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Two new species of *Dendrophthora* (Viscaceae) from the Venezuelan Andes

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Abstract

Two new species of *Dendrophthora* Eichler (Viscaceae) from northwestern Venezuela are described and illustrated. Both mistletoes, *D. apiculata* Canelón et al., **sp. nov.** and *D. coronata* Canelón et al., **sp. nov.**, are confined to subpáramo and páramo ecosystems of the Venezuelan Andes and are, at present, only known from Guaramacal National Park (Portuguesa and Trujillo states). Ecological aspects and possible taxonomic affinities are discussed. A distribution map also is presented.

Resumen

Se describen e ilustran dos nuevas especies de *Dendrophthora* Eichler (Viscaceae) del noroeste de Venezuela. Ambos muérdagos, *D. apiculata* Canelón et al., **sp. nov.** y *D. coronata* Canelón et al., **sp. nov.**, se limitan a los ecosistemas subpáramo y páramo de los Andes venezolanos y, en la actualidad, solo se conocen en el Parque Nacional Guaramacal (estados Portuguesa y Trujillo). Se discuten aspectos ecológicos y posibles afinidades taxonómicas. También se presenta un mapa de distribución.

Keywords

Flora of Venezuela, Guaramacal National Park, Mistletoe, Muérdago, Páramo, Parque Nacional Guaramacal, Subpáramo

Introduction

Dendrophthora Eichler (Viscaceae) is the second most diverse genus of mistletoe in the New World comprising over 125 species distributed in Mexico, Central and South America, and the Caribbean (Nickrent in press). We follow Nickrent et al. (2010, 2019) in placing *Dendropthora* in the Viscaceae, even though Stevens (2020) places the genus in a more broadly construed Santalaceae (tribe Visceae Horan.). In South America, the vast majority of *Dendrophthora* species are found at high elevations along the Cordillera de los Andes, ranging from Colombia and Venezuela south to Ecuador, Peru, and Bolivia (Kuijt 1961). Approximately 28 species of *Dendrophthora* have been reported from Venezuela, including 13 endemic species (Rizzini 1982, Kuijt 2008, Tropicos 2019). For Guaramacal National Park, which is located in the northeastern portion of the Venezuelan Andes (Portuguesa and Trujillo states, Fig. 1), Dorr et al. (2000) reported three species of *Dendrophthora*: *D. ambigua* Kuijt, *D. elliptica* (Gardner) Krug & Urb. and an unidentified species. Continued research and collection, focusing on the flora of Guaramacal National Park, revealed an additional unidentified species. Inasmuch as both unnamed species do not match any previously published description of *Dendrophthora* found in the Andes of Venezuela or in the rest of the Americas, we describe them as new and provide information on their

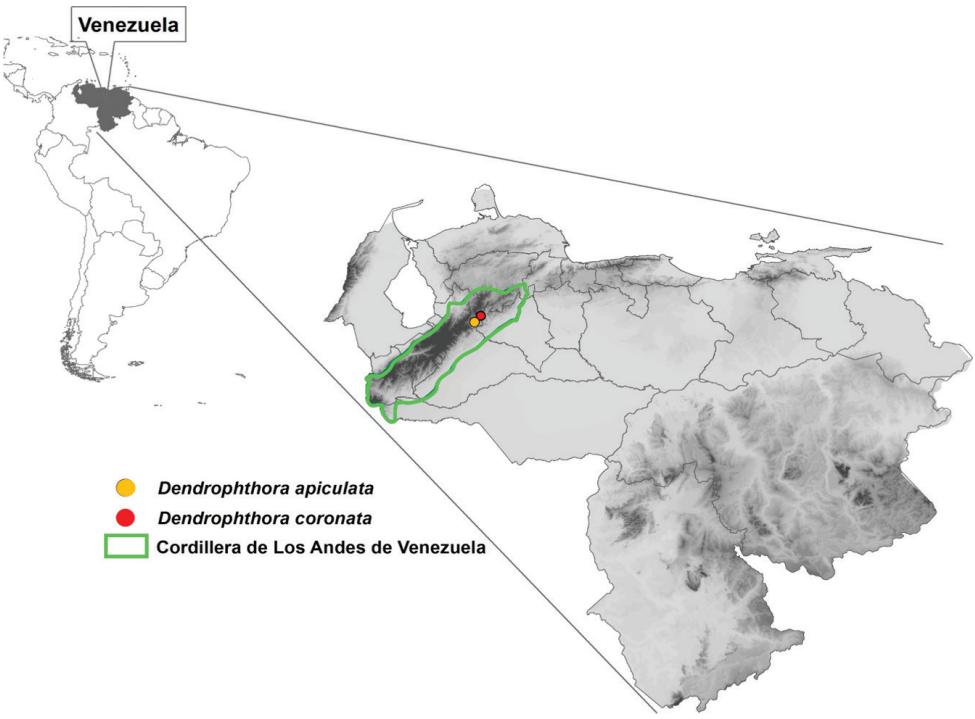

Figure 1. Distribution of *Dendrophthora apiculata* and *D. coronata* in the Andes of Venezuela. (Source: Centro Cartográfico, UNELLEZ-VPA, 2019).

habitats and known hosts. Both species apparently are restricted to the subpáramo and páramo ecosystems of the Venezuelan Andes and are, at present, only known from Guaramacal National Park.

Materials and methods

We studied herbarium specimens of *Dendrophthora* collected in the Venezuelan Andes and deposited in the following herbaria: PORT (Portuguesa, Venezuela) and US (Smithsonian Institution). We also examined the type collection of the latter herbarium, examined specimens in COL (Universidad Nacional de Colombia herbarium, Bogotá, Colombia), and accessed specimens at MO (Missouri Botanical Garden) via the Global Plants JSTOR (2019) and Tropicos (2019) online portals. We also exhaustively reviewed the many articles, monographs, and checklists treating *Dendrophthora* published by Kuijt (1961, 2000, 2003, 2008, 2011, 2016) and our terminology follows his.

Species descriptions were made combining information from fresh and dried specimens, with inflorescences and flowers rehydrated using Aerosol OT solution (Ayensu 1967).

Taxonomic treatment

1. *Dendrophthora apiculata* Canelón, S.M.Niño, Dorr & Caraballo, sp. nov.
urn:lsid:ipni.org:names:77205497-1
Figures 2, 3

Type. Venezuela. Trujillo: Municipio Boconó, Parque Nacional Guaramacal, Páramo de Guaramacal, from summit of Boconó-Caserío de Guaramacal road to the television towers, 2900–3100 m, 13 June 2001, *L.J. Dorr 8953*, with B. Stergios & S.M. Niño (holotype: PORT!; isotypes: K, MO, US-00662868!).

Diagnosis. *Dendrophthora apiculata* is distinguished from congeners by its rough striate stems; minute, ca. 0.5 mm long cataphylls surrounding all nodes; leaf blades 5–20 × 3–6 mm, apex apiculate with an apiculum 0.2–0.5 mm long; inflorescences usually 1(2) per leaf axil, staminate inflorescences triseriate and pistillate ones uniseriate; flowers ca. 1 mm long; and mature fruits globose-compressed, ca. 0.8–2 × 2–3 mm when dried.

Description. *Aerial parasitic shrubs*, monoecious; yellowish-green when fresh and drying dark brown. *Stems* woody; erect branches 20–30+ cm long; mature nodes at 2–3 cm long intervals, dichotomous, with multiple branches; coarse, longitudinal striations along principal branches with some transversal striations in basal branches (striations not visible in distal branchlets), minute papillose trichomes dispersed or absent; some lenticels present; cataphylls at nodes ca. 0.5 mm long. *Leaves* opposite, coriaceous; petioles winged, 2–5 mm long, indistinct; blades obovate, 5–20 × 3–6 mm, base cuneate, apex apiculate with an apiculum 0.2–0.5 mm long, margin entire;

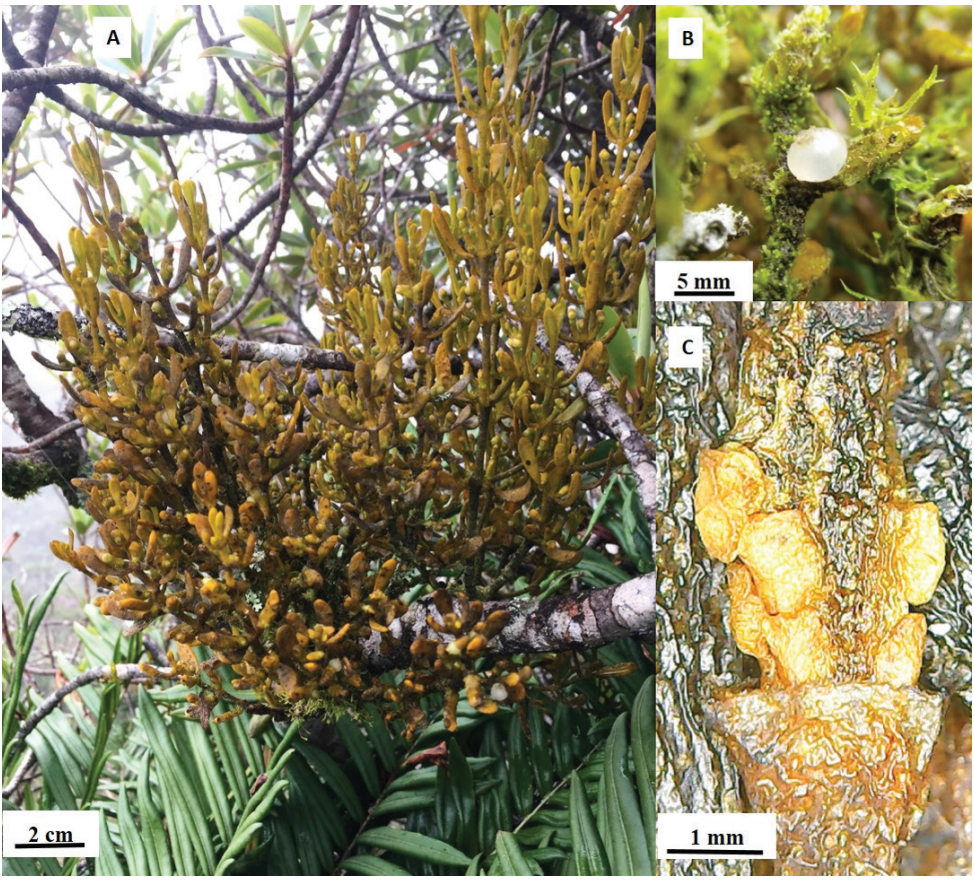

Figure 2. *Dendrophthora apiculata*. **A** Habit **B** fruit **C** basal flowers (staminate) (Photograph: D. Canelón).

veins reticulate with midvein evident on the adaxial side when fresh and inconspicuous when dried. Pistillate and staminate *inflorescences* separate, alternate on the same branch or branches either predominantly staminate or predominantly pistillate, usually 1 inflorescence per leaf axil, sometimes 2; fertile internodes usually 1(2), 7–18 mm long; *staminate inflorescences* triseriate; peduncles simple, 1–3 mm long, rugose; cup subtending inflorescence 1.5–1.8 × 2–2.5 mm, edge of cup papillose; basal portion of staminate inflorescences with 9–12 flowers per segment (see Fig. 2C); flowers ca. 1 mm in diameter; embedded in an alveolus (sunken receptacle), emerging up to 2/3 during anthesis; *pistillate inflorescences* uniseriate; peduncles simple, 2–4 mm long, rugose; cup (sensu Kuijt) subtending inflorescence 0.8–1 × 1–2.5 mm, edge of cup papillose, with 3–5 flowers per segment, sometimes more (Fig. 3), flowers adjacent (sometimes touching each other); petals 3(4), triangular, glabrous. *Fruits* globose-compressed, ca. 0.8–2 × 2–3 mm when dried, ripening white-translucent, crowned by persistent petals.

Distribution and hosts. *Dendrophthora apiculata* is known only from Guaramacal National Park (Trujillo state) between 2600–3100 m on both its northeastern and

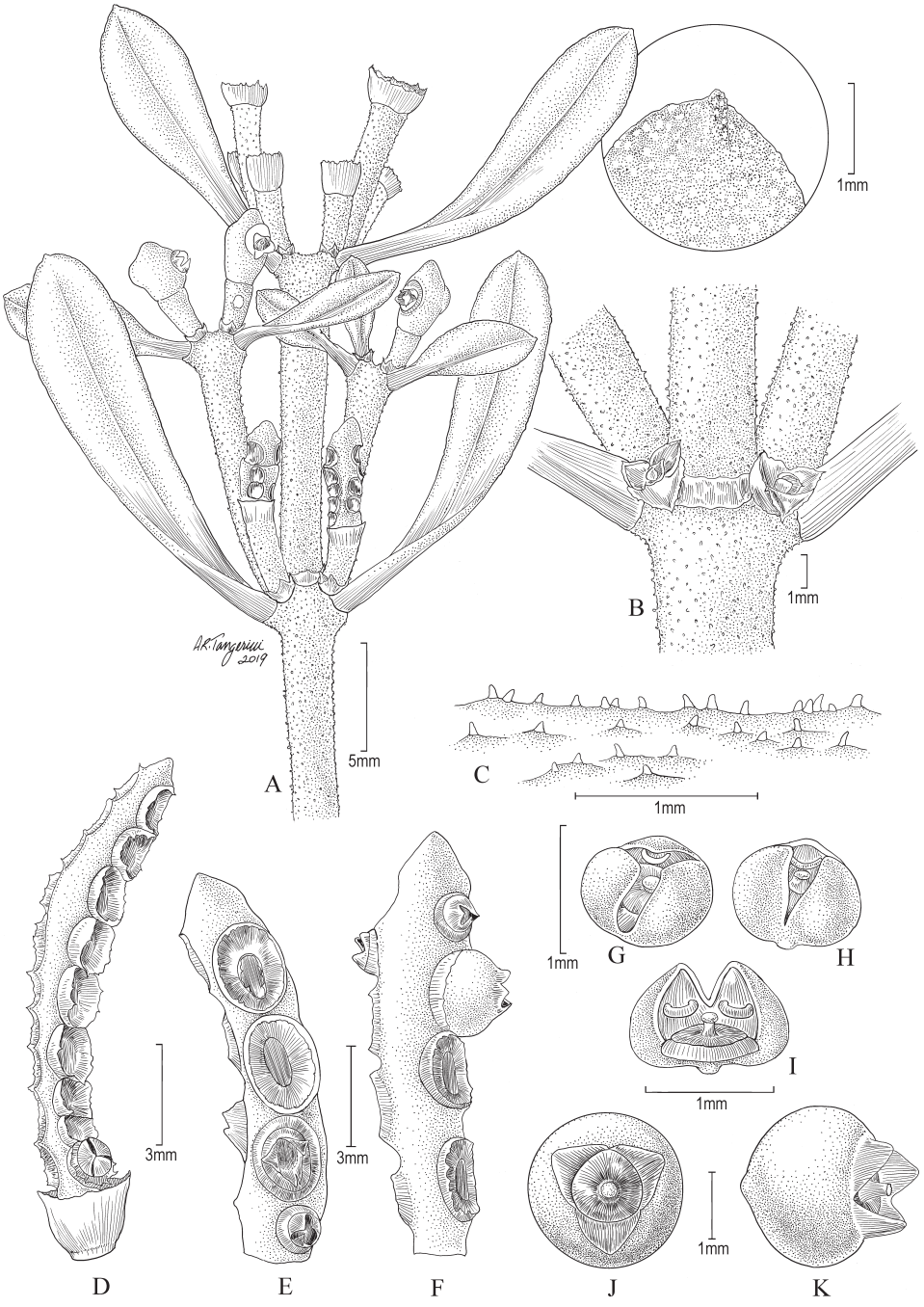

Figure 3. *Dendrophthora apiculata*. **A** Terminal branches showing inflorescences and leaves, including an enlargement of a leaf apex showing the apiculum (striations are not visible in these terminal branches) **B** Cataphylls at the base of a node **C** Papillose indumentum **D–F** Segments of pistillate inflorescences **G–I** Pistillate flowers **J, K** Mature fruits. (Source: *Stergios et al. 20126*, US).

southwestern mountain slopes. This mistletoe grows in open areas of the páramo and evidently is uncommon throughout its distributional range. Its host range seems to be limited, as the only hosts recorded so far are the shrubs *Cybianthus marginatus* (Benth.) Pipoly (Primulaceae), *Hypericum juniperinum* Kunth (Hypericaceae), and *Espeletia griffinii* Ruiz-Teran & López-Fig. (Asteraceae). Interestingly, the mistletoes found so far on the first host listed were observed on lower and middle branches, while in the last one they were found at the tips of branches.

Phenology. Reproductive individuals of *Dendrophthora apiculata* have been observed with flowers and fruits all year round, and the fruits seem to be an important source of food for the bird fauna present in the páramo and subpáramo habitats.

Etymology. The specific epithet is derived from "apiculate," which describes the minute sharp apiculum observed at the apex of leaves.

Discussion. *Dendrophthora apiculata* is similar to *D. lindeniana* Tiegh., but the latter has stems up to 1 m long, dense papillose trichomes covering the entire plant, leaves with rounded apices with papillose edges, and the cup subtending the inflorescence is usually bifid. In contrast, *D. apiculata* has stems up to 40 cm long, coarsely striate stems with scarce papillose indumentum or papillae absent; leaves with a smooth margin and a persistent, minute apiculum 0.2–0.5 mm long at the apex; and a cup subtending the inflorescence that is usually whole (or rarely rounded).

Regarding their distributions, *Dendrophthora apiculata* is found in the Páramo de Vicuyal (ca. 2730 m) in Guaramacal National Park (Trujillo state), while *D. lindeniana* grows in the Páramo de Portachuelo (2860 m) (Táchira state), near the border with Colombia. The known localities for these two species are separated one from the other by ca. 240 km.

Additional specimens examined. Venezuela. Trujillo: Municipio Boconó, Páramo de Guaramacal, SE of Boconó, 09°10–14'N, 70°11–15'W, 2600–3100 m, 18 July 1990, *L.J. Dorr, L.C. Barnett, W. Diaz, G. Aymard, F. Ortega & N. Murakami 7377* (NY, PORT); Carretera de tierra vía hacia las antenas, 09°14'29.0"N, 70°11'65.0"W, 2800 m, 23 Sep. 2000, *M. Niño, A. Licata & L. Linárez 1385* (US); Sector El Campamento, UTM: 19 368148-1022056 [9.244052N, -70.200324W], 2600 m, 13 Apr. 2019, *S. Niño & D. Canelón 6112* (PORT, US); Páramo de Guaramacal, 3000–3100+ m, July 2002, *B. Stergios & R. Caracas 19754* (PORT, US-00728477); Parque Nacional Guaramacal, Páramo Vicuyal, UTM: 1014040 N, 362685 E [9.171395N, -70.249794W], 2730 m, 11 Apr. 2003, *B. Stergios, L.J. Dorr, S.M. Niño & R. Caracas 20126* (PORT, US-00728399).

2. *Dendrophthora coronata* Canelón, S.M.Niño, Dorr & Caraballo, sp. nov.
urn:lsid:ipni.org:names:77205498-1
Figure 4

Type. Venezuela. Trujillo: Municipio Boconó, Parque Nacional Guaramacal, trail from antennas on the summit of Páramo de Guaramacal, NE to Fila Los Recostaderos [sic, Recortaderos] (UTM: 19 369344E, 1023140N) [9.253891N, -70.189471W],

páramo and subpáramo vegetation, 2677–3100 m, 14 June 2001, *L.J. Dorr 8988* with S.M. Niño & R. Caracas (holotype: US-00662772!; isotype: PORT).

Diagnosis. *Dendrophthora coronata* is distinguished from congeners by its stems and young branches with longitudinal parallel striations; coroniform trichomes (i.e., papillae crowned by 2–6 minute, simple hairs) covering the entire plant; small, ca. 0.5 × 1 mm cataphylls present at all nodes and sometimes found 1–2 cm above nodes on older branches; petioles 0.5–1 mm long, leaf blades 1–1.5 × 1–1.2 cm, apex rounded and margin slightly crenulate and papillose; uniseriate inflorescences with 5–9 flowers per series; and fruits globose-compressed, 2–3 × 2 mm when mature, white.

Description. *Aerial parasitic shrubs*, monoecious; yellow-orange when fresh, drying blackish or dark green. *Stems* woody, with multiple branches, 30–45 cm long, terete, surface coarse with parallel striations, with a dense layer of coroniform trichomes covering the entire plant; mature nodes separated by 2–3.5 cm long intervals, dichotomous; cataphylls 0.5–1 mm long at nodes, found 1–2 cm above nodes as branches became older. *Leaves* opposite, coriaceous; petioles flattened, 0.5–2 mm long; blades orbicular to elliptic, 1–1.5 × 1–1.2 cm, base slightly cuneate, apex rounded, margin indistinctly crenulate and papillose, surface rough on both sides, veins obscure in dry leaves (Fig. 4). *Inflorescences* completely pistillate within a branch, occasionally with staminate flowers at the base of the inflorescence (completely staminate inflorescences not seen); 1 per leaf axil, 1–3 fertile segments, each 4–10 mm long, uniseriate, greenish when dry; peduncles simple, 6–9 mm long, cup subtending inflorescence 1–1.5 × 3–3.5 mm, almost always forked with a papillate apical edge. *Flowers* 3–9 per segment, 1–1.4 × 0.7–1 mm. *Fruits* globose-compressed, 2–3 × 2 mm, ripening white- or purplish-translucent, surface granulose, tip protruding and crowned with persistent petals.

Distribution and habitat. This species has been found in the transition between cloud forest and subpáramo in Guaramcal. This vegetation is influenced by multiple factors including high rainfall (3200+ mm/year), elevation above sea level (2400–3100 m), as well as relative humidity (100% for most of the year) (Cuello and Cleef 2011). *Dendrophthora coronata* is found in these Andean/high Andean forests characterized by woody vegetation of low stature with numerous individual shrubs and small trees, and a thin understory with a carpet of thick leaf litter. The canopy can reach between 6–14 m tall, with some emerging trees that reach 16 m, among them: *Ilex guaramacalensis* Cuello & Aymard (Aquifoliaceae), *Miconia jahnii* Pittier (Melastomataceae), *Myrsine dependens* (Ruiz & Pav.) Spreng. (Primulaceae), and *Symplocos tamana* Steyerm. (Symplocaceae) (Cuello and Cleef 2011).

Phenology. As with most other species of tropical mistletoe, *Dendrophthora coronata* can be observed bearing flowers and fruits throughout the year. Its white fleshy fruits seem to be an important food source for forest birds.

Etymology. The species name is derived from the coroniform trichomes (i.e., papillae crowned by 2–6 minute, simple hairs) that cover the entire plant and that resemble small crowns.

Discussion. At first sight, *Dendrophthora coronata* resembles *D. apiculata* and *D. lindeniana*. However, *D. coronata* can be readily recognized by its marked parallel

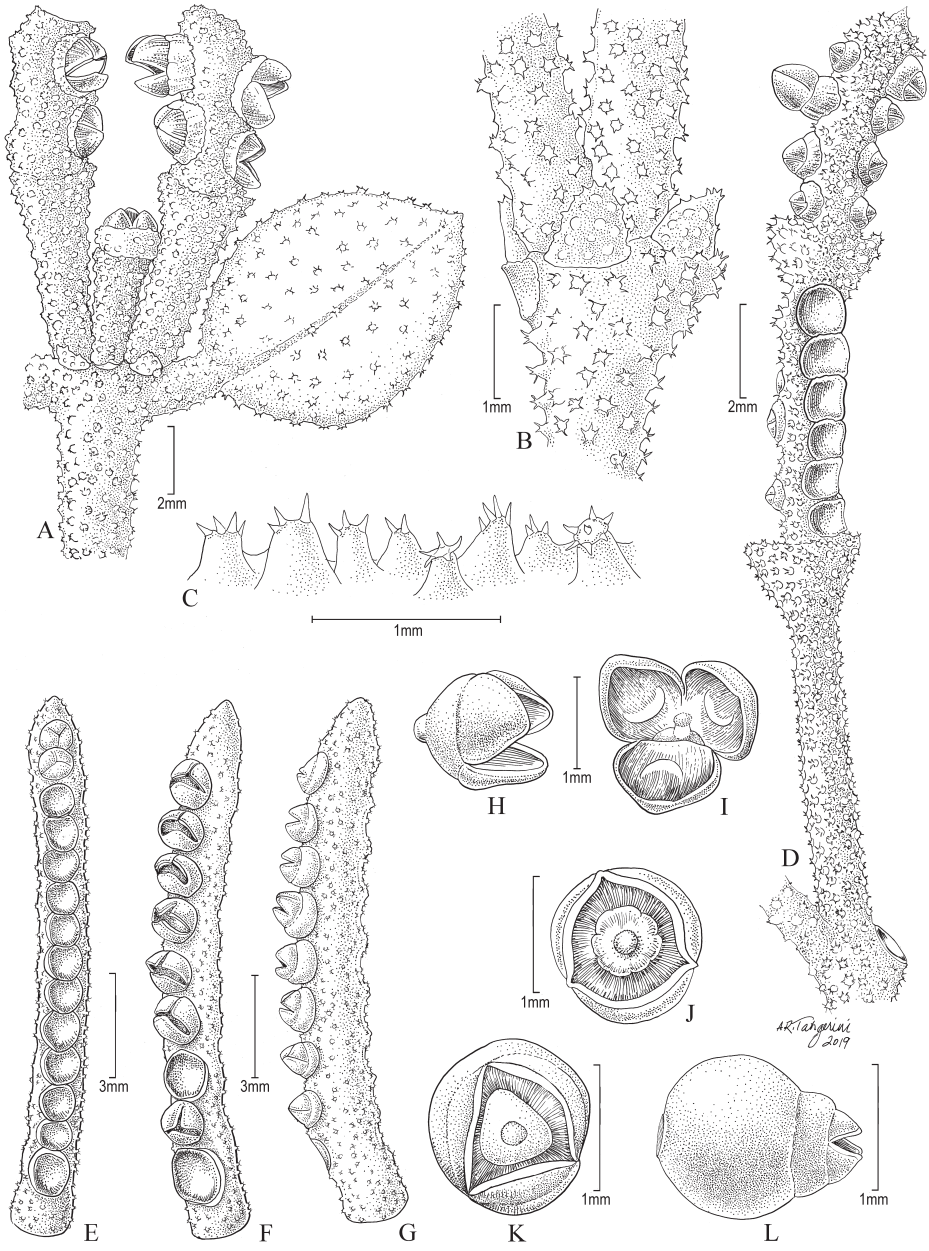

Figure 4. *Dendrophthora coronata*. **A** Leaf and terminal inflorescences **B** Cataphylls at the base of a node **C** Coroniform trichomes **D** Complete pistillate inflorescence **E–G** Segments of a pistillate inflorescence **H, I** Flowers with vestigial anthers **J–L** Mature fruits. (Source: *Dorr et al. 8988*, US).

striations along the stems, small, 0.5–1 mm long basal cataphylls, which sometimes are found 1–2 cm above nodes in old branches, and its dense layer of predominantly coroniform trichomes that cover the entire plant. In contrast, *D. apiculata* and

Table 1. Morphological characters distinguishing *Dendrophthora apiculata*, *D.coronata*, and *D. lindeniana.*

	D. apiculata	***D. coronata***	***D. lindeniana***
Plant height	20–30+ cm.	30–45 cm.	Up to 100 cm.
Indumentum	Entire plant sparsely papillate.	Entire plant abundantly covered with coroniform trichomes.	Entire plant abundantly papillate.
Stem	With longitudinal striations (not pronounced).	With pronounced striations (furrows).	Without striations.
Cataphylls	Cataphylls surrounding nodes 0.2–0.5 mm long; only located at the base of a node.	Cataphylls in basal branches 0.5–1 mm long; found 1–2 cm above a node.	Cataphylls not present.
Leaf apex	Apiculate; apiculum 0.2–0.5 mm long.	Not apiculate.	Not apiculate.
Petiole	Petiole 2–3 mm long.	Petiole 0.5–2 mm long.	Petiole up to 1 mm long.
Leaf margin	Entire.	Slightly crenulate.	Entire.
Staminate inflorescence	Triseriate.	Uniseriate.	Uniseriate.

D. lindeniana have stems sparsely covered by simple trichomes and lack basal cataphylls (or rarely have a few very small, 0.5 mm long cataphylls and then always at the nodes) (Table 1).

Additional specimens examined. Venezuela. Trujillo: Municipio Boconó, Sector El Campamento, UTM: 19 368148 E, 1022056 N [9.244052N, -70.200324W], 2600, 13 Apr. 2019, *S. Niño & D. Canelón 6111* (US); Parque Nacional Guaramacal, sector Vertiente Sur, carretera al caserío Guaramacal, 2000–2750 m, Dec. 1996, *B. Stergios & A. Licata 16813* (US-00656274).

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RESEARCH ARTICLE

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Molecular phylogenetic study of the tribe Tropidieae (Orchidaceae, Epidendroideae) with taxonomic and evolutionary implications

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Abstract

The orchid tribe Tropidieae comprises three genera, *Tropidia*, *Corymborkis* and *Kalimantanorchis*. There are three fully mycoheterotrophic species within Tropidieae: *Tropidia saprophytica*, *T. connata* and *Kalimantanorchis nagamasui*. A previous phylogenetic study of *K. nagamasui*, based only on plastid *matK* data, placed *K. nagamasui* outside the clade of *Tropidia* and *Corymborkis* without support. In this study, we performed phylogenetic analyses using a nuclear ribosomal DNA spacer (ITS1-5.8S-ITS2), a low-copy nuclear coding gene (*Xdh*) and a mitochondrial intron (*nad1b-c* intron) to study the phylogenetic relationships within Tropidieae. We included six photosynthetic and all three fully mycoheterotrophic Tropidieae species. The resulting phylogenetic trees placed these fully mycoheterotrophic species inside the *Tropidia* clade with high support. In our trees, these three species do not form a monophyletic group together, because the photosynthetic *T. graminea* is nested amongst them. Our results also suggest that the loss of photosynthetic ability occurred at least twice in *Tropidia*.

Keywords

Corymborkis, *Kalimantanorchis*, mycoheterotrophy, phylogeny, *Tropidia*

Introduction

The tribe Tropidieae is one of the earliest diverging clades in subfamily Epidendroideae (Orchidaceae) and currently contains three genera: *Corymborkis* Thouars, *Tropidia* Lindl. and *Kalimantanorchis* Tsukaya, M.Nakaj. & H.Okada (Tsukaya et al. 2011; Freudenstein and Chase 2015; Koch et al. 2016). *Corymborkis* comprises eight species and has a pantropical distribution (Rasmussen 1977; Govaerts et al. 2019). About 30 species are recognised in *Tropidia* and most of the species occur in tropical Asia and Australasia; the Neotropical *T. polystachya* (Sw.) Ames is the only species outside Asia and Australasia (Jones and Bolger 1988; Kumar et al. 2015; Koch et al. 2016; Ormerod 2018). *Kalimantanorchis* is the most recently established genus in the tribe with the single fully mycoheterotrophic species *K. nagamasui* Tsukaya, M.Nakaj. & H.Okada from Sabah and West Kalimantan in Borneo (Tsukaya et al. 2011; Suetsugu et al. 2017a). Two other fully mycoheterotrophic species are reported in Tropidieae; both belong to *Tropidia*: *T. saprophytica* J.J.Sm. and *T. connata* J.J.Wood & A.L.Lamb. *Tropidia saprophytica* has been recorded from Sabah and Sarawak and *T. connata* from Sabah and West Kalimantan (Wood 1988; Kikuchi and Tsukaya 2017). Wood (1984) established the fully mycoheterotrophic genus *Muluorchis* J.J.Wood together with the description of *Muluorchis ramosa* J.J.Wood. He later found that the species is conspecific with *T. saprophytica* and synonymised *Muluorchis* with *Tropidia* (Wood 1988). Wood and Lamb later described another fully mycoheterotrophic species, *T. connata* (Wood and Cribb 1994). *Tropidia connata* is easily distinguished from *T. saprophytica* by the zigzag inflorescence, connate lateral sepals and shortly spurred lip (Wood and Cribb 1994). More recently, *Kalimantanorchis* was established as a new genus, based on its morphological characters and molecular phylogenetic data (Tsukaya et al. 2011). The subterranean tuberous structure of *Kalimantanorchis* had never been observed in the other two fully mycoheterotrophic *Tropidia* species, although we now know that a tuberous structure occurs at least in *T. connata* as well (Tsukaya et al. 2011; Kikuchi and Tsukaya 2017). Kikuchi and Tsukaya (2017) suggested that the tuberous structures of the fully mycoheterotrophic Tropidieae species may be equivalent to the tuber-like nodules of other leafy *Tropidia* (Yeh et al. 2009, Chun-Kuei et al. 2013). On the other hand, Tsukaya et al. (2011) provided a molecular phylogenetic tree based only on plastid *matK* data, in which *Kalimantanorchis* was placed outside the clade comprising *Tropidia* and *Corymborkis*.

The study of mycoheterotrophy is hampered by two major obstacles (Merckx et al. 2013a). One is the rarity of many fully mycoheterotrophic species: several have been found only once or twice in the field. Due to the scarcity of adequate material, for some of the species, information is lacking even at the basic level of morphology and anatomy. The other major obstacle is the elevated plastid DNA substitution rates usually occurring in fully mycoheterotrophic plants. In these plants, the leaves are normally reduced to scales or sheaths and they are achlorophyllous (Tsukaya 2018). Most of the plastid genes of fully mycoheterotrophic species are, supposedly, not functioning anymore as the plants have lost the ability to conduct photosynthesis and often experienced higher mutation rates and deletions in these genes (Freudenstein and Senyo

2008; Delannoy et al. 2011). Although some fully mycoheterotrophic species still retain amplifiable plastid DNA regions, they can be difficult to align and analyse due to these elevated substitution rates (Merckx et al. 2013a). Since most of the phylogenies of the photosynthetic, presumably autotrophic or partially mycoheterotrophic relatives are at least partly based on plastid regions, it is sometimes impossible to relate the fully mycoheterotrophic species properly to other lineages. Therefore, the phylogenetic placement of fully mycoheterotrophic plants has often been difficult. These problems also apply to the fully mycoheterotrophic Tropidieae species.

In 2011 and 2012, multiple specimens of *Tropidia connata* were collected by the last author (HT) in Borneo. These specimens included underground tuberous structures, which were not described in the original description (Wood and Cribb 1994) due to the incompleteness of the examined material. The examination of tuberous structures in *T. connata* clearly indicates that the tuber is a shared character between *Kalimantanorchis nagamasui* and *T. connata*, suggesting that *Kalimantanorchis* may not be distinct from *Tropidia* (Kikuchi and Tsukaya 2017) (Fig. 1). As already mentioned, Tsukaya et al. (2011) provided a phylogenetic analysis based on a plastid region (*matK*), which placed *Kalimantanorchis* outside the *Tropidia* + *Corymborkis* clade. However, this result had low support, possibly because of a highly elevated substitution rate of *matK* in *Kalimantanorchis*.

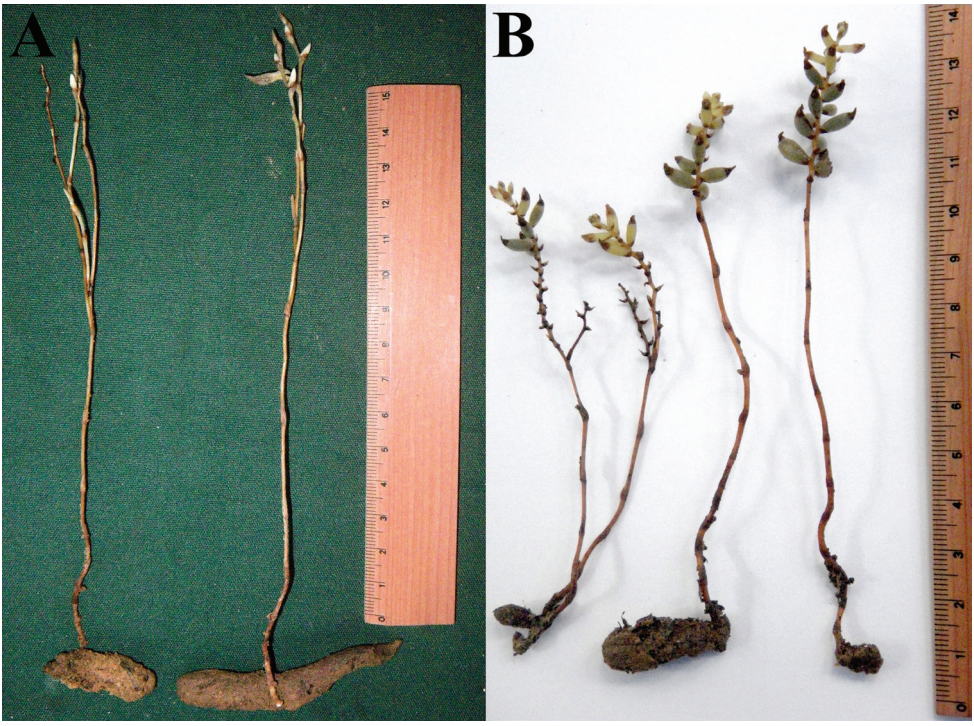

Figure 1. *Tropidia connata* J.J.Wood & A.L.Lamb and *Kalimantanorchis nagamasui* Tsukaya, M.Nakaj. & H.Okada. **A** Gross morphology of *T. connata* individual (specimen number 1040, collected in January, 2011 by H. Tsukaya, H. Okada and A. Soejima). **B** Gross morphology of fruiting *K. nagamasui* individual (specimen number HT1035, collected in January, 2011 by H. Tsukaya, H. Okada and A. Soejima). Scale in cm.

Based only on the morphological findings of Kikuchi and Tsukaya (2017), Ormerod and Juswara (2019) synonymised *Kalimantanorchis* with *Tropidia*; however, they did not provide molecular evidence to counter the findings of Tsukaya et al. (2011). In this study, we were able to include all three fully mycoheterotrophic species within a broader sampling of Tropidieae compared to the previous phylogenetic analysis of Tsukaya et al. (2011) and conducted molecular phylogenetic analyses using a nuclear ribosomal DNA spacer (ITS1-5.8S-ITS2), a low-copy nuclear coding gene (*Xdh*) and a mitochondrial intron (*nad1b-c* intron) to clarify the placement of the fully mycoheterotrophic species.

Methods

Taxon sampling and plant material

Nine species of Tropidieae were included in this study (six species of *Tropidia*, one of *Kalimantanorchis* and two of *Corymborkis*). We sampled all three fully mycoheterotrophic species. Apart from *Tropidia polystachya* occurring in the Neotropics, all sampled *Tropidia* species are native to Asia. We selected *Sobralia rosea* Poepp. & Endl. (Epidendroideae: Sobralieae) as an outgroup taxon for the phylogenetic analyses considering that Sobralieae is sister to the rest of Epidendroideae, except for Neottieae (Freudenstein and Chase 2015; Givnish et al. 2015). We obtained three sequences of *Sobralia rosea* from GenBank. We used five DNA samples from the DNA Bank of the Royal Botanic Gardens, Kew. Four samples were collected in the field and extracted in this study. We provided the sample and voucher information in Table 1.

DNA extraction, PCR amplification and sequencing

DNA extraction was conducted for the four samples obtained in the field using FTA cards (Whatman, Tokyo, Japan) and DNeasy kit (Qiagen, Hilden, Germany). Amplification for the nuclear ITS, *Xdh* and mitochondrial *nad1b-c* intron, was performed for nine samples using TaKaRa ExTaq polymerase (TaKaRa Bio, Shiga, Japan) and DreamTaq DNA Polymerases (Thermo Fisher, Epsom, UK). The following primer sets were used: ITS1 and ITS4 for ITS1-5.8S-ITS2 (White et al. 1990), X502 and X1599R for *Xdh* (Górniak et al. 2010) and nad1 exon B and nad1 exon C for *nad1 b-c* intron (Demesure et al. 1995). For ITS, the thermal cycling protocol began with 3 min initial denaturation at 94°C, followed by 35 amplification cycles, each with 15 s at 94°C, 30 s at 44°C and 40 s at 72°C, which was concluded by a final extension at 72°C for 7 min. For *Xdh,* the thermal cycles were as follows: initial denaturation at 94°C for 2 min, 6 touchdown cycles of 45 s at 94°C, 45 s at 53°C and 90 s at 72°C, reducing one degree per cycle, which was followed by 28 cycles of 45 s at 94°C, 45 s at 47°C and 90 s at 72°C and a final extension at 72°C for 5 min. For *nad1 b-c* intron, the thermal

Table 1. Voucher information and GenBank accessions of samples used in phylogenetic analyses. Herbarium codes follow Thiers (2019). Samples with NA were extracted in this study, except for *S. rosea*. Sequences of *S. rosea* were obtained from GenBank.

Taxon	Voucher information	Kew DNA Bank ID	ITS	Xdh	nad1 b-c intron
Corymborkis corymbis Thouars	10041 (REU)	23681	MH596711	MH594866	MH594874
Corymborkis veratrifolia (Reinw.) Blume	Tsukaya et al., B200608289 (BO)	NA	MH596710	MH594865	MH594875
Kalimantanorchis nagamasui Tsukaya, M.Nakaj. & H.Okada	Tsukaya et al., T31 (BO, TI)	NA	MH596709	MH594864	MH594873
Sobralia rosea Poepp. & Endl.	Romano, Sob5 and Szlachetko	NA	KT923827	KT923862	EF464132
Tropidia bambusifolia (Thwaites) Trimen	without number	17721	MH572221	MH594863	MH594872
Tropidia connata J.J.Wood & A.L.Lamb	Tsukaya et al., 224 (TI)	NA	MH596708	MH594862	MH594871
Tropidia graminea Blume	Duangjai, 40 (BRUN, K)	21775	MH596707	MH594861	MH594870
Tropidia nipponica var. *hachijoensis* F.Maek. & Yokota	Matsumoto, 466 (TNS)	NA	MH596706	MH594860	MH594869
Tropidia polystachya (Sw.) Ames	89001 (MWC)	O-211	MH596705	MH594859	MH594868
Tropidia saprophytica J.J.Sm.	Cameron	O-798	MH596704	MH594858	MH594867

cycles were set as follows: pre-melting for 3 min at 94°C, followed by 35 cycles of 15 s at 94°C, 30 s at 50°C and 2 min at 72°C and a final extension at 72°C for 7 min. Cycle sequencing was conducted using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR reactions. We additionally used nad1M as an internal primer for sequencing *nad1 b-c* intron (see Freudenstein and Chase 2001). Newly generated 27 sequences were deposited in GenBank and their accession numbers are listed in Table 1 with the accession numbers of the three sequences of *Sobralia*, which were obtained from GenBank.

Phylogenetic analyses

In total, 30 sequences were edited and assembled using Geneious Prime 2019.2.1 (https://www.geneious.com) (Kearse et al. 2012). Sequence alignments for each region were generated with the MUSCLE alignment tool (Edgar 2004), installed in Geneious Pro using the default setting. Phylogenetic relationships were inferred for the concatenated alignments of the three markers (ITS, *nad1b-c* intron, *Xdh*). The best partitioning scheme was selected by the Bayesian Information Criterion (BIC) using PartitionFinder 2.1.1 (Guindon et al. 2010; Lanfear et al. 2016). The best partitioning and substitution models for each partition were estimated as follows: ITS (K80+G), *nad1 b*-c intron (K81UF), *Xdh* first and second codon (K80), *Xdh* third codon (HKY). The Maximum Likelihood (ML) analysis was conducted using IQ-TREE 1.6.12 (Nguyen et al. 2015). We obtained branch supports by the ultrafast bootstrap with 1000 replicates (Hoang et al. 2018). The Bayesian Inference (BI) analysis was performed using

MrBayes 3.2.7a (Ronquist et al. 2012). The BI analysis was run for 50000 generations. Trees were sampled every 100 generations of the MCMC chain. By default, MrBayes discarded the first 25% samples from the cold chain. The average standard deviation of split was checked at the end of the run. Using the sump command, the model parameters were also checked for the convergence diagnostic, the Potential Scale Reduction Factor (PSRF). All the PSRF values were close to 1.0. A 50% majority rule consensus tree was estimated using the sumt command to obtain the posterior probabilities for each clade. Generated trees were visualised using FigTree 1.4.3 (Rambaut 2017). Clades with over 85% bootstrap (BS) value or 0.95 Bayesian Posterior Probability (PP) were considered strongly supported. Clades with over 75% BS or 0.90 PP support were considered moderately supported. Clades with lower support values were not regarded as reliably supported clades.

Results

The aligned sequence lengths for each region were 669 bp (ITS), 1389 bp (*nad1b-c* intron) and 843 bp (*Xdh*). We separately conducted ML analyses for the three regions and none of the incongruences in topology was significantly supported between the trees. Therefore, the combined usage of the three markers was justified.

The positions of all the taxa were consistent in both trees resulting from the ML and BI analyses for the combined alignments. As the topology was consistent with strong support, we added the bootstrap values obtained from the BI analysis to the ML tree, which is shown in Figure 2.

In both the ML and BI trees, *Corymborkis* species form a monophyletic clade (BS = 100%, PP = 1). *Tropidia* species also form a monophyletic clade with the Neotropical *T. polystachya* as the first diverging species with a moderately high PP support (0.91) but with a low BS support (65%) (Fig. 2). In our trees, *T. polystachya* is sister to all Asian *Tropidia* species. In this Asian *Tropidia* clade, the East Asian *Tropidia nipponica* var. *hachijoensis* F.Maek. & Yokota is the first diverging species, followed by the Sri Lankan *Tropidia bambusifolia* (Thwaites) Trimen. The three fully mycoheterotrophic Tropidieae species form a clade (clade Myco, hereafter) with the photosynthetic *Tropidia graminea* Blume. In clade Myco, the clade which includes *K. nagamasui* and *T. saprophytica* (clade A, hereafter) is sister to the clade formed by *T. connata* and *T. graminea* (clade B, hereafter) with high support (BS = 95%, PP = 1).

Discussion

Monophyly of Corymborkis and Tropidia

Corymborkis and *Tropidia* were each inferred as monophyletic in our study. In the comparative morphological study of *Corymborkis* by Rasmussen (1977), *Tropidia polystachya* was mentioned as an exceptional *Tropidia* species because of the type of inflo-

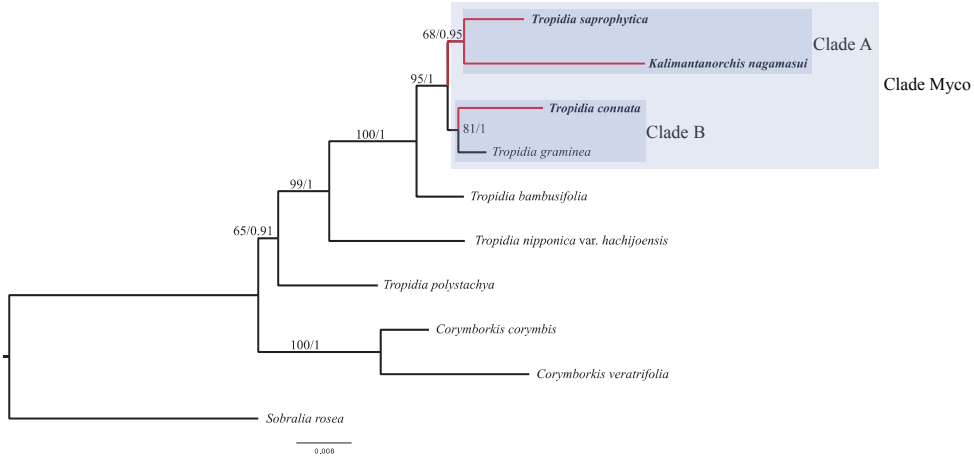

Figure 2. Phylogenetic relationships of Tropidieae based on Maximum Likelihood analysis on three non-plastid regions (ITS, *nad1 b-c* intron and *Xdh*). Numbers at nodes are Bootstrap Percentages obtained by Maximum Likelihood analysis and Bayesian Posterior Probabilities, respectively. Fully mycoheterotrophic species are shown in bold. Red branches indicate mycoheterotrophic origins. The scale bar below the tree indicates the substitution rate.

rescence feature, which is shared with *Corymborkis*: branched inflorescence when fully developed. As *T. polystachya* is placed as the first diverging species of *Tropidia* in our trees, we suggest that the branched inflorescence may be a symplesiomorphic character in *Corymborkis* and *T. polystachya*. As the morphology of these orchids is still little studied, it would be desirable to analyse character evolution in more detail using a broader sampling of Tropidieae.

