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Iron islands in the Amazon: investigating plant beta diversity of canga outcrops

Caroline Oliveira Andrino[1,2], Rafael Gomes Barbosa-Silva[1,2], Juliana Lovo[1,2], Pedro Lage Viana[2], Marcelo Freire Moro[3], Daniela Cristina Zappi[1,2]

1 *Instituto Tecnológico Vale, Belém, Pará, Brazil* **2** *Museu Paraense Emílio Goeldi, Coordenação Botânica, Belém, Pará, Brazil* **3** *Instituto de Ciências do Mar (Labomar), Universidade Federal do Ceará, Fortaleza, Ceará, Brazil*

Corresponding author: Caroline Oliveira Andrino (coliveiraandrino@gmail.com)

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Abstract

The world's largest mineral iron province, Serra dos Carajás, is home to an open vegetation known as canga, found on top of isolated outcrops rising out of the Amazon rainforest. Over one thousand vascular plants species have been recorded in these canga sites, including 38 edaphic endemics. A new survey adds to our investigation of biogeographic relationships between sixteen canga outcrops and the effect of the distance between site pairs on the number of shared species, regional species turnover and species distribution patterns. Plant collecting expeditions to the westernmost site, the Serra de Campos of São Félix do Xingu (SFX), were carried out followed by the identification of all collected specimens and the creation of a species database, built to perform biogeographical analyses. Floristic relationships among the sites were investigated regarding their similarity, using multivariate analyses. The correlation between canga areas and species richness was tested, as well as the geographical distance between pairs of outcrops and their shared species. Vascular plants at SFX total 254 species including 17 edaphic endemics. All canga sites are grouped with 25% of minimum similarity, and the SFX falls within a large subgroup of outcrops. The total species number shared between site pairs does not change significantly with geographical distance but is positively correlated with the area of each outcrop. Meanwhile, shared endemic species numbers between site pairs decline when geographical distance increases, possibly imposed by the barrier of the rainforest. Our data suggest higher shared similarity between the largest and species-richest sites as opposed to geographically nearby sites, and provide useful insight for drafting conservation and compensation measures for canga locations. The size of the canga outcrops is associated to higher floristic diversity but connectivity among islands also plays a role in their similarity.

Keywords

campo rupestre, edaphic endemism, island-like habitats, Neotropical mountains, plant species diversity, rainforest, vascular plant survey

Introduction

Mountaintops are often compared to sky-islands, as their vegetation is often distinct from the surrounding lowlands (Alves and Kolbek 2010; Barres et al. 2019). Montane habitats have been scrutinized due to their high species richness and complexity (Särkinen et al. 2012; Antonelli 2015; Kok et al. 2017), arousing scientific interest and have been featured since the first biogeographic studies (Humboldt 1805). In the Amazonian context, open vegetation predominates on exposed rocky surfaces on mountaintops, as opposed to the surrounding lowland rainforest. This vegetation may occur on isolated granite and gneiss inselbergs and quartzitic tepuis, usually above 900 m a.s.l. (Prance 1996; Riina et al. 2019), or over iron-ore conglomerates in the campo rupestre on canga (CRC), found between 600 and 800 m a.s.l. (Viana et al. 2016; Mota et al. 2018; Zappi et al. 2019). There are also island-like lowland ecosystems, such as white sand campinaranas, savannas, and low elevation granitic domes or inselbergs, associated with arenitic and often waterlogged soil in the Amazon region (Gröger and Huber 2007; Adeney et al. 2016; Costa et al. 2019; Henneron et al. 2019; Devecchi et al. 2020).

Canga is the lateritic duricrust that covers a supergene iron ore, with poorly developed soil and moderately hard rocks that are very resistant to erosion and permeable (Gagen et al. 2019). The iron-rich canga presents a series of restrictions to plant establishment, including shallow and rocky soils, high insolation levels, elevated temperatures at ground level, extreme water regime – waterlogged soil alternating with up to five months of drought, added to the presence of metals at potentially toxic concentrations (Schettini et al. 2018). The vegetation in the canga has specific strategies to survive in these stressful edaphic conditions (Gagen et al. 2019), and these conditions have favoured the diversification of edaphic endemic species that are exclusive to the CRC associated with the iron-rich substrate (Giulietti et al. 2019).

Species isolation caused by environmental conditions contrasting with the surrounding forests and associated with the mosaic of different geomorphological situations in the canga creates also an abundance of micro-habitats (Jacobi et al. 2007; Mota et al. 2015; Silva et al. 2020). It is known that such micro-habitats may be linked to multiple speciation events, and the occurrence of endemism (Bonatelli et al. 2014; Leal et al. 2016; Fiorini et al. 2019; Perrigo et al. 2019; Mota et al. 2020).

The first botanical studies on the iron islands of the Serra dos Carajás began in the late 1960s. However, the floristic knowledge was not synthetized and organized until the Flora of the canga of the Serra de Carajás (FCC) project was completed in 2018 (Viana et al. 2016; Mota et al. 2018). This recent flora increased the number of recorded species to 1042 vascular plants (Mota et al. 2018; Salino et al. 2018), and a number of species were confirmed as endemic to the local canga habitat, with 38 species occurring exclusively on this substrate in an area of occupancy of less than 150 km2 (Giulietti et al. 2019). In terms of phytophysiognomies, three major groups were defined by Mota et al. (2015) for Carajás: canga vegetation (scrub, bare slab, nodular canga and low forest grove), hydromorphic vegetation (bogs, temporary lagoons, permanent lakes, temporary streams, buriti palm lakes, swampy forest) and other associated forests (mostly at the edge of canga outcrops).

Due to historic reasons, collection efforts of the FCC project prioritized some areas of canga, while others still lack in-depth studies. For instance, a research in the canga of the Serra Arqueada (SA) in the municipality of Ourilândia do Norte has recently been completed (Fonseca-da-Silva et al. 2020), while the outcrops located within the recently created Parque Nacional dos Campos Ferruginosos (PNCF) are still in need of further investigation (Zappi et al. 2019). Giulietti et al. (2019) mentioned the existence of an interesting, isolated area of canga located c. 160 km southwest of the area studied by the FCC known as Serra de Campos, in the municipality of São Félix do Xingu (SFX).

This study aims to investigate plant distribution and biogeographical patterns that connect the island-like habitats of canga outcrops isolated within an Amazonian rainforest matrix. We evaluated species distribution in the different sites in order to observe whether canga vegetation has elevated levels of beta diversity and whether the flora of each outcrop will be more dissimilar to other outcrops as the geographical distance increases. We provided the first checklist of vascular plants growing on canga at the Serra de Campos of São Félix do Xingu (SFX), to add to the dataset we built to investigate the floristic relationship between canga areas, aiming to improve our understanding of the rich and diverse flora of the region.

Methods

Characterization of the overall study area

The CRC are found in the region of Carajás, located in the southeast part the State of Pará (Viana et al. 2016; Zappi et al. 2019), one of the largest mineral provinces in the world (Ab'saber 1986). At the Serra dos Carajás, the CRC appears atop a series of outcrops that form discontinuous island-like habitats of open, shrubby or grassy vegetation within a dense matrix of rainforest in the southeastern Amazon basin (Mota et al. 2018).

Most of the ferruginous island complex in the southeastern Amazon is within areas protected at different levels. The Serra Norte (SN1, SN2, SN3, SN4, SN5, SN6, SN7, SN8), the Serra Sul (S11A, S11B, S11C S11D) are located in the Floresta Nacional de Carajás, which is an area of sustainable use and thus subject to anthropogenic pressures, and iron ore mining currently occurs in areas SN4, SN5 and S11D. The Serra da Bocaina and Serra do Tarzan are the only fully protected areas, and are both inserted within the Parque Nacional dos Campos Ferruginosos (PNCF). However, the Serra Arqueada and Serra de Campos of São Félix do Xingu have no legal protection.

Floristic list of Serra de Campos

The Serra de Campos (SFX) is a canga outcrop found in the municipality of São Félix do Xingu, southeastern Pará state, Brazilian Amazon. It represents the westernmost limit of the Serra dos Carajás, a complex of ferruginous highland outcrops that extends eastwards to the Municipality of Curionópolis, totalling 126 km2. The plateaus previously studied in the scope of the FCC project (Viana et al. 2016) are found in the Municipalities of Parauapebas (Serra Norte – SN1 to SN8), and Canaã dos Carajás (Serra Sul – S11, Serra do Tarzan – ST and Serra da Bocaina – SB). The SFX comprises two plateaus measuring c. 9 km2, distant about 1 km from each other, known as SFX1 and SFX2 (Fig. 1). The largest of the two plateaus, known as SFX2, extends for 8.5 km and covers an area of 7.6 km2, while SFX1 is 2.5 km long, measuring 1.4 km2. The plateaus are located at 6°23'41"S, 51°52'25"W, with altitudes ranging from 580 to 730 m. a.s.l. (Fig. 1). Distant about 80 km west from SA, the SFX can be accessed through the Municipality of São Felix do Xingu first by crossing the Rio Fresco then taking a road that goes through farmland, leading, after a steep climb, to the canga plateaus.

Botanical specimens from SFX deposited in herbaria prior to this study were located through an online search at the Herbarium of the Museu Paraense Emílio Goeldi (MG) and Herbário Ezechias Paulo Heringer (HEPH) (acronyms according to Thiers, continuously updated). Prior to our expeditions, specimens at MG were collected in the 1990´s by João Batista Fernandes da Silva and include the type of *Mimosa dasilvae* A.S.L. Silva & Secco and several gatherings of Orchidaceae, while HEPH currently holds collections made by Annajulia Elizabeth Heringer Salles and J.B.F. Silva in 2001. All materials available in these collections were analyzed and included in this study.

Four plant collecting expeditions were carried out between 2016 and 2019 (May 2016, April 2017, March 2018, October 2019), aiming to collect fertile material of all vascular species. Collecting method followed Filgueiras et al. (1994) with random walks covering the accessible parts of both plateaus, attempting to stop every 1 km to sample the vegetation and collect fertile specimens. We aimed to visit diverse vegetation types, including open canga slabs, nodular canga, canga scrub, palm swamps (buritizais) and temporary lagoons (Mota et al. 2015)

The samples collected were identified to species by comparing their macroscopic and microscopic morphological features with available bibliography, against herbarium collections (physically and on-line) and also consulting key family specialists. Voucher specimens were deposited at MG. Only one collection number per taxon is cited in the present floristic list. A full specimen list is provided in supplement S1. Species names follow Flora do Brasil online (Flora do Brasil under construction), family delimitation followed APG IV (Angiosperm Phylogeny Group 2016) and author abbreviations follow IPNI (2019).

Database of the distribution of the flora of Serra dos Carajás

Seed plant species distribution data were assembled from the FCC project (Mota et al. 2018), with the compilation of a database comprising 3228 occurrences of 823 species (Zappi et al. 2019). The updates included 23 recent new occurrences for SN1, SN4, SN5, SN7, S11D, and the Serra da Bocaina based on recently collected herbarium material; 149 species for SA (Fonseca-da-Silva et al. 2020); and the newly prepared dataset of SFX. The assembled database comprises 909 seed plant species recorded in CRC at the Carajás

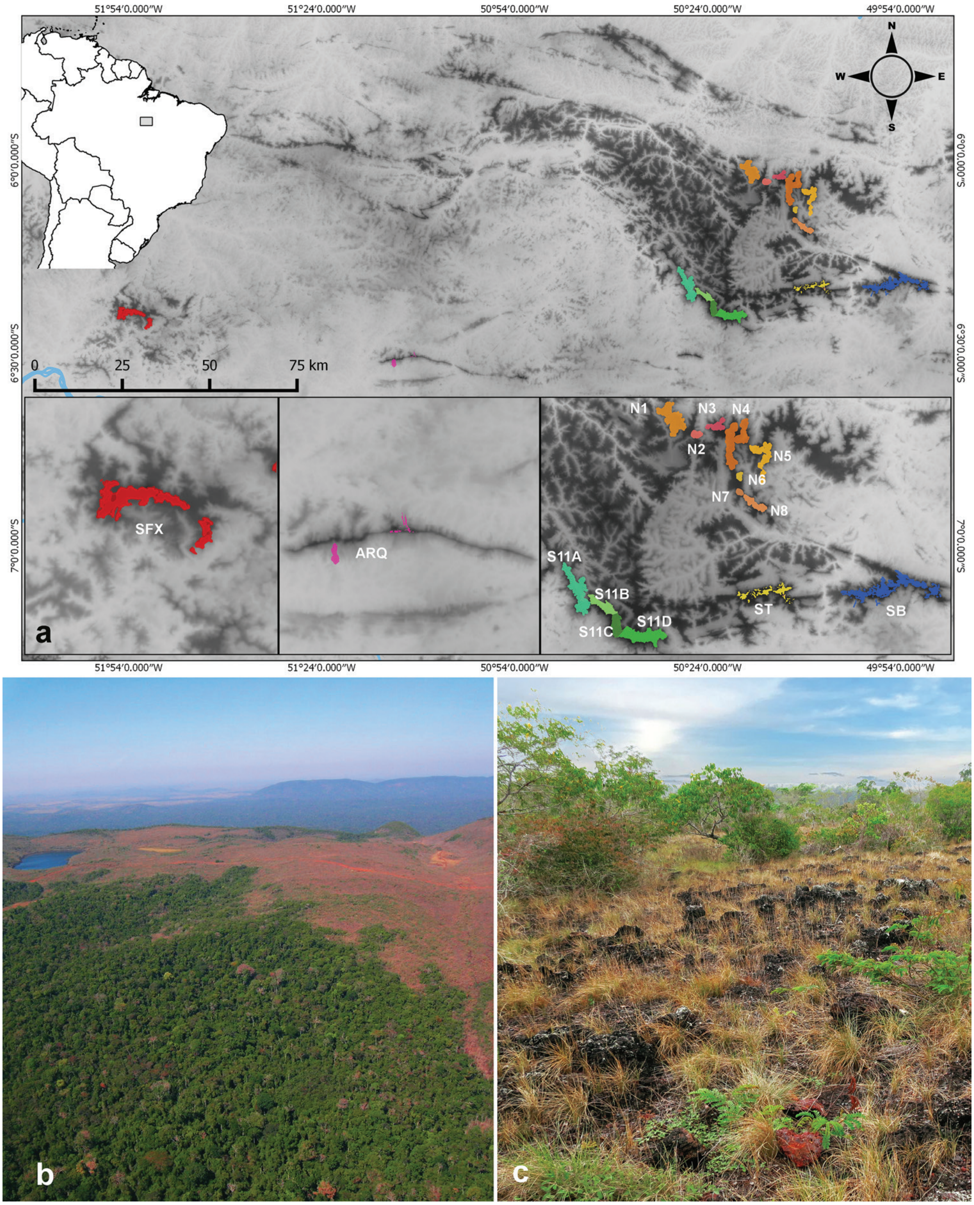

Figure 1. a Geographic location of the present study site at SFX and the other study areas from Carajás complex **b** aerial view of an island of *canga* vegetation surrounding by the rainforest (Photo: Leonardo Vianna) **c** *Serra de Campos* of *São Félix do Xingu* (SFX) phytophysiognomy with shrubby and grassy vegetation.

complex, including 16 sites (SN1, SN2, SN3, SN4, SN5, SN6, SN7, SN8, S11A, S11B, S11C, S11D, ST, SB, SA and SFX). For the purpose of our analyses, exotic, invasive and weedy species were removed from the dataset as identified in (Giulietti et al. 2018), resulting in 893 species analysed. The code assigned for each site is found in Table 2.

Biogeographical analyses of the flora of canga sites in the Carajás complex

To perform the biogeographical analysis of the CRC of the Carajás complex, the species database was used to investigate the floristic similarity and shared endemicity between different mountaintops across canga sites. Invasive exotic species recorded in each site were excluded from this analysis, as well as specimens with imprecise identification, Lycophytes, and Monilophytes. Floristic similarity between sites was calculated using a presence-absence Matrix (S2, Suppl. material 1) to perform multivariate analysis using ordination and group multivariate methods using the Vegan package in R (Oksanen et al. 2010). We constructed a matrix showing the presence of each species in each site and subjected it to ordination and grouping analyses using a Non-metric Multidimensional Scaling (NMDS) and Unweighted Pair Group Method with Arithmetic mean (UPGMA), respectively. Both analyses used Sorensen (Bray-Curtis) index (Legendre and Legendre 2012) to reflect beta diversity between sites.

To investigate the floristic richness of sites in relation to the size of each outcrop we used the species count for each canga outcrop and, employing GIS, we calculated the area of each outcrop in square kilometres. A linear model of the recorded richness versus area of each outcrop using the 'glm' function with Gaussian model was prepared in R. Because the outcrops were subjected to a large collecting effort during the 'Flora of Carajás' Project, we assumed that they were adequately sampled. We also evaluated whether the total number of species and of endemic species shared between sites were significantly related with the geographical distance between them. We computed the centroid of each outcrop using GIS and calculated the geographical distance between the centroids of all outcrop pairs. We tested the normality of the residuals of the models with the Shapiro-Wilk test to see whether the residuals significantly departed from normality. If these did not significantly differ from normality, we accepted the p value of the model. If the residuals differed from normality, we analysed the data using non parametric Spearman's correlation to evaluate if the correlation was significant.

Results

Plant species in canga vegetation at Serra de Campos

This study recorded a total of 254 species, of which 248 are seed plants, five ferns and one lycophyte in the SFX (Table 1). The richest families recorded are Fabaceae (22 species), Poaceae (21 spp.), Cyperaceae (15 spp.), Orchidaceae (12 spp.) and Rubiaceae (12 spp.). The five richest genera are *Mimosa* (Fabaceae), with 5 species, *Cyperus* and *Rhynchospora* (Cyperaceae), with 4 species each, and *Borreria* (Rubiaceae) and *Aechmea* (Bromeliaceae), with 3 species each. Thirty-seven species are new records for the CRC of the *Carajás* complex. From these new records, seven belong to the family Orchidaceae, five are new records of Fabaceae, three Annonaceae, and three Sapindaceae. A yet undescribed species of Lauraceae was found in SFX, belonging to the genus *Dicypellium* (*Dicypellium* aff. *caryophyllaceum* (Mart.) Nees – PLV 6100, Table 1; Fig. 2).

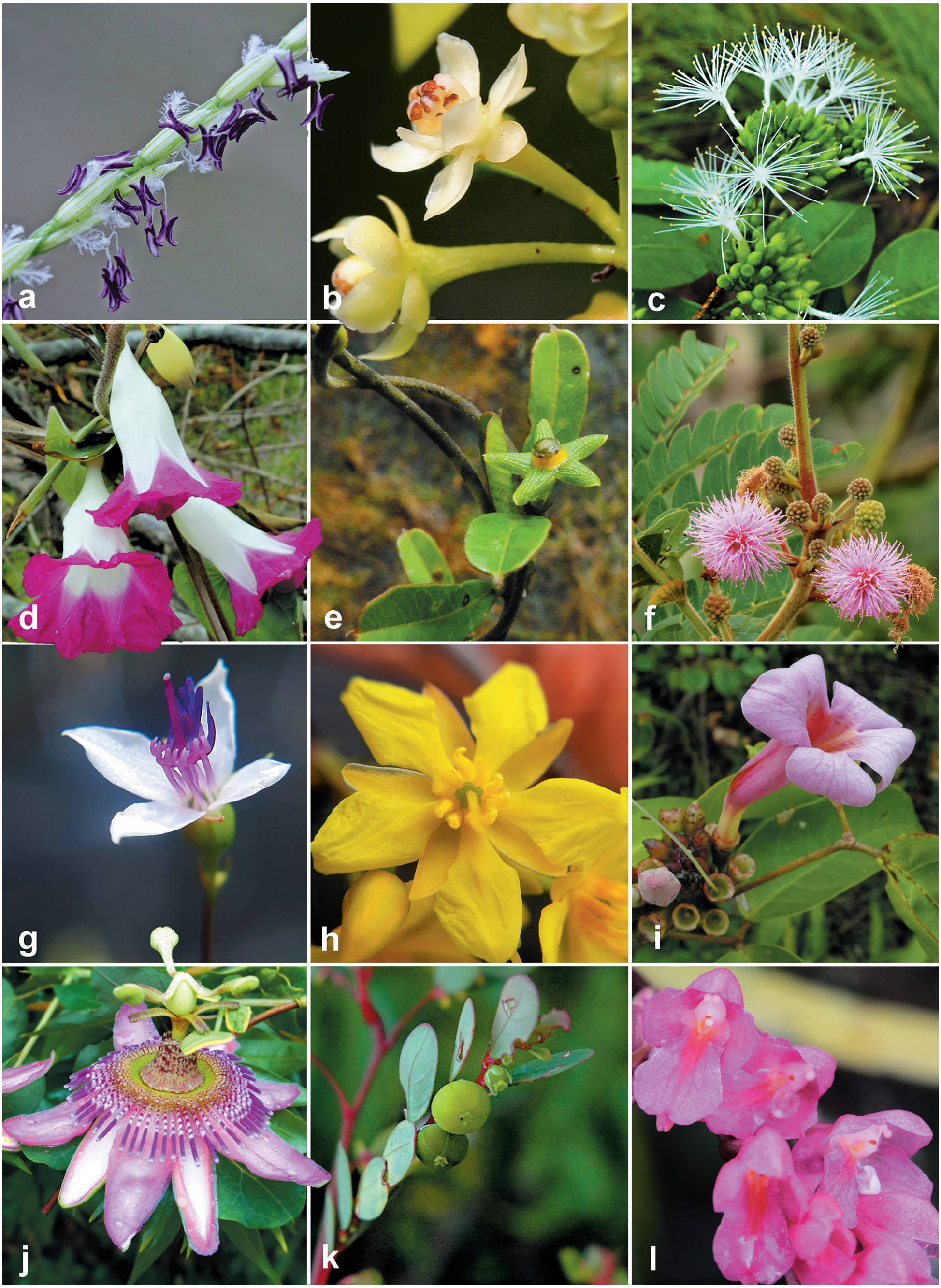

Figure 2. Representative species of canga in new dataset, SFX **a** *Axonopus longispicus* (Döll) Kuhlm **b** *Dicypellium* aff. *caryophyllaceum* (Mart.) Nees **c** *Inga heterophylla* Willd **d** *Ipomoea decora* Meisn **e** *Matelea microphylla* Morillo **f** *Mimosa dasilvae* A.S.L. Silva & Secco **g** *Nepsera aquatica* (Aubl.) Naudin **h** *Ouratea cearensis* (Tiegh.) Sastre & Offroy **i** *Pachyptera incarnata* (Aubl.) Francisco & L.G. Lohmann **j** *Passifora picturata* Ker Gawl. **k** *Phyllanthus minutulus* Mull.Arg. **l** *Rodriguezia lanceolata* Ruiz & Pav.

Table 1. Vascular plant species from Serra de Campos of São Félix do Xingu (SFX), discriminated by novelties for Flora of the canga of Carajás according to Mota et al. (2018) and Fonseca-da-Silva et al. (2020) endemism in canga outcrops according to Giulietti et al. (2019); endemism in Serra de Campos, and life form and voucher information for each species. Collectors: AHS: Anajulia Heringer Salles; BF: Bruno Fernandes Falcão; COA: Caroline Oliveira Andrino; DCZ: Daniela Cristina Zappi; JBFS: João Batista da Silva; MN: Matheus Nogueira; MP: Mayara Pastore; PLV: Pedro Lage Viana. *Invasive exotic species.

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Lycophyte					
Selaginellaceae					
Selaginella radiata (Aubl.) Spring.				Herb	DCZ 4055
Monilophytes					
Dennstaedtiaceae					
Pteridium arachnoideum (Kauf.) Maxon				Herb	DCZ 4002
Polypodiaceae					
Microgramma persicariifolia (Schrad.) C.Presl				Herb	DCZ 4066
Pleopeltis polypodioides (L.) Andrews & Windham				Herb	DCZ 3922
Serpocaulon triseriale (Sw.) A.R.Sm.				Herb	DCZ 4037
Pteridaceae					
Doryopteris collina (Raddi) J.Sm.				Herb	DCZ 4040
Spermathophytes					
Acanthaceae					
Justicia birae A.S.Reis, F.A.Silva, A.Gil & Kameyama				Herb	MP 600
Alismataceae					
Helanthium tenellum (Mart. ex Schult & Schult.f.) Britton				Herb	MP 613
Limnocharis flava (L.) Buchenau	X			Herb	PLV 6149
Anacardiaceae					
Anacardium occidentale L.				Treelet	DCZ 3923
Spondias mombin L.	X			Treelet	DCZ 3921
Annonaceae					
Annona sericea Dunal	X			Shrub	DCZ 4051
Annona exsucca DC.				Tree	COA 658
Guatteria procera R.E.Fr.	X			Tree	DCZ 4050
Xylopia aromatica (Lam.) Mart.				Treelet	DCZ 3970
Apocynaceae					
Himatanthus cf. *articulatus* (Vahl) Woodson				Tree	COA 676
Mandevilla scabra (Hoffmanns. ex Roem. & Schult.) K. Schum.				Liana	DCZ 3880
Mandevilla tenuifolia (J.C. Mikan) Woodson				Herb	DCZ 3885
Matelea microphylla Morillo		X		Herb	DCZ 3942
Tabernaemontana flavicans Willd. ex Roem. & Schult.				Treelet	COA 613
Tabernaemontana macrocalyx Müll. Arg.				Treelet	COA 605
Araceae					
Anthurium gracile (Rudge) Lindl.				Herb	DCZ 5017
Anthurium sp.1		X		Herb	DCZ 3898
Arecaceae					
Mauritia flexuosa Mart.				Palm	DCZ 3961
Mauritiella armata (Mart.) Burret				Palm	DCZ 3960
Oenocarpus distichus Mart.				Palm	DCZ 3948
Syagrus cocoides Mart.				Palm	DCZ 3892
Asteraceae					
Emilia fosbergii Nicolson				Herb	DCZ 4046
Ichthyothere terminalis (Spreng.) S.F. Blake				Shrub	DCZ 3868
Monogereion carajensis G.M. Barroso & R.M. King		X		Herb	DCZ 3861
Riencourtia pedunculosa (Rich.) Pruski				Herb	DCZ 3924
Tilesia baccata (L.f.) Pruski				Herb	DCZ 3980
Unxia camphorata L.f.				Herb	DCZ 3941
Begoniaceae					
Begonia humilis Dryand				Herb	DCZ 3973

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Bignoniaceae					
Adenocalymma schomburgkii (DC.) L.G.Lohmann				Liana	COA 611
Amphilophium mansoanum (DC.) L.G.Lohmann				Liana	DCZ 4025
Anemopaegma carajasense A.H. Gentry ex Firetti-Leggieri & L.G. Lohmann		X		Shrub	DCZ 3914
Anemopaegma longipetiolatum Sprague				Liana	DCZ 3867
Jacaranda ulei Bureau & K.Schum.				Shrub	DCZ 3945
Pachyptera incarnata (Aubl.) Francisco & L.G. Lohmann				Liana	DCZ 4061
Pleonotoma melioides (S.Moore) A.H.Gentry				Liana	COA 638
Pleonotoma orientalis Sandwith				Liana	DCZ 3883
Bixaceae					
Cochlospermum orinocense (Kunth) Steud.				Treelet	DCZ 3875
Boraginaceae					
Cordia nodosa Lam.				Tree	COA 641
Bromeliaceae					
Aechmea castelnavii Baker				Herb	COA 670
Aechmea mertensii (G.Mey.) Schult. & Schult.f.				Herb	COA 673
Aechmea tocantina Baker				Herb	AHS 2194
Ananas ananassoides (Baker) L.B. Sm.				Herb	DCZ 3891
Dyckia duckei L.B.Sm.				Herb	DCZ 3872
Tillandsia adpressiflora Mez	X			Herb	DCZ 4034
Burmanniaceae					
Burmannia capitata (Walter ex J.F.Gmel.) Mart.				Herb	MP 644
Burmannia flava Mart.				Herb	DCZ 3903
Cabombaceae					
Cabomba furcata Schult. & Schult.f.				Herb	DCZ 3963
Commelinaceae					
Commelina erecta L.				Herb	DCZ 4058
Dichorisandra hexandra (Aubl.) C.B. Clarke				Liana	DCZ 3858
Connaraceae					
Rourea ligulata Baker				Shrub	COA 666
Convolvulaceae					
Distimake macrocalyx (Ruiz & Pav.) A.R. Simões & Staples	X			Liana	MP 660
Ipomoea decora Meisn.				Liana	DCZ 4057
Ipomoea marabaensis D.F.Austin & Secco				Liana	DCZ 3873
Ipomoea rubens Choisy	X			Liana	MP 672
Cucurbitaceae					
Gurania sinuata (Benth.) Cogn.				Herb	AHS 2167
Cyperaceae					
Bulbostylis conifera (Kunth) C.B. Clarke				Herb	COA 624
Cyperus aggregatus (Willd.) Endl.				Herb	DCZ 3865
Cyperus laxus Lam.				Herb	DCZ 3957
Cyperus sesquiflorus (Torr.) Mattf. & Kük.				Herb	DCZ 4031
Cyperus sphacelatus Rottb.				Herb	DCZ 4042
Diplasia karatifolia Rich. in Pers.	X			Herb	DCZ 4032
Eleocharis flavescens (Poir.) Urb.				Herb	MP 627
Eleocharis pedrovianae C.S. Nunes, R. Trevis. & A. Gil		X		Herb	DCZ 4027
Eleocharis plicarhachis (Griseb.) Svenson				Herb	COA 678
Rhynchospora barbata (Vahl) Kunth				Herb	COA 657
Rhynchospora filiformis Vahl				Herb	DCZ 3930
Rhynchospora holoschoenoides (Rich.) Herter				Herb	MP 608
Rhynchospora seccoi C.S.Nunes, P.J.S. Silva Filho & A.Gil				Herb	DCZ 3905
Scleria cyperina Willd. ex Kunth				Herb	DCZ 3925
Scleria microcarpa Nees ex Kunth				Herb	COA 650
Dioscoreaceae					
Dioscorea piperifolia Humb. & Bonpl. ex Willd.				Liana	DCZ 3884
Dioscorea trilinguis Griseb.	X			Liana	DCZ 3934
Eriocaulaceae					
Eriocaulon carajense Moldenke		X		Herb	DCZ 3936

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Eriocaulon cinereum R.Br.				Herb	DCZ 4049
Paepalanthus fasciculoides Hensold				Herb	DCZ 3878
Syngonanthus discretifolius (Moldenke) M.T.C. Watanabe		X		Herb	PLV 6119
Syngonanthus heteropeplus (Körn.) Ruhland				Herb	MP 659
Erythroxylaceae					
Erythroxylum nelson-rosae Plowman		X		Shrub	COA 672
Erythroxylum rufum Cav.				Shrub	COA 637
Euphorbiaceae					
Alchornea discolor Poeppig				Shrub	DCZ 3886
Aparisthmium cordatum (A. Juss.) Baill.				Tree	DCZ 3997
Astraea lobata (L.) Klotzsch				Shrub	DCZ 3955
Mabea angustifolia Spruce ex Benth.				Shrub	DCZ 3987
Manihot quinquepartita Huber ex D.J.Rogers				Shrub	DCZ 3954
Manihot tristis Müll.Arg.				Shrub	MP 666
Maprounea brasiliensis A.St.-Hil.	X			Shrub	DCZ 3991
Fabaceae					
Abrus melanospermus Hassk.				Liana	DCZ 3912
Aeschynomene sensistiva var. *hispidula* (Kunth) Rudd				Subshrub	DCZ 4024
Bauhinia pulchella Benth.				Shrub	DCZ 3869
Camptosema ellipticum (Desv.) Burkart				Shrub	DCZ 3907
Centrosema carajasense Cavalcante				Herb/Liana	DCZ 4007
Chamaecrista desvauxii (Collad.) Killip				Subshrub	DCZ 3946
Clitoria falcata Lam.				Liana	DCZ 3917
Crotalaria maypurensis Kunth				Shrub	DCZ 3881
Dioclea apurensis Kunth				Liana	DCZ 3919
Inga calantha Ducke	X			Tree	COA 600
Inga heterophylla Willd	X			Tree	DCZ 4036
Inga leiocalycina Benth.	X			Tree	MP 598
Mimosa dasilvae A.S.L. Silva & Secco	X	X	X	Subshrub	COA 622
Mimosa guilandinae var. *spruceana* (Benth.) Barneby				Shrub	COA 668
Mimosa skinneri Benth. var. *carajarum* Barneby		X		Herb	DCZ 3860
Mimosa somnians Humb. & Bonpl. ex Willd.				Subshrub	DCZ 3876
Mimosa xanthocentra Mart.				Tree	PLV 6158
Parkia platycephala Benth.				Shrub	DCZ 4013
Periandra mediterranea (Vell.) Taub.				Shrub	DCZ 3902
Senegalia multipinnata (Ducke) Seigler & Ebinger				Treelet	COA 603
Stylosanthes capitata Vogel				Subshrub	DCZ 3977
Tachigali vulgaris L.F.G.Silva & H.C.Lima				Tree	COA 655
Gentianaceae					
Schultesia benthamiana Klotzsch ex Griseb.				Herb	DCZ 3928
Heliconiaceae					
Heliconia psittacorum L.f.	X			Herb	MP 671
Hypericaceae					
Vismia gracilis Hieron				Treelet	COA 654
Iridaceae					
Cipura xanthomelas Maxim. ex Klatt				Herb	DCZ 3899
Lamiaceae					
Amasonia lasiocaulos Mart. & Schau ex Schau.				Subshrub	DCZ 3947
Hyptis atrorubens Poit.				Herb	DCZ 3981
Mesosphaerum pectinatum (L.) Kuntze				Herb	MN 697
Mesosphaerum suaveolens (L.) Kuntze				Herb	DCZ 4048
Vitex panshiniana Moldenke	X			Tree	DCZ 4053
Lauraceae					
Cassytha filiformis L.				Parasite	DCZ 3874
Dicypellium aff. *caryophyllaceum* (Mart.) Nees	X		X	Shrub	PLV 6100
Mezilaurus itauba (Meisn.) Taub. ex Mez				Shrub	DCZ 4001
Rhodostemonodaphne praeclara (Sandwith) Madriñán	X			Tree	DCZ 3983

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Lentibulariaceae					
Utricularia neottioides A.St-Hil & Girard				Herb	MP 664
Utricularia pusilla Vahl				Herb	DCZ 3904
Utricularia subulata L.				Herb	PLV 6139
Loranthaceae					
Passovia pedunculata (Jacq.) Kuijt				Parasite	DCZ 3909
Psittacanthus eucalyptifolius (Kunth) G. Don				Parasite	DCZ 4056
Lythraceae					
Cuphea annulata Koehne				Subshrub	DCZ 3864
*Cuphea carajasensi*s Lourteig		X		Shrub	COA 616
Malpighiaceae					
Banisteriopsis malifolia (Nees & Mart.) B.Gates				Shrub	MN 743
Banisteriopsis stellaris (Griseb.) B.Gates				Liana	DCZ 3863
Byrsonima chrysophylla Kunth				Shrub	DCZ 3929
Heteropterys nervosa A.Juss.				Liana	COA 645
Malvaceae					
Waltheria indica L.	X			Shrub	DCZ 4064
Marantaceae					
Monotagma plurispicatum (Körn.) K.Schum.				Herb	DCZ 4000
Marcgraviaceae					
Norantea guianensis Aubl.				Shrub	DCZ 3887
Melastomataceae					
Bellucia grossularioides (L.) Triana	X			Shrub	DCZ 3995
Brasilianthus carajensis Almeda & Michelangeli				Herb	DCZ 3877
Clidemia capitellata (Bonpl.) D.Don				Shrub	DCZ 4020
Miconia alternans Naudin				Shrub	DCZ 4021
Miconia heliotropoides Triana				Shrub	DCZ 4008
Nepsera aquatica (Aubl.) Naudin				Herb	COA 649
Pleroma carajasense K.Rocha, R.Goldenb. & F.S.Mey		X		Shrub	DCZ 3910
Pterolepis trichotoma (Rottb.) Cogn.				Herb	DCZ 4019
*Tibouchina edmundo*i Brade				Shrub	DCZ 3932
Menispermaceae					
Abuta grandifolia (Mart.) Sandwith				Shrub	COA 646
Cissampelos andromorpha DC. .				Liana	COA 663
Metteniusaceae					
Emmotum nitens (Benth.) Miers				Shrub	MP 601
Myrtaceae					
Eugenia punicifolia (Kunth) DC.				Shrub	DCZ 3894
Myrcia cuprea (O.Berg.) Kiaersk.				Shrub	COA 639
Myrcia splendens (Sw.) DC.				Shrub	DCZ 3965
Myrciaria floribunda (H.West ex Willd.) O.Berg				Shrub	DCZ 3915
Myrciaria glomerata O.Berg	X			Shrub	DCZ 4010
Ochnaceae					
Ouratea castaneifolia (DC.) Engl.				Treelet	DCZ 3920
Ouratea cearensis (Tiegh.) Sastre & Offroy	X			Shrub	COA 604
Ouratea racemiformis Ule				Shrub	DCZ 4033
Onagraceae					
Ludwigia cf. *latifolia* (Benth.) H.Hara	X			Subshrub	DCZ 3967
Ludwigia nervosa (Poir.) H.Hara				Shrub	COA 674
Orchidaceae					
Catasetum boyi Mansf.	X			Herb	JBFS 648
Catasetum discolor (Lindl.) Lindl.				Herb	DCZ 4030
Cyrtopodium andersonii (Lamb. ex Andrews) R.Br.				Herb	COA 643
Encyclia chloroleuca (Hook.) Neum.	X			Herb	JBFS 540
Epidendrum strobiliferum Rchb.f.	X			Herb	COA 667
Erycina pusilla (L.) N.H.Williams & M.W.Chase				Herb	JBFS 498
Habenaria nuda Lindl				Herb	MP 609

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Habenaria orchiocalcar Hoehne	X			Herb	JBFS 219
Polystachya concreta (Jacq.) Garay & H.R.Sweet				Herb	COA 669
Rodriguezia lanceolata Ruiz & Pav.	X			Herb	COA 665
Scaphyglottis cf. *livida*				Herb	COA 671
Sobralia liliastrum Salzm. ex Lindl.				Herb	DCZ 3888
Orobanchaceae					
Buchnera carajasensis Scatigna & N.Mota		X		Herb	DCZ 3931
Passifloraceae					
Passiflora ceratocarpa F. Silveira				Liana	DCZ 4060
Passiflora picturata Ker Gawl.	X			Liana	DCZ 3976
Passiflora tholozanii Sacco				Liana	COA 612
Phyllanthaceae					
Phyllanthus hyssopifolioides Kunth.				Herb	DCZ 4028
Phyllanthus minutulus Müll.Arg.				Herb	DCZ 4026
Phytolaccaceae					
Phytolacca thyrsiflora Fenzl ex J. Schmidt				Herb	DCZ 4041
Piperaceae					
Peperomia albopilosa D. Monteiro		X		Herb	PLV 6169
Peperomia magnoliifolia (Jacq.) A.Dietr.				Herb	COA 647
Plantaginaceae					
Scoparia dulcis L.				Herb	DCZ 4065
Poaceae					
Acroceras zizanioides (Kunth) Dandy				Herb	DCZ 4022
Andropogon bicornis L.				Herb	DCZ 3950
Axonopus cf. *longispicus* (Döll) Kuhlm.				Herb	DCZ 4023
Axonopus rupestris Davidse				Herb	DCZ 3896
Eleusine indica (L.) Gaertn.*				Herb	DCZ 4045
Hildaea parvispiculata C. Silva & R.P. Oliveira				Herb	PLV 6124
Ichnanthus calvescens (Nees ex Trin.) Döll				Herb	DCZ 4011
Luziola peruviana Juss. ex J.F.Gmel.				Herb	DCZ 3918
Melinis minutiflora P.Beauv.*				Herb	COA 640
Mesosetum cayennense Steud.				Herb	PLV 6117
Oryza glumaepatula Steud.				Herb	BFF 634
Paspalum axillare Swallen				Herb	PLV 6130
Paspalum foliiforme S.Denham				Herb	DCZ 3916
Paspalum reticulinerve Renvoize				Herb	PLV 6166
Rhytachne gonzalezii Davidse				Herb	PLV 6127
Rugoloa pilosa (Sw.) Zuloaga				Herb	DCZ 3964
Steinchisma laxum (Sw.) Zuloaga				Herb	COA 677
Taquara micrantha (Kunth) I.L.C.Oliveira & R.P.Oliveira				Herb	DCZ 3999
Trachypogon spicatus (L.f.) Kuntze				Herb	DCZ 3944
Trichanthecium cf. *arctum* (Swallen) Zuloaga & Morrone				Herb	DCZ 3913
Urochloa maxima (Jacq.) R.D. Webster*				Herb	DCZ 3951
Polygalaceae					
Bredemeyera divaricata (DC.) J.F.B. Pastore				Shrub	DCZ 3911
Caamembeca spectabilis (DC.) J.F.B. Pastore				Subshrub	COA 642
Polygala adenophora DC.				Herb	DCZ 3900
Portulacaceae					
Portulaca sedifolia N.E.Br.				Herb	DCZ 3862
Primulaceae					
Cybianthus detergens Mart.				Shrub	DCZ 4062
Proteaceae					
Roupala montana Aubl.				Shrub	DCZ 4063
Rhamnaceae					
Gouania pyrifolia Reissek	X			Liana	DCZ 3953
Rubiaceae					
Alibertia edulis (Rich.) A. Rich. ex DC.				Shrub	DCZ 4035

Taxa	New for Carajás Flora	Endemic canga	Endemic SFX	Life form	Voucher
Borreria alata (Aubl.) DC.				Herb	DCZ 3866
Borreria carajasensis E.L. Cabral & L.M. Miguel		X		Subshrub	DCZ 3859
Borreria semiamplexicaulis E.L.Cabral				Herb	DCZ 3938
Cordiera myrciifolia (K.Schum.) C.H.Perss. & Delprete				Shrub	DCZ 3971
Coutarea hexandra (Jacq.) K.Schum.	X			Shrub	COA 610
Guettarda argentea Lam.				Shrub	COA 602
Palicourea guianensis Aubl.				Treelet	DCZ 4052
Perama carajensis J.H. Kirkbr.		X		Herb	DCZ 3879
Psychotria colorata (Willd. ex Schult.) Mull. Arg.				Herb	DCZ 4017
Psychotria hoffmannseggiana (Willd. ex Schult.) Mull. Arg.				Subshrub	COA 601
Sabicea grisea Cham. & Schltdl.				Liana	DCZ 3901
Rutaceae					
Dictyoloma vandellianum A. Juss.				Treelet	DCZ 3975
Ertela trifolia (L.) Kuntze				Subshrub	COA 607
Pilocarpus microphyllus Stapf ex Wardlew.				Shrub	COA 653
Salicaceae					
Casearia arborea (Rich.) Urb.				Tree	DCZ 3982
Casearia javitensis Kunth				Shrub	DCZ 4014
Sapindaceae					
Allophylus semidentatus (Miq.) Radlk.	X			Shrub	DCZ 3959
Paullinia stellata Radlk.	X			Liana	DCZ 4044
Pseudima frutescens (Aubl.) Radlk.	X			Shrub	PLV 6151
Serjania lethalis A.St.-Hil.				Liana	DCZ 3996
Sapotaceae					
Pouteria ramiflora (Mart.) Radlk.				Treelet	DCZ 3969
Simaroubaceae					
Simaba guianensis Aubl.				Shrub	DCZ 3984
Simarouba amara Aubl.				Shrub	DCZ 3985
Siparunaceae					
Siparuna ficoides S.S.Rener & Hausner				Treelet	COA 660
Smilacaceae					
Smilax irrorata Mart. ex Griseb				Liana	DCZ 3935
Solanaceae					
Solanum americanum Mill.				Herb	DCZ 4059
Solanum crinitum Lam.				Treelet	COA 623
Trigoniaceae					
Trigonia nivea Cambess.				Liana	COA 651
Turneraceae					
Turnera glaziovii Urb				Shrub	DCZ 4012
Turnera laciniata Arbo				Herb	DCZ 3993
Turnera melochioides Cambess.				Shrub	PLV 6160
Urticaceae					
Cecropia palmata Willd.				Tree	COA 664
Velloziaceae					
Vellozia glauca Pohl				Herb	DCZ 3890
Verbenaceae					
Lantana trifolia L.	X			Shrub	MN 755
Lippia grata Schauer				Shrub	DCZ 3871
Stachytarpheta cayennensis (Rich.) Vahl				Subshrub	COA 608
Vitaceae					
Cissus erosa Rich.				Liana	DCZ 3882
Vochysiaceae					
Qualea parviflora Mart.				Tree	MP 624
Xyridaceae					
Xyris brachysepala Kral		X		Herb	PLV 6125
SPECIES TOTAL (254)	**36**	**17**	**2**		

Table 2. Areas compared by this study, respective area codes used in the multivariate analysis and number of angiosperms species recorded for each area. Serra de Campos of São Félix do Xingu (SFX) data is produced by this study, ARQ-CAN data is available in Fonseca-da-Silva et al. (2020) and Flora of the canga of the Serra de Carajás (FCC) data is available in Mota et al. (2018).

Area code	Area	Species	Cumulative species
ARQ	Serra Arqueada	149	149
S11A	Serra dos Carajás – Serra Sul 11A	230	535
S11B	Serra dos Carajás – Serra Sul 11B	201	
S11C	Serra dos Carajás – Serra Sul 11C	180	
S11D	Serra dos Carajás – Serra Sul 11D	428	
SN1	Serra dos Carajás – Serra Norte 1	383	643
SN2	Serra dos Carajás – Serra Norte 2	125	
SN3	Serra dos Carajás – Serra Norte 3	218	
SN4	Serra dos Carajás – Serra Norte 4	308	
SN5	Serra dos Carajás – Serra Norte 5	293	
SN6	Serra dos Carajás – Serra Norte 6	99	
SN7	Serra dos Carajás – Serra Norte 7	112	
SN8	Serra dos Carajás – Serra Norte 8	101	
SB	Serra dos Carajás – Serra da Bocaina	223	336
ST	Serra dos Carajás – Serra do Tarzan	211	
SFX	Serra de Campos – São Félix do Xingu	248	248

Among the 38 edaphic endemic species of canga, defined according to Giulietti et al. (2019), 17 (c. 50%) were recorded in SFX. Two of these, *Erythroxylum nelson-rosae* Plowman (Erythroxylaceae) and *Matelea microphylla* Morillo (Apocynaceae) were not previously recorded for SFX in the list of endemic edaphic species of the canga of Carajás (Giulietti et al. 2019). One species, *Mimosa dasilvae* (Fabaceae), is only known to occur in SFX.

Around 25% (60) of the 248 angiosperms registered for SFX are restricted to the Amazonian Rainforest biome, but the majority of the flora is widely distributed in open habitats throughout South America.

The vegetation of the Serra de Campos

Regarding the phytophysiognomies listed by Mota et al. (2015) for the region, the canga vegetation of the SFX has a predominance of vast spreads of scrub composed of closely disposed treelets and shrubs. Amongst them, treelets and shrubs such as *Byrsonima chrysophylla* Kunth, *Cordiera myrciifolia* (K.Schum.) C.H.Perss. & Delprete, *Anemopaegma carajasense* A.H. Gentry ex Firetti-Leggieri & L.G. Lohmann*, *Cuphea annulata* Koehne, *Lippia grata* Schauer, *Erythroxylum nelson-rosae* Plowman*, *Syagrus cocoides* Mart., as well as several species of *Myrcia* and *Eugenia*, the palm *Syagrus cocoides* Mart. and scramblers and climbers such as *Norantea guianensis* Aubl., *Cissus erosa* Rich., *Mandevilla scabra* (Hoffmanns. ex Roem. & Schult.) K. Schum. and *Smilax irrorata* Mart. ex Griseb. On more exposed, bare canga slabs, the plants grow mostly in rock crevices with presence of monocots such as *Vellozia glauca* Pohl, *Sobralia liliastrum* Salzm. ex Lindl., *Dyckia duckei* L.B. Sm. and the tuberous, low growing *Mandevilla tenuifolia* (J.C. Mikan) Woodson, as well as the herbaceous *Borreria semiamplexicaulis* E.L.Cabral, *Perama carajensis* J.H.Kirk.*, *Begonia humilis* Dryand and *Brasilianthus carajensis* Almeda & Michelangeli*. The nodular canga has more or less continuous covering of grass and sedge, with occasional specimens of *Riencourtia pedunculosa* (Rich.) Prusky. During the expeditions we did not come across low forest groves, and our impression was that between the canga edge and the surrounding rainforest there was not much transition but a sharp substitution of the open vegetation by the associated forest types. Regarding the hydromorphic vegetation found in SFX, temporary shallow ponds with *Utricularia* species, *Burmannia flava* Mart., *Cabomba furcata* Schult. & Schult. f., *Syngonanthus caulescens* (Poir.) Ruhland and *Xyris brachysepala* Kral.* were visited. However, perennial, larger ponds of the magnitude found in the *Serra Sul* were lacking and temporary streams were not observed. There were also Palm swamps (*buritizais*), with margins occupied by *Mauritia flexuosa* Mart. and *Mauritiella armata* (Mart.) Burret, harbouring aquatic *Oryza glumaepatula* Steud., *Helanthium tenellum* (Mart. ex Schult. & Schult.f.) Britton and *Eleocharis* spp. (edaphic endemic species marked with *).

Database of the flora of Serra dos Carajás complex

The biogeographical database from the CRC of the Carajás complex was updated by our study (see supplementary data) and includes now a total of 893 angiosperms distributed in 121 families and 441 genera. For the Carajás flora (FCC), Poaceae was the most species-rich family (75 species in the FCC), followed by Fabaceae (66 spp.), Cyperaceae (57 spp.), Rubiaceae (49 spp.), and Melastomataceae (40 spp.). The richest genera were *Rhynchospora* (24 spp.), *Miconia* (18 spp.), *Paspalum* and *Solanum* (17 spp. each), *Myrcia* and *Ipomoea* (13 spp. each), while 64% (284 genera) were represented by only a single species. The inclusion of SFX in our database increased the number of known taxa by 18 genera and 37 species not previously recorded for the canga of Carajás.

Biogeography of the Campos Rupestres on Canga of the Carajás complex

The mean angiosperm species richness for each outcrop of the Serra dos Carajás was 218 species. The NMDS and UPGMA analyses included 3451 records of 893 species across 16 sites (Fig. 3a, b). The UPGMA analyses produced statistically significant clusters (Fig. 3b) with the same major groups found by Fonseca-da-Silva et al. (2020), one comprising four of the eight areas of the Serra Norte (SN2, SN6, SN7, and SN8), while the remaining four (SN1, SN3, SN4, and N5) appear closer to the areas of Serra Sul (S11A, S11B. S11C, S11D), along with SB and ST. SA also emerged as the least similar to the Carajás complex, and SFX was found to be more similar to the group comprising SB, ST, Serra Sul and the four most species rich sites in Serra Norte (SN1, SN3, SN4, and SN5). A similar result was obtained by the NMDS analysis (Fig. 3a), also showing SA as the most dissimilar from other areas.

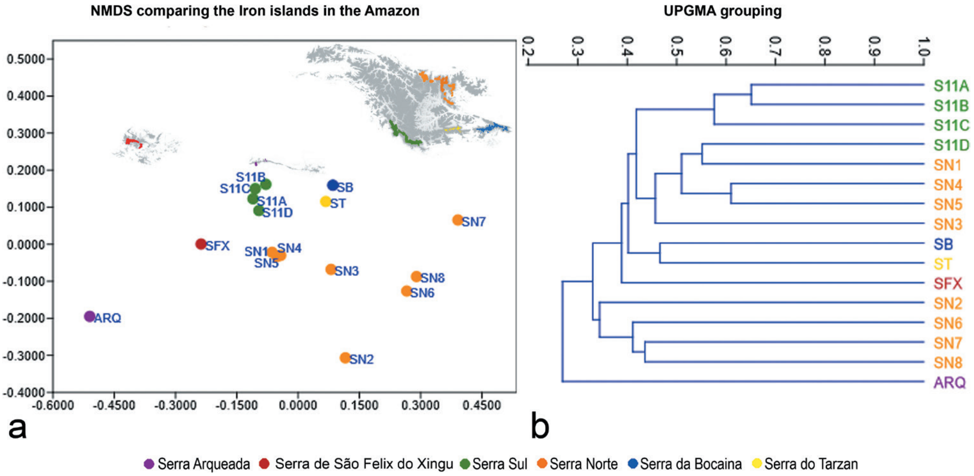

Figure 3. UPGMA (**a**) and NMDS (**b**) multivariate analysis clustering areas from FCC and SFX (see Table 2 for area codes). UPGMA cophenetic coefficient: 0.902. b. NMDS stress: 0.1859.

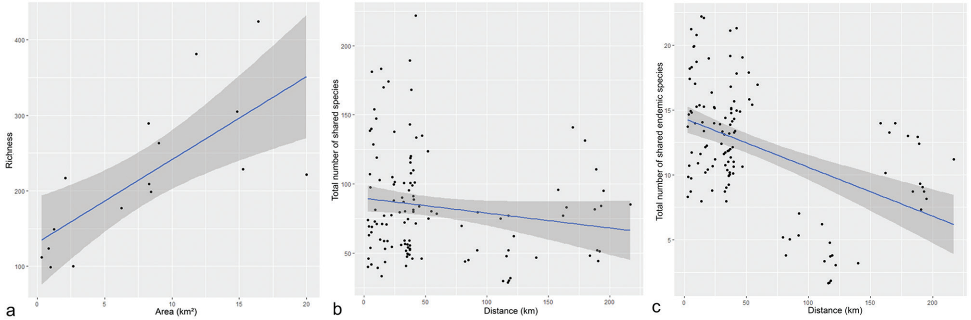

Figure 4. a Species richness plotted against area of Carajás. Pearson correlation coefficients: $r = 0.806094$, $P = 0.001548$ **b** the number of species shared between site pairs does not change significantly with geographical distance between regions. $r = -0.16$; $P = 0.08$ **c** the number of shared endemic species between site pairs declines with geographical distance between regions. $r = -0.45872$; $P = 1.37e\text{-}07$.

Species richness was significantly correlated with site area ($r = 0.806094$, $P = 0.001548$). The larger the area of each individual mountaintop (site), the larger the number of species recorded. The total number of shared species between mountaintop outcrops did not differ significantly with geographical distance across sites ($r = -0.16$; $P = 0.08$). There was a tendency of distant sites to share less species, but this trend was not significant. When the residuals of this model were evaluated they significantly departed from normality. Spearman's correlation was not significant either (*p-value* = 0.2972). However, when focusing on the number of shared endemic edaphic species versus the geographical distance between sites, we found a significant correlation, where closer sites shared more edaphic endemic species than with more distant sites ($r = -0.45872$; $P = 1.37e\text{-}07$) (Fig. 4).

Regarding the total of species of the canga, the Carajás iron islands share an average of 40% of their flora with each other. SFX has, on average, 30% of shared species with each other area. The percentage of similarity between sites was a minimum of 30% and a maximum of 55%.

Discussion

Floristic composition of Serra de Campos × other canga outcrops

The most species-rich families and genera found in the SFX coincide with those found in the Flora das cangas de Carajás (Mota et al. 2018) and SA (Fonseca-da-Silva et al. 2020), where Cyperaceae, Fabaceae, Poaceae, and Rubiaceae are among the richest plant families. Interestingly, SFX has a much higher number of Orchidaceae species than other surveys of canga in the Amazon (Koch et al. 2018; Mota et al. 2018; Fonseca-da-Silva et al. 2020). The participation of botanical specialists during collecting expeditions enhances floristic studies in the Amazon (Medeiros et al. 2014) and elsewhere, and the high number of Orchidaceae in SFX possibly reflects the specific search for this group by J.B. Silva in the region from the 1990's onwards, which may have resulted in a greater sampling effort for this group when compared to other areas.

There is a large turnover of species between outcrops (Zappi et al. 2019; Fonseca-da-Silva et al. 2020) and very few species are widely distributed across these disjunct, isolated habitats. Similar to what was found by (Costa et al. 2019) in Amazonian White Sand Campinas, the isolation of the patchy canga outcrops limits dispersal and increases floristic differentiation, and the adverse conditions, such as high temperature, extreme exposure to sunlight and winds, and a relatively well defined dry season represent ecological filters for the species that occupy the canga, partly explaining the high number of endemic species in the CRC of Carajás.

As an example, only three species were recorded in all surveyed areas: the widely distributed *Riencourtia pedunculosa*, an Asteraceae common in open areas in the Amazon (Flora do Brasil under construction, Bringel 2014), and two species associated with Amazonian canga outcrops: *Brasilianthus carajensis* and *Perama carajensis*. *Perama carajensis* is a confirmed canga edaphic endemic species, and *Brasilianthus carajensis* has been collected also on granite, being locally endemic to Carajás, but not a canga edaphic endemic (Giulietti et al. 2019; Silva et al. 2020). Other four species also present wide occurrence across *campos rupestres* on canga of Carajás: *Bulbostylis conifera* (Kunth) C.B. Clarke, *Rhynchospora barbata* (Vahl) Kunth, *Rhynchospora seccoi* C.S.Nunes et al., and *Syngonanthus discretifolius* (Moldenke) M.T.C. Watanabe were recorded for SFX and many other FCC areas, except for one of them missing in SN3, SN7, SN7 and SA, respectively. Their absence in these four sites may be related to the more modest canga surface found in these areas.

Some widely distributed species from the canga of Carajás, found at more than 10 of the 16 sites surveyed, were not recorded at SFX. The absence of the common treelets *Callisthene microphylla* Warm. and *Mimosa acutistipula* var. *ferrea* Barneby (Mota et al.

2015) at SFX may be partially explained by differences in the micro-habitats between SFX and the other canga outcrops considered here. For *Brasilianthus carajensis*, distinct adaptive genetic clusters have been found in the SFX (see Silva et al. 2020), increasing the argument for the protection of the site.

The canga is typically a mosaic of different vegetation types (Mota et al. 2015, Viana et al. 2016). Some of these vegetation types are infrequent in SFX, as for example low forest groves (Mota et al. 2015), and in consequence some of the species found in these groves elsewhere are absent at SFX: *Callisthene microphylla*, *Mimosa acutistipula* var. *ferrea*, and *Cereus hexagonus* (L.) Mill. Although forest groves are closely associated with the lower scrub vegetation, the latter is more abundant in the canga plateau of SFX than the former. In plateau SFX2 of SFX the shrubby vegetation is dominant, and there are large stands of *Syagrus cocoides* Mart., a palm emerging from the impenetrable shrubbery. In the context of CRC of Carajás, this palm forms large populations only in SA and SFX.

Despite having the lowest number of species registered in the FCC, the hydromorphic vegetation found atop the plateaus is the habitat with the highest proportion of exclusive species (Pereira et al. 2016; Mota et al. 2018). Seasonal lakes and palm lakes in the SFX ensure the presence of annual aquatic species such as *Eriocaulon carajense* Moldenke, *Oryza glumaepatula* Steud., *Syngonanthus caulescens* (Poir.) Ruhland, and *Xyris brachysepala* Kral.

As a relatively large canga site isolated from the active iron mines further to the east, the SFX has been found to harbour a rich and unique vegetation, representing a suitable area for the implementation of conservation strategies. On the other hand, this canga outcrop is currently threatened by surrounding deforestation, land transformation and frequent fires, and is not included within any type of protected area.

Iron islands of Carajás and their floristic connections

The mosaic of landscapes typical of CRC of Carajás may also explain the low floristic similarity between the sites. The number of shared species represents less than half the local richness from each site separately. This brings attention to the high beta diversity among sites (Zappi et al. 2019), with a large species turnover across these disjunct outcrops. Habitat diversity associated with the size of the island-like habitats is also related to the beta diversity in French Guiana´s inselbergs (Henneron et al. 2019), similarly to what is found in Andean alpine flora (Sklenář et al. 2014) and South American tepuis (Riina et al. 2019). This confirms the association between area and habitat diversity found here for the canga vegetation as an important factor for determining plant biodiversity.

The greater similarity between SFX, SB and ST, along with *Serra Sul* (S11A, S11B, S11C, and S11D) and SN1, SN3, SN4 and SN5 reflected in the UPGMA clustering patterns (Fig. 3b) suggests there is more similarity of species richness between the largest sites rather than among geographically closest areas, as observed by Fonseca-da-Silva et al. (2020) for SA. In fact, the correlation between the shared species of each canga site and their geographical distance was significant. Considering the size of each of these areas and their positive correlation with floristic richness (Fig. 4), we interpret the canga's overall surface as being more important for floristic composition than the distance between sites in the Serra dos Carajás. Thus, the larger a canga outcrop is, the greater the number of micro-habitats it can harbour, reflecting an increased species richness and unique floristic composition of each canga site. On the other hand, that relationship (distance between areas vs shared flora) holds true when analysing shared endemic species, where shared endemic species decrease with distance at different rates (Fig. 4C).

The low number of species restricted to the Amazon (25%) and the high number of species widely distributed in South America (75%) recorded at SFX, may explain the discrepancy in the correlation between shared species and distance being negative when all species are considered, whereas it is positive for endemic species only. On a macro-scale, the majority of the species recorded in SFX have a broad distribution, occurring beyond the Amazon Rainforest, and the distance factor between different outcrops may not matter so much. On the other hand, when observing only the species endemic to Carajás, and especially edaphic endemic species, the trend is the opposite, possibly due to the local scale of observation, as elsewhere the distance between areas tends to affect the floristic similarity between island vegetations (Sklenář et al. 2014; Schrader et al. 2020). A genomic study revealed that gene flow in two endemic species of Carajás is mainly influenced by geographic distance between mountain pairs, as the rainforest surrounding different mountaintops constitutes an important barrier (Carvalho et al. 2019). Therefore, gene flow also decreases with the increase of the barrier represented by the rainforest (Carvalho et al. 2019).

Another factor that may have an impact on the contrasting effects of floristic similarity vs. distance from canga islands is the different environmental requirements of herbs, shrubs and trees, that shape their biogeographical patterns and affect species-area and richness-environment relationships (Schrader et al. 2020). Herbs, shrubs and trees have contrasting strategies in different environmental conditions with potential implications for community assemblage on islands. For example, herbs can form larger populations on small islands due to their smaller size, and as a result face less risk of extinction and greater dispersal capacity (Moles 2005; Thomson et al. 2010), while shrubs are associated with more stable environmental conditions, and therefore have more success on larger islands (Chiarucci et al. 2017).

Recent analyses of open vegetation in the Amazon reinforce the insular character of Amazonian canga and their low similarity to other vegetation types in the Amazonian biome (Devecchi et al. 2020). While there is some evidence that canga in Southeastern Brazil may be influenced by the surrounding Atlantic Rainforest and Cerrado (Zappi et al. 2017) these biomes are known to have a more varied life-form balance (respectively 1: 4 and 1: 7 proportion of trees over other life forms) than the Amazon Rainforest, where the life form balance is less extreme (1: 2) (Brazil Flora Group [BFG] 2015), thus it may have less floristic influence over the open vegetation found in the CRC of Carajás (Zappi et al. 2019). Therefore, in order to colonize the Amazonian CRC, shrubby or herbaceous plant species may have to come from further afield through long distance dispersal, and, if established, they may remain genetically isolated from their original populations, leading over a period of time to the patterns of endemism observed today.

Different evolutionary processes of the species occurring in CRC may also have led to different floristic composition in the outcrops. Although evolutionary studies involving species of canga in the Brazilian Amazon are just beginning (Zappi et al. 2017), the phylogeography of a species of Gesneriaceae distributed in humid rock formations in the Cerrado reveals its recent expansion into CRC vegetation during the Pleistocene (Fiorini et al. 2020). Recent and rapid radiations have been observed in mountaintops ecosystems (Salerno et al. 2012; Pirie et al. 2016; Vasconcelos et al. 2020) but more phylogenetic and phylogeographic studies are necessary to establish dating for plants species groups found in the CRC in order to understand their diversification and colonization processes.

Table 3. Species richness of the iron islands outcrops of Carajás complex (bold diagonal) along with the number of shared species (above diagonal) and distance in kilometres (below diagonal) between the centroid sites; an estimated area for each site is provided.

Sites	Area (km^2)	SB	ST	ARQ	S11A	S11B	S11C	S11D	SFX	SN1	SN2	SN3	SN4	SN5	SN6	SN7	SN8
SB	19.98	**221**	100	47	79	80	75	135	85	124	46	84	108	101	56	57	56
ST	8.3	24	**209**	48	88	90	80	138	84	119	59	87	102	105	55	59	53
ARQ	1.27	140	116	**149**	52	44	45	80	70	75	30	52	77	62	30	29	32
S11A	15.27	59	24	92	**228**	139	119	170	96	143	59	89	116	101	56	54	53
S11B	8.44	54.6	30.8	82	4.5	**199**	107	147	77	120	53	81	96	99	49	52	48
S11C	6.26	52.5	28.8	85	10	4.5	**177**	140	83	110	46	72	101	91	49	41	50
S11D	16.41	47	24.4	92.3	15.7	9.8	5.7	**424**	141	222	80	134	189	168	75	80	72
SFX	9.04	217	193	79.5	158	162	165	170	**239**	131	48	82	111	95	52	44	51
SN1	11.81	52	37.7	111	37	38	40	42	180	**381**	98	154	183	174	77	71	78
SN2	0.86	46.8	32.8	113	36.8	37.1	39.3	40	184	5.18	**124**	69	73	71	40	34	44
SN3	2.1	44.7	32	117.5	40.2	40.1	42	42.2	188	8.1	3.8	**217**	129	103	71	60	59
SN4	14.83	38	25	117.4	37.5	36.4	37.7	37	189	13.7	8.6	7.4	**305**	181	74	65	81
SN5	8.26	32.36	22.75	122	41	39	40	38.53	195	19.78	14.6	12.4	6.2	**289**	63	54	69
SN6	0.97	35.29	22.46	118	37.3	35.8	36.7	35.7	190	16	11	10	3	4	**99**	40	42
SN7	0.34	33	19	117	35.7	33.8	34	33.1	190.5	18	14	13	6	5	3	**112**	46
SN8	2.69	30	17	119	37	34.7	35	33	192	22	17	16	8.8	6	5.7	3.3	**100**

Table 4. Endemic edaphic species of the iron islands outcrops of Carajás complex (bold diagonal) along with the number of shared endemic species (above diagonal) and distance in kilometres (below diagonal) between the centroid sites.

Sites	SB	ST	ARQ	S11A	S11B	S11C	S11D	SFX	SN1	SN2	SN3	SN4	SN5	SN6	SN7	SN8
SB	**20**	15	3	17	15	16	19	11	18	11	15	15	13	11	11	12
ST	24	**16**	2	14	13	14	15	9	15	9	12	11	11	9	10	10
ARQ	140	116	**7**	5	4	5	7	5	6	3	4	5	3	2	2	4
S11A	59	24	92	**24**	17	21	22	14	21	10	16	17	13	11	9	12
S11B	54.6	30.8	82	4.5	**18**	18	19	10	15	14	14	13	12	10	8	10
S11C	52.5	28.8	85	10	4.5	**21**	21	13	11	10	15	15	13	10	9	12
S11D	47	24.4	92.3	15.7	9.8	5.7	**25**	14	21	11	18	19	14	12	12	14
SFX	217	193	79.5	158	162	165	170	**17**	13	9	13	12	8	9	7	9
SN1	52	37.7	111	37	38	40	42	180	**29**	15	20	22	19	13	12	16
SN2	46.8	32.8	113	36.8	37.1	39.3	40	184	5.18	**16**	15	14	14	11	8	12
SN3	44.7	32	117.5	40.2	40.1	42	42.2	188	8.1	3.8	**23**	20	15	15	12	15
SN4	38	25	117.4	37.5	36.4	37.7	37	189	13.7	8.6	7.4	**24**	18	14	12	17
SN5	32.36	22.75	122	41	39	40	38.53	195	19.78	14.6	12.4	6.2	**20**	11	9	15
SN6	35.29	22.46	118	37.3	35.8	36.7	35.7	190	16	11	10	3	4	**15**	8	10
SN7	33	19	117	35.7	33.8	34	33.1	190.5	18	14	13	6	5	3	**14**	10
SN8	30	17	119	37	34.7	35	33	192	22	17	16	8.8	6	5.7	3.3	**17**

Conclusions

This is the most complete study analysing a database of canga outcrop islands in the Amazon thus far. Our data suggest higher shared similarity between largest sites and higher species richness. We show that species richness in these vegetation islands reveals complex biogeographic patterns and relatively high beta diversity. Outcrop size seemed to be more important than geographical proximity between outcrops, and this should be taken into account when drafting conservation and compensation measures for the canga. There are still inaccessible canga outcrops towards the north of the state of Pará that remain unexplored, and their study would certainly yield interesting information to be added to the present findings.

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Supplementary material 1

Investigating plant beta diversity of canga outcrops

Authors: Caroline Oliveira Andrino, Rafael Gomes Barbosa Silva, Juliana Lovo, Pedro Lage Viana, Marcelo Freire Moro, Daniela Cristina Zappi

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New records and key to *Poa* (Pooideae, Poaceae) from the Flora of Southern Africa region and notes on taxa including a diclinous breeding system in *Poa binata*

Robert J. Soreng[1], Steven P. Sylvester[2], Mitsy D.P.V. Sylvester[2], Vincent Ralph Clark[3]

1 *Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington DC 20560, USA* **2** *College of Biology and the Environment, Nanjing Forestry University, Long Pan Road No. 159, Nanjing, 210037, China* **3** *Afromontane Research Unit and Department of Geography, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, 9866, South Africa*

Corresponding author: Robert J. Soreng (sorengr@si.edu)

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Abstract

Four species of *Poa* L. are newly reported for sub-Saharan Africa and southern Africa, *Poa compressa* L., *P. iconia* Azn., *P. infirma* Kunth and *P. nemoralis* L. This is the first report of *P. iconia* from Africa. Vouchers at PRE of *P. bulbosa* L. all belong to var. *vivipara* Koeler, those of *P. iconia* belong to var. *iconia* and the one of *P. trivialis* L. belongs to var. *trivialis*. Two subspecies are recognised in *P. pratensis* L.: subsp. *irrigata* (Lindm.) H.Lindb. and subsp. *pratensis*. We also designate a lectotype for *P. iconia* and second-step lectotype for *P. leptoclada* Hochst. ex A.Rich. and report the first recording of a diclinous breeding system in *P. binata* Nees. Our account updates the treatment in Identification Guide to Southern African Grasses (Fish et al. 2015) including a key to the taxa and notes on infrageneric taxonomy, DNA subtypes, ecology, chromosome numbers and breeding systems.

Keywords

Afro-alpine grassland, bluegrass, breeding systems, invasive species, Lesotho, Namibia, South Africa

Introduction

The genus *Poa* L. includes over 580 species (RJS count 2020) and occurs on all continents. In Africa, 38 species are reported (Valdés and Scholz 2009; Clayton et al. 2016; Plants of the World Online 2020), 14 of which extend to Africa from their primary distributions in Europe and/or Southwest Asia. Twenty-four species are endemic. Of the endemics, eight are confined to northwest Africa (Libya westwards), nine to Ethiopia and Eritrea (Phillips 1995), three to Madagascar and one to the Canary Islands. Of the five endemic or indigenous to Africa in Tropical East Africa (Clayton and Renvoize 1982), *P. schimperiana* Hochst. ex A. Rich. and *P. leptoclada* Hochst. ex A. Rich. are more wide ranging in eastern Africa (both reaching the Arabian Peninsula) and *P. leptoclada* is reported from the Canary Islands (Valdés and Scholz 2009; although RJS thinks those specimens represent *P. flaccidula* Boiss. & Reut.; RJS pers. obs.). In the Flora of Southern Africa region (Botswana, Lesotho, Namibia, South Africa and Eswatini a.k.a. Swaziland; FSA), Fish et al. (2015) recorded six species of *Poa*, four of which are introduced from the temperate northern hemisphere (*P. annua* L., *P. bulbosa* L., *P. pratensis* L. and *P. trivialis* L. without noting any infraspecies) and none of which is endemic to the FSA (*P. binata* Nees near-endemic to the FSA being also found in Zimbabwe). *Poa binata* and *P. leptoclada* are the only indigenous species.

There is a strong association of FSA *Poa* with southern Africa's mountains: three species are closely aligned in distribution with the rugged, moist eastern Escarpment (*P. annua*, *P. binata* and *P. leptoclada*); one with the eastern Escarpment and Cape Flora (*P. pratensis*) and one with arid western Escarpment (*P. bulbosa* var. *vivipara* Koeler). *Poa leptoclada*, in the FSA region only known from a few collections from the Maloti-Drakensberg (MD), also occurs naturally in the eastern African mountains and into Yemen (Fish et al. 2015).

Although the FSA Poaceae flora is relatively well known, the grass flora of the MD remains incompletely known, especially in more poorly-botanised areas, such as the Eastern Cape Drakensberg (including the former Transkei) and the alpine zone across the MD (Pooley E, pers. comm.). Given the immense grazing pressure that the MD is under from communal rangeland use and associated ecological degradation, it is imperative that the taxonomic status of these natural montane rangelands – where they still exist – are carefully documented. In addition, the FSA region still has many questions and complexities as to the accurate identity and taxonomic status of mountain-associated genera, such as *Festuca* L. (Sylvester et al. in press), *Trisetopsis* Röser & A. Wölk (our species included in *Helichtotrichon* Besser by Fish et al. 2015, revised *Trisetopsis* by Mashau et al. 2020), *Poa* and others; these temperate, usually C_3 groups, are essential components in the functional ecology of these mountains as indigenous pastures, particularly in the alpine zone of the MD.

From February-March 2020, a comprehensive survey of MD Poaceae in the alpine zone was undertaken by SPS, MDVPS and RJS. During fieldwork, two previously-unreported species of *Poa* were recorded for the FSA region (Fish et al. (2015):

Poa compressa L. and *P. nemoralis* L. In addition, while identifying the grass collections at the South African National Herbarium in Pretoria (PRE), three additional collections of *P. compressa* and two more introduced species of *Poa* (*P. iconia* Azn. (var. *iconia*) and *P. infirma* Kunth) were discovered amongst herbarium collections. Infraspecific determinations of certain taxa were also made for the first time, with *P. bulbosa* identified to var. *vivipara*, *P. pratensis* identified to subsp. *irrigata* (Lindm.) H.Lindb. and subsp. *pratensis* and *P. trivialis* identified to subsp. *trivialis*.

Accordingly, here we present:

1. Details on these new records to FSA;
2. An updated key for the *Poa* of the FSA region;
3. Taxonomic notes on *Poa* of the FSA region, including reporting a diclinous breeding system in *P. binata*. Aside from *P. trivialis* L., which was reported to be self-incompatible and sexually reproducing (Connor 1979), the other introduced species are either inbreeders or known for apomictic reproduction.

Materials and methods

Extensive field collecting was conducted by SPS, RJS and MDPVS throughout the MD between 1 Feb and 9 Mar 2020, with specimens deposited in the US [first set, pending export permits], PRE and NU herbaria (Herbarium acronyms follow Thiers [continuously updated]). Study was also conducted at the PRE herbarium between 13 and 20 Mar 2020. Visits to other national herbaria in southern Africa (e.g. NU) were not possible due to the onset of the Covid-19 pandemic. We follow Fish et al. (2015) for country and province distributions and only report vouchers renamed at PRE to the newly-reported species and those of our new collections of *Poa* from the MD. Collection records used to plot species dot maps in Fish et al. (2015) are available online from SANBI (South African National Biodiversity Institute) − PRECIS (National Herbarium Pretoria [PRE] Computerized Information System) which covers NBG, PRE and UDW herbaria; http://www.sanbi.org. These data are also reflected by GBIF; http://www.gbif.org.

Taxonomic treatment

New FSA records

Four new species records are presented for the FSA: *Poa compressa*, *P. iconia* var. *iconia*, *P. infirma* and *P. nemoralis*. New infraspecific records are also presented for the FSA, with *P. trivialis* identified to subsp. *trivialis*, *P. bulbosa* identified to var. *vivipara* and two subspecies are recognised in *P. pratensis*: subsp. *irrigata* and subsp. *pratensis*.

Key to *Poa* in the Flora of Southern Africa region

The following presents a key to all the *Poa* species and infraspecies that are currently known to occur in the FSA region. ‘Glabrous’ means without pubescence, ‘smooth’ means without prickle hairs/hooks.

1 Plants with bulbous-based vegetative shoots; flowering shoots usually producing leafy bulbils within spikelets which may or may not have somewhat normal appearing floret proximally or occasionally throughout some spikelets (rarely all spikelets normal-flowered within a plant) **2**

2 Ligules of lowest leaves mostly (0.8–)1–2 mm long, as long or longer than wide, apically obtuse to acute, usually smooth, rarely a lightly scabrous; ligules of bulbil leaflets decurrent along sheathlet margins; longest blades of basal tufts mostly less than 4 cm long; sheaths usually smooth, rarely sparsely hispidulous; prophylls proximally retrorsely scabrous, distally mixed directionally; callus of quite normal lemmas with a dorsal tuft of hairs; panicles more or less tightly contracted. .... ***P. bulbosa* var. *vivipara***

2' Ligules of lowest leaves < 1 mm long, shorter than wide, apically truncate to obtuse, no longer than broad, abaxially usually more or less scabrous or strigulose; ligules of bulbil leaflets not decurrent along sheathlet margins; longest blades of basal tufts mostly 4–15 cm long; sheaths and blades of lowest leaves abaxially sometimes obscurely strigulose to hispidulous; prophylls antrorsely scabrous; callus of (rare) normal lemmas glabrous; panicles more or less loosely contracted .... ***P. iconia* var. *iconia***

1' Plants without bulbous bases; flowering shoots producing normal spikelets (rarely with bulbils in a few spikelets or inflorescences) **3**

3 Annuals; branches, spikelet bracts smooth, palea keels softly villous/pilose; anthers 0.2–1 mm long (those of the uppermost florets, often sterile rudiments); floret callus glabrous; lemmas usually softly villous at least on the keel and marginal veins **4**

4 Anthers 0.2–0.5(–0.55) mm long; panicle branches ascending, spikelets crowded; lemmas usually prominently villous on 5 veins; leaves light green; spring ephemerals .... ***P. infirma***

4' Anthers (0.55–)0.6–1 mm long; panicle branches ascending to spreading, spikelets more loosely arranged; lemmas prominently villous on 3 or 5 veins; leaves darker green; spring ephemerals to long lasting annuals ....***P. annua***

3' Perennials; branches smooth or scabrous, spikelet bracts distally scabrous at least along keels; anthers 0.5–3 mm long; callus glabrous or with a dorsal tuft of hairs separated from those on the lemma keel (webbed) and usually longer than those (hairs sometimes a bit diffuse on the callus in *P. binata*); lemmas glabrous or softly sericeous to villous on the keel and often on the marginal veins........**5**

5 Lemma intermediate veins faint (sometimes distinct in *P. compressa*); sheath margins of upper culm leaves fused < 1/5(– ¼) the length; all shoots flower-

ing in a given season, all shoots extravaginal with cataphylls proximally, with rudimentary prophylls at shoot junctures; first glumes 3-veined.................. **6**

6 Plants strongly rhizomatous, shoots mostly isolated; culms and nodes strongly compressed (cannot roll them between your fingers), often geniculate proximally with lower nodes exposed; ligules truncate to obtuse to 2 mm long; uppermost leaf blades shorter than their sheaths .... ***P. compressa***

6' Plants tufted or a bit loose with some basal branching; culms and nodes round (easily rolled between your fingers), not geniculate except at very base with lower nodes sometimes covered by their sheaths; ligules truncate 0.2–0.8 (–1) mm long; uppermost leaf blades usually longer than their sheaths......... ***P. nemoralis***

5' Lemma intermediate veins distinct, sometimes quite pronounced; sheath margins of upper culm leaves fused > ¼ the length; some or many shoots vegetative (non-reproductive) in a given season, all shoots extravaginal with cataphylls and rudimentary prophylls at shoot junctures or some or all shoots intravaginal with well-developed prophylls at shoot junctures; first glumes 1- or 3-veined **7**

7 Anthers 0.5–1 mm long; panicles contracted in age, branches appressed, longest branches sometimes shorter than their axis internodes, spikelets crowded, (2.1–)3–4.5(–6) mm long, usually with 50 or more spikelets per panicle; florets glabrous or sericeous on keel and marginal veins and sometimes between them; callus glabrous or webbed; plants small tufted, sometimes straggling, without rhizomes; leaf blades flat, tender .... ***P. leptoclada***

7' Anthers (0.8–)1–3 mm long; panicles loosely contracted to open in age, longest branches as long or longer than their axis internodes, spikelets crowded or dispersed, 2.5–6(–7) mm long, with 20 to 100+ spikelets per panicle; florets glabrous or variously sericeous to villous on keel and marginal veins; callus glabrous or webbed; plants small to large (broad) tufted or loosely tufted, sometimes sprawling or straggling, with or without rhizomes **8**

8 Ligules as long as wide or longer than wide, acute to acuminate, upper culm ones 4–6(–8) mm long; callus webbed; lemmas prominently 5-veined, sericeous on the keel, marginal veins glabrous or sericeous proximally for less than ¼ the length; first glume 1-veined, often sickle shaped; sheaths more or less retrorsely scaberulous; plants small tufted, erect to sprawling or straggling and somewhat stooling; leaf blades flattish, tender, dark green; anthers (0.8–)1–1.8 mm long .... ***P. trivialis* subsp. *trivialis***

8' Ligules shorter than wide, mostly truncate to obtuse, upper culm ones 0.5–2(–3) mm long; callus glabrous or webbed; lemmas distinctly to prominently 5-veined, glabrous to sericeous or villous on the keel and marginal vein and sometimes between them; first glume 1- or 3-veined, lanceolate; sheaths smooth or retorsely scaberulous or strigulose; plants small to large tufted or loosely spreading with isolated culms and vegetative shoots, erect, rhizomatous or not; leaf blades flat or more often V-shaped or folded, tender or firm; bluish to dark green; normal anthers 1.4–3 mm long.................................. **9**

9 Lemmas glabrous or variously sericeous, to villous as above, sometimes with hairs on the intermediate veins and between the veins; callus glabrous, with a dorsal web or hairs slightly diffused dorsally; plants without or with some short rhizomes, forming small to large (broad) tussocks; basal sheaths more or less fibrous in age (often burned); leaf blades all alike; blades adaxially glabrous, somewhat thick with thick margins; first glume 3-veined; anthers 1.5–3 mm long, often sterile/rudimentary in upper flowers of spikelets or sometimes in all spikelets, particularly so in the lower spikelets of a panicle............***P. binata***

9' Lemmas villous on the keel and marginal veins only; callus with a prominent dorsal web; plants strongly rhizomatous, forming small tufts, turf or with isolated flowering and vegetative shoots; basal sheaths not fibrous in age; leaf blades all alike or dimorphic, with long slender vegetative leaves and shorter broader culm leaves; blades adaxially glabrous or often with few to many strigulose hairs, not noticeably thick with thick margins; first glume 1- or 3-veined; anthers 1.4–2.5 mm long, infrequently some aborted in age.............**10**(*P. pratensis s.l.*)

10 Leaf blades all more or less alike in form, mostly 1.5–3 mm wide, mostly flat or folded; collars often ciliolate on the margins, hairs sometimes extending down the sheath margins and sometimes the upper surfaces; first glume (1-)3-veined..***P. pratensis* subsp. *irrigata***

10' Leaf blades of two forms, vegetative blades slender and elongated, ca. 0.5–1 mm wide as folded or involute, culm blades shorter and broader and flatter; collars and sheaths usually glabrous; first glume 1- or 3-veined..... ***P. pratensis* subsp. *pratensis*** (if the lateral shoots occur in tight, intravaginally originating fascicles and the blades are fairly firm (with veins pronounced abaxially, strigulose hairs common adaxially), the plants belong to *P. pratensis* subsp. *angustifolia* [L.] Lej.)

Taxon notes

For full explanation of genotype coding in *Poa*, see Soreng et al. (2010, 2020). For genotypes, the first letter indicates the plastid clade and the second letter the nuclear ribosomal internal and external transcribed spacer clade. The 2*n* chromosome number modes are in *italic*, main modes are **bold**.

Poa annua L. Sp. Pl. 1: 68 1753.

Ochlopoa annua (L.) H. Scholz, Ber. Inst. Lanschafts-Pflanzenokologie Univ. Hohenheim Beih. 16: 58. 2003.

Type. Habitat in Europa ad vias. (lectotype, designated by Soreng 2020: 254: LINN (LINN-87.17!, right-hand plant)).

Many heterotypic synonyms. – P. sect. *Micrantherae* Stapf, Fl. Brit. India 7(22): 343. 1897 [1896]. Type, *P. annua* L.

Distribution. widespread in Lesotho and South Africa. Introduced, native to Eurasia and North Africa, now worldwide.

Ecology. weedy in temperate climates.

Flowering. anytime.

Economics. common, a pesky garden, lawn and trail weed of little consequence.

Vouchers. Lesotho. Menoaneng Pass, on road between Rafolatsane and Thaba-Tseka, S29.427251 E28.947895, 3086 m alt., basaltic substrate, Afro-alpine grassland, 24 Feb 2020, S.P. Sylvester et al. 3609 (PRE, US); Sani Pass area, close to Sani river northwest of Sani Mountain Lodge, S29.562993 E29.246806, 2803 m alt., basaltic substrate, short Afro-alpine grassland, close to a pool of water, frequently to heavily grazed, 26 Feb 2020, S.P. Sylvester et al. 3625 (NU, PRE, US). **South Africa**. Eastern Cape: Naudes Nek pass, near Rhodes, in grassland next to radio tower, S30.764488 E28.090455, 2607 m alt., basaltic substrate, overgrazed alpine grassland with some low *Erica* and *Helichrysum* shrubs, gently sloping, moderately deep soil, 13 Feb 2020, S.P. Sylvester et al. 3503 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, S30.675222 E27.959111, 2529 m alt., basaltic substrate, heavily-grazed livestock paddocks amongst alpine grassland, 12 Feb 2020, S.P. Sylvester et al. 3472 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, S30.675667 E27.958950, 2532 m alt., basaltic substrate, grazed alpine grassland next to livestock paddocks, 12 Feb 2020, S.P. Sylvester et al. 3477 (NU, PRE, US).

Notes. *Poa annua* is a tetraploid species derived from hybridisation, somewhere around the Mediterranean Sea, between two diploids that overlap in that region: *P. infirma* (maternal parent, contributor of the plastid genotype) and *P. supina* Schrad. (paternal parent, providing the nuclear ribosomal internal [and also external] transcribed spacer genotypes) (Soreng et al. 2010). It is sometimes considered the world's most widespread weed. *Poa annua* grades in form in the directions of both parents, sometimes making it tricky to differentiate, especially from *P. infirma*. Hybrids with *P. supina*, called *P.* × *nannfeldtii* (H. Scholz ex Val.N.Tikhom.) Nosov, exhibit a C-value indicative of triploidy (Soreng, pers. obs.). Self-compatible, mostly inbreeding, it is gynomonoecious. The upper florets within spikelets being pistillate facilitates outcrossing. 2*n* = ***28***. − $\mathbf{M}^{i}\mathbf{M}^{u}$ genotype.

Poa binata Nees, Fl. Afr. Austral. Ill. 378. 1841.

Fig. 1

= *Poa atherstonei* Stapf, Fl. Cape. 7: 713. 1900. Type: SOUTH AFRICA. Central Region: Graaff Reinet. Div., summit of Compass Berg, Atherstone 46 (holotype: K (K000345194 [image!]); isotype: PRE fragm. ex K!).

= *Poa bidentata* Nees, Fl. Afr. Austral. Ill. 3–379. 1841. Type: SOUTH AFRICA. (without precise location), Zeyher s.n. [1832] (holotype: K (K000345195 [image!]); isotype: PRE fragm. ex K!).

= *Poa heterogama* Hack., Rec. Albany Mus. 1: 112. 1904. Type: SOUTH AFRICA. Kentani, [valleys after grass fire, 1000 ft [305 m], frequent], Aug 1902 [1904 on

Figure 1. *Poa binata*. **A** whole plants **B** basal part of plant showing fibrous basal sheaths **C, D** portions of inflorescence. Image **A** of S.P. Sylvester et al. 3489 (US) **B** of S.P. Sylvester et al. 3412 (US) **C** of S.P. Sylvester et al. 3518 (US) **D** of S.P. Sylvester et al. 3677 (US).

BM and BOL isotypes; 4 Oct 1904 on GRA isotype; Oct 1904 on K isotypes], Mis Alice Pegler No. 50 (holotype: W (W19160014385 [image!); isotypes: BM (BM000922785 [image!]), BOL (BOL139269 [image!]), GRA (GRA0000194-0 [image!]), K (K000345191 [image!], K000345192 [image!]), PRE (PRE0029722!), US (US00956065 fragm. ex W!)).

Type. [South Africa. Eastern Cape:] In montibus inter Katrivier et Klipplaatarivier flumina locis graminocis et paludosis alt. 4000–5000' [1219–1525 m], atque in monte Los Tafelberg. alt. 6000' [1829 m], Drège s.n. (lectotype, “9/11 32. [9 Nov 1832] Sumpf auf Gras[flächen {or} plätzen?].auf dem Katberg, 4000–5000' [1219–1525 m],

| af (I af." {original Drège ticket}, {second ticket:} "&. c. 389b | *Poa binata* N.ab. E. | 27)" (lectotype, **designated here:** P (P00434748 [image!])).
– sect. unplaced.

Distribution. Lesotho, South Africa, reaching Zimbabwe. Native, endemic to southern Africa.

Ecology. cool temperate grasslands.

Flowering. December to March.

Economics. common, an important component of high elevation grasslands.

Vouchers. Lesotho. AfriSki area, in valley adjoining and northwest of the valley of the AfriSki resort, on the north side of the A1 highway, S28.808394 E28.708658, 3104 m alt., basaltic substrate, dry upper slopes above valley, 27 Feb 2020, S.P. Sylvester et al. 3653 (NU, PRE, US); Bokong Nature Reserve, ca. 350 m north from the information centre, S29.067203 E28.421496, 2972 m alt., basaltic substrate, Afro-alpine grassland dominated by *Lachnagrostis barbuligera* var. *barbuligera* with moderately-controlled grazing and burning, 2 Mar 2020, S.P. Sylvester et al. 3677 (NU, PRE, US); Bokong Nature Reserve, east of Mafica Lisiu Pass, below the ridge south of the road, S29.066689 E28.40595, 3100 m alt., basaltic substrate, Afro-alpine grassland E, facing burned slope, dominant grass, rich organic topsoil, with many orchids and *Senecio macrocephalus*, 3 Mar 2020, S.P. Sylvester et al. 3698 (NU, PRE, US); Matebeng Pass, below highest summit close to the pass, S29.868524 E28.976439, 3125 m alt., basaltic substrate, "Lesotho Highland Basalt Grassland" with clear elements of "Drakensberg Afro-alpine Heathland" with *Erica* and *Helichrysum* shrubs dominating the landscape, heavy horse grazing, 22 Feb 2020S.P. Sylvester et al. 3582b (NU, PRE, US); Menoaneng Pass, on road between Rafolatsane and Thaba-Tseka, S29.427403 E28.951124, 3039 m alt., basaltic substrate, Afro-alpine grassland, windy ridge, grazed by horses down to low turf, 24 Feb 2020, S.P. Sylvester et al. 3598 (PRE, US); Sani Pass area, ca. 250 m east of Sani Mountain Lodge, S29.584906 E29.291216, 2882 m alt., basaltic substrate, short Afro-alpine grassland, frequently to heavily grazed, soil gravelly loam to 5 cm deep, 25 Feb 2020, S.P. Sylvester et al. 3616 (NU, PRE, US); Sehlabathebe National Park, lower end of the park on the border, S29.860061 E29.095497, 2719 m alt., basaltic substrate, wet Afro-alpine tussock grassland, soil damp, under dripping crag, heavily grazed, close to livestock paths, 19 Feb 2020, S.P. Sylvester et al. 3525 (NU, PRE, US); Sehlabathebe National Park, lower end of the park on the border, S29.877593 E29.086461, 2606 m alt., basaltic substrate, wet Afro-alpine tussock grassland, soil damp, not grazed recently, 20 Feb 2020, S.P. Sylvester et al. 3541 (NU, PRE, US); Tsehlanyane National Park, along path next to 'Black Pool', S28.900154 E28.452053, 2120 m alt., basaltic substrate, *Leucosidea* woodland, S facing slope, 4 Mar 2020, S.P. Sylvester et al. 3705 (NU, PRE, US). **South Africa**. Eastern Cape: Barclay Pass area, Mountain Shadows Hotel, in grassy field behind guest bungalows, S31.203522 E27.838044, 2052 m alt., basaltic substrate, remnant patch of ungrazed native upland grassland, on east facing slope, 14 Feb 2020, S.P. Sylvester et al. 3518 (NU, PRE, US); Eastern Cape: Naudes Nek pass, near Rhodes, S30.764792 E28.105164, 2589 m alt., basaltic substrate, alpine tussock grassland, gently sloping, good soil, 13 Feb 2020, S.P. Sylvester et al. 3489 (US

[3 sheets]); Eastern Cape: Tiffindell Ski Area, S30.649239 E27.928720, 2845 m alt., basaltic substrate, alpine grassland, 10 Feb 2020, S.P. Sylvester et al. 3448 (US); Eastern Cape: Tiffindell Ski Area, next to ski lift, S30.651034 E27.925149, 2778 m alt., basaltic substrate, alpine grassland, annually burnt, appears to be seeded with exotic species, 10 Feb 2020, S.P. Sylvester et al. 3453 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, Ben Macdhui summit, S30.647683 E27.934042, 2995 m alt., basaltic substrate, alpine grassland, 11 Feb 2020, S.P. Sylvester et al. 3458a (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, Ben Macdhui summit, S30.647683 E27.934042, 2995 m alt., basaltic substrate, alpine grassland, 11 Feb 2020, S.P. Sylvester et al. 3458b (US); Eastern Cape: Tiffindell Ski Area, S30.676696 E27.958347, 2522 m alt., basaltic substrate, alpine tussock grassland, 12 Feb 2020, S.P. Sylvester et al. 3481a (NU, PRE, US); Free State: Sentinel trail before reaching the chain ladders that take you up to Amphitheatre, S28.740954 E28.886656, 2857 m alt., basaltic substrate, ungrazed mesic alpine grassland on steep N-facing slope, 5 Feb 2020, S.P. Sylvester et al. 3412 (NU, PRE, US); Kwazulu-Natal: Amphitheatre, slopes near the Tugela waterfall, S28.754008 E28.893853, 2983 m alt., basaltic substrate, alpine grassland, 5 Feb 2020, S.P. Sylvester et al. 3404 (PRE, US); Kwazulu-Natal: Amphitheatre, slopes near the Tugela waterfall, S28.754498 E28.892780, 2979 m alt., basaltic substrate, alpine grassland, 5 Feb 2020, S.P. Sylvester et al. 3407 (US); Kwazulu-Natal: Sani Pass area, below southwest facing cliffs to the southeast of Sani Mountain Lodge, S29.585365 E29.290839, 2866 m alt., basaltic substrate, short Afro-alpine grassland, frequently to heavily grazed, 26 Feb 2020, S.P. Sylvester et al. 3638 (PRE, US).

Notes. *Poa binata* is a common species in the upper Maloti-Drakensberg mountains. In areas with enough moisture and low grazing pressure, the species can be the dominant grass species, forming dense tussocks to 0.5 m diameter. As in many large grass tussocks, a few shoots can appear to be rhizomatous, but are actually stooling shoots as in *P. bidentata* (see below). Under high grazing pressure, plants become smaller and weaker and sparsely distributed. Plants seem to tolerate burning well. The species displays unusual diversity in lemma pubescence, varying from glabrous to pubescent on three veins, to pubescent on five veins and sometimes between veins and callus hairs may be present or absent. Flowers are pistillate and/or perfect within plants, anthers are 1.5–2.7 mm long or vestigial. $2n$ = 28, 42, 56. – **Ha** genotype (Gillespie and Soreng, unpublished).

The species exhibits a diclinous breeding system. Most species of *Poa* are hermaphroditic. Dicliny occurs in about one quarter of the species of *Poa* examined and ranges from simple gynomonoecy to full dioecy (Soreng et al. 2020). In *P. binata*, many plants have spikelets with pistillate upper flowers. Other plants exhibit more pistillate flowers within spikelets and wholly pistillate spikelets. The latter are concentrated on the lower branches of panicles. Some plants were judged to be completely pistillate. The sterile rudiments of anthers (staminodes), present in pistillate flowers, are believed to result from genetic control, not from apomixis. All other florets, spikelets and sometimes whole plants examined were perfect-flowered. The breeding system of *P. binata* needs further study, but seems to match sequential gynomonoecy as described by Soreng and Keil (2003). This breeding system is estimated by RJS to occur in 28 species equally divided between the Americas and east Asia (Soreng et al. 2020), almost all of which have anthers averaging 2 mm long or longer.

The lectotype at P is selected as it is one of two sheets with Drège's original handwritten location and date, the other original set of tickets being destroyed (Gunn and Codd 1981). The lectotype is clearly distinct from all the others, which may or may not be duplicates of the second collection cited by Nees ab Esenbeck (1841). Other syntypes or original material have only secondary notes from Ernst Meyer's distribution of Drège sets (in 1837, 1840, 1847; Meyer 1837, 1840, 1847) or guessed at from other duplicates, some of which may actually have been collected by Zeyer (who joined the Drège brothers in 28 Nov 1832 into early December and then collected on his own for some months before departing South Africa, for example, the K000345193 sheet which originally said Zeyer, but that was crossed out and replaced by Dredge and a location where they collected together). For further reading, see Gunn and Codd (1981). Some of the other distributions say Tafelberg 6000–7000 ft [1829–2134 m], but these may be tertiary writings or collections not used in the protologue, as the protologue did not mention anything above 6000 ft [1819 m]. We have located various specimens:

.... 7/12 32. [7 Dec 1932] Unter den Hängen vom Los-Tafelberg, 5000–6000' [1524–1829 m], | b (I af. {original Drège label} (syntype, P000434747 [image!])

.... "*Poa binata* N.ab.E. a" {**original ticket from E. Meyer distribution**}, Los Tafelberg, in dem Kranzen und auf feuchten und felsigen, Bergplatte, 6000–7000 fuss [1829–2134 m], December, J.F. Drège {penned by someone} (E00200327 [image!])

.... "*Poa binata* N.ab.E. a" {**original ticket from E. Meyer distribution**} J.F. Drège {stamped on that}, in monte Tafelberg 6000' [1829 m], J. F. Drège {typed later} (HAL [image!])

.... "*Poa binata* N.ab.E. a" {**original ticket from E. Meyer 1840 distribution**}, Afr. Austr., Drège, 1840, {old note, year 1840 presumably referring to E Meyer distribution of Drège set}, Hb. Benth. Table Mountain, Queenstown Div. 6000–7000 ft {penned by someone} [1829–2134 m] (K000345242 [image!])

.... "*Poa binata* N.ab.E. a" {**original ticket from E. Meyer distribution**}, *Poa binata* N.ab.E., Gramin Africa p. 378 No 2., Africa Austr. Drège No. {**original duplicate ticket from E. Meyer?**} (BM ex hb. Shuttleworth)

.... "*Poa binata* N.ab.E. a 1840, 324" {**original ticket from E. Meyer 1840 distribution**}, "Los Tafelberg, in den Kranzen und auf der feuchten und felsigen Bergplatte, 6000–7000 fuss [1829–2134 m], December" {typed ticket} (S-C-4936)

.... "*Poa binata* N.ab.E. a" {**original ticket from E. Meyer distribution**}, 210 *Poa binata* N. ab. E. 117.11 ex Bernhardi herbarium (MO2112449 (bc) 2397251)

Poa bidentata Nees is usually placed in *P. pratensis*, but in our opinion, it is merely a stooling example of *P. binata*. It has lemmas that, in addition to having pubescence like *P. pratensis*, are quite scabrous in the margins and between the veins, ruling out *P. pratensis*. There are various sheets and fragments of *P. atherstonei* (= *P. binata*) at PRE, collected by Ms. Pelger between 1901 and 1914, but only one that matches the date and cited by Hackel (1904) . That one has lemmas that are glabrous or sparsely pubescent on the keel and marginal veins, web short and scant or absent.

Poa bulbosa L. Sp. Pl. 1: 70. 1753. var. *vivipara* Koeler, Descr. Gram. 189. 1802.

Poa bulbosa subsp. *vivipara* (Koeler) Arcang., Comp. Fl. Ital. 785. 1882.

Type. [Germany. Mainz:] Prope Moguntiam in arenosis (specimen not found).

− *P.* sect. *Arenariae* (Hegetschw.) Stapf s.s., Fl. Brit. India 7(22): 338. 1897 [1896]. Type *P. bulbosa* L.

Distribution. native to Eurasia and northwest Africa. Introduced/possibly arrived via long-distance-dispersal, but that seems unlikely for the bulbils are bulky and have no special dispersal mechanisms.

Ecology. hemicryptophyte, geophyte, with bulbous based shoots that store hemicellulose. Well-adapted to temperate climates with winter rains and dry summers.

Flowering. winter and spring green, flowering in mid-spring and quickly going dormant, flowers mostly forming bulbils. Apomictic.

Economics. common, excellent early spring forage for sheep, but invasive and can become dominant.

Vouchers. no new records.

Notes. All the specimens reviewed at PRE were pseudoviviparous, at least in part. More or less normal-looking lemmas are commonly present in the lower one or two florets of bulbiferous spikelets. The very normal-looking lemmas will have soft hairs on the keel and marginal veins and a tuft of longer hairs on the dorsal side of the callus. The normal florets are thought to be fertile to some degree, although RJS has rarely observed seed in these. Some plants produce more normal florets and more normal spikelets than others, but the main mode of dispersal and establishment is by leafy bulbils that readily root and grow with the next seasons' rains. Some taxonomists decline to recognise infraspecies here, but for purposes of natural history research, it is useful to identify plants with any bulbiferous spikelets as var. *vivipara*. Apomictic via bulbifery. $2n$ = 21, 28, 29, 31, 32, 33, 34, 35, 37, *42*, 44, 46, 48, 49. – **Aa** genotype.

Poa compressa L., Sp. Pl. 1: 69. 1753.

Fig. 2

Type. Habitat in Europae and Americae septentrionalis (lectotype, designated by Soreng in 2000: 255: LINN (LINN-87.41!)).

Many heterotypic synonyms. − P. sect. *Tichopoa* Asch. & Graebn., Syn. Mitteleur. Fl. 2: 419. 1900. Type, *P. compressa* L.

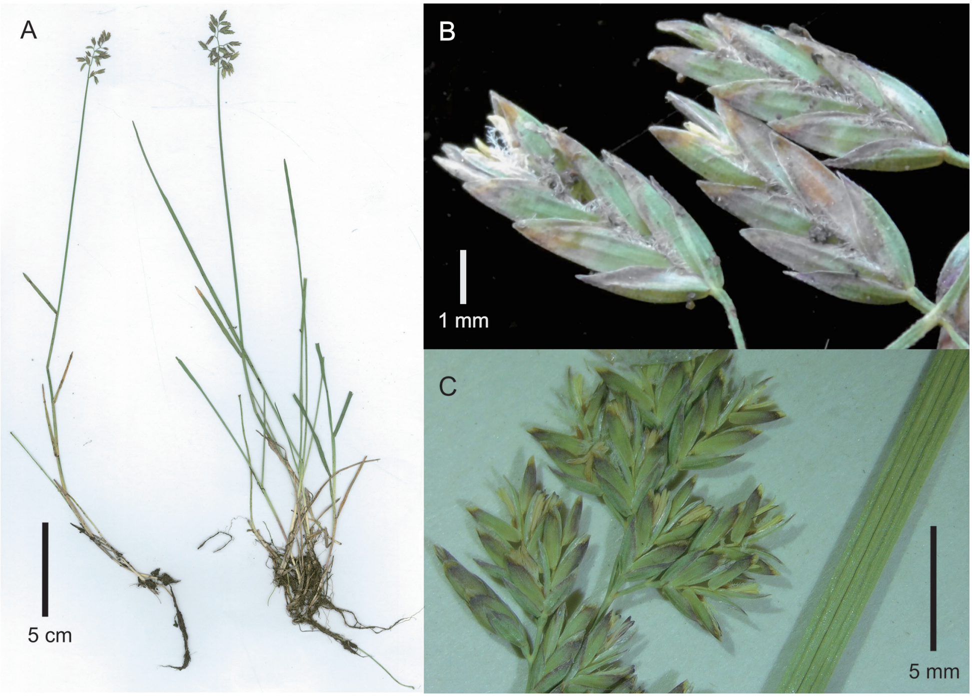

Figure 2. *Poa compressa*. **A** whole plant **B** spikelets, lateral view **C** portion of inflorescence and leaf blade. Image **A** of S.P. Sylvester et al. 3439 (US) **B** of S.P. Sylvester et al. 3439 (PRE) **C** of J.P.H. Acocks 20227 (PRE).

Distribution. Lesotho, Sehlabathebe N. P., South Africa EC. Introduced, panboreal native of Eurasia, NW Africa and North America.

Ecology. wet grasslands at high elevations.

Flowering. summer and autumn.

Economics. infrequent, useful for soil stabilisation in wet soils.

Vouchers. LESOTHO. West of Sehlabathebe National Park, under large dripping roof/cave above the Leqooa-Legowa river, S29.858547 E29.055979, 2330 m alt., sandstone substrate, below W facing cliffs, soil very wet and dominated by *Lachnagrostis lachnantha*, 21 Feb 2020, S.P. Sylvester et al. 3573 (NU, PRE, US). SOUTH AFRICA. Eastern Cape: about 15 km east of Rhodes in Kloppershoekspruit valley, Mavis Bank Farm, stream, on wet mud on streamside, 7 Dec 1999, L. Smook 10245 (PRE); Eastern Cape: Barkly East, Morriston above Barkly P., Marg. Dohne Sour V., 6700' [1829 m], 17 Jan 1959, J.P.H. Alcocks 20227 ("= Alcocks 12124 from Tarka") (PRE); Eastern Cape: between Casrlisleshoekspruit Pass and Tiffindell Ski Area, S30.677202 E27.956643, 2526 m alt., basaltic substrate, Afro-alpine riparian wetland, 10 Feb 2020, S.P. Sylvester et al. 3439 (NU, PRE, US).

Notes. First reports for South Africa and Lesotho. Apparently, it is well established in the southern Drakensberg, where it was collected previously in 1959 and three times

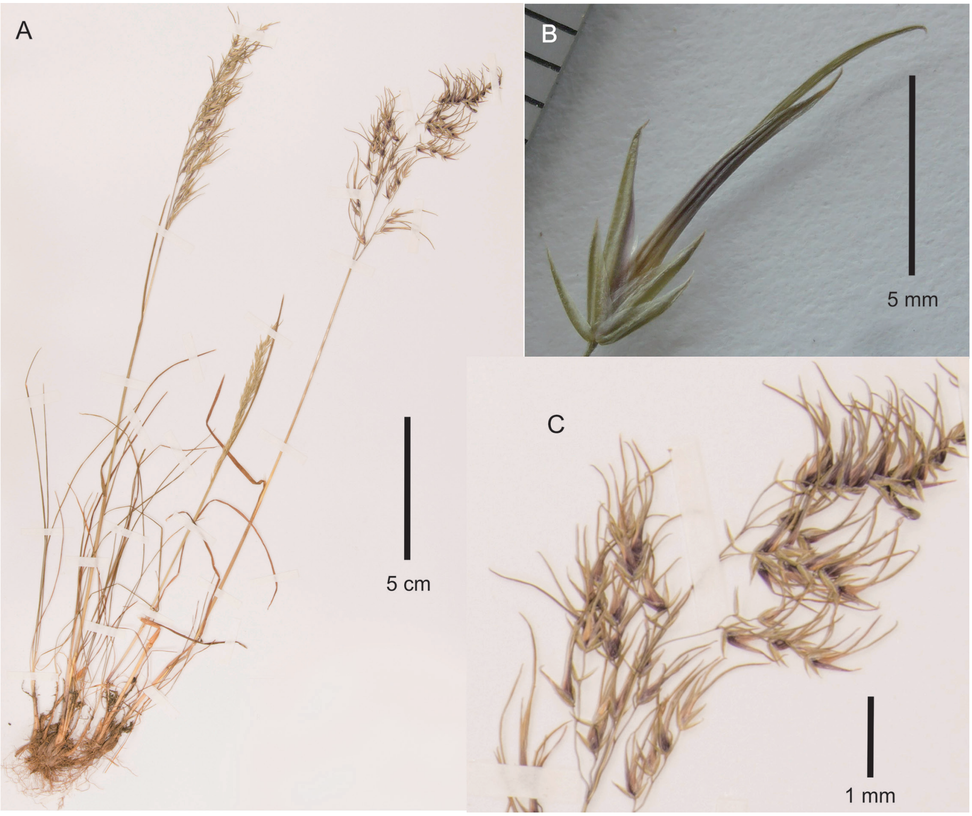

Figure 3. *Poa iconia* var. *iconia*. **A** whole plant **B** spikelet, lateral view **C** close-up of inflorescence. Images **A, C** of V.R. Clark & C. Kelly 269 (GRA0009104) **B** of V.R. Clark & C. Kelly 269 (PRE).

since. At PRE, it has passed under the determinations as *Poa pratensis* and *Poa* sp. Now it is also known from south-eastern Lesotho. Apomixis is known. $2n$ = 35, ***42***, 45, 49, 50, 56. – **Ss** genotype.

Poa iconia Azn., Magyar Bot. Lapok 1918, xvii. 67. 1919 var. *iconia*

Fig. 3

Type. Turkey. Anatolia centralis [Lycaonia:] Mont Hagios [‘Agios’ on G isotypes] Philippos, pres de Konia, 30 Apr 1913, B.V.D. Post (lectotype, designated here: Post B 53, E (E00367667!); isolectotypes: G (G00308664 [image!], G00386674 [image!])). – sect. unplaced.

Distribution. Cape Province, mainly Asia Minor and SW Europe. Introduced rare in South Africa, originating from Asia Minor and SW Europe.

Ecology. similar to *Poa bulbosa*. Mediterranean climate.

Flowering. Spring.

Economics. One collection site known from 2007, likely more common by now, good spring forage, but potentially invasive.

Voucher. South Africa. Northern Cape: Sutherland District, Komsberg Farm Schietfontein 179, 32°40'29"S, 20°48'51"E, open shrubland, level, along drainage line (moist), sandstone gravel, abundant, 1474 m alt., 28 Sep 2007, V.R. Clark & C. Kelly 269 (PRE8610990).

Notes. First report for the African continent and South Africa and Lesotho. *Poa iconia* was recognised as *Poa pelasgis* H. Scholz (Scholz 1985), a synonym of *Poa iconia* var. *pelasgis* (H. Scholz) Soreng (Soreng and Simmons 2018), its normal-flowered counterpart. The species genotype markers suggest it is only remotely related to *P. bulbosa* (Cabi et al. 2016). Aznavour (1918) did not state a collection number or herbarium. Only three sheets have been located that match the protologue, all Post B 53 (the E sheet originally had B29, but that was crossed out and replaced by 53), all three are viviparous. We select the E sheet where Aznavour's herbarium and types are kept as the lectotype. Apomictic via bulbifery. 2*n* = unknown (possibly, in a few cases, counted as *P. bulbosa* var. *vivipara*.) – **Nn** genotype (Cabi et al. 2016).

Poa infirma Kunth, Nov. Gen. Sp. 1: 158. 1816.

Fig. 4

Megastachya infirma (Kunth) Roem. & Schult., Syst. Veg. [Sprengel] 2: 585. 1817. *Eragrostis infirma* (Kunth) Steud., Nomencl. Bot. (ed. 2) 1: 563. 1840. *Ochlopoa infirma* (Kunth) H. Scholz, Ber. Inst. Lanschafts – Pflanzenokologie Univ. Hohenheim Beih., 16: 59. 2003.

= *Poa annua* var. *exilis* Tomm. ex Freyn, Verh. K. K. Zool.-Bot. Ges. Wien. 27: 469. 1878. *Poa exilis* (Tomm.) Murb. ex Asch. & Graebn. Acta Univ. Lund. 4: 73. 1905. Type protologue: S Europe: Istria: Langs der Kust von Fasana bis Medolino, auch auf S. Marina, 1872, *Tommasini s.n.* Lectotype: Italy. S. Marina, 24 Mar. 1873, *Tommasini s.n.* (lectotype, designated by Soreng and Fulvio Tomsich Caruso in Sylvester et al. 2020: TSM!).

– *P.* sect. *Micrantherae* Stapf

Type. Colombia. Crescit in frigidis regni Novogranatensis, inter Fonibon, Suba et Santa Fe de Bogota, 1360 hexap. [2448 m], floret. Aug, *Humboldt & Bonpland s.n.* (lectotype, designated by Sylvester et al. 2020: P (P00669436!, herb. Humboldt & Bonpland Ameriqui Ecuatorial; isolectotypes: P (P00128983!), US (US1851276! fragm. ex P, US2851277! {134; Aug 1801; Colombia [ex P-Bonpl.]})).

Distribution. Introduced to the FSA region and found in Namibia and the Western Cape Province of South Africa. Native to the Mediterranean Sea region of Europe, North Africa and western Asia.

Ecology. ruderal.

Flowering. early spring.

Figure 4. *Poa infirma*. **A** whole plants **B–D** close-up of inflorescence showing spikelets generally in lateral view and miniscule anthers (a). Image **A, B** of Smook 3576 (PRE0652756-0) **C, D** of Smook 3672 (PRE).

Economics. infrequent, insignificant.

Vouchers. **Namibia**. Noordoewer: Motel flower beds, 14 Sep 1981, L. Smook 3576 (PRE). **South Africa**. Western Cape: Swellendam District, Sep 1962, L.C.C. Liebenberg 6495 (PRE); Western Cape: Porterville, Dasklip pans, wet gully up pass, 7 Oct 1981, L. Smook 3672 (PRE).

Notes. First report for South Africa and sub-Saharan Africa. *Poa infirma* is one of the diploid parents of the tetraploid species, *Poa annua*. The species is self-compatible, inbreeding. 2*n* = ***14***. − **M^{i}M^{i}** genotype.

Poa leptoclada Hochst. ex A.Rich., Tent. Fl. Abyss. 2: 422. 1851 [1850].

Type. Ethiopia. [Tigray:] Crescit in montibus prope Cojeta, provinciae Schire, [et in regno Choa (ant. Petit)], 16 Oct 1840, G.H.W. Schimper 1826 (first-step lectotype, designated by Clayton 1970: 47: TUB; second-step lectotype, **designated here**: TUB (TUB009107 [image!]); isolectotypes: BM (BM000922778 [image!], BM000922779 fragm. [image!]), BR (BR0000008255792 [image!]), G (G00022704 [image!]), K (K000345208 [image!]), P (P02610380 [image!]), S (S-G-6769 [image!]), TUB (TUB009108 [image!], TUB009109[image!]); syntypes: ETHIOPIA. Choa, A.

Petit s.n.(P (P02619542 [image!])); ERITREA. 12 Sep 1902, A. Pappi 1543 (MO (MO1660901 [image!]), PRE (PRE0676737-0 [image!]))).

8 heterotypic synonyms. – sect. unplaced

Distribution. for the FSA region, found in Lesotho and the Kwazulu Natal Province of South Africa. Native, endemic to and widespread mainly in the mountains of tropical eastern Africa and adjacent Arabian Peninsula.

Ecology. wet places in high Maloti-Drakensberg.

Flowering. around July.

Economics. rare, insignificant.

Vouchers. no new records.

Notes. *Poa leptoclada* exhibits a wide variation in floret pubescence. Callus hairs may be present or absent and lemma hairs, when present, occur on the keel only, the keel and marginal veins and sometimes between them. Infrequently, florets are entirely glabrous and callus and lemma hairs occur in different combinations of presence and absence. We did not have time to evaluate the case in Drakensberg plants. Presumably it is self-compatible and mostly self-fertilising. Clayton (1970: 47) incompletely lectotypified *P. leptoclada* on a Schimper 1826 TUB collection, although without mentioning which specimen or leaving annotations on any of the three duplicates at TUB. We second-step lectotypify to the TUB009107 collection as this is presumably the sheet Clayton (1970) considered as "holotype", as it is the only sheet which displayed Hochstetter's handwritten diagnosis and was photographed for K (K negative No. 10325, 23 Sep 1968). 2*n* = 28, *42*. − **Hh** genotype (Gillespie and Soreng, unpublished).

Poa nemoralis L., Sp. Pl. 1: 69. 1753.

Fig. 5

Type. Habitat in Europa ad radices montium umbrosas, (lectotype, designated by Soreng in Cafferty et al. 2000: 255: Scheuchzer. Agrostogr. Helv. Prodr. t. 2 (1708); epitype: SWEDEN. Uppland: Danmark Parish, Linnés Hammarby, 14 June 1933, N. Hylander s.n. (epitype, designated by R.J. Soreng and J.R. Edmonson in Cafferty et al. 2000: 255: BM!).

Many heterotypic synonyms. − P. sect. *Stenopoa* Dumort., Observ. Gramin. Belg. 110, 112. 1823 [1824]. Type, *P. nemoralis* L.

Distribution. Lesotho, Sehlabathebe National Park. Presumably introduced, native to Eurasia and northwest Africa.

Ecology. bases of basaltic cliffs and shady high-elevation slopes.

Flowering. late summer, early autumn.

Economics. rare, little potential in the region.

Vouchers. Lesotho. Sehlabathebe National Park, lower end of the park on the border, S29.877392 E29.088250, 2653 m alt., basaltic substrate, base of S facing escarpment, soil damp, growing with *Bromus catharticus*, *Myosotis* and *Melica*, 20 Feb 2020, S.P. Sylvester et al. 3555 (NU, PRE, US); Sehlabathebe National Park, lower end of the park on the border, on a small grassy pass between large rock escarpments, S29.875613

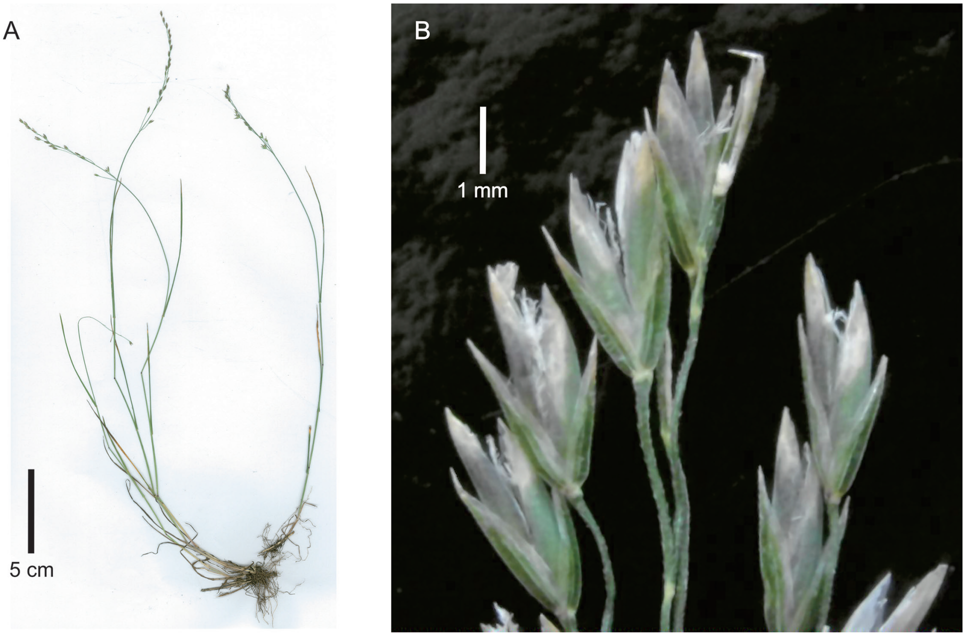

Figure 5. *Poa nemoralis*. **A** whole plant **B** close-up of inflorescence with spikelets in mostly lateral view. Image **A** of S.P. Sylvester et al. 3555 (US) **B** of S.P. Sylvester et al. 3555 (PRE).

E29.087374, 2750 m alt., basaltic substrate, crest of narrow defile between cliffs, grassy area with damp soil, 20 Feb 2020, S.P. Sylvester et al. 3561 (NU, PRE, US).

Notes. This is the first report of *P. nemoralis* for Lesotho and sub-Saharan Africa. Populations were found at two locations within 1 km of each other. Mostly hexaploid. Apomixis known. 2*n* = *28*, 33, 35, ***42***, 48, 50, 56, 70. – **Ss** genotype.

Poa pratensis L., Sp. Pl. 1: 67–68, 1753.

Type. Russia. Prov. Sanct-Petersburg: 5 km australi-occidentum, 26 June 1997, N.N. Tzvelev N-257 (conserved type, designated by Soreng and Barrie 1999: 157: BM!; isotypes: B!, C!, CAN!, CONC!, H!, K!, KW!, L!, LE!, LIV!, MA!, MO!, MW!, NSW!, P!, PE!, PR!, S!, SI!, TNS!, US (US3456252!), W!).

Many heterotypic synonyms. – *P.* sect. *Poa*. Type, *P. pratensis* L. (Type of genus *Poa* L.)

Distribution. widespread in Lesotho and South Africa. Introduced, mainly from European sources, native and widespread in Eurasia (also native in part in North America) and now around the world.

Ecology. cool temperate to subarctic, mesic habitats.

Flowering. late spring early summer, to late summer at high elevations.

Vouchers. Lesotho. West of Sehlabathebe National Park, on grassy slopes above the Leqooa-Legowa river, S29.859179 E29.055580, 2310 m alt., sandstone substrate, mesic soil on steep grassy W facing slopes, 21 Feb 2020, S.P. Sylvester et al. 3571 (US). **South Africa**. Eastern Cape: Naudes Nek pass, near Rhodes, in grassland next to radio tower, S30.765121 E28.092349, 2585 m alt., basaltic substrate, alpine tussock grassland transitioning to low shrubland dominated by *Erica* and *Helichrysum*, fairly heavily grazed by sheep and cattle, gently sloping, moderately deep soil, 13 Feb 2020, S.P. Sylvester et al. 3499 (PRE, US).

Notes. There are three major subspecies recognised in *Poa pratensis*: subsp. *angustifolia*, *irrigata* and *pratensis*. Intermediate specimens are common and difficult to place. Cope and Gray (2009) provide a good account of the distinctions, also see Soreng (2007). Facultatively apomictic. 2*n* = 21−147 (including nearly every number in between). − **Php** genotype.

Poa pratensis subsp. *irrigata* (Lindm.) H. Lindb. Sched. Pl. Finland. Exs. 2: 20. 1916.

Fig. 6

≡ *Poa irrigata* Lindm., Bot. Not. 1905: 88, f. 1. 1905, nom. illeg.

≡ *Poa irrigata* fo. *ehrhartii* Lindm., Bot. Not. 1905: 89, 1905. *Poa humilis* Ehrh. ex Hoffm., Deutschl. Fl. 1: 45. 1800.

≡ *Poa pratensis* var. *humilis* (Ehrh. ex Hoffm.) Spenn, Fl. Friburg. 1: 130. 1825.

= *Poa subcaerulea* Sm., Engl. Bot. 14: t. 1004. 1802. *Poa pratensis* subsp. *subcaerulea* (Sm.) Hiitonen, Suom. Kasvio 205, f. 5. 1933 (based on *Poa subcaerulea*). Type: UK. Anglesea, on the mountains of Westmoreland and Cumberland, [June 1801], *Rev. H. Davies s.n.* [EBot. 1004] [E: B: A. 1004] (lectotype, designated by R.J. Soreng and Mark A. Spencer in Sylvester et al. 2020: BM (BM001168037 [image!] ex Sowerby's Herbarium; isolectotypes: K (K000641177 [image!], LINN (LINN-HS127-53 [image!])).

Type. Sweden. Upsaliae, Ehrhart 115 (lectotype, designated by Sylvester et al. 2020: LINN (LINN-HS127-54 [image!]); isolectotypes: LE (LE00009654 [image!], LE00009655 [image!], LE00009656 [image!], LE00009657 [image!], LE00009658 [image!] plant B on sheet), LE-TRIN-2598.02 [not seen], O! [plant B on E. Fries, Hb. Norm. 9: 93a, from "Upsaliae"], UPS [not seen], W (W0029751 [image!])).

Economics. possibly frequent but rarely collected, often seeded for lawns and soil stabilisation.

Vouchers. South Africa. Eastern Cape: between Casrlisleshoekspruit Pass and Tiffindell Ski Area, S30.677202 E27.956643, 2526 m alt., basaltic substrate, Afromontane riparian wetland, 10 Feb 2020, S.P. Sylvester et al. 3437 (NU, PRE, US); Eastern Cape: Tiffindell Ski Area, S30.674511 E27.959358, 2521 m alt., basaltic substrate, heavily grazed livestock paddocks amongst alpine grassland, 12 Feb 2020, S.P. Sylvester et al. 3471 (NU, PRE, US).

Notes. Sometimes passing under other names: *Poa humilis* Ehrh. ex Hoffm., *P. subcaerulea* Sm. This subspecies is highly favoured for growing dense soft, dark green, durable, lawn turf. 2*n* = 54, *56*, *65*, *84*, *98*, *105*, *112*, *119*, *140*, 82–147.

Figure 6. *Poa pratensis* subsp. *irrigata*. **A** whole plant **B** junction of sheath and blade of tiller leaf showing collar with ciliolate margin **C** part of inflorescence with spikelets in lateral view. Images of S.P. Sylvester et al. 3437 (US).

Poa pratensis subsp. *pratensis*

Fig. 7

Economics. frequent, often seeded for lawns, pastures and soil stabilisation, mainly as the "field form" in subsp. *angustifolia*.

Voucher. South Africa. Eastern Cape: Lundeans Nek, top of pass, S30.647517 E27.741630, 2170 m alt., basaltic substrate, Afro-montane grading into Afro-alpine vegetation dominated by short shrubs, 9 Feb 2020, R.J. Soreng et al. ZA-33 (NU, PRE, US).

Notes. Subspecies *pratensis* is often confused with subspecies *angustifolia*, which has denser fascicles of shoots of intravaginal origin and firmer vegetative leaf blades that are involute, with veins distinctly expressed abaxially, in those fascicles. $2n$ = 42, 43, 44, 48, 49, 50, 51, 52, 53, 54, 55, *56*, 58, 59, 62, 65, 66, 67, 88, 89, 91, 95 (counts may represent other subspecies, particularly subsp. *irrigata*).

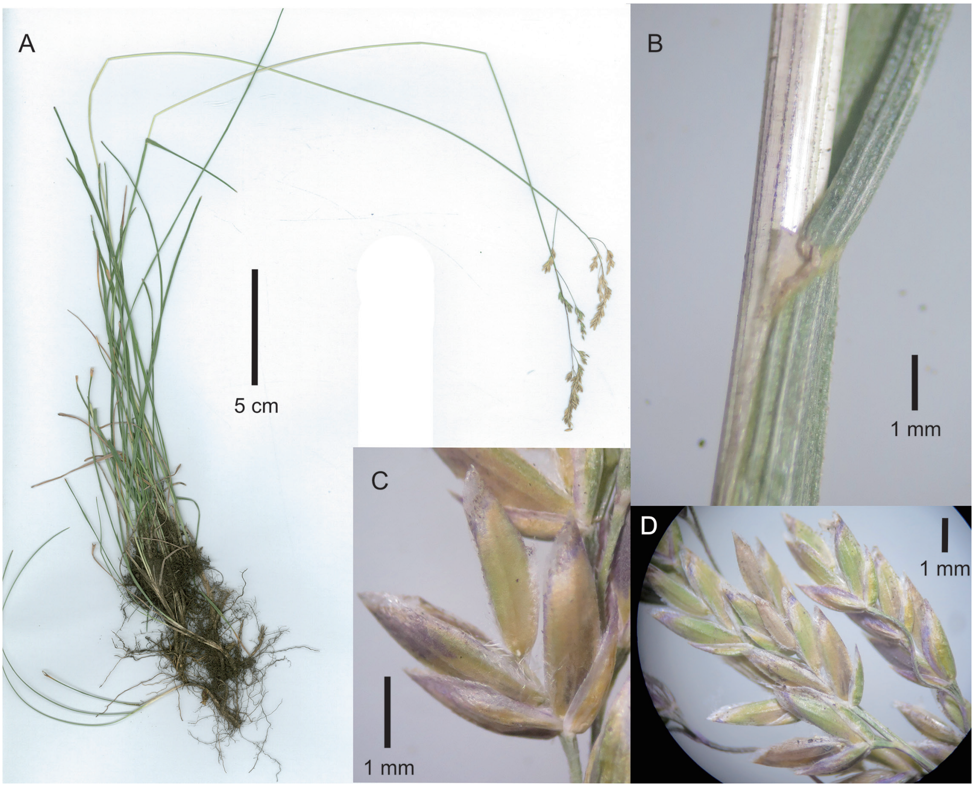

Figure 7. *Poa pratensis* subsp. *pratensis*. **A** whole plant **B** junction of sheath and blade of tiller leaf showing glabrous collar **C** spikelet, lateral view **D** part of inflorescence with spikelets mostly in lateral view. Images of R.J. Soreng et al. ZA-33 (US).

Poa trivialis **L., Sp. Pl. 1: 67. 1753. subsp.** ***trivialis***

Fig. 8

Type. Habitat in Europae pascuis, no date, Hudson 16 (neotype, designated by Soreng in Cafferty et al. [2000: 256]: LINN (LINN-87.9!)).

Many heterotypic synonyms. – P. sect. *Pandemos* Asch. & Graebn., Syn. Mitteleur. Fl. 2: 425. 1900. Type, *P. trivialis* L.

Distribution. South Africa, Gautan Province. Introduced, native to western Eurasia and North Africa, introduced to sub-Saharan Africa in Zimbabwe and South Africa.

Ecology. ruderal of temperate climates.

Flowering. spring.

Economics. rarely collected. Sometimes seeded for pastures, invasive.

Vouchers. **South Africa.** Gauteng: Johannesburg, Rosebank 50 Bath Ave., 28 Dec 1962, Meredith s.n. (PRE0021311-0); Gauteng: Johannesburg, Hort. Rosebank, Mar 1965, Meredith s.n. (PRE0029743-0).

Figure 8. *Poa trivialis*. **A** whole plant **B** part of inflorescence with spikelets mostly in lateral view. Images of Meredith s.n. (PRE0021311-0).

Notes. *Poa trivialis* subsp. *trivialis* is reputedly self-incompatible and sexually reproducing (Connor 1979). It can be quite invasive in temperate climates with a cool wet season. Aesthetically, it makes a poor lawn grass due to its sprawling habit when mown. Valdés and Scholz (2009) recorded it only for Algeria in North Africa. The second major subspecies, *P. t.* subsp. *sylvicola* (Guss.) H. Lindb., has bead-like swellings along the stolons and more hair on the lemma marginal veins and is more tolerant of drought: It is infrequently found outside of the Mediterranean basin and Irano-Turainian floristic region, but is reported across northern Africa (Valdés and Scholz 2009). 2*n* = ***14***, 14 + 1 − 2B, 15, 27, 28 (27 and 28 counts not confirmed to subspecies may represent subsp. *sylvicola*). − **Vv** genotype.

Acknowledgements

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RESEARCH ARTICLE

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Two new species of *Codonoboea* (Gesneriaceae) from Kenaboi State Park, Peninsular Malaysia

Suhaimi Syahida-Emiza[1], Yen Yen Sam[1], Mat Yunoh Siti-Munirah[1]

1 *Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia*

Corresponding author: Syahida-Emiza Suhaimi (syahida@frim.gov.my)

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Abstract

Two new *Codonoboea* species, *C. kenaboiensis* Syahida-Emiza, Y.Y.Sam & Siti-Munirah and *C. ruthiae* Syahida-Emiza, Y.Y. Sam & Siti-Munirah were discovered from the Kenaboi State Park, Peninsular Malaysia. Descriptions, illustrations, colour plates and provisional conservation status are provided.

Keywords

Codonoboea kenaboiensis, *Codonoboea ruthiae*, conservation status, endemic, Negeri Sembilan

Introduction

Kenaboi State Park is the first and only state park in Negeri Sembilan, Peninsular Malaysia. The park is located within the greater Kenaboi Forest Reserve which lies at the south end of the Titiwangsa Range, Peninsular Malaysia's granite mountain range. With 9,036 ha of tropical evergreen rain forest, the park includes pristine lowland dipterocarp and bamboo forests at low elevation with seraya ridges and hill dipterocarp forests at the higher elevation (Latiff and Faridah-Hanum 2014). The highest peak in Negeri Sembilan, Gunung Besar Hantu, at 1,462 m and the tallest waterfall in Negeri Sembilan, known as Lata Kijang falls 115 m, are both located within the park. Three main rivers, namely Sungai Kenaboi, Sungai Semong, and Sungai Kering and their tributaries flow through the reserve (Ramli et al. 2009) supplying clean water to the states of Negeri Sembilan and Selangor. Before the park was established, part of the forest was classified as water catchment, recreational, educational and wildlife forest in accordance with the National Forestry Act 1984.

In 2010, a scientific expedition to Gunung Besar Hantu was carried out to document the biodiversity at high elevation about 1,400 m. Seven years later in 2017, another expedition called the Lembah Jemaloi Scientific Expedition covered the remaining areas at lower elevations, especially the valley at Lembah Jemaloi. During the expeditions, two unknown species of *Codonoboea* were discovered in the lowland forest below 400 m elevation. After careful examination, the collections were shown to have a unique combination of characters that do not match any existing described species and hence, both are described here as new.

Codonoboea kenaboiensis Syahida-Emiza, Y.Y.Sam & Siti-Munirah is a small rheophyte discovered on the rocky riverbank of Sungai Kenaboi. This species is unique in its narrowly elliptic leaves with serrate margins. The plants are usually overshadowed by other larger species and are inconspicuous unless their purplish tubular flowers are blooming. On the other hand, *Codonoboea ruthiae* Syahida-Emiza, Y.Y.Sam & Siti-Munirah was found on the forest floor near one of the tributaries of Sungai Kenaboi. Its distant pairs of unequal leaves and attractive maroon flowers distinguish it from other ground flora.

Peninsular Malaysia is the centre of diversity for the genus *Codonoboea*, the most speciose genus in the Gesneriaceae family of Peninsular Malaysia, with at least 95 species recorded so far (Kiew and Lim 2011, 2019; Lim and Kiew 2014). New taxa continue to be discovered and named as botanical collecting ventures into unexplored localities (Kiew and Lim 2019). *Codonoboea* is commonly found in primary forest from lowlands up to mountains growing on granite, sandstone and quartz-derived soils or rocks (Kiew and Lim 2011). Its distribution ranges from Peninsular Thailand, Sumatra, Singapore, Batam and Lingga Islands, Borneo, Palawan (the Philippines), Sulawesi and New Guinea (Lim and Kiew 2014).

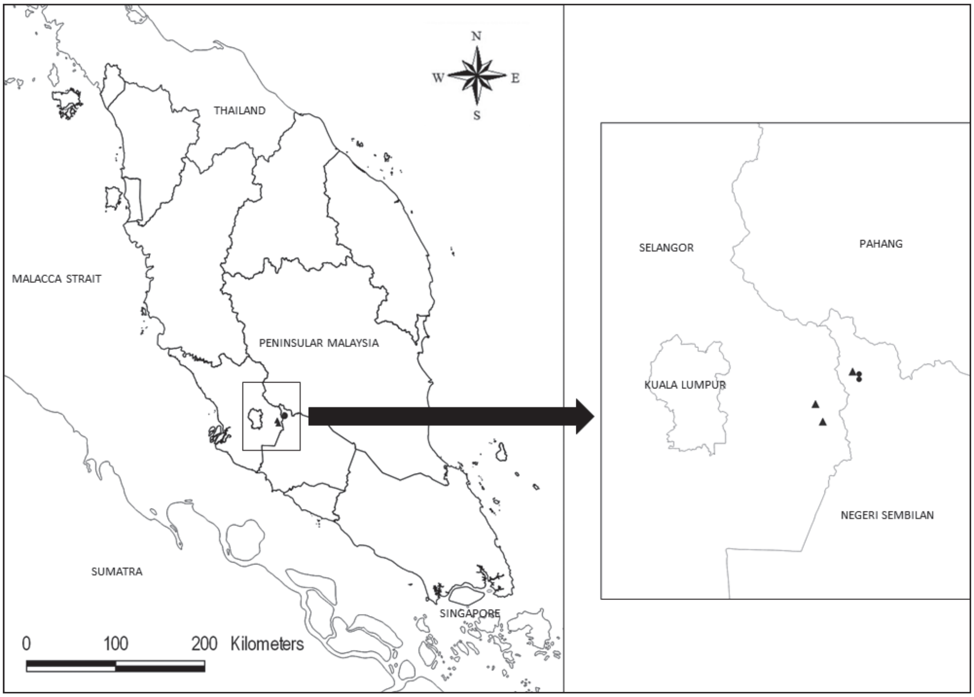
Taxonomy

Codonoboea kenaboiensis Syahida-Emiza, Y.Y.Sam & Siti-Munirah, sp. nov.

urn:lsid:ipni.org:names:77212567-1
Figs 1, 2, Map 1

Diagnosis. Similar to *Codonoboea rheophytica* Kiew in its rheophytic habit, distinct narrow leaves, serrate leaf margin and numerous lateral veins but it differs in its cymose inflorescence with 2–3 flowers (*C. rheophytica* has one-flowered inflorescences), small tubular flowers (1–1.3 cm vs. 3–3.5 cm long) and flower colour, violet not white as in *C. rheophytica*.

Type. Peninsular Malaysia. Negeri Sembilan: Jelebu, Kenaboi Forest Reserve, 3°10'N, 101°59'E, 11 July 2019, Syahida Emiza, S., Sam, Y.Y. & Angan, A. FRI 87630 (holotype: KEP; isotype: K, SING).



Map 1. Distribution of *Codonoboea kenaboiensis* (circle) and *Codonoboea ruthiae* (triangle) in Peninsular Malaysia.

Description. Rheophyte, 10–20 cm tall. ***Stems*** woody, erect, wiry, unbranched. ***Leaves*** decussate, pairs spaced up to 0.5 cm apart, denser to the apex; petioles slender, 0.5–1 cm long, *c.* 0.1 cm diameter; laminas narrowly elliptic, 4.5–10 × 0.8–1 cm, coriaceous, mid-green above, pale green beneath, base attenuate, apex acute to attenuate, margin serrate; midrib and lateral veins sunken above, glabrous, prominent beneath, puberulous, lateral veins up to 30 pairs, generally opposite or alternately arranged, intercostal veins reticulate, slightly prominent. ***Inflorescences*** at upper leaf axils, erect, 1 per axil, cymes, 2–3-flowered; indumentum of floral parts a combination of glandular and simple hairs; peduncle 4–8 cm, purplish-maroon, densely hairy; bracts 3–3.5 × *c.* 1 mm, densely hairy, lanceolate, apex blunt. ***Flowers*:** pedicels 2–3 mm long, purplish, densely hairy; calyx mid-green, densely hairy, 5-lobed, narrowly lanceolate, lobes 1.2–1.5 × 0.2–0.8 mm, apex blunt; corolla tubular, 10–13 mm long, base 2 mm wide, dilating to 3 mm at the mouth, outside whitish to violet, sparsely hairy, inside whitish, glabrous, veins conspicuous; corolla lobes deeper violet, unequal in size, oblong, *c.* 3 × 2 mm, apex blunt; stamens 2, 3–3.5 mm long, filaments erect, *c.* 2.5 mm long, glabrous, anthers versatile, *c.* 0.8 mm long, *c.* 1 mm wide, white; nectary annular, *c.* 0.6 mm tall, rim toothed, glabrous; pistil yellowish-cream, *c.* 8 mm long, ovary *c.* 3.5 × 0.7 mm, sparsely hairy, style *c.* 4.5 mm long, white, sparsely hairy, stigma peltate, *c.* 0.7 mm across, papillose. ***Capsules*** cylindrical, 18–20 mm long, *c.* 1 mm wide at base, green, splitting adaxially when mature, calyx persistent. ***Seeds*** elliptic-oblong, *c.* 0.2 × 0.15 mm.

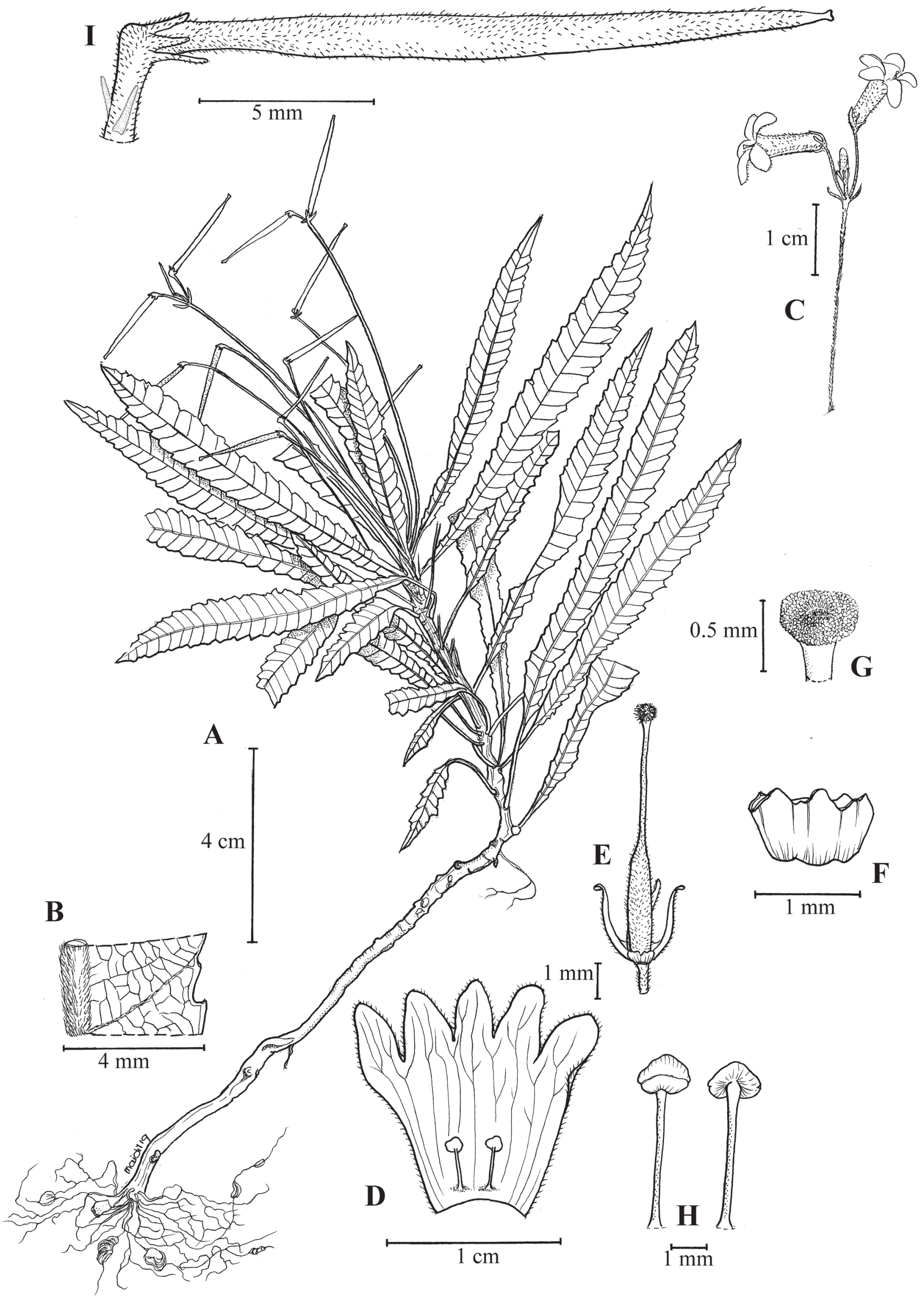


Figure 1. Illustration of *Codonoboea kenaboiensis* Syahida-Emiza, Y.Y.Sam & Siti-Munirah, sp. nov. **A** habit **B** leaf section show midrib and reticulate vein **C** inflorescence **D** flower opened to show position of stamens **E** calyx with pistil **F** nectary annular **G** stigma **H** stamens **I** fruit (All from *FRI 87630*, drawn by Mohamad Aidil Noordin).

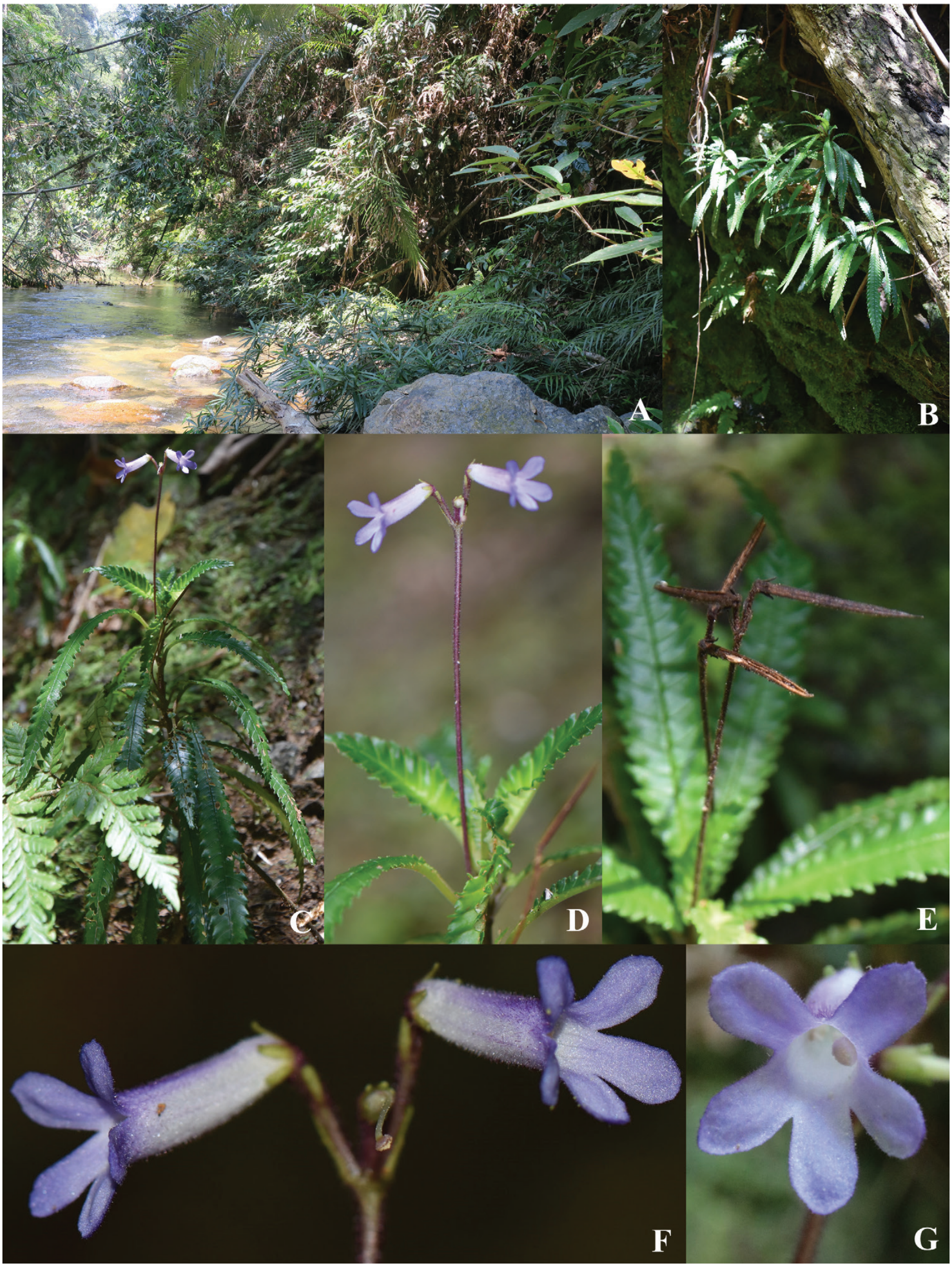


Figure 2. *Codonoboea kenaboiensis* Syahida-Emiza, Y.Y.Sam & Siti-Munirah, sp. nov. **A, B** habitat **C, D** habit **E** fruits **F** flowers **G** corolla lobes (front view).

Other specimen examined. Peninsular Malaysia. Negeri Sembilan: Jelebu, Kenaboi Forest Reserve, 6 May 2010, Mohd. Hairul, M.A. et al. FRI 70988 (KEP).

Distribution. Endemic in Negeri Sembilan, Peninsular Malaysia. Currently known only from the type locality.

Ecology. Lowland dipterocarp forest, on rocks or steep earthy river banks, at 275–315 m altitude. Flowering from May to July. *Codonoboea kenaboiensis* grows on the lower levels of the riparian zone to about 1.5 m above the normal water level. Conditions are cool and damp with a thick layer of mosses covering the ground and shrubs and trees on higher ground leaning towards the river providing partial shade to the vegetation underneath. Such conditions are ideal for many plants but they are subjected to regular flooding events such as flash floods and annual floods during monsoon seasons. Only plants with rheophytic adaptations like *C. kenaboiensis* can survive the swift moving flood water. It has narrow leaves and roots that secure it firmly to the ground, preventing it from being uprooted by water currents. However, more frequent and intense flooding as a result of climate change might affect its long-term survival at Sungai Kenaboi.

Etymology. The epithet refers to the Kenaboi State Park, its only known locality.

Conservation status. Vulnerable, VU D2. *Codonoboea kenaboiensis* is hyper-endemic with a very small and restricted population at Sungai Kenaboi, Kenaboi State Park. Its small population and position in the flood zone is threatened by the increasing records of flood and extreme weather events caused by climate change. Tang (2019) has projected more rainfall events of high intensity and tropical storms in Malaysia as climate change intensifies. Under such circumstances, the seeds and seedlings of *C. kenaboiensis* will be washed away before they can firmly establish to the substrate thereby affecting its regeneration. Following the IUCN Standards and Petitions Committee (2019), *C. kenaboiensis* qualifies for VU D2 because the increased frequency of floods is expected to cause a population reduction and, due to its small population, the species could become Critically Endangered or Extinct in a very short period of time.

Discussion. *Codonoboea kenaboiensis* belongs to *Codonoboea* sect. *Pectinati*, characterised by narrow and serrate to deeply toothed leaves and small tubular flower (Ridley 1923; Lim and Kiew 2014). It closely resembles *C. rheophytica*, a recently described rheophyte from Terengganu (Kiew and Lim 2019). Both have distinctly narrow leaves (0.8–1 cm in *C. kenaboiensis* and 0.8–1.3 cm in *C. rheophytica*), serrate leaf margins and numerous lateral veins (30 pairs in *C. kenaboiensis* and 33–36 pairs in *C. rheophytica*). However, they are distinguished by the inflorescence and floral structures. *Codonoboea kenaboiensis* has simple cymes bearing 2–3 tubular flowers whereas *C. rheophytica* has solitary trumpet-shaped flowers. In addition, the violet flowers of *C. kenaboiensis* are much smaller (*c.* 1–1.3 cm vs 3–3.5 cm in *C. rheophytica*) than the white flowers of *C. rheophytica*.

Codonoboea species are common on stream and river banks but very few are true rheophytes. Other than *C. rheophytica* and *C. kenaboiensis*, *C. densifolia* (Ridl.) C.L.Lim and *C. salicina* (Ridl.) C.L.Lim are two other rheophytes found in Peninsular Malaysia (Kiew 1987). The narrow leaves of *C. densifolia* and *C. salicina* have entire to serrulate margins and fewer lateral veins (up to 20 pairs) that clearly distinguish them from *C. kenaboiensis*. In terms of inflorescence structure, both *C. densifolia* and *C. salicina* have cymose inflorescences similar to *C. kenaboiensis* but their campanulate flowers are distinct from the tubular flowers of *C. kenaboiensis*.

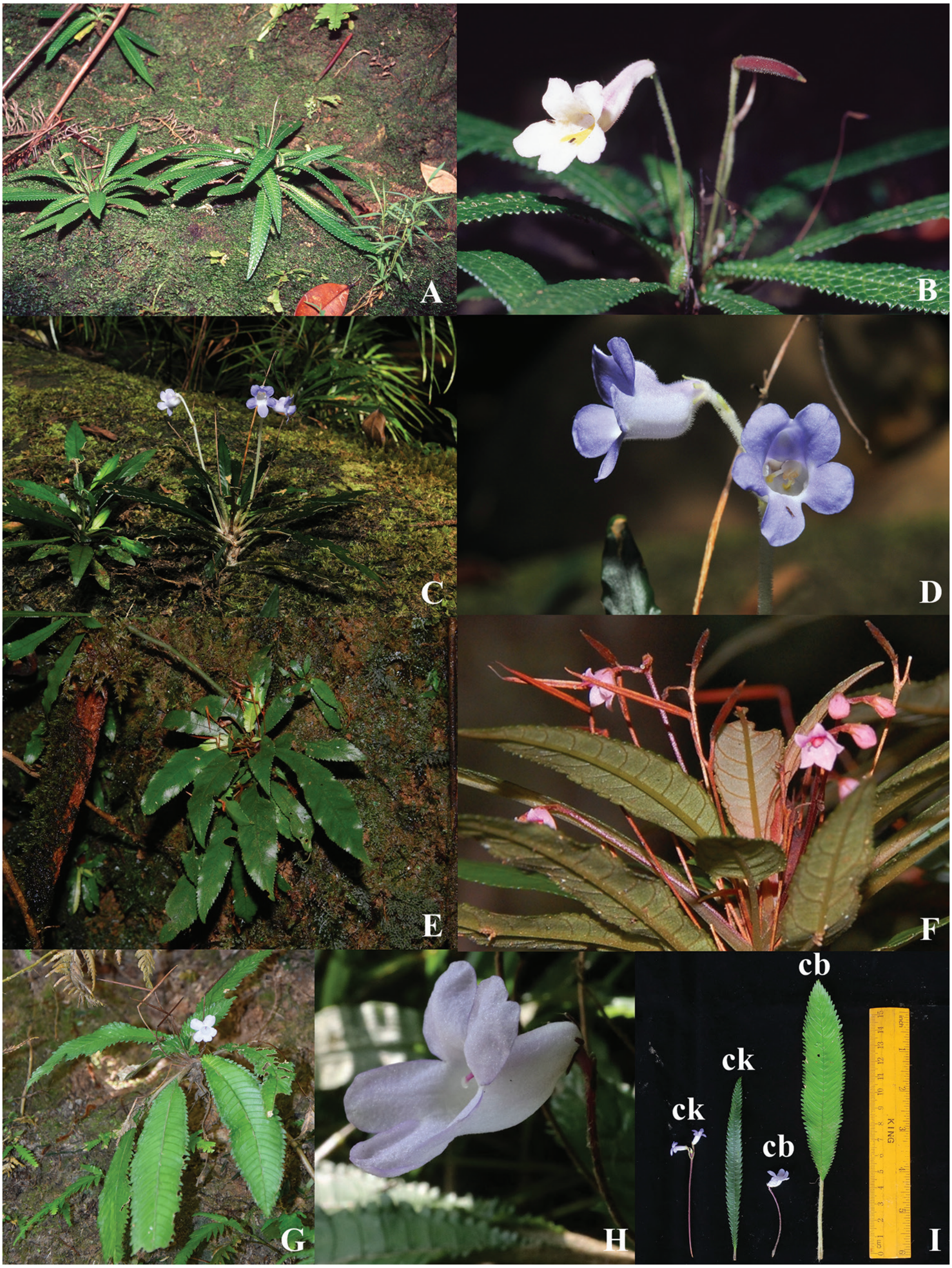


Figure 3. A, B *Codonoboea rheophytica* **C, D** *Codonoboea densifolia* **E, F** *Codonoboea salicina* **G, H** *Codonoboea breviflora* **I** ck = *Codonoboea kenaboiensis*, cb = *Codonoboea breviflora*.

At Sungai Kenaboi, there is another *Codonoboea* species growing on the steep riverbanks. *Codonoboea breviflora* can easily be mistaken as a larger form of *C. kenaboiensis* that also has long narrow leaves and serrate leaf margin. However, the leaves of *C. kenaboiensis* are conspicuously smaller with shorter petioles (0.5–1 cm vs 1–2.5 cm long) and smaller laminas (4.5–10 × 0.8–1 cm vs 9–20 × 2.5–5.5 cm) compared to *C. breviflora* (Fig. 3). Upon closer examination, the leaf venation of *C. kenaboiensis* also proves to be distinct from *C. breviflora*. *Codonoboea kenaboiensis* has craspedodromus lateral veins (the veins run directly from midrib to the margin) whereas in *C. breviflora* the veins branch before reaching the margin. In addition, the single campanulate flower of *C. breviflora* instantly differentiates it from *C. kenaboiensis*.

Both *C. kenaboiensis* and *C. breviflora* grow at Sungai Kenaboi, but based on observations they occupy different sections of the flood zone. *Codonoboea kenaboiensis* resides on the lower levels of the river banks that are less than 1.5 m above the normal water level whereas *C. breviflora* is found at slightly higher levels more than 2 m above the water level. The higher ground is less affected by floods and supports a denser vegetation compare to the site of *C. kenaboiensis*.

Codonoboea ruthiae **Syahida-Emiza, Y.Y.Sam & Siti-Munirah, sp. nov.**

urn:lsid:ipni.org:names:77212568-1
Figs 4, 5, Map 1

Diagnosis. Amongst species in *Codonoboea* sect. Didymanthus, *Codonoboea ruthiae* is most similar to *C. ramosa* (Ridl.) Kiew, but can be distinguished by having a larger lamina (9.5–15.5 × 3.5–6.2 cm vs 5.1–7.6 × *c*. 3.8 cm) with more lateral veins (10–12 vs *c*. 7 pairs); the inflorescences in *C. ruthiae* consists of 5–6 flowers (only 2 in *C. ramosa*) with larger flowers (corolla tube 1.7–1.9 cm long vs c. 1.3 cm long) and flower colour (maroon vs greenish yellow)

Type. Peninsular Malaysia. Negeri Sembilan: Jelebu, Kenaboi Forest Reserve, 3°10'48.6"N, 101°58'19.9"E, 30 October 2017, Syahida Emiza, S., Sam, Y.Y., Yap, J.W., Angan, A. & Markandan, M. FRI 86960 (holotype: KEP)

Description. Erect, perennial herb, 20–50 cm tall. ***Stems*** slender-branched, 3–4 mm diameter, semi-woody at base. ***Leaves*** decussate, pairs spaced up to 4.5 cm apart, very unequal; petioles slender, grooved above, pale green, puberulous to glabrescent; smaller leaves less than half the size of larger leaves, petioles 0.5–1.5 cm, *c*. 0.1 cm diameter, laminas 5.5–7.5 × 1.5–3 cm; larger leaf with petioles 1.0–3.2 cm long, *c*. 0.1 cm diameter, laminas lanceolate to ovate, 9.5–15.5 × 3.5–6.2 cm, asymmetric, thinly coriaceous, mid-green above, glabrous, pale green beneath, sparsely puberulous to glabrescent, base oblique, apex acute, margin serrulate, sparsely puberulous; midrib and lateral veins raised on both surfaces, sparsely puberulous to glabrescent, lateral veins 10–12 pairs, alternately arranged, intercostal veins reticulate, slightly prominent. ***Inflorescences*** at upper most leaf axils, erect, pair-flowered cyme twice branched with 5–6 flowers; indumentum of floral parts a combination of glandular and simple hairs, simple hairs 0.75–1 mm long; peduncle 4–5 cm, reddish green, densely hairy; bracts and bracteoles lanceolate, bracts *c*. 3.2 × 0.7 mm, bracteoles *c*. 2.5 × 0.5 mm. ***Flowers*****:** pedicels 2–10 mm long, greenish maroon, densely hairy; calyx mid-green, densely hairy, 5-lobed, calyx lobes three quarters of the calyx length, lobes narrowly acute, 2.1–2.5 × 0.4–0.5 mm, apex slightly

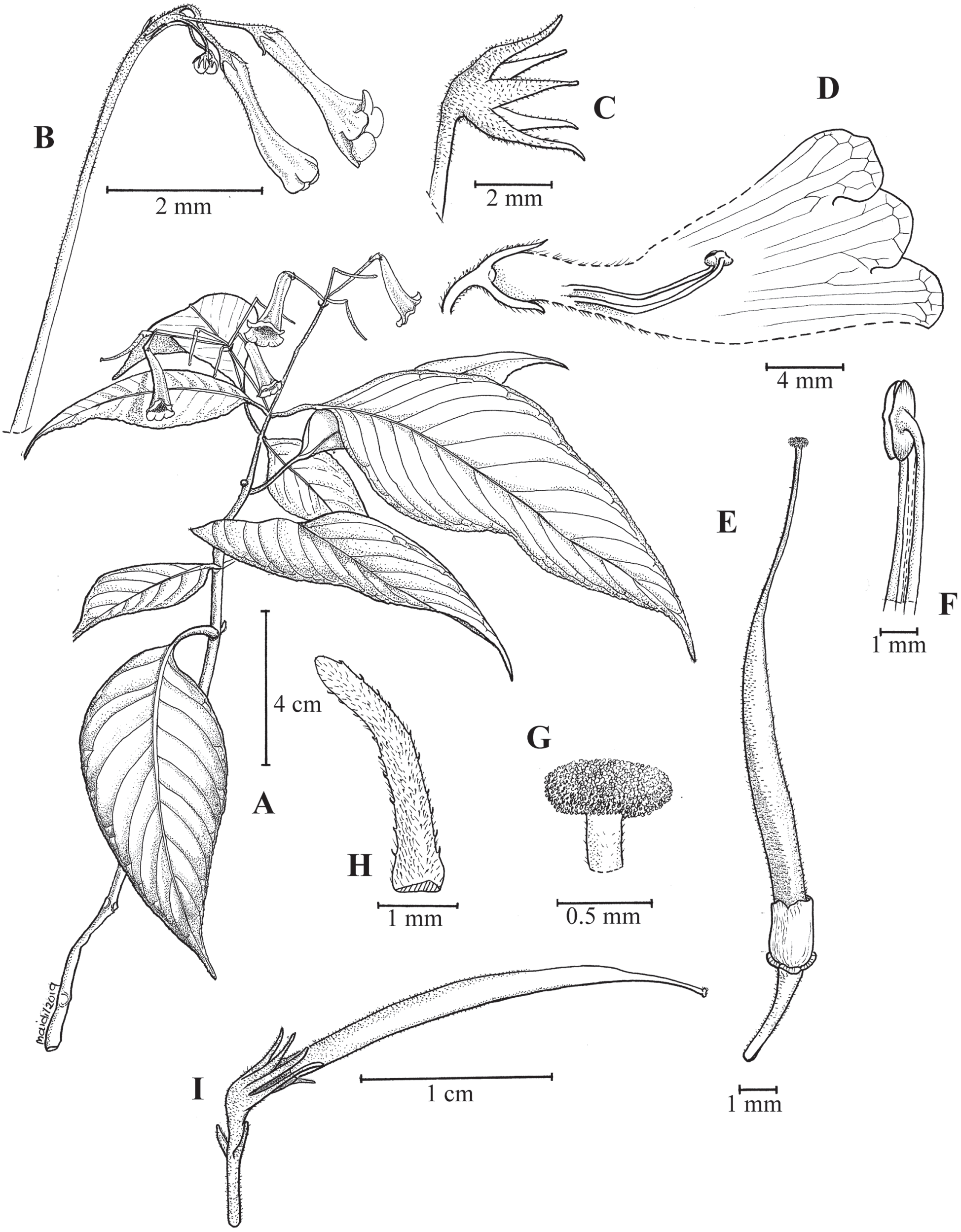


Figure 4. Illustration of *Codonoboea ruthiae* Syahida-Emiza, Y.Y.Sam & Siti-Munirah, sp. nov. **A** habit **B** inflorescence **C** calyx **D** flower opened to show position of stamens **E** pistil **F** anthers **G** stigma **H** bract **I** fruit (All from *FRI 86960*, drawn by Mohamad Aidil Noordin).

blunt; corolla trumpet-shaped, 20–24 mm long, base narrow, 1–1.5 mm wide, dilating to 7–8 mm wide at mouth, tube 17–19 mm long, veins conspicuous, outside pale maroon, sparsely hairy and hirsute, inside with deep maroon streaks, throat with two yellow nectar guides, veins conspicuous, pubescent from base up to stamens attach, upper lobes

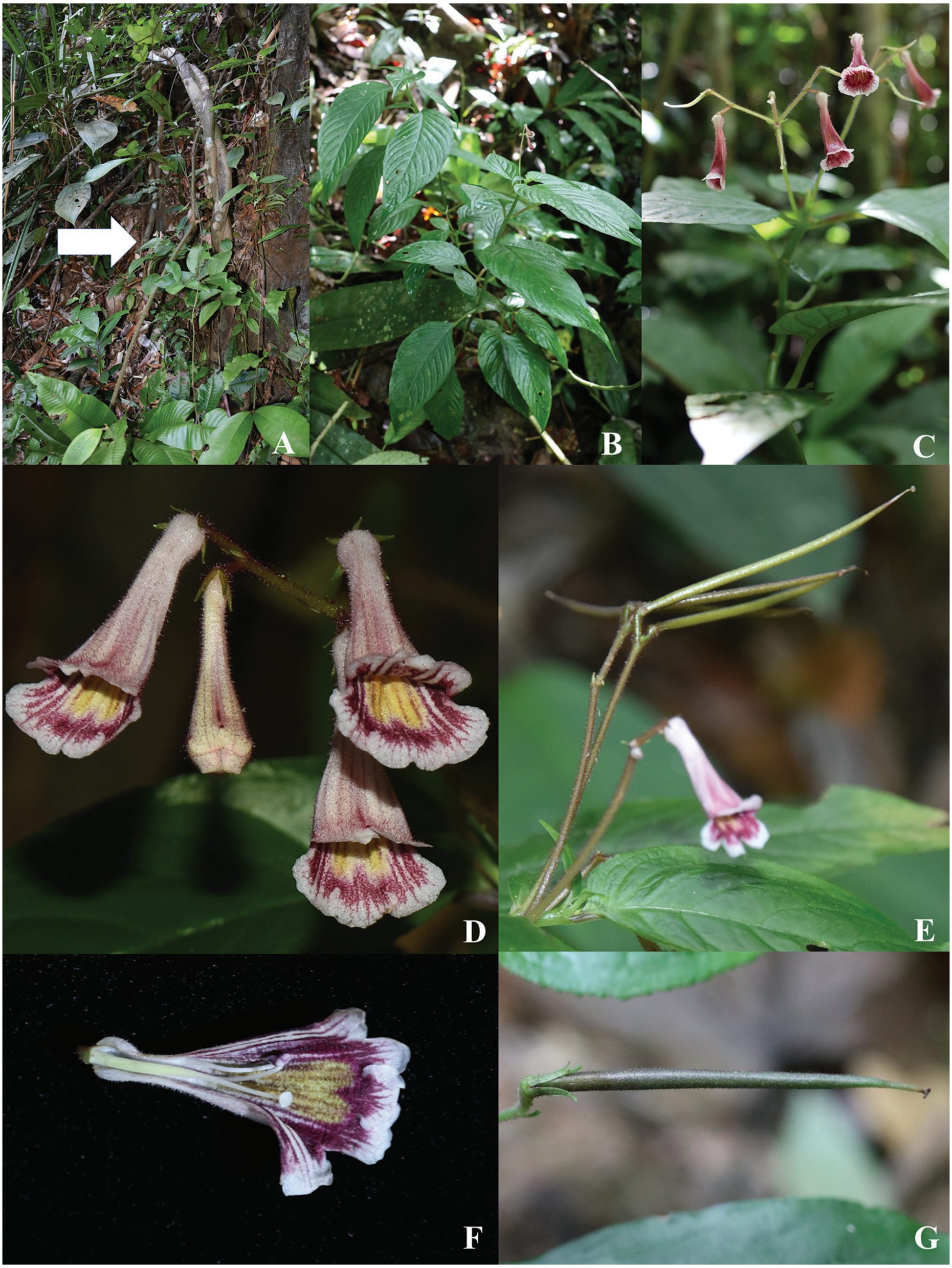


Figure 5. *Codonoboea ruthiae* Syahida-Emiza, Y.Y. Sam & Siti-Munirah. **A** habitat **B** habit **C** inflorescence with flower and pistil (corolla fallen) **D** flowers from front view **E** flower and fruits **F** longitudinal section of flower **G** capsule.

nearly rounded, *c.* 4 × 5 mm, slightly reflexed, side lobes rounded *c.* 5 × 5 mm, lower lobe near transverse elliptic, *c.* 5 × 7 mm; stamens 2; filaments slender, *c.* 5.3 mm long, glabrous, slightly curved upward, attached to corolla tube at *c.* 7 mm from base, anthers

Table 1. Differences between *Codonoboea ruthiae* and *C. ramosa*.

Character	*Codonoboea ruthiae*	*Codonoboea ramosa*
Lamina size of larger leaf (cm)	9.5–15.5 × 3.5–6.2	5.1–7.6 × *c.* 3.8
Lateral veins (pairs)	10–12	*c.* 7
Peduncle (cm)	4–5	2.5–3.2
Number of flowers	5–6	2
Flower colour	Maroon	Greenish yellow
Corolla tube (cm)	1.7–1.9	*c.* 1.3

reniform, small, *c.* 2 mm long, 0.6 mm wide, white, dorsifixed, fused face-to-face; nectary annular, *c.* 1.3 mm tall, rim undulate, glabrous; pistil whitish-cream, *c.* 13 mm long, ovary 7–8 × *c.* 0.6 mm, glabrous; style 5–6 mm long, white, sparsely hirsute; stigma peltate, *c.* 0.8 mm across, papillose. ***Capsules*** cylindrical, very slender, 25–40 mm long, 1–1.5 mm wide, maroon-green, densely hairy, splitting adaxially when mature; calyx persistent. ***Seeds*** elliptic-oblong, *c.* 0.3 mm × 0.25 mm.

Other specimens examined. Peninsular Malaysia. Selangor: Hulu Langat, Sungai Lalang Forest Reserve, Compartment 42, 29 June 2004, Sam Y.Y. et al. FRI 47248 (KEP), Compartment 43, 13 February 2019, Sam Y.Y. et al. FRI 69261 (KEP, K, L, SAN, SAR, SING).

Distribution. Peninsular Malaysia, recorded in Negeri Sembilan and Selangor.

Ecology. In lowland dipterocarp forest, on forest floor, hill side or slope near shaded small stream at 130–335 m altitude. Flowering in February, June and October.

Etymology. Named after Dr. Ruth Kiew, a prominent botanist, plant taxonomist and conservationist. She is well known for her work on herbaceous plants such as begonias and gesneriads, and also limestone and montane flora.

Conservation status. Least Concern (LC). *Codonoboea ruthiae* is well protected within the Protected Area Network. Its existence in Kenaboi State Park and also at the water catchment forest in Sungai Lalang Forest Reserve, Selangor, which is categorised as a Protection Forest, is legally secured under the National Forestry Act 1984 (Ministry of Water, Land and Natural Resources 2019). Furthermore, no threat to the species population has been identified.

Discussion. *Codonoboea ruthiae* belongs to *Codonoboea* sect. *Didymanthus* (Lim and Kiew 2014) characterised by opposite and well-spaced petiolate leaves. It closely resembles *C. ramosa* in its slender-branched stem, distant pairs of leaves, one of each pair much smaller, oblique lamina base and trumpet-shaped flowers (Ridley 1923). However, *C. ruthiae* can be distinguished from *C. ramosa* by a combination of characters shown in Table 1.

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RESEARCH ARTICLE

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A reappraisal of *Adinobotrys* Dunn (Fabaceae) with two new combinations

James A. Compton[1], Brian D. Schrire[2]

1 *Spilsbury Farm, Tisbury, SP3 6RU, UK* **2** *Comparative Plant and Fungal Biology Department, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK*

Corresponding author: James A. Compton (jamiecompton@madasafish.com)

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Abstract

Two new species from Borneo that have been described in the genus *Callerya* are shown here to belong in *Adinobotrys*. The new combinations *A. katinganensis* and *A. sarawakensis* have consequently been made, bringing the total number of species in the genus to four. A morphological key and taxonomic conspectus is provided for all species.

Keywords

Adinobotrys, *Callerya*, Leguminosae, morphological key, Tribe Wisterieae

Introduction

Schot (1994: 15) included *Pongamia atropurpurea* Wall., in her revision of the genus *Callerya* Endl. (Endlicher 1843) as *Callerya atropurpurea* (Wall.) Schot., the species having already been segregated from *Millettia* (Wight & Walker-Arnott 1834) and *Padbruggea* Miq. (Miquel 1855) into the genus *Adinobotrys* Dunn, on the basis of apparently having a stipitate ovary and one-seeded pods (Dunn 1911: 194). Schot regarded *Adinobotrys* to be synonymous with *Padbruggea* Miq. The distinction between *Adinobotrys* and the genera *Padbruggea* and *Whitfordiodendron* Elmer, 1910 was fully discussed in Compton et al. (2019: 35). *Adinobotrys* was typified on the species *A. atropurpureus* (Wall.) Dunn by Geesink (1984: 83) based on *Pongamia atropurpurea* Wall. (Wallich 1830).

Research based on morphology, NrDNA and CpDNA sequence data has led to the redefinition of the *Callerya* Group of genera within the Inverted Repeat-lacking clade (IRLC) of legumes as an enlarged Tribe Wisterieae Zhu (Zhu 1994) comprising 36 species within 13 genera (Compton et al. 2019). The same research revealed that the genus *Adinobotrys* (Dunn 1911) was sister to *Glycyrrhiza* L., (Linnaeus 1753) and Tribe Wistereae and that another tree species *Callerya vasta* (Kosterm.) Schot, also belonged in *Adinobotrys* (Compton et al. 2019: 20).

Adinobotrys as circumscribed by Compton et al. (2019) comprised two species; *A. atropurpureus* (Wall.) Dunn and *A. vastus* (Kosterm.) J.Compton & Schrire. Adema (2019) has subsequently described two new tree species in *Callerya sens.lat.* (Schot 1994) from Borneo, which are placed here in *Adinobotrys*, bringing the total to four species.

Several morphological characters separate *Adinobotrys* from *Callerya sens.str.* (Compton et al. 2019: 35): *Adinobotrys* comprises species of evergreen trees (vs. lianes in all genera of Tribe Wisterieae); its bracteoles are persistent, placed on the calyx (vs. mostly caducous and placed on the pedicel in *Callerya sens.str.*); its standards are glabrous (vs. sericeous in *Callerya sens.str.*) and its wing petals are ± equal to the keel in length (vs. much shorter than the keel in *Callerya sens.str.*).

Although the position of *Adinobotrys* with respect to its sister genus *Glycyrrhiza* and the other genera in tribe Wisterieae is not yet fully resolved (see discussion in Compton et al. 2019), it is nevertheless fully supported in being excluded from Tribe Wisterieae and is morphologically distinct from *Callerya sens.str.* as noted above. Accordingly we make the two new combinations here in *Adinobotrys* with a new key emended from Adema (2019).

Taxonomic conspectus

Adinobotrys Dunn, Bull. Misc. Inform. 1911: 194 (1911)

≡ *Millettia* Sect. *Nothomillettia* Miq., Fl. Ned. Ind., Eerste Bijv. 2: 301 (1861).
≡ *Millettia* subgen. *Nothomillettia* (Miq.) Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 45(2): 273 (1876).

Key to species of *Adinobotrys*

1 Leaves with 5–9 leaflets, lateral leaflets ± equal-sided at base; flowers 12–17 mm long; pod not inflated, flattened, narrowly elliptic to narrowly obovate; seeds 2–4, flattened lenticular, 3–9 mm thick (unknown for *A. vastus*) **2**
– Leaves with 7–11 leaflets; lateral leaflets oblique at base; flowers 17–20 mm long; pod inflated, elliptic to obovate, 7–20 × 3–6 cm; seeds 1–2, ovoid, 20–26 mm thick..***A. atropurpureus***
2 Indumentum grey; bracteoles at base of calyx..**3**
– Indumentum brown; floral bracts 1.6 mm long; bracteoles halfway along calyx; pod 14–23 × 2.5–3 cm...***A. katinganensis***

3 Floral bracts 2.8–5 mm long; calyx sericeous; disk c. 2 mm high; pod 19–24 × 2.5 cm .... ***A. sarawakensis***

– Floral bracts 1.2 mm long; calyx almost glabrous; disk c. 0.5 mm high; pod 23–24 × 4–4.5 cm .... ***A. vastus***

Adinobotrys atropurpureus **(Wall.) Dunn, Bull. Misc. Inform. 1911(4): 194 (1911)**

≡ *Pongamia atropurpurea* Wall. Pl. As. Rar. 1(4): 70 t. 78 (1830). Type: Myanmar. “Martaban [Mottama] ad Amherst [Kyaikkami] 15 July 1827”, *Wallich* Cat. No. *5910*, holotype K! [K-000881026]; isotypes BM! [BM-000997335]; BO n.v.; CAL x 2 n.v.; P! [P-02141756]

≡ *Millettia atropurpurea* (Wall.) Benth. Pl. Jungh. [Miquel] 2: 249 (1852).

≡ *Phaseoloides atropurpureum* (Wall.) Kuntze, Revis. Gen. Pl. 1: 201 (1891).

≡ *Whitfordiodendron atropurpureum* (Wall.) Dunn, Bull. Misc. Inform. Kew 1912(8): 364 (1912).

≡ *Callerya atropurpurea* (Wall.) Schot, Blumea 39(1–2): 15 (1994).

= *Millettia paniculata* Miq., Fl. Ned. Ind., Eerste Bijv. 2: 301 (1861). Type: Indonesia, Sumatra “Sumatra orient. in prov. Palembang prope Kebur Lahat (T.)” 3675 H.B. Leguminosae, Masiboengan, Hortus Botanicus 023149 Utrecht, *J.E.Teijsmann s.n.*, holotype U! [U-0003669].

= *Padbruggea pubescens* Craib, Bull. Misc. Inform. Kew 1927(2): 61 (1927) Type: Thailand, Prov. Nakawn Panom [Nakhon Phanom], Ta Uten, elev. 1200 m, 15 February 1924, tree, fls pink. Ki Mo., *A.F.G.Kerr 8457*, holotype K! [K-000881016]; isotypes ABD n.v.; BM! [BM-000997332]; E! [E00275433].

≡ *Whitfordiodendron pubescens* (Craib) Burkill, Bull. Misc. Inform. Kew 1935(5): 319 (1935).

≡ *Callerya atropurpurea* (Wall.) Schot var. *pubescens* (Craib) P.K.Lôc, Bot. Zhurn. (Moscow & Leningrad) 81(10): 98 (1996).

Illustrations. Lôc and Vidal in Fl. Cambodge, Laos & Vietnam 30: 34, t. 8 [9 – 11] (2001). https://singapore.biodiversity.online (in Home Page enter *Callerya atropurpurea*).

Distribution. Cambodia; India; Indonesia (Java, Sumatra); Laos, Malaysia (Malay Peninsula); Myanmar; Thailand and Vietnam.

Habitat. A component of evergreen forests from sea level to 1200 m.

Adinobotrys katinganensis **(Adema) J.Compton & Schrire, comb. nov.**

urn:lsid:ipni.org:names:77212569-1

≡ *Callerya katinganensis* Adema, Blumea 64(3): 275 (2019). Type: Indonesia, Borneo, Kalimantan, Upper Katingan river, c. 96 km west of Batu Badingding, K. T. C. logging area west of base camp, elev. c. 200 m. 20 December 1982. *J.P.Mogea* 4276 holotype L! [L-0772470]; isotype L! [L-0772469]; BO n.v.

Note. For a full description, details of habitat and ecology see Adema (2019: 275).

***Adinobotrys sarawakensis* (Adema) J.Compton & Schrire, comb. nov.**
urn:lsid:ipni.org:names:77212570-1

≡ *Callerya sarawakensis* Adema, Blumea 64(3): 276 (2019). Type: Malaysia, Borneo, Sarawak, third division, Bukit Raya, Kapit elev. 300 m. 26 November 1963. *P.P.K.Chai S-18911* holotype L! [L-0772465]; isotypes BO! [BO-1711732]; BO! [BO-1714048]; KEP! [KEP-195256]; MEL n.v.; SING! [SING-0263900]; SING! [SING-0263901].

Note. For a full description, details of habitat and ecology see Adema (2019: 276).

***Adinobotrys vastus* (Kosterm.) J.Compton & Schrire, Phytokeys 125: 50 (2019)**

≡ *Millettia vasta* Kosterm., Reinwardtia 5: 349 (1960). Type: Indonesia, Kalimantan [Borneo], Belajan River near Muara Lempong, June 1956, *A.J.G.H.Kostermans 12516A*, holotype BO! [BO-1249898]; isotypes BM! [BM-000997327]; K! [K-000880991]; L! [L-0018805]; P! [P-03081895]
≡ *Callerya vasta* (Kosterm.) Schot, Blumea 39(1–2): 36 (1994).

Distribution. Borneo: Brunei; Indonesia (Kalimantan); Malaysia (Sabah, Sarawak).
Habitat. Component tree in woods and forests from sea level to 250 m.

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SHORT COMMUNICATION

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Deparia ×nanakuraensis K.Hori (Athyriaceae), a new hybrid pteridophyte from Japan

Kiyotaka Hori[1]

[1] *The Kochi prefectural Makino Botanical Garden, Kochi, Japan*

Corresponding author: Kiyotaka Hori (khori@makino.or.jp)

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Abstract

I describe *Deparia ×nanakuraensis* **hyb. nov.** and discuss differences in morphological characteristics between parental species *D. pterorachis* and *D. viridifrons* with chromosome counting, plastid, and nuclear DNA markers. The new hybrid is endemic to the eastern and northern parts of Japan. Based on the criteria of the International Union for Conservation of Nature and Natural Resources, this new species is here considered Data Deficient. The ploidy level is diploid sterile.

Keywords

Athyriaceae, *Deparia*, new hybrid, Japan

Introduction

The genus *Deparia* Hook. & Grev. is one of the largest groups in the Athyriaceae family. It contains 60–90 species mostly in East Asia, with some species distributed in Africa, western Indian Ocean, northeastern North America, the Hawaiian Islands, Australia, New Zealand, and South Pacific Islands (Kato 1984; Rothfels et al. 2012; He et al. 2013; Kuo et al. 2016, 2018; PPG I 2016; Moran et al. 2019).

The genus is characterized by hair-like scales and disconnected grooves between rachises and costae (Kato 1973, 1977, 1984; Rothfels et al. 2012; Sundue and Rothfels 2014; Kuo et al. 2018). These two features have not been observed in the genera *Anisocampium* C.Presl, *Athyrium* Roth., *Diplazium* Sw., *Ephemeropteris* R.C.Moran & Sundue, and *Pseudathyrium* Newman but in some species in the Athyriaceae family

(Kato 1973; Rothfels et al. 2012; Moran et al. 2019). In addition, narrowly U-shaped rachis grooves are also a unique character of the genus *Deparia* (Kuo et al. 2018). The basic chromosome number of *Deparia* is 40, contrary to *Diplazium* of 41 (Sano et al. 2000; Rothfels et al. 2012).

In Japan, several hybrids of the genus *Deparia* have been described: *D.* ×*birii* Fraser-Jenk. (Fraser-Jenkins 2008), *D.* ×*kiyozumiana* (Sa.Kurata) Y.Shimura (Shimura 1980), pentaploid sterile *D. lancea* (Thunb.) Fraser-Jenk. (Nakato and Mitui 1979), *D.* ×*lobatocrenata* (Tagawa) M.Kato (Kato 1984; Ebihara 2017), *D.* ×*musashiensis* (H.Ohba) Seriz. (Serizawa 1981), pentaploid sterile *D. petersenii* (Kunze) M.Kato (Shinohara et al. 2003), *D.* ×*togakushiensis* Otsuka & Fujiw. (Otsuka and Fujiwara 1999), and *D.* ×*tomitaroana* (Masam.) R.Sano (Sano et al. 2000). Furthermore, Ebihara (2017) mentioned several combinations of hybrids that are not still described.

The *Deparia okuboana* complex (Athyriaceae) is recently defined by Ebihara (2017) as consisting of *D. okuboana* (Makino) M.Kato (apogamous triploid; Hirabayashi 1970), *D. coreana* (Christ) M.Kato (sexual tetraploid, Nakato and Ebihara 2018), *D. henryi* (Baker) M.Kato (apogamous triploid, Nakato and Ebihara 2018), *D. viridifrons* (Makino) M.Kato (sexual diploid; Hirabayashi 1970), *D. unifurcata* (Baker) M.Kato (apogamous triploid; Hirabayashi 1970), *D. pterorachis* (Christ) M.Kato (sexual diploid; Hirabayashi 1970). There is continuous morphological variation between *D. coreana*, *D. henryi*, *D. okuboana*, and *D. unifurcata* (Ebihara 2017). Kuo et al. (2018) identified that these members belong to sect. *Dryoathyrium*. Hori (2018) reported there were reticulate relationships in the *D. okuboana* complex with sect. *Lunathyrium* (Kuo et al. 2018) based on plastid and nuclear DNA marker. In addition, Ebihara (2017) mentioned undescribed diploid sterile hybrid between *D. pterorachis* and *D. viridifrons* based on morphology and ploidy level. This study described this new hybrid of *D. pterorachis* and *D. viridifrons*, *Deparia* ×*nanakuraensis* K.Hori, based on morphological characteristics, chromosome number, plastid, and nuclear DNA marker.

Materials and methods

Plant materials, Chromosome count, and DNA extraction

In this study, *Deparia viridifrons*, *D.* ×*nanakuraensis*, and *D. pterorachis* were investigated in molecular DNA analysis. Other members of the *D. okuboana* complex (*D. coreana*, *D. henryi*, *D. okuboana*, *D. unifurcata*) and Japanese members of the sect. *Lunathyrium* (*D. pycnosora* var. *albosquamata* M.Kato, *D. pycnosora* (Christ) M.Kato var. *pycnosora*, *D. pycnosora* var. *mucilagina* M.Kato) were also used as materials. Voucher information for all samples is listed in Appendix I. All voucher specimens have been deposited in the Makino Herbarium of Tokyo Metropolitan University (MAK), and/or the Kochi Prefectural Makino Botanical Garden (MBK). The DNA sequences of *Athyrium melanolepis* Christ, *A. crenulatoserrulatum* Makino, *A. opacum*

Copel., *Diplazium chinense* (Baker) C.Chr., *Di. esculentum* (Retz.) Sw., *Di. wichurae* (Mett.) Diels were used as outgroups, quoted from the Genbank database.

Additionally, specimens from the Collection Database and Materials of TNS (http://db.kahaku.go.jp/webmuseum/), PE (http://pe.ibcas.ac.cn/en/), TAIF (http://taif.tfri.gov.tw/search.php), and from the JSTOR Global Plants (https://plants.jstor.org/) as well as from the Global Biodiversity Information Facility (GBIF: https://www.gbif.org) database were checked.

For the conservation assessment, the area of occupancy (AOO) and extent of occurrence (EOO) were estimated using GeoCAT (Bachman et al. 2011), default settings for grid size were applied. In addition, mitotic chromosomes from *D.* ×*nanakuraensis* were counted.

To observe mitotic chromosomes, root tips were collected in the field, and pretreated with 0.004 M 8-hydroxyquinoline for 6 h at approximately 17–20 °C. After fixation in ethanol and acetic acid (3:1) for 15–30 min, the root tips were hydrolyzed in 1 N HCl at 60 °C for 1–3 min and then squashed in 2% aceto-orcein solution. The chromosomes were observed under a microscope (Leica DM2500) and then photographed by using a digital camera (Leica MC170 HD).

For the molecular analyses, total DNA was extracted from silica-dried leaves using cetyltrimethylammonium bromide solution, according to Doyle and Doyle (1990).

Plastid and nuclear DNA sequencing

trnL-F was used as the maternally-inherited (Gastony and Yatskievych 1992; Kuo et al. 2018) plastid DNA marker (F: 5'-ATTTGAACTGGTGACACGAG-3' and FernL 1 Ir1: 5'-GGYAATCCTGAGCAAATC-3'; Taberlet et al. 1991; Li et al. 2009). *AK1* (AK4F: 5'-GATGAAGCCATCAAGAAACCA-3' and AKR2: 5'-ATGGATCCAGCGACCAGTAA-3'; Hori and Murakami 2019) was used as a biparentally-inherited nuclear marker for polymerase chain reaction-single-strand conformation polymorphism (PCR–SSCP) analysis, which was used to determine allelic variation in each individual (Hori and Murakami 2019).

PCR amplification was performed using PrimeSTAR Max DNA Polymerase (Takara, Kyoto, Japan). PCR entailed an initial denaturation step at 95 °C for 10 min, followed by 35 cycles of denaturation, annealing, and elongation steps at 98 °C for 10 s, 55 °C for 5 s, and 72 °C for 5 s, respectively, using a Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Gel electrophoresis of *AK1* PCR products was performed using gels of 50% MDE gel solution (Lonza) containing 2% glycerol at 15 °C for 16 h at 300 V, followed by silver staining. For sequencing of the bands separated on the gels, the polyacrylamide gel was dried after silver staining by sandwiching the gel between Kent paper and a cellophane sheet on an acrylic backplate at 55 °C for 4 h. To extract the DNA, a piece of the DNA band was peeled from the dried gel using a cutter knife and incubated in 50 μL of Tris-EDTA buffer (10-mM Tris-HCl and 1-mM EDTA, pH 8.0) at 4 °C overnight. The supernatant solution was used as a template for further PCR amplification with the same primer set employed for initial PCR amplification.

PCR products were purified using Illustra ExoStar 1-Step (GE Healthcare, Wisconsin, USA) and used as templates for direct sequencing. Reaction mixtures for sequencing were prepared using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). The reaction mixtures were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems).

Molecular analysis

The accession numbers of DNA sequences in the datasets were shown in Appendix I. The sequences were aligned using MUSCLE (Edgar 2004) and assessed with Bayesian inference (BI) analysis using MrBayes 3.2.6 (Ronquist et al. 2012), maximum parsimony (MP), and maximum likelihood (ML) analysis using the MEGA X software (Kumar et al. 2018). Indels were treated as missing characters in all analyses. In the BI analysis, the best-fit model (*trnL-F*: HKY+I model; *AK1*: HKY model) of sequence evolution for each DNA region was selected using jModelTest 2.1.10 (Darriba et al. 2012). Four Markov chain Monte Carlo chains were run simultaneously and sampled every 100 generations for 1 million generations in total. Tracer 1.7.1 (Rambaut et al. 2018) was used to examine the posterior distribution of all parameters and their associated statistics, including estimated sample sizes. The first 2,500 sample trees from each run were discarded as burn-in periods. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm (Nei and Kumar 2000) at search level 3, at which the initial trees were obtained by the random addition of sequences (100 replicates). The confidence level of the monophyletic groups was estimated with 1,000 MP bootstrap pseudo-replicates. In ML analysis, the best-fitting model of sequence evolution for each marker was selected using MEGA; Tamura 3-parameter + I model was used for *trnL-F* and HKY model for *AK1*. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log likelihood value. The bootstrap method with 1,000 replications was employed to estimate the confidence levels of monophyletic groups in MP and ML analysis.

Results

Chromosome count

Mitotic metaphase chromosome number observed in an individual of *D.* ×*nanakuraensis* (*Hori 3391*) was $2n = 80$ (Figure 1). This individual had shrunken sporangium with no spores. The basic chromosome numbers of the genus *Deparia* is x= 40 (Sano et al. 2000; Rothfels et al. 2012), and suitably, this sample was found to be a sterile diploid.

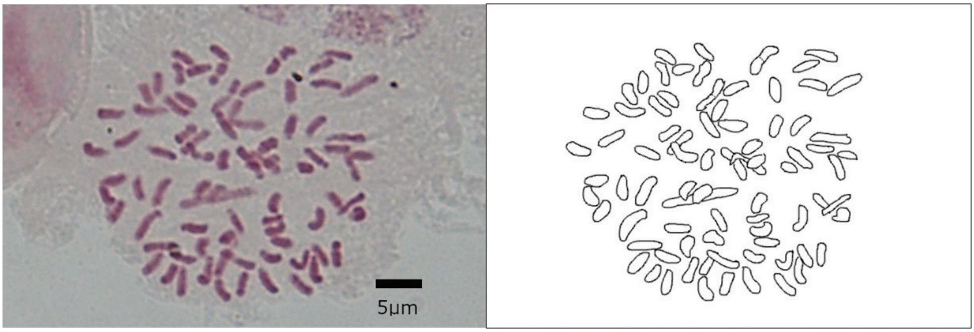

Figure 1. Photograph and sketch of mitotic metaphase chromosomes (2*n* = 80) of *D.* × *nanakuraensis* (*Hori 3391*).

Plastid and nuclear DNA phylogenetic trees

We sequenced 653–746 bp of the *trnL-F* intergenic spacer from different specimens. The aligned *trnL-F* matrix was 765 bp, of which 121 characters (15%) were parsimony-informative. For the *AK1* intron, we sequenced 338–590 bp of the intron for each specimen, yielding a 604 bp aligned matrix, of which 74 characters (12%) were parsimony-informative.

The ML trees according to the sequences of *trnL-F* (ln L = -2309.05) and *AK1* (ln L = -1616.59) with bootstrap percentages (BPs), Bayesian posterior probabilities (PP) were shown in Figures 2, 3, respectively. In the *trnL-F* phylogeny, the haplotype of *D. pterorachis* and *D. viridifrons* composed different clades with *D. coreana*, *D. henryi*, and *D. okuboana* which were supported by BP (>70) and PP (>0.90) values. In the *AK1* phylogeny, the two clades containing *D. pterorachis* and *D. viridifrons* were supported by BP, but *D. viridifrons* was not supported by PP value. *Deparia* ×*nanakuraensis* had the same haplotype of *D. pterorachis* and *D. viridifrons* in both *trnL-F* and *AK1* phylogenies. Other members of the *D. okuboana* complex (*D. coreana*, *D. henryi*, *D. okuboana*, *D. unifurcata*) shared the same alleles with *D. viridifrons* partly (Hori 2018), but the combination of alleles was different from *D.* ×*nanakuraensis*. Japanese members of the sect. *Lunathyrium* (*D. pycnosora* var. *albosquamata*, *D. pycnosora* var. *pycnosora*, *D. pycnosora* var. *mucilagina*) did not share any alleles with *D.* ×*nanakuraensis*. Therefore, *D.* ×*nanakuraensis* can be of origin hybrid from *D. pterorachis* and *D. viridifrons*.

Taxonomic treatment

***Deparia* ×*nanakuraensis* K.Hori, hyb. nov.**
urn:lsid:ipni.org:names:77212571-1
Figure 4

Type. Japan. Honshu: Akita prefecture, Noshiro city, Futatsui town, Nanakura-shrine, 40°12'9.48"N, 140°15'29.82"E, alt. 23 m, deciduous forest containing *Acer miyabei*

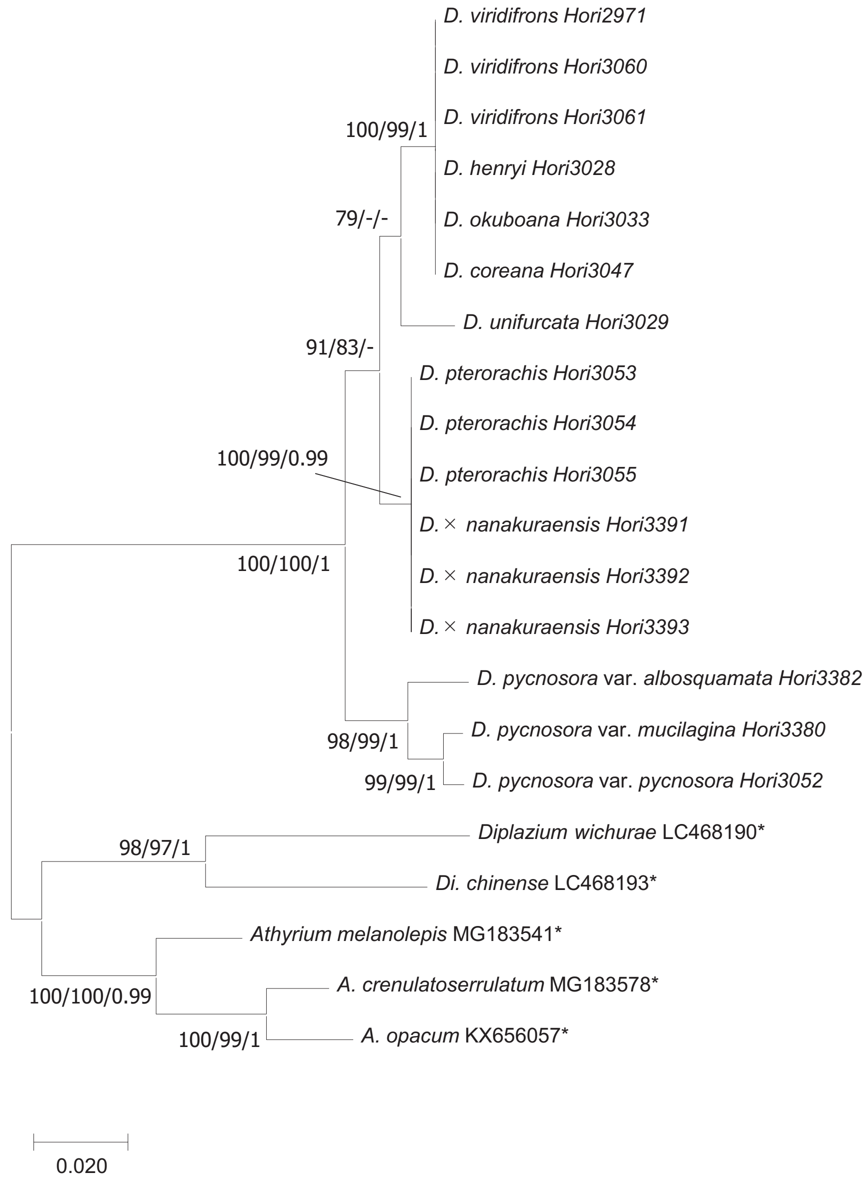

Figure 2. The ML tree based on the sequence variation of the gene *trnL-F* (ln *L* = -2309.05) with PP (>0.90) and BP (>70) of ML/MP/BI analyses on each branch. The sequences with asterisks were quoted from Genbank.

Maxim., *Aesculus turbinata* Blume, *Cercidiphyllum japonicum* Siebold & Zucc., *Cryptomeria japonica* (Thunb. ex L.f.) D.Don, *Dryopteris monticola* (Makino) C.Chr., and *Pachysandra terminalis* Siebold & Zucc., on soil, 7 Jul 2020, *K. Hori 3391* (holotype: MAK467056; isotype: MBK).

Description. *Terrestrial, summer green fern. Rhizomes* creeping, occasionally branched, with buds, stramineous, 15–25 × 4–7 cm, closely set with roots and persis-

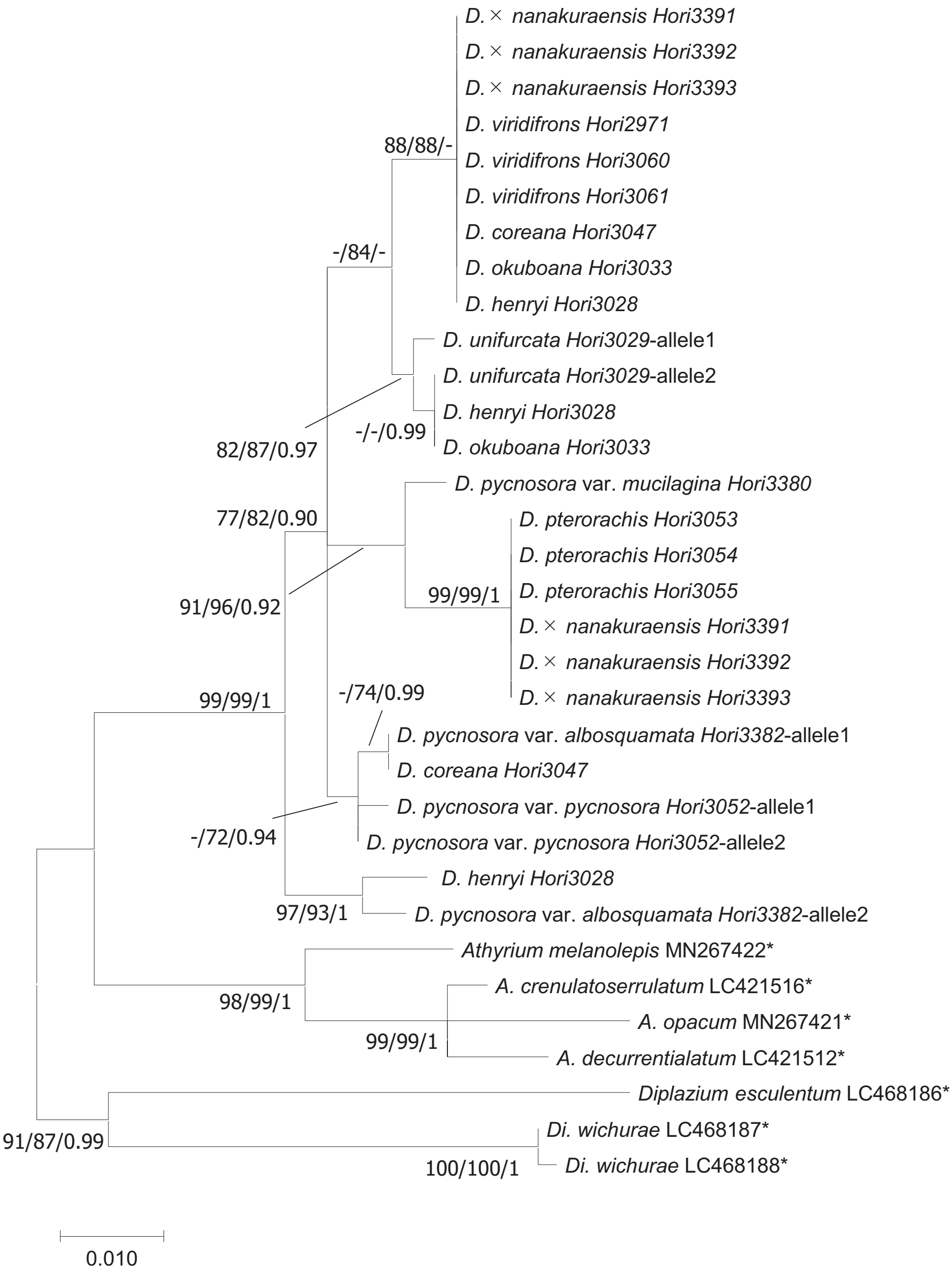

Figure 3. The ML tree based on the sequence variation of the gene *AK1* (ln *L* = -1616.59) with PP (>0.90) and BP (>70) of ML/MP/BI analyses on each branch. The sequences with asterisks were quoted from Genbank.

tent, densely clothed by old stipe bases, glabrous; *fronds* 4–6 per rhizome; *stipes* whitish green, 30–40 × 0.8–1.5 cm, sparsely clothed with stramineous scales at the base (1–1.5 × 0.5–1 cm), ovate; *blades* yellowish green adaxially, 3-pinnate-pinnatifid at the

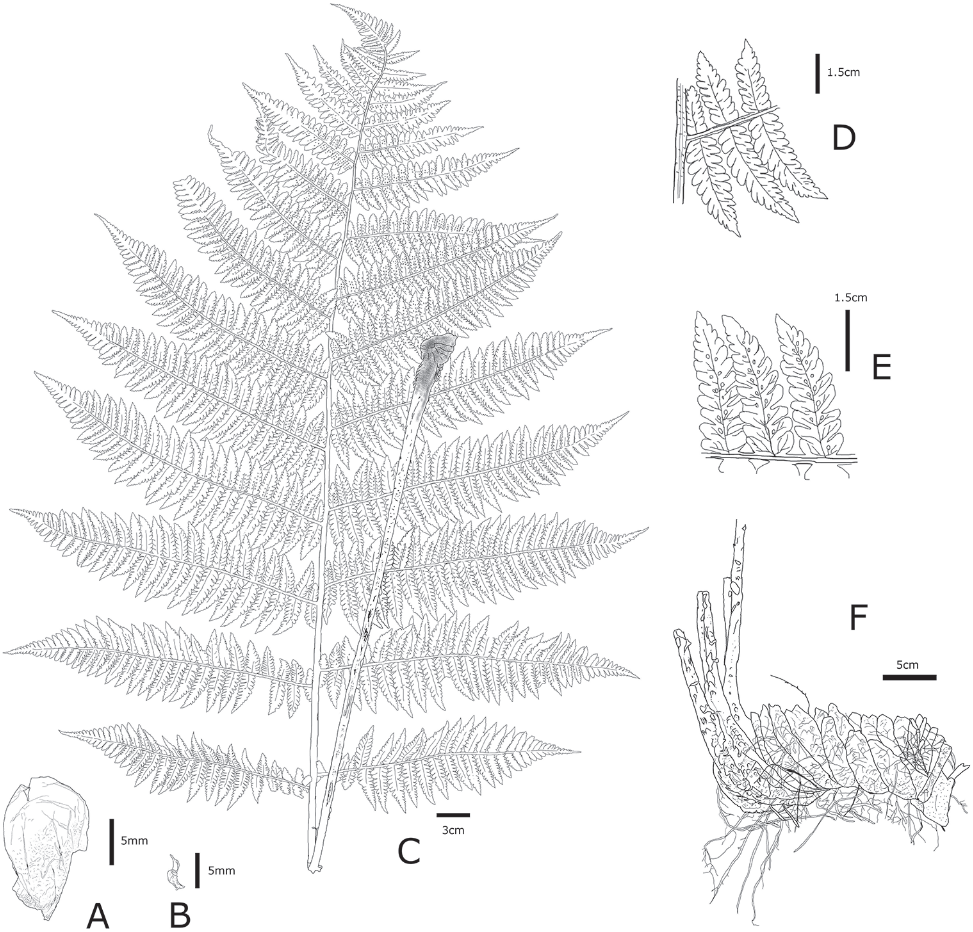

Figure 4. *D.* ×*nanakuraensis* K.Hori **A** lower stipe scale **B** upper stipe scale **C** abaxial surface of frond and stipe **D** detail of adaxial pinnule **E** detail of abaxial pinnule, and **F** rhizome and base of stipes. **A–F** from the holotype (MAK467056) (illustration by K. Hori).

base, in the middle to upper section, 2-pinnate at the apex, 40–70 × 30–40 cm, deltoid; *rachises* whitish green, glabrous, sparsely clothed with stramineous scales (2–5 × 1–2 mm) and black hairs adaxially; *pinnae* 10–15 pairs, ascending, lanceolate, shrunken at base, alternate, petiolated (2–5 mm), sessile near the apex, lowest pinnae slightly reduced, second lowest pair usually the largest, 25–30 × 4–8 cm; *pinnules*, alternate on the basal and middle sections of the blade, 20–30 pairs on the basal and middle sections of the blade, 15–20 pairs on the apex of the blade, reduced distally, lanceolate, deeply serrated, vein-free, close to or reaching to the margin, 10–15 pairs in the middle lobe; *sori* brown, tending to appear on the abaxial surface of the middle or upper part of blades, oblong- to J-shaped, 1.5–3 mm long, on the apex or middle of veinlets, 5–10 per ultimate segment, persistent; *indusium* entire to serrated on margin, *sporangium* shrunken, spores absent.

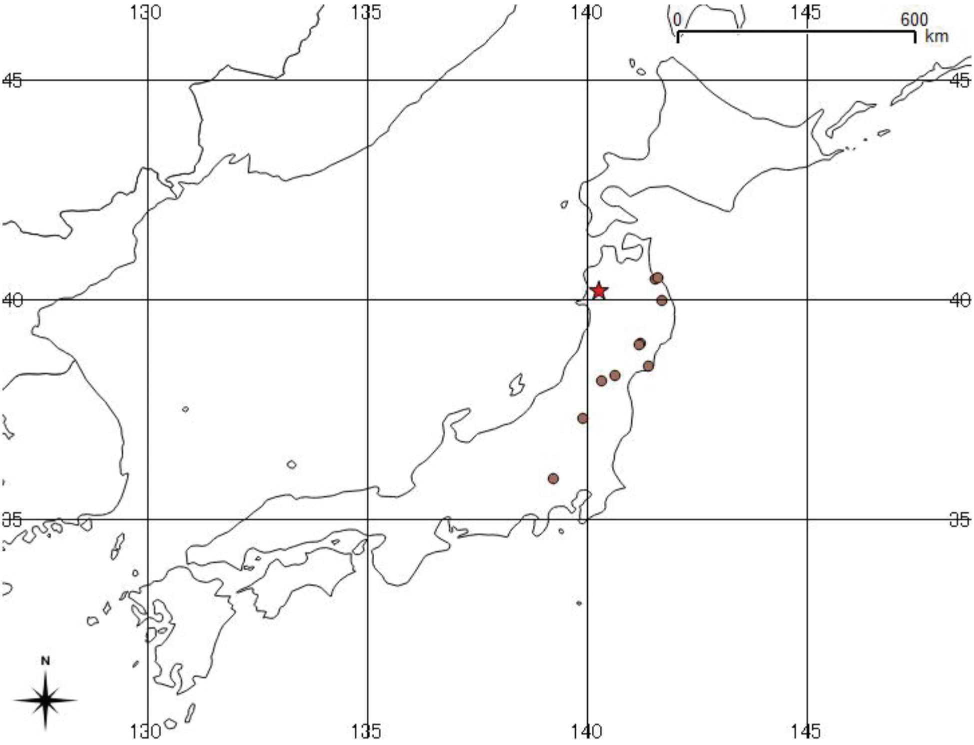

Figure 5. Map showing the known distribution of *D. ×nanakuraensis* in Japan. Red star indicates type locality, other circles indicate examined specimens.

Etymology. The name derives from Nanakura-shrine, Futasui town, Noshiro City, Akita prefecture, northeast Japan, where *Deparia ×nanakuraensis* was first found.

Specimens examined. Japan. Honshu: Akita pref., Noshiro city, Futatsui town, Nanakura-shrine, 40°12'9.48"N, 140°15'29.82"E, alt. 23 m, 7 Jul 2020, *K. Hori 3392, loc. cit., K. Hori 3393, loc. cit., K. Hori 3394, loc. cit.*, 10 Jul 2012, *Y. Horii* 35548 (TNS 01167830), *loc. cit., Y. Horii* 35549 (TNS 01167829); Aomori pref., Hachinohe city, Same town, Kamikoswa, alt. 100 m, 23 Aug 1975, coll. *M. Neichi* (TNS 1170337, image!); *loc. cit.*, Kitsunetai, alt. 30m, 9 Jul 2005, coll. *M. Neichi* (TNS 01183638, image!); Iwate pref., Iwaizumi town, Atsuka, Matsugasawa, alt. 350 m, 18 Jul 1981, coll. *M. Neichi* (TNS 01161869, image!); *loc. cit.*, Ichinoseki city, Higashiyama cho, Nagasaka, Nagahira, alt. 180 m, 22 Aug 1987, coll. *M. Suzuki* (TNS 932028, image!); *loc. cit.*, Maikawa, Ohira, alt. 120 m, 22 Sep 1986, coll. *M. Suzuki* (TNS 9320284image!); Miyagi pref., Ishinomaki city, Mano, Uchihara, alt. 70 m, 25 May 1990, coll. *K. Shogo* (TNS01184195, image!); *loc. cit.*, Sendai city, Akiu town, Baba, alt. 200 m, 15 Oct 1983, coll. *K. Shogo* (TNS01184194, image!); Yamagata pref., Kamiyama city, Takano, alt. 250 m, 5 Jun 1983, coll. *N. Sakawa* (TNS01161877, image!); Fukushima pref., Minamiaizu county, Shimosato town, Yunokami, alt. 500 m, 8 Sep 1972, coll. *T. Waku* (TNS01161873, image!); Saitama pref., Hannnou city, Kasasugitouge, alt. 500 m, 21

Figure 6. Wild plant of *D.* ×*nanakuraensis* in type locality.

Figure 7. Juvenile of *D.* ×*nanakuraensis*.

Figure 8. Indefinite growth through bud (red arrow) on rhizome of *D.* ×*nanakuraensis*.

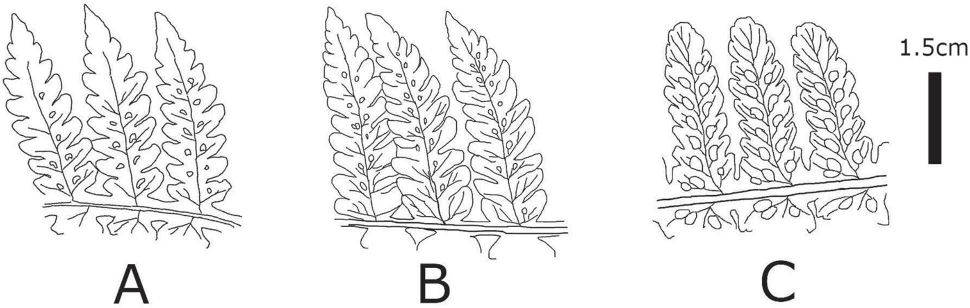

Figure 9. Abaxial surface of pinnule and sori of **A** *D. viridifrons* **B** *D.* ×*nanakuraensis*, and **C** *D. pterorachis* (illustration by K. Hori).

Aug 1984, coll. *T. Iwata* (TNS01140142, image!); *loc. cit.*, 14 Sep 1980, coll. *Y. Kobayashi* (MBK0233005); *loc. cit.*, 14 June 1981, coll. *Y. Kobayashi* (MBK0232983).

Distribution and ecology. *Deparia* ×*nanakuraensis* is known from the eastern and northern part of Honshu in Japan (Figure 5). It was observed to grow on soil under deciduous forest (Figure 6) or planted coniferous forest containing *Cryptomeria japonica*. This hybrid is endemic to Japan. In the type locality, this hybrid comprised a population of over 30 individuals with juveniles (Figure 7) although parents of *D. viridifrons* and *D. pterorachis* were both absent, and sporangium had no spores. However, it is expected that *Deparia ×nanakuraensis* can reproduce young individuals from buds on its rhizome (Figure 8).

Conservation status. IUCN Red List Category. Based on estimates from GeoCAT, the EOO of *D. ×nanakuraensis* was 46,321 km^2. The known AOO of *D. ×nanakuraensis* was 44 km^2. The localities correspond to less than 20 points, but I could not check the population size on each locality. Therefore, available information is inadequate to support the assessment of its extinction risk. According to the IUCN (2012) criteria, the category of Data Deficient (DD) is appropriate.

Discussion

Deparia ×nanakuraensis presents almost intermediate morphologies between *D. viridifrons* and *D. pterorachis* species. *Deparia viridifrons* is characterized by having deltoid-ovate or ovate-lanceolate fronds, reniform to U-shaped sori, pinnules with costal wing, rounded serration of pinnules, and acute apex of pinnules. In contrast, *D. pterorachis* has oblong fronds, oblong to J-shaped sori, pinnules truncated to costa; truncate serration of pinnules, and obtuse apex of pinnules (He et al. 2013; Ebihara 2017). *Deparia ×nanakuraensis* has deltoid fronds, oblong to J-shaped sori, pinnules with narrow costal wing, rather rounded serration of pinnules, and a rather acute apex of pinnules (Figure 9, Table 1).

Kuo et al. (2018) classified *D. viridifrons* and *D. pterorachis* as the members of sect. *Dryoathyrium* because lateral pinnules are not auricled, and these are closely related in plastid DNA phylogeny (Kuo et al. 2018). Therefore, *Deparia ×nanakuraensis* is infra section hybrid in the sect. *Dryoathyrium*.

The ploidy level of this hybrid is the same as its parents because *D. viridifrons* and *D. pterorachis* are both sexual diploid (Kurita 1963; Mitui 1966, 1968, 1970; Hirabayashi 1970). In addition, this can be the first report of a diploid sterile hybrid of the genus *Deparia* from Japan although several hybrids have been described (Ebihara 2017).

In conclusion, this study described *Deparia ×nanakuraensis* based on morphology, cytology, and molecular DNA analysis. The morphological characteristics were intermediate between its parents *D. viridifrons* and *D. pterorachis*. This hybrid can produce young individuals from buds on its rhizome. Based on the criteria of the International Union for Conservation of Nature and Natural Resources, this new species is here

Table 1. Morphological comparison among *D. ×nanakuraensis* and related species.

Characteristics	Shape of frond	Shape of sori	Margin of indusium	Base of pinnule	Serration of pinnules	Apex of pinnules
D. viridifrons	deltoid-ovate or ovate-lanceolate	reniform to U-shaped	serrated	with costal wing	rounded	acute
D. ×nanakuraensis	deltoid	oblong to J-shaped	entire to serrated	with narrow costal wing	rather rounded	rather acute
D. pterorachis	oblong	oblong to J-shaped	entire	truncated to costa	truncate	obtuse

considered Data Deficient. This hybrid can be the first report of diploid sterile hybrid of the genus *Deparia* from Japan. In future studies, it is expected that more hybrids of the genus *Deparia* will be discovered and described from Japan.

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Appendix I

Voucher specimens for DNA analysis in this study. Data are in the order: Species name –locality voucher (Herbarium); haplotype of plastid *trnL-F*; allele of nuclear *AK1*.

***Deparia ×nanakuraensis* K.Hori**– JAPAN. Akita pref., Noshiro city, Futatsui town, Nanakura-shrine, 23m alt., 40°12'9.48"N, 140°15'29.82"E, 7 Jul 2020, *K. Hori 3391* (MAK, MBK); MT898446 (*trnL-F*); MT887301, MT887307 (*AK1*). ibid., *K. Hori 3392* (MAK, MBK); MT898447 (*trnL-F*); MT887302, MT887308 (*AK1*). ibid., *K. Hori 3393* (MAK, MBK); MT898448 (*trnL-F*); MT887303, MT887309 (*AK1*).

***D. pterorachis* (Christ) M.Kato**– JAPAN. Hokkaido Pref., Sapporo city, Minami-ku, Jouzannkei, 530m alt., 42°55'36.8"N, 141°10'6.1"E, July 30 2018, *K. Hori 3053* (MBK); MT898441 (*trnL-F*); MT887299 (*AK1*). ibid., Ebetsu city, Nopporo nature park, July 30 2018, *K. Hori 3054* (MBK); MT898442 (*trnL-F*); MT887300 (*AK1*). ibid., *K. Hori 3055* (MBK); MT898443 (*trnL-F*); LC421964 (*AK1*, Hori 2018).

***D. viridifrons* (Makino) M.Kato**– JAPAN. Kochi pref., Takaoka county, Ochi town, Mt. Yokogura, May 30 2018, *K. Hori 2971* (MBK); LC421960 (*trnL-F*, Hori and Murakami 2019); LC468191 (*AK1*, Hori 2018). ibid., Oct 17 2018, *K. Hori*

3060 (MAK); MT898444 (*trnL-F*); MT887305 (*AK1*). ibid., Oct 17 2018, *K. Hori 3061* (MAK); MT898445 (*trnL-F*); MT887306 (*AK1*).

D. coreana **(Christ) M.Kato**– JAPAN. Aomori Pref., Kamikita county, Shichinohe town, Jul 26 2018, *Hori 3047* (MBK); MW051518 (*trnL-F*); MW051522, MW051523 (*AK1*).

D. henryi **(Baker) M.Kato**– JAPAN. Kyoto Pref., Kyoto City, Jul 14 2018, *Hori 3028* (MBK); MW051514 (*trnL-F*); MW051527, MW0515278, MW051529 (*AK1*).

D. okuboana **(Makino) M.Kato**– JAPAN. Kyoto pref., Kyoto city, Jul 14 2018, *Hori 3033* (MBK); MW051515 (*trnL-F*); MW051530, MW051531 (*AK1*).

D. pycnosora **(Christ) M.Kato var. *albosquamata* M.Kato–** JAPAN. Nagano Pref., Nagano city, Togakushi shrine, Okusha, Jul 9 2020, *K. Hori 3382* (MAK); MW051519 (*trnL-F*); MW051520, MW051521 (*AK1*).

D. pycnosora **(Christ) M. Kato var. *mucilagina* M.Kato–** JAPAN. Nagano Pref., Nagano city, Togakushi shrine, Okusha, Jul 9 2020, *K. Hori 3380* (MAK); MW051516 (*trnL-F*); MW051526 (*AK1*).

D. pycnosora **(Christ) M. Kato var. *pycnosora* M.Kato–** JAPAN. Aomori Pref., Kamikita county, Touhoku town, Otsutomo, Jul 26 2018, *K. Hori 3052* (MAK); MW051517 (*trnL-F*); MW051524, MW051525 (*AK1*).

D. unifurcata **(Baker) M.Kato–** JAPAN. Kyoto Pref., Kyoto city, Jul 14 2018, *K. Hori 3029* (MBK); LC468192 (*trnL-F*, Hori and Murakami 2019); LC421961, LC421962 (*AK1*, Hori 2018).

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Zehneria grandibracteata (Cucurbitaceae), an overlooked new species from western Kenyan forests

Neng Wei[1,2,3], Zhi-Xiang Zhong[1,2], David Kimutai Melly[1,2,3], Solomon Kipkoech[1,2,3], Benjamin Muema Watuma[1,2,3], Veronicah Mutele Ngumbau[1,2,3,4], Peris Kamau[4], Guang-Wan Hu[1,2], Qing-Feng Wang[1,2]

1 *CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, CN-430074, China* **2** *Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan, CN-430074, China* **3** *University of Chinese Academy of Sciences, Beijing, CN-100049, China* **4** *East Africa Herbarium, National Museums of Kenya, P.O. Box 451660-0100, Nairobi, Kenya*

Corresponding author: Guang-Wan Hu (guangwanhu@wbgcas.cn)

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Abstract

Zehneria grandibracteata, a new species of Cucurbitaceae from western Kenya, is described here, based on morphological and molecular data. It has long been misidentified as the widely-distributed species *Z. scabra*. However, it differs by its ovate leafy probract at the base of the inflorescences, subglabrous condition of the entire plant, shorter receptacle-tube and filaments, as well as denser and sessile inflorescences. Furthermore, the molecular phylogenetic analysis of *Zehneria*, based on nrITS sequences, further supports the argument that *Z. grandibracteata* should be segregated from *Z. scabra*.

Keywords

East Africa, Flora of Kenya, phylogeny, taxonomy, *Zehneria scabra*

Introduction

Zehneria Endlicher (1833: 69) is a genus of Cucurbitaceae. It contains over 60 species, which are mainly distributed in tropical and subtropical Africa, Madagascar and southeastern Asia (Schaefer and Renner 2011a; Dwivedi et al. 2018). *Zehneria* is characterised by male flowers largely with the three stamens all 2-thecate, the thecae ± erect, straight or little curved (Simmons and De Wilde 2000; Schaefer and Renner 2011a). De Wilde and Duyfjes (2006a, b, 2009a, b) split several genera from *Zehneria* s.l. (in the sense of Jeffrey), with only the type species, *Zehneria baueriana* Endlicher (1833: 69) remaining in *Zehneria* s.s.. Besides, De Wilde and Duyfjes (2006a) proposed morphological characters including leaf drying colour, stamen insertion, presence or absence of staminode, presence or absence of probract and shape of stigmatic lobes, disc and seed, in their circumscription of *Zehneria* s.s. and the related genera. Nevertheless, this treatment is not supported by the molecular phylogeny inferred by Schaefer et al. (2009), Schaefer and Renner (2011a, b) and Dwivedi et al. (2018), who argued against over-splitting of the group. East Africa has been recognised as a neglected diversity centre for *Zehneria* (Wei et al. 2017), with several new taxa discovered and named in recent years (Zhou et al. 2016; Wei et al. 2017; Watuma et al. 2019; Ngumbau et al. 2020). Besides, Africa was also referred to as the origin centre (Schaefer et al. 2009; Dwivedi et al. 2018), followed by recent long-distance dispersal to other continents and islands.

During field investigations of the Kenyan flora in 2016, a *Zehneria* species with evident leafy probracts attracted the authors' attention for the first time. Herbarium specimens had been identified as *Z. scabra* Sond. in Harvey and Sonder (1862: 486), a widespread species with great morphological variability. In the following years, more specimens were collected and detailed morphological studies were conducted. Measurements of morphological characters, as well as molecular phylogenetic analysis, based on nrITS, all support the segregation of this *Zehneria* from *Z. scabra*. Hence, we describe it as *Z. grandibracteata* below.

Materials and methods

Morphology

Specimens of East African *Zehneria* deposited in the herbaria of K, EA and HIB were studied, as well as relevant digitised specimens from online databases, including specimens from the herbaria B, BR, BM, E and P (herbarium acronyms follow Thiers (2020)). Morphological measurements of the details given in the description are based on living materials during the field trips, except tendrils and seeds confirmed by specimen observations at herbaria. The detailed morphological comparison between *Z. scabra* and our collection was initially made. Given *Z. longiflora* G.W. Hu & Q.F. Wang in Wei et al. (2017: 89) has largely overlapped the distribution area with our collection, as well as the great similarity with the latter, *Z. longiflora* was also included for morphological comparison.

Molecular phylogeny

Aiming to delimitate the phylogenetic position of our *Zehneria* collections, a total of 63 sequences were used to infer a phylogenetic tree. Amongst these sequences, 60 accessions representing 38 *Zehneria* species were included and another three accessions from *Cucumis*, *Coccinia*, *Benincasa* were treated as outgroups, according to Schaefer et al. (2009) and Dwivedi et al. (2018). Nineteen sequences of African *Zehneria* species were newly generated in this study, while the other sequences were downloaded from GenBank. The source of the materials and the corresponding GenBank accession numbers were given in Table 1. Total genomic DNA was extracted from silica gel-dried material using a modified CTAB protocol (Doyle and Doyle 1987) (see Suppl. material 1). The primers of nrITS region were obtained from White et al. (1990). PCR amplification, sequencing and data analysis were performed according to Dwivedi et al. (2018). Forward and reverse sequences were manually checked and edited where necessary. Sequences were aligned by MAFFT v. 7 (Katoh and Standley 2013). Gblocks (Talavera and Castresana 2007) was used to trim with the default setting to remove any ambiguous alignment. Additionally, these alignments were visually inspected in Geneious 8.0.2 (Kearse et al. 2012) and manually adjusted where needed. The best-fit model for Bayesian Inference (BI) and Maximum Likelihood (ML) analyses was estimated by ModelFinder (Kalyaanamoorthy et al. 2017) under the Bayesian Information Criterion (BIC). ML analyses were inferred by IQ-TREE v.1.6.8 (Nguyen et al. 2015) under the Ultrafast bootstrapping algorithm (Guindon et al. 2010) with 1000 bootstrap replicates. BI analyses were performed with MrBayes 3.2.7 (Ronquist et al. 2012). Two independent Markov Chain Monte Carlo analyses (MCMC) were run with four simultaneous chains of 10 million generations sampling one tree every 1000 generations with the initial 25% discarded as burn-in. The remaining trees were then used to construct majority-rule consensus trees. The average deviation of split frequencies was verified by reaching a value below 0.01 at the end of MCMC analyses. The effective sample sizes (ESS) for all parameters and statistics were assessed using Tracer version 1.7.1 (Rambaut et al. 2018). The phylogenetic tree was visualised using the online tool iTOL (Letunic and Bork 2007).

Results

Morphological comparison

The Table 2 distinguishes morphological characters of these three species, mainly based on Jeffrey (1967, 1978), Wei et al. (2017) and observations on specimens. Our collection can be readily recognisable by its large leafy probract. Besides, it also differs from the other two species by morphological characters including thick stem, subglabrous leaf blade, sessile inflorescence and size of perianth, pedicel, filament, style and fruit.

Phylogenetic analysis

In total, 60 sequences representing 38 *Zehneria* species were included in our dataset. Multiple sequences per species were identical as to some species, like *Z. grandibracteata*, *Z. anomala*, *Z. tuberifera* and *Z. longiflora*. They might, however, be different regard-

Table 1. GenBank accession numbers for sequence data used in this study.

Species and specimen-voucher	Accession No.
Benincasa hispida, *Renner et al. 2760* (M)	KJ467162
Coccinia grandis, *DeWilde & Duyfjes 22270* (L)	HQ608207
Cucumis melo, *Mitchell & Schaefer 68* (TUM)	KY434575
Neoachmandra boholensis, *Ramos 2-107/37215* (US)	KY523290
Neoachmandra capillacea, *Achigan-Dako 07nia757*	AM981144
Neoachmandra capillacea, *Wieringa 11246* (M)	KY523291
Neoachmandra cunninghamii, *Telford 12489* (M)	KY523292
Neoachmandra filipes, *Brass 31994* (US)	KY523293
Neoachmandra gilletii, *De Wilde 11246* (L)	KY523280
Neoachmandra hallii, *Achigan-Dako 91sn003*	AM981143
Neoachmandra hermaphrodita, *Phonsena 440938* (K)	KY523281
Neoachmandra japonica, *Su EM0045T001*	MK771856
Neoachmandra japonica, *Zhang 1518* (M)	KY523294
Neoachmandra leucocarpa, *Junghuhn s.n.* (U)	KY523295
Neoachmandra odorata, *He s.n.* (K)	KY523307
Neoachmandra odorata, *Wallich 6706* (M)	KY523297
Neoachmandra pentaphylla, *Guillaumin 8611* (US)	KY523286
Neoachmandra pentaphylla, *McKee 3504* (US)	KY523300
Neoachmandra samoensis, *Sykes 170278* (L)	KY523301
Neoachmandra samoensis, *Whistler W2908* (B)	MG680626
Neoachmandra thwaitesii, *Pallithanam 3637* (BLAT)	KY523314
Neoachmandra wallichii, *Fujikawa 053262* (TUM)	KY523310
Zehneria anomala, *Gilbert 1681* (EA)	MT733849
Zehneria anomala, *Gillett 16503* (M)	KY523289
Zehneria baueriana, *McKee 38396* (GH)	KY523288
Zehneria baueriana, *Sykes 533* (US)	KY523284
Zehneria bodinieri, *Dwivedi 1004* (DUH)	KY523266
Zehneria bodinieri, *Tanaka 080913* (MBK)	KY523267
Zehneria emirnensis, *Mitchell & Schaefer 25* (TUM)	KY523268
Zehneria grandibracteata, *SAJIT 6670* (EA/HIB)	MT733851
Zehneria grandibracteata, *SAJIT 6966* (EA/HIB)	MT733852
Zehneria grandibracteata, *SAJIT 6968* (EA/HIB)	MT733850
Zehneria guamensis, *Perlman 14* (US)	KY523273
Zehneria longiflora, *SAJIT 6669* (EA/HIB)	MT733853
Zehneria longiflora, *SAJIT 6672* (EA/HIB)	MT733854
Zehneria marlothii, *Merxmueller & Giess 30031* (M)	KY523283
Zehneria maysorensis, *CALI 10625*	KY523386
Zehneria maysorensis, *Dwivedi 1002* (DUH)	KY523256
Zehneria microsperma, *Loveridge 64* (GH)	KY523274
Zehneria minutiflora, *SAJIT 8861* (EA/HIB)	MT733855
Zehneria minutiflora, *Stolz 1139* (M)	KY523296
Zehneria monocarpa, *SAJIT 7172* (EA/HIB)	MT733856
Zehneria monocarpa, *SAJIT 7173* (EA/HIB)	MT733857
Zehneria oligosperma, *Luke 11710* (EA)	MT733858
Zehneria pallidinervia, *Holstein 52* (M)	KY523287
Zehneria pallidinervia, *SAJIT 6241* (EA/HIB)	MT733859
Zehneria perpusilla, *Santapau 13074* (BLAT)	KY523255
Zehneria perrieri, *Mitchell & Schaefer 10* (TUM)	KY523270
Zehneria pisifera, *Hoogland & Pullen 5926* (GH)	KY523275
Zehneria polycarpa, *Mitchell & Schaefer 36* (TUM)	KY523276
Zehneria racemosa, *Mendes 1841* (M)	KY523298
Zehneria scabra, *Schaefer 05/317*	HQ202009
Zehneria scabra, *SAJIT 6501* (EA/HIB)	MT733860
Zehneria scabra, *SAJIT 6554* (EA/HIB)	MT733861
Zehneria scabra, *SAJIT 6736* (EA/HIB)	MT733863
Zehneria scabra, *SAJIT 6873* (EA/HIB)	MT733865

Species and specimen-voucher	Accession No.
Zehneria scabra, *Schaefer s.n.*	KY523278
Zehneria scrobiculata, *Bolus 11558* (M)	KY523285
Zehneria scrobiculata, *Schimper 164* (M)	KY523299
Zehneria tahitensis, *Sachet 2662* (US)	KY523313
Zehneria tridactyla, *Espirito 3053* (M)	KY523321
Zehneria tuberifera, *SAJIT-6350* (EA/HIB)	MT733866
Zehneria tuberifera, *SAJIT-W0044* (EA/HIB)	MT733867

Table 2. Dissimilar characters to distinguish *Zehneria grandibracteata*, *Z. longiflora* and *Z. scabra*, based on Jeffrey (1967, 1978), Wei et al. (2017) and own observations.

Character	*Z. grandibracteata*	*Z. scabra*	*Z. longiflora*
Stem	Thick, up to 2.5 cm in diam., subglabrous	Thick, up to 1.5 cm in diam., puberulous	Thin, up to 0.8 cm in diam., subglabrous
Leaf blade	Membraneous, deeply cordate to subtruncate at the base, subglabrous, with sparsely scabrid setulose on both sides	Membraneous to subcoriaceous, deeply cordate to subtruncate at the base, puberulous on both sides or sparsely scabrid-setulose on the veins beneath	Slightly fleshy, membraneous, subglabrous, cordate to subtruncate at the base, with sparsely scattered bristles on adaxial surface only
Male inflorescence	Sessile, subumbelliform	Subumbelliform or shortly racemiform sessile or pedunculate axillary clusters	Sessile or pedunculated, subumbelliform or racemiform
Probract	Well-developed, leafy, ovate, up to 18 × 12 mm, incurved, beak-like, persistent	Linear, hooked or curly, minute, caduceus	Linear, hooked or curly, less than 10 mm long, minute, caduceus
Perianth	Receptacle-tube 1.8–3 mm long, hairy only on inner surface, petal lobes ca. 1.8 mm long	Receptacle-tube 2.0–5.5 mm long, hairy on both inner and outside surface, petal lobes 1.5–3.5 mm long	Receptacle-tube 6.0–7.5 mm long, hairy only on inner surface, petal lobes 2.0–3.0 mm long reflexed
Pedicle	3–12 mm long in male, 4–6 mm long in female	1.5–10 mm long in male, 0.4–11.0 (20.0) mm long in female	4–20 mm long in male, 8–25 mm long in female
Filament length	ca. 1.5 mm	1–2.5 mm	ca. 3.5 mm
Style length	2–3.5 mm long, stigma ca. 1.5 mm in diam.	2–4 mm long, stigma ca. 2 mm in diam.	6–7 mm long, stigma ca. 2 mm in diam.
Ovary	Glabrous, subglobose, with neck up to 1 mm long	Puberulous, subglobose to fusiform to beaked, with neck up to 2 mm long	Glabrous, subglobose, with neck up to 3.5 mm long
Fruit	2–16 in clusters, sparsely covered with tiny protuberances, subglobose, 8–10 mm in diam.	1–10 in clusters, usually glabrous, globose, 8–13 mm in diameter, or ellipsoid, 10–12 × 7–8 mm	2–8 in clusters, densely covered with tiny protuberances, globose, 9–11 mm in diam.

ing the other species, such as *Z. scabra*, Z. *pallidinervia* and *Z. minutiflora*. The final trimmed alignment of 63 sequences has 721 columns, with 92 parsimony-informative sites. *Z. grandibracteata* differs in the 71th position (G vs. A) and 208th position (A vs. T) of ITS1 alignment from other *Zehneria* species. HKY+F+G4 was selected as the best-fit model to infer the Maximum Likelihood tree and Bayesian tree. As shown in Figure 1, three accessions of *Z. grandibracteata* clustered together with robust support (PP = 0.99; BS = 98%). Then, it joined the other three East African taxa group (*Z. oligosperma*, *Z. tuberifera* and *Z. longiflora*), which offers morphological synapomorphies and a conclusive biogeographic scenario of its evolution. These four species formed a monophyly with high support (PP = 0.99; BS = 96%). However, accessions of *Z. scabra* did not form a monophyly as expected (newly-sequenced accessions are monophyletic, but two previously-published accessions are nested in *Z. monocarpa*). Despite the new species being closely related to *Z. scabra*, they are not recognised as monophyletic in our phylogenetic tree.

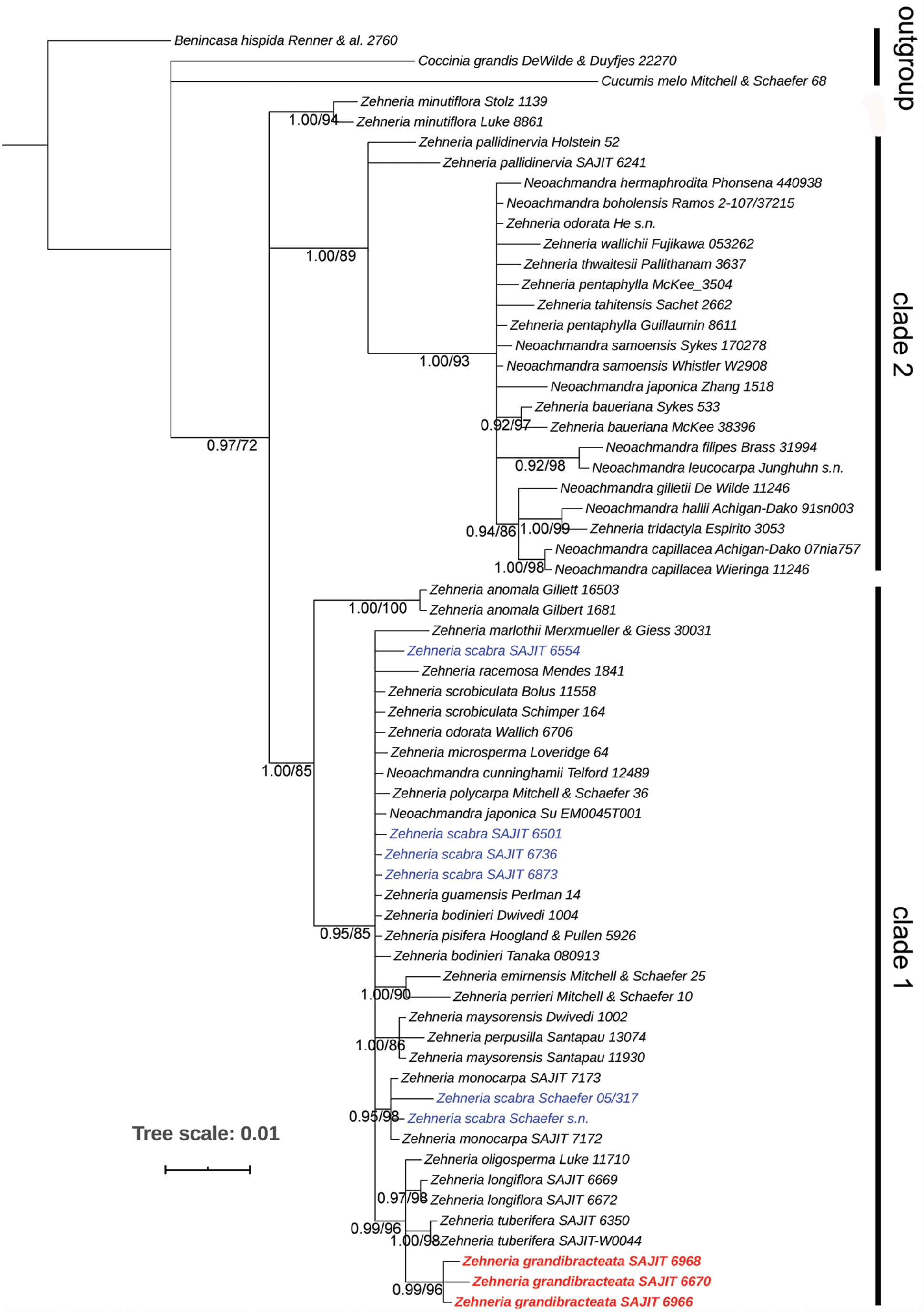

Figure 1. Bayesian tree inferred from the nrITS sequences dataset to elucidate the phylogenetic position of *Zehneria grandibracteata*. Bayesian posterior probability values > 0.9 and bootstrap values ≥70% are shown below the branches. The new species is highlighted in bold and red colour and *Z. scabra* is noted in blue colour.

Taxonomic description

Zehneria grandibracteata G.W. Hu, Neng Wei & Q.F. Wang, sp. nov.

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Figures 3, 4

Diagnosis. It is close to *Z. scabra*, but differs by its consistently ovate leafy probracts (linear minute or even absent in *Z. scabra*), subglabrous condition of the entire plant (puberulous in *Z. scabra*), shorter receptacle-tube (1.8–3 mm long vs. 2–5.5 mm in *Z. scabra*) and filaments (ca. 1.5 mm long vs. 1–2.5 mm in *Z. scabra*), as well as sessile and denser inflorescences (cluster of 8–30 in male, 6–22 in female vs. 2–60 in male, 1–16 in female in *Z. scabra*) (Table 2).

Type. Kenya. Nandi County, South Nandi Forest, Morongiot area, 0°04'N, 35°00'E, elev. 1980 m, 20 April 2018, *Sino-Africa Joint Investigation Team* (*SAJIT*) *006973* (Female) (holotype HIB!; isotype EA!, HIB!)

Description. Perennial climber, 8 m or longer; rhizome robust, woody when old, up to 2.5 cm in diam., roots slender, branched; stem many-branched, grooved, usually contorted when aged, sparsely puberulous except densely hairy at nodes. Leaves simple, petioles 2–7 cm long, grooved adaxially, subglabrous; blades 38–65 × 28–46 mm, ovate-cordate in outline, shallowly 3-lobed occasionally, membraneous, subglabrous, deeply cordate to subtruncate at base, margin slightly sinuate-toothed, apex acuminate and apiculate; scabrid-punctate above, 3–11 main veins sunken adaxially and protrudent abaxially, with sparsely-scattered bristles on both sides, especially on veins and margins; tendrils simple, up to 15 cm long. Dioecious. Inflorescence base with a well-developed leafy probract, up to 18 × 12 mm, ovate, incurved, beak-like, persistent, 2–3 main veins from base, base cordate, apex acuminate. Male inflorescences axillary, sessile, subumbelliform, 8- to 30-flowered, pedicels 3–12 mm long; receptacle-tube 1.8–3 mm long, campanulate, greenish-cream, turning into orange when aged, inner surface densely hairy, outside surface glabrous; sepal lobes 5, ca. 1 mm long, dentiform, pale green; petal lobes 5, ca. 1.8 × 1.5 mm, triangular-ovate, white, turning cream to orange when aged. Stamens 3, inserted in middle of tube; filaments ca. 1.5 mm long, subglabrous, lower half fused with tube; anthers ca.1 mm long, ellipsoid, 2-thecae; thecae 1 mm long, vertical, slightly curved, connective elliptic, with finely papillose hairs; disc ca. 1 mm in diam., depressed globose, obscurely trilobed, elevated. Female inflorescences axillary, sessile, 6- to 22-flowered in umbelliform clusters; pedicel 4–6 mm long; perianth similar to male flowers; ovary subglobose, glabrous, with evident neck up to 1 mm long; style 2–3.5 mm long, glabrous, stigma ca. 1.5 mm in diam., with 3 down-curved papillose lobes; staminodes 3, ca. 1.5 mm long, linear, glabrous, at base of the tube; disc ca. 1.8 mm in diam., annular, 3-lobed, surrounding base of style, free from tube. Fruits clustered, 8–10 mm in diam., subglobose, subglabrous, sparsely covered with tiny protuberances, turning from green to orange when mature; pedicel 5–10 mm long. Seed ovate in outline, narrowly bordered, lenticular, compressed.

Distribution and ecology. Numerous populations of this new species have been documented in the western parts of Kenya's forests, including Morongiot and Kobujoi

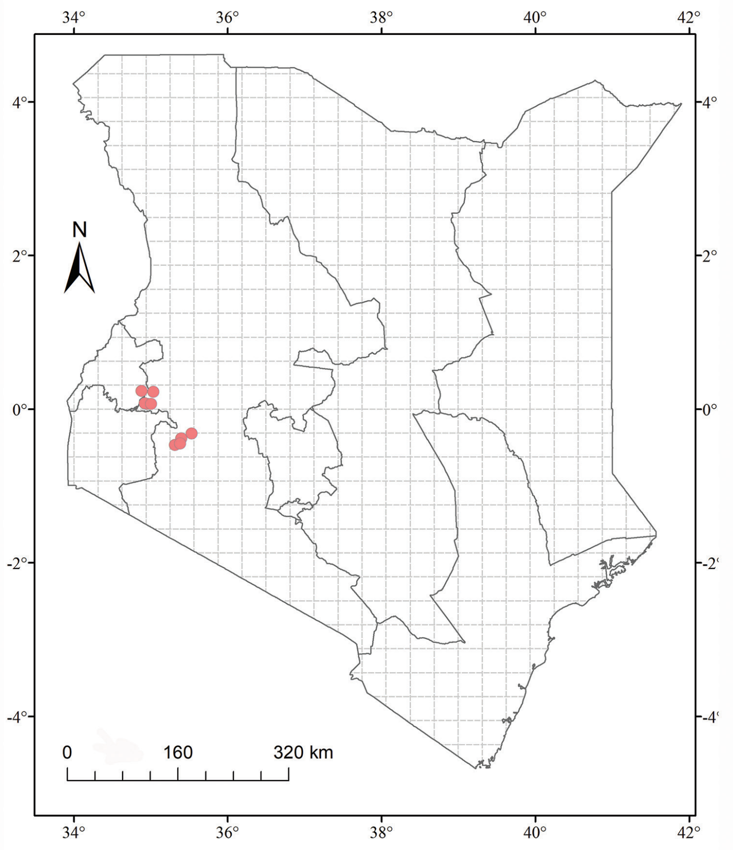

Figure 2. Distribution map of *Zehneria grandibracteata* in Kenya. Red dots indicate its documented localities.

areas of South Nandi Forest, Kapsasur area of Nandi Centre, Yale River Trail of Kakamega Forest, Timbilil and Sambret Catchment area of south-western Mau Forest. It usually climbs over tree trunks or twines around shrubs in moist forests or at forest margin at elevations of 1950–2230 m.

Conservation status. This new species was found in the western Kenyan forests with numerous localities. It is locally quite common in the wild and frequently grows in forests or at forest margins. Thus, we assess it to be "Least Concern" (LC) based on IUCN Red List Categories and Criteria (IUCN 2001).

Phenology. Flowering and fruiting from April to July and November to January, corresponding to the wet seasons of the bimodal rainfall pattern of this region.

Etymology. The epithet "*grandibracteata*" refers to the fairly large leafy probract of this new species.

Additional specimens examined (Paratypes). Kenya. Nandi County, South Nandi Forest, Kobujoi area, 34°57'E, 0°04'N, elev. 1970 m, 11 December 2016, *SA-*

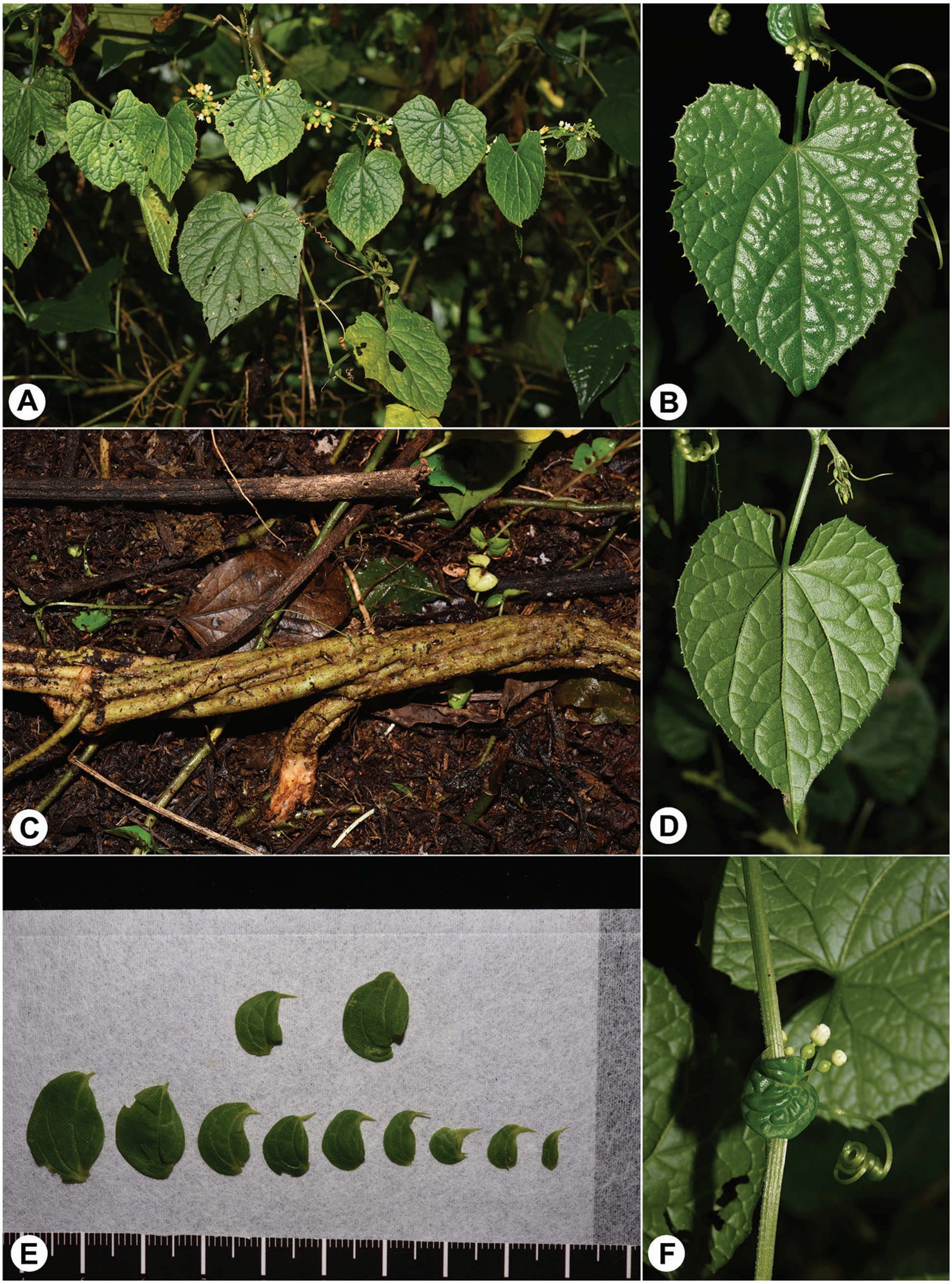

Figure 3. Photographs showing vegetative characters of *Zehneria grandibracteata* **A** climbing stem of female plant in habitat **B** adaxial lamina **C** creeping stem **D** abaxial lamina **E** probracts at different developing stages **F** tendril and probract at base of female inflorescence. Scale in picture **E** represents cm.

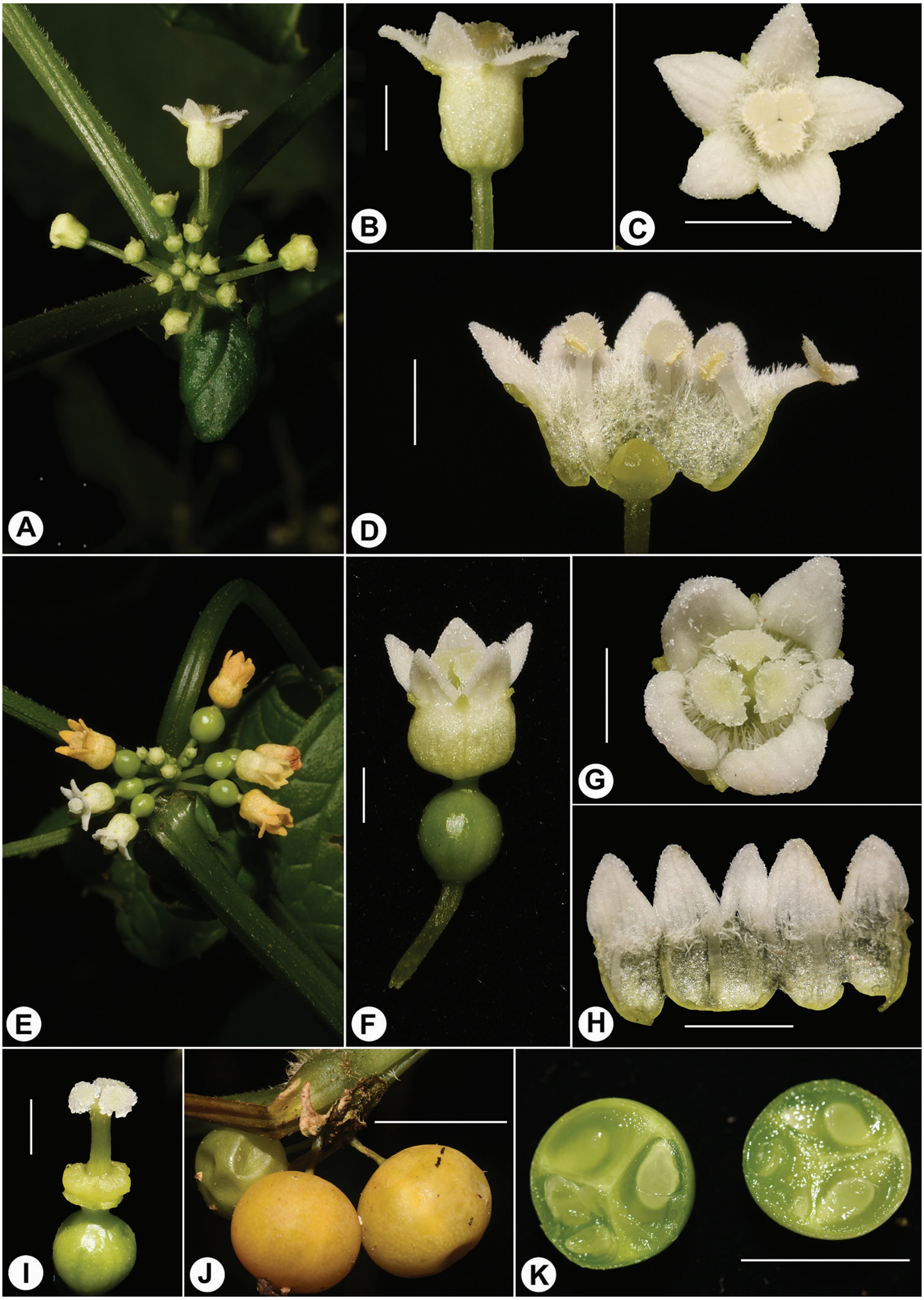

Figure 4. Photographs showing reproductive characters of *Zehneria grandibracteata* **A** male inflorescence **B** male flower, side view **C** male flower, top view **D** dissected male flower showing disc and stamens **E** female inflorescence **F** female flower, side view **G** female flower, top view **H** dissected female flower showing staminodes **I** pistil and disc **J** infructescence **K** cross-section of fruit. Scale bars: 2 mm (**B–D, F–I**); 1 cm (**J, K**).

JIT 006670 (EA! HIB!); Nandi County, South Nandi Forest, Morongiot area, 0°04'N, 34°55'E, elev. 1980 m, 19 April 2018, *SAJIT 006966* (EA! HIB!) and *SAJIT 006968* (EA! HIB!); Nandi County, Nandi Centre, Kapsasur area, elev. 1970 m, 18 April 2018, *SAJIT s.n.* (HIB!); Kakamega County, Kakamega Forest, Yale River Trail, 0°16'N, 34°52'E, 7 January 2017, *SAJIT s.n.* (HIB!); Kericho County, Changana Tea Estate, 5.3 miles south of Kericho Town, 0°27'S, 35°18'E, 22 November 1967, *Perdue R.E. and Kibuwa S.P. 9179* (BR! EA! K!); Kericho County, Sambret Catchment of south-western Mau Forest, 0°22'S, 35°23'E, 2160 m, 5 July 1962, *Kerfoot O. 3375* (EA! K!); Kericho County, Sambret Catchment of Southwestern Mau Forest, 0°26'S, 35°22'E, 2230 m, 16 Jan 1963, *Kerfoot O. 4696* (EA!); Kericho County, Timbilil of southwestern Mau Forest, 0°18'S, 35°31'E, 2130 m, Jan 1963, *Kerfoot O. 4708* (EA!).

Discussion

Our *Z. grandibracteata* collections are recognised as monophyletic, separated from the related *Z. scabra*. The possible reasons to explain the paraphyly of *Z. scabra* in our phylogeny are 1) the nrITS provides limited phylogenetically-informative sites in *Zehneria* and mutations on few loci produced inconsistent phylogenetic topology; 2) the two accessions collected by Schaefer here probably should be *Z. monocarpa*, which was separated from *Z. scabra* recently (Ngumbau et al. 2020). Furthermore, we also found that species of *Neoachmandra* in the sense of De Wilde and Duyfjes (2006a) and De Boer et al. (2015), are paraphyly. In line with the conclusion made by Dwivedi et al. (2018), the whole genus tended to be separated into two major clades (clade 1 and clade 2), with African taxa being the basal lineages. Even though the morphological characters proposed by De Wilde and Duyfjes (2006a) are not suitable for splitting groups (Dwivedi et al. 2018), they are still important and helpful characters when identifying at the species level. The ovate leafy probracts in our new species are readily distinguishable, while probracts on other East African taxa tend to be minute linear hooked or even caducous. Geographically, it is only documented in western Kenyan forests (Figure 2), while *Z. scabra* is widely distributed in the pantropical Old World region. Furthermore, the molecular phylogenetic analysis of *Zehneria*, based on nrITS sequences, also supports the segregation of *Z. grandibracteata* from *Z. scabra*. Combined with morphological and phylogenetic analyses, *Z. grandibracteata* is confirmed as new to science.

The broadly circumscribed concept of *Zehneria* may represent a better natural group, while there is no comprehensive classification system for this group until now. Jeffrey (1962) tried to divide *Zehneria* into two subgenera, namely subg. *Zehneria* and subg. *Pseudokedrostis* (Harms 1923: 616) Jeffrey (1962: 368) (largely accord with clade 1 and clade 2 here), mainly based on the position of stamen insertion, the thecae and connective of anther and length of pedicel. Viewing from the phylogenetic tree inferred by Dwivedi et al. (2018), as well our tree here, Jeffrey's morphological summaries mostly work well. Besides, the two fruit shapes, short (sub)globose and long fusiform/ellipsoid, largely fit in with clade 1 and clade 2, respectively, though several taxa with round fruits could also be found in clade 2. All these characters would provide insights into building a classification system within the genus *Zehneria*. Future biogeographical analysis, based on a robust phylogenic framework, would substantially improve our understanding towards its origin and dispersal history.

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Supplementary material 1

Modified CTAB protocol on the base of Doyle and Doyle (1987)

Authors: Neng Wei, Zhi-Xiang Zhong, David Kimutai Melly, Solomon Kipkoech, Benjamin Muema Watuma, Veronicah Mutele Ngumbau, Peris Kamau, Guang-Wan Hu, Qing-Feng Wang

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Phylogenetic relationships of '*Polyalthia*' in Fiji

Bine Xue[1], Yanwen Chen[2], Richard M.K. Saunders[2]

1 *College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, Guangdong, China* **2** *Division of Ecology & Biodiversity, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China*

Corresponding author: Bine Xue (xuebine@zhku.edu.cn)

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Abstract

The genus *Polyalthia* (Annonaceae) has undergone dramatic taxonomic changes in recent years. Nine *Polyalthia* species have historically been recognized in Fiji, all of which have subsequently been transferred to three different genera, viz. *Goniothalamus*, *Huberantha* and *Meiogyne*. The transfer of six of these species has received strong molecular phylogenetic support, although the other three species, *Polyalthia amoena*, *P. capillata* and *P. loriformis* [all transferred to *Huberantha*], have never previously been sampled in a phylogenetic study. We address this shortfall by sampling available herbarium specimens of all three species and integrating the data in a molecular phylogenetic analysis. The resultant phylogeny provides strong support for the transfer of these species to *Huberantha*. The taxonomic realignment of all nine Fijian species formerly classified in *Polyalthia* is also clearly demonstrated and supported by the resultant phylogeny. The updated taxonomic treatments of the nine species, a key to the three genera and a key to the Fijian *Huberantha* species are provided.

Keywords

Annonaceae, Fiji, *Huberantha*, molecular phylogeny, *Polyalthia*

Introduction

The genus *Polyalthia* Blume (Annonaceae) has historically been the source of considerable taxonomic confusion (van Setten and Koek-Noorman 1992; Doyle and Le Thomas 1994; Doyle et al. 2000). Recent molecular phylogenetic studies have confirmed its polyphyletic status, and accelerated the segregation of disparate elements from *Polyalthia* s.l., with distantly related species transferred to various genera, including *Fenerivia* Diels (Saunders et al. 2011), *Goniothalamus* (Blume) Hook.f. & Thomson (Tang et al. 2013), *Huberantha* Chaowasku (Chaowasku et al. 2012; Chaowasku et al. 2015), *Maasia* Mols, Keßler & Rogstad (Mols et al. 2008), *Marsypopetalum* Scheffer (Xue et al. 2011), *Meiogyne* Miquel (Xue et al. 2014), *Monoon* Miquel (Xue et al. 2012), *Polyalthiopsis* Chaowasku (Chaowasku et al., 2018; Xue et al., 2020a), *Wangia* X.Guo & R.M.K.Saunders (Xue et al. 2016), and *Wuodendron* B.Xue, Y.H.Tan & T.Chaowasku (Xue et al. 2018).

In Fiji, ten species were published under the name *Polyalthia* (Seemann 1861; Gillespie 1931; Smith 1936, 1950, 1978). In Smith's (1981) revision of Fijian *Polyalthia*, nine species were accepted: *P. amoena* A.C.Sm., *P. amygdalina* (A.Gray) Gillespie, *P. angustifolia* A.C.Sm., *P. capillata* A.C.Sm., *P. habrotricha* A.C.Sm., *P. insularis* (A.C.Sm.) A.C.Sm., *P. laddiana* A.C.Sm., *P. loriformis* Gillespie, and *P. vitiensis* Seem. The tenth species, *P. pedicellata* A.C.Sm., was treated as a synonym of *P. vitiensis* (Smith 1981). Among the nine accepted species, *P. insularis* was later recognized as *Meiogyne stenopetala* (F.Muell.) Heusden subsp. *insularis* (A.C.Sm.) Heusden (van Heusden 1994), although this was recently elevated to species rank as *Meiogyne insularis* (A.C.Sm.) D.C.Thomas, B.Xue & R.M.K.Saunders (Xue et al. 2014). Three other Fijian *Polyalthia* species were subsequently transferred to *Meiogyne*: *P. amygdalina* as *Meiogyne amygdalina* (A.Gray) B.Xue & R.M.K.Saunders; *P. habrotricha* as *Meiogyne habrotricha* (A.C.Sm.) B.Xue & R.M.K.Saunders; and *P. laddiana* as *Meiogyne laddiana* (A.C.Sm.) B.Xue & R.M.K.Saunders. The above treatments were based on combined molecular and morphological evidence (Xue et al. 2014). A fifth species, *Polyalthia angustifolia*, was transferred to *Goniothalamus* as *G. angustifolius* (A.C.Sm.) B.Xue & R.M.K.Saunders (Tang et al. 2013); although the lack of flowers in the type specimen precluded an identification as *Goniothalamus* based on the connivent inner petals, the evidence from an unpublished molecular phylogeny based on sequences of the type specimen was strong enough to support the transfer (Xue 2013). The transfer was later supported in a published phylogenetic analysis with a larger taxon sampling of *Goniothalamus* (Tang et al. 2015). The remaining four species–*P. amoena*, *P. capillata*, *P. loriformis*, and *P. vitiensis*–were transferred to *Huberantha* based on a morphological study as *Huberantha amoena* (A.C.Sm.) Chaowasku, *H. capillata* (A.C.Sm.) Chaowasku, *H. loriformis* (Gillespie) Chaowasku, and *H. vitiensis* (Seem.) Chaowasku (Chaowasku et al. 2015) [initially under the generic name *Hubera* Chaowasku (Chaowasku et al. 2012), although this name was considered illegitimate (Chaowasku 2013; Applequist 2014)]. All nine Fijian *Polyalthia* species have therefore been realigned to three different genera.

Turner and Utteridge (2017) recently reviewed the taxonomy and distribution of Pacific Annonaceae and incorporated the above-mentioned treatments of Fijian *Polyalthia* species. It is noteworthy that although the treatment of five of the Fijian *Polyalthia* species has been supported by molecular studies (Xue 2013; Xue et al. 2014; Tang et al. 2015), the transfer of the other four species to *Huberantha* was only based on morphological comparison (Chaowasku et al. 2012, 2015). Thomas et al. (2015) sampled *Huberantha vitiensis* (as '*Hubera vitiensis*') in their phylogenetic study while studying the origins of intercontinental disjunctions in Annonaceae, and confirmed its taxonomic affinity with *Huberantha*. The other three species have never previously been sampled in a phylogenetic study.

As the genus *Huberantha* is taxonomically challenging and difficult to recognize, the transfer for some species based on limited collections may be problematic in the absence of molecular evidence. One example is *Polyalthia floribunda* Jovet-Ast from Vietnam (Jovet-Ast 1940), which was transferred to *Huberantha* based on its cuneate, symmetrical leaf bases, single ovule per carpel and leaf venation pattern (Turner 2016). A recent molecular phylogenetic study has revealed that the species is not congeneric with *Huberantha*, however, but is sister to *Miliusa*, although without statistical support (Chaowasku et al. 2018). A new genus, *Polyalthiopsis* Chaowasku, was therefore erected to accommodate it (Chaowasku et al. 2018). The sister relationship between *Polyalthiopsis* and *Miliusa* was later supported by Xue et al. (2020a, b) and Chaowasku et al. (2020), redefining the long-recognized sister relationship between *Huberantha* and *Miliusa* in previous studies (Mols et al. 2008; Saunders et al. 2011; Xue et al. 2011, 2012; Chaowasku et al. 2012, 2014).

As nomenclatural transfers based solely on morphological data can sometimes be misleading, molecular phylogenetic data can provide invaluable evidence for confirming correct taxonomic placement. To avoid such errors, we have therefore sampled the remaining three Fijian *Huberantha* species and undertaken a phylogenetic study to confirm their taxonomic placements.

Materials and methods

Three Fijian *Huberantha* species that lack DNA sequence data–*H. amoena*, *H. capillata* and *H. loriformis*–were sampled in this study to verify their generic position. The other six previously recognized Fijian '*Polyalthia*' species were also included in this study. Sequence data for three commonly used chloroplast regions (*matK*, *rbcL* and *trnL-F*) were newly generated for the three *Huberantha* species. Sequences for other taxa were downloaded from the nucleotide database of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The final data matrix comprised a total of 77 Annonaceae species, representing the major clades in the family. The samples, localities and GenBank accession numbers are listed in the Appendix 1.

The phylogenetic trees were reconstructed using Bayesian Inference (BI) and maximum likelihood (ML) methods. Detailed information regarding DNA extraction, PCR amplification, and primer sequences are available (Xue et al. 2011, 2012), as is information on sequence alignment, model selection of the sequence matrix constructed and methods in tree reconstruction (Xue et al. 2018).

Results

The concatenated alignment of the 77-taxon dataset consisted of 3,659 aligned positions (*trnL-F*: 1,475 bp; *matK*: 834 bp; and *rbcL*: 1,350 bp). The Bayesian and ML analyses resulted in similar topologies. The 50% majority-rule consensus tree resulting from the Bayesian analysis under the three-partitioned model is shown as Fig. 1. The results are consistent with previous phylogenetic analyses of the family in which the backbone of the tribe Miliuseae remains largely unresolved.

The Fijian species previously assigned to *Polyalthia* are retrieved in three distinct clades (Fig. 1). *Huberantha amoena*, *H. capillata*, *H. loriformis*, and *H. vitiensis* form a well-supported clade (PP = 1; ML BS = 100%) nested within *Huberantha* (PP =1; ML BS = 100%), with *H. nitidissima* (Dunal) Chaowasku and *H. jenkinsii* (Hook.f. & Thomson) Chaowasku forming the sister clade (PP =1; ML BS = 75%). *Polyalthiopsis* is recovered as sister to *Miliusa* instead of *Huberantha*, although lacking statistical support. *Meiogyne amygdalina*, *M. habrotricha*, *M. laddiana*, and *M. insularis* form a well-supported clade (PP = 1; ML BS = 97%) nested within *Meiogyne* (PP = 1; ML BS = 70%), with *Meiogyne amicorum* (A.C.Sm.) B.Xue & R.M.K.Saunders from Tonga being the closest sister clade (PP = 0.99; ML BS = 78%). *Goniothalamus angustifolius* is nested within the *Goniothalamus* clade (PP = 1; ML BS = 100%) and closely related to another *Goniothalamus* species in Fiji, *G. monospermus* (A.Gray) R.M.K.Saunders (PP = 1; ML BS = 85%).

Discussion

The transfer of *Polyalthia amoena*, *P. capillata* and *P. loriformis* to *Huberantha* is supported here in a molecular phylogenetic analysis for the first time. The four Fijian *Huberantha* species form a well-supported clade that shows a close affinity with *H. nitidissima* (distributed in Papua New Guinea, Australia, and New Caledonia) and *H. jenkinsii* (distributed in continental Asia and western Malesia). The clade comprising *Polyalthia nitidissima* and the Fijian '*Polyalthia*' species is well separated from its sister clade consisting of species from continental Asia, Africa and Madagascar; this is consistent with the phylogeny including only one Fijian *Huberantha* species (*H. vitiensis*) published by Thomas et al. (2015).

Huberantha can be distinguished from other closely related genera by a combination of characters, including leaves with reticulate tertiary venation, axillary inflorescences, a single ovule per ovary (and therefore single-seeded monocarps), seeds with a flat to slightly raised raphe, spiniform(-flattened peg) ruminations of the endosperm, and pollen with a finely and densely granular infratectum (Chaowasku et al. 2012). It resembles *Polyalthia* and *Polyalthiopsis* in having brochidodromous foliar venation with reticulate tertiary veins. *Polyalthia* differs in having a generally asymmetrical leaf base and ovaries with 2–6 ovules (Xue et al. 2012). *Polyalthiopsis* differs in having foliar glands, petioles

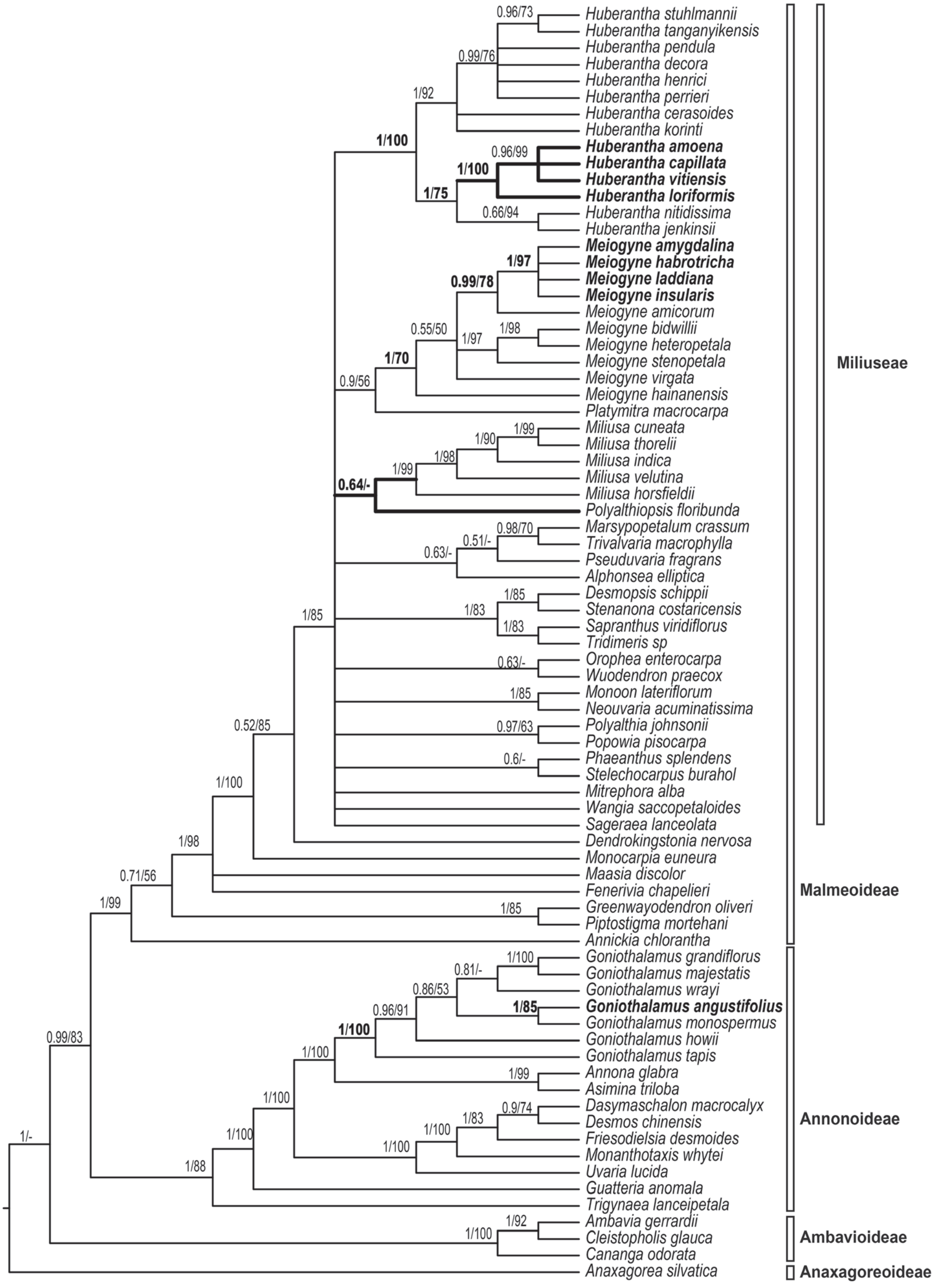

Figure 1. Bayesian 50% majority-rule consensus tree inferred from combined data of *matK*, *rbcL* and *trnL-F* under three-partitioned models. Numbers at the nodes indicate BI posterior probabilities and ML bootstrap values (> 50%). Species names of previous Fijian *Polyalthia* are in bold.

with transverse striations when dry, a leaf midrib that is raised adaxially in vivo, ovaries with 1–2 ovules, and lamelliform endosperm rumination (Chaowasku et al. 2018; Xue et al. 2020a). These four Fijian *Huberantha* species all possess a symmetrical leaf base, a flat leaf midrib adaxially, axillary inflorescences and single-seeded monocarps (Fig. 2). It is noteworthy that the monocarps are much larger, however, especially in *P. capillata* (25–30 cm long, 0.5–1cm broad; Fig. 2B), *P. loriformis* (up to 4 cm long, 1 cm broad; Fig. 2C) and *P. vitiensis* (up to 4.5 cm long, 1.5 cm broad; Fig. 2D) (Gillespie 1931, Smith 1950). Other *Huberantha* species, including *H. nitidissima* and *H. jenkinsii*, have much smaller monocarps, with the largest dimension rarely exceeding 1 cm. Seed size is possibly correlated with various factors, including overall plant size and architecture, dispersal agents, habitat and insularity (Bellot et al. 2020): the larger fruits of these Fijian *Huberantha* species probably reflect adaptations to alternative dispersal vectors.

The taxonomic placement of *Goniothalamus angustifolius* and the four *Meiogyne* species (*M. amygdalina*, *M. habrotricha*, *M. insularis* and *M. laddiana*) are confirmed in our study (Fig. 1): *G. angustifolius* is sister to another Fijian *Goniothalamus* species, *G. monospermus*; and the four Fijian *Meiogyne* species form a well-supported clade, although the relationship among these species is not well resolved since our analysis is based on only three chloroplast regions. The topology is consistent with a better-resolved topology based on seven markers reconstructed by Thomas et al. (2012) and Xue et al. (2014), however, with the Fijian clade sister to *M. amicorum* from Tonga, together forming a well-supported clade within the Australian-Pacific clade of *Meiogyne* species (Thomas et al. 2012, Xue et al. 2014).

Conclusions

The transfer of *Polyalthia amoena*, *P. capillata* and *P. loriformis* to *Huberantha* is supported here in a molecular phylogenetic study for the first time. The phylogenetic analyses of previous Fijian *Polyalthia* species confirm that this group is a highly heterogeneous assemblage, with nine species now divided into three distantly related genera, viz. *Goniothalamus*, *Huberantha* and *Meiogyne*. The updated taxonomic treatments of the nine species, a key to the three genera and a key to the four *Huberantha* species, are provided below.

Taxonomic treatment of the nine previous Fijian *Polyalthia* species

Goniothalamus angustifolius (A.C.Sm.) B.Xue & R.M.K.Saunders, PhytoKeys 32: 33. 2013.

Basionym. *Polyalthia angustifolia* A.C.Sm., Bull. Torrey Bot. Club 70: 538. 1943.

Type. Fiji, Viti Levu, Naitasiri Province, Tamavua woods, 7 miles from Suva, 9 Aug. 1927, *J.W. Gillespie 2198* (holotype: A[A00039617]; isotypes: GH[GH00039618], BISH).

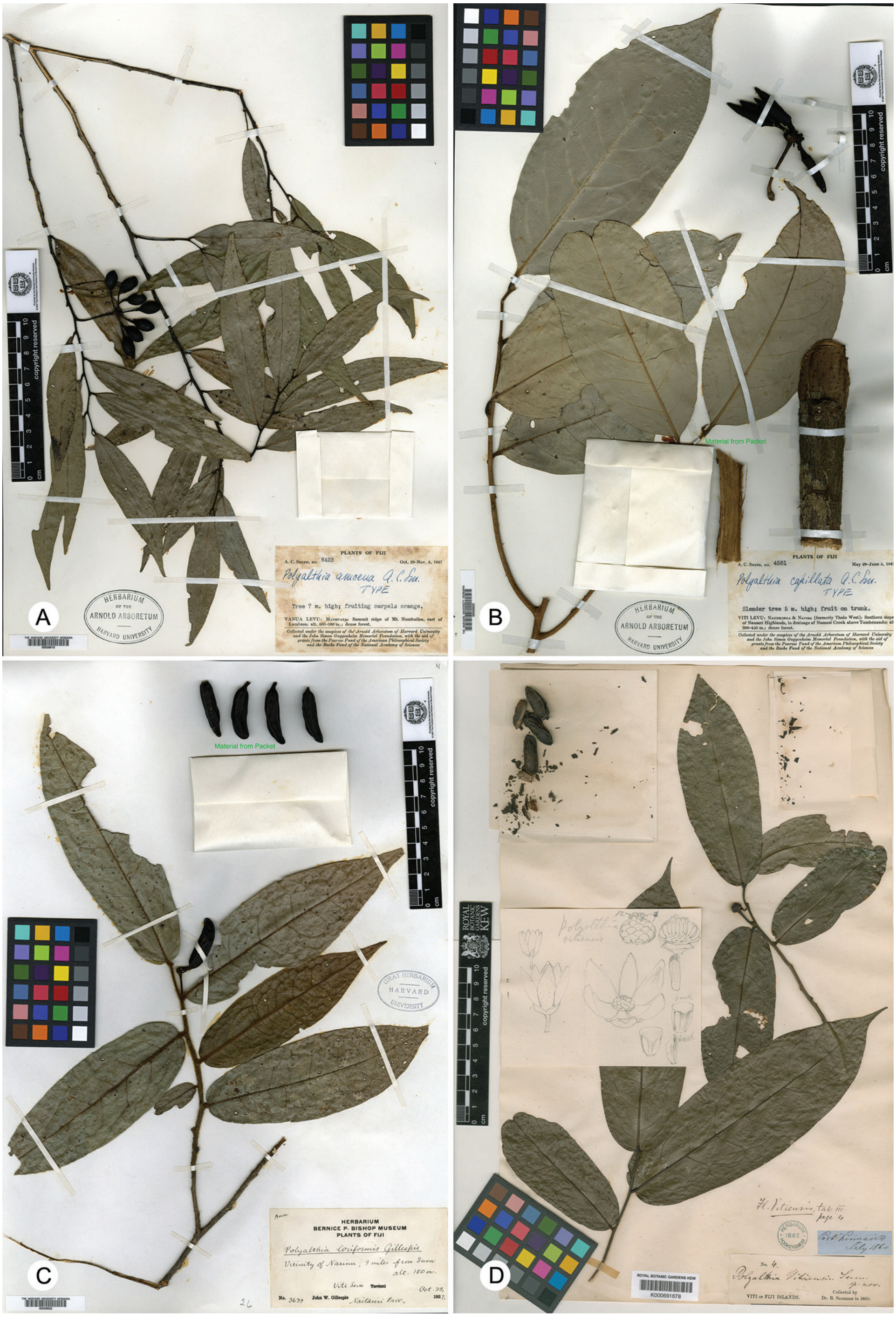

Figure 2. Type specimens of the four Fijian *Huberantha* species **A** *Huberantha amoena* (*A.C. Smith 6423*, A) **B** *H. capillata* (*A.C. Smith 4581*, A) **C** *H. loriformis* (*J.W. Gillespie 3639*, GH) **D** *H. vitiensis* (*B. Seemann 4*, K).

Huberantha amoena (A.C.Sm.) Chaowasku, Kew Bulletin 70(2)–23: 2. 2015.

Basionym. *Polyalthia amoena* A.C.Sm., Journal of the Arnold Arboretum 31: 159. 1950.

Homotypic synonym. *Hubera amoena* (A.C.Sm.) Chaowasku, Phytotaxa 69: 47. 2012.

Type. Fiji, Vanua Levu, Mathuata Province, east of Lambasa, on the summit ridge of Mt. Numbuiloa, 29 Oct. 1947, *A.C. Smith 6423* (holotype: A[A00039619]; isotypes: BISH, BRI[BRI-AQ0211645], K[K000691676], L[L0038107], P[P00636930], S[S-G-7470], US[US00098656]).

Huberantha capillata (A.C.Sm.) Chaowasku, Kew Bulletin 70(2)–23: 2. 2015.

Basionym. *Polyalthia capillata* A.C.Sm., Journal of the Arnold Arboretum 31: 158. 1950.

Homotypic synonym. *Hubera capillata* (A.C.Sm.) Chaowasku, Phytotaxa 69: 47. 2012.

Type. Fiji, Viti Levu, Nandronga & Navosa Province, on the southern slopes of the Nausori Highlands, in the drainage of Namosi Creek, above Tumbenasolo, 29 May 1947, *A.C. Smith 4581* (holotype: A[A00039620]; isotypes: BISH, BRI[BRI-AQ0332771], K[K000691675], US[US00098658]).

Huberantha loriformis (Gillespie) Chaowasku, Kew Bulletin 70(2)–23: 3. 2015.

Basionym. *Polyalthia loriformis* Gillespie, Bulletin of the Bernice P. Bishop Museum 83: 4, fig. 1. 1931.

Homotypic synonym. *Hubera loriformis* (Gillespie) Chaowasku, Phytotaxa 69: 49. 2012.

Type. Fiji, Viti Levu, Naitasiri Province, in the vicinity of Nasinu, 29 Oct. 1927, *J.W. Gillespie 3639* (holotype: BISH[BISH1011147]; isotypes: BISH[BISH1011148], GH[GH00039622], NY[NY00026209]).

Huberantha vitiensis (Seem.) Chaowasku, Kew Bulletin 70(2)–23: 3. 2015.

Basionym. *Polyalthia vitiensis* Seem., Flora Vitiensis 1: 4, pl. 3. 1865.

Homotypic synonym. *Hubera vitiensis* (Seem.) Chaowasku, Phytotaxa 69: 51. 2012.

Heterotypic synonym. *Polyalthia pedicellata* A.C.Sm., Bulletin of the Bernice P. Bishop Museum 141: 61, fig. 29. 1936.

Type. Fiji, Ovalau, near Port Kinnaird, Jul. 1860, *B. Seemann 4* (holotype: K[K000691678]).

Meiogyne amygdalina **(A.Gray) B.Xue & R.M.K.Saunders, Syst. Bot. 39(2): 401. 2014.**

Basionym. *Uvaria amydalina* A.Gray, Bot. U.S. Expl. Exped. 1: 31. 1854.

Homotypic synonym. *Polyalthia amygdalina* A.Gray Gillespie, Bernice P. Bishop Mus. Bull. 83: 4. 1931.

Heterotypic synonym. *Desmos leucanthus* A.C.Sm., J. Arnold Arbor. 31 (2): 156. 1950.

Type. Fiji, Ovalau, 1840, *Wilkes Explor. Exped. s.n.* (hololectotype, designated by Smith (1936: 60): GH[GH00039616]; isolectotype: US[US00104128]).

Meiogyne habrotricha **(A.C.Sm.) B.Xue & R.M.K.Saunders, Syst. Bot. 39(2): 401. 2014.**

Basionym. *Polyalthia habrotricha* A.C.Sm., J. Arnold. Arbor. 31: 157–158. 1950.

Type. Fiji, Viti Levu, Nandronga & Navosa Province, on the northern portion of the Rairaimatuku Plateau, between Nandrau and Rewasau, 11 Aug. 1947, *A.C. Smith 5614* (holotype: A[A00019830]).

Meiogyne insularis **(A.C.Sm.) D.C.Thomas, B.Xue & R.M.K.Saunders, Syst. Bot. 39(2): 401. 2014.**

Basionym. *Desmos insularis* A.C.Sm., Sargentia 1: 31–32. 1942.

Homotypic synonyms. *Polyalthia insularis* (A.C.Sm.) A.C.Sm., Allertonia 1: 351. 1978. *Meiogyne stenopetala* subsp. *insularis* (A.C.Sm.) Heusden, Blumea 38: 507. 1994.

Type. Fiji, Viti Levu, Mba Province, east of Tavua, near Korovou, 1 Apr. 1941, *O. Degener 14968* (holotype: A[A00019829]; isotypes: BISH[BISH1000666], F, K[K000691250], L[L0037996], MICH, P[P00636931], S, US, WIS).

Meiogyne laddiana **(A.C.Sm.) B.Xue & R.M.K.Saunders, Syst. Bot. 39(2): 401. 2014.**

Basionym. *Polyalthia laddiana* A.C.Sm., Bernice P. Bishop Mus. Bull. 141: 60–61, fig. 28. 1936.

Type. Fiji, Fulanga, 22 Feb. 1934, *A.C. Smith 1147* (holotype: BISH; isotypes: GH[GH00039621], K[K000691674], NY[NY00026208], P[P00636929], S[S07-13360], US[US00098666], WIS[WIS00000302MAD]).

Key to *Goniothalamus*, *Huberantha* and *Meiogyne* in Fiji

1 Flowers with inner petals connivent, forming a mitriform dome over the reproductive organs .... ***Goniothalamus***

– Flowers with inner petals spreading **2**

2 Inner petals adaxially grooved at the base; staminal connectives with a tongue-shaped apical prolongation in innermost stamens; 1 to many seeds per monocarp .... ***Meiogyne***

– Inner petals not grooved; staminal connectives of innermost stamens not expanded; 1 seed per monocarp .... ***Huberantha***

Key to species of *Huberantha* in Fiji

1 Leaf blade narrowly lanceolate; monocarps ellipsoid; stipe c. 10–20 mm long .... ***H. amoena***

– Leaf blade ovate or broadly lanceolate; monocarps oblong; stipe less than 10 mm long **2**

2 Leaf base obtuse, petiole 8–12 mm long .... ***H. capillata***

– Leaf base rounded or subcordate, petiole 2–6 mm long **3**

3 Young branches and leaves often persistently yellowish-hirsute .... ***H. loriformis***

– Young branches and leaves glabrous .... ***H. vitiensis***

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Appendix 1

Voucher information and GenBank accession numbers for samples used in this study (—, missing data; *, newly generated sequences). Voucher data are given for accessions for which DNA sequences were newly obtained, using the following format: species, origin, voucher and Genbank accession numbers for *matK*, *rbcL* and *trnL-F*. For DNA sequences published in previous studies, voucher information is available from GenBank.

Alphonsea elliptica Hook.f. & Thomson, AY518807, —, AY319078; ***Ambavia gerrardii*** (Baill.) Le Thomas, AY220435, —, AY220411(intron), AY220358(spacer); ***Anaxagorea silvatica*** R.E.Fr., AY743477, AY743439, AY743458; ***Annickia chlorantha*** (Oliv.) Setten & Maas, AY841393, AY841594, AY841671; ***Annona glabra*** L., DQ125050, AY841596, AY841673; ***Asimina triloba*** (L.) Dunal, AY743479, AY743441, AY743460; ***Cananga odorata*** (Lam.) Hook.f. & Thomson, AY841394, AY841602, AY841680; ***Cleistopholis glauca*** Pierre ex Engl. & Diels, AY841395, AY841603, AY841681; ***Dasymaschalon macrocalyx*** Finet & Gagnep., EF179277, AY841610, AY841688; ***Dendrokingstonia nervosa*** (Hook.f. & Thomson) Rauschert, KJ418392, KJ418382, KJ418407; ***Desmopsis schippii*** Standl., AY518805, AY319060, AY319174; ***Desmos chinensis*** Lour., JQ768567, JQ762414, JQ762415; ***Fenerivia chapelieri*** (Baill.) R.M.K.Saunders, JF810375, JF810387, JF810399; ***Friesodielsia desmoides*** (Craib) Steenis, JQ768577, JQ768696, JQ768738; ***Goniothalamus angustifolius*** (A.C.Sm.) B.Xue & R.M.K.Saunders, KM818569, KM818797, KM818878; ***Goniothalamus grandiflorus*** (Warb.) Boerl., KM818587, KM818802, KM818851; ***Goniothalamus howii*** Merr. & Chun, KM818590, KM818833, KM818886; ***Goniothalamus majestatis*** P.J.A.Kessler, KM818598, KM818788, KM818903; ***Goniothalamus monospermus*** (A.Gray) R.M.K.Saunders, KM818601, KM818790, —; *Goniothalamus tapis* Miq., DQ125058, AY841622, AY841700; *Goniothalamus wrayi* King, KM818630, KM818803, KM818859; ***Greenwayodendron oliveri*** (Engl.) Verdc., AY743489, AY743451, AY743470; ***Guatteria anomala*** R.E.Fr., AY740913, AY740962, AY741011; ***Huberantha amoena*** (A.C.Sm.) Chaowasku, Fiji, Vanua Levu, A. C. Smith 6423 (A), MW024830*, —, MW024834*; ***Huberantha capillata*** (A.C.Sm.) Chaowasku, Fiji, Vanua Levu, A. C. Smith 4581 (A), MW024831*, —, MW024835*; ***Huberantha cerasoides*** (Roxb.) Chaowasku, AY518854, AY319017, AY319131; ***Huberantha decora*** (Diels) Chaowasku, —, —, JX544869; ***Huberantha henrici*** (Diels) Chaowasku, —, —, JX544870; ***Huberantha jenkinsii*** (Hook.f. & Thomson) Chaowasku, —, —, JX544803; ***Huberantha korinti*** (Dunal) Chaowasku, EU522234, EU522289, EU522179; ***Huberantha loriformis*** (Gillespie) Chaowasku, Fiji, Vanua Levu, J. W. Gillespie 2055 (NY), MW024832*, MW024833*, MW024836*; ***Huberantha nitidissima*** (Dunal) Chaowasku, KF682110, KF682103, KF682105; ***Huberantha pendula*** (Capuron ex G.E.Schatz & Le Thomas) Chaowasku, AY518852, AY319030, AY319144; ***Huberantha perrieri*** (Cavaco & Keraudren) Chaowasku, —, —, JX544871; ***Huberantha stuhlmannii*** (Engl.) Chaowasku, AY518853, —, AY319149; ***Huberantha tanganyikensis*** (Vollesen) Chaowasku, —, —, JX544872; ***Huberantha vitiensis*** (Seem.) Chaowasku, KM924849, KM924919, KM924950; ***Maasia discolor*** (Diels) Mols, P.J.A.Kessler & Rogstad, AY518872, AY319021, AY841584; ***Marsypopetalum crassum*** (R.Parker) BXue & R.M.K.Saunders, HQ286571, HQ286577, HQ286583; ***Meiogyne amicorum*** (A.C.Sm.) B.Xue, D.M.Johnson & R.M.K.Saunders, KF301021, —, KF573503; ***Meiogyne amygdalina*** (A.Gray) B.Xue, D.M.Johnson & R.M.K.Saunders, KF301022, —, KF573497; ***Meiogyne bidwillii*** (Benth.) D.C.Thomas, Chaowasku & R.M.K.Saunders, JQ723764, JQ723851, JQ723904; ***Meiogyne habrotricha*** (A.C.Sm.) B.Xue, D.M.Johnson & R.M.K.Saunders, KF301025, —, KF573498; ***Meiogyne hainanensis*** (Merr.) Bân, JQ723773, JQ723860, JQ723913; ***Meiogyne heteropetala*** (F. Muell.) D.C.Thomas, Chaowasku & R.M.K.Saunders, JQ723766, JQ723853, JQ723906; ***Meiogyne insularis*** (A.C.Sm.) D.C.Thomas, B.Xue & R.M.K.Saunders, KF301028, —, KF573502; ***Meiogyne laddiana*** (A.C.Sm.) B.Xue, D.M.Johnson & R.M.K.Saunders, KF301026, —, KF573499; ***Meiogyne stenopetala*** (F.Muell.) Heusden, JQ723779, JQ723866, JQ723919; ***Meiogyne virgata*** (Blume) Miq., AY518798, AY318982, AY319094; ***Miliusa cuneata*** Craib, AY518844, —, AY319097; ***Miliusa horsfieldii*** (Benn.) Pierre, AY518849, —, AY319098; ***Miliusa indica*** Lesch. ex A.DC., JQ723781, JQ723868, JQ723921; ***Miliusa thorelii*** Finet & Gagnep., AY518846, —, AY319104; ***Miliusa velutina*** (Dunal) Hook.f. & Thomson, AY518847, AY318993, AY319105; ***Mitrephora alba*** Ridl., AY518855, AY318994, AY319106; ***Monanthotaxis whytei*** (Stapf) Verdc., EF179278, AY841635, AY841713; ***Monocarpia euneura*** Miq., AY518865, AY318998, AY319111; ***Monoon lateriflorum*** (Blume) B.Xue & R.M.K. Saunders, JQ723783, JQ723870, JQ723923; ***Neo-uvaria acuminatissima*** (Miq.) Airy–Shaw, AY518793, AY318999, AY319112; ***Orophea enterocarpa*** Maingay ex Hook.f. & Thomson, AY518815, —, AY319119; ***Phaeanthus splendens*** Miq., AY518864, JX544754, AY319126; ***Piptostigma mortehani*** De Wild., AY743492, AY743454, AY743473; ***Platymitra macrocarpa*** Boerl., AY518812, AY319013, AY319127; ***Polyalthia johnsonii*** (F.Muell.) B.Xue & R.M.K.Saunders, JQ723767, JQ723854, JQ723907; ***Polyalthiopsis floribunda*** (Jovet-Ast) Chaowasku, Chaowasku 168, MG264583, MG264580, MG264575; ***Popowia pisocarpa***

(Blume) Endl., AY518862, AY319044, AY319158; ***Pseuduvaria fragrans*** Y.C.F.Su, Chaowasku & R.M.K.Saunders, JQ723784, JQ723871, JQ723924; ***Sageraea lanceolata*** Miq., AY518799, AY319050, AY319164; ***Sapranthus viridiflorus*** G.E.Schatz, AY743493, AY319051, AY319165; ***Stelechocarpus burahol*** (Blume) Hook.f. & Thomson, AY518803, AY319053, AY319167; ***Stenanona costaricensis*** R.E.Fr., AY518801, AY319069, AY319183; ***Tridimeris*** sp., JX544750, JX544753, JX544782; ***Trigynaea lanceipetala*** D.M.Johnson & N.A.Murray, AY743487, AY743449, AY743468; ***Trivalvaria macrophylla*** (Blume) Miq., HQ286576, HQ286582, HQ286588; ***Uvaria lucida*** Benth., AY238966, AY238957, EF179319; ***Wangia saccopetaloides*** (W.T.Wang) X.Guo & R.M.K.Saunders, KF680920, KF680926, KF680930; ***Wuodendron praecox*** (Hook.f. & Thomson) B.Xue, Y.H.Tan & X.L.Hou, MF687367, MF687373, MF687375.

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A new species of *Thinouia* (Paullinieae, Sapindaceae) from the Amazon and its phylogenetic placement

Herison Medeiros[1,3], Jenifer de Carvalho Lopes[1],
Pedro Acevedo-Rodríguez[2], Rafaela Campostrini Forzza[3]

1 *Universidade de São Paulo, Instituto de Biociências, Departamento de Botânica, Rua do Matão, 277, 05508-090 São Paulo, SP, Brazil* **2** *Department of Botany, MRC-166 Smithsonian Institution, P.O. Box 37012, Washington D.C. 20013-7012, USA* **3** *Jardim Botânico do Rio de Janeiro, Pacheco Leão, 915, 22460-030, Rio de Janeiro, Rio de Janeiro, Brazil*

Corresponding author: Herison Medeiros (medeiros.herison@gmail.com)

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Abstract

Thinouia is a Neotropical genus of lianas with approximately 12 species and is the only genus in tribe Paullinieae with actinomorphic flowers. During a taxonomic revision of the genus and fieldwork in southwestern Amazonia, we found a new species that appears similar to *Thinouia trifoliata* (ex *Allosanthus*) because of its racemiform inflorescence. However, before describing the new species, we had to confirm that *Allosanthus* was congeneric with *Thinouia* so we could place the new species in the correct genus. The results of the phylogenetic analysis, based on molecular data (*trnL* intron and ITS sequences), show that *Allosanthus* should be included in *Thinouia*. Thus, the new taxon is described here as *Thinouia cazumbensis* **sp. nov**. The new species is described, illustrated and phylogenetic trees showing relationships within supertribe Paulliniodae and *Thinouia* and the congeneric *Allosanthus* are given.

Keywords

Allosanthus, Amazonia, Brazil, lianas, neotropical biodiversity, Paulliniodae, Paullinieae, Sapindales, Sapindaceae, taxonomy, *Thinouia*

Introduction

Thinouia is a neotropical genus of lianas that includes around 12 species, of which *T. myriantha* Planch. & Triana is widely distributed, including records from Mexico, Central America and northern South America (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2011). The remaining species are distributed in Brazil, Bolivia, Paraguay and Peru, except for *T. tomocarpa* Standl. which is restricted to Mexico, Belize and Guatemala. Most *Thinouia* species occur in rainforest; a few species occur in savannah (BFG 2015).

Thinouia was proposed by Triana and Planchon (1862). It is characterised by the presence of umbelliform and racemiform thyrses, actinomorphic flowers with marginal or bifid petal appendages, an annular disc and schizocarpic fruits that split into three mericarps, each with a distal wing (Ferrucci and Somner 2008; Acevedo-Rodríguez et al. 2017).

Molecular phylogenetic studies show that *Thinouia* is a monophyletic group in Sapindaceae. In the most recent phylogenetic study, tribe Paullinieae (i.e. *Cardiospermum*, *Lophostigma*, *Paullinia*, *Serjania*, *Thinouia* and *Urvillea*) is a well-supported clade with *Thinouia* sister to the remaining genera (Acevedo-Rodríguez et al. 2017). In the same work, the monospecific genus *Allosanthus* (*A. trifoliatus* Radlk.) was maintained as a synonym of *Thinouia*, based on morphological characters. The only differentiating character (i.e. a racemiform inflorescence) was not considered worthy of generic recognition (Acevedo-Rodríguez et al. 2011, 2017).

During a taxonomic revision of the genus and fieldwork in south-western Amazonia, we found a new species of *Thinouia* that is similar to *Thinouia trifoliata* (Radlk.) Acev.-Rodr. & Ferrucci because of its racemiform inflorescence. Since we now have high-quality DNA material for the taxa previously assigned to *Allosanthus*, we re-analysed the placement of *Allosanthus* within *Thinouia* and further tested the monophyly of *Thinouia s.l.*, which revealed the correct position of the new species.

Material and methods

Plant material

We collected the new species in Reserva Extrativista do Cazumbá-Iracema in Sena Madureira, Acre, Brazil. The collection was pressed and dried for vouchers, leaves were collected in silica gel for DNA extraction and reproductive structures were fixed in 70% alcohol for morphological analyses, which were performed using a stereomicroscope. The morphological structures were described using the terminology in Radford et al. (1974) and Weberling (1989). The herbarium abbreviations cited in the text follow Thiers (2020, cont. upd.).

Phylogenetic analysis

The phylogenetic analysis included the same taxa and molecular markers of Acevedo-Rodríguez et al. (2017), 93 taxa, plastid marker trnL intron and nuclear ribosomal internal transcribed spacer, ITS. Six samples (*Allosanthus* sp., *Allosanthus trifoliatus*, *Thinouia mucronata*, *T. myriantha*, *T. obliqua* and *Thinouia* sp.), including the new species, were added to the analysis, using the same molecular markers. For these additional taxa, approximately 60 mg of leaf tissue were pulverised with Tissuelyzer (Qiagen, Duesseldorf, Germany) for 3 min at 60 hz. The DNA extraction used the DNA NucleoSpin Plant II kit (Machery-Nagel, GmbH & Co. KG, Dueren, Germany) following the manufacturer's protocol. Primers and the PCR amplification were used, as described in Acevedo-Rodríguez et al. (2017). Products were purified and sequenced by Macrogen (Seoul, South Korea). All sequences, vouchers and GenBank accession numbers are summarised in Appendix I.

The alignments were performed using MAFFT (Katoh et al. 2002) using the default parameters implemented in Geneious 2020.0.5 (Kearse et al. 2012). Poorly-aligned regions were removed and adjusted manually. We used jModelTest 2.0 (Guindon et al. 2010; Darriba et al. 2012) and the Akaike Information Criterion (AIC) to select the best-fit model of nucleotide substitution for each dataset. The GTR+I+G was selected as the best model for the ITS dataset, whereas the GTR+G was selected as the best model for the trnL dataset. Bayesian Inference (BI) analyses were conducted using MrBayes 3.2.2 (Ronquist et al. 2012) in the online CIPRES Science Gateway interface (Miller et al. 2015) with four Markov Chain Monte Carlo (MCMC) runs using a random starting tree and 10 million generations, with a sampling frequency of one every 1000 generations. We used Tracer 1.7 (Rambaut et al. 2018) to check for convergence of the MCMC and to check for stationarity. We discarded 25% of the trees as burn-in.

Phylogenetic trees were plotted and built inside the R environment (R Core Team 2020), version 3.6.2, using the packages ggplot2 (Wickham et al. 2020), ggtree (Yu et al. 2017; Yu and Lam 2020) and cowplot (Wilke 2019).

Results

Phylogenetic results

The ITS dataset included 99 terminals and 876 bp, the trnL dataset included 99 terminals and 727 bp and the combined dataset included 99 terminals and 1604 bp. Phylogenetic trees from the analyses of the combined dataset showed high posterior probability values (PP > 0.8). Only the topology from the combined analysis is described here (Fig. 1). Separate analyses of each locus did not reveal any strong groupings that would indicate incongruences.

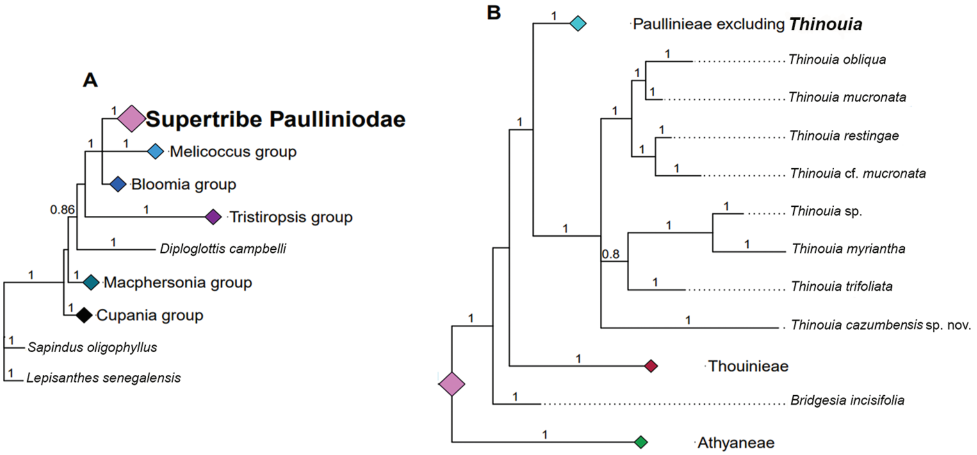

Figure 1. A bayesian 50% majority-rule consensus tree from a Bayesian analysis of the combined, two-marker dataset for Paulliniodae and outgroups **B** relationships of *Thinouia* and the congeneric *Allosanthus* [(=*Thinouia trifoliata* (Radlk.) Acev.-Rodr. & Ferrucci], including the newly-described *Thinouia cazumbensis* sp. nov. Bayesian posterior probability values are indicated above the branches.

Supertribe Paulliniodae is strongly supported as monophyletic (Fig. 1 A, PP = 1.0). The tribe Paullinieae is also strongly supported as monophyletic (PP = 1.0) and the genus *Thinouia* (including *Allosanthus*) is recovered as the clade, sister to the remaining genera of the tribe Paullineae (PP = 1.0). *Thinouia* species are grouped in two main clades that are in a polytomy with the new species *Thinouia cazumbensis*. The first clade (PP = 1.0) includes *Thinouia obliqua*, *T. mucronata*, *T. restingae* and *T.* cf. *mucronata* species. The second one (PP = 0.8) includes *Thinouia* sp., *T. myriantha* and *T. trifoliata* (= *Allosanthus trifoliatus* Radlk.) (Fig. 1).

Taxonomic treatment

Thinouia cazumbensis Medeiros, sp. nov.

urn:lsid:ipni.org:names:77212573-1
Figure 2

Diagnosis. The new species differs from *Thinouia trifoliata* by the 5-lobed floral disc, fruits with trichomes and basal petal appendages smaller than the petals, versus annular disc, glabrous fruits and marginal petal appendages larger than the petals.

Type. Brazil. Acre. Sena Madureira. Reserva Extrativista do Cazumbá-Iracema, Núcleo Cazumbá, castanhal coletivo, floresta ombrófila aberta com bambu, 9°8'30"S, 68°56'23"W, 20 Jul 2018, *H. Medeiros, M. Silveira & E.M. Soares 3401,* (holotype RB!; isotypes: INPA!, SPF!, UFACPZ!, US!).

Description. Tendrilled liana 6–8 m long; stem puberulent, with yellowish to whitish indumentum, lenticellate; cross section simple, cylindrical. Leaves trifoliol-

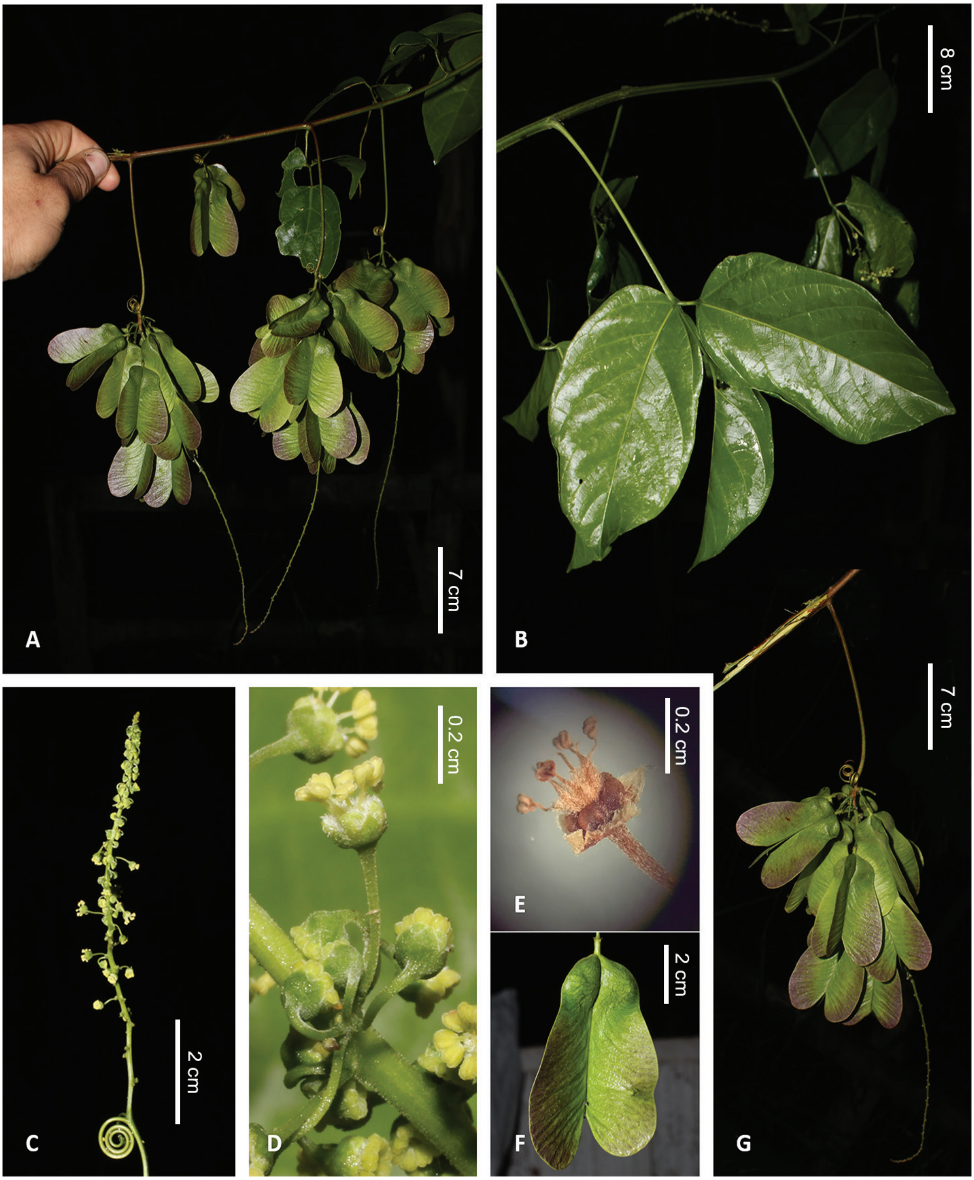

Figure 2. *Thinouia cazumbensis* **A** fruiting branch **B** detail of leaf, abaxial view **C** racemiform inflorescence with a pair of basal tendrils **D** detail of inflorescence (cincinnus) **E** flower with removed petals showing a 5-lobed nectary disc **F** detail of fruit **G** infructescence (**A–G**) from H. Medeiros 3401 (RB). Photos by H. Medeiros.

ate; stipules ca. 2 mm long, hirsute-tomentose, linear triangular to lanceolate; petiole 2–8.5 cm long, canaliculate; petiolules of lateral leaflets 0.2–0.8 cm long; leaflets 7–14 × 3–9 cm, oblong to ovate- rhomboidal, apex acute, mucronate, margins entire to dentate-serrate, with 2–4 teeth reduced to inconspicuous glands, ciliate, base trun-

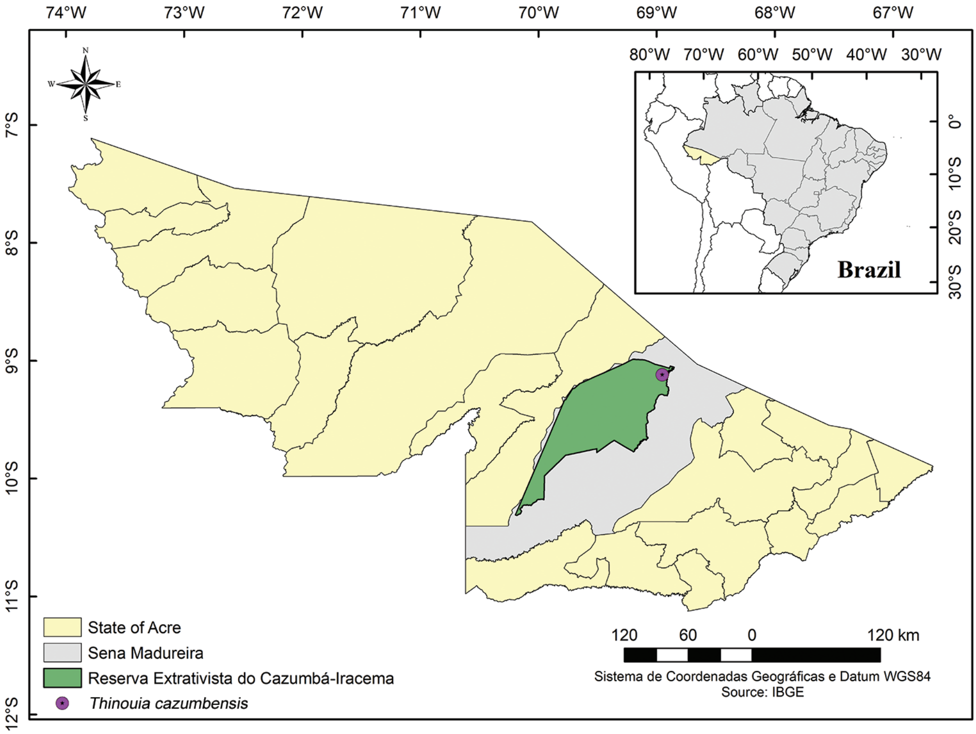

Figure 3. Geographic distribution of *Thinouia cazumbensis*.

cate, rounded to obtuse, sometimes cuneate on the distal leaflet, glabrous on both surfaces, domatia sometimes in the axils of abaxial secondary veins. Thyrses axillary, racemiform, ca. 8.5–16 cm long, peduncle 1.1–2.8 cm long, rachis of 7.5–16 cm long; numerous cincinnus, sessile. Flowers ca. 2 mm long, pedicel ca. 0.5 mm long; sepals 5, ca. 1 mm long, fused at the base, lobes ovate, acute, glabrous and with prominent veins on the internal surface, external surface villous; petals 5, ca. 1.5 mm long, obovate, obtuse, clawed, villous on the central part and margins, the rest glabrous; petal appendages rudimentary, bifid, smaller than the petals, basally adnate, villous; nectary disc glabrous, 5-lobed, lobes ca. 1 mm long; staminate flower: stamens 8, 1.5 mm long, filaments villous for more than half of their length, anthers glabrous, pistillode ca. 1.5 mm long; pistillate flower: staminodes ca. 1 mm long, pistil ca. 1.5 mm long, style 0.5 mm long, with 3 stigmas, ovary ca. 1 mm long. Fruits ovate, chartaceous, 5–5.5 × 2–2.3 cm; cocci slightly inflated, 1.2–1.4 × 1.1–1.4 cm, including the ca. 2–3 mm long stipe constricted at junction with wing; epicarp densely strigose (simple trichomes of same length) on cocci, strigose on wings; endocarp glabrous. Seeds trigonous ovoid, ca. 6 × 4 mm, basally attached, glabrous, mature embryo not observed.

Thinouia cazumbensis is differentiated from most species of *Thinouia* by the thyrses axillary, racemiform (Fig. 2A, C) and the 5-lobed nectary disc, a character that is unique and for the first time recorded in the genus (Fig. 2E). The lobed nectary disc within *Thinouia* should be further investigated through morpho-anatomical studies to understand how nectaries evolved within the genus.

Distribution and ecology. *Thinouia cazumbensis* is known only from the Reserva Extrativista do Cazumbá-Iracema (Fig. 3) where it is an infrequent liana that reaches the canopy of the open rainforest with abundant bamboo (*Guadua* spp.) (Silveira 2005).

Phenology. Collected in flower and fruit during July.

Etymology. The epithet *cazumbensis* refers to Reserva Extrativista do Cazumbá-Iracema, where the species was collected. In the 1980s, local rubber tappers and extractivists fought against the area becoming a rural settlement and on 19 September 2002 succeeded in getting the area designated as a conservation unit (ICMBio 2007). Situated in the State of Acre between the municipalities of Sena Madureira and Manoel Urbano, the Reserva Extrativista do Cazumbá-Iracema covers an area of 750,794.70 hectares of the Western Amazon Corridor, one of the seven major ecological corridors proposed for Brazil (Ricardo and Lima 2004).

Conservation status. The species is only known from one locality in Acre and is categorised as Data Deficient (DD) according to IUCN (2019). Further field studies are needed to evaluate its conservation status more accurately.

Discussion

The broader relationships that we recovered within supertribe Paulliniodae largely agree with those in Acevedo-Rodríguez et al. (2017). Additionally, with the inclusion of new sequences of *Thinouia* in this study merged with sequence data from Acevedo-Rodríguez et al. (2017), our results recovered the same clades in tribe Paullinieae, where *Thinouia* forms a clade that is the earliest diverging lineage. Therefore, our phylogenetic results reinforce including *Allosanthus* in *Thinouia* as proposed by Acevedo-Rodriguez et al. (2011), based on morphological characters. The only differentiating morphological character (i.e. the racemiform inflorescence) was not considered worthy of generic recognition by Acevedo-Rodriguez et al. (2011, 2017) and the molecular data in the present study corroborate this conclusion. The position of the new species as a member of *Thinouia* is strongly supported albeit its relationship to other species is not fully resolved, perhaps because of our limited sampling of *Thinouia* or because only two markers have been sequenced.

Conclusion

Thinouia cazumbensis is supported as a distinct taxon, based on morphological and molecular sequence data. Its position within the genus is still undetermined, highlighting the need for in-depth taxonomic studies on this genus. Ongoing systematics studies, based on molecular and morphological analyses of *Thinouia*, should provide additional insights into the evolution and biogeographic history of this neotropical genus (H. Medeiros et al. in prep.).

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Appendix I

Voucher and GenBank information for the taxa included in the phylogenetic analyses. Listed as: taxon, collection, herbarium, place of origin and GenBank accession numbers (ITS, trnL intron). Herbarium acronyms follow Index Herbariorum (Thiers, continuously updated).

Allophylastrum frutescens Acev.-Rodr., Lima 812 (K), Brazil, KX584885, KX584982. *Allophylus abyssinicus* (Hochst.) Radlk., Desissa & Binggeli DD-318 (MO), Ethiopia, KX584886, KX584983. *Allophylus africanus* