

Glossoloma wiehleri (Gesneriaceae), a new species from the northwestern Andes of Ecuador

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Abstract

A new species of *Glossoloma* is described from the western Andean slopes of the Pichincha Province in northern Ecuador. *Glossoloma wiehleri* J.L.Clark & Tobar is differentiated from all other congeners by an epiphytic habit, elongate scandent shoots that exceed four meters in length, and coriaceous leaves with a velutinous indument on the lower leaf surface. The new species is illustrated, featured with field images from recent expeditions, and assigned the category of Endangered (EN) according to IUCN Criteria.

Resumen

Se describe una nueva especie de *Glossoloma* de las laderas occidentales de la provincia Pichincha en el norte de Ecuador. *Glossoloma wiehleri* J.L.Clark & Tobar se diferencia de todos sus otros congéneres por su hábito epifito, ramas escandentes alargadas que superan los cuatro metros de longitud y hojas coriáceas con un indumento velutino en la superficie inferior de la hoja. La nueva especie se ilustra con imágenes de campo de expediciones recientes y se le asigna la categoría de En Peligro (EN) de acuerdo a los criterios de la IUCN.

Keywords

Ecuador, Gesneriaceae, *Glossoloma*, taxonomy

Introduction

The flowering plant family Gesneriaceae, with over 3400 species and 150+ genera (Weber 2004; Weber et al. 2013), is in the order Lamiales. The family is divided into three

subfamilies and seven tribes (Weber et al. 2013, 2020), which represent monophyletic lineages (Ogutcen et al. 2021). The majority of New World members are in the subfamily Gesnerioideae and are represented by 1200+ species and 77 genera (Clark et al. 2020). *Glossoloma* Hanst. is classified in the tribe Gesnerieae and subtribe Columneinae (Weber et al. 2013, 2020).

The genus *Glossoloma* is defined by resupinate (upside down) flowers, a feature that was recently documented and discovered as a morphological synapomorphy (Clark and Zimmer 2003; Clark et al. 2006; Clark 2009; Clark et al. 2012). *Glossoloma* corollas are usually tubular, apically pouched and appear laterally compressed. Most *Glossoloma* are unbranched subshrubs with terminally clustered leaves, but some are scandent terrestrial subshrubs or facultative epiphytes with horizontal stems. The new species described here is unusual for its habit of being epiphytic with elongate horizontal scandent stems. Three other species in *Glossoloma* that have elongate scandent stems are *G. chrysanthum* (Planch. & Linden) J.L.Clark, *G. penduliflorum* (M.Freiberg) J.L.Clark, and *G. scandens* J.L.Clark. Several other species of *Glossoloma* are facultative epiphytes, but their stems are erect or non-scandent. Differences between *Glossoloma wiehleri* and closely-related congeners are discussed below.

Glossoloma ranges from southern Mexico to Panama, northwestern South America, and south to Bolivia. The center of diversity of *Glossoloma* is the western lowland forests in Ecuador and Colombia where 15 species occur. *Glossoloma* was monographed by Clark (2009) and included 27 species. An additional species was described by Rodas and Clark (2014) from the Cordillera Central of the Colombian Andes. The description of *Glossoloma wiehleri* brings the total number of *Glossoloma* species to 29.

Taxonomic treatment

***Glossoloma wiehleri* J.L.Clark & Tobar, sp. nov.**

urn:lsid:ipni.org:names:77233918-1

Figs 1, 2

Diagnosis. Differs from all other congeners by the presence of elongate scandent shoots that exceed four meters in length, coriaceous leaves that are velutinous on lower surface, and a corolla tube that is broadly ampliate on the dorsal surface.

Type. ECUADOR. Pichincha: Quito towards Chiriboga, past San Juan and El Sigal, kilometer #40, 27 Apr 1993, *H. Wiehler & Gesneriad Research Foundation Study Group 93228*. (holotype: SEL [095415]).

Description. Scandent subshrub with elongate horizontal shoots, sparingly branched, to 4 m long, to 1.5 cm in diameter, subwoody; internodes 3.8–8 cm long, subquadrangular, brown velutinous. Leaves opposite, isophyllous, coriaceous; blade symmetric, ovate to broadly ovate, 9.3–12.5 × 5–6.7 cm, base truncate to slightly cordate, apex acute, margin serrulate, adaxially light green, densely pilose, abaxially uniformly dark red to green with red venation, densely villous, lateral veins 6–9, pri-

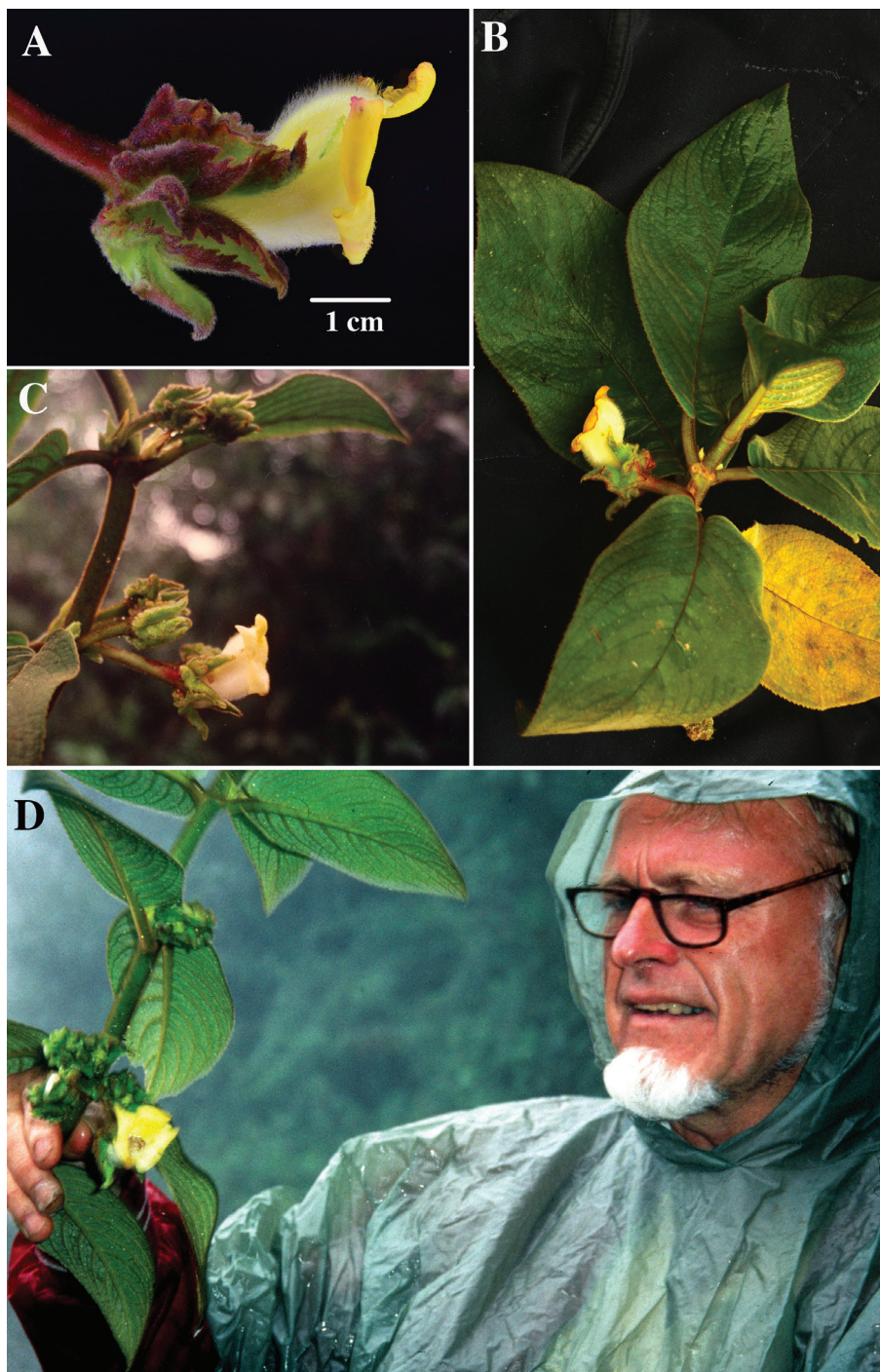


Figure 1. *Glossoloma wiehleri* J.L. Clark & F. Tobar. **A** Mature flower **B** Stem with foliage **C** Stem with axillary clusters of flowers **D** Hans Wiehler holding the holotype (**A, B** from Tobar & Gavilanes 3521 **C, D** from H. Wiehler *et al.* 93228). Photos **A, B** by F. Tobar, **C** by M. Riley **D** by G. Robinson.

mary and secondary veins occasionally red. Inflorescence reduced to a single axillary flower (rarely 2–3); peduncles absent or highly reduced (< 2 mm); bracts absent or caducous, 2 × 3 mm. Flowers resupinate, subtended by elongate pedicels, 1.2–4.5 cm long, densely pilose, oriented horizontal relative to shoot, becoming more pendent during anthesis; calyx lobes 5, nearly free, mostly equal in size and shape, dorsal lobe slightly smaller, lobes appressed to flower when immature and spreading during anthesis, mostly green with red margins, 1.6–3.0 × 1.0–1.3 cm, broadly ovate, apex acute to acuminate, margin with 5–10 deep serrations (ca. 4 mm long teeth), densely pilose on both surfaces; corolla tubular, broadly ampliate on dorsal surface (not ampliate on ventral surface), posture horizontal relative to calyx, corolla tube 2.4–2.6 cm long, outer surface densely pilose, uniformly bright yellow on the inside and white suffused with yellow on the outside, lobes 3–5 × 4–6 mm. Androecium of 4 stamens, filaments connate at the base and forming a filament curtain for 3–4 mm, free portion of filaments 2–2.5 cm long, glabrous; anthers longer than broad, ca. 2 × 1.5 mm, dehiscing by longitudinal slits; staminode lanceolate 3–5 × 1–2 mm; nectary a bilobed dorsal gland, sometimes appearing truncate, glabrous; ovary superior, densely pilose, 2–4 × 2 mm, style ca. 2.0 cm long, glabrous, stigma included and shallowly bifid. Immature fruit cone-shaped, densely pilose, 1.4 × 0.9 cm. Mature fruit not observed.

Phenology. Collected in flower during February, April, and July. Immature fruits observed in February.

Etymology. The specific epithet is in reference to Hans Wiehler (1930–2003). Wiehler was a practicing Mennonite from East Prussia (now Poland) and immigrated to the USA in the 1950s. He attained a Bachelor's degree from the Eastern Mennonite College in 1954 and a Bachelor of Divinity degree in 1956 from Goshen College in Goshen, Indiana (Clark 2003). He eventually left the Mennonite church and pursued his passion for botany. Wiehler earned a Master's degree from Cornell and obtained his Ph.D. in Botany from the University of Miami. Wiehler's advanced degrees focused on the taxonomy and classification of Gesneriaceae. Wiehler was one of the first botanists hired by the Marie Selby Botanical Gardens where he served as the associate editor and business manager of the garden's journal, *Selbyana* (1975–1981). He left Selby in 1982 and established the Gesneriad Research Foundation (GRF) in Sarasota, Florida where he directed annual seminars that were attended by horticulturists, taxonomists, students and plant enthusiasts. Wiehler also organized and directed 14 study trips to South and Central America, including the 1993 expedition that resulted in the discovery of *Glossoloma wiehleri*. The first author met Hans Wiehler in 1994 and corresponded with him regularly until he died in 2003. Wiehler's passion for Gesneriaceae was contagious.

Distribution and preliminary assessment of conservation status. *Glossoloma wiehleri* is endemic to the Pichincha Province on the western slopes of the Ecuadorian Andes and is known from three localities. The type locality is the old highway between Quito and Santo Domingo (via Chiriboga). In 2020, Tobar located an extant population of *G. wiehleri* near kilometer #40 (San Juan) where Hans Wiehler made the initial discovery in 1993 (Wiehler 1993). An additional population is supposedly from the Bombolí Cloud Forest, near kilometer #20 on the highway Quito–Santo Domingo



Figure 2. Holotype of *Glossoloma wiehleri* J.L. Clark & F. Tobar (*H. Wiehler & Gesneriad Research Foundation Study Group* 93228, SEL).

(via Alóag). Clark facilitated a visit for Brian K. Schuetz in 2005 to the Smithsonian Institution's National Herbarium (US). During that time, Schuetz was a graduate student at the Idaho State University (Pocatello, ID) and was completing research for his Master's thesis on the woody flora of the Bombolí Cloud Forest. Schuetz had an unmounted specimen of *Glossoloma wiehleri* (B. Schuetz 600) that was supposedly from 2955 m above sea level inside the Bombolí Cloud Forest. Schuetz's dissertation (Schuetz 2004) provides longitude and latitude for most of his collections. *Glossoloma wiehleri* is featured with images and a description (Schuetz 2004), but lacks detailed locality data (e.g. it is one of the only species in Schuetz's floristic study that does not include longitude and latitude). Schuetz did not deposit specimens in an Ecuadorian herbarium. Likewise, specimens of *G. wiehleri* were not deposited at the Idaho Museum of Natural History (IDS). A third population was documented by Tobar in 2019 from the Bosque Protector Pacaya, a Reserve that is managed by the community Alaspungo. Bosque Protector Pacaya is adjacent to El Pahuma Orchid Reserve (Ceiba Foundation for Tropical Conservation). The forests in Bosque Protector Pacaya are mostly above 3,000 meters and the forests in El Pahuma Orchid Reserve are mostly below 3,000 meters. It is likely that populations of *G. wiehleri* are limited to forests above 3,000 meters and that is why it has not been documented in the lower elevation forests of El Pahuma Orchid Reserve. According to the IUCN Red List Criteria (IUCN 2012; IUCN Standards and Petitions Committee 2019) for limited geographic range (B1 = $EOO < 5,000 \text{ km}^2$) and associated subcriteria, including occurrence at less than five locations (B2a) and continuing decline of Andean forests (B2b), *Glossoloma wiehleri* should be listed in the category Endangered (EN).

Comments. Most *Glossoloma* are terrestrial woody subshrubs with an unbranched primary stem. An epiphytic habit is unusual in *Glossoloma*, especially with elongate or scandent stems. *Glossoloma chrysanthum*, *G. penduliflorum*, *G. scandens*, and *G. wiehleri* are the only known species of *Glossoloma* with an epiphytic habit and elongate scandent stems. Some species, such as *G. altescandens* (Mansf.) J.L.Clark or *G. herthae* (Mansf.) J.L.Clark are facultative epiphytes, but their stems are erect and non-scandent. The population of *Glossoloma wiehleri* from the type locality was observed to have stems that exceed four meters in length. The longest recorded stem in the genus is *G. chrysanthum* that exceeded five meters in length (Fig. 3E). *Glossoloma chrysanthum* is endemic to Venezuela and is differentiated from *G. wiehleri* by a corolla tube that is apically constricted (Fig. 3A) vs. apically amplified (Fig. 1A). In addition, *G. wiehleri* differs by the presence of a velutinous indument on the lower leaf surface (vs. hirsute to pilose in *G. chrysanthum*) and coriaceous leaves (vs. papyraceous in *G. chrysanthum*). The mature resupinate flowers of *G. wiehleri* are inflated on the upper surface (i.e. ampliate or gibbous) and straight on the lower surface (i.e. not ampliate or gibbous). *Glossoloma penduliflorum* is readily differentiated from all other members of the genus by the presence of solitary axillary flowers with elongate slender pedicels that are 10–15 cm long, the longest known pedicels for any member of *Glossoloma*. *Glossoloma scandens* differs from *G. penduliflorum* by the presence of three flowers per axil and relatively short pedicels (< 1 cm long).

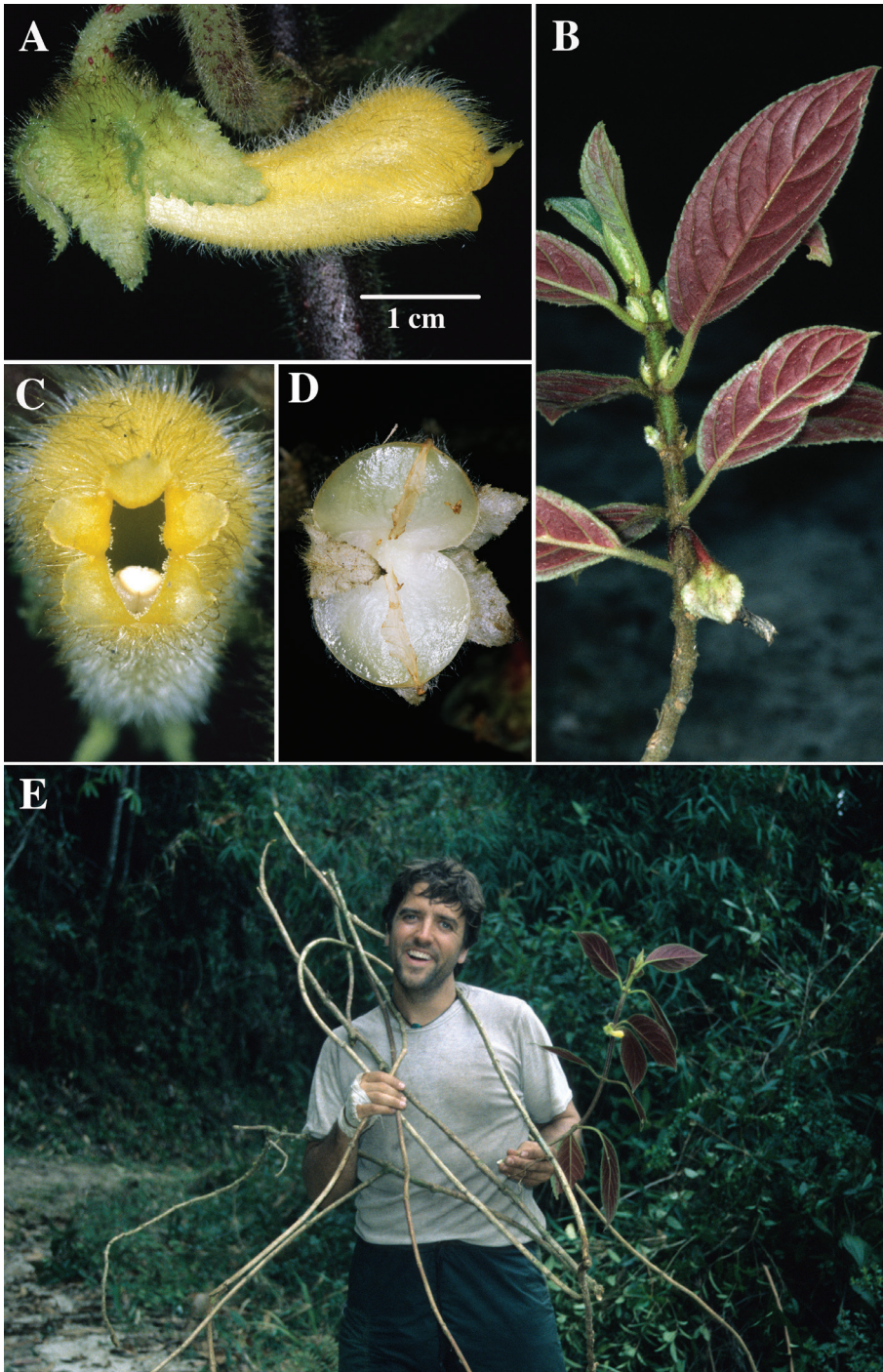


Figure 3. *Glossoloma chrysanthum* (Planch. & Linden) J.L. Clark. **A** Mature flower **B** Stem with foliage **C** Front view of corolla **D** Mature capsule **E** Elongate scandent shoots held by John L. Clark during an exploratory research expedition in Venezuela (**A–E** from J.L. Clark 6872). Photos by J.L. Clark.

Specimens examined. ECUADOR. Pichincha: cantón Quito, distrito Metropolitano de Quito, Chillogallo, road San Juan–Chiriboga, near San Juan, 0.416333°N, 78.6580°W, 3004 m alt., 20 Feb 2020, *Tobar & Gavilanes 3521* (QCNE); distrito Metropolitano de Quito, Nono, comunidad de Alaspungo, Bosque Protector Pacaya, 0.002320°N, 78.631260°W, 3000 m alt., 15 Jul 2019, *Tobar, Marayata & Gavilanes 3399* (QCNE, US).

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Materials to the revision of the genus *Cranichis* (Orchidaceae) in Bolivia

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Abstract

The diversity of *Cranichis* in Bolivia is evaluated. An updated key for identifying species is provided. Morphological characteristics of 15 species of Bolivian *Cranichis* are presented together with illustrations of their floral segments. The occurrence of *C. diphylla*, *C. lehmannii*, and *C. muscosa* in this country was not confirmed. In our opinion the previously published Bolivian record for *C. polyantha* is doubtful. For the first time, *C. badia* and *C. longipetiolata* are reported in this country. Two new species of *Cranichis* are described.

Keywords

Cranichidinae, diversity, new species, taxonomy

Introduction

Bolivian Orchidaceae are the least studied in terms of biodiversity (Vásquez et al. 2003), however, novelties are being reported (e.g. Dalström 2006; Pupulin and Moreno 2018; Kolanowska et al., 2019; Pace 2020). A preliminary revision of Bolivian material has revealed some interesting discoveries within the Cranichidinae (Kolanowska et al. 2020), which are a significant element of the terrestrial neotropical flora (Salazar et al. 2009).

According to Vásquez et al. (2014) there are six genera of *Cranichidinae sensu* Dressler (1993; *Baskervilla* Lindl., *Cranichis* Sw., *Ponthieva* R.Br., *Pseudocentrum* Lindl., *Pterichis* Lindl., and *Solenocentrum* Schltr.) and six of *Prescottiiinae* (Dressler 1990; *Aa* Rchb.f., *Altensteinia* Kunth, *Gomphichis* Lindl., *Myrosmodes* Rchb.f., *Prescottia* Lindl., and *Stenoptera* C.Presl.) present in Bolivia.

Cranichis was described by Swartz in 1788 and typified with *C. muscosa* Sw. over 150 years later by Acuña (1939). Species of *Cranichis* are usually terrestrial or lithophilic plants characterized by petiolate leaves, non-resupinate flowers, petals much narrower than the sepals, and cochleate lip that is often conspicuously veined or ornamented with nodules. The gynostemium of *Cranichis* is massive, often swollen at the apex with thick, massive, spacious clinandrium and elongated, digitate, thick hamulus (Szlachetko and Rutkowski 2000; Kolanowska and Szlachetko 2015). The species grow in various habitats at altitudes ranging from 350 to over 3000 m (Carnevali and Ramírez-Morillo 2003; Cribb 2003). The geographical range of *Cranichis* extends from USA (Florida) south to Bolivia and Argentina.

Vásquez et al. (2014) report nine species of *Cranichis* occurring in Bolivia and listed *C. castellanosii* L.O. Williams as unconfirmed taxon. However, *C. fertilis* (F. Lehm. & Kraenzl.) Schltr. catalogued by the authors, has been earlier included (as a synonym of *Ophrys parviflora* Presl) in the genus *Exalaria* Garay & G.A. Romero (Garay and Romero 1999). Moreover, Vásquez et al. (2014) accepted the broad concept of *C. ciliata* (Kunth) Kunth and *C. diphylla* Sw. In this recognition, the authors included several synonyms of the former species (e.g. *C. atrata* Schltr., *C. pleioneura* Schltr., *C. polyblephara* Schltr., *C. sororia* Schltr., and *C. mandonii* Schltr). *Cranichis nigrescens* Schltr., *C. ovatilabia* Schltr., and *C. stictophylla* Schltr. are accepted as synonyms of *C. diphylla*. In addition, three species of *Cranichis*, *C. lehmannii* Rchb. f., *C. polyantha* Schltr., and *C. pulvinifera* Garay were reported as occurring in Bolivia by Jiménez-Pérez (2011).

Recent research on *Cranichis* resulted in the description of numerous new species from the Northern Andes (e.g. Kolanowska and Szlachetko 2014; Kolanowska and Szlachetko 2019; Szlachetko and Kolanowska 2019) and in this study the diversity of this genus in Bolivia has been evaluated.

Materials and methods

Herbaria acronyms used in this paper follow Thiers (2020). During the research on *Cranichis* over 400 specimens deposited in the herbaria: AAU, AMES, BM, C, CAY, CUV, COL, FMB, K, LPB, MO, NY, P, PSO, RENZ, RPSC, UGDA, US, VALLE, and W were examined. Morphological characteristics of Bolivian species were prepared based exclusively on material collected in Bolivia and deposited in LPB, MO, and AMES. The morphological variation of Colombian and Ecuadorian species of *Cranichis* has been described by Szlachetko and Kolanowska (2019). Specimens ex-

amined from outside Bolivia are listed in Kolanowska and Szlachetko 2013, 2019; Szlachetko and Kolanowska 2013, 2019, and books of Szlachetko 2016; Szlachetko and Kolanowska 2020. The list of species of Bolivian *Cranichis* examined in this study is provided in Supplementary Information (Annex 1). Information on the habitats of Bolivian *Cranichis* was gathered during field studies and from the data on herbarium labels. Characteristics of species that are reported from Bolivia, but not confirmed or examined in this study, were prepared based on specimens collected in other regions, as well as the literature.

All herbarium specimens were examined in the standard way. The size and shape of the leaves and length of the scape were studied first. Then the details of the inflorescence (e.g. form of the floral bracts and ovaries) were examined. Three flowers from the middle part of the inflorescence were studied. The floral segments were observed under a stereoscopic microscope, after softening the flowers in boiling water.

Only those localities that could be identified based on information on the labels of the herbarium specimens were included in the distribution maps compiled using ArcGis 10.6 (Esri, Redlands, CA, USA).

Nomenclature

The electronic version of this article in portable document format is a published work according to the International Code of Nomenclature for algae, fungi and plants (Turland et al. 2018) and hence the new names contained in the electronic version are effectively published under that Code. In addition, new names included in this work that were issued with identifiers by IPNI will eventually be made available to the Global Names Index. The IPNI Life Science Identifiers (LSIDs) can be obtained and the associated information viewed using any standard web browser by appending the LSID contained in this publication to the prefix <http://ipni.org/>. The online version of this paper is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

Results

Here the characteristics of 15 Bolivian *Cranichis* are presented. The occurrence of *C. diphylla*, *C. lehmannii*, and *C. muscosa* in this country was not confirmed as we were not able to find reference material in the collection of Vasquez deposited in LPB. Moreover, in our opinion the previously published Bolivian record for *C. polyantha* is doubtful and is discussed below. Two new species of *Cranichis* are described in this paper and for the first time we are reporting the occurrence of *C. badia* and *C. longipetiolata* in Bolivia. While both Schweinfurth (1958) and Vásquez et al. (2014) accepted the name *Cranichis multiflora* (Poepp. & Endl.) Cogn., in our opinion this taxon should be classified within *Ponthieva* R.Br.

Taxonomic treatment

Key to Bolivian *Cranichis*

1. Margins of lateral lobes of lip irregularly erose to erose-lancinate..... 2
 - Lip margin entire 3
2. Petals linear-ligulate *C. pulvinifera*
 - Petals obliquely oblanceolate to clavate..... *C. garayana*
3. Petals glabrous..... 4
 - Petals ciliate, ciliate-papillate or pilose..... 12
4. Lip lacks nodules on inner surface..... 5
 - Lip with nodules on inner surface 8
5. Petals oblanceolate-linear 6
 - Petals linear to oblong-ligulate 7
6. Leaf petiole up to 14 cm long, petals obliquely linear-lanceolate.... *C. longipetiolata*
 - Leaf petiole up to 7.5 cm long, petals elliptical or ligulate from a short claw *C. polyantha* (excluded)
7. Lip base cuneate..... *C. silvicola*
 - Lip base unguiculate *C. badia*
8. Dorsal sepal 3-veined *C. diphylla* (not confirmed)
 - Dorsal sepal 1-veined 9
9. Petals spatulate-obovate..... *C. beckii*
 - Petals lanceolate, linear-lanceolate or linear-oblanceolate..... 10
10. Ovary glandular-ciliate..... *C. stictophylla* Schltr.
 - Ovary glabrous or almost so..... 11
11. Inflorescence conical *C. lehmannii* (not confirmed)
 - Inflorescence cylindrical *C. cylindrostachys*
12. Lip with nodules on inner surface *C. muscosa* (not confirmed)
 - Lip lacks nodules on inner surface..... 13
13. Sepals sparsely pubescent..... *C. mandonii*
 - Sepals glabrous..... 14
14. Lateral sepals 2- or 3-veined *C. ciliata*
 - Lateral sepals 1-veined..... 15
15. Petals ligulate-oblanceolate, ciliate along both margins..... *C. atrata*
 - Petals lanceolate-ovate, obtuse, 1-veined, ciliate on the basal 2/3.... *C. maldonadoana*

1. *Cranichis atrata* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 58. 1920.

Type. COLOMBIA. *Madero* 3 (B†; lectotype, designated by Garay (1978: 191): AMES–drawing).

Diagnosis. Plants 24 cm tall, erect. Leaf 1, basal, petiolate; petiole 12 cm long, narrow, canaliculate; blade 9 cm long, 7 cm wide, obliquely elliptical, acuminate, base

cordate. Scape glabrous, enclosed in 4 non-foliaceous sheaths. Inflorescence 2.5 cm long, subdensely many-flowered. Flowers small, glabrous. Floral bracts 4.8 mm long, lanceolate, obtuse. Pedicellate ovary 5.0 mm long, glabrous. Dorsal sepal 3.7 mm long, 1.0 mm wide, oblong-elliptical, obtuse, 1-veined. Petals 3.1 mm long, 1.1 mm wide, obliquely ligulate-oblancoate, obtuse, long cilia on both margins, 1-veined. Lateral sepals 3.1 mm long, 1.5 mm wide, obliquely elliptic-ovate, subacuminate, 1-veined. Lip 2.8 mm long, 2.1 mm wide, concave in the centre, subsessile, elliptic-obovate, minutely apiculate; disc with 3 thickened, dendritic branching veins. Gynostemium 1.3 mm long. Fig. 1.

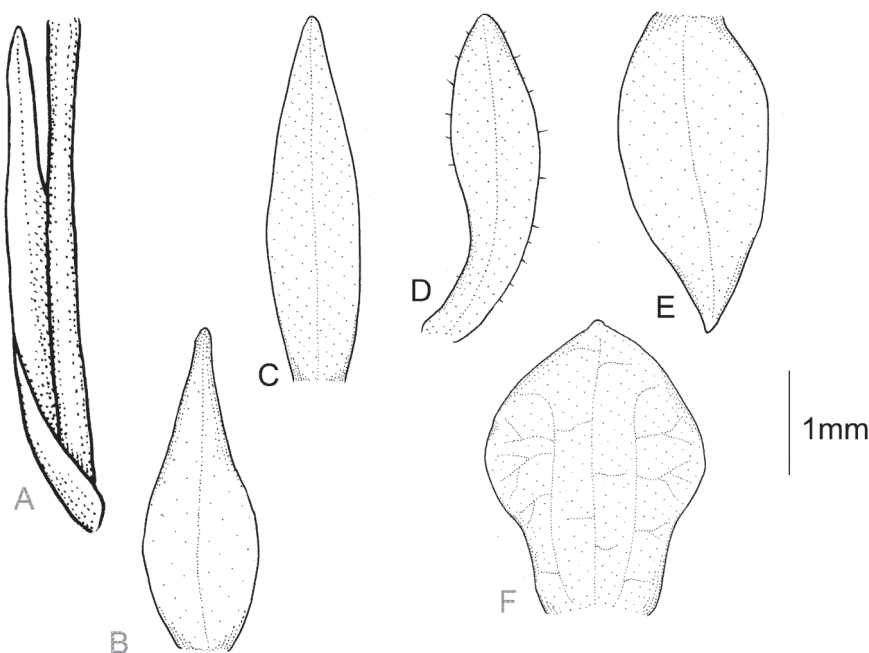


Figure 1. *Cranichis atrata* Schltr. **A** Ovary and floral bract. **B** Floral bract. **C** Dorsal sepal. **D** Petal. **E** Lateral sepal. **F** Lip. Drawn by P. Baranow from *R. Vazquez et al. 1429* (LPB).

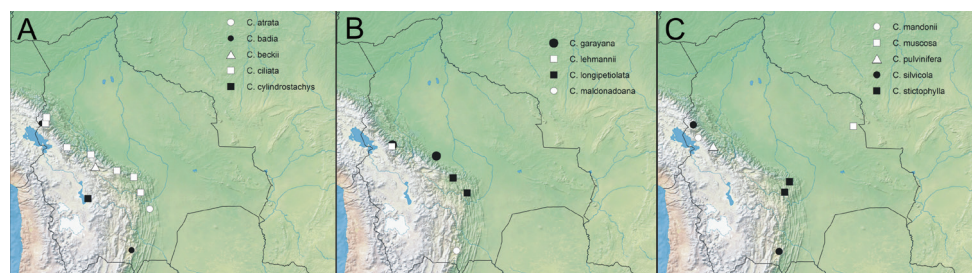


Figure 2. Distribution of *Cranichis* species in Bolivia. **A** *C. atrata*, *C. badia*, *C. beckii*, *C. ciliata*, *C. cylindrostachys*. **B** *C. garayana*, *C. lehmannii*, *C. longipetiolata*, *C. maldonadoana*. **C** *C. mandonii*, *C. muscosa*, *C. pulvinifera*, *C. silvicola*, *C. stictophylla*. Base map provided by Natural Earth (www.naturalearthdata.com).

Habitat and ecology. Terrestrial plants growing in subhumid Tucumano-Boliviano forest at an altitude of 2200 m. Flowers in February.

Representative specimen. **BOLIVIA. Santa Cruz:** Prov. Vallegrande. Río San Lorenzo, entre Piraimiri y Masicurí, 2220 m. 23 February 1991, *R. Vasquez et al.* 1429 (LPB!). Fig. 2.

Notes. This species is usually considered to be a synonym of *C. ciliata* (e.g. Garay 1978; Hamer 1985; Christenson 1991; Bogarín et al. 2014), however, the venation of the lateral sepals is a constant character that can be used to distinguish these two taxa. Lateral sepals of *C. atrata* are always 1-veined (vs. 2- or 3-veined).

2. *Cranichis badia* Renz ex Kolan. & Szlach., *Nordic J. Bot.* 32(3): 289. 2014.

Type. VENEZUELA. *Renz 6065* (holotype: RENZI!; isotypes: RENZI!).

Diagnosis. Plants 28–60 cm tall. Leaf 1, basal, petiolate; petiole 10–19 cm long, narrow, canaliculate; blade 7–11 cm long, 5 cm wide, ovate-elliptical, acuminate, cuneate at the base. Scape sparsely pubescent on upper part, enclosed in 4–5, non-foliaceous sheaths. Inflorescence 6–8 cm long, cylindrical, subdensely many-flowered. Flowers brownish or greenish-yellow. Floral bracts 4.0–4.5 mm long, lanceolate, acuminate, glabrous. Pedicellate ovary 5.3–6.2 mm long, glabrous. Dorsal sepal 3.3–4.0 mm long, 1.5 mm wide, ovate, obtuse, 3-veined. Petals 3.7–4 mm long, 0.6–0.7 mm wide, falcately linear to linear-lanceolate, apex rounded, 1-veined, glabrous. Lateral sepals 4.0–

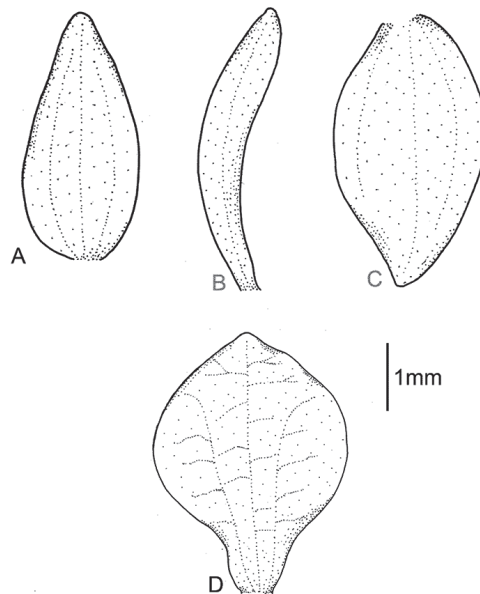


Figure 3. *Cranichis badia* Renz ex Kolan. & Szlach. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip. Drawn by P. Baranow from *L. Cayola et al.* 3657 (LPB).

4.1 mm long, 1.5–2.1 mm wide, obliquely elliptical, obtuse, 2- or 5-veined. Lip 2.9–3.6 mm long, 2.3–2.6 mm wide, concave, unguiculate, elliptic-suborbicular above, obtuse; disc 3-veined, midvein anastomosing, lateral veins branching. Gynostemium 1.5–2 mm long. Fig. 3.

Habitat and ecology. Terrestrial in Yungas montane humid forest with *Weinmannia* L. (Cunoniaceae), *Clethra* L. (Clethraceae), *Ocotea mandonii* Mez (Lauraceae), numerous plants of *Chusquea* Kunth (Poaceae) and *Piper* L. (Piperaceae). It occurs at altitudes between 2150–2890 m. Flowers in March and May.

Representative specimens. **BOLIVIA. La Paz:** Prov. B. Saavedra. Area Natural de Manejo Integrado Apolobamba, Wayrapata. 15°06'45"S 68°55'05"W, 2889 m. 8 May 2010. *L. Cayola et al.* 3657 (LPB!). **Tarija:** Prov. Cercado, cerca Victoria, 2150 m. 3 March 1986. *E. Bastian* 937 (LPB!). Fig. 2.

Notes. Bolivian plants are somewhat different from typical plants of *C. badia*. Lateral sepals of *Cayola et al.* 3657 are 2-veined and lateral sepals of *Bastian* 937 are 5-veined, whereas typically *C. badia* has 3 veins. *Cranichis badia* was described relatively recently (Kolanowska and Szlachetko 2014) and its morphological variation requires further study.

3. *Cranichis beckii* Kolan., Baranow, S. Nowak & A. Fuentes, sp. nov.

urn:lsid:ipni.org:names:77233921-1

Type. BOLIVIA. *Beck* 313 (holotype: LPB!).

Diagnosis. Species similar to *C. lehmannii*, but distinguished by larger leaves up to 20 cm long, 1-veined lateral sepals, spathulate-obovate petals and ciliate ovary.

Plants 50 cm tall. Leaves 3, basal, petiolate; petiole 8–10 cm long, narrow; blade 11–20 cm long, 4–6 cm wide, ovate, acute. Scape erect, enclosed in about 6, foliaceous sheaths. Inflorescence 16 cm long, conical, subclaxly many-flowered. Flowers yellowish, glabrous. Floral bracts 6.2 mm long, lanceolate, acute, microscopically ciliate. Pedicellate ovary 9.5 mm long, microscopically ciliate. Dorsal sepal 3.5 mm long, 1.5 mm wide, oblong-ovate, acuminate, obtuse, 1-veined. Petals 3.2 mm long, 1.3 mm wide, obliquely spathulate-obovate, apiculate, 1-veined. Lateral sepals 3.5 mm long, 1.7 mm wide, obliquely oblong-elliptical, subapiculate, concave near base, 1-veined. Lip 3 mm long, 2.1 mm wide, concave, sessile, elliptical to oblong-elliptical in outline, apiculate at apex; disc deeply concave in the centre with numerous, irregularly subglobose thickenings on inner surface, veins 3, thickened. Gynostemium 2 mm long. Fig. 4.

Etymology. Dedicated to Stephan G. Beck, who collected the type specimen and is a former director of Herbario Nacional de Bolivia.

Habitat and ecology. Terrestrial in Yungas humid, secondary montane forest at an altitude of ca. 1730 m. Flowers in February.

Representative specimen. **BOLIVIA. La Paz:** Prov. Nor Yungas. Cotapata. Estación Biológica de Tunkini. A media hora de la EBT, cruzando l río, 1735 m. 2 February 2002. *S. G. Beck* 313 (LPB!). Fig. 2A, 4.

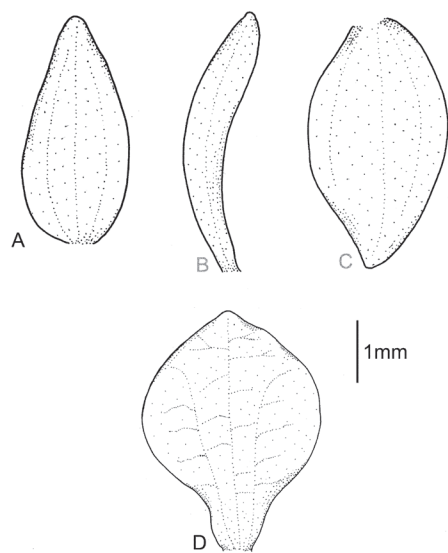


Figure 4. *Cranichis beckii* sp. nov. **A** Ovary and gynostemium. **B** Floral bract. **C** Dorsal sepal. **D** Petal. **E** Lateral sepal. **F** Lip, front view. **G** Lip, side view. Drawn by P. Baranow from S. G. Beck 313 et al. (LPB).

Notes. This species resembles *C. lehmannii* in general flower morphology, but its petals are spatulate-obovate, widest apically and similar in shape to those of *C. diphylla*. Leaves of *C. lehmannii* are smaller (up to 11 cm long), its ovary is glabrous (microscopically ciliate in *C. beckii*), lateral sepals are 2-veined (vs 1-veined in *C. beckii*)

Table 1. Comparative morphology of *C. beckii* and *C. lehmannii*.

Character	<i>C. beckii</i>	<i>C. lehmannii</i>
Leaves	3, petiole 8–10 cm long; blade 11–20 x 4–6 cm, ovate, acute	1–3, petiole 3–4(8) cm long; blade 6.5–11 x 2.8–5 cm, ovate, acute
Inflorescence	16 cm, conical, sublxly many-flowered	2.5–10.5 cm long, conical, sublxly many-flowered
Ovary	9.5 mm long, microscopically ciliate	6–9 mm long, almost glabrous
Floral bracts	6.2 mm long, lanceolate, acute, microscopically ciliate	4.5–8 mm long, lanceolate, acute, glabrous
Dorsal sepal	3.5 x 1.5 mm, oblong-ovate, acuminate, obtuse, 1-veined	3–4 mm x 1–1.1 mm, oblong-lanceolate to oblong ovate, acuminate, obtuse, concave, 1-veined
Lateral sepals	3.5 x 1.7 mm, obliquely oblong-elliptic, subapiculate, concave near the base, 1-veined	3.5–4 x 1.5–1.7 mm, obliquely elliptic-ovate to elliptic-lanceolate, subacute to subapiculate, concave in the center, obscurely 2-veined
Petals	3.2 x 1.3 mm, obliquely spatulate-obovate, apiculate, 1-veined	2.5–3.5 x 0.5–1.2 mm, lanceolate, somewhat oblique at base, subobtuse, 1-veined
Lip shape	3 x 2.1 mm, elliptic to oblong-elliptic in outline, apiculate at apex	3–3.3 x 1.6–2.3 mm, elliptic to oblong-elliptic in outline, obtuse at apex, lateral margins reflexed
Lip disc	with numerous, irregularly subglobose thickenings on the inner surface, veins thickened	with numerous, irregularly subglobose thickenings on the inner surface, veins thickened

and petals are lanceolate. The comparative morphology of *C. beckii* and *C. lehmannii* is presented in Table 1.

4. *Cranichis ciliata* (Kunth) Kunth, Syn. Pl. 1: 324. 1822.

Ophrys ciliata Kunth, Nov. Gen. Sp. (quarto ed.) 1: 334, t. 74. 1816.

Type. VENEZUELA. *Humboldt s.n.* (lectotype, designated by Garay (1978: 191): W!).

Diagnosis. Plants 26–54 cm tall. Leaves 1–2, basal, petiolate; petiole 5–19 cm long, canaliculate; blade 4–15 cm long, 3–7.5 cm wide, oblong-ovate to elliptical, acute to acuminate, broadly rounded to subcordate at the base. Scape glabrous in lower part, glandular-pubescent above, enclosed in 3–6 non-foliaceous sheaths. Inflorescence 3.5–17 cm long, cylindrical, subclaxly to subdensely many-flowered. Flowers whitish marked with green or purple-brown, with reddish or brown lip. Floral bracts 4.2–6.0 mm long, ovate-lanceolate, ovate, acuminate to acute, glabrous. Pedicellate ovary 5.0–8.5 mm long, glabrous. Dorsal sepal 3.0–4.1 mm long, 1.2–2.0 mm wide, oblong-elliptical to ovate, obtuse, 3-5-veined (rarely 1-veined). Petals 2.8–4.1 mm long, 0.4–1.0 mm wide, obliquely narrowly-ligulate to oblanceolate, obtuse, 1-veined, margins ciliate. Lateral sepals 3.0–4.5 mm long, 1.2–2.0 mm wide, obliquely oblong-ovate to elliptic-ovate, subobtuse, 2- or 3-veined. Lip 2.5–3.2 mm long, 2.1–3.0 mm wide, gibbose at base, cochleate above, obovate to suborbicular above the base, rounded or obtuse at apex; disc with 3 thickened, dendritic branching veins. Gynostemium 1.2–2 mm long. Fig. 5-6.

Habitat and ecology. Terrestrial in Yungas secondary submontane humid forest, montane, cloud forest, secondary forest with *Tibouchina* Aubl. (Melastomataceae) and *Miconia* Ruiz & Pav. (Melastomataceae), forest with *Clusia* L. (Clusiaceae) and *Weinmannia* L. (Cunoniaceae) and in Tucumano-Boliviano secondary submontane humid forest with *Myrcianthes* O. Berg. (Myrtaceae). Flowers in March, April, May and June. The populations of this species were recorded growing at altitudes between 1900–3000 m. According to Vásquez et al. (2014) this species occurs in the Yungas ecoregion at altitudes between 1000–3000 m.

Representative specimens. **BOLIVIA. Cochabamba:** Cochabamba: Prov. Carrasco. La Siberia. January 1983. *R. Vásquez et al.* 792 (Herbarium Vasquezianum–Dodson and Vásquez. 1989); Sehuencas, PN Carrasco, 2100 m. 5 April 1996. *P. Ibish & C. Ibish* 96.0020 (LPB!), Prov. Ayopaya, 10 km Cocapata-Cotacajes, 3000 m. 9 May 1997. *M. Kessler et al.* 9412 (LPB!). **La Paz:** Prov. Nor Yungas. 2.4 km below Chusipata on road to Chulumani, 2950 m. 4 March 1983. *J. C. Solomon* 9678 (LPB!). Prov. B. Saavedra. Area Natural de Manejo Integrado Apolobamba. Chulina, al frente de estancia Wikjelani, pasando por el río Sillaca. 15°07'57"S 68°52'57"W, 2760 m. 17 April 2010. *A. Fuentes & R. Rodas* 16115 (LPB!), Area Natural de Manejo Integrado Apolobamba. Kazu, cruzando el río Sillaca, 30 minutos bajando por el río Sillaca, al

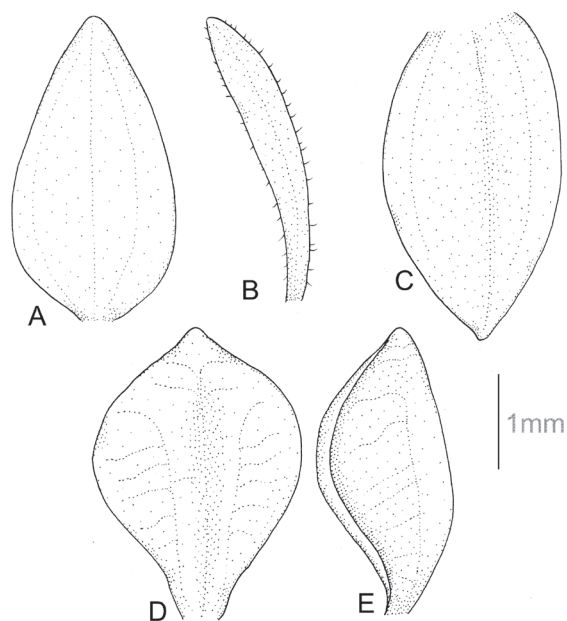


Figure 5. *Cranichis ciliata* (Kunth) Kunth. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip, front view. **E** Lip, side view. Drawn by P. Baranow from *I. Loza et al. 1621A* (LPB).



Figure 6. *Cranichis ciliata* (Kunth) Kunth (photo by A. Fuentes).

frente de la loma Wakelli. 15°07'53"S 68°53'08"W, 2788 m. 17 April 2010. *I. Loza et al.* 1621A (LPB!), Area Natural de Manejo Integrado Apolobamba. Paian, río Silliaca, sector Kumamita. 15°06'47"S 68°55'04"W, 2659 m. 20 April 2010. *I. Loza et al.* 1698 (LPB!), Prov. Franz Tamayo. Parque Nacional Madidi. Sector campamento Tanhuara, por el antiguo camino Pelechuco-Apolo, pasando el río Pelechuco. 14°44'59"S 68°56'57"W, 1905 m. 28 June 2009. *A. Fuentes & D. Alanes* 15018 (LPB!). **Santa Cruz:** Prov. Vallegrande. Vallegrande, aprox. 10 km al S por el camino a Pucará, 2260 m. 31 March 2001. *A. Fuentes* 2903 (LPB!). Fig. 2.

Notes. This is a widely distributed species reported from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Ecuador, Peru, Bolivia and Argentina. Various authors have synonymized *C. ciliata* with different species, e.g. *C. antioquiensis* Schltr. (Davidse et al. 2020), *C. atrata* (Garay 1978; Christenson 1991; Bogarín et al. 2014), *C. curtii* Schltr. (Davidse et al. 2020), *C. irazuensis* Schltr. (Davidse et al. 2020), *C. mandonii* (Schweinfurth 1958; Bogarín et al. 2014), *C. pachnodes* Løjtnant (Bogarín et al. 2014), *C. pleioneura* (Bogarín et al. 2014), *C. polyblephara* Schltr. (Brako and Zarucchi 1993; Bogarín et al. 2014), *C. schlimii* Rchb. f. (Bogarín et al. 2014) and *C. sororia* Schltr. (Garay 1978; Bogarín et al. 2014). The actual taxonomic position of most of them requires further study.

5. *Cranichis cylindrostachys* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 59. 1920.

Type. COLOMBIA. *Madero* 14 (B†, lectotype, designated by Garay (1978: 199): AMES!–drawing).

Diagnosis. Plants 26–29 cm tall. Leaves 2–3, basal, petiolate; petiole 3–8 cm long, narrow, canaliculate; blade 2–6 cm long, 1.6–4 cm wide, ovate, acute, base obliquely cordate to cuneate. Scape glabrous, remotely 4–5-sheathed. Inflorescence 5–12 cm long, cylindrical, subdensely many-flowered. Flowers small, glabrous. Floral bracts 3.8 mm long, ovate-lanceolate, acuminate, glabrous. Pedicellate ovary 5.5 mm long, glabrous. Dorsal sepal 3 mm long, 1.3 mm wide, ovate-lanceolate, subobtuse, 1-veined. Petals 3.1 mm long, 0.7 mm wide, obliquely lanceolate to linear-oblongate, subobtuse, glabrous on margins, 1-veined. Lateral sepals 3.6 mm long, 2.1 mm wide, obliquely elliptic-ovate, slightly concave at base, obtuse, 2-veined. Lip 3.1 mm long, 2 mm wide, somewhat concave, subsessile, oblong-elliptical, shortly apiculate; disc with 3 thickened, dendritic branching veins with prominent nodules. Gynostemium 1.8 mm long. Fig. 7.

Habitat and ecology. Terrestrial in Yungas montane secondary forest at an altitude of 1980 m. Flowers in March.

Representative specimen. BOLIVIA. **La Paz:** Prov. Yungas, Challapata, pasando ladera quemada más alla de las Masdevallias, 1981 m. 4 March 2006. *F. Miranda et al.* 1236 (LPB!). Fig. 2.

Notes. *Cranichis cylindrostachys* is often considered to be a synonym of *C. lehmannii* (e.g. Garay, 1978). The two species differ in leaf petiole length (usually 3–4 cm long

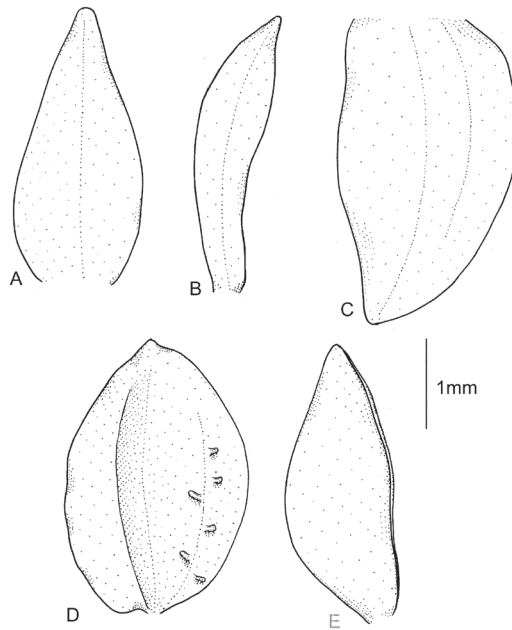


Figure 7. *Cranichis cylindrostachys* Schltr. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip, front view. **E** Lip, side view. Drawn by P. Baranow from *F. Miranda 1236 et al.* (LPB).

in *C. lehmannii*) and inflorescence architecture (conical in *C. lehmannii*), but whether they are different species is doubtful and further molecular studies are needed to clarify the situation.

6. *Cranichis diphylla* Sw., Prodr. 120. 1788.

Type. JAMAICA. *Swartz s.n.* (lectotype, designated by Garay (1978: 192): BM!; isoelectotypes, LD, S!, UPS, W!; AMES! -drawing).

Diagnosis. Plants up to 40 cm tall. Leaves 1–3, basal, often variegated, petiolate; petiole rather variable in length, up to 3 cm; blade up to 9 cm long, 4 cm wide, ovate to ovate-lanceolate, acute to subacuminate, subcordate at base. Scape slender, erect, remotely few-sheathed, glabrous below, glandular-pubescent above. Inflorescence up to 6.5 cm long, cylindrical, loosely to subdensely many-flowered. Flowers white with green veins. Floral bracts 4 mm long, ovate-lanceolate, acuminate, sparsely glandular. Pedicellate ovary up to 6 mm long, cylindrical, more or less glandular. Dorsal sepal up to 3.5 mm long, 1.6 mm wide, erect, elliptical, subacute to subobtus, 3-veined, occasionally sparsely pubescent dorsally. Petals up to 3.1 mm long, 1 mm wide, near apex linear-oblongate, acute to obtuse, 1-veined, glabrous along margins. Lateral sepals up to 4 mm long, 1.6 mm wide, spreading, obliquely ovate to ovate-elliptical, acute to obtuse, 2-veined, occasionally sparsely pubescent dorsally. Lip up to 3.6 mm long,

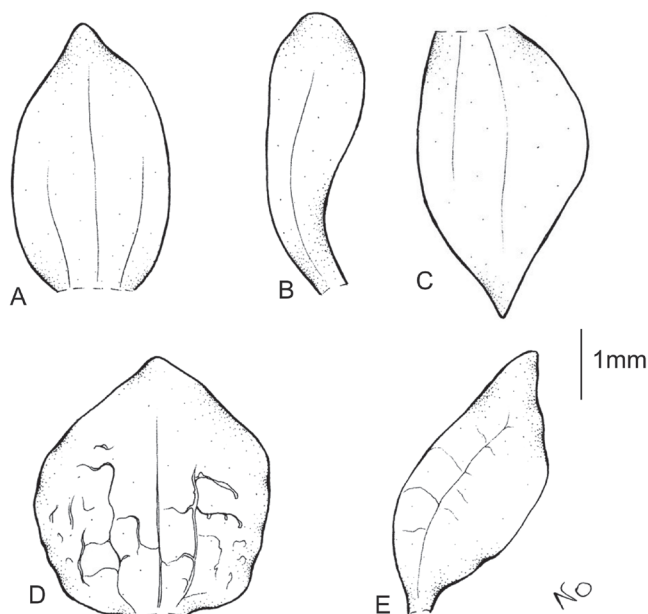


Figure 8. *Cranichis diphylla* Sw. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip, front view. **E** Lip, side view. Drawn by N. Olędzżyńska from Killip & Smith 15946 (AMES).

3.2 mm wide, concave, inconspicuously subcordate at base, ovate to broadly elliptical in outline, subacute to subobtuse; disc obcordately papillose-thickened with three branching, often glandular (with nodules) veins from base to middle of lip. Gynostemium 1.3–2 mm long. Fig. 8.

Habitat and ecology. According to Vásquez et al. (2014) this species occurs in the Yungas ecoregion at altitudes between 1000–1500 and 2500–3500 m.

Representative specimen. **BOLIVIA. Santa Cruz.** *R. Vásquez C. 2180* (herb. Vásquez, LPB).

Notes. The specimen cited by Vásquez et al. (2014) was not found in the Orchid collection in LPB and we were not able to confirm the occurrence of this species in Bolivia. The characteristics presented above were prepared based on those of Ecuadorian and Colombian plants (Szlachetko and Kolanowska 2019).

7. *Cranichis garayana* Dodson & R. Vásquez, *Icon. Pl. Trop.*, ser. 2. 3: pl. 210. 1989.

Type. **BOLIVIA.** *Vásquez Ch. 612* (holotype MO).

Diagnosis. Plants 20–40 cm tall. Leaves 2, basal, petiolate; petiole 4–6 cm long, narrow, canaliculate; blade 2.8–8.0 cm long, 3.0–5 cm wide, ovate, acuminate. Scape glabrous, enclosed in about 4–5 non-foliaceous sheaths. Inflorescence 4.0–10 cm long, cylindrical, sub-laxly few- to many-flowered. Flowers whitish-brown, glabrous. Floral

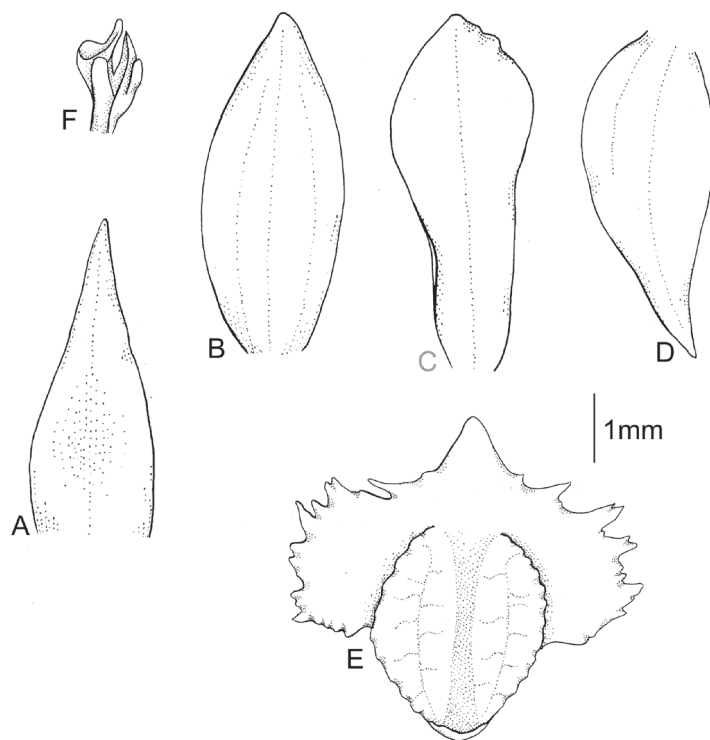


Figure 9. *Cranichis garayana* Dodson & R. Vásquez. **A** Floral bract. **B** Dorsal sepal. **C** Petal. **D** Lateral sepal. **E** Lip. **F** Gynostemium. Drawn by P. Baranow from *I. Jimenez 3854* & *Miranda F* (LPB).

bracts 5.0 mm long, lanceolate, acute. Pedicellate ovary 7.5 mm long, almost glabrous. Dorsal sepal 5.0–6.0 mm long, 2.3–3.0 mm wide, oblong-elliptical to ovate, obtuse, 3-veined. Petals 4.5–5.0 mm long, 1.7–2.0 mm wide, obliquely oblanceolate to spatulate, obtuse, 1-veined, glabrous. Lateral sepals 5.1–6.0 mm long, 2.3–3.5 mm wide, obliquely ovate, obscurely 2-veined. Lip 4.5–5.0 mm long, 4.5–5.0 mm wide, concave, subsessile, 3-lobed above the elliptical base, lateral lobes subquadrate, deeply laciniate, middle lobe ovate, rounded or obtuse; disc with 5 dendritic branching veins. Gynostemium 2.9 mm long. Fig. 9.

Habitat and ecology. Terrestrial or epiphytic in Yungas montane wet forest at altitudes between 1880–2240 m. Flowers in March. According to Vásquez et al. (2014) this species occurs in Yungas ecoregion at altitudes between 1500–2500 m.

Representative specimens. **BOLIVIA. Cochabamba:** Chapare, km 100, Cochabamba to Villa Tunari, 1880 m. 22 March 1981. *R. Vásquez Ch. 612* (MO). **La Paz:** Prov. Nor Yungas. PN-ANMI Cotapata, sendero Sandillani al segundo campamento, 2240 m. 4 March 2006. *I. Jimenez* & *F. Miranda 3854* (LPB!). Fig. 2.

Notes. *Cranichis garayana* is a Bolivian endemic easily distinguished from other species by its 3-lobed lip above an elliptical base with subquadrate, deeply laciniate, lateral lobes and ovate, rounded middle lobe.

8. *Cranichis lehmannii* Rchb. f., Otia Bot. Hamburg. 1: 4. 1878.

Type. ECUADOR. *Lehmann* 77 (lectotype, designated by Garay (1978: 199): W!; AMES!—drawing, UGDA-DLSz!—drawing).

Diagnosis. Plants 26–60 cm tall. Leaves 1–3, basal, petiolate; petiole 3–4(8) cm long, narrow, canaliculate; blade 6.5–11 cm long, 2.8–5 cm wide, ovate, acute. Scape erect, enclosed in 6–9 sheaths. Inflorescence 2.5–10.5 cm long, conical, sub-laxly many-flowered. Flowers small, glabrous. Floral bracts 4.5–8 mm long, lanceolate, acute. Pedicellate ovary 6–9 mm long, almost glabrous. Dorsal sepal 3–4 mm long, 1–1.1 mm wide, oblong-lanceolate to oblong ovate, acuminate, obtuse, concave, 1-veined. Petals 2.5–3.5 mm long, 0.5–1.2 mm wide, lanceolate, somewhat oblique at base, subobtuse, 1-veined. Lateral sepals 3.5–4 mm long, 1.5–1.7 mm wide, obliquely elliptic-ovate to elliptic-lanceolate, subacute to subapiculate, concave in the centre, obscurely 2-veined. Lip 3–3.3 mm long, 1.6–2.3 mm wide, concave, subsessile, elliptical to oblong-elliptical in outline, obtuse at apex, lateral margins reflexed; disc with numerous, irregularly subglobose thickenings on the inner surface, veins thickened with dendritic branching. Gynostemium 1.2–1.5 mm long. Fig. 10.

Habitat and ecology. According to Vásquez et al. (2014) this species grows as an epiphyte in the Yungas ecoregion at altitudes between 2500–3500 m.

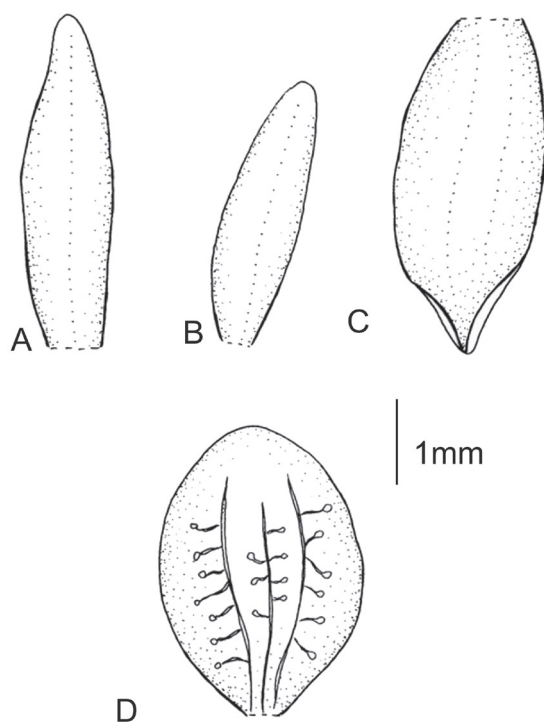


Figure 10. *Cranichis lehmannii* Rchb. f. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip. Redrawn by A. Król from Garay's illustration of specimen collected by *Lehmann* 77 (W).

Representative specimen. BOLIVIA. La Paz: PN-ANMI Cotapata, sendero Chojllapata, poco antes de llegar al codo del sendero. 16°14'S, 67°52'O, 2670 m. *I. Jiménez* 5579 (LPB–Jiménez-Pérez 2011). Fig. 2.

Notes. The specimen cited by Jiménez-Pérez (2011) was not found in the Orchid collection deposited in LPB and we were not able to confirm the occurrence of this species in Bolivia. The characteristics presented above are based on those of Ecuadorian and Colombian plants (Szlachetko and Kolanowska 2019).

9. *Cranichis longipetiolata* C. Schweinf., Amer. Orchid Soc. Bull. 21: 268. 1952.

Type. PERU. *Ferreyra* 3120 (lectotype, designated by Garay (1978: 200): AMES!, isoelectotype: USM; UGDA-DLSz!–drawing).

Diagnosis. Plants up to 29–37 cm tall. Leaf 1, basal, petiolate; petiole 10.5–14 cm long; blade 8–10 cm long, ca. 5.0 cm wide, oblong-elliptical to ovate, oblique, acute or shortly acuminate, cuneate to subcordate at base. Scape glabrous below, finely pubescent or ciliate above, with 3–5, non-foliaceous sheaths. Inflorescence 5–7 cm long, conical, subdensely many-flowered. Flowers greenish or greenish-white. Floral bracts 4.3

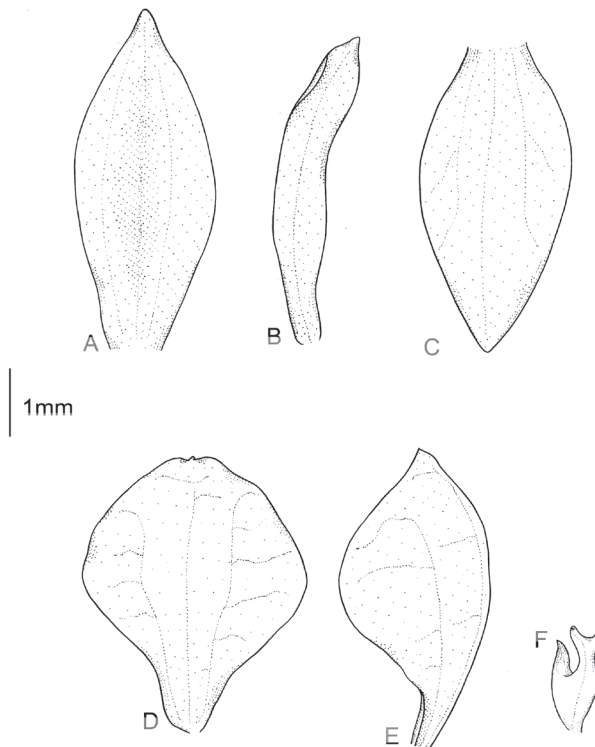


Figure 11. *Cranichis longipetiolata* C. Schweinf. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip, front view. **E** Lip, side view. **F** Gynostemium. Drawn by P. Baranow from *M. Mendoza* & *S. Acebo* 912 (LPB).

mm long, lanceolate, glabrous. Pedicellate ovary 6.2 mm long, glandular. Dorsal sepal 4.1–5.0 mm long, 1.2–2.1 mm wide, ovate-lanceolate to narrowly elliptical, subacute, concave, 3-veined. Petals 3.3–4.0 mm long, 0.3–0.7 mm wide, obliquely linear-lanceolate, subobtusate, more or less oblique or curved, 1-veined, glabrous. Lateral sepals 4.2 mm long, 1.6–2 mm wide, obliquely elliptical, subacute to obtuse, 2–3-veined, margin very sparsely ciliate. Lip 3.2–3.6 mm long, 1.8–3.0 mm wide, deeply concave, basally gibbous, shortly unguiculate, obovate to suborbicular, apex rounded with a minute apiculus; disc with three transversely anastomosing veins. Gynostemium 1.8–2.0 mm long. Fig. 11.

Habitat and ecology. Terrestrial in Yungas montane humid, and cloud forest, also in montane Tucumano-Boliviano forest, at altitudes between 2380–3000 m. Flowers in March.

Representative specimens. Bolivia. Santa Cruz: J.M. Caballero, Comunidad Siberia, ca. 1–2 km arriba del pueblo de Siberia, sobre un camino vecinal, entrando hacia el Parque Nacional Amboro. 17°49.36'S, 64°45.14'W, 3001 m. 26 March 2004. *M. Mendoza* & *S. Acebo* 912 (LPB!), Vallegrande. Tucumano-Boliviano. 18°34'28"S 64°02'33"W, 2387 m. *Parada et al.* 4208 (LPB!). Fig. 2.

Notes. The lip of the specimen collected by *Parada et al.* 4208 (LPB) differs somewhat in shape from the typical form of that of *C. longipetiolata*, which is obovate rather than suborbicular in outline.

10. *Cranichis maldonadoana* Kolan., Baranow, S. Nowak & A. Fuentes, sp. nov.

urn:lsid:ipni.org:names:77233922-1

Type. BOLIVIA. *Bastian* 937 (holotype LPB!; isotype LPB!).

Diagnosis. Species similar to *C. pleioneura*, but distinguished by 1-veined sepals, lanceolate-ovate petals that are sparsely ciliate in the lower part and by subsessile, suborbicular-obovate lip with apiculate apex.

Plants 30–40 cm tall, erect. Leaf 1, basal, petiolate; petiole 11–14 cm long, narrow, canaliculate; blade 7.8–10 cm long, 4.2–6 cm wide, ovate, apex not preserved. Scape enclosed in 4–5 sheaths, glandular towards apex. Inflorescence 6–7 cm long, cylindrical, densely many-flowered. Flowers greenish-yellow, glabrous. Floral bracts 3.3 mm long, lanceolate, acuminate, glabrous. Pedicellate ovary 4.2 mm long, glabrous. Dorsal sepal 3.7 mm long, 1.5 mm wide, oblong-elliptical, obtuse, 1-veined. Petals 3.2 mm long, 1 mm wide, obliquely lanceolate-ovate, obtuse, 1-veined, ciliate on basal 2/3. Lateral sepals 4.2 mm long, 1.3 mm wide, obliquely elliptic-ovate, subacuminate, subobtusate, 1-veined. Lip 2.4 mm long, 2 mm wide, lower part concave, subsessile, suborbicular-obovate, apex apiculate; disc with 3 thickened, dendritic branching veins. Gynostemium 2.3 mm long. Fig. 12.

Etymology. Dedicated to Carla Maldonado, the director of Herbario Nacional de Bolivia, for her great support during our studies in Bolivia.

Habitat and ecology. Terrestrial plants growing in Tucumano-Boliviano pine (Podocarpaceae) forest at an altitude of 2100 m. Flowers in March.

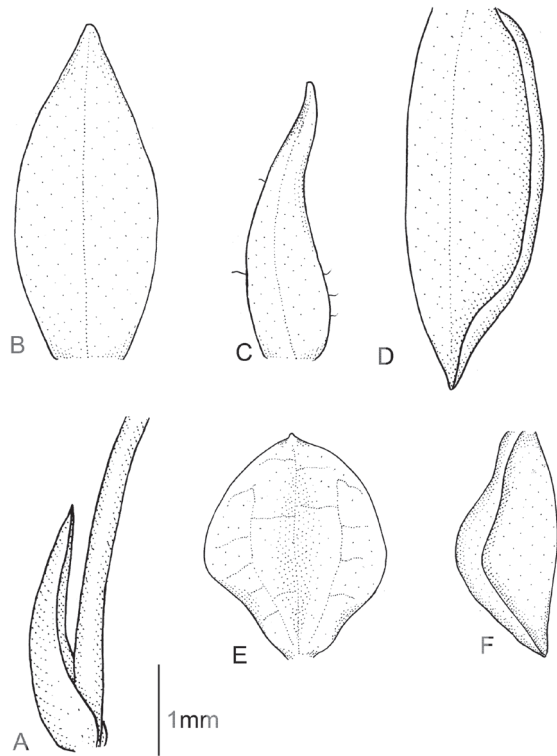


Figure 12. *Cranichis maldonadoana* sp. nov. **A** Ovary and floral bract. **B** Dorsal sepal. **C** Petal. **D** Lateral sepal. **E** Lip, front view. **F** Lip, side view. Drawn by P. Baranow from *E. Bastión* 937 (LPB).

Representative specimen. BOLIVIA. Tarija: Prov. Cercado, cerca Victoria, 2150 m. 3 March 1986. *E. Bastian* 937 (LPB!). Fig. 2B, 12.

Notes. This species resembles *C. pleioneura* and *C. barkleyi* Szlach. & Kolan., from which it differs in its lanceolate-ovate petals (vs. linear-ob lanceolate to linear-ligulate), which are rather sparsely ciliate on the lower part (vs. equally ciliate or pilose along whole length). Unlike in *C. pleioneura* those of *C. maldonadoana* are 1-veined. *Crani-chis atrata* Schltr. differs from *C. maldonadoana* by having ligulate-ob lanceolate petals, which are ciliate along both margins (vs. lanceolate-ovate, obtuse, 1-veined, ciliate on the basal 2/3). The comparative morphology of *C. maldonadoana*, *C. barkleyi* and *C. pleioneura* is presented in Table 2.

Table 2. Comparative morphology of *C. maldonadoana*, *C. barkleyi* and *C. pleioneura*.

Character	<i>C. maldonadoana</i>	<i>C. barkleyi</i>	<i>C. pleioneura</i>
Leaves	1, petiole 11–14 cm long; blade 7.8–10 x 4.2–6 cm, ovate, apex not preserved	1, petiole 9–17 cm long; blade 9–10 x 4–7 cm, ovate, ovate-elliptic, acute or acuminate	1, petiole 8–15 cm long; blade 7.5–15 x 3.5–7.5 cm, oblong-ovate, acute to acuminate
Inflores- cence	6–7 cm long, cylindric, densely many-flowered	3–4 cm long, cylindric, loosely many-flowered	5–12 cm long, cylindric, densely many-flowered

Character	<i>C. maldonadoana</i>	<i>C. barkleyi</i>	<i>C. pleioneura</i>
Ovary	4.2 mm long, glabrous	8–9 mm long, glabrous	5–9 mm long, glabrous
Floral bracts	3.3 mm long, lanceolate, acuminate, glabrous	5–8 mm long, lanceolate, acuminate, glabrous	4.5–5 mm long, lanceolate, acuminate, glabrous
Dorsal sepal	3.7 x 1.5 mm, oblong-elliptic, obtuse, 1-veined	3–4.5 x 1.1–2 mm, elliptic-ovate to oblong ovate, subacute, 1-veined	3–3.8 x 1.1–1.5 mm, oblong-elliptic to elliptic-ovate, subobtuse, 3-veined
Lateral sepals	4.2 x 1.3 mm, obliquely elliptic-ovate, subacuminate, subobtuse, 1-veined	3.5–4 x 1.6–2 mm, oblong ovate, subacute, 1-veined	3–4 x 1.3–2 mm, obliquely ovate to elliptic-ovate, slightly concave at the base, subacuminate, subobtuse, 3-veined
Petals	3.2 x 1 mm, obliquely lanceolate-ovate, obtuse, 1-veined, ciliate in the basal 2/3	2.8–4 x 0.4–0.8 mm, narrowly linear to narrowly ligulate, somewhat falcate, subobtuse, 1-veined, margins sparsely pilose	2.8–3.2 x 0.4–0.8 mm, obliquely linear-oblancoate to linear-ligulate, apex obtuse to truncate, 1-veined, ciliate along margins
Lip shape	2.4 x 2 mm, concave in the lower part, subsessile, suborbicular-obovate, apex apiculate	2.5–3.5 x 2–2.8 mm, gibbose at the base, cochleate above, shortly unguiculate, obovate in outline, rounded at apex	2.5–3.2 x 2–2.6 mm, concave in the lower part, subsessile, suborbicular-obovate, apex rounded
Lip disc	with 3 thickened, dendritic branching veins	with 3 thickened, dendritic branching veins	with 3 thickened, dendritic branching veins

Specimen *Bastian 937* is heterotypic. Two specimens from this collection, which are currently included in the general collection of LPB, are *C. maldonadoana*. Plants deposited in the boxes with the undetermined materials in the same herbarium fit the characteristic of *C. badia* Renz ex Kolan. & Szlach.

11. *Cranichis mandonii* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 10: 38. 1922.

Type. BOLIVIA. *Mandon 1163* (lectotype, designated by Szlachetko and Kolanowska (2019: 12): AMES!; isolectotypes, BM, G, GH, NY, P, S).

Diagnosis. Plants 40–60 cm tall. Leaves 1–2, basal, petiolate; petiole 13–16 cm long, narrow, canaliculate; blade 6–8.5 cm long, 3.7–4.4 cm wide, oblong to ovate, slightly oblique, base cordate. Scape delicate, terete, enclosed in 5–6 acuminate sheaths, apically glandular-pilose. Inflorescence 12 cm long, cylindrical, subdensely many-flowered. Flowers with tepals maroon at base, white at apex. Floral bracts about 5–5.5 mm long, narrowly lanceolate, acuminate. Pedicellate ovary about 5–5.5 mm long, fusiform-cylindrical, sparsely glandular. Sepals sparsely pubescent on the outer surface. Dorsal sepal 4.5 mm long, 1.5 mm wide, lanceolate-ovate to oblong ovate, subacute to subobtuse, 3-veined. Petals 4 mm long, 1 mm wide, oblong ligulate to oblong oblanceolate, obtuse to rounded at apex, 1-veined, margins minutely ciliate-papillate. Lateral sepals 4.5 mm long, 2 mm wide, elliptical to elliptic-ovate, subacute, 3-veined. Lip 3 mm long and wide, basally gibbose, subsessile, suborbicular-obovate to suborbicular, apically rounded; disc 3-veined, veins somewhat thickened, dendritic branching, without any nodules. Gynostemium 2 mm long. Fig. 13.

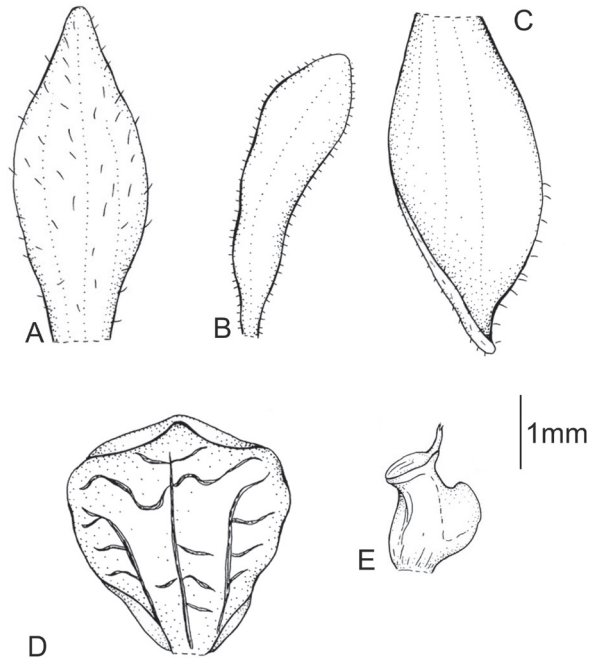


Figure 13. *Cranichis mandonii* Schltr. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip. **E** Gynostemium. Redrawn by A. Król from Garay's illustration of specimen collected by *Mandon 1163* (AMES).

Habitat and ecology. Terrestrial plants growing in Yungas montane cloud forest at altitudes between 2600–3100 m. Flowers in April and May.

Representative specimens. **BOLIVIA.** **Larecaja:** Sorata, 2650–3100 m. Apr–May 1860. *G. Mandon 1163* (AMES!, BM, G, GH, NY, P, S). Fig. 2.

Notes. *Cranichis mandonii* is often considered as conspecific with *C. ciliata* (e.g. Schweinfurth 1958), however, the two species differ in the ornamentation on the sepals, which in *C. mandonii* is sparsely pubescent and in *C. ciliata* glabrous.

12. *Cranichis muscosa* Sw., Prodr. 120. 1788.

Cranichis ovata Wikstr., Kongl. Vetensk. Acad. Handl. 73. 1920.

Type. JAMAICA. *Swartz s.n.* (lectotype, designated by Garay (1978: 202): BM!; isolectotypes, LD, S!, W!).

Diagnosis. Plants up to 25 cm tall, erect, strict or flexuose. Leaves 3–5, basal, rosulate, petiolate; petiole 2–3 cm long; blade 2.5–3 cm long, up to two cm wide, ovate, elliptic-ovate to oblong, acute to subobtuse. Scape slender, enclosed in five sheaths. Inflorescence up to 7.5 cm long, cylindrical, subdensely many-flowered. Flowers small, white. Floral bracts four to five mm long, lanceolate to ovate-lanceolate, acuminate.

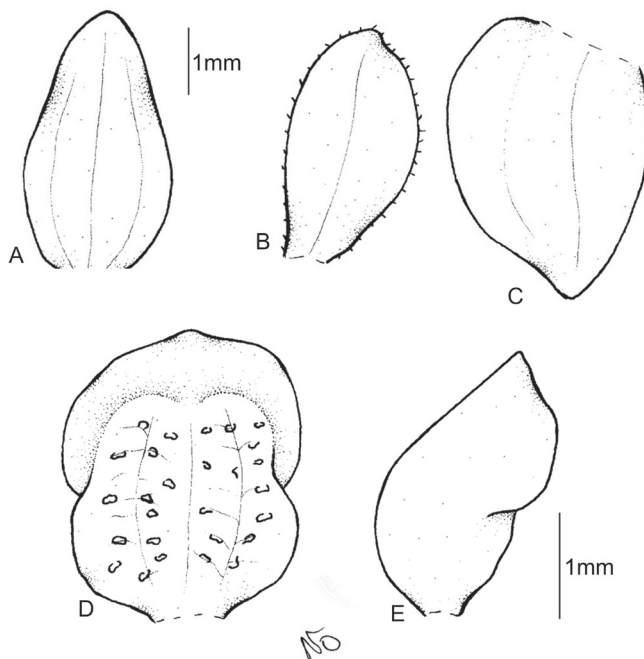


Figure 14. *Cranichis muscosa* Sw. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip, front view. **E** Lip, side view. Drawn by N. Olędryńska from *Garay & Sweet 1057* (AMES).

Pedicellate ovary 5–6 mm long, glabrous. Dorsal sepal 2.2 mm long, one mm wide, oblong-lanceolate to oblong-ovate, acute, 3-veined. Petals 2 mm long, 0.6 mm wide, linear-ligulate to narrowly oblanceolate, obtuse, subfalcate, margins ciliate, 1-veined. Lateral sepals 3 mm long, 1.8 mm wide, obliquely oblong-ovate to elliptic-ovate, acuminate, acute, obscurely 2-veined. Lip 2.2 mm long, 1.87 mm wide, concave, sessile, ovate to suborbicular-ovate, shortly apiculate to acute; disc with irregular knob-like projections in the centre. Gynostemium 2 mm long. Fig. 14.

Habitat and ecology. According to Vásquez et al. (2014) this species grows as an epiphyte in humid amazon forest at altitudes between 500–1000 m. Flowers in April.

Representative specimen. **BOLIVIA. Santa Cruz:** Velasco. Parque Nacional Noel Kempff Mercado. Campamento Las Gamás. Bosque de colina, 900 m. 4 April 1993. *T. Killeen et al.* 5050 (herb. Vásquez, MO, USZ–Vásquez et al. 2014). Fig. 2.

Notes. This is a widely distributed species. Its geographical range extends from USA (Florida) to Brazil and Bolivia. It is recognized by its foliaceous scape, minutely ciliate petals and lip with membranous margin.

13. *Cranichis pulvinifera* Garay, Fl. Ecuador 9: 204. 1978.

Type. COLOMBIA. *Bristol* 1227 (holotype AMES!).

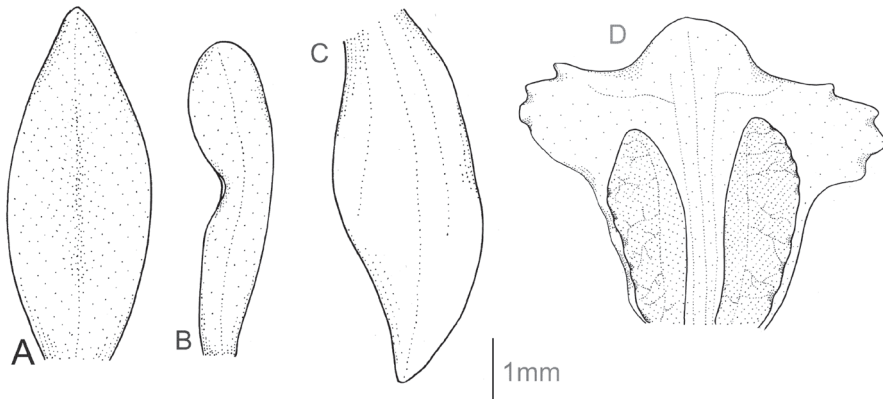


Figure 15. *Cranichis pulvinifera* Garay. **A** Dorsal sepal. **B** Petal. **C** Lateral sepal. **D** Lip. Drawn by P. Baranow from M. López & G. Villegas 74 (LPB).

Diagnosis. Plants 42 cm tall. Leaves 5, basal, petiolate; petiole 4–6.5 cm long, narrow; blade 4–6 cm long, 2.2–3 cm wide, ovate to elliptical, acute, with rounded base. Scape erect, slender, ca. 5-sheathed, the lowermost foliaceous. Inflorescence 8 cm long, cylindrical, loosely many-flowered. Flowers with beige sepals, salmon petals and brown lip, greenish. Floral bracts up to 8 mm long, lanceolate, acuminate, glabrous. Pedicellate ovary 9–11 mm long, glabrous. Dorsal sepal 5.1 mm long, 2 mm wide, narrowly elliptical, obtuse, 1-veined. Petals 4.5 mm long, 1.3 mm wide, linear-ligulate, rounded, 1-veined, glabrous. Lateral sepals 5.3 mm long, 2.2 mm wide, obliquely ovate-elliptical, obtuse, obscurely 3-veined. Lip 4.2 mm long, 4.5 mm wide, navicular or subsaccate, sessile, triangular-ovate, truncately 3-lobed in front with triangular, obtuse middle lobe and ovate, erose lateral lobes; disc with a pair of inflated cushions which are sparsely covered with large papillae. Gynostemium 2 mm long. Fig. 15.

Habitat and ecology. Terrestrial in humid montane forest. It grows at an altitude of ca. 2400 m. Flowers in April. According to Vásquez et al. (2014) this species grows in the Yungas ecoregion at altitudes between 2000–3000 m.

Representative specimen. **BOLIVIA. La Paz:** Prov. Nor Yungas. Parque Nacional Cotapata. Bajada Hornuni. 16°12'40"S 67°53'26"W, 2420 m. 5 April 2006. M. López & G. Villegas 74 (LPB!). Fig. 2.

Notes. This species is recorded in Colombia, Ecuador and Bolivia. Surprisingly, so far, it has not been reported from Peru.

14. *Cranichis silvicola* Renz ex Kolan. & Szlach., *Nordic J. Bot.* 32(3): 296. 2014.

Type. VENEZUELA. *Renz 6139* (holotype RENZ!).

Diagnosis. Plants 34–40 cm tall. Leaves 1–2, basal, petiolate; petiole 4–12 cm long, narrow, canaliculate; blade 6–22 long, 2.4–6 cm wide, elliptical, shortly acuminate, cuneate at base. Scape enclosed in 4–5 non-foliaceous sheaths, microscopically ciliate on

upper half. Inflorescence 5(18) cm long, cylindric-conical, rather laxly many-flowered. Flowers white with green veins and lip, glabrous. Floral bracts 3.4–5 mm long, lanceolate to ovate lanceolate, acute or acuminate, sparsely glandular to almost glabrous. Pedicellate ovary up to 5.5 mm long, glabrous. Dorsal sepal 3.0–3.5 mm long, 1.5–1.7 mm wide, narrowly elliptic-obovate, obtuse, 3-veined. Petals 3.4–3.5 mm long, 0.8–1.0 mm wide, oblong-ligulate to linear-oblongate, obtuse, 1-veined, glabrous. Lateral sepals 3.0–3.5 mm long, 1.5–2.0 mm wide, obliquely oblong-ovate to ovate-elliptical, obtuse, 3-veined (sometimes obscurely 3-veined). Lip about 3.0 mm long, 2.1–2.2 mm wide, cochleate, sessile, from the cuneate base suborbicular to broadly obovate, subacute at apex; disc with 3 veins, lateral veins branching, middle vein sometimes only extends to the middle part of the lip. Gynostemium 1.8–2 mm long. Fig. 16.

Habitat and ecology. Terrestrial plants growing in Yungas montane humid forest with *Weinmannia* L. (Cunoniaceae) and *Miconia* Ruiz & Pav. (Melastomataceae), and in cloud forest at an altitude of ca. 3088 m. Also reported from a lower altitude of 2100 m. Flowers in April and December.

Representative specimens. BOLIVIA. La Paz: Prov. B. Saavedra. Area Natural de Manejo Integrado Apolobamba, Paján, sector Cochapata. 15°07'13"S 68°53'45"W, 3088 m. 22 April 2010. *A. Fuentes* & *A. Machaca* 16283 (LPB!). **Tarija:** Prov. Cercado. Cerca Victoria, 2180 m. 24 December 1985. *E. Bastian* 265 (LPB!). Fig. 2.

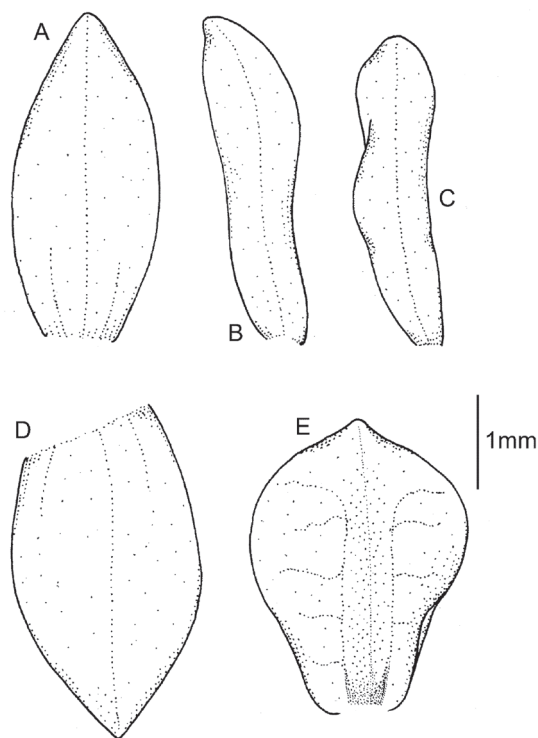


Figure 16. *Cranichis silvicola* Renz ex Kolan. & Szlach. **A** Dorsal sepal. **B–C** Petal. **D** Lateral sepal. **E** Lip. Drawn by P. Baranow from *A. Fuentes* & *A. Macacha* 16283 (LPB).

Notes. This species resembles *C. badia*, but differs in having a subsessile, cuneate lip base (unguiculate in *C. badia*) and floral bracts equal in length to pedicellate ovary (vs. much shorter). Unlike in the type material the margins of the lip of Bolivian specimens are not undulate.

15. *Cranichis stictophylla* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 62. 1920.

Type. COLOMBIA. *Madero s.n.* (B[†]).

Diagnosis. Plants 33–36 cm tall. Leaf 1, basal, petiolate; petiole 3–4 cm long; blade 4.5–6.5 cm long, 2–3.5 cm wide, ovate, acuminate, base subrounded-cuneate, white spotted. Scape glandulous-pilose toward the apex, enclosed in 5–6 sheaths. Inflorescence 3–6 cm long, cylindrical, subaxly to subdensely 15-flowered. Flowers greenish-white, glabrous. Floral bracts 2.1–4 mm long, ovate-lanceolate, acuminate, sparsely glandular. Pedicellate ovary 7–7.5 mm long, densely glandular-ciliate. Dorsal sepal 2.9–3 mm long, 0.8–1.2 mm wide, oblong elliptical, obtuse, 1-veined. Petals 2.9–3 mm long, 0.7–0.8 mm wide, obliquely linear-ob lanceolate, obtuse, glabrous, 1-veined. Lateral sepals 3 mm long, 1.2–1.5 mm wide, obliquely elliptic-ovate, slightly concave at base, subacuminate, obtuse, obscurely 2-veined. Lip 2.5 mm long, 2.0–1.9 mm wide, slightly concave, sessile, ovate, rounded or obtuse; disc with 3 or 5 dendritic thick branching veins, with prominent nodules. Gynostemium 1–1.3 mm long. Fig. 17.

Habitat and ecology. Terrestrial in Tucumano-Boliviano cloud forest and disturbed forest with *Prumnopitys* Phil. (Podocarpaceae), Myrtaceae, *Dicksonia* L'Hér. (Dicksoniaceae, and *Cyathea* Kaulf. (Cyatheaceae) at altitudes between 2100–2200 m. Flowers in June.

Representative specimens. **BOLIVIA. Santa Cruz:** Prov. Florida, 7 km NE of Mairana. Southern limit of expanded Parque Nacional Amboró, entering from Mairana, 2200 m. 2 June 1991. *M. Nee* 40653 (LPB!), Prov. Vallegrande, 5 km de Loma Larga a Vallegrande. 7 June 1996. *M. Kessler et al.* 6352 (LPB!). Fig. 2.

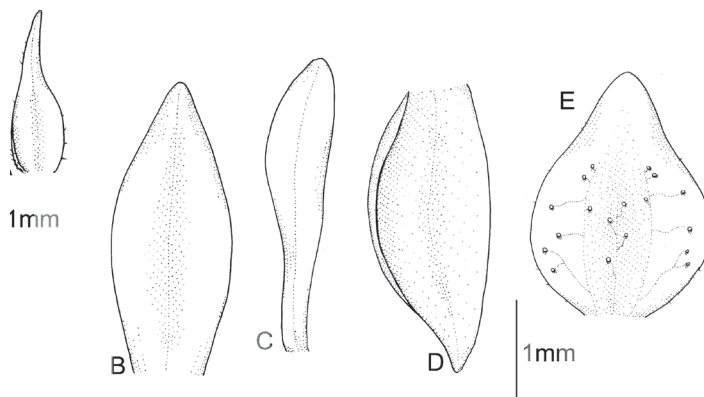


Figure 17. *Cranichis stictophylla* Schltr. **A** Floral bract. **B** Dorsal sepal. **C** Petal. **D** Lateral sepal. **E** Lip. Drawn by P. Baranow from *M. Nee* 40653 (LPB).

Notes. This species is often considered as conspecific with *C. diphylla* (e.g. Garay 1978) from which it differs, e.g., in having a 1-veined dorsal sepal.

Excluded species

***Cranichis multiflora* (Poepp. & Endl.) Cogn., Fl. Bras. 3(4): 248. 1895.**

Type. PERU. *Poeppig 1724* (?). *Ponthieva multiflora* Poepp. & Endl., Nov. Gen. Sp. Pl. 2: 16, t. 123. 1838.

Notes. This species was included by Vásquez et al. (2014) in their list of Bolivian orchids as a species of *Cranichis*, however, the original illustration of *Ponthieva multiflora* shows that the petal, gynostemium and lip are basally fused, a character not recorded for *Cranichis*, but is present in *Ponthieva*. Unfortunately, we were not able to find the specimen *Vásquez C. et al. 670* to confirm its generic placement in *Ponthieva*.

***Cranichis polyantha* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 7: 61. 1920.**

Type. COLOMBIA. *Madero 22* (B†, lectotype, designated by (Garay 1978: 203): AMES!—drawing).

Notes. We examined *Jimenez 5547 et al.* (LPB) cited as reference material for this species in Bolivia by Jiménez-Pérez (2011) and Vásquez et al. (2014) and in our opinion it is not *C. polyantha* (Fig. 18).

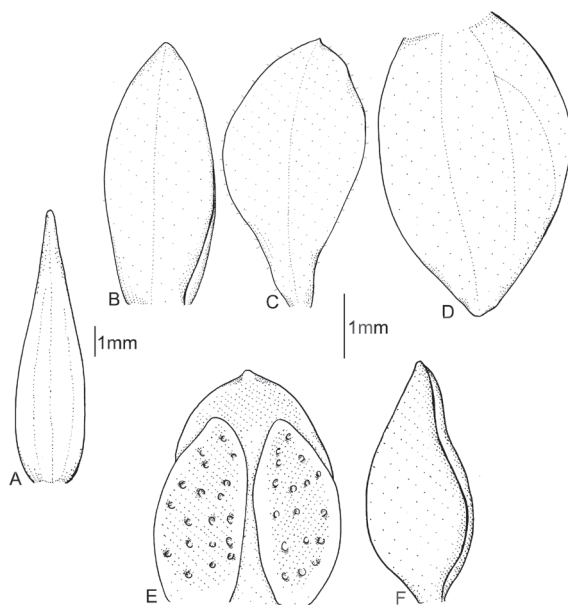


Figure 18. *Cranichis* sp. **A** Floral bract. **B** Dorsal sepal. **C** Petal. **D** Lateral sepal. **E** Lip, front view. **F** Lip, side view. Drawn by P. Baranow from *I. Jimenez 5547 et al.* (LPB).

The only information about the original collection of this species is the incomplete illustration deposited in AMES. While Schlechter (1920) did not describe any ornamentation on the petals or lip of *C. polyantha*, both Garay (1978) and Bennett and Christenson (1995) identified this species based on its ciliate petals and lip covered with numerous nodules or papillose-verrucose.

Incertae sedis

I. Jimenez 5547 et al. (LPB!): this specimen was cited by Jiménez-Pérez (2011) as *C. polyantha*, however, several characters of this specimen do not fit the morphological characteristic of this species (Fig. 18). In *Jimenez 5547 et al.* the lip is covered with small nodules and the margins of the petals are glandular-ciliate. Currently, with only a single specimen in LPB and discrepancies between the original description of *C. polyantha* and the treatment of this taxon by Garay (1978) and Bennett and Christenson (1995), we prefer not to describe a new species based on this specimen.

Discussion

As summarized by Mace (2004) and Dubois (2003) effective conservation of species requires a good taxonomic basis. Unfortunately, the number of taxonomic studies that estimate the actual (alpha) diversity of ecosystems is declining (Wägele et al. 2011). Vásquez et al. (2003) note that there is a need for further research on orchids in Bolivia as the actual number of Orchidaceae occurring in this country is most probably much higher than currently recorded. In this paper we provide the most complete data on Bolivian *Cranichis* published so far. The species characteristics and dichotomous key presented will simplify the process of identification of *Cranichis* by local botanists and improve the quality of regional checklists.

Based on this study, details of perianth segments are the most useful diagnostic characters in *Cranichis*, however, the proper identification of some taxa is difficult without information about leaf petiole size (*C. longipetiolata*, *C. polyantha*) or inflorescence shape (*C. lehmannii*, *C. cylindrostachys*). Only two Bolivian *Cranichis* (*C. garayana*, *C. pulvinifera*) are characterized by a distinctly 3-lobed lip with irregularly erose to erose-lancinate margins. The lip form and ornamentation, together with shape, size and ornamentation on the margins of the tepals, can be used to identify most species in this genus in Bolivia. Petals of Bolivian *Cranichis* are 1-veined, whereas sepals can be 1-, 2-, 3- or 5-veined. Tepals can be glabrous or variously ciliate, papillate or pilose. While extensive studies on *Cranichis* (e.g. Kolanowska and Szlachetko 2014; Szlachetko and Kolanowska 2019) indicate that the number of veins is constant within a species we noted a variation in venation in Bolivian populations, which correspond to *C. badia*. There is a possibility that they represent an

undescribed species of *Cranichis*, but we prefer not to delimit any new species based solely on the number of veins.

Similar to many other new species of orchids (e.g. Averyanov et al. 2015; Baquero et al. 2018; Lin et al. 2020) the new taxa described here are based on single specimens. This is not surprising as numerous tropical orchids are local endemics and their geographical ranges are often limited to small patches of forests or single valleys (Koopowitz 1992; Vermeulen and Lamb 2011). Among the numerous factors that affect the geographical distribution and diversity of orchids (Dodson 2003), local radiations resulting in the evolution of numerous species of orchids in relatively small areas seems to be the most important (e.g. Jost 2004). Describing new species based on limited data is obviously risky as there is no information on the intraspecific variation in such taxa. However, considering the ongoing loss of habitats for orchids (Wraith et al. 2020) it is important to identify novel orchids before they go extinct (Swarts and Dixon 2009; Vermeulen et al. 2014), even though based on incomplete data on their morphological variation.

Conclusions

Here we present synopsis of Bolivian species of *Cranichis*. Morphological characteristics of all the species together with the illustrations of the perianth segments and the identification key will be useful for local botanists collecting orchids. The proper documentation of the distribution of rare and endangered orchids in Bolivia will help in the establishment of more advanced nature management programs.

The occurrence of 12 species of *Cranichis*, including two new species and two new records, in Bolivia was confirmed. We did not validate the presence of *C. diphylla*, *C. lehmannii* and *C. muscosa* in this country. Moreover, in our opinion, the previously published Bolivian record for *C. polyantha* is doubtful and the specimen collected is an undescribed species. However, due to incongruities between the original description of *C. polyantha* and concept of this orchid presented by Garay (1978) and Bennett and Christenson (1995), we prefer not to delineate a new taxon.

Acknowledgements

We are grateful to Carla Maldonado for her assistance during our studies on the orchid flora of Bolivia. This work was supported by the Ministry of Education, Youth and Sports of CR within the CzeCOS program, grant number LM2018123 and by the National Science Centre, Poland (PRELUDIUM nr 2016/21/N/NZ8/01601). We would like to thank Anna Król and Natalia Olędryńska for preparing some of the line drawings presented in this paper.

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Supplementary material I

Annex1

Authors: Kolanowska et al.

Data type: Collections list

Explanation note: Herbarium collection list

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Link: <https://doi.org/10.3897/phytokeys.186.71499.suppl1>

Taxonomic studies on the genus *Isotrema* (Aristolochiaceae) from China III: *I. pseudohei*, a new species from Yunnan, Southwest China

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Abstract

Isotrema pseudohei, a new species from Yunnan, Southwest China, is described and illustrated. It is morphologically similar to *I. hei* and *I. moupinense*, but differs from the former in the colour of flower and throat, the size of throat and the shape of gynostemium lobes, and from the latter in the shape of lamina and gynostemium lobes.