Evolution of non-photosynthetic feature

The evolutionary pathway to full mycoheterotrophy is hypothesised to be irreversible (Barrett et al. 2014). Once the ability to perform photosynthesis is lost, the photosynthetic genes are not expected to be regained (Lam 2016). In our results, *Tropidia graminea*, a photosynthetic species with well-developed leaves, is placed as a sister taxon to fully mycoheterotrophic *T. connata* in clade B, which is sister to clade A. Granted that the evolution of the non-photosynthetic feature is irreversible, we hypothesise that the ancestral character of clade A and clade B is photosynthetic. Therefore, this result indicates that non-photosynthetic features independently evolved at least twice from photosynthetic ancestors in the *Tropidia* clade (Fig. 2). However, the branch lengths of the basal nodes of clade A and clade B are short and it might be the case that long branch attraction or the incongruences amongst datasets influenced this topology. We can only test this if we increase the number of sampled species from Asian *Tropidia* and include more markers to conduct phylogenetic analyses.

Flowering plant genera with multiple transitions to full mycoheterotrophy have rarely been reported to date. *Hexalectris* Raf. (Orchidaceae) and *Burmannia* L. (Burmanniaceae) are two of the few genera known to have multiple transitions to full mycoheterotrophy (Merckx et al. 2008; Barrett et al. 2019).

In Orchidaceae, more than 230 species in 43 genera are assumed to be non-photosynthetic and thus fully mycoheterotrophic (Merckx et al. 2013b). It is estimated that within Orchidaceae, full mycoheterotrophy has evolved about 30 times independently, possibly from partially mycoheterotrophic ancestors (Freudenstein and Barrett 2010). Partially mycoheterotrophic species still retain the ability to photosynthesise at the same time gaining carbon also from the mycorrhizal fungal partners and this nutritional mode of life has been suggested to be more widespread in Orchidaceae than previously assumed (Gebauer et al. 2016). Partial mycoheterotrophy has been hypothesised to be an intermediate evolutionary step from initial mycoheterotrophy towards full mycoheterotrophy in Orchidaceae (Merckx et al. 2013a). For instance, it is likely that in *Cymbidium* Sw. fully mycoheterotrophic species evolved from partial mycoheterotrophic ancestors (Motomura et al. 2010). Partial mycoheterotrophy has been confirmed for many photosynthetic orchid species, mainly in subfamily Epidendroideae through isotopic analyses (Julou et al. 2005; Cameron et al. 2009; Suetsugu et al. 2017b).

So far, the occurrence of partial mycoheterotrophy in *Tropidia* has not been examined. However, all species of *Tropidia* usually grow in deep shade in the forest understorey, which supports the hypothesis that they might be partially mycoheterotrophic (Rasmussen 2005). In order to elucidate the evolutionary pathway from photosynthetic plants to fully mycoheterotrophic organisms in *Tropidia*, isotopic analyses on photosynthetic *Tropidia* species would be indispensable.

Kalimantanorchis, a synonym of Tropidia

Kalimantanorchis is nested in the Asian *Tropidia* clade with high support in our study. This suggests that the previous molecular phylogenetic analysis, based only on plastid *matK* (Tsukaya et al. 2011), failed to show the phylogenetic relationships. The *matK* sequence of *Kalimantanorchis* used in that study was extremely short (501 bp) and we suspect that it also had a particularly high substitution rate, which possibly made the alignment problematic and thus caused the unsupported placement of *Kalimantanorchis* outside the *Tropidia* + *Corymborkis* clade. According to our results and the finding of the tuberous underground structures being a shared character with *T. connata* (Kikuchi and Tsukaya 2017), we cannot support the recognition of *Kalimantanorchis* as a distinct genus.

Conclusions

According to morphological data by Kikuchi and Tsukaya (2017) and the highly supported positions of *Kalimantanorchis* inside *Tropidia* in our phylogenetic trees, we reject both grounds (i.e. the unique subterranean tuberous structure of *Kalimantanorchis*

and the result of the molecular phylogenetic analysis based on *matK*) for the establishment *Kalimantanorchis* as a distinct genus suggested by Tsukaya et al. (2011). We support the synonymisation of *Kalimantanorchis* with *Tropidia,* as proposed by Ormerod and Juswara (2019).

Our results suggest that the loss of photosynthetic ability occurred at least twice in *Tropidia*. Genera with multiple transitions to non-photosynthetic full mycoheterotrophy have rarely been reported. These genera may play an important role in future studies of the evolution of mycoheterotrophy, as they may provide insights into the drivers of such transitions.

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RESEARCH ARTICLE

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Lysimachia xiangxiensis (Primulaceae), a new species from limestone area in Hunan Province, central China

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Abstract

A new species of *Lysimachia*, *L. xiangxiensis* (Primulaceae), is described and illustrated from western Hunan, central China. The species is similar to *L. melampyroides* in plant densely strigillose, leaves subglabrous adaxially, and flowers usually solitary in axils of upper leaves, but differs by the succulent leaves, the creeping or ascending stems 15–25 cm long, and the suborbicular to broadly elliptic corolla lobes. This new species is also supported by a molecular phylogenetic analysis of some *Lysimachia* species based on ITS sequence data.

Keywords

Lysimachia, *L. xiangxiensis*, new species, taxonomy, western Hunan

Introduction

The genus *Lysimachia* L., a large genera of Primulaceae s. l. (APG III 2009), consists of over 180 species of annual or perennial herbs (Hu and Kelso 1996). *Lysimachia* has a nearly cosmopolitan distribution, mainly occurring in the temperate and subtropi-

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cal parts of the northern hemisphere, with a few species in Africa, Australia and South America (Hu and Kelso 1996, Liu et al. 2014a). Southwestern China and its neighboring region of Indochina Peninsula have an extremely high species diversity with ca. 130 species and have been considered to be the diversity center of the genus (Yan et al. 2017).

During our expedition in 2017 and 2019 to the Youshui River valley in western Hunan, China, an unusual population of *Lysimachia*, with the plants having revolute succulent leaves, caught our attention. After consulting the relevant literature (Chen et al. 1989, Hu and Kelso 1996, Yan and Hao 2012, Liu et al. 2014a, Liu et al. 2014b, Zhou et al. 2015, Wang et al. 2018) and checking relevant specimens, we determined that the population represents a new species. Additionally, the new species is supported by a molecular phylogenetic analysis of some *Lysimachia* species based on ITS sequence data.

Materials and methods

Taxon sampling and morphological analysis

The type specimens and fresh materials of the new species were collected from Huayuan County and Jishou City, Hunan Province, central China. Morphological observations and measurements were randomly made on flowering and fruiting plants. We examined related specimens kept in JIU and HUN and also specimen images in the online database of Chinese Virtual Herbarium (http://www.cvh.ac.cn) and JSTOR Global Plants (https://plants.jstor.org).

A total of 39 nuclear ribosomal ITS sequences for 34 species (Appendix S1) were downloaded from GenBank, following a study of *Lysimachia* (Zhang et al. 2011, Zhou et al. 2015). Two accessions of the putatively new species were sequenced for this study (GenBank Acc. No.: MN647744, MN647745). *Ardisia verbascifolia* Mez was selected as outgroup following Zhang et al. (2011). Voucher specimens of those specimens of the new species used for sequencing were deposited in JIU.

Molecular analyses

Total genomic DNA of the two accessions of the putatively new species was isolated from silica gel-dried leaves using a modified cetyltrimethylammonium bromide procedure (Doyle and Doyle 1987). The ITS region was amplified and sequenced by method of Zhang et al. (2011).

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The models determined for the datasets using the Akaike information criterion (Burnham and Anderson 2003) as implemented in MrModeltest 2.3 (Nylander 2004). ML trees were generated in RAxML 7.2.6 (Stamatakis 2006) with 1000 bootstrap replicates. BI trees were inferred in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Four chains, each starting with a random tree, were run for 1,000,000 generations with trees sampled every 1000 generations. The convergence

of the two runs was accessed with the average standard deviation of split frequencies less than 0.01. After the first ca. 25% discarded as burn-in, the remaining trees were imported into PAUP* v.4.0b10 (Swofford 2002) and a 50% majority rule consensus tree was produced to obtain posterior probabilities (PP) of the clades.

Results and discussion

Morphological comparisons

According to the key in Hu and Kelso (1996), the new species is positioned to "Key 2" by flowers 5-merous, homomorphic, corolla yellow, anthers shorter than filaments, and further to "19a" by anthers distinctly dorsifixed (1b), inflorescences not paniculate(3b), stems more than 5 cm and leaves opposite (5b), corolla subfunnelform, filaments connate 1/3–1/2 into a tube (7b), flowers axillary and solitary or in terminal clusters with bracts leaflike (12b), inflorescences not capitate (17a), leaf blade not connate-perfoliate (18b), flowers solitary and axillary or in terminal racemes, plants strigillose (19a).

Morphologically, the new species is most similar to *L. melampyroides* R. Knuth in Engler with which it shares such features as the plants densely strigillose, leaves subglabrous adaxially, and flowers that are usually solitary in axils of upper leaves. However, the new species differs from *L. melampyroides* by the succulent leaves, the creeping or drooping stems 15–25 cm long, and the suborbicular to broadly elliptic corolla lobes. A morphological comparison between the new species and *L. melampyroides* is presented in Table 1.

Phylogenetic position

The aligned lengths of ITS are 655 bp with gaps treated as missing data. BI and ML analyses produced similar topology and only the BI tree was presented in Figure 1.

Table 1. Morphological comparison between *Lysimachia xiangxiensis* sp. nov. and its similar species.

Character	*L. xiangxiensis* sp. nov.	*L. melampyroides*
Stems	creeping or drooping.	erect or ascending.
Plant height	15–25 cm	15–50 cm
Petiole	not auriculate at base	dilated and auriculate at base
Blades of lower leaves	succulent, rhomboid-ovate to ovate, the basal 1 or 2 pairs scale-like	papery, ovate to linear-lanceolate
Blades of upper leaves	succulent, ovate to elliptic-lanceolate, 2–5.5 cm × 1–2.3 cm	papery, ovate to linear-lanceolate, 1.5–9 × 0.3–2.5 cm
Secondary veins	blurry or invisible on adaxial surface, slightly raising on abaxial surface	visible on both surfaces
Glandular dots on leaves	Absent	transparent, sparse
Corolla lobes	suborbicular to broadly elliptic, apex cuspidate or emarginated, 7–9 mm long and wide	obovate-elliptic, apex rounded, 6–7 × 4–6 mm
Calyx lobes	costa indistinct	costa distinct

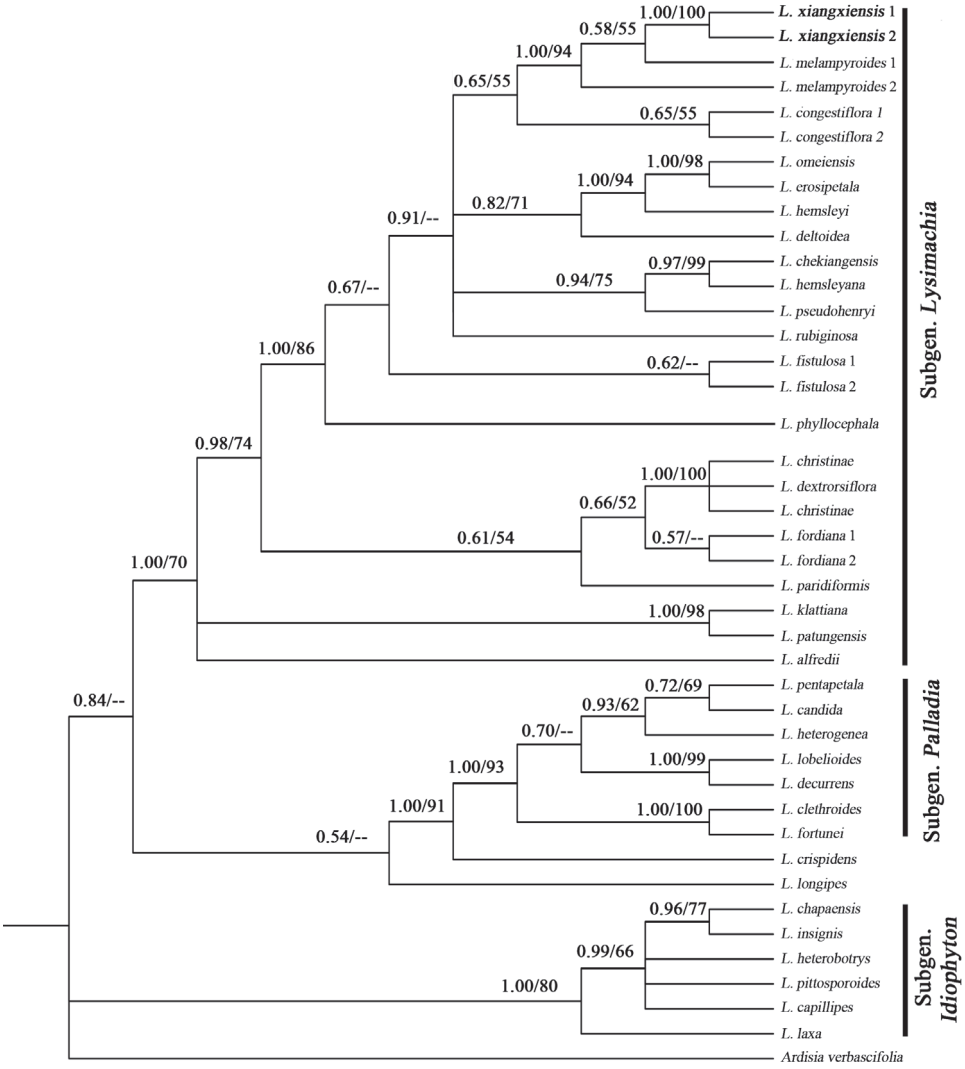

Figure 1. The phylogram of Bayesian inference (BI) tree from the ITS sequence data, showing the phylogenetic position of *Lysimachia xiangxiensis* sp. nov. (shown in bold). Values above the branches represent Bayesian posterior probabilities (PP) and bootstrap values (LP) for maximum likelihood, respectively; the dash (–) indicates LP < 50%.

The phylogenetic results indicate that two samples of the new species were grouped together with a strong support (PP = 1.00, LP = 100%) and closely related to *L. melampyroides* (PP = 1.00, LP = 94%).

On the basis of the classification in Handel-Mazzetti (1928), Chen and Hu (1979) divided the genus into five subgenera as well as many series, Subgen. *Lysimachia*, Subgen. *Palladia* (Moench) Hand.-Mazz, Subgen. *Idiophyton* Hand. -Mazz., Subgen. *Naumburgia* (Moench) Klatt and Subgen. *Heterostylandra* (Hand.-Mazz.) Chen et C.

M. Hu. In this topology, all *Lysimachia* species form three main clades: Subgen. *Lysimachia* (PP = 1.00, LP = 70%), Subgen. *Palladia* (PP = 1.00, LP = 93%) and Subgen. *Idiophyton*. (PP = 1.00, LP = 80%). In addition, *L. crispidens* (Hance) Hemsley in F. B. Forbes & Hemsley of Subgen. *Heterostylandra* is close to Subgen. *Palladia* (PP = 1.00, LP = 91%) and *L. longipes* Hemsley is assigned to Subgen. *Lysimachia* with weak supported (PP = 0.54) in a neutral position between Subgen. *Lysimachia* and *L. crispidens*. But classification of series are not well reflected in this analysis.

Taxonomic treatment

***Lysimachia xiangxiensis* D.G.Zhang & C.Mou, Y.Wu, sp. nov.**
urn:lsid:ipni.org:names:77206205-1
Figure 2–4

Type. CHINA. Hunan Province, Huayuan County, Buchou Town, Da-long-dong, cliff of a valley, 28°19'06.42"N, 109°30'03.22"E, alt. 295 m, 26 August 2019, D. G. Zhang 0826075 (holotype: JIU!; isotype: JIU!).

Diagnosis. The new species differs from *L. melampyroides* by the succulent leaves; the creeping or drooping stems (15–25 cm long); and the suborbicular to broadly elliptic corolla lobes.

Description. Terrestrial, perennial herbs. Rhizome brown, reduced to a small tuber or rarely creeping, with sparse fibrous roots. ***Stems*** creeping or drooping on cliffs, 15–25 cm long, clustered, branched at base, unbranched or rarely branched from the middle, terete, purple-red, densely strigillose, the internodes usually 3–7 cm long. ***Leaves*** petiolate, opposite. Petioles 5–7 mm long, with a furrow on adaxial side, green or purple-red, strigillose. Leaf blade succulent; blade of lower leaves rhomboid-ovate to ovate, with 1 or 2 pairs of basal leaves scalelike (much smaller); blade of upper leaves ovate to elliptic-lanceolate, 2–5.5 cm × 1–2.3 cm, base cuneate, apex acuminate or acute to subobtuse, margin entire and revolute, adaxially dark green, shiny, subglabrous, abaxially

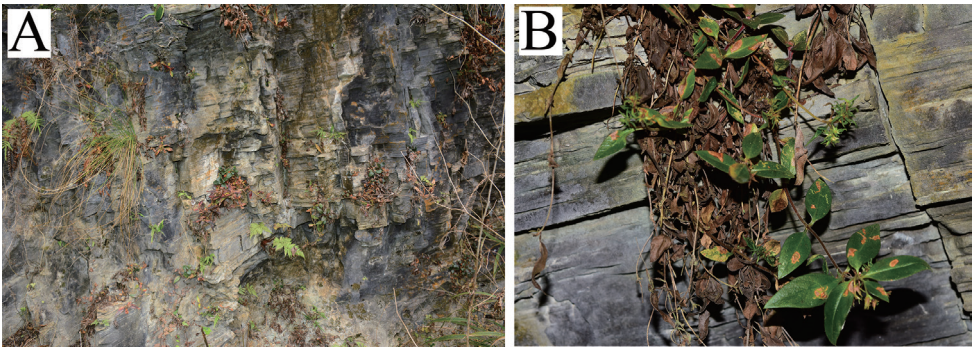

Figure 2. *Lysimachia xiangxiensis* sp. nov. in the wild **A** habitat (dry limestone cliff) **B** stems drooping on the cliff.

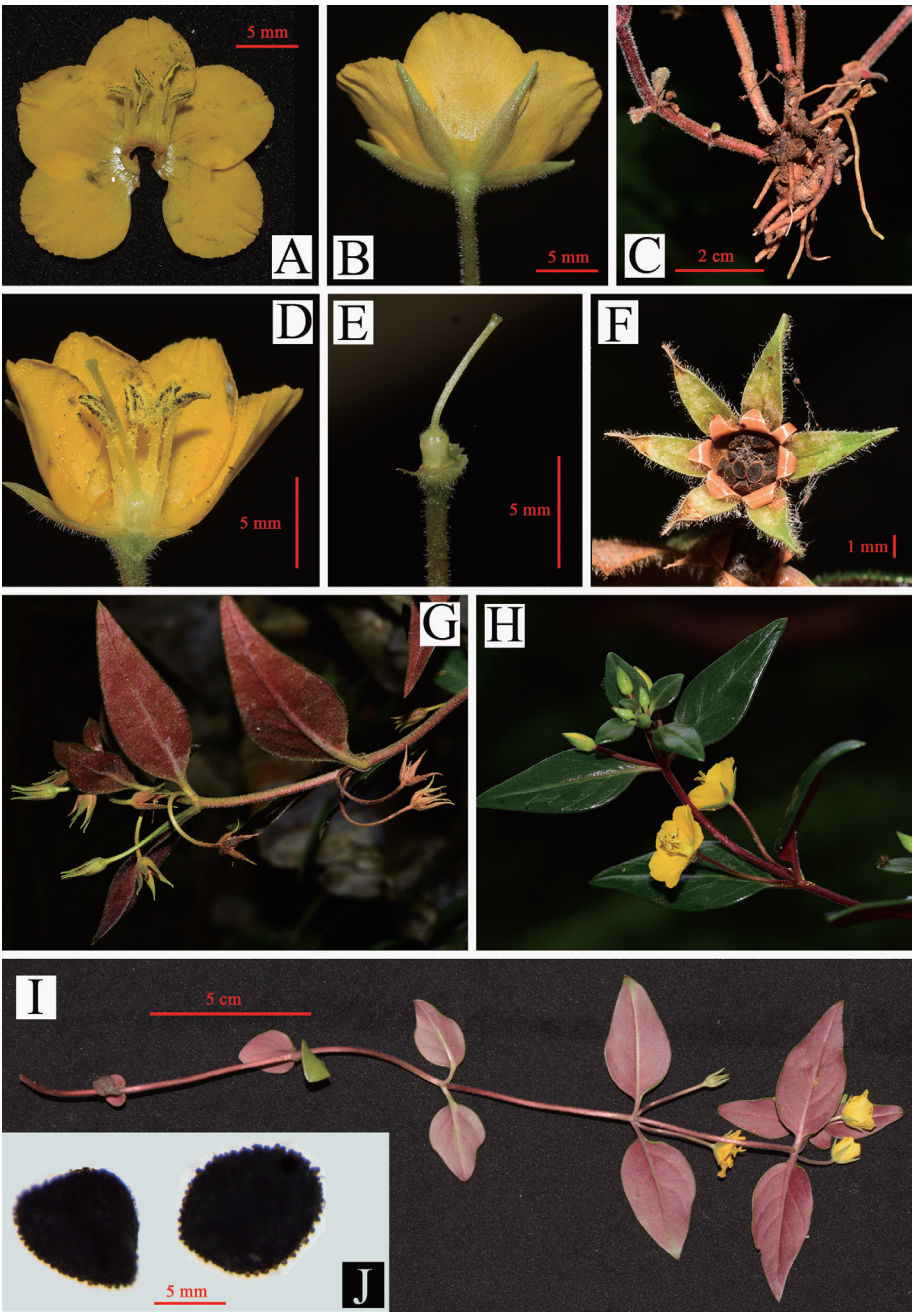

Figure 3. *Lysimachia xiangxiensis* sp. nov. in the field **A** corolla opened, showing the suborbicular lobes **B** flower (lateral view), showing the lanceolate calyx lobes indistinctly costate **C** proximal stems and underground part, showing stems clustered, rhizome, sparse fibrous roots, and 1 or 2 pairs of scalelike basal leaves **D** longitudinal section of flower, showing filaments connate basally into a tube **E** pistil, showing strigillose hairs on apex of ovary and base of style **F** dehiscent capsule **G** plant in fruiting, showing the recurved pedicels **H** plant in flowering, showing the solitary flowers in axils of upper leaves **I** plant in flowering, showing the reduced basal leaves **J** papillate seeds

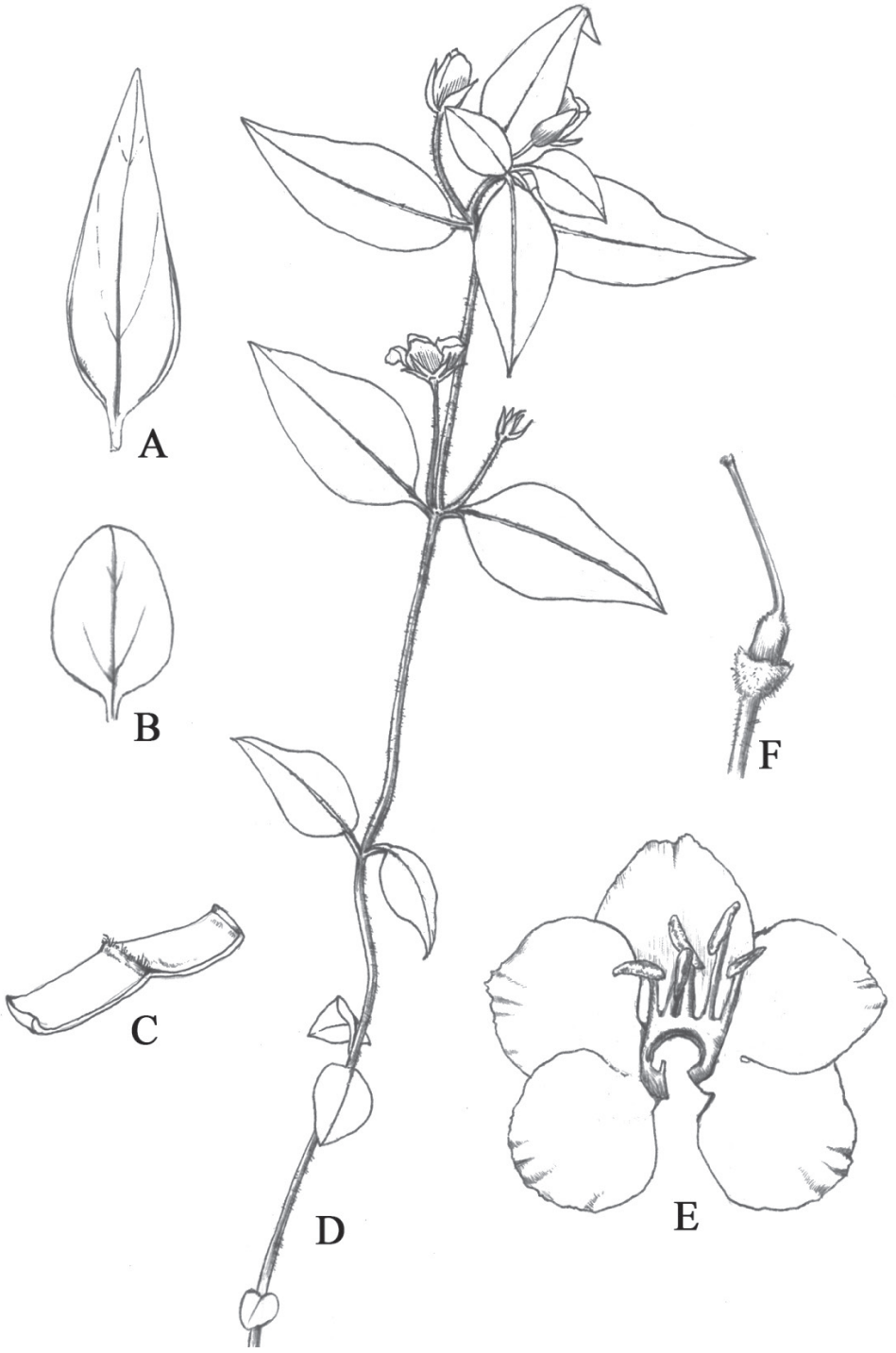

Figure 4. *Lysimachia xiangxiensis* sp. nov. **A** upper stem leaf (abaxial surface), showing revolute margins **B** lower stem leaf (adaxial surface) **C** portion of a leaf (abaxial surface), showing the revolute margins and strigillose midrib **D** plant in flowering **E** corolla lobes **F** pistil.

purple-red (in arid places) or light green (in moist places), densely strigillose along the midrib, not glandular on both surfaces; secondary veins 3–4 pairs, blurry or invisible adaxially, slightly raising abaxially, veinlets invisible. ***Flowers*** bisexually, solitary in axils of upper leaves, occasionally in terminal racemes with bractlike leaves. ***Pedicels*** 1.5–3 cm long, gradually reduced toward stem apex, purple-red or light purple-red, densely strigillose, recurved in fruit. ***Calyx*** lobes 5, rarely 6, persistent, lanceolate with indistinct costa, 6–8 mm × 1.5–2 mm, apex acuminate-subulate, inside glabrous and with 3–4 veins, outside purple-red or green, densely strigillose. ***Corolla*** yellow, tube 1–2 mm long, actinomorphic, contorted; lobes 5, 7–9 mm × 7–9 mm, suborbicular to broadly elliptic, apex cuspidate or rounded, erose above the middle. ***Stamens*** 5, yellow, opposite to corolla lobes; ***filaments*** connate basally into a tube ca. 2.5 mm high, free parts 3.5–4.5 mm; ***anthers*** ca. 2 mm long, dorsifixed, opening by lateral slits. ***Style*** ca. 6 mm long, apex slightly expanded, strigillose on lower part. ***Ovary*** cylindrical, ca. 1.5 cm in diam., strigillose on apex, superior. Capsule brown, subglobose, 3–4 mm in diam., densely strigillose, dehiscing by valves. Seeds small, black, angular, papillate.

Phenology. Flowering May–June, fruiting July–August.

Distribution and habitat. This new species is currently known from Huayuan County and Jishou City in western Hunan Province, central China. It usually grows on limestone cliffs in valleys (Figure 2), and is associated with e.g. *Eriophorum comosum* (Wallich) Nees in Wight, *Pteris vittata* Linnaeus, *Pteris deltodon* Baker, and *Dryopteris* sp.

Etymology. The specific epithet "*xiangxiensis*", literally meaning western Hunan, refers to the Xiangxi Tujia and Miao Autonomous Prefecture in central China, to which Huayuan County and Jishou City belong. The Chinese name of the *Lysimachia xiangxiensis* is xiang xi guo lu huang in Pinyin.

Conservation status. *Lysimachia xiangxiensis* usually grows on limestone cliffs in valleys so we suggest its placement in the Data Deficient category of IUCN (2017)

Additional collection. CHINA. Hunan Province, Jishou City, Aizhai Town, National Forest Park, cliff of a valley, 31 May 2019, Y. Wu 0531001(paratype, JIU!).

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Appendix

Appendix S1. Accessions of the genus *Lysimachia* L. examined in this study.

Taxon	GenBank Acc. No.	Voucher	Locality
L. alfredii Hance	JN638405	Hao394	Lianping, Guangdong, China
L. candida Lindley	JF976885	Ge2010001	Yangchun, Guangdong, China
L. capillipes Hemsley in F. B. Forbes & Hemsley	JF976886	Y2009200	Jiujiang, Jiangxi, China
L. chapaensis Merrill	JF976888	GBOWS878	Hekou, Yunnan, China
L. chekiangensis C. C. Wu	JF976891	Y2009263-1	Longquan, Zhejiang, China
L. christiniae Hance	JF976894	Y2009244	Lin'an, Zhejiang, China
	JF976896	Y2009209	Jiujiang, Jiangxi, China
L. clethroides Duby in A. de Candolle	JF976899	Y2009157	Tongbai, Henan, China
L. congestiflora Hemsley in F. B. Forbes & Hemsley	JF976902	GBOWS262	Malipo, Yunnan, China
	JF976903	Y2009266	Longquan, Zhejiang, China
L. crispidens (Hance) Hemsley in F. B. Forbes & Hemsley	JF976906	Hao212	Yichang, Hubei, China
L. decurrens G. Forster	JF976908	GBOWS1234	Hekou, Yunnan, China
L. deltoidea Wight	JF976909	GLM081121	Zhongdian, Yunnan, China
L. dextrosiflora X. P. Zhang, X. H. Guo & J. W. Shao	JF976913	Y2009265-1	Longquan, Zhejiang, China
L. erosipetala F. H. Chen & C. M. Hu	JF976914	Y2010037-2	Emeishan, Sichuan, China
L. fistulosa Handel-Mazzetti	JF976916	Ning20101	Jinggangshan, Jiangxi, China
	JF976917	Ye et al. 3561	Lianshan, Guangdong, China
	JF976919	Y2009285	Ruyuan, Guangdong, China
	JF976920	Ye et al. 3940	Lianshan, Guangdong, China
L. fortune Maximowicz	JF976925	Y2009195	Jinggangshan, Jiangxi, China
L. hemsleyana Maximowicz ex Oliver	JF976932	Guo20001	Ningguo, Anhui, China
L. hemsleyi Franchet	JF976935	Hao713	Huili, Sichuan, China
L. heterobotrys F. H. Chen & C. M. Hu	JF976936	Y2010053-2	Ningming, Guangxi, China
L. heterogenea Klatt	JF976939	Y2009199	Jiujiang, Jiangxi, China
L. insignis Hemsley	JF976945	Hao245	Napo, Guangxi, China
L. klattiana Hance	JF976947	Y2010014-1	Tongbai, Henan, China
L. laxa Baudo	JF976949	Han longran6	Puer, Yunnan, China
L. lobelioides Wallich in Roxburgh	JF976951	Hao303	Menglian, Yunnan, China
L. longipes Hemsley	JF976952	Guo xinhu200012	Shitai, Anhui, China
L. melampyroides R. Knuth in Engler	JF976955	Dengyunfei15945	Xinning, Hunan, China
	JF976956	Lichanghan8174	Shangzhi, Hunan, China
L. omeiensis Hemsley	JF976958	Y2010033	Emeishan, Sichuan, China
L. paridiformis Franchet	JF976962	Y2010044	Emeishan, Sichuan, China
L. patungensis Handel-Mazzetti	JF976964	Ye et al. 3851	Lianshan, Guangdong, China
L. pentapetala Bunge	JN638407	Y2010013-1	Tongbai, Henan, China
L. phyllocephala Handel-Mazzetti	JF976969	Y2010030	Emeishan, Sichuan, China
L. pittosporoides C. Y. Wu	JF976970	Hao248	Malipo, Yunnan, China
L. rubiginosa Hemsley in F. B. Forbes & Hemsley	JF976972	Hao419	Dujiangyan, Sichuan, China
Lysimachia xiangxiensis D.G.Zhang & C.Mou, Y.Wu, sp. nov.	MN647745	Y. Wu 0531001	Jishou, Hunan, China
	MN647744	D. G. Zhang 0826075	Huayuan, Hunan, China
Ardisia verbascifolia Mez	JN638408	GBOWS1216	Hekou, Yunnan, China

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Taxonomic notes on *Scutellaria taipeiensis* (Lamiaceae) from morphological and molecular data

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Abstract

The genus *Scutellaria* comprises eight species distributed from 50 to 2000 m in Taiwan. Amongst them, *S. barbata* and *S. taipeiensis* are very similar on the basis of morphological and plastid DNA sequence information. Therefore, a comprehensive study of the taxonomic status of *S. taipeiensis* is necessary. We reviewed the herbarium sheets, related literature and protologues and compared morphologies of these two species, as well as their phylogenetic relationships. All evidence, including the diagnostic characters between *S. taipeiensis* and *S. barbata*, suggest that they belonged to a single species rather than two. As a result, *S. taipeiensis* is treated as a synonym of *S. barbata*.

Keywords

Lamiaceae, Scutellarioideae, plant taxonomy, Taiwan

Introduction

The genus *Scutellaria* L. is composed of approximately 360 species worldwide (Paton 1990; Li and Hedge 1994; Harley et al. 2004). This genus is characterised by being non-aromatic, having simple leaves with entire to pinnatifid margins, a terminal or axillary raceme-like thrysoid inflorescence with single-flowered cymes, a two-lobed calyx with a scutellum on the upper lobe and a two-lobed corolla with an often saccate or spurred base, anterior anthers dimidiate due to aborted development of upper thecae and the ovary being borne on a peg-like gynophore (Paton 1990; Li and Hedge 1994).

The Taiwanese *Scutellaria* were revised in the 1990s, based on morphology and palynology and five species were recognised (Hsieh and Huang 1995). Later, a new species, *S. austrotaiwanensis* T. H. Hsieh & T. C. Huang was described (Hsieh and Huang 1997), resulting in a total of six species recorded in the second edition of the Flora of Taiwan (Huang et al. 1998). Two new species, *S. taipeiensis* T. C. Huang, A. Hsiao & M. J. Wu and *S. hsiehii* T. H. Hsieh, were described subsequently (Huang et al. 2003; Hsieh 2013). A genetic study of *S. barbata* D. Don and *S. taipeiensis* was conducted by Hsiung et al. (2017) and the data showed no remarkable divergence between these two species. These results attracted our attention to verify their findings. Therefore, we revised the taxonomic status of *S. taipeiensis* after re-evaluating morphological and plastid DNA sequence evidence in this study.

Materials and methods

Morphological comparison

Study materials were obtained from herbarium sheets of the HAST, TAI and TAIF herbaria and from living plants (herbarium acronyms follow Index Herbariorum (Thiers 2019, continuously updated). Type specimens of *S. taipeiensis*, deposited in the herbarium of the National Taiwan University (TAI), were also examined. Voucher specimens were deposited in the herbarium of the Taiwan Forestry Research Institute (TAIF). We examined leaf, floral and fruit morphology from dried and living materials. For living materials, we observed four populations of *S. barbata* and two of *S. taipeiensis*, including the type locality. *Scutellaria barbata* is widespread in Taiwan. Hence, herbarium sheets complement the fresh material gathered so that the variation, present in Taiwan, was represented in the study. For the population of *S. taipeiensis*, only few populations, including the type, have been recorded. All of these populations were located in Taipei City. Thus, our observation covered all populations in Taiwan. Observation of the nutlet sculpture of Hsiung et al. (2017) was applied here as a reference. The identification of *S. barbata*, *S. taipeiensis* and other Taiwanese species was according to the protologues of Huang et al. (2003) and other related literature (Hsieh and Huang 1995; Huang et al. 1998).

Molecular analysis

In order to revise the taxonomic state of *S. taipeiensis*, phylogenetic trees were reconstructed. The species, selected for analysis, were from Chiang et al. (2012) and *Holmskioldia sanguinea* was applied as outgroup, since it was closely related to *Scutellaria* (Bendiksby et al. 2011). Two nuclear (CAD, CHS) and three chloroplast DNA fragments (*mat*K, *ndhF-rpl32 and rpl32-trnL*) were used by Chiang et al. (2012), amongst them, *ndhF-rpl32* and *rpl32-trnL* being also applied in the study of Hsiung et al. (2017). Two chloroplast regions (*ndhF-rpl32* and *rpl32-trnL*) were applied in the phylogenetic analysis of this study. In addition to the sequences from Chiang et al. (2012) and Hsiung et al. (2017),

Table 1. Sequences and accession number of sequences applied in this study. Sequences generated for this study are marked *. Other sequences were sourced from Genbank.

Scientific name	*ndh*F-*rpl*32	*rpl*32-*trn*L
Scutellaria barbata	KY458956.1	KY458962.1
	KY458957.1	KY458963.1
	KY458958.1	KY458965.1
	KY458959.1	KY458966.1
S. alpina	JX981401.1	JX981439.1
S. baicalensis	JX981400.1	JX981443.1
S. altissima	JX981404.1	JX981440.1
S. zhongdianensis	JX981405.1	JX981441.1
S. diffusa	JX981406.1	JX981442.1
S. galericulata	MN720754*	MN720750*
S. incana	MN883839*	MN883840*
S. lateriflora	JX981403.1	JX981444.1
S. indica	JX981422.1	JX981387.1
	JX981423.1	JX981388.1
S. austrotaiwanensis	JX981421.1	JX981386.1
	JX981429.1	JX981394.1
	JX981430.1	JX981393.1
	JX981431.1	
	JX981432.1	
S. tashiroi	JX981433.1	
S. playfairii	JX981424.1	JX981389.1
	JX981425.1	JX981390.1
	JX981426.1	JX981391.1
		JX981392.1
S. salviifolia	JX981402.1	JX981438.1
	JX981427.1	MN720752*
S. taiwanensis	JX981428.1	MN720753*
	KY458960.1	KY458964.1
S. taipeiensis	KY458961.1	KY458967.1
Holmskioldia sanguinea	MN720755*	MN720751*

we sequenced the chloroplast DNA fragments of *ndh*F-*rpl*32 spacer from *S. galericulata*, *S. incana* and *H. sanguinea* and *rpl*32-*trn*L spacer from *S. galericulata*, *S. incana*, *S. taiwanensis* and *H. sanguinea*. These newly generated sequences were amplified following the procedure of Hsiung et al. (2017). All sequences, applied in this study, are listed in Table 1. These sequences were used for phylogeny reconstruction by Bayesian Inference (BI), Maximum Likelihood (ML) and Neighbour-Joining (NJ) approaches. The variable sites, parsimony-informative sites and substitution model were checked and selected by MEGA 7 (Kumar et al. 2016). The optimal model with the highest BIC and AIC values was selected for BI and ML analyses (Kumar et al. 2016) (Table 2). The BI reconstruction was conducted using Mr. Bayes 3.2.6 (Ronquist et al. 2012). Two independent runs were conducted with 10,000,000 generations, sampled every 1000 generations and a 10% dataset was discarded as burn-in. ML analysis was performed by PhyML 3.1 (Guindon et al. 2010). The substitution model of the two loci was the same as for BI analysis and the gamma distribution parameter was fixed at 1.52 and 0.35, respectively, according to the

Table 2. Summary of the sequence information of two plastid region applied in phylogenetic analysis.

	*ndh*F-*rpl*32	*rpl*32-*trn*L
Aligned length (bp)	536	546
No. of variable characters	195	120
No. of parsimony-informative characters	117	58
Substitution model	HKY+G	HKY+G

results of model selection. The tree topology search operation was set as the best of NNI and SPR (Guindon et al. 2010). The approximate likelihood ratio test non-parametric branch support was based on a Shimodaira-Hasegawa-like procedure (Guindon et al. 2010). NJ analysis was conducted using MEGA 7, with 1000 bootstrap resamplings. All of the phylogenetic trees were summarised and output by FigTree 1.4.4 (Rambaut 2012).

Results

Diagnostic characters of *S. barbata* and *S. taipeiensis*

Leaves

Leaf morphology had been regarded as a diagnostic character for distinguishing *S. barbata* from *S. taipeiensis* (Huang et al. 2003). Leaf shape of *S. barbata* varies from suborbicular to narrowly lanceolate (Fig. 1A–D); in contrast, the leaf shape of *S. taipeiensis* varies from ovate to broadly ovate (Fig. 1E, F). The leaves of both species had sparse pubescence on the abaxial surface. The leaves of *S. barbata* are 1.1–2.8 cm long and 0.9–1.4 cm wide, the length-width-ratio from 1.1 to 2.0, while *S. taipeiensis* leaves are 1.0–1.7 cm long and 0.5–1.1 cm wide, the length-width-ratio from 1.5 to 2.0. The shapes and sizes of the leaves overlapped between the two species and thus were difficult for use as a diagnostic character to distinguish species.

Inflorescence and flowers

The floral morphology of *S. barbata* (Fig. 2A, C) was very similar to that of *S. taipeiensis* (Fig. 2B, D). They both had terminal inflorescences and bilabiate flowers that were only slightly curved near base (Fig. 3), while other species of Taiwan have geniculate (e.g. *S. austrotaiwanensis*, *S. indica* etc.) or a strongly curved corolla (e.g. *S. tashiroi*). The corolla was bluish-purple, 0.8–1.3 cm long and pubescent on the outer surface.

Nutlets

According to the observations of Hsiung et al. (2017), the sculpture of *S. barbata* and *S. taipeiensis* are rounded concentric type and no other difference is found between them.

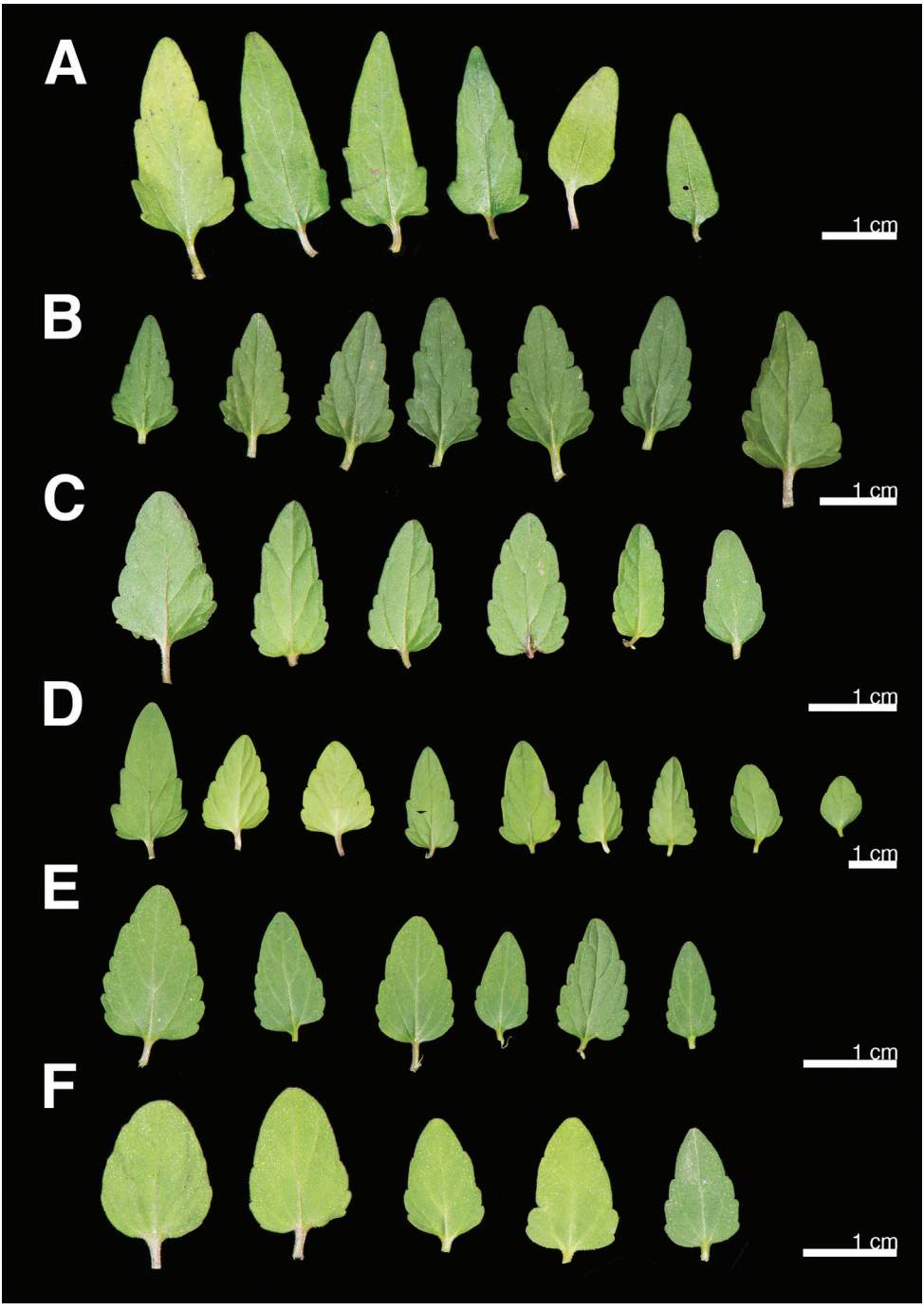

Figure 1. Leaf variation of *S. barbata* (**A–D**) and *S. taipeiensis* (**E, F**). **A** New Taipei City, Gueishan rd. (Chao 4768) **B** Taipei City, Hsichou street (Chao 4762) **C** Ilan County, Ilan City (Chao 4787) **D** Ilan County, Sanhsing Township (Chao 4789) **E** Taipei City, campus of NCCU (Chao 4837) **F** Taipei City, Maukong (type locality, Chao 4838).

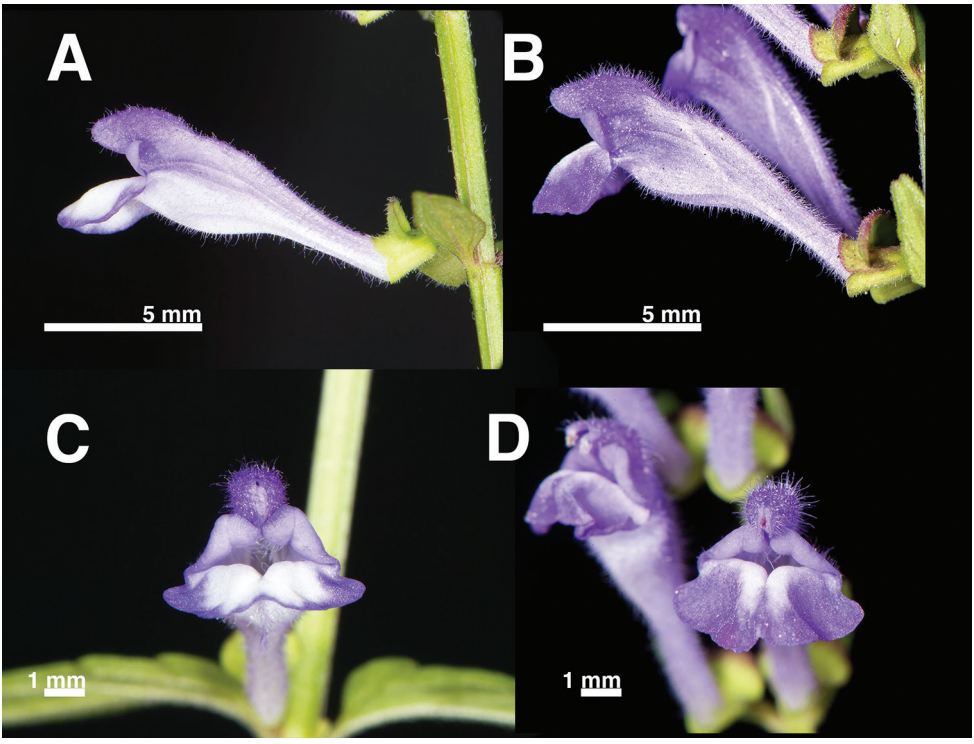

Figure 2. Flower morphology of *S. barbata* (**A, C**)(Chao 4762) and *S. taipeiensis* (**B, D**)(Chao 4838). **A, B** lateral view **C, D** front view.

