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

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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. fuchsiflora*, *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* 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-
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Distribution 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 µm), small (10–25 µm), medium (25–50 µm), large (50–100 µm), very large (100–200 µm) and giant (> 200 µm). 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. fuchsiiflora* Hemsl., *P. jussieui* 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.


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 5.6–15 × 2.7–7.9 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; sepsals 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 µm), prolate spheroidal, 6-corporate, colpi long, narrow, three endoapertures lalongate (ca. 6.2 × 8.1 µm), unique for each pair of ectoaperture, sexine reticulate, heterobrochate, with muri (ca. 1.2 µm), muri simple columellate, sinuous, continuous, with perforations, without high columellae, not apparent, tectum surface mostly curved, lumina ornamented, small (ca. 6.7 µm diam.) (Fig. 3A, B).

**Specimens examined.** BRAZIL. Amapá: Amapari, Cabeceras of the Rio Amapari, on the left bank of the rio Anacuí, trail 5, 01°50’41”N, 52°44’29”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. *Botryastrae* (Harms) Killip, ser. *Carneae* Feuillet. It is morphologically most similar to *P.* *fuchsii-flora*, 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-corporate, colpi long, narrow, three endoapertures lalongate (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 Uatumá, grid of PPBio, 2°2’4”S, 60°1’30”W, 16 Apr 2007 [fl, fr], S. Sakagawa et al. 248 (INPA); Rebio Uatumá, 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 Uatumá, 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. *Astrophea*, sect. *Botryastrophea*, 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.

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, exerted, apex crenulate; trochea 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 Balerio, 7 km from the village Campo Novo, 10°35’0S, 63°39’0W, 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. *Astrophea* 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-capitelate and straight filaments and the sixth series with hair-like and reflexed filaments. The pollen grains of the closely related *P. serradensis* 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. serradensis* are included in the type III pollen group (Mezzonato-Pires et al. 2017).
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)

Figs 2G, H, 3G, H

**Descriptions.** **Lianas;** tendrils not seen. **Stipules** diminue, 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** diminue, 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; opeculum straight, tubular, exerted, 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 longate (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 colomellae, 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:** Acrelandia, 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:** Sao Gabriel da Cachoeira: Highway BR-307, SGC-Cucui km 50, [0°16’25”N, 66°39’35”W], 27 Oct 2008 [fl], S. Sakagawa et al. 668 (INPA); Highway BR-307, SGC-Cucui 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 Acrelandia) and Amazonas (municipality of Sao 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 opeculum 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, opeculum 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 funnelform; 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 Uatumá, 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 Figueirredo, Rebio Uatumá, 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 Figueirredo. 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. *Leptopoda* 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 dolabriliform, 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 µm), prolate spheroidal, 6-corporate, colpi short, narrow, three endoaperture lalongate (ca. 8.7 × 17.0 µm) unique for each pair of ectoaperture, sexine reticulate, heterobrochate; muri (ca. 2.0 µm) duplicolumellate, sinuous, continuous, without perforations, columnellae high, apparent, tectum surface mostly slightly curved, lumina ornamented with pila, large (ca. 15.2 µm diam.) (Fig. 3H, I).

**Specimens examined.** BRAZIL. Roraima: Cantá, Serra Grande, 2°32′53″S, 60°47′10″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).

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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** 6.2–11 × 4–8 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** 6.3–6.4 × 2.3–2.4 cm, ellipsoid, puberulous.

**Palynology.** Pollen grains medium-sized (ca. 46.0 µm), oblate spheroidal, 6-corporate, colpi long, narrow, three endoaperture lalongate (ca. 4.3 × 20.4 µm) 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


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

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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. dahuoaiensis 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/).
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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, \textit{rbcL} and \textit{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+\(\gamma\) 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 long, 2.1–2.4 cm in diam. vs. 0.6–0.8 cm long, 1.2–1.5 cm in diam.).
Lithocarpus vuquangensis (Fagaceae), a new species from Vu Quang National Park...