Keywords

Aristolochia, clarification, morphology, taxonomy

Introduction

Isotrema Raf. was previously treated as a subgenus of *Aristolochia* L. and was recently reinstated as an independent genus base on both molecular data and morphological evidence (Zhu et al. 2019a). It can be distinguished from the genus *Aristolochia* in the strongly curved perianth with a 3-lobed limb, and especially the 3-lobed gynostemium with a pair of anthers on the outer surface of each gynostemium segment. Comprising 109 species,

† These authors contributed equally to this work.

the genus mainly occurring in East and South Asia, with a few species disjunctly distributed in North and Central America (Zhu et al. 2019a). Currently, 71 species of *Isotrema* are recorded in China, of which 57 are endemic (Hwang et al. 2003; Lai et al. 2019; Li et al. 2019; Peng et al. 2019; Zhou et al. 2019; Zhu et al. 2019a, 2019b, 2019c, 2019d, 2019e; Cai et al. 2020a, 2020b; Luo et al. 2020; Wang et al. 2020a, 2020b; Liao et al. 2021).

During a field expedition to Yunnan, Southwest China, some unknown specimens of *Isotrema* were discovered. After careful comparison with previously known species and study of related literature (Hwang 1988; Ma 1989a, 1989b; Tao 1997; Hwang et al. 2003; Do et al. 2015; Do and Nghiem 2017; Yang et al. 2018; Zhu et al. 2019a, 2019d; Cai et al. 2020a, 2020b; Wang et al. 2020a, 2020b), we confirm it as a new species, describe and illustrate it below.

Material and methods

Specimens of *Isotrema* from 36 herbaria (A, BM, BR, CDBI, CSFI, CSH, E, EMA, GXMI, HAST, HENU, HHBG, HIB, HITBC, IBK, IBSC, K, KUN, L, LBG, LE, NAS, NTUF, P, PE, PEM, QTPMB, SM, SNU, SYS, TAI, W, WCU, WU, WUK, YUKU; acronyms follow Thiers 2021) and our collections in the field all over Asia were examined. Meanwhile, the literature, in particular the protologues of published names, was collated and reviewed. The geographic distribution has been compiled from literatures and complemented by the analyzed specimens and color photos. Photographs of plants were taken in the wild for each species and the terminology of description was mainly based on the Flora of China (Hwang et al. 2003).

Taxonomy

Isotrema pseudohei X.X.Zhu, Jun Wang bis & G.D.Li, sp. nov.

urn:lsid:ipni.org:names:77234089-1

Figures 1–3, 4A–C

Type. CHINA. Yunnan: Yuxi City, Xinping County, near Atizuo Village, 101°55'51"E, 23°58'46"N, 1823 m a.s.l., 11 September 2018, X. X. Zhu et al. ZXX18249 (holotype: CSH [CSH-0157854!]; isotypes: CSH!, KUN!).

Diagnosis. Similar to *Isotrema hei* Lei Cai & X.X.Zhu and *I. moupinense* (Franch.) X.X.Zhu, S. Liao & J.S.Ma, but significantly differs in the following characters: laminas lanceolate to narrowly lanceolate, basal tube of calyx ca. 1.5 cm long, inside dark purple at base and yellowish white above base; upper tube of calyx ca. 2.3 cm long, inside yellowish white, getting yellow in upper portion; inner surface of limb yellow with purplish red patches; throat yellow, suborbicular, 7–9 mm in diameter; apex of gynostemium lobes acute.

Description. Climbing shrubs. Stems terete, brown pubescent when young, old branchlets glabrous. Petioles 1–2 cm long, appressed villous; laminas lanceolate to nar-

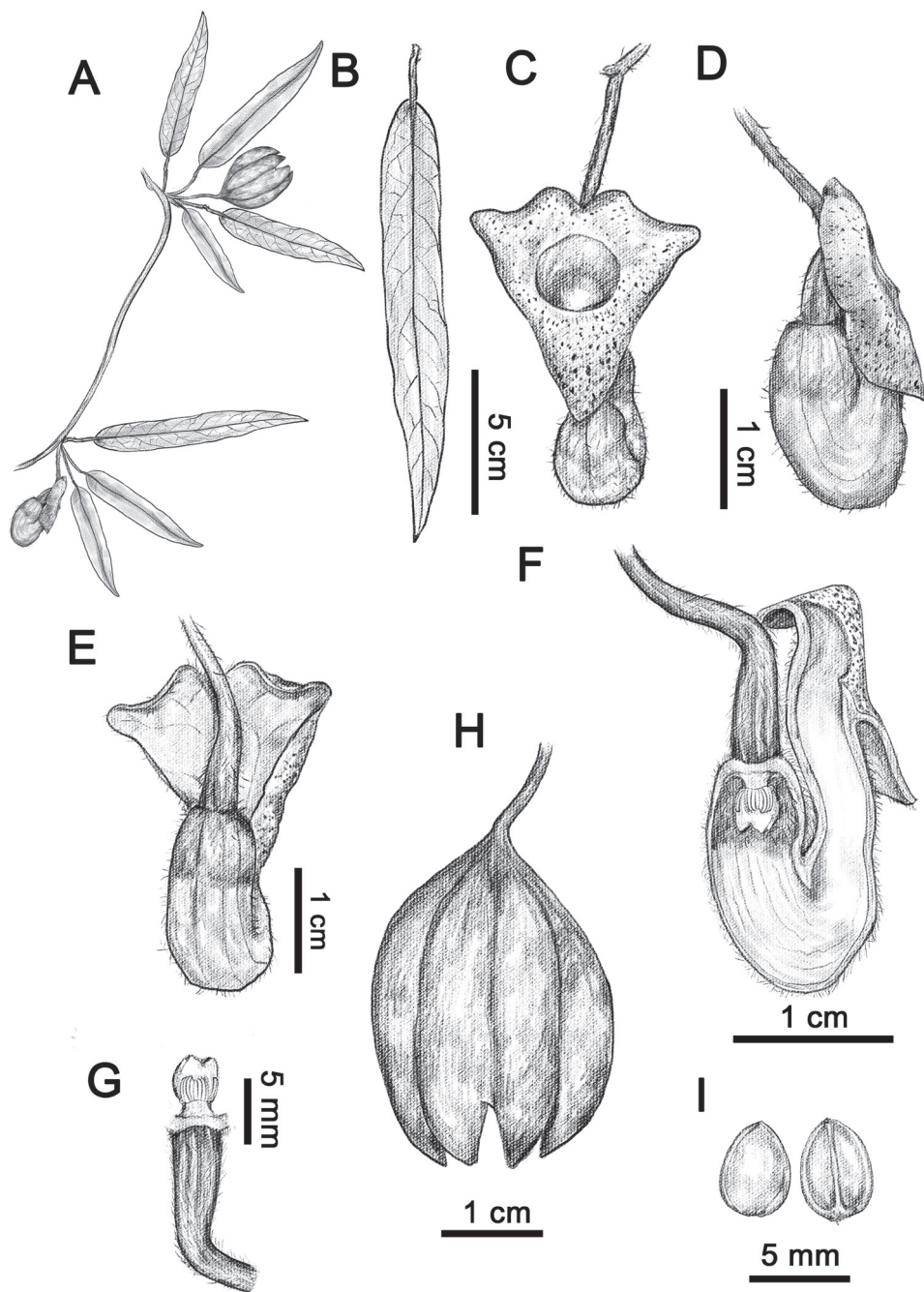


Figure 1. Line drawing of *Isotrema pseudohei* X.X.Zhu, Jun Wang bis & G.D.Li **A** habit **B** leaf **C** flower (frontal view) **D** flower (lateral view) **E** flower (dorsal view) **F** dissected flower (showing the inside structure) **G** anthers and gynostemium **H** dehiscent capsule **I** seeds. Illustrated by Shi-Zhen Qiao.

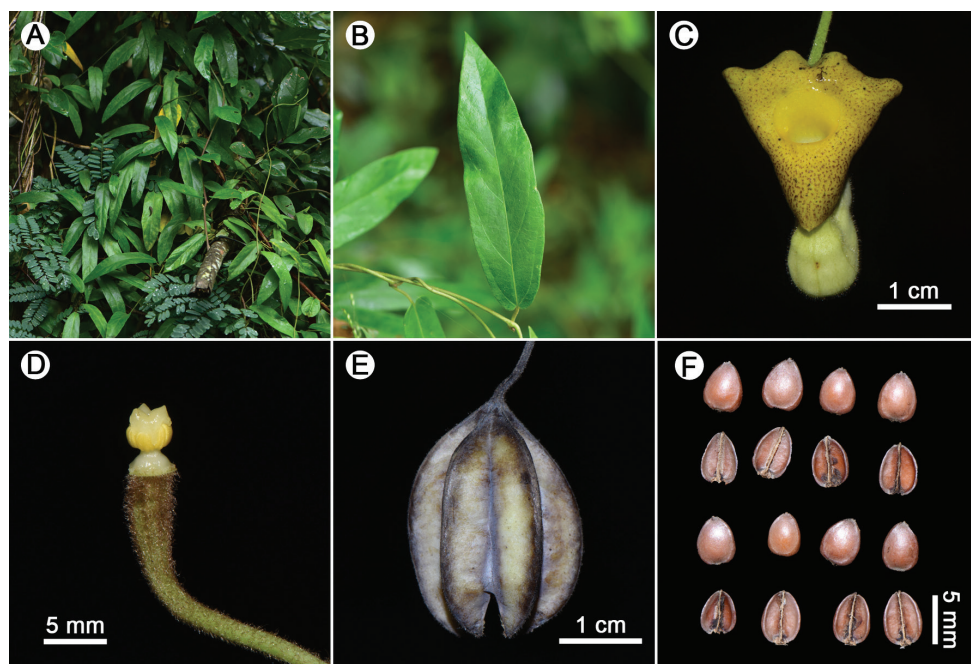


Figure 2. *Isotrema pseudohei* X.X.Zhu, Jun Wang bis & G.D.Li from the type locality **A** habit **B** leaves **C** flower (frontal view) **D** anthers and gynostemium **E** fruit **F** seeds. Photographed by Xin-Xin Zhu.

rowly lanceolate, 6.5–17.5 × 2.6–4.5 cm, base round to shallowly cordate, margin entire, apex acute, adaxially sparsely pubescent, abaxially densely villous, especially on veins, lateral veins 4–6-paired. Flower solitary, axillary or on stems; pedicels ca. 3 cm long, densely rusty villous; bractlet 1, lanceolate to elliptic, 3–4 mm long, adaxially subglabrous, abaxially densely rusty villous, inserted on middle part of pedicel. Calyx tube geniculately curved, abaxially yellowish white, densely villous; basal tube ca. 1.5 cm long, inside dark purple at base and yellowish white above base; upper tube ca. 2.3 cm long, inside yellowish white, getting yellow in upper portion; limb discoid, ca. 2.1 cm wide, shallowly 3-lobed, lobes broadly triangular, inner surface yellow with purplish red patches; throat suborbicular, yellow, 7–9 mm in diameter. Anthers 6, oblong, ca. 1.6 mm long, adnate in 3 pairs to base of gynostemium, opposite to lobes. Gynostemium 4–5 mm long, 3-lobed, apex of lobes acute. Ovary terete, ca. 8 mm long, densely brown villous. Capsule cylindric, six arrises, ca. 3 × 2.2 cm. Seeds ovate to elliptic, 3.5–5 × 3–4.5 mm, adaxially deeply concave, abaxially convex, glabrous on both sides.

Phenology. Flowering and fruiting specimens of the new species were collected in September.

Etymology. The specific epithet refers to the similarity between the new species and *Isotrema hei* in the morphology of lamina and flower. The Chinese name is given as “拟何氏关木通”.

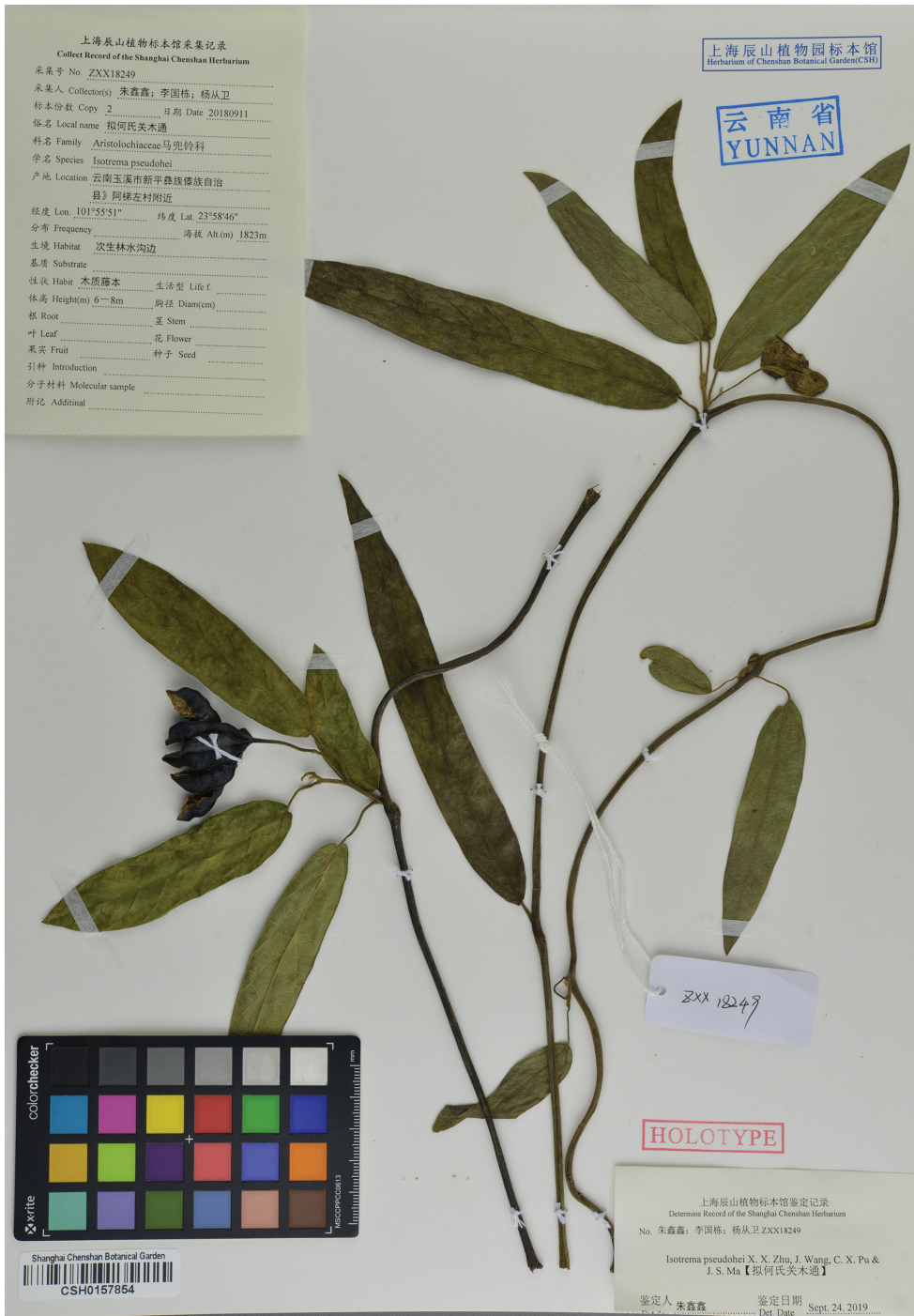


Figure 3. Holotype of *Isotrema pseudohei* X.X.Zhu, Jun Wang bis & G.D.Li. (CSH-0157854).

Distribution and habitat. *Isotrema pseudohei* is distributed in Xinping County of Yunnan Province, China. It grows on the roadside or in mixed forests on sunny slopes at an elevation between 1820 m and 2045 m, together with other taxa as *Castanopsis* sp. (Fagaceae), *Commelina undulata* R. Br. (Commelinaceae), *Disporum* sp. (Colchicaceae), *Lycianthes macrodon* (Wall.) Bitter (Solanaceae), *Rubus* sp. (Rosaceae), etc.

Preliminary conservation status. *Isotrema pseudohei* is currently only known from two populations in Xinping County, with fewer than five individuals at each site. Based on the present study, its Extent of Occurrence (EOO) is less than 100 km² and the known Area of Occupancy (AOO) is less than 20 km². Furthermore, the habitat is being destroyed by road construction and small-scale agriculture. Besides, the root of *Isotrema* itself is often harvested for medicinal purposes by the locals. Although further investigation is necessary to fully map its distribution, it is considered to be Vulnerable (VU) status, based on the criteria D2 of IUCN according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee 2019).

Additional specimens of *Isotrema pseudohei* examined (paratypes). CHINA. Yunnan: Yuxi City, Xinping County, 2045 m a.s.l., 11 September 2018, X. X. Zhu et al. ZXX18250 (CSH, KUN).

Specimens of *Isotrema hei* examined. China. Yunnan: Wenshan City, 12 May 2018, X. X. Zhu ZXX18072 (CSH), 20 April 2018, Lei Cai et al. CL115 (KUN).

Specimens of *Isotrema moupinense* examined. CHINA. Chongqing: Nanchuan District, 27 May 1957, T. H. Hsiung & Z. L. Chou 91052 (IBSC), 11 July 1957, S. Y. Chen et al. 95126 (NAS), 12 July 1957, T. H. Hsiung & Z. L. Chou 91972 (IBSC), 25 July 1957, T. H. Hsiung & Z. L. Chou 92285 (IBSC), 17 May 2007, Z. B. Feng et al. 20070508 (PE, WCSBG); Sichuan: Baoxing County, 4 August 1936, K. L. Qu 3410 (IBSC), 1954, T. P. Soong, 38982 (IBSC, NAS), 20 May 1958, Sichuan Agricultural College 4867 (IBSC); Dayi County, 26 May 2015, X. X. Zhu & Z. X. Hua ZH082 (CSH), 26 May 2015, X. X. Zhu & Z. X. Hua ZH083 (CSH); Emeishan City, 1952, C. H. Hsiung et al. 30475 (IBK, IBSC, NAS), 24 May 1952, C. H. Hsiung et al. 30750 (IBSC), 6 June 1960, Sichuan Med. Pl. Exped. 12729 (NAS), 29 April 2015, X. X. Zhu & Z. X. Hua ZH046 (CSH); Jinyang County, 29 April 2013, C. Du & Y. Wang DC-325 (CSH); Luding County, 14 July 2006, X. M. Gao G06117 (WCSBG); Yingjing County, 20 May 1940, K. L. Chu 6901 (NAS); Yunnan: Gongshan County, 25 May 2011, J. Cai et al. 11CS2767 (KUN); Yunlong County, 24 April 2019, X. X. Zhu et al. ZXX19356 (KUN).

Discussion

Characterized by a horseshoe-shaped perianth and a 3-lobed gynostemium with each lobe consisting of two oblong stamens, the new species is shown to be a member of *Isotrema* (Zhu et al. 2019a). *Isotrema pseudohei* is similar to *I. hei* in the shape of lamina (Fig. 4A & D), but differs in the morphology of flowers, the inner surface of limb is yellow with purplish red patches and throat suborbicular, yellow, and 7–9 mm in diameter in *I. pseudohei* (Figure 4C), whereas the inner surface of limb yellowish

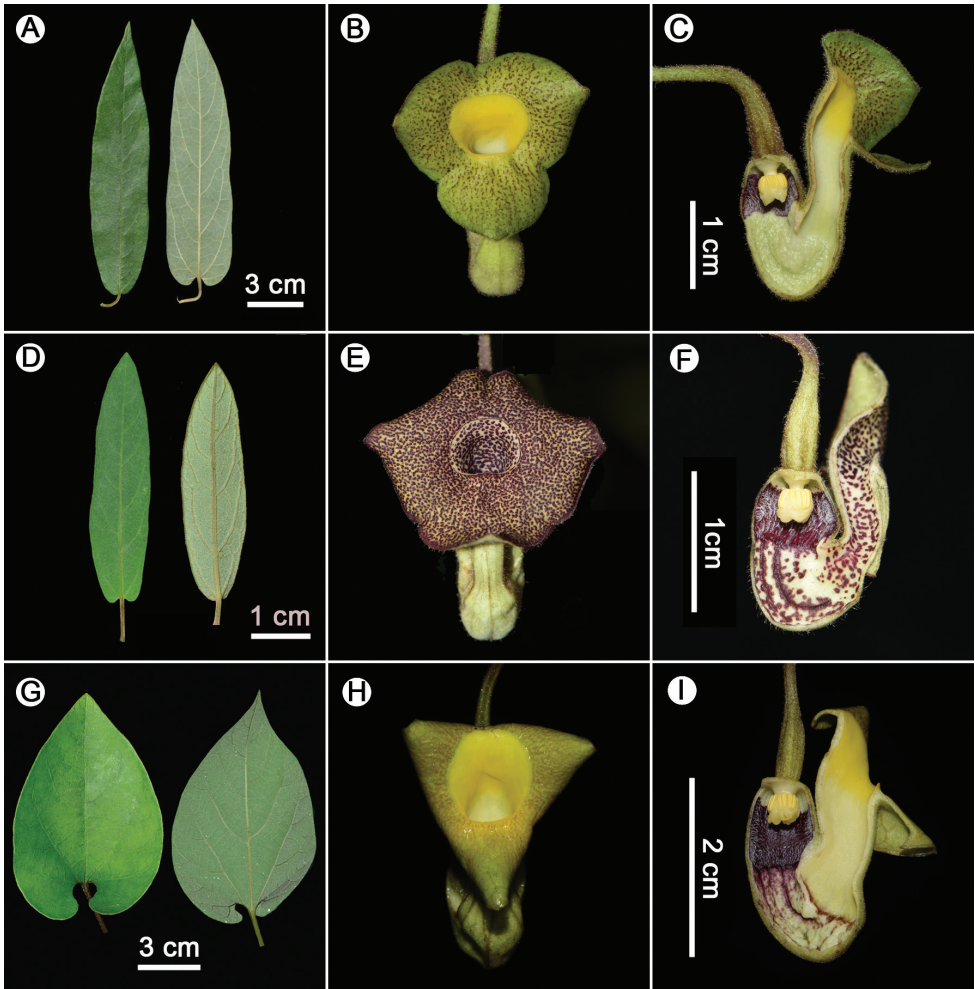


Figure 4. Morphological comparisons of the leaves and flowers of *Isotrema pseudohei* (A–C), *I. hei* (D–F) and *I. moupinense* (G–I). Photographed by Xin-Xin Zhu.

green and densely covered with purple papillae and throat circular, yellowish green with purple patches, and 4–5 mm in diameter in *I. hei* (Figure 4F). Though sharing similar morphology of flowers (Fig. 4B, C, H, J), *I. pseudohei* can be easily distinguished from *I. moupinense* by the shape of lamina, which is lanceolate in the new species (Figure 4A), but ovate in *I. moupinense* (Figure 4G). Moreover, *I. pseudohei* also differs in the gynostemium lobes acute at apex (Figures 2D & 4C), whereas round at apex in *I. hei* (Figure 4F) and *I. moupinense* (Figure 4I). Detailed morphological comparisons are provided in Table 1 and shown in Figure 4. Geographically, the new species is currently only known from Xinping County in central Yunnan, *I. hei* is restricted to Wenshan City in Southeast Yunnan, while *I. moupinense* is widely distributed in Southwest China (Figure 5).

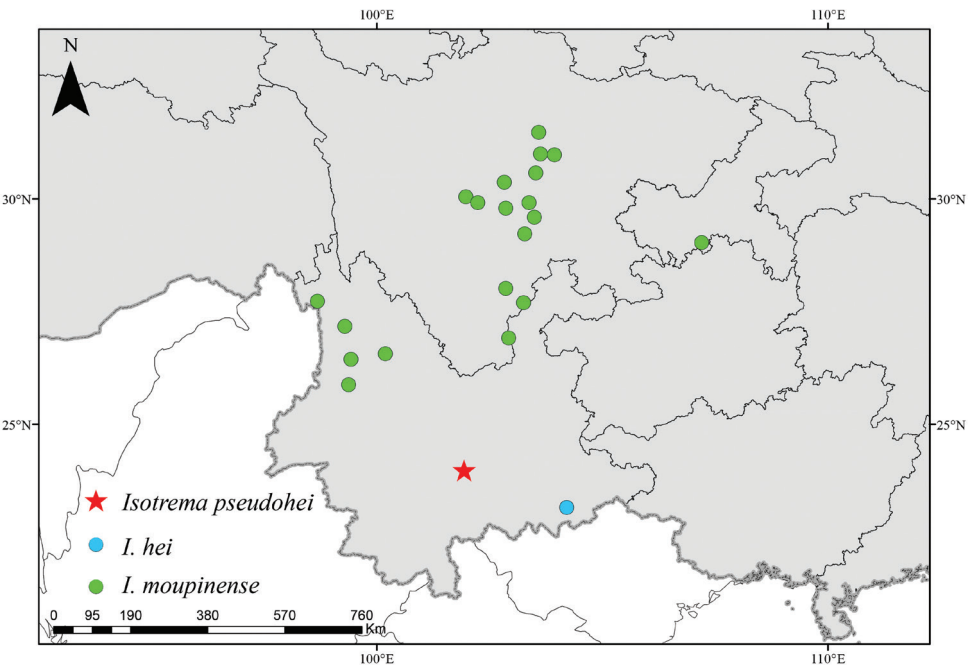


Figure 5. Distribution of *Isotrema pseudohei*, *I. hei* and *I. moupinense* based on field observation, specimens, color photos and literatures examined.

Table 1. Morphological comparisons of *Isotrema pseudohei*, *I. hei* and *I. moupinense*.

Character	<i>I. pseudohei</i>	<i>I. hei</i>	<i>I. moupinense</i>
Lamina	lanceolate to narrowly lanceolate, 6.5–17.5 × 2.6–4.5 cm	lanceolate to narrowly lanceolate or narrowly elliptic, 2.5–12.5 × 1–4.5 cm	ovate, 6–16 × 5–12 cm
Basal tube of calyx	inside dark purple at base and yellowish white above base	inside dark purple at base and yellowish white with dark purple stripes and patches above base	inside dark purple at base and yellowish with purple stripes and patches above base
Upper tube of calyx	inside yellowish white, getting yellow in upper portion	inside yellowish white with reddish purple patches	inside yellowish white, getting yellow in upper portion
Limb	ca. 2.1 cm wide; inner surface yellow with purplish red patches	ca. 2.4 cm wide; inner surface yellowish green and densely covered with purple papillae	2–2.5 cm wide; inner surface yellow to dark red, sometimes with red or yellow spots
Throat	suborbicular, yellow, 7–9 mm in diameter	circular, yellowish green with purple patches, 4–5 mm in diameter	circular, yellow, 8–11 mm in diameter
Gynostemium	apex of lobes acute	apex of lobes round	apex of lobes usually bilobate, rarely obtuse

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Notes on Brazilian *Pachira* (Malvaceae, Bombacoideae) II: Additional typifications and new synonymies

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Abstract

The typification and status of the names of 14 species of *Pachira* (Malvaceae: Bombacoideae) found in Brazil are discussed, including type material from Brazil, the Guianas, Colombia, Venezuela, and cultivated in Algeria. We designate 11 lectotypes, three neotypes, and four epitypes for these names. Six names are newly considered to be synonyms of the species accepted here. The results support a forthcoming taxonomic treatment of *Pachira* for the Flora of Brazil.

Keywords

Bombacoideae, *Bombax*, Brazil, *Carolinea*, Malvaceae, *Pachira*

Introduction

Pachira Aubl. is the most species-rich genus among the 17 genera of Bombacoideae (Malvaceae) (Carvalho-Sobrinho et al. 2016) and it consists of trees distributed primarily in wet forest in northern South America (Robyns 1963; Alverson 1994; Fernández-Alonso 1998, 2003; Carvalho-Sobrinho et al. 2014). About one-hundred names have been published in *Pachira* (IPNI 2021; Tropicos 2021), but they probably

represent only about 50 species in this genus. The genus was last revised by Robyns (1963). Since then, generic concepts have changed radically and a contemporary revision of *Pachira* would resolve not only questions about species richness, but also clarify evolutionary relationships and biogeography.

Pachira includes several Neotropical taxa originally described in other genera, including *Bombacopsis* Pittier, *Bombax* L. (Carvalho-Sobrinho and Dorr 2020), *Carolinea* L. f., *Pochota* Ram. Goyena, *Pseudobombax* Dugand, and *Rhodognaphalopsis* A. Robyns (Cuatrecasas 1954; Robyns 1963, 1967; Steyermark and Stevens 1988). During the preparation of a taxonomic treatment of *Pachira* for the Flora of Brazil 2020 project (<http://floradobrasil.jbrj.gov.br>), we encountered several names in *Bombax*, *Carolinea*, *Pachira*, and *Rhodognaphalopsis* that require typification and/or clarification.

Herein we discuss the typification and status of the names (and some of their synonyms) of 14 species of *Pachira* found in Brazil. Type material is from Brazil (including Brazilian material cultivated in Algeria), the Guianas, Colombia, and Venezuela. We designate 11 lectotypes, three neotypes, and four epitypes for these names. Notably, an epitype is designated here for *P. aquatica* Aubl., the type of the genus. Six names are newly considered to be synonyms of the species accepted here. Most significantly, *P. nitida* Kunth, a name often used in checklists of the Brazilian flora (see e.g., Duarte 2010; BFG 2015), is revealed to be a synonym of *P. minor* (Sims) Hemsl. Similarly, *P. dolichocalyx* A. Robyns, previously considered to be endemic to French Guiana, is shown to be a synonym of *P. macrocalyx* (Ducke) Fern. Alonso that was described from Brazil.

Materials and methods

Protologues of names of *Pachira* taxa found in Brazil (and their basionyms) were examined along with relevant revisionary and floristic literature in order to determine what constituted original material, the identities of these taxa, and to establish whether these names had been typified. The specimens cited as types were either examined by us in person or via digital proxies. Herbarium acronyms for these specimens follow Thiers (2021). The notation “F neg. no.” refers to the “Berlin Negatives” of the Field Museum (F), a unique type-photographic collection of European herbaria that J. Francis Macbride began assembling in 1929 (<https://www.fieldmuseum.org/node/5186>). These photographic images frequently are the only records of types or original material that was destroyed during WWII and these images are commonly found in northern hemisphere herbaria.

For the sake of brevity, we do not provide complete synonymies for the *Pachira* taxa that we discuss, but rather list only names that have not been, or were previously incorrectly, typified. In a few instances, we include names that were typified earlier, but whose citations require clarification. We also list names of taxa that are considered here to be new synonyms irrespective of their typification status.

Typification

***Pachira aquatica* Aubl., Hist. Pl. Guiane 2: 726, tt. 291, 292. 1775.**

Figs 1, 2A

Carolinea princeps L. f., Suppl. Pl.: 314. 1782 [1781], nom. illeg. superfl. *Pachira carolinea* Dum. Cours., Bot. Cult., ed. 1, 3: 84. 1802, nom. illeg. superfl. *Bombax aquaticum* (Aubl.) K. Schum., in Engler & Prantl, Nat. Pflanzenfam. 3(6): 62. 1895.

Carolinea pompalis Moc. & Sessé ex DC., Prodr. 1: 478. 1824, nom. nud., pro syn.

Pachira grandiflora Tussac, Fl. Antill. 4: 12, tt. 3, 4. 1827. *Carolinea grandiflora* (Tussac) Spach, Hist. Nat. Vég. 14: 206. 1847 [1848]. Type: "ANTILLES." Lectotype, designated by Yoshikawa et al. (in press): Tussac (1827, t. 3).

Carolinea macrocarpa Schltdl. & Cham., Linnaea 6: 423. 1831. *Pachira macrocarpa* (Schltdl. & Cham.) Walp., Repert. Bot. Syst. 1(2): 329. 1842. *Pachira longifolia* Hook., Bot. Mag., ser. 3, 6: t. 4549. 1850, nom. illeg. superfl. *Bombax macrocarpum* (Schltdl. & Cham.) K. Schum., in Engler & Prantl, Nat. Pflanzenfam. 3(6): 62. 1895. Type: MEXICO. Veracruz, Papantla, Feb 1829 (lf, fl bud), *F. Deppe & C.J.W. Schiede 1312* (lectotype, designated by Yoshikawa et al. (in press): BM barcode 000645670 [s.n.]; isolectotypes: HAL barcode 0098398, HAL barcode 0128027 (2 sheets), HAL barcode 0128028 [s.n.] (2 sheets), LE n.v., W n.v.).

Carolinea lovisa-carolina L. ex B.D. Jacks., Index Linn. Herb.: 52. 1912, nom. nud.

Type. FRENCH GUIANA. Cayenne, 1775 (lf), *F. Aublet s.n.* (lectotype, as "holotype," designated by Robyns 1963, pp. 238, 242: BM barcode 000645671). Epitype: FRENCH GUIANA. Tour de l'Île River, 0–10 m, 04°47' 36"N, 052°22' 38"W, 18 Oct 1991 (lf, fl), *S.A. Mori et al. 22126* (epitype, designated here: US barcode 00636701; isoepitypes: CAY barcode 155330, NY barcode 402480).

Note. Yoshikawa et al. (in press) typified several names that are synonyms of *Pachira aquatica*, but omitted mention of *nomina nuda*. Despite their lack of nomenclatural standing, these *nomina nuda* associated with *P. aquatica* do appear in standard indices (e.g., IPNI 2021; Tropicos 2021) and we include them to clarify their identification. Likewise, we discuss two names typified by Yoshikawa et al. (in press) in order to clarify their synonymies and bibliographic citations that are confused in standard indices.

The lectotype of *Pachira aquatica* consists solely of detached leaflets and a sterile twig. Consequently, a flowering collection from French Guiana is designated here as an epitype (Fig. 1).

"*Carolinea pompalis fl. mex. ic. ined.*" was cited originally as a synonym of *C. minor* Sims (≡ *Pachira minor* (Sims) Hemsl.). Robyns (1963) did not agree with this interpretation and placed the *nomen nudum* among his "*Species dubiae incertae sedis.*" McVaugh (2000, p. 88) discussed the sources of the material that de Candolle (1824) examined, which included a plate in G (see F neg. no. 30513) and presumably another illustration now in the Torner Collection (Hunt Institute for Botanical Documentation acces-



Figure 1. Epitype of *Pachira aquatica* Aubl. (US barcode 00636701).

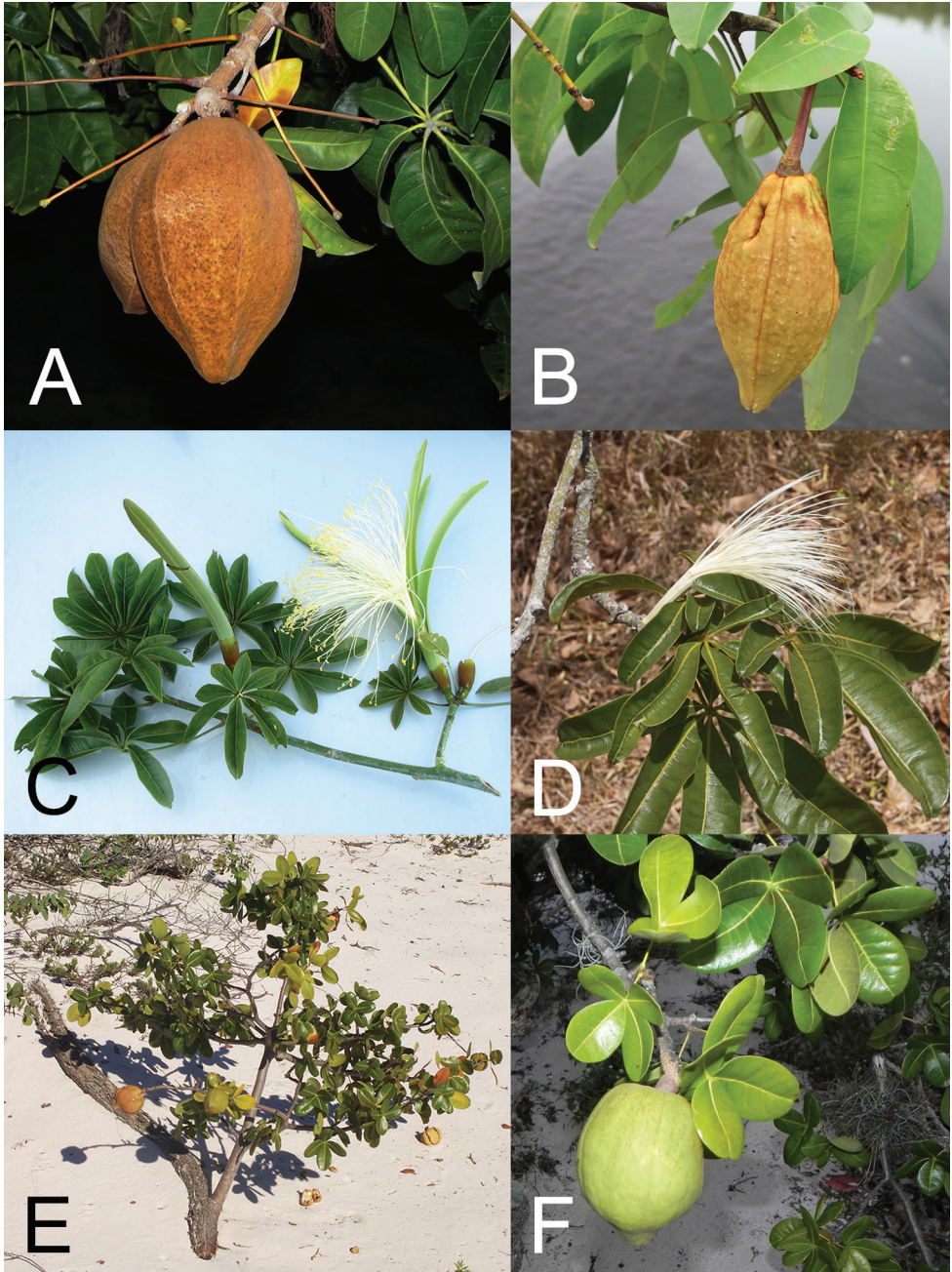


Figure 2. *Pachira* species **A** *P. aquatica* fruit **B** *P. duckei* fruit **C** *P. endecaphylla* leaves and flower **D** *P. glabra* leaves and flower **E** *P. retusa* habit **F** *P. retusa* leaves and fruit. Photographs **A**, **D** J.G. Carvalho-Sobrinho **B** C.E. Zartman **C** B.A.S. Pereira **E** M.C. Machado **F** L.P. Queiroz.

sion No. 6331.0864; see also No. 6331.1977). These images depict *P. aquatica*, which occurs in Mexico and South America. *Pachira minor* is restricted to South America, which was not visited by the Sessé and Mociño Expedition.

Several indices (e.g., IPNI 2021; Tropicos 2021) state incorrectly that the combination *Carolinea grandiflora* (Tussac) Spach was made in 1834, but Spach (1834, p. 426) did not then definitely associate the epithet “*grandiflora*” with the genus *Carolinea* stating simply “*Carolinéa* de Tussac. – *Pachira grandiflora* Tussac” (see Turland et al. 2018; Art. 35.2). The combination was made in 1847 in an index to the larger work when Spach (1847, p. 206) wrote “[*Carolinea*] *grandiflora*, Tuss. .. [Vol.] 3 [Pag.] 426”: the volume and page numbers providing an indirect reference to the basionym (see Turland et al. 2018; Art. 41.3).

Carolinea macrocarpa was described from Mexican material cultivated in Berlin. The protologue gives the type locality as “*Ad ripas fluminum et rivulorum Papantlensium et Tecolutensium. Papantlae.*” Robyns (1963, p. 239) selected “*Schiede et Deppe 1312*” as “*typus*” and he cited specimens at BM, LE and W, but not HAL. The labels on the HAL specimens, however, suggest the collecting combination may have been “*Deppe & Schiede*” and not “*Schiede & Deppe.*”

Pachira longifolia is a superfluous name for *P. macrocarpa* ($\equiv$ *Carolinea macrocarpa*). The epithet of this superfluous name is often miscited as “*longiflora*” (see e.g., Robyns 1963; Alverson in Berry et al. 1997), which is perhaps understandable given that Hooker (1850) called it the “Long-flowered *Pachira*.”

***Pachira calophylla* (K. Schum.) Fern. Alonso, Anales Jard. Bot. Madrid 56: 308. 1998.**

Bombax calophyllum K. Schum., in Martius, Fl. Bras. 12(3): 227. 1886. *Bombacopsis calophylla* (K. Schum.) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 201. 1963.

Bombax stenopodum Ulbr., Notizbl. K. Bot. Gart. Mus. Berlin 6: 55. 1914. Type: BRAZIL. Rio de Janeiro, s.d. (lf, fl), *L. Riedel s.n.* (neotype, designated here: LE barcode 00003676; isoneotype: LE barcode 00003677; possible isoneotype: S-PLE-E4208 n.v.).

Type. BRAZIL. Rio de Janeiro, s.d. (lf, fl), *L. Riedel s.n.* (lectotype, designated here: LE barcode 00003676; isolectotype: LE barcode 00003677; possible isolectotype: S-PLE-E4208 n.v.).

Note. Robyns (1963, p. 203) designated “*Riedel s.n. (LE)*” as lectotype of *Bombax calophyllum*. When Fernández-Alonso (1998) made the combination in *Pachira*, he accepted Robyns’s type designation, but stated that he had not seen the lectotype. Robyns annotated the two Riedel specimens in St. Petersburg (LE) cited above as “*Bombacopsis calophylla* (K. Schum.) A. Robyns, *comb. nov.*” and wrote “*lectotypus*” on the one specimen that has a handwritten label indicating the type locality as “*Brasiliae: R. Janeiro,*” locality information that matches the protologue. The ICN (Turland et al. 2018; Art.

7.10) requires that a type designation be effectively published and the mere annotation of a herbarium sheet does not meet this requirement. Our lectotypification (second step; see Turland et al. 2018; Art. 9.17) narrows the type to a single element, a gathering that has loose leaves and a fragmented flower (calyx, ovary, and androecium). The isolectotype is sterile.

The protologue of *Bombax stenopodum* cites a single collection, “E. Ule n. 4631,” which was deposited in Berlin (B†; see F neg. no. 9545). Inasmuch as the Berlin specimen was destroyed in WWII and no duplicate material has been found, we designate a neotype for this name. Our choice makes this name an obligate synonym of *Pachira calophylla*.

***Pachira duckei* (A. Robyns) Fern. Alonso, Anales Jard. Bot. Madrid 56: 310. 1998.**
Fig. 2B

Rhodognaphalopsis duckei A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 275, fig. 14. 1963.

Rhodognaphalopsis duckei var. *obtusifolia* A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 278. 1963, **syn. nov.** Type: BRAZIL. Amazonas, Marmellos, Rio Madeira, Mar 1902 (lf, fl), *E. Ule* 6077 (holotype: G barcode 00177546; isotypes: K barcode 000382340, L barcode 0012900, RB barcode 00534490, RB barcode 00534520).

Type. BRAZIL. Amazonas, Manaus, Igarapé da Raiz, 30 Aug 1946 (lf, fl), *A. Ducke* 310 (IIa. col.) (holotype: MG barcode 018577 [= MG n.° 18.577]; isotypes: IAC 13840, IAN, SP barcode SP 003366).

Note. When Robyns (1963) described *Rhodognaphalopsis duckei* and *R. d.* var. *obtusifolia* he had very little material available. In fact, he cited fewer than a dozen collections for both taxa. The characters that he used to distinguish var. *obtusifolia* from the nominate variety were subtle and overlap with specimens he cited as paratypes of the latter. We cannot distinguish the obtuse leaflet bases of *Ducke s.n.* [RB 23484] (see e.g., K barcode 000913930), a paratype of *R. duckei*, from those of *Ule* 6077 (see e.g., K barcode 000382340), the type of *R. d.* var. *obtusifolia*. For this reason and because the two taxa lack discrete ranges, we propose these names should be treated as synonyms.

***Pachira endecaphylla* (Vell.) Carv.-Sobr., Taxon 62: 816. 2013.**
Fig. 2C

Bombax endecaphyllum Vell., Fl. Flumin.: 288. 1829 [1825], Ibid., Fl. Flumin. Icones 7: t. 50. 1831 [1827]. *Pseudobombax endecaphyllum* (Vell.) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 60. 1963.

Pachira stenopetala Casar., Nov. Stirp. Bras.: 21. 1842. *Bombax stenopetalum* (Casar.) K. Schum., in Martius, Fl. Bras. 12(3): 226, t. 45. 1886. *Bombacopsis stenopetala*

(Casar.) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 221. 1963. Type: BRAZIL. “Occurrit passim prope Rio de Janeiro,” s.d. [Nov 1838] (fl, fl), *Casaretto Herb. No. 581* (lectotype, designated by Delprete et al. 2019, p. 802): TO [sheet No. 2] n.v.). *Pachira elegans* Hooibr. ex Planch., Hort. Donat. 23. 1858, **syn. nov.** Type: Hort. Paris, 1854 [lf], without collector (lectotype, designated here: MPU barcode 761966).

Type. Lectotype, designated here: “Monad. Polyand. *BOMBAX endecaphyllum* tab. 50” (original pen and ink drawing for the *Flora Fluminensis* preserved in the Manuscript Section, Biblioteca Nacional do Brasil, Rio de Janeiro [cat. no.: mss1198656_054]). Epitype: BRAZIL. Espírito Santo, Santa Leopoldina, Morro Agudo, propr. Dona Maria, 20°05'51"S, 40°25'44"W, 28 Jan 2008 (lf, fl), V. Demuner et al. 4907 (epitype, designated here: HUEFS; isoeotypes: MBML, HVASF n.v.).

Note. Carvalho-Sobrinho et al. (2013) accepted the “lectotype” designation of Robyns (1963, p. 61) for the name *Bombax endecaphyllum*, but Robyns selected one of the plates published in the *Flora Fluminensis Icones* (1831), which is not original material and thus his designation can be superseded (Turland et al. 2018; Art. 9.19(a)). The published Vellozo plates were prepared and issued posthumously and there is no evidence that Vellozo ever saw them. Original pen and ink drawings that serve as the basis for these published plates, however, are archived in the Biblioteca Nacional do Brasil, Rio de Janeiro (see http://objdigital.bn.br/acervo_digital/div_manuscritos/mss1198656/mss1198656.htm).

Delprete et al. (2019) re-examined the typification of *Pachira stenopetala* and determined that the holotype cited by Carvalho-Sobrinho et al. (2013) did not agree with the protologue and they further established that the only original material available is the specimen in the Casaretto Herbarium (TO) that they designated as lectotype.

The specimen designated here as lectotype of *Pachira elegans* is labeled “*Pachira? elegans*, Daniel” in Planchon’s hand. It was collected in 1854 before the name was published and it is now in the Herbarium Planchon (MPU). It therefore can be considered original material (see Turland et al. 2018; Art. 9.4(a)) even though it is labeled “Hort. Paris” and not Hortus Donatensis. In addition, although the specimen is sterile the description of setiform mucros terminating leaflet apices matches the physical specimen. The protologue also cites “Brésil. Rio de Janeiro, *Gaudich.* in Herb. Mus. Par.,” which almost certainly is a reference to two collections made by Ch. Gaudichaud that have leaves that are superficially similar to *Pachira endecaphylla*. These collections (*Gaudichaud 945* and *945bis*) were discussed in detail by Carvalho-Sobrinho et al. (2013) who considered them both to be mixtures and who identified their flowers as *Pseudobombax grandiflorum* var. *majus* A. Robyns and their leaves as *Jacaratia spinosa* (Aubl.) A. DC. (Caricaceae). Since flowers are not mentioned in the protologue of *Pachira elegans* and the leaflets of the Gaudichaud collections do not have setiform mucros (and are from a different plant family), we do not think either Gaudichaud collection is suitable as a lectotype. Planchon (1858, p. 232) identified “Daniel” as the horticulturist Daniel Hooibrenk (1813–1895).

***Pachira glabra* Pasq., Rendiconto Reale Accad. Sci. Fis. 7: 18. 1868.**

Fig. 2D

Bombax glabrum (Pasq.) A. Robyns, Bull. Jard. Bot. État Bruxelles 30: 474. 1960.*Bombacopsis glabra* (Pasq.) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 207.1963. *Pochota glabra* (Pasq.) Bullock, Kew Bull. 20: 528. 1966.*Pachira oleagina* Decne., Ann. Gén. Hort. 23: 49. 1881? [1880]. *Bombax oleaginum*

(Decne.) A. Robyns, Bull. Jard. Bot. État Bruxelles 29: 26. 1959. Type: ALGERIA.

Jardin du Hamma, près d'Algers, 1879 (lf, fl), *Ch. Rivière s.n.* (neotype, desig-

nated here: P barcode 02286303; isoneotypes: P barcode 02286301, P barcode

02286302 [= F neg. no. 35351]).

Bombax anisophyllum Buxb., Oesterr. Bot. Z. 73: 121. 1924. Type: BRAZIL. Paraná,Antonina, 1904 (lf, fl), *M. Wacket s.n.* (lectotype, designated here: WU 0073137;

isolectotypes: W barcode 19240009283, WU 0073138).

Type. ALGERIA. Jardin du Hamma, près d'Algers, 1879 (lf, fl), *Ch. Rivière s.n.* (neotype, designated here: P barcode 02286303; isoneotypes: P barcode 02286301, P barcode 02286302 [= F neg. no. 35351]).**Note.** Robyns (1960) established that there is no extant original material of *Pachira glabra*. He then neotypified (first step) the names *P. glabra* and *P. oleaginea* with the same collection (i.e., “Rivière s.n.”). This collection is represented by three sheets in Paris (P), all three of which were labeled “Neotypus” by Robyns. The three sheets are not marked as being part of the same specimen and we interpret them as duplicates of a single gathering (see Turland et al. 2018; Art. 8.3). Accordingly, we designate here one of the three sheets as the neotype (second step) for the names *P. glabra* and *P. oleaginea*. One of the isoneotypes (P barcode 02286301) has a faint pencil sketch of the fruit that was labeled by Decaisne “fructus maturus magnif. nat.”The labels on the neotype and one of the two isoneotypes indicate that the collection was made by “Cl. Ch. Rivière” where “Cl.” is Latin for *clarissimus* (i.e., distinguished). The latter label also has the initial “A.” added below this name, presumably a reference to A. Rivière who, however, was deceased before the collection was made. The horticulturists and brothers [Marie] Auguste Rivière (1821–1877) and Charles Marie Rivière (b. 1845) were successive directors of the Jardin du Hamma, the latter succeeding the former (Stafleu and Cowan 1983).***Pachira humilis* Spruce ex Decne., Ann. Gén. Hort. 23: 52. 1881? [1880].***Bombax humilis* (Spruce ex Decne.) K. Schum., in Martius, Fl. Bras. 12(3): 224. 1886.*Rhodognaphalopsis humilis* (Spruce ex Decne.) A. Robyns, Bull. Jard. Bot. ÉtatBruxelles 33: 294. 1963. *Pochota humilis* (Spruce ex Decne.) Steyerl. & W.D.

Stevens, Ann. Missouri Bot. Gard. 75: 397. 1988.

Pachira humilis Spruce ex Benth., J. Proc. Linn. Soc., Bot. 6: 108. 1862, nom. nud.

Type. VENEZUELA. Amazonas, [prope] San Carlos, [ad Rio Negro], Nov 1853 (lf, fl), *R. Spruce 3135* (lectotype, as “holotypus,” designated by Robyns 1963, p. 296): K barcode 000382334 [handwritten label]; isolectotypes: BM barcode 000645664 [1853–54], BR barcode 0000006961398 [1853–54], E barcode 00285199 [1853–54; handwritten label], F barcode F0052164F [s.d.; fragment], GH barcode 00071941 [s.d.], LD barcode 730579 [1853–54], NY barcode 00133523 [1853–54], P barcode 02285964 [locality illegible; Nov 1854 (sic)], S-PLE-E4502 n.v., TCD barcode 0000900 [1853–54], W barcode 18890017581 [1853–54; handwritten label], W barcode 18890123939 [1853–54; handwritten label]).

