

Taxonomy, palynology and distribution notes of seven species of *Passiflora* L. (Passifloraceae s.s.) newly recorded from Brazil

Ana Carolina Mezzonato-Pires¹, Michaela Alvim Milward-de-Azevedo²,
Cláudia Barbieri Ferreira Mendonça¹, Vania Gonçalves-Esteves¹

1 Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Botânica, Quinta da Boa Vista, São Cristóvão, CEP: 20940-040, Rio de Janeiro, RJ, Brasil **2** Universidade Federal Rural do Rio de Janeiro, Instituto Três Rios, Departamento de Ciências do Meio Ambiente, Avenida Prefeito Alberto da Silva Lavinas 1847, Centro, CEP: 25802-100, Três Rios, RJ, Brasil

Corresponding author: Ana Carolina Mezzonato-Pires (carolina.mezzonato@gmail.com)

Academic editor: Ricarda Riina | Received 17 November 2017 | Accepted 20 January 2018 | Published 30 January 2018

Citation: Mezzonato-Pires AC, Milward-de-Azevedo MA, Mendonça CBF, Gonçalves-Esteves V (2018) Taxonomy, palynology and distribution notes of seven species of *Passiflora* L. (Passifloraceae s.s.) newly recorded from Brazil. PhytoKeys 95: 1–14. <https://doi.org/10.3897/phytokeys.95.22342>

Abstract

Passiflora subgenus *Astrophea* is one of the five recognised subgenera of *Passiflora*. Brazil presents ca. 26 species of this subgenus with the majority distributed in the Amazon Basin. During the ongoing taxonomic revision of the Brazilian species of subg. *Astrophea*, seven species previously unknown for the country were recorded: *Passiflora amoena*, *P. fuchsiiiflora*, *P. jussieui*, *P. ovata*, *P. plumosa*, *P. quelchii*, and *P. tessmannii*. The new records expand the species distribution ranges, especially for *P. plumosa*, which was exclusively known from its type locality and *P. quelchii*, which was known only for southeastern Guyana. The authors provide taxonomic and palynological descriptions, distribution maps and illustrations for these species, in the hope that the knowledge and understanding of Brazilian Passifloraceae s.s. will be improved.

Keywords

Amazon basin, *Astrophea*, new records, Passifloraceae, pollen, taxonomy

Introduction

Passiflora L. is by far the largest genus in the Passifloraceae s.s., with ca. 400 accepted species and a pantropical distribution (Feuillet and MacDougal 2007). The genus is currently divided into five subgenera: *Passiflora* subg. *Astrophea* (DC) Mast., *P.* subg. *Deidamioides* (Harms) Killip, *P.* subg. *Decaloba* (DC.) Rchb., *P.* subg. *Passiflora*, and *P.* subg. *Tetrapathea* (DC.) P.S.Green (Feuillet and MacDougal 2003, Krosnick et al. 2009).

Passiflora subg. *Astrophea* is a mainly neotropical group, with ca. 60 accepted species. The diversity of the subgenus is concentrated in lowland forest formations in the Amazon and the Guyana Shield regions (Ulmer and MacDougal 2004). Feuillet and MacDougal (2003) divided *P.* subg. *Astrophea* into two supersections: the supersection *Astrophea*, with three sections (*Astrophea*, *Capreolata* J.M. MacDougal & Feuillet and *Leptopoda* Killip ex Feuillet & Cremers) and the supersection *Pseudoastrophea* (Harms) Feuillet & MacDougal, with two sections (*Pseudoastrophea* (Harms) Killip and *Botryastrophea* (Harms) Killip) and two series (*Botryastrophea* (Harms) J.M. MacDougal & Feuillet and *Carneae* Feuillet). The subgenus is characterised by its well-developed woody stems generally reaching the canopy way above 30 m, entire leaves, the presence of two petiolar glands and diminute stipules and bracts. Difficulties in the collection of plant material due to the lianoid habit of these species are reflected in the small number of collections available in herbaria.

Brazil is the most species-rich country with ca. 26 species of *P.* subg. *Astrophea* (Flora do Brasil under construction). The majority of the Brazilian species in this subgenus are recorded for the Amazon biogeographical domain (ca. 15 species), which represents 70% of the total for the country. Colombia is the second most species-rich country with 24 accepted species, including the recently described *P. gironensis* C.Aguirre, M.Bonilla & A.Rojas (Aguirre-Morales et al. 2016). Nonetheless, the species of *Passiflora* from the Amazon domain are currently poorly understood, with *P.* subg. *Astrophea* being especially problematic.

Pollen morphology is important in Passifloraceae species delimitation. This was also confirmed for *P.* subg. *Astrophea* by Mezzonato-Pires et al. (2017), who found that amongst all pollen characters, the most significant was the sexine ornamentation.

As a result of an ongoing taxonomic revision of the Brazilian species of subgenus *Astrophea*, seven species were recorded for the first time in the Brazilian territory. The new records expand the distribution range for these taxa, especially for *P. plumosa*, which was exclusively known from its type locality and *P. quelchii*, which was known only from southeastern Guyana. Morphological descriptions, distribution maps and illustrations have been provided for these species, aiming to provide a better understanding of the Brazilian Passifloraceae.

Methods

The specimens analysed were deposited in the following herbaria: HAMAB, IAN, INPA, MBM, MG, NY, R, RB, SP, SPF, UB, UEC and UPCB, acronyms following Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>). For each species, descriptions, distri-

bution maps, illustrations of leaves, flowers and pollen grains were provided. The pollen material was processed with the acetolysis method established by Erdtman (1952) and using light microscopy (LM) for pollen grain observations and measurements. Size of pollen grains were classified according to Erdtman (1952) as follows: very small ($<10\text{ }\mu\text{m}$), small ($10\text{--}25\text{ }\mu\text{m}$), medium ($25\text{--}50\text{ }\mu\text{m}$), large ($50\text{--}100\text{ }\mu\text{m}$), very large ($100\text{--}200\text{ }\mu\text{m}$) and giant ($>200\text{ }\mu\text{m}$). For scanning electron microscope (SEM) analysis observed with a Jeol JSM-6510, anthers were macerated and non-acetolysed pollen grains were sprayed on stubs covered with carbon tape (Melhem et al. 2003). For sectional classification of the species, the authors followed Radford et al. (1974) and Feuillet and MacDougal (2003). For the description of structures, the terminology used by Punt et al. (2007) and Mezzonato-Pires et al. (2017) was also used. Field photographs were only available for *P. amoena*.

Results

Seven species are recorded for the first time for Brazil: *Passiflora amoena* L.K.Escobar, *P. fuchsiflora* Hemsl., *P. jussieu* Feuillet, *P. ovata* Jos.Martin ex DC., *P. plumosa* Feuillet & Cremers, *P. quelchii* N.E.Br. and *P. tessmannii* Harms. The distribution of each species in the Brazilian territory is shown in Figure 1.

***Passiflora amoena* L.K. Escobar, Systematic Botany 19(2): 203–205, f. 1. 1994.**
Figs 2A, B, 3A, B, 4A, D

Descriptions. *Lianas*; tendrils thin to robust, glabrous. *Stipules* not seen. *Petioles* with two glands on the terminal end of the adaxial side. *Blades* $5.6\text{--}15 \times 2.7\text{--}7.9$ cm, chartaceous, elliptic or ovate, apex acute-mucronate or obtuse or emarginate, base cuneate, glabrous on both sides, discolorous, abaxial side greyish-brown, adaxial side purplish-vinaceous; margins non-undulate, glandular, 2–6 glands distributed on the abaxial side of the blade; 8–14 pairs of secondary veins, arcuate. *Bracts* diminute, triangular. *Flowers* arranged in racemose inflorescences, hypanthium cylindrical; sepals oblong, dark pink; petals oblong, pink; corona with 3 series of filaments, yellow to orange-yellow, filaments of first series with dolabriform-triangular, filaments of the second series with tuberculate wavy margins; operculum straight, non-tubular, included, ligulate, apex plicate; trochlea absent on the androgynophore; ovary ellipsoid, glabrous. *Fruits* 6.45×2.24 cm, ellipsoid, glabrous.

Palynology. Pollen grains medium-sized (ca. $42.8\text{ }\mu\text{m}$), prolate spheroidal, 6-colporate, colpi long, narrow, three endoapertures lalongate (ca. $6.2 \times 8.1\text{ }\mu\text{m}$), unique for each pair of ectoaperture, sexine reticulate, heterobrochate, with muri (ca. $1.2\text{ }\mu\text{m}$), muri simple columellate, sinuous, continuous, with perforations, without high columellae, not apparent, tectum surface mostly curved, lumina ornamented, small (ca. $6.7\text{ }\mu\text{m}$ diam.) (Fig. 3A, B).

Specimens examined. BRAZIL. Amapá: Amapari, Cabeceiras of the Rio Amapari, on the left bank of the rio Anacuí, trail 5, $01^{\circ}50'41''\text{N}$, $52^{\circ}44'29''\text{W}$, 7 Mar 2006 [fl],

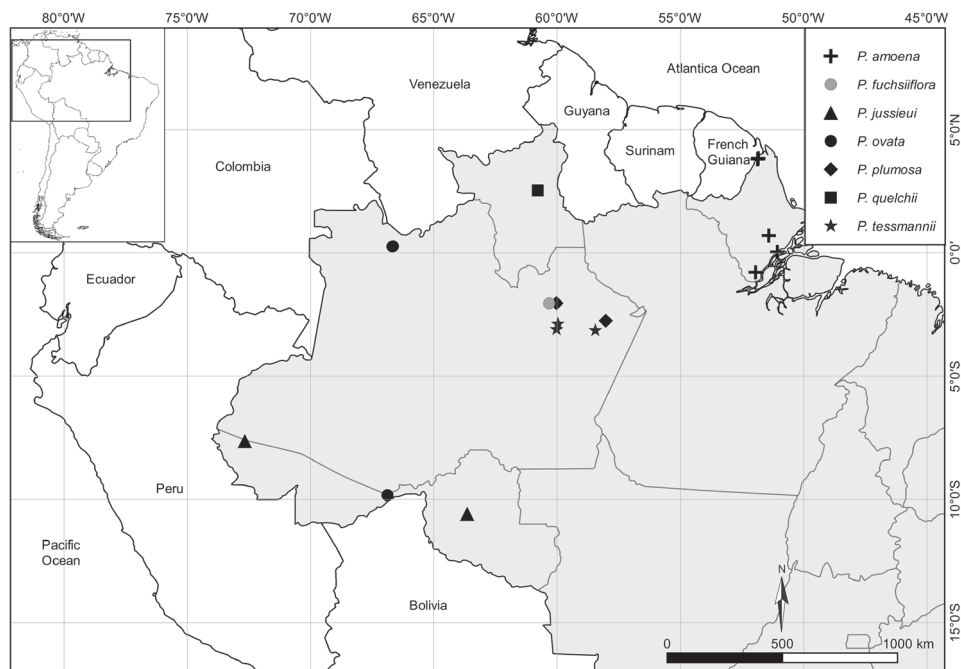


Figure 1. Distribution map of new plant records of species of *P.* subg. *Astrophea* for Brazil.

M.O. Hamada et al. 151 (HAMAB, INPA, R); Clevelândia do Norte, Rio Oiapoque, along the road between Oiapoque and Clevelândia, 3°48'48"N, 51°51'38"W, 20 Jul 1960 [fr], B. Maguire, J.M. Pires and C.K. Maguire 47085 (NYBG); Igarapé Ponta-Narri, third waterfall, 08 Oct 1949, [fl], G.A. Black 49-8461 (IAN); Macapá, Colônia do Torrão, 0°2'20"N, 51°3'59"W, 29 Aug 1962 [bt, fl], J.M. Pires & P.B. Cavalcante 52667 (IAN); Oiapoque, BR156, road between Calçoene and Oiapoque, 17 km southeast of Oiapoque, ca. 3°50'35"N, 51°50'6"W, 3 Dec 1984 [fl], S.A. Mori, J. Reitsma and R. Cardoso 17157 (HAMAB); Rio Oiapoque, about 1 km west of Cachoeira Utussansain, 2°8'N, 52°55'W, 8 Sep 1960 [fl], H.S. Irwin et al. 48080 (IAN); Rio Oiapoque, upper slopes and hilltop Tipac, 3°36'N, 51°19'W, 200-250m, 15 Oct 1960 [bt, fl], H.S. Irwin 48731 (IAN); Porto Grande, Floresta Nacional do Amapá, rio Mutum, conglomerate 02, 0°42'48"N, 51°24'48"W, 20 Feb 2009 [fl], S.V. Costa Neto et al. 2818 (HAMAB).

Distribution and ecology. It is known to occur in Guyana, French Guiana, Suriname and Brazil. It is recorded for the state of Amapá, growing near river banks, in *Floresta de Terra Firme* and in periodically flooded areas called *Floresta de Várzea*.

Taxonomic comments. *Passiflora amoena* belongs to *P.* subg. *Astrophea*, sect. *Botryastrophea* (Harms) Killip, ser. *Carneae* Feuillet. It is morphologically most similar to *P. fuchsiflora*, being differentiated from the latter by its flowers with yellow to orange-yellow corona and dolabriform-triangular outer corona filaments. Both species possess pollen grains of the IV type (Mezzonato-Pires et al. 2017) due to their reticulate sexine and small lumina.

***Passiflora fuchsiiflora* Hemsl., Icon. Pl. 26: pl. 2553. 1898.**

Figs 2C, D, 3C, D

Descriptions. *Lianas*; tendrils robust, glabrous. *Stipules* not seen. *Petioles* with two glands on the terminal end of the adaxial side. *Blades* 13.2–15.2 × 11.2–13.5 cm, chartaceous, widely ovate, apex round to slightly emarginate, base slightly to deeply cordate, glabrous on both sides; margins non-undulate, glandular; 11–14 pairs of secondary veins, arcuate. *Bracts* diminute, linear-setaceous, alternate. *Flowers* arranged in a racemose inflorescence, hypanthium cylindrical; sepals linear-oblong, pink; petals linear-oblong, pink; corona with 3 series of filaments, dark purple, filaments of first series subdolabriform, laterally attached filaments, filaments of second series tuberculate to tuberculate-triangular, filaments of third tuberculate; operculum straight, membranous, non-tubular, included, apex fimbriate; trochlea absent on the androgynophore; ovary obovoid to oblongoid, glabrous. *Fruits* not seen.

Palynology. Pollen grains medium-sized (ca. 38.4 µm), prolate spheroidal, 6-colporate, colpi long, narrow, three endoapertures alongate (ca. 7.6 × 11.0 µm), unique for each pair of ectoaperture, sexine reticulate, heterobrochate; muri (ca. 1.2 µm) simple columellate, sinuous, continuous, with perforations, without high columellae, not apparent, tectum surface mostly curved, lumina slightly ornamented, small (ca. 4.0 µm diam.) (Fig. 3C, D).

Specimens examined. **BRAZIL. Amazonas:** Presidente Figueiredo, Rebio Uatumã, grid of PPBio, 2°2'4"S, 60°1'30"W, 16 Apr 2007 [fl, fr], S. Sakagawa et al. 248 (INPA); Rebio Uatumã, Igarapé access to camp 2, left bank rising, 2°2'4"S, 60°1'30"W, 27 Sep 2008 [fl], S. Sakagawa and J.R.M. Ferreira 512 (INPA).

Distribution and ecology. It is known to occur in Guyana, French Guiana, Suriname, Venezuela and Brazil. A single specimen collected in 1993 at the Indigenous Reserve of Yanomani, state of Amazonas, by *W. Miliken 1801*, was originally identified as *P. fuchsiiflora*. Until the present contribution, this was the only specimen of *P. fuchsiiflora* known for Brazil. Nonetheless, this specimen actually belongs to *P. balbis* Feuillet. Two specimens from the state of Amazonas, municipality of Presidente Figueiredo, at the Biological Reserve of Uatumã, are here correctly identified as *P. fuchsiiflora*. Thus, this species is recorded for the first time in Brazil based on the aforementioned specimens. In Brazil, *P. fuchsiiflora* is restricted to the state of Amazonas, growing in *Igarapé* formations.

Taxonomic comments. *Passiflora fuchsiiflora* belongs to *P.* subg. *Astropheae*, sect. *Botryastropheae*, ser. *Carneae*. It possesses widely ovate and chartaceous leaf-blades, with round to slightly emarginate apex, added to the three filament series of the corona, with subdolabriform outer filaments and the two inner series with tuberculate filaments. The pollen grains possess reticulate sexine with small lumina and, for this reason, the pollen was included by Mezzonato-Pires et al. (2017) in the type IV pollen group.

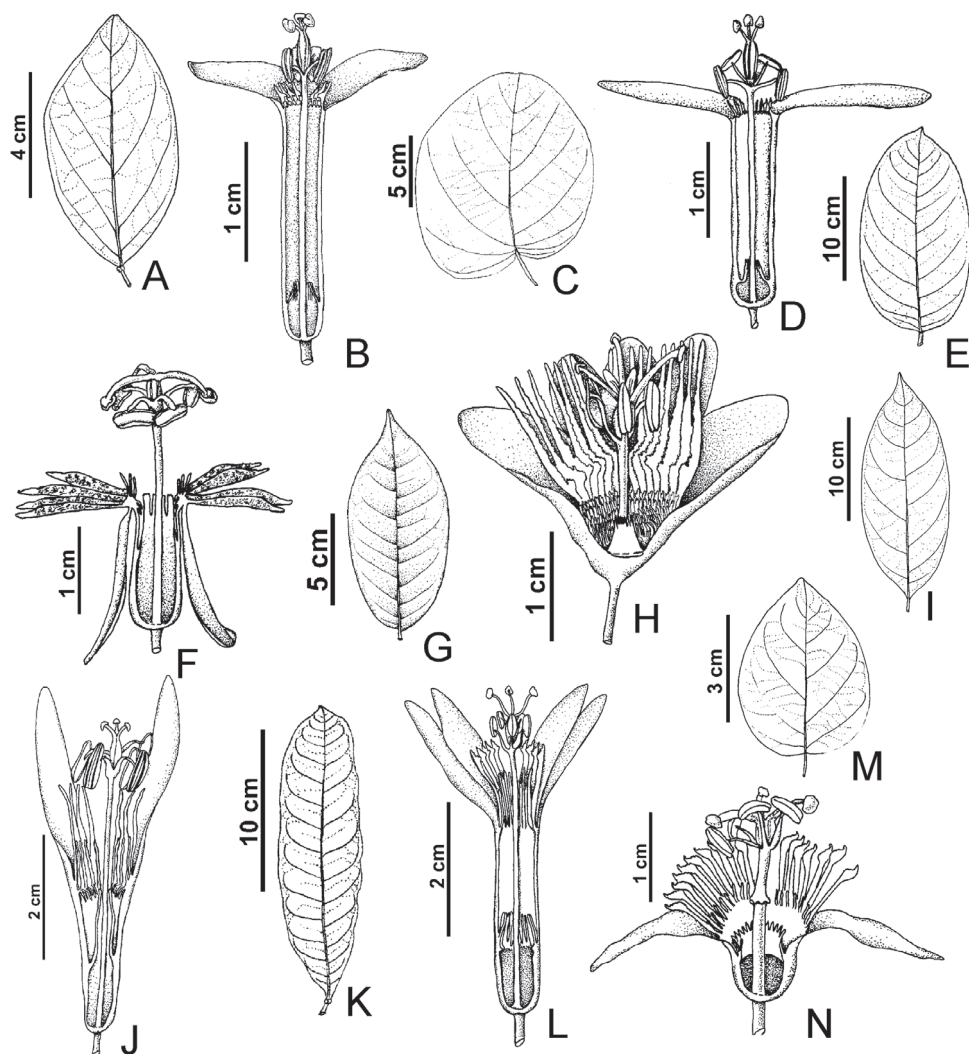


Figure 2. Illustrations of leaves and flowers in longitudinal section. **A, B** *Passiflora amoena* (S.V. Costa Neto et al. 2818, HAMAB) **C, D** *Passiflora fuchsiiiflora* (S. Sakagawa et al. 248, INPA) **E, F** *Passiflora jussieu* (D.C. Daly et al. 7423, UPCB) **G, H** *Passiflora ovata* (S. Sakagawa et al. 668, INPA) **I, J** *Passiflora plumosa* (C.A. Cid et al. 561, MG) **K, L** *Passiflora quelchii* (R.C. Forzza et al. 8321, RB) **M, N** *Passiflora tessmannii* (C.A. Sothers & E.C. Pereira 612, INPA).

Passiflora jussieu Feuillet, *Journal of the Botanical Research Institute of Texas* 4(2): 611, f. 1. 2010.

Figs 2E, F, 3E, F

Descriptions. *Lianas*; tendrils slightly robust, glabrous to slightly puberulous. *Stipules* not seen. *Petioles* with two glands on the terminal end of the adaxial side.

Blades 15–23 × 8.4–12.7 cm, coriaceous, ovate to ovate-oblong to oblong to widely oblong, apex acuminate, base truncate to round, glabrous on both sides; margins conspicuous, undulate, brown to brownish-green, glandular, with 2–3 glands; 16–23 pairs of secondary veins, arcuate. **Bracts** diminute, linear-setaceous, alternate. **Flowers** arranged in a racemose inflorescence, hypanthium cylindrical; sepals linear-oblong, greenish; petals linear-oblong, white with lilac spots; corona with 4–6 series of filaments, greenish-yellow with dark red to purplish spots, filaments of first series subdolabriform, straight, laterally attached, filaments of second, third and fourth series with linear-capitate, straight, filaments of fifth series linear-capitate to hair-like, semi-straight or reflexed, filaments of sixth series hair-like, reflexed; operculum straight, tubular, membranous, exserted, apex crenulate; trochlea absent on the androgynophore; ovary narrowly oblongoid to narrowly ovoid, densely velutine. **Fruits** not seen.

Palynology. Pollen grains large-sized (ca. 67.6 µm), prolate spheroidal, 6-colporate, colpi short, narrow, three endoaperture lalongate (ca. 5.5 × 9.2 µm) unique for each pair of ectoaperture, sexine partially tectate as small pieces of remnant muri can be observed; muri (ca. 2.8 µm) duplicolumellate, columellae high, apparent, without perforations and most of the tectum surface curved or with spines (ca. 4.8 × 4.6 µm), not forming lumina. A large part of the surface is ornamented with sparsely distributed bacula and conspicuous pila (Fig. 3E, F).

Specimens examined. **BRAZIL. Acre:** Cruzeiro do Sul: Reserva Extrativista do Alto Juruá, Rio Juruá, Seringal São João, placing Tapaúna, [7°37'52"S, 72°40'12"W], 14 Mar 1992 [fl], D.C. Daly et al. 7423 (INPA, UPCB). **Rondônia:** Serra do Bala-terio, 7 km from the village Campo Novo, 10°35'0S, 63°39'0"W, 24 Apr 1987 [fl], C.A. Cid et al. 8915 (INPA).

Distribution and ecology. It is known to occur in French Guiana, Suriname and Brazil, being also cultivated at the ORSTOM Botanical Garden in Cayenne, in the UK by R.J.R. Vanderplank (Feuillet 2010) and in the USA by L. Gilbert. It is recorded for the states of Acre (municipality of Cruzeiro do Sul) and Rondônia (municipality of Porto Velho). According to Feuillet (2010), *P. jussieui* can be found growing in lowland rainforests, whereas in Brazil, it is found in *Floresta de Terra Firme* formations, in sandy and rocky soils.

Taxonomic comments. *Passiflora jussieui* belongs to *P.* subg. *Astropheia* sect. *Capreolata* J.M. MacDougal & Feuillet. It can be characterised by its leaf-blades with conspicuous margins, flowers with corona arranged in 4–6 filament series with dark red to purplish spots, the outer series with subdolabriform filaments, the inner series with linear-capitellate and straight filaments and the sixth series with hair-like and reflexed filaments. The pollen grains of the closely related *P. cerradensis* Sacco present semitectate exine and reticulate sexine with large and ornamented lumina; which differ greatly from the mostly non-TECTATE exine, not producing lumina, pollen grains of *P. jussieui*. The pollen grains of *P. jussieui* are included in the type II pollen group, while the ones of *P. cerradensis* are included in the type III pollen group (Mezzonato-Pires et al. 2017).

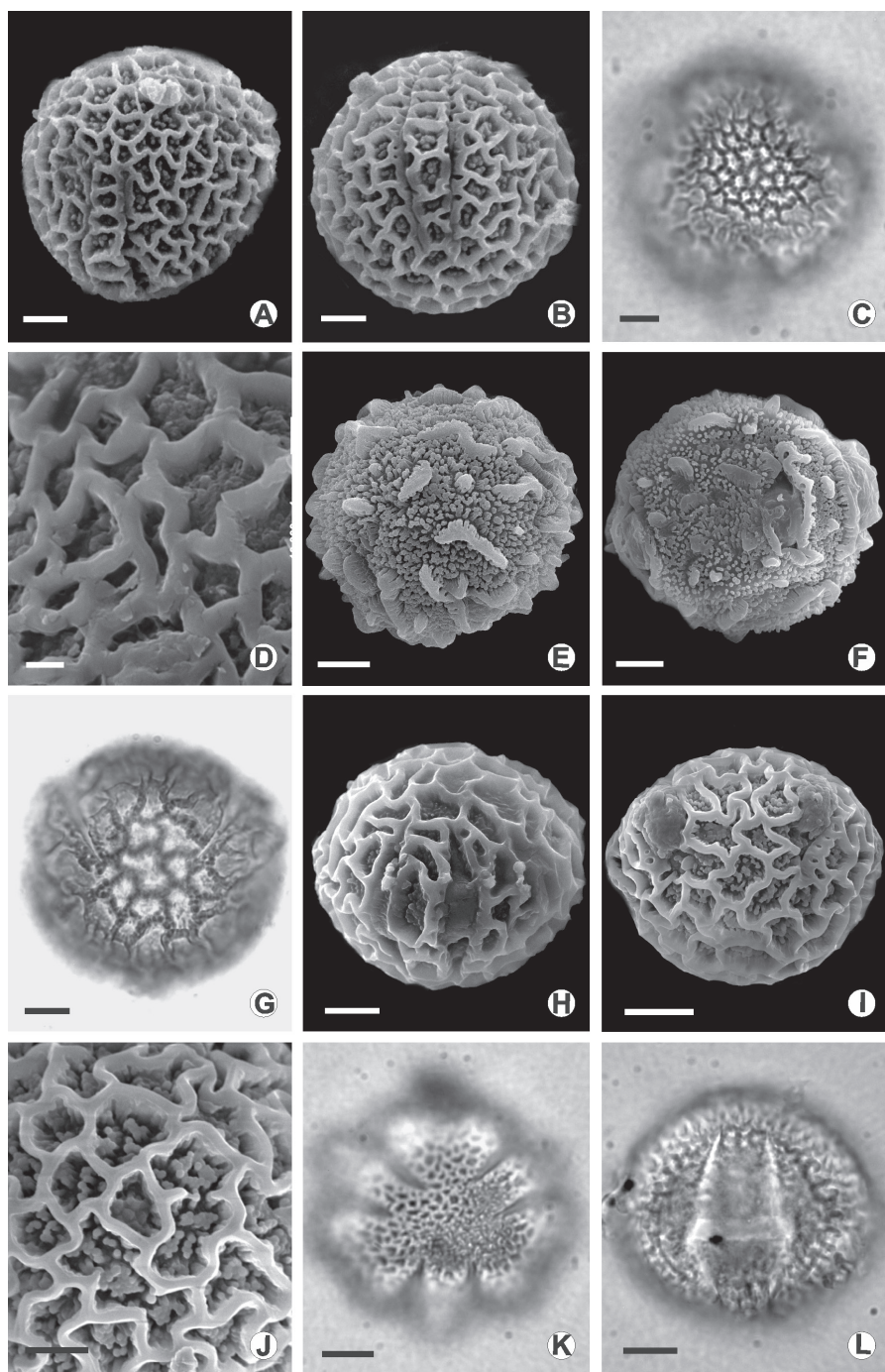


Figure 3. Photomicrographs and electron photomicrographs of pollen grains of the species of *Passiflora*. **A, B** *Passiflora amoena* **C, D** *Passiflora fuchsiiflora* **E, F** *Passiflora jussieui* **G, H** *Passiflora ovata* **I, J** *Passiflora quelchii* **K, L** *Passiflora tessmannii* **A, C, E, G, K** general aspect of polar view **B, F, H, I, L** general aspect of equatorial view, **D** and **J** ornamentation detail. Scale bars: 10 µm (**C, E, F, G, H, I, K, L**); 5 µm (**A, B, J**); 1 µm (**D**).



Figure 4. *Passiflora amoena* L.K. Escobar. **A** habit **B** inflorescence **C** flower **D** fruits. Scale bars: 10 mm (**A**, **C**, **D**); 2 mm (**B**). Photographs by S.C. Neto (S.V. Costa Neto et al. 2818, HAMAB)

***Passiflora ovata* Jos. Martin ex DC., Prodr. 3: 322. 1828. *Astrophea ovata* (Jos. Martin ex DC.) M. Roem. Familiarum Naturalium Regni Vegetabilis Synopses Monographicae 2: 151. 1846.**

Figs 2G, H, 3G, H

Descriptions. *Lianas*; tendrils not seen. *Stipules* diminute, linear to linear-falcate. *Petioles* with two glands on the terminal end of the adaxial side. *Blades* 9.5–19.3 × 4.3–8.7 cm, chartaceous, oblong to obovate, apex attenuate to abruptly attenuate, base obtuse to round, glabrous on both sides; margins slightly undulate, with 4–6 glands; 22–25 pairs of secondary veins. *Bracts* diminute, linear to linear-falcate, alternate. *Flowers* solitary, hypanthium widely campanulate; sepals oblong, light green; petals oblong, white; corona with 4–6 series of filaments, filaments of first series dolabriform, with apex narrowly linear, margins slightly undulate, yellow below the inflated portion of the filaments, orange-yellow in the inflated portion, filaments of second series linear, filaments of third and fourth series with hair-like, filaments of fifth and sixth series with hair-like, reflexed; operculum straight, tubular, exserted, filamentous with a fimbriate apex, papillose; trochlea absent on the androgynophore; ovary obovoid to oblong-ovoid, densely tomentose. *Fruits* 5.5 × 3.5 cm, ellipsoid, glabrous.

Palynology. Pollen grains large-sized (ca. 56.6 µm), prolate spheroidal, 6-colporate, colpi short, narrow, three endoaperture lalongate (ca. 10.0 × 15.5 µm) unique for each pair of ectoaperture, sexine reticulate, heterobrachate; muri (ca. 1.9 µm) duplicolumellate, sinuous, continuous, without perforations, without high columellae, not apparent, tectum surface mostly slightly curved, lumina slightly ornamented with pila, large (ca. 13.4 µm diam.) (Fig. 3G and H).

Specimens examined. **BRAZIL. Acre:** Acrelândia, PAE Porto Dias (placing Bibi), 9°49'40"S, 66°53'0"W, 09–15 Nov 2006 [fl, fr], F. Obermuller et al. 102 (RB). **Amazonas:** São Gabriel da Cachoeira: Highway BR-307, SGC-Cucuí km 50, [0°16'25"N, 66°39'35"W], 27 Oct 2008 [fl], S. Sakagawa et al. 668 (INPA); Highway BR-307, SGC-Cucuí km 50, [0°15'49"N, 66°40'56"W], 22 Apr 2008, R.L. Assis et al. 93 (INPA).

Distribution and ecology. It is known to occur in French Guiana, Venezuela and Brazil. It is recorded here for the states of Acre (municipality of Acrelândia) and Amazonas (municipality of São Gabriel da Cachoeira), growing in *Floresta Ombrófila Densa* formations, along roadsides, reaching up to 10 m high.

Taxonomic comments. *Passiflora ovata* belongs to *P.* subg. *Astrophea* sect. *Pseudoastrophea* (Harms) Killip. It can be easily differentiated due to its oblong to obovate leaf-blades, glands restricted to the abaxial side of the petiole near the blade, flowers with widely campanulate hypanthium, and dolabriform filaments on the first corona series. According to Escobar (1990), the straight and tubular operculum of *P. ovata*, probably favours hummingbird pollination. The most morphologically similar species is *P. costata*, which can be easily distinguished from *P. ovata* by the presence of a trochlea in the androgynophore, operculum declinate at base with a straight upper part and corona with non-reflexed filaments in the inner series. According to Mezzonato-Pires et al. (2017), *P. ovata* is included in the type III pollen group, due to its reticulate sexine and large lumina.

Passiflora plumosa Feuillet & Cremers, Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C: Biological and Medical Sciences 87(3): 381, f. 2. 1984.

Fig. 2I, J

Descriptions. *Lianas*; tendrils thin, glabrous. *Stipules* diminute, linear. *Petioles* with two glands on the terminal end of the adaxial side. *Blades* 14.2–22 × 6.3–8.5 cm, membranous, oblong, apex attenuate, base obtuse, glabrous on both sides; margins inconspicuous, with two glands at base; 13–19 pairs of secondary veins, arcuate. *Bracts* diminute, deltoid, alternate. *Flowers* solitary or arranged in racemose inflorescences, hypanthium cylindrical to slightly funnellform; sepals linear-oblong; petals linear-oblong; corona with 4 series of filaments, white, filaments of first series liguliform, filaments of second series linear or liguliform, filaments of third and fourth series liguliform, plumose, reflexed or not; operculum straight, tubular exserted, apex crenulate; trochlea absent in the androgynophore; ovary oblongoid, densely velutine. *Fruits* not seen.

Palynology. Pollen grains not seen.

Specimens examined. BRAZIL. Amazonas: Itapiranga: rio Uatumã, the left bank, in front of the Rio Pitinga, [2°44'56"S, 58°1'19"W], 27 Aug 1979 [fl], C.A.Cid et al. 561 (MG); Presidente Figueiredo, Rebio Uatumã, 1°00'S, 59°00"W, grid of PPBio s.n., portion L3/500, [2°2'4"S, 60°1'30"W], 11 Jul 2008 [fl], F.A. Carvalho 31UAT (INPA).

Distribution and ecology. The species was hitherto known to occur exclusively in its type locality, in the central-southern forests of French Guiana. Two specimens were identified by the authors, occurring in the state of Amazonas, municipalities of Itapiranga and Presidente Figueiredo. The new-found specimens were collected growing in *Floresta Ombrófila Densa* and *Floresta de Terra Firme* formations, on river edges with clay soil, at 600 m elevation.

Taxonomic comments. *Passiflora plumosa* belongs to *P.* subg. *Astrophea* sect. *Lep-topoda* Killip ex Feuillet & Cremers. It can be differentiated by its glabrous and membranous leaf-blades, corona with four series of filaments, all of them being liguliform in shape and the second and fourth or the third and fourth series with reflexed filaments. The apex of the operculum of *P. plumosa* can easily distinguish this species from *P. cauliflora* Harms and *P. cerradensis*, due to its crenulate apex.

Passiflora quelchii N.E. Br., Transactions of the Linnean Society of London, Botany 6: 31, pl. 3. 1901.

Figs 2K, L, 3H, I

Descriptions. *Shrubs*; tendrils absent. *Stipules* diminute, narrowly triangular, glabrous. *Petioles* with two glands on the terminal end of the adaxial side. *Blades* 11.3–18.8 × 2.6–4.8 cm, chartaceous, narrowly oblong to oblong-lanceolate, apex acute, base cuneate, glabrous on both sides; margins strongly undulate, with ca. three glands; 29–33 pairs of secondary veins, arcuate, conspicuous. *Bracts* diminute, triangular, ver-

ticillate, with marginal glands. **Flowers** arranged in racemose inflorescences, hypanthium cylindrical; sepals oblong-lanceolate; petals oblong-lanceolate; corona with 2 series of filaments, filaments of first series dolabriform, filaments of second series hair-like, apex entire or bifid, straight; operculum straight, non-tubular, included, filamentous with a fimbriate apex, 3.4–3.5 cm long; trochlea absent on the androgynophore; ovary oblongoid, glabrous. **Fruits** ca. 3.11×2.93 cm, orbicular, glabrous, green.

Palynology. Pollen grains medium-sized (ca. $47.8 \mu\text{m}$), prolate spheroidal, 6-colporate, colpi short, narrow, three endoaperture lalongate (ca. $8.7 \times 17.0 \mu\text{m}$) unique for each pair of ectoaperture, sexine reticulate, heterobrochate; muri (ca. $2.0 \mu\text{m}$) duplicolumellate, sinuous, continuous, without perforations, columellae high, apparent, tectum surface mostly slightly curved, lumina ornamented with pila, large (ca. $15.2 \mu\text{m}$ diam.) (Fig. 3H, I).

Specimens examined. BRAZIL. Roraima: Cantá, Serra Grande, $2^{\circ}32'53''\text{S}$, $60^{\circ}47'10''\text{W}$, 554 m, 12 Nov 2014 [fl], R.C. Forzza et al. 8321 (RB).

Distribution and ecology. Found in savannahs from southwestern Guyana, being here recorded in the state of Roraima, municipality of Cantá, in the Serra Grande inselberg, around 554 m elevation. Beside the type specimens and the one recorded for Brazil, *P. quelchii* is only known by another specimen (i.e., *Graham 342*) from the Ireng District in Guyana (Escobar 1990).

Taxonomic comments. *Passiflora quelchii* belongs to *P.* subg. *Astrophea* sect. *Capreolata*. It can be distinguished by its shrubby habit and leaf-blades with undulate margins and inconspicuous *in sicco*. The included, non-tubular, filamentous operculum with fimbriate apex is characteristic to *P. quelchii* and easily differentiates it from closely related species. *Passiflora quelchii* possess type III pollen grains, with reticulate sexine forming large lumina (Mezzonato-Pires et al. 2017).

***Passiflora tessmannii* Harms, Notizbl. Bot. Gart. Berlin-Dahlem 9: 978. 1926.**

Figs 2M, N, 3K, L

Descriptions. **Lianas;** tendrils absent. Stipules not seen. **Petioles** with two glands on the terminal end of the adaxial side. **Blades** $6.2\text{--}11 \times 4\text{--}8$ cm, chartaceous, elliptic to ovate to oblong-elliptic, apex emarginate, mucronate, base cuneate to round, abaxially puberulous, adaxially glabrous, except for the midvein; margins revolute, non-undulate, glands not seen; 10–14 pairs of secondary veins, arcuate. **Bracts** diminute, triangular, alternate. **Flowers** arranged in pairs, hypanthium campanulate-cylindric; sepals linear-lanceolate, green; petals linear-lanceolate, white; corona with 2 series of filaments, white at base, yellowing at mid length, with vinaceous spots at apex, filaments of first series linear, apex widely falcate, attenuate, filaments of second series subulate, apex acute; operculum straight, with irregular slits, non-tubular, included, filaments with fimbriate apex, papillose; androgynophore with trochlea; ovary oblongoid to obovoid, velutine to pilose, with hairs restricted to the veins. **Fruits** $6.3\text{--}6.4 \times 2.3\text{--}2.4$ cm, ellipsoid, puberulous.

Palynology. Pollen grains medium-sized (ca. $46.0 \mu\text{m}$), oblate spheroidal, 6-colporate, colpi long, narrow, three endoaperture lalongate (ca. $4.3 \times 20.4 \mu\text{m}$) unique for each pair

of ectoaperture, sexine reticulate, heterobrochate; muri (ca. 1.0 μm) simple columellate, sinuous, continuous, with perforations, without high columellae, not apparent, tectum surface slightly curved, lumina not ornamented, small (ca. 2.4 μm diam.) (Fig. 3K, L).

Specimens examined. **BRAZIL. Amazonas:** Manaus, Igarapé do Crespo, [3°6'7"S, 60°1'30"W], 04 Sep 1945 [fl], A. Ducke 1749 (IAN, NYBG, R); Itacoatiara-Manaus, Reserva Florestal Ducke, km 26, 2°53'0"S, 59°58'0"W, 10 Oct 1995 [bt, fl], C.A. Sothers and E.da C. Pereira 612 (INPA, MBM, MG, UEC); km 26, 2°53'0"S, 59°58'0"W, 27 Nov 1996, [bt, fl], M.J.G. Hopkins et al. 1609 (IAN, INPA, NYBG, SP, UB); next to road the Acará, [3°8'35"S, 58°26'39"W], 19 Dec 1997 [bt, fl], M.A.D. Souza and M.J.G. Hopkins 514 (INPA, SPF).

Distribution and ecology. It is known for Ecuador and Peru, being herein recorded for the state of Amazonas, municipality of Manaus, localities of Igarapé do Crespo and Adolpho Ducke Forest Reserve. It is found growing in lowland forests and *Terra Firme* and *Campinarana* formations, in sandy soils, reaching 18 m.

Taxonomic comments. *Passiflora tessmannii* belongs to *P.* subg. *Astrophea* sect. *Pseudoastrophea*. Until the present study, the Brazilian specimens of *P. tessmannii* were erroneously identified as *P. hexagonocarpa*, due to conflicting taxonomic characters. This confusion caused this species to be treated as *P. hexagonocarpa* in the Flora da Reserva Ducke (Hopkins and Sousa 1999). *Passiflora tessmannii* possesses conical trochlea with undulated margins, corona clearly composed of two series, outer filaments linear in shape, with apex widely falcate and attenuate and inner filaments subulate. The pollen grains possess reticulate sexine with small lumina, similarly to most species of *P.* subg. *Astrophea* sect. *Pseudoastrophea* and, according to Mezzonato-Pires et al. (2017), the pollen is included in the type IV pollen group.

Acknowledgements

The first author thanks the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for a Doctoral scholarship. All researchers thank the FAPERJ (APQ1-E-26/111.214/2014) for financial support, Rodrigo Theófilo Valadares for the map, Monique Goes for the illustration and Marcelo Sales for his help with the SEM photomicrographs. V. Gonçalves-Esteves and C.B.F. Mendonça thank the CNPq for a Productivity Grant. We thank Pablo Muñoz-Rodríguez and Ricarda Riina for their helpful comments and suggestions.

References

- Aguirre-Morales AC, Bonilla-Morales MM, Rojas A (2016) *Passiflora gironensis* a new species of *Passiflora* subgenus *Astrophea* (Passifloraceae) from Santander, Colombia. *Phytotaxa* 280(3): 278–284. <http://dx.doi.org/10.11646/phytotaxa.280.3.6>
- Erdtman G (1952) Pollen morphology and plant taxonomy angiosperms. Almqvist & Wiksells Press, Stockholm, 539 pp.

- Escobar LA (1990) Una Revision Taxonomica de *Passiflora* subgenero *Astrophea*, Passifloraceae. PhD Thesis, Universidad de Antioquia.
- Feuillet C (2010) Folia taxonomica 18. The status of *Passiflora citrifolia* and a new species in subgenus *Astrophea* (Passifloraceae), *Passiflora jussieu*. Journal of the Botanical Research Institute of Texas 4: 609–614.
- Feuillet C, MacDougal J (2003) A new infrageneric classification of *Passiflora* L. (Passifloraceae). *Passiflora: The Journal & Newsletter of Passiflora Society International* 13: 34–38.
- Feuillet C, MacDougal J (2007) Passifloraceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Springer-Verlag, 270–280. https://doi.org/10.1007/978-3-540-32219-1_35
- Flora do Brasil 2020 (2017) *Passiflora*. Jardim Botânico do Rio de Janeiro. <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB86373> [accessed 14.07.2017]
- Hopkins MJG, Souza MAD (1999) Passifloraceae. In: Ribeiro JES, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR, Procópio LC (Eds) Flora da Reserva Ducke: Guia de Identificação das Plantas Vasculares de uma Floresta de Terra-Firme na Amazônia Central. INPA, Manaus, 299–306.
- Killip EP (1938) The American species of Passifloraceae. Field Museum of Natural History, Botanical Series 19: 1–613. <https://doi.org/10.5962/bhl.title.2269>
- Krosnick SE, Ford AJ, Freudenstein JV (2009) Taxonomic revision of *Passiflora* subgenus *Tetrapathea* including the monotypic genera *Hollrungia* and *Tetrapathea* (Passifloraceae), and a new species of *Passiflora*. Systematic Botany 34: 375–385. <https://doi.org/10.1600/036364409788606343>
- Melhem TS, Cruz-Barros MAV, Corrêa AMS, Makino-Watanabe H, Silvestre-Capelato MSF, Gonçalves-Esteves VL (2003) Variabilidade polínica em plantas de Campos de Jordão. Boletim do Instituto de Botânica (São Paulo) 16: 9–104.
- Mezzonato-Pires AC, Gonçalves-Esteves V, Bernacci LC (2016) A new species of *Passiflora* subgenus *Astrophea* (Passifloraceae) from the Brazilian Amazon. Phytotaxa 288: 077–084. <https://doi.org/10.11646/phytotaxa.288.1.8>
- Mezzonato-Pires AC, Mendonça CBF, Milward-de-Azevedo MA, Gonçalves-Esteves V (2017) The systematic value of pollen morphology of *Passiflora* subgenus *Astrophea* (Passifloraceae). Phytotaxa 298(1): 1–19. <https://doi.org/10.11646/phytotaxa.298.1.1>
- Punt W, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Review of Paleobotany and Palynology 143: 1–81. <https://doi.org/10.1016/j.rev-palbo.2006.06.008>
- Radford AE, Dickison WC, Massey JR, Bell CR (1974) Vascular Plant Systematics. Harper & Row Publishers, New York, 891 pp.
- Ulmer T, MacDougal JM (2004) Passiflora: Passionflowers of the World. Timber Press, Cambridge, 430 pp.

Lithocarpus vuquangensis (Fagaceae), a new species from Vu Quang National Park, Vietnam

Ngoc Nguyen Van^{1,2}, Hung Nguyen Viet³, Binh Hoang Thi^{1,2}, Shuichiro Tagane⁴, Hironori Toyama⁴, Hoang Thanh Son⁵, Ha Tran Viet⁶, Tetsukazu Yahara^{1,4}

1 Laboratory of Ecological Sciences, Graduate School of Systems Life Sciences, Kyushu University, 744 Motoooka, Fukuoka, 819-0395, Japan **2** Department of Biology, Dalat University, 01 – Phu Dong Thien Vuong, Dalat, Vietnam **3** Vu Quang National Park, Ha Tinh, Vietnam **4** Centre for Asian Conservation Ecology, Kyushu University, 744 Motoooka, Fukuoka, 819-0395, Japan **5** Silviculture Research Institute, Vietnamese Academy of Forest Sciences, Ha Noi, 10999, Vietnam **6** Vietnam National University of Forestry, Xuan Mai, Chuong My, Ha Noi, Vietnam

Corresponding author: *Nguyen Van Ngoc* (ngocnv@dlu.edu.vn)

Academic editor: *H. Schaefer* | Received 24 October 2017 | Accepted 20 January 2018 | Published 30 January 2018

Citation: Nguyen Van N, Nguyen Viet H, Hoang Thi B, Tagane S, Toyama H, Son HT, Tran Viet Ha, Yahara T (2018) *Lithocarpus vuquangensis* (Fagaceae), a new species from Vu Quang National Park, Vietnam. *PhytoKeys* 95: 15–25. <https://doi.org/10.3897/phytokeys.95.21832>

Abstract

Lithocarpus vuquangensis Ngoc & Hung is described from Vu Quang National Park, North Central Vietnam. The morphological comparison and phylogenetic analysis based on *rbcL*, *matK* and ITS provided evidence that the new species was not assignable to any of the previously known taxa in Vietnam and its surrounding countries. The description, photographs, preliminary conservation status and DNA barcode sequences are also provided for the new species.