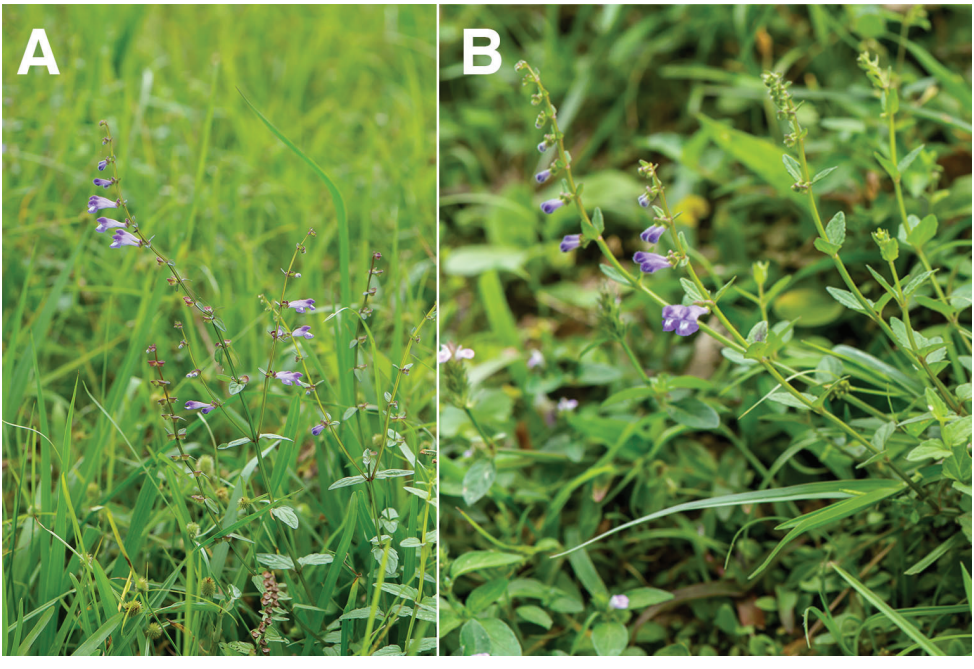

Figure 3. Inflorescence morphology of *S. barbata* (**A**) and *S. taipeiensis* (**B**).

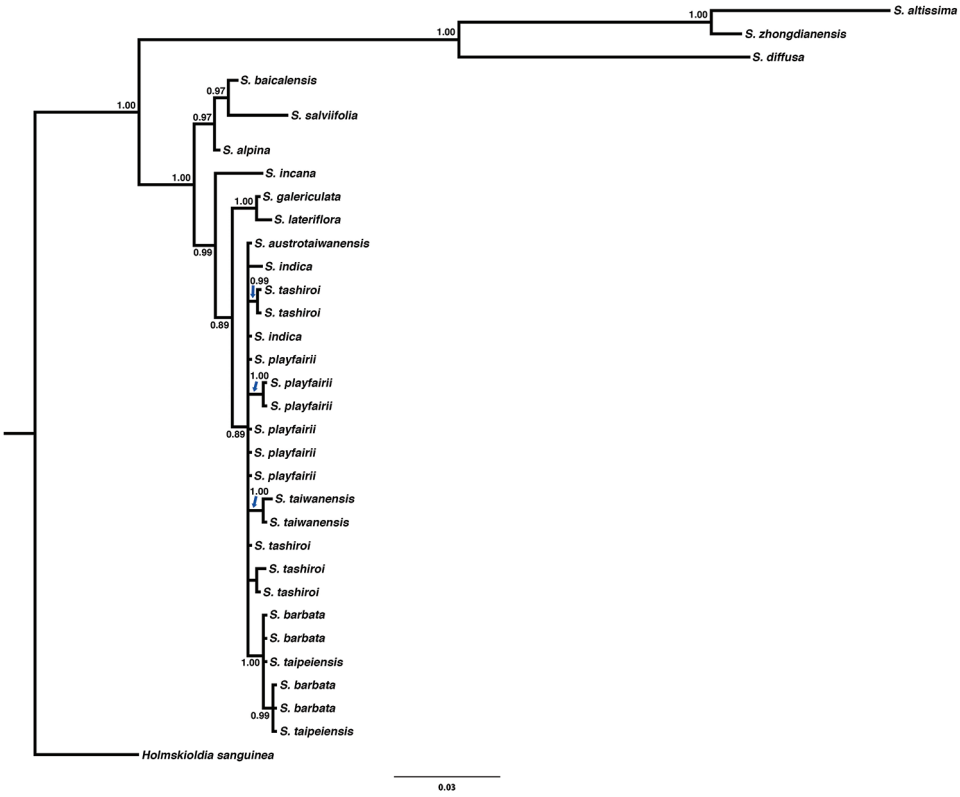

Figure 4. Phylogenetic tree reconstructed by Bayesian inference from chloroplast DNA sequence *ndh*F-*rpl*32. Only posterior probability > 0.85 was labelled on the branch. Scale bar represent substitutions.

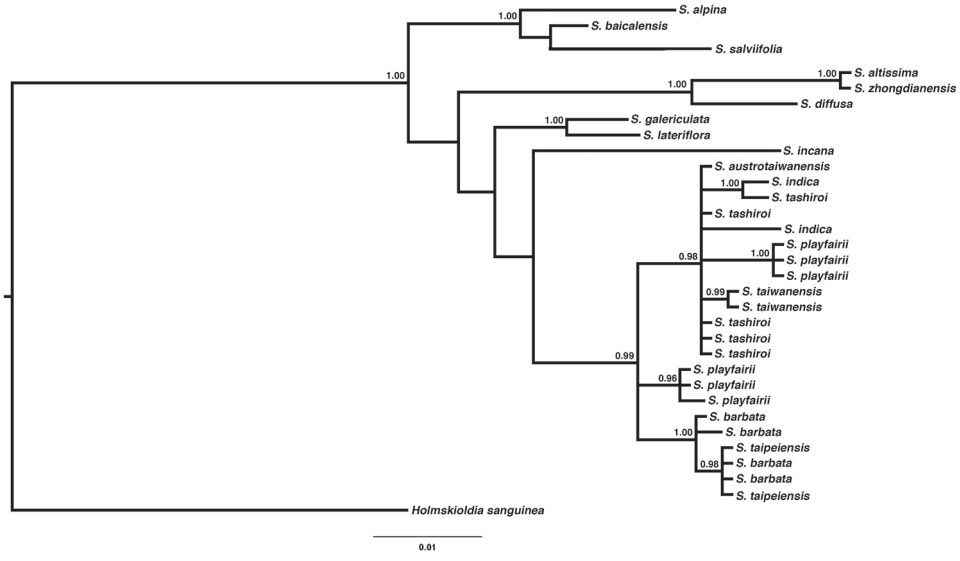

Figure 5. Phylogenetic tree reconstructed by Bayesian inference from chloroplast DNA sequence *rpl*32-*trn*L. Only posterior probability > 0.85 was labelled on the branch. Scale bar represent substitutions.

Molecular phylogeny

The best substitution model for both fragments, *ndh*F-*rpl*32 and *rpl*32-*trn*L, was HKY+G. The phylogenetic trees, reconstructed by ML, NJ and BI, revealed similar topologies with slight differences in the arrangement of non Taiwanese species (Figs 4, 5; Suppl. materials 1–4). In all analyses, all Taiwanese species formed a clade with moderate to high support (PP = 0.99, bootstrap = 0.65–0.91). Amongst them, *S. barbata* and *S. taipeiensis* formed a highly supported monophyletic group (PP = 1.0, bootstrap = 0.87–0.95), but neither *S. barbata* nor *S. taipeiensis* formed a single clade. Instead, *S. taipeiensis* was nested with *S. barbata* in the phylogenetic tree, i.e. neither species being monophyletic as currently delimited.

Distribution and habitat

Scutellaria barbata grows throughout low altitudes, ca. 50–500 m, in Taiwan, but lower in southern parts. *Scutellaria taipeiensis* is found in Taipei City and New Taipei City. The two species were growing in similar habitats, such as grassland, roadside, riverbank or forest margin and often with high humidity. Some references regarded *S. barbata* as an aquatic plant in a broad sense (Chen 1990) due to its wetland habitat. This could also be true for *S. taipeiensis*, according to the field investigation. No apparent differentiation was observed in the distribution and habitat types between these two species.

Discussion

The taxonomic status of *S. taipeiensis*

Scutellaria taipeiensis was first described by Huang et al. (2003), based on the morphology of the leaves and the nutlets. According to the original description, the leaves of *S. taipeiensis* were triangular-ovate to broadly ovate and less than twice as long as the width. To confirm the similarity of the leaves, we collected and compared leaf morphology of the two species amongst several populations. The results showed that the variation within *S. barbata* was larger than the difference between *S. barbata* and *S. taipeiensis*. Additionally, the length-width-ratio of leaves is the same in both species. Therefore, leaf morphology could not be regarded as a diagnostic character for these two species.

With regard to the nutlets, the coat had also been used to distinguish *S. barbata* from *S. taipeiensis*. The former had a radiating, umbrella-like shape, while the latter was a rounded concentric type (Huang et al. 2003). However, Hsiung et al. (2017) reviewed this character on a population level and found no remarkable difference between the two species. The mature nutlets appear to the rounded concentric type in both species, such state was stable amongst populations (Hsiung et al. 2017). The umbrella-like appendage was found in immature nutlets only, which meant that it was a transitional state during nutlet development and could not provide a valid taxonomic value.

We further looked at other characters to separate them, such as floral morphology and DNA sequence data. Different sequence data revealed some phylogenetic incongruence amongst lineages *S. alpina*, *S. altissima*, *S. bicalensis*, *S. diffusa*, *S. galericulata*, *S. salviifolia* and *S. zhongdianensis*. Such incongruence may be due to uneven sampling, but the relationship of these species was not a concern in this study. Therefore, we will not discuss the evolutionary relationship between this group of species here. All Taiwanese species formed a highly supported clade in all trees. *Scutellaria barbata* and *S. taipeiensis*, which had very similar inflorescences and floral morphology, are phylogenetically nested within a monophyletic clade. Based on this evidence, *S. taipeiensis* was treated as a synonym of *S. barbata*, rather than a distinct species or on an intraspecific level.

Taxonomic treatment

According to the results and discussions, we established the following taxonomy:

Scutellaria barbata D. Don in Prodromus Florae Nepalensis 109–110. 1825.

S. taipeiensis T. C. Huang, A. Hsiao & M. J. Wu in Taiwania 48:133. Type: Taiwan. Taipei City, growing on exposed rocks or soils adjacent rocks along sunny roadside between Maukong to Chihnan Temple T. C. Huang and A. Hsiao 18104 (Holotype: TAI!, Isotype: TAI!) syn. nov.

Distribution. *Scutellaria barbata* is widely distributed in southern and eastern Asia (Li and Hedge 1994). In Taiwan, this species is found in low altitude from 50 to 500 m, in wet grasslands, riverside and margins of forest.

Specimens examined. Specimens marked with an asterisk (*) denote material *S. taipeiensis* following.the concept of Huang et al. (2003) on the labels.

Taiwan. Changhua County: Lukang, at road mileage sign 35 km along Provincial Highway 17, 18 Apr 1999, K. F. Chung 1147 (HAST). **Hsinchu County:** Hengshan, Peiwuo, 245 m a.s.l., 28 Apr 1994, C. M. Wang 763 (HAST). **Hualien County:** *Fengping Township, a public cemetery, 0–50 m a.s.l., 1 May 2015, S. W. Chung 12187 (TAIF); Patu, 9 Jul 2008, M. J. Jung 3055 (TAIF); Fuli Township, 22 May 2012, S. H. Chen s.n. (TAIF); Juisuei Township, 28 Jan 1987, S. H. Chen s.n. (TAIF); Kaoliao, 12 Feb 1990, J. P. Lin 421 (TAIF). **Ilan County:** Shuanglien Pond, 250–300 m a.s.l., 10 Apr 2009, W. Y. Wang 153 (TAIF); Pitou Lake, 1 Apr 2012, S. Z. Tsai & Y. S. Liang TSY265 (TAIF); Tungshan Township, 6 Oct 1991, Y. H. Liou Liu9110A-027 (TAIF); Tali, 20 Apr 1962, C. C. Chuang 2171 (TAI); Kanchiaokeng, 8 Feb 2001, H. Y. Chen & K. L. Jien 1601 (TAI); Meihuahu, 50 m a.s.l., 23 May 2000, C. H. Lin 352 (HAST); Ilan City, Huanhe N. Rd., out of river bank, 23 Mar 2019, C. T. Chao 4787 (TAIF); Annong river flood diversion weir park, 23

Mar 2019, C. T. Chao 4789 (TAIF); Ilan, 100 m a.s.l., 22 Mar 1987, S. Y. Lu 21257 (TAIF); Panomakutao, 350 m a.s.l., 2 Apr 2005, W. F. Ho 1735 (TAIF); Shuanglien Pond, 250–300 m a.s.l., 10 Apr 2009, W. Y. Wang 153 (TAIF); Dongshan river, 50 m a.s.l., 13 Feb 2012, S. W. Chung 10589 (TAIF); Shan-shin, 7 Apr 1982, M. T. Kao 9656 (TAI). **Kaohsiung City:** Lienhuachih, 30 Aug 1991, L. Y. Tseng 509 (TAIF). **Keelung City:** *Tienwaitien Landfill Site, 150 m a.s.l., 25 Apr 2014, P. F. Lu 26638 (TAIF); Patu, 9 Jul 2008, M. J. Jung 3055 (TAIF). **Miaoli County:** Miaoli, 17 Apr 1970, T. C. Chuang 5277 (TAI). **Nantou County:** Chungyuan neighbourhood, 24 Jan 1988, S. M. Li 76 (TAIF). **New Taipei City:** *Mt. Erhke, 8 May 2011, M. J. Jung 5453 (TAIF); *Mt. Chungling, 400–600 m a.s.l., 10 Jan 2015, P. F. Lu 27688 (TAIF); Hsiaokotou-Kankou, 27 Dec 1968, C. C. Hsu 5213 (TAI); Wazihwei, 0–20 m a.s.l., 16 Apr 2004, S. C. Liu 1711 (TAIF); Sanchakang Village, 100 m, 4 Oct 2008, P. F. Lu 16991 (TAIF); Fujen Catholic University, 22 Dec a.s.l. 2002, C. L. Hu s.n. (TAIF); Santiaoling, 60 m a.s.l., 20 Apr 2012, S. W. Chung 10815 (TAIF); Menghu Rd., 26 Mar 2012, C. F. Chen 3306 (TAIF); Sanhsia, 5 Apr 1994, T. H. Hsieh 1194 (TAI); Hsiaokengkou, 50–100 m a.s.l., 19 May 2000, H. Y. Chen 1398 (TAIF); Shihting to Huangtitien, 1 Jun 2003, T. C., L. C. & R. P. Huang 18105 (TAI); Sshlioufennz, 300–370 m a.s.l., 4 Apr 1985, C. I Peng 7551 (HAST); Hsinshang-Menghu, 350–400 m a.s.l., 16 May 1993, C. C. Wang 1363 (HAST); Hsichou Street, 26 Feb 2019, C. T. Chao 4762 (TAIF); Gueishan rd., 15 Mar 2019, C. T. Chao 4768 (TAIF); Hsiunghustien to Peihsinchuang, 100 m a.s.l., 23 Mar 2001, S. M. Kuo 216 (TAIF); Shuangshi, 100 m a.s.l., 12 Jul 2003, P. F. Lu 5134, 6255 (TAIF); Yunhsien garden, 700–800 m a.s.l., 29 Mar 2000, Y. P. Cheng 2911 (TAIF); Gungliau, Waiwenshiouk, 50 m a.s.l., 9 Apr 2000, H. M. Chang 3110 (TAIF); HuangTiTien, 150–250 m a.s.l., 16 Apr 2011, P. F. Lu 21794 (HAST); Tali, 20 Apr 1962, C. C. Chuang 2171 (HAST); Chuwei, 26 Feb 1989, T. Y. Yang & C. C. Wang 4474 (TAI); Shihting, 20 Apr 1991, M. J. Wu 1303 (TAI); Yinhoton, 28 Aug 1970, M. T. Kao 7621 (TAI); Pinlin, 1 Apr 1977, C. M. Kuo 8119 (TAI). **Taichung City:** Pingting, 18 Jul 1968, C. C. Hsu 9128 (TAI); Fengyuan, along a steep trail between Panchang and Fengyuan Golf Club, 250–400 m a.s.l., 20 Dec 1985, C. I Peng 12165 (HAST); Ta-chia, 28 Apr 1982, M. T. Kao 9677 (TAI). **Taipei City:** *Huajiang Wild Duck Nature Park, 12 Apr 2010, M. J. Jung 4918; *same loc., 20 Apr 2010, M. J. Jung 4933 (TAIF); NTU farm, 14 Dec 1960, M. T. Kao 7633 (TAI); Shuiyuanti, H. Simizu 210 (TAI); Mucha, 25 May 1975, C. I Peng 1464 (TAI); *Maokun, 200 m a.s.l., 13 Apr 2003, T. C. Huang 18103 (TAI); at Tachia Riverfront Park, 15 m a.s.l., 21 Mar 2007, C. I Huang 3094 (HAST); Taipei, 10 Jul. 1908, Y. Simada s.n. (TAIF); same loc., Dec. 1909, Y. Kawakami & S. Sasaki s.n. (TAIF); Neihu, 16 Apr 1974, C. M. Kuo 4820 (TAI); Campus of NCCU, 13 May 2019, C. T. Chao 4837 (TAIF); Section 3 of Chinan Rd., 13 May 2019, C. T. Chao 4838 (TAIF). **Taitung County:** Provincial Rd. No. 11, 29 Mar 2003, Y. C. Liu s.n. (TAIF). **Taoyuan City:** Jeiyuan neighbourhood, 17 Dec 2005, I W. Deng s.n. (TAIF); Jenmei, 1 May 1975, C. M. Guo 6119 (TAI); Chungyuan Univ., 17 Mar 1976, C. M. Kuo 6613 (TAI);

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Supplementary material 1

Phylogenetic tree reconstructed by maximum likelihood analysis from chloroplast DNA sequence *ndh*F-*rpl*32. Only SH-like support value > 0.85 was labelled on the branches. Scale bar represent substitution

Authors: Chien-Ti Chao, Bing-Hong Huang, Jui-Tse Chang, Pei-Chun Liao

Data type: molecular data.

Link: https://doi.org/10.3897/phytokeys.140.48578.suppl1

Supplementary material 2

Phylogenetic tree reconstructed by maximum likelihood analysis from chloroplast DNA sequence *rpl*32-*trn*L. Only SH-like support value > 0.85 was labelled on the branches. Scale bar represent substitution

Authors: Chien-Ti Chao, Bing-Hong Huang, Jui-Tse Chang, Pei-Chun Liao

Data type: molecular data.

Link: https://doi.org/10.3897/phytokeys.140.48578.suppl2

Supplementary material 3

Phylogenetic tree reconstructed by neighbour-joining analysis from chloroplast DNA sequence *ndh*F-*rpl*32. Only bootstrap support value > 0.85 was labelled on the branches

Authors: Chien-Ti Chao, Bing-Hong Huang, Jui-Tse Chang, Pei-Chun Liao

Data type: molecular data.

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Supplementary material 4

Phylogenetic tree reconstructed by neighbour-joining analysis from chloroplast DNA sequence *rpl*32-*trn*L. Only bootstrap support value > 0.85 was labelled on the branches

Authors: Chien-Ti Chao, Bing-Hong Huang, Jui-Tse Chang, Pei-Chun Liao

Data type: molecular data.

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RESEARCH ARTICLE

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Drymaria veliziae (Caryophyllaceae), a new species from the Andes of Cajamarca (North Peru)

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Abstract

A new species from the Northern Peruvian Andes (Cajamarca department), *Drymaria veliziae* **sp. nov.**, is proposed in the present paper. It grows in the high-elevation montane grasslands and it is morphologically similar to *D. auriculipetala* from which it differs in having elliptic-ovate leaves, blade margin bases glandular, large number of stipules arranged in a pedicel form at the leaf axis and by the short and glandular pedicels. A detailed description, original photographs and a location map are provided, as well as an updated diagnostic key of *Drymaria* Ser. Frutescens. The IUCN status of the new species is assessed as Endangered (EN).

Keywords

Andes, Cajamarca, new species, Caryophyllaceae

Introduction

The genus *Drymaria* Willd. ex Schult. (Caryophyllaceae Juss.) contains 48 species mainly distributed in subtropical regions of the Western Hemisphere (see the most recent revision of the genus by Duke 1961), whereas one species (*Drymaria cordata*

Willd. ex Schult.) is widespread, occurring in Asia, Africa, Oceania, and Madagascar (Villarreal and Estrada 2008). Duke (1961) recognised 17 Series but they were not validly published because Latin diagnoses were not given (*nomina nuda*; see Art. 38.2 Ex. 1 of ICN, Turland et al. 2018) (see Hartman 2005 and Villarreal and Estrada 2008). After Duke (l.c.), no studies have been made on Peruvian *Drymaria* taxa.

Concerning the molecular data, those available for *Drymaria* are included in the large phylogenetic study of Caryophillaceae by Greenberg and Donoghue (2011), but here no Andean species of *Drymaria* were involved.

On the basis of some authors (Macbride 1937; Brako and Zarucchi 1993) and our ongoing studies (Montesinos-Tubée in prep.), 24 *Drymaria* species (including 18 infraspecific taxa) are expected to occur in the Peruvian Andes.

As part of the ongoing floristic and taxonomic studies on Peruvian Flora (Montesinos-Tubée 2013; Montesinos-Tubée and Kool 2015; Montesinos-Tubée et al. 2018), we found an interesting population belonging to the genus *Drymaria* which, however, cannot be identified with any of the currently known species. We, therefore, decided to propose a new species for Science.

Material and methods

Specimens of *Drymaria*, housed in many South American and other herbaria (B, CUZ, F, HSP, HUT, HUSA, K, L, LP, LPB, MOL, P, SI, SGO, USM, WAG; acronyms according to Thiers 2019+), were studied by the first author (DBM-T). Additionally, field surveys were carried out. Specimen information (including digital images) were searched using online sources such as GBIF (2019), JSTOR Global Plants (2019), Tropicos (2019) and herbarium databases of several herbaria.

Morphological characters were studied using a NSZ-405 1X-4.5X stereomicroscope and an AmScope M100CLED 40×-1000× compound microscope. Conservation assessments were undertaken using the IUCN Red List Criteria (IUCN 2019). The monograph by Duke (1961) was used as the basic reference to describe the new species.

Results and discussion

Drymaria veliziae **Montesinos, sp. nov.**
urn:lsid:ipni.org:names:77206319-1
Figs 2, 3

Type. Peru. Cajamarca: Cajamarca: Encañada: Chanta baja, on sandy clay loam soils amongst shrub species and tussock grasslands, close to agricultural lands, 3295 m elev., slope of 60% and rock cover of 35%, 6°49’56”S, 78°30’20”W (DMS). 06 June 2009, *C. Tovar 1058* (holotype CPUN–22705!).

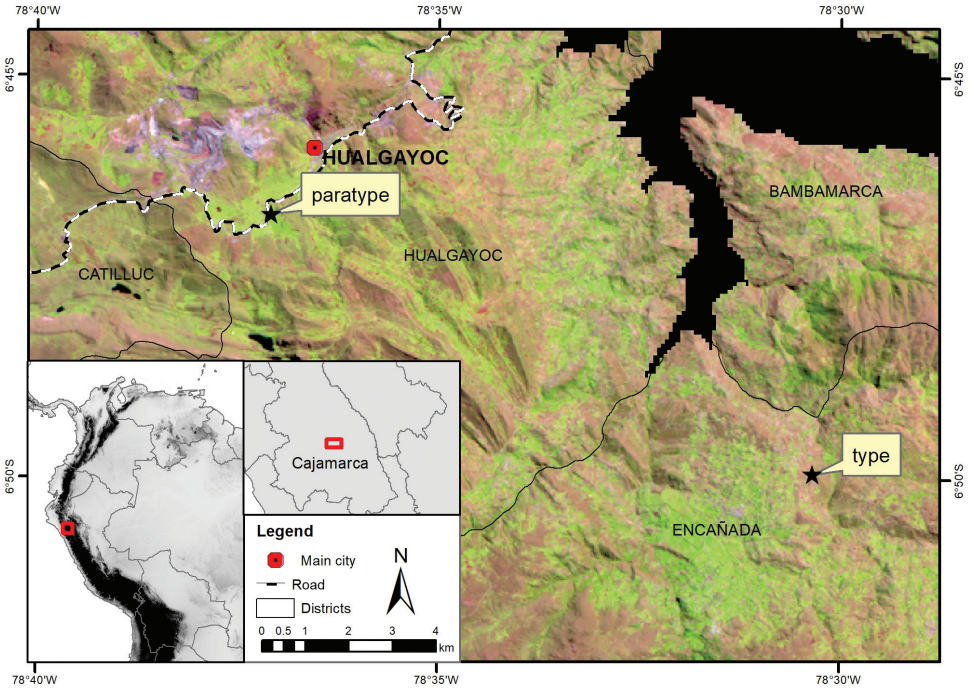

Figure 1. Location map of the type and paratype collection localities. Background: LANDSAT 5TM satellite image (June 2007) where light brown-pink areas represent Jalca grasslands, light green areas are agricultural fields and purple represents mining areas.

Diagnosis. *Drymaria veliziae* is similar to *D. auriculipetala* Mattf. from which it differs in having glands covering the stems and pedicels, leaves with elliptic-ovate form, shorter in size (4–5.5 mm vs. 5–15 mm in *D. auriculipetala*), by the leaves arranged in fascicules (vs. simple opposite leaves), stipules in numbers of 14–20 per axis (vs. 2–4 in *D. auriculipetala*), pedicel size (1–2 mm long vs. 5–40 mm) and by the capsule size being smaller (1.4–1.6 mm vs. 3–4 mm).

Description. Perennial herb, the taproot woody, stems originating from the root brow spreading or ascending, rarely decumbent, of 20–35(–50) cm long. Stems rigid, greenish-lilac, densely glandular, of about 0.05–0.3 mm long, persistent on mature stems and having scattered plicate trichomes of 1–3 mm long on young stems. Internodes 0.2–5.5 cm long. Leaves opposite, usually forming short fascicules; petioles 0.3–0.9 mm long, partially glaucous, scarcely covered with glands in the margins; blades elliptic to ovate, 4–5.5 mm long × 1.2–2 mm width, coriaceous, the bases cuneate, decurrent to the petiole, the apex aristate, 1–1.5 mm long, narrowly bearing short glands along the margin, midrib nearly inconspicuous; leaf margins lustrous, revolute and glabrous except at the base; stipules aciculate to linear-lanceolate, aristate, 1.5–3 mm long × 0.1–0.4 wide, shorter or equalling the length of the leaves, with glabrous margins and usually verticillate, in numbers of 14–20 per axis, persistent, white trans-

Figure 2. Holotype of *Drymaria veliziae* (CPUN-22705!).

lucid to brownish with age, rarely bifid or trifid; bracts opposite, 2.5–3 mm long × 2 mm width, involute, cupuliform, irregularly ovate, margins covered with scattered glands, surface white coloured with lilac blotches. Flowers except the first formed, axilar and solitary at the end of the branches, base protected by the bracts (in pairs 1 or 2). Pedicels 1–2 mm long, densely glandular and covered with carinate plicate trichomes, rarely aereal, of about 0.2–0.4 mm. Calyx cylindrical-campanulate; sepals 5, equal,

Figure 3. A. Internode and leaf arrangements **B** glandular stems **C** crown of stipules at the leaf axis **D** leaves (adaxial side) **E** leaves (abaxial side) **F** axis of leaves **G** bracts at the leaf axis **H** mature and immature flowers **I** flower detail **J** sepals (adaxial side) **K** sepals (abaxial side) **L** single petal with bifid apex portion **M** flower with ovary, stamens (indicated with dark lines) and style **N** ovary detail with a trifurcated style **O** seeds.

Figure 4. Quadrat where the new species was found. Chanta baja, Encañada District, 3295 m elev. Photo by Carolina Tovar.

5–6 mm long × 2–2.2 mm width, glabrous, elliptic-ovate, apex apiculate and aristate, basally truncate, 3–5 nerved; petals 5, 5–7 mm long × 1.8–2.2 mm width, bifid about half their length, elliptic, apex rounded, 1–1.2 mm width, 8–10 nerved, constricted at the junction of the lobes; stamens 5, 2–2.2 mm long, anthers oblong, 0.2–0.3 mm long; ovary roundish, 1–1.4 mm long, slightly exceeded by the anthers; style 1–1.2 mm long, trifid about half its length, stigmatic branches twisted or coiled. Capsule ovoid, 1.4–1.6 mm long, 5–8 seeded. Seeds roundish, reniform, 0.6–0.9 mm long × 0.6–0.8 mm wide, granulate, ventral surface with roundish-acute tubercles, black to dark brown in colour.

Etymology. The epithet "*veliziae*" honours Claudia Véliz Rosas (1978–2019), a passionate biologist who devoted her research efforts to the study Peruvian biodiversity. Her deep love of nature, people and travelling inspired her to work throughout Peru, studying freshwater, marine and mountain ecosystems. Her research contributed to the establishment of protected areas and the development of management plans. Claudia dedicated many years to study taricaya turtles in the Amazon, helping local human communities to improve taricayas' management and conservation. She was an excellent and supportive friend, a talented amateur painter and dancer and a keen cyclist.

Paratype: Peru: Cajamarca: Hualgayoc: Hualgayoc, less than 1 km from the Goldfield mine, surrounded by agricultural fields downslope, found on sandy clay loam soils, 3715 m elev., 6°46'43"S (DMS) and 78°37'5"W (DMS), 100% slope and 5% rock cover. 01 June 2009, *C. Tovar 909* (CPUN–22858!).

Ecology and distribution: *Drymaria veliziae* grows on steep mountain cliffs (slope 60–100%) on sandy clay loam soils at an elevation of 3295–3715 m on the eastern slopes of the Jalca, on the headwaters of the Llaucano River, tributary of the Marañon River. Climatic characteristics for the localities of the type and paratype, extracted from the CHELSA climatology (Karger et al. 2017), show mean annual temperatures in these areas are 8.5–11.5°C, with minimum temperatures estimated between 1.8 and 5°C. Total annual precipitation ranges from 900 to 1200 mm with driest months receiving 16–28 mm. Other species found in the two localities were *Hieracium peruanum* Fr. (Asteraceae), *Hypochaeris taraxacoides* (Meyen & Walp.) Ball (Asteraceae) and *Calamagrostis* spp. (Poaceae). In the type locality of *Drymaria veliziae* (Fig. 4), it has been observed that it grows associated with shrubs (e.g. *Coreopsis senaria* S.F. Blake & Sherff (Asteraceae), *Achyrocline celosioides* (Kunth) DC. (Asteraceae), *Ageratina cutervensis* (Hieron.) R.M. King & H. Rob. (Asteraceae)), tussock grasses (*Calamagrostis* and *Festuca* spp. (Poaceae)) and an orchid (e.g. *Masdevallia* spp.), amongst others. The locality of the paratype, being at higher altitude, had less species richness and associated species with *D. velizzii* were *Geranium peruvianum* Hieron. (Geraniaceae), *Calceolaria concava* Molau (Calceolariaceae), *Chrysactinium acaule* (Kunth) Wedd. (Asteraceae), *Euphorbia huanchahana* (Klotzsch & Garcke) Boiss. (Euphorbiaceae), amongst others.

Taxonomical notes. On the basis of the classification proposed by Duke (1961), *Drymaria veliziae* would belong to the ser. *Frutescens* Duke sharing the leaf shape (linear to lanceolate) glandular pedicels, the number of sepal nervadures (3–5) and petals bifid which are not tapered to the claw.

Drymaria veliziae is morphologically similar to *D. auriculipetala* Mattf. (1936: 438–439) but differs in having glands covering the stems and pedicels, leaves with elliptic-ovate form, shorter in size, by the leaves arranged in fascicules (vs. simple opposite leaves), stipules larger numbers per axis, shorter pedicel size and smaller capsule size.

Furthermore, *Drymaria veliziae* differs from *D. stereophylla* Mattf. (1936: 436–437) by the plant habit, the glabrous surface of the leaves (vs. presence of glands and puberulent trichomes in *D. stereophylla*), bifid or trifid stipules (vs. entire), shorter stamen size (2–2.2 mm vs. 4–6 mm), shorter style size (1–1.2 mm vs. 1–2.5 mm), capsule size shorter (1.4–1.6 vs. 2.5–3.5 mm) and seed size (0.6–0.9 vs. 0.9–1.3 mm in *D. stereophylla*).

The new species is further differentiated from *D. stellarioides* Willd. ex Schult. (1819: 406) by the stipule form (bifid to trifid vs. entire), shorter bract size (2.5–3 mm vs. 3–5 mm in *D. stellarioides*), sepals glabrous (vs. glabrous to densely glandular-puberulent) and shorter capsule size and form (1.4–1.6 mm, ovoid vs. 3–5 mm long, ellipsoid).

An updating of the diagnostic key for the ser. *Frutescens*, as proposed by Duke (1961: 214) follows:

1 Pedicels and sepals present and glabrous, sepals (3-)5-nerved **2**
– edicels and sepals present or absent, glabrous or glandular, sepals 3-nerved.... **3**
2 Leaves imbricate, closely appressed to the stems, 2–6 mm long, 1–1.5 mm broad, basally clasping and pungently acute; the sepals mostly 5-nerved; petals tapered to the claw..... ***D. frutescens***
– Leaves not imbricate, 4–12 mm long, 2–6 mm broad, apically acute to aristately acuminate; the sepals 3–4-nerved; petals not tapered to the claw.... ***D. stereophylla***
3 Pedicels and sepals usually glandular, leaves glabrous, apically aristate-attenuate, aristate and basally cuneate; stipules present; seeds with domical or conical tubercles **4**
– Pedicels and sepals glabrous to densely glandular-puberulent; leaves apically acute and marginally entire, densely glandular-puberulent; stipules entire, apparently fused or occasionally absent; seeds without domical tubercles..... ***D. stellarioides***
4 Bracts absent, simple opposite leaves, pedicels 5–40 mm long, stipules copiously shorter than the leaves; glabrous pedicels and stems, short number of pedicels per axis (2–4)..... ***D. auriculipetala***
– Bracts present; leaves arranged in fascicules; pedicels 1–2 mm long, glands covering the stems and pedicels; leaves with elliptic-ovate form, large number of pedicels per axis (14–20)..... ***D. veliziae***

Conservation status. Only the two localities referring to holotype and paratype are currently known for *Drymaria veliziae* (these localities are separated by about 12 km). The surrounding areas are characterised by various types of human activities, for example, agriculture, land conversion, forestry with exotic species, slash burning, natural resource extraction, amongst others (Figure 1). Land use change occurred between 1987 and 2007 with a reduction of the 25% of grasslands and an increasing of landscape fragmentation (see Tovar et al. 2013). The type specimen was collected on a Jalca patch surrounded by agricultural fields (*Vicia faba* L. (Fabaceae), *Solanum tuberosum* L. (Solanaceae), *Zea mays* L. (Poaceae)), while the paratype was collected in a smaller patch less than 1 km distant from a mining area developed after 1987. A total of 110 vegetation plots were sampled across the Jalca in 2007 (Tovar et al. 2012) and the new species was found in only two of them. Using the criteria B1a and B1b of the IUCN (2019), we assessed *D. veliziae* as Endangered species (EN).

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RESEARCH ARTICLE

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Four new species of *Acalypha* L. (Euphorbiaceae, Acalyphoideae) from the West Indian Ocean Region

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Abstract

Four new species of *Acalypha* (Euphorbiaceae, Acalyphoideae) from the Western Indian Ocean Region, based on morphological and molecular evidence, are described, illustrated, and mapped. *Acalypha gillespieae* sp. nov., *A. leandrii* sp. nov. and *A. nusbaumeri* sp. nov. are endemic to Madagascar, and *A. mayottensis* sp. nov. is known only from Mbouzi islet (Mayotte), in the Comoros Archipelago. We also describe for the first time in *Acalypha* the presence of membranous or chartaceous perules covering the axillary buds. Preliminary conservation assessments of the new species are also provided.

Keywords

Comoros, endemic, Madagascar, Mayotte, taxonomy

Introduction

This paper follows the revisionary work on *Acalypha* L. for the West Indian Ocean Region (Madagascar, the Comoros Archipelago, the Mascarene island, Scattered islands and Seychelles Archipelago) that was begun by Montero Muñoz et al. (2018a, b, in press).

Acalypha, with around 500 species, is the third largest genus in the family Euphorbiaceae, after *Euphorbia* L. (Riina et al. 2013) and *Croton* L. (Berry et al. 2005). It includes mainly small trees and shrubs of tropical and subtropical distribution, and some herbs that extend to temperate regions. About 65 species are found in continental Africa (Cardiel and Montero Muñoz 2018), and 39 species were previously known from West Indian Ocean Region (WIOR), most of them endemic there (Montero Muñoz et al. 2018a, b, 2020). A description of the main characteristics of the *Acalypha* species in WIOR, as well as the previous revisionary works in this region, can be found in Montero Muñoz et al. (2018a, b).

Acalypha is included in the subfamily Acalyphoideae, the most diverse in the Euphorbiaceae (Hayden and Hayden 2000). Although this subfamily is considered to be paraphyletic, the core group of included taxa (Acalyphoideae sensu stricto) is clearly monophyletic. Molecular analyses also support the monophyly of *Acalypha* (Tokuoka 2007, Wurdack and Davis 2009). Preliminary results of *Acalypha* molecular phylogeny (Levin et al. 2005, Sagun et al. 2010) suggest that the genus first evolved in Africa, where it is morphologically most diverse. Currently, we are working on the molecular phylogeny of *Acalypha* species from the WIOR (Montero Muñoz et al. in press) in the context of the phylogeny of the whole genus (Levin et al. in prep.). The preliminary results of this work also confirm the species here described as new. In all four newly described species, the axillary buds are covered by a pair of membranous or chartaceous perules; which are especially conspicuous in deciduous specimens. We have seen perules in other species of Acalypha from the WIOR region, usually associated with seasonally dry habitats. This is the first published report of the presence of perules in *Acalypha*.

Materials and methods

The taxonomic status of the new species is based on morphological evidence, supported by geographical and ecological data. The descriptions and illustrations provided are based on field images and herbarium specimens located in CAN, G, HKM, ILLS, K, MAO, MO, P and TAN (abbreviations following Thiers 2020). Specimens seen by the authors are indicated with an exclamation mark (!). Herbarium barcodes are included when they are known. Specimens have been studied using a binocular microscope. Information about habit, plant size, and habitat are based on field notes on the specimen labels. The field photographs provided were made by the late Jean-Noël Labat and downloaded from the Muséum National d'Histoire Naturelle (Paris) website. The distribution maps were prepared using QGIS Desktop 3.2.2. Conservation assessments are based on the IUCN Red List Categories and Criteria (IUCN 2012, 2017). Area of occupancy (AOO) and extent of occurrence (EOO) were calculated with GeoCAT, a geospatial conservation assessment tool (Bachman et al. 2011; http://geocat.kew.org/), using a 2 × 2 km grid cell size as recommended by IUCN (2012, 2017).

All the taxonomic and biogeographical information about *Acalypha* is available online in the regularly updated "Acalypha Taxonomic Information System" website (Cardiel et al. 2020; www.acalypha.es).

Taxonomic treatment

1. *Acalypha gillespieae* G.A.Levin & I.Montero, sp. nov.
urn:lsid:ipni.org:names:77206320-1

Diagnosis. *Acalypha gillespieae* G.A.Levin & I.Montero is morphologically most similar to *A. humbertii* Leandri, but differs from it by having spherical axillary buds with imbricate perules (vs. pyriform buds with superposed perules), elliptic to obovate leaf blades (vs. ovate leaf blades), inflorescences c. 1 cm long with the fertile part of the male segment c. 1.5 mm long (vs. inflorescences c. 2.5 cm long with the male segment c. 20 mm), and mature female bracts subreniform with entire margins (vs. bracts suborbicular with dentate margins).

Type. Madagascar. Reg. Diana [Prov. Antsirinana]: Montagne des Français, E of Antsirinana (Diego Suarez), 12°19'26.4"S, 49°20'16.6"E, 258 m, 31 Oct 2012, *L. J. Gillespie, G. A. Levin, J. Andriatiana, & W. M. Cardinal-McTeague 10692* (holotype: MO!; isotypes: CAN!, K!, P!, TAN!). Fig. 1.

Description. ***Shrubs*** to 3 m high, intricately branched, deciduous, monoecious. ***Young branches*** slender, densely pubescent with short, simple, straight, antrorsely appressed trichomes proximally, and antrorsely curved trichomes distally; older branches glabrescent. ***Axillary buds*** spherical, c. 2 mm diam., perules 2, imbricate, chartaceous, glabrous. ***Stipules*** deciduous, 2–3.5 mm long, subulate, densely pubescent with short, simple, spreading-ascending trichomes. ***Petioles*** slender, 2–5 mm long, densely pubescent with antrorsely curved trichomes. ***Leaf blades*** 1.5–4 × 1–3 cm, elliptic to obovate, membranous, unlobed or (2–)3-lobed; base rounded to broadly obtuse; margins crenate; apex obtuse; upper surface sparsely pubescent with simple, straight, erect to antrorse trichomes; lower surface with indumentum similar to that found on upper surface, but denser; venation actinodromous, somewhat prominent on both surfaces, with 3 veins at the base, secondary veins 2–3 per side. ***Stipels*** absent. ***Inflorescences*** androgynous, axillary, c. 1 cm long, spiciform, with one female bract near the base, and a male segment distally; peduncle thin, 2–3 mm long, densely pubescent with antrorsely curved trichomes; male segment persistent, sterile axis 1–2 mm, fertile portion c. 1.5 mm long, densely pubescent with simple, slender, flexuous trichomes. ***Female bracts*** sessile, enlarging in fruit to 5 × 9 mm, subreniform, sparsely pubescent with simple, straight, antrorse trichomes; margins entire. ***Bracteoles*** absent. ***Male flowers*** not seen (only the pedicels present). ***Female flowers*** solitary, sessile; sepals 3, slightly connate at base, c. 0.75 mm long, broadly triangular, ciliate with simple, slender, flexuous trichomes c. 0.5 mm long; ovary not seen; styles 3, persistent in fruit, c. 2 mm long, slightly connate at base, rachis stout, pubescent with short, simple, straight, antrorse trichomes, each

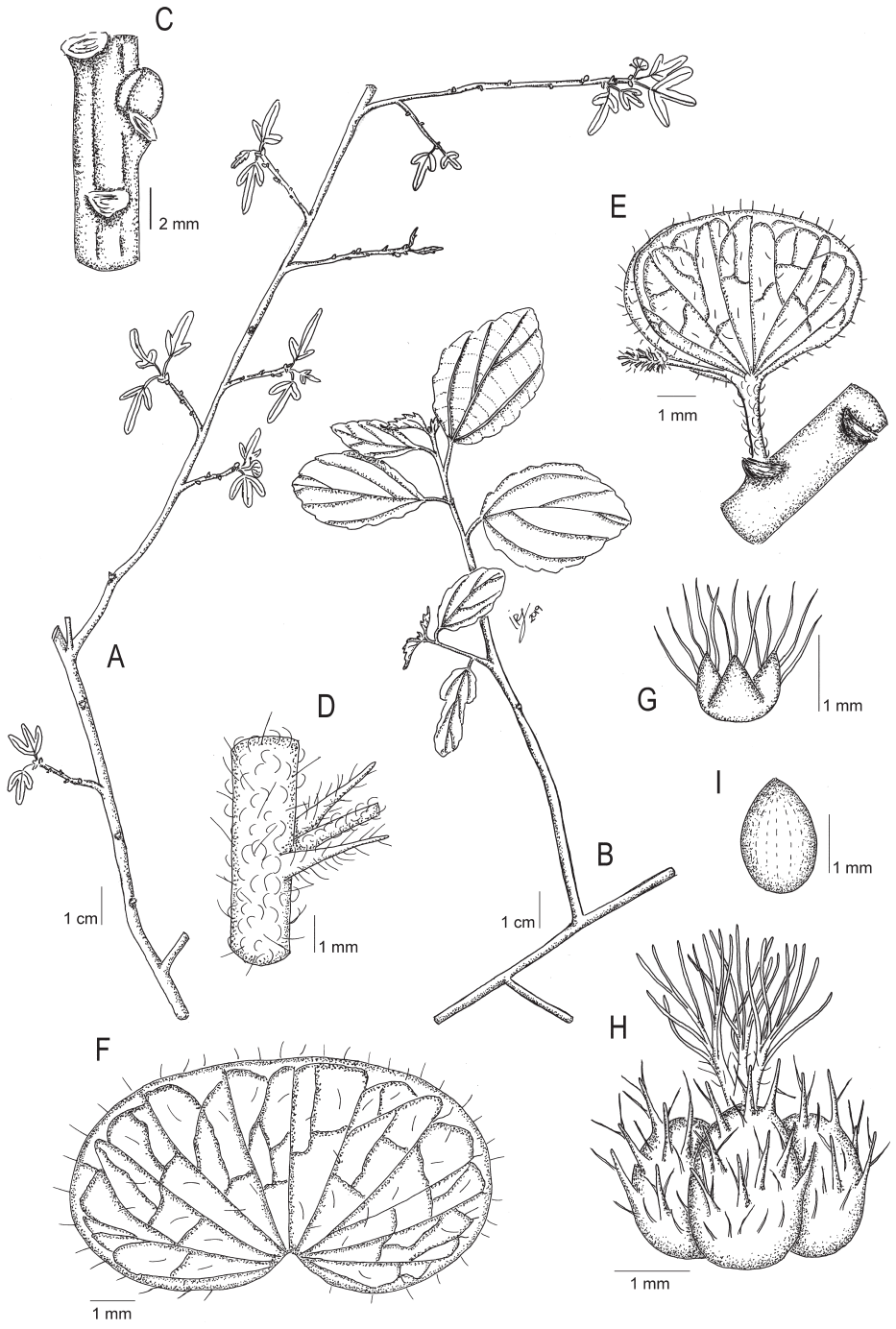

Figure 1. *Acalypha gillespieae* **A** flowering branch with young leaves **B** flowering branch with mature leaves **C** detail of node with axillary bud **D** detail of node, stipules, and petiole base **E** detail of the androgynous inflorescence **F** mature female bract **G** calyx of the female flower **H** capsule with persistent styles **I** seed. Based on *L. Gillespie 4079* (**A, C, E**), *Gillespie et al. 10693* (**B, D**), and *Gillespie et al. 10692* (**F–I**). Illustration: Iris Montero Muñoz.

style divided into 5–8 slender, fimbriate segments. ***Capsules*** 3-locular, c. 3 mm diam., echinate and hispid, with simple, straight, erect to antrorse trichomes c. 0.5 mm long, and conical projections c. 0.75 mm long. ***Seeds*** pyriform, 2 × 1.5 mm, smooth.

Distribution and habitat. *Acalypha gillespieae* is known only from a small area between 200 and 300 m elevation on the north side of Montagne des Français (Fig. 2). This limestone massif, including the area where *A. gillespieae* grows, is covered with dry deciduous forest (Moat and Smith 2007, Goodman et al. 2018).

Etymology. The proposed epithet honors the botanist Lynn J. Gillespie, research scientist at the Canadian Museum of Nature. In addition to studying Arctic plants and Poaceae, she has worked on the systematics of Euphorbiaceae worldwide, including in Madagascar. She collected all the known specimens of this species, either alone or as leader of a team of botanists.

Conservation status. *Acalypha gillespieae* is known from three collections from the same locality. Montagne des Français has been relatively well collected (P. Lowry, pers. comm.), so the dearth of collections suggests this species is rare, even there. Its apparent rarity could also, at least in part, reflect it being quite inconspicuous and thus easily overlooked. The extent of occurrence (EOO) could not be calculated. Its area of occupancy (AOO) is estimated to be 8000 km^2. Montagne des Français is a category V protected area (Dudley 2008). Its habitat is somewhat threatened by wood-cutting, primarily for charcoal, but mainly on its lower slopes, below where *A. gillespieae* is found. *Acalypha gillespieae* is assigned a preliminary IUCN conservation status of Critically Endangered: CR B2ab(ii,iii,iv).

Additional specimens examined (paratypes). Madagascar. Reg. Diana [Prov. Antsirinana]: Montagne des Français, E of Antsirinana (Diego Suarez), 12°19'26.4"S, 49°20'16.6"E, 258 m, 31 Oct 2012, *L. J. Gillespie*, *G. A. Levin*, *J. Andriatiana, & W. M. Cardinal-McTeague 10693* (CAN!, MO!, P!, TAN!); 12°19'S, 49°20'E, 200–300 m, 2 Dec 1990, *L. J. Gillespie 4097* (ILLS!, MO!, P[P00324524]!, TAN!).

Notes. *Acalypha gillespieae* is very unusual among *Acalypha* species in having some lobed leaves. The proportion of lobed leaves varies among collections from about 10% in *Gillespie et al. 10693* to about 20% in *Gillespie et al. 10692* and 70% in *Gillespie 4097*. The lateral lobes range from much smaller than the central lobe to almost equal to it. The lobes, if present, arise near the base of the blade, with the basal veins becoming the midveins of the lobes. Like the very similar *Acalypha humbertii*, *A. gillespieae* flowers when the plants are leafless, probably in late August or September. By the time the leaves emerge in late October, the male flowers have been shed and the female bracts and capsules are mature.

