

A taxonomic synopsis of *Virola* (Myristicaceae) in Mesoamerica, including six new species

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Abstract

A taxonomic synopsis of *Virola* (Myristicaceae) is presented for Mesoamerica. Fourteen species are recognised, amongst them six are described and published as new, based on morphology: *V. allenii* D.Santam. & Aguilar, **sp. nov.** from Costa Rica, *V. otobifolia* D.Santam., **sp. nov.** from Panama and *V. amistadensis* D.Santam., **sp. nov.**, *V. chrysocarpa* D.Santam. & Aguilar, **sp. nov.**, *V. fosteri* D.Santam., **sp. nov.** and *V. montana* D.Santam., **sp. nov.** from both Costa Rica and Panama. Additionally, a lectotype is designated for *V. koschnyi*, accompanied by an epitype in view of the fragmentary material. Finally, we recognise *V. laevigata* and *V. nobilis* as morphologically distinct species, though these are frequently considered synonyms of *V. guatemalensis* and *V. surinamensis*, respectively. Of the fourteen accepted species, twelve of them are endemic to Mesoamerica, while the remaining two species (*V. elongata* and *V. sebifera*) extend into South America. Illustrations, species diagnoses and distribution maps for each species are provided, as is an identification key to all species.

Keywords

Costa Rica, Flora Mesoamericana, hallucinogenic, Magnoliales, nutmeg, Panama, taxonomy

Introduction

Myristicaceae, a member of the magnoliid order Magnoliales (APG IV 2016), comprises 21 genera and nearly 500 species of woody trees, shrubs and, occasionally, lianas (i.e. *Pycnanthus* spp.) By far the most economically important species of the family is *Myristica fragrans* Houtt., the source of the common spices nutmeg and mace. Myristi-

caceae has a broad pantropical distribution, though most species are found in lowland rainforest habitats. The family is represented in America by about 101 species (Ulloa Ulloa et. al. 2017) in six endemic genera: *Bicuiba* W. J. de Wilde (1 sp.), *Compsonura* (A. DC.) Warb. (19 spp.), *Iryanthera* Warb. (~20 spp.), *Osteophloeum* Warb. (1 sp./ 2 subsp.; as *Osteophloem* in Smith and Wodehouse 1938), *Otoba* (A. DC.) Karsten (~10 spp.) and *Virola* Aubl. (~60 spp.). Species richness of American Myristicaceae is highest in the lowland forest of the Amazon, where it is considered a hyperdominant family (Smith and Wodehouse 1938; de Wilde 1991; Kühn and Kubitzki 1993; Janovec 2000; Jaramillo et al. 2004; ter Steege et al. 2013; Cardoso et al. 2017).

Virola is the largest genus of Myristicaceae in America and, with about 60 species, it is the fourth largest genus in the family. The plants have two particularly distinctive characteristics: their growth form and the mature fruit. The growth form, sometimes called myristicaceous branching (e.g. Gentry 1993), corresponds largely with Massart's Model (Hallé et al. 1978), which is characterised by a monopodial, orthotropic principal shoot with plagiotropic lateral branches that are developed in regular tiers by rhythmic branching of the main axis (Kühn and Kubitzki 1993). Fruits of *Virola* are distinctive and striking: they dehisce by two valves when ripe, displaying their single seed, which is covered by a brightly coloured lacinate aril that is rich in proteins, lipid and sugars. Birds and monkeys are primary dispersers of these seeds (Howe and Vande Kerckhove 1980; Howe 1981, 1982, 1993; Sabatier 1997; Moreira et al. 2017). Not surprisingly, given the importance of their fruits as a food for wildlife and its abundance in many lowland forests, the ecological importance of *Virola* has been extensively documented (e.g. Frankie et al. 1974; Pitman et al. 2008; Steeves 2011; Riba Hernández 2017). Below, we provide a review of the morphology, biogeography, taxonomic history and ethnobotanical uses of *Virola*.

Morphology

As with many magnoliids, *Virola* has aromatic tissues. Unlike other magnoliids, though, when the bark is cut or a twig is broken, it yields an exudate that is initially watery and clear and quickly changes to red or pinkish. The leaves, which are exstipulate and simple with entire margins and occasionally have pellucid punctuation, are distichous. Most surfaces of the plant, including leaves, are usually covered with stellate or dendritic trichomes. Staminate inflorescences are paniculate, with few to many branches. Flowers, sometimes reported as fragrant, are unisexual, ebracteolate and inserted on a receptacle; they are produced in lax to dense clusters. Their yellow or brown perianth is connate to varying degrees and uniseriate, usually with 3-lobes. The androecium is compound, with filaments fused into a column, with 3– (4–6) anthers fused to the column. The pistillate inflorescences are shorter than staminate inflorescences. Pistillate flowers have a perianth that is more carnosose than those of the staminate flowers and their gynoeceia are pubescent and sessile or short-stipitate, topped by a stigma that is usually bilobed (Smith and Wodehouse 1938; de Wilde 1991; Kühn and Kubitzki 1993; Jaramillo et al. 2004).

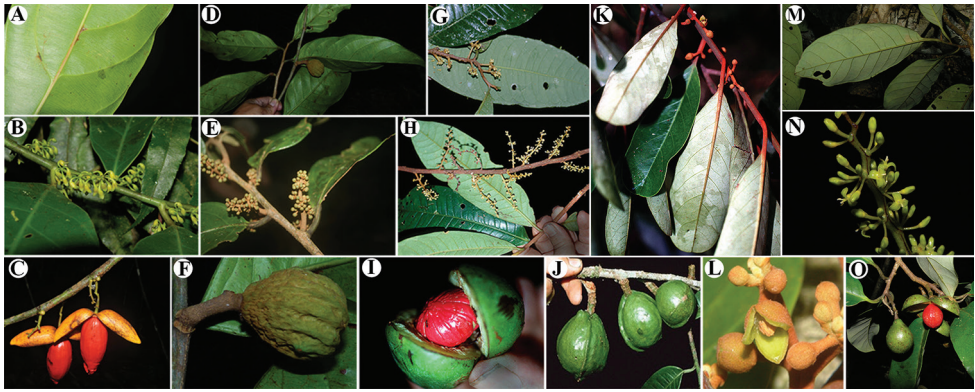


Figure 1. Genera of Myristicaceae present in Mesoamerica **A–C** *Compsononeura* sect. *Compsononeura* (*C. excelsa*) **D–F** *Compsononeura* sect. *Hadrocarpa* (*C. capitellata*) **G–J** *Iryanthera* (**G–I** *Iryanthera* spp.; **J** *I. megistocarpa*) **K–L** *Osteophloeum platyspermum* **M–O** *Otoba novogranatensis*. Photos by Reinaldo Aguilar (**A–C**, **M–O**), Benjamin Chambi (**E**), Jason D. Wells (**D**, **F** from <http://atrium.andesamazon.org/>); Robin Foster (**G–I**, **K** from <https://plantidtools.fieldmuseum.org/en/nlp>); Alwyn H. Gentry (**J** from <http://www.tropicos.org>); and David A. Neill (**L**).

In Mesoamerica, where five of the six native genera of American Myristicaceae occur (Fig. 1), *Virola* can be identified using the following key:

- 1 Leaf blades with tertiary veins conspicuous and more or less parallel and perpendicular to the lateral veins.....***Compsononeura*** (Fig. 1A–F)
- Leaf blades with tertiary veins inconspicuous or, when conspicuous, not as above2
- 2 Leaf blades with malpighiaceous trichomes on abaxial surface.....3
- Leaf blades with stellate, dendritic, or irregularly stellate trichomes on abaxial surface4
- 3 Vernation convolute; abaxial surface of leaf blades without hyaline crystals; flowers with bracteoles; fruits transversally ellipsoid, if globose, the pericarp 7–8 mm thick; seeds not gibbose at the apex***Iryanthera*** (Fig. 1G–J)
- Vernation conduplicate; abaxial surface of leaf blades with hyaline crystals; flowers without bracteoles (in Mesoamerica); fruits longitudinally globose to subglobose; seeds usually gibbose near the apex ***Otoba*** (Fig. 1M–O)
- 4 Trichomes on abaxial leaf surface stellate, concolorous; inflorescences sessile, with 1–2 (–3) main branches; flowers with bracteoles; stamens 10 (in Mesoamerica); fruits transversally ellipsoid.....***Osteophloeum*** (Fig. 1K–L)
- Trichomes on abaxial leaf surface dendritic, irregularly stellate or if stellate, the central part of the trichome reddish to reddish-clear, contrasting in colour with the hyaline branches; inflorescences distinctly pedunculate, with 1 main branch; flowers without bracteoles; stamens 3–7; fruits ellipsoid, globose, ovoid or subglobose***Virola***

Biogeography

Virola is found throughout tropical America. Species have been collected from Mexico to southern Brazil, though are notably absent in El Salvador. Occurrence in the Caribbean is limited: one species is found in the West Indies (*V. surinamensis* [Rol. ex Rottb.] Warb.), though a fossil *Virola* flower, recently described from Dominican Republic (Poinar and Steeves 2013), suggests that genus has persisted over geological time in the region. Species richness is highest in western Amazonia, with the greatest concentration of species in Brazil (~35 spp.), Colombia (~29 spp.) and Peru (~23 spp.), where they are found mainly in humid forests, though some species grow in dry formations (e.g. *V. sessilis* [A. DC.] Warb., *V. subsessilis* [Benth.] Warb.) (Rodrigues 1980, 2015; Gradstein 2016; Vásquez et al. 2018).

Within Mesoamerica, the greatest concentration of *Virola* species occurs in Costa Rica and Panama. Together, these two countries contain 10 and 11, respectively, of the 14 Mesoamerican species (Fig. 2). Of the 14 species treated in this synopsis, 12 are endemic to Mesoamerica, while *V. elongata* (Spruce ex Benth.) Warb. occurs from Panama to Brazil and *V. sebifera* Aublet from Honduras to Brazil; both of these species are widespread in South America. The majority of *Virola* species occupy lowland wet forest below 800 m elevation, with a few species reaching montane forests up to 2000 m elevation (e.g. *V. montana*). A single montane species of *Virola* can occur on both slopes, though, within a country or region, there is a tendency to find them on only one, more often the Caribbean slope (e.g. *V. fosteri*, *V. amistadensis*, *V. multiflora*). A list of the current species of *Virola* in Mesoamerica with geographic distribution by country, elevations and slope is given in Table 1.

Ethnobotany

Virola has diverse ethnobotanical uses and there are many reports of local use of several species of *Virola* for many non-medicinal purposes. For example, the oils from the seeds of *Virola* (e.g. *V. guatemalensis*, *V. sebifera*) are used as lubricant in machinery and to make soap and candles that emit intense light, produce little smoke and smell pleasant; Pesce 2009). Instead of using the extracted oils, indigenous people of the Talamanca mountains of Costa Rica (Pittier 1978) and the Cuna tribe of Panama (Duke 1975) burn whole seeds as candles. Wood from many species, including *Virola fosteri*, *V. kotschyi*, *V. sebifera* and *V. surinamensis*, is used for construction and carpentry, to make matchsticks, as pulp for paper and to manufacture plywood (Rodrigues 1972; Flores-Vindas and Obando-Vargas 2014; Pérez and Condit 2018). The seeds and arils of *Virola guatemalensis*, a common shade tree in coffee plantations in Veracruz, Mexico (Sánchez Hernández et al. 2017), are used to flavour chocolate drinks and other beverages (Warburg 1897; Standley and Steyermark 1946; Pennington and Sarukhán 2016).

Virola also produces many chemicals that are biologically active in humans. For example, *Virola* is perhaps best known for its hallucinogenic properties, which are often



Figure 2. Geographic distribution of *Virola* in Mesoamerica, with the number of species native to each country indicated in parentheses below the country’s name.

Table I. List of the 14 accepted species of *Virola* in Mesoamerica with countries of occurrence, slope preference and elevation within Mesoamerica.

| Species | Distribution | Slope | Elevations (m) |
|---|--|---|-----------------|
| <i>V. allenii</i> D. Santam. & Aguilar. | Costa Rica | Pacific | 0–350 (–1350) |
| <i>V. amistadensis</i> D. Santam. | Costa Rica, Panama | Caribbean | 650–1200 |
| <i>V. chrysocarpa</i> D. Santam. & Aguilar. | Costa Rica, Panama | Pacific | 0–700 |
| <i>V. elongata</i> (Benth.) Warb. | Panama | Caribbean | 50–450 |
| <i>V. fosteri</i> D. Santam. | Costa Rica, Panama | Caribbean | 0–350 (–800) |
| <i>V. guatemalensis</i> (Hemsl.) Warb. | Mexico, Guatemala, Honduras | Both slopes | 150–1250 |
| <i>V. koschnyi</i> Warb. | Mexico, Guatemala, Belize, Honduras, Nicaragua, Costa Rica, Panama | Both slopes, mainly on the Caribbean side | 10–1000 (–1700) |
| <i>V. laevigata</i> Standl. | Costa Rica, Panama | Pacific | 0–500 (1600?) |
| <i>V. megacarpa</i> A. H. Gentry. | Panama | Caribbean | 50–550 |
| <i>V. montana</i> D. Santam. | Costa Rica, Panama | Both slopes | 700–2000 |
| <i>V. multiflora</i> (Standl.) A. C. Sm. | Belize, Honduras, Nicaragua, Costa Rica | Caribbean | 0–650 (–1400) |
| <i>V. nobilis</i> A. C. Sm. | Costa Rica, Panama | Both slopes | 0–400 (–1300) |
| <i>V. otobifolia</i> D. Santam. | Panama | Caribbean | 50–850 |
| <i>V. sebifera</i> Aubl. | Honduras, Nicaragua, Costa Rica, Panama | Both slopes | 0–1000 |

incorporated into indigenous cultural practices of South America. The hallucinogen is usually obtained from the exudate of the inner bark of several species, including: *Virola calophylla* Warb., *V. calophylloidea* Markgr., *V. duckei* A. C. Sm., *V. elongata*, *V. sebifera*, *V. surinamensis* and *V. theiodora* Warb.; it has also been documented in *Iryanthera* and *Osteophloeum platyspermum* (Spruce ex A. DC.) Warb. (Schultes 1954, 1969, 1979; Schultes and Holmstedt 1968; Prance 1970; Soares Maia and Rodrigues 1974; Bennett and Alarcón 1994). In addition to being psychoactive, the reddish exudate of various *Virola* species is thought to have medicinal properties. For example, the sap of *V. melinonii* (Benoist) A. C. Sm. and *V. surinamensis* is applied to treat skin conditions, alleviate tooth pain, soothe colic and as a styptic (i.e. a substance used to stop bleeding) to treat ulcerating sores and wounds, haemorrhoids and other sources of bleeding (Plotkin and Schultes 1990). The sap of an undetermined species of *Virola* has been reported to be used as a contraceptive by Indians on the Rio Negro of Brazil and is reputedly effective for a period of two or three years (Plotkin and Schultes 1990). Finally, the oil obtained from the seeds of *Virola officinalis* Warb., *V. sebifera* and *V. surinamensis* has many medical folk uses, including treatments for asthma, rheumatism, tumours of the joints, intestinal worms, skin diseases, erysipelas, haemorrhoids and bad breath (Rodrigues 1972, 1980; Plotkin and Schultes 1990).

Taxonomic history

Virola was first described by Aublet in “Histoire des plantes de la Guiane Française”, based on *V. sebifera* Aublet (Aublet 1775). It is likely that the etymology of the generic name is derived from one of the common names mentioned in the protologue, which stated: “Nomen Caribaeum Oyapocenfibus *VOIROUCHI*; Sinemarienfibus *VIROLA*; Gallium *JEJEAMADOU*” (Aublet 1775). Subsequent authors have attributed this etymology to a Galibi or Sinemari vernacular name for *V. sebifera* in French Guiana (Rodrigues 1972, 1980; Cardona et al. 2010; Mari Mut 2018). However, others believe that the name is derived from the Latin *viriola* (bracelet), in reference to the aril that surrounds the seed (e.g. Flores 2010).

There is a long taxonomic history to the American species of Myristicaceae. This began with George Bentham (1853), who was the first to publish on these species in “Notes on the American Species of Myristica”. In this treatment, he recognises 12 species of *Myristica* (nine of them new) and places these in three different groups, characterised by differences in the androecium (i.e. number of anthers and shape, thickness and length of the column) and leaf venation (i.e. number of veins and their degree of separation). Alphonse de Candolle published the second and broadest treatment of Myristicaceae, including species from both the New and Old World (de Candolle 1856). He recognised ca. 84 species, which he divided into 13 sections in a single genus, *Myristica*, which included two sections that contain species today recognised as *Virola*: *Virola* (Sect. III/ 8 spp.) and *Sychoneura* (Sect. IV/10 spp.). In this work, he described 12 new taxa that now belong to *Virola*. The British botanist William Bot-

ting Hemsley was the earliest author to publish an account of the Mesoamerican species of Myristicaceae in “Biologia centrali-americana” (Hemsley 1882). In this work, three species are newly described: *Myristica guatemalensis* Hemsl., *M. mexicana* Hemsl. [= *Compsonura mexicana* (Hemsl.) Janovec] and *M. panamensis* Hemsl. The most comprehensive treatment of Myristicaceae during this era was produced by the German botanist and agriculturalist Otto Warburg in his “Monographie der Myristicaceen” (Warburg 1897). Warburg recognised 240 species in 15 genera, including *Virola* (recognised for the first time as a genus). Warburg’s concept of *Virola* included 27 species in two sections: *Oxyanthera* Warb. (13 spp.; anthers longer than the column) and *Amblyanthera* Warb. (14 spp.; anthers equal to or shorter than the column). Amongst the *Virola* species that Warburg recognised were two previously recognised as *Myristica*: *V. guatemalensis* (Hemsl.) Warb. and *V. panamensis* (Hemsl.) Warb. Following the publication of Warburg’s treatment, generic recognition of *Virola* became broadly accepted and, subsequently, five new names were published for Mesoamerica: *V. koschnyi* Warb. (Warburg 1905), *V. merendonis* Pittier, *V. warburgii* Pittier (Pittier 1916, 1922), *V. laevigata* Standl. and *V. brachycarpa* Standl. (Standley 1929, 1932). These names and species identities are followed in regional taxonomic treatments (e.g. Standley 1928, 1930, 1931). “The American species of Myristicaceae” (Smith and Wodehouse 1938) by Albert C. Smith, with contributions of pollen analysis by Roger P. Wodehouse was the next contribution to the taxonomy of Mesoamerican Myristicaceae. These authors proposed the division of *Virola* into six groups: *Mollissimae* (I), *Sebiferae* (II), *Calophyllae* (III), *Rugulosae* (IV), *Surinamenses* (V), and *Subsessilis* (VI). The characters used to delimit these groups included trichome type, degree of perianth connation, spacing of secondary leaf veins, column shape and anther apex shape. Of the 38 species of *Virola* recognised in Smith and Wodehouse (1938), the following proposals pertain to Mesoamerican species: (1) *V. panamensis*, *V. warburgii*, *V. merendonis* and *V. laevigata* were sunk into synonymy with *V. sebifera*, *V. sebifera*, *V. koschnyi* and *V. guatemalensis*, respectively; (2) the description of *V. nobilis* A. C. Smith from Panama; and (3) the transfer of *Dialyanthera multiflora* Standl. to *Virola multiflora* (Standl.) A. C. Smith and the designation of *V. brachycarpa* Standl. as synonymous with this species.

Since then, 17 new species of *Virola* have been published from South America (Ducke 1945; Smith 1953, 1956; Williams 1964; Little 1970; Rodrigues 1977, 1989; Sabatier 1997; Jaramillo et al. 2000) and one from Mesoamerica: *V. megacarpa* A. H. Gentry (Gentry 1975). Several important local floristic accounts of *Virola* have also been published, some of which made important taxonomic changes for their region (South America: Cogollo 2011, Gradstein, 2018 [Colombia]; Achá and Liesner 2014 [Bolivia]; Rodrigues 1980, 2010, 2015 [Brazil]; Jaramillo et al. 2004 [Ecuador]; Mitchell 2002 [central French Guiana]; Aymard et al. 2007 [Guiana Shield]; Vásquez Martínez 1997, 2010, Vásquez et al. 2018 [Peru]; Rodrigues 2008, Rodrigues et al. 2001 [Venezuela]; Mesoamerica: Acevedo-Rodríguez and Strong 2012 [West Indies]); (Balick et al. 2000 [Belize]; Jiménez 2007 [Costa Rica]; Nelson 2008 [Honduras]; Villaseñor 2016 [Mexico]; Gentry 2001 [Nicaragua]; Duke 1962, Correa et al. 2004 [Panama]). Perhaps the most substantial of these is “Revisão taxonômica das espécies

de *Virola* Aublet (Myristicaceae) do Brasil” (Rodrigues 1980), which provided a treatment for Brazil and refuted the infrageneric proposals by previous authors.

Virola has been the subject of limited phylogenetic analysis. The first molecular phylogenetic study of *Virola*— and the only to date— demonstrated that the genus is divided into two large subclades, informally called “Multinervae” and “Sebiferae” (Steeves 2011). Morphologically, Multinervae is composed of tall emergent canopy trees that typically have more numerous secondary leaf veins, thicker pericaps and arils that are laciniate throughout their length, compared to the sub-canopy to canopy species of Sebiferae that have fewer secondary veins, thin pericarps and arils that are only laciniate for half of their length. This phylogeny, or future improved phylogenetic hypotheses, has the potential to provide information for future infrageneric reclassification of *Virola*.

Materials and methods

This work was undertaken as part of the “Flora Mesoamericana” project (<http://www.tropicos.org/Project/FM>) by the first author. However, our definition of “Mesoamerica” is different than that used in the *Flora*; here, it refers to the portion of Central America and southern Mexico from the Isthmus of Tehuantepec in Mexico in the north to the Panamanian-Colombian border in the south.

Approximately 500 physical herbarium specimens of Mesoamerican *Virola* were examined for this study from the following herbaria: CR (including ex INB), LSCR, LSU, MO, NO and USJ (acronyms follow Thiers [continuously updated]), though specimens from MO represent the majority of the material studied. All type specimens, as well as general collections, hosted by virtual herbaria, were consulted, including those maintained by: the Field Museum (**F**; <http://emuweb.fieldmuseum.org/botany/taxonomic.php>), Instituto Nacional de Pesquisas da Amazônia (**INPA**; <http://inct.florabrasil.net/en/>), JSTOR Global Plants (<http://plants.jstor.org>), Museum of Natural History, Paris (**P**; <http://www.mnhn.fr>), Oberösterreichischen Landesmuseums (**LI**; <https://www.europeana.eu/portal/es>), University of Maryland (**MARY**; <http://intermountainbiota.org/portal/>), Universidad Nacional Autónoma de México (**MEXU**; <https://datosabiertos.unam.mx/biodiversidad/>) and the National Herbarium of The Netherlands (**U**; <http://herbarium.naturalis.nl/>). Images of specimens not available online were provided by: EAP, F, HUH, NY, PMA, SCZ and US. When specimens were only studied digitally, they are denoted by an asterisk following the herbarium acronym (e.g. EAP!*). Label information of specimens studied and housed at Museo Nacional de Costa Rica (**CR**) and Missouri Botanical Garden (**MO**) are available online via the ECOBIOSIS (<http://ecobiosis.museocostarica.go.cr/index.aspx>), HERBARIO (<http://www.specify7.museocostarica.go.cr:8080/specify-solr/Herbario/>) and TROPICOS (<http://www.tropicos.org/>) databases.

Species descriptions are based primarily on herbarium specimens, though observations during fieldwork by the first and second authors, especially in the Osa Peninsula of Costa Rica, were also important. If necessary and material permitted, flowers from

herbarium specimens were rehydrated before measurement. A ruler was used to measure leaves and inflorescences; a digital Neiko caliper was used to measure fruits and seeds, as well as the thickness of the twigs, petioles and peduncles; and, finally, flowers, trichomes and thickness of the pericarp were measured with a micrometer calibration tool (1div = 1mm) under a dissecting stereoscope (Bausch & Lomb).

The preliminary conservation status of each new species was assessed using quantitative criteria recommended by the IUCN Red List (IUCN Standards and Petitions Subcommittee 2014). Georeferenced specimen data were used to determine the area of occupancy (AOO) and the extent of occurrence (EOO), which in turn were used to determine threat status. All analyses were performed in the R package conR (Dauby et al. 2017). When the recommendation differed between AOO and EOO assessments for a given species, we opted to conservatively recommend the more vulnerable status, following Knapp (2013).

Specimens cited are listed first by country in a north to south sequence. Within country, specimens are listed alphabetically by major division and then alphabetically by province or department and, finally, in alphabetical order by the collector's surname. When the coordinates and/or elevation were not included on the herbarium label, but were present in the TROPICOS database, the values from TROPICOS are included. Dot-distribution maps were compiled from studied specimens and generated with SimpleMappR (Shorthouse 2010).

In the nomenclatural section for each new species, we cite both accession numbers and barcodes when present. Barcodes are included in square brackets and follow the format of a series of numbers preceded by a herbarium acronym (e.g. [GH00039891]); all other numbers correspond to accession numbers.

Knowledge of the group was improved by numerous field trips since 1991 throughout Costa Rica, especially in the Osa Peninsula and La Selva Biological Station, by the second author and, more recently, by the first author in the same region.

Key to the species of *Virola* in Mesoamerica

- 1 Abaxial leaf surface with pediculate trichomes (sometimes sessile in *V. sebifera* and with short pedicle in *V. elongata*) (Fig. 3C, G, I, J, N) **2**
- Abaxial leaf surface with sessile trichomes (Fig. 3 A, B, D, E, F, H, K–M, O).... **5**
- 2 Leaf blades with 9–17 lateral veins per side, free or slightly anastomosing near the margin and without forming a very marked intramarginal vein, indument on abaxial surface caducous; petiole slightly canaliculate; staminate flower with the filament column 0.1–0.4 mm long, anthers with apiculus 0.1–0.2 mm long; fruits 1.1–1.7 (–2.3) × 0.8–1.3 (–1.6) cm, globose to subglobose (Fig. 4K); pericarp 0.3–0.6 (–0.9) mm thick. ***V. sebifera***
- Leaf blades with (16–) 20–50 lateral veins per side, anastomosing near the margin and forming a conspicuous intramarginal vein, indument on abaxial surface persistent; petiole markedly canaliculate; staminate flower with the

- filament column 0.7–1.5 mm long, anthers without apiculus or this very inconspicuous; fruits 1.9–5.7 × 1.5–2.9 cm, ellipsoid, ovoid-ellipsoid or sometimes subglobose (Fig. 4 E, F, P); pericarp 1.2–6 mm thick.....**3**
- 3 Leaf blades with (32–) 40–50 lateral veins per side; fruits 4–5.7 × 2–2.9 cm, the apex acuminate to rostrate (Fig. 4P); pericarp 3–6 mm thick *V. megacarpa*
- Leaf blades with (16–) 20–35 lateral veins per side; fruits 1.9–3.1 × 1.5–1.9 cm, the apex acute to apiculate or obtuse; pericarp 1.2–3.1 mm thick**4**
- 4 Mature leaf blades on adaxial surface pubescent and asperous to the touch in dry specimens, abaxial surface densely hirsute to hirsutulous, trichomes with 3–6 branches, the branches 0.2–0.6 mm long; staminate flowers with filament column 1.3–1.5 mm long..... *V. chrysocarpa*
- Mature leaf blades on adaxial surface glabrous to glabrescent and smooth to the touch in dry specimens, abaxial surface densely tomentose, trichomes with 4–10 branches, the branches 0.1–0.2 mm long; staminate flowers with filament column 0.7–0.9 (–1.4) mm long..... *V. koschnyi*
- 5 Leaf blades with 9–20 lateral veins per side, (0.8–) 1–3 cm apart; staminate flowers with perianth infundibuliform, the filament column 0.2–0.9 (–1) mm long; fruits green when mature; aril laciniate in very narrow bands distally when dry, membranaceous.....**6**
- Leaf blades with 10–34 lateral veins per side, 0.2–1.1 (–1.5) cm apart; staminate flowers with perianth campanulate, globose or subglobose, the filament column 0.6–1.3 mm long; fruits yellow, orange or a combination of these colours when mature; aril laciniate in narrow bands distally when dry, coriaceous.....**9**
- 6 Abaxial leaf surface sparsely pubescent, trichomes concolorous, the trichome branches 0.1–0.2 mm long; peduncle of staminate inflorescences 0.07–0.15 cm thick; staminate flower with the perianth 1.5–1.9 mm long; fruits 1.6–1.9 × 0.9–1.1 cm (Fig. 4L), the line of dehiscence canaliculate or smooth; pericarp 0.5–0.7 mm thick..... *V. elongata*
- Abaxial leaf surface densely pubescent, trichomes dicolorous (the central part usually reddish, contrasting in colour with the hyaline to pale reddish branches), the trichome branches ± 0.01–0.05 mm long; peduncle of staminate inflorescences 0.09–0.34 cm thick; staminate flower with the perianth (1.5–) 2–2.8 mm long; fruits 2.1–4.5 × 1.5–2.9 cm (Fig. 4A–C), the line of dehiscence (generally) carinate; pericarp 1–4.7 mm thick**7**
- 7 Leaf blades elliptic to widely elliptic; staminate flowers with the filament column 0.2–0.4 mm long; fruits with pericarp 1–2 mm thick..... *V. amistadensis*
- Leaf blades oblong-elliptical or rarely elliptical; staminate flowers with the filament column 0.5–1 mm long; fruits with pericarp (2.7–) 3–4.7 mm thick.....**8**
- 8 Leaf blades 3.2–7.3 cm wide; lateral veins 15–20 per side, with 4–5 per 5 cm, 1.2–1.8 cm apart; staminate flowers in dense terminal fascicles; staminate

- perianth lobes glabrous or sparsely pubescent on the adaxial margin; filament column 0.5–0.6 mm long, thin throughout its length (sometimes slightly thickened at the base), not constricted at the apex; mature fruits with trichomes that fall like dust *V. allenii*
- Leaf blades (4.1–) 7.3–14.2 cm wide; lateral veins 10–16 per side, with 2–4 per 5 cm, 1.7–3 cm apart; staminate flowers in lax terminal fascicles; staminate perianth lobes pubescent adaxially; filament column 0.9–1 mm long, conspicuously thickened throughout its length, except constricted at the apex; mature fruits with persistent trichomes *V. otobifolia*
- 9 Abaxial leaf surface with caducous trichomes or practically glabrous or, if some persistent trichomes, then along the veins, never on the entire surface **10**
- Abaxial leaf surface with trichomes persistent throughout **11**
- 10 Twigs and petioles glabrous; leaf blades with 12–20 lateral veins per side; staminate flowers with the filament column 0.8–1.3 mm long; fruits 1.8–2.9 × 1.5–1.8 cm (Fig. 4M), glabrous to glabrescent or very sparsely pubescent, the line of dehiscence canaliculate or smooth; pericarp 1.8–2.8 mm thick; 0–500 (1600?) m elevation *V. laevigata*
- Twigs and petioles pubescent; leaf blades with (15–) 18–30 lateral veins per side; staminate flowers with the filament column 0.6–0.9 mm long; fruits (2.8–) 3–3.6 × 2–2.5 cm (Fig. 4H), densely tomentose to tomentulose, the line of dehiscence carinate; pericarp 3.2–5 mm thick; 700–2000 m elevation *V. montana*
- 11 Leaf blades 1.4–3.6 (–4.8) cm wide **12**
- Leaf blades (2.4–) 3.8–7.1 (–8.9) cm wide **13**
- 12 Leaf blades with the base revolute; lateral veins 16–24 per side, with 10–15 veins per 5 cm, 0.2–0.5 (–0.7) cm apart; staminate flowers with the filament column 0.9–1.3 mm long, anthers 0.6–0.9 mm long; fruits 1.5–2.3 × 1.2–1.8 cm (Fig. 4I), pericarp 1.5–2.5 mm thick *V. fosteri*
- Leaf blades with the base not revolute; lateral veins 10–18 per side, with (6–) 8–10 veins per 5 cm, 0.4–0.8 (–1.2) cm apart; staminate flowers with the filament column 0.7–1 mm long, anthers 0.3–0.6 mm long; fruits 1.3–1.9 × 0.9–1.2 (–1.4) cm (Fig. 4J), pericarp 0.7–1 mm thick *V. multiflora*
- 13 Lateral veins 13–21 per side, slightly elevated or flat on the abaxial leaf surface, tertiary veins almost indistinct or very slightly visible on both surfaces (Fig. 3F) ; fruits commonly glabrous or glabrous distally and pubescent at base or scattered and inconspicuously pubescent; pericarp 0.4–1 (–2.5) mm thick *V. guatemalensis*
- Lateral veins 20–30 (–32) per side, markedly elevated on the abaxial leaf surface, tertiary veins usually distinct on both surfaces (especially abaxially) (Fig. 3L); fruits densely tomentose to tomentulose; pericarp 2.3–3.5 (–4.2) mm thick *V. nobilis*

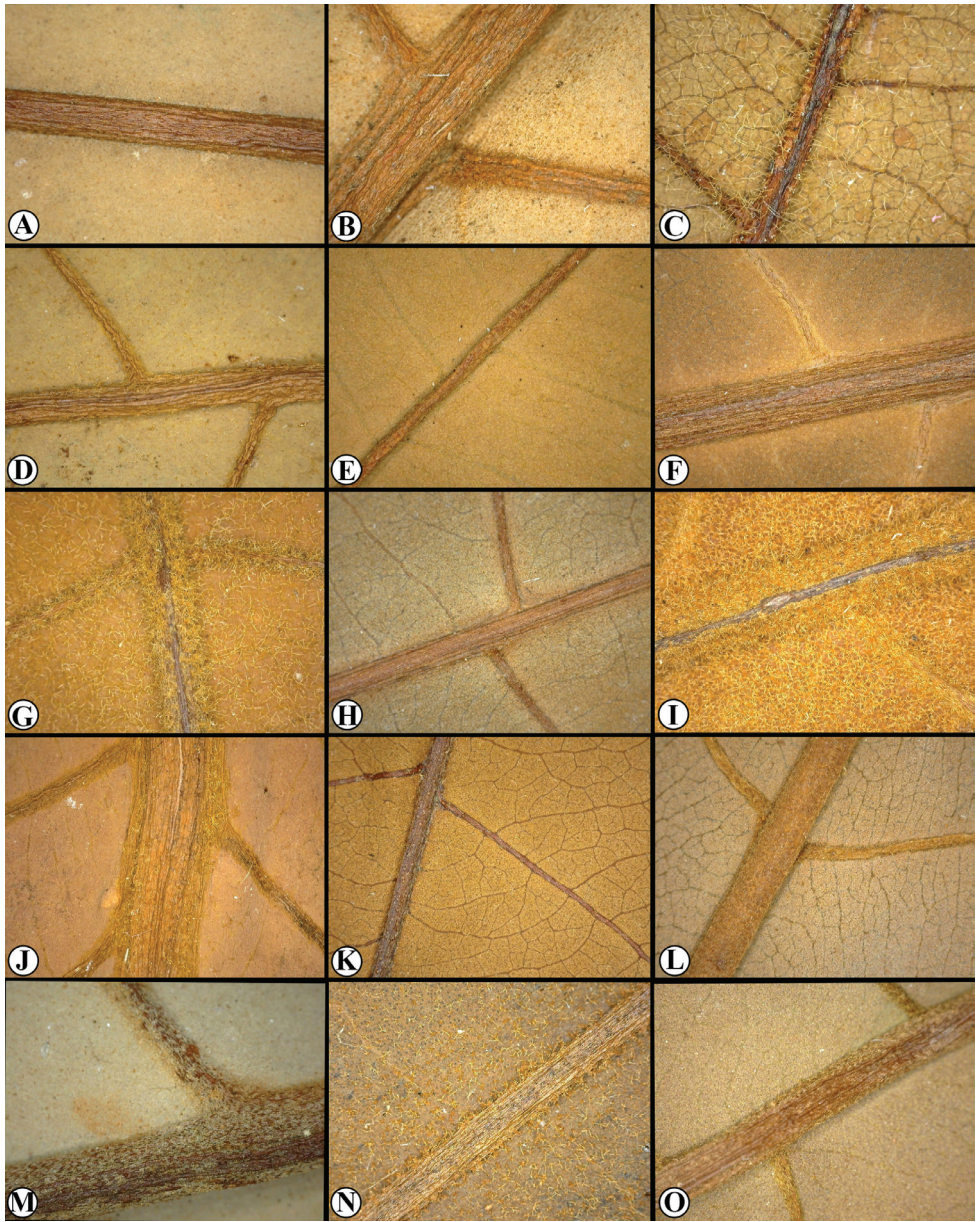


Figure 3. Trichomes in Mesoamerican *Virola* **A** *Virola allenii* (R. Aguilar 2224, MO) **B** *V. amistadensis* (G. McPherson 8703, MO) **C** *V. chrysocarpa* (O. Valverde 970, MO) **D** *V. elongata* (G. McPherson 13674, MO) **E** *V. fosteri* (G. McPherson 20913, MO) **F** *V. guatemalensis* (R. Cedillo 3349, MO) **G** *V. koschnyi* (R. E. Gereau et al. 3455, MO) **H** *V. laevigata* (R. Aguilar 2004, MO) **I** *V. megacarpa* (J. A. Duke 15261, MO) **J** *V. montana* (E. Bello 855, MO) **K** *V. multiflora* (R. Rueda et al. 8196, MO; inset, J. C. Sandino 3302, MO) **L** *V. nobilis* from Barro Colorado (R. J. Schmalzel 320, MO) **M** *V. otobifolia* (G. de Nevers & H. Herrera 7056, MO) **N** *V. sebifera* (F. Almeda et al. 5553, MO) **O** *V. nobilis* from Osa Peninsula (R. Aguilar 11186, MO).

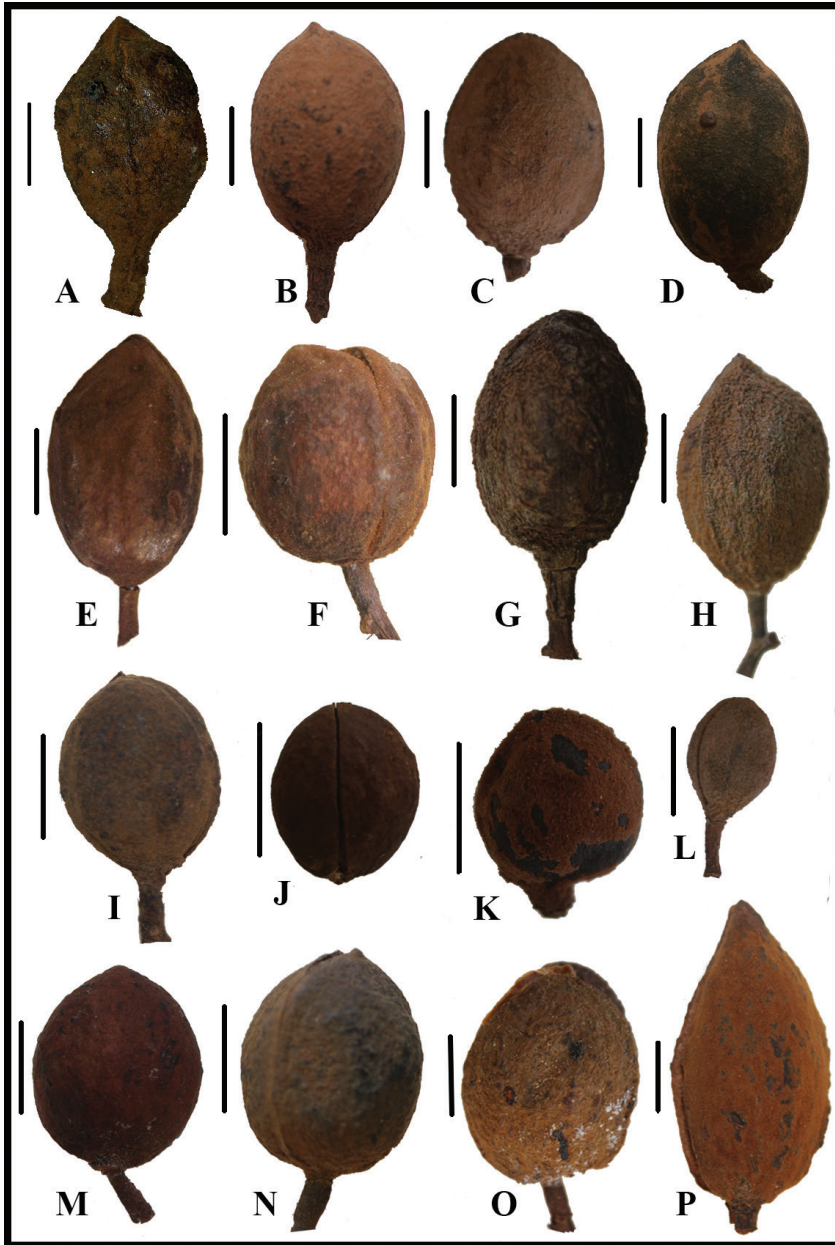


Figure 4. Fruits of Mesoamerican *Virola*, organised in groups of morphologically similar species **A** *V. allenii* (P. H. Allen 6727, GH) **B** *V. amistadensis* (G. McPherson 9715, MO) **C** *V. otobifolia* (A. Gentry & S. Mori 14199, MO) **D** *V. macrocarpa* (A. E. Lawrance 675, MO) **E** *V. chrysocarpa* (B. Hammel et al. 16864, MO) **F** *V. koschnyi* (R. Robles 1587, MO) **G** *V. guatemalensis* (S. Sinaca 1542, MO) **H** *V. montana* (J. Utley & K. Utley 5054, MO) **I** *V. fosteri* (S. Mori & J. Kallunki 4891, MO) **J** *V. multiflora* (R. Rueda et al. 9960, MO) **K** *V. sebifera* (T. B. Croat 5959, MO) **L** *V. elongata* (J. D. Dwyer & M. Correa 8420, MO) **M** *V. laevigata* (R. Aguilar 1585, MO) **N** *V. nobilis* from Barro Colorado (T. B. Croat 8090, MO) **O** *V. nobilis* from the Osa Peninsula (G. Herrera 4222, MO) **P** *V. megacarpa* (G. de Nevers 5184, MO). Scale bars: 1 cm.

Taxonomy

1. *Virola allenii* D.Santam. & Aguilar, sp. nov.

urn:lsid:ipni.org:names:77202542-1

Figs 5, 6, 7A

Diagnosis. Species resembling *Virola macrocarpa* in its leaf blades that are whitish on the abaxial side and covered with stellate, sessile trichomes with the centre reddish and contrasting with the hyaline branches to reddish-clear in colour and lateral veins that are not densely arranged, as well as large fruits that are covered with ferruginous trichomes. It differs in its narrow leaf blades (3.2–7.3 cm vs. 7–11 cm wide) with acute or obtuse to rounded bases (vs. broadly obtuse), fruits with thick pericarp (3.2–3.8 mm vs. 1.8–3 mm thick) and preference for humid lowland forests at 0–350 (–1350) m elevation (vs. montane forests in Andes of Colombia at around 1100 m elevation).

Type. COSTA RICA. Puntarenas: Esquinas forest preserve, 0 m, 10 Jan 1951 (♂ fl), P. H. Allen 5763 (holotype: F-2 sheets* [1394346!, 1679106!]; isotype: USJ [9016]).

Description. *Tree* 13–30 m × 10.4–50 cm DBH; bark sometimes described as smooth and reddish or dark brown. *Exudate* sometimes described as abundant and reddish or watery, but without specifying from where or red in the trunk. *Twigs* 0.16–0.22 cm thick, terete to slightly flattened laterally, puberulent, trichomes stellate to irregularly stellate, ferruginous. *Leaves:* petiole 0.5–1.4 × 0.13–0.24 cm, slightly canaliculate, tomentose, the trichomes stellate to irregularly stellate; *leaf blades* 16.2–29.2 × 3.2–7.3 cm, oblong-elliptic or rarely elliptic; adaxial surface of mature leaves olive or light brown (sometimes shining) when dry, glabrous or with scattered stellate trichomes, the surface smooth; abaxial surface pale brown to whitish when dry, densely but inconspicuously pubescent, trichomes stellate, sessile, the central part of the trichome reddish and contrasting in colour with the hyaline branches to reddish-clear, with 4–10 branches, the branches ± 0.01–0.05 mm long, persistent; lateral veins 15–20 per side, 4–5 veins per 5 cm, 1.2–1.8 cm apart, the same colour as the adaxial surface or slightly transparent, on adaxial side flat to sunken, on abaxial side slightly elevated, arcuate-ascending, slightly anastomosing near the margin and without forming a very marked intramarginal vein; tertiary veins barely visible on both sides; mid-vein adaxially slightly elevated (sometimes flat, distally), abaxially raised, rounded to somewhat triangular, tomentose to glabrate; base acute or obtuse to rounded, not revolute, flat; margin flat; apex acuminate or rarely rounded. *Staminate inflorescences* 3.5–5.5 cm long, axillary, axes flattened, tomentose, with trichomes irregularly stellate, ferruginous; peduncle 1.2–1.9 × ca. 0.1 cm long; bracts not seen; terminal fascicles dense, with 15–40+ flowers. *Staminate flowers* with the pedicel 0.3–1.2 mm long; receptacle 1.2–2 mm wide; perianth 2–2.8 mm long, infundibuliform, yellow when fresh, connate by 1.1–1.7 (–2.3) mm long, external surface pubescent, with brown trichomes, internal surface glabrous or with few trichomes close to the margin of the lobes; lobes 3 (4), 0.8–1.5 × 0.6–0.9 (–1.2) mm; stamens 3, the filament column 0.5–0.6 mm long, glabrous, straight, thin, sometimes slightly thickened at the base, not

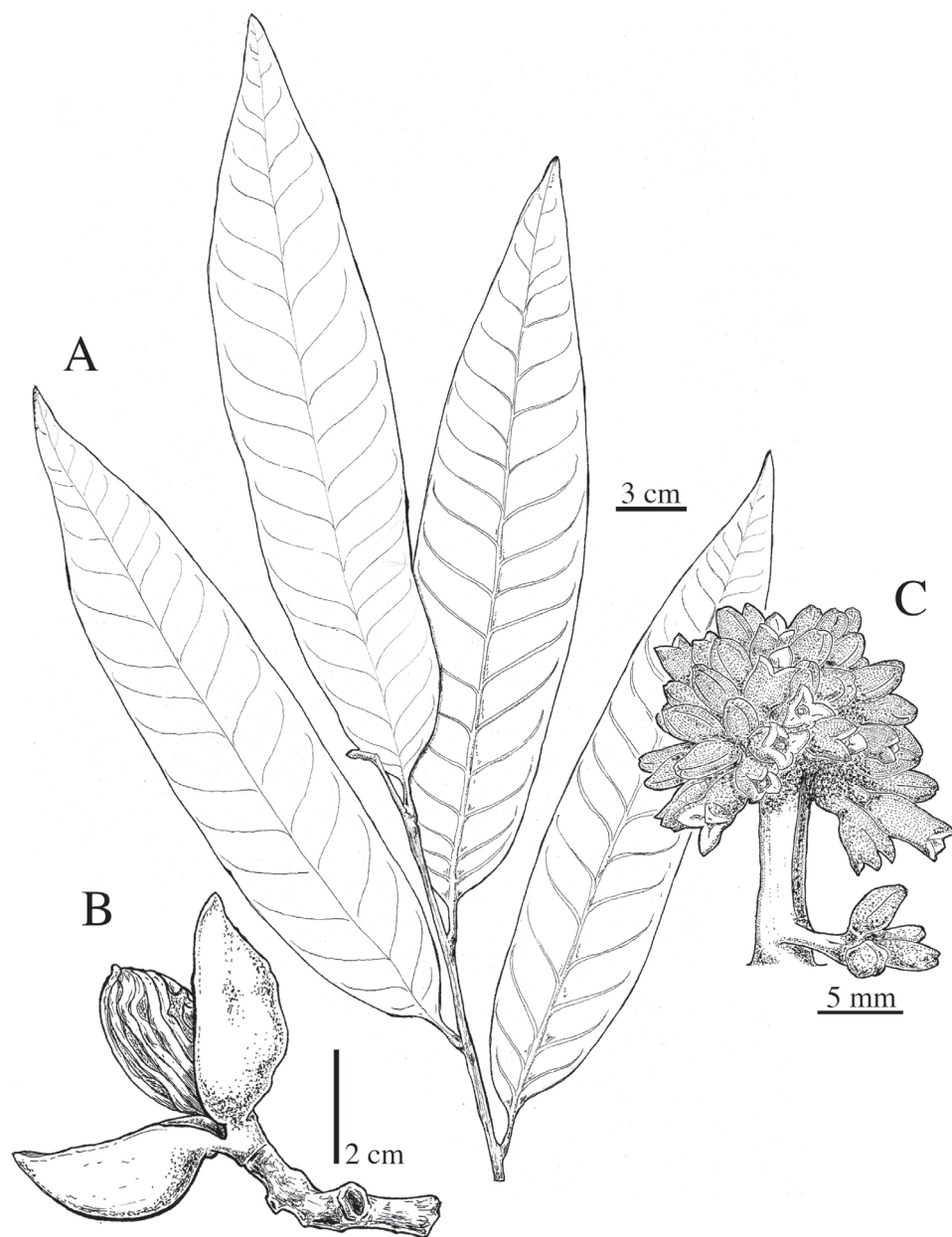


Figure 5. *Viola allenii* **A** leaves **B** fruit **C** partial inflorescences. Drawn by Pedro Juárez based on *P. H. Allen* 6727 (**A**), *R. Aguilar* 2224 (**B**) and *R. Aguilar* (**C**) from a photo, physical specimen not seen.

constricted at the apex; anthers 0.6–0.9 mm long; apiculus 0.06–0.1 mm long, acute to apiculate, connate. **Pistillate inflorescences** and **pistillate flowers** not seen. **Infructescence** 2.5–7.5 cm long, with 2–13 fruits, peduncle 2–3.5 × ca. 0.47 cm. **Fruits** 2.7–3.5 × 1.5–2.5 cm, usually ellipsoid or rarely ovoid, stipitate, densely tomentose,

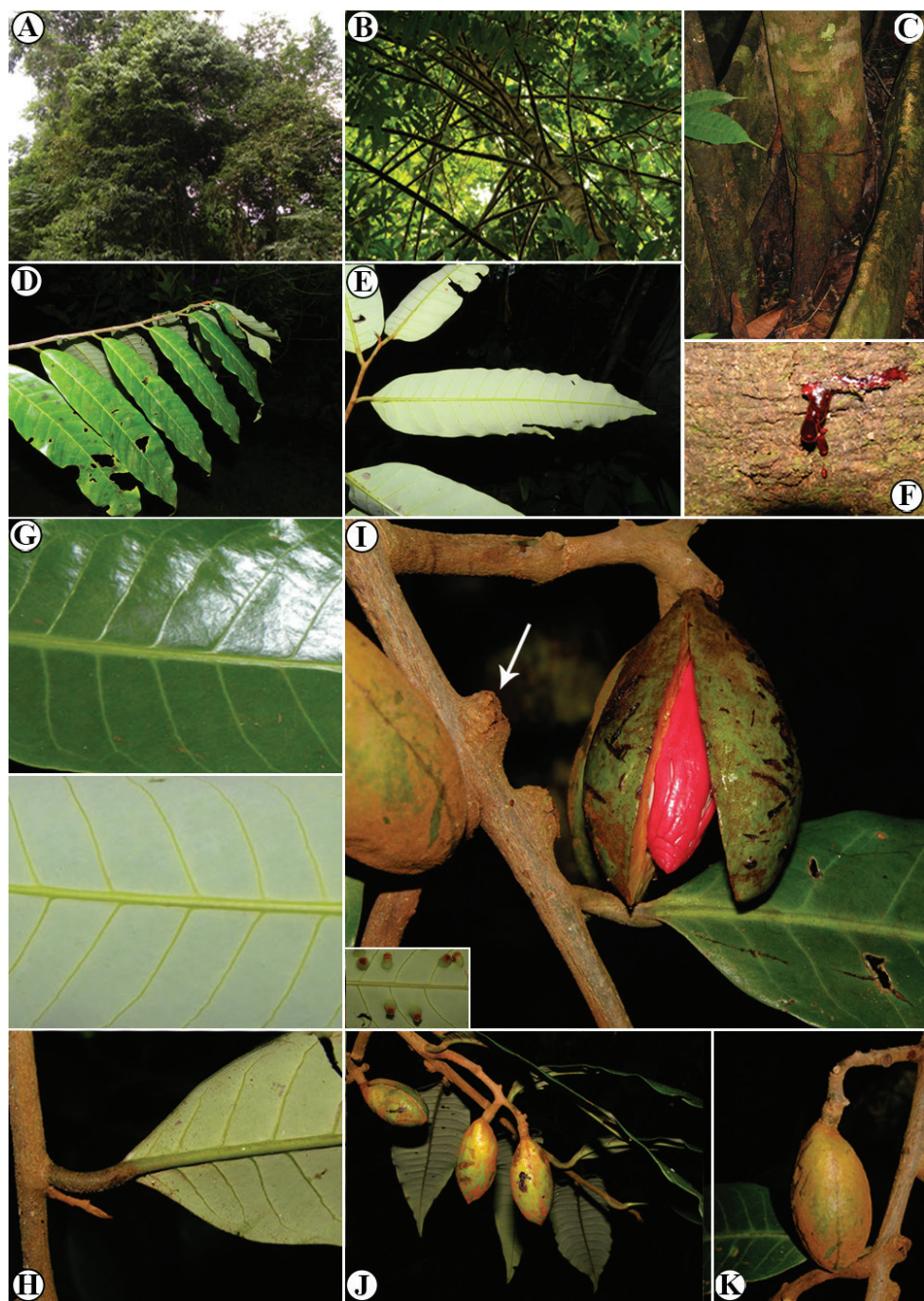


Figure 6. *Virola allenii* **A** treetop **B** branching **C** lower trunk and buttress **D** branch with leaves, showing the adaxial surface **E** leaf blades on abaxial surface **F** exudate of the trunk **G** leaf blade surfaces, adaxial (above) and abaxial (below) **H** petiole and leaf base **I** mature fruit, inset left and arrow showing the galls on leaves and branches, respectively **J** fruits **K** fruit close-up. Photos by Reinaldo Aguilar.

the trichomes dendritic, ferruginous and falling very easily to the touch (as dust), the surface rugose or smooth when dry, the line of dehiscence usually carinate, but not very conspicuous, the base obtuse, the apex acute to obtuse, green or golden and ferruginous by the pubescence when fresh; pericarp 3.2–3.8 mm thick; pedicel 0.4–0.7 cm long; seed ca. 2.5×1.3 cm, the testa when dry whitish-greyish, markedly grooved; aril usually described as red when fresh, pale brown when dry, membranaceous, the texture dry and thin, lacinate in narrow bands distally. **Germination** epigeal, seedling cryptocotylar, the first pair of leaves (sub)opposite (Ley López and Chacón Madrigal 2017; as *V. macrocarpa*).