Keywords

Fagaceae, *Lithocarpus*, new species, phylogeny, taxonomy, Vietnam, Vu Quang National Park

Introduction

It has been known that species richness of the genus *Lithocarpus* Blume (Fagaceae Dumorier) is high in Vietnam where 120 species and two varieties have been reported including the recently published species, *L. dabuoiensis* Ngoc & L. V. Dung (Ban 2003, Ho 2003, Ngoc et al. 2016). Here, an additional new species of *Lithocarpus* is

described from Vu Quang National Park located in Ha Tinh Province, North Central Vietnam (Figure 1).

Vu Quang National Park covers an area of ca. 56,000 ha from lowlands (alt. 10–300 m) to the highlands (the highest peak of Rao Co, alt. 2,286 m). Two new species of mammals (Sao La - *Pseudoryx nghetinhensis*, Artiodactyla and the world's largest muntjac - *Muntiacus vuquangensis*, Cetartiodactyla) were discovered from this national park in the 1990s (Dung et al. 1993, 1994). The vegetation is diverse along the elevation gradient and five major forest types are recognised: lowland forests (alt. 10–300 m), hill forest (alt. 300–1,000 m), medium montane forest (alt. 1,000–1,400 m), montane forest (alt. 1,400–1,900 m) and upper montane forest (alt. 1,900–2,100 m) (Kuznetsov 2001, Vu Quang National Park Management Board 2014). Until now, 1,678 species of vascular plants including many endemic and rare species have been reported (Vu Quang National Park Management Board 2014, Tagane et al. 2016). As for Fagaceae, one species of *Castanea* Mill, nine species of *Castanopsis* (D. Don) Spach., 12 species of *Quercus* L. and 37 species of *Lithocarpus* Blume have been recorded from the National Park, amongst which 10 species have been listed in Viet Nam Red Data Book (Ban et al. 2007, Hung et al. 2014). In addition, natural populations of *Trigonobalanus verticillata* Forman were discovered during the authors' recent botanical surveys in the National Park in 2016 (voucher specimens: *Yahara et al.* V5764 & V5766, DLU, FU, the herbarium of Vu Quang National Park), which brings the number of Fagaceae genera in the region up to five.

From 2015 to 2016, floristic expeditions were carried out in Vu Quang National Park and trees of the genus *Lithocarpus* were discovered that did not match any described species. Here, the authors describe and name it as *Lithocarpus vuquangensis* Ngoc & Hung, sp. nov. accompanied with its photographs and the morphological comparison with related species. In addition to the morphological examination, DNA sequences and phylogenetic analysis are extremely helpful for identifying and delimiting species (Hebert and Gregory 2005, Dick and Webb 2012). Here, parts of the DNA barcode regions *rbcl*, *matK* (CBOL Plant Working Group 2009) and ITS (China Plant BOL Working Group 2011) were sequenced and the phylogenetic relationship of *L. vuquangensis* and its related taxa were examined.

Materials and methods

Morphological observations

The morphological traits of the new species were compared with its putative relatives based on systematic literature (Camus 1948, Huang et al. 1999, Ban 2003, Ho 2003, Phengklai 2008) and more than three hundreds dried specimens kept in the following herbaria were also examined: BKF, DLU, FOF, HN, KYO, P, RUPP, TI and VNM as well as digitised plant specimen images available on the web of JSTOR Global Plants (<https://plants.jstor.org/>), Muséum National d'Histoire Naturelle (<https://science.mnhn.fr/>) and Chinese Virtual Herbarium (<http://www.cvh.org.cn/>).

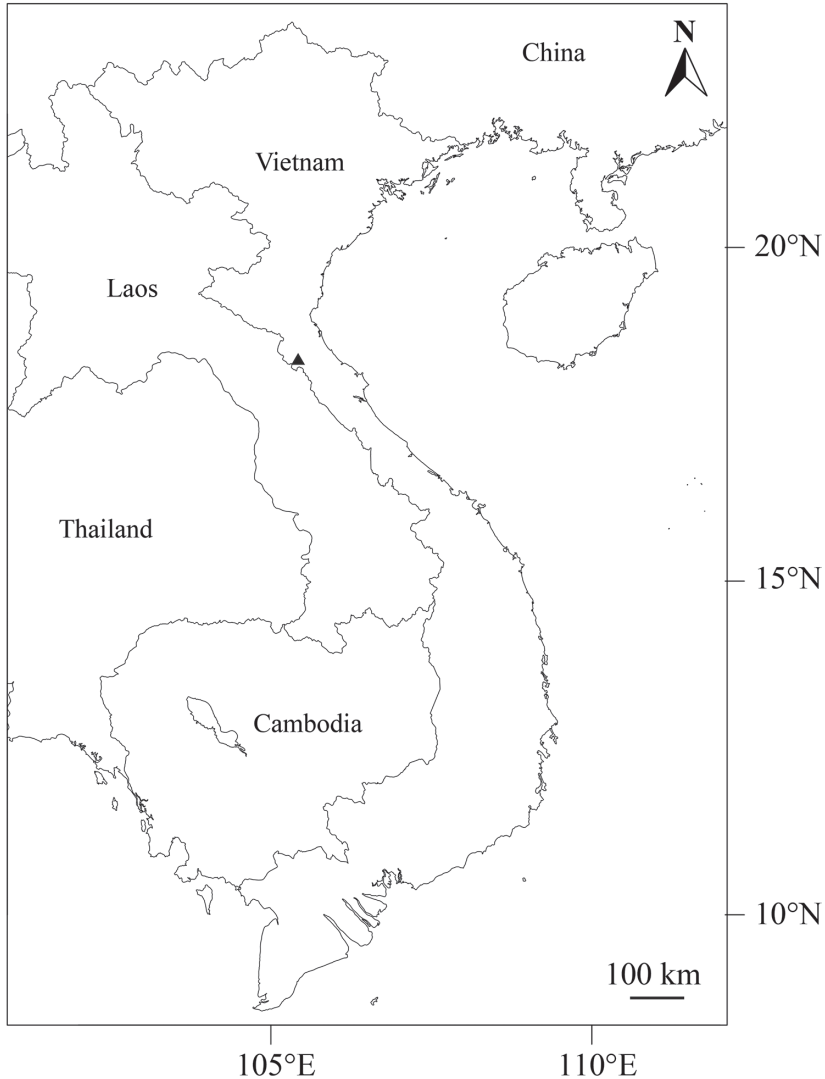


Figure 1. Location of Vu Quang National Park (Black triangle), type locality of *Lithocarpus vuquangensis*.

DNA extraction and sequencing

Total DNA was extracted from 17 silica-gel dried leaf pieces collected in the field. DNA extraction was performed using the CTAB method (Doyle and Doyle 1987) with minor modifications described in Toyama et al. (2015). Two chloroplast DNA barcode regions, *rbcL* and *matK*, were amplified and sequenced following published protocols (Kress et al. 2009, Dunning and Savolainen 2010). In addition, the internal transcribed spacer (ITS) region was sequenced using the protocol of Rohwer et al. (2009) with a minor modification in PCR amplification using the Tks GflexTM DNA Polymerase (Takara Bio Inc., Japan).

Phylogenetic analysis

A total of 16 accessions representing 15 species of *Lithocarpus*, collected throughout Vietnam, were analysed (Table 1). In addition, *Trigonobalanus verticillata* Forman was used as an outgroup in the phylogenetic analysis. The sequence alignment was performed by ClustalW with default parameters implemented in MEGA v 7.0.25 (Kumar et al. 2016) and subsequently adjusted manually.

Bayesian Inference (BI) of phylogeny was performed on the concatenated data set of three genes (*rbcL*, *matK* and ITS) using MrBayes v. 3.2 (Huelsenbeck and Ronquist 2001, Ronquist et al. 2012). The hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC) were used to select the best model of evolution using MrModeltest v. 2.3 (Nylander 2004). The nucleotide substitution model was set to GTR+ γ as selected by MrModeltest. Four independent Markov Chain Monte Carlo (MCMC) runs of four chains each were run for 10,000,000 generations sampling every 1,000 generations. The programme Tracer v. 1.6 (Rambaut et al. 2014) was used to examine marginal prior and posterior densities of MCMC outputs. Each run produced 10,001 trees and a relative burnin of 25% was used for diagnostics. Consequently, 7,501 trees of each run were sampled to generate the summary tree and posterior probabilities distributions. The summary tree was visualised and edited with FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

The morphological comparison showed that *Lithocarpus vuquangensis* is most similar to *L. nantoensis* (Hayata) Hayata distributed in Taiwan, in having entire leaf margin, mostly solitary, rarely 2 or 3 clustered cupules, cupules not completely enclosing nut and glabrous nut. The Vietnamese species sharing the above diagnostic feature of *L. vuquangensis* are *L. hongiaoensis*, *in ined.* (Ngoc et al. in review) and *L. vinhensis* A. Camus. However, the new species is clearly different from all three in the following points: *L. vuquangensis* is distinguished from *L. nantoensis* by its fewer secondary veins (7–10 pairs vs. 10–15 pairs), shorter infructescences (4–7 cm long vs. 16 cm long), longer fruiting stalks (4–6 mm long vs. almost sessile), larger nut size (1.7–2.0 cm high by 2.1–2.4 cm in diam. vs. 1.4–1.7 cm high by 1.5–1.6 cm in diam.) and larger basal scar of the nut (ca. 1.1 cm in diam. vs. 0.5–0.8 cm in diam.). *Lithocarpus vuquangensis* is distinct from *L. hongiaoensis* by its shorter petioles (1–1.5 cm long vs. 2.1–3 cm long), shorter infructescences (4–7 cm long vs. 10 cm long), longer fruiting stalks (4–6 mm long vs. almost sessile), arrangement of scales on the cupule (scales arranged into concentric rings vs. imbricate, not forming rings) and larger nut size (1.7–2.0 cm long, 2.1–2.4 cm in diam. vs. 0.6–0.8 cm long, 1.2–1.5 cm in diam.). The new species differs from *L. vinhensis* in having fewer secondary veins (7–10 pairs vs. 11–12 pairs), shorter infructescences (4–7 cm long vs. 10 cm long) and larger nut size (1.7–2.0 cm

Table 1. List of taxa used in this study with vouchers and GenBank accession number.

Species	Vouchers	GenBank accession number		
		<i>rbcL</i>	<i>matK</i>	ITS
<i>Lithocarpus aggregatus</i>	Tagane et al. V6288 (DLU, FU)	LC318967	LC318550	MF770309
<i>Lithocarpus bidoupensis</i>	Tagane et al. V4320 (DLU, FU, VNM)	LC318961	LC318547	KY940070
<i>Lithocarpus coalitus</i>	Tagane et al. V4191 (DLU, FU, VNM)	LC318959	LC318545	MF770305
<i>Lithocarpus dabuoiensis</i>	Ngoc et al. V3194 (DLU, FU, HN, K, KYO, P, VNM)	LC318953	LC318551	KY436002
	Ngoc et al. V5404 (DLU, FU)	LC318964	LC318548	MF770307
<i>Lithocarpus gigantophyllus</i>	Ngoc et al. V3185 (DLU, FU)	LC318951	LC318538	MF770299
<i>Lithocarpus hancei</i>	Ngoc et al. V5111 (DLU, FU)	LC318963	LC318970	MF952868
<i>Lithocarpus hongiaoensis</i>	Ngoc et al. V3235 (DLU, FU)	LC318956	LC318542	KY851759
<i>Lithocarpus lemeeanus</i>	Tagane et al. V4273 (DLU, FU)	LC318960	LC318546	MF770306
<i>Lithocarpus licentii</i>	Ngoc et al. V3205 (DLU, FU)	LC318954	LC318540	MF770301
<i>Lithocarpus longipedicellatus</i>	Nguyen et al. V3813 (DLU, FU)	LC318958	LC318544	MF770304
<i>Lithocarpus ombrophilus</i>	Yahara et al. V3000 (DLU, FU)	LC318949	LC318420	MF770297
<i>Lithocarpus pseudomagneinii</i>	Ngoc et al. V3223 (DLU, FU)	LC318955	LC318541	MF770302
<i>Lithocarpus stenopus</i>	Ngoc et al. V3187 (DLU, FU)	LC318952	LC318539	MF770300
<i>Lithocarpus vinhensis</i>	Nguyen et al. V3787 (DLU, FU)	LC318957	LC318543	MF770303
<i>Lithocarpus vuquangensis</i>	Yahara et al. V5743 (DLU, FU)	LC319671	LC319670	KY786083
<i>Trigonobalanus verticillata</i>	Yahara et al. V5764 (DLU, FU)	LC318965	LC318549	MF770308

long, 2.1–2.4 cm in diam. vs. 0.9–1 cm long, 1 cm in diam.). A more detailed comparison amongst these four species is shown in Table 2.

In the molecular phylogenetic tree (Fig. 2), *L. vuquangensis* is sister to *L. hongiaoensis* with the posterior probability of 0.94. One nucleotide substitution in *rbcL*, six in *matK* and six in ITS were found between these two species. On the other hand, *L. vinhensis*, another Vietnamese species most similar to *L. vuquangensis*, is placed in a separated clade which includes *L. longipedicellatus*, *L. ombrophilus*, *L. gigantophyllus*, *L. licentii*, *L. pseudomagneinii* and *L. lemeeanus*, with a posterior probability 0.93.

Both *Lithocarpus vuquangensis* and *L. vinhensis* were collected in Vu Quang National Park, but these two species occur at different altitudes: *L. vuquangensis* was found between 1,500 m and 1,700 m altitude, while *L. vinhensis* was found at a lower elevation, below 1,100 m.

Discussion

Phylogenetically, *L. vuquangensis* is sister to *L. hongiaoensis* *in ined.* collected from Lam Dong Province located in southern Vietnam. These two species are morphologically

Table 2. Morphological comparison of *Lithocarpus vuquangensis* with three related species: The measurements of *L. nantoensis* is derived from Hayata (1911), Liao (1996), Huang et al. (1999) and from digitised type specimen image (*Kawakami & Mori 1157*, TI); The measurements of *L. vinhensis* and *L. hongiaoensis* are derived from Camus (1948) and Ngoc et al. (in review), respectively.

Characters	<i>L. vuquangensis</i>	<i>L. nantoensis</i>	<i>L. hongiaoensis</i>	<i>L. vinhensis</i>
Leaf margin	Entire	Entire	Entire	Entire
Leaf surface	Glabrous adaxially, adaxially white farinose	Abaxially glaucous to light green and with adherent, waxy scalelike trichomes	Glabrous upper, adherent waxy scale abaxially	Glabrous adaxially, covered with very short white villi abaxially
Leaf blade size	7.5–11 × 2.3–3.6 cm	12–16 × 2.5–3.5 cm	9.6–14.5 × 2.5–3.8 cm	7.5 cm × 3 cm
Petiole length	1–1.5 cm long	0.7–1.3 cm long	2.1–3 cm long	1 cm long
Number of secondary veins	7–10 pairs	10–15 pairs	8–11 pairs	11–12 pairs
Infructescences length	4–7 cm long	16 cm long	12.5–16.5 cm long	10 cm long
Fruiting stalk length	4–6 mm long, 4–7 mm in diam.	Almost sessile	Sessile to 2 mm long	5–6 mm long
Cupule	Solitary, 0.6–0.9 cm high by 1.8–2.2 cm in diam.	Solitary, 1.2–1.5 cm in diam.	Solitary, 1–1.2 cm high by 1.8–2.1 cm in diam.	Solitary, 1.2–1.3 cm high by 0.8–1 cm in diam.
Scale arrangement	Arranged into concentric rings	Arranged into concentric rings	Imbricate	Arranged into concentric rings
Nut size	1.7–2.0 cm high by 2.1–2.4 cm in diam.	1.4–1.7 cm high by 1.5–1.6 cm in diam.	0.6–0.8 cm high by 1.2–1.5 cm in diam.	0.9–1 cm high by 1 cm in diam.
Nut enclosure by cupule	Only basal to 1/4 of the nut	Only basal part of the nut	Enclosing ca. 1/3–1/2 of the nut	Enclosing ca. 1/3–1/2 of the nut
Basal scar of the nut	Concave, ca. 1.1 cm in diam.	Concave, 0.5–0.8 cm in diam.	Slightly concave, 1.2–1.4 cm in diam.	Nearly flat

distinguished in their length of infructescences and fruiting stalk, the arrangement of cupule bracts, nut size and other characteristics as summarised in Table 2. Further molecular phylogenetic studies, using additional DNA markers, are needed to clarify the relationship between *L. vuquangensis* and *L. hongiaoensis*. However, morphological differences are sufficiently distinct to distinguish them as different species.

Lithocarpus vuquangensis is also morphologically similar to *L. vinhensis* in having an entire leaf margin, solitary cupules not completely enclosing nut, scales arranged into concentric rings and glabrous nut, but these two species are not closely located in the phylogeny. This morphological similarity may have evolved in the similar habitat of the montane evergreen forest in Vu Quang National Park. Whereas *L. vuquangensis* and *L. vinhensis* were collected at 1,518 m and 1,062 m, respectively, altitudinal distributions of the two species may overlap in the montane evergreen forest.

The morphological comparison provided evidence to distinguish *L. vuquangensis* from a Taiwanese species, *L. nantoensis*, although the relationship between them remains to be clarified by further molecular phylogenetic studies.

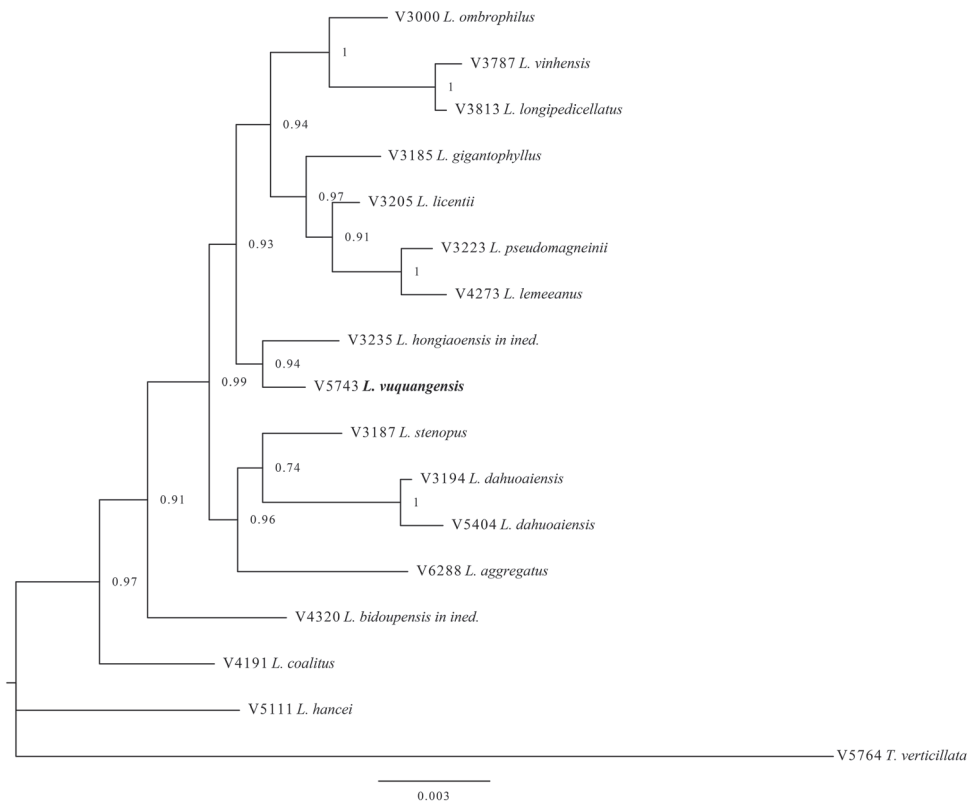


Figure 2. Bayesian phylogeny estimate of 15 taxa of *Lithocarpus* and one *Trigonobalanus verticillata* (as an outgroup) based on combined *rbcl*, *matK* and ITS sequences. Branches are labelled with posterior probabilities greater than 0.7.

Taxonomy

Lithocarpus vuquangensis Ngoc & Hung, sp. nov.

urn:lsid:ipni.org:names:60475914-2

Figure 3

Diagnosis. Similar to *Lithocarpus nantoensis*, *L. hongiaoensis* and *L. vinhensis*, but distinguished from *L. nantoensis* mainly by its fewer secondary veins, shorter infructescences, longer fruiting stalk, larger nut size and larger scar size of the nut, from *L. hongiaoensis* by its much shorter petioles and infructescences, longer fruiting stalk, scales united into concentric rings and much larger nut size and from *L. vinhensis* by having fewer secondary veins, shorter infructescences and much larger nut size (Table 2).

Type. VIETNAM. Ha Tinh Province, Vu Quang National Park, in lower montane forest, along trail to the summit of Mt. Rào Cồ, alt. 1518 m, 18°12'12.2"N, 105°23'15.3"E,

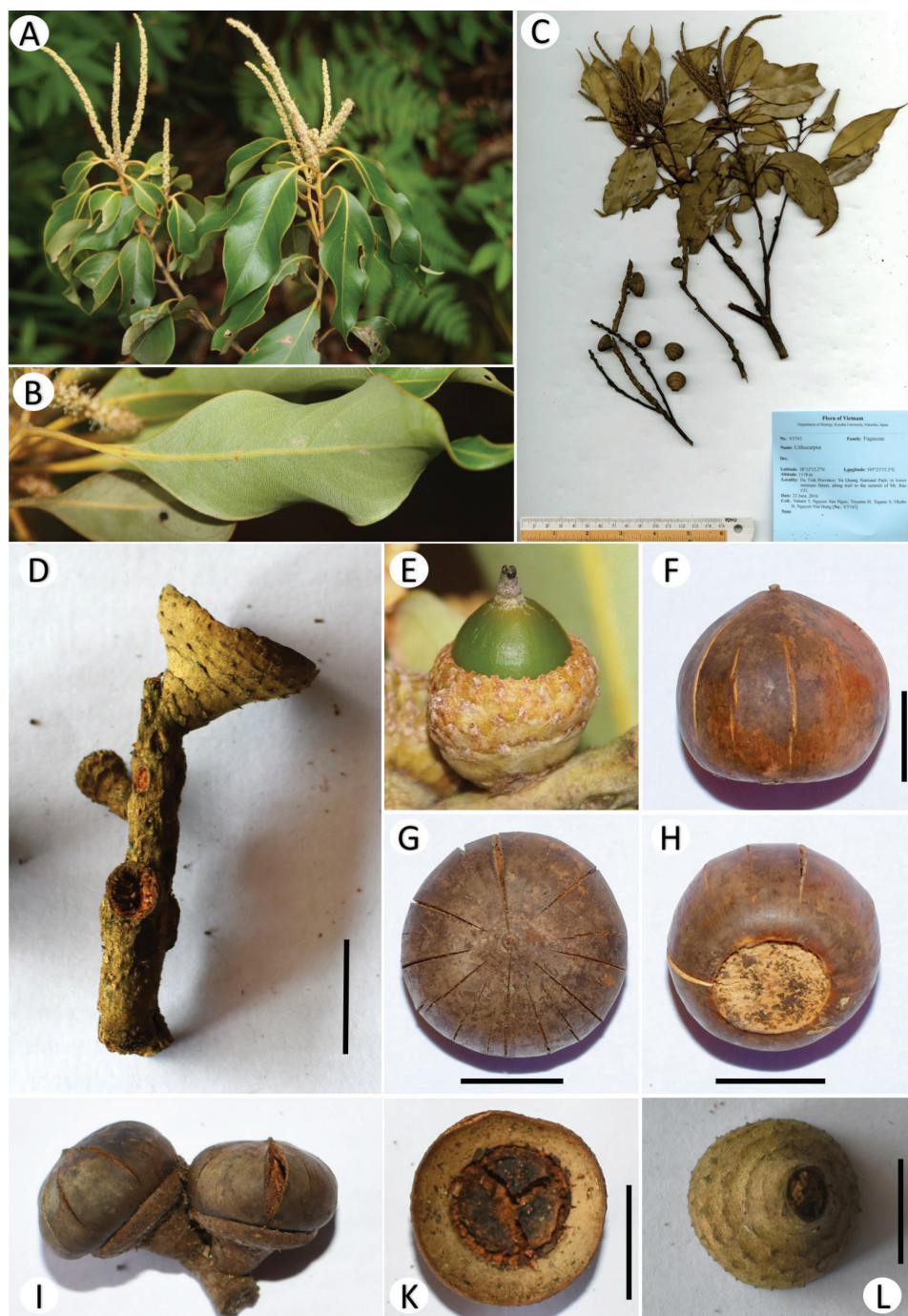


Figure 3. *Lithocarpus vuquangensis* Ngoc & Hung: **A** Branch with male inflorescences **B** Lower leaf surface **C** Holotype (KYO) **D** Spike with cupule **E** Young acorn **F–H** Side view, top view and scar of the mature nut, respectively **I** A part of infructescence **K, L** Inside and outside of the cupule. **C, D, F–L** From Yahara et al. V5743. Scale bars: 2 cm (**D**), 1 cm (**F–H, K, L**).

22 June 2016, Yahara T., Nguyen Van Ngoc, Toyama H., Tagane S., Okabe N., Nguyen Viet Hung V5743 (holotype: KYO!; isotypes: DLU!, FU!, HN!, KI, P!, VNM!).

Description. Trees, to 20 m tall; young branches mostly glabrous, yellowish *in vivo*, reddish-brown *in sicco*. Leaves alternate, spirally arranged, blade narrowly elliptic to lanceolate, 7.5–11 × 2.3–3.6 cm, crunchy, glabrous adaxially, white farinose abaxially, apex long acuminate, acumen up to 1.2 cm long, base cuneate to attenuate, margin entire and wavy; midrib flat or slightly prominent near base adaxially, prominent abaxially, greenish-yellow *in vivo*, reddish-brown *in sicco*, secondary veins 7–10 pairs, at an angle of 40–50 degrees from the midrib, prominent abaxially, tertiary veins scalariform, faintly visible to invisible on both sides; petiole 1–1.5 cm long, glabrous, terete. Male inflorescence a spike, 7–8.5 cm long. Male flower solitary; calyx 6-lobed, lobes ovate, 0.5–0.6 mm × 0.4–0.5 mm, pubescent on both surfaces; stamens 12, 0.7–0.9 mm long, anthers 0.1–0.15 mm long. Infructescences erect, woody spike, up to 7 cm long, axis ca. 2 mm thick at base, greyish-brown, lenticellate. Cupule solitary, broadly obconical to saucer-shaped, 1.4 cm long, 1.8 cm in diam., enclosing only basal to 1/4 of the nuts; scales triangular, arranged into 4–5 concentric rings, apex shortly acuminate, densely covered with tawny minute hairs; fruiting stalk ca. 4–6 mm long, 4–7 mm in diam. Nut obovoid or globose, 1.7–2.0 cm long, 2.1–2.4 cm in diam., glabrous, dehiscent; basal scar concave, ca. 1.1 cm in diam.

Phenology. Mature fruits were collected in June.

Distribution. Vietnam (so far known only from Vu Quang National Park, Ha Tinh Province) (Figure 1).

Etymology. The specific epithet is derived from its type locality, Vu Quang National Park.

GenBank accession No. *Yahara et al. V5743*: LC319671 (*rbcl*), LC319670 (*matK*), KY786083 (ITS).

Preliminary conservation status. Critically Endangered (CR). In the field observation, less than 10 individuals were found along the trail to the summit of Mt. Rào Cô, in lower montane forest. The habitat is inside the protected areas of Vu Quang National Park, but based on criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR. Further intensive inventories are needed to find additional populations in Vu Quang National Park and its surrounding areas.

Acknowledgements

The authors thank the Director of Vu Quang National Park for providing us with opportunities to undertake field surveys in the protected area. We thank the curators and staff of the following herbaria BKF, DLU, FOF, HN, KYO, P, RUPP, TI and VNM for making their materials accessible. The present study was supported by the Environment Research and Technology Development Fund (S9 & 4–1601) of the Ministry of the Environment, Japan and MEXT/JSPS KAKENHI (Grant Number JP15H02640 and JP16H02553).

References

- Ban NT (2003) Fagaceae. In: Ban NT (Ed.) Checklist of plant species of Vietnam 2. Agricultural Publishing House, Hanoi, 227–271.
- Ban NT, Ly DT, Tap N, Dung VV, Thin NN, Tien VN, Khoi KN (2007) Vietnam Red Data Book Part II. Plants. Natural Sciences and Technology Publishers, Hanoi, 563 pp.
- Camus A (1948) Les Chênes: Monographie du genres *Quercus* et *Lithocarpus*. *Chênes Atlas* Volume 3. Paul Lechevalier & fils, 1–1314.
- CBOL Plant Working Group (2009) A DNA barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America 106: 12794–12797. <https://doi.org/10.1073/pnas.0905845106>
- China Plant BOL Working Group (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. Proceedings of the National Academy of Sciences 108(49): 19641–19646. <https://doi.org/10.1073/pnas.1104551108>
- Dick CW, Webb CO (2012) Plant DNA barcodes, taxonomic management, and species discovery in tropical forests. In: Kress WJ, Erickson DL (Eds) DNA barcodes: methods and protocols. Methods in Molecular Biology, vol. 858, 379–393. https://doi.org/10.1007/978-1-61779-591-6_18
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Dung VV, Giao PM, Chinh NN, Tuoc D, Arctander P, Mackinnon J (1993) A new species of living bovid from Vietnam. *Nature* 363: 443–445. <https://doi.org/10.1038/363443a0>
- Dung VV, Giao P, Chinh N, Tuoc D, MacKinnon J (1994). Discovery and conservation of the Vu Quang ox in Vietnam. *Oryx* 28(1): 16–21. <https://doi.org/10.1017/S0030605300028246>
- Dunning LT, Savolainen V (2010) Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164: 1–9. <https://doi.org/10.1111/j.10958339.2010.01071.x>
- Hayata B (1911) Materials for a Flora of Formosa. The journal of the College of Science, Imperial University of Tokyo, Japan 30 (Art. 1): 1–471.
- Hebert PD, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic biology* 54(5): 852–859. <https://doi.org/10.1080/10635150500354886>
- Ho PH (2003) An Illustrated Flora of Vietnam Vol. 2. Young Publishing House, Ho Chi Minh City, 951 pp.
- Huang CJ, Zhang YT, Bartholomew B (1999) Fagaceae. In: Zhengyi W, Raven PH, Deyuan H (Eds) Flora of China. Volume 4, 333–369. <http://www.efloras.org>
- Hung NV, Son NT, Toan TC, Phien DD, Son MT, Anh PNQ, Anh TD (2014) Results on species composition of Fagaceae at the Vu Quang National Park, Ha Tinh province. *Vietnam Forest Sciences Journal* 1: 3095–3100.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- IUCN (2012) IUCN Red List Categories and Criteria. Version 3.1. Second edition. IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK.

- Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O, Bermingham E (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences of the United States of America* 106(44): 18621–18626. <https://doi.org/10.1073/pnas.0909820106>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kuznetsov A (2001) The forests of Vu Quang Nature Reserve: a description of habitats and plant communities. WWF, Hanoi, 1–102.
- Liao JC (1996) Fagaceae. In: Huang TC (Eds) *Flora of Taiwan*. Volume 2, 51–123.
- Ngoc NV, Dung LV, Tagane S, Binh HT, Son HT, Trung VQ, Yahara T (2016) *Lithocarpus dahuoaiensis* (Fagaceae), a new species from Lam Dong Province, Vietnam. *PhytoKeys* 69: 23–30. <https://doi.org/10.3897/phytokeys.69.9821>
- Ngoc NV, Binh HT, Tagane S, Toyama H, Dang VS, Naiki A, Nagamasu H, Yahara T (in review) Two new species of *Lithocarpus* (Fagaceae) from Bidoup-Nui Ba National Park, Vietnam.
- Nylander JAA (2004) MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Phengklai C (2008) Fagaceae. In: Santisuk T, Larsen K (Eds) *Flora of Thailand* 9(3). The Forest Herbarium, Bangkok, 179–410.
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. <http://tree.bio.ed.ac.uk/software/tracer/>
- Rohwer JG, Li J, Rudolph B, Schmidt SA, van der Wer H, Li HW (2009) Is *Persea* (Lauraceae) monophyletic? Evidence from nuclear ribosomal ITS sequences. *Taxon* 58(4): 1153–1167.
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Tagane S, Nguyen VH, Ngoc NV, Son HT, Toyama H, Yang C-J, Yahara T (2016) *Homalium glandulosum* (Salicaceae), a new species from Vu Quang National Park, North Central Vietnam. *PhytoKeys* 58: 97–104. <https://doi.org/10.3897/phytokeys.58.6816>
- Toyama H, Kajisa T, Tagane S, Mase K, Chhang P, Samreth V, Ma V, Sokh H, Ichihashi R, Onoda Y, Mizoue N, Yahara T (2015) Effects of logging and recruitment on community phylogenetic structure in 32 permanent forest plots of Kampong Thom, Cambodia. *Philosophical Transactions of the Royal Society B: Biological Sciences* 370(1662): 20140008. <https://doi.org/10.1098/rstb.2014.0008>
- Vu Quang National Park Management Board (2014) Planning for conservation and development of Vu Quang National Park 2015–2020. [In Vietnamese; published by author]

Lobelia hongiana (Campanulaceae), a new species from Guangxi, China

Zhi-Zhong Li^{1,2,3,*}, Neng Wei^{1,2,3,*}, Yan Liu⁴, Jin-Ming Chen^{1,2},
Guang-Wan Hu^{1,2}, Qing-Feng Wang^{1,2}

1 Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, CN-430074, China **2** Sino-Africa Joint Research Centre, Chinese Academy of Sciences, Wuhan, CN-430074, China **3** University of Chinese Academy of Sciences, Beijing, CN-100049, China **4** Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, CN-541006, China

Corresponding authors: Guang-Wan Hu (guangwanhu@wbcas.cn); Qing-Feng Wang (qfwang@wbcas.cn)

Academic editor: G.G. del Galdo | Received 18 September 2017 | Accepted 24 January 2018 | Published 30 January 2018

Citation: Li Z-Z, Wei N, Liu Y, Chen J-M, Hu G-W, Wang Q-F (2018) *Lobelia hongiana* (Campanulaceae), a new species from Guangxi, China. PhytoKeys 95: 27–36. <https://doi.org/10.3897/phytokeys.95.20245>

Abstract

Lobelia hongiana, a new species of Campanulaceae from Guangxi, South China, is described and illustrated here. This new species is most similar to *L. chinensis* and *L. loochooensis*, but differs by its elliptic-obovate or oblanceolate leaf, 2.5–3 mm long greenish-carmine hypanthium, 5 or 6 calyx lobes, purplish-white corolla, with yellowish-green blotches at the base of lower lobes, glabrous filaments, 7–8 mm long broadly obconic capsule. Molecular phylogenetic analysis has been conducted based on ITS and two chloroplast sequences (*atpB* and *rbcL*) and 14 taxa in *Lobelia* are included. *L. hongiana* is well supported as a new species by the evidence from both morphology and molecular phylogeny.

Keywords

Hypsela, *Lobelia chinensis*, *Lobelia loochooensis*, Southern China

Introduction

Lobelia Linnaeus (1753: 929) (Campanulaceae) is mainly distributed in tropics and subtropics (Lammers 2011). Wimmer (1943, 1953, 1968) proposed the first comprehensive classification system of this genus, which was mainly based on some morphological characters. Subsequently, Murata (1995) and Lammers (2011) improved

* These authors contributed equally to the work.

the classification system using more morphological characters. With over 400 species, *Lobelia* is the second largest genus in Campanulaceae (*Campanula* Linnaeus (1753: 163) is the largest one) and it was classified as 18 sections based on the morphological characters and molecular analyses (Lammers 2011; Chen et al. 2016). Of these, there are 23 species (with six endemic species), belonging to five sections, which have been recorded in China (Hong and Lammers 2011).

During a fieldwork in Huixian town of Guangxi Zhuang Autonomous Region in June 2016, some interesting specimens of *Lobelia* were collected near a local crops field. The leaf shape and flower characters of these individuals were distinctly different from those of the other described *Lobelia* in China. Besides the collected specimens of this unknown *Lobelia*, some individuals were also transplanted in the greenhouse of Wuhan Botanical Garden for further observations. Based on careful observation on morphological characters, literature consulting and specimen comparisons, it was found that these specimens should be a new species, belonging to *L. sect. Hypsela* (C. Presl) Lammers in Hong and Lammers (2011: 555). Morphologically, this new species is similar to *L. chinensis* Loureiro (1790: 514) as well as *L. loochoensis* Koidzumi (1929: 406) that is endemic to Okinawa, Japan. A molecular phylogeny using the combined ITS, *atpB* and *rbcL* dataset also supported these specimens as a separate species. In this study, therefore, the new species was named as *Lobelia hongiana* Q.F.Wang & G.W.Hu.

Materials and methods

Morphological observation

Morphological descriptions and comparisons are based on observations of *Lobelia* specimens from the herbaria of GXMG, HIB, IBSC, KUN, PE and literature. There are eight species of *L. sect. Hypsela* recorded in Southern and Eastern Asia (Lammers 2011), *L. archboldiana* (Merr. & L.M. Perry) Moeliono (1960: 131), *L. brachyantha* Merr. & L.M. Perry (1941: 385), *L. conferta* Merr. & L.M. Perry (1949: 59), *L. donanensis* P. Royen (1966: 305), *L. nummularia* Lam. (1792: 589), *L. victoriensis* P. Royen (1978: 118), *L. chinensis* and *L. loochoensis*. The taxonomic status of the eight species was examined by checking the type specimens from JSTOR Global Plants (<http://plants.jstor.org/>) and the protologue.

Phylogenetic analysis

Two individuals were used from Huixian town, Guangxi Zhuang Autonomous Region, China to assess the phylogenetic position. A total of 14 closed taxa as ingroups and one *Trachelium* species as outgroup were used in phylogenetic analyses. Total genomic DNA was extracted from the fresh material according to Chen et al. (2016). Six sequences from two individuals of the new species were newly generated in this

Table 1. GenBank accession numbers for sequence data of *Lobelia hongiana* used in this study. (“–” indicates not accessible)

Species	ITS	atpB	rbcL
<i>Rachelium caeruleum</i>	DQ304570	EU437661	EU713435
<i>Lobelia urens</i>	–	–	HM850130
<i>Lobelia linnaeoides</i>	–	EF694723	EF694723
<i>Lobelia macrodon</i>	AY568734	EF694736	EF694736
<i>Lobelia roughii</i>	–	EF694738.1	EF694738.1
<i>Lobelia oligophylla</i>	–	–	DQ356159
<i>Lobelia angulata</i>	AY362767	AJ235524.1	MF061180
<i>Lobelia nummularia</i>	–	MF061203.1	AB645964
<i>Lobelia purpurascens</i>	AY568729	–	DQ356160
<i>Lithotoma petraea</i>	–	KY354215	KY354215
<i>Lobelia chinensis</i>	KT957582	KC146452	KC146532
<i>Lobelia arnhemiaca</i>	–	EF694737	EF694737
<i>Isotoma fluviatilis</i>	AY644648	EF999977	DQ356161
<i>Lobelia loochooensis</i>	–	–	AB645961
<i>Lobelia hongiana_1</i>	MF580388	MF580392	MF580390
<i>Lobelia hongiana_2</i>	MF580389	MF580393	MF580391

study and the other sequences were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) (Table 1). The primers were obtained following Haberle et al. (2009, *atpB* and *rbcL*) and Chen et al. (2016, ITS). PCR amplification, sequencing and sequence assembly were implemented following Chen et al. (2016). The best model of nucleotides substitution was selected using jModeltest 2.1.4 (Darriba et al. 2012) with the Akaike Information Criterion (AIC). The Maximum Likelihood (ML) analysis was obtained using RAxML version 8.1.24 (Stamatakis 2006), with separate partitions for the nuclear and plastid data using 1000 bootstrap replicates. The Bayesian Inference (BI) was performed by MrBayes version 3.2.6 (Ronquist and History 2015). Monte Carlo Markov chains were run for 10 million generations with sampling every 5,000 generations. The default setting was used for chain heating (temp = 0.2). The first 10 % of trees were discarded as burn-in and the remaining trees were combined to estimate posterior probability (PP) and other settings following Jin et al. (2016).

Results and discussion

The Bayesian tree showed that the new species is well supported as sister to *L. loochooensis* (ML bootstrap values = 96, PP = 1.00) which placed it in *L.* sect. *Hypsela*. Evidence from molecular phylogeny supports *L. hongiana* as an independent taxon, with *L. loochooensis* as the sister taxon. This study also made the supposition that *L.* sect. *Hypsela* originated from Australia and dispersed to New Zealand, Ryukyus and Southern China (Kokubugata et al. 2012, Chen et al. 2016).

Lobelia hongiana has the following characters, including its solitary flowers in the axils of leaves, a sub-bilabiate corolla with lobes longer than the tube, anthers with a single elongate bristle at the apex of each of the ventral pairs and seed coat reticulate. All of these characters group it into *L.* sect. *Hypsela*. In this section, this new species is most similar to *L. chinensis* and *L. loochooensis*, but the differences amongst them are also dominant (Table 2). Compared with *L. chinensis*, it has a smaller leaf, shorter hypanthium, calyx lobe 5 or 6, shorter than hypanthium, purplish-white corolla, corolla lobe not spreading in a plane on anterior side and shorter glabrous filaments. Compared with *L. loochooensis*, it has a prostrate stem, elliptic-obovate or oblanceolate leaf, longer pedicels, longer greenish-carmine hypanthium, its calyx lobes are shorter than hypanthium, longer corolla, bearing tufts of filiform hairs at 3 dorsal anther tubes and longer broad obconic capsule. Combined with morphological and phylogenetic analyses, *L. hongiana* is confirmed as new to science.

Taxonomic description

Lobelia hongiana Q.F.Wang & G.W.Hu, sp. nov.

urn:lsid:ipni.org:names:60475915-2

Figure 1, Table 2

Diagnosis. The new species is distinguished from *L. chinensis* and *L. loochooensis* by its elliptic-obovate or oblanceolate leaves, usually sinuate-dentate margin; hypanthium 2.5–3 mm long, greenish-carmine; calyx lobes 5 or 6, shorter than hypanthium; corolla purplish-white, yellowish-green blotches at the base of lower lobes; glabrous filaments; broadly obconic capsule, 7–8 mm long; flowering time from May to July.

Type. CHINA. Guangxi: Guilin City, Lingui District, Huixian Town, Huixian Wetland, 25°06.158'N, 110°12.563'E, elev. 140 m, 21 June 2016, G. W. Hu & Z. Z. Li HGW-01120 (holotype HIB!; isotype GXMG!, HIB!).

Description. Herbs, perennial. Stems decumbent, creeping and branched, slender, green or purple, up to 25 cm or higher, glabrous, lower nodes rooted. Leaves alternate, in 2 rows, sessile or petiole up to 2 mm; blade elliptic-obovate, or oblanceolate, thick, 2.2–9 × 1.6–5 mm, glabrous, green or purple beneath, base rounded, obtuse or broadly cuneate, margin usually coarsely sinuate-dentate or occasionally entire, apex obtuse. Flowers solitary, axillary; pedicels slender, 1.3–4.4 cm; hypanthium narrowly obconical, base attenuate, not well distinguished from pedicel, 2.5–3 mm, glabrous, greenish-carmine; calyx lobes 5 or 6, lanceolate, occasionally unevenly bifid, 1.4–2 mm long, shorter than tube, margin with 1 or 2 pair of denticles, occasionally entire. Corolla white, purplish, 9–13 mm, outside glabrous, densely white villous below throat; sub-bilabiate, lobes 5, monomorphic, ovate-lanceolate, 5–7 mm long, longer than the tube, gradually recurved outwards when open, tube split not to base on dorsal side; lower lobes 3, covered sparsely white villous, with yellowish-green blotches at the base; upper lobes 2, glabrous except occasionally covered rarely white villous; filament ca.

Table 2. Characters distinguishing *Lobelia hongiana*, *L. chinensis* and *L. loochooensis*. (The character information of *L. loochooensis* is mainly based on Murata (1992); “–” indicates the description of *L. loochooensis* is not yet accessible.)