Note. A collection, “*Pachira humilis*, Spruce, *Mss.* (exsicc. 3135),” is cited in the protologue, but Decaisne (1881) gives no explicit indication as to where he examined material. The description of this species (and others in the same article) ends with the phrase “H. Mus. Par.” Presumably this is a reference to material in the herbarium and not the gardens (“hortus”) in Paris since Ch. Rivière, Director of the Jardin du Hamma in Algeria is acknowledged by Decaisne for providing him with fresh flowers and fruit of *Pachira* species.

The synonymy and authorship of *Pachira humilis* Spruce ex Decne. have been confused because of the mistaken belief that Bentham (1862) transferred the species to *Bombax*. Bentham cited the unpublished name “*Pachira humilis*, Spruce” in a discussion regarding the distinction between *Bombax* and *Pachira*, but he did not definitely associate the final epithet “*humilis*” with *Bombax* (see Turland et al. 2018; Art. 35.2). Schumann (1886, p. 224) was the first to make the combination in *Bombax*, albeit inadvertently. He recognized “*Bombax humile*, Benth.” and he cited the basionym when he wrote “*Pachira humilis* Spruce *Msc.! in schedulis; Dcne. Miscell. bot. 1880, p. 10.*” The subordinate phrase is a reference to “*Miscellanea botanica*,” which reprints Decaisne’s (1881) article on *Bombax* and *Pachira* including the original description of *P. humilis*.

***Pachira insignis* (Sw.) Sw. ex Savigny, in Lamarck, Encycl. 4: 690. 1798.**

Carolinea insignis Sw., Prodr. 101. 1788. *Pachira loddigesii* Decne., Ann. Gén. Hort. 23: 51. 1881? [1880], nom. illeg. superfl. *Bombax insigne* (Sw.) K. Schum., in Engler & Prantl, Nat. Pflanzenfam. 3(6): 62. 1895, non Wall., Pl. Asiat. Rar. 1: 71, tt. 79, 80. 1830. *Bombax spectabile* Ulbr., Bot. Jahrb. Syst. 49: 544. 1913, nom. nov. *Carolinea affinis* Mart., Nov. Gen. Sp. Pl. 1: 85. 1826 [1824]. *Pachira affinis* (Mart.) Decne., Ann. Gén. Hort. 23: 52. 1881? [1880]. *Bombax affine* (Mart.) Ducke, Arch. Jard. Bot. Rio de Janeiro 5: 162. 1930. Type: BRAZIL. Pará, Habitat in aquaticis Archipelagi Paraënsis, s.d. (lf, fl), *C.F.P. von Martius s.n.* (lectotype, designated here: M barcode 0211673; isolectotype: M barcode 0211672).

Pachira macrantha Spruce ex Decne., Ann. Gén. Hort. 23: 47. 1881? [1880], nom. nud., pro syn.

Pachira spruceana Decne., Ann. Gén. Hort. 23: 46. 1881? [1880]. *Bombax spruceanum* (Decne.) Ducke, Arch. Jard. Bot. Rio de Janeiro 4: 126. 1925. Type: BRAZIL. Amazonas, Panure ad Rio Uaupes, Oct 1852-Jan 1853 (lf, fl), *R. Spruce 2884* (lectotype, designated here: P barcode 04694521 [= F neg. no. 35353]; isolecotypes: BM barcode 000645672, BR barcode 0000006960704, BR barcode 0000006962050, E barcode 00285198, F n.v., G n.v., K barcode 000382357, K barcode 000382358, LD barcode 1758563, LD barcode 1758627, LE n.v., NY n.v., P barcode 06715161, P barcode 06623110, P barcode 06623111, P barcode 04694522, RB barcode 00059374 (2 sheets), W n.v.).

Type. BRAZIL. Amazonas, Paraná da Eva, Rio Amazonas, abaixo de Manaus, 27 Mar 1943 (lf, fl), *A. Ducke 1211* (neotype, designated by Robyns 1963, pp. 250, 252: MO barcode 309160; isoneotypes: K barcode 000382356, MG n.v., NY n.v., R barcode 000055277, R barcode 000055277, R barcode 000055277a, S-PLE-E4290 n.v., S-PLE-E4291 n.v., S-PLE-E4292 n.v., US barcode 00101951, US barcode 00901732, US barcode 00901733).

Note. *Pachira loddigesii* is an illegitimate renaming of *Carolinea insignis* Sw. In his protologue, Decaisne (1881) cites “*Carolinea insignis*, Lodd., Bot. Cab., 1004, (*non Swartz*).” The “name” ascribed to Loddiges, however, has no standing. Loddiges (1825) simply illustrated *C. insignis* Sw. without explicitly stating that he was illustrating the species previously described by Swartz. Robyns (1963, p. 250) designated a lectotype (as “*holotypus*”) for *P. loddigesii*, but this was incorrect since the name is typified automatically by the type of the name which ought to have been adopted under the rules (Turland et al. 2018; Art. 7.5). Similarly, Turner (2016, p. 1115) argued that “*Carolinea insignis* G. Lodd.” is a valid name, even though he noted that Loddiges cited Swartz in his description and thus provided an indirect reference to *C. insignis* Sw. Turner’s lectotypification of this “name” incorrectly attributed to Loddiges also is unnecessary.

A lectotype (second step) is designated here for the name *Carolinea affinis* because although Robyns (1963, p. 251) stated the “*holotypus*” was in Munich (M) there are two sheets in that herbarium. Robyns annotated both sheets as *Pachira insignis*, but did not indicate that either was type material. The specimen with label data that matches the type locality given in the protologue is designated here as the lectotype.

We also designate here a lectotype for *Pachira spruceana*. Neither Ducke (1925) nor Robyns (1963) selected one for this name. We have chosen a specimen deposited at Paris (P) that has handwriting on Spruce’s label that matches the type locality and collecting date (“Prope Panure ad rio Uaupes, Oct 1852-Jan 1853”) cited in the protologue.

Bombax spectabile was proposed as a replacement name for *B. insigne* (Sw.) K. Schum., which is a later homonym of *B. insigne* Wall. *Bombax insigne* Wall. is a Palearctic species found in India, south-central China, and south-east Asia.

***Pachira macrocalyx* (Ducke) Fern. Alonso, Anales Jard. Bot. Madrid 56: 310. 1998.**

Bombax macrocalyx Ducke, Arch. Jard. Bot. Rio de Janeiro 4: 124. 1925. *Bombacopsis macrocalyx* (Ducke) A. Robyns, Bull. Jard. Bot. État 33: 203. 1963.

Pachira dolichocalyx A. Robyns, Bull. Jard. Bot. Belg. 58: 535, fig. 1988, **syn. nov.**
Type: FRENCH GUIANA. Piste de St. Elie, km 16, à proximité de nos carbeta bota [Sinnamary], 28 May 1980 (fl, fr), *M.F. Prévost 840* (holotype: CAY [now P barcode 0007210]; isotypes: P barcode 00077211, P barcode 05273658, U barcode 0000784, U barcode 0000785).

Type. BRAZIL. Pará, Rio Xingú, margem do Rio Tucuruhy (curso superior), 24 Aug 1919 (lf, fl), *A. Ducke s.n.* [RB 11417] (holotype: RB barcode 00534493; isotypes: B† [= F neg. no. 9536], S-PLE-E4252 n.v., S-PLE-E4253 n.v., S-R-11282, U barcode 0000771).

Note. When Robyns (1988) described *Pachira dolichocalyx* he compared it to *P. aquatica* and *P. insignis*, which according to his concept of the Bombacoideae were the only two species that comprised *Pachira*. We assume he failed to compare the material to *Bombacopsis macrocalyx* because he considered *Bombacopsis* to be a different genus.

In the protologue of *Pachira dolichocalyx*, the petals are described as greenish outside and reddish-purple inside “*in vivo*.” This information could only have come from whatever may have been inferred from examining the pressed and dried material and information on the type label, which simply states that the petals are wine red (“rouges lie de vin”). Robyns (1963, p. 204) described the petals of *P. macrocalyx* (as *Bombacopsis macrocalyx*) as pale yellow outside and whitish-puberulent inside. Yet many specimen labels of *P. macrocalyx* report red petals. Additionally, staminal-tube length, fruit and seed morphology (mainly dimensions), and habitat are similar for both species supporting our decision to consider them synonymous.

***Pachira minor* (Sims) Hemsl., Biol. Cent.-Amer., Bot. 1: 124. 1879.**

Carolinea minor Sims, Bot. Mag. 34: t. 1412. 1811. *Bombax minus* (Sims) Ducke, Arch. Jard. Bot. Rio de Janeiro 6: 65. 1933. *Rhodognaphalopsis minor* (Sims) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 278. 1963. *Pochota minor* (Sims) Steyerl. & W.D. Stevens, Ann. Missouri Bot. Gard. 75: 397. 1988.

Pachira nitida Kunth, in H.B.K., Nov. Gen. Sp. (quarto ed.) 5: 302. 1822 [1821], Ibid. (folio ed.) 5: 235. 1822 [1821], **syn. nov.** *Rhodognaphalopsis nitida* (Kunth) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 282. 1963. *Pochota nitida* (Kunth) Steyerl. & W.D. Stevens, Ann. Missouri Bot. Gard. 75: 397. 1988. Type: VENEZUELA. Amazonas, Caño de Pimichin, s.d. (fl), *F.W.H.A. von Humboldt & A.J.A. Bonpland 987* (lectotype, as “*holotypus*,” designated by Ro-

byns 1963, p. 284): P barcode 00679764 [= F neg. No. 35354]; isoelectotype: P barcode 04694524).

Type. Lectotype, designated here: Sims (1811, t. 1412). Epitype: GUYANA. From the interior woods of Guiana, s.d. (lf, fl), *Alex. Anderson s.n.* (epitype, designated here: BM barcode 000645662).

Note. In the protologue of *Carolinea minor*, Sims (1811) states that he received this plant from “Messrs. Loddiges and Sons, under the name of *Bombax Carolinoides*, an appellation given it by Dr. Anderson of the Botanic Garden at St. Vincent’s.” Thus, original material that can be considered for a lectotype is either a cultivated specimen (or specimens) from Loddiges nursery in Hackney (now London) or the plate (t. 1412) illustrating *Carolinea minor*. Robyns (1963, p. 280) stated that the “*holotypus*” was at BM (“*Anderson* (321 ?) (BM)”), but while it is clear that Anderson conveyed seed to Loddiges, Sims’s description was not based on Anderson’s wild-collected herbarium material and such specimens are not original material. Robyns’s “*holotypus*” cannot therefore be corrected to neotype (see Turland et al. 2018; Art. 9.10).

Robyns (1963, p. 284) effectively designated a lectotype for the name *Pachira nitida* when he stated that a collection from Caño de Pimichin made by F.W.H.A. von Humboldt and A.J.A. Bonpland was the “*holotypus*.” The handwriting on the label of this specimen (P barcode 00679764) is that of Kunth (see Stauffer et al. 2012) and the fragmentary nature of the flower agrees with statements in the protologue (here translated) that declare “Only fragments available to us. A description of a flower from the label of Bonpland.” The handwriting on the label of the isoelectotype (P barcode 04694524) is that of Bonpland (see Stauffer et al. 2012). This specimen was originally identified as *Carolinea princeps* L.f. ($\equiv$ *P. aquatica*), which also is noted in the protologue where *P. aquatica* is listed as a synonym of *P. nitida* with doubt. The morphology of the leaves (leaflet shape, venation, and strongly raised midribs on abaxial surfaces) and of the flower (calyx shape and dimensions and stamen length) of the type of *P. nitida* matches that of the type of *P. minor*.

***Pachira nervosa* (Uittien) Fern. Alonso, Anales Jard. Bot. Madrid 56: 310.1998.**

Bombax nervosum Uittien, Recueil Trav. Bot. Néerl. 22: 364. 1925. *Bombacopsis nervosa* (Uittien) A. Robyns, Bull. Jard. État Bruxelles 33: 199. 1963.

Type. SURINAME. Boschreserve (forest reserve), Sectie O, Boomnummer (Tree Number) 628, 8 May 1910 [sic, 1916 in protologue and on field ticket] (lf), *Forestry Bureau 1901* (lectotype, designated by Robyns 1963, p. 201: U barcode 0000772). Epitype: BRAZIL. Manaus, 1 Oct 1946 (lf, fl, fr), *A. Ducke 2001* (epitype, designated here: US barcode 01226557; isoeptypes: A n.v., IAN 20135, MG n.v., NY barcode 01539149, RB barcode 00054274, RB barcode 00059756, RB barcode 00775585, S-PLE-E4214 s.n.).

Note. Robyns (1963, p. 201) designated “For. Bur. 1901 (U)” as the lectotype of *Bombax nervosum*. He annotated the specimen as “*Bombacopsis nervosa* (Uitt.) A. Robyns, *comb. nov.*” and as “*lectotypus!*” Fernández-Alonso (1998, p. 310) stated that he had not seen the lectotype when he transferred *Bombax nervosum* to *Pachira*. The lectotype of *B. nervosum*, however, is sterile and to avoid any ambiguity about its identity, an epitype is herein selected. The epitype has flowers, a determination label in Robyns’s hand, and it was cited in his revision (Robyns 1963, p. 201).

***Pachira obtusa* Spruce ex K. Schum., in Martius, Fl. Bras. 12(3): 232. 1886.**

Bombax obtusum (Spruce ex K. Schum.) Bakh., Bull. Jard. Bot. Buitenzorg, sér. 3, 6: 181. 1924.

Bombax poissonianum K. Schum., in Martius, Fl. Bras. 12(3): 225. 1886, **syn. nov.**
Type: BRAZIL. Rio Negro, s.d. (lf, fl), *sine collector* (holotype: P barcode 02285965 [= F neg. No. 35362]).

Bombax utiarityi Hoehne, Arch. Bot. São Paulo 1: 567, t. 10. 1927, **syn. nov.** *Pachira utiarityi* (Hoehne) Toledo & Handro, in Hoehne, Relat. Anual Inst. Bot. 1943: 39. 1944. *Pachira utiarityi* (Hoehne) Hoehne, Indice Bibliogr. Num.: 280. 1951, nom. inval. *Rhodagnaphalopsis nitida* var. *utiarityi* (Hoehne) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 284. 1963. Type: BRAZIL. Mato Grosso, Salto do Utiarityi, Rio Papagaio, Apr 1918 (lf, fl), *J.G. Kuhlmann 2145* (lectotype, as “*holotypus*,” designated by Robyns 1963, p. 285: RB barcode 00534522; isoelectotypes: R barcode 000027315, R barcode 000027315a, S-PLE-E4219 n.v., SP barcode SP002722).

Type. BRAZIL. Amazonas, Prope San Gabriel da Cachoeira, ad Rio Negro, Brasiliae borealis, Jan-Aug 1852 [Feb. 1852] (lf, fl), *R. Spruce 2150* (lectotype, designated here: K barcode 000382337 [“São Gabriel, Feb. 1852”; handwritten label]; isoelectotypes: B†, BM barcode 000778668, E barcode 00285197, FI barcode 006090, G n.v., K barcode 000382338, LE n.v., M barcode 0211650 [= F neg. no. 19668], NY barcode 00133532, P barcode 05273649 [handwritten label], RB barcode 00060397, TDC [= TCD?] n.v., W barcode 18890017579).

Note. The protologue of *Pachira obtusa* cites a single collection, “Spruce n. 2150,” which we assume Schumann examined in Berlin (B) and which was subsequently destroyed in WWII. In his revision, Robyns (1963, p. 284) failed to select a lectotype since he made no distinction between duplicates of this collection deposited in various herbaria. We designate here as lectotype of the name a sheet deposited in Kew (K) that has a handwritten locality as well as a narrower collecting date (“Feb. 1852”) than the majority of duplicates that have printed labels and an eight-month range for the collecting date (“Jan.-Aug. 1852”).

In the protologue of *Bombax poissonianum*, Schumann (1886) states that he thinks his new species might be the same as the *Pachira nitida* of Decaisne or Kunth (viz., “*Pachira nitida* Dcne.! *Miscell bot.* 1880. p. 9, an Kunth?”). *Bombax poissonianum*, however, is not a superfluous renaming of *P. nitida* because Schumann (1886, p. 225) included an expression of doubt (see Turland et al. 2018; Art. 52.2, Note 1). There is nothing to tell us who collected the type specimen nor when it was collected. The epithet and author (viz., “*nitida* Kth.”) written on the holotype label appears to be a later addition (the handwriting differs from that of whomever wrote the locality). The specimen was annotated “det. Schumann in Fl. Bras.” and is undoubtedly the one Schumann (1886, p. 225) mentioned in the protologue.

In the protologue of *Bombax utiarityi*, Hoehne (1927) cites a single collection, “Kuhlmann 2145,” but he does not indicate where it was deposited. When Toledo and Handro in Hoehne (1944, p. 39) proposed a new combination for this species, they mentioned a Kuhlmann specimen housed at SP (as “Inst. Bot. 11.914, leg. J.G. Kuhlmann”), but they did not provide a collection number and failed to use the word “type” or an equivalent (Turland et al. 2018; Art. 7.11). When Robyns (1963, p. 285) proposed a new combination and status for *B. utiarityi*, he also effectively selected a lectotype (Turland et al. 2018; Art. 9.10). The lectotype he designated has an original handwritten label. Inasmuch as *Bombax utiarityi* agrees with both *B. poissonianum* and *Pachira obtusa* morphologically and the habitat of occurrence (flooded forest in Amazonia and adjacent Savanna) of all three are the same, we consider *B. utiarityi* to be a synonym of *P. obtusa*.

The designation *Pachira utiarityi* (Hoehne) Hoehne (Hoehne 1951) is not validly published. It was proposed as an alternative for *Bombax utiarityi*, which was the name Hoehne accepted (see Turland et al. 2018; Art. 36.1(a)).

***Pachira paraensis* (Ducke) W.S. Alverson, Novon 4: 7. 1994.**

Bombax paraense Ducke, Arch. Jard. Bot. Rio de Janeiro 4: 124. 1925. *Bombacopsis paraensis* (Ducke) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 213. 1963.

Type. BRAZIL. Pará, Itaituba, Rio Tapajoz, 26 Aug 1923 (lf, fl), A. Ducke s.n. [RB 18094] (lectotype, designated here: RB barcode 00534489; isolectotypes: B† [= F neg. no. 9540], G barcode 00177452, K barcode 000382352, RB barcode 00534497 [without original label], S-R-11283, U barcode 0008391, U barcode 0008392, US barcode 00101944).

Note. Three syntypes are cited in the protologue. Robyns (1963) selected one, “Ducke 18094,” as the lectotype (first step) of *Bombax paraense* and stated that it was deposited in RB. There are, however, two sheets in RB of this collection. Our lectotypification (second step) narrows Robyns’s choice to a single specimen and it is the same one he annotated as “*lectotypus*.”

***Pachira retusa* (Mart.) Fern.Alonso, Revista Acad. Colomb. Ci. Exact. 27(102): 36. 2003.**

Fig. 2E, F

Bombax retusum Mart., Flora 8: 28. 1825, Ibid., Nov. Gen. Sp. Pl. 1(4): 92–93, t. 59. 1826 [1824]. *Bombacopsis retusa* (Mart.) A. Robyns, Bull. Jard. Bot. État Bruxelles 33: 205. 1963.

Type. BRAZIL. Minas Gerais, In deserto Serro Frio, [1817–20] (fl), *C.F.P. von Martius s.n.* (lectotype, designated here: M barcode 0211681; isolectotype: M barcode 0211682; possible isolectotype: S-PLE-E4218 n.v.).

Note. When Robyns (1963, p. 207) stated that a Martius specimen in Munich (M) was the “*holotypus*” of the name *Bombax retusum*, he effectively selected a lectotype (Turland et al. 2018; Art. 9.10). However, there are at least two specimens with identical label data in that herbarium that must be considered syntypes as well as a copy of the plate cited in the protologue (“tab. 60,” sphalm. pro 59), but published a year later (Martius 1826). Robyns wrote “*holotypus*” on one of the two specimens and annotated all three elements as “*Bombacopsis retusa* (Mart. et Zucc.) A. Robyns, *comb. nov.*” The ICN (Turland et al. 2018; Art. 7.10) requires that a type designation be effectively published and the mere annotation of a herbarium sheet does not meet this requirement. Our lectotypification (second step; see Turland et al. 2018; Art. 9.17) narrows Robyns’ selection to the specimen with the best flowering material.

Robyns (1963, p. 205) attributed the basionym to “Mart. et Zucc.,” but according to Stafleu and Cowan (1981, p. 329) authorship is to be attributed to von Martius alone. Fernández-Alonso (2003, p. 36) also included J.G. Zuccarini as a co-author of this species name. Interestingly, Schumann (1886, p. 226) recognized “*Bombax retusum* Mart.” while citing “*Bombax retusum* Mart. et Zucc.! Nov. gen. et spec. I. 92. t. 59.” The article that includes the validating description of *B. retusum* (Martius 1825) clearly states that Martius collected and described (“collegit et descriptis”) the plants while Zuccarini organized the plates, etc. (“*Pingendas curavit et secundum auctoris schedulas digessit*”).

***Pachira sordida* (R.E. Schult.) W.S. Alverson, Novon 4: 8. 1994.**

Bombax sordidum R.E. Schult., Bot. Mus. Leaf. 16: 75. 1953. *Rhodognaphalopsis coriacea* var. *sordida* (R.E. Schult.) A. Robyns, Bull. Jard. Bot. État Bruxelles 63: 292. 1963. *Pochota sordida* (R.E. Schult.) Steyerf. & W.D. Stevens, Ann. Missouri Bot. Gard. 75: 398. 1988.

Type. COLOMBIA. Vaupés, Río Negro, San Felipe (El Castillo), below confluence of Ríos Guainía and Casiquiare, 12 Dec 1947 (lf, fr), *R.E. Schultes & F. López 9342* (lectotype, designated here: GH barcode 00066501; isolectotypes: GH barcode 00066502, GH barcode 00066503).

Note. A single collection, “R.E. Schultes & F. López 9342,” is cited in the protologue of *Bombax sordidum*. When Robyns (1963, p. 292) proposed a new combination and status for this name, he indicated that the holotype was deposited in the Gray Herbarium: “Colombie: Vaupes: *Schultes & F. López, 9342* (f., fr., *holotypus* GH)”. There are, however, three sheets of this collection in that herbarium, and although one was annotated as the holotype by Robyns, the ICN (Turland et al. 2018; Art. 7.10) requires that a type designation be effectively published. The mere annotation of a herbarium sheet does not meet this requirement, hence our lectotypification (second step). We designate here the specimen that has the greatest abundance of indumentum on the abaxial surfaces of the leaflets because this character is considered diagnostic by Robyns (1963, p. 292, viz. “*a specie foliolorum lamina infra densissime et sordide pulveraceo-lepidota sat differt*”).

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Morphological and molecular evidence reveals three new species of *Lithocarpus* (Fagaceae) from Bidoup-Nui Ba National Park, Vietnam

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Abstract

Three new species, *Lithocarpus bidoupensis* Ngoc & Tagane, *L. congtrouensis* Ngoc & Yahara, and *L. hongiaoensis* Ngoc & Binh are described from Bidoup-Nui Ba National Park, Central Highland of Vietnam. Morphological analyses and Maximum likelihood tree based on genome-wide SNPs support the distinction of those species from the previously known taxa in the region. The three new species are considered to be endemic to the Bidoup-Nui Ba National Park and the preliminary conservation status for each species is evaluated as Critically Endangered.

Keywords

Fagales, Lam Dong Province, MIG-seq, phylogeny, taxonomy

Introduction

Fagaceae are highly diversified in Vietnam and 216 species of 6 genera have been reported in various forest types, from dry evergreen forest at lowlands to montane evergreen forest in the higher elevation (Ho 2003; Ban 2005; Ngoc et al. 2016). Recently, 10 species of Fagaceae were newly described from Vietnam: *Castanopsis grandicaticata* N.H.Xia & D.H.Vuong, *C. multiporcata* N.H.Xia & D.H.Vuong (Vuong and Xia 2014), *Lithocarpus dahuoaiensis* Ngoc & L.V.Dung (Ngoc et al. 2016), *L. vuquangensis* Ngoc & V.H.Nguyen (Ngoc et al. 2018), *Quercus baolamensis* H.T.Binh & Ngoc (Binh et al. 2018a), *Q. bidoupensis* H.T.Binh & Ngoc (Binh et al. 2018a), *Q. honbaensis* H.T.Binh, Tagane & Yahara (Binh et al. 2018a), *Q. trungkhanhensis* H.T.Binh & Ngoc (Binh et al. 2018b), *Q. xuanlienensis* H.T.Binh, Ngoc & T.N.Bon (Binh et al. 2018c), and *Q. ngochoaensis* Binh & Son (Binh et al. 2021).

Lithocarpus Blume is the largest genus of the family Fagaceae in Vietnam, including 119 species and two varieties, among which 44 species are endemic (Ho 2003; Ban 2005; Linh et al. 2013; Ngoc et al. 2016, 2018). The previous taxonomic treatments of *Lithocarpus* in Vietnam were mostly based on Camus' studies using the specimens collected by French botanists, the results of which were documented in the part of *Flore générale de l'Indo-Chine* (Hickel and Camus 1930) or *Chênes Atlas* (Camus 1948). All these early studies relied only on morphological features to identify and construct the keys to species level, so the taxonomic circumscriptions were sometimes inaccurate, leading to continued uncertainty in the taxonomic status and relationship among species.

The phylogenetic approach has become a widespread and efficient way to identify and delimit species, but there is only one study for *Lithocarpus* in Vietnam (Ngoc et al. 2018). The MIG-seq is a PCR-based method used to identify large numbers of genetic markers throughout the genome (Suyama and Matsuki 2015) that is highly applicable for use in phylogenetic studies (Binh et al. 2018a; Okabe et al. 2021). Recently, a growing number of taxonomic studies of *Lithocarpus* have yielded new species (Ngoc et al. 2016, 2018), but we often find *Lithocarpus* material that is difficult to identify to species level. Here we applied MIG-seq for phylogenetic reconstruction to accurately assess the diversity and taxonomy of *Lithocarpus* species.

Bidoup-Nui Ba National Park (Fig. 1) is in the core zone of UNESCO Langbiang Biosphere Reserve, which is in Lam Dong Province in the central highland of Vietnam. The national park, with the area of 70,038 ha covering almost the entire Langbiang Plateau, harbors 1933 species of vascular plants (Bidoup-Nui Ba National Park 2021) including 62 threatened species (Ban et al. 2007; IUCN 2012), and 42 endemic species (Tagane et al. 2017, 2020; Bidoup-Nui Ba National Park 2021). For Fagaceae, 25 species of *Lithocarpus*, nine species of *Castanopsis*, eleven species of *Quercus*, and one species of *Trigonobalanus* have been recorded from Bidoup-Nui Ba National Park (Dung 2005; Ngoc et al. 2016; Binh et al. 2018a).

During our floristic research in Bidoup-Nui Ba National Park from 2015 to present, we found some individuals of the genus *Lithocarpus* that could not be identified to species level. We here describe them as *Lithocarpus bidoupensis* Ngoc & Tagane, sp. nov., *Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov., and *Lithocarpus congtrioensis*

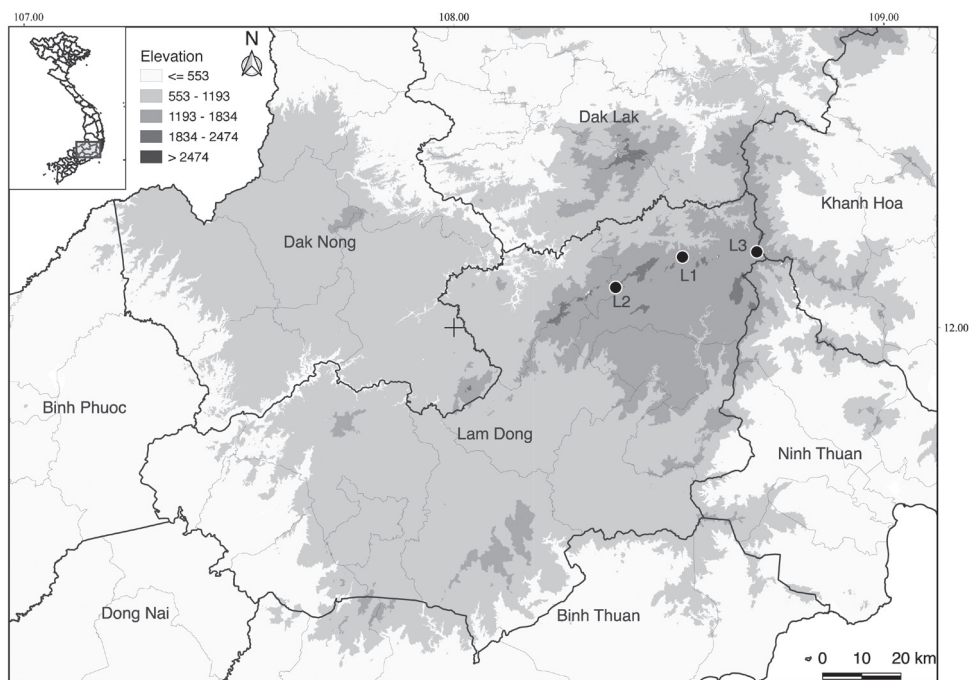


Figure 1. Type locality of the new species (Black dots): L1: *L. bidoupensis*, L2: *L. congtraiensis*, and L3: *L. hongiaoensis*.

Ngoc & Yahara, sp. nov., based on comparisons of morphology with related species and provide molecular phylogenetic evidence using the MIG-seq method (Suyama and Matsuki 2015).

Materials and methods

Taxon sampling

In the present study, we conducted botanical inventories in Bidoup-Nui Ba National Park and the other protected areas in Vietnam and collected a total of 63 samples consisting of 23 species including sixteen samples of three unknown species. Five samples of *Lithocarpus balansae* (Drake) A.Camus, which is morphologically distinct from the other species of the genus, were included as an outgroup for the phylogenetic analysis. Localities and voucher specimens of these materials are listed in Table 1.

Morphological analysis

We compared morphological traits of three unknown species with those of related species using taxonomic literature (Camus 1931, 1938, 1942, 1943, 1945, 1948; Huang et al. 1999; Ho 2003; Ban 2005; Phengklai 2008), specimens kept in the herbaria

Table 1. List of vouchers specimen that were used in this study.

Species	Vouchers	Localities
<i>L. annamitorius</i>	Nguyen et al. V3214	Bidoup-Nui Ba NP
<i>L. bidoupensis</i>	Tagane et al. V4320, Yahara et al. V7850, V8190, V8417, V9940 (DLU, FU, KAG); Ngoc et al. NAF122, NAF185 (DLU)	Bidoup-Nui Ba NP
<i>L. blaensis</i>	Nguyen et al. V3176, V3176A (DLU, FU)	Bidoup-Nui Ba NP
<i>L. coalitus</i>	Tagane et al. V4191, Yahara et al. V10140 (DLU, FU),	Bidoup-Nui Ba NP
<i>L. congtrioensis</i>	Ngoc et al. NAF200 (DLU); Nguyen et al. V3205, Tagane et al. V9102, Tagane et al. V9470, Tagane et al. V9492, Yahara et al. V9555 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. encleisocarpus</i>	Tagane et al. V1627; Nguyen et al. V3263 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. dahuoiensis</i>	Nguyen et al. V3194 (DLU, FU)	Bidoup-Nui Ba NP
	Ngoc et al. V5404, V5404A (DLU, FU)	Dong Nai NR
<i>L. dalatensis</i>	Tagane et al. V9106 (DLU, FU, KAG)	Bidoup-Nui Ba NP
<i>L. dealbatus</i>	Ngoc et al. V3258, Tagane et al. V4357 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. hancei</i>	Ngoc et al. V5111, V4918, SP008 (DLU, FU)	Hoang Lien NP
<i>L. honbaensis</i>	Tagane et al. V0003, V207; Ngoc et al. V5540 (DLU, FU)	Hon Ba NR
<i>L. hongiaensis</i>	Nguyen et al. V3235 (DLU, FU); Ngoc et al. NAF123, NAF192 (DLU)	Bidoup-Nui Ba NP
<i>L. laoticus</i>	Nguyen et al. V3193 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. lemecanus</i>	Tagane et al. V4523 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. licentii</i>	Tagane et al. V6261, V6400 (DLU, FU)	Ngoc Linh NR
<i>L. sp1</i>	Nguyen et al. V3171, V3171A (DLU, FU)	Bidoup-Nui Ba NP
<i>L. parvulus</i>	Yahara et al. V8636, V9720, V10068, V10077, V10164 (DLU, FU, KAG)	Bidoup-Nui Ba NP
<i>L. pseudomagneinii</i>	Nguyen et al. V3183, V3223 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. syncarpus</i>	Nguyen et al. V3188, V3188A, V3246, V3250 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. vinhensis</i>	Nguyen et al. V3591, V3787 (DLU, FU)	Vu Quang NP
<i>L. vuquangensis</i>	Yahara et al. V5743, V5938 (DLU, FU)	Vu Quang NP
<i>L. xylocarpus</i>	Tagane et al. V4337; Ngoc et al. V8464 (DLU, FU)	Bidoup-Nui Ba NP
<i>L. balansae</i> (outgroup)	Yahara et al. V2938 (DLU, FU)	Bach Ma NP
	Nguyen et al. V3177, Ngoc et al. V8467 (DLU, FU)	Bidoup NP
	Nguyen et al. V5447, V5512 (DLU, FU)	Pu Mat NP

NP = National Park; NR = Nature Reserve.

ANDA, BKF, DLU, HN, KAG, KYO, P, and VNM, and digitized plant specimen images available on the web of JSTOR Global Plants (<https://plants.jstor.org/>) and Chinese Virtual Herbarium (<http://www.cvh.org.cn/>).

The ImageJ software (Schneider et al. 2012) was used to measure the following characters of the new species and related species based on images of type specimens: length, width, aspect ratio and circularity of leaf blade, petiole length, and size of cupules. Aspect ratio and circularity are defined as length/width of leaf blade and $4\pi \times (\text{area}/\text{perimeter squared})$, respectively. Analysis of variance (ANOVA) and post hoc Tukey’s honestly significant difference test (Tukey’s HSD) (Tukey 1953) were applied to reveal the mean difference among species. All statistical analyses were performed in R version 4.0.5 (R Core Team 2021) with R-Sutido ver. 1.4.1106 (R-Studio Team 2021).

DNA extraction and sequencing

Leaf pieces were dried using silica-gel in the field, and DNA was isolated with the CTAB method (Doyle and Doyle 1987) with minor modifications described in Toyama et al. (2015). The extracted DNA was diluted to 10 ng/μl and used as templates to amplify thousands of short sequences (loci) from a wide variety of genomes with a standard PCR

protocol according to Suyama and Matsuki (2015). MIG-seq library was constructed as described in Suyama and Matsuki (2015) with a minor update by using dual-indexed primers (Suyama et al. 2021). The 1st PCR, multiple non-repetitive regions from various inter-simple-sequence repeats (ISSRs) were amplified from genomic DNA by multiplexed PCR with tailed ISSR forward and reverse primers sets. The first PCR products were diluted and used as the templates for the 2nd PCR with dual indexed primers sets. Then, 3 µl of each 2nd PCR product was pooled in equimolar concentrations as single mixture library. The mixture was then purified and the size range of 350–800 bp were isolated by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Quantitative PCR was performed to measure final concentration of size-selected library with approximately 10 pM and then used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Phylogenetic analysis

A total of 50 samples of 22 species of *Lithocarpus* including samples of unknown species were sequenced (except *NAF122*, *NAF123*, *NAF185*, *NAF192*, *V3205*, *V9470*, *V9492*, *V9555*), of which five samples of *L. balansae* were used as an outgroup. The low-quality reads and primer sequences were eliminated from raw data by using the trimmomatic software version 0.40 (Bolger et al. 2014). The quality-filtered sequence data were demultiplexed and filtered through the software Stacks v1.46 (Catchen et al. 2011; Catchen et al. 2013) following the parameters set as described by Takata et al. (2019) with minor modifications: in the U-stacks, the option settings of ‘maximum distance allowed between stacks (M)’ = 4, ‘maximum distance allowed to align secondary reads to primary stacks (N)’ = 4; in the population program, the minimum percentage of individuals required to process a locus across all data (r) was set at 10% and the minimum minor allele frequency required to process a nucleotide site at a locus (min_maf) = 0.005, the maximum observed heterozygosity required to process a nucleotide site at a locus (max_obs_het) = 0.6.

Phylogenetic analyses were conducted using maximum likelihood method on SNPs data set. The model of sequence evolution was set to GTR+G as selected by jMrModeltest 2.1.10 (Darriba et al. 2012). Maximum Likelihood analyses were implemented using the RAxML ver. 8.2 (Stamatakis 2014). The topological reliability of the maximum likelihood tree was evaluated with 1000 bootstrap replicates.

Results

Morphological analyses

After the morphological examination and taxonomic review in *Lithocarpus* of Vietnam and its surrounding countries, sixteen unknown samples of *Lithocarpus* were not assignable to any of the species recognized in the region. Hence, hereafter we named

these samples as (1) *Lithocarpus bidoupensis* Ngoc & Tagane, sp. nov. for Ngoc et al. NAF125, NAF185; Tagane et al. V4320; Yahara et al. V7850, V8190, V8417, and V9940; (2) *Lithocarpus congtrioensis* Ngoc & Yahara, sp. nov. for Ngoc et al. NAF200; Nguyen et al. V3205, Tagane et al. V9102, V9470, V9492; Yahara et al. V9555; and (3) *Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov. for Nguyen et al. V3235; Ngoc et al. NAF123, NAF192.

Lithocarpus bidoupensis is most similar to *L. blaoensis* in having completely entire leaf margin, leaf blade width 3–5 cm, 10–12 pairs of secondary veins, cupules clustered in sets of three, and a concave nut scar, but ANOVA with a post-hoc Tukey HSD test showed significant differences ($p < 0.05$) between species (Table 2 and Table 3). Specifically, *L. bidoupensis* significantly differed in much shorter petioles (0.5 ± 0.1 cm long in *L. bidoupensis* vs. 1.89 ± 0.23 cm long in *L. blaoensis*), shorter leaf blades (9.74 ± 1.12 cm long vs. 13.66 ± 1.89 cm long), bigger cupules (0.98 ± 0.19 cm high, 2.47 ± 0.2 cm in diam. vs. 0.64 ± 0.06 cm high, 1.58 ± 0.11 cm in diam.) *Lithocarpus bidoupensis* also has a larger scar of the nut than *L. blaoensis* (1.4 – 1.9 cm in diam. vs. 1 – 1.2 cm in diam.). *Lithocarpus bidoupensis* is also similar to *L. licentii*. Both have a completely entire leaf margin, glossy green leaf blades, a leaf blade length of 7.2 – 11.6 cm, and a cupule height of 0.7 – 1.4 cm. However, *L. bidoupensis* has a significantly shorter and wider leaf blade (9.74 ± 1.12 cm $\times$ 4.5 ± 0.59 cm in *L. bidoupensis* vs. 10.76 ± 2.01 cm $\times$ 3.46 ± 0.53 cm in *L. licentii*), lower leaf blade aspect ratio (2.17 ± 0.15 vs. 3.1 ± 0.31), higher leaf blade circularity (0.71 ± 0.03 vs. 0.55 ± 0.05), shorter petioles (0.5 ± 0.1 vs. 0.81 ± 0.14), and bigger cupules size (0.98 ± 0.19 cm high, 2.47 ± 0.2 cm in diam. vs. 0.88 ± 0.15 cm high, 2.17 ± 0.13 cm in diam.) (Table 2 and Table 3). It also has fewer secondary veins (10–12 pairs in *L. bidoupensis* vs. 12–15 pairs in *L. licentii*), much shorter infructescences (8.4 – 11.5 cm long vs. 15 – 20 cm long), cupules clustered in sets of three (vs. solitary in *L. licentii*), a cupule covering less than $1/3$ of the nut (vs. covering $1/2$ – $2/3$ of the nut), and a concave basal scar (vs. convex).

Lithocarpus congtrioensis is morphologically similar to *L. dahuoaiensis* in having a completely entire leaf margin, blade broadly elliptic, glabrous adaxially, petioles length 1 – 1.8 cm long, and concave nut scar, but significantly differed in shorter leaf blades (14.83 ± 1.6 cm $\times$ 5.3 ± 0.84 cm in *L. congtrioensis* vs. 19.4 ± 3.45 cm $\times$ 8.06 ± 1.48 cm in *L. dahuoaiensis*), higher leaf blades aspect ratio (2.86 ± 0.34 vs. 2.41 ± 0.17), lower leaf blade circularity (0.59 ± 0.06 vs. 0.7 ± 0.04), bigger cupules (2.99 ± 0.28 vs. 2.24 ± 0.19) (Table 2 and Table 3), and also differs in having more secondary veins (13–15 pairs vs. 11–12 pairs), shorter infructescences (10 – 15 cm long vs. 20 – 25 cm long), cupule clustered of three (vs. solitary). *Lithocarpus congtrioensis* is also similar to *L. honbaensis* in having completely entire leaf margin, adaxially glabrous lamina, long petioles, cupule clustered of three, and concave nut scar, but significantly differed in smaller leaf blades (14.83 ± 1.6 cm $\times$ 5.3 ± 0.84 cm in *L. congtrioensis* vs. 20.39 ± 3.44 cm $\times$ 8.84 ± 1.66 cm in *L. honbaensis*), higher leaf blade aspect ratio (2.86 ± 0.34 vs. 2.32 ± 0.16), lower leaf blade circularity (0.59 ± 0.06 vs. 0.69 ± 0.04), shorter petioles (1.42 ± 0.19 vs. 2.11 ± 0.36) (Table 2 and Table 3), and also differed in having more

Table 2. The comparisons of mean (X) and standard deviation (SD) value of the leaf blade and cupule size between *L. bidoupensis*, *L. congtrouensis*, and *L. hongiaoensis* with related species.¹Derived from type specimens, ²Derived from this study collections, n = number of leaf or cupule were measured in this study.

Parameters (cm)	<i>L. bidoupensis</i> ¹			<i>L. blaensis</i> ^{1,2}			<i>L. licentii</i> ^{1,2}		
	X	SD	n	X	SD	n	X	SD	n
Leaf blade length	9.74	1.12	23	13.66	1.89	22	10.76	2.01	20
Leaf blade width	4.5	0.59	23	4.41	0.51	22	3.46	0.53	20
Leaf blade aspect ratio	2.17	0.15	23	3.11	0.36	22	3.1	0.31	20
Leaf blade circularity	0.71	0.03	23	0.58	0.05	22	0.55	0.05	20
Petiole length	0.5	0.1	23	1.89	0.23	22	0.81	0.14	20
Cupule high	0.98	0.19	27	0.64	0.06	22	0.88	0.15	16
Cupule diameter	2.47	0.2	27	1.58	0.11	22	2.17	0.13	16
<i>L. congtrouensis</i> ¹				<i>L. dabuoiensis</i> ^{1,2}			<i>L. bonbaensis</i> ^{1,2}		
	X	SD	n	X	SD	n	X	SD	n
Leaf blade length	14.83	1.6	22	19.4	3.45	22	20.39	3.44	20
Leaf blade width	5.3	0.84	22	8.06	1.48	22	8.84	1.66	20
Leaf blade aspect ratio	2.86	0.34	22	2.41	0.17	22	2.32	0.16	20
Leaf blade circularity	0.59	0.06	22	0.7	0.04	22	0.69	0.04	20
Petiole length	1.42	0.19	22	1.4	0.14	22	2.11	0.36	20
Cupule high	1.10	0.18	21	1.23	0.14	28	-	-	-
Cupule diameter	2.99	0.28	21	2.24	0.19	28	-	-	-
<i>L. hongiaoensis</i> ¹				<i>L. vinhensis</i> ^{1,2}			<i>L. vuquangensis</i> ^{1,2}		
	X	SD	n	X	SD	n	X	SD	n
Leaf blade length	10.81	1.93	29	8.42	2.26	22	7.49	1.32	25
Leaf blade width	3.26	0.6	29	2.97	0.95	22	2.39	0.32	25
Leaf blade aspect ratio	3.33	0.33	29	2.9	0.4	22	3.13	0.33	25
Leaf blade circularity	0.49	0.04	29	0.57	0.07	22	0.5	0.05	25
Petiole length	2.59	0.49	29	0.74	0.12	22	1.3	0.23	25
Cupule high	1.01	0.15	25	0.57	0.05	19	0.86	0.26	18
Cupule diameter	2.06	0.28	25	1.31	0.14	19	1.63	0.18	18

secondary veins (13–15 pairs vs. 10–11 pairs), shorter infrutescences (10–15 cm long vs. 15–24 cm long), and shorter acorn-stalk (0.2–0.4 cm long vs. 0.5–0.8 cm long).

Lithocarpus hongiaoensis is most similar to *L. vinhensis* in having blades narrowly elliptic to lanceolate, completely entire leaf margins, adaxially glabrous and abaxially hairy leaf surface, solitary cupules, and concave nut scar, but ANOVA analysis with post-hoc Tukey HSD test showed that *L. hongiaoensis* significantly differed from *L. vinhensis* in having longer leaf blade length (10.81 ± 1.93 cm long in *L. hongiaoensis* vs. 8.42 ± 2.26 cm long in *L. vinhensis*), higher leaf blade aspect ratio (3.33 ± 0.33 vs. 2.9 ± 0.4), lower leaf blade circularity (0.49 ± 0.04 vs. 0.57 ± 0.07), much longer petioles (2.59 ± 0.49 vs. 0.74 ± 0.12), bigger cupules (1.01 ± 0.15 cm high, 2.06 ± 0.28 cm in diam. vs. 0.57 ± 0.05 cm high, 1.31 ± 0.14 cm in diam.) (Table 2 and Table 3). *Lithocarpus hongiaoensis* is morphologically almost identical with *L. vuquangensis* such as blade narrowly elliptic to lanceolate, completely entire leaf margin, midrib flat or slightly prominent, adaxially glabrous and abaxially hairy leaf surface, solitary cupules, and concave nut scar, but the new species significantly differed in having bigger leaf blades (10.81 ± 1.93 cm $\times$ 3.26 ± 0.6 cm in *L. hongiaoensis* vs. 7.49 ± 1.32 cm $\times$ 2.39 ± 0.32 cm in *L. vuquangensis*), longer petioles (2.59 ± 0.49 vs. 1.3 ± 0.23), bigger cupules (1.01 ± 0.15 cm high, 2.06 ± 0.28 cm in diam. vs. 0.86 ± 0.26 cm high, 1.63

Table 3. Differences between the species for morphological characters and their levels of significance determined by TukeyHSD Test.

Comparisons	Leaf blade length		Leaf blade width		Aspect ratio		Circularity		Petiole length		Cupule high		Cupule diameter	
	diff.	P	diff.	P	diff.	P	diff.	P	diff.	P	diff.	P	diff.	P
<i>L. bidoupensis</i> – <i>L. blaoensis</i>	3.92	0	-0.09	0.85	0.93	0	-0.13	0	1.39	0	-0.35	0	-0.89	0
<i>L. bidoupensis</i> – <i>L. licentii</i>	1.01	0.13	-1.04	<0.001	0.90	0	-0.16	0	0.31	0	-0.1	0.07	-0.29	0
<i>L. congtrouensis</i> – <i>L. dabuaoiensis</i>	4.57	<0.001	2.76	0	-0.45	<0.001	0.11	0	-0.02	0.97	0.13	<0.01	-0.75	0
<i>L. congtrouensis</i> – <i>L. honbaensis</i>	5.56	<0.001	3.53	0	-0.09	0	0.09	0	0.69	0	-	-	-	-
<i>L. hongiaoensis</i> – <i>L. vinhensis</i>	-2.39	<0.001	-0.29	0.26	-0.43	<0.001	0.08	<0.001	-1.85	0	-0.44	0	-0.75	0
<i>L. hongiaoensis</i> – <i>L. vuquangensis</i>	-3.33	0	-0.58	<0.001	-0.20	0.09	0.02	0.55	-1.29	0	-0.15	0.012	-0.43	<0.001

Note: diff. = mean difference; **Bold** font indicates statistically significant differences, (-) not available.

± 0.18 cm in diam.), and also differs in having longer infrutescences (12.5–16.5 cm long vs. 5–7 cm long), larger nut scar (1.2–1.4 cm in diam. vs. 1–1.1 cm in diam.) (Table 2 and Table 3).

Phylogeny inference

The Maximum likelihood tree based on data set of 4962 genome-wide SNPs strongly supports two sister clades, clade 1 and 2, with 100% bootstrap value (Fig. 2). Clade 1 is divided into two subclades, 1a and 1b, each with 100% bootstrap value. Clade 2 is divided into four subclades of 2a, 2b, 2c, and 2d, each with 100% bootstrap value. The three candidates for new species were included in the clade 2 and supported the monophyly of *L. congtrouensis* and *L. bidoupensis* with 100% bootstrap value. The specimen here described as *Lithocarpus hongiaoensis* is sister to two specimens of *L. vuquangensis*.

Lithocarpus bidoupensis (clade 2a) was clearly separated from the morphologically similar species of *L. licentii* (Clade 1a) and *L. blaoensis* (Clade 1b) and is sister to many different species in clade 2b. *Lithocarpus congtrouensis* is included in a clade with *L. encleisocarpus*, *L. lemeeanus* and *L. pseudomagneinii* (clade 2.b2) with 97% bootstrap support, but it is well supported as monophyletic. The morphologically similar *L. honbaensis* is in a different clade (clade 2.b1).

Lithocarpus hongiaoensis (clade 2.c1) was clearly separated from the morphologically similar species of *L. vinhensis* (clade 2.b3) but showed a sister relationship with *L. vuquangensis* in the clade 2.c1 with a strongly bootstrap value (100%). Those three species share the character of solitary cupules but the distribution of *L. hongiaoensis* is narrowly restricted and apart from the two species: *L. vinhensis* and *L. vuquangensis* are distributed in Nghe An and Ha Tinh Provinces, which are located in the north of the Central Coast of Viet Nam, whereas *L. hongiaoensis* was found only in Bidoup-Nui Ba National Park, Lam Dong Province about 1000 km further south.

