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Three new species of *Begonia* (Begoniaceae) from Bahia, Brazil

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Abstract

The taxonomic treatment of Begoniaceae for the state of Bahia, Brazil, led to the recognition of three new species of *Begonia* with narrow distributions, which are described and illustrated here: *B. delicata* Gregório & J.A.S. Costa, **sp. nov.** is a herb restricted to the region of the Recôncavo; *B. elianeae* Gregório & J.A.S. Costa, **sp. nov.** is a shrub endemic to the Atlantic forest of the southern part of the state; and *B. paganuccii* Gregório & J.A.S. Costa, **sp. nov.** is a subshrub known only from the type material, collected in the Piedmont of Paraguaçu. Notes on morphology, comparisons with morphologically similar species, etymology, geographic distribution, habitat and phenological data for each species are also presented. Furthermore, keys are provided as an aid to separating the new species from congeneric species that occur in their surroundings. Due to the sparse knowledge of the new species, there is as yet insufficient data to accurately assess their conservation status.

Resumo

O tratamento taxonômico das Begoniaceae do estado da Bahia, Brasil, resultou no reconhecimento de três espécies novas de *Begonia* com distribuição restrita, as quais são descritas e ilustradas aqui: *B. delicata* Gregório & J.A.S. Costa, **sp. nov.** é uma erva restrita ao Recôncavo; *B. elianeae* Gregório & J.A.S. Costa, **sp. nov.** é um arbusto endêmico da Mata Atlântica do sul do estado; e *B. paganuccii* Gregório & J.A.S. Costa, **sp. nov.** é um subarbusto, conhecido apenas pelo espécime-tipo, coletado no Piemonte do Paraguaçu. São apresentados comentários morfológicos, comparações com espécies semelhantes, etimologia,

distribuição geográfica, hábitat e dados fenológicos para cada espécie. Além disso, são fornecidas chaves de identificação para separá-las de espécies congenéricas que ocorrem na circunvizinhança dessas espécies. Devido ao conhecimento esparso das novas espécies, os dados ainda são insuficientes para classificá-las acuradamente quanto ao estado de conservação.

Keywords
Atlantic forest, Piedmont of Paraguaçu, Recôncavo, South coast, taxonomy

Palavras-chave
Mata Atlântica, Piemonte do Paraguaçu, Recôncavo, Litoral Sul, taxonomia

Introduction

Begonia L. is one of the largest genera of Angiosperms (~1,500 species), known worldwide as ornamentals, with numerous hybrids and cultivars popular in the horticultural market (Neale et al. 2006). The genus probably arose in Africa but is most diverse in the Americas and Asia (Goodall-Coopestake et al. 2010), occurring in a variety of habitats, but mainly in moist and shady forests (Clement et al. 2004). Taxonomically, it is arranged in more than 60 sections (Doorenbos et al. 1998). Nevertheless, these sections are not morphologically consistent and diagnostic features of one section are often found in members of other sections (Forrest et al. 2005). Although phylogenetic studies in *Begonia* have been based on low density, world-wide sampling (e.g., Forrest et al. 2005) or focused only on species of certain Old World regions (e.g., Plana 2003; Thomas et al. 2011), several sections of *Begonia* were already shown to be poly- or paraphyletic, and the sectional circumscription of Neotropical groups appears to be highly problematic (Dewitte et al. 2011).

In the course of preparing the taxonomic treatment of *Begonia* for the state of Bahia, Brazil (Gregório 2014), in addition to field work in different habitats, specimens from 24 Brazilian herbaria—ALCB, BAH, BHCB, BRBA, CEN, CEPEC, HB, HEPH, HRB, HST (Herbário Sérgio Tavares), HUEFS, IBGE, IPA, MBM, MBML, PEUFR, R, RB, RBR, SP, SPF, UB, UFP and UPCB (acronyms according to Thiers et al. 2014)—and photos of specimens from seven herbaria from other countries (B, G, K, M, NY, P and US) were examined. This inventory recorded 37 species of *Begonia* for the state and recognised ten new synonyms in the genus. More than 80% of these species occur in Atlantic forest, 14 are endemic to Bahia and according to Jacques et al. (2013) six are endangered. In addition to *Begonia obdeltata* Gregório & E.L. Jacques, which also occurs in the state of Pernambuco (Gregório et al. 2014), three undescribed species of *Begonia* were discovered during the inventory. They have a narrow distribution and are described and illustrated here: *B. delicata* Gregório & J.A.S. Costa, endemic to the Recôncavo; *B. elianeae* Gregório & J.A.S. Costa, endemic to southern Bahia, and *B. paganuccii* Gregório & J.A.S. Costa, known only from the type specimen, collected in seasonal forest in the region of the Piedmont of Paraguaçu.

Since the sectional classification of *Begonia* is morphologically inconsistent and phylogenetically unsatisfactory, and Neotropical species have been poorly sampled and appear to be phylogenetically unresolved, we provide a key to separate them from species that occur in the same surroundings. However, the new species are compared with morphologically similar species and their likely sections (*sensu* Doorenbos et al. 1998) are suggested. Data available for the three new species is still sparse and insufficient to assess them as to their conservation status. Thus, we chose to regard them as Data Deficient (DD; IUCN 2001, 2013) until more information on their ecology and demography is made available.

Taxonomic treatment

Begonia delicata **Gregório & J.A.S. Costa, sp. nov.**
urn:lsid:ipni.org:names:77144526-1
Figures 1, 2

Note. *Begonia delicata* is similar to *Begonia alchemilloides* A. DC., differing by the presence of a ring of trichomes at the apex of the petiole, stipules and first order bracts with entire margin (vs. laciniate) and staminate flowers with 2 (vs. 4) tepals.

Type. BRAZIL. Bahia: São Felipe, Serra da Copioba, 12°50'50"S, 39°05'22"W, Jun 1953 (fl, fr), *G. Pinto 53–55* (holotype: ALCB!).

Description. *Annual herb*, 11–15.5 cm high, monoecious, villous to glabrescent, provided with three types of trichomes, simple, slender trichomes, 1–2.6(–4.5) mm long, trichomes with thickened base, 0.3–0.8 mm long and microscopic, and sparse glandular trichomes. *Stem* 6–8 mm diam., rhizomatous, prostrate, fleshy, pilose, covered by stipules; internodes 1–3 mm long. *Stipules* 0.7–0.75 × 0.3–0.35 cm, ovate, apex long-apiculate, margin entire, with minute hairs to essentially glabrous, carinate, appressed, persistent. *Leaves*: petiole 3.5–9 cm long, cylindrical, villous to glabrescent, ring of trichomes at apex ca. 4 mm long; blade 3.5–7.8 × 4–9.2 cm, reniform, entire, symmetric to slightly asymmetric, basifixed; base cordate; apex rounded; margin crenate, ciliate; sparsely pilose to glabrescent on both surfaces, trichome scars with thickened base, concolorous, light green; venation actinodromous, 7–9 veins at base, membranaceous. *Inflorescence*: dichasial cyme 9–20 cm long, 4–14-flowered; peduncle 7–17,5 cm long, pilose and glandular; first order bracts ca. 1.5 × 0.8 mm, lanceolate, apex apiculate, margin entire, carinate, persistent. *Staminate flowers*: pedicel 9–12 mm long, sparsely glandular to glabrous; tepals 2, white, 6–7 × 5.5–6 mm, ovate to elliptic, apex acute to obtuse, margin entire, glandular on abaxial surface; androecium actinomorphic, stamens 16–22, filaments 0.2–0.4 mm long, free, anthers 1.5–2 mm long, rimose, connective prolonged. *Pistillate flowers*: tepals 5, [only seen in bud]: bracteoles 2, opposite, at base of ovary, lanceolate, persistent [only seen in bud]; styles 3, ca. 0.5 mm long, bifid, branches spirally-arranged, stigmatic papillae covering branches, stigmatic surface papillose, yellow; ovary 7.5–8.2 mm long, trilocular, placentation axile, placenta bifid [obtained from capsules].

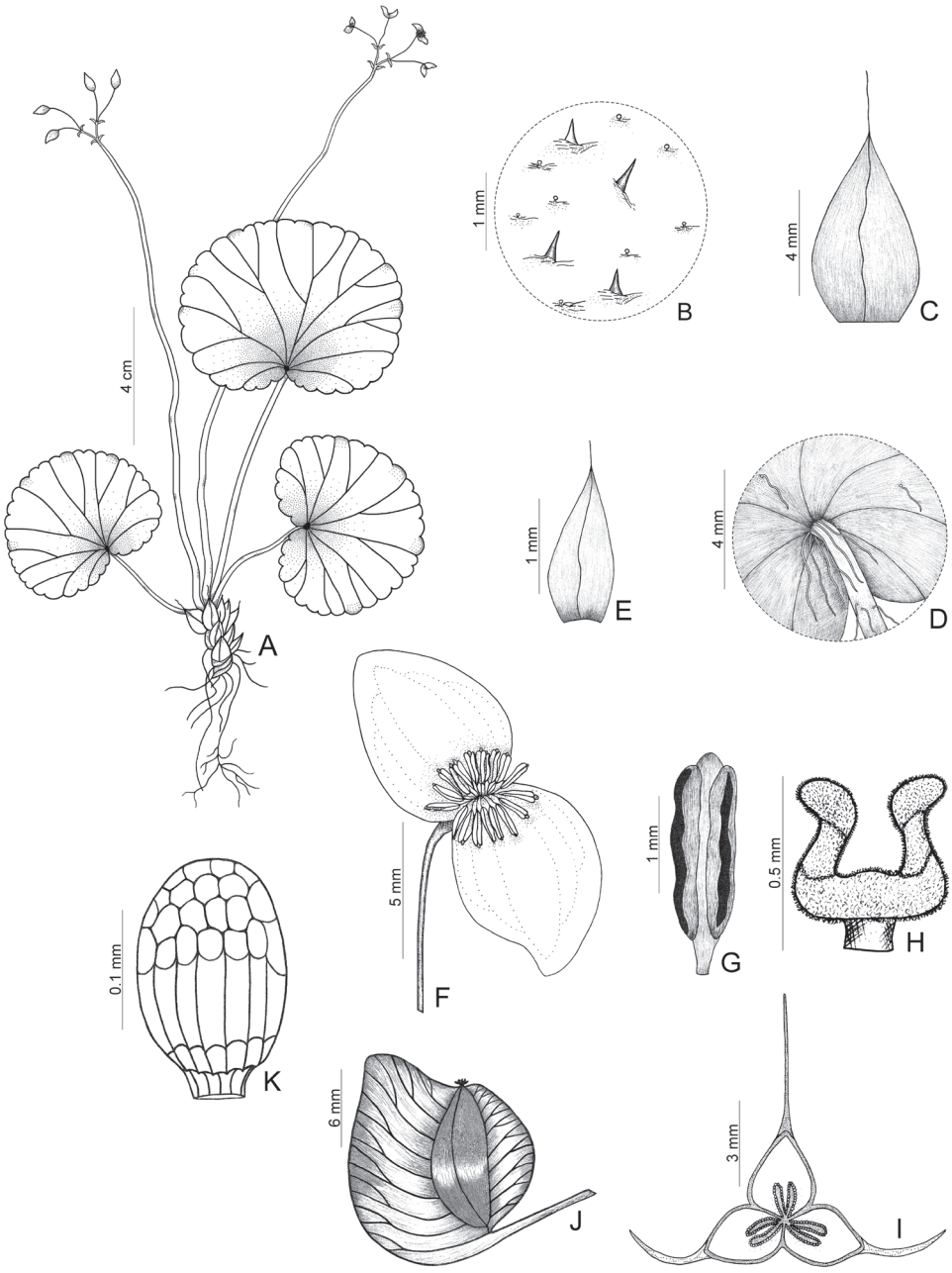

Figure 1. *Begonia delicata*. **A** Habit **B** Detail of indumentum on adaxial surface of leaf-blades **C** Stipule, seen from dorsal side **D** Detail of the ring of trichomes at the apex of the petiole **E** First order bract **F** Staminate flower **G** Stamen **H** Style-branch **I** Ovary, transverse cut, showing placenta **J** Capsule **K** Seed [**A–G** *Pinto 587* (RB); **H–K** holotype *Pinto 53–55* (ALCB); drawn by Bernarda Gregório].

Capsules ca. 12 × 13 mm [including wings], three-winged, sparsely glandular, dehiscing at the basal portion; wings unequal, larger ones ca. 14 × 5 mm, apex rounded, smaller ones ca. 12 × 3 mm, rounded. *Seeds* ca. 0.2 mm long, elliptic to oblong.

Specimen examined (paratype). BRAZIL. Bahia: São Felipe, Serra da Copioba, 12°50'50"S, 39°05'22"W, Oct 1950 (fl), *G. Pinto 587* (RB!).

Etymology. The epithet refers to the fragility and delicacy of the plant.

Distribution and habitat. *Begonia delicata* occurs exclusively in the Recôncavo region (Fig. 2). It is known by only two collections, both from Serra da Copioba, the most recent made in 1953, growing on rocks covered by moss. It has not been found in conservation unit.

Phenology. Found flowering in June and October, and with fruits in June.

Discussion. *Begonia delicata* is a small herb easily recognised by the rhizomatous stem covered in stipules, the petioles with a ring of trichomes at the apex, and by the reniform leaf-blades, with crenate margins. Few Brazilian *Begonia* are delicate herbs and, amongst those species, *B. alchemilloides* and *B. hoehneana* Irmsch. (state of São Paulo) are those that most resemble the new species. *Begonia delicata*, however, can easily be distinguished from both species by the presence of a ring of trichomes at the apex of the petiole and by the staminate flowers with fewer tepals (2 vs. 4). Moreover, the stipules and first order bracts are entire, whereas in *B. alchemilloides* they are laciniate, and the leaves are crenate whereas in *B. hoehneana* they are dentate. Among the species that occur in Bahia, *B. hirtella* Link most closely resembles *B. delicata* (see the key below), but can be distinguished by its habit (prostrate in *B. delicata* vs. erect in *B. hirtella*), the stipules and first order bracts (entire vs. fimbriate), the ring of trichomes at the apex of the petiole (present vs. absent) and the shape of the leaf-blades (reniform vs. ovate). According to the sectional classification of Doorenbos et al. (1998), *B. delicata* would belong to the sect. *Doratometra* (Klotzsch) A. DC., which consists of approximately ten annual species, with inconspicuous flowers in relative small inflorescences and two bracteoles below ovary.

Key to *Begonia* from the Recôncavo region

1 Stipules with fimbriate margins
2 Seeds fusiform ..... ***B. fischeri* Schrank**
2' Seeds oblong..... ***B. hirtella* Link**
1' Stipules with entire margins
3 Leaf-blades with craspedodromous venation ..... ***B. ulmifolia* Willd**
3' Leaf-blades with actinodromous venation
4 Stem prostrate; internodes inconspicuous; stipules persistent; ring of trichomes at apex of petiole; leaf-blades with crenate margins; staminate flowers with 2 tepals ..... ***B. delicata* Gregório & J.A.S. Costa**
4' Stem erect; internodes conspicuous; stipules caducous; ring of trichomes absent from apex of petiole; leaf-blades with serrulate margins; staminate flowers with 4 tepals ..... ***B. reniformis* Dryand**

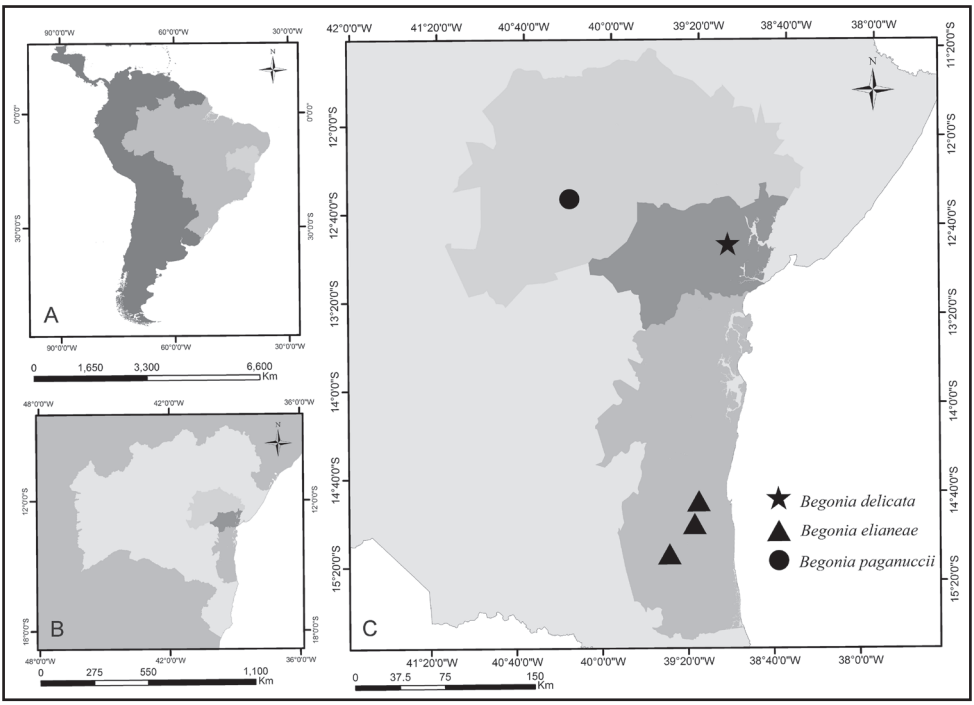

Figure 2. Geographical distribution of three new species of *Begonia*. **A** Latin America showing Brazil and Bahia State **B** Bahia State showing the three political-economic regions of Bahia with new species of *Begonia* **C** Political-economic regions of Bahia showing the occurrence of the three new species.

Begonia elianeae **Gregório & J.A.S. Costa, sp. nov.**
urn:lsid:ipni.org:names:77144527-1
Figures 2, 3

Note. *Begonia elianeae* is similar to *B. besleriifolia* Schott, but is easily distinguished from that species by leaf-blades glabrescent to glabrous on the abaxial surface (vs. sericeous); dichasial cyme with 4 to 8 flowers (vs. 40 to 60); tepals of staminate flowers larger (outer ones: ≥ 15 × 15 mm vs. ≤ 7.5 × 5.3 mm long; inner ones: ≥ 12 × 3 mm vs. ≤ 6 × 3 mm long); filaments shorter (≤ 1.2 mm vs. ≥ 1.5 mm long) and anthers larger (≥ 2 mm vs. ≤ 1.5 mm long); capsules larger (≥ 1.8 cm vs. ≤ 1.5 cm long), with larger wing (≥ 2 cm vs. ≤ 1.2 cm long).

Type. BRAZIL. Bahia: Jussari, ca. 9 km North of Jussari, east off of road to Palmira on farm road past cattle farm of Alciato Carvalho, 15°06'58"S, 39°31'58"W, 10 May 1995 (fl, fr), *W.W. Thomas et al. 10863* (holotype: CEPEC!).

Description. *Shrub*, 1–2.5 m high, monoecious, with sparse minute, simple hairs and microscopic glandular hairs to essentially glabrous. *Stem* erect to scandent, fleshy, sparsely pilose, longitudinally striate in herbarium specimens; internodes 1.7–5 cm long. *Stipules* 1.7–3.5 × 0.6–0.8 cm, lanceolate, apex apiculate, margin entire, with minute hairs to essentially glabrous, appressed, persistent. *Leaves*: petiole 1.3–3.3 cm

long, cylindrical, with minute hairs to essentially glabrous; blade 13–18.2 × 6.2–8 cm, oblong to obovate, entire, asymmetrical, basifixed; base oblique; apex acuminate; margin entire to slightly undulate, glabrescent to glabrous on both surfaces, discolorous, adaxial surface green, abaxial surface light green to vinaceous; venation craspedodromous, thickened. *Inflorescence*: dichasial cyme 9–15 cm long, 4–8-flowered; peduncle 4,5–6 cm long, with minute hairs to essentially glabrous, vinaceous; first order bracts ca. 15 × 6 mm, obovate, apex rounded, margin entire, caducous. *Staminate flowers*: pedicel 10–14 mm long, glandular; tepals 4, white, the outer pair larger, 15–17 × 15–17 mm, orbicular to ovate, apex rounded, margin entire, concave, glabrescent on abaxial surface, the inner pair 12–14 × 3–4 mm, elliptic to oblanceolate, apex acute to obtuse, margin entire, concave, glabrous; androecium actinomorphic, stamens 26–34, filaments 0.1–1.2 mm long, free, anthers 2–3 mm long, rimose, connective not prolonged. *Pistillate flowers* [only seen in bud]: bracteoles 2, opposite, borne on pedicel, just below the ovary, caducous [not seen; inferred by pedicel scars on flower bud]; pedicel of floral bud ca. 1 cm long; tepals of floral bud 5, 9.2–10 × 5–7 mm, three slightly larger ones, elliptic, apex acute to obtuse, margin entire, glabrescent on the abaxial surface, white [styles damaged]; ovary ca. 7.5 mm long, trilocular, placentation axile, placenta bifid [obtained from capsules]. *Capsules* 1.8–2 × 2.7–3.7 cm [including wings], three-winged, glabrescent, light green, young wings vinaceous, becoming brown at maturity, dehiscing at the basal portion; wings unequal, larger ones 2–2.3 × 1.7–2.1 cm, apex obtuse to rounded, smaller ones 1.5–1.8 × 0.6–0.8 cm, rounded. *Seeds* ca. 0.3 mm long, oblong.

Specimens examined (paratypes). BRAZIL. Bahia: Buerarema, estrada São José da Vitória-Buerarema, ramal à direita, ca. 1 km de São José. Estrada de acesso para Pedra Branca, 15°05'00"S, 39°19'00"W, 15 Oct 2003 (fl, fr), *P. Fiaschi et al. 1704* (CEPEC!; SPF!); Itabuna, fazenda Santa Clara, distrito Ribeirão dos Cachorros, entrada 200 m após a ponte da Bananeira da rodovia BR-101, 12°31'39"S, 40°18'25"W, 21 Aug 1972 (fl), *R.S. Pinheiro 1930* (CEPEC!).

Etymology. The specific epithet is given in honour of Dra. Eliane de Lima Jacques, a botanist who has contributed extensively to our knowledge of *Begonia* from Brazil.

Distribution and habitat. *Begonia elianeae* was found in three localities in southern Bahia, in areas of Atlantic rainforest (Fig. 2), growing on rocks, near pastures or eventually supported by tree trunks, on the edge of trails or disturbed forests. It has not been found in conservation units.

Phenology. Flowering in May, August and October, and with fruits in May and October.

Discussion. *Begonia elianeae* is a shrub characterised by the large oblong to obovate leaf-blades, and by the large, yet few-flowered inflorescences and few-fruited infructescences. In *Begonia*, shrubby species are not so common and only two species with this habit occur in the state of Bahia: *B. elianeae* and *B. besleriifolia*. Both have craspedodromous leaves and can be distinguished from all other species from southern Bahia with this type of venation using the key below. The most likely section of *B. elianeae* is the sect. *Ruizopavonia* A. DC., which consists of suffrutescent plants, with woody stem, straight, craspedodromous leaves, and bracts and styles caducous in fruit.

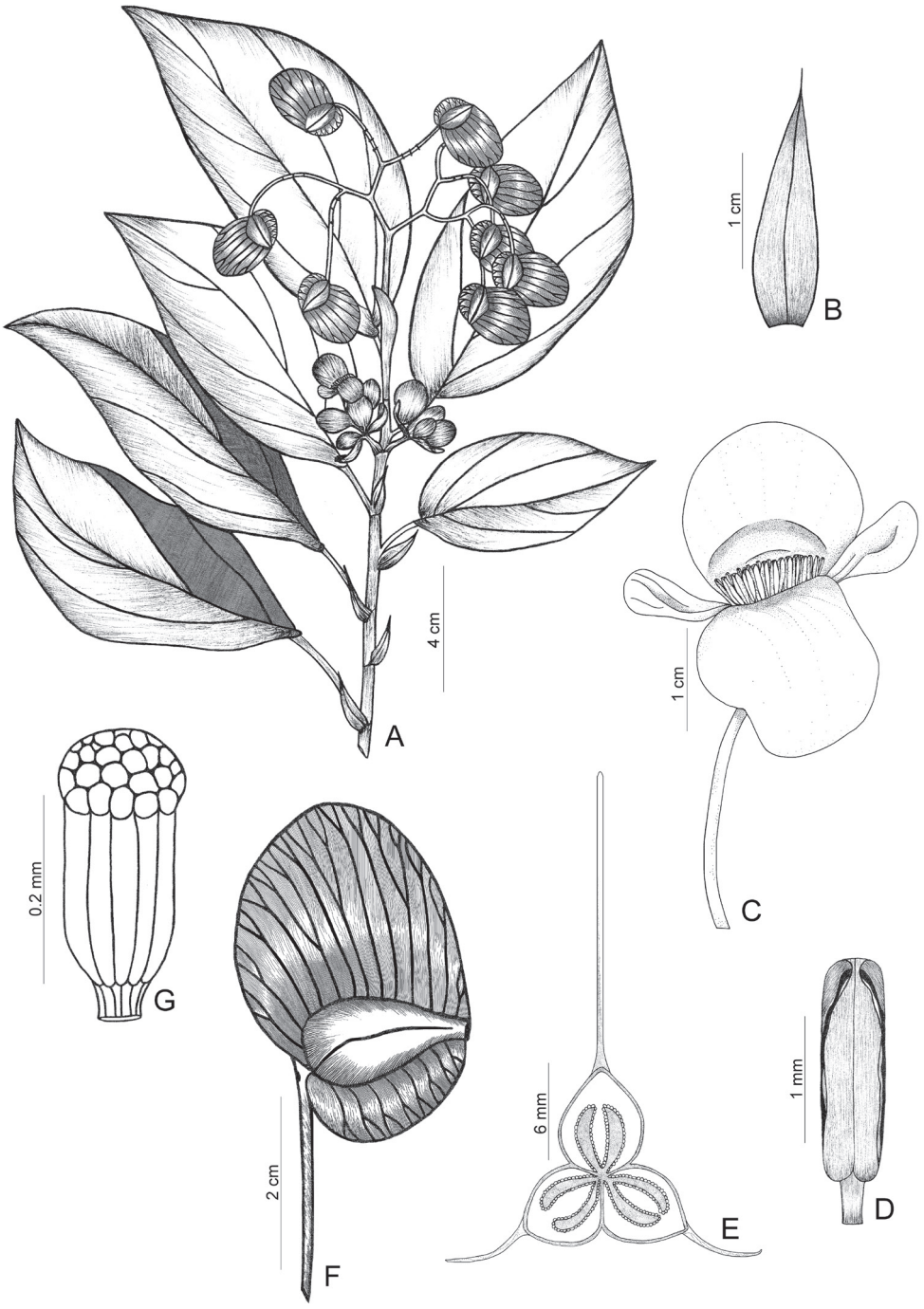

Figure 3. *Begonia elianeae*. **A** Flowering branch **B** Stipule, dorsal side **C** Staminate flower **D** Stamen **E** Ovary, transverse cut, showing placenta **F** Capsule **G** Seed [**A–D** holotype *Thomas 1086* (CEPEC); **E–G** *Fiaschi 1704* (SPF); drawn by Bernarda Gregório].

Key to the species of *Begonia* from Southern Bahia with craspedodromous leaves

1 Shrubs
2 Leaf-blades sericeous on abaxial surface; dichasial cyme with > 40 flowers; staminate flowers: the outer pair of tepals ≤ 7.5× 5.3 mm, the inner pair of tepals ≤ 6 × 3 mm; filaments ≥ 1.5 mm long, anthers ≤ 1.5 mm long; capsules ≤ 1.5 cm long, larger wing ≤ 1.2 cm long.....***B. besleriifolia*** **Schott**
2' Leaf-blades glabrescent to glabrous on abaxial surface; dichasial cyme with 4 to 8 flowers; staminate flowers: the outer pair of tepals ≥ 15× 15 mm, the inner pair of tepals ≥ 12 × 3 mm; filaments ≤ 1.2 mm long, anthers ≥ 2 mm long; capsules ≥ 1.8 cm long, larger wing ≥ 2 cm long ....***B. elianeae*** **Gregório & J.A.S. Costa**
1' Climbers
3 Stipules lanceolate; leaf-blades with serrate margins; capsules ≤ 1.2 cm wide [including wings], wings equal.....***B. fruticosa*** **A. DC.**
3' Stipules ovate; leaf-blades with entire to sparsely dentate margins; capsules ≥ 1.5 cm wide [including wings], wings unequal, one larger than the others.... .....***B. polygonifolia*** **A. DC.**

Begonia paganuccii **Gregório & J.A.S. Costa, sp. nov.**
urn:lsid:ipni.org:names:77144529-1
Figures 2, 4

Note. *Begonia paganuccii* is similar to *B. gardneri* A. DC. However, it can be easily distinguished by the indumentum of dendritic trichomes (vs. simple trichomes); stipules lanceolate and pubescent (vs. ovate and glabrous); staminate flowers with the outer pair ovate to elliptic and the inner pair oblong to oblanceolate (vs. both pairs obovate); endemic to the State of Bahia (vs. endemic to the State of Minas Gerais State).

Type. BRAZIL. Bahia: Itaberaba, fazenda Gameleira, entre as fazendas Monte Verde e Leão dos Brejos, 12°24'44"S, 40°32'12"W, 19 Aug 2005 (fl, fr), *L.P. Queiroz et al. 10790* (holotype: HUEFS!; isotypes: CEPEC!, K!, RB!).

Description. *Subshrub*, ca. 3 m high, monoecious, pubescent, with both dendritic greyish trichomes, 0.1–0.4 mm long, and microscopic glandular trichomes. *Stem* erect, fleshy, pubescent; internodes 1–3.5 cm long. *Stipules* 2.5–3 × 0.7–1.5 cm, lanceolate, apex apiculate, margin entire, pubescent, carinate, appressed, caducous. *Leaves*: petiole 6.3–11.6 cm long, cylindrical, pubescent; blade 13–18 × 19–28 cm, transversally elliptic, deeply lobed (lobes approximately half the length of their main vein), 6 or 7 lobes, asymmetric, basifixed; base cordate; lobes with acute apex; margin serrulate; pubescent on both surfaces, more densely so on abaxial surface, discolorous, adaxial surface green, abaxial surface green-cinereous; venation actinodromous, 6 or 7 veins at base, slightly thickened. *Inflorescence*: dichasial cyme 32–39 cm long, ca. 180 flowers; peduncle 23.5–27 cm long, cinereous; first order bracts 4–6 × 1.5–2.5 mm, lanceolate, apex acuminate,

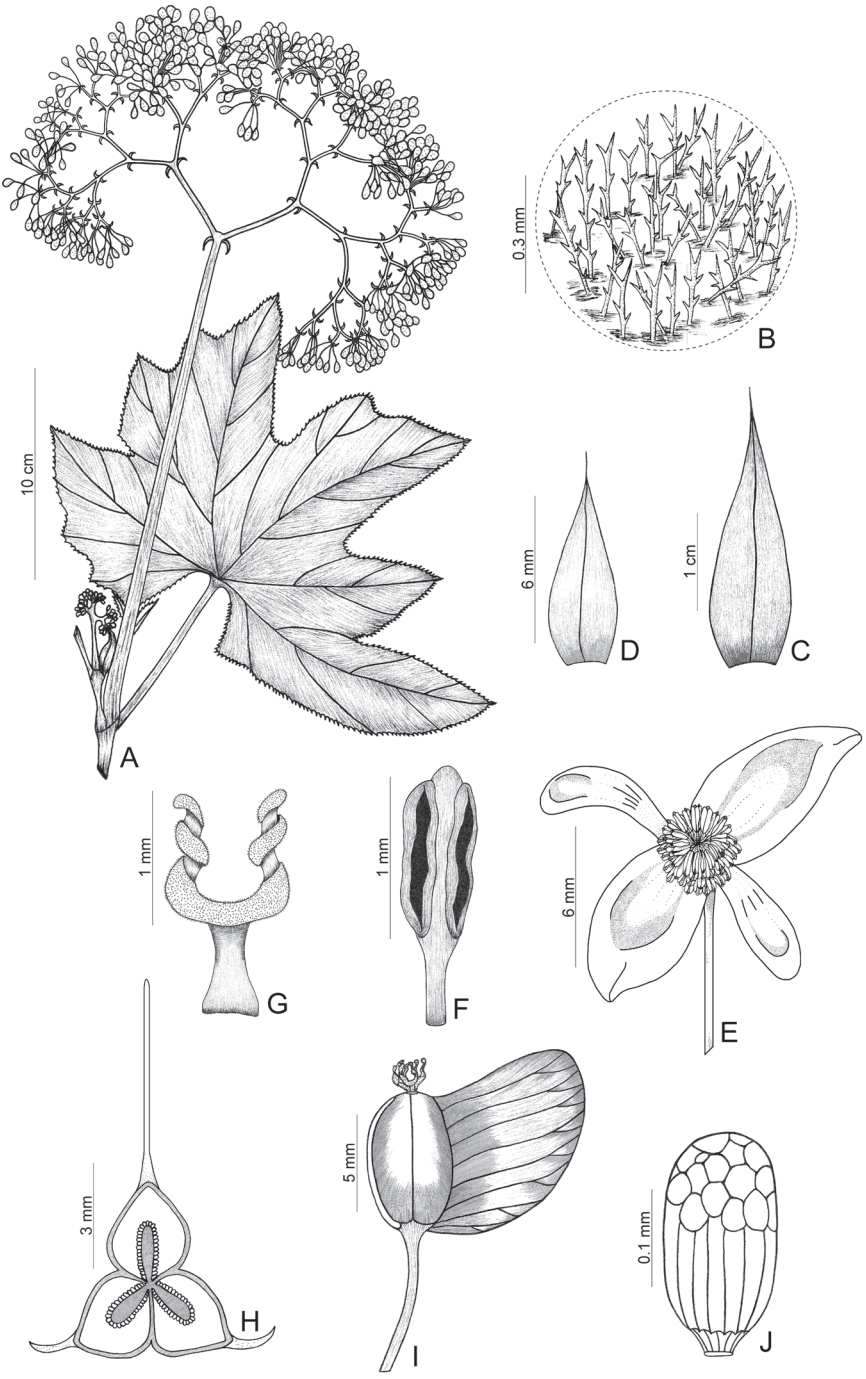

Figure 4. *Begonia paganuccii*. **A** Flowering stem **B** Detail of leaf, showing the dendritic trichomes **C** Stipules, seen from dorsal side **D** First order bract **E** Staminate flower **F** Stamen **G** Style-branch **H** Ovary, transverse cut, showing placenta **I** Capsule **J** Seed [**A–J** holotype *Queiroz 10790* (HUEFS); drawn by Bernarda Gregório].

margin entire, caducous. *Staminate flowers*: pedicel 1–1.4 cm long, pilose; tepals 4, white, the outer pair larger 6–7.2 × 3–4 mm, ovate to elliptic, apex acute to obtuse, margin entire, concave, glabrescent on abaxial surface, the inner pair 5–6.2 × 1.8–2.3 mm, oblong to oblanceolate, apex obtuse to rounded, margin entire, concave, glabrous; androecium actinomorphic, stamens 32–48, filaments 0.2–0.9 mm long, free, anthers 1–1.3 mm long, rimose, connective prolonged. *Pistillate flowers* [not seen]: bracteoles 2, opposite, borne on pedicel, just below ovary, caducous [scars seen on the pedicel from capsules]; styles 3, 1.6–2 mm long, bifid, branches spirally-arranged, stigmatic papillae covering branches, stigmatic surface papillose, yellow [obtained from capsules]; ovary 5–6.7 mm long, trilocular, placentation axile, placenta entire [observed from capsules]. *Capsules* 6–7.5 × 11–14.6 mm [including wings], three-winged, glabrescent, brown when mature, dehiscing at the basal portion; wings unequal, larger one 5–7 × 6–7 mm, apex obtuse to rounded, smaller ones 5.8–7 × 0.6–1.6 mm. *Seeds* ca. 0.3 mm long, oblong.

Etymology. This species is named in honour of Dr. Luciano Paganucci de Queiroz, a great expert on the flora of Bahia, who collected the type material.

Distribution and habitat. *Begonia paganuccii* is known from a single collection from the Área de Relevante Interesse Ecológico (ARIE), a protected area in the municipality of Itaberaba (Fig. 2), region of the Piedmont of Paraguaçu, growing in seasonal forest at 783 m a.s.l. Nevertheless, agriculture and livestock are common around and within the conservation unit.

Phenology. Flowering and fruiting in August.

