 1998). However, the Chinese described new taxa of Gesneriaceae in China using molecular evidence starting around 2010. The first one was a new genus, *Litostigma* Y.G.Wei, F.Wen & M.Möller. It was confirmed and published using classical taxonomy, palynology, and phylogenetic analysis (Wei et al. 2010). Since then, some redefined genera, such as *Hemiboea* Clarke (Weber et al. 2011a), *Loxostigma* Clarke (Möller et al. 2014), *Oreocharis* (Möller et al. 2011), *Paraboea* (Clarke) Ridley (Puglisi et al. 2011), *Petrocodon* Hance (Weber et al. 2011b), *Primulina* Hance (Weber et al. 2011c), etc., and newly divided or restored genera, for example, *Glabella* Mich.Möller & W.H.Chen (Möller et al. 2014) and *Bournea* Oliv. (Chen et al. 2020), were confirmed by the molecular and morphological evidence.

In 2017, a plant enthusiast, Mr. Yi Huang, sent the authors some photos of a rare and distinct Gesneriaceae plant, and we considered it a new taxon but could not undertake further studies since no material was collected at that time. Coincidentally, in

2019, one of the authors, Mr. Hui-Feng Wang, collected this species while undertaking a field trip in southern Guangdong, China.

After a careful morphological comparison, we could not place it into any genus of Gesneriaceae despite it sharing some similarities with *Boeica* or *Leptoboëa*. To better understand the generic placement of this species, molecular phylogenetic analysis was also performed. After consulting the relevant literature (Wang et al. 1998; Li and Wang 2005; Wei et al. 2010; Wei 2018; Wen et al. 2019) and the molecular evidence, we concluded that this new species was assignable to a new genus, *Actinostephanus* gen. nov.

Materials and methods

Ethics statement

The only known location where this new species was found and collected was in the Qixingkeng provincial natural reserve, Enping, Guangdong. Two authors, Ms. Xiao-Yun Chen and Mr. Jun-Jie Liang, are staff at this natural reserve. They helped us get specific permission to enter the reserve and collect specimens. Our field studies did not involve any endangered or protected species. Further, special permits to conduct this research were not required.

Material collection

This new species/genus has been monitored in the field by staff from Qixingkeng provincial natural reserve and grown by the authors at the nursery of the Gesneriad Conservation Center of China (GCCC) and National Gesneriaceae Germplasm Resources Bank of Guangxi Institute of Botany (GXIB) since the plants were collected. We also collected leaf materials of this proposed new species, using silica gel to dry them in the field for DNA extraction.

Genomic DNA extraction and sequencing

Leaf material for DNA extraction was dried using silica gel (Chase and Hills 1991). Genomic DNA was extracted using the CTAB protocol (Doyle and Doyle 1987). The total gDNA sample was sent to Majorbio (<http://www.majorbio.com/>, China) for library construction and next-generation sequencing. Short-insert (350 bp) paired-end read libraries preparation and 2×150 bp sequencing were performed on an Illumina (HiSeq4000) genome analyzer platform. Approximately 6 Gb of raw data for the new species was filtered using the FASTX-Toolkit to obtain high-quality clean data by removing adaptors and low-quality reads (http://hannonlab.cshl.edu/fastx_toolkit/download.html).

Whole plastid genome assembly and annotation

Clean reads were paired and imported in Geneious Prime (Kearse et al. 2012). For plastid genome assembly, the clean reads were mapped to published plastid genome

sequence (*Petrocodon jingxiensis*, Genbank accession number: NC_044477.1) as reference (Xin et al. 2019) using the Fine Tuning option in Geneious Prime (iterating set as 10 times) to exclude nuclear and mitochondrial reads. Then, de novo assembly was performed using Geneious Prime with a medium-low sensitivity setting to assemble the plastid genome sequence. The clean reads mapped the generated contigs using the Fine Tuning option in Geneious Prime (iterating set as 10 times) to fill gaps. Contigs could be concatenated using the Repeat Finder option implemented in Geneious Prime until a ~130 kb contig (including SSC, IR, and LSC) was built. The Inverted Repeat (IR) region was determined by the Repeat Finder option in Geneious Prime and was reverse copied to obtain the complete plastid genome. The annotation approach of the plastid genome was performed using CPGAVAS2 and PGA (Qu et al. 2019; Shi et al. 2019).

Phylogenetic analyses

To confirm the placement of this new plant, we reconstructed phylogenetic trees using the dataset of 80 encoded protein genes of the whole plastid genome. The new plant is morphologically similar to *Boeica* or *Leptoboëa*, both of which belong to Subtr. Leptobaeinae C.B. Clarke (Clarke 1883). Therefore, we sampled all genera within this subtribe except for *Championia* Gardner and representatives of other subtribes within the Gesneriaceae as in-group, and 11 species represented other families as out-group. Consequently, 11 accessions were newly generated, while 36 accessions were downloaded from NCBI. Sequences obtained from this study and their information are listed in Appendix I.

All gene sequences were extracted using the PhyloSuite v1.2.2 (Zhang et al. 2020) and aligned by MAFFT v7.4 (Katoh and Standley 2013). The aligned sequences were then concatenated with PhyloSuite v1.2.2 (Zhang et al. 2020). Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inferences (BI), respectively. For the BI tree, we employed MrBayes v3.2.6 (Ronquist et al. 2012) to obtain a maximum clade credibility (MCC) tree. The parameters set as follows: nst = 6, rates = invgamma. Bayesian inference was performed with the concatenate sequence, using two million generations, two runs, four chains, a temperature of 0.001, and 25% trees were discarded as burn-in, and trees were sampled every 1,000 generations. Then, we used ModelFinder (Kalyaanamoorthy et al. 2017) to find the best fit model for ML analysis and further conducted the ML tree using IQ-TREE v2.1.2 (Nguyen et al. 2014) with 1000 bootstrap replicates. Tree visualization was achieved in Figtree v1.4.3.

Results

Characteristics of the complete plastid genome and ribosomal DNA

The complete plastid genome of *Actinostephanus enpingensis* comprised 154,315 – 154,344 bp (Fig. 1). The characteristics and statistics of the plastid genome are summarized in Tables 1, 2.

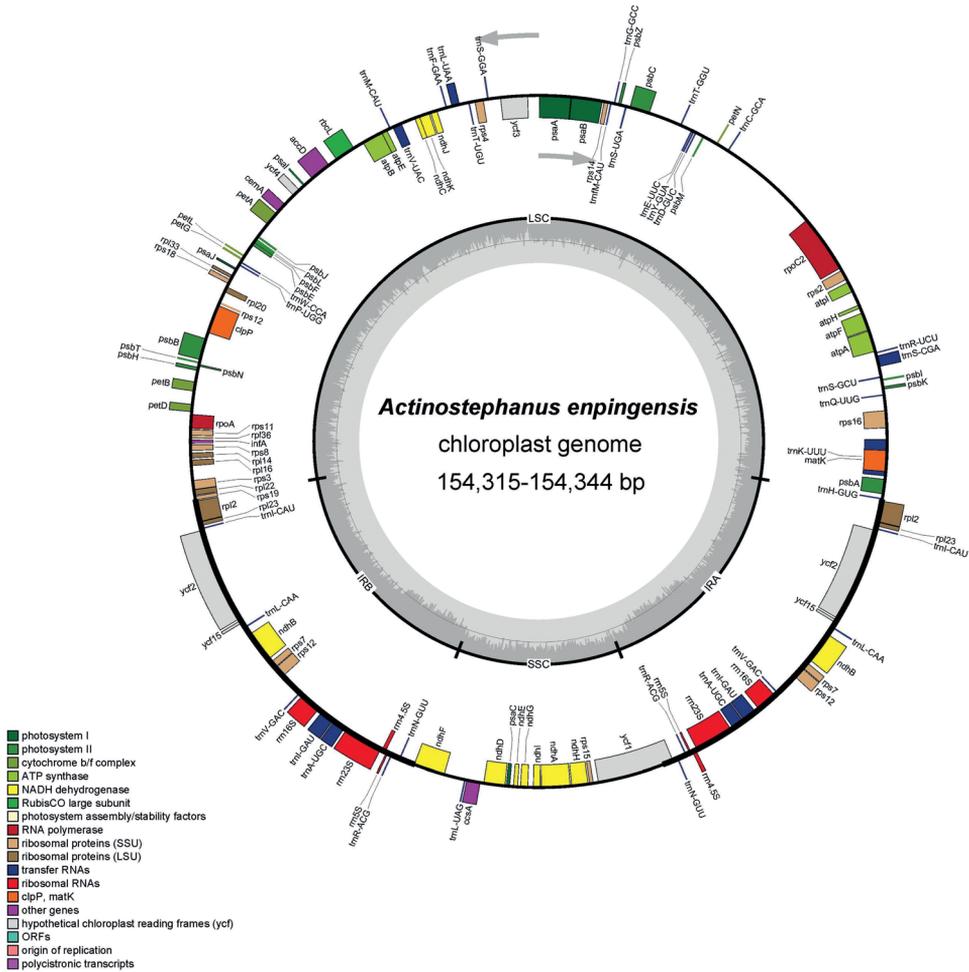


Figure 1. Plastid genome map of *Actinostephanus enpingensis*. The thick lines on the outer complete circle identify the inverted repeat regions (IRa and IRb). The innermost track of the plastome shows the GC content. Genes on the outside and inside of the map are transcribed in clockwise and counter directions, respectively.

Table 1. Summary of plastid genome of *Actinostephanus enpingensis*.

Characteristic	<i>Actinostephanus enpingensis</i>
Size (base pair, bp)	154,315-154,344
LSC length (bp)	85,450-85,479
SSC length (bp)	17,887-17,891
IR length (bp)	25,489-25,493
Number of genes	111-113
Protein-coding genes	77-79
rRNA genes	4
tRNA genes	30
LSC GC%	35.52%
SSC GC%	31.50%
IR GC%	43.20%

Table 2. Genes encoded in plastid genome of *Actinostephanus enpingensis*.

Group of genes	Gene name
tRNA genes	<i>trnA-UGC</i> (×2), <i>trnC-GCA</i> , <i>trnD-GUC</i> , <i>trnE-UUC</i> , <i>trnF-GAA</i> , <i>trnG-GCC</i> , <i>trnH-GUG</i> , <i>trnI-CAU</i> (×2), <i>trnI-GAU</i> (×2), <i>trnK-UUU</i> , <i>trnL-CAA</i> (×2), <i>trnL-UAA</i> , <i>trnL-UAG</i> , <i>trnM-CAU</i> , <i>trnN-GUU</i> (×2), <i>trnP-UGG</i> , <i>trnQ-UUG</i> , <i>trnR-ACG</i> (×2), <i>trnR-UCU</i> , <i>trnS-CGA</i> , <i>trnS-GCU</i> , <i>trnS-GGA</i> , <i>trnS-UGA</i> , <i>trnT-GGU</i> , <i>trnT-UGU</i> , <i>trnV-GAC</i> (×2), <i>trnV-UAC</i> , <i>trnW-CCA</i> , <i>trnY-GUA</i> , <i>trnfm-CAU</i>
rRNA genes	<i>rrn16</i> (×2), <i>rrn23</i> (×2), <i>rrn4.5</i> (×2), <i>rrn5</i> (×2)
Ribosomal small subunit	<i>rps16*</i> , <i>rps2</i> , <i>rps14</i> , <i>rps4</i> , <i>rps18</i> , <i>rps12**</i> (×2), <i>rps11</i> , <i>rps8</i> , <i>rps3</i> , <i>rps19</i> , <i>rps7</i> (×2), <i>rps15</i>
Ribosomal large subunit	<i>rpl33</i> , <i>rpl20</i> , <i>rpl36</i> , <i>rpl14</i> , <i>rpl16*</i> , <i>rpl22</i> , <i>rpl2*</i> (×2), <i>rpl23</i> (×2)
DNA-dependent RNA polymerase	<i>rpoC2</i> , <i>rpoC1*</i> , <i>rpoB</i> , <i>rpoA</i>
Photosystem I	<i>psaB</i> , <i>psaA</i> , <i>psaI</i> , <i>psaJ</i> , <i>psaC</i>
Large subunit of rubisco	<i>rbcL</i>
Photosystem II	<i>psbA</i> , <i>psbK</i> , <i>psbI</i> , <i>psbM</i> , <i>psbC</i> , <i>psbZ</i> , <i>psbG</i> , <i>psbJ</i> , <i>psbL</i> , <i>psbF</i> , <i>psbE</i> , <i>psbB</i> , <i>psbT</i> , <i>psbN</i> , <i>psbH</i>
NADH dehydrogenase	<i>ndhJ</i> , <i>ndhK</i> , <i>ndhC</i> , <i>ndhB*</i> (×2), <i>ndhF</i> , <i>ndhD</i> , <i>ndhE</i> , <i>ndhG</i> , <i>ndhI</i> , <i>ndhA*</i> , <i>ndhH</i>
Cytochrome b/f complex	<i>petN</i> , <i>petA</i> , <i>petL</i> , <i>petG</i> , <i>petB*</i> , <i>petD*</i>
ATP synthase	<i>atpA</i> , <i>atpF*</i> , <i>atpH</i> , <i>atpI</i> , <i>atpE</i> , <i>atpB</i>
Maturase	<i>matK</i>
Translational initiation factor	<i>infA</i>
Subunit of acetyl-CoA carboxylase	<i>accD</i>
Envelope membrane protein	<i>cemA</i>
Protease	<i>clpP**</i>
C-type cytochrome synthesis	<i>ccsA</i>
Conserved open reading frames (ycf)	<i>ycf3**</i> , <i>ycf4</i> , <i>ycf2</i> (×2), <i>ycf1</i> , <i>ycf15</i> (×2)

Notes: Genes with one or two introns are indicated by one (*) or two asterisks (**), respectively. Genes in the IR regions are followed by the (×2) symbol.

Molecular phylogenetic studies

BI and ML analyses of the dataset of 80 encoded protein genes of whole plastid genome resulted in the identical tree topologies that both indicate the three accessions of the new plant formed a strongly supported clade that was recovering in a polytomy including *Boeica*, *Rhynchotechum*, and *Leptoboaea* in the clade of Subtr. Leptobaeinae (posterior probability (PP) = 1, bootstrap support (BS) = 100%) (Fig. 2).

Ecology

Plants of the new taxon were primarily accessible in the Qixingkeng provincial natural reserve, growing on nearly vertical or steep slopes of montane yellow soil under tropical evergreen broad-leaved forest. Voucher specimens were made in the usual way (Bridson and Forman 1998) from some accessible plants that could be reached from the type locality. The conservation assessment was prepared following IUCN (2019).

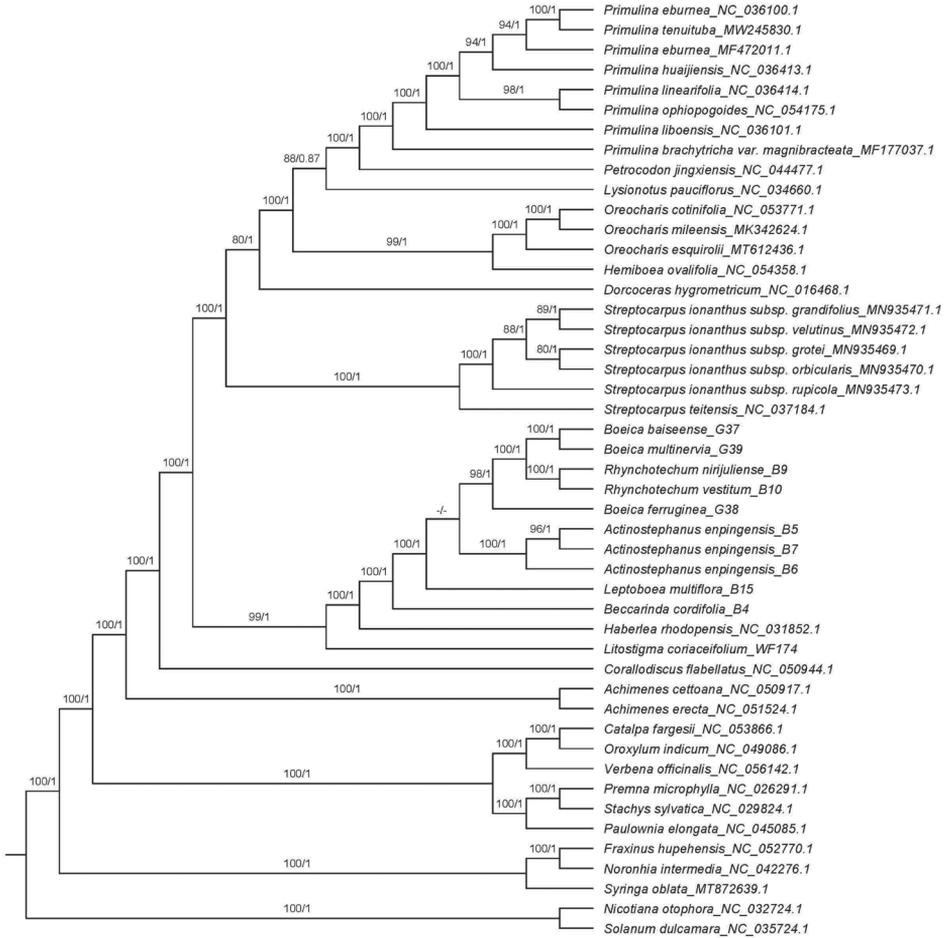


Figure 2. Phylogenetic tree of Gesneriaceae generated from maximum likelihood (ML) based on the dataset of whole-chloroplast protein-coding genes. Numbers on the branches indicate the bootstrap support (>70%) of the ML and the posterior probability (>0.8) of BI analyses.