Distinctive characters. *Virola allenii* is recognised by its narrow leaf blades with lateral veins that are well separated (Fig. 8A) with a whitish abaxial surface and covered with stellate, sessile trichomes with the central portion of the trichome reddish and contrasting in colour with the hyaline branches to reddish-clear (Figs 3A, 6F); the staminate flowers with the lobes of the perianth almost glabrous on the inner surface, the column of filaments straight and not constricted at the apex, anthers that are usually longer (0.6–0.9 mm long) than the column of the filaments (0.5–0.6 mm long) and an apiculus that is 0.06–0.1 mm long. It is also distinctive for its large, usually ellipsoid fruits (Figs 4A, 6I–K) with thick pericarp that are green when ripe and covered by ferruginous trichomes that fall very easily to the touch (Fig. 6K inset); and seeds with the testa markedly ribbed.

Etymology. The specific epithet honours the collector of the type specimen, Paul H. Allen (1911–1963), who was probably the first person to collect this species 67 years ago (*P. H. Allen* 5763; 10 Jan 1951). During his five-year residency in Palmar Norte, Puntarenas, Costa Rica (Grayum et al. 2004), Allen made important collections and publications in this region (e.g. The Rain Forests of Golfo Dulce, Allen 1956).

Distribution. *Virola allenii* is known only from Costa Rica (Puntarenas and San José) (Fig. 9A). It is found on the Pacific slope, at 0–350 (–1350) m elevation.

Preliminary conservation status. *Virola allenii* is Vulnerable following IUCN criteria B1a and B2a. Justifying its status, it is known from seven localities and has an EOO of 3,424 km² and an AOO of 40 km². Specimens have been collected regularly since the 1990s during botanical expeditions in the Osa Peninsula of Costa Rica, though only 22 specimens have been verified.

Common names. None recorded.

Phenology. Flowering of *Virola allenii* has been recorded in January, March, April and December. Fruits have been observed in January, August to October. Pistillate flowers were not seen in the studied material.

Field characters. The bark is described as brown and smooth or as peeling in small pieces, sometimes with a strong, spicy scent. Twigs and leaves often have galls (e.g. Fig. 6I). Leaf blades are adaxially lustrous and abaxially whitish. Staminate flowers are yellow and fragrant. Fruits, which are ca. 5×3.2 cm when fresh, are green at maturity and covered with brown trichomes that fall very easily when touched and have a pericarp that is ca. 6 mm thick. The aril of mature fruits is red and white in immature fruits. Seeds have a white testa.

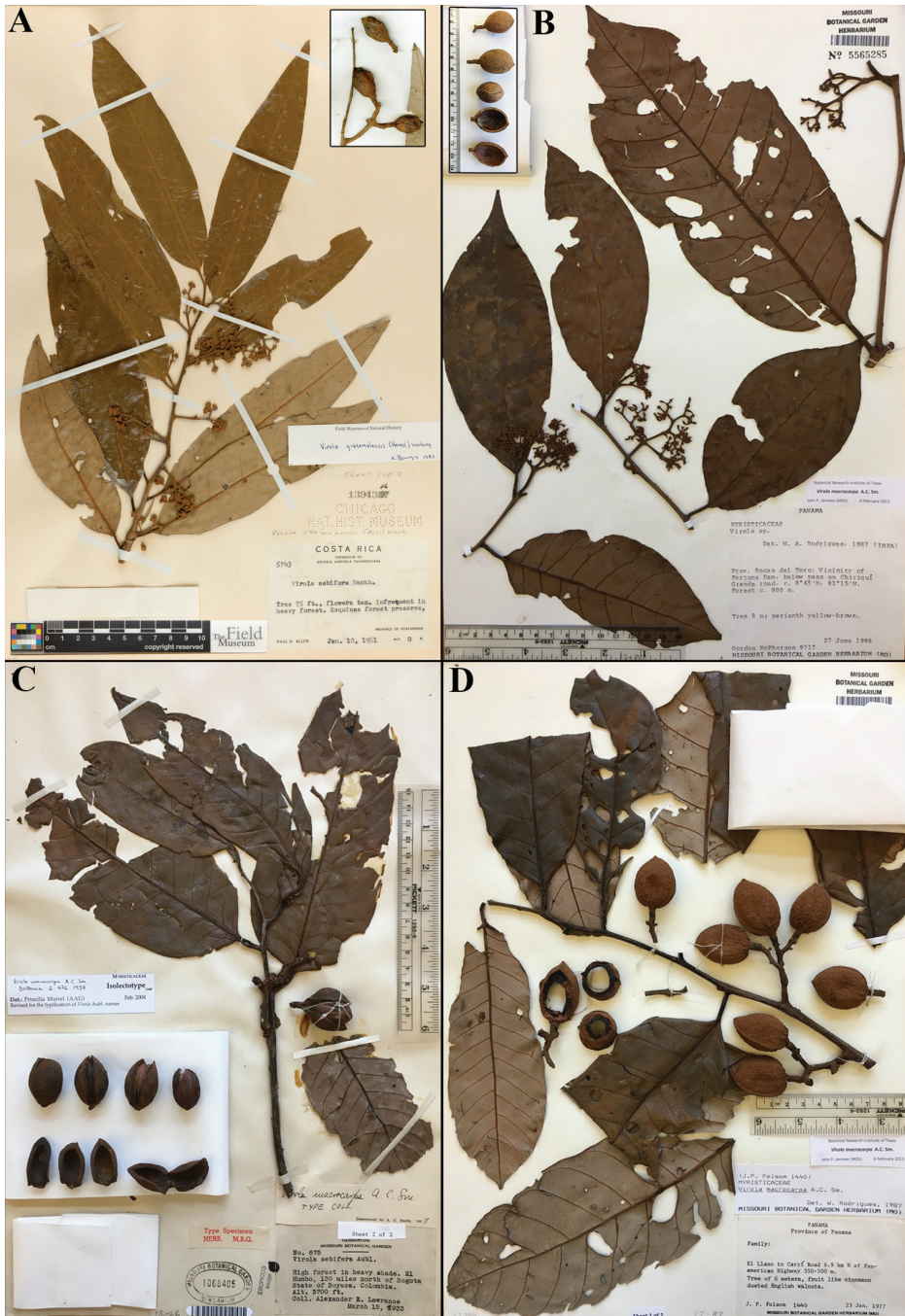


Figure 7. Comparisons of *Virola macrocarpa* with similar species in Mesoamerica **A** *V. allenii* (P. H. Allen 5763, F; inset fruits, from P. H. Allen 6727, GH) **B** *V. amistadensis* (G. McPherson 9717, MO; inset fruits, from G. McPherson 9715, MO) **C** *V. macrocarpa* (A. E. Lawrence 675, MO) **D** *V. otobifolia* (J. P. Folsom 1440, MO). Image courtesy of Field Museum (A).

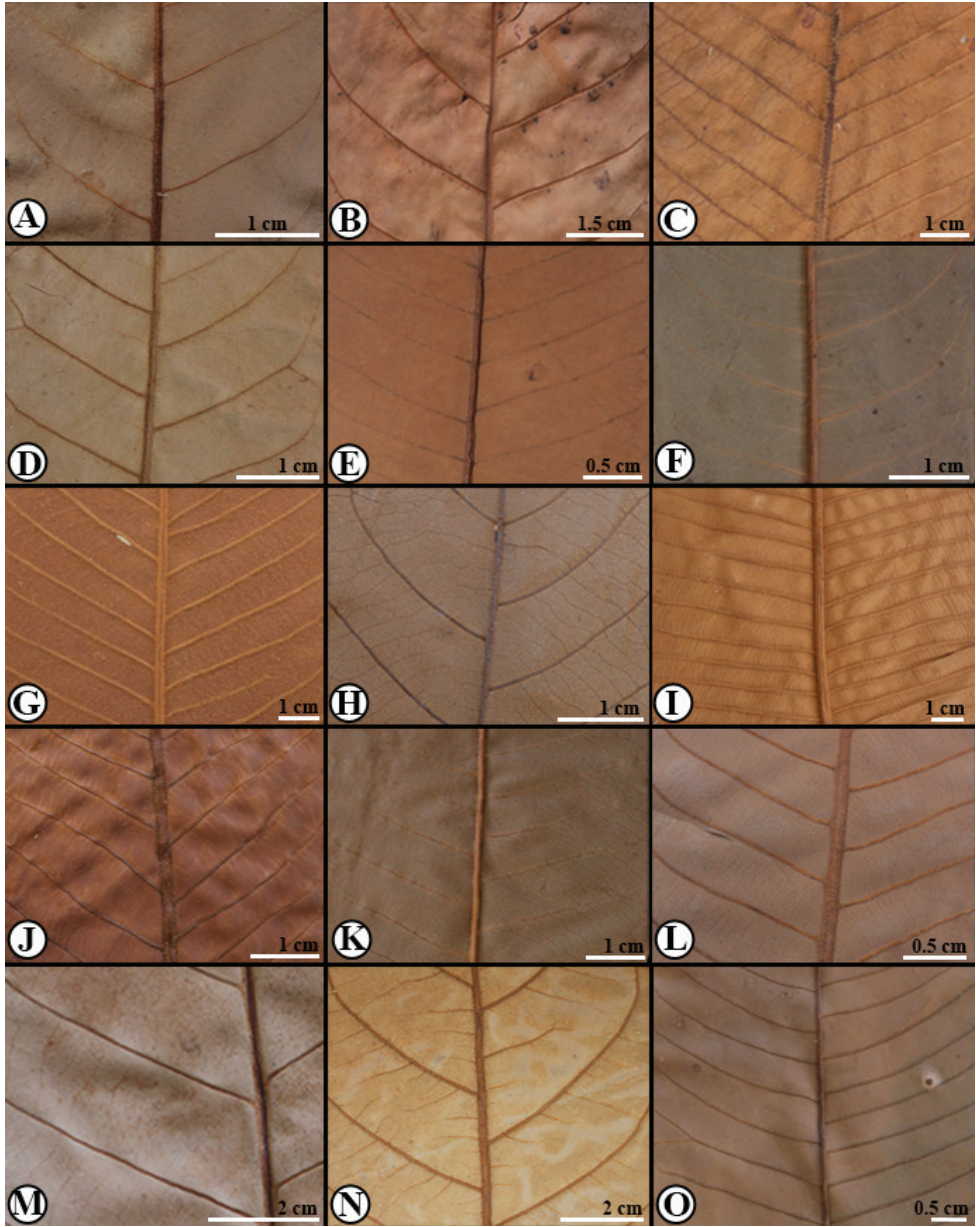


Figure 8. Pattern of the lateral veins in Mesoamerican *Virola* **A** *Virola allenii* (K. Thomsen 1284, NO) **B** *V. amistadensis* (G. McPherson 8703, MO) **C** *V. chrysocarpa* (B. Hammel et al. 16864, MO) **D** *V. elongata* (M. Correa & R. L. Dressler 1078, MO) **E** *V. fosteri* (G. de Nevers 7226, MO) **F** *V. guatemalensis* (G. Ibarra 957, MO) **G** *V. koschnyi* (J. Miller & J. C. Sandino 1110, MO) **H** *V. laevigata* (N. Zamora & T. D. Pennington 1583, MO) **I** *V. megacarpa* (G. de Nevers 5184, MO) **J** *V. montana* (E. Lépiz & J. F. Morales 284, MO) **K** *V. multiflora* (J. Manzanares 3561, MO) **L** *V. nobilis* from Barro Colorado (R. J. Schmalzel 320, MO) **M** *V. otobifolia* (G. de Nevers et al. 7530, MO) **N** *V. sebifera* (T. B. Croat 5959, MO) **O** *V. nobilis* from Osa Peninsula (R. Aguilar 11186, MO).

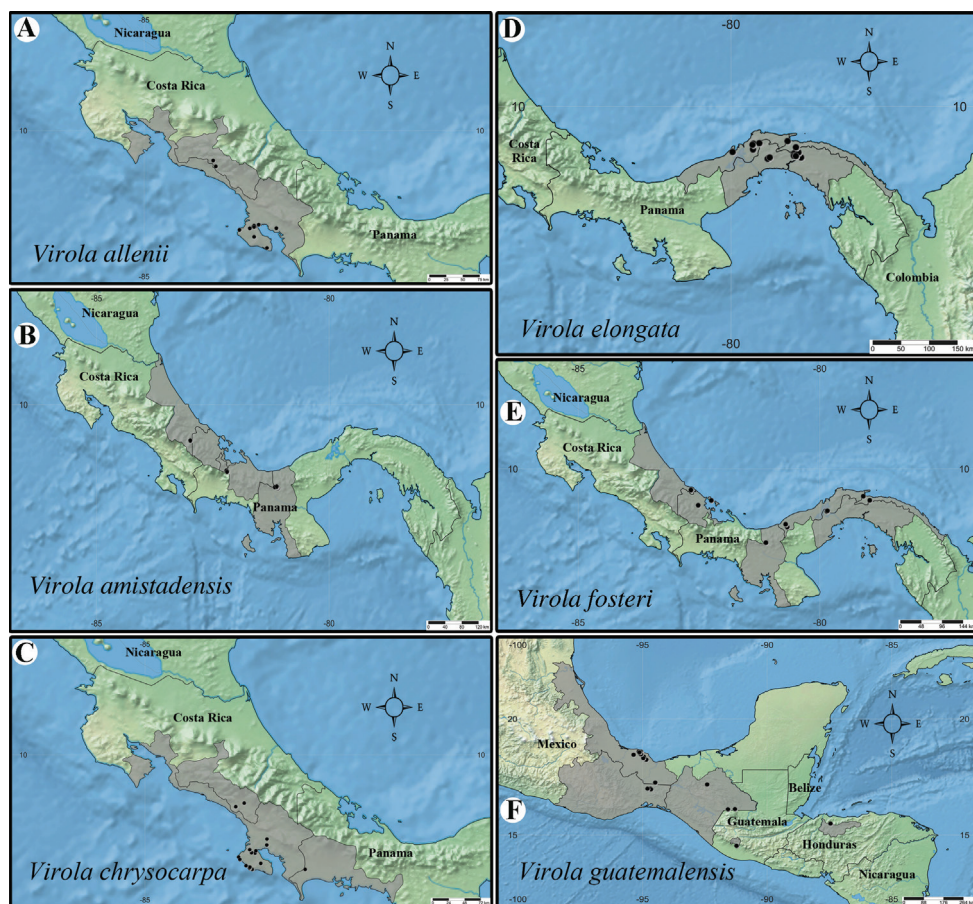


Figure 9. Geographic distribution of *Virola allenii* (A), *V. amistadensis* (B), *V. chrysocarpa* (C), *V. elongata* (D), *V. fosteri* (E) and *V. guatemalensis* (F).

Discussion. *Virola allenii* is most similar to *V. macrocarpa*, a species from montane forests at 1100 m elevation in the Andes of Colombia (Boyacá) and this name has been previously applied to the species described here (e.g. Jiménez 2007; Cornejo et al. 2012; Aguilar et al. 2017 onwards). It is differentiated from *V. macrocarpa* by the characteristics presented in the diagnosis and in Table 2.

The comparison presented hereafter for *Virola macrocarpa* (Fig. 7C) is strictly based on the protologue (except for the pericarp thickness), from which we have been able to study two physical duplicates deposited at MO (*A. Lawrance* 675, MO-2 sheets!, fr [Fig. 4D]) and the images at A! (st), F! (2-sheets, fr), G! (2-sheets, st.), K! (st), S (fr) and US! (2-sheets, fruits likely in the packet, but not seen). We confirmed all measurements from the protologue on *Lawrance* 675 (MO) and found them consistent with the exception of pericarp thickness; while 2–4 mm was stated in the protologue, our measurements ranged from 1.8–3 mm, which we present in Table 2 below. In our estimation, all other observed South American specimens, annotated with this name, represent an amalgamation of different identities (D. Santamaría-Aguilar, in prep.).

Table 2. Comparison of *Virola allenii* with the morphologically most similar species.

| Character | <i>V. allenii</i> | <i>V. calophylla</i> | <i>V. calophylloidea</i> * | <i>V. macrocarpa</i> |
|---|---|--|---|---------------------------------------|
| Leaf blade | 16.2–29.2 × 3.2–7.3 cm; base acute or obtuse to rounded | (15–) 20–60 × 10–16 cm; base (usually) deeply cordate to truncate (obtuse) | 16–33 × 4.5–8 cm; base cordate to rounded or broadly obtuse | 20–40 × 7–11 cm; base broadly obtuse† |
| Length of staminate inflorescences | 3.5–5.5 cm | 6–30 cm | 1–4 cm | Unknown |
| Length of perianth of staminate flowers | 2–2.8 mm | 1–2.1 mm | 1.7–2 mm | Unknown |
| Filament column | 0.5–0.6 mm long; not constricted at apex | 0.2–0.6 mm long; constricted at apex | 0.7–0.8 mm long; abruptly narrowed at apex | Unknown |
| Anther length | 0.6–0.9 mm | 0.4–0.5 mm | 0.5–0.6 mm | Unknown |
| Fruit | 2.7–3.5 × 1.5–2.5 cm; base obtuse | 2.5–3 × 1.2–2.5 cm; base usually truncate | 1.8–2.1 × 0.8–1.1 cm; base obtuse | 2.7–3.3 × 2–2.3 cm; base obtuse† |
| Pericarp thickness | 3.2–3.8 mm | 0.5–5 mm | 0.5–0.8 mm | 1.8–3 mm |

*From Smith 1938.

Table 3. Comparison of *Virola allenii* with the two other morphologically most similar species in Mesoamerica.

| Characters | <i>V. allenii</i> | <i>V. amistadensis</i> | <i>V. otobifolia</i> |
|---|-------------------------------------|--------------------------------------|--|
| Leaf blades | 16.2–29.2 × 3.2–7.3 cm | 12.3–22.8 (–27) × 4.4–9.5 (–12.5) cm | (14–) 18.2–42.5 × (4.1–) 7.3–14.2 cm |
| Lateral veins | 15–20 per side | 9–15 per side | 10–16 per side |
| Length of staminate inflorescence | 3.5–5.5 cm | 3–7.5 cm | 3.5–9.5 cm |
| Length of perianth of staminate flowers | 2–2.8 mm | 1.5–2.2 mm | 2.5–2.8 mm |
| Filament column | 0.5–0.6 mm; not constricted at apex | 0.2–0.4 mm; constricted at the apex | 0.9–1 mm; constricted at the apex |
| Anther length | 0.6–0.9 mm | 0.6–0.7 mm | 0.6–1 mm |
| Fruit | 2.7–3.5 × 1.5–2.5 cm (Fig. 4A) | 2.1–3.8 × 1.7–2 cm (Fig. 4B) | (2.7–) 3.5–4.5 × (1.9–) 2.3–2.9 cm (Fig. 4C) |
| Pericarp thickness | 3.2–3.8 mm | 1–2 mm | (2.7–) 3–4.7 mm |
| Seed | ca. 2.5 × 1.3 cm | 1.6–2.2 × 1.4–1.6 cm | 2.5–2.8 × 1.5–1.7 cm |

Based on a number of features listed in the diagnosis of *V. allenii*, including colour of the abaxial leaf blade, sessile trichomes, degree of separation of lateral veins and the length of the anther apiculus, *V. allenii* is similar to *V. calophylla* (Fig. 10A, B) and *V. calophylloidea* from South America; the latter was recently included as a synonym of *V. calophylla* (see notes below). Furthermore, *Virola allenii* also shares narrow oblong-elliptic leaf blades (3.2–8 cm broad) and short inflorescences with *V. calophylloidea*. However, it is distinguished from both species by the filament column that is constricted at or towards the apex (vs. not constricted in *V. allenii*) and the tendency towards short anthers (0.4–0.7 mm vs. 0.6–0.9 mm long). Additional differences amongst these three species are presented in the Table 2.

In Mesoamerica, *Virola allenii* can be confused with *V. amistadensis* and *V. otobifolia* (which are formally described as new below). All these species have lateral veins that are well spaced (Fig. 8A, B, M) and an abaxial leaf surface that is usually whitish with sessile stellate trichomes; for differences between these species see Table 3.

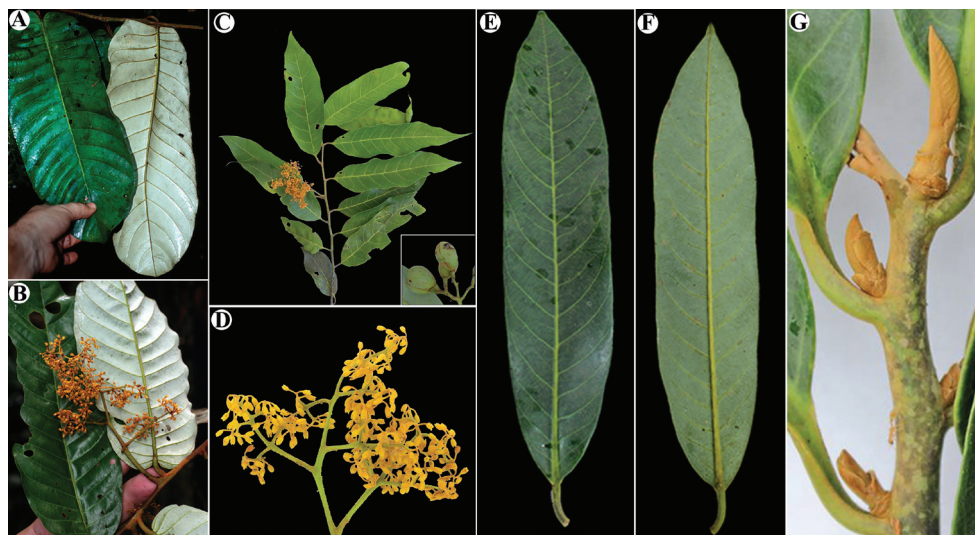


Figure 10. *Virola calophylla* **A** branch with leaves, showing both sides **B** branch with inflorescences. *Virola elongata* **C** leafy branch with inflorescences, inset fruit **D** inflorescences. *Virola guatemalensis* **E, F** leaf blades showing adaxial (**E**) and abaxial (**F**) surface. **G** young twigs and petioles. Photos by Robin Foster (**A, B**), from <https://plantidtools.fieldmuseum.org/en/nlp>; Steven Paton (**C, D**), Rolando Pérez (**C** inset), from <https://stricollections.org/portal/index.php> and Angela Rojas (**E–G**).

Some of the first specimens of this new species were confused with other taxa, though they differ based on their trichomes: *Otoba* (e.g. *L. J. Poveda* 887, CR!) with malpighiaceous trichomes (vs. stellate trichomes), *Virola sebifera* (e.g. *N. Zamora et al.* 1440, CR!) with dendritic to dendritic-stellate and generally pediculate trichomes (vs. stellate and sessile) (Fig. 3A, N); or *V. guatemalensis* (e.g. *P. H. Allen* 6727, F, GH) with the central part of the trichome colourless (vs. reddish) and with more compressed lateral veins (0.6–1.1 cm vs. 1.2–1.8 cm of separation between veins) (Figs 3A, 8A, F).

Notes. The holotype, deposited at Field Museum (F), represents two sheets with hand written annotation (“Sheet 1 of 2,” “Sheet 2 of 2”), which suggests that they represent a multi-sheet specimen of the same plant (ICN Art. 8.3) (Turland et al. 2018).

The specimen *B. Hammel et al.* 24041 (CR!; fr) from 1370 m elevation in the Tarrazú region of San José province (Costa Rica) differs from other members of this new species by its more rounded and pubescent fruits and its occurrence at a higher elevation than other specimens. It is included here with some reservation. This specimen is most similar to *E. Alfaro* 492 (CR!, LSU!, MO!; ♂ fl), which was also collected in montane forests on the Pacific slope of the Cordillera de Talamanca (1240 m elevation). *E. Alfaro* 492 differs from the rest of *V. allenii* in its larger staminate perianth (ca. 3.8–4 mm vs. 2–2.8 mm long) that is fleshy with dense pubescence on the entire adaxial surface (vs. glabrous or with sparse trichomes close to the margin of the lobes in *V. allenii*) and the column of the filaments (ca. 0.5–0.7 mm vs. 0.5–0.6 mm long) that is shorter than the anthers (ca. 1.2 mm vs. 0.6–0.9 mm long). This specimen was also discussed by Jiménez (2007) as *V. macrocarpa*.

The specimens [*P. H.*] *Allen* 5763 (type; F, USJ) and [*P. H.*] *Allen* 6727 (F, GH), cited as *V. guatemalensis* in *The Rain Forests of Golfo Dulce* (Allen, 1956), correspond with this new species.

Virola calophylloidea has recently been considered synonymous with *V. calophylla* (e.g. Rodrigues 1980; Jaramillo et al. 2004; ter Steege et al. 2019). However, here, it is treated as a morphologically distinct species. This is due to its smaller leaves, more compact staminate and pistillate inflorescences and infructescences, staminate flowers with the filament column longer than the anthers and smaller fruits (see Table 2). Some representative collections of *V. calophylloidea* include:

BRAZIL. Acre: Rio Jurua & Rio Moa, Serra da Moa, 30 Apr 1971 (♂ fl), *P. J. M. Maas et al.* P12659 (MO). **Amazonas:** Rio Urubú, 04 Aug 1979 (♂ fl), *C. E. Calderon et al.* 2922 (MO); Km 500 on Manaus-Humaitá road, 17 Sep 1980 (fr), *S. R. Lowrie et al.* 54 (MO); Km. 133, Manaus-Itacoatiara Road, 11 Sep 1974 (♂ fl), *T. D. Pennington & O. P. Monteiro* P22638 (MO); Rio Cuieras, 12 Sep 1973 (♂ fl), *G. T. Prance et al.* 17790 (MO); Reserva Experimental Station of INPA, 30 Aug 1974 (♂ fl), *G. T. Prance et al.* 21689 (MO); Rio Javari, Rio Curaçá, 8 miles above mouth, 26 Oct 1976 (♀ fl), *G. T. Prance et al.* 24133 (MO). **Pará:** Km 133, Madeira-Mamore Railway, 15 Sep 1963 (♂ fl), *B. Maguire et al.* 56666 (MO); Itaituba, Km 60 da estrada Itaituba, 16 Nov 1978 (fr), *M. G. da Silva & C. S. Rosário* 3775 (MO). **Rondônia:** Basin of Rio Madeira, 23 Nov 1968 (fr), *G. T. Prance et al.* 8775 (MO).

Specimens examined. COSTA RICA. Puntarenas: Golfito, alrededores de la estación Agujas, 300 m elev., 22 May 2000 (st), *L. Acosta et al.* 1389 (CR!); Golfito, Piro, 100 m elev., 14 Oct 1991 (st), *R. Aguilar* 511 (CR!); Golfito, estación Los Patos, 200 m elev., 02 Sep 1993 (imm fr), *R. Aguilar* 2224 (CR-2 sheets!, LSU!, MO!); Osa, Bahía Chal, La Parcela, 150 m elev., 12 Dic 1996 (st), *R. Aguilar* 4735 (CR!, MO!); Península de Osa, Rancho Quemado, sendero a Cerro Brujo, 343 m elev., 30 Jul 2013 (st), *R. Aguilar* 14519 (CR!); area between Rio Esquinas & Palmar, 60 m elev., 18 Feb 1963 (fr, dupl. in GH), *P. H. Allen* 6727 (F!*, GH!*); Osa, Sierpe, 1 km antes de la Villa de Banegas, 55 m elev., 14 Oct 2007 (fr), *E. Chacón et al.* 885 (USJ!); Osa, fila Casa Loma, Aguabuena Sur, 07 Oct 1992 (fr), 50–150 m elev., *A. Fernández* 410 (CR!, MO!); Osa, Playa Campanario o San Josecito, 1–10 m elev., 29 Dec 1991 (fl bud), *P. Harmon* 291 (CR-2 sheets!); Golfito, bosque de los Austriacos, La Gamba, 300 m elev., 09 Jan 1994 (fr), *W. Huber & A. Weissenhofer* 136 (CR!, LI*); Golfito, near the Tropenstation La Gamba on the fila, 200 m elev., 27 Jan 2002 (fl), *H. Huber* 3012 (LI-2 sheets!*); Golfito, Jiménez, Piro, no elev., 20 Jan 2012 (fr), *J. M. Ley-López* 69 (USJ!); Golfito, montaña aladaña al campo de aterrizaje, 07 Jun 1994 (st), *L. J. Poveda* 887 (CR!); Osa, 3 km después de la quebrada Banegas, camino a Rancho Quemado, 200 m elev., 10 Jan 2018 (fr), *D. Santamaría & R. Aguilar* 9865 (CR!); Península de Osa, Aguabuena, 3.5 km W of Rincón, 350 m elev., 14 Jun 1993 (st), *K. Thomsen* 746 (CR!); Península de Osa, Aguabuena, 3 km W of Rincón, 130 m elev., 15 Apr 1993 (♂ fl), *K. Thomsen* 857 (CR!, MO!, NY!*); Península de Osa, Aguabuena, 3 km W of Rincón, 130 m elev., 05 May 1993 (st), *K. Thomsen* 915 (CR!); Península de Osa, Aguabuena, 3.5 km W of Rincón, 150 m elev., 06 Oct 1994 (fr), *K. Thomsen* 1049

(CR!); Península de Osa, Aguabuena, 2.5 km W of Rincón, 150 m elev., 10 Mar 1995 (fl), *K. Thomsen* 1283 (NY!*); Península de Osa, Aguabuena, 2.5 km W of Rincón, 150 m elev., 10 Mar 1995 (♂ fl), *K. Thomsen* 1284 (NO!, NY!*). **San José:** Tarrazú, del puente de San Marcos 18 km camino hacia Quepos, 1370 m elev., 13 Jan 2006 (fr), *B. Hammel et al.* 24041 (CR!); Esquipulas, base del Cerro San Isidro, alrededores del río Naranjo, 400 m elev., 28 Aug 1987 (fr), *N. Zamora et al.* 1440 (CR!, MO!).

2. *Virola amistadensis* D.Santam., sp. nov.

urn:lsid:ipni.org:names:77202543-1

Figs 7B, 11

Diagnosis. Similar to *Virola calophylla* in its abaxial leaf surface that is whitish and covered with stellate, sessile trichomes whose centre is reddish-clear to reddish and contrasting in colour with the hyaline branches and the lateral veins that are well-spaced. *Virola amistadensis* can be distinguished from *V. calophylla* by its shorter leaf blades [12.3–22.8 (–27) cm vs. (15–) 20–60 cm long] with a usually acute or sometimes attenuate base (vs. usually deeply cordate to truncate), shorter staminate inflorescence (3–7.5 cm vs. 12–30 cm long) and fruits with an obtuse base (vs. usually truncate).

Type. PANAMA. Bocas del Toro: Vicinity of Fortuna Dam, below pass on Chiriquí Grande road, ca. 800 m elev., 27 Jun 1986 (♀ fl), *G. McPherson* 9717 (holotype: MO! [5565285, MO281258]; isotypes: INPA!* [144165], PMA!* [046113, PMA36179]).

Description. *Tree* 4–13 m tall, no recorded DBH; bark and exudate not described in herbarium specimens. *Twigs* 0.18–0.42 cm thick, inconspicuous but densely strigulose, glabrescent, trichomes irregularly stellate to dendritic, ferruginous to whitish. **Leaves:** petiole 1.4–2.3 × 0.18–0.23 cm, slightly canaliculate, pubescent, trichomes stellate; **leaf blades** 12.3–22.8 (–27) × 4.4–9.5 (–12.5) cm, elliptic to widely elliptic; adaxial surface leaves pale to dark brown (sometimes shining) when dry, glabrous or occasionally with scattered stellate trichomes; abaxial surface usually whitish-greyish when dry, but can be very light to dark brown, densely but inconspicuously pubescent, trichomes stellate, sessile, ferruginous or sometimes with the central part of the trichome reddish, contrasting in colour with the hyaline branches to reddish-clear, with 4–8 branches, the branches ca. 0.02 mm, persistent (the surface with a dense layer of squamiform hyaline structures, especially obvious at high resolution); lateral veins 9–15 per side, 3–5 veins per 5 cm, (1.2–) 1.4–2.5 cm apart, the same colour as the adaxial surface, on adaxial surface flat to slightly elevated or slightly sunken, on abaxial surface raised, free or slightly anastomosing near the margin and without forming a very marked intramarginal vein; tertiary veins barely visible or indistinct on both surfaces; midvein adaxially raised, slightly sunken in a channel, abaxially raised, rounded, tomentose, adpressed pubescent to glabrate; base usually acute or sometimes attenuate, not revolute, flat; margin not revolute; apex acuminate. **Staminate inflorescences** 3–7.5 cm long, axillary, axes flattened, pubescent, with trichomes stellate, ferruginous; peduncle 1–2 × 0.09–0.16 cm; bracts not seen; terminal fascicles lax, with 2–11 flow-

ers. **Staminate flowers** with the pedicel 0.7–1 mm long; receptacle sometimes ca. 1 mm wide; perianth 1.5–2.2 mm long, infundibuliform, brown when fresh (possibly due to indumentum), connate for ca. 0.8 mm of length, abaxial surface pubescent with brown trichomes, adaxial surface pubescent, especially on the lobes and margins; lobes 3, ca. 1.2×0.7 mm; stamens 3 (–6), the filament column 0.2–0.4 mm long, glabrous, straight, conspicuously thickened throughout its length, constricted at the apex; anthers 0.6–0.7 mm long; apiculus 0.1–0.15 mm long, apiculate, connate or slightly separated at the apex. **Pistillate inflorescences** 4.3–5 cm, similar to staminate inflorescences; peduncle $1.5\text{--}1.8 \times 0.18\text{--}0.25$ cm; bracts not seen; terminal fascicles of 4–10 flowers. **Pistillate flowers** in terminal fascicles of 4–10 flowers; with the pedicel 1.5–2 mm long; perianth 3.4–3.8 mm long, infundibuliform, brown-yellow when fresh, connate for 2.2–2.5 mm of length, abaxial surface pubescent, with brown trichomes, adaxial surface pubescent, especially on the lobes and sparsely pubescent basally; lobes 3, $1\text{--}1.4 \times 1\text{--}1.2$ mm; gynoecium $1.8\text{--}2 \times 0.8\text{--}1.1$ mm, densely pubescent, ovate, sessile; stigmatic lobes 0.4–0.5 mm, erect. **Infructescence** 2.7–6.3 cm long, with 1–5 fruits, peduncle $1\text{--}2.5 \times 0.25\text{--}0.5$ cm. **Fruits** $2.1\text{--}3.8 \times 1.7\text{--}2$ cm, ovoid to globose, shortly stipitate, densely tomentose, the trichomes irregularly stellate, ferruginous, persistent, the surface smooth to rugulate when dry, the line of dehiscence slightly carinate, the base obtuse, the apex acute to obtuse, brown to brown-yellow when fresh; pericarp 1–2 mm thick; pedicel 0.4–0.9 cm long; seed $1.6\text{--}2.2 \times 1.4\text{--}1.6$ cm, the testa when dry pale brown; aril described as red, pale brown or blackish when dry, membranaceous, dry in texture, thin, lacinate in narrow bands.

Distinctive characters. *Viola amistadensis* is recognised by its elliptical to widely elliptical leaf blades with lateral veins that are well separated (Fig. 8B) and abaxially covered with stellate, sessile trichomes (Fig. 3B) and a dense layer of squamiform structures that are hyaline in colour; the small staminate flowers with a thickened filament column that is markedly constricted at the apex and shorter (0.2–0.4 mm long) than the anthers (0.6–0.7 mm), which are apiculate at the apex; and the ovoid to globose fruits (Fig. 4B) with relatively thin pericarp (1–2 mm thick) (Figs 7B inset, 11D) that are covered by a dense, but inconspicuous layer of ferruginous trichomes. It is also distinctive for being a small tree (4–13 m tall) and its preference for premontane forests.

Etymology. The specific epithet, *amistadensis*, refers to Parque Internacional La Amistad, a UNESCO World Heritage Site, shared between Costa Rica and Panama where the holotype and some of the paratypes of this species were collected.

Distribution. *Viola amistadensis* is known from Costa Rica (Limón) and Panama (Bocas del Toro and Veraguas; Fig. 9B). It is found on the Caribbean slope at 650–1200 m elevation.

Preliminary conservation status. *Viola amistadensis* is Endangered following IUCN criteria B1a and B2a. This species is known from 4 localities and has an EOO of 2,573 km² and an AOO of only 28 km². Only nine specimens were verified in this study. While it occurs in protected areas, its montane habitat is particularly prone to habitat disturbance.

Common names. None recorded.

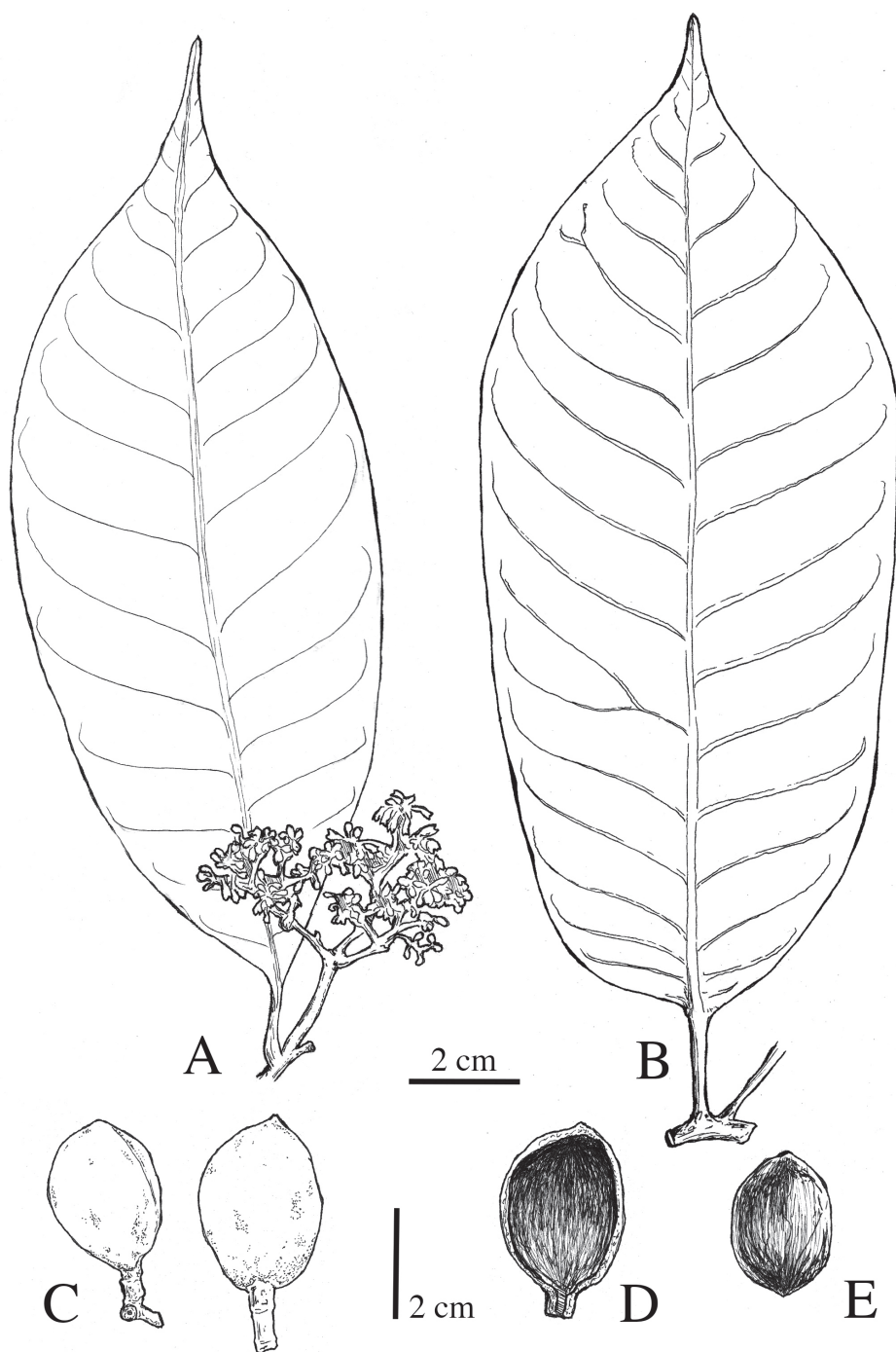


Figure 11. *Virola amistadensis* **A** leaf with staminate inflorescences **B** leaf **C** fruits **D** pericarp **E** seed. Drawn by Pedro Juárez, based on G. McPherson 9717 (**A**) and G. McPherson 9715 (**B–E**).

Table 4. Comparison of *Viola amistadensis* with the morphologically most similar species.

| Characters | <i>V. amistadensis</i> | <i>V. macrocarpa</i> |
|--------------------------------|--|---|
| Leaf blades | 12.3–22.8 (–27) × 4.4–9.5 (–12.5) cm; on abaxial side, densely but inconspicuously pubescent | 20–40 × 7–11 cm; on abaxial side, inconspicuously puberulent* |
| Lateral veins | 9–15 per side, 3–5 veins per 5 cm, (1.2–) 1.4–2.5 cm apart | 14–16 per side*, 4–5 veins per 5 cm, 0.8–1.5 cm apart |
| Petiole | 0.18–0.23 cm thick | 0.3–0.5 cm thick |
| Fruit size | 2.1–3.8 × 1.7–2 cm (Fig. 4B) | 2.7–3.3 × 2–2.3 cm* (Fig. 4D) |
| Infructescence peduncle length | 1–2.5 cm | 2.5–3 cm |
| Pedicle length in fruit | 0.4–0.9 cm | 0.3–0.5 cm* |
| Pericarp | 1–2 mm thick, with persistent trichomes | 1.8–3 mm thick, with caducous trichomes |
| Seed size | 1.6–2.2 × 1.4–1.6 cm | 2.2–2.5 × 1.5–1.7 cm* |
| Tree size | 4–13 m | 20–30 m† |

* From Smith 1938.

Phenology. Flowering of *V. amistadensis* has been recorded in April, June and July and fruiting in January to March, May, June and December.

Field characters. Plants are trees that are 4–13 m tall. Flowers have a yellow-brown perianth and brown fruits.

Discussion. Herbarium specimens of this new species usually have been identified as *Viola calophylla* (Figs 10A, B) or *V. macrocarpa* (Fig. 7C), probably because its leaves are abaxially whitish and covered with stellate, sessile trichomes with a reddish-clear to reddish centre that contrasts in colour with the hyaline branches and lateral veins that are well-spaced. Differences between *V. amistadensis* and *V. calophylla* are enumerated in the diagnosis, while those separating it from *V. macrocarpa* are listed in Table 4.

In Mesoamerica, *Viola amistadensis* is similar to *V. allenii* (Figs 6, 7A) and *V. otobifolia* (Figs 7D, 23A–E), two species from the lowland wet forest of Costa Rica and Panama. Their similarities include the characteristics of the leaf blades mentioned above. Their differences are summarised in Table 3.

Notes. Specimens from Veraguas Province (Panama), have smaller leaf blades and lateral veins that are more deeply sunken on the adaxial surface than the specimens from Limón and Bocas del Toro provinces.

Specimens examined. **COSTA RICA. Limón:** Parque Internacional La Amistad, subiendo por la fila entre la margen derecha del Río Uren y la Quebrada Crori, Croriña, 650 m elev., 17 Jul 1989 (♂ fl), *A. Chacón 194* (CR, MO!). **PANAMA. Bocas del Toro:** Along pipeline road in area of Fortuna Dam, 900–950 m elev., 08 Mar 1986 (fr), *G. McPherson 8703* (INPA!*, MO!, PMA!*); vicinity of Fortuna Dam, below pass on Chiriquí Grande road, 800 m elev., 27 Jun 1986 (fr), *G. McPherson 9715* (INPA!*, MO!); along old pipeline road from continental divide, 900 m elev., 27 Dec 1986 (imm fr), *G. McPherson & J. Aranda 10169* (INPA!*, MO!, PMA!*). **Veraguas:** Vicinity of Escuela de Agricultura Alto Piedra, near Santa Fé, 2800–3200 ft [850–975 m] elev., 03 Apr 1980 (♂ fl), *T. Antonio 4011* (INPA!*, MO!); vicinity of Cerro Tute,

850–1000 m elev., 19 Mar 1987 (fr), *G. McPherson* 10687 (MO!); near Cerro Tute-Arizona, above Santa Fe and Alto de Piedra, 850–1100 m elev., 05 Feb 1988 (fr), *G. McPherson* 12047 (MO!); vicinity of Santa Fe on slopes of Cerro Tute-Arizona above school at Alto Piedra, 900–1100 m elev., 29 Jan 1989 (fr), *G. McPherson* 13669 (INPA!*, MO!); Cerro Tute, 1 km beyond Escuela Agrícola Alto Piedra above Santa Fe, 900–1200 m elev., 14 May 1981 (fr), *K. Sytsma* & *L. Andersson* 4653 (MO!).

3. *Virola chrysocarpa* D.Santam. & Aguilar, sp. nov.

urn:lsid:ipni.org:names:77202545-1

Figs 12–14

Diagnosis. Species similar to *Virola koschnyi* due to many characteristics of the leaf, including overall shape, number of lateral veins and stalked trichomes. It differs in leaf blades with pubescent adaxial surfaces that are rough to the touch in herbarium specimens (vs. adaxial surface glabrous to glabrescent and smooth) and abaxial surfaces that are hirsute to hirsutulous (vs. tomentose) with trichomes that have few (3–6 vs. 4–10), but long branches (0.2–0.6 mm vs. 0.1–0.2 mm long), staminate flowers with a longer filament column (1.3–1.5 mm vs. 0.7–0.9 [–1.4]) mm long) and fruits with an acute to apiculate apex (vs. typically obtuse).

Type. COSTA RICA. Puntarenas: Golfito, Parque Nacional Corcovado, Estación Sirena, 10 m elev., 06 Feb 1994 (♂ fl), *R. Aguilar* 3082 (holotype: CR! [9864]; isotypes: CR! [201389], LSU! [0193694, LSU00199098], MO! [5551151, MO280080], USJ! [60813]).

Description. *Tree* 15–45 m × 25–50 cm DBH; bark sometimes described as reddish to reddish-brown. *Exudate* described as light red but without specifying from which part or red from the trunk. *Twigs* 0.18–0.28 cm thick, terete, flattened laterally to slightly angulate, hirsute tomentose, trichomes dendritic, yellowish or very pale brown. *Leaves:* petiole 1–1.6 (–2) × 0.15–0.28 cm, canaliculated, pubescent, the trichomes dendritic; *leaf blades* (17.5–) 24.2–28.8 × 7.6–10 cm, obovate to oblong; adaxial surface of mature leaves olivaceous, brown to greyish when dry, hirsute to hirsutulous, asperous (in new leaves hirsute, the trichomes dendritic-stellate, pediculate, asperous to the touch); abaxial surface similar in colour to the adaxial surface when dry, densely hirsute to hirsutulous, trichomes dendritic to dendritic-stellate, yellowish to pale brown, pediculate, with 3–6 branches, the branches 0.2–0.6 mm long, persistent; lateral veins 28–32 per side, with 5–7 (–11) veins per 5 cm, (0.5–) 0.7–1.3 cm apart, the same colour as the adaxial surface or sometimes contrasting in colour, on adaxial surface flat to slightly sunken, on abaxial surface conspicuous and raised, straight to slightly arcuate, anastomosing near the margin, forming an intramarginal vein; tertiary veins usually inconspicuous adaxially, conspicuous abaxially; midvein adaxially flat, pubescent, abaxially raised, rounded, pubescent; base usually markedly cordate, not revolute, flat; margin flat, sometimes ciliolate; apex acuminate. *Staminate inflorescences* 4–8.5 cm long, usually at nodes lacking leaves or, on few occasions, in

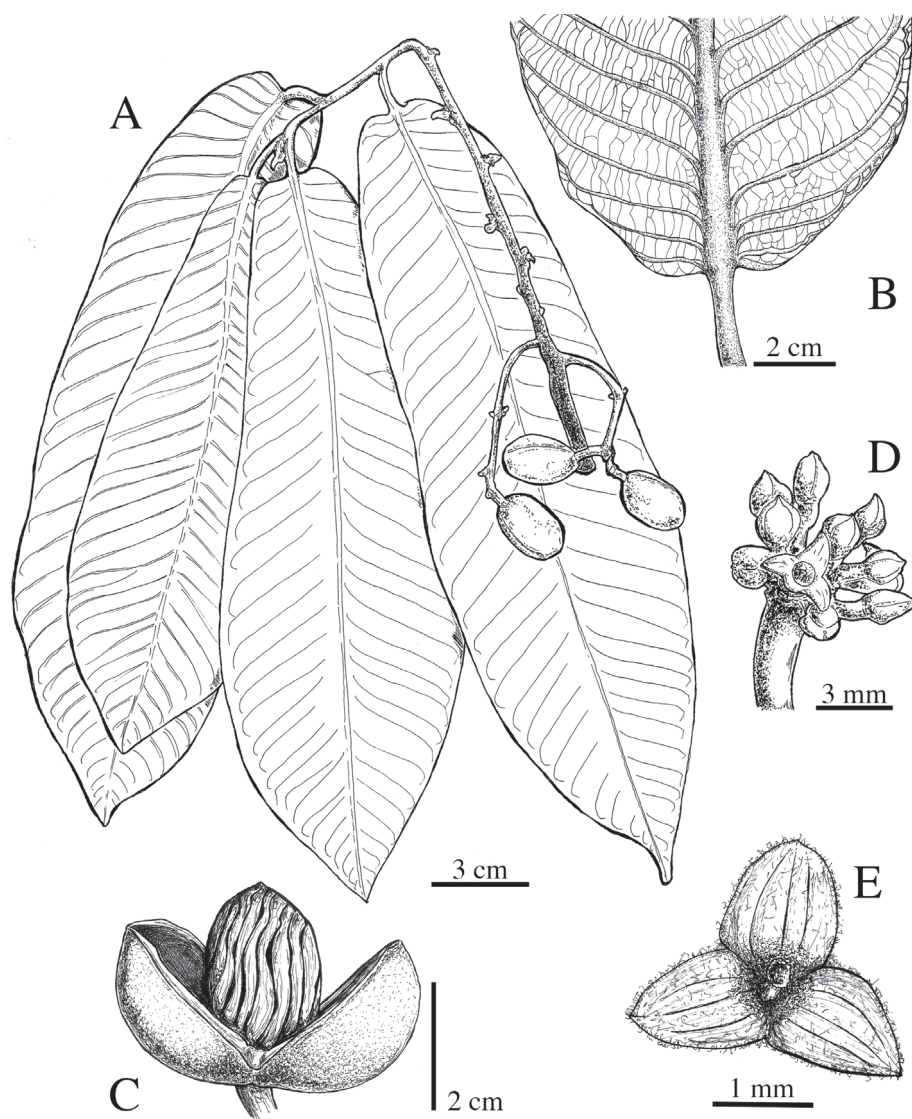


Figure 12. *Virola chrysocarpa* **A** branch with leaves and fruits **B** base of young leaf **C** fruit **D** partial staminate inflorescences **E** staminate flower. Drawn by Pedro Juárez, based on *R. Aguilar* 11190 (**A–C**) and *R. Aguilar* 11569 (**D, E**).

the axis of leaves, axes slightly flattened, densely pubescent, the trichomes dendritic, yellowish to pale brown; peduncle $1.5\text{--}4 \times 0.13\text{--}0.19$ (–0.3) cm; bracts $0.5\text{--}0.8 \times 0.3\text{--}0.5$ cm, pubescent on both sides, caducous; terminal fascicles dense, with 15–30 + flowers. **Staminate flowers** with the pedicel 2.8–3.5 mm long; receptacle 2–4 mm wide; perianth 2–3 mm long, subglobose to rhomboid, yellow when fresh, connate for 0.6–1.5 mm of length, abaxial surface pubescent, with pale brown, yellowish or golden

trichomes, adaxial surface with few scattered trichomes, especially on the lobes; lobes 3, $1.5\text{--}2.3 \times 0.6\text{--}1.5$ mm; stamens 3, the filament column $1.3\text{--}1.5$ mm long, thin, not constricted at the apex; anthers 0.6 mm long; apiculus apparently absent, the apex obtuse; pollen $28\text{ }\mu\text{m}$, with bilateral symmetry, boat shaped to elliptic grain, exine reticulate, exine structure tectate-perforate (based on Lambright 1981; *Skutch* 4260, US).