Discussion. *Begonia paganuccii* is characterised by a dendritic indumentum, stipules lanceolate, and transversally elliptic leaf-blades, 6- or 7-lobed. Trichomes are quite important in the taxonomy of Begoniaceae when combined with other morphological information (Jacques 2002). Some species in Brazil have dendritic trichomes, such as *Begonia egregia* N.E. Br and *B. lindmanii* Brade. *Begonia paganuccii* differs from *B. egregia* by the basifixed, lobed and transversally elliptic (vs. peltate, entire and ovate to elliptic) leaf-blade, staminate flowers with 4 tepals (vs. 2) and pistillate flowers with trilocular ovary and 3 styles (vs. ovary tetralocular and with 4 styles). It also differs from *B. lindmanii* by the lobed (vs. entire) leaf-blade, as well as by the many-flowered dichasial cyme (ca. 180 flowers vs. 10–15 flowers) and pistillate flowers with 2 bracteoles (vs. 3 bracteoles). This species can be distinguished from the remaining species of *Begonia* from the region where it occurs using the key below. Due to the leaves with cystoliths and the entire placenta, it most likely belongs to the sect. *Pritzelia* (Klotzsch) A. DC.

Key to the species of *Begonia* from Itaberaba, Piedmont Region of the Paraguaçu basin

1 Leaf-blades with craspedodromous venation ..... ***B. ulmifolia* Willd**

1' Leaf-blades with actinodromous venation.

2 Stem rhizomatous, prostrate or decumbent; internodes inconspicuous; stipules persistent; leaf-blades with margin entire or slightly undulate .... ***B. pernambucensis* Brade**

2' Stem not rhizomatous, erect; internodes conspicuous; stipules caducous; leaf-blades with serrulate margin.

3 Plants covered by microscopic simple and glandular trichomes; stipules triangular; leaf-blades superficially 3–6-lobed, lobes shorter than half the length of their main vein ..... ***B. reniformis*** **Dryand**

3' Plants covered by microscopic glandular and dendritic trichomes; stipules lanceolate; leaf-blades deeply 6- or 7-lobed, lobes approximately half the length of their main vein..... ***B. paganuccii*** **Gregório & J.A.S. Costa**

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Phylogenetic relationships of *Zieria* (Rutaceae) inferred from chloroplast, nuclear, and morphological data

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Abstract

Zieria Sm. (Rutaceae, Boronieae) is predominantly native to eastern Australia except for one species, which is endemic to New Caledonia. For this study, sequence data of two non-coding chloroplast regions (*trnL-trnF*, and *rpl32-trnL*), one nuclear region (ITS region) and various morphological characters, based on Armstrong's (2002) taxonomic revision of *Zieria*, from 32 of the 42 described species of *Zieria* were selected to study the phylogenetic relationships within this genus. *Zieria* was supported as a monophyletic group in both independent and combined analyses herein (vs. Armstrong). On the basis of Armstrong's (2002) non-molecular phylogenetic study, six major taxon groups were defined for *Zieria*. The Maximum-parsimony and the Bayesian analyses of the combined morphological and molecular datasets indicate a lack of support for any of these six major taxon groups. On the basis of the combined Bayesian analysis consisting of molecular and morphological characters, eight major taxon groups are described for *Zieria*: 1. *Z. cytisoides* group, 2. *Z. granulata* group, 3. *Z. laevigata* group, 4. *Z. smithii* group, 5. *Z. aspalathoides* group, 6. *Z. furfuracea* group, 7. *Z. montana* group, and 8. *Z. robusta* group. These informal groups, except for of the groups *Z. robusta* and *Z. cytisoides*, correspond to the clades with posterior probability values of 100.

Keywords

Zieria, Rutaceae, Boronieae, Australia, conservation

Introduction

Zieria Sm. (Rutaceae, Boronieae) comprises 42 species. Six major taxonomic groups were defined based on non-molecular characters, according to the most recent classification by Armstrong (2002). Within Armstrong's (2002) tribal concept of the Boronieae, *Zieria* forms a distinct clade with *Boronia* Sm. s. l., *Boronella* Baill., *Brombya* F. Muell., *Medicosma* Hook.f., *Neobyrnesia* J.A. Armstr. and *Euodia* J.R. Forst. & G. Forst. s. s.; this clade is characterized by the presence of foliar sclereids.

Zieria consists of prostrate shrubs to small trees, with opposite and trifoliolate, or rarely unifoliolate leaves. Inflorescences are axillary, with four-merous, white or pink flowers. The fruits are comprised of one to four basally connate cocci, which dehisce explosively along the adaxial and apical margins. The seeds are usually one (often by abortion of one ovule) per fruit, with a thin brittle testa that is irregularly sculptured. In general, *Zieria* is distinguished from other genera of the Australian Rutaceae by the combination of opposite leaves, the conspicuous and 4-merous flowers, free petals, four stamens, free filaments, a deeply four-lobed disc, and dry, dehiscent fruits. This genus is predominantly native to eastern Australia, with the exception of the one species, *Z. chevalieri* Virot., which is endemic to New Caledonia. The distribution in eastern Australia extends from northeastern Queensland to Tasmania and as far west as Kangaroo Island in South Australia.

Sir James E. Smith first described the genus in 1798, in memory of Jan Zier, a Polish botanist. In 1810, H.C. Andrews described the first species, *Zieria smithii* Andrews, in H.C. Andrew's Botanist's Repository. In 1815, Bonpland published the descriptions of four species and soon after, in 1818, J.E. Smith described five more species. Bentham in his Flora Australiense (1863) described 11 new taxa and provided the first comprehensive key, with descriptions, synonyms and distribution data. For almost 136 years very little taxonomy was completed apart from C.T. White's descriptions of five new taxa in 1942, and Virot's (1953) circumscription of the endemic species from New Caledonia. It was not until 2002 that Armstrong reassessed and revised the classification, including defining six major taxonomic groups within *Zieria*. Accordingly, the nomenclature used in this paper is that of Armstrong (2002) and incorporates the morphological phylogenetic characters from that study (cf. Table 1). This study will be the first to test the monophyly of *Zieria* and its six major taxonomic groups using molecular data.

A subfamilial phylogenetic analysis was completed for Rutaceae by Chase et al. (1999), Groppo et al. (2008, 2012), Poon et al. (2007), Bayly et al. (2013), and Morton and Telmer (2014), using evidence from *rbcL* and *atpB*, *rps16* and *trnL-trnF* and *trnL-F*, *xdh*, and ITS sequence variation. All of the above authors, except for Bayly et al. (2013), did not include taxa from either *Zieria* or *Neobyrnesia* (sister genus to *Zieria*). Bayly et al. (2013) only included three *Zieria* species and *Neorbyrnesia*, and therefore, their relationships to each other and to other taxa of Rutaceae based on molecular techniques need to be examined for the degree of congruence with morphological characters. Of the 32 species used in this study, 21 are considered endangered or vulnerable according to the Environment Protection and Biodiversity Conservation

(EPBC) Act (http://www.environment.gov.au/cgi-bin/sprat/public/spratlookupspecies.pl?name=zieria&searchtype=Wildcard).

Molecular studies can produce effective and practical solutions for conservation biology to taxonomic uncertainties with respect to rare and threatened taxa and, in light of the high proportion of endangered taxa and overlying distribution patterns for a number of these taxa, examinations should be conducted on *Zieria*.

The goals of this study are (1) to test the monophyly of the genus *Zieria* and to identify its closest relatives; (2) to evaluate the six taxonomic groups within *Zieria* as recognized in the most recent revision (Armstrong 2002); and (3) to examine the relationship based on distribution patterns and molecular change of the endangered or vulnerable taxa of *Zieria*.

Methods

For this study, two non-coding chloroplast regions (*trnL-trnF*, and the *rpl32-trnL*) were selected, as well as the Internal Transcribed Spacer (ITS) of the nuclear region and various morphological characters. The *trnL-trnF* region consists of the *trnL* intron and the *trnL-trnF* intergenic spacer (Taberlet et al. 1991). The *rpL32-trnL* intergenic spacer is in the SSC (small single copy) region of the chloroplast genome. The *rpl32-trnL* was first used for phylogenetic studies by Shaw et al. (2005). Various workers have found that both of these sequences provided good resolution at the generic and species level (e.g. Wallander and Albert 2000; Baker et al. 2000). The ITS region of the *18S-26S* nuclear ribosomal DNA (nrDNA) consists of three genes that code for the *18S*, *5.8S* and *26S* ribosomal subunits. The three genes are separated by two internal transcribed spacers, ITS1 between *18S* and *5.8S* and ITS2 between *5.8S* and *26S*. Morphological characters were taken from information in Armstrong's (2002) taxonomic revision of *Zieria* (Table 1).

Taxon sampling & DNA extraction

Vouchers for the 33 species used in this study along with the GenBank accession numbers are listed in the Appendix 1. The total genomic DNA was extracted from (0.5—1.0 g) fresh or dried leaf material. Leaves were ground with a mortar and pestle and subsequently treated with the DNEasy plant DNA extraction kit from Qiagen (Qiagen, Valencia, California, USA) following the manufacturer's protocol. Alignments were made using the Sequencher software program (Gene Codes Corporation, Ann Arbor, MI), for each marker for 32 *Zieria* and 1 *Neobyrnesia* species and also a broader *trnL-F* alignment with sampling across all Rutaceae subfamilies including Meliaceae and Simaroubaceae as outgroups. All GenBank accessions numbers for the additional sequences can be found in Morton and Telmer (2014) with the exception of *Boronia* (EU853780), and *Medicosma* (EU853806) and *Euodia* (EU493243).

Table 1. The six taxonomic groups within *Zieria* as defined by Armstrong (2002).

Zieria, Group A
Z. adenodonta (F. Muell.) J.A. Armstr.
Z. adenophora Blakely
Z. buxijugum J.D. Briggs & J.A. Armstr.
Z. collina C.T. White
Z. floydii J.A. Armstr.
Z. formosa J.D. Briggs & J.A. Armstr.
Z. furfuracea R.Br. ex Benth.
Z. granulata C. Moore ex Benth.
Z. hindii J.A. Armstr.
Z. obcordata A. Cunn.
Z. parrisiae J.D. Briggs & J.A. Armstr.
Z. robusta Maiden & Betche
Z. tuberculata J.A. Armstr.
Z. verrucosa J.A. Armstr.
Zieria, Group B
Z. arborescens Sims
Z. caducibracteata J.A. Armstr.
Z. lasiocaulis J.A. Armstr.
Z. oreocena J.A. Armstr.
Z. southwelli J.A. Armstr.
Zieria, Group C
Z. chevalieri Virot
Z. fraseri Hook.
Z. laevigata Bonpl.
Z. laxiflora Domin
Zieria, Group D
Z. montana J.A. Armstr.
Z. prostrata J.A. Armstr.
Z. robertsiorum J.A. Armstr.
Z. smithii Andrews
Zieria, Group E
Z. aspalathoides A. Cunn. ex Benth.
Z. citriodora J.A. Armstr.
Z. ingramii J.A. Armstr.
Z. minutiflora (F. Muell.) Domin
Z. obovata (C.T. White) J.A. Armstr.
Z. odorifera J.A. Armstr.
Z. pilosa Rudge
Z. rimulosa C.T. White
Zieria, Group F
Z. baeuerlenii J.A. Armstr.
Z. covenyi J.A. Armstr.
Z. cytisoides Sm.
Z. involucrata R.Br. ex Benth.
Z. littoralis J.A. Armstr.
Z. murphyi Blakely
Z. veronicea (F. Muell.) Benth.

rpl32-trnL

The *rpl32-trnL* gene in 33 species was amplified using the primer pair *rpl32F/trnL* (Shaw et al. 2005) to acquire the entire region. The final PCR cocktail of 50 µl contained the following: 38 µl water, 5 µl of 10% Mg free buffer solution, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 0.25 µl *Taq* polymerase, and 0.5 µl of each primer. The amplifying reactions were run for 25 cycles of denaturing for 30 s at 95 °C, primer annealing for 50 s at 57 °C, and elongation for 2 min at 72 °C.

trnL-trnF

The *trnL* intron and the *trnL-trnF* intergenic spacer for 33 species were PCR-amplified using the universal primers trn-c, trn-d, trn-e, and trn-f as described by Taberlet et al. (1991). For some samples the entire *trnL* intron/*trnL-trnF* spacer region was amplified with *trn-c* and *trn-f*. In others, two separate amplifications were performed, one to amplify the *trnL* intron with trn-c and trn-d and the other to amplify the *trnL-trnF* spacer with *trn-e* and *trn-f*. In general each 50 µl amplification reaction contained the same proportions as in the *rp16* reactions. PCR amplification used a 7-min denaturing step at 94 °C followed by 30 cycles of denaturing for 1 min at 94 °C, primer annealing for 1 min at 45 °C, and elongation for 1 min at 72 °C, with a final 7-min elongation step at 72 °C.

ITS

The amplification of the ITS was performed successfully on 33 species using oligonucleotide primers ITS1/ITS4 (White et al. 1990) to acquire the entire region. The DNA fragment amplified using these two primers is approximately 800 bp long and includes ITS1, ITS2 and the *5.8S* ribosomal gene. The basic mix contained the following: 38 µl of water, 5 µl of 10% Mg free buffer solution, 3–6 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 0.5 µl of each primer (10 nM), and 0.25 µl *Taq* DNA along with 1.5 µl of DNA template for each reaction. The thermal cycler was programmed to perform an initial 1 cycle of denaturation at 95 °C for 2 min, followed by 24 cycles of 30 seconds at 55 °C, 72 °C for 90 seconds and 95 °C for 30 seconds. This was followed by a 10 min. extension at 72 °C to allow completion of unfinished DNA strands.

Cycle sequencing

The PCR products were cleaned using the QIAGEN QIAquick PCR purification kit (QIAGEN Inc., Chatsworth, California, USA) following the protocols provided by the manufacturer. Cleaned products were then directly sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Kit with AmpliTaq DNA Polymerase (Applied

Biosystems Inc., Foster City, California, USA). Unincorporated dye terminators were removed using the QIAGEN DyeEx dye-terminator removal system (QIAGEN Inc.) following the manufacturer's recommendations. Samples were then loaded into an ABI 3100 DNA Sequencer. The sequencing data was analyzed and edited using the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Morphological characters

A morphological dataset of 48 characters was constructed. Twenty-eight characters were coded as unordered binary and 20 as multistate. All but two characters (4-types of pubescence on young branches and 12-presence or absence of revolute lamina margins) were variable within *Zieria*. The invariant characters were included because they were thought to be important in testing the monophyly of the genus. All analyses were conducted as stated in the analysis section. Character states of taxa were taken from Armstrong (2002: 291–294).

Phylogenetic analysis

Boundaries of the *trnL* intron, *rpl32-trnL*, and the ITS nuclear gene were determined by comparison with sequences in GenBank. The following two alignment criteria and methodology were used: (1) when two or more gaps were not identical but overlapping, they were scored as two separate events and (2) phylogenetically informative indels (variable in two or more taxa) were scored as one event at the end of the data set. All DNA sequences reported in the analyses have been deposited in GenBank (Appendix 1).

Maximum-parsimony (MP) analyses of all single markers as well as the combined datasets were performed in PAUP* 4.0b8 (Swofford 2002) using the heuristic search option and with uninformative characters excluded. Searches were conducted with 100 random-taxon-addition replicates with TBR branch swapping, steepest descent, and MulTrees selected with all characters and states weighted equally and unordered. All trees from the replicates were then swapped onto completion, all shortest trees were saved, and a strict consensus or majority rule tree was computed. Relative support for individual clades was estimated with the bootstrap method (Felsenstein 1985). One thousand pseudoreplicates were performed with uninformative characters excluded. Ten random-taxon-addition heuristic searches for each pseudoreplicate were performed and all minimum-length trees were saved for each search. To reduce bootstrap search times, branches were collapsed if their minimum length was zero ("amb-").

The Bayesian analysis of the combined molecular and morphological analysis used a mixed-model approach (Mr Bayes 3.1.2 Ronquist et al. 2005). MrModelTest v2.3 (Posada and Crandall 1998, 2001; Nylander 2004) was used to choose the best evolutionary model, as selected by the Akaike Information Criterion. Four independent analyses were run, each performing 10 million generations, sampling every 1000th generation and using 3 heated and 1 cold chain, and other default settings. Tracer v1.4.1.

(Rambaut and Drummond 2007) was used to assess convergence of the runs and to discard the initial 20% of the trees as a burn-in. Branch lengths are averaged from the distribution of trees and the posterior probability values (BPP) for the branches reported (Nylander et al. 2004). Morphological state changes were examined on the combined tree by using MacClade 4.0 (Maddison and Maddison 2000).

To determine the combinability of the data sets, their data structure was compared using methods outlined by Mason-Gamer and Kellogg (1996), who discussed various ways to assess conflict between data sets. In one method the combination of independent data sets is possible if the trees do not conflict or if conflict receives low bootstrap support. Therefore, each node on the independent trees is tested for congruence against the other. If the nodes do not contain conflicting information, they are congruent and the data sets are combinable. Where there are incongruent nodes, the bootstrap values for each node are examined. If the support is less than 70%, there is no hard conflict and the incongruence is interpreted as being due to chance. In this study the different data sets were analyzed in combination to see how each data set changed or confirmed the tree topologies of each other and to adopt a hypothesis of phylogenetic relationships for the genus.

Conservation

Morton and Schlesinger (2014) found that species with low genetic diversity are less able to respond to environmental change; therefore this information can be informative and has been considered.

This study examined the following 15 of the 21 endangered or vulnerable species (*Z. adenophora*, *Z. baeuerlenii*, *Z. buxijugum*, *Z. citriodora*, *Z. collina*, *Z. convenyi*, *Z. formosa*, *Z. granulata*, *Z. ingramii*, *Z. murphyi*, *Z. obcordata*, *Z. parrisiae*, *Z. prostrata*, *Z. verrucosa*, and *Z. tuberculata*). An examination for similarity was made using the distribution patterns and the number of bp changes within all three genes for the taxa in clades that had strong posterior probabilities.

Results

The inclusion of gap coding in all data sets containing molecular data resulted in more homoplasy and lack of resolution; therefore, gap coding was not used in the following results. GenBank sequences EU281855–EU281953 were specifically generated for this study.

Larger trnL-trnF Family Analysis

Multiple sequence alignment of *Zieria* and *Neobyrnesia* with 44 other Rutaceae and closely related taxa resulted in a data matrix of 1038 characters. No regions were

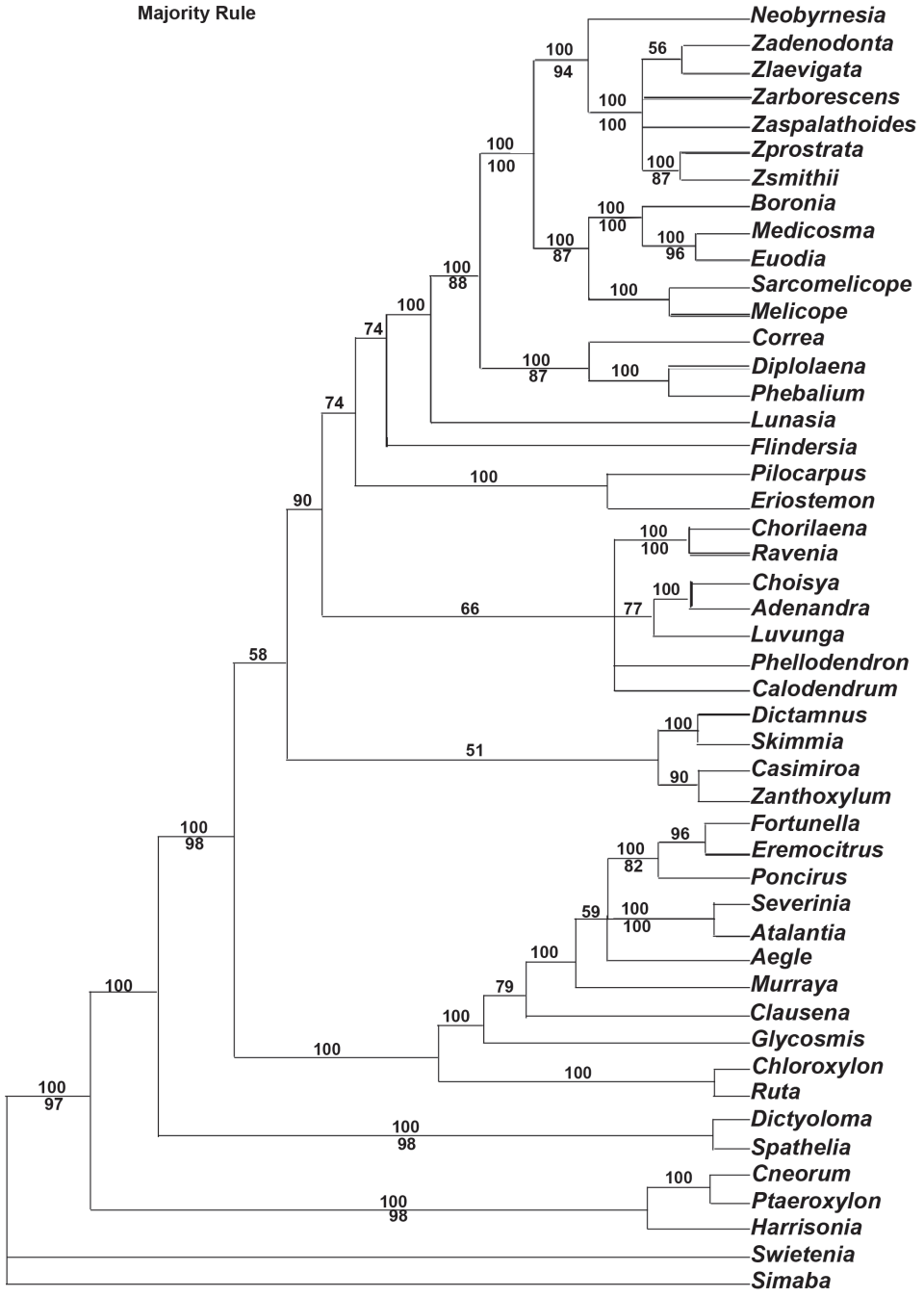

Figure 1. MP majority rule consensus tree of the expanded *trnL-trnF* dataset using a broad sampling of genera of Rutaceae as well as *Simaba* (Simaroubaceae) and *Swietenia* (Meliaceae) as outgroups. Numbers below nodes are bootstrap values.

excluded. Of the 1038 positions constituting the aligned *trnL-trnF* sequences, 357 (34%) were variable and 408 (39%) were parsimony-informative. The analysis recovered 4,383 equally optimal trees of 1037 steps (CI = 0.57, RI = 0.72; Fig. 1).

Zieria are supported as a monophyletic clade in the strict consensus tree (BS 100%). Sister to six species of *Zieria* is the genus *Neobyrnesia* (BS 94%). Sister to this grouping is (((*Medicosma* and *Euodia* (BS 96%)) *Boronia* (BS100%)) (*Sarcomelocope* and *Melicope* (BS 100)) (BS 87%)) followed by the remaining taxa. *Neobyrnesia* was therefore selected as the outgroup for this study.

trnL-trnF

Multiple sequence alignment of *Zieria* and *Neobyrnesia* resulted in a matrix of 1035 characters. A total of 10 gaps were required for proper alignment of the *trnL-trnF* sequences. These gaps ranged from one to 15 bps. No regions were excluded. Mean percentage G + C content was 56%. Of the 1035 positions, 127 (12.3%) were variable and 33 (3.2%) were parsimony-informative. The analysis recovered 35,458 equally optimal trees of 71 steps (CI = 0.59, RI = 0.69).

Zieria was supported as monophyletic in the strict consensus trees (BS 100). Most of *Zieria* consists of an unsupported grade or small polytomies except for one minor clade with bootstrap support of 75% (*Z. furfuracea* R.Br. ex Benth. and *Z. laxiflora* Domin).

Rpl32-trnL

Multiple sequence alignment of *Zieria* and *Neobyrnesia* resulted in a matrix of 1180 characters. Approximately 14 gaps were required for proper alignment of the *rpL32-trnL* sequences. These gaps ranged from one to 49 bps. No regions were excluded. Mean percentage G + C content was 30%. Of the 1180, 236 (20%) were variable and 46 (3.9%) were parsimony-informative. The analysis recovered 87,213 equally optimal trees of 77 steps (CI = 0.69, RI = 0.90).

Zieria was supported as monophyletic in the strict consensus trees (BS 100). The tree mainly consists of a polytomy except for one minor clade with bootstrap support greater than 75% (*Z. furfuracea* and *Z. laxiflora* (BS 95%)).

ITS

Multiple sequence alignment of *Zieria* and *Neobyrnesia* resulted in a data matrix of 714 characters. Approximately five gaps were required for proper alignment of the ITS sequences. These gaps ranged from one to 16 bps. No regions were excluded. Mean percentage G + C content was 36%. Of the 714, 207 (29%) were variable and 82 (11.5%)

were parsimony-informative. The analysis recovered 7,259 equally optimal trees of 169 steps (CI = 0.72, RI = 0.84). *Zieria* is supported as a monophyletic clade in the strict consensus tree (BS 100%). Basal within this clade is *Z. citriodora* J.A. Armstr., which is sister to *Z. aspalathoides* A. Cunn. Ex Benth. and *Z. ingramii* J.A. Armstr. (BS 88%). The backbone phylogeny of the genus remained unresolved, however a number of minor clades were inferred. Clades that contain bootstrap support greater than 75% starting from the base of the tree include: 1) a clade containing *Z. arborescens* Sims sister to a polytomy of *Z. covenyi* J.A. Armstr., *Z. murphyi* Blakely and *Z. odorifera* J.A. Armstr. (BS 88%); 2) a clade containing *Z. montana* J.A. Armstr. and *Z. southwelli* J.A. Armstr. (BS 100%); 3) a clade containing a polytomy of *Z. adenophora* Blakely, *Z. furfuracea* and *Z. laxiflora* (BS 100%); 4) a clade containing *Z. fraseri* Hook. and *Z. laevigata* Bonpl. (BS 100%); 5) a clade containing *Z. pilosa* Rudge and *Z. verrucosa* J.A. Armstr. (BS 100%); and 6) a clade containing ((*Z. collina* C.T. White and *Z. prostrata* J.A. Armstr. (BS 89%)) sister to *Z. adenodonta* (F. Muell.) J.A. Armstr. (BS 77%)).

Phylogenetic utility of the three genes (*trnL-trnF*, *rpl32-trnL*, and ITS) in *Zieria*

The respective numbers of variable and potentially phylogenetically informative characters in each dataset, the consistency indices and the numbers of branches with bootstrap support above 75% can be found in Table 2. The ITS sequences produced the most parsimony-informative characters for similar taxon sampling when compared with the other regions: *trnL-trnF* (33), *rpl32-trnL* (46), and ITS (82). The *trnL-trnF* gene produced the fewest parsimony-informative characters. The ITS gene also had the highest number of resolved nodes at or above 75% bootstrap support when compared with all other genes: *trnL-trnF* (2), *rpl32-trnL* (2), and ITS (9). The combined parsimony analysis had 7 nodes at or above 75% bootstrap support whereas in the Bayesian analyses 13 branches had posterior probability values higher than 93%. There was no correlation between the increase of the CI and RI values and the increase in the number of informative characters.

Combined molecular MP analysis

Following the methods outlined by Mason-Gamer and Kellogg (1996) and applied by Eldenäs and Linder (2000), the data sets were considered combinable. Within each gene analysis, *trnL-trnF*, *Rpl32-trnL* and ITS, the genus was monophyletic with 100% bootstrap support. Among the molecular trees there were no conflicting nodes with bootstrap support greater than 75%; therefore congruence exists between the data sets and a combined molecular analysis was completed.

Multiple sequence alignment of *Zieria* and *Neobyrnesia* resulted in a matrix of 2929 characters, of which (32.7%) include at least one accession with a gap. Mean

Table 2. Genetic statistics for genes and genic regions utilized in the individual genic analyses, and in the combined molecular and morphological datasets.

Results	trnL	rpl32	ITS	molecular	morphology	Total data
Gaps	10	14	5	957		
Range of Gaps	1–15	1–49	1–16			
Excluded	none	none	none	none	none	none
	56	30	36	40		
Length	1035	1180	714	2929	48	2977
Informative characters	33	46	82	161	45	209
Variable characters	127	236	207	570	48	618
Trees	35458	87213	7259	2301	591	555
Steps	71	77	169	378	278	1177
CI (consistency index)	59	69	72	57	30	62
RI (retention index)	69	90	84	74	57	59
BB (branch and bound) above 75%	2	2	9	7	0	6

percentage G + C content is 40%. Of the 2929, 570 (19.5%) were variable and 161 (5.5%) were parsimony informative. The analysis recovered 2,301 equally optimal trees of 378 steps (CI = 0.57, RI = 0.74; Fig. 2 majority rule tree).

Zieria was supported as monophyletic in the strict consensus trees (BS 100)

Internally, *Zieria* consists of mainly a polytomy except for several minor clades with bootstrap support greater than 75%. Clades that contain bootstrap support greater than 75% starting from the base of the tree include: 1) a clade containing *Z. prostrata* and *Z. smithii* (BS 94%); 2) a clade containing *Z. fraseri* and *Z. laevigata* (BS 100%); 3) a clade containing a polytomy of *Z. arborescens*, *Z. covenyi*, *Z. murphyi* Blakely and *Z. odorifera* A. Cunn. (BS 76%); 4) a clade containing *Z. furfuracea* and *Z. laxiflora* (BS 99%) sister to *Z. adenophora* (BS 99%); and 5) a clade containing *Z. collina* and *Z. adenodonta* (BS 95%).

Morphological-based MP analysis

Of the 48 characters constituting the non-molecular dataset, 48 were variable and 45 (93.8%) were parsimony-informative. The analysis recovered 591 equally optimal trees of 278 steps (CI = 0.30, RI = 0.57). *Zieria* was monophyletic in the strict consensus of these trees (BS 100%). The in-group topology consisted of a large grade with only one clade that contained bootstrap support greater than 75% (*Z. laxiflora* and *Z. laevigata* (BS 75%)).

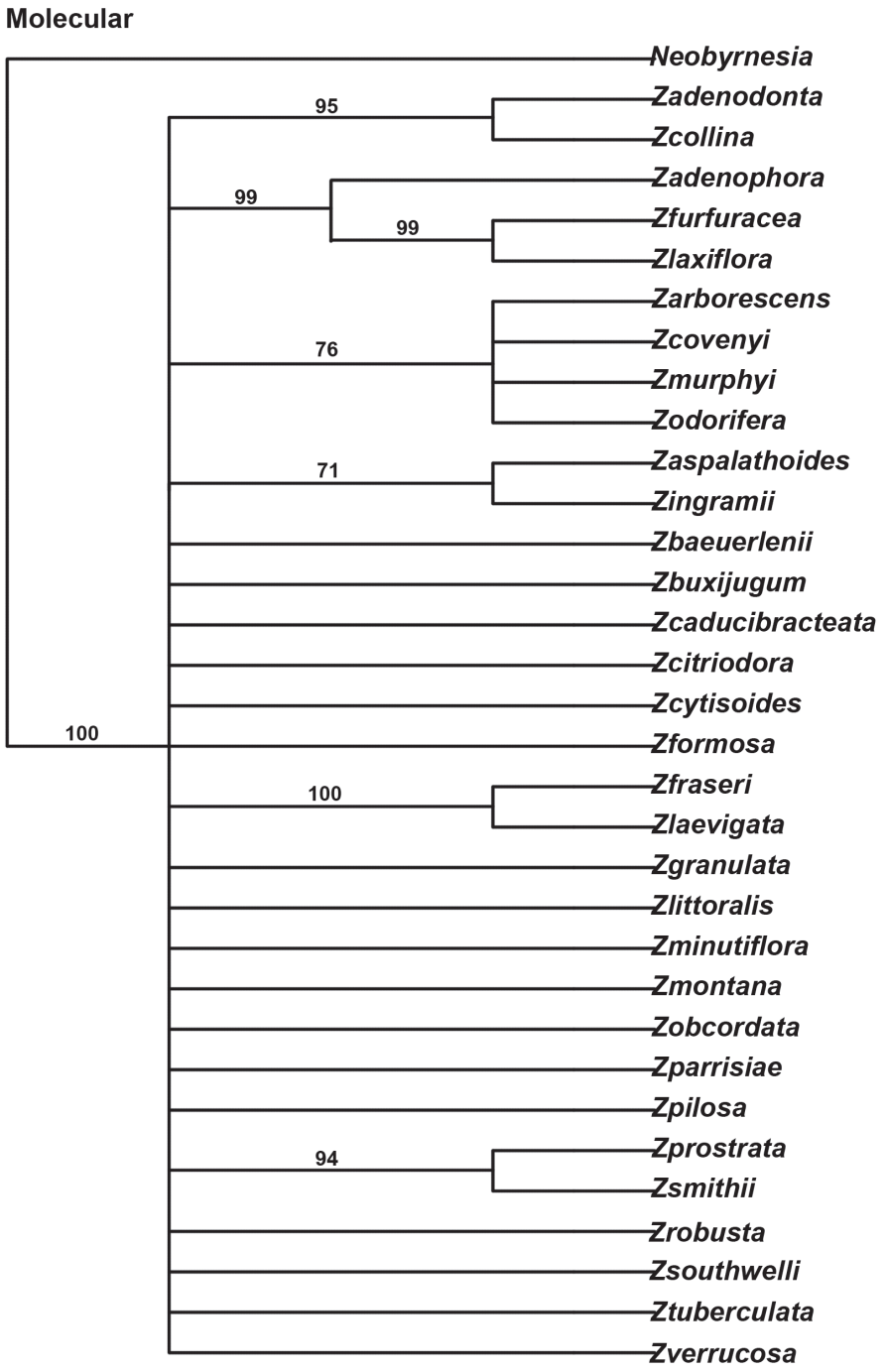

Figure 2. The strict MP consensus tree (L. = 749 steps, CI = 0.57, RI = 0.39) obtained from all molecular data. Numbers above nodes are bootstrap values.

Combined molecular and morphological data

Following the methods outlined by Mason-Gamer and Kellogg (1996), the molecular and morphological data sets contained only one potential hard conflict between a clade containing *Z. fraseri* and *Z. laevigata* (BS 100%) in the molecular data set and a clade containing *Z. laxiflora* and *Z. laevigata* (BS 75%) sister to *Z. fraseri* in the morphology data set. The positions of these three taxa have interchanged among the three separate molecular data sets and this is reflected in the morphology matrix having all three grouped together. The conflict appears to be due to a lack of resolution within the independent molecular dataset or that some of the morphological characters are homoplasious; therefore congruence exists between the data sets and a combined analysis was completed.

Multiple sequence alignment of *Zieria* and *Neobyrnesia* resulted in a matrix of 2977 characters, of which 28% include at least one accession with a gap. Of the 2977 positions constituting the aligned sequences, 618 (%) were variable and 209 (%) were parsimony informative. The analysis recovered 555 equally optimal trees of 1177 steps (CI = 0.62, RI = 0.59; Fig. 3 majority rule tree).

Zieria was supported as monophyletic in the strict consensus trees (BS 100).

Zieria consists mainly of grades except for several minor clades with bootstrap support greater than 75%. Clades that contain bootstrap support greater than 75% starting from the base of the clade include: 1) a clade containing *Z. furfuracea* and *Z. laxiflora* (BS 76%); 2) a clade containing *Z. fraseri* and *Z. laevigata* (BS 100%); 3) a clade containing *Z. prostrata* and *Z. smithii* (BS 95%); 4) a clade containing a polytomy of (*Z. buxijugum* J.D. Briggs & J.A. Armstr., *Z. formosa* J.D. Briggs & J.A. Armstr.), *Z. granulata* C. Moore ex Benth., *Z. littoralis* J.A. Armstr., *Z. parrisiae* J.D. Briggs & J.A. Armstr., *Z. tuberculata* J.A. Armstr., and *Z. verrucosa* (BS 93%); and 5) a clade containing *Z. collina* and *Z. adenodonta* (BS 92%).