2. *Acalypha leandrii* I.Montero & Cardiel, sp. nov.

urn:lsid:ipni.org:names:77206321-1

Diagnosis. *Acalypha leandrii* I.Montero & Cardiel is morphologically similar to *A. radula* Baker, but differs from it mainly by having leaf blades broadly ovate-lanceolate, not bullate, and petioles 2–8 cm long (vs. leaf blades usually narrowly triangular-

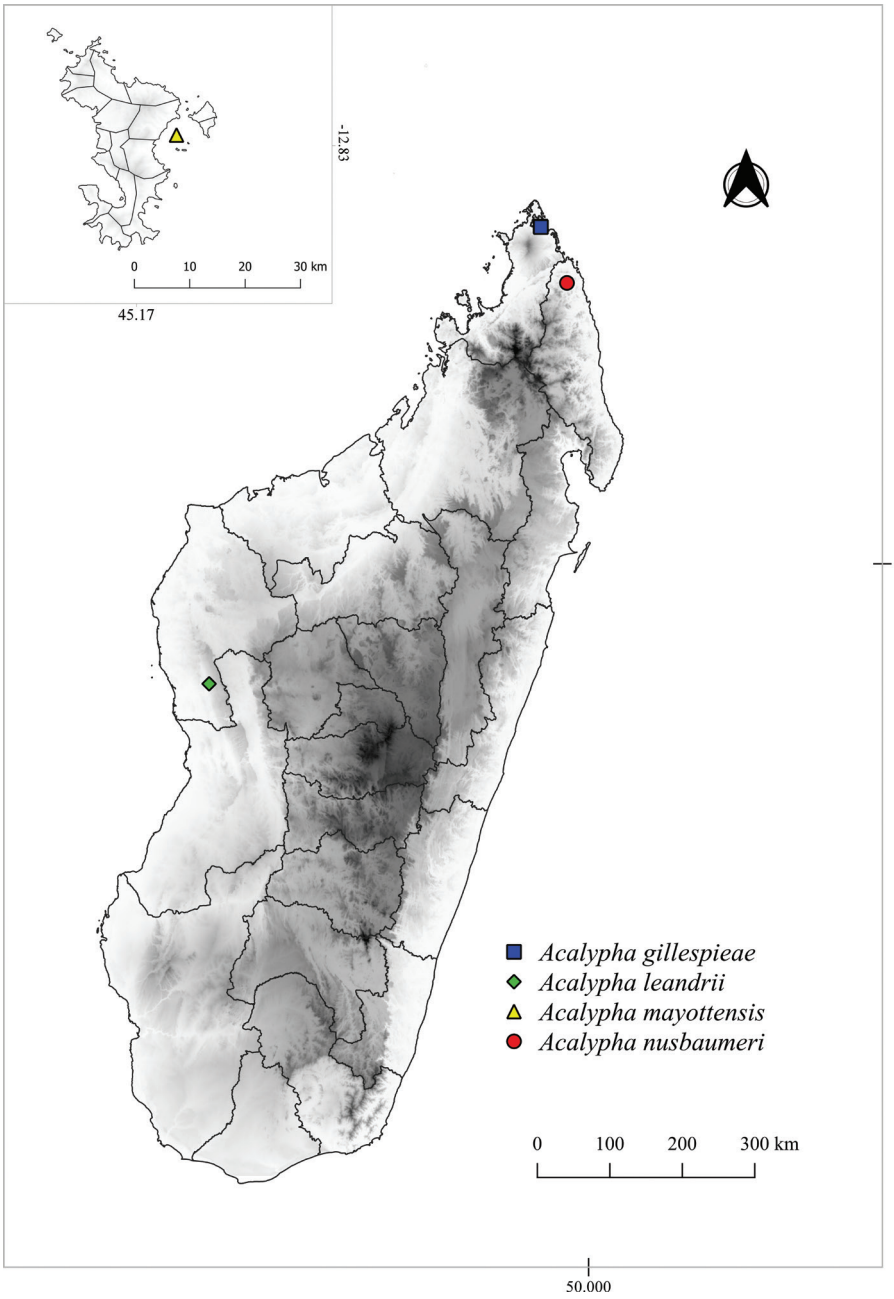

Figure 2. Map of Madagascar and the Mayotte island showing the distributions of *Acalypha gillespieae* (square), *A. leandrii* (rhombus), *A. mayottensis* (triangle) and *A. nusbaumeri* (circle).

lanceolate, bullate, and petioles 1.5–1.8 cm long), mature female bracts with entire margins (vs. mature female bracts with dentate margins), and capsules with simple trichomes (vs. capsules with simple and glandular trichomes).

Type. Madagascar, Reg. Melaky [Prov. Mahajanga]: Antsalova, vers Ambodiriana (E. d'Antsalova), 18°40'0.12"S 44°43'59.879"E, 100–150 m, 06 Dec 1952, *J. Leandri, R. Capuron & A. Razafindrakoto 2037* (holotype: P [P05547059!]). Fig. 3.

Description. ***Shrubs*** or ***subshrubs*** (probably sprawling or clambering) evergreen [height unknown], probably dioecious. ***Young branches*** densely pubescent, with short, simple, antrorsely curved trichomes; older branches glabrous. ***Stipules*** c. 7 mm long, oblong-lanceolate, with scarious margins and a central rib; midrib appressed-pubescent, margins ciliate with thin trichomes mixed with minute glands. ***Axillary buds*** ovoid, c. 2 × 1 mm, perules 2, valvate, membranous, pubescent with short, simple trichomes. ***Petioles*** 2–8 cm long, indumentum similar to that found on the young branches, glabrescent. ***Leaf blades*** 8–12 × (3.5–) 4.5–9 cm, broadly ovate-lanceolate, membranous; base rounded to cordate; margins serrate, teeth acute, slightly calloseedged; apex acuminate to caudate, acumen acute, c. 2.5 cm long, mucronate; both surfaces laxly pubescent with simple, erect trichomes, also with short, antrorsely curved trichomes on veins; venation actinodromous, prominent in both surfaces, with 3 or 5 veins at the base, secondary veins 7–9 per side. ***Stipels*** triangular, c. 0.7 mm long, ciliate, mixed with glandular trichomes. ***Inflorescences*** unisexual, axillary, in terminal nodes. ***Male inflorescences*** spiciform, c. 8 cm long, peduncle c. 2 cm long, indumentum similar to that found on the young branches. ***Female inflorescences*** spiciform, with up to 18 bracts, c. 8.5 cm long, peduncle c. 2.5 cm long, indumentum similar to that found on the young branches. ***Female bracts*** sessile, enlarging in fruit to 6 × 12 mm, subreniform, with prominent veins on adaxial surface, laxly pubescent with erect, simple trichomes and thick glandular trichomes c. 1 mm long; margins entire. ***Male flowers*** inconspicuous, pedicel c. 0.5 mm long, sparsely hairy; buds c. 0.7 mm diameter, glabrous, papillose. ***Female flowers*** solitary, sessile; sepals 3, slightly connate at base, c.1 mm long, oblong-lanceolate, ciliate with simple, erect trichomes c. 0.5 mm long; ovary 3-locular, c. 1 mm diameter, densely hispid; styles 3, c. 5 mm long, slightly connate at base, each divided into 5 slender segments, glabrous. ***Capsules*** c. 3 mm diam., papillose-hispid, with papillae c. 0.5 mm long, each ending in a simple, erect trichome c. 1 mm long. ***Seeds*** pyriform, c. 2 × 1.6 mm, minutely foveolate.

Distribution and habitat. *Acalypha leandrii* is known from two localities in western Madagascar, in the area east of Antsalova. They are both from the karstic massif of Mesozoic limestones known as Tsingy de Bemaraha, in the Melaky Region. This region has dry climates, and the primary vegetation is dry deciduous forest (Schatz 2000, Moat and Smith 2007, Goodman et al. 2018). The altitudinal range of *A. leandrii* is from 100 to 300 m. (Fig. 2).

Etymology. The proposed epithet honors the French botanist Jacques Désiré Leandri (1903–1982). He worked extensively in the Euphorbiaceae family from Madagascar, including writing the last taxonomic treatment of *Acalypha* from the island, in which he described numerous new species (Leandri 1942). Leandri collected the type specimen of this species.

Conservation status. *Acalypha leandrii* is known from three collections. The extent of occurrence (EOO) could not be calculated. Its area of occupancy (AOO) is

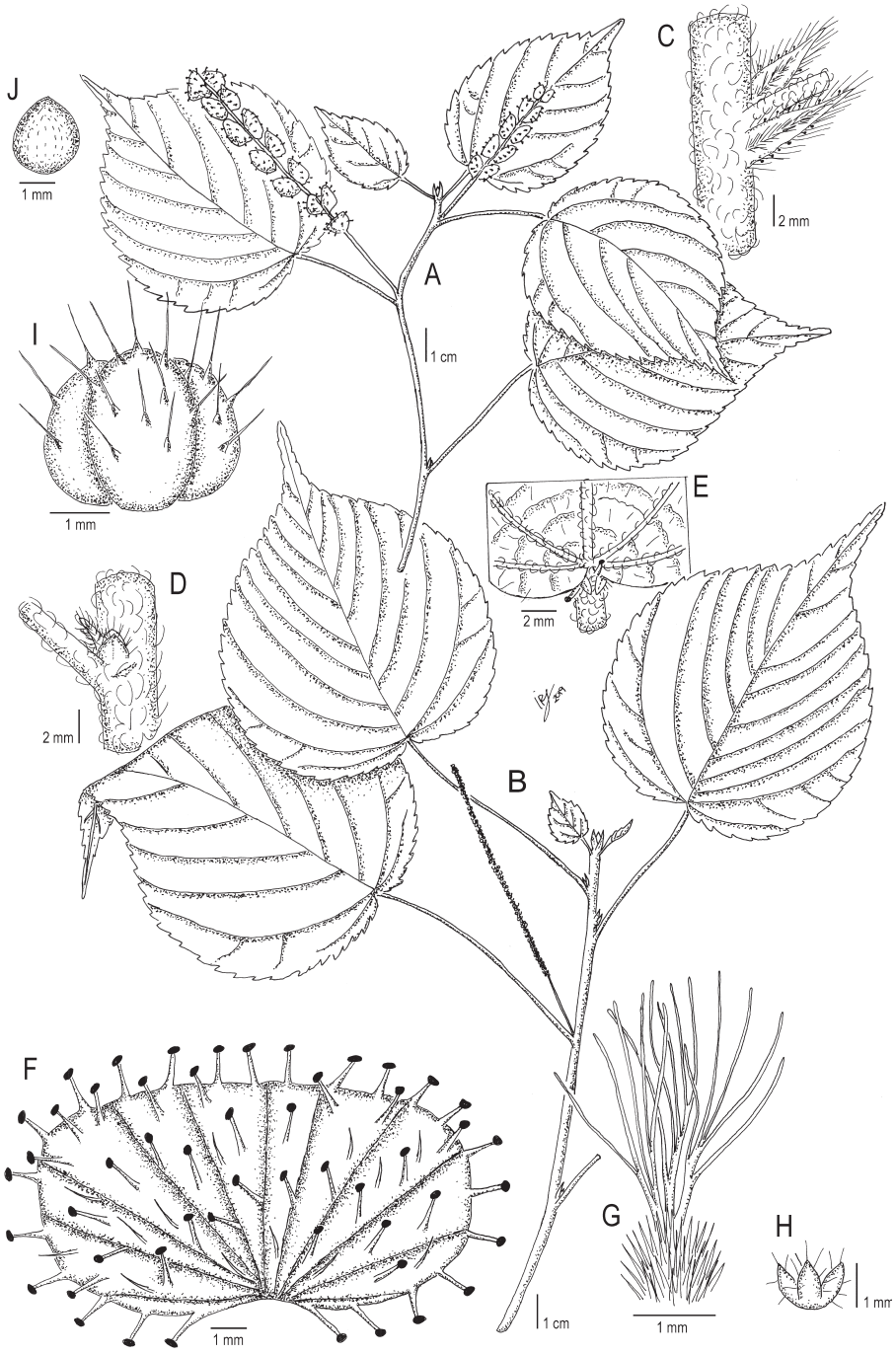

Figure 3. *Acalypha leandrii* **A** flowering female branch **B** flowering male branch **C** detail of node, stipules, and petiole base **D** detail of node with axillary bud **E** detail of upper leaf surface showing the leaf base and stipels **F** mature female bract **G** ovary and styles **H** calyx of the female flower **I** capsule **J** seed. Based on *J. Leandri, R. Capuron & A. Razafindrakoto 2037* (**A, C–J**) and *J. Leandri & P. Saboureau 2996* (**B**). Illustration by Iris Montero Muñoz.

estimated to be 8 km^2. The Tsingy de Bemaraha lies within a national park and a nature reserve that has been IUCN category II and Ia protected areas (Dudley 2008, Goodman et al. 2018) since 1927 and a UNESCO World Heritage Site since 1990 (Goodman et al. 2018). The forest of this area has local anthropogenic pressures such as fire associated with the renewal of zebu (cattle) pastures, logging for construction and deforestation for new agricultural lands. Bemaraha has lost more forest habitat from 2006 to 2016 compared to 1996 to 2006 (Goodman et al. 2018). No specimens of this species have been collected for 60 years, so we cannot rule out that this species has become extirpated from one or both areas. In conclusion, due to habitat loss and the absence of recent collections, *A. leandrii* is assigned a preliminary IUCN conservation status of Critically Endangered: CR B2ab(ii,iii).

Additional specimen examined (paratypes). Madagascar. Reg. Melaky [Prov. Mahajanga]: Calcaires de l'Antsingy, vers Andobo (E. d'Antsalova), en remontant vers Tsiandro, 18°40'0.12"S, 44°43'59.879"E, 05–08 Feb 1960, 300 m, *J. Leandri & P. Saboureau 2996* (K!, P [P05543680!, P00324506!], MO [MO-3025001!], TAN); *J. Leandri & P. Saboureau 3016* (G!, P [P05547274!], MO [MO-2966304!]).

3. *Acalypha mayottensis* I.Montero & Cardiel, sp. nov.

urn:lsid:ipni.org:names:77206322-1

Diagnosis. *Acalypha mayottensis* I.Montero & Cardiel is morphologically similar to *A. humbertii* Leandri, but differs from it mainly by having ovoid axillary buds with imbricate perules (vs. pyriform buds with superposed perules), triangular-lanceolate stipules c. 6 mm long (vs. linear stipules c. 3 mm long), and mature female bracts to 19 × 21 mm with crenate to subentire margins (vs. bracts to 6 × 8 mm with dentate margins).

Type. Mayotte. Mamoudzou commune: Îlot M'bouzi, 12°48'50"S, 45°14'08"E, 10–50 m, 22 Nov 2000, *J.-N. Labat, F. Barthelat, C.M. Hladik & A.B. Sifary 3268*. (holotype: G [G00034240!]; isotypes: K!, MAO, MO [MO-2965774!], P [P00209719!]). Figs 4, 5.

Description. ***Shrubs*** to 5 m high, deciduous, monoecious. ***Young branches*** laxly pubescent, with simple, erect trichomes c. 1 mm long; ***Older branches*** glabrous. ***Axillary buds*** ovoid, c. 3 × 2.3 mm, perules 2, imbricate, blackish, chartaceous, glabrous. ***Stipules*** caducous, c. 6 mm long, linear to triangular-lanceolate, becoming filiform when mature, sparsely hairy, glabrescent, margins translucent, with some glands. ***Petioles*** slender, (2–) 3–5 (–6) cm long, pubescent with simple, antrorsely curved, trichomes. ***Leaf blades*** 5–10 × 3–6 cm, ovate-lanceolate to elliptic-lanceolate, membranous; base rounded to subcordate; margins crenate-serrate to subentire, slightly revolute, teeth minute, rounded, sinuses ciliate; apex subacuminate to acuminate, acumen c. 1.5 cm long, rounded; upper surface pubescent with simple, thin, patent, trichomes, glabrescent; lower surface with indumentum similar to that found on upper surface, but more dense; axils of the secondary veins with minute, sparsely hairy, pocket-shaped domatia, sometimes only hair-tuft domatia; venation actinodromous, with 3 veins at the base, secondary veins 4–6 per side. ***Stipels absent. Inflorescences***

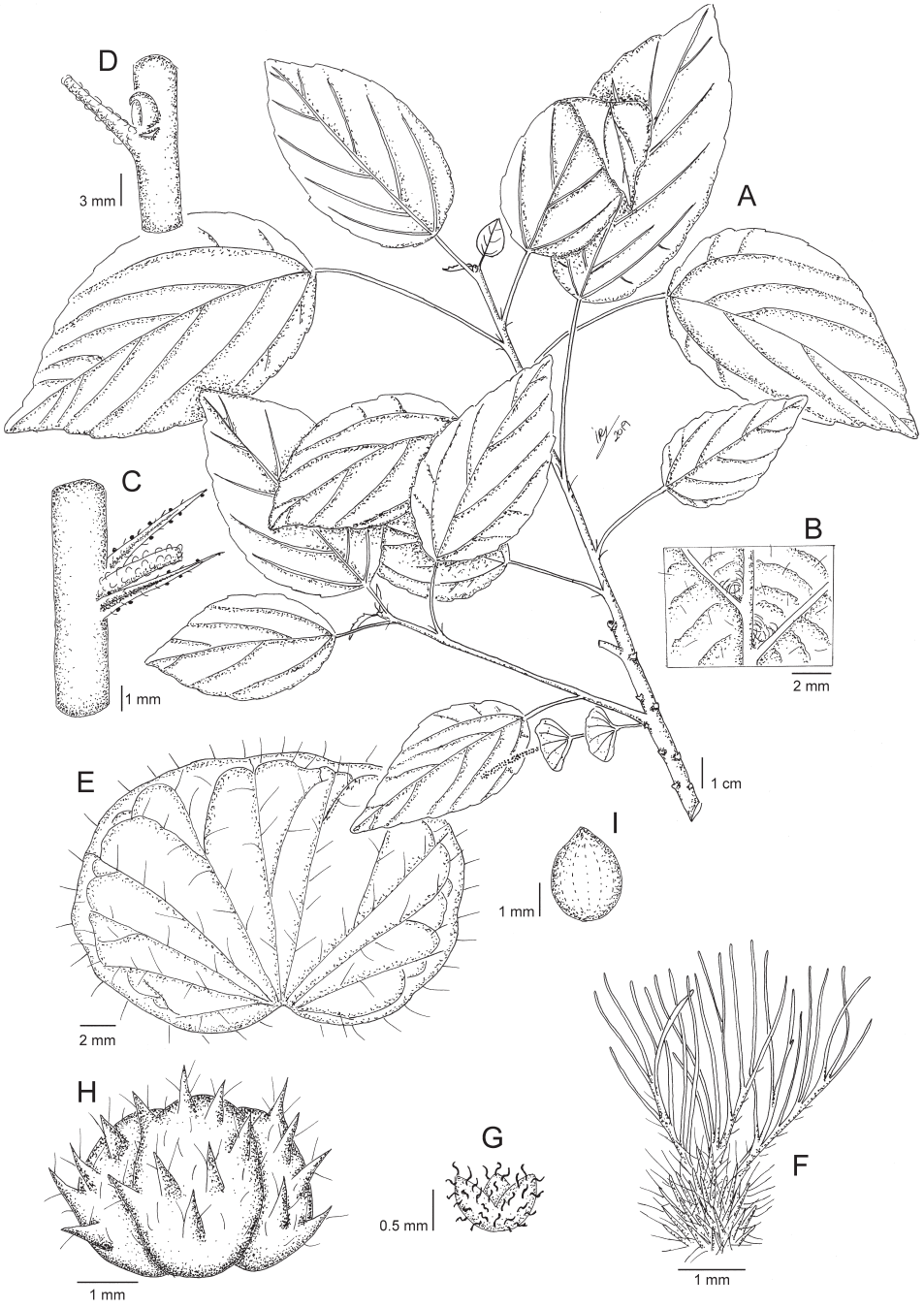

Figure 4. *Acalypha mayottensis* **A** flowering branch **B** detail of lower leaf surface showing the domatia **C** detail of node, stipules, and petiole base **D** detail of node with axillary bud **E** mature female bract **F** ovary and styles **G** calyx of the female flower **H** capsule **I** seed. Based on *J.-N. Labat, F. Barthelat, C.M. Hladik & A.B. Sifary 3268* (**A–C**), and *J.-N. Labat, F. Barthelat, C.M. Hladik & A.B. Sifary 3272* (**D–I**). Illustration by Iris Montero Muñoz.

androgynous, axillary, to 6 cm long, spiciform, with 1–2 female bracts near the base and a male segment distally; peduncle thick, c. 1.5 cm long, laxly pubescent, trichomes similar to those found on the young branches, glabrescent; male segment c. 4 cm long. ***Female bracts*** sessile, enlarging in fruit to 19 × 21 mm, subreniform, sparsely hairy with simple, erect trichomes c. 1.5 mm long on veins and margins, glabrescent; margins crenate to subentire, sometimes dentate in young bracts. ***Male flowers*** inconspicuous, pedicel c. 1 mm long, glabrous; buds c. 0.7 mm diameter, sparsely hairy, with arachnoid trichomes. ***Female flowers*** solitary, sessile; sepals 3[4], connate at base, c. 0.7 mm long, ovate-triangular, sparsely hairy with simple, arachnoid trichomes; ovary 3-locular, c. 1 mm diameter, echinate and hispid; styles 3, c. 3 mm long, free at the base, rachis thick, appressed-pubescent, each divided into 8–10 segments. ***Capsules*** to 4 mm diameter, echinate and hispid, with simple, erect trichomes c. 1 mm long, and conical projections c. 1 mm long, subacute. ***Seeds pyriform***, 2.5 × 2 mm, minutely foveolate.

Distribution and habitat. *Acalypha mayottensis* is endemic to Mayotte, a French overseas department in the Comoros Archipelago, and presumably restricted to the Mbouzi islet (Fig. 2). Mbouzi is a small, volcanic, unoccupied islet, of 82 ha, located east of the main island (Grande-Terre). It has a tropical humid climate, with two seasons: one cool and dry, the other hot and wet, resulting from shifts in the Intertropical Convergence Zone. Mbouzi is mainly covered by secondary dry deciduous forest (Boullet and Traclet 2018). According to the specimens' labels, *A. mayottensis* is a common deciduous bush on the islet, growing in deciduous forest, in ravines and stony areas, from 10 to 90 m elevation.

Etymology. The proposed epithet refers to Mayotte island, to which the small Mbouzi islet belongs.

Conservation status. *Acalypha mayottensis* is only known from Mbouzi islet. The extent of occurrence (EOO) is estimated to be 0.017 km^2. Its area of occupancy (AOO) is estimated to be 8 km^2. Mbouzi islet was declared a "Réserve Naturelle Nationale" in 2007, a category IV protected area (Dudley 2008). In the 1990s the islet had lost 70% of its original forests due to agricultural activities. Mbouzi currently conserves 10% of its natural and subnatural forest (Boullet and Traclet 2018). Currently, the most serious threat is invasive species, both animals, such as *Eulemur fulvus*, and plants, such as *Antigonon leptopus*, *Lantana strigocamara*, *Leucaena leucocephala*, *Litsea glutinosa*, *Spathodea campanulata* and *Furcraea foetida* (Boullet and Traclet 2018, Quintard et al. 2019). *A. mayottensis* is assigned a preliminary IUCN conservation status of Critically Endangered: CR B1ab(i,iii) + B2ab(ii,iii).

Additional specimen examined (paratypes). Mayotte. Mamoudzou commune: Îlot M'Bouzi, 12°48'57"S, 45°14'06"E, 90 m, 22 Nov 2000, *J.-N. Labat, F. Barthelat, C.M. Hladik & A.B. Sifary 3272* (G [G00034255!], K!, MAO, MO [MO-2966248!], P [P00209724!, P00209725!]); Îlot M'Bouzi, 12°48'39"S, 45°14'06"E, 26 Dec 2002, *F. Barthelat, A. de Vanssay & G. Rembert 1112* (MAO, P [P00339165!]); precise location unknown, probably from M'Bouzi islet, 01 Jan 2010, *G. Viscardi 310* (HKM, P [P02439826!]).

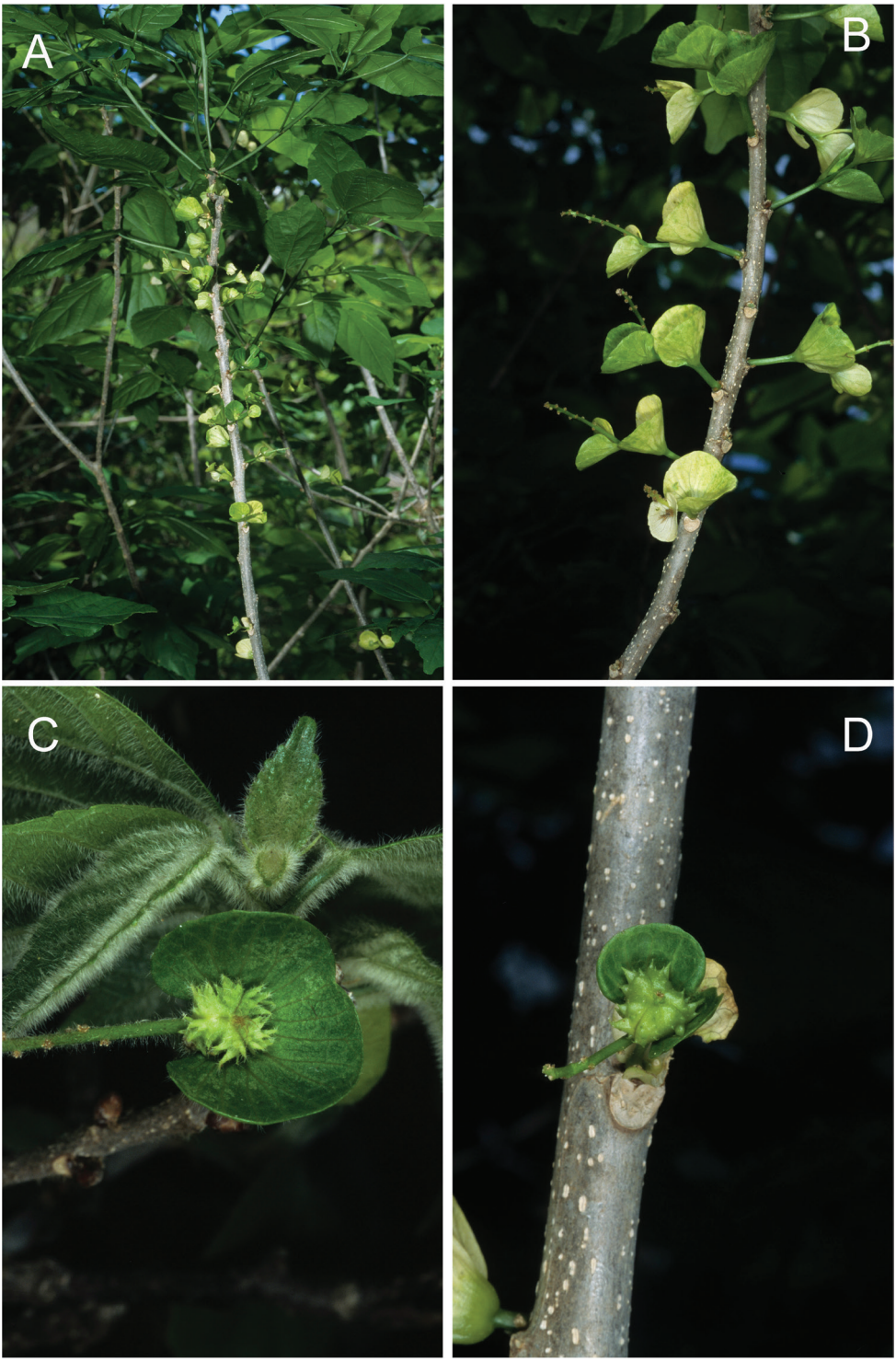

Figure 5. *Acalypha mayottensis* **A** habit **B** flowering branch **C** female flower subtended by mature bract **D** capsule subtended by mature bract. Field images of type specimen. Photographs by Jean-Noël Labat.

Notes. Five other species of *Acalypha* are known from Mayotte: *Acalypha chibomboa* Baill., *A. indica* L., *A. lanceolata* (Müll.Arg.) Radcl.-Sm., *A. paxii* Aug.D.C., and *A. richardiana* Baill. *A. mayottensis* does not strongly resemble any of them. The only other *Acalypha* species known from Mbouzi islet is *A. richardiana*, which differs mainly by having sessile androgynous inflorescences and mature female bracts subrounded, c. 7 × 6 mm (vs. pedunculate androgynous inflorescences and mature female bracts subreniform, c. 19 × 21 mm in *A. mayottensis*). The herbarium specimens of *A. mayottensis* had been previously identified as *A. claoxyloides* Hutch., endemic to the Seychelles Archipelago, but it clearly differs by having flattened resinous glands on lower leaf surface, female bracts and flowers, and smooth capsules (vs. resinous glands absent and echinate capsules in *A. mayottensis*).

4. *Acalypha nusbaumeri* I.Montero & Cardiel, sp. nov.
urn:lsid:ipni.org:names:77206323-1

Diagnosis. *Acalypha nusbaumeri* I.Montero & Cardiel is morphologically similar to *A. perrierii* Leandri, but differs from it mainly by having leaf blades with subacuminate apices (vs. leaf blades with caudate apices), inflorescences to 1.7 cm long (vs. inflorescences to 3 cm long), and mature female bracts 2 × 2.5 mm, translucent, with crenate margins and two basal bracteoles (vs. bracts 7 × 12 mm, opaque, with entire margins and no bracteoles).

Type. Madagascar. Reg. Sava [Prov. Antsiranana]: sous-préfecture de Vohemar. Commune rurale de Daraina, forêt de Bekaraoka, partie nord, 13°06'S, 49°42'E, 177 m, 13 Feb 2004, *L. Nusbaumer & P. Ranirison LN1169.* (holotype: G [G00028080!]; isotypes: K!, MO!, P [P05547228!, P01152829!]). Fig. 6.

Description. ***Shrubs*** to 0.8 m high, probably deciduous, monoecious. ***Young branches*** very slender, divaricate, blackish, pubescent with simple, antrorsely curved trichomes; older branches glabrous. ***Axillary buds*** spherical, c. 1 mm diameter, perules 2, imbricate, membranous, external perule dentate, sparsely hairy. ***Stipules*** caducous, c. 2 mm, triangular-lanceolate, with a prominent central rib, sparsely hairy with simple, short, erect trichomes. ***Petioles*** slender, 2–3.5 (–4.5) cm long, with indumentum similar to that found on young branches. ***Leaf blades*** 5–8.5 × 2.5–5 cm, ovate-lanceolate to elliptic-lanceolate, thin-membranous; base obtuse to rounded; margins crenate-serrate; teeth rounded; apex subacuminate, acumen c. 0.5 mm long, rounded and mucronate at apex; upper surface subglabrous, with simple, short, antrorsly curved trichomes on veins; lower surface with indumentum similar to that found on upper surface, axils of the secondary veins with pocket-shaped, sparsely hairy domatia; venation actinodromous, with 3 or 5 veins at the base, secondary veins 3–4 per side. ***Stipels*** absent**.** ***Inflorescences*** inconspicuous, androgynous, axillary, c. 1.7 cm long, spiciform, mostly male with 1 female bract near the base; peduncle thick, c. 0.5 cm long, with indumentum similar to that found on the young branches; male segment c. 7 cm long. ***Female bracts*** sessile, translucent, enlarging in fruit to 2 × 2.5 mm, orbic-

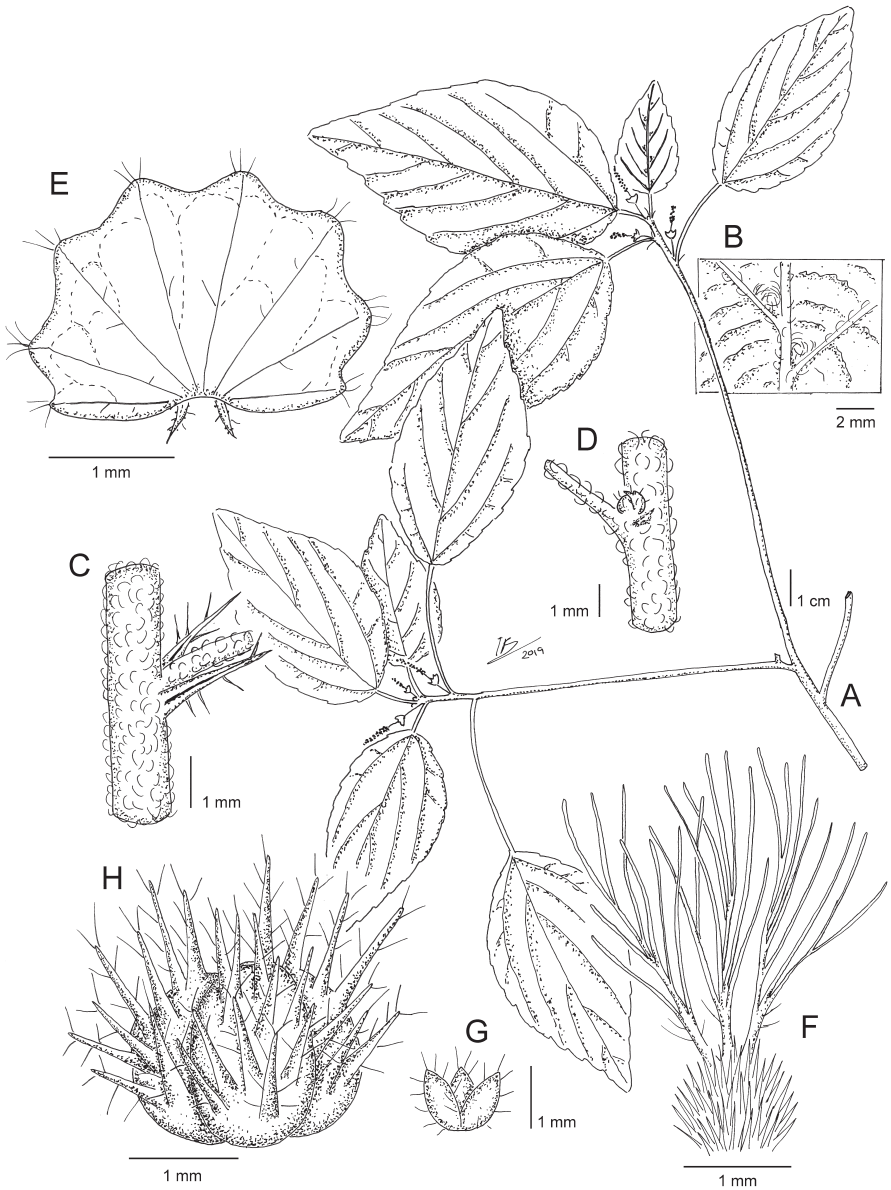

Figure 6. *Acalypha nusbaumeri* **A** flowering branch **B** detail of lower leaf surface showing the pocket-shaped domatia **C** detail of node, stipules, and petiole base **D** detail of node with axillary bud **E** mature female bract with bracteoles **F** ovary and styles **G** calyx of the female flower **H** capsule. Based on *L. Nusbaumer & P. Ranirison LN1169*. Illustration by Iris Montero Muñoz.

ular-reniform, subglabrous, with short, simple trichomes on teeth and veins, margins crenate. ***Bracteoles*** linear-lanceolate, c. 0.5 mm long, sparsely hairy, with short, simple trichomes, and some sessile glands on the margins. ***Male flowers*** inconspicuous, pedicel c. 0.5 mm long, sparsely hairy; buds c. 0.8 mm diameter, glabrous, papillose.

Female flowers solitary, sessile; sepals 3, free at base, c. 1 mm long, lanceolate, ciliate with short, simple trichomes; ovary 3-locular, c. 1 mm diameter, densely hispid; styles 3, c. 3.5 mm long, free at the base, each divided into 8–10 slender segments, rachis sparsely hairy. ***Capsules*** (immature) to 2 mm diam., echinate, with projections c. 1 mm long, pubescent with simple, short erect trichomes c. 0.5 mm long. ***Seeds*** too young to describe.

Distribution and habitat. *Acalypha nusbaumeri* is only known from Bekaraoka forest, in the Loky-Manambato protected area, in Sava Region, northern Madagascar (Fig. 2). This area has a seasonally dry climate (Schatz 2000; Goodman et al. 2018). Regarding its vegetation, Loky-Manambato is a special area because it is between the Eastern Humid and Western Dry phytogeographic domains and so has many types of vegetation (Nusbaumer et al. 2010). Bekaraoka forest has dry deciduous forest on basement rocks (Moat and Smith 2007, Goodman et al. 2018), which seems to be the characteristic habitat of *A. nusbaumeri*.

Etymology. The proposed epithet honors Louis Nusbaumer, researcher and curator of Conservatoire et Jardin botaniques de la Ville de Genève, Switzerland. He works on the systematics, phylogeny, biogeography, and conservation of Malagasy plants. Nusbaumer is also the collector, with Patrick Ranirison, of the type specimen of this species.

Conservation status. *Acalypha nusbaumeri* is only known from one collection. The extent of occurrence (EOO) could not be calculated. Its area of occupancy (AOO) is estimated to be 8 km^2. Loky-Manambato is a category V (Goodman et al. 2018) protected area since 2005. The forest in this region has been degraded and continues to be threatened by slash and burn agriculture, fires to clear land for grazing, illegal cutting of precious woods, and in some areas, as Bekaraoka, gold extraction (Vargas et al. 2002, Rakotondravony 2009, Goodman et al. 2018). *Acalypha nusbaumeri* is assigned a preliminary IUCN conservation status of Critically Endangered: CR B2ab(ii,iii).

Acknowledgments

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RESEARCH ARTICLE

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An integrative taxonomic approach reveals a new species of *Eranthis* (Ranunculaceae) in North Asia

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Abstract

A new endemic species, *Eranthis tanhoensis* **sp. nov.**, is described from the Republic of Buryatia and Irkutsk Province, Russia. It belongs to *Eranthis* section *Shibateranthis* and is morphologically similar to *E. sibirica* and *E. stellata*. An integrative taxonomic approach, based on cytogenetical, molecular and biochemical analyses, along with morphological data, was used to delimit this new species.

Keywords

Biochemistry, cytology, integrative taxonomic approach, morphology, phylogeny, Ranunculales, Russia

Introduction

The genus *Eranthis* L. (Ranunculaceae) consists of eight to ten species distributed in southern Europe and temperate Asia (Lee et al. 2012; Park et al. 2019). Most species have narrow distributions and only one European species, *E. hyemalis* (L.) Salisb., has been widely cultivated in gardens and become naturalised in Britain (Boens 2014) and North America (Parfitt 1997). *Eranthis* are perennial herbs with tuberous rhizomes, basal long-petiolate leaves with the blades divided into several or many palmate segments (leaflets) that are entire or lobate; unbranched scapes carrying a solitary, bisexual and actinomorphic flower supported by three verticillate leaf-like bracts forming an involucre; (4–)5–8 yellow, white or pink, caducous sepals; 5–10(–15) yellow or white, bifid petals shorter than sepals; nectaries located at the middle or upper part of the petals; > 10 stamens; and 3–10 follicles with several smooth seeds in each fruitlet (Parfitt 1997). All species are early-blooming plants, with anthesis from March to May (depending on the altitude), but *E. hyemalis* has been found at full anthesis in mid-January in gardens (Sukhorukov, pers. obs. in Mainz, Germany, 2019 and Leiden, Netherlands, 2020).

On the basis of morphology, the genus has been divided into two sections: *E.* sect. *Eranthis* and *E.* sect. *Shibateranthis* (Nakai) Tamura (Tamura 1987). The type section is characterised by annual tubers, yellow sepals and emarginate or slightly bilobate upper petal margins without swellings (nectaries), whereas the members of section *Shibateranthis* have long-lived tubers, white sepals and bilobate or forked petal margins with swellings (Tamura 1995). Molecular phylogenetic analysis. based on nrITS and chloroplast *trn*L-*trn*F interspacer region, supports the subdivision of the genus into these sections (Park et al. 2019). Furthermore, they are geographically separated, with section *Eranthis* occurring in Europe (*E. hyemalis*) and SW & W Asia (*E. cilicica* Schott & Kotschy, *E. longistipitata* Regel) and section *Shibateranthis* distributed in temperate N & E Asia (*E. albiflora* Franch., *E. byunsanensis* B.Y.Sun, *E. lobulata* W.T.Wang, *E. pinnatifida* Maxim., *E. pungdoensis* B.U.Oh, *E. sibirica* DC. and *E. stellata* Maxim.: Park et al. 2019). Two additional species with yellow sepals, *E. bulgarica* (Stef.) Stef. (Stefanoff 1963) and *E. iranica* Rukšāns & Zetterl. (Rukšāns and Zetterlund 2018), have been described from Bulgaria and Iran, respectively, but have not yet been included in molecular analysis.

Recent studies have revealed the genetic diversity, phylogeny and presumed origin of some narrowly distributed Korean and Japanese species with further conclusions about their taxonomic status (Lee et al. 2012; Oh and Oh 2019). The taxonomic and genetic diversity of *Eranthis* in the Asiatic part of Russia is insufficiently studied. To date, only two species have been found in Russia: *E. sibirica* and *E. stellata* (both belonging to sect. *Shibateranthis*) from South Siberia and Far East Russia (Malyshev 2005). High genetic polymorphism of *E. sibirica* across populations near Baikal Lake was discovered only recently (Protopopova et al. 2015) and this fact has inspired us to conduct a new study of *Eranthis* in the Asiatic part of Russia.

The aim of the present study was to investigate the morphological, molecular, biochemical and cytogenetic heterogeneity of the Baikal populations to determine wheth-

er any undescribed species were present there. The relationship between *E. sibirica*, *E. stellata* and a new species, described and named below as *Eranthis tanhoensis* Erst, sp. nov. is explored here.

Materials and methods

Plant material

More than 300 herbarium specimens were collected during field investigations in the Republics of Khakassia and Buryatia and the Irkutsk Province during 2018 and 2019. Fieldwork was conducted during different seasons to observe the species in both their flowering and fruiting stages. The specimens were deposited in the E and NS herbaria (herbarium abbreviations according to Thiers 2019+). Revision of herbarium materials was undertaken in the herbaria at IRK, LE, MW, NS, NSK, PE, VBGI and VLA. Drawings of the new species, *Eranthis tanhoensis*, are based on images of the type specimen (NS-0000948!) and paratype (NS-0000949!). The flowering and fruiting times and habitats are provided as cited on the collectors' labels. Maps of records were made with SimpleMappr (http://www.simplemappr.net). Conservation analysis was performed using criteria from the International Union for the Conservation of Nature (IUCN 2019). The Extent of Occurrence (EOO) and Area of Occupancy (AOO) of each species were estimated using GeoCat (Bachman et al. 2011).

Molecular analysis

We sampled 15 individuals of *E. tanhoensis* and six of *E. sibirica*. Two individuals of *E. stellata* and one each of *E. pinnatifida* and *E. longistipitata* were also included. The details of the samples are presented in Suppl. material 1: Table S1. Six nuclear and plastid DNA regions (ITS, *trn*L-F, *trn*H-*psb*A, *rps16*, *mat*K and *rbc*L) were included in the molecular analysis. Total genomic DNA was extracted from silica gel-dried leaves or herbarium specimens using DNeasy Mini Plant Kits (Tiangen Biotech, Beijing, China) following the protocol specified by the manufacturer. Sequencing reactions were conducted using BigDyeTM Terminators (Applied Biosystems Inc., Foster City, CA, USA). Sequences were read using an automated ABI 3730xl DNA Analyzer. Geneious v8.0.4 (Kearse et al. 2012) was used to evaluate the chromatograms for base confirmation and to edit contiguous sequences. We first used the Maximum Likelihood (ML) method to perform non-parametric bootstrap analyses for each DNA region in RAxML v7.0.4 (Stamatakis 2006). No significant bootstrap support for conflicting nodes was evident amongst individual DNA regions (here considered to exceed 70%) and the six-locus datasets were therefore combined for subsequent analyses. Phylogenetic analyses of the combined dataset were conducted using ML and Bayesian Inference (BI) methods. RAxML was conducted with the GTR + Γ substitution model for each region with the fast bootstrap

option using 1000 replicates. BI analysis was conducted in MrBayes v3.2.1 (Ronquist et al. 2012). Data partitioning and nucleotide substitution models were determined using PartitionFinder 2.1.1 (Lanfear et al. 2016). Two independent analyses, consisting of four Markov Chain Monte Carlo chains were run, sampling one tree every 1000 generations for 10 million generations. Runs were completed when the average standard deviation of split frequencies reached 0.01. The stationarity of the runs was assessed using Tracer v1.6 (Rambaut et al. 2014). After removing the burn-in period samples (the first 25% of sampled trees), a majority rule (> 50%) consensus tree was constructed.

Morphological analysis

The morphology of vegetative and reproductive structures was examined on well-developed specimens. For numerical analysis, 25 specimens at flowering and 25 specimens at fruiting stages were examined for each species (more than 150 specimens altogether). For each species, we studied different populations from across the range, including populations from the type localities of *E. stellata* and *E. sibirica*. As *E. stellata* often does not produce basal leaves at flowering, we studied this character in a limited number of samples. The morphological characters were measured using AxioVision 4.8 software (Carl Zeiss, Munich, Germany).

The missing values in the original data table were restored using multidimensional linear regression, in accordance with recommendations of Myers (2000) and Lee and Carlin (2010). A one-way analysis of variance (ANOVA), according to Chambers et al. (1992), was used to identify the distinguishing morphometric features of each species. The differences were considered significant at P-value < 0.05. As multiple statistical testing was performed, the calculated P-value was adjusted using the procedure proposed by Benjamini and Hochberg (1995). The principal component analysis was used to visualise the distribution of the analysed individuals over the space of morphometric characters. This method was employed only for those characters that displayed significant intergroup differences, according to the results of the ANOVA. For scale adjustment, the logarithmic transformation of data was used. The results of the principal component analysis were visualised using the Factoextra package (Kassambara and Mundt 2017).

Cytogenetic analysis

Somatic chromosomes were studied in root tip cells. Tubers were germinated in wet moss at ~15 °C for 2–4 weeks. Newly formed 1–2 cm long roots were excised and pretreated in a 0.5% colchicine solution for 2–3 h at 15 °C. Roots were fixed in a mixture of 96% ethanol and glacial acetic acid (3:1). Root tips were stained with 1% aceto-haematoxylin and the squash method was employed for investigation of the karyotype (Smirnov 1968).

Chromosomes were counted in 50–100 mitotic cells for each population. Mitotic metaphase chromosome plates were observed using an Axio Star microscope (Carl

Zeiss, Munich, Germany) and photographed using an Axio Imager A.1 microscope (Carl Zeiss, Germany) with AxioVision 4.7 software (Carl Zeiss, Germany) and AxioCam MRc5 CCD–camera (Carl Zeiss, Germany) at 1000× magnification in the Laboratory for Ecology, Genetics and Environmental Protection (Ecogene) of the National Research Tomsk State University. KaryoType software (Altinordu et al. 2016) was used for karyotyping, whereas Adobe Photoshop CS5 (Adobe Systems, USA) and Inkscape 0.92 (USA) were used for image editing. Karyotype formulae were based on measurements of mitotic metaphase chromosomes taken from photographs. The measurements were performed on 5–10 metaphase plates. The symbols used to describe the karyotypes followed those of Levan et al. (1964): m = median centromeric chromosome with arm ratio of 1.0–1.7 (metacentric chromosome); sm = submedian centromeric chromosome with arm ratio of 1.7–3.0 (submetacentric chromosome); st = subterminal centromeric chromosome with arm ratio of 3.0–7.0 (subtelocentric chromosome); t = terminal centromeric chromosome with arm ratio of 7.0–∞ (acrocentric chromosome); T = chromosome without obvious short arm, i.e. with arm ratio of ∞.