Taxonomic treatment

Subfam. Didymocarpoideae

***Actinostephanus* F.Wen, Y.G.Wei & L.F.Fu, gen. nov.**

urn:lsid:ipni.org:names:77296131-1

Diagnosis. *Actinostephanus* F.Wen, Y.G.Wei & L.F.Fu resembles two small genera, *Boeica* C.B.Clark and *Leptoboea* Benth. according to the molecular evidence and some morphological data, but differs from the latter two by the following distinguishing characters: leaves in whorls of three, all closely clustered at the top; corolla bowl-shaped, 5-lobed, actinomorphic; capsule hard, oblong-ovoid, short, 3–4 mm long, densely

appressed villous, wrapped by persistent densely pubescent calyx lobes, style persistent. The detailed distinguishing characters of this new genus and its congeners are listed in Table 1.

Type and only known species. *Actinostephanus enpingensis* F.Wen, Y.G.Wei & Z.B.Xin, sp. nov.

Description. Herbs, perennial, acaulescent, or forming elongated rhizome slightly fleshy growing after some years, rhizomes cylindrical, surface densely brown pubescent, fibrous root filiform, forming adventitious buds and plantlets in the middle or at the end of the fibrous root. Leaves all basal, whorls of three, sometimes opposite, all closely clustered at the top, forming a rosette, or clustered forming a rosette at the top of the rhizome after years of growth. Leaf-blades obovate elliptic, asymmetric, rarely symmetric, attenuate to base and base usually oblique, rarely aequilateral. Bracts 2. Calyx actinomorphic, 5-parted to the base. Corolla actinomorphic, bowl-shaped; tube very short, shallow bowl-shaped; limb quinquelobate, lobes equal. Stamens 4, separated, anthers dorsifixed, free, dehiscing longitudinally. Disc glabrous, margin crenulate. Ovary conical, stigma punctate. Capsule oblong-ovoid, appressed villous, wrapped by persistent calyx lobes, and the abaxial surfaces of calyx lobes covered densely pubescent. The number of seeds per capsule fewer. Seeds bigger, elliptic, both ends pointed.

Etymology. The genus name, “*Actinostephanus*”, consists of two parts, both derived from the Greek. The front part, “*Actino-*” is derived from ἀκτίς (aktís, “ray, beam”), means radiating; the latter half, “*-stephanus*”, is derived from Στέφανος (Stéphanos, “crown”), is also closely associated in ἄνθος (ánthos, “flower, blossom, bloom”), hints corolla. The combined Greek word-roots characterize the uncommon corolla characteristic of the new genus and species. The character of the corolla, in China’s Gesneriaceae, is rare. Only three species belonging to two genera were known to have actiniform corolla in China, namely *Bournea sinensis* Oliv., *B. leiophylla* (W.T.Wang) W.T.Wang & K.Y.Pan ex W.T.Wang and *Oreocharis esquirolii* H.Lév. before this new genus was discovered.

Vernacular name of the new genus. Chinese mandarin: Fú Guàn Jù Tái Shǔ (辐冠苣苔属).

Distribution and habitat. Endemic to Enping county, Guangdong province, China, under evergreen broadleaved forests in a montane mountain yellow soil area at 170–250 m altitude.

***Actinostephanus enpingensis* F.Wen, Y.G.Wei & Z.B.Xin sp. nov.**

urn:lsid:ipni.org:names:77296132-1

Figs 3–4

Type. China. Guangdong province, Enping city, Naji town, Qixingkeng provincial natural reserve, growing on the cliffs or slopes of montane yellow soil and sandy loam near a stream. 22°11.1808'N, 112°5.841'E, ca. 153 m, *Chen Xiaoyun & Liang Junjie 210519-01* (holotype: IBK!, isotypes IBK!).

Description. Herbs, perennial, acaulescent, or forming elongated rhizome slightly fleshy after some years, rhizomes cylindrical, surfaces densely brown pubescent,



Figure 3. Photos of *Actinostephanus* F.Wen, Y.G.Wei & L.F.Fu gen. nov. (*A. enpingensis* F.Wen, Y.G.Wei & Z.B.Xin sp. nov.), the individuals in natural habitat. **A** habitat **B** habit **C** plant in flowering **D** plant in fruiting.

5–15 mm long, 3–8 mm in diam., fibrous root filiform, 4–6 cm long, up to 10 cm, forming adventitious buds and plantlets in the middle or at the end of fibrous root. Leaves all basal, whorls of three, sometimes opposite, all closely clustered at top forming rosette, or clustered forming rosette at the top of rhizome after years of growth, (8)9–(16)18 or more, subsessile or shortly petiolate, short petiole cylindrical, 6–15 × 3.5–4.8 mm, densely brown villous. Leaf blades chartaceous to thickly herbaceous, thin chartaceous when dried, obovate elliptic, occasionally obovate lanceolate, greenery to green, dark green after a year of growth, 7.5–15.0 × 3.5–6.0 cm, asymmetric, rarely symmetric, attenuate to base and base usually oblique, rarely aequilateral, apex rounded, obtuse to subacute, margin numerous crenulate, adaxial surfaces of young leaf blades sparsely puberulent, subsequently gradually deciduous, adaxial surfaces of mature leaf blades nearly glabrous, but abaxial surfaces of young and mature leaf blades pubescent, covered by long and obvious strigose hairs along the main and lateral veins; venation alternate along main vein, lateral veins 7–9 on each side of the midrib, main and lateral veins on adaxial surface obviously sunken and on abaxial surface distinctly

prominent. Inflorescence dichasium, 4–8, axially, 8–14-flowered, rarely 4–5-flowered and occasionally more than 14, 1–2-branched; peduncle sturdier, 2.2–4.5 cm long, 1.0–1.5 mm in diam., brownish-green to brownish-red, densely upward short strigose, the hairs brownish red, occasionally pink to pinkish white. Bracts 2, brownish-green, opposite, linear-lanceolate, ca. 6.0×1.0 mm, adaxial surface appressed pubescent, abaxial surface puberulent, apex acute, margin entire; pedicel 4.0–9.0 mm long, ca. 1.0 mm in diam., pale brownish-green to green, pubescent. Calyx actinomorphic, 5-parted to the base, segments pinkishwhite, pale pinkish-purple to pale brownish-red, equal, lanceolate, $3.5\text{--}4.0 \times 1.2\text{--}1.4$ mm, apex acute, margin entire, abaxial surface puberulent, adaxial surface glabrous, but persistent in the fruiting period. Corolla pale purple to pale bluish-purple, actinomorphic, bowl-shaped, 4.0–5.0 mm long/high, 65–75 mm in diam., outside puberulent, inside nearly glabrous and sparsely very few glandular-puberulent; tube very short, 1.5–2.0 mm long/high, shallow bowl-shaped; limb quinquelobate, lobes equal, half elliptic, the major axis ca. 3.5 mm long, the minor axis ca. 1.2 mm long, apex cambered, margin revolute. Stamens 4, separated, filaments nearly slender cylinder, glabrous, two longer and two shorter, longer pairs ca. 1.5 mm long, shorter pairs ca. 1.0 mm long, the four adnate to the base of corolla tube, anthers dorsifixed, free, cordate, yellowish-brown to pale greenish-brown, ca. 1.0 mm height, ca. 1.0 mm across at the bottom of the cordate shape, dehiscing longitudinally, glabrous. Disc wax yellow, ca. 1.0 mm high, glabrous, smooth, margin crenulate. Pistil 4.5–4.8 mm long, ovary pale pink, conical, sparsely inconspicuously puberulent, ca. 0.9 mm long, ca. 1.0 mm across at bottom, style translucent to white, 3.8–4.0 mm long, stigma punctate, yellow. Capsule oblong-ovoid, 4.5–5.0 mm long, 1.2–1.3 mm across, appressed villous, wrapped by persistent calyx lobes and the abaxial surfaces of calyx lobes densely pubescent; capsule hard when mature, style usually persistent, rarely dehiscent, occasionally split into 4-valves. The number of seeds per capsule fewer than 100, only 50–80, the macroaxis of seeds bigger, ca. 0.5×0.3 mm, brownish-black, not appendant, elliptic, both ends pointed.

Preliminary conservation status. Based on the result of our joint field surveys in the type locality and adjacent regions, the EOO and AOO of *Actinostephanus enpingensis* are about 79.5 km² and 0.1 km², respectively. So far, only one population of this species has been recorded along the local stream in the Qixingkeng provincial natural reserve, Enping city, Guangdong province, southern China, but we believe that more *A. enpingensis* populations can be found in the hills of Enping and its adjacent counties. If that is the case, the Extent of Occurrence (EOO) and Area of Occupancy (AOO) of this species will increase. Because the flowers and leaves of this species are inconspicuous, and after learning from some local people that it has no known medicinal value we feel that this species faces little risk. Moreover, almost all of these plants are growing in the protected areas of this reserve so that the species are well protected. According to the Guidelines for Using the IUCN Red List Categories and Criteria (IUCN 2019), we assess this taxon as a Least Concern species (LC).

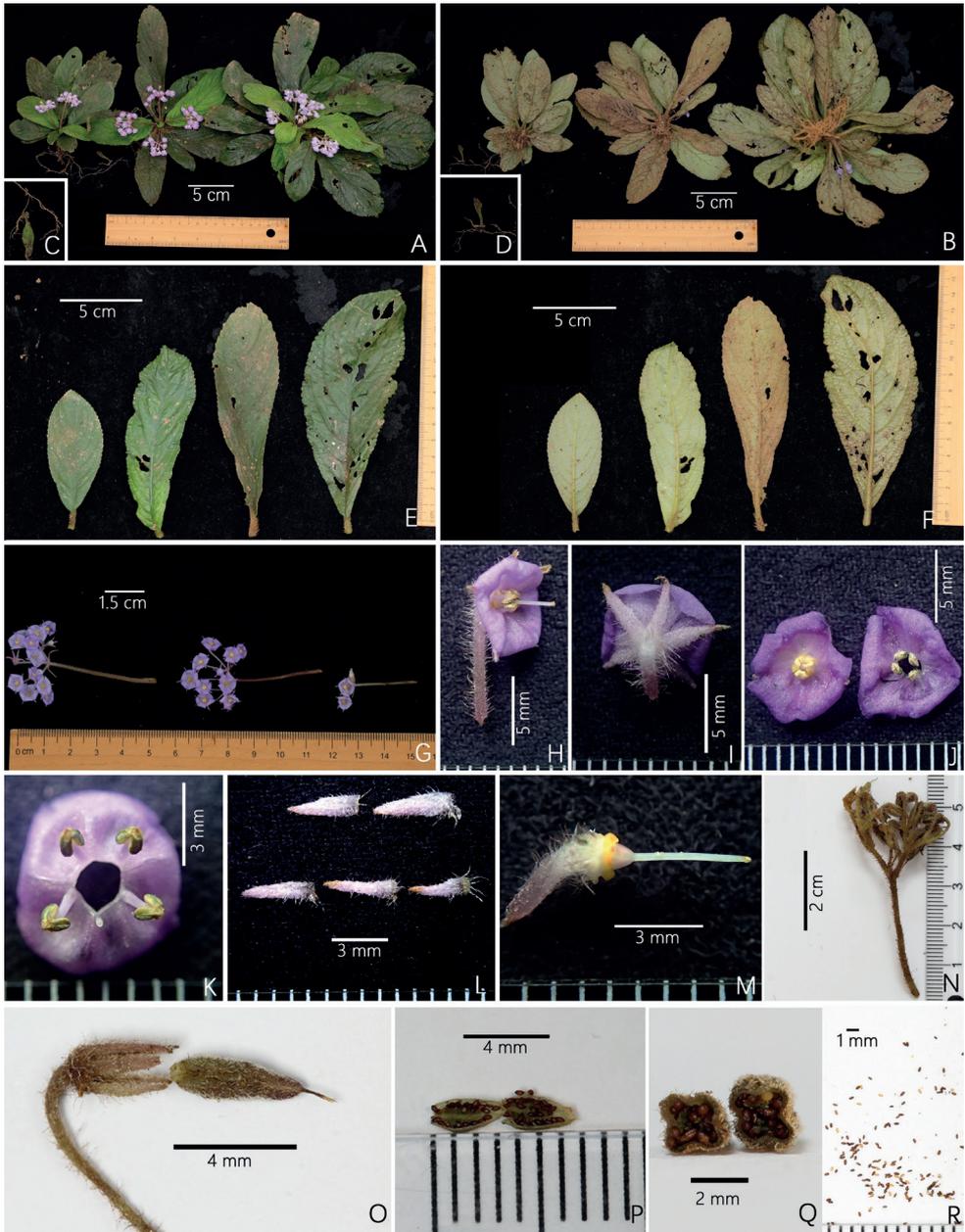


Figure 4. Photos of *Actinostephanus* F.Wen, Y.G.Wei & L.F.Fu gen. nov., the individuals in natural habitat. *A. enpingensis* F.Wen, Y.G.Wei & Z.B.Xin sp. nov. **A** top view of plant **B** upward view of plant for showing root system **C** top view of bud at the end of root **D** upward view of bud at the end of root **E** adaxial surfaces of leaves **F** abaxial surfaces of leaves **G** cymes **H** lateral view of flower **I** posterior view of flower **J** frontal view of corolla **K** stamens and staminodes **L** abaxial surfaces of calyx lobes **M** pistil **N** infructescence **O** capsule and persistent calyx lobes **P** opened capsule **Q** cross-section of capsule **R** seeds.

Discussion

Our phylogenetic studies revealed that the new plant fell into Subtr. Leptoboeinae C.B.Clarke (PP = 1, BS = 100%). This subtribe belongs to Tribe Trichosporeae Nees, Subfamily Didymocarpoideae. At present, six genera have been included in this subtribe (Möller et al. 2017). Although their morphologies from different genera of this Subtribe are heterogeneous, several characters, such as the absence of large flowers, the inconspicuous to capitate stigma, straight but no-twisted fruits, and commonly 4-valved and dehiscent capsules or fleshy berries were concluded as common ones (Weber et al. 2013, Weber et al. 2020). The new plant is morphologically congruent with these characters that further indicate the monophyly of Subtr. Leptoboeinae. Within Subtr. Leptoboeinae, the new plant, was recovering in a polytomy including *Boeica*, *Rhynchotechum*, and *Leptoboea*. Two *Boeica* spp. are most closely related to *Rhynchotechum*, and both sisters to the type species of *Boeica* (*B. furruginea*) indicated that *Boeica* is not monophyletic. This relationship was congruent with previous studies (Yang et al. 2020). Expanding the sampling, and exploring key characters, is needed to re-define the *Boeica*. Despite this, the new genus is morphologically similar to these genera based on phyllotaxis and inflorescence cyme. However, it can be easily distinguished by corolla bowl-shaped, 5-lobed, actinomorphic, capsule densely appressed villous, wrapped by persistent densely pubescent calyx lobes and style persistent (Table 3). The most distinct characters of the new plant are its actinomorphic corolla, tiny fruit hard when mature, rarely dehiscent, occasionally split into 4-valves, style usually persistent, which are likely to be derived characters or autapomorphies. We, therefore, based on the molecular and morphological evidence, treat it as a new genus, namely, *Actinostephanus*.

The high levels of plant species diversity and endemism in southern and southwestern China are more and more renowned, especially in karst regions. Nevertheless, it is evident that the geographic accessibility of those mountainous areas (including townships, villages, and surrounding regions) has been hindered by terrible transport problems. It also seriously affected the understanding of plant diversity in South and Southwest China. But with the fast development of the Chinese economic and construction systems, more and more road construction projects are being carried out, forming a relatively completed road transportation system in China. Thus, there are more opportunities to discover many taxa new to science. The people have easy access to those places that were difficult to reach in past decades.

Not only do more and more taxonomists focus on the biodiversity of Gesneriaceae in China, but plant enthusiasts are also making an enormous contribution to help botanists discover rare and new Gesneriads. As previously mentioned, Mr. Yi Huang, a plant enthusiast, found the interesting *Boeica*-like species of Gesneriaceae in South China, and he offered this critical information to us. Thus, we will be more conscious of this uncertain species over the next few years. Therefore, we propose that plant enthusiasts, especially Gesneriad fans, are playing an increasingly important role in the process of new taxa-discoveries.

Several new species, for example, *Primulina papillosa* Z.B.Xin, W.C.Chou & F.Wen (Xin et al. 2021), *P. purpureokylin* F.Wen, Yi Huang & W.C.Chou, *P. niveolanosa* F.Wen, S.Li & W.C.Chou, *P. persica* F.Wen, Yi Huang & W.C.Chou (Li et al. 2019), were

Table 3. Comparison of morphological characters of *Actinostephanus*, *Boeica* and *Leptoboaea*.