Bayesian analysis of molecular and morphological data

In the Bayesian analysis (Fig. 4) *Zieria* is resolved as a monophyletic group, which consists mainly of a grade with the following clades containing posterior probability values higher than or equal to 95%: 1) a clade containing *Z. montana* and *Z. southwelli* (100); 2) a clade containing ((*Z. furfuracea* and *Z. laxiflora* (100)) (*Z. adenophora* (100))); 3) a clade containing *Z. aspalathoides* and *Z. ingramii* (100); 4) a clade containing *Z. prostrata* and *Z. smithii* (100); 5) a clade containing *Z. fraseri* and *Z. laevigata* (100); 6) a clade containing a grade of ((*Z. buxijugum*, *Z. formosa* (99)), *Z. parrisiae*, *Z. tuberculata* (98), *Z. granulata* (99)), sister to ((*Z. littoralis*, *Z. caducibracteata* J.A. Armstr., and *Z. verrucosa*)) (100); 7) a clade containing *Z. collina* and *Z. adenodonta* (100); and 8) clades in number 6 and 7 along with *Z. minutiflora* and *Z. obcordata* (100). There are no hard conflicts between the supported clades of the

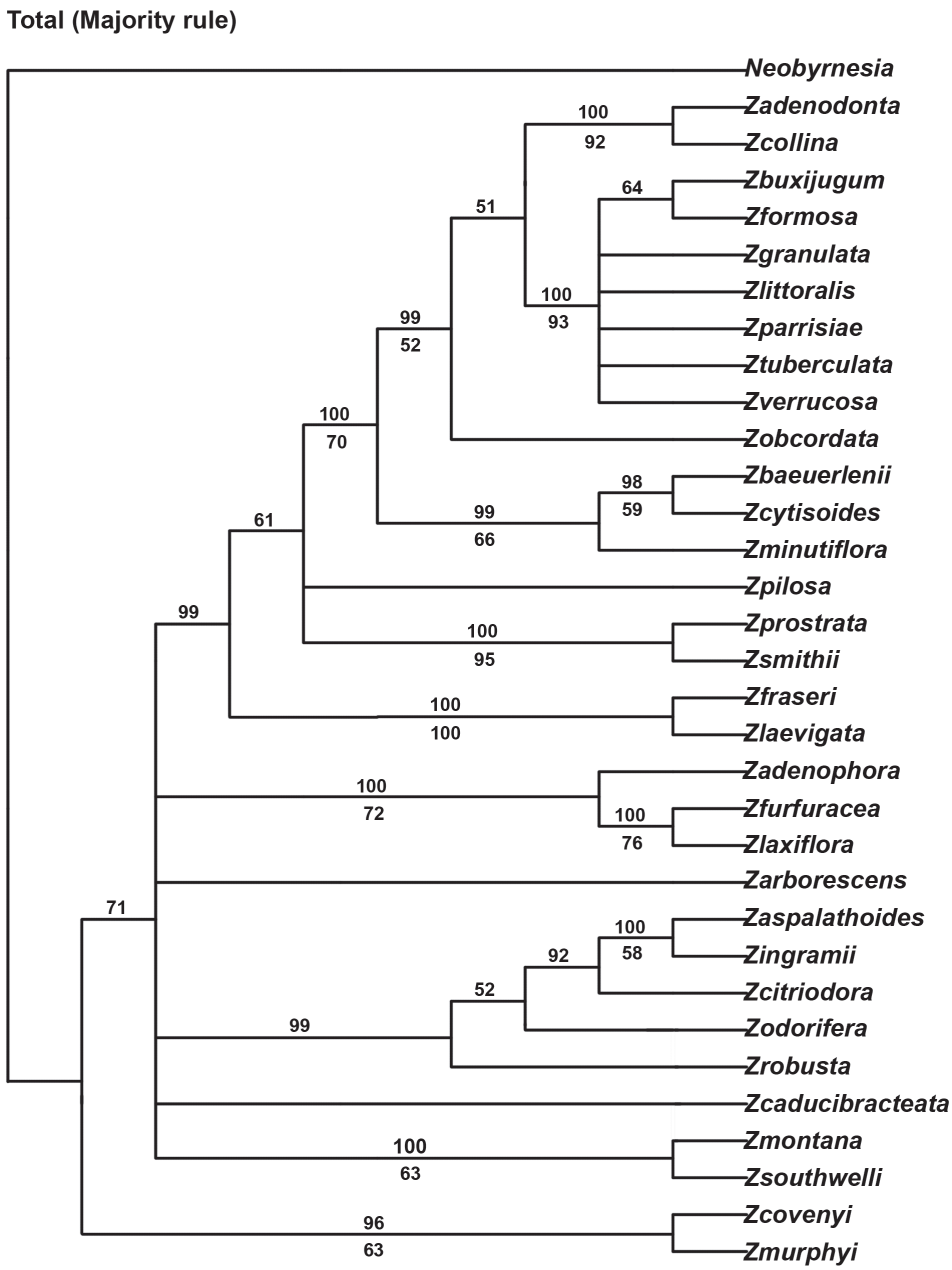

Figure 3. MP majority rule consensus tree using molecular and morphological data. Numbers below nodes are bootstrap values.

Bayesian and the parsimony topologies; in fact they are very similar except for the position of *Z. caducibracteata*, which is just a matter of resolution. An examination of the 48 morphological characters revealed no unambiguous synapomorphies.

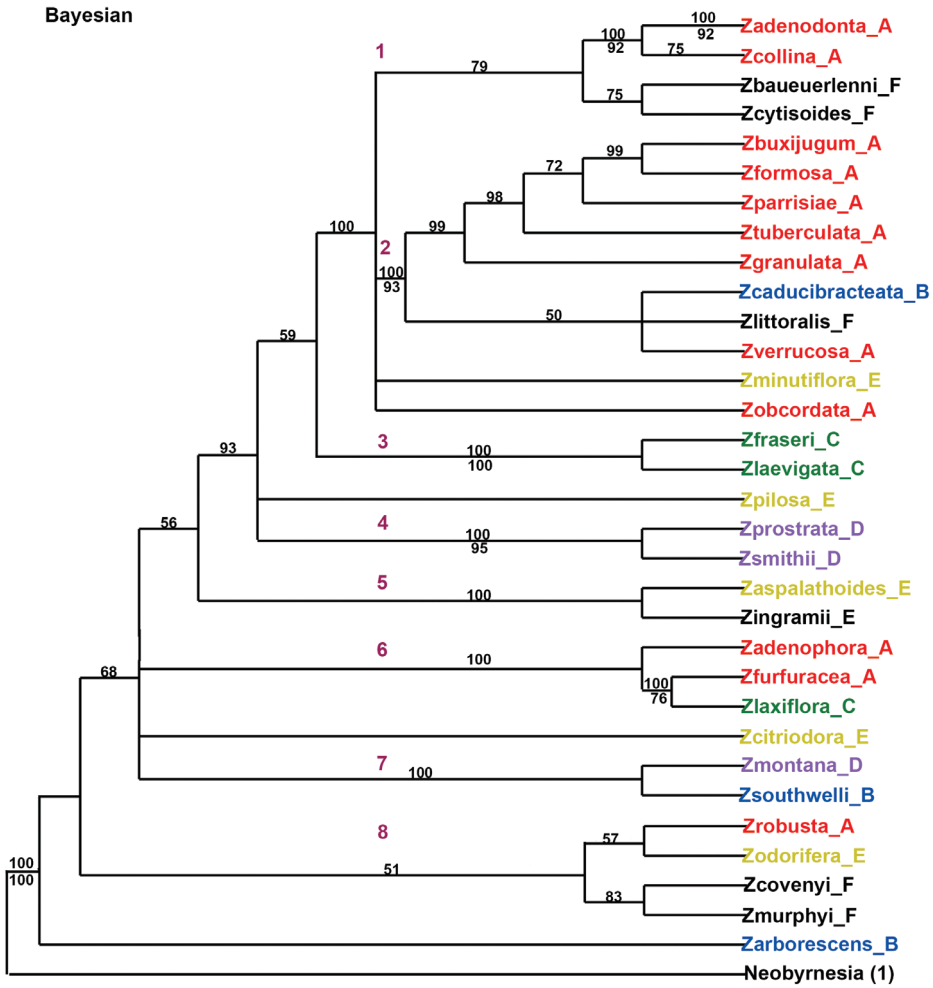

Figure 4. Bayesian majority rule consensus tree using molecular and morphological data. Numbers above the nodes are posterior probability values. **A–F** at the end of the taxa names corresponds to Armstrong (2002) classification system. The 1-8 listed on the tree corresponds to this study's finding.

Conservation

This study examined 15 of the 21 endangered or vulnerable species found in *Zieria* for similarity in their distribution patterns and for the number of bp changes within all three genes inside clades that had strong posterior probabilities.

The first clade containing *Z. adenodonta* and *Z. collina* (BPP 100 and BS 92%) have similar distribution patterns, however two of the three genes indicated had numerous bp changes (over 10 bps), indicating the taxa are distinct species.

The second clade contains eight species, one species being *Z. buxijugum* (BPP 100 and BS 93%), and although the species all occurred mostly in the southeastern

territory (New South Wales, Victoria and Tasmania), they had numerous bp changes between taxa.

Within the clade consisting of *Z. aspalathoides*, and *Z. ingramii* (BPP 100), there is distributional overlap, however there are over 30 bp changes among the taxa.

Although *Z. adenophora* has a non-overlapping distribution pattern from *Z. furfuracea*, and *Z. laxiflora*, the latter two taxa are very similar in distribution pattern. All three taxa have numerous bp differences, however *Z. furfuracea* and *Z. laxiflora* only had 2 solid bp differences.

The third clade consisted of *Z. prostrata* and *Z. smithii* (BPP 100 and BS 76%) these taxa have non-overlapping distribution patterns and two of the three genes had numerous bp changes (over 10 bps).

Z. covenyi and *Z. murphyi* (BPP 83), are from the same area and only had 3 bp changes among all three genes.

Discussion

Monophyly of *Zieria* and its closest relatives

We assembled a *trnL-F* dataset including 44 taxa of Rutaceae to determine the outgroup relationship of *Zieria* (Fig. 1). Based on this analysis six species of *Zieria* form a strongly supported clade with *Neobrynesia* (BS 94%). The monophyly of *Zieria* is also suggest by Bayly et al. (2013) and Appelhans et al. (2014). Bayly et al. (2013) using only *rbcL* and *atpB* also included *Z. chevalieriiii* from New Caledonian, the only disjunct species within *Zieria* to support not only the monophyly of *Zieria* but also the outgroup relationship with *Neobrynesia.*

Sister to this grouping is a clade containing the following taxa: *Medicosma*, *Euodia*, *Boronia*, *Sarcomelicope* and *Melicope* (see results for BS values and clade arrangements). We therefore used *Neobrynesia* as the outgroup for this study. Armstrong (2002), using morphological features, found that *Zieria*, together with *Boronia* s. l., *Brombya*, *Medicosma*, *Neobyrnesia,* and *Euodia* s. s., formed a distinct clade that is characterized by the presence of foliar sclereids. Although we did not include a species of *Brombya* the remaining members of the above group, plus *Sarcomelicope* and *Melicope*, are represented in the clade.

Circumscription of *Zieria*

Both independent and combined analyses of the molecular and morphological data supported the monophyly of *Zieria* (Figs 2, 3 and 4), as previously postulated by Armstrong (2002). The present study examined forty-eight morphological characters, including vegetative, floral, and fruit features (Armstrong 2002). Only one character, leaves palmately trifoliolate, provided a synapomorphy for *Zieria* (excluding *Z. mur-*

phyi). Other morphological characters that had been used to define the genus were examined (e.g. opposite leaves, 4-merous flowers, free petals, four stamens, free filaments, four-lobed disc and dehiscent fruits). Many of these morphological characters (e.g. opposite leaves, 4-merous flowers, four stamens, free filaments, and dehiscent fruits) that were used to define the genus are also found in the outgroup *Neobyrnesia* and in other genera of Australasian Rutaceae, and therefore, are not generic synapomorphies of *Zieria* (Armstrong and Powell 1980). The only other potential synapomorphy of *Zieria* is the intrafloral disc with "distinct antesepalous lobes", which in *Neobyrnesia* is entire. This study confirms the need to identify additional morphological characters that provide synapomorphies for classification at the generic level.

Circumscriptions of the six major groups of *Zieria*

On the basis of Armstrong's (2002) non-molecular phylogenetic study, six major taxon groups were defined for *Zieria*. The MP and the Bayesian analyses of the combined non-molecular and molecular datasets indicate a lack of support for any of these six groups (see Table 1 and Figs 2, 3 and 4).

The MP trees (strict-consensus trees from the independent, the combined molecular, and the non-molecular datasets) are poorly resolved and thus do not allow conclusive evaluation of the classification of Armstrong's (2002) six taxon groups. The Bayesian tree from the combined molecular and morphological datasets provides groupings with high support; therefore this dataset is used to discuss these relationships (Fig. 4).

Characters that support the six major taxon groups defined by Armstrong (2002) are as follows:

Group A contains 14 species and is characterized by having distinctly tuberculate younger branches, peduncles, petioles, midveins, and fruits.

Group B contains five species. The characteristics include younger branches slightly ridged or terete, primary inflorescence bracts boat-shaped and deciduous leaving a scar, and the abaxial surface of the calyx lobes with stellate hairs.

Group C consists of four species defined by having younger branches distinctly ridged with prominent glabrous leaf decurrencies, lower lamina surface velvet like, midveins glabrous with pellucid glands, inflorescences equal to or longer than the leaves, apex of calyx lobes curved inward adaxially, anthers prominently sharply pointed, and fruits with pellucid glands.

Group D comprises four species with the following characteristics: younger branches distinctly ridged with prominent glabrous leaf decurrencies; lower lamina surface glabrous and with pellucid glands that turn black on drying and become sunken; petiole either with pellucid glands or tuberculate; midvein glabrous with pellucid glands; and fruit with pellucid glands.

Group E is composed of eight species with younger branches densely pubescent, upper lamina surface with simple hairs, lamina lower surface and midvein hirsute, fila-

ments warty towards the apex, anthers prominently sharply pointed, ovary pubescent, cocci sharply pointed, and fruits glabrous or pubescent.

Group F, the final group, consists of seven species. The characteristics include upper lamina surfaces that are velvet like, inflorescences equal to or longer than the leaves, primary bracts that are boat-shaped and fruits that are pubescent.

In examining the Bayesian clade the following three mixed clades indicate that none of Armstrong's (2002) groups are monophyletic (Fig. 4). 1) *Z. montana* from Group D forms a sister grouping with *Z. southwelli* from Group B (BPP 100). 2). *Z. furfuracea* from Group A forms a sister grouping with *Z. laxiflora* from Group C (BPP 100). 3). *Z. minutiflora* (F. Muell.) Domin from Group E forms a well-supported polytomy with taxa from Groups A, B, and F (BPP 100).

Tentative new groups for *Zieria*

On the basis of the combined Bayesian analysis based on three genes (two-cholorplast and one-nuclear) and a morphological matrix (48 features), eight major taxon groups are distinguishable within *Zieria*. All of these informal groups, except for Groups 1 and 8, correspond to the clades with posterior probability values of 100 (Fig. 4). The make-up of these Groups are as follows:

The examination of the 48 morphological characters within the Bayesian tree revealed no unambiguous synapomorphies. However, sets of morphological synapomorphies in combination provide unique groups of characters to define a clade.

Z. cytisoides Group 1: four species — *Z. adenodonta*, *Z. baeuerlenii* J.A. Armstr., *Z. collina*, and *Z. cytisoides* Sm. This group contained the following synapomorphies: young branches densely pubescent and abaxial lamina surface not tuberculate.

Z. granulata Group 2: eight species — *Z. buxijugum*, *Z. caducibracteata*, *Z. formosa*, *Z. granulata*, *Z. littoralis*, *Z. parrisiae*, *Z. tuberculata*, and *Z. verrucosa*. Morphological characters that were found to be synapomorphic for this clade include: abaxial lamina surface and midvein tuberculate.

Z. laevigata Group 3: two species — *Z. fraseri* and *Z. laevigata*. These taxa had a number of morphological synapomorphies including: young branches, petioles and midveins not tuberculate, lamina suface and filaments pubescent, and calyx lobes glaucous and apex inflexed adaxially.

Z. smithii Group 4: two species — *Z. prostrata* and *Z. smithii*. Morpholoigcal characters that were found to be synapomorphic for this clade include: lamina surface and peduncles glabrous, lamina surface without black pellucid glands, midveins with pellucid oil glands, and inflorescences containing 10–50 flowers.

Z. aspalathoides Group 5: two species — *Z. aspalathoides* and *Z. ingramii*. Several morphogical characters are shared by these taxa such as: young branches distinctly ridged and densely pubescent, lamina surface with simple hairs, lamina margins revolute, filaments prominently dilated at base and anthers slightly apiculate.

Z. furfuracea Group 6: three species — *Z. adenophora*, *Z. furfuracea*, and *Z. laxiflora*. Only one morphological synapomorphy was found for this grouping: filaments not prominently dilated at base.

Z. montana Group 7: two species — *Z. montana* and *Z. southwelli*. One synapomorphy was found for these taxa: pubescence consisting of stellate trichomes.

Z. robusta Group 8: four species — *Z. covenyi*, *Z. murphyi*, *Z. odorifera* and *Z. robusta* Maiden & Betche. This group had several synapomorphies including the lamina surface containing pellucid oil glands, and the few flowered inflorescences being equal to or longer than the leaves.

Because of the lack of resolution, five taxa, *Z. citriodora*, *Z. arborescens*, *Z. minutiflora*, *Z. obcordata* and *Z. pilosa*, will remain unplaced until additional studies are completed. DNA for the following species were not examined and therefore these taxa will not be placed into groups until sequencing and analysis is completed: *Z. chevalieriiii*, *Z. floydii*, *Z. hindii*, *Z. involucrata*, *Z. lasiocaulis*, *Z. obovata*, *Z. oreocena*, *Z. rimulosa*, *Z. robertsiorum*, and *Z. veronicea*. Although six of the eight groups have strong posterior probabilities the relationships between these clades remain uncertain. The monophyly of the genus and of six of these groups appears unamibiguous; however, additional molecular and morphological studies are needed to further define the groupings and internal relationships.

Endangered taxa and conservation issues

Many *Zieria* taxa are considered endangered or vulnerable (Briggs and Leigh 1988, 1996; EPBC Act; current website http://www.environment.gov.au/cgi-bin/sprat/public/spratlookupspecies.pl?name=zieria&searchtype=Wildcard). Of the 51 taxa recognized by Armstrong (2002), the following 21 are considered endangered or vulnerable: *Z. adenophora*, *Z. baeuerlenii*, *Z. bifida*, *Z. buxijugum*, *Z. citriodora*, *Z. collina*, *Z. convenyi*, *Z. floydii*, *Z. formosa*, *Z. granulata*, *Z. ingramii*, *Z. involucrae*, *Z. lasiocaulis*, *Z. murphyi*, *Z. obcordata* A. Cunn., *Z. obovata*, *Z. parrisiae*, *Z. prostrata*, *Z. rimulosa*, *Z. verrucosa*, and *Z. tuberculata*.

This study examined 15 of the 21 endangered or vulnerable taxa found in *Zieria* for similarity in their distribution patterns and for the number of bp changes within all three genes inside clades that had strong posterior probabilities.

Z. covenyi and *Z. murphyi* (BPP 83), are from the same area and only had 3 bp changes among all three genes. Both taxa have several solid morphological differences such as leaves pubescent or glabrous, inflorescence numerous or few and filaments dilated or not dilated respectively. Because of these solid morphological differences these species appear distinct.

Z. furfuracea, and *Z. laxiflora*, (BPP 100) were very similar in pattern and had only 2 bp differences. Once again an examination of the non-molecular features revealed a number of differences such as the leaves having stellate-pubescence vs. being glabrous;

flowers ranging from 21–125 vs. commonly 9; petals valvate vs. imbricate; and flowering from spring to early summer vs. late winter to spring, to name a few.

Taxa in clades with strong posterior probabilities, with similar distribution patterns and low genetic variation, need to be closely examined before conservation management decisions are made to assure that they are unique species.

Conclusion

Zieria as currently circumscribed (Armstrong 2002) is monophyletic. This is supported by the molecular phylogenetic analysis and by one morphological synapomorphy: distinct antesepalous lobes of the gynoecium. This study found that the previous six species groups considered by Armstrong (2002) are not monophyletic, and confirmed that *Neobyrnesia* is the closest relative to *Zieria*. The analyses identified eight groups within *Zieria* and six of the eight groups have strong posterior probabilities.

Based on the number of informative characters and the number of branches with supported, ITS is an excellent candidate for higher-level analysis. In addition, ITS produced very few alignment difficulties within the ingroup and outgroup, and its tree topology remained consistent with that of the other genes.

Of the 32 taxa used in this study, 21 are considered endangered or vulnerable according to the EPBC. Several taxa grouped together and formed clades with strong posterior probabilities. Further examination revealed that two of these groups had similar distribution patterns and low genetic variation but solid differences in non-molecular characters. The taxonomic relationships of these taxa should be closely examined as further conservation management decisions are made.

The phylogenetic analysis presented here provides the first study within *Zieria* using both chloroplast and nuclear datasets, as well as a morphological dataset. Topics to be addressed in a future study include the determination of tribal and subtribal groupings and the use of additional taxa and genes to elucidate the biogeographic history of the genus.

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Appendix 1

Zieria species sequenced for the present study, with *Neobyrnesia* as outgroup. Collection data for accession vouchers and GenBank accession numbers are given below (see Materials and Methods). The country of origin for all specimens is Australia and specimens were collected from the associated botanical garden[1].

Species	Herbarium voucher	GenBank Accession Numbers		
		rpl32-trnL	***trnL-trnF***	***ITS***
Neobyrnesia suberosa J.A. Armstr.	*R. Mueller s.n. Dec. 2 1982* (CBG-8316286)	EU281888	EU281921	EU281855
Zieria adenodonta (F. Muell.) J.A. Armstr.	*F. A. Zich 453* (CANB 653334)	EU281889	EU281922	EU281856
Zieria adenophora Blankly	*J. A. Armstrong et al. 5097a* (CBG-8805884)	EU281890	EU281923	EU281857
Zieria arborescens Sims.	*I. R. Telford 3134* *(CBG-54528) *S. R. Donaldson & S. Golson 3594* **(CANB-748530)	EU281891	EU281924	EU281858
Zieria aspalathoides A. Cunn. ex Benth	*D. L. Jones & C.H. Broers 7814* (CBG-9109508	EU281892	EU281925	EU281859
Zieria baeuerlenii J.A. Armstr.	*S. Donaldson 111A* CBG-9104885	EU281893	EU281926	EU281860
Zieria buxijugum J.D. Briggs & *J.A. Armstr.*	*M. Parris* et al. *9018a* CBG-8602343	EU281894	EU281927	EU281861
Zieria caducibracteata J.A. Armstr.	*J. A. Armstrong & R. Coveny 744* CBG-8208729	EU281895	EU281928	EU281862
Zieria citriodora J.A. Armstr.	*I.R. Telford & S. Corbett 7346* CBG- 8001161	EU281896	EU281929	EU281863
Zieria collina C.T. White	*M. Parris 8847* (CBG- 8413675	EU281897	EU281930	EU281864
Zieria covenyi J.A. Armstr.	*P. Beesley et al. 285* (CBG-8411672)	EU281898	EU281931	EU281865
Zieria cytisoides Sm.	*F. A. Zich 405* CANB-643984 (CANB-629784)	EU281899	EU281932	EU281866
Zieria formosa J.D. Briggs & J.A. Armstr.	*M. Parris & N. Fisher 9151a* CBG-8604998	EU281900	EU281933	EU281867
Zieria fraseri Hook.	*I.R. Telford & S. Donaldson 12120* (CANB-9613250)	EU281901	EU281934	EU281868
Zieria furfuracea R.Br. ex Benth.	*A. M. Lyne et al. 2143* (CBG-9705354)	EU281902	EU281935	EU281869
Zieria granulata C. Moore ex Benth.	*K. Mills 2A* CBG-8501509 (CBG-9505133)	EU281903	EU281936	EU281870
Zieria ingramii J.A. Armstr.	*K. M. Groeneveld 89*A CBG-8800001	EU281904	EU281937	EU281871
Zieria laevigata Bonpl.	*F. A. Zich 448* CANB 653329	EU281905	EU281938	EU281872

Species	Herbarium voucher	GenBank Accession Numbers		
		rpl32-trnL	***trnL-trnF***	***ITS***
Zieria laxiflora Domin	*S. Fethers et al. 11* (CANB-617460)	EU281906	EU281939	EU281873
Zieria littoralis J.A. Armstr.	*M. Parris & N. Fisher 9240* CBG-8703977	EU281907	EU281940	EU281874
Zieria minutiflora (F. Muell.) Domin	*P. Beesley & P. Ollerenshaw 959* CBG-8604299	EU281908	EU281941	EU281875
Zieria montana J.A. Armstr	*F. A. Zich 462* CANB 653343	EU281909	EU281942	EU281876
Zieria murphyi Blakely	*A. M. Lyne et al. 325* CBG-9101073	EU281910	EU281943	EU281877
Zieria obcordata A. Cunn.	*J. D. Briggs 2376* CANB-389372	EU281911	EU281944	EU281878
Zieria odorifera J.A. Armstr. subsp. williamsii Duretto & P.I. Forst.	*I. Southwell H85-039* CBG-8505944	EU281912	EU281945	EU281879
Zieria parrisiae J.D. Briggs & J.A. Armstr.	*M. Parris 9145B* CBG-8604990	EU281913	EU281946	EU281880
Zieria pilosa Rudge	*D. L. Jones & C. Broers 6063* CBG-9010362)	EU281914	EU281947	EU281881
Zieria prostrata J.A. Armstr.	*S. Myers ANGB 2134a* (CBG-8802463)	EU281915	EU281948	EU281882
Zieria robusta Maiden & Betche	*M. D. Crisp 4397* CBG-7809037	EU281916	EU281949	EU281883
Zieria smithii Andrews	*S. Pedersen 16* CBG-9705152	EU281917	EU281950	EU281884
Zieria southwelli J.A. Armstr.	*I. R. Telford 3298* CBG- 54531	EU281918	EU281951	EU281885
Zieria tuberculata J.A. Armstr.	J. D. Briggs 2344 CANB 387032	EU281919	EU281952	EU281886
Zieria verrucosa J.A. Armstr.	*P. Beesley & P. Ollerenshaw 970A* CBG-8604310	EU281920	EU281953	EU281887

[1]The herbarium holdings of the Australian National Botanic Gardens (CBG) were combined in 1993 with those of the Australian National Herbarium (CANB) as part of the Centre for Plant Biodiversity Research, now the Centre for Australian National Biodiversity Research, (CANB), was adopted as the herbarium abbreviation for the combined collections; however, specimens originally from CBG continue to be cited as CBG. *Asterisks indicate which sample was used for each gene.

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RESEARCH ARTICLE

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Four new non-spiny *Solanum* (Solanaceae) species from South America

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Abstract

Four new species of "non-spiny" *Solanum* from South America are described. *Solanum longifilamentum* Särkinen & P.Gonzáles, **sp. nov.** (Morelloid clade) is widespread from Ecuador to Bolivia and is most similar to *S. macrotonum* Dunal from Central and northern South America. *Solanum antisuyo* Särkinen & S.Knapp, **sp. nov.** (Morelloid clade) is found on the eastern Andean slopes in Ecuador, Peru and Bolivia and is most similar to the widespread lower elevation species *S. polytrichostylum* Bitter. *Solanum arenicola* Särkinen & P.Gonzáles, **sp. nov.** (Morelloid clade) is found in low elevation habitats on the eastern Andean slopes and in Amazonia of Peru and Bolivia and is most similar to the higher elevation species *S. aloysiifolium* Dunal of Bolivia and Argentina. *Solanum mariae* Särkinen & S.Knapp, **sp. nov.** (Potato clade) is endemic to Cajamarca Department in Peru, and is most similar to the widespread *S. caripense* Dunal. Complete descriptions, distributions and preliminary conservation assessments of all new species are given.

Resumen

Se describen cuatro species nuevas de *Solanum* de América del Sur. *Solanum longifilamentum* Särkinen & P. Gonzáles, **sp. nov.** (clado Morelloide) se encuentra de Ecuador a Bolivia y es muy similar a *S. macrotonum* Bitter de Centroamerica y la parte norte de América del Sur. *Solanum antisuyo* Särkinen & S.Knapp, **sp. nov.** (clado Morelloide) se encuentra en las vertientes orientales de los Andes en Ecuador, Peru y

Bolivia y es similar a *S. polytrichostylum* Bitter, una especie de amplia distribución de elevaciones menores. *Solanum arenicola* Särkinen & P. Gonzáles, **sp. nov.** (clado Morelloide) se encuentra en las vertientes orientales de los Andes y la selva baja de Peru y Bolivia, es muy parecida a una especie que se encuentra a mayor altitud en Bolivia y Argentina, *S. aloysiifolium* Dunal. *Solanum mariae* Särkinen & S.Knapp, **sp. nov.** (clado Potato) es endémica del departamento de Cajamarca (Perú), y es similar a *S. caripense* Dunal, una especie de amplia distribución en los Andes. Se presenta descripciones completas, mapas de distribuciones y evaluaciones preliminares de conservación de todas las especies descritas aquí.

Keywords

Tropical Andes, Solanaceae, Peru, endemism, Morelloid clade, *Solanum* section *Solanum*, Potato clade, *Solanum* section *Basarthrum*

Palabras clave

Andes tropicales, Solanaceae, Perú, endemismo, clado Morelloide, *Solanum* sección *Solanum*, clado Potato, *Solanum* sección *Basarthrum*

Introduction

Solanum is one of the largest of flowering plant genera (Frodin 2004) and its centre of diversity is in South America (Knapp 2002). The Andes are a hot-spot for *Solanum* diversity and *Solanum* is one of the most species-rich vascular plant genera in the Andes (Jørgensen et al. 2011). In Peru alone 272 species of *Solanum* occur, of which 83 are currently listed as endemic (Knapp et al. 2006; Särkinen et al., in review). Many new species continue to be described (e.g., Anderson et al. 2006; Stern and Bohs 2010; Knapp 2010 a,b; Farrugia and Bohs 2010; Tepe et al. 2012; Särkinen et al. 2013) from the Andean region, discovered both in the field and in the herbarium. *Solanum* comprises 13 major clades of which the spiny solanum clade corresponding to subgenus *Leptostemonum* is the largest (Särkinen et al. 2013). The "non-spiny" solanums are distributed amongst a series of monophyletic groups, some of which have been treated recently (e.g., Knapp 2002, 2013; Tepe et al. 2011) while others are in the process of revision. Traditional sectional names for groups of non-spiny solanums are still in wide use, but many of these groups are very different in circumscription to these traditional concepts. Stern et al. (2011) provided an assessment of the clades of spiny solanums in the New World, but the "non-spiny" solanums have not been similarly treated.

We here describe four new species that came to light on recent field work as part of a project investigating the distributional ranges of Solanaceae in this megadiverse region. Descriptions are based on field work and examination of herbarium specimens from 20 herbaria (BH, BM, COL, CORD, CPUN, DUKE, E, F, GH, GOET, HUSA, HUT, K, MO, MOL, NY, S, UDBC, US, USM). All specimens are cited in the text and full data are provided in the supplemental file and on Solanaceae Source (http://www.solanaceaesource.org).

Taxonomic treatment

The Morelloid clade

The Morelloid clade is one of the larger monophyletic groups of "non-spiny" solanums, and contains the type of the genus (*Solanum nigrum* L., a European hexaploid species). The clade comprises five groups, four of which only have a few species (e.g., section *Campanulisolanum* Bitter, Barboza 2005). The largest group of species (ca. 52 species) are those related to *S. nigrum* that are often referred to as section *Solanum*. Members of this group occur worldwide with a centre of diversity in the Andes. This large group of species is morphologically relatively homogenous and is distinguished by its herbaceous habit, inflorescences usually positioned along the internodes, small flowers and fruits, and the usual possession of stone cells in the fruits (Bitter 1911). Stone cell aggregates are small, seed-like structures that are usually spherical (rather than flattened as are the seeds) and can most easily be seen in pressed specimens through the fruit wall. Although some studies have been done to clarify the taxonomy of the Old World and North American species (Edmonds 1977, 1978; Schilling 1981), monographic treatment of the entire section is needed to aid species identification and to clarify synonymy for the ca. 580 published names for these taxa. This is especially the case for South America, where most species diversity within the section lies (Edmonds 1972; Barboza et al. 2013), and where many taxa remain to be re-circumscribed and new species described. Recent work towards a taxonomic revision of this complex group has resulted in the description of two new species in this group (Särkinen et al. 2013; Manoko et al. 2013), both of which had previously been subsumed within other widespread taxa. The three species described here were similarly considered as part of poorly understood widespread taxa, but field and herbarium studies have shown them to be distinct.

***Solanum longifilamentum* Särkinen & P. Gonzáles, sp. nov.**
urn:lsid:ipni.org:names:77144531-1
Figs 1–2

Diagnosis. Like *Solanum macrotonum* Bitter, but differing in consistently narrower oblong-lanceolate leaves, few-flowered inflorescences, longer calyx lobes which elongate in fruit, filaments a minimum of half the length of anthers, and less exerted style exceeding to only 1 mm beyond the anther cone.

Type. Peru. Pasco: Prov. Oxapampa, Dist. Huancabamba, Parque Nacional Yanachaga-Chemillén, sector Tunqui, riberas del rio Muchuymayo, alrededores del hito PNYC, 1790 m, 22 Oct 2008 (fl, fr), *M. Cueva, A. Peña, R. Rivera & M. Moens 276* (holotype: USM [USM-00268971]; isotypes: HOXA, HUT, MO [acc. 6455431]).

Description. Delicate herb to small subshrub, woody at base, 20–100 cm tall, single stemmed or occasionally branching at the base. Stems 2–4 mm in diameter at the base, terete to ridged, often purple-tinged, sparsely pubescent with appressed

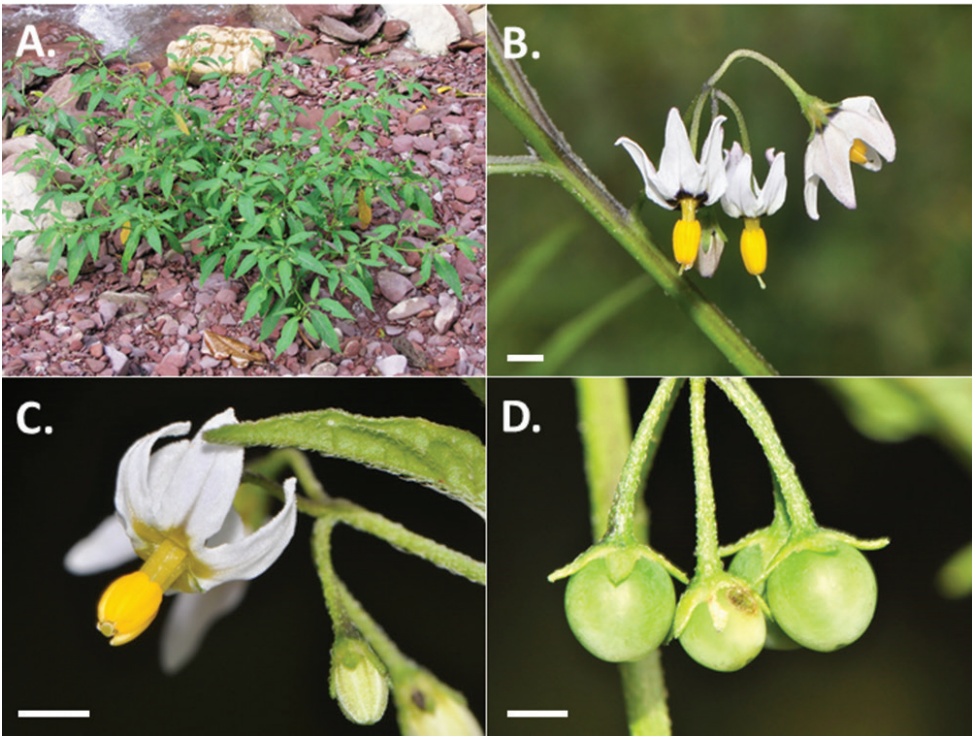

Figure 1. Photos of *Solanum longifilamentum*. **A** Habit (*Cueva et al.* 276) **B** Flowers at full anthesis (*Särkinen et al.* 4030) **C** Buds and flowers, floral type without black central star (*Knapp et al.* 10545) **D** Fruits with spreading calyx lobes (*Knapp et al.* 10545). Scale bars = 2 mm. Photos by S. Knapp (**C, D**), M. Cueva (**A**), and T. Särkinen (**B**).

1–2-celled simple uniseriate trichomes ca. 0.2 mm long. Sympodial units difoliate, not geminate. Leaves simple, 2.5–12.0 cm long, 1.0–4.0 cm wide, ovate-lanceolate; adaxial surface glabrous; abaxial surface with appressed 1–2-celled simple uniseriate trichomes like those of the stem along the veins; primary veins 4–8 pairs; base cuneate to attenuate, slightly unequal and oblique; margins entire; apex acuminate; petiole 0.5–1.0 cm long, sparsely pubescent with simple uniseriate trichomes like those of the stems and leaves, especially on young growth. Inflorescences lateral and internodal, 1.5–3.0 cm long, simple, with 3–5(6) flowers often all apparently arising from the same place, sparsely pubescent with simple uniseriate trichomes like those of the stems and leaves; peduncle 1.0–1.5 cm long, often tinged with purple; pedicels 0.5–0.6 cm long, ca. 0.4 mm in diameter at the base and 0.5 mm at apex, straight and spreading at anthesis, articulated at the base; pedicel scars closely spaced a maximum of 1 mm apart. Buds conical, white, occasionally purple-tinged towards the base, the corolla strongly exerted from the calyx tube long before anthesis. Flowers 5-merous, all perfect; calyx tube ca. 1.5–2.0 mm long, the lobes 1.0–1.5 mm long, deltate to traingular with acute apices, slightly reflexed at anthesis, sparsely pubescent with simple uniseriate trichomes

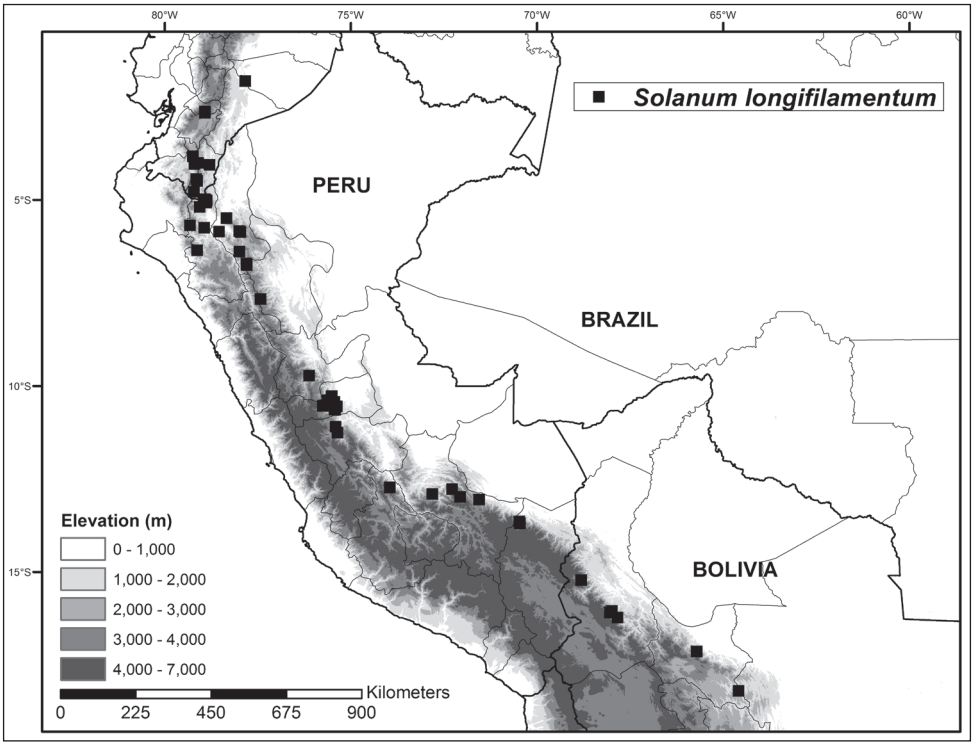

Figure 2. Distribution map of *Solanum longifilamentum* along eastern flanks of Central Andes in southern Ecuador and Peru.

like those of the stems and leaves; corolla 5–6 mm in diameter, stellate, whitewith a yellow, purple or black central star at the base, lobed 2/3 to nearly to the base, the lobes ca. 3.0–3.5 mm long, 1.5–2.0 mm wide, strongly reflexed at anthesis, later spreading, purple towards tips, densely pubescent abaxially with 1–2-celled simple uniseriate trichomes, these usually shorter than the trichomes of the stems and leaves; filament tube 1.0–1.2 mm long, pubescent with a few scattered 3–5-celled trichomes at the base adaxially; free portion of the filaments ca. 1.1–1.4 mm long, pubescent like the tube; anthers (1.7-)3.0–3.4 mm long, 0.8–0.9 mm wide, ellipsoid, yellow, poricidal at the tips, the pores lengthening to slits with age; ovary globose, glabrous; style 3.5–4 mm long, exerted only to 0.5–1.0 mm beyond the anther cone, densely pubescent in lower ¼ with 2–3-celled simple uniseriate trichomes; stigma globose, minutely papillate, pale yellow in live plants. Fruit a globose berry, 6–7 mm in diameter, green at maturity or green and turning purplish black when ripe, the surface shiny; fruiting peduncle same as in flower; fruiting pedicels 1.0–1.2 cm long, ca. 0.6 mm in diameter at the base, 0.9 mm at apex, spreading; fruiting calyx lobes 1.8–3.5 mm long, spreading, the tips reflexed. Seeds 35–45 per berry, c. 1.2 mm long, c. 1.1 mm wide, concave-reniform, narrower at one end, brownish orange, the sub-lateral hilum positioned towards the narrower end of the seed, the testal cells pentagonal in outline; stone cells few per fruit.

Distribution. Ecuador, Peru, and Bolivia on the eastern slopes of the Andes growing in mid-elevation montane forests in moist areas, along roadsides, often amongst mosses and small herbs, associated with Ericaceae and Asteraceae shrubs and herbs, Lauraceae, *Alnus acuminata* Kunth , *Cecropia* (Urticaceae), *Clusia* (Clusiaceae), *Fuchsia* (Onagraceae), *Hedyosmum* (Chloranthaceae), *Weinmannia* (Cunoniaceae), *Miconia* (Melstomataceae), and tree ferns; between (800-) 1,000–2,800 (-3,500) m elevation.

Ecology. Flowering and fruiting throughout the year, with a peak of fruiting in March–July.

Common names. Mortiño (Spanish; *Särkinen et al. 4577*); Wampishkúr (Shuar Jívaro; *Lewis 14172*).

Uses. Stems and leaves crushed and applied with achiote (*Bixa orellana* L., Bixaceae) warm to treat skin irritations (papera) (*Lewis 14172*).

Etymology. The species is named based on its uniquely long filaments in relation to the anthers which distinguish it from the closely related *S. macrotonum*.

Conservation status. The preliminary conservation status (IUCN 2010) of *S. longifilamentum* is here considered of least concern (LC) based on the relatively large extent of the species occurrence (EOO, c. 781,800 km^2), although the actual area of occupancy (AOO) is small (228 km^2) and would merit status as endangered (EN). Many recent collections exist, indicating that populations are not in decline, and as are most members of the Morelloid clade, *S. longifilamentum* is a weedy plant of disturbed areas.

Specimens examined. BOLIVIA. Cochabamba: Chapare: Along highway from Villa Tunari to Cochabamba, 43 km SW of bridge over Río Espírito Santo, 17°14'42"S, 65°71'17"W, 1320m, 1 May 2007, *M. Nee 55273* (MO); **La Paz:** Bautista Saavedra: Area Natural de Manejo Integrado Apolobamba, carretera entre Charazani y Camata, 15°12'48"S, 68°49'08"W, 1870m, 19 Aug 2008, *A. Fuentes 13261* (NY,USM); Murillo: 44.0 km below Lago Zongo dam, vicinity of Cahua hydroelectric plant, 16°05'00"S, 68°01'67"W, 1200m, 12 Sep 1983, *J.C. Solomon 10806* (MO); Murillo: Valle del Río Zongo, 40.2 km al N (abajo) de la cumbre, 16°04'S, 68°02'W, 1600m, 30 Apr 1990, *J.C. Solomon 18869* (G, MO); Nor Yungas: Carretera 3, 16.2 km SW of Yolosa jct toward Unduavi, 16°13'15"S, 67°49'58"W, 2078m, 12 Nov 1976, *C. Davidson 4976* (MO); **Santa Cruz**: Within the Flora de la Region del Parque Nacional Amboró, but above the 700m contour, 18°06'30"S, 063°57'00"W, 1465m, *M. Nee 37245* (MO); **ECUADOR. Cañar:** Iganilla Pass on Pan America Highway between Cañar and Biblian, 2°37'S, 78°55'W, 3500m, 12 Dec 1980, *L.B. Holm-Nielsen 28998* (BM,MO); Iganilla, S of the pass on Pan American Highway between Cañar and Biblian, 2°37'S, 78°55'W, 3400m, 12 Dec 1980, *L.B. Holm-Nielsen 29039* (BM, MO); Carretera Cañar-Azoguez, desvio a Moloboc-Grande-Molon-Ventana, 1°46'00"S, 78°36'W, 3200–3450m, 14 Aug 1987, *V. Zak 2446* (MO); **Loja**: Reserva Ecológica Tapichalaca (Fundación Jocotoco), between Yangana and Valledolid, 4°29'42"S, 79°07'55"W, 2500m, 3 Apr 2005, *L. Bohs 3408* (NY, QCNE); Rd from Loja to Yangana, 4°12'00"S, 79°10'12"W, 11 Jul 1986, *W.G. D'Arcy 16452A* (MO); 20 kms from Yangana, 4°25'59"S, 79°10'00"W, 2743m, 12 Jul 1986, *W.G. D'Arcy 16476* (MO); Parque Educacional y Recreacional Universidad Nacional de Loja, vicinity of Loja, 4°02'02"S,

79°11'87"W, 2100–2300m, 28 Oct 1994, *S. Knapp 9092* (BM, MO); between Loja and Saraguro, 2316m, 14 Jul 1984, *W.G. D'Arcy 15746* (MO, NY); **Pastaza:** Tsurakú (Pitirishca), km 51 S on rd from Puyo to Macas, 1°51'S, 77°48'W, 800m, 1 Aug 1988, *W.H. Lewis 14172* (MO); **Pichincha:** Along rd from Tandayapa to Nono, 4.1 km S of Tandayapa, 0°00'36"S, 78°39'23"W, 1828m, 4 Sep 2007, *T.B. Croat 98274* (MO); **Zamora-Chinchipe:** Fundación Arco Iris, between Loja and Zamora, trail from field station to Río San Francisco, 3°59'20"S, 79°05'35"W, 2200m, 5 Apr 2005, *L. Bohs et al. 3434* (BM, QCNE); 10 km S of Namirez and Rio Zimora, vicinity of Nambija, along rd to mine headquarters ca. 5 km long, just south of Nambija, 4°03'44"S, 78°47'29"W, 1779m, 23 Jul 2004, *T.B. Croat 92009* (BM); Prov. Chinchipe, region of Guaramizal, Parroquia Zumba, Quebrada Tarrangamí, near cabin of Sandy León, W of Escuela Byron Jiménez, just S of Las Pircas, 4°46'50"S, 79°12'33"W, 2000m, 29 Mar 2005, *L. Bohs et al. 3354* (BM, QCNE, UT); **PERU. Amazonas:** Prov. Bagua, third camp SE of la Peca, 1867–2179m, 10 Oct 1978, *P. Barbour 3958* (USM); Prov. Bongará, outside of main entrance to privately owned bird sanctuary just outside of the Alto Mayo protected area, ca. 2 km E of Area de Conservación Privada Arba Patricia-Alto Nieva, 13 Oct 2010, *F. Farruggia et al. 2753* (BM, HAO, NY, UT); Prov. Chachapoyas, Torrecilla, nr Turbaco, 2300m, 31 Mar 1979, *C.M. Ochoa 13254* (US); Dist. Leymebamba, Bosque Alto Atuen, 6°45'20"S, 77°48'07"W, 2583m, 27 Jun 2009, *R.W. Bussmann et al. 15823* (HUT); Prov. Luya, km 18–20 on rd from Nuevo Tingo to Kuelap, just before bridge at Choctamal, 6°23'13"S, 77°59'10"W, 2610m, 19 Apr 2013, *T. Särkinen et al. 4611* (USM); **Ayacucho:** Prov. La Mar, Aina, between Huanta and Río Apurimac, 12°43'40"S, 73°57'07"W, 1883m, 7 May 1929, *E.P. Killip & A.C. Smith 23116* (US); Dist. Anco, alrededores de Buena Gana, ca. 8.5 km lineales al WNW de San Antonio, 1775m, 21 Apr 2007, *J. Roque 5484* (USM); **Cajamarca:** Prov. Chota, a 1 km de Paraguay (Queroto-La Granja), 2250m, 7 Aug 1994, *S. Leiva et al. 1390* (F); Dist. Querocoto, Hacienda La Granja, quebrada San Lorenzo, 6°22'34"S, 73°08'18"W, 2100–2500m, 2 Dec 2012, *P. Gonzáles et al. 2108* (USM); Prov. Jaén, Sallique, camino entre El Espino y Tablón, 5°41'S, 79°19'W, 2500m, 25 Jul 1998, *J.Campos et al. 5379* (MO, USM); Prov. San Ignacio, San José de Lourdes, Santo Tomás, 5°01'S, 78°52'W, 1900m, 7 Apr 1997, *J. Campos & S. Corrales 3796* (BM, F, MO, USM); San José de Lourdes, localidad Laurel, 5°01'S, 78°56'W, 1500–1600m, 17 May 1997, *J. Campos & W. Vargas 3908* (MO, MOL, USM); San José de Lourdes, 5°45'S, 78°56'W, 1020m, 18 Feb 2000, *J. Campos & R. Vásquez 6472* (MO, MOL, NY, USM); Dist. Huarango, Nuevo Mundo, Caseiro Pisaguas, a 2 horas del poblado y al N, margen derecha quebrada Santa Rosa, 5°51'08"S, 78°32'12"W, 1700m, 11 Nov 1997, *E. Rodríguez R. 1909* (BM, HUT, MO); Dist. San Ignacio, El Chaupe, 5°11'12"S, 79°03'12"W, 1880m, 10 Oct 2010, *F. Farruggia et al. 2720* (BM, HAO, NY, UT); San José de Lourdes, campamento Zural, camino al cerro Picorana, 4°59'15"S, 78°54'03"W, 2010m, 29 Jan 1999, *C.Díaz et al. 10555* (MO, MOL, USM); Tabaconas, Santuario Nacional Tabaconas-Namballe, Quebrada Chichilapa grande, 2190m, 11 Nov 1998, *C. Díaz 9972* (MO,NY,USM); Along mud track between San Jose de Lourdes and Monterey de le Frontera, past the village of Diamantes, 4°59'03"S, 78°55'41"W, 1587m, 16 Apr 2013,

T. Särkinen et al. 4577 (USM); **Cusco:** Prov. Calca, ca. 2 km up Lares branch of Calca-Quellouno rd (from junction of the Amparaes branch and the Lares branch), just above large bridge nr rd junction on way to Lares, 12°58'27"S, 72°03'32"W, 2589m, 16 Mar 2012, *S. Knapp et al. 10454* (BM, USM); Prov. La Convención, Dist. Ocobamba, Versalles, 12°46'37"S, 72°16'58"W, 1853m, 21 Nov 2007, *L. Valenzuela et al. 10353* (BM); Prov. Paucartambo, entre los km 128–131 de la carretera Paucartambo-Pilcopata, 2350–2400m, 4 Jul 1992, *A. Cano & O. Riofrío 5402* (USM); Entre los km 126–128 de la carretera Paucartambo-Pilcopata, 2500–2600m, 5 Jul 1992, *A. Cano & O. Riofrío 5516* (USM); Entre los km 128–131 de la carretera Paucartambo-Pilcopata, 2350–2400m, 4 Jul 1992, *A. Cano & O. Riofrío 5455* (USM); 13°03' S, 71°33' W, 1500m, Aug 2010, *P. Chambi & J. Chambi 21* (USM); **Huánuco:** Prov. Chinchao, San Pedro de Carpish, arriba del tunel, camino a la torre eletrica chica, 2770–2820m, 22 May 2002, *I. Salinas 336* (USM); Dept. Chinchao, San Pedro de Carpish, arriba del tunel, 2770–2820m, 3 May 2005, *I. Salinas & H. Beltrán 1144* (USM); **Junín:** Prov. Chanchamayo, on rd from San Ramon to Puyusacha, a private conservation area, 11°05'31"S, 75°24'25"W, 949m, 30 May 2013, *T. Särkinen et al. 4820* (USM); Rd from San Ramón past Vitoc to Union Mantish, past Rio Chilpes, 11°15'05"S, 75°21'22"W, 1309m, 2 Jun 2013, *T. Särkinen et al. 4834* (USM); **Pasco:** Prov. Oxapampa, 2–4 km N of Mallampampa, 10°32'S, 75°45' W, 2200–2400m, 22 Jan 1984, *D.N. Smith & J. Canne 5857* (MO,USM); Parque Nacional Yanachaga-Chemillén, sector San Daniel, zona de amortiguamiento, 10°25'36"S, 75°26'35"W, 2387m, 28 Dec 2008, *M. Cueva & A. Peña 418* (HUSA,USM); Parque Nacional Yanachaga-Chemillén, sector San Tunqui, camino hacia Maria Puñis, 10°16'19"S, 75°30'35"W, 1895m, 6 Feb 2009, *M. Cueva & A. Peña 444* (HUSA); Dist. Huancabamba, Zona de amortiguamiento del Parque Nacional Yanachaga-Chemillén, Rio Chillcatambo, parte media, 10°17'28"S, 75°30'32"W, 1852m, 18 Jul 2008, *A. Monteagudo et al. 16710* (USM); Dist. Huancabamba, Parque Nacional Yanachaga-Chemillén, parte alta de la trocha Tunqui-Cajonpata, sector Tunqui, 10°16'15"S, 75°30'23"W, 1950m, 31 Oct 2007, *A. Monteagudo et al. 15798* (USM); at Ulcumano Ecolodge nr Oxapampa, 10°38'11"S, 75°26'05"W, 2239m, 9 Jun 2013, *T. Särkinen et al. 4856* (USM); Dist. Huancabamba, Sector Tunqui, parte media de la quebrada Muchuy Mayo, 10°17'30"S, 75°31'05"W, 1800m, 29 Oct 2007, *A. Monteagudo et al. 15724* (BM, USM); Dist. Oxapampa, parte media de la quebrada San Alberto (zona de amortiguamiento), 10°33'00"S, 75°22'39"W, 2135m, 8 May 2007, *A. Monteagudo et al. 13936* (BM); Dist. Huancabamba, carretera San Daniel-Tunqui, 10°23'06"S, 73°32'54"W, 1645m, 25 May 2009, *R. Vásquez et al. 35721* (USM); Dist. Huancabamba, sector Tunqui, Parque Nacional Yanachaga-Chemillén, camino hacia Maria Punis, 10°16'31"S, 75°30'59"W, 1895m, 18 Oct 2008, *M. Cueva et al. 216* (USM); Dist. Huancabamba, sector Tunqui, 10°16'42"S, 75°31'01"W, 1784m, 11 Nov 2008, *J.R. Ayerbe & D. Heredia 194* (USM); Dist. Huancabamba, sector San Daniel, Parque Nacional Yanachaga-Chemillén, 10°26'27"S, 75°26'30"W, 2240m, 24 Feb 2009, *M. Cueva & R. Rivera 474* (USM); **Puno:** Prov. Carabaya, Ollachea to San Gaban, 1000–2000m, 17 Jul 1978, *M. Dillon et al. 1115* (MO); Prov. Carabaya, km 258–255 on rd to Juliaca from San Gaban, just before Charcaneque, 13°38'30"S,

70°28'12"W, 1550m, 19 Mar 2012, *T. Särkinen et al. 4029* (USM); Km 250 on rd to Juliaca from San Gaban, just before Uruhuasi, 13°41'23"S, 70°27'26"W, 1918m, 19 Mar 2012, *T. Särkinen et al. 4030* (BM, USM); Prov. Sandia, 2100–2200m, 14 May 1966, *R.Ferreyra 16721* (USM); **San Martín:** Prov. Mariscal Cáceres, between the la playa and Las Papayas camps, 7°00'S, 77°00'W, 2700m, 18 Aug 1986, *K. Young 4265* (USM).

Discussion. *Solanum longifilamentum* is most similar to *S. macrotonum* Bitter of Central and northern South America but these species can be distinguished based on calyx lobes (size and shape) in flower and fruit, anther: filament length ratio, and the length of the style. *Solanum longifilamentum* has oblong, calyx lobes 1.0–1.5 mm long that are slightly spreading in fruit, while *S. macrotonum* has smaller, 0.5–1.0 mm long, triangular lobes that are tightly appressed to the mature fruit. Filaments are a minimum of half the length of anthers in *S. longifilamentum* compared to *S. macrotonum* where filaments are always clearly smaller in relation to anthers. Styles are exerted to only 0.5–1.0 mm beyond the anther cone in *S. longifilamentum,* but extend 1.5–3.5 mm beyond the anthers in *S. macrotonum*. Although leaf shape is generally variable within most *Solanum* species, *S. longifilamentum* has consistently narrower, oblong-lanceolate leaves compared to the more ovate leaves of *S. macrotonum*. Other members of the Morelloid clade with which *S. longifilamentum* could be confused include *S. americanum* Mill. and *S. pseudoamericanum* Särkinen, P. Gonzáles & S.Knapp both of which have smaller anthers c. 1.0–1.5 mm long and curved or included styles, and *S. zahlbruckneri* Bitter that is a larger, broadly spreading shrub up to 2 m high, with larger, violet corollas up to 2 cm in diameter.

Some infraspecific variation in anther size and pubescence can be noted within *S. longifilamentum*. Some specimens from throughout the species range have shorter and slightly narrower anthers 1.7-2.0 mm long and 0.6-0.7 mm wide (*Smith & Canne 5857; Salinas 1144; Knapp et al. 10454; Campos 5379; Cueva & Rivera 474; Gonzáles et al. 2108*). These specimens also have denser leaf pubescence on both surfaces, especially on young growth, and more broadly ovate leaves. The variation appears to correlate with environmental conditions rather than geography, where with denser pubescence and smaller anthers seem to grow in drier habitats based on herbarium specimen labels plants.

Specimens belonging to this new taxon have been identified under various names, including *S. macrotonum* and *S. nigrescens* Martens & Galeotti, neither of which occur in Peru.

***Solanum antisuyo* Särkinen & S.Knapp, sp. nov.**
urn:lsid:ipni.org:names:77144534-1
Figs 3–4

Diagnosis. Like *Solanum polytrichostylum* Bitter, but differing in having either simple or once-branched inflorescences with pedicels spaced c. 1–3 mm apart along the rachis,

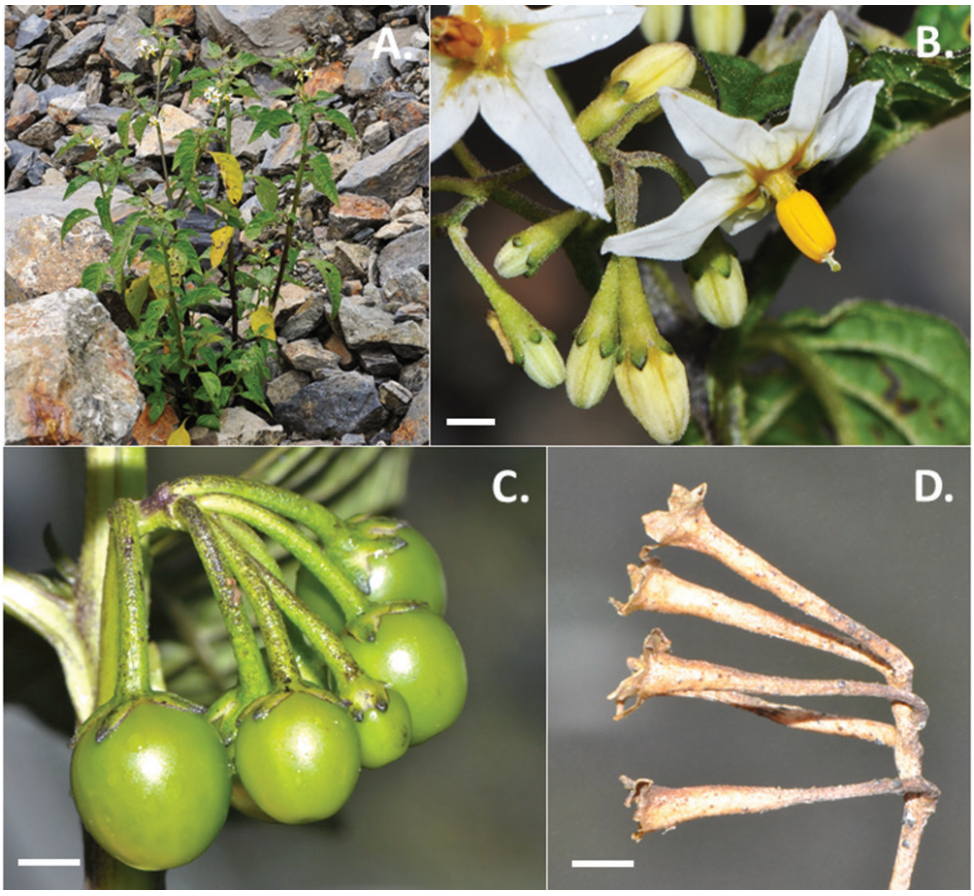

Figure 3. Photos of *Solanum antisuyo*. **A** Buds and flowers, showing the distinct calyx with long tube and minute but thick purple coloured lobes (*Knapp et al.* 10399) **B** Habit, growing in a rocky land slide in gravel (*Knapp et al.* 10399) **C** Woody pedicels of the infrutescence, distinct character in herbarium specimens (*Knapp et al.* 10401) **D** Ellipsoid fruits with reflexed pedicels, and the characteristic appressed calyx lobes that split into two in fruit (*Knapp et al.* 10435). Scale bars = 2 mm. All photos by S. Knapp.

more reduced, minute calyx lobes, ellipsoid rather than spherical fruits, and larger brown-coloured seeds.

Type. Peru. Cusco: Prov. Paucartambo, 1 km from Puesto de Vigilancia of Parque Nacional de Manu on rd from Paucartambo to Pilcopata coming from Puesto, 13°12'05"S, 71°37'21"W, 3480 m, 15 Mar 2012 (fl,fr), *S. Knapp, P. Gonzáles, A. Matthews & T. Särkinen 10435* (holotype: USM; isotypes: BM [BM001114929], F, HUSA, HUT, MO).

Description. Stout herb to a shrub up to 1.5 m tall, much branching at base, the individual branches up to 1m long. Stems 2-ridged or slightly winged especially towards base, 0.4–0.6 cm in diameter, purple-coloured especially at leaf nodes, nearly glabrous, sparsely pubescent with simple uniseriate, much reduced 1–3-celled trichomes

especially on the often purple coloured young growth. Sympodial units difoliate, not geminate. Leaves simple, 2–17 cm long, 1.2–8.4 cm wide, broadly ovate-lanceolate, membranous to somewhat fleshy; adaxial and abaxial surfaces sparsely pubescent with more or less appressed 1–3-celled simple uniseriate trichomes 0.1–0.2 mm long; primary veins 7–10 pairs; base rounded, decurrent on the petiole; margins entire, often purple tinged; apex acute to acuminate; petiole 0.3–1.2 cm long, occasionally narrowly winged, sparsely pubescent with simple uniseriate trichomes like those of the stems and leaves. Inflorescences 1.4–4.0 cm long, lateral and internodal, simple or once-branched, with 5–14 flowers arising very close together, sparsely pubescent with appressed 1–2-celled simple uniseriate trichomes similar to those on stem and leaves; peduncle 1.0–3.3 cm long, if the inflorescence branched then the peduncle rachis 0.2–0.4 cm long, short and congested; pedicels 1.0–1.2 cm long, 0.5–0.6 mm in diameter at the base tapering gradually to 1.0–1.2 mm in diameter at apex, straight and spreading at anthesis, recurving and becoming woody in fruit, not dehiscing; pedicel scars spaced 0–2 mm apart. Buds conical-ellipsoid, cream-coloured, the corolla strongly exerted from the calyx tube before anthesis. Flowers 5-merous, all perfect; calyx tube 1.5–2.0 mm long, green, the lobes 0.7–0.9 mm long, broadly deltate with rounded apices, purple coloured, sparsely pubescent with 1-celled simple uniseriate trichomes; corolla 12–24 mm in diameter, stellate, white or rarely lilac with a yellow to yellow-green central star at the base, lobed slightly less than halfway to the base, the lobes ca. 9–15 mm long, 4–5 mm wide, spreading to reflexed at anthesis, pubescent abaxially with 1–3-celled simple uniseriate trichomes shorter than the trichomes of the stems and leaves, sparsely pubescent adaxially at base near the filaments with 5–7-celled simple uniseriate trichomes; filament tube ca. 2 mm long, adaxially pubescent with 5–7-celled simple uniseriate trichomes; free portion of the filaments ca. 2 mm long, sometimes slightly longer in two lowermost anthers at anthesis (elongating after anthesis?), pubescent like the tube; anthers ca. (2.8)3.0–3.4 mm long, 1 mm wide, ellipsoid, yellow, poricidal at the tips, the pores lengthening to slits with age; ovary cylindrical, pubescent 2/3 from the base with 2–3-celled simple uniseriate trichomes; style 6 mm long, exerted (0.5)1–2 mm beyond the anther cone, densely pubescent up to 2/3 of the length with 2–3-celled simple uniseriate trichomes at the base; stigma globose, minutely papillate, pale yellow in live plants. Fruit an ellipsoid berry, 8–11 mm in diameter, green turning translucent green-orange when ripe (purple-black in *Knapp et al. 10404* but these affected by pathogens?), the surface smooth and shiny when young, with relatively thick pericarp ca. 0.1 mm; fruiting peduncle woody; fruiting pedicels 11–22 mm long, purple coloured, ca. 1 mm in diameter at the base and 1.5 mm at apex, reflexed and woody in fruit, remaining on the plant after fruit drops; fruiting calyx lobes tightly appressed to the berry, purple-coloured, calyx often splitting into two larger lobes. Seeds 35–45 per berry, ca. 1.1 mm long, ca. 1.7 mm wide, concave-reniform, narrower at one end, brown, the hilum positioned sub-laterally towards the narrower end, the testal cells pentagonal in outline; stone cells few per fruit.

Distribution. Andean Ecuador, Peru, and Bolivia; growing in secondary vegetation, disturbed roadsides, landslides, and gravely slopes in ceja de Selva, montane cloud

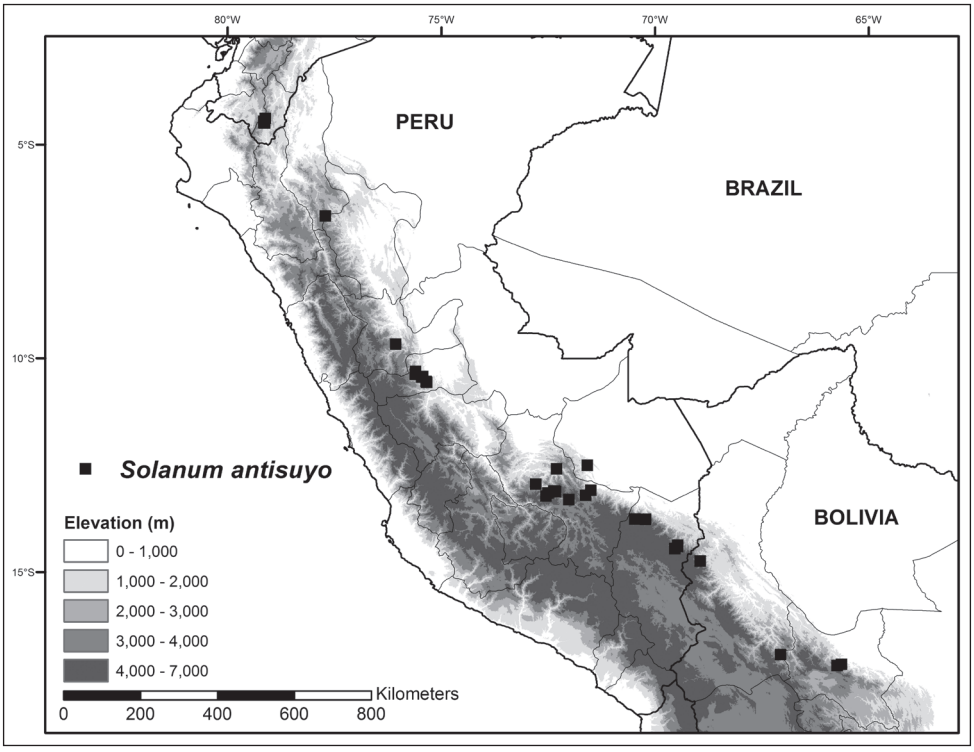

Figure 4. Distribution map of *Solanum antisuyo*, endemic species to Peru.

forest and *Polylepis* forests, associated with *Chusquea* (Poaceae). *Gunnera* (Gunneraceae), *Cecropia* (Urticaceae) and *Weinmannia* (Cunoniaceae); 2,000–3,600 (-3,900) m in elevation.

Ecology. Flowering and fruiting throughout the year, peak in March and June–July.

Etymology. The species name refers to Antisuyo (also Antisuyu), Quechua, for the eastern (*anti*) region (*suyo/suyu*) of the Inca territory, that referred to the lands on the eastern Andean slopes. The species is most diverse along the eastern flanks of the Andes in southern Peru, and the name is chosen to reflect this.

Conservation status. The preliminary conservation status (IUCN 2010) of *S. antisuyo* is here considered of least concern (LC) based on the relatively large EOO (ca. 692,500 km^2), although the AOO (136 km^2) would merit listing as endangered (EN). The species grows readily in disturbed sites and combined with its wide range, it appears to have relatively low threat status despite the generally increasing human pressure and habitat destruction in the Andes.