Pistillate inflorescences and flowers not seen. **Infructescence** $3.2\text{--}7.5$ cm long, 1–2 fruits (sometimes 4 in an immature infructescence), peduncle $1.2\text{--}5 \times 0.18\text{--}0.27$ cm.

Fruits $2.4\text{--}2.9 \times 1.7\text{--}1.8$ cm, ellipsoid, sessile, densely tomentose to glabrate, the trichomes dendritic, brown to brown-reddish, the surface smooth to rugulose, the line of dehiscence smooth, canaliculate, to slightly carinate, the base rounded, the apex acute to apiculate, yellow, orange or ferruginous (possibly by the indumentum) when fresh; pericarp $1.8\text{--}2.5$ mm thick; pedicel $0.5\text{--}0.8$ cm long; seed ca. $1.7\text{--}2.1 \times 1.3\text{--}1.4$ cm, the testa pale brown to blackish when dry, slightly grooved to almost smooth; aril usually described as red or pink when fresh, brown or yellowish when dry, oily, thick, lacinate in narrow bands or wide distally. Germination epigeal, seedling cryptocotylar (Ley López and Chacón Madrigal 2017; as *V. koschnyi*).

Distinctive characters. *Viola chrysocarpa* is distinguishable for its leaf blades with pubescent adaxial surfaces that are rough to the touch in mature leaves (at least in herbarium specimens) and abaxial surfaces that are hirsute to hirsutulous with trichomes with long branches ($0.2\text{--}0.6$ mm long) (Fig. 3C), numerous lateral veins (28–32 per side), tertiary veins that are usually conspicuous on both surfaces (Figs 3C, 8C) and with a base that is usually markedly cordate; staminate inflorescences that are little-branched (Fig. 14D) with flowers with filament columns that are much longer ($1.3\text{--}1.5$ mm) than the anthers (0.6 mm); and fruits that are acute to apiculate at the apex (Fig. 14E, F, H). Additionally, as far as we are aware, this is the only Mesoamerica species that is completely deciduous (i.e. all leaves fall off the tree) (Fig. 13A).

Etymology. The specific epithet, *chrysocarpa*, is derived from the Greek *chryso* (gold) and *carpo* (fruit). This is in reference to its common name, “fruta dorada” (golden fruit), which is used by locals of the Osa Peninsula, Costa Rica, where this species is frequent.

Distribution. *Viola chrysocarpa* is known from Costa Rica (Puntarenas and San José) and Panama (Chiriquí) (Fig. 9C). It is found in the Pacific slope at 0–700 m in elevation.

Preliminary conservation status. Possible Near Threatened: This species has a small estimated AOO (60 km^2), though a relatively large estimated EOO of $5,334\text{ km}^2$. Its eighteen known specimens represent eleven localities. This limited number of specimens warrants a Possible NT status, though additional collection efforts may demonstrate the lack of conservation threat for this poorly known species.

Common names. Costa Rica: fruta dorada. Panama: bogamani.

Phenology. Herbarium specimens of flowering *Viola chrysocarpa* have been collected in December to March and fruiting specimens from March to June. Herbarium specimens with pistillate flowers were not observed. In the Osa Peninsula, leaves fall completely during the dry season, which occurs in November to February (Allen 1956; Quesada Quesada et al. 1997; and R. Aguilar pers. obs., 2015, 2017, 2018, 2019).

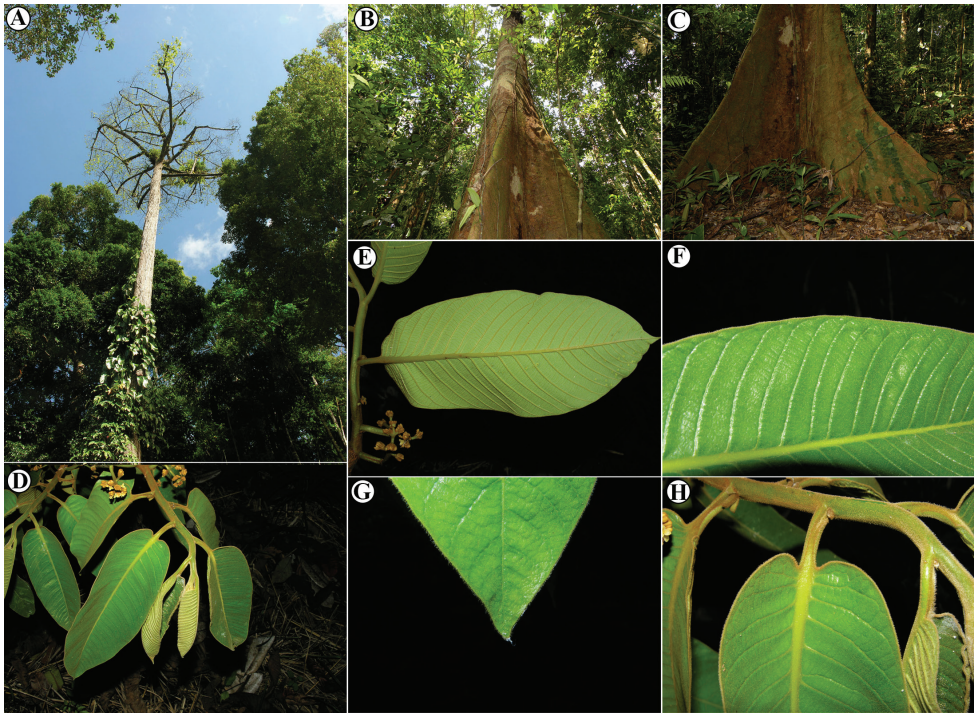


Figure 13. Vegetative characteristics of *Virola chrysocarpa* **A** tree showing deciduous nature of the species **B** trunk **C** buttress **D** branch showing new leaves **E** leaf blades on abaxial surface **F** leaf blades on abaxial surface, showing margin detail and venation **G** leaf apex **H** new branch and leaf base. Photos by Reinaldo Aguilar.

A study of vegetative, flowering and fruiting phenology has been published by Lobo et al. (2008; as *V. koschnyi*) in the Osa Peninsula and Golfo Dulce, Costa Rica. In this study, flowering was documented in January and February, when the canopy was deciduous. Fruiting occurred in the rainy season from June to August.

Field characters. Plants are large trees with boles that are straight and do not begin to branch until they reach a great height, with buttresses, up to 1.6–2.5 m tall. Bark is sometimes described as finely fissured. The new leaves are lime green in colour. Twigs, petioles and leaf blades on both surfaces (especially the youngest ones) are covered with golden, brown-reddish to rusty-red trichomes. Flowers have yellow or yellow-cream perianth and anthers. Mature fruits are yellow, orange or ferruginous (possibly due to their indumentum). Seeds are brown or blackish and covered with a red to scarlet aril.

Discussion. *Virola chrysocarpa* resembles a morphological group of species from South America that includes *V. caducifolia* W. A. Rodrigues, *V. decorticans* Ducke, *V. guggenheimii* W. A. Rodrigues, *V. multicostata* Ducke, *V. multinervia* Ducke, *V. polynura* W. A. Rodrigues and *V. rugulosa* (Spruce) Warb. These species are characterised by having leaves that are evidently pubescent, some with dendritic to irregularly dendritic pediculate trichomes on the abaxial surface and leaf blades with numerous, conspicuous and comparatively dense lateral veins; staminate flowers with anthers that

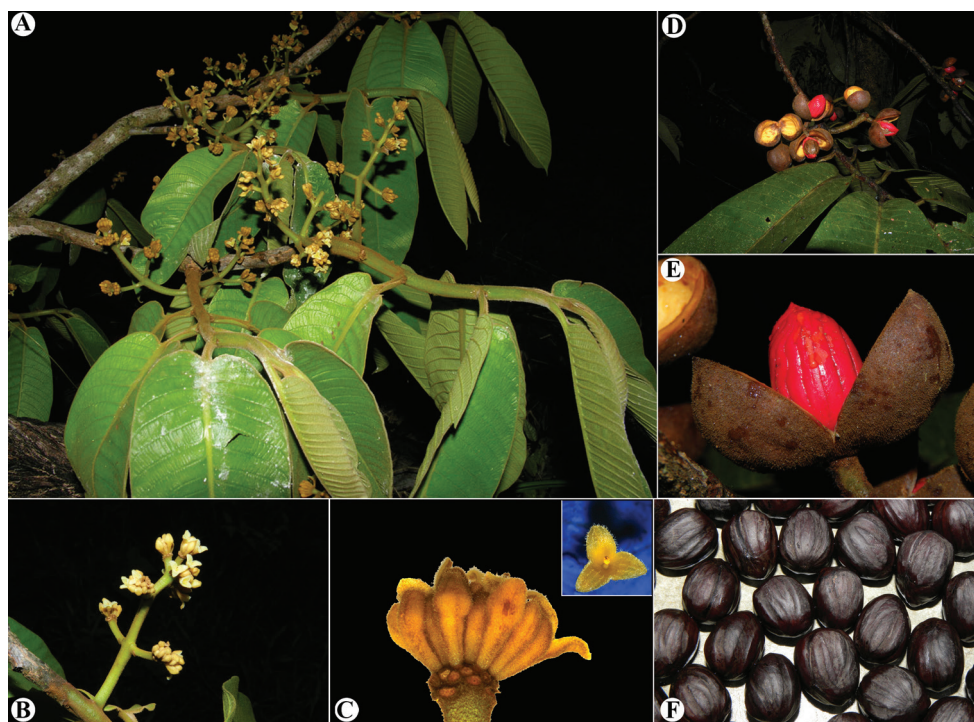


Figure 14. Fertile characteristics of *Virola chrysocarpa* **A** branch with staminate inflorescences **B** staminate inflorescences **C** staminate flower buds and open flower **D** infructescence **E** open fruit **F** seeds. Photos by Reinaldo Aguilar.

are subequal to or shorter than the filament column; and fruits with thick pericarp. Additionally, these species tend to be large, sometimes deciduous trees with cordate leaf bases and staminate flowers with the anthers that are obtuse at the apex. Table 5 presents the differences between these species and *V. chrysocarpa*.

In Mesoamerica, *Virola chrysocarpa* resembles and has been confused with, *V. koschnyi* (e.g. Allen 1956; Quesada Quesada et al. 1997; Jiménez 2007; Aguilar et al. 2017 onwards) (Figs 3G, 4F, 17), from which it differs by the characteristics included in the diagnosis. Additionally, *V. chrysocarpa* is a deciduous (vs. evergreen) species of the Pacific slope (vs. Caribbean slope). In the region, *Virola chrysocarpa* can also be confused with *V. megacarpa* from Panama for its leaf blades with dense lateral veins and a prominent marginal vein, as well as pediculate trichomes on the abaxial leaf surface; however, *V. megacarpa* has more lateral veins [(32–) 40–50 vs. 28–32 per side], the fruits are larger (4–5.7 × 2–2.9 cm vs. 2.4–2.9 × 1.7–1.8 cm) and with an acuminate to rostrate apex (vs. apex acute to apiculate) (Fig. 4E, P) and thick pericarp (3–6 mm vs. 1.8–2.5 mm).

Notes. The illustration presented in *Manual de Plantas de Costa Rica* (Jiménez 2007) as *V. koschnyi* is a mix of these two species. The branch with leaves and inflorescences (and, most likely, the trichomes) are based on material that represents *V.*

Table 5. Comparison of *Virola chrysocarpa* with the most morphologically similar species.

| Character | <i>V. chrysocarpa</i> | <i>V. caducifolia</i> [‡] | <i>V. decorticans</i> [‡] | <i>V. koschnyi</i> | <i>V. guggenheimii</i> [‡] | <i>V. multicostata</i> [‡] | <i>V. multinervia</i> [‡] | <i>V. polyneura</i> [‡] | <i>V. rugulosa</i> [‡] |
|---------------------------|-------------------------------------|------------------------------------|------------------------------------|---------------------------|-------------------------------------|---|------------------------------------|-------------------------------------|-------------------------------------|
| Petiole length | 1–1.6 (–2) cm | 0.5–0.35 cm | 0.7–2 cm | 0.5–1.5 cm | 0.5–1 (–2) cm | 0.2–0.4 cm | 0.4–1.5 cm | 1–2.5 cm | 0.8–1.1 cm [†] |
| Leaf size | (17.5–) 24.2–28.8 × 7.6–10 cm | 10–42 × 3.5– 12.5 cm | 25–60 × 11– 21 cm | 14.1–29.9 × 4.2–8.7 cm | 5–22 (–25.5) × 2–6.5 (–10) cm | 20–28 × 4–10 cm | 25–45 × 8–16 cm | 5.5–11 × 4–8.5 cm | 20–27 × 7–9.5 cm |
| Adaxial pubescence | Pubescent | Glabrous | Pubescent (pilose) | Glabrous | Pubescent (sparsely strigulose) | Glabrous or pubescent on the veins [†] | Glabrous | Glabrous (pubescent on the midvein) | Glabrous (pubescent on the midvein) |
| Trichomes on abaxial side | Pediculate | Sessile | Pediculate | Pediculate | Pediculate | Pediculate [†] | Pediculate | Pediculate | Pediculate |
| No. of lateral veins | 28–32 | 48–60 (–69) | 45–60 | (16–) 20–35 | 24–58 | 50–60 | 40–60 | 30–50 | 23–27 |
| Staminate infls. length | 4–8.5 cm | 18 cm | 22 cm | 5–11 cm | 14 cm | ca. 15 cm | 15–20 cm | 5 cm | 25 cm |
| Staminate perianth length | 2–3 mm | 1–1.4 mm | 1.5–1.8 mm | 2–2.5 mm | 1–1.5 mm | ca. 1 mm | 1.2–1.5 mm | None recorded | 1.3–1.5 mm [†] |
| Filament column length | 1.3–1.5 mm | 0.7–0.9 mm | 0.3–0.4 mm | 0.7–0.9 (–1.4) mm | 0.3–0.4 mm | None recorded | ca. 0.6 mm [§] | None recorded | Not described |
| Anther length | 0.6 mm | 0.4 mm | 0.5–0.6 mm | 0.5–0.7 (–1) mm | 0.4–0.5 mm | None recorded | ca. 0.5 mm [§] | None recorded | Not described |
| Fruit size | 2.4–2.9 × 1.7– 1.8 cm | 2.5–3 × 1.3– 2.5 cm | 2.7–3.5 × 1.7– 2.2 cm | 1.9–3.1 × 1.5– 1.9 cm | 2–2.8 × 1.5– 2 cm | 2–3.5 × 1.8– 2.5 cm | 2–3 × 1.5– 2.5 cm | 2–2.3 × 1.5– 1.8 cm | 1.5–2 × 1.5– 1.7 cm |
| Pericarp thickness | 1.8–2.5 mm | 2–3 mm | Not described | 1.2–3.1 mm | 2–4 mm | 2–5 mm | 1.5–4 mm | 1–2 mm | 3 mm |
| Leaves phenology | Deciduous | Deciduous | Evergreen | Evergreen | Evergreen | Deciduous | Deciduous | Evergreen | Evergreen |

[†]From Rodrigues 1980, except where it is specified otherwise that it comes from [‡]Smith 1938 and [§]Jaramillo et al. 2004.

chrysocarpa (R. Aguilar 3082, 3125), while the other parts of this illustration (staminate flowers and fruits) represent *V. koschnyi* and are based on *F. Araya* 197 (fl), and *U. Chavarria* 1918 (fr) (B. Hammel pers. comm., Feb 2019).

Specimens examined. **COSTA RICA. Puntarenas:** Osa, Parque Nacional Corcovado, Estación San Pedrillo, 10–100 m elev., 19 Feb 1994 (♂ fl), *R. Aguilar* 3125 (CR-2 sheets!, MO!); Osa, Reserva Forestal Golfo Dulce, Mogos, a 20 km. de Chacarita, 17 Apr 2008 (fr), *R. Aguilar* 11190 (NY!*, USJ!); Rincón, Banegas centro del pueblo, 49 m elev., 15 Dec 2008 (♂ fl), *R. Aguilar* 11569 (MO!, NY n.v., PMA!*); forest below Esquinas Experiment Station Residence, area between Río Esquinas and Palmar Sur de Osa, 100 ft [30 m] elev., 30 May 1950 (fr), *P. H. Allen* 5554 (CR-2 sheets!, MEXU!*, MO-2 sheets! [photo & dried specimen], PMA!*); Golfito, Estación Agujas, 300 m elev., 18 Feb 1998 (♂ fl), *A. Azofeifa* 683 (CR-2 sheets!, MO!); Osa Península, Rancho Quemado, ca. 15 km W of Rincón, 200–400 m elev., 28 May 1988 (fr), *B. Hammel et al.* 16864 (CR!, INPA!*, MO!, PMA!*); Rancho Quemado, a lo largo de Río Riyito en la pura entrada al valle, 200 m elev., 31 Mar 1991 (imm fr), *B. Hammel et al.* 18186 (CR!, MEXU!*, MO!, USJ!); Parque Nacional Corcovado, Pavo Forest, 0–150 m elev., 16 Jun 1988 (fr), *C. Kernan & P. Phillips* 582 (CR!, INPA!*, MO!); Parque Nacional Corcovado, Ollas trail, 0–100 m elev., 09 Jan 1989 (fl bud), *C. Kernan et al.* 876 (CR!, MEXU!*, MO!, USJ!); Parque Nacional Corcovado, Llorona Forest, 0 m elev., 16 Jan 1989 (♂ fl), *C. Kernan & P. Phillips* 913 (CR!, INPA!*, MO!, USJ!); Parque Nacional Corcovado, Sirena, 1–20 m elev., 15 Jun 1990 (fr), *G. Maass* 34 (CR!, MO!); Golfito, Parque Nacional Corcovado, sendero Las Ollas, 100–150 m elev., 21 Mar 1995 (fr), *J. F. Morales* 3690 (CR!, LSU!, MO!); Aguabuena, 3 km W of Rincón, 120 m elev., 06 May 1993 (fr), *K. Thomsen* 371 (CR-2 sheets!). **San José:** [Pérez Zeledón], Vicinity of El General, 700 m elev., n.d., Feb 1939 (♂ fl), *A. F. Skutch* 4241 (MO!); [Pérez Zeledón], Vicinity of El General, 740 m elev., n.d. Mar 1939 (♂ fl), *A. F. Skutch* 4260 (MO!, US n.v.); Tarrazú, San Lorenzo, camino entre cerro Pito y cerro Toro, 600–700 m elev., 26 May 1998 (fr), *O. Valverde* 970 (CR!, MO!, USJ!). **PANAMA. Chiriquí:** Progreso, no elev., Jul–Aug 1927 (fr, at GH, NY), *G. P. Cooper & G. M. Slater* 175 (F!*, GH!*, NY!*).

4. *Virola elongata* (Benth.) Warb.

Fig. 10C–D

Virola elongata (Benth.) Warb. Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 68: 178. 1897.

Myristica elongata Benth. Hooker's J. Bot. Kew Gard. Misc. 5: 5. 1853. Type. Brazil. "Brasilia prope Borba", Aug. 1828, [L.] *Riedel* 116 or s.n. (holotype: K; isotypes: B destroyed, BM, C, G, LE, P, S; fide Jaramillo et al. 2004).

Distinctive characters. *Virola elongata* it is recognized by its relatively small leaf blades [10.8–18.5 × 2.7–3.7 (–5.1) cm] with a sparsely pubescent abaxial surface with stellate to

dendritic-stellate trichomes that are usually sessile (Fig. 3D) and few lateral veins (9–14 per side) that are well separated [(0.8–) 1–1.7 cm apart] (Fig. 8D); staminate inflorescences with thin axes and flowers with short filament columns (0.3–0.4 mm long), anthers that are apiculate at the apex (apicula 0.1–0.2 mm long) and more than twice the length of the column (0.6–0.9 mm long); and its small fruits (1.6–1.9 × 0.9–1.1 cm) (Fig. 4L) that are green when ripe (drying light brown in herbarium specimens with the surface blistering to rough), inconspicuously to sparsely pubescent (densely pubescent when young) with trichomes that are dendritic and brown to ferruginous in colour (when present), pericarp that is 0.5–0.7 mm thick and an aril that is thin and lacinate in narrow bands.

Distribution. In Mesoamerica, *Virola elongata* is only known in Panama (Colón, Panamá and San Blas) (Fig. 9D). It has been recorded from between 50–450 m elevation.

Common names. None recorded in Mesoamerica.

Phenology. Specimens of *Virola elongata* with flowers were collected in July, October and September and with fruits in January, February, April, July, August and October. Collections with pistillate flowers were not seen.

Field characters. Plants are trees between (3–) 7–15 m tall and 0.7–12 cm DBH. Bark cuts are sometimes aromatic. Leaf blades are light green or whitish abaxially. Flowers have a yellow or yellow-orange perianth.

Selected specimens seen. **PANAMA. Colón:** Camino a la zona maderera de Santa Rita, no elev., 03 Oct 1968 (♂ fl), *M. D. Correa & R. L. Dressler 1078* (MO!); Santa Rita, East ridge, no elev., 23 Jan 1968 (fr), *J. D. Dwyer & Correa 8420* (MO!); near Rio Boqueron, no elev., 11 Oct 1974 (♂ fl), *S. Mori & J. Kallunki 2423* (MO!); Santa Rita Ridge, 1000–1200 ft [305–365 m] elev., 25 Sep 1985 (♂ fl), *K. J. Sytsma 1346* (MO!). **Panamá:** Una milla después del Lago Goofy, no elev., 04 Jan 1968 (imm fr), *M. D. Correa & R. L. Dressler 585* (MO!); El Llano-Carti road, 350 m elev., 16 Jul 1987 (fl bud), *G. McPherson 11275* (MO!); Cerro Azul, [600 m elev.], 06 Aug 1961 (fl bud, imm fr), *J. D. Dwyer 1383* (MO!); Canal Zone, between Chilibre and Madden Dam, no elev. and date (fr), *J. D. Dwyer 8420A* (MO!); vicinity of El Llano, no elev., 07–08 Sep 1962 (♂ fl), *J. A. Duke 5508* (MO!); along Rio Terable, [50 m elev.], 14–15 Sep 1962 (♂ fl), *J. A. Duke 5667* (MO!); West of El Llano, [20–50 m elev.], 03 Oct 1972 (imm fr, ♂ fl), *E. L. Tyson 6866, 6867* (MO!). **San Blas:** San Blas–Panama border, 300 m elev., 01 Feb 1989 (fr), *G. McPherson 13674* (MO!); Río Cangandi, 100 m elev., 17 Feb 1985 (fr), *G. de Nevers 4891* (MO!).

5. *Virola fosteri* D.Santam., sp. nov.

urn:lsid:ipni.org:names:77202547-1

Figs 15, 16

Diagnosis. Species resembling *Virola multiflora* due its small leaf blades and fruits, similar leaf shape and inconspicuous stellate, sessile trichomes on the abaxial leaf surface. Both species also occur on the Caribbean slope of Mesoamerica. They differ in the shape of the leaf base (revolute in *V. fosteri* vs. not revolute in *V. multiflora*), the length

of the filament column (0.9–1.3 mm vs. 0.7–1 mm long) and anthers (0.6–0.9 mm vs. 0.3–0.6 mm long) and thickness of the pericarp (1.5–2.5 mm vs. 0.7–1 mm thick).

Type. PANAMA. Bocas del Toro: Isla Colón, Aprox. a 8 km al NE de los laboratorios del Instituto Smithsonian de Investigaciones Tropicales, Big Creek, 5 m elev., 23 Apr 2009 (♂ fl), *C. Galdames*, *M. Stapf*, *K. Toribio* & *Arsenio* 6422 (holotype: PMA!* [094201, PMA92162]; isotypes: MO! [6421737, MO-2504180], SCZ!* [17752, SCZ17684]).

Description. *Tree* (15–) 20–35 m × 35–60 cm DBH; bark brown or reddish. *Exudate* described as watery-reddish possibly from the bark, damage to any part of the plant causes the flow of a watery exudate that turns reddish moments later. *Twigs* 0.12–0.24 cm thick, terete to slightly angulate, puberulent, trichomes stellate, yellowish to pale brown. *Leaves:* petiole 0.4–0.7 (–1) × 0.07–0.12 cm, canaliculate, densely tomentose to sparsely pubescent, the trichomes stellate; leaf blades 7.8–12 × 1.4–2.7 cm, narrowly elliptical or oblong to oblanceolate; adaxial surface dark brown, light brown or blackish when dry, glabrous, the surface smooth; abaxial surface pale brown to reddish-brown when dry, puberulent, trichomes stellate, sessile, yellowish to pale brown, with 4–8 branches, each branch ± 0.03–0.05 mm long, persistent; lateral veins 16–24 per side, 10–15 veins per 5 cm, 0.2–0.5 (–0.7) cm apart, the same colour as the adaxial surface, on adaxial surface sunken, on abaxial surface flat to slightly elevated, arcuate-ascending, slightly anastomosing near the margin and not forming a marked intramarginal vein; tertiary veins adaxially almost indistinct to slightly sunken, abaxially almost indistinct; midvein adaxially canaliculate, glabrous, abaxially raised, laterally compressed and sometimes resembling a cutting edge, tomentose to sparsely pubescent; base attenuate, revolute; margin revolute (especially near the base) or flat; apex acute to acuminate. *Staminate inflorescences* 2.5–5.3 cm long, axillary, usually in the axil of terminal leaves, axes flattened to irregularly angled, tomentose, with trichomes stellate, yellowish to pale brown; peduncle 0.9–17 × 0.13–0.25 cm; bracts 2–5 × ca. 2.5 mm, tomentose on both surfaces, the indumentum more clustered on the external side, caducous; terminal fascicles dense, with 5–15+ flowers. *Staminate flower* with the pedicel 1–2 mm long; receptacle 1.5–2.3 mm wide; perianth 2–2.5 (–3) mm long, subglobose, yellow, orange or yellow-orange when fresh, connate for 0.5–0.8 mm of length, abaxial pubescent, with golden to yellowish trichomes, adaxial surface glabrous somewhat pubescent near the lobes; lobes 3, 1.5–2.6 × (0.6–) 1.3–1.8 mm; stamens 3 (–6), the filament column 0.9–1.3 mm long, glabrous, straight or rarely thickened near the base in some flowers (*McPherson* 20148), thin, not constricted at the apex; anthers 0.6–0.9 mm long; apiculus small enough to as appear absent, acute to obtuse. *Pistillate inflorescences* 1.3–3.9 cm long, axillary, with trichomes on the axes similar to those of the staminate inflorescences; peduncle 0.7–2.2 × 0.08–0.14 cm; bracts not seen; terminal fascicles of 4–7 flowers. *Pistillate flowers* with the pedicel 1.5–2.5 mm long; perianth 2–3 mm long, subglobose, yellow when fresh, connate by 0.6–0.8 mm long, abaxial surface pubescent with golden to yellowish trichomes, adaxial surface sparsely pubescent, the indumentum on the lobes; lobes 3, 1.2–1.5 (–2.5) × 0.7–2.1 mm; gynoecium 1.6–2.4 × 1.1–1.4 mm, densely pubescent, globose to subglobose, stipitate; stigmatic lobes ca. 0.6 mm, erect. *Infructescence* 2.5–3 cm long, with 1–3 fruits, peduncle 1.5–1.7 ×

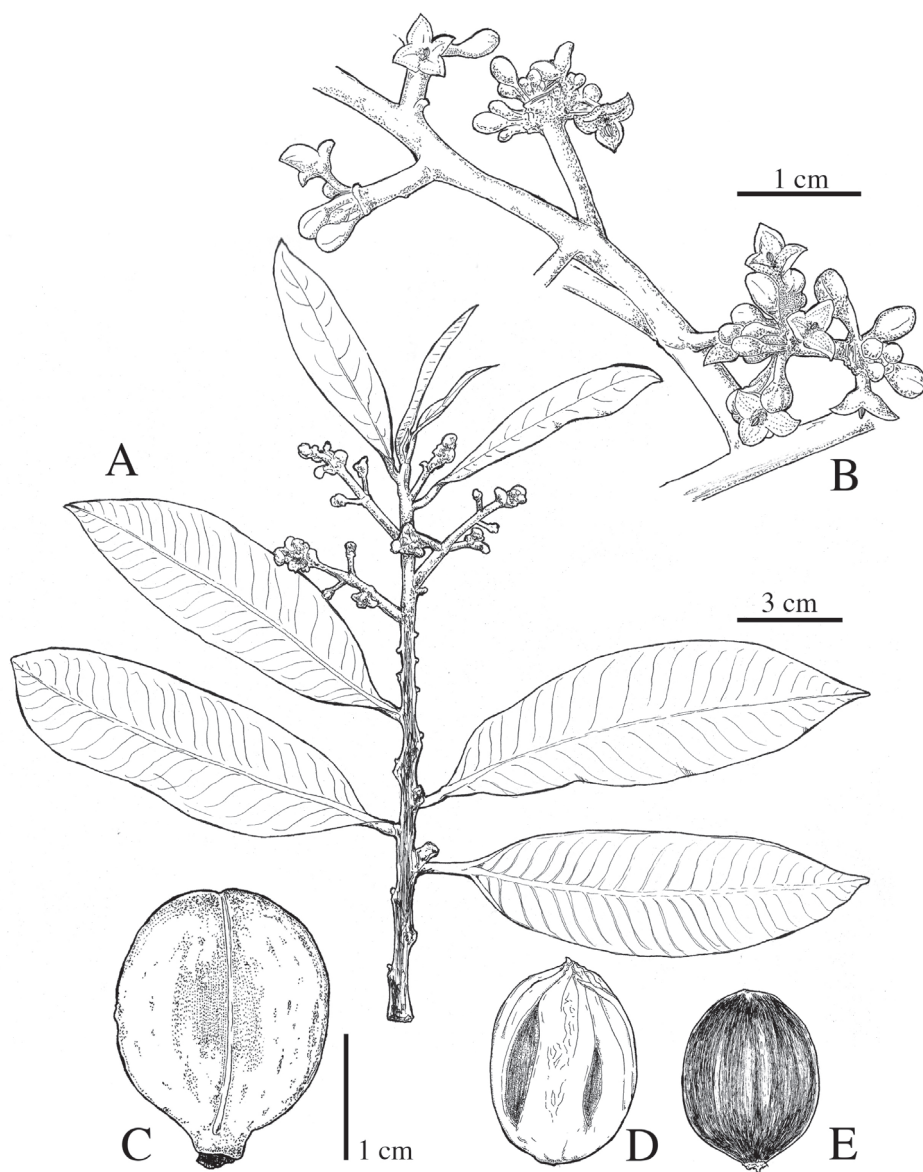


Figure 15. *Viola fosteri* **A** branch with leaves and inflorescences **B** partial inflorescences **C** fruit **D** seed with aril **E** seed. Drawn by Pedro Juárez, based on C. Galdames *et al.* 6422 (**A–E**).

0.15–0.38 cm. **Fruits** 1.5–2.3 × 1.2–1.8 cm, ovoid, sessile or very shortly stipitate, tomentose, the trichomes stellate, reddish-brown, the surface rugose when dry, the line of dehiscence canaliculate or smooth, the base obtuse to rounded, the apex obtuse, yellow, orange or golden brown when fresh; pericarp 1.5–2.5 mm thick; pedicel 0.4–0.5 cm long; seed ca. 1.6 × 0.9 cm, the testa pale brown when dry, very slightly grooved; aril usually described as red when fresh, reddish-brown when dry, coriaceous, oily, some-

what thick, laciniate in narrow bands. Germination epigeal, seedling cryptocotylar, epicotyl hairy, moderately dense, stellate and sessile (Garwood 2009; as *V. multiflora*).

Distinctive characters. *Virola fosteri* is recognised by its small leaf blades (7.8–12 × 1.4–2.7 cm) and fruits (1.5–2.3 × 1.2–1.8 cm) (Figs 4I and 16F–H), as well the stellate, sessile trichomes on the abaxial surface of the leaf (Fig. 3E). It is also distinguished by its leaf blades that have numerous lateral veins (16–24 per side) that are prominent on adaxial surface, the revolute leaf margin and the base (Fig. 16C), the midvein that is laterally compressed adaxially and sometimes resembling a cutting edge; staminate flowers with a filament column (0.9–1.3 mm long) that is longer than the anthers (0.6–0.9 mm long); and its thick pericarp (1.5–2.5 mm).

Etymology. The specific epithet honours one of its collectors, Robin B. Foster (1945–), ecologist and botanist at Field Museum in Chicago (F) who pioneered the cataloguing of the flora of Barro Colorado Island (BCI) in Panama, where *V. fosteri* occurs. Robin noted on one of his collections (*R. B. Foster 2931*) that it could represent a new species. In addition, on the same herbarium sheet, he observed one of the taxonomic characters that we here use to distinguish this as a new species: “*Leaves are consistently small throughout the tree and on juvenile plants.*”

Distribution. *Virola fosteri* is known from Costa Rica (Limón) and Panama (Bocas del Toro, Colón, Panamá, San Blas and Veraguas) (Fig. 9E). It is found on the Caribbean slope from 0–350 (–800) m elevation.

Preliminary conservation status. *Virola fosteri* is Vulnerable following IUCN criterion B2a. While the EOO for this species is large (25,645 km²), the small AOO (40 km²) with only eight known localities warrants its conservative status.

Common names. Panama: bogamani, fruta dorada.

Phenology. Flowering of *Virola fosteri* has been recorded in January to April, June and October and production of fruits in January to April.

Field characters. Plants are large trees with tall buttresses. Bark exudes reddish watery exudate when damaged. Their small leaves are white or grey below. Flowers have pale orange or yellow perianth. The mature fruit is yellowish or golden brown with a red aril and brown seed.

Discussion. In addition to the characteristics presented in diagnosis, *Virola fosteri* tends to have a higher number of and sunken (vs. plane) lateral veins per side than *V. multiflora* (16–24 vs. 10–18 per side), denser trichomes with longer branches on the abaxial leaf surface (Fig. 3E, K) and larger fruits [1.5–2.3 × 1.2–1.8 cm vs. 1.3–1.9 × 0.9–1.2 (–1.4) cm] (Fig. 4I, J).

It is also comparable to *V. micrantha* A. C. Sm. from Colombia due to the similar size of the leaf blades, which also have sessile stellate trichomes on the abaxial surface and short staminate inflorescences. *Virola micrantha* is a name apparently ignored in recent publications (ter Steege et al. 2016, 2019; Ulloa Ulloa et al. 2017; Gradstein 2016). Until recently, *V. micrantha* was only known from the type specimen (*R. E. Schultes & G. A. Black 46-377, US**); however, via a herbarium study at MO, we identified additional Colombian material with staminate flowers (*R. Jaramillo et al. 7846, MO-2 sheets!*) and can also extend its distribution to Venezuela (*E. Marín 571 [MO!]*),

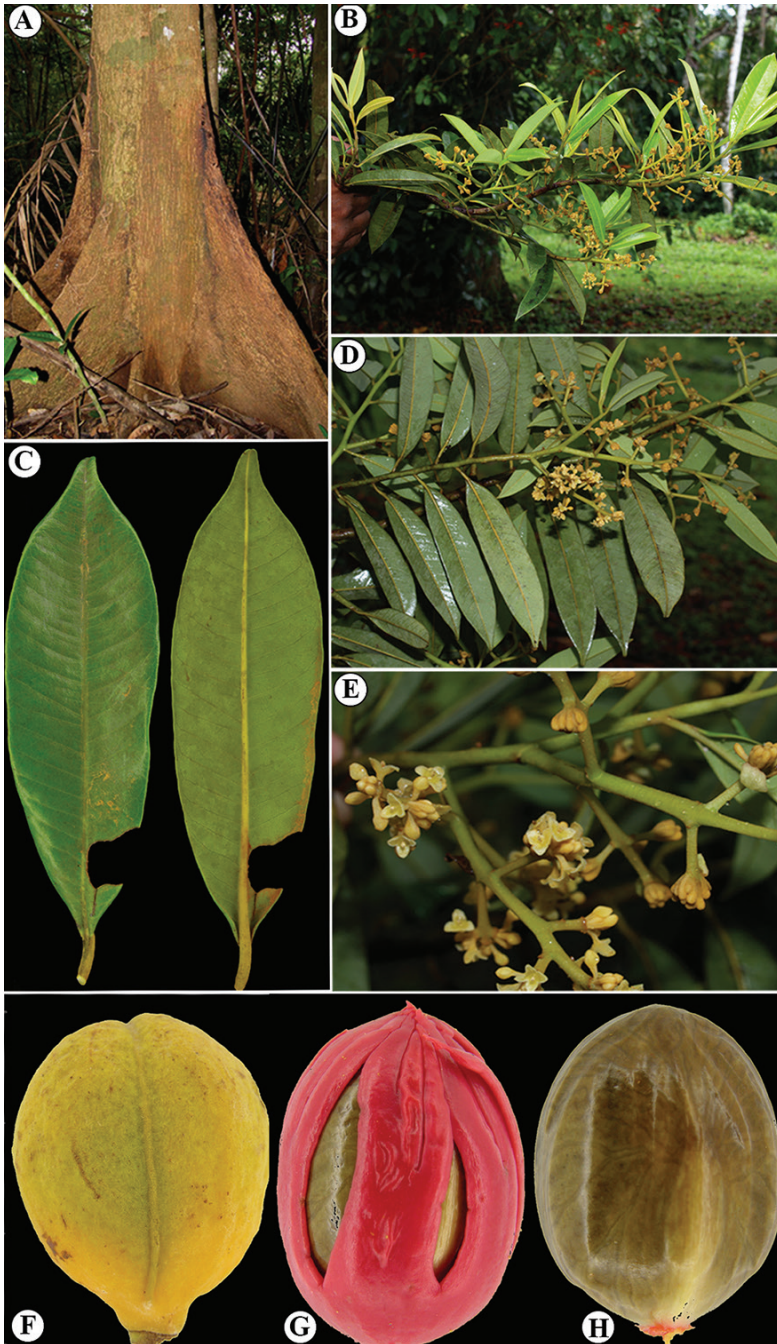


Figure 16. *Virola fosteri* **A** lower trunk and buttresses **B** branch with staminate inflorescences **C** leaf blades showing adaxial (left) and abaxial (right) surface; also demonstrating the revolute base **D** branch with staminate inflorescences and leaf blades on abaxial surface **E** close up of staminate inflorescences **F** fruit **G** aril covering the seed **H** seed Photos by Rolando Pérez (**A**), Carmen Galdames (**B, D, E**), Steven Paton (**C, F, G, H**); all photos from <https://stricollections.org/portal/index.php>.

R. L. Liesner 6778 [MO!]) and *J. Velazco* 851 [MO!]). *Virola fosteri* differs from this species by its attenuate leaf base and acute to acuminate apex (vs. obtuse on both sides, also mucronulate at the apex), longer perianth of staminate flowers (2–2.5 [–3] mm vs. ca. 1 mm long) and longer anthers (0.6–0.9 mm vs. ca. 0.3 mm long) (Smith 1953).

Virola coelhoi W. A. Rodrigues (Colombia; *S. Defler* 411, MO!, Peru; *C. Grández & N. Jaramillo* 2787, MO!) from Brazil, and *V. parvifolia* Ducke (Brazil) are other species with similarly-sized leaves with revolute margins, traits shared with *V. fosteri*. Additionally, with *V. coelhoi*, which it is more likely to be confused, *V. fosteri* shares overall leaf shape and the type of trichomes on the abaxial surface (i.e. stellate, sessile and yellowish). The new species is distinguished from *V. coelhoi* by the abaxial surface that is pale brown to reddish-brown when dry and puberulent (vs. abaxial surface yellowish, very densely pubescent), staminate inflorescences with small bracts (2–5 × ca. 2.5 mm vs. 2.5–9 × 4–6 mm), and staminate flowers with longer filament columns [0.9–1.3 mm vs. (0.3–) 0.6–0.7 long] and anthers (0.6–0.9 mm vs. 0.4–0.5 mm long). It can be differentiated from *V. parvifolia* by its leaf blades and inflorescences that are glabrous or nearly so (vs. pubescent in *V. fosteri*) (Ducke 1936); additionally, Ducke (1936) mentions that *V. parvifolia* has numerous small granules or tubercles on the branches, leaf blades and peduncles that are lacking in *V. fosteri*.

Jiménez (2007; as *Virola* sp. B) mentions that the new species is similar to *Virola pavonis* (A. DC.) A. C. Sm. from South America. This is probably because both species have sessile and stellate trichomes on the abaxial surface of the leaf, sometimes a similar leaf size, a similar number of lateral veins (though there is a tendency towards higher numbers of veins in *V. pavonis*) and the length of the filament column and anthers. However, the new species differs in its shorter staminate inflorescences [2.5–5.3 cm vs. (3–) 7–15 cm long], smaller fruits (1.5–2.3 × 1.2–1.8 cm vs. 2.5–5 × 1.5–2.5 cm) and in the canaliculate or smooth line of dehiscence (vs. carinate).

In Mesoamerica, other species with leaf blades that are covered with stellate and sessile trichomes on the abaxial surface (Fig. 3E, F, L) and a filament column that is longer than the anthers are *V. guatemalensis* and *V. nobilis*. However, these two species have larger leaf blades (12.3–17.2 [–27.5] cm vs. 7.8–12 cm long) and fruits ([2.1–] 2.3–2.7 [–3.1] cm vs. 1.5–2.3 cm long). Similarities with *V. nobilis*, specifically, include their distribution pattern (at least in Panama), leaf blades with more lateral veins per side (20–30 [25–32] vs. 16–24) that are markedly elevated abaxially (vs. flat to slightly elevated), the leaf margin and base that are usually not revolute (vs. revolute) and fruits that generally have thick pericarp (2.5–3.5 mm vs. 1.5–2.5 mm).

Notes. The species referred to as *Virola* sp. B in the *Manual de Plantas de Costa Rica* (Jiménez 2007) and as *V. multiflora* (*G. de Nevers* 7608, MO!) in the *Catálogo de las plantas vasculares de Panamá* (Correa et al. 2004) correspond to *V. fosteri*.

Specimens examined. COSTA RICA. Limón: Talamanca. San Miguel, Asacode, sendero a San Miguel, 30–100 m elev., 18 Jan 1997 (♂ fl), *J. González et al.* 1632 (CR!, MEXU!*, MO!); Lomas Mreduk (La Pera), antiguo campo de exploración petrolera, 300–350 m elev., 06 Oct 2002 (fl), *J. Gómez-Laurito et al.* 13903 (USJ!); cerros al sur del camino entre Puerto Viejo y Manzanillo por un camino nuevo hacia Bribri, 100

m elev., 18 Jan 1992 (fr), *B. Hammel* 18392 (CR-2 sheets!, MEXU!*, MO!). **PANAMA.** **Bocas del Toro:** Parcela ubicada a 10 km de la desembocadura de la quebrada Boca Chica en la margen izquierda del río Changuinola, 550 m elev., 23 Oct 2007 (fl), *R. Aizprúa et al.* 3398-RA (PMA!*, US!*); *ibid.*, 23 Oct 2007 (fl), *N. Daguerre et al.* 660-ND (PMA!*); along road to Chiriquí Grande, ca. 1.5 miles along side road east of highway, 250–300 m elev., 24 Jun 1986 (♂ fl), *G. McPherson & B Allen* 9646 (MO!). **Colón:** San Lorenzo, no elev., 15 Jun 2009 (fr), *J. Lezcano & E. Spear* 593 (PMA!*); Donoso, Teck Cominco Petaquilla mining concession, 300 m elev., 22 Feb 2008 (♂ fl), *G. McPherson & M. Merello* 20148 (MO!, PMA!*); Donoso, westernmost part of province, site of proposed copper mine (INMET), 150 m elev., 12 Apr 2009 (imm fr), *G. McPherson* 20913 (MO!, PMA!*); camino viejo de Piñas-Sherman, no elev., 22 Sep 2013 (fl), *R. Pérez et al.* 1130 (MO n.v., PMA!*, SCZI*). **Panamá:** Zona del Canal, Barro Colorado Island, slope between AVA 7 and FD 5, [10–100 m elev.], 30 Mar 1979 (fr), *R. B. Foster* 2931 (MO!, PMA!*); Barro Colorado Island, Wetmore trail, [10–100 m elev.], n.d. 1980 (♂ fl), *R. B. Foster* 2946 (CR!, F!*, MO!, PMA!*, U!*, US!*); Barro Colorado Island, Drayton, Drayton 18–19, [10–100 m elev.], 31 Mar 1988 (fr), *N. Garwood* 2301A (PMA!*). **San Blas:** El Llano Cartí road, km 26.5, no elev. 10 Apr 1985 (fr), *G. de Nevers et al.* 5285 (INPA!*, MEXU!*, MO!, PMA!*); El Llano–Cartí road, km 32.3, 200 m elev., 02 Mar 1986 (♀ fl, imm fr), *G. de Nevers* 7226 (INPA!*, MO!, PMA!*); Cangandi, 30 m elev., 27 Mar 1986 (♀ fl, imm fr), *G. de Nevers et al.* 7608 (MO!). **Veraguas:** Santa Fe, near the entrance to the agriculture school, Alto de Piedra, [800 m elev.], 26 Feb 1975 (fr), *S. Mori & J. Kallunki* 4891 (MO!).

6. *Virola guatemalensis* (Hemsl.) Warb.

Fig. 10E–G

Virola guatemalensis (Hemsl.) Warb. Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 68: 220. 1897.

Myristica guatemalensis Hemsl. Biol. Cent.-Amer., Bot. 3: 66–67. 1882. Type. Guatemala. [no specific data in location], [no date], [seeds], [*G. U.*] *Skinner s.n.* (holotype: K!*)

Distinctive characters. *Virola guatemalensis* is distinguished by many characters of its leaf, including overall size [12.3–17.5 (–24.1) × (2.4–) 3.8–5.5 (–8.9) cm], inconspicuous pubescence of tiny stellate and sessile trichomes on abaxial surfaces (Fig. 3F), 13–21 lateral veins on each side of the leaf that are barely distinctive abaxially (Figs 8F, 10F) and slightly revolute margins. Additionally, the staminate flowers have a filament column that is longer (1–1.2 mm long) than the anthers (0.5–0.8 mm long) and fruits that are 2.7–3.4 × 1.7–2.3 cm, ellipsoid (Fig. 4G), commonly glabrous or glabrous distally and with pubescence at the base with the line of dehiscence slightly carinate or smooth and a thin pericarp [0.4–1 (–2.5) mm thick].

Distribution. *Virola guatemalensis* is known from Mexico (Chiapas, Oaxaca and Veracruz), Guatemala (Sololá) and Honduras (Yoro) (Fig. 9F). It has been recorded

between 150–1250 m elevation. Standley and Steyermark (1946) mention that it also occurs in Alto Verapaz, Suchitepéquez, San Marcos and Huehuetenango in Guatemala, while Standley (1931) indicates that it occurs in Lancetilla (Honduras), where it is noted to be one of the most common tree species. While Gentry (2001) postulated that *V. guatemalensis* is an expected species for Nicaragua, no Nicaraguan collections of this species are known.

Common names. Mexico: cacao, cacao volador (Chiapas), cacaotillo, cedrillo (Veracruz), k'ik' che' [Lacandon name]. Guatemala: chucul, palo de sebo, cacao volador, cacao cimarrón. Honduras: sangre.

Phenology. Flowering of *Virola guatemalensis* has been recorded in April and May and fruit production in January, March, August, October and December.

Field characters. Plants are trees between 12–35 m high and 45–130 cm DBH with a straight trunk, sometimes with moderately sized buttresses. The bark is variously described as smooth or fissured and scaly and is brown to greyish-brown in colour and exudes watery reddish transparent sap when damaged. Flowers have yellow, green-yellowish or brown perianth. The mature fruit is yellow with a red aril.

Discussion. While the name *Virola guatemalensis* has been applied to herbarium specimens from Costa Rica and Panama in the past, those are here interpreted as a distinct species, *V. montana*. Based on our interpretation, *V. guatemalensis* is restricted to Mexico, Guatemala and Honduras. It is distinguished from *V. montana* by a series of characters, described below. While *V. laevigata* was typified with material from the Pacific slope in Chiriquí, Panama and is frequently considered a synonym of *V. guatemalensis* (e.g. Smith and Wodehouse 1938; Standley and Steyermark 1946; Duke 1962), we treat these as morphologically distinct; differences between these two species are discussed under *V. laevigata*.

Notes. The specimens, identified in Gómez-Laurito and Ortiz (2004), Haber (2014) and Monro et al. (2017) as *V. guatemalensis* or *V. surinamensis*, correspond to *V. montana* (R. Aguilar 1131, [E.] Bello 470, M. Chinchilla 3, J. Gómez-Laurito 11846, 11970, J. González 823) or *V. amistadensis* (A. Chacón 194). Additionally, a specimen cited as *V. guatemalensis* in *Flora of Panama* (Duke 1962) corresponds with the type of *V. laevigata* (G. P. Cooper & G. M. Slater 308).

The specimen J. A. Steyermark 47624 (MO-3 sheets!) seems to represent two different individuals – two of the sheets have staminate flowers, while the third has immature fruits. The duplicate of this collection at the Field Museum (digital image) has two sheets, both from a pistillate individual: one sheet carries flowers and the other immature fruits.

Virola guatemalensis, along with another Mesoamerican species, *V. koschnyi* (see below), produce the largest grains of pollen in *Virola* (Walker and Walker 1979).

Selected specimens seen. MEXICO. Chiapas: Tila, Chewupaj, 1000 m elev., 10 Dec 1982 (fr), A. Méndez 5223 (MEXU!*, MO!); Peltalcingo, slope of Ahk'ulbal Nab above Peltalcingo, 1700 m elev., 27 Feb 1981 (fr), D. Breedlove 49868 (MEXU!*); La Trinitaria, 10 km east northeast of Dos Lagos above Santa Elena, 1170 m elev., 19 Jan 1982 (fr), D. Breedlove & F. Almeda 57553 (MO!); La Independencia, ridge, 45–50 km E of Lagos de Montebello National Park on road to Ixcán from Santa

Elena, 760 m elev., 22 Jan 1982 (fr), *D. Breedlove & F. Almeda 57735* (MO!). **Oaxaca:** Matías Romero Avendaño, ± 11 Km. al S de Aserradero La Floresta, ± 24 Km. al S de Esmeralda, lomas al S del Río Verde, 300 m elev., 24 Apr 1981 (fl), *T. Wendt et al. 3235* (MEXU!*). **Veracruz:** Cima del cerro Vigía, 450 m elev., 19 Apr 2005 (σ fl), *E. Velasco-Sinaca 678* (MEXU!*, MO!); La Escondida, 3 km NO de Estación de Biología Tropical Los Tuxtlas, 200 m elev., 05 Apr 1983 (σ fl), *G. Ibarra 604* (MEXU!*, MO!); Estación de Biología Tropical Los Tuxtlas, 200 m elev., 29 Oct 1983 (fr), *G. Ibarra 957* (MO!); camino Laguna Escondida, 2 km NW de la Estación de Biología Tropical Los Tuxtlas, 200 m elev., 08 May 1984 (σ fl), *G. Ibarra & G. Gómez 1607* (MO!); Ocotal Grande, 5 km N de Mecayapan, 1000 m elev., 14 Mar 1985 (fr), *G. Ibarra et al. 2357* (MEXU!*, MO!, NO!); 2 km al NW del rancho Rubén Sánchez, 250–350 m elev., 28 May 1985 (σ fl), *G. Ibarra 2450* (MO!); Santiago Tuxtla, Alta Luz, 540 m elev., 11 May 1968 (σ fl), *M. Sousa 3680* (MO!); Catemaco, Tebanca, no elev., 20 Oct 1971 (fr), *J. I. Calzada 615* (MO!); Cerro El Vigía de Santiago Tuxtla, 800 m elev., 18 Feb 1967 (fr), *R. Cedillo 9* (MO!); Catemaco, El Chinchero, 14.2 km al SE de Tebanca camino al río Huacinapan, no elev., 19 Dec 1984 (fr), *R. Cedillo & G. Pérez 2958* (MEXU!*, MO!, NO!); Hidalgotitlán, lomas al SE de Poblado 6, 150 m elev., 27 Apr 1982 (σ fl), *T. Wendt et al. 3898* (LSU!, MO!). **Guatemala. Sololá:** Bordering bar-ranco on Finca Olas de Mocá, just west of Finca Mocá, south-facing slopes of Volcán Atitlán, 1000–1100 m elev., 15 Jun 1942 (σ fl, imm fr), *J. A. Steyermark 47624* (F!*, MO-3 sheets!). **Honduras. Yoro:** Cascada de Río Guán Guán, 400–440 m elev., 20 Apr 1995 (σ fl), *T. Hawkins & M. Merello 768B* (MO!).