Characters	<i>Actinostephanus</i>	<i>Boeica</i>	<i>Leptoboaea</i>
Habit	perennial herb but acaulescent, or elongated rhizome slightly fleshy growing after some years	subshrub, or perennial herb	Subshrub
Stem	Acaulescent	erect aerial stem, sometimes stolon, stem more or less lignified	erect aerial stem, lignified
Stolon and root system	no stolon, fibrous root filiform, at the end of root with ability for cloning	stolon or no stolon; roots no fecundity	no stolon; roots no fecundity
Leaf	whorls of three, all closely clustered at top	alternative	branches and leaves opposite, usually clustered at annual shoots
Inflorescence	cyme, corymbose, 1- or 2-branched; peduncle sturdier	cyme, multi-branched, occasionally no-branched; peduncle sturdier	cyme, corymbose, multi-branched; peduncle and pedicel slender similar to filiform
Corolla	actinomorphic, bowl-shaped, limb and tube nearly isometric, lobes deflexed	campanulate, corolla tube shorter than limb; limb slightly bilabiate, 5-lobed, lobes equal or slightly unequal	campanulate, small; limb slightly bilabiate, 5-lobed, lobes nearly equal
Capsule	oblong-ovoid, short, appressed villous, wrapped by persistent calyx lobes, and the calyx lobes also outside covered densely pubescent; hard when mature, style usually persistent, rarely dehiscent, occasionally split into 4-valves	linear, long, glabrous, apex pointed, style no persistent	long linear, long, glabrous, style no persistent
Seed	the number of seeds per capsule fewer, only 50–80	the number of seeds per capsule numerous, hundreds	the number of seeds per capsule numerous, hundreds

discovered by Mr. Wei-Chuen Chou, who is passionate about collecting *Primulina* species. He has also registered a number of new ornamental varieties by hybridization with the Gesneriad Society, the International Registration authority for Gesneriad horticultural variety. Other examples are *P. longii* (Z.Y.Li) Z.Y.Li and *P. leiyyi* F.Wen, Z.B.Xin & W.C.Chou; the scientific names paid homage to the discoverers and collectors, Mr. Guang-Ri Long and Mr. Yu-Yang Lei (Li 2002; Li et al. 2019). Since 2011, at least 14 taxa in China (including those five new species above-mentioned) were discovered and published with the assistance of domestic Gesneriad enthusiasts (non-professionals/botanists/taxonomists, who are not associated with any university, institute or botanical garden), based on our statistics. They are *Primulina spiradiclioides* Z.B.Xin & F.Wen (Xin et al. 2020a), *P. hochiensis* var. *ochroleuca* F.Wen, Y.Z.Ge & Z.B.Xin (Ge et al. 2020), *P. anisocymosa* F.Wen, Xin Hong & Z.J.Qiu (Hong et al. 2019), *P. wuae* F.Wen & L.F.Fu (Li et al. 2017), *P. qintangensis* Z.B.Xin, W.C.Chou & F.Wen (Xin et al. 2020b), *P. titan* Z.B.Xin, W.C.Chou & F.Wen (Xin et al. 2020c), *P. bipinnatifida* var. *zhoui* (F.Wen & Z.B.Xin) W.B.Xu & K.F.Chung, *P. huangii* F.Wen & Z.B.Xin (Xin et al. 2018), *P. duanensis* F.Wen & S.L.Huang (Huang et al. 2015), *P. moi* F.Wen & Y.G.Wei (Zhou et al. 2015), *Oreocharis aimodisca* Lei Cai, Z.L.Dao & F.Wen, *O. longipedicellata* Lei Cai & F.Wen (Cai et al. 2020), *O. panzhouensis* Lei Cai, Y.Guo & F.Wen (Cai et al. 2019), *Didymocarpus dissectus* F.Wen, Y.L.Qiu, Jie Huang & Y.G.Wei (Wen et al. 2013).

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Zhou SB, Hong X, Wei YG, He LP, Wen F (2015) *Primulina moi* sp. nov. (Gesneriaceae) from a limestone area in northern Guangdong, China. *Nordic Journal of Botany* 33(4): 446–450. <https://doi.org/10.1111/njb.00725>

Appendix I

The following is a list of used samples that are ordered alphabetically by taxon with their GenBank accession number of whole plastid genome sequences respectively. The samples with newly generated sequences are listed with the complete voucher information.

*Actinostephanus enpingensis*_B5 Qixingkeng Provincial Nature reserve, Enping City, Guangdong, WF210730-02, IBK!, OM176663; *Actinostephanus enpingensis*_B6 Qixingkeng Provincial Nature reserve, Enping City, Guangdong, FZY210502-01, IBK!, OM176664; *Actinostephanus enpingensis*_B7 Qixingkeng Provincial Nature reserve, Enping City, Guangdong, WF210730-01, IBK!, OM176665; *Boeica baiseense*_G37 Liujun village, Youjiang District, Baise City, Guangxi, WYG180520-05, IBK!, OM176669; *Boeica ferruginea*_G38 Pu Luong Nature Reserve, Thanh Hoa Province, Vietnam, WF21030328-01, IBK!, OM176670; *Boeica multinervia*_G39 Jingping County, WF190814-01, IBK!, OM176671; *Beccarinda cordifolia*_B4 Dulongjiang, Gongshan County, Yunnan, XZB2104, IBK!, OM176662; *Leptoboea multiflora*_B15 Kauai National Forest, Thailand, WF180508-05, IBK!, OM176668; *Litostigma coriaceifolium*_WF174 Maling Gorge, Xingyi City, Guizhou, FLF170420-01, IBK!, OM176672; *Rhynchochum nirijuliense*_B9 Beibeng to Mihan, Medog County, Xizang, WF200910-06, IBK!, OM176666; *Rhynchochum vestitum*_B10 Beibeng to Mihan, Medog County, Xizang, WF200910-22, IBK!, OM176667; *Achimenes cettoana* NC_050917; *Achimenes erecta* NC_051524.1; *Catalpa fargesii* NC_053866.1; *Chirita eburnea* NC_036100.1; *Corallodiscus flabellatus* NC_050944.1; *Dorcoceras hygrometricum* NC_016468.1; *Fraxinus hupehensis* NC_052770.1; *Haberlea rhodopensis* NC_031852.1; *Hemiboea ovalifolia* NC_054358.1; *Lysionotus pauciflorus* NC_034660.1; *Nicotiana otophora* NC_032724.1; *Noronhia intermedia* NC_042276.1; *Oreocharis cotinifolia* NC_053771.1; *Oreocharis esquirolii* MT612436.1; *Oreocharis mileensis* MK342624.1; *Oroxylum indicum* NC_049086.1; *Paulownia elongata* NC_045085.1; *Petrocodon jingxiensis* NC_044477.1; *Premna microphylla* NC_026291.1; *Primulina brachytricha* var. *magnibracteata* MF177037.1; *Primulina eburnea* MF472011.1; *Primulina huaijiensis* NC_036413.1; *Primulina liboensis* NC_036101.1; *Primulina linearifolia* NC_036414.1; *Primulina ophiopogoides* NC_054175.1; *Primulina tenuituba* MW245830.1; *Solanum dulcamara* NC_035724.1; *Stachys sylvatica* NC_029824.1; *Streptocarpus ionanthus* subsp. *grandifolius* MN935471.1; *Streptocarpus ionanthus* subsp. *grotei* MN935469.1; *Streptocarpus ionanthus* subsp. *orbicularis* MN935470.1; *Streptocarpus ionanthus* subsp. *rupicola* MN935473.1; *Streptocarpus ionanthus* subsp. *velutinus* MN935472.1; *Streptocarpus teitensis* NC_037184.1; *Syringa oblata* MT872639.1; *Verbena officinalis* NC_056142.1.

Thorea baiyunensis sp. nov. (Thoreaales, Rhodophyta) and *T. okadae*, a new record from China

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Abstract

The freshwater red algal order Thoreaales has a triphasic life history, of which the “Chantransia” phase is a small filamentous sporophyte. The “Chantransia” stage is difficult to distinguish from species in the genus *Audouinella* by its morphological characteristics. In this study, five “Chantransia” isolates (GX41, GX81, GD224, GD225, GD228) were collected from Guangxi Zhuang Autonomous Region and Guangdong Province in China. Based on morphological data, all five isolates were similar to *A. pygmaea*, whereas sequence data from the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) gene and the 5' region of the mitochondrial cytochrome oxidase I gene (COI-5P) determined that these specimens represented the “Chantransia” stage of two species in the genus *Thorea* rather than *Audouinella*. Phylogenetic analyses of the concatenated genes supported the proposal of a new species, *T. baiyunensis*, and a new geographic record of *T. okadae*, a species previously described only in Japan. Therefore, combined with previous records, four species of this genus are now recognized in China, including *T. hispida*, *T. violacea*, *T. baiyunensis* and *T. okadae*.

Keywords

COI-5P, freshwater Rhodophyta, new taxon, phylogeny, *rbcL*, *Thorea*

Introduction

Currently, more than 7,000 red algal species are reported worldwide, of which freshwater species only account for 3%. In the Rhodophyta, only four orders (Balbianiales, Batrachospermales, Compsopogonales, and Thoreaales) have all members that are strictly freshwater species. Among the freshwater red algae, the genus *Thorea* Bory was established by Bory de Saint-Vincent (1808), since when its taxonomic status has undergone numerous changes. Based on the pit plug structure, Pueschel and Cole (1982) removed the Thoreaceae and Batrachospermaceae from the Nemaliales and established the order Batrachospermales. However, molecular research showed that species in the Thoreaceae were distantly related to the other Batrachospermales taxa (Vis et al. 1998). Subsequently, based on DNA sequence data, secondary structure of the SSU gene and characteristics of the outer layer of the pit plug, Müller et al. (2002) established the Thoreaales. Species of this order are characterized by having multiaxial gametophytes, a uniaxial “Chantransia” stage, and pit plugs with two cap layers, the outer one of which is usually plate-like.

As currently recognized, the Thoreaales contain a single family Thoreaceae with two genera, *Thorea* and *Nemalionopsis*. The main difference between the genera *Thorea* and *Nemalionopsis* is that *Thorea* has assimilatory filaments that are not contained in a common gelatinous matrix with reproductive structures (carpogonia, spermatangia, carposporangia and monosporangia) at their base and a lower ratio of sporangial branch to assimilatory filament length and loose aggregation (Skuja 1934; Sheath and Cole 1993). In a recent review of *Thorea*, sixteen species were recognized worldwide (Johnston et al. 2018). However, only two species (*T. hispida* (Thore) Desvaux, *T. violacea* Bory) of this genus have been reported in China (Shi 2006). The type species of the genus, *T. hispida*, was widely distributed in China, while *T. violacea* was only reported in Guizhou Province (Xie and Shi 2003; Shi 2006). Asexual reproduction by monosporangia is commonly reported in *Thorea*, while sexual reproduction is observed in a few species, including *T. hispida*, *T. conturba* Entwisle & Foard, *T. okadae* Yamada, *T. bachmannii* C.Pujals ex R.G.Sheath, M.L.Vis & K.M.Cole, *T. kokosinga-pueschelii* E.T.Johnston & M.L.Vis, and *T. mauritukitukii* E.T.Johnston, K.R.Dixon, J.A.West & M.L.Vis (Kumano 2002; Johnston et al. 2018). Among them, *T. okadae* is widely distributed in Japan (Yamada 1949; Johnston et al. 2018), but so far, this species has not been reported in other countries and regions. In addition, according to Kumano (2002), *T. okadae* is the largest species in the genus *Thorea*, and the length of its gametophyte often exceeds 1 m, and may reach 3 m.

Like other sexually reproductive species of freshwater red algae, *Thorea* species have a triphasic life history, including gametophyte, carposporophyte, and a diminutive diploid sporophyte termed “Chantransia” stage. Recently, several studies have focused on the phylogenetic affinities of the “Chantransia” stages of *Thorea* and the relationship between the isolates in this stage and a phylogenetically distant genus *Audouinella* Bory (Necchi and Oliveira 2011; Han et al. 2021). Based on these studies, new species were proposed, new distributions were found, and higher *Thorea* species diversity was

revealed (Johnston et al. 2018; Han et al. 2021). In terms of morphological characters, the “Chantransia” stages of the Thoreales are very similar to those of *Audouinella* taxa, although some studies have indicated that thallus color can be used as a reliable character (Zucchi and Necchi 2003) to distinguish true *Audouinella* (reddish) from “Chantransia” (bluish). However, “Chantransia” stages of the Thoreales can be brownish (Chiasson et al. 2007), and some species of *Sheathia* can be brownish to reddish in addition to bluish (Han et al. 2020). Thus, morphological characteristics that can unequivocally distinguish them have not been found (Pueschel et al. 2000; Chiasson et al. 2005). It was not until the emergence of molecular data that the “Chantransia” stage could be used for species identification. Recently, researchers have shown that some “Chantransia” isolates of *Thorea* were misclassified as *Audouinella* (Han 2012; Chen et al. 2014; Nan et al. 2016). Based on different gene markers, Han (2012), Chen et al. (2014) and Nan et al. (2016) proposed that *A. sinensis* C.-C.Jao and *A. heterospora* S.L.Xie & Y.J.Ling were “Chantransia” of *T. hispida* rather than belong to *Audouinella*. Besides, numerous surveys (Pueschel et al. 2000; Zucchi and Necchi 2003; Chiasson et al. 2005, 2007; Necchi and Oliveira 2011; Han et al. 2020) demonstrated that eleven species, including members of the Thoreales (*T. hispida* and *N. tortuosa* Yoneda & Yagi), were associated with “*A. pygmaea*”. Furthermore, the molecular phylogeny based on the *rbcl*, COI-5P and the plastid 23S rRNA (UPA) genes unequivocally demonstrated that a sample morphologically similar to *A. macrospora* (Wood) Sheath & Burkholder represented the “Chantransia” phase of *T. hispida* (Han et al. 2021).

It is therefore evident that the morphological difference between “Chantransia” stages and *Audouinella* is not clear. Relying solely on traditional morphological methods can often cause confusion in the classification and identification of *Audouinella*-like freshwater red algae. All samples in this study are “Chantransia” stages, meaning that key diagnostic morphological characters were unavailable. Therefore, the present work has attempted to use two molecular markers (*rbcl* and COI-5P) to infer the phylogenetic position of all isolates in this study. In addition, a secondary aim of this investigation is to provide reference for local resource survey of freshwater red algae biodiversity in China.

Methods

Five “Chantransia” specimens (GX41, GX81, GD224, GD225 and GD228) were collected from Guangxi Zhuang Autonomous Region and Guangdong Province in China (Fig. 1, Table 1). Handheld meters (YSI Professional Plus Multiparameter Water Quality Instrument 19E102487, YSI Incorporated, Brannum Lane Yellow Springs, Ohio, USA) were used to measure water quality parameters, including water temperature and pH. Materials were picked up carefully using a knife and tweezers and transferred to the laboratory as soon as possible. After transfer to the laboratory, the samples were rinsed with sterile water to remove impurities. Other algae attached to the samples were carefully removed using tweezers and other tools. Then microscopic

examination was undertaken to ensure that all epiphytic algae were removed. Morphological and genetic analysis was performed for each sample. For observations and measurements, we used a BX-51 Olympus microscope equipped with a charge-coupled device (DP72; Olympus, Tokyo, Japan).

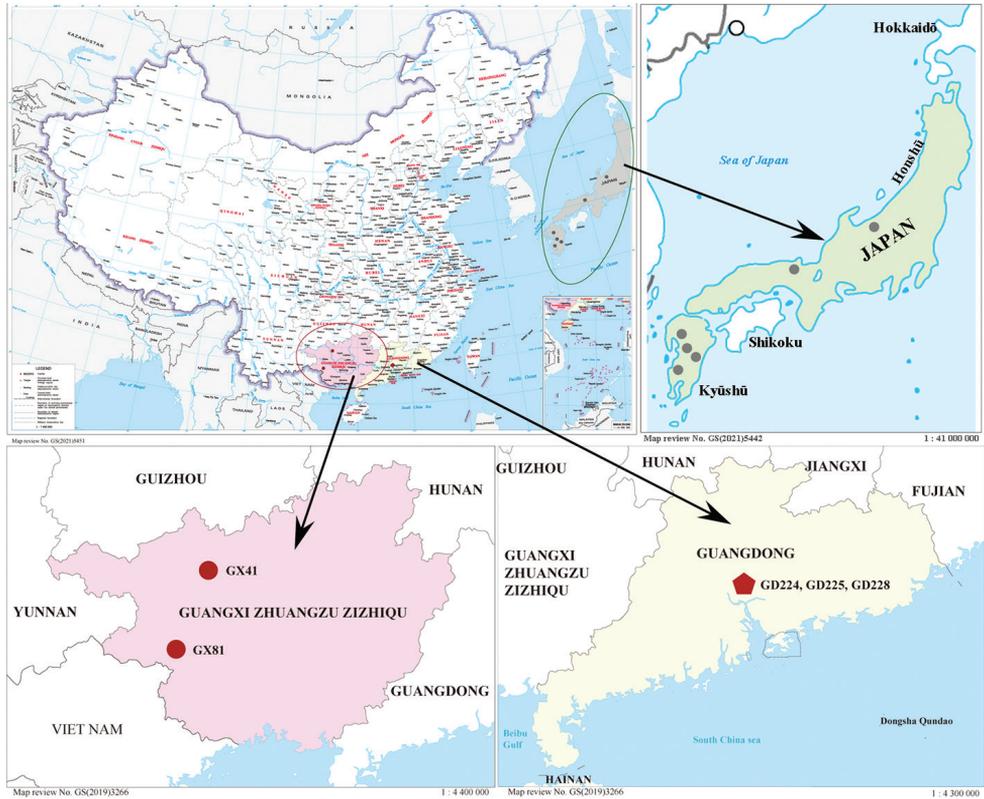


Figure 1. Map showing approximate locations of samples investigated in this study. More detailed location information is provided in Table 1. The red circles and the green circles indicate the locations of *Thorea okadae* in China and Japan, respectively.

Table 1. Collection information and sequence accession numbers for taxa analyzed in this study.

Isolate	Locality with longitude and latitude	Collection date	Collector	Voucher number
GX41	Baimo Cave, Bama County, Guangxi Zhuang Autonomous Region, China (24°18.03'N, 107°05.96'E)	22 December 2019	Kunpeng Fang	GX19041
GX81	Tongling Great Falls, Jinxi County, Guangxi Zhuang Autonomous Region, China (23°43.10'N, 106°39.77'E)	23 December 2019	Kunpeng Fang	GX19081
GD224	Baiyun Mountain, Guangzhou, Guangdong province, China (23°36.00'N, 113°49.53'E)	22 November 2020	Jinfen Han and Kunpeng Fang	GD20224
GD225	Baiyun Mountain, Guangzhou, Guangdong province, China (23°36.00'N, 113°49.53'E)	22 November 2020	Jinfen Han and Kunpeng Fang	GD20225
GD228	Baiyun Mountain, Guangzhou, Guangdong province, China (23°36.00'N, 113°49.53'E)	22 November 2020	Jinfen Han and Kunpeng Fang	GD20228

Table 2. GenBank accession numbers of *rbcl* and COI-5P sequences generated in this study.