Specimens examined. BOLIVIA. Cochabamba: Chapare: along highway from Villa Tunari to Cochabamba, at Rio San Jacinto bridge, 54.5 km SW of bridge over Rio Espirito Santo, 17°11'09"S, 65°44'49"W, 1915m, 1 May 2007, *M. Nee 55288* (NY); Chapare: New highway to the Chapare, ca. 4 km beyond Laguna Corani, 3140m, 15 Jul 1994, *N. Ritter 1231* (NY); Chapare: El Limbo, 17°09'38"S, 65°38'22"W,

2900m, 18 Dec 2007, *J. Terán 1757* (NY); **La Paz:** Franz Tamayo: Madidi, Tanhuara Area Natural de Manejo Integrado Apolobamba, bajando del campamento Tanhuara cruzando el Río Pelechuco, 14°44'47"S, 68°56'38"W, 2291m, 11 Jul 2009, *I. Loza 1086* (NY); Inquisivi: Following the slopes E of Communidad Micayani to the Río Khokhoni more or less to the junction with a fork flowing down from Communidad Yamora, and following the Río Khokhoni upstream 1 km from this point, ca. 4 km SE from Inquisivi, 16°91'67"S, 67°10'00"W, 14 Jan 1989, *M. Lewis 35076* (G,MO); Nor Yungas: Unduavi, 3200m, Feb 1914, *O. Buchtien 463* (BM, G, US). **ECUADOR. Loja:** Loja: Parroquia Yangana, rd descending from Cerro Toledo antennas toward Yangana, 4°23'02"S, 79°06'58"W, 3050m, 27 Mar 2005, *L. Bohs 3321* (BM, LOJA, NY, QCNE, UT); Loja: Rd from Yangana to boundary of Zamora-Chinchipe, 2560m, 13 Jul 1984, *W.G. D'Arcy 15728* (NY); **Napo:** Napo-Pastasa, E.N.E of Cayambe Mountain, Oriente trail, 3261m, 6 Dec 1961, *P.C.D. Cazalet 5523* (K); between Tena and Papallacta, 12 Jan 1981, *W.G. D'Arcy 14108* (NY); **Pichincha:** Quito: Termas de Papallacta Hot Springs, 67 km E of Quito nr the Hwy, 0°20'S, 78°10'W, 3300m, 18 Aug 2005, *J.L. Clark 9444* (BM, NY, QCNE, US); Quito: Parroquia, Nono, Reserva Yanacocha, Fundacion de Conservacion Jocotoco, 0°07'00"S, 78°35'10"W, 3500m, 13 May 2007, *J.L. Clark 9534* (NY); **Zamora-Chinchipe**: Rd from Yangana to Zumba, 2438m, 13 Jul 1984, *W.G. D'Arcy 15734* (NY); Rd from Vilcabamba to Valladolid, 27 km from Yangana, 2682m, 12 Jul 1986, *W.G. D'Arcy 16468* (BM,NY); Parque Nacional Podocarpus, km 26 on rd Yangana-Valladolid, 4°29'S, 79°09'W, 2550m, 2 Dec 1988, *J.E. Madsen 75741* (BM). **PERU. Amazonas:** Prov. Chachapoyas, Dist. Leymebamba, Cordillera Yasgolga, slopes W of summit El Rayo, 6°40'01"S, 77°43'05"W, 3287m, 24 Jun 2009, *R.W. Bussmann et al. 15721* (HUT); Prov. Bagua, SE of la Peca, 2362–2461m, 16 Oct 1978, *P. Barbour 4088* (USM); **Cusco:** Prov. La Convención, Dist. Echarate, Quebrada Lorohuachana, sector Laco, Santuario Nacional Megantoni, 3400m, 21 Jun 2008, *L. Hernani A 1037* (HUSA); Dist. Echarate, Quebrada Lorohuachana, sector Laco, Santuario Nacional Megantoni, 3533m, 18 Jun 2008, *L. Hernani A 968* (HUSA); Dist. Echarate, Quebrada Lorohuachana, sector Laco, Santuario Nacional Megantoni, 3400m, 21 Jun 2008, *L. Hernani A 1033* (HUSA); Bosque del Chuyapi, 12°56'33"S, 72°47'04"W, 2100m, 19 Jul 2006, *L. Valenzuela et al. 7282* (USM); Bosque del Chuyapi, 12°56'33"S, 72°47'04"W, 2100m, 19 Jul 2006, *L. Valenzuela et al. 7284* (USM); Ca. 51 km from Ollantaytambo on rd over Abra Malaga to Quillabamba and Quelluno, ca. 10 km below Abra Malaga, Amazon slope, 13°07'15"S, 72°19'34"W, 3877m, 13 Mar 2012, *S. Knapp et al. 10399* (BM, USM); Ca. 57 km from Ollantaytambo on rd over Abra Malaga to Quillabamba and Quelluno, ca. 16 km below Abra Malaga, Amazon slope, 13°06'30"S, 72°21'04"W, 3579m, 13 Mar 2012, *S. Knapp et al. 10401* (BM, USM); Ca. 61 km from Ollantaytambo on rd over Abra Malaga to Quillabamba and Quelluno, ca. 21 km below Abra Malaga, Amazon slope, 13°06'15"S, 72°22'03"W, 3448m, 13 Mar 2012, *S. Knapp et al. 10404* (BM, USM); Ca. 61 km from Ollantaytambo on rd over Abra Malaga to Quillabamba and Quelluno, ca. 21 km below Abra Malaga, Amazon slope, 13°06'15"S, 72°22'03"W, 3448m, 13 Mar 2012, *S. Knapp et al. 10406*

(BM, USM); Prov. Paucartambo, 3500–3600m, 14 Jul 1990, *A. Cano 3636* (USM); Parque Nacional de Manu, 3500–3600m, 11 Jul 1990, *A. Cano 4327* (USM); Parque Nacional de Manu, 3600–3700m, 6 Mar 1991, *A. Cano 4593* (USM); Prov. Urubamba, Aguas Calientes, Quebrada Alccamayo, 13°09'01"S, 72°30'17"W, 2050–2200m, 29 Aug 2002, *I. Huamantupa & G. Calatayud 2258* (MO, USM); **Huánuco:** Prov. Huánuco, along rd between Huánuco and Tingo María, 1.1 km N of Carpish Tunnel, ca. km 455, 9°40'S, 76°04'W, 2680m, 1 Jun 1998, *T.B.Croat & M. Sizemore 81568* (BM, MO, USM); Carpish, above Acomayo, lower ceja, 2800m, 17 Jul 1964, *P.C. Hutchison et al. 5931* (MO); Carpish, carretera Huanuco-Tingo Maria, 2700–2900m, 3 Oct 1950, *R. Ferreyra 8164* (MOL, USM); North of Carpish Pass, rd from Huánuco to Tingo Maria, 50–52 km NE of Huánuco, 2350–2430m, 6 Dec 1981, *T. Plowman & P.M. Rury 11141* (HB,USM); Prov. Pachitea, 2450m, 24 Dec 1979, *HHV 001519* (USM); Prov. Tingo Maria, 2900m, 21 Jun 1980, *HHV 3050* (USM); **Junín:** Prov. Huancayo, 3200m, 17 Jun 1972, *D. Tovar T. 5* (USM); **Pasco:** Prov. Oxapampa, Dist. Oxapampa, Parque Nacional Yanachaga-Chemillén, sector San Alberto, alrededores del Refugio el Cedro, 10°32'26"S, 75°21'18"W, 2483m, 26 Apr 2009, *M. Cueva & R. Rivera 625* (HUSA, USM); Dist. Huancabamba, Parque Nacional Yanachaga-Chemillén, sector San Daniel, 10°26'09"S, 75°27'07"W, 2192m, 29 Feb 2008, *R. Vásquez et al. 33798* (USM); Parque Nacional Yanachaga-Chemillén, quebrada diablo fuerte, trocha hacia la parcela Oso-Playa, 10°18'14"S, 75°36'14"W, 2398–2500m, 23 Jun 2008, *A. Monteagudo et al. 16477* (USM); Dist. Huancabamba, Zona de amortiquamiento del P.N. Yanachaga-Chemillén, sector Milpo, 10°22'20"S, 75°36'29"W, 2800m, 23 Sep 2004, *A.Monteagudo et al. 7326* (K); Dist. Huancabamba, Parque Nacional Yanachaga-Chemillén, sector San Daniel, 10°25'48"S, 75°27'00"W, 2500–3500m, 1 Mar 2009, *M. Cueva et al. 492* (USM); **Puno:** Prov. Carabaya, margen derecha del rio que pasa frente al campamento Chacayage, 13°45'48"S, 70°13'07"W, 2600m, 10 Mar 2004, *S. Vilca C. et al. 71* (HUSA); km 238–239 on rd to Juliaca from San Gaban, ca. 8–9 km before Ollachea and Puente Chillichaca, 13°45'30"S, 70°28'09"W, 2451m, 19 Mar 2012, *T. Särkinen et al. 4035* (USM); Prov. Sandia, 8 km from Sandia on rd to Cuyocuyo, 14°22'08"S, 69°28'23"W, 2709m, 21 Mar 2012, *T. Särkinen et al. 4048* (BM, USM); 16 km from Sandia on rd to Cuyocuyo, 14°24'06"S, 69°28'25"W, 2990m, 21 Mar 2012, *T. Särkinen et al. 4049* (BM, USM); in Cuyocuyo outside of a house on main rd to Sandia, 14°27'09"S, 69°32'03"W, 3364m, 21 Mar 2012, *T. Särkinen et al. 4053* (BM, USM).

Discussion. *Solanum antisuyo* is morphologically most similar to *S. polytrichostylum* Bitter with which it has been conflated in the past. It can be distinguished by simple or once-branched inflorescences where pedicels are spaced ca. 1–3 mm apart along the short rachis compared to consistently branched inflorescences with the flowers congested at the branch tips in *S. polytrichostylum*, and ellipsoid fruits as compared to the spherical fruits of *S. polytrichostylum*. *Solanum antisuyo* has a longer calyx tube with more reduced, poorly developed purple-tinged calyx lobes compared to the shorter calyx tubes with slightly larger, triangular calyx lobes in *S. polytrichostylum*, and larger brown coloured seeds compared to smaller yellow seeds in *S. polytrichostylum*. Further-

more, styles are always more exerted (2–4 mm versus 1–2 mm beyond the anther cone) in *S. polytrichostylum*. The two species are also ecologically somewhat distinct, with *S. polytrichostylum* restricted to streams and moist road sides, and *S. antisuyo* is found in drier areas in gravel, disturbed areas, and landslides. Other members of the Morelloid clade in Peru without glandular trichomes with which *S. antisuyo* could be confused include *S. probolospermum* Bitter that has smaller, spherical fruits, larger violet corollas that are more rotate in outline, and denser indumentum with longer 3–7-celled simple hairs, and *S. pallidum* Rusby (incl. *S. planifurcum* Bitter) that is similar to *S. probolospermum* but has branched rather than simple hairs.

Variation in growth form and flower colour can be observed in the field, where individuals growing in more humid conditions grow into stout herbs to ca. 1.5 m tall, whilst individuals in drier, higher elevation habitats in rocky landslides are stunted herbs reaching only ca. 40 cm in height. Colour variation in corolla is common within Morelloids and *Solanum* species in general: most specimens of *S. antisuyo* have creamy white petals, but occasional specimens with lilac corollas are known (e.g., *Särkinen et al. 4048, 4049*, and *4053*).

Solanum arenicola **Särkinen & P. Gonzáles, sp. nov.**
urn:lsid:ipni.org:names:77144535-1
Figs 5–6

Diagnosis. Like *Solanum aloysiifolium* Dunal, but differing in having simple, subumbellate inflorescences, and a dense indumentum of multicellular glandular-tipped trichomes; also similar to *Solanum subtusviolaceum* Bitter, but differing in having internodal inflorescences, much reduced calyx lobes to only 0.5 mm long, corolla deeply lobed to 2/3 of the way to the base, and a more exerted style extending 2–3 mm beyond the anther cone at anthesis.

Type. Peru. Madre de Dios: Prov. Tambopata, in the boat harbor of Infierno, c. 20 km SW by road from Puerto Maldonado, 12°44'06"S, 69°13'47"W, 186 m, 3 Aug 2014 (fl,fr), *T. Särkinen & A. Balarezo 4866* (holotype: USM; isotypes (to be distributed): BM, E, F, GHMDD, HOXA, MO, MOL).

Description. Herb or vigorous, weak-stemmed shrub 0.2–1.5 m tall. Stems angled, sparsely to densely pubescent with simple, translucent, uniseriate 3–8-celled trichomes 0.8–2 mm long with glandular tips; new growth densely pubescent with spreading glandular trichomes like those of the stem. Sympodial units difoliate, not geminate. Leaves simple, 2.6–13 cm long, 0.8–5 cm wide, ovate to broadly ovate, membranous; adaxial surface glabrous; abaxial surface paler or tinged with purple, sparsely pubescent with simple uniseriate trichomes like those of the stem restricted to the veins; primary veins 5–7 pairs; base acute to cuneate and decurrent on the petiole; margins variable in shape from entire to undulate to shallowly lobed; apex acute-acuminate; petiole 0.5–5.0 cm long, sparsely to densely pubescent with glandular trichomes like those of the stems. Inflorescences 2.0–3.5 cm long, lateral and internodal, simple, with 3–8(9)

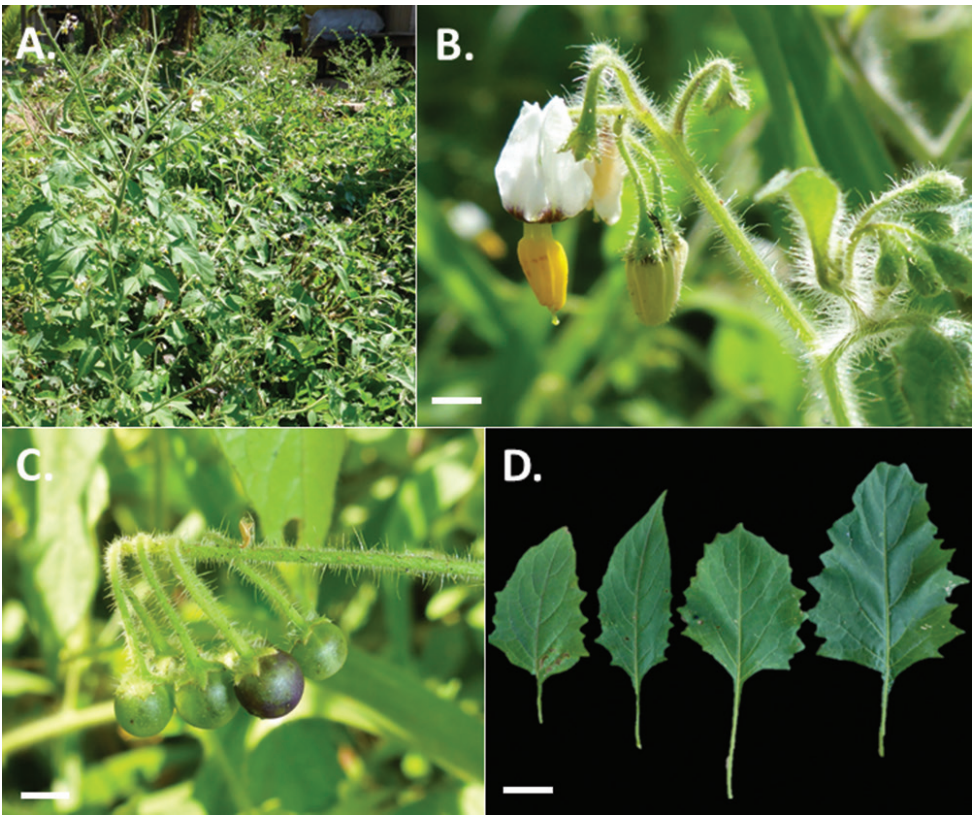

Figure 5. Photos of *Solanum arenicola*. **A** Habit **B** Buds and flowers, showing the dense indumentum of glandular-tipped, multi-cellular hairs throughout **C** Maturing fruits, showing reflexed pedicels in infrutescence **D** Leaf size and shape variation present within individuals as observed in the field (**A–D** *Särkinen & Balarezo 4866*). Scale bars = 1 mm. All photos by T. Särkinen.

flowers, sparsely to densely pubescent with spreading glandular trichomes like those of the stem; peduncle 1.0–2.4 cm long; pedicels 0.5–0.7 cm long, ca. 0.3 mm in diameter at the base and 0.4 mm at apex, straight and spreading, articulated at the base; pedicel scars unevenly spaced 1.0–2.5 mm apart. Buds ellipsoid, the corolla strongly exerted from the calyx tube long before anthesis. Flowers 5-merous, all perfect; calyx tube ca. 1 mm long, shallow, the lobes 0.2–0.5 mm long, triangular with acute apices, sparsely to densely pubescent with glandular trichomes like those of the stem; corolla 8–12 mm in diameter, stellate, white with a purple-yellow or yellow-green central eye at the base, lobed 2/3 to the base, the lobes ca. 3.5–4.0 mm long, 1.0–1.5 mm wide, strongly reflexed at anthesis, later spreading, densely pubescent abaxially with glandular trichomes like those of the stems, glabrous adaxially; filament tube 1.0–1.2 mm long; free portion of the filaments slightly unequal in length, the lower two ca. 1.5 mm long, the upper three ca. 1.0–1.2 mm long, sparsely pubescent with simple uniseriate 1–3-celled trichomes on the side facing the ovary; anthers 3.0–4.0 mm long, 0.8–0.9 mm wide

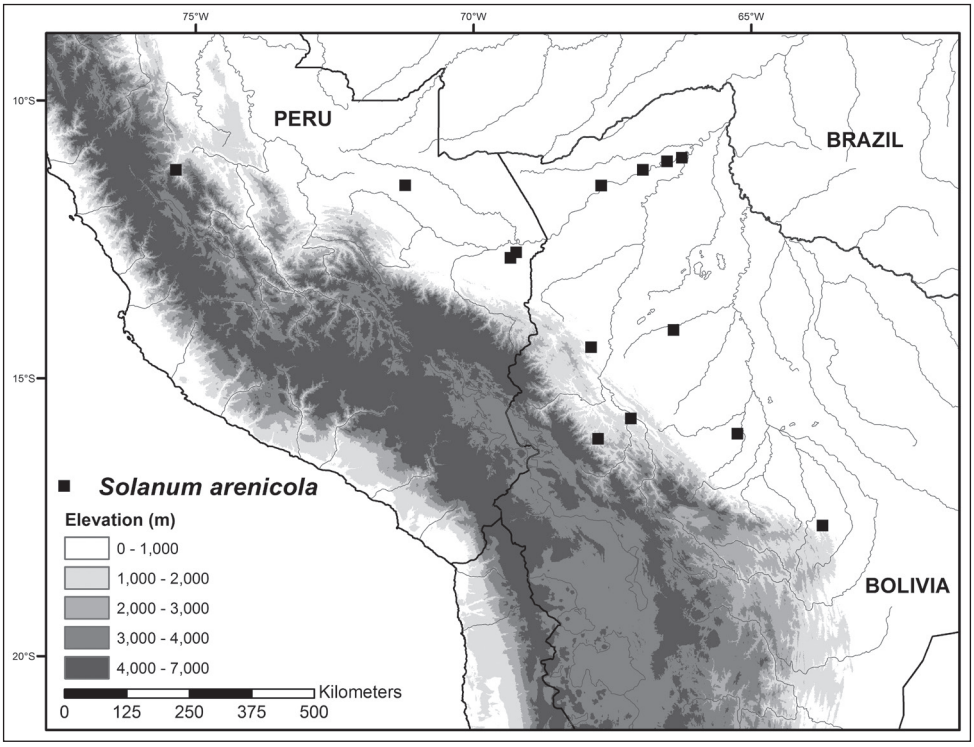

Figure 6. Distribution map of *Solanum arenicola* in lowlands of central and southern Peru, and northern Bolivia.

at base and 0.5–0.6 mm wide at apex, cylindrical, narrowing towards the apex, yellow, poricidal at the tips, the pores lengthening to slits with age; ovary ellipsoid, glabrous; style 4–5.7 mm long, exerted 2.0–3.0 mm beyond the anther cone, densely pubescent up to 2/3 of the length with 1–6-celled simple uniseriate trichomes, these longer at the base and becoming gradually shorter towards the middle; stigma clavate, minutely papillate. Fruit a globose berry, 3.5–7.0 mm in diameter, green, turning black when ripe; fruiting peduncle 2.0 cm long; fruiting pedicels 1.0–2.0 cm long, ca. 0.5 mm in diameter at the base and 0.6 mm at apex, strongly recurved; fruiting calyx lobes appressed to the berry, the tips not reflexed. Seeds 35–45 per berry, ca. 0.8 mm long, ca. 0.6 mm wide, flattened-reniform, narrowing towards one end, yellow, the sub-laterally positioned hilum positioned towards the narrower end, the testal cells pentagonal in outline; stone cells few per fruit.

Distribution. In lowland Bolivia and Peru; in lowland moist rain forest in sandbanks and river margins, tree fall gaps, and in disturbed sites near housing and fields in open, sandy soil, with occasional records from seasonally dry semi-deciduous forests with *Hura crepitans* L. (Euphorbiaceae), *Gallesia integrifolia* (Spreng.) Harms (Phytolaccaceae), *Bougainvillea modesta* Heimer (Nyctaginaceae), and *Anadenanthera colubrina* (Vell.) Brenan (Amaranthaceae); most commonly associated with lowland rain

forest pioneer species, including *Salix humboldtiana* Willd. (Salicaceae), *Tessaria integrifolia* Ruiz & Pav. (Asteraceae), *Cecropia* spp. (Urticaceae), *Calliandra* sp. (Fabaceae), *Neea* spp. (Nyctaginaceae), *Garcinia* spp. (Clusiaceae), and *Jacaratia digitata* (Poepp. & Endl.) Solms (Caricaceae), and annual herbs such as *Glinus radiatus* (Ruiz & Pav.) Rohrb. (Molluginaceae), *Physalis angulata* L., *P. peruviana* L., and *Solanum americanum* Mill. (Solanceae); 0–600 (1,300) m elevation.

Ecology. Flowering January–February and August–October, fruiting September–October.

Etymology. *Solanum arenicola* is named for its habitat preference as deduced from both field observations and specimen labels. The species prefers growing on sand (*cola* = "liver on", and *arena* = "sand") and is most commonly collected from sand bars, loose sandy soils on land slides, at the base of tree falls on open, loosened sandy soil, or in open-sandy soils in disturbed areas around houses and cultivated plots.

Conservation status. The preliminary IUCN (2010) conservation status of *S. arenicola* is here considered of least concern (LC) based on the relatively large EOO (c. 412,600 km^2), although the small AOO (56 km^2) would merit listing with endangered (EN) status. The species grows in disturbed sites along rivers, tree falls, and cultivations where bare sandy soils are available, and its association with other pioneer species indicates that the species is not sensitive to human disturbance from expanding construction and agriculture.

Specimens examined. BOLIVIA. Beni: Prov. Ballivian, Carmen Florida, Rio Beni, 7 km upstream from Rurrenabaque, 320m, 13 Sep 1989, *D.E. Williams 960* (US); **La Paz:** Prov. Abel Iturralde, Parque Nacional Madidi, laguna Chalalan, Río Yariapo, c. 14°26'41"S, 67°53'05"W, 275m, 26 Sep 2006, *A. Araújo-M et al. 3130* (NY); Prov. Franz Tamayo, Tuichi, Río San Juan, Buenahora., 14°12'01"S, 68°39'21"W, 840m, 1 Oct 2005, *A. Araújo-M et al. 2071* (BM); **Pando:** Prov. Manuripi, large sandbar in Río Madre de Dios, 8 km (by air) NNE of Nueva Etea, 11°15'S, 66°57'W, 125m, 22 Aug 1985, *M. Nee 31497* (K); Prov. Madre de Dios, Puerto Candelaria, along the Rio Madre de Dios, 21 km (by air) WSW of Riberalta, c. 11°02'00"S, 66°15'00"W, 125m, 18 Aug 1985, *M. Nee 31398* (NY); Prov. Manuripi, Conquista, Embarcadero sobre el Madre de Dios, 150m, 2 Feb 1983, *J. Fernández Casas 8591* (NY); Along Rio Madre de Dios between Trinidadcito and San Miguel, 50 km (by air) WSW of Riberalta, c. 11°06'S, 66°31'W, 125m, 21 Aug 1985, *M. Nee 31483* (NY); Along Rio Madre de Dios, Camacho, 11°32'S, 67°42'W, 135m, 2 Sep 1985, *M. Nee 31724* (NY); **Santa Cruz:** Ichilo: Parque Nacional Amboró, along Rio Saguayo, 0.5 km NE of entrance into first Andean foothills, 1465m, 22 Dec 1988, *M. Nee 37350* (MO, US); **PERU. Cusco:** Prov. Paucartambo, 6 Aug 1974, *R.B. Foster et al. 3021* (USM); **Junín:** Chanchamayo: La Merced, 610m, 10 Aug 1923, *J.F. Macbride, 5314* (F); Chanchamayo: Rd from San Ramon past Vitoc to Union Mantish, past Rio Chilpes, 11°15'08"S, 75°21'36"W, 1309m, 2 Jun 2013, *T. Särkinen et al. 4831* (USM); **Madre de Dios:** Prov. Tambopata, Explorer'S, Inn, nr the confluence of Rio Tambopata and Rio La Torre, 39 km SW of Puerto Maldonado, 400m along the Laguna Chica Trail, c. 12°50'S, 69°20'W, 17 Jan 1989, *S.F. Smith et al. 1354* (NY).

Discussion. *Solanum arenicola* can be easily distinguished from *S. americanum* Mill., the only other similar Morelloid species found in lowland Amazonia; it has larger anthers which are 3.0–3.2 mm long in *S. arenicola* as compared to the minute anthers < 1.5 mm of *S. americanum*. Specimens without locality information can be easily confused with *S. nigrescens* M.Martens & Galeotti of Central and northern South America, *S. aloysiifolium* Dunal of middle to high elevation Argentina and Bolivia or *S. subtusviolaceum* Bitter of low to middle elevation Peru and Bolivia. Both *S. arenicola* and *S. nigrescens* have simple inflorescences, but *S. arenicola* differs in having longer anthers (3.0–3.2 mm) compared to *S. nigrescens* (1.5–2.2 mm) and in the possession of glandular hairs (*S. nigrescens* is eglandular). The anthers are similar in size and shape to those of *S. aloysiifolium*, but *S. arenicola* has simple inflorescences and glandular-tipped hairs, while *S. aloysiifolium* has branched inflorescences (sometimes many branched) and lacks glandular hairs. *Solanum subtusviolaceum* possesses the same dense, glandular-haired indumentum as *S. arenicola*, but differs from it in having inflorescences with the flowers clustered near the tips positioned opposite the leaves rather than arising along the internodes, longer calyx lobes (2–3 mm versus 0.2–0.5 mm), more rotate corollas lobed only halfway to the base, and less exerted styles (to a maximum of 1 mm versus exerted to 3 mm beyond the anther cone).

Solanum arenicola is one of the few morelloids known from lowland humid forests in South America. Most morelloid species grow > 2,000 m or in drier habitats along the western slope of the Andes or in the inter-Andean valleys, whilst *S. arenicola* is restricted to humid forests on the eastern side of the Andes below 1,200 m elevation. Currently, the species is known from central and southern Peru and from Bolivia, but it is likely that the species also occurs in adjacent areas of Brazil in the state of Rondônia, where the Río Madre de Dios and Río Beni join and cross the Brazilian border, especially considering habitat preferences of *S. arenicola* for disturbed, sandy soils along river banks.

The Potato clade

Within the non-spiny taxa of *Solanum*, the Potato clade forms a strongly supported monophyletic group (Särkinen et al. 2013) that comprises the potatoes, tomatoes and a series of smaller groups (e.g., section *Pteroidea* Dunal, Knapp and Helgason 1998, Tepe et al. 2010 and section *Herpystichum* Bitter, Tepe et al. 2011). *Solanum* sect. *Basarthrum* (Bitter) Bitter is one of these smaller groups and is distinguished by the presence of distinctive uniseriate few-celled trichomes termed bayonet hairs (sensu Seithe and Anderson 1982) and basal pedicel articulation. The taxonomy and reproductive biology of these species have been treated by Anderson and colleagues (Anderson 1975, 1976, 1977, 1979; Anderson et al. 1991; Seithe and Anderson 1982), but new species continue to be discovered in the Andes, a centre of species diversity in the group (Anderson et al. 2006; Prohens et al. 2006). The new species described here is clearly a member of this group, but has unusual calyx morphology and growth form.

***Solanum mariae* Särkinen & S.Knapp, sp. nov.**
urn:lsid:ipni.org:names:77144536-1
Figs 7–8

Diagnosis. Like *Solanum caripense* Dunal, but differing in having distinctive calyx lobes 2.0–2.5 mm long and 1.8–2.0 mm wide, broadly ovate in shape and spreading in buds and flowers, and in having fruits fully enclosed in an accrescent calyx.

Type. Peru. Cajamarca: Prov. San Marcos, Dist. Chancay, 14 km from San Marcos, just S of Chancay, on road from San Marcos to Cajabamba, 7°24'20"S, 78°07'05"W, 2606 m, 9 May 2013 (fl,fr), *S. Knapp, T. Särkinen, H.M. Baden, P. Gonzáles & E. Perales 10571* (holotype: USM; isotypes: BM [BM001034677], CPUN, E [E00700640], HUT, MOL).

Description. Trailing herbs, stems to 20–30 cm tall arising from woody trailing stems that root at nodes, the individual stems up to 5 m long. Stems terete, 1.5–2.5 mm in diameter, moderately to densely pubescent with spreading bayonet hairs (uniseriate, 2-celled hairs with an elongate, thicker-walled basal cell capped by a short acuminate cell) and with simple, 2–4-celled uniseriate glandular-tipped finger hairs c. 0.5 mm long; new growth densely pubescent with trichomes like those of the stems; bark of older stems grey-brown, smooth. Sympodial units plurifoliate, not geminate. Leaves simple, 1.4–3.5 cm long, 1.0–1.6 cm wide, ovate-lanceolate; adaxial surface moderately pubescent with bayonet hairs like those on the stems, and with simple, 2-celled uniseriate glandular-tipped hairs c. 0.3 mm long; abaxial surface more densely pubescent with trichomes like those of the upper surface; primary veins 4–6 pairs; base acute to obtuse; margins entire; apex rounded; petiole 0.5–1.2 cm long, moderately to densely pubescent with trichomes like those of the stems. Pseudostipules in pairs, simple, 5 mm long, 3 mm wide, ovate-lanceolate, tip acute, resembling leaves in shape and appearance. Inflorescences 1.5–2.7 cm long, lateral and internodal, simple, with 3–5 flowers in the distal half, moderately to densely pubescent with spreading trichomes like those of the stems; peduncle 0.4–1.6 cm long; pedicels 0.6–0.7 cm long, ca. 0.3 mm in diameter at the base and apex, straight, curved at the tip, articulated at the base; pedicel scars spaced ca. 1 mm apart. Buds globose, the corolla only exerted from the calyx tube just before anthesis. Flowers 5-merous, all perfect; calyx tube ca. 1.5–2.0 mm long, the lobes 2.0–2.5 mm long, 1.8–2.0 mm wide, broadly deltate, with acute apices, spreading in bud and flower, moderately to densely pubescent; corolla 1.2–1.5 cm in diameter, shallowly stellate, white, lobed halfway to slightly less than halfway to the base, the lobes ca. 4–5 mm long and 4–5 mm wide, spreading at anthesis, moderately to densely pubescent abaxially with trichomes like those of the stem, glabrous adaxially; filament tube minute, glabrous; free portion of the filaments ca. 1.0–1.2 mm long, glabrous; anthers 2.7–3.0 mm long, ca. 2.5 mm wide, ellipsoid, yellow, poricidal at the tips, the pores lengthening to slits with age; ovary conical, glabrous; style 5–6 mm long, exerted 1.5–2.0 mm beyond the anther cone, glabrous; stigma clavate, minutely papillate, yellow-green in live plants. Fruit

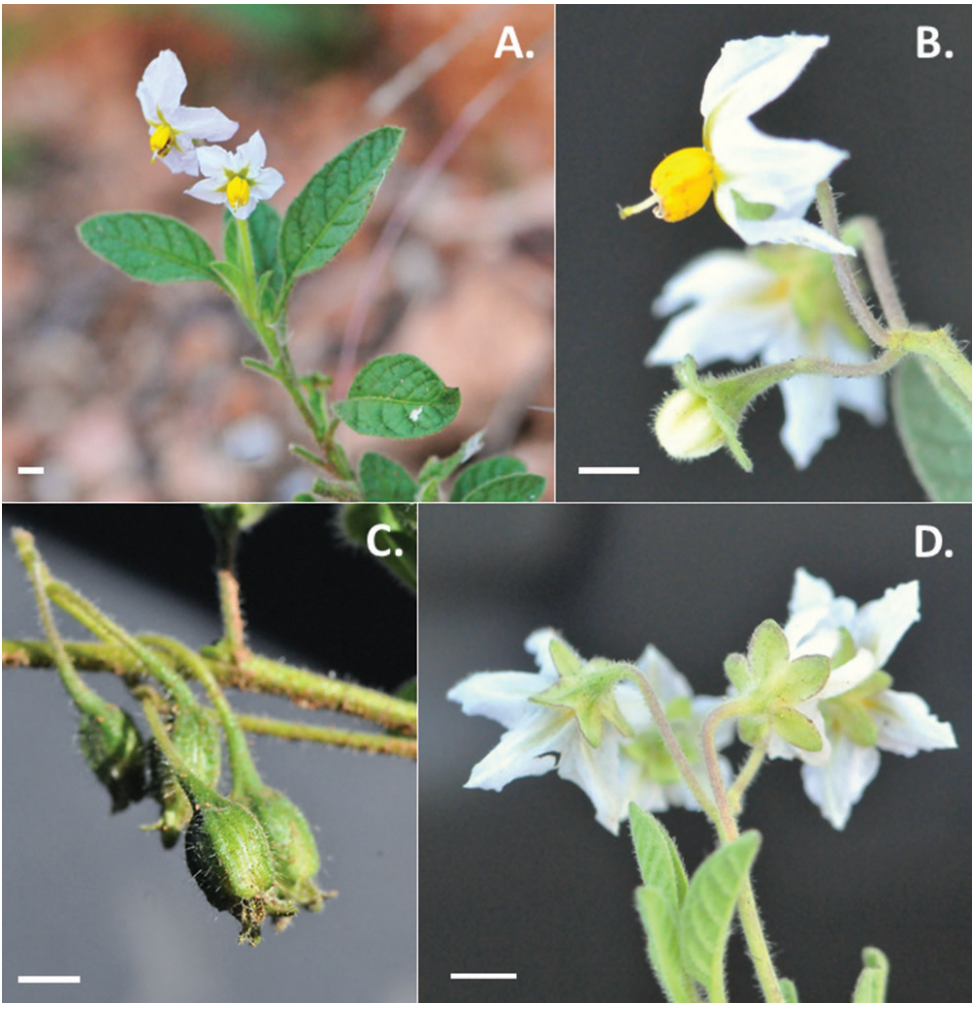

Figure 7. Photos of *Solanum mariae*. **A** General habit **B** A floral bud and a flower at full anthesis **C** Maturing fruits enclosed in calyx **D** Distinct calyx lobes spreading in flower (**A–D** *Särkinen & Baden 4651*). Scale bars = 2 mm. All photos by T. Särkinen.

(immature) an ellipsoid berry, 8–9 mm long and 6.8 mm wide when developing, with the mesocarp ca. 0.2 mm wide, green, fully enclosed in the accrescent calyx, glabrous, mature fruits not seen; fruiting peduncle 1.3–2.2 cm long; fruiting pedicels 1.8–2.3 cm long, 0.3–0.5 mm in diameter at the base and 0.5–1.8 mm at apex, reflexed 180° in fruit; fruiting calyx 8–9 mm long, 3.5–4.0 mm wide and still developing, appressed to and enclosing the entire berry, the calyx lobes spreading at the mouth of enclosing tube. Seeds 30–40 per berry, 1.2–1.5 mm long, 1.0–1.2 mm wide, flattened-reniform, yellowish, the surfaces minutely pitted, the hilum positioned laterally in the middle, the testal cells pentagonal in outline.

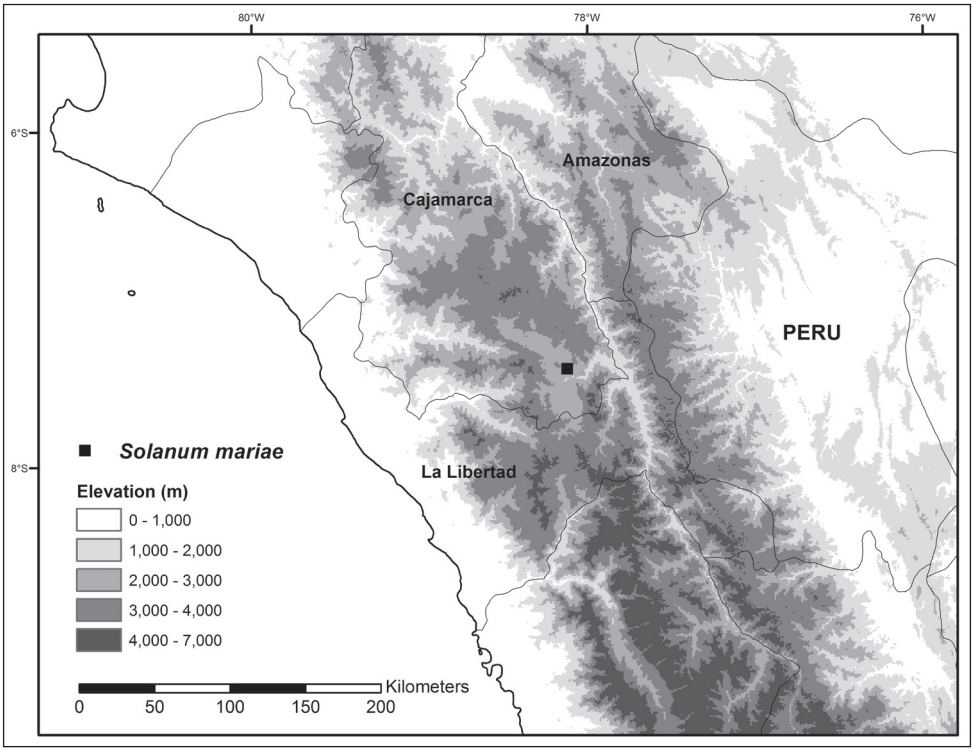

Figure 8. Distribution map of *Solanum mariae,* a narrow endemic from northern Peru.

Distribution. Endemic to Peru; growing along north facing banks in loamy soils in along roadsides, not in full sun, associated with *Lycianthes lycioides* (L.) Hassl. (Solanaceae) and various grasses; only known from a single population at 2,600 m elevation from San Marcos Province in the Department of Cajamarca.

Ecology. Flowering and fruiting April–May.

Etymology. The species is named after biologist Maria Baden who collected the first specimen in Cajamarca in 2013 with the authors. Many of the collections made in our field trips in Peru since 2006 would not have been made without her, and to honour the number of hours spend on plant spotting from fast moving vehicles and driving carefully through recent landslides, we name this shy and stunningly beautiful *Solanum* species in her honour.

Conservation status. The IUCN (2010) threat status of *S. mariae* is here considered of critically endangered (CR) based on only a few known occurrence points near Chancay in the San Marcos province, Department of Cajamarca, Peru, with AOO of 4 km^2. The species appears to have specialist habitat requirements, preferring north-facing shady cliff sides. The known populations are both small, and vulnerable to grazing pressures.

Specimens examined. PERU. Cajamarca: Prov. San Marcos, c. 14 km from San Marcos towards Cajabamba, 7°24'20"S, 78°07'05"W, 2604m, 25 Apr 2013, *T. Särkinen & H.*M. Baden 4651 (USM).

Discussion. *Solanum mariae* can be easily distinguished from other section *Basarthrum* species based on the large, broadly ovate and spreading calyx lobes in flower that become to enclose the entire fruit that are not known from any other species of the section. Further distinguishing characters include the combination of trailing stems that root along nodes, the relatively dense pubescence of long, 2–4-cellular glandular-tipped finger hairs throughout the plant, and the strictly simple leaves. In Peru, the species is most similar to *S. caripense* Dunal and closely allied species, but differs in having simple leaves combined with the glandular-tipped finger hairs throughout mature plants and larger calyx lobes that are spreading in flower and are accrescent and enclose the fruit. Anderson and Bernardello (1991) provide a key to the members of series *Caripensia* Correll to which the new species clearly belongs based on morphology.

Based on the style extension well beyond anthers in the newly described species, *S. mariae* is likely to be self-incompatible. Style extension has been found to indicate self-incompatibility in sect. *Basarthrum* in previous studies (e.g., Anderson 1979).

The trailing growth form of the new species, where roots are formed at leaf nodes, has been observed in other members of sect. *Basarthrum*, as well as in the closely related sect. *Anarrhichomenum* Bitter (Tepe et al. 2012), whose members are distinguished from those of sect. *Basarthrum* by the presence of pseudostipules, winged seeds, and fruits maturing red or orange.

The central Andes region is a centre of diversity for *Solanum* sect. *Basarthrum* (Stern et al. 2008; Anderson 1979; Anderson et al. 2006; Särkinen et al. in prep.), and the new species described here adds to the growing list. *Solanum mariae* remains poorly understood based on our two collections from the single known population from the San Marcos Province of the Department of Cajamarca. No further specimens have been seen in local herbaria in Trujillo and Cajamarca. The locality lies within the watershed of the Río Marañón, and further field work in the same watershed in the Province of San Marcos is a priority in order to increase knowledge of this rare species, including the morphology of the fully mature fruits.

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Supplementary material 1

Occurrence records of the four new Solanum species

Authors: Tiina Särkinen, Paúl Gonzáles, Sandra Knapp

Data type: occurence

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Species delimitation and recognition in the *Pediomelum megalanthum* complex (Fabaceae) via multivariate morphometrics

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Abstract

Pediomelum is a genus endemic to North America comprising about 26 species, including the *megalanthum* complex, which consists of *P. megalanthum* and its varieties *retrorsum* and *megalanthum*, *P. mephiticum*, and the recently described *P. verdiense* and *P. pauperitense*. Historically, species of the *megalanthum* complex have been variably recognized at the species or variety levels, dependent upon the relative importance of morphological characters as diagnostic of species. Ten quantitative morphological characters regarded as diagnostic at the species level were analyzed using multivariate morphometrics across these taxa in order to examine the discriminatory power of these characters to delineate species and to aid in species delimitation. The analyses support the recognition of *P. megalanthum*, *P. mephiticum,* and *P. verdiense* at the species level, *P. retrorsum* as a variety under *P. megalanthum*, and suggest the sinking of *P. pauperitense* into *P. verdiense*. The findings of the present study help quantify the power of certain characters at delimiting taxa and provide a basis for taxonomic revision of the *P. megalanthum* complex.

Keywords

Pediomelum, principal component analysis, multivariate morphometrics, species delimitation, cluster analysis, Fabaceae

Introduction

Pediomelum Rydb. (Psoraleeae; Leguminosae) includes about 26 species, all native to North America (Rydberg 1919). The genus radiated recently and rapidly, with diversification shifts taking place within the last 2 mya, likely due to the impacts of Pleistocene glaciations (Egan and Crandall 2008a). This recent radiation is illustrated in the shallow branch lengths within phylogenies, relative to related genera within the Psoraleeae tribe, even phylogenies based on a combined dataset of eight DNA markers (Egan and Crandall 2008b). This is also reflected in the disparate taxonomic views among botanists, with several taxa variably recognized at specific or varietal levels. The most taxonomically contested group of species lie within subgenus *Disarticulatum sensu* Grimes (1990), which includes 10 species, with most variably restricted to areas of Texas and the deserts of the southwest U.S.

An example of these contrasting taxonomic views can be found within the subspecific classification of *P. megalanthum* (Wooton & Standl.) Rydb. Some botanists have recognized this as having three varieties (e.g. Grimes 1990; Isely 1998): var. *megalanthum* is found mostly in Uintah, Grand, and San Juan counties down the eastern border of Utah as well as neighboring counties in Colorado; var. *epipsilum* (Barneby) Grimes is endemic to the Dixie Corridor of Kane Co., UT and neighboring Coconino Co., AZ; var. *retrorsum* (Rydb.) Grimes is restricted to Nye, Lincoln, and Clark Co, NV, Mohave, Coconino, and disjunct in Graham Co AZ. Other botanists treat each as a separate species (e.g. Rydberg 1919; Welsh et al. 1993). Grimes (1990) favors varietal ranking based on overlapping qualitative and quantitative morphological characters, with no clear diagnostic features enabling species distinction. Isely (1998) even throws *P. mephiticum* (S. Watson) Rydb. into the '*megalanthum-mephiticum-epipsilum*' complex. On the other hand, Welsh et al. (1993) utilized directionality of pedicel and peduncle hairs (*P. retrorsum* Rydb. vs. *P. megalanthum*) and absence of pubescence on the upper leaf surface (*P. epipsilum* (Barneby) S.L.Welsh) as diagnostic characters to maintain their distinctiveness.

Given the narrow distributions of these taxa and the disparate views on the usefulness of certain morphological characters for species delimitation in this group, many might see this as an example of the age old war between lumpers, those who tend to recognize fewer species, often allowing considerable breadth of morphological variation as inherent in species concepts, and splitters, those who split species based on more minute morphological differences, sometimes down to the population level. Hewitt C. Watson eloquently exemplified this war in a letter to Charles Darwin dated 13 August 1855 wherein he wrote "Taking J. D. Hooker & Jordan as representative men for the opposite factions in botany,—'lumpers & splitters', the former would reduce the species of vascular plants to three score thousand, or perhaps much fewer;—while Jordan would raise them to three hundred thousand." (Darwin Correspondence Database, 25 Sept 2014).

Whilst preparing the treatment of *Pediomelum* for the Flora of North America, I was confronted with the question of where I lie on the spectrum of lumpers vs. split-

ters, for *P. megalanthum* and its varieties, and in particular, in regards to two recent species described by Welsh and Licher (2010): *P. pauperitense* S.L.Welsh, Licher, & N.D. Atwood, described based on six collections, and *P. verdiense* S.L.Welsh & Licher, described from four specimens. These species are said to differ from *P. mephiticum* in the directionality of pedicel and peduncle hairs as well as the shape of the lateral and upper calyx teeth. The species are said to differ distinctly from each other in the size of pedicels, bracts, seeds, and flower lengths, as well as flower color and peduncle length. They join the ranks of what I refer to as the *P. megalanthum* complex in subgenus *Disarticulatum*.

The rapid radiation of the genus coupled with the variable recognition of specific or varietal ranking and the contrasting opinions of the relative discriminatory power and usefulness of several morphological characters invites an approach using multivariate morphometric analysis as a means of delimiting species or varieties among contested taxa. This is particularly so in the case of the newly described *P. pauperitense* and *P. verdiense*. This work will additionally examine the relative power of certain quantitative morphological characters historically used to delimit species in this group. Here, I aim to objectively delimit species within the *P. megalanthum* complex using a multivariate morphometric approach, incorporating the morphological diversity of *P. megalanthum* (vars. *megalanthum* and *retrorsum*), *P. mephiticum*, *P. verdiense*, and *P. pauperitense*. I have chosen to recognize *P. epipsilum* at the specific level (discussed below) and so it is not included in these analyses.

Methods

Plant material – Twenty-seven herbarium specimens from Utah and Arizona (deposited at ARIZ, ASC, BRY, and US) were chosen for study and tentatively identified as *P. mephiticum*, *P. megalanthum* var. *megalanthum*, *P. m.* var. *retrorsum*, *P. pauperitense*, and *P. verdiense*. A list of the specimens included in the morphometric analyses, with voucher information and origin, are given in Table 1. Effort was made to obtain all identified specimens of *P. verdiense* and *P. pauperitense* through a request to ARIZ, ASC, and BRY for such, especially those listed in Welsh and Licher (2010). However, some specimens were not available for loan. Because of this, comparatively few specimens for these taxa were available. So as not to over-weigh the analyses with specimens from *P. mephiticum* and *P. megalanthum* and its varieties, and thereby introducing sampling bias, a comparable number of these specimens were used as well, resulting in a set of specimens smaller than that used in some other studies. However, trends were still very visible in these data. Most specimens were collected or annotated by botanists having authority on the genus, including S.L. Welsh, J.W. Grimes, A.N. Egan, M. Licher, and N.D. Atwood, and taxonomic identifications were initially accepted according to the most recent annotation on the specimens. Those recognized at the specific vs. subspecies levels with the same epithet were analyzed together, i.e. *P. retrorsum* was grouped in analyses with *P. megalanthum* var. *retrorsum*.

Table 1. Specimens and their source herbaria used for morphometric analyses. Only first collectors listed. *specimens listed as paratypes by Welsh and Licher (2010).

P. megalanthum var. *megalanthum*: (BRY) -Belnap 244, Licher 1915, Welsh 22771, Welsh 27822, Welsh 22787
P. megalanthum var. *retrorsum*: (BRY) - Bundy 140, Atwood 4798, Hughes 3
P. mephiticum: (BRY) - Baird 3080, Welsh 23478, Atwood 5148, Egan 126, Neese 16864; (US) - Atwood 3903, Holmgren 3290, Jones 5095, Jones 5064b
P. pauperitense: (BRY) - Higgins 23137*, Atwood 18013
P. verdiense: (ARIZ) - Wojciechowski 212*, Harbison 41.312*, Demaree 43938; (ASC) - Rink 1840*, Licher 2347; (BRY) - Licher 2009, Licher 2015, Licher 2007.

Table 2. Morphometric characters used in this study.

Character acronym	Detailed description of the character
flower length	from the base of the calyx to the tip of the banner
calyx length	from the base of the calyx to the tip of the lower calyx tooth
calyx tube	from the base of the calyx to the beginning of the calyx teeth
lower calyx tooth	from point of attachment on calyx to tip
stipules	from point of attachment to tip
petioles	from point of attachment to base of petiolule
leaflets	from point of attachment to petiolule to tip of terminal leaflet
bracts	from point of attachment to tip
peduncle	from point of attachment on stem to base of first pedicel
pedicel	from point of attachment to peduncle to base of calyx

Characters scored – Ten quantitative morphological characters (Table 2) were scored. Characters were chosen based on those quantitative traits widely used in flora and monographic works to distinguish species, particularly flower and calyx characters, as well as those characters used by Welsh and Licher (2010) as diagnostic of species. Other studies have used a similar number of characters for morphometric analysis within species complexes with positive information content (e.g. Kaplan and Marhold 2012; Lihová et al. 2004). Characters were scored for between two and five sites per specimen using digital calipers. While there are a few qualitative characters – such as direction of pedicel and peduncle hairs – that are used by some to differentiate taxa (i.e. *P. megalanthum* varieties), these were not included here. Different researchers have debated the relative utility and importance of directionality of hairs as diagnostic of species or lower-level taxa (see discussion above). I chose explicitly not to include these characters because my focus is on the use of quantitative (continuous) morphological traits with the aim to determine whether quantitative traits can separate taxa along similar lines as some previous researchers do for species vs. subspecies based on the qualitative character of vestiture (e.g. *P. megalanthum* varieties).

Multivariate morphometric analyses – A combination of multivariate analyses and hierarchical clustering were employed to investigate species limits in this group. All statistics were computed in the statistical package JMP v. 11.1.1 (SAS Institute Inc.,

Cary, NC). As an initial step, correlation coefficients were computed on the total dataset and on each species' dataset to reveal any highly correlated character pairs that may distort downstream analyses. In addition, departure from a normal distribution for each character within each species was tested using the Shapiro-Wilk goodness of fit test (Shapiro and Wilk 1965).

Morphometric multivariate analyses were conducted on values of individual measurements without averaging across multiple observations per specimen. This was done because of the limited number of specimens available for *P. pauperitense* and *P. verdiense*. Use of all observations both within and across specimens will likely provide a better view of the intraspecific variation within a character. This is akin to Pedersen's (2010) justification for using values measured on each individual plant as opposed to using a population mean.

A hierarchical cluster analysis (HCA) was performed to investigate how specimens would group based on overall morphological similarity using Ward's minimum variance method with the data standardized by standard deviation (Ward Jr 1963). This method groups specimens by minimizing the increase in the error sum of squares upon each addition of a cluster. Because the use of multiple methods is recommended to ascertain the robustness of clusters (Marhold 2011), UPGMA (unweighted pair-group method using arithmetic averages) with data standardized by standard deviation was also employed (Sokal and Michener 1958).

Principal component analysis (PCA; Sneath and Sokal 1973) was used to delineate patterns of morphological variation across the *P. megalanthum* complex. This method is a good first tool for investigating overall patterns in morphology as each character is weighted the same. This was first applied to the complete dataset with all characters and species included. For greater resolution among the main groups, PCA was then conducted on two subgroups: i) *P. mephiticum*, *P. verdiense*, and *P. pauperitense;* ii) *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*.

Canonical discriminant analyses (CAN) were employed to investigate the spread of means across each species group and determine how well the characters (Y) predicted the separation of species based on means. This method measures the distance of each point from the centroid, or multivariate mean, of its group as defined previously by species or subspecies. The distance measure is based on the Mahalanobis distance, which incorporates the variances and covariances between variables. CAN is classically implemented using a linear method, which assumes that Y variables are normally distributed with the same variances and covariances, or a quadratic method in which covariances can be different across groups. Because not all of the character distributions were normal, I employed a regularized, compromise method, which is a mixture between the linear and quadratic methods (Friedman 1989). The regularized method incorporates two parameters: lambda deals with the shrinkage to a common covariance and ranges from 0 = quadratic to 1 = linear; gamma deals with the shrinkage to diagonal and ranges from 0 = no shrinkage to 1 = diagonals only. A low gamma is suggested when variables are correlated. Here I used a lambda proportional to the number of non-normal character distributions by species ($\lambda = 0.8$) and a gamma of 0.

Among the multivariate analyses employed here, several have been used by other researchers to determine those variables causally impacting the separation of species. As a variable reduction technique, PCA helps to discern which characters are responsible for grouping individuals. However, it does not assume an underlying causal model. For determining those characters responsible for delineating species, factor analysis may be a more appropriate method as this technique makes the explicit assumption of an underlying causal model (Jolliffe 2005; Suhr 2005). Factor analysis was performed using principal components and the varimax rotation (FAPC) as well as the maximum likelihood framework with varimax rotation (FAML). Similar to PCA, CAN reduces the variable space to those discriminants that are responsible for assigning individuals to previously defined groups, and is often employed to determine those variables with the most discriminatory power (e.g. Koch et al. 2013; Semple and Chmielewski 1987). Stepwise discriminant analysis (SDA) is a method designed to order variables by discriminatory power, adding variables in a stepwise fashion according to the amount of correlation of the variable to the reduced eigenvectors. SDA was compared to results from PCA, CAN, and factor analyses to provide a robust investigation into which of the morphological characters are most informative at delineating species or taxa.

Each analysis was conducted on a series of three data sets or levels: (1) the first dataset included all species, (2) the second dataset includes only data from *P. mephiticum*, *P. verdiense*, and *P. pauperitense* (the MVP group), (3) the third data set includes the *P. megalanthum* varieties, *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*. Datasets are thus notated by the type of analysis and the dataset used such as CAN1, HCA2, PCA3, and so on.

Results

Following Kaplan and Marhold (2012), a cutoff value of 0.90 for the correlation between characters was used to ascertain exclusion of characters from the data analyses. Correlation coefficients did not exceed 0.90 for any character pair within any species, and so all characters were used in subsequent analyses. Only three character pairs across all species had correlation coefficients above 0.8 in either the positive or negative direction: *P. verdiense* had a correlation coefficient of 0.8775 for calyx:lower calyx tooth; *P. m.* var. *retrorsum* exhibited a negative correlation of -0.8108 for pedicel:bract; *P. pauperitense* exhibited a correlation of 0.8783 for flower:bract. For all species, the correlation between calyx:lower calyx tooth was between 0.6 and 0.8, an expected result considering the overlapping nature of these characters. However, inclusion of both incorporates the plasticity of calyx morphology into the overall analysis – a key character used for species delimitation – and thus all characters are kept for further analyses.

The vast majority of character distributions fit a normal curve, with three rejecting the null hypothesis of a normal fit only marginally ($0.04 < p < 0.05$; *P. m.* var.

retrorsum:lower calyx tooth, *P. mephiticum*:peduncle & petioles), three rejecting moderately ($0.02 < p < 0.04$; leaflets for *P. verdiense* & *P. pauperitense*), three rejecting strongly ($0.001 < p < 0.01$; *P. pauperitense*:calyx tube*, *P. m.* var. *megalanthum*:flowers & petioles*), and one rejecting very strongly ($p=0.0003$; *P. mephiticum*:pedicel*), those distribution with outliers detected are notated with an asterisk. Summary statistics for each character by species are given in Table 3.

Ward's cluster analysis of all specimens (HCA1) produced a dendrogram with two main groups: one comprised of entirely *P. mephiticum*, *P. verdiense*, and *P. pauperitense* with the exception of a single *P. m.* var. *megalanthum* data point, and the other group comprised of three subgroups, two comprising mixtures of the two *P. megalanthum* varieties and one comprised mainly of *P. verdiense* (Fig. 1). UPGMA cluster analysis (data not shown) produced three main clusters, two comprised of a mixture of *P. megalanthum* varieties, one with a few *P. verdiense* or *P. mephiticum* specimens included, and one comprised wholly of the *P. mephiticum-verdiense-pauperitense* complex (hereafter denoted as the MVP group), again with exception of the single *P. m.* var. *megalanthum* data point. Considering the strong support for the MVP group, a separate hierarchical analysis was conducted on the MVP group only (HCA2). This analysis suggests two main clusters, one cluster almost entirely of *P. mephiticum* data points with two *P. verdiense* data points included therein, and a second main cluster mostly comprised of *P. verdiense* and *P. pauperitense* with three *P. mephiticum* data points scattered throughout (Fig. 2). A cluster analysis of the *P. megalanthum* varieties (HCA3) showed no clear division between taxa (data not shown).

The ordination diagram from the principal component analysis based on all specimens (PCA1; Fig. 3) also suggested two main groups. Specimens of *P. mephiticum*, *P. verdiense*, and *P. pauperitense* were separated from the *P. megalanthum* varieties along the first axis with all characters contributing to this division. The second axis separated *P. mephiticum* from a mixture of *P. verdiense* and *P. pauperitense* but did not separate the *P. megalanthum* varieties from each other. Floral characters (flower, calyx, calyx tube, lower calyx tooth, pedicel) vs. vegetative characters (peduncle, petiole, bracts, stipules, and leaflets) separated *P. verdiense* and *P. pauperitense* from *P. mephiticum* along the second axis, with vegetative characters contributing more to differentiation along the second component (Fig. 3; Table 5).

Independent principal component analyses were also conducted on the two main subgroups defined by PCA1. PCA2, comprising the MVP group, showed good separation along the first axis of *P. mephiticum* from *P. verdiense* and *P. pauperitense*, with floral vs. vegetative characters strongly affiliated with this break (Fig. 4A). However, *P. verdiense* and *P. pauperitense* created a mixed group with most of the *P. pauperitense* data points clustering below the second axis (capturing 20% of the variation in the data) amidst *P. verdiense*, suggestive of a lack of differentiation normally found between species. The same result is found in PCA3, the analysis of the differentiation between *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*, illustrating a mixture of *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum* data points (Fig. 4B). Contributions of characters to each multivariate axis for each of the three PCA analyses are listed in Table 4.

Table 3. Summary statistics for each character by species. number of observations (n), minimum (min) and maximum (max) values, mean, standard deviation (st. dev.), and 5th and 95th percentiles.

Taxon	Parameter	flower	calyx	calyx tube	lower calyx tooth	stipules	petiole	leaflets	bracts	peduncle	pedicel
P. megalanthum	Min–max	12–18	11.8–16.2	4.8–8.1	6.5–10.2	6.8–12.4	44–150	16–33	5.7–13	8.5–80	3–6.2
var. *megalanthum*	Mean/St.dev.	16/1.716	13.81/1.213	6.28/0.802	8.08/1.103	9.57/1.538	72.05/23.125	25.53/5.265	9.34/1.930	34.15/21.185	4.07/0.896
n=20	5-95 Quantiles	12.03–17.98	11.81–16.16	4.81–8.07	6.5–10.19	6.81–12.38	44.1–147.25	16.15–32.95	5.74–12.98	8.59–79.5	3–6.17
P. mephiticum	Min–max	9.4–12.6	8.2–11.7	2.6–4.1	5.3–8.3	7.4–14	32–120	15–35	6.8–12.5	19.7–65	2.3–5.7
n=34	Mean/St.dev.	10.94/0.583	10.12/0.798	3.52/0.399	6.74/0.734	9.69/1.427	64.76/23.745	24.36/5.195	10.1/1.428	40.64/13.099	3.09/0.734
	5-95 Quantiles	9.93–12.15	8.43–11.48	2.75–4.1	5.45–8.15	7.85–12.58	32.75–113.25	16.5–34.25	7.78–12.43	20.68–64.25	2.3–4.8
P. pauperitense	Min–max	10.7–12.2	9.8–12.1	4.2–5.5	5.5–7.9	8.7–13	57–98	14–28	3.6–7.3	17–54	3–4.2
n=10	Mean/St.dev.	11.53/0.585	10.84/0.869	4.59/0.465	6.49/0.791	10.54/1.377	71.7/14.345	19.6/5.211	5.83/1.218	33.7/11.814	3.62/0.405
	5-95 Quantiles	10.7–12.2	9.8–12.1	4.2–5.5	5.5–7.9	8.7–13	57–98	14–28	3.6–7.3	17–54	3–4.2
P. megalanthum	Min–max	14.8–19.8	12.2–16.2	6.2–7.7	6.4–9.6	7.6–12.9	40–94	18–29	6.8–12.3	19–62	3–5.1
var. *retrorsum*	Mean/St.dev.	17.78/1.402	14.45/1.19	6.94/0.533	7.69/1.154	10.2/1.818	65.42/15.785	22.23/3.265	9.53/1.745	38.4/15.995	3.82/0.667
n=12	5-95 Quantiles	14.8–19.8	12.2–16.2	6.2–7.7	6.4–9.6	7.6–12.9	40–94	18–29	6.8–12.3	19–62	3–5.1
P. verdiense	Min–max	10.3–15	8.5–13	3–4.9	4.5–9	6–11.7	22–95	14–30	5–10	11–35	2.3–6
n=34	Mean/St.dev.	12.2/0.983	10.82/1.216	4.09/0.412	6.88/1.239	8.9/1.37	52.63/15.876	20.75/4.465	7.13/1.092	22.53/7.184	3.93/0.912
	5-95 Quantiles	10.43–14.03	8.7–13	3.2–4.84	4.89–9	6.13–11.7	23.95–84.6	14.65–30	5.46–9.35	11.65–34.35	2.56–6

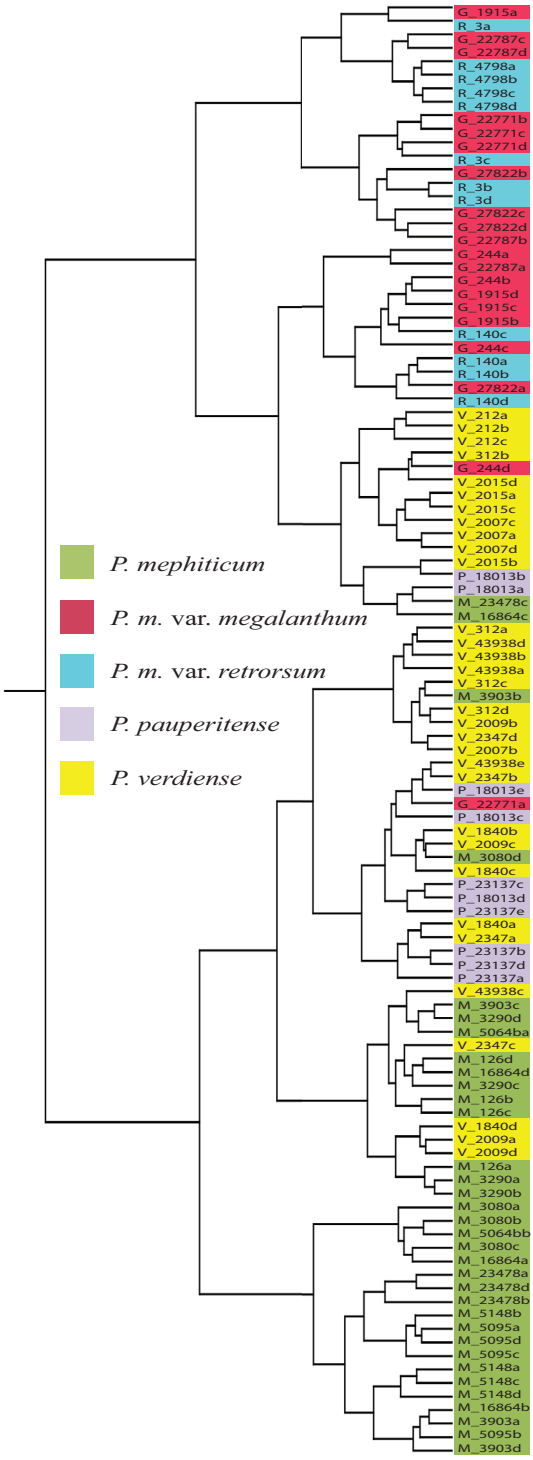

Figure 1. Dendrogram based on all characters for all specimens using Ward's cluster analysis.

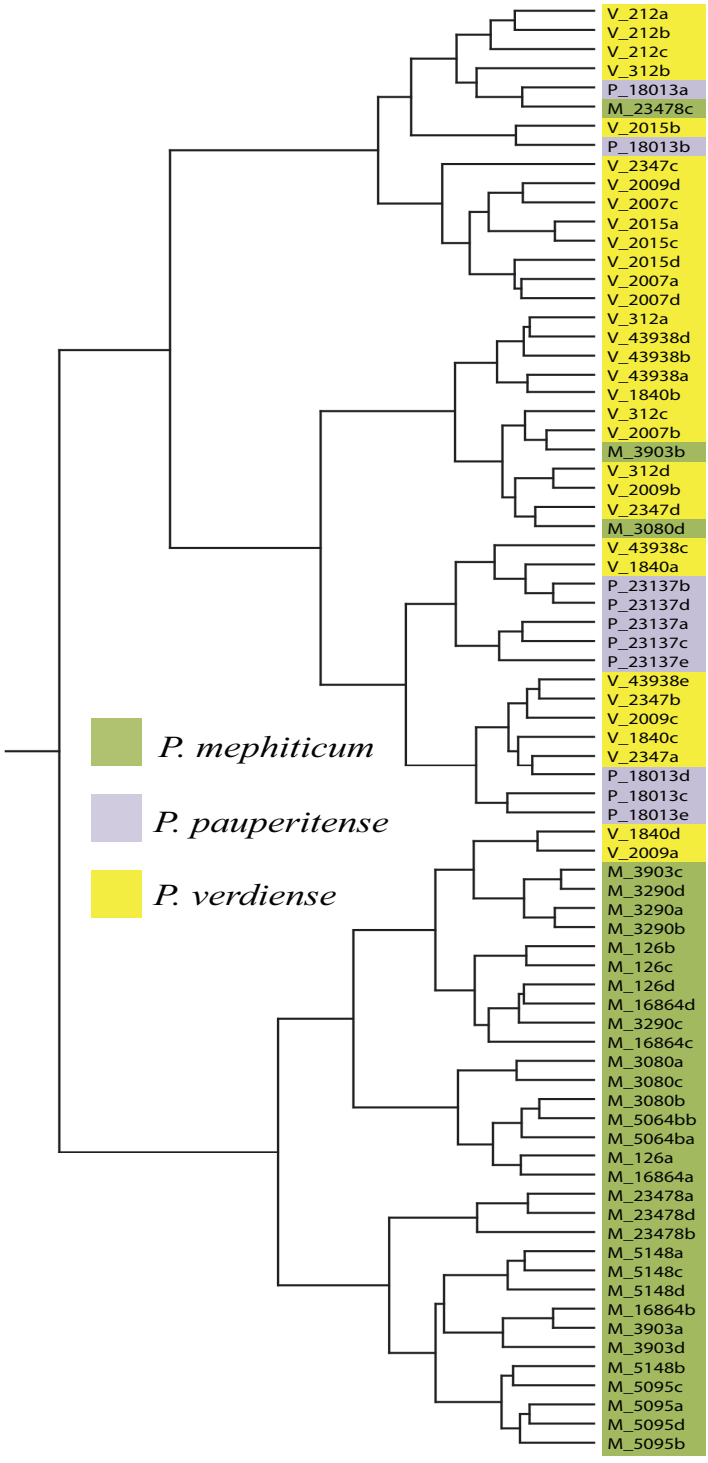

Figure 2. Dendrogram using Ward's cluster analysis for *P. mephiticum*, *P. verdiense*, and *P. pauperitense*.

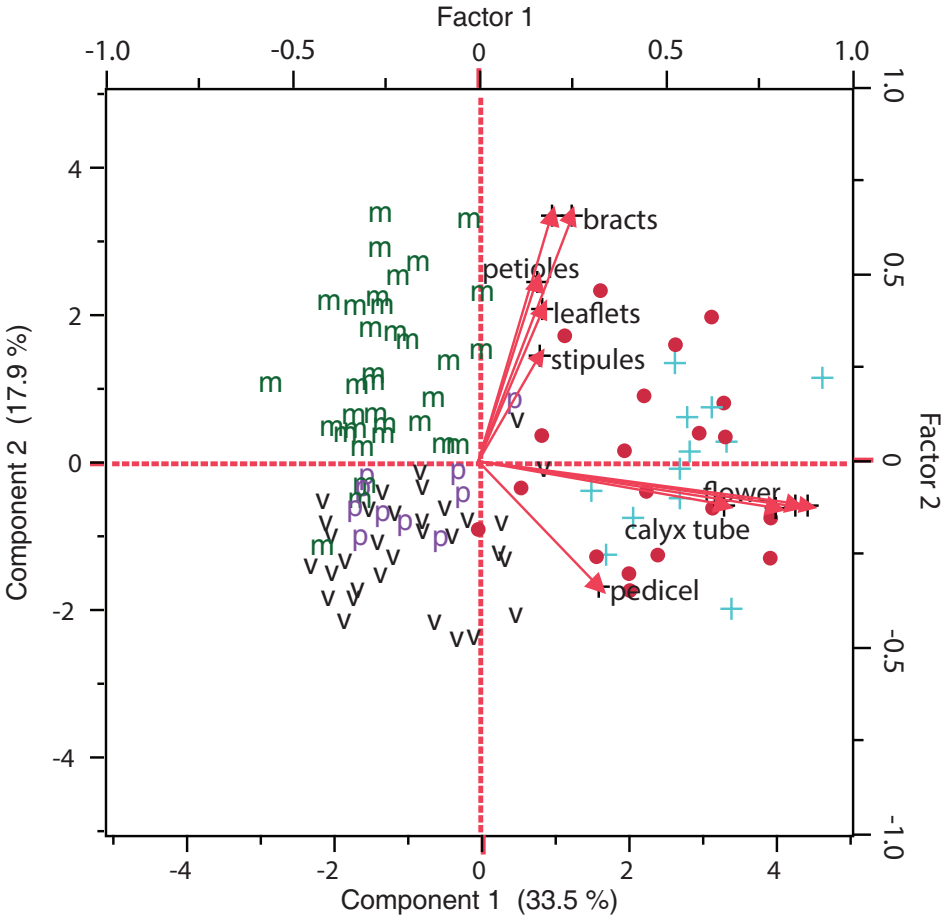

Figure 3. Principal component analysis ordination diagram incorporating all specimens with symbols according to initial identifications. **m** *P. mephiticum*, **v** *P. verdiense*, **p** *P. pauperitense*, ● *P. megalanthum* var. *megalanthum*, **+** *P. megalanthum* var. *retrorsum*. Principal component scale on the left and bottom; unrotated factor loading scale on the top and right. Exact loading matrix vector lengths are listed in Table 5.

Canonical and classificatory discriminant analyses were also conducted to investigate the spread of means per species group. In accordance with HCA and PCA analyses, CAN1 shows *P. mephiticum* largely distinct from the others, with the two *P. megalanthum* taxa creating one group while *P. verdiense* and *P. pauperitense* another (Fig. 5). The inner circles of each species represent the 95% confidence region for the true mean (of all characters taken together) of the species. The 95% confidence region for *P. verdiense* and *P. pauperitense* are overlapping, as are those of *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*, suggesting that the overall mean for these species based on all characters is not statistically different.

Factor analyses and PCA were taken together to help elucidate those characters most influential in separating predefined groups based on expert identification (Table 5). For

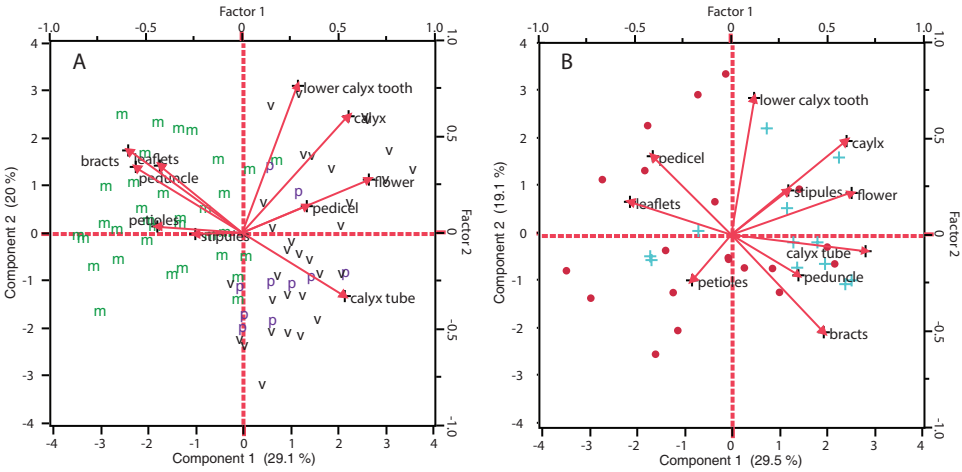

Figure 4. Principal component analysis ordination diagram for **A**) PCA2 and **B**) PCA3. **m** *P. mephiticum,* **v** *P. verdiense*, **p** *P. pauperitense*, ● *P. megalanthum* var. *megalanthum*, **+** *P. megalanthum* var. *retrorsum*. Principal component scale on the left and bottom; unrotated factor loading scale on the top and right. Exact loading matrix vector lengths are listed in Table 5.

Table 4. Eigenvector contributions (PC) of each character from the first two axes of the principal component analyses based on all morphological characters incorporating all species (PCA1), the *P. mephiticum-P. verdiense-P. pauperitense* (MVP) complex only (PCA2), and for the two varieties of *P. megalanthum* (PCA3). Characters are described in Table 2.

	PCA1 (Fig. 3)		PCA2 (Fig. 4A)		PCA3 (Fig. 4B)	
Character	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
flower	0.502	-0.098	0.432	0.218	0.424	0.186
calyx	0.522	-0.095	0.362	0.484	0.405	0.413
calyx tube	0.472	-0.101	0.350	-0.262	0.473	-0.072
lower calyx tooth	0.390	-0.094	0.189	0.608	0.082	0.600
stipules	0.097	0.229	-0.162	-0.004	0.201	0.195
petioles	0.092	0.391	-0.292	0.024	-0.137	-0.198
leaflets	0.103	0.330	-0.282	0.277	-0.360	0.145
bracts	0.150	0.536	-0.389	0.342	0.324	-0.426
peduncle	0.116	0.536	-0.366	0.270	0.235	-0.176
pedicel	0.191	-0.272	0.220	0.111	-0.278	0.345

the all species dataset, FAPC1, FAML1, and PCA1 all suggested that the separation of MVP from the *P. megalanthum* taxa calculated along the first factor or component are most highly influenced by floral traits (flower, calyx, calyx tube, and lower calyx tooth). The second factor or component axis largely separates *P. mephiticum* from *P. verdiense* and *P. pauperitense* and is most strongly associated with various vegetative characters (Table 5). This same association between discriminatory power, axes, and characters follows into analyses conducted on the MVP group only (FAPC2, FAML2, and PCA2),

Table 5. Relative distinguishing power of characters between species by factor or PCA analyses. Bold factors are those highly associated with the corresponding loading.

	FAPC-rotated loadings		FAML-rotated loadings			PCA-loading matrix	
All specimens	**FA1 (32.2%)**	**FA2 (19.3%)**	**FA1 (26.6%)**	**FA2 (12.8%)**	**FA3 (12.5%)**	**PC1 (33.5%)**	**PC2 (17.9%)**
	mvp|gr	**m|vp**	**mvp|gr**	**m|vp**	**m|vp**	**mvp|gr**	**m|vp**
flower	**0.917418**	0.146258	**0.951893**	0.091466	0.02346	**0.91974**	-0.13088
calyx	**0.951527**	0.160732	**0.914948**	0.135937	0.192597	**0.9566**	-0.12712
calyx tube	**0.865426**	0.125783	**0.838602**	0.109935	**0.475039**	**0.86402**	-0.13511
lower tooth	**0.718706**	0.090375	0.243157	-0.207947	0.216766	**0.71338**	-0.12566
stipules	0.078849	0.345099	0.044324	**0.807081**	0.096593	0.17714	0.30648
petioles	0.006661	**0.549323**	0.003746	**0.629476**	0.071232	0.16841	**0.52291**
leaflets	0.04924	**0.477942**	0.087371	0.278027	-0.074971	0.18804	**0.44215**
bracts	0.051288	**0.765723**	-0.028996	0.236017	0.206967	0.27489	**0.71652**
peduncle	-0.008032	**0.748081**	0.125539	0.146123	-0.014782	0.21301	**0.71716**
pedicel	**0.440783**	-0.24418	0.357629	0.015441	**0.933736**	0.34914	-0.36334
MVP only	**FA1 (25.5%)**	**FA2 (23.7%)**	**FA1 (20.3%)**	**FA2 (19%)**	**FA3 (12.9%)**	**PC1 (29.1%)**	**PC2 (20%)**
	m|vp	**vp|v**	**m|vp**	**m~vp**	**p~v**	**m|vp**	**vp|v**
flower	-0.374642	**0.705445**	**0.991576**	0.090467	-0.092824	**0.73706**	0.30781
calyx	-0.044509	**0.920401**	**0.783954**	-0.304062	-0.105493	**0.61815**	**0.68338**
calyx tube	**-0.697581**	0.092945	**0.574663**	0.254593	-0.285595	**0.59828**	-0.37056
lower tooth	0.29626	**0.868977**	0.206384	0.173869	0.009934	0.32207	**0.85975**
stipules	0.209798	-0.179713	0.176519	**0.973894**	0.144094	-0.27618	-0.00589
petioles	**0.407106**	-0.289188	0.046761	-0.393911	**0.898281**	**-0.49816**	0.03461
leaflets	**0.620859**	-0.002673	-0.131126	-0.151598	**0.410291**	**-0.48171**	0.3917
bracts	**0.819303**	-0.046928	-0.038605	**-0.658242**	0.367456	**-0.66321**	**0.48334**
peduncle	**0.724966**	-0.100322	-0.054292	0.073884	0.210615	**-0.62414**	0.38222
pedicel	-0.190591	0.359369	0.027441	-0.371378	0.117906	0.37528	0.15697
***P. megalanthum* varieties**	**FA1 (25.2%)**	**FA2 (23.4%)**	**FA1 (21%)**	**FA2 (19%)**		**PC1 (29.5%)**	**PC2 (19.1%)**
	g~r	**gr**	**g~r**	**gr**		**g~r**	**gr**
flower	0.392436	**0.664925**	**0.745911**	0.264933		**0.7282**	0.25664
calyx	0.16657	**0.885112**	**0.614819**	-0.08418		**0.69691**	**0.57051**
calyx tube	**0.68603**	**0.446127**	**0.535314**	0.386092		**0.81223**	-0.09975
lower tooth	**-0.425283**	**0.725083**	0.318941	0.092851		0.14083	**0.82872**
stipules	0.091589	**0.427999**	-0.043631	-0.156071		0.34545	0.26877
petioles	-0.003729	-0.36035	0.279887	**0.927344**		-0.23466	-0.27349
leaflets	**-0.602025**	-0.244695	-0.361722	**0.823352**		**-0.61834**	0.19993
bracts	**0.805107**	-0.091753	0.188143	0.265238		**0.55739**	**-0.58816**
peduncle	**0.465463**	0.074153	**-0.493267**	-0.003841		**0.40407**	-0.24265
pedicel	**-0.672394**	0.057123	**-0.538243**	-0.170644		**-0.47806**	**0.47628**

FAPC, factor analysis on principal components. FAML, factor analysis via maximum likelihood. PCA, principal component analysis. FA1, factor 1. FA2, factor 2. FA3, factor 3. PC1, principal component 1. PC2, principal component 2. (%), amount of variance explained by that factor or component. m, *P. mephiticum*. v, *P. verdiense*. p, *P. pauperitense*. g, *P. megalanthum* var. *megalanthum*. r, *P. megalanthum* var. *retrorsum*. Symbols signify a strong (|) or moderate (~) split between indicated taxa.

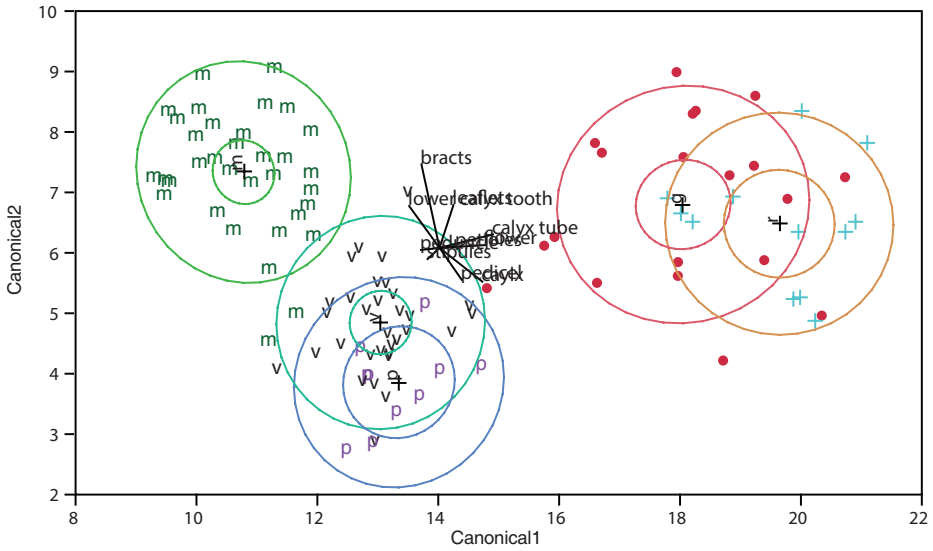

Figure 5. Canonical plot of points and means from linear discriminant analysis of all species by all characters with groups defined as: **m** *P. mephiticum,* **v** *P. verdiense*, **p** *P. pauperitense*, ● *P. megalanthum* var. *megalanthum*, **+** *P. megalanthum* var. *retrorsum*. Inner circles by group are the 95% confidence region for containing the true overall mean of the group; the outer circles by group are the normal 50% contours, the normal ellipse region that contains 50% of the population for each group. Rays show the coordinate directions in canonical space.

Table 6. Rank order of relative discriminatory power of characters for distinguishing species as ascertained by stepwise discriminatory analysis. F-ratio and probability are calculated based on the stepwise inclusion into the set of characters ranked previously.

	All specimens			MVP only			*P. megalanthum* varieties		
rank	**Character**	**F-ratio**	**Prob>F**	**Character**	**F-ratio**	**Prob>F**	**Character**	**F-ratio**	**Prob>F**
1	**calyx tube**	156.911	0.0000	**bracts**	66.759	0.0000	**flower**	9.19	0.0050
2	**bracts**	28.938	0.0000	**flower**	25.432	0.0000	leaflets	2.32	0.1385
3	**flower**	13.322	0.0000	**pedicel**	12.814	0.0000	lower tooth	1.711	0.2015
4	**leaflets**	5.107	0.0009	**peduncle**	10.466	0.0001	bracts	1.299	0.2643
5	**pedicel**	3.527	0.0098	**calyx tube**	4.649	0.0128	calyx	1.957	0.1737
6	**peduncle**	3.769	0.0068	**stipules**	3.539	0.0345	petioles	0.144	0.7072
7	**stipules**	3.497	0.0103	petioles	1.543	0.2213	peduncle	0.062	0.8055
8	petioles	1.681	0.1607	leaflets	1.339	0.2690	calyx tube	0.052	0.8215
9	lower tooth	1.445	0.2252	calyx	1.475	0.2363	stipules	0.009	0.9257
10	calyx	2.015	0.0986	lower tooth	0.722	0.4897	pedicel	0	0.9864

MVP, the dataset including only *P. mephiticum*, *P. verdiense*, and *P. pauperitense*. Bold characters are those showing significant discriminatory power as ranked in the inclusion set.

but with less distinction between the relative discriminatory power for vegetative vs. floral traits. Analyses of only *P. megalanthum* varieties showed no clear pattern between floral or vegetative traits as being most discriminatory, but showed several associations

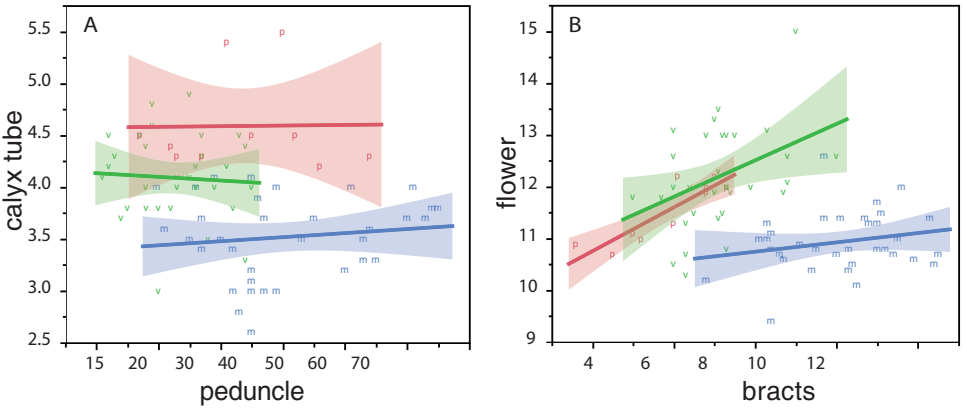

Figure 6. Scatterplot and linear regression of key distinguishing character traits for the MVP group. Fit of **A**) peduncle by calyx tube and **B**) bracts by flower sizes for *P. mephiticum* (m, blue color), *P. verdiense* (v, green color), and *P. pauperitense* (p, red color). All measurements in mm. Line is regression by taxon with accompanying 95% confidence band shaded.

that differed across analytical method (Fig. 5). Stepwise discriminatory analysis showed flower length to be the only significant trait offering some measure of discriminatory power between *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum* (SDA3; Table 6) with all other characters not significant. For MVP only, SDA2 determined that bracts were the most important factor in discriminating between species, followed by flower length, pedicel length, peduncle, calyx tube, and stipules (Table 6). Scatterplots showing the fit of peduncle × calyx tube lengths (Fig. 6A) and bract × flower lengths (Fig. 6B) show the range and distinguishing power of these traits for MVP. For all species analyzed together, SDA1 calyx tube length was strongly correlated with separation of groups, followed by bract and flower lengths; leaflets, pedicel, peduncle, and stipules were also associated with discriminatory power across these groups, but less so (Table 6).

Discussion

Species recognition often relies on deciphering nonoverlapping patterns in morphology between biological entities (Davis and Heywood 1963; Mayr 1942), with these gaps in morphology used as a means of delimiting species or lower taxa. Recognition of taxa at the specific vs. varietal or subspecific level can often be a difficult choice to make, especially for plants. Ideally, subspecies or varieties should be characterized by some cohesive trait along side morphology, such as geography, ecology, or phylogenetic traits (Hamilton and Reichard 1992). Often this is exacerbated by a disagreement concerning the relative importance of various morphological characters as being diagnostic of species or varieties or the lack of a clear supporting character not of the morphological type. This battle is evident in the genus *Pediomelum*, particularly for species of the intermountain west.

Relationships among the species or varieties of the *Pediomelum megalanthum* complex have been debated among botanists, largely due to differing opinions as to which morphological characters are most important for distinguishing species. In his key to species of *Pediomelum*, Rydberg (1919) emphasized the pubescence of the peduncle (hairs appressed vs. hairs spreading or retrorse) as diagnostic between *P. megalanthum* and *P. mephiticum* or *P. retrorsum*. Welsh et al. (1993) followed Rydberg (1919). Ockendon (1965) commented extensively on the *P. megalanthum* complex, suggesting that this group of species required an extensive review coupled with reproductive studies to truly decipher amongst species. He considered the pubescence character to be useful, but recognized that use of pubescence on the peduncle could be problematic, favoring that of the petioles as being more diagnostic and leading to his recognition of *Psoralea megalantha* Wooton & Standl. and *Ps. mephitica* S.Watson, with specimens relegated to *retrorsum* being subsumed under *Ps. mephitica*. Ockendon (1965) also recognized the potential of flower size as a distinguishing character and suggested that new species might be recognized in the future. Ultimately, Ockendon (1965) concluded that "Rather than altering the taxonomy of this group in a piecemeal fashion, it seems best to wait until a more unified treatment is possible."

Grimes (1990) took up Ockendon's challenge and produced the most recent treatment of the genus, taking a more quantitative approach emphasizing flower and calyx lengths. This relative increase in the importance of flower size as delimiting between taxa in this species group admitted less variability in both *P. mephiticum* and *P. megalanthum*, these being distinguished by those plants having calyx tubes 4.5 mm or shorter vs. those 6 mm or longer, respectively. This shifted specimens previously referred by Ockendon (1965) to *Ps. mephitica* being now recognized as a variety of *P. megalanthum* due to flower size and other overlapping quantitative characters (see Table 5 of Grimes 1990).

The morphometric analyses conducted herein largely support the use of flower and calyx sizes as being useful characters for species delimitation. All level 1 morphometric analyses involving all species in the complex, *P. mephiticum*, *P. verdiense*, *P. pauperitense*, and the two varieties of *P. megalanthum*, illustrated a clean break in overall morphological variation between *P. mephiticum*, *P. verdiense*, and *P. pauperitense* (the MVP group) and the *P. megalanthum* varieties (HCA1, PCA1; Figs. 1, 3). Canonical discriminant analysis (CAN1; Fig. 5) also illustrated a strong break between the MVP group and the *P. megalanthum* varieties, with no overlap in overall species means. Flower size, calyx, and calyx tube were most strongly associated with the first canonical axis, while calyx tube and bracts showed the strongest association with the second canonical axis.

In most analyses, floral characters separated species with the greatest discriminatory power along the first component or axis of the analysis, whereas the suite of vegetative characters contributed more to the second component divisions (Fig. 3; Table 5). These results are not unexpected, as most current species determinations were based on Grimes' (1990) criteria of flower and calyx morphology as being most distinguishing of species. The stepwise discriminatory analysis for all taxa (SDA1) suggested that the strongest discriminatory character amongst taxa was calyx tube length (Table 6),

a finding in line with most researchers who use this character in dichotomous keys to separate species (Grimes 1990; Isely 1998; Welsh et al. 1993) and is here largely responsible for the separation of the *megalanthum* varieties from the MVP group. Bract size is the second most discriminatory character (Table 6), responsible largely for the separation of *P. mephiticum* from *P. verdiense* + *P. pauperitense*. Flower size is the third most discriminating character for distinguishing amongst the previously determined species groups.

The distinction of large and small flowered forms within the *megalanthum* complex has been recognized by previous researchers (Grimes 1990; Isely 1998). The larger-flowered group comprising the *P. megalanthum* varieties has been another battleground for taxonomists in this group. *Pediomelum m.* var. *retrorsum* has variably been recognized at the varietal level (Grimes 1990; Isely 1998), at the specific level as *P. retrorsum* (Rydberg 1919; Welsh et al. 1993), or included as either *Psoralea megalantha* or *Ps. mephitica* (Rydberg 1919), depending on the relative importance of flower size vs. pubescence type and direction of peduncular and petiolar hairs deemed by the researcher. That said, the analyses herein do not support a clear distinction between species designated as *P. retrorsum* vs. *P. megalanthum* based on the quantitative characters employed, by either principal component analyses (PCA1, PCA3; Figs. 3, 4B) or canonical discriminant analysis (CAN1; Fig. 5). In fact, all of the ten characters employed here present overlapping character ranges (Table 3), a finding illustrated best by the canonical discriminant analysis that shows strong overlap in the 50% normal contours, the normal ellipse region (outer circle) that contains 50% of the species' quantitative morphological diversity based on the 10 characters included here (Fig. 5). In addition, there is an overlap, albeit slight, in the 95% confidence region between *megalanthum* and *retrorsum*, suggesting that the overall morphological means are not statistically different. Grimes (1990) presented a comparison of 14 quantitative characters across the three varieties of *P. megalanthum* and concluded something very similar to these findings: "…the varieties overlap in most qualitative and quantitative characters, and the diagnostic characters for all three varieties overlap so much as to make specific status untenable." (see Table V of Grimes 1990: 81).

That said, there is some quantitative morphological and geographic separation evident between *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*. The varieties are fairly distinct geographically, with *megalanthum* primarily of eastern Utah, western Colorado, and northwest New Mexico and *retrorsum* of southern Nevada, northwestern Arizona, and sporadically along the Gila River drainage elsewhere in Arizona. Flower length was the only character having significant discriminatory power in the stepwise discriminant analysis (SDA3; Table 6), suggesting that flower length may be the only causal quantitative character offsetting the separation of the population normal contours in the canonical discriminant analysis. This suggests that perhaps there is ongoing differentiation among the *megalanthum* varieties, perhaps spurred by geographic separation that may in time lead to species differentiation. The lack of quantitative separation from each other argues against recognizing these taxa as separate varieties and instead lumping them under *P. megalanthum*. However, some qualitative differ-

ence in the directionality of prevailing hair types and geographic separation exists, providing some justification for recognition and separation at the varietal level. In an effort to favor tradition and lessen the upset to prevailing taxonomic concepts, I am in favor of recognizing these taxa at the varietal level as *P. megalanthum* var. *megalanthum* and *P. megalanthum* var. *retrorsum*, largely following the concept of Grimes (1990).

I agree with Grimes' conclusions to a point with the recognition of *P. m.* var. *megalanthum* and *P. m.* var. *retrorsum*. However, after careful comparison of the character ranges of *P. m.* var. *epipsilum* with the others as ascertained by Grimes, I find sufficient distinguishing characters that separate *P. epipsilum* from the other varieties, including having leaflets smaller and glabrate above or sparingly strigose along veins (vs. leaflets larger and pubescent above and below in vars. *megalanthum* and *retrorsum*), and bracts larger and caudate (vs. smaller, acuminate, acute, or shortly caudate in the others). Indeed, Grimes' quantitative character comparison shows non-overlapping ranges for leaflet and bract size, separating *P. epipsilum* from the others. Barneby (1943), in his description of the species as *Psoralea epipsila* Barneby, states that it differs from *Ps. mephitica* by its caulescent nature and conspicuous bicolored leaves and that it is intermediate between *Ps. mephitica* and its variety *Ps. m.* var. *retrorsa* (Rydb.) Kearney & Peebles in flower size. He also states that the banner is barely exserted from the calyx, whereas the others are well exserted. This preponderance of both quantitative and qualitative differences, coupled with the fact that *P. epipsilum* is set apart in phylogenetic analyses (Egan and Crandall 2008b), lead me to recognize *P. epipsilum* at the species level, as others before have also done (Barneby 1943; Welsh et al. 1993).

During an examination of *Pediomelum* in Arizona, Welsh and Licher (2010) discovered some collections that did not key out well based on flower size and peduncular pubescence. All these offending specimens had small flowers and ascending hairs on pedicels and peduncles, a character combination at odds with the prevailing concepts of *P. megalanthum* and *P. mephiticum*. Those plants having flowers with a banner that is purple or white suffused with purple that is not strongly contrasting with the wings or keel in color and found mainly from the Verde Limestone Formation were described as *P. verdiense* whilst those plants from near Poverty Mountain having a banner and wings of white or cream that contrasts strongly to the purple color of the keel, with leaves that tend to exceed inflorescence in height were described as *P. pauperitense*. For comparison, *P. mephiticum* has a white or cream banner with wings and keel purple or white suffused with purple and is present in the extreme northwest corner of Arizona and adjacent areas in Utah and Nevada.

Furthermore, Welsh and Licher (2010) stated that *P. verdiense* corresponded to those plants with pedicels 3–3.5(–5) mm long, bracts 5–8 mm long; flowers 10–11.3 mm long whereas *P. pauperitense* corresponded to those plants with pedicels 1.5–2.5(–3) mm long, bracts 3–5 mm long; and flowers 7.3–10 mm long. These ranges suggest a clean break between these species and were used to key out specimens. However, my examination and measuring of specimens used in this study, many of which were also cited by Welsh and Licher or were paratypes thereto, gave a very different picture (Table 3). In fact, of these characters, my measurements for *P. pauperitense* pedicel and

flower length were completely non-overlapping with the ranges suggested by Welsh and Licher (pedicels 3–4 mm and flowers 10.7–12.2, as compared to those above). This made me question the authenticity of these species.

The multivariate morphometric analyses on the MVP group only (level 2 analyses) were very telling. There seems to be a distinct separation between *P. mephiticum* and the other two species, as evidenced by all methods applied herein, but strong overlap between *P. verdiense* and *P. pauperitense*. This is perhaps best illustrated by the hierarchical cluster analysis (HCA2) which shows two main clusters, one cluster almost entirely of *P. mephiticum* and a second main cluster mostly comprised of *P. verdiense* and *P. pauperitense* (Fig. 2).

As in the case with the *P. megalanthum* varieties, canonical discriminant analysis (Fig. 5) shows strong overlap in the 50% normal contours as well as a large overlap in the 95% confidence region between *P. verdiense* and *P. pauperitense*, suggesting that the overall morphological means are not statistically different. Lastly, principal component analyses (Figs. 3, 4A) showed separation of *P. mephiticum* from the others along the first component. Factor analysis suggested that flower, calyx tube, peduncle and bracts contributed most to the separation along the first axis. A linear regression of peduncle vs. calyx tube (Fig. 6A) and of bracts vs. flower (Fig. 6B) supports the distinction between *P. mephiticum* and *P. verdiense*+*P. pauperitense* and illustrates the lack of distinction between the latter two taxa. This is evident in the overlapping 95% confidence bands between *P. verdiense* and *P. pauperitense* in both linear regressions, with no overlap with *P. mephiticum*. SDA2 suggests that bracts are the most distinguishing character in the MVP group, followed by flower, pedicel, peduncle and calyx tube in rank order (Table 6).

Given the overlap in continuous character distributions between several species or taxa in this study, some researchers may invoke hybridization as one reason behind overlapping morphology. Traditionally, hybridization is said to create morphological intermediacy (Anderson 1949). However, several studies have shown that hybridization does not always result in morphological intermediacy, but that it can, in fact, produce parental and even novel morphological characters or combinations (e.g. Rieseberg 1995; Rieseberg and Ellstrand 1993). Furthermore, the use of multivariate morphometric techniques for detecting hybridization has been called into question, as these methods cannot distinguish between divergence and hybridization (Wilson 1992). With these caveats in mind, the presence of hybridization within or between taxa in this complex cannot be proven nor ruled out. Indeed, it is possible that *P. verdiense* or *P. pauperitense*, or any of the species in this group, could be hybrids involving one or more of the other species in the complex. However, this study cannot address this at this time. The role of hybridization in this complex may best be addressed using molecular or genomic methods spanning the species and population levels.

Taken together, the results of this study argue for the recognition of *P. mephiticum* and *P. verdiense* at the specific level, but do not support *P. pauperitense* as its own species. Some researchers might suggest that *P. pauperitense* be recognized as a variety of

P. verdiense based on geographic separation, differences in peduncle length relative to petiole length, or flower color. However, given the few numbers of populations and specimens relegated to *P. verdiense* and *P. pauperitense*, I deem it premature to make this distinction, especially considering the lack of any non-overlapping quantitative morphological character to justify this separation.

Now, with all this said and done, I revisit the initial question posed to myself: where do I lie on the spectrum of lumpers vs. splitters? Considering my conclusions in the paragraph above, I think me a lumper – at least in the case of *Pediomelum*. And yet, my initial inclination – prior to this analytical undertaking – was to synonymize both *P. verdiense* and *P. pauperitense* under *P. mephiticum*. This exercise convinced me to do otherwise – to recognize *P. verdiense* at the species level. This is more leaning towards a splitter mentality. The problem? Not knowing the dimensions of the spectrum! I guess I lie somewhere in the middle…

Conclusions

Given the conglomeration of past research with current findings shown herein, I support the recognition of *P. megalanthum* as having varieties *megalanthum* and *retrorsum*. I also recognize the specific status of *P. mephiticum*. As per the sinking of *P. pauperitense* under *P. verdiense*, a new description of *P. verdiense* is given below, along with a key to the taxa investigated or discussed herein.

Key to the species

1	Calyx tube less than 5.5 mm long **2**
2	Bracts (7–)8–12.5 mm long; calyx tube 2.5–4 mm long; plants of sw UT, nw AZ, se NV ..... ***P. mephiticum***
2'	Bracts (3–)4–8(–9) mm long; calyx tube (3.5–)4–5.5 mm long; plants of Mohave and Yavapai Cos, AZ ..... ***P. verdiense***
1'	Calyx tube more than 5.5 mm long **3**
3	Bracts caudate, 13–18×6–9 mm; Upper surfaces of leaflets glabrous to pubescent only along base of veins ..... ***P. epipsilum***
3'	Bracts not caudate, or if caudate, not as large, 5–7×2.5–6 mm; Upper surfaces of leaflets pubescent throughout **4**
4	Peduncle hairs shorter, appressed to incurved-ascending hairs and longer erect ones or sometimes with sparse, long curly hairs going in all directions.. ..... ***P. megalanthum* var. *megalanthum***
4'	Peduncle hairs mostly long straight erect or reflexed hairs, or rarely of short and long hairs, but then both erect ..... ***P. megalanthum* var. *retrorsum***

Pediomelum verdiense S.L.Welsh & Licher

Western North American Naturalist 70: 12 (2010). *Type:* USA, Arizona, Yavapai Co., on the flats above a wash just north of Middle Verde exit from I-17, 18 April 2008, M. Licher 1911 (holotype BRY; isotype ASC).

P. pauperitense S.L.Welsh, Licher, & N.D.Atwood, Western North American Naturalist 70: 14 (2010). *Type:* USA, Arizona, Mohave Co., SW of Poverty Mountain, near Dewdrop Spring, 25 May 2001, L.C. Higgins 23135 (holotype BRY; isotypes distributed previously as *P. mephiticum*).

Plant acaulescent to short caulescent, 4.5–13(–15) cm tall, essentially glandular and pubescent throughout, from underground caudex branches arising from a deep, tuberous root. **Stems** 0–4(–6) cm, spreading white hairy; pseudoscapes 0–3, up to 6 cm, mainly subterranean; cataphylls 0–5 mm, glabrous to pubescent. **Leaves** clustered, palmately (3)5-foliolate; petioles 2–10(–11.5) cm long, with hairs appressed-ascending, jointed basally; stipules lanceolate to elliptic, scarious, 4–16 × 2–6 mm, tardily deciduous to persistent; petiolules 2–3 mm, pubescent; leaflet blades cuneate-obovate, (0.8–)1.2–3 × 0.7–1.8(–2.2) cm, cuneate basally, broadly acute to rounded or retuse apically, glandular and pubescent with more hairs along veins above and on lower surface, gray-green below, green to yellow-green above. **Inflorescence** globose, 1.5–3 cm long, with (1–)2–4(–6) nodes and (2)3-flowers per node; peduncles 0.5–4.5(–6) cm long, shorter than the petioles, spreading or spreading-ascending white-hairy, sometimes with longer spreading white hairs; bracts tardily deciduous to persistent, elliptic, 3.5–8.5(–10) × 2–6 mm. **Pedicels** filiform, 2.5–4.5(–6) mm long. **Flowers** (8–)10–13.5(–15) mm long, calyx (7.5–)8.5–12(–13) mm long, calyx-tube (2.5–)3.5–5 mm long, glandular, teeth lanceolate to oblong or elliptic, upper teeth 4–7(–8) × 1–2.5 mm, lower tooth (4–)5–9 × (1.5–)2.0–3.5 mm, gibbose-campanulate in fruit; petals white to purple, the banner white, cream, purple or suffused with pale purple, the wings and keel dark purple, with the wings sometimes lighter in color; 9–12(–14) × 6–8 mm with claw 2–5 mm, wings 10–13 × 2–3 mm with claw 4–5 mm, keel 8–10 × 2–3.5 mm with claw 3–5 mm; filaments 7–8.5 mm; anthers elliptic, 0.33 mm; ovary glabrous to pubescent apically, style concomitantly so basally. **Fruits** pubescent, eglandular, round to ovoid, body 5–7 × 3.5–5 mm, beak 1–4 mm, not exerted beyond calyx. **Seeds** oval to reniform, 3.5–5 mm × 2.5–3 mm, olive to gray brown and with or without purple mottling.

Flowering spring to summer. On limestone soils of the Verde Formation in Yavapai Co. and near Poverty Mountain in Mohave Co, Arizona.

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RESEARCH ARTICLE

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Chapelieria magna, a new species of Rubiaceae from eastern Madagascar

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Abstract

A new species of *Chapelieria* was discovered during a recent field trip to the Masoala National Park in eastern Madagascar, and is described here as *Chapelieria magna* Kainul., **sp. nov.** This species is readily distinguishable from previously described species of the genus by its quadrangular shoots, triangular-calyptrate stipules, sessile leaves, pubescent styles, and ridged fruits. It also differs in the larger number of ovules and the much larger size of leaves and fruits.

Keywords

Chapelieria, Madagascar, Octotropideae, Rubiaceae

Introduction

Chapelieria A. Rich., is a genus endemic to Madagascar that belongs to tribe Octotropideae (Rubiaceae; subfamily Ixoroideae). The taxonomic history of the genus is complex (Madagascar Catalogue 2014). *Chapelieria madagascariensis* A. Rich. was originally described by Richard (1830), commemorating Louis Armand Chapelier who had collected the type material in eastern Madagascar. However, the name was first published by De Candolle in September 1830, citing Richard's (1830) manuscript that was not published until December that same year (Stearn 1957). De Candolle's

(1830) description is essentially identical, but with an added note on the similarity of the plant habit to that of an Apocynaceae. Baillon (1880), considered *Chapelieria* and *Tamatavia* Hook.f. as congeneric. *Tamatavea melleri* had been described by Hooker (1871: pl 1090) just a few years earlier although with some reservation as to its novelty: "I advance this genus as new with some hesitation, because it may prove to be one of the several Madagascarian genera which are so imperfectly or incorrectly characterized in systematic works, that it is impossible to recognize them by their description". Schumann (1891), included *Tamatavia melleri* in *Chapelieria* as *C. melleri*, and Chevalier (1942) subsequently synonymized the two names.

In a revision of Malagasy Apocynaceae, Pichon (1949) noted that the type material of *Chapelieria madagascariensis* was mixed and included both Apocynaceae and Rubiaceae material, and consequently he synonymized the name under *Carissa edulis* Vahl *var. septentrionalis* Pichon. The Apocynaceae specialists Markgraf (1976), and Leeuwenberg and van Dilst (2001), also considered *Chapelieria* a synonym of *Carissa*. Recently, however, Davies and Davis (2014), emended the description of *Chapelieria madagascariensis* and specified one of the Chapelier specimens as the holotype (a paper to clarify the issue of the typification is in preparation, Davis AP, pers. comm.). They also described two new species of *Chapelieria* (*C. multiflora* N.M.J. Davies & A.P. Davis and *C. septentrionalis* N.M.J. Davies & A.P. Davis), and estimated the total number of species in Madagascar to be about ten.

During a recent field trip to southern Masoala National Park, we collected an unknown *Chapelieria* and it is here described as a new species. Morphologically, the plant conforms to the characterization of tribe Octotropideae by Tosh et al. (2008), having articulated petiole bases with distinct sutures, paired supra-axillary inflorescences, hermaphroditic flowers with secondary pollen presentation, funnelform corollas with left-contorted aestivation, 2-locular ovaries with axile placentation, pendulous ovules, and striate pattern of the seed coat. Characters that support a placement in *Chapelieria* as described by Davies and Davis (2014), include the sessile inflorescences, sessile flowers, 5-merous flowers, and seeds with entire endosperm. In contrast, the new species does not have grooved/ridged styles, and further broadens the generic description of *Chapelieria* (Davies and Davis 2014) by having stipules fused to a cap that cover the apical buds, sessile leaves, simple styles (not club-shaped), and in the larger number of ovules per locule (16 vs. 3–7). Furthermore, the styles of this species are sparsely pubescent, and the fruits are distinctly ribbed. The latter two traits are also be found in the genus *Flagenium* Baill. Characters that distinguish *Flagenium* from *Chapelieria* include the presence of both erect and pendulous ovules, and the absence of articulated petioles (Ruhsam and Davis 2007). Preliminary molecular phylogenetic analyses of both cpDNA and rDNA data support a position of the new species in *Chapelieria* (Kainulainen et al. unpublished data).

Flower buds of *Chapelieria* are enclosed by calyptrate bracts (Chevalier 1947), and this is also the case in *Chapelieria magna*. The conical sheath formed by the fused bracts is split by the expanding flower buds, but the bracts persist as an asymmetric triangular sheath around the inflorescence branches. Lateral buds appear to form continuously,

and many buds of varying levels of development are found within the cymose inflorescences. However, because of the congested nature of the inflorescence, branchlets with primordial buds may appear as single bracteolate flowers.

Taxonomy

Chapelieria magna **Kainul., sp. nov.**
urn:lsid:ipni.org:names:77144550-1
Figures 1, 2

Diagnosis. Differs from previously described species of *Chapelieria* (*C. madagascariensis*, *C. multiflora*, and *C. septentrionalis*) by its quadrangular shoots; triangular-calyptrate stipules; sessile leaves (vs. petiole 5–11 mm); simple, terete, sparsely pubescent styles (vs. club-shaped, grooved/ridged, glabrous styles); ovule number (ca. 16 vs. 3–7 per locule); distinctly ridged fruits (vs. ±smooth fruits); and the much larger size of leaves (up to 42 × 12.2 cm vs. <16.6 × 7.8 cm), and fruits (up to 45 × 20 mm vs. <13 × 7.0 mm).

Type. MADAGASCAR. Toamasina Province: Analanjirofo Region, Maroantsetra District, Masoala National Park, 15°41.910'S; 49°57.815'E, 115 m altitude, 15 January 2013 (fl.), *S.G. Razafimandimbison et al. 1240* (holotype S!, isotype, TAN!).

Description. Treelet, to 4 m tall, all vegetative parts glabrous; with decussate, horizontal branches; branchlets quadrangular, 4.0–7.0 mm in diameter, bark drying brown. Stipules ca. 25–30 mm long, initially calyptrate and covering the apical bud, subsequently interpetiolar, triangular, with raised median line and apiculate apex; persistent. Leaves: sessile, narrowly obovate, ca. 39.0–42.0 × 10.5–12.2 cm; bases acute–auriculate; apices acute; adaxial surface: green when fresh, drying pale brownish-gray, smooth, secondary veins brochidodromus, obvious, curved, 15–20 pairs; midribs prominent, pale green when fresh, ±the same colour of the leaf when dry; abaxial surface: pale green when fresh, pale brown when dry, veins reddish-brown. Inflorescences ±sessile, many-flowered (although only 1–few flowers may be mature at any given time); bracts initially calyptrate and covering the flower buds, subsequently splitting unequally to asymmetric, ±triangular sheaths, ca. 18 × 21 mm (1st order bracts), pale green–bright reddish pink, adaxially glabrous, abaxially densely strigose (hairs ca. 0.9 mm), bracteoles reduced; Flowers: hypanthium narrowly urceolate, ca. 6.7 × 2.0 mm; calyces greenish white–bright reddish pink; calyx tubes 3.0–5.0 mm long, externally glabrous, but with hairs (ca. 0.5–1.0 mm long) and colleters on the lower inner surface; calyx lobes ca. 7.5 × 1.3 mm, narrowly triangular, with ciliolate margins (hairs ca. 0.5–2.5 mm); corollas white, funnelform, ±curved; corolla tubes ca. 15 mm long, externally and internally glabrous; corolla lobes ca. 10 × 4.6 mm long, acute, recurved at anthesis; stamens: sessile, attached ca. 3 mm below corolla sinus; anthers white, ca. 7.9 × 0.8 mm, linear, medifixed, exserted for ca. 0.5–1.0 mm; styles simple, ca. 16.5 mm long, sparsely pubescent (hairs ca. 0.5 mm long); stigmas shortly bifid (lobes ca. 0.5 mm long); exserted for ca. 0.5–1.0 mm; ovary ellipsoid, 2-locular, ovules arranged

Figure 1. *Chapelieria magna*. **A** Habit and habitat **B** Flowering branch. Note the apical calyptrate stipules (one leaf removed) **C** Flower buds, and fruits in longitudinal and transversal sections, on leaf (×1.5) **D** Fruits **E** Inflorescence on leaf (×1.5). Photographs by Kent Kainulainen.

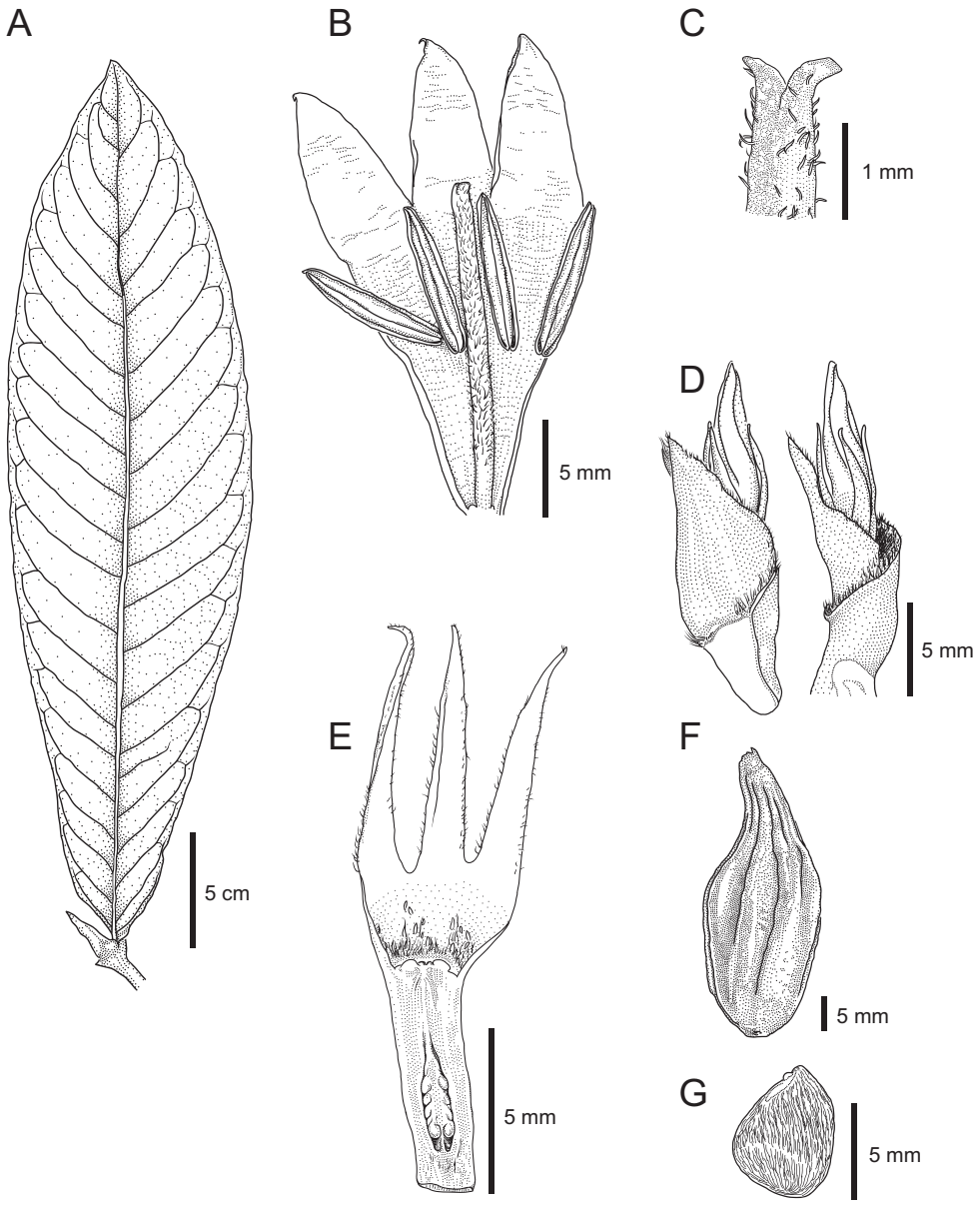

Figure 2. *Chapelieria magna*. **A** Leaf **B** Longitudinal section of corolla. **C** Stigma **D** Part of an inflorescence with flower buds and bracts **E** Longitudinal section of ovary and calyx **F** Fruit **G** Seed. Drawings from the holotype: *Razafimandimbison et al. 1240*, by Kent Kainulainen.

in two series, pendulous, ca. 16 ovules per locule; Fruits: mature fruits red, ca. 36–45 × 14–20 mm, glabrous, fleshy-indehiscent, fusiform, and apically elongated, with distinctive longitudinal grooves/ridges; calyx lobes persistent. Seeds: maturing at ± same rate, ca. 4.8–6.8 × 4.0–6.0 mm, compressed and angular.

Distribution and habitat. *Chapelieria magna* is only known from the type collection, made from a small stand of understory treelets in the rainforest of southern Masoala National Park. Notably, *Chapelieria madagascariensis* also occurs in this area. Although previously only known from the (eastern) Masoala peninsula by a collection made in 1951 (A. Tata 3404-RN; Davies and Davis 2014), we collected a specimen 4.7 km south of the *C. magna* locality in the nearby Tampolo littoral forest (*Razafimandimbison et al. 1217A*; S, TAN). However, whereas *Chapelieria madagascariensis* was found on sandy soil (cf. Davies and Davis 2014), the habitat of *C. magna* was on lateritic soil.

Phenology. Both flowers and fruits were found when we collected *Chapelieria magna* in mid-January. This is during the rainy season in Madagascar.

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RESEARCH ARTICLE

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Psoralea diturnerae and *P. vanberkelae* (Psoraleeae, Fabaceae): two new species restricted to the Core Cape Region of South Africa

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Abstract

Two new species of *Psoralea* L. are described: *Psoralea diturnerae* A. Bello, C.H. Stirt. & Muasya, **sp. nov**. and *P. vanberkelae* C.H. Stirt., A. Bello & Muasya, **sp. nov**. *Psoralea diturnerae* is endemic to the Outeniqua mountains (Camferskloof) and is characterised by a mass of numerous basal shoots out of which emerge 2–3 woody stems up to 2 m tall, 3-foliolate needle-like leaflets at the base of the seasonally growing shoot reducing to one towards the apex and bearing numerous 1–3-flowered axillary inflorescences along its length; each mauve to purple and white flower subtended by a trifid cupulum. *Psoralea vanberkelae* is characterised by its spreading mounding habit, short tightly packed fleshy leaves, with large impressed papillae, densely glandular short broadly triangular stipules, pale to intense mauve to deep blue flowers, standard with a dark purple central blotch above a M-shaped white patch situated above claw, and khaki seeds with purple flecks.

Keywords

Fabaceae, Leguminosae, New species, Endemic, *Psoralea*, Psoraleeae, South Africa, Taxonomy

Introduction

The predominantly southern African genus *Psoralea* L. is a young lineage (ca. 2 million years old, ± 75 species) which has diversified rapidly within the Fynbos Biome and related habitats in South Africa (Dludlu et al. 2013). New species in this genus are discovered regularly as remote areas are being explored (Stirton et al. 2011, 2012) and many species remain undescribed (Stirton and Schutte 2012). The genus is commonly found in mountain fynbos along drainage systems (river beds, stream banks, seepage areas), occurring frequently on sandstone derived soils across the Greater Cape Floristic Region (Stirton and Schutte 2000, 2012). However, there are a number of species, marginal to the main generic distribution, that have adapted to surviving in drier conditions along the arid Fynbos-Succulent Karoo boundary (e.g. *P. angustifolia* Jacq., *P. glaucescens* Eckl. & Zeyh., *P. karooensis* C.H. Stirt., Muasya & Vlok, *P. tenuifolia* L., and *P. verrucosa* Willd.) (Stirton et al. 2012). Two new species also occurring on specialised substrates are reported from the Core Cape Region and are described below. *Psoralea diturnerae* is endemic to arid fynbos on the northern slopes of the Outeniqua mountains in the Camferskloof area, on acidic lithosol (Glenrosa and Mispah forms) sandstone of the Table Mountain group of soils (Rebelo et al. 2006) while *P. vanberkelae* is endemic to the rocky quartzitic outcrops of the South Outeniqua Sandstone Fynbos vegetation at the Fynbos Private Nature Reserve (Robberg Coastal Corridor, Rooikrans).

All *Psoralea* species bearing (3)5–11(19)-foliolate leaves tend to be lumped together as the *Psoralea pinnata* species complex. This complex is thought to contain at least 28 species among which only 10 have been described formally. The complex includes all members of the genus *Psoralea* L. that fall within the broad concept of *P. pinnata* as described by Linnaeus (1753). The major features of Linnaeus' concept of *P. pinnata* are: arborescent or shrubby, densely branched, pubescent or glabrous, leaves imparipinnate, in 3–5 pairs, linear or linear lanceolate, acute, very narrow, pedicels axillary, long or short, bracteolate (now cupulum) beyond the middle, calyx very variable in incision and pubescence. At the time of Linnaeus' description, only 5 species were recognised. However, several species were described later by various authors including de Candolle, Jacquin, Poiret and Harvey. The following two new species are part of this complex as expanded progressively by these authors. Our current phenetic analysis, results not reported here, shows that only 24 species can be recognise as members of this complex. Most of the undescribed species are known only by their informal names in herbaria, and in publications describing the plants of the region e.g., Manning and Godlblatt (2012). Some of these have been included as 'sp. nov' in the Red Data list of southern African plants (Raimondo et al. 2009). These undescribed species will be described in a separate paper.

In the descriptions below, reference is made of a cupulum; an unusual and unique structure in the Fabaceae. In *Psoralea* there are no free bracts or bracteoles on or below the calyx. Instead these are fused into a complex cupulate structure which may occur anywhere along the peduncle between its base and apex. The cupulum is a diagnostic feature of most species (Tucker and Stirton 1981).

Species treatment

***Psoralea diturnerae* A. Bello, C.H. Stirt. & Muasya, sp. nov.**
urn:lsid:ipni.org:names:77144551-1

Note. Similar to *Psoralea pinnata* L., but differs in being a resprouter with massed short shoots from a woody rootstock (versus much-branched reseeder with single stem in *P. pinnata*); grooved 3-foliolate leaves (versus 7–9-foliolate); flowers 1–3 per axil (versus 1–6).

Type. SOUTH AFRICA**.** Western Cape, Oudtshoorn (–3322), Northern foothills of Outeniqua mountains, Camfersklоof (–CD), 33°50'42.20"S, 22°25'8.93"E, 14 February 2014, *A. Bello, A.M. Muasya, & C.H. Stirton 41* (holotype: BOL!; isotypes: K!, NBG!, PRE!).

Description. *Habit* an erect shrub up to 2 m tall, resprouter. *Stems* 1–3, bare with bursts of seasonal shoots in upper parts, with wide internodes; brown, covered in white storied lenticels; young seasonal shoots green, glabrous, glandular; flowering shoots produced seasonally on old stems, leafy along their entire length; plants also produce numerous sterile "water" shoots up to 1 m tall giving the plant an untidy restioid appearance. *Leaves* 3-foliolate at the base of each seasonal shoot, reducing to 1-foliolate thereafter, glabrous; leaf size variable, larger on water shoots from the rootstock (30–45 mm long, 30–40 mm wide); petiole 2–3 mm long; terminal leaflet of flowering shoots longest (20–40 mm long), basal pair (25–35 mm long), all 1.0–1.3 mm wide; glabrous, dark green; grooved, apex acuminate, base rounded; stipules 2–3 mm long, fused for half their length to the petiole, rigid, triangular, semi-patent, those on water shoots are longer, green and arching, rapidly senescent on flowering shoots. *Inflorescences* axillary along the length of seasonal shoots; flowers 1–3 per axil; peduncles absent or <1 mm long, terminated by a tri-toothed cupulum; cupulum lower tooth longest, acuminate, upper two teeth fused for half their length, yellowish, rapidly senescent, 1.0–1.2 mm long; pedicels 1–2 mm long *Flowers* 10–12 mm long, mauve to purple and white, borne 1–3 in leaf axils along seasonal flowering shoots. *Calyx* 5–6 mm long, 4 mm wide, pale green; tube 4 mm long, glabrous, ribbed; teeth triangular, equal, shorter than the tube, 2 mm long, carinal tooth cucullate at apex; glandular, margins ciliate with black hairs, inner face of teeth densely black-haired. *Standard petal* broadly elliptic to broadly ovate, 9–10 mm long, 8–10 mm wide; claw 2–3 mm long; mauve to purple, nectar "guide" situated above the strongly developed free appendages above the apex of the claw and comprised of a basal white area from which emerges a trifid purple flash that bleeds off into purple veins. *Wing petals* 9–11 mm long, 4 mm wide; claw 3 mm long; locked into keel indentation but not fused with it; longer than the keel; petal sculpturing present, upper basal, comprising 7–8 transcostal parallel lamellae. *Keel* 6 mm long, 3 mm wide; claw 5 mm long; apex dark purple. *Androecium* 9 mm long; tenth stamen free; sheath split adaxially, fenestrate; nectarial ring present, 0.5 mm high. *Pistil* 9 mm long; ovary 1.5 mm long, stipitate, glabrous but sparsely covered near distal end in curved stalked glands; ovules 1; style thickened at point of

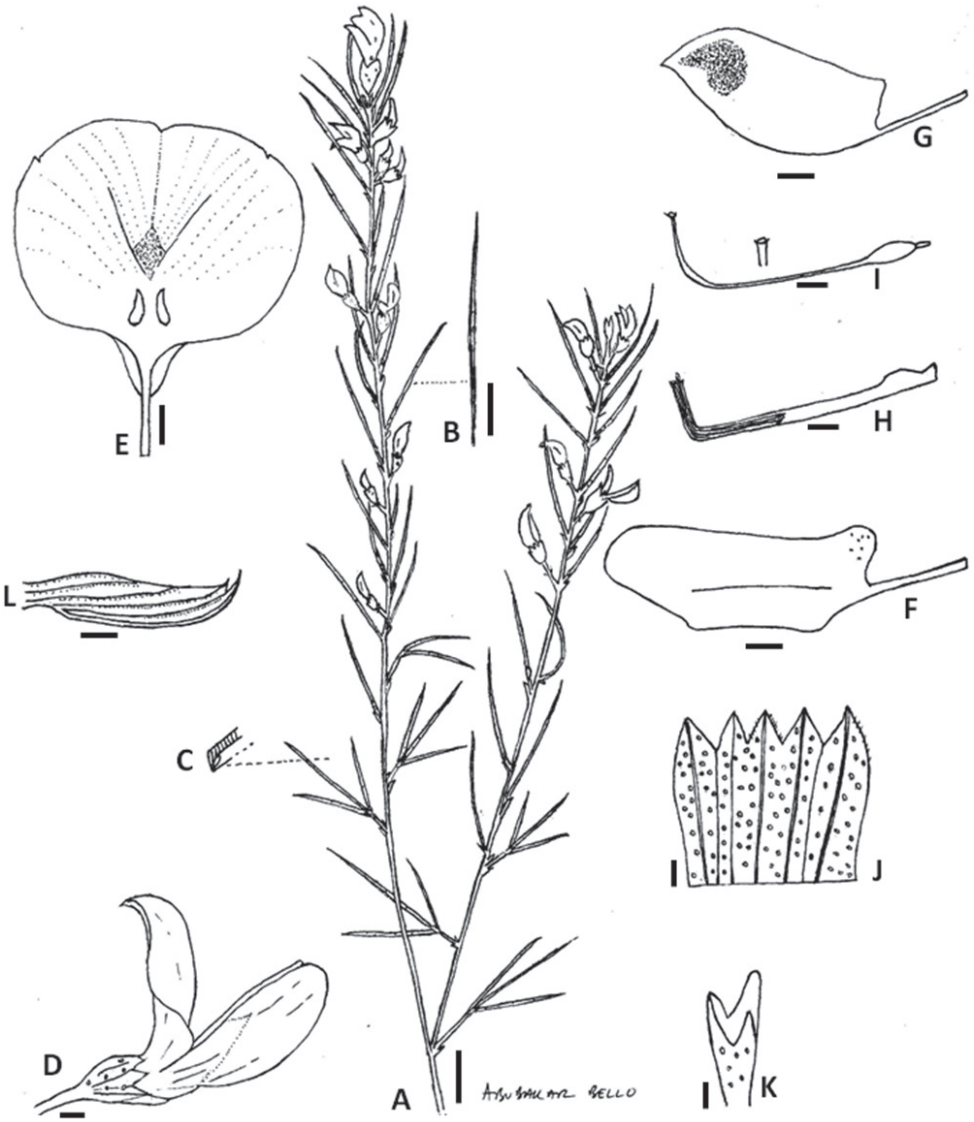

Figure 1. *Psoralea diturnerae* A. Bello, C.H. Stirt. & Muasya **A** flowering branch **B** leaf **C** transverse section of the leaflets **D** flower side view **E** standard petal **F** wing petal **G** keel petal **H** androecium **I** gynoecium showing the stigma **J** outer surface of calyx opened out **K** trifid cupulum **L** bud. Scale bars: **A, B**=1 cm; **D–K**=1 mm. Line drawing by Abubakar Bello from voucher *A. Bello, C.H. Stirton & A.M. Muasya 41* (BOL).

flexure, height of curvature 2 mm; stigma erect, penicillate. *Fruits* unknown. *Seeds* unknown (Fig. 1, Plate 1).

Habitat. This species occurs in a small area of arid fynbos on the acidic lithosol soils (Glenrosa and Mispah forms) of North Outeniqua Sandstone Fynbos vegetation

Plate 1. *Psoralea diturnerae* A. Bello, C.H. Stirt. & Muasya **A** front view of flower **B** side view of flower **C** apex of flowering shoot **D** seasonal flowering shoot **E** habit with C.H. Stirton **F** leaf **G** short flowering shoot. Photographs Nicky van Berkel (**A–C, F & G**), Abubakar Bello (**D**) and Sandra Falanga (**E**). Voucher *A. Bello, A.M. Muasya & C.H. Stirton 41* (BOL).

(FFs 18; Rebelo et al. 2006). The plant grows along streams or near water in the valley bottom, but is also found higher up the lower slopes along seepages.

Flowering time. December to February.

Altitude. Known from 620–667 m.

Distribution. *Psoralea diturnerae* is narrowly endemic to the northern slopes of the Outeniqua mountains in the Camferskloof area, George, Western Cape Province of South Africa (Fig. 3). Unlike other species of *Psoralea* (e.g. *P. odoratissima* Jacq., *P. pinnata* L., and *P. speciosa* Eckl. & Zeyh.) that are colonial, this species is occasional in the landscape across a wider area.

Etymology. The specific epithet *diturnerae* honours Ms. Di Turner, ispotter (http://www.ispot.org.za/user/10170), the leader of the Outramps group of the Custodians of Rare and Endangered Wild flowers (C.R.E.W., South Africa) and her merry band of walkers who brought this species to our attention and sent us reference material and photographs. Her energy and drive has made her group the most active C.R.E.W. group in South Africa.

Conservation status. *Psoralea diturnerae* is very rare and has only been found in the Camferskloof area on the northern slopes of Outeniqua mountains. So far only

eight live individuals have been recorded in its habitat which is in a privately protected area but under possible threat from the nearby alien pine plantations. It is, however, possible that other individuals will emerge after the next fire as the plants we saw were old and leggy. Further surveys are being planned to find more individuals. We therefore assess this species to be Vulnerable under the South African Red List categories and criteria (VU D2, von Staden et al. 2009, IUCN 3.1, 2012a, 2012b).

Discussion. *Psoralea diturnerae* is a recent discovery and is part of the *Psoralea pinnata* complex. It is a suffrutex with a restioid appearance of massed short shoots arising from a woody rootstock and from which emerge 1–3 long shoots that branch in their upper parts. It also has grooved 3-foliolate clasping glabrous needle-like leaves with the terminal leaflet longest. It bears rigid, triangular, semi-patent, rapidly senescent stipules, 1–3-flowered axillary inflorescences produced for long lengths of the flowering shoot and a white to purple standard petal with a white trifid central flash above the strongly developed auricles. It can also be recognised by its glabrous pale green calyx with purple flushes. *Psoralea pinnata* by contrast is a much-branched reseeding shrub to small tree up to 5 m tall with 7–9-foliolate linear, villoso-pubescent spreading leaves with the terminal leaflet shortest; has subulate recurved persistent stipules that become woody when leaves are shed, pale mauve or pale blue flowers borne along flowering shoots in pseudo-inflorescences, hidden within the subtending leaves, and the yellowish green, white (mostly) and black-haired calyces (Table 1). The species are allopatric.

Additional specimens examined. Camferskloof, northern slopes of the Outeniqua Mountains, 33°50'56.9"S, 22°24'54.7"E (3322CD), Sandstone Fynbos, 622 m, 14 February 2014, *A. Bello, C.H. Stirton & A.M. Muasya 43* (BOL).

Camferskloof, northern slopes of the Outeniqua Mountains, 33°51'07.2"S, 22°25'04.7"E (3322CD), Sandstone Fynbos, 667 m, 23 January 2013, *Nicky van Berkel 1120* (BOL).

Psoralea vanberkelae **C.H. Stirt., A. Bello & Muasya, sp. nov.**
urn:lsid:ipni.org:names:77144552-1

Note. Similar to *Psoralea pinnata* L., but differs in being a short (less than 1 m) resprouter with sprawling and mounding habit (versus tall reseeder to 5 m with single stem in *P. pinnata*); short 5-foliolate leaves (versus 7–9-foliolate); flowers solitary per axil (versus 1–6).

Type. SOUTH AFRICA**.** Western Cape, Knysna (–3423), Robberg Coastal Corridor, Fynbos Private Nature Reserve (–AB), 34°05'51.72"S, 23°17'5.82"E, 134 m, 18 October 2013, *N. van Berkel 1118* (holotype: BOL!; isotypes: GRA!, K!, NBG!, SCHG!, PRE!).

Description. *Habit* a small sprawling and mounding shrub to 60 cm tall and up to 1.5 m wide, resprouter. *Stems* 1–10, branching in upper parts of stems; branches erect, rough, grey, mostly bare except for upper parts; young seasonal shoots rough,

Table 1. Some diagnostic characters distinguishing the two new species from *P. pinnata*.

S/No	Characteristics	*P. pinnata*	*P. diturnerae*	*P. vanberkelae*
1	Habit	tall shrub to 5 m	small shrub to 2 m	shrub, less than 1 m
2	Regeneration strategy	reseeder	resprouter	resprouter
3	Appearance	erect, multi-branched in upper parts	erect, less branched in upper parts with numerous sterile short basal shoots	sprawling and mounding, multi-branched
4	Leaves	7–9-foliolate, 20–45 mm long	1–3-foliolate, 30–45 mm long	5-foliolate, 9–11 mm long
5	Inflorescence	flowers 1–6 per axil, pale mauve or pale blue, 14–18 mm long, hidden within the subtending leaflets	flowers 1–3 per axil, mauve to purple and white, 10–12 mm long, exposed within the subtending leaflets	flowers solitary per axil, pale to intense mauve to blue, 10–11 mm long, exposed above the subtending leaflets
6	Calyx	8–9 mm long, yellowish green	5–6 mm long, pale green	6 mm long, pale green
7	Cupulum	trifid, lobes equal, overlapping the calyx	trifid, lobes unequal, free from the calyx	trifid, lobes equal, free from the calyx

blackish, hairy. *Leaves* 9 mm long, 10 mm wide, pinnately 5-foliolate, linear oblong, petiolate, fleshy, basal leaves of seasonal shoots smallest, patent to semi-erect, surface bumpy, glabrous; glands raised, hyaline but drying reddish brown to black, rachis grooved; basal leaflet pair 10 mm long, 0.5 mm wide, equal to or slightly shorter than terminal leaflet; terminal leaflet 10–11 mm long, 0.5 mm wide, flat on adaxial surface with a distinct furrow; stipules 2 mm long, 1 mm wide, straight, fused, joined by a bridge of tissue, glabrescent, teeth broadly triangular, apex acute, fleshy, persistent, becoming prominent and woody when leaves are shed, hairy, hairs short and stubby, covered densely with large raised glands. *Inflorescences* axillary in upper nodes of short seasonal shoots; peduncle short, 2 mm long, hairy; peduncle bracts paired, minute; cupulum 1 mm long, pale green, trifid, shortly triangular, lobes equal, black-haired, covered in large glands, drying reddish brown; pedicel 2 mm long. *Flowers* 10–11 mm long, pale to intense mauve to blue, borne solitary per axil. *Calyx* 6 mm long, 4 mm wide; tube 4 mm long, ribbed; teeth equal, shorter than tube, 2 mm long, pale green, sparsely covered in small black flat hairs and densely encrusted with mixed sized glands on outside; margins of teeth densely black ciliate, inside of teeth densely stubby black-haired; vexillar teeth scarcely fused above tube. *Standard petal* 9–10 mm long, 7–8 mm wide; claw 2–3 mm long, flattened, erect; very broadly ovate, reflexed to 90 degrees, apex rounded; mauve but dark purple in central area above the M-shaped white nectar "guide", venation purple; callosities above the claw absent. *Wing petals* 6–7 mm long, 3–4 mm wide; claw 4–5 mm long; longer than keel petals, strongly folded once along middle, slightly billowy near apex, held parallel to keel, strongly auriculate; sculpturing present, upper basal comprised of 4–5 transcostal lamellae. *Keel* 5–6 mm long, 3–4 mm wide; claw up to 5 mm long. *Androecium* 7 mm long; tenth stamen free; sheath

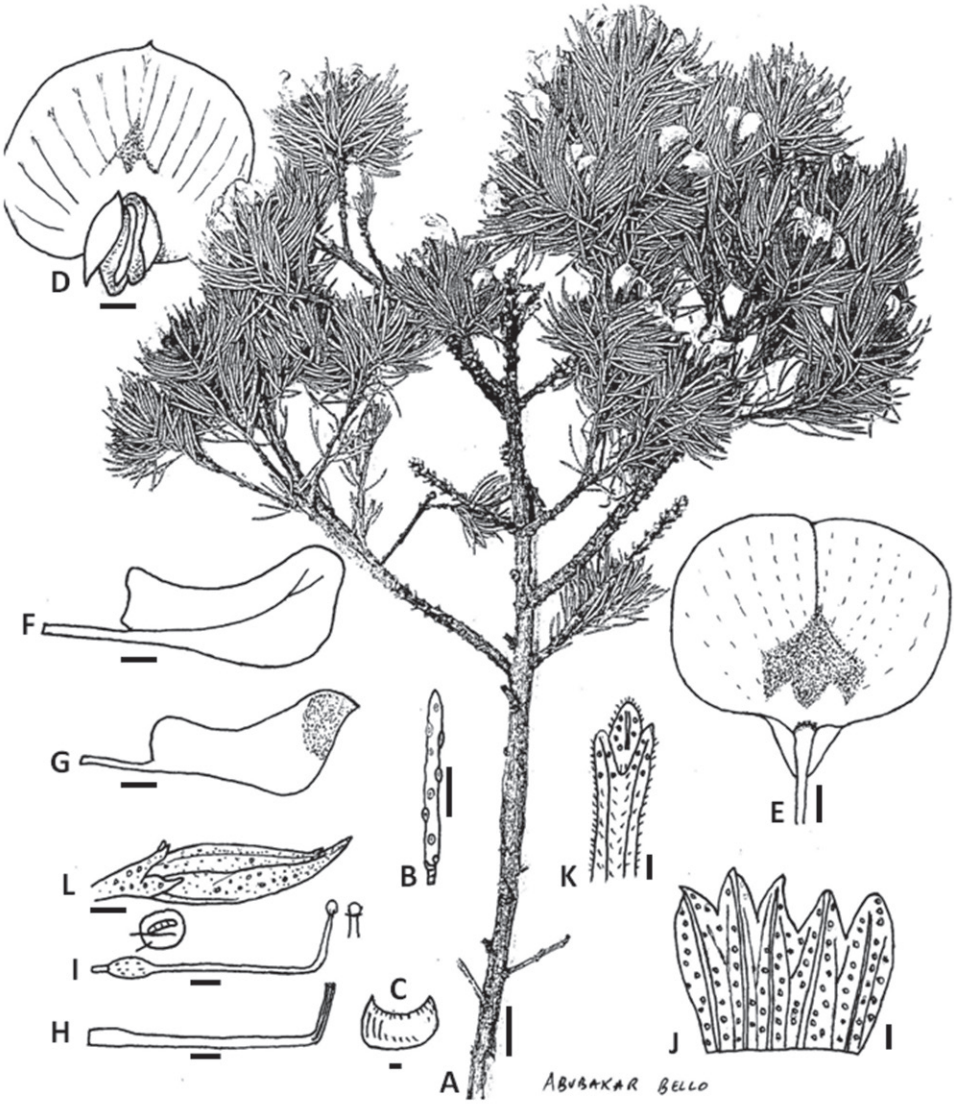

Figure 2. *Psoralea vanberkelae* C.H. Stirt., A. Bello & Muasya **A** flowering branch **B** leaf **C** stipule **D** flower viewed from the front **E** standard petal showing the M-shaped nectar patch **F** wing petal **G** keel petal **H** androecium **I** gynoecium showing the stigma **J** outer surface of calyx opened out **K** trifid cupulum **L** bud. Scale bars: **A, B**=1 cm; **C–L**=1 mm. Line drawing by Abubakar Bello from voucher *N. van Berkel 1118* (BOL).

split abaxially, fenestrate; nectarial ring present, 0.3 mm high. *Pistil* 7 mm long; ovary 2 mm long, stipitate, glabrous but sparsely covered in curved stalked glands across sides; ovules 1; stigma penicillate. *Fruits* 1, 5 mm long, 3 mm wide, papery, rugose, reticulate, brown. *Seeds* 4 mm long, 2.5 mm wide, oblong-elliptic, khaki with black mottles and flecks, hilum central (Fig. 2, Plate 2).

Plate 2. *Psoralea vanberkelae* C.H. Stirt., A. Bello & Muasya **A** details of glands on leaflets **B** base view of flowers showing cupulums below calyces **C** flower **D** short seasonal shoot with flower **E** habit. Photographs Nicky van Berkel. Voucher *N. van Berkel 1118* (BOL).

Habitat. Endemic to South Outeniqua Sandstone Fynbos (FFs19, Rebelo et al. 2006). The vegetation type is a mixture of Eastern Fynbos and Renosterveld. It grows in full sun on sandy soils over Peninsula formation quartzite on a gentle slope. The area was subjected to a controlled burn in April 2008, so plants are becoming old and starting to die back.

Flowering time. August to November.

Altitude. 70–150 m.

Distribution. *Psoralea vanberkelae* is a narrow endemic. It is known from some hundreds of individuals in an area of 500 × 500 m along the George to Knysna coastal stretch of the Indian Ocean and also from Cairnbrogie (Nicky van Berkel pers. comm., photographs) all in Western Cape Province of South Africa (Fig. 3).

Etymology. The specific epithet *vanberkelae* honours Ms. Nicky van Berkel, a C.R.E.W. volunteer and iSpotter ("Nicky"; http://www.ispot.org.za/user/10095), who brought this species to our attention and sent us reference material and photographs. Like many plant enthusiasts from C.R.E.W. she plays a valuable role in establishing the conservation status of plants in her area. The plant is a beautiful flagship species for a very threatened habitat.

Conservation status. *Psoralea vanberkelae* is locally abundant in its habitat and the main population is protected by private ownership (Fynbos Private Nature Reserve).

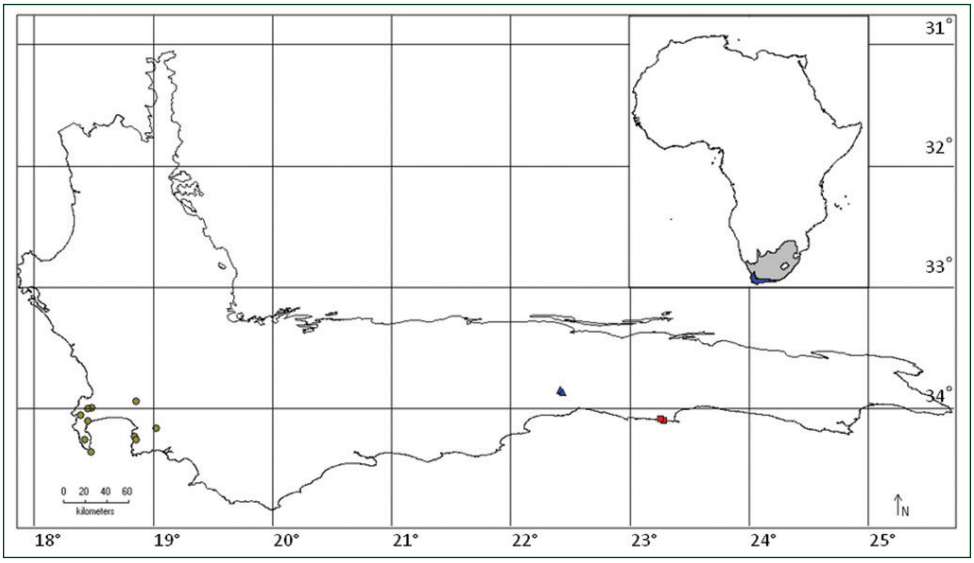

Figure 3. Distribution of *Psoralea pinnata* (circles), *P. diturnerae* (triangles) and *P. vanberkelae* (squares). The top right map of Africa shows the position of the Core Cape Region (blue) in South Africa (grey).

It is, however, restricted by a narrow range of distribution (area less than 20 km^2). The coastal stretches where the plants occur are all on private land with limited access. The cliff edges rise sharply from the sea and their escarpments are not easy to access. We therefore assess this species to be Vulnerable under the South African Red List categories and criteria (VU D2, von Staden et al. 2009, IUCN 3.1, 2012a, 2012b).

Discussion. *Psoralea vanberkelae* is a recent discovery and is part of the *Psoralea pinnata* complex. It is a small, colonial, resprouting, sprawling and mounding shrub to 60 cm tall and up to 1.5 m wide. It has clasping, tightly packed, 10–11 mm long leaves on short shoots. It also has glabrous leaflets with large round impressed glands. Its terminal leaflet is in most cases longest. It has pale to intense mauve to blue flowers borne at the end of short flowering shoots in pseudo-inflorescences and held above the subtending leaves. *Psoralea pinnata* on the other hand is a taller much-branched reseeding shrub to small tree up to 5 m tall with 7–9-foliolate linear, 20–45 mm long, villoso-pubescent spreading leaves with the terminal leaflet shortest (Table 1).

Additional specimens examined. Robberg Coastal Corridor, Fynbos Private Nature Reserve Section, Knysna, 34°05'53.84"S, 23°17'4.56"E (3423AB), South Outeniqua Sandstone Fynbos, 134 m, 14 February 2014, *A. Bello, C.H. Stirton & A.M. Muasya 53* (BOL).

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