Flow cytometry

Flow cytometry with propidium iodide (PI) staining was used to determine the absolute DNA content. The relative DNA content in the nucleus (C-value) in representatives of three *Eranthis* species – *E. stellata*, *E. sibirica* and *E. tanhoensis* from different populations, was determined in this study. In total, more than 70 samples from 15 populations were studied (see Suppl. material 1: Table S1). Silica gel-dried leaf material (0.5–1.0 cm^2) was chopped with a sharp razor blade in a 1 ml cold nuclei extraction buffer composed of 50 mM Hepes, 10 mM sodium metabisulphite, 10 mM $MgCl_2$, 0.5% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.3% Tween20, 0.2% Triton X-100, 50 μg/ml RNase, 1 μg/ml β-mercaptoethanol and 50 μg/ml propidium iodide (PI). The samples were filtered through 50 μm nylon membranes into sample tubes and incubated in the dark at 4 °C for 15 min. Samples were measured using a Partec CyFlow PA flow cytometer equipped with a green laser, at 532 nm wavelength. The absolute nuclear DNA content, the 2C-value according to Greilhuber et al. (2005), was calculated as the ratio of the mean fluorescence intensity of the nuclei of the sample to that of an external standard multiplied by the total nuclear DNA content of the standard. The possible effect of secondary metabolites on the binding of the intercalating dye was evaluated by measuring the fluorescence of *Allium fistulosum* L. leaf samples prepared as described above, but with the addition of the supernatant from *Eranthis* samples, centrifuged without PI. The samples were measured three times at 10 min intervals. If no variation in the average values of the detection channels was observed for the *A. fistulosum* peak, the effect of secondary metabolites was considered negligible.

The 1Cx-value (monoploid DNA content *sensu* Greilhuber et al. 2005) was calculated by dividing the 2C-value by the ploidy level of the species. The species, used as external standards, were *Zamioculcas zamiifolia* Engl., 2C = 48.35 pg and *Vicia faba*

L. 'Inovec' 2C = 26.90 pg (Doležel et al. 1992; Skaptsov et al. 2016). We used the Statistica 8.0 software (StatSoft, Inc.), Flowing Software 2.5.1 (Turku Centre for Biotechnology) and CyView software (Partec, GmbH) for data analyses. Flow cytometry was performed at the Laboratory for Bioengineering of the South-Siberian Botanical Garden, Altai State University (Barnaul, Russia).

High-performance liquid chromatography (HPLC) analysis of individual phenolic compounds in ethanol leaf extracts

In order to determine the composition of phenolic compounds, air-dried plant material was mechanically ground to obtain a homogenous powder and then samples of ~0.2 g were extracted three times using 70% aqueous ethanol solution for 30 min in a water bath at 72 °C. Next, the combined extract was concentrated in porcelain dishes to 5 ml. The solutions were filtered and stored at 4 °C until analysis. Analysis of phenolic components was performed using an Agilent 1200 HPLC system equipped with a diode array detector and a ChemStation system for the collection and processing of chromatographic data (Agilent Technology, Palo Alto, CA, USA). The separation was performed on a Zorbax SB-C18 column (5 μm, 4.6 × 150 mm) at 25 °C. The methanol content of the mobile phase in an aqueous solution of phosphoric acid (0.1%) varied from 50–52% over 56 min (van Beek 2002). The eluent flow rate was 1 ml/min. Detection wavelengths were 255, 270, 340 and 360 nm. Groups of phenolic substances were identified by their spectral characteristics (Bate-Smith 1962; Mabry et al. 1970). For identification of the phenolic components in plant extracts, standard samples of salicylic and chlorogenic acids, quercetin, kaempferol, orientin (Sigma-Aldrich Chemie GmbH, Munich, Germany), gentisic and caffeic acids (Serva Heidelberg, Germany), hyperoside and vitexin (Fluka Chemie AG, Buchs, Switzerland) were used. The samples were analysed twice.

Results and discussion

Molecular phylogenetic analysis

Bayesian and ML analyses of the combined dataset produced highly consistent topologies. *Eranthis sibirica* and the new species *E. tanhoensis* formed a sister clade of that of *E. pinnatifida*. The monophyly of each species, *E. tanhoensis* sp. nova, *E. sibirica* and *E. stellata*, was strongly supported (Fig. 1).

Morphological analysis

The morphological analysis revealed that *E. sibirica* was not homogeneous across its distribution area. We compared 41 characters to distinguish *E. sibirica*, *E. tanhoensis* and

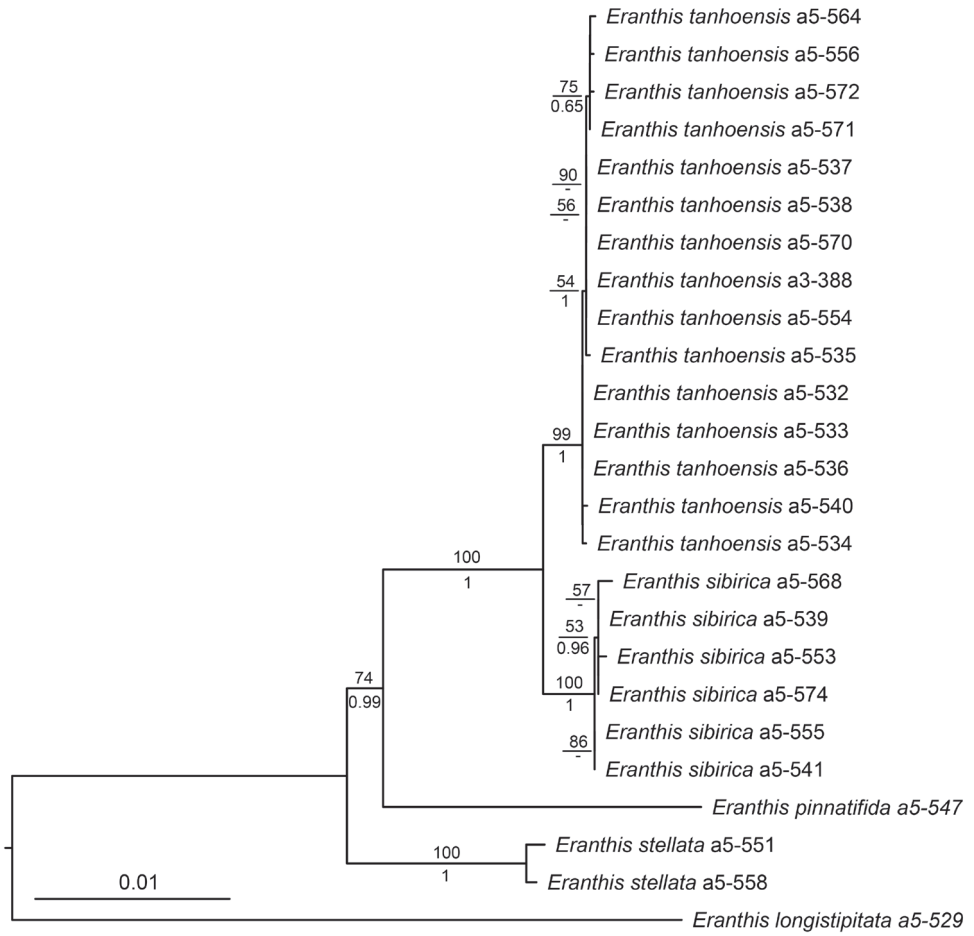

Figure 1. ML tree inferred from the combined cpDNA and ITS data. The numbers above branches are bootstrap values (BS > 50%) and numbers under branches are Bayesian posterior probabilities (PP > 0.50).

E. stellata (Suppl. material 1: Table S2). The basal and involucral leaves in *Eranthis* spp. undergo changes at fruiting and, for this reason, the lengths of all leaves, their segments and segment lobes were measured both at the flowering and fruiting stages. In Suppl. material 1: Table S2, an asterisk (*) indicates the characters used in the numerical analysis. An ANOVA was conducted only for quantitative characteristics. As basal leaves are often absent at the time of flowering and there were no samples with basal leaves in herbarium collections, there were limited data on these characteristics of *E. stellata*.

The ANOVA of morphometric characters showed significant differences amongst the studied species in characters (1), (9), (16), (18), (22), (24), (29), (30), (31) and (32) at the flowering stage and (6), (10), (14), (17), (19), (23), (25), (40) and (41) at the fruiting stage (Suppl. material 1: Tables S3, S4). In total, significant differences amongst the species were found in 10 out of 15 morphometric parameters measured at flowering

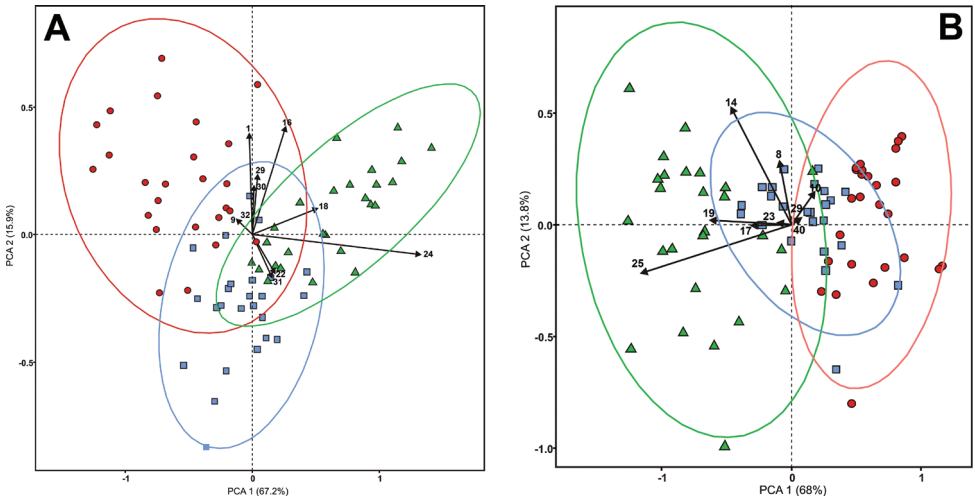

Figure 2. Scatter point diagram in the space of the first two main components for *Eranthis sibirica* (red dots), *Eranthis tanhoensis* (green triangles) and *Eranthis stellata* (blue squares) **A** at flowering and **B** at fruiting stages. Ellipses enclose the regions of the space that contain each of the plant species with a 95% probability (95% confidence ellipses).

and in 9 out of 13 parameters at fruiting. The principal component analysis revealed that the first two main components accounted for 83.1% and 81.8% of the variance in the entire data array of the parameters measured at flowering and fruiting, respectively and showed the best species discrimination. The highest variability of morphometric characters was found at flowering in *E. sibirica* (Fig. 2A) and at fruiting in *E. tanhoensis* (Fig. 2B). As signified by the directions of the vectors indicating the gradients in the character values, at flowering, *E. sibirica* differed from *E. tanhoensis* by having lower values for characters (18), (22), (24) and (31) and a higher value for character (9). At fruiting, *E. sibirica* was characterised by having lower values for parameters (19), (17), (23) and (25) and higher values for parameters (10), (40) and (29), in comparison with those of *E. tanhoensis*. *E. sibirica* differed from *E. stellata* by having higher values for characters (1), (16), (29), (30) and (32) at flowering and (10) and (14) at fruiting. The pattern of overlap between the species differed between flowering and fruiting plants. For instance, *E. tanhoensis* was reliably distinguished from *E. sibirica* only at fruiting (the ellipses enclosing the samples did not overlap; Fig. 2B). In addition to numerical parameters, the new species was also distinguished by qualitative characters.

Cytogenetic analysis

The karyotypes of three related species, *E. sibirica*, *E. tanhoensis* and *E. stellata*, were investigated (Table 1), those of *E. sibirica* and *E. tanhoensis* being studied for the first time. The chromosomes of each species were medium or large in size (from 5 to 11–12 μm) and belonged to the R-type (Langlet 1932). The vouchers are listed in Suppl. material 1: Table S1.

Table 1. Chromosome numbers (2*n*), ploidy level (n*x*), karyotype formulas, and C-values (C ± SD) of the three studied *Eranthis* species.

Voucher number	Species	Voucher information	2*n*	n*x*	Karyotype formulae	2C±SD, pg	1Cx±SD, pg
1	*E. sibirica*	Republic of Khakassia, Bolshoi On river	28	4*x*	2*n* = 20m (2 sat) + 2m/sm + 6 sm	38.83±1.03	9.71±0.26
2	*E. sibirica*	Irkutsk Province, Kuitun river	28	4*x*	2*n* = 20m (2 sat) + 2m/sm + 6 sm	38.19 ± 0.28	9.55 ± 0.14
3	*E. sibirica*	Irkutsk Province, Slyudyanka river	42	6*x*	2*n* = 30m + 12sm (2 sat)	55.75±0.28	9.23±0.14
4	*E. sibirica*	Irkutsk Province, Burovschina river	42	6*x*	2*n* = 30m + 12sm (2 sat)	55.76±0.47	9.27±0.23
5	*E. sibirica*	Irkutsk Province, Utulik river	42	6*x*	2*n* = 30m + 12sm (2 sat)	55.31±0.45	9.22±0.25
6	*E. tanhoensis*	Irkutsk Province, Mamai river	14	2*x*	2*n* = 10m (2sat) + 4sm	24.88±0.54	12.44±0.27
7	*E. tanhoensis*	Republic of Buryatia, Duliha river	14	2*x*	2*n* = 10m (2sat) + 4sm	24.97±0.43	12.49±0.22
8	*E. tanhoensis*	Republic of Buryatia, Tolbazikha river	14	2*x*	2*n* = 10m (2sat) + 4sm	24.77±0.52	12.38±0.26
9	*E. tanhoensis*	Irkutsk Province, Malye Mangaly river	14	2*x*	2*n* = 10m (2sat) + 4sm + 0–8B	24.15±0.11	12.07±0.06
10	*E. tanhoensis*	Irkutsk Province, Semirechka river	14	2*x*	2*n* = 10m (2sat) + 4sm	25.31±0.15	12.41±0,29
11	*E. tanhoensis*	Republic of Buryatia, Osinovka river (Tanhoi village)	14	2*x*	2*n* = 10m (2sat) + 4sm	25.11±0.32	12.56±0.16
12	*E. tanhoensis*	Republic of Buryatia, Mishiha river	14	2*x*	2*n* = 10m (2sat) + 4sm + 0–4B	25.25±0.15	12.07±0.07
13	*E. tanhoensis*	Republic of Buryatia, Shestipalikha river	14	2*x*	2*n* = 10m (2sat) + 4sm	25.53±0.18	12.77±0.09
14	*E. stellata*	Primorsky Krai, Vladivostok, Studencheskaya railway station	16	2*x*	2*n* = 16 = 10m + 4sm (2sat) + 2t	31.76±0.61	15.88±0.31
15	*E. stellata*	Primorsky Krai, Malaya Sedanka river	16	2*x*	2*n* = 16 = 10m + 4sm (2sat) + 2t	31.88±0.67	15.94±0.34
16	*E. stellata*	Primorsky Krai, "13th km" railway station	16	2*x*	2*n* = 16 = 10m + 4sm (2sat) + 2t	–	–
17	*E. stellata*	Primorsky Krai, Russkiy Island	16	2*x*	2*n* = 16 = 10m + 4sm (2sat) + 2t	28.47±0.46	14.23±0.23

Eranthis sibirica. Two cytotypes, with basic chromosome number *x* = 7, were revealed. *Eranthis sibirica* from the Republic of Khakassia (1) and Irkutsk Province (2) were tetraploid with 2*n* = 4*x* = 28 (Fig. 3A, B). Three populations from the Irkutsk Province (3, 4 and 5) were hexaploid with 2*n* = 6*x* = 42 (Fig. 3C). Metacentric and submetacentric chromosome types were present in all examined *E. sibirica* specimens. The karyotype formula of tetraploid plants was 2*n* = 20m(2sat) + 2m/sm + 6sm and 2n = 30m + 12sm(2sat) in hexaploid plants. No B chromosomes were identified in this species.

Eranthis tanhoensis. We determined the chromosome numbers in specimens of eight populations of *E. tanhoensis*. All plants studied were diploid, with 2*n* = 2*x* = 14 (Table 1 and Fig. 3D, E). Metacentric and submetacentric types of chromosomes were found (Fig. 3D, E). The two populations examined (9, 12) were characterised by the presence

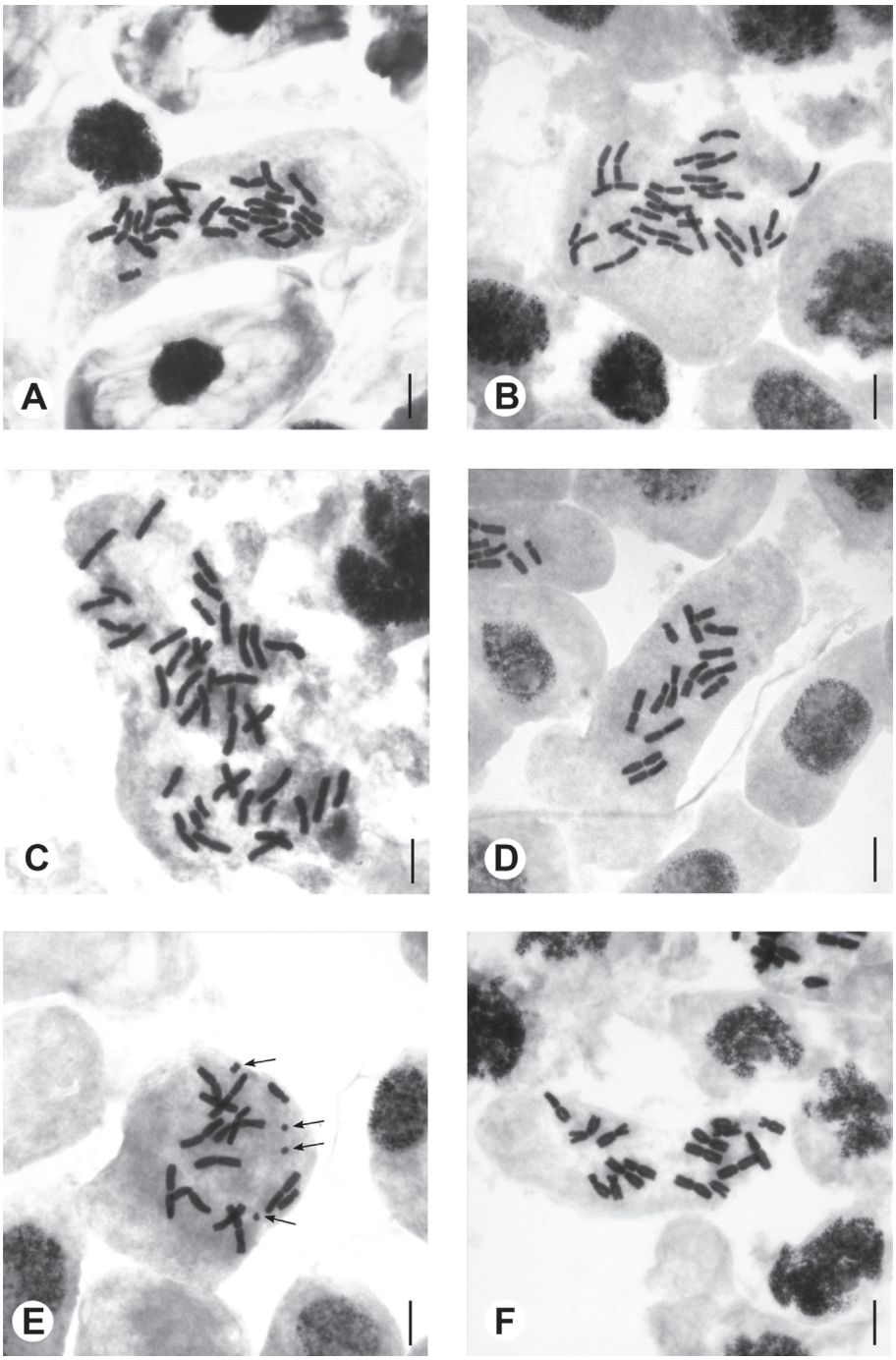

Figure 3. Mitotic metaphase chromosomes. **A** *Eranthis sibirica* (voucher 1 in Table 1), $2n = 28$ **B** *Eranthis sibirica* (voucher 2), $2n = 28$ **C** *Eranthis sibirica* (voucher 3), $2n = 42$ **D** *Eranthis tanhoensis* (voucher 11), $2n = 14$ **E** *Eranthis tanhoensis* (voucher 9), $2n = 14 + 0–8$ B (arrows point at B chromosomes) **F** *Eranthis stellata* (voucher 14), $2n = 16$. Scale bars: 10 μm.

of B chromosomes. The maximum number of B chromosomes appeared to be eight (9). B chromosomes in this species were represented by two types: small (2.3–2.5 μm) metacentrics and dot-shaped 1.3–1.5 μm long chromosomes, which were obviously acrocentric. The karyotype formula of *E. tanhoensis* was $2n$ = 10m (2sat) + 4sm + 0–8B.

Eranthis stellata. In all four studied populations of *E. stellata*, the basic chromosome number was x = 8. This species was diploid with $2n = 2x = 16$, which is typical of the genus (Table 1; Fig. 3F). Five pairs of chromosomes were metacentric, two pairs were submetacentric and one pair was acrocentric (Fig. 3F). The karyotype formula of *E. stellata* was $2n$ = 10m + 4sm (2sat) + 2t. No B chromosomes were observed in this species.

The basic chromosome number x = 8 has been reported for the entire genus *Eranthis* (Langlet 1932; Kurita 1955; Tak and Wafai 1996; Gömürgen 1997; Yuan and Yang 2006; Kim et al. 2011; Marhold et al. 2019). Our results are consistent with previously published data (Yuan and Yang 2006), with insignificant differences in the karyotype formula. However, we showed, for the first time, that *E. sibirica* and *E. tanhoensis* are distinguished from other species of the genus by the basic chromosome number x = 7. Such differences in basic chromosome numbers (x = 7 and x = 8) have been found in some other genera of Ranunculaceae, for example, *Anemone* L. and *Ranunculus* L. (Rice et al. 2015). Our results regarding the chromosome numbers in *E. sibirica* ($2n$ = 28 and $2n$ = 42) differed from the data reported by other researchers for this species ($2n$ = 32: Krogulevich (1976) or $2n$ = 16: Gnutikov et al. (2016, 2017)). *Eranthis tanhoensis* was found to have $2n$ = 14. Based on the incongruence of the chromosome data with previous and recent analyses, we assume that some populations of *E. sibirica* and *E. tanhoensis* may have diverse cytotypes. Both species clearly differed from *E. stellata* by the absence of acrocentrics. All three species were characterised by five metacentrics and two submetacentrics per monoploid chromosome set.

Flow cytometry

The average absolute DNA content of hexaploid samples of *E. sibirica* was 2C = 55.33 ± 0.52 pg and that of tetraploid samples was 2C = 38.19 ± 0.28 pg. In diploid *E. tanhoensis*, the average absolute DNA content was 2C = 25.02 ± 0.28 pg. The average absolute DNA content of diploid *E. stellata* was 2C = 31.47 ± 0.46 pg. The monoploid DNA content of the *E. sibirica* cytotypes was similar: 1Cx = 9.55 ± 0.14 pg in tetraploids and 1Cx = 9.25 ± 0.20 pg in hexaploids. The monoploid DNA content of *E. tanhoensis* was 1Cx = 12.49 ± 0.16 pg and that of *E. stellata* was 1Cx = 15.77 ± 0.20 pg.

Tetraploids and hexaploids of *E. sibirica* exhibited insignificant differences in DNA content (9.25 pg for 6x and 9.55 for 4x), whereas diploids of *E. tanhoensis* showed a higher 1Cx level (12.49 pg), which may indicate a relatively ancient diversification of these species. Data on the 1Cx level of *E. stellata* (15.77 pg) indicated the independent or parallel evolution of genome size in this species. According to flow cytometry, variations in 1Cx levels between diploid samples of *E. tanhoensis* and hexaploids and tetraploids of *E. sibirica* were in accordance with the hypothesis of genome downsizing in polyploid flowering plants (Leitch and Bennett 2004).

HPLC analysis of individual phenolic compounds

Phenolic compounds are often used in chemotaxonomic studies owing to their wide distribution in plants, structural diversity and chemical stability (Braunberger et al. 2015; Radušienė et al. 2018). They have also been reported as promising chemotaxonomic markers for Ranunculaceae (Hao 2018). However, data on the significance of these substances for the taxonomy of *Eranthis* is still insufficient. Only a few studies of the phytochemical characteristics of certain *Eranthis* species, considered as medicinal plants, have been published (Djafari et al. 2018; Hao 2018; Watanabe et al. 2003, 2019).

Twenty four phenolic compounds were detected in 70% ethanol extracts of plant leaves of the three *Eranthis* species (*E. sibirica*, *E. stellata* and *E. tanhoensis*) using HPLC (Fig. 4). Phenolic acids (chlorogenic, gentisic, caffeic and salicylic acids), flavonols (quercetin, kaempferol and hyperoside) and flavones (orientin and vitexin) were identified amongst these compounds. All three species were very similar in the composition of the phenolic compounds extracted from their leaves; however, there were specific compounds for each taxon. The common compounds present in all studied plants were chlorogenic acid, phenolic acids (Fig. 4, peak 3: t_R, min = 10.0, λ_{max}, nm = 250, 290 sh, 335; peak 12: t_R, min = 20.9, λ_{max}, nm = 240, 290 sh, 335 and peak 23: t_R, min = 44.3, λ_{max}, nm = 255, 300, 330) and flavonols (Fig. 4, peak 9: t_R, min = 15.1, λ_{max}, nm = 255, 360 and peak 15: t_R, min = 32.7, λ_{max}, nm = 270, 310, 365). Almost all plants contained kaemp-

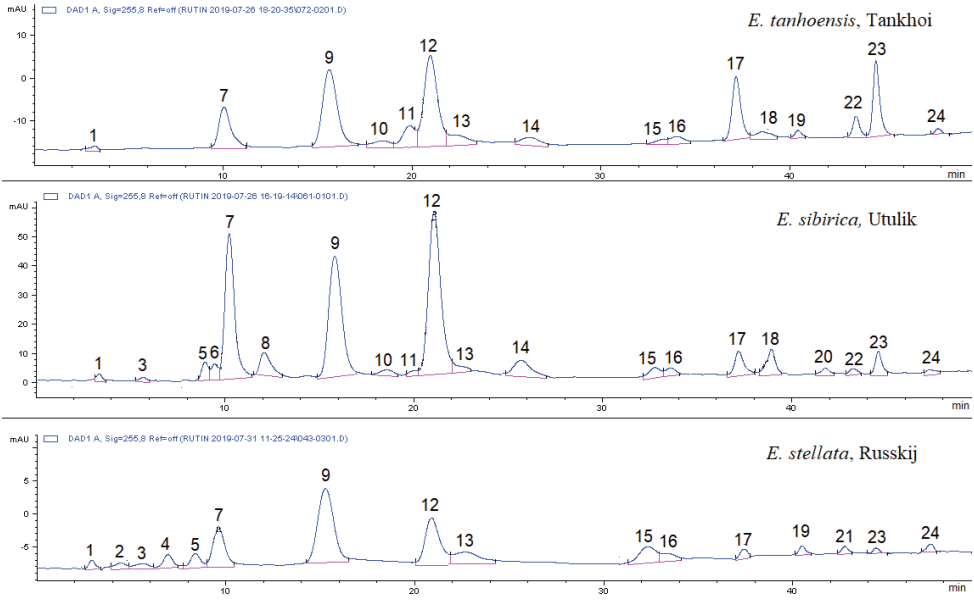

Figure 4. HPLC chromatograms of 70% water-ethanol extracts of *Eranthis* leaves detected by HPLC-DAD at 255 nm. The X-axis displays the retention time, min; Y-axis – the detector signal in optical density units. The identified peaks are **1.** chlorogenic acid, **2.** gentisic acid, **3.** caffeic acid, **5.** orientin, **8.** vitexin, **10.** hyperoside, **11.** salicylic acid, **19.** quercetin and **24.** kaempferol.

ferol, phenolic acids (Fig. 4, peak 14: t_R, min = 25.4, λ_{max}, nm = 255, 300 and peak 16: t_R, min = 34.5; λ_{max}, nm = 250, 290, 330) and flavonols (Fig. 4, peak 13: t_R, min = 22.3, λ_{max}, nm = 255, 360 and peak 18: t_R, min = 38.7, λ_{max}, nm = 255, 305, 360). *Eranthis sibirica* leaves contained about 15 to 20 phenolic compounds, whereas, in *E. stellata* leaves, their number varied from 16 to 18. Phenolic compounds were less diverse in *E. tanhoensis* leaves than in leaves of other species, whose numbers varied from 13 to 16 substances.

The chromatographic profile of *E. sibirica* differed from that of *E. tanhoensis* in the presence of caffeic acid, orientin, vitexin and flavone (peak 6: t_R, min = 9.4, λ_{max}, nm = 270, 310) in 70% ethanol leaf extracts (Fig. 4). Caffeic acid, orientin and flavone (peak 6) were generally absent from leaves of *E. tanhoensis*, whereas vitexin was found in some samples in trace amounts. The leaves of *E. tanhoensis* from almost all the studied populations contained quercetin, which was not detected in *E. sibirica*. Distinguishing compounds in leaf extracts of *E. stellata* were gentisic acid, phenolic acid (Fig. 4, peak 4: t_R, min = 7.1, λ_{max}, nm = 250, 300) and flavone (Fig. 4, peak 21: t_R, min = 42,2; λ_{max}, nm = 210, 310), which were absent from the two other species. Vitexin, hyperoside and salicylic acids were not found in *E. stellata* leaves. All samples of *E. stellata* contained orientin and caffeic acid, which were characteristic of *E. sibirica* and quercetin, which was typical of *E. tanhoensis*.

Taxonomy

The analysis of the data presented above allowed us to distinguish a new species from specimens previously identified as *E. sibirica*.

***Eranthis tanhoensis* Erst, sp. nov.**
urn:lsid:ipni.org:names:77206949-1
Figs 5, 6A–D, 7B

Type. Russia, Republic of Buryatia, Kabansky district, Osinovka River near Tanhoi village, 51°33'06.2"N, 105°05'34.7"E, 458 m a.s.l., 01 May 2019, *A.S. Erst*, *D.A. Krivenko*, & *O.A. Chernysheva s.n.* (holotype, NS-0000948!, isotypes TK, IRK, E).

Description. *Herb* perennial, 12.0–23.0 cm long at flowering and 18.0–40.0 cm long at fruiting. *Tubers* subglobose, not or slightly branching, 1.2–3.3 cm diam., producing thin fibrous roots. *Basal leaf* single, long-petiolate, green; petioles 5.0–6.0 cm long at flowering and 23–25 cm at fruiting; blades 2.5–3.8 × 2.5–3.5 cm at flowering and 7.5–12 × 7.5–12 cm at fruiting, deeply palmately divided into 5 segments (maximum length of segment dissection 2.3 cm at flowering (3.5 cm at fruiting)); leaf blade segments rounded or widely rhombic, 0.8–2.5 × 0.4–1.8 cm at flowering (1.7–8.5 × 1.2–7.5 cm at fruiting), unlobed or dissected into 1–2 lobes at both flowering and fruiting stages; segment of basal leaves with 5–19 acute teeth at apex at flowering, 6–25 teeth at fruiting. *Involucre* present, 1.1–5.5 cm diam. at flowering (7–11 cm at fruiting stage); involucral bracts (cauline leaf) sessile, laciniate, similar to basal leaf, divided into

Figure 5. General habit of *Eranthis tanhoensis*. Scale bar: 1 cm.

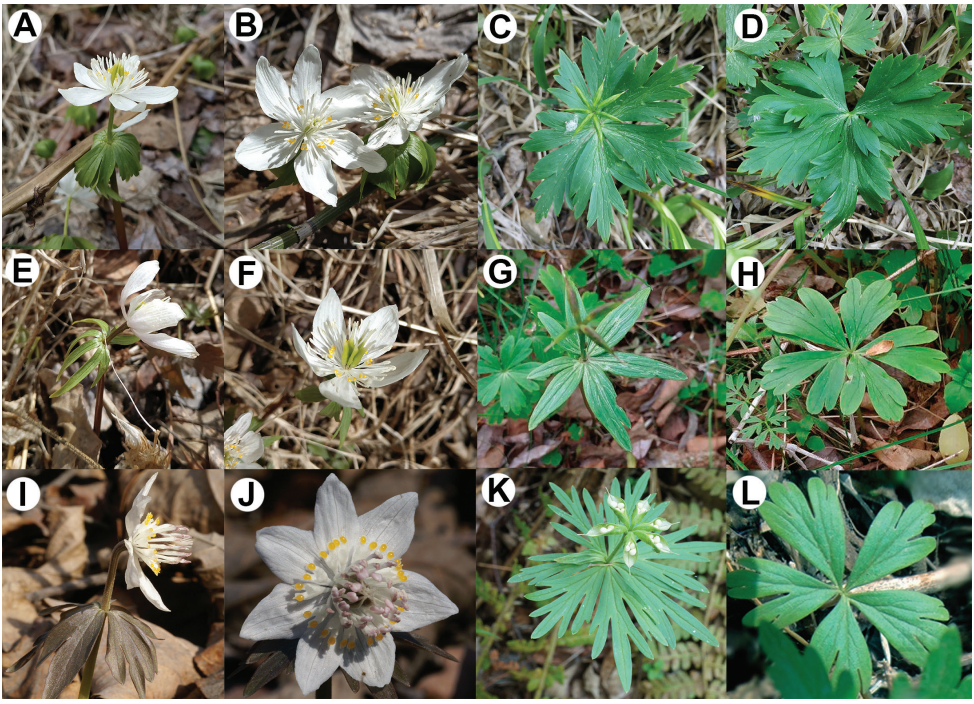

Figure 6. Morphological differences amongst **A–D** *Eranthis tanhoensis* **E–H** *Eranthis sibirica*; and **I–L** *Eranthis stellata* **A, E, I** flower position **B, F, J** flowers **C, G, K** involucral bracts and follicles **D, H, L** basal leaves.

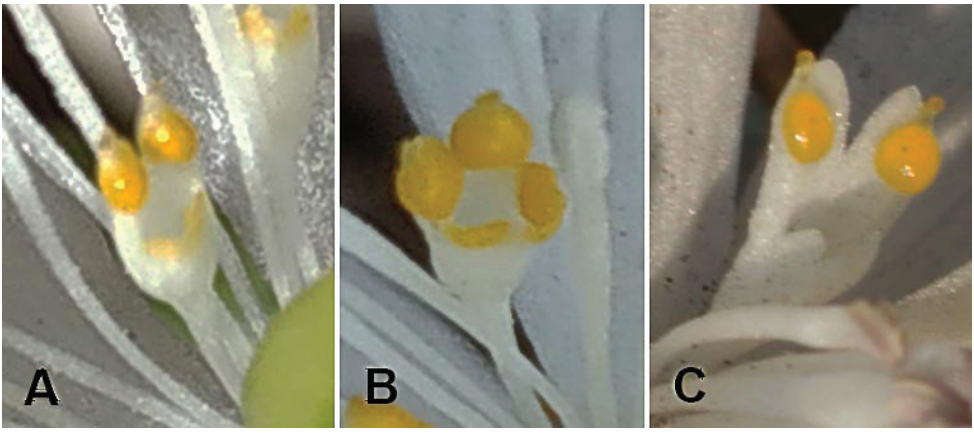

Figure 7. Petals. **A** *Eranthis sibirica* **B** *Eranthis tanhoensis* **C** *Eranthis stellata*.

5 trifid leaf-like segments (maximum length of segment dissection is 1.6 cm at flowering (4.0 cm at fruiting)); segments rounded or widely rhombic, 1.1–3.0 × 0.5–2.5 cm at flowering (3.3–6.4 × 1.4–5.3 cm at fruiting), unlobed or dissected into 2 lobes both

at flowering and fruiting stages; each segment with 5–21 teeth (at both flowering and fruiting stages), acute at the apex. *Pedicels* 0.5–1.5 cm long, elongated in fruiting (3.5–5.5 cm long), densely covered with papillate and large hemispherical trichomes. *Flowers* bisexual, actinomorphic, solitary, erect, 2–4 cm diam. *Sepals* 4–7, deciduous in fruit, white or light pink at margin, flat, narrowly obovate or elliptic, 1.1–2.6 × 0.5–1.3 cm. *Petals* 5–15 × 0.6–0.8 cm long, bicoloured, white, tubular, two-lipped with bilobate or forked lips, each lobe of abaxial lip acute at the apex and with globular yellow swellings (nectaries: Fig. 7B). Stamens 36–45, 0.7–1.1 cm long; filaments filiform, white; anthers white. Follicles 3–10, 0.8–1.4 cm long, on short (0.3–0.5 mm) stalks, divergent towards the end of fruiting; stylodium 0.1–0.3 mm long, straight or slightly curved.

Notes. Turczaninow (1842) described the species *E. uncinata* Turcz., growing at higher altitudes and distinguished from *E. sibirica* by the number of petals (5–6, not strictly 5), by the shape of the stylodium (recurved rather than straight), smaller flowers and more dissected leaf blades. However, our studies have shown that these morphological characters are variable and all variations can be found both in the foothill and alpine plants. Shipchinskiy (1937) merged *E. uncinata* with *E. sibirica.* However, he described two varieties: *E. sibirica* DC. var. *nuda* Schipcz. with glabrous pedicels (= *E. sibirica* var. *sibirica*) and *E. sibirica* DC. var. *glandulosa* Schipcz. with glandular-pubescent pedicels. These varieties were not validly published under ICN Article 39.1 (Turland et al 2018). Nakai (1937) attributed *E. sibirica* and *E. uncinata* to the genus *Schibateranthis* Nakai (≡ *Eranthis* sect. *Schibateranthis* (Nakai) Tamura).

Affinity. The new species belongs to *E.* sect. *Shibateranthis* (Nakai) Tamura and it is sister to *E. sibirica*, according to the results of molecular phylogenetic analysis (Fig. 1). *E. tanhoensis* is morphologically similar to *E. sibirica* and *E. stellata* (Figs 5–9) in having white sepals, tubular two-lipped petals with bilobate or forked lips, apically acute lobes with abaxial lip and globular yellow swellings (nectaries) at the top or in the central part. The differences amongst the three species are presented in Table 2.

The new species differs from other related species by dense glandular pubescence of the flower stems, rounded or widely rhombic (not obovate or lanceolate) leaf blade segments, acute, rather than rounded teeth apices of the basal and stem leaves, a large number of teeth and width of the segments of the basal and stem leaves (see also 2). Additionally, all three species growing in Russia have different distribution patterns (Figs 10, 11).

Phenology. Flowering time: April–early May; fruiting time: late May–June.

Distribution (Fig. 10): *Eranthis tanhoensis* is endemic to southern Baikal (Khamar-Daban range of the Republic of Buryatia and Irkutsk Province).

Habitat and ecology. *Eranthis tanhoensis* can be found at 350–2400 m a.s.l., where it grows in fir, Siberian pine, spruce and birch forests, on riverbanks, beside streams (up to 1500 m a.s.l.) and in subalpine meadows (at higher altitudes).

Etymology. The specific epithet of the new species is derived from the type locality, Tanhoi village, Republic of Buryatia, Russia.

Additional specimens examined. Russia: Republic of Buryatia: Kabansky district, Osinovka river (Tanhoi village), 51°33'06.2"N, 105°05'34.7"E, 458 m a.s.l., 20 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva s.n.* (NS-

Table 2. Morphological differences among *E. sibirica*, *E. tanhoensis*, and *E. stellata*.

Character	*E. sibirica*	*E. tanhoensis*	*E. stellata*
Leaf colour at flowering	green	green	coppery or green
Teeth at the apex of basal leaf segments	rounded	acute	rounded
Maximum dissection of the basal leaf segments (at flowering), cm	1.0	2.3	0.4–?
Maximum dissection of the basal leaf segments (at fruiting), cm	2.3	3.5	1.3
Number of teeth on the segments of the basal leaf (at fruiting)	3–12	6–25	3–5
Apex of involucral leaves	rounded	acute	rounded
Width of the involucral leaf segments (at fruiting), cm	0.4–1.2	1.4–5.3	0.5–2.3
Maximum dissection of the involucral leaf segments (at flowering), cm	1.6	1.6	1.0
Maximum dissection of the involucral leaf segments (at fruiting), cm	2.1	4.0	1.7
Number of teeth on the segments of the involucral leaf (at flowering)	1–5	5–21	3–9
Number of teeth on the segments of the involucral leaf (at fruiting)	2–5	5–21	3–8
Flower position	erect	erect	recurved
Scape pubescence	glabrous or with papillate trichomes	large hemispherical and papillate trichomes	glandular and stellate trichomes
Sepal number	5–7	4–7	5–8
Shape of petals	narrow urn-shaped	broadly urn-shaped	funnelform
Swellings (nectaries) position	at the apex	at the apex	in medium part
Apex colour of adaxial lip	yellow	yellow	white
Apex colour of abaxial lip	yellow	yellow	white
Margin colour between abaxial and adaxial lips	white	yellow	white
Stamen colour	white	white	violet, pink or white
Stylodium length, cm	0.2–0.5	0.1–0.3	0.2–0.4

0000949!); Kabansky district, Mishikha river, 51°37'46.7"N, 105°32'05.2"E, 480 m a.s.l., 01 May 2019, *A.S. Erst*, *D.A. Krivenko* & *O.A. Chernysheva 31* (NS-0000950!); Kabansky district, Mishikha river, 51°37'46.7"N, 105°32'05.2"E, 480 m a.s.l., 01 May 2019, *A.S. Erst*, *D.A. Krivenko & O.A. Chernysheva 31a* (NS-0000951!); Kabansky district, Mishikha river, 51°37'32.6"N, 105°32'03.4"E, 478 m a.s.l., 20 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva s.n.* (NS-0000952!); Kabansky district, Dulikha river, 51°32'04.9"N, 105°01'43.2"E, 461 m a.s.l., 01 May 2019, *A.S. Erst*, *D.A. Krivenko & O.A. Chernysheva 14* (NS-0000953!); Kabansky district, Dulikha river, 51°32'04.9"N, 105°01'43.2"E, 461 m a.s.l., 20 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva* (NS-0000954!); Kabansky district, Shestipalikha river, 51°32'46.4"N, 105°04'28.9"E, 465 m a.s.l., 01 May 2019, *A.S. Erst*, *D.A. Krivenko & O.A. Chernysheva s.n.* (NS-0000955!); Kabansky district, Shestipalikha river, 51°32'46.4"N, 105°04'28.9"E, 465 m a.s.l, 21 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva* (NS-0000956!); Kabansky district, Tolbazikha river,

Figure 8. General habit of *Eranthis sibirica*. Scale bar: 1 cm.

Figure 9. General habit of *Eranthis stellata*. Scale bar: 1 cm.

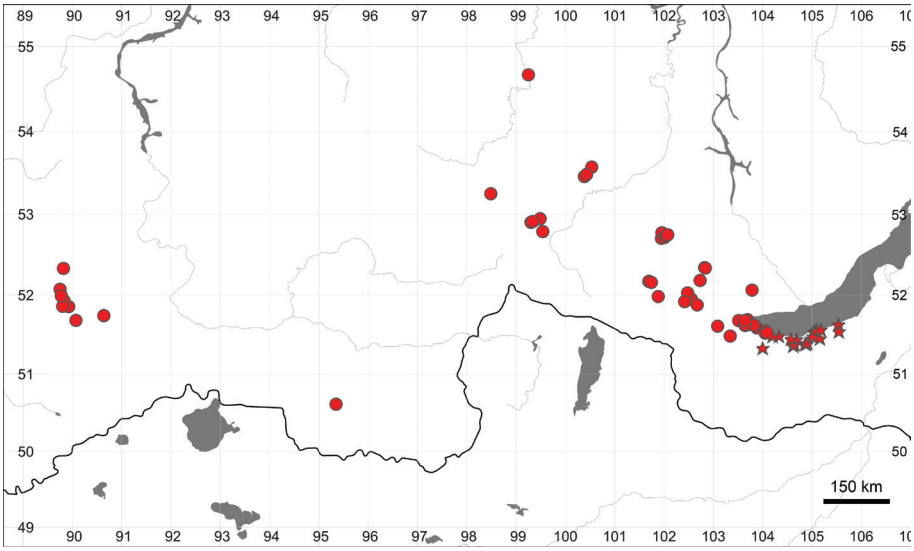

Figure 10. General distribution of *Eranthis sibirica* (dots) and *E. tanhoensis* (stars), based on herbarium materials.

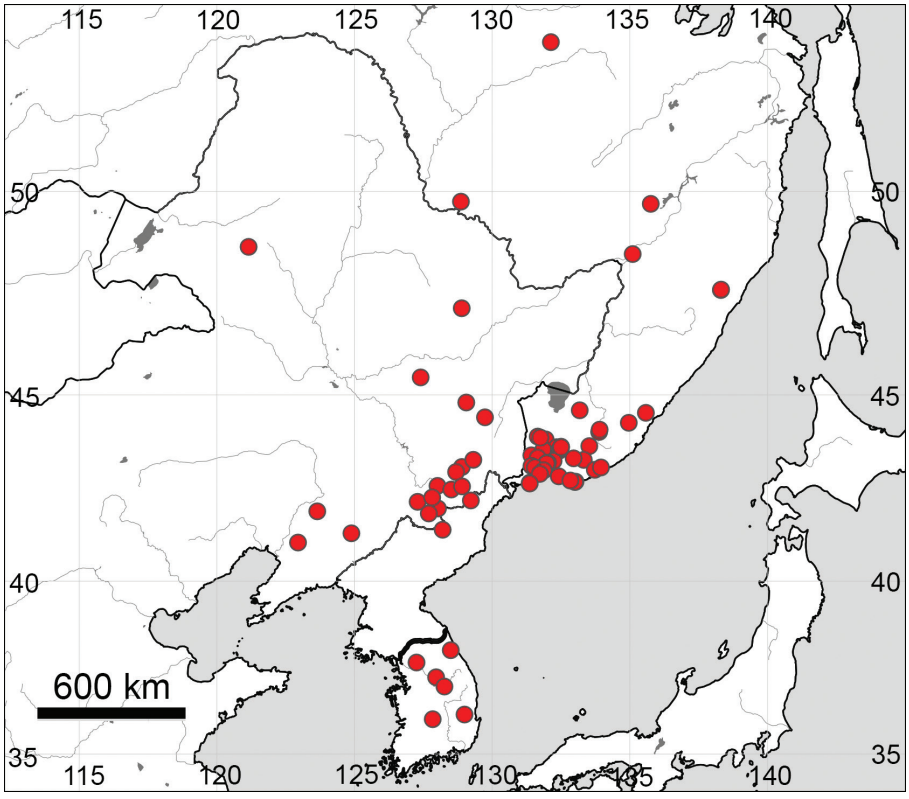

Figure 11. General distribution of *Eranthis stellata*, based on herbarium materials and data in the literature (Oh and Oh 2019; Park et al. 2019).

51°26'21.06"N, 104°41'09.82"E, 471 m a.s.l., 02 May 2019, *A.S. Erst*, *D.A. Krivenko & O.A. Chernysheva s.n.* (NS-0000957!); Kabansky district, Tolbazikha river, 51°26'21.06"N, 104°41'09.82"E, 471 m a.s.l., 20 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva s.n.* (NS-0000958!); Irkutsk Province: Slyudyansky district, Semirechka river, 51°28'56.92"N, 104°19'43.47"E, 470 m a.s.l., 02 May 2019, *A.S. Erst*, *D.A. Krivenko & O.A. Chernysheva 048* (NS-0000959!); Slyudyansky district, Semirechka river, 51°28'56.92"N, 104°19'43.47"E, 470 m a.s.l., 21 Jun 2019, *A.S. Erst*, *D.A. Krivenko*, *E.Yu. Mitrenina & O.A. Chernysheva s.n.* (NS-0000960!).

Preliminary conservation status. Although the species seems to have a small distribution area in southern Baikal Lake, the populations observed in 2018 and 2019 consisted of numerous individuals producing viable fruits and no threats to the habitats were observed in the field studies. The EOO of *E. tanhoensis* was estimated for an area of more than 1372 km^2, while the AOO was 72 km^2. Preliminary conservation status, according to IUCN's Extent of Occurrence criteria indicates the species as Endangered (EN) (IUCN 2019).

Key to the *Eranthis* species growing in Asiatic Russia

1 Maximum dissection of basal leaf segments ~0.4 cm long at flowering stage, 1.3 cm long at fruiting stage; scape with stellate hairs; involucral leaves green or coppery at flowering; maximum dissection of the involucral leaves 1.7 cm long at fruiting; flowers recurved; petals narrowly funnelform, swellings (nectaries) located in medium part of adaxial lip lobes, apex of abaxial and adaxial lips white; anthers violet, pink or white ..... ***E. stellata***

– Maximum dissection of basal leaf segments at least 1.0 cm long at flowering, 2.3 cm long at fruiting stage; scape without stellate hairs; involucral leaves green at flowering; maximum dissection of the involucral leaves 2.1 cm long or more at fruiting; flowers erect, petals urn-shaped, swellings (nectaries) located at the apex of adaxial lip lobes, apex of abaxial and adaxial lips yellow; anthers white **2**

2 Apex of basal and involucral leaves rounded; maximum dissection of basal leaf segments 1.0 cm long at flowering and 2.3 cm long at fruiting; segments of involucral leaves at fruiting 0.4–1.2 cm wide; maximum dissection of the involucral leaves at fruiting 2.1 cm long; each segment of involucral leaves with 1–5 teeth; scape glabrous or papillate; petals narrowly urn-shaped, margins between abaxial and adaxial lips white ..... ***E. sibirica***

– Apex of basal and involucral leaves acute; maximum dissection of basal leaf segments 2.3 cm long at flowering and 3.5 cm long at fruiting; segments of involucral leaves at fruiting 1.4–5.3 cm wide; maximum dissection of the involucral leaves at fruiting 4.0 cm long; each segment of involucral leaves with 5–21 teeth; scape papillate and with large hemispherical glands; petals broadly urn-shaped, margins between abaxial and adaxial lips yellow..... ***E. tanhoensis***

Acknowledgements

We thank Mark Newman, Marco Pellegrini, Andriy Novikoff, Colin Pendry, Christoph Dobeš and Johannes Walter for discussion of some parts of the manuscript and valuable comments, the staff of the herbaria visited, as well as Valentin Yakubov for the images of *Eranthis stellata* and Roman Annenkov for preparing Fig. 3. We are indebted to Natalya Pridak for all the black and white drawings. The samples of *E. longistipitata* were kindly provided by Evgeny Boltenkov. The research was supported by the Russian Foundation for Basic Research, grant 18-34-20056 mol_a_ved. The work of Alexander Sukhorukov and Maria Kushunina was also supported by a Moscow State University (MSU) Grant for Leading Scientific Schools "Depository of the Living Systems" in the framework of the MSU Development Programme.

