

***Novitates neocaledonicae* X: A very rare and threatened new microendemic species of *Acropogon* (Malvaceae, Sterculioideae) from New Caledonia**

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Abstract

A new species, *Acropogon horarius* Gâteblé & Munzinger, **sp. nov.** (Malvaceae, Sterculioideae), is described from New Caledonia. It is known only from two very small subpopulations in the rainforests of the Petchécara Pass between Thio and Canala, in the southeast of Grande-Terre, New Caledonia's main island. This shrub to small tree has hastate leaves and minute sessile tubular whitish-yellowish flowers and is strikingly different from all other members of the genus. The type locality is geologically complex and located within one of only four amphibolite lenses known in New Caledonia. A line drawing and colour photos are provided for the new species, along with a preliminary risk of extinction assessment, which indicates that the species is Critically Endangered.

Keywords

Acropogon, geology, Malvaceae, New Caledonia, new species, Sterculioideae, taxonomy, threatened species

Introduction

The endemic New Caledonian genus *Acropogon* Schltr. (Malvaceae, Sterculioideae) comprises 26 currently recognised species (Munzinger and Gâteblé 2017). A putatively undescribed species of *Acropogon* was recently found by Jean-Jacques Villegente and Jacqueline Ounémoa in the rainforests of the Petchécara Pass and reported to the

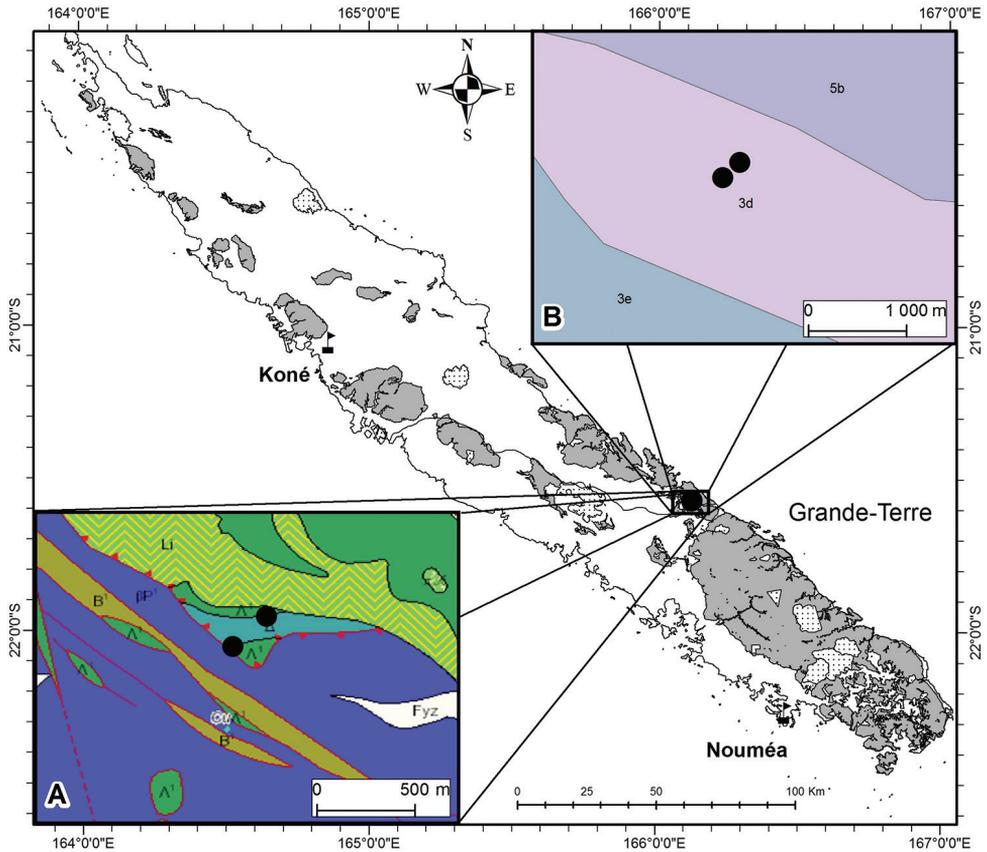


Figure 1. Distribution of *Acropogon horarius* Gâteblé & Munzinger sp. nov. mapped on the geological (A) and soil (B) GIS layers of the Petchécara Pass. Insert A, geology from Gouvernement de la Nouvelle-Calédonie (2018): Λ^1 , serpentinites; Δ , amphibolites; B^1 , undifferentiated poly-metamorphic substrate; βP^1 , undifferentiated basalts and dolerites; Fyz, recent and extant alluvial; Li, listwanites. Insert B, soils from Fritsch (2012): 3d, haplic cambisol combined with lithic leptosol on peridotites; 3e, ferralic cambisol combined with haplic ferralsol on volcano-sedimentary and metamorphic rocks; 5b, posic ferralsol on peridotites.

first author. This part of New Caledonia, along the locally famous and last remaining “scheduled road” or “route à horaire” along which traffic between the towns of Thio and Canala and above the Douthio river alternates one direction then the other, is not very well known from a botanical perspective. The place where the putative new species grows is also interesting geologically as it is at the edge of a peridotite massif, on one of only four rare amphibolite lenses known in New Caledonia (Cluzel et al. 2012) and close to or within a serpentinite vein according to the geological map of the territory (Gouvernement de la Nouvelle-Calédonie 2018; Fig. 1A). In such places of geological complexity, a large diversity of soils derived from the initial parent material can be found and it is often difficult to characterise the soils precisely (Fritsch 2012; Fig. 1B). However, it is important to conserve these contact zones between soil types to maintain evolutionary processes such as speciation and hybridisation (Pillon et al. 2009).

In this paper, we describe and illustrate a morphologically distinctive new *Acropogon* species. We also provide a risk of extinction assessment based on the IUCN Red List Categories and Criteria (IUCN 2017).

Materials and methods

In order to determine whether the material from the Petchécara Pass belonged to a currently recognised species or represented a new member of the genus, we carefully examined and measured living specimens and herbarium material. We focused on morphological characters recognised by previous authors (Schlechter 1906; Morat 1986, 1988; Morat and Chalopin 2003, 2005, 2007; Callmander et al. 2015) as being taxonomically informative within the genus. Undetermined herbarium specimens from MPU, NOU and P were studied to assess whether additional material had also been collected previously. The new species was also observed in the field and under cultivation. In order to obtain specimens of the minute and fragile flowers, a fertile branch (cutting) with flower buds was placed in a propagation house (22 °C ± 2 °C, 95% relative humidity) for one month to gather flowers in alcohol as soon as they opened. Descriptions of colour pertain to material seen in vivo, unless otherwise noted.

Taxonomy

Acropogon horarius Gâteblé & Munzinger, sp. nov.

urn:lsid:ipni.org:names:77191339-1

Figures 2, 3

Diagnosis. *Acropogon horarius* Gâteblé & Munzinger differs from all other members of the genus by the combination of its long and thin petioles, hastate and cordate leaves and minute sessile tubular whitish-yellowish flowers.

Type. NEW CALEDONIA. Province Sud: Thio, Col de Petchécara, route à horaire, 200 m alt., 21°34'41.01"S, 166°07'22.41"E, 25 Aug 2016, G. Gâteblé, J. Ounémoa, M. Moenteapo & E. Poitchili 806 (holotype: P00722668; isotypes: K, MPU311373, NOU088956, P00722670).

Description. Monoecious shrub to small tree up to 6 m tall, sparsely branched with usually a main trunk less than 7 cm in d.b.h.; bark brown, with conspicuous scars left by the caducous cataphylls and sometimes petioles of upper leaves subtending the terminal bud. Leaves clustered at the apex of branches on adult plants, branches 4–5 mm in diameter; petioles light green to yellow (greyish to orange in herbarium material), strongly different in colour than the branch, glabrous, (6.5–)9.0–12.0(–15.5) cm long, 1–2 mm in diameter, elliptical in cross section (in vivo), slightly striate (in herbarium material), enlarged (3–4 mm) proximally and distally, the pulvini slightly pruinose (in vivo); blades simple, coriaceous, flat, slightly discoloured, glabrous on both surfaces, unlobed to slightly hastate on juvenile plants (Gâteblé et al. 804), rarely unlobed to strongly trilobed or

hastate on adult plants, (10.5–)12.5–15.0(–16.0) cm long, (6.0–)11.5–18.0(–23.5) mm wide, base cordate (rarely truncate), apex of the lobes broadly acute to rounded; generally with 3 strong primary palmate veins, primary and secondary veins prominent abaxially, conspicuously different in colour (yellow-orange in herbarium material and light green to yellowish in vivo) than the blade, reticulum visible abaxially (and adaxially in herb.), secondary veins 2–5 pair; tertiary and quaternary veins finely reticulate with scattered crateriform glands 25–35 μm . Inflorescence a reduced spike-like raceme, axillary within, above or just below the terminal cluster of leaves, up to 3.5 cm long, 2.5–3.5 mm in diameter, axes greenish-yellowish, covered with a dense rust-brown indumentum composed of minute stellate trichomes ca. 90 μm x 90 μm , bracts covered with rust-brown tomentum adaxially. Pedicels minute. Male and female flowers seemingly randomly distributed within the inflorescence, solitary, of the same size. Calyx tubular, 4–5 mm long, 2–2.5 mm in diameter, yellowish and with scattered rust-brown stellate trichomes outside, whitish and glabrous inside, lobes 5, triangular, 1–1.5 mm long, ending in an apical appendage 0.5–1 mm long, interior margins of the lobes and appendages covered with papillose glandular trichomes 35–55 μm long, 15–20 μm in diameter. Male flowers: androecium ca. 4 mm long; androphore tubular, ca. 2 mm long, 0.7–1 mm in diameter, with a few scattered glands; stamens 6–8, ca. 1.7 mm long, inserted at apex of androphore; anther dehiscence longitudinal, extrorse. Female flowers: gynophore ovoid, ca. 1 mm in diameter, with a few scattered glands; staminodes 5–7, composed of sessile, sterile anthers, ca. 1.3 mm long, inserted at the base of the gynophore; ovary ovoid, 1.5–2 mm in diameter, with 3–4 carpels, covered by a dense indumentum of stellate trichomes; style ca. 1 mm long, with scattered stellate trichomes; stigmas 3 or 4 ovoid, 0.4–0.5 mm long. Infructescence 7–12 cm wide, borne on a peduncle 0.5–3 cm long, 2.5–3.5 mm in diameter. Fruit comprising 1–4 follicle(s), each borne on a pedicel 0.5–0.8 cm long, 0.2–0.3 cm in diameter at maturity, green and turning greenish-yellow towards maturity, covered by sparse, erect, stellate trichomes, each follicle ellipsoid to ovoid, 3.5–4.5 cm long, 2.5–3 cm wide, with a woody pericarp ca. 0.2 cm thick in dry material (ca. 0.3 cm in alcohol), apex apiculate. Seeds 4–6 per locule, ellipsoid, white when immature, light brown to black at maturity, 10–12 x 4–6 mm in diameter.

Distribution and ecology. The new species is only known from the south-eastern part of the Grande-Terre, at the Petchécara Pass between Thio and Canala (Fig. 1), where it grows on slopes in rainforest on a soil of complex geological origin.

Cultivation note. Young plantlets of *Acropogon horarius* are fairly easy to grow in nursery conditions. Shrubs have been grown in a private nursery (Eriaxis nursery) and at the research station of Institut Agronomique néo-Calédonien located in Saint-Louis, Mont-Dore.

Etymology. The plant is named after the last of New Caledonia's scheduled roads or "route à horaire" along which it grows.

Discussion. With its hastate and cordate leaves and its long and thin petioles, its minute sessile tubular whitish-yellowish flowers, *Acropogon horarius* cannot be confused with any other member of this endemic genus. In fact, the morphological characters of its inflorescence [reduced spike-like raceme of solitary (sub)sessile flowers versus true racemes and panicles in other species] and flowers (tubular versus

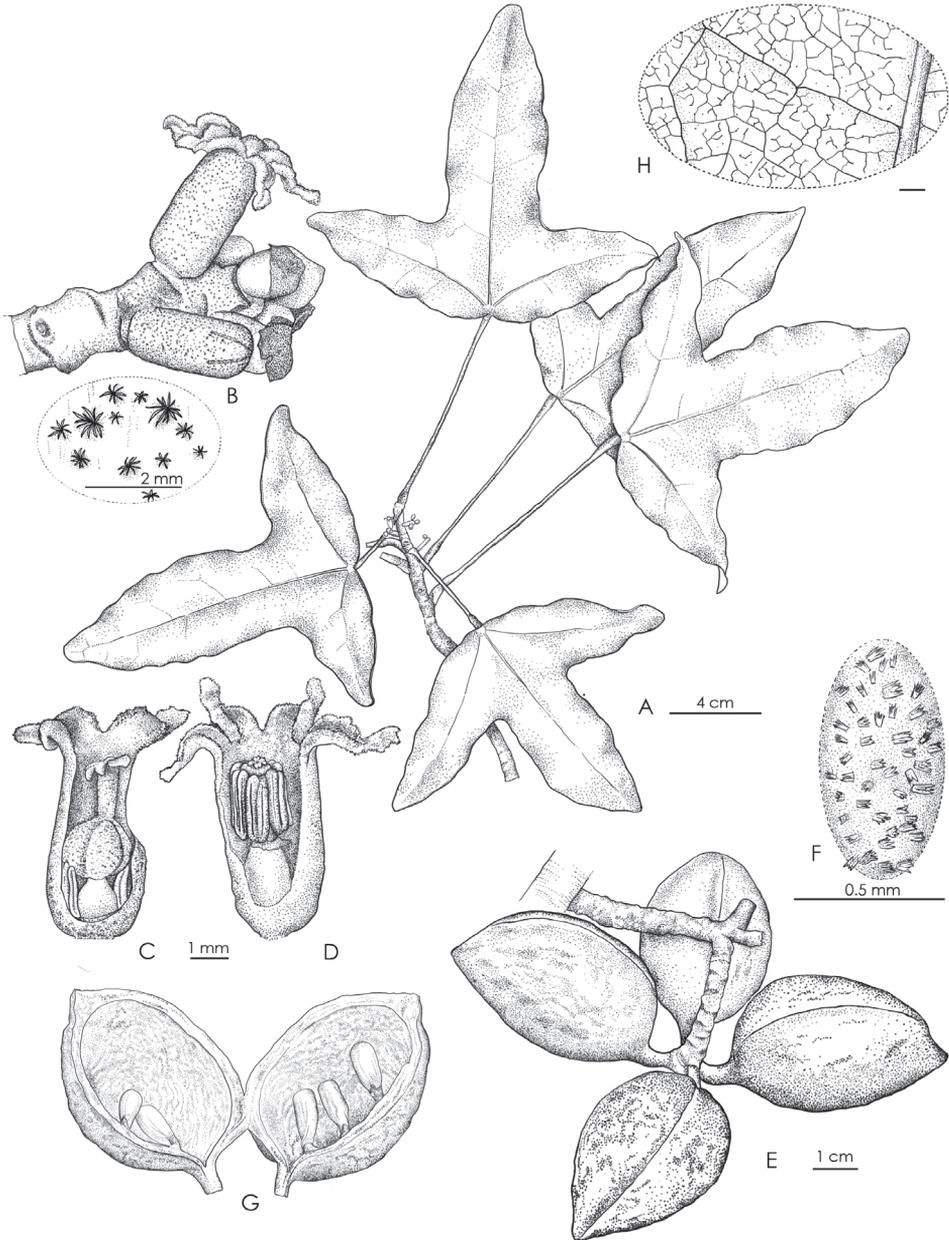


Figure 2. *Acropogon horarius* Gâteblé & Munzinger sp. nov. **A** flowering branch **B** inflorescence and a zoom on stellate trichomes of the flower tube **C** female flower **D** male flower **E** infructescence **F** detail of erect stellate trichomes on the follicle's surface **G** detail of an open follicle **H** close-up of the abaxial leaf reticulum. Drawings by Laurence Ramon (A–H Gâteblé *et al.* 806).

cup shaped in the other taxa) appear to be unique in the genus. A phylogenetic study would be worthwhile to determine whether *A. horarius* belongs to a distinct clade within the genus. The very slow development of the inflorescence axis (or brachy-

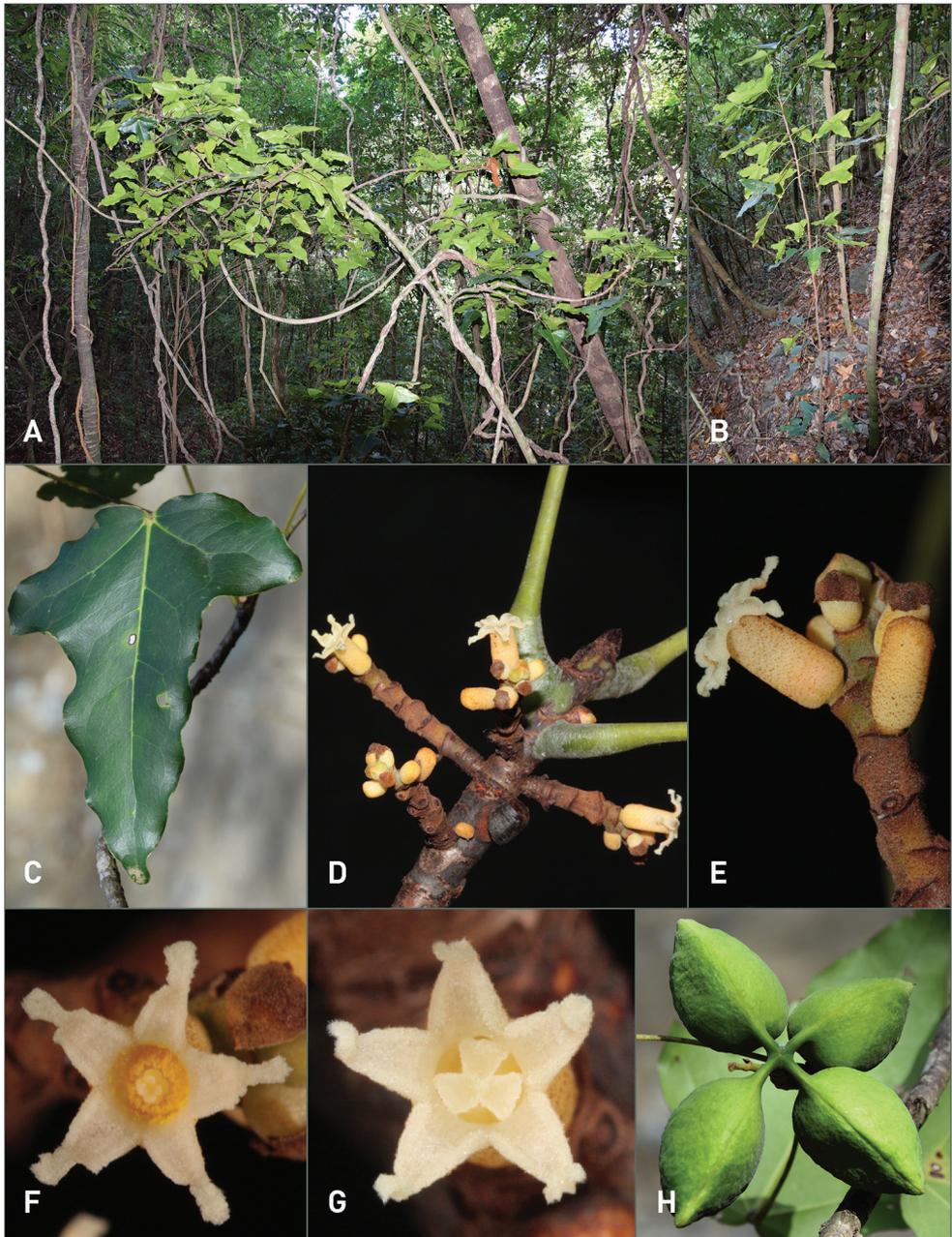


Figure 3. *Acropogon horarius* Gâteblé & Munzinger sp. nov. **A** overview of a single mature shrub in habitat **B** juvenile plant **C** leaf **D** flowering branch **E** inflorescence **F** male flower **G** female flower **H** follicles. Photographs by G. Gâteblé (**A** Gâteblé et al. 803 **B** Gâteblé et al. 804 **C–H** Gâteblé et al. 806).

blast), which takes several months, is also unusual as it appears to produce flowers sequentially throughout its growth, perhaps until there is a successful fruit set or until the axis becomes too long (3.5 cm) to produce more flowers, as opposed to the

much more nearly synchronous flowering in all other species. The combination of a prolonged duration of flowering and the unusual morphology of the minute tubular flowers suggests that the breeding mechanism in *A. horarius* may be different from that of other members of the genus.

Preliminary conservation status (IUCN 2017). *Acropogon horarius* is known from only two very small subpopulations on both sides of the road from Thio to Canala and distant from each other by less than 300 m. In the upper subpopulation, about 10 mature individuals were seen whereas only 5 were recorded in the lower subpopulation. During fieldwork, invasive deer (*Rusa timorensis*) were observed in the area and regeneration on the dry forest floor of the upper population is very poor. Deer do not seem to graze the leaves of this *Acropogon* but the habitat is threatened by erosion and reduced regeneration. The lower subpopulation does not seem to be as badly impacted by deer as regeneration is better there, erosion is lower and the forest floor is more moist. However, in the lower subpopulation some rat predation of immature fruits and seeds was observed directly on the trees (see predation marks on fruits of Gâteblé *et al.* 805) and black rat (*Rattus rattus*) predation of fruits was recently reported to be a threat for most *Acropogon* species (Munzinger and Gâteblé 2017). Inasmuch as the two subpopulations are so close to each other, they can be considered as a single location with respect to the main threat (deer). With an EOO and AOO respectively much smaller than 100 km² and 10 km² and with a projected decline in habitat quality, number of subpopulations and mature trees, the species qualifies as Critically Endangered for the IUCN criterion B. Additionally, given that fewer than 50 mature individuals are known, this *Acropogon* also qualifies as CR under criterion C and also under criterion D when combined with a predicted decline in the number of mature individuals. *Acropogon horarius* is therefore assigned a preliminary status of Critically Endangered, CR B1ab(iii,iv,v)+2ab(iii,iv,v), C1+2a(i), D, based on the IUCN Red List Categories and Criteria (IUCN 2017).

Additional specimens examined. NEW CALEDONIA. Province Sud: Thio, Col de Petchécara, route à horaire, 200 m alt., 21°34'41.01"S, 166°07'22.41"E, 25 Aug 2016, G. Gâteblé, J. Ounémoa, M. Moenteapo & E. Poitchili 805 (MPU311374, NOU088958, P00722669), Ibid., 270 m alt., 21°34'36"S, 166°07'28"E, 25 Aug 2016, G. Gâteblé, J. Ounémoa, M. Moenteapo & E. Poitchili 804 (NOU088959, P00722672), Ibid., 25 Aug 2016, G. Gâteblé, J. Ounémoa, M. Moenteapo & E. Poitchili 803 (K, MEL, MPU311375, NOU088960, P00722671), Ibid., 200 m alt., 21°34'41.01"S, 166°07'22.41"E, 3 Aug 2017, G. Gâteblé & J. Taramoin 981 (MO, MPU311376, NOU088957, P00722674).

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A Linnaeus NG interactive key to the species of *Glomera* (Orchidaceae, Coelogyninae) from Southeast Asia

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Abstract

We present a multilingual interactive key available online (<http://glomera.linnaeus.naturalis.nl>) that can be used on any web browser without the need for installing additional software. The key includes 169 species of *Glomera*, a genus within the necklace orchids (Coelogyninae) not yet comprehensively treated in any recent field guide or web-based survey. With this key, plants can be identified using a combination of vegetative and floristic characters in addition to distribution and ecology as a first step to further taxonomic revisions. We urge anyone with an interest in wild orchids in Southeast Asia to contribute new observations to update current information on the distribution of these overlooked plants as a first step for a taxonomic revision and to gain more insight into their conservation status.

Keywords

Cybertaxonomy, epiphytes, Indonesia, monitoring, necklace orchids, Papua New Guinea

Abstrak

Studi ini menyajikan kunci identifikasi interaktif multibahasa yang dapat diakses secara online (<http://glomera.linnaeus.naturalis.nl>) dan dapat digunakan pada berbagai jenis peramban web tanpa perlu menggunakan aplikasi tambahan. Kunci identifikasi ini terdiri dari 169 jenis *Glomera* dalam genus anggrek kalung (Coelogyninae) yang belum pernah dibahas secara menyeluruh dalam panduan lapangan atau survei berbasis online. Tumbuhan dapat diidentifikasi menggunakan kombinasi karakter vegetatif dan bunga juga distribusi dan ekologi sebagai langkah pertama untuk revisi taksonomi lebih lanjut. Kami menghimbau bagi

siapapun yang mempunyai ketertarikan dengan anggrek liar di Asia Tenggara untuk berkontribusi memberikan informasi terbaru dan menambahkan data distribusi dari spesies ini sebagai langkah awal untuk merevisi taksonomi dan untuk mendapatkan lebih banyak informasi tentang status konservasi spesies ini.

Kata kunci

Taksonomi Siber, epifit, Indonesia, pengamatan, anggrek kalung, Papua New Guinea

Introduction

Southeast Asia is one of the richest biodiversity regions on earth. Its complex geological history contributed to unique biota and high concentration of endemic species (Myers et al. 2000). The region also suffered the highest rate of habitat loss and associated biodiversity due to deforestation and global warming (Carr 2004; Sodhi and Brook 2006; Sodhi et al. 2010). More than 7 million hectares of forests were lost per year between 2000 and 2010 to meet rising demands for food, fuel and fibres (FAO 2016). Global warming accelerates the current biodiversity crisis that will especially lead to the extinction of those species living on mountain tops (Spehn et al. 2010). To measure the impact of deforestation and global warming on the species level, biodiversity indicators are very useful to monitor local biodiversity losses (Caro and O'Doherty 1999; Lindenmayer et al. 1999; Soberón et al. 2000; Kati et al. 2004). Orchids are an ideal flagship group to investigate biodiversity changes because of their enormous popularity amongst plant enthusiasts worldwide and widespread distribution (Newman et al. 2007). Unfortunately, the number of professional orchid taxonomists is dwindling. On the other hand, wildlife photography has been on the rise in the last decades because of the improved technology of cameras, lower costs and more accessible web-based portals to store and share photographs online. Free web-based portals such as Pbase, Facebook, Flickr, Pinterest, Instagram, Google+, SmugMug and other sites provide a platform to exchange photographs accessible to anyone, anywhere and anytime. Portals such as Google+, Yahoo's Flickr and SmugMug also provide features like geotagging, enabling users to add additional data such as when and where a photograph was made in the field.

The downside of photographs uploaded by orchid enthusiasts is that plants are often incompletely or wrongly identified, especially when it concerns orchids for which no comprehensive, up-to-date taxonomic information is available. To correctly identify a plant species, plants need to be keyed out with the help of field guides, containing a description or key of all species known to occur in an area. Plant identification can be challenging, especially for novices when they have to use dichotomous keys filled with specialistic terms (Mangold 2013). With the onset of the internet era, online and real-time information can be shared, including interactive online keys for species identification. Several software packages for making interactive online keys by converting paper-printed dichotomous keys into computer-aided interactive keys are already available such as LINNAEUS 2.0, Lucid or FRIDA (Martellos 2010; Lindsay and Middleton

2009; Farr 2006). For Orchidaceae, online identification keys have already been developed for species of European orchids and the genera *Cypripedium* L. and *Vanda* Jones ex R.Br. using the Lucid3 and Xper3 platforms at the University of Basel (<https://orchid.unibas.ch/index.php/en/orchidinfos/orchid-keys>). The interactive keys produced with these programmes are much more user-friendly than traditional keys. They can therefore be used by a broad range of users ranging from novice plant enthusiasts up to professionals. With more accessible identification keys, it will become increasingly easy for novice users to identify plants photographed in the wild correctly.

Better identification of plants in the wild is especially needed for overlooked taxa. A prime example of such are the necklace orchids (Coelogyninae), a popular group often seen in cultivation because of their showy flowers. They belong to subfamily Epidendroideae and comprise a total of 16 genera (Gravendeel et al. 2001; Gravendeel et al. 2005; Kosina and Szkudlarek 2015). *Glomera* Blume is one of the least known genera of the necklace orchids. Species of this genus are rarely cultivated. When not in flower, most species resemble a small ericaceous shrub rather than an orchid and, regarding biomass, *Glomera* is one of the predominant orchid genera in the montane forests of New Guinea (www.orchids.naturalis.nl). A total of 169 species are known of *Glomera* after 27 species of *Glossorhyncha* Ridl. were united under *Glomera* in 2016 (Shaw 2016; Govaerts et al. 2018). The key characteristics of the genus are the elongated, often branching stem with many leaves that are enveloped by warty sheaths at the base. These species mainly occur in New Guinea but some have expanded their distribution up to Fiji, the Philippines and the New Hebrides. Most are epiphytes or terrestrials in either lowland or montane rainforest up to subalpine environments. Flowers are mostly white, but some species have orange, salmon-pink or green-coloured flowers. Inflorescences are usually single-flowered, but some species have multiple flowered inflorescences.

Traditional keys that already exist for *Glomera* are in the English and German languages only and either restricted to genus level or specific geographical regions. Examples include keys for Fiji (Kores 1989) and Papua New Guinea (van Royen 1979; www.orchids.naturalis.nl). With this publication, we present an up-to-date and accessible multilingual interactive key using the Linnaeus NG platform for identification of all species of *Glomera* in Southeast Asia.

Software technical specification

Linnaeus NG (<http://linnaeus.naturalis.nl/>) is a web-based species information management system. Linnaeus NG has several modules such as species and additional features such as media (in which distribution maps and illustrations can be found) and two types of keys. For this study, a single entry key and multi-entry key were built. Linnaeus NG has been developed using open source techniques (PHP, MySQL) and is hosted in a Linux environment. On the client-side, project administrators interact with the programme through a web browser. A recent version of all major browsers

Table 1. The 169 species of *Glomera* and their distributions included in the keys in alphabetical order.

Species	Distributions
<i>Glomera acicularis</i> Schltr.	Papua New Guinea
<i>G. acuminata</i> J.J.Sm	Indonesia, Papua New Guinea
<i>G. acutiflora</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. adenandroides</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. adenocarpa</i> (Schltr.) J.J.Sm.	Indonesia, Papua New Guinea
<i>G. affinis</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. albiviridis</i> P.Royen	Indonesia, Papua New Guinea
<i>G. altigena</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. altomontana</i> (Gilli) J.M.H. Shaw	Papua New Guinea
<i>G. amboinensis</i> (Ridl.) J.J.Sm.	Indonesia, Papua New Guinea, Bismarck Islands
<i>G. ambricaulis</i> (P.Royen) J.M.H. Shaw	Papua New Guinea
<i>G. ambuensis</i> (P.Royen) J.M.H. Shaw	Papua New Guinea
<i>G. angiensis</i> J.J.Sm.	Indonesia
<i>G. antaresensis</i> (P.Royen) J.M.H. Shaw	Indonesia, Papua New Guinea
<i>G. appendiculoides</i> Ormerod.	Papua New Guinea
<i>G. asperata</i> Schltr.	Papua New Guinea
<i>G. aurea</i> Schltr.	Indonesia, Papua New Guinea
<i>G. bambusiformis</i> Schltr.	Papua New Guinea
<i>G. bismarckiensis</i> J.J.Sm.	Papua New Guinea
<i>G. bougainvilleana</i> Ormerod	Papua New Guinea
<i>G. brachychaete</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. brassii</i> Ormerod.	Papua New Guinea
<i>G. brevipetala</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. caespitosa</i> (P.Royen) J.M.H. Shaw	Papua New Guinea
<i>G. calocephala</i> Schltr.	Papua New Guinea
<i>G. carnea</i> J.J.Sm.	Indonesia
<i>G. carolinensis</i> L.O. Williams	Republic of Kiribati
<i>G. celebica</i> (Schltr.) J.J.Sm.	Indonesia
<i>G. chlorantha</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. compressa</i> J.J.Sm.	Papua New Guinea
<i>G. confusa</i> J.J.Sm.	Papua New Guinea
<i>G. conglutinata</i> J.J.Sm.	Indonesia
<i>G. crispa</i> (P.Royen) J.M.H.Shaw	Indonesia
<i>G. cristata</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. cyatheicola</i> P.Royen	Papua New Guinea
<i>G. dekokkii</i> J.J.Sm.	Papua New Guinea
<i>G. dentifera</i> J.J.Sm.	Indonesia
<i>G. dependens</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. diffusa</i> (P.Royen) J.M.H.Shaw	Indonesia
<i>G. diosmoides</i> (Schltr.) J.J. Sm.	Papua New Guinea
<i>G. dischorensis</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. distichifolia</i> Ormerod	Vanuatu
<i>G. dubia</i> J.J.Sm.	Indonesia
<i>G. elegantula</i> (Schltr.) J.J.Sm.	Indonesia, Papua New Guinea
<i>G. emarginata</i> Kores	Fiji
<i>G. ericifolia</i> Ridl.	Indonesia
<i>G. erythrosma</i> Blume	Indonesia
<i>G. flaccida</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. flamulla</i> Schltr.	Papua New Guinea
<i>G. fluviatilis</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. fransseniana</i> J.J.Sm.	Indonesia
<i>G. fruticula</i> J.J.Sm.	Papua New Guinea
<i>G. fruticulosa</i> Schltr.	Papua New Guinea
<i>G. fusca</i> Schltr.	Papua New Guinea
<i>G. fuscetosa</i> Schuit. & de Vogel	Papua New Guinea

Species	Distributions
<i>G. gamosepalata</i> P.Royen	Indonesia
<i>G. geelvinkensis</i> J.J.Sm.	Indonesia
<i>G. geminata</i> Ormerod.	Indonesia
<i>G. glomeroides</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. goliathensis</i> J.J.Sm.	Indonesia
<i>G. graminifolia</i> Schltr.	Papua New Guinea
<i>G. grandiflora</i> J.J.Sm.	Indonesia
<i>G. grandilabella</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. hamadryas</i> (Schltr.) J.J.Sm.	Indonesia, Papua New Guinea
<i>G. hubrechtiana</i> J.J.Sm.	Indonesia
<i>G. hunsteiniana</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. imitans</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. inconspicua</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. inflata</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. jabiensis</i> J.J.Sm.	Indonesia
<i>G. kamay-nolomi</i> Ormerod.	Papua New Guinea
<i>G. kaniensis</i> Schltr.	Papua New Guinea
<i>G. kanke</i> P.Royen	Indonesia, Papua New Guinea
<i>G. kerewensis</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. keysseri</i> (Schltr.) J.M.H.Shaw	Papua New Guinea
<i>G. keysiana</i> J.J.Sm.	Indonesia
<i>G. kuperensis</i> Ormerod.	Papua New Guinea
<i>G. lancipetala</i> J.J.Sm.	Indonesia
<i>G. latilinguis</i> J.J.Sm.	Indonesia
<i>G. latipetala</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. ledermannii</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. leucomela</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. longa</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. longicaulis</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. macdonaldii</i> (Schltr.) J.J.Sm.	Papua New Guinea, New Caledonia, New Hebrides, Fiji
<i>G. macrantha</i> J.J.Sm.	Papua New Guinea
<i>G. macrophylla</i> Schltr.	Papua New Guinea
<i>G. manicata</i> J.J.Sm.	Papua New Guinea
<i>G. mayuensis</i> Ormerod	Papua New Guinea
<i>G. melanocaulon</i> Schltr.	Papua New Guinea
<i>G. merrillii</i> Ames	The Philippines
<i>G. microphylla</i> J.J.Sm.	Indonesia
<i>G. minjensis</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. minutigibba</i> J.J.Sm.	Indonesia
<i>G. montana</i> Rehb.f.	Papua New Guinea, Solomon, Fiji, Samoa, Vanuatu
<i>G. monticuprina</i> (P.Royen) J.M.H.Shaw	Indonesia
<i>G. muscicola</i> (P.Royen) J.M.H.Shaw	Indonesia
<i>G. myrtilus</i> (Schltr.) Schuit. & de Vogel	Papua New Guinea
<i>G. nana</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. neohibernica</i> Schltr.	Papua New Guinea
<i>G. nigricans</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. nigrilimbata</i> P. Royen	Papua New Guinea
<i>G. nigrimarginata</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. noroma</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. obovata</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. obtusa</i> Schltr.	Papua New Guinea
<i>G. oligantha</i> Schltr.	Indonesia
<i>G. palustris</i> J.J.Sm.	Indonesia, Papua New Guinea, Vanuatu, Solomon
<i>G. palustris</i> var. <i>subintegra</i> J.J.Sm.	Papua New Guinea
<i>G. papuana</i> Rolfe	Papua New Guinea
<i>G. parviflora</i> J.J.Sm.	Indonesia
<i>G. patens</i> Schltr.	Papua New Guinea

Species	Distributions
<i>G. pendulosa</i> J.M.H.Shaw	Papua New Guinea
<i>G. pensilis</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. pilifera</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. pinifolia</i> (P.Royen) J.M.H. Shaw	Indonesia
<i>G. platypetala</i> Schltr.	Indonesia
<i>G. pleiotricha</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. plumosa</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. polychaete</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. pseudomonanthos</i> Ormerod	Indonesia, Papua New Guinea
<i>G. pteropetala</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. pullei</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. pumilio</i> J.J.Sm.	Indonesia
<i>G. pungens</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. retusa</i> J.J.Sm.	Indonesia
<i>G. retusimentum</i> J.J.Sm.	Indonesia
<i>G. rhombea</i> J.J.Sm.	Indonesia
<i>G. rigidula</i> J.J.Sm.	Papua New Guinea
<i>G. rubroviridis</i> J.J.Sm.	Indonesia
<i>G. saccharipanis</i> Ormerod.	Papua New Guinea
<i>G. saccosepala</i> J.J.Sm.	Papua New Guinea
<i>G. salicornioides</i> J.J.Sm.	Indonesia
<i>G. salmonea</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. sandaveri</i> Ormerod.	Papua New Guinea
<i>G. scandens</i> J.J.Sm.	Indonesia
<i>G. schlechteriana</i> Mansf.	Papua New Guinea
<i>G. schultzei</i> Schltr.	Papua New Guinea
<i>G. scopulata</i> (P. Royen) J.M.H. Shaw	Indonesia
<i>G. secunda</i> J.J.Sm.	Indonesia
<i>G. sepalosiphon</i> Schuit. & de Vogel	Papua New Guinea
<i>G. similis</i> J.J.Sm.	Indonesia
<i>G. sororia</i> J.J.Sm.	Indonesia
<i>G. squamulosa</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. stenocentron</i> (Schltr.) J.J.Sm.	Indonesia, Papua New Guinea
<i>G. stolonifera</i>	Papua New Guinea
<i>G. subciliata</i> J.J.Sm.	Indonesia
<i>G. sublaevis</i> J.J.Sm.	Indonesia
<i>G. subnivalis</i> J.M.H.Shaw	Indonesia
<i>G. subpetiolata</i> Schltr.	Papua New Guinea
<i>G. subracemosa</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. subulata</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. subuliformis</i> J.J.Sm.	Indonesia
<i>G. tamiana</i> J.J.Sm.	Papua New Guinea
<i>G. tenuis</i> (Rolfe) J.J.Sm.	Papua New Guinea
<i>G. terrestris</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. torricellensis</i> Schltr.	Papua New Guinea
<i>G. tortuosa</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. transitoria</i> J.J.Sm.	Indonesia
<i>G. triangularis</i> J.J.Sm.	Papua New Guinea
<i>G. tubisepala</i> (P.Royen) J.M.H.Shaw	Papua New Guinea
<i>G. umbrosa</i> P.Royen	Indonesia
<i>G. uniflora</i> J.J.Sm.	Indonesia
<i>G. verrucifera</i> Schltr.	Papua New Guinea
<i>G. verrucosissima</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. verruculosa</i> (Schltr.) J.J.Sm.	Papua New Guinea
<i>G. versteegii</i> J.J.Sm.	Indonesia, Papua New Guinea
<i>G. viridis</i> (Schltr.) J.J.Sm.	Papua New Guinea

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User interface

Users can access the key at <http://glomera.linnaeus.naturalis.nl> and it can be used online using any web browser. No additional software is required. The interface was designed to be able to access from any device with flexible layout. The navigation menu is shown on the left side. The menu includes an index, species list, single-access key, multi-access key, two language options (English or Bahasa Indonesia) and a glossary. A user can directly search for a species by using the search box on the top. If the user does not yet have a clue about the identity of the species, a single-access key is available with 166 steps to help with the identification process. A multi-access key is also provided, in which remaining choices with 100% fit only are indicated at every step. A glossary is present to help novice users to understand terms used in the descriptions and keys.