<table>
<thead>
<tr>
<th>Species</th>
<th>Vouchers</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rbcL</td>
</tr>
<tr>
<td>Lithocarpus aggregatus</td>
<td>Tagane et al. V6288 (DLU, FU)</td>
<td>LC318967</td>
</tr>
<tr>
<td>Lithocarpus bidoupensis</td>
<td>Tagane et al. V4320 (DLU, FU, VNM)</td>
<td>LC318961</td>
</tr>
<tr>
<td>Lithocarpus coalitus</td>
<td>Tagane et al. V4191 (DLU, FU, VNM)</td>
<td>LC318959</td>
</tr>
<tr>
<td>Lithocarpus dahuoaensis</td>
<td>Ngoc et al. V3194 (DLU, FU, VNM)</td>
<td>LC318953</td>
</tr>
<tr>
<td>Lithocarpus gigantophyllus</td>
<td>Ngoc et al. V3185 (DLU, FU)</td>
<td>LC318951</td>
</tr>
<tr>
<td>Lithocarpus hancei</td>
<td>Ngoc et al. V5111 (DLU, FU)</td>
<td>LC318963</td>
</tr>
<tr>
<td>Lithocarpus hongiaoensis</td>
<td>Ngoc et al. V3235 (DLU, FU)</td>
<td>LC318956</td>
</tr>
<tr>
<td>Lithocarpus licentii</td>
<td>Tagane et al. V4273 (DLU, FU)</td>
<td>LC318960</td>
</tr>
<tr>
<td>Lithocarpus lemeeanus</td>
<td>Ngoc et al. V3205 (DLU, FU)</td>
<td>LC318954</td>
</tr>
<tr>
<td>Lithocarpus longipedicellatus</td>
<td>Nguyen et al. V3813 (DLU, FU)</td>
<td>LC318958</td>
</tr>
<tr>
<td>Lithocarpus ombrophilus</td>
<td>Yabara et al. V3000 (DLU, FU)</td>
<td>LC318949</td>
</tr>
<tr>
<td>Lithocarpus pseudomagneinii</td>
<td>Ngoc et al. V3223 (DLU, FU)</td>
<td>LC318955</td>
</tr>
<tr>
<td>Lithocarpus stenopus</td>
<td>Ngoc et al. V3187 (DLU, FU)</td>
<td>LC318952</td>
</tr>
<tr>
<td>Lithocarpus vinhensis</td>
<td>Nguyen et al. V3787 (DLU, FU)</td>
<td>LC318957</td>
</tr>
<tr>
<td>Trigonobalanus verticillata</td>
<td>Yabara et al. V5764 (DLU, FU)</td>
<td>LC318965</td>
</tr>
</tbody>
</table>

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 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.
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.

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

The 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.
**Lithocarpus vuquangensis** (Fagaceae), a new species from Vu Quang National Park...

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 Cô, 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).

**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. Infrafructescences 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.


**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).
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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

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

**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

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* 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. loochooensis* 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**


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

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-ovovate 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-ovovate 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-ovovate, 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.)

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

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
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, dehiscing 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.

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**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.
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

<p>| | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fruit berry, purple-red, ellipsoid or globose; stem villous, rarely glabrous; petiole puberulent.</td>
<td><strong>L. nummularia</strong></td>
</tr>
<tr>
<td>–</td>
<td>Fruit capsule, green, subglobose or obconic; stem glabrous; petiole glabrous.</td>
<td><strong>2</strong></td>
</tr>
<tr>
<td>2</td>
<td>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.</td>
<td><strong>L. loochooensis</strong></td>
</tr>
<tr>
<td>–</td>
<td>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.</td>
<td><strong>3</strong></td>
</tr>
<tr>
<td>3</td>
<td>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 subbilabiate, 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.</td>
<td><strong>L. hongiana</strong></td>
</tr>
<tr>
<td>–</td>
<td>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.</td>
<td><strong>L. chinensis</strong></td>
</tr>
</tbody>
</table>

### 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


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

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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 \textit{rbcL}, \textit{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: \textit{Q. baolamensis sp. nov.}, \textit{Q. bidoupensis sp. nov.} and \textit{Q. honbaensis sp. nov.} These new species are all phenotypically similar to \textit{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, \textit{Q. bidoupensis} is not close to any other species. In the Bayesian tree, \textit{Q. honbaensis} is sister to both \textit{Q. baolamensis} and \textit{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 Reverse, 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
A taxonomic study of *Quercus langbianensis* complex based on morphology...