Characters	<i>Lobelia hongiana</i>	<i>L. chinensis</i>	<i>L. loochooensis</i>
Stem	Decumbent	Decumbent	Prostrate
Leaf	Elliptic-obovate or oblanceolate, slightly thick, 2.2–9 × 1.6–5 mm, sessile or to 2 mm, apex obtuse, margin usually sinuate-dentate or sub-entire	Narrowly elliptic, elliptic or lanceolate, thin, 7–26 × 1.5–7 mm, sessile or to 1 mm, apex acute or acuminate, margin entire or obviously serrate at upper part	Orbicular to broadly obovate to sub-obtriangular, thick, 5–7 mm in diam., almost sessile, apex rounded, margin entire or tridentate at upper part
Flower	Solitary, pedicel 1.3–4.4 cm long	Solitary at upper part of stem, pedicel 1.2–6.5 cm long	Solitary, pedicel 1–2.5 cm long
Hypanthium	2.5–3 mm long, greenish carmine	3–5 mm long, green	Ca. 1 mm long, yellowish green
Calyx lobes	5 or 6, lanceolate or sometimes unevenly bifid, 1.4–2 mm long, shorter than hypanthium, 1 or 2 pair(s) of denticles	5, lanceolate, 3–5 mm long, as long as hypanthium, 1 pair of denticles	5, narrowly triangular, 1.5 mm long, longer than hypanthium, –
Corolla	Purplish-white, 9–13 mm long, sub-bilabiate, lobes 5, gradually recurved when open, lobes equal or subequal, ovate-lanceolate	Rose, white or bluish, 10–15 mm long, unilabiate, lobes 5, all spreading in a plane on anterior side, lateral 2 lanceolate or oblanceolate, central 3 elliptic	White to pale violet, 8–9 mm long, sub-bilabiate, lobes 5, gradually recurved when open, lobes equal or subequal, oblong-lanceolate
Lower/Central lobes	Yellowish-green blotches at the base, apex recurved, covered sparsely white villous, without vein	Green blotches with yellow margin at the base, apex incurved, glabrous, with purple veins	Blue blotches at the base, apex recurved, glabrous, with blue veins
Filament	Ca. 4 mm long, glabrous	6–8 mm long, the two anterior ones hairy	Ca. 3 mm long, sparsely hairy
Anther	Tube 1.1–1.5 mm, bearing tufts of filiform hairs at 3 dorsal anther tubes	Tube 2–2.5 mm, 3 dorsal anther tubes sparsely villous or glabrous	Tube ca. 1 mm long, 3 dorsal anther tubes glabrous
Pistil	Style glabrous, stigma puberulous	Lower half style hairy, stigma puberulous	–
Fruit	Capsule broadly obconic, 7–8 mm long	Capsule narrowly obconic, 6–7 mm long	Capsule sub-globose, laterally compressed, ca. 4 mm long
Flowering time	May to July	May to September	July to September

4 mm long, adnate to corolla tube on lower third, glabrous, connate above middle, filament tube ca. 1.5 mm; anther tube 1.2–1.5 mm, anther tube bearded with tufts of filiform hairs, ventral anthers bearing two awns, ca. 0.5 mm long. Style enclosed at

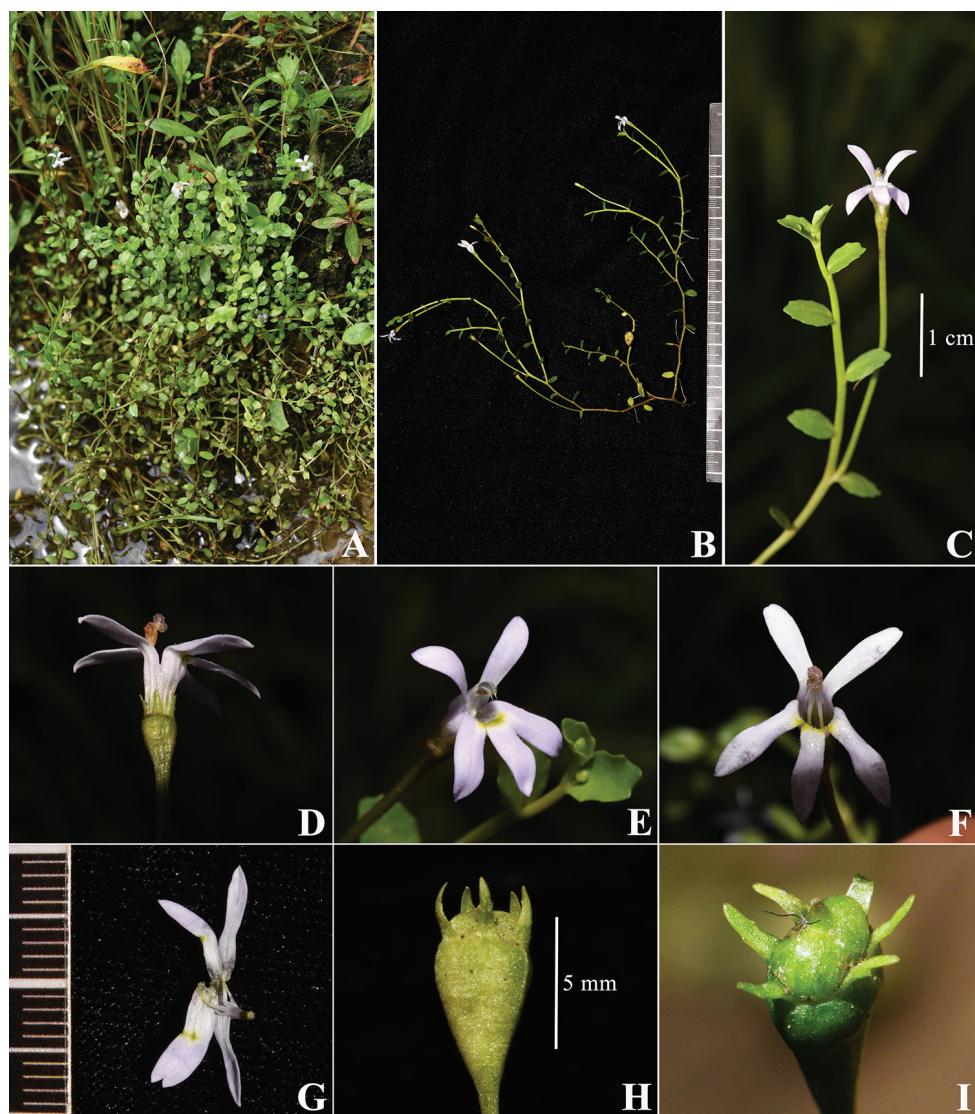


Figure 1. Photos of *Lobelia hongiana* Q.F.Wang & G.W.Hu: morphology. **A** Habitat **B** Part of one individual **C** A stem bearing leaves and a flower **D–G** Flower viewed from different orientations **H–I** Fruit viewed from different orientations.

connate filaments, glabrous, protruded and curved once mature; stigma bifid, puberulous. Ovary 2-locular, ovules numerous. Capsule obconic, apically 2-valved, 6–8 mm long, dehiscent loculicidally, calyx lobes persistent. Seeds narrowly elliptic, terete.

Distribution and ecology. The new species has been found in Huixian Wetland, Guangxi Zhuang Autonomous Region in China, with only two populations. There is a high probability that *L. hongiana* is also distributed at adjacent areas, given its vegetative propagation traits. Its living environment is wetland and farmland.

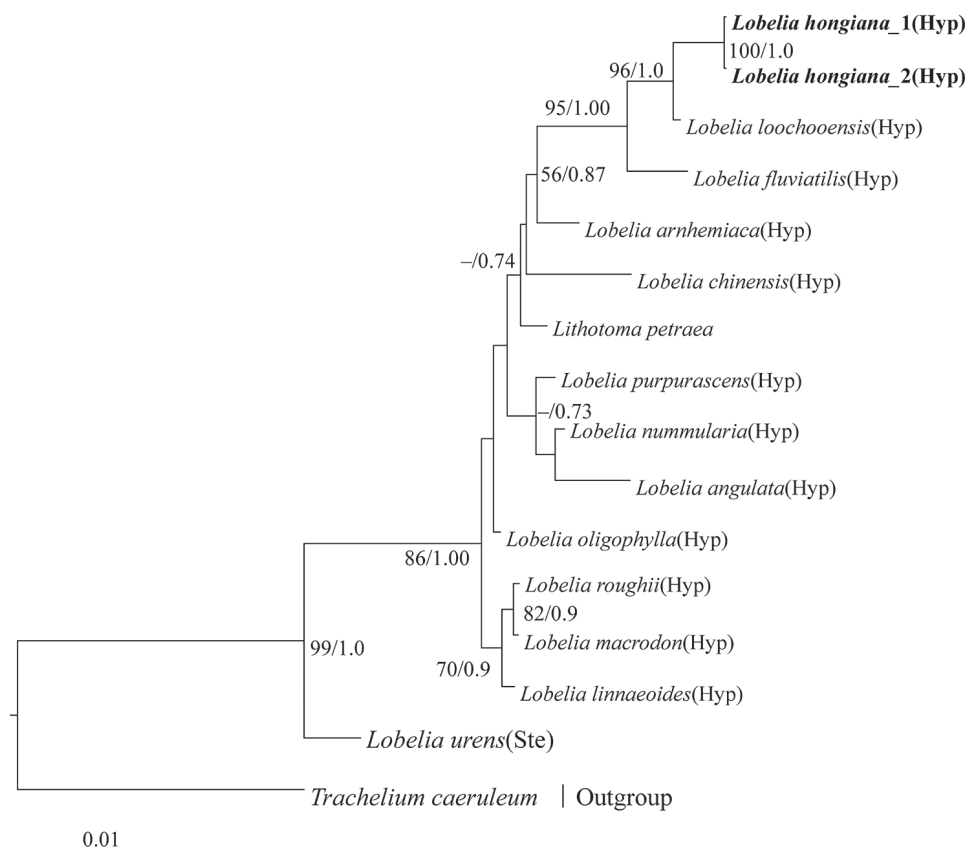


Figure 2. Bayesian tree inferred using the combined ITS, *atpB* and *rbcL* dataset showing the phylogenetic position of *Lobelia hongiana* and its sister species. Maximum likelihood bootstrap values ≥ 50 and PPs ≥ 0.70 are shown at the nodes. The abbreviations, Hyp and Ste, represented the sections of *Hypsela* and *Stenotium*, respectively. The new species is shown in bold.

Phenology. The new species was found in flower from May to July.

Etymology. Species epithet, “*hongiana*”, is in honour of Prof. De-Yuan Hong who made a significant contribution to the authors’ knowledge of Campanulaceae.

Conservation status. This new species was only found at Huixian Wetland in Guangxi Zhuang Autonomous Region, China, although it might also be distributed in adjacent areas. Until now, about 200 individuals were found in each population. Since there is not enough information on population size and dynamics, an assessment of the current conservation status of this species cannot be given. Therefore, it is suggested that the species be evaluated as Data Deficient (DD) according to the IUCN Red List Categories and Criteria (IUCN 2001).

Other specimens examined (paratypes). CHINA, Guangxi, Guilin City, Lingui District, Huixian Town, elev. 140 m, 8 June 2016, *G. W. Hu & Z. Z. Li* HGW-01117 (HIB!)

Key to the *Lobelia* sect. *Hypsela* in East Asia

- 1 Fruit berry, purple-red, ellipsoid or globose; stem villous, rarely glabrous; petiole puberulent.....*L. nummularia*
- Fruit capsule, green, subglobose or obconic; stem glabrous; petiole glabrous..... **2**
- 2 Stem prostrate; leaves thick, pedicel under 2.5 cm long; hypanthium no more than 1 mm long, calyx lobes longer than hypanthium, corolla under 9 mm long; all 5 filaments sparsely hairy; capsule subglobose, no more than 5 mm long.....*L. loochooensis*
- Stem decumbent; leaves thin or slightly thick, pedicel up to 4 cm or longer; hypanthium more than 2.5 mm, calyx lobes not longer than hypanthium, corolla over 9 mm long; not all 5 filaments hairy; capsule obconic, more than 6 mm long..... **3**
- 3 Leaves slightly thick, under 1 cm long, apex obtuse; hypanthium 2.5–3 mm long, calyx lobes 5 or 6, 1.4–2 mm long, shorter than hypanthium, corolla sub-bilabiate, corolla lobes gradually recurved when open, covered sparsely white villous, without vein; filament ca. 4 mm long, glabrous, anther tube 1.1–1.5 mm; style glabrous; capsule broadly obconic, 7–8 mm long.....*L. hongiana*
- Leaves thin, 0.7–2.6 cm long, apex acute or acuminate; hypanthium 3–5 mm long, calyx lobes 5, 3–5 mm long, as long as hypanthium, corolla unilabiate, corolla lobes all spreading in a plane on anterior side, glabrous, with purple veins; filament 6–8 mm long, the two anterior filaments hairy, anther tube 2–2.5 mm; style hairy; capsule narrowly obconic, 6–7 mm long.....*L. chinensis*

Acknowledgments

We would like to thank Prof. De-Yuan Hong for encouraging us in publishing this new species. Thanks are also given to Mr. Andrew Wanyoike Gichira for revising the beginning of the manuscript. The research was supported by grants from the National Natural Science Foundation of China (31270244), the Backbone Talents Project of Wuhan Botanical Garden, CAS (Y655301M01) and Sino-Africa Joint Research Centre, CAS (SAJC201614).

References

- Chen LY, Wang QF, Renne SS (2016) East Asian Lobelioideae and ancient divergence of a giant rosette *Lobelia* in Himalayan Bhutan. *Taxon* 65(2): 293–304. <http://doi.org/10.12705/652.6>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): 772. <http://doi.org/10.1038/nmeth.2109>
- Haberle RC, Dang A, Lee T, Penafior C, Cortes-Burn H, Oestreich A, Raubenson L, Cellinese N, Edwards EJ, Kim ST, Eddie WMM, Jansen RK (2009) Taxonomic and biogeographic

- implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. *Taxon* 58(3): 715–734. <http://www.jstor.org/stable/27756940>
- Hong DY, Lammers TG (2011) *Lobelia*. In: Wu ZY, Peter HR (Eds) *Flora of China*, Vol. 19. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 554–562.
- IUCN (2001) IUCN Red List Categories and Criteria, Version 3.1. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, United Kingdom, 30 pp.
- Jin X, Chen Y, Lee J, Qi Z, Liu L, Li P (2016) A new species of *Smilax* (Smilacaceae) from Yunnan, China. *Phytotaxa* 275(2): 159–167. <http://doi.org/10.11646/phytotaxa.275.2.7>
- Kokubugata G, Nakamura K, Forster PI, Hirayama Y, Yokota M (2012) Antitropical distribution of *Lobelia* species (Campanulaceae) between the Ryukyu Archipelago of Japan and Oceania as indicated by molecular data. *Australian Journal of Botany* 60(5): 417–428. <http://doi.org/10.1071/BT11316>
- Lamarck J (1792) *Lobelia nummularia* Lam. In: Lamarck J, Poiret J (Eds) *Encyclopédie méthodique, Botanique*, Vol. 3. Panckoucke & Plomteux, Paris & Liège, 589. <http://doi.org/10.5962/bhl.title.824>
- Lammers TG (2011) Revision of the infrageneric classification of *Lobelia* L. (Campanulaceae: Lobelioideae). *Annals of the Missouri Botanical Garden* 98(1): 37–62. <http://doi.org/10.3417/2007150>
- Linnaeus C (1753) *Species Plantarum*, Vol. 1. Laurentius Salvius, Stockholm, 1–560. <http://doi.org/10.5962/bhl.title.59734>
- Linnaeus C (1753) *Species Plantarum*, Vol. 2. Laurentius Salvius, Stockholm, 561–1200. <http://doi.org/10.5962/bhl.title.59735>
- Loureiro J de (1790) *Lobelia chinensis*. In: Loureiro J de (Ed.) *Flora cochinchinensis: sistens plantas in regno Cochinchina nascentes*, Vol. 2, T.2. Typis, et expensis academicis, Ulysipone, 514. <http://doi.org/10.5962/bhl.title.560>
- Merrill ED, Perry LM (1941) *Plantae papuanae archboldianae*, VII. *Journal of the Arnold Arboretum* 22: 375–388. <http://doi.org/10.5962/bhl.title.480>
- Merrill ED, Perry LM (1949) *Plantae archboldianae*, XVIII. *Journal of the Arnold Arboretum* 30: 39–63. <http://doi.org/10.5962/bhl.title.480>
- Moeliono B, Tuyn P (1960) In: Steenis CGG J van (Ed.) *Flora Malesiana*, Series 1, Spermatophyte, Being an Illustrated Systematic Account of the Malaysian Flora. Noordhoff-Kolff, Djakarta, 107–142. <http://doi.org/10.5962/bhl.title.40744>
- Murata J (1992) Morphology and Chromosome Number of *Lobelia loochooensis* Koidz. *Journal of Japanese Botany* 67(5): 282–285. http://www.jjbotany.com/pdf/JJB_067_282_285.pdf
- Murata J (1995) A revision of the infrageneric classification of *Lobelia* (Campanulaceae: Lobelioideae) with special reference to seed coat morphology. *Journal of the Faculty of Science, University of Tokyo, Section III, Botany* 15: 349–371.
- Ronquist F, Huesenbeck J (2015) MrBayes: Bayesian Inference of Phylogeny. <http://mrbayes.sourceforge.net/> [Accessed 19 May 2015]
- Royen P (1966) A new species of *Lobelia* L. from New Guinea. *Kew Bulletin* 20(2): 305–307. <http://doi.org/10.2307/4107805>
- Royen P (1978) Two new Campanulaceae from New Guinea. *Botanical Journal of the Linnean Society* 77(2): 117–123. <https://doi.org/10.1111/j.1095-8339.1978.tb01378.x>

- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57(5): 758–771. <http://doi.org/10.1080/10635150802429642>
- Wimmer FE (1943) Campanulaceae–Lobelioideae. In: Engler HGA, Diels FLE, Stubbe H, Noack K (Eds) *Das Pflanzenreich*, Vol. 106. Akademie-Verlag, Berlin, 1–260.
- Wimmer FE (1953) Campanulaceae–Lobelioideae. In: Engler HGA, Diels FLE, Stubbe H, Noack K (Eds) *Das Pflanzenreich*, Vol. 107. Akademie-Verlag, Berlin, 261–814.
- Wimmer FE (1968) Campanulaceae–Lobelioideae Supplementum et Campanulaceae–Cyphioideae. In: Stubbe H (Ed.) *Das Pflanzenreich*, Vol. 108. Akademie-Verlag, Berlin, 815–1024.

A taxonomic study of *Quercus langbianensis* complex based on morphology and DNA barcodes of classic and next generation sequences

Hoang Thi Binh^{1,2}, Nguyen Van Ngoc^{1,2}, Shuichiro Tagane³, Hironori Toyama³,
Keiko Mase³, Chika Mitsuyuki⁴, Joeri Sergej Strijk^{5,6},
Yoshihisa Suyama⁴, Tetsukazu Yahara^{1,3}

1 Graduate School of Systems Life Sciences, Kyushu University, 744 Motoooka, Fukuoka, 819-0395, Japan
2 Department of Biology, Dalat University, 01 – Phu Dong Thien Vuong, Dalat, Vietnam **3** Centre for Asian Conservation Ecology, Kyushu University, 744 Motoooka, Fukuoka, 819-0395, Japan **4** Kawatabi Field Science Centre, Graduate School of Agricultural Science, Tohoku University, 232-3 Yomogida, Naruko-onsen, Osaki, Miyagi 989-6711, Japan **5** Biodiversity Genomics Team, Plant Ecophysiology & Evolution Group, Guangxi Key Laboratory of Forest Ecology and Conservation (under state evaluation status), College of Forestry, Daxuedonglu 100, Nanning, Guangxi, 530005, PR China **6** State Key Laboratory for Conservation and Utilisation of Subtropical Agro-bioresources, College of Forestry, Guangxi University, Nanning, Guangxi 530005, PR China

Corresponding author: Hoang Thi Binh (binhht@dlu.edu.vn)

Academic editor: H. De Boer | Received 21 September 2017 | Accepted 28 January 2018 | Published 7 February 2018

Citation: Binh HT, Ngoc NV, Tagane S, Toyama H, Mase K, Mitsuyuki C, Strijk JS, Suyama Y, Yahara T (2018) A taxonomic study of *Quercus langbianensis* complex based on morphology and DNA barcodes of classic and next generation sequences. *PhytoKeys* 95: 37–70. <https://doi.org/10.3897/phytokeys.95.21126>

Abstract

The taxonomy of *Quercus langbianensis* and its relatives in Vietnam and Cambodia have been revised based on evidence obtained from field observations, morphological comparison of herbarium specimens and molecular analyses using both classic and next generation DNA markers. Based on Bayesian inference using *rbcl*, *matK* and ITS regions and Neighbour-joining tree using genome-wide sequences amplified with multiplexed inter-simple sequence repeat (ISSR) primers (MIG-seq), the authors recognised ten species in the complex in Vietnam and Cambodia, three of which are newly described in this paper: *Q. baolamensis* **sp. nov.**, *Q. bidoupensis* **sp. nov.** and *Q. honbaensis* **sp. nov.** These new species are all phenotypically similar to *Q. langbianensis* *s. str.* in having lanceolate to oblanceolate leaf shape, upper 4–5/6–serrated leaf margin, acute or acuminate leaf apex and bracts of cupule arranged in 5–9 rings but distinguished both morphologically and phylogenetically. In molecular phylogenetic reconstructions, *Q. bidoupensis* is not close to any other species. In the Bayesian tree, *Q. honbaensis* is sister to both *Q. blaensis* and *Q. camusiae*

that are found in the same locality but morphologically distinct and those three species are sister to *Q. langbianensis* s. str., while *Quercus baolamensis* is not sister to *Q. langbianensis* s. str. in both the Bayesian tree and MIG-seq tree. In addition, *Q. cambodiensis* and *Q. baniensis* previously reduced to *Q. langbianensis* s. lat. have been recognised as distinct species. Six species were in need of lectotypification and that is undertaken herein.

Keywords

DNA barcoding, Fagaceae, MIG-seq, *Quercus*, taxonomy, Vietnam

Introduction

The genus *Quercus* L., with 400–500 species, is the largest genus in the family Fagaceae (Nixon 1993, Valencia-A et al. 2016). The genus is widely distributed in the northern Hemisphere including tropical montane forests in South East Asia and often dominant in temperate deciduous forests in East Asia, Europe and North America and desert scrubs in the Mediterranean (Nixon 1993, Hubert et al. 2014, Valencia-A et al. 2016). In Vietnam, 45 species of the genus *Quercus* have been recognised (Ban 2003, Ho 2003, Binh et al. in press) but taxonomic identities of some species remain to be revised. One of them is *Quercus langbianensis* Hickel & A.Camus (1921), described from Mt. Langbian of Lam Dong Province, southern Vietnam. Following previous studies including Deng et al. (2010), The Plant List (2013) adopted a broad concept of this species by treating the following seven names as synonyms: *Q. baniensis* A.Camus, *Q. blaoensis* A.Camus, *Q. camusiae* Trel. ex Hickel & A.Camus (a replacement name of *Q. geminata* Hickel & A.Camus), *Q. dilacerata* Hickel & A.Camus and *Q. donnaiensis* A.Camus from Vietnam, *Q. cambodiensis* Hickel & A.Camus from Cambodia and *Cyclobalanopsis faadoouensis* Hu from mainland of China. However, the authors' recent comparison based on the collections of *Q. camusiae* and *Q. cambodiensis* from their type localities revealed that both *Q. camusiae* and *Q. cambodiensis* are distinct species from *Q. langbianensis* s. str. This finding triggered the re-examination of the taxonomy of *Q. langbianensis* s. lat. hereafter designated as “*Q. langbianensis* complex” and its similar species such as *Q. auricoma* A.Camus in which *Q. cambodiensis* was recently included (Tagane et al. 2017). Deng et al. (2010) studied the relationship of *Q. camusiae*, *Q. cambodiensis* and *Q. langbianensis* and concluded that the three species are phenotypically indistinguishable. However, their study was based on the comparison of a limited number of herbarium specimens.

In this study, specimens of the *Q. langbianensis* complex were observed and collected more widely: Mt. Hon Ba of Khanh Hoa Province (the type locality of *Q. camusiae*), some localities of Lam Dong Province (near the type locality of *Q. langbianensis* s. str.), Mt. Ba Na (the type locality of *Q. baniensis*) and Mt. Bokor of Cambodia (the type locality of *Q. cambodiensis*). In Mt. Hon Ba, *Q. camusiae* was found at the higher elevation whereas two additional morphologically similar but distinct species were found at the lower elevation. Observations in the field revealed that two neighbouring provinces of southern Vietnam, Khanh Hoa Province and Lam Dong Province, harbour

the highest diversity of the *Q. langbianensis* complex including three unknown species. However, those species are phenotypically very similar to each other and evidence based on molecular analyses is needed to elucidate their identities and relationships.

Recently, molecular studies of the genus *Quercus* have succeeded in elucidating phylogenetic relationships within the genus by using multiple gene markers (Hubert et al. 2014, Simeone et al. 2016) or RAD-seq (Hipp et al. 2014, Cavender-Bares et al. 2015, Fitz-Gibbon et al. 2017). In this study, both classic multiple gene markers (*rbcL*, *matK* and ITS) and genome-wide markers have been employed using the next generation sequencing platform (MIG-seq; Suyama and Matsuki 2015) to clarify relationships of the species within *Q. langbianensis* complex. As in RAD-seq, MIG-seq provides genetic markers of relatively short sequence reads determined by the next generation sequencer, but it is obtained with a PCR-based procedure without restriction enzyme digestion steps and is widely applicable to field samples even with low-quality DNA and/or small quantities of DNA (Suyama and Matsuki 2015).

The purpose of this paper is to revise the taxonomy of the *Q. langbianensis* complex based on evidence obtained from field observations, morphological studies and molecular data from both classic and next generation DNA markers. In conclusion, 10 species in the *Q. langbianensis* complex, including the seven species treated as synonyms of *Q. langbianensis* (The Plant List 2013) and the remaining three undescribed species have been distinguished. The latter three species are described as *Q. baolamensis*, sp. nov., *Q. bidoupensis* sp. nov. and *Q. honbaensis* sp. nov.

Materials and methods

Observations and collections in the field

The field surveys were carried out in 13 conservation areas (national parks, nature reserves and conservation areas) in Vietnam and one national park in Cambodia (Fig. 1). In Hon Ba Nature Reserve, Khanh Hoa Province, southern Vietnam, eight rectangular plots of 100 m × 5 m were placed at various locations from 225 m to 1,498 m altitude and girth and height for all the individual trees above 4 m tall within the plots were recorded (Table 1). The authors also recorded trees in the same way for the following localities: two plots at 1,553 m and 1,807 m in Bidoup-Nui Ba National Park, Lam Dong Province; three plots at 1,850 m, 2,225 m and 2,933 m in Hoang Lien National Park (Mt. Fan Si Pan), Lao Cai Province; four plots at 86 m, 650 m, 1,420 m and 1,720 m in Vu Quang National Park, Ha Tinh Province; two plots at 450 m and 1,274 m in Bach Ma National Park, Thua Thien Hue Province; and three plots at 833 m, 1,070 m and 1,376 m in Ngoc Linh Nature Reserve, Kon Tum Province. In these localities, general collections of vascular plants outside of the plots were also made, with particular attention to the species of Fagaceae. In addition to the above 6 conservation areas, general sampling of Fagaceae was undertaken in the following 6 conservation areas: Ba Na Nature Reserve, Da Nang Province; Ba Vi National Park,

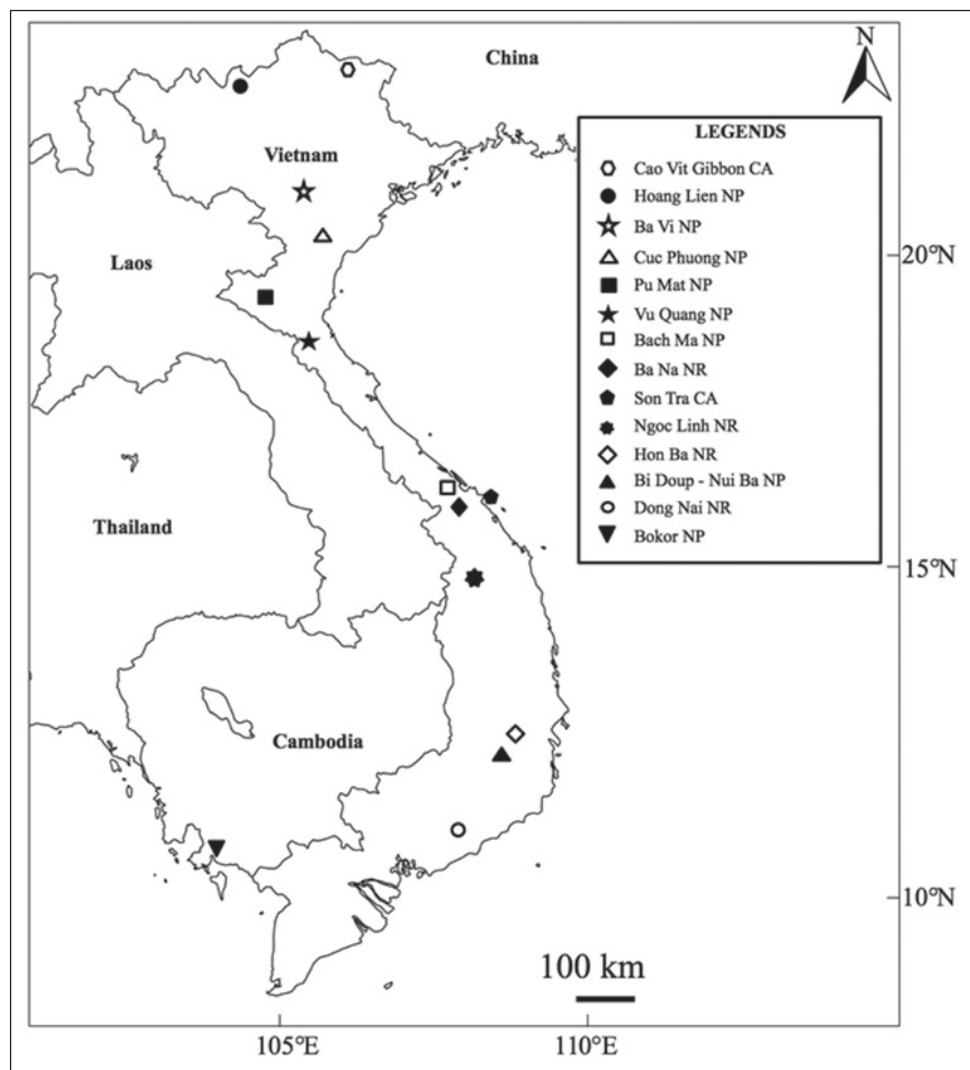


Figure 1. Collection sites in Vietnam and Cambodia in this study, including eight national parks, four nature reserves and two conservation areas.

Ha Noi Capital; Cuc Phuong National Park, Ninh Binh Province; Dong Nai Nature Reserve, Dong Nai Province; Pu Mat National Park, Nghe An Province; Son Tra Conservation Area; and Cao Vit Gibbon Conservation Area, Cao Bang Province. In Mt. Bokor, Cambodia, 20 plots from 266 m to 1,048 m altitude were established and *Q. cambodiensis* was sampled (Zhang et al. 2016, Tagane et al. 2017). For each specimen collected, photographs were taken in the field and samples of silica gel-dried leaf pieces for DNA isolation were gathered.

Amongst the collections of *Quercus*, the authors regarded species having the following traits as members of the *Q. langbianensis* complex: mature leaves are 12–17 cm

Table 1. Altitudinal distribution of *Quercus* spp. found in Mt. Hon Ba.

Altitude (m)	<i>Quercus</i> trees found in each plot (tree height, girth)
1498	<i>Q. camusiae</i> (16 m, 88.5 cm)
1336	<i>Q. camusiae</i> (4.5 m, 4.8 cm)
1204	<i>Q. poilanei</i> (25 m, 86.5 cm; 25 m, 114 cm)
1021	No <i>Quercus</i> species
919	No <i>Quercus</i> species
617	<i>Quercus honbaensis</i> (12 m, 51.4 cm)
400	<i>Quercus honbaensis</i> (8 m, 19 cm; 18 m, 88 cm; 5 m, 10 cm)
225	<i>Quercus honbaensis</i> (4 m, 6.8 cm), <i>Q. blaoensis</i> (14 m, 50.5 cm; 11 m, 15.4 cm)

long, 3–5 cm wide, serrated along the upper 5/6 to 1/3 margin (although young leaves of *Q. camusiae* are often almost entire), acute or acuminate at apex, cuneate at base and hairy when young but almost glabrous when mature; cupule obconical or bowl- or cup-shaped, bracts of cupule arranged in 5–9 rings and covers 1/4 to 2/3 of a nut that is ovoid or subglobose to ellipsoid. *Q. auricoma* in the *Q. langbianensis* complex was not included because mature leaves have entire margin and smaller size (5.5–7 cm long, 2–2.7 cm wide, from *E. Poilanei* 13098 (P)).

In this study, 46 samples including 9 species of the *Quercus langbianensis* complex (*Q. baniensis*, *Q. baolamensis*, *Q. bidoupensis*, *Q. blaoensis*, *Q. cambodiensis*, *Q. camusiae*, *Q. donnaiensis*, *Q. honbaensis*, *Q. langbianensis* s. str.) and ten species of non-*Quercus langbianensis* complex (*Q. annulata*, *Q. auricoma*, *Q. austrocochinchinensis*, *Q. braianensis*, *Q. djiringensis*, *Q. helferiana*, *Q. kerrii*, *Q. macrocalyx*, *Q. neglecta* and *Q. poilanei*) were used for morphological and DNA studies. One species of *Trigonobalanus*, *T. verticillatus* Forman was also analysed as an outgroup in phylogenetic analyses. Three to four sets of voucher specimens were collected from each locality and deposited in FU and herbaria of each protected area, DLU and VNM.

DNA extraction

DNA was isolated from each silica-gel dried sample by the CTAB method (Doyle and Doyle 1987) with the following modifications: dried leaf material was milled by QIAGEN TissueLyser to obtain fine powder and washed three times in a 1 ml buffer (including 0.1 M HEPES, pH 8.0; 2% Mercaptoethanol; 1% PVP; 0.05 M Ascorbic acid) as in Toyama et al. (2015).

Classic DNA sequencing

DNA regions of the large subunit of ribulose-1,5-biphosphate carboxylase oxygenase (*rbcL*), maturase K (*matK*) and the internal transcribed spacer (ITS) were amplified

with the following primer sets (sequence: 5' to 3'): *rbcl*-F (ATGTCACCACAAACA-GAGACTAAAGC, Levin et al. 2003), *rbcl*-724r (TCGCATGTACCTGCAGTAGC, Fay et al. 1997); *matK*-XF (TAATTTACGATCAATTCATTC, Ford et al. 2009), *matK*-1326R (TCTAGCACACGAAAGTCGAAGT, Cuénoud et al. 2002); ITS-18F (GTCCACTGAACCTTATCATTTAGAGG, Rohwer et al. 2009) and ITS-26R (GCCGTTACTAAGGGAATCCTTGTTAG, Rohwer et al. 2009). The sequences of *rbcl*, *matK* and ITS were amplified with Tks Gflex™ DNA Polymerase (Takara Bio, Kusatsu, Japan) following the protocols of Kress et al. (2009), Dunning and Savolainen (2010) and Rohwer et al. (2009), respectively. The PCR product was cleaned with 0.5 µl of ExoSap-IT enzyme (GE Healthcare, Little Chalfont, UK) and 1.5 µl of distilled water and incubated at 37°C for 30 min and subsequently at 80°C for 15 min for deactivation of the enzyme. Sequence reactions were continued using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The reaction mixtures were analysed in an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Next generation DNA sequencing – MIG-seq

For 105 samples, thousands of short sequences (loci) were amplified from each genome using primers designed for “multiplexed ISSR genotyping by sequencing” (MIG-seq, Suyama and Matsuki 2015) and presence/absence of each sequence (amplicon) were used in each sample for phylogenetic tree reconstruction regardless of whether it has SNP or not, as sequence-based dominant markers. The experimental standard conditions were performed following Suyama and Matsuki (2015). The 1st PCR step was performed to amplify ISSR regions from genomic DNA with MIG-seq primer set-1. The products of the 1st PCR were diluted 50 times for each 1st PCR product with deionised water. The 2nd PCR step was conducted independently to add individual indices to each sample using indexed primers. Then, 3 µl of each 2nd PCR product was pooled as a single mixture library. The mixture was purified and fragments in the size range 350–800 bp were selected by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Finally, the concentration of size-selected library was measured by using a SYBR green quantitative PCR assay (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) with approximately 10 pM of libraries that were used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Phylogenetic analyses

For classical phylogenetic analyses, a phylogenetic tree was constructed by combining nucleotide sequences of the three DNA regions comprising *rbcl*, *matK* and ITS for 30 samples of 29 *Quercus* species and one *Trigonobalanus verticillatus* (as an outgroup).

All DNA sequences were newly generated in this study. The sequences were aligned by MEGA v7.0 (Kumar et al. 2016). For reconstructing phylogeny, a Bayesian method implemented in the programme BEAST v1.8.4 (Drummond et al. 2012) was used. The GTR + γ model of molecular evolution and an uncorrelated lognormal (UCLN) relaxed-clock model were selected to infer relative divergence times. In computation, the programme was started with a random tree and a tree prior that was useful for species-level was set according to a Yule process (Drummond and Rambaut 2007). Five independent chains of 100 million generations each were run with sampling every 10,000 generations. The first 1,000 trees were discarded as burn-in from each run. The remaining trees from each run were combined by using LogCombiner v 1.6.1 (Drummond and Rambaut 2007). Amongst the posterior distribution of 9,000 trees, the maximum clade credibility tree was identified using TreeAnnotator v 1.6.1 (Drummond and Rambaut 2007) with a posterior probability limit of 0.5 and median node heights. The congruence amongst *rbcL*, *matK* and ITS trees was tested using the incongruence length difference test (Farris et al. 1994) implemented in PAUP* 4.0b10 (Swofford 2003). As the incongruence was rejected ($p=0.06$), a combined tree using concatenated sequences was constructed.

For MIG-seq, raw data were pretreated from 105 samples and quality control was completed following Suyama and Matsuki (2015). The programme 'fastx_trimmer' in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) was used to trim read 2 sequences including 12 bases of SSR region and two bases of anchor sequences in the 1st primers. The authors used option 'quality_filter' of FASTX-Toolkit to select reads in which 40% or more sequences had quality scores Q30 or more. Then the TagDust programme (Lassmann et al. 2009) was used to remove the reads derived from extremely short library entries and to trim read 1 and read 2 sequences. Then, loci were assembled from the quality-filtered reads data with the *de novo* map pipelines (ustacks, cstacks, sstacks) in Stacks software package version 1.35 (Catchen et al. 2011) and then a table prepared of presence/absence of loci in each individual from the outputs of the populations pipeline of Stacks 1.35. Using ustacks, homologous sequences (loci) were assembled in each individual with the following settings: minimum depth of coverage (m) = 10, maximum distance allowed between stacks (M) = 1, maximum distance allowed to align secondary reads to primary stacks (N) = 1 and maximum gaps = 2. Using cstacks, a catalogue of consensus loci was built for all the individuals by assembling loci in each individual assembled using ustacks, with the number of mismatches allowed between sample loci (n) = 2. Using sstacks, IDs of loci were associated in each individual with IDs of the consensus loci. Finally, presence/absence of loci were determined in each individual from a haplotypes list obtained using the populations pipeline. The populations pipeline output file haplotypes.tsv provides genotypes of individuals at each locus. For each individual, the authors recorded a locus that had genotype information as "1" and a locus that had no genotype information as "0". The authors obtained a list of loci that were detected in at least one individual ($1/105 = 0.01$) with the following settings: all samples belong to the same population and threshold frequency of haplotype count in a population (r) = 0.001, a threshold

one-order higher than 0.01. Using presence/absence (1/0) data of loci, the authors computed distance matrix, constructed a neighbour-joining (NJ) tree and examined the reliability of tree topology by bootstrapping with 1000 replicate using PHYLIP ver. 3.695 (Shimada and Nishida 2017) as follows; 1000 times re-sampling with Seqboot, distance computation with Restdist, tree construction with Neighbour and consensus tree construction with Censense. The resulted tree was visualised with FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). A phylogenetic analyses was first made for 105 samples including more *Quercus* species and then the sample size reduced to 31 by focusing on the *Q. langbianensis* complex. A total of 16,809 loci were used for the final phylogenetic tree.

Morphological and taxonomic comparison

The collections contain considerable numbers of sterile specimens including those from young trees that are often morphologically different from adult trees. Thus, after phylogenetic trees were obtained, morphological traits of leaves and shoots were carefully re-examined as well as reproductive organs if available and species were distinguished. If two OTUs are morphologically distinguishable and also not monophyletic on phylogenetic trees, these were regarded as two distinct species. Then, these were identified by a thorough literature review and comparisons with type specimen images available online (e.g. JSTOR Global Plants, <http://plants.jstor.org/>). In *Q. langbianensis* complex, lectotypification was needed for *Q. baniensis*, *Q. blaoensis*, *Q. cambodiensis*, *Q. camusiae*, *Q. dilacerata* and *Q. donnaiensis*. One of the co-authors, J.S. Strijk, examined specimens at P for lectotypification; selected for each species, was one of the specimens cited in the original description, which best represents the diagnostic traits of each species.

Results

Observation in the field

In Hon Ba Nature Reserve, tree diversity was examined in eight plots of 100 m × 5 m and four species of *Quercus* (Table 1) were found including *Q. poilanei* and three species of the *Q. langbianensis* complex: *Q. blaoensis*, *Q. camusiae* and an undescribed species, *Q. honbaensis*. *Quercus camusiae* was found in the two plots at 1,336 m and 1,498 m altitude and one of canopy trees in the latter. *Quercus honbaensis* was found in three plots at 225 m, 400 m and 617 m altitude and occurred sympatrically with *Q. blaoensis* in the plot at 225 m altitude. *Quercus honbaensis* was one of the canopy trees at both 225 m and 400 m altitude (Table 1). In late February of 2014, *Q. honbaensis* had mature fruits and *Q. blaoensis* had young fruits. Two species were distinct in pubescence on young shoots (*Q. honbaensis* has long, very thin and curly hairs vs. *Q. blaoensis* has

short, thicker and straight hairs). *Quercus camusiae* was distinct from *Q. honbaensis* and *Q. blaoensis* in that shoots and leaves were golden tomentose when young.

In Bidoup-Nui Ba National Park, approximately 100 km west of Mt. Hon Ba, tree diversity was examined in two plots at 1,553 m and 1,807 m altitude and *Q. langbianensis* s. str. was found at 1,553 m altitude. *Quercus langbianensis* s. str. was similar to *Q. camusiae* in having golden tomentose cupules, but different in distinctly toothed leaves and longer nuts (vs. almost entire or with only a few low teeth in *Q. camusiae*). The flora was surveyed above 800 m altitude in Bidoup-Nui Ba National Park and *Q. camusiae* and *Q. honbaensis* were not found. On the other hand, two additional and unknown species of the *Q. langbianensis* complex were found: *Q. bidoupensis* and *Q. donnaiensis*. *Quercus bidoupensis* was distinct from *Q. langbianensis* s. str. in having oblong-lanceolate leaves, acuminate and slightly caudate at apex and undulate and distinctly serrate along the upper half of the margin. *Quercus donnaiensis* was similar to *Q. bidoupensis* in leaf shape but differs in its margin not being undulate, serrated only near the apex and with 3-5 teeth. From the general collection in Lam Dong Province, three species of the *Q. langbianensis* complex were collected: *Q. bidoupensis* and *Q. donnaiensis* in Lam Thanh District and another undescribed species, *Q. baolamensis*, in Bao Lam District.

In Ba Na Nature Reserve and Son Tra Natural Conservation Area, central Vietnam, *Q. baniensis* of the *Q. langbianensis* complex and *Q. poilanei* and *Q. auricoma* of non-*Q. langbianensis* complex were found.

In the top plateau of Mt. Bokor, Cambodia, *Q. cambodiensis* of the *Q. langbianensis* complex and *Q. augustinii* of non-*Q. langbianensis* complex were collected.

A phylogenetic tree combining three DNA regions (*rbcl*, *matK*, and *ITS*)

A total of 2,034 bases consisting of three DNA regions (657 bp for *rbcl*, 834 bp for *matK* and 543 bp for *ITS*) included 142 variable sites, amongst which 56 bases were parsimony-informative (Table 2). According to the Bayesian tree combining the three regions (Fig. 2), two major clades were supported by posterior probabilities higher than 80%: Clade 1 with 85 % posterior probability consists of five species of non-*Quercus langbianensis* complex (*Q. poilanei*, *Q. kerrii*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis*) and Clade 2 with 82 % posterior probability including seven species of the *Q. langbianensis* complex (*Q. cambodiensis* and six Vietnamese species) and five species of non-*Quercus langbianensis* complex (*Q. neglecta* nested with the *Q. langbianensis* complex and *Q. annulata*, *Q. auricoma*, *Q. djiringensis* and *Q. macrocalyx*). In Clade 2, *Q. cambodiensis* was sister to *Q. neglecta* with 81% posterior probability and clearly separated from the Vietnamese species of the *Q. langbianensis* complex (*Q. langbianensis* s. str., *Q. baniensis*, *Q. blaoensis*, *Q. honbaensis*, *Q. baolamensis* and *Q. camusiae*). *Quercus langbianensis* s. str. was sister to *Q. blaoensis*, *Q. camusiae* and *Q. honbaensis* with a strong branch support (PP = 1.00). *Quercus camusiae* was sister to *Q. blaoensis* with a high branch support (PP = 0.99). *Quercus baolamensis* and *Q. baniensis* were clustered together, but with weak branch support (PP = 0.64).

Table 2. Summary statistics of datasets used for phylogenetic inference comprising *rbcL*, *matK* and ITS sequences.

Regions	<i>rbcL</i>	<i>matK</i>	ITS	Combined data
Aligned sequence length	657	834	543	2034
Variable DNA sites	9	35	98	142
Parsimony-informative sites	3	9	44	56

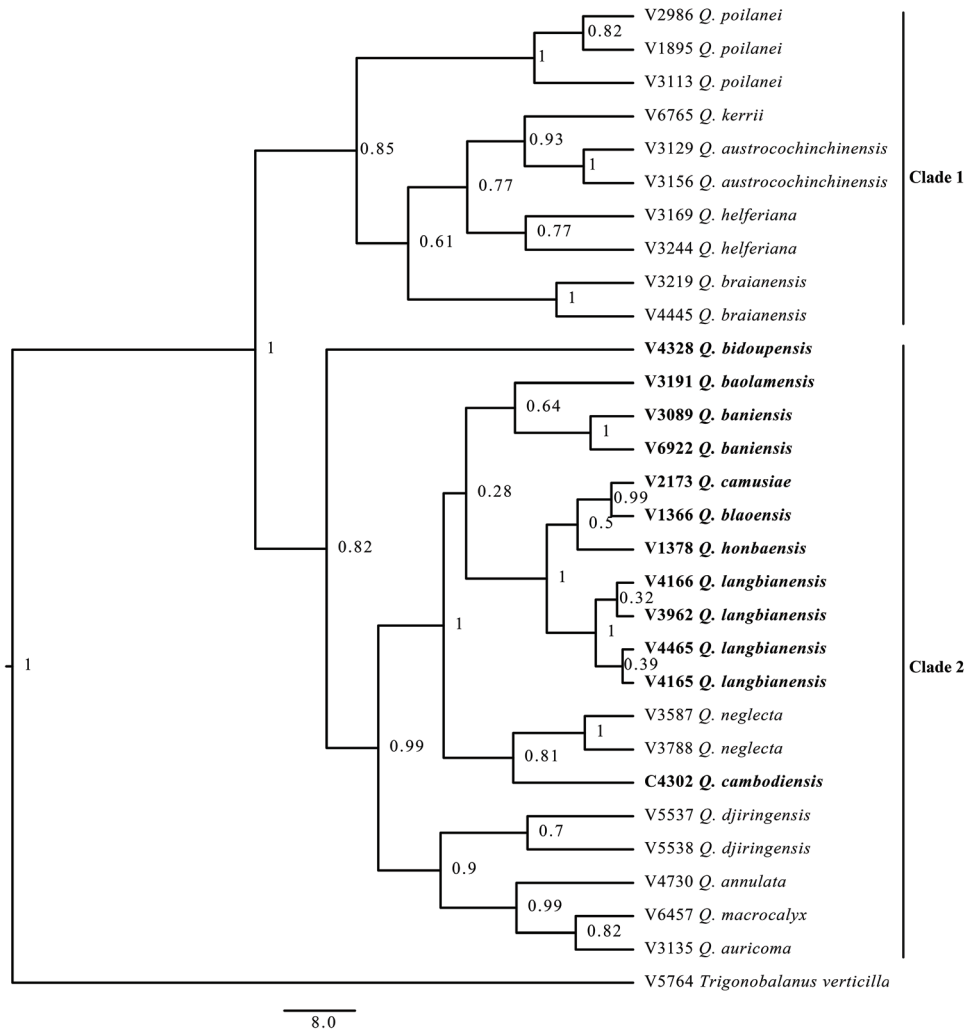


Figure 2. Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on *rbcL*, *matK* and ITS sequences. Braches are labelled with posterior probabilities.

Trees based on single gene sequences gave lower resolution but the ITS tree (see Suppl. material 1: Figure 1) supported the following points: (1) A clade, consisting of seven species of the *Q. langbianensis* complex and *Q. neglecta*, was supported by

100 % PP. (2) *Quercus bidoupensis* was not clustered with the other seven species of the *Q. langbianensis* complex. (3) A clade, including five species of non-*Q. langbianensis* complex, was supported by 70 % PP. In the cpDNA tree (see Suppl. material 1: Figure 2), neither the seven species of the *Q. langbianensis* complex nor the five species of non-*Q. langbianensis* complex was monophyletic. Neither *Q. poilanei*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis* was monophyletic whereas those four species were monophyletic in the ITS tree.