Data

Morphological characters used in the interactive key (Figure 1; Table 2) were initially selected from already existing dichotomous keys. Not all characters turned out to be clear to both advanced and novice users, though, so the number was reduced to a final selection after tests of preliminary versions of the key by students of the annual Orchid Biology Course taught at Basel University in 2017. Students were provided with a similar set of specimens and we monitored if they were able to come up with the correction identification within half an hour and which characters were considered easy to use and which were not. Both keys were constructed independently. For the single entry key, it is possible to start at any step and, if you want to start again, you have to click on step 1. For the multi-entry key, you have to select the option 'Start all over' if you want to start again. All character states used are illustrated by images.

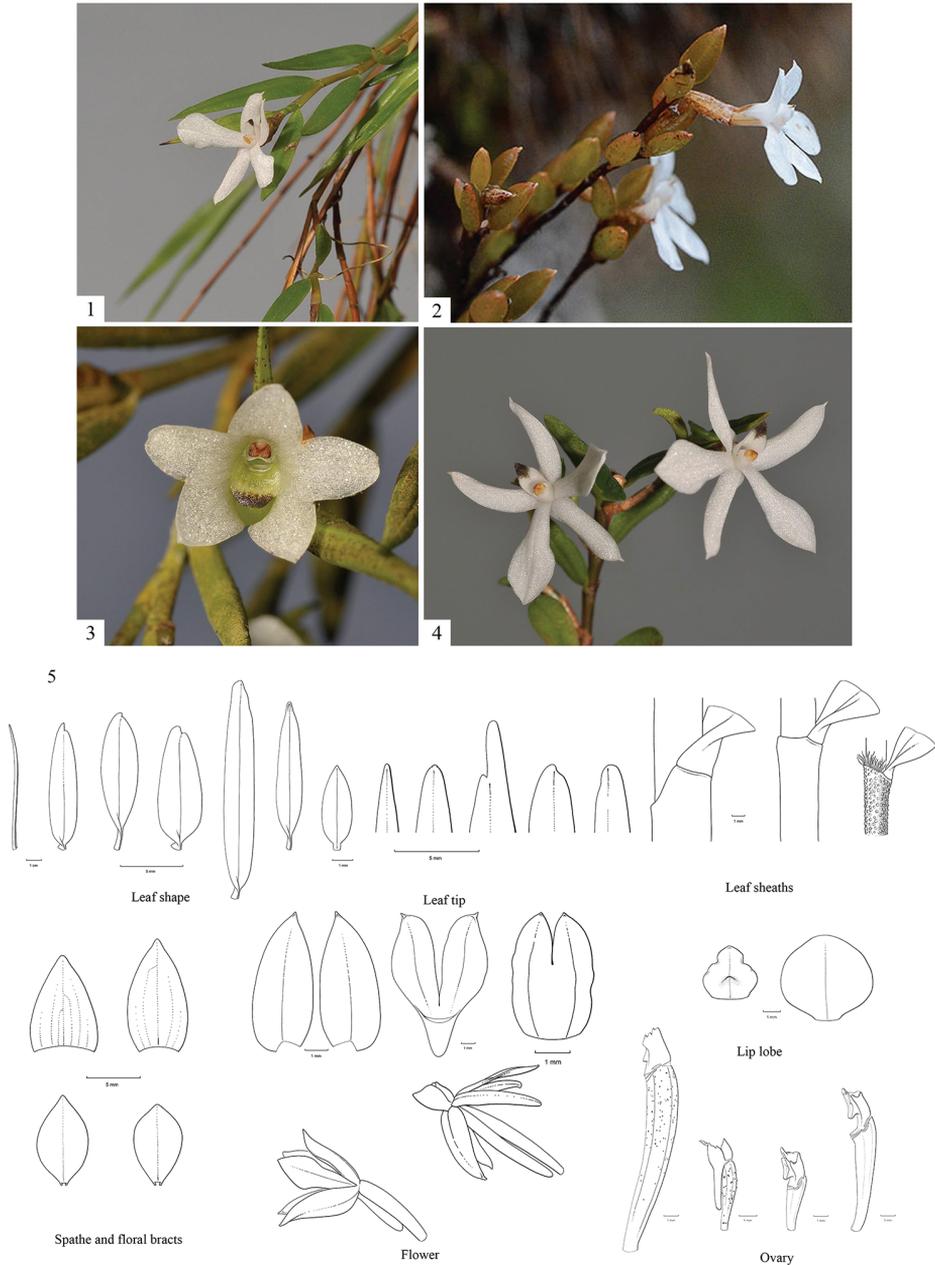


Figure 1. Illustrations of a selection of key characters used in the identification keys. **1** *Glomera acutiflora* (Schltr.) J.J.Sm. with green leaves (photograph by Rogier van Vugt) **2** *Glomera* sp. with reddish-brown leaves (photograph by fotosynthesys deposited on FLICKR) **3** *Glomera pungens* (Schltr.) J.J.Sm. with upright flowers (photograph by Rogier van Vugt) **4** *Glomera hamadryas* (Schltr.) J.J.Sm. with flowers turned up-side-down (photograph by Rogier van Vugt) **5** Various shapes of the leaf blade, leaf tip, leaf sheath, leaf spathe, floral bract, entire flower, sepals, petals, lip and ovary present in *Glomera* and *Glossorhyncha* (illustrations by Esmée Winkel).

Table 2. Morphological characters and their states used in the keys.

Plant part	Character	States
Rhizome	Division	Heavily branched; not or only sparsely branched
Leaf blade	Color	Green; reddish-brown
	Lamina	Fleshy; not fleshy
	Dots	With brown dots; without brown dots
	Tip	One lobe (obtuse or acute); two lobes (acute-acute, obtuse-obtuse or acute-obtuse)
Leaf sheath	Color	Green; black
	Tooth	With tooth; without tooth
	Notch	Notched; not notched
	Bristles	With bristles; without bristles
	Warts	With warts; without warts
Spathe	Warts	With warts; without warts
	Hairs	With hairs; without hairs
	Dots	With brown dots; without brown dots
Floral bract	Warts	With warts; without warts
	Hairs	With hairs; without hairs
	Dots	With brown dots; without brown dots
	Size	Longer than spathe; shorter than spathe
Inflorescence	Number of flowers	One; more than one
Flower	Color	White; green; pinkish-salmon; orange; red
	Orientation	Upright; up-side-down
	Spur length	Shorter than 10 mm; longer than 10 mm
	Spur tip	One-lobed; two-lobed
	Lateral sepals	Free; fused for more than two-thirds
	Lip	With glands on tip; without glands on tip
	Lip tip color	White; black; green; red; grey; pink
	Odor	Fragrant; not fragrant
	Sepal orientation	Straight; bent backward
	Column foot	Present; absent
	Ovary ribs	With ribs; without ribs
	Ovary dots	With brown dots; without brown dots
	Ovary warts	With warts; without warts

Table 3. Non-morphological characters and their states used in the keys.

Character group	Character	States
Ecology	Lifeform	Epiphyte; terrestrial
Flowering season	Months	January; February; March; April; May; June; July; August; September; October; November; December
Global distribution	Country	Indonesia; Papua New Guinea; Fiji; New Hebrides; Philippines
Distribution in Indonesia	Island	Papua; Java; Moluccas; Sulawesi
Occurrence over the elevational gradient	Altitude	Lowland; midland; highland

Apart from morphology, geographical distribution and ecology can be informative as well. The key therefore also includes a few of these non-morphological characters (Table 3).

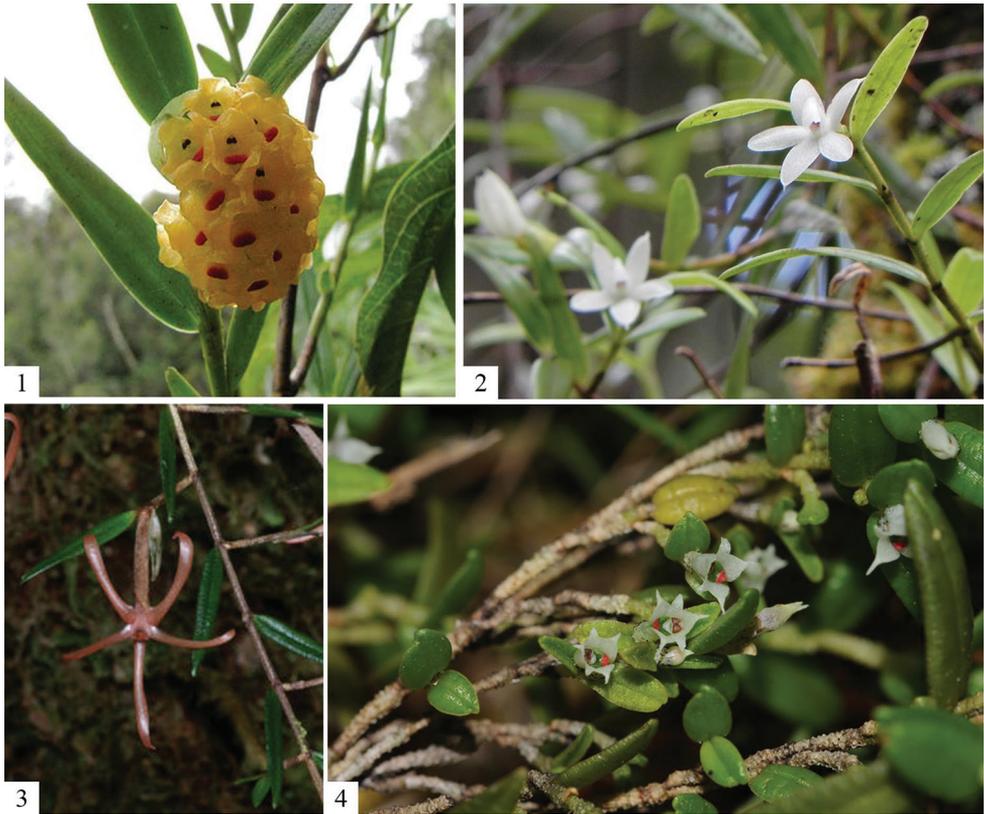


Figure 2. Photographs of *Glomera* species collected from online platforms. **1** *Glomera aurea* (photograph by Mehd Halaouate) **2** *Glomera macdonaldii* (photograph by Benoit Henry) **3** *Glomera tubisepala* (photograph by Gary Yong Gee) **4** *Glomera glomeroides* (photograph by S.A. James).

Discussion

The interactive key presented here for *Glomera* of Southeast Asia encompasses more species and geographic areas than any existing key currently available for this genus. Next to English, it was also written in a language commonly used in Southeast Asia, Bahasa Indonesia, which enables a much wider group consisting of both novice and advanced users in the region to identify these orchids correctly. The main challenges to construct this key consisted of the fact that type descriptions were often rather vague and that many type collections were lost after the bombing of the herbarium of the Botanic Garden and Botanical Museum in Berlin in the second world war. Of the 169 species, a total of 52 types were lost. We therefore studied a lot of additional collections, all listed under Species, option Collection specimens on the website, to verify character states.

Compared with traditional dichotomous keys, interactive keys can be used much more easily by relatively novice users (Jacquemart et al. 2016). Users of this key can

quickly survey images of remaining species after selection of a first set of characters such as flower colour and distribution. This key therefore enables much faster (i.e. seconds rather than minutes) identification of the best candidate from the remaining choice than working through a traditional key until the final result appears with little indication of the remaining potential outcomes during the identification process. When using a conventional dichotomous key, a user is not provided with an indication of the remaining taxa during the identification process and can only hope that the final outcome matches the candidate. Professional taxonomists often rely on extensive previous exposure to species during the identification process but novice users cannot fall back on this.

Our key will hopefully urge the users to further enrich the database and help update the distribution of species or detect possible new species. Pictures on the web placed there by enthusiasts are considered to be an essential source for the discovery of new data (Marshal 2008). Use of our key by more wildlife photographers will help to record the presence of species in geographic regions where they were previously overlooked. The idea of democratisation of taxonomy by involving the general public (hobbyists, naturalists, tourists) to this field could trigger higher interest in currently unexplored taxa. Recently, the taxonomy and biogeography of diving beetles in Bali could, for instance, be improved by using citizen scientists and social networks such as Facebook and WhatsApp (Suprayitno et al. 2017).

A first indication that the same might happen for *Glomera* orchids is illustrated by the fact that we could combine historical, literature-based data and recent photographs of plants taken by wildlife photographers of *G. aurea* Schltr., *G. macdonaldii* (Schltr.) J.J. Sm., *G. tubisepala* (P.Royen) J.M.H. Shaw and *G. glomeroides* Schltr. Pictures of flowering plants, taken in Papua of the first species, the Solomon islands of the second and Papua New Guinea of the third and the fourth and deposited on Flickr and SmugMug, came to our attention during this study. Once contacted by us, the photographers provided more detailed locality data and dates, which enabled us to update the distribution maps and also the flowering time of these species.

We also used our key to assign a name to a yet unknown *Glomera* species photographed. For example, on the photograph of *Glomera* sp. 2010-064 uploaded in the username PNG Collection of Smugmug, we could see details such as the shape of the leaf sheath, tooth and warts, colour and shape of the leaves and leaf apex and the colour of the flower (white with a green lip and red lip tip). After selecting all the characters that could be identified from the photograph and additional data such as location and altitude, we could reduce the number of species from 169 to 10. We ended up with identifying it as cf. *G. glomeroides* by comparing the photograph to a drawing made by Friedrich Richard Rudolf Schlechter in 1923 that accompanied the type description. The type of this species was lost in the herbarium of Berlin and no documented photograph has yet been published. The photographer could provide us with locality data in the Madang province of Papua New Guinea. With the aid of our interactive key, we could therefore simplify the process of identification of this unidentified species.

Conclusions

We expect that the interactive key presented here for *Glomera* of Southeast Asia will enable a higher number of people to collect more precise and more reliably identified observations of species of this overlooked orchid genus. It is user-friendly due to the many illustrations and colour photographs, encompassing a combination of historical, literature-based and recent, web-mined data of all species rather than subsets only and written in a language commonly spoken in parts of the world where these orchids occur in the wild. The key was designed for efficient use by both inexperienced and advanced orchidologists. This publication accompanies the release of version 1.0. We encourage all users to provide feedback to improve and further expand this version by contacting us by email to gain more insight into the current distribution of these overlooked orchids. This will enable us to accurately assess their conservation status. By obtaining more knowledge of the regions of distributions but without disclosing too detailed locality data, we hope to prevent extinction of these orchids in the wild.

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Begonia yenyeniae (Begoniaceae), a new species from Endau Rompin National Park, Johor, Malaysia

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Abstract

Begonia yenyeniae is a new species of horticultural value known only from the Endau Rompin National Park, Peninsular Malaysia. It is similar to *Begonia rajah* with which it had previously been confused in the number of tepals and leaf characters. The new species is compared with three similar species, *B. foxworthyi*, *B. rajah* and *B. reginula* and photographs of all four species and descriptions of *B. yenyeniae* and *B. rajah* are provided. Molecular analysis using the *ndhF-rpl132* chloroplast marker confirms the four species as distinct. Amongst native species, the three variegated species, *B. yenyeniae*, *B. rajah* and *B. reginula*, are some of the most popular Malaysian begonias in cultivation. Based on its restricted distribution, *Begonia yenyeniae*, under the IUCN Red List Categories and Criteria, is assessed as Critically Endangered.

Keywords

new species, *Begonia yenyeniae*, *Begonia rajah*, *Begonia reginula*, ornamental, conservation

Introduction

Asian begonias are prized for the eye-catching variety of colours and patterns of their variegated leaves and so are targets for horticultural interest as a genetic source for new hybrid cultivar development. However, a vast array of begonias is of conservation importance either because they have restricted distributions, being known from very few

localities or they are confined to a specific habitat niche. For example, of the 54 native taxa in Peninsular Malaysia, 57% are threatened with 24 taxa assessed as Critically Endangered and 21 species are known from a single locality (Chua et al. 2009). The great majority grows in primary forest, frequently on rocks beside streams or waterfalls, where they are threatened by loss of habitat. Forest cover declined rapidly from 70% in 1960 to 41% in 2000 in Peninsular Malaysia due to massive land development schemes (Chua 2010), disturbance from eco-tourism activities and for pretty species, collecting from wild populations for cultivation or sale. At least one begonia species is extinct (Kiew 1989a) and several have not been found since their original discovery. Ironically, some species, that may be extinct in the wild, live on because they are popular with plant enthusiasts and are available from commercial growers.

As pristine forest diminishes in the face of extraction of timber or land clearance for the planting of oil palm and other crops and for infrastructure development, forests are becoming valued for the ecological services they provide and as a source of medicinal and ornamental plants or minor forest products, as well as for tourism and recreation. However, the commercial value of native species is often realised outside the country of origin so minimal benefit is generated locally. In Malaysia, the introduction of wild native species into horticulture has been haphazard. In recognising this, the Forest Research Institute Malaysia (FRIM) started a plant domestication project to trial attractive wild species for their horticultural potential with the aims of not only enabling Malaysia to benefit commercially from its rich biodiversity (Tan 2009), but also to illustrate that other species, besides timber trees, are of commercial value and that their habitat should also be protected. In addition, making attractive species readily available would help to take pressure off collecting, often illegally, from wild populations.

This new species was one of the candidates of this project. It was discovered in 2002 by Dr Sam Yen-Yen in the Endau-Rompin National Park in the southern state of Johor. It proved to be amenable to cultivation and, unlike many native begonias that need constant high humidity, it does not need to be grown in a covered terrarium. So the species was 'bulked up' and offered to the horticulture trade to test its potential. Unfortunately, the local horticultural market was reluctant to invest in the marketing of this species on a commercial scale but, based on information available on social media and websites, it spread widely amongst specialist growers. Individual plants from the original Johor collection continued to be grown for further studies in the Kepong Herbarium (KEP) nursery. The Johor plants have been identified as *Begonia rajah* Ridl. because they have the same habit, number of tepals (four in the male flower and three in the female) and similar attractive leaf colour and surface (Kiew 2005). Comparison between these plants and those of *B. rajah*, obtained commercially, showed important differences and indicated that they were different taxa. To test this supposition, molecular phylogenetic analysis of the new species, *B. rajah* and *B. reginula*, was performed.

Begonia rajah is one of the most decorative Malaysian begonias due to its striking and uniquely contrasting bronzy-green leaf colour. It was discovered in 1892 and gained fame in 1894 when it was sent to England and won a First Class Certificate from the Royal Horticultural Society (Anonymous 1894a). After more than a cen-

tury, it remains popular in cultivation today (Thompson 1976, Kiew 1989b, Tebbitt 2005). However, its origin is still shrouded in mystery. Ridley (1892) recorded only that it was collected by a native collector from Tringganu (the Malaysian state of Terengganu). Despite continuous botanical exploration, it has never been re-found, although another species, *Begonia reginula* Kiew (Figure 4), similar in leaf characters, was discovered in Terengganu, but it proved to be a distinct species based on the different number of tepals (Kiew 2005, Tan 2016).

With recent publications of higher level (sectional) molecular phylogenies of *Begonia* (Moonlight et al. 2018, Tebbitt et al. 2006, Rajbhandary et al. 2011), especially involving species from South East Asia (Thomas et al. 2011, 2012), it is now timely to perform a molecular phylogenetic study that includes greater representation of *Begonia* species from Peninsular Malaysia.

Methods

The description and measurements of the new species are based on herbarium specimens deposited in the Kepong Herbarium, Forest Research Institute Malaysia (KEP) and Singapore Botanic Gardens Herbarium (SING) and fresh material cultivated in the KEP nursery. The diagnostic characters of the new species were compared with those of the type specimen of *B. rajah* in the Herbarium of Royal Botanic Gardens, Kew (K), the watercolour painting made from the original *B. rajah* plant (Figure 1) and early botanical descriptions. As *B. rajah* was confused with the new species (Kiew 2005), a new description is provided here. Other similar species, *B. reginula* Kiew and *B. foxworthyi* Burkill ex Ridl. are shown by molecular data to share a close relationship and are compared with the new species based on herbarium specimens in KEP, protologues (Kiew 2005) and living material available in the KEP nursery. The conservation status of the new species is assessed following the standard IUCN Categories & Criteria (IUCN 2012).

Species relationships amongst *B. yenyeniae*, *B. foxworthyi*, *B. rajah* and *B. reginula*

Interspecific relationships amongst the proposed new species (*B. yenyeniae*), *B. foxworthyi*, *B. rajah* and *B. reginula* were inferred from our current, on-going phylogenetic study that focuses on *Begonia* species from Peninsular Malaysia. DNA sequences for the chloroplast *ndhF-rpl32* intergenic spacer region were generated for 48 taxa of *Begonia* which included species mostly from Peninsular Malaysia (Suppl. material 1).

DNA Extraction, PCR amplification and DNA sequencing

DNA of *Begonia* species was extracted from living material or silica gel dried material using the NucleoSpin Plant II kit following the manufacturer's protocols (Macherey

Nagel, Germany). Amplification of the chloroplast *ndhF-rpl32* intergenic spacer was done using the forward and reverse primers *ndhFBeg-F* and *trnLBeg-R*, respectively, designed by Thomas et al. (2011). Each PCR reaction contained 12.50 µl of 2X Go-Taq Green Master Mix (Promega, U.S.A.), 1 µl of each forward and reverse primer (10 µM) (Integrated DNA Technologies, U.S.A.), 1 µl of bovine serum albumin (New England BioLabs Inc., U.S.A.), 7.50 µl of RNase free water (Bio Basic Inc., Canada) and 2 µl (~ 100 ng) of DNA template to make up 25 µl total volume. The following PCR profile was used: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 2 min; followed by a final extension step at 72 °C for 5 min. Amplicons were visualised using 1.0% agarose gel electrophoresis stained with SYBR Safe DNA Gel Stain (Invitrogen, U.S.A.). Samples with the expected size (~ 750 bp) were purified using the Wizard SV Gel and PCR Clean-Up Kit (Promega, USA) before being sent for DNA sequencing using both the forward and reverse primers by First BASE Laboratories Sdn Bhd (Selangor, Malaysia). Both the forward and reverse sequences were manually edited (cleaned) and aligned to generate a consensus sequence for each sample using the programme BioEdit. Multiple sequence alignment of the generated sequences together with the *ndhF-rpl32* sequences of 79 Asian *Begonia* species (from an earlier phylogenetic study by Thomas et al. 2011) downloaded from GenBank was performed using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) using default parameters. The resulting alignment file was then manually edited before analysis and is available from the corresponding author upon request.

Data analysis

The final alignment file, comprising of 126 taxa and 1244 characters, was subjected to Bayesian analysis performed using MrBayes 3.2.6 on XSEDE via the CIPRES portal (<https://www.phylo.org/>). Following Thomas et al. (2011), the GTR + G model was selected for the analysis with the following MCMC parameters: number of generations = 1,000,000; number of runs = 2; number of chains = 4; temperature parameter = 0.20; sample frequency = 1000; and burn-in fraction = 0.25. The resulting 50% majority rule consensus tree with node support expressed as posterior probabilities was viewed using FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>). The programme MEGA 6 (Tamura et al. 2013) was used to compute values of genetic distance (gd) using the Kimura 2 parameter (K2P) model for interspecific comparisons between the three species, *B. yenyeniae*, *B. rajah* and *B. reginula*.

Results

Results of our on-going study from Bayesian analysis of 47 taxa of *Begonia* from Peninsular Malaysia, which included accessions of *B. rajah*, *B. reginula* and the new species

B. yenyeniae, together with 79 other Asian species using the *ndhF-rpl32* chloroplast marker, showed that all three species resolved together in a strongly supported polytomous clade (Bayesian posterior probability (BPP) support = 1) where *B. yenyeniae* clearly separated in its own subclade (BPP support = 0.98) with accessions of *B. foxworthyi* and *B. nurii*. The tree shows that the *B. yenyeniae* accession resolved in a relatively long branch which lends support to its separation as a proposed new species. Part of the tree highlighting relationships amongst *B. rajah*, *B. reginula*, *B. foxworthyi* and *B. yenyeniae* are presented in Figure 5. Values of genetic distance (gd) computed under the Kimura 2 parameter (K2P) model for interspecific comparisons amongst the three species showed the gd between *B. yenyeniae* and *B. rajah* as 0.024; *B. yenyeniae* and *B. reginula* of 0.021 and *B. rajah* and *B. reginula* of 0.01. The two *B. foxworthyi* samples (from Bentong and Merapoh, respectively, that resolved in the same subclade as *B. yenyeniae*) recorded the same gd values from *B. reginula* at 0.006; whilst the gd between the two *B. foxworthyi* samples and *B. yenyeniae* are 0.015. The gd values between *B. foxworthyi* (Bentong) and *B. foxworthyi* (Merapoh) from *B. rajah* are 0.01 and 0.012, respectively.

The *ndhF-rpl32* DNA sequences of *B. yenyeniae*, *B. rajah* and *B. reginula* have been deposited to GenBank (accession numbers MH454102, MH454103 and MH454104, respectively). Thus, molecular data support the close relationships of these species and the genetic distinction of *B. yenyeniae*. It is noted that the phylogenetic tree shows that a single chloroplast marker has limited power to resolve interspecific relationships in this group and, clearly, additional molecular markers are required as certain accessions of the same species did not resolve in clearly differentiated clades.

Taxonomy

Begonia yenyeniae J.P.C.Tan, sp. nov.

urn:lsid:ipni.org:names:77191563-1

Figure 2

Section. *Jackia* M.Hughes

Diagnosis. Similar to *Begonia rajah* Ridl. (1894:213) in its handsome leaves, striking brownish-pink to brownish-red with greenish-yellow veins in young leaves becoming bronzy variegation at maturity; in its creeping growth habit, number of tepals in male (4 tepals) and female flowers (3), palmate leaf venation and many-flowered cymes, but several notable characters distinguish the new species, including its orbicular-reniform leaf blades (vs. subrotund with an abruptly acute apex in *B. rajah*), smaller stipules, 9–12 × 3–4 mm, three times longer than wide (vs. 15–20 × 10–12 mm, less than twice as long as wide), smaller ovate or obovate bracts 2–3 × 1.5–2 mm (vs. bracts bowl-shaped, wide ovate, 5–8 × 7.5–8 mm), margin shallowly crenate (vs. margin angular), bullate leaf surface (vs. conspicuously pronounced bullate). Furthermore, the leaf colour of cultivated *B. rajah* is more vivid and, in contrast, its tepals, stipules and bracts are also a deeper shade of pink, compared to those of *B. yenyeniae*.



Figure 1. Watercolour painting of *Begonia rajah* of an original wild-collected plant grown in the Botanic Gardens, Singapore. (Reproduced with permission of the Singapore Botanic Gardens)

It is also similar to *B. reginula* Kiew (2005: 218) in its habit, leaf colour, less pronounced bullate blade, palmate venation, bracts, ovary with 3 equal wings of similar shape, but *B. yenyeniae* is different in its relatively smaller and narrower tepals 5–7 × 6 mm (vs. 6–10 × 9–11 mm in *B. reginula*), 3 in male or 4 tepals in female flowers (vs. 2 tepals in both male and female flowers) and rounded base (vs. subcordate), stipules with prominently keeled (vs. keel absent), and apex rounded (vs. apex attenuate).

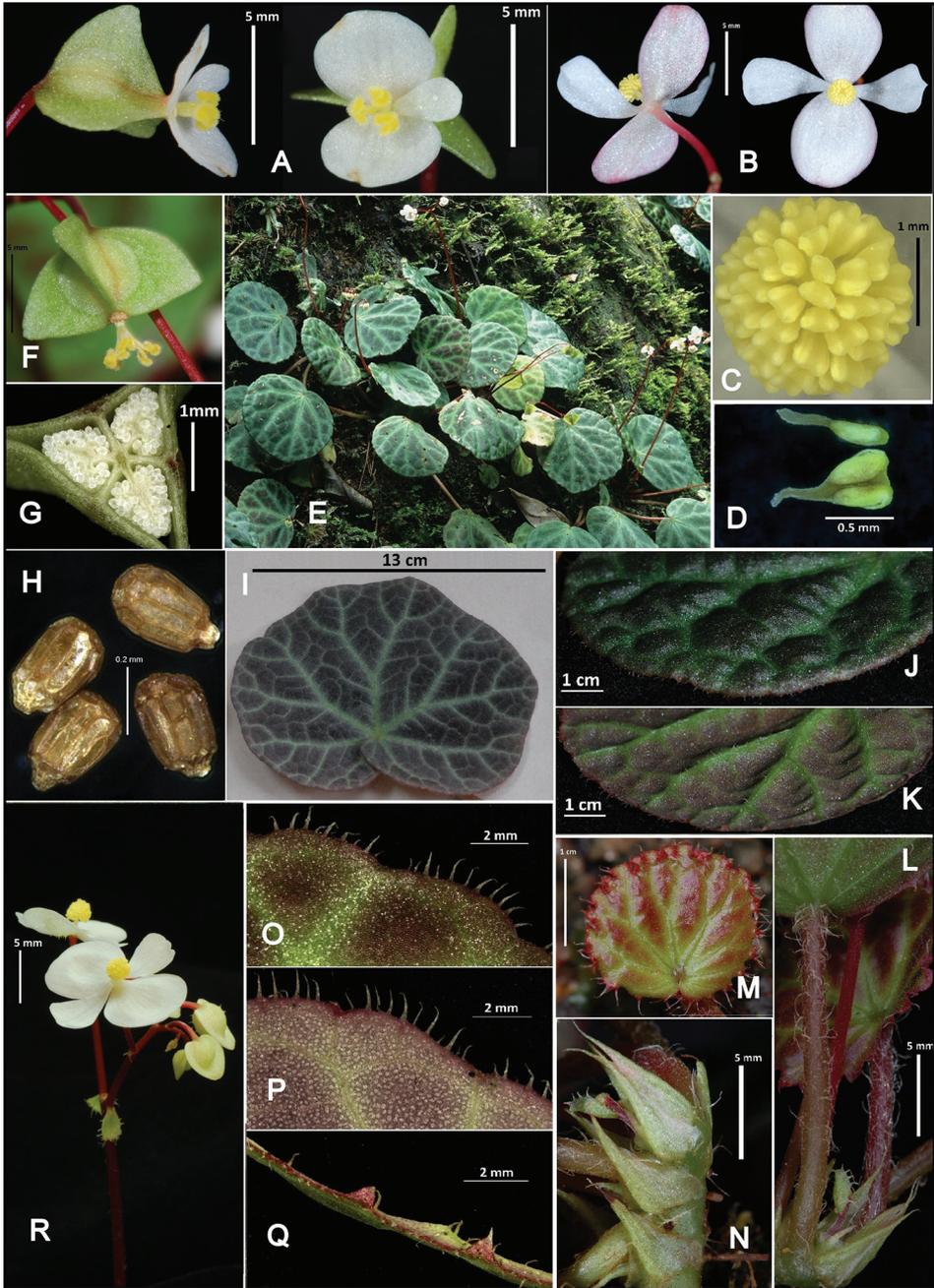


Figure 2. *Begonia yenyeniae* J.P.C.Tan, sp. nov. **A** Side and front view female flower **B** Back and front view of male flower **C** Stamen mass **D** Anthers **E** Habitat: moss-covered rocky slope by waterfall **F** Young fruit with stigma still attached **G** Transverse section of fruit **H** Seeds **I** Mature leaf **J** Upper leaf surface (moderately bullate) **K** Veins completely prominent on lower leaf surface **L** Petiole **M** Young blade **N** Stipules **O–Q** Upper, lower and side view of leaf margin **R** A pair of bracts and bracteole at peduncle and rachis; hairs scarcely on ventral surface of outer tepals. (Photographs by E.Y.Y. Sam, D.P.T. Ong)

Although molecular data indicate a close relationship to *B. foxworthyi* ((1925: 311), the latter species is morphologically distinct in its conspicuously oblique leaf with an acute to acuminate apex (vs. orbicular-reniform with a rounded apex), its entire margin (vs. crenate) and plain green, non-bullate leaf surface (vs. purplish-green to brownish-purple and bullate) and its male flowers with 2 tepals (vs. 4 tepals). In addition, it usually grows on limestone substrates (vs. confined to granite substrates).

Type. Peninsular Malaysia. Johor, Mersing, Endau-Rompin National Park, Sungai Selai, 14 April 2018, Kiew FRI 81950 (holotype E!; iso-: K!, SAR!, SING!).

Description. Lithophytic herb with rhizomatous stem. Indumentum of soft hairs, usually cream- coloured, sparse on stipules (mostly on the keel and 0.5–1.5 mm long), petioles more densely hairy towards blade (2–3 mm), leaf margin (hairs ca. 1 mm) and veins beneath (ca. 1 mm) but sparse (ca. 0.5 mm) or absent on ventral surface of outer tepals and on peduncle hairs hardly visible to the naked eye. **Stems** creeping, apex usually slightly erect, branched, 5–9 mm thick, light yellowish-green; stipules 3 per node, two triangular, abruptly narrowed to an attenuate apex terminating in a hair, keeled from base to apex, margin entire and translucent, 9–12 × 3–4 mm, pale yellowish-green, one narrowly lanceolate, ca. 3 mm long, persistent. **Leaves** tufted, alternate, 2–3 mm apart; petioles terete, 2.5–3.5 mm across, up to 12.5 cm long, pale brownish-pink to darker pink towards blade; blades thinly succulent, glabrous, orbicular-reniform, asymmetric, 8–11.5 × 9.5–13.5 cm, scarcely angular, margin somewhat crenate with acute teeth bent abruptly downwards between teeth, ciliate, basal lobes cordate, overlapping when mature, moderately raised between veins, light purplish-green to dull brownish-purple, young blades brownish-pink to brownish-red, paler beneath; veins palmate, slightly prominent towards the base but impressed where branched towards the margin, prominent beneath, lateral veins ca. 2–3 pairs, greenish-yellow when young and whitish-green when mature. **Inflorescences** axillary, more or less erect, 10–23 cm long, brownish-red, peduncles 8–18.5 cm long, two main branches 2–3 cm long, pedicels 6–9 mm; bracts in pairs at node of peduncle, glabrous, elliptic-ovate or obovate, margin towards apex lacinate, 2–3 × 1.5–2 mm, light yellowish-green sometimes with a faint tinge of pink, persistent. **Male flowers** with 4 tepals, margin entire, 1–1.2 × 1.1–1.4 cm; outer 2 tepals rotund, concave at centre, 5–7 × 6 mm, pale greenish-white or light pink or pale pinkish-white, inner 2 tepals obovate, apex rounded or sometimes retuse and impressed along centre, ca. 5 × 3 mm, white; stamens numerous, torus ca. 1 mm long, stamen mass globose, symmetric, ca. 2.5 mm across; anthers obovoid-oblong, tip emarginate, ca. 0.5 mm long, dehiscing through 2 longitudinal slits. **Female flowers** with a light yellowish-green ovary sometimes with faint light pink tinge, ca. 5.5 × 9 mm, locules 3, wings 3 equal, placentation axile, 1 placenta per locule, each placenta usually with 2 minute branches at the base; usually tepals 3, outer 2 tepals, rotund, concave at centre, ca. 4 × 4.5 mm, white with faint green tinge; styles and stigmas 3, 1.5–2 mm, style light greenish-yellow, stigma pale yellow, papillose, spiral band. **Capsules** ca. 6 × 12 mm, locules 3, splitting longitudinally between locules, wing 3 equal, ca. 4 mm wide, surface glabrous, styles and stigmas persisting after

tepals have fallen, dangling on a fine, thread-like pedicel ca. 5.5 mm long. **Seeds** numerous, barrel-shaped, 0.3–0.34 × 0.2–0.22 mm, collar cells slightly more than half the seed length, surface sculptured.

Distribution. Endemic in Peninsular Malaysia, Johor, Mersing District, Endau-Rompin National Park, Sungai Selai. It is apparently a rare species as it is known only from the type locality in Endau Rompin National Park.

Etymology. Named after Dr Sam Yen-Yen, Malaysian botanist, specialist in Zingiberaceae who first discovered the species and recognised its potential as an ornamental plant.

Conservation status. Critically Endangered CR B2ab(iii, v), D1. Although the type locality is within a Totally Protected Area, it is known from only one population about 1.5 km² and its habitat is threatened by ecotourism activity and illegal collecting. The area of observed population covers about 1.5 km² (P.T. Ong pers. comm.).

Ecology. In primary lowland mixed dipterocarp forest, growing on moss-covered rocks, rarely epiphytic, near a waterfall in deep shade.

Note. The ovary of species in section *Jackia* have three locules, each with an unbranched placenta, but in this species two vestigial branches are present near the base of the placenta (Figure 2G). This is also seen, but is less pronounced, in *B. rajah*.

Other specimens examined. Peninsular Malaysia. Johor: Mersing, Endau-Rompin National Park, Sungai Selai, 15 Aug 2002, Sam et al. FRI 47082 (KEP!), 24 July 2012, Hairul et al. FRI 78570 (KEP!, SING!).

Begonia rajah Ridl. ex Rolfe

Figure 1, 3

Gardeners' Chronicle XVI (1894) 213, nom. nud.; Rolfe, Bulletin of Miscellaneous Information (1914) 327; Irmscher, Mitteilungen aus dem Institut für allgemeine Botanik in Hamburg 8 (1929) 96; Tebbitt, Begonias: Cultivation, Identification, and Natural History (2005) 198; Kiew, Begonias of Peninsular Malaysia (2005) 216, pro parte.