7. *Viola koschnyi* Warb.

Fig. 17

Viola koschnyi Warb. Repert. Spec. Nov. Regni Veg. 1: 71. 1905. Type. Costa Rica [Costa Rica]. [Alajuela] San Carlos, *Th. Koschny s.n.* (lectotype, here designated: F!*, fragment with B photo [649050, F0360191F]); Costa Rica. Heredia. [Sara-piqui] Parque Nacional Braulio Carrillo, bosque primario frente al Puesto La Ceiba, 450 m elev., 23 Dec 1988 (σ fl), *M. Ballesterio 72* (epitype, designated here: CR! [16354]; isoeotypes: CR! [156734], MO! [5550256, MO-299037]).

Viola merendonis Pittier., Contr. U.S. Natl. Herb. 20: 453. 1922. Type. [Honduras]. [Copán:] Collected in the forests of Cuchillitas, between Arranca Barba Hills and Mohanes, in the Cordillera de Merendon, borders of Guatemala and Honduras, in fruit, [10–] 18 May 1919 [fr], *H. Pittier 8530* (holotype: US!*; isotype: NY!*).

Viola costaricensis nomen nudum

Distinctive characters. *Viola koschnyi* can be recognised by the densely tomentose leaf undersides with pediculate, dendritic trichomes (Fig. 3G) that are soft to the touch, the numerous lateral veins [(16–) 20–35] that form a clear submarginal vein and tertiary veins that are usually inconspicuous abaxially (Figs 3G, 8G). Adaxially, the new leaves

are densely covered with dendritic sessile or subsessile trichomes that are deciduous at maturity, resulting in glabrous to glabrescent and smooth adxial surface on mature leaves. The staminate flowers have filament columns that are 0.7–0.9 (–1.4) mm long with anthers 0.5–0.7 (–1) mm long. Its fruits are 1.9–3.1 × 1.5–1.9 cm and ellipsoid or subglobose (Fig. 4F), with a pericarp 1.2–3.1 mm thick.

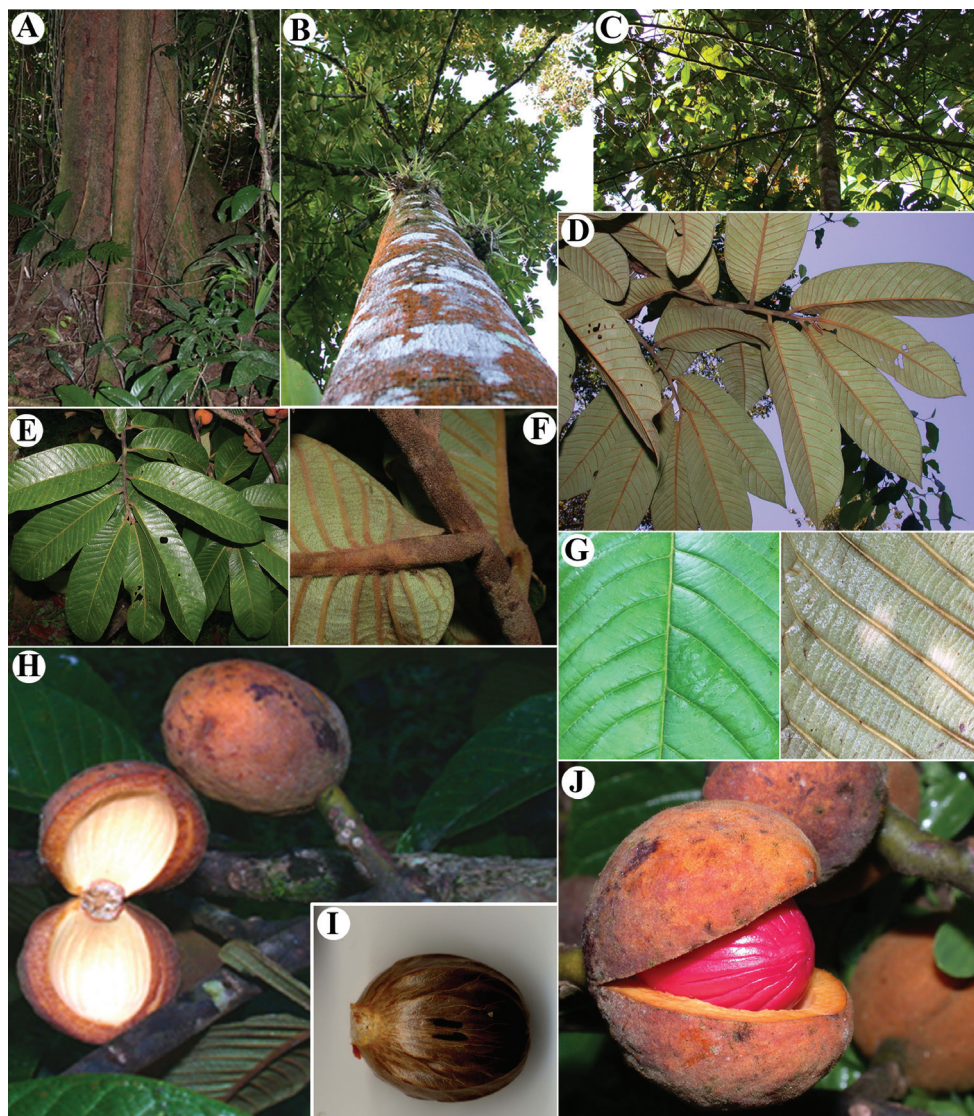


Figure 17. *Virola koschnyi* **A** lower trunk and buttresses **B** trunk **C** branching **D** leaf blades on abaxial surface **E** leaf blades on adaxial surface **F** twig, petiole and leaf base **G** leaf venation on adaxial surface (left) and abaxial surface (right) **H** branch with fruits, showing the interior part of the fruit **I** seed **J** mature fruit. All photos by Reinaldo Aguilar from <https://sura.ots.ac.cr/florula4/index.php>, except **G** by D. Santamaría-Aguilar.

Distribution. *Viola koschnyi* is known from Mexico (Chiapas), Guatemala (Izabal and Petén), Belize (Cayo, Toledo and Stann Creek), Honduras (Atlántida, Cortés, Gracias a Dios and Olancho), Nicaragua (Atlántico Norte, Atlántico Sur, Jinotega and Río San Juan), Costa Rica (Alajuela, Guanacaste, Heredia, San José and Limón), and Panamá (Bocas del Toro) (Fig. 18A). It is found mainly on the Caribbean slope, where it has been recorded between 10–1000 (–1700) m elevation.

We consider this species restricted to Mesoamerica. The collections identified as *V. koschnyi* from Ecuador in *Catalogue of the Vascular Plants of Ecuador* (i.e. C. H. Dodson *et al.* 6465 [RPSC, SEL]; Jørgensen and León-Yáñez 1999) correspond to *V. multinervia* Ducke (Jaramillo *et al.* 2004). Determinations of *V. koschnyi* cited for Colombia by Cogollo *et al.* (2007) are doubtful and do not cite voucher specimens, while those of Gradstein (2016) could not be confirmed.

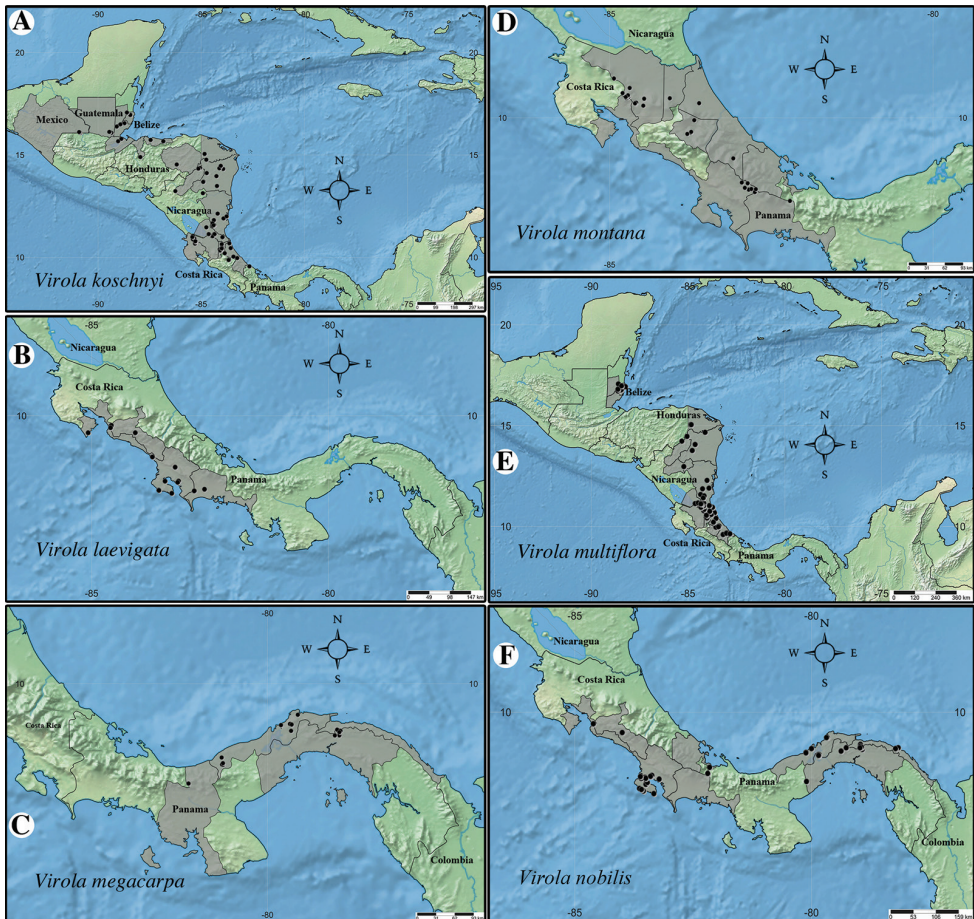


Figure 18. Geographic distribution of *Viola koschnyi* (A), *V. laevigata* (B), *V. megacarpa* (C), *V. montana* (D), *V. multiflora* (E) and *V. nobilis* (F).

Common names. Guatemala: Cedrillo, drago, sangre. Belize: banak, black banak. Honduras: bának, sebo, sangre de montaña, sangre real. Nicaragua: banak blanco, banak colorado, cebo, fruta dorada, sebo. Costa Rica: achiotillo, fruta dorada.

Phenology. Flowering of *Viola koschnyi* has been recorded in January, March and May, July, September, October and December. Only three herbarium specimens with pistillate flowers were seen, two from Costa Rica and one from Nicaragua. Fruits are produced throughout the year, though most often collected in March, May and June.

Field characters. Plants are trees (4–) 10–70 m tall and 12–80 cm DBH, with a trunk that is straight with well-developed buttresses. The bark is described as scaly and falling off in small plates or as smooth, red-brown or whitish in colour; clear, red or reddish exudate is released when damaged. Flowers usually have yellow perianth, though it can sometimes be white, brown, or orange. The mature fruit is yellow, orange or brown, with a red or pink-red aril. The testa of the seed is black, brown or white.

Typification. *Viola koschnyi* was described by Otto Warburg based on a collection made by Theodor Koschny (Warburg 1905). Warburg indicated in the protologue “Costarica: San Carlos, leg. Th. Koschny,” but did not mention where the type was housed. The type is presumed to have been housed at Berlin (B) and subsequently destroyed during World War II.

The only known original material of *Viola koschnyi* is a fragmentary specimen accompanied with a photograph at the Field Museum. The photo shows the original specimen was composed of three fertile branches with leaves (two with inflorescences and one with fruit), with a handwritten annotation of “*V. costaricensis* Warb”, presumably by Warburg, though this name was never validly published. The fragment material consists of pieces of leaves, two inflorescence branches with a few immature flowers and a broken fruit comprising pericarp, testa and seed. Since they are mounted with the photo of the Berlin specimen, these fragments presumably originated from the holotype. Although it is not ideal type material due to its fragmentary nature, the specimen at F appears to be the only extant original material and is sufficient to confirm that it coincides with the protologue and concept of the species used and so is here designated as lectotype. In order to ensure the precise application of the name, given the fragmentary type material, an epitype was selected from the studied specimens.

Notes. Most of the specimens from the Pacific slope of Costa Rica and Panama previously identified as *V. koschnyi* are here interpreted as *V. chrysocarpa*. The similarities and differences between *V. koschnyi* and *V. chrysocarpa* are discussed under the latter species. While most fruiting specimens of *V. koschnyi* have obtuse to rounded apices, *G. Herrera* 1092 (CRI, MO!) from Costa Rica and *R. Rueda* & *H. Mendoza* 17069 (MO!) from Nicaragua have apiculate apices. Aside from the fruit apex, these specimens conform to the species concept adopted here in all other characters (e.g. number of lateral veins, submarginal vein and tertiary veins that are usually inconspicuous abaxially and pediculate, dendritic trichomes).

Selected specimens seen. MEXICO. Chiapas: Ocosingo, En ejido Chaju, 150 m elev., 17 Mar 1993 (fl), *E. Martínez* & *C. H. Ramos* 26336 (MEXU-4 sheets!*); Boca

de Chajul, 800 m al SE del poblado, 350 m elev., 21 May 1992 (fr), *G. Domínguez* 438 (MEXU!*). **BELIZE.** **Cayo:** At mile 28.5 m on Hummingbird highway, 200–300 ft [60–90 m] elev., 14–21 Jun 1973 (fr), *T. B. Croat* 24562 (MO!). **Stann Creek:** Mountain Cow, [200 m elev.], 25 Mar 1940 (imm fr), *P. H. Gentle* 3277 (MO!); Big Eddy Ridge, [50–200 m elev.], 21 May 1940 (fr), *P. H. Gentle* 3347 (MO!); 22 miles Stann Creek, 200 ft [60 m] elev., 27 Jun 1927 (fr), *W. A. Schipp* 949 (MO-2 sheets!). **Toledo:** Bladen Nature Reserve, 20 m from the Bladen River, 45 m elev., 27 Feb 1997 (♂ fl), *S. W. Brewer* 178 (MO!); Southern Maya mountains, Bladen Nature Reserve, 250 m elev., 23 May 1996 (fr), *G. Davidse* 36211 (MO!). **GUATEMALA.** **Izabal:** Between Seja and Fronteras, no elev. 08 May 1971 (fl bud), *E. Contreras* 10751 (MO!); Livingston, Creek Jute, Biotopo Chocón Machacas, [0–5 m elev.], 28 Jul 1988 (fr), *P. Tenorio et al.* 14953 (MEXU!*, MO!). **Petén:** Chinchila, Sebol road, [20 m elev.], 16 May 1967 (fr), *E. Contreras* 6924 (MO!); La Cumbre, [300 m elev.], 30 Jul 1969 (fr), *E. Contreras* 8790 (MEXU!*, MO!). **HONDURAS.** **Atlántida:** Lancetilla S of Tela, 20–50 m elev., 25 Jun 1970 (fr), *G. Davidse & R. Pohl* 2178 (MO!). **Cortés:** [Santa Cruz de Yojoa], 660 m elev., 13 Feb 1952 (fl), *P. H. Allen* 6456 (GH!*, EAP-2 sheets!*). **Gracias a Dios:** La Mosquitia, Ahuas Bila, 100 m elev., 5–13 May 1985 (fr), *C. Nelson & G. Cruz* 9484 (MO!). **Olancho:** San Esteban, montaña El Carbón, 460 m elev., 02 Feb 1994 (fl bud), *A. Meras* 02 (MO); a 3 km comunidad El Carbón, 430 m elev., 12 Mar 1997 (fl), *I. Rivas* 08-C1 (EAP!*). **NICARAGUA.** **Atlántico Norte:** Bonanza, Musawas, 50–150 m elev., 17 Oct 2002 (♂ fl), *C. Aker et al.* 624 (MO!); Siuna, Waspado, 100–120 m elev., 06 Oct 1982 (imm fr), *F. Ortiz* 252 (MO!); sur de río Wawa, 40 m elev., 16 Mar 1971 (imm fr), *E. L. Little Jr.* 25164 (MO!). **Atlántico Sur:** Kurinwac[s]ito, 80–100 m elev., 18–22 Mar 1984 (♀ fl, fr), *P. P. Moreno* 23682 (MO), *P. P. Moreno* 23701 (MO!); El Zapote, 40 km NE de Nueva Guinea, 130–150 m elev., 29 Feb 1984 (♂ fl), *J. C. Sandino* 4808 (MO!). **Estelí:** El Zacatón, entre Mesas Plan Helado y la laguna de Miraflor, 1300–1400 m elev., 29 Mar 1983 (imm fr), *P. P. Moreno* 23788 (MO!). **Jinotega:** Cua Bocay, Reserva de Bosawas, comunidad de San Andrés, 180 m elev., 30 Jun 2005 (fr), *I. Coronado et al.* 1951 (MO!). **Río San Juan:** El Castillo, Reserva Indio Maíz, 100–200 m elev., 15 Mar 1999 (♂ fl), *R. Rueda et al.* 10319 (MO!); San Juan del Norte, Reserva Indio Maíz, 30 m elev., 18 May 2002 (fr), *R. Rueda & H. Mendoza* 17069 (MO!); río Sábalos, 2 km de Santa Eduvigis, 80 m elev., 18 Feb 1984 (♂ fl), *P. P. Moreno* 23042 (MO!). **COSTA RICA.** **Alajuela:** Parque Nacional Rincón de La Vieja, 900–1000 m elev., 07 Mar 1988 (♀ fl), *G. Herrera* 1607 (CR!, MO!); Upala, Dos Ríos, 500 m elev., 02 Nov 1987 (fr), *G. Herrera* 1092 (CR!, MO!); Upala, Estación San Ramón, 550 m elev., 27 Jan 1995 (fr), *F. Quesada* 243 (CR!, LSU!, MO!); Upala, Finca San Gerardo, 600 m elev., 20 Feb 1998 (fl bud), *F. Quesada et al.* 577 (CR-2 sheets!, MO!, USJ!). **Cartago:** Turrialba, CATIE, 650 m elev., 08 Feb 1975 (fr), *C. Vaughan* 93 (USJ!); Bosque de Florencia de Turrialba, 650 m elev., 31 Jul 1969 (fr), *R. Ramalho* 7990 (USJ!). **Guanacaste:** Santa Cruz, road from Santa Cecilia to La Esperanza, 340 m elev., 29 Dec 1989 (fr), *R. E. Gereau et al.* 3455 (CR-2!, MO!); Parque Nacional Guanacaste, Estación Pitilla, 700

m elev., 02 Mar 1991 (♂ fl), *P. Ríos* 309 (CR-2 sheets!, MO!). **Heredia:** Parque Nacional Braulio Carrillo, Estación El Ceibo, 500–600 m elev., 01 Oct 1989 (fl bud), *R. Aguilar* 9 (MO!, LSU!, USJ!); Los Arbolito, al N de Puerto Viejo, 20 m elev., 09 Mar 1993 (♂ fl), *F. Araya* 197 (CR-2 sheets!, LSU!, MO!); between La Selva entrance and bridge before Puerto Viejo, [100 m elev.], 18 Apr 1981 (fr), *J. Folsom* 9791 (MO!). **Limón:** Refugio de Vida Silvestre Gandoca Manzanillo, sendero Cerillo, 1 m elev., 03 Mar 1999 (fr), *U. Chavarría* 1918 (CR-2 sheets!, MO!); Cordillera de Talamanca, ridge separating Río Madre de Dios from Quebrada Cañabral, 440 m elev., 02 Sep 1988 (♀ fl), *M. Grayum et al.* 8692 (CR-2 sheets!, MO-2 sheets!, USJ!); Sixaola, Quebrada Mata de Limón, 20–40 m elev., 27 Jan 1987 (fr), *M. Grayum et al.* 8004 (CR!, MO!); Río Peje, no elev., 14 Jun 1979 (fr), *R. Ocampo* 2582 (CR!); Parque Nacional Tortuguero, Estación Agua Fría, 40 m elev., 02 Feb 1988 (fr), *R. Robles* 1587 (CR!, MO!); Cerro Coronel, 20–170 m elev., 16–23 Jan 1986 (♂ fl), *W. D. Stevens* 23768 (CR, MO). **San José:** Z. P. [Zona Protectora] La Cangreja, Santa Rosa de Puriscal, 350 m elev., 01 Oct 1992 (fr), *J. F. Morales* 764 (CR). **PANAMA. Bocas del Toro:** Isla Colon, camino central, unos 4 km de Boca de Drago, 2–25 m elev., 16 Mar 1993 (fr), *R. Foster et al.* 14549 (F!*).

8. *Virola laevigata* Standl.

Fig. 19

Virola laevigata Standl. Publ. Field Mus. Nat. Hist., Bot. Ser. 4(8): 209. 1929. Type. Panama. Province of Chiriquí, Progreso, [July–Aug.] 1927 [♂ fl], *G. P. Cooper & G. M. Slater* 308 (holotype: F!*; isotypes: NY!*, WIS!*, US!*).

Distinctive characters. *Virola laevigata* is distinguished by its glabrous or nearly glabrous vegetative parts (i.e. twigs, mature leaf blades on both surfaces [Fig. 3H], petioles); when trichomes are present, they are primarily on the leaf buds or very new leaves. Additionally, leaves have 12–20 lateral veins and margins and bases that are slightly revolute, staminate flowers have a straight filament column that is longer (0.8–1.3 mm) than the anthers (0.5–0.7 mm) and relatively small fruits (1.8–2.9 × 1.5–1.8 cm) (Fig. 4M) with pericarp that is 1.8–2.8 mm thick.

Distribution. *Virola laevigata* is known from Costa Rica (Puntarenas and San José) and Panama (Chiriquí) (Fig. 18B). It is found on the Pacific slope, where it has been recorded between 0–500 (1600?) m elevation. Jiménez (2007) suggested that the maximum elevation for this species in Costa Rica is 1600 m, potentially based on *L. González* 3089 from Cerro Turrubares (San José province) (B. Hammel pers. comm., Aug. 2019); however, we have not found any specimen that occurs this high.

Common names. Costa Rica: fruta dorada. Panama: bogamani.

Phenology. Flowering of *Virola laevigata* has been recorded in January, May, July and November. Fruits are produced from December to February. Pistillate flowers were not present on herbarium sheets studied.

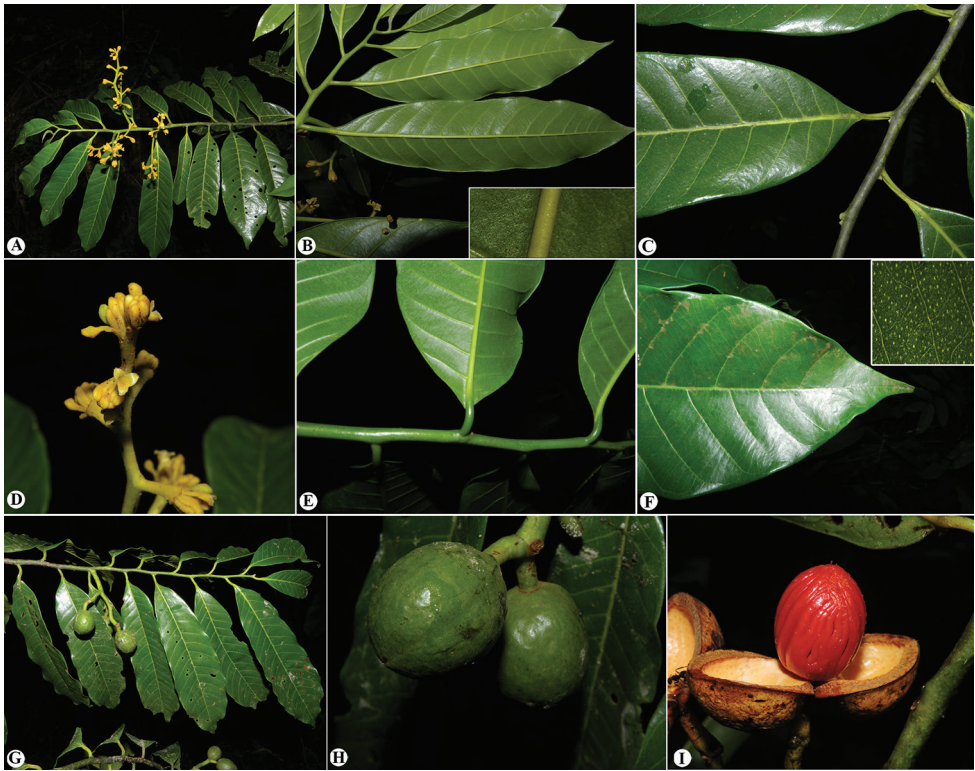


Figure 19. *Virola laevigata* **A** branch with staminate inflorescences **B** leaf blades on abaxial surface, inset showing the glabrous midvein **C** twig, petiole and leaf on adaxial surface **D** close up of staminate inflorescences **E** leaf base **F** leaf apex, inset showing leaf punctations **G** branch with immature fruits **H** close-up of immature fruit **I** open fruit, showing the aril. Photos by Reinaldo Aguilar.

Field characters. Plants are trees 9–40 m tall and 35–60 cm DBH with a straight trunk and small (ca. 20 cm tall), triangular buttresses. The bark is described as finally grooved, smooth, flaking in vertical strips or scaly and is grey, blackish or reddish in colour, with exudate that is reddish or colourless and oxidising to reddish-cream. The leaves are bright green on both sides and have numerous pellucid dots that are most visible against the light. Flowers have yellow, yellow-brown or yellowish perianth, sometimes with a slight aroma in staminate flowers (*N. Zamora & T. D. Pennington 1583*, but the specimen label states pistillate flower). The mature fruit is yellow with a red aril (when immature, it is white). In the Osa Peninsula, where this species is frequent, it prefers riparian habitats.

Discussion. *Virola laevigata* has traditionally been considered a synonym of *V. guatemalensis* (e.g. Smith and Wodehouse 1938; Standley and Steyermark 1946; Duke 1962), likely due to limited material. However, with new herbarium specimens, both species can be clearly distinguished vegetatively and with fruit characters. Vegetative material of *V. laevigata* can be distinguished by petioles and mature leaf blades that are abaxially glabrous (vs. diminutively pubescent with tiny stellate, sessile trichomes in

V. guatemalensis). Their fruits also differ in size, shape and thickness of the pericarp; in *V. laevigata*, they are smaller ($1.8\text{--}2.9 \times 1.5\text{--}1.8$ cm), ovoid or subglobose and with thick pericarp ($1.8\text{--}2.8$ mm); while in *V. guatemalensis*, they are large (vs. $2.7\text{--}3.4 \times 1.7\text{--}2.3$ cm), ellipsoid and with a thin pericarp [$0.4\text{--}1$ (-2.5) mm].

In addition to *Virola guatemalensis*, herbarium specimens of *V. laevigata* have been determined as *V. surinamensis* (interpreted here as *V. nobilis*). However, *V. laevigata* is distinguished by its glabrous or almost glabrous abaxial leaf surface (Fig. 3H, O) and mature fruits (vs. pubescent) and its tendency towards thinner pericarp [$1.8\text{--}2.8$ mm vs. $2.5\text{--}3.5$ (-4.2) mm thick]. In the Osa Peninsula, where these two species grow together, they can be easily distinguished in the field: *V. laevigata* prefers riparian habitats, does not usually develop tall buttresses and the external bark has a greenish tone, while *V. nobilis* grows far from bodies of water, has tall buttresses and the external bark is reddish to brown. For a description and comparison of the bark of these two species, see Moya Roque et al. (2014), as *Virola* sp. A and *V. surinamensis*.

Notes. The seedlings of *V. laevigata* are described by Ley López and Chacón Madrigal (2017) (though as “*Virola* sp. A”). Additionally, the species presented as “*Virola* sp. A” in Jiménez (2007) and as *V. surinamensis* in Jiménez Madrigal and Grayum (2002; RZ [=R. *Zuñiga*] 459) corresponds with *V. laevigata*. The illustration in Quesada Quesada et al. (1997) as *V. guatemalensis* is potentially also *V. laevigata*.

Selected specimens seen. **COSTA RICA. Puntarenas:** Golfito, 1 km antes de llegar a La Palma, 8 m elev., 16 Jan 1993 (fr), *R. Aguilar* 1585 (CR-2 sheets!, LSU!, MO!); fila Carbonera, cabo Matapalo, 300 m elev., 16 Jul 1993 (♂ fl), *R. Aguilar* 2004 (CR!, MO!); estación Sirena, 10 m elev., 12 Oct 1993 (fl bud, fr), *R. Aguilar* 2490 (CR-2 sheets!, LSU!, MO!); Golfito, orillas del camino a las torres de comunicación, 450–500 m elev., 12 Jan 1999 (fr), *J. Gómez-Laurito & V. Mora* 13191 (CR!); Playa Cacao, Fila entre quebrada Nazanero y el mar en Punta Voladora, 200 m elev., 26 May 1994 (♂ fl), *G. Herrera & G. Rivera* 7076 (CR!); Parque Nacional Corcovado, Sirena, 10 m elev., 28 Feb 1989 (fr), *C. Kernan & P. Phillips* 962 (CR-2 sheets!, MO!); Parque Nacional Corcovado, Sirena, Ollas Trail, 1–20 m elev., 19 Oct 1989 (fr), *C. Kernan & G. Fonseca* 1288 (CR-2 sheets!, INPA!*, MO!); Jiménez, Piro, camino a Laguna Silvestre, [0–100 m elev.], 01 Feb 2012 (fr), *J. M. Ley-López* 74 (US); Osa, fila Esquinas, 200 m elev., 26 May 1993 (♂ fl), *M. Segura & F. Quesada* 69 (CR!, LSU!, MO!); Rincón de Osa, in vicinity of airstrip, 40 m elev., 25 Jul 1974 (fl bud), *J. Utley & K. Utley* 1236 (CR!); Garabito, por Carara, cerca de la toma de agua, 97 m elev., 22 Nov 2006 (fl bud), *L. D. Vargas & D. Castillo* 1870 (CR!); Osa, Uvita, cerca de Dominical, 200 m elev., 28 Jan 1991 (♂ fl), *N. Zamora & T. D. Pennington* 1583 (CR-2 sheets!, INPA!*, MEXU!*, MO!); Puntarenas, Montezuma, camino a Cóbano por el río Montezuma, ca. 1.5 km oeste de la intersección con camino a Cabuya, 101 m elev., 11 Dec 2005 (fr), *B. Hammel & I. Pérez* 23944 (CR!); Montezuma, por el Canopy, 100 m elev., 08 Jul 2006 (fl bud), *B. Hammel & I. Pérez* 24149 (CR!). **San José:** Carara, sector Agrominas, sitio Carretera Costanera, 100 m elev., 20 Sep 1991 (fr), *R. Zuñiga* 459 (CR-sheets!, LSU!). **PANAMA. Chiriquí:** Río Platanal-Bugaba, 10 Dec 1975 (fr), *M. M. Gutiérrez* 21 (MO!).

9. *Virola megacarpa* A. H. Gentry.

Fig. 20A, B

Virola megacarpa A. H. Gentry. Ann. Missouri Bot. Gard. 62(2): 474. 1975. Type. Panama. Colón: Santa Rita Ridge, 23 Mar 1972 [fr], [A. H.] Gentry & [J. D.] Dwyer 4804 (holotype: MO!; isotypes: BM!*, MEXU!*, WIS!*).

Distinctive characters. *Virola megacarpa* can be recognised by its large and oblong leaf blades (20.3–37 × 7–13 cm) with numerous [(32–) 40–50 per side], dense lateral veins and a densely pubescent abaxial surface with dark brown to ferruginous dendritic trichomes (Fig. 3I). It is also the species with the largest fruits (4–5.7 × 2–2.9 cm) in the region and likely the genus; these are also densely pubescent with an acuminate to rostrate apex (Fig. 4P) and thick pericarp (3–6 mm).

Distribution. *Virola megacarpa* is only known from Panama (Colón, Panamá, San Blas and Veraguas) (Fig. 18C) from 50–550 m elevation.

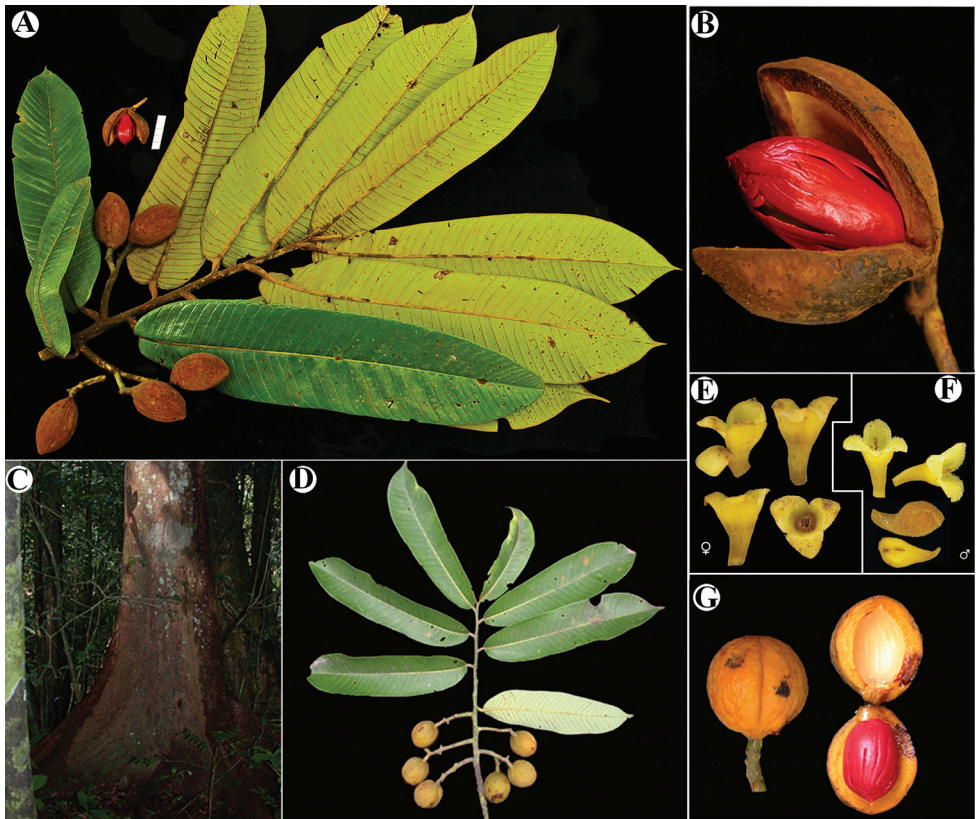


Figure 20. A. *Virola megacarpa* A branch with fruits, both leaf blade surfaces shown B fruit. *Virola nobilis* from Barro Colorado C lower trunk and buttress D branch with fruits, leaf blades on both surfaces E pistillate flowers F staminate flower and bud G fruits. Photos by Carmen Galdames (A, B), Rolando Pérez (C, D, G), Steven Paton (E, F); all photos from <https://stricollections.org/portal/index.php>.

This species is attributed to Colombia in Cogollo (2011), based on the specimen *J. Brand 1252* (JAUM!*; fr). It is also mentioned as occurring in Colombia in Gradstein (2016) and Ulloa Ulloa et al. (2017), though these references do not mention a voucher specimen (likely they refer back to the same specimen cited by Cogollo [2011]). The first author has seen a digital image of this specimen (*J. Brand 1252*, JAUM) and it appears that the leaf undersurface and fruits are scarcely pubescent, as well as smaller. This specimen clearly corresponds to a species of the group *Surinamenses* sensu Smith and Wodehouse (1938) and not *V. megacarpa*. For that reason, *V. megacarpa* is considered restricted to Mesoamerica.

Common names. None recorded.

Phenology. The only observed herbarium specimen with flowers (these staminate) was collected in August. Fruits were collected in February and March and August to November.

Field characters. Plants are trees 12–30 m tall and 21.5–53 cm DBH. Damaged bark releases exudate that is red or that oxidises reddish-brown. Flowers have pale yellow perianth. Fruits are densely pubescent with brown trichomes and a red aril.

Discussion. Vegetatively, *Virola megacarpa* can be confused with *V. koschnyi* and some specimens have been identified as the latter (e.g. *G. de Nevers* & *H. Herrera 7917*, MO). Both species share leaf blades with numerous and conspicuous lateral veins and pediculate trichomes on the abaxial surface. However, *V. megacarpa* has more lateral veins per side [(32–) 40–50 vs. (16–) 20–35] and these are more closely spaced (Figs 8G and I) and fruits are larger (4–5.7 × 2–2.9 cm vs. 1.9–3.1 × 1.5–1.9 cm) (Fig. 4F, P).

Specimens examined. **PANAMA. Colón:** East of Portobelo, 50–100 m elev., 12 Oct 1992 (fr), *G. McPherson* & *M. Richardson 15873* (MO!); Teck Cominco Petaquilla mining concession, 220 m elev., 20 Feb 2008 (fr), *G. McPherson* & *M. Merello 20081* (MO!); East ridge, no elev., 23 Feb 1968 (fr), *J. A. Duke 15261* (MEXU!*, MO!). **Panamá:** [Chepo] El Llano-Cartí road, 5 km N of Pan-American Highway at El Llano, 300 m elev., 10–11 Nov 1973 (imm fr), *M. Nee 7920* (MEXU!*, MO!); El Llano-Cartí road, 16–18.5 km by road N of PanAmerican Hwy, at El Llano, 400–450 m elev., 28 Mar 1974 (fr), *M. Nee* & *E. Tyson 10983* (CR!, F!*, INPA!*, MO-2 sheets!). **San Blas:** El Llano-Cartí Road, Km 19.1, 350 m elev., 19 Mar 1985 (fr), *G. de Nevers 5184* (INPA!*, MEXU!*, MO!). **Veraguas:** Santa Fe, Valley of Río Dos Bocas along road between Escuela Agrícola Alto Piedra and Calovebora, 450–550 m elev., 31 Aug 1974 (♂ fl), *T. B. Croat 27785* (INPA!*, MO!).

10. *Virola montana* D.Santam., sp. nov.

urn:lsid:ipni.org:names:77202550-1

Figs 21, 22A–D

Diagnosis. Species most similar to *Virola guatemalensis*, from which it differs by the mostly caducous (vs. persistent) trichomes on the abaxial leaf surface that have 3–10

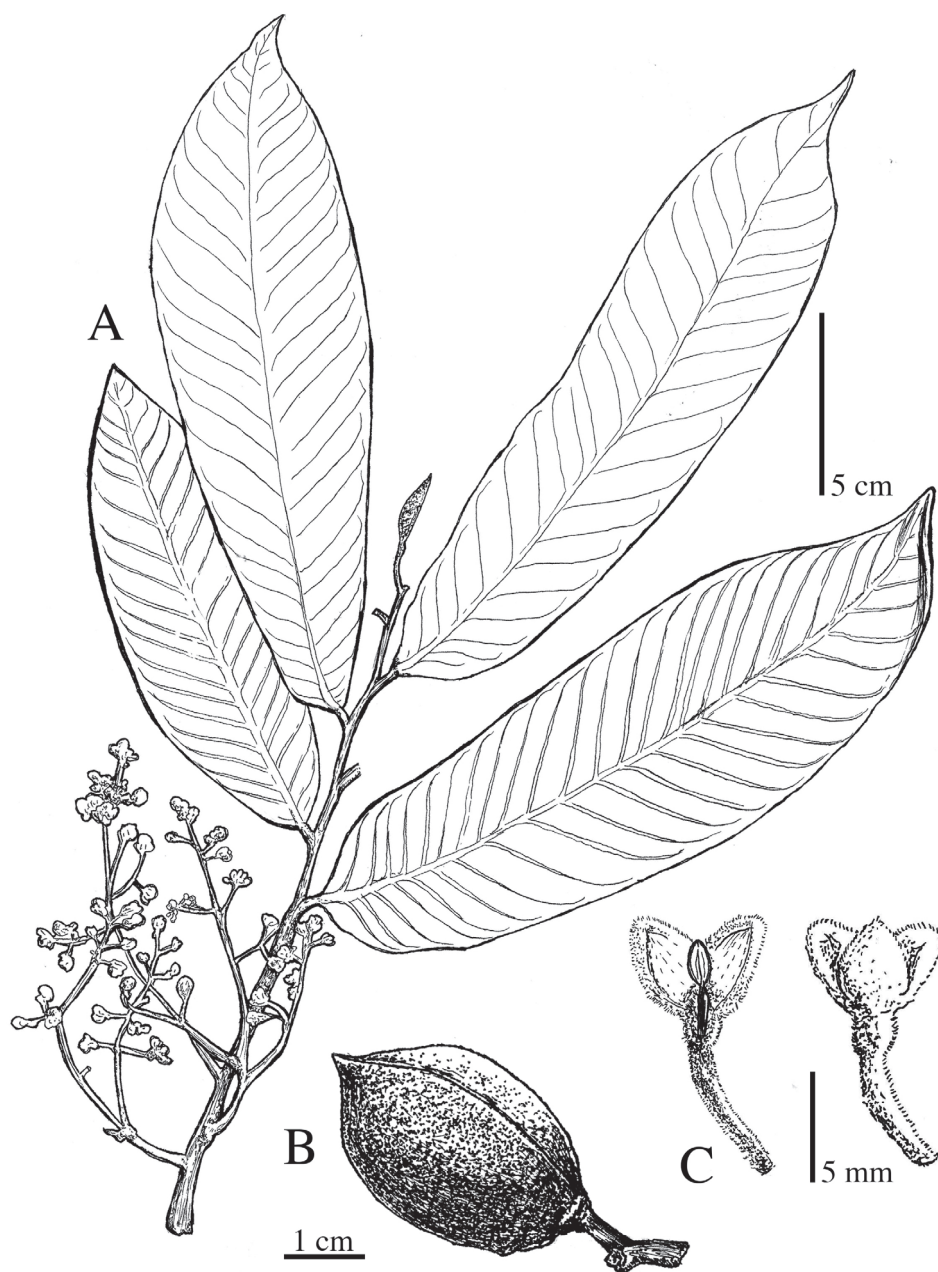


Figure 21. *Virola montana* **A** branch with leaves and inflorescences **B** fruit **C** flowers. Drawn by Pedro Juárez based on *G. Herrera et al.* 514 (**A–C**), and *R. Lent* 3899 (**B**).

branches and are 0.2–0.6 mm long (vs. 3–6 branches 0.05–0.1 mm long), staminate flowers with a shorter filament column (0.6–0.9 mm vs. 1–1.2 mm long) and fruits with a thicker pericarp (3.2–5 mm vs. 0.4–1 [–2.5] mm thick).

Type. COSTA RICA. Cartago: Jiménez, Taus de Pejibaye, 900 m elev., 06 Apr 1994 (♂ fl), *E. Lépiz & J. F. Morales* 284 (holotype: CR! [201663]; isotypes: CR! [CR1578073], LSU! [0193696, LSU00199100], MO! [5551138, MO280083], USJ! [75968]).

Description. *Tree* 6–35 m × (4–) 10–50 cm DBH; bark not described. *Exudate* described on one occasion as orange in bark, branches and fruits. *Twigs* 0.13–0.4 cm thick, angulate to lightly compressed, densely tomentose to puberulent, trichomes dendritic, yellowish, brownish to ferruginous. *Leaves:* petiole 0.5–1.2 × 0.16–0.3 cm, canaliculate, densely to sparsely pubescent, the trichomes dendritic to irregularly stellate; leaf blades (11.2–) 15–30.5 × (3.9–) 4.5–7.4 cm, oblong-elliptic; adaxial surface on mature leaf blades dark brown when dry, reddish-brown or greyish-blackish, glabrous or with trichomes very sparse and scattered, the surface smooth; abaxial surface pale brown, dark brown or whitish-greyish when dry, sparsely pubescent, but usually densely to sparsely pubescent along the lateral veins and midvein (in new leaves, blades with a dense layer of trichomes that fall readily when touched, covering the entire surface), trichomes dendritic or rarely irregularly stellate, sessile, yellowish to pale brown, with 3–10 branches 0.2–0.6 mm long, caducous; lateral veins (15–) 18–30 per side, (4–) 6–9 veins per 5 cm, 0.5–0.9 (–1.5) cm apart, the same colour as the adaxial surface or sometimes lighter, flat or very slightly sunken on adaxial surface, raised on abaxial surface, straight to slightly arcuate (especially towards the distal part), slightly anastomosing near the margin and without forming a very marked intramarginal vein; tertiary veins usually visible on both surfaces; midvein adaxially flat to slightly canaliculate, glabrous or with scattered trichomes, sometimes densely pubescent at the base, abaxially raised, rounded, densely tomentose (with trichomes that fall very easily to the touch) to glabrescent; base acute to rounded, not revolute, flat; margin flat; apex acute to acuminate. *Staminate inflorescences* 3–9 cm long, axillary either at the junction with a leaf or at leafless nodes, axes slightly flattened to irregularly angled, densely tomentose, with trichomes dendritic, brown to yellowish-brown; peduncle 1.7–3.5 × 0.05–0.12 cm; bracts 3–7 × 1.9–4 cm, pubescent on both sides, caducous; terminal fascicles dense, with 7–20 + flowers. *Staminate flowers* with the pedicel 1.7–3.4 mm long; receptacle 1.5–3 mm wide; ; perianth (1.6–) 2–2.7 mm, subglobose, yellow, greenish-white or brown, possibly by the indumentum), connate by (0.2–) 0.5–0.8 mm long, abaxial surface pubescent, with brown to ferruginous trichomes, adaxial surface glabrous at the base, sparsely pubescent on the lobes; lobes 3, 1.5–2 × 1–1.4 (–1.8) mm; stamens 3 (–6), the filament column 0.6–0.9 mm long, straight or sometimes slightly thickened at the base and somewhat narrow at the apex, thin, not constricted at the apex; anthers 0.5–0.8 mm long; apiculus ca. 0.07–0.1 mm, inconspicuously apiculate. *Pistillate inflorescences* 3.5 cm long, at leafless nodes, with trichomes on the axes similar to those of the staminate inflorescences; peduncle 1.8–2.5 × 1–2 cm; bracts not seen; terminal fascicles of 5–6 flowers. *Pistillate flowers* with the pedicel 3.5–4 mm long; perianth 3.5–4.6 mm long, globose to subglobose, pale brown when fresh (possibly by the indumentum), connate by 1–1.5 mm long, abaxial surface pubescent, with brown trichomes, adaxial surface sparsely pubescent; lobes 3, 2.5–3.5 × 1.5–2.2 mm; gynoecium 2–2.7 × 1.5–1.6 mm, densely pubescent, subglobose, stipitate; stigmatic lobes ca. 0.3 mm,

erect. **Infructescence** 3–6.2 cm long, with 1 (–2) fruits, peduncle $2\text{--}3.3 \times 0.19\text{--}0.3$ cm. **Fruits** (2.8–) $3\text{--}3.6 \times 2\text{--}2.5$ cm, ovoid-ellipsoid, sessile, densely tomentose to tomentulose, the trichomes dendritic to irregularly stellate, pale brown to ferruginous, the surface commonly rugulose or smooth when dry, the line of dehiscence carinate, the base obtuse, the apex acute, orange, golden or yellowish-brown when fresh; pericarp 3.2–5 mm thick; pedicel 0.7–1 cm long; seed $2.2\text{--}2.5 \times \text{ca. } 1.5$ cm, the testa dark brown, almost smooth; aril usually described as red when fresh, yellowish- or reddish-brown when dry, coriaceous, oily in texture, thick, lacinate in narrow bands distally.

Distinctive characters. *Virola montana* is recognised by twigs, new leaf undersurfaces, petioles and inflorescences covered in indument of dendritic to irregularly stellate, caducous trichomes, with long branches; on the underside of the leaf, this indument is mainly found on the midvein and the lateral veins (Fig. 3J). Additional traits that distinguish this species include leaf blades with numerous lateral veins [(15–) 18–30 per side; Fig. 22C, D]), the length of the filament column (0.6–0.9 mm long), nearly the same size as the anthers (0.5–0.8 mm long), fruits with thick pericarp that are densely tomentose (Fig. 4H) and carinate in the line of dehiscence (3.2–5 mm), as well as its montane habitat.

Etymology. The specific epithet refers to the montane habitat where the species has been collected.

Distribution. *Virola montana* is known from Costa Rica and Panama, where it has been collected on the Caribbean slope in the provinces of Alajuela, Cartago, Guanacaste, Heredia and Limón in Costa Rica and Bocas del Toro in Panama; it has only been collected on the Pacific slope in Puntarenas (Costa Rica) (Fig. 18D). It has been recorded between 700–2000 m elevation.

Preliminary conservation status. *Virola montana* is of Least Concern following IUCN guidelines. It has both a large EOO ($14,606 \text{ km}^2$) and AOO (104 km^2) and is known from nineteen localities.

Common names. Costa Rica: fruta dorada.

Phenology. Flowering of *Virola montana* has been recorded in January, March to May, November and December; only two herbarium specimens with pistillate flowers were seen. Fruits have been collected in March and June to December.

Table 6. Morphological differences between *Virola montana* and *V. guatemalensis*.

| Character | <i>V. montana</i> | <i>V. guatemalensis</i> |
|---|---|---|
| Leaf blades size | (11.2–) 15–30.5 \times (3.9–) 4.5–7.4 cm | 12.3–17.5 (–24.1) \times (2.4–) 3.8–5.5 (–8.9) cm |
| Indument and trichomes on abaxial surface | Sparsely pubescent, but usually densely to sparsely pubescent primarily along the lateral veins and midvein; trichomes with 3–10 branches, the branches 0.2–0.6 mm long, caducous (Fig. 3J) | Puberulent, trichomes over the entire surface; trichomes with 3–6 branches, the branches 0.05–0.1 mm long, persistent (Fig. 3F) |
| Lateral veins | (15–) 18–30 per side, on abaxial side raised (Figs 8J, 22D) | 13–21 per side, on abaxial side slightly raised or flat (Figs 8F, 10F) |
| Filament column | 0.6–0.9 mm long | 1–1.2 mm long |
| Pericarp thickness and presence of a carina | 3.2–5 mm thick, carinate | 0.4–1 (–2.5) mm thick, smooth or slightly carinate |

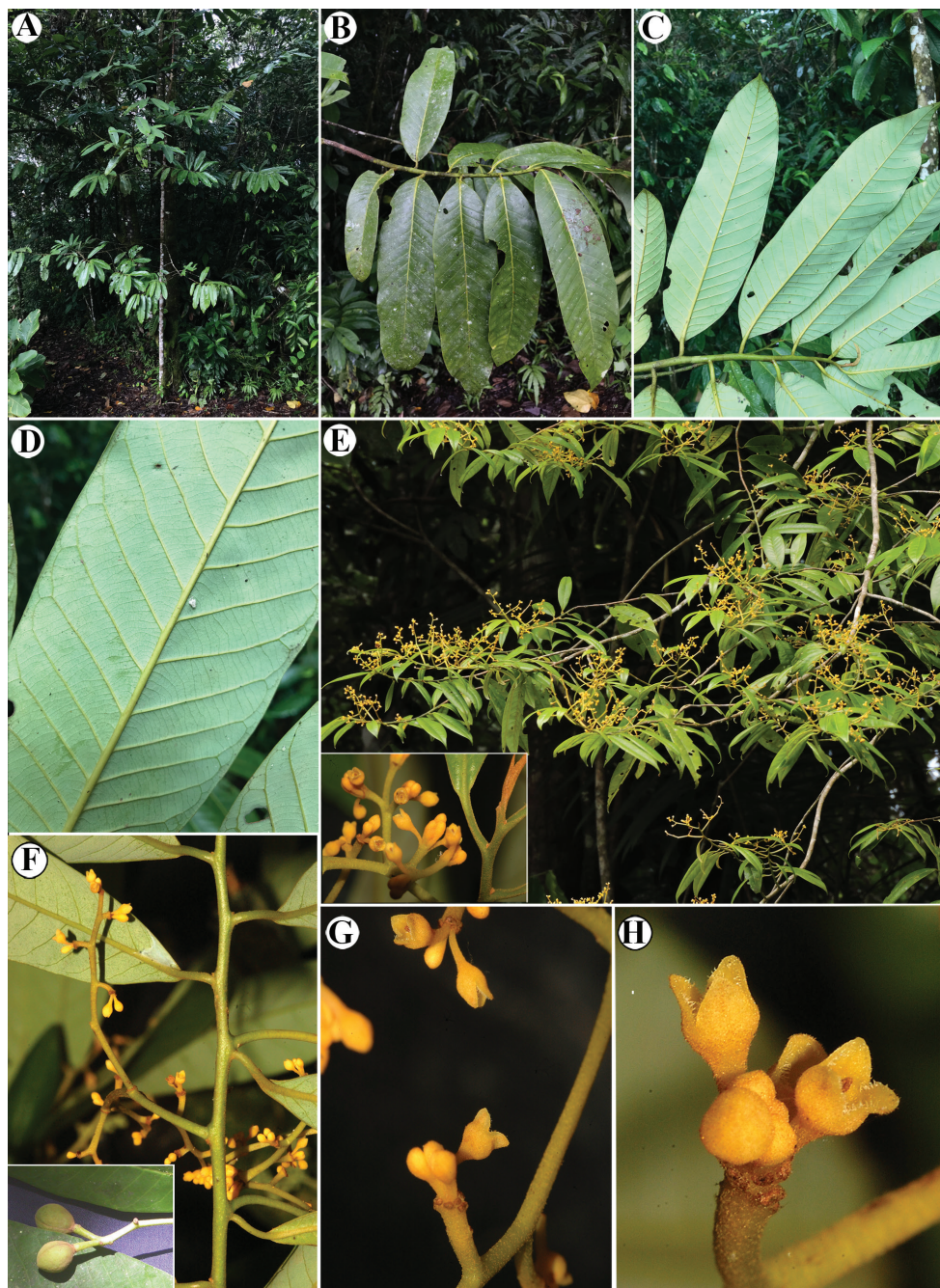


Figure 22. *Virola montana* **A** juvenile tree showing branching pattern **B** leaf blades on adaxial side **C** leaf blades on abaxial side **D** venation. *Virola multiflora*. **E** Branch with staminate inflorescences, inset showing twig and flowers **F** twig, leaf blade on abaxial surface and inflorescences, inset showing immature fruit **G** lateral view of a perianth **H** close-up of staminate flowers. Photos by J. Esteban Jiménez (**A–D**); B. Hammel (**E–H**), Indiana Coronado (**F**, inset).