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Supplementary material 1

Tables S1–S4

Authors: Andrey S. Erst, Alexander P. Sukhorukov, Elizaveta Yu. Mitrenina, Mikhail V. Skaptsov, Vera A. Kostikova, Olga A. Chernisheva, Victoria Troshkina, Maria Kushunina, Denis A. Krivenko, Hiroshi Ikeda, Kunli Xiang, Wei Wang

Data type: measurement.

Explanation note: **Table S1.** List of samples characters used in molecular (M), cytogenetical (C) and biochemical (B) analyses. **Table S2.** Morphological characters of Russian *Eranthis* species. An asterisk indicates characters used in the numerical analysis. **Table S3.** The results of the variance analysis for plant characters in the flowering stage. The values in parentheses are adjusted P-values; the characters in bold are those without significant interspecific differences. **Table S4.** The results of the variance analysis for plant characters in the fruiting stage. The values in parentheses are adjusted P-values; the characters in bold are those without significant interspecific differences.

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RESEARCH ARTICLE

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A new species of *Phoebe* (Lauraceae) from south-western China

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Abstract

Here *Phoebe hekouensis* Bing Liu, W.Y. Jin, L.N. Zhao & Y. Yang from south-eastern Yunnan Province of China is described as new to science. This species is morphologically similar to *P. megacalyx* H.W. Li in the twigs being robust and brownish tomentose, the ovary densely pubescent and the tepals longer than 1 cm, but differs from the latter species by the leaves being broader, up to 18 cm (vs. 4.5–11.5 cm), the inflorescences shorter, ca. 10–15 cm long (vs. up to 23 cm), the ovary completely and densely pubescent (vs. pubescent only at the apical portion) and the stigma conspicuous (vs. inconspicuous). The new species also resembles *P. macrocarpa* C.Y. Wu, but differs from the latter by the tepals being longer, 9–13 mm long (vs. ca. 4 mm).

Keywords

China, Lauraceae, *Phoebe*, taxonomy, Yunnan

Introduction

The genus *Phoebe* Nees of the Lauraceae contains about 100 currently recognised species and is widely distributed in tropical and subtropical Asia (van der Werff 2001, Wei and van der Werff 2009). Members of this genus are usually trees, with pinnately-veined leaves usually obovate to oblanceolate and slightly clustered at the tips of branches and trimerous bisexual flowers with nine 4-loculed fertile stamens and persistent tepals clasping the

* These authors contributed equally to the work.

base of the fruit. Recent molecular systematic studies have suggested that this genus appears to be monophyletic (Rohwer et al. 2009, Li et al. 2011a, 2011b, Song et al. 2017).

Traditionally, the genus *Phoebe* is classified into two sections, based on the pubescence of the tepals and inflorescences: sect. *Phoebe* possessing glabrous or appressed puberulent tepals and inflorescences and sect. *Caniflorae* Meisn. having densely pubescent/tomentose tepals and inflorescences (Lee and We 1982). This classification, however, is not supported by molecular phylogenetic studies, sect. *Caniflorae* being paraphyletic because a few species of this section actually fall within the clade of sect. *Phoebe* to which the type species belongs (Li et al. 2011a, 2011b, Song et al. 2017). The genus is in need of reclassification based on further molecular study involving more extensive sampling and examination of morphological characters.

A few species of the sect. *Caniflorae* do comprise a robust clade, for example, *P. macrocarpa* C.Y. Wu, *P. megacalyx* H.W. Li, *P. glaucophylla* H.W. Li and *P. hungmaoensis* S. Lee (Song et al. 2017). These species usually have robust twigs with dense indumentum, long leaves up to 40 cm (except for *P. hungmaoensis* that has shorter leaves ca. 10–15 cm), paniculate inflorescences possessing a long peduncle and branched only in the distal portion, pubescent ovaries and large fruits. We collected a specimen of *Phoebe* in south-eastern Yunnan Province, China, in April 2014. Further phylogenetic studies based on nuclear ITS and chloroplast *psb*A-*trn*H suggests that this plant belongs to the clade of *P. megacalyx* and *P. macrocarpa* (Jin 2017), but it clearly differs from all known species of this clade. As a result, we here describe this species as new to science. For identification purposes, we also provide a key to all known members of this particular clade.

Materials and methods

We conducted field investigations during 2010 and 2014. Photographs were taken in the field. Morphological observations and measurements of the new species were made, based on both living plants and dry specimens.

Taxonomy

Phoebe hekouensis **Bing Liu, W.Y. Jin, L.N. Zhao & Y. Yang, sp. nov.**
urn:lsid:ipni.org:names:77206950-1
Figs 1, 2

Type. China. Yunnan: Hekou Yao Minority Autonomous County, Nanxi Town, Hua-Yu-Dong, alt. ca. 140 m elev., 5 Apr 2014, *Bing Liu, Y. Yang, Q.W. Lin, L. Jiang & X.J. Li 1988* (Holotype: PE; Isotypes: PE).

Diagnosis. This new species resembles *P. megacalyx* in having tomentose twigs and large tepals, but differs from the latter species by broader leaves (12–18 cm vs. 4.5–11.5 cm), shorter inflorescences (10–15 cm vs. up to 23 cm), densely pubescent ovary

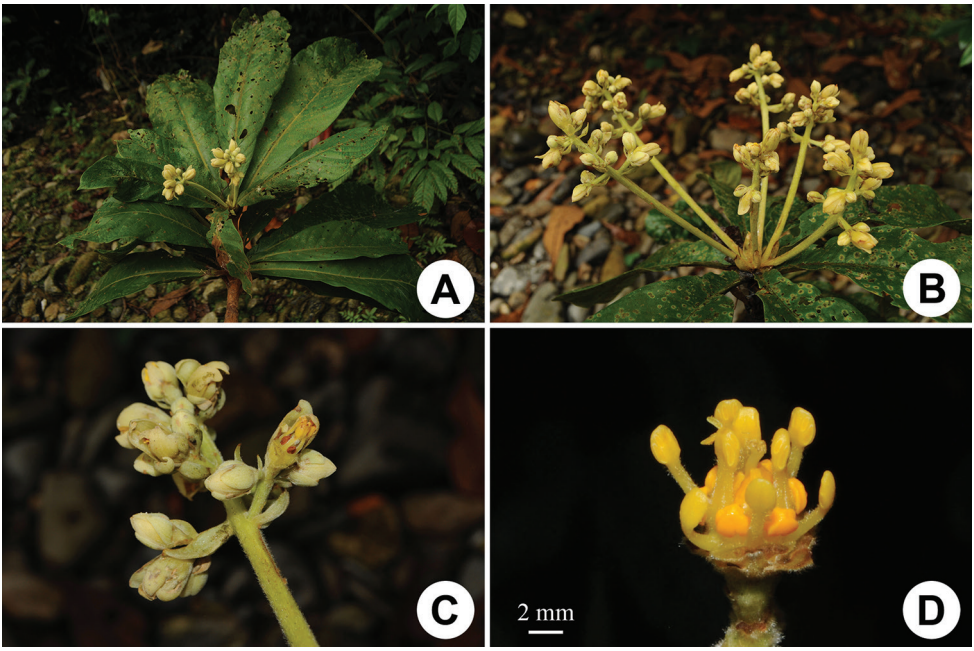

Figure 1. Morphology of *Phoebe hekouensis*. **A** Flowering branch, showing oblanceolate leaves and terminal inflorescences **B, C** inflorescences, these being panicles with long peduncles and branched only in the distal portion **D** flower with tepals removed, showing stamens, glands and staminodes.

(vs. only pubescent at the apical portion) and the enlarged stigma (vs. inconspicuous); also similar to *P. macrocarpa* in that the twigs of both being robust and tomentose, but distinguished by the longer tepals ca. 9–13 mm (vs. ca. 4 mm).

Description. Trees, ca. 12 m tall, bark greyish-brown. Branchlets robust, ca. 9 mm in diam., ridged, densely brown tomentose, possessing prominent dispersed leaf scars and clustered bud scale scars. Leaves alternate, usually clustered to somewhat verticillate at the apex of branches, coriaceous, oblanceolate, 25–45 × 12–18 cm, apex acuminate, base acute, upper surface glabrous, midrib impressed on the upper surface, principal lateral vein 18–30 pairs, immersed in the upper surface, both the midvein and the lateral veins prominently elevated on the lower surface, yellowish pubescent; petioles 1–2 cm long, brown tomentose. Inflorescences paniculate, 2–6 clustered at the apex of branches in between the clustered leaves; panicles robust, many-flowered at the apex, 10–15 cm long, densely yellow-brown tomentose, not branched in the lower half, usually few-branched in the distal portion and the flowers appearing to be clustered at the apex; peduncles 7.5–8.5 cm long, more than 2/3 of the total length, tomentose; bracts 2 cm long, tomentose. Flowers yellowish-white; subsessile. Bracts linear, brownish tomentose. Tepals in two whorls, subequal in length, elliptic, tepals of the outer whorl 9–13 mm long, ca. 6 mm broad, those of the inner whorl linear and narrower, ca. 4 mm broad, brownish tomentose on both sides. Fertile stamens 9, 4-loculed, the four locules arranged in trapezoid pattern; filaments 4–6 mm long,

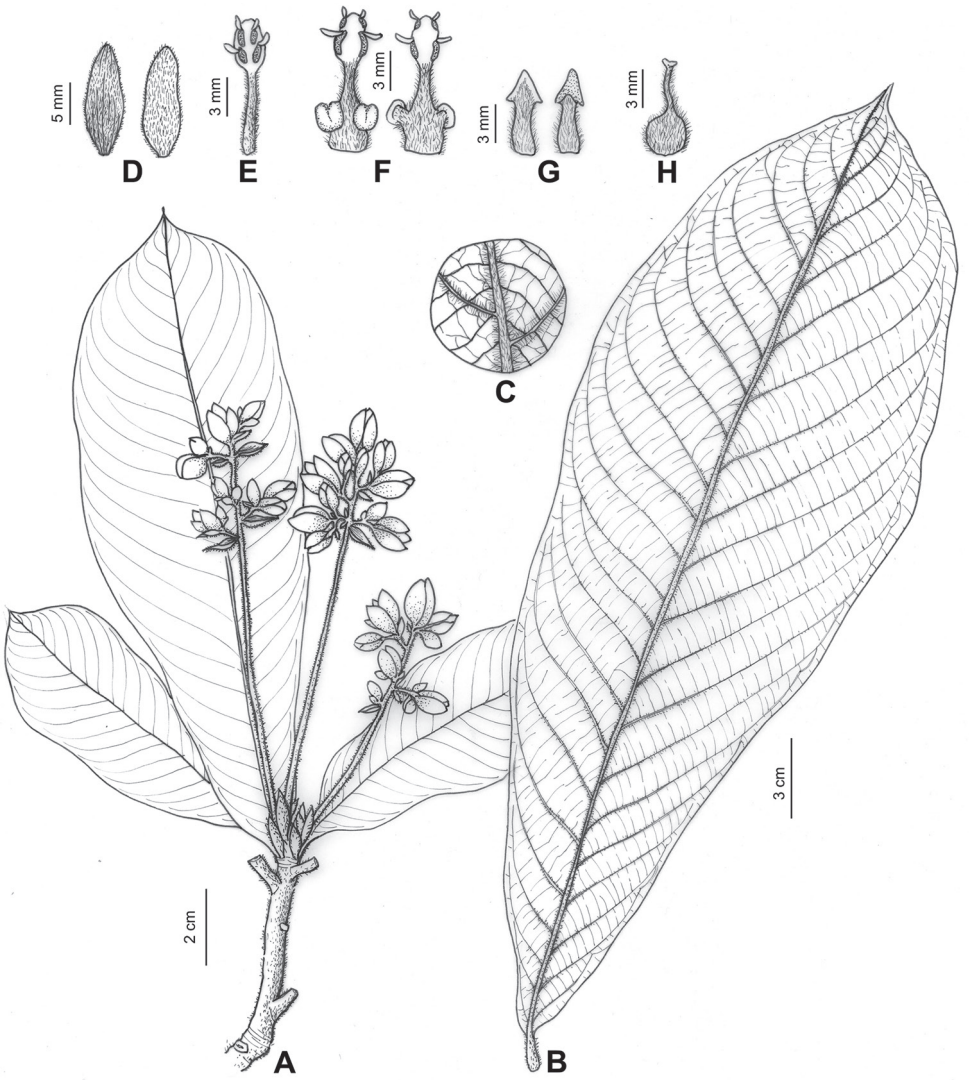

Figure 2. Illustration of *Phoebe hekouensis* to show morphological details. **A** Flowering branch **B** leaf, showing the oblanceolate shape and the ascending principal lateral veins **C** leaf portion magnified to show veinlet reticulations **D** tepals, depicting shape and pubescence of adaxial and abaxial side **E** fertile stamen of the first and second whorls **F** fertile stamens of the third whorl **G** staminodes, note sagittate head and pubescent stalk **H** pistil, showing pubescence.

brownish pubescent, each filament of the third whorl bearing two yellow glands at the base; glands ovoid, stalked, stalks pubescent. Staminodes sagittate, ca. 4–6 mm long, possessing pubescent stalks. Ovary obovoid, brown tomentose; style straight and thread-like, ca. 3 mm long, pubescent, glabrescent toward distal end; stigma enlarged, disc-shaped. Flowers collected in April. Fruit not seen.

Distribution. China. Yunnan, Hekou Yao Minority Autonomous County (Fig. 3).

Habitat. In limestone ravines, near water.

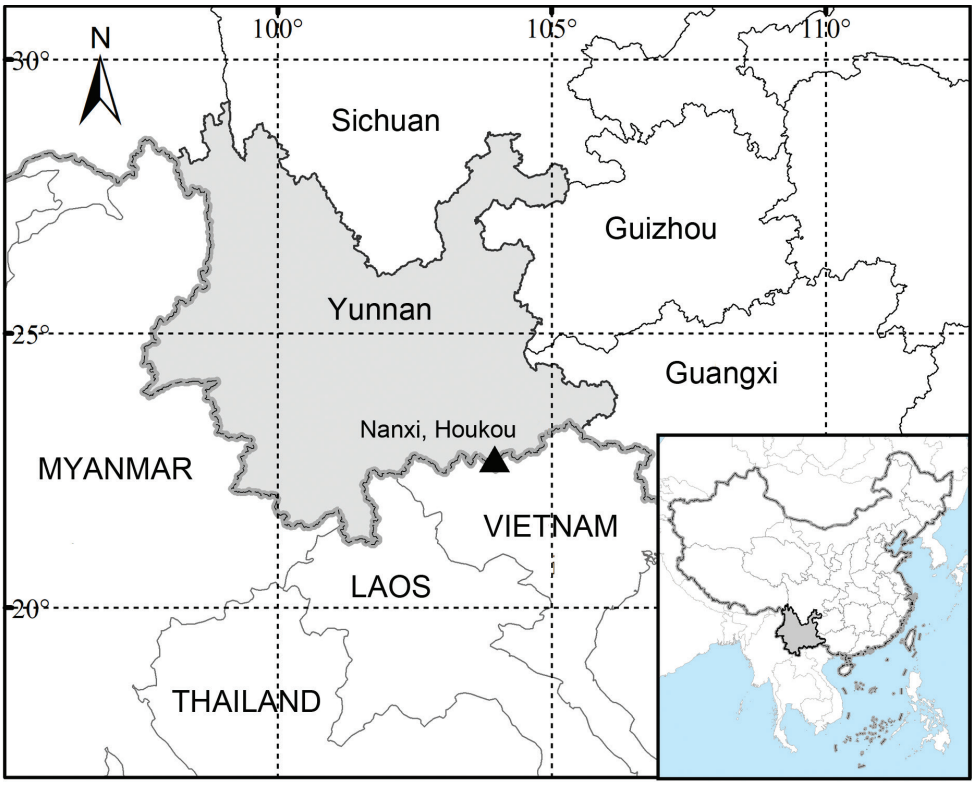

Figure 3. Distribution map showing the only known locality of *Phoebe hekouensis* (▲).

Etymology. The epithet "*hekouensis*" is after the type locality Hekou Yao Minority Autonomous County of Yunnan Province, south-western China.

Preliminary conservation status. We have conducted field investigations in south-eastern Yunnan Province of China for ten years, but have found only one tree at the type locality, and no fruiting specimens were observed. It is uncertain if the species is endemic to China or is also distributed in adjoining Vietnam due to lack of field investigations in Vietnam. Based on IUCN Red List Categories and Criteria (IUCN 2012), we considered the new species as Critically Endangered (CR) in China. To conserve the species, we propose to take actions on reproduction of the tree in botanical gardens in the future.

Key to the closely related species of *Phoebe* in the clade to which *P. hekouensis* belongs

1 Tepals usually 10 mm or longer **2**
– Tepals shorter, ca. 4 mm **3**
2 Leaves relatively narrow, 4.5–11.5 cm wide; inflorescences up to 23 cm long; stigma inconspicuous, punctiform ..... ***P. megacalyx***
– Leaves relatively broad, 12–18 cm wide; inflorescences 10–15 cm long; stigma conspicuous, disc-like..... ***P. hekouensis***

3 Fruits 3–4 cm long ..... ***P. macrocarpa***
– Fruits 1–2 cm long **4**
4 Twigs usually glabrous; fruits ca. 1.8 cm long ..... ***P. glaucophylla***
– Twigs stout, pubescent; fruits shorter than 1.5 cm **5**
5 Leaves relatively large, 12–23×5–9 cm long; pubescence brownish; fruits ovoid ..... ***P. puwenensis* W.C. Cheng**
– Leaves relatively small, 10–15×2–4.5 cm long; pubescence rusty; fruits ellipsoid..... ***P. hungmaoensis***

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We thank Mr. X.J. Guo for his kind help during fieldwork, and Mr. Y.F. Li for the line-drawing. We are also grateful to Dr. L. Li of Xishuangbanna Tropical Botanical Garden and two anonymous reviewers for their valuable suggestions.

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RESEARCH ARTICLE

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Pinguicula rosmarieae Casper, Bussmann & T.Henning (Lentibulariaceae), a new butterwort from the Amotape-Huancabamba Zone (northern Peru)

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Abstract

The insectivorous genus *Pinguicula* occurs along the whole Andean mountain chain from Colombia-Venezuela in the north to Tierra del Fuego in the south with a short interruption in the Peruvian-Chilean desert range. This paper describes a new and striking species of *Pinguicula* that occurs in the south-eastern part of the Amotape-Huancabamba Zone in north Peru. It grows either as a lithophyte on moist rocks or as an epiphyte on *Polylepis multijuga* Pilg. in the wet highlands of the Cordillera Central. *Pinguicula rosmarieae* Casper, Bussmann & T.Henning, **sp. nov.** is clearly distinguished by a basal rosette of ovate-obovate leaves spread out flat on the ground and especially by a two-partite corolla with a straight uniform tube-spur complex, two features unknown from other Andean *Pinguicula* species. The morphological similarity to *P. calyptrata* Kunth is discussed and the habitat and distribution of *P. rosmarieae* are characterised.

Resumen

El género insectívoro *Pinguicula* se encuentra a lo largo Andes desde Colombia y Venezuela en el norte hasta Tierra Fuego en el sur, con una breve interrupción en el los desiertos peruano-chilenos. Este artículo describe una nueva y distintiva especie de *Pinguicula* que se encuentra en la parte sur de la zona Amotape-Huancabamba en el norte del Perú. Puede crecer tanto como litófita sobre rocas húmedas o como epífita sobre *Polylepis multijuga* Pilg. en las tierras altas y húmedas de la Cordillera Central. *Pinguicula rosmarieae* Casper, Bussmann & T.Henning, **sp. nov.** se distingue claramente por tener una roseta basal de hojas ovadas-obovadas, postradas sobre el suelo y, especialmente, por la corola bipartida con un espolón uniforme recto, una combinación de características desconocidas de otras especies andinas de *Pinguicula*. Se discute la similitud morfológica con *P. calyptrata* Kunth y se caracterizan el hábitat y la distribución de *P. rosmarieae.*

Keywords
Lentibulariaceae, *Pinguicula*, *Pinguicula rosmarieae*, Peru, Amotape-Huancabamba Zone, Cordillera Central, endemic, taxonomy, new species, distribution

Introduction

In South America, the insectivorous genus *Pinguicula* (Lentibulariaceae) is represented by seven at first sight ± closely related, although not well-known taxa: *P. antarctica* Vahl, *P. calyptrata* Kunth, *P. elongata* Benj., *P. involuta* Ruiz & Pav., *P. jarmilae* Halda & Malina, *P. nahuelbutensis* Gluch and the here newly described *P. rosmarieae* Casper, Bussmann & T.Henning. Forming a nearly continuous chain of ± vicarious species, the taxa occur over a distance of 8,500 km in the Andes from the north (Colombia, Santa Marta) via Venezuela, Ecuador, Peru, Bolivia and Chile to the extreme south (Chile, Argentina: Tierra del Fuego), with a gap between ~30°S and ~36°S in the Peruvian-Chilean arid region. This "Atacama"-gap has been bypassed (perhaps in former cooler and wetter periods). In northern Peru (~ 05°30'–06°30'S), the 'páramo-butterwort' *P. calyptrata* is more or less replaced by the litho-/epiphyte *P. rosmarieae* and, further to the south, the 'Jalca-Puna'-butterworts *P. involuta* and *P. jarmilae* close the gap.

Collection history

In 2017, searching for material of *P. involuta* during his study of the South American *Pinguicula* taxa, the first author came across the Catalogue of the Flowering Plants and Gymnosperms of Peru (Peru Checklist) (https://www.tropicos.org/Project/PEC). At first sight, he believed that the specimen MO 6607881 (Paniagua Zambrana, Bussmann & Vega Ocaña 8586), designated as *Pinguicula*, could represent a new species, but more information, especially photos and, ideally, additional material was needed to confirm this initial assumption. The plants were gathered in November 2012 in the eastern Andes of north Peru in the area surrounding the Laguna Huayabamba ("Huayllabamba") and described as growing on a vertical rock wall in the spray of a small waterfall. The collectors located the sampled population in the Department La Libertad, but, instead, they were already in the adjacent Department San Martín.

Further investigations drew the attention to another, earlier collection (May 2001: T. Henning and C. Schneider 275) deposited in the Berlin herbarium (B) at the BGBM. They collected a violet-white flowering *Pinguicula* growing in large stands on moist rock surfaces in the cliffs forming the southern limit of the Laguna de los Cóndores (~06°51'S, ~77°42'W), ca. 20 km east of Leymebamba (B 100136109, B 100136110; duplicate specimens in HUT). The original label indicates "Departamento Amazonas, Province Chachapoyas". However, the Laguna and the adjacent area are part of the Department San Martín and situated some 4–5 km east of the border to the Department Amazonas. These specimens enabled the first author to conduct

morphological comparisons with the material at MO for a thorough investigation. The collections showed the same taxon from a different population some 15 km further north in the same mountain range. The morphological differences to the other Peruvian *Pinguicula* species proved to be stable, at least in these two populations sampled independently and, together with the reported peculiar habitat preferences, enough to justify the description of a new species.

Finally, just very recently (2019), a third population has been reported and documented photographically by Lázaro Santa Cruz Cervera (USM) from the province of Bongará in the Department Amazonas, some 100 km further north.

Taxonomic background

During the process of describing the new species, the most important Peruvian herbaria in Lima (USM) and Trujillo (HUT) have been contacted in order to locate the duplicates and gather information about potential additional collections and records. After a review of all herbarium material that we could get access to, it became apparent that most of the *Pinguicula*-collections from north Peru have been misidentified as *Pinguicula involuta*. Instead, all collections made north of the Department Huánuco in central Peru belong to *P. calyptrata*, a species traditionally referred to as a Colombian-Ecuadorean taxon. This common misconception is due to the inadequate original description and the therein indicated distribution of these two Andean butterworts. Whereas *P. calyptrata* was collected by Humboldt and Bonpland (Humboldt et al. 1817) in Saraguro in the southern Ecuadorean province Loja, *P. involuta* was described by Ruiz and Pavon (1798) from Huánuco in central Peru and especially the latter is insufficiently characterised in the protologue (Casper and Hellwig 2019).

However, the distributional patterns revealed from the two previously described and the undescribed species draw a much clearer picture corresponding to the phytogeographic characteristics of the so-called Amotape-Huancabamba Zone (hereinafter: AHZ) (Weigend 2002). Instead of the (simplified) subdivision into a northern (*P. calyptrata*) and southern (*P. involuta*) taxon whose limits roughly correspond to the border between Peru and Ecuador, *Pinguicula* is present in the AHZ with two taxa, one occurring in the west stretching northwards (*P. calyptrata*) and one endemic to the eastern slope of the Peruvian Andes (*P. rosmarieae*). South of the AHZ, they are replaced by the widespread *P. involuta* whose distribution extends into Bolivia. The new taxon, *Pinguicula rosmarieae* Casper, Bussmann & T.Henning spec. nov. is here described as new to science. The morphology of the new species and its affinities to related taxa are illustrated and discussed. The coarse biogeographical patterns observed for the Peruvian species are outlined and explained in the context of the characteristics of the AHZ. A distribution map, based on collection data from revised herbarium material and a key to the Peruvian *Pinguicula*-species, is provided to enable a reliable determination of existing and future collections which is a crucial component of floristic studies as basis for urgent conservation efforts.

The present study is the first of a series of contributions at different stages of completion (Casper; Casper et al. in prep.), each dealing with a certain taxonomic or nomenclatural problem that became apparent in anticipation of a comprehensive synopsis of the South-American *Pinguiculas* that is in preparation and will be published soon. Therein, all questions regarding the historical biogeography as well as nomenclatural and taxonomic issues will be discussed extensively.

Materials and methods

The first author examined herbarium specimens of *Pinguicula* L. from South America in preparation for a revision of the Andean *Pinguiculas* at HUT, USM, MO, B and M (Thiers 2019) and from specimen scans using online databases (www.tropicos.org). This study combines the results of the herbarium studies, the experiences of the collectors (R.W. Bussmann and T. Henning) in the natural habitat and observations and reports kindly received from the Peruvian colleagues.

Results

Key to the Peruvian species of *Pinguicula*

1 Foliage not star-like, leaf blades oblong-obovate-ovate, with margins slightly (mostly ~2 mm) curled up; corolla bi-partite, i.e. divided in the lip and the straight, more or less uniform tube-spur complex (the funnel-shaped tube merges into the conical blunt spur, with little to no angle); living on water-rinsed sandstone rocks or as epiphyte on *Polylepis* twigs; cloud forest (eastern North-Peru) ..... ***P. rosmarieae***

– Foliage star-like ("stellate"), leaf blades ovate, with margins distinctly curled up (appearing boat-shaped); corolla tri-partite, i.e. divided distinctly in lip, tube and spur, i.e. the tube distinctly angled with the spur..... **2**

2 Corolla with nearly equal-sized notched lobes (subisolobate, i.e. lobes of the upper-lip only slightly smaller than those of the lower lip); ~2 mm behind the lower lip middle lobe base, a prominent clapper-like palate covered by yellow hairs inserted; the tube typical funnel-shaped; the spur short, conical, blunt; páramo–jalca (North-Peru) ..... ***P. calyptrata***

– Corolla with distinctly unequal-sized notched lobes, (i.e. lobes of the upper-lip lobes distinctly smaller than those of the lower-lip); the lower-lip middle lobe dominating the lip, often distinctly bent down; no distinct palate inserted; the tube cylindrical, nearly as long as wide; the spur slender, sickle-shaped, pointed; puna (Central- and South-Peru) ..... ***P. involuta***

Taxonomic treatment

***Pinguicula rosmarieae* Casper, Bussmann & T.Henning, sp. nov.**
urn:lsid:ipni.org:names:77206951-1
Figs 1 A–H, 3A

Type. Peru: Dept. San Martín, Prov. Huallaga ["Dept. Amazonas, Prov. Chachapoyas" (sic!)], Lagunas de los Cóndores (06°50'40.5"S, 77°41'52.2"W), 3,000 m a.s.l. Violet-white flowers, diameter up to 100 mm. Lithophyte, 24 May 2001, T. Henning & C. Schneider 275. (***Holotype:*** HUT 41126!: det. Pinguicula spec. – Figs 1H, 3A; ***Isotypes:*** B: B 100136110 (Fig. 2A)! B 100136109!)

Diagnosis. Herba perennis rosulata, rosula ca. 100 mm in diametro; lamina foliorum circuito suborbiculata vel obovata, margine vix (~3 mm) involuta, ca. 30–40 mm longa ac lata, solum plusminusve adpressa; scapus 1(–4), erectus, 20–40 mm altus, teres; flores 1, parvi, ~8–10 mm longi (tubo-calcari incluso), bilabiati; calyx lobis oblongis, lobis superis usque ad basin fere divisis, lobis inferis usque ad dimidium divisis, ad angulum ~45° divaricatis; corolla lobis 5, oblongis, ~5–7 mm longis, subisolobatis, apice valde emarginatis; corollae tubum infundibuliformi-cylindraceum cum calcari conico ± uniformem rectum coniunctionem formans, 5–6 mm longum apice obtusum.

Habitatio in locis apertis et humidis montium Andinensium regionis Peruviae septentrionalis usque ad 3.100 m supra mare, praesertim ad rupes et saxa. Habitu Pinguiculae Andinensium simili, praecipue differt tubo-calcari-coniunctio recto uniformi.

Description. Perennial rosette leaved herb with 1 (–4) flowered scapes. *Rhizome* ~ 10 mm long, with numerous adventitious fibrous roots. *Leaves* (4–) 6–10, flat on the ground, ± succulent (dried translucent-membranous), adult (20–) 30–40 (–50) mm long, nearly as long as wide, the blades ovate-obovate-oblong in outline, rounded at the tip attenuated to the base into a short petiole, the margins weakly (up to 3 mm) curled up, yellowish-green, upper surface of lamina covered with sessile glandular hairs. *Hibernacula* (winter buds, dormant buds) absent. *Scapes* 1–2 (–4), erect, (20–) 30 (–40) mm tall, terete, filiform (0.5–1 mm thick), one–flowered, green to reddish-brown, scattered with glandular hairs, often becoming glabrous or nearly so. *Flowers* small, ~8–10 (–11) mm long (including tube-spur-complex). *Calyx* two-lipped, green to pale brown to purple, upper surface of sepals scattered with stalked glandular hairs; upper lip divided deeply into three nearly equal-sized oblong lobes, at apex pointed; lower lip up to ½ divided into two oblong lobes, at apex pointed. *Corolla* two-lipped, bluish-magenta to white-violet; upper lip two-lobed, lobes obovate, ~5–6 mm long and ~2–3.5 mm wide, shallowly notched at the apex; lower lip larger and longer than the upper lip, with three oblong to obovate-oblong lobes (the median lobe somewhat larger than the two lateral ones), 4.5–5 mm wide, each distinctly (~1/5 to 1/3 of its length) notched. *Tube* (*tube-spur-complex*) at the throat funnel-shaped, on both sides broader than the *spur*, on the back side higher than the spur, proximally cylindrical

Figure 1. *Pinguicula rosmarieae* (**A–H**) *and P. calyptrata* (**I, J**).

(nearly as long as wide), on the ventral side merging without any sharp angle into the cylindrical to cone-like stubby, at apex rounded, yellow-greenish spur; tube and spur forming a more or less uniform funnel- to cone-like straight, from the ventral side appearing as a box-like '*tube-spur-complex*', ~6 mm long; the tube-spur-complex externally dark blue to purple lengthwise-striped by parallel veins. *Palate* bipartite, weakly developed (not clapper-like), inserted immediately behind (~1–2 mm) the corollas´ lower-lip middle lobe, yellow, set with short-stalked glandular hairs, proximally elongated into a short ventral hair strip; each of the two lateral corolla lobes with a small

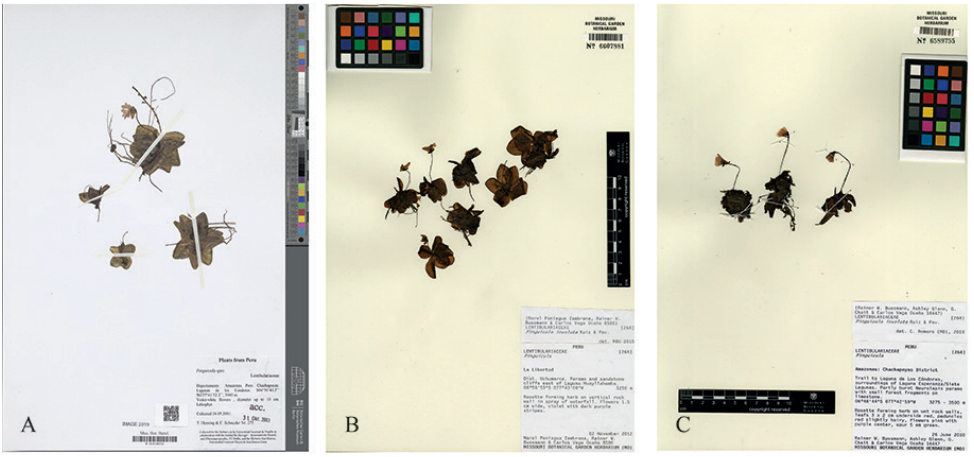

Figure 2. Specimen scans of *Pinguicula rosmarieae* and *P. calyptrata*. **A** Isotype of *P. rosmarieae* – Scan (Henning & Schneider 275, B100136110), with permission **B** paratype, from vertical sandstone cliff east of the Laguna Huayllabamba, 3,250 m a.s.l., the three flowers (left) showing corollas with their significant straight uniform tube-spur-complex. The rosette is composed of flat outspread leaves. – Scan of MO 6607881, with permission **C** *P. calyptrata*, the leaves have curled up margins and the corollas are typical tri-partite in corolla, tube and spur. The corolla lobes are nearly equal-sized, the tube funnel-shaped and the spur short and angled with the tube. – Scan MO 6589755; with permission.

Figure 1. Continued. **A** *P. rosmarieae*, 2-scaped; upper flower opened, in profile view, lower one in bud; epiphytic on *Polylepis multijuga* Pilg., in *Polylepis multijuga-Iochroma stenanthum* S.Leiva, Quip. & N.W.Sawyer – dominated cloud forest. Peru, Department San Martín, close to 'El Jardín'(Inca-hut and surrounding area east of the Laguna Huayabamba), 3,090 m a.s.l., 06°56'044"S, 077°41'54"W **B** ditto, flower, profile view **C** ditto, flower, semi-ventral view **D** ditto, flower, dorsal view **E** *P. rosmarieae* rosette from the northernmost known habitat, "Hatumpampa" Department Amazonas, Province Bongará (no voucher specimen) **F** ditto, flower, semi-ventral view **G** ditto, flower ventral view **H** corolla in frontal view, lower-lip lobes to ¼ of its length notched, throat without distinct palate. Peru, Department San Martín, Laguna de los Cóndores (Henning & Schneider 275) **I** *P. calyptrata*, corolla in frontal view, lobes with lateral margins slightly covering each other, lower-lip lobes to 1/6 of its length notched, throat with clapper-like yellow palate. Peru, Department San Martín, Sphagnum-bog, 3,000 m a.s.l. above the Laguna de los Cóndores (Bussmann, A. Glenn, G. Chait & C. Vega Ocaña 16447) **J** flowering stand of *P. calyptrata* near Pulan, Cajamarca. (Credits: photographs **A–D, I** R. W. Bussmann **E–G, J** L. Santa Cruz Cervera **H** T. Henning).

yellow hair bubble at their base, stretching proximally along on each side of the inner tube wall. *Indumentum* (apart from that visible in the photographs), *stamens, pollen grains*, *ovary, stigma, capsule, seeds* not seen. *Chromosome number* unknown.

Pinguicula rosmarieae is distinguished by its notable uniform funnel-cone-shaped straight tube-spur-complex, a feature unknown in any other Andean *Pinguicula* taxon. It is an endemic species, restricted to the eastern slopes of the Cordillera Central within the Amotape-Huancabamba-Zone of northern Peru (Departments Amazonas and San Martín; Fig. 4). It grows in rocky habitats, either under moving water or in the spray of small waterfalls and occasionally epiphytic on moss-covered twigs of *Polylepis multijuga*, at altitudes of about 2500 m–3100 m a.s.l. (Fig. 3).

Etymology. The new species is named after Dipl.-Biol. Rosmarie Casper, beloved wife and steady companion of the scientific efforts of S. J. Casper and mother of their children.

Discussion

Affinities

Based on current floristic literature, *Pinguicula involuta* was considered as the only butterwort native in northern Peru. However, a revision of the available herbarium material in this study has revealed that this simple assumption, according to the historical taxonomic treatments, *P. involuta* is native to Peru whilst *P. calyptrata* is native to Ecuador, is largely incorrect. Furthermore, the "northern" *Pinguicula* of Humboldt and Bonpland (i.e. *P. calyptrata*), that was first discovered in the Saraguro range in southern Ecuador, has repeatedly been collected in northern Peru. We have seen specimens of *P. calyptrata* from the Peruvian Departments Piura, Amazonas, San Martín, Lambayeque and Cajamarca (Fig. 4, cf. the list of specimens below). In turn, *P. involuta*, so far, has never been collected in these Departments, although misidentified collections and erroneous floristic literature suggest that. Based on our observations and fieldwork, the northernmost locality of *P. involuta* is in the Department Huánuco in central Peru, some 350 km further south of the southernmost *P. calyptrata* localities (Province Pachitea, Panao; road from Chaglia [Chaglla] to Rumichaca [Tambo de Vaca], km 81, 09°51'S, 75°53'W), leg. M. Weigend, K. Weigend, T. Henning, & Ch. Schneider 5426, Fig. 4). At present, the exact distribution limits of *P. involuta* and *P. calyptrata* remain uncertain.

Morphological data indicate that the new taxon is distinct from *P. involuta* and *P. calyptrata*. A thorough study of South American *Pinguicula* is in preparation and will elucidate the morphological affinities amongst all relevant taxa.

Foliage: The leaf blades of *P. rosmarieae* are ovate-obovate-oblong, dried membranous-translucent (Fig. 2A) and spread out, i.e. they are not boat-like and they lack heavily curled-up margins (Fig. 1A, E): The whole rosette (up to 100 mm across) is not star-shaped compared to *P. calyptrata* (Figs 1J, 2C) and *P. involuta* (field name of the latter in the original collection was *P. stellata*, see Casper and Hellwig 2019). The foliage shape of *P. rosmarieae* is similar to the geographically-distant *P. albida* Wright that occurs in Cuba and

may represent an individual adaptation to the rocky, exposed habitats. Both habitats share nutrient-poor and often acidic white-sand-soils (for Peru: M. Weigend, pers. comm.). However, this observation is contradicted by the fact that the rosettes of *P. calyptrata* and *P. involuta* retain their star-like appearance on relatively open stands. Shape and posture of the foliage are, therefore, taxonomically meaningful for species delimitation.

Corolla: The violet-white to pale-bluish corolla is similar to that of most Andean *Pinguicula*: its lips are nearly equal-lobed (subisolobate). The two lobes of the upper-lip are (mostly only shallowly) notched at the distal margins (Fig. 1B–D, F–H), the three lobes of the lower-lip are longer and at its distal margins deeply notched (up to ¼ –1/3 of its length; Fig. 1G–H). The most striking feature that separates *P. rosmarieae* from other Andean *Pinguicula* taxa is the more or less uniform straight tube-spur-complex that appears as almost entire. The typical tri-partite divided *Pinguicula* corolla (i.e. into corolla lobes, tube and spur) appears nearly bi-partite here and is divided only into the corolla lobes and the tube-spur complex. The funnel-shaped, cylindrical tube merges ventrally into the short and comparatively wide cone-shaped spur, lacking a sharp angle (Fig. 1A, B). The spur is stubby, rounded at the apex and light yellow-green. The back of the tube only weakly protrudes from the spur: from this point, the stubby spur slightly attenuates proximally and ends in an obtuse apex. Looking at the corolla from a lateral (Fig. 1B) or semi-ventral (Fig. 1C) perspective, the tube and spur are not markedly separated. The tube-spur-complex is lengthwise dark parallel-veined. A tri-partite yellow to white palate is only weakly developed (Fig. 1H, i.e. non clapper-like as, for example, in *P. calyptrata* – Fig. 1I) and placed immediately behind the corolla lower-lip middle lobe (that appears as a shallow yellowish-greenish shimmering dent on the ventral tube-side directly behind the base of the middle lobe).

To illustrate the differences that can be observed on herbarium specimens, we have chosen *Pinguicula calyptrata* (Bussmann et al. 16447 MO, barcode: MO 6589755, Fig. 2C) collected in the immediate neighbourhood of the *P. rosmarieae*-type population (Fig. 2A). The sheet shows three well-preserved, single-scaped flowering specimens with the flowers in lateral profile view. The leaf rosette with its curled-up leaf margins measures up to ~30 mm in diameter. The flowering scapes are up to ~50 mm tall; the flowers are up to ~12 mm long (spur included), the corolla is distinctly tri-partite into the lip, tube and spur; its lobes are nearly equal-sized (subisolobate), the lower-lip middle lobe is only slightly larger than the lateral lobes, ~5 mm long; the corolla tube is distinctly funnel-shaped (at throat widest, ~5 mm), about as long as the corolla lobes, ~5 mm long; the spur is distinctly separated from the tube, short, ~2.5 mm long, thin, at apex rounded (sometimes almost imperceptibly thickened), angled at about 60°–90° with the length axis of the tube. Overall, in *P. calyptrata* as in the other Andean *Pinguicula*-taxa, the corolla is distinctly divided into three parts: lip, tube and angled spur.

Contrarily, in *P. rosmarieae*, the corolla appears bi-partite: tube and spur form a straight uniform funnel-cone-shaped tube-spur-complex, i.e. the spur is not angled with the tube. These features are clearly visible on the specimen collected by Paníagua-Zambrana, Bussmann & Vega Ocaña 8586 (MO 6607881; Fig. 2B), gathered in the surroundings of the Laguna Huay(ll)abamba (Department San Martín). The plants

were found growing either as lithophytes on a vertical rock wall in the spray of a small waterfall or as epiphytes on twigs of *Polylepis multijuga*. They are distinguished by a spread-out 6–8 leaved rosette appressed to the ground. Its flower shows the striking straight uniform tube-spur-complex which we also observed in the Laguna de los Cóndores-*Pinguicula* (see the three specimens on the left-hand side of the sheet). Photographs (Fig. 1H, I) of the corollas (frontal view) support the deep morphological disparity between the two taxa.

In *P. rosmarieae* (Fig. 1H), the corolla is widely open and appears radially symmetrical at first sight. The corolla lobes are spread out, the two lobes of the upper lip are smaller than those of the lower-lip, their distal margins are shallowly notched (to ~1/6 of their length). The lower-lip lobes are much larger, deeply notched (to ~¼ –1/3 of their length). The throat and the adjacent tube portion are not dark, a pronounced palate is not developed; it is replaced by a weak yellowish shimmering patch at the base of the corolla lower-lip middle lobe, continuing proximally (to the middle of the tube-spur-complex) lengthwise in two white hairy stripes.

In *P. calyptrata* (Fig. 1I), the corolla is widely open and also appears radially symmetrical. The corolla lobes are spread out, covering each other slightly with their lateral margins. They are nearly equal in size, except for the middle-lobe of the lower-lip that dominates the corolla to a certain degree. The distal margins of all corolla lobes are only shallowly notched, the throat being dark-purple coloured. Nearly 2 mm behind (proximally) the middle lobe, at the base of the lower-lip, a pronounced yellow clapper-like palate is inserted, from which two white hairy stripes stretch out into the tube.

Habitat

The Departments Amazonas and San Martín in northern Peru partly occupy the Sierra zone between the dry coastal region (Costa) and the upper Amazon river lowlands (Selva) and are largely characterised by extensive and very species-rich, cloud forests and wet subalpine grasslands (páramos). In contrast to *Pinguicula calyptrata*, which is largely found on wet, often peaty, soils, in the páramo region, *Pinguicula rosmarieae* occupies a completely different, even wetter, habitat. The species has been found either growing on steep, often vertical, rock-walls, normally on sandstones, in the spray of waterfalls (Fig. 3A, B) or rarely as an epiphyte in dense moss layers on *Polylepis multijuga* (Fig. 3C). Both represent equally extreme habitats, with extremely wet, nutrient-poor and acidic conditions and considerable mechanical stress.

The population at the type location grew in full sunlight on a steep sandstone cliff immediately above the famous tombs built by the Chachapoyas culture (AD ca. 800–1500). The tombs were built underneath natural overhangs, thereby allowing dry storage of the mummies. The type population (Henning & Schneider 275) grows above these overhangs exposed to constant dripping water and the general high precipitation typical for the eastern slopes of the Andes in this region (Fig. 3A).

Figure 3. Habitats of *Pinguicula rosmarieae* in the Department San Martín. **A** Large stands at the type locality above the Laguna de los Cóndores **B** sandstone rock walls with small waterfall near 'El Jardín' **C** 'El Jardín' *Polylepis multijuga* stands with *P. rosmarieae* growing as an epiphyte. (Credits: **A** T. Henning, **B, C** R. W. Bussmann).

Distribution

Pinguicula rosmarieae is endemic to the northernmost foothills of the Cordillera Central in northern Peru. The herbarium specimens, known so far, are from three nearby collections in the same mountain range southeast of Leymebamba, stretching over some 15 km in a north-south direction. Both expedition teams have mistakenly located the collection sites in the adjacent western Departments (Amazonas and La Libertad, respectively), since the border runs along the pass of the Cordillera. According to current online map-sources (Google maps), all populations, deposited in herbaria so far, were found some 4–6 km east of the border to San Martín (Fig. 4). The presumed narrow endemism has just recently been rebutted by the report of the new taxon from the Province of Bongará in the Department Amazonas some 100 km further north. Photographs made by L. Santa Cruz Cervera clearly show the characteristic rosette and flower patterns of *P. rosmarieae*, photographed in a similar open habitat near the famous Gocta falls. However, the taxon seems restricted to the northern branches of the Cordillera Central, but reaches further into the northernmost foothills. The collection data indicate that *P. calyptrata* and *P. rosmarieae* show distributional overlap over the entire range of the latter. True sympatry is nevertheless prevented by the different habitat requirements of the two taxa.

The area lies well in the so-called Amotape-Huancabamba Zone, an important biodiversity-hotspot that spans from the Pacific coast over the cordilleras to the tropical lowlands of southern Ecuador and large parts of northern Peru (Fig. 4, for details see: Weigend 2002, 2004;). Many plant groups have a centre of diversity here (Weigend et al. 2005; Struwe et al. 2009; Deanna et al. 2018) and show a concentrated occurrence of narrow-endemic taxa in that region (Berry 1982; Ayers 1999; Weigend 2002; Henning and Weigend 2009, 2009a; Henning et al. 2019). While the vegetation and flora of the inner-Andean valleys and the western slopes are relatively well-investigated, the eastern flanks of the Cordillera Central facing Amazonia are still under-collected in many areas. Especially, the areas east of Leymebamba (Dept. Amazonas) in the north and Buldibuyo (Dept. La Libertad) in the south remain largely unexplored, since the eastern slopes can only be reached by foot. Single collection trips have yielded unexpected, supposedly narrowly endemic taxa in other plant groups, although only a tiny fraction of the area could be sampled to date (e.g. Loasaceae: *Nasa rugosa* subspp. *gracilipes* and *pygmaea*; Henning et al. 2011).

The whole region is characterised by great geological diversity, with large areas of dolomitic karst, caused by the exceptionally high rainfall (the indigenous Chachapoya were often called "warriors of the clouds"), interspersed with small areas of sandstone outcrops and metamorphic rocks. The vegetation of the sandstone areas is particularly intriguing, with many endemic species and unique vegetation types (e.g. *Weinmannia* sp. and *Polylepis multijuga-Iochroma stenanthum* dominated forests). *P. rosmarieae* has been found exclusively in the spray of waterfalls on sandstone cliffs and as an epiphyte on *Polylepis* – both systems characterised by extreme moisture and almost permanent cloud cover, a fact that is recognised in the local topographic maps, which often simply indicate "clouds" in these areas.