Section. *Jackia* M. Hughes

Type. Peninsular Malaysia. Terengganu, 1892, *Native collector s.n.* (holotype SING ex K!). **Description.** Dwarf herb with rhizomatous stem. Indumentum of soft hairs, usually pale pink, dense on stipule (1.5–2 mm long), petiole (cream-coloured, 2–4 mm), leaf margin (1–1.5 mm) and veins beneath (2–2.5 mm), peduncle (cream-coloured, 2–2.5 mm). **Stems** creeping, ca. 9 mm thick, light yellowish-green; stipules lanceolate-oblong or triangular, 15–20 × 6–10 mm, apex attenuate terminating in a hair, keeled from base to apex, margin entire and opaque, brownish-pink to scarlet, persistent. **Leaves** tufted, alternate, ca. 5 mm apart; petioles suberect, terete, ca. 5 mm across, 8–25 cm long, light yellowish-pink; blades rigid, succulent, glabrous, broadly ovate with an abruptly acute apex or radical oblique cordate, 7–15 × 6–15 cm, prominently bullate, margin ciliate, angular with abruptly acute teeth, basal lobes cordate, slightly overlapping when mature, notably dark brownish-purple blotches, polished shining sur-

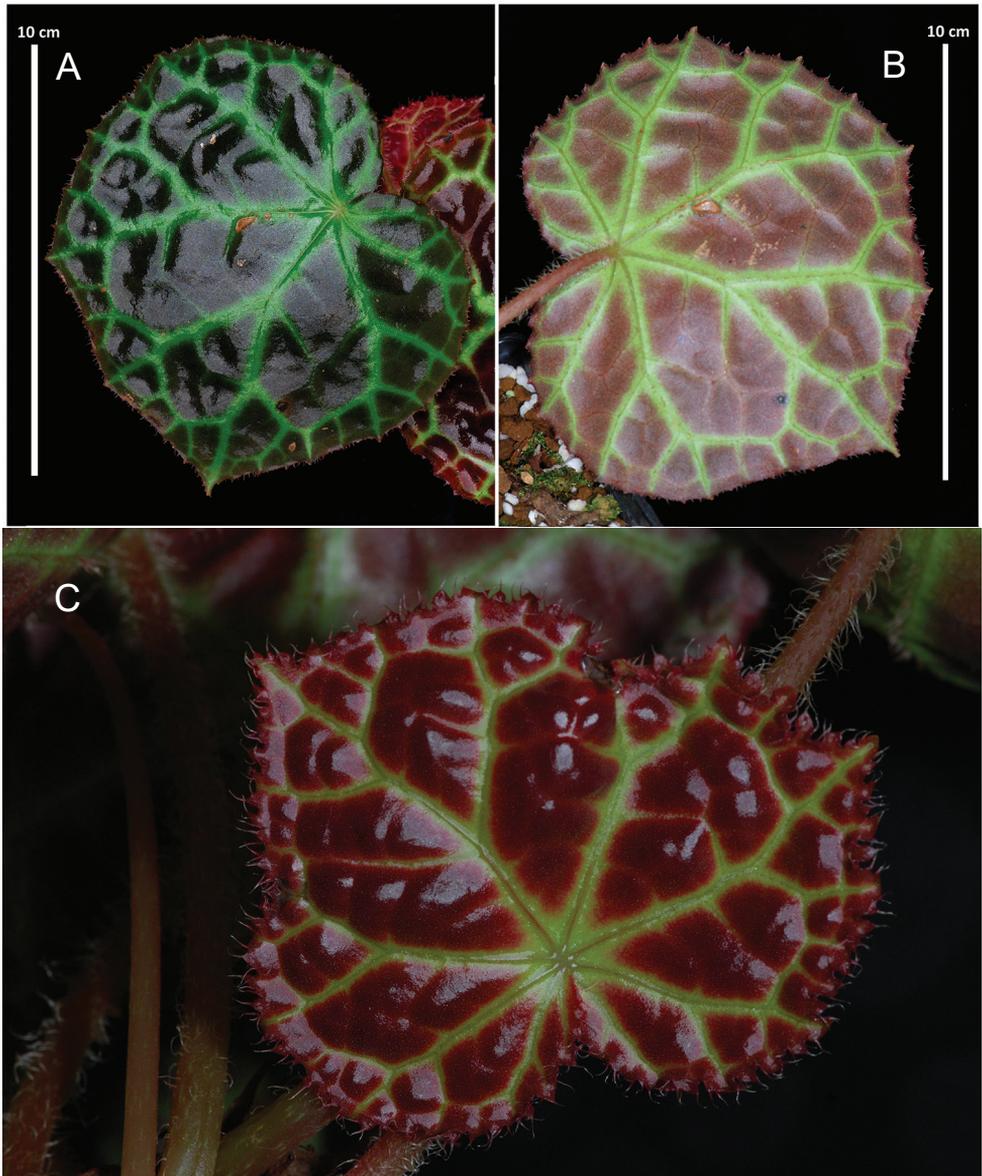


Figure 3. *Begonia rajah* Ridley (cultivated) **A** Upper leaf surface **B** under surface **C** Young blade.

face, young blades brownish-pink to vivid brownish-red, paler beneath; veins palmate, prominent towards the base and slightly impressed near margin, prominent beneath, lateral veins ca. 1–3 pairs, greenish-yellow when young, yellowish-green when mature. **Inflorescences** axillary, almost erect, many-flowered cyme, 20–25 cm long, peduncles 7–12 cm long, brownish-pink, two main branches ca. 1.3 cm long, pedicels slender, ca. 1 cm; bracts in pairs at node of peduncle, glabrous, broadly ovate, bowl-shaped,



Figure 4. *Begonia reginula* Kiew **A** *In situ*: clinging to moss-covered granite boulders **B** Young blade **C** Mature leaf of coloured form.

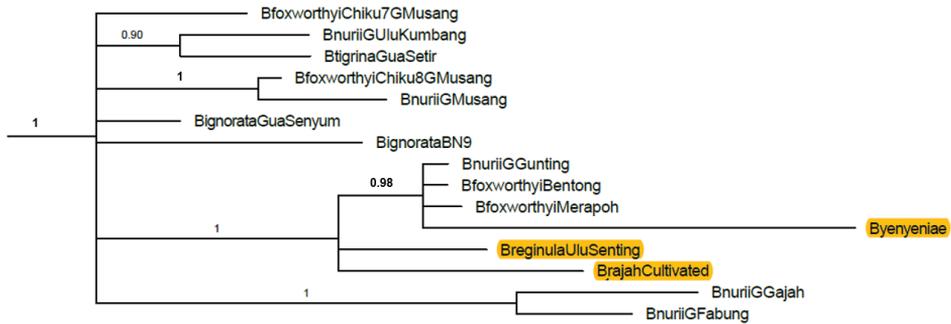


Figure 5. Part of the Bayesian 50% majority rule consensus tree from the analysis of chloroplast *ndhF-rpl32* region depicting relationships amongst *B. foxworthyi*, *B. rajah*, *B. reginula* and *B. yenyeniae*. The locality is indicated at the end of the species name for species with multiple samples. Numbers on the branches indicate Bayesian posterior probability (BPP) support values.

subacute, margin lacinate 5–8 × 7.5–8 mm, light yellowish-pink, persistent; bracteole narrowly ovate, 2–3 mm long, pale yellowish-pink. **Male flowers** with 4 tepals, margin entire, ca. 1.8 cm across, rose pink; outer tepals 2, obovate-orbicular or elliptic-ovate, 6–8 × 5–8 mm, inner 2 tepals obovate or narrow oblong, apex rounded or sometimes retuse and impressed along centre, ca. 5 × 3 mm; stamen mass globose, symmetric, 3 × 2.5 mm; anthers broadly obovoid-oblong, tip rounded, ca. 0.7 mm long, dehiscing through 2 longitudinal slits. **Female flowers** with a pale pink ovary with darker pinkish margin, 7–8 mm, locules 3, placentation axile, 1 placenta per locule, each placenta usually with 2 minute branches at the base, usually 3 tepals rarely 2, outer 2 tepals widely elliptic-ovate, concave at centre, ca. 6–7 mm, pale pink; styles and stigmas 3, 2–2.5 mm, style light greenish-yellow, stigma pale yellow, papillose, spiral band. Capsules ca. 1.2 cm across, locules 3, splitting longitudinally between locules, surface glabrous, dangling on a fine, thread-like pedicel 1 cm long. **Seeds** not seen by us.

Distribution. Endemic in Peninsular Malaysia, Terengganu (without specific locality). Apparently restricted in its distribution because, despite continuous botanical collecting in Terengganu and elsewhere in Peninsular Malaysia, it has not been re-found since it was first collected in 1892 (Kiew, 2005).

Etymology. From a Sanskrit word *raja* = king, presumably referring to it as the most beautiful begonia species.

Conservation status. Extinct in the Wild (EW). However, it survives in cultivation since its first introduction into cultivation in 1894 (Anonymous 1894b, 1895).

Ecology. Its natural habitat is not known but judging from its growth requirements in cultivation, it probably grew in cool and shaded conditions. **Other specimens examined. Peninsular Malaysia.** Herbarium specimens made from cultivated plants in Royal Botanic Gardens, Kew Anonymous s.n. 1903 (K!); Sander F. et al., s.n. 1913 (K!); Anonymous s.n. August 1913 (K!).

Table 1. *Begonia rajah*, *B. yenyeniae*, *B. reginula* and *B. foxworthyi* compared.

Character	<i>Begonia rajah</i>	<i>Begonia yenyeniae</i> sp. nov.	<i>Begonia reginula</i>	<i>Begonia foxworthyi</i>
Leaf: texture	thickly succulent	thinly succulent	thinly succulent	thinly succulent
Surface	polished shining prominent bullate	moderately raised in between veins	slightly raised in between veins	glossy, veins slightly impressed
Leaf shape	subrotund apex abruptly acute angular	orbicular-reniform apex rounded scarcely angular	broadly ovate apex abruptly attenuate angular	almost rotund apex short- pointed not angular
Tepal no. in male flower	4	4	2	2
Tepal no. in female flower	3	3	2	2
Outer tepal size (mm)	6–8 × 5–8	5–7 × 6	6–10 × 9–11	4–6 × 4–6
Outer tepal: shape	widely ovate or cordate	rotund	widely ovate or rotund	broadly ovate to rotund rounded
apex	obtuse	rounded	rounded or acute subcordate	subcordate
base	subcordate	rounded		
Stipule: shape	lanceolate-oblong or triangular	narrowly triangular,	narrowly triangular,	narrowly triangular
size (mm)	15–20 × 10–12	9–12 × 3–4	5–7 × 2–3	7–12 × 2–5
colour	Brownish-pink to scarlet	light yellowish- green sometimes with a faint pink tinge	pale greenish yellow or reddish yellow	red
Keel on stipules	from base to apex	from base to apex	absent	from base to apex
Bract: shape	broadly ovate, bowl- shaped	elliptic-ovate or obovate, curved or flat	obovate, curved or flat	obovate, flat
size (mm)	5–8 × 7.5–8	2–3 × 1.5–2	3–4 × 1–3	4–6 × 2–4
Hair density:				
petioles	hairy	sparsely hairy, more hairy near leaf base	sparsely hairy	densely hairy
stipules	densely hairy	sparsely hairy	hairy	densely hairy
peduncles	hairy	sparsely covered, hairs inconspicuous	without hairs	sparsely hairy
Ovary colour	pink or pale pink	light yellowish- green	light yellowish- green	reddish or dark pink

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Supplementary material I

List of taxa with accession and location information for the 48 samples of Begonia included in the Bayesian analysis using *ndhF-rpl32* intergenic spacer sequences

Authors: Joanne Pei-Chih Tan, Sheh May Tam, Ruth Kiew

Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.110.25846.suppl1>

Coccomyxa greatwallensis sp. nov. (Trebouxiophyceae, Chlorophyta), a lichen epiphytic alga from Fildes Peninsula, Antarctica

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Abstract

A single-celled green alga *Coccomyxa greatwallensis* Shunan Cao & Qiming Zhou, **sp. nov.**, isolated from a specimen of Antarctic lichen *Psoroma hypnorum* (Vahl) Gray, is described and illustrated based on a comprehensive investigation of morphology, ultrastructure, ecology and phylogeny. The cells of *C. greatwallensis* are ovoid to long ellipsoidal and measured 3–5 µm × 6–12 µm. The new species has distinct ITS rDNA and SSU rDNA sequences and differs from the phylogenetic closely related species *C. antarctica*, *C. arvernensis* and *C. viridis* in cell size, distribution and habitat.

Keywords

Lichen epiphyte, Morphology, TEM, Phylogeny

Introduction

The coccoid green algal genus *Coccomyxa* Schmidle (1901) is well known for its diversified ecological habitats and worldwide distribution. Algae of this genus has been reported as free living (Blanc et al. 2012), endophytic (Tremouillaux-Guiller et al. 2002; Zuykov et al. 2014) and lichen photobionts (Zoller and Lutzoni 2003; Muggi et al. 2010). *Coccomyxa* can survive under extremely harsh environments, such as in the spent fuel cooling pond of a nuclear reactor (Rivasseau et al. 2016), in a highly acidic lake (pH=2.6) (Hrdinka et al. 2013), as well as in polar regions (as low as -88 °C) (Blanc et al. 2012).

Based on the mucilaginous colonies' structure, cell length and width variability details, Jaag (1933) delimited 33 species of this genus, including 14 free-living, 13 lichenised and 6 lichen epiphytic species. Subsequently, only seven species were well recognised based on some morphological characters, such as cell shape and size, chloroplasts numbers and mucilage properties (Ettl and Gärtner 1995). However, the morphological characters of *Coccomyxa* depend on the culture conditions, for example, salinity influenced the phenotypic plasticity significantly (Darienko et al. 2015) and nutrient availability influenced the presence of mucilaginous sheaths (Malavasi et al. 2016). As the instability of morphological features led to a problematic morphological delineation of the genus *Coccomyxa*, a DNA-barcode based method has been developed and seven distinct species were subdivided (Darienko et al. 2015). Malavasi et al. (2016), combining morphological characters, ecological features and DNA sequences of *Coccomyxa*, recognised 27 species scenarios. Subsequently, *Coccomyxa antarctica* Shunan Cao & Qiming Zhou, 2018 was described as a new epiphytic species living with lichen *Usnea aurantiacoatra* on King George Island (Cao et al. 2018)

Currently, 28 species scenarios have been accepted, amongst which *C. actinabiotis* Rivasseau, Farhi & Couté, 2016, *C. antarctica*, *C. avernensis* Jaag, 1933, *C. polymorpha* T. Darienko & T. Pröschold, 2015, *C. subellipsoidea* E. Acton, 1909 and undescribed *Coccomyxa* spp., belonging to Clade I, Clade KL and Clade N according Malavasi et al. (2016), are the eight epiphytic species scenarios. Meanwhile, the species *C. avernensis* and *C. subellipsoidea* are also reported as lichen photobionts. The other lichenised species scenarios include *C. dispar* Schmidle, 1901, *C. solorinae* Chodat, 1909, *C. viridis* Chodat, 1913 and *Coccomyxa* Clades A, D and F (Malavasi et al. 2016). The species *C. antarctica*, *C. dispar*, *C. subellipsoidea* and *C. simplex* Mainx, 1928 show the Antarctic distribution, amongst which *C. simplex* is the only free living one (Holm-Hansen 1964; Darienko et al. 2015; Borchhard et al. 2017; Cao et al. 2018).

The Fildes Peninsula undergoes a typical sub-Antarctic oceanic climate with relatively high precipitation (89%) with 56–64 mm rainfall, wind blowing from west through northwest with a speed of 6.8–7.4 m/s and the average temperature ranging from 0.5–1.8 °C in summer (Yang et al. 2013). About 127 lichen species have been recorded in Fildes Peninsula (http://www.aari.aq/KGI/Vegetation/lst_lichens.html). The lichen *Psoroma hypnorum* (Vahl) Gary, one of the four *Psoroma* spp. found in this region, is characterised by its squamulose thallus without secondary products, dull brown discs, apothecia margin without or with very short hairs (Øvstedal and Smith 2001). Both cyanobacteria and green algae have been reported as photosynthetic part-

ners of *P. hypnorum* (Holien and Jørgensen 2000; Øvstedal and Smith 2001; Wirtz et al. 2003; Ekman et al. 2014).

In the current study, a lichenicolous single cell green alga was isolated from *P. hypnorum*. Based on the comprehensive analysis approach, including morphology, ultrastructure, ecology and phylogeny, the green alga is demonstrated to be new to science.

Material and methods

Isolation and culture

The lichen specimen (collection No. 274) of *Psoroma hypnorum* was collected from Fildes Peninsula, King George Island, Antarctica (62°12.69'S, 58°55.70'W) during the 30th Chinese National Antarctic Research Expeditions in summertime (1 February 2014–15 March 2014). The specimen was kept in the Resource-sharing Platform of Polar Samples which includes samples of Biology, Ice-snow, Rock, Deep-space and Sediment (BIRDS ID 2131C0001ASBM100076) at 4 °C till the isolation was processed.

A single algal cell was obtained following a modified aseptic isolation procedure (Cao et al. 2018). The isolations, cultured on a petri-dish with PDA and BBM medium in an illumination incubator (4 °C, 12 hr light/12 hr dark), were deposited in the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB) as an open collection (FACHB-2139).

Light and electron microscopy

For observing and photographing the algal cultures, compound microscopes Nikon Eclipse 80i and Nikon ACT-1 V2.70 were used.

After fixing with 2.5% glutaraldehyde buffer, the algal cells were used for transmission electron microscopy (TEM). The procedures and reagents (including 2.5% glutaraldehyde buffer) used followed Cao et al. (2018). The 70 nm cell sections, cut by a Leica EM UC6 ultramicrotome and stained with 3% uranyl acetate and lead citrate, were observed using a Jeol JEM1230 transmission electron microscope at 80–120 kV. The micrographs were captured using iTEM software by an Olympus SIS VELETA CCD camera.

DNA extraction, amplification, sequencing and analysis

A modified CTAB method (Cao et al. 2015) was used to extract the alga genomic DNA. Primer pairs NS1, NS4; NS3, NS6; NS5, NS8 (White et al. 1990) and primer pair ITS5, O2 (Cao et al. 2015) were used to amplify the SSU rDNA and ITS rDNA, respectively. A 50 µl volume PCR reaction was selected, PCR application and products verification followed Cao et al. (2015) and double-stranded PCR products were sequenced by an ABI3730XL sequencer.

SEQMAN programme within Lasergene v.7.1 software (DNASTAR Inc.) was selected to check the double-directional ITS rDNA and SSU rDNA sequences. These two regions were overlapped into one single contig and the flanking regions were trimmed off. The sequence representing the new species was submitted to GenBank (MF465899).

ClustalW algorithm, including in MEGA 7 (Kumar et al. 2016), was performed to align the sequences with default parameters (Higgins et al. 1994) and then adjusted manually. The Neighbour-Joining (NJ) was selected to calculate the ITS phylogenetic structures,

Table 1. *Coccomyxa* spp. sequences used in the present study.

Species	Collection No.	GenBank No.	
		ITS rDNA	SSU rDNA
Clade B* <i>Coccomyxa</i> sp.	GA5a	AB917140	AB917140
Clade D* <i>Coccomyxa</i> sp.	CCAP 216/24	FN298927	FN298927
	CCAP 812/2A	HG972992	HG972992
Clade E* <i>Coccomyxa</i> sp.	IB-GF-12	–	KM020052
Clade E* <i>Coccomyxa subellipsoidea</i>	CCAP 812/3	HG972972	HG972972
Clade H* <i>Coccomyxa</i> sp.	KN-2011-U5	HE586557	–
Clade I* <i>Coccomyxa</i> sp.	KN-2011-T3	HE586515	HE586515
	KN-2011-T1	HE586550	–
Clade K* <i>Coccomyxa</i> sp.	KN-2011-C4	HE586508	HE586508
Clade L* <i>Monodus</i> sp.	UTEX B SNO83	–	HE586506
Clade M* <i>Monodus</i> sp.	CR2-4	HE586519	HE586519
Clade N* <i>Coccomyxa viridis</i> 3	CAUP H5103	HG973007	HG973007
	SAG 2040	HG973004	HG973004
<i>Coccomyxa actinabiotis</i>	216-25	FR850476	FR850476
	KN-2011-T4	HE586516	HE586516
<i>Coccomyxa antarctica</i>	FACHB-2140	MF465900	MF465900
<i>Coccomyxa arvernensis</i>	SAG 216-1	–	HG972999
	Wien C19	HG973000	HG973000
<i>Coccomyxa dispar</i>	SAG 49.84	HG972998	HG972998
<i>Coccomyxa elongata</i>	CAUP H5107	HG972981	HG972981
	SAG 216-3b	HG972980	HG972980
<i>Coccomyxa galuniae</i>	CCAP 211/97	FN298928	FN298928
	SAG 2253	HG972996	HG972996
<i>Coccomyxa greatwallensis</i> sp. nov.	FACHB-2139	MF465899	MF465899
<i>Coccomyxa melkonianii</i>	SCCA048	KU696488	KU696488
<i>Coccomyxa onubensis</i>	ACCV1	HE617183	HE617183
<i>Coccomyxa polymorpha</i>	CAUP H5101	HG972979	HG972979
	KN-2011-T2	HE586514	HE586514
<i>Coccomyxa simplex</i>	CAUP H 102	HE586504	HE586504
	SAG 216-2	HG972989	HG972989
<i>Coccomyxa solorinae</i>	SAG 216-12	HG972987	HG972987
	SAG 216-6	HG972988	HG972988
<i>Coccomyxa subellipsoidea</i>	SAG 216-7	HG972976	HG972976
	Wien C20	HG972975	HG972975
	CAUP H5105	HG972974	–
<i>Coccomyxa vinatzeri</i>	ASIB V16	HG972994	HG972994
<i>Coccomyxa viridis</i>	SAG 216-14	HG973002	HG973002
	SAG 216-4	HG973001	HG973001
<i>Elliptochloris bilobata</i>	SAG 245.80	HG972969	HG972969
<i>Hemichloris antarctica</i>	SAG 62.90	HG972970	HG972970

* Clades referred after Malavasi et al. (2016).

as well as Maximum Likelihood (ML) method for SSU sequences. Pairwise distances of ITS rDNA and SSU rDNA sequences were calculated using MEGA 7. A 1000 resamplings bootstrap was tested for the reliability of the inferred trees. In total, 42 sequences, which have been confirmed by Malavasi et al. (2016), were retrieved from GenBank (Table 1).

Results

Coccomyxa greatwallensis Shunan Cao & Qiming Zhou, sp. nov.

Figures 1, 2

Holotype. Strain FACHB-2139, Freshwater Algae Culture Collection, the Institute of Hydrobiology (FACHB-Collection) (Fig. 1a).

Type locality. Antarctic, Fildes Peninsula, on soil (62°12.69'S, 58°557.70'W), 40 m a.s.l.; isolated from the Antarctic lichen *Psoroma hypnorum* (collection No. 274, BIRDS ID: 2131C0001ASBM100076) on 14 February 2014.

Habitat. Epiphytic green alga, living with lichen *Psoroma hypnorum* in Sub-Antarctic climate.

Description. Single-celled green alga, ovoid to long ellipsoidal, asymmetrical, measured 3–5 μm \times 6–12 μm , some cells nearly rounded in nutrient-rich PDA medi-

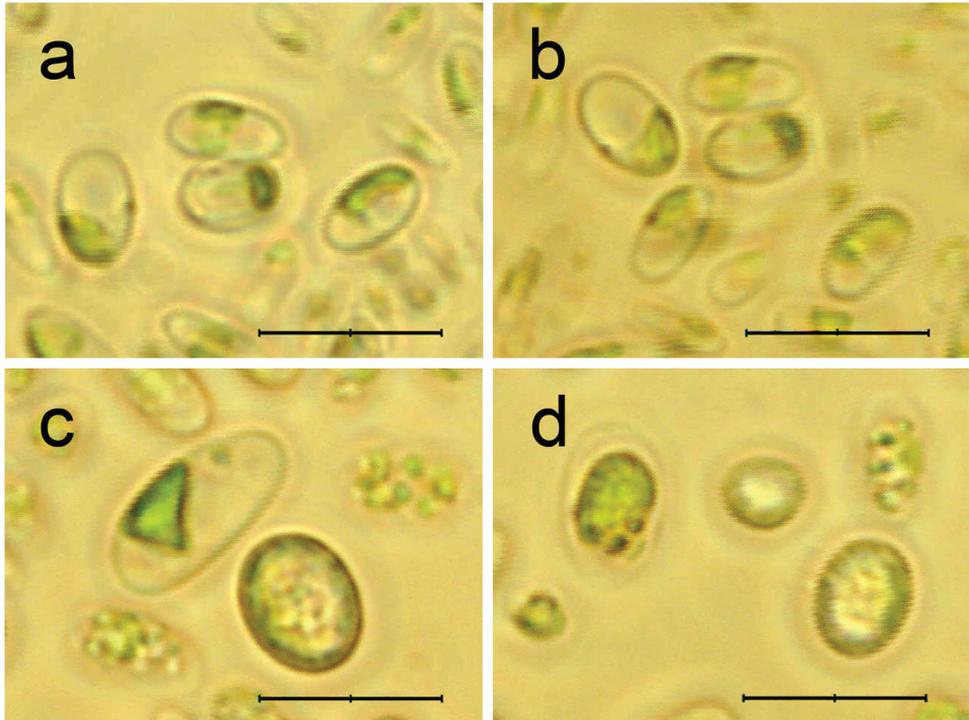


Figure 1. *Coccomyxa greatwallensis* Shunan Cao & Qiming Zhou, sp. nov., light microphotographs. Cells cultured in BBM medium (**a, b**) and in PDA medium (**c, d**). Scale bar: 10 μm .

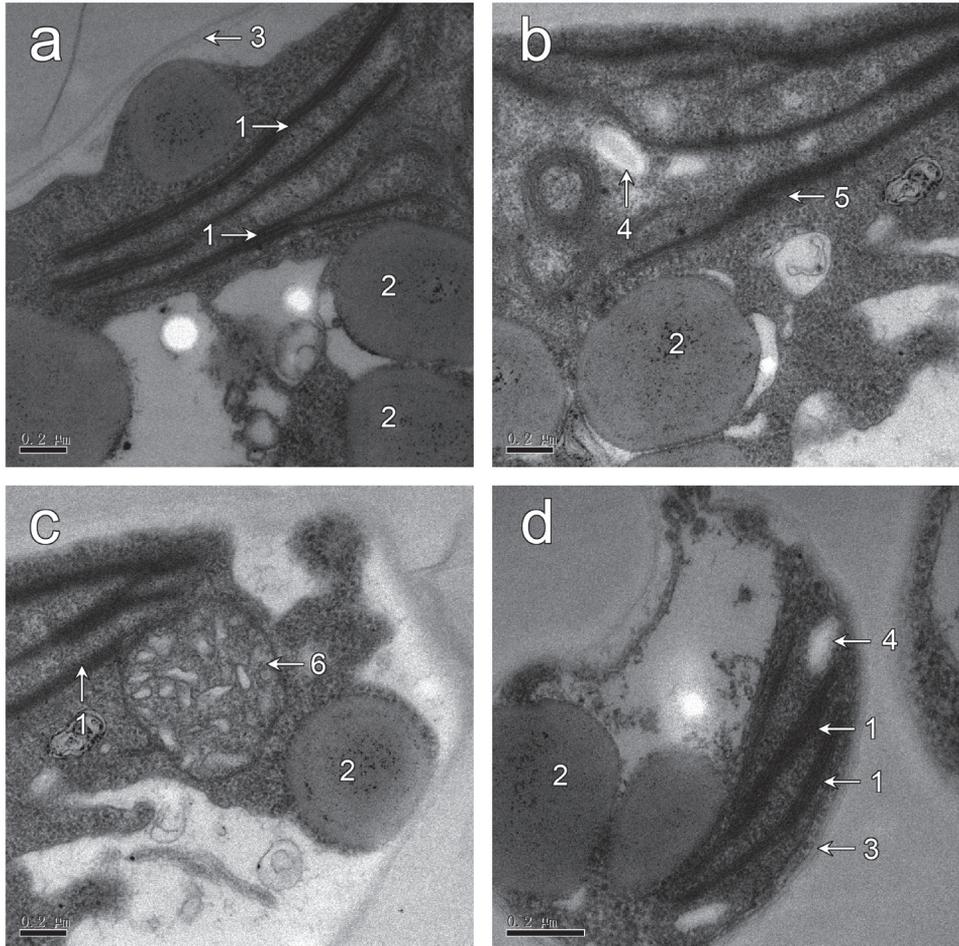


Figure 2. Ultrastructure of *Coccomyxa greatwallensis* Shunan Cao & Qiming Zhou, sp. nov. in PDA medium; note: chloroplast (2), plastoglobuli (2), cell wall (3), starch granules (4), thylakoids (5) and mitochondria (6). Scale bar: 0.2 μm .

um; cells without mucilaginous sheath (Fig. 1). Cell wall smooth, three layers in ultrastructures. Protoplast filled with lipid droplets. Chloroplast parietal, without pyrenoid and with starch granules in the inter thylacoidal spaces. One nucleus in the central part of the cell present. Reproductive process not observed (Fig. 2).

Molecular analyses

The pairwise distance analysis of ITS rDNA sequences shows that the overall mean distance is 0.171 ± 0.015 . The pairwise distance between our algal strain FACHB-2139 and the other species of *Coccomyxa* ranged from 0.253 to 0.022, of which *C. arvern-*

ensis shows the minimum distance with our isolate of 0.022 followed by *Coccomyxa* sp. Clade N of 0.030 (Suppl. material 1: Table S1). The pairwise distance analysis of SSU rDNA sequences shows that the overall mean distance is 0.017 ± 0.002 . In addition, the pairwise distance between alga strain FACHB-2139 and the other species of *Coccomyxa* ranged from 0.025 to 0.001, amongst which both *C. arvernensis* and *C. viridis* show the minimum distance of 0.001 with our sample (Suppl. material 1: Table S1). That indicated that alga FACHB-2139 is closely related to *C. arvernensis* and *C. viridis*.

For the ITS rDNA, all the *Coccomyxa* sequences clustered into one group supported with bootstrap value 100 and within *Coccomyxa*, six subgroups have been clustered. The alga FACHB-2139 together with *C. antarctica*, *C. arvernensis*, *C. viridis*, *Coccomyxa* spp. of clade KL, Clade M and Clade N clustered as a subgroup, were supported with a bootstrap value 100; but the newly isolated strain FACHB-2139 differs from the other species clearly, no well supported clade for FACHB-2139 and species mentioned above were formed (Fig. 3a). In the SSU rDNA phylogenetic result, the sequences of *Coccomyxa* clustered into five subgroups and the alga FACHB-2139, *C. antarctica*, *C. arvernensis*, *C. viridis*, *Coccomyxa* spp. Clade K, Clade L, Clade M and Clade N lay in the same subgroup whose bootstrap value was 97. Though alga strain FACHB-2139 and *C. arvernensis* formed a monophyletic group, this clade was supported by a low bootstrap value 53, which indicated that these two species were insufficiently supported statistically (Fig. 3b).

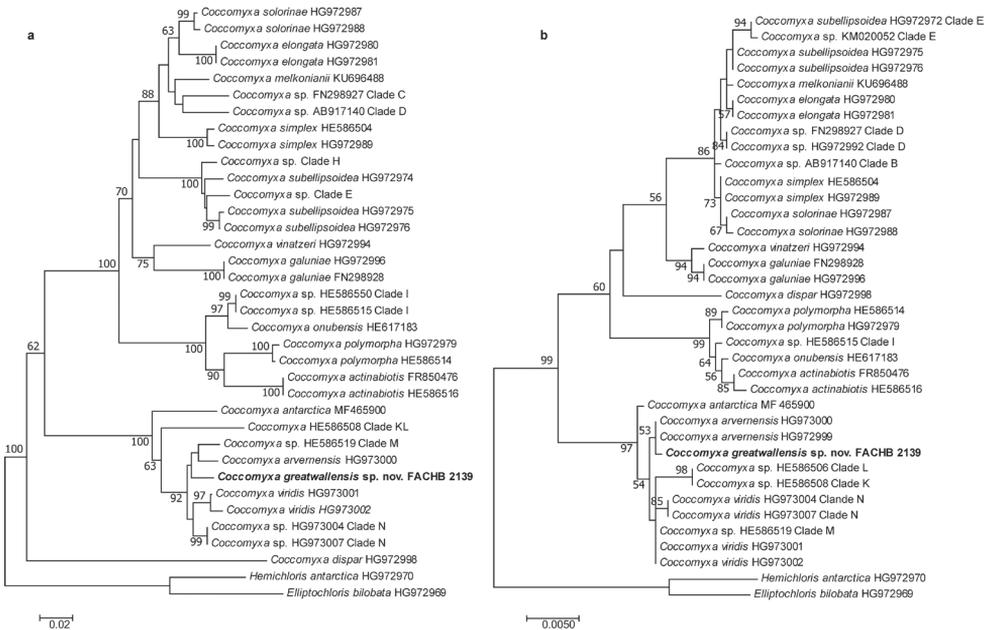


Figure 3. The NJ tree based on ITS rDNA (a) and the ML tree based on SSU rDNA (b) sequences phylogenetic analyses. The sequences marked with *Coccomyxa* clade A–N referred after Malavasi et al. (2016).

Diagnosis

Morphologically, our sample FACHB-2139 can be distinguished from its phylogenetically close congeners *C. viridis* (1.8–3.6 $\mu\text{m} \times$ 4.7–8.4 μm) and *C. arvernensis* (3–4 $\mu\text{m} \times$ 6–8 μm) (Müller 2005, Hodač 2015) by its larger cells and from *C. antarctica* (4–7 $\mu\text{m} \times$ 8–12 μm) by its smaller cells (Cao et al. 2018). The cell sizes of the above species were recorded when cultured in BBM medium. In addition, both *C. viridis* and *C. arvernensis* are lichenised or are epiphytic species and have not been recorded as an Antarctica distribution.

Our molecular and morphological analyses indicate that algal isolate FACHB-2139 represents a new *Coccomyxa* species which we named *Coccomyxa greatwallensis* Shunan Cao & Qiming Zhou sp. nov.

Discussion

Coccomyxa greatwallensis Shunan Cao & Qiming Zhou sp. nov., isolated from Antarctic squamulose lichen *P. hypnorum*, is one of the *Coccomyxa* species, which is characterised by ovoid to ellipsoidal single cells. The usage of molecular barcode provides an effective and stable tool to identify and classify the species of *Coccomyxa* (Darienko et al. 2015; Malavasi et al. 2016). In the current study, both ITS and SSU rDNA were used and a comparison with the closely related species had been listed in Suppl. material 3:Table S3. The minimum pairwise distance was calculated between *C. arvernensis* and *C. greatwallensis* using ITS rDNA sequences, but the bootstrap value, which was lower than 50, did not support these two species as a monophyletic group. A similar result was also obtained using SSU rDNA sequences.

Though some *Coccomyxa* species could be the photosynthetic partner of lichens (Honegger and Brunner 1981), due to the lichen mycobiont's selectivity to its photobiont partner, one photobiont group occurs within relative stable lichen groups (Tschermak-Woess 1988; Cao et al. 2015); for example, *Coccomyxa* is known as the photobiont of lichenised ascomycots belonging to Peltigerales (i.e. *Nephroma* Müll. Arg., *Peltigera* Willd. and *Solorina* Ach.), Baeomycetales (*Baeomyces* Pers., *Dibaeis* Clem., *Orceolina* Hertel and *Placynthiella* Gyeln.), Pertusariales (*Icmadophila* Trevis.), Agaricales (*Lichenomphalia* Redhead, Lutzoni, Moncalvo & Vilgalys), Lecanorales (*Micarea* Fr.) and Cantharellales (*Multi clavula* R.H. Petersen) (Poulsen et al. 2001; Smith et al. 2009; Wirth et al. 2013), as well as the basidiomycots belonging to Agaricales (*Omphalina* Qué.) (Jaag 1933; Zoller and Lutzoni 2003). In addition, *Coccomyxa* is optionally lichenised with the fungus *Schizoxylon albescens* Gilenstam, H. Döring & Wedin (Ostropales) (Muggi et al. 2010). Furthermore, there is also evidence to support the photosynthetic partner of Antarctic lichen *P. hypnorum* is cyanobacteria or the green algae *Myrmecia* Printz (Brodo et al. 2001; Øvstedal and Smith 2001; Wirtz et al. 2003; Ekman et al. 2014) but not the species of *Coccomyxa*. We therefore conclude that the newly described green alga *C. greatwallensis* is an epiphytic alga of lichen *P. hypnorum*.

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Supplementary material I

Table S1. Pairwise distance calculated using ITS rDNA sequences

Authors: Shunan Cao, Fang Zhang, Hongyuan Zheng, Fang Peng, Chuanpeng Liu, Qiming Zhou

Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.110.26961.suppl1>

Supplementary material 2

Table S2. Pairwise distance calculated using SSU rDNA sequences

Authors: Shunan Cao, Fang Zhang, Hongyuan Zheng, Fang Peng, Chuanpeng Liu, Qiming Zhou

Data type: molecular data

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Link: <https://doi.org/10.3897/phytokeys.110.26961.suppl2>

Supplementary material 3

Table S3. Comparison of the closely related species

Authors: Shunan Cao, Fang Zhang, Hongyuan Zheng, Fang Peng, Chuanpeng Liu, Qiming Zhou

Data type: species data

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Link: <https://doi.org/10.3897/phytokeys.110.26961.suppl3>

Phylogeny, infrageneric classification and species delimitation in the Malagasy *Impatiens* (Balsaminaceae)

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Abstract

The species-rich genus *Impatiens* (Balsaminaceae) is represented in Madagascar by no less than 260 species. We conducted molecular phylogenetic analyses of the Malagasy *Impatiens* based on nuclear and plastid data and 52 accessions (representing 33 species) to: 1) reassess the monophyly of the Malagasy *Impatiens*; 2) assess the monophyly of the sections *Preimpatiens* (*Humboldtianae* and *Vulgare* groups) with spurs and *Trimorphopetalum* without spurs as delimited by Perrier de la Bâthie, as well as that of the subgenera *Impatiens* and *Trimorphopetalum* as defined by Fischer and Rahelivololona; 3) infer the current status of some morphologically variable species; and 4) test whether the species of *Impatiens* from the Marojejy National Park form a monophyletic group. The Malagasy *Impatiens* are further confirmed to be paraphyletic with respect of the Comorian *I. auricoma*. The present sectional and subgeneric classifications of the Malagasy *Impatiens* are partly supported, with strong support for the monophyly of the sect. or subgen. *Trimorphopetalum*. Section *Preimpatiens* was not supported as monophyletic and neither the *Humboldtianae* group nor the *Vulgare* group is monophyletic. *Impatiens elatostemmoides*, *I. "hammarbyoides"*, *I. inaperta*, *I. lyallii* and *I. manaharensis* are either para- or polyphyletic and may represent morpho-species. The *Impatiens* species from the Marojejy National Park do not form a monophyletic group and therefore are suggested to be derived from numerous independent colonisation events from all over Madagascar followed by subsequent diversifications.

Keywords

Balsaminaceae, infrageneric classification, *Impatiens*, Madagascar, monophyly, species delimitation, systematics, taxonomy

Introduction

The genus *Impatiens* L. (Balsaminaceae) is a monophyletic and diverse genus with more than 1000 species (e.g. Yuan et al. 2004, Janssens et al. 2009) and is represented by at least 260 endemic species in Madagascar (e.g. Fischer and Rahelivololona 2002, 2004a, b, 2015a, b, c, 2016, Fischer et al. 2003, 2017). This continental island is one of the centres of species diversity for the genus, which is the largest flowering plant genus on the island (Perrier de la Bâthie 1934, 1948, Humbert 1955, 1956, Fischer and Rahelivololona 2002, 2004a, b, 2007a, b, 2015a, b, c, 2016, Fischer et al. 2003, 2017) (Figs 1, 2). Fischer and Rahelivololona (e.g. 2002, 2004a, b) initiated the taxonomic studies of the Malagasy and Comorian members of *Impatiens* in an attempt to produce an updated Flora of the family Balsaminaceae for Madagascar and the Comoros. Since then, 75 new species have been described and at least another 75 new species will be published in the near future (E. Rahelivololona and E. Fischer, unpubl. data). The majority of the Malagasy *Impatiens* occurs in the montane regions of northern and eastern Madagascar (e.g. Tsaratanana National Park with 36 species, Marojejy National Park with 48 species, Masoala National Park with 59 species) (Figs 1, 2).

Warburg and Reiche (1895) provided the first global infrageneric classification for *Impatiens* based solely on morphological data. Since then, a number of infrageneric classifications of the genus have been proposed for some tropical regions (including Madagascar). Perrier de la Bâthie (1934) established the first sectional classifications for the Malagasy and Comorian *Impatiens*, placing the species with obvious spurs and anthers with apical dehiscence in his section *Preimpatiens*. The author subdivided sect. *Preimpatiens* into two groups: the *Vulgare* group with pink, purple, white or orange flowers with shorter and slender spurs and the *Humblotianae* group with red, yellow or orange flowers with larger and broader spurs. Furthermore, Perrier de la Bâthie (1934) classified the Malagasy *Impatiens* species with anthers dehiscing apically but without spurs on the low sepals into two sections: the monotypic sect. *Impatientella* with deltoid and sharp anthers and sect. *Trimorphopetalum* with obtuse or truncate anthers (Fig. 2), both endemic to Madagascar. Fischer and Rahelivololona (2002) recognised Perrier de la Bâthie's sect. *Impatiens* with spurs (Fig. 1) and sect. *Trimorphopetalum* without spurs (Fig. 4) at subgeneric level. They formally subsumed sect. *Impatientella* into subgen. *Trimorphopetalum*.