Field characters. Leaf blades are lustrous, dark green above and whitish or silver below. Flowers have yellow, cream, greenish-white or brown perianth. Mature fruits are yellow, pale brown, brown yellow or orange. The seed is brown with a red aril.

Discussion. As far as we know, the first specimen of this species was collected 115 years ago by Henri F. Pittier (1857–1950) in the mountains of El Rosario de Orosi, Cartago, Costa Rica (*H. Pittier 16628*, NY-2 sheets!*). Paul C. Standley treated this specimen as *V. koschnyi* in “Flora of Costa Rica” (Standley 1937). Shortly after, Albert C. Smith and Roger P. Wodehouse (1938) included the Pittier specimen under *V. guatemalensis* and this remains the name that is most frequently misattributed to specimens of *V. montana* (e.g. Standley and Steyermark 1946; Jiménez 2007). However, *V. guatemalensis*, as interpreted here, is restricted to northern Mesoamerica (Mexico, Guatemala and Honduras). In addition to the differences given in the diagnosis, the *V. montana* can be distinguished morphologically from *V. guatemalensis* by the differences summarised in Table 6. Although it is difficult to quantify, specimens of *V. montana* commonly have twigs, petioles and inflorescences covered with more trichomes with long branches (see Fig. 3F, J).

In addition to *Virola guatemalensis*, herbarium specimens of this new species have been determined as three other species: *V. koschnyi*, *V. sebifera* and *V. surinamensis* (here treated as *V. nobilis*). Vegetatively, *V. montana* can be distinguished from these species by its mature leaves that are abaxially sparsely pubescent (vs. covering the entire surface of the leaf blades abaxially). The first two species are distinguished by pediculate trichomes on abaxial surface of leaf blades (vs. sessile in *V. montana*, these primarily present in young leaves). Finally, *V. nobilis* has trichomes with short branches [0.05–0.1 mm (Fig. 3L) vs. 0.2–0.6 mm long (Fig. 3J)], tends to have more lateral veins [25–34 vs. (15–) 18–30 per side] and has a preference for lower elevation habitats [0–500 (–1300) m vs. 700–2000 m elevation].

Based on the number of herbarium specimens collected, *Virola montana* is the most common montane species of *Virola* in southern Mesoamerica and it is usually the only species where it occurs. However, three fruiting specimens of *Virola* sp., represented by *R. Aguilar et al. 4327* (CR!, MO!), *G. McPherson 8723* (MO!) and *G. McPherson 10514* (MO!), have been collected in the general vicinity of *V. montana* in its preferred elevational range (1050–1500 m). These specimens clearly differ from *V. montana* in their small leaf blades [$10.5\text{--}12.5 \times 2.3\text{--}3.5$ cm vs. $(11.2\text{--}) 15\text{--}30.5 \times (3.9\text{--}) 4.5\text{--}7.4$ cm] with trichomes on the abaxial surface that have short branches (0.08–0.1 mm vs. 0.2–0.6 mm long) and cover the entire surface (vs. sparsely pubescent, but usually densely to sparsely pubescent along the lateral veins and midvein in *V. montana*). These specimens share characteristics with two lowland species of *Virola*: *V. nobilis* and *V. fosteri*, including sessile, stellate trichomes on the abaxial surface of leaves. Additionally, these three specimens share their small leaves with *V. fosteri*, though not *V. nobilis* $(9\text{--}17.2 [27.5] \times 2.5\text{--}5 [-4.7\text{--}7.1]$ cm vs. $10.5\text{--}12.5 \times 2.3\text{--}3.5$ cm). Further, the fruits of *V. fosteri* are smaller $(1.5\text{--}2.3 \times 1.2\text{--}1.8$ cm vs. $2.8\text{--}3.2 \times 2.2\text{--}2.7$ cm). Additional collection and study is necessary to determine the identity of these specimens.

Specimens examined. COSTA RICA. Alajuela: Reserva Biológica Monteverde, Río Peñas Blancas, parcela de Badilla, 800 m elev., 23 Oct 1988 (fr), *E. Bello* 470 (CR-2 sheets!, INPA!*, LSU!, MO!); Reserva Biológica Monteverde, Parcela de Jesús Rojas, 850–900 m elev., 05 May 1989 (♂ fl), *E. Bello* 855 (CR-2 sheets!, INPA!*, MEXU!*, MO!); Reserva Biológica Monteverde, Ref. Aleman, 900 m elev., 10 Nov 1993 (♂ fl), *E. Bello* 5404 (CR!, LSU!, MO!); Reserva Forestal de San Ramón, 850–1150 m elev., 12–14 Mar 1987 (♂ fl), *W. Burger et al.* 12152 (CR!, F!); San Ramón, Reserva Biológica Alberto Brenes, Estación San Lorencito, 900 m elev., 03 May 2000 (♂ fl), *K. Caballero* 2 (CR-2 sheets!, MO!); Reserva Forestal de San Ramón, 900–1200 m elev., 12–15 Mar 1987 (♂ fl), *J. Gómez-Laurito* 11446 (CR!, USJ!); Reserva Forestal de San Ramón, 900–1200 m elev., 16–19 Apr 1987 (♂ fl), *J. Gómez-Laurito* 11484 (CR!, F-2 sheets!); Reserva Forestal de San Ramón, Estación Río San Lorencito, 850–900 m elev., 18 Oct 1989 (fr), *J. Gómez-Laurito et al.* 11846 (CR!, MEXU!*, MO!, USJ!); La Balsa, 1100 m elev., 02 Jul 1990 (st), *J. Gómez-Laurito & J. A. López* 11970 (USJ!); Reserva Biológica Monteverde, Río Peñas Blancas, 850 m elev., 31 Mar 1987 (♂ fl), *W. Haber & E. Bello* 6978 (CR!, F!*, INPA!*, MEXU!*, MO!); Reserva Biológica Monteverde, finca Wilson Salazar, 800 m elev., 07 Nov 1987 (fr), *W. Haber & E. Cruz* 7698 (CR!, INPA!*, MO!, USJ!); Río San Lorenzito, 800–1000 m elev., 30 Mar 1987 (♂ fl), *G. Herrera et al.* 514 (CR!, INPA!*, LSU!, MO!); La Fortuna, Finca El Jilguero, 1160 m elev., 20 Nov 1992 (fr), *G. Herrera* 5544 (CR!); 2 km N.E of La Balsa de San Ramón, 900 m elev., 26 Sep 1976 (fr), *R. Lent* 3899 (CR-3 sheets!, INPA!*, MARY!*, MEXU!*, MO!, PMA!*, U!); Reserva Forestal San Ramón, Estación Río San Lorenzo, sendero al Volcán Muerto, 1100 m elev., 26 Apr 1993 (♀ fl), *F. Quesada* 20 (CR!, LSU!, MO!); Lago Coter [Cote], Hotel Eco-Lodge, 700 m elev., 09 Apr 1997 (♂ fl), *J. Rivera & B. Petruzzi* 2933 (CR!). **Cartago:** Taus, faldas del Cerro Alto El Humo, 900–1200 m elev., 06 Apr 1994 (♂ fl), *J. F. Morales & E. Lépez* 2654 (CR!, MO!); El Rosario de Orosi, no date, Jan 1903 (fr), 1120 m elev., *H. Pittier* 16628 (NY-2 sheets!); Turrialba, Monumento Nacional Guayabo, Santa Teresita, sobre los ríos Guayabo, Lajas y Torito, 700–1800 m elev., 08 May 1992 (♂ fl), *G. Rivera* 1659 (CR!, MO!, USJ!); límite Sur del Monumento Nacional Guayabo, sector Las Ventanas, 1100 m elev., 07 Jul 1992 (fr), *G. Rivera* 1910 (CR!, F!*, MO!, USJ!); [Paraíso] 5 km N.W of Río Grande de Orosi at Tapanti, 1300 m elev., 27 May 1976 (♂ fl), *J. Utley & K. Utley* 5054 (CR-2 sheets!, MEXU-2 sheets!*, MO-2 sheets!). **Guanacaste:** San Gerardo, 1000 m elev., 21 Nov 1998 (fr), *E. Bello* 553 (CR!, INPA!*, MO!). **Heredia:** Sarapiquí, Parque Nacional Braulio Carrillo, puesto El Ceibo, 750 m elev., 05 Mar 1994 (st), *B. Boyle et al.* 2938 (MO!); Cariblanco, 800 m elev., n.d. Mar 1950 (fl bud), *J. León* 2368 (USJ!); Cariblanco, 800 m elev., 10 Aug 1950 (fr), *LRH* [*L. R. Holdridge*] 2618 (USJ!). **Limón:** Talamanca, Bratsi, Alto Lari, Kivut, 1300–1500 m elev., 15 Mar 1992 (fr), *R. Aguilar & H. Schmidt* 1131 (CR!, MO!). **Puntarenas:** Coto Brus, Zona Protectora Las Tablas, sitios Las Juntas, 1500 m elev., 03 Jul 1999 (fr), *E. Alfaro* 2357 (CR-2 sheets!, MO!); [Coto Brus], Las Tablas, río Cotoncito, [1300–1500 m elev.], 10 Dec 1983 (fr), *I. Chacón et al.* 1819 (INPA!*, LSU-2 sheets!, MO!); Parque Internacional La Amistad, Estación Pittier, 1650 m elev., 28 Jan 1995 (♀ fl), *M. Chinchilla & IV curso de para-*

toxónomos 3 (CR-2 sheets!, LSU!, MO!); Zona Protectora Las Tablas, 2000 m elev., 01 Sep 1992 (fr), *A. Fernández* 354 (CR!); Fila Tigre, SE of Las Alturas, 1350–1450 m elev., 29 Aug 1983 (fr), *G. Davidse* 24178 (CR!, INPA!*, MEXU!*, MO!); Parque Internacional La Amistad, Santa María de Pittier, 1700 m elev., 13 Jun 1995 (fr), *J. González* 823 (CR-2 sheets!); Coto Brus, Zona Protectora Las Tablas, Hacienda La Amistad, 1600–2000 m elev., 28 Dec 2003 (♂ fl), *R. Kriebel et al.* 4174 (CR-2!, MO!). **PANAMA. Bocas del Toro:** Bocas del Toro-Chiriquí border above Fortuna Dam, 1200 m elev., 04 Dec 1985 (imm fr), *G. McPherson* 7756 (INPA!*, MO!, PMA!*).

11. *Virola multiflora* (Standl.) A. C. Sm.

Fig. 22E–H

Virola multiflora (Standl.) A. C. Sm. Brittonia 2: 499. 1938.

Dialyanthera multiflora Standl. Publ. Field Mus. Nat. Hist., Bot. Ser. 8: 12. 1930. Type. [Belize] British Honduras. In jungle, Stann Creek Railway, alt. 30 m, 16 Jul 1929 [♂ fl], *W. A. Schipp* 279 (holotype: F!*; isotypes: A!*, BM!*, G-2 sheets!*, GH!*, K!*, MO!, S!*).

Virola brachycarpa Standl. Publ. Field Mus. Nat. Hist., Bot. Ser. 11: 131. 1932. Type. [Belize] British Honduras. Stann Creek Valley, 13 Jan 1932 [fr], *J. A. Burns* 20 (holotype: F!*; isotypes: BKL!*, G!*, US!*, WIS!*).

Distinctive characters. *Virola multiflora* is recognised by its usually small and narrow leaf blades [$5.5\text{--}15.5 \times 1.5\text{--}3.6$ (–4.8) cm] with an inconspicuously pubescent abaxial surface with stellate and sessile trichomes (Fig. 3K), lateral veins that are not very prominent (Fig. 8K) and long and thin petioles [$0.5\text{--}0.9$ (–1.1) cm long]; staminate flowers with the filament column longer (0.7–1 mm long) than the anthers (0.3–0.6 mm long); and for its small fruits [$1.3\text{--}1.9 \times 0.9\text{--}1.2$ (–1.4) cm] (Fig. 4J) with the pericarp 0.7–1 mm thick.

Distribution. *Virola multiflora* is known from Belize (Cayo, Stann Creek and Toledo), Honduras (Gracias a Dios), Nicaragua (Atlántico Norte, Atlántico Sur, Jinotega, Matagalpa and Río San Juan) and Costa Rica (Alajuela, Cartago, Heredia and Limón) (Fig. 18E). Throughout its range in Mesoamerica, it is only known from the Caribbean slope. It has been recorded from between 0–650 (–1400) m elevation.

While *V. multiflora* is not documented from Guatemala in herbaria, Standley and Steyermark (1946) postulated that the species is to be expected to occur in Izabal (Guatemala) and Jiménez (2007) attributes it to that country. Conversely, while it is mentioned as occurring in Peru by Jiménez (2007), specimens could not be located. The Peruvian specimen attributed to *V. multiflora* (*R. Vásquez & C. Grández* 17507, MO!; fr) in Vásquez M. et al. (2018) corresponds to a species related to *V. multinervia*. Finally, the report presented in the *Nuevo Catálogo de la Flora Vascular de Venezuela* (Rodríguez 2008) is erroneous. Based on this evidence, *V. multiflora* is considered as restricted to Mesoamerica.

Common names. Belize: banak, bastard banak. Honduras: asang banak, bának, banak almuk, báhanak luhpia, sangre, sebo álmot, sebo negro. Nicaragua: conchillo, samo. Costa Rica: fruta dorada.

Phenology. Flowering of *Virola multiflora* has been recorded in March to August, with a noted peak in July; just one herbarium specimen with pistillate flowers was seen and it is from Nicaragua. Fruits were collected in December through April.

Field characters. Plants are trees between 6–30 m tall and 17–35 cm DBH. Bark exudes latex red. The leaf blades are sometimes whitish abaxially. The flowers usually have yellow, golden or orange perianth. The mature fruit is yellow or orange with a red aril.

Notes. We were not able to locate any Panamanian specimens of *V. multiflora*: all fertile Panamanian specimens identified as *V. multiflora* that the first author has studied are actually *V. fosteri*. However, *V. multiflora* is to be expected in Panama because it occurs in physiognomically similar forests in Costa Rica near the border. The similarities and differences between *V. multiflora* and *V. fosteri* (which is formally described above) are discussed under the latter species.

Selected specimens seen. **BELIZE.** **Cayo:** Hummingbird Highway south of Belmopan, 200–300 ft [60–90 m] elev., 26 Jun 1973 (♂ fl), *A. Gentry* 8615 (MO!). **Stann Creek:** Big Eddy Ridge, [50–200 m elev.], 12 May 1940 (fl bud), *P. H. Gentle* 3333 (MO!); Cockscomb Basin Wildlife Sanctuary, 80 m elev., 17 Jan 2007 (fr), *P. Hechenleitner* 289 (MO!). **Toledo:** Between Rancho Chico and Cockscomb, no elev., 26 Mar 1943 (fl bud), *P. H. Gentle* 4342 (MO!); Big Creek, 100 ft [30 m] elev., 02 Dec 1931 (fl bud), *W. A. Schipp* 858 (MO-2 sheets!). **HONDURAS.** **Gracias a Dios:** 1 km al sureste de Krausirpe, pie de montaña de Wimpi, 90 m elev., 18 Feb 1994 (fr), *P. R. House* 1888 (MO!); transecto Botánico Cerro Krautara, 120 m elev., 16 Mar 1995 (♂ fl), *P. R. House* 2308 (MO!). **NICARAGUA.** **Atlántico Norte:** El Salto, along Río Pis Pis, 100 m elev., 27 Feb 1979 (fr), *J. J. Pipoly* 3615 (MO!); 13 km above Kururia on road to San Jerónimo, 200 m elev., 02 Mar 1979 (♂ fl), *J. J. Pipoly* 3846 (MO!). **Atlántico Sur:** Nueva Guinea, Reserva Indio-Maíz, 200–300 m elev., 05 Jan 1999 (fr), *R. Rueda et al.* 9857 (MO!); 2 km de Colonia Serrano, Comarca El Escobillo, 80–100 m elev., 28 Jul 1982 (♀ fl), *J. C. Sandino* 3302 (MO!). **Jinotega:** Cua Bocay, Reserva de Bosawas, 278 m elev., 03 Oct 2005 (imm fr), *I. Coronado et al.* 2306 (MO!). **Matagalpa:** Río Blanco, Reserva Natural Cerro Musún, 500–1400 m elev., 15 Jul 2000 (♂ fl), *R. Rueda & O. Caballero* 14294 (MO!). **Río San Juan:** 1 km al NW del Río Santa Cruz, 60 m elev., 22 Feb 1984 (fr), *P. P. Moreno* 23253 (MO!); sobre el río Sábalo, 40 m elev., 07 Jul 1984 (♂ fl), *P. P. Moreno & W. Robleto* 25984 (MO!); Boca Negra, 120 m elev., 14 Feb 1990 (fr), *P. P. Moreno* 27266 (MO!, P!*); Estación Biológica Bartola, 50–100 m elev., 26 Jul 1998 (♂ fl), *R. Rueda et al.* 8196 (MO!); Estación Experimental La Lupe, 100 m elev., 22 Nov. 2000 (fr), *R. Rueda & W. Velásquez* 15004 (MO!). **COSTA RICA.** **Alajuela:** Corredor Fronterizo Costa Rica-Nicaragua, 2 km antes de Boca San Carlos, 0–100 m elev., 15 Mar 2004 (♂ fl), *J. F. Morales* 10322 (CR!, MO!). **Cartago:** 24 km northeast of Turrialba on highway to Limón, 450–525 m elev., 10 May 1983 (♂ fl), *R. Liesner et al.* 15390 (MO!). **Heredia:** Puerto Viejo de Sarapiquí, camino a Cerros Sardinal, Caño Negro, 71 m elev., 25 Jul 2016 (♂ fl), *B. Hammel & I. Pérez* 27148 (CR!); along

Guácimo ridge trail, 315 m elev., 18 Jan 1983 (fr), *G. S. Hartshorn 2553* (CR!, MO-2 sheets!). **Limón:** Siquirres, Fila Mirador, camino a Las Brisas de Pacuarito, 400 m elev., 13 Feb 2000 (fr), *M. Blanco & A. Vega 1460* (USJ!); Bajo Telire, 400–600 m elev., n.d. July 1984 (♂ fl), *L. D. Gómez 24151* (CR!, MO!); Talamanca, Finca La Culebra, a 1.5 km de camino Bribri, 0–200 m elev., 26 Oct 1992 (fl), *J. Gómez-Laurito & H. Gómez 12340* (USJ!); Matina, intersección de Río Barbilla y Quebrada Cañabral, 100–200 m elev., 11 Oct 1988 (imm fr), *G. Herrera 2167* (MO!); Parque Nacional Tortuguero, Lomas de Sierpe, 100 m elev., 15 Aug 1988 (♂ fl), *R. Robles et al. 2063* (MO!); Sixaola, San Miguel, camino entre Fila Tsipubeta y Cerro Mirador, 200–300 m elev., 12 Nov 1999 (fl), *O. Valverde & S. Hernández 1237* (CR!, USJ!).

12. *Virola nobilis* A. C. Sm.

Figs 20C–G, 24B

Virola nobilis A. C. Sm. Brittonia 2: 490. 1938. Type. Panama. Canal Zone, Barro Colorado Island, 07 Jan. 1932 [imm fr], [*R. H. Wetmore*, [*E. C. Abbe & O. E. Shattuck 155* (holotype: GH!*; isotypes: A!*, F!*, MO!)]

Distinctive characters. *Virola nobilis* is recognised by its narrow, oblong leaf blades (9–17.2 [–27.5] × 2.5–5 [–4.7–7.1] cm) with numerous lateral veins (20–30 [25–32] per side) corresponding to 8–11 (5–7 [–9]) veins per 5 cm, as well the stellate, tertiary veins that are slightly sunken above (Fig. 3L) and sessile trichomes scattered on underside of the leaf (Fig. 3L); the staminate flowers with the filament column longer (0.8–1.1 [1.2–1.3] mm long) than the anthers (0.6–0.8 mm long); and usually ellipsoid or ovoid fruits (2.3–2.7 × 2.2–2.4 cm [2.1–3.1 × 1.6–2.5 cm]) (Fig. 4N) with thick pericarp (2.3–3.5 mm [2.5–4.2 mm]) and aril.

Distribution. *Virola nobilis* is known from the Pacific slope of Costa Rica (Puntarenas, San José) and the Caribbean slope of Panama (Bocas del Toro, Colón, Panamá, San Blas). It is recorded from 0–400 (–1300) m elevation (Fig. 18F). Jiménez (2007) reported this species (as *V. surinamensis*) from just one locality (Chitaría) on the Caribbean slope in Costa Rica; however, we have not examined the specimen (*Poveda 144*, CR).

Common names. Costa Rica: Fruta dorada. Panama: bogamani, coton cuinur gia, sabdurgia (Kuna name).

Phenology. Flowers have been collected in January to April, July, August, November and December and fruits in almost all months except October.

Field characters. Plants are large trees between (5–) 15–40 m tall and 20–70 cm DBH. The trunk is straight, usually with conspicuous buttresses and does not begin to branch until it reaches a great height. The bark is reddish and releases red exudate when damaged. The leaves are whitish with inconspicuous trichomes on the abaxial surface. Flowers have yellow perianth. The mature fruit is yellow to orange with a red aril.

Discussion. This species has usually been treated, identified and included as a synonym of *Virola surinamensis* (e.g. Gentry 1975; Croat 1978; Correa et al. 2004;

Jiménez 2007), which has similar leaf morphology (i.e. number of lateral veins, stellate and sessile trichomes scattered on the abaxial surface of the leave blades). However, *Virola surinamensis* (Figs 23G–J, 24A) is characterised by having a shorter perianth that is also somewhat fleshy to submembranous and smaller fruits that are also ovoid to subglobose, glabrescent and with a thin pericarp (Figs 23I–J, 24A inset; also see illustration in Rodrigues 1972, 1980). Although both species may have similarly-sized leaves, they tend to be smaller in *V. surinamensis*, which also has inflorescence axes that are longer and with many more flowers (Figs 23F, 24A).

Notes. *Virola nobilis* exhibits complex variation that requires more fieldwork, ideally in combination with molecular phylogenetic analysis, to clarify species boundaries. For example, specimens from the Pacific slope in Costa Rica (see specimens cited below and Figs 24C, 25) are included, with reservation, under *V. nobilis*. These specimens differ from other collections of *V. nobilis*, including the type and additional specimens from Barro Colorado Island (the type locality), in having wider leaf blades, fewer lateral veins, tertiary veins that are less inconspicuous below (Fig. 3L, O), a longer filament column and ovoid fruits with obtuse to rounded apices (Fig. 4N, O). These specimens also resemble *V. reidii* Little, a species from Colombia and Ecuador (Jaramillo et al. 2004). The measurements presented in square brackets [] correspond with the Costa Rican material under question.

Other material that potentially belongs to *V. nobilis* includes a few collections from Panama, including both infertile and fruting specimens from El Llano Cartí and others from Cerro Jefe (e.g. *G. de Nevers* & *H. Herrera* 4333, imm fr, MO!; *R. L. Liesner* 657, fr, MO!; *E. L. Tyson et al.* 3353, fr, MO!). These differ from *V. nobilis* by having the abaxial surface of the leaf blade covered by a dense and a very inconspicuous layer of stellate, sessile and yellowish trichomes and fruits with a more or less smooth surface and that are not carinate in the line of dehiscence. Finally, *R. Aguilar* 3408 (CR-3 sheets!, MO!) from the Osa Peninsula of Costa Rica and *G. McPherson* 11474 (MO!; fr) from Panama differ from the species concept that we adopt in having trichomes on the abaxial leaf side with long branches (ca. 0.2 mm long) that are often shortly pediculate; and small fruits (ca. 2.1×1.6 –1.7) that are ovoid to subglobose and apiculate at the apex.

Selected specimens. **COSTA RICA. Puntarenas:** Golfito, camino a Piro, finca de Adrian, 50 m elev., 16 Apr 2005 (fr), *R. Aguilar* & *X. Cornejo* 9739 (MO!); Osa, Punta Pargos, 0 m elev., 17 Apr 2008 (fr), *R. Aguilar* 11186 (MO!, USJ!); Osa, Rancho Quemado, siguiendo el nuevo camino a Drake, 400 m elev., 20 Jun 1990 (fr), *G. Herrera* 4222 (MO!); Trocha de La Tarde rd. 10 km SW of La Palma, 150–200 m elev., 28 Apr 1988 (fr), *B. Hammel* & *R. Robles* 16722 (MO!); Osa, Fila Ganado, 350 m elev., 15 Dec 1990 (♂ fl), *B. Hammel et al.* 18017 (CR-2 sheets!, MO!); Osa, Fila Ganado hasta Guerra, 1–300 m elev., 28 Mar 1991 (fr), *B. Hammel et al.* 18170 (MO!). **San José:** Tarrazú, 900–1300 m elev., 19 Aug 1997 (fr), *O. Valverde* & *A. Estrada* 135 (CR!). **PANAMA. Bocas del Toro:** North of Fortuna Dam, on road to Chiriquí Grande, 500 m elev., 18 Jan 1986 (fr), *G. McPherson* 8098 (MO!). **Colón:** Carretera Gatún-Piñas, 0–50 m elev., 26 Jul 1994 (fr), *C. Galdames et al.* 1415 (CR!, MO!). **Panamá:** Barro Colorado Island, [10– 100 m elev.], 23 Jan 1969 (fl bud), *T. B. Croat* 7488 (MO!); ibid, 22 Feb 1969 (fr), *T. B. Croat* 8090 (MO!); El Llano Cartí, 1100–1200 m

[335–365 m] elev., 28 Dec 1974 (fr), *S. Mori & J. Kallunki 4151* (CR!). **San Blas:** El Llano-Cartí, 100–350 m elev., 03 Nov 1985 (imm fr), *G. de Nevers et al. 6172* (MO!).

13. *Virola otobifolia* D.Santam., sp. nov.

urn:lsid:ipni.org:names:77202553-1

Figs 7D, 23A–E, 26

Diagnosis. Species most similar to *Virola macrocarpa* in having leaf blades that are discolorous abaxially with stellate, sessile trichomes with a reddish central portion and similar number of lateral veins (10–16) that are not densely spaced. However, it differs in its leaves with dense, but inconspicuously pubescent leaf undersides (vs. inconspicuously puberulent) with 2–4 veins per 5 cm that are 1.7–3 cm apart (vs. 4–5 veins per 5 cm, 0.8–1.5 cm apart) and fruits that are (2.7–) 3.5–4.5 × (1.9–) 2.3–2.9 cm, densely tomentose with persistent trichomes, a rugose surface when dry and a line of dehiscence that is conspicuously carinate (vs. 2.7–3.3 × 2–2.3 cm, tomentellous, with caducous, dust-like trichomes, a smooth surface when dry, the line of dehiscence slightly carinate), as well as the production of thicker pericarp [(2.7–) 3–4.7 mm vs. 1.8–3 mm thick].

Type. PANAMA. [Panamá: Chepo], El Llano Cartí road 6.9 km N of Panamerican Highway, 350–500 m elev., 23 Jan 1977 (fr), *J. P. Folsom 1440* (holotype: MO-2 sheets! [2601823, MO117643, 5551400, MO299335]; isotype: PMA!* [101093, PMA111119]).

Description. *Tree* 6–30 m × 24.9–37.3 cm DBH; bark not described. *Exudate* from the trunk sometimes described as red, watery-red, or black-reddish. *Twigs* 0.23–0.34 cm, terete to slightly flattened, glabrescent to puberulent, trichomes stellate to irregularly stellate, ferruginous to greyish. *Leaves:* petiole (1–) 1.3–2 × 0.19–0.44 cm, flat to very slightly canaliculate, tomentose, the trichomes stellate; leaf blades (14–) 18.2–42.5 × (4.1–) 7.3–14.2 cm, oblong to elliptic; adaxial surface of mature leaf blades brown (sometimes shining) when dry, glabrous or sometimes with scattered stellate trichomes (especially along the veins), the surface smooth; abaxial surface when drying whitish-greyish or sometimes very light brown, dense but inconspicuously pubescent, trichomes stellate, sessile, the central part of the trichome usually reddish, contrasting in colour with the hyaline branches to reddish-clear, with 4–11 branches, the branches ± 0.01–0.05 mm, persistent; lateral veins 10–16 per side, 2–4 veins per 5 cm, 1.7–3 cm apart, the same colour as the adaxial surface, on adaxial surface flat to slightly sunken, on abaxial surface slightly elevated, arcuate, slightly anastomosing near the margin and without forming a very marked intramarginal vein; tertiary veins barely visible on both surfaces; midvein adaxially elevated, but submerged in a channel, abaxially raised, triangular to rounded, tomentose; base acute to obtuse, sometimes slightly cordate, not revolute, flat; margin flat to slightly revolute; apex acuminate. *Staminate inflorescences* 3.5–9.5 cm long, axillary either at a leaf or at a leafless node, axes flattened or irregularly flattened, tomentose, with trichomes irregularly stellate to irregularly dendritic, brown to ferruginous; peduncle 1.4–2.2 × 0.22–0.34 cm long; bracts not seen; terminal fascicles lax, with 5–13 flowers. *Staminate flower* with the pedicel 1.5–2 mm long;

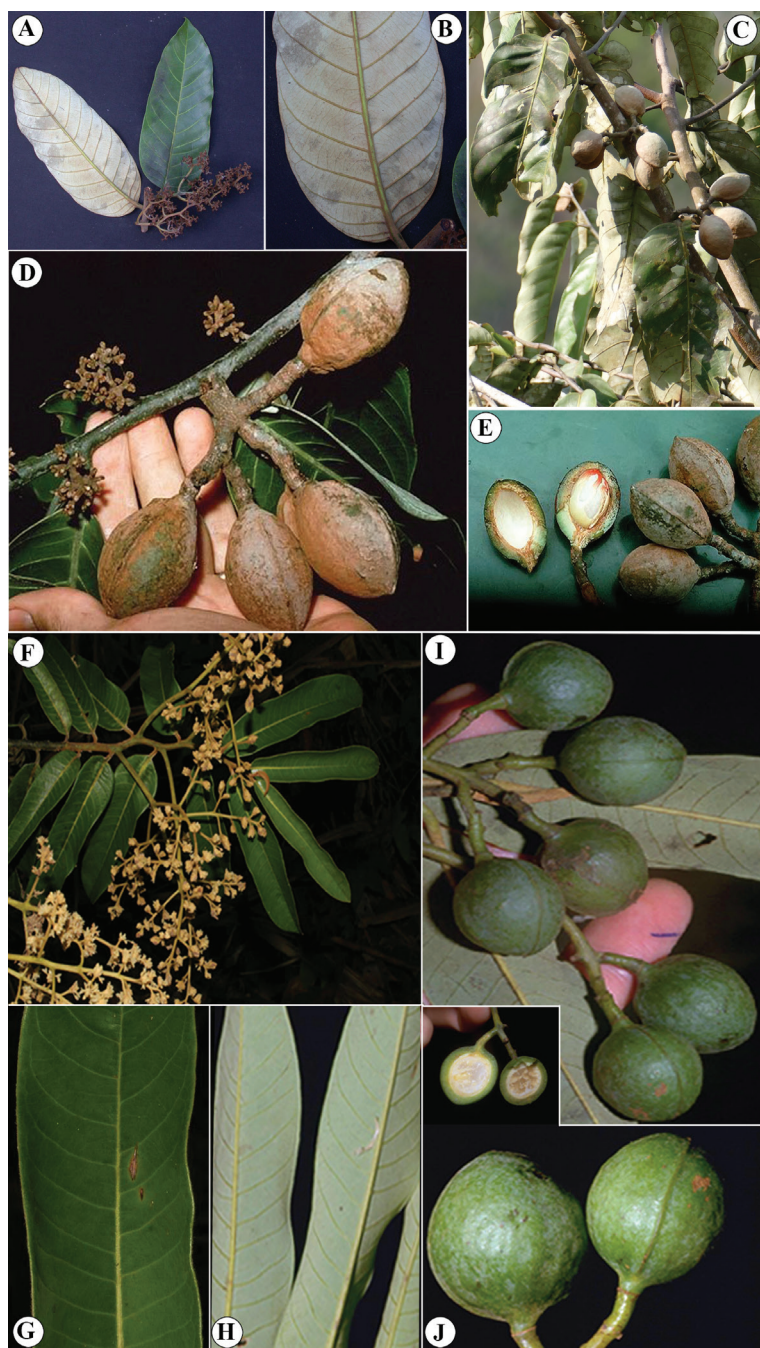


Figure 23. *Virola obovifolia* **A** branch with leaf blades showing both surfaces and inflorescences **B** leaf blade, venation and base on abaxial surface **C** infructescence **D** fruits **E** detail of fruit, showing an aril of an immature fruit. *Virola surinamensis*. **F** Branch with inflorescences **G**, **H** leaf blade on adaxial (**G**) and abaxial surface (**H**). **I** Infructescence **J** detail of fruits, including a longitudinal section of an immature fruit. Photos by Rolando Pérez (**A**, **B** from <https://stricollections.org/portal/index.php>); Jerry Harrison (**C**), Alwyn H. Gentry (**D**, **E** from <http://www.tropicos.org>) and John P. Janovec (**F–J** from <http://atrium.andesamazon.org/>).

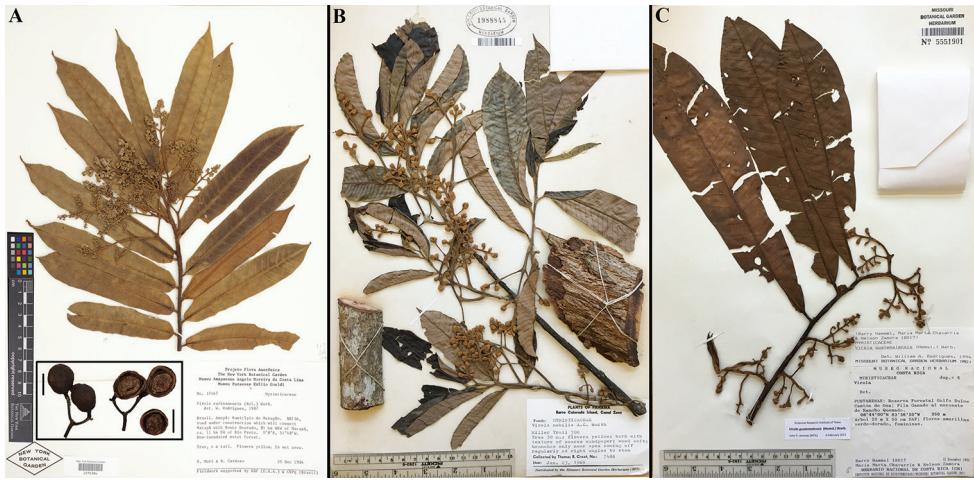


Figure 24. Comparisons of *Virola surinamensis* with collections of *V. nobilis* **A** *V. surinamensis* (S. Mori & R. Cardoso 17467, NY; inset showing fruits from W. E. Broadway 5264a, MO) **B** *V. nobilis* (T. B. Croat 7488, MO) **C** *V. nobilis* from Costa Rica (B. Hammel 18017, MO). **A**, image courtesy of New York Botanical Garden.

receptacle 0.7–1.5 mm wide; ; perianth 2.5–2.8 mm long, infundibuliform, yellow or brown when fresh, connate by 0.9–1.5 mm long, abaxial surface pubescent with brown trichomes, adaxial surface glabrous or with few trichomes close to the base; lobes 3 (4), 1.2–1.5 × 0.9–1.1 mm; stamens 3 (–6), the filament column 0.9–1 mm long, straight and very thickened throughout its length, fleshy, constricted at the apex; anthers 0.6–1 mm long; apiculus ca. 0.1 mm long, acute to apiculate, connate or slightly separate. **Pistillate inflorescences** 3.2–4 cm long (immature), axillary, axes flattened or irregularly flattened, tomentose, with trichomes stellate, brown to ferruginous; peduncle 0.6–1.2 × ca. 3.2 cm long; bracts not seen. **Pistillate flowers** not seen. **Infructescence** 3.2–5 cm long, with (1–) 2–4 fruits, peduncle 1–3.5 × 0.35–0.53 cm. **Fruits** (2.7–) 3.5–4.5 × (1.9–) 2.3–2.9 cm, ellipsoid, shortly stipitate, densely tomentose, the trichomes stellate, ferruginous, persistent, the surface rugose when dry, the line of dehiscence conspicuously carinate, the base obtuse to subtruncate, the apex acute to acuminate, brown (possibly by pubescence), although presumably green when fresh; pericarp (2.7–) 3–4.7 mm thick; pedicel 0.5–0.8 (–1) cm long; seed 2.5–2.8 × 1.5–1.7 cm, the testa dark brown when dry, markedly grooved; aril described as red when fresh, brown to blackish when dry, membranaceous, with a dry texture, thin, lacinate in narrow bands distally.

Distinctive characters. *Virola otobifolia* is recognised by its large leaf blades with well-spaced lateral veins (Fig. 8M) and a whitish abaxial surface that is covered with stellate, sessile trichomes (Fig. 3M); staminate flowers that have a filament column that is straight and very thickened throughout its length, except where it is constricted at the apex and anthers that are almost the same length as the filament column; and its large fruits with thick pericarp that are densely tomentose and with a conspicuously carinate line of dehiscence (Fig. 4C).

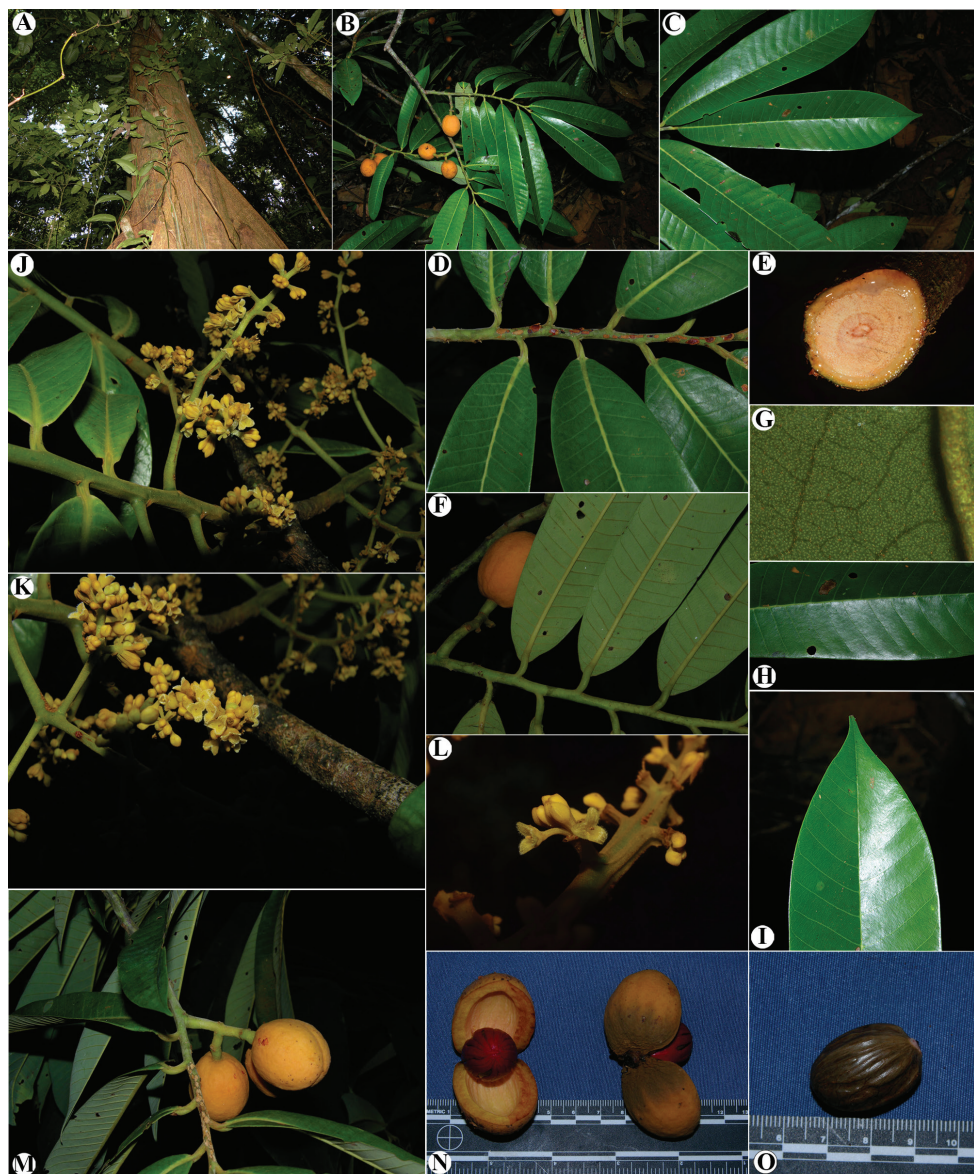


Figure 25. *Virola* cf. *nobilis* from the Osa Peninsula, Costa Rica **A** trunk **B** branch with fruits **C** leaf blades on adaxial surface **D** twig, petiole and leaf base on adaxial surface **E** exudate on a twig **F, G** leaf blades on abaxial surface and trichomes (**G**). **H** Leaf margin **I** leaf apex **J** staminate inflorescences **K** detail of staminate inflorescences **L** detail of staminate flower **M** branch with fruits **N** fruits **O** seed. Photos by Reinaldo Aguilar.

Etymology. The specific epithet refers to the similarity of the leaf blades with *Otoba*, another member of Myristicaceae. This epithet was, in part, inspired by the fact that some of the first collections of this new species were initially confused with this genus (as *Dialyanthera* Warb.; e.g. A. Gentry & S. Mori 14199, MO).

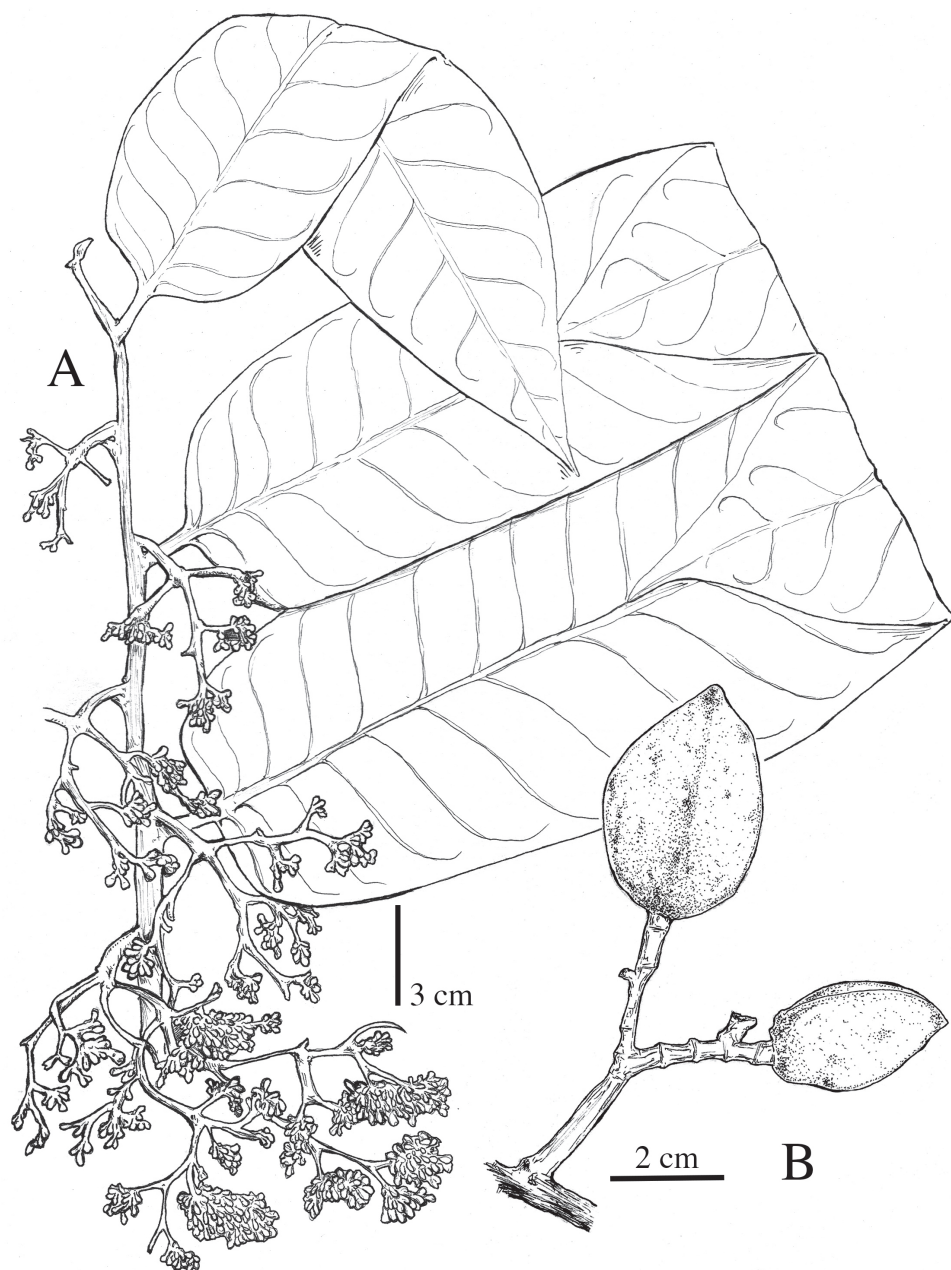


Figure 26. *Virola otobifolia* **A** branch with leaves and inflorescences **B** fruits. Drawn by Pedro Juárez, based on S. Mori & J. Kallunki 5542 (**A**) and J. P. Folsom 1440 (**B**).

Distribution. *Virola otobifolia* is only known in Panama (Colón, Panamá and San Blas), where it is found on the Caribbean slope (Fig. 27A). It has been recorded between 50–850 m elevation.

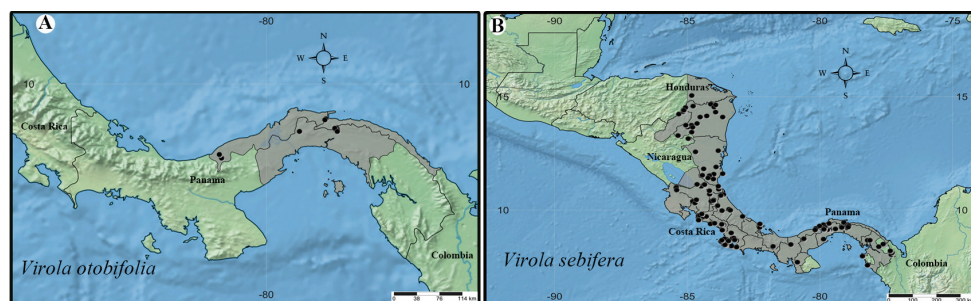


Figure 27. Geographic distribution of *Virola otobifolia* (A) and *V. sebifera* (B).

Preliminary conservation status. *Virola otobifolia* is Endangered following IUCN criteria B1a and B2a. Justifying this status, it has both a small EOO (3,269 km²) and AOO (36 km²) and is known from only five localities.

Common names. Panama: velario, miguelario; cuinur burwi, putmas (Kuna).

Phenology. *Virola otobifolia* has been recorded with flowers and fruits in February to April and one collection with fruits was made in October.

Field characters. Exudate is slow to appear and is watery and red-black. Leaf blades are whitish abaxially. Flowers have yellow perianth. Fruits are green, but appearing brown (possibly due to pubescence).

Discussion. Specimens of *Virola otobifolia* have been confused with and identified as *V. macrocarpa* (e.g. Correa et al. 2004; Condit et al. 2011) (Figs 4D, 7C), probably due to the similar size of the fruits and the leaf morphology (i.e. large discolorous leaf blades, with stellate, sessile trichomes with a reddish centre, and lateral veins that are well-separated). However, it is easily distinguished by the leaf width, pubescence on abaxial surface of the leaves, the number of lateral veins, fruit morphology and habitat; a comparative table of these two species is presented in Table 7.

Two similar species from Mesoamerica that resemble *Virola otobifolia* are: *V. allenii* (Figs 6, 7A), which occurs in the lowland wet forest on the Pacific slope of Costa Rica and *V. amistadensis* (Fig. 7B) from montane forests of Costa Rica and Panama on the Caribbean slope. Morphological comparison between these species is presented in Table 3.

Notes. The holotype, deposited at Missouri Botanical Garden (MO), represents a single collection mounted on two sheets that are clearly labelled (“Sheet 1 of 2,” “Sheet 2 of 2”) as being parts of the same specimen (ICN Art. 8.3) (Turland et al. 2018).

Specimens examined. **PANAMA. Colón:** Teck Cominco Petaquilla, 200 m elev., 21 Feb 2008 (fl bud), *G. McPherson* 20135 (MO!); [Donoso] Westernmost part of province, site of proposed copper mine (INMET), 150 m elev., 12 Apr 2009 (fr), *G. McPherson* 20905 (MO!). **Panamá:** [Chepo] El Llano Cartí road, near El Llano, [460 m elev.], 27 Mar 1976 (fr), *T. B. Croat* 33725 (MO!); Cerro Jefe, Parque Nacional Chagres, [985 m elev.], 17 Jan 2002 (fr), *N. Flores & R. Aizprúa* B3156 (MO!); [Chepo] El Llano Cartí road 18 km from Pan-American Highway, 330–370 m elev., 14 Feb 1975 (fr), *A. Gentry & S. Mori* 14199 (MO!, SCZ!*); El Llano-Cartí road, 12.7 km N from Pan American Highway, 350 m elev., 15 Feb 1975 (fl bud), *A. Gentry &*

Table 7. Morphological comparison between *Virola otobifolia* and *V. macrocarpa*.

| Characters | <i>V. otobifolia</i> | <i>V. macrocarpa</i> |
|---------------|---|---|
| Leaf blades | (14–) 18.2–42.5 × (4.1–) 7.3–14.2 cm; dense, but inconspicuously pubescent on abaxial side | 20–40 × 7–11 cm; inconspicuously puberulent on abaxial side* |
| Lateral veins | 10–16 per side, 2–4 veins per 5 cm, 1.7–3 cm apart | 14–16 per side*, 4–5 veins per 5 cm, 0.8–1.5 cm apart |
| Petiole | (1–) 1.3–2 × 0.19–0.44 cm | 1.5–2.3* × 0.18–0.23 cm |
| Fruit | (2.7–) 3.5–4.5 × (1.9–) 2.3–2.9 cm; densely tomentose, with persistent trichomes; the surface rugose when dry, the line of dehiscence conspicuously carinate. | 2.7–3.3 × 2–2.3 cm*; tomentellous, with cauducous trichomes that are dust-like when they fall; the surface smooth when dry, the line of dehiscence slightly carinate. |
| Pericarp | (2.7–) 3–4.7 mm thick | 1.8–3 mm thick |
| Habitat | Lowland or premontane rain forest, Central Panama (Panamá, San Blas) at 50–850 m elevation | Montane forests at high elevations Andes of Colombia (Boyacá) at 1100 m elevation* |

* From Smith and Wodehouse 1938 (except the pericarp).

S. Mori 14213 (MO!); below Cerro Jefe, along road to Río Pacora, 850 m elev., 09 Jan 1986 (fr), *G. McPherson* 7946 (INPA!*, MO!, PMA!*). **San Blas:** El Llano-Cartí road, 24.5–25 km from PanAmerican highway, [250–350 m elev.], 12 Apr 1975 (♂ fl), *S. Mori & J. Kallunki* 5542 (MO!); El Llano-Cartí road, 350 m elev., 01 Oct 1984 (fr), *G. de Nevers & H. Herrera* 3981 (MO!, MEXU!*, PMA!*); Cangandí, 30 m elev., 10 Feb 1986 (fr), *G. de Nevers & H. Herrera* 7056 (MO!, INPA!*, PMA!*); ibid, 10 Feb 1986 (fl bud), *G. de Nevers & H. Herrera* 7068 (MO!, INPA!*); Cangandí, 30 m elev., 27 Mar 1986 (fr), *G. de Nevers et al.* 7430 (MO-2 sheets!, PMA!*); ibid, 27 Mar 1986 (fl bud), *G. de Nevers et al.* 7448 (MO!); ibid, 27 Mar 1986 (♂ fl), *G. de Nevers et al.* 7530 (MEXU!*, MO!, PMA!*); ibid, 27 Mar 1986 (fl bud), *G. de Nevers et al.* 7605, 7607 (MEXU!*, MO!, PMA!*).

14. *Virola sebifera* Aubl.