Taxon	Isolate	<i>rbcl</i> accession No.	COI-5P accession No.
<i>Thorea okadae</i>	GX41	MZ648088	MZ676778
<i>T. okadae</i>	GX81	MZ648089	MZ676779
<i>T. baiyunensis</i>	GD224	MZ648090	MZ676780
<i>T. baiyunensis</i>	GD225	MZ648091	MZ676781
<i>T. baiyunensis</i>	GD228	MZ648092	MZ676782

Total DNA was extracted following the protocol originally described by Saunders (1993) and revised by Vis and Sheath (1997). Two molecular markers, *rbcl* and COI-5P, were amplified using the primers and protocols described by Vis et al. (1998) and Saunders (2005). The PCR products with their amplification primers were sent to BGI Tech Corporation (Beijing, China) for sequencing on an ABI 3730XL sequencer. Sequences generated in this study were submitted to the GenBank databases (Table 2). Additional related sequence data of the Thoreales order and outgroup taxa *Batrachospermum* were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/>) (Suppl. material 3: Table S1). The 58 *rbcl*, and 47 COI-5P sequences were aligned by CLUSTAL-X 2.0 (Thompson et al. 1997) and MEGA 5.0 (Tamura et al. 2011). Pairwise distance and the number of nucleotide variances for the taxa's molecular markers were calculated in MEGA 5.0. For phylogenetic analyses, the appropriate models for the sequence evolution were determined by MODELTEST 3.7 (Posada and Buckley 2004). The parameters for the concatenated sequences (*rbcl* and COI-5P) maximum likelihood (ML) analyses were as follows: GTR+I+G model; gamma distribution=0.5411; proportion of invariable sites=1.1344; base frequencies A=0.3352, C=0.1247, G=0.1641, and T=0.3760; and rate matrix A-C=3.7852, A-G=9.4223, A-T=1.1874, C-G=0.6077, and C-T=20.8378. PHYML software (Felsenstein 1981; Guindon and Gascuel 2003) was utilized to construct the ML trees with 1,000 replicates of bootstrap analysis. Bayesian Inferences (B.I.) were performed in MRBAYES VERSION 3.1.2 (Ronquist and Huelsenbeck 2003) and runs 5,000,000 generations sampling every 1,000 generations until the standard error was lower than 0.01. The resulting phylogenetic trees were edited using FIGTREE 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

Molecular analysis

The *rbcl* data matrix included 56 specimens of Thoreales and 2 outgroup taxa, consisting of 1203 characters, of which 410 (34.08%) were variable and 380 (31.59%) were parsimony informative. The *rbcl* *p*-distance among the five "Chantransia" isolates and other specimens of order Thoreales (Suppl. material 4: Table S2) showed that divergences between the isolates (GX41 and GX81) collected from Guangxi Zhuang Autonomous Region and five *T. okadae* specimens previous reported from Japan were 0%–1%, corresponding to 4–6-bp

differences, the average distance between these two samples (GX41 and GX81) and the other species of genus *Thorea* was 8.5%. These results supported the close relationship between the two isolates (GX41 and GX81) and *T. okadae* and further substantiated that GX41 and GX81 were the “Chantransia” of *T. okadae*. However, three other isolates (GD224, GD225, and GD228) collected from Guangdong Province had unique sequences. The distances between them and the other species of *Thorea* were higher than the intraspecific distances of this genus (10.88% VS 8.28%).

The COI-5P sequence determined for the five “Chantransia” isolates from this study were 676 bp, of which 297 (43.93%) were variable and 273 (40.38%) were parsimony informative. The pairwise distance among the “Chantransia” isolates collected in this study and other Thoreales taxa (Suppl. material 5: Table S3) showed that isolates GX41 and GX81 were closely related to *T. okadae* from Japan. The mean *p*-distance between samples from China and Japan was 3%. The intraspecific *p*-distances of species in this genus were 0–6%, whereas the interspecies *p*-distances of COI-5P sequences among the species of *Thorea* were 7.3–18.0%. For the remaining three isolates GD224, GD225, and GD228, their pairwise distances with other Thoreales specimens also supported the unique taxonomic status of GD224, GD225 and GD228: the *p*-distance between them and the other species of *Thorea* was 14.17%, which was larger than the intraspecific distance of this genus (0–6%).

Phylogenetic analyses based on single gene and concatenated genes produced trees with similar tree topologies, such that only the concatenated B.I. tree with supporting values calculated from two methods was displayed (Fig. 2). The single gene phylogenetic trees based on *rbcl* and COI-5P are shown in the supporting materials (Suppl. material 1, 2: Figs S1, S2).

The phylogenetic analyses strongly supported the monophyly of the Thoreales, *Nemalionopsis* and *Thorea* (Fig. 2). The *Thorea* clade contained two major subclades, one comprising four species: *T. quisqueyana* E.T.Johnston & M.L.Vis, *T. riekei* Bischoff, *T. gaudichaudii* C.Agardh, and *T. maunitukitukii* (1.00/99.9); and the second subclade including six species (*T. baiyunensis*, *T. bachmannii*, *T. kokosinga-pueschellii*, *T. okadae*, *T. indica* Necchi, E.K.Ganesan & J.A.West, and *T. hispida*) and an undetermined species (1.00/90.3). Isolates from this study were in the second subclade and formed two distant clusters. The isolates GX41 and GX81 were within a clade with five *T. okadae* specimens collected from Japan. This relationship was well supported by Bayesian posterior probabilities (100%) and ML bootstrap (100%). The remaining three isolates, GD224, GD225, and GD228 were an independent clade distantly related from any of the previously described species with high support values (1.00/90.3).

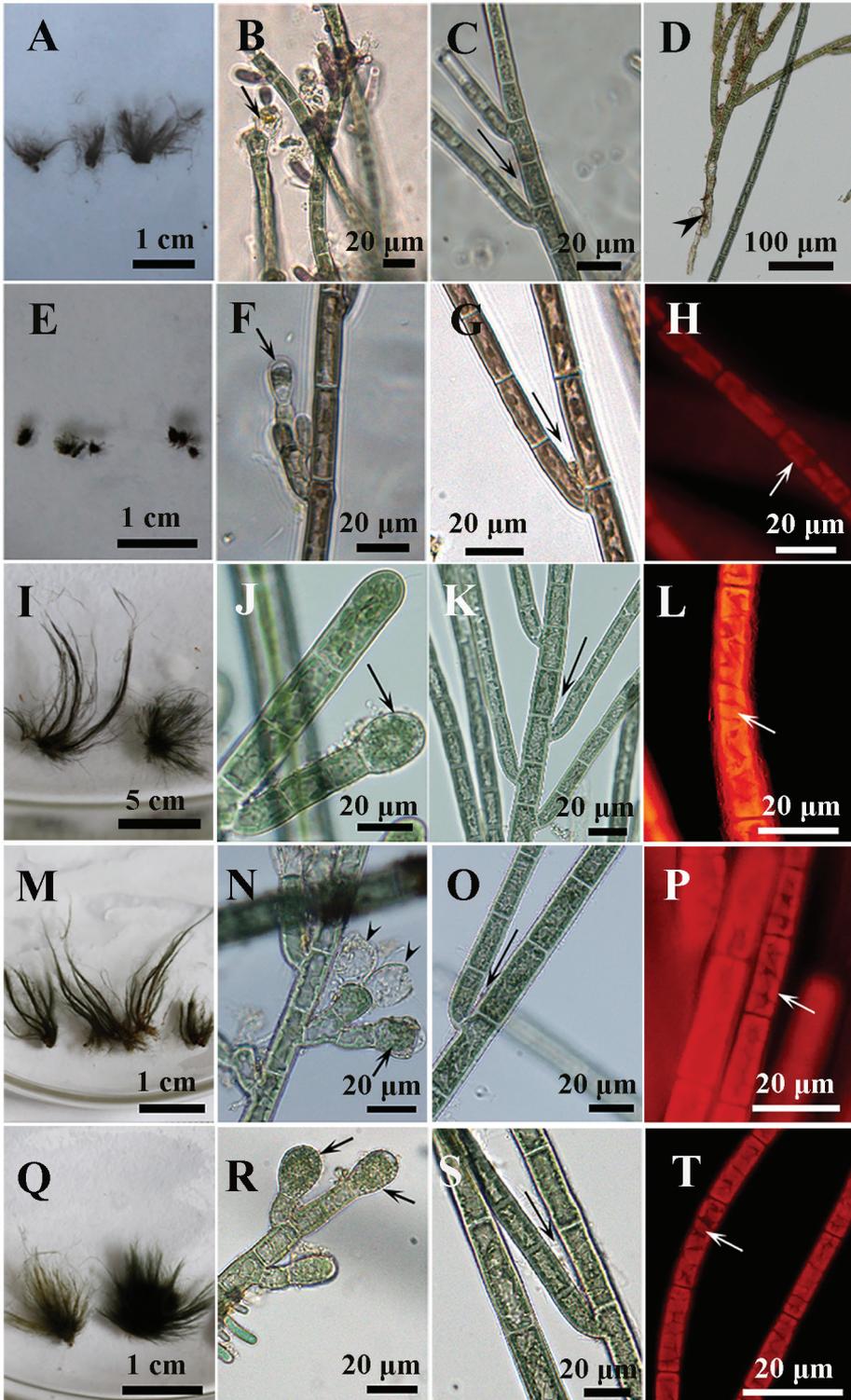
Morphological observations

The morphometric data showed that all samples used in this study fit the morphological description of *Audouinella pygmaea* (Roth) Duby, although the tuft length of GX81 is significantly smaller than others (Fig. 3A–T, Table 3). Their characteristics are as follows: tuft-shape, 1.2–25.9 mm length, bluish or brownish in color; basal portion consisting

of an irregular prostrate system of densely aggregated filaments; erect filaments dense, irregular branched, apical cells obtuse, without hair; vegetative cells of main branches cylindrical, 8.2–68.2 μm in length and 7.2–22.7 μm in diameter; monosporangial branches short, mostly grow at the tip of vegetative branch, with few small branches; monosporangia are obovoidal, 11.8–22.7 μm in length and 7.2–17.3 μm in diameter.



Figure 2. Bayesian inference tree for *Thorea* and *Nematolopsis* based on concatenated sequences from *rbcL* and *COI-5P* genes. Support values for all analyses are shown as follows: Bayesian posterior probabilities/ML bootstrap. ‘-’ denotes <50% support for those analyses at that node. All new sequences generated in this study are indicated in red boxes.



Taxonomic proposals

The genetic distance and phylogenetic analysis based on *rbcL*, COI-5P and the concatenated genes all supported the identification of new species described below.

Thorea baiyunensis Han, Nan & Xie, sp. nov.

Fig. 3I–T

Description. Known only from the “Chantransia” sporophyte generation. Plant macroscopic, up to 25.9 mm, bluish; basal portion consisting of an irregular prostrate system of densely aggregated filaments; lateral branches developing at angle < 25°. Vegetative cells of main branches cylindrical, (12.7) 13.6–63.6 (68.2) μm long and (8.2) 10.9–20.9 (22.7) μm in diameter. Monosporangia numerous, mostly grow at the tip of 1–5-celled, short branchlets, singly or in clusters, obovoidal, (11.8) 12.7–21.8 (23.6) μm long and (10.9) 11.8–16.4 (17.3) μm in diameter. Chloroplasts laminate or irregularly lobed, 2–4 per cell.

Diagnosis. Diagnostic DNA sequence: *rbcL* and COI-5P (accession number: MZ648090, MZ648091, and MZ648092 for *rbcL* and MZ676780, MZ676781, and MZ676782 for COI-5P).

Type. CHINA, Guangdong Province, Baiyun Mountain, 540 m alt., 23°36.00'N, 113°49.53'E: epilithic on the rocks in spring water, November 2020, J.F. Han & K.P. Fang (Holotype: SXU-SAS18040; Paratype: SXU-SAS18041). Deposited in Herbarium of Shanxi University (SXU), Shanxi University, Taiyuan, Shanxi Province, China.

Habitat and distribution. Baiyun Mountain (23°36.00'N, 113°49.53'E), Guangdong Province, China: on the rocks in spring water; water temperature 17.3–20.6 °C, pH 6.5–7.6 (Figs 1, 4A–C).

Etymology. The species epithet refers to the type locality (Baiyun Mountain, China).

Authentic strain. SXU-GD20224.

Figure 3. Morphological characters of samples investigated in this study **A–D** sample GX41 **A** morphological observation of the tufts of erect filament, bluish in color **B** monosporangial branch with ovoid monosporangium (black arrow) **C** filament showing branch angle ≤ 25° (black arrow) **D** erect filaments arise from basal system consisting of a prostrate mass with sparse rhizoids (black arrowhead) **E–H** sample GX81 **E** morphological observation of the tufts of erect filament, brownish in color **F** monosporangial branch with ovoid monosporangium (black arrow) **G** filament showing branch angle < 25° (black arrow) **H** cells have parietal laminate or irregularly lobed Chloroplast (white arrow) **I–L** sample GD224 **I** morphological observation of the tufts of erect filament, bluish in color **J** monosporangial branch with obovoidal monosporangium (black arrow) **K** filament showing branch angle < 25° (black arrow) **L** cells have parietal laminate or irregularly lobed Chloroplast (white arrow) **M–O** sample GD225 **M** morphological observation of the tufts of erect filament, bluish in color **N** monosporangial branch with obovoidal monosporangium (black arrow) **O** filament showing branch angle < 25° (black arrow) **P** cells have parietal laminate or irregularly lobed Chloroplast (white arrow) **Q–T** sample GD228 **Q** morphological observation of the tufts of erect filament, bluish in color **R** monosporangial branch with obovoidal monosporangium (black arrow) **S** filament showing branch angle < 25° (black arrow) **T** cells have parietal laminate or irregularly lobed Chloroplast (white arrow).

Table 3. Morphological characteristics of specimens in this study.

Thalli characteristics	GX41	GX81	GD224	GD225	GD228
Color	Bluish	Brownish	Bluish	Bluish	Bluish
Height (mm)	(4.8) 5–9.4 (10.0)	(1.2) 1.5–3.5 (4.4)	(3.4) 4.1–9.3 (10.8)	(4.1) 5.4–22.9 (25.9)	(8.5) 8.8–12.9 (13.6)
Branch angle*	≤ 25°	< 25°	< 25°	< 25°	< 25°
Vegetative cells	(8.2) 10–28.2 (31.8)	(20.9) 22.7–54.5	(12.7) 13.6–63.6	(21.8) 22.7–	(22.7) 23.6–43.6
Length (µm)		(59.1)	(68.2)	59.1(60.9)	(45.5)
Vegetative cells	(7.2) 8.2–10.9 (11.8)	(8.2) 9.1–10.9 (11.8)	(8.2) 10.9–15.5	(10.9) 11.8–15.5	(11.8) 12.7–20.9
Diameter (µm)			(16.4)	(16.4)	(22.7)
Monosporangia	Obovoidal	Obovoidal	Obovoidal	Obovoidal	Obovoidal
Shape					
Monosporangia	(12.7) 13.6–14.5	(12.7) 13.6–18.2	(11.8)	(12.7) 13.6–17.3	(13.6) 14.5–21.8
Length (µm)	(15.5)	(19.1)	12.7–21.8 (22.7)	(19.1)	(23.6)
Monosporangia	(7.2) 8.2–14.5 (15.5)	(8.2) 9.1–12.7 (13.6)	(10.9) 11.8–15.5	(10.9)	(10.9) 11.8–16.4
Diameter (µm)			(16.4)	11.8–13.6 (14.5)	(17.3)
Chloroplast	2–4	2–4	2–4	2–4	2–4
number					
Chloroplast	lamine or	lamine or	lamine or	lamine or	lamine or
shape	irregularly lobed	irregularly lobed	irregularly lobed	irregularly lobed	irregularly lobed

* Definition follows Necchi and Zucchi (1995).

Discussion

Several studies have confirmed that it is difficult to distinguish “Chantransia” stages from true *Audouinella* based only on morphological characteristics, which often hinders species identification and brings confusion to the classification system of freshwater red algae (Chiasson et al. 2005; Necchi and Oliveira 2011; Han et al. 2020). More surprising is that even individuals with completely different gametophyte morphology, belonging to different species, genera or orders, may also produce “Chantransia” stages with extremely similar morphology. In this study, morphological data showed that all samples collected from South China were similar and within the circumscription of *A. pygmaea*. However, molecular analysis confirmed that they were not *Audouinella* taxa but the “Chantransia” stages of two different *Thorea* species, *T. okadae* and *T. baiyunensis*. Based on sequence data and culture studies (Pueschel et al. 2000; Zucchi and Necchi 2003; Chiasson et al. 2005, 2007; Necchi and Oliveira 2011; Han et al. 2020), eleven species of orders Batrachospermales and Thoreaales have been confirmed to form “Chantransia” stages similar to *A. pygmaea* in morphology. Thus, at least thirteen species of the orders Batrachospermales and Thoreaales are associated with “*A. pygmaea*” so far. It is clear that the morphological characteristics of the “Chantransia” stages cannot be used to determine the identity individual species (Necchi and Oliveira 2011; Han et al. 2021). However, how genetic information and environmental factors regulate the morphological expression of “Chantransia” stages remain unclear.