The category of species is widely accepted as the basic or working unit of biological classification (Rosell et al. 2010, Hohenegger 2014). From a phylogenetic point of view, a species of traditional taxonomy is often viewed as a species hypothesis tested by recovering either monophyletic or non-monophyletic units. Most species concepts (e.g. Baum and Shaw 1995) consider monophyly to be congruent with species hypotheses, while almost all species concepts view polyphyly as a rejection of species hypotheses (e.g. Rosell et al. 2010). Previous and contemporary workers (e.g. Perrier de la Bâthie 1934, Fischer and Rahelivololona 2002, Fischer et al. 2003, 2017), dealing with species circumscription in the Malagasy *Impatiens*, have encountered difficulties in deciding whether a taxon represents a “real” species or a morpho-species. Several species (e.g. *I. elatostemmoides* H.Perrier, *I. “hammarbyoides”* Eb.Fisch. & Raheliv. (nomen

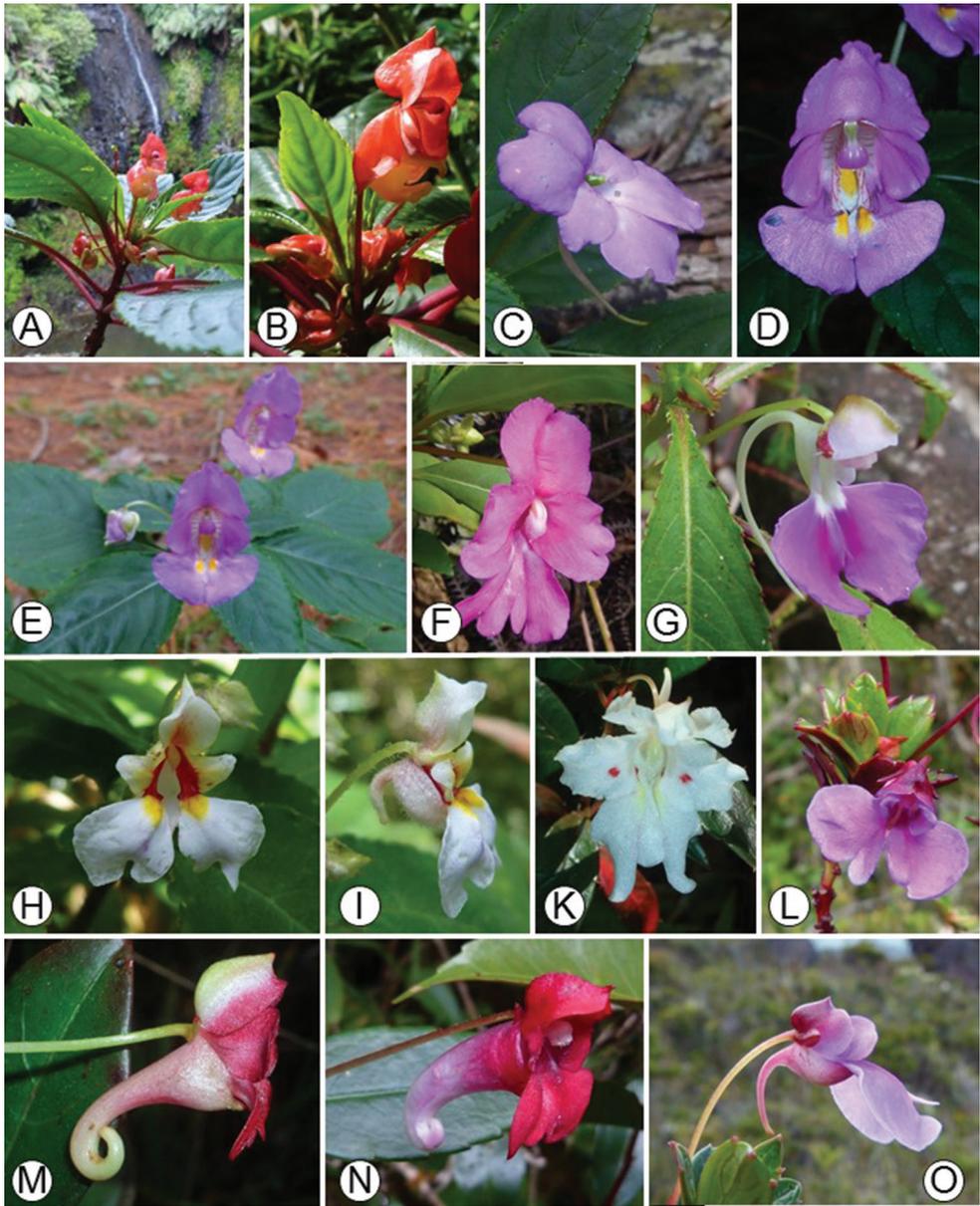


Figure 1. Representatives of *Impatiens* subgen. *Impatiens*. **A, B** *Impatiens bicaudata*, Montagne d'Ambre **C** *I. lyallii*, Montagne d'Ambre **D, E** *I. bisaccata*, Montagne d'Ambre **F** *I. max-huberi*, Marojejy **G** *I. nomenyae*, Marojejy **H, I** *I. masoalensis*, Marojejy **K** *I. cf. manaharensis*, Marojejy **L, O** *I. marojejyensis*, Marojejy **M** *I. susan-nathansoniae*, Marojejy **N** *I. hendrikii*, Marojejy. Photos: E. Fischer.

provisorium, not yet published), *I. inaperta* (H.Perrier.) H.Perrier, *I. lyallii* H.Perrier and *I. manaharensis* Baill.) are known to be morphologically variable and this raises doubts as to whether these species deserve specific status. Molecular trees (phylogenetic

hypotheses) can identify mono-, para- or polyphyletic taxa and can therefore be an important tool for assessing species delimitation. In addition, phylogenetic trees can also be used for assessing the various infrageneric classifications of the Malagasy *Impatiens*.

The first molecular phylogenetic study of the family Balsaminaceae by Yuan et al. (2004), based on nuclear ribosomal ITS (nrITS) sequence data and including 18 Malagasy *Impatiens* species (six species from subgen. *Trimorphopetalum* and 12 species from subgen. *Impatiens*), indicated that the Malagasy *Impatiens* species were polyphyletic, as they were resolved into three groups: a *Humboldtianae-Vulgare-Trimorphopetalum* clade, a lineage with *I. baroni* Baker of sect. *Impatiens* and a *Humboldtianae-Vulgare* clade consisting of seven Malagasy species of sect. *Impatiens* (*I. anovensis* H.Perrier to *I. vilersii* Costantin & Poiss.). The authors also showed the monophyly of the Malagasy sect. *Trimorphopetalum* only if sect. *Impatientella*, containing the spurless and entirely cleistogamous species *I. inaperta*, is included. Their results suggested a Malagasy origin of the Comorian species. In addition, each of the Malagasy *Impatiens* clades was nested within an African *Impatiens* lineage, suggesting multiple African origins of the Malagasy *Impatiens*. Moreover, subgen. *Impatiens* was not monophyletic. In contrast to Yuan et al. (2004), Janssens et al. (2006, 2007, 2009), who included representatives of subgen. *Impatiens* and *Trimorphopetalum*, strongly supported the monophyly of the Malagasy *Impatiens* (including the Comorian *I. auricomma*). These last three studies also confirmed a single African origin of the Malagasy representatives. However, all of the above-mentioned molecular studies (Yuan et al. 2004 with 17 species; Janssens et al. 2006, 2007 and 2009 with six species) used a very limited sampling of the Malagasy *Impatiens* and, therefore, the monophyly of subgen. *Trimorphopetalum* was in need of being tested with a much larger sampling effort. More recently, based on both morphological and molecular evidence, Yu et al. (2015) divided the genus *Impatiens* into two subgenera, subgen. *Clavicarpa* and subgen. *Impatiens*. Moreover, the authors delineated seven sections in subgenus *Impatiens*: sect. *Semeiocardium*, sect. *Tuberosae*, sect. *Racemosae*, sect. *Impatiens*, sect. *Scorpioidae*, sect. *Fasciculatae* and sect. *Uniflorae*. Of these, the latter is characterised by short fusiform capsules and includes all Malagasy species of *Impatiens*, as well as several African and Asian species.

The Marojejy National Park is located in north-eastern Madagascar within the SAVA Region. With its tallest peak rising to 2137 m, the area is home to a diverse flora of upland species. The wide range of elevations and rugged topography of Marojejy create diverse habitats, which transition quickly with changes in altitude. There are four types of forests within the park: lowland rainforest below 800 m (Fig. 3); moist montane rainforest between 800 and 1400 m (Fig. 3); sclerophyllous montane cloud forest between 1400 and 1800 m; and ericoid shrub above 1800 m (Fig. 4) (Humbert 1955). The higher summits are covered by subalpine grassland with small ericaceous shrubs (Fig. 4), and are home to numerous local endemic species of *Impatiens* and of other large genera, such as *Streptocarpus* (Gesneriaceae) and *Helichrysum* (Asteraceae). The park has been recognised as a marked centre of plant endemism. For example, 32 palm species found in the Marojejy area are endemic to Madagascar and seven of them are restricted to the park. Of the 18 species of tree ferns, inventoried in the

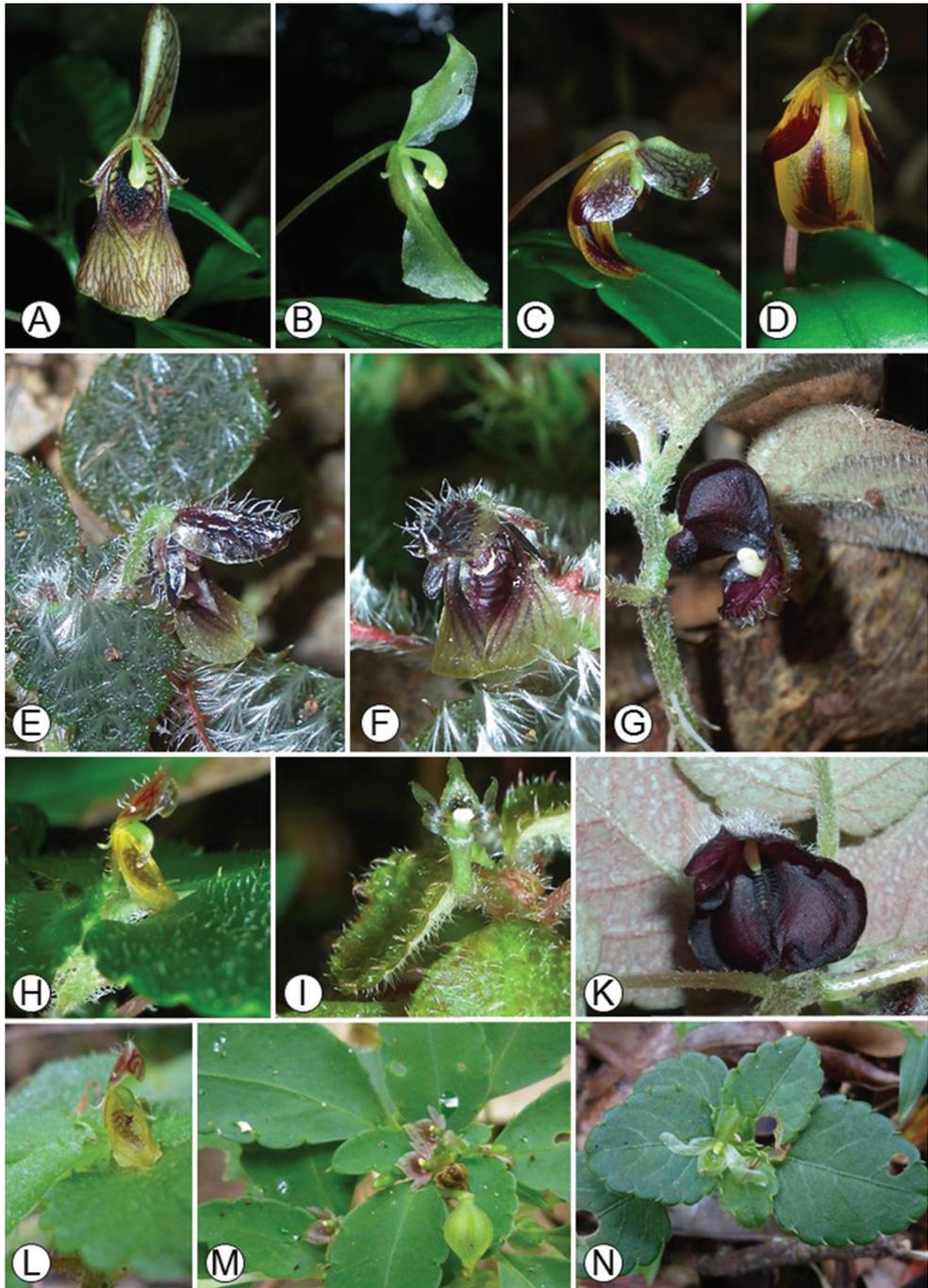


Figure 2. Representatives of *Impatiens* subgen. *Trimorphopetalum*. **A** *Impatiens lutzii*, Montagne d'Ambre **B** *I. galactica*, Marojejy **C, D** *I. "capuronii"*, Marojejy **E, F** *I. furcata*, Marojejy **G, K** *I. navicula*, Marojejy **H, L** *I. "humillima"*, Marojejy **I** *I. "hammarbyoides"*, Marojejy **M** *I. elatostemmoides*, Marojejy **N** *I. sp. nov. aff. elatostemmoides* 3, Montagne d'Ambre. Photos: E. Fischer.



Figure 3. **A, B** Lowland rainforest of the Marojej National park **A** ca. 400 m **B** ca. 490 m **C** Moist montane rainforest of the Marojej National park at ca. 1100 m. Photo: E. Fischer.



Figure 4. **A** Sclerophyllous montane cloud forest of the Marojej National park; 1500 m **B** Subalpine grassland, Marojej at ca. 2100 m. Photo: E. Fischer.

rainforests of Marojejy, seven are endemic to the area (Madagascar Catalogue 2017). Whether the endemic species of *Impatiens* from the Marojejy form a monophyletic group or are the result of a mixture of colonisation events from other regions through time have yet to be assessed.

The main objective of this study was to reconstruct a new and larger phylogeny of the Malagasy *Impatiens*, with a particular emphasis on taxa collected from Marojejy, using two nuclear *AP3/DEF* homologues (*ImpDEF1* and *ImpDEF2*) and the plastid *atpB-rbcL* spacer. The resulting phylogeny was subsequently used to (i) reassess the monophyly of the Malagasy *Impatiens* as stated by Janssens et al. (2006, 2007, 2009); (ii) assess the monophyly of the sections *Preimpatiens* (*Humblotianae* and *Vulgare* groups) and *Trimorphopetalum* as delimited by Perrier de la Bâthie (1934), as well as that of the subgenera *Impatiens* and *Trimorphopetalum* (including sect. *Impatientella*) as defined by Fischer and Rahelivololona (2002); (iii) assess the current species status of the morphologically variable species *I. elatostemmoides*, *I. "hammarbyoides"*, *I. inaperta* and *I. manaharensis*, using monophyly as the primary criterion (Backlund and Bremer 1998); and iv) test whether the species of *Impatiens* from the Marojejy National Park form a monophyletic group. The sectional classification proposed by Yu et al. (2015) could not be assessed, as our sampling was solely addressing the Malagasy and Comorian *Impatiens* species.

Methods

Taxon sampling

The taxon sampling for this study was focused on the Malagasy representatives of the genus *Impatiens*. We expanded the previous dataset of Yuan et al. (2004) and Janssens et al. (2009) with 29 new accessions (Table 1). In total, 52 accessions were included in our analyses, representing 48 Malagasy specimens (representing about 31–33 species), two Comorian accessions (representing one species) and two African species (Table 1). This sampling represented the three major groups defined by Perrier de la Bâthie (1934) as occurring in Madagascar and the Comoros: 31 accessions from sect. *Preimpatiens* (Perrier de la Bâthie, 1934) or subgen. *Impatiens* (Fischer & Rahelivololona, 2002) (10 accessions representing six species of the *Humblotianae* group; 21 accessions representing 11 or 12 species of the *Vulgare* group); and 22 accessions representing about 14 species from sect. *Trimorphopetalum* (Perrier de la Bâthie, 1934) or subgen. *Trimorphopetalum* (Fischer & Rahelivololona, 2002). The species showing some morphological variation were represented by more than one individual and, thus, were the subject of a test for monophyly. Twenty-seven accessions, represented by at least 17 species, were from the Marojejy National Park. *Impatiens cecilii* and *I. hydrogetonoides*, both from Africa, were used as outgroup based on Janssens et al. (2006, 2009).

Molecular protocols

Total genomic DNA was isolated from silica-dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987), which was optimised for *Impatiens* by Janssens et al. (2006, 2009). The two nuclear *AP3/DEF* homologues (*ImpDEF1* and *ImpDEF2*) and the plastid *atpB-rbcL* intergenic spacer were amplified following Janssens et al. (2007) and Janssens et al. (2006). PCR reactions for all three gene markers investigated in this study consisted of 2 min initial denaturation at 94 °C and 30 cycles of 30 s denaturation at 94 °C, 30 s primer annealing at primer specific temperature and 1 min extension at 72 °C. Primer annealing for *ImpDEF1*, *ImpDEF2* and *atpB-rbcL* were at 57 °C, 55.5 °C and 51 °C, respectively. Amplification reactions were carried out on a Gene Amp PCR system 9700 (Applied Biosystems). Purified amplification products were sent to Macrogen, Inc. (Seoul, South Korea) for sequencing. Sequences obtained in this study will be deposited at GenBank (Table 1).

Data analyses

Contiguous sequences were assembled using Geneious v7.0.6 (Biomatters, New Zealand). Automatic alignment of the datasets was carried out with MAFFT (Kato et al. 2002) under an E-INS-i algorithm, a 100PAM/k=2 scoring matrix, a gap open penalty of 1.3 and an offset value of 0.123. Subsequent manual fine-tuning of the aligned dataset was done in Geneious v7.0.6. Congruency between the nuclear and chloroplast datasets was inferred by a partition homogeneity test as implemented in PAUP*4.0b10a (Swofford 2000). The best-fit nucleotide substitution model for each plastid and nuclear dataset was determined using jModel Test 2.1.4 (Posada 2008) under the Akaike information criterion (AIC). The GTR+I+G model was found as best fit for *ImpDEF1*, whereas the GTR+G model was calculated as best substitution model for *ImpDEF2* and *atpB-rbcL*. A mixed-model approach was used in which the combined dataset was partitioned in order to apply a different model of evolution on each DNA region (Ronquist and Huelsenbeck 2003). Bayesian Inference (BI) analyses were conducted with MrBayes v3.1 (Huelsenbeck and Ronquist 2001) on three individual data partitions and a combined data matrix. Each analysis ran two times for 10 million generations. Trees were sampled every 2500 generations. Inspection of chain convergence and ESS parameters was done with TRACER v1.4 (Rambaut and Drummond 2007). Bayesian posterior probability (BPP) values between 0.50 and 0.95 were considered to be weakly supported, whereas BPP values above or equal to 0.95 were taken into consideration to indicate well-supported branches (Suzuki et al. 2002, Alfaro et al. 2003). Maximum Likelihood analyses were carried out on the CIPRES web portal using RAxML v7.2.8 (Stamatakis et al. 2008) under the GTR-GAMMA model. Non-parametric ML bootstrapping analysis was calculated with 1000 bootstrap replicates.

Table 1. List of taxa studied, voucher information and accession numbers of the selected markers. ‘–’ refers to a missing sequence.

Taxa	Voucher information	nrITS	atpB-rbcL	ImpDEF1	ImpDEF2
<i>Impatiens andringirensis</i> H.Perrier	Bot. Gard. Bonn 36655 (BONN), Madagascar	–	MH157104	MH157123	–
<i>Impatiens auricomma</i> Baill. 1	Bot. Gard. Bonn 34154 (BONN), Comores	–	DQ147815	EF133562	EF133615
<i>Impatiens auricomma</i> Baill. 2	E. Fischer 1270 (Bot. Gard. Zürich, E.Fischer s.n.) (BONN), Comores	MH881113	MH881068	–	–
<i>Impatiens bicaudata</i> H.Perrier 1	E. Fischer 1340 (Bot. Gard. Bonn 36586) (BONN), Madagascar	MH881114	MH881069	–	MH881160
<i>Impatiens bicaudata</i> H.Perrier 2	E. Fischer 1437 (BONN), Madagascar	MH881115	MH881070	MH881199	MH881161
<i>Impatiens bicaudata</i> H.Perrier 3	Bot. Gard. Zürich, E.Fischer s.n. (BONN), Madagascar	–	–	MH881198	–
<i>Impatiens bisaccata</i> H.Perrier 1	E. Fischer 1271 (Bot. Gard. Bonn 36496) (BONN), Madagascar	MH157152	MH881071	–	–
<i>Impatiens bisaccata</i> H.Perrier 2	E. Fischer 1435 (BONN), Madagascar	MH881117	MH881072	MH881200	–
<i>Impatiens “capuronii”</i> Humb. ex Eb.Fisch. & Raheliv. ined.	E. Fischer 1432 (Bot. Gard. Bonn 36427) (BONN), Madagascar	MH157171	MH157106	MH157127	MH157135
<i>Impatiens catati</i> H.Perrier 1	E. Fischer 1347 (Bot. Gard. Bonn 35920) (BONN), Madagascar	MH881120	MH881074	–	–
<i>Impatiens catati</i> H.Perrier 2	E. Fischer 1278 (Bot. Gard. Bonn 28424) (BONN), Madagascar	MH157142	FJ826634	FJ826686	–
<i>Impatiens cecili</i> N.E.Br.	Knox 4353 (LV), Zimbabwe	–	FJ826635	FJ826687	FJ826741
<i>Impatiens</i> cf. <i>manabarensis</i> Baill.2	E. Fischer 1427 (BONN), Madagascar	MH881123	MH881078	MH881204	MH881166
<i>Impatiens</i> cf. <i>manabarensis</i> Baill. 3	E. Fischer 1348 (Bot. Gard. Bonn 36384)(BONN), Madagascar	MH881139	–	–	MH881182
<i>Impatiens elatostemmoides</i> H.Perrier 1	E. Fischer 1284 (Bot. Gard. Bonn 26821) (BONN), Madagascar	MH157156	MF567403	–	MF567460
<i>Impatiens elatostemmoides</i> H.Perrier 2	E. Fischer 1420 (BONN), Madagascar	MH881124	MH881080	MH881205	–
<i>Impatiens</i> sp. nov. aff. <i>elatostemmoides</i> H.Perrier 3	E. Fischer 1439 (BONN), Madagascar	MH881110	MH881065	MH881194	MH881156
<i>Impatiens elatostemmoides</i> H.Perrier 4	E. Fischer 1429 (BONN), Madagascar	MH881121	MH881076	–	–
<i>Impatiens elianae</i> S.Abrahamczyk & Eb.Fisch	E. Fischer 1326 (Bot. Gard. Bonn 36144) (BONN), Madagascar	MH157157	MF567404	–	MF567461
<i>Impatiens eriosperma</i> H.Perrier	E. Fischer 1342 (Bot. Gard. Bonn 35921) (BONN), Madagascar	MH157158	MF567414	–	MF567466
<i>Impatiens furcata</i> H.Perrier	E. Fischer 1441 (BONN), Madagascar	MH881127	MH881083	MH881206	MH881170
<i>Impatiens galactica</i> Eb.Fisch., Raheliv. & S.Abrahamczyk 1	E. Fischer 1319 (Bot. Gard. Bonn 36393) (BONN), Madagascar	MH881153	MH881107	–	–
<i>Impatiens galactica</i> Eb.Fisch., Raheliv. & S.Abrahamczyk 2	E. Fischer 1426 (BONN), Madagascar	MH881128	MH881108	MH881225	MH881192
<i>Impatiens „hammarbyoides”</i> Eb.Fisch. & Raheliv. 1 ined.	E. Fischer 1430 (BONN), Madagascar	MH157165	MF567417	MF567445	MF567469
<i>Impatiens “hammarbyoides”</i> Eb.Fisch. & Raheliv.2 ined.	E. Fischer 1447 (Bot. Gard. Bonn 37437) (BONN), Madagascar	MH157144	MH157099	MH157121	–

Taxa	Voucher information	nrITS	atpB-rbcL	ImpDEF1	ImpDEF2
<i>Impatiens hendrickii</i> Eb.Fisch. & Raheliv. 1	E. Fischer 1445 (BONNN), Madagascar	MH881130	MH881086	MH881209	MH881173
<i>Impatiens hendrickii</i> Eb.Fisch. & Raheliv. 2	E. Fischer 1440 (BONNN), Madagascar	MH881129	MH881085	MH881208	MH881172
<i>Impatiens "humillima"</i> Humb. Eb.F. isch. & Raheliv. ined.	E. Fischer 1431 (BONNN), Madagascar	MH881131	MH881087	MH881210	MH881174
<i>Impatiens hydrogetonoides</i> Launert	Dessein 719 (BR), Zambia	–	FJ826648	FJ826699	FJ826755
<i>Impatiens inaperta</i> (H-Perr.) H.Perrier 1	E. Fischer 1346 (Bot. Gard. Bonn 27467) (BONNN), Madagascar	–	MH157109	–	–
<i>Impatiens inaperta</i> (H-Perr.) H.Perrier 2	E. Fischer 1357 (BONNN), Madagascar	MH881132	MH881089	–	–
<i>Impatiens inaperta</i> (H-Perr.) H.Perrier 3	E. Fischer 1448 (BONNN), Madagascar	MH881133	MH881090	MH881213	MH881177
<i>Impatiens laurentii</i> Eb.Fisch. & Raheliv.	E. Fischer 1293 (Bot. Gard. Bonn 36132) (BONNN), Madagascar	MH157159	–	MH157120	–
<i>Impatiens luzizi</i> Eb.Fisch. & Raheliv. 1	E. Fischer 1318 (Bot. Gard. Bonn 36381) (BONNN), Madagascar	MH881135	MH881092	–	MH881179
<i>Impatiens luzizi</i> Eb.Fisch. & Raheliv. 2	E. Fischer 1438 (BONNN), Madagascar	MH881136	MH881093	MH881214	MH881180
<i>Impatiens yallii</i> H.Perrier 1	E. Fischer 1294 (Bot. Gard. Bonn 152a) (BONNN), Madagascar	MH157169	MF567420	MF567448	MF567471
<i>Impatiens yallii</i> H.Perrier 2	E. Fischer 1341 (Bot. Gard. Bonn 152b) (BONNN), Madagascar	MH881138	MH881094	–	–
<i>Impatiens manuharensis</i> Baill. 1	E. Fischer 1434 (Bot. Gard. Bonn 36384) (BONNN), Madagascar	MH881139	MH881077	MH881203	MH881182
<i>Impatiens mandrakae</i> Eb.Fisch. & Raheliv.	E. Fischer 1345 (Bot. Gard. Bonn 26822) (BONNN), Madagascar	MH157166	MF567421	–	MF567472
<i>Impatiens marojejyensis</i> Humbert & H.Perrier	E. Fischer 1444 (BONNN), Madagascar	MH881141	MH881096	MH881215	MH881184
<i>Impatiens masoalensis</i> H.Perrier 1	E. Fischer 1443 (BONNN), Madagascar	MH881143	–	MH881216	–
<i>Impatiens masoalensis</i> H.Perrier 2	E. Fischer 1424 (Bot. Gard. Bonn 36386) (BONNN), Madagascar	MH157161	MF567422	MF567449	MF567473
<i>Impatiens masoalensis</i> H.Perrier 3	E. Fischer 1424 (BONNN), Madagascar	MH881144	–	MH881217	MH881186
<i>Impatiens max-huberti</i> Eb.Fisch. & Raheliv.	E. Fischer 1421 (Bot. Gard. Bonn 36428) (BONNN), Madagascar	MH157147	MH157110	MH157116	MH157137
<i>Impatiens navicula</i> Eb.Fisch. & Raheliv. 1	E. Fischer 1422 (BONNN), Madagascar	MH881147	MH881101	MH881220	MH881189
<i>Impatiens navicula</i> Eb.Fisch. & Raheliv. 2	E. Fischer 1446 (BONNN), Madagascar	MH881146	MH881100	MH881219	MH881188
<i>Impatiens nomenya</i> Eb.Fisch. & Raheliv.	E. Fischer 1425 (BONNN), Madagascar	MH881148	MH881102	MH881221	–
<i>Impatiens renae</i> Eb.Fisch. & Raheliv.	E. Fischer 1442 (BONNN), Madagascar	MH881149	MH881103	MH881222	–
<i>Impatiens rutenbergii</i> O.Hoffm	E. Fischer 1310 (Bot. Gard. Bonn 37463) (BONNN), Madagascar	MH881150	MH881104	–	MH881190
<i>Impatiens scripta</i> H.Perrier	E. Fischer 1423 (BONNN), Madagascar	MH881151	MH881105	MH881223	MH881191
<i>Impatiens</i> sp. nov. aff. <i>yallii</i>	E. Fischer 1428 (BONNN), Madagascar	MH881152	MH881106	MH881224	–
<i>Impatiens susan-nathansoniae</i> Eb.Fisch. & Raheliv.	E. Fischer 1433 (BONNN), Madagascar	MH881155	MH881109	MH881226	MH881193

Results

The aligned *atpB-rbcL* and *ImpDEF1/ImpDEF2* matrices contained 924 bp and 812 bp, respectively. The phylogenetic tree, based on the combined data, is shown in Figure 5. The monophyly of the Malagasy *Impatiens* was not supported by our analyses, as the Comorian species *I. auricoma* was deeply nested within the Malagasy *Impatiens* Clade I (BS: 99; BPP: 1). Therefore, the Malagasy *Impatiens* are paraphyletic, unless the Comorian *Impatiens* species are included. The sect. *Preimpatiens* sensu Perrier de la Bâthie (1934)/subgen. *Impatiens* sensu Fischer and Rahelivololona (2002) was not resolved as a monophyletic group. Neither the *Humbotianae* group nor the *Vulgare* group was supported as monophyletic (Fig. 5). However, sect. *Trimorphopetalum* sensu Perrier de la Bâthie (1934)/subgen. *Trimorphopetalum* sensu Fischer and Rahelivololona (2002) was strongly supported as a monophyletic group (BS: 92; BPP: 1). The earliest diversified lineages in the Malagasy *Impatiens* clade fell into a large polytomy containing five groups (Fig. 5): *I. marojejensis* Humbert & H.Perrier (member of the *Vulgare* group), *Impatiens* Clade I (formed by the representatives of the *Humbotianae* and *Vulgare* groups) (BS: 99; BPP: 1), *Impatiens* Clade II (also formed by the representatives of the *Humbotianae* and *Vulgare* groups) (BS: 59; BPP: 0.87), *I. nomenyae* Ed.Fisch. & Raheliv. and a strongly supported Clade III (BS: 92; BPP: 1) (formed by the representatives of the *Trimorphopetalum*). The phylogenetic relationships amongst these major lineages were unresolved. Within the *Impatiens* Clade II, the morphologically variable *I. manaharensis* was not supported as monophyletic. Within the *Impatiens* Clade III, the morphologically variable species *I. elatostemmoides* appeared polyphyletic, while *I. "hammarbyoides"*, *I. lyallii* and *I. inaperta*, also variable, seemed paraphyletic. Finally, the sampled species of *Impatiens* from the Marojejy National Park did not form a monophyletic group, as they were scattered across the tree (Fig. 5).

Discussion

The present analyses confirm the paraphyly of the Malagasy *Impatiens* with respect to the Comorian *I. auricoma*. This is consistent with Janssens et al. (2006, 2007, and 2009) but inconsistent with the polyphyly of the Malagasy *Impatiens* as shown by Yuan et al. (2004). The monophyly of sect. *Trimorphopetalum* (*I. inaperta* included) suggested by Yuan et al. (2004) is further strongly supported (BS: 92; BPP: 100) by the present study. In other words, subgen. *Trimorphopetalum*, as delimited by Fischer and Rahelivololona (2002), is supported. Spurless, greenish, brown to blackish or yellowish (never white, pink or purple) flowers with boat-shaped lower sepals, obtuse or truncate and apically dehiscent anthers and the lack of extrafloral nectaries on leaf lamina and petioles are the synapomorphic characters for this lineage, which seems to have evolved from a common ancestor with spurs (Fig. 5). In Yuan et al. (2004), the *Trimorphopetalum* clade was resolved as the most derived within *Impatiens*. The authors argued that this spurless lineage could not be recognised at sectional or subgeneric level, as proposed by Perrier

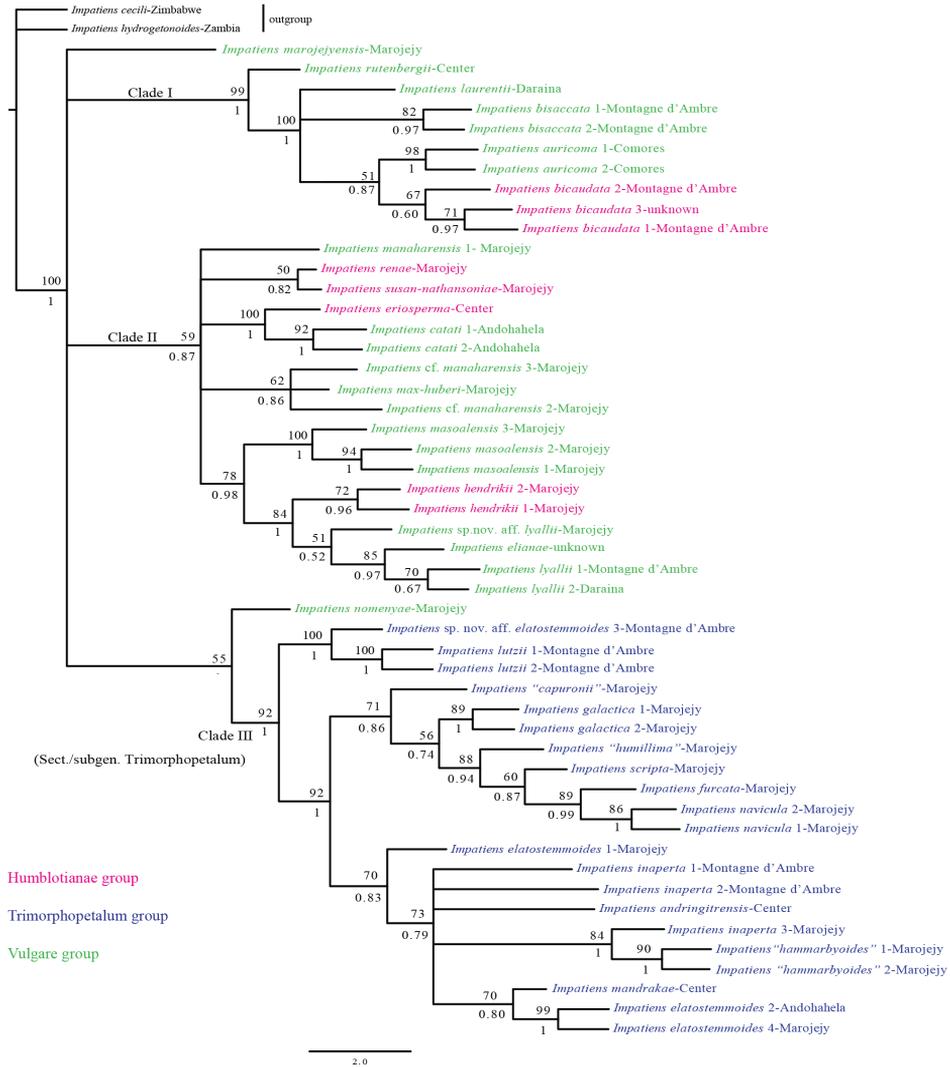


Figure 5. Maximum likelihood tree based on the combined nuclear-plastid data. Bootstrap support values and Bayesian posterior probabilities are above and below nodes, respectively.

de la Bâthie (1934) and Fischer and Rahelivololona (2002), respectively, because this taxonomic decision seems to make sect. *Preimpatiens* sensu Perrier de la Bâthie (1934) or *I.* subgen. *Impatiens* sensu Fischer and Rahelivololona (2002) paraphyletic. Our results do not support or reject Yuan et al. (2004)'s claims, as the Malagasy *Impatiens* clade (including the Comorian *I. auricoma*) is largely unresolved (Fig. 5). Neither the sampled species from the *Vulgare* group nor those from the *Humblotianae* group form a monophyletic group, a result consistent with Yuan et al. (2004). Therefore, our results provide no support for the *Vulgare* group characterised by shorter and slender spurs or for the *Humblotianae* group defined by larger and broader spurs, as delimited by Perrier

de la Bâthie (1934). Furthermore, our analyses do not support or reject the monophyly of sect. *Preimpatiens* sensu Perrier de la Bâthie (1934) or subgen. *Impatiens* sensu Fischer and Rahelivololona (2002). To summarise, this study partly supports the sectional and subgeneric classifications of the Malagasy *Impatiens* proposed by Perrier de la Bâthie (1934) and Fischer and Rahelivololona (2002). More molecular data are needed to further assess the monophyly of sect. *Preimpatiens* or subgen. *Impatiens*.

In addition, results of this molecular phylogenetic study further highlight the difficulties that the *Impatiens* taxonomists have faced when dealing with the species delimitation of the Malagasy *Impatiens* (e.g. Perrier de la Bâthie 1934; Humbert 1956; Fischer and Rahelivololona 2002, 2004a, 2007, 2015a, b, c). *Impatiens manaharensis* seems polyphyletic and this supports our suspicion in the field that at least two taxa with very different morphology could be distinguished within this variable species. *Impatiens elatostemmoides* seems polyphyletic, while *I. inaperta*, *I. "hammarbyoides"* and *I. lyallii* (Fig. 5) appear paraphyletic. Therefore, these morphologically variable species may well represent morpho-species, meaning that they represent a group of several different species or are parts of a species complex. As a consequence, this study indicates that the current species delimitation of these para- or polyphyletic species is in need of revision.

Finally, the *Impatiens* taxa from the Marojejy National Park do not form a monophyletic group, as they are spread across the tree (Fig. 5). This suggests that they are the result of numerous independent colonisation events from elsewhere in Madagascar, followed by subsequent diversifications. In other words, they seem to have had multiple origins.

Conclusions

The Malagasy *Impatiens* are paraphyletic with respect of the Comorian *I. auricoma*. The present subgeneric and sectional classifications of the Malagasy *Impatiens* are partly supported, with strong support for the monophyly of subgen. *Trimorphopetalum*. Neither the *Humblotianae* group nor the *Vulgare* group forms a monophyletic group. *Impatiens elatostemmoides*, *I. "hammarbyoides"*, *I. inaperta* and *I. manaharensis* are either para- or polyphyletic and may represent morpho-species. The *Impatiens* species from Marojejy do not form a natural group. A further study based on a much larger molecular data set and sampling from the entire geographic ranges of *Impatiens* in Madagascar is needed to produce a well-resolved phylogeny. This will hopefully allow for a retest of the monophyly of sect. *Preimpatiens* sensu Perrier de la Bâthie (1934) or subgen. *Impatiens* sensu Fischer and Rahelivololona (2002), as well as molecular dating and biogeographic analyses of the Malagasy *Impatiens*.

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Hedychium viridibracteatum X.Hu, a new species from Guangxi Autonomous Region, South China

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Abstract

Hedychium viridibracteatum X.Hu, a new species from Guangxi Zhuang Autonomous Region, South China, is described and illustrated. *Hedychium viridibracteatum* X.Hu is included in the short-anther group of *Hedychium* and is most similar to *Hedychium villosum* Wall. var. *tenuiflorum* Voigt ex Baker, *H. villosum* Wall. var. *villosum* Wall., and *H. chingmeianum* N. Odyuo & D. K. Roy.

Keywords

Hedychium, Zingiberaceae, new species, Guangxi Autonomous Region

Introduction

Hedychium J. Koenig, 1783 (Zingiberaceae, Zingibereae) is a genus with about 50–80 species mainly distributed in tropical Asia (Jain and Prakash 1995, Wu and Larsen 2000). With pleasant fragrances and beautiful flower forms, *Hedychium* species are used as cut flowers or to decorate landscapes.