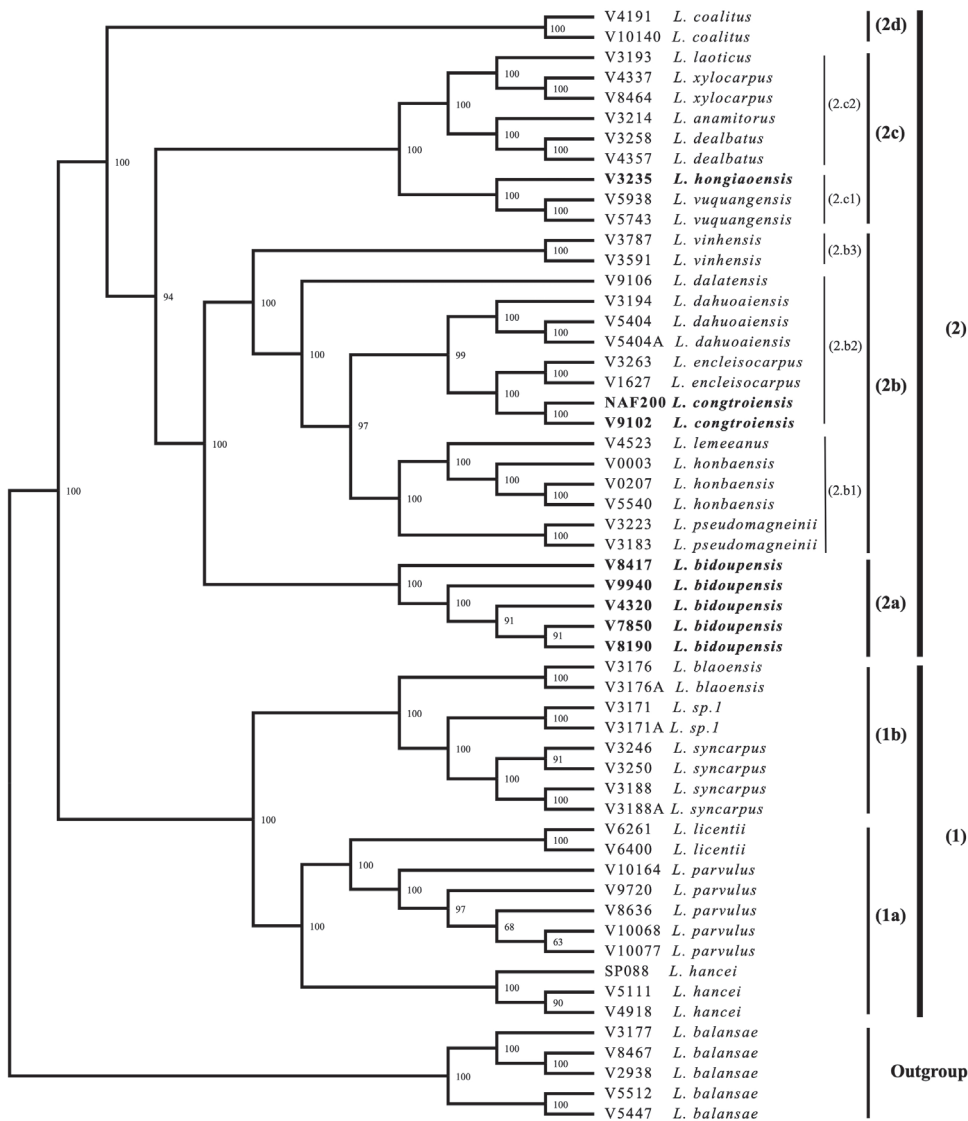


Figure 2. Maximum likelihood tree of three new species (**Bold**) with their related based on SNPs data from MIG-seq.

Discussion

The morphological comparison and phylogenetic analysis provided evidence of the validity of three new species. *Lithocarpus bidoupensis* is most similar to *L. blaoensis* that occurred in the same locality with *L. bidoupensis*, and also similar to *L. licentii* that was collected in Kon Tum Province, which is the type locality of *L. licentii*. However, the new species is clearly different from both in many morphological traits (Table 2 and Table 3) as well as shown in the phylogenetic results (Fig. 2). The molecular phylogenetic tree

strongly supports this disjunction in that the monophyly of *L. bidoupensis* was supported by 100% bootstrap value, while *L. blaoensis* and *L. licentii* were placed in another clade.

Lithocarpus congtrioensis is placed in the same clade with *L. encleisocarpus* and *L. dahuoaiensis* (2.b2), but the morphology was clearly distinct. *Lithocarpus congtrioensis* is distinguished from *L. encleisocarpus* by its greater number of secondary vein (13–15 pairs in *L. congtrioensis* vs. 8–10 pairs in *L. encleisocarpus*), cupules with tiny imbricate scales, enclosing 1/3–1/2 of the nut (vs. the scales forming 5–7 dimly concentric flanges, cupules completely enclosing the nut). Especially, the cupule of *L. congtrioensis* usually clustered of three, while the cupules of *L. encleisocarpus* is solitary. The morphological distinctness between *L. congtrioensis* and *L. dahuoaiensis* is clearly shown in Tables 2 and 3.

Although we could not collect any specimens of *L. honbaensis* with mature fruits, the morphological analysis of leaf and cupule characters provided enough evidence to distinguish species from *L. congtrioensis*. In addition, the molecular phylogenetic tree showed that *L. honbaensis* has a close genetic relationship with *L. lemeeanus* and *L. pseudomagneinii* than *L. congtrioensis* (clade 2b).

Lithocarpus hongiaoensis is most similar to *L. vinhensis* and *L. vuquangensis*, of which the latter showed the sister relationship to *L. hongiaoensis* in the molecular phylogenetic tree (Fig. 2, clade 2.c1). However, *L. hongiaoensis* is narrowly endemic to the Hon Giao area of Bidoup-Nui Ba National Park, Lam Dong Province, in the southern part of Vietnam while *L. vuquangensis* is endemic to Vu Quang National Park of Ha Tinh Province, north-central coast of Vietnam. From 2015 to 2017 we conducted three field trips at Bach Ma National Park, Ba Na Nature Reserve and Ngoc Linh National Park. These protected areas are located between Lam Dong and Ha Tinh Province, but we did not find any individual of *Lithocarpus* similar to *L. hongiaoensis* or *L. vuquangensis*. Also, morphological differences are distinct enough to distinguish them as different species (Table 2 and 3). While *L. hongiaoensis* was collected in Lam Dong Province, *L. vinhensis* occurred in Nghe An Province, the province located in the north central coast of Vietnam. The genetic differences between *L. hongiaoensis* and *L. vinhensis* were presented in the phylogenetic tree, *L. hongiaoensis* and *L. vuquangensis* a sister to each other (2.c1), while the two samples of *L. vinhensis* formed a clade not closely related to these two species (2.b3).

Taxonomic treatments

Lithocarpus bidoupensis Ngoc & Tagane, sp. nov.

urn:lsid:ipni.org:names:77234073-1

Fig. 3

Type. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, in hill evergreen forest dominated by the species of Fagaceae, 1698 m elev., 12°09'52.95"N, 108°32'00.38"E, 24 February 2016, S. Tagane, H. Toyama, H. Nagamasu, A. Naiki, V.S. Dang, N.V. Ngoc, J. Wai V4320 [fr.] (holotype DLU!, isotypes FU!, HN!, KYO!, VNM!).

Diagnosis. *Lithocarpus bidoupensis* is most similar to *L. blaensis* but differs in its shorter leaf blades, petioles and infructescences, and bigger cupules and nuts. The new species is also similar to *L. licentii* but distinguished from *L. licentii* by its shorter petioles, fewer secondary veins (10–12 pairs in *L. bidoupensis* vs. 12–15 pairs in *L. licentii*), much shorter infructescences, clustered cupules (vs. solitary), cupule covering less than 1/3 of the nut (vs. 1/2–2/3 of the nut), and concave basal scar of the nut (vs. convex) (Table 2).

Description. Evergreen tree, up to 27 m tall. Branches yellowish green when young, turning greyish brown when old, glabrous, sparsely lenticellate. Terminal and lateral buds ovoid, up to 7 mm long. Leaves alternate; petiole 0.3–0.4 cm long, glabrous; blade elliptic to oblong-elliptic, obovate-elliptic, 6–11.6 × 2.8–5.3 cm, coriaceous, glabrous, glossy green on both surfaces, apex acuminate or attenuate, acumen up to 0.9 cm long, base cuneate, rarely obtuse, margin completely entire, midrib prominent on both surfaces, secondary veins 10–12 pairs, prominent abaxially, at an angle of 55–70° from the midrib, tertiary veins scalariform-reticulate, visible abaxially. Inflorescences not seen. Infructescences a woody spike, 8.4–11.5 cm long. Cupules sessile, usually in cluster of 3, fused at the base each other, depressed obconical or saucer-shaped, 0.71–1.40 cm high, 2.13–2.80 cm in diam., enclosing 1/4–1/3 of the nut, pubescent with short grayish indumentum outside; wall woody, ca. 2 mm thick, with brown triangular scales outside, the scales up to 4 × 4 mm, imbricate, arranged in 3 or 4 interrupted concentric rings. Nut broadly ovoid-conical to depressed ovoid-globose, 1.5–1.6 cm high, 2.1–2.3 cm in diam., glabrous, brown to blackish brown; basal scar slightly concave, 1.4–1.9 cm in diam.

Phenology. Unknown. Fallen fruits were collected in February.

Distribution. Vietnam (Khanh Hoa and Lam Dong provinces) (Fig. 1).

Etymology. The specific epithet is derived from the type locality, Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam.

Vernacular name. Dé đá Bidoup

Additional specimens examined. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park; 1602 m elev., 12°09'27.6"N, 108°32'06.6"E, 24 Mar. 2018, *T. Yahara, H. Nagamasu, H. Toyama, M. Zhang, A. Nagahama, N.V. Ngoc, K. Tsuchiya* V7850 [ster.] (DLU!, FU!); *ibid.*, 1656 m elev., 12°09'36.61"N, 108°32'11.16"E, 24 Mar. 2018, *T. Yahara, S. Tagane, M. Zhang, A. Nagahama, K. Tsuchiya, N.V. Ngoc, H.T. Binh, T.Q. Cuong* V8190, V8417 [ster.] (DLU!, FU!); *ibid.*, 1669 m elev., 12°09'36.65"N, 108°32'11.18"E, 29 Apr. 2019, *N.V. Ngoc, H.T. Binh, N.V. Duy, T.T. Nhung* NAF122 [ster.] (DLU!); *ibid.*, 1669 m elev., 12°09'36.62"N, 108°32'11.25"E, 29 Apr. 2019, *N.V. Ngoc, H.T. Binh, N.V. Duy, T.T. Nhung* NAF185 [ster.] (DLU!). Khanh Hoa Province: Son Thai Commune, Khanh Vinh District, in evergreen; 1430 m elev., 12°10'42.09"N, 108°43'32.59"E, 23 Apr. 2019, *T. Yahara, S. Tagane, A. Nagahama, N. Komada, V.N. Ngoc, H.V. Thanh* V9940 [ster.] (DLU!, FU!).

Conservation status. Critically Endangered (CR). From our intensive field survey in Bidoup-Nui Ba National Park and its vicinities from 2015 to present (Tagane et al. 2017 & 2020, Binh et al 2018b), *Lithocarpus bidoupensis* was found only in a narrow area within the protected areas of Bidoup-Nui Ba National Park, and its adjacent area of Son Thai Commune at the elevation range between 1400 and 1669 m. In the area,

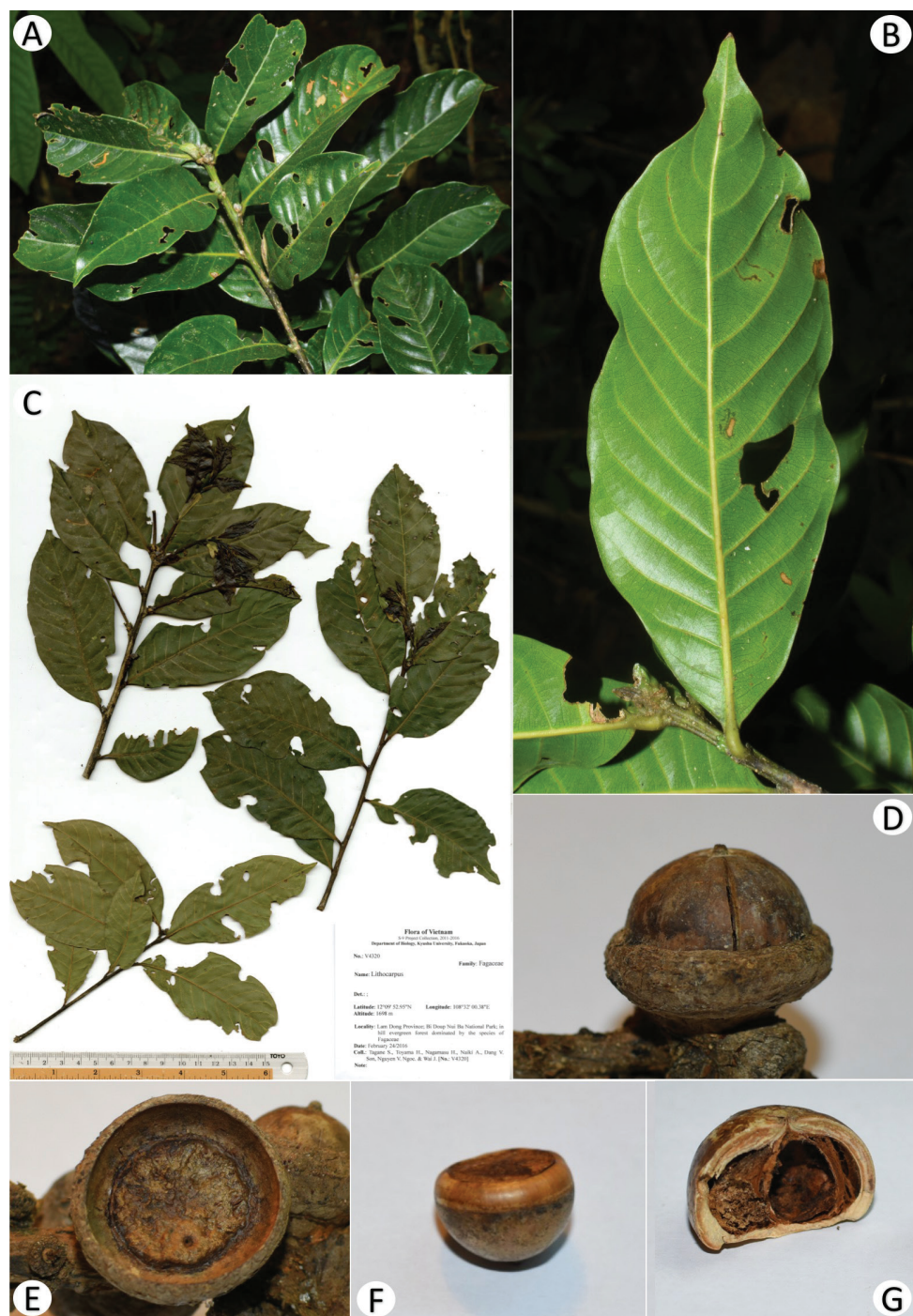


Figure 3. *Lithocarpus bidoupensis* Ngoc & Tagane **A** leafy twig **B** abaxial leaf surface **C** holotype (Tagane et al. V4320, DLU) **D** mature fruit **E** cupule **F** mature nut **G** section of mature nut.

we observed fewer than 50 mature individuals. Based on criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR.

***Lithocarpus congtrouiensis* Ngoc & Yahara, sp. nov.**

urn:lsid:ipni.org:names:77234074-1

Fig. 4

Type. VIETNAM. Lam Dong Province, Bidoup-Nui Ba National Park: Cong Troi, at edge of evergreen forest, roadside, 1752 m elev., 12°05'37.3"N, 108°22'38.8"E, 11 July 2018, S. Tagane, A. Nagahama, K. Tsuchiya, N.V. Ngoc., T.Q. Cuong V9102 [fr.] (holotype DLU!; isotypes FU!, HN!, KYO!, VNM!).

Diagnosis. *Lithocarpus congtrouiensis* is most similar to *L. dahuoiensis* but differs by its smaller leaf blades, more secondary veins, shorter infructescences, cupules clustered of three (vs. solitary), and bigger nut size. It is also similar to *L. honbaensis* but distinguished mainly by its shorter petioles, smaller leaf blade, more secondary veins, shorter infructescences, and shorter fruiting stalk (Table 2).

Description. Evergreen tree, up to 25 m tall. Twigs blackish gray, glabrescent. Stipules caducous, not seen. Leaves alternate; petioles 1.1–1.8 cm long, glabrous; blades broadly elliptic, 12–18 × 4.2–7.2 cm, coriaceous, glabrous on both sides, apex acuminate, acumen 0.7–1.2 cm long, base acute to cuneate, margin entire, midrib flat or slightly prominent adaxially, strongly prominent abaxially, yellowish *in vivo*, brown *in sicco*, secondary veins 13–15 pairs, prominent abaxially, at an angle of 40–50° from the midrib, tertiary veins parallel, faintly visible abaxially. Inflorescence a terminal or axillary spike, 8–12 cm long, erect, male and female flowers separate or female below. Male flowers white, in 1–3-flowered cluster, calyx 6-lobed, lobes ovate, 0.4–0.7 mm × 0.5–0.7 mm; stamens 12, 1–1.2 mm long, anthers 0.15–0.20 mm long. Female flower always cluster of three, styles 3, stigmata pointed. Infructescences terminal, erect, 10–15 cm long, rachis grayish brown, lenticellate. Cupules clustered of three, 0.2–0.4 cm long stalked, bowl-shaped, 0.7–1.4 cm high, 2.5–3.6 m diam., enclosing 1/3–1/2 of the nut; wall woody, with tiny imbricate scales; scales triangular, obscure, covered with white-grayish indumentum. Nut broadly conical or globose, 2.1–2.6 cm high, 2.3–3.1 cm in diam., outer surface densely white tomentose; wall woody, crackled; apex shortly acuminate; basal scar slightly concave, 1.5–1.8 cm in diam.

Phenology. Flowers were collected in December and mature fruits were collected from June to July.

Distribution. Vietnam (so far known only from Mt. Cong Troi and Mt. Langbiang of Bidoup-Nui Ba National Park, Lam Dong Province). (Fig. 1)

Etymology. The specific epithet is derived from the type locality, Mt. Cong Troi of Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam.

Vernacular name. Dé đá Công Trói.

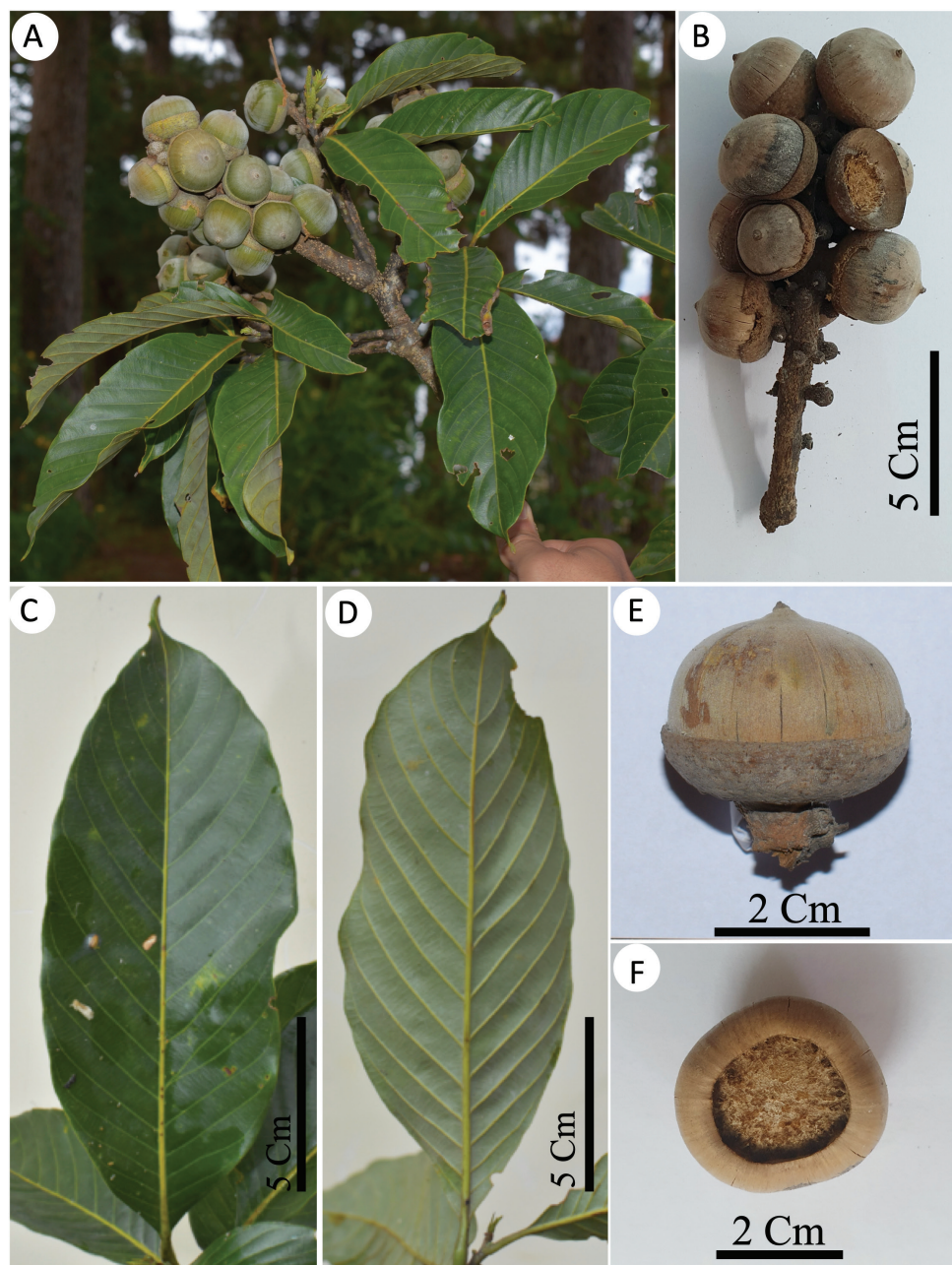


Figure 4. *Lithocarpus congtruiensis* Ngoc & Yahara **A** twig with infructescence **B** infructescence **C, D** leaves adaxially and abaxially, respectively **E** side view of mature cupule and nut **F** bottom view of nut with basal scar.

Additional specimens examined. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, Cong Troi area; 1750 m elev., 12°04'08.5"N, 108°21'55.5"E, 18 June 2015, *N. Nguyen, D. Luong, B. Hoang* V3205 [fr.] (DLU!, FU!); *ibid.*, 1860

m elev., 12°06'06.85"N 108°23'00.32"E, *S. Tagane*, *T. Yahara*, *A. Nagahama*, *M. Zhang*, *K. Tsuchiya*, *T. Nguyen*, *C.T. Nguyen* V9470 [fr.] (DLU!, FU!); 1790 m elev., 12°06'03.86"N, 108°23'39.73"E, 20 Dec. 2018, *T. Yahara*, *S. Tagane*, *A. Nagahama*, *K. Tsuchiya*, *C.T. Quong*, *P. Chhang* V9555 [male and female fl.] (DLU!, FU! KAG [KAG127308]); *ibid.*, 1864 m elev., 12°04'08"N, 108°21'54.5"E, 15 Jun. 2019, *N.V. Ngoc*, *H.T. Binh*, *N.V. Duy*, *T.T. Nhung* NAF200 [fr.] (DLU!); Mt. Langbiang; 1918 m elev., 7 Oct. 2018, 12°02'48.13"N, 108°26'06.67"E, *S. Tagane*, *T. Yahara*, *A. Nagahama*, *M. Zhang*, *K. Tsuchiya*, *T. Nguyen*, *C.T. Nguyen* V9492 [ster.] (DLU!, FU!).

Conservation status. Critically Endangered (CR). We found around ten individuals of *L. congtrouensis* along the road and inside the permanent plot at Cong Troi area, and three individuals in Mt. Langbiang, both located inside the protected area of Bidoup-Nui Ba National Park. Based on criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR. The new species is endemic to Bidoup-Nui Ba National Park, Lam Dong Province.

***Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov.**

urn:lsid:ipni.org:names:77234075-1

Fig. 5

Type. VIETNAM. Lam Dong Province, Bidoup-Nui Ba National Park, Hon Giao, at edge of evergreen forest, roadside, 1580 m elev., 12°10'35.9"N, 108°42'25.1"E, 19 June 2015, *N. Nguyen*, *D. Luong*, *B. Hoang* V3235 [young male fl. and fruits] (holotype DLU!; isotypes FU!, HN!, KYO!, VNM!).

Diagnosis. *Lithocarpus hongiaoensis* is similar to *L. vinhensis* but differs in having much longer petioles, fewer secondary veins, longer infructescences, bigger cupules, and bigger nuts. It is also similar to *L. vuquangensis* but differs in having much longer petioles, more secondary veins, longer infructescences, and bigger cupules (Table 2).

Description. Evergreen tree, up to 25 m tall. Twigs blackish gray, glabrescent, densely lenticellate. Stipules narrowly triangular, ca. 5 × 1 mm, densely covered with indumentum abaxially, almost glabrous adaxially. Leaves alternate; petioles 1.7–3.5 cm long, glabrous; blades narrowly elliptic to lanceolate, 7.6–14.7 × 2.3–4.6 cm, coriaceous, glabrous adaxially, covered with adherent waxy scales abaxially, apex acuminate, acumen up to 1.5 cm long, base attenuate and decurrent on petiole, margin entire, midrib flat or slightly prominent near base adaxially, prominent abaxially, greenish yellow *in vivo*, reddish brown *in sicco*; secondary veins 8–11 pairs, prominent abaxially, at an angle of 35–45° from the midrib, tertiary veins scalariform, faintly visible or invisible on both sides. Young male inflorescences terminal, ca. 5–7 cm long, densely covered with white indumentum. Infructescences terminal, erect, 12.5–16.5 cm long, rachis 0.4–0.6 cm thick at base, grayish brown, lenticellate, covered with indumentum. Cupules solitary, sessile, obconical to saucer-shaped, 0.8–1.2 cm high, 1.7–2.6 cm in diam., enclosing 1/3–1/2 of the nut; wall woody, ca. 2 mm thick, with triangular scales not united into concentric rings; scales up to 4 mm long, apex shortly acuminate, covered with dense grayish indumentum outside. Nut strongly depressed ovoid,

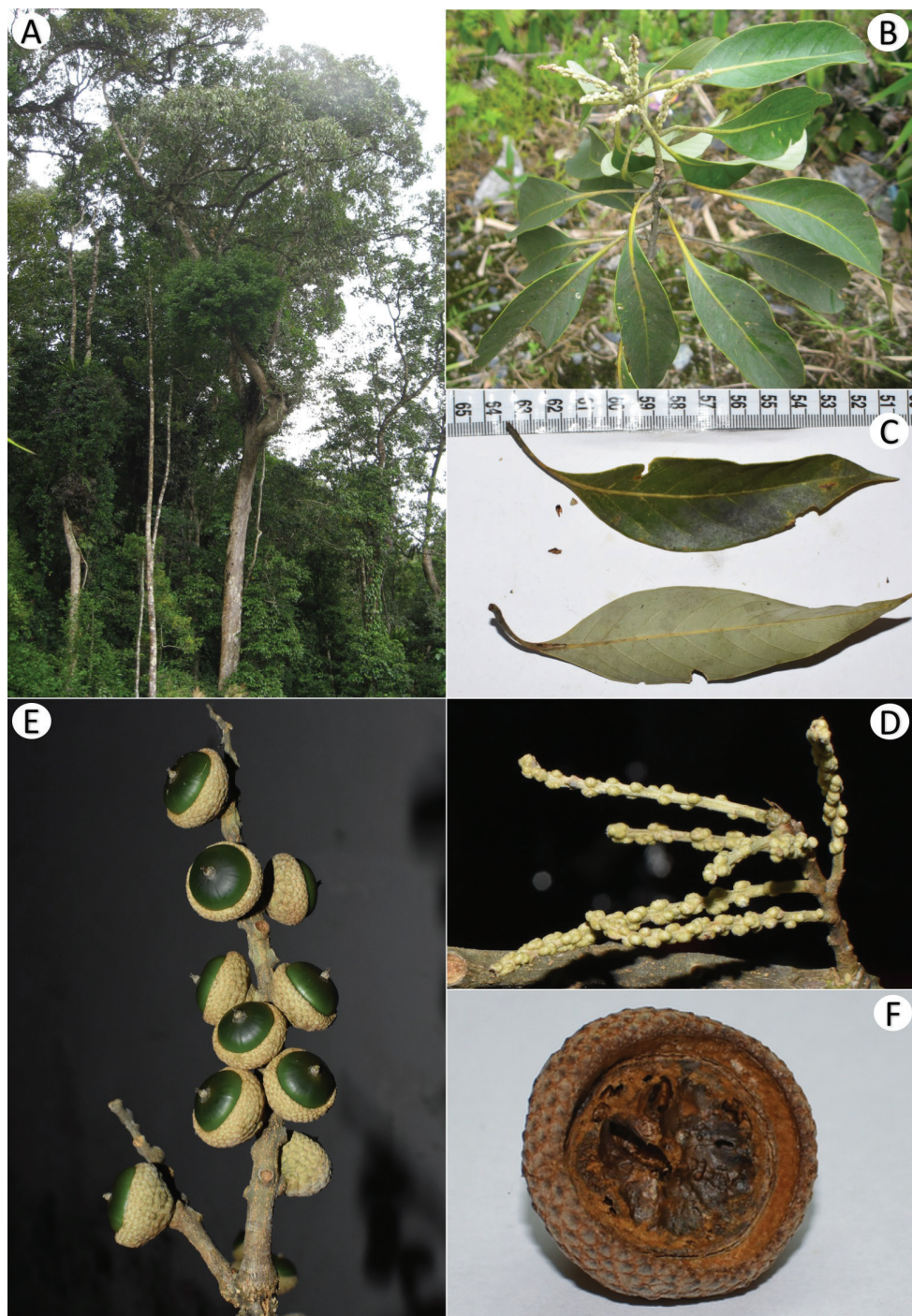


Figure 5. *Lithocarpus hongiaoensis* Ngoc & Binh **A** habit **B** twig with young inflorescences **C** leaves **D** young male inflorescence **E** infructescence **F** inside of mature cupule.

0.6–1.1 cm high, 1.2–1.5 cm in diam., glabrous, reddish brown to grayish brown *in sicco*, tomentose with soft white hairs near apex; basal scar slightly concave, 1.2–1.4 cm in diam.

Phenology. Young male flowers and mature fruits were collected in May and June.

Distribution. Vietnam (so far known only from Hon Giao area of Bidoup-Nui Ba National Park, Lam Dong Province). (Fig. 1).

Etymology. The specific epithet is derived from the type locality, Hon Giao area of Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam.

Local name. Dé đá Hòn Giao.

Conservation status. The new species is narrowly endemic to Hon Giao area of Bidoup-Nui Ba National Park, Lam Dong Province. During our floristic expedition from 2015 to present, we found only five mature individuals of *Lithocarpus hongiaensis* at the road in montane evergreen forest which is located within the protected area of the national park. According to criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR.

Additional specimens examined. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, Hon Giao, 1574 m elev., 12°10'34.5"N, 108°42'25.5"E, 15 May 2019, *N.V. Ngoc, H.T. Binh, N.V. Duy, T.T. Nhung* NAF122 [fr.] (DLU!); *ibid.*, 1574 m elev., 12°10'35.5"N, 108°42'25.9"E, 11 June 2020, *N.V. Ngoc, H.T. Binh, N.V. Duy, T.T. Nhung* NAF192 [fr.] (DLU!).

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***Coleus* (Lamiaceae) in Peninsular Malaysia including two new species**

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Abstract

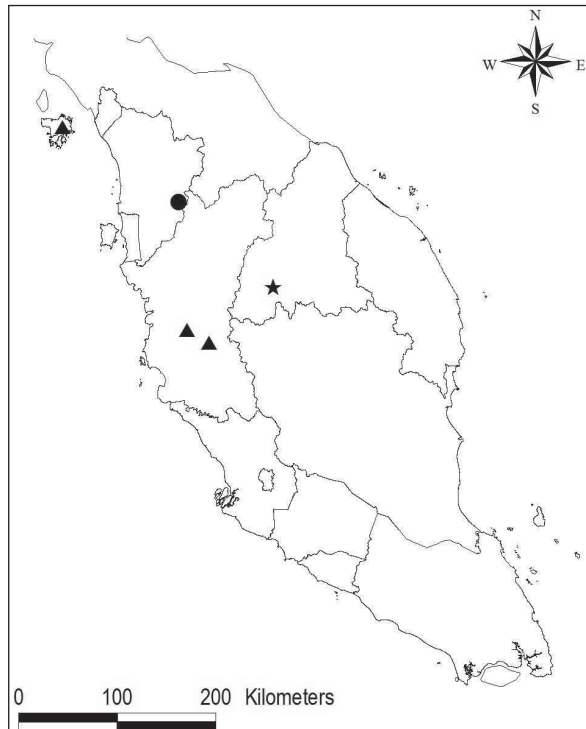
In Peninsular Malaysia, *Coleus* is represented by five species. Two, *C. hairulii* Kiew and *C. rafidahiae* Kiew, are new species. Both are narrowly endemic and restricted to limestone hills as is *C. kunstleri* (Prain) A.J.Paton. All three are Critically Endangered. *Coleus scutellarioides* (L.) Benth., although widespread, is probably not indigenous. It is also a common ornamental, while *C. monostachyus* (P.Beauv.) A.J.Paton is a recent introduction that has spread rapidly and threatens to become a troublesome weed.

Keywords

Conservation status, limestone hills, *Plectranthus*, *Solenostemon monostachyus*, *Solenostemon scutellarioides*

Introduction

The flora of Peninsular Malaysia now includes five species of *Coleus*, compared with the two previously recorded under *Plectranthus* by Keng (1978) and Bramley (2019). *Coleus* has recently been recognised as distinct from *Plectranthus* (Paton et al. 2019). *Coleus scutellarioides* (L.) Benth. is a widespread lowland species, often associated with villages, and *C. kunstleri* (Prain) A.J. Paton is very rare and known from just two limestone localities. Recent exploration of limestone karsts in Peninsular Malaysia has led to the discovery of the two new species that are described here. *Coleus monostachyus* (P.Beauv.) A.J. Paton is an African weed recently introduced and now, after a decade, has rapidly become widespread and is found everywhere in ruderal habitats (Kiew 2016). The three limestone species are endemic and restricted to karst limestone (Map 1).



Map I. Distribution of *Coleus hairulii* (circle), *C. kunstleri* (triangle) and *C. rafidahiae* (star) in Peninsular Malaysia.

Conservation

Most of the 445 or so limestone karst hills or islands in Peninsular Malaysia are small with a basal area of about 1 km² (Liew et al. 2016). Together they cover about 0.2% of land area but support about 14% of the Peninsula's flora of vascular plants (Chin 1977). About 20% of these species are restricted to limestone substrates. The great majority of these karst limestone hills lie outside the network of Totally Protected Areas that include National and State Parks, Wildlife Sanctuaries and Permanent Reserved Forests, so are particularly vulnerable to disturbance from mining, fire, ecotourism and cave temples. Nationally, limestone hills are classified as Environmentally Sensitive Areas.

Both the new species described below are each known from a single karst limestone hill. *Coleus kunstleri* is also restricted to karst limestone but is known from two widely separated localities. All three are endemic in Peninsular Malaysia. For assessing conservation status, the IUCN criteria and categories (IUCN Standards and Petitions Committee 2019) are followed. Extent of Occupancy (EOO) for species restricted to limestone habitats is assumed to be the same as its Area of Occupancy (AOO) due to substrate restriction. *Coleus hairulii*, *C. kunstleri* and *C. rafidahiae* are assessed as Critically Endangered because the EOO and AOO are below 100 km² and 10 km² respectively and none lies within a Totally Protected Area, which means that they are vulnerable to habitat disturbance or loss. In addition, *Coleus hairulii* and *C. rafidahiae*

are each known from a single locality, while *C. kunstleri*, although known from two localities (Kuala Dipang, Perak and Pulau Langgun, Langkawai, Kedah) is unlikely to still grow in Kuala Dipang because it has been, and is, heavily disturbed by mining and, although the area has been regularly visited for botanical collecting, this species has not been recollected for more than 130 years. Today, it is therefore extant at a single locality. In contrast, *C. scutellarioides* does not grow on limestone and is widespread, though not common, on the fringes of lowland forest. It is not endemic. For endemic species, the conservation status is the global conservation status, while for *C. scutellarioides*, it is the regional conservation status that applies only to the Peninsular Malaysian population (Chua and Saw 2006).

Botanical exploration has shown that many species (about 15%) of the limestone flora have extremely local distributions (Kiew et al. 2017). One hundred taxa (species, subspecies or varieties) are known from a single karst hill, while a further 92 are known from two to four karst hills or islands. These species are particularly endangered by extinction. The three *Coleus* species that are endemic and restricted to limestone are further examples of this phenomenon.

Taxa cultivated in Peninsular Malaysia

Coleus, *Coleus scutellarioides*, is widely available in nurseries and popular as an ornamental plant for its incredible variety of leaf colour (reds, purples and yellows) and patterns of variegation. In Peninsular Malaysia, it seldom flowers. Its local Malay name is *pokok ati-ati*. Several minor medicinal uses have been ascribed to it (see below under species).

Indian borage, *Coleus amboinicus* Lour., said to be native to India, is commonly grown in pots in home gardens for its medicinal value (for a dry cough a decoction of the leaves is drunk to sooth the throat). It rarely flowers and does not fruit. It is readily distinguished from the other species by its thick, hairy, succulent, harshly aromatic leaves. Its local Malay name is *daun bangun-bangun*.

Plectranthus ‘Mona Lavender’ is currently a very popular floriferous species with deep purple flowers that grows well in the highlands at Cameron Highlands, Pahang, and has caused confusion with the public because it is marketed as a true lavender (Kiew 2016). It is a hybrid developed in the 1990s in the Kirstenbosch Botanic Gardens, Cape Town, South Africa.

The edible *C. rotundifolius* (Poir.) A.Cheval & Perrot, although once reported from Peninsular Malaysia (Burkill 1966), is today not planted even as a curiosity (Kiew 2016).

Excluded species

Suddee et al. (2004) reported *Plectranthus glabratus* (Benth.) Alston (now known as *Coleus paniculatus* Benth.) from Penang, Peninsular Malaysia, based on a single specimen, *Haniff & Nur 4020* (BM, K). However, examination of the specimen label shows that it was in fact collected from ‘Rilau Seng Penong, Lower Siam’. At the time of col-

lection (12 December 1918) Haniff and Nur were collecting plants from Pulau Panji and Pulau Pungah in southern Thailand. This species is therefore excluded from the Peninsular Malaysian flora.

Taxonomy

Key to *Coleus* species in Peninsular Malaysia

- 1 Inflorescences spike-like with distinct condensed cymes.....2
- Inflorescence thyrsoid, flowers in lax cymes, single or in 2–3s3
- 2 Inflorescences (14–)20–38 cm long. Lower median lobes of the fruiting calyx fused, obovate with two apiculate teeth.....3. *C. monostachyus**
- Inflorescences 5–10(–25) cm long. Lower, median lobes of the fruiting calyx fused, spatulate and deeply bifid5. *C. scutellarioides*
- 3 Lamina 2–3 times longer than wide. Flowers yellowish white....4. *C. rafidabiae*
- Lamina less than twice as long as wide. Flowers purple or greenish white....4
- 4 Leaves 5–6 × 3.3–4.5 cm. Branches from the inflorescence axis 3–5 times shorter than the 13–18 cm-long inflorescence. Cymes lax with 6–8 flowers, flowers ca. 20 mm long, lower lip deep purple1. *C. hairulii*
- Leaves 6–12 × 4–6 cm. Branches from the inflorescence axis 2–3 times shorter than the 5–6 cm-long inflorescence. Cymes single-flowered, c. 10 mm long, greenish white or pale pinkish.....2. *C. kunstleri*

Species

1. *Coleus hairulii* Kiew, sp. nov.

urn:lsid:ipni.org:names:77234076-1

Diagnosis. Most similar to *Coleus paniculatus* Benth. (as *Plectranthus glabratus* (Benth.) Alston in Suddee et al. 2004) from southern Thailand in its habit (both are low, little branched or unbranched herbs), in their inflorescences with conspicuous side branches and cymules with more than 3 flowers, but *Coleus hairulii* (Figure 2) is different (Table 1) in its short branches from the inflorescence axis 2.5–4 cm long, i.e. 3–5 times shorter than the inflorescence length (vs. branches from the inflorescence axis 5.5–10 cm long and 2–3 times shorter than the inflorescence), and its calyx 6–7 mm long in fruit (vs. 4–6 mm long).

* Note. In life, *Coleus monostachyus* is immediately recognised by its erect inflorescences (Figure 3). It begins to flower when just 15 cm tall. In contrast, *C. scutellarioides* in Peninsular Malaysia seldom flowers even in cultivation. Its inflorescences too are shorter. *Coleus scutellarioides* is conspicuous in being the only Malaysian species with coloured leaves. Vegetatively, it is extremely variable due to the myriad of selections for leaf shape, size and colour. It is for this reason that the key uses inflorescence and calyx characters.

Table 1. *Coleus hairulii* and *C. paniculatus* compared.

Character	<i>C. hairulii</i>	<i>C. paniculatus</i>
Stem		
height (cm)	15–30(–40)	to 50
diameter (mm)	ca. 2	3–4
Leaves		
petiole length (cm)	2–6.5	4–7
lamina length × width (cm)	5–6 × 3.3–4.5	1.5–12 × 1–7.5
margin	shallowly crenate	regularly crenate
vein no.	4–5	ca. 6
Inflorescences		
total length (cm)	13–18	17–30
length side branches (cm)	2.5–4	5.5–10
Flowers		
calyx length in fruit (mm)	6–7	4–6
length anterior corolla lobe (mm)	9–14	5–8
inner surface of anterior lobe	densely hirsute	pubescent

Type. MALAYSIA. Peninsular Malaysia: Kedah, Baling District, Gunung Pulai, Kepala Gajah, trail to summit 5°40.05'N, 100°53.03'E, 19 Nov 2019, Wan Syafiq & R. Kiew FRI 99402 (holotype KEP!; isotypes K!, SAN!, SAR!, SING!).

Description. Soft unbranched herb, 15–30(–40) cm tall, erect becoming decumbent, not aromatic, stem quadrangular, ca. 2 mm thick, fleshy, glabrous. **Leaves** glabrous; petiole slender, 2–6.5 cm long, decreasing in length in the upper leaves; lamina thinly membranous, ovate to broadly ovate, 5–6 × 3.3–4.5 cm, base rounded to truncate, margin shallowly crenate, apex acute to acuminate, pale green beneath; veins 4–5 on either side of midrib. **Inflorescence** thyrsoid, terminal, erect, 13–18 cm long, shortly glandular pubescent, rachis and branches pale reddish, peduncle 7–9.5 cm long, 3–4 tiers of short side branches from the inflorescence axis, 2.5–4 cm long; cymes single-flowered; bracts sessile, foliose, lanceolate, apex acute, 6–7 × 2–3 mm; pedicels attached slightly above centre of calyx, 2.5–4 mm long at anthesis, 4–5 mm in fruit. **Flower** pendent; **calyx** obliquely campanulate, reddish brown, posterior lip rosy red, 2–3 mm long at anthesis, 10-veined, unequally 5-lobed, minutely puberulent, in fruit 6–7 mm long, 2.5–3 mm wide, posterior lip spatulate, apex subacute, 2–2.5 × 3 mm, anterior lip with 2 lateral and lower lobes sharply acute, slightly upcurved, lateral lobes ca. 2–2.5 mm long, slightly longer than the lower 2.5 mm; **corolla** (9–)20 mm long, minutely puberulent outside, pale or bluish purple except for the white posterior lip, tube sigmoid, basal ca. 3 mm very narrow ca. 1 mm wide, abruptly decurved and dilating to 3–4 mm wide across the mouth, posterior lip erect, ca. 8 × 6 mm, almost rounded with a broad median lobe deeply notched at apex, lateral lobes narrow, slightly longer than the median lobe, anterior lip boat-shaped and enclosing the stamens and style, 9–14 mm long, 5–6 mm deep and wide with a dense tuft of long translucent hairs in the inner lower half; **stamens** hardly exerted, only anther tips visible, in 2 pairs, filaments 7 and 8 mm long, anthers slightly obovoid, ca. 0.5 mm long; stigma slightly bilobed, slightly exerted from tip of lower corolla lip. **Nutlets** orbicular in outline, bilaterally flattened, ca. 1 mm long; testa reddish brown, smooth.



Figure 1. *Coleus hairulii* Kiew, sp. nov. **A** Mohd. Hairul bin Mohd. Amin with a flowering specimen **B** habit and limestone habitat **C** inflorescence and flower enlarged. (Photographs: **A** by AR Ummul-Nazrah **B** by Wan Mohamad Syafiq bin Wan Putra and **C** by AR Rafidah).

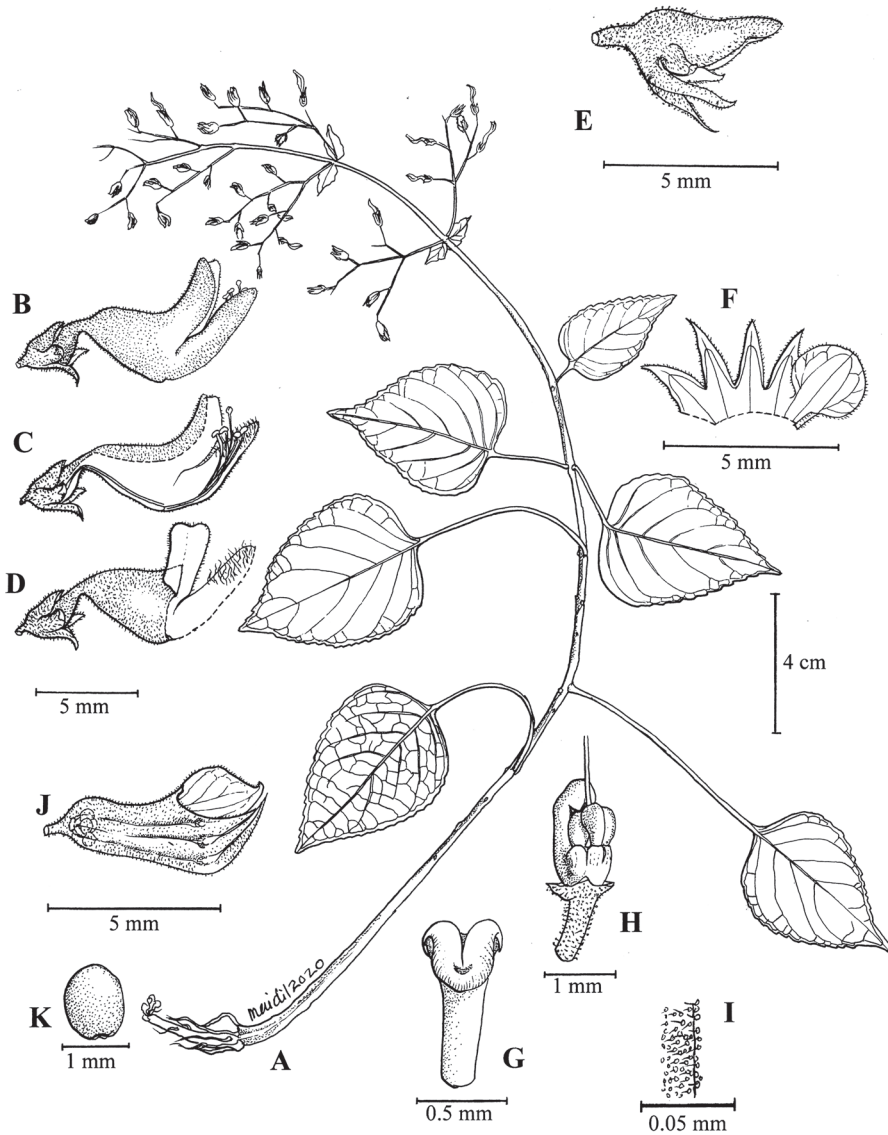


Figure 2. *Coleus hairulii* Kiew **A** flowering plant **B** flower side view **C** flower side view with corolla cut away to show stamens **D** flower side view to show hirsute patch on anterior lip **E** calyx in flower **F** calyx opened **G** stamen apex **H** ovary side view **I** glandular hairs of the pedicel **J** calyx in fruit **K** seed. (All from FRI 94402. Drawn by N. Mohamad Aidil).

Distribution. Endemic in Peninsular Malaysia, Kedah, Gunung Pulai, known only from two populations (trail to Gunung Pulai and Kampung Sungai Limau) at a single locality (Map 1).

Provisional conservation status. Critically Endangered B1ab(i,iii,iv). The species is known only from one locality, with a low population size, estimated at less than a

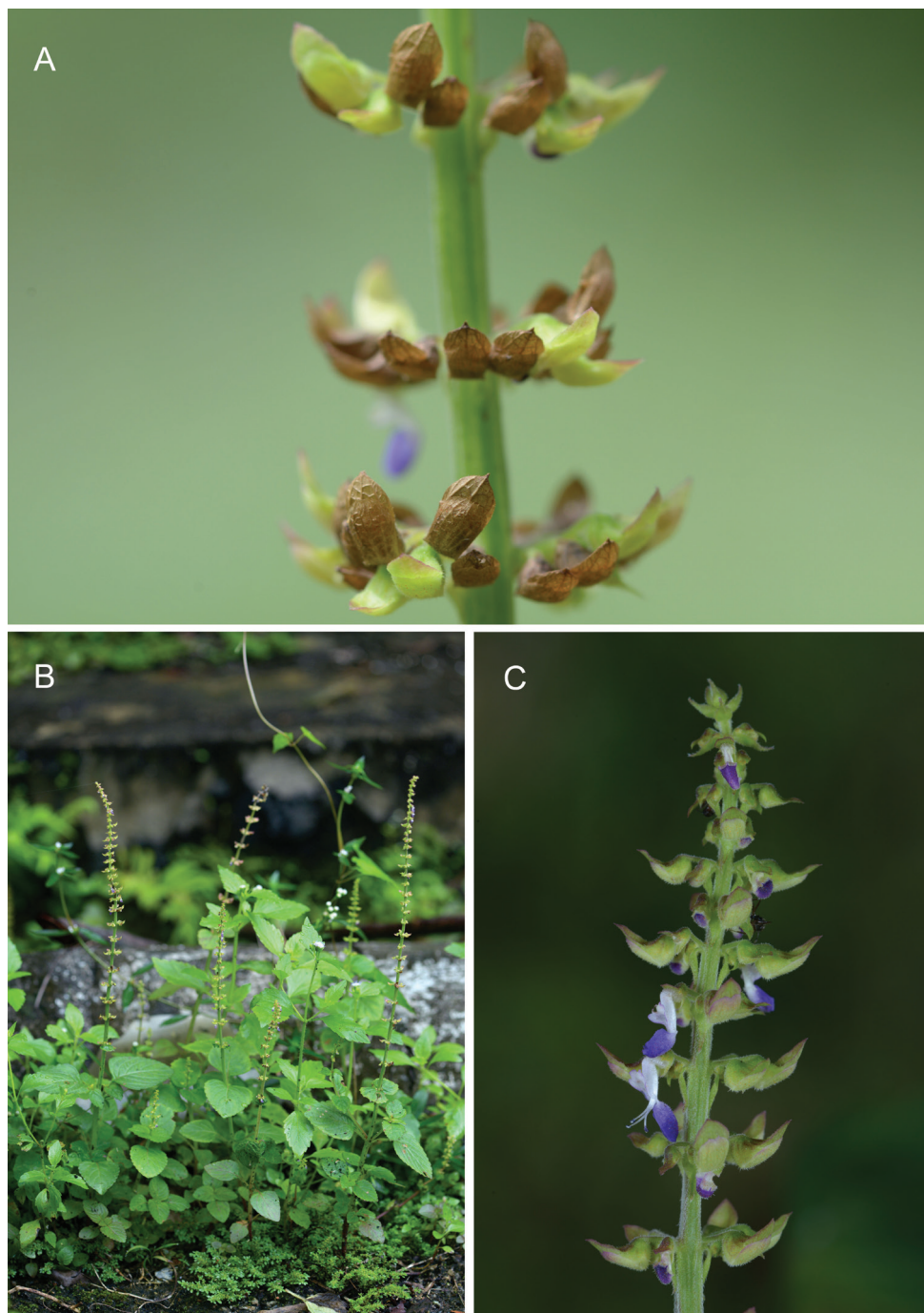


Figure 3. *Coleus monostachyus* (P.Beauv.) A.J.Paton subsp. *monostachyus* **A** portion of fruiting spike **B** habit **C** top of flowering spike. (Photographs by PT Ong).

hundred individuals. The hill is threatened by a proposal for a cement quarry. (Assessed by A.R. Rafidah).