Fig. 28

Virola sebifera Aubl. Hist. Pl. Guiane. 2: 904. 1775. Type. French Guiana. Cayenne, 1775, [*J. Aublet s.n.*, (holotype: BM!*; isotypes: NY photograph of BM holotype). Fide Jaramillo et al. 2004.

Myristica panamensis Hemsl. Biol. Cent.-Amer., Bot. 3: 67. 1882. *Virola panamensis* (Hemsl.) Warb., Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 68: 185. 1897. Syntypes: Panama. shady forest near Cruces, n.d. (fls.), *Seemann* 545 (BM!*, K!*); [Canal Area], Lion Hill station, [28 Mar 1862, leaves, excl. fr], *S. Hayes* 618 (K!*).

Virola warburgii Pittier. Contr. U.S. Natl. Herb. 18: 143. 1916. Type. Panama. Panamá, collected in forests along the Chagres River above Alhajuela, 12 May 1911 [fls], *H. Pittier* 3505 (holotype: US; isotype: BM).

Distinctive characters. *Virola sebifera* is recognised by its dense pubescence of pediculate and ferruginous trichomes (Fig. 3N) that cover almost all parts of the plant; leaf blades with few lateral veins (9–17 per side), well-spaced between them [3–4 (–5) veins per 5 cm, 1.3–2.7 cm apart] (Fig. 8N); the long staminate inflorescences that

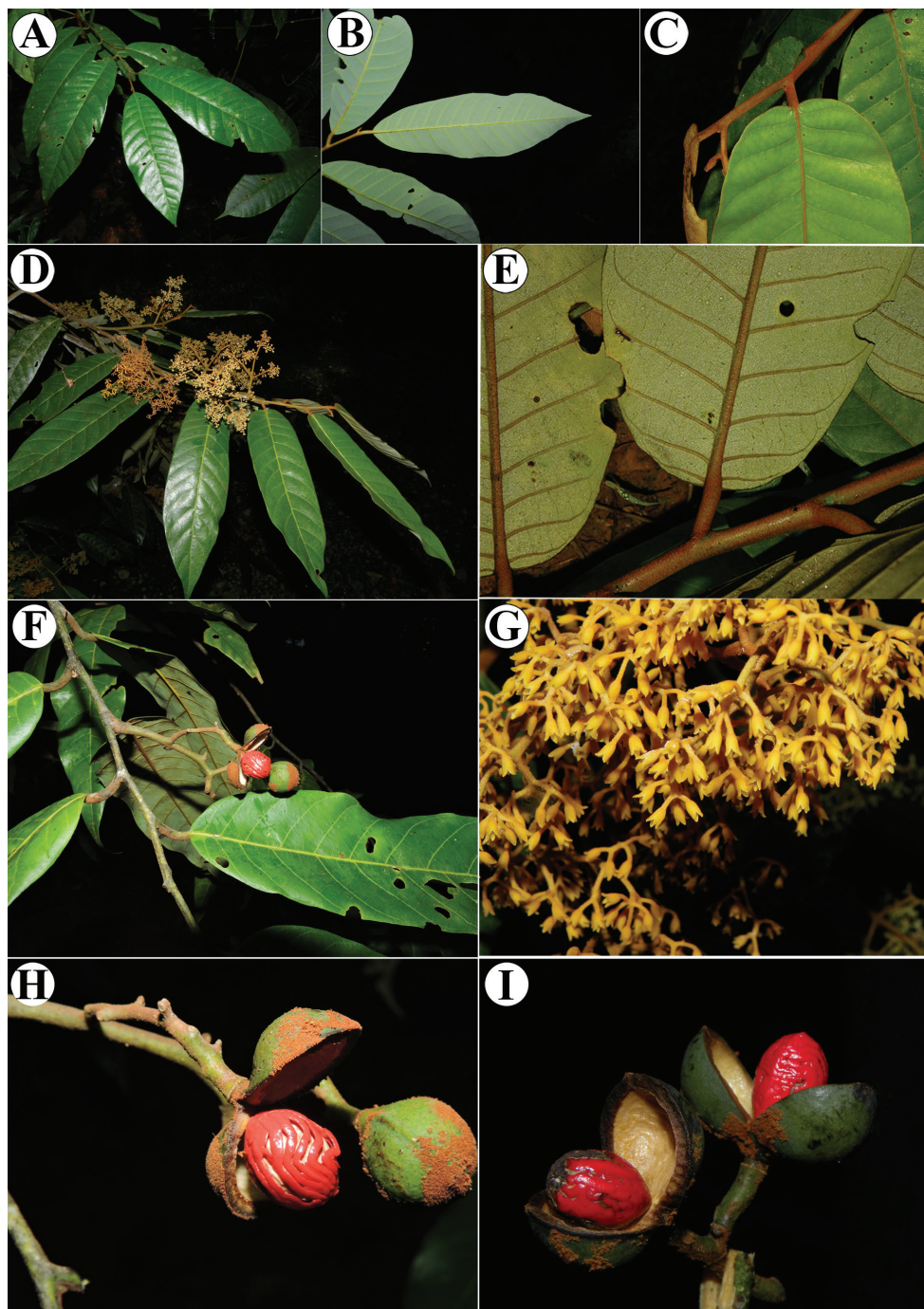


Figure 28. *Virola sebifera* **A** leaf blade on adaxial surface **B** leaf blade on abaxial surface **C**, **E** leaf base on both sides **D** flowering branch **G** staminate flowers **H** infructescence **I** fruits and aril. Photos by Reinaldo Aguilar.

are very branched; the staminate flowers with the filament column short (0.1–0.4 mm long) and the anthers more than twice the length of the column of the filaments (0.6–0.8 mm long) and apiculate at the apex; its small fruits [1.1–1.7 (–2.3) × 0.8–1.3 (–1.6) cm] that are globose to subglobose (Fig. 4K), covered by a dense layer of trichomes that fall easily and a thin pericarp [0.3–0.6 (–0.9) mm]; and the thin aril that is lacinate in narrow bands. There are also usually many fruits per infructescence.

Distribution. *Virola sebifera* is the most collected and widespread species in Mesoamerica. It is known from Honduras (Gracias a Dios), Nicaragua (Atlántico Norte, Atlántico Sur, Jinotega, Matagalpa and Río San Juan), Costa Rica (Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas and San José) and Panamá (Bocas del Toro, Chiriquí, Coclé, Colón, Darién, Herrera, Panamá, San Blas and Veraguas) (Fig. 27B). It has been recorded between 0–1000 m elevation.

Common names. Honduras: walus, banak. Nicaragua: banak, cebo, cebo macho, cebo sirio, sangre drago. Costa Rica: coton, fruta dorada, miguelario. Panama: bogamani, copidijo, fruta dorada, gorgoran, malagueta de montaña, mancha, sangre, tabegua, velario colorado.

Phenology. Herbarium specimens of flowering and fruiting individuals have been collected throughout the year. In the Osa Peninsula and Golfo Dulce, Costa Rica, flowering occurs in December to May.

Field characters. Plants are shrubs or trees between 8–25 (–40) m tall and 7–40 cm DBH. Trunks are straight, sometimes with small buttresses and with long and horizontal branches. Bark is described as dark, minutely fissured vertically or flaking off in small pieces and aromatic. Exudate from different parts of the plant is red or reddish. The leaf blades are bright green above and whitish to greyish below in living material. Flowers have brown, cream, orange, yellow-brown or yellowish perianth and are sometimes fragrant, with a lemon or sweet aroma. The mature fruit is green and covered by a dense layer of trichomes, with a white seed with a red aril.

Notes. The leaves of this species are very variable in shape and size. Sometimes, a single individual may have leaves of different sizes. Individuals with small leaf blades from Panama (e.g. *G. McPherson* 15341, MO; *G. McPherson* 20671, MO), that are sparsely pubescent and with sessile trichomes on the abaxial surface of the leaf, may be difficult to distinguish from *V. elongata*. However, specimens of *V. sebifera* tend to have thicker twigs, petioles and floriferous and fruiting peduncles.

Selected specimens seen. **HONDURAS. Gracias a Dios:** Monte Alto de Anastasio, 90 m elev., 17 Mar 1994 (♂ fl), *P. R. House* 2019 (MO!); al sur de Krausirpe, 25 Mar 1995 (♀ fl), *P. R. House* 2351 (MO!); 30 minutos al sur de Krausirpe, 11 May 1994 (fr), *P. R. House* 2814 (MO!). **NICARAGUA. Atlántico Norte:** 13 km above Kururia on road to San Jerónimo, 200 m elev., 02 Mar 1979 (♂ fl), *J. J. Pipoly* 3844 (MO!); Siuna, La Pimienta, [150 m elev.], 01 Jun 1984 (fr), *F. Ortiz* 1971 (MO!). **Atlántico Sur:** El Zapote, 40 km NE de Nueva Guinea, 130–150 m elev., 27 Feb 1984 (♂ fl), *J. C. Sandino* 4767 (MO!); Boca de Sábalo, 70 m elev., 24 Mar 1985 (♀ fl), *P. P. Moreno* 25639 (MO!). **Jinotega:** Wiwili, zona de amortiguamiento Bosawas, 170 m elev., 18 Jan 2006 (♀ fl), *I. Coronado et al.* 2949 (MO!); Wiwili, Reserva de Bosawas, 220 m elev., 09 Feb

2006 (♂ fl), *I. Coronado et al.* 3388 (MO!); Wiwili, Reserva de Bosawas, 170 m elev., 09 Jun 2007 (fr), *I. Coronado et al.* 3969 (MO!). **Río San Juan:** 2 km al NW del Río Santa Cruz, 70 m elev., 24 Feb 1984 (♀ fl), *P. P. Moreno* 23328 (MO!); El Castillo, Finca de Madrid, 100 m elev., 18 Jan 2005 (♂ fl), *R. Loredó* 2348 (MO!); entre el pueblo de San Juan del Norte Nuevo y la casa de Ramón Castillo, 0–100 m elev., 07 Jul 1994 (fr), *R. Rueda et al.* 1851 (MO!). **COSTA RICA. Alajuela:** Reserva Biológica de Monteverde, río Caño Negro, 800 m elev., 19 Oct 1989 (fr), *E. Bello* 1402 (MO!); Upala, Estación San Ramón, 550 m elev., 16 Mar 1993 (♂ fl), *R. Espinoza* 803 (MO!); San Carlos, Fortuna, 255–400 m elev., 27 May 2004 (fr), *A. Rodríguez* 9046 (MO!). **Cartago:** Turrialba, El Zapote Nature Reserve, 700 m elev., 01 Mar 1987 (imm fr), *W. Burger & J. Gómez-Laurito* 12030 (CR!, MEXU!*). **Guanacaste:** Finca La Pasmompa, 400 m elev., 10 Dec 1990 (fl bud), *C. Moraga* 227 (MO!). **Heredia:** Tirimbina, Istarú farm, 220 m elev., 28 Jan 1972 (♂ fl), *R. W. Lent* 2329 (MO!); Finca La Selva, [90 m elev.], 09 Jul 1984 (fr), *D. Smith* 1088 (MO!). **Limón:** Parque Nacional Barbilla, 300–400 m elev., 22 May 2000 (fl bud), *E. Mora* 1155 (MO!); Cerro Coronel, 20–170 m elev., 16–23 Jan 1986 (♂ fl), *W. D. Stevens* 23823 (MO!). **Puntarenas:** Golfito, Mata Palo, 160–200 m elev., 09 Sep 1991 (fr), *R. Aguilar* 363 (MO!); Golfito, 1 km antes de llegar a la Palma, 8 m elev., 16 Jan 1993 (♂ fl), *R. Aguilar* 1586 (MO!); Punta Burica, 0–100 m elev., 22 Aug 1988 (fr), *M. M. Chavarria et al.* 292 (MO-2 sheets!). **San José:** Puriscal, San Martín, 800 m elev., 20 Nov 1993 (fr), *J. F. Morales* 2022 (MO!); vicinity of El General, 915 m elev., n.d. May 1937 (♂ fl), *A. F. Skutch* 3118 (MO!); Turrubares, San Rafael, 600–700 m elev., 08 Dec 2004 (fr), *A. Soto & D. Santamaría* 409 (MO!). **PANAMA. Bocas del Toro:** Isla Bastimentos, Bocatorito, 30 m elev., 15 Feb 1989 (♀ fl), *P. M. Peterson & C. R. Annble* 6872 (MO!). **Chiriquí:** 17 km NE of San Felix, 1000 m elev., 18–19 Mar 1974 (fr), *M. Nee* 10664 (MO!). **Coclé:** Caribbean side of divide at El Copé, 200–400 m elev., 04 Feb 1983 (fr), *C. Hamilton & G. Davidse* 2756 (MO!). **Colón:** Area near Guasimo, no elev., 22 Apr 1970 (♂ fl), *T. B. Croat* 9950 (MO!); Santa Rita ridge, 500 m elev., 13 Jan 1987 (fr), *G. McPherson* 10265 (MO!). **Darién:** Vicinity of Cana, 1750 ft [530 m] elev., 24 Jun 1959 (♂ fl), *W. L. Stern* 512 (MO!); serranía de Sapo, 300–800 m elev., 26 Nov 1990 (fr), *H. Herrera & J. Polanco* 793 (MO!). **Herrera:** Road between Las Minas and El Toro, 700–800 m elev., 23 Jan 1987 (fr), *G. McPherson* 10273 (MO!). **San Blas:** El Llano Cartí road, 350 m elev., 06 May 1985 (fr), *G. de Nevers* 5609 (MO!). **Panamá:** Barro Colorado Island, [10–100 m elev.], 13 Dec 1967 (fr), *T. B. Croat* 4275 (MO!); along Pipeline Road, 125 m elev., 10 Mar 1974 (♂ fl), *M. Nee* 10445 (MO!); Llano Cartí road, 350 m elev., 26 Jan 1986 (fr), *G. McPherson & M. Merello* 8148 (MO!). **Veraguas:** Cerro Hoya, subiendo por Cobachón, [750 m elev.], 21 Apr 1997 (fl), *J. Deago* 499 (PMA!*).

Conclusions

In this synopsis of Mesoamerican *Virola*, 14 species are recognised. Twelve of these are restricted to Mesoamerica, while the other two species extend into South America. Six of the treated species are new; these are all from Costa Rica and Panama, countries with

the highest species diversity of *Virola*, with 10 and 11 species, respectively. We clarified the application of some names, the resurrection of others and the distribution of some species previously considered to occur in Mesoamerica (e.g. South American *V. macrocarpa*) or South America (e.g. the Mesoamerican *V. multiflora*). This has resulted in a much modified taxonomy for *Virola*, summarised here: Mesoamerican collections previously identified as *V. calophylla* and/or *V. macrocarpa* are here interpreted as three new species: *V. alleni*, *V. amistadensis* and *V. otobifolia*, while Mesoamerican specimens previously determined as *V. surinamensis* are treated as *V. nobilis*. As a result of this, *V. calophylla* and *V. macrocarpa* are now restricted to South America and *V. surinamensis* to South America and the Antilles. Additionally, two new species are identified within what was previously *V. guatemalensis*: montane collections from the southern range now belong to *V. montana* and we have resurrected *V. laevigata*, previously considered a synonym. Under our concept, *Virola guatemalensis* has a restricted range, found only in northern Mesoamerica. Finally, the majority of *V. koschnyi* specimens from the Pacific coast of Costa Rica and Panama correspond to *V. chrysocarpa*, while most of Panama's collections, identified as *V. multiflora*, are now considered *V. fosteri*.

These contributions came to light via detailed comparisons across the complete range of these species thanks to hundreds of collections made by numerous botanists and herbaria who care, maintain and provide access to the specimens, including making them available online.

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Supplementary material I

List of exsiccatae

Authors: Santamaría-Aguilar Daniel, Aguilar Reinaldo, Lagomarsino Laura Penolope

Data type: specimens data

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Defining phylogenetic relationship of *Nepeta* × *tmolea* and its parents via DNA barcoding

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Abstract

Nepeta viscida and *N. nuda* subsp. *nuda* and *N.* × *tmolea* were examined in this study. Mainly fresh leaf pieces, dried with silica grains, were used for DNA extraction procedures via DNA isolation kits. Standard PCR techniques were executed using three different primer sets (one nuclear DNA region (nrITS) and two chloroplast DNA regions (*rpL32-trnL* and *trnA(Leu)-trnA(Phe)-trnL-F*). DNA sequences were analysed and evaluated using different molecular approaches and software. Consequently, the inconstant molecular structure and hybrid nature of *N.* × *tmolea* specimens were shown and interpreted in this study. According to our result, *N.* × *tmolea* have some intermediate characters compared to its parents. nrITS data give more information phylogenetically, and also the most polymorphic loci are seen in nrITS data. Morphological and molecular data contribute to define separation of *N.* × *tmolea*. Consequently, the inconstant molecular structure and hybrid nature of *N.* × *tmolea* specimens were shown and interpreted in this study.

Keywords

hybrid, molecular, *Nepeta*, phylogeny, Turkey

Introduction

Lamiaceae family – the mint family – members are well known for their medicinal and aromatic properties in the pharmaceutical industry. The *Nepeta* L. genus is mainly native to Europe, Western Siberia, Far East and North Asia and consists of approximately 300 species with its being one of the largest genera in Lamiaceae (Pojarkova 1954; Hedge 1986; Jamzad et al. 2000, 2003b; Tzakou et al. 2000; Mojab et al. 2009). In

recent studies, Turkish *Nepeta* members have been represented by 44 species. Twenty-two of these species are endemic to Turkey (Aytaç and Yıldız 1996; Güner et al. 2000; Dirmenci 2003) with the distribution areas of the species being mainly in east Anatolia and the Taurus Mountains in Turkey (Dirmenci 2005). *Nepeta nuda* L. subsp. *nuda* is a widespread and well-known subspecies of *N. nuda* in Turkey with its distinguishing characters of violet-blue calyx and corolla (Hedge and Lamond 1982; Dirmenci 2003). *Nepeta nuda* subsp. *nuda* and *N. viscida* Boiss. are members of Group A, according to the Flora of Turkey classification (Hedge and Lamond 1982; Dirmenci 2003). *Nepeta viscida* is readily separated from *N. nuda* subsp. *nuda* by its viscous glandular trichomes and general habit.

It is mentioned in the Flora of Turkey that *N. viscida* hybridises with *N. nuda* in overlapping areas and forms the hybrid described as *N. × tmolea* Boiss. (Hedge and Lamond 1982). In the field trips during this study, we found some *N. nuda* subsp. *nuda* and *N. viscida* individuals that reflect their typical characters. Some individuals had, however, some intermediate morphological characters: they were not viscid and their stem, leaf and corolla colours were quite different from *N. nuda* subsp. *nuda* and *N. viscida*. Thus, we recognised these specimens as *N. × tmolea*. Some *N. × tmolea* hybrid individuals were more similar to *N. viscida* in terms of general habits, calyx and leaf characters; on the other hand, some samples were more similar to *N. nuda* subsp. *nuda* in terms of their bluish colour on the verticillasters and their having no adhesive glandular trichomes.

According to literature, trichome types, density, presence/absence etc. are very important characters for identifying different taxa in the Lamiaceae family (Husain et al. 1990; Ecevit-Genç et al. 2015, 2017, 2018; Krawczyk and Głowacka 2015; Sajna and Sunojkumar 2018) and, of course, the genus *Nepeta* (Kolalite 1988; Dirmenci 2003; 2005; Kaya et al. 2007; Açar et al. 2011; Yarmooammadi et al. 2017; Özcan 2019). Additionally, it is mentioned in the studies that, although the type and density of trichomes are distinctive amongst species, they can vary in different organs of the same individual.

DNA barcoding methods have been frequently used in differentiating taxa in recent years (Hebert et al. 2003). Specimens can be separated by obtaining a standard DNA region using a very small sample (Kress and Erickson 2007). According to Jamzad et al. (2003a), nuclear ITS DNA sequences are correlated with some morphological characters and, thus, this region can be helpful in defining the phylogenetic positions of the *Nepeta* species. Molecular approaches are also used to reveal heterozygotic and polymorphic structures of some hybrid taxa belonging to the Lamiaceae family in literature (Bariotakis et al. 2016; Kokubugata et al. 2011; Jedrzejczyk 2018; Dirmenci et al. 2018a, 2018b, 2019a). Some Single Nucleotide Polymorphisms (SNPs), which are the most common type of genetic variation among plants and meaning replacing of a nucleotide (i.e. C) to another (i.e. T) in a certain stretch of DNA, were identified in this study.

This research aimed to reveal the phylogenetic relationships and heterozygous DNA structure of *Nepeta nuda* subsp. *nuda*, *N. viscida* and their hybrid *N. × tmolea*. The internal transcribed spacers of nuclear ribosomal DNA (nrITS), *trnL-F* and *rpl32* regions from chloroplast DNA were examined to define heterozygosity of DNA sequences amongst parents and hybrid specimens.

Materials and methods

Plant materials

The different individuals of *N. viscida*, *N. × tmolea* and *N. nuda* subsp. *nuda* were collected during the field trips (2016–2018) from their natural habitats in Balıkesir (Dursunbey-Çamlık) (Fig. 1), İzmir (Ödemiş-Bozdağ) and from Kütahya in 2002. Voucher specimens are deposited in the Herbarium of Necatibey Education Faculty of Balıkesir University in Balıkesir, Turkey.

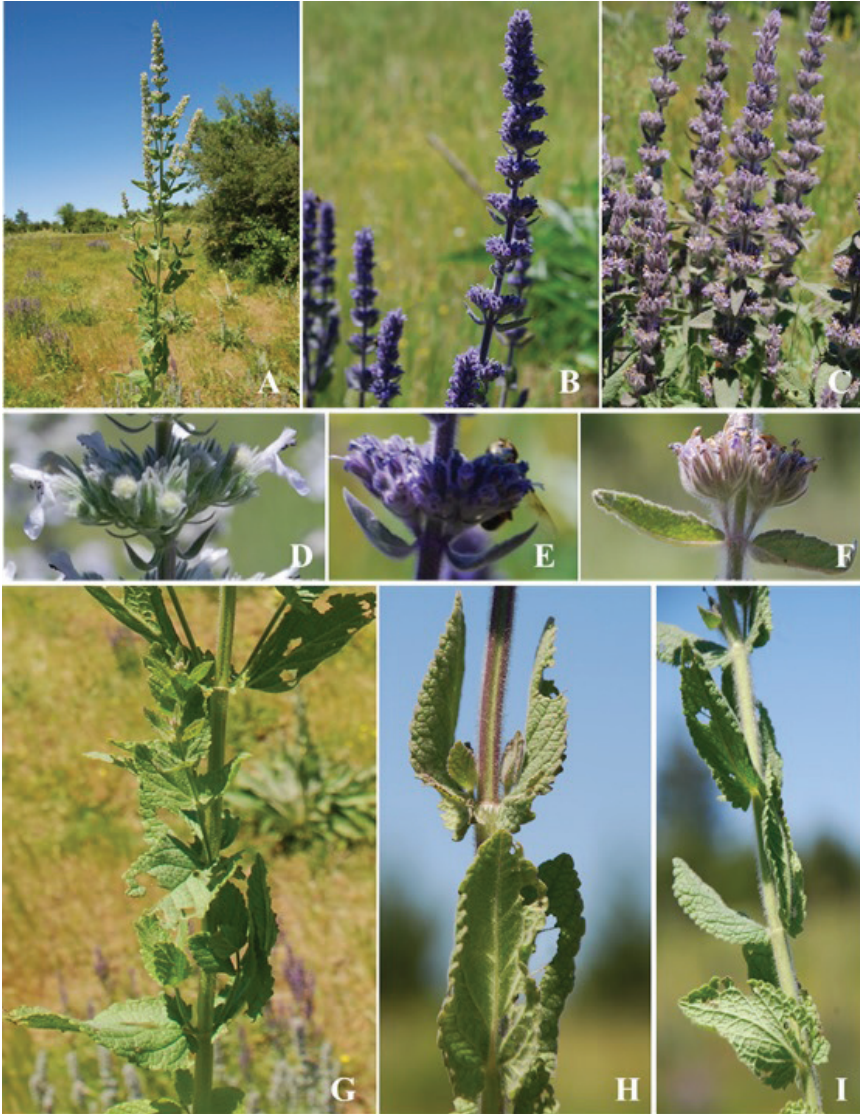


Figure 1. General habit, inflorescence and lower parts of *N. nuda* subsp. *nuda* (A, D, G); *N. × tmolea* (B, E, H) and *N. viscida* (C, F, I).

DNA isolations

DNA isolations were performed using the DNeasy Plant Mini Kit (QIAGEN, Germany), following the manufacturer's instructions with some modifications. Eight different *N. × tmolea* specimens and different specimens of *N. viscida* and *N. nuda* subsp. *nuda* were used for DNA isolations. Taxon name, voucher number and localities are given in Table 1.

PCR amplification

In this study, molecular analyses of *N. × tmolea*, *N. viscida* and *N. nuda* subsp. *nuda* were carried out using three different DNA regions: the nuclear internal transcribed spacer (nrITS), trnA (Leu)-trnA (Phe) (trnL-F) and rpl32-trnL regions of the chloroplast DNA (cpDNA). PCR amplification of the ITS nrDNA were performed using ITS5a (5'-CCT TAT CAT TTA GAG GAA GGA G-3') (Stanford et al. 2000) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990) primers. The rpl32- trnL cpDNA amplifications were performed using rpl32-F (5'-CAG TTC CAA AAA AAC GTA CTT C-3') (Shaw et al. 2007) and trnL (UAG) (5'-CTG CTT CCT AAG AGC AGC GT-3') (Shaw et al. 2007) primers and the trnL-F amplifications were performed with trnL-c (5'-CGA AAT CGG TAG ACG CTA CG-3') (Taberlet et al. 1991) and trnL-f (5'-ATT TGA ACT GGT GAC ACG AG-3') (Taberlet et al. 1991) primers.

DNA data analysis

The PCR products that were successfully amplified and checked on agarose gel were sent to Genoks (Gene Research and Biotechnology Company, Turkey) for sequencing. Raw sequenced DNA data files were edited via Sequencer version 5.4 (Gene Codes Corporation, Ann Arbor, MI, USA) and edited sequences were aligned using Bioedit 7.2.5 (Hall 1999). The polymorphic sequence loci and heterozygous structure of *N. viscida*, *N. × tmolea* and *N. nuda* subsp. *nuda* were identified and polymorphisms of these specimens were demonstrated by selected software. Successfully sequenced specimens were given in Table 1. Phylogenetic cladograms were constructed using PAUP* 4.0a165 (Swofford 2003) and Dendroscope (Huson and Scornavacca 2012), and a Neighbour-Net split graph was conducted using SplitsTree 4.14 (Huson and Bryant 2006). A data matrix was constructed according to discriminative characters belonging to *rpl32* DNA data. In addition, discriminant analysis was carried out with PAleontoSTatistics (PAST) (Hammer et al. 2001) to show the position of individuals in these studies.

Table 1. Voucher information of *Nepeta* species examined for DNA extractions.

| Taxon | Voucher number | Locality |
|--------------------------------------|----------------|---|
| <i>N. viscida</i> | 4759 | Balıkesir: Dursunbey, Alaçam Mount., Gölcük, around Karaveli Hill, 39.42650N, 28.53057E, 4970 ft alt., 19/06/2017. |
| | 4762 | Balıkesir: Dursunbey, Alaçam Mount., Sokuldak area, 39.43673N, 28.51373E, 4790 ft alt., 19/06/2017. |
| | 4766 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 4768 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 5024 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42625N, 28.53123E, 4930 ft alt., 11/06/2018. |
| | 5027 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42682N, 28.52975E, 4960 ft alt., 11/06/2018. |
| | 5030 | Balıkesir: Dursunbey, Alaçam Mount., Sokuldak area, 39.43662N, 28.51364E, 4780 ft alt., 11/06/2018. |
| <i>N. × tmolea</i> | 4758 | Balıkesir: Dursunbey, Alaçam Mount., Gölcük, around Karaveli Hill, 39.42650N, 28.53057E, 4970 ft alt., 19/06/2017. |
| | 4761 | Balıkesir: Dursunbey, Alaçam Mount., Sokuldak area, 39.43673N, 28.51373E, 4790 ft alt., 19/06/2017. |
| | 4765 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 4770 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 5023 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42625N, 28.53123E, 4930 ft alt., 11/06/2018. |
| | 5026 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42682N, 28.52975E, 4960 ft, 11/06/2018. |
| | 5029 | Balıkesir: Dursunbey, Alaçam Mount., Sokuldak area, 39.43662N, 28.51364E, 4780 ft alt., 11/06/2018. |
| | 1073 | Balıkesir: Dursunbey, above Tahtalık Hill, 5413 ft alt., 07/05/2000. |
| <i>N. nuda</i> subsp. <i>nuda</i> | 4757 | Balıkesir: Dursunbey, Alaçam Mount., Gölcük, around Karaveli Hill, 39.42650N, 28.53057E, 4970 ft alt., 19/06/2017. |
| | 4764 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 4769 | İzmir: Ödemiş, around Bozdağ ski resort, 20/06/2017. |
| | 5021 | Balıkesir: Dursunbey, Alaçam Mount., Soğucak area, 39.45649N, 28.53786E, 3818 ft alt., 11/06/2018. |
| | 5022 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42625N, 28.53123E, 4930 ft alt., 11/06/2018. |
| | 5025 | Balıkesir: Dursunbey, Alaçam Mount., around Karaveli Hill, 39.42682N, 28.52975E, 4960 ft alt., 11/06/2018. |
| | 5028 | Balıkesir: Dursunbey, Alaçam Mount., Sokuldak area, 39.43662N, 28.51364E, 4780 ft alt., 11/06/2018. |
| | 1940 | Kütahya: Radar road, 3935–4920 ft alt., 07/10/2002. |

Results

N. viscida is easily morphologically distinguished from *N. nuda* subsp. *nuda* by its sticky stem and leaves. These sticky structures, resulting from viscous glandular trichomes, are highly characteristic for *N. viscida* in *Nepeta* genus (Dirmenci 2003; Özcan 2019). Although this morphological feature is very significant, *N. viscida* and *N. nuda* subsp. *nuda* taxa are classified under Group A (Hedge and Lamond 1982; Dirmenci 2003). According to the Flora of Turkey, *N. viscida* and *N. nuda* belong to Group A along with *N. cataria* L., *N. isaurica* Boiss. & Heldr. ex Benth. and *N. caeserea* Boiss. Özcan (2019)

stated that *N. × tmolea* is quite different from its parents but micromorphologically more similar to *N. nuda* subsp. *nuda*. The indumentum is a distinctive character for distinguishing *N. viscida* and *N. nuda* subsp. *nuda*. As with many hybrids (Clevinger and Panero 2000; Baumel et al. 2002; Lowe and Abbott 2004; Liu et al. 2017; Szczecińska et al. 2017; Jąźwa et al. 2018; Van Valkenburg et al. 2018; Dirmenci et al. 2019b), *N. × tmolea* hybrid individuals show transition/intermediate characteristics between *N. viscida* and *N. nuda* subsp. *nuda*. However, some specimens of *N. × tmolea* have higher similarities to *N. nuda* subsp. *nuda*, while others have high morphological similarities to *N. viscida*. Namely, *N. × tmolea* is distinguished from *N. nuda* subsp. *nuda* by its bracteoles 5–10 mm (not 2.5–4 mm), calyces 6–9 mm (not 3.5–4 mm), calyx teeth 3.5–5 mm (not 1.5–2 mm), corolla 7.5–10 mm (not 5.5–6.5 mm). Also, it differs from *N. viscida* by its bracteoles 5–10 mm (not 8.5–11 mm), calyces 6–9 mm (not 8.5–12 mm), calyx teeth 3–5 mm (not to 6.5 mm), and corolla 7.5–10 mm (not 9–13 mm).

We used three different loci, one nuclear DNA loci-nrITS- and two DNA loci from chloroplast genome-*rpl32-trnL* and *trnA(Leu)-trnA(Phe)*- in this study.

Nuclear DNA data

In total, 21 taxa were sequenced for the ITS sequence matrix. In the parsimony heuristic search, consistency, retention and homoplasy indices were identified as 0.75, 0.78 and 0.25, respectively. According to Fig. 2, *N. viscida* and *N. nuda* subsp. *nuda* are sister taxa and belong to the same clade in comparison to other Group A members with a strong bootstrap value (86). When Fig. 1B is analysed, the *N. viscida*-*N. nuda* subsp. *nuda* group has a close relationship with *N. kurdica* Hausskn. & Bornm., *N. fissa* C.A.Mey, *N. scrophularioides* Rech.f. and *N. lamiifolia* Adam ex G.F.Hoffm. When the nrITS sequences of *N. × tmolea* and its parents are compared, *N. × tmolea* has 8 single nucleotide polymorphisms (Table 2). As mentioned above, *N. × tmolea* has some intermediate characters between its parents, such as leaf size and indumentum density, and our DNA data contribute further with the morphological characters. *N. nuda* subsp. *nuda* (1940) and *N. nuda* subsp. *nuda* (4764 and 4769) individuals (distributing in Ödemiş, see Table 1) differed the specimens from Balıkesir-Dursunbey (4757 and 5021). Thus, nrITS data also gave us intra-individual differentiations.

All the nrITS DNA data included 594 characters; 579 of 594 characters were constant, 6 variable characters were parsimony uninformative and 9 of the rest were parsimony informative (Table 2). *Nepeta viscida* 5024-4, 5024-2 and 5030-1 specimens have different nucleotides at the nucleotide positions of 11, 353, 420, and 462 in comparison to *N. viscida* 5024-1, 5030-3 and 5024-3 specimens, which are distributing in the same location (Dursunbey). In addition, the most heterozygous individual, *N. viscida* 5024-3 has heterozygote nucleotide polymorphisms at positions 355, 420 and 462. The most polymorphic locus is seen at position 421 (C-T nucleotide heterozygous - in bold characters) for all the specimens. On the other hand, all the examined taxa have polymorphic loci, according to nrITS data. These heterozygote sequences may be the result of continuous crossing between *N. viscida* and *N. nuda* subsp. *nuda*

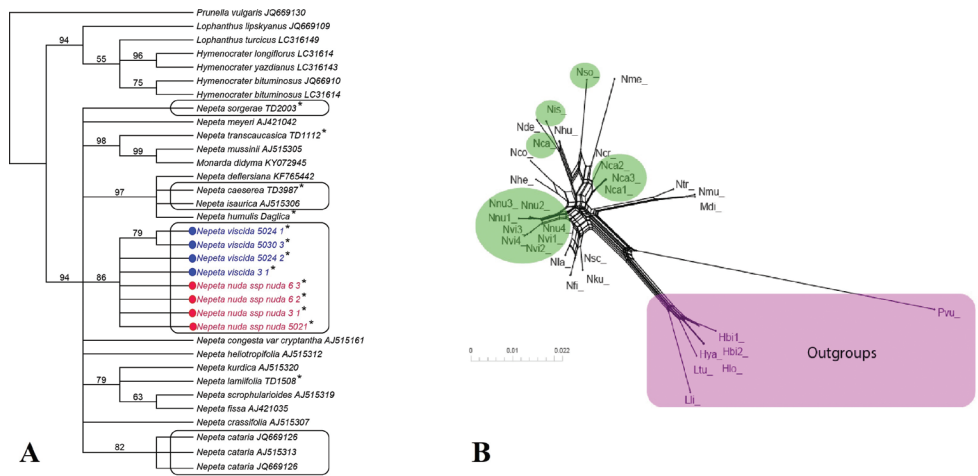


Figure 2. Phylogenetic position of *N. viscida* and *N. nuda* subsp. *nuda* amongst different *Nepeta* species and outgroups (based on nrITS sequences and Maximum Likelihood phylogram (A) and Neighbour-Net Diagram (B) without hybrids.(* examined taxa in this study).

Table 2. Separated loci of *N. viscida*, *N. nuda* subsp. *nuda* and *N. × tmolea* based on nrITS data.

| | 1 | 4 | 1 | 3 | 4 | 4 | 4 | 4 | 5 |
|--|---|---|---|-----|-----|-----|-----|-----|---|
| | 1 | 3 | 2 | 5 | 2 | 2 | 6 | 7 | 3 |
| | | | 0 | 5 | 0 | 1 | 2 | 4 | 1 |
| <i>Nepeta viscida</i> 5024 4 | C | C | T | T | G | T | T | G | C |
| <i>Nepeta viscida</i> 5024 2 | C | C | G | T | G | C/T | T | G/T | C |
| <i>Nepeta viscida</i> 5030 1 | C | C | G | T | G | C/T | T | T | C |
| <i>Nepeta viscida</i> 5024 1 | A | C | G | A | T | T | A | G | C |
| <i>Nepeta viscida</i> 5030 3 | A | A | G | A | T | T | A | G | C |
| <i>Nepeta viscida</i> 5024 3 | A | C | G | A/T | G/T | T | A/T | G | C |
| <i>Nepeta viscida</i> 4759 | A | C | G | A/T | G | T | T | G | C |
| <i>Nepeta × tmolea</i> 4758 | A | C | G | A | G | C/T | A/T | G | T |
| <i>Nepeta × tmolea</i> 5023 3 | C | C | T | T | G | T | T | G | C |
| <i>Nepeta × tmolea</i> 5023 2 | C | C | T | T | G | T | T | G/T | C |
| <i>Nepeta × tmolea</i> 4761 | C | C | G | T | G | C/T | T | G/T | C |
| <i>Nepeta × tmolea</i> 1073 | C | A | G | T | G | C/T | T | T | C |
| <i>Nepeta × tmolea</i> 5029 2 | C | A | G | T | G | C/T | T | T | C |
| <i>Nepeta nuda</i> subsp. <i>nuda</i> 5021 | C | C | T | T | G | T | T | G | C |
| <i>Nepeta nuda</i> subsp. <i>nuda</i> 4757 | C | C | G | A/T | G | T | A/T | G | T |
| <i>Nepeta nuda</i> subsp. <i>nuda</i> 1940 | C | C | G | A/T | G | T | A/T | G | T |
| <i>Nepeta nuda</i> subsp. <i>nuda</i> 4769 | C | C | G | T | G | C/T | T | G/T | C |
| <i>Nepeta nuda</i> subsp. <i>nuda</i> 4764 | C | A | G | A/T | G | C/T | T | G/T | C |

and backcrossing amongst the parents and *N. × tmolea*. Additionally, it can be seen from the Table 1 that, (* not only studied *N. viscida* members (7 specimens), but also *N. nuda* subsp. *nuda* (5 specimens) members have heterozygous structures, not only constant characters, at the given nucleotide positions.

According to nrITS sequences, different *N. × tmolea* specimens are classified with different parents (Fig. 3). Five main clades can be seen in Fig. 2. Two parents and their

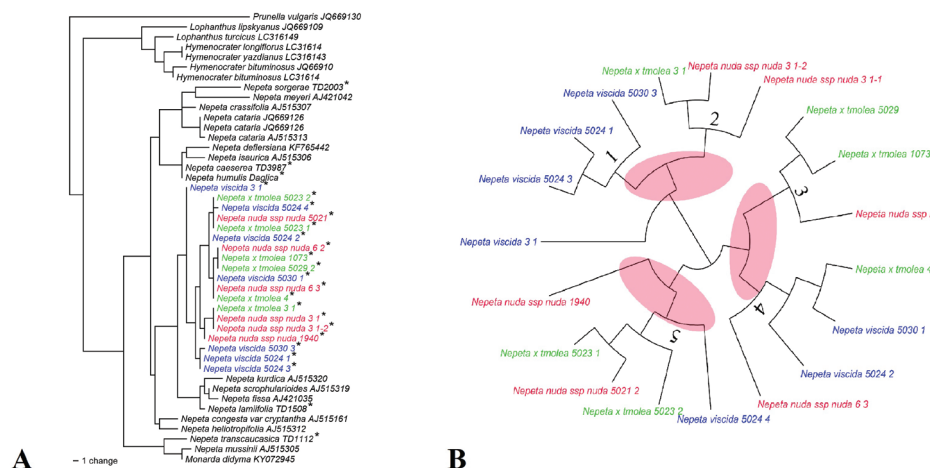


Figure 3. Phylogenetic relationship of *N. viscida*, *N. nuda* subsp. *nuda* and *N. × tmolea* with some *Nepeta* members and outgroups (based on nrITS sequences and Neighbour Joining phylogram **(A)** and Dendroscope diagram **(B)**. (* examined taxa in this study).

putative hybrid specimens share the same clade at clades 4 and 5, *N. nuda* subsp. *nuda* and *N. × tmolea* are more similar at clades 2 and 3. Therefore, it can be considered that the phylogenetic position of *N. × tmolea* is not constant and that ancestral species show transitions in different clades. When the hybrid individuals are not included in the phylogenetic analysis, *N. viscida* and *N. nuda* subsp. *nuda* tend to be closer to individuals of their own species, but ancestral species are divided into different clades after adding hybrid sequences in the analysis.

Chloroplast DNA data

rpl32-trnL and *trnL-F* DNA regions were examined from the chloroplast genome. The longest data of studied regions were obtained from *rpl32-trnL* sequences. A total of 891 nucleotides were obtained from 29 specimens belonging to the parents and hybrid taxa; 855 of 891 characters were constant and parsimony-informative characters were 31. On the other hand, 847 characters were obtained from 32 specimens belonging to the parents and hybrid taxa, 833 of which were constant and 10 characters of the rest of the sequences were parsimony-informative for the *trnL-F* region.

When we analyse Fig. 4, the phylogenetic tree and PCA diagram show us the transition amongst the species and hybrid individuals. This means that neither *N. viscida* nor *N. nuda* subsp. *nuda* specimens are monophyletic. Some clades have only one putative ancestor and hybrid and some of them have parents and hybrid taxa. These three taxa are mixed together and grouped at different clades in the cladogram (Fig. 4A) or at different regions in the PCA diagram (Fig. 4B). In addition, three *N. × tmolea* samples have completely similar DNA sequences with three *N. nuda* subsp. *nuda* samples and this can also be seen from the PCA diagram (with black arrows) (Fig. 4B).

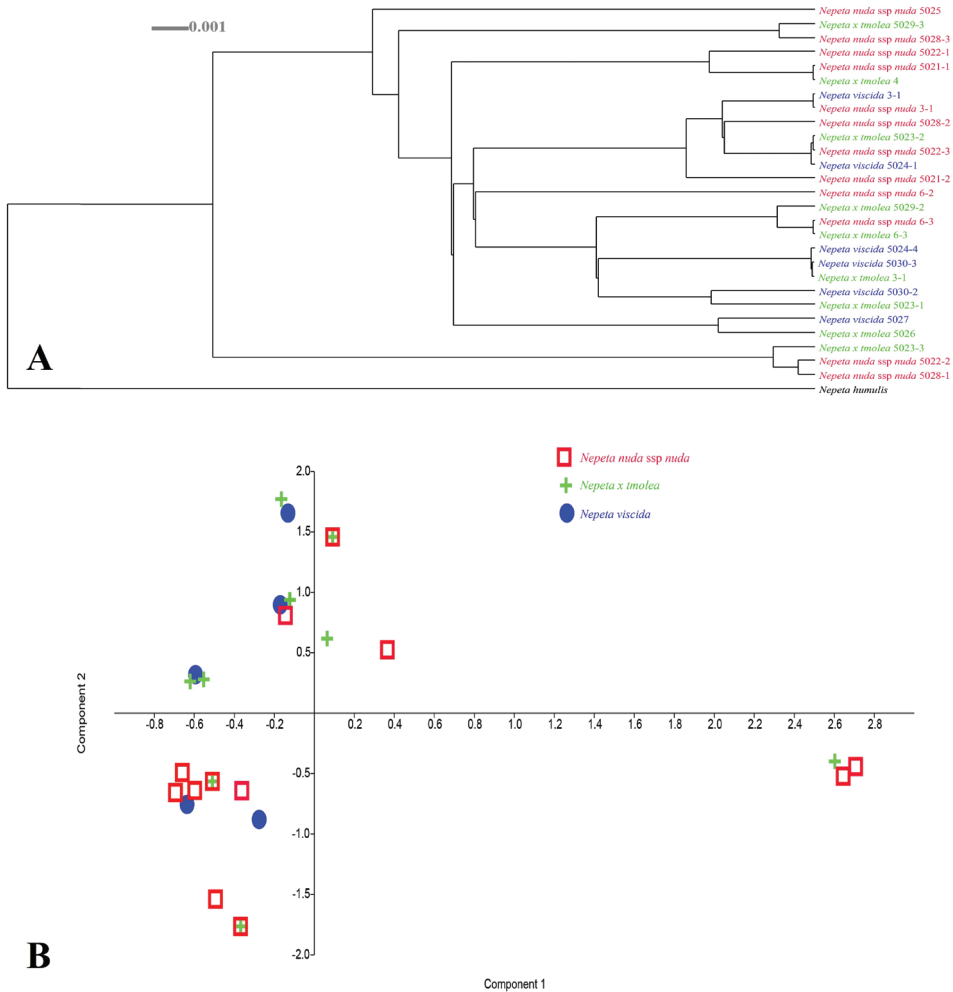


Figure 4. Dendroscope cladogram and PCA diagram based on *rpl32-trnL* data.

Single nucleotide polymorphisms (SNPs) were mostly seen in *rpl32* data. G-T polymorphisms at positions 22, 41, 135, A-G polymorphisms at positions 24, 160, 311, A-C polymorphisms at positions 45, 331, 334 and C-T polymorphism at position 758 are significant for distinguishing specimens. Insertion-deletion sites are very significant, especially at the nucleotide positions between 140–150, 312–314, 325–328, 340–353, 603–608 and the longest one between positions 764–810 (Fig. 5).

trnL-F has also some SNPs at the nucleotide positions of 244, 596 and 696. Insertion-deletion (I-D) sites in *trnL-F* data are shorter than *rpl32* data. There are three parsimony-informative I-D regions around the nucleotides 260, 410 and 600 (Fig. 6). Unfortunately, insertion or deletion sites were not parsimony informative for our finding out phylogenetic position of the species.

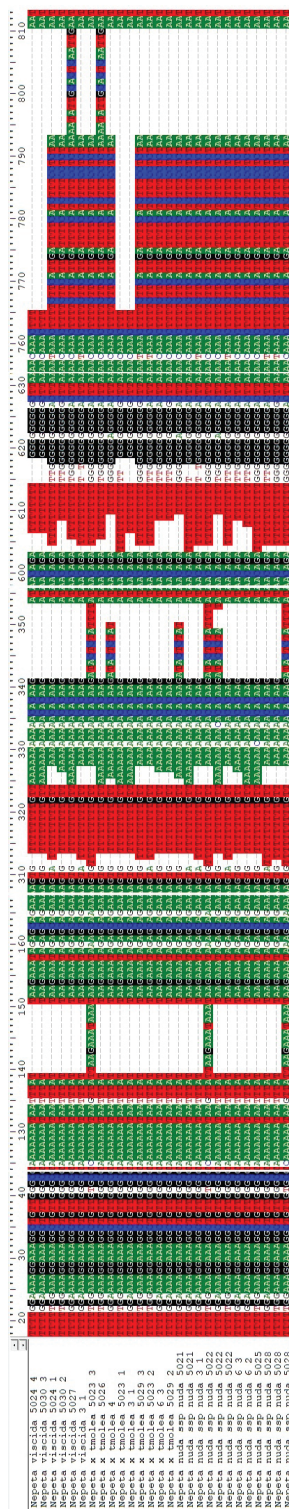


Figure 5. Insertions, deletions and single nucleotide polymorphisms based on *rpl32-trnL* sequences.

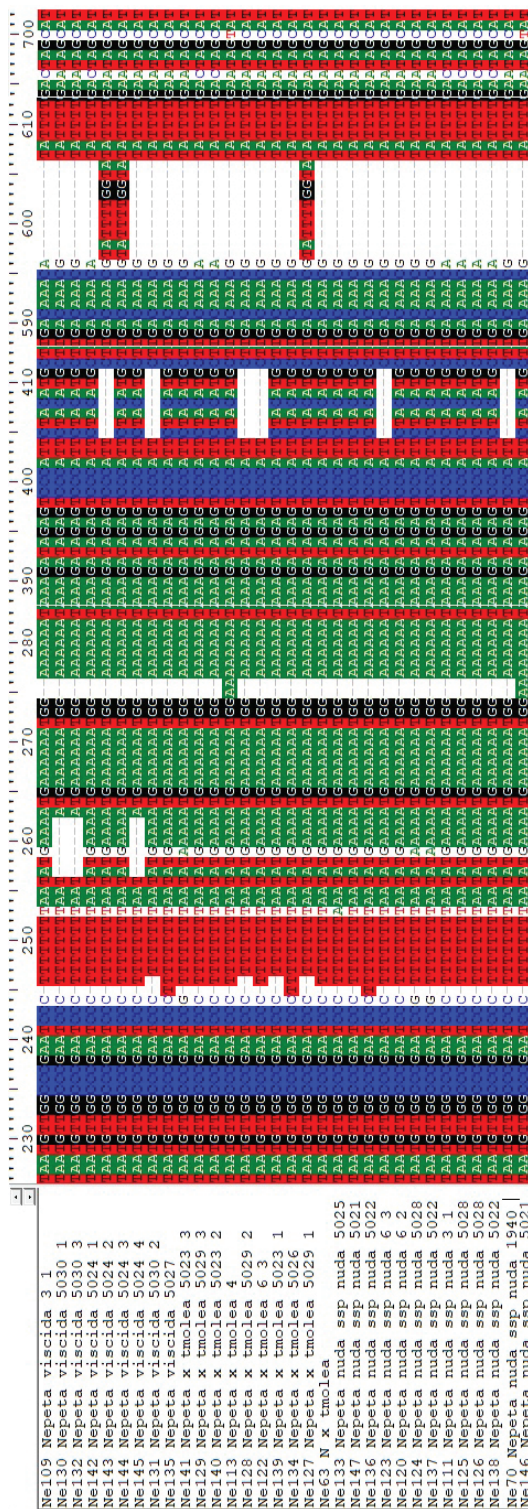


Figure 6. Insertions, deletions and single nucleotide polymorphisms based on *trnL-F* sequences.

Conclusions

Possible hybridisation between *N. nuda* subsp. *nuda* and *N. viscida* was estimated by Boissier (1859) for the first time but *N. × tmolea* was not presented as a hybrid. According to morphological studies, although general habitus, calyx and leaf characters of *N. × tmolea* are more similar to *N. viscida*, its indumentum (especially glandular trichome) is very different and separated. Molecular data overlaps with morphological data. As in the morphological data, hybrid individuals have intermediate characters in DNA sequences, and these characters occur as polymorphic loci.

DNA sequences, especially nrITS data, have been used by many scientists to discover the phylogenetic position and relationship of numerous species in literature. In this study, nrITS gave information about SNPs and *rpl32-trnL* and *trnL-F* were used to specify the parents' taxa *N. × tmolea*. Having some polymorphic loci of *N. nuda* subsp. *nuda* (Table 2) has probably caused introgression. Hybrid forming areas (Dursunbey and Ödemiş districts) of *N. nuda* subsp. *nuda* and *N. viscida* are mostly contacted and formed *N. × tmolea*. In these hybrid swarm regions, *N. × tmolea* individuals possibly do backcrossing with its parents. Additionally, because of this backcrossing, some *N. nuda* subsp. *nuda* specimens have different nucleotides from the other *N. nuda* subsp. *nuda* samples which are the original parental individuals. According to literature, while chloroplast DNA gives us information about maternal or paternal inheritance, this study did not provide a completely reasonable result based on *rpl32-trnL* and *trnL-F* data.

In addition, we could not see logical clustering among the specimens growing in the same location (Dursunbey or Ödemiş), and nrITS data also gave us intra-individual differentiations of *N. viscida* and *N. nuda* subsp. *nuda*.

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New taxa of *Rhododendron tschonoskii* alliance (Ericaceae) from East Asia

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Abstract

Three new taxa, *Rhododendron sohayakiense* Y.Watan. & T.Yukawa (Ericaceae), and its two varieties, var. *kiusianum* Y.Watan., T.Yukawa & T.Minamitani and var. *koreanum* Y.Watan. & T.Yukawa are described and illustrated from Japan and South Korea. They can be distinguished from each other and from the other members of the *R. tschonoskii* alliance, i.e. *R. tschonoskii*, *R. tetramerum*, *R. trinerve* and *R. tsusiophyllum*, through their combination of leaf size, leaf morphologies including lateral nerves on abaxial leaf surface, corolla morphologies including number of corolla lobes, style length and anther form. Phylogenetic inferences based on chloroplast DNA and genome-wide sequences revealed that each of the three new taxa is monophyletic and they further form a clade. Distributions of the three taxa are also clearly separated from each other and also from the other members of the *R. tschonoskii* alliance.

Keywords

Ericaceae, new species, phylogeny, *Rhododendron*

Introduction

The genus *Rhododendron* L. (Ericaceae) is morphologically diverse, comprising about 1,000 woody species (Chamberlain et al. 1996). The genus is mostly distributed across the Northern Hemisphere and members of subgenus *Vireya* extend into the South-

ern Hemisphere via the Indo-Australian Archipelago. The subgenus *Tsutsusi* is mostly found in East Asia (Yamazaki 1996; Kron and Powell 2009). Although most species of this subgenus occur in warm-temperate to subtropical regions, some are present in cold and alpine regions.