Twelve species of *Hedychium* have been reported in Guangxi Zhuang Autonomous Region, South China (Wu and Larsen 2000). According to Wu and Larsen (2000) *Hedychium villosum*, has two varieties, *H. villosum* Wall. with large flowers and *H. villosum* var. *tenuiflorum* Wall. ex Baker with small flowers. Yu et al. (2010) proposed that the two varieties should be recognised as distinct species on the basis of their discernible

morphological characteristics, different polyploid levels and flowering time, which contribute to complete reproductive isolation. Sanoj et al. (2013), having compared the original specimens and descriptions, concluded that the plant with small flowers was the true *H. villosum* Wall. var. *villosum* Wall., and the one with large flowers was *H. villosum* Wall. var. *tenuiflorum* Voigt ex Baker. Concurring with Yu et al. (2010), Gao et al. (2014) referred to *H. villosum* var. *tenuiflorum* Wall. ex Baker (Wu and Larsen 2000) as an independent species, *H. tenuiflorum* (Baker) K. Schum. In this paper, the treatment of *H. villosum* Wall. and its related species follows Sanoj et al. (2013).

In the autumn of 2008 we received a flowering plant of *Hedychium* from a local person in Napo, Guangxi Autonomous Region, South China. The plant resembled *H. villosum* Wall. var. *tenuiflorum* (Wall. ex Voigt) Wall. ex Baker (Sanoj et al. 2013) in having long filaments and sagittate anthers but could be distinguished from the above by its pure white flower, broad leaves, short ligule, short green bracts and bracteoles. After a further field investigation, morphological studies, flow cytometric analysis of nuclear DNA content, and comparison with published protologues and descriptions (Baker 1890–1892; Schumann 1904; Schilling 1982; Ridley 1898; Larsen 1965; Jain and Prakash 1995; Wu and Larsen 2000; Pornpimon 2008; Yu et al. 2010; Sanoj et al. 2013; Odyuo and Roy 2017), the plant in question was confirmed to be a new species of *Hedychium*.

Materials and methods

Morphological comparison and distribution mapping

In October 2011 we carried out a field survey and found the new plant in the wild in the vicinity of the Nongyi village, Napo County, Guangxi Zhuang Autonomous Region. The new plant grows on steep rocks under the forest canopy, at about 600 m a.s.l. Before that field trip, we had collected *Hedychium villosum* Wall. in the field from different part of China between 2006 and 2010. Based on those field surveys and collections, the affinities between the new species and its relatives, *H. villosum* Wall., were compared to living plants and specimens. Guided by the collection site of several suspected specimens, we made further field investigations after 2012 to gain a better understanding of the distribution range of the new plant. By combining the geographical distribution data collected from field investigations, specimens (GH, GXMI, HITBC, IBK, IBSC, KUN, PE) and references (Yu et al. 2010), we made a distribution map of the new plant and the related species using DIVA-GIS (version 7.5) (<http://www.diva-gis.org/>).

Ploidy level analysis of the new plant and its related species

Flow cytometric analysis of nuclear DNA content of the new species and its relatives was performed according to Sakhanokho et al. (2009). Ploidy levels were determined for young leaf tissues. A piece of leaf tissue of about 1 cm² was chopped with a sharp

razor blade in a petri dish containing 0.4 ml nuclei extraction buffer. After a 3 min incubation with gentle agitation, the extract was poured through a 50 µm mesh sieve. DNA fluorochrome, a nucleus staining buffer, was added to the extract buffer in the ratio of 4:1 and the sample was analysed immediately for the DNA content of the nuclei. Buffers were supplied as part of the Cystain ultraviolet Precise T reagent kit (Partec GmbH, Münster, Germany). The fluorescence of the nuclei was measured using a Partec Cy Flow Space flow cytometer (Partec GmbH, Münster Germany). Sample measurements were replicated three times for each plant. The results were displayed as histograms showing the number of nuclei grouped in peaks of relative fluorescence intensity, which is proportional to the DNA content. To determine the standard peak of diploid cells (2C DNA), leaf tissues were collected from young leaves of diploid *Hedychium coronarium* plants (Hu et al. 2011; Ramachandran 1969). The instrument gain was adjusted so that the peak of nuclei isolated from diploid (*H. coronarium*) was set at channel 50 and this calibration was checked periodically to minimise variation resulting from runs. Therefore, peaks representing nuclei from samples with diploid and tetraploid levels were expected at channels 50 and 100, respectively.

Results

Hedychium viridibracteatum X.Hu, sp. nov.

urn:lsid:ipni.org:names:77191580-1

Figures 1–3

Diagnosis. *Hedychium viridibracteatum* X.Hu, sp. nov. is morphologically similar to *H. villosum* Wall. var. *tenuiflorum* Voigt ex Baker by having sagittate anther, long filament, relatively thick and small leaves, more than two flower per-bract, but can be easily distinguished from it by its green (vs. brown) shorter bracts (1.3–1.5 × 0.4–0.5 cm vs. 2.7–2.9 × 1.1–1.2 cm) and bracteoles (1.0–1.1 × 0.3–0.4 cm vs. 2.1–2.2 × 0.7–0.75 cm), pure white flowers (vs. white with red stamen), dentate (vs. acute) tips to the lateral staminodes, and the apex of the labellum incised to the middle (vs. deeply divided).

Type. China, Zhuang Autonomous Region, Napo county, Nongyi village, on rocks, 22°57.727'N, 106°00.265'E, alt. 696 m, 6 October 2011, *Xiu Hu* 267 (holotype: IBSC; isotype: MO).

Description. Epilithic, evergreen perennial rhizomatous herbs. Rhizome purplish brown outside, sheathed, creamy white inside, 2–4 cm in diameter. Leaf stems slender, 60–100 × 0.5–0.7 cm, glabrous, 7–10 leaves. Ligule oblong-ovate, green or purple, glabrous, 1.8–2.3 cm × 1.2–1.5 cm, membranous. Leaf blades elliptic, 15–25 × 5–8 cm, base attenuate into a short petiole, 0.5 cm long, apex caudate, glabrous on both sides and dark green above, purple beneath, especially the leaves in the lower part of the stem. Inflorescence a terminal spike, erect, 10–15 cm long, lax flowered; peduncle 1.5–2.0 cm long, green; rachis green, pubescent. Bracts oblong-ovate, apex acute, green, 1.3–1.5 × 0.4–0.5 cm, coriaceous, pubescent outside, glabrous inside, 2–4 flowered. Bracteoles



Figure 1. *Hedychium viridibracteatum* X.Hu, sp. nov., holotype, **A** upper leaves and inflorescence **B** flower **C** bract **D** ovary and glands **E** calyx tube **F** floral tube with calyx tube wrapped outside **G** corolla lobe **H** lateral staminodes **I** labellum **J** stamen **K** anther **L** stigma. Drawings Y. X. Liu.

tubular, apex acute, green, coriaceous $1.0\text{--}1.1 \times 0.3\text{--}0.4$ cm, pubescent outside, glabrous inside. Flowers pure white, 10–11 cm long, lightly fragrant. Calyx tubular, green, $2.5\text{--}2.8 \times 0.12\text{--}0.15$ cm, apex obtusely 3-toothed, pubescent. Floral tube pure white,

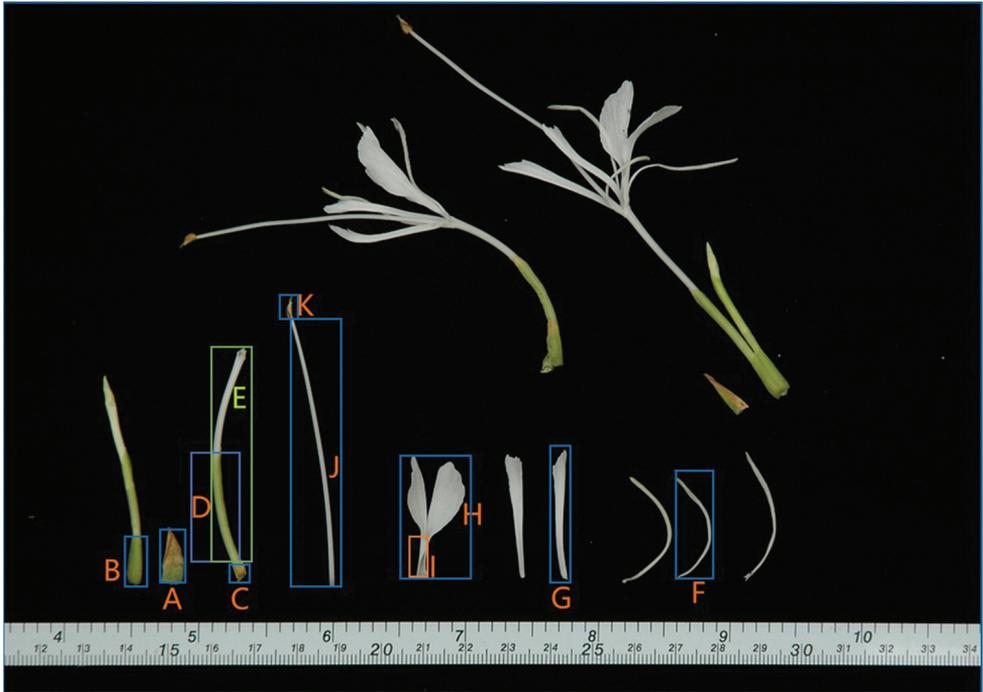


Figure 2. *Hedychium viridibracteatum* X.Hu, sp. nov., flowers and their parts: **A** bract **B** bracteole **C** ovary **D** calyx tube **E** floral tube **F** corolla lobe **G** lateral staminodes **H** labellum **I** labellum claw **J** filament **K** anther.

4.0–5.0 cm long, slender; lobes glabrous, pure white, reflexed, linear, dorsal lobe 3.1–3.3 × 0.12 cm, lateral lobes 2.9–3.1 × 0.12 cm. Lateral staminodes linear-ridged, pure white, 3.0–3.3 cm long, dentate at apex. Labellum ovate, pure white, 2.8–3.0 × 1.2–1.4 cm, base attenuate into a claw, 0.8–1.0 × 0.2 cm, apex incised to the middle, white. Filament 6.0–6.8 cm long, white; anther dorsifixed, sagittate, 3.5–4 mm long, glabrous, light yellow. Ovary 0.4–0.5 × 0.2–0.4 cm, densely silky hairy, trilocular, placentation axile; epigynous glands 2, slender, ca. 0.2 cm long, yellow. Stigma green, ciliate. Tetraploid.

Flowering. September–October.

Fruiting. Unknown.

Etymology. The new species is named after its green bracts and pure white flowers which are highly diagnostic.

Habitat. This species is currently found on limestone rocks in Guangxi Autonomous Region (Napo, Longzhou and Jingxi Counties) mainly growing under forest at altitudes of 600–800 m.

Ploidy level analysis. The results (Fig. 4) show that, when the nuclei of the diploid control (Fig. 4A), *Hedychium coronarium* (Hu et al. 2011; Ramachandran 1969) were set to channel 50, the new plant, *H. viridibracteatum* X.Hu (Fig. 4B) resolved at channel 100. This means that the new plant is a tetraploid which shares the same level of ploidy with *H. villosum* Wall. var. *tenuiflorum* Voigt ex Baker (Fig. 4C) but differs from *H. villosum* var. *villosum* Wall. (Fig. 4D).

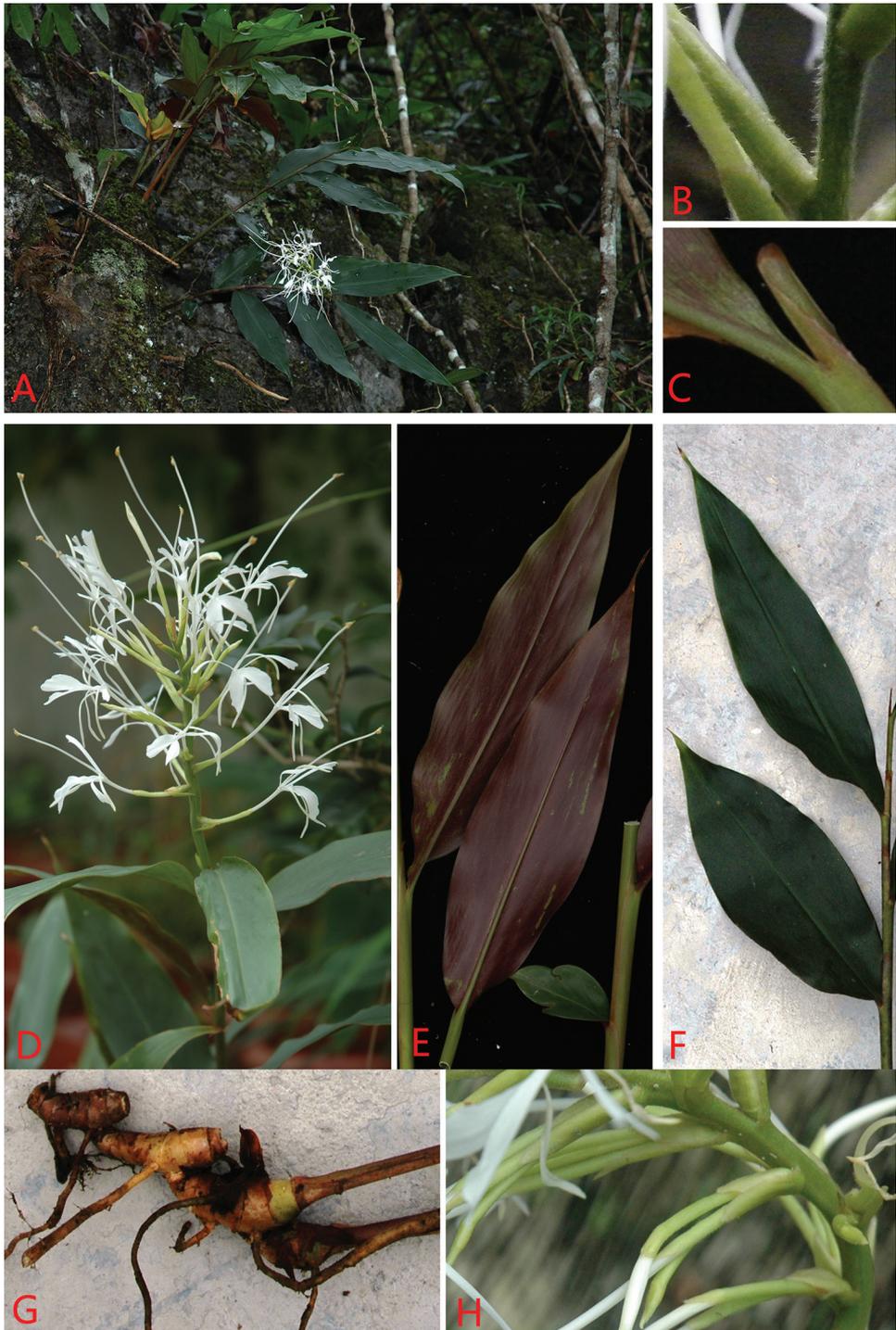


Figure 3. *Hedychium viridibracteatum* X.Hu sp. nov. **A** habit (growing on rocks) **B** rachis and bracts (pubescent) **C** ligule (glabrous) **D** inflorescence at anthesis **E** leaf blade surface (adaxial view) **F** leaf blade surface (abaxial view) **G** rhizome **H** portions of inflorescence (2–4 flowers per bract).

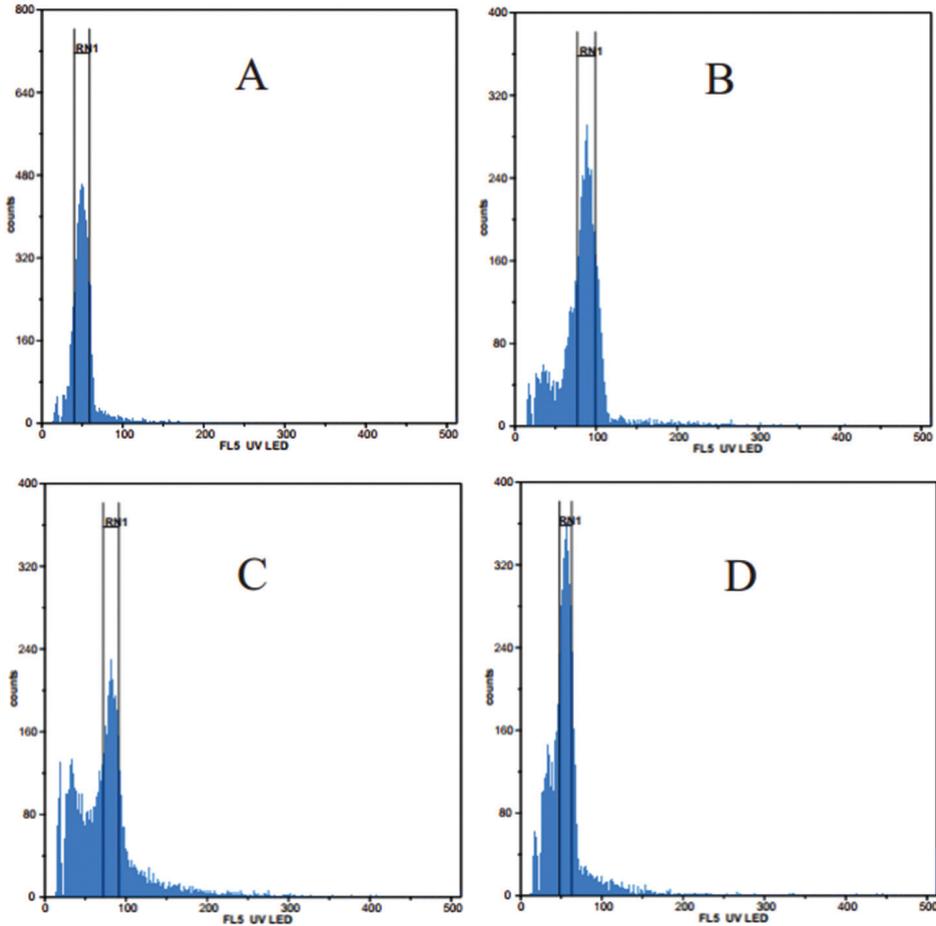


Figure 4. Histogram of relative DNA content of nuclei isolated from the leaves of the new plant and related *Hedychium* species. **A** *H. coronarium* (control) **B** *H. viridibracteatum* **C** *H. villosum* var. *tenuiflorum* **D** *H. villosum* var. *villosum*.

Distribution. In addition to the type location, the new species is found in the adjacent area, Longzhou and Jingxi Counties (Fig. 5). *Hedychium viridibracteatum* can be easily distinguished from related species, even from dried specimens. The following sites were discovered by examining specimens and confirmed by our field investigations from 2012–2017: 20 September 1935, X. P. Gao 55777, Guangxi Autonomous Region, Jingxi County, Ande district (IBSC 0022836); 13 September 2007, W. B. Xu & Y. Y. Liang 0144, Guangxi Autonomous Region, Jingxi County, Bangliang National Nature Reserve (IBK 00223131); 29 September 2007, W. B. Xu & Y. Y. Liang B0065, Guangxi Autonomous Region, Jingxi County, Bangliang National Nature Reserve (IBK 00223137); 16 September 2010, Y. S. Huang et al. LYJX0450, Guangxi Autonomous Region, Jingxi County, Bangliang National Nature Reserve (IBK 00224854); 27 October 2010, W. H. Wu W0286, Guangxi Autonomous Region, Longzhou county, Nonggang National Nature Reserve (IBK 00216857).

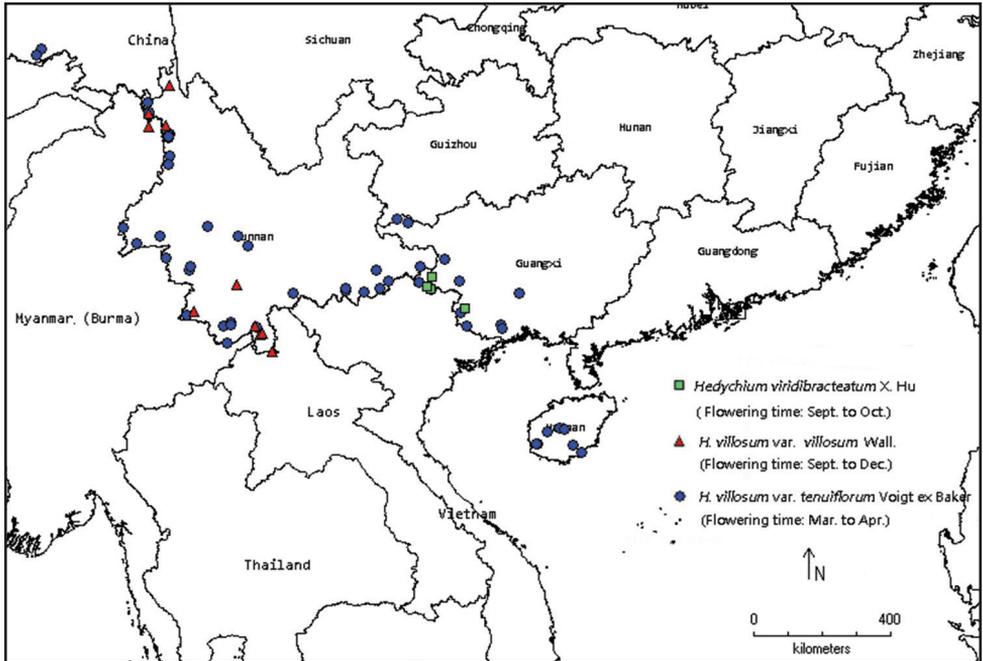


Figure 5. Distribution and flowering of *Hedychium viridibracteatum* X. Hu, sp. nov. and the related species *H. villosum* var. *tenuiflorum* and *H. villosum* var. *villosum*.

Other *Hedychium* spp. examined

Hedychium villosum Wall. var. *tenuiflorum* Voigt ex Baker

CHINA. **Guangxi:** Baise County: Daleng, 800 m, 26 Mar 1975, *D. Fang* 36943 (GXMI); Longguang, 900 m, 14 Jan 1956, *Baise Group* 1914 (IBK); Longlin County: Dee, Yanyu, 933 m, 9 Sep 1977, *J.F. Wei & D. Fang* 3–1145 (GXMI); Longlin County: Zhelang, Hanshan, 1000 m, 24 Mar 1975, *D. Fang* 25610 (GXMI); Longzhou County: Binqiao, Anzhen village, 273 m, 29 Apr 1956, *Y.K. Li* 212 (IBK); Napo County: Pingmeng, Mengda, back Mountain, 600 m, 16 Apr 1976, *D. Fang* 22291 (GXMI); Pingmeng, Nonghua to Sheng village, 700 m, 12 May 1989, *South China team* 702 (IBSC); Pingmeng, Nongyi, Stone Mountain, 646 m, 1 Mar 2006, *M. Liu* 3 (HITBC); Nanning County: Maoqiao, Medicinal Garden, 80 m, 3 Mar 1975, *D. Fang & X.X. Chen* 78882 (GXMI); Shangsi County: Fenghuang, Longshan Mid-range, 811 m, 4 Mar 1944, *S.Q. Chen & S.H. Chun* 4628 (IBSC); Fulongai, back valley, 607 m, 23 May 1966, *T.J. Wang* 5393 (GXMI); Tiandeng County: Fuxin, Miao village, 502 m, 18 Sep 1977, *Tiandeng County Investigation Team* 2–233 (GXMI); **Hainan:** Changjiang County: Bawang Mountain, Dongsi Forest Farm, 1400 m, 20 Apr 1988, *Z.X. Li & F.W. Xing* 3526 (IBSC); Bawang Mountain, Dongwu Forest Farm, 1015 m, 27 Mar 1983, *G.Y. Fu* 3418 (IBSC); Baoting County: Diaoluo Mountain, Xin'an village, 84 m, 24 Dec 1954, *Diaoluo Mountain Team* 3280 (IBSC); Qizhi Mountain,

185 m, 9 Jan 1934, *Z. Huang & C. Wang 36223* (IBSC); Jianfeng Mountain, 830 m, 15 Apr 1982, *Q. Huang 820154* (IBSC); **Xizang**: Motuo County: Damu to Gedang, 850 m, 25 Mar 1993, *H. Sun & Z.K. Zhou & H.Y. Yu 5043* (KUN); **Yunnan**: Fugong County: Pihe, east coast of Nu River, 1100 m, 30 May 1978, *Bijiang Investigation Team 0257* (KUN); along Nu River, Stone Mountain, 1262 m, 9 Apr 2008, *F. Yu 19* (HITBC); Diao Ga Guo Zhai, 25 Apr 2004, *D. Heng 49837* (GH); Funing County: Banlun, 728 m, 10 Apr 1940, *Q.W. Wang 88283* (KUN); Gengma County: 1670 m, 1 Apr 1936, *Q.W. Wang 72918* (IBSC); along the Gengma River, 1280 m, 7 Mar 2008, *F. Yu 20* (HITBC); Gongshan County: Dulongjiang, Longyuan, 1772 m, 12 Apr 1994, *Dulongjiang Investigation Team 5582* (KUN); Hekou County: Dawei Mountain, Laozhai, 1338 m, 6 Mar 1940, *X. Wang & X.P. Gao & X.Q. Liu 100235* (IBSC); Qiaotou, 958 m, 1 Apr 2008, *F. Yu 21* (HITBC); Jingdong County: Dongchuan River, 1097 m, 26 Mar 1940, *M.G. Li 1900* (KUN); Wuliang Mountain, 1500 m, 19 Mar 2008, *F. Yu 22* (HITBC); Jinghong County: Damenglong Mingsong Mountain, 1800 m, 29 Nov 2006, *S.S. Zhou 3409* (HITBC); Liuku County: Nu River, along the road, 1241 m, 8 Apr 2008, *F. Yu 18* (HITBC); Mang County: Mengjiu, 1601 m, 28 Jul 1984, *Q.G. Wu 96* (IBSC); Lvchun County: Huanglianshan national Nature Reserve area, 1500 m, 28 Apr 1984, *S.Q. Dong & A.M. Li 24905* (HITBC); Malipo County: Huangjinyin, 1455 m, 13 Jan 1940, *Q.W. Wang 83189* (KUN); Nanwen River, Laojun Mountain, 1600 m, 1 Jun 1983, *S.Q. Dong 32927* (HITBC); Menghai County: Mingsong, Manjin, 1221 m, 27 Mar 1957, *Zhongsu Team 5469* (IBSC); Mengla County: Menglun Tropical Botanical Garden, 564 m, 25 Jan 2007, *L.Q. He 119* (HITBC); Xiangming, 900 m, 26 Mar 1984, *S.Q. Dong & A.M. Li 24897* (HITBC); Pingbian County: 1677 m, 9 Mar 1934, *Y. Qian 13443* (IBSC); Yanshan County: Shuitouzhai, 1200 m, 19 Oct 1939, *Q.W. Wang 84527* (KUN); Yingjiang County: Tongbiguan, 1400 m, 9 Apr 1985, *Examination Team 85–200* (KUN); Zhenkang County: Bainiu village, 1593 m, Mar 1936, *Q.W. Wang 72237* (KUN).

Hedychium villosum var. *villosum* Wall

CHINA. Yunnan: Fugong County: Maji Township, 1337 m, 4 Nov 1990, *Dulong River Investigation Team 140* (KUN); Gongshan County: Dulong River to Meilin River, 1400–1420 m, 11 Jul 1979, *Q. Lin & X.F. Deng 2731* (KUN); Dulong River East Coast, 2200–2400 m, 20 Sep 1940, *G.M. Feng 2168* (KUN); Dulong River West Coast, 1300 m, 20 Nov 1990, *Dulong River Investigation Team 637* (KUN); Dulong River West Coast, Four village, 1300 m, 25 Nov 1959, *G.M. Feng 24396* (KUN); Mengla County: Menglun, 800 m, 6 Dec 1972, *Y.H. Li 8466* (HITBC); Menglun, 26 Jan 1979, *J.Y. Cui 14683* (HITBC); Menglun, Silver Factory, limestone mountains, 860 m, 6 Jan 1960, *Y.H. Li 2731* (HITBC); Menglun, limestone mountains, 1200 m, 23 Nov 2005, *S.S. Zhou 3320* (HITBC); Mengyuan, Nov 1982, *Expedition team 34279* (HITBC); Shangyong, 900 m, 18 Oct 1974, *Z.H. Yang 10983* (HITBC); Yiwu Dt.: Manpi, 1000 m, 8 Sep 1959, *S.J. Pei 9993* (HITBC); Menglian County: 957 m, 2 Oct 2006, *X. Hu 001* (IBSC).

Table 1. A morphological comparison of *Hedychium viridibracteatum* with related species.

Characters	<i>H. viridibracteatum</i>	<i>H. villosum</i> var. <i>villosum</i>	<i>H. villosum</i> var. <i>tenuiflorum</i>
Ligule	Oblong-ovate, green or purple, 1.8–2.3 × 1.2–1.5 cm, glabrous	Oblong, pale pink, 1.8–2.1 × 0.7–0.8 cm, pubescent externally	Oblong, pale pink, 2.9–3.4 × 1.3–1.6 cm, densely pubescent externally
Leaf blades	Elliptic, purple beneath, 15–25 × 5–8 cm, glabrous on both sides.	Elliptic-lanceolate, pale green below, 14–20 × 3.7–4.5 cm, midrib pubescent below	Elliptic-lanceolate, pale green below, 34–40 × 8–9 cm, midrib pubescent below
Bract	Oblong-ovate, green, 1.3–1.5 × 0.4–0.5 cm, pubescent outside, 2–4 flowered	Elliptic, brown, 1.2–1.4 × 0.4–0.5 cm, densely hairy externally; 2–3-flowered	Lanceolate, brown, 2.7–2.9 × 1.1–1.2 cm, densely hairy externally; 3–4 flowered
Bracteole	Green, 1.0–1.1 × 0.3–0.4 cm, pubescent outside	Brown, 1–1.3 × 0.25 cm, densely hairy externally	Brown, 2.1–2.2 × 0.7–0.75 cm, densely hairy externally
Flowers	White, 10.0–11.0 cm long, lightly fragrant.	Pale yellow, 4.7–5.2 cm, highly fragrant	White with red stamen, 11.8–12.2 cm long, mildly fragrant
Calyx	Pale green, 2.5–2.8 × 0.12–0.15 cm, pubescent outside	Pale green, 1.6–1.8 × 0.15 cm, densely pubescent externally, hairs brown	Pale green, pink tinged towards tip, 3.3–3.7 × 0.2–0.3 cm, densely pubescent externally
Floral tube	Pure white 4.0–5.0 cm long,	Pale yellow, 1.9–2.1 cm long	Creamy-white, 5–5.2 cm long
Corolla lobes	Linear, pure white dorsal lobe 3.1–3.3 × 0.15 cm; lateral lobes 2.9–3.1 × 0.15cm	Oblong, pale yellow; dorsal lobe 1.6–1.7 × 0.15–0.2 cm; lateral lobes 1.5–1.6 × 0.15 cm	Oblong, greenish-white, dorsal lobe 4.3–4.4 cm long; lateral lobes 4–4.1 cm long
Lateral staminodes	Linear-ridged, pure white 3.0–3.3 × 0.2–0.4 cm, tip dentate	Linear, 1.3–1.5 × 0.15 cm; tip acute	Linear, white with a yellow tinge at base, tip acute, rarely forked 3.3–3.5 × 0.1–0.15 cm
Labellum	Ovate, pure white, 2.8–3.0 × 1.2–1.4 cm, base attenuate into 8–10 × 2 mm claw, apex incised to the middle	Elliptic, pale yellow, 1.3–1.4 × 0.5 cm, apex deeply divided (sinus 0.8–0.9 cm deep); claw 1 mm wide	Oblanceolate, white with a yellow tinge at base, 3.1–3.5 × 1.2–1.5 cm, apex deeply divided (sinus 1.9–2.2 cm deep; claw 4–4.5 mm wide
Filament	Pure white, 6.0–6.8 cm long	Scarlet, 2.4–2.6 cm long	Red, light red towards tip, 5.3–6.3 cm long
Anther	Light-yellow, 3.5–4 mm long	Brown, 1.5 × 1.5 mm long	Brown, 3.5 × 2.5–3 mm long
Flowering time	Sept–Oct	Sep–Dec	Mar–Apr
Ploidy	Tetraploid	Diploid	Tetraploid

Discussion

Hedychium viridibracteatum X.Hu is included in the short-anther group of *Hedychium*. It is similar to *H. villosum* in having visible rachis, non-imbricate bracts, more than one flower per bract, long filament and short, dorsifixed anther. It differs from *H. villosum* var. *tenuiflorum* in having short green ligules (vs. long and pale pink), elliptic glabrous leaf blade (vs. elliptic-lanceolate, midrib pubescent), short green (vs. brown) bracts and bracteole, flowers without much fragrance (vs. mildly fragrant), green calyx (vs. pink tinged towards tip), pure white short floral tube and filament (vs. creamy-white floral tube and red filament), pure white corolla lobes (vs. greenish-white lobe), pure white lateral staminodes with dentate tip (vs. a yellow tinge at base, tip acute lateral staminodes), pure white ovate labellum, apex incised to the middle (vs. white with a yellow tinge at base ob lanceolate labellum, apex deeply divided) blooming in September to October (vs. in March–April) (see Table 1).

Although *Hedychium viridibracteatum* and *H. villosum* var. *tenuiflorum* have the same ploidy level the two species differ in their flowering time which ensures that they are reproductively isolated. *Hedychium viridibracteatum* shares the same flowering time with *H. villosum* var. *villosum*, but they can be distinguished by their different flower form, flower size (Table 1), ploidy level (Fig. 4), and are geographically isolated (Fig. 5). The new species is also similar to the newly published *H. chingmeianum* N. Odyuo & D. K. Roy, 2017 (Odyuo and Roy 2017) but can be distinguished by having green bract (vs. purplish-brown), larger flowers (10.0–11.0 cm long vs. 4–5 cm long), a longer filament (6.0–6.8 cm vs. 2.8–3.0 cm) with white colour (vs. brownish-red). Thus, we confirm that *H. viridibracteatum* is a new species.

Acknowledgements

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Two new species of *Hiptage* (Malpighiaceae) from Yunnan, Southwest of China

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Abstract

Hiptage pauciflora Y.H. Tan & Bin Yang and *Hiptage ferruginea* Y.H. Tan & Bin Yang, two new species of Malpighiaceae from Yunnan, South-western China are here described and illustrated. Morphologically, *H. pauciflora* Y.H. Tan & Bin Yang is similar to *H. benghalensis* (L.) Kurz and *H. multiflora* F.N. Wei; *H. ferruginea* Y.H. Tan & Bin Yang is similar to *H. calcicola* Sirirugsa. The major differences amongst these species are outlined and discussed. A diagnostic key to the two new species of *Hiptage* and their closely related species is provided.

Keywords

Hiptage, Malpighiaceae, samara, Yunnan, China

Introduction

Hiptage Gaertn. (Gaertner 1791) is one of the largest genera of Malpighiaceae with about 30 species of woody lianas and shrubs growing in forests of tropical South Asia, Indo-China Peninsula, Indonesia, Philippines and Southern China, including Hainan and Taiwan islands (Chen and Funston 2008, Ren et al. 2013). There are currently ten species of *Hiptage* recorded in Thailand (Sirirugsa 1987, 1991), ten species with seven endemic ones in China (Chen and Funston 2008) and 9 species in India (Srivastava 1992). So far, Ren (2015) recognised a total of 29 species in this genus.

The genus name, *Hiptage*, is derived from the Greek “hiptamai” which means to fly and refers to its distinctive three-winged fruit samaras, i.e. a larger middle wing and two smaller lateral wings, sometimes the dorsal wing is absent or present as a small crest, most species bear an elongated commissural gland on the calyx (Srivastava 1992, Anderson et al. 2006). *Hiptage* is also unique for having mirror-image flowers, with 1 long and 9 short unequal stamens (Ren et al. 2013, Qian et al. 2016). *H. benghalensis* (L.) Kurz is the most well-known species due to its attractive and fragrant flowers; it is often cultivated as a tropical ornamental plant in gardens (Anderson et al. 2006).

During floristic surveys in southern and south-western Yunnan from 2009 to 2017, some populations of *Hiptage* were discovered and some plants were cultivated in Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (CAS). Based on detailed examination on the morphological and anatomical characters of the living plants and specimens and comparing them with the possible closely similar species, we found these populations representing two species distinct from the known 29 species so far (Siriruga 1991, Srivastava 1992, Chen and Funston 2008, Ren 2015). We draw a conclusion that those two species are new to science. Therefore, we described and illustrated the new species and compare them morphologically to its most similar congener.

Material and methods

Measurements and assessments of morphological characters of the two possible new species *H. pauciflora* Y.H. Tan & Bin Yang and *H. ferruginea* Y.H. Tan & Bin Yang were based on dried specimens in herbarium and fresh materials in field observations and cultivation plants flowered and fruited in XTBG. They were compared with the morphologically similar species *H. benghalensis* (Siriruga 1991, Chen and Funston 2008), *H. multiflora* (Wei 1981, Chen and Funston 2008) and *H. calcicola* (Siriruga 1987, 1991) in affinities inferred from protologues, type specimens and cultivation plants in XTBG. Images of type specimens were gathered from JSTOR Global Plants (<http://plants.jstor.org>) and Chinese Virtual Herbarium (<http://www.cvh.ac.cn>). Conservation status evaluations of the new species were based on the International Union for Conservation of Nature guidelines (IUCN 2012).

Taxonomy

***Hiptage pauciflora* Y.H. Tan & Bin Yang, sp. nov.**

urn:lsid:ipni.org:names:77191587-1

Figure 1

Vernacular name. Shao hua feng zheng guo (少花风筝果) (Chinese)

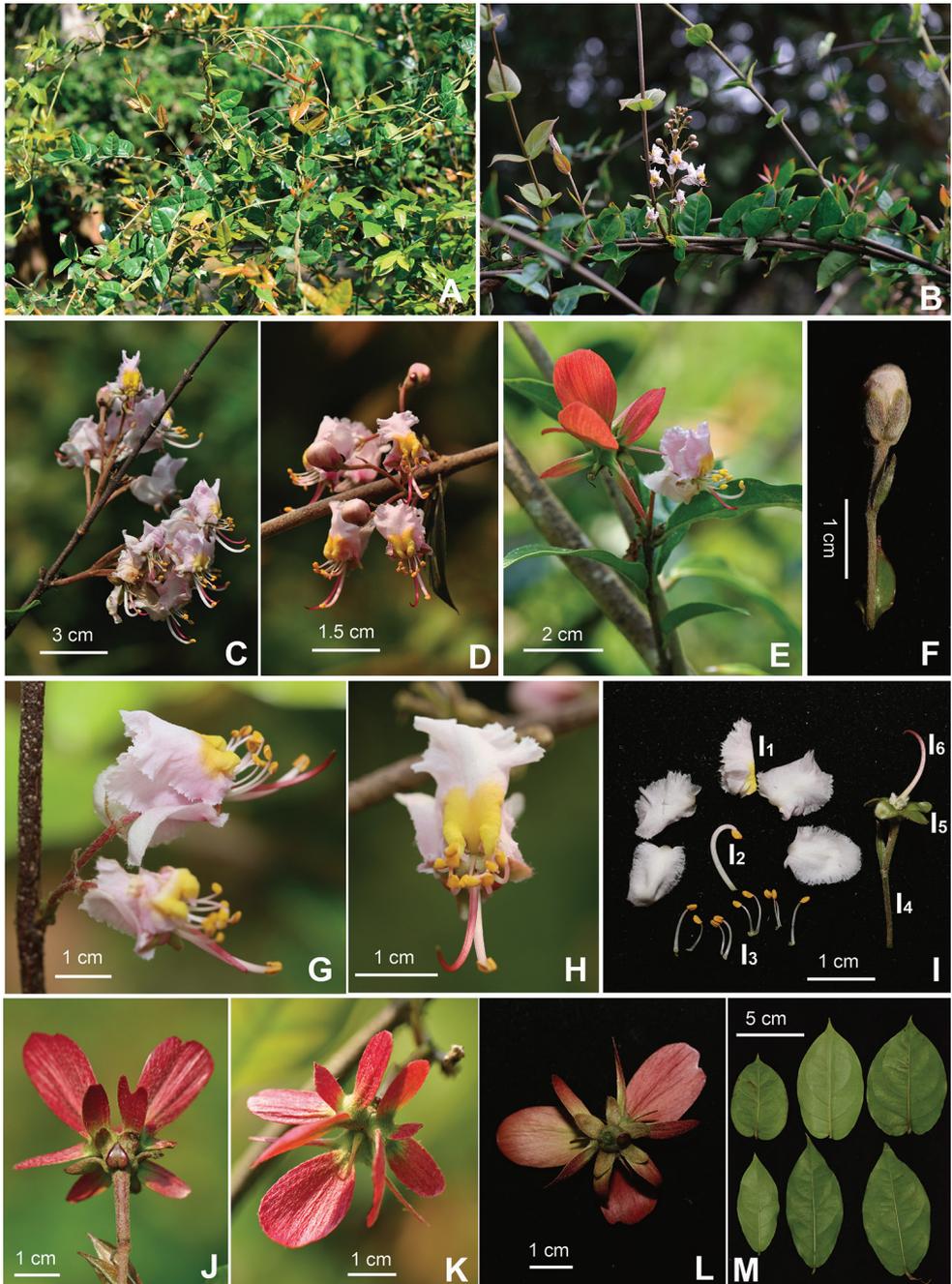


Figure 1. *Hiptage pauciflora* Y.H.Tan & Bin Yang, sp. nov. **A, B** Habit **C** Inflorescence (lateral view) **D** Inflorescence (frontal view) **E** Flowering branch **F** Flower bud **G** Flowers (lateral view) **H** Flower (frontal view) **I** Flower (I1 Petals; I2 the longest stamen; I3 the 9 short stamens; I4 Pedicel; I5 Calyx; I6 Style) **J** Samaras (lateral view and showing calyx gland) **K** Samaras (vertical view) **L** Samaras (dorsal view) **M** Leaves (adaxial view). Photographed by Y.H. Tan, H.B. Ding and B. Yang.

Diagnosis. *Hiptage pauciflora* is similar to *H. benghalensis* in elliptic-oblong leaf blades, petal shape and size and also shares similarities with *H. multiflora* in having leaf marginal gland dots, sub-orbicular calyx glands and not decurrent to pedicel, obovate middle wing of samara, but differs from the former by lacking basal glands at the leaf base, calyx glands ovate or sub-orbicular to cordate, scarcely decurrent to pedicel, middle wing of the samara obovate-elliptic, lanceolate bracteoles 7–11 mm (vs. 1 mm); and differs from the latter by its fewer flowers, longer pedicels, without basal glands at the leaf base and elliptic sepals (vs. ovate).

Type. CHINA. Yunnan Province, Menglian, Chengzi, limestone forest, the voucher from a cultivated plant at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 1 March 2017, *Y.H. Tan & B. Yang, XTBG-0013* (holotype, HITBC!).

Description. Woody climbing shrubs. Young branches pubescent, older twigs with rounded lenticels, rough warts dot-like. Leaf blades ovate or ovate-elliptic to elliptic-oblong, 6.0–10.5 × 2.5–12.0 cm, base subcordate to cordate, apex attenuate to acuminate, lateral veins 5–8 pairs, without basal glands; young leaves silver-grey pubescent; mature leaves with upper surface glabrous, lower surface sparsely pubescent, conspicuous along the midvein, with 3–6 pairs marginal gland dots. Petiole 4–7 mm long, pubescent. Racemes axillary or terminal, 5–10 cm, silver-grey pubescent; peduncle 0.5–2 cm. Flowers pink; Bracts triangular to ovate-triangular, 1.5–2 mm, bracteoles lanceolate, 7–11 × 1–3 mm; Pedicels 1.8–2.9 cm long, pubescent, articulate at the middle to a little above the middle; Calyx with 1 gland, prominent, ovate or sub-orbicular to cordate, scarcely decurrent to pedicel; Sepals elliptic to ovate-elliptic, ca. 5–7 × 2.5–3 mm, apex obtuse, base truncate, pubescent outside; Petals 5 per flower, pink, with yellowish blotches, ovate-oblong to suborbicular, 1.2–1.4 × 0.8–1.1 cm, glabrous, base rounded to subcordate, apex rounded, margin fringed, claw 1–1.5 mm; Stamens 10, differing in size, the longest filaments 12–13 mm, circinate at apex, the short ones 5–8 mm, anther ca. 1.5–2 mm long. Ovary ca. 2.5–3 mm in diam., pilose; style 1.2–1.4 cm long, glabrous, circinate at apex. Mericarps hairy, middle wing obovate-elliptic, puberulous, apex rounded, base obtuse, margin sometimes repand, 1.7–2.5 × 1–1.3 cm, lateral wings oblong, apex obtuse or rounded, margin sometimes crested, 1.3–1.6 × 0.3–0.5 cm; dorsal wing present or not, 2–4 × 1–2 mm.

Phenology. *Hiptage pauciflora* has been observed in flower at Xishuangbanna Tropical Botanic Garden under cultivation from the beginning of March and in fruit from March to April.

Etymology. The species epithet refers to its inflorescence with fewer flowers, usually 1–8 flowers.

Distribution and habitat. According to the introduction record, *H. pauciflora* was originally collected from Menglian, south-western Yunnan. Additional collections in the future may help to clarify its full distribution.

Conservation status. Due to insufficient field surveys so far, very few details about its natural distribution and population status are currently known. The lack of suf-

ficient data does not allow a final risk evaluation and the species might be regarded as data deficient (DD) according to the IUCN Red List Categories (IUCN 2012).

Specimen examined (paratypes). CHINA. Yunnan, Menglian, Chengzi, limestone forest, 7 February 1976, *G. D. Tao 9082* (HITBC!, IBSC!). Menglian, from Mangxin to Chahe, 22°12.985'N, 99°35.292'E, alt. 987m, limestone forest, 29 March 2010, *E. D. Liu, W. Fang, W. Z. Ma & H. J. Dong 2376* (KUN!). Menglian, Chengzi, the voucher from a cultivated plant at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 8 March 2018, *B. Yang & H.B. Ding, XTBG 0044* (HITBC!).

***Hiptage ferruginea* Y.H.Tan & Bin Yang, sp. nov.**

urn:lsid:ipni.org:names:77191588-1

Figure 2

Vernacular name. Xiu mao feng zheng guo (锈毛风箏果) (Chinese)

Diagnosis. *Hiptage ferruginea* is similar to *H. calcicola* in elliptic leaf shape, hairy pedicels and calyx without glands, suborbicular petals; but differs in having marginal gland dots, without laminal gland dots (vs. without marginal gland dots, with laminal gland dots), pink petals (vs. white) larger size, claw 2.5–4 mm (vs. 1–2 mm), middle wings of samara obovate (vs. oblong).

Type. CHINA. Yunnan, Mengla, Menglun, tropical seasonal moist limestone forest, 690 m a.s.l, *B. Yang & X.D. Zeng*, 4 March 2017, *XTBG0022* (holotype, HITBC!).

Description. Scrambling shrubs. Young branches glabrescent, older twigs with rounded lenticels, rough, axillary buds sparsely pubescent. grey blades elliptic or elliptic-oblong, 6.0–7.5 × 2.2–2.8 cm, glabrous, surface without larmlinal gland dots, base cuneate to obtuse, with 1 pair of dark-coloured marginal glands near base, apex acute to attenuate, midrib prominent beneath, lateral veins 5–6 pairs. Petiole 5–7 mm long, glabrous. Racemes axillary or terminal, multiflorous, rust-coloured hairy, up to 6 cm. Flowers pink to pinkish-white; Bracts triangular to lanceolate, 1.5–2 mm, bracteoles ovate-triangular, ca.1 mm long; Pedicels 0.5–1.3 cm long, rust-coloured hairy, articulate below the middle; Calyx glands absent; Sepals elliptic to ovate-elliptic, ca. 2.5 × 2 mm, apex obtuse, base truncate, pubescent outside; Petals 5 per flower, spreading, suborbicular, 7–8 × 6–7 mm, pubescent outside, densely near base, glabrous inside, base rounded to truncate, apex rounded, margin fimbriate, claw 2.5–4 mm, adaxial pubescent; Stamens 10, differing in size, the longest filaments ca. 8–9 mm, the short ones 5–6 mm, anther ca. 1 mm long. Ovary ca. 2 mm in diam., style ca. 9–10 mm long, glabrous. Mericarps dark puberulous, middle wing obovate, puberulous. apex rounded, margin repand, 1.7–2 × 1–1.2 cm, lateral wings oblong, apex margin crest, 0.9–1.1 × 0.5–0.7 cm.

Phenology. Flowering in March and fruiting occurs from March to April.

Etymology. The specific epithet is derived from its inflorescence rust-coloured hairy, ferruginous appearance.

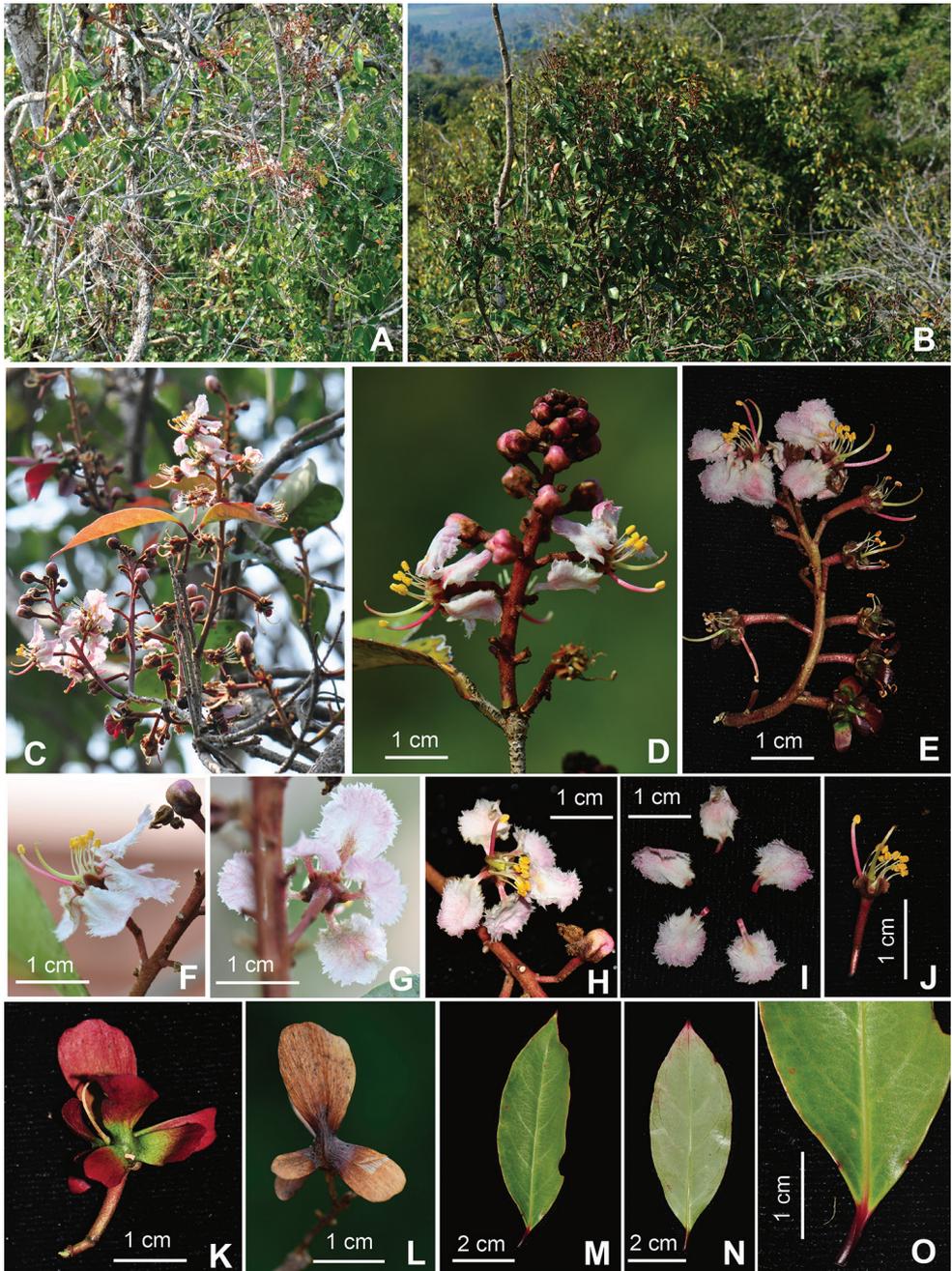


Figure 2. *Hiptage ferruginea* Y.H. Tan & Bin Yang, sp. nov. **A, B** Habit **C** Flowering branch **D, E** Inflorescence **F** Flower (lateral view) **G** Flower (dorsal view) **H** Flower (frontal view) **I** Petals **J** Flower (picked petals) **K, L** Samaras **M** Leaf (abaxial view) **N** Leaf (adaxial view) **O** Leaf base (showing the marginal glands). Photographed by B. Yang.

Distribution and habitat. There is only one population and less than 10 individuals known from the limestone areas in the Menglun Natural Reserve.

Conservation status. Since we have not discovered the wild population of this species in other places, very few details about its natural distribution and population status are currently obtained and the information is too inadequate to assess its risk of extinction. At present, it is proposed that it be considered as a taxon under the Data Deficient (DD) category on the basis of current IUCN Red List Categories and Criteria (IUCN 2012).

Key to the species of *Hiptage pauciflora*, *H. ferruginea* and their closely related species

- 1 Calyx glands conspicuous 2
- Calyx glands inconspicuous or absent 4
- 2 Calyx glands more or less decurrent on the pedicel, middle wings of samara 3–5 cm long *H. benghalensis*
- Calyx glands scarcely decurrent to pedicel, middle wings of samara less than 3 cm long 3
- 3 Leaf blade ovate or ovate-elliptic to elliptic-oblong, less than 10 cm long, bracteoles lanceolate, 7–11 mm, leaf basal gland dots absent, inflorescence with fewer flowers, usually 1–8 flowers, pedicels 1.8–2.9 cm .. *H. pauciflora*
- Leaf blade oblong, 12–13 cm long, bracteoles small, ca. 1mm, leaf basal gland dots 1 pair, inflorescence with many flowers, pedicels ca.1 cm *H. multiflora*
- 4 Leaf marginal gland dots absence and with 1–2 pairs larmental gland dots *H. calcicola*
- Leaf marginal gland dots conspicuous, usually 1 pair and larmental gland dots absent *H. ferruginea*

Discussion

The zygomorphic or mirror-image flower of the *Hiptage* is a unique feature of evolutionary and biological importance that enhances adaptations (Ren et al. 2013, Qian et al. 2016). The presence or absence of calycinal glands calyx is an important characteristic and feature of the glands' shape and number, and whether it is adnate to pedicel or not is also a key diagnostic for species identification (Siriruga 1991, Srivastava 1992, Chen and Funston 2008). Morphologically, *H. pauciflora* shares certain characteristics with *H. benghalensis* in having elliptic-oblong leaf blades and sub-orbicular petals and also shares similarities with *H. multiflora* in having leaf marginal gland dots, sub-orbicular calyx glands and not decurrent to pedicel, obovate middle wing of samara. *Hiptage ferruginea* is similar to *H. calcicola* in elliptic leaf shape, hairy pedicels and hairy calyxes without glands,

Table 1. Morphological comparison of key characters in *Hiptage pauciflora*, *H. ferruginea* and the morphological similar taxa.

Character	<i>Hiptage pauciflora</i>	<i>H. benghalensis</i>	<i>H. multiflora</i>	<i>H. ferruginea</i>	<i>Hiptage calcicola</i>
Leaf blade	ovate or ovate-elliptic to elliptic-oblong, 6.0–10.5 × 2.5–12.0 cm	oblong, elliptic-oblong, or ovate-lanceolate, 9–18 × 3–7 cm	oblong, 12–13 × 5–5.5 cm	elliptic or elliptic-oblong, 6.0–7.5 × 2.2–2.8 cm	elliptic or ovate, 3–10.5 × 1–4.5 cm
Leaf marginal gland dots	3–6 pairs	2–8 pairs	2–4 pairs	1 pair	absent
Leaf basal gland dots	absent	1 pair	1 pair	absent	absent
Larminal gland dots	absent	absent	absent	absent	1–2 pairs
Bracteoles	lanceolate, 7–11 × 1–3 mm	acute, ca. 1 mm	ovate-triangular, ca. 1 mm	ovate-triangular, ca. 1 mm	acute, ca. 1 mm
Flowers	pink	white	white?	pink to pinkish-white	white
Pedicels	pubescent, 1.8–2.9 cm	pubescent, 0.8–2.5 cm	puberulent, ca. 1 cm	dark red hairy, 0.5–1.3 cm	pubescent, 0.4–2 cm
Calyx glands	ovate or sub-orbicular to cordate, scarcely decurrent to pedicel	elliptic, oblong, triangular, lanceolate to oblanceolate; more or less decurrent on the pedicel	sub-orbicular; not decurrent to pedicel	absent	inconspicuous or absent
Sepals	elliptic to ovate-elliptic	broadly elliptic or ovate	ovate	elliptic to ovate-elliptic	elliptic
Petals	ovate-oblong to sub-orbicular	ovate-oblong to suborbicular, glabrous	unknown	suborbicular, 7–8 × 6–7 mm	suborbicular, 3.5–4 × 3.5–4 mm
Claw	1–1.5 mm	1–2 mm, glabrous	unknown	2.5–4 mm, pubescent	1–2 mm, hairy
Middle wings of Samara	obovate-elliptic, 1.7–2.5 × 1–1.3 cm	oblong, elliptic or obovate-lanceolate, 3–5 × 1–1.6 cm	obovate, 2.2–2.5 × 0.7–1.0 cm	obovate, 1.7–2 × 1–1.2 cm	oblong, 2–2.5 × 0.5–0.8 cm

suborbicular petals. The detailed comparisons of the morphological differences amongst these taxa are given in Table 1 and evidence from morphological analysis supports the recognitions of *H. pauciflora* and *H. ferruginea* as two distinct species, respectively.

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A new large-flowered species of *Andeimalva* (Malvaceae, Malvoideae) from Peru

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Abstract

Andeimalva peruviana Dorr & C.Romero, **sp. nov.**, the third Peruvian endemic in a small genus of five species, is described and illustrated from a single collection made at high elevation on the eastern slopes of the Andes. Molecular phylogenetic analyses of nuclear ribosomal ITS sequence data resolve a group of northern species of *Andeimalva* found in Bolivia and Peru from the morphologically very different southern *A. chilensis*. The new species bears the largest flowers of any *Andeimalva* and is compared with Bolivian *A. mandonii*. A revised key to the genus is presented.

Keywords

Andeimalva, Andes, Malvaceae, Malvoideae, Peru, phylogeny

Introduction

The genus *Andeimalva* J.A. Tate (Malvaceae, Malvoideae) was created to accommodate four species found in the Andes of South America from northern Peru to central Chile and includes three species previously placed in *Tarasa* Phil. and one in *Mala-cothamnus* Greene (Tate 2003). Krapovickas (1965) described two of these species, *T. machupicchensis* Krapov. and *T. spiciformis* Krapov., and allied them with a third,

T. mandonii (Baker f.) Kearney, because he observed that the three differed from all other species of *Tarasa* in having entire lanceolate leaves more than three times as long as wide and awned mericarps with smooth (versus reticulate) sides. Despite the distinctiveness of this group, Krapovickas (1965) chose not to recognize it formally. The fourth species of *Andeimalva*, endemic to Chile, was long known as *M. chilensis* (Gay) Krapov. even though its placement in *Malacothamnus* was problematic based on its morphology, cytology, and geography (Bates 1963; Bates and Blanchard 1970; Tate and Simpson 2003). Tate (2003), combining molecular, cytological and morphological data, transferred these three species of *Tarasa* and one species of *Malacothamnus* to the newly described genus *Andeimalva*. The Chilean species of *Andeimalva* is anomalous morphologically in that it differs from the group recognized by Krapovickas (1965) in having suborbicular, slightly 3–5-lobed leaves and awnless mericarps. Nonetheless, molecular and cytological data indicate that it is closely related to the other three species found in Bolivia and Peru (Tate and Simpson 2003).

Recent floristic exploration in the northern Andes of Peru yielded a Malvaceae collection (*Vega Ocaña 419*) of uncertain initial generic affiliation. With further study and molecular phylogenetic analyses, we determined it to represent a new species of *Andeimalva*, which is described here as *A. peruviana* Dorr & C. Romero, and the third one endemic to Peru. We are amazed that a shrub with such conspicuous flowers is not known from earlier collections and can only speculate that either its range is very restricted in a botanically poorly explored area or it might simply have passed unnoticed due to a limited flowering period.

Materials and methods

We sought molecular data both to confirm the generic identity of the new collection and to provide a phylogenetic context within the ITS (Internal Transcribed Spacer region of the nuclear ribosomal repeat) data set of Tate and Simpson (2003). Leaf tissue with abaxial trichomes scraped off was ground in a bead mill and extracted with the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California, USA) following an extended digestion with the AP1 buffer emended with 1.0 mg proteinase K (PCR grade solution, Roche Applied Science, Germany) and 1.8% v/v 2-mercaptoethanol. To reduce systemic contamination risk, all equipment was dedicated to “antique DNA” in a pre-PCR extraction laboratory and no other Malvales were co-extracted or amplified at the same time. Despite being a 2.5-year old collection with reasonably good preservation (i.e., retaining some color and alcohol treated), the resulting extraction only yielded highly degraded DNA (< 400 bp on an agarose test gel). Amplifications of ITS were attempted using a series of nested primers for decreasing amplicon sizes, and only a 302 bp fragment of ITS1 could be recovered with primers 1m (CGTAG-GTGAACCTGCGGAAGGATC, newly modified from ITS1) and P2 (GCCRAGATATCCGTTGCCGAG; Cheng et al. 2016). Amplification reactions were conducted in a final volume of 15 µl with 1× Bioline reaction buffer, 8 mM dNTPs, 1.75 mM

magnesium chloride, 2 nM of each primer, 0.1 mg/ml of bovine serum albumin, 0.025 U/ μ l of Biolase DNA polymerase (Bioline USA, Taunton, Massachusetts, U.S.A.), 1 M betaine, and 1 μ l of DNA extraction. Sequencing was with BigDye Terminator v3.1 chemistry (Thermo Fisher Scientific, Waltham, Massachusetts) on an ABI 3730xl DNA Analyzer (Thermo Fisher Scientific). The new ITS1 sequence (GenBank MK044847, from *Vega Ocaña 419*) was manually aligned using a similarity criterion with the Tate and Simpson (2003) data set archived in TreeBASE (<http://purl.org/phylo/treebase/phylovs/matrix/TB2:M516>). Small changes were made to the source matrix including converting mixed bases to ambiguity codes and improving the alignment within *Fuertesimalva* Fryxell (i.e., inconsistencies relative to positions 114–155 in our alignment), which reduced local branch lengths relative to prior analyses. The 53-tip matrix has an aligned length of 696 bp and relatively few, mostly small 1–4 bp indels (except *Fuertesimalva* spp. sharing a 42 bp deletion); we did not exclude any data in the analyses. We could not obtain any new plastid data and thus did not analyze the plastid data set of Tate and Simpson (2003), which the authors did not combine with ITS due to incongruence. Parsimony (MP) analyses were conducted with PAUP* 4.0a build 163 (Swofford 2002) using 1000 random addition replicates with TBR branch swapping and no search limits, and 1000 bootstrap (MP-BS) replicates, each with two random additions and a search limit of 10,000 trees per iteration (MulTrees = 10,000). Maximum likelihood (ML) analyses were conducted under a GTR + I + Γ model with GARLI 2.01 (Zwickl 2006) on CIPRES XSEDE (<https://www.phylo.org/>), using 100 replicates for optimal trees, and 1000 ML bootstrap replicates (ML-BS), each with two random additions and the automated stopping criterion.

Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Digital Repository at: <https://doi.org/10.5061/dryad.44dm150>

Results

Suspicions that *Vega Ocaña 419* belonged with *Andeimalva* were confirmed by molecular data where BLAST searches of our ITS1 sequence against GenBank yielded best matches with that genus. This affiliation was confirmed by phylogenetic analyses (Fig. 1) that placed it as strongly supported (BS 95–100) within a nested subclade of *Andeimalva*, although the exact sister relationship resolved with *A. mandonii* was weakly supported (ML-BS 75, MP-BS < 50). Parsimony analysis yielded a single island with 180 trees (length = 346 steps, CI = 0.682 or 0.613, excluding uninformative characters; 128 parsimony informative characters) and a topology similar to prior phylogenies (i.e., Tate 2003; Tate and Simpson 2003). The topology of our ML tree (best score of 100 trees, $-\ln L = -3137.458$) was also similar but not identical to any of

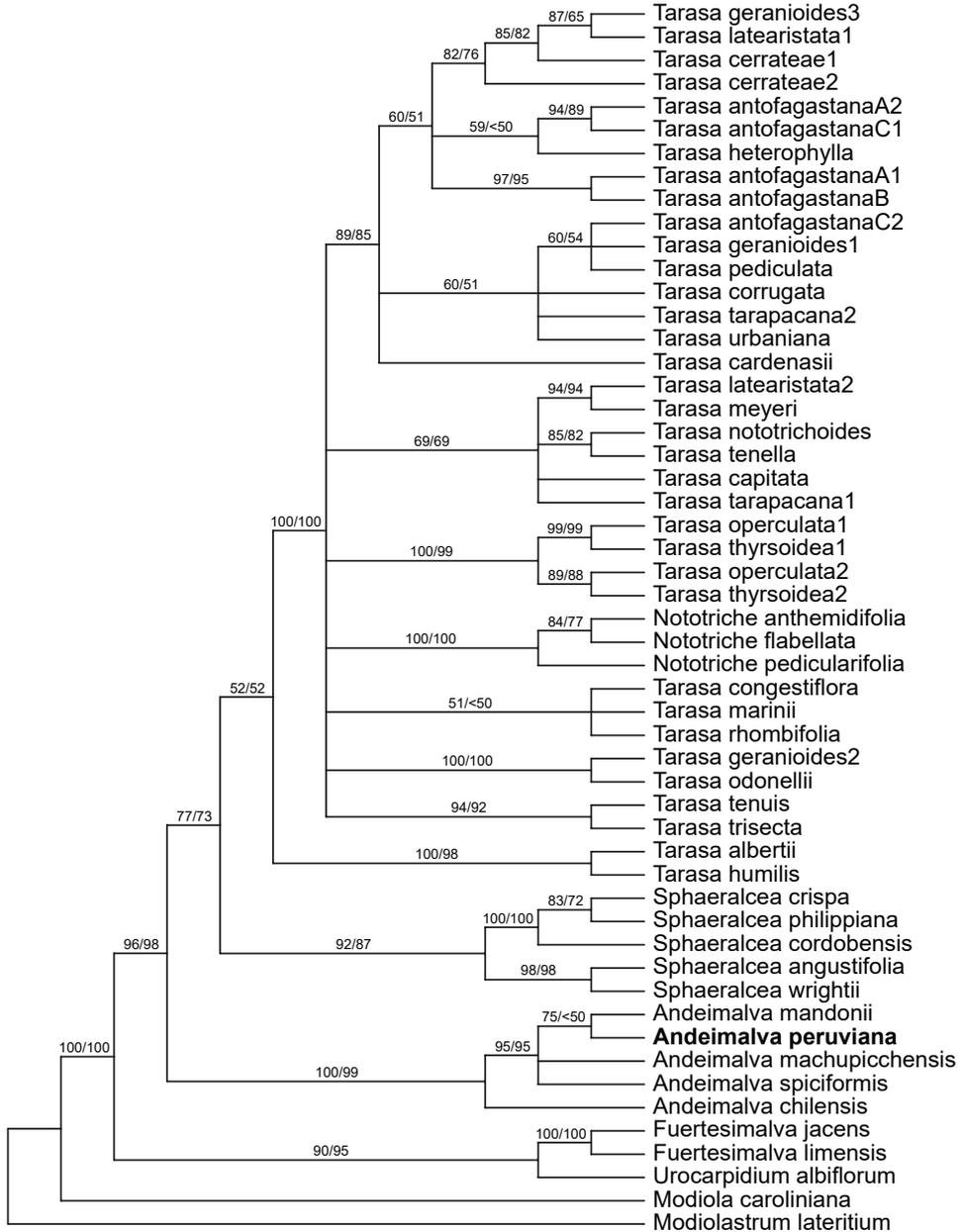


Figure 1. Maximum likelihood (ML) bootstrap 50% majority-rule consensus tree based on ITS data for *Andeimalva* and its Malvoideae relatives. ML/parsimony bootstrap values are indicated above branches.

the parsimony trees. There is a 3-bp deletion (loss of tandem GTT repeat at positions 280–282 in our alignment) only possessed by *A. chilensis* (Gay) J.A. Tate and *A. machupicchensis* (Krapov.) J.A. Tate, but this is not reflected in the recovered relationships and would not appear to be a structural synapomorphy.

Taxonomy

Andeimalva peruviana Dorr & C.Romero, sp. nov.

urn:lsid:ipni.org:names:77191589-1

Figure 2

Type. PERU. La Libertad: Bolivar, alpine bogs around Rio Negro and Pampa Uchulala along road to Bambamarca, 07°13'51"S, 077°38'21"W, 3750 m, 3 June 2015 (fl), *C. Vega Ocaña* 419 with R.W. Bussmann, N. Paniagua Zambrana, F. Díaz Llajo & F. Díaz Vega (holotype: MO-2423556!; isotypes: HAO, US-01184179!, USM).

Diagnosis. Differs from *Andeimalva mandonii* (Baker f.) Kearney in having larger stipules (13–15 × 5–8 mm versus 5–12 × 1–2 mm) that are broadly subulate (not filiform), and larger calyx lobes (1.7–2.1 × 1–1.8 cm versus 0.5–1 × 0.3–0.5 cm) and petals (3.5–4.5 × 2.5–3.2 cm versus 1.5–3 × 1.5–2 cm).

Description. Shrubs, 1.5–3 m tall; young stems densely lanate and appearing white, glabrescent and dark brown to almost black in age. Stipules subulate with broader cordate base, 13–15 mm long, 5–8 mm wide, lanate with appressed stellate hairs, venation with 3 prominent parallel primaries running their length and up to 8 at base, persistent beyond life of leaf. Leaves simple, spirally arranged, petiolate, petioles 4–6 mm long, lanate; blades lanceolate to narrowly lanceolate, 5–8.2 cm long, 0.8–1.8 cm wide (lower leaf measurements due to serial size reduction toward the apex of flowering branches), base cordate, slightly asymmetric, apex acute, discolorous, drying dark green to brown above and whitish-tan below, bullate above with scattered sessile stellate hairs of varying sizes, the arms on the larger hairs ascending, midrib above densely pubescent and appearing white, densely lanate below with sessile stellate hairs and larger stalked, multi-rayed stellate hairs, the arms on the larger hairs spreading, stalks dark-colored, margin crenate-serrate, venation pinnate with 13–18 secondaries per side. Flowers solitary or in 2–3(–4)-flowered axillary or pseudo-terminal cymes, ca. 5–7 cm in diameter at anthesis, pedicellate, pedicels to 0.5 cm long; involucre bracts (2–)3, 1–1.8 cm long, 0.5–0.8 cm wide, inserted just below calyx, densely stellate pubescent, hairs near margin long-stalked and multi-rayed. Calyx lobes broadly deltoid, acuminate, slightly unequal in size, 1.7–2.1 cm long, 1–1.8 cm wide, pubescent within especially toward base, stellate-pubescent on the outside with small and large multi-rayed hairs. Petals obovate, unequal in size within the flower, 3.5–4.5 cm long, 2.5–3.2 cm wide, slightly to markedly asymmetric, deep mauve or dark magenta-purple, apex slightly undulate, unguiculate, glabrous except appressed simple hairs near base within and claw margins densely pubescent. Staminal column 2.8–3.2 cm long, sparingly pubescent throughout with simple hairs; anthers numerous (100+), 0.7–1.4 mm long, clustered at upper half of column; free portion of filaments (1–)1.6–5(–6) mm long. Carpels 10, cells uniovulate. Stigmas 20, capitate, scarcely exceeding the anthers at anthesis. Mericarps and seeds unknown.

Distribution and ecology. At present, known only from the type locality where it occurs in alpine bogs at 3750 m.

On the label of the type specimen there is a slight discrepancy regarding the political subdivision for the locality. Google Earth Pro mapping (8 June 2011 imagery, <https://>

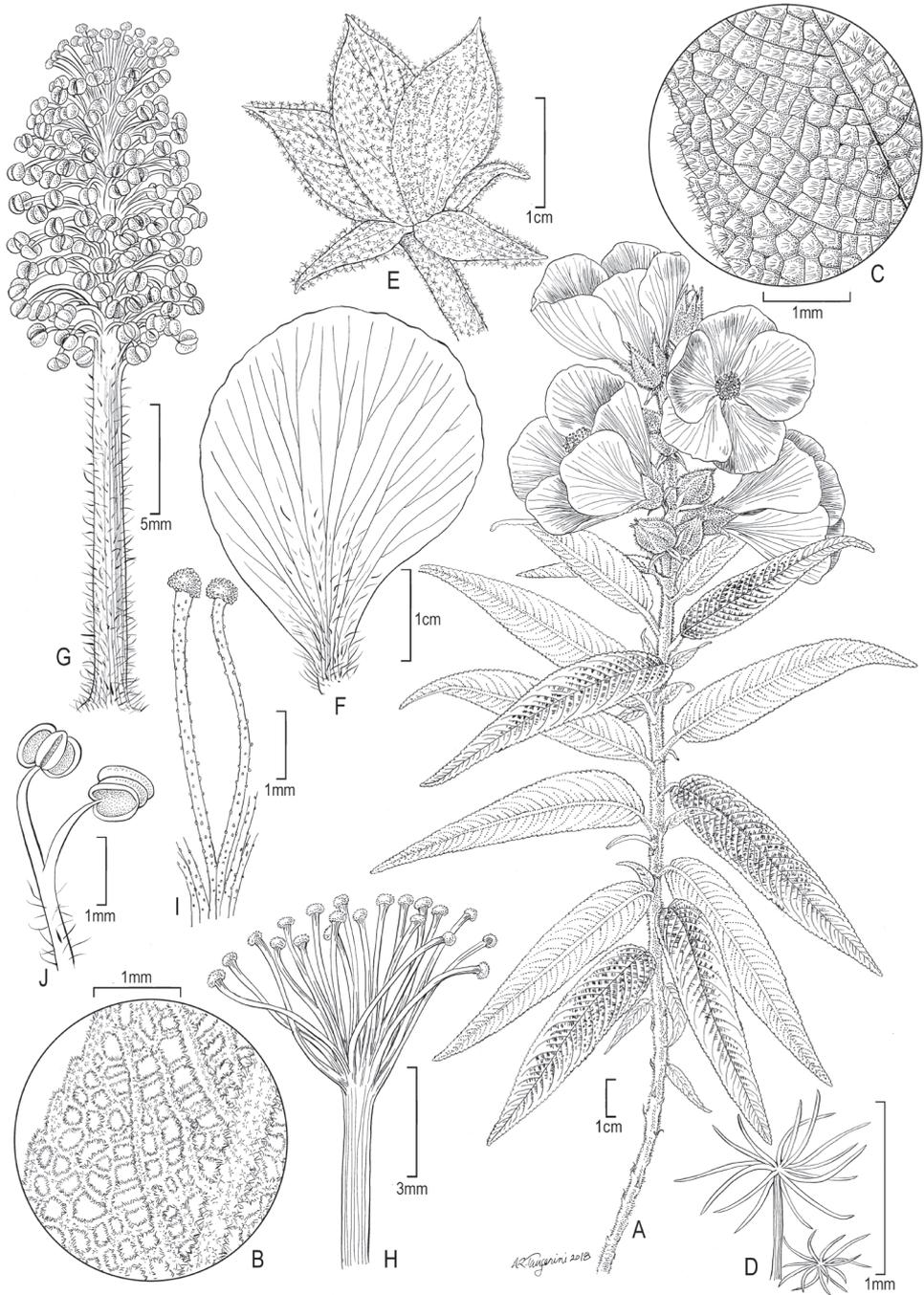


Figure 2. Illustration of *Andeimalva peruviana*. **A** Habit **B** detail of leaf below **C** detail of leaf above: note bullate surface **D** stalked and sessile stellate trichomes from leaf blade below **E** involucrel bracts subtending calyx (only 3 calyx lobes visible) **F** petal **G** staminal column: note stigmas scarcely exerted at apex **H** style with 20 capitate stigmas **I** detail showing two stigmas **J** detail showing two anthers (Source: Vega Ocaña 419, US).

www.google.com/earth/) places the coordinates cited on the label (presumably derived from a GPS device and for a logical locality near a road crossing of a wet drainage) in San Martín, and just over the eastern border delineated for La Libertad. However, we cannot verify how well Google Earth Pro finely draws political boundaries in relation to those that might appear on official government maps of such remote regions.

Etymology. The specific epithet is derived from the name of the country (Peru) where the new species is found.

Preliminary conservation status. Following the criteria and categories of IUCN (2017), *Andeimalva peruviana* is given a preliminary status of Vulnerable (VU D2) due to population very small or restricted (area of occupancy < 20km² and number of locations < 5). While the type of locality is quite remote and unexplored habitat occurs nearby, the general area has no protected regions and might be subject to habitat degradation from grazing or changes in hydrology. The latter includes human modification to its drainage system or climate change affecting montane precipitation.

Discussion

Morphologically *Andeimalva peruviana* appears to be closer to the three anomalous species of *Tarasa* first united by Krapovickas (1965) and later transferred to *Andeimalva* by Tate (2003), which share leaf morphology and mericarps with apical awns. *Andeimalva chilensis*, sister to the crown group (Fig. 1), has greater morphological divergence. However, its lack of awns relative to the other species of *Andeimalva* (uncertain in *A. peruviana*) may be an autapomorphic loss given that related genera such as *Tarasa* mostly possess awns (see Tate 2003). The chromosome number of our new species is unknown, but it would prove informative for generic affiliation. Optimal trees place *A. peruviana* as sister to *A. mandonii*, which has ML but not parsimony bootstrap support. This affinity is reflected in the morphology with shared generally larger floral features relative to the other species, but notably differs in stipule morphology (see Key below and Diagnosis). The large stipules of *A. peruviana* with prominent parallel major veins differ from those of the other species that are narrow in width and attachment.

Most species of *Andeimalva* are geographically well separated. However, *A. spiciformis* (Krapov.) J.A. Tate and *A. peruviana* are nearly sympatric on a gross scale with both occurring at high elevation in La Libertad, Peru. Nonetheless, the two species appear to be separated by altitude, hydrology, and a physical barrier. *Andeimalva spiciformis* with its scattered distribution in northwestern Peru at 2400–3200 m, is known in La Libertad from a single collection (*A. López M. 1439*, US; see Tate 2003: 15) made at 2600 m. In contrast, the type locality of *A. peruviana*, about 70 km distant near the border of La Libertad, was made at 3750 m and is physically well separated by an east-west divide created by the deep valley of the Rio Marañón. The boggy habitat of *A. peruviana* in a region rich in small alpine lakes near the wet eastern side of the Andes generally appears much wetter than where *A. spiciformis* grows on drier mountain areas to the west where the Andean rainfall shadow has greater influence.