A phylogenetic tree using MIG-seq

A neighbour-joining (NJ) tree based on MIG-seq for 31 samples of *Quercus* recognised three major clades excluding an outgroup of *Trigonobalanus* (Fig. 3). Clade M1 includes single species, *Q. bidoupensis*. Clade M2 with a 100 % bootstrap value consists of five species of non-*Q. langbianensis* complex (*Q. poilanei*, *Q. kerrii*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis*). Clade M3 with 100 % bootstrap value includes *Q. neglecta*, *Q. macrocalyx*, *Q. auricoma* and eight species of the *Q. langbianensis* complex. Within this clade, *Q. cambodiensis* was sister to *Q. neglecta* with a 74 % bootstrap value. *Quercus honbaensis* and *Q. baolamensis* were monophyletic with a bootstrap value of 100 %. *Quercus donnaensis* and *Q. camusiae* were also monophyletic with a bootstrap value of 75 %. *Quercus blaoensis* and *Q. langbianensis* s. str. of the *Q. langbianensis* complex are clustered with *Q. baniensis*, *Q. auricoma*, *Q. macrocalyx*, forming a clade with 82 % bootstrap value.

Discussion

The results of the three gene tree (Bayesian tree) and MIG-seq tree (NJ tree) were mostly consistent. First, five species of non-*Q. langbianensis* complex (*Q. poilanei*, *Q. kerrii*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis*) formed a highly supported clade, Clade 1 or Clade M2. This clade was supported also in the ITS tree. Second, three gene and MIG-seq trees matched in Clade 2 and Clade M3. Third, the species of the *Q. langbianensis* complex except *Q. bidoupensis* formed a highly supported clade (also in ITS tree) and *Q. auricoma*, *Q. macrocalyx* and *Q. neglecta* of non-*Q. langbianensis* complex were included in this clade (Fig. 4). Fourth, *Q. cambodiensis* was sister to *Q. neglecta* and separated from the Vietnamese species of the *Q. langbianensis* complex by relatively high supports (posterior probability 0.81 and bootstrap probability 74%). Fifth, *Q. bidoupensis* was placed in Clade 2 or Clade M1 and not close to the other species of the *Q. langbianensis* complex (also in ITS tree). The cpDNA tree did not support monophilies of *Q. poilanei*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis* that were monophyletic in the ITS tree, three gene tree and MIG-seq tree and thus the cpDNA tree alone provides less reliable evidence.

The consistent topology of three gene and MIG-seq trees (Figs 2-4) provided reliable evidence to resolve taxonomy of “species” currently treated as synonyms of *Q. lang-*

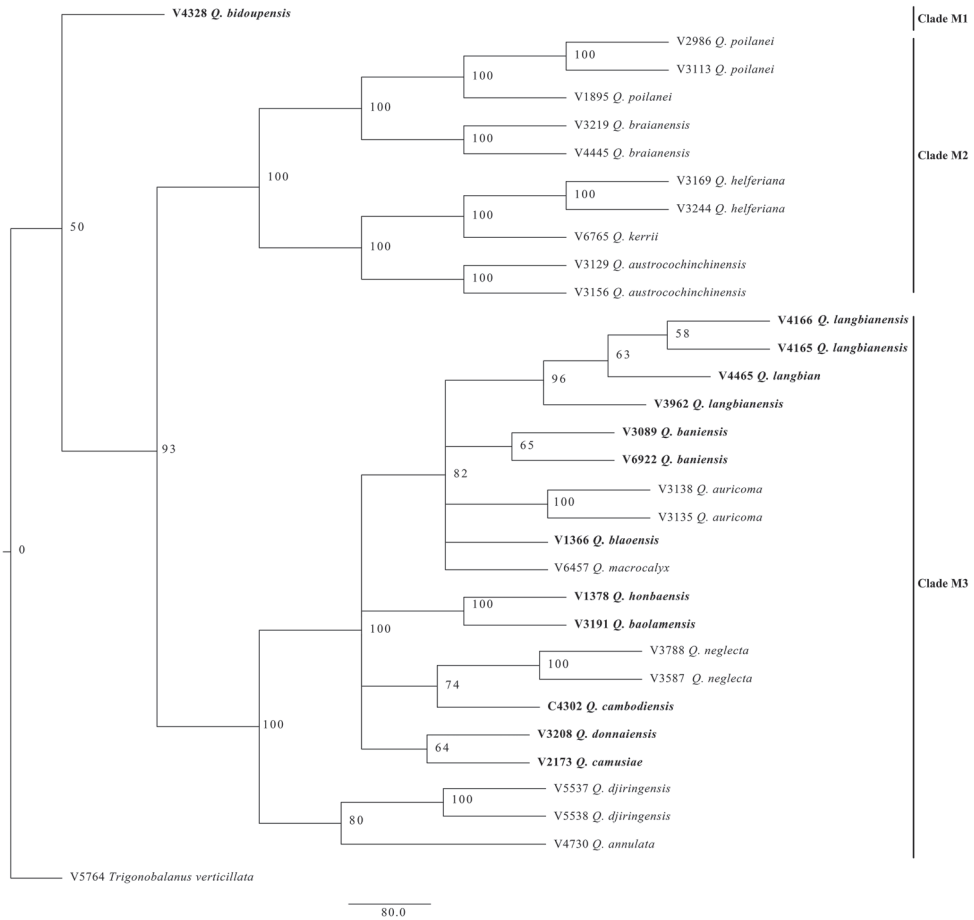


Figure 3. NJ tree of 31 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on presence/absence data of 16,809 MIG-seq loci. Branches are labelled with bootstrap supports (% of 1000 replicates).

bianensis s. lat. (*Q. camusiae*, *Q. blaoensis*, *Q. cambodiensis* and *Q. baniensis*). First, *Q. cambodiensis* is separated as a species because it is sister to *Q. neglecta* that is morphologically distinct in linear leaves and small nuts and has been treated as a distinct species in the Flora of China (Huang et al. 1999 as *Cyclobalanopsis neglecta*). Amongst the others, both *Q. camusiae* and *Q. blaoensis* are native in the Hon Ba Nature Reserve where *Q. camusiae* occurs at the higher elevation and *Q. blaoensis* occurs at the lower elevation (Table 1). As *Q. camusiae* and *Q. blaoensis* are sister to each other in the MIG-seq tree and not sympatric but paratactic in the distribution, those can be treated as two infraspecific taxa (varieties or subspecies) or two different species. Considering the morphological distinction described above, the latter treatment has been adopted. *Quercus blaoensis* co-occurs with another undescribed species: *Q. honbaensis*. As *Q. blaoensis* and *Q. honbaensis* are sympatric and morphologically distinct, those are recognised as different species. The monophyly of *Q. honbaensis* and *Q. baolamensis* was strongly supported in the

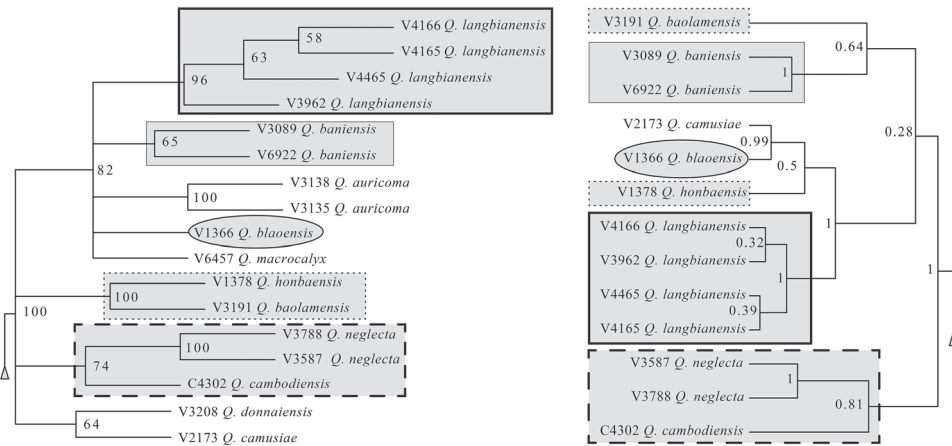


Figure 4. Comparison of *Q. langbianensis* complex between NJ tree (left, Clade M3 of Fig. 3) and Bayesian tree (right: Clade 2 of Fig. 2).

MIG-seq tree with a bootstrap value of 100%. While *Q. honbaensis* occurs at an elevation lower than 617 m in Hon Ba Nature Reserve, Khanh Hoa Province, *Q. baolamensis* is collected at 1000 m from the Lam Dong province. Considering this distinction in cupule and nut morphology (Figs 6, 12), they are treated as two distinct species. While *Q. baniensis* is found in Da Nang Province of central Vietnam, the other five species (*Q. baolamensis*, *Q. blaoensis*, *Q. camusiae*, *Q. honbaensis* and *Q. langbianensis* s. str.) occur in Khanh Hoa or Lam Dong Province of southern Vietnam. From phylogenetic trees and morphological observations, it is difficult to relate *Q. baniensis* to any of the five species. In particular, a sister relationship between *Q. langbianensis* s. str. and *Q. baniensis* is not strongly supported (Fig. 4). Thus, *Q. baniensis* is treated as a distinct species.

Although the topologies of the three gene and MIG-seq tree are mostly consistent, there are some notable differences, particularly in Clade 2 and Clade M3 containing the *Q. langbianensis* complex (Fig. 4). In the Bayesian tree, based on the three regions of *rbcL*, *matK* and ITS, the monophyly of the Vietnamese species of the *Q. langbianensis* complex was only weakly supported (PP = 0.28), whereas it was strongly supported in MIG-seq tree (bootstrap value 100 %). This higher support in MIG-seq tree was obtained because MIG-seq provided more informative sites for constructing phylogenetic relationships amongst the species in the *Q. langbianensis* complex. *Quercus auricoma* and *Q. macrocalyx* were included in a clade of the *langbianensis* complex in the MIG-seq tree but clustered with *Q. annulata* in the Bayesian tree. Further studies using more gene markers are needed to derive a conclusion on the placement of these two species.

A comparison, based on morphological characters both in the field and from dried specimens of the herbarium and the molecular evidence for the *Q. langbianensis* complex, revealed that *Q. baniensis*, *Q. blaoensis*, *Q. cambodiensis*, *Q. camusiae* and *Q. langbianensis* s. str. are all distinct species (Table 3). In addition, it is concluded that three species amongst the *Q. langbianensis* complex are undescribed and below they are described

Table 3. Morphological comparison of *Quercus langbianensis* complex.

Characters	<i>Q. bidoupensis</i>	<i>Q. camusiae</i>	<i>Q. cambodiensis</i>	<i>Q. baniensis</i>	<i>Q. honbaensis</i>	<i>Q. dilacerata</i>	<i>Q. blaensis</i>	<i>Q. donnaiensis</i>	<i>Q. baolanensis</i>	<i>Q. langbianensis</i>
Young shoot	Almost glabrous		Golden tomentose		Curly hairy	Golden tomentose	Straight hairy	Golden tomentose	Almost glabrous	
Leaf margin	Undulate, distinctly serrate in upper 1/2	Not undulate, almost entire or with a few low teeth in upper 1/4	Not undulate, distinctly serrate in upper 1/3	Not undulate, distinctly serrate in upper 1/3	Not undulate, distinctly serrate in upper 5/6–3/4(–2/3)	Not undulate, distinctly serrate in upper 1/3		Not undulate, distinctly serrate in upper 1/3	Not undulate, distinctly serrate in upper 1/2	Not undulate, distinctly serrate in upper 1/3
Length of petioles	1.3–2 cm	1–1.6 cm		1.2–2(–2.9) cm	0.8–1 cm				1–1.4 cm	0.9–1.8 cm
Number of secondary veins	10–13 pairs	8–13 pairs	7–11 pairs		(9–)10–14(–16) pairs	12–14 pairs	8–13 pairs	9–12(–14) pairs	(7–)10–13 pairs	10–12 pairs
Cupule shape	Obconical	Cup-shaped			Obconical	Bowl-shaped	Cup-shaped			
Cupule coverage	Enclosing 1/3 of the nut	Enclosing <1/2 of the nut		Enclosing 2/3 of the nut	Enclosing 1/3–1/2 of the nut	Enclosing 2/3 of the nut		Enclosing 1/2 of the nut	Enclosing 1/3 of the nut	
Cupule bract	5–6 rings	6 rings	7–8 rings	6–8 rings		Distinctly toothed in all rings		5–6 rings	6–9 rings	
Cupule bract margin	Entire	Sparsely dissected in the lower rings	Distinctly toothed in two lower rings		Undulate			Nearly entire, not undulate		
Nut shape	Ovoid	Subglobose		Ovoid			Ovoid to ellipsoid			
Nut scar	Convex		Flat		Convex		Convex			
Nut hairiness	Glabrous	Densely hairy			Sparsely hairy		Densely hairy	Sparsely hairy		Densely hairy

as *Q. baolamensis*, *Q. bidoupensis* and *Q. honbaensis*. In this study, only sterile specimens of *Q. donnaiensis* were collected and the sequence of ITS for *Q. donnaiensis* could not be determined due to low DNA quality. In the MIG-seq tree, *Q. donnaiensis* and *Q. camusiae* were monophyletic with a bootstrap value of 64 %. In vegetative traits, *Q. donnaiensis* is distinguished from *Q. camusiae* in having distinct serrations on the upper 1/3 of the leaf margin (vs. almost entire in *Q. camusiae*). Additional materials having fruits need to be examined to conclude whether those two are distinct species or infraspecific taxa. In the following taxonomic section, they are tentatively treated as two species. Amongst species treated as synonyms of *Q. langbianensis* s. lat. (Plant List 2013), *Q. dilacerata* is morphologically distinct as described in the following taxonomic section, but DNA samples of this species could not be obtained. Further studies using phylogenetic analyses are required to clarify the identity of *Q. dilacerata*.

While *Q. cambodiensis* is treated as a synonym of *Q. auricoma* by Tagane et al. (2017), those two species are not sister to each other in both Bayesian and MIG-seq trees. The treatment of Tagane et al. (2017) is based on the broad concept of *Q. auricoma* adopted in the Flora of Thailand (Phengklai 2008) in which a species morphologically similar to *Q. cambodiensis* in northern and north-eastern Thailand is treated as *Q. auricoma*. However, after examining the collection of *Q. auricoma* from Son Tra (V3135, V3138) that is morphologically identical with the species of the type specimen of *Q. auricoma*, it is concluded that the species treated as *Q. auricoma* in the Flora of Thailand (Phengklai 2008) is different from genuine *Q. auricoma*, in that leaves are serrate along the upper 1/2–1/3 margin (vs. completely entire in *Q. auricoma*), nuts ovoid to oblong (vs. suborbicular) and cupules densely hairy (vs. less hairy). As far as is known, *Q. auricoma* is endemic to Vietnam. Further studies are needed to elucidate the identity of the species called “*Q. auricoma*” in Thailand.

Key to the species of *Quercus langbianensis* complex in Vietnam and Cambodia

- 1 Leaves undulate, distinctly serrate in the upper 1/2. Cupules obconical, enclosing 1/3 of the nut, bracts set in 5–6 rings, margin entire. Nut ovoid, scar convex ***Q. bidoupensis***
- Leaves not undulate **2**
- 2 Leaves almost entire or with a few low teeth. Cupule cup-shaped, enclosing <1/2 of nut, bracts set in 6–8 rings, margin undulate at least in the lower rings. Nut subglobose, scar convex or flat..... **3**
- Leaves distinctly serrate in the upper 5/6 to 1/3 of margin..... **4**
- 3 Cupule distinctly narrowed at base, bracts set in 6 rings, sparsely dissected in the lower rings. Nut scar convex ***Q. camusiae***
- Cupule not distinctly narrowed at base, bracts set in 7–8 rings, margin distinctly toothed in two lower rings. Nut scar flat ***Q. cambodiensis***
- 4 Cupule obconical **5**
- Cupule cup-shaped or bowl-shaped **6**

- 5 Margin distinctly serrate in the upper 1/3; secondary veins 7–11 pairs; petioles 1.2–2(–2.9) cm long; Cupules enclosing 2/3 of the nut. Nut ovoid *Q. baniensis*
- Margin distinctly serrate in the upper 5/6–3/4(–2/3); secondary veins (9–)10–14(–16) pairs; petioles 0.8–1 cm long. Cupules enclosing 1/3–1/2 of the nut. Nut obovoid to ellipsoid..... *Q. honbaensis*
- 6 Cupule bowl-shaped, enclosing about 2/3 of nut, bracts set in 7 rings, bract margin distinctly toothed in all rings. Nut subglobose *Q. dilacerata*
- Cupule cup-shaped, enclosing 1/3–2/3 of nut, bracts set in 5–9 rings, bract margin nearly entire.....7
- 7 Cupules enclosing 2/3 of the nut. Nut ovoid. Young shoots covered with straight whitish hairy. Leaves distinctly serrate in the upper 1/3... *Q. blaoensis*
- Cupules enclosing 1/3–1/2 of the nut. Nut subglobose, ovoid-ellipsoid, obovoid to ellipsoid. Young shoots covered with golden tomentose or almost glabrous. Leaves regularly distinctly serrate in the upper 1/3–1/28
- 8 Cupules enclosing 1/2 of the nut, bracts set in 5–6 rings. Leaves regularly distinctly serrate in the upper 1/3. Nut subglobose, scar convex..... *Q. donnaiensis*
- Cupules enclosing 1/3 of the nut, bracts set in 6–9 rings. Leaves regularly distinctly serrate in the upper 1/2 or upper 1/3. Nut scar flat or convex.....9
- 9 Leave regularly distinctly serrate in the upper 1/2; petiole 0.4–1 cm long. Nut ovoid–ellipsoid, scar flat, sparsely hairy *Q. baolamensis*
- Leave regularly distinctly serrate in the upper 1/3; petiole 1–2 cm long. Nut obovoid to ellipsoid, scar convex, densely hairy..... *Q. langbianensis* s. str.

Taxonomic treatments of *Quercus langbianensis* complex in Vietnam and Cambodia

Quercus baniensis A.Camus

Fig. 5

Quercus baniensis A.Camus, Chênes Atlas 2: 123, pl. 231 (1935–1936), nom. nud.; Bull. Soc. Bot. France 83: 343 (1936).

Type. VIETNAM. “Mont Bani, in the main coast range about 25 kilometres from Tourane”, 4–13 June, 1927, *J. & M.S. Clemens* 3455 (lectotype: P [P00753998!]; isolectotype: BM [BM000839274, BM000839274, image!], MICH [MICH1210512, image!], U [U0238780, image!], US [US00089422, image!], designated here).

Distribution and habitat. VIETNAM. Da Nang Province: Ba Na Nature Reserve. In this study, this species was found along the roadside and edge of evergreen forest, at 707 and 789 m altitude.

Additional specimen examined. VIETNAM. Ba Na Nature Reserve, 16°00'07.30"N, 108°01'33.90"E, alt. 707 m, 29 May 2015, *Tagane S., Toyama H., Nguyen N., Nguyen C.*

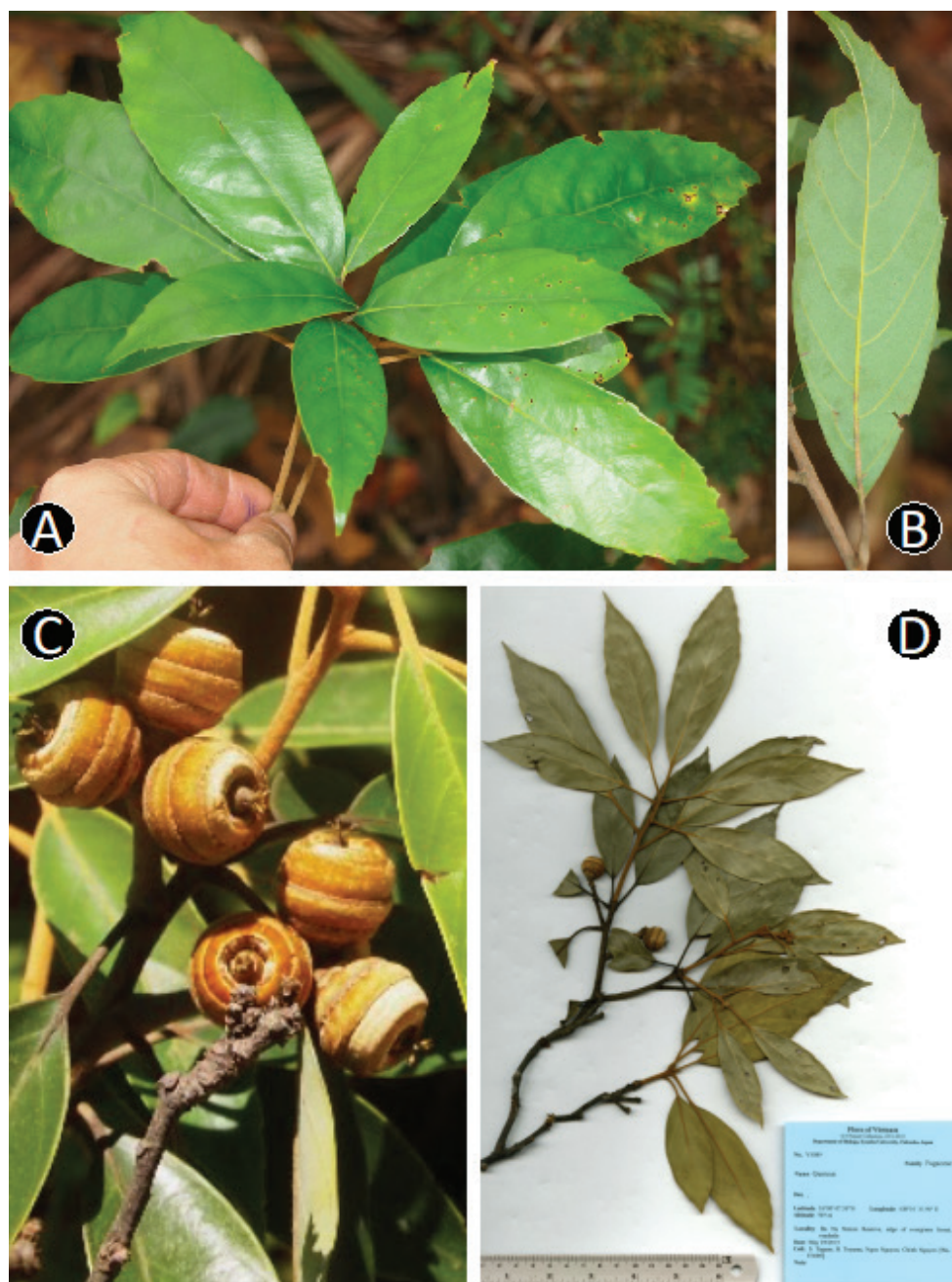


Figure 5. *Quercus baniensis* A.Camus. **A** Leafy twig **B** Abaxial side of mature leaf **C** Infructescence and young fruits **D** Dried specimen. Materials: **A, B** from Hoang T.S. & Tagane S. V6922 **C, D** from Tagane et al. V3089.

V3089 [young fr.] (DLU, FU); *ibid.*, 16°00'10.0"N, 108°02'17.8"E, alt. 789 m, 19 Feb. 2017, *Hoang T.S. & Tagane S. V6922* (DLU, FU).

Note. Camus (1935) illustrated *Quercus baniensis* in Chênes Atlas 2 (Pl.231) and later Camus (1936) effectively described this species based on the specimen *Clemens 3455* collected from mountain Bani, Vietnam. The authors examined six specimens of *Clemens 3455* in P, BM, MICH, U and US directly or by using digitised images on the web. Amongst them, *Clemens 3455* in P [P00753998] was selected as the lectotype of *Q. baniensis* because the trait of a nut is well represented in this specimen.

***Quercus baolamensis* Binh & Ngoc, sp. nov.**

urn:lsid:ipni.org:names:77175738-1

Fig. 6

Diagnosis. *Quercus baolamensis* is most similar to *Q. langbianensis* s. str., but differs in having the leaf margin regularly distinctly serrate in the upper 1/2 (vs. serrate in the upper 1/3) and shorter petioles 0.4–1 cm long (vs. 1–1.8 cm long).

Type. VIETNAM. Lam Dong Province: Bao Lam District, B40 Pass, roadside and edge of evergreen forest, 11°43'37"N, 107°42'34.5"E, alt. 1,000 m, 13 June 2015, with fruits, *Ngoc N.V., Binh H.T., Dung L.V., Truong N.K. V3191* (holotype: KYO!; isotypes: FU!).

Description. Tree, 6–8 m tall. Young twigs almost glabrous except near bud, 1–1.2 mm in diam., sometimes sulcate. Old twigs glabrous, brownish-black when dry, lenticellate. Stipules linear, 3–5 mm long, hairy on both surfaces, caducous. Leaf blades elliptic to elliptic-lanceolate or rarely oblanceolate, (5.2–)9–15 × 1.7–4.5 cm, thinly coriaceous, glossy adaxially, pale green abaxially, acuminate at apex, cuneate at base, margin regularly distinctly serrate in the upper 1/2, having 9–12 teeth per side, glabrous on both surfaces; midrib slightly prominent adaxially, prominent abaxially, lateral veins (7–)10–13 pairs, straight and running into the teeth of margin, slightly prominent adaxially, prominent abaxially, at an angle of 40–45 degrees from midrib, tertiary veins scalariform-reticulate, visible on both surfaces; petioles 0.4–1 cm long, whitish hairy when young, glabrescent. Male and female inflorescences not seen. Infructescences axillary or terminal, erect spike, rachis 0.5–1.4 cm long, 1–3 mm in diam., tomentose when young, glabrescent when mature. Mature fruits ca. 2.9 cm high (including cupule), usually 1 (or 2) per infructescence, sessile; cupules obconical, 1.2 cm high, 1.5 cm in diam., enclosing 1/3 of the nut, wall comprising bracts, arranged in 7 rings, margin of rings nearly entire; nut ovoid-ellipsoid, 2.5 cm high, 1.5 cm in diam., apex nearly flat, sparsely hairy except densely appressed hairy around stylopodia, stylopodia up to 4 mm long, basal scar flat, 0.8 cm in diam., glabrous.

Phenology. Fruiting specimens were collected in June.

Distribution and habitat. VIETNAM. Lam Dong Province: Bao Lam District. At present, this species is known only from the type locality. Only one individual was found along the roadside and edge of evergreen forest, at 1,000 m altitude.

Etymology. The specific epithet is derived from the name of its type locality, Bao Lam District.



Figure 6. *Quercus baolamensis* Binh & Ngoc. **A** Leafy twig **B** Abaxial side of mature leaf **C** Mature fruit **D** Inside of cupule **E** Nut. Materials: **A–E** from Ngoc *et al.* V3191.

***Quercus bidoupensis* Binh & Ngoc, sp. nov.**

urn:lsid:ipni.org:names:77175739-1

Fig. 7

Diagnosis. Similar to *Quercus langbianensis* s. str. in leaf shape, the number of secondary veins and basal scar of the nut convex, but distinguished in having bud oblong to ellipsoid (vs. globose to broadly ovoid), undulate and distinctly serrate leaf margin along the upper half (vs. regularly distinctly serrate in the upper 1/3), obconical cupules (vs. cup-shape), bracts of cupule arranged in 5–6 rings (vs. 6–9 rings), and nut ovoid (vs. obovoid to ellipsoid).

Type. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, hill evergreen forest dominated by the species of Fagaceae, 12°09'52.95"N, 108°32'00.38"E,

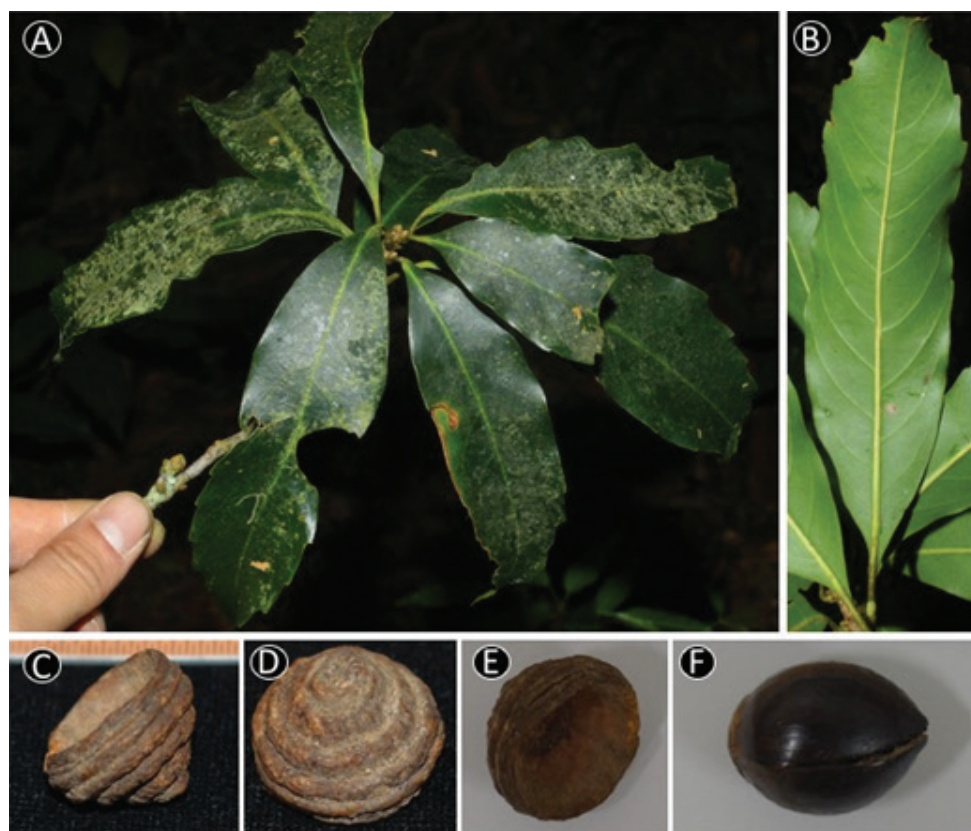


Figure 7. *Quercus bidoupensis* Binh & Ngoc. **A** Leafy twig **B** Abaxial side of mature leaf **C, D** Side view and base view of the cupule, respectively **E** Inside of cupule **F** Nut. Materials: **A–F** from *Tagane et al.* V4328.

alt. 1,698 m, 24 Feb. 2016, *Tagane S., Toyama H., Nagamasu H., Naiki A., Dang Son, Nguyen V. Ngoc, Wai J.* V4328 (holotype: KYO!; isotypes: DLU!, the herbarium of Bidoup-Nui Ba National Park).

Description. Tree, 8 m tall. Buds oblong to ellipsoid, ca. 2–4 mm high, ca. 1–2 mm in diam., scales 6–7 rows, imbricate, ovate-triangular, ca. 3×2.5 mm, apex obtuse, margin ciliate, densely hairy or glabrous outside, glabrous inside. Twigs greyish, glabrous, lenticellate. Leaf blades oblong-lanceolate, (7.5–)10–13 \times 2.5–4 cm, thinly coriaceous, blackish-brown adaxially, pale brown abaxially when dry, glabrous on both surfaces, acuminate at apex, cuneate at base, margin undulate, distinctly serrate in the upper 1/2; midrib sunken adaxially, prominent abaxially, lateral veins 10–13 pairs, slightly prominent adaxially, prominent abaxially, at an angle of 45–50 degrees from midrib and running into the teeth of margin, tertiary veins scalariform-reticulate, slightly prominent, visible on both surfaces; petioles 1.3–2 cm long, blackish when dry, glabrous. Male and female inflorescences and

infructescences not seen. Fruits 2.6 cm high (including cupule); cupules obconical, 1.3–1.5 cm high, 1.3–1.7 cm in diam., enclosing 1/3 of nut when mature, outside tomentose with whitish hairs to glabrous, inside villous with erect whitish hairs, wall ca. 2–3 mm thick, bracts arranged in 5–6 rings, margin of rings entire (without scale-like structure); nut ovoid, 2.2 cm high, 1.4 cm in diam., blackish, apex acute, basal scar 0.9 cm in diam., convex, glabrous. Fruits characters were obtained from the fallen materials.

Phenology. Unknown. Fallen fruits were collected in February.

Distribution and habitat. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park. At present, this species is known only from the type locality.

Additional specimens examined. Vietnam. Lam Dong Province, Lan Tranh, 12°04'08.5"N, 108°21'55.5"E, alt. 1,695 m, 18 June 2015, *N. Nguyen, D. Luong, B. Hoang V3202* (DLU, FU).

Etymology. The specific epithet "*bidoupensis*" is derived from its type locality.

Quercus blaoensis A.Camus

Fig. 8

Quercus blaoensis A.Camus, Chênes Atlas 2: 121, pl. 229 (1935–1936), nom. nud.; Les Chênes 1: 293 (1935).

Type. VIETNAM. "Station agricole de Blao, province du haut Donai", 800 m, 25 Apr. 1933, *E. Poilane 22372* (lectotype: P [P00754000!]; isolectotypes: P [P00753999!], K [K000832201, K000832202, K000832203, K000832204, image!], G [G00358072, image!], designated here).

Distribution and habitat. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. This species was found in hill evergreen forest at 225 m and 1067 m altitude.

Additional specimens examined. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, *Toyama H., Dang V Son, Tagane S., Fuse K., Yahara T., Nagamasu H., Hop Tran V1366* (FU, VNM, the herbarium of Hon Ba Nature Reserve); Kon Tum Province: Ngoc Linh Nature Reserve, 15°10'05.7"N, 107°45'23.6"E, alt. 1067 m, 11 Feb. 2017, *Tagane S., Nagamasu H., Nguyen N., Hoang B., Hoang S., Yang CJ. V6136* (DLU, FU, the herbarium of Ngoc Linh Nature Reserve).

Note. Camus (1935) described *Quercus blaoensis* based on the specimen *Poilane 22372* from Vietnam. The authors examined specimens of *Poilane 22372* in P ([P00754000], [P00753999]) and the digitised images of the specimens in K ([K000832201, K000832202, K000832203, K000832204]) and G [G00358072]. Amongst them, only two specimens in P are fertile and only P00754000 represents diagnostic traits of a nut with cupule. Thus, the specimen *Poilane 22372* deposited in P [P00754000] was selected as the lectotype for *Q. blaoensis*.

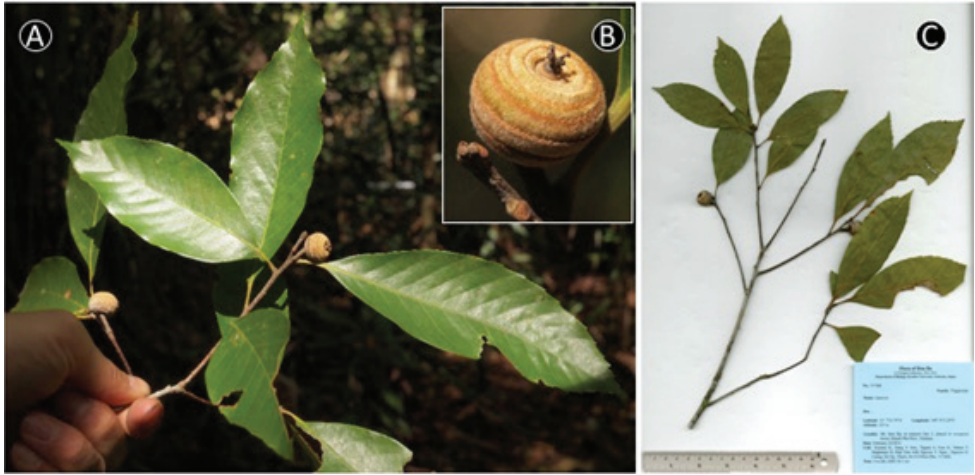


Figure 8. *Quercus blaoensis* A.Camus **A.** Branch with fruits **B** Young fruit **C** Dried specimen Materials: **A–C** from Toyama et al. V1366.

Quercus cambodiensis Hickel & A.Camus

Fig. 9

Quercus cambodiensis Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat. 29: 600 (1923); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 946 (1929). *Quercus auricoma* auct. non A.Camus; Tagane et al., Tree Fl. Bokor National Park: 277 (2017).

Type. CAMBODIA. “Mont. De Elephant, sol argileux tourbeu”, 1,000 m, 6 Aug. 1919, *E. Poilane 215* (lectotype: P [P00379257!]; isolectotypes: P [P00379258!], NY [00253790, image!], designated here).

Distribution and habitat. CAMBODIA. Kampot Province, Bokor National Park. *Quercus cambodiensis* is common in moist evergreen forest on the top plateau of Mt. Bokor.

Additional specimens examined. CAMBODIA. Kampot Province, Bokor National Park: 10°37'32.30"N, 104°05'15.84"E, alt. 844 m, 17 Oct. 2012, *Tagane S., Fuse K., Choeung HN. C4302* [fr.] (FU, the herbarium of Forest Administration of Cambodia); 10°37'15.48"N, 104°05'10.71"E, 888 m, 9 Dec. 2011, *Toyama H., Tagane S., Ide T., Phourin C., Nagamasu H., Yahara T. 1834* [fr.] (FU, the herbarium of Forest Administration of Cambodia); 10°38'12.59"N, 104°02'06.37"E, 1014 m, 4 Dec. 2011, *Toyama H., Tagane S., Kajisa T., Sakata K., Nobayashi M., Mihara N., Ide T., Chhang P., Nagamasu H. 1458 & 1541* (FU, the herbarium of Forest Administration of Cambodia); ; 10°37'16.77"N, 104°01'52.32"E, 1043 m, 17 Dec. 2013, *Toyama H., Fuse K., Iwanaga F., Rueangruea S., Suddee S., Kim W., Loth M. 6276* (FU, the herbarium of Forest Administration of Cambodia); 10°38'12.59"N, 104°02'06.37"E, 1000 m, 12 Dec. 2013, *Fuse K., Suddee S., Rueangruea S., Iwanaga F., Loth M. Fuse K. 6342* (FU, the herbarium of Forest Administration of Cambodia).

Note. Hickel and Camus (1923) described *Quercus cambodiensis* Hickel & A.Camus based on two specimens collected by E. Poilane (*Poilane 215* and *Poilane*

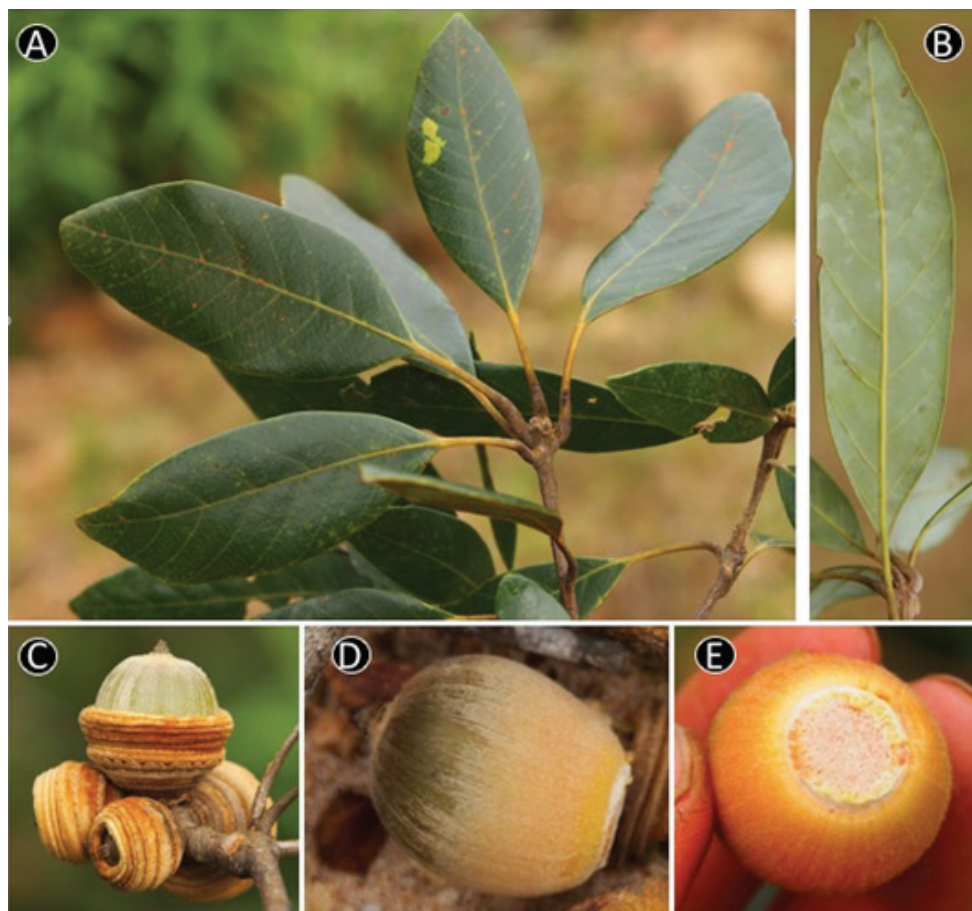


Figure 9. *Quercus cambodiensis* Hickel & A.Camus. **A** Leafy twig **B** Abaxial side of mature leaf **C** Infructescence and fruits **D** Nut **E** Basal scar of the nut. Materials: **A–E** from Tagane *et al.* 4302.

270) from Cambodia. Deng *et al.* (2010) selected *Poilane 215* as the lectotype for *Q. cambodiensis*. However, there are three specimens of *Poilanei 215* in P [P00379257, P00379258] and NY [00253790], amongst which only one specimen [P00379257] represents the diagnostic traits of nuts and cupules. Thus, specimen [P00379257] was selected as the lectotype of *Q. cambodiensis*.

***Quercus camusiae* Trel. ex Hickel & A.Camus**

Fig. 10

Quercus camusiae Trel. ex Hickel & A.Camus, Fl. Indo-Chine 5: 957 (1929). *Quercus geminata* Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat.: 599 (1923), nom. illegit. *Cyclobalanopsis camusiae* (Trel. ex Hickel & A.Camus) Y.C.Hsu & H.W.Jen, J. Beijing Forest. Univ. 15(4): 44 (1993).

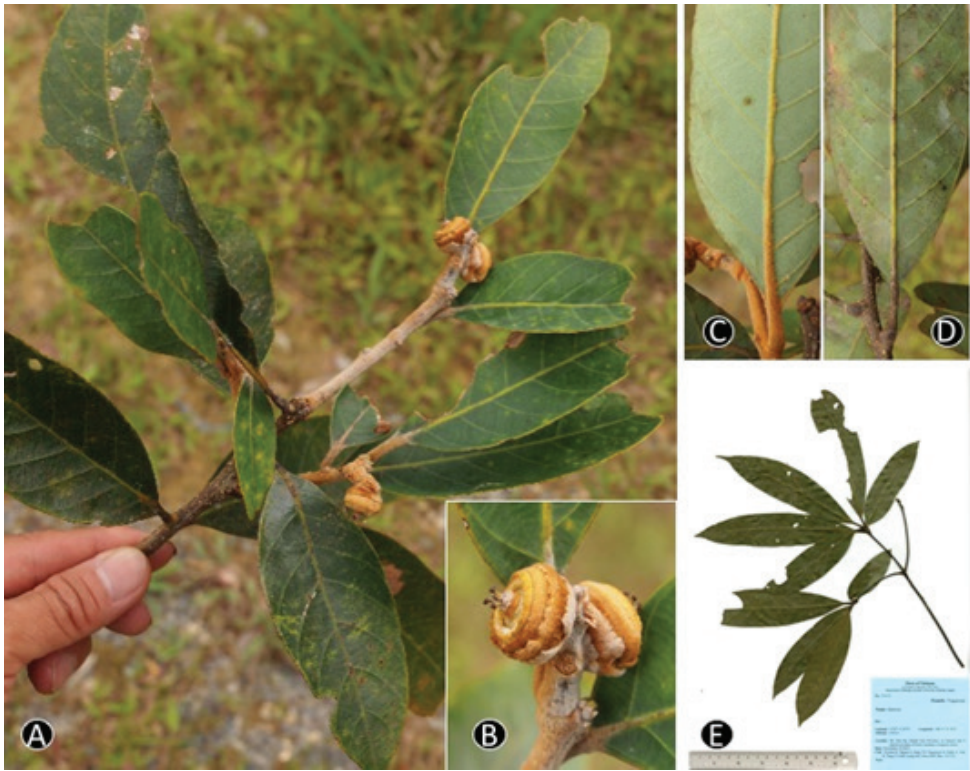


Figure 10. *Quercus camusiae* Trel. ex Hickel & A. Camus. **A** Branch with young fruit, **B**. Infructescence and young fruits **C, D** Abaxial side of young and mature leaf, **E**. Dried specimen. Materials: **A–D** from Tagane et al. V342 **E** from Toyama et al. V2173.

Type. VIETNAM. Annam [Trung Ky]: “Pres de Nha-trang, massif de Honba,” 1,000–1,500 m, 18–20 Sep. 1918, *A. Chevalier* 38650 (lectotype: P [P00379252!]; isolectotype: P [P00379253!], designated here).

Distribution and habitat. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. *Quercus camusiae* was found on slope of lower montane evergreen forest at 995 m, 1336 m and 1498 m altitude.

Additional specimens examined. VIETNAM. Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'08.64"N, 108°56'51.99"E, alt. 1,498 m, 18 July 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Son VD.* V290 (FU, the herbarium of Hon Ba NR, VNM); 12°07'10.02"N, 108°56'51.71"E, alt. 995 m, 19 July 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Son VD.* V342 [young fr.] (FU, the herbarium of Hon Ba NR, VNM); 12°07'11.42"N, 108°57'25.76"E, alt. 1336 m, 25 Nov. 2014, *Toyama H., Tagane S., Dang VS., Nagamasu H., Naiki A., Tran H., Yang CJ.* V2173 (FU, VNM, the herbarium of Hon Ba Nature Reserve).

Note. *Quercus camusiae* Trel. ex Hickel & A. Camus was described by Hickel and Camus (1929) based on a specimen collected by A. Chevalier from Vietnam (“An-

nam: Pres de Nha-trang, massif de Honba”) to replace the name *Q. geminate* Hickel & Camus (1923) without any collection number. Later, Camus (1938) cited specimen *Chevalier 38650* from Vietnam as the type specimen of *Q. camusiae*. Two specimens of *Chevalier 38650* were found in P comprising [P00379252] and [P00379253], between which only one specimen [P00379252] represents the diagnostic traits of nuts and cupules. Thus, specimen [P00379252] was selected as the lectotype of *Q. camusiae*.

***Quercus dilacerata* Hickel & A.Camus**

Quercus dilacerata Hickel & A.Camus, [P. H. Lecomte et al.] Fl. Indo-Chine 5: 960 (1929).

Type. VIETNAM. “Tonkin: Km. 8 du col de Lo qui Ho près de Chapa”, 1800 m, 29 July 1926, *E. Poilane 12645* (lectotype: P [P00753996!]; isolectotype: P [P00753997!], designated here).

Distribution and habitat. VIETNAM. Lao Cai Province: Lo Qui Ho Pass, Chapa.

Note. In the original publication of *Quercus dilacerata*, Hickel and Camus (1929) cited the specimen collected by E. Poilane from Tonkin, Km. 8 du col de Lo qui Ho près de Chapa, Vietnam without any collection number. Two specimens of *Quercus* collected by Poilane from Tonkin, Km. 8 du col de Lo qui Ho près de Chapa, Vietnam were found in P with collector’s number 12645 (P [P00753996], [P00753997]). Both specimens are fertile and consistent with the description of Hickel and Camus (1929). Here, the specimen [P00753996] with more nuts was designated as the lectotype for *Q. dilacerata*.

***Quercus donnaiensis* A.Camus**

Fig. 11

Quercus donnaiensis A.Camus, Chênes Atlas 2: 119, pl. 227 (1935–1936), nom. nud.; Les Chênes 1: 190 (1935).

Type. VIETNAM. “Annam: Près de Sapoum, près station agricole de Blao, prov. du Haut Donai”, 1000–1100 m, 9 Jan. 1935, *E. Poilanei 23732* (lectotype: P [P00753995!]; isolectotype: P [P00753994!], designated here).

Distribution and habitat. VIETNAM. Lam Dong Province: Lan Tranh wards. In this study, *Q. donnaiensis* was found in lower montane evergreen forest, beside a stream at 1489 m altitude and at the edge of evergreen forest, at 1695 m altitude.

Additional specimens examined. VIETNAM. Lam Dong Province, Lan Tranh wards, 12°04'08.5"N, 108°21'55.5"E, alt. 1,695 m, 18 June 2015, *N. Nguyen, D. Luong, B. Hoang V3208* (DLU, FU); Lam Dong Province, Bi Doup-Nui Ba National Park, 12°11'19.8"N, 108°40'48.3"E, alt. 1,489 m, 25 Feb. 2016, *Tagane S., Wai J. V4398* (FU, DLU, the herbarium of Bidoup-Nui Ba National Park).

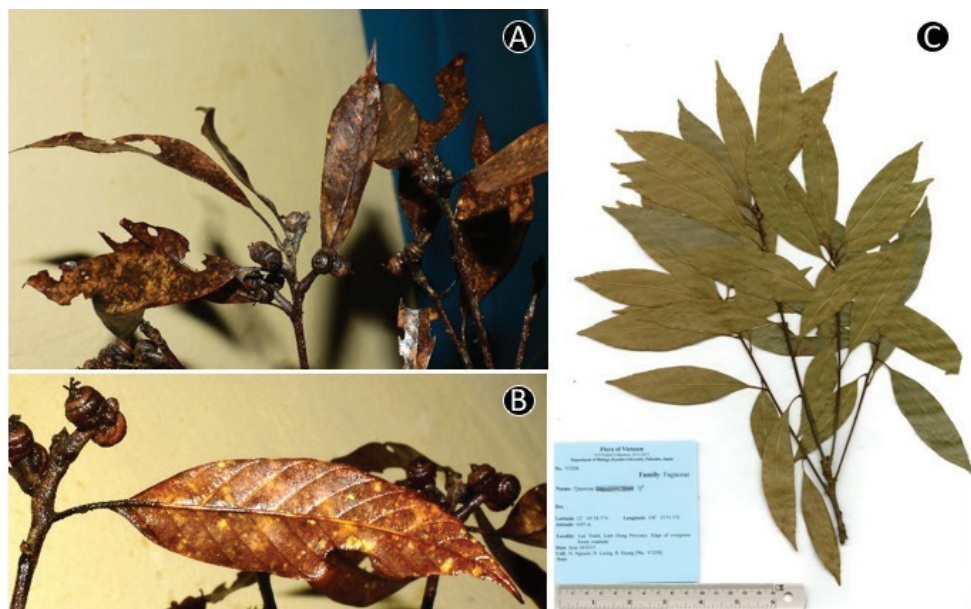


Figure 11. *Quercus donnaiensis* A.Camus. **A** Leafy twig **B** Infructescence, young fruits and abaxial side of mature leaf **C** Dried specimen. Materials: **A, B** from Tagane S., Wai J. V4398 **C** from Ngoc et al. V3208.

Note. *Quercus donnaiensis* was described by Camus (1935), based on the specimen *Poilanei* 23732 collected from Vietnam and then illustrated by Camus (1935–1936). Two specimens of *Poilanei* 23732 deposited in P have acorns and are consistent with the illustration and description of Camus (1935a, b). Amongst them, the specimen *Poilane* 23732 in P [P00753995] with nuts, as it better represents the diagnostic traits, was designated as the lectotype of *Q. donnaiensis*.

***Quercus honbaensis* Binh, Tagane & Yahara, sp. nov.**

urn:lsid:ipni.org:names:77175740-1

Fig. 12

Diagnosis. *Quercus honbaensis* is distinguished from *Q. langbianensis* s. str. in having shorter petiole of 0.8–1 cm long (vs. 1–1.8 cm long), more secondary veins ((10–)14–16 pairs vs. 10–12 pairs) and obconical cupules (vs. cup-shaped).

Type. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, evergreen forest along river, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, Toyama H., Dang V Son., Tagane S., Fuse K., Yahara T., Nagamasu H., Hop Tran V1378 (holotype: KYO!; isotypes: FU!, VNM!).

Description. Tree, 12 m tall. Buds ovoid, ca. 3–4 mm high, ca. 2–3 mm in diam., scales in 4–6 rows, imbricate, ovate-triangular, ca. 1 × 1.5 mm, apex obtuse, margin yel-

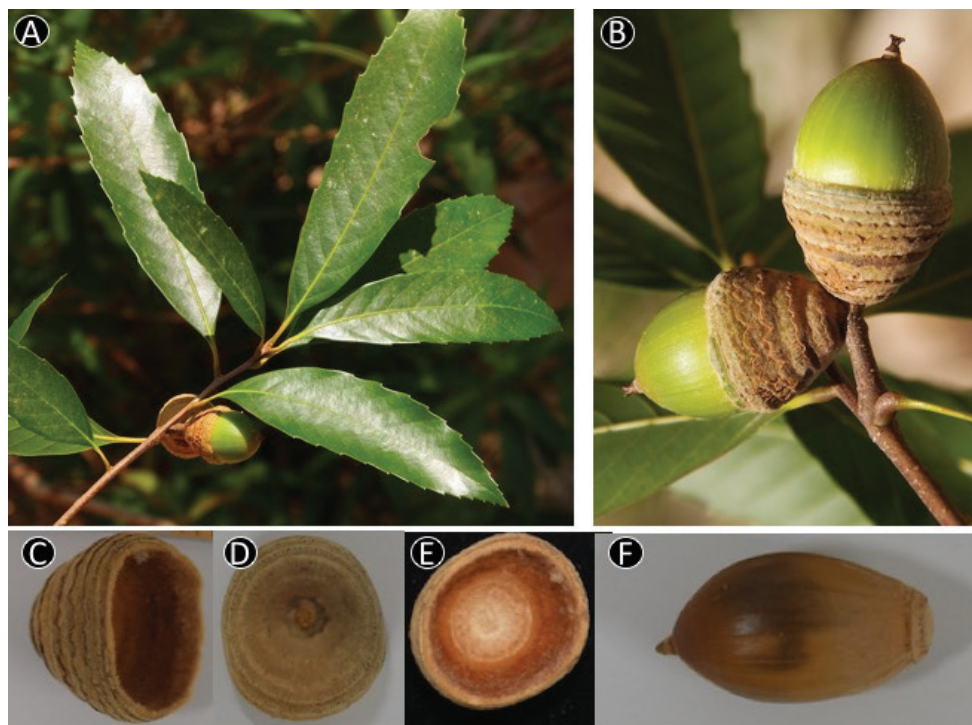


Figure 12. *Quercus honbaensis* Binh, Tagane & Yahara. **A** Leafy twig **B** Infructescence and mature fruits, **C, D** Side view and base view of the cupule, respectively, **E**. Inside of cupule, **F**. Nut. Materials: **A–F** from Toyama *et al.* V1378.

lowish-brown ciliate, appressed hairy on both surfaces. Young twigs greyish-brown, 1–1.2 mm in diam., curly hairy, sometimes sulcate, old twigs greyish-brown, glabrous, lenticellate. Leaf blades lanceolate to oblanceolate, (3.6–)11–16.5 × (1.4–)2–5.2 cm, acute at apex, cuneate at base, margin regularly distinctly serrate in the upper 5/6–3/4(–2/3), glabrous on both surfaces; midrib slightly prominent adaxially, prominent abaxially, lateral veins (9–)10–14(–16) pairs, straight and running into the teeth of margin, slightly prominent adaxially, prominent abaxially, at an angle of 40–45 degrees from midrib, tertiary veins scalariform-reticulate, faintly visible on both surfaces; petioles 0.8–1 cm long, tomentose when young, soon glabrous. Male and female inflorescences not seen. Infructescences axillary, erect, rachis 0.5–1.4 cm long, 1–2 mm in diam., glabrous. Mature fruits 2–3.5 cm high (including cupule), usually 1 (or 2) per infructescence, sessile; cupules obconical, 1.4–1.6 cm high, 1.5–1.8 cm in diam., enclosing 1/3–1/2 of the nut, wall covered with densely whitish- to yellowish-brown hairs, bracts arranged in 6–8 rings, margin of the ring undulate; nut obovoid to ellipsoid, 2.3–2.8 cm high, 1.3–1.7 cm in diam., apex obtuse, sparsely hairy except densely appressed hairy around stylopodia and basal scar, stylopodia up to 3 mm long, basal scar 0.7–0.8 cm in diam., convex.

Phenology. Fruiting specimens were collected in February.

Distribution and habitat. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. This species is known only from the type locality. A few individuals were found in evergreen forest from 225–617 m elevation.

Additional specimens examined. VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 12°06'33.41"N, 108°59'24.89"E, alt. 367 m, 22 July 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Dang V.S.*, V744 (FU, VNM, the herbarium of Hon Ba NR); 12°06'39.77"N, 108°58'59.23"E, alt. 617 m, 22 Feb. 2014, *Toyama H., Dang V.S., Tagane S., Fuse K., Yahara T., Nagamasu H., Tran H., Nguyen V.N., Nguyen Q.C., Do N.T., Ho N.P.H.*, V1200 (FU, VNM, the herbarium of Hon Ba NR); 12°06'31.2"N, 108°59'14.1"E, alt. 400 m, 13 July 2014, *Tagane S., Kanemitsu H., Dang V.S., Tran H., Hanh N., Loi X.N., Thach N.D., Dinh N., Hieu P.N.H.*, V1548 (FU, VNM, the herbarium of Hon Ba NR); 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 15 July 2014, *Tagane S., Kanemitsu H., Dang V.S., Tran H., Loi X.N., Thach N.D., Dinh N., Hieu P.N.H.*, V1662 (FU, VNM, the herbarium of Hon Ba NR).

Etymology. The specific epithet “*honbaensis*” is derived from its type locality, Mt. Hon Ba.

Quercus langbianensis Hickel & A.Camus

Fig. 13

Quercus langbianensis Hickel & A.Camus, Ann. Sci. Nat., Bot. 10, 3: 382 (1921); [P.H. Lecomte et al.] Fl. Indo-Chine 5: 950 (1929).

Type. VIETNAM. “Annam: massif du Lang-Bian, grand Piton Lang-Bian, près du village de Beneur”, 1500–2000 m, 15 Feb. 1914, *A.J.B Chevalier 30029* (holotype: P [P00379254!]; isotypes: P [P00379255! P00379256!]).

Distribution and habitat. VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park. In this study, *Quercus langbianensis* s. str. is known only from Lang-Bian Mountain of the national park, in lower montane evergreen forest at 1472 m and 1533 m altitude.

Additional specimen examined. VIETNAM. Bidoup-Nui Ba National Park, 12°10'34.04"N, 108°40'28.93"E, alt. 1,472 m, 29 Feb. 2016, *Tagane S., Son V.D., Wai J.* V4465 (DLU, FU, the herbarium of Bidoup-Nui Ba National Park); ibid. 12°10'34.09"N, 108°41'04"E, alt. 1,533 m, 22 Feb. 2016, *Tagane S., Nagamasu H., Naiki A., Dang V. Son, Nguyen V. Ngoc., Binh T. Hoang* V4165, V4166 (DLU, FU, the herbarium of Bidoup-Nui Ba National Park); 12°10'34.7"N, 108°41'08.4"E, alt. 1,533 m, 21 Feb. 2016, *Tagane S., Nagamasu H., Naiki A., Dang V. Son, Nguyen V. Ngoc., Binh T. Hoang* V3962 (DLU, FU, the herbarium of Bidoup-Nui Ba National Park).

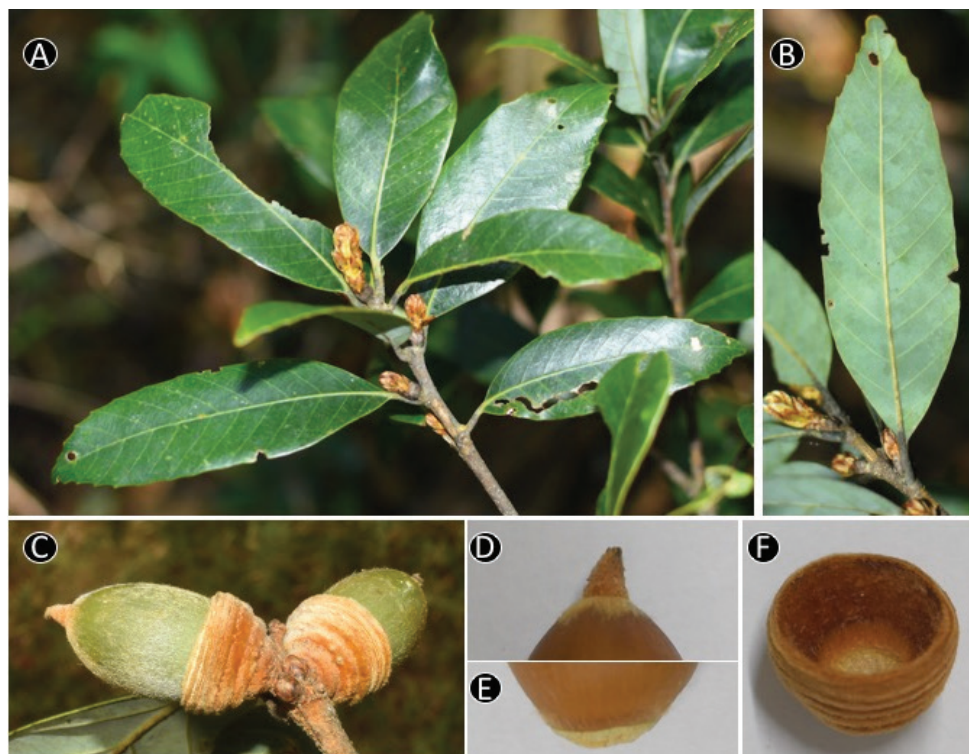


Figure 13. *Quercus langbianensis* Hickel & A.Camus. **A** Leafy twig **B** Abaxial side of mature leaf **C** Infructescence and mature fruits **D** Apex of the nut **E** Basal scar of the nut **F** Inside of cupule. Materials: **A, B** from Tagane et al. V 4165 **C–F** from Tagane et al. V4166.

Acknowledgements

We thank the curators and staff of the following herbaria DLU, FU, HN, HUH, P, PE, VNM for making their materials accessible. This study was supported by the Environment Research and Technology Development Fund (S9 & 4-1601) of the Ministry of the Environment, Japan and MEXT/JSPS KAKENHI (Grant Numbers JP15H02640 & 16H02553) and JSPS Core-to-Core Program, A. Advanced Research Networks. Funding was provided to JSS through the Flore du Cambodge, du Laos et du Viêt Nam programme (MNHN, Paris & RBGE, Edinburgh) to study specimens in P.

References

Ban NT (2003) Vietnam plant checklist, Vol. 2. Agriculture Publishers, Hanoi National University. [In Vietnamese]

- Binh HT, Ngoc NV, Tai VA, Son HT, Tagane S, Yahara T (in press) *Quercus trungkhanhensis* (Fagaceae), a new species from Cao Vit Gibbon Conservation Area, Cao Bang Province, north-eastern Vietnam. *Acta Phytotaxonomica et Geobotanica*.
- Camus A (1935) Les Chênes. Monographie du Genre *Quercus* Tome 1. Paul Lechevalier. Paris, 190–293.
- Camus A (1935–1936) Les Chênes. Monographie du Genre *Quercus* Tome 2. Paul Lechevalier. Paris, 79–236.
- Camus A (1936) Quelques Fagacées nouvelles de l'Inde et de l'Indo-Chine. *Bulletin de la Société Botanique de France* 83(4–5): 343. <https://doi.org/10.1080/00378941.1936.10836359>
- Camus A (1938) Les Chênes. Monographie du Genre *Quercus* Tome 1, Paul Lechevalier. Paris.
- Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH Stacks (2011) Building and genotyping loci de novo from short-read sequences. *G3 Genes, Genomes, Genetics* 1(3): 171–182. <https://doi.org/10.1534/g3.111.000240>
- Cavender-Bares J, Gonzalez-Rodriguez A, Eaton DAR, Hipp AAL, Beulke A, Manos PS (2015) Phylogeny and biogeography of the American live oaks (*Quercus* subsection *Virentes*): a genomic and population genetics approach. *Molecular Ecology* 24(14): 3668–3687. <https://doi.org/10.1111/mec.13269>
- Cuénoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* 89(1): 132–144. <https://doi.org/10.3732/ajb.89.1.132>
- Deng M, Zhou ZK, Coombes A (2010) Lectotypification and new synonymy in *Quercus* subg. *Cyclobalanopsis* (Fagaceae). *Novon: A Journal for Botanical Nomenclature* 20(4): 400–405. <https://doi.org/10.3417/2004208>
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Drummond AJ, Rambaut A (2007) Beast: Bayesian evolutionary analysis by sampling trees. *BMC evolutionary biology* 7(1): 214. <https://doi.org/10.1186/1471-2148-7-214>
- Drummond AJ, Suchard MA, Dong Xie, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular biology and evolution* 29(8): 1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Dunning LT, Savolainen V (2010) Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164(1): 1–9. <https://doi.org/10.1111/j.1095-8339.2010.01071.x>
- Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics* 10(3): 315–319. <https://doi.org/10.1111/j.1096-0031.1994.tb00181.x>
- Fay MF, Swensen SM, Chase MW (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin*, 111–120. <https://doi.org/10.2307/4117844>
- Fitz-Gibbon S, Hipp A, Pham K, Manos P, Sork VL (2017) Phylogenomic inferences from reference-mapped and de novo assembled short read sequence data using RADseq sequencing of California white oaks (*Quercus* subgenus *Quercus*). *Genome* 60(9): 743–755. <https://doi.org/10.1139/gen-2016-0202>

- Ford CS, Ayres KL, Toomey N, Haider N, Van Alphen Stahl J, Kelly LJ, Cowan RS (2009) Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159(1): 1–11. <https://doi.org/10.1111/j.1095-8339.2008.00938.x>
- Hickel MR, Camus A (1921) Les Chênes d'Indo-Chine. *Annales des Sciences Naturelles, Series* 10(3): 377–409.
- Hickel MR, Camus A (1923) Fagacées nouvelles Hipd'Indo-China: Genre *Quercus* L. *Bulletin du Muséum national d'histoire naturelle* 29: 598–601.
- Hickel MR, Camus A (1929) Fagaceae. In: Lecomte H (Ed.) *Flore générale de l' Indo-Chine*. Paris, volume 5, 937–1033.
- Hipp AL, Eaton DA, Cavender-Bares J, Fitzek E, Nipper R, Manos PS (2014) A framework phylogeny of the American oak clade based on sequenced RAD data. *PLoS One* 9(4): e93975. <https://doi.org/10.1371/journal.pone.0093975>
- Ho PH (2003) *An Illustrated Flora of Vietnam*, Vol. 2. Young Publishers, Ho Chi Minh City. [In Vietnamese]
- Huang CJ, Zhang YT, Bartholomew B (1999) Fagaceae. In: Zhengyi W, Raven PH, Deyuan H (Eds) *Flora of China*. Volume 4, 333–369. <http://www.eoras.org>
- Hubert F, Grimm GW, Joussetin E, Berry V, Franc A, Kremer A (2014) Multiple nuclear genes stabilize the phylogenetic backbone of the genus *Quercus*. *Systematics and Biodiversity* 12(4): 405–423. <https://doi.org/10.1080/14772000.2014.941037>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjurjo O, Bermingham E (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences of the United States of America* 106(44): 18621–18626. <https://doi.org/10.1073/pnas.0909820106>
- Lassmann T, Hayashizaki Y, Daub CO (2009) TagDust—a program to eliminate artifacts from next generation sequencing data. *Bioinformatics* 25(21): 2839–2840. <https://doi.org/10.1093/bioinformatics/btp527>
- Levin RA, Wagner WL, Hoch PC, Nepokroeff M, Pires JC, Zimmer EA, Sytsma KJ (2003) Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany* 90(1): 107–115. <https://doi.org/10.3732/ajb.90.1.107>
- Nixon KC (1993) Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. *Annales des Sciences Forestières* 50: 25s–34s. <https://doi.org/10.1051/forest:19930701>
- Phengklae C (2008) Fagaceae. *Flora of Thailand* 9(3): 179–410.
- Rohwer JG, Li J, Rudolph B, Schmidt SA, van der Wer H, Li HW (2009) Is *Persea* (Lauraceae) monophyletic? Evidence from nuclear ribosomal ITS sequences. *Taxon* 58(4): 1153–1167.
- Shimada MK, Nishida T (2017) A modification of the PHYLIP program: A solution for the redundant cluster problem, and an implementation of an automatic bootstrapping on trees inferred from original data. *Molecular Phylogenetics and Evolution* 109: 409–414. <https://doi.org/10.1016/j.ympev.2017.02.012>

- Simeone MC, Grimm GW, Papini A, Vessella F, Cardoni S, Tordoni E, Piredda R, Franc A, Denk T (2016) Plastome data reveal multiple geographic origins of *Quercus* Group Ilex. *PeerJ* 4: e1897. <https://doi.org/10.7717/peerj.1897>
- Suyama Y, Matsuki Y (2015) MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Scientific Reports* 5: 16963. <https://doi.org/10.1038/srep16963>
- Swofford DL (2003) PAUP*: phylogenetic analysis using parsimony, version 4.0 b10.
- Tagane S, Toyama H, Fuse K, Chhang P, Naiki A, Nagamasu H, Yahara T (2017) A picture guide of forest trees in Cambodia IV– Bokor National Park. Center for Asian Conservation Ecology, Kyushu University, Fukuoka, Japan, 776 pp. <https://sites.google.com/site/pictureguides/home/cambodia/bokornational-park> [accessed 1 May 2017].
- The Plant List (2013) Version 1.1. Published on the Internet. <http://www.theplantlist.org/> [accessed 15th June, 2017]
- Toyama H, Kajisa T, Tagane S, Mase K, Chhang P, Samreth V, Ma V, Sokh H, Ichihashi R, Onoda Y, Mizoue N, Yahara T (2015) Effects of logging and recruitment on community phylogenetic structure in 32 permanent forest plots of Kampong Thom, Cambodia. *Philosophical Transactions of the Royal Society B: Biological Sciences* 370(1662): 20140008. <https://doi.org/10.1098/rstb.2014.0008>
- Valencia-A S, Rosales JLS, Arellano OJS (2016) A new species of *Quercus*, section *Lobatae* (Fagaceae) from the Sierra Madre Oriental, Mexico. *Phytotaxa* 269(2): 120–126. <https://doi.org/10.11646/phytotaxa.269.2.5>
- Zhang M, Tagane S, Toyama H, Kajisa T, Chhang P, Yahara T (2016) Constant tree species richness along an elevational gradient of Mt. Bokor, a table-shaped mountain in southwestern Cambodia. *Ecological Research* 31(4): 495–504.

Supplementary material I

Figure S1, S2

Authors: Hoang Thi Binh, Nguyen Van Ngoc, Shuichiro Tagane, Hironori Toyama, Keiko Mase, Chika Mitsuyuki, Joeri Sergej Strijk, Yoshihisa Suyama, Tetsukazu Yahara
Data type: Phylogenetic trees

Explanation note: Figure S1. Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on ITS sequences. Branches are labeled with posterior probabilities. Figure S2. Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on concatenated *rbcL* and *matK* sequences. Branches are labeled with posterior probabilities.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/phytokeys.95.21126.suppl1>

Appendix I

Voucher information and GenBank accession numbers for samples used in this study (newly sequenced data).

Species	Vouchers	Elevation	GenBank accession no.			MIG-seq
			<i>rbcL</i>	<i>matK</i>	ITS	
<i>Quercus annulata</i>	Tagane et al. V4730	1850 m	LC318796	LC318516	MF770291	+
<i>Quercus auricoma</i>	Ngoc et al. V3135	518 m	LC318778	LC318498	MF770277	+
<i>Quercus auricoma</i>	Ngoc et al. V3138	556 m	LC318779	LC318499	–	+
<i>Quercus austrocochinchinensis</i>	Ngoc et al. V3129	249 m	LC318777	LC318497	MF770276	+
<i>Quercus austrocochinchinensis</i>	Ngoc et al. V3156	340 m	LC318780	LC318500	MF770278	+
<i>Quercus baniensis</i>	Tagane et al. V3089	707 m	LC318775	LC318495	MF770274	+
<i>Quercus baniensis</i>	Hoang & Tagane V6922	789 m	LC318802	LC318522	MF770296	+
<i>Quercus baolamensis</i>	Ngoc et al. V3191	1000 m	LC318782	LC318502	MF770280	+
<i>Quercus bidoupensis</i>	Ngoc et al. V3202	1695 m	LC318783	LC318503	–	–
<i>Quercus bidoupensis</i>	Tagane et al. V4328	1698 m	LC318793	LC318513	MF770288	+
<i>Quercus blaensis</i>	Toyama. et al. V1366	225 m	LC318768	LC318488	MF770269	+
<i>Quercus blaensis</i>	Tagane et al. V6136	1067 m	LC318799	LC318519	–	+
<i>Quercus braianensis</i>	Ngoc et al. V3219	1535 m	LC318785	LC318505	MF770281	+
<i>Quercus braianensis</i>	Ngoc et al. V4445	1464 m	LC318794	LC318514	MF770289	+
<i>Quercus cambodiensis</i>	Tagane et al. 1834	888 m	–	–	–	–
<i>Quercus cambodiensis</i>	Tagane et al. 1458	1014 m	–	–	–	–
<i>Quercus cambodiensis</i>	Tagane et al. 1541	1014 m	–	–	–	–
<i>Quercus cambodiensis</i>	Tagane et al. 6276	1043 m	–	–	–	–
<i>Quercus cambodiensis</i>	Tagane et al. 6342	1000 m	–	–	–	–
<i>Quercus cambodiensis</i>	Tagane et al. 4302	844 m	LC318766	LC318445	MF770268	+
<i>Quercus camusiae</i>	Tagane et al. V290	1498 m	–	–	–	–
<i>Quercus camusiae</i>	Tagane et al. V342	1511 m	LC318787	LC318507	–	–
<i>Quercus camusiae</i>	Toyama. et al. V2173	1336 m	LC318773	LC318493	MF770272	+
<i>Quercus donnaiensis</i>	Ngoc et al. V3208	1695 m	LC318784	LC318504	–	+
<i>Quercus donnaiensis</i>	Tagane & Wai 4398	1489 m	–	–	–	–
<i>Quercus djiringensis</i>	Dung et al. V5537	N/A	LC318797	LC318517	MF770292	+
<i>Quercus djiringensis</i>	Dung et al. V5538	N/A	LC318798	LC318518	MF770293	+
<i>Quercus helferiana</i>	Ngoc et al. V3169	1400 m	LC318781	LC318501	MF770279	+
<i>Quercus helferiana</i>	Ngoc et al. V3244	1580 m	LC318786	LC318506	MF770282	+
<i>Quercus honbaensis</i>	Tagane et al. V744	367 m	–	–	–	–
<i>Quercus honbaensis</i>	Toyama. et al. V1200	617 m	LC318767	LC318446	–	–
<i>Quercus honbaensis</i>	Tagane. et al. V1548	400 m	LC318770	LC318490	–	–
<i>Quercus honbaensis</i>	Tagane. et al. V1662	225 m	LC318771	LC318491	–	–
<i>Quercus honbaensis</i>	Toyama. et al. V1378	225 m	LC318769	LC318489	MF770270	+
<i>Quercus kerrii</i>	Tagane et al. V6765	833 m	LC318801	LC318521	MF770295	+
<i>Quercus langbianensis</i>	Tagane et al. V3962	1533 m	LC318790	LC318510	MF770285	+
<i>Quercus langbianensis</i>	Tagane et al. V4165	1533 m	LC318791	LC318511	MF770286	+
<i>Quercus langbianensis</i>	Tagane et al. V4166	1533 m	LC318792	LC318512	MF770287	+
<i>Quercus langbianensis</i>	Tagane et al. V4465	1472 m	LC318795	LC318515	MF770290	+
<i>Quercus macrocalyx</i>	Tagane et al. V6457	1376 m	LC318800	LC318520	MF770294	+

Species	Vouchers	Elevation	GenBank accession no.			MIG-seq
			<i>rbcL</i>	<i>matK</i>	ITS	
<i>Quercus neglecta</i>	<i>Ngoc et al. V3587</i>	546 m	LC318788	LC318508	MF770283	+
<i>Quercus neglecta</i>	<i>Ngoc et al. V3788</i>	1062 m	LC318789	LC318509	MF770284	+
<i>Quercus poilanei</i>	<i>Toyama. et al. V1895</i>	1644 m	LC318772	LC318492	MF770271	+
<i>Quercus poilanei</i>	<i>Yahara et al. V2986</i>	1412 m	LC318774	LC318494	MF770273	+
<i>Quercus poilanei</i>	<i>Tagane et al. V3113</i>	1310 m	LC318776	LC318496	MF770275	+
<i>Trigonobalanus verticillata</i> (Outgroup)	<i>Ngoc et al. V5764</i>	1735 m	LC318965*	LC318549*	MF770380*	+

(+): Used for analyses in this paper; (–): Not used for analyses in this paper. (*) From GenBank.

Two new species and two new records of *Artabotrys* (Annonaceae) from Thailand

Junhao Chen¹, Piya Chalermglin², Richard M.K. Saunders¹

1 School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, P. R. China

2 Agricultural Technology Department, Thailand Institute of Scientific & Technological Research, 35 Technopolis, Liap Khlong Ha Road, Khlong Luang District, Pathum Thani Province 12120, Thailand

Corresponding author: Richard M.K. Saunders (saunders@hku.hk)

Academic editor: T. Couvreur | Received 5 January 2018 | Accepted 23 January 2018 | Published 7 February 2018

Citation: Chen J, Chalermglin P, Saunders RMK (2018) Two new species and two new records of *Artabotrys* (Annonaceae) from Thailand. *PhytoKeys* 95: 71–81. <https://doi.org/10.3897/phytokeys.95.23434>

Abstract

Two new species of *Artabotrys* are described from Thailand. *Artabotrys tanaosriensis* J.Chen, Chalermglin & R.M.K.Saunders, **sp. nov.**, is similar to *A. oblancoelatus* Craib but differs in its symmetrical, cuneate or decurrent leaf base, externally distinct outer petal blades and claws, deltoid and undulate outer petal blades, rhomboid and undulate inner petal blades and shorter, subsessile and slightly beaked monocarps. *Artabotrys spathulatus* J.Chen, Chalermglin & R.M.K.Saunders, **sp. nov.**, is most similar to *A. tanaosriensis* but differs in having flat outer petal blades, broadly rhomboid outer petal claws, broadly spathulate and strongly concave inner petal blades and strongly beaked monocarps. Two new records for the Flora of Thailand are furthermore reported here: *A. punctulatus* C.Y.Wu ex S.H.Yuan and *A. byrsophyllus* I.M.Turner & Utteridge, which were previously confused with *A. aeneus* Ast and *A. grandifolius* King, respectively. A key to *Artabotrys* species indigenous to Thailand is provided here.

Keywords

Annonaceae, *Artabotrys*, new records, new species, Thailand

Introduction

Artabotrys R.Br. is one of the largest palaeotropical genera in the Annonaceae, with ca. 105 species (Guo et al. 2017). The majority of the species occur in Asia, with only ca. 30 species in Africa (Mabberley 2008). *Artabotrys* species are woody climbers that

are unique among climbing members of the Annonaceae in possessing specialised and persistent inflorescence hooks (Fig. 2B, D) to assist climbing; these hooks are derived from peduncles and bear flowers and fruits (Posluszny and Fisher 2000). Although these inflorescence hooks allow easy recognition of the genus, identification at the species level is often challenging (Turner 2009; Turner and Utteridge 2015). The leaf lamina is often decurrent to the petiole and the midrib is often raised on the adaxial surface of leaves. The inflorescences often bear several to many flowers, but are single-flowered in some species.

The underlying floral Bauplan of *Artabotrys* species is rather uniform, with the exception of two African species, viz. *A. thomsonii* Oliv. (Le Thomas 1969) and *A. brachypetalus* Benth. (Robson 1960), which have been shown to be early divergent lineages in molecular phylogenetic reconstructions (Chen et al. unpubl.). Individual flowers possess one whorl of three sepals and two whorls of three petals each, with the sepals much smaller than the petals and with the two petal whorls subequal. Each petal has a distinct upper laminar blade and a basal concave claw, usually with a constriction at the junction between the two regions (Figs 1A, B, 2B, D). The inner petals are basally connivent, forming a dome that covers the reproductive organs, with three lateral apertures at the base of the dome, enabling pollinator access. The three inner petals abscise as a single unit after anthesis. The flowers are hermaphroditic, with numerous stamens and carpels. Each carpel contains two ovules with basal placentation. The fruits are apocarpous, with “monocarps” (derived from individual carpels after fertilisation) that are usually sessile or borne on very short stipes; the generic name is derived in part from the Greek word “*botrys*”, which alludes to its grape-like fruits.

Although *Artabotrys* is comparatively well-studied in Thailand, many names have been misapplied and several taxonomic and nomenclatural misunderstandings persist. Craib (1925) listed 11 species in his checklist of the Thai flora, viz. *A. brevipes* Craib, “*A. burmanicus* A.DC.” (a misapplied name, for which the name *A. siamensis* Miq. should be applied), *A. harmandii* Finet & Gagnep., *A. oblanceolatus* Craib, *A. hexapetalus* (L.f.) Bhandari (as “*A. odoratissimus* R.Br.”), *A. scortechinii* King, *A. siamensis*, *A. spinosus* Craib, *A. suaveolens* Blume (Blume), *A. vanprukii* Craib and *A. venustus* King. Chalermglin (2001, 2005) published a photographic guide to Thai Annonaceae that included 10 species, viz. “*A. burmanicus*” (a misapplied name), “*A. grandifolius*” (a misapplied name: see new records for Thailand below) *A. harmandii*, *A. hexapetalus*, “*A. oblanceolatus*” (a misapplied name), *A. siamensis*, *A. spinosus*, *A. suaveolens*, *A. vanprukii* and an unnamed species (which represents “true” *A. oblanceolatus*). More recently, Thongpairroj (2008) recognised 20 species in her unpublished PhD thesis, including four new records (*A. multiflorus* C.E.C.Fisch., *A. lowianus* Scort. ex King, *A. oxycarpus* King and *A. uniflorus* (Griff.) Craib) and six unnamed species. Insura (2009) subsequently recognised 15 species in his unpublished MSc thesis, of which four are new records, viz. “*A. aereus* Ast” (a misapplied name: see new records for Thailand below), “*A. blumei* Hook.f. & Thomson” (a misapplied name, for which the name *A. tipuliferus* I.M. Turner & Utteridge should be applied: Turner and Utteridge 2015), “*A. havilandii*

Ridl.” (a misapplied name, for which the name *A. oxycarpus* should be applied: Thongpairaj 2008) and *A. sumatranus* Miq. Several species cited by these authors do not occur naturally in Thailand: *A. hexapetalus* is widely cultivated in Thailand but is likely to be of South Indian or Sri Lankan origin, for example; and Craib (1925) recorded *A. scortechinii* from Langkawi, an island that was previously under Siamese rule but now part of Peninsular Malaysia. No specimens of *A. scortechinii* from Thailand or Langkawi have been seen; *A. scortechinii* is likely to be endemic to Peninsular Malaysia and Singapore (Chen et al. in press). Additionally, three other species (*A. lowianus*, *A. sumatranus* and *A. vanprukii*) are poorly known owing to the absence of flowers and/or fruits in the type specimens, limited available material to assess variation and the problematic treatment of these taxa by previous authors. It is beyond the scope of this paper to clarify these taxonomic problems, however.

Examination of herbarium specimens and fieldwork in Thailand has revealed two new *Artabotrys* species, which are formally described here. In addition, two new records for Thailand are reported and past taxonomic errors rectified. A total of 15 species are recognised (excluding the aforementioned problematic taxa) and a key to the native species of *Artabotrys* in Thailand is provided.

Materials and methods

The material studied comprises herbarium specimens of *Artabotrys* species from Thailand and neighbouring regions from the following herbaria: A, E, KUFF, KUN, L, NY, QBG, SING and US; high-resolution digital images of specimens (especially types) from JSTOR Global Plants (<https://plants.jstor.org/>) and other virtual herbarium websites; as well as fresh material collected during fieldwork in Thailand. Species delimitation was based on discontinuities (gaps) in morphological variation. The morphological gap is an indirect assessment of the underlying reproductive isolation because the lack of gene flow prevents two lineages from homogenising (Coyne and Orr 2004; Rieseberg et al. 2006). Morphological measurements were taken from dried herbarium specimens unless otherwise stated.

Taxonomy

***Artabotrys tanaosriensis* J.Chen, Chalermglin & R.M.K.Saunders, sp. nov.**

urn:lsid:ipni.org:names:77175741-1

Fig. 1

Diagnosis. Similar to *Artabotrys oblanceolatus* Craib except with cuneate or decurrent (vs. rounded) leaf base, symmetrical (vs. asymmetrical) leaf base, externally distinct outer petal blades and claws, deltoid and undulate (vs. ovate and flat) outer petal

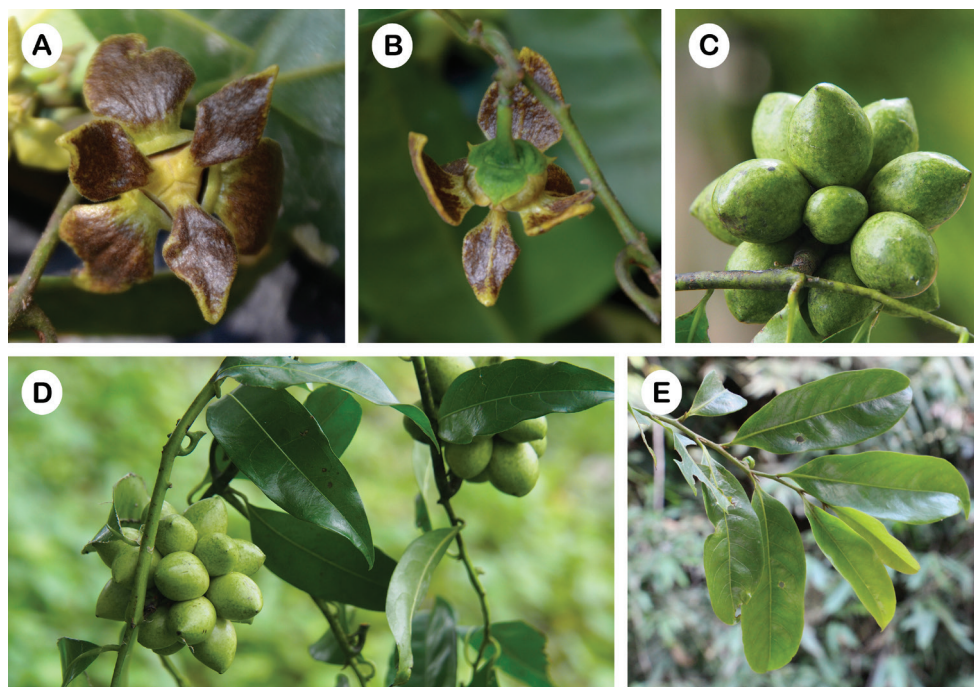


Figure 1. *Artabotrys tanaosriensis* **A** Flower with undulate petal blades and narrow constriction at the junction between petal blades and claws **B** Flower with rhomboid inner petal blades **C** Fruit with weakly beaked monocarps **D** Habit **E** Leaves (Photos: P. Chalermglin).

blades, rhomboid and undulate (vs. ovate and flat) inner petal blades, shorter monocarps (1.5–2 cm vs. 2–2.5 cm), slightly beaked (vs. sharply beaked) monocarps and monocarps with shorter stipes (ca. 2 mm vs. ca. 4 mm).

Type. THAILAND: Phetchaburi Province, Kaeng Krachan National Park, Phaenon Thung Ranger Station, 1000 m elev., 25 Aug 2004, *I.C. Nielsen* 1911 (holotype: L! [barcode L 3729228]; isotype: AAU, n.v.; SING! [barcode SING 0115634]).

Description. Climbers, to 20 m in height. Twigs drying grey-brown, glabrous to sparsely hairy. Leaf laminas 9.5–15.5 cm long, 2.3–4.0 cm wide, oblong-elliptic, apex acuminate, base cuneate or decurrent, chartaceous, glabrous both ab- and adaxially; midrib glabrous to sparsely hairy abaxially, glabrous adaxially, prominent and raised on both surfaces; secondary veins 8–12 pairs per leaf, visible on both surfaces; tertiary venation reticulate, visible on both surfaces; petioles 2–5 mm long, ca. 1 mm in diameter, glabrous to sparsely hairy. Inflorescence hooks recurved, laterally compressed, with 1–3 flowers; flowering pedicels 7–9 mm long, ca. 1 mm in diameter, glabrous. Sepals ca. 4 mm long, 4–6 mm wide, triangular, subglabrous both ab- and adaxially, venation indistinct. Outer petals with externally distinct blades and claws; blades 10–12 mm long, 7–8 mm wide, deltoid, undulate, sparsely hairy both ab- and adaxially; claws ca. 4 mm long, 6–8 mm wide, broadly ovate, densely hairy abaxially, subglabrous adaxially. Inner petals with externally

distinct blades and claws; blades 7–10 mm long, 4–5 mm wide, rhomboid, undulate, sparsely hairy both ab- and adaxially; claws ca. 6 mm long, ca. 4 mm wide, rhomboid, densely hairy abaxially, glabrous adaxially. Stamens ca. 90 per flower, ca. 1 mm long, ca. 1 mm wide; apex of connectives truncate. Carpels 24–30 per flower, ca. 1 mm long, ca. 1 mm wide; ovaries ovoid; stigmas ellipsoid, extending centrifugally. Fruiting pedicels 10–11 mm long, ca. 2 mm wide, glabrous. Monocarps 8–30 per fruit, 15–20 mm long, 9–13 mm wide, ellipsoid, glabrous, slightly beaked (beak less than 1 mm long), drying with longitudinal ridges, subsessile or with stipes up to ca. 2 mm long. Seeds 2 per monocarp, plano-convex, ca. 16 mm long, ca. 8 mm wide.

Phenology. Flowering specimens collected in February and August; fruiting specimens collected in October.

Distribution and habitat. So far only known from Thailand (Fig. 5), but possibly also occurring in the adjacent Tanintharyi National Park in Myanmar. Inhabits tropical rain forests on lateritic soil; 150–1000 m elev.

Etymology. The specific epithet alludes to “Tanao Sri”, the Thai name of the Bilaukaung subrange of the Tenasserim Range where this species occurs.

Local name. Karawek Tanao Sri.

Additional specimens examined (paratypes). Thailand: Prachuap Khiri Khan Province, Hua Hin District, Huai Sat Yai, Pa La-U Village, 17 Oct 2016, *P. Chalermglin* 591017 (BKF, HKU, L, QBG); Phetchaburi Province, Kaeng Krachan District, Huai Mae Phriang Village, 14 Feb 2015, *P. Chalermglin* 580214 (BKF, HKU, L, QBG).

***Artabotrys spathulatus* J.Chen, Chalermglin & R.M.K.Saunders, sp. nov.**

urn:lsid:ipni.org:names:77175742-1

Fig. 2

Diagnosis. Similar to *Artabotrys tanaosriensis* J.Chen, Chalermglin & R.M.K.Saunders except with flat (vs. undulate) outer petal blades, broadly rhomboid (vs. broadly ovate) outer petal claws, broadly spathulate and strongly concave (vs. rhomboid and undulate) inner petal blades and strongly beaked monocarps.

Type. THAILAND: Krabi Province, Mueang Krabi District, Phruksa Sawan limestone hill, 100 m elev., 15 Mar 2015, *P. Chalermglin* 580315 (holotype: BKF!; isotypes: L, QBG!).

Description. Climbers, to 20 m in height. Twigs drying brown, glabrous to sparsely hairy. Leaf laminas 9.6–18.1 cm long, 3–4.5 cm wide, oblong-elliptic, apex acuminate, base cuneate or decurrent, chartaceous, subglabrous both ab- and adaxially; midrib glabrous to sparsely hairy abaxially, glabrous adaxially, prominent and raised on both surfaces; secondary veins 6–14 pairs per leaf, visible on both surfaces; tertiary venation reticulate, visible on both surfaces; petioles 2–6 mm long, ca. 1 mm in diameter, glabrous to sparsely hairy. Inflorescence hooks recurved, laterally compressed, with 1 or 2 flowers; flowering pedicels ca. 10 mm long, ca. 1 mm in diameter, subgla-

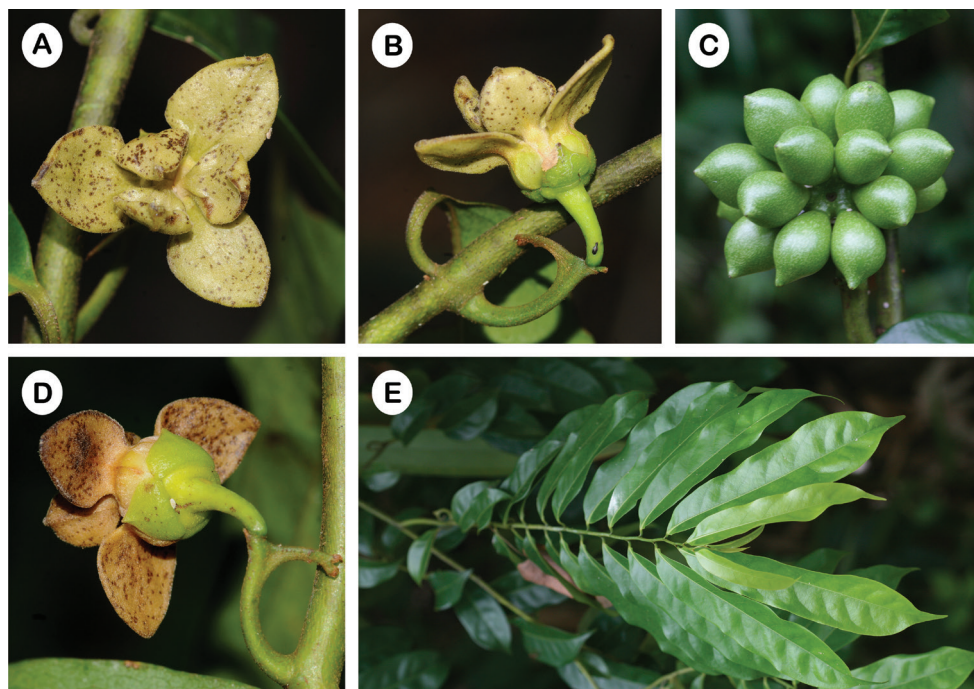


Figure 2. *Artabotrys spathulatus*. **A** Flower with flat outer petal blades and broadly spathulate and strongly concave inner petal blades **B** Inflorescence with hooked peduncle and flower with externally distinct inner petal claw and blade **C** Fruit with distinctly beaked monocarps **D** Flower with externally distinct outer petal blade and claw **E** Leaves and habit (Photos: P. Chalermglin).

brous. Sepals 4–5 mm long and wide, triangular, subglabrous both ab- and adaxially, venation indistinct. Outer petals with externally distinct blades and claws; blades 8–10 mm long, 7–8 mm wide, deltoid, flat, sparsely hairy both ab- and adaxially; claws ca. 5 mm long and wide, broadly rhomboid, densely hairy abaxially, glabrous adaxially. Inner petals with externally distinct blades and claws; blades 6–7 mm long, 4–5 mm wide, broadly spathulate, strongly concave, sparsely hairy both ab- and adaxially; claws ca. 5 mm long, ca. 3 mm wide, narrowly rhomboid, densely hairy abaxially, glabrous adaxially. Stamens numerous per flower, ca. 1 mm long, ca. 1 mm wide; apex of connectives truncate. Carpels ca. 30 per flower, ca. 1 mm long, ca. 1 mm wide; ovaries ovoid; stigmas ellipsoid, extending centrifugally. Fruiting pedicels unknown. Monocarps (in fresh material) up to 30 per fruit, 19–26 mm long, 14–18 mm wide, obovoid, strongly beaked (beak ca. 2 mm long), sessile. Seeds 2 per monocarp, plano-convex, 12–14 mm long, 9–11 mm wide.