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

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

...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 langbiangensis* 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 (ATGTCACCACAAAACAGAGACTAAAGC, Levin et al. 2003), *rbcL*-724r (TCGCATGTACCTGCAGTAGC, Fay et al. 1997); *matK*-XF (TAATTTACGATCAATTCAATC, Ford et al. 2009), *matK*-1326R (TCTAGCACAGAAAAAGTCCAGAGT, Cuénoud et al. 2002); ITS-18F (GTCCACTGAACCTTATCATTGAAGG, Rohwer et al. 2009) and ITS-26R (GCCGTTACTAAGGGAAATCCTTGTTTAG, Rohwer et al. 2009). The sequences of *rbcL*, *matK* and ITS were amplified with Tks GflexTM 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 + $\gamma$ 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 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
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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 Qu. 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 Tranh 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. helfe- riana 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.

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

Figure 2. Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on *rbcL*, *matK* and ITS sequences. Branches 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
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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. austrocochinensis*, *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. austrocochinensis*, *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 donnaienis* 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. austrocochinensis*, *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. austrocochinensis*, *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).

*b. 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
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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.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>Q. bidoupensis</em></th>
<th><em>Q. camusiae</em></th>
<th><em>Q. cambodiensis</em></th>
<th><em>Q. baniensis</em></th>
<th><em>Q. honbaensis</em></th>
<th><em>Q. dilacerata</em></th>
<th><em>Q. blaoensis</em></th>
<th><em>Q. donnaiensis</em></th>
<th><em>Q. baolamensis</em></th>
<th><em>Q. langbianensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Young shoot</td>
<td>Almost glabrous</td>
<td>Golden tomentose</td>
<td>Curly hairy</td>
<td>Golden tomentose</td>
<td>Straight hairy</td>
<td>Golden tomentose</td>
<td>Almost glabrous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf margin</td>
<td>Undulate, distinctly serrate in upper 1/2</td>
<td>Not undulate, almost entire or with a few low teeth in upper 1/4</td>
<td>Not undulate, distinctly serrate in upper 1/3</td>
<td>Not undulate, distinctly serrate in upper 5/6–3/4(–2/3)</td>
<td>Not undulate, distinctly serrate in upper 1/3</td>
<td>Not undulate, distinctly serrate in upper 1/2</td>
<td>Not undulate, distinctly serrate in upper 1/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of petioles</td>
<td>1.3–2 cm</td>
<td>1–1.6 cm</td>
<td>1–2.2 cm</td>
<td>1.2–2(–2.9) cm</td>
<td>0.8–1 cm</td>
<td>1–1.4 cm</td>
<td>0.9–1.8 cm</td>
<td>1–2 cm</td>
<td>0.4–1 cm</td>
<td>1–2 cm</td>
</tr>
<tr>
<td>Number of secondary veins</td>
<td>10–13 pairs</td>
<td>8–13 pairs</td>
<td>7–11 pairs</td>
<td>9(–)10–14(–10) pairs</td>
<td>12–14 pairs</td>
<td>8–13 pairs</td>
<td>9–12(–14) pairs</td>
<td>(7–)10–13 pairs</td>
<td>10–12 pairs</td>
<td></td>
</tr>
<tr>
<td>Cupule shape</td>
<td>Obconical</td>
<td>Cup-shaped</td>
<td>Obconical</td>
<td>Bowl-shaped</td>
<td>Cup-shaped</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cupule coverage</td>
<td>Enclosing 1/3 of the nut</td>
<td>Enclosing &lt;1/2 of the nut</td>
<td>Enclosing 2/3 of the nut</td>
<td>Enclosing 1/3–1/2 of the nut</td>
<td>Enclosing 2/3 of the nut</td>
<td>Enclosing 1/2 of the nut</td>
<td>Enclosing 1/3 of the nut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cupule bract margin</td>
<td>5–6 rings</td>
<td>6 rings</td>
<td>7–8 rings</td>
<td>6–8 rings</td>
<td>5–6 rings</td>
<td>6–9 rings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nut shape</td>
<td>Ovoid</td>
<td>Subglobose</td>
<td>Ovoid</td>
<td>Obovoid to ellipsoid</td>
<td>Subglobose</td>
<td>Ovoid</td>
<td>Subglobose</td>
<td>Ovoid-ellipsoid</td>
<td>Obovoid to ellipsoid</td>
<td></td>
</tr>
<tr>
<td>Nut scar</td>
<td>Convex</td>
<td>Flat</td>
<td>Convex</td>
<td>Convex</td>
<td>Flat</td>
<td>Convex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nut hairiness</td>
<td>Glabrous</td>
<td>Densely hairy</td>
<td>Sparsely hairy</td>
<td>Densely hairy</td>
<td>Sparsely hairy</td>
<td>Densely hairy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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
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 ovoid to ellipsoid............................. Q. honbaensis