Key to Species of *Andeimalva* (modified from Tate 2003)

- 1 Leaves suborbicular, slightly 3–5-lobed; mericarps without an apical awn; central and southern Chile.....*A. chilensis*
- Leaves lanceolate, not lobed; mericarps with prominent apical awn (or character state unknown); Bolivia, Peru.....**2**
- 2 Flowers in dense axillary glomerules; petals notched apically; mericarp awns 1–1.5 mm long; Peru.....*A. spiciformis*
- Flowers solitary or in few-flowered axillary cymes; petals not notched apically; mericarp awns 1.5–4 mm long (or character state unknown).....**3**
- 3 Staminal column < 2 mm long; calyx lobes 0.4–0.5 cm long; petals < 2 cm long; Peru.....*A. machupicchensis*
- Staminal column > 2 mm long; calyx lobes 0.5–2.1 cm long; petals 1.5–4.5 cm long.....**4**
- 4 Petioles 3–12(–20) mm long; stipules 5–12 × 1–2 mm, filiform; staminal column 0.3–0.8 cm long; calyx lobes 0.5–1 cm long; petals 1.5–3 cm long; Bolivia.....*A. mandonii*
- Petioles 4–6 mm long; stipules 13–15 × 5–8 mm, broadly subulate; staminal column 2.8–3.2 cm long; calyx lobes 1.7–2.1 cm long; petals 3.5–4.5 cm long; Peru.....*A. peruviana*

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Poa secunda J. Presl (Poaceae): a modern summary of infraspecific taxonomy, chromosome numbers, related species and infrageneric placement based on DNA

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Abstract

Poa secunda J. Presl. s.l. is a morphologically highly variable bunchgrass that is a valuable forage species in western North America. There has been much controversy as to whether multiple taxa should be recognised and at what rank in this taxonomically challenging apomictic complex. Here we propose an infraspecific classification for *Poa secunda* of six varieties within two subspecies, *juncifolia* and *secunda*. New combinations are *P. secunda* vars. *ampla*, *gracillima*, *juncifolia*, *nevadensis* and *scabrella*. Conflicting plastid and nrDNA phylogenies show that *P. sect. Secundae* is of ancient hybrid origin. Based on this and its distinct morphology, the section is raised to the rank of subgenus. A key is presented for *P. secunda* infraspecies and closely related non-arctic species. Suppl. materials are provided of chromosome counts for *Secundae* taxa and D.D. Keck specimen annotations of taxa here included in *P. secunda*.

Keywords

Apomixis, hybridisation, *Poa secunda*, Poaceae, polyploidy, reticulation, *Secundae*, taxonomy

Introduction

Poa secunda J. Presl. s.l. is a morphologically highly variable species found primarily in western North America. It is common to dominant in grasslands extending from Alaska to Northern Mexico and eastwards in the northern Great Plains and scattered

more eastern locations to the Gaspé Peninsula in Quebec (Marsh 1952, Kellogg 1985a, Soreng 2007). The species is disjunct but not common in South America, occurring in Patagonian Argentina and Chile (presumed origin of the type collection of *P. secunda*). This perennial bunchgrass is a valuable forage species that greens up and flowers in early spring and is abundant across western grasslands, coastal chaparral, Great Basin steppe, uplands of the Mojave Desert and dry forests surrounding these and reaches into alpine meadows. It exhibits diverse ecotypes across this wide geographic and climatic range. *Poa secunda* s.l. is abundantly represented in herbaria across the United States; for example, the United States National Herbarium (US) has more than three very full herbarium cases of specimens for the United States and Canada. It is distinguished from most *Poa* species by its obscurely keeled lemmas, calluses often with a minute crown of hairs surrounding the base of the lemma (in subsp. *secunda*) and elongated spikelets (mostly 3.5–5 × longer than wide).

Differing taxonomies of the *Poa secunda* J. Presl complex continually appear in the literature. There are some 45 formal names applied to *P. secunda* s.l. Some taxonomists follow A.S. Hitchcock's (1935, 1951) taxonomic revision in the *Manual of the Grasses of the United States*, in recognising his two informal groups of species, "Scabrellae" and "Nevadenses". In the former group, Hitchcock included *P. scabrella* (Thurb.) Benth. ex Vasey, *P. gracillima* Vasey, *P. secunda* and *P. canbyi* (Scribn.) Howell; in the latter group *P. nevadensis* Vasey ex Scribn., *P. curtifolia* Scribn., *P. juncifolia* Scribn. and *P. ampla* Merr. Most of these taxa are still often recognised as species, subspecies or varieties (e.g. Keck 1950, 1959, Hitchcock et al. 1969, Dorn 1977, 1988). Keck (1950, 1959) additionally recognised *P. tenerrima* Scribn., a serpentine endemic of the Sierra Nevada foot hills and *P. incurva* Scribn. & T.A. Williams, a western sub-alpine/alpine element, within the Scabrellae group. Keck (1950, 1959) furthermore treated North American *P. secunda* as *P. sandbergii* Vasey, restricting *P. secunda* to South America and Hitchcock et al. (1969) accepted this split. Arnow (1981) concluded *P. secunda* was the correct name for plants of the *P. sandbergii* form. Hitchcock (1935, 1951) included *P. sandbergii* and *P. incurva* in *P. secunda*. Marsh (1950, 1952) and Kellogg (1985a, b) quite independently lumped most of these taxa in *P. secunda* without any infraspecies (followed by Lesica 2012, for Montana). Kellogg's work, based on morphometric analyses, separated *P. curtifolia*; Marsh maintained that and also *P. tenerrima*. Soreng (1991b) divided *P. secunda* s.l. into two subspecies, subsp. *secunda* and subsp. *juncifolia* (Scribn.) Soreng, corresponding to the taxa in the Scabrellae and Nevadenses groups, respectively and accepted *P. curtifolia* and *P. tenerrima*. Dorn (1988) accepted as full species the two subspecies that Soreng recognised and established a few varieties within those. Skinner (2010) followed the subspecies split, but provided separate pages for each of the common names that Hitchcock (1951) applied to species aligned within the two subspecies: Sandberg bluegrass (*P. secunda* var. *secunda*), Sandberg bluegrass [*P. secunda* var. *incurva* (Scribn. & T.A. Williams) Beetle], Pine bluegrass (*P. scabrella*), Canby bluegrass (*P. canbyi*), Nevada bluegrass (*P. nevadensis*), Big bluegrass (*P. ampla*) and Alkali bluegrass (*P. juncifolia*). Several cultivars correspond to these different forms (Alderson and Sharp 1995). Thus, there appears to be a consistent desire and effort to maintain some or all of the diversity of forms commonly recognised, at some taxonomic rank.

Molecular (Patterson et al. 2005) and cytogenetic (Hiesey and Nobs 1982) studies show that *P. secunda* s.l. is a highly complex, apomictic species. Apomixis, although it is facultative to varying degrees (Clausen 1961, Kellogg 1987), is apparently the main mode of reproduction in *P. secunda* (Kellogg 1987). This has led to the production of various “strains”, ecotypes and races that are fairly monomorphic, but also to much intermediacy. Taxonomy in apomictic complexes is notoriously difficult, as is evident in the case of *P. secunda* s.l. However, we concur with Marsh (1952) and Kellogg (1985a, b) that the rank of species is not justifiable for most of the *Scabrellae* and *Nevadenses* taxa.

Poa secunda s.l. belongs to *P.* sect. *Secundae*, a primarily North American section of about eight species (Soreng 1991a, b, 1998, 2007, Soreng et al. 2003a, b, Gillespie et al. 2007). Soreng (1991a) established sect. *Secundae* with two subsections to accommodate species that share similar, apparently plesiomorphic Poae traits, along with a derived chloroplast type (Soreng 1990) and suggested the section was of hybrid origin (Soreng 1990, 1991a, b). Our phylogenetic studies, based on plastid and nuclear ribosomal DNA, demonstrate that sect. *Secundae* is reticulate in origin (Gillespie et al. 2008, Cabi et al. 2017, Soreng et al. 2017). *Secundae* species exhibit several atavistic traits (Soreng 1991a, Soreng et al. 2015) that are otherwise odd in *Poa*, including; a crown of hairs around the base of the callus, upper culm leaf sheaths margins often free more than 80–90% of their length and lemmas that are often somewhat rounded on the back. These traits are common in genera outside of *Poa* and have led to many names for *Secundae* taxa being published in *Atropis*, *Glyceria* and *Puccinellia*. *Secundae* species are tufted (rhizomes occur in some putative hybrids, see Species hybrids involving *Poa secunda* in the Taxonomy section) and perfect flowered, with anthers ranging from 1.2–3 mm long. Soreng (1991a, 2007) recognised two subsections: *Secundae* and *Halophytæ* V.L. Marsh ex Soreng. In addition to *P. curtifolia*, *P. secunda* s.l. and *P. tenerima*, subsection *Secundae* also includes two Arctic species, *P. ammophila* A.E. Porsild and *P. hartzii* Gand. (Soreng 1991b) and is characterised by elongate, weakly compressed spikelets and lemmas rounded on the back. Species of subsection *Halophytæ* (including *P. napensis* Beetle, *P. stenantha* Trin., *P. unilateralis* Scribn. ex Vasey) commonly have distinctly keeled lemmas and sometimes a papilliate epidermis on pedicels and leaf blades (Soreng 1991a, b, 2007).

Our goals in this paper are: 1) to provide a current overview of the taxonomy of *P. secunda* s.l. and present an up-to-date infraspecific classification including new combinations for the forms often recognised as species and 2) to document what we know of the relationships and hybrid origin of *P. secunda* s.l. and sect. *Secundae*. We also provide a review of the cytology of *P. secunda* s.l. and other species in sect. *Secundae*.

***Poa secunda* s.l.: a revised infraspecific classification**

In the interest of facilitating land-managers, ecologists, plant breeders, seed-storage facilities and collections managers in maintaining the understanding of variations in morphological forms in *P. secunda* s.l. that are often recognised as species, we here

provide varietal names within *P. secunda* s.l. subspecies. This would also maintain herbarium collections that are organised or understood along A. S. Hitchcock's taxonomic concepts, in which the taxa can be viewed to correspond to ecotypes or ecologically adapted apomictic lineages within *P. secunda*. There is extensive intermediacy between these taxa and, thus, the rank of species is viewed as untenable (Marsh 1950, 1952, Kellogg 1985a, b, Soreng 1991b, 1993, 2007, Soreng et al. 2003a, b). RJS's revised classification of *P. secunda* s.l. is presented in the Taxonomy section below.

As a whole, *P. secunda* s.l. is relatively easily split into two subspecies (*juncifolia* and *secunda*, Fig. 1), but finer distinctions are often inconstant and overlapping. Within subspecies *secunda*, it is a futile exercise to attempt to consistently separate *P. canbyi*, *incurva*, *sandbergii* and *secunda* forms and so these are all included in var. *secunda*. The epithet *gracillima* is often misapplied to plants RJS would classify as var. *secunda*. Both *gracillima* and *scabrella* are extreme forms that many botanists seem to think are worthy of taxonomic recognition at some level. Kellogg (1985a, b) concluded that the *P. ampla* form was the most distinct element within *P. secunda* s.l., but the difficulty noted in the literature (see Hitchcock et al. 1969) of separating that from subspecies *juncifolia* s.s., leads RJS to treat it as a variety in the subspecies. Range maps of the two *P. secunda* subspecies are provided in Fig. 2.

An exhaustive summary of the nomenclature, protologues and types for the more than 45 names included in *P. secunda* s.l. is beyond the scope of the present paper, but names of synonyms are listed. Hitchcock's and some other historical synonymies for the nominal taxa can be found in his widely available "Manual of the Grasses of the United

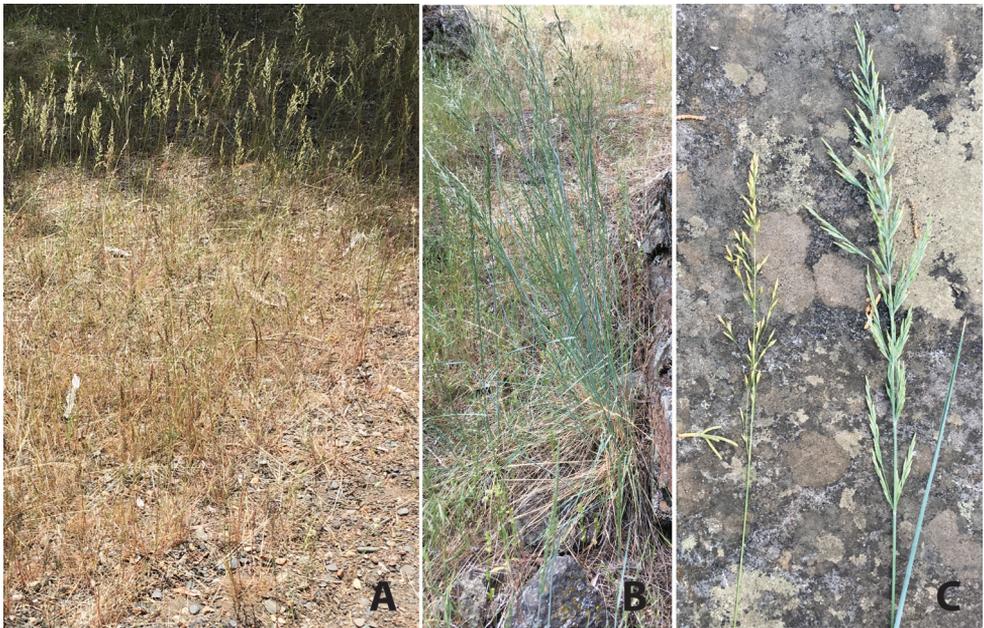


Figure 1. *Poa secunda* habit and panicles: **A** *P. s.* subsp. *secunda* var. *secunda* (Soreng 9359) **B** *P. s.* subsp. *juncifolia* var. *ampla* (Soreng 9358) **C** Panicles of subsp. *secunda* var. *secunda* (left) and *juncifolia* var. *ampla* (right) (Photos. RJS, Deschutes River near Madras, Jefferson Co., Oregon).

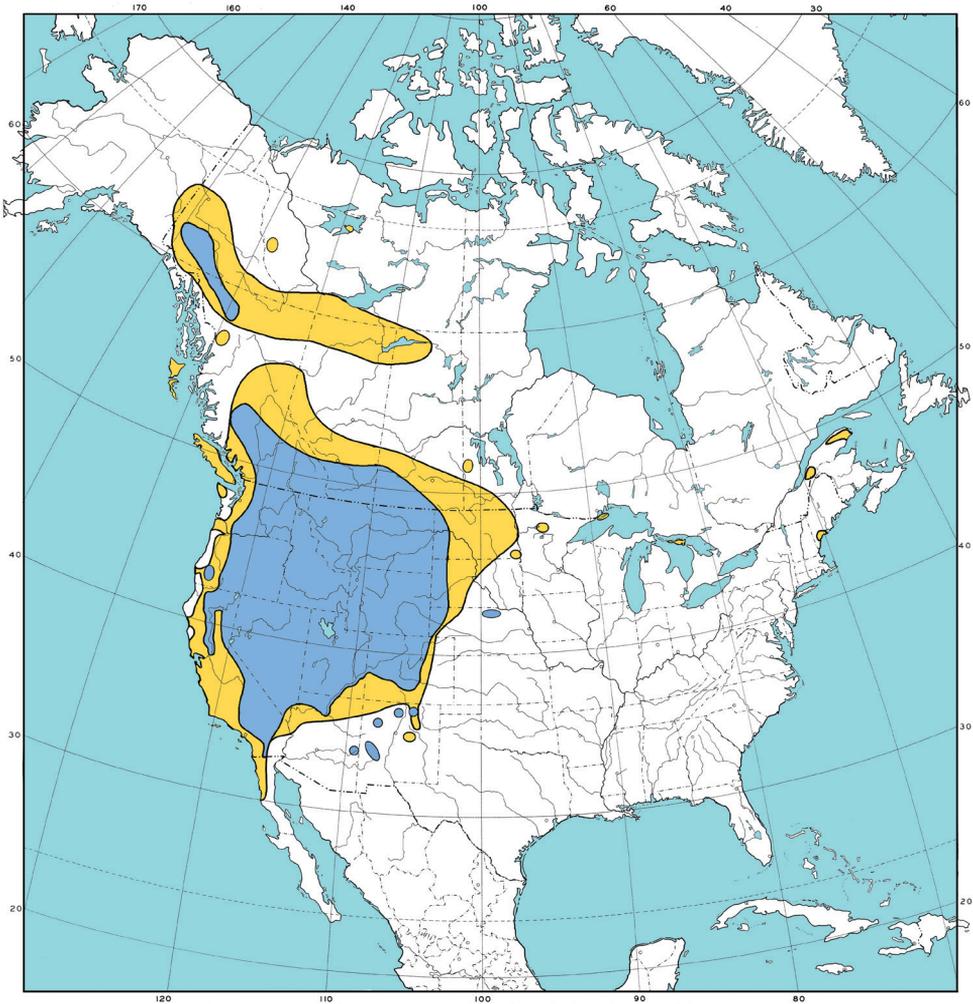


Figure 2. Distribution of *Poa secunda* subspecies in North America: subsp. *secunda* (yellow); subsp. *juncifolia* (blue).

States" (Hitchcock 1951) and on-line: <http://tropicos.org>. See also the "Catalogue of New World Grasses" (Soreng et al. 2003a, and on-line Soreng et al. 2003b: <http://tropicos.org/NameSearch.aspx?projectid=10>) for synonymy for the two subspecies accepted.

A compilation of chromosome numbers for *Poa secunda* s.l. and other species in sect. *Secundae* (*P. hartzii*, *P. napensis*, *P. stenantha*, *P. tenerrima* and *P. unilateralis*) is presented in Suppl. material 1: Table S1. This shows that *Poa secunda* s.l. and all other taxa in sect. *Secundae* (for which chromosome numbers are known) are polyploid with a hexaploid base chromosome number of $2n = 42$. There are many higher numbers, particularly in *P. secunda* ($2n = 42$ to 105 or 106; some 140 counts). Subsp. *secunda* has a mode at $2n = 84$, while subsp. *juncifolia* has a mode at $2n = 63$ ($2n = 42$ to ca. 100)

(Soreng 1991b). Maintenance of a dysploid - nanaploid mode of ca. $2n = 63$ in subsp. *juncifolia* suggests this subspecies is highly apomictic.

Suppl. material 2 provides Keck's annotations for each of the nominal taxa he accepted that are here included in *P. secunda*. David Daniels Keck (1903–1995) had a deep interest in *Poa secunda* s.l. and other western North American species of *Poa* and, in 1986, he kindly gave RJS his manuscript on those, as well as his extensive specimen annotation lists for *Poa* of North America. He worked at the Carnegie Institute of Washington, at Stanford University, with J. Clausen and W. Hiesey for more than two decades (up to 1950), on the nature of species. He ceased his work on the lists by 1958 when he retired from the New York Botanical Garden. His lists focused on western Continental United States species, but included some mainly non-arctic Alaskan and Canadian and Mexican (Baja California) records, along with representative records of eastern United States species. Keck's annotations represent hundreds of historical collections widely distributed in herbaria as vouchers for *P. secunda* infraspecies. His annotations are considered to be sound by RJS, although here we recognise the taxa at the rank of variety. Copies of the full lists are stored in the reprint files in the Grass Lab in the Department of Botany, Smithsonian Institution. The lists are reproduced here in a semi-searchable form.

Phylogenetic relationships of *Poa secunda* s.l. and sect. *Secundae*

Methods

Phylogenetic analyses were performed on 78 samples (73 *Poa* and five outgroups) (Suppl. material 3: Table S2.) using sequences of three plastid markers (matK, rpoB-trnC and trnT-trnL-trnF) and two nuclear ribosomal DNA (nrDNA) markers (ITS and ETS). Methods follow Soreng et al. (2017) and clade designations follow Soreng et al. (2010, 2017) (in the text, bold capital letters represent plastid types, bold small capital letters represent nrDNA types). Bayesian and maximum parsimony (MP) analyses were performed on the separate nuclear and plastid datasets. Branches having MP bootstrap support (BS) > 85% and Bayesian posterior probability (pp) support > 95% were considered strongly supported. See the following publications for genotype notation in *Poa*: Soreng et al. (2010, 2017), Nosov et al. (2015), Cabi et al. (2016, 2017).

Results

Separate plastid and nuclear Bayesian trees are presented in Fig. 3 (summary statistics are given in Suppl. material 4: Table S3). In the plastid analysis *Secundae* members form a strongly supported subclade (pp = 1, BS = 97%) within the **N** clade (pp = 1, BS = 61%), whereas in the nuclear analysis members are intermixed with species of *P.* sects. *Abbraviatae* Nannf. ex Tzvelev, *Tichopoa* Asch. & Graebn. and *Stenopoa* Dumort.

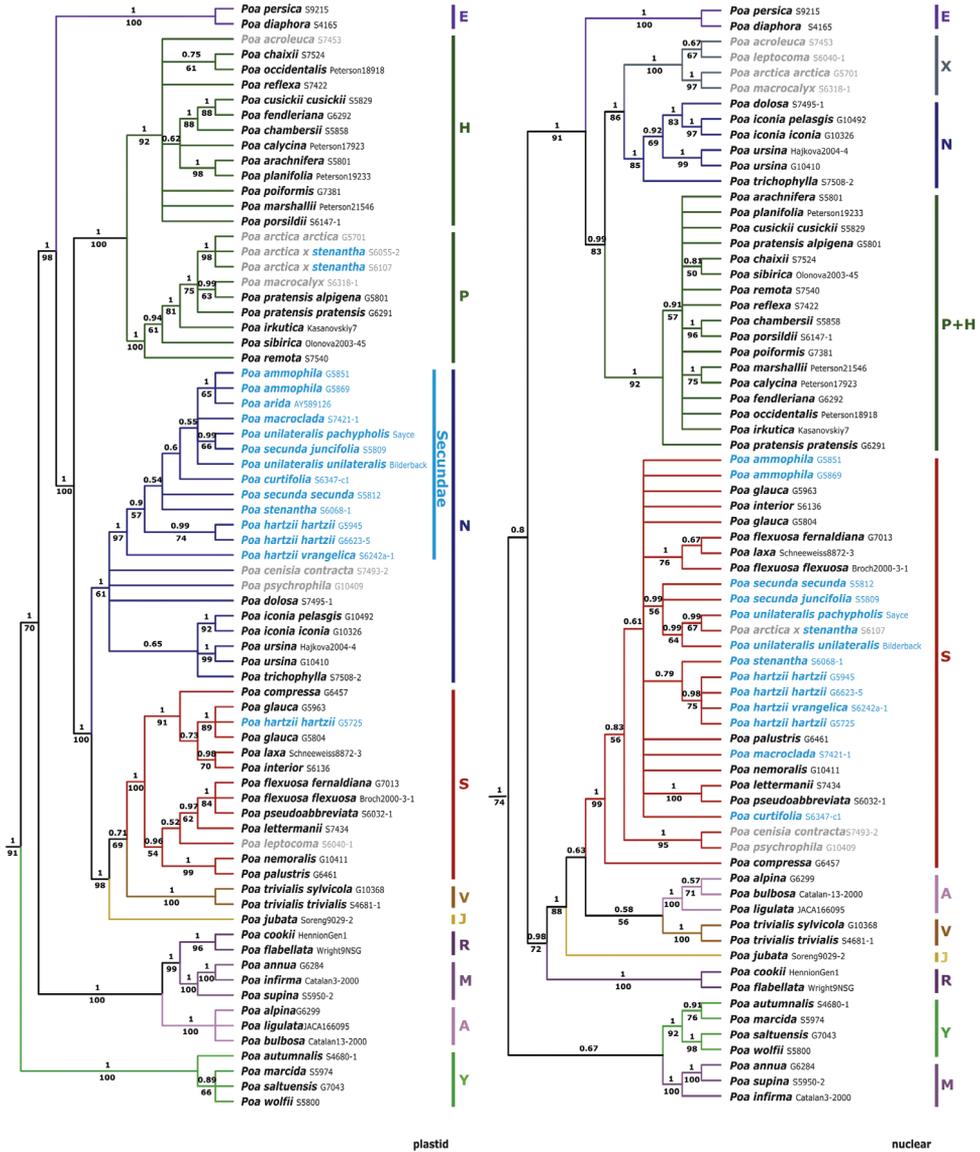


Figure 3. Bayesian 50% majority rule consensus trees of *Poa* based on plastid (trnT-trnL-trnF, rpoB-trnC, MatK) data (left) and nrDNA ITS and ETS data (right). Bayesian posterior probabilities are shown above branches, maximum parsimony bootstrap values below branches. Outgroups are not shown. Major clades are indicated by colour and capital letters. Taxa shown in blue belong to *P.* subg. *Secundae*; those in grey are other taxa of putative hybrid origin that belong to different major clades in plastid and nrDNA trees.

in the strongly supported s clade (pp = 1, BS = 99%). The postulated hybrids *P. arctica* R. Br. × *P. stenantha* (Soreng 6055-2, 6107) resolved with *P. arctica* (pp = 1, BS = 98%) in the P clade (pp = 1, BS = 100%) in the plastid tree and with *P. unilateralis* in the s clade in the nuclear tree (pp = 0.99, BS = 64%) (Soreng 6055-2 not included since sequence unreadable due to multiple sequence copies).

Relationships amongst taxa within *Secundae* are mostly poorly supported and not congruent between plastid and nuclear trees. *Poa unilateralis* subspecies and *P. arctica* × *stenantha* form a clade and these form a clade with both *P. secunda* subspecies in the nuclear tree. In contrast in the plastid tree, *P. unilateralis* subsp. *pachypholis* (Piper) D.D. Keck and *P. secunda* subsp. *juncifolia* are sister taxa, whereas conspecific subspecies are not together. *Poa hartzii* formed a moderately supported subclade (pp = 0.98, BS = 0.75) within the s clade in the nuclear tree, but one of four samples (Gillespie 5725) resolved outside the N clade, amongst *P. glauca* Vahl samples in the S clade, in the plastid tree. *Poa macroclada* Rydb., currently considered a synonym of *P. stenantha* (Soreng et al. 2003b, 2007), resolved in *Secundae* but did not resolve with the latter species in either tree.

Discussion

The whole section *Secundae* is shown to be of reticulate origin (Ns plastid / nrDNA genotype combination) consistent with our previous results based on fewer samples (Gillespie et al. 2008, Cabi et al. 2017, Soreng et al. 2017). All sampled taxa of the section have the N plastid genotype marker that is otherwise known only from Europe and SW Asia (sect. *Nanopoa* J. R. Edm. and unclassified species) and the s nrDNA genotype of subg. *Stenopoa* (Dumort.) Soreng & L.J. Gillespie. While it remains a mystery how the N plastid type came to North America, it is evident that the s nrDNA type came from *P.* subg. *Stenopoa* (Ss genotype combination), either from sect. *Stenopoa* or, as we predict, from the primarily western North American sect. *Abbreviatae* (where *P. hartzii* was historically placed; see notes in Soreng 1991b) or possibly from the morphologically related Russian *P.* sect. *Kolymenses* Prob. (still lacking DNA data). Since N genotypes are otherwise absent from South America and S genotypes are otherwise rare there, we believe *Secundae* (*P. secunda* subsp. *secunda*, subsp. *juncifolia* and *P. stenantha*) arrived there secondarily by long distance dispersal from North America.

This ancient hybrid origin, together with its unusual morphology, make *P.* sect. *Secundae* rather difficult to place within *Poa*. To better accommodate *P.* sect. *Secundae* within the infrageneric classification of *Poa*, we here raise the section to the rank of subgenus (see Taxonomy section below).

Curiously, *Poa cenisia* All. and *P. psychophila* Boiss. & Heldr. (*P.* sect. *Cenisiae* Asch. & Graebn.) also have an Ns genotype combination. This species group is confined to alpine habitats in central and southern Europe and Anatolian Turkey. The taxa are rhizomatous, with extravaginal branching, have a strongly keeled lemma and a dorsal tuft of cobwebby hairs (web) on the callus lemma. Morphological characteristics lead us to hypothesise that this group of species is derived from a cross between NN taxa of Europe and Ss taxa from *P.* subg. *Stenopoa* sect. *Stenopoa*, whereas we predict *Secundae* originated from a cross with *P.* subg. *Stenopoa* sect. *Abbreviatae* Ss taxa. The N and s genotypes are slightly different between *Secundae* and *Cenisiae*, but if they

prove to have a common origin, sect. *Cenisiae* may be better placed within subgenus *Secundae* than with species they are usually associated with in *P.* sect. *Malacanthae* (Roshev.) Olonova (type: *P. malacantha* Kom.), which exhibit **Px** genotypes (e.g. *P. arctica*, *P. macrocalyx* Trautv. & C.A. Mey., *P. smirnowii* Roshev.).

Poa hartzii, an Arctic species distributed from Wrangel Island across the North American Arctic to Svalbard, represents an example in *Secundae* of more recent secondary reticulation. Subspecies *hartzii* was shown previously (and again here) to include individuals with two different plastid types (**N** and **S**), interpreted as a case of recent and localised chloroplast introgression from *P. glauca* (Gillespie et al. 1997, 2007, Gillespie and Boles 2001). The sample with **S** plastid type, clearly has a *P. hartzii* nrDNA type.

Poa arida Vasey, a rhizomatous species of the Great Plains and eastern slopes of the Rocky Mts., was suggested by Soreng (2007) to have arisen from hybridisation of *P. secunda* and some element of *P.* sect. *Poa*. The species appears to have the typical *Secundae* **Ns** genotype; it is shown here to have an **N** plastid genotype, whereas its nrDNA type is likely to be the **s** type as cloned nuclear DNA genes of *P. arida* track with *P. secunda* (Patterson et al. 2005). So, the postulated origin of rhizomes in *P. arida* deriving from *Poa* sect. *Poa* may be wrong, but deeper genetic analyses would be more convincing. Two collections, Soreng & Soreng 6055 and 6107, from Alaska, are viviparous plants identified by RJS as *P. arctica* × *P. stenantha*. Both form a clade with *P. arctica* (sect. *Malacanthae*) in the plastid tree. In the nuclear tree, while the single sample included (6107, from the Alaska Range) resolves amongst *Secundae* taxa, it forms a clade with *P. unilateralis* and these with *P. secunda*, rather than *P. stenantha*. Based on geography, the paternal parent could not be *P. unilateralis* (coastal Washington to California) and is unlikely to be *P. secunda* (occurs in SE Alaska, but not in the Alaska Range); the only co-occurring *Secundae* is *P. stenantha*. Further research is needed, including deeper sampling in *P. stenantha* to determine the precise origin of this common viviparous Alaskan form.

Given that *P.* subg. *Secundae* is an apomictic, polyploid (often with high and odd sets of seven and further dysploid chromosome numbers), hybridising complex of reticulate origin, it is not surprising that the detected phylogenetic structure within the group is mostly not well resolved nor supported. Neither the plastid nor nrDNA trees support the current division into two subsections, *Secundae* and *Halophytæ*. Several clades that are weakly to moderately supported may be informative of possible changes to the taxonomy. One example is the status of *P. macroclada*. This taxon was considered as a southern variant of *P. stenantha*, subsequently tentatively treated as a separate species in *P.* sect. *Stenopoa* (Soreng et al. 2003a) and then most recently as a synonym of *P. stenantha* (Soreng et al. 2003b, 2007). Our data suggest that it may be a distinct species in subg. *Secundae*. Such taxonomic changes may be considered in the future, but we feel making changes within *Secundae*, based on the current phylogenetic trees, to be premature, especially given the small sample set within *P. secunda* s.l. and the nature of the beast.

Taxonomy

***Poa* subg. *Secundae* (V.L. Marsh ex Soreng) Soreng & L.J. Gillespie, comb. et stat nov.**
 urn:lsid:ipni.org:names:77191590-1

Basionym. *Poa* sect. *Secundae* V.L. Marsh ex Soreng, Syst. Bot. 16(3): 511, 523. 1991a.

Type species. *Poa secunda* J. Presl.

Species included. *Poa ammophila*, *P. curtifolia*, *P. hartzii*, *P. napensis*, *P. secunda*, *P. stenantha* (including *P. macroclada*?), *P. tenerrima*, *P. unilateralis*.

Notes. The section *Secundae* was originally suggested to belong to *P.* subg. *Poa* (Soreng 1991a), but, in subdividing the genus into more subgenera based on our plastid phylogeny (Gillespie et al. 2007), the section was moved to subg. *Stenopoa* (Dumort.) Soreng & L.J. Gillespie. We subsequently discovered that the set of plastid *Secundae* genotypes resolved in a clade with three European diploid species with NN genotypes, but the nrDNA genotypes of *Secundae* were like those of subg. *Stenopoa* (Ss) (Cabi et al. 2017, Soreng et al. 2017 and results presented above). The apparently ancient reticulate origin of the group, along with its odd morphology within *Poa*, now leads us to raise the section to the rank of subgenus.

***Poa secunda* J. Presl, Reliq. Haenk. 1(4–5): 271, 1830**

Range. CANADA: Alberta, British Columbia, Manitoba (sw), Northwest Territories (sw), Ontario (Manitoulin Island), Quebec (Gaspé Peninsula), Saskatchewan, Yukon (s). UNITED STATES: Alaska (se interior border), Arizona (n), California, Colorado, Idaho, Montana, North Dakota, Nevada, New Mexico (n), Oregon, South Dakota (w), Utah, Washington, Wyoming, with outlying populations in Nebraska (w), Oklahoma (panhandle), Michigan (Isle Royal) and sporadic in Illinois and Maine, Massachusetts.

The geographic range of *P. secunda* in North America north of Mexico is mapped in Fig. 2. Subspecies *secunda* occurs throughout the species range and also reaches into Baja California Norte, Mexico (Fig. 2A). The range of subspecies *juncifolia* is almost completely within that of subsp. *secunda*, except for several scattered localities in Arizona, New Mexico and Nebraska; it is absent from Mexico and mostly absent from the Pacific Coast and coastal mountains (Fig. 2B). The ranges of varieties are broadly overlapping within their subspecies. Both subspecies have disjunct populations in the Patagonian Andes of Argentina and Chile.

***Poa secunda* subsp. *juncifolia* (Scribn.) Soreng, Phytologia 71(5): 401. 1991b [1992]**

Basionym. *Poa juncifolia* Scribn., Bull. Div. Agrostol., U.S.D.A. 11: 52, pl. 8, 1898.

***Poa secunda* subsp. *juncifolia* var. *ampla* (Merr.) Soreng, comb. et stat nov.**

urn:lsid:ipni.org:names:77191591-1

Figs 1B, C, 3A

Basionym. *Poa ampla* Merr., *Rhodora* 4(43): 145, 1902.

Synonyms. *Poa ampla* Merr., *P. confusa* Rydb., *P. juncifolia* var. *ampla* (Merr.) Dorn, *P. laeviculmis* T.A. Williams, *P. truncata* Rydb.

Habitat and range. Open upland forests, mountain steppe, generally in light, well-drained soils to somewhat heavy soils. Range of the subspecies (Fig. 2B), but mostly absent from west side of the Sierra Nevada and westwards. Minor outlying occurrences in Arizona and New Mexico of subsp. *juncifolia* (Fig. 2B) mostly represent var. *ampla* (Soreng 1985), likely introduced by seeding.

Chromosome numbers. Numbers reported as *P. ampla*: $2n = 61, 62(x3), 63(x11), \approx 63(x3), 63-64(x2), 64(x6), \approx 65, 70-71, \approx 97, \approx 100$.

***Poa secunda* subsp. *juncifolia* var. *juncifolia* (Scribn.) Soreng, comb. et stat nov.**

urn:lsid:ipni.org:names:77191594-1

Fig. 4B.

Basionym. *Poa juncifolia* Scribn., *Bull. Div. Agrostol., U.S.D.A.* 11: 52, pl. 8, 1898.

Synonyms. *Poa brachyglossa* Piper, *P. fendleriana* var. *juncifolia* (Scribn.) M.E. Jones, *P. juncifolia* Scribn., *P. juncifolia* subsp. *juncifolia*, *P. juncifolia* subsp. *porteri* D.D. Keck (?), *P. nevadensis* var. *juncifolia* (Scribn.) Beetle.

Notes. The type and other material identified as *Poa juncifolia* subsp. *porteri* by Keck combine pubescent lemmas with narrow panicles, firm blades and short ligules on lateral shoots and appear to RJS to be intermediate in form between varieties *juncifolia* and *secunda* (*canbyi* form).

Habitat and range. Pine forests and steppe, riparian and alkali meadows, in well-drained to poorly-drained, light to heavy, often alkaline or saline soils. Range of the subspecies (Fig. 2B), but infrequent in the California Floristic Province and south-western states.

Chromosome numbers. Numbers reported as *P. juncifolia*: $2n = 42, 60, 62, 63, 63-64, 78, 84$. The one $2n = 42$ count was originally published by Hartung (1946) as *P. cusickii*, but RJS re-determined the California voucher as *P. secunda* subsp. *juncifolia* var. *juncifolia*.

***Poa secunda* subsp. *juncifolia* var. *nevadensis* (Vasey ex Scribn.) Soreng, comb. et stat nov.**

urn:lsid:ipni.org:names:77191595-1

Fig. 4C

Basionym. *Poa nevadensis* Vasey ex Scribn., *Bull. Torrey Bot. Club* 10: 66, 1883.

Synonyms. *Atropis nevadensis* (Vasey ex Scribn.) Beal, *Atropis pauciflora* Thurb., *Panicularia thurberiana* Kuntze, *Poa nevadensis* Vasey ex Scribn., *Poa pauciflora* (Thurb.) Benth. ex Vasey, *Poa tenuifolia* var. *scabra* Vasey ex Scribn. (nom. inval.), *Poa thurberiana* (Kuntze) Vasey, *Puccinellia nevadensis* (Vasey ex Scribn.) Ponert.

Habitat and range. Open forests and steppe, in light, well-drained to heavier soils. Range of the subspecies (Fig. 2B), but mostly absent from west side of the Sierra Nevada and westward. Collected once in New Mexico (Soreng 1985).

Chromosome numbers. Numbers reported as *P. nevadensis*: $2n = 62$ (x2), 62–63, 63 (x7), 64, 64–66 (x2), ≈ 65 , 70.

Poa secunda subsp. *secunda*

Poa secunda subsp. *secunda* var. *gracillima* (Vasey) Soreng, comb. et stat nov.

urn:lsid:ipni.org:names:77191596-1

Fig. 4D

Basionym. *Poa gracillima* Vasey, Contr. U.S. Natl. Herb. 1(8): 272, 1893.

Synonyms. *Poa gracillima* var. *gracillima*, *P. gracillima* Vasey, *P. invaginata* Scribn. & T.A. Williams.

Habitat and range. Open forests, moist cliffs and rocks and subalpine to alpine meadows, in well-drained acid soils that are consistently moist through the growing season of the variety. Pacific Northwest and northern California.

Chromosome numbers. Numbers reported as *P. gracillima*: $2n = 81$, ≈ 81 , ≈ 84 , 86, ≈ 91 .

Poa secunda subsp. *secunda* var. *scabrella* (Thurb.) Soreng, comb. et stat nov.

urn:lsid:ipni.org:names:77191598-1

Fig. 4E

Basionym. *Atropis scabrella* Thurb., Bot. California 2: 310–311, 1880. *Poa scabrella* (Thurb.) Benth. ex Vasey, Grass. U.S. 42, 1883.

Synonyms. *Atropis californica* Munro ex Thurb., *Atropis scabrella* Thurb., *Panicularia scabrella* (Thurb.) Kuntze, *Poa acutiglumis* Scribn., *Poa californica* (Munro ex Thurb.) Scribn., *Poa capillaris* Scribn., *Poa nudata* Scribn., *Poa orcuttiana* Vasey, *Poa scabrella* (Thurb.) Benth ex Vasey, *Puccinellia scabrella* (Thurb.) Ponert, *Sclerochloa californica* Munro ex Benth.

Habitat and range. Open pine forests, coastal scrub and coastal and Central Valley grasslands, in well-drained or heavier soils. Mainly in the California Floristic Province, but extending northwards in the Pacific North West and southeast into the Mojave Desert, where it is largely replaced by var. *secunda*.