Ecology. Restricted to a limestone karst hill at ca. 90–155 m on lower slopes of the hill, growing in soil-filled cracks in the limestone rocks on or near wet, shaded vertical cliffs or between deep pinnacles on a low summit, locally common in a restricted area. It is not known if it is short-lived or an annual plant.

Etymology. Named for Mohd. Hairul b. Mohd. Amin (b. 1984), field staff in the KEP Herbarium, Forest Research Institute Malaysia, who first collected this species (Figure 1).

Additional specimen examined. PENINSULAR MALAYSIA, **Kedah:** Baling Hill, Kampung Sungai Limau, 5°40.38'N, 100°53.23'E, 17 Nov 2011, Mohd. Hairul et al. FRI 54055 (K!, KEP!, SING!).

Note. In discussions with AJ Paton (*pers. comm.*), he pointed out that the type of *Plectranthus glabratus* (Benth.) Alston (now *C. paniculatus*) from Chennai (Madras) is a more robust plant with a thicker stem, bigger leaves and relatively shorter branches of the inflorescence compared with the three specimens from southern Thailand. *Coleus hairulii* resembles specimens from southern Thailand that are also all from limestone rocks (Suddee et al. 2004).

2. *Coleus kunstleri* (Prain) A.J. Paton in Paton et al., *Phytokeys* 129 (2019) 63.

Homotypic synonym: *Plectranthus kunstleri* Prain, J. Asiat. Soc. Bengal 74, 2: 706 (1907); Ridley, Fl. Mal. Penin. 2: 646 (1923); Keng, Gard. Bull. Singapore 24: 151 (1969), Fl. Malesiana Ser. I, 8: 392 (1978); Chin, Gard. Bull. Singapore 32: 160 (1979); Turner, Gard. Bull. Singapore. 47: 272 (1996 '1995'); Bramley, Fl. Malesiana Ser. I, 23: 293 (2019). Type: MALAYSIA. Peninsular Malaysia: Perak, Kuala Dipang, top of limestone hills, 130–200 m. Sept. 1885. *Kunstler* (*King's collector*) 8240 (holotype CAL; isotypes BM!, K!).

Description. Erect herb, almost shrubby, 0.5–1.2(–1.5) m high. **Stem** and branches finely puberulous, angular. **Leaves** with petiole 1–4.5 cm; lamina membranaceous, ovate to broadly ovate, 6–10(–13) × 4–6 cm, base truncate or shortly cuneate, margin elsewhere remotely crenate, apex acute, very sparsely pubescent on the main and secondary veins on both surfaces, sometimes with a whitish line down midrib. **Inflorescence** terminal, paniculate, 10–20 cm long, the lower branches of the inflorescence axis 5–6 cm, decreasing in length towards apex. Bracts ovate, acute, 2–3 mm long, caducous. Pedicels 3–4 mm long, glandular-puberulent. **Flowers** spreading, (not pendent); *calyx* obliquely campanulate, 1–2(–2.5) mm long, in fruit 5–7 mm, shortly hirsute and sparingly gland-dotted, unequally 5-lobed; upper lip ovate, broadly ovate or sub-rounded, lower lip with two lateral ovate-acute lobes shorter (about $\frac{3}{4}$) than the lower central lobes but later almost equal in fruit, the two central lobes subulate, connate

beneath; *corolla* waxy white or greenish white (*Kunstler* 8240) or purplish pink (*Chin* 1760), 7–8(–10) mm long, puberulent, tube sigmoid, decurved, only slightly gibbous near the base, posterior lip short, erect, anterior lip concave, boat-shaped, c. 4 mm long; *stamens* not exerted, lying within the anterior lip, free above point of insertion on the corolla tube. *Nutlets* oblong ovoid, black, *ca.* 1 mm long.

Distribution. Endemic in Peninsular Malaysia, Perak – Kinta (no specific locality) and Kuala Dipang; and Kedah – Langkawi, Pulau Langgun (Map 1).

Provisional conservation status. Critically Endangered B1ab(i,iii). Limestone hills in Perak are regularly visited for botanical collecting but this species has not been recollected there for over a hundred years and the majority of karst limestone hills in the Kinta Valley are heavily disturbed by quarries. The species was last collected in the 1970s from Pulau Langgun. (Assessed by A.R. Rafidah).

Ecology. Restricted to limestone rocks, in shade, at 50–200 m elevation, either in open habitats on top of a limestone hill to 200 m (*Kunstler* 8240) or on huge boulders rooting in rock crevices and on humus accumulated on boulders under shade of scattered trees at ca. 50 m (*Chin* 1760).

Etymology. H.H. Kunstler, a German botanist, who collected plants in 1880–1886 mostly from Perak, Peninsular Malaysia, for Sir George King, Director of Calcutta Botanic Garden. He is also recorded on herbarium labels as ‘King’s Collector’.

Additional specimens examined. PENINSULAR MALAYSIA. Perak: Kinta (*Kunstler* 7143, BM!). Kedah: Langkawi, Pulau Langgun (*Chin* 1760, KLU!).

3. *Coleus monostachyus* (P.Beauv.) A.J.Paton, *Phytokeys* 129 (2019): 76.

Homotypic synonym: *Plectranthus monostachyus* (P.Beauv.) B.J. Pollard, *Kew Bull.* 56: 980 (2001); *Solenostemon monostachyus* (P.Beauv.) Briq. in H.G.A.Engler & K.A.E.Prantl, *Nat. Pflanzenfam.* 4(3a): 359. 1897. Basionym: *Ocimum monostachyum* P.Beauv., *Fl. Oware* 2: 60, t. 95 (1818). Type: Africa, Benin, *Palisot de Beauvois s.n.* (holotype G, *n.v.*).

Subsp. *monostachyus*. Chung et al., *Nature in Singapore* 8: 1 (2015); Kiew, *Conservation Malaysia* 23: 6 (2016).

Note. The description is based on specimens from Peninsular Malaysia.

Description. Erect herb, almost shrubby, not aromatic, without tubers. *Stem* quadrangular, densely hairy on the angles, hairs descending ca. 0.5 mm long, green, woody but brittle, to 30 cm tall, ca. 4 mm diameter, at first unbranched, flowering at ca. 20 cm tall, soon branching near the base, internodes (2.5–)4.5–6.5 cm, branches ascending, to 29 cm long. *Leaves* held horizontally; petiole 2–5.6 cm long, narrowly winged in the upper third, grooved above, densely hairy on the angles; lamina broadly ovate, (3.5–)6–13.5 × (2.5–)4.5–11.5 cm, base rounded or slightly truncate, shortly decurrent into

the petiole, margin shallowly crenate, apex acute, tip rounded, membranous, glabrous and completely dull green above, pale beneath, lateral veins ca. 4 on either side of the midrib, impressed above, beneath prominent and finely short hairy. **Inflorescence** terminal on stem and branches, spicate, ca. 14 cm long in unbranched plants, in branched plants 21–38 cm, often with a subsidiary pairs at the base ca. 16.5–23 cm long, peduncle short 3–5 cm, 8–8.5 cm in inflorescences from the lower branches; peduncle and rachis quadrangular, finely pubescent on the angles; verticils 1–2 cm apart at the base, ca. 0.5 cm apart near the apex, each verticil with two sessile, condensed cymes each with 3–5 flowers. Bracts broadly ovate apex caudate, keeled, ca. 4 × 2.5 mm, pale green, deflexed and appressed to rachis. Pedicels reddish on the upper side, slightly excentrically attached behind the posterior lip, minutely pubescent, 1.5–2 mm long. **Flower** with *calyx* funnel-shaped, pale green, densely pubescence 2–3.5 mm long, in fruit 5–5.5 mm long, upper lip curved upwards, oval, minutely punctate at the apex, ca. 4–4.5 mm long, lower lip broadly oval with two fine apical teeth, curved upwards and closing the throat; **corolla** 8.5–10 mm long, minutely pubescent outside, tube abruptly decurved above the calyx, dilating to the mouth, white except for the upper lip and lateral lobes outlined in deep purple and the deep purple lower lip 3–4 mm long; *stamens* fused at base, filament white, glabrous, anthers, ca. 0.25–0.3 mm long, deep purple; *stigma* ca. 0.4 mm long, positioned above the anthers. **Nutlets** 4, plain brown, broadly ovoid, almost 1 mm long, producing mucilage when wet.

Distribution. Native in tropical West Africa, this is a recent introduction into Peninsular Malaysia and Singapore, probably an escape from the horticultural trade (Kiew 2016). First collected in Peninsular Malaysia in 2003, it is now naturalised and since about 2010 has rapidly become widespread. Apparently, it has not yet been recorded from Southeast Asia (Suddee et al. 2004).

Ecology. In Peninsular Malaysia, it is found in light shade to fully exposed conditions in almost all lowland habitats associated with disturbance, e.g. roadsides, plantations, farms and gardens. It begins to flower at 20 cm tall, and its many-flowered spikes produce fruits that shatter at the slightest touch, scattering hundreds of seeds. This makes it a weed that is extremely difficult to eradicate. The seeds are sticky and may be dispersed by animals or water but long distance dispersal is probably effected by soil on vehicles or in planting material. It therefore threatens to become a noxious weed in nurseries and gardens where it cannot be exterminated by herbicides.

Etymology. Latin, *mono-* = one or single; *stachys* = ear of corn or spike, referring to the inflorescence.

Additional Peninsular Malaysian specimens examined. **JOHOR:** Senterre et al. s.n. 30 Sept 2003 (KEP!). **KELANTAN:** Felda Chiku, Nazrul et al. FRI 83177 31 August 2015 (KEP!). **SELANGOR:** Bukit Nanas, Norzielawati et al. FRI 83050 (KEP!), Kepong, Forest Research Institute Malaysia, Kiew FRI 81947 13 Jan 2016 (KEP!); Rasa, Kiew FRI 655542 17 April 2010 (KEP!); Subang Rafidah et al., FRI 75694 4 April 2013 (KEP!).

4. *Coleus rafidabiae* Kiew, sp. nov.

urn:lsid:ipni.org:names:77234077-1

Diagnosis. Unusual among Malaysian *Coleus* species in its branched inflorescence with yellowish white flowers (Figure 5). It resembles *C. calcicola* Murata from Thailand in its branched thyrsoid inflorescence with 1-flowered spaced cymes, in its calyx and yellowish white corolla but it is different in its larger laminas, 8.5–13.5 × 4–5.5 cm with longer petioles 2.5–8 cm long (vs. laminas 3–8 × 1.5–3 cm with petioles 0.5–1.5 cm long in *C. calcicola*).

Type. PENINSULAR MALAYSIA. Kelantan: Kuala Betis, Gunung Biol, 4°54.01'N, 101°45.33'E, 19 July 2017, Imin, Ong & Wan Shafiq FRI 87271 (holotype KEP!; isotype K!).

Description. Erect perennial, rather shrubby herb, to ca. 1 m tall. **Stems** brown and woody, branched, quadrangular, internodes 2–3 cm apart, ca. 4 mm thick, glabrous. **Leaves:** petiole slender, (2.5–)5.5–8 cm long, glabrous; lamina broadly elliptic to slightly ovate, 8.5–13.5 × 4–5.5 cm, base cuneate to truncate, margin crenate, apex acuminate, acumen 0.5–1.5 cm long, puberulous and densely dotted with minute glands on both sides; midrib white; veins inconspicuous beneath, 6–7 pairs on either side of midrib, midrib and veins slightly impressed above, slightly prominent beneath. **Inflorescence** terminal, branched lax thyrses, to 19 cm long, peduncle ca. 3.5 cm long, branches 5–6 cm long, 1.2–2.5 cm apart, glabrescent with a few minute hairs persisting near the apex, cymes 1-flowered, spaced on the branches. Bracts sessile, ovate, apex acute, 2–3 mm long, ciliate, pubescent, early caducous. Pedicels pubescent with gland-tipped hairs, ca. 2 mm long at anthesis, 3–4 mm in fruit. **Flower** with *calyx* campanulate, yellowish, pubescent with gland-tipped hairs, ca. 3.5 mm long at anthesis, in fruit 6–8 mm long, posterior lip erect, ovate, shortly acuminate at apex, constricted at base, 2–2.5 × 3–3.5 mm, margin entire; anterior lip 3.5–4 × 2–2.5 mm deeply divided into two teeth equal in length, ca. 2 mm long, slightly longer than posterior lip, lateral teeth obovate, 2–3 × 2–3 mm, spreading; tube 10-veined; *corolla* yellowish white, 7–12 mm long, minutely pubescent; posterior lobes minute rounded at apex; anterior lip ovate-oblong, 3–5 mm long, concave, shortly pubescent inside; tube ca. 7 mm long, not abruptly decurved above the calyx, dilated towards the throat; *stamens* included in anterior corolla lip; *style* bifid, subequal to anterior stamens; nectary exceeding ovary. **Nutlets** brown, ellipsoid, 1.75 mm long, 1.25 mm wide, smooth.

Distribution. Endemic in Peninsular Malaysia, Kelantan: Kuala Betis, Gunung Biol. Known only from two specimens from the type locality (Map 1).

Provisional conservation status. Critically Endangered B1ab(i,iii,iv). The species occurs on a single karst limestone hill that lies outside the network of Totally Protected Areas. It is known from a single small population. (Assessed by A.R. Rafidah).

Ecology. Near the summit of a karst limestone hill at 325 m altitude.

Etymology. Rafidah binte Abdul Rahman (b. 1981), KEP botanist, active contributor to revisions for the Flora of Peninsular Malaysia and the team leader of the project



Figure 4. *Coleus rafidabiae* Kiew, sp. nov. **A** AR Rafidah on limestone hill with a *Monophyllaea* **B** inflorescence **C** flowering plant.

‘Towards a Conservation Strategy/Policy for Limestone Hills in Peninsular Malaysia: Understanding and Documenting Plant Biodiversity with Focus on Kelantan and Perlis Limestone Hills’ funded by the National Conservation Trust Fund (Figure 4).

5. *Coleus scutellarioides* (L.) Benth.

in Wall., Pl. Asiat. Rar. 2: 15 (1830); Keng, Gard. Bull. Singapore 24: 51 (1969). *Coleus scutellarioides* var. *scutellarioides* Keng, Gard. Bull. Singapore 24: 53 (1969). Basionym: *Ocimum scutellarioides* L., Sp. Pl. ed. 2, 2: 834 (1763) (as *Ocimum*). Type: Majana (alba et rubra) *in* Rumphius, Herb. Amboin. 5: 291, t. 101 (1747), (lectotype, designated by Merrill, Interpr. Rumph. Herb. Amboin.: 460 (1917).) Homotypic synonyms: *Plectranthus scutellarioides* (L.) R.Br., Prodr. Fl. Nov. Holl.: 506 (1810); Blume, Bijdr. Fl. Ned. Ind.: 837 (1826); Keng, Fl. Malesiana Ser. I, 8: 389 (1978); Suddee et al., Kew Bull. 59: 410 (2004); Kiew *in* Henderson’s Malaysian Wild Flowers Dicotyledons: 298 (2014); Bramley, Fl. Malesiana Ser. I, 23: 296 (2019). *Solenostemon scutellarioides* (L.) Codd, Bothalia 11: 439 (1975); Turner, Gard. Bull. Singapore 47: 273 (1996 ‘1995’). Heterotypic synonyms: *Coleus atropurpureus* Benth. *in* Wall., Pl. Asiat. Rar. 2: 16 (1830); Prain, J. Asiat. Soc. Beng. 74, 2: 706 (1907); Ridley, Fl. Mal. Penins. 2: 646 (1923). Syntypes: Singapore Wall. Cat. 2733A (syntypes K!, K-W!). *Coleus blumei* Benth., Labiat. Gen. Sp.: 56 (1832). Type: Java, *Blume s.n.* (lectotype L, designated by Suddee et al., Kew Bull. 59: 410 (2004).)

Note. Here are included only references that cite Malaysian specimens. For synonymy under *Coleus* see Paton *et al.* Phytokeys 129 (2019) 1–158, for *Plectranthus* for continental South East Asia see Suddee et al. (2004) and for Malesia see Bramley (2019).

The description provided here refers only to wild plants from Peninsular Malaysia.

Description. Erect or ascending sparsely branched herb, 30–60 cm tall, aromatic, without tubers. **Stem** and branches finely pubescent to glabrous. **Leaves** with petiole 1–5 cm; lamina ovate, (2.5–)4–7.5 × (2–)3–4 cm, plain dark green, base rounded or sometimes truncate, margin crenate with rounded teeth, apex acute, tip rounded, upper surface glabrous or with short hairs, lower surface pubescent on the main and secondary veins. **Inflorescences** terminal, unbranched spike or sometimes branched at base, to 40 cm long, flowers in few- to many-flowered verticils or in irregularly branched cymes, peduncles of the lateral cymes short or elongated. Bracts narrowly ovate to ovate, apex acute, pubescent, 2–8 mm long, caducous. Pedicels 1–2 mm long in flower, extending in fruit. **Flower:** calyx campanulate, 2–3 mm long, in fruit 4–8 mm, minutely pubescent; posterior lobe broadly ovate, subacute, recurved and erect, two lateral lobes of anterior lip short, oblong-obtuse, truncate or rounded, rarely mucronate with a tiny apiculate apex, about half as long as central lobes of anterior lip, these oblong to subulate, connate for two thirds their length, acuminate at apex, longer than posterior lobe, anterior lobe divided into 2 points; corolla 8(–13)–16 mm

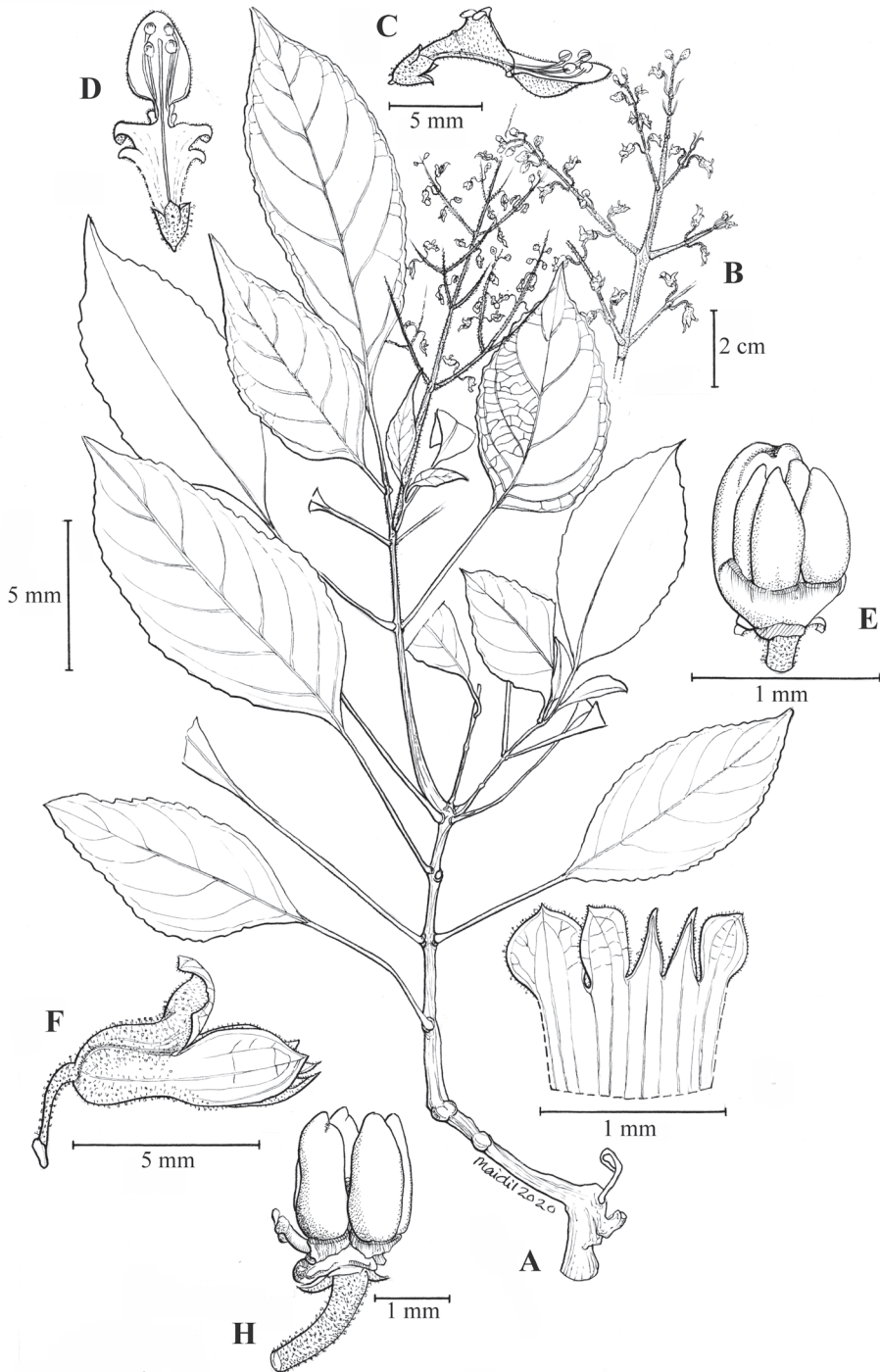


Figure 5. *Coleus rafidabiae* Kiew **A** flowering plant **B** portion of inflorescence **C** flower side view **D** flower from above **E** ovary **F** calyx in fruit **G** calyx opened **H** seeds side view. (All from FRI 87271. Drawn by N. Mohamad Aidil).

long, tube abruptly decurved above the calyx, white, 4–5, ca. 7.5 mm long, dilating widely to the throat, with scattered hairs; posterior lip with lobes pubescent, the two central lobes rounded, anterior lip deep or rosy violet, 4–5(–9) mm long; *stamens* not exerted from anterior lip. **Nutlets** broadly ovate or orbicular, brown to black, shining, 1–1.2 mm long, minutely tuberculate, mucilaginous when wet.

Note. Ridley (1923) and Burkill (1966) considered the coleus of gardens (i.e. *Coleus blumei*) was introduced from Java and was distinct from Malayan *C. atropurpurea*. These two species are recognized as conspecific and are currently known as *Coleus scutellarioides* (Paton et al. (2019) with the result that the description has expanded to include the huge range in leaf size, shape and colour of the ornamental forms that are not seen in the wild form of Malaysian population. The description above is based on the wild form that has smaller, plain green leaves. Unlike *Scutellaria discolor* Wall. ex Benth., it is not found deep in forest and is seldom found in flower. That it is usually associated with forest edge, often close to habitation, suggests that many of these populations have been established for their medicinal properties.

On account of it being extremely variable, Keng (1978) and Bramley (2019) did not recognise subspecific taxa, there being too many intermediate specimens. A view that is followed here.

Distribution. SE Asia (India, Myanmar, Thailand, Indo-China, S. China, Taiwan, throughout Malesia to Australia, Melanesia (Solomon Is.) and Polynesia. In Peninsular Malaysia, widespread (Johor, Melaka, Penang, Perak, Pahang and Selangor).

Provisional conservation status. Least Concern. (Assessed by A.R. Rafidah).

Ecology. In Peninsular Malaysia, from forest edge and often near villages throughout the lowlands to 350 m elevation (Kiew 2014), sometimes in forest by streams but not from limestone hills as reported by Keng (1978). That the forest localities are often close to villages and it has medicinal uses suggests these populations may have been planted, particularly because this species seldom flowers but can easily be propagated by stem cuttings.

Etymology. Greek *-oides* = resembling; *Scutellaria* L., a genus in Lamiaceae.

Uses. In Peninsular Malaysia, it has always been commonly known as ‘coleus’ and in Malay as *pokok ati-ati*. Cultivated forms are popular ornamental plants and come in a great variety of multicoloured, variegated foliage. In Cameron Highlands, they are grown in great quantities for home gardens. The colour of plants grown in the highlands, ca. 2000 m, is more vivid than those grown in the lowlands.

It is a minor medicinal plant used to cure a wide range of ailments. Burkill (1966, pg. 643) reported it was a remedy for heart disease, heart burn, inflammation of heart; sensitive skin, stimulates digestion and for congestion of the liver that causes swellings of the hands and feet, amongst other ailments. Among the aboriginal population, the Besis people (now more commonly known as the Mah Meri) call it *torek*, and plant it around their graves. They also traditionally use it as the brush used for sprinkling holy rice-gruel over a new clearing (Burkill 1966).

Peninsular Malaysia specimens examined (* indicates specimens collected in villages that are presumed to be cultivated): **JOHOR:** Pulau Aor *Fielding s.n. 1892*. **MELAKA:**

Griffith 3957* 1845 (K), *Burkill* 35515*. **PAHANG:** Bentong *Burkill & Md Haniff* SFN 16536; Pekan *Burkill & Md Haniff* SFN 17728; Sungai Telom Poore 538 roadside; Tapah *Burkill & Md Haniff* SFN 13527. **PENANG:** *Wallich s.n.** 1829 (K); *Curtis* 466 Paya To-bong 1892; *Sinclair* SFN 39391* village, Pulau Betong. **PERAK:** Bagan Datuk *Md Haniff* SFN 16273; Batu Kurau *Md Haniff & Sa'at* SFN 13272; Gerik *Burkill & Md Haniff* SFN 13647; Kampung Gajah *Md Haniff* SFN 16299; Kota Setir *Md Haniff* SFN 15924; Kuala Kangsar to Kota Lama, *Md Haniff* SFN 15562; Lubuk Merbok, *Md Haniff* SFN 15985, *Md Haniff* SFN 16004; Tanjung Malim – *Burkill & Md Haniff* SFN 13500; Telok Anson *Burkill & Md Haniff* SFN 15945, *Md Haniff* SFN 10315. **SELANGOR:** Ulu Gombak *T & B* 2823 roadside [leaves multicoloured], *Md Nur* SFN 34232 by stream; Kuala Lumpur *Ridley s.n.** 1890 bunga ati-ati merah. **TERENGGANU:** Bundi *Rostado s.n.*

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Dipteris shenzhenensis, a new endangered species of Dipteridaceae from Shenzhen, southern China

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Abstract

Dipteris shenzhenensis, a new species of ferns from Shenzhen, Guangdong, southern China, is identified and described. It closely resembles *D. chinensis* but possesses several unique traits, such as long rhizome scales, castaneous stipe, and abaxially pale fronds with two fan-shaped fronds connected by a broad wing. Molecular evidence showed that *D. shenzhenensis* is allied to *D. conjugata*, whereas it has morphologically significant differences ($P < 0.05$) on the basis of quantitative trait statistical analysis. Overall, the morphological evidence, taken together with the result of cpDNA indicated that *D. shenzhenensis* is a distinct species.

Keywords

fern, Gleicheniales, morphology, phylogeny, quantity traits, taxonomy

Introduction

Dipteris Reinw. is one of two genera in Dipteridaceae (Zhang et al. 2013; PPG I 2016), and is considered as an early-diverging leptosporangiate fern lineage related to the Gleicheniaceae (Schuettpelz and Pryer 2007; Lehtonen 2011). The genus has rare components consisting of about eight species, and is restricted to Indo-Malaysian Islands, including north-eastern India, southern China, and from the southern Ryukyus to north-east Queensland (Australia) and Fiji (Kramer 1990; Zhou et al. 2016; Choo and Escapa 2018; Zhang et al. 2013). The morphology of *Dipteris* is characterized by having long creeping rhizomes and fan-shaped fronds possessing elaborately anastomosing veins with free veinlets in the areoles (Bomfleur and Kerp 2010; Tidwell and Ash 1994).

In August 2020, during botanical research on Mt. Qiniangshan in Shenzhen, Guangdong, southern China, a unique species of *Dipteris* was documented on rocks in evergreen broad-leaf forest. The species is so similar to *D. chinensis* Christ that it has always been interpreted as the latter (Yan 2017). Upon closer carefully specimen identification and comparison with other species in *Dipteris*, we found that this unknown species possesses several unique characteristics, the most striking of which is awfully long rhizome scales. Furthermore, we constructed the molecular phylogeny of *Dipteris* to obtain a phylogenetic insight into the species. The morphological evidence taken together with the result of cpDNA validated it as a new species.

Materials and methods

Morphological analyses

The features of rhizome scales were obtained using a Leica M205A dissecting microscope. Morphology of spores was observed with Phenom Pro scanning electron microscope after being sputter-coated with gold. Measurements were made from mature and intact specimens. For length and width of lobes, each specimen was measured six times using ImageJ software (Collins 2007), followed by taking an average. All images of specimens were provided by the National Specimen Information Infrastructure (<http://www.nsii.org.cn>), Global Biodiversity Information Facility (<https://www.gbif.org/>), and JSTOR (<https://plants.jstor.org/>). Voucher specimens were deposited in the National Orchid Conservation Center of China (NOCC) and Shenzhen Fairy Lake Botanical Garden Herbarium (SZG).

Phylogenetic analyses

Ten samples, representing five species were used in this study. Apart from the sequences of *Dipteris conjugata* Reinw. (Metzgar et al. 2008) and the outgroup *Cheiropleuria integrifolia* (D. C. Eaton ex Hook.) M. Kato, Y. Yatabe, Sahashi & N. Murak. (Schuettpelz and Pryer 2007) were downloaded from GenBank, all other sequences were newly generated (Table S1). Five chloroplast DNA (cpDNA) regions (*rbcL*, *atpA*, *rps4*, *rbcL-accD* and

Table 1. List of primers used in the study.

	Primer	Sequence	Reference
<i>rbcL</i>	ESRBCL1F	ATGTCACCACAAACGGAGACTAAAGC	Schuettpelz and Pryer (2007)
	ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz and Pryer (2007)
<i>atpA</i>	ESATPF412F	GARCARGTTTCGACAGCAAGT	Schuettpelz et al. (2006)
	ESTRNR46F	GTATAGGTTTCRARTCCTATTGGACG	Schuettpelz et al. (2006)
<i>rps4</i>	RPS5*	ATGTCCCGTTATCGAGGACCT	Nadot et al. (1994)
	TRNS*	TACCGAGGGTTCGAATC	Souza-Chies et al. (1997)
<i>trnG-trnR</i>	TRNG1F ^a	GCGGGTATAGTTTAGTGGTAA	Korall et al. (2007)
	TRNR22R ^a	CTATCCATTAGACGATGGACG	Korall et al. (2007)
<i>rbcL-accD</i>	RBCL1187F ^a	GGAACYTTGGGACATCCTTGG	Korall et al. (2007)
	ACCD816R ^a	CCATGATCGAATAAAGATTGACG	Ebihara et al. (2003)

Table 2. Best nucleotide substitution model in phylogenetic analyses.

Partition names	MrBayes	Sites
<i>atpA</i> , <i>trnG-trnR</i>	GTR	2 098
<i>rbcL</i> , <i>rps4</i>	GTR+G	1 279
<i>rbcL-accD</i>	GTR+I+G	806

trnG-trnR) were extracted, amplified and sequenced following Wei et al. (2021). Primers used for polymerase chain reaction (PCR) amplification and sequencing are shown in Table 1. All sequences newly generated in this study were deposited in GenBank (see Table S1 for accession numbers). The cpDNA sequences were assembled and edited using SeqMan v.7.1.0 (DNASTAR, USA), then aligned using MEGA v.7.0 (Kumar et al. 2016). Alignments of five genes were concatenated using PhyloSuite (Zhang et al. 2020), and best nucleotide substitution model (Table 2) was used on the basis of Akaike Information Criterion with PartitionFinder2 (Lanfear et al. 2017) integrated into PhyloSuite. Bayesian analysis was constructed using MrBayes v.3.2.6 (Ronquist et al. 2012) with four Markov chains for 1,000,000 generations, sampling every 100 generations. Standard deviation of split frequencies was controlled within 0.01 to ensure the convergence of the independent runs. The majority-rule consensus tree and estimation of the posterior probabilities (PP) were performed with the first 25% of samples discarded as burn-in.

Results and discussion

Morphological comparison

Dipteris shenzhenensis has been confused with *D. conjugata* and *D. chinensis* because of similar gross morphology. This is especially true of dried herbarium specimens. Most specimens of *D. shenzhenensis* were formerly identified as *D. chinensis* in herbaria because of the presence in similar fronds morphology. We studied most online specimens of these three species and conducted quantitative trait statistical analysis. The result showed that *D. conjugata* displayed significant differences compared to *D. shenzhenensis* and *D. chinensis* in the length of lobes (LL) ($P < 0.0001$), the width of lobes (WL)

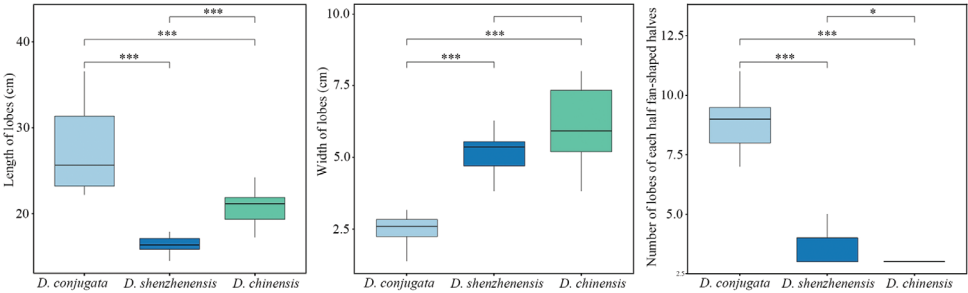


Figure 1. Boxplot (median and interquartile values) for the length of lobes (left), the width of lobes (center), and the number of lobes of each half of the fan-shaped fronds (right) amongst *Dipteris conjugata*, *D. shenzhenensis* and *D. chinensis*. Significant differences between species are represented with * ($P < 0.05$), ** ($P < 0.001$), and *** ($P < 0.0001$).

Table 3. Comparisons of morphological characters and elevational range of *Dipteris shenzhenensis* and *D. chinensis*.

Character	<i>D. shenzhenensis</i>	<i>D. chinensis</i>
Rhizome	Long and creeping	Creeping
Rhizome scales	Dark brown to black, 6.8–8.0 × 0.06–0.27 mm	Brown, 3.74–4.00 × 0.04–0.26 mm
Fronds	Abaxially pale; base with broad wings	Abaxially green; base without wings
Lobes	4–6 × 14.5–18.0 cm	4–8 × 17–23 cm
Stipe	Castaneous, 40–85 cm	Stramineous to brown, 25–50 cm
Spores	18.5–19 × 37.5–39 μm	21.5– 25.5× 32.5–39 μm
Elevation	70–200 m	500–2100 m

($P < 0.0001$), and the number of lobes for each half of the fan-shaped fronds (NL) ($P < 0.0001$) (Fig. 1). *Dipteris shenzhenensis* and *D. chinensis* were indistinguishable from the WL ($P > 0.05$), with the significant difference being in the LL ($P < 0.0001$) and the NL ($P < 0.05$) (Fig. 1). The former was also readily distinguished from the latter by having stiffer rhizome scales and castaneous stipe, as well as being pale abaxially (Fig. 2, Table 3). Micromorphological comparison indicated that the rhizome scale length of *D. shenzhenensis* was twice that of *D. chinensis* (Fig. 2). Most notably, the two fan-shaped fronds of *D. shenzhenensis* were connected by broad wings at the base in contrast to these of *D. conjugata*, *D. chinensis*, and other species in *Dipteris* (Figs. 2 and 3).

Phylogenetic analyses

To further determine the relationships among the three species, we conducted Bayesian analysis using the five chloroplast gene regions (*rbcL*, *atpA*, *rps4*, *rbcL-accD*, and *trnG-trnR*). With the *Cheiropleuria integrifolia* as outgroup, the phylogram showed that *Dipteris* can be classified into four well-supported clades. *Dipteris shenzhenensis* was typically well supported as monophyletic and strongly supported as sister to *D. conjugata* (PP = 1.0) (Fig. 4). Although *D. shenzhenensis* has been misidentified as *D. chinensis*, the relationship between the two species was not close. In addition, because there were missing data form many samples, the several clades showed a relatively low resolution

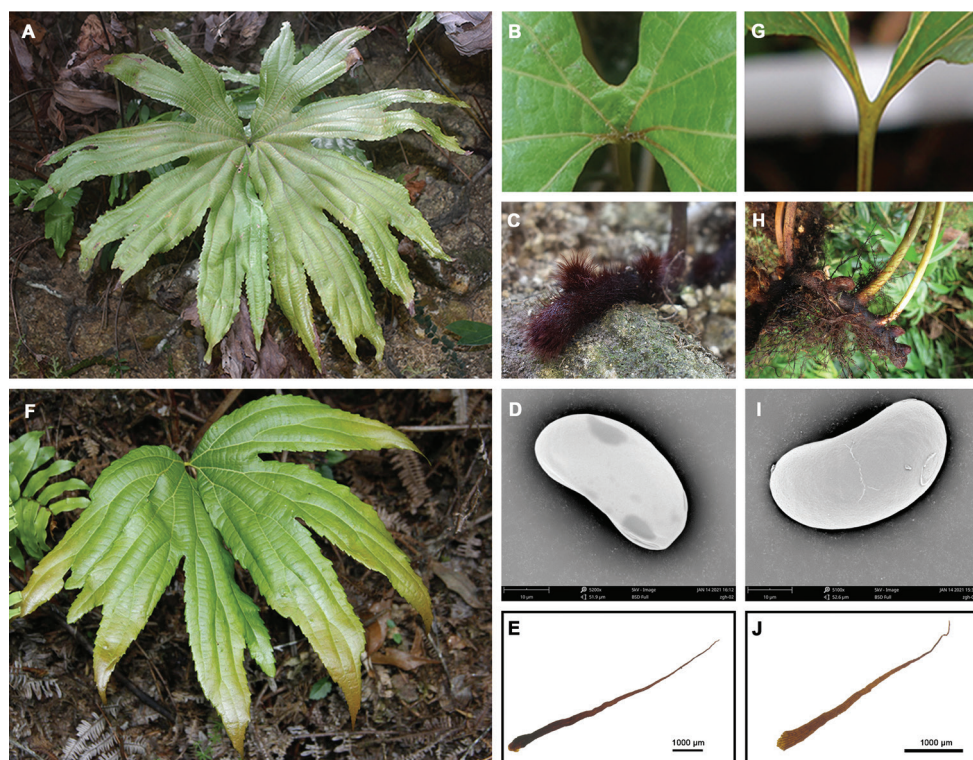


Figure 2. Morphological features of *Dipteris shenzhenensis* Y.H.Yan & Z.Y.Wei (A–E) and *D. chinensis* (F–J). **A** fan-shaped frond of *D. shenzhenensis* **B** the frond base with broad wing of *D. shenzhenensis* **C** rhizome of *D. shenzhenensis* **D** spore of *D. shenzhenensis* **E** rhizome scale of *D. shenzhenensis* **F** fan-shaped frond of *D. chinensis* **G** the frond base without wing of *D. chinensis* **H** rhizome of *D. chinensis* **I** spore of *D. chinensis* **J** rhizome scale of *D. chinensis*.

in Bayesian phylogenetic analyses. We will, in future, use more molecular markers or utilize high-throughput sequencing to obtain a better topology with resolution.

Overall, based on the above morphological comparison and molecular phylogenetic analyses, *D. shenzhenensis* is clearly different from *D. conjugata* and *D. chinensis*. We therefore here describe *D. shenzhenensis* as a new species.

Taxonomic treatment

Dipteris shenzhenensis Y.H.Yan & Z.Y.Wei, sp. nov.

urn:lsid:ipni.org:names:77234195-1

Figs 2 and 3

Diagnosis. The new species is similar to *D. chinensis*, but differs in rhizome scales being longer (6.8–8.0 mm vs. 3.74–4.00 mm), in the base and color of fronds (base with broad wings, abaxially pale vs. base without wings, abaxially green), and in stipe color (castaneous vs. stramineous to brown).

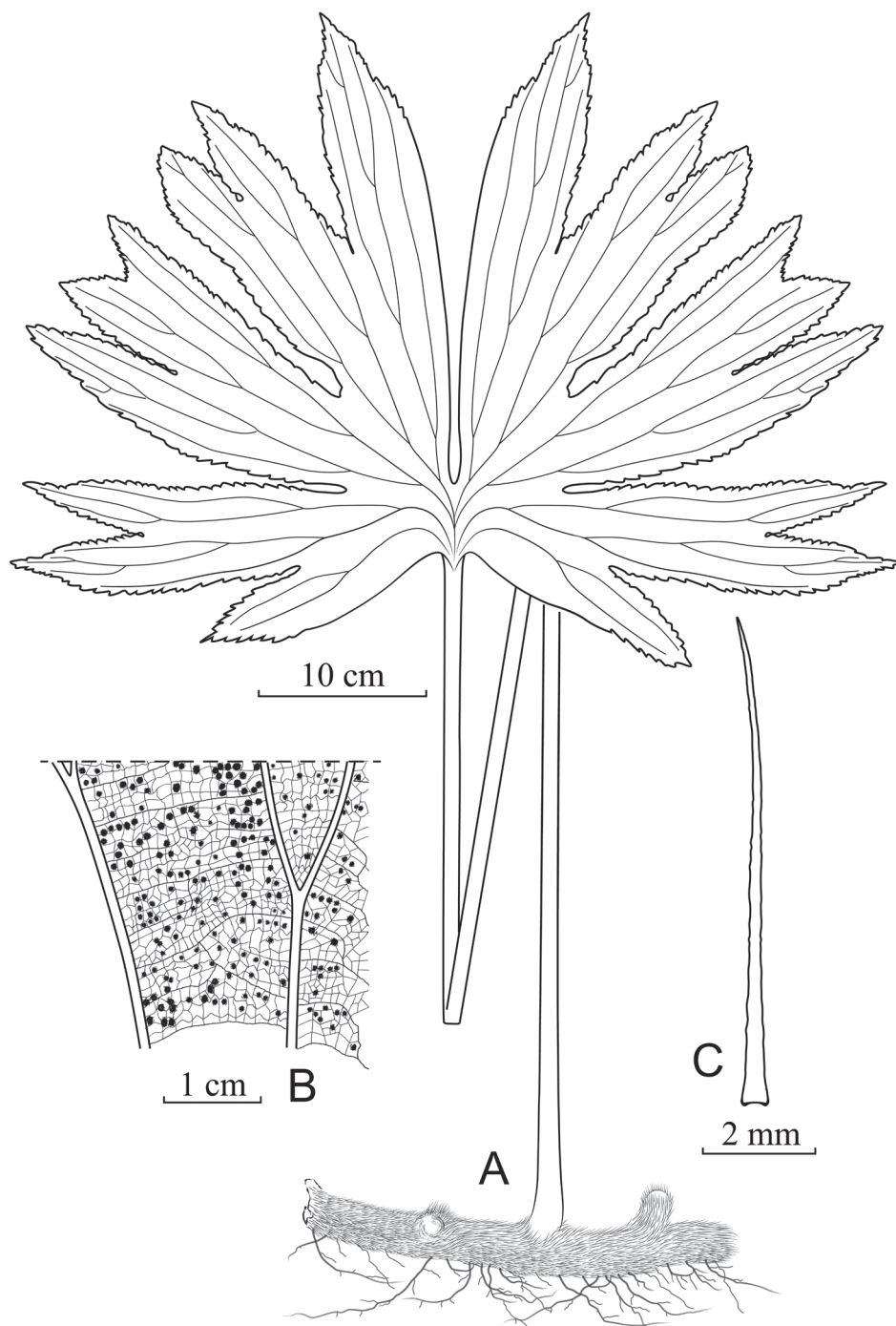


Figure 3. *Dipteris shenzhenensis* Y.H.Yan & Z.Y.Wei **A** habit **B** details of a lamina showing the venation and the distribution of sori **C** rhizome scale showing the profile and length (drawn by Zuo-Ying Wei & Li-Jun Chen, based on the type material at SZG).



Figure 4. The phylogenetic tree inferred by the Bayesian inference with posterior probabilities above the branches.

Type. CHINA. Guangdong Province: Shenzhen City, Mt. Qiniangshan, elev. ca. 82 m, 16 August 2020, Y. H. Yan et al. YYH15638 (*holotype*: SZG!; *isotype*: NOCC!)

Description. Plants. terrestrial on rocks, 0.5–1.0 m tall. **Rhizome.** long-creeping, ca. 1 cm in diam., densely scaly. **Rhizome scales.** spreading, dark brown to black, stiff, margin almost entire, $6.8\text{--}8.0 \times 0.09\text{--}0.27$ mm, lanceolate, apex long acuminate, acumens up to 2–3 mm long; **Stipe.** glabrous except at the very base, castaneous, 30–85 cm. **Fronds.** slightly funnel-shaped, divided into 2 fan-shaped fronds, each half deeply divided into 4 to 5 unequal lobes, lobes shallowly divided one or more times, with 8–10 ultimate lobes in each half of lamina, abaxially glabrous and pale; base with broad wings; venation reticulate, visible on both surfaces, prominent abaxially. **Lobes.** margins serrate, apices acute, $4.5\text{--}12.0 \times 10.5\text{--}17.0$ cm, reticulated venation network. **Spores.** spreading, monolete, $18.5\text{--}19.0 \times 37.5\text{--}39.0$ μm , glabrous.

Distribution and habitat. So far only known from Shenzhen City, Guangdong Province, southern China. It is distributed in Mt. Qiniangshan, growing on rocks at elevation of 70–200 m in evergreen broad-leaf forest.

Chinese name. Shen-zhen-shuang-shan-jue (深圳双扇蕨).

Etymology. *Dipteris shenzhenensis* was discovered in the City of Shenzhen located in Guangdong Province, southern China. The specific epithet, therefore, is from this city name.

Conservation status. *Dipteris shenzhenensis* is currently found in only one location in Mt. Qiniangshan, Shenzhen City, Guangdong Province, southern China. The predicted Area of Occupancy (AOO) for the species is no more than 5,000 m². This species prefers to grow in low and opening mountain areas and is very likely to experience human disturbance. Over the past 20 years, the authors have observed that *D. shenzhenensis* showed signs of decline with the recovery of macrophanerophytes in Mt. Qiniangshan. Following the International Union for Conservation of Nature (IUCN) Categories and Criteria (IUCN 2019), we regard the newly discovered *D. shenzhenensis* as of Critically Endangered (CR) (B1a; B2ab).

Additional specimens examined. CHINA. Guangdong Province, Shenzhen City, Mt. Qiniangshan, elev. ca. 90 m, 16 August 2020, Y. H. Yan et al. YYH15637

(NOCCI!); loc. cit., elev. ca. 200 m, 27 December 2003, *Y. H. Yan* 1937 (HUST!); loc. cit., elev. ca. 150 m, 8 November 2002, *Y. H. Yan* 885 (HUST!); loc. cit., 17 August 2002, *S. Z. Zhang* et al. 011036-A1 (SZG, photo!); loc. cit., elev. ca. 75.38 m, 18 November 2015, *L. Jiang* & *Y. P. Chen* JL00328 (KUN, photo!); loc. cit., elev. ca. 70 m, 22 February 2003, *S. Z. Zhang* et al. 012037-A (SZG, photo!); loc. cit., elev. ca. 70 m, 22 February 2003, *S. Z. Zhang* et al. 012037-B (SZG, photo!); loc. cit., 31 March 2000, *F. W. Xing* & *Y. X. Zhang* 12374 (IBSC, photo!). s. coll. 0685742 (IBSC, photo!)

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<https://doi.org/10.1016/j.palwor.2015.11.008>

Supplementary material I

Table S1. List of species and GenBank accession numbers used in the present study

Authors: Zuo-Ying Wei, Yu-Feng Gu, Zeng-Qiang Xia, Li-Jun Chen, Ting Wang, Shou-Zhou Zhang, Guo-Hua Zhao, Jian-Bing Chen, Jian-Guo Cao, Yue-Hong Yan

Data type: molecular data

Explanation note: Dash (—) indicates unavailable data.

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Link: <https://doi.org/10.3897/phytokeys.186.73739.suppl1>

Supplementary material 2

Table S2. Specimen information used for morphological comparison

Authors: Zuo-Ying Wei, Yu-Feng Gu, Zeng-Qiang Xia, Li-Jun Chen, Ting Wang, Shou-Zhou Zhang, Guo-Hua Zhao, Jian-Bing Chen, Jian-Guo Cao, Yue-Hong Yan

Data type: species data

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Systematic reinstatement of highly sacred *Ficus krishnae* based on differences in morphology and DNA barcoding from *Ficus benghalensis* (Moraceae)

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Abstract

Ficus krishnae is considered as native to India and is well-known for the peculiarity in nature of its cup-shaped leaves where both the vernacular name (Krishna Fig) and specific epithet were derived. The taxonomic status of *Ficus krishnae* is still unclear and currently treated as a subspecies or variety under *Ficus benghalensis*. In the present study, morphological characters and molecular analysis were employed to address their species delimitation. The spacer markers ITS2 and *trnH-psbA* were used for constructing phylogenetic trees along with morphometric analysis. *Ficus krishnae* distinctly differs from *Ficus benghalensis* by having cup-forming leaves and the nature of the aerial roots, stipules, petioles, ostiolar bracts of the receptacle, DNA content, chromosome differences and nodal anatomy. The results showed that the highest divergence is observed in *trnH-psbA* (20.8 ± 12.2), followed by ITS2 (5.7 ± 3.2). The phylogenetic tree construction using Bayesian analysis showed a divergent boundary between the two species suggesting that *F. krishnae* could be an independent species, not a variety of *F. benghalensis*. The present study's findings support the view that these two floras can be treated as different species.

Keywords

Bayesian analysis, conspecifics, *Ficus*, ITS2 region, species delimitation

Introduction

Ficus L. (Fig) is one of the largest and most diverse genera of angiosperms in the family Moraceae, comprising 750 species, mainly distributed in tropical and subtropical regions of the world (Berg 1989; Berg and Corner 2005; Pederneiras et al. 2015). Fig trees were characterized by a unique kind of closed inflorescence named syconia and provide a breeding place for fig-wasp pollinators (Wiebes 1979). Recently, the checklist of Indian *Ficus* species was updated, and reported about 89 species and 26 infraspecific taxa, mainly distributed in north Eastern states, peninsular regions, and Andaman and Nicobar Islands (Chaudhary et al. 2012). The major flaw in the systematics of this genus is the high degree of intra-specific variability, which often leads to misidentification and closely related species treated as different taxa (Weiblen 2000; Chantarasuwan et al. 2015). Those species showing morphological similarity are considered as Taxonomically Complex Group Species (TCGs). The morphological characteristics of *Ficus* are not natural and revealed several parallel transitions in growth habits and breeding systems (Machado et al. 1996; Weiblen 2000; Jousselin et al. 2003; Rønsted et al. 2005; Rønsted et al. 2008). The species with uniparental reproduction (e.g. self-fertilization, apomixes, gynogenesis and hybridogenesis) also leads to species complexity and misidentification. Differences in the breeding system among the species of *Ficus*, which is the leading cause for the complexity related to ascertaining the taxonomic status of *Ficus* species, remain poorly understood (Chantarasuwan et al. 2013; Lu et al. 2016).