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

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 tomenose or almost glabrous. Leaves regularly distinctly serrate in the upper 1/3–1/2............... 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

Leaves regularly distinctly serrate in the upper 1/2; petiole 0.4–1 cm long. Nut ovoid–ellipsoid, scar flat, sparsely hairy................................. Q. baolamensis

Leaves regularly distinctly serrate in the upper 1/3; petiole 1–2 cm long. Nut ovoid 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


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 specimens examined. VIETNAM. BaNa 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.
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*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.  


**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-riangular, 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 × 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


**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


**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.* 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 216*)...
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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

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!]; isolecotype: 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-

A taxonomic study of *Quercus langbianensis* complex based on morphology...

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


**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


**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).
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-
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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.


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

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**Quercus langbianensis Hickel & A.Camus**  
Fig. 13


**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 VD., 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).

**Acknowledgements**

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**References**


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**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.

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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).

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<th>GenBank accession no.</th>
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(+) : 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

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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. oblanceolatus* 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 spatulate 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. brachy- petalus 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 placentaion. 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. ob lanceolatus Craib, A. hexa- petalus (L.f.) Bhandari (as “A. odoratissimus R.Br.”), A. scortechnii 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. ob lanceolatus” (a misapplied name), A. siamensis, A. spinosus, A. suaveolens, A. vanprukii and an unnamed species (which represents “true” A. ob lanceolatus). More recently, Thongpairoj (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
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Ridl.” (a misapplied name, for which the name *A. oxycarpus* should be applied: Thongpairoj 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
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).


**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, sub-glabrous 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

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**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).
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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 Bilaulktaung 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).


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 spatulate 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, subglu-
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 spatulate, 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.
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**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, ndbF, 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


**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), Thongpaipairoj (2008, as “*A. grandiflorus*”) and Insura (2009), for which the name *A. byrsophyllus*
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


**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. Thongpairoj 235 (CMU).

**Notes.** Specimens of this species were mistakenly recognised as a new species (“*A. sp. 5 (Kao Yai)”*) by Thongpairoj (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 hairy ............2
   - Young branches glabrous to sparsely hairy; abaxial surface of leaves glabrous to sparsely hairy ......................................................3

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 flower .........................4
   - Pedicels slightly shorter, as long as, or longer than flower ..............6

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 wide ................ *A. brevipes*

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. oxycarpus*
   - 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. harmandii*

6. Stamen connectives apiculate ...................................................................7
   - Stamen connectives truncate .............................................................8

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. punctulatus*
   - 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 ......

8. Petal blades terete; monocarps 1–2(–4) per fruit .................................. *A. suaveolens*
   - Petal blades not terete; monocarps 4–30 per fruit .........................9

9. Inflorescences with 10–20 flowers; monocarps not beaked ..................10
   - Inflorescences with up to 4 flowers; monocarps beaked .............11

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 spatulate ...........................................12
    - Outer petals without externally distinct blades and claws; inner petal blades ovate or triangular ........................................13
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 spathulate and strongly concave; monocarps strongly beaked (beak ca. 2 mm long) .......................................................... *A. spathulatus*

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 ..........

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*

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

**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.

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Chalermglin P (2005) Family Annonaceae (2nd edn). Ban and Suan, Bangkok. [In Thai]
Paraboea wenshanensis, a new species of Gesneriaceae from Yunnan, China

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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).

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).
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*.

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

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-ombicular, 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,
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.
Paraboea wenshanensis, a new species of Gesneriaceae from Yunnan, China

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

**Vernacular name.** Wén Shān Zhū Máo Jù Tái (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.

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Map 1. The geographical distribution of *P. wenshanensis* sp. nov., *P. angustifolia*, *P. martinii* and *P. glutinosa*. 
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)”.

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


Triboun P (2013) Paraboea middletonii (Gesneriaceae), a new species from Thailand. Thai Forest Bulletin (Botany) 41: 45–47.