Rhododendron tschonoskii Maxim., *R. tetramerum* (Makino) Nakai, *R. trinerve* Franch. ex Boissier and *R. tsusiophyllum* Sugim. are closely related species placed within the subgenus *Tsutsusi*, which grow on exposed rocks or open sites in slopes and ridges on mountains. Among them, *R. tsusiophyllum* was originally described as a species of an independent genus *Tsusiophyllum tanakae* Maxim. (1870), because anthers of this species open through longitudinal slits while anthers of all other species in *Rhododendron* open through apical pores. The first taxonomic reappraisal of this group except *R. tsusiophyllum* was conducted by Takahashi (1975) in which he recognized the aforementioned four taxa at species rank. Subsequently, Yamazaki (1996) treated these two taxa as varieties of *R. tschonoskii*, i.e. *R. tschonoskii* var. *tetramerum* (Makino) Komatsu and *R. tschonoskii* var. *trinerve* (Franch. ex Boissier) Makino. In this study, we tentatively adopt Takahashi's (1975) concept.

Rhododendron tschonoskii sensu Takahashi (1975) is widely distributed across the Japanese Archipelago and extends to the southern part of the Korean Peninsula. Despite the wide distribution, the species is absent from the central part of the Japanese Archipelago. As pointed out by Takahashi (1975) and Minamitani (1993), morphological characters of the species are distinct between eastern and western parts of the Japanese Archipelago. To evaluate these previous observations, we investigate morphological and macromolecular characters of *R. tschonoskii* and its related species by using the samples covering the entire species ranges.

Methods

The morphological characters were observed and measured based on living materials in the field and herbarium specimens listed in the sections "Additional specimens examined".

Samples for DNA analyses were collected from three individuals for *Rhododendron tschonoskii*, *R. tetramerum*, *R. trinerve*, *R. tsusiophyllum* and new entities, respectively. Three samples for each species and two entities and two samples for one entity (see Results and Discussion) were selected for covering entire range, and a holotype for each entity was included (Table 1). In addition, one individual for each species was collected from other relatives, i.e. *R. dilatatum*, *R. kaempferi*, *R. macrosepalum*, *R. reticulatum*, *R. serpyllifolium* and *R. tashiroi* belonging subgenus *Tsutsusi* (Kron and Powell 2009). For phylogenetic analysis, genomic DNA was extracted from silica-dried leaf samples using a DNeasy Plant mini kit (Qiagen, Hilden, Germany) after treatment with sorbitol buffer (Wagner et al. 1987). Five non-coding regions of chloroplast DNA (*trnL-F*, *trnL* intron, *trnS-G*, *trnG* intron and *rpl32-trnL*) were amplified and sequenced following the protocols described in Yoichi et al. (2017). The sequences were assembled

Table 1. Locations of samples used for phylogenetic analyses.

| Species | Code | Locality | Latitude / Longitude | Haplotype | Voucher |
|--|------|--|----------------------|-----------|--------------------------------------|
| <i>R. sohayakiense</i> var. <i>sohayakiense</i> (Type 1) | Syk | Mt. Syakagadake, Nara, Japan | 34.1145, 135.9020 | H1 | Y. Watanabe & K. Yukitoshi s.n. |
| | Miu | Mt. Miune, Tokushima, Japan | 33.8398, 133.9877 | H1 | Y. Watanabe & T. Fukuda s.n. |
| | Ttj | Mt. Tsutsujyo, Ehime, Japan | 33.7333, 133.1593 | H1 | Y. Watanabe & M. Takahashi Ttj02 |
| <i>R. sohayakiense</i> var. <i>kiusianum</i> (Type 2) | Ici | Mt. Ichifusa, Kumamoto, Japan | 32.3124, 131.1010 | H2 | Y. Watanabe Ici01 |
| | Mks | Mt. Mukousaka, Kumamoto, Japan | 32.5842, 131.1054 | H2 | Y. Watanabe Mks04 |
| | Sob | Mt. Sobo, Miyazaki, Japan | 32.8114, 131.3470 | H3 | Y. Watanabe & T. Oi s.n. |
| <i>R. sohayakiense</i> var. <i>koreanum</i> (Type 3) | Gom | Gonam, Jeollabuk-do, South Korea | 35.4744, 127.5002 | H4 | Y. Watanabe, S. Hwang & N. Yun Gom01 |
| | Wol | Mt. Wolbong, Gyeongsangnam-do, South Korea | 35.7476, 127.7094 | H4 | Y. Watanabe, S. Hwang & N. Yun Wol01 |
| <i>R. trinerve</i> | Snp | Mt. Sanpouiwa, Ishikawa, Japan | 36.2586, 136.8441 | H5 | Y. Watanabe Snp02 |
| | Sad | Sado Island, Niigata, Japan | 37.9280, 138.4534 | H5 | H. Abe s.n. |
| | Iid | Mt. Iide, Niigata, Japan | 37.9188, 139.5849 | H6 | Y. Watanabe s.n. |
| <i>R. tetramerum</i> | Utk | Mt. Utsukushigahara, Nagano, Japan | 36.2277, 138.0975 | H7 | Y. Watanabe s.n. |
| | Abe | Abe-touge pass, Shizuoka, Japan | 35.3135, 138.3605 | H7 | Y. Watanabe Abe01 |
| | Kmg | Mt. Kamagatake, Mie, Japan | 35.0012, 136.4212 | H7 | Y. Watanabe & T. Oi s.n. |
| <i>R. tschonoskii</i> | Zao | Mt. Zao, Miyagi, Japan | 38.1105, 140.4553 | H8 | Y. Watanabe Zao01 |
| | Mus | Mt. Musadake, Hokkaido, Japan | 43.6741, 144.8850 | H8 | Y. Watanabe s.n. |
| | Tor | Mt. Toraidake, Aomori, Japan | 40.4505, 141.0100 | H9 | Y. Watanabe s.n. |
| <i>R. tsusiophyllum</i> | Hkn | Mt. Hakone-komagatake, Kanagawa, Japan | 35.2235, 139.0233 | H10 | Y. Watanabe Hkn01 |
| | Kdz | Kouzushima Island, Tokyo, Japan | 34.13103, 139.0912 | H11 | H. Abe s.n. |
| | Tng | Mt. Tengu, Nagano, Japan | 35.99188, 138.5689 | H11 | Y. Watanabe s.n. |

Haplotype, Haplotype codes detected by chloroplast DNA sequences, corresponding to those in Fig. 3.

Specimens of all of the analysed samples were deposited in TNS.

using DNA Baser 4 (Heracle BioSoft, Pitești, Romania) and aligned using the MUSCLE algorithm implemented in MEGA 7 (Edgar 2004; Kumar et al. 2016).

In addition, genome-wide SNPs were identified from two double digest restriction-site associated DNA (ddRAD) libraries using Peterson et al. (2012) protocol with some modifications. To fragment DNA sequences, 10 ng of genomic DNA was digested with *EcoRI* and *BglII*. Digestion and ligation were performed at 37 °C for 16 h in a 10 µL volume containing 20–40 ng of genomic DNA, 0.5 µL of each 10U/µL *EcoRI* and *BglII* enzyme (Takara, Kyoto, Japan), 1 µL of ×10 NEB buffer 2, 0.1 µL of ×100 BSA (New England Biolabs, Ipswich, USA), 0.4 µL of each 5 µM *EcoRI* and *BglII* adapter, 0.1 µL of 100 mM ATP and 0.5 µL of T4 DNA

ligase (Enzymatics, Beverly, USA). The ligated product was purified with AMPure XP (Beckman Coulter, Brea, USA). The purified adaptor-ligated DNA was subsequently amplified by PCR. The PCR was performed in a 10 μ L volume containing 2 μ L of adaptor-ligated DNA, 2 μ L of 5 μ M index primer including 6-mer variable sequences for identifying different samples, 1 μ L of 10 μ M TruSeq universal primer, 5 μ L of \times 2 KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, USA). The PCR was performed with an initial denaturation for 4 mins at 94 $^{\circ}$ C, followed by 20 cycles of 10 s at 98 $^{\circ}$ C, 15 s at 65 $^{\circ}$ C and 15 s at 68 $^{\circ}$ C. The PCR products from different samples were pooled and purified again with AMPure XP. Fragments of 350–400 bp in the purified DNA solution were retrieved by electrophoresis using a 2.0% of E-Gel SizeSelect (Life Technologies, Carlsbad, USA). After quantity assessment using a QuantiFluor dsDNA System (Promega, Madison, USA) and quality assessment using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), the libraries were sequenced with 51-bp single-end reads in two lanes of an Illumina HiSeq2000 (Illumina, San Diego, USA). After removing reads containing low-quality bases and adaptor sequences from the raw data using Trimmomatic v. 0.33 (Bolger et al. 2014), sequences with polymorphic SNPs were assembled by pyRAD v. 3.0 (Eaton 2014). Parameters for the assembly were set as follows: the minimum depth coverage for creating a cluster from reads was set to 6, the similarity threshold of clusters within and across individuals was set to 0.85, the maximum number of samples with shared heterozygous sites in a locus for filtering potential paralogs was set to 3, and polymorphic loci sequenced in more than half of the samples were finally exported as consensus sequences (Eaton 2014).

The phylogenetic relationships were inferred from two data sets, which were obtained from chloroplast DNA sequences and RAD-seq, based on the maximum likelihood method using RAxML v. 8.2.0 (Stamatakis 2014). In the analyses, the GTR-GAMMA model was used as a substitution model, and node supports were assessed by bootstrap analysis with 1000 replicates. Phylogenetic relationships among individuals for *Rhododendron tschonoskii*, *R. tetramerum*, *R. trinerve*, *R. tsusiophyllum* and new entities based on RAD-seq were further evaluated by constructing a neighbor-net based on *p*-distance using SplitTree4 (Huson and Bryant 2006).

Results and discussion

Morphological differences

We found three new entities (Types 1, 2 and 3) that have previously been included within *Rhododendron tschonoskii* (Fig. 2). However, corolla lobes for the three types, *R. trinerve* and *R. tetramerum* are tetramerous; contrastingly *R. tschonoskii* and *R. tsusiophyllum* are pentamerous. It is noteworthy to mention that the number of corolla lobes is sometimes variable within individuals. Further, they are distinguished from each other and from the other members of the *R. tschonoskii* alliance, i.e. *R. tschonoskii*, *R. tetramerum*, *R. trinerve* and *R. tsusiophyllum* through their leaf size, leaf morphologies

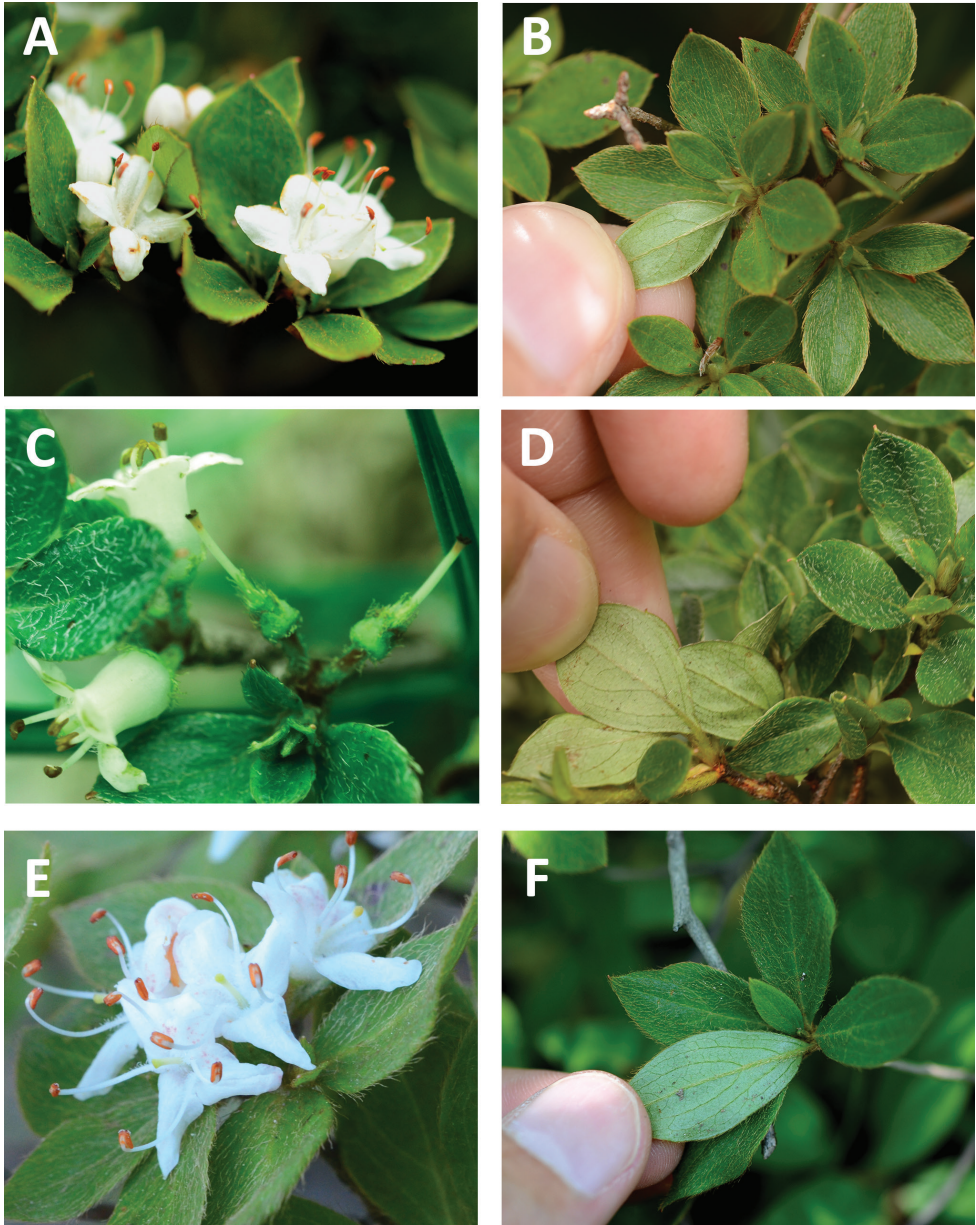


Figure 1. Photographs of flowers and leaves for the new taxa described in this study. **A, B** *Rhododendron sohayakiense* var. *sohayakiense*, Mt. Tsutsujo, Ehime Prefecture, Japan **C, D** *Rhododendron sohayakiense* var. *kiusianum*, Mt. Mukousaka, Kumamoto Prefecture, Japan **E, F** *Rhododendron sohayakiense* var. *koreanum*, Mt. Wollong, Gyeongsangnam-do, South Korea. Photographs by Yoichi Watanabe.

including lateral nerves on abaxial leaf surface, corolla morphologies and style length (Table 2, Figs 1, 2). Leaf sizes of Types 1, 2 and *R. tschonoskii* are medium (5–30 mm long), while Type 3 and *R. trinerve* are large (10–50 mm long). Lateral nerves on the abaxial surface of the leaf are pinnate but obscure for Type 1, while 1–3 pairs of pin-

Table 2. Diagnostic characters among the *Rhododendron tschonoskii* alliance.

| Character | <i>R. sobayakiense</i> var. <i>sobayakiense</i> (Type 1) | <i>R. sobayakiense</i> var. <i>kiusianum</i> (Type 2) | <i>R. sobayakiense</i> var. <i>koreanum</i> (Type 3) | <i>R. tschonoskii</i> | <i>R. tsusiophyllum</i> | <i>R. trinerve</i> | <i>R. tetramerum</i> |
|----------------------------------|---|---|---|--|--|--|---|
| Leaf length (mm) | 10–20 | 10–20 | 10–35 | 10–25 | 5–13 | 15–50 | 5–20 |
| Leaf width (mm) | 4–7 | 5–10 | 5–15 | 5–12 | 2–5 | 5–20 | 5–10 |
| Nerve on abaxial surface of leaf | pinnate 2–3 pairs, obscurely raised | pinnate 2–3 pairs, raised | pinnate 1–3 pairs, raised | nervules reticulate | nervules obscurely reticulate | pinnate 1–2 pairs, prominent, nervules reticulate | nervules obscurely reticulate |
| Hair of leaf | densely strigose on adaxial surface, glabrous or sparsely strigose on abaxial surface | densely strigose on adaxial surface, sparsely strigose on abaxial surface | densely strigose on adaxial surface, sparsely strigose on abaxial surface | strigose on both surfaces | densely strigose on adaxial surface, glabrous on abaxial surface | strigose on both surfaces | densely strigose on adaxial surface, glabrous or sparsely strigose on abaxial surface |
| Corolla form | tubular-funnelform | tubular-funnelform | tubular-funnelform | tubular-funnelform | tubiform | tubular-funnelform | tubiform |
| Number of corolla lobes | 4 | 4 | 4 | 5 | 5 | 4 | 4 |
| Corolla tube length (mm) | 2–3 | 3–4 | 2–3 | 2–4 | 5–7 | 2–4 | 3.5–4.5 |
| Corolla lobe length (mm) | 3–5 | 2–5 | 3–5 | 4–6 | ca. 2 | 4–6 | 2–3 |
| Style length (mm) | 4–10 | 3–4 | 5–6 | 6–13 | 4–5 | 3–7 | 2.5–3.5 |
| Style condition | exserted | included | exserted | exserted | included | exserted | included |
| Anther | opening by apical pores | opening by apical pores | opening by apical pores | opening by apical pores | opening by longitudinal slits | opening by apical pores | opening by apical pores |
| Distribution | Japan: Honshu (Kii Peninsula) and Shikoku | Japan: Kyushu | South Korea: Gyeongsang and Jeolla provinces | Japan: Honshu (Kanto and Tohoku districts) and Hokkaido. Russia: Kunashir Island | Japan: Honshu (Chubu and Kanto districts) | Japan: Honshu (Kinki, Chubu, Kanto and Tohoku districts) | Japan: Honshu (Kinki, Chubu and Kanto districts) |

nate lateral nerves are raised for Types 2 and 3. Corolla tube lengths of Types 1, 3, *R. tschonoskii* and *R. trinerve* are short (2–4 mm long), while Type 2, *R. tetramerum* and *R. tsusiophyllum* are long (3.5–7 mm long). Styles of Types 1, 3, *R. tschonoskii* and *R. trinerve* are longer than the corolla tube and exserted from the corolla, while those of Type 2, *R. tetramerum* and *R. tsusiophyllum* are similar or shorter than the corolla tube and included within the corolla.

The three types share the following combination of characters as commonly derived character states, which can be distinguished from the other members of the *R. tschonoskii* alliance. The corolla form of the three types are tubular-funnelform and corolla lobes are tetramerous; in addition, lateral nerves on abaxial leaf surface are raised or obscure raised, and not prominent, such as *R. trinerve*.

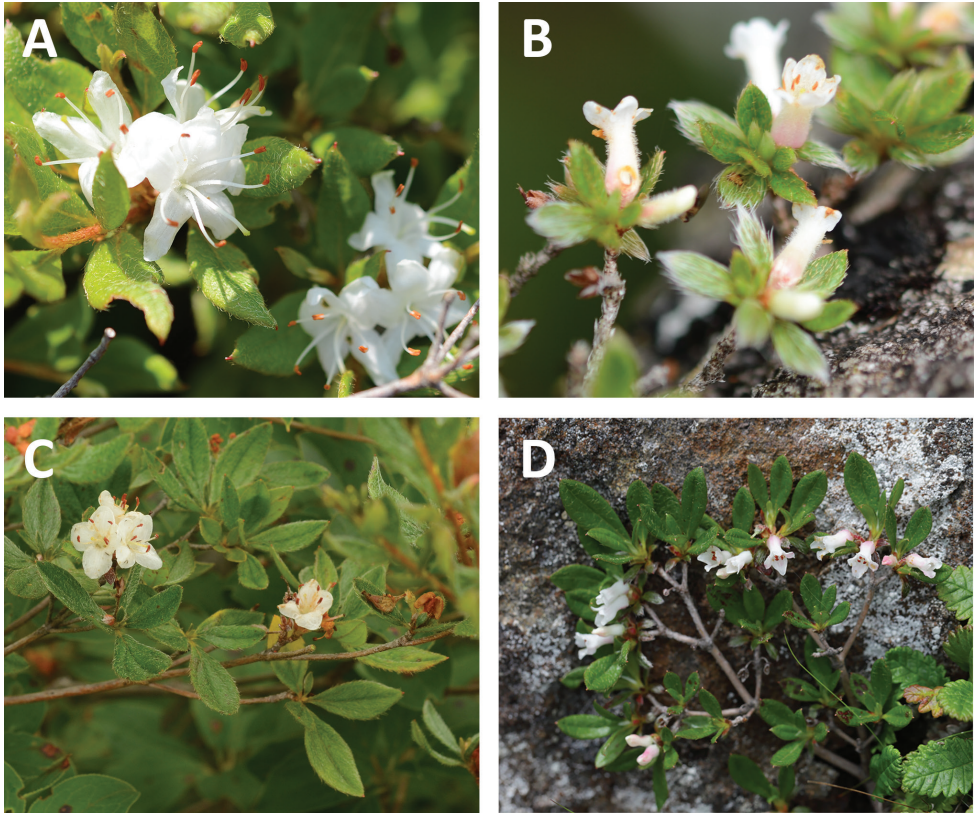


Figure 2. Photographs of flowers for the other members of *Rhododendron tschonoskii* alliance. **A** *Rhododendron tschonoskii*, Mt. Bandai, Fukushima Prefecture, Japan **B** *Rhododendron tsusiophyllum*, Mt. Kobushigatake, Yamanashi Prefecture, Japan **C** *Rhododendron trinerve*, Mt. Sanpouiwa, Ishikawa Prefecture, Japan **D** *Rhododendron tetramerum*, Mt. Yatsugatake, Nagano Prefecture, Japan. Photographs **A–C** by Yoichi Watanabe **D** courtesy of Osamu Takahashi.

Phylogenetic relationships

Phylogenetic relationships of the *R. tschonoskii* alliance based on chloroplast DNA sequences (2,847 bp with 76 polymorphic sites) and genome-wide sequences (RAD-seq, 316,455 bp with 37266 SNPs) were almost concordant including outgroup species (Fig. 3). The *R. tschonoskii* alliance formed a monophyletic group (95% for chloroplast DNA and 100% for RAD-seq). The chloroplast DNA sequences identified four haplotypes from the three types, Types 1 and 3 had one haplotype respectively and Type 2 had two haplotypes. Although the monophyly of the clade comprising Types 1, 2 and 3 was supported with high bootstrap probability (80%), the monophyly of two haplotypes in Type 2 was not supported. The monophyly of the clade comprising Types 1, 2 and 3 and the monophyly of each type were supported with the highest bootstrap probabilities (100%) based on RAD-seq. The neighbor-net for a data set, which in-

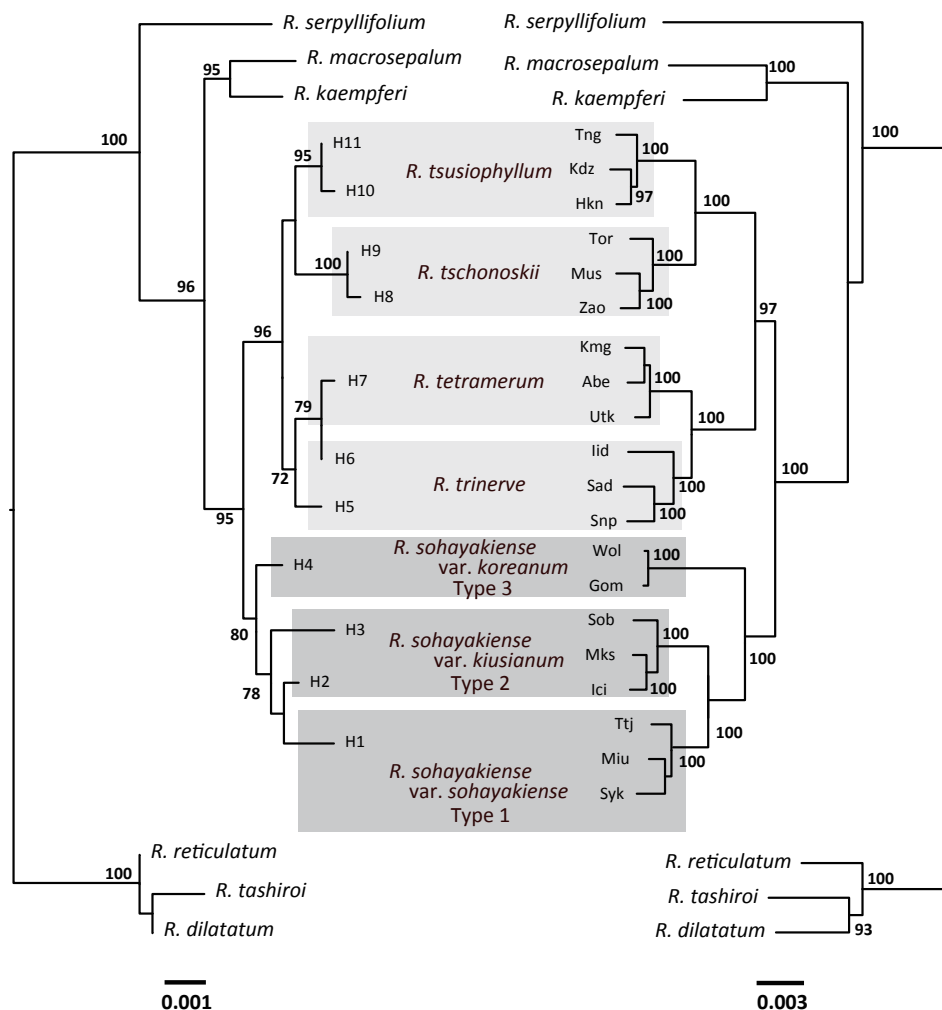
A Chloroplast DNA**B Genome-wide**

Figure 3. Comparative phylogenies for **A** haplotypes based on chloroplast DNA sequences and **B** genotypes based on restriction site associated DNA sequences (RAD-seq). Bootstrap probabilities (> 70%) are shown above nodes. Gray boxes indicate three new taxa described in this study and light gray boxes indicate the other members of the *Rhododendron tschonoskii* alliance.

cluded only the *R. tschonoskii* alliance, identified three groups corresponding to Types 1, 2 and 3, which can be clearly distinguished from each other (Fig. 4).

Thus the three types can be distinguished from each other and also from the other members of the *R. tschonoskii* alliance. The results indicate that the three types should be treated as different taxa but these are more closely related than the others in the *R. tschonoskii* alliance. Since we confirmed the independent state of the three types from morphological and phylogenetic characteristics, we hereby describe them as a new species and its two varieties.

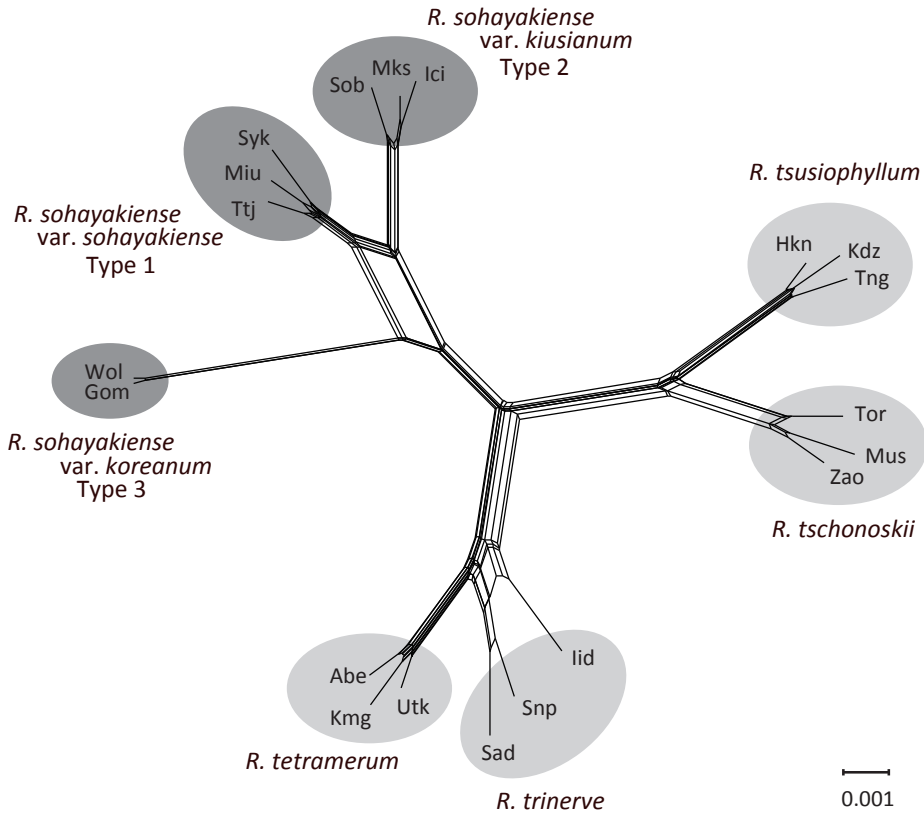


Figure 4. Neighbor-net for members of the *Rhododendron tschonoskii* alliance reconstructed from p -distance among individuals based on RAD-seq.

Taxonomic treatments

***Rhododendron sohayakiense* Y.Watan. & T.Yukawa, sp. nov.**

urn:lsid:ipni.org:names:77202559-1

Figs 1, 5, 6

Diagnosis. This species is similar to *Rhododendron tschonoskii* Maxim, but is distinguishable through its 4 corolla lobes and its pinnate nerves on the adaxial leaf surface.

Type. JAPAN. Shikoku: Ehime Pref., Kumakogen Town, Mt. Tsutsujo-yama, 33°44'00.01"N, 133°09'33.40"E, on ridge of the mountain, 1800 m, 20 July 2016 (fl), Y. Watanabe and M. Takahashi Ttj02 (holotype TNS; isotypes TNS, KYO).

Additional specimens examined. JAPAN. Kii Peninsula, Honshu: Nara Pref., Yoshino County, Kamikitayama Village, Mt. Oodaigahara, Daijyagura, 1500 m, 23 Aug 1956 (fr), G. Murata 10133 (KYO); Nara Pref., Yoshino County, Kamikitayama Village, Mt. Oodaigahara, Daijyagura, 1600 m, 18 Jul 2012 (fl), K. Yamawaki 4869 (KYO); Nara Pref., Yoshino County, Shimokitayama Village, Mt. Kujiyaku, 1800 m, 17 Jul 1954 (fl), G. Murata & T. Shimizu 104 (KYO); **Shikoku:** Tokushima Pref.,

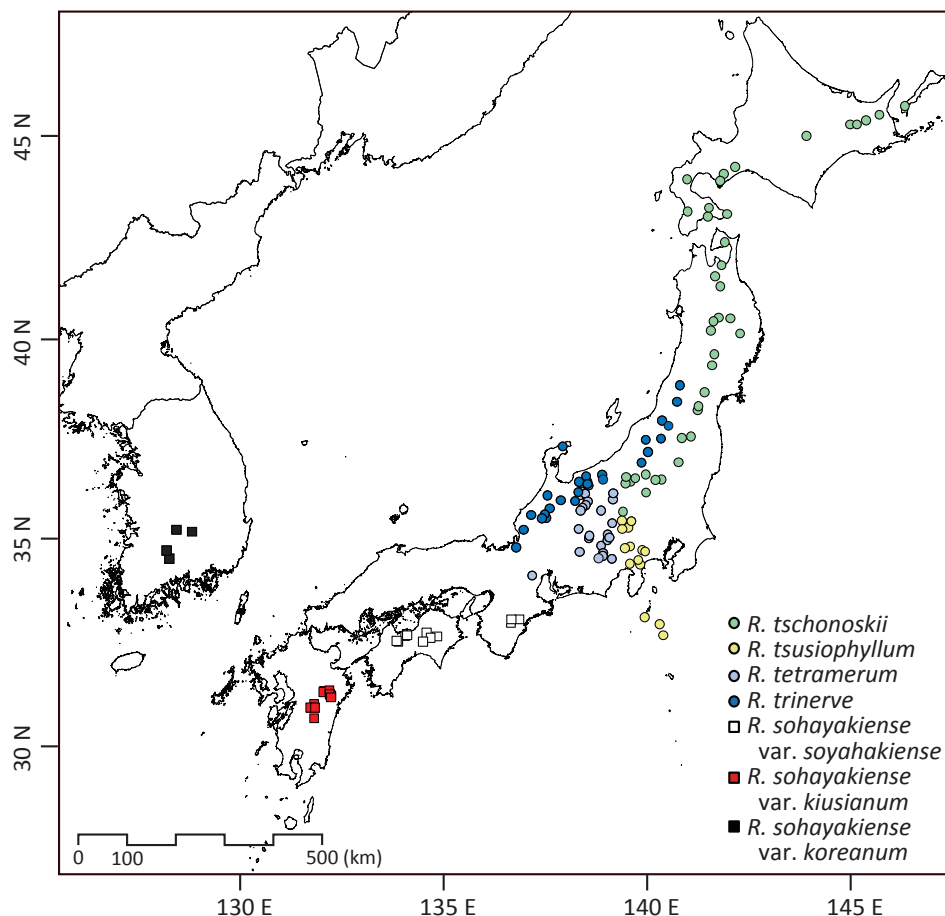


Figure 5. Distribution of the *Rhododendron tschonoskii* alliance. Circles and squares showed locations of herbarium specimens (KYO, TNS).

Miyoshi County, Higashiiya Village, Mt. Tsurugi, 1950 m, 25 Jul 1986 (fl), G. Murata et al. 45946 (KYO); Tokushima Pref., Miyoshi County, Higashiiya Village, Mt. Tsurugi, 1700m, 22 Oct 2012 (fr), Y. Katayama 32 (KYO); Tokushima Pref., Miyoshi County, Nishiiyayama Village, Mt. Nakatsu, 1400 m, 10 Aug 1954, G. Murata 7728 (KYO); Kochi Pref., Nagaoka County, Ootoyo Village, Mt. Kajigamori, 1200 m, 22 Aug 1964, G. Murata 18671 (KYO); Ehime Pref., Kamiukena County, Kumakougen Town, Mt. Ishizuchi, between Dogamori and summit, 1600–1980 m, 27 Jul 1983 (fl), G. Murata 44754 (KYO); Ehime Pref., Saijyo City, Mt. Ishizuchi, between starting point and summit, 1400–1850 m, 17 Jul 1992 (fl), T. Minamitani 43440 (TNS); Ehime Pref., Niihama City, Mt. Douzanmine, 1300 m, 15 Jul 1980 (fl), K. Tsuchiya 491 (KYO); Ehime Pref., Uma County, Mt. Higashiakaisi and Mt. Futatsudake, 1600 m, 8 Sep 1961 (fr), G. Murata 14993 (KYO); Ehime Pref, Niihama City,

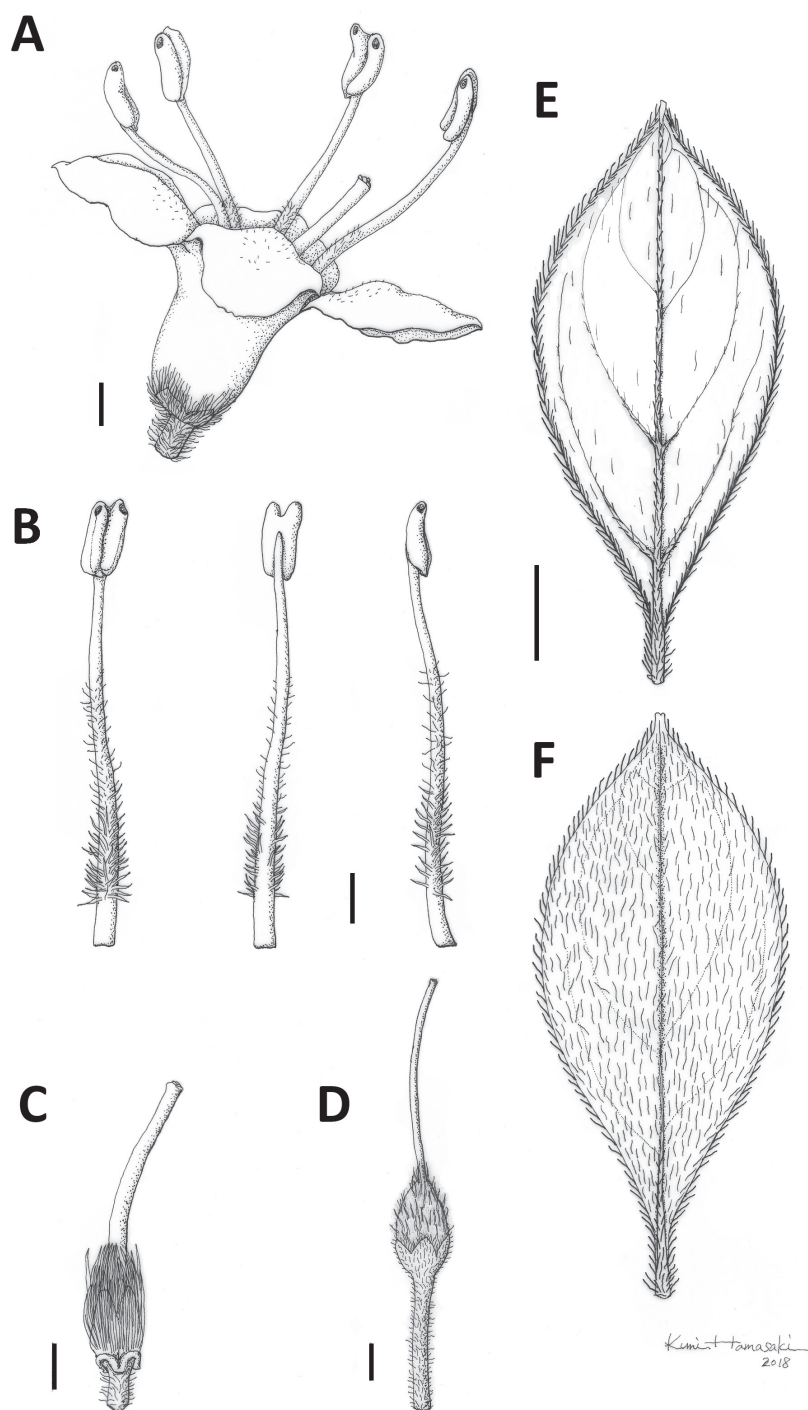


Figure 6. *Rhododendron sohayakiense* var. *sohayakiense*. **A** Flower, side view **B** Stamen **C** Pistil **D** Fruit **E** Abaxial and **F** adaxial sides of leaf. Scale bars: 1 mm (**A–D**); 3 mm (**E, F**). Drawings by Kumi Hamasaki from Y. Watanabe & M. Takahashi Tj02 (holotype, TNS).

Mt. Higashiakaishi, 33°52'31.03"N, 133°22'26.31"E, 1700 m, 15 Jul 2017 (fl), Y. Watanabe Hga03 (TNS).

Description. Much branched semi-evergreen shrubs 1–1.5 m tall. Branchlets and petioles with dense appressed flattened brownish strigose hairs. Spring leaves scattered or crowded on upper branchlets; petioles 0.5–1 mm long; blade thick chartaceous, oblong, 10–20 mm long (at maximum within each individual), 4–7 mm wide, apex acute and terminating in a gland, base acute, adaxial surface green, abaxial surface pale green, densely strigose on adaxial surface, glabrous or sparsely strigose on abaxial surface without midrib; midrib prominent abaxially; lateral nerves pinnate, 2–3 paired, obscure raised abaxially. Summer leaves oblanceolate, 5–10 mm long, 1–6 mm wide, densely strigose on both surfaces. Flower buds terminal, single, broadly ovoid, acute, ca. 2 mm long, 2 mm wide; scales widely ovate, densely strigose on upper outer surface. Inflorescences umbel-like, 2–4 flowers. Pedicel 2–4 mm long at flowering, densely appressed hirsute. Calyx saucer-shaped, ca. 1.5 mm in diam., densely strigose, shallowly 4-lobed; lobes semiorbiculate, ca. 0.5 mm long. Corolla white, no blotches, openly tubular-funnelform, 8–12 mm long and wide, dissected 1/2 corolla length into 4 lobes; tube 2–3 mm long, ca. 2 mm wide, glabrous outside, pilose on upper inside; lobes elliptic to oblong, rounded, 3–5 mm long, 2–4 mm wide. Stamens 4, subequal, 5–8 mm long, exserted; filaments densely pilose on lower three-quarters; anthers yellow, oblong, ca. 1 mm long. Ovary ovoid, densely soft strigose, ca. 1.5 mm. Style 4–10 mm long, exserted, glabrous. Capsule ovoid, 2–5 mm long, 2–3 mm wide, densely strigose.

Distribution. JAPAN: Honshu (Kii Peninsula), Shikoku.

Ecology. The plants inhabit sunny places and grow on mountain ridges and slopes at altitudes over 1000 m above sea level. In such places, there are few trees and established communities of shrubs and dwarf bamboos (*Sasa* sp.). Flowering specimens have been collected from July to August; fruiting specimens have been collected from October to November. Bumblebees are frequent visitors to the flowers, suggesting that they are pollinators of the species.

Etymology. The specific epithet refers to ‘Sohayaki’ a floristic region in Japan that covers Kii Peninsula of Honshu, Shikoku and Kyushu (Koidzumi 1931), where the new species is distributed.

***Rhododendron sohayakiense* var. *kiusianum* Y.Watan., T.Yukawa & T.Minamitani, var. nov.**

urn:lsid:ipni.org:names:77202565-1

Figs 1, 5, 7

Diagnosis. This variety is similar to *Rhododendron sohayakiense* var. *sohayakiense* Y. Watan. & T. Yukawa and *R. sohayakiense* var. *koreanum* Y. Watan. & T. Yukawa, but is distinguishable through its longer corolla tube and its shorter style included within the corolla. Further, it differs from the former through its raised lateral nerves on the abaxial leaf surface and from the latter through its small leaf size.

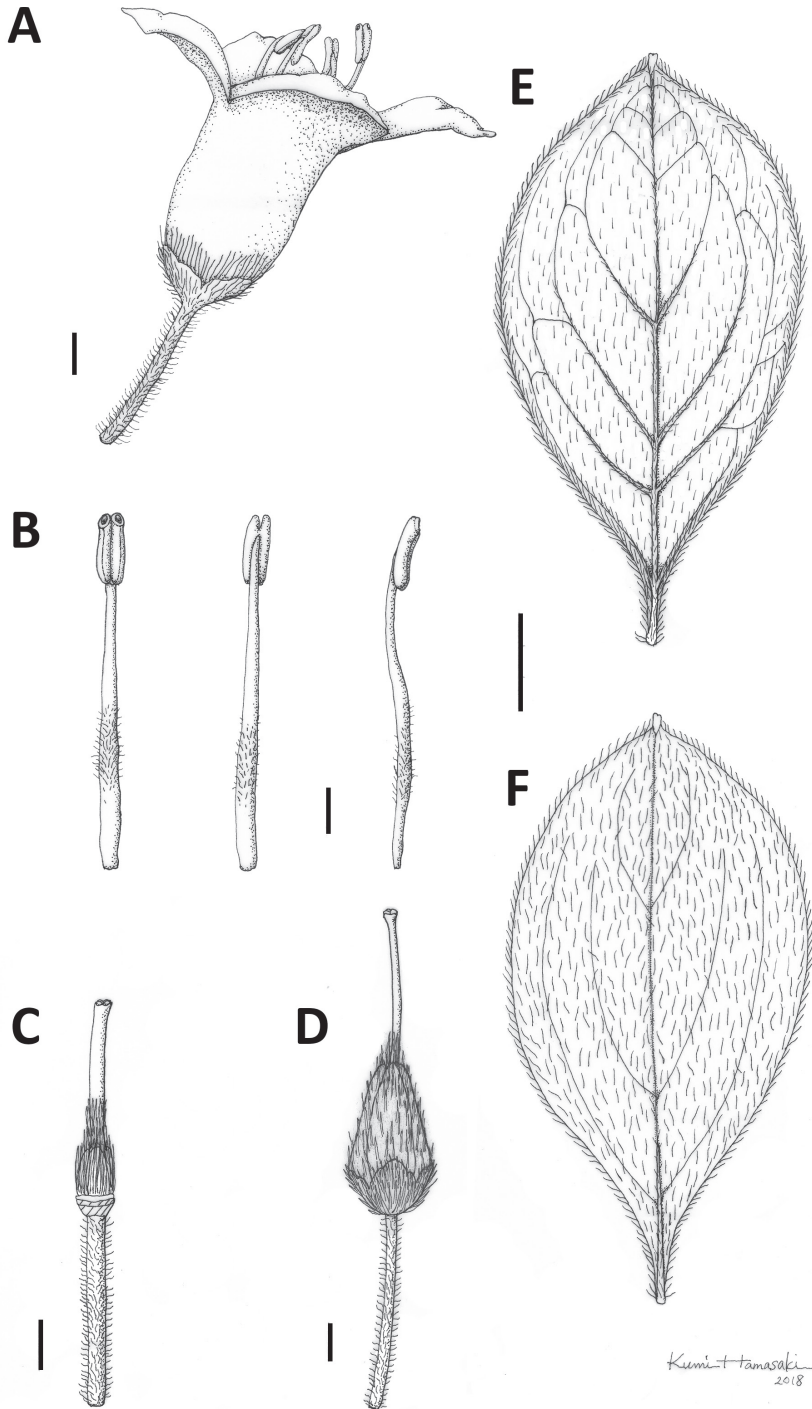


Figure 7. *Rhododendron sohayakiense* var. *kiusianum*. **A** Flower, side view **B** Stamen **C** Pistil **D** Fruit **E** Abaxial and **F** adaxial sides of leaf. Scale bars: 1 mm (**A–D**); 3 mm (**E, F**). Drawings by Kumi Hamasaki from *Y. Watanabe Mks04* (holotype, TNS).

Type. JAPAN. Kyushu: Kumamoto Pref., Kamimashiki County, Yamato Village, Mt. Mukosaka, 32°35'03.22"N, 131°06'19.30"E, on rocky ridge of the mountain, 1500 m, 23 July 2016 (fl), Y. Watanabe Mks04 (holotype: TNS; isotypes: TNS, KYO).

Additional specimens examined. JAPAN. Kyushu: Ooita Pref., Mt. Katamuki, 2 Aug 1921 (fl), Z. Tashiro (KYO); Miyazaki Pref., Higashi-Usuki County, Shiiba Village, Mt. Eboshi, 1690 m, 18 Jul 2007 (fl), T. Minamitani (TNS); Miyazaki Pref., Higashi-Usuki County, Shiiba Village, Mt. Ougi, 1200–1661 m, 7 Jul 1994 (fl), T. Minamitani 049666 (TNS); Miyazaki Pref., Nishi-Usuki County, Hinokage Town, Mt. Goyou, 1570 m, 11 Aug 1990 (fr), T. Minamitani (TNS); Miyazaki Pref., Nishi-Usuki County, Hinokage Town, Mt. Katamuki, 1500 m, 25 Aug 1970 (fr), T. Minamitani (TNS); Miyazaki Pref., Nishi-Usuki County, Takachiho Town, Mt. Tengu, 1700 m, 24 Jun 1993 (fl), T. Minamitani (TNS); Miyazaki Pref., Nishi-Usuki County, Takachiho Town, Mt. Tengu, 1600–1700 m, 16 Aug 1992, T. Minamitani 44119 (TNS); Miyazaki Pref., Nishi-Usuki County, Takachiho Town, Mt. Shoji, 1600–1700 m, 16 Aug 1992, T. Minamitani 44128 (TNS); Miyazaki Pref., Nishi-Usuki County, Gokase Town, Mt. Mukosaka, Tsutsujigaoka, 1570 m, 12 Jul 1993 (fl), T. Minamitani (TNS); Miyazaki Pref., Nishi-Usuki County, Gokase Town, Mt. Mukosaka, Kita, 1550 m, 24 Sep 1996 (fl, fr), T. Minamitani B-T-052838 (TNS); Miyazaki Pref., Higashi-Usuki County, Kitakata Town, Mt. Hoko, 1100–1200 m, 26 Jun 1994 (fl), T. Minamitani (TNS); Miyazaki Pref., Higashi-Usuki County, Kitakata Town, Mt. Hoko, 1270 m, 23 Sep 1989 (fr), T. Minamitani (TNS); Miyazaki Pref., Nobeoka City, Kitakata, Mt. Ohkue, Kozumidaki, 1340 m, 6 Aug 1992 (fr), T. Minamitani (TNS); Kumamoto Pref., Kuma County, Mt. Ichifusa, 1700 m, 6 Aug 1960 (fl), M. Tagawa & K. Iwatsuki 3676 (KYO); Kumamoto Pref., Kuma County, Yunomae Town, Mt. Ichifusa, 26 Jul 1992 (fl), T. Minamitani 43478 (TNS); Kumamoto Pref., Kuma County, Mizukami Village, Mt. Ichifusa, 32°18'44.65"N, 131°06'03.50"E, on summit of the mountain, 1600 m, 24 July 2016 (fl), Y. Watanabe Ici02 (TNS).

Description. Spring leaves scattered or crowded on upper branchlets; petioles 0.5–1 mm long; blade thick chartaceous, oblong-ovate, 10–20 mm long (at maximum within each individual), 5–10 mm wide, apex acute and terminating in a gland, base acute, strigose on both surfaces; midrib prominent abaxially; lateral nerves pinnate, 2–3 paired, raised abaxially. Summer leaves oblanceolate, 3–10 mm long, 1–5 mm wide, densely strigose on both surfaces. Calyx saucer-shaped, ca. 1.5 mm in diam., densely soft strigose, shallowly 4-lobed; lobes semiorbiculate, ca. 0.5 mm long. Corolla white, no blotches, tubular-funnelform, 7–13 mm long and wide, 4 lobes; tube 3–4 mm long, ca. 3 mm wide, glabrous outside, pilose on upper inside; lobes elliptic to oblong, rounded, 2–5 mm long, ca. 2 mm wide. Stamens 4, irregular, 3–5 mm long, as long as or shorter than corolla; filaments densely pilose on lower half; anthers yellow, oblong, ca. 1 mm long. Ovary ovoid, densely soft strigose, ca. 1.5 mm. Style 3–4 mm long, glabrous, shorter than corolla. Capsule ovoid, 3–4 mm long, 2.5 mm wide, densely strigose.

Distribution. JAPAN: Kyushu.

Ecology. The plants inhabit sunny and rocky mountain ridges and slopes at altitudes over 1000 m above sea level. Flowering specimens have been collected from July to August; fruiting specimens have been collected from October to November.

Etymology. The specific epithet refers to 'Kyushu' where the new variety is distributed.

Note. Although the style of this variety is included within the corolla, this part is exerted from the corolla in individuals from Mt. Ichifusa.

***Rhododendron sohayakiense* var. *koreanum* Y.Watan. & T.Yukawa, var. nov.**

urn:lsid:ipni.org:names:77202566-1

Figs 1, 5, 8

Diagnosis. This variety is similar to *Rhododendron sohayakiense* var. *sohayakiense* Y. Watan. & T. Yukawa and *R. sohayakiense* var. *kiusianum* Y. Watan., T. Yukawa & T. Minamitani, but is distinguished by its large leaf size. Further, it differs from the former through its raised lateral nerves on the abaxial leaf surface and from the latter through its shorter corolla tube and by its longer style exerted from the corolla.

Type. SOUTH KOREA. Gyeongsangnam-do: Hamyang County, Mt. Wolbong, 35°44'51.31"N, 127°42'33.99"E, on slope of the mountain, 1000 m, 15 June 2017 (fl), Y. Watanabe, S. Hwang and N. Yun Wol06 (holotype: TNS; isotypes: TNS, KB).

Additional specimens examined. SOUTH KOREA. Jeollabuk-do: Namwon City, Mt. Gonam, 35°28'27.74"N, 127°30'00.82"E, 800 m, 15 Jun 2017 (fl), Y. Watanabe, S. Hwang and N. Yun Gom01 (TNS); Mt. Jirisan, Banyabong peak, 1700 m, 20 Aug 1982 (fr), T. Yamazaki & F. Yamazaki 3294 (KYO); Gyeongsangnam-do: Mt. Gayasan, 23 Aug 1935 (fr), G. Koidzumi (KYO).

Description. Spring leaves scattered or crowded on upper branchlets; petioles 0.5–1 mm long; blade thick chartaceous, oblong, 10–35 mm long (25–35 mm long at maximum within each individual), 5–15 mm wide, apex acute and terminating in a gland, base acute, strigose on both surfaces; midrib prominent abaxially; lateral nerves pinnate, 1–3 paired, raised abaxially. Summer leaves oblanceolate, 6–20 mm long, 1–8 mm wide, densely strigose on both surfaces. Calyx saucer-shaped, ca. 1.5 mm in diam., densely soft strigose, shallowly 4-lobed; lobes semiorbicular, ca. 0.5 mm long. Corolla white, no blotches, openly tubular-funnelform, 8–12 mm long and wide, dissected 1/2 corolla length into 4 lobes; tube 2–3 mm long, ca. 2 mm wide, glabrous outside, pilose on upper inside; lobes elliptic to oblong, rounded, 3–5 mm long, 2–4 mm wide. Stamens 4, subequal, 6–9 mm long, exerted; filaments densely pilose on lower three-quarters; anthers yellow, oblong, ca. 1 mm long. Ovary ovoid, densely soft strigose, ca. 2 mm. Style 5–6 mm long, exerted, glabrous. Capsule ovoid, 3–4 mm long, 2.5 mm wide, densely strigose.

Distribution. SOUTH KOREA: Jeollabuk-do, Gyeongsangnam-do.