With the widespread application of molecular data in the taxonomy of freshwater red algae, the view that morphologically simple organisms have considerable genetic diversity and species richness has been widely recognized (Johnston et al. 2018; Han et al. 2020, 2021). In the past few years, several new species of the Batrachospermales and

Thoreales were proposed based on the “Chantransia” sporophyte generation, such as *T. quisqueyana*, *Sheathia shimenxiaensis* J.-F.Han, F.-R.Nan et S.-L.Xie, *S. jiugongshanensis* J.-F.Han, F.-R.Nan et S.L.Xie, and *S. qinyuanensis* J.-F.Han, F.-R.Nan et S.L.Xie (Johnston et al. 2018; Han et al. 2020, 2021). In this article, based on the *rbcl* and COI-5P sequences, all five samples utilized in this study were unquestionably proved to be the “Chantransia” stages of two species of genus *Thorea*, whose gametophytes have never been reported in China before. Therefore, taking into account the two stages, the geographical distribution of genus *Thorea* would be larger, and the number of species would be more than that identified only by morphological data. Combined with previous reports in China, four species of genus *Thorea* have been recognized, including *T. hispida*, *T. violacea*, *T. baiyunensis*, and *T. okadae*. At the same time, the number of the *Thorea* distribution sites is proposed to have expanded to eight, including Shanxi, Jiangsu, Yunnan, Guizhou, Hunan, Henan, Guangdong provinces and Guangxi Zhuang Autonomous Region. This study reinforces the importance of collecting and sequencing specimens of “Chantransia” stages as a tool to reveal the hidden diversity of freshwater red algae of the orders Batrachospermales and Thoreales in different regions of the world.

In 1949, Yamada reported a new species, *T. okadae*, when studying the specimens of genus *Thorea* in Kagoshima prefecture, Japan (Yamada 1949). According to Kuman (2002) and Kozono (2020), *T. okadae* is the largest species known in the genus *Thorea* and is widely distributed in different areas of Honshu Island and Kyushu Island in Japan. This species has long been considered endemic to Japan. However, in this study we reported two “Chantransia” stages (GX41 and GX81) of *T. okadae* from Guangxi Zhuang Autonomous Region, China. Sequence analysis based on *rbcl* and COI-5P showed that there were only a few base differences between *T. okadae* from China and Japan. As shown in Fig. 1, the distribution of *T. okadae* in China (red circle) is far away from that in Japan (green circle). However, these regions all belong to the subtropical monsoon climate, which means that these places have similar climatic conditions, including light, temperature and precipitation. According to Higa et al. (2007), both gametophytes and “Chantransia” stage specimens of *T. okadae* were observed in Kikuchi River, Japan where water pH is 6.6–7.5, temperature is 26.3 °C in summer and 9.5 °C in winter. In addition, the mean day length of this place ranged from 9.9 h in December to 14.3 h in June. In this study, the “Chantransia” isolates of *T. okadae* were collected in Baimo Cave and Tongling Great Falls (Fig. 4D, E). The habitat characteristics of these two places are as follows: mean water temperature ranged from 14 °C to 21 °C and pH values fluctuated between 7.2 and 7.8, while the day length is about 10.7 h in December and 13.5 in June. The environmental factors of Baimo Cave and Tongling Great Falls were similar to those of Kikuchi River, thus, it is not surprising that *T. okadae* was found in Guangxi Zhuang Autonomous Region, China. It is therefore reasonable to infer that *T. okadae* also occurs in other areas of China with the same regional climate.

In a study on the seasonality of gametophyte occurrence, maturation and fertilization of the freshwater red alga *T. okadae*, Higa et al. (2007) pointed out

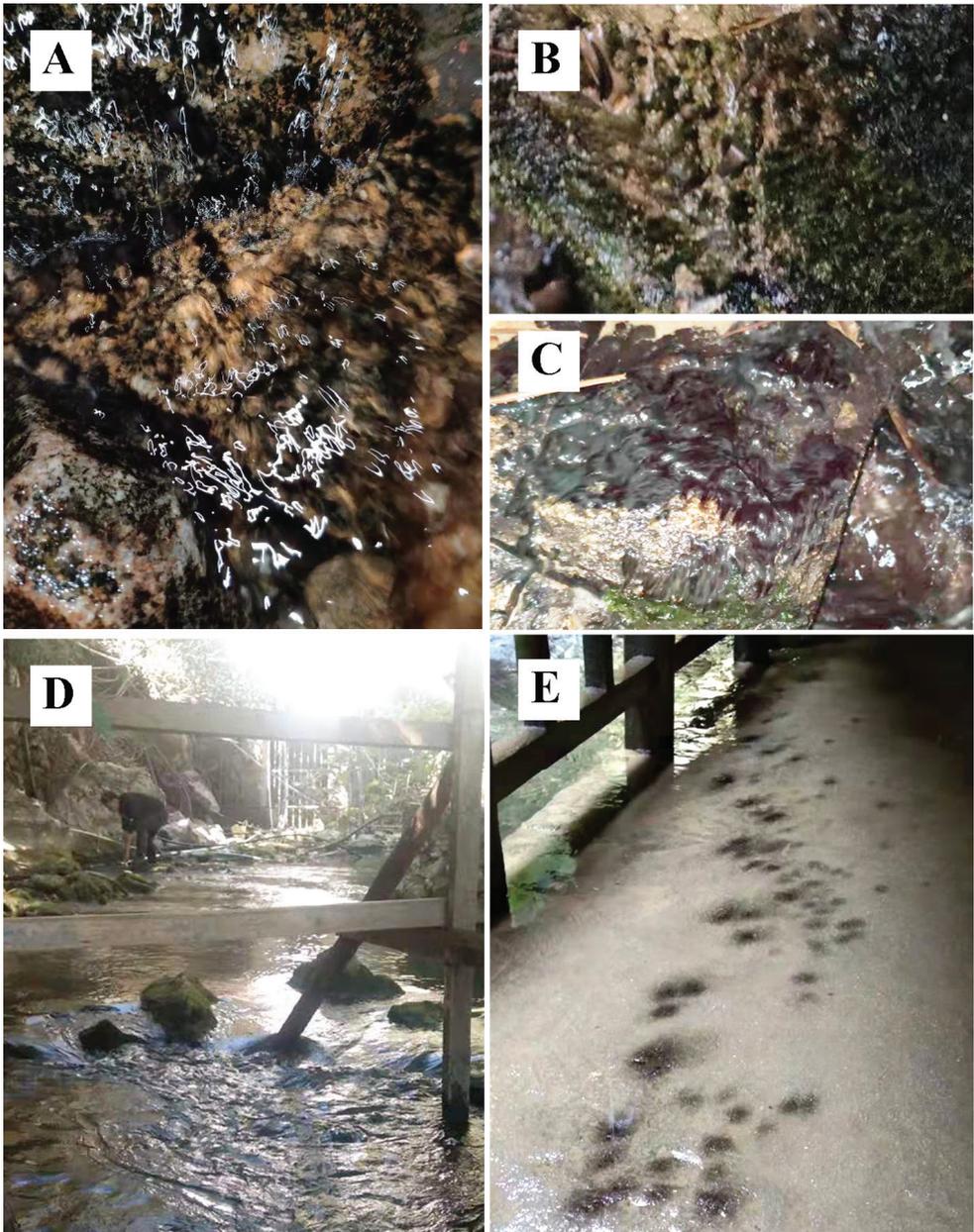


Figure 4. Habitat of *Thorea baiyunensis* and *T. okadae* **A–C** habitat of *Thorea baiyunensis* **D, E** habitat of *T. okadae*.

that gametophytes generally form from December and can be observed from early autumn to late spring, while “Chantransia” stages (sporophytes) last throughout the year. The “Chantransia” stages of *T. okadae* in this study were collected in December 2019, but we did not find its gametophytes. We speculate that this may be due to

the following reasons: firstly, since the samples were only collected in December, the gametophytes of *T. okadae* may have just begun to form. In this case, the small number of gametophytes and the short length of assimilatory filaments make them difficult to detect; additionally, the absence of the gametophytes of *T. okadae* may also be due to the environmental conditions that were not suitable for inducing their formation.

Acknowledgements

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Supplementary material I

Figure S1

Authors: Jinfen Han, Fangru Nan, Jia Feng, Junping Lv, Qi Liu, Xudong Liu, Shulian Xie
Data type: COL

Explanation note: Bayesian inference tree based on the *rbcL* gene sequences. Support values for all analyses are shown as follows: Bayesian posterior probabilities/ML bootstrap. ‘-’ denotes <50% support for that analyses at that node. All new sequences generated in this study are indicated in red boxes.

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Link: <https://doi.org/10.3897/phytokeys.193.79667.suppl1>

Supplementary material 2

Figure S2

Authors: Jinfen Han, Fangru Nan, Jia Feng, Junping Lv, Qi Liu, Xudong Liu, Shulian Xie

Data type: COL

Explanation note: Bayesian inference tree based on the COI-5P gene sequences.

Support values for all analyses are shown as follows: Bayesian posterior probabilities/ML bootstrap. ‘-’ denotes <50% support for that analyses at that node. All new sequences generated in this study are indicated in red boxes.

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Link: <https://doi.org/10.3897/phytokeys.193.79667.suppl2>

Supplementary material 3

Table S1

Authors: Jinfen Han, Fangru Nan, Jia Feng, Junping Lv, Qi Liu, Xudong Liu, Shulian Xie

Data type: docx

Explanation note: Specimen information of sequences downloaded from the GenBank database. “–” denotes no related information for the specimen.

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Link: <https://doi.org/10.3897/phytokeys.193.79667.suppl3>

Supplementary material 4

Table S2

Authors: Jinfen Han, Fangru Nan, Jia Feng, Junping Lv, Qi Liu, Xudong Liu, Shulian Xie

Data type: xls

Explanation note: Pairwise distance (lower-left matrix) and number of nucleotide variance (upper-right matrix) of *rbcl* sequence among the taxa in this study.

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Link: <https://doi.org/10.3897/phytokeys.193.79667.suppl4>

Supplementary material 5

Table S3

Authors: Jinfen Han, Fangru Nan, Jia Feng, Junping Lv, Qi Liu, Xudong Liu, Shulian Xie

Data type: xls

Explanation note: Pairwise distance (lower-left matrix) and number of nucleotide variance (upper-right matrix) of *COI-5P* sequence among the taxa in this study.

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A new species of *Ranunculus* (Ranunculaceae) from Western Pamir-Alay, Uzbekistan

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Abstract

New data on the phylogeny of four rare and endemic species of *Ranunculus* L. (sect. *Ranunculastrum* DC.) of western Pamir-Alai, one of which is new to science, have been obtained. *Ranunculus tojibaevii* sp. nov., from the Baysantau Mountains, Western Hissar Range of Uzbekistan, is described. The new species is closely related to *R. botschantzevii* Ovcz., *R. convexiusculus* Kovalevsk. and *R. alpinus* Kom., but differs in the blade of the radical leaves, which is rounded-reniform, segments 3–5-dissected, each 2–5-partite with elongated, rounded apical lobes. A phylogenetic analysis, using both the nuclear ribosomal internal transcribed spacer (ITS) and cpDNA (*matK*, *rbcL*, *trnL-trnF*), was informative in placing *R. tojibaevii* in context with its most closely-related species. Discussion on the geographic distribution, updated identification key, a detailed description, insights about its habitat and illustrations are provided.

Keywords

Endemic, Hissar Range, Pamir-Alay, phylogenetic analysis, Ranunculales, *Ranunculastrum*

Introduction

Ranunculus L., the largest genus in the Ranunculaceae Juss., includes ca. 600 genetically diverse species (Tamura 1995). The genus is distinguished by its high ecological-geographical diversity over a wide zonal spectrum ranging from the Arctic tundra through varied forests, steppes, deserts to exclusively aquatic habitats and high-altitude communities on nearly all continents (Paun et al. 2005). However, the main centres of speciation of *Ranunculus* are often in large mountain systems, where the formation of species is not only directly related to isolation, but also significantly depends on specific conditions of the highlands (Emadzade et al. 2015; Fernández Prieto et al. 2017; Shchegoleva 2018; Shchegoleva et al. 2020; Zverev et al. 2020).

More than 90 species of *Ranunculus* are distributed in Central Asia (Kovalevskaya 1972). Differentiation in the genus and the formation of locally endemic species are closely related to the history of the Tian Shan and Pamir–Alay Mountain formations. Here, more than half of the members of the genus are autochthonous representatives of the mountainous Central Asian flora, which arose in the process of regional adaptive diversification from ancient Mediterranean predecessors (Ovchinnikov 1971; Kamelin 1973). In the dry Central Asian seasonal climate, the features of these species are observed only in the short-term hydrothermal period of active vegetative growth.

Ranunculus tojibaevii was first discovered in 2013 on the Baysuntau Highlands (Khodzha-Gurgur-ata Mountain) on the south-western spur of the Hissar Range (Pamir–Alay). The populations were detected again in 2019 while working on the *Flora of Uzbekistan Project* (Sennikov et al. 2016). It should be noted that the flora of the Western Pamir–Alay is characterised by a high taxonomic diversity (Kamelin 1973; Vasilchenko and Vasileva 1985; Tojibaev et al. 2016; Makhmudjanov et al. 2019; Yusupov et al. 2020).

The morphological features indicated that the unknown plants belonged to *R.* subg. *Ranunculus* sect. *Ranunculastrum* (Hörandl and Emadzade 2012; Baltisberger and Hörandl 2016). The main differences between sect. *Ranunculastrum* and other sections of *Ranunculus* are the presence of a triangular beak equal to or longer than the achene body, a receptacle completely glabrous, a taproot partly tuberous and a mostly elongate fruit (Hörandl and Emadzade 2012).

The unknown plants closely resembled *R. botschantzevii* Ovcz. (Ovchinnikov 1941) and *R. convexiusculus* Kovalevsk. (Kovalevskaya 1972), as well as *R. alpigenus* Kom. (Komarov 1896) in their main morphological features. The molecular results presented here also clearly substantiated its independent taxonomic status. In this study, we present a morphological description of these plants, which we named *Ranunculus tojibaevii* Schegol. & Turginov. Figures showing its features, a map of its distribution, taxonomy and an identification key separating it from the most closely-related species are also provided.

Materials and methods

Morphological observations and measurements of *R. tojibaevii* were made on two populations; in total, 34 individuals were compared. Voucher specimens have been deposited in the National Herbarium of Uzbekistan – TASH (Tashkent, Uzbekistan). Additionally, two closely-related species, *R. convexiusculus* and *R. botschantzevii*, from the same territory and high-altitude regions were examined. Herbarium specimens at TASH, TAD, LE, FRU, AA, MW, LE and TK were also compared. Studies of closely related species were performed during field expeditions to Uzbekistan, Tajikistan and Kyrgyzstan, from 2017–2021 (Shchegoleva et al. 2020). The distribution map was generated in ESRI ArcGIS 10 software using GPS coordinates (www.esri.com). The conservation status was established, based on IUCN Criteria (IUCN 2019).

Molecular methods

DNA extraction, amplification and sequencing

DNA isolation was performed using a Plant Genomic DNA Kit (TIENGEN Biotech, Beijing, China) according to the manufacturer's protocol. CTAB extraction protocol with some modifications was used to extract genomic DNA from herbarium specimens of *R. alpigenus* (Doyle and Doyle 1987).

Selected nuclear DNA regions ITS1-ITS2 (for herbarium specimen *R. alpigenus*) and ITS1-ITS4; plastid DNA regions *matK*, *rbcL* and *trnL-F* were amplified on a thermal cycler (BioRad) using the 2X PCR Taq Plus MasterMix with dye (Applied Biological Materials Inc., Canada). Amplification of the DNA regions was carried out by using primers of the forward and reverse primer sets (TsingKe, China) (Table 1).

To obtain sequences of the genes of interest, PCR amplification was carried out according to the following parameters (except *R. alpigenus*): for ITS1-ITS4, initial denaturation for 3 min at 94 °C, followed by 35 amplification cycles: 30 s at 94 °C, 30 s at 50–54 °C, 1 min at 72 °C; elongation 7 min at 72 °C; for *matK*– an initial denaturation for 3 min 94 °C, followed by 35 amplification cycles: 30 s 94 °C, 1 min 51 °C, 1 min 72 °C; final extension 10 min at 72 °C; for *rbcL*, an initial denaturation for 4 min 95 °C, followed by 34 amplification cycles: 1 min 94 °C, 1 min 50 °C, 1 min 72 °C; final extension 10 min at 72 °C; for *trnL-F*, an initial denaturation for 3 min 94 °C, followed by 32 amplification cycles: 45 s 94 °C, 45 s 50 °C, 1 min 72 °C; final extension 8 min at 72 °C.

PCR amplification for *R. alpigenus* was performed for ITS1-ITS2, with the following programme: initial denaturation at 94 °C/5 min; 35 amplification cycles at 94 °C/30 s, at 54 °C/30 s, at 72 °C/ 45 s; elongation at 72 °C/7 min; for *rbcL*, 94 °C/4 min, 34 cycles: 94 °C/30 s, 54 °C/ 45 s, 72 °C/45 s; final extension at 72 °C/10 min.

Table 1. Primers used in this study.