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

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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, Amblysperma 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 (Katina 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 Aisan section Isanthus (Less.) C. Jeffry (7 species), the Madagas- car section Pseudoseris (Baill.) C. Jeffry (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, Amblysperma, 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 ml 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>
<thead>
<tr>
<th>Species</th>
<th>Section</th>
<th>Locality</th>
<th>Voucher information</th>
<th>Adaxial leaf</th>
<th>Bracts on scape</th>
<th>Inner rays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stomata</td>
<td>Trichome</td>
<td></td>
</tr>
<tr>
<td>Gerbera viridifolia (DC.) Sch.Bip.</td>
<td>Lasiopus</td>
<td>Kenya</td>
<td>T.H. Trinder-Smith s.n. (US)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. jamesonii Adlam</td>
<td>Lasiopus</td>
<td>Cultivar</td>
<td>V.A. Funk s.n. (US)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
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<td>G. aurantiaca Sch.Bip.</td>
<td>Lasiopus</td>
<td>South Africa</td>
<td>Bayliss 2505 (US)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. ambiguus Sch.Bip.</td>
<td>Lasiopus</td>
<td>South Africa</td>
<td>M. Koekemoer 2097 (US)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. piloselloides Cass.</td>
<td>Piloselloides</td>
<td>Swaziland</td>
<td>M. Koekemoer 2590 (US)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. cordata Less.</td>
<td>Piloselloides</td>
<td>Madagascar</td>
<td>T.B. Croat 29083 (MO)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. perrieri Humbert</td>
<td>Pseudoseris</td>
<td>Madagascar</td>
<td>L. Gautier 3110 (MO)</td>
<td>+</td>
<td>★</td>
<td>–</td>
</tr>
<tr>
<td>G. crocea Kuntze</td>
<td>Gerbera</td>
<td>South Africa</td>
<td>M. Koekemoer 2029 (US)</td>
<td>+</td>
<td>≈</td>
<td>+</td>
</tr>
<tr>
<td>G.wrightii Harv.</td>
<td>Gerbera</td>
<td>South Africa</td>
<td>P. Goldblatt 5287 (US)</td>
<td>+</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>G. serrata Druce</td>
<td>Gerbera</td>
<td>South Africa</td>
<td>M. Koekemoer 2001 (PRE)</td>
<td>+</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>G. gossypina Beauverd</td>
<td>Isanthus</td>
<td>India</td>
<td>W.N. Koelz 4828 (US)</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>G. maxima Beauverd</td>
<td>Isanthus</td>
<td>India</td>
<td>D.H. Nicolson 2755 (US)</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>G. delavayi Franch.</td>
<td>Isanthus</td>
<td>China</td>
<td>X. Xu 1102 (KMUST)</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>G. nivea Sch.Bip.</td>
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<td>China</td>
<td>J.F. Rock 6430 (US)</td>
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<td>–</td>
<td>+</td>
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<tr>
<td>G. henryi Dunn</td>
<td>Isanthus</td>
<td>China</td>
<td>W.B. Hemsley 1903 (US)</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>G. hieracioides (Kunth) Zardini</td>
<td>?</td>
<td>Ecuador</td>
<td>P.M. Peterson 9287 (US)</td>
<td>–</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>G. hieracioides (Kunth) Zardini</td>
<td>?</td>
<td>Peru</td>
<td>R. Ferrerya 15362 (US)</td>
<td>–</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Chatpatila pringlei Greene</td>
<td>N</td>
<td>Mexico</td>
<td>Rzedowski 34853 (US)</td>
<td>–</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>C. mandonii Burkart</td>
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<td>Argentina</td>
<td>P.M. Simón 438 (US)</td>
<td>–</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>C. m. Blake</td>