Ecology. The plants inhabit mountain ridges and slopes at altitudes over 800 m above sea level. Flowering specimens have been collected from June to August; fruiting

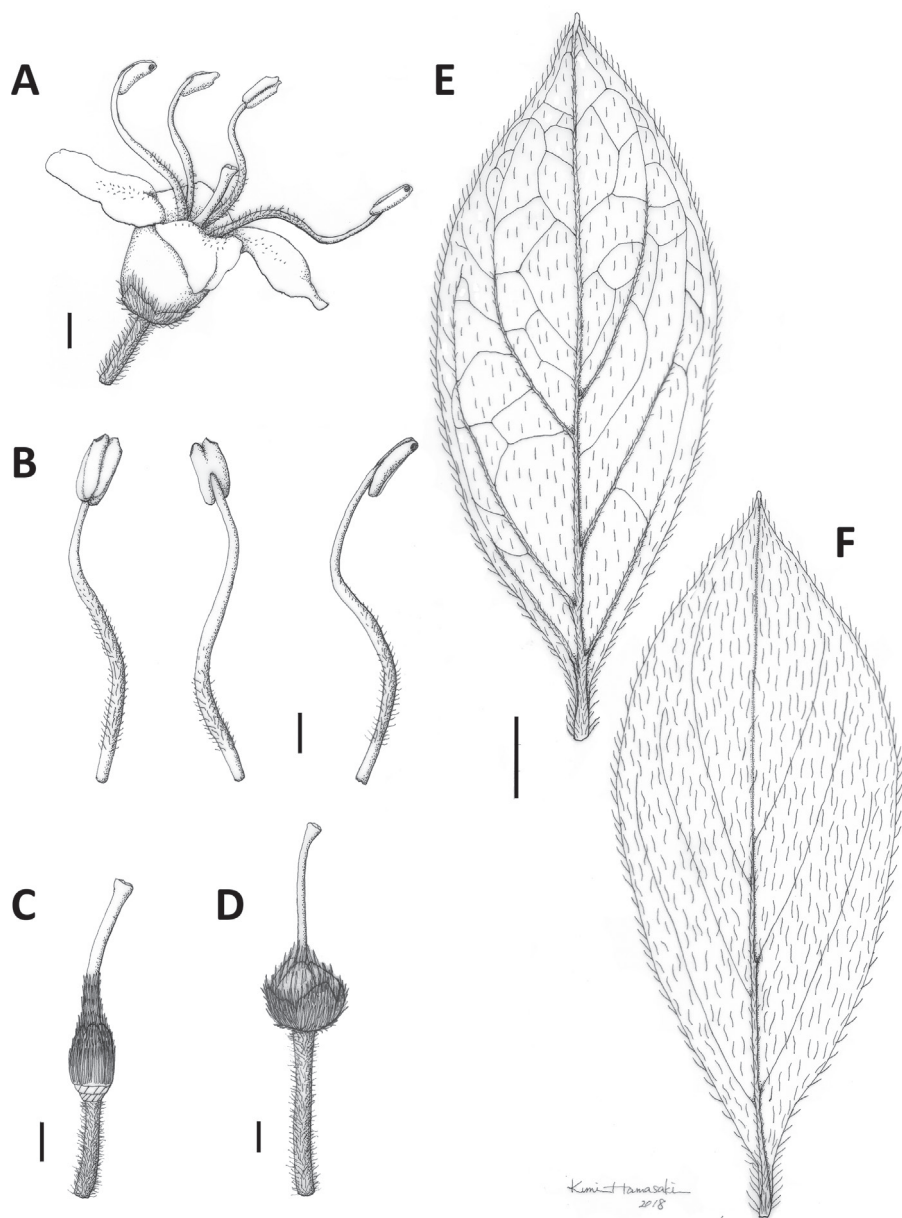


Figure 8. *Rhododendron sohayakiense* var. *koreanum*. **A** Flower, side view **B** Stamen **C** Pistil **D** Fruit **E** Abaxial and **F** adaxial sides of leaf. Scale bars: 1 mm (**A–D**); 3 mm (**E, F**). Drawings by Kumi Hamasaki from Y. Watanabe, S. Hwang and N. Yun W0101 (holotype, TNS).

specimens have been collected from October. Honeybees are frequent visitors to the flowers, suggesting that they are pollinators of the variety.

Etymology. The specific epithet refers to ‘Korea’ where the new variety is distributed.

Key to the *Rhododendron tschonoskii* alliance, expanded from Yamazaki (1996)

- 1 Corolla 4 lobes.....2
- Corolla 5 lobes.....6
- 2 Corolla tubiform.....*R. tetramerum*
- Corolla tubular-funnelform3
- 3 Spring leaves 25–50 mm long at maximum within each individual.....4
- Spring leaves 10–20 mm long at maximum within each individual.....5
- 4 Lateral nerves of spring leaf 1 paired, prominently raised abaxially, grooved adaxially, nervules reticulate*R. trinerve*
- Lateral nerves of spring leaf 1–3 paired, raised abaxially, not grooved adaxially*R. sobayakiense* var. *koreanum*
- 5 Style 4–10 mm long, exserted from corolla; lateral nerves of spring leaf obscurely raised.....*R. sobayakiense* var. *sobayakiense*
- Style 3–4 mm long, included within corolla; lateral nerves of spring leaf raised*R. sobayakiense* var. *kiusianum*
- 6 Corolla tubular-funnelform, tube 2–4 mm long; style 6–13 mm long, exserted from corolla.....*R. tschonoskii*
- Corolla tubiform, tube 5–7 mm long; style 4–5 mm long, included within corolla.....*R. tsusiophyllum*

Data accessibility

DNA sequences of chloroplast DNA haplotypes reported in this study were deposited in GenBank under accession numbers; LC499847–LC499863 for *trnL-F*, LC499864–LC499880 for *trnL* intron, LC499830–LC499846 for *trnS-G*, LC499813–LC499829 for *trnG* intron, LC499796–LC499812 for *rpl32-trnL*. Genotype data for RAD-seq were deposited in Dryad: <https://doi.org/10.5061/dryad.5tm6680>.

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

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Abstract

Isotrema cangshanense X.X.Zhu, H.L.Zheng & J.S.Ma, a new species from western Yunnan, China, is described and illustrated here. It is similar to *I. utriforme*, *I. forrestianum*, *I. cucurbitoides* and *I. obliquum*. The major differences between them are outlined and discussed. A detailed description, along with line drawings, photographs, habitat and distribution, as well as a comparison to morphologically similar species, is also provided. Meanwhile, the new taxon is assessed as Vulnerable (VU D2), according to the IUCN Red List criteria.

Keywords

Aristolochia, *Isotrema*, morphology, subgenus *Siphisia*, taxonomy

Introduction

Aristolochia L. (s. l.) consists of more than 550 species (González 2012; Zhu et al. 2019a) and is the largest genus in Aristolochiaceae (Hwang et al. 2003). Three subgenera: subgenus *Aristolochia*, subgenus *Siphisia* (Duch.) Schmidt and subgenus *Pararistolochia* (Hutch. & Dalziel) Schmidt are recognised, based on morphological and mo-

lecular data (Wanke et al. 2006). Recently, one of the subgenera, *Aristolochia* subgen. *Siphisia* was reinstated to be an independent genus, *Isotrema* Raf., with morphological synapomorphies, such as strongly curved perianth, 3-lobed gynostemium, anthers paired on the outer surface of each gynostemium segment (Zhu et al. 2019a). In China and neighbouring countries, several species belonging to *Isotrema* have been described in recent years (Liu and Deng 2009; Xu et al. 2011; Yao 2012; Huang et al. 2013, 2015; Wu et al. 2013, 2015; Do et al. 2014, 2015a, b, c, d, 2016, 2017, 2019; Huong et al. 2014; Lu and Wang 2014; Ohi-Toma et al. 2014; Zhu et al. 2015, 2016, 2017a, b, 2018, 2019b; Gong et al. 2018; Yang et al. 2018; Li et al. 2019; Peng et al. 2019; Zhou et al. 2019). Additionally, a useful key to Asian species of *Isotrema* (*Aristolochia* subgenus *Siphisia*) is provided by Do et al. (2015a).

During an expedition to Yangbi County, western Yunnan, an unknown species of *Isotrema* was collected. Subsequent examination of herbarium specimens and study of the related literature (Hwang 1988; Ma 1989a, b; Tao 1997; Hwang et al. 2003; Do et al. 2015a; Do and Nghiem 2017; Yang et al. 2018; Zhu et al. 2019a) reveals that it is a new species described and illustrated here.

Taxonomy

***Isotrema cangshanense* X.X.Zhu, H.L.Zheng & J.S.Ma, sp. nov.**

urn:lsid:ipni.org:names:77202594-1

Figures 1–3, 4A–C

Type. CHINA. Yunnan: Yangbi County, The Cangshan Mountain, Sancha River, 25°41'49"N, 100°02'55"E, 2239 m a.s.l., 23 April 2019, X. X. Zhu et al. ZXX19353 (holotype: CSH [CSH-0164770!]; isotypes: CSH!, KUN!).

Diagnosis. *Isotrema cangshanense* is morphologically similar to *Isotrema utriforme* (S. M. Hwang) X. X. Zhu, S. Liao & J. S. Ma, *I. forrestianum* (J. S. Ma) X. X. Zhu, S. Liao & J. S. Ma, *I. cucurbitoides* (C. F. Liang) X. X. Zhu, S. Liao & J. S. Ma and *I. obliquum* (S. M. Hwang) X. X. Zhu, S. Liao & J. S. Ma (Zhu et al. 2019a), but is distinguishable from these species by the following diagnostic characters: laminas oblong-lanceolate; calyx outside light yellow; limb narrow-ovoid, 2.4–3 × 0.9–1 cm, asymmetric, forming an acute angle with the upper part of the tube, 3-lobed, upper part separated to the middle, lower part shallowly lobed, inside black purple, net-shaped protruding stripes; throat ca. 5 mm in diam. Detailed morphological comparisons are shown in Table 1 and Figure 4.

Description. Woody liana. Stems terete, young shoots pubescent. Petioles 0.7–3.5 cm long, pubescent to almost glabrous; laminas oblong-lanceolate, 6–20 × 1–7 cm, adaxially almost glabrous, abaxially villous, base auriculate, sinus 0.7–1.8 cm deep, apex acute, margin entire; basal veins 2–3 pairs, palmate, 2–3 pairs from base, lateral veins 12–18 pairs, pinnate. Flowers in axils of leafy shoots or, on older stems, solitary or in fascicles, each fascicle with 2–6 flowers; pedicels 1.5–4 cm, pubescent partly villous; bracteole 1, lanceolate, 2–5 × 1–2 mm, adaxially glabrous, abaxially densely villous, inserted below the middle of pedicel. Calyx tube geniculately curved,

Table 1. Morphological comparisons of *Isotrema cangshanense* with *I. utrifforme*, *I. forrestianum*, *I. cucurbitoides* and *I. obliquum*.

| Characters | <i>I. cangshanense</i> | <i>I. utrifforme</i> | <i>I. forrestianum</i> | <i>I. cucurbitoides</i> | <i>I. obliquum</i> |
|--------------------------|---|--|---|---|--|
| Lamina | oblong-lanceolate, 6–20 × 1–7 cm, base auriculate, sinus 0.7–1.8 cm deep | ovate-lanceolate, 10–17 × 3–4 cm, base cordate, sinus 1–1.5 cm deep | ovate to narrowly ovate, 7–21 × 3–10.5 cm, base cordate, sinus 1.5–2 cm deep | trullate-lanceolate, ovate-lanceolate or lanceolate, 12–22 × 2.5–4.5 cm, base auriculate, sinus 1–2 cm deep | oblong-lanceolate to narrowly ovate, 12–16 × 4–6.5 cm, base cordate, sinus 1–1.5 cm deep |
| Inflorescence and flower | flowers in axils of leafy shoots or, on older stems, solitary or in fascicles, each fascicle with 2–6 flowers | flowers in axils of leafy hoots, solitary | flowers in axils of leafy shoots, solitary or, on older stems, solitary or in fascicles, each fascicle with 2–4 flowers | flowers in axils of leafy shoots, solitary | flowers in axils of leafy shoots, solitary |
| Calyx outside | light yellow | light yellow | light brown or purple | undocumented | yellowish white or pinky white |
| Limb shape | saccate, narrow-ovoid, asymmetric, forming acute angle with upper tube | saccate, ovoid, slightly asymmetric, straight extended from upper tube | saccate, cylinder, asymmetric, forming almost right angle with upper tube | cylindric, straight extended from upper tube | not saccate or cylindric, forming right angle with upper tube |
| Limb size | 2.5–3 × 0.7–0.8 cm | 1–2 × 0.5–1.5 cm | 6–8 × 1.5–2 cm | 2 × 0.2–0.3 cm | 1.2–1.3 × 0.8–0.9 cm |
| Limb lobes | 3-lobed, upper part separated to the middle, lower part shallowly lobed, inside black purple, net-shaped protruding stripes | 3-lobed, shallowly lobed, inside black purple, sparse processes | 3-lobed, upper part separated to middle, lower part shallowly lobed, inside black purple, densely spinous outgrowths | 3-lobed, shallowly lobed, inside undocumented | 3-lobed, deeply lobed, slightly asymmetric, inside light brown, smooth |
| Throat | ca. 5 mm in diam. | ca. 1 mm in diam. | ca. 3 mm in diam. | ca. 1 mm in diam. | ca. 6 mm in diam. |

outside light yellow, abaxially sparsely pubescent partly villous; basal tube 1.8–2.5 × 0.5–0.6 cm, inside black purple, densely villous at base; upper tube 1.3–1.6 × 0.6–0.8 cm, inside black purple at base, light yellow towards apex, light red at upper part; limb saccate, narrow-ovoid, asymmetric, forming acute angle with upper part of the tube, 2.5–3 × 0.7–0.8 cm, 3-lobed, upper part separated to the middle, lower part shallowly lobed, upper two lobes triangular-lanceolate, 1.2–1.5 × 0.4–0.5 cm, lower lobe triangle, 0.4–0.7 × 0.4–0.5 cm, inside black purple, net-shaped protruding stripes; throat ca. 5 mm in diam. Anthers 6, oblong, ca. 1.5 mm long, adnate in 3 pairs to base of gynostemium, opposite to lobes. Gynostemium ca. 2.7 × 2.5 mm, 3-lobed. Ovary terete, ca. 10 mm long, densely villous. Capsule obovate-elliptic, ca. 4.5 × 2 cm. Seeds obovate-elliptic, ca. 5 × 4 mm, not winged, the adaxial surface deeply concave and the abaxial surface convex, both surfaces glabrous.

Phenology. Flowering from April to May, fruiting is predicted from July to August (we have seen is just two residual fruit from last year).

Etymology. The specific epithet derives from the type locality, The Cangshan Mountain, Yangbi County, western Yunnan, south-western China. The Chinese name is given as “苍山关木通”.

Distribution and habitat. The new species is currently known only from the Cangshan Mountain, Yangbi County, Yunnan, China. It grows in forests at an elevation between 2239 m and 2379 m, together with *Castanopsis* sp. (Fagaceae), *Disporum* sp. (Colchicaceae), *Notochaete hamosa* Benth. (Lamiaceae), *Photinia* Lindl. (Rosaceae), *Rubus* sp. (Rosaceae) etc.

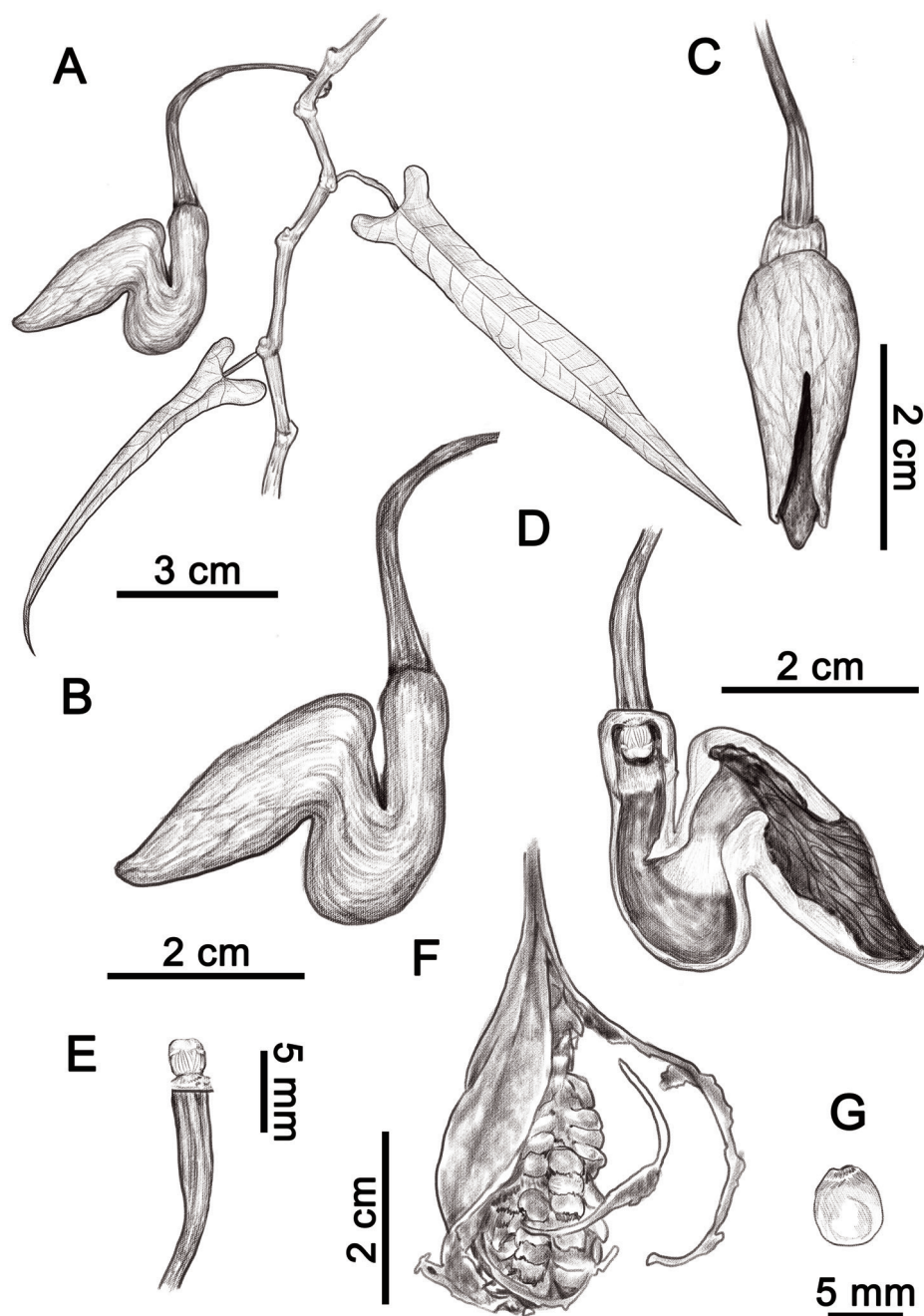


Figure 1. *Isotrema cangshanense* X.X.Zhu, H.L.Zheng & J.S.Ma, sp. nov. **A** habit **B** flower (lateral view) **C** flower (front view) **D** opened flower (showing the inner structure) **E** anthers and gynostemium **F** the dehiscent capsule **G** seed. Illustration by Shizhen Qiao.

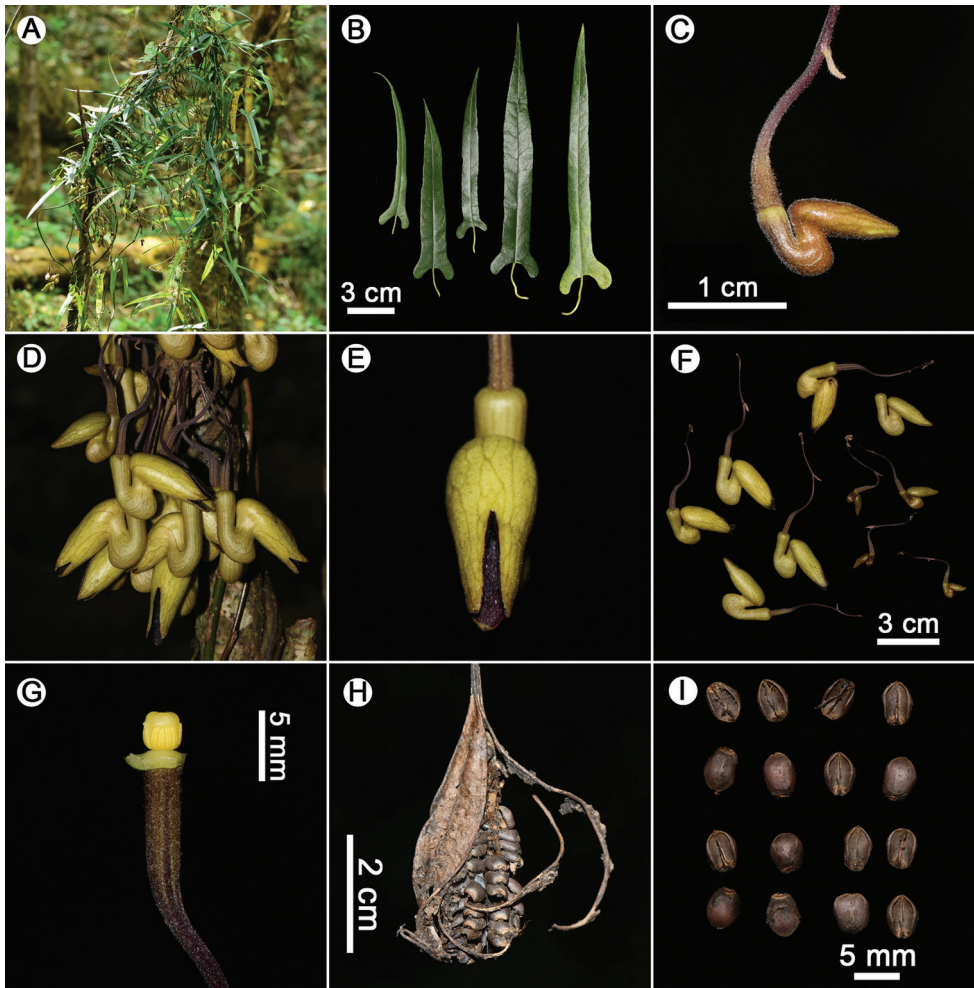


Figure 2. *Isotrema cangshanense* X.X.Zhu, H.L.Zheng & J.S.Ma, sp. nov. **A** habit **B** leaves **C** flower bud **D** inflorescence **E** flower (front view) **F** flowers (lateral view) **G** anthers and gynostemium **H** the dehiscent capsule **I** seeds. Photographed by Xinxin Zhu.

IUCN Red List category. Since *Isotrema cangshanense* is known from one population only, with fewer than ten individuals, the new species is assigned a preliminary status of Vulnerable (VU D2) according to IUCN Red List Criteria (IUCN 2012), indicating a population with a very restricted area of occupancy (typically less than 20 km²) or number of locations (typically five or fewer). Although the area is under protection as a national nature reserve, habitat disturbance brought about by human activities, such as grazing and felling, may have a negative impact on the new species.

Specimens examined (Paratypes). CHINA. Yunnan: Yangbi County, The Cangshan Mountain, 2300 m a.s.l., 23 April 2019, X. X. Zhu *et al.* ZXX19354 (CSH!); the same location, 2379 m a.s.l., 23 April 2019, X. X. Zhu *et al.* ZXX19355 (CSH!).



Figure 3. Holotype of *Isotrema cangshanense* X. X.Zhu, H.L.Zheng & J.S.Ma, sp. nov. (CSH-0164770).

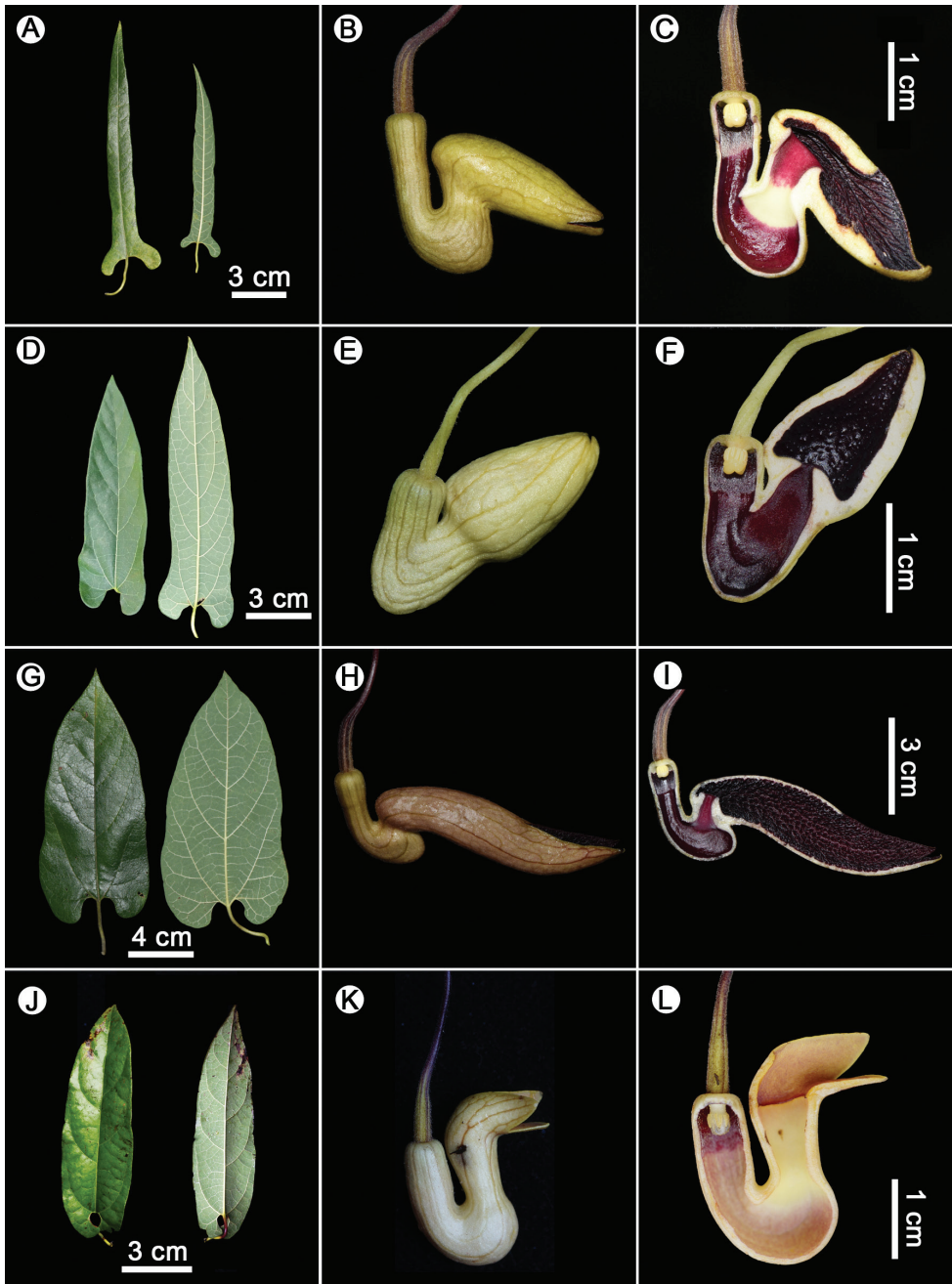


Figure 4. **A–C** *Isotrema cangshanense* X.X.Zhu, H.L.Zheng & J.S.Ma, sp. nov. **A** leaves **B** flower (lateral view) **C** longitudinal section of flower (showing the inside structure) **D–F** *Isotrema utrifforme* (S. M. Hwang) X. X. Zhu, S. Liao & J. S. Ma **D** leaves **E** flower (lateral view) **F** longitudinal section of flower (showing the inside structure) **G–I** *I. forrestianum* (J. S. Ma) X. X. Zhu, S. Liao & J. S. Ma **G** leaves **H** flower (lateral view) **I** longitudinal section of flower (showing the inside structure) **J–L** *I. obliquum* (S. M. Hwang) X. X. Zhu, S. Liao & J. S. Ma **J** leaves **K** flower (lateral view) **L** longitudinal section of flower (showing the inside structure). **A–C, G–I** Photographed by Xinxin Zhu **D–F** photographed by Lei Cai; **J–L** photographed by Yuan Wang.

Discussion

Isotrema cangshanense has a horseshoe-shaped perianth, a 3-lobed gynostemium, each lobe fused with one pair of oblong stamens which are characteristics for the genus *Isotrema* (Zhu et al. 2019a). The new discovery, along with many new species recently discovered from China (Liu and Deng 2009; Xu et al. 2011; Huang et al. 2013, 2015; Wu et al. 2013, 2015; Lu and Wang 2014; Do et al. 2015a; Zhu et al. 2015, 2016, 2017a, b, 2018, 2019b; Gong et al. 2018; Yang et al. 2018; Li et al. 2019; Peng et al. 2019; Zhou et al. 2019), provide evidence that the genus *Isotrema* is very diverse in China. Moreover, referring to Ohi-Toma et al. (2014), Do et al. (2015a), Li et al. (2019), Peng et al. (2019), Zhou et al. (2019) and Zhu et al. (2019a, b), there are 64 species and one subspecies of *Isotrema* in China. It is predicted that more new species of *Isotrema* will be discovered when more field investigations are conducted in this region.

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***Sedum lipingense* (Crassulaceae) identifying a new stonecrop species in SE Guizhou, China, based on morphological and molecular evidence**

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Abstract

We describe and illustrate *Sedum lipingense* (Crassulaceae), a new species of stonecrop found in the limestone areas of SE Guizhou, China. Based on the presence of adaxially gibbous carpels and follicles, this taxon belongs to sect. *Sedum* S.H. Fu. The new species superficially resembles *S. subtile* Miquel and *S. bulbiferum* Makino but differs from these two taxa in its development of a basal leaf rosette during florescence. The nrDNA internal transcribed spacer (ITS) sequences also support the claim that this plant is a new species in the *Sedum* genus.

Keywords

flora of Guizhou, karst, limestone flora, new taxon, *Sedum lipingense*

Introduction

Sedum Linnaeus is the largest genus in the Crassulaceae family, containing about 430 species, with the greatest diversity centering in eastern Asia (Thiede and Eggli 2007, Ito et al. 2017a). Approximately 121 *Sedum* species (91 endemics) occur in China, and 49 of these species (34 endemics) belong to sect. *Sedum*, a subclass which possess adaxially gibbous carpels and follicles (Wu et al. 2013). There are 23 species within five genera of Crassulaceae found in the Guizhou Province (Li et al. 1985). From 2005, a number of new species of *Sedum* were reported across mainland China, in areas includ-

ing Zhejiang (Wang et al. 2005, Jin et al. 2010, 2013), Anhui (Xie et al. 2014, Chen et al. 2017) and the Guizhou Province (Yang et al. 2012). In China, only a few species in this genus retain rosette leaves during florescence, such as *S. balfourii* Hamet and *S. drymarioides* var. *saxifragiforme* X. F. Jin & H. W. Zhang. *Sedum balfourii* was formerly placed in sect. *Aizoon*, within the genus *Sedum* (Fu and Fu 1984), but was then moved to the genus *Ohbaea* (Raymond-Hamet) V. V. Byalt & I. V. Sokolova (Fu and Ohba 2001) based on its conspicuous lateral flowering stems that derive from rosettes during florescence.

During our fieldwork, a new species of *Sedum* was discovered in Liping County, Qiandongnan Prefecture, Guizhou Province, China. This particular species has conspicuous rosettes during florescence, an attribute similar to *O. balfourii*. However, the new species differs from *O. balfourii* as it possesses central flowering stems rather than lateral ones (Fig. 2D). It also differs from *S. drymarioides* var. *saxifragiforme*, a species which is glandular-pubescent throughout, despite its rosette leaves. Based on its adaxially gibbous carpels, we place the new species in Sect. *Sedum*. Macro-morphological character studies indicated that this species is also somewhat similar to *S. subtile* Miquel and *S. bulbiferum* Makino, sharing a number of traits with these species, including opposite leaves on proximal stems and alternate leaves mainly on distal stems. We conducted morphological comparisons and molecular phylogenetic analysis to elucidate the presumed new *Sedum* species.

Materials and methods

All morphological characters were measured using dissecting microscopes. Specimen checking was done at PE, IBK, ZY, with the additional use of some web database, including the Plant Photo Bank of China (<http://ppbc.iplant.cn/>) and Global Plants (<http://plants.jstor.org/>).

Leaf material from the presumed new species was collected in the field, and immediately dried in silica gel for DNA extraction. The nuclear ribosomal internal transcribed spacer (ITS) regions were used as molecular markers. ITS-F (TGAACCTGCGGAAGGATCAT) and ITS-R (GGTAGTCCCGCCTGACCTG) primers (Wu et al. 2013) were selected to amplify the ITS sequences. DNA extraction and PCR amplification of the new species followed the procedure of Wu et al. (2013). Primer synthesis and PCR product sequencing were carried out at the Shanghai Sangon Biotech Institute, China.

The ITS sequence of the new species, as well as the ITS sequences of the congeners downloaded from GeneBank (Table 1), were aligned using MEGA7 and then manually adjusted. Bayesian inference was implemented using MrBayes v3.2.6. Prior to the Bayesian analysis, the Akaike information criterion (AIC) implemented in mrModelTest v1.0 was used to select the best-fit model (GTR+I+G) of molecular evolution. For the BI analyses, four Markov Chain Monte Carlo (MCMC) chains were run, sampling one tree every 100 generations for 2,000,000 generations starting with a random tree (Xie et al. 2014). When the log-likelihood scores were found to have stabilized, a

Table 1. Accession information relating to internal transcribed spacer (ITS) sequences downloaded from GeneBank.

| Species | Voucher | Accession no. |
|--------------------------------|---------------------------|---------------|
| <i>Aeonium lancerottense</i> | MEM 1518 | AY082143 |
| <i>Aeonium viscatum</i> | MEM 1432 | AY082154 |
| <i>Greenovia aizoon</i> | MEM 1425 | AY082112 |
| <i>Sedum alfredii</i> | WUK415208 | FJ919953 |
| <i>Sedum baileyi</i> | LBG0064555 | FJ919935 |
| <i>Sedum bergeri</i> | Ni et al. | AY352897 |
| <i>Sedum bulbiferum_416</i> | Ito416 | LC229234 |
| <i>Sedum bulbiferum_bs41</i> | 130514bs41 | KM111166 |
| <i>Sedum bulbiferum_qz09</i> | 130524qz09 | KM111165 |
| <i>Sedum emarginatum</i> | 130512hs27 | KM111145 |
| <i>Sedum erici-magnusii</i> | Ito 2077 | LC229235 |
| <i>Sedum erythrospermum</i> | Tsutsumi 504 | AB906473 |
| <i>Sedum formosanum</i> | Ito 1260 | LC229279 |
| <i>Sedum hakonense</i> | S. Mayuzumi C00005 | AB088625 |
| <i>Sedum hangzhouense</i> | Ito2604 (TNS) | LC260130 |
| <i>Sedum japonicum</i> | Kokubugata 16749 | AB906475 |
| <i>Sedum jiulungshanense</i> | CMQ20150076 | LC229243 |
| <i>Sedum kiangnanense</i> | Ito 1030 | LC229244 |
| <i>Sedum lineare</i> | Mayuzumi C00120 | AB088623 |
| <i>Sedum lipingense</i> | ZRB1479 | MN150061 |
| <i>Sedum lungtsuanense</i> | Ito3563 | LC260131 |
| <i>Sedum makinoi</i> | Kokubugata 16730 | AB906476 |
| <i>Sedum mexicanum</i> | Ito 647 | LC229247 |
| <i>Sedum morrisonense</i> | Ito2765 | LC229290 |
| <i>Sedum multicaule</i> | Miyamoto et al. TI9596136 | AB088631 |
| <i>Sedum nagasakianum</i> | Ito2064 | LC229249 |
| <i>Sedum nokoense</i> | Kokubugata 10426 | AB906478 |
| <i>Sedum oligospermum</i> | CMQ 74 | LC229250 |
| <i>Sedum oreades</i> | G. Y. Rao 090803-03 | KF113733 |
| <i>Sedum polytrichoides</i> | CMQ1057 | LC229251 |
| <i>Sedum rupifragum</i> | Ito 2070 | LC229254 |
| <i>Sedum sarmentosum</i> | Ito 978 | LC229255 |
| <i>Sedum satumense</i> | Ito2295 | LC229256 |
| <i>Sedum trullipetalum</i> | 9420132 | AB088630 |
| <i>Sedum subtile_1999</i> | A. Shimizu 1999 | AB088622 |
| <i>Sedum subtile_2259</i> | Ito2259 | LC229257 |
| <i>Sedum subtile_624</i> | Ito 624 | AB930277 |
| <i>Sedum taiwanianum</i> | Ito2770 | LC229297 |
| <i>Sedum tetractinum</i> | Ito3623 | LC260135 |
| <i>Sedum tianmushanense</i> | LP 67 | LC229261 |
| <i>Sedum tosaense</i> | Kokubugata 16726 | AB906483 |
| <i>Sedum triactina</i> | 9596091 | AB088629 |
| <i>Sedum tricarpum</i> | Ito 2269 | LC229259 |
| <i>Sedum trullipetalum</i> | Miyamoto et al.9420132 | AB088630 |
| <i>Sedum truncastigmum</i> | Ito3254 | LC229306 |
| <i>Sedum yabeianum</i> | S. Mayuzumi C00029 | AB088626 |
| <i>Sedum zentaro-tashiroi</i> | H. Ohba 1998 | AB088619 |

consensus tree was calculated after omitting 5,000 sampled trees as burn-in. *Aeonium lancerottense*, *A. viscatum* and *Greenovia aizoon* were selected as the outgroups referring to Ito et al. (2017b).

Results

Molecular analyses

In this study, the sequences of 40 species (44 samples) were treated as ingroups. Sequence length was 584 bp for the ITS region, of which 234 characters were constant, 45 characters were parsimony-uninformative and 305 characters were parsimony-informative.

The sequence of the ITS region taken from *S. lipingense* aligned with the genus *Sedum*, confirming its generic identity (Fig. 1). The new species was resolved as sister to *S. bulbiferum* (Bayesian posterior probabilities (PP) was 97) but turned out to be

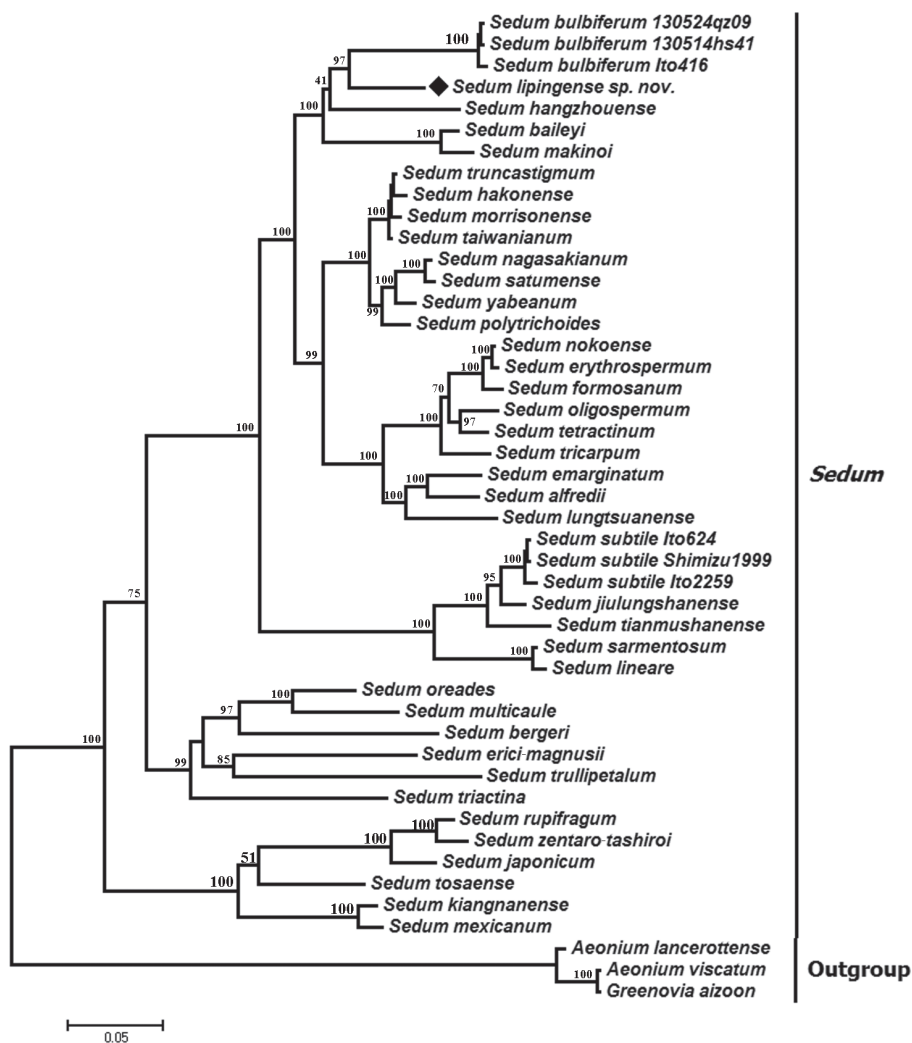


Figure 1. Bayesian phylogenetic tree based on ITS sequence for genus *Sedum* related to *S. lipingense* and three outgroups. Bayesian posterior probabilities are shown.

genetically distant from *S. subtile*. There were 50 nucleotides differ between *S. lipingense* and *S. bulbiferum*, suggesting the high variation compared to the closest relatives was remarkable.

S. lipingense and *S. bulbiferum* were found to be nested with *S. hangzhouense* (PP = 41, suggesting a weak support), and then to be nested with *S. baileyi* and *S. makinoi* (PP = 100), all species with alternate or opposite stem leaves. Except for *S. lipingense*, the above four (or perhaps two-three) species were also clustered as a distinct clade (Wu et al. 2013, Xie et al. 2014, Ito et al. 2017a), suggesting that the four species are closely related. *Sedum lipingense* is a close member to this clade, but these species form a polytomy and it is hard to say for sure, which one is the closest relative of *S. lipingense*. *Sedum subtile* is not within the same clade as *S. bulbiferum*, *S. hangzhouense*, *S. baileyi*, and *S. makinoi* (Wu et al. 2013) or with *S. hangzhouense*, *S. baileyi*, and *S. makinoi* (Ito et al. 2017a), suggesting that the relationship between *S. subtile* and *S. lipingense* is relatively distant.

Taxonomy

***Sedum lipingense* R.B. Zhang, D. Tan & R.X. Wei, sp. nov.**

urn:lsid:ipni.org:names:77202732-1

Figs 2–4

Diagnosis. *S. lipingense* can be distinguished from the closely related *S. subtile* and *S. bulbiferum* by the presence of rosettes, absent sterile shoots and bulbils, subequal lanceolate-oblong sepals, and other traits (Table 2).

Type. CHINA. Guizhou Province, Kaili City, Liping County, Mengyan Township, on moist rocks, 26°07'N, 108°42'E, 800 m alt., 13 April 2019, ZRB1479 (fl., holotype ZY!, isotype IBK!), 16 June 2019, ZRB1495 (fr., paratype ZY!)

Description. Biennial (or perennial?) herb. **Sterile stems** absent. **Rosette** present during florescence; rosette leaves alternate, broadly obovate, base attenuated and shortly spurred, 0.5–1.5 × 0.4–0.7 cm. **Flowering stems** 1 to 3 (–4), erect, slender, 3–7 cm; single stems shoot from rosette centers, others shoot from the rosette leaf axils; lateral proximal leaves sometimes opposite, akin to rosette leaves but smaller, 0.6–0.8 × 0.3–0.5 cm, base shortly spurred; distal leaves alternate, spatulate-obovate to spatulate-ob lanceolate, 0.7–1.2 × 0.3–0.4 cm, apex obtuse, base shortly spurred. **Cymes** scorpioid, 2 to 3 branched; branches 1 to 2 flowered; bracts obliquely oblanceolate, apex obtuse, 4–9 × 2–4 mm. **Sepals** 5, lanceolate-oblong, subequal, ca. 2 mm, base shortly spurred, apex obtuse. **Petals** 5, yellow, broadly lanceolate, ca. 4 mm, apex mucronate. **Stamens** 10; antesealous one ca. 3 mm; antepetalous one inserted ca. 1 mm above petal base, slightly shorter than the antesealous stamens. **Nectar scales** broadly cuneate, ca. 0.6 × 0.4 mm, apex truncate. **Carpels** erect, lanceolate, ca. 3.5 mm, base connate for ca. 1 mm. **Styles** slender, ca. 1 mm. **Follicles** stellately divergent at maturity. **Seeds** oblong, ca. 0.6 mm, papillate.

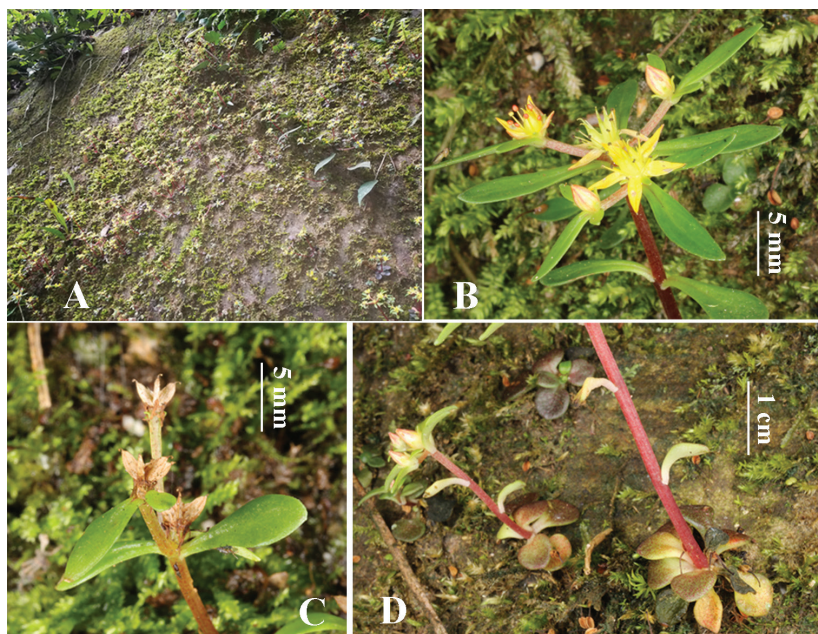


Figure 2. *Sedum lipingense* **A** natural habitat **B** 3-branched scorpioid cyme **C** follicles and bracts **D** single flowering stems derived from rosette centers. Charted by Ren-Bo Zhang.

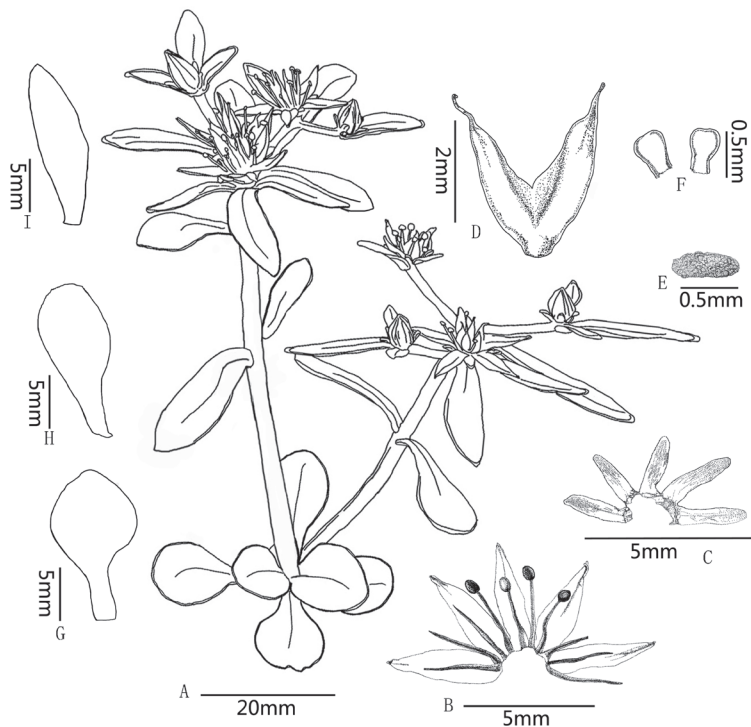


Figure 3. *Sedum lipingense* **A** flowering plant **B** opened corolla **C** sepals **D** two follicles **E** seed **F** nectar scales **G** rosette leaf **H** distal leaf **I** bract of flower. Drawn by Tan Deng.

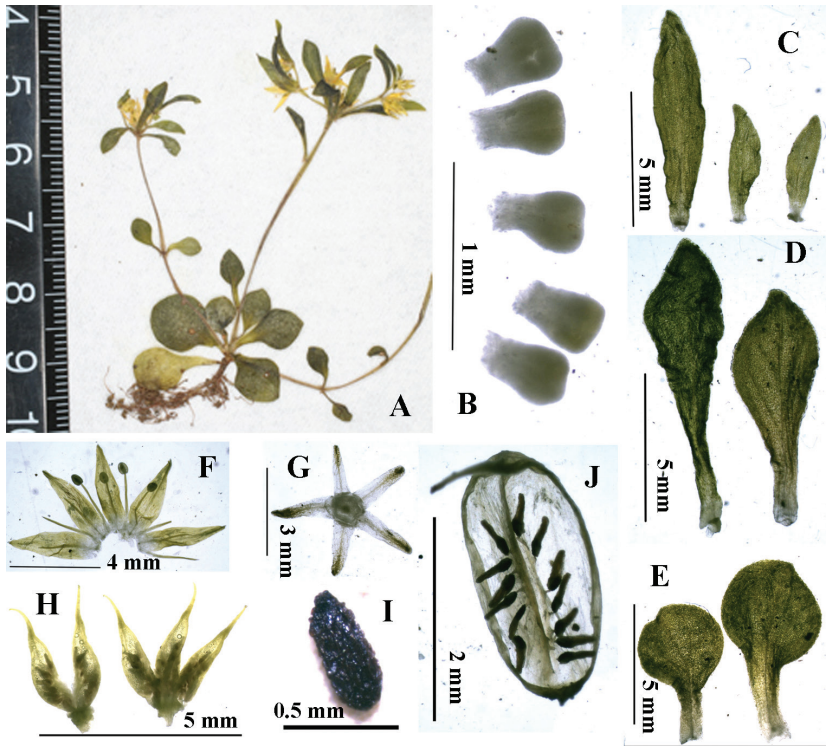


Figure 4. *Sedum lipingense* **A** rosette, central and lateral flowering stems **B** nectar scales **C** bracts of flowers **D** distal leaves **E** rosette leaves **F** opened corolla **G** sepals **H** split carpels **I** seed. Charted by Ren-Bo Zhang.

Table 2. Comparing the diagnostics of *Sedum lipingense* sp. nov., *S. subtile* and *S. bulbiferum*.

| Traits | | <i>S. lipingense</i> | <i>S. subtile</i> | <i>S. bulbiferum</i> |
|-----------------------------------|------------------|---|--|-------------------------------------|
| Rosette leaves during florescence | | present | absent | absent |
| Sterile shoots | | absent | present | absent |
| Flowering stem | | 3–7 cm | 5–10 cm | 7–22 cm |
| Proximal stem leaves | Phyllotaxy | alternate, sometimes opposite on lateral flowering stem | opposite or 3–6-verticillate | opposite |
| | Leaf blade | broadly obovate | obovate | ovate-spatulate |
| Distal stem leaves | Phyllotaxy | alternate (sometimes subopposite) | alternate | alternate |
| | Leaf blade | spatulate-oblancoelate | oblancoelate-linear | spatulate-oblancoelate |
| | Bulbils in axils | absent | absent | present |
| Cymes | Branches | (2-) 3 | 2- or 3-branched | 3-branched, branches 2-forked |
| | Branch flowers | 1- to two | 3- to several | many |
| Sepals | | lanceolate-oblong, subequal | broadly linear to narrowly lanceolate, unequal | lanceolate to oblancoelate, unequal |
| Nectar scales | | broadly cuneate, ca. 0.6 × 0.4 mm, apex truncate | broadly cuneate, ca. 0.4 × 0.5 mm, apex truncate | obovate, ca. 0.6 mm |
| Carpels | | ca. 3.5 mm base connate for ca. 1 mm | ca. 5 mm base connate for ca. 2 mm | ca. 4 mm base connate for ca. 1 mm |
| Styles | | ca. 1 mm | ca. 2 mm | ca. 1 mm |
| Fl. | | Apr–May | Apr–Jun | Apr–May |
| Fr. | | May–Jun | Jul–Aug | Jun–Jul |

Distribution and habitat. At this time, based on our field observations, *Sedum lipingense* is only known to occur in Longxi village, Mengyan town, Liping County, Guizhou Province. It grows on moist limestone rocks, at ca. 800 m altitude, in groups of several hundred individuals.

Conservation status. This species is currently known to occur in a single valley and we suggest its placement in the Data Deficient category of IUCN (2017).

Phenology. This new species was observed flowering from April to May and fruiting from May to June.

Etymology. The specific epithet '*lipingense*' is derived from the plant's locality, Liping County, Guizhou Province, China.

Acknowledgments

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