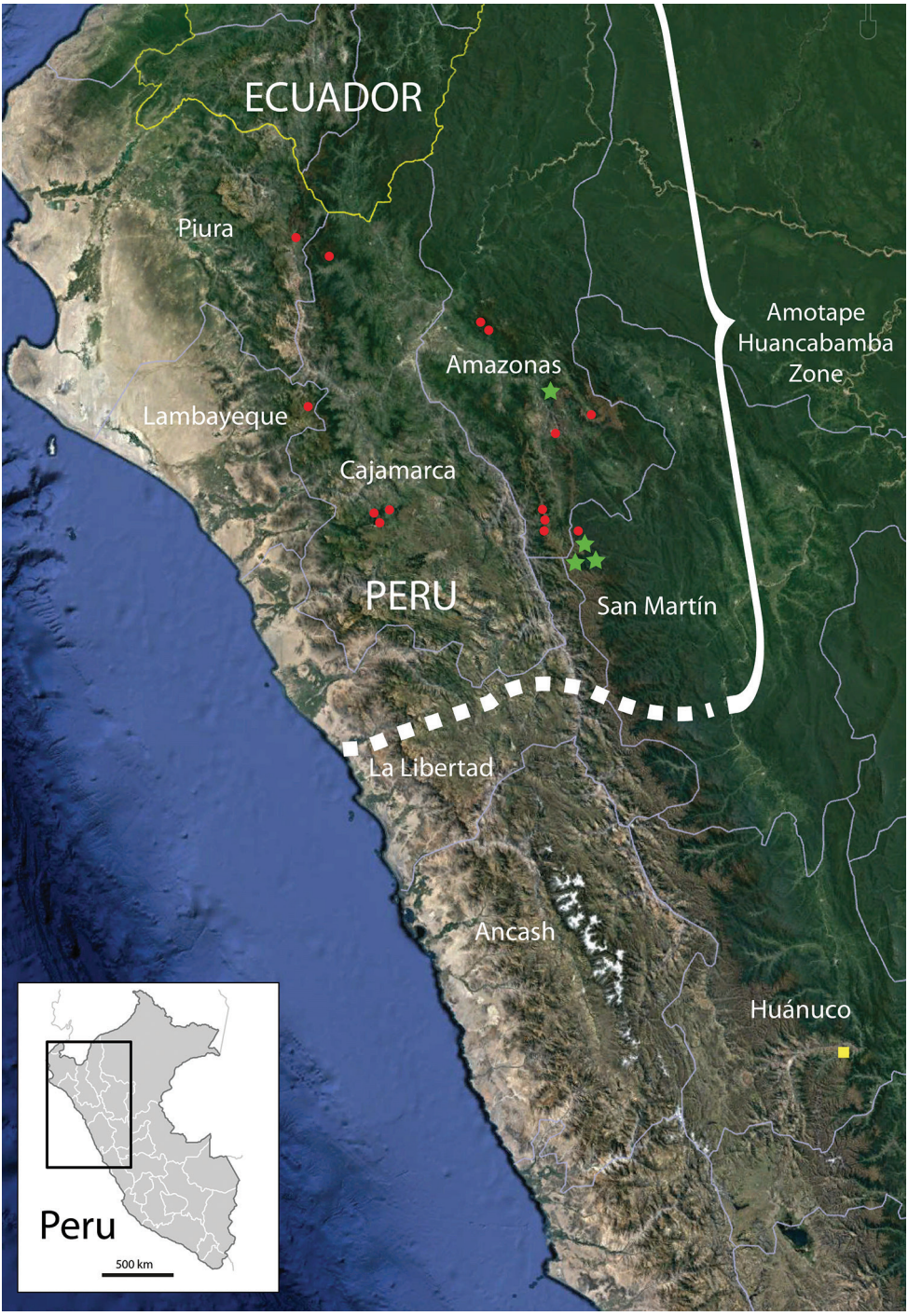

Figure 4. Distribution of *Pinguicula rosmarieae* and related taxa based on geo-referenced collections. Green stars: *P. rosmarieae*, red dots: *P. calyptrata*, yellow square: *P. involuta*, dotted line: northern and southern limits of the Amotape-Huancabamba-Zone.

Pinguicula is yet another example of a plant group that is present in the Amotape-Huancabamba Zone with an endemic taxon, whereas its widespread relatives have their distribution limits at the zonal boundary, either seen from within (*P. calyptrata*) or outside (*P. involuta*). Since *Pinguicula rosmarieae* has been collected in those areas of the overall region that are comparatively easy to access, it cannot be ruled out that there are additional taxa awaiting discovery. However, the nondescript shape of the insectivorous butterwort, represented by only a few, widespread taxa outside of that region (Fig. 4), does not encourage gathering.

On a broader scale, the endemism of *P. rosmarieae* is by no means a unique phenomenon amongst the South American *Pinguicula*. *P. jarmilae* Halda & Malina from southeast Bolivia (Department Chuquisaca, municipio Villa Serrano, village Nuevo Mundo), for example, is only known from the type locality (Halda et al. 2007). Although it grows on vertical sandstone (laterite) rock faces under flowing/moving water, its morphology is quite different from that of *P. rosmarieae*: the superficial similarity is likely coincidental and does not reflect a close relationship between the two taxa.

Preliminary conservation status

Due to the aforementioned lack of a continuous botanical exploration of the region, we have to consider *P. rosmarieae* as Data Deficient (DD), according to the IUCN threatened species assessment guidelines (2001, 2017). However, within its distributional range, it seems to be limited to the easternmost ridge of the Cordillera Central. As an insectivorous plant, it seems to be adapted to special habitat types, characterised by 'open' vegetation, high precipitation and nitrogen-poor soils. These habitats might only be available in a narrow altitudinal band along the mountain chain. As our observations suggest, such sites are not present at similar altitudes in the west and it is very likely that suitable habitats are only available a few kilometres further east up to the lower altitudes of the Andes. This might indicate that *P. rosmarieae* is generally a rare taxon and might, thus, be vulnerable to habitat destruction. The lack of additional collections emphasises this assumption. This delicate butterwort might be in danger of extinction, but more data is needed to better assess the entire distribution and abundance, especially to the east of the known populations.

Annotated list of *P. rosmarieae* specimens (paratypes)

Peru. "Dept. Amazonas: Distr. Chachapoyas" (sic!) = Dept. **San Martín**, Prov. Huallaga, Lei[y]mebamba, Oseres [10 km east of Leymebamba]. Bosque Montano, 2542 m a.s.l. (~06°58'S, ~77°40'W), 22 May 2015. *C. Vega Ocaña, L. Cotrina P., J. Valle, R. W. Bussmann, & N. Paniagua Zambrana 247* – HAO, MO 2852736. dp! [Det. *Pinguicula* RBU 2015; *involuta* E. Feltz (Ma), 2016]. – Duplicate: MO 6726506. "Dept. La Libertad, Distr. Uchumarca" (sic!) = Dept. San Martín, Prov. Huallaga, páramo and

sandstone cliffs east of Laguna Huay(ll)abamba (06°58'53"S, 77°43'09"W) 3250 m a.s.l., 02 Nov 2012. – *N. Paníagua Zambrana, R. W. Bussmann & C. Vega Ocaña 8586* [det. *Pinguicula involuta* RBU 2015]. – MO 6607881. dp!

List of *P. calyptrata* specimens in the research region (used for Fig. 4)

Peru: Dept. **Piura**: [Prov.] Huancabamba, Lomas Redonda (Sapalache-Chinguelas) 2400 m (05°09'S, 79°26'W) a.s.l., 15 Ago 1981. *A. Sagástegui Alva & al. 10187* – MO 2940293 [Barcode 348636], MO 4025844 [Barcode 348635] (dp!), HUT (dp!) – (det. *P. involuta* P. Taylor & M. Cheek). Dept. **Lambayeque**: [Prov.] Ferreñafe, [Distr.] Incahuasi cerca a la laguna Tembladera. Vegetación de jalca, zonas humedas. 3300 m a.s.l. (~06°09'S, ~79°19'W). 08 Oct 1989. *S. Llatas Quiroz 2606* – F 2051195 (dp!). (det. *P. involuta* P. Taylor 1992). Dept. **Cajamarca**: Prov. San Ignacio, Distr. Tabaconas, Local. Santuario Nac. Tabaconas-Namballe, alrededores de las lagunas Coyona (Arrebiatadas), 05°14'S, 79°16'W (Arriba Laguna Lagartocha), 3140 m–3180 m a.s.l.. *S. M. Baldéon Malpartida 5108 & L. Adriazon Ocupa*. – USM 00266675 dp! (det. *P. involuta*). – Cajamarca, km 30 de la carretera Cajamarca-Bambamarca Jalca, estepa de gramineas, sobre un afloramiento rocoso. 3600 m a.s.l., 23 Mar 1985. – *Sánchez-Vega, I. M., U. Molau & L. Ohmann 3756.* – F 2216084 (Barcode V0469923F - dupl. CPUN; dp! det. *Pinguicula* L.) – [Prov.] Santa Cruz, [Distr.] Pulán, [caserio] El Molino, 2,500 m a.s.l. (06°46'S, 78°55'W), 12 Feb 2007. *L. Santa Cruz, M. Chocce & M. Beltrán 996*– USM 241552 (dp!), HUT 50771 (dp!). – [Prov.] Santa Cruz, [Distr.] Pulán, Pampa el suro, 2500 m a.s.l. (06°50'S, ~78°54'W), 31 Ene 2008. *L. Santa Cruz 2098* – HAO, USM 240752 (dp!). – Prov. San Miguel, distrito Tongod, Bosque San Pedro Norte (06°45'S, 78°49'W), 03 Nov 2001. *I. M. Sánchez Vega & M. Sánchez M. 11122* – F 2245015 [V 0410057F] (dp! det. *P. involuta*). Dept. **Amazonas**: [Prov.] Bagua, [Santuario Nacional] Cordillera [de] Colan-La Peca, 9600 ft a.s.l. (05°35'S, 78°14'W), 29 Aug 1978. *Ph. J. Barbour 3222* – USM 53585 (pd!), MO 2798203 (dp! – Barcode MO 348631, det. *P. involuta* P. Taylor 1992). – Cordillera Colan-La Peca, 9600–11075 ft a.s.l. (05°35'S, 78°16'W), 08 Sep 1978. Ph. J. Barbour 3425 – F 1909251 (V0469936F; dp!), MO 2789874 (pd! det. *P. involuta* Taylor). – Amazonas: 3000–4000 m a.s.l. (~06°07'S, ~77°39'W), 09 Nov 2012. *H. van der Werff, L. Valenzuela, G. Shareva & A. Reyes Barrantes 25401* – MO. – Cerro de Fraijaca (Huaui-Huni) n. e. Tambo de Ventilla. Jul 07 1948. *A. W. Pennell 15875* USM 90946 (det. Pinguicula - dp!). – Prov. Chachapoyas, declives superiors de [Cerro] Puma-Urcú, [~2 km] este-sureste de [ciudad] Chachapoyas (~06°14'S, ~77°52'W), alt. 2700 m. – 3000 m., Jun 01 1962. *J. J. Wurdack 679*. – USM 90948 (dp!), COL (dupl. dp! det. *P. antarctica*, by Fernandes-Pérez 1964), NY. – Prov. Chachapoyas, bosque bajo y húmedo al lado de (moist scrub forest on south side of) Molinopampa-Diosan pass, alt. 2700 m – Balsa road to Leymebamba, just below Abra Callacalla (= Alba Barro Negro) on the slope towards Leymebamba, 3559 m a.s.l. (06°44'S, 77°53'W), 19.10.2000 – *M. Weigend, E. Rodríguez R., H. Förther & N. Dostert 867* – USM 166766 (dp! det. *Pinguicula* spec.) – [Prov.] Chachapoyas,

[Distr.] Balsas, En el Paso de Calla Calla (06°48'S, 77°53'W), 07 Oct 2001. *I. M. Sãnchez-Vega, M. Sãnchez, M. 11061* – F. – Cerros [Cordillera] Calla Calla. 26 km above Leimebamba, road to Balsas. Km 403, 3360 m a.s.l. (~06°48'S, ~77°53'W), 16 Oct 1964. *P. C. Hutchison & J. K. Wright 6993*. – MO 2233627 (GBIF: as *P. antarctica*); USM 90947 (dp!). – Prov. Chachapoyas, Distr. Chachapoyas. Trail to Laguna de Los Cóndores, surroundings of Laguna Esperanza/Siete Lagunas (06°49'S, 77°43'W) 3275 m–3500 m a.s.l., Jun 26 2010. *R. W. Bussmann, A. Glenn, G. Chait & C. Vega Ocaña 16447*. Dept. **La Libertad**: Distr. Uchumarca, páramo in the surroundings of Vira Vira/Lagunas La Quinuas (07°00'S, 77°45'W), 3050 m a.s.l. – Photograph: *R. W. Bussmann* (det *P. involuta* = *P. calyptrata*).

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Capsicum carassense (Solanaceae), a new species from the Brazilian Atlantic Forest

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Abstract

Capsicum carassense Barboza & Bianchetti **sp. nov.**, a species from mid-elevation of the Atlantic Forest (Minas Gerais, Brazil) is described and illustrated. This endemic new species is distinguished from the most similar *C. mirabile* Mart. by its moderate to dense general pubescence, narrowly elliptic leaves and larger calyx appendages and corollas. A key for the native Brazilian species of *Capsicum* growing in the state of Minas Gerais is also provided.

Keywords

Brazil, *Capsicum*, Minas Gerais, new species, taxonomy

Introduction

Capsicum L. (Capsiceae, Solanaceae) comprises ca. 41 species of mostly shrubs or subshrubs with axillary sessile inflorescences, truncate calyces or appendages borne below the margin, corollas usually stellate or campanulate and variously coloured and usually pungent fruits (Hunziker 2001, Barboza et al. 2016). Some species have pharmaco-

logical properties (Papoiu and Yosipovitch 2010, Al-Snafi 2015, Parvez 2017) and several constitute major vegetable and spice crops worldwide (Jarret et al. 2019), mainly due to the presence of compounds unique to *Capsicum*, the capsaicinoids, which are responsible for the pungency of the fruits. The genus is native to tropical and temperate Central and South America, Mexico and the West Indies (Barboza et al. 2019), with five species widely cultivated throughout Europe, the southern United States, Africa, India, China and South America (Pickersgill 1997, Basu and De 2003, Libreros et al. 2014, Scaldaferro et al. 2018).

There are two main centres of diversity for *Capsicum*, the Andes and Brazil (Barboza et al. in prep.). For both centres, new species have been described in recent years (Barboza and Bianchetti 2005, Nee et al. 2006, Barboza 2011, Barboza et al. 2011, Barboza et al. 2019). Brazil is an extensive country that hosts the most diverse flora within the Americas (Ulloa Ulloa et al. 2017, BFG 2018) and *Capsicum* has 50% of its species growing in its territory. Additionally, Brazil's Atlantic Forest is the fourth leading hotspot in terms of endemic plants (2.7% of global total, Myers et al. 2000), where ten *Capsicum* species are endemic (Barboza et al. 2011), some with very restricted distributions (e.g. *C. friburgense* Bianchetti & Barboza and *C. hunzikerianum* Barboza & Bianchetti).

While working with the genus on the Flora do Brasil 2020 project, we revised several Brazilian herbaria and visited the Serra do Caraça, a historical natural reserve with remnants of semi-deciduous montane forest in the Iron Quadrangle, in Minas Gerais state. There, we found some populations that were recognised at first glance as belonging to *C. mirabile* Mart., a species that typically occurs in montane rainforests in south-eastern Brazil. *Capsicum* species from different regions of the Atlantic forest were included in the broad molecular phylogeny of the genus, based on nuclear and chloroplast markers (Carrizo García et al. 2016). The new species, denoted as *Capsicum* aff. *mirabile* in Carrizo García et al. (2016) was not sister to the *C. mirabile* accession included in these analyses, suggesting that it may be an undescribed taxon. Then, we reviewed in detail the morphology of the *Capsicum* species of the Brazilian interior and coastal forests and determined that the populations from Serra do Caraça and surroundings represent a distinct species of *C. mirabile.* Here, we describe and illustrate this new species and provide comments on taxonomy, ecology and conservation, taking into account its occurrence in a region heavily threatened by mining activities.

Material and methods

The description is based on observations and data taken from specimens collected in the field between 1986 to 2019, mainly in Serra do Caraça (Minas Gerais, Brazil) and examination of herbarium specimens from 13 herbaria (BHCB, BHZB, BM, CEN, CORD, ESA, JPB, M, MBM, RB, SP, UEC and UT). Specimens have been accessed *in situ* or through digital images via INCT Herbário Virtual (http://inct.splink.org.br), Herbário Virtual Reflora (http://reflora.jbrj.gov.br/reflora/herbarioVirtual) or Global

Plants (https://plants.jstor.org/) databases. Measurements were made from preserved material in FAA solution (formaldehyde –acetic acid – ethanol) or from living material using a Zeiss Stemi 2000-C stereomicroscope at 6.5–50× magnification. Information on corolla and fruit colour and pungency of fruit was recorded from living material in the fieldwork. Illustrations were made by composite line drawings from preserved material. Photographs were taken during fieldwork by the authors; images were edited using Adobe PhotoshopVR.

To compare this new species with its morphologically similar congener (*C. mirabile*), about 200 specimens of *C. mirabile* were analysed (see Suppl. material 1), using the same methods (fieldwork and examination of living and herbarium collections). To assess *C. carassense* and *C. mirabile* distributions in Minas Gerais, latitude and longitude data indicated on the labels were directly mapped; other localities were georeferenced by searching the locality by GeoLoc tools (https://splink.cria.org.br/geoloc). QuantumGis (QGIS V. 2.18) was used to build the distribution map. Conservation status was assessed using primarily the IUCN criteria B, geographic range in the form of B1 (extent of occurrence, EOO) and B2 (area of occupancy, AOO) (IUCN 2019). The EOO and AOO were calculated using the Geospatial Conservation Assessment Tool, GeoCAT (Bachman et al. 2011) and AOO was based on a defined cell width of 2 km.

Taxonomic treatment

Capsicum carassense **Barboza & Bianchetti, sp. nov.**
urn:lsid:ipni.org:names:77206952-1
Figs 1–3

Diagnosis. *Capsicum carassense* is morphologically most similar to *C. mirabile* Mart., but differs in its moderate to dense pubescence, narrowly elliptical to lanceolate leaf blade with acute to obtuse apices, longer calyx appendages (up to 5 mm) and larger corollas (up to 20 mm in diameter).

Type. Brazil. Minas Gerais: Catas Altas, RPPN Serra do Caraça, trilha da gruta de Lourdes, após a capelinha, 20°05'41"S, 43°28'52"W, 1386 m elev., 26 Oct 2014 (fl), *J.R. Stehmann, L.L. Giacomin, G.E. Barboza & S. Knapp 6347* (***holotype*** [two sheets]: BHCB acc.#174038 [BHCB0019940_1!, BHCB0019940_2!]; ***isotypes:*** CORD [CORD00006968!], RB [RB 01220059, acc. # 674586!]; MBM).

Description. Shrubs (0.8–) 1–2 (–3) m tall, with the main stem somewhat thick and sparsely branched, the branches dichotomous and spreading horizontally. Stems hollow, angled with ridges; young stems green, striate, moderately to densely pubescent with simple, uncinate and antrorse uniseriate 3–5 (–6)-celled eglandular trichomes 0.2–0.7 mm long, yellowish-brown when dried, the nodes green or purple; bark of older stems brown, pubescent, striate; lenticels absent. Sympodial units difoliate, the leaves geminate or the leaves solitary in the bifurcation of the branches; the leaves of

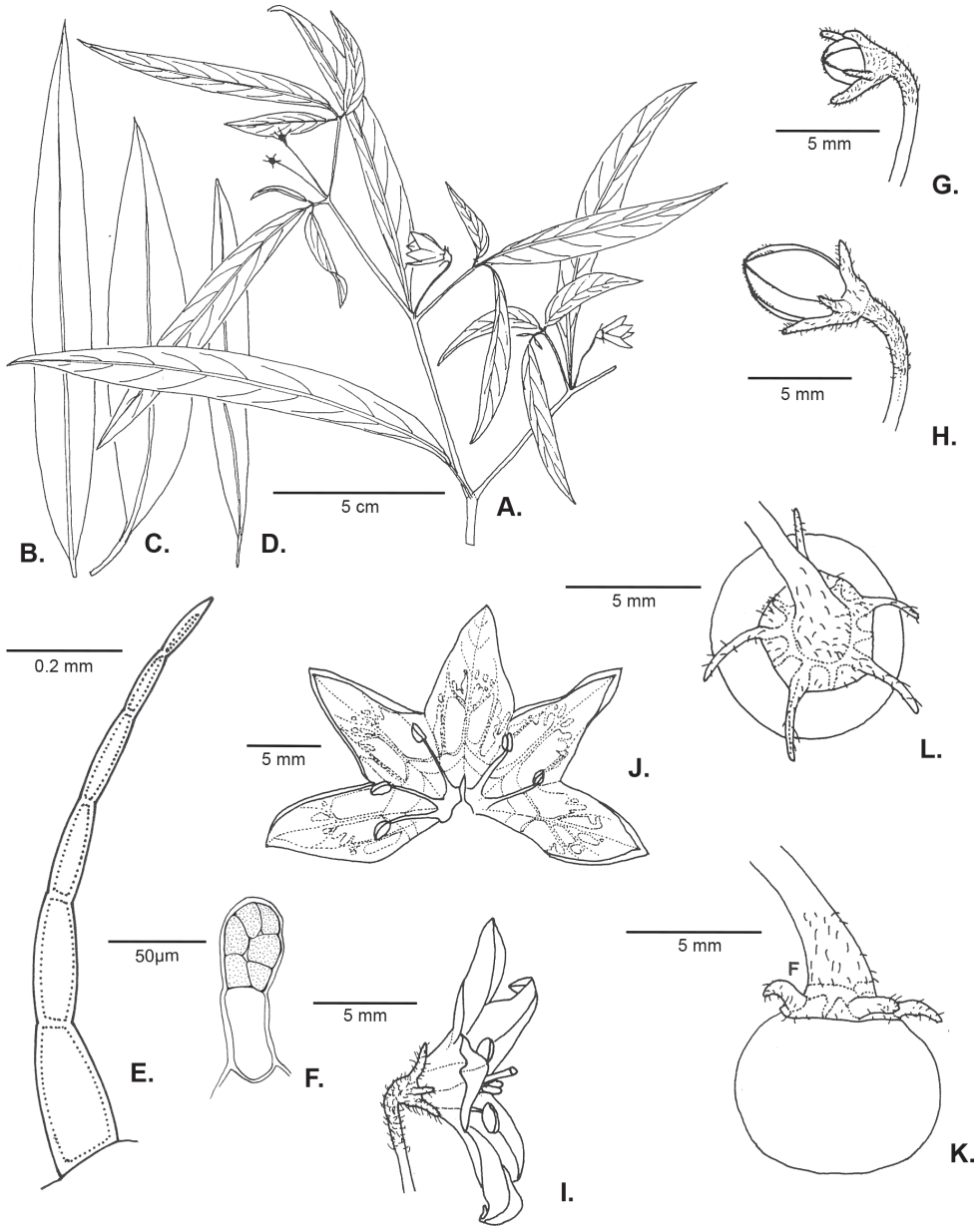

Figure 1. *Capsicum carassense* Barboza & Bianchetti **A** flowering branch **B–D** leaf morphology **E** eglandular trichome of the stem **F** glandular trichome of the calyx **G, H** flower buds in different stages of development **I** flower in anthesis (note the geniculate pedicel) **J** opened corolla **K** fruit **L** fruiting calyx **A–K** from Bianchetti et al. 1364. Drawn by L. Bianchetti.

a geminate pair anisophyllous in size. Leaves simple, membranaceous to chartaceous, discolourous, dark green above, paler beneath; adaxial surface moderately pubescent with simple trichomes like those of the stem, especially on the veins; abaxial surface

Figure 2. *Capsicum carassense* Barboza & Bianchetti **A** habit, showing the typical lanceolate leaves **B** inflorescence with geniculate pedicels **C** flower, in frontal view **D** globose-depressed fruit **A–C** from Stehmann 6347 **D** from Agra 7268. Photos by J.R. Stehmann. Scale bars: 2 cm (**A**); 1 cm (**B**); 5 mm (**C–D**).

moderately pubescent like the adaxial surface, but with less frequent glandular trichomes with a unicellular stalk and a multicellular head; major leaves with blades 6–16 cm long, 0.9–2.5 cm wide, narrowly elliptic to lanceolate; the major veins 4–6 on each side of midvein, the midvein prominent and the secondary veins obscure, the base attenuate, the margins entire and moderately pubescent, the apex acute to obtuse; petioles 0.2–0.6 cm long, moderately pubescent; the minor leaves 2.9–3.9 cm long, 0.5–0.8 cm wide, narrowly elliptic; the major veins 2–3 (–4) on each side of midvein, the base attenuate, the margins entire, moderately pubescent, the apex obtuse; petioles

0.2–0.4 cm long, moderately pubescent. Inflorescence with the flowers in fascicles of 2–4; pedicels (1.2–) 1.5–2 (–2.2) cm long, slightly angled, erect to oblique, green, geniculate at anthesis, moderately pubescent. Buds ellipsoid, cream with greenish-yellow pigmentation. Flowers 5-merous, all perfect. Calyx 1.2–1.6 mm long, 2.5–3 mm wide, cup-shaped, thin, light green to cream, the margin truncate, pubescent with abundant antrorse curved 3–5-celled eglandular trichomes and sparse short glandular trichomes with a dark elongate, multicellular head and short unicellular stalk (see Fig. 1E, F), the calyx appendages 5, (2.5–) 3–4 (–5) mm long, green, thick, erect, cylindrical, inserted very close to the margin, with the same indument as the calyx tube. Corolla (8–) 10–12 mm long, 13–20 mm in diameter, stellate, thick, with abundant interpetalar tissue, white near the lobe margins and greenish-yellow in the middle and base without, white with 5 purple spots covering the base of the lobes and the throat with a cream centre within, lobed 1/2 or less to the base, the tube 4.5–5 mm long; pubescent in the throat and the base of the lobes with long glandular trichomes with a globose peltate unicellular head and a 2-3-celled stalk inside, the lobes 4.5–6.5 mm long, 5–8 mm wide, broadly triangular to triangular, the tips cucullate, the margins densely pubescent. Stamens subequal; filaments 2.7–3.1 (–4.1) mm long, white, glabrous, inserted on the corolla ca. 1 mm from the base, with inconspicuous auricles; anthers 1.5–1.9 mm long, elliptic, the thecae blue, the pollen whitish-cream. Ovary 1.3–1.5 mm long, ca. 1.2 mm diam., light green, subglobose to ovoid, glabrous; nectary ca. 0.3 mm high, conspicuous; style 4.3–5 (–7) mm long, white, clavate, glabrous; stigma ca. 0.2 mm long, ca. 0.7 mm wide, cream, discoid. Fruit a globose-depressed berry 6–7 mm in diameter, green when immature, yellowish-green when mature, glabrous, pungent, the pericarp hyaline with very long giant cells, the endocarp alveolate; stone cells absent; fruiting pedicels 1.8–2.5 cm long, pendent and slightly curved, slightly angled and widened at the apex; fruiting calyx ca. 4 mm in diameter, persistent, not accrescent, discoid, yellowish-green, the appendages spreading, green, fleshy and cylindrical. Seeds 7–13 per fruit, 3.5–4 mm long, 2.5–3 mm wide, ellipsoidal to reniform, brownish-black to black, the seed coat deeply reticulate, with small spine-like projections. Chromosome number not known.

Distribution. *Capsicum carassense* is endemic to south-eastern Minas Gerais (Fig. 3), growing mainly in the Serra do Caraça and other nearby mountainous areas (Serra do Gandarela, Serra Geral, Serra do Capanema, Serra São Geraldo), between 1000–1390 m elevation.

Ecology. The population studied in the field at Serra do Caraça inhabits the understorey of the semi-deciduous montane Atlantic Forest, in a shaded and moist environment. Information about pollination and dispersal is not yet known.

Phenology. In flower from October to January, also in May; fruiting in December, February and April.

Etymology. The new species is named in allusion to its restricted habitat in the Serra of Caraça and surrounding areas (Minas Gerais, Brazil).

Preliminary assessment of conservation status. Following the IUCN Red List Criteria (IUCN 2019), this species is considered Endangered (EN) B2 a,b (iii, iv).

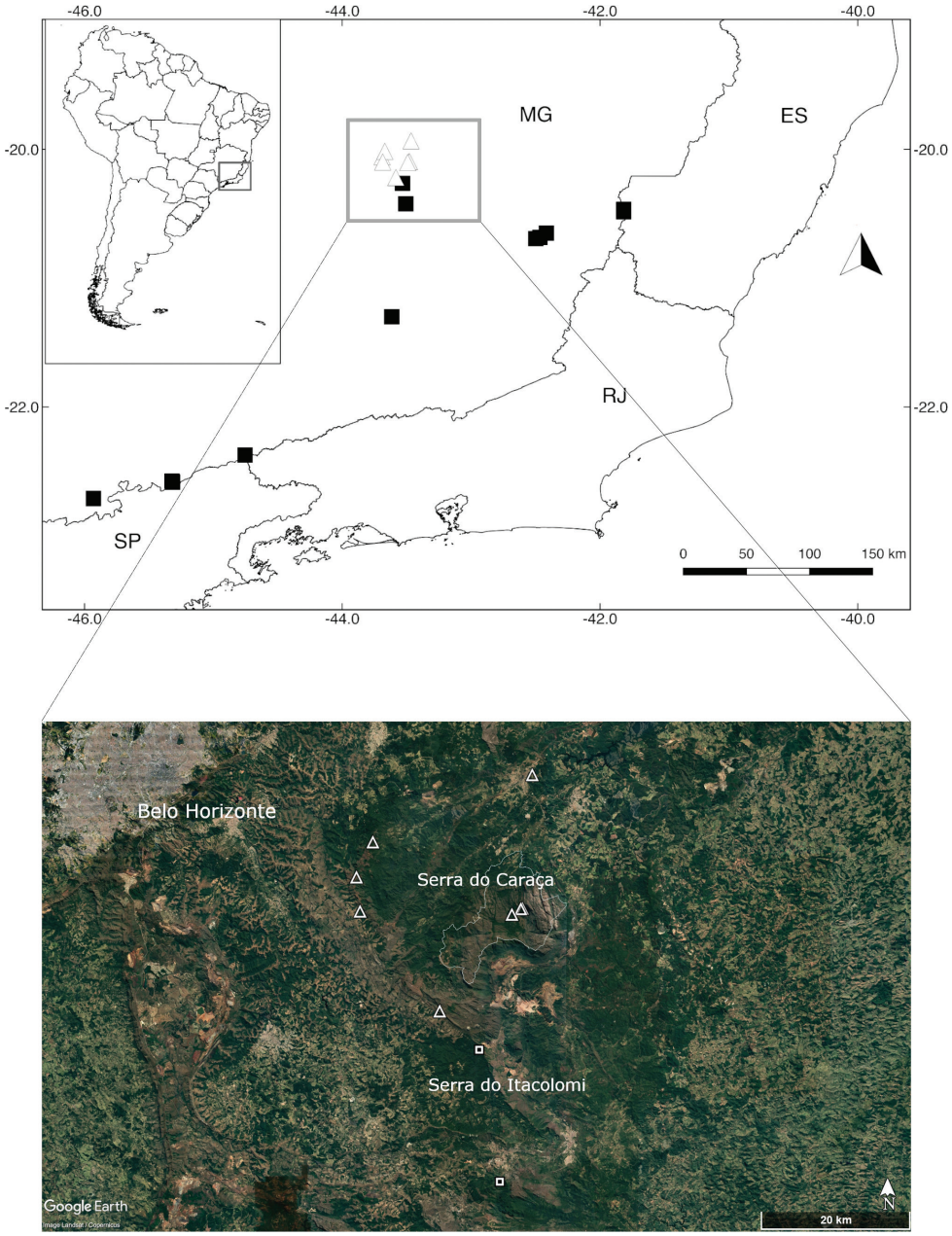

Figure 3. Map showing the distribution of *Capsicum carassense* (triangle) and *C. mirabile* (square) in south-eastern Brazil. Colour map was reproduced and adapted from Google Earth Pro. Abbreviations. MG, Minas Gerais; ES, Espirito Santo; RJ, Rio de Janeiro; SP, São Paulo.

We suggest this category, because of the species' very restricted geographic distribution (EOO < 483.4 km^2, AOO < 32 km^2), as well as the increasingly degraded habitat quality, especially associated with the extensive iron mining activities in the region (see below).

Specimens examined. Brazil. Minas Gerais: Mun. Catas Altas, Serra do Caraça, near Santa Barbara, trilha a Capela y Gruta de Lourdes, 20°05'39"S, 43°28'45"W, 1430 m elev., 26 Apr 2010 (fr), *M.F. Agra et al. 7268* (BHCB, JPB, RB, UT); Serra do Caraça, ca. 70 km sudeste de Belo Horizonte, próximo ao Mosteiro do Caraça, 17 Nov 1977 (fl), *N.D. da Cruz et al. 6291* (SP, RB, UEC); Barão de Cocais, Parque Natural do Caraça (PNC), a 150 m do monastério, perto da Fonte do Bode, 1250 m elev., 19°56'S, 43°28'W, 22 Apr 1986 (fr), *L. Bianchetti et al. 512* (CEN); Barão de Cocais, PNC, 31 May 1992, *L. Bianchetti et al. 1363, 1364, 1367, 1368* (CEN); PNC, muy cerca del monasterio, en el bosquecillo vecino al Fonte de Bode, ca. 1250 m elev., 21 Apr 1986 (fr), *A.T. Hunziker et al. 25206* (CORD); PNC, muy cerca del monasterio, 1250–1300 m elev., 10 Dec 1986 (fl), *A.T. Hunziker et al. 25256* (CORD, BM); Serra do Caraça, 4 Dec 1999 (fl), *R.C. Mota 106* (BHCB); at the same locality, 18 Dec 2002 (fl), *R.C. Mota 2260* (BHCB); at the same locality, 5 Jan 2005 (fl), *R.C. Mota 2653* (BHCB); RPPN Caraça, Trilha para Tanque Grande, 03 Dec 2013 (fr), *J. Ordones et al. 2229* (BHZB); Serra do Caraça, 1600 m elev., 12 Sep 1990 (fl), *J.R. Stehmann et al. s.n.* (ESA 33757); Serra do Caraça, Tanque Grande, 16 Feb 2012 (fr), *J.R. Stehmann & F.S. Faria 6269* (BHCB); na trilha para o Tanque Grande, 20°06'05"S, 43°29'33"W, 1249 m elev., 26 Oct 2014 (fl), *J.R. Stehmann et al. 6344* (BHCB, RB); Mun. Ouro Preto, Mina da Capanema (Mina da Serra Geral), RPPN Vale, near town of Glaura, trail to Cabeza do Macaco, 20°13'04"S, 43°35'05"W, 1691 m elev., 29 Apr 2011 (fr), *M.F. Agra et al. 7340* (BHCB); estrada da torre-Samarco Mineração-Antonio Pereira, 16 Dec 1996 (fl, fr), *M. Brosehel & J. Craig 406* (RB); RPPN Capanema, 19 Oct 2015 (fl), *M.O. Pivari et al. 2768* (BHCB); Mun. Santa Barbara, Serra de Gandarela/C2, 20°3'24"S, 43°41'28.60"W, 1637 m elev., 26 Nov 2008 (fl), *F.F. Carmo & L.C. Ribeiro 3527* (BHCB); Santa Barbara, 20°00'52"S, 43°40'13"W, 1497 m elev., 17 Dec 2014 (fl), *F.D. Gontijo et al. 589* (BHCB); Rio Acima, Serra do Gandarela, 20°05'52"S, 43°41'12"W, 12 Dec 2011 (fl), *C.V. Vidal & R.L. de Paula 1157* (BHCB); Without municipality: habitat in irriguis lapidosis Serra do S. Geraldo, w/d, *C.F.P. von Martius s.n.* (M 0171537).

Discussion. *Capsicum carassense* belongs to the Atlantic Forest clade (sensu Carrizo García et al. 2016, as *Capsicum* aff. *mirabile*). For many years, this species has long been confused with *C. mirabile* in herbaria (Bianchetti 1996, Barboza per. obs.). Both species share similar traits, such as habit, geniculate pedicels at anthesis, number of calyx appendages, the shape and colour of the corolla, colour and pungency of the fruits and blackish seeds. They can be easily distinguished by the indumentum, shape of the major leaf and its length/width ratio, length of the calyx appendages, corolla size and ecology and distribution (see Table 1 for contrasting details).

Capsicum carassense is a pubescent low shrub with very narrow leaves and large white corollas with purple-spots. The shape and length/width ratio of the leaves of *C. carassense* are very close to the description of *C. mirabile* var. *grandiflorum* Sendtn. (Sendtner 1846) but Sendtner also stated that this variety had "floribus majoribus" and "planta glaberrima". The diameter of the corolla measured in three flowers in the F neg. 2871 of the destroyed varietal holotype (Sellow 209, Herb. Reg. Berolinense) is

Table 1. Differences between *C. carassense* and *C. mirabile*. Abbreviations. BA: Bahia, ES: Espírito Santo, RJ: Rio de Janeiro, SP: São Paulo, MG: Minas Gerais.

Character	*Capsicum carassense*	*Capsicum mirabile*
Indumentum	Moderately to densely pubescent	Glabrate to sparsely pubescent
Major leaf shape	Narrowly elliptical to lanceolate, apex acute to obtuse	Elliptical to ovate, rarely narrowly elliptical, apex acuminate to long acuminate
Major leaf length/width ratio	(4–) 5–10 (–16)	(2–) 2.5–4 (–4.9)
Buds colour	Cream with greenish-yellow tones	Purple
Calyx appendages	Long appendages (2.8–) 3–4 (–5) mm	Short to long appendages (0.4–) 0.5–1.5 (–3) mm
Corolla size	(8–) 10–12 mm long 13–20 mm in diameter	(6–) 7.5–12 mm long (9–) 10–13 mm in diameter
Distribution and ecology	Endemic to Serra do Caraça and surrounding areas (MG); mostly in semi-deciduous montane forests	Widely distributed in eastern and southern Brazil (BA, ES, RJ, SP, MG); mostly in dense ombrophilous montane forests

not more than 1 cm (https://collections-botany.fieldmuseum.org/project/6454), thus these two traits, size of the corolla and lack of pubescence fit with the concept of *C. mirabile* rather than *C. carassense*.

Capsicum carassense and *C. mirabile* differ in geographic distribution, with the former inhabiting mostly the understorey of the semi-deciduous montane forests of the southernmost areas of the Espinhaço Range in Minas Gerais, while the latter has a wider distribution (Barboza and Bianchetti 2005), growing mostly along the dense ombrophilous montane forest of south-eastern Brazil, an area characterised by high rainfall and humidity and the absence of a pronounced dry season (Silva Magnago et al. 2007). Both species have a contact zone at the municipalities of Mariana and Ouro Preto, in the Serra do Itacolomi, where *C. carassense*, as well as two other species, *C. mirabile* and *C. villosum* Sendtn., were recorded. There is no information about edaphic preferences for these species, nor possible events of hybridisation in this contact area.

All collections of the new species come from the Iron Quadrangle in Minas Gerais, except one historical Martius specimen at M [M!, photo nº 6522 at F!], collected in the Serra de São Geraldo between Mariana and Presídio de São João Batista (today the municipality of Visconde do Rio Branco). This material, a syntype of *C. mirabile* (Sendtner 1846), was examined when Barboza (2011) lectotypified *C. mirabile.* She stated that "the third syntype […] is unusually pubescent for this species". Here, we re-examined this specimen housed at M (M–0171537) and concluded that it actually belongs to *C. carassense*.

The relationships amongst the species belonging to the Atlantic Forest clade appear to be fully resolved and an apparent phase of rapid speciation has been suggested for this lineage (Carrizo García et al. 2016). In spite of the morphological similarity between *C. carassense* and *C. mirabile*, these species are not closely related phylogenetically, as *C. carassense* (as *Capsicum* aff. *mirabile* in Carrizo García et al. 2016) is not sister to *C. mirabile*, but occurs in a different subclade of the Atlantic Forest clade. In the analysis of Carrizo García et al. (2016), *C. mirabile* appears closest to *C. villosum* var. *muticum* Sendtn.

The new species deserves conservation attention, because few populations are known and most of them are distributed in the Iron Quadrangle and associated with remnants of native forests. This area was assessed as priority for conservation in the state of Minas Gerais (Drummond et al. 2005), with high animal and plant diversity and extensive threats, especially from iron mining activities (Jacobi et al. 2011, Salles et al. 2018). The impacts on native vegetation, especially forests, are high because of the activities associated with iron and bauxite mining as the building of dams and urban expansion, all increase deforestation pressures at regional-scale (Sonter et al. 2014).

Artificial key to the 11 native Brazilian species growing in the state of Minas Gerais (excluding cultivated species)

1 Calyx without teeth or with 5 very short appendages delimiting a pentagonal outline **2**
– Calyx with 5 well-developed appendages, these being 0.4–5 mm long **6**
2 Pedicels at anthesis non-geniculate, pendent **3**
– Pedicels at anthesis geniculate, erect **5**
3 Leaves elliptic to narrowly elliptic, coriaceous, glabrous; corolla mostly white with purple pigmentation within; fruits greenish-yellow at maturity ..... ***C. pereirae*** **Barboza & Bianchetti**
– Leaves elliptic to ovate, membranaceous, glabrescent to moderately pubescent; corolla mostly white or purple with yellowish-green pigmentation within; fruits red at maturity **4**
4 Inflorescence with (1–) 2–3 (–6) flowers; corolla white with 5 yellowish-green spots at the base of the lobes and throat within; seeds blackish-brown..... ***C. flexuosum*** **Sendtn.**
– Inflorescence with 5–13 (–20 or more) flowers; corolla mostly purple with yellowish-green centre and white margin within; seeds pale yellow..... ***C. caatingae*** **Barboza & Agra**
5 Corolla 4.5–6.5 (–8) mm long, with golden spots in the base of the lobes and throat within, purple pigmentation absent; ovules 2 per locule; fruits globose-compressed ..... ***C. campylopodium*** **Sendtn.**
– Corolla 7–8 (–10) mm long, with an obvious purple or brownish zone over the greenish-yellow spots within (occasionally purple pigmentation absent); ovules more than 2 per locule; fruits globose or globose-depressed ..... ***C. schottianum*** **Sendtn.**
6 Corolla rotate, rotate-pentagonal or stellate-rotate; fruiting pedicels always erect, red, usually globose or less frequently ellipsoid; seeds pale yellow **7**
– Corolla stellate; fruiting pedicels always pendent, greenish-yellow, globose; seeds brown to black **8**
7 Corolla mostly white with greenish-yellow spots and white centre, lilac or purple pigmentation absent..... ***C. baccatum*** **L. var.** ***baccatum***
– Corolla mostly lilac or purple with greenish-yellow spots and cream centre..... ***C. praetermissum*** **Heiser & P.G. Sm.**

8 Pedicels non-geniculate, pendent; major leaves small to medium-sized 2.5–6.5 (–9) cm long ..... ***C. parvifolium* Sendtn.**
– Pedicels geniculate, erect to oblique; major leaves medium- to large-sized (5–) 6–17 (–25.5) cm long **9**
9 Plants densely pubescent on stems, petioles, pedicels and sometimes also on the leaf nerves beneath, the trichomes spreading; leaves ovate .... ***C. villosum* Sendtn.**
– Plants glabrate to densely pubescent on stems, leaves and pedicels, the trichomes antrorse; leaves elliptic to very narrowly elliptic or lanceolate, less commonly ovate **10**
10 Plants glabrate to sparsely pubescent; major leaves elliptic to ovate, rarely narrowly elliptic (length/width ratio: (2–) 2.5–4 (–4.9), apex acuminate to long acuminate; calyx appendages (0.4–) 0.5–1.5 (–3) mm; corolla (6–) 7.5–12 mm long, (9–) 10–13 mm in diameter ..... ***C. mirabile* Mart.**
– Plants moderately to densely pubescent; major leaves narrowly elliptic to lanceolate (length/width ratio: (4–) 5–10 (–16), apex acute to obtuse; calyx appendages (2.8–) 3–4 (–5) mm; corolla (8–) 10–12 mm long, 13–20 mm in diameter..... ..... ***C. carassense* Barboza & Bianchetti**

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Supplementary material 1

Searchable CSV file of all specimens examined for *C. carassense* and *C. mirabile*

Authors: Gloria E. Barboza, Luciano de Bem Bianchetti, João Renato Stehmann

Data type: CSV file

Link: https://doi.org/10.3897/phytokeys.140.47071.suppl1

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RESEARCH ARTICLE

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Cryptocarya kaengkrachanensis, a new species of Lauraceae from Kaeng Krachan National Park, southwest Thailand

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Abstract

A new species of Lauraceae, *Cryptocarya kaengkrachanensis* M.Z.Zhang, Yahara & Tagane, from Kaeng Krachan National Park, Phetchaburi Province, southwestern Thailand, is described and illustrated. This species is morphologically most similar to *C. amygdalina* in that its leaves are pinnately veined, leathery, and apparently glabrous (but microscopically hairy) abaxially, twigs are yellowish brown hairy, and fruits are 1.36 to 1.85 times longer than width. However, *C. kaengkrachanensis* is distinguished from *C. amygdalina* in having the leaves of ovate and elliptic (vs. oblong-lanceolate) with leaf aspect ratio (length:width) from 1.38 to 2.28 (vs. 2.46–3.43), and ovoid fruits (vs. ellipsoid) with stalk distinctly swollen (vs. not or only slightly swollen). In addition, phylogenetic trees constructed based on internal transcribed spacer sequences (ITS) and genome-wide SNPs using MIG-seq showed that *C. kaengkrachanensis* is not sister to *C. amygdalina* and is distinct from all the other *Cryptocarya* species hitherto recognized in Thailand. Analysis including other species demonstrates that *C. floribunda* should be a synonym of *C. amygdalina*, but we recognize *C. scortechinii* as a distinct species.

Keywords

Cryptocarya, flora, Laurales, Lauraceae, new species, taxonomy, Thailand

Introduction

Lauraceae, a plant family widely distributed across the world, contain an estimated 2500–3500 species in about 50 genera, and its highest species richness is found in the tropical forests of Southeast Asia and the Americas (Rohwer 1993, Li et al. 2008, Rohwer et al. 2014, Yahara et al. 2016). In Southeast Asia, trees of Lauraceae occur widely from lowlands to high elevations (van der Werff 2001, Ngernsaengsaruay et al. 2011, Wuu-Kuang 2011, de Kok 2015, 2016a, 2016b, Yahara et al. 2016), and are often among the most dominant components of the canopy in montane forests (Ohsawa 1991, Tagawa 1995, Sri-Ngernyuang et al. 2003). Reflecting their species diversity and dominance, many taxonomic studies have been published for Lauraceae of Southeast Asia, including a classic monograph by Liou (1934), a series of publications by Kostermans (1968, 1969, 1970, 1974, 1988), floristic treatments of Lecomte (1914), Kochummen (1989), and Hô (1999), and more recent publications of new species by various authors (Nishida 2008, Liu et al. 2013, Tagane et al. 2015, de Kok 2016a, 2016b, Yahara et al. 2016, Mitsuyuki et al. 2018). In spite of all these studies, the species-level taxonomy of Lauraceae is still in need of critical scrutiny in many parts of Southeast Asia (Yahara et al. 2016).

Among Southeast Asian genera of Lauraceae, the genus *Cryptocarya* is particularly well studied. de Kok (2015) revised the taxonomy of *Cryptocarya* of Indochina and Thailand and enumerated 16 species, among which six species are endemic to Indochina and Thailand. Subsequently, de Kok (2016a) revised *Cryptocarya* of Peninsular Malaysia and recognized 17 species, among which three species are endemic to Peninsular Malaysia. However, during our field surveys in Cambodia, Laos, Vietnam, Thailand, Myanmar, Malaysia and Indonesia, we collected specimens of *Cryptocarya* that are difficult to identify using the classifications of de Kok (2015, 2016a). By combining molecular phylogenetic evidence, comparative morphological studies, and field observations on sympatric occurrences of different entities, we concluded that some of these represent new species. In this paper, we document three species of *Cryptocarya* as occurring in Kaeng Krachan National Park, Phetchaburi Province, southwest Thailand, namely *C. amygdalina* Nees, *C. pustulata* Kosterm. and a new species, described below.

According to a taxonomic treatment of de Kok (2015, 2016a), two entities we collected in Kaeng Krachan National Park, excluding *C. pustulata*, keyed out as *C. amygdalina*. This name is arrived at because (1) the leaves are elliptic, pinnately veined and leathery; (2) the mature lower leaf surface is apparently glabrous except on veins (but microscopically hairy), (3) young twigs are covered with yellowish brown hairs, and (4) mature fruits are ellipsoid or ovoid (not globose) and smooth (not ridged). *Cryptocarya amygdalina s. str.* is a species described from India, but de Kok (2015, 2016a) proposed a broader concept of *C. amygdalina* by including *C. floribunda* Nees described from Bangladesh and *C. scortechinii* Gamble described from Peninsular Malaysia. However, the discovery of what turned out to be two co-occurring species in Kaeng Krachan National Park identified as "*C. amygdalina*" following the classification system of de Kok (2015, 2016a) lead us to reassess his broader concept of "*C. amygdalina*". Here, we

show that the two species from Kaeng Krachan National Park identified as "*C. amygdalina*" are not sister to each other in a phylogenetic tree constructed by ITS sequences and genome-wide SNPs of MIG-seq (Suyama and Matsuki 2015). By combining the molecular evidence with morphological and field observations, we revise the broader concept of "*C. amygdalina*" of de Kok (2015, 2016a) by recognizing three species, *C. amygdalina* s. str., *C. scortechinii*, and a taxon from Kaeng Krachen National Park.

Materials and methods

Field observations

In Kaeng Krachan National Park (Fig. 1), we established five 100 m × 5 m plots at elevations of 360 m (12°48'11.4"N, 99°26'31.6"E, surveyed on 5 Oct. 2012), 540 m (12°48'18.48"N, 99°25'07.12"E, surveyed on 27 May 2014), 680 m (12°48'25.6"N, 99°24'24.0"E, surveyed on 25 Oct. 2013), 850 m (12°49'03.5"N, 99°22'53.5"E, surveyed on 28 Oct. 2013), and 960 m (12°49'19.7"N, 99°21'57.7"E, surveyed on 21 Oct. 2013). All vascular plants were recorded in each plot. For trees 4 m or taller, we recorded girth and height of trunks. For trees lower than 4 m and herbs, we recorded presence/absence in each of ten 10 m × 5 m sections. For all the species distinguished in the field, we collected voucher specimens and sampled some pieces of leaves for DNA isolation (vouchers were deposited at BKF and FU). Each sample collected for DNA isolation was dried with silica gel in a zipper storage bag. In addition to plants recorded in the five plots, we also collected additional specimens with flowers or fruits from outside the plots.

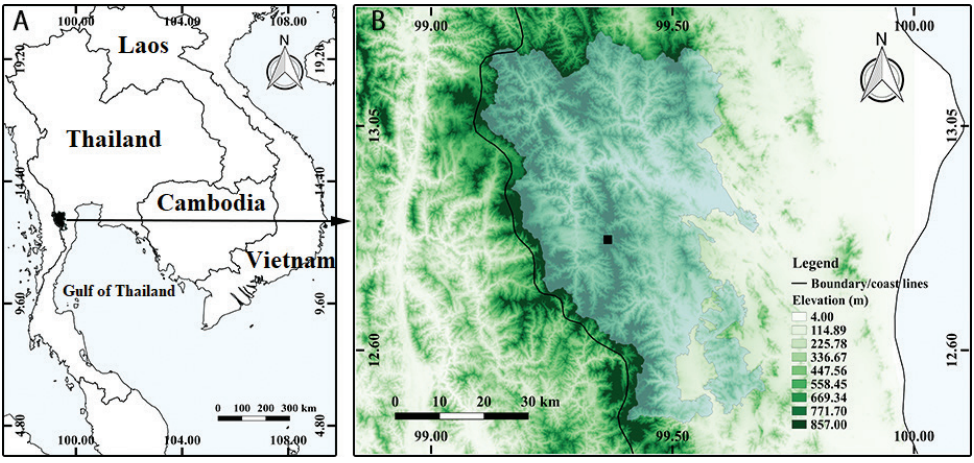

Figure 1. Study area **A** location of Kaeng Krachan National Park **B** topographical map of Kaeng Krachan National Park; solid square indicates the type locality (elevation, 960 m) of *Cryptocarya kaengkrachanensis* M.Z. Zhang, Yahara & Tagane.

Morphological observation

We scanned dried herbarium specimens and specimen images were measured for leaf length, leaf width, aspect ratio and circularity using ImageJ (Schneider et al. 2012); aspect ratio and circularity are defined as leaf length/leaf width and $4\pi \times$ (area/perimeter squared), respectively. To identify species, we examined images of type specimens using JSTOR Global Plants (https://plants.jstor.org/). We also examined specimens kept at the herbaria BKF, BO, FOF, FU, KAG, KYO, RUPP, SAR, SNP and VNM, and reviewed taxonomic literature of *Cryptocarya* in Southeast Asia (Lecomte 1914, Liou 1934, Kostermans 1988, Kochummen 1989, Hô 1999, Dy Phon 2000, Newman et al. 2007, Li et al. 2008, de Kok 2015, 2016a, Liu et al. 2017).

DNA barcoding

For DNA extraction, we milled the dried leaf material into fine powder by QIAGEN TissueLyser and the powder was washed three times with 1 ml buffer solution (including 0.1 M HEPES, pH 8.0; 2% Mercaptoethanol; 1% PVP; 0.05 M Ascorbic acid) (Toyama et al. 2015). DNA was then isolated from the washed powder by using the CTAB method (Doyle and Doyle 1987) with a slight modification (Toyama et al. 2015).

We determined partial sequences of the internal transcribed spacer (ITS region) of ribosomal DNA using the following primer sets of Rohwer et al. (2009): ITS18-F (5'-GTCCACTGAACCTTATCATTTAGAGG-3') and ITS26-R (5'-GCCGTTACTAAGGGAATCCTTGTTAG-3') and Tks Gflex DNA Polymerase (Takara Bio, Kusatsu, Japan) (Binh et al. 2018). The PCR reaction was carried out following the published protocols of Kress et al. (2009) with some modification by setting PCR cycling conditions as [95 °C 4 min (94 °C 30 sec, 55 °C 1 min, 72 °C 1 min) 25 cycles, 72 °C 10 min]. The PCR products were purified by a diluted mixture of ExoSap-IT (GE Healthcare, Little Chalfont, UK). Using the purified PCR products, forward and reverse sequencing was carried out separately by adding BigDye terminator sequencing mixture (BigDye Terminator v3.1; Applied Biosystems) and setting cycling conditions as [96 °C 1 min (96 °C 30 sec, 50 °C 30 sec, 60 °C 4 min) 25 cycles]. The BigDye reaction products were finally read with ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

We determined ITS sequences for 29 samples of *Cryptocarya* (Table1) including two samples (numbered as T1883 and T2069) of the Kaeng Krachan taxon and 27 samples of eleven known species. All of these sequences are deposited into the DDBJ database with accession numbers in Table 1. In addition, we downloaded one sequence of a *Beilschmiedia* sp. collected in Vietnam from the NCBI database (http://www.ncbi.nlm.nih.gov) for outgroup comparison (Table 1).

Table 1. Sample lists for genotyping.

Country	Area	Sample ID	DDBJ Acc. No.	Specimen	Species or variety
Cambodia	Cardamon	625	LC479107	625 (FU)	*C. concinna*
	Cardamon	657	LC479108	657 (FU)	*C. concinna*
	Bokor	1839	LC479106	1839 (FU)	*C. concinna*
	Bokor	6217	LC479111	6217 (FU)	*C. concinna*
Laos	Nam Kading	L21	LC477686	L21 (FU)	*C. sublanuginosa*
	Nam Kading	L26	LC477687	L26 (FU)	*C. sublanuginosa*
	Nam Kading	L49	LC477688	L49 (FU)	*C. sublanuginosa*
Myanmar	Tanintharyi	MY479	LC477685	MY479 (FU)	*C. amygdalina*
Thailand	Doi Inthanon	T5	LC479104	T5 (FU)	*C. kurzii*
	Doi Inthanon	T16	LC479117	T16 (FU)	*C. densiflora*
	Doi Inthanon	T1373	LC479118	T1373 (FU)	*C. densiflora*
	Khao Soi Dao	T1545	LC479098	T1545 (FU)	*C. pustulata*
	Kaeng Krachan	T1883	LC405942	T1883 (FU)	*C. kaengkrachanensis*
	Kaeng Krachan	T2069	LC405941	T2069 (FU)	*C. kaengkrachanensis*
	Kaeng Krachan	T2195	LC479099	T2195 (FU)	*C. pustulata*
	Khao Soi Dao	T2838	LC479097	T2838 (FU)	*C. chanthaburiensis*
	Kaeng Krachan	T2971	LC479100	T2971 (FU)	*C. pustulata*
	Kaeng Krachan	T3090	LC477684	T3090 (FU)	*C. amygdalina*
	Phu Kradueng	T3589	LC479102	T3589 (FU)	*C. pallens*
	Khao Luang	T3902	LC479101	T3902 (FU)	*C. albiramea*
	Khao Luang	T3944	LC479116	T3944 (FU)	*C. densiflora*
	Phu Kradueng	T4471	LC479105	T4471 (FU)	*C. kurzii*
	Phu Kradueng	T4507	LC479103	T4507 (FU)	*C. kurzii*
	Khao Luang	T4796	LC479115	T4796 (FU)	*C. scortechinii*
Vietnam	Bach Ma	V2462	LC479112	V2462 (FU)	*C. concinna*
	Bach Ma	V3287	LC479109	V3287 (FU)	*C. concinna*
	Vu Quang	V3518	LC479114	V3518 (FU)	*C. concinna*
	Vu Quang	V3566	LC479113	V3566 (FU)	*C. concinna*
	Vu Quang	V5615	LC479110	V5615 (FU)	*C. concinna*
Vietnam	–	HG315547.1	HG315547.1	–	*Beilschmiedia* sp.

Next generation DNA sequencing – MIG-seq

We amplified thousands of short sequences by using the primers of "multiplexed ISSR (inter simple sequence repeats) genotyping by sequencing" (MIG-seq, Suyama and Matsuki 2015) for 24 samples of *Cryptocarya*, following the protocol of Suyama and Matsuki (2015). Two steps of PCR were performed; for the 1st PCR step, we amplified ISSR regions from genomic DNA with MIG-seq tailed ISSR primer set-1 and diluted 50 times for each 1st PCR product with deionized water (Suyama and Matsuki 2015, Binh et al. 2018). The 2nd PCR step was conducted with common and indexed primers. The 2nd PCR products were then pooled in equimolar concentrations as a single mixture library. Fragments of size range 350–800 bp were isolated from the purified mixture of 2nd PCR products by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA) The concentration was measured by quantitative PCR (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) and then sequenced by Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA) with a MiSeq Reagent Kit v3 (150 cycle, Illumina) (Suyama and Matsuki 2015, Binh et al. 2018).

Phylogenetic tree reconstruction

For DNA barcoding analysis, MEGA X (Kumar et al. 2018, http://www.megasoftware.net/) was used to assemble the ITS sequences of 30 samples including 29 of *Cryptocarya* spp. and an additional sample of *Beilschmiedia* sp.; MAFFT ver. 7 (http://mafft.cbrc.jp) was used to align ITS sequences. We reconstructed a phylogenetic tree by the maximum likelihood method with Tamura 3-parameter model using MEGA X with a bootstrap test of 1500 replicates. In addition, we drew a TCS haplotype network (Clement et al. 2000) among 29 samples of *Cryptocarya* using POPART ver.1.7 (Leigh and Bryant 2015).

For MIG-seq, we pretreated the raw data of *Cryptocarya* samples following the quality control protocol of Suyama and Matsuki (2015) and Binh et al. (2018). We then assembled homologous sequences (designated as loci below) with the *de novo* map pipelines (ustacks, cstacks, sstacks) using Stacks ver. 1.48 (Catchen et al. 2011). First, we assembled loci by ustacks with the following settings: m = 3, M = 3, N = 2, and maximum gaps = 2 (where "m" is the minimum depth of coverage, "M" is maximum distance allowed between stacks, and "N" is the maximum distance allowed to align secondary reads to primary stacks). We then used cstacks to build a catalogue of consensus loci by assembling loci from ustacks, by setting the parameter of "number of allowed mismatches between sample (n)" as 2. Second, by using the sstacks, we associated all stacks created by ustacks with the catalog produced by cstacks. Third, we got an output vcf file containing genotypes of individuals at each locus. Subsequently, we used the vcf2phylip program (Ortiz 2019) to convert the vcf file to a phylip type file. Finally, we constructed a maximum likelihood tree with RAxML ver. 8.2 (Stamatakis 2014) and examined its reliability by bootstrapping using 1500 replicates.

Results

Field observation

In the plot at an elevation of 360 m, we recorded three sterile trees of *C. pustulata* and collected a specimen (voucher specimen number T0524) from one of these trees. In the plot at 540 m, we found no trees of *Cryptocarya*. However, a sterile specimen of *C. pustulata* was collected along the roadside at 550 m (T2971). In the plot at 680 m, we recorded two sterile trees of *C. pustulata* for which we recorded girth × height as 110.7 cm × 25 m and 11.3 cm × 5.5 m, respectively. In addition, we collected a sterile specimen (T2195) from a tree lower than 4 m. Along the roadside at 709 m, we collected a fruiting specimen of *C. amygdalina* (T3090) on 30 May 2014. In the plot at 850 m, we recorded two sterile trees of *Cryptocarya*, both of

which were lower than 4 m. However, we could not identify these trees and vouchers were not collected. In the plot at 960 m (Fig. 1), we recorded girth × height for two trees of the Kaeng Krachan taxon as 24.3 cm × 5 m and 15.7 × 4.5 m, respectively. In addition, in the vicinity of the plot, a fruiting specimen (T2069) was collected from a tree 12 m tall on 23 Oct. 2013. Young trees of the Kaeng Krachan taxon lower than 4 m were found in all ten sections of 10 m × 5 m in the 100 m × 5 m plot at the elevation of 960 m.

Morphological observation

In fruiting specimens, the Kaeng Krachan taxon (T2069; Fig. 2) has relatively shorter and broader leaves than *C. amygdalina* (T3090; Fig. 3), but the ranges are largely overlapping: the range (and average±SD) of leaf length (cm) is 2.6–10.3 (7.2±2.5) in the Kaeng Krachan taxon (n=17) vs. 9.4–14.1 (11.6±1.4) in *C. amygdalina* (n=10); the range of leaf width (cm) is 1.5–6.4 (4.2±0.6, n=17) vs. 3.5–5.2 (4.1±1.2, n=10). On the other hand, the two species are distinct in aspect ratio: 1.38–2.28 (1.79±0.25) in the Kaeng Krachan taxon vs. 2.46–3.43 (2.88±0.37) in *C. amygdalina*. The two taxa were also different in circularity, but with overlapping values: 0.55–0.77 (0.69±0.07) vs. 0.42–0.61 (0.51±0.06). Figure 4 shows that the Kaeng Krachan taxon is distinguishable from *C. amygdalina* by having a lower aspect ratio and larger circularity.

The abaxial leaf blade surface of the Kaeng Krachan taxon is sparsely covered with minute hairs that are almost invisible to the naked eye or hand lens (10 ×), but visible under a microscope (25 ×). Similarly, the lower leaf surface of *C. amygdalina* (T3090) is sparsely covered with minute hairs that are visible only under a microscope (25 ×). Both *C. amygdalina* and the Kaeng Krachan taxon have scalariform to scalariform-reticulate tertiary veins and it is difficult to distinguish between the two species by their venation.

The specimen T2069 of the Kaeng Krachan taxon had smaller fruits than the specimen T3090 of *C. amygdalina*: the range (and average±SD) of fruit length (mm) is 9.88–13.82 (11.49±1.28, n=14) vs. 22.07–28.1 (25.67±2.3, n=6), and the range (average±SD) of fruit width (mm) is 5.81–9.67 (7.53±2.36, n=14) vs. 11.27–12.63 (12.03±0.49, n=6). However, the fruits of T2069 (Fig. 2E) and T3090 (Fig. 3E) were green and not fully matured when collected. While the fruits of the Kaeng Krachan taxon are ovoid (Fig. 2E, F), the fruits of *C. amygdalina* are ellipsoid (Fig. 3E, F) but fruits of both specimens are still immature. Fruit stalks of *C. amygdalina* (T3090) are smooth and not or only slightly swollen (Fig. 3E, F) as in the lectotype (*Wallich Cat. 2585*, K001116509) and isolectotype (*Francis 990*, E00393147) of *C. amygdalina*. On the other hand, fruit stalks of the Kaeng Krachan taxon (T2069) are rough and swollen (Fig. 2E).

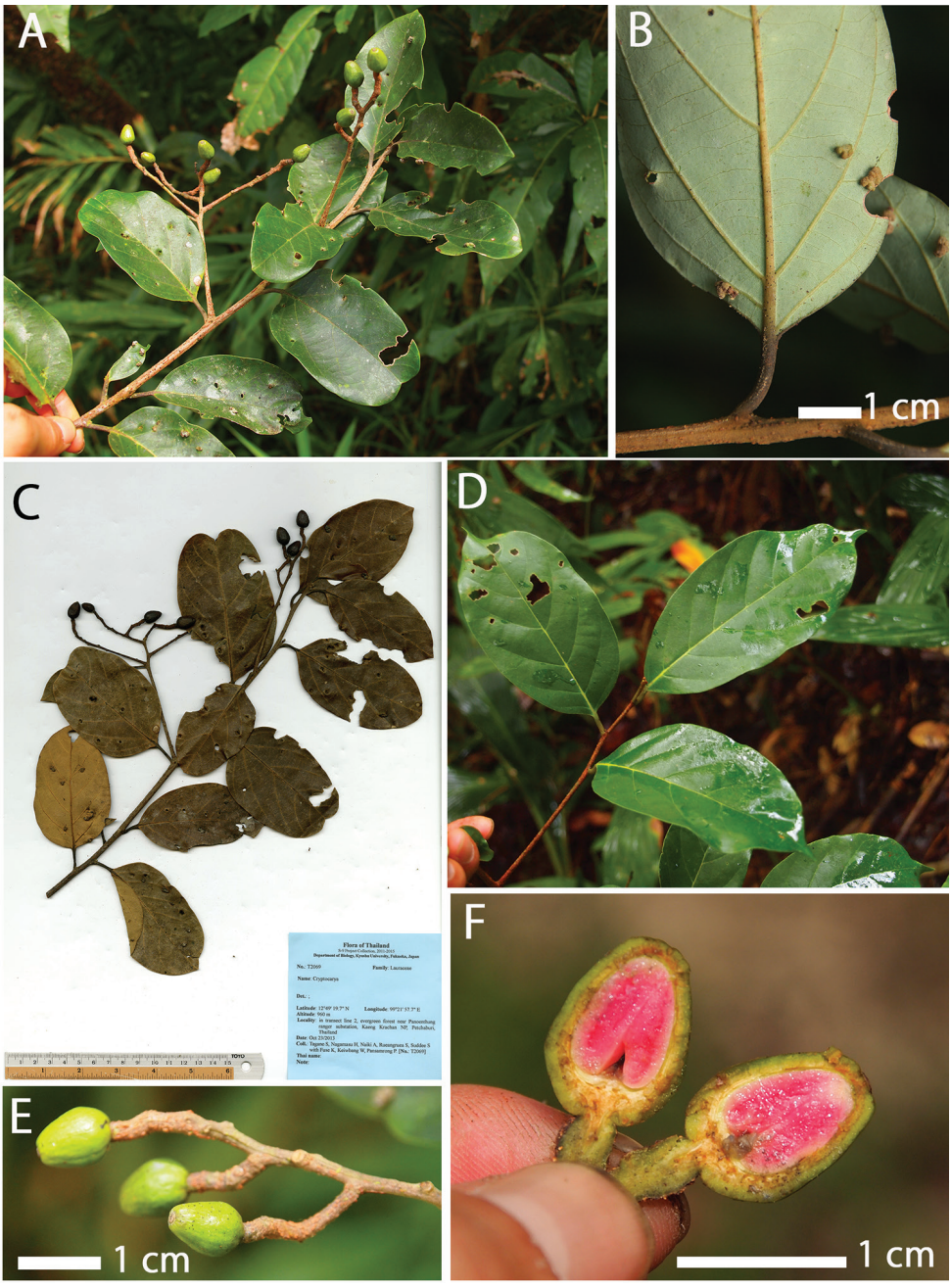

Figure 2. *Cryptocarya kaengkrachanensis* M.Z. Zhang, Yahara & Tagane **A** branch with immature fruit **B** lower leaf surface **C** holotype: *Tagane et al. T2069* (KYO) **D** young branchlet **E** part of an infructescence with immature fruits **F** longitudinal sections of an immature fruit.

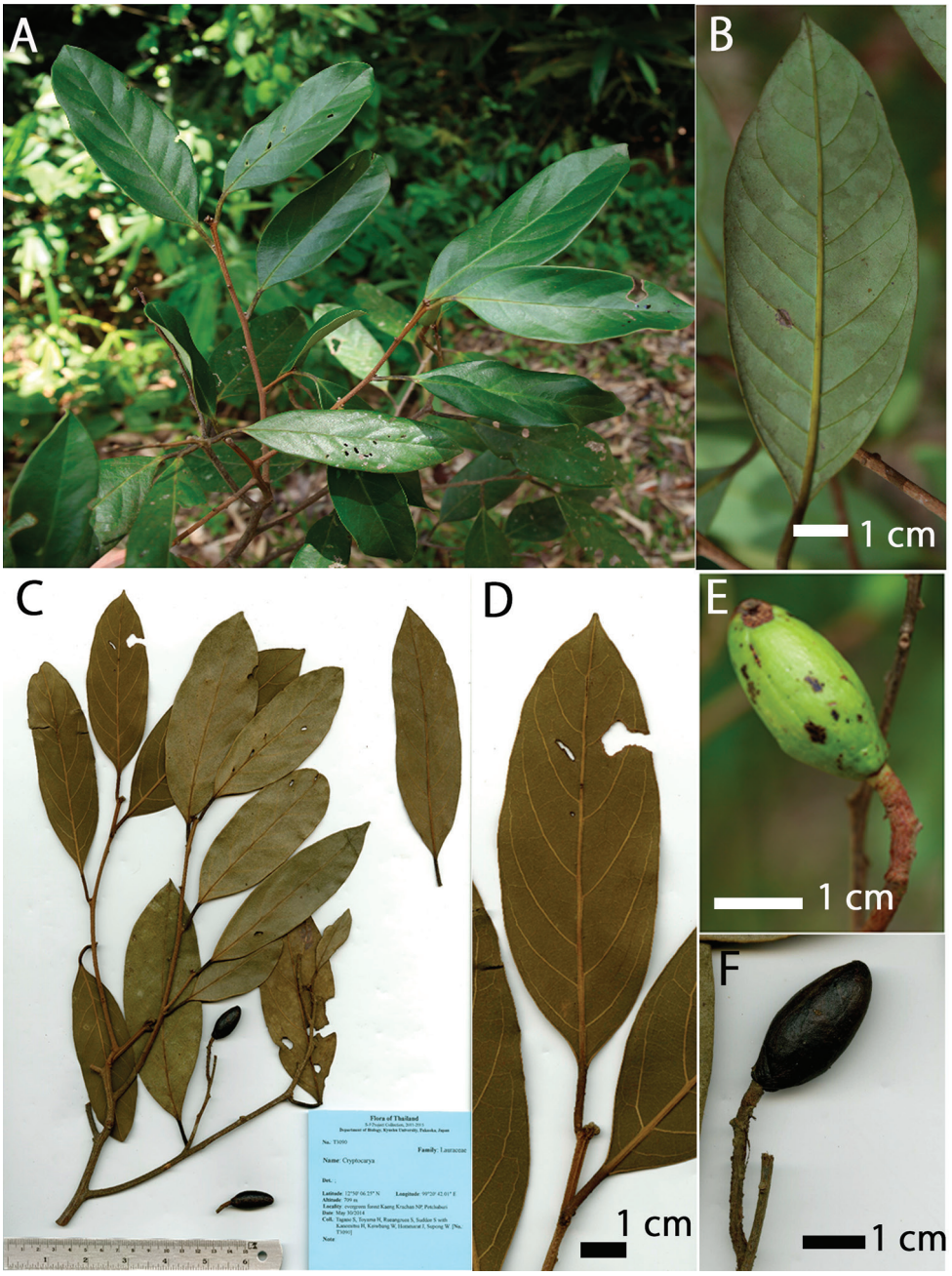

Figure 3. *Cryptocarya amygdalina* **A** leafy branchlet **B** lower leaf surface **C** specimen *Tagane et al. T3090* (KYO) **D** lower leaf surface (dry) **E** fresh immature fruit **F** dried fruit.

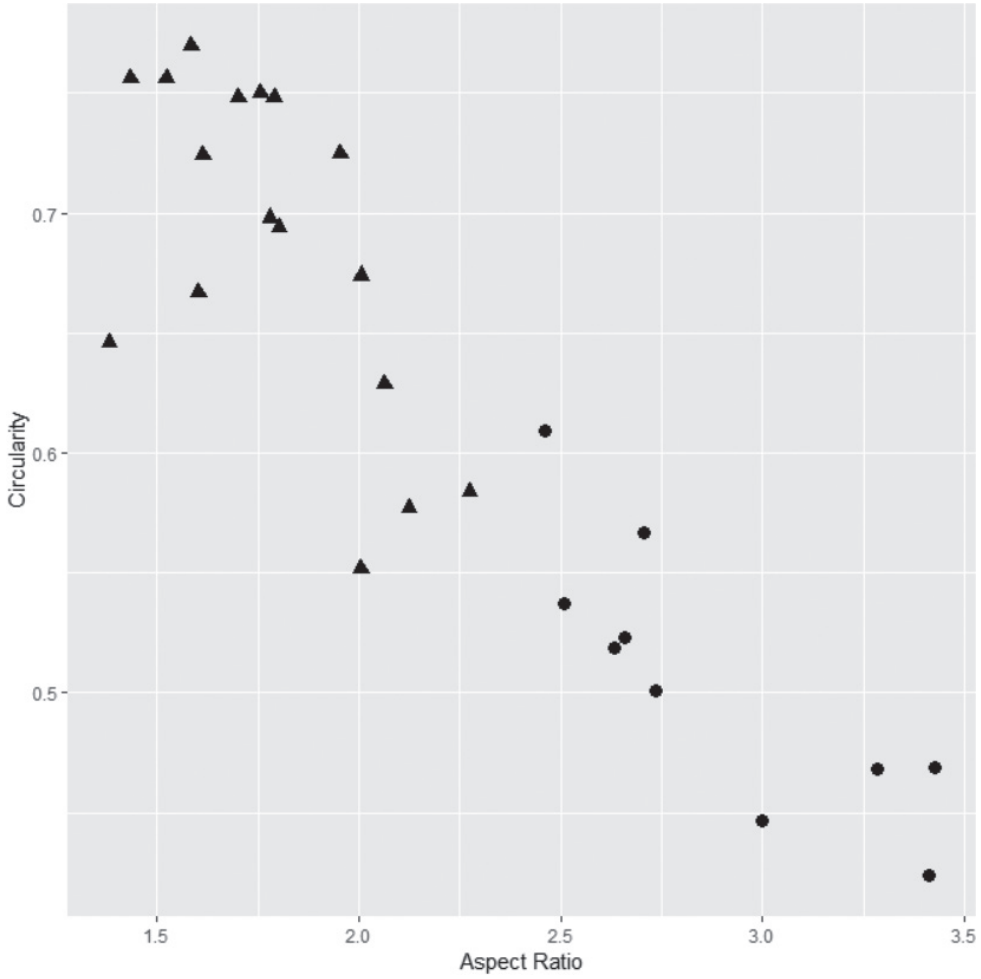

Figure 4. Scatter plot of leaf aspect ratio (horizontal axis) and circularity (vertical axis). Solid circles: *Cryptocarya amygdalina*, solid triangles: *C. kaengkrachanensis*.

Phylogenetic analysis

A phylogenetic tree constructed from ITS sequences with length of about 670 bp (Fig. 5) showed that *C. amygdalina* is close to *C. albiramea* Kosterm. (T3902) and *C. pustulata*, and the bootstrap support for the monophyly of the clade including these species was 84%. For the ITS sequence, two samples initially identified as *C. amygdalina* (T3090 collected from Kaeng Krachan, Thailand and MY479 collected from Myanmar) were identical in the ITS sequences determined. Also, two samples of *C. pustulata* (T2195 of Kaeng Krachan and T1545 collected from Kao Soi Dao, Chanthaburi, Thailand, the type locality) were identical but another ITS sequence of *C. pustulata* (T2971) differed from T2195 in one base pair (Fig. 6). On the other hand, *C. amygdalina* is one species in a well supported clade with

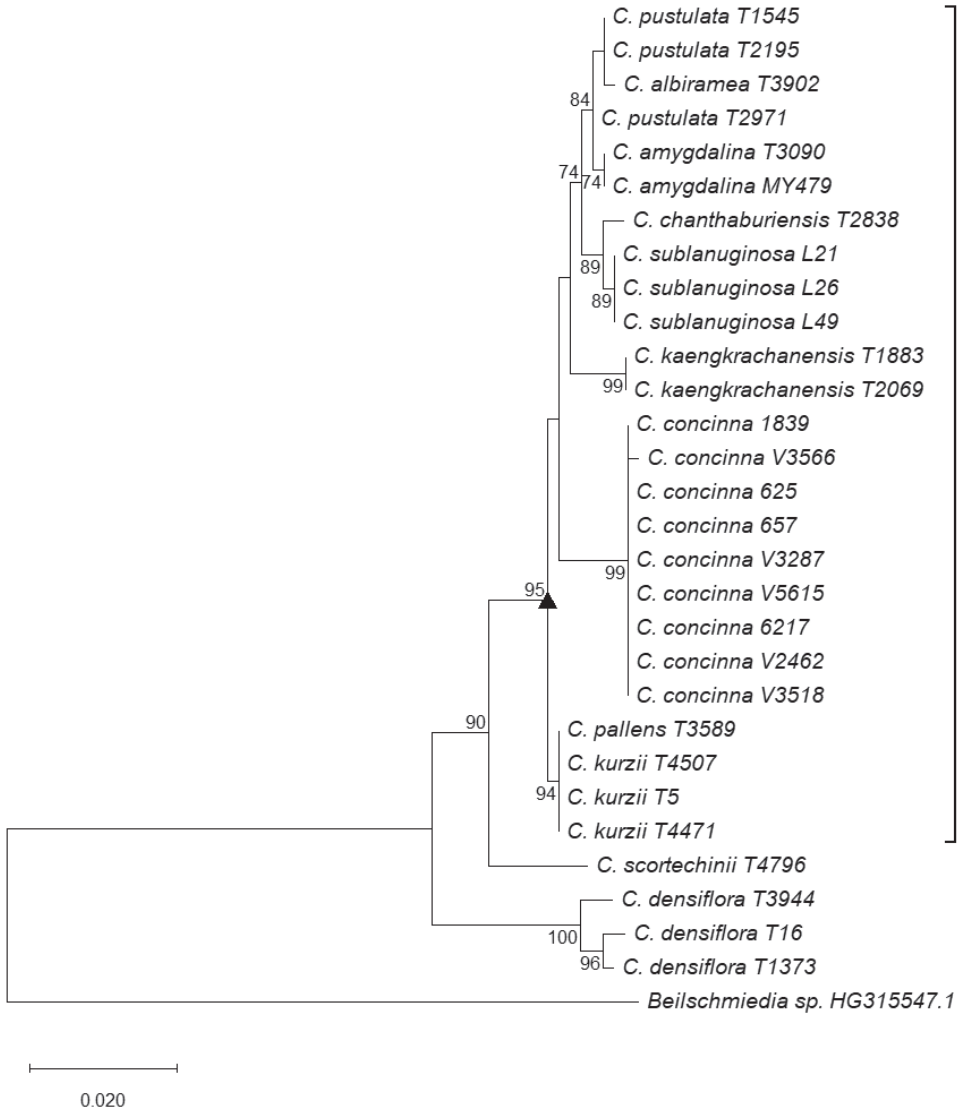

Figure 5. Maximum likelihood tree of *Cryptocarya* species from Thailand reconstructed from ITS sequences. Numbers: bootstrap values; Scale bar: mean number of nucleotide substitutions per site; solid triangle and square bracket: clade of 95% bootstrap value.

the plants of the Kaeng Krachan taxon sister to this clade, differing by 10 base pairs in the ITS sequences (Fig. 6) and are distinct from each other in the ITS haplotype network (Fig. 6).

For 24 of the samples that belonged to a clade supported by 95% bootstrap value in the ITS tree (Fig. 5), we constructed a MIG-seq tree in which conspecific clusters of *C. amygdalina* (T3090, MY0479), the Kaeng Krachan taxon (T1883, T2069) and *C. pustulata* (T2195, T2971, T1545) were supported by 100% bootstrap values (Fig.

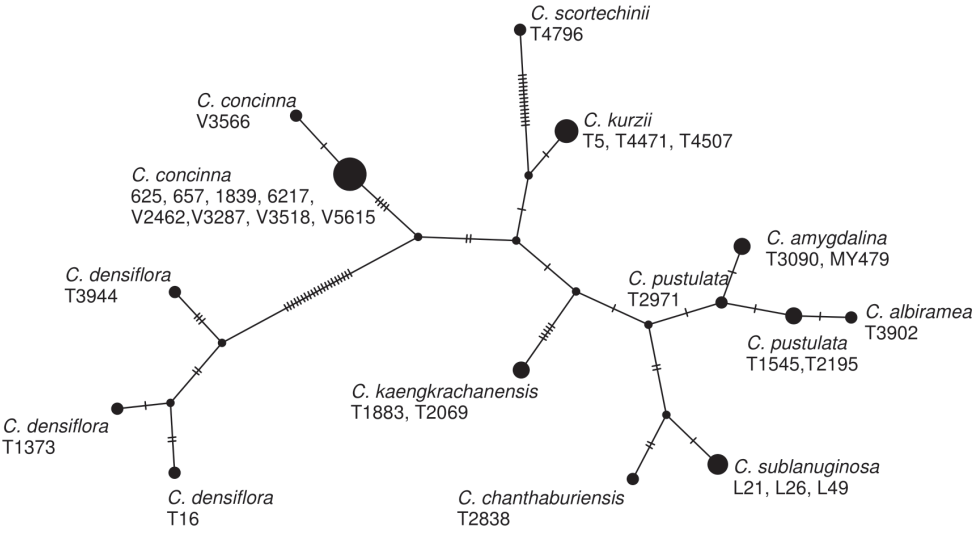

Figure 6. A haplotype network of *Cryptocarya* species from Thailand constructed from the ITS sequences.

7). While *C. amygdalina* and *C. pustulata* were sister to each other, the Kaeng Krachan taxon was not sister to either of these species, but instead to *C. albiramea* (Fig. 7), with 99% bootstrap (BS).

Based on the evidence of morphology and phylogenetic analysis presented above, the two samples (T1883 and T2069) collected from Kaeng Krachan national park clearly represent a distinct and new species, which is named as *Cryptocarya kaengkrachanensis*.

Taxonomy

Cryptocarya kaengkrachanensis **M.Z.Zhang, Yahara & Tagane, sp. nov.**
urn:lsid:ipni.org:names:77206953-1
Fig. 2

Diagnosis. *Cryptocarya kaengkrachanensis* resembles *C. amygdalina* in having pinnately veined, leathery leaves apparently glabrous (microscopically hairy) below, young twigs with yellowish brown hairs and fruits 1.36–1.85 times longer than width. However, *C. kaengkrachanensis* differs from *C. amygdalina* (Fig. 3) in having the leaves ovate and elliptic to narrowly elliptic (vs. oblong-lanceolate) with leaf aspect ratio from 1.38 to 2.28 (vs. 2.46–3.43) (Fig. 4), and fruits ovoid (vs. ellipsoid) with the stalk distinctly swollen (vs. not or only slightly swollen). While *C. kaengkrachanensis* was sister to *C. albiramea* in MIG-seq tree, *C. kaengkrachanensis* is distinguished from *C. albiramea* by elliptic leaves with leaf aspect ratio less than 2.5 (vs. oblong-lanceolate leaves with leaf aspect ratio more than 2.5).

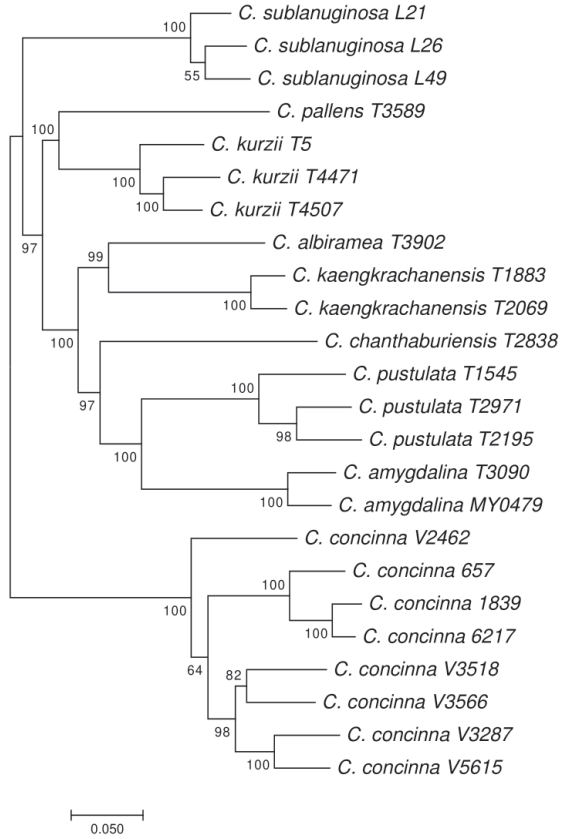

Figure 7. A maximum likelihood tree of *Cryptocarya* species from Thailand. reconstructed using MIG-seq data. Numbers: bootstrap values; Scale bar: mean number of nucleotide substitutions per site;

Type. THAILAND. Phetchaburi Province: Kaeng Krachan National Park, 960 m elev., 12°49'19.7"N, 99°21'57.7"E, 23 Oct. 2013, *Tagane S.*, *Nagamasu H.*, *Naiki A.*, *Rueangruea S.*, *Suddee S.*, *Fuse K.*, *Keiwbang W.*, *Pansamrong P. T2069* [fr.] (holotype KYO!, isotypes BKF!, FU!, KAG!).

Description. Trees up to 12 m tall. Young twigs densely covered with appressed short yellowish to brown hairs, old twigs lenticellate, terete and slightly hairy. Leaves alternate; blade leathery, ovate, elliptic to narrowly elliptic, the range (and average±SD) of leaf length (cm) is 2.6–10.3 long (7.2±2.5, n=17), the range (and average±SD) of leaf width (cm) is 1.5–6.4 (4.2±0.6, n=17) wide leaf aspect ratio from 1.38 to 2.28, obtuse or retuse at apex in adult trees, acuminate in young trees, broadly cuneate at base, green and not lustrous above and slightly glaucous below when fresh, brown above and grey brown below when dry, apparently glabrous but microscopically sparsely hairy below; pinnately veined, midrib sunken above, raised below, secondary veins 6 or 7 pairs, slightly sunken above, raised below, tertiary veins scalariform-reticulate, faintly visible

above, raised below; the range (and average±SD) of petiole length (cm) is 0.7–1.5 long (1.1±0.22, n=10), flat above, rounded below, dark brown when dry, covered with short yellowish hairs. Inflorescences and flowers not seen. Infructescence axillary, 4–17 cm long (8.4±3.4, n=16) (the range, average±SD), rachis hairy, lenticellate; bracteoles not seen. Immature fruits ovoid, 9.9–13.8 mm long (11.49±1.28, n=14), 5.8–9.7 mm wide (7.53±2.36, n=14) with aspect ratio 1.36–1.85 (1.54±0.14, n=14). yellow green when fresh, dark brown when dry, shortly hairy. Fruiting stalk slightly swollen, rough, light brown when fresh, dark brown when dry. Mature fruits not seen.

Other specimens of *C. kaengkrachanensis* examined. THAILAND. Phrae Province: between Ban Nam Krai and Pha Tuem, 16 Apr 1970, *Smitinand T.*, *Cheke A.S. 10817* [BKF 46511!]. Phetchaburi Province: Kaeng Krachan National Park, 960 m elev., 12°49'19.7"N, 99°21'57.7"E, 23 Oct. 2013, *Tagane S.*, *Nagamasu H.*, *Naiki A.*, *Rueangruea S.*, *Suddee S.*, *Fuse K.*, *Keiwbang W.*, *Pansamrong P. T1883* (BKF!, FU!). Kanchanaburi Province: Thong Pha Phum District, Pilok, at the Thai-Burmanese border. C. 900 m. 14 41.0'N, 98 21.8'E, tree 12m, 25 January 2009 [fr.], *Middleton D.J.*, *Karaket P.*, *Lindsay S.*, *Suddee S. 4785* [BKF 182421!].

Distribution. Endemic to Thailand. The new species is currently only known in a few protected areas of Phrae, Phetchaburi and Kanchanaburi Provinces including Kaeng Krachan National Park.

Etymology. The specific epithet *kaengkrachanensis* is derived from the name of the national park from which the species has first been recorded.

Conservation status. Least Concern (IUCN 2012, 2017). This species occurs in hill evergreen forests of some protected areas including Kaeng Krachan National Park and there is no sign of declining trends.

Discussion

In Kaeng Krachan National Park, we found three species of *Cryptocarya* that grew at different elevations. While *C. pustulata* was collected at lower elevations, 360 m, 550 m and 680 m, *C. amygdalina* and *C. kaengkrachanensis* were collected at higher elevations, 709 m and 960 m. *Cryptocarya pustulata* is a canopy tree constituent and attains a height of 25 m and we were unable to collect fertile material of this species. On the other hand, *C. kaengkrachanensis* is a subcanopy tree and we collected fruits from a tree 12 m tall. This species was common in the hill evergreen forest at an elevation of 960 m. *Cryptocarya amygdalina* and *C. kaengkrachanensis* are suspected to flower in different seasons because we collected a fruiting specimen of *C. amygdalina* (T3090) on 30 May 2014, and a fruiting specimen of *C. kaengkrachanensis* (T2069) on 23 Oct. 2013. The above observations in the field supported our hypothesis that there are three ecologically distinct species of *Cryptocarya* in Kaeng Krachan National Park.

In addition to ecological differences, the three species are genetically well differentiated. In particular, *C. amygdalina* and *C. kaengkrachanensis* differed by 10 base pairs

of the ITS sequences and are placed in distant positions on both the ITS and MIG-seq trees. While *C. kaengkrachanensis* was sister to *C. albiramea* in MIG-seq tree, *C. kaengkrachanensis* is distinguished from *C. albiramea* by having elliptic leaves with leaf aspect ratio less than 2.5 (vs. oblong-lanceolate leaves with leaf aspect ratio more than 2.5).

To apply names to the species of *Cryptocarya* in Kaeng Krachan National Park, we examined the images of the lectotype and isolectotype of *C. amygdalina* and noticed that the description of the fruit morphology of *C. amygdalina* by de Kok (2015) does seem to not agree with the type material of *C. amygdalina*. While de Kok (2015) described the fruit stalk of *C. amygdalina* as "red, strongly swollen when mature" and used this state to distinguish *C. amygdalina* from morphologically similar species in the key, the type of *C. amygdalina* has fruit stalks not or only slightly swollen, as in our collection T3090. On the other hand, the fruit stalks of *C. kaengkrachanensis* are somewhat swollen, and brownish rather than red. The fruit stalks of *C. scortechinii* Gamble in Malay Peninsula are red and strongly swollen (e.g. G. Kedah, *T. Witmore FRI 4683*, KEP!). We suggest that the concept of *C. amygdalina* adopted by de Kok (2015) is a heterogeneous one that includes *C. amygdalina* s. str., *C. scortechinii* (see below) and *C. kaengkrachanensis.* In fact, the following specimen cited under *C. amygdalina* by de Kok (2015) is identical to *C. kaengkrachanensis* in leaf morphology; Phrae: between Ban Nam Krai and Pha Tuem, 16 Apr 1970, *Smitinand T, Cheke A.S. 10817* [BKF 46511]).

Before concluding that T2069 was an undescribed species, we needed to compare it with the type material of *C. floribunda* Nees and *C. scortechinii* Gamble, two names that were treated as synonyms of *C. amygdalina* by de Kok (2015). The type specimens of *C. floribunda* [Wallich Cat. n. 2593, BM000880687, K000768399, MEL2390468, MEL2390469, MEL2390467, MNHN-P-P02010447] have only flowers and we cannot verify the fruit characters. However, these specimens are most similar to the lectotype and isolectotype of *C. amygdalina* in floral and vegetative morphology. Thus, we agree with the earlier treatment of de Kok (2015, 2016a) that *C. floribunda* is a synonym of *C. amygdalina*. We collected a specimen (T4796) morphologically similar to the type specimens of *C. scortechinii* [King's collector 6297, L0036248-lectotype, MEL2386583-isolectotype] at the elevation of 322 m at Khao Luang, peninsular Thailand. Although our collection is sterile, it is identified as *C. scortechinii* based on its leaf size, shape, and venation as well as its distribution in peninsular Thailand. As is shown in Fig. 5 and 6, *C. scortechinii* was placed in a distant position from *C. amygdalina*. Thus, our evidence does not support the treatment of de Kok (2015, 2016a) that *C. scortechinii* is a synonym of *C. amygdalina*. *Cryptocarya kaengkrachanensis* is easily distinguished from *C. scortechinii* by its elliptic leaves (aspect ratio lower than 2.5) that are obtuse at the apex and not lustrous above. Based on the evidence provided above, we here concluded that *C. kaengkrachanensis* is an undescribed species.

While de Kok (2015) included *C. amygdalina* in the group characterized by "Mature lower leaf surface glabrous, except on veins", both *C. amygdalina* and *C. kaengkrachanensis* have minute hairs on the lower surface of leaves that are almost invisible to the naked eye and hand lens (10 ×), but clearly visible under magnification (25 ×). Be-

cause most species of *Cryptocarya* are more or less hairy on the lower blade surface and hairiness of leaves is very variable, we do not use the hairiness trait in the following key.

In his key, de Kok (2015) characterized *C. amygdalina* as "Tertiary veins scalariform" and other species as "Tertiary veins reticulate to scalariform", but the specimen T3090 of *C. amygdalina* has undulate scalariform veins that are connected by finer reticulate veins. Among Thai species of *Cryptocarya*, *C. chanthaburiensis* Kosterm., *C. concinna* Hance and *C. densiflora* Blume are characterized by reticulate tertiary veins, but other species including *C. amygdalina* and *C. kaengkrachanensis* have more or less scalariform tertiary veins connected with finer reticulate veins. Thus, we used only two categories of venations, reticulate and scalariform, in the key that follows. In de Kok (2015), *C. diversifolia* Blume, *C. ferrea* Blume, *C. laotica* (Gagnep.) Kosterm., *C. nitens* (Blume) Koord. & Valeton, and *C. rugulosa* Hook.f. from Thailand, but these species are not included in the following key because we could not confirm the distribution of these species in Thailand.

Identification Key to the species of *Cryptocarya* in Thailand

1 Leaf aspect ratio less than 2.5 ..... **2**
– Leaf aspect ratio more than 2.5 ..... **3**
2 Basal lateral veins attaining to 1/3 to 1/2 of leaf blade; tertiary veins reticulate; fruits globose..... ***C. densiflora***
– Basal lateral veins attaining less than 1/4 of leaf blade; tertiary veins scalariform; fruits ovoid..... ***C. kaengkrachanensis***
3 Leaves (dried) distinctly glaucous below..... **4**
– Leaves (dried) not or only slightly glaucous below..... **5**
4 Leaves lustrous above when fresh, distinctly foveolate above when dried ..... ***C. albiramea***
– Leaves not lustrous above when fresh, not foveolate above when dried.... ***C. kurzii***
5 Tertiary veins mostly reticulate..... **6**
– Tertiary veins scalariform **7**
6 Leaves (thinly) leathery; lamina oblong, oblong-lanceolate, (5.5–)10–19 × (2.6–)3–4.6 cm; petiole 0.8–1.5 cm long ..... ***C. chanthaburiensis***
– Leaves papery; lamina elliptic to elliptic-oblong, (3–)5–10(–13) × (1.5–)2–3(–6) cm; petiole 0.4–0.8(–1) cm long..... ***C. concinna***
7 Inflorescences longer than leaves; fruits ellipsoid **8**
– Inflorescences shorter than leaves; fruits globose or unknown (for *C. pustulata*) **9**
8 Leaves lustrous above when fresh; fruit stalks thickly swollen ..... ***C. scortechinii***
– Leaves not lustrous above; fruit stalks not or slightly swollen..... ***C. amygdalina***
9 Leaves waxy below, light brown when dried ..... ***C. pallens***
– Leaves not waxy below, dark brown when dried **10**
10 Finely reticulate veins raised on the upper surface of dried leaves .... ***C. pustulata***
– Finely reticulate veins visible but not raised above..... ***C. sublanuginosa***

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