<td>N</td>
<td>Venezuela</td>
<td>L. Aristeguieta 2591 (US)</td>
<td>–</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Trichocline cineraria Hook. &amp; Am.</td>
<td>N</td>
<td>Argentina</td>
<td>A.R. Cezza 20mz398 (US)</td>
<td>+</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>T. catharinensis Cabrera</td>
<td>N</td>
<td>Brazil</td>
<td>L.B. Smith 11376 (US)</td>
<td>+</td>
<td>=</td>
<td>+</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Voucher information</th>
<th>ITS</th>
<th>ETS</th>
<th>trnL–trnF</th>
<th>trnL–rpB2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gerbera viridiflora</em> (DC.) Sch. Bip.</td>
<td>South Africa</td>
<td>T.H. Trinder-Smith s.n. (US)</td>
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<td>MG661588*</td>
<td>MG661639*</td>
<td>MG661670*</td>
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<tr>
<td><em>G. crocea</em> Kuntze</td>
<td>South Africa</td>
<td>M. Koekemoer 2029 (US)</td>
<td>MG661709*</td>
<td>MG661606*</td>
<td>MG661645*</td>
<td>MG661683*</td>
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<tr>
<td><em>G. delatyu</em> Franch.</td>
<td>China</td>
<td>X. Xu 1102 (KMUST)</td>
<td>MG661708*</td>
<td>MG661605*</td>
<td>MG661659*</td>
<td>MG661682*</td>
</tr>
<tr>
<td><em>G. henryi</em> Dunn</td>
<td>China</td>
<td>X. Xu 1103 (KMUST)</td>
<td>MG661706*</td>
<td>MG661602*</td>
<td>MG661655*</td>
<td>MG661681*</td>
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<tr>
<td><em>G. nivea</em> Sch. Bip.</td>
<td>China</td>
<td>Y.S. Chen 2674 (PE)</td>
<td>MG661703*</td>
<td>MG661598*</td>
<td>MG661648*</td>
<td>MG661678*</td>
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<tr>
<td><em>G. aurantiaca</em> Sch.Bip.</td>
<td>South Africa</td>
<td>Bayliss 2505 (US)</td>
<td>MG661711*</td>
<td>MG661610*</td>
<td>MG661637*</td>
<td>MG661687*</td>
</tr>
<tr>
<td><em>G. ambigua</em> Sch. Bip.</td>
<td>South Africa</td>
<td>M. Koekemoer 2097 (US)</td>
<td>MG661712*</td>
<td>MG661611*</td>
<td>MG661636*</td>
<td>MG661688*</td>
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<tr>
<td><em>G. jamesonii</em> Adlam</td>
<td>Cultivar</td>
<td>T. Derby s.n. (US)</td>
<td>MG661704*</td>
<td>MG661599*</td>
<td>MG661638*</td>
<td>MG661679*</td>
</tr>
<tr>
<td><em>G. cordata</em> Less.</td>
<td>South Africa</td>
<td>J. Wen 10067 (US)</td>
<td>N</td>
<td>MG661608*</td>
<td>MG661661*</td>
<td>MG661685*</td>
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<tr>
<td><em>G. piloselloides</em> Cass.</td>
<td>Swaziland</td>
<td>M. Koekemoer 2590 (US)</td>
<td>MG661701*</td>
<td>MG661592*</td>
<td>MG661650*</td>
<td>MG661675*</td>
</tr>
<tr>
<td><em>G. wrightii</em> Harv.</td>
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<td>P. Goldblatt 5287 (US)</td>
<td>MG661695*</td>
<td>MG661587*</td>
<td>MG661642*</td>
<td>N</td>
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<td><em>G. serrata</em> Druce</td>
<td>South Africa</td>
<td>M. Koekemoer 2001 (PRE)</td>
<td>MG661697*</td>
<td>MG661590*</td>
<td>MG661656*</td>
<td>MG661671*</td>
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<tr>
<td><em>G. hieracioides</em> (Kunth) Zardini</td>
<td>Ecuador</td>
<td>P.M. Peterson 9287 (US)</td>
<td>MG661705*</td>
<td>MG661601*</td>
<td>MG661657*</td>
<td>MG661680*</td>
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<tr>
<td><em>G. hieracioides</em> (Kunth) Zardini</td>
<td>Peru</td>
<td>J. Campos 5255 (US)</td>
<td>N</td>
<td>MG661600*</td>
<td>N</td>
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<tr>
<td><em>Amblysperma scapigera</em> Benth.</td>
<td>Australia</td>
<td>A. Morrison s.n. (US)</td>
<td>MG661713*</td>
<td>MG661612*</td>
<td>N</td>
<td>MG661689*</td>
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<tr>
<td><em>Adenocaulon chilense</em> Less.</td>
<td>Chile</td>