Primer name	Sequences (forward / reverse)	DNA fragment size, bp	Primer source
ITS1-18S	5'-TCCGTAGGTGAACCTGCGG-3'	- 700 bp	White et al. (1990)
ITS4-26S	5'-TCCTCCGCTTATTGATATGC-3'		
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	- 650 bp	White et al. (1990)
ITS2	5'-GCTGCGTTCTTCATCGATGC-3'		
<i>matK-390F</i>	5'-CGATCTATTCAATCAATATTTTC-3'	- 900 bp	Cuenoud et al. (2002)
<i>matK-1326R</i>	5'-TCTAGCACACGAAAGTCGAAGT-3'		
<i>trnL-F_F</i>	5'-CGAAATCGGTAGACGCTACG-3'	- 900 bp	Taberlet et al. (1991)
<i>trnL-F_R</i>	5'-ATTTGAACTGGTGACACGAG-3'		
<i>rbcLaF</i>	5'-ATGTCACCACAAAACAGAGACTAAAGC-3'	- 600 bp	Kress and Erickson (2007)
<i>rbcLaR</i>	5'-GTAAAATCAAGTCCACCRCG-3'		

Taxon sampling

To determine the taxonomic status and systematic position of *R. tojibaevii*, we sampled 24 species of *Ranunculus*. New nDNA (ITS) and cpDNA intergenic spacers (*matK*, *rbcL*, *trnL-trnF*) sequences for nine species were generated. We also used available sequences of 15 *Ranunculus* species from GenBank (www.ncbi.nlm.nih.gov/Genbank) (Table 2). We used *Ranunculus* subgenus *Auricomus* as the outgroup (Hörandl and Emadzade 2012; Almerikova et al. 2020).

Phylogenetic analyses

Sequence alignments were performed using ClustalW (Thompson et al. 2002) as implemented in MEGA X software (Kumar et al. 2018). The best partitioning scheme for the combined dataset contained two partitions: the ITS data; and the three plastid sequences data (*matK*, *rbcL*, *trnL-trnF*). Phylogenetic reconstruction was first conducted separately, based on the nuclear and the plastid data. Visual inspection determined that differences between the nuclear and the plastid trees were solely due to resolved/collapsed clades. No topological incongruence with a high support value (posterior probabilities and bootstrap percentages) was found. To further test whether the nuclear and plastid data could be combined for phylogenetic reconstruction, the incongruence length difference (ILD, Farris et al. 1995) test was conducted in PAUP* 4.0a169 (current) by using only the informative sites, heuristic search, tree-bisection-reconnection (TBR) branch-swapping algorithm, simple addition sequence and 1,000 replicates. The ILD test between the nuclear and the plastid data found $p = 0.322$, indicating insignificant support for incongruence between the two datasets. Therefore, the nuclear and the plastid sequences were combined into one dataset for phylogenetic analyses using SequenceMatrix software (Vaidya et al. 2011).

Phylogenetic trees were reconstructed using Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI). For ML, we employed raxmlGUI 2.0 (Edler et al. 2020), with 1,000 bootstrap replicates and, for BI, we used MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) with 10,000,000 generations with random trees sampled every 1,000 generations. In the latter analysis, after discarding the first 25% trees as burn-in, a 50% majority-rule consensus tree was constructed from the remaining trees to estimate Posterior Probabilities (PP). For analyses, a model of nucleotide substitution was selected, based on the Akaike Information Criterion (AIC) using jModelTest2 on XSEDE (www.phylo.org). Phylogenetic analyses were also performed with the MP method using PAUP* 4.0a169. The MP bootstrap analysis was performed with heuristic search, TBR branch-swapping, 1,000 bootstrap replicates, random addition sequence with ten replicates, a maximum of 1,000 trees saved per round. Trees were visualised in FigTree v.1.4.0 (Rambaut 2012).

Table 2. Accession numbers of samples used for phylogenetic analyses of *Ranunculus* (* newly-generated sequences).

Species	GenBank accession number			
	ITS	<i>matK</i>	<i>rbcl</i>	<i>trnL-F</i>
<i>Ranunculus acris</i>	AY680167	AY954199	HQ590232	–
<i>R. alpigenus</i> *	OM283824	–	OM287560	–
<i>R. arvensis</i>	HQ650550	HQ650551	MK925091	–
<i>R. auricomus</i>	FM242803	FM242739	JN893758	–
<i>R. botschantzevii</i> *	MW540744	MW748677	MW748685	MW748693
<i>R. convexiusculus</i> *	MW540743	MW748676	MW748684	MW748692
<i>R. flammula</i>	AY680185	AY954204	MK526480	–
<i>R. glaberrimus</i>	KP687273	JF509974	MG247649	–
<i>R. inamoenus</i>	KP687279	KP687302	MG249011	–
<i>R. japonicus</i>	EU591982	AY954200	MH657741	DQ410744
<i>R. leptorrhynchus</i> *	MW737444	MW748673	MW748681	MW748689
<i>R. linearilobus</i> *	MW737445	MW748674	MW748682	MW748690
<i>R. lingua</i>	AY680184	AY954206	JN892742	–
<i>R. muricatus</i>	DQ410718	AY954191	HM850296	DQ410740
<i>R. paucidentatus</i> *	MW540747	MW748679	MW748687	MW748695
<i>R. pygmaeus</i>	KP687287	KP687310	KC483860	–
<i>R. regelianus</i> *	MW737446	MW748675	MW748683	MW748691
<i>R. repens</i>	MT271835	HM565166	MK925397	EU382995
<i>R. sceleratus</i>	MT271836	GU257993	AB517148	DQ410746
<i>R. sulphureus</i>	JF509969	JF509983	KC483870	–
<i>R. talassicus</i> *	MW540748	MW748680	MW748688	MW748696
<i>R. tojibaevii</i> *	MW540745	MW748678	MW748686	MW748694
<i>R. tojibaevii</i> *	OM278385	OM287558	OM287559	–
<i>R. trichophyllus</i>	KC620483	AY954133	L08766	–
<i>R. turneri</i>	FM242817	FM242741	MG249550	–

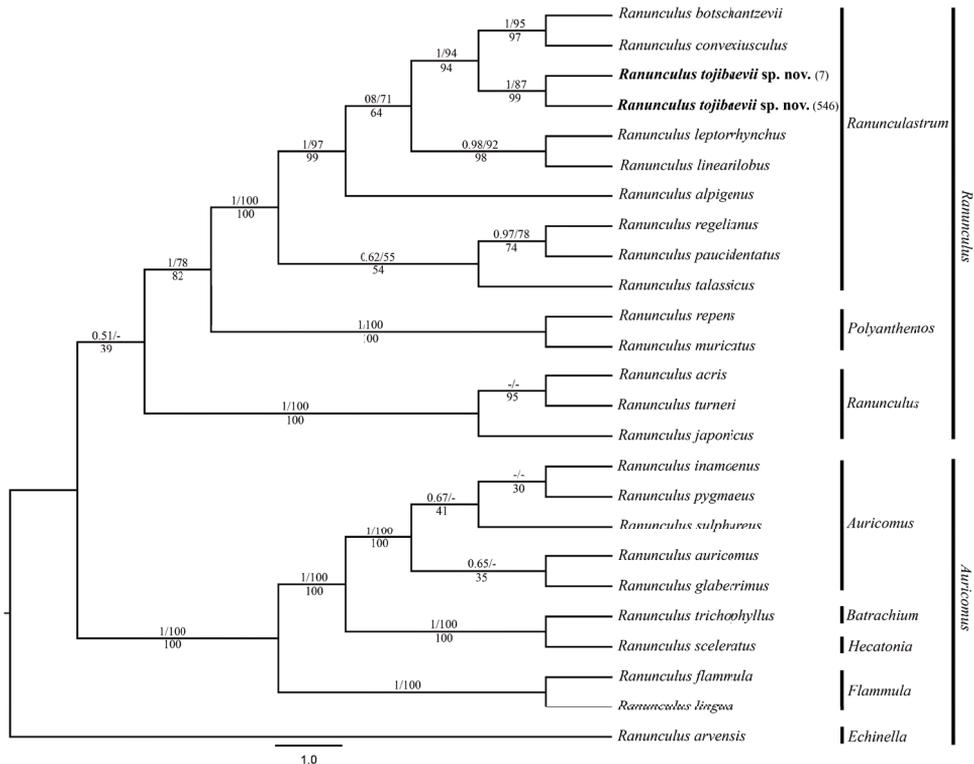


Figure 1. Bayesian tree based on combined nuclear (ITS) and plastid (*matK*, *rbcL*, *trnL-trnF*) sequence data showing phylogenetic position of *Ranunculus tojibaevii* Schegol. & Turginov in *R.* sect. *Ranunculastrum*. Bayesian Posterior Probability (PP) / Maximum Parsimony (MP) is given on each branch, respectively; Maximum Likelihood (ML) is below branches. The classification is according to Hörandl and Emadzade (2012). * denotes the *Ranunculus* species analysed in this study. The new species is highlighted in bold.

Results and discussion

The phylogenetic tree, based on the nuclear and plastid sequences (Fig. 1), showed that *R. tojibaevii* is sister to *R. convexusculus* and *R. botschantzevii* with high support values PP = 1, MP = 94% and ML = 94%. *Ranunculus tojibaevii*, *R. convexusculus* and *R. botschantzevii* formed a clade with well supported values (PP = 0.8, MP = 71% and ML = 64%).

The results of the phylogenetic analysis were similar to the results of Hörandl and Emadzade (2012) and Almerikova et al. (2020). Species of *R.* sect. *Ranunculastrum* are of particular interest. In our case, these native Asian species are mainly limited to the foothills and mountains of Central Asia (southern Kazakhstan, Uzbekistan, Kyrgyzstan, Tajikistan and Turkmenistan), as well as to the mountains of Afghanistan, Iran and Pakistan.

It is obvious that *R.* sect. *Ranunculastrum* in Central Asia is heterogeneous. The species forming sub-clusters in this section differ in their area of distribution, altitude confinement and time of origin, thereby confirming the neoendemic nature and origin of *R. tojibaevii*.

Taxonomy

Ranunculus tojibaevii Schegol. & Turginov, sp. nov.

urn:lsid:ipni.org:names:77296907-1

Figs 2, 3

Diagnosis. Similar to *R. botschantzevii*, *R. convexiusculus* and *R. alpigenus* morphologically, but differing in the rounded-reniform radical leaves dissected into 3–5 segments, each 2–5-partite into elongated lobes rounded at the apex (Fig. 4). *Ranunculus tojibaevii* differs from *R. alpigenus* in having fewer levels of leaf blade dissection. It differs from *R. convexiusculus* in the dissection of the radical leaf blades, larger flowers and having somewhat white, bristle-like hairs on the root collar and also from *R. botschantzevii* by the rounded apical lobes of the basal leaves and more xeromorphic habit.

Type. UZBEKISTAN. Hissar Ridge, Baysuntau, Khodzha-Gurgur-ata Mountains, vicinity of the Village Gumatag, 38°22.2888'N, 67°21.0834'E, 2482 m a.s.l., 30 May 2019, N. Shchegoleva, A. Jabborov, O. Turginov (holotype: TASH-003743; isotypes: TASH-003748, TASH-003749, TASH-003750, TK-002339).

Description. *Herbs* perennial. *Roots* dimorphic, some roots subulate, up to 0.5 mm thick, storage roots palmately-thickened, ca. 2.5 mm thick; root collar with milk-white bristle-like hairs. *Stems* 10–12(–15) cm tall, up to 2 mm diam., erect, branched, pubescent with white curly hairs, 1–3-flowered. *Leaves* dimorphic, radical leaves 2–3, 1.6–2 × 1.5–2.2 cm, blade rounded-reniform, 3–5-dissected, segments 2–5-partite, elongate, lobes apically rounded; cauline leaves 1–2, petiole short, slender, blade trisected, lobes 0.6–0.9 × 0.1–0.2 cm, oblong-lanceolate. *Flowers* 1.6–2.4 cm diam., sepals 0.4–0.6 mm long, ovate-concave, sparsely white pubescent; petals 0.9–1.2 cm long, well-developed, ovate, apex rounded. Inflorescence globose-ovoid; receptacle oblongoid, glabrous; achenes 1–1.8 mm long, with white bristle-like hairs; beak hamate-curved.

Specimen seen (paratype). UZBEKISTAN. Pamir-Alay, South-western spurs of the Hissar Ridge, Baysuntau, vicinity of the Village Gumatag, amongst the stones, 4 June 2013, O. Turginov (TASH-003754).

Phenology. Flowering in May. Fruiting in May and June.

Distribution. *Ranunculus tojibaevii* is distributed in the Khodzha-Gurgur-ata of the Baysuntau Mountains area of Hissar Ridge (Fig. 5). The closely-related *R. convexiusculus* is endemic to Central Asia and *R. botschantzevii* is endemic to the Western Pamir-Alay.

Habitat and plant associations. *Ranunculus tojibaevii* is rupicolous on southern and western exposed limestone outcrops and in cracks and crevices of large boulders at 2,450–2,500 m. a.s.l. The region is alpine and rather xerophytic. The common taxonomic composition of phytocenoses includes *Cerasus amygdaliflora* Nevski (Rosaceae), *Corydalis ledebouriana* Kar. & Kir. (Papaveraceae), *Cousinia regelii* C. Winkl. (Asteraceae), *Eremurus regelii* Vved. (Asphodelaceae), *Gagea gymnopoda* Vved.



Figure 2. *Ranunculus tojibaevii* Schegol. & Turginov (Shchegoleva, Jabborov, Turginov, holotype TASH-003743).

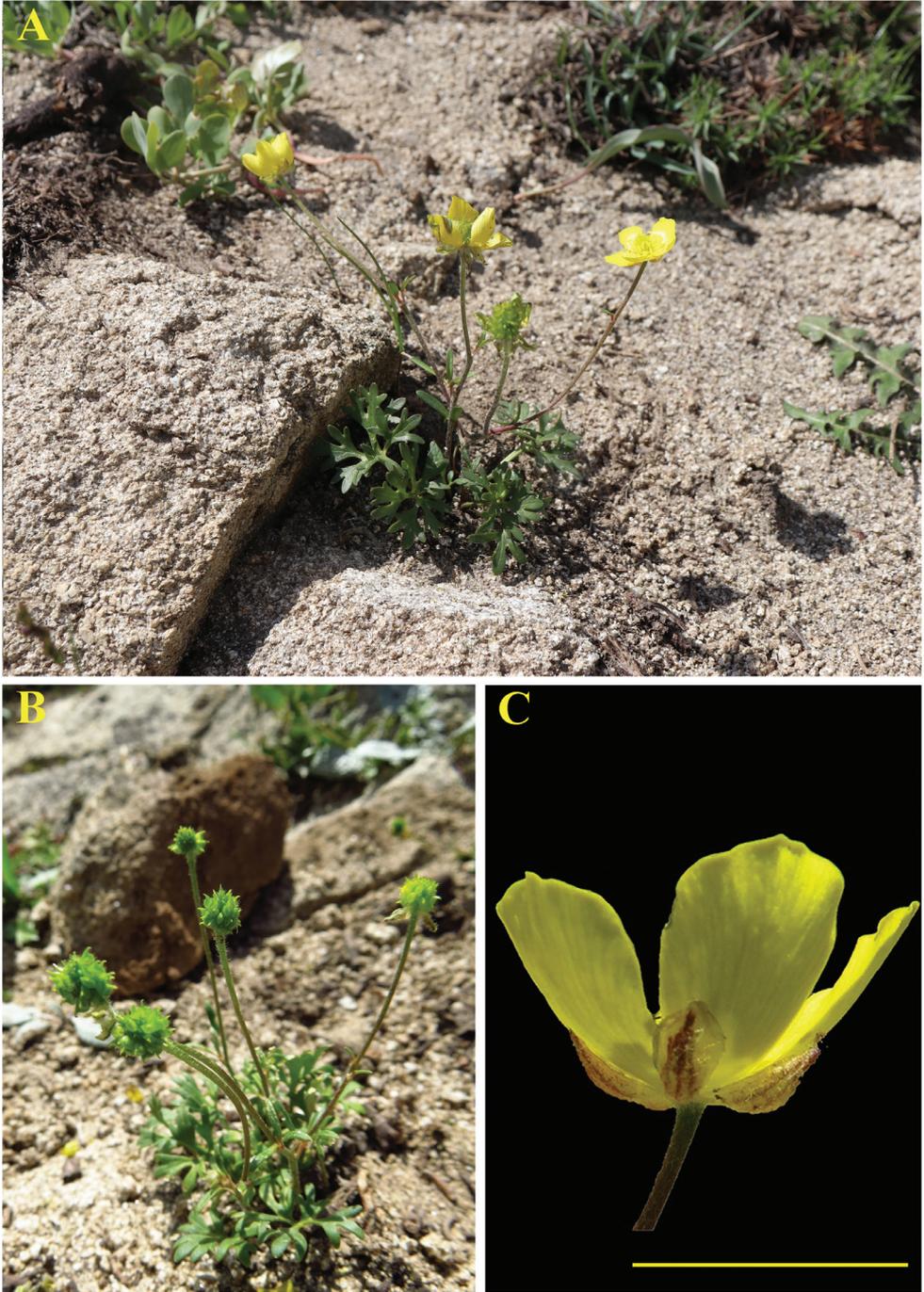


Figure 3. *Ranunculus tojibaevii* Schegol. & Turginov. Habitat (**A** flowering **B** fruiting) and flower (**C**). Scale bar: 1 cm.

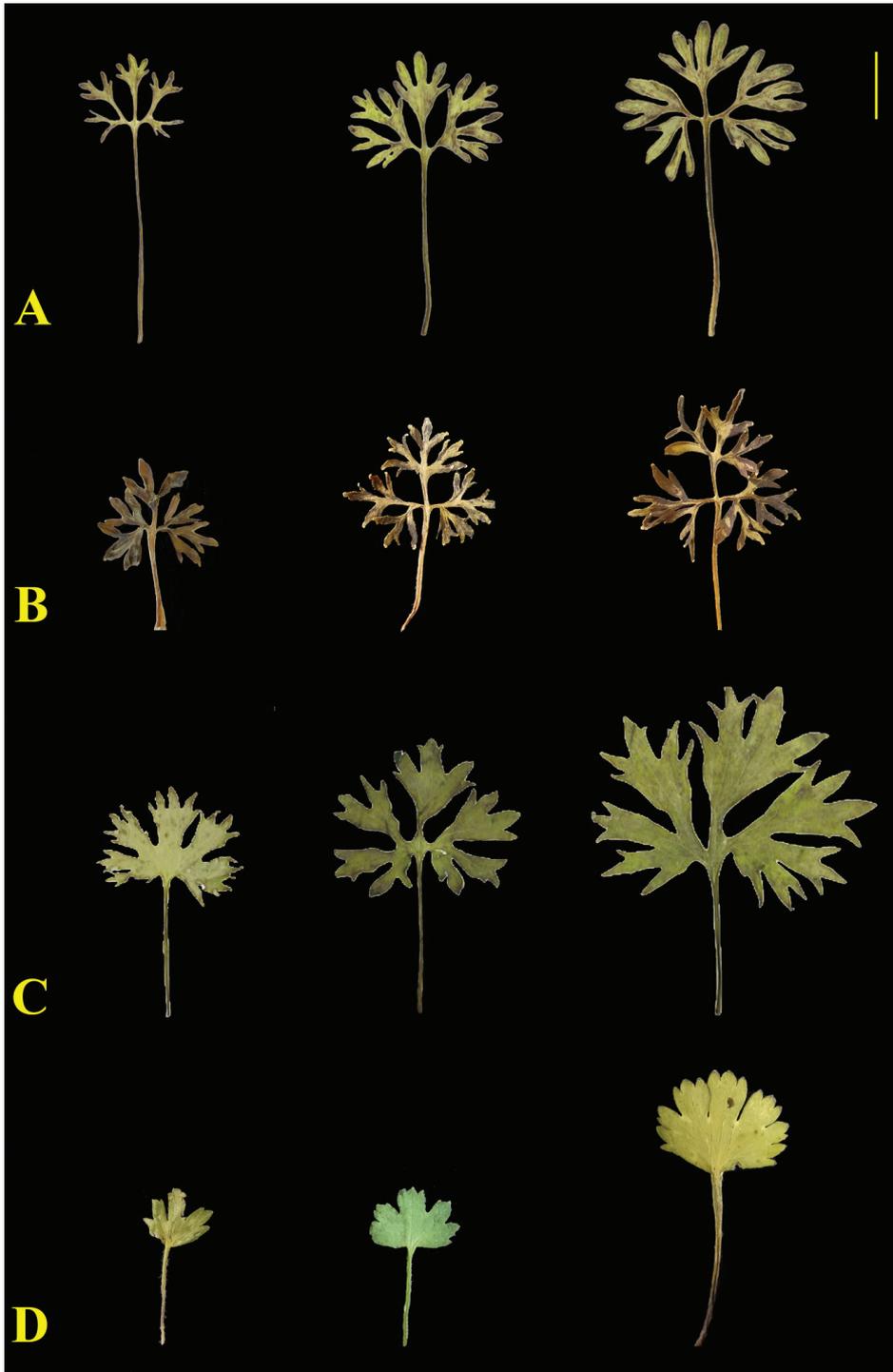


Figure 4. Series of basal leaves in related species **A** *Ranunculus tojibaevii* (from the holotype) **B** *R. alpigenus* **C** *R. botschantzevii* **D** *R. convexusculus*. Scale bar: 1 cm.

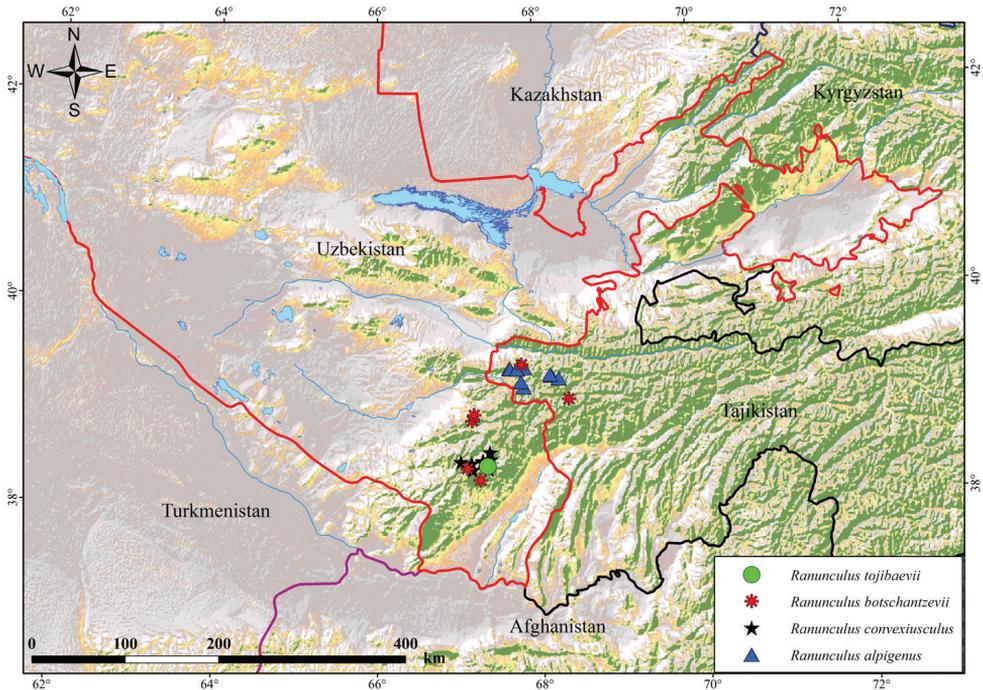


Figure 5. Distribution of *Ranunculus tojibaevii*, *R. alpigenus*, *R. botschantzevii* and *R. convexiusculus*.

(Liliaceae), *Iris khassanovii* Tojibaev & Turginov, *I. parvula* (Vved.) T.Hall & Seisums, *I. stolonifera* Maxim. (all Iridaceae), *Rheum maximowiczii* Losinsk. (Polygonaceae), *Tulipa lanata* Regel (Liliaceae) and *Ziziphora pamiroalaica* Juz. (Lamiaceae).

Conservation status. *Ranunculus tojibaevii* is a local, narrowly distributed endemic, represented by two or three individuals per m² within an area of < 500 m². It should therefore be assigned the status EN (Endangered), Criteria B1 ab(i, ii, iii)+ B2 ab(i, ii, iii), following the IUCN Standards and Petitions Committee (IUCN 2019).

Notes. *Ranunculus tojibaevii* differs from closely-related species by its habitat on well-heated limestone outcrops, as well as in cracks and crevices of large boulders at ca. 2,500 m a.s.l., which is atypical of related species. *Ranunculus convexiusculus* is on clayey-stony soil, less often on slopes of fine earth, at 2,000–2,600 m a.s.l. *Ranunculus botschantzevii* is hygrophilous in wet mountain meadows with melting snow and on slopes of fine clayey soil at 2,400–3,500 m a.s.l. *Ranunculus alpigenus* grows on slopes of fine soil of the alpine belt at 2,800–4,000 m a.s.l. All these species are endemic to the western Pamir-Alay. The vicariant species to *R. alpigenus* is *R. badachschanicus* Ovcz. & Koch. from the western Pamirs.

Etymology. *Ranunculus tojibaevii* is named after Komiljon Tojibaev, a leading botanist, professor and academician from Uzbekistan who actively promotes the botanical sciences in Central Asia.

Key to *Ranunculus tojibaevii* and similar species (Table 3)

- 1 Blades of basal leaves broadly ovate, dissected into pinnatipartite segments, with tripartite-oblong terminal lobules *R. alpigenus*
 – Radical leaves 3–5-dissected or lobed-incised **2**
 2 Blade of radical leaves 1/3 or nearly 1/2 unequally partite into broad incised-dentate lobes.....*R. convexiusculus*
 – Blade of radical leaves 3–5-dissected **3**
 3 Blade of basal leaves triangular-reniform, 3–5-dissected almost to the base, wedge-shaped segments, unequally sharp-toothed..... *R. botschantzevii*
 – Blade of radical leaves is round-reniform, 3–5-dissected, each section divided into 2–5 elongated lobules; apex of lobules rounded.....*R. tojibaevii*

Table 3. Comparison of *R. tojibaevii*, *R. botschantzevii*, *R. convexiusculus* and *R. alpigenus*.

	<i>R. tojibaevii</i>	<i>R. botschantzevii</i>	<i>R. convexiusculus</i>	<i>R. alpigenus</i>
Blade of radical leaves	rounded-ovate, 3–5-dissected, segments further 2–5-dissected, lobules elongated, rounded at apex	triangular-reniform, 3–5-partite, segments narrowly cuneate, unequally and subacutely dentate	reniform, dissected 1/3 to nearly 1/2 of its length, lobes broad incised-dentate	broadly ovate, dissected into pinnatipartite segments, segments tripartite, terminal lobules oblong
Sepals	narrowly elliptic, concave, less than half as long as petals, with long reclinate hairs	elliptic, concave, some shorter than the petals, with sparse, long reclinate hairs	elliptic, concave, half as long as petals, with short reclinate spreading hairs	elliptic, concave, with scattered hairs
Petals	oblong-obovate, greenish-yellow, base cuneate, margin undulate	obovate, bright yellow, becoming dark when dry, base broadly cuneate, margin undulate	very broadly ovate, golden yellow, base cuneate, margin slightly undulate	oblong-ovate, yellow-green, base narrowly cuneate, margin undulate
Achenes	1.0–1.8 mm long, asymmetrically ovate, slightly convex, with semi-appressed hairs	2.2–2.5 mm long, oblong, slightly laterally compressed, with appressed hairs	1.8–2.5 mm long, oblong, slightly convex, with appressed hairs	1.5–2.0 mm long, asymmetrically obovate, laterally compressed, with scattered not appressed hairs

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years “Grid mapping of the flora of Uzbekistan” and “The creation of DNA bank and barcoding of endemic plants of Uzbekistan”, as well as in accordance with the current State Assignment of Central Siberian Botanical Garden at Siberian Branch of Russian Academy of Sciences (AAAA-A21-121011290026-9).

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Rubus dianchuanensis sp. nov. (Rosaceae) from Sichuan and Yunnan, southwest China

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Abstract

Rubus dianchuanensis, a new name for the species previously named as *R. vicarius* by W. O. Focke in 1911, is proposed. A detailed description, illustrations, and remarks on its phenology, ecology, and geographic distribution are provided. This raspberry (subg. *Idaobatus*) is endemic to China and was only found in Sichuan and Yunnan, southwest China. Morphologically, it is most similar to *R. ovatisepalus* but clearly differs from the latter by having leaf densely white or grey tomentose abaxially, usually leaf-like bracts at the base of inflorescence, 1–6 cm long pedicels, and triangular-ovate sepals with acute to caudate apex.

Keywords

Endemism, later homonym, *Rubus subornatus*, synonymy, taxonomy

Introduction

The genus *Rubus* was established by Linnaeus (1753) in his *Species Plantarum* and ten species were described there. Currently, about 700 species of *Rubus* are recognized, making it one of the largest genera of Rosaceae (Robertson 1974; Lu and Boufford 2003). Plants of this genus are usually shrubs, rarely subshrubs or perennial herbs, and more or less prickly. Their leaves are compound or simple, flowers are pentamerous and mostly bisexual, fruits are fleshy aggregates of drupelets, and tori are usually convex, conical or cylindrical (Kalkman 1993; Lu and Boufford 2003; Wang and Wang 2019).

Members of *Rubus* are distributed nearly worldwide except for Antarctica and can be found in most types of land biomes from tropical to subarctic regions (Gustafsson 1942; Spies and Du Plessis 1984; Hummer 1996; Lu and Boufford 2003). There are more than 250 species of *Rubus* in East Asia, and this region is the center of diversity for the subgenera *Malachobatus* and *Idaeobatus* (Wang and Wang 2019). More than 200 species are recorded in China, and most of them occur in the southern and southwestern provinces (Lu and Boufford 2003). Recently, new species and nomenclatural changes of *Rubus* in China have been constantly reported (e.g., Huang and Hu 2009; Byalt 2011; Sun and Boufford 2012; Wang et al. 2013, 2017, 2019; Wang and Wang 2019).

During our fieldwork and the herbarium studies on a taxonomic revision for the Chinese species of *Rubus*, we encountered a raspberry difficult to assign to any species recognized by Yu and Lu (1985) and Lu and Boufford (2003). Further research showed that it should be identified as *R. vicarius* Focke, which had been synonymized with *R. subornatus* Focke previously (Yu and Lu 1985; Lu and Boufford 2003). This plant represents a separate species, therefore, should be resurrected. Nevertheless, Focke's name is a later homonym of *R. vicarius* Sudre (1902); consequently, a new name for this distinctive species is required.

Materials and methods

We studied the newly named species both in the field and the herbaria. Type specimens (or type photos) of accepted names and their synonyms in *Rubus* subg. *Idaeobatus* were extensively examined and compared, as well as herbarium materials from CDBI, IBSC, KUN, P, PE, PYU and YUKU (acronyms after Thiers 2022). Pertinent taxonomic literature (e.g., Focke 1877, 1910, 1911, 1914; Yu and Lu 1985; Lu and Boufford 2003) were extensively consulted. Morphological studies were carried out on dried material under a stereomicroscope, and measurements were conducted using a ruler or a metric vernier calliper.

Taxonomy

Rubus dianchuanensis Huan C. Wang & Q. P. Wang, sp. nov.

urn:lsid:ipni.org:names:77296909-1

Figs 1, 2, 3A1–A5

Type. China. Sichuan Province: Liangshan Prefecture, Muli County, on the way from Wujiao to Yiji, 27°58'21.73"N, 100°41'51.20"E, 3300–3500 m a.s.l., 23 July 2021, Q. P. Wang et al. ML12992 (holotype YUKU!, isotypes YUKU!).

Rubus vicarius Focke in Sargent, Pl. Wils. 1: 56. 1911, *nom. illeg.*, non Sudre (1902: 12). Type: China. Sichuan Province, Leshan City, Wa Shan, in thickets, 1500–2100 m a.s.l., July to August 1908, E. H. Wilson 948 (BM!, NYBG!, US!).

Diagnosis. *Rubus dianchuanensis* is most similar to *R. ovatisepalus* Huan C. Wang, but clearly differs from the latter by its leaf abaxially densely white or grey tomentose, bracts in the inflorescence often leaf-like, pedicels 1–6 cm long, sepals triangular-ovate and with acute to caudate apex.

Description. Arching shrubs, 1 to 2 m high, deciduous or semi-evergreen. Stems with dense ferruginous glandular hairs and soft eglandular hairs. Branchlets cylindric, grey-green to brown, villous, with curved prickles and nearly straight, ferruginous, 1–2 mm long, glandular hairs. Leaves imparipinnate, usually 3-foliolate, rarely 5-foliolate. Stipules persistent, linear, 5–8 mm long, ca. 1.5 mm wide, pubescent, with glandular hairs, base shortly adnate to petioles. Petioles 0.5–4 cm long, petiolule of

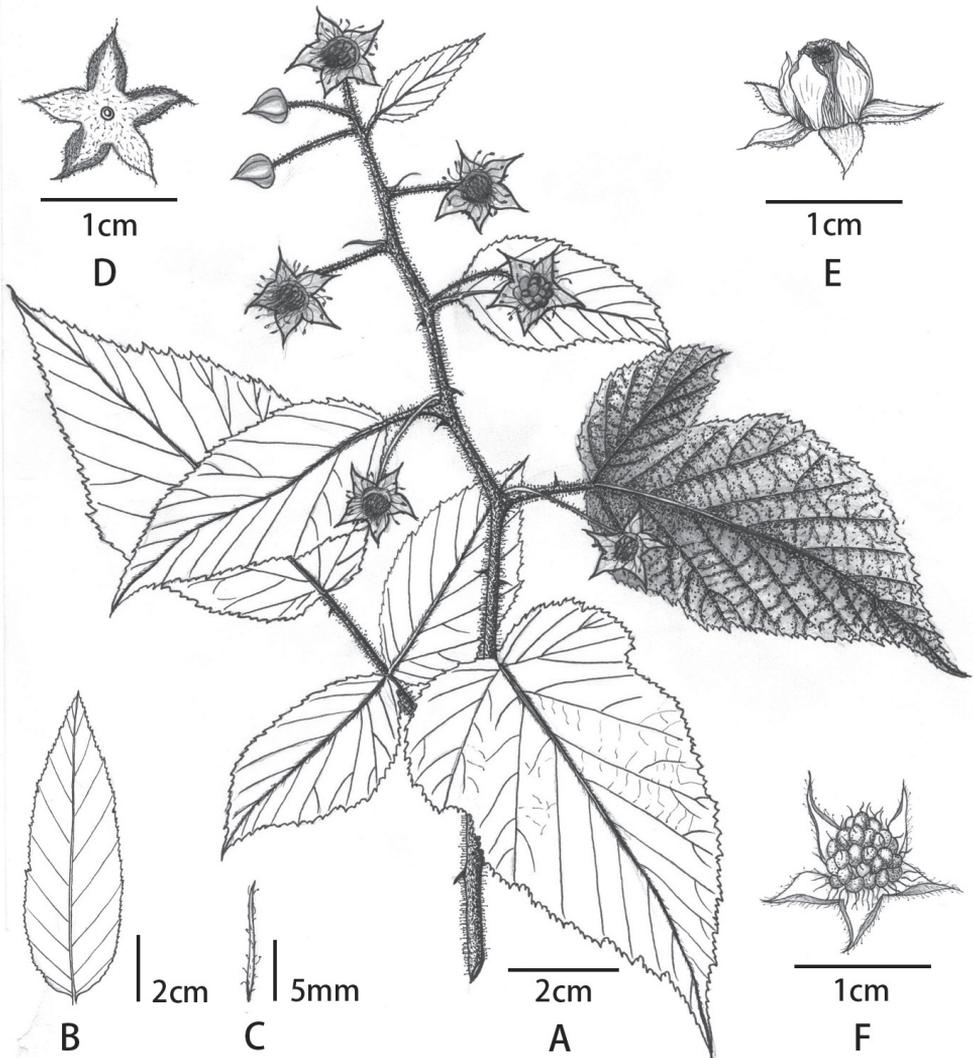


Figure 1. *Rubus dianchuanensis* **A** habit **B** bract at the base of inflorescence **C** bract at the upper part of inflorescence **D** calyx **E** flower (side view) **F** aggregate fruit with persistent calyx.