Chromosome numbers. Numbers reported as *P. scabrella*: $2n = 44+f$, 61–63, ≈ 62 , 63 (x4), 64, ≈ 66 , ≈ 68 , 81 (x2), 82 (x3), 84 (x10, ≈ 84 , 84+f, 86, ≈ 86 (x2), ≈ 88 , ≈ 91 , 104.

Poa secunda* subsp. *secunda* var. *secunda

Figs 1A, C, 4F, G

Synonyms. *Aira brevifolia* Pursh, *Aira missurica* Spreng. ex B.D. Jacks., *Airopsis brevifolia* (Pursh) Roem. & Schult., *Atropis canbyi* (Scribn.) Beal, *Atropis laevis* Beal, *Atropis laevis* var. *rigida* Beal, *Atropis tenuifolia* Thurb., *Atropis tenuifolia* var. *stenophylla* Vasey ex Beal (*incurva* form), *Festuca oregona* Vasey, *Festuca patagonica* Phil., *Festuca spaniantha* Phil., *Glyceria canbyi* Scribn., *Panicularia nuttalliana* Kuntze, *Poa andina* Nutt. ex S. Watson, *Poa buckleyana* Nash, *Poa buckleyana* var. *buckleyana*, *Poa buckleyana* var. *elongata* (Vasey) M.E. Jones, *Poa buckleyana* var. *sandbergii* (Vasey) M.E. Jones, *Poa buckleyana* var. *stenophylla* (Vasey ex Beal) M.E. Jones, *Poa canbyi* (Scribn.) Piper, *Poa fulvescens* Trin., *Poa gracillima* var. *saxatilis* (Scribn. & T.A. Williams) Hack., *Poa helleri* Rydb., *Poa incurva* Scribn. & T. A. Williams, *Poa laevigata* Scribn., *Poa laevis* Vasey, *Poa leckenbyi* Scribn., *Poa lucida* Vasey, *Poa nevadensis* var. *laevigata* (Scribn.) M.E. Jones, *Poa nevadensis* var. *leckenbyi* (Scribn.) M.E. Jones, *Poa sandbergii* Vasey, *Poa saxatilis* Scribn. & T.A. Williams (toward *gracillima* form), *Poa secunda* var. *elongata* (Vasey) Dorn (= *canbyi* form), *Poa secunda* var. *incurva* (Scribn. & T.A. Williams) Beetle, *Poa secunda* var. *stenophylla* (Vasey ex Beal) Beetle, *Poa stenantha* var. *sandbergii* (Vasey) B. Boivin, *Poa tenuifolia* Buckley, *Poa tenuifolia* Nutt. ex S. Watson, *Poa tenuifolia* var. *elongata* Vasey, *Poa tenuifolia* var. *oregona* (Vasey) Vasey, *Poa wyomingensis* Scribn., *Puccinellia canbyi* (Scribn.) Ponert, *Puccinellia laevis* (Beal) Ponert

Notes. There are various variety names included in the autonymic var. *secunda*. Variety *sandbergii* is *secunda* s.s.; vars. *elongata*, *leckenbyi*, *laevigata* and *rigida* represent the *canbyi* form; and vars. *incurva*, *saxatilis*, *buckleyana* and *stenophylla* represent the *incurva* form. Varieties *incurva* and *saxatilis* are generally subalpine to alpine with slightly open panicles; Hitchcock (1951) included them within *P. gracillima*, but Keck (1959 and annotations) treated them as *P. incurva*. Vars. *buckleyana* and *stenophylla* are intermediate to var. *scabrella*, but are too smooth to be included in the latter variety. Hitchcock (1951) equated *stenophylla* with var. *gracillima*, but we agree with Keck (Suppl. material 2) in equating it with the *incurva* form. *Poa tenuifolia* Nutt. ex S. Watson (1871), nom. illeg., was described from heterogeneous original material (at US, distributed as *S. Watson 1318*): E. Humboldt Mts. = var. *juncifolia*; Virginia Mts. = var. *secunda*; Diamond Mts. = var. *scabrella*. However, Watson also cited “*Poa tenuifolia* Nutt., ms. In Herb.” The Nuttall specimen with that name is at PH (PH00020091) and *P. tenuifolia* Buckley (1862) is based on that specimen. Therefore, Watson’s taxon name is superfluous.

Habitat and range. Open forests, steppe, and alpine, generally in light, well-drained soils. Range of the subspecies (Fig. 2B), but mostly replaced by var. *scabrella* on west side of the Sierra Nevada and westwards and in the Mojave Desert.

Chromosome numbers. Numbers reported as *P. secunda* and avowed synonyms: $2n = 42, 56, \approx 68, 70 (x2), \approx 70 (x2), \approx 74, \approx 78 (x2), 81, 81, 82 (x2), 84 (x2), 84-88+II, \approx 84, 85-87, 86 (x2), \approx 87, \approx 99, 104$. The one count of $2n = 42$ was reported by Bowden (1961) originated from Alberta. Numbers originally reported as *P. canbyi*: $2n = 56, 70 (x4), \approx 72, \approx 82 (x2), \approx 83, 84 (x4), \approx 84 (x2), 85, \approx 86$. Keck (in Munz 1959) attributed higher numbers to *P. incurva*. Numbers secondarily reported as *P. incurva*: $2n = 90, 93, 94, 99, 105-106$.

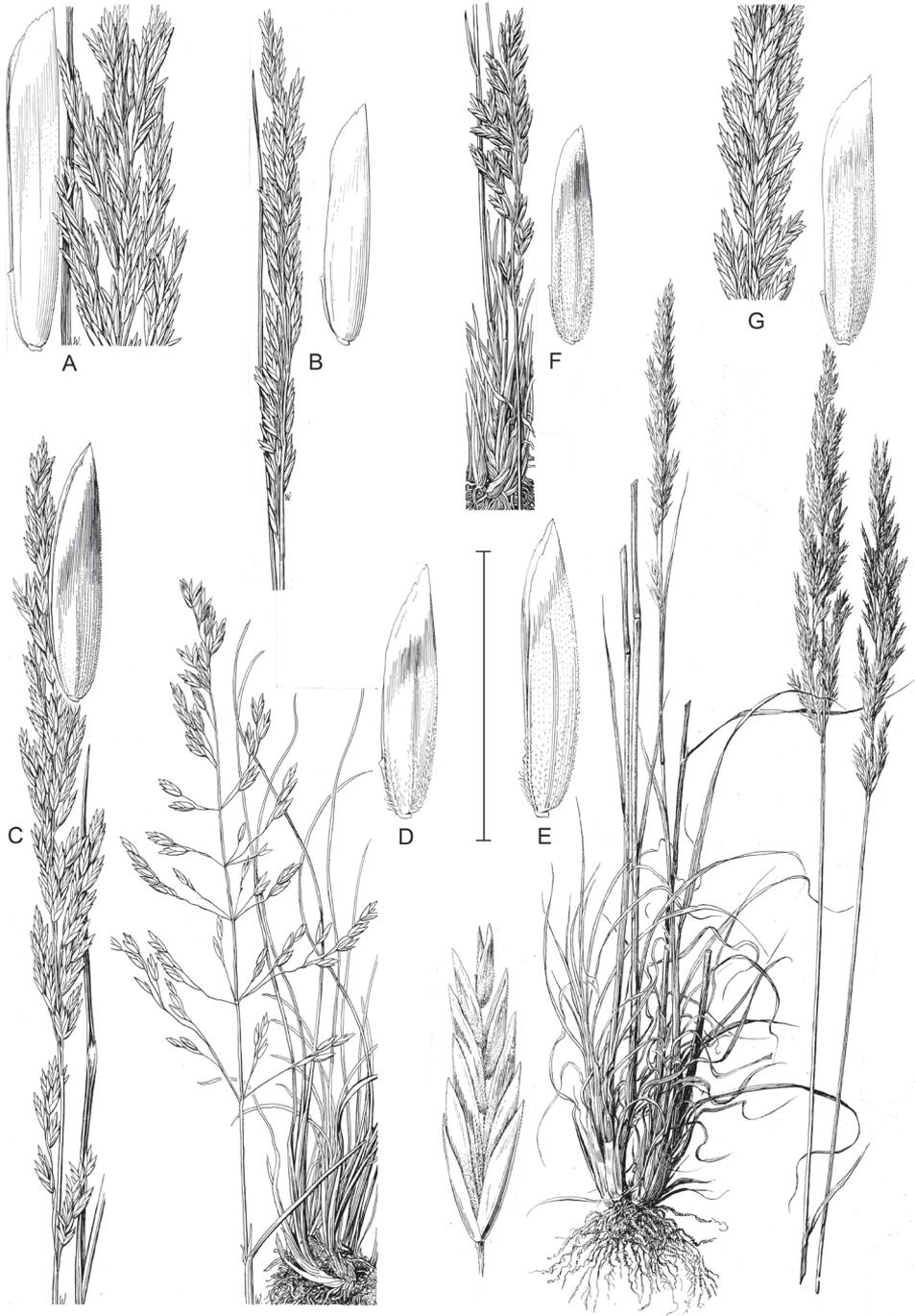


Figure 4. *Poa secunda* infraspecies illustrations (reproduced from Hitchcock 1935): **A** *P. s.* var. *ampla* panicle and floret **B** *P. s.* var. *juncifolia* panicle and floret **C** *P. s.* var. *nevadensis* panicle and floret **D** *P. s.* var. *gracillima* habit, panicle and floret **E** *P. s.* var. *scabrella* habit, panicles, spikelet and floret **F, G** *P. s.* var. *secunda* habit, panicle and floret variations **A, B, C** = subsp. *juncifolia* **D, E, F, G** = subsp. *secunda*. Scale bar: 5 mm for florets, 10 mm for spikelet, 5 cm for habits and panicles (10 cm for **E** habit and panicles).

Key to *Poa secunda*, *P. curtifolia* and *P. tenerrima* (adapted from Hitchcock 1951) (non-Arctic taxa of *P.* subg. *Secundae* with rounded lemma keels)

- 1 Lemmas more or less crisp-puberulent on the lower half or basal portion (sometimes obscurely so in *P. secunda* var. *scabrella*); ligules of lower culm usually well developed and acute to acuminate (short in *P. tenerrima*); tillers strictly intravaginal, cataphylls absent, prophylls well developed, mostly over 1 cm long; leaf blades commonly withering early, long-cells all or mostly fusiform and smooth-walled . 2
- Lemmas glabrous, smooth or scabrous (except in “*P. juncifolia* subsp. *porteri*” form, but then plants from the plains of the eastern slope of the Rocky Mts.); ligules of lower culm and lateral shoot leaves truncate to rounded (acute in var. *nevadensis*); tillers intravaginal and sometimes extravaginal, the latter with cataphylls and reduced prophylls (mostly less than 2 mm long); leaf blades more or less persisting in form, long-cells mostly rectangular and sinuous-walled 6
- 2 Leaf blades short (mostly 1–3 cm long), (1–) 1.5–3 mm wide, flat, with prominent white, cartilaginous margins; plants of serpentine rocks in the Wenatchee Range of the Cascade Mts., Washington State..... ***P. curtifolia***
- Leaf blades of various lengths and widths, but not short and flat, without prominent cartilaginous margins 3
- 3 Sheaths scabrous, at least on the margins; ligules scabrous; panicle branches scabrous, often densely so; plants mainly of California Floristic Province and Mojave Desert....4
- Sheaths smooth; ligules smooth or lightly scabrous; panicle branches smooth or scabrous; plants mostly of the eastern slope of the western Cordilleras and eastward... 5
- 4 Ligules of culm leaves well developed (2–6 mm long), acute to acuminate; blades filiform or broader; panicles branches capillary or thicker, appressed to ascending (rarely spreading); plants widespread; chromosomes $2n = 63$ and higher ***P. secunda* subsp. *secunda* var. *scabrella***
- Ligules of culm leaves short [0.5–1.5 (–2.5) mm long], truncate to obtuse (acute); blades filiform; panicle branches capillary, widely spreading; plants of serpentine barrens in central foothills of west slope of the Sierra Nevada; chromosomes $2n = 42$ ***P. tenerrima***
- 5 Panicles open, the branches spreading to patent, divergent more than 45° at anthesis and remaining open with spikelets absent in the lower half; plants of moist often shady places ***P. secunda* subsp. *secunda* var. *gracillima***
- Panicles usually loosely to tightly contracted at maturity, branches sometimes ascending but branches finally divergent by less than 45°, spikelets from near the base or lower 1/3rd; plants mostly of more open places.....***P. secunda* subsp. *secunda* var. *secunda***
- 6 Sheaths scabridulous; ligules elongated, acute, decurrent..... ***P. secunda* subsp. *juncifolia* var. *nevadensis***
- Sheaths smooth; ligules of lower culm and basal leaf short, obtuse to truncate, not decurrent.....7
- 7 Blades involute; plants of open riparian and alkali or saline meadows..... ***P. secunda* subsp. *juncifolia* var. *juncifolia***
- Blades flat; plants of mountain meadows and forests ***P. secunda* subsp. *juncifolia* var. *ampla***

Species hybrids involving *Poa secunda*

Poa secunda is known or postulated to hybridise widely (see Hiesey & Nobs, 1982). Many artificial crosses were produced by Carnegie Institute of Washington researchers between *P. secunda* races and between *P. secunda* and other species including *P. pratensis*. One of their expectations was that apomixis would stabilise new strains of super-grasses that could be used to re-vegetate overgrazed rangelands, but most of the inter-specific hybrids they produced, some being quite vigorous, were sterile or produced few seeds. Soreng (2007) discussed two named nothospecies, *P. × multinomae* (p. 545) and *P. × limosa* (p. 600–601), and *P. arida* Vasey (p. 599–601), which he considered a full species of reticulate origin. Two collections represented in our trees (Fig. 3), Soreng & Soreng 6055 and 6107, from Alaska, are viviparous plants that we hypothesise involved hybridisation between *P. arctica* (6107 and 6055) of sect. *Malacanthae* and *P. stenantha*.

Poa secunda × *nervosa* (Hook.) Vasey = *Poa* × *multinomae* Piper

Notes. This putative nothospecies is restricted to the Columbia River Gorge, between the Sandy River and Hood River, where the putative parents are parapatric. Unlike *P. secunda* subsp. *secunda*, the plants exhibit extravaginal branching, truncate ligules and open panicles with scabrous branches. Synonyms are: *Sporobolus bolanderi* Vasey, *Poa gracillima* var. *multinomae* (Piper) C.L. Hitchc. and *Poa alcea* Piper.

Poa secunda subsp. *juncifolia* × *pratensis* = *Poa* × *limosa* Scribn. & T.A. Williams

Notes. The types of *P. limosa* and its synonym *P. fibrata* Swallen, are putatively naturally occurring examples (according to Soreng 1993, 2007) of artificially produced hybrids (Hiesey and Nobs 1982) of *P. secunda* × *pratensis*. The plants are short rhizomatous and inhabit sub-alkaline and saline meadows, but other than rhizomes, do not exhibit characters of *P. pratensis*.

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Supplementary material I

Table 1. Chromosome numbers in taxa of *Poa* subg. *Secundae*

Authors: Robert John Soreng, Lynn J. Gillespie

Data type: species data

Explanation note: Chromosome numbers in taxa of *Poa* subg. *Secundae*, with RJS' subspecies determinations and original determinations as published or found on herbarium sheets, literature reference, number of counts, voucher collection, country and state or province abbreviation and herbarium where deposited, if known. CI = Carnegie Institution. These numbers show the hexaploid nature of the species complex and the wide and differing ranges of chromosome numbers in each of the *P. secunda* subspecies.

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Link: <https://doi.org/10.3897/phytokeys.110.27750.suppl1>

Supplementary material 2

David D. Keck's annotations of taxa here included in *Poa secunda*

Authors: Robert John Soreng, Lynn J. Gillespie

Data type: species data

Explanation note: The following taxa recognised by Keck, but included by us in *P. secunda* s.l., are given in the specimen annotation lists: *P. ampla*, *P. canbyi*, *P. gracillima*, *P. incurva*, *P. juncifolia*, *P. nevadensis*, *P. sandbergii* and *P. scabrella*. His lists focused on western Continental United States species but included some mainly non-arctic Alaskan, Canadian and Mexican (Baja California) records and some records of eastern United States species. Copies of the original typed lists are stored in the reprint files in the Grass Lab in the Department of Botany, Smithsonian Institution. Optical character recognition (OCR) was performed on the present selection to allow the lists to be searchable to a large degree. Keck's annotations are considered to be sound and to represent hundreds of historical collections widely distributed in herbaria as vouchers for *P. secunda* infraspecies. We treat *P. ampla*, *P. juncifolia* and *P. nevadensis* as varieties of *P. secunda* subsp. *juncifolia* (vars. *ampla*, *juncifolia* and *nevadensis*, respectively) in our revised classification. The remaining taxa are treated as varieties of *P. secunda* subsp. *secunda*, as follows: var. *gracillima* (*P. gracillima*), var. *scabrella* (*P. scabrella*) and var. *secunda* (*P. canbyi*, *P. incurva*, and *P. sandbergii*).

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Supplementary material 3

Table 2. *Poa* and outgroup samples used in the phylogenetic analyses

Authors: Robert John Soreng, Lynn J. Gillespie

Data type: phylogenetic data

Explanation note: *Poa* and outgroup samples used in the phylogenetic analyses with subgeneric classification (subtribe for outgroups), voucher information and GenBank Accession numbers for each of the five DNA regions (ITS, ETS, trnT-trnL-trnF, matK, rpoB-trnC).

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Link: <https://doi.org/10.3897/phytokeys.110.27750.suppl3>

Supplementary material 4

Table 3. Data partition characteristics, summary statistics and models of the phylogenetic analyses

Authors: Robert John Soreng, Lynn J. Gillespie

Data type: phylogenetic data

Explanation note: For each data partition (ITS, ETS, trnT-trnL-trnF, rpoB-trnC, MatK) and concatenated dataset (plastid and nuclear), the following are given: number of sequences, number of characters, number of parsimony informative (PI) characters, % parsimony informative characters, maximum parsimony tree length (L), number of most parsimonious trees, consistency index excluding uninformative characters (CI) and retention index (RI). Also given are the models used in the Bayesian analyses as determined using the Akaike information criterion (AIC) in jModeltest.

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Vernonia subgenus *Austrovernonia*, a new subgenus from South America (Compositae, Vernonieae, Vernoniinae)

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Abstract

Vernonia incana Less. and *V. echioides* Less. are two semi-aquatic species from southern South America that are referred to as “the semi-aquatic *Vernonia* of South America” and they have been, until now, retained as members of *Vernonia* mostly because each had some unusual characters that made them hard to place. Based on an ongoing molecular study, we can now say that *V. incana* is the sister taxon to all true *Vernonia* and can therefore be responsibly left in *Vernonia* but, because of its morphological and geographic differences, it is now assigned to its own subgenus: *Vernonia* subg. *Austrovernonia*. *Vernonia echioides* is not closely related to *V. incana* and is now part of a separate investigation. This placement of *V. incana* as sister to the true *Vernonia* species brings up the possibility of a South American root for the *Vernonia* clade and perhaps indicates a propensity in the lineage for growing in or near water. The species *V. incana* is lectotypified.

Keywords

Asteraceae, corolla ducts, DNA GenBank, lectotype, North America, semi-aquatic plants, South America, *Vernonia*, *Vernonia* subg. *Austrovernonia*

Introduction

Revisions of the concept of *Vernonia* Schreb. during the last 30 years, as summarised in the treatments of American Vernonieae (Robinson 1999b; also see discussion on global distribution in Keeley and Robinson 2009), have excluded the non-North American species once placed in the genus. The genus *Vernonia* Schreb. currently contains 20 species, 17 are

native to eastern and central North America (Strother 2006), three of which cross the border into Coahuila, Mexico (Table 1). Additionally, there is one species in Eastern Mexico, *V. greggii* A.Gray with 2–3 varieties and two from South America: *V. echioides* Less. and *V. incana* Less. (Table 1). The South American species share several features with other members of typical *Vernonia*: rhizomiform rootstocks, totally cymiform inflorescences and sublophate pollen (shared by all members of the subtribe Vernoniinae). Due to the shared habits and similarity in pollen, there was no reason to remove the two species from *Vernonia*. However, the question of where to place these two somewhat odd South American species continued to puzzle students of the tribe. The situation remained unchanged as attention turned to the greater problems found in the Old World Vernoniaceae (Robinson 1999a, Robinson et al. 2016, Bunwong et al. 2014, Funk and Robinson 2017).

Recently, this relationship was called into question as a result of a study of corolla lobe anatomy in which a brief observation of the two species was included (Robinson and Yankowski 2016). The corolla lobe feature of “multiple longitudinal ducts” was first noted in the description of *Trepadonia* H. Rob. (1994) and was recognised in the Robinson (1999b) treatment, as being characteristic of the typical element of the Vernoniaceae. The feature was found in the corolla lobes of *Vernonia*, *Vernonanthura* H. Rob. and *Trepadonia* H. Rob. and was illustrated schematically in the Robinson



Figure 1. Habitat of *Vernonia incana* and *V. echioides* taken with a drone at the Environmental Protection Area *Rincon de Franquía*, Cuareim River, Artigas, Uruguay: insert shows in red the areas where the semi-aquatic *Vernonias* of South America can be found. (photos by J.M. Bonifacino, MVFA).

Table 1. Species of true *Vernonia*. Names in bold indicate that they have been sequenced. Two species, *V. arkansana* and *V. lettermannii* have recently been collected and will be added to future sequencing efforts.

Species	Locality
<i>Vernonia</i> in USA (Robinson 1999; Strother 2006)	
<i>V. acaulis</i> (Walter) Gleason	Carolinas & Georgia
<i>V. angustifolia</i> Michx.	Southeast US
<i>V. arkansana</i> DC.	Central US
<i>V. baldwinii</i> Torr.	Central US
<i>V. blodgettii</i> Small	Florida & the Bahamas
<i>V. fasciculata</i> Michx.	Central US
<i>V. flaccidifolia</i> Small	Southeast US
<i>V. gigantea</i> (Walter) Trel.	Eastern US
<i>V. glauca</i> (L.) Willd.	Eastern US
<i>V. larseniae</i> B.L.King & S.B.Jones	Texas & Coahuila Mexico
<i>V. lettermannii</i> Engelm. ex A.Gray	Arkansas & Oklahoma
<i>V. lindheimeri</i> A.Gray & Engelm.	Texas & Coahuila Mexico
<i>V. missurica</i> Raf.	Central US
<i>V. marginata</i> (Torr.) Raf.	Southcentral US & Coahuila Mexico
<i>V. noveboracensis</i> (L.) Michx.	Eastern US
<i>V. texana</i> (A.Gray) Small	Southcentral US
<i>V. pulchella</i> Small	Georgia
<i>Vernonia</i> only in Mexico (Turner 2007)	
<i>V. greggii</i> A.Gray	Mexico, Sierra Madre Oriental
<i>V. ervendbergii</i> A.Gray [= <i>V. greggii</i>]	
<i>V. greggii</i> var. <i>schaffneri</i> A.Gray [previously <i>V. schaffneri</i> A.Gray]	
<i>Vernonia</i> in South America	
<i>V. incana</i> Less.	Argentina, southern Brazil, Uruguay
<i>V. echioides</i> Less.	Argentina, southern Brazil, Uruguay

(1999a) treatment and with photographs in the Robinson and Yankowski (2016) treatment. The feature was seen as a potential defining characteristic for the *Vernonia* typical group. However, in the study by Robinson and Yankowski (2016), the corolla lobes were examined in full anatomical detail and in only that study were the corolla lobes of the two South American species examined for the characteristic ducts. *Vernonia echioides* and *V. incana*, as seen at that time, did not show the multiple longitudinal ducts. Thus, on the basis of the remote geographical location, details cited below and the apparent lack of multiple ducts in the species, it was suggested that the species are not closely related to *Vernonia* and needed further study.

Vernonia incana and *V. echioides* inhabit hydromorphic soils always growing along river banks in open patches of *Coleataenia prionitis* (Nees) Soreng and other grasses forming tall grass associations locally known as “pajonales” (Fig. 1; JM Bonifacino, pers. comm.). The soil in which they grow is sufficiently soft that the roots are often extracted with the plants.

This study was designed to determine where in the Vernoniae to place these two semi-aquatic species. It was hoped that results of the DNA sequencing would fully resolve the issue.

Materials and methods

We were fortunate that our colleagues Sterling Keeley, Tim Gallaher and Jason Cantley were just finishing a project that included an updated molecular phylogeny of the entire Vernoniae tribe (Keeley et al., in prep.). We were even more fortunate that they were willing to share their published (Keeley et al. 2007) and unpublished data. They did not sequence either of the semi-aquatic taxa, so, as part of this study, those two taxa were sequenced using the same molecular markers as Keeley et al. (in prep.) (*ITS1*, *ITS2*, *5pt8s*, *trnL*, *ndbF*-in part) and the sequences from these two species were combined with 125 taxa from Keeley et al. (in prep.), mostly from the Americas. Extractions were made from one specimen of *V. incana* housed in the U.S. National Herbarium (US). The *5pt8s* marker was not informative within the true *Vernonia* so its use was not continued.

Sequencing and alignment

Samples were prepared by hand grinding leaf material with liquid N in a mortar and pestle followed by extraction using DNeasy Plant Mini Kit following the manufacturer's protocols (Qiagen, Valencia, California, USA).

Methods followed those of Keeley et al. (2007) so that the data could be combined. The full nuclear ITS region, part of the chloroplast gene *ndbF* and the non-coding spacer region *trnL-F* were used for the analyses. Sequencing was done at the Laboratory of Analytical Biology, NMNH, Smithsonian Institution. The resulting sequences were added to the previously selected taxa from the existing nexus file generated by Keeley et al. (in prep) and aligned by eye. Preliminary phylogenetic trees were produced using RAxML (Randomized Axelerated Maximum Likelihood; Stamatakis 2014).

Two phylogenies were generated using two different versions of the dataset. As all of the taxa in the database had ITS but some of the other markers were missing for some of the taxa, especially a group of Brazilian taxa that were of particular interest, the data were analysed twice. The first analysis included all the taxa and only used the ITS data. The second analysis included just the taxa that had all (or nearly all) of the data. The placement of *Vernonia incana* was the same in both analyses so we are confident that we have the correct sister group relationship for this taxon. However, *Vernonia echioides* was not an immediate relative of *V. incana* and its placement was ambiguous, thus necessitating the addition of more species and it will be dealt with in a later publication.

The GenBank numbers and voucher information for *Vernonia incana* are listed in Table 2. The numbers for the remaining taxa were either published in Keeley et al. 2007 or will be published in Keeley et al. (in prep.).

Table 2. Voucher information and GenBank numbers for *Vernonia incana* Less. Voucher: Paraguay, Central: Estero del Ypoa, Villeta - Puerto Guyrati, 11 km S of Villeta, east of trail to Villa Oliva. Inundated savannah on clay soil with patches of forest, Zardini & Aquino 4305, 16 Dec 1992, housed at US [US Catalog No.: 3299682; Barcode: 01627756]. Available on line at <https://collections.nmnh.si.edu/search/botany/> [search on collecting number].

Gene/gene region	GenBank numbers
ITS1&2	MH933736
<i>trnLF</i>	MH933737
<i>ndhF</i>	MH933738

Results

The results show that while both species remain in the sub-tribe Vernoniinae, they are not in the same clade. *Vernonia incana* is the sister group of the clade consisting of the true *Vernonia*. Although the phylogeny cannot be presented until the Keeley et al. paper is published, it is possible to visualise the placement of *V. incana* by examining the published trees of Keeley et al. (2007). In that publication (Keeley et al. 2007), the phylogenies contained 13 samples representing 11 of the 17 species of true *Vernonia*, the same ones that are used in the current Keeley et al. (in prep) article. The placement of *V. incana* in our phylogeny was in clade number 1 in Keeley et al. (2007) as the sister taxon of the group containing all of the 11 North American species. Figure 2 in this paper is a portion of one of the figures from Keeley et al. (2007) and is used to indicate where *V. incana* is placed.

The suggested placement of *V. incana* is in conflict with the findings of Robinson and Yankowski (2016) which suggested exclusion from the immediate relationship to *Vernonia*. The nearest relatives in the subtribe Vernoniinae, *Vernonia s.s.*, *Vernonanthura* and *Trepadonia*, all have obvious multiple longitudinal ducts in the corolla lobes. A careful re-examination of the corolla lobes of *V. incana* confirmed that ducts were not evident under the microscope in whole mounts of the corollas. If any ducts are present, they are too obscure to be seen in this way. It is possible that the more semi-aquatic habitat of these species has somehow suppressed the production of the ducts. Thus, while being a character of some value, the ducts are not 100% reliable for defining the subtribe Vernoniinae.

Pollen morphology, that is so helpful in many other members of the Vernonieae (Bunwong and Chantaranothai 2008; Robinson and Skvarla 2010, Robinson et al. 2016), is not useful because pollen of all the genera involved here have the structure that, in the Vernonieae, is called sublophate. This sublophate pollen type has a moderately uneven distribution of spines and muri on the grains with a continuous perforated tectum covering all the non-colpate surfaces and is common in the tribe. Even the pollen sizes are alike, being 40–50 µm in diameter (in fluid).

One of the features that the two South American species share is semi-aquatic habit. The habit is clearly stated in the label data of many of the specimens. Labels for

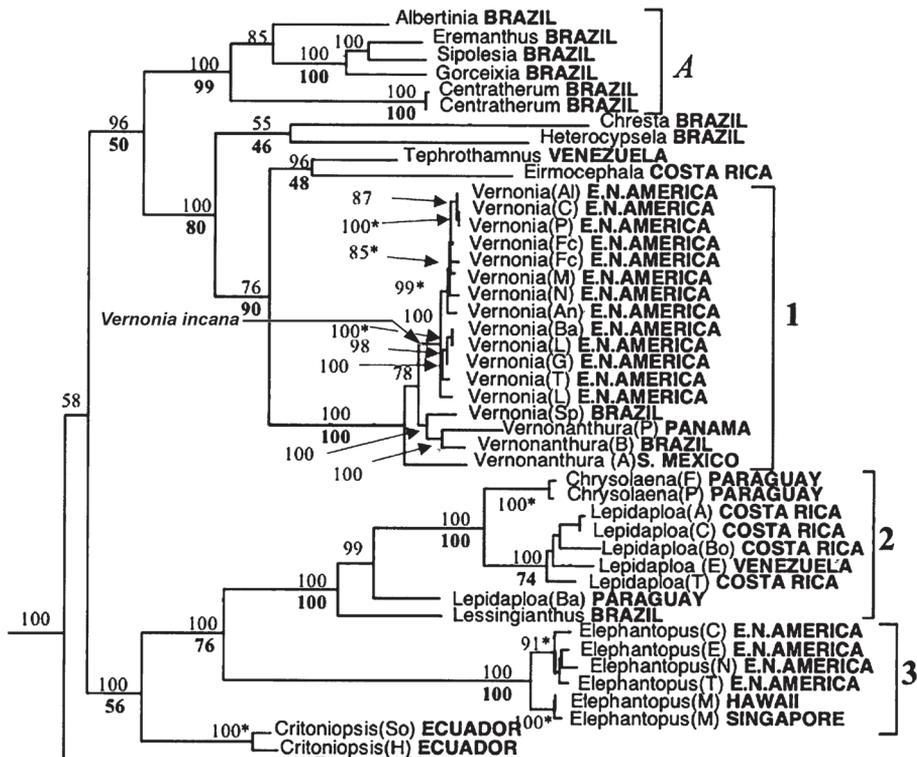


Figure 2. Phylogeny with an indication of where *Vernonia incana* Less. is placed (arrow). The phylogeny is modified from Keeley et al. 2017 and does not take into account all the taxa that were in the analysis performed by the authors using data from Keeley et al. (in prep.).

V. incana cite: gallery forest, inundated savannah, flooded field, laguna margin, marsh or emerging from water. One specimen, [Mary A. Walter 118, Paraguay, Esterito, Dist. Yataity, Dept. Ñeembucú, no. 15, Jan 1975, from the Herbarium Univ. Florida Agricultural Experimental Station] has an additional label in the packet stating: “Coastal for nearly all marshes – common ... leaves emerge above water level, often dense”. The rhizomes of the species have fleshy lateral roots apparently a specialisation for their habitat (Fig. 3) and are easily extracted from the soil in contrast to the North American *Vernonia* which have deep thick roots that are extremely difficult to extract. Indeed, recent fieldwork in the central USA showed that all seven species collected had large underground root systems that defied normal extraction procedures (Funk pers. comm.). The semi-aquatic habit of the South American species is especially interesting because the North American species of the true *Vernonia* are all spatially related to water in that they grow near water (river banks, near boat ramps, in boggy areas or in moist soil) but not in standing water as do many of the South American collections. This propensity



Figure 3. Image of US herbarium sheet of *Vernonia incana* Less. which shows the two types of roots: fleshy and fibrous. [photo courtesy of I. Lin, US].

to grow in or near water may indicate a *prope aquam* origin for the genus which is in contrast with the other members of the subtribe Vernoniinae: *Vernonanthura* is often in open areas requiring fire-survival strategies and *Trepadonia* is a climbing plant.

Based on the results of the DNA analysis, both of these species belong in the subtribe Vernoniinae. Of the two species, the position of *V. incana* is separate from *V. echioides* and is the sister group of *Vernonia s.s.* but geographically remote from the rest of the genus, without obvious corolla lobe ducts and also without a basal node on the style. The basal node has been observed on the style in all examined species of *Vernonia s.s.*, *Vernonanthura* and *Trepadonia* (Robinson 1999b, table 2). These differences between *V. incana* and its more northern relatives indicate that it is not a recent introduction from the north. It is interesting to consider whether or not this placement indicates a possible location for the origin of the *Vernonia s.s.* clade: all of the related clades in the Vernoniinae are rooted in either in Meso- or South America.

The morphological differences and geographical separation support a new subgeneric position for *V. incana*.

The new subgenus is named for its geographic position with regard to typical *Vernonia* of North and Central America.

Taxonomy

Family: Compositae (Asteraceae)

Subfamily: Cichorioideae

Tribe: Vernonieae

Subtribe: Vernoniinae

Genus: *Vernonia*

***Vernonia* subg. *Austrovernonia* H. Rob. & V.A. Funk, subg. nov.**

urn:lsid:ipni.org:names:77191608-1

Subgeneritype. *Vernonia incana* Less.

Diagnosis. Semi-aquatic herbs 1.0–1.5 m tall, with base an apparently unbranched contorted taproot or rhizome, with fleshy lateral roots rather easily extracted from the soil. Stems striated, subsericeous with appressed T-shaped trichomes. Leaves alternate, sessile with narrow bases; blades linear to elliptical, margins sub-entire with sparse minute denticulations, apices narrowly acute, both surfaces with glandular dots, sub-sericeous with appressed T-shaped trichomes, abaxial surface somewhat paler; venation pinnate with usually 12 or more curving secondary veins on each side. Inflorescences cymbiform, with basal capitulum often appearing sessile as a result of 2–3 or more immediately subtending lateral branches, with branches loosely seriate-cymose. Capitula mostly appearing pedunculate as a result of lowered positions of subtending lateral branches; involucre sub-imbricate with 25–35 gradate involucre bracts in 3–5 series; receptacle flat or slightly convex, epaleate, ridges fringed with min-

ute trichomes; florets ca. 17 in capitula, corollas lavender to reddish, with basal tube narrowly cylindrical below, throat short, lobes narrowly lanceolate, without obvious multiple longitudinal ducts; anther thecae and apical appendages bearing glandular dots, bases spurred, acute, not tailed; style without enlarged basal node or disk, with sweeping hairs extending strongly on to upper shaft. Achenes cylindrical, ca. 2 mm long, 10-ribbed, short twin-hairs dense on ribs, glands in furrows, with scattered idioblasts amongst elongate surface cells, walls with sub-quadrate raphids; pappus of ca. 40 scabrid capillary bristles ca. 7 mm long, with outer series vestigial and bristleform. Pollen ca. 40–50 μ in diam. in fluid, tricolporate, sublophate with continuous perforated tectum between colpi.

Type material. The single species is as follows: *Vernonia incana* Less., *Linnaea* 4: 277. 1829. TYPE: “Brasilia meridionalis legit Sello[w]”, [between Rio Grande do Sul, São Gabriel and Uruguay, Catalán Area]: Friedrich Sello[w] 3379, *s.d.* [1826]. **Lectotype here designated: LL 00373309**; isosyntypes: **BR** 0000005536627, **GDC** G00327411, **HAL** 0114069, **K** 000497031, **K** 001066026 (a mixed sheet with a Tweedie *s.n.*, and Gillies 154-2 collection also mounted with the Sello[w] collection), **P** 00682761 and **P** 00682762. None of the isosyntypes has the collecting number so they cannot be considered isolectotypes. A photograph of a specimen of this gathering from B is mounted and filed at **F** 0BN014552. There may be other isosyntypes that are not available on line [Images of type material cited above can be found online at JSTOR-Plants, continuously updated].

Synonymy.

Vernonia immunis Griseb., *Symb. Fl. Argent.* 163. 1879.

Cacalia immunis (Griseb.) Kuntze, *Revis. Gen. Pl.* 2: 970. 1891.

Cacalia incana (Less.) Kuntze, *Revis. Gen. Pl.* 3(2): 138. 1898.

Remarks. Lessing worked at Berlin (B) and all of the Compositae in that herbarium were destroyed during WWII (Hiepko 1987). There are seven isosyntypes in JSTOR from which one may select a Lectotype. There is also a photograph at F of a B specimen that may have been some of the material Lessing studied but it is not indicated on the sheet. The LL specimen (now housed at TEX) was selected as the Lectotype (Fig. 4) because the label had the most complete information, including the collecting number and because the label information indicates that it had formerly been in the Berlin Herbarium. It was, at some point, sent to S.F. Blake (BARC) who worked at the herbarium of the US Department of Agriculture but also spent most of his free time at the US National Herbarium (US). In 1959, after his death, the Blake family sold his personal herbarium and library to C.L. Lundell (1959) who later transferred it to TEX [all of the Lundell specimens should be cited as LL]. The K specimen and one of the P specimens have a larger portion of the root than the others but they have less label information than the LL sheet. According to Vegter (1986), Friedrich Sello[w] lived from 1789 to 1831 and his original herbarium was at B. In TL II, Staffleu and Cowan (pg. 500–501, 1985) indicate that Sello changed the spelling to Sellow in 1914 but later his family dropped the ‘w’ and returned to the original spelling. It seems best to acknowledge the differing opinions and list him as Sello[w].



Figure 4. Image of the selected lectotype for *Vernonia incana* Less. There are now six isosyntypes in European herbaria: all the specimens are similar, but the LL specimen was selected as the lectotype because it had the most complete information, including the collecting number and the label indicates that it had been in the Berlin herbarium before it was sent to S.F. Blake. [photo courtesy of JSTOR-Plants, continuously updated].

The collection date and location were determined by consulting Herter (1945) and Herter and Rambo (1953) which together provide a detailed guide to Sello[w]'s itinerary. Number 3379 was collected in 1826. The data from Herter (1945) and Herter and Rambo (1953) say that the numbers 3331–3623 were collected between Rio Grande do Sul, São Gabriel (Brazil) and Catalán, Uruguay. Given the low collecting number, it would seem fairly certain that this collection was made in 1826 in southern Brazil between São Gabriel in the state of Rio Grande do Sul (Brazil) and the Catalán area in the department of Artigas just south of the city of Artigas (Uruguay) and just across the Uruguay River from Brazil. Both of the maps (Herter 1945 and Herter and Rambo 1953), however, show all 1826 collections as being south of the Uruguay river in what is now Uruguay. As a result, we are continuing to list the locality between Rio Grande do Sul, São Gabriel (Brazil) and Catalán, Uruguay.

According to J. Mauricio Bonifacino (MVFA), in the early 19th Century, Brazil had control of Uruguay and some collections made at that time (from what is now southern Brazil and Uruguay) were labelled as “*Brasilia meridionalis*”.

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