<td>G.L. Sobel 2558 (US)</td>
<td>MG661714*</td>
<td>N</td>
<td>N</td>
<td>MG661690*</td>
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<tr>
<td><em>Gerbena maxima</em> Beauverd</td>
<td>Australia</td>
<td>F. Kingdom 18199 (NY)</td>
<td>KX349402</td>
<td>N</td>
<td>KX349371</td>
<td>N</td>
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<tr>
<td><em>G. gossypina</em> Beauverd</td>
<td>India</td>
<td>W. Koelz 4294 (US)</td>
<td>GU126777</td>
<td>N</td>
<td>N</td>
<td>GU126755</td>
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<tr>
<td><em>Adenocaulon chilense</em> Less.</td>
<td>Argentina</td>
<td>J.M. Bonifacio 3997 (LP)</td>
<td>KX349359</td>
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<td>KX349360</td>
<td>N</td>
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<tr>
<td><em>Chaptalia nutans</em> (L.) Polák</td>
<td>Argentina</td>
<td>P.M. Simon 477 (US)</td>
<td>GU126772</td>
<td>N</td>
<td>N</td>
<td>GU126751</td>
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<tr>
<td><em>C. pringlei</em> Greene</td>
<td>Mexico</td>
<td>G. Nesom 4405 (US)</td>
<td>GU126773</td>
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<td>N</td>
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<tr>
<td><em>C. runcinata</em> Kunze</td>
<td>Argentina</td>
<td>P.M. Simon 415 (US)</td>
<td>GU126774</td>
<td>N</td>
<td>N</td>
<td>GU126752</td>
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<tr>
<td><em>C. chapadensis</em> D.J.N. Hind</td>
<td>Argentina</td>
<td>Roque &amp; al. 2188 (ALCB)</td>
<td>KF989508</td>
<td>N</td>
<td>KF989614</td>
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<td><em>C. similis</em> R.E. Fr.</td>
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<td>P.M. Simon 711 (US)</td>
<td>GU126775</td>
<td>N</td>
<td>N</td>
<td>GU126753</td>
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<tr>
<td><em>C. tomentosa</em> Vent.</td>
<td>USA</td>
<td>V.A. Funk 12303 (US)</td>
<td>GU126776</td>
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<td>N</td>
<td>GU126754</td>
</tr>
<tr>
<td>Species</td>
<td>Locality</td>
<td>Voucher information</td>
<td>ITS</td>
<td>ETS</td>
<td>trnL–trnF</td>
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</tr>
<tr>
<td><em>C. piloselloides</em> (Vahl) Baker</td>
<td>Brazil</td>
<td><em>E. Pasini</em> 1021 (ICN)</td>
<td>KX349357</td>
<td>N</td>
<td>KX349358</td>
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<tr>
<td><em>Trichocline auriculata</em> Hieron</td>
<td>Argentina</td>
<td><em>H. Simón &amp; J.M. Bonifacino</em> 633 (US)</td>
<td>KX349386</td>
<td>N</td>
<td>KX349387</td>
<td>N</td>
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<tr>
<td><em>T. catharinensis</em> Cabrera</td>
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<td><em>E. Pasini</em> 915 (ICN)</td>
<td>KX349388</td>
<td>N</td>
<td>KX349389</td>
<td>KX349411</td>
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<td><em>T. caulescens</em> Phil.</td>
<td>Chile</td>
<td>V.A. Funk &amp; al. 13055 (US)</td>
<td>KX349390</td>
<td>N</td>
<td>KX349391</td>
<td>KX349406</td>
</tr>
<tr>
<td><em>T. cineraria</em> Hook. &amp; Arn.</td>
<td>Argentina</td>
<td><em>E. Pasini &amp; E. Torchelsen</em> 1027 (ICN)</td>
<td>KX349392</td>
<td>N</td>
<td>KX349393</td>
<td>KX349407</td>
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<tr>
<td><em>T. plicata</em> Hook. &amp; Arn.</td>
<td>Argentina</td>
<td><em>E. Pasini &amp; E. Torchelsen</em> 1023 (ICN)</td>
<td>KX349396</td>
<td>N</td>
<td>KX349397</td>
<td>KX349409</td>
</tr>
<tr>
<td><em>T. reptans</em> (Wedd.) Hieron</td>
<td>Argentina</td>
<td><em>E. Pasini &amp; E. Torchelsen</em> 1025 (ICN)</td>
<td>KX349398</td>
<td>N</td>
<td>KX349399</td>
<td>KX349410</td>
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</tbody>
</table>

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

**Table 3.** Primers and amplification protocols for all markers.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Primers and sequences 5′–3′</th>
<th>PCR protocol: initial pre-heating; DNA denaturation; primer annealing; DNA extension; final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS</td>
<td>ITS5A: GGAAGGAGAAGTCGTAACAAGG ITS4: TCCTCCGCTTTATGTGCATGC</td>
<td>95 °C 1 min; 54 °C 1 min; 72 °C 1 min; 72 °C 10 min; 40 cycles</td>
</tr>
<tr>
<td>ETS</td>
<td>18s-ETS: ACTTACATGCATGGCTTAATCT ETS-Hel-1: GCTCCTTGCTTGGCGAACAACF</td>
<td>94 °C 0:30 min; 60 °C 0:40 min; 72 °C 1:20 min; 72 °C 5 min; 30 cycles</td>
</tr>
<tr>
<td>trnL–trnF</td>
<td>trnL-Fc: CGAATCGTACGACGCTACG trnL-Ff: ATTTGAACGTTGGTACAGG</td>
<td>94 ° C 1 min; 53 ° C 1 min; 72 ° C 2 min; 72 ° C 10 min; 35 cycles</td>
</tr>
<tr>
<td>trnL–rpB2</td>
<td>trnL: TACCGATTTCACCATAGCGG rpB2: AGGAAAGGATATTGGCGG</td>
<td>95 ° C 3 min; 51 ° C 40 s; 72 ° C 1:20 min; 72 ° C 5 min; 40 cycles</td>
</tr>
</tbody>
</table>
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.
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

*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 *trn* L–*trn* F and *trn* L–*rpl* 32. 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


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


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

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References


A new lectotype for *Passiflora laurifolia* L.

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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*, *Astropeia* (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 corymbose 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.
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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 membranéuses"]. 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 foltis’* 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).
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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 Thijsse and Veldkamp (2003), there is no voucher material of *Passiflora laurifolia*.

Linnaeus (1749) named *Passiflora laurifolia* as ‘*Passiflora foliis indivisis integerrimis, involucris dentatis*’ (*Passiflora* with undivided leaves and dentate involucres). 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 *murucuia* 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
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
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A new lectotype for *Passiflora laurifolia* L. (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-
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);
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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 transluscent 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**

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References


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Merian MS (1705) Metamorphosis Insectorum Surinamensium. G. Valk, Amsterdam.


Roussel HFA (1806) Flore du Calvados et Terrains Adjacents, Composée Suivant la Méthode de Jussieu. Poisson, Caen.


Appendix 1

Selected specimens examined


**Passiflora nitida** (herbarium specimens): **BRAZIL.** Amazonas: Manaus, Rio Negro, Dec 1901, Ule 5974 (K); São Carlos, April 1854, Spruce 3472 (P, K); Street of Alexis, 27 Dec 1973, Ramos P20141 (K); El Marco, North of Leticia-Tabatinga Road, 24 July 1973, Prance 16843 (NY); Island of Ínamá, 19 Nov 1952, Romero-Castanedo 3633 (NY); **RONDÓNIA.** Porto Velho, 30 March 2011, Simon 1280 (CEN). **COLOMBIA.** Amazonas: Santa Isabel, Indigenous Reserve Mirana, 25 May 1984, La Rotta 419 (COAH); Igaraj Parana, Milan, 28 Aug 1987, Henao 34 (COAH); about 6 km north of Leticia at Santa Isabel, 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,
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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); Mataruku 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, Rio Momón, 30 Jan 1985, Rimachi 7714 (USM); Iquitos, near Versalles, 25 Feb 1969, Plowman 2567 (USM); Prov. Maynas, Rio Nanay, 6 sept 1974, Foster 4066 (F); **Madre de Dios**: Rio 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, Washhausen 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 Ypacana 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 139 (LYJB), Rome 130 (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).
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

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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 Ophiorhiza 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.

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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 (longistylos flower: 2.5–2.7 mm and 7–8 mm vs. 4.5–5.5 mm and 9–11 mm; brevistylos 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 longistyloous flower, showing stamen and style position F dissected brevistyloous flower, showing stamen and style position G pistil of brevistyloous 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 longistyloous flowers E dissected longistyloous flower F pistil of longistyloous flower G inflorescence, showing brevistyloous flowers H dissected brevistyloous flower I pistil of brevistyloous flower.
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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 × 0.8–1.1 mm, glabrescent adaxially, puberulent abaxially, persistent. Flowers distylos. 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, funnelform to tubular-funnelform, longitudinally winged, glabrous outside; tube 8–9 mm long, villous inside; corolla lobes triangular to ovate, ca. 4 × 2 mm, villous inside, dorsally ridged. Longistylos 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. Brevisstylos 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 × 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).

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References