Phenology. Flowering and fruiting specimens collected in March.

Distribution and habitat. So far only known from Thailand (Fig. 5). Inhabits tropical rain forests at the base of limestone hills; ca. 100 m elev.

Etymology. The specific epithet reflects the morphology of the inner petals.

Local name. Karawek Phruksa Sawan.

Notes. The fruit and seeds are only known from fresh material and have not been preserved due to fungal infection.

The distinction between *A. spathulatus* and *A. tanaosriensis* is corroborated by unpublished phylogenetic analysis of combined chloroplast (*matK*, *ndhF*, *psbA-trnH*, *trnL-F*) and nuclear (*AP3*, *carboxymethylenebutenolidase*, *GI*, *HMGS*, *LFY*, *mag1*, *ncpGS*, *NIA*, *PhyA*, *RPB2*) DNA sequence data, which retrieved *A. spathulatus* as sister to *A. uniflorus*, with *A. tanaosriensis* sister to *A. suaveolens* (J. Chen et al. unpublished data).

New records for Thailand

Artabotrys byrsophyllus I.M.Turner & Utteridge

Fig. 3

Artabotrys byrsophyllus I.M.Turner & Utteridge, Nordic J. Bot. 33: 562 (2015). – TYPE: Malaysia, Peninsular Malaysia, Kelantan, Ulu Sungei Aring near Kuala Tapah, 21 Sep 1967, *P.F. Cockburn FRI 7151* (holotype: K! [2 sheets, barcodes K 000607815, K 000607816], isotype: KEP, n.v.).

Distribution and habitat. Northern Peninsular Malaysia (Kedah, Kelantan, and Trengganu) and southern Thailand (Narathiwat and Songkhla), in lowland and hill rain forests (Fig. 5).

Local name. Karawek Bai Yai.

Specimens examined. Thailand: Narathiwat Province, Kaluwotai, Khao Chana, 21 Sep 1985, *C. Niyomdham et al.* 1065 (AAU, BKF); Waeng district, *T. Insura* 77 (KUFF); idem, *T. Insura* 78 (KUFF); idem, *T. Insura* 90 (KUFF); idem, *A. F. G. Kerr* 14474 (BM); idem, *S. Phusomsaeng* 389 (BKF).

Notes. The name *A. grandifolius* is misapplied by Chalermglin (2001), Thongpairoj (2008, as “*A. grandiflorus*”) and Insura (2009), for which the name *A. byrsophyllus*

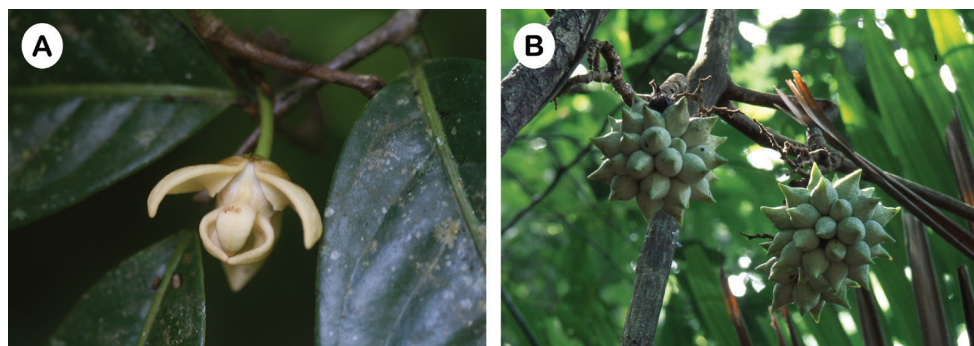


Figure 3. *Artabotrys byrsophyllus*. **A** Flower **B** Fruit (Photos: P. Chalermglin).

should be applied; *A. grandifolius* sensu King is restricted to Peninsular Malaysia. *Artabotrys byrsophyllus* can be distinguished from *A. grandifolius* by reference to its leathery leaves, reticulate tertiary leaf venation that is indistinct on both surfaces, shorter petals (outer petals 13–16 mm vs. ca. 21 mm; blade of inner petals ca. 8 mm vs. 16–17 mm) and subsessile monocarps with marked apiculum.

***Artabotrys punctulatus* C.Y.Wu ex S.H.Yuan**

Fig. 4

Artabotrys punctulatus C.Y.Wu ex S.H.Yuan, Acta Bot. Yunnan. 4: 260 (1982). – TYPE: China, Yunnan, Jinghong, 1015 m, 9 Apr 1957, *Exped. Biol. Sino-Ross. ad prov. Yunnan* 7654 (holotype: KUN! [barcode KUN 0045889]; isotypes: IBSC, n.v., PE! [barcode PE 00934500]).

Distribution and habitat. Southern China (Yunnan) and Thailand (Khao Yai National Park and Phu Kradueng National Park), in lower montane forests, 1000–1500 m elev. (Fig. 5).

Local name. Karawek Kra.

Specimens examined. Thailand: Loei Province, Phu Kradueng National Park, 24 Dec 1971, 1300 m, *van Beusekom et al.* 4555 (L); Nakhon Ratchasima Province, Pak Chong District, Khao Yai National Park, 1200 m, *T. Insura* 43; idem, *T. Insura* 101; idem, *T. Insura* 102; idem, *T. Insura* 103; idem, *T. Insura* 104 (KUFF); idem, *U. Thongpaiboj* 235 (CMU).

Notes. Specimens of this species were mistakenly recognised as a new species (“*A.* sp. 5 (Kao Yai)”) by Thongpaiboj (2008) and misidentified as “*A. aereus*” by Insura (2009). *Artabotrys punctulatus* can be distinguished from *A. aeneus* (which is only known from Vietnam) by its chartaceous leaves, sparsely pubescent (vs. densely villose) flowering pedicels, sepals and petals, smaller sepals and its elongated, raised rim between the claw and blade on the adaxial surface of the inner petals.

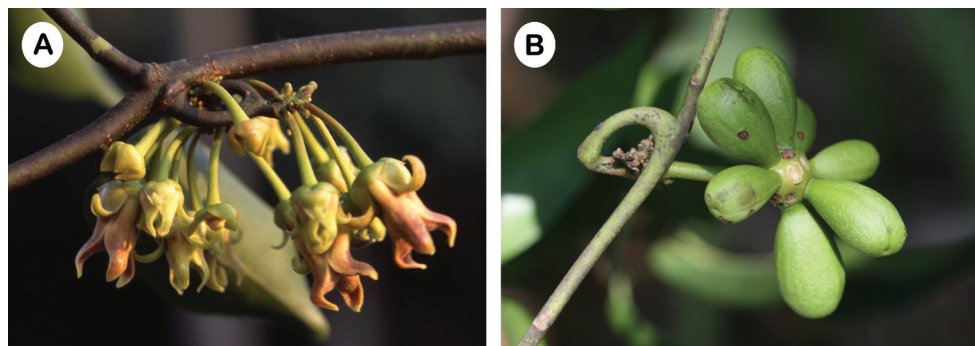


Figure 4. *Artabotrys punctulatus*. **A** Inflorescence **B** Fruit (Photos: P. Chalermglin).

Key to the native *Artabotrys* species in Thailand

- 1 Young branches velutinous; abaxial surface of leaves densely hairy2
- Young branches glabrous to sparsely hairy; abaxial surface of leaves glabrous to sparsely hairy3
- 2 Leaf apex acuminate; leaf laminas broadly elliptic and coriaceous; petal blades 10–15 mm wide *A. siamensis*
- Leaf apex caudate; leaf laminas oblong and chartaceous; petal blades 2–5 mm wide *A. uniflorus*
- 3 Pedicels less than a quarter of the length of the flower4
- Pedicels slightly shorter, as long as, or longer than flower6
- 4 Petal blades linear, 1–2 mm wide; sepals ca. 3 mm long, ca. 2.5 mm wide; leaf laminas 10.5–19 cm long, 4–9 cm wide *A. tipuliferus*
- Petal blades oblong-elliptic, 7–12 mm wide; sepals 4–10 mm long, 4–8 mm wide; leaf laminas 7–14 cm long, 2.5–4.2 cm wide5
- 5 Inflorescences with up to 5 flowers; petal blades chartaceous, 2.8–3.3 cm long, 0.7–1 cm wide; monocarps distinctly stipitate (stipes 3–5 mm long) and slightly beaked (beak 1–2 mm long) *A. brevipes*
- Inflorescences with a solitary flower; petal blades coriaceous, 3.5–4 cm long, ca. 1.2 cm wide; monocarps sessile and sharply beaked (beak up to 1 cm long) *A. oxycarpus*
- 6 Stamen connectives apiculate7
- Stamen connectives truncate8
- 7 Young branches often with spines; leaf apex retuse, truncate or mucronate; leaf laminas 6–8 cm long, 2.5–4.5 cm wide; monocarps ellipsoid, 3.5–4.5 cm long, 1–2 cm wide *A. spinosus*
- Young branches without spines, leaf apex acuminate; leaf laminas 9–15 cm long, 3–7 cm wide; monocarps obovoid, 3–3.5 cm long, 2–2.5 cm wide *A. harmandii*
- 8 Petal blades terete; monocarps 1–2(–4) per fruit *A. suaveolens*
- Petal blades not terete; monocarps 4–30 per fruit9
- 9 Inflorescences with 10–20 flowers; monocarps not beaked10
- Inflorescences with up to 4 flowers; monocarps beaked11
- 10 Petals chartaceous, yellow (in fresh material); adaxial surface of inner petals with a short, raised rim between claw and blade (ca. 1 mm long); leaf laminas coriaceous *A. multiflorus*
- Petals coriaceous, green to beige with maroon patches (in fresh material); adaxial surface of inner petals with an elongated, raised rim between claw and blade (ca. 5 mm long); leaf laminas chartaceous *A. punctulatus*
- 11 Outer petals with externally distinct blades and claws; inner petal blades rhomboid or spatulate12
- Outer petals without externally distinct blades and claws; inner petal blades ovate or triangular13

- 12 Outer petal blades undulate; outer petal claws broadly ovate; inner petal blades rhomboid and undulate; monocarps slightly beaked (beak less than 1 mm long)..... *A. tanaosriensis*
- Outer petal blades flat; outer petal claws broadly rhomboid; inner petal blades broadly spatulate and strongly concave; monocarps strongly beaked (beak ca. 2 mm long) *A. spathulatus*
- 13 Leaf laminas chartaceous, 3–4 cm wide; leaf base asymmetrical; flowering pedicels 4–6 mm long; monocarps 2–2.5 cm long, 0.8–1.2 cm wide..... *A. oblanceolatus*
- Leaf laminas coriaceous, 4–13.5 cm wide; leaf base symmetrical; flowering pedicels 10–25 mm long; monocarps 3–4.5 cm long, 2–3.5 cm wide..... 14
- 14 Leaf laminas 9–15.5 cm long, 4–7 cm wide; outer petals 1.5–2 cm long; monocarps ca. 6 per fruit, 4–4.5 cm long, 2–3.5 cm wide *A. venustus*
- Leaf laminas 21–32 cm long, 8.5–13.5 cm wide; outer petals 1.3–1.6 cm long; monocarps ca. 20 per fruit, 3–3.5 cm long, ca. 2 cm wide..... *A. byrsophyllus*

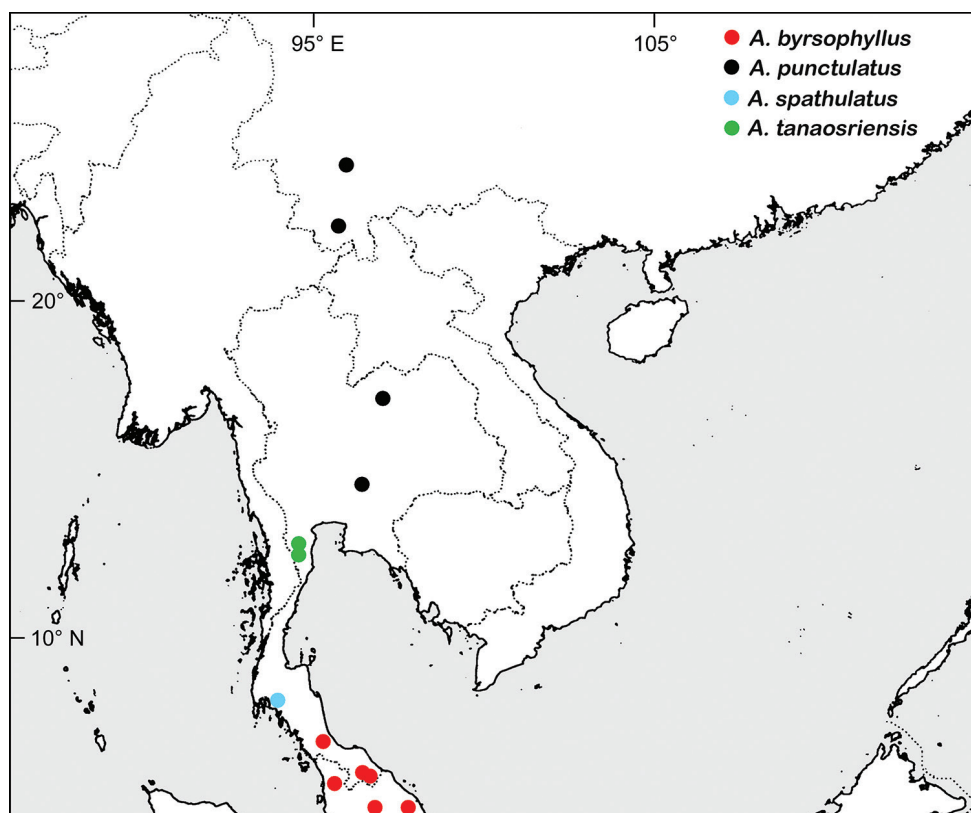


Figure 5. Distributions of *A. byrsophyllus*, *A. punctulatus*, *A. spathulatus* and *A. tanaosriensis* in Thailand and surrounding areas. Localities of *A. byrsophyllus* in Peninsular Malaysia were retrieved from Turner and Utteridge (2015). Localities of *A. punctulatus* in Yunnan were retrieved from georeferenced herbarium records in GBIF (<https://www.gbif.org/>).

Acknowledgements

We are grateful to: the directors of A, AAU, BKF, E, IBSC, K, KUFF, KUN, L, NY, PE, QBG, SING, U and US herbaria for the loan of, or access to, specimens; and Alicia Musson (K), Dr Ende Liu (KUN) and Siti Nur Bazilah Mohamed Ibrahim (SING) for access to high-resolution digital images of selected specimens. This research was funded by project 17109417 from the Hong Kong Research Grants Council, awarded to RMKS.

References

- Chalermglin P (2001) Family Annonaceae. Ban and Suan, Bangkok. [In Thai]
- Chalermglin P (2005) Family Annonaceae (2nd edn). Ban and Suan, Bangkok. [In Thai]
- Chen J, Turner IM, Saunders RMK, Thomas DC (in press) *Artabotrys scortechinii* (Annonaceae): an augmented species description and a new record for Singapore. Gardens' Bulletin Singapore.
- Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland, Massachusetts, 1–545.
- Craib WG (1925) Florae Siamensis Enumeratio: a list of the plants known from Siam with records of their occurrence, Vol. 1. Siam Society, Bangkok.
- Guo X, Tang CC, Thomas DC, Couvreur TLP, Saunders RMK (2017) A mega-phylogeny of the Annonaceae: taxonomic placement of five enigmatic genera and support for a new tribe, Phoeniciantheae. Scientific Reports 7: art. 7323. <https://doi.org/10.1038/s41598-017-07252-2>
- Insura T (2009) Systematics and some ecological characteristics of *Artabotrys* R.Br. (Annonaceae) in Thailand. Masters Thesis, Kasetsart University, Thailand. [In Thai with English abstract]
- Le Thomas A (1969) Annonaceae. In: Aubreville A (Ed.) Flore du Gabon 16. Muséum National d'Histoire Naturelle, Paris, 1–371.
- Mabberley DJ (2008) Mabberley's plant-book: a portable dictionary of plants, their classifications, and uses. Cambridge University Press, Cambridge, 1021 pp.
- Posluszny U, Fisher JB (2000) Thorn and hook ontogeny in *Artabotrys hexapetalus* (Annonaceae). American Journal of Botany 87: 1561–1570. <https://doi.org/10.2307/2656731>
- Rieseberg LH, Wood TE, Baack EJ (2006) The nature of plant species. Nature 440: 524–527. <https://doi.org/10.1038/nature04402>
- Robson NKB (1960) Annonaceae. In: Exell AW, Wild H (Eds) Flora Zambesiaca 1. Crown Agents for Overseas Governments and Administrations, London, 104–149.
- Thongpaiboj U (2008) Taxonomy and molecular phylogeny of *Artabotrys* R. Brown and palynology of tribe Unoneae (Annonaceae) in Thailand. PhD Thesis, Chiang Mai University, Thailand.
- Turner IM (2009) *Artabotrys* (Annonaceae) in Borneo: new species and new synonyms. Folia Malaysiana 10: 59–88.
- Turner IM, Utteridge TMA (2015) *Artabotrys byrsophyllus* and *A. tipuliferus* spp. nov. (Annonaceae) from Peninsular Malaysia and Thailand. Nordic Journal of Botany 33: 562–566. <https://doi.org/10.1111/njb.00791>

Paraboea wenshanensis, a new species of Gesneriaceae from Yunnan, China

De-Ming He¹, Yan-Fei Feng¹, Fu-Zhuan Pan², Xin Hong², Fang Wen³

1 Administration of Wenshan National Nature Reserve, Wenshan Zhuang and Miao Autonomous Prefecture, CN-663000, Yunnan, China **2** School of Resources and Environmental Engineering, Anhui University, CN-230601, Hefei, China **3** Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, CN-541006 Guilin, China

Corresponding author: Xin Hong (hongxin200710084@126.com)

Academic editor: A. Paton | Received 15 October 2017 | Accepted 4 February 2018 | Published 9 February 2018

Citation: He D-M, Feng Y-F, Pan F-Z, Hong X, Wen F (2018) *Paraboea wenshanensis*, a new species of Gesneriaceae from Yunnan, China. *PhytoKeys* 95: 83–91. <https://doi.org/10.3897/phytokeys.95.21586>

Abstract

Paraboea wenshanensis is a new species of Gesneriaceae from Yunnan, China and is described and illustrated here. It is morphologically similar to *P. angustifolia*, *P. martinii* and *P. glutinosa*, but the congeners of this new taxon can be distinguished by several salient characters. A description of *P. wenshanensis*, together with illustrations and photographs, a distribution map and conservation assessment are presented.

Keywords

Limestone flora, karst, new taxon

Introduction

Southern and south-western China has extensive areas of karst topography (Chenet et al. 2008) so that seed plants in this region show extreme diversity and are endemic on alkaline limestone substrates (Xu 1993), a typical example being Gesneriaceae (Fang et al. 1995, Wang et al. 1998, Li and Wang 2004, Wei et al. 2010, Ai et al. 2015).

Paraboea (C.B. Clarke) Ridley has recently been redefined to accommodate *Phylloboea* Benth and *Trisepalum* C.B. Clarke (Middleton et al. 2010, Puglisi et al. 2011), distributed from south to southwest China, Indo-China Peninsula to Malaysia, Indonesia and the Philippines (Burt 1984, Xu and Burt 1991, Xu et al. 2008, Triboun and Middleton 2012).

Many new taxa of this genus have been discovered and published in recent years (Chen et al. 2008, Kiew 2010, Wei et al. 2010, Chen et al. 2012, Wen et al. 2013, Wen and Wei 2016). *Paraboea* is restricted to Asia and includes about 130+ species (Weber 2004, Wen and Wei 2016).

In 2009, one of the authors (WF) encountered a *Paraboea* species with last year's fruits when collecting plants specimens endemic to karst landforms in Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province. Then, in the course of floristic surveys in Wenshan National Nature Reserve between 2012 and 2014, the same species was again collected by the authors. After thorough comparisons of diagnostic morphological and anatomical features of similar taxa from China, Vietnam and Thailand (Barnett 1961, Wang 1990, 1998, Xu and Burtt 1991, Xu 1994, Pham-Hoang 2000, Burtt 2001, Li and Wang 2004, Xu et al. 2008), it was concluded that it was a species new to science and thus it is described and illustrated here.

Material and methods

Measurements and morphological character assessments of the putative new species were undertaken and described using specimens worked on by the current authors and living material observed in the field and at the Gesneriad Conservation Centre of China. All available specimens of *Paraboea* stored in the following herbaria in China, Vietnam, the United States and the United Kingdom were examined (codes according to Thiers 2015): E, GH, HN, IBK, K, KUN, MO, PE, PH, US and VNMN. In addition, images of other type specimens were obtained from Tropicos (<http://www.tropicos.org>), JSTOR Global Plants (<http://plants.jstor.org>) and the International Plant Names Index (<http://www.ipni.org>). All morphological characters were studied under dissecting microscopes and are described using the terminology presented by Wang et al. (1998).

Taxonomic treatment

Paraboea wenshanensis X.Hong & F.Wen, sp. nov.

urn:lsid:ipni.org:names:60476045-2

Figures 1 and 2

Diagnosis. *Paraboea wenshanensis* is similar to *P. martinii* (H. Lév. & Vaniot) B.L. Burtt and *P. glutinosa* (Hand.-Mazz.) K.Y. Pan in having similar corolla shape and colour, but can be distinguished by its oblong-ovate to elliptic leaf blade, crenate margin, lateral veins 4–8 on each side of midrib, petiole subsessile or up to 3 cm long, broadly obovate, glabrous bracts, 6–8 mm long, glabrous membranous calyx and capitate staminodes. It also morphologically resembles *P. angustifolia* Yan Liu & W.B. Xu, but can be easily distinguished by the oblong-ovate to elliptic leaf blade, broadly obovate, glabrous bracts, oblong to oblanceolate, glabrous membranous calyx, sparsely glandu-



Figure 1. *Paraboea wenshanensis* X.Hong & F.Wen. **A** Habitat in fruiting period **B** Inflorescences **C** Dissection of a flower showing corolla, stamens and staminodes **D** Calyx and pistil, stamen (showing the glandular-puberulous, inflated and strongly geniculate).



Figure 2. *Paraboea wenshanensis* X.Hong & F.Wen. **A** Habitat **B** Adaxial surface view of leaf blade **C** Abaxial surface view of leaf blade **D** Cyme with flowers, showing wide campanulate **E** Frontal view of corolla **F** Calyx lobes **G** Opened corolla for showing stamens and pistil **H** Pistil with calyx lobes and stamens, showing anthers and strongly geniculate filaments **I** Infructescence with many capsules.

lar puberulent filaments; capitate staminodes and twisted capsule. A morphological comparison between *P. wenshanensis* and congeners: *P. angustifolia*, *P. martinii* and *P. glutinosa* is provided in Table 1. (see also Fig. 3).

Type. CHINA. Yunnan Province: Shaka County, Gumu Town, Wenshan Zhuang and Miao Autonomous Prefecture, 23°9'22.5"N, 104°12'16.59"E, a.s.l. 1,500 m, 4 Jul 2014, flowering, *D.M. He & Y.F. Feng* WSLJS646 (holotype: KUN; isotype: AHU, IBK).

Description. *Terrestrial* lithophilic, perennial rosulate herbs. *Stems* subterete, 5–10 cm long, 5–9 mm in diameter. *Leaves* 6–20, congested at the apex of the stem, subsessile or up to 3 cm long, leaf blade oblong-ovate to elliptic, (5–)8–18 × 1–3 cm, coriaceous, bases strongly oblique and asymmetrically attenuate, margins crenate sometimes with a woolly strip, apices acute to obtuse, upper leaf surfaces with arachnoid covering when young, becoming glabrescent with age, lower leaf densely appressed greyish arachnoid hairs; lateral veins 4–8 on each side of midrib, convex

Table 1. Diagnostic character differences amongst *Paraboea wenshanensis* sp. nov., *P. angustifolia*, *P. martinii* and *P. glutinosa*.

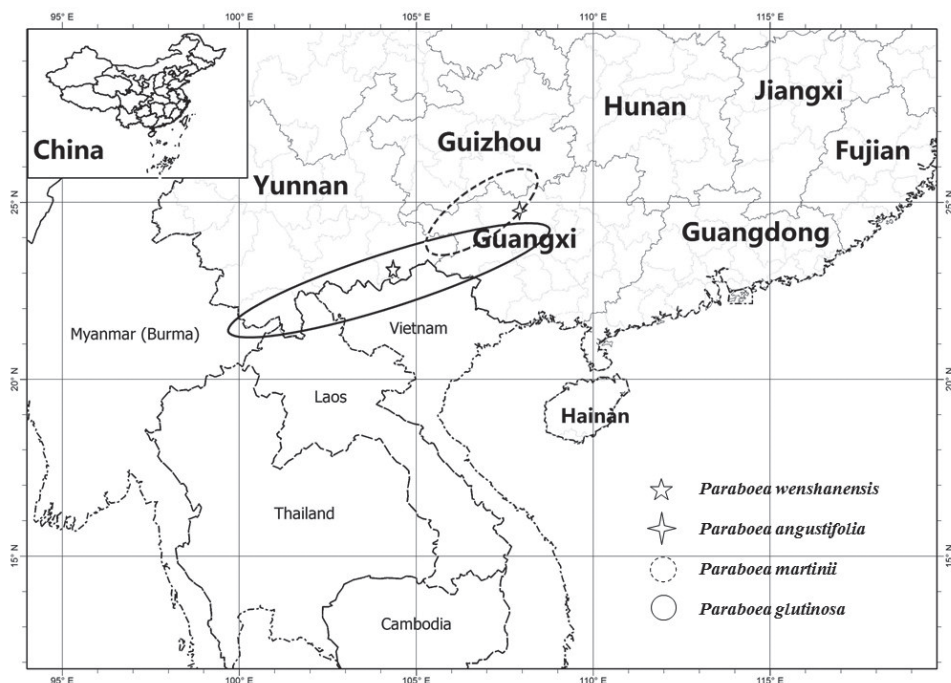
Characters	<i>P. wenshanensis</i>	<i>P. angustifolia</i>	<i>P. martinii</i>	<i>P. glutinosa</i>
Petiole	subsessile or up to 3 cm long	subsessile or up to 2 cm long	2–10 cm long	3–7 cm long
Shape of leaf blade	oblong-ovate to elliptic	linear-oblanccolate	elliptic to ovate or oblanceolate	obovate to elliptic, ovate or oblong
Margin of leaf blade	crenate	serrulate	serrulate to crenulate	serrate to subentire
Number of lateral veins on each side of midrib	4–8	4–8	7–11	10–14
Shape of Bracts	broadly obovate	linear-lanceolate	lanceolate to ovate,	narrowly ovate to obovate
Indumentum of bracts	glabrous	outside pannose	outside pannose	outside pannose
Shape of Calyx	oblong to oblanceolate	linear-lanceolate	narrowly oblong to narrowly triangular	lanceolate to narrowly triangular
Texture of calyx	membranous	thick papery	papery	papery
Indumentum of calyx	glabrous	pannose	sparsely puberulent	glandular puberulent to glabrous
Indumentum of filaments	sparsely glandular puberulent	glabrous	bearded	glabrous to glandular puberulent
Staminodes	capitate, ca. 0.2 mm long	linear, ca. 4 mm long,	linear, ca. 3 mm long,	linear, 1.2–2 mm long
Capsule	twisted	straight	twisted	twisted

and densely appressed brown to greyish woolly hairs along the abaxial veins. *Cymes* dichotomous, axillary or subterminal, dichasia 1–3(–5), (2–)4–16-flowered; peduncles 3–10 cm long, ca. 5 mm in diameter, sparsely greyish matted woolly hairs, green; bracts 2, opposite, broadly obovate, 7–9 × ca. 5 mm, margins entire, apices blunted to obtuse, glabrous, whitish to purple; pedicels 0.8–1 cm long, ca. 2 mm in diameter, sparsely greyish matted woolly, greenish. *Calyx* membranous, 5-parted to the base, lobes oblong to oblanceolate, 6–8 × ca. 2.6 mm, glabrous, margins entire, apex obtuse or rounded, white to purplish. *Corolla* zygomorphic, 1–1.5 cm long, purple outside, bluish or purplish inside, glabrous; tube obliquely wide campanulate, 0.6–1.2 cm long, ca. 1 cm in diameter at the mouth; the limb two-lipped; adaxial lip 2-lobed to near base, lobes semi-orbicular, apex rounded, 2–4 × ca. 3 mm, abaxial lip 3-lobed to base, central lobe ovate, lateral lobes obliquely ovate, the apex of 3 lower lobes rounded, 5–6 × ca. 3 mm. *Stamens* 2, included, adnate to abaxial side of corolla tube near base; filaments 4–5 mm long, inflated and strongly geniculate on the upper part, wide along its length and narrowly constricted at base, white, sparsely purple glandular–puberulous near the base; anthers dorsifixed, ca. 3.5 mm long; transversely spindle-shaped, coherent at the lateral sides, dehiscing longitudinally, white, glabrous; *staminodes* 2, capitate,



Figure 3. **A:** *Paraboea wenshanensis* X.Hong & F.Wen. **A-1** plant in habitat **A-2** corolla face view **B** *P. angustifolia* Yan Liu & W.B. Xu **B-1** habitat **B-2** flowering habit, **B-3** corolla face view **C** *P. martinii* (Lévl.) Burt **C-1** flowering habit **C-2** corolla face view **D** *P. glutinosa* (Handel-Mazzetti) K. Y. Pan **D-1** plant in habitat **D-2** inflorescence, showing corolla face view.

ca. 0.2 mm long, adnate to ca. 1.5 cm above the corolla tube base. *Pistil* glabrous; ovary narrowly ovoid to conical, ca. 1 cm long, ca. 1.1 mm in diameter, placentas 4, axile, undivided; style ca. 6 mm long, stigma capitate, with numerous papillae. *Capsule* linear, spirally twisted, ca. 3 cm long, 0.4–0.6 cm in diameter, glabrous, slightly curved, dehiscing loculicidally to base.



Map I. The geographical distribution of *P. wenshanensis* sp. nov., *P. angustifolia*, *P. martinii* and *P. glutinosa*.

Etymology. The specific epithet is derived from the type locality, Wenshan National Nature Reserve, Yunnan Province, China.

Vernacular name. Wén Shān Zhǔ Máo Jù Tǎi (Chinese pronunciation); 文山蛛毛苣苔 (Chinese name).

Distribution and habitat. To date, *Paraboea wenshanensis* is locally abundant and endemic to south-western China, from type locality: Wenshan Nature Reserve, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan province. This species grows on moist shady cliffs of limestone hills, at an elevation of 1,500 m a.s.l. The average temperature is 14.5 °C, the average annual precipitation has been calculated as ca. 1,022 mm. The forest is a subtropical monsoon climate evergreen broad-leaved forest, with main community types of *Ilex polyneura* (Hand.-Mazz.) S.Y. Hu, *Triadica rotundifolia* (Hemsl.) Esser and *Debregeasia orientalis* C.J. Chen.

Conservation status. Current information for this new species is only known from very few collections and details on the size of the population are known in Wenshan Nature Reserve, where the plants' protected status is guaranteed. Based on five careful field investigations in the past years, this species appears to be locally abundant. Considering that not enough is known about the population, it is proposed that *Paraboea wenshanensis* should currently be classed as data deficient (DD) (IUCN 2016).

Notes. The geographical distributions of *P. wenshanensis* and its similar species are identified in Map 1.

Acknowledgments

The authors are grateful to Prof. Yi-Bo Luo and Dr. Wei-Bin Xu for the photographs of *Paraboea martinii* and *P. angustifolia* and Miss Le-Ping He and Miss Wen Ma for the beautiful hand-drawing. This study was financially supported by the Anhui University Doctor Startup Fund, Key University Science Research Project of Anhui Province (No. KJ2017A022), Fund of Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain (16-B-01-01), Plant germplasm resources projects of the germplasm bank of Wild species of Kunming Institute of Botany, Chinese Academy of Sciences (WGB-1411), the Chinese Academy of Sciences under the Guangxi Natural Science Foundation (2015GXNSFBB139004) and the Chinese Academy of Sciences under the STS initiative “Development of Chinese Union of Botanical Gardens (KFJ-1W-NO1)”.

References

- Ai B, Gao Y, Zhang X, Tao J, Kang M, Huang H (2015) Comparative transcriptome resources of eleven primulina species, a group of ‘stone plants’ from a biodiversity hot spot. *Molecular Ecology Resources* 15(3): 619–632. <https://doi.org/10.1111/1755-0998.12333>
- Barnett EC (1961) Contribution to the Flora of Thailand: LV. *Kew Bulletin* 15: 249–259. <https://doi.org/10.2307/4109363>
- Burtt BL (1984) Studies in the Gesneriaceae of the Old World, XLVII: Revised generic concepts for Boea and its allies. *Notes from the Royal Botanic Garden Edinburgh* 41: 401–452
- Burtt BL (2001) Flora of Thailand: annotated checklist of Gesneriaceae. *Thai Forest Bulletin (Botany)* 29: 81–109.
- Chen WH, Möller M, Shui YM, Zhang MD (2008) A new species of *Paraboea* (Gesneriaceae) from a karst cave in Guangxi, China and observations on variations in flower and inflorescence architecture. *Botanical Journal of the Linnean Society* 158: 681–688. <https://doi.org/10.1111/j.1095-8339.2008.00873.x>
- Chen WH, Möller M, Zhang MD, Shui YM (2012) *Paraboea hekouensis* and *P. manhaoensis*, two new species of Gesneriaceae from China. *Annales Botanici Fennici* 49(3): 179–187. <https://doi.org/10.5735/085.049.0304>
- Fang D, Qin DH, Rao WY, Zeng L (1995) New plants of Gesneriaceae from Guangxi and Guizhou of China (Cont. II). *Acta Phytotaxonomica Sinica* 33: 602–607.
- IUCN (2016) Guidelines for Using the IUCN Red List Categories and Criteria. Version 12. Prepared by the Standards and Petitions Subcommittee. Available from: <http://www.iucn-redlist.org/documents/RedListGuidelines.pdf> [accessed 15 April 2016]
- Kiew R (2010) Two new species of *Paraboea* (Gesneriaceae) from Peninsular Malaysia and Thailand. *Edinburgh Journal of Botany* 67(2): 209–217. <https://doi.org/10.1017/S0960428610000107>
- Li ZY, Wang YZ (2004) Plants of Gesneriaceae in China. Science and Technology Publishing House: Zhengzhou, Henan, 305–332.

- Middleton DJ, Puglisi C, Triboun P, Möller M (2010) Proposal to conserve *Paraboea* against *Phylloboea* and *Trisepalum* (Gesneriaceae). *Taxon* 59: 1603.
- Pham–Hoang H (2000) An illustrated flora of Vietnam 3. Youth Publishing House, Ho Chi City, 12–29.
- Puglisi C, Middleton DJ, Triboun P, Möller M (2011) New insights into the relationships between *Paraboea*, *Trisepalum* and *Phylloboea* (Gesneriaceae) and their taxonomic consequences. *Taxon* 60: 1693–1702.
- Thiers B (2015) [continuously updated] Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden. <http://sweetgum.nybg.org/ih/> [accessed 3 Feb 2015]
- Triboun P, Middleton DJ (2012) Twenty new species of *Paraboea* (Gesneriaceae) from Thailand. *Gardens' Bulletin Singapore* 64(2): 333–370.
- Triboun P (2013) *Paraboea middletonii* (Gesneriaceae), a new species from Thailand. *Thai Forest Bulletin (Botany)* 41: 45–47.
- Wang WT (1990) Gesneriaceae. In: Wang WT (Ed.) *Flora Reipublicae Popularis Sinicae* 69. Science Press, Beijing, 460–472.
- Wang WT, Pan KY, Li ZY, Weitzman AL, Skog LE (1998) Gesneriaceae. In: Wu ZH, Raven PH (Eds) *Flora of China*, Vol. 18. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, 362–367.
- Weber A (2004) Gesneriaceae. In: Kubitzki K, Kadereit JW (Eds) *The Families and Genera of Vascular Plants volume 7: Dicotyledons, Lamiales*. Springer, Berlin/Heidelberg, 63–158. https://doi.org/10.1007/978-3-642-18617-2_8
- Wei YG, Wen F, Möller M, Monro A, Zhang Q, Gao Q, Mou HF, Zhong SH, Cui C (2010) Gesneriaceae of South China. Guangxi Science and Technology Publishing House, Nanning, Guangxi, 608–646.
- Wen F, Wei YG (2016) *Paraboea yunfuensis*: a new calcicolous species of Gesneriaceae from Yunfu, Guangdong Province, China. *Telopea* 19: 125–129.
- Wen F, Hong X, Chen LY, Zhou SB, Wei YG (2013) A new species of *Paraboea* (Gesneriaceae) from a karst limestone hill in Southwestern Guangdong, China. *Phytotaxa* 131(1): 1–8. <https://doi.org/10.11646/phytotaxa.131.1.1>
- Xu WB, Huang YS, Wei GF, Tan WN, Liu Y (2012) *Paraboea angustifolia* (Gesneriaceae): a new species from limestone areas in northern Guangxi, China. *Phytotaxa* 62(1): 39–43. <https://doi.org/10.11646/phytotaxa.62.1.8>
- Xu ZR (1993) A study of the limestone forest flora of southern and south-western China: floristics, ecology, conservation and taxonomy. *Guihaia Additamentum* 4: 5–54.
- Xu ZR (1994) A new species of *Paraboea* Ridley from Thailand. *Acta Phytotaxonomica Sinica* 32: 359–361.
- Xu ZR, Burtt BL (1991) Towards a revision of *Paraboea* (Gesneraceae): I. *Edinburgh Journal of Botany* 48(1): 1–18. <https://doi.org/10.1017/S0960428600003541>
- Xu ZR, Burtt BL, Skog LE, Middleton DJ (2008) A revision of *Paraboea* (Gesneriaceae). *Edinburgh Journal of Botany* 65(2): 161–347. <https://doi.org/10.1017/S0960428608005106>

Home at Last II: *Gerbera hieracioides* (Kunth) Zardini (Mutisieae, Asteraceae) is really a *Chaptalia*

Xiaodan Xu¹, Wei Zheng², Vicki A. Funk³, Jun Wen³

1 Faculty of Art and Communication, Kunming University of Science and Technology, Kunming 650500 China **2** Faculty of Architecture and City Planning, Kunming University of Science and Technology, Kunming 650500 China **3** Smithsonian Institution, Department of Botany, MRC 166, Washington, D.C. 20013-7012 USA

Corresponding author: Jun Wen (wenj@si.edu)

Academic editor: A. Sukhorukov | Received 13 December 2017 | Accepted 30 January 2018 | Published 9 February 2018

Citation: Xu X, Zheng W, Funk WA, Wen J (2018) Home at Last II: *Gerbera hieracioides* (Kunth) Zardini (Mutisieae, Asteraceae) is really a *Chaptalia*. *PhytoKeys* 95: 93–106. <https://doi.org/10.3897/phytokeys.95.22916>

Abstract

Gerbera hieracioides (Kunth) Zardini of the *Gerbera*-complex (Mutisieae, Asteraceae/Compositae) is distributed in Ecuador and Peru. This perennial herb was first named as *Onoseris hieracioides* Kunth and was later recognised as *Trichocline hieracioides* (Kunth) Ferreyra. Now it is generally treated as *Gerbera hieracioides* (Kunth) Zardini but it has never been included in any section of *Gerbera*. In this study, the position of *Gerbera hieracioides* is assessed based on morphology and a molecular phylogeny that includes *G. hieracioides* and 28 other species from the *Gerbera*-complex. Morphologically, *G. hieracioides* bears leaves with the adaxial epidermal surface without stomates but with soft thin trichomes, bracteate scapes, trimorphic capitula and inner ray florets with the corolla shorter than the style. These characters suggest that the species is most closely related to *Chaptalia* rather than to *Gerbera* or *Trichocline*. Furthermore, the phylogenetic results based on two nuclear (ITS and ETS) and two chloroplast (*trnL-trnF* and *trnL-rpl32*) sequences strongly support the placement of *G. hieracioides* nested within *Chaptalia*. As both morphological characters and the molecular phylogenetic results support the transfer of *G. hieracioides* to *Chaptalia*, this enigmatic taxon is recognised as *Chaptalia hieracioides* (Kunth) X.-D. Xu & W. Zheng.

Keywords

Compositae, *Gerbera hieracioides*, *Trichocline hieracioides*, *Chaptalia hieracioides*, *Gerbera*-complex, SEM, stomata, South America, Africa, Asia

Introduction

Gerbera hieracioides (Kunth) Zardini (Mutisieae, Asteraceae) is a species belonging to the *Gerbera*-complex (*Gerbera* L., *Leibnitzia* Cass., *Uechtritzia* Freyn, *Amblyserma* Benth., *Chaptalia* Vent., *Trichocline* Cass., *Perdicium* L. and *Lulia* Zardini). The species is distributed in Ecuador and Peru. This perennial herb was first named as *Onoseris hieracioides* Kunth in 1818. It was transferred to *Trichocline hieracioides* (Kunth) Ferreyra in 1944. In 1974, Zardini moved this species out of *Trichocline* because it did not have the characters which were used to define that genus. The apex of achenes is truncate in *Trichocline* but tapering or beaked in *G. hieracioides* (Zardini 1974, Hansen 1990). Zardini (1974, 1975) moved it into *Gerbera* because it had bracteate scapes, uniseriate ray florets, achenes rostrate at the apex and slender achene hairs. However, *Gerbera* and *Chaptalia* were found to share the same traits such as achenes rostrate at the apex (Katinas 2004) and the transfer of *Trichocline hieracioides* to *Gerbera* remained controversial (Hansen 2006).

Gerbera currently contains about 32 species, which belong to six sections: the three African sections: *Gerbera* (8 species), *Parva* H.V.Hansen (1 species) and *Lasiopus* (Cass.) Sch.Bip. (6 species), the Asian section *Isanthus* (Less.) C. Jeffrey (7 species), the Madagascar section *Pseudoseris* (Baill.) C. Jeffrey (8 species) and section *Piloselloides* Less. (2 species, one of which is widespread from Asia, Africa and Australia: Hansen 1985a, 1985b, 1988, Johnson et al. 2014, Funk et al. 2016). However, Zardini (1974) did not include *G. hieracioides* in any section of *Gerbera*, she only compared it with two species in sect. *Lasiopus* (Hansen 1988): *G. jamesonii* Bolus ex Adlam and *G. ambigua* Sch. Bip. Although *G. hieracioides* has the trimorphic capitula similar to those of *G.* sect. *Lasiopus* (Hansen 1985a) from Africa, it has bracteate scapes, suggesting that it is perhaps related to *Gerbera* sect. *Isanthus* (Hansen 1988) from Asia. Furthermore, the SEM studies showed that the achene hairs of *G. hieracioides* possess a significantly lower L/W ratio than that in either sect. *Isanthus* or sect. *Lasiopus* of *Gerbera* (Hansen 1990). Therefore, it was still difficult to place *G. hieracioides* into an existing section (or a new section) of the genus *Gerbera*.

Gerbera is an Old World genus, whereas *Chaptalia*, *Trichocline* and the enigmatic *G. hieracioides* are New World groups (Nesom 2004b, 1995). Recently, phylogenetic analyses of the *Gerbera*-complex based on molecular data showed that *Chaptalia* was placed between *Trichocline* and *Gerbera* (Baird et al. 2010, Funk et al. 2014, Pasini et al. 2016). This suggested to the authors that the New World *G. hieracioides* may be a species of *Chaptalia*.

In this study, the authors seek to determine the correct generic placement of *G. hieracioides* by sampling 28 congeneric species using both molecular (two nuclear and two chloroplast markers) and morphological data (leaf adaxial surface, scape and floral morphology).

Materials and methods

A total of 29 species from four genera (*Gerbera*, *Amblyserma*, *Chaptalia* and *Trichocline*) of the *Gerbera* complex and *Adenocaulon chilense* (outgroup) were sampled for

this study. Most of the specimens were sampled from the United States National Herbarium (US) of the Smithsonian Institution (Tables 1, 2).

Adaxial leaf epidermal morphology. Lamina (0.5–1.0 cm²) were placed with the adaxial side exposed on carbon tape over stubs for the scanning electron microscopy (SEM), without soaking the material in different solutions prior to SEM. The stubs bearing leaves were treated with gold-palladium to 16.6 µm thickness and were examined under a Philips XL-30 scanning electron microscope at the SEM Lab of the National Museum of Natural History (NMNH). The 22 samples were subsequently observed and photographed under SEM. Images of the leaves were captured using the proprietary software associated with the Philips SEM. Images of at least 15 different areas of the adaxial leaf surface were captured.

Floret morphology. The florets and scapes of 20 herbarium specimens were examined in the United States National Herbarium, Smithsonian Institution, using an optical microscope.

DNA extraction, amplification and sequencing. The molecular work was performed in the Laboratory of Analytical Biology (LAB) of NMNH (Smithsonian Institution). DNAs of 16 samples (15 species, including two samples of *Gerbera hieracioides*) were extracted using the AutoGen. Herbarium leaf samples, along with 1.0 and 2.3 mm diameter beads, were dipped in liquid nitrogen then immediately shaken for 30 seconds at 18000 rpm. About 500 µl of CTAB was added to the tubes, vortexed and incubated overnight (500 rpm at 45 °C). Then 300 µl of the supernatant was transferred to an AutoGen plate. AutoGen was run according to the manufacturer's default settings (AutoGen, Inc., Holliston, MA, USA).

Four markers including two nuclear ribosomal (ITS and ETS) and two chloroplast intergenic spacers (*trnL–trnF* and *trnL–rpl32*) were amplified. The ITS primers were designed by Downie and Katz-Downie (1996) and White et al. (1990), ETS primers by Baldwin and Markos (1998), *trnL–trnF* primers by Taberlet et al. (1991) and *trnL–rpl32* spacer primers by Timme et al. (2007) (Table 3). The PCR reaction mixture had a total volume of 25 µl, comprising 14.05 µl nuclease free water, 2.5 µl 10× buffer, 2 µl dNTPs, 1.25 µl MgCl₂, 1 µl of both forward and reverse primers, 0.5 µl BSA, 0.2 µl Taq DNA polymerase and 2.5 µl of template DNA. The amplified products were purified with ExoSapIT enzyme with activation at 37 °C and deactivation at 95 °C. 4 µl of the purified product and same primers (1 µl, 1 µM) were cycle-sequenced in a mixture containing 0.8 µl Big Dye (Applied Biosystems, Foster City, USA) and 2.0 µl 5× Big Dye buffer and 4.2 µl water.