Ficus krishnae C. De Candolle (1906: t. 8092) belongs to subsection *Conosycea* under the section *Cordifoliae* G.Don of the subgenus *Spherosuke* Raf. (Chaudhary et al. 2012; Pederneiras et al. 2015). This species was highly sacred due to the cup-shaped leaf bases, which were considered “Krishna as the Divine Child, sucking his toe and lying on a banyan leaf.” The species precisely differentiated from similar species *F. benghalensis* Linnaeus (1753: 1059) predominantly based on leaf peculiarities and in addition to the differences in tomentum, aerial roots, stipules, petiole, ostiolar bracts, stamens, stigma and pollinator wasps (Prain 1906; Weiblen 2000). Moreover, a detailed taxonomic description of *F. krishnae* was published in ‘Curtis Botanical Magazine’ (Candolle 1906). Molisch (1930) and Biswas (1932, 1935) described that the peculiar nature of its leaves is due to the bud mutation in *F. benghalensis*. Berg and Corner (2005) had subsumed *F. krishnae* with *F. benghalensis* based on morphological characteristics without considering the leaf characters. Corner (1960a, 1960b, 1961, 1962, 1965) and Chaudhary et al. (2012) have treated *F. krishnae* as a taxonomic variety of *F. benghalensis*. In an earlier study, Priyadarsanan (1999) suggests that the two species are pollinated by different species of wasps, indicating the taxonomic dissimilarity between them based on the pollination system. In 2009, Rout and Aparajita analyzed ISSR markers of 23 *Ficus* species and reported that the two species are formed in the same cluster (Rout and Aparajita 2009). Recent studies of *F. krishnae* species based on morphology and anatomy revealed the characteristics such as habit, plant height, presence of aerial roots, leaves structure, stipules, lamina, receptacle, male flower and stigma of female flowers are distinct (Tiwari et al. 2015). Anand et al. (2016) reported

non-divergence in nuclear ITS sequence between the species, while chloroplast DNA (*rps16* and *atpB*) shows slow divergence among both species.

The emergence of a fast, accurate and efficient technique named “DNA barcoding” has proven reliable for identifying both intraspecific and interspecific species and for population studies (Hebert et al. 2003). In plants, several coding and noncoding regions of chloroplast DNA (*rbcL*, *matK*, *ycf1*, *ycf5*, *trnL*, *rpoc1*, *trnH-psbA*) (Dong et al. 2015) and internal transcribed spacer (ITS) region of nuclear ribosomal DNA have been recommended as a potential DNA barcode candidate (Baldwin 1992). In 2009, CBOL-Plant Working Group suggests *rbcL* and ITS are the core barcode candidates for all plants species and *trnH-psbA* have been recommended as supplementary barcode candidate. In the case of *Ficus* species, only a few reports assert the molecular studies and suggests nuclear internal transcribed spacer (ITS2) and plastid intergeneric spacer (*trnH-psbA*) are the efficient barcode candidates for differentiating *Ficus* species as it had more variable regions. Besides, intergeneric spacer *trnH-psbA* shows high variable regions, especially in closely allied species (Weiblen 2000; Rønsted et al. 2008; Olivar et al. 2014; Castro et al. 2015 Chantarasuwan et al. 2015 and Mahima et al. 2020).

Meanwhile, there is a brewing conflict among taxonomists regarding the taxonomic status of the two species because of the enormous scarcity value attached to the taxa and their high cultural importance, especially in India. Hence we propose that the species complex needs to be evaluated using morphological characteristics and molecular markers to identify the species delimitation and genetic divergence.

Materials and methods

Sample collection

Fresh leaf samples of both species were collected from A. J. C. Bose Indian Botanic Garden, Howrah, West Bengal; Coimbatore and Calicut University Botanical Garden, Calicut during the period of 2016-17 (Fig. 1). The voucher specimens of *F. benghalensis* and *F. krishnae* (JV135330, JV135335, JV135440, and JV135445) were generated and deposited at the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India.

DNA Extraction, PCR Amplification and DNA Sequencing

About 100 mg of leaf tissue from each sample was used for genomic DNA extraction using Nucleospin Plant II mini spin DNA extraction kit (Macherey-Nagel, Germany), following the manufacturers’ protocol. The purity and quantity of genomic DNA were determined using a spectrophotometer (Nanodrop 2000, Thermo Scientific, USA). The Polymerase Chain Reaction (PCR) was performed for ITS2 (nrDNA) and *trnH-psbA* (cpDNA) in a thermal cycler (Bio-Rad, USA) with 1 µL (50 ng) of DNA product, 10 µL of EmeraldAmp GT PCR master mix (TaKaRa, Bio USA, Inc.), 2 µL each of 10 µM primers (forward and reverse), and 7 µL of nuclease-free H₂O, in a total

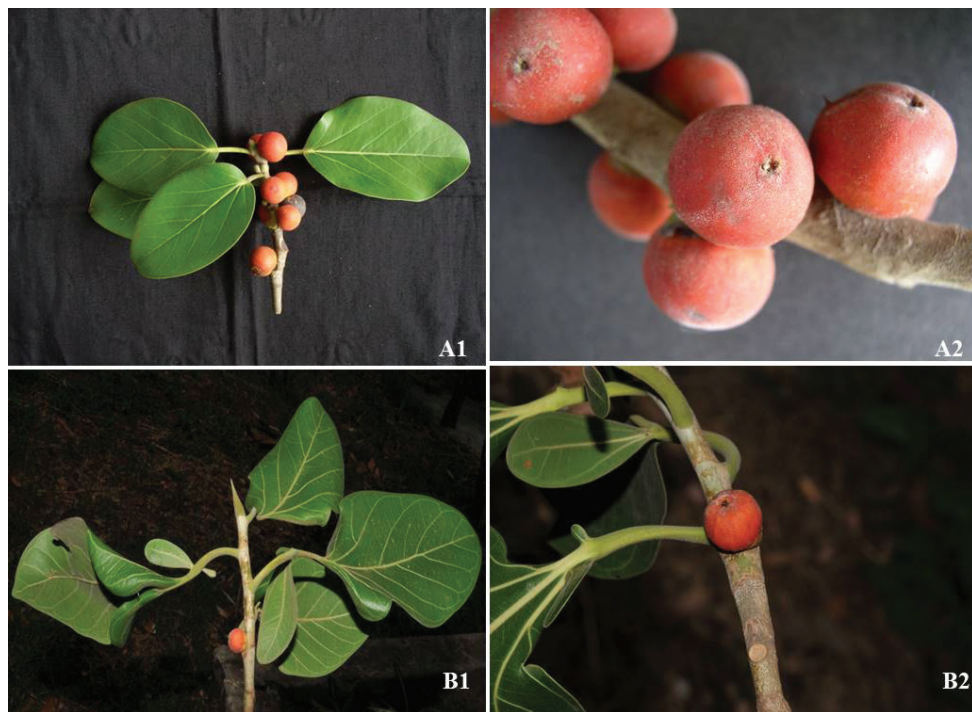


Figure 1. Typical morphology of *Ficus benghalensis* and *Ficus krishnae*. **A1, A2** *Ficus benghalensis* twig and figs (PC: Jana Venkata Sudhakar) **B1, B2** *Ficus krishnae* twig and figs (PC: Jana Venkata Sudhakar)

volume of 20 μ L. The employed primer sets and PCR programs are summarized in (Suppl. material 1: Table S1). The amplified PCR products were inspected on 1% TAE agarose gels and were purified and sequenced on 3730 XL-automated DNA Sequencer by Bioserve Biotechnologies Pvt. Ltd., Hyderabad, Telangana, India. Sequencing was done with both the primers to retrieve the entire length of the desired region.

DNA sequence alignments

Sequences were initially edited and assembled using Codon Code Aligner version 7.1 (Codon Code Corporation, USA). This software automatically removes the low-quality sequence at the start and end of the sequence using sequence quality scores to identify the low-quality regions. The Phred score of 20 or above was set for the quality check of the sequences. Both forward and reverse primers were trimmed from the assembled sequences and the edited sequences were then aligned using the MUSCLE algorithm in the MEGA 7.0 package (Kumar et al. 2016). BLAST analyses were performed for all the assembled sequences obtained from GenBank to check the potential contamination with non-*Ficus* species DNA and the threshold value of 99% identity was set for

the top match (Altschul et al. 1990). The gaps were treated as missing data and indels were excluded from the alignments since they were not informative. Both the strands were sequenced for multiple accessions per species and other *Ficus* species sequences were deposited in GenBank, USA, with the accession number listed in (Suppl. material 2: Table S2). The additional ITS2 and *trnH-psbA* sequences for the following species are *F. racemosa*, *F. religiosa*, *F. drupacea* and *F. elastica*, and were retrieved from the GenBank. Information pertaining to the sequences data of the present study is provided in Suppl. material 3: Table S3 (Roy et al. 2010; Li et al. 2012; Ando et al. 2016; Williams et al. 2017; Zhang et al. 2018). *F. racemosa* was used as outgroup (Roy et al. 2010).

Morphological data

In total, 48 qualitative and quantitative characters were selected and coded for the analysis (Suppl. material 4: Table S4). The distinctive characters of *F. krishnae* and *F. benghalensis* are tabularized in (Table 1). The data matrix with multi-state characters are summarized in (Suppl. material 5: Table S5). All characters were treated as unordered and of equal weight, missing data were coded as unknown. The morphological data matrices were constructed using the recent taxonomic revision of *F. krishnae* and *F. benghalensis* (Tiwari et al. 2015). The specimens used in the revision were also the primary source for the compiling data matrix.

Table 1. Morphological characteristics feature in *Ficus krishnae* and *Ficus benghalensis* species

S.No	Characters	<i>F. krishnae</i> C.DC.	<i>F. benghalensis</i> L.
1	Habitat	Only in cultivation	Wild as well as in cultivation
2	Plant height	10-15 m tall	25-30 m tall
3	Aerial roots	Few, delicate, thin, do not touch the ground and not forming any accessory trunks	Numerous, strong, thick touch the ground and forming numerous accessories pillar like trunks
4	Stipule	2-6.8 cm long	2.5-3 cm long
5	Petiole	4.5-10 cm long, terete in upper portion and subflat and pulvinous towards base	2-4 cm long, grooved above throughout
6	Leafy appendages on petiole	Generally present	Always present
7	Lamina	Generally, with cup shaped structure on lower surface at base, cuneate at base, entire or sub undulate along margins, downy	Never with cup like structures, acute, obtuse or sub cordate at base, entire along margins, puberulous
8	Receptacle (fig body)	Slightly projected at apex	Dispersed at apex
9	Male flower	Chiefly confined to ostiolar region	Scattered throughout
10	Stigma of female flowers	Linear, feathery or flattened	Linear and swollen, never feathery
11	Chromosome numbers	2n =26 with 1-2 small euchromatic accessory chromosomes	2n=26
12	DNA content	1.47 pg	1.45 pg
13	Nodal anatomy	Multilacunar (7-8 nodal lacunae and their respective traces) and variable	Pentacular (5-nodal lacunae and their respective traces) and constant
14	Size of stomata	Smaller	Larger
15	Size of parenchyma cells	Smaller	Larger

Phylogenetic analysis

In total, five analyses were done for eight species (three accessions per species), including morphological characteristics evaluation, individual marker ITS2 and *trnH-psbA*, combined marker (ITS2+*trnH-psbA*) were performed with Bayesian Inference (BI) methods. Further, morphological datasets combined with molecular data (morphology+combined markers) as the 'total evidence' approach. The morphological dataset was analyzed under the Maximum Parsimony (MP) method using PAUP v4.0b10 (Swofford 2002). Molecular-based analyses were performed in Bayesian Inference (BI) method using MrBAYES v 3.1.2 (Ronquist et al. 2012). For the phylogenetic tree construction, two individual alignment matrices (ITS2 and *trnH-psbA*) and the combined matrices (ITS2+*trnH-psbA*) were aligned separately using the MUSCLE algorithm in the MEGA 7.0 package (Kumar et al. 2016) and checked manually. Model selection for Bayesian analysis was conducted using j Model Test 2.1.7 (Darriba et al. 2012). The models were selected based on BIC scores (Bayesian Information Criterion) and it resulted as HKY for ITS2, TPM3uf+G for *trnH-psbA* and F81+G for combined ITS2+*trnH-psbA*. MP analyses for morphological characters were run in PAUP 4.0b10. In Bayesian phylogenetic analysis, the default values of four chains (three heated and one cold chain) and two independent runs of Markov Chain Monte Carlo (MCMC) for ten million generations with sampling every 1000 generations were performed on the concatenated data matrix. The chain convergence was performed based on the average standard deviation of split frequencies and estimated sample size (ESS) values. A majority-rule consensus tree was then calculated after discarding the first 25% trees as burn-in. The Potential Scale Reduction Factors (PSRF) in the MrBayes SUMP output was one or close to one, describing precise convergence. Bayesian inference produces a moderately higher posterior probability than the equivalent bootstrap frequencies (Erixon et al. 2003); thus, we used only posterior probabilities (PP) above 0.9 as high support. The posterior probabilities values (PP) between 50 and 70 were considered weak support, with percentages between 71 and 89 as moderate, while more than 90 as high support. Fig tree v.1.4.3 annotator was used to visualize and annotate the Maximum Clade Credibility (MCC) tree from the run subsequently (Rambaut 2018). In addition, interspecies and intra-species divergences were estimated based on the Kimura 2-parameter distance method in the MEGA 7.0 package (Kumar et al. 2016).

Results

Species divergence

The amplification and sequence success rate of *F. krishnae* and *F. benghalensis* for the ITS2 and *trnH-psbA* was 100%. The length of the ITS2 and *trnH-psbA* sequences had an average of 328 bp and 317 bp, respectively. The variable informative sites and parsimony-informative sites were high in *trnH-psbA* (13.5%, 15.5%) as compared to

ITS2 (7.4%, 8.4%). In order to estimate the genetic divergences between the species, three matrices (mean inter-specific distances, mean intra-specific distances and theta prime) were used; the worked-out results showed that *trnH-psbA* exhibited significant divergences, which aided to distinguish the two species. The mononucleotides 'A' or 'T' in the *trnH-psbA* sequence did not affect the sequencing and sequence length, respectively. In addition, the calculation of genetic divergence for individual markers in both species showed the absence of any intra-specific divergence in *F. benghalensis* and *F. krishnae*. However, the highest divergence in total inter-specific divergence was observed in *trnH-psbA* (20.8 ± 12.2), followed by ITS2 (5.7 ± 3.2) (Table 2). The multiple sequence alignments of these samples revealed the sequence divergence of *trnH-psbA* to be higher in both species than ITS2, as shown in Suppl. material 6: Fig. S1 and Suppl. material 7: Fig. S2.

Analysis of morphological data

The morphological differences between *F. krishnae* and *F. benghalensis* are tabularized in Table 2. A total of 48 morphological characters, including quantitative and qualitative characters, were coded for analysis. The Maximum Parsimony shown in 1105 trees with a length = 276, consistency index (CI) = 0.27 and retention index (RI) = 0.73. The resulting strict consensus tree presented that two species are partially distinct in morphological characters (Suppl. material 8: Fig. S3).

Phylogenetic analysis

In the present study, a total of 24 taxa (3 accessions per species) with 697 characters, 649 combined ITS2 and *trnH-psbA* sequences data and 48 morphological characters were used for phylogenetic tree construction. The tree of individual marker ITS2 and

Table 2. Properties of the two candidate DNA barcoding loci in *Ficus krishnae* and *Ficus benghalensis*

Parameter	ITS2	<i>trnH-psbA</i>
PCR Success (%)	100	100
Sequencing success (%)	100	100
Conserved Sites (%)	91	84.5
Variable informative sites (%)	7.4	13.5
Parsimony-informative sites (%)	8.4	15.5
Aligned length (bp)	309	382
No: of Indels	1	26
Identical sites (%)	76.6	29.5
Transition and Transversion bias (R)	0.446	0.51
Mean inter-specific distance (%)	5.7 ± 3.2	20.8 ± 12.2
Mean intra-specific distance (%)	0.2 ± 0.1	0.3 ± 0.1
Theta prime	0.161 ± 0.069	0.494 ± 0.321
BIC model	HKY	TPM3uf+G
Resolution of species (%)	100	100

trnH-psbA was generated with low discrimination species resolution (Suppl. material 9: Fig. S4; Suppl. material 10: Fig. S5). The trees of a combined dataset of ITS2+*trnH-psbA* were discriminated with high bootstrap supports (Fig. 2). Considering the conflict between the species, the two markers and morphological characters were combined to analyse Bayesian Inference (BI). The cladogram shows that *F. krishnae* was clearly differentiated from *F. benghalensis*, which formed a separate clade. The branch support with high PP values (100%) indicates that *F. benghalensis* and *F. krishnae* are two distinct lineages and the individuals of the same were bifurcated into two different clades (Fig. 3). Accessions of *F. religiosa*, *F. middletonii*, *F. drupacea*, *F. drupacea* var. *pubescens*, *F. elastica* and *F. racemosa* were with high posterior probabilities (PP=100), where *F. racemosa* was used as outgroup in Bayesian analysis.

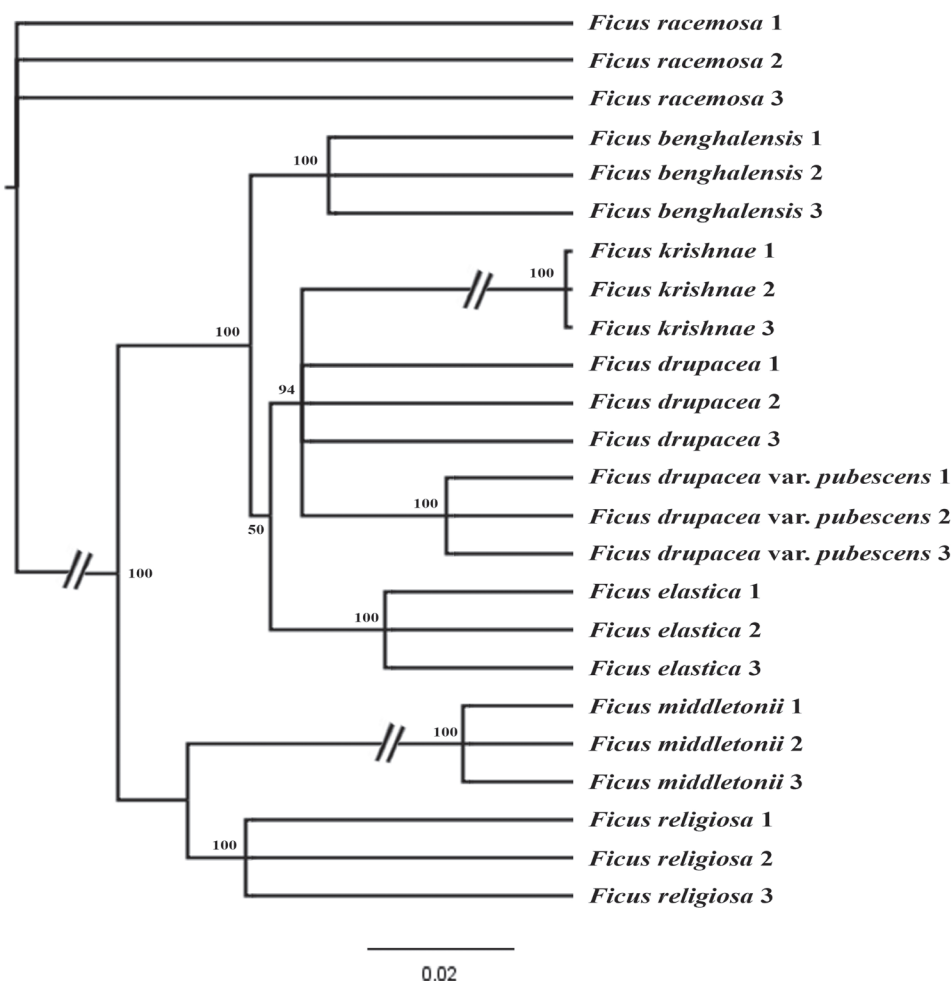


Figure 2. Maximum Clade Credibility (MCC) tree from Bayesian analysis using two DNA barcode markers (ITS2+*trnH-psbA*) with posterior probabilities values in percentage that are shown at nodes.

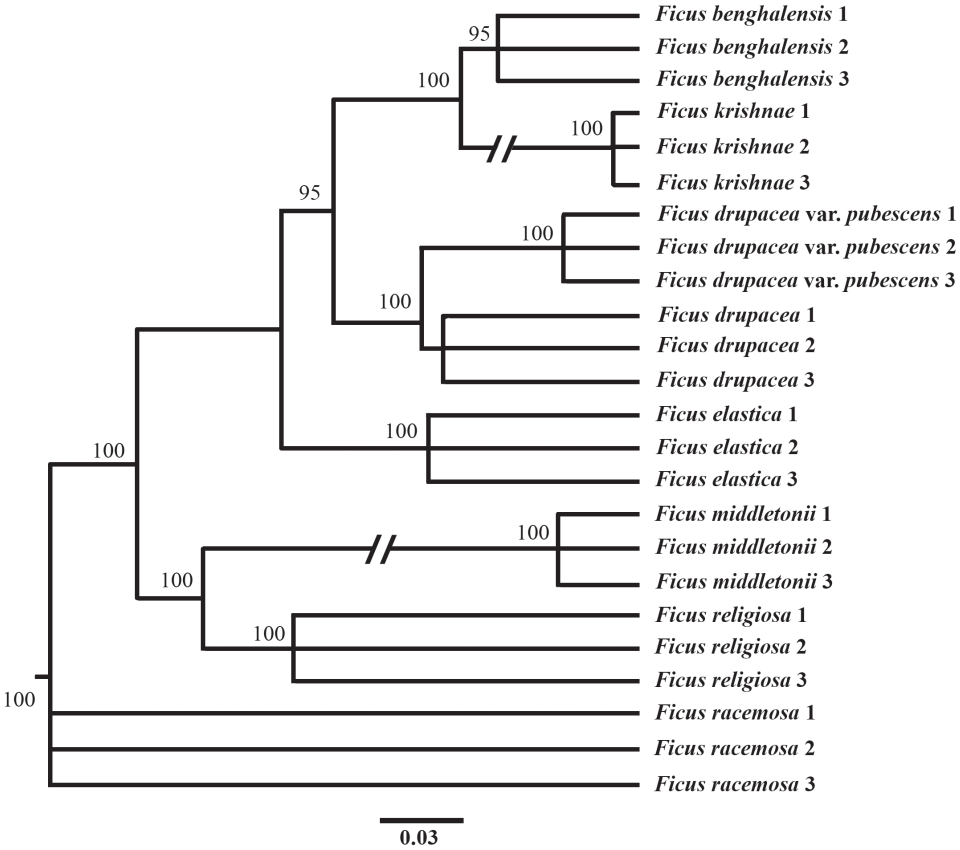


Figure 3. Total evidence MCC tree from Bayesian analysis of the two DNA markers and morphology. The Posterior Probabilities values in percentage are shown at the nodes.

Discussion

We observed that *F. krishnae* and *F. benghalensis* are morphologically distinct in their characters, such as aerial roots, stipules, petioles, lamina shape, male flower, the stigma of the female flower, and receptacle, but information on their genetic variations is lacking. Recently, DNA barcoding has been implied to identify intra-specific delimitations and resolve taxonomic-complexity based on genetic divergence (Kress et al. 2005; Ragupathy et al. 2009; Nithaniyal and Parani 2016; Sivaraj et al. 2018). Thus, we have also employed a phylogenetic tool to resolve species complexity in *F. benghalensis* with its counterpart *F. krishnae*, based on spacer markers. The morphological characters of *F. krishnae*, such as the height of plants, aerial roots, stipules, petioles and ostiolar bracts of the receptacle, show differentiation from *F. benghalensis* (Tiwari et al. 2015). Other reports supported to the species differentiation in *F. krishnae* as a distinct species from *F. benghalensis*—based on the karyotype (Joshi and Raghuvanshi 1970), DNA contents

(Ohri and Khoshoo 1987), stomatal, parenchymatous cells and nodal anatomy (Chatopadhyay and Maiti 2006). Tiwari et al. 2015 considered *F. krishnae* as a distinct species against the merger with *F. benghalensis* under its variety (Berg and Corner, 2005).

Regarding the conflict between species' distinctness, our results of individual DNA markers (ITS2 and *trnH-psbA*) have shown that the two species are entirely distinct with significant sequence variations (Suppl. material 6: Fig. S1 and Suppl. material 7: Fig. S2). The combined marker studies also revealed the species differences in both species (Fig. 2). Further, we combined the morphology and molecular data to construct a phylogenetic tree, which strongly supports that the two species are distinct (Fig. 3). Thus, morphology and molecular data strongly support each other and derived the concordance. On the other hand, Biswas (1935) reported that the variations in *F. krishnae* were due to bud mutations. Some reports in *Ficus* species show that the evaluation of morphological characteristics combined with molecular analysis is necessary for exact *Ficus* species identification and classification (Chantarasuwan et al. 2015). Lu et al. 2016 reported the taxonomic delimitation of the Hairy-Fig complex species using phylogenetic analysis and results showed that *F. hirta*, *F. esquiroliana*, *F. simplicissima* and *F. fulva*, with continuously variable morphological characteristics and conspecific based on their genetic traits. In addition, Zhang et al. (2018) studied the *F. auriculata* complex, which contained five species, and resolved the species boundaries using molecular markers and SSR analysis. So hence, we propose that *F. krishnae* is a distinct species among *F. benghalensis* complex due to the possession of high divergence and lineated as sister to the same; it was not nested within *F. benghalensis*. The synonymy of the *F. krishnae* under the *F. benghalensis* complex can be illegitimate for their nomenclature.

Conclusion

The present study showed that ITS2 and *trnH-psbA* DNA barcode markers could be used as taxon-specific markers for *Ficus* to confirm the species identity. In addition, DNA barcodes will be much more helpful and have been proved to be an effective taxonomic tool for reliable identification, discrimination and resolution among the two closely related *Ficus* L. species. So we suggest that the combination of these two markers with morphology can be used in species delimitation studies to resolve the complexity.

Acknowledgments

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Supplementary material I

Table S1. The PCR reaction conditions for the barcoding loci

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.186.74086.suppl1>

Supplementary material 2

Table S2. List of *Ficus* species collected from different parts of India

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.186.74086.suppl2>

Supplementary material 3

Table S3. List of *Ficus* sequences retrieved from GenBank

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: molecular data

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Supplementary material 4

Table S4. Vegetative and floral characters

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: species data

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Supplementary material 5

Table S5. The data matrix of vegetative and floral characters

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Data type: species data

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Supplementary material 6

Figure S1. Multiple sequence alignment of ITS2 in *Ficus krishnae* and *Ficus benghalensis*

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.186.74086.suppl6>

Supplementary material 7

Figure S2. Multiple sequence alignment of *trnH-psbA* in *Ficus krishnae* and *Ficus benghalensis*

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.186.74086.suppl7>

Supplementary material 8

Figure S3. Parsimony distribution of the morphological characters and their status on the MCC Bayesian tree

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Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.186.74086.suppl8>

Supplementary material 9

Figure S4. Maximum Clade Credibility (MCC) tree from Bayesian analysis using the ITS2 DNA barcode marker with posterior probabilities values in percentage that are shown at nodes

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: molecular data

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Supplementary material 10

Figure S5. Maximum Clade Credibility (MCC) tree from Bayesian analysis using the *trnH-psbA* DNA barcode marker with posterior probabilities values in percentage that are shown at nodes.

Authors: Karthikeyan Mahima, Senthilkumar Umapathy, Jana Venkata Sudhakar, Ramalingam Sathishkumar

Data type: NEX file.

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On the identity of *Thymus humifusus* var. *aureopunctatus* (Lamiaceae) and taxonomic notes on the *Th. richardii* complex

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Abstract

The name *Thymus humifusus* var. *aureopunctatus*, described from Bosnia and Herzegovina, is lectotypified, and its taxonomic value is discussed. *Thymus richardii* subsp. *richardii* is currently considered an endemic subspecies common to Mallorca (Balearic Islands) and Bosnia and Herzegovina from the Balkan Peninsula. Specimens identified as *Th. richardii* from both Balearic Islands and Bosnia and Herzegovina were studied to determine if they are indeed the same taxonomic entity. Detailed micromorphological observations and morphometric analysis, suggest that the Balkan plants (*Th. humifusus* var. *aureopunctatus*) and the Majorcan populations (*Th. richardii* subsp. *richardii*) are clearly separate entities. For the former name, based on morphological, phytochemical, biogeographical and present results, we propose the subspecific rank, as *Th. richardii* subsp. *aureopunctatus* **comb. & stat. nov.** Full descriptions of all five subspecies currently accepted within *Th. richardii* are provided.

Keywords

Balkan Peninsula, Mediterranean, nomenclature, original material, taxonomy, typification

Introduction

The western Mediterranean Basin is one of the most important regions where the genus *Thymus* L. has diversified (Morales 1997). *Thymus* sect. *Serpyllum* (Mill.) Benth. is a difficult group taxonomically that includes the largest number of species of the genus (Morales 1997). One of the species included in this section is *Thymus richardii* Pers. a diploid ($2n = 28, 30$) Mediterranean species with a strongly fragmented distribution (Morales 1997, 2010; Bartolucci 2018; Bartolucci et al. 2018). This species represents an aggregate of allopatric subspecies: *Th. richardii* subsp. *richardii* ($2n = 28, 30$), occurring in Mallorca and Bosnia and Herzegovina (Balkans), *Th. richardii* subsp. *ebusitanus* (Font Quer) Jalas, endemic to Ibiza ($2n = 30$), *Th. richardii* subsp. *vigoii* Riera, Güemes & Rosselló, endemic to eastern Spain (Valencia and Alicante provinces) and *Th. richardii* subsp. *nitidus* (Guss.) Jalas, endemic of Marettimo Island (Sicily; $2n = 28$) (Riera et al. 2007; Morales 2010; Bartolucci et al. 2013; Brullo and Brullo 2020).

As currently circumscribed, *Th. richardii* subsp. *richardii* presents a striking distribution pattern, since the isolation between both areas (Mallorca and the Bosnia) is remarkable, and there are no other cases of shared endemism between these areas. Furthermore, i) the differences between the habitat occupied by *Th. richardii* subsp. *richardii* in both areas, ii) the differences in the composition of essential oils (Llorens et al. 2014), and iii) the fact that the Bosnian and Herzegovinian population was initially recognised as a separate taxon (*Th. humifusus* var. *aureopunctatus* Beck) by several authors (Malý 1908, 1923; Ronniger 1930a, b) invites a re-evaluation of the inclusion of the latter taxon within the synonymy of *Th. richardii* subsp. *richardii* as originally proposed by Jalas (1971).

In order to elucidate the taxonomic identity of *Th. humifusus* var. *aureopunctatus*, we have sampled specimens from the Bosnian and Herzegovinian and Balearic populations of *Th. richardii* in the field for a detailed comparison. On the other hand, the morphological characters used to separate the rest of the subspecies recognised in *Th. richardii* have also been analysed in detail. Finally, a multivariate morphometric analysis based on quantitative traits was carried out to clarify the relationships among taxa within the *Th. richardii* complex.

Material and methods

Plant material

This study is based on analysis of relevant literature, field surveys and examination of herbarium specimens kept in BC, BCN, COI, HBJS, MA, P, PAL, SARA, VAL, ZA, ZAGR (herbarium codes according to Thiers 2021) and the Herbarium of the Balearic Islands University. For the typification purposes, herbarium specimens deposited at BC, BM, L, P, and PRC were studied using the online herbarium databases or images were requested.

Furthermore, the plant material of recently collected samples of *Th. humifusus* var. *aureopunctatus* and *Th. richardii* subsp. *richardii* from Bosnia and Herzegovina and

Mallorca was analysed, too (Fig. 1A). In total, 61 individuals from seven populations (see Appendix 1) were surveyed for micromorphology and quantitative morphometry.

Morphological characters recognised as taxonomically discriminant within the *Th. richardii* complex (Jalas 1972; Morales 2010; and our own observations) were scored either in the field or in the herbarium specimens (Table 1, 3). Morphological observations of materials were carried out under a Zeiss Stemi DV4 binocular stereoscopic microscope. We scored both qualitative and quantitative traits in evaluation of taxa, the latter ones were used to describe the pattern of morphological variation and relationships among taxa.

Micromorphology was observed on calyces, which were glued directly to aluminium stubs, coated with 40–50 nm gold, and examined with a scanning electron microscopy (SEM) (Zeiss Merlin FE-SEM) at 5 kV.

Data analysis

Descriptive statistics (mean, minimum and maximum value, standard deviation and coefficient of variation for each of the studied characters at the taxon level) and univariate statistics (one-way ANOVA followed by Tukey's test) were calculated to test the significance of differences between taxa within the complex. Overall morphological variation of quantitative traits and relationships of the sampled taxa was evaluated using Principal Component Analysis (PCA). *Thymus richardii* subsp. *vigoi* was excluded from analysis due to distinct characters in relation to the other taxa (see Identification key). Means of averaged and standardised values of individuals were used as a matrix data in PCA. PCA was computed on the correlation matrix data of all scored traits. The axes with Eigen values > 1 were used in analysis. PCA computation, descriptive and univariate statistics were run in PAST ver. 3.14 (Hammer 2016).

Results and discussion

The variation based on SEM micromorphology, univariate and multivariate morphometrics (PCA) of taxa included within *Th. richardii* complex is described and their taxonomic value of the characters is here discussed.

Variation of individual quantitative morphological traits within the *Th. richardii* complex

Mean values of the analysed traits go in favour of morphological differentiation among taxa (Table 1). In general, coefficients of variation (CV) did not exceed 20% (Table 1). The values in *Th. richardii* subsp. *vigoi* should be treated with caution because their calculations were based on three individuals. In most cases the coefficients of variation had low (CVs $\leq$ 10%, 29 cases) and moderate values (CVs from 10 to 20%, 36 cases) (Table 1). High values of coefficients of variation were observed for the trait *length of longer cilia of upper calyx teeth* (LCU) in each taxon and for the trait *longer inflorescence length* (IL). The traits *calyx length* (CL) and *calyx tube length* (CTL) showed the lowest values of coefficients of variation (Table 1).

Table 1. Descriptive parameters of the analysed traits of the *Thymus richardii* complex: minimum and maximum values in brackets, mean value with standard deviations and coefficients of variation (%) in brackets.

Trait	subsp. <i>richardii</i>	subsp. <i>aureopunctatus</i>	subsp. <i>ebusitanus</i>	subsp. <i>nitidus</i>	subsp. <i>vigoii</i>
leaf base	cuneate	cuneate	cuneate	cuneate	cordate
leaf margin	entire	entire	entire	entire	denticulate
leaf (blade) length (mm)	(7.0–2.03) 9.45 ± 1.40 (14.81)	(4.83–7.4) 6.22 ± 0.72 (13.74)	(7.0–12.03) 8.62 ± 1.40 (14.80)	(6.43–9) 7.66 ± 1.05 (13.74)	(7.4–9.738.62 ± 1.17 (13.57)
leaf width (mm)	(4.3–7.07) 5.52 ± 16.85) 0.93)	(3.06–4.93) 3.67 ± 0.59 (15.40)	(4.53–6.23) 5.64 ± 0.46 (8.18)	(3.1–4.1) 3.67 ± 0.43 (11.83)	(5.76–7.96) 6.53 ± 1.24 (19.01)
ratio LL/LW	(1.21–2.14) 1.73 ± 12.96) 0.22)	(1.34–2.01) 1.62 ± 0.17 (10.55)	(1.23–1.87) 1.53 ± 0.21 (13.84)	(1.98–2.26) 2.11 ± 0.12 (6.13)	(1.22–1.51) 1.37 ± 0.15 (11.72)
longer inflorescence length (mm)	(15.0–30.0) 20.71 ± 18.39) 3.81)	(8.0–16.0) 11.25 ± 2.35 (20.97)	(19–62) 33.66 ± 1.50 (31.20)	(22–25.33) 23.33 ± 1.41 (6.06)	(15.0–34.0) 23 ± 9.84 (42.82)
pedicel length (mm)	(2.97–4.50) 3.58 ± 11.95) 0.43)	(1.23–2.1) 1.60 ± 0.23 (14.83)	(2.0–3.5) 2.73 ± 0.55 (20.12)	(2.23–2.66) 2.40 ± 0.20 (8.37)	(3.7–4.66) 4.05 ± 0.53 (13.10)
bract length of larger bracts (mm)	(6.40–10.50) 7.80 ± 12.44) 0.97)	(3.73–5.83) 4.77 ± 0.45 (9.53)	(5.85–9.73) 7.02 ± 0.86 (12.30)	(5.73–6.76) 6.23 ± 0.45 (7.30)	(6.0–7.33) 6.78 ± 0.69 (10.30)
bracts width of larger bracts (mm)	(3.57–7.40) 4.70 ± 16.66) 0.78)	(2.2–4.66) 2.97 ± 0.49 (16.64)	(3.43–6.93) 4.79 ± 0.70 (14.65)	(2.76–3.33) 3.03 ± 0.25 (8.26)	(2.9–6.5) 5.12 ± 1.94 (37.93)
calyx: stipitate glandular hairs	usually absent	absent	usually abundant	abundant	few or absent
calyx tube hairiness (eglandular hairs)	glabrescent (sometimes glabrous)	glabrescent to sparsely hairy	densely hairy	sparsely hairy	sparsely hairy
calyx length (mm)	(6.30–7.66) 6.85 ± 0.33 (4.83)	(3.5–4.442) 3.99 ± 0.20 (5.03)	(4.82–6.36) 5.49 ± 0.40 (7.32)	(5.06–5.44) 5.25 ± 0.21 (4.17)	(6.5–6.74) 6.58 ± 0.13 (2.02)
upper (middle) calyx teeth length (mm)	(1.44–1.98) 1.70 ± 10.99) 0.19)	(0.84–1.26) 1.0 ± 0.10 (10.76)	(1.04–1.54) 1.21 ± 0.13 (10.76)	(1.06–1.12) 1.09 ± 0.02 (2.29)	(2.26–2.36) 2.30 ± 0.05 (2.18)
lower calyx teeth length (mm)	(2.92–3.58) 3.13 ± 4.65) 0.15)	(1.84–2.26) 2.00 ± 0.11 (5.88)	(2.26–2.82) 2.59 ± 0.16 (6.28)	(2.02–2.54) 2.33 ± 0.23 (10.23)	(3.24–3.34) 3.29 ± 0.05 (1.52)
length of longer cilia of upper calyx teeth (mm)	(0.02–0.14) 0.08 ± 45.74) 0.04)	(0.02–0.22) 0.12 ± 0.06 (55.23)	(0.52–1.72) 0.68 ± 0.29 (42.65)	(0.04–0.28) 0.21 ± 0.11 (54.11)	(0.2–0.24) 0.22 ± 0.02 (9.09)
length of longer cilia of lower calyx teeth at middle length (mm)	(0.16–0.26) 0.22 ± 13.88) 0.03)	(0.34–0.46) 0.39 ± 0.04 (9.74)	(0.62–0.84) 0.75 ± 0.05 (7.87)	(0.38–0.4) 0.39 ± 0.01 (2.96)	(0.38–0.5) 0.46 ± 0.06 (15.06)
calyx tube length (mm)	(2.34–3.00) 2.61 ± 6.48) 0.17)	(1.42–2.0) 1.61 ± 0.15 (9.88)	(2.04–2.5) 2.32 ± 0.12 (5.28)	(2.1–2.2) 2.16 ± 0.04 (2.04)	(2.5–2.7) 2.59 ± 0.10 (3.88)
length of longer eglandular hair of calyx tube (mm)	(0.16–0.26) 0.22 ± 13.11) 0.03)	(0.28–0.4) 0.34 ± 0.03 (9.95)	(0.6–0.88) 0.74 ± 0.007 (9.90)	(0.3–0.38) 0.33 ± 0.03 (10.19)	(0.24–0.4) 0.30 ± 0.08 (29.05)

One-way ANOVA displayed significant differences between mean values of quantitative traits for all subspecies ($p \leq 0.01$). The Tukey’s test revealed significant differences among subspecies for the most of the studied traits (Table 2). The highest number of observed differences was 13 (*Th. richardii* subsp. *ebusitanus* vs. *Th. humifusus* var. *aureopunctatus*) and 12 (*Th. richardii* subsp. *richardii* vs. *Th. humifusus* var. *aureopunctatus* and *Th. richardii* subsp. *richardii* vs. *Th. richardii* subsp. *nitidus*). A considerable level of significant differences (10) was observed between *Th. richardii* subsp. *richardii* vs. *Th. richardii* subsp. *ebusitanus* and *Th. richardii* subsp. *ebusitanus* vs. *Th. richardii* subsp. *nitidus* (9). The smallest number of significant differences (7) was evidenced between Sicilian population of *Th. richardii* subsp. *nitidus* and the Balkan *Th. humifusus* var. *aureopunctatus* (Table 2).

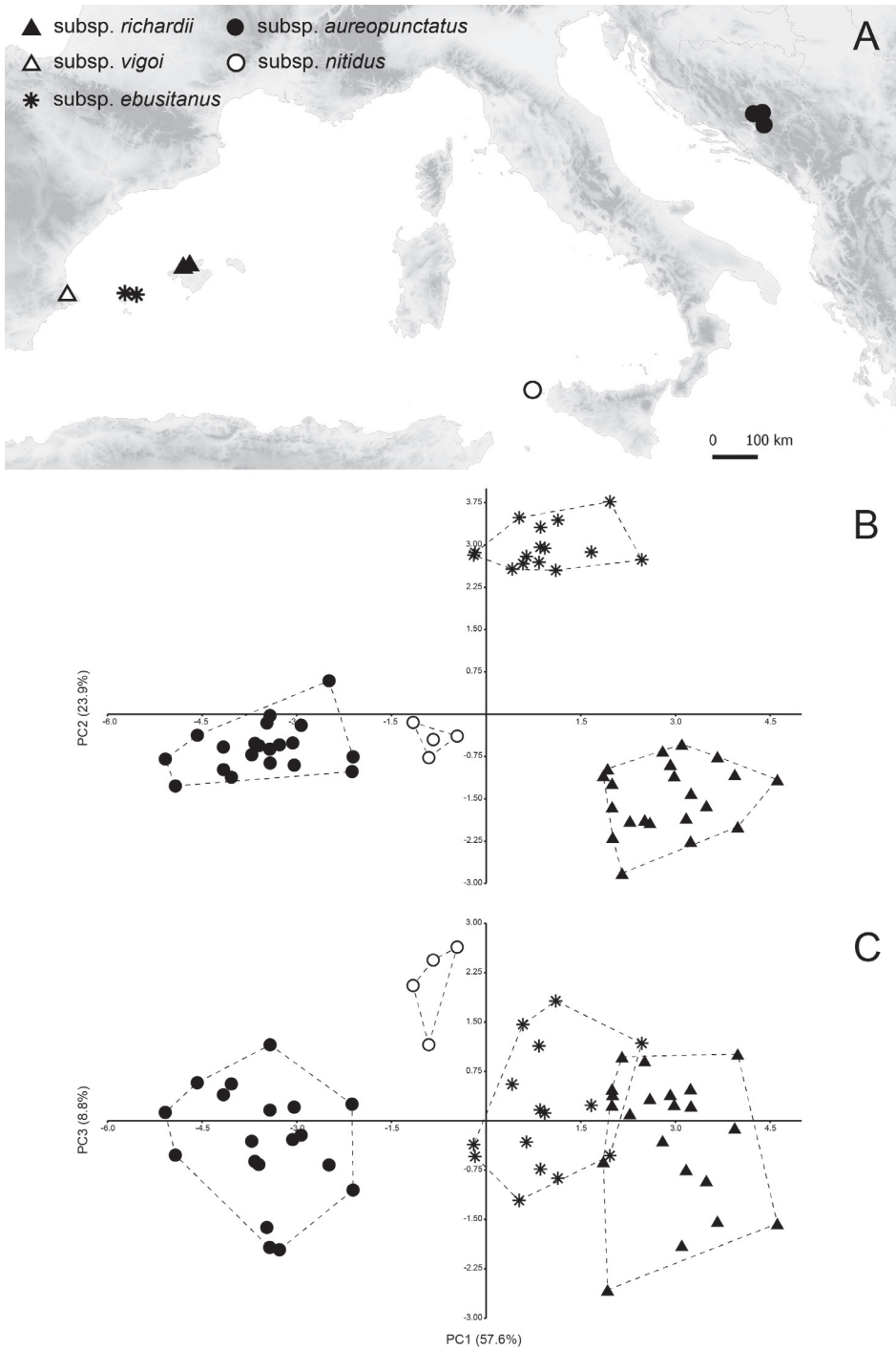


Figure 1. Principal Component Analysis (PCA) of 61 individuals of the *Thymus richardii* complex **A** distribution of the studied samples **B** plot of analysed samples by first and second principal components. **C** plot of analysed samples by first and third principal components.

Table 2. The studied traits differentiating between taxa based on result of the Tukey’s t tests ($p = 0.01$) (abbreviations are as in Table 3).

Taxon	<i>richardii</i>	<i>ebusitanus</i>	<i>aureopunctatus</i>
<i>ebusitanus</i>	LL/LW, IL, PL, BL, CL, UTL, LTL, LCU, LCL, CTL, LEH		
<i>aureopunctatus</i>	LL, LW, IL, PL, BL, BW, CL, UTL, LTL, LCU, LCL, CTL, LEH	LL, LW, IL, PL, BL, BW, CL, UTL, LTL, LCU, LCL, CTL, LEH	
<i>nitidus</i>	LL, LW, LL/LW, PL BL, BW, CL, UTL, LTL, LCL, CTL, LEH	LW, LL/LW, BW, CL, LTL, LCU, LCL, CTL, LEH	LL/LW, IL, PL, BL, CL, LTL, CTL

Morphometric relationships among taxa within the *Th. richardii* complex

Morphological variation was explained by three principal components with Eigen values > 1 which clearly separated four morphological clusters corresponding to *Th. richardii* subsp. *richardii*, *Th. humifusus* var. *aureopunctatus*, *Th. richardii* subsp. *ebusitanus* and *Th. richardii* subsp. *nitidus* (Table 3, Fig. 1B, C). The first three components accounted for 90.3% of the total variance (PC1 = 57.6%, PC2 = 23.9% and PC3 = 8.8%). The taxa were separated mostly along the first and second axis (Fig. 1B). *Thymus richardii* subsp. *nitidus* was a neighbouring group to *Th. humifusus* var. *aureopunctatus*. A plot onto PC 1 and PC 3 revealed that *Th. richardii* subsp. *nitidus* also represented a distinct cluster within the complex (Fig. 1C). Following characters (CL, LTL, CTL, and BL) with moderate coefficients of correlations were associated with PC1 (Table 3). The characters LEH, LCL and LCU contributed to the PC2. The highest correlations with the PC3 showed derived trait L/W and leaf width (LW) which contributed to the separation of *Th. richardii* subsp. *nitidus* (Table 3). Principal component analysis of quantitative morphological data demonstrates that allopatric populations of the *Th. richardii* complex are clearly distinguishable according to their taxonomic affiliation.

Variation in particular morphological traits indicated a similar pattern observed in PCA, confirming a high level of morphological differentiation between the studied taxa. High levels of both morphological and genetic differentiation within plant complexes are not surprising in the Mediterranean. This pattern of variation, which often results in endemism, is particularly pronounced for populations inhabiting the Mediterranean islands (Thompson 2020). Due to different geological and biogeographical processes, long-term isolation, adaptation and specialization to contrasting habitats, the ancestral populations of *Th. richardii* diverged into distinct entities across the Mediterranean and the Balkans.

General habit

All the taxa included within the *Thymus richardii* complex are woody perennials with young or flowering stems with hairs on all faces, more or less evenly distributed. These hairs are eglandular, usually retrorse, up to 0.2 mm long (0.4 mm long in *Th. richardii* subsp. *ebusitanus*), intermixed with sessile glands. According to Morales (2010), the plant length separates the populations from Mallorca from those of Ibiza (7–13 cm vs. 10–24 cm, respectively), but in

Table 3. Principal components revealed by the PCA for the *Thymus richardii* complex.

Trait		Component		
		PC 1	PC 2	PC 3
LL	leaf (blade) length	0.307	0.032	0.143
LW	leaf (blade) width	0.275	0.181	-0.372
L/W	ratio L/W	0.032	-0.241	0.818
IL	longer inflorescence length	0.246	0.311	0.157
PL	pedicel length	0.321	-0.023	0.088
BL	bract length of larger bracts	0.332	0.036	0.059
BW	bracts width of larger bracts	0.296	0.163	-0.178
CL	calyx length	0.347	-0.062	0.043
UTL	upper (middle) calyx teeth length	0.316	-0.167	-0.094
LTL	lower calyx teeth length	0.339	-0.067	0.033
LCU	length of longer cilia of upper calyx teeth	0.002	0.472	0.250
LCL	length of longer cilia of lower calyx teeth at middle length	-0.108	0.502	0.117
CTL	calyx tube length	0.338	-0.029	0.064
LEH	length of longer eglandular hair of calyx tube	-0.072	0.519	0.098
Eigenvalue		7.990	3.353	1.132
Contribution		0.576	0.239	0.088
Cumulative (%)		0.576	0.815	0.903

our opinion this character is rather variable and has no taxonomic significance. We have collected Majorcan plants of *Th. richardii* subsp. *richardii* that measure up to 40 cm in length.

Leaves

All the studied taxa have flat leaves, not ciliate at base, with entire margins, except in *Th. richardii* subsp. *vigoi*, which has denticulate leaves. On the basis of leaf morphology (Riera et al. 2007) *Th. richardii* subsp. *vigoi* is easily separable from the rest of the members of the *Th. richardii* complex. Leaves shape varies from ovate to elliptical. Jals (1972) attributed to *Th. richardii* subsp. *nitidus* leaves more than twice as long as wide. Certainly *Th. richardii* subsp. *nitidus* usually has leaves with a higher length / width ratio than the rest of the taxa (Table 1), but we have studied plants of the island of Marettimo with leaves less than twice as long as wide. On the other hand, some Majorcan specimens of *Th. richardii* subsp. *richardii* have leaves more than twice as long as wide.

The leaves have spheroidal yellowish-reddish glands, and sometimes scattered hairs exist in several taxa of this complex. Some specimens of *Th. richardii* subsp. *ebusitanus*, *Th. richardii* subsp. *vigoi* and *Th. richardii* subsp. *nitidus* have a hairy main midrib in its basal half; this hairiness sometimes extending towards adjacent areas of the blade. Nevertheless, this character seems not to be sufficiently constant for taxonomic purposes.

Inflorescence

Flowers are arranged in distinct inflorescences, usually capitate to more or less elongate (up to 62 mm long in *Th. richardii* subsp. *ebusitanus*, Table 1). Bracts are similar to leaves, but smaller, and the bracteoles linear to linear-lanceolate. Pedicels are somewhat longer than documented for the species (Morales 2010), since in Majorcan plants of *Th. richardii* subsp. *richardii* can reach up to 5 mm long.

Calyx

Upper calyx-teeth are conspicuously different from lower. The upper lip teeth are usually narrower in *Th. richardii* subsp. *vigoi*. The calyx is green to purplish-green or to purple-violet. This colour variation can be observed within the same population, and the purplish coloration usually occurs in specimens that grow in more exposed places.

Regarding the calyx length, *Th. humifusus* var. *aureopunctatus* shows the lowest values, whereas the longest are those of the Majorcan populations of *Th. richardii* subsp. *richardii* (Table 1; Fig. 2). On the other hand, the length of the lower teeth of the calyx also allows separating the previous taxa (Table 1, 2). The presence of shorter calyces in *Th. humifusus* var. *aureopunctatus* was documented by Morales (2010), but so far this variation had not been quantified. From our point of view, the calyx length is a diagnostic character to separate the Balkan and the Balearic populations, together with other morphological characters (Table 1, 2).

The calyces are more or less hairy, with spheroidal yellowish-reddish glands. Our results show that the characters related to the hairiness of the calyx have taxonomic relevance in the *Th. richardii* complex. Calyx indumentum in *Th. richardii* subsp. *ebusitanus* is dense, with long eglandular hairs (up to 1 mm long), mainly on the margins of the lower teeth of the calyx and the ventral part of the calyx tube (Figs 2, 3). On the contrary, the calyx in the Majorcan populations of *Th. richardii* subsp. *richardii* is glabrescent (the upper lip and the dorsal surface of the calyx tube are glabrous or glabrescent) and the hairs are much shorter (Table 1). *Thymus humifusus* var. *aureopunctatus* has glabrescent to sparsely hairy calyces, but the eglandular hairs are usually more abundant and longer than in the Majorcan plants of *Th. richardii* subsp. *richardii*.

Stipitate glandular hairs are found in calyces (tube, teeth and even on the adaxial surface of upper teeth) of several taxa (Table 1). As noted by Jalas (1972) these glandular hairs are particularly abundant in *Th. richardii* subsp. *nitidus* (Figs 2, 3). However, stipitate glandular hairs are also usually found (in variable density) in *Th. richardii* subsp. *ebusitanus*, while in Majorca the specimens having these glandular hairs are rather rare but are observed on specimens from Coma de n'Arbona (BC 651145). These glandular hairs were not documented for Balearic plants of *Th. richardii* by Morales (2010). This character seems to be variable in the Balearic populations, since in the same locality there are plants without these glandular hairs.

Corolla

The upper lip is emarginate and the lower has 3 subequal lobes (middle lobe somewhat longer). The corolla is more or less hairy on the outer surface, with spheroidal yellowish-reddish glands. Its colour varies from pale rose (sometimes whitish or cream in *Th. richardii* subsp. *vigoi*) to pinkish-purple. The coloration is somewhat variable within the different taxa and in our opinion has no taxonomic significance.

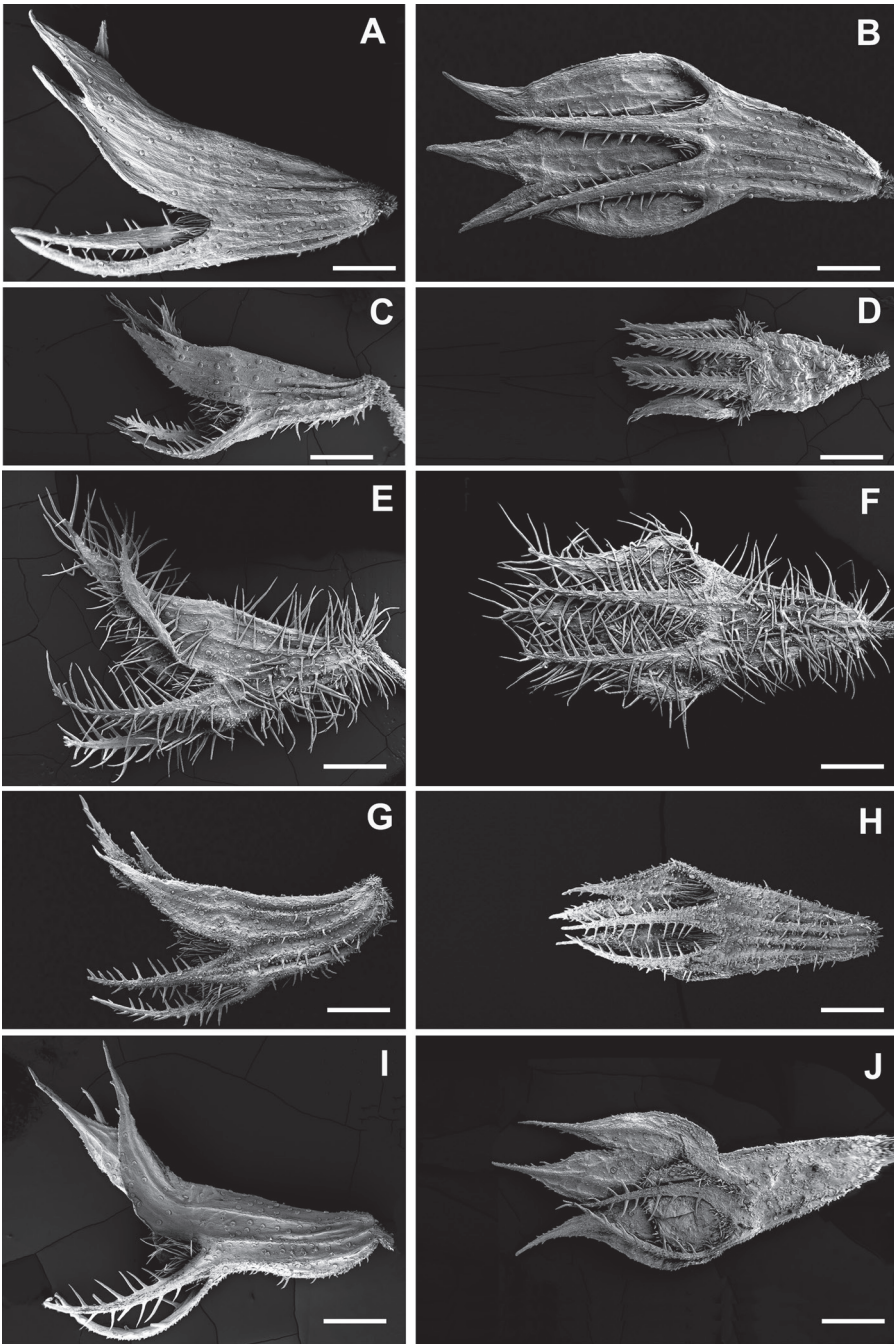


Figure 2. Calyx morphology for *Thymus richardii* and *Th. humifusus* var. *aureopunctatus*. For each taxon lateral (left) and ventral (right) views are shown. *Thymus richardii* subsp. *richardii* (**A, B** Spain, Mallorca); *Th. humifusus* var. *aureopunctatus* (**C, D** Bosnia and Herzegovina, Dužani); *Th. richardii* subsp. *ebusitanus* (**E, F** Eivissa, Ses Balandres); *Th. richardii* subsp. *nitidus* (**G, H** Sicily, Marettimo); *Th. richardii* subsp. *vigoi* (**I, J** Spain, Valencia, La Safor). Scale: 200 micrometres.

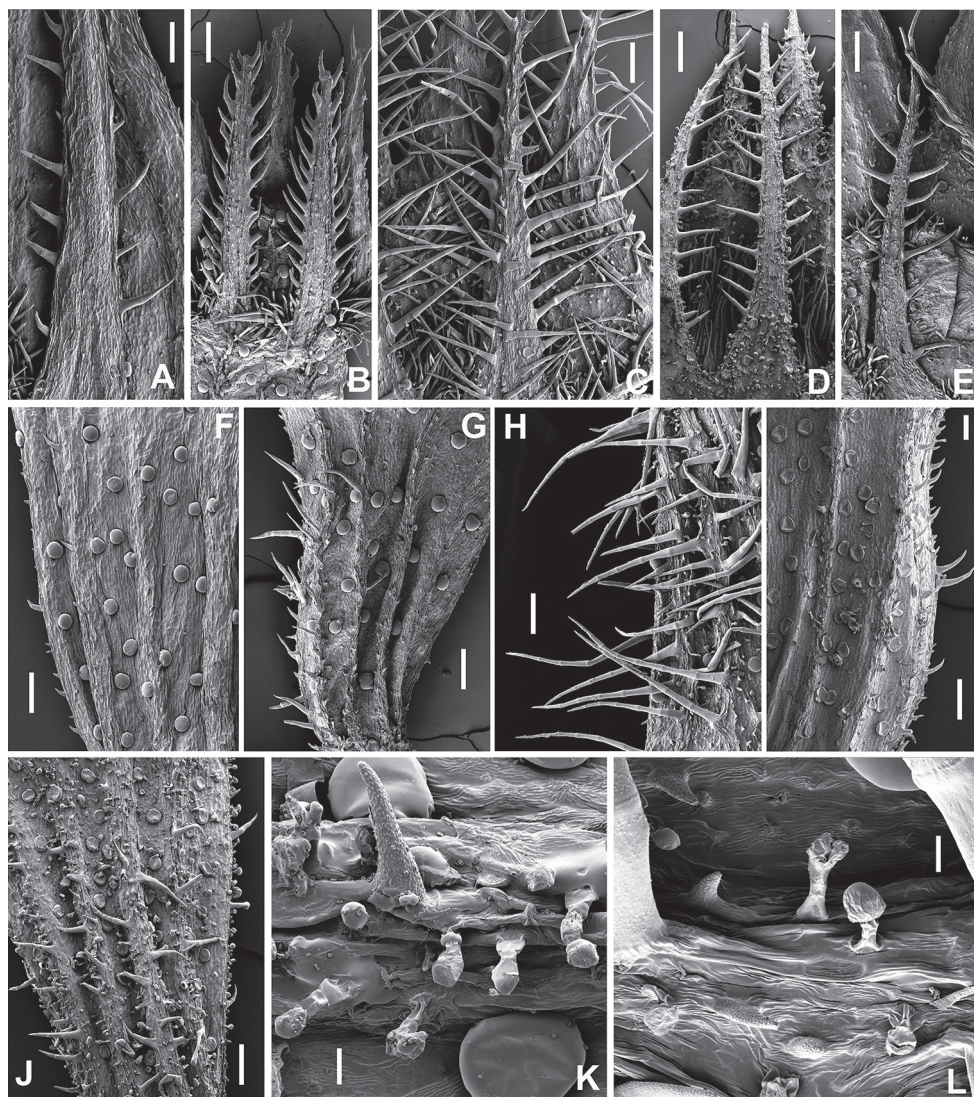


Figure 3. Detail of lower teeth and tube of the calyx and detail of glandular hairs of calyx tube in *Thymus richardii* subsp. *richardii* (**A, F** Spain, Mallorca, Puig Major, Es Bufador); *Th. humifusus* var. *aureopunctatus* (**B, G** Bosnia and Herzegovina, Dužani); *Th. richardii* subsp. *ebusitanus* (**C, H, L** Balearic Island, Eivissa); *Th. richardii* subsp. *nitidus* (**D, J, K** Italy, Sicily, Marettimo); *Th. richardii* subsp. *vigoi* (**E, I** Spain, Valencia, La Safor). Scales: 200 micrometers (**A–J**); 20 micrometers (**K–L**).

Taxonomic treatment

The Majorcan and the Balkan populations, which were included within typical *T. richardii* (Jalas 1971, 1972; Morales 2010) are morphologically distinct; they differ in several characters including calyx size, lower calyx teeth length, length of hairs on the calyx tube, length of pectinate hairs of lower calyx teeth and indumentum density on the calyx (Figs 1–3; Table 1, 3). The Majorcan plants have, compared to those from Bosnia

and Herzegovina, longer and less hairy calyces, with shorter hairs and longer lower calyx teeth with shorter (and less dense) pectinate hairs. Examination of herbarium specimens from five populations (16 specimens from Mallorca, 21 from Bosnia and Herzegovina) plus other specimens (see additional specimens examined) revealed that the diagnostic characters are constant within each geographic group. The morphological and biochemical (Llorens et al. 2014) differentiation between the Majorcan and the Balkan populations and their allopatric distribution (they are separated by a gap of ca. 1.300 km) firmly support the recognition of two subspecies, since the level of morphological differentiation between the two taxa does not meet the criteria commonly used to delimit species in *Thymus*. Certainly, further research using molecular markers is needed to reveal genetic relationships and biogeographic history of the *Th. richardii* complex.

1. *Thymus richardii* Pers., Syn. Pl. 2: 130. 1806 subsp. *richardii*

≡ *Thymus serpyllum* var. *richardii* (Pers.) Knoche, Fl. Balear. 2: 354. 1922.

≡ *Th. serpyllum* subsp. *richardii* (Pers.) Malag., H. Bianor, Educador Botánico Baleares: 150. 1971.

Type. Holotype (see Rosselló and Sáez 2001: 109): P-Lamarck.

Description. Stems up to 47 cm long, procumbent to reptant. Leaf blade up to 13 × 7.7 mm, broadly ovate to elliptical, entire. Inflorescence 15–30 mm long, capitate to oblong; bracts up to 11 × 7.8 mm, similar to leaves, entire, glabrous. Calyx 6–8 mm long, glabrescent (sometimes glabrous), with eglandular hairs up to 0.3 mm long, occasionally with scattered stipitate glandular hairs; calyx tube 2.2–3.2 mm long, glabrescent (sometimes glabrous on the dorsal surface), with eglandular hairs up to 0.3 mm long on the ventral surface; central tooth of upper lip 1.3–2.2 mm long, lower teeth 2.8–3.8 mm long, with pectinate hairs up to 0.3 mm long. Corolla 7–11 mm long, rose to pinkish-purple (Fig. 4, C, E).

Chromosome number. $2n = 30$ (Morales 1995).

Distribution. Endemic to Mallorca, Eastern Balearic Islands (Spain).

Habitat. Cliffs, on humid and north-facing limestone rocks, 250–1430 m a.s.l.

Remarks. This is a rare plant, documented from three localities in the north of Mallorca (Ternelles mountain, Formentor peninsula and Puig Major) of which we have only been able to verify its presence in the last locality, growing on cliffs with very difficult access. This taxon could be facing a population decline. Bianor (1917) at the beginning of the 20th century, considered it as abundant in the Puig Major [“Abondant dans les endroits peu accessibles”]. In fact, there are dozens of specimens from this mountain and which are preserved in various herbaria; mostly collected in the late 19th and early 20th centuries. Currently, *Th. richardii* subsp. *richardii* is very scarce at the same locality where it was reported by Bianor (1917) and the plants are practically inaccessible if climbing techniques are not used. Another population located on a different slope of the same massif is also scarce and very difficult to access. This possible population decline could be due to a loss of potential habitat and intense predation by feral goats (*Capra hircus*).

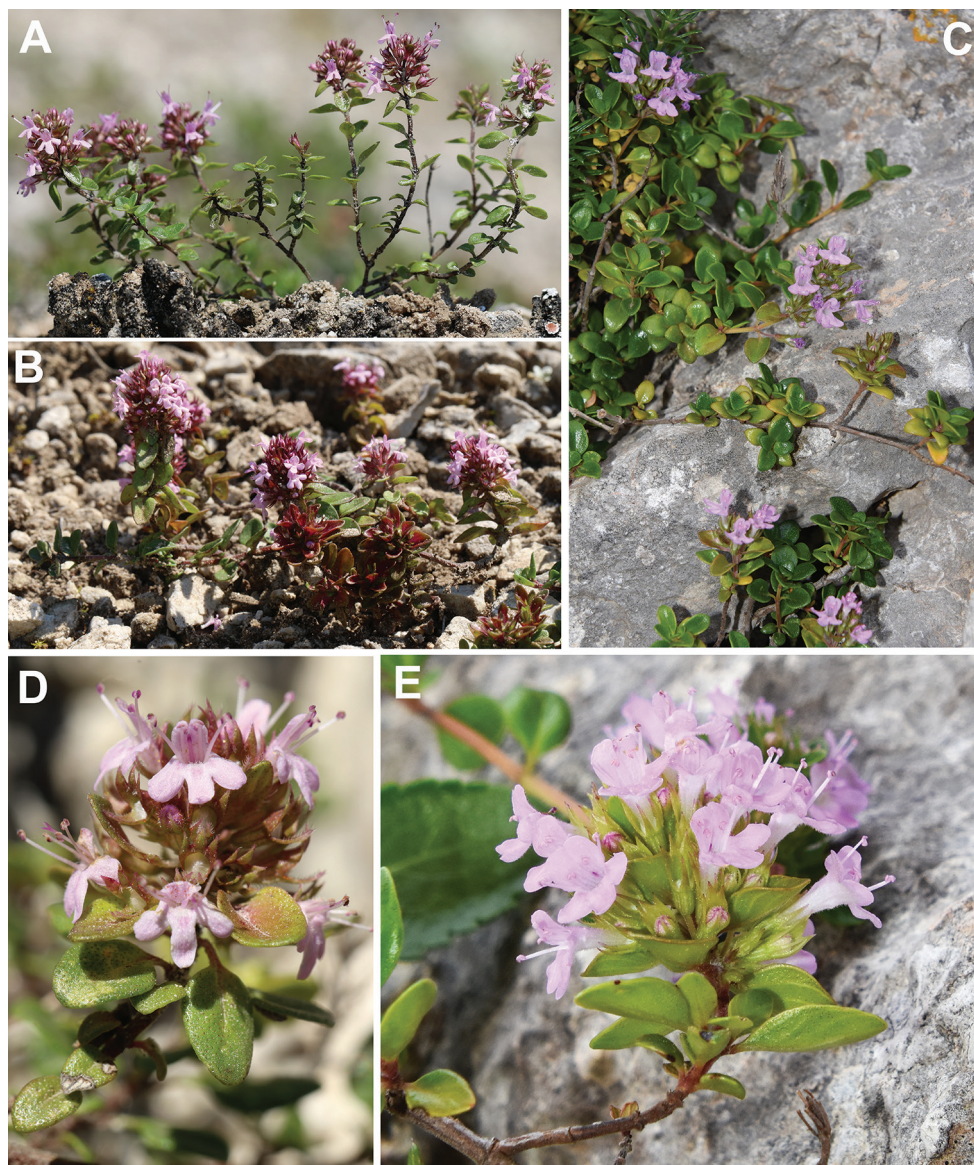


Figure 4. Habit and detail of the inflorescence of *Thymus humifusus* var. *aureopunctatus* **A, D** from Bosnia and Herzegovina, Dužani, 3 July 2020; **B** from Bosnia and Herzegovina Džepi, 10 July 2020 (photo F. Bogunić), and of *Th. richardii* subsp. *richardii* **C, E** from Spain, Balearic Islands, Mallorca, Coma de N'Arbona, 17 June 2021 (photo L. Sáez).

Specimens examined. **SPAIN.** Balearic Islands, Mallorca: Comma de n'Arbona, Puig Major, 12 June 1852, *G. Vigineix* (P 04436032, P 04436034); Majorque, 19 June 1869, *Bourgeau* (P 04436046); rochers des Arbonas [n'Arbona], 17 Apr 1870, *F. Barceló* (COI 00045051, P 03389631); Coma de n'Arbona et Puig Major de Son Torrella, 1000–1300 m, 24 June 1885 and 29 July 1885, *Porta & Rigo* (P 04436045); Mallorca: Coma

de n'Arbona, Sóller, 30 June 1879, *A. Crespi* (BC 651145, P 04407218); Puig Major, 1000–1400 m, 12 July 1917, *F. Bianor* (BC 50119); Féntes des rochers, Puig Major, 1000–1450 m, 12 July 1918, *F. Bianor* (BC 50118); Puig Major, Féntes des rochers, 1000–1500 m, 7 July 1919, *F. Bianor*, Pl. Espagne F. Sennen 3768 (BC 50123); Coma de n'Arbona, 18 June 1920, *Gros* (BC 859198, P 04407218); Coma de n'Arbona, 4 July, 1936, *Kennedy* 48 (BC 103732); Sóller, esclatxes dels espadats de la Coma de n'Arbona, July, 1958, *L. Garcías Font* (BC 145169); Puig Major, Coma de n'Arbona, 27 June 1985, *T. Rabassa* (HBSJ 5700); Puig Major, Escorca, 10 July 1986, *L. Sáez* (MA 592837); Puig Major de Son Torrella, c. via des Bufador, Escorca, 31SDE8207, 1200 m, 14 June 2006, *L. G. Valle & L. Sáez LS-6445* (*L. Sáez*, herb. pers.); Escorca, Puig Major, Penyal des Bufador, 31DE8206, 1340 m, 30 June 2020, *L. Sáez* (*L. Sáez*, herb. pers.); Fornalutx, Coma N'Arbona, 31SDE8105, 1100 m, 17 June 2021, *L. Sáez* (*L. Sáez*, herb. pers.).

2. *Thymus richardii* subsp. *aureopunctatus* (Beck) L.Sáez, Bogunić & Bogdanović, comb. & stat. nov.

urn:lsid:ipni.org:names:77234197-1

≡ *Thymus humifusus* var. *aureopunctatus* Beck, Ann. Naturhist. Mus. Wien 2: 142. 1887, basionym.

≡ *Th. aureopunctatus* (Beck) K. Malý, Prilozi za floru Bosne i Herzegovine: 557. 1908.

Type. Herc. [Herzegovina], Nächst Konjica, 8 July 1885, *G. Beck* (lectotype: PRC 455886! designated here, Fig. 5).

Description. Stems up to 45 cm long, procumbent to reptant. Leaf blade up to 7.7 × 5.3 mm, suborbicular to elliptical, entire. Inflorescence 8–21 mm long, capitate; bracts up to 6.5 × 5 mm, similar to leaves, entire, usually hairy at margin (eglandular hairs up to 1 mm long). Calyx 3–5 mm long, glabrescent to sparsely hairy, with eglandular hairs up to 0.5 mm long, without stipitate glandular hairs; calyx tube 1.3–2.2 mm long, sparsely hairy, with eglandular hairs up to 0.5 mm long on the ventral surface; central tooth of upper lip 0.7–1.6 mm long, lower teeth 2–3 mm long, with hairs pectinate up to 0.5 mm long. Corolla 6–9 mm long, rose (Fig. 4, A, B, D).

Chromosome number. $2n = 28$ (Kaleva 1969).

Distribution. Endemic to surroundings of Konjic (Podorašac, Koznik, Dužani, Dudle, Džepi, Zlatar, Borci, Spiljani, Glavatičevo, Pribilja, Repovica), northern Herzegovina. The taxon covers an area of c. 280 km².

Habitat. Sandy dolomites and dolomitic rocky places, 400–1040 m a.s.l.

Remarks. Beck (1887) described *Th. humifusus* var. *aureopunctatus* from “In saxosis prope Konjicam” [Bosnia and Herzegovina] and related this new variety to *Thymus humifusus* Bernh. ex Link, which is currently regarded a synonym of the tetraploid *Th. praecox* Opiz (Jalas 1971; Euro+Med 2006; Plant List 2021; WFO 2021). Günther Beck (1856–1931) was a Bohemian botanist, and his herbarium is currently kept at PRC and W (Stafleu and Cowan 1976). We have been able to locate original material of *Th. humifusus* var. *aureopunctatus* at PRC. This is a well-prepared specimen; it

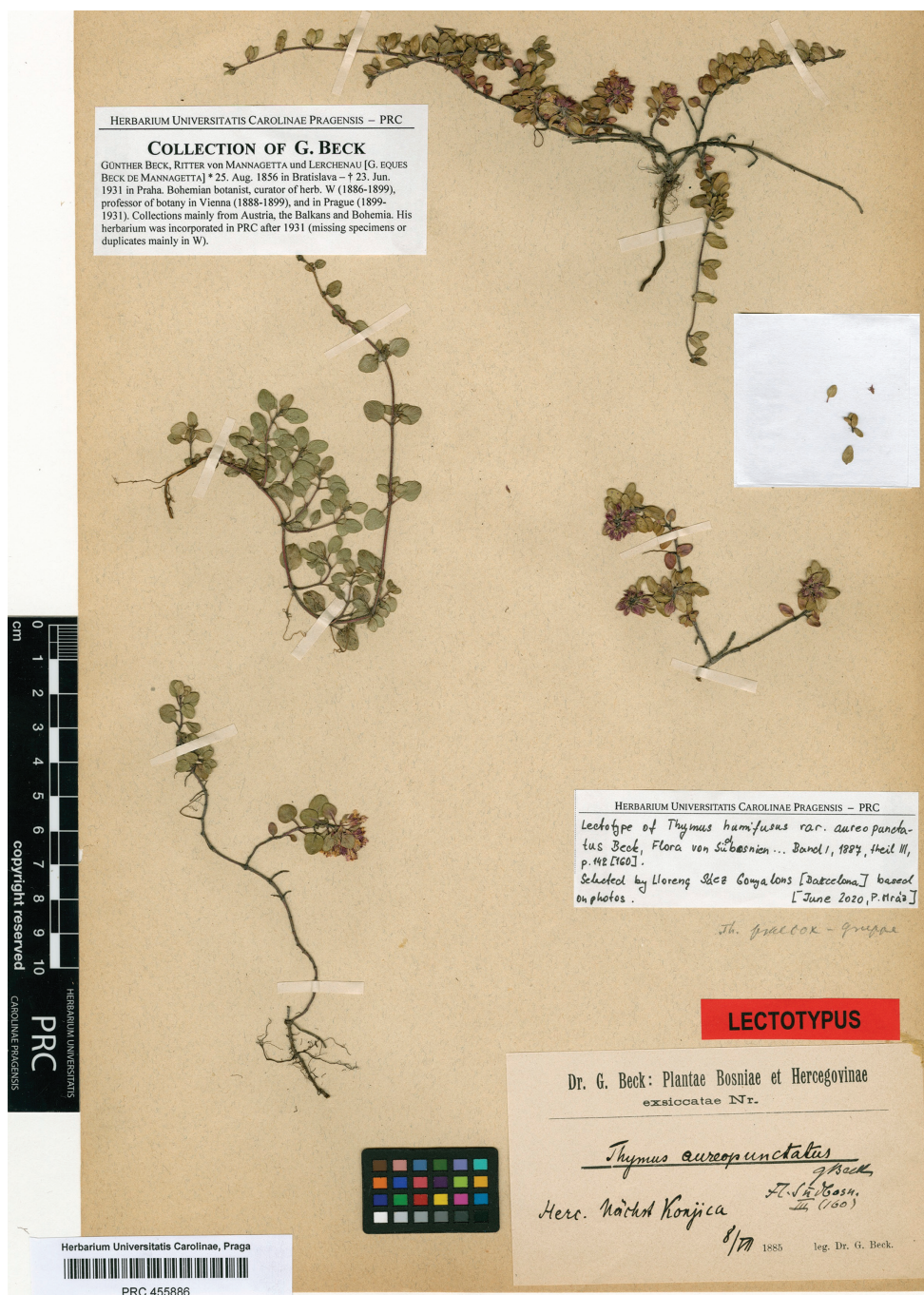


Figure 5. Lectotype of *Thymus humifusus* var. *aureopunctatus* (PRC 455886).

matches the description and the provenance indicated in the protologue. Therefore, we designate the specimen with barcode PRC 455886 as the lectotype of the name *Th. humifusus* var. *aureopunctatus* (Fig. 5). The taxon occurs in fragmented subpopulations in

Bosnia and Herzegovina. Their habitats are threatened by forest succession and canopy closure, but frequent fire incidences represent the most serious threat to its subpopulations. However, the overall population trend of *Th. richardii* subsp. *aureopunctatus* is inferred to be generally stable (F. Bogunić, pers. observ.).

Specimens examined. BOSNIA AND HERZEGOVINA. Konjic, 8 July 1885, *G. Beck* (PRC 455886!, lectotype); Konjic, Dužani, 43.509894N 18.152114E, 830 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.); Konjic, Džepi, 43.675506N 18.011992E 757 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.); Bosnia and Herzegovina, Dudle, 43.540567N 18.121261E, 1034 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.); Flora Herzegovinae. In pineti (*Pinus nigra*) inter Pričepa-Bigolje; solo dolomitico, 720 m, 9 August 1908, *K. Maly* (ZA); Flora Hercegovinae. In saxosis dolomiticis ad Repovica prope Konjic, 12 July 1931, *V. Loschingg* (ZA).

3. *Thymus richardii* subsp. *ebusitanus* (Font Quer) Jalas, Bot. J. Linn. Soc. 64(3): 264. 1971

≡ *Thymus richardii* var. *ebusitanus* Font Quer, Cavanillesia 7: 77. 1935;

≡ *Th. ebusitanus* (Font Quer) Romo, Fl. Silvestres Baleares: 266. 1994.

Type. Eivissa, cala de les Torretes, 29 May 1918, *Font Quer & Gros* (lectotype: BC 50117! designated by Jalas 1971: 264).

Description. Stems up to 54 cm long, more or less reptant to suberect. Leaf blade up to 11 × 8 mm, suborbicular to elliptical, entire. Inflorescence 19–62 mm long, oblong; bracts up to 8.3 × 7.3 mm, similar to leaves, entire; glabrous to hairy at margin and midrib (eglandular hairs up to 1 mm long). Calyx 4.5–6.9 mm long, densely hairy, sometimes hirsute, with eglandular hairs up to 1 mm long, usually with stipitate glandular hairs; calyx tube 1.8–2.7 mm long, densely hairy, with eglandular hairs up to 1 mm long on the ventral surface; central tooth of upper lip 0.9–1.9 mm long, lower teeth 1.9–3.1 mm long, with pectinate hairs up to 1 mm long. Corolla 6–8.5 mm long, pale rose.

Chromosome number. $2n = 30$ (Morales 1990).

Distribution. Endemic to northern Eivissa, Western Balearic Islands (Spain).

Habitat. Limestone rocky places, 5–370 m a.s.l.

Specimens examined. SPAIN. Balearic Islands, Eivissa: cala de les Torretes, 29 May 1918, *Font Quer & Gros* (BC 50117, lectotype); Santa Agnès, a la Cala de les Torretes, 15 June 1918, *Gros* (BC 50116); Cala de'n Damià, 10 July 1920, *Gros* (BC 858975, P 04438273); cala de les Torretes, 8 July 1920, *Gros* (BC 859210, P 04438274); cala de Santa Agnès, 5 m, July 1935, *Gros* (BC 87078, BC87079); Cala Aubarca, 1 Aug 1974, *J.Y. Lesouëf* (MA 620032); vicum Sant Mateu, cala d'Aubarca, 31SCD52, 50 m, 23 June 1979, *Fernández Casas* 2883 (BC 633215); Cala den Sardina, 2 June 1981, *Cardona & al.* (BC 644574); Cala den Sardina, 20 June 1983, *L. Llorens* (Herb. Univ. Illes Balears); cingles d'en Recó, 8 June 1997, *N. Torres, M. Mayol & L. Sáez* (MA 592780); Ses Balandres, 31SCD5523, 131 m, 3 June 2010, *C. Benedí & L. Sáez* (L. Sáez, herb. pers.).

4. *Thymus richardii* subsp. *nitidus* (Guss.) Jalas, Bot. J. Linn. Soc. 64: 264. 1971

≡ *Thymus nitidus* Guss., Fl. Sicul. Syn. 2(1): 97. 1844;

≡ *Th. serpyllum* var. *nitidus* (Guss.) Bég. in Fiori & Béguinot, Fl. Italia 3: 66. 1903. -
Th. sensu lucidus Guss., Fl. Sicul. Prodr., Suppl.: 198. 1843

Type. Marettimo, 10 May 1829, Herb. Gussone Sicilia *s.c.*, bottom-right specimen (Lectotype: NAP-Gussone!, designated by Bartolucci et al. 2013: 1310).

Description. Stems up to 25 cm long, procumbent or suberect. Leaf blade up to 10 × 4.5 mm, elliptical, entire. Inflorescence 8–30 mm long, subcapitate to oblong; bracts up to 7 × 4 mm, similar to leaves, entire, glabrous to hairy at margin and midrib (eglandular hairs up to 0.4 mm long, mixed with stipitate glandular hairs). Calyx 4.5–6.3 mm long, densely covered by stipitate glandular hairs and sparse eglandular hairs up to 0.5 mm long; calyx tube 1.9–2.5 mm long, with eglandular hairs up to 0.5 mm long on the ventral surface; central tooth of upper lip 0.8–1.5 mm long, lower teeth 2–3 mm long, with pectinate hairs up to 0.5 mm long. Corolla 6.5–9.5 mm long, pale rose.

Chromosome number. $2n = 28$ (Morales 1997)

Distribution. Endemic to Island of Marettimo, Sicily (Italy).

Habitat. Limestone rocky places, 10–600 m a.s.l.

Specimens examined. ITALY. Sicily, Marettimo, *sine leg.* (PAL); Isola di Marettimo, rupi di P. Anzine, 21 July 2007, *Scuderi* (VAL 184304).

5. *Thymus richardii* subsp. *vigo*i Riera, Güemes & Rosselló, Fl. Montiber. 37: 78. 2007

Type. Spain, Valencia, Villalonga, La Safor, ad l'Orxa, 30SYJ3706, 600 m, 4 July 2000, *J. Riera & J. Güemes* (holotype: VAL 185406!; isotype: MA 757804!; Riera et al. 2007).
= *Thymus richardii* var. *valentinus* O. Bolòs & Vigo, Collect. Bot. (Barcelona) 14: 95. 1983

Type. Spain, Valencia province, Valentia, c. Gandia, 15 Sept 1950, *P. Cañigüeral* (holotype: BC 119858!; Bolòs and Vigo 1983).

Description. Stems up to 16 cm long, suberect to erect. Leaf blade up to 11 × 8.1 mm, ovate-triangular, denticulate. Inflorescence 15–34 mm long, usually oblong; bracts up to 8 × 7 mm, similar to leaves, denticulate, usually glabrous. Calyx 5.8–7 mm long, sparsely hairy, with eglandular hairs up to 0.7 mm long, sometimes with sparse stipitate glandular hairs; calyx tube 2.1–2.9 mm long, with eglandular hairs up to 0.5 mm long on the ventral surface; central tooth of upper lip 1.8–2.6 mm long, lower teeth 3–3.5 mm long, with pectinate hairs up to 0.7 mm long. Corolla 7–10 mm long, whitish to pale rose, sometimes cream.

Chromosome number. Unknown.

Distribution. Endemic to Alicante and Valencia provinces (Spain).

Habitat. Open scrub, on limestone soil, 130–600 m a.s.l.

Remarks. Plants which were considered to be hybrids between *Th. richardii* subsp. *vigoii* and *T. piperella* L. have been called *T. × bolosii*. The hybrid has been reported from a small area of Serra de la Safor, eastern Spain (Riera et al. 2020).

Specimens examined. SPAIN. Alicante province: La Vilallonga, La Safor, 136 m, 22 June 1984, *J.B. Peris & G. Stübing* (BC 674556); Valencia province, Valentia, c. Gandia, 15 Sept 1950, *P. Cañigüeral* (BC 119858); Villalonga, La Safor, ad l'Orxa, 30SYJ3706, 600 m, 4 July 2000, *J. Riera & J. Güemes* (VAL 185406!).

Identification key for *Thymus richardii* complex

We propose the following key for the subspecies of the *Thymus richardii* complex in order to include the new proposed subspecies.

- 1 Leaves denticulate, cordate at base **subsp. *vigoii***
- Leaves entire (rarely slightly denticulate) cuneate at base **2**
- 2 Calyx glabrescent (sometimes glabrous) to sparsely hairy; glandular hairs usually scarce or absent **3**
- Calyx hairy to densely hairy; glandular hairs usually present **4**
- 3 Calyx 3–5 mm long **subsp. *aureopunctatus***
- Calyx 6–8 mm long **subsp. *richardii***
- 4 Calyx with abundant eglandular hairs, usually with sparse to dense glandular stipitate hairs; lower calyx teeth with pectinate pluricellular eglandular hairs up to 1 mm long **subsp. *ebusitanus***
- Calyx densely covered by glandular hairs, mixed with sparse eglandular hairs; calyx teeth with pectinate eglandular pluricellular hairs up to 0.5 mm long .
..... **subsp. *nitidus***

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Appendix I. List of specimens included in morphometric analyses.

Thymus richardii subsp. *richardii*. SPAIN. Mallorca: Coma de n'Arbona, Sóller, 30 June 1879, *A. Crespi* (BC 651145) [1 specimen]; Fentes des rochers, 1000–1150 m, 12 July 1918, *Bianor* (BC 50118) [1 specimen]; *Ibidem* 7 July, 1919, *Bianor* (BC 50123) [1 specimen]; *Ibidem* 12 July, 1917, *Bianor* (BC 50119) [1 specimen]; Coma de n'Arbona, 18 June 1920, *Gros* (BC 859198) [1 specimen]; Col de n'Arbona, 4 July, 1936, *Kennedy* 48 (BC 103732) [1 specimen]; Espadats de la coma de n'Arbona, July, 1958, *Garcias Font* (BC 145169) [1 specimen]; Coma de n'Arbona, 27 June 1985, *T. Rabassa* (HBJS 5700) [1 specimen]; Coma N'Arbona, 31SDE8105, 1100 m, 17 June 2021, *L. Sáez* (L. Sáez, herb. pers.) [4 specimens]. Puig Major, via des Bufador, Escorca, 31SDE8207, 1200 m, 14 June 2006, *L.G. Valle & L. Sáez LS-6445* (L. Sáez, herb. pers.) [1 specimen]; Puig Major, Penyal des Bufador, 31DE8206, 1330–1350 m, 30 June 2020, *L. Sáez* (L. Sáez, herb. pers.) [8 specimens].

***Thymus richardii* subsp. *ebusitanus*. SPAIN.** Balearic Islands, Eivissa: cala de les Torretes, 29 May 1918, *Font Quer & Gros* (BC 50117, lectotype) [2 specimens]; Santa Agnès, a la Cala de les Torretes, 15 June 1918, *Gros* (BC 50116) [1 specimen]; cala de les Torretes, 8 July 1920, *Gros* (BC 859210) [1 specimen]; Cala de'n Damià, 10 July 1920, *Gros* (BC 858975) [1 specimen]; cala de Santa Agnès, 5 m, July 1935, *Gros* (BC 87078, 87079) [2 specimens]; vicum Sant Mateu, cala d'Aubarca, 31SCD52, 50 m, 23 June 1979, *Fernández Casas 2883* (BC 633215) [1 specimen]; Cala den Sardina, 2 June 1981, *Cardona & al.* (BC 644574) [1 specimen]; Cala den Sardina, 20 June 1983, *L. Llorens* (Herb. Univ. Illes Balears) [1 specimen]; Ses Balandres, 31SCD5523, 131 m, 3 June 2010, *C. Benedí & L. Sáez* (L. Sáez, herb. pers.) [5 specimens].

***Thymus richardii* subsp. *aureopunctatus*. BOSNIA AND HERZEGOVINA.** Konjic, Dužani, 43.509894N 18.152114E, 830 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.) [7 specimens]; Konjic, Džepi, 43.675506N 18.011992E 757 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.); [7 specimens]; Dudle, 43.540567N 18.121261E, 1034 m, 10 July 2020, *F. Bogunić* (SARA, ZAGR, L. Sáez herb. pers.) [7 specimens].

***Thymus richardii* subsp. *nitidus*. ITALY.** Sicily, Marettimo s.d., s.r. (PAL) [3 specimens]; Isola di Marettimo, rupi di P. Anzine, 21 July 2007, *Scuderi* (VAL 184304) [1 specimen].

Phelypaea boissieri f. *lutea* (Orobanchaceae), a peculiar new form from Turkey and typification of the name of this species

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Abstract

The genus *Phelypaea* includes three holoparasite species with one of the most intense red flowers among all plants worldwide. So far, there are few references to other colour taxa of this genus. We describe a new yellow-coloured form, *Phelypaea boissieri* f. *lutea*, from Hakkari province in the Cilo Mountains of SE Turkey, found at an altitude of 2,470 m. In typical *P. boissieri*, here typified by us, the flowers are deeply red, and stems, calyx and scales are red to brown, or rarely pale-brown, whereas in the entire population of f. *lutea* the corolla, calyx, and scales are yellow to orange with black folds in the corolla, while only the stem is brownish.

Keywords

Forma nova, lectotype, Orobanchaceae, *Phelypaea*, SE Turkey, synonyms, typification

Introduction

The genus *Phelypaea* L. ($\equiv$ *Diphelypaea* Nicolson, nom. illeg.) (Orobanchaceae) includes three holoparasite species: *P. coccinea* (M. Bieb.) Poir., *P. boissieri* (Reut.) Stapf, and *P. tournefortii* Desf., whose parasitise Asteraceae hosts. *P. coccinea* occurs in the

Caucasus and Crimea, and is a parasite of *Psephellus* Cass. and *Centaurea* L., rarely *Klasea* Cass., while *P. tournefortii* occurs in the Caucasus and Turkey, and is a parasite of *Tanacetum* L. (Sánchez Pedraja et al. 2016; Piowarczyk et al. 2019). Meanwhile, *P. boissieri* shows a different distribution; it occurs in the Balkans (Albania, Greece, North Macedonia), and Western Asia (Turkey, Iraq and Iran), and parasitises *Centaurea* and occasionally *Cousinia* Cass. in Iraq (Sánchez Pedraja et al. 2016; Piowarczyk et al. 2019). *P. boissieri* is molecularly, morphologically and regarding host preferences similar to *P. coccinea* (Piowarczyk et al. 2019, 2021). The morphological features that separate *P. boissieri* and *P. coccinea* are as follows: corolla - tube short and cup-shaped; corolla - lobes broadly obovateorbicular to orbicular, overlapping; anthers - hairy (Stapf 1915; Nicolson 1975; Cullen 2010); however, sometimes apparently intermediate features are also observed (Piowarczyk et al. 2019). Therefore, further research into the variability of *P. boissieri* and the inclusion of more samples for molecular analysis are required (Piowarczyk et al. 2021).

Species from the genus *Phelypaea* are achlorophyllous and possess one of the most intense red flowers among all plants worldwide. A recent study on *P. tournefortii* showed that anthocyanins were found in unprecedented large quantities in the flowers, as well as large amounts of polyphenols, especially eukovoside (Piowarczyk et al. 2020).

So far, there have been few references to colour forms other than red in the genus *Phelypaea* in the literature. One of them is *P. coccinea* f. *aurantiaca* Beck in Engl., Pflanz. 96: 261 (1930), which was described from Karabakh in the Caucasus based on material collected by Radde as having an orange corolla (“aurantiaco”). Another non-red colour form is *P. helenae* Popl., described from Alushta in Crimea (“corolla orange-yellow”) and, according to Novopokrovsky and Tzvelev (1958: 28), it is synonymous with the species mentioned before and does not constitute a different species (but rather a case of polychromism) with regard to *P. coccinea* (“corolla bright-red”), and both yellow and red forms occur together in Crimea. In *P. coccinea*, sometimes the corolla in the lower-side is orangish or yellow, with the upper side in the typical red color preserved (Piowarczyk et al. 2019). Within one population, there may be individuals with a corolla which is red on both sides, as well as with one yellow-orange side.

In this paper, we typify *P. boissieri* and indicate its synonyms, and besides, describe a new yellowish-coloured form from Turkey.

Typification of *Phelypaea boissieri*

Phelypaea boissieri (Reut.) Stapf in Bull. Misc. Inform. Kew 1915, 6: 291 (1915 [17 Aug 1915]).

Basionym: *Anoplangthus biebersteinii* var. *boissieri* Reut. in DC., Prodr. 11: 42 (1847 [25 Nov 1847]).

Type: lectotype (here designated): 1. “*Orobanche* / Cadmus [Mount Cadmus / Topçambaba Dağı / Baba Dağı / Baba Dagħ, Aydın Province? or, more likely, mont Honaz / mont Cadmus / Honaz Dağı, Denizli Province?] ad or. Denisleh [to the east

Denizli Jun" [m. Boissier]. – 2. "*A. coccineus*". – 3. "Syntypes / *Anoplanganthus biebersteinii* / var. *boissieri* Reut." (G-Boiss G00150150 [Fig. 1, the sheet contains four specimens, the lectotype is formed by the three specimens of a single gathering, located in the lower left of the sheet]).

Isolectotypes: idem (G-Boiss G00150150 [Fig. 1, the isolectotype is a single specimen in the upper right corner]); "*Anoplanganthus biebersteinii* var. *boissieri* Reut. / Cadmus supra Denisleh [Denizli], ad *Centaurea* / *Boissier*, [6 Jun 1842] (JE000000021 – hb. Haussknecht [two specimens of a single gathering]); "Herb. E. Boissier / *Anoplanganthus Biebersteinii* Reut. / β *Boissieri* Reut. / Mesogis [Messogis Mountains / Mesogis Mountains, Aydın Dağları (ancient Lydia), near Aydın / Güzelhisar / Tralles, Aydın Province] et Cadmus [Mount Cadmus / Topçambaba Dağı / Baba Dağı / Baba Dagh, Aydın Province? or mont Honaz / mont Cadmus / Honaz Dağı, Denizli Province?] / Jun. 1842" (P02970934 - Boissier 1842 [the three specimens of a single gathering on the lower right corner close to Boissier's label]); idem (P02970938 – hb. E. Cosson [the two specimens of a single gathering on the upper left corner close to Boissier's label]).

Isosyntypes: 1. "Caria [Karia], *C. Pinard*, 1843". – 2. "Syntypes / *Anoplanganthus biebersteinii* / var. *boissieri* Reut." (G-Boiss G00150149 [two specimens of a single gathering, which are attached to its host-plant]); 1. "Caria [Karia], *C. Pinard*, 1843". – 2. "*A. Biebersteinii*". – 3. "*Anoplanganthus Biebersteinii* Reut. / in DC. prod. 11 p. 42 / *Phelipaea biebersteinii* Fisch. / *Anoplon* — C.A. Mey. / *Orobancha coccinea* Pers. / *Lathraea phelipaea* β Linn. / — β . *Boissieri* Reut. in DC. prod.". – 4. "Syntypes / *Anoplanganthus biebersteinii* / var. *boissieri* Reut." (G00096074 - hb. Reuter-Barbey [Fig. 2, three specimens of a single gathering, two of them are attached to their host-plant]).

Homotypic synonyms: *Diphelypaea boissieri* (Reut.) Nicolson in Taxon, 24 (5–6): 654 (1975), nom. illeg.

Phelypaea coccinea var. *boissieri* (Reut.) Beck in Engl., Pflanzenr. 96: 43 (1930)

Diphelypaea coccinea var. *boissieri* (Reut.) Uhlich in Kochia 9: 83 (2015), nom. illeg.

Heterotypic synonyms: *Anoplanganthus coccineus* var. *nigrovittatus* Boiss., Fl. Orient. 4(2): 494 (1879 [Apr-May 1879]) ["*A. coccineus* γ *nigrovittatus*"] (Stapf 1915: 290, 293). *Ind. loc.*: "Hab. in graminosis montis Pir Omar Gudrum [Chiya-i Piromar / Chiyā-i Pīrōmar / Jabal Biru Mar / Jabal Bīrū Mār / Pīr `Umar, Bīrūmā / Piromar / Pīrōmar, Iraqi Kurdistan, Iraq] 5–6000' (*Hausk!*)". *Lectotype* (designated here, or perhaps holotype): 1. "711 / *Anoplanganthus Biebersteinii* Reuter / fl sanguin., vitta nigra lata / In gramin. 5-6000' / Haussknecht, It. Orient, Pir Omar Gudrum. Jun. — 1867". – 2. "*A. coccineus* γ *vittatus*". – 3. "*Anoplanganthus coccineus* γ *vittatus*" (G-BOIS G00768924 - n° SIB 436114/1 (Stapf 1915: 293) [five specimens of a single gathering]).

Phelypaea coccinea var. *boissieri* f. *septemfida* Gilli in Feddes Repert. 46: 47 (1939) ["Calyx irregulariter septemfidus"]. *Ind. loc.*: "Nord-Iran [Iran / Persia]: Nördlich vom Kendewan-Paß [Kendavan Pass / Gardaneh-ye Kandovān, 36°9'0"N, 51°18'0"E, Māzandarān, Alborz / Alburz / Elburz / Elborz Mountains] an einem Abhang unweit des Tunnelbaues, 1800 m; 8.VII.1936."

The species typified here was described by Reuter, with the rank of var., on the basis of collections made in western Anatolia (Turkey): Mount Cadmus by Boissier



Figure 1. Lectotype and isolectotype (G00150150 - G-Boiss) of *Phelypaea boissieri*.



Figure 2. Isosyntype (G00096074 - hb. Reuter-Barbey) of *Phelypaea boissieri*.

and Caria by Pinard (Reuter 1847: 42). Later, Boissier (1879: 494) included it within his *Anoplanganthus coccineus*, which would be the origin of future confusions between the two species. In addition, he described two new varieties, the var. *peduncularis* (*P. tournefortii* Desf.) and the var. *nigrovittatus* (*P. boissieri* (Reut.) Stapf). Stapf (1915) raised the variety of Reuter to the rank of species and clarified the differences between the three taxa, so that nowadays we believe that they form this genus. Beck (1930), in a monograph on the family, preferred to continue treating our species with the rank of var. within *P. coccinea*, but created some confusion when giving the distribution of this species where he included the var. described by Reuter. Finally, Cullen (2010) complements the description of *P. boissieri*, giving good illustrations with analysis, providing a new key for the three species, and indicating the distribution of the species treated here.

A new form of *Phelypaea boissieri*

Phelypaea boissieri f. *lutea* Ü. Subaşı, R. Piwowarczyk, Ó. Sánchez Pedraja, f. nov.
Figure 3

Diagnosis. This new taxon is very similar to typical *Phelypaea boissieri*, and is compliant with morphometric characteristics in the description of the plant after Reuter (1847: 42, sub *Anoplanganthus biebersteinii* var. *boissieri*), Stapf (1915), Nicolson (1975), Cullen (2010) but differs significantly in color and, usually, a higher stem. In typical *P. boissieri*, flowers are deeply red, and stem, calyx and scales are red to brown, or rarely pale-brown, whereas in the f. *lutea* corolla, calyx, and scales are yellow to orange with black folds in the corolla, with only the stem being brownish (Fig. 3).

Type. TURKEY. C10 Hakkâri: Merkez district, Kırıkdağ village, Gümüşlü location (Kurdish: Spixane). Cilo Mountains, subalpine grasslands and meadows, ca. 2,470 m alt., 10 June 2021, Ü. Subaşı s.n. (holotype and isotypes ANK!).

Distribution and ecology. This taxon is known exclusively from the type locality. The Cilo (Glacial) Mountains (Cilo (Buzul) Dağı) are part of the Hakkâri Dağları range and include the third highest peak of Turkey with an altitude of 4,135 meters. Turkey's Eastern Anatolia Region, the easternmost extension of the Southeast Taurus Mountains, is located within the borders of Hakkari province. The locality of *P. boissieri* f. *lutea* is located in Hakkari province, Kırıkdağ village, Gümüşlü location (Kurdish: Spixane). This area is located around Mt Uludoruk (Reşko 4,135 m), at an altitude of approximately 2,470 meters. In this area dominated by high mountain steppe plants, the bedrock consists mainly of tectonic deposits and limestone. It is approximately 20 km from any settlements. Parasitising *Centaurea*, probably *C. persica* Boiss. (new host-plant). Phenology - flowering end of June to July, fruiting July to August. The population size is small, over a dozen individuals. The number of individuals counted in the area is fewer than 100. The entire population in this locality is formed by higher plants than the type with yellow corolla. In the future, research into the cause, phytochemical composition, ecological importance and adaptation, and



Figure 3. Habitat and general habit of *Phelypaea boissieri* f. *lutea* at 2,470 m alt. in Cilo Mountains, Hakkari province, SE Turkey. Phot. Ü. Subaşı.

also into pollinators of typical red *Phelypaea* individuals and yellow-orange ones could shed new light on this topic.

Etymology. The form name originated from the yellow to orange colouring of plants.

Note. There are also photos of a yellow flower belonging to *P. boissieri* in Internet sources [<https://www.flickr.com/photos/camerar/2887571252/> and <http://www.agacilar.net/forum/1296397-post1573.htm>]. The photos, respectively, were taken in SE Turkey by Karen Nichols in 26 June 2008, possibly in the same Hakkari province, but no more detailed location data is available, and Hakkari-Mergan-Yaylası (Mergan Plateau) by Güngör Salman in 16 June 2014 [<http://www.agacilar.net/forum/1296397->

post1573.htm], as well as from Yüksekova/Hakkari by Leoš Smutný in 13 May 2014 [<https://www.inaturalist.org/observations/71616618>]. We are also aware of a near mention (“Yüksekova [Gever / Gawar], 1950 m, 23 v 1966, *Eiselt!*”) (Gilli 1982: 2, sub *P. coccinea*); this record probably corresponds to the same f. previously described.

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