terminal leaflets 1–3.5 cm long, lateral leaflets sessile or subsessile; petiolule and rachis with glandular hairs, intermixed pubescence and erect or recurved prickles. Leaf blades cordate or ovate-cordate in outline, papery, adaxially pubescent and with sparse glan-

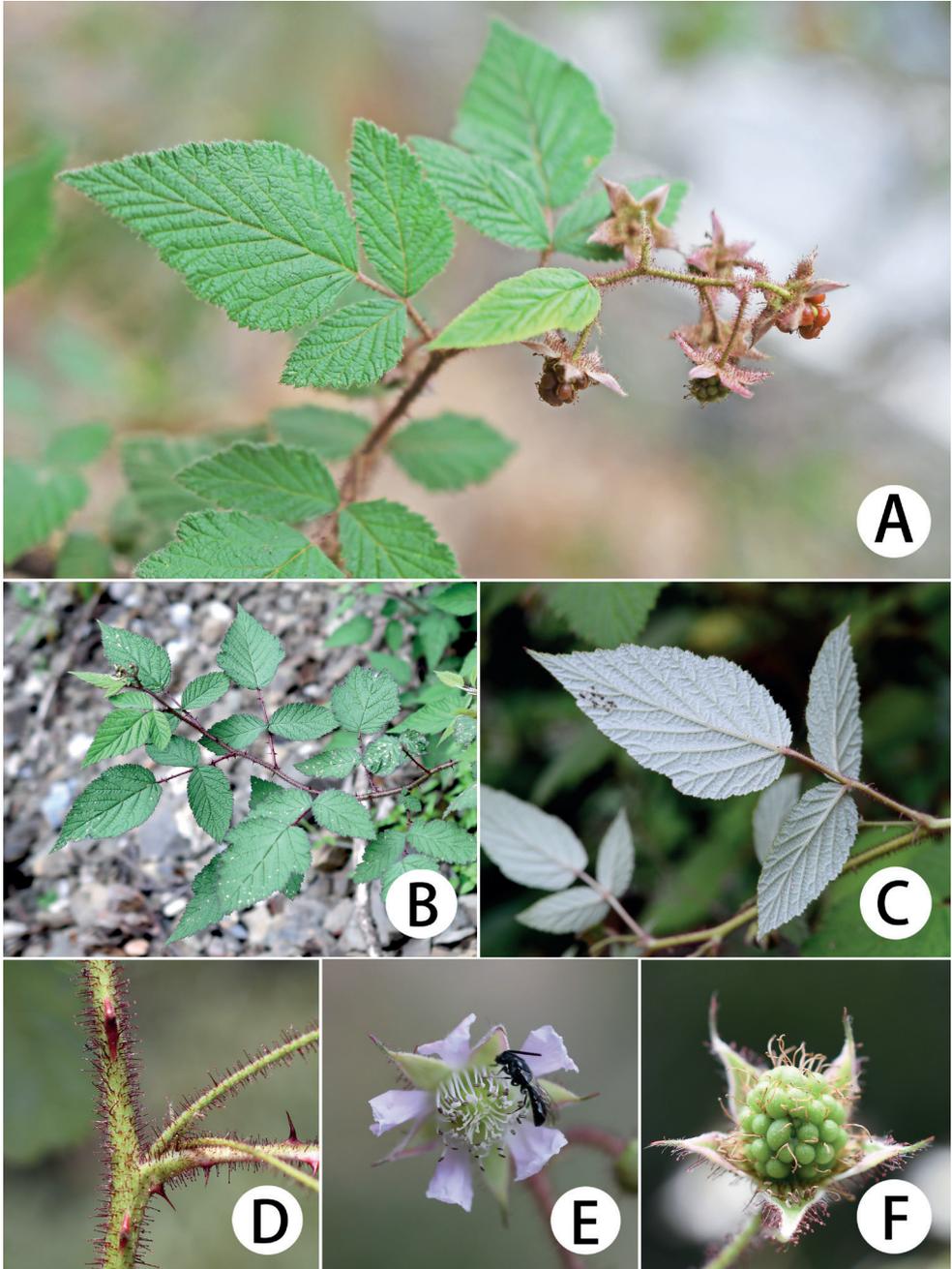


Figure 2. *Rubus dianchuanensis* **A, B** habit **C** abaxial surfaces of leaves **D** portion of branchlet showing glandular hairs **E** flower **F** an immature aggregate fruit with calyx.

dular hairs, abaxially densely white or grey tomentose throughout, with sparse glandular hairs along veins. Terminal leaflets cordate, broadly ovate or ovate, 3–11 cm long, 2–7 cm wide, apex acute to acuminate, base rounded to subcordate; margin slightly lobed or not, double serrate; nervation pinnate, with 6–9 lateral veins on each side of the midrib. Lateral leaflets ovate or elliptic, apex acute, base cuneate to round, slightly oblique, 1.5–7 cm long, 1–5 cm wide, lateral veins 5–7 paired. Terminal inflorescences racemose-cymose, 4–10-flowered, 5–15 cm long; bracts at the base usually leaf-like, simple, ovate, ovate-lanceolate or lanceolate, with similar indumentum as the leaves,



Figure 3. *Rubus dianchuanensis* (A1–A5) and *R. subornatus* (B1–B5) A1 a flowering branch showing terminal inflorescence A2 portion of stem showing indumentum A3 bract at the base of inflorescence A4 flower (side view) A5 mature aggregate fruit B1 habit B2 portion of the stem B3 bracts B4 flower (side view) B5 mature aggregate fruit.

2.5–11 cm long, 1–5 cm wide, apex acute to acuminate, base rounded to subcordate; bracts at the upper portion linear, 4–12 mm long, ca. 1 mm wide, pubescent, with glandular hairs. Axillary flowers usually solitary, rarely 2–3-flowered. Pedicels 1–6 cm in length, densely pilose, with dense glandular hairs and curved prickles. Flowers 1–1.5 cm in diameter. Calyx grey-green or reddish, abaxially with soft hairs and glandular hairs; sepals triangular-ovate, erect or spreading after anthesis, 5–10 mm long, 2–4 mm wide, margin grey tomentose and entire, apex acute to caudate. Petals pink to white, obovate, 5–8 mm long, 4–5 mm broad, apex repand, base shortly clawed. Stamens numerous in 2 whorls; filaments linear, glabrous, ca. 5 mm long. Pistils numerous; ovaries sparsely pilose, styles glabrous. Aggregate fruit ovoid, orange-red to red.

Taxonomic notes. *Rubus dianchuanensis* was firstly collected by Ernest Henry Wilson in 1908 from Wa Shan (Leshan City) in western Sichuan, southwest China. Based on Wilson's collection, Focke (1911) published "*R. vicarius* n. form. (?)" with a Latin description in his monograph *Species Ruborum*. However, the name *R. vicarius* Focke was not validly published there under Article 36.1 of the Shenzhen Code (Turland et al. 2018). Shortly afterwards, in July 1911, the name *R. vicarius* Focke was definitely accepted by Focke (in Sargent 1911) and accompanied by a complete and direct reference, namely "Bibl. Bot. LXXII 211 (Spec. Rub.) (1911)", to his previous Latin description, it was therefore validated. Unfortunately, the name *R. vicarius* had been previously used by Sudre (1902) for a European species; thus, Focke's name as a later homonym was illegitimate (Article 53.1 of the Shenzhen Code).

Morphologically, *Rubus dianchuanensis* is most similar to *R. ovatisepalus* Huan C. Wang (Fig. 4), a species described recently from northwestern Yunnan and southeastern Xizang, southwest China (Wang and Wang 2019), in having dense glandular hairs throughout the plant and the racemose-cymose terminal inflorescences. However, *R. dianchuanensis* differs markedly from the latter by its leaf abaxially densely white or grey tomentose (*vs.* sparsely pubescent, with glandular hairs), bracts at base of the inflorescence usually leaf-like, ovate, ovate-lanceolate or lanceolate (*vs.* lanceolate to linear), 2.5–11×1–5 cm (*vs.* 0.7–1.2×0.1–0.2 cm), flower usually larger, 1–1.5 cm (*vs.* 0.8–1.2 cm) in diameter, pedicels 1–6 cm (*vs.* 0.7–1.5 cm) long, apex of sepals acute to caudate (*vs.* long acuminate to caudate).

Rubus dianchuanensis is also similar to *R. subornatus* Focke (including its variety *R. subornatus* var. *melanodenus* Focke) (Fig. 3B1–B5), with which *R. vicarius* Focke had been erroneously synonymized by Yu and Lu (1985) as well as Lu and Boufford (2003). Nevertheless, *R. dianchuanensis* differs from it by its not glaucous stems usually covered with dense ferruginous glandular hairs and soft eglandular hairs (*vs.* more or less glaucous, glabrous), terminal inflorescences racemose-cymose (*vs.* corymbose), bracts at base of the inflorescence often leaf-like, rarely trifoliolate, ovate, ovate-lanceolate or lanceolate (*vs.* linear) (Fig. 3: A3, B3), pedicels usually longer, 1–6 cm (*vs.* 1–2.5 cm) long, calyx abaxially with grey pubescent (*vs.* intermixed tomentose) and dense ferruginous glandular hairs (*vs.* sparse or not), without needle-like prickles (*vs.* with needle-like prickles), and slightly pink to white (*vs.* purplish-red) petals. Some specimens of *R. dianchuanensis* had been identified as *R. phoenicolasius* Maxim., but it



Figure 4. *Rubus ovatisepalus* **A** habit **B** abaxial surfaces of leaflets **C** mature aggregate fruit.

is well differentiated from the latter by stems and branches with short glandular hairs (*vs.* long glandular hairs) and without bristles (*vs.* dense bristles), terminal inflorescence racemose-cymose (*vs.* short racemes) 5–15 cm (*vs.* 1–6 cm) long, pedicels 1–6 cm (*vs.* 0.5–1.5 cm) long, flowers 1–1.5 cm (*vs.* 0.6–1.5 cm) in diameter, calyx without bristles (*vs.* with dense bristles), sepals triangular-ovate (*vs.* lanceolate). A detailed morphological comparison between these four species is summarized in Table 1.

Phenology. *Rubus dianchuanensis* flowering from June to August, fruiting from July to September.

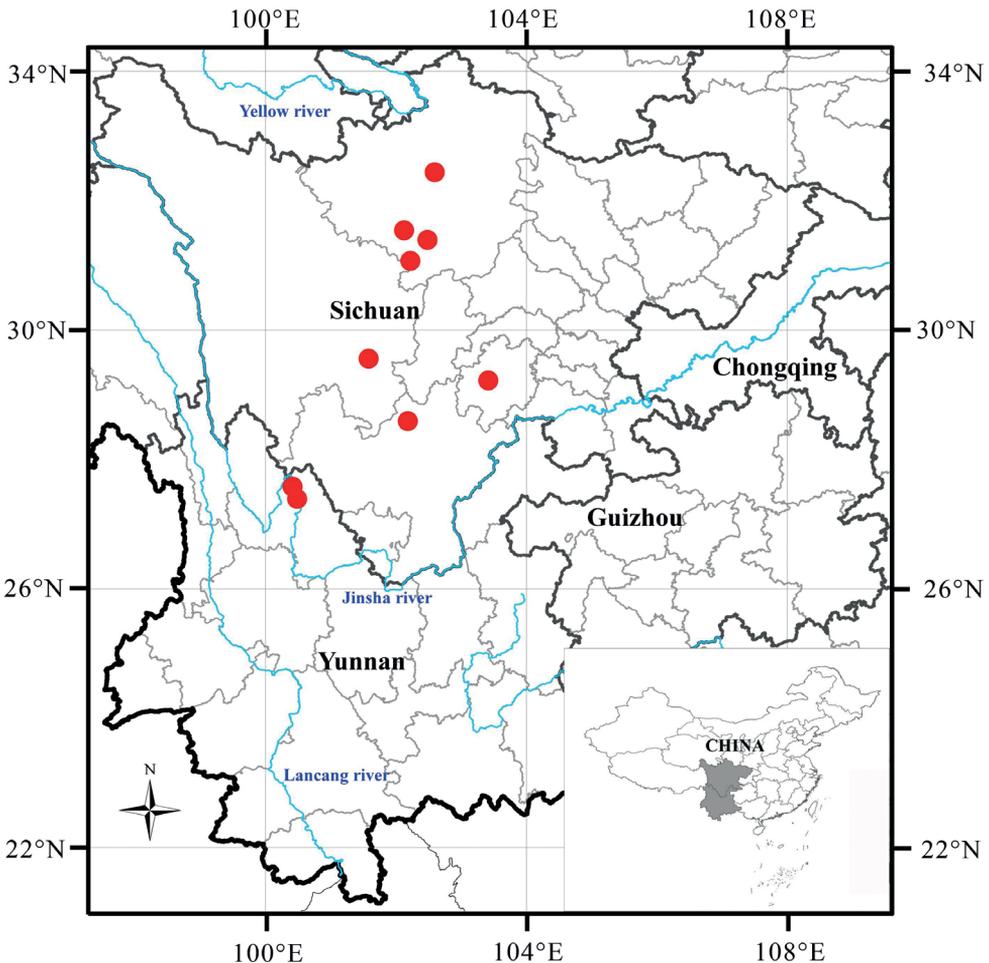
Etymology. The specific epithet “dianchuanensis” refers to the Yunnan (called *dian* for short in Chinese) and Sichuan (called *chuan* for short in Chinese) provinces, where this species occurs.

Distribution and habitat. *Rubus dianchuanensis* is endemic to southwest China, where it has been collected from western Sichuan and northwestern Yunnan (Fig. 5). It usually occurs at elevations ranging from 2500–3600 meters and grows in open woods and thickets.

Additional specimens examined (Paratypes): CHINA. **Sichuan:** Muli County, Damadian, 3000 m a.s.l., 16 Aug 1937, *T. T. Yu* 7740 (PE); Shimian County, Liziping Country, 2700 m a.s.l., 28 Jul 1978, *Shimian Team* 78-0875 (SM); Shimian County, 1955, *C. C. Hsieh* 41198 (IBSC, PE); Kangding City, Zheduotang village, 3100 m a.s.l., 1 Aug 1963, *K. C. Kuan et all* 1218 (PE); same location, 3450 m a.s.l., 5 July 1953, *X. L. Jiang* 36185 (IBK, IBSC, PE); same location, 3600 m a.s.l., 16 Jul 1953, *W. P. Fang & X. L. Jiang* 36323 (IBK, ISBC, PE); Kangding County, 2750 m a.s.l., 24 Jun 1980, *Z. J. Zhao* 112962 (CDBI) and *Z. J. Zhao* 119262 (PE); Kangding

Table 1. A morphological comparison of *Rubus dianchuanensis* with its relatives.

	<i>R. dianchuanensis</i>	<i>R. ovatisepalus</i>	<i>R. subornatus</i>	<i>R. phoenicolasius</i>
Indumentum of stems	dense glandular hairs	dense glandular hairs	glabrous	dense glandular hairs and bristles
Abaxial indumentum of leaf blade	densely grey tomentose	sparsely pubescent, with glandular hairs	densely grey tomentose	densely grey tomentose
Terminal Inflorescence	racemose-cymose	racemiform cymes	corymbose	short racemes
Length of pedicel	1–6 cm	0.7–1.5 cm	1–2.5 cm	0.5–1.5 cm
Diameter of flower	1–1.5 cm	0.8–1.2 cm	2–3 cm	0.6–1.5 cm
Petal colour	white or slightly pink	white or slightly pink	purplish-red	white
Petal vs. sepal	petal slightly longer than sepals	petal shorter than sepals	petal shorter than sepals	petal much shorter than sepals

**Figure 5.** Geographical distribution of *Rubus dianchuanensis* (red dots).

City, Yajiageng, Laoyunshachang, 3318 m a.s.l., 101°58'17"E, 29°56'00"N, 28 Aug 2008, *Y. L. Peng & W. G. Tu Gaoxf-0856* (KUN); Xiaojin County, 3500 m a.s.l., 1 Jul 1959, *Z. G. Liu 0412* (CDBI, PE); Lixian County, 26 Aug 1957, *X. Li 74160* (IBSC, PE, NAS); Lixian County, Miyaluo village, 25 Jul 1958, *Z. L. Wu 33375* (PE; CAF);

Barkam City, Barkam County, Dalangjiao River, 2300–2900 m a.s.l., 12 Jul 1960, *Sichuan Medicine Source Survey Team 22297* (NAS, SM); Barkam City, 2800 m a.s.l., 11 Jul 1957, *H. F. Zhou & Z. Y. Zhang 22772* (IBSC, NAS, KUN, PE); Barkam City, Dalangzugou, 2700 m a.s.l., 27 Aug 1957, *X. Li 72288* (IBSC, NAS, PE); Heishui County, Naizigou, 2900 m a.s.l., 22 Jul 1957, *X. Li 73260* (IBSC, NAS, KUN, PE). **Yunnan:** Ninglang County, Lugu Lake, 27°39'21"N, 100°48'36"E, 2500–2600 m a.s.l., 6 Aug 2015, *H. C. Wang et al. LGH8164* (YUKU).

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