The cycle sequencing programme was 30 cycles of 95 °C for 30 s, 50 °C for 30 s and 60 °C for 4 min. The resultant product was sephadex filtered and sequenced through an ABI 3730 automated sequencer (Applied Biosystems, Foster City, USA). The PCR reactions were performed in a Veriti PCR Thermal Cycler. The amplification protocols for all markers are summarised in Table 3. Sequences were aligned by using MAFFT (Katoh and Standley 2013) using Geneious 10.0.9. (Biomatters Ltd., Auckland, New Zealand) and checked manually. A total of 57 newly generated sequences from the 16 samples were deposited in GenBank (Table 2).

Table 1. Voucher information and morphological characters of *Gerbera hienatioides* and the related species.

Species	Section	Locality	Voucher information	Adaxial leaf		Bracts on scape	Inner rays
				Stomata	Trichome		
<i>Gerbera viridifolia</i> (DC.) Sch.Bip.	<i>Lasiopus</i>	Kenya	<i>T.H. Trinder-Smith s.n.</i> (US)	+	★	-	+
<i>G. jamesonii</i> Adlam	<i>Lasiopus</i>	Cultivar	<i>V.A. Funk s.n.</i> (US)	+	★	-	+
<i>G. aurantiaca</i> Sch.Bip.	<i>Lasiopus</i>	South Africa	<i>Bayliss 2505</i> (US)	+	★	-	+
<i>G. ambigua</i> Sch.Bip.	<i>Lasiopus</i>	South Africa	<i>M. Koekemoer 2097</i> (US)	+	★	-	+
<i>G. piloselloides</i> Cass.	<i>Piloselloides</i>	Swaziland	<i>M. Koekemoer 2590</i> (US)	+	★	-	+
<i>G. cordata</i> Less.	<i>Piloselloides</i>	Madagascar	<i>T.B. Croat 29083</i> (MO)	+	★	-	+
<i>G. perrieri</i> Humbert	<i>Pseudoseris</i>	Madagascar	<i>L. Gautier 3110</i> (MO)	+	★	-	+
<i>G. crocea</i> Kuntze	<i>Gerbera</i>	South Africa	<i>M. Koekemoer 2029</i> (US)	+	≈	+	-
<i>G. wrightii</i> Harv.	<i>Gerbera</i>	South Africa	<i>P. Goldblatt 5287</i> (US)	+	≈	+	-
<i>G. serrata</i> Druce	<i>Gerbera</i>	South Africa	<i>M. Koekemoer 2001</i> (PRE)	+	≈	+	-
<i>G. gossypina</i> Beauverd	<i>Isanthus</i>	India	<i>W.N. Koelez 4828</i> (US)	-	-	+	-
<i>G. maxima</i> Beauverd	<i>Isanthus</i>	India	<i>D.H. Nicolson 2755</i> (US)	-	-	+	-
<i>G. delavayi</i> Franch.	<i>Isanthus</i>	China	<i>X. Xu 1102</i> (KMUST)	-	-	+	-
<i>G. nivea</i> Sch.Bip.	<i>Isanthus</i>	China	<i>J.F. Rock 6430</i> (US)	-	-	+	-
<i>G. henryi</i> Dunn	<i>Isanthus</i>	China	<i>W.B. Hemsley 1903</i> (US)	-	-	+	-
<i>G. hienatioides</i> (Kunth) Zardini	?	Ecuador	<i>P.M. Peterson 9287</i> (US)	-	≈	+	+
<i>G. hienatioides</i> (Kunth) Zardini	?	Peru	<i>R. Ferreyra 15362</i> (US)	-	≈	+	+
<i>Chaptalia pringlei</i> Greene	N	Mexico	<i>Rzedowski 34853</i> (US)	-	≈	+	+
<i>C. mandonii</i> Burkart	N	Argentina	<i>P.M. Simón 438</i> (US)	-	≈	+	+
<i>C. meridensis</i> S.F. Blake	N	Venezuela	<i>L. Aristeguieta 2591</i> (US)	-	≈	+	+
<i>Trichoclina cineraria</i> Hook. & Arn.	N	Argentina	<i>A.R. Cuezco 20mz398</i> (US)	+	≈	+	-
<i>T. catharinensis</i> Cabrera	N	Brazil	<i>L.B. Smith 11376</i> (US)	+	≈	+	-

Notes: + designates those mentioned present; - designates those mentioned absent; ★ designates rigid, straight and upright trichomes present on the adaxial leaf surface; ≈ designates soft thin trichomes present on the adaxial leaf surface; N designates data not available.

Table 2. Voucher information and GenBank accessions of *Gerbera hieracioides* and the related species.

Species	Locality	Voucher information	ITS	ETS	<i>trnL-trnF</i>	<i>trnL-rpβ2</i>
<i>Gerbera viridifolia</i> (DC.) Sch. Bip.	South Africa	<i>T.H. Trinder-Smith s.n.</i> (US)	MG661696*	MG661588*	MG661639*	MG661670*
<i>G. crocea</i> Kuntze	South Africa	<i>M. Koekemoer 2029</i> (US)	MG661709*	MG661606*	MG661645*	MG661683*
<i>G. delavayi</i> Franch.	China	<i>X. Xu 1102</i> (KMUST)	MG661708*	MG661605*	MG661659*	MG661682*
<i>G. henryi</i> Dunn	China	<i>X. Xu 1103</i> (KMUST)	MG661706*	MG661602*	MG661655*	MG661681*
<i>G. nivea</i> Sch. Bip.	China	<i>Y.S. Chen 2674</i> (PE)	MG661703*	MG661598*	MG661648*	MG661678*
<i>G. aurantiaca</i> Sch.Bip.	South Africa	<i>Bayliss 2505</i> (US)	MG661711*	MG661610*	MG661637*	MG661687*
<i>G. ambigua</i> Sch. Bip.	South Africa	<i>M. Koekemoer 2097</i> (US)	MG661712*	MG661611*	MG661636*	MG661688*
<i>G. jamesonii</i> Adlam	Cultivar	<i>T. Derby s.n.</i> (US)	MG661704*	MG661599*	MG661638*	MG661679*
<i>G. cordata</i> Less.	South Africa	<i>J. Wren 10067</i> (US)	N	MG661608*	MG661661*	MG661685*
<i>G. piloselloides</i> Cass.	Swaziland	<i>M. Koekemoer 2590</i> (US)	MG661701*	MG661592*	MG661650*	MG661675*
<i>G. wrightii</i> Harv.	South Africa	<i>P. Goldblatt 5287</i> (US)	MG661695*	MG661587*	MG661642*	N
<i>G. serrata</i> Druce	South Africa	<i>M. Koekemoer 2001</i> (PRE)	MG661697*	MG661590*	MG661656*	MG661671*
<i>G. hieracioides</i> (Kunth) Zardini	Ecuador	<i>P.M. Peterson 9287</i> (US)	MG661705*	MG661601*	MG661657*	MG661680*
<i>G. hieracioides</i> (Kunth) Zardini	Peru	<i>J. Campos 5255</i> (US)	N	MG661600*	N	N
<i>Amblyosperma scapigera</i> Benth.	Australia	<i>A. Morrison s.n.</i> (US)	MG661713*	MG661612*	N	MG661689*
<i>Adenocaulon chilense</i> Less.	Chile	<i>G.L. Sobel 2558</i> (US)	MG661714*	N	N	MG661690*
<i>Gerbera maxima</i> Beauverd	India	<i>F. Kingdom 18199</i> (NY)	KX349402	N	KX349371	N
<i>G. gossipina</i> Beauverd	India	<i>W. Koelz 4294</i> (US)	GU126777	N	N	GU126755
<i>Adenocaulon chilense</i> Less.	Argentina	<i>J.M. Bonifacio 3997</i> (LP)	KX349359	N	KX349360	N
<i>Chaptalia nutans</i> (L.) Polák	Argentina	<i>P.M. Simon 477</i> (US)	GU126772	N	N	GU126751
<i>C. pringlei</i> Greene	Mexico	<i>G. Nesom 4405</i> (US)	GU126773	N	N	N
<i>C. runcinata</i> Kuntze	Argentina	<i>P.M. Simon 415</i> (US)	GU126774	N	N	GU126752
<i>C. chapadensis</i> D.J.N. Hind	Argentina	<i>Roque & al. 2188</i> (ALCB)	KF989508	N	KF989614	N
<i>C. similis</i> R.E. Fr.	Argentina	<i>P.M. Simon 711</i> (US)	GU126775	N	N	GU126753
<i>C. tomentosa</i> Vent.	USA	<i>V.A. Funk 12303</i> (US)	GU126776	N	N	GU126754

Species	Locality	Voucher information	ITS	ETS	<i>trnL-trnF</i>	<i>trnL-rpB2</i>
<i>C. piloselloides</i> (Vahl) Baker	Brazil	<i>E. Pasini 1021</i> (ICN)	KX349357	N	KX349358	KX349403
<i>Trichoclina auriculata</i> Hieron	Argentina	<i>H. Simón & J.M. Bonifacio 633</i> (US)	KX349386	N	KX349387	N
<i>T. catharinensis</i> Cabrera	Brazil	<i>E. Pasini 915</i> (ICN)	KX349388	N	KX349389	KX349411
<i>T. caulescens</i> Phil.	Chile	<i>V.A. Funk & al. 13055</i> (US)	KX349390	N	KX349391	KX349406
<i>T. cineraria</i> Hook. & Arn.	Argentina	<i>E. Pasini & F. Torchelsen 1027</i> (ICN)	KX349392	N	KX349393	KX349407
<i>T. plicata</i> Hook. & Arn.	Argentina	<i>E. Pasini & F. Torchelsen 1023</i> (ICN)	KX349396	N	KX349397	KX349409
<i>T. reptans</i> (Wedd.) Hieron	Argentina	<i>E. Pasini & F. Torchelsen 1025</i> (ICN)	KX349398	N	KX349399	KX349410

Notes: * designates the new sequences from this study; N designates data not available.

Table 3. Primers and amplification protocols for all markers.

Marker	Primers and sequences 5'-3'	PCR protocol: initial pre-heating; DNA denaturation; primer annealing; DNA extension; final extension
ITS	ITS5A: GGAAGGAGAAGTCGTAAACAAG ITS4: TCCTCCGCTTATGATATGC	95 °C 1 min; 54 °C 1 min; 72 °C 1 min; 72 °C 10 min; 40 cycles
ETS	18s-ETS: ACTTACACATGCATGGCTTAATCT ETS-Hel-1: GCTCTTTGCTTGCGCAACAAC	94 °C 0:30 min; 60 °C 0:40 min; 72 °C 1:20 min; 72 °C 5 min; 30 cycles
<i>trnL-trnF</i>	<i>trnL</i> -Fc: CGAAATCGGTAGACGCTACG <i>trnL</i> -Ff: ATTGAACTGGTGACACGAG	94 °C 1 min; 53 °C 1 min; 72 °C 2 min; 72 °C 10 min; 35 cycles
<i>trnL-rpB2</i>	<i>trnL</i> : TACCGATTTCACCATAGCGG <i>rpB2</i> : AGGAAAGGATATTGGGCGG	95 °C 3 min; 51 °C 40 s; 72 °C 1:20 min; 72 °C 5 min; 40 cycles

A total of 37 sequences of 16 species were retrieved from NCBI for the related taxa within the tribe Mutisieae (Table 2). Phylogenetic relationships were inferred based on the concatenated ITS+ETS+trnL-rpl32+trnL-trnF data with MrBayes v. 3.2.2 (Ronquist et al. 2012) by using the substitution model of GTR based on the best-fitting model determined using jModelTest 2.1.6 (Posada 2008), the chain length of 10,000,000, rate variation of gamma, gamma categories of 4, heated chains of 4, heated chain temp. of 0.2, subsampling freq. of 200 and burn-in length of 100,000. Tracer v. 1.5 (Rambaut and Drummond 2009) was used to confirm that the effective sample size (ESS) for all relevant parameters was > 200. After discarding the trees as burn-in, a 50 % majority-rule consensus tree and posterior probabilities (PP) for node support were calculated using the remaining trees.

Results

Adaxial leaf epidermal morphology. The results of the SEM work (Table 1) showed that the two tested samples of *Gerbera hieracioides* have no stomates but have soft, thin and appressed trichomes on the adaxial leaf surface (Figure 1G). These adaxial leaf morphological traits differ from the *Gerbera* species: (1) they are different from *Gerbera* sections sampled [sect. *Lasiopus* (4 species), sect. *Piloselloides* (2 species) and sect. *Pseudoseris* (1 species)] which have stomates and stiff, straight, upright trichomes. Figure 1 has images of one sample for each section: *G. ambigua* (Fig. 1A), *G. piloselloides* (Fig. 1B) and *G. perrieri* (Fig. 1D), respectively. (2) they are different from the members of *Gerbera* sect. *Gerbera* which have stomates and soft, thin and appressed trichomes. Three species from South Africa were examined and represented by *G. crocea* (Fig. 1C). (3) they are different from the Asian *Gerbera* sect. *Isanthus* which have no stomates and no trichomes based on this study of five species of sect. *Isanthus* that were examined in the study and are represented by *G. maxima* (Fig. 1E): the authors' observations agree with Lin et al. (2008) for the Asian species *G. delavayi*. Additionally, the morphological traits of *G. hieracioides* differ significantly from those of the *Trichocline* species, which have many stomates with guard cells sunken on the leaf surface, illustrated by *T. catharinensis* (Fig. 1H). However, the two tested *G. hieracioides* samples share the same adaxial leaf epidermal characters such as soft, thin and appressed trichomes, epidermal cell shape and striations and absence of stomates, with the three examined *Chaptalia* species, as represented by *C. pringlei* (Fig. 1F). Therefore, based on the adaxial leaf epidermal morphology, *G. hieracioides* is most closely related to *Chaptalia* rather than to *Gerbera* or *Trichocline*.

Scape and floret morphology. The results (Table 1) showed that the two examined samples of *Gerbera hieracioides* have bracteate scapes and trimorphic capitula which have the inner rays with corollae shorter than the styles (Fig. 2G, H). The above morphological traits also differ from those of the *Gerbera* species: (1) *Gerbera* sect. *Lasiopus*, sect. *Piloselloides* and sect. *Pseudoseris* have ebracteate scapes and trimorphic capitula and the inner rays have corollae as long as the styles or longer. *Gerbera jamesonii* (Fig. 2A) and *G.*

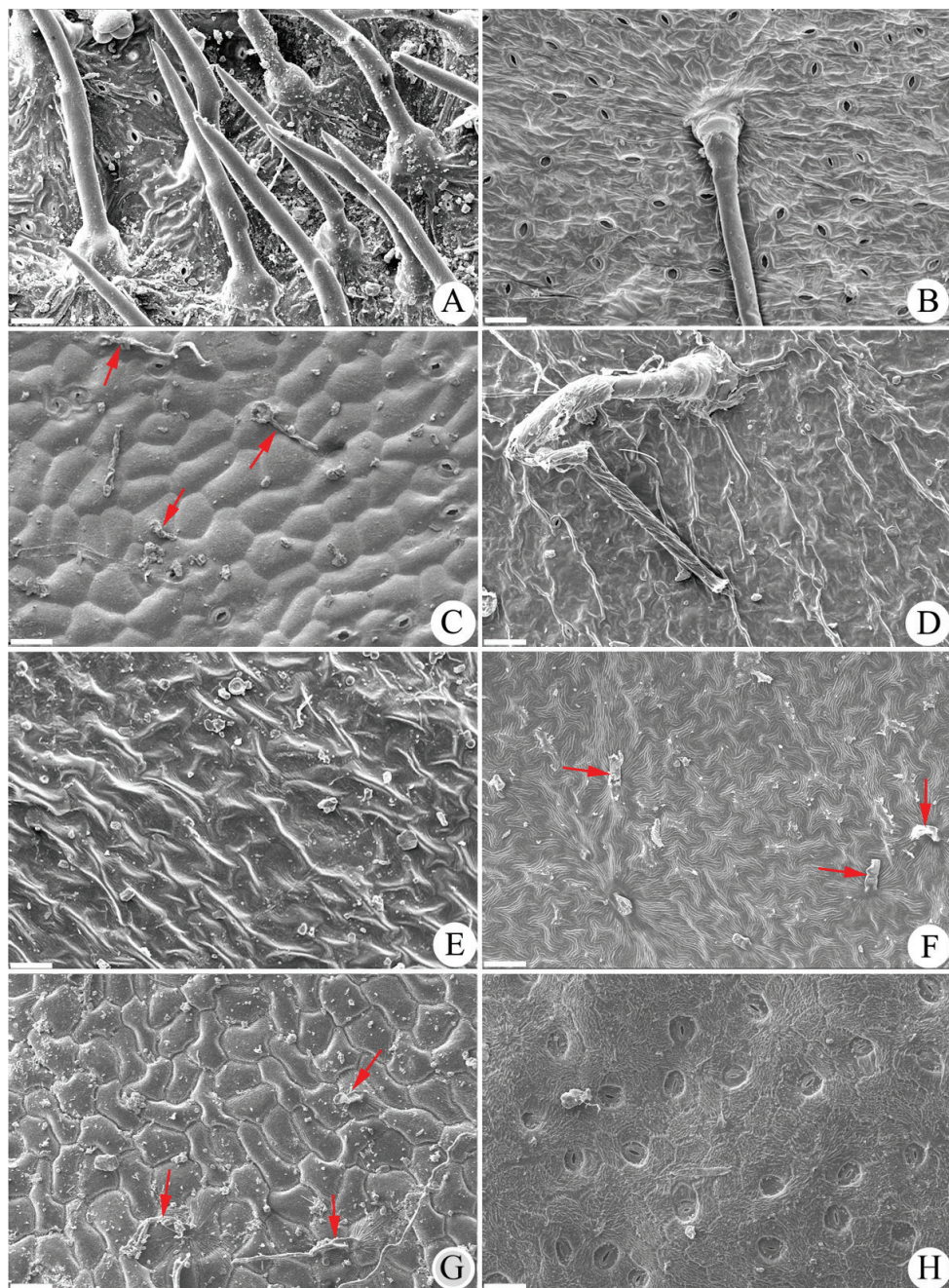


Figure 1. Adaxial leaf epidermal surface morphology of *Gerbera hieracioides* and the related species. **A** *G. ambigua* (sect. *Lasiopus*) **B** *G. piloselloides* (sect. *Piloselloides*) **C** *G. crocea* (sect. *Gerbera*) **D** *G. perrieri* (sect. *Pseudoseris*) **E** *G. maxima* (sect. *Isanthus*) **F** *Chaptalia pringlei* **G** *G. hieracioides* **H** *Trichocline catharinensis*. Arrows point to the soft thin trichomes. Scale bar=50 μm .

ambigua (Fig. 2B) belong to sect. *Lasiopus* and *G. cordata* (Fig. 2C) for sect. *Piloselloides*. (2) they are different from *Gerbera* sect. *Gerbera* and sect. *Isanthus*, which have bracteate scapes but dimorphic capitula without inner rays of florets. Three South African species and five Asian species were examined and are illustrated by *G. crocea* (Fig. 2D), *G. nivea* (Fig. 2E) and *G. gossypina* (Fig. 2F). The two tested *G. hieracioides* samples share the traits of bracteate scapes and trimorphic capitula which have inner rays with corollae shorter than the styles with the three tested *Chaptalia* species, represented by *C. meridensis* (Fig. 2I) and *C. mandonii* (Fig. 2J). Therefore, based on the scape and floret morphology, *G. hieracioides* should be best considered as a species of *Chaptalia* rather than *Gerbera*.

Phylogenetic analysis. The MrBayes analysis of the combined nuclear markers and two plastid genes showed four clades of the sampled species of the *Gerbera*-complex, all with a strong biogeographic signal (Fig. 3): (1) the African and Australian species of the *Gerbera* complex (African *Gerbera* species are sister to the Australian *Amblysperma*), (2) the American genus *Chaptalia* and the South American *Gerbera hieracioides*, (3) the Asian *Gerbera* species and (4) the South American genus *Trichocline*. However, there is no well-supported resolution amongst the first three clades mentioned above, so no conclusions can be made about the monophyly of *Gerbera* at this time.

Both samples of *Gerbera hieracioides* were nested within the *Chaptalia* clade. *Gerbera hieracioides* is sister to *Chaptalia pringlei*; then the *G. hieracioides*-*C. pringlei* clade is sister to the other *Chaptalia* species with strong support (posterior probability of 1.00). Therefore, the molecular data also support the placement of *G. hieracioides* in *Chaptalia*.

Discussion

The molecular phylogeny of the *Gerbera*-complex showed that *G. hieracioides* did not group with *Trichocline* (Fig. 3) but was nested inside *Chaptalia*. Furthermore, the leaf adaxial epidermis of *G. hieracioides* has no stomates, while that of *Trichocline* usually has many stomates (Fig. 1). In addition, Katinas (2004) presented a key to distinguish the genera of the *Gerbera*-complex and *Gerbera* and *Chaptalia* were found to share the same trait of achenes rostrate at the apex but this is not found in *Trichocline*.

The confusion about the placement of *Gerbera hieracioides* is no doubt the result of the morphology falling between that of *Gerbera* and *Chaptalia*. A good case concerning this point is the inner ray florets of the trimorphic capitula: *Gerbera* has a corolla as long as the style or longer and the staminodes are present, whereas *Chaptalia* has the corolla shorter than the style and without staminodes (Katinas 2004). As for *G. hieracioides*, the inner ray florets have moderately reduced stamens (Fig. 2G, H) which are different from both *Gerbera* and *Chaptalia* species. Although the stamen morphology of *G. hieracioides* is not identical to *Chaptalia*, their moderately reduced corollae (Fig. 2G, H) are similar to those of *Chaptalia* rather than those of *Gerbera*, according to Katinas (2004). Furthermore, the characters of leaf adaxial epidermis of *G. hieracioides* including the lack of stomates and the presence of soft thin trichomes, as well as bracteate scapes and cell shape and striations, all suggest that the species is closest to



Figure 2. Scape and floret morphology of *Gerbera hieracioides* and the related species. **A** *G. jamesonii* (sect. *Lasiopus*) **B** *G. ambigua* (sect. *Lasiopus*) **C** *G. cordata* (sect. *Piloselloides*) **D** *G. crocea* (sect. *Gerbera*) **E** *G. nivea* (sect. *Isanthus*) **F** *G. gossypina* (sect. *Isanthus*) **G** *G. hieracioides* (Ecuador) **H** *G. hieracioides* (Peru) **I** *Chaptalia meridensis* **J** *C. mandonii*. The arrows mark the styles of inner ray florets.

Chaptalia. Additionally, Hansen (1990) stated that the achene hairs of *G. hieracioides* are sub-inflated with a lower L/W-ratio than that of *Gerbera*. Therefore, the morphological data support the transfer of *G. hieracioides* to *Chaptalia* that was consistent with the molecular phylogeny (Fig. 3) based on both nuclear ITS and ETS and chloroplast *trnL-trnF* and *trnL-rpl32*. This transfer is in agreement with the geographic distribution (Fig. 3), because *G. hieracioides* is from South America and all the other *Chaptalia* species are from the New World (Nesom 2004b, 1995).

Chaptalia is a New World genus and contains about 70 species in the Americas (Funk et al. 2016). Although there are partial regional treatments, there is no comprehensive monograph of the genus (e.g. Burkart 1944, Cabrera and Nesom 2003, Nesom 2004a, b). Hansen (2006) argued that the most significant problem of the *Gerbera*-complex is the lack of a revisionary treatment of *Chaptalia* and argued for further studies to test whether *Chaptalia* is monophyletic. In the molecular analysis (Fig. 3), the nine *Chaptalia* samples (including *G. hieracioides*) grouped into two well-supported clades. This result indicates that *Chaptalia* seems to be monophyletic when *G. hieracioides* is included. *Chaptalia* is typically characterised by differentiated and reduced rays (Hansen 1990): the inner ray florets with corolla strongly reduced, filiform (irregularly tubular, ligulate or bilabiate), shorter than the style and without staminodes (Katinas 2004). The inner ray florets of *G. hieracioides* with moderately reduced corollae and stamens suggest that the inner ray florets of trimorphic capitula may be a key morphological character for the further revisionary treatment of *Chaptalia*.

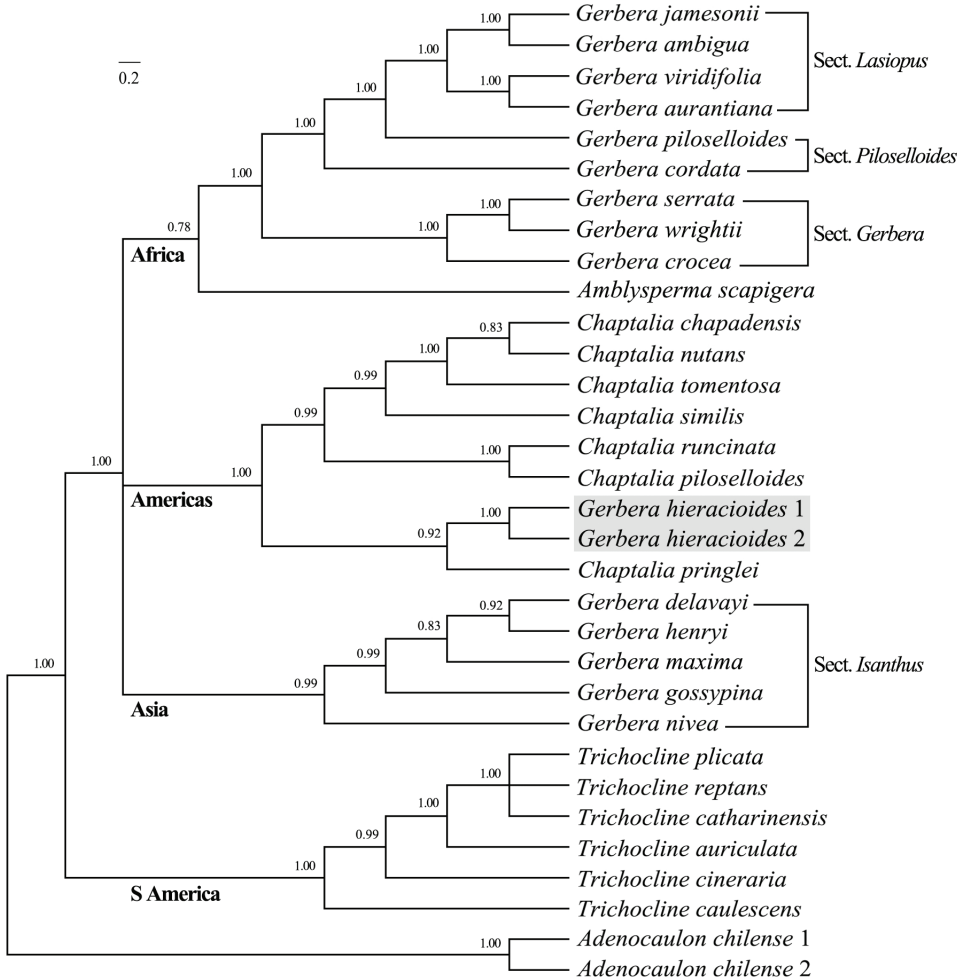


Figure 3. Phylogeny of *Gerbera hieracioides* and the related species. The phylogeny is based on the MrBayes analysis of the combined ITS and ETS, *trnL-trnF* and *trnL-rpl32* markers. The posterior probabilities support values are shown next to branches.

As for *Gerbera*, this study showed that it falls into two distinct clades, one from Africa which is the sister group of the Australian genus *Amblysperma* and the other contains all the Asian *Gerbera* (Fig. 3). However, the two *Gerbera* clades are in a trichotomy with the *Chaptalia* clade. It is clear that, based on the sampling, the Asian taxa may be best separated out into a separate genus then *Amblysperma* is the sister genus of African *Gerbera*. If the two clades of *Gerbera* form a single clade, then *Amblysperma* will most likely be nested within that clade. The decision must wait for ongoing studies using additional data. However, it is clear that *Gerbera hieracioides* should be considered within *Chaptalia*.

Taxonomic treatment

***Chaptalia hieracioides* (Kunth) X.-D.Xu & W.Zheng, comb. nov.**

urn:lsid:ipni.org:names:60476046-2

Basionym: *Onoseris hieracioides* Kunth, Nov. Gen. Sp. [H. B. K.] 4 (ed. folio): 5, Tab. 304. 1818; 4 (ed. quarto): 7, Tab. 304. 1820. **Type:** Ecuador: “Alousi”, A.J.A. Bonpland 3233 (Lectotype: P00322236, here designated).

Trichocline peruviana Hieron., Bot. Jahrb. Syst. 21: 368. 1895. [according to IPNI]

Trichocline hieracioides (Kunth) Ferreyra, J. Arnold Arbor. 25: 394. 1944, comb. illeg. non Baker (1884).

Gerbera hieracioides (Kunth) Zardini, Bol. Soc. Argent. Bot. 16(1–2): 105. 1974.

Trichocline beckeri (as ‘beckeri’) H.Rob., Phytologia 65(1): 47. 1988.

Conclusions

The placement of *Gerbera hieracioides* within *Chaptalia* is strongly supported by both the molecular sequence data (two nuclear markers ITS and ETS and two chloroplast markers *trnL–trnF* and *trnL–rpl32*) and the morphology of the scape, capitula and the leaf adaxial epidermal surface. Therefore, *Gerbera hieracioides* has been transferred to *Chaptalia* and it is recognised as *Chaptalia hieracioides* (Kunth) X.-D. Xu et W. Zheng.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 31560086), the China Scholarship Council, the Social Science Foundation of Kunming University of Science and Technology (no. kkz3201655009) and the Laboratory of Analytical Biology of the Smithsonian Institution. We are grateful to Marinda Koekemoer (South African National Biodiversity Institute) and Yousheng Chen (Institute of Botany, the Chinese Academy of Sciences, China) for providing samples and AJ Harris, Stanley Yankowski, Scott Whittaker, Carol Kelloff, Harold Robinson and Gabriel Johnson (all of the Smithsonian Institution) and Yuan Xu (South China Botanical Garden, China), Sayed Afzal Shah (Quaid-i-Azam University, Pakistan) and Zhumei Ren (Shanxi University, China) for their assistance with experiments, data analyses and helpful discussions. We also appreciate the suggestions and advice from two reviewers and the subject editor Alexander Sukhorukov.

References

- Baird KE, Funk VA, Wen J, Weeks A (2010) Molecular phylogenetic analysis of *Leibnitzia* Cass. (Asteraceae: Mutisieae: *Gerbera*-Complex): An Asian-North American disjunct genus. *Journal of Systematics and evolution* 48: 161–174. <https://doi.org/10.1111/j.1759-6831.2010.00077.x>
- Baldwin BG, Markos S (1998) Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463. <https://doi.org/10.1006/mpev.1998.0545>
- Burkart A (1944) Estudio del género de Compuestas *Chaptalia* con especial referencia a las especies argentinas. *Darwiniana* 6: 505–594.
- Downie SR, Katz-Downie DS (1996) A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 83: 234–251. <https://doi.org/10.1002/j.1537-2197.1996.tb12701.x>
- Ferreira R (1944) Revisión del género *Onoseris*. *Journal of the Arnold Arboretum* 25: 349–395.
- Funk VA, Pasini E, Bonifacino JM, Katinas L (2016) Home at last: The enigmatic genera *Eriachaenium* and *Adenocaulon* (Compositae, Mutisioideae, Mutisieae, Adenocaulinae). *PhytoKeys* 60: 1–19. <https://doi.org/10.3897/phytokeys.60.6795>
- Funk VA, Sancho G, Roque N, Kelloff CL, Ventosa-Rodríguez I, Diazgranados M, Bonifacino JM, Chan R (2014) A phylogeny of the Gochnatieae: Understanding a critically placed tribe in the Compositae. *Taxon* 63: 859–882. <https://doi.org/10.12705/634.27>
- Hansen HV (1985a) A taxonomic revision of the genus *Gerbera* (Compositae–Mutisieae) sections *Gerbera*, *Parva*, *Piloselloides* (in Africa) and *Lasiopus*. *Opera Botanica* 78: 5–36.
- Hansen HV (1985b) Notes on *Gerbera* sect. *Pseudoseris* (Compositae, Mutisieae). *Nordic Journal of Botany* 5: 451–453. <https://doi.org/10.1111/j.1756-1051.1985.tb01675.x>
- Hansen HV (1988) A taxonomic revision of the genera *Gerbera* sect. *Isanthus*, *Leibnitzia* (in Asia), and *Uechtrizia* (Compositae, Mutisieae). *Nordic Journal of Botany* 8: 61–76. <https://doi.org/10.1111/j.1756-1051.1988.tb01707.x>
- Hansen HV (1990) Phylogenetic studies in the *Gerbera*-complex (Compositae, tribe Mutisieae, subtribe Mutisiinae). *Nordic Journal of Botany* 9: 469–485. <https://doi.org/10.1111/j.1756-1051.1990.tb00537.x>
- Hansen HV (2006) Comments on the *Gerbera*-complex (Asteraceae: Mutisieae). *SIDA, Contributions to Botany* 22: 539–543. [jstor.org/stable/41968606](https://www.jstor.org/stable/41968606)
- Johnson IM, Crouch NR, Edwards TJ (2014) *Gerbera sylvicola* (Asteraceae: Mutisieae), a new forest species from KwaZulu-Natal, South Africa. *Phytotaxa* 186: 229–235. <https://doi.org/10.11646/phytotaxa.186.4.7>
- Katinas L (2004) The *Gerbera*-complex (Asteraceae, Mutisieae): To split or not to split. *SIDA, Contributions to Botany*: 935–940. [jstor.org/stable/41968349](https://www.jstor.org/stable/41968349)
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>

- Kunth KS (1818) *Onoseris hieracioides* Kunth. In: Humboldt A, Bonpland A, Kunth KS (Eds) *Nova Genera et Species Plantarum*, vol. 4 (ed. folio), Maze, Paris, [i–vii], [1]–247, pl. 301–412.
- Lin NN, Wang H, Li DZ (2008) Comparative morphology of leaf epidermis of the tribe Mutisieae (Compositae) in East Asia and its related groups. *Acta Botanica Yunnanica* 30: 51–63. <https://doi.org/10.3969/j.issn.2095-0845.2008.01.009>
- Nesom GL (1995) Revision of *Chaptalia* (Asteraceae: Mutisieae) from North America and continental Central America. *Phytologia* 78: 153–188.
- Nesom GL (2004a) Generic placement of *Chaptalia hintonii* (Asteraceae: Mutisieae). *Sida* 21: 929–933. [jstor.org/stable/41968348](https://www.jstor.org/stable/41968348)
- Nesom GL (2004b) Response to “The *Gerbera* complex (Asteraceae: Mutisieae): to split or not to split” by Liliana Katinas. *Sida* 21: 941–942.
- Pasini E, Funk VA, de Souza-Chies TT, Miotto STS (2016) New insights into the phylogeny and biogeography of the *Gerbera*-Complex (Asteraceae: Mutisieae). *Taxon* 65: 547–562. <https://doi.org/10.12705/653.7>
- Posada D (2008) jModelTest, phylogenetic model averaging. *Molecular Biology and Evolution* 257: 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut A, Drummond AJ (2009) Tracer v1.5. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. <https://doi.org/10.1007/BF00037152>
- Timme R, Kuehl EJ, Boore JL, Jansen RK (2007) A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identification of divergent regions and categorization of shared repeats. *American Journal of Botany* 94: 302–313. <https://doi.org/10.3732/ajb.94.3.302>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (Eds) *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Zardini EM (1974) About the presence of the genus *Gerbera* in America. *Boletín de la Sociedad Argentina de Botánica* 16: 103–108.
- Zardini EM (1975) Revisión del género *Trichocline* (Compositae). *Darwiniana* 19: 618–733.

A new lectotype for *Passiflora laurifolia* L.

Maxime Rome¹, Geo Coppens d'Eeckenbrugge^{2,3}

1 Université Grenoble Alpes, CNRS, SAJF, F-38000 Grenoble, France **2** CIRAD, UMR AGAP, Avenue Agropolis, 34398 Montpellier, France **3** AGAP, Université de Montpellier, CIRAD, INRA, Montpellier SupAgro, Montpellier, France

Corresponding author: Geo Coppens d'Eeckenbrugge (geo.coppens@cirad.fr)

Academic editor: Y. Mutafchiev | Received 16 November 2017 | Accepted 8 February 2018 | Published 15 February 2018

Citation: Rome M, d'Eeckenbrugge GC (2018) A new lectotype for *Passiflora laurifolia* L.. PhytoKeys 95: 107–120. <https://doi.org/10.3897/phytokeys.95.22324>

Abstract

From the "Description des plantes d'Amérique" by Plumier, in 1693, to the "Species Plantarum" by Linnaeus in 1753, several dubious synonymies of *Passiflora laurifolia* L. were proposed, generating a persisting confusion. A revision of the process, which led to the Linnaean description of this species, shows that the type finally chosen by Cusset does not match the original description. A new lectotype for the species and a more complete description are proposed from field observations, herbarium and bibliographic data.

Keywords

Passifloraceae, subgenus *Passiflora*, series *Laurifoliae*

Introduction

Amongst Passifloraceae Jussieu (1805) ex Roussel (1806), *Passiflora* Linnaeus (1753) is the most important genus with about 576 species, mostly distributed in tropical America (Krosnick et al. 2013). It includes lianas with tendrils, trees and shrubs, with alternate leaves, axillary stipules, extra-floral nectaries on the petiole and/or the surface of the leaves or even bracts and flowers with crowns of filaments and an androgynophore (Ulmer and MacDougal 2004). Five subgenera are currently recognised: *Passiflora*, *Astrophea* (Candolle, 1822) Masters (1872), *Decaloba* (Candolle, 1822) Reichenbach

(1828), *Deidamioides* (Harms, 1925) Killip (1938) and *Tetrapathea* (Candolle, 1822) P.S. Green (1972) (Krosnick et al. 2009), each one subdivided into supersections, sections and/or series.

Supersection *Laurifolia* (Cervi, 1997) Feuillet and MacDougal (2003) is part of subgenus *Passiflora*. It includes series *Laurifoliae* Killip (1938) ex Cervi (1997), which forms a morphologically very homogenous group, with a very difficult taxonomy. It is composed of 24 species including glabrous to pubescent plants, with stems that are terete to angular and sometimes corky on old parts; leaves that are entire, oblong-lanceolate, not peltate, with entire to glandular-serrulate margins, biglandular petioles; and stipules that are setaceous or linear, early deciduous. Their three bracts, free at the base, with entire or serrulate-glandular margins, are more than 1 cm long. Their flowers are pendent, usually large and showy, often fragrant with a short hypanthium and two campanulate series of long external filaments and a variable number of series of reduced internal filaments (Rome and Coppens d'Eeckenbrugge 2017). They are valued for their soft and sweet pulp and some species are grown commercially, including *P. laurifolia*.

Passiflora laurifolia Linnaeus (1753) was one of the earliest passion flowers to be described and, logically, the first one in the series *Laurifoliae*. It was first mentioned and illustrated in the “Description des plantes d'Amérique” by Plumier (1693), amongst 12 passion flower species from the Caribbean, under the polynomial *Clematis indica, fructu citriformis, foliis oblongis* (climbing plant from the Indies, with lemon-shaped fruits and oblong leaves). Linnaeus (1753) recognised Plumier's polynomial, as well as several other polynomials and illustrations under his *P. laurifolia*; however, as stated by Killip (1938), this species is not represented by a true type in the Linnaean Herbarium and several Linnaean specimens correspond to the very similar *P. nitida* Kunth (1817), another member of the series *Laurifoliae*. Thus, when Cusset (1967) finally typified *P. laurifolia*, he chose, amongst Linnaean materials, an illustration on plate 21 by Merian (1705) representing in fact a specimen of *P. nitida* from Suriname.

Here, the pre-Linnaean and Linnaean treatments of *P. laurifolia* are revised to support the choice of Plumier's illustration as a new lectotype for this species. Furthermore, the authors compare specimens from the Antilles and South-America to ascertain that they are conspecific and correspond to the successive descriptions of *P. laurifolia*, discarding any geographical reason for the ancient confusion. A range of materials of *P. nitida* is also examined. From these observations, a complete description for *P. laurifolia* is presented.

Materials and methods

Three specimens from the herbarium of the London Linnaean Society (LINN) were observed: Linnaeus (*P. laurifolia*), not numbered, of unknown origin, number 1070.2 in the herbarium of the London Linnaean Society; Linnaeus 74 (*P. nitida*), from Suriname; and Linnaeus 152, (*Passiflora* sp.), of unknown origin.

Other materials of *P. laurifolia* examined include 28 herbarium specimens from the Antilles and South America, as well as ten living specimens of *P. laurifolia* with flowers and fruits in Martinique and Guadeloupe. Other materials of *P. nitida* examined include 50 herbarium specimens from South America and living plants with flowers and fruits from eight populations in French Guiana. Examined materials are presented in more details in Appendix 1 and Appendix 2.

Results and discussion

Pre-Linnaean treatment

The authors transcribe here the long and precise description of *P. laurifolia* in French by Plumier (1693) giving the colour and size of each part of the plant. The plant is woody with only one leaf or fragrant flower at each node. The leaves are ovate-elongated, slightly pointed at apex and indented at base, with two glands at the petiole apex. The flowers are enclosed in three green membranaceous bracts [“dans trois feuilles vertes membranées”]. The perianth is white, densely dotted with brown red inside. The two outer series of filaments are not equal, the innermost being about 3.75 cm long and the outermost over 1.25 cm long. They are purple on the distal half and striped with red and purple on their proximal half. Inside the flower, there are two other, very short, slender and whitish series of filaments. The androgynophore is yellowish, marbled with red, with three red styles, three yellow stigmas, a yellow ovary and five stamens with whitish anthers. The fruit is the size of a chicken egg, with three longitudinal ribs, turning orange with many tiny dots at maturity. Plumier describes its pericarp as thin, leathery, [“de l'épaisseur et la consistance d'un gros cuir molasse indiquant que l'enveloppe du fruit n'est pas très épaisse”] and pubescent [“écorce cotonnée par dehors”]. The whitish pulp is mucilaginous and sweet and contains black heart-shaped seeds. The species is cultivated in home gardens and the fruits are ripe from April to May. Plumier's description of *P. laurifolia* was well illustrated by the drawing presented at Figure 1.

In addition, Plumier (1693) pointed out that the fruit is very similar to those of the “murucuia guaçu” (meaning large passion fruit) of Georg Marcgrave in Ray (1686) and the “murucuia guaçu” of Piso (1648), raising the possibility that they could belong to the same species. However, these two species do not have simple leaves and they cannot even match any species description within series *Laurifoliae*.

Plukenet (1696) considered Plumier's ‘*Clematis indica, fructu citriformis, foliis oblongis*’ a synonym of ‘*Passiflora arborea Laurinis foliis*’ represented in table 211 in the book of Plukenet (1692). Indeed, the latter includes a drawing of a passion flower with oblong leaves and two glands at the petiole apex; however there is no textual description of the species and the absence of flowers in the drawing allows no further identification of the species. Additionally, Plukenet (1696) considered two other synonyms: Marcgrave's ‘*Murucuja* 4.s. *Pyriformis altera*’ and an illustrated description of ‘Quauh Chichic Patli’ by Hernández de Toledo (1651). However, while the synonymy of the

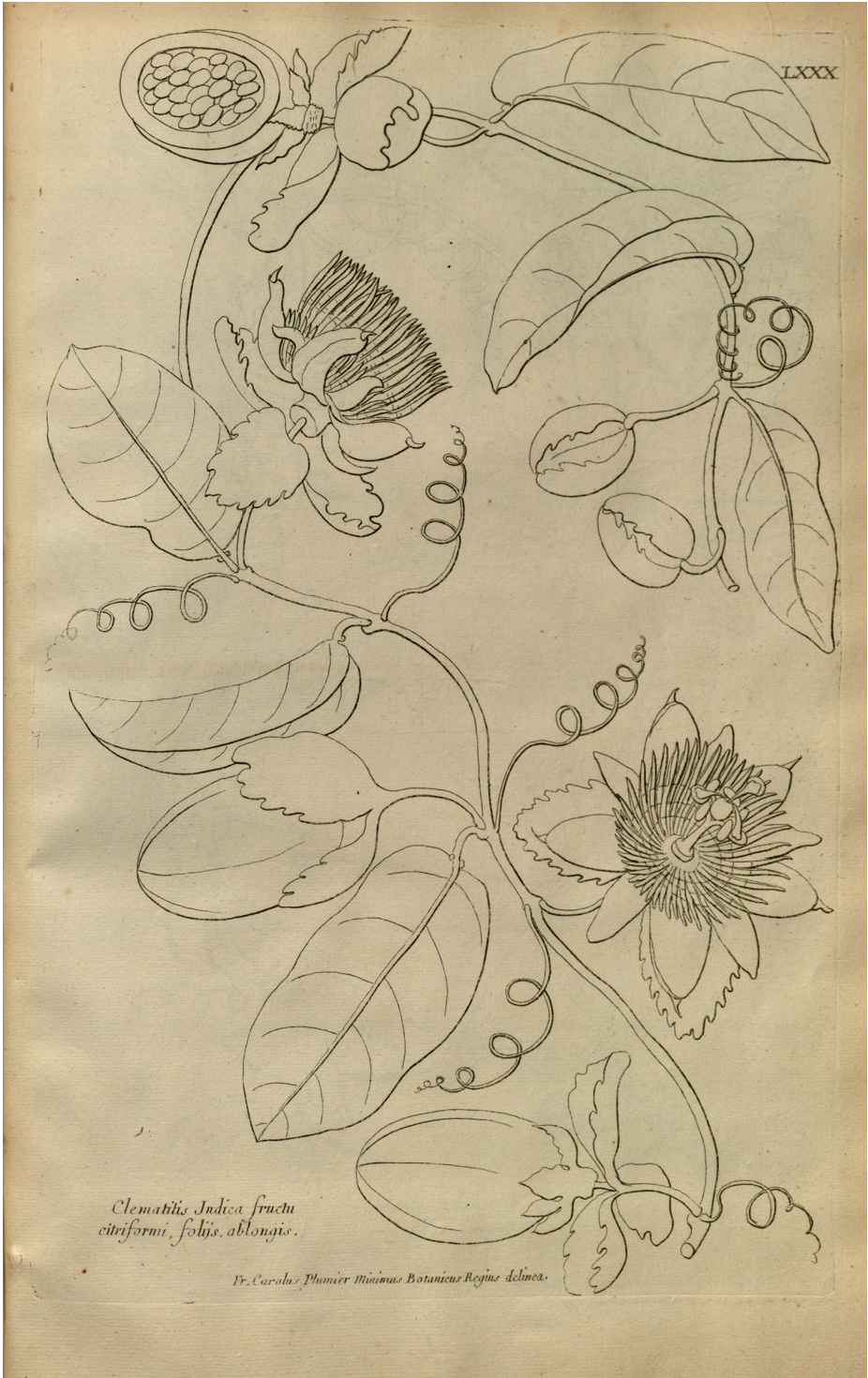


Figure 1. Illustration of *Passiflora laurifolia* in “Description des Plantes d’Amérique” by Plumier (1693).

former cannot be verified because it gives no indication of the position of petiolar glands, the drawing of the latter shows a plant with opposite leaves, which cannot belong to the genus *Passiflora*.

Van Royen (1740) described several species from the Leiden Botanical Garden and its surroundings. Amongst these, *Passiflora laurifolia* sensu Plumier (1693) was renamed '*Passiflora foliis solitariis oblongis intergerrimis, floribus solitariis, involucro tripartite dentate*'. Van Royen (1740) added 'Haec duas habet sub basi glandulas convexas'. In his collection deposited at the National Herbarium of the Netherlands and inventoried by Thijssen and Veldkamp (2003), there is no voucher material of *Passiflora laurifolia*.

Linnaeus (1749) named *Passiflora laurifolia* as '*Passiflora foliis indivisis intergerrimis, involucris dentatis*' (*Passiflora* with undivided leaves and dentate involucre). Amongst synonyms, he quoted the above-mentioned polynomials of Plumier (1693), Plukenet (1692) and van Royen (1740), as well as '*Granadilla fructu citriformi, foliis oblongis*' of Tournefort (1700) and an illustration by Merian (1705, pl. 21), where plants are drawn with their pollinator and predator insects. In the legend, Merian (1705) named the plant under the vernacular name '*marquiaas*', which is related to the generic vernacular terms *muruciá* and *maracuyá* (cf. Cuvier, 1823), used for all passion fruits across South America. On the drawing by Merian (1705), presented in Figure 2, several diagnostic traits are inconsistent with the description of Plumier (1693): flowers with white perianth (instead of flowers densely dotted with brown red inside) and equal (instead of unequal) outer series of blue corona filaments. The ovoid fruit is uniform yellow or green when unripe (instead of dotted with darker ribs), with a thick mesocarp (instead of a thin one).

Linnaeus (1749) specified that *P. laurifolia* has glabrous and undulate to flat leaves and that bracts are of the same length as the flowers [*"involucrum magnitudine floris"*], which was a new observation in relation to Plumier's description.

Linnaean treatment

In *Species Plantarum*, Linnaeus (1753) maintained his description of *P. laurifolia* from *Amoenitates academicae*, with the same synonyms but he did not designate a type of this species. He considered the species as only native to Suriname although he based his species' description on that of Plumier from Caribbean specimens. Moreover, the white perianth, the two equal outer series of filaments and the uniformly green immature fruit with a thick pericarp, show that Merian's '*marquiaas*' belongs to *P. nitida*.

Killip (1938) specified that *P. laurifolia* is not represented by a true type in the Linnaean Herbarium even if the latter includes three specimens identified as belonging to this complex group. Only one of them corresponds unambiguously to *P. laurifolia*; this is Linnaeus nn (no collection number; number 1070.2 in the herbarium of the London Linnaean Society), determined as *P. pallida* by Linnaeus, with a note from the hand of J.E. Smith: "*laurifolia* Jacq. non Linn.". Thus, Smith indicated that the herbarium specimen does not match the description of *P. laurifolia* by Linnaeus but



Figure 2. Illustration of the 'marquiaas' in "Metamorphosis Insectorum Surinamensium" by Merian (1705).

corresponds to that of Jacquin (1776) in *Hortus Vindobonensis* where this author specifies that *P. laurifolia* has two series of filaments, the outer series being shorter. Below the first annotation, "*pallida*" can be read, hand-written by Linnaeus and next to that

name, another annotation from Smith: “non Plumeri icon”). Here, Smith referred to an illustration of Plumier, cited in Linnaeus’ description of *Passiflora pallida* Linnaeus (1753), a species of subgenus *Decaloba*. This suggests that Linnaeus was not comfortable with this group of plants.

The specimen Linnaeus 152, collected in Suriname, was determined by Linnaeus as “*Passiflora dubia*” (doubtful *Passiflora*) in *Plantae Surinamensis* (1775). In this book, he gave a brief description of the plant that does not allow the determination of the species in the absence of bracts. The specimen Linnaeus 74, from Suriname, also noted as “*Passiflora dubia*” in Linnaeus’ *Plantae Surinamenses*, is in fact *P. nitida* (the fourth inner series of filaments close to the nectary chamber of the flower is easily observable on the specimen), the species described by Kunth in 1817.

Post-linnean treatment

In his *American species of Passifloraceae*, Killip (1938) simply followed Linnaeus, maintaining the contradiction between the description of *P. laurifolia* taken from Plumier and Merian’s illustration representing *P. nitida*. Later, Cusset (1967) mentioned *P. laurifolia* in *Flore du Cambodge du Laos et du Vietnam*, considering Merian’s drawing as the type of this species.

Analysis of herbarium and living specimens

The authors’ literature review and observations confirm that *P. laurifolia* is present in all of the Caribbean islands; in fact, it is the only species of series *Laurifoliae* in the Antilles. Regarding its presence on the South American continent, the examined herbarium specimens document its presence in Suriname, Guyana, Venezuela and Brazil. A detailed analysis of the morphology of *P. laurifolia* from the Caribbean and South America shows that the internal structure of the flower is identical in both regions (Figure 3).

A comparison of the successive descriptions of *P. laurifolia* shows that the original one, provided by Plumier (1693) is the most complete and comprehensive and constitutes the common reference linking all subsequent descriptions of the species. Even now, it allows the discrimination of *P. laurifolia* amongst the 24 species composing series *Laurifoliae* (Rome and Coppens d’Eeckenbrugge 2017). The species described by Piso (1648), Hernández de Toledo (1651) and Marcgrave in Ray (1686) are to be invalidated and the corresponding polynomials cannot be considered as earlier synonyms. The descriptions of van Royen (1740) and Plukenet (1696) are too imprecise, their unique interest being that they refer to that of Plumier (1693). Linnaeus’ descriptions (1749, 1753) of *P. laurifolia* were based on the morphology of two different species, the one described by Plumier (1693) and an iconography of a plant that turned out to be *Passiflora nitida*. This confusion has persisted until now, with the treatments of Killip (1938) and Cusset (1967). According to the article 9.19(b) of the Interna-

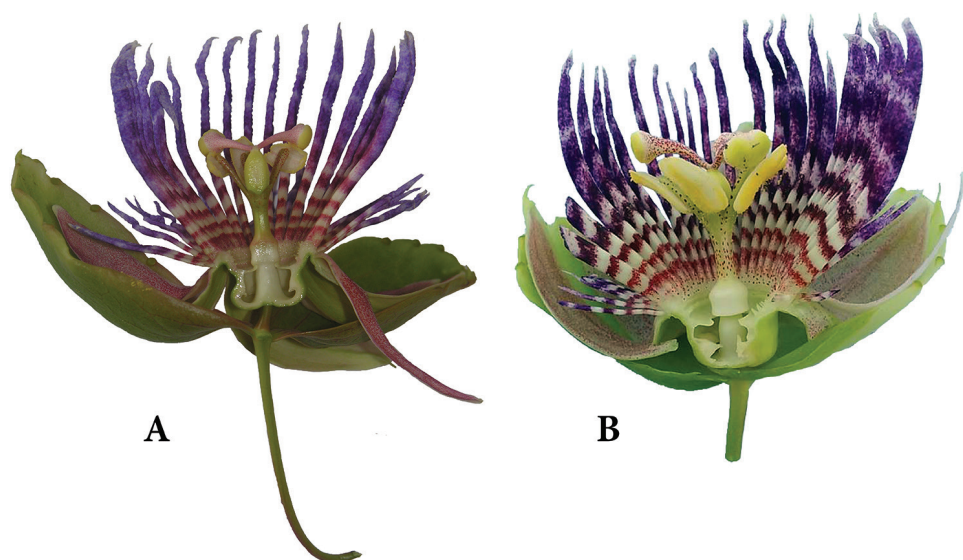


Figure 3. *Passiflora laurifolia*. **A** Flower from Trujillo, Venezuela (photograph courtesy of M. Molinari) **B** Flower from Le Moule, Guadeloupe (photograph courtesy of F. Booms).

tional Code of Nomenclature (McNeill et al. 2012), the choice of the lectotype may also be superseded if one can show that it is in serious conflict with the protologue and another element is available that is not in conflict with the protologue. Thus, the iconotype of Merian (1705) is replaced here by that of Plumier (1693). A complete description of *P. laurifolia* is also presented here, based on that of Plumier and examination of materials from the Antilles and the Guianas.

***Passiflora laurifolia* L. Sp. Pl. 956. 1753**

Lectotype. Plum. PL. Amer. Pl. 80. 1693, from the Antilles, very probably the Martinique island where Plumier worked. Designated here.

Description. Liana strong. Stem terete, glabrous and green; internodes 3.5–14 cm long. Tendrils cone-shaped, glabrous. Stipules linear, falcate, green yellowish to green brown, eglandular to glandular (0–2 glands), glabrous, 5.5–13.2 × 0.4–1 mm (including an arista, 0–1.8 mm long), deciduous. Petiole 1.1–2.9 cm long, green to dark green, slightly caniculate adaxially, glabrous, with two oval sessile glands situated at petiole apex. Leaves simple, 7.3–14.2 × 4.4–7.5 cm, glabrous throughout, green to dark green, adaxial surface lustrous, rounded to cordate at base, acute (angle within 45 to 90°) at apex, slightly acuminate and mucronate; leaf margin entire or glandular (7–25 marginal nectaries). Peduncles 1.5–7.4 cm long, terete, green, glabrous to slightly pubescent, strong (diameter about 1.3–2.4 mm);

pedicel 6.7–15 mm long. Bracts permanent (until fruit maturity), slightly pubescent on both sides, green, concave, 2.8–5.5 cm long, 2.2–4.9 cm wide (same length as flowers), with 4–11 marginal nectariferous sessile glands in distal half. Flowers axillary, pendulous, 22–33 mm long (from the nectary chamber to the ovary apex). Hypanthium slightly pubescent, green outside and white inside, 2.59–5.94 mm, with a diameter of 10.35–16.64 mm at the base of sepals. Nectary chamber slightly pubescent, green outside and white inside, 3.1–5.9 mm long, with a diameter of 8.8–17.9 mm. Sepals glabrous, oblate, 2.9–5.1 cm long, 1–1.7 cm wide, adaxial surface white with a sparse to very dense red punctuation, abaxial surface green with red-brown dots, slightly keel-shaped in distal half with a short awn (1–2 mm long). Petals glabrous, oblate, 2.8–4.9 cm long, 0.7–1.2 cm wide, white with a sparse to very dense red punctuation. Corona filaments in 5–7 series, banded white and red to dark purple (purple on the distal half and striped with red to purple on their proximal half); two major series, slightly curved, the outer series 12–30 mm long, the second series 24–43 mm long; others series about 1 mm long. Ovary pubescent, yellow to green, 7–11 mm long; styles, whitish with red purple dots, 8–13 mm long, stigmas light yellow to green. Stamens 7–11 mm long. Androgynophore glabrous, white greenish with red dots, 10–16 mm long with an enlarged base about 10 mm wide. Operculum membranaceous, 0.5–1.1 mm long, recurved, shortly fimbriated at margin. Fruit ovoid, pubescent, 4.7–8.4 cm long, 3.8–6.3 cm in diameter, round to triangular transversal section, epicarp about 0.5–0.9 cm thick; immature fruits green with white dots and with six longitudinal ribs (three of them conspicuous); mature fruits yellowish orange with many tiny light orange dots. Pulp translucent and sweet. Seeds heart-shaped, black.

Conclusion

The original description of *P. laurifolia* by Plumier (1693) is the common reference linking all subsequent descriptions of the species. Linnaeus' descriptions (1749, 1753) involved two iconographies: the one from Plumier (1693) and one from Merian (1705) representing the closely related *Passiflora nitida*, described by Kunth (1817). The latter was mistakenly chosen by Cusset as the lectotype of *P. laurifolia*. To restore consistency, the iconotype of Merian has been replaced by that of Plumier (1693) and a more complete description of *P. laurifolia* has been presented from field observations, herbarium and bibliographic data.

Acknowledgements

The authors thank R. Govaerts of the Royal Botanic Garden, Kew, for his helpful suggestions and M. Molinari and F. Booms for their photographs of *Passiflora laurifolia* flowers.

References

- Candolle AP de (1821) 3. *Passiflora ligularis* (Juss.). Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 1: 434–436.
- Cervi AC (1997) Passifloraceae do Brasil: estudo do gênero *Passiflora* L. subgênero *Passiflora*. Fontqueria 45(1): 1–92.
- Cusset G (1967) Passifloraceae. In: Tardieu-Blot M-L, Cusset G (Eds) Flore du Cambodge, du Laos et du Viêt Nam. Tome 5. Muséum national d'Histoire naturelle, Paris, 101–155.
- Cuvier MF (1823) Dictionnaire des Sciences Naturelles. Tome 29. FG Levrault and LeNormant, Strasbourg and Paris. <https://doi.org/10.5962/bhl.title.42219>
- Feuillet C, MacDougal JM (2003) A new infrageneric classification of *Passiflora* L. (Passifloraceae). *Passiflora* 13(2): 34–38.
- Green PS (1972) *Passiflora* in Australasia and the Pacific. *Kew Bulletin* 26: 539–558. <https://doi.org/10.2307/4120317>
- Harms H (1925) Passifloraceae. In: Engler A, Prantl K (Eds) Die Natürlichen Pflanzenfamilien. Vol 21. Engelmann, Leipzig, 470–507.
- Hernández de Toledo F (1651) Rerum Medicarum Novae Hispaniae Thesaurus. Vitalis Mascardi, Rome. <https://doi.org/10.5962/bhl.title.53514>
- Jacquin NJ von (1776) Hortus Botanicus Vindobonensis seu Plantarum Rariorum. Vol 2. Typis Leopoldi Joannis Kaliwoda, Aulæ Imperialis Typographi, Wien. <https://doi.org/10.5962/bhl.title.531>
- Jussieu AL de (1805) Premier mémoire sur quelques espèces du genre *Passiflora*, et sur la nécessité d'établir une famille des passifloracées. *Annales du Muséum d'Histoire Naturelle*, 6: 102–116.
- Killip EP (1938) The American species of Passifloraceae. Part 2. Field Museum of Natural History, Chicago, 360–376.
- Krosnick SE, Ford AJ, Freudenstein JV (2009) Taxonomic revision of *Passiflora* subgenus *Tetrapathea* including the monotypic genera *Hollrungia* and *Tetrapathea* (Passifloraceae), and a new species of *Passiflora*. *Systematic Botany* 34(2): 375–385. <https://doi.org/10.1600/036364409788606343>
- Krosnick SE, Porter-Utley KE, MacDougal JM, Jørgensen PM, McDade LA (2013) New insights into the evolution of *Passiflora* subgenus *Decaloba* (Passifloraceae): phylogenetic relationships and morphological synapomorphies. *Systematic Botany* 38(3): 692–713. <https://doi.org/10.1600/036364413X670359>
- Kunth KS (1817) *Passiflora nitida*. In: Humboldt FWHA, Bonpland AJA, Kunth KS (Eds) Nova Genera et Species Plantarum (quarto ed.). Tome 2. Gide Filium, Bibliopolam, Paris. <https://doi.org/10.5962/bhl.title.640>
- Linnaeus C (1749) *Amoenitates Academicæ*. Volume 1. Cornelium Haak, Leiden. <https://doi.org/10.5962/bhl.title.910>
- Linnaeus C (1753) *Species Plantarum*. Volume 2. Impensis Laurentii Salvii, Stockholm. <https://doi.org/10.5962/bhl.title.727>
- Linnaeus C (1775) *Plantæ Surinamensis*. Typis Edmannianis, Uppsala.

- Masters MT (1872) Passifloraceae. In: Martius CFP, Eichler AG (Eds) *Flora Brasiliensis* 13. Fleischer, Leipzig.
- McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen S, Knapp S, Marhold K, Prado J, Prud'Homme Van Reine F, Smith GF, Wiersema JH (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), 18th International Botanical Congress Melbourne, Australia, July 2011 (electronic ed.). International Association for Plant Taxonomy, Bratislava. <http://www.iapt-taxon.org/nomen/main.php>
- Merian MS (1705) *Metamorphosis Insectorum Surinamensium*. G. Valk, Amsterdam.
- Piso W (1648) De Murucuja, variisque illius speciebus, & facultatibus (Cap. LXXIII). In: Piso W, Marcgrave G (Eds) *Historia naturalis Brasiliae*. Leiden et Lud. Elzevirium, Amsterdam, 106–107. <https://doi.org/10.5962/bhl.title.565>
- Plukenet L (1692) *Phytographia*. Volume 3. Sumptibus Autoris, London.
- Plukenet L (1696) *Almagestum Botanicum*. Sumptibus Autoris, London.
- Plumier C (1693) *Description des Plantes de l'Amérique*. Imprimerie Royale, Paris. <https://doi.org/10.5962/bhl.title.911>
- Ray J (1686) *Historia Plantarum*. Tomus primus. Clark, London.
- Reichenbach HGL (1828) C. Passifloreae, p. 132. *Conspectus Regni Vegetabilis per Gradus Naturales Evoluti*. Cnobloch, Leipzig.
- Rome M, Coppens d'Eeckenbrugge G (2017) Delimitation of the series *Laurifoliae* in the genus *Passiflora* (Passifloraceae). *Phytotaxa* 309(3): 245–252. <https://doi.org/10.11646/phytotaxa.309.3.5>
- Roussel HFA (1806) *Flore du Calvados et Terrains Adjacents, Composée Suivant la Méthode de Jussieu*. Poisson, Caen.
- Van Royen A (1740) *Florae Leydensis prodromus Exhibens Plantas quae in Horto Academico Lugduno-Batavo Aluntur*. Samuelen Luchtmans Academiae Typographum, Leiden, 1–532. <https://doi.org/10.5962/bhl.title.693>
- Thijssse G, Veldkamp JF (2003) *The Van Royen herbarium (short guide to the microform collection, including the supplement)*. IDC publishers, Leiden.
- Tournefort JP de (1700) *Institutiones Rei Herbariae*. 1. Typographia Regia, Paris. <https://doi.org/10.5962/bhl.title.713>

Appendix I

Selected specimens examined

Passiflora laurifolia (examined on herbarium specimens): **ANTIGUA**. Macarthy Hills, 28 Aug 1938, Box 1524 (MO). **CUBA**. Guantánamo, Rio Navas, Mar 1910, Shafer 4411 (MO). **DOMINICAN REPUBLIC**. Rancho la Cumbre, July 1978, Liogier 27709 (NY). **DOMINICA**. Hamlet of Concord, 0.9 km E of the Pagua River, 14 Aug 1992, Higgins 35 (FTG). **GRENADA**. St Patrick, N of Prospect, 3 Dec 2002, Hawthorne 970 (FHO). **GUADELOUPE**. Petit Bourg, 22 April 1988, Fournet 4368 (P). Basse Terre, 1893, Duss 604 (P), 1894, Duss 3249 (P). **MARTINIQUE**. Carbet, Case Pilote, 1901, Duss 4634 (P); Presqu'île de la Caravelle, 16 Feb 1977, Sastre 5302 (P); Saint Pierre, Le Marin, 1877, Duss 883 (NY). **PUERTO RICO**. Jussieu 16670 (P); Río Piedras, 8 Aug 1979, Woodbury nn (MO). **SAINT EUSTATIUS**. Top of the Quill, 4 June 1906, Boldingh 249 (U). **SAINT LUCIA**. Matout Road to Quillesse, 1 Feb 1985, Howard 20002 (NY). **SAINT VINCENT**. Jan 1890, Eggers 6958 (P). **TRINIDAD**. San Fernando Hill, 18 July 1926, Broadway 6368 (K). **U.S. VIRGIN ISLANDS**. St Croix, Scenic Drive, west slope of Mt. Eagle, 12 Jan 1980, Fosberg 59228 (FTG); St John, Maho Bay Quarter, 23 August 1987, Acevedo-Rodríguez 1924 (NY); Tortola, Sage Mt above Carrot Bay, 9 Feb 1966, D'Arcy 702 (MO); Saint Thomas, nn (P), August 1882, Drake 765 (P). **BRAZIL**. **Maranhão**: Island of São Luiz, Fe. 1939, Froes 11514 (NY); **Roraima**: SEMA ecological Reserve, Ilha de Maracá, Milliken 49 (INPA). **Unknown origin**: Linnaeus nn, number 1070.2 in the herbarium of the London Linnean Society (LINN). **GUYANA**. Rupununi Distr., N of Shea, 18 Jan 1994, Jansen-Jacobs 3240 (K); Western extremity of Kanaku Mountains, in drainage of Takutu River, 4 March 1938, Smith 3157 (K). **SURINAME**. Mts Bakhuis, 5 April 2006, Bordenave 8382 (CAY), 11 April 2006, Bordenave 8464 (CAY). **VENEZUELA**. **Bolívar**: Municipio Piar, isla en el Lago de Guri, 23 May 1989, Aymard 7610 (MO); **Anzoátegui**: Ijigua, Headwaters of Rio León, north-east of Bergantín, 27 Feb 1945, Steyermark 61250 (US).

Passiflora nitida (herbarium specimens): **BRAZIL**. **Amazonas**: Manaus, Rio Negro, Dec 1901, Ule 5974 (K); São Carlos, April 1854, Spruce 3472 (P, K); Street of Aleixo, 27 Dec 1973, Ramos P20141 (K); El Marco, North of Leticia-Tabatinga Road, 24 July 1973, Prance 16843 (NY); Island of Inambú, 19 Nov 1952, Romero-Castañedo 3633 (NY); **Rondônia**: Porto Velho, 30 March 2011, Simon 1280 (CEN). **COLOMBIA**. **Amazonas**: Santa Isabel, Indigenous Reserve Mirana, 25 May 1984, La Rotta 419 (COAH); Igará Paraná, Milan, 28 Aug 1987, Henao 34 (COAH); about 6 km north of Leticia at Santa Isabella, 28 Feb 1974, Gillett 16526 (COL); Río Igaraparano, 10 June 1942, Schultes 3950 (K); Corregimiento de Tarapacá, 31 Aug 2004, López 8578 (COAH); **Caquetá**: Municipality of Doncello, 21 May 2003, Castaño 1649 (COAH); **Meta**: municipality Vista Hermosa, 6 Jan 2006,

Betancur 12050 (COL); **Guaviare:** Municipality San José del Guaviare, 25 Aug 1995, Cárdenas 6535 (COAH); **Vaupés:** Raudal Jirijirimo, Pacoa, 22 Mar 2008, Betancur 13611 (COL); Municipality of Mitú, 22 Mar. 2003, Betancur 10030 (COL). **FRENCH GUIANA.** Indigenous village of Mana, Feb 1856, Sagot nn (P); Crique Petit Laussat, Bassin de la Mana, 9 Feb 1990, Cremers 11336 (CAY); Savane Gabrielle, 22 April 1979, Prevost 564 (CAY). **GUYANA.** Konashen area, Essequibo River, 24 Sept 1989, Jansen-Jacobs 1804 (P, K, CAY); Basin of Essequibo River, near mouth of Onoro Creek, 15 Dec 1937, Smith 2820 (K); Mataruki River, upper Essequibo, 4 Dec 1935, Myers 5826 (K); Christianburg, Demerara River, March 1916, Persaud nn (K); North West District, Barama River, Kariako, 10 July 1996, Van Andel 875 (U); Mabura Hill Region, Ekuk compartment, 17 Feb 1993, Ek 734 (U); Potaro River, Kaieteur Plateau, 9 March 1962, Cowan 2080 (US); Naamryck Canal, 14 April 1989, Gillespie 1017 (US); Mabaruma, 14 July 1934, *Archer* 2305 (K); Mabaruma, Aruka River, 7 March 1945, Fanshawe 5108 (K, P); Coast lands, June 1886, Jenman 5406 (K); Rupununi district, Kuyuwini River, 9 Feb. 1991, Jansen-Jacobs 2482 (K, P, U); Potaro-Siparuni Region, Pakaraima Mountains, 24 Oct 1994, Mutchnick 286 (US). **PERU.** **Loreto:** District of Iquitos, trail Iquitos to San Juan, 7 Feb 1932, Mexia 6488 (K); prov. Mariscal Ramón Castilla, 7 April 2003, Beltrán 5633 (USM); road of Santo Tomás, south of Iquitos, 12 Aug 1972, Croat 19107 (USM); Province of Requena, District of Jenaro Herrera, 17 Feb 2010, Torres LAT331 (USM); Iquitos region, Road to Santa Clara, 14 June 1966, Martin 1012 (K); Maynas, department of Iquitos, Road of Santo Tomás, 11 Feb 1983, Rimachi 6559 (USM); Department of Iquitos, Río Momón, 30 Jan 1985, Rimachi 7714 (USM); Iquitos, near Versalles, 25 Feb 1969, Plowman 2567 (USM); Prov. Maynas, Río Nanay, 6 sept 1974, Foster 4066 (F); **Madre de Dios:** Río Manú, Pakitza station, 20 Nov 1980, Foster 5779 (USM); Manú National Park, 15 Aug 1983, Gentry 43605 (MO); along banks of Río La Torre, 27 Jan 1989, Smith 1637 (USM); **Pasco:** Province of Oxapampa, District of Palcazu, Iscozacín, 16 Nov 2007, Rodríguez 33 (MO); **Cuzco:** Quispicanchis, Limonchayoc, 25 April 1984, Knapp 6404 (USM); Quispicanchis, along trail 25 km SW of Quincemil, 8 Oct 1976, Wasshausen 739 (USM). **SURINAME.** Linnaeus 74 (LINN). Forest of the Station, Groningen, 10 May 1916, Samuels 124 (K). **VENEZUELA.** **Amazonas :** Río Orinoco, along river just below mouth of Yapacana cano, 18 June 1959, Wurdack 43025 (K). Wankéhe, Cano Marneto, Lower Ventuari, 30 July 1976, Lister 606 (K).

Passiflora nitida (observations on living materials): **FRENCH GUIANA.** Road of Apatou, 26 Nov 2009, Rome 234 (LYJB); Road of Cacao, May 2008, Rome 67 (LYJB); Road of Cacao, 25 Nov 2009, Rome 207 (LYJB); Escol, May 2008, Rome 17 (LYJB); Road of St Georges, May 2008, Rome 49 (LYJB), Rome 130 (LYJB), Rome 139 (LYJB); Road of Tonnegrande, Dec 2009, Rome 203 (LYJB).

Passiflora sp.: Linnaeus 152 (LINN).

Appendix 2

Index to numbered collections

- Acevedo-Rodríguez, P. 1924 (laurifolia).
 Andel (van), T. 875 (nitida).
 Archer, W. A. 2305 (nitida).
 Arcy (D'), W. G. 702 (laurifolia).
 Aymard, G. 7610 (laurifolia).
 Beltrán, H. 5633 (nitida).
 Betancur, B. 10030, 12050, 13611 (nitida).
 Boldingh, I. 249 (laurifolia).
 Bordenave, B. 8382, 8464 (laurifolia).
 Box, H.E. 1524 (laurifolia).
 Broadway, W. E. 6368 (laurifolia).
 Cárdenas, D. 6535 (nitida).
 Castaño, N. 1649 (nitida).
 Cowan, R.S. 2080 (nitida).
 Cremers, G. 11336 (nitida).
 Croat, T. 19107 (nitida).
 Duss, A. 604, 883, 3249, 4634 (laurifolia).
 Drake, E. 765 (laurifolia).
 Eggers (von), H.F. A. 6958 (laurifolia).
 Ek, R.C. 734 (nitida).
 Fanshawe, D.B. 5108 (nitida).
 Fournet, A. 4368 (laurifolia).
 Fosberg, F.R. 59228 (laurifolia).
 Foster, R.B. 4066, 5779 (nitida).
 Froes, R. de Lemos 11514 (laurifolia).
 Gentry, A. 43605 (nitida).
 Gillett, J.M. 16526 (nitida).
 Gillespie, L.J. 1017 (nitida).
 Hawthorne, W. 970 (laurifolia).
 Henao, C.I. 34 (nitida).
 Higgins, J. 35 (laurifolia).
 Howard, R.A. 20002 (laurifolia).
 Jansen-Jacobs, M.J. 3240 (laurifolia);
 1804, 2482 (nitida).
 Jenman, G. S. 5406 (nitida).
 Jussieu (de), A. 16670 (laurifolia).
 Knapp, S. 6404 (nitida).
 La Rotta, C. 419 (nitida).
 Linnaeus, C. nn (laurifolia) ; 74 (nitida) ;
 152 (sp.).
 Liogier, A.H. 27709 (laurifolia).
 Lister, J.R.A. 606 (nitida).
 López, R. 8578 (nitida).
 Martin, R.T. 1012 (nitida).
 Mexia, Y. 6488 (nitida).
 Milliken, W. 49 (laurifolia).
 Mutchnick, P. 286 (nitida).
 Myers, J.G. 5826 (nitida).
 Persaud, N. nn (nitida).
 Plowman, T. 2567 (nitida).
 Prance, G.T. 16843 (nitida).
 Prevost, M.F. 564 (nitida).
 Ramos, J.F. 20141 (nitida).
 Rimachi, Y. 6559, 7714 (nitida).
 Rodríguez, D. 33 (nitida).
 Rome, M. 17, 49, 67, 130, 139, 203, 207,
 234 (nitida).
 Romero-Castañedo, R. 3633 (nitida).
 Sagot, P.A. nn (nitida).
 Samuels, J.A. 124 (nitida).
 Sastre, C. 5302 (laurifolia).
 Schultes, R. E. 3950 (nitida).
 Shafer, J.A. 4411 (laurifolia).
 Simon, M.F. 1280 (nitida).
 Smith, S.F. 1637, 2820 (nitida).
 Spruce, R. 3472 (nitida).
 Steyermark, J.A. 61250 (laurifolia).
 Torres, L. 331 (nitida).
 Ule, E.H. G. 5974 (nitida).
 Wasshausen, D.C. 739 (nitida).
 Woodbury, R.O. nn (laurifolia).
 Wurdack 43025 (nitida).

Ophiorrhiza guizhouensis (Rubiaceae), a new species from Guizhou Province, southwestern China

Chuan-Dong Yang^{1,2}, Xuan-Ze He³, Guang-Qian Gou³

1 Tongren University, Tongren 554400, Guizhou, China **2** Fanjingshan National Nature Reserve Administration of Guizhou, Tongren 554400, Guizhou, China **3** College of Life Sciences, Guizhou University, Guiyang 550025, Guizhou, China

Corresponding author: Guang-Qian Gou (ggqian106@163.com)

Academic editor: Y. Mutařchiev | Received 24 November 2017 | Accepted 21 February 2018 | Published 6 March 2018

Citation: Yang C-D, He X-Z, Gou G-G (2018) *Ophiorrhiza guizhouensis* (Rubiaceae), a new species from Guizhou Province, southwestern China. *PhytoKeys* 95: 121–126. <https://doi.org/10.3897/phytokeys.95.22506>

Abstract

In this study, *Ophiorrhiza guizhouensis*, a new species of Rubiaceae from south-western China, is described and illustrated. The new species is morphologically similar to *O. japonica*, but differs from the latter by having terete stems which are densely hirtellous, usually persistent ciliate stipules with well-developed colleters inside the base of the stipule, shorter corolla tubes and shorter stamens and styles.

Keywords

Ophiorrhiza, Guizhou, China, new taxa, distyly

Introduction

Ophiorrhiza L. is a member of tribe Ophiorrhizeae, subfamily Rubioideae, Rubiaceae (Bremer and Eriksson 2009), including more than 300 species worldwide (WCSPF 2017). The genus is mainly distributed in wet tropical forests of South-East Asia, extending to Australia, New Guinea and the Pacific Islands (Darwin 1976, Chen and Taylor 2011). *Ophiorrhiza* is a taxonomically difficult genus and is poorly known in

South-East Asia (Chen and Taylor 2011). In China, 68 species, including 47 endemics, are recorded (Chen and Taylor 2011, Deng and Huang 2012, Wu et al. 2017a, b) and most of them are distributed in the region south of the Changjiang River, especially the provinces of Yunnan and Guangxi (Lo 1999).

During field work in north-eastern Guizhou, China, some specimens of *Ophiorrhiza* were collected. After carefully examining the specimens and living materials and reviewing the relevant literature (Lo 1990, 1999, Chen and Taylor 2011), it was concluded that the newly found plants represented an undescribed species. Here, the new species is described and illustrated.

Materials and methods

Specimens were collected during February 2017. Additionally, some flowers were also collected and preserved in FAA for subsequent observations. The photographs were taken in the field. Morphological observations and measurements of the new species were carried out based on living plants, dry specimens and preserved materials.

Taxonomy

***Ophiorrhiza guizhouensis* C.D.Yang & G.Q.Gou, sp. nov.**

urn:lsid:ipni.org:names:60476091-2

Figs 1, 2

Diagnosis. Similar to *O. japonica* Blume, but distinguished from the latter by the terete, densely hirtellous stems (vs. stems subterete to slightly compressed, glabrous or with 2 hirtellous or pilosulous lines), the usually persistent and ciliate stipules (vs. caducous glabrescent stipules), the well-developed colleters inside the base of stipule (vs. without colleters), the shorter corolla tubes (8–9 mm vs. 9–14 mm) and the shorter stamens and styles (longistylous flower: 2.5–2.7 mm and 7–8 mm vs. 4.5–5.5 mm and 9–11 mm; brevistylous flower: 3.6–4.2 mm and ca. 2.5 mm vs. 4.5–5 mm and ca. 3 mm).

Type. CHINA. Guizhou: Tongren, Jiangkou County, Dewang Town, Miaowangpo, in broad-leaved forest, elevation 868 m, 27°46'31.87"N, 108°33'0.84"E, 19 Feb. 2017, C.D. Yang 092 (Holotype: GACP!; Isotypes: GACP!, PE!, KUN!)

Description. Perennial herbs, ascending, 40–60 cm tall; stems branched, terete, the lower stems prostrate, rooting from the node, the upper stems erect, densely hirtellous. Leaves in subequal pairs; petiole 1.3–3 cm, hirtellous; leaf blade drying papery, ovate, broadly ovate or elliptic, 3–6 cm × 1.8–3 cm, drying brown adaxially, purple abaxially, sparsely hirtellous on both surfaces, densely hirtellous on the midrib abaxially; base cuneate to obtuse, apex acute to obtuse; margin flat to crisped; secondary veins 5–8 on each side of the midrib; stipules persistent, rarely caducous, ovate-triangular, 1.3–1.6 mm × 0.9–1.2 mm, glabrescent adaxially, puberulent abaxially, margin ciliate,

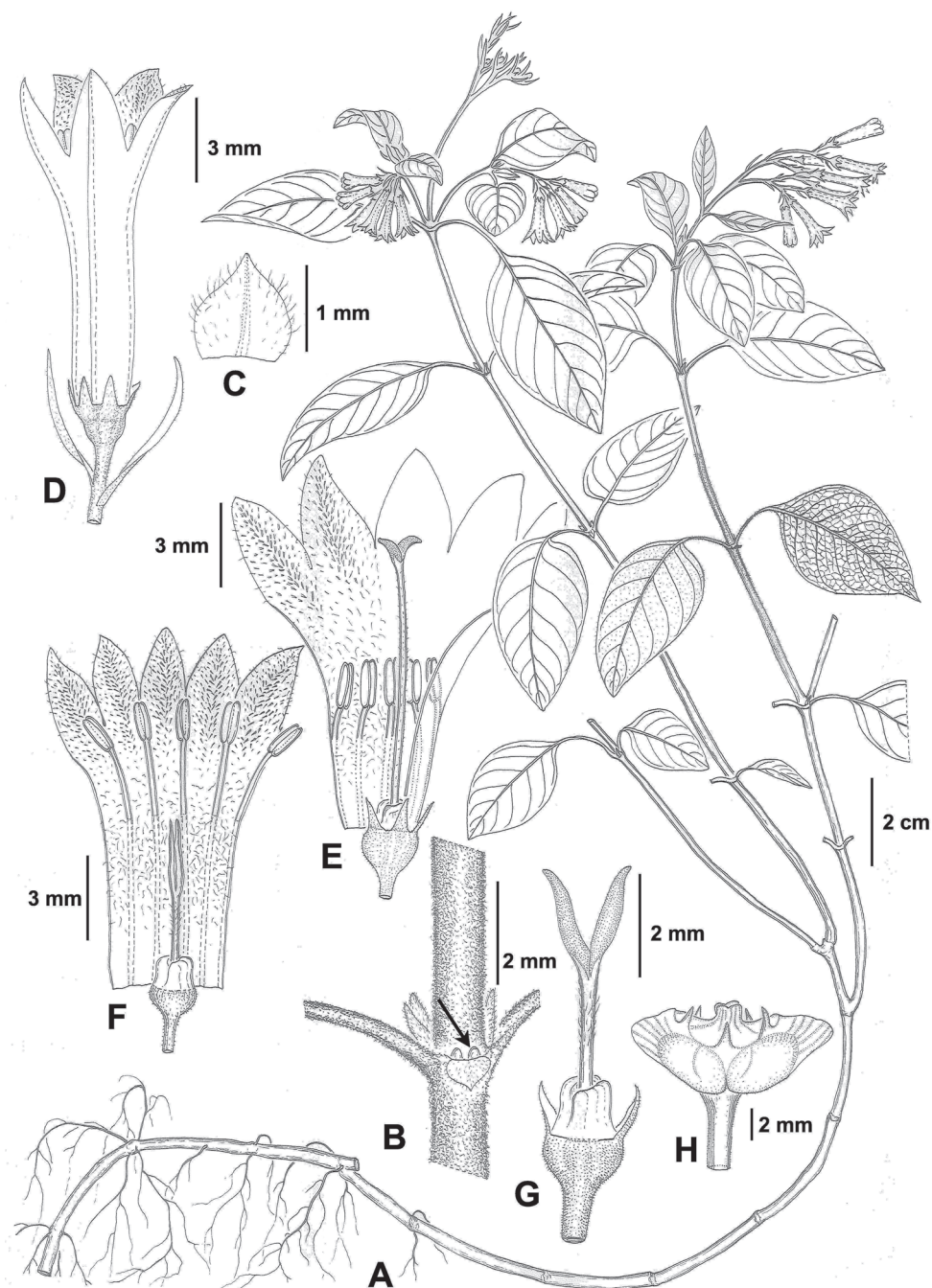


Figure 1. *Ophiorrhiza guizhouensis* **A** habit **B** stem, showing a stipule with two colleters (arrow) at the base inside **C** stipule, abaxial view **D** flower and bracts **E** dissected longystylous flower, showing stamen and style position **F** dissected brevistylous flower, showing stamen and style position **G** pistil of brevistylous flower **H** fruit. Drawn by Xiao-Yu Wang from the holotype.

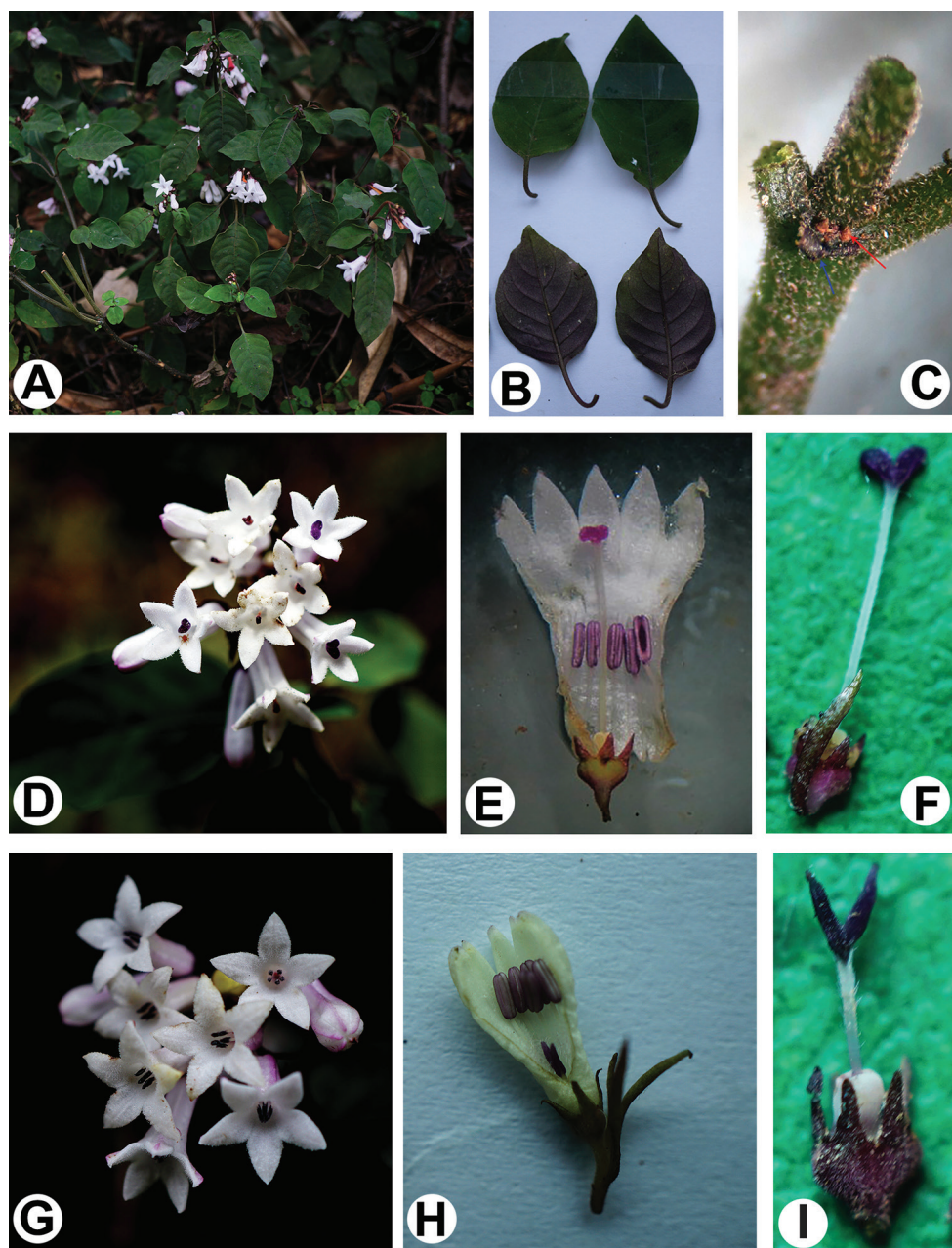


Figure 2. *Ophiorrhiza guizhouensis* **A** habit **B** leaves **C** node, showing persistent stipule (blue arrow) and colleters (red arrow), also showing stem pubescence **D** inflorescence, showing longistylous flowers **E** dissected longistylous flower **F** pistil of longistylous flower **G** inflorescence, showing brevistylous flowers **H** dissected brevistylous flower **I** pistil of brevistylous flower.

with two well-developed colleters inside the base of stipules, apex acute to acuminate. Inflorescence terminal, congested-cymose to cymose, 5–30-flowered, hirtellous; peduncle 1–2 cm, densely hirtellous. Bracts linear, 4–5 mm \times 0.8–1.1 mm, glabrescent adaxially, puberulent abaxially, persistent. Flowers distylous. Pedicel 1–3 mm long, pubescent. Calyx densely puberulent; hypanthium subturbinate, 0.8–1.1 mm long, 5-ribbed; lobes triangular, 1–1.5 mm long. Corolla purple in bud, white at anthesis, drying pink, funnellform to tubular-funnelform, longitudinally winged, glabrous outside; tube 8–9 mm long, villous inside; corolla lobes triangular to ovate, ca. 4 \times 2 mm, villous inside, dorsally ridged. Longistylous flower: stamens included, inserted in middle lower part of the corolla tube; filaments ca. 1 mm long; anthers linear, 1.4–1.7 mm long; style filiform, 7–8 mm long, sparsely puberulent; stigmas 2-lobed, lobes ovate, ca. 1.2 mm long, glabrous. Brevistylous flower: stamens exerted, inserted at the middle of the corolla tube; filaments 1.8–2.1 mm long; anthers oblong linear, subequal to filament; style filiform, ca. 2.5 mm long, sparsely puberulent; stigmas deeply 2-lobed, linear-lanceolate, ca. 2 mm long, glabrous. Capsules obcordate in outline, 3.5–4.5 \times 6.5–8 mm, subglabrous. Seeds small, angular, numerous, pale yellow to brown.

Phenology. Plants were observed in full bloom on 19 February 2017. It can be expected that flowering time of the new species is from January to March; fruiting time needs further observations.

Distribution and habitat. *Ophiorrhiza guizhouensis* is currently only known from Jiangkou County, Guizhou, south-western China, where at least 300 individuals are found. The species grows in evergreen broad-leaved forest or bamboo forest at elevations between 850–1,000 m, along with *Chimonobambusa angustifolia* C.D. Chu & C.S. Chao, *Lindera communis* Hemsl., *L. pulcherrima* var. *hemsleyana* (Diels) H.B. Cui, *Clematis henryi* Oliv. and *Ophiopogon bodinieri* H. Lév.

Etymology. The specific epithet refers to Guizhou, a province of south-western China in which the new species was collected.

Preliminary conservation status. The new species is currently only known from the type locality. More explorations are needed to fully understand its distribution and to assess its conservation status. Based on the available data, the new species is treated as “Data Deficient (DD)” according to the IUCN Red List Categories and Criteria guidelines (IUCN 2012).

Acknowledgements

We thank Xiao-Yu Wang for his line drawing of the new species. We are also grateful to the editor and three reviewers for critical comments and helpful suggestions. This work was supported by the national science and technology project of rural areas in the Twelfth Five Year Plan (2015BAD04B00) and by the Guizhou Education Department ([2012]018).

References

- Bremer B, Eriksson T (2009) Time tree of Rubiaceae: phylogeny and dating the family, sub-families and tribes. *International Journal of Plant Sciences* 170(6): 766–793. <https://doi.org/10.1086/599077>
- Chen T, Taylor CM (2011) *Ophiorrhiza*. In: Wu CY, Raven PH (Eds) *Flora of China*, Vol. 19. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 258–282.
- Darwin SP (1976) The Pacific species of *Ophiorrhiza* L. (Rubiaceae). *Lyonia* 1(2): 48–101.
- Deng YF, Huang YF (2012) *Ophiorrhiza loana*, a new name for *Ophiorrhiza longipes* H.S. Lo (Rubiaceae). *Phytotaxa* 49(1): 34. <http://dx.doi.org/10.11646/phytotaxa.49.1.5>
- IUCN (2012) IUCN Red List Categories and Criteria, Version 3.1 (2nd edn). Gland and Cambridge, 1–32.
- Lo HS (1990) Taxonomic revision of the Chinese species of *Ophiorrhiza* (Rubiaceae). *Bulletin of Botanic Research* 10(2): 1–82.
- Lo HS (1999) *Ophiorrhiza*. In: Lo HS (Ed.) *Flora Republicae Popularis Sinicae*. Vol. 71(1). Science Press, Beijing, 110–174.
- WCSPF (2017) World Checklist of Selected Plant Families: Royal Botanic Gardens, Kew. <http://apps.kew.org/wcsp/qsearch.do> [accessed 20 Oct. 2017]
- Wu L, Hareesh VS, Deng YF (2017a) Excluding *Ophiorrhiza mungos* (Rubiaceae) from Flora of China. *Phytotaxa* 309(2): 184–188. <https://doi.org/10.11646/phytotaxa.309.2.11>
- Wu L, Hareesh VS, Yu YL (2017b) The taxonomic identity of *Ophiorrhiza ravior* and *O. mycetiifolia* (Rubiaceae). *Phytotaxa* 299(2): 261–266. <https://doi.org/10.11646/phytotaxa.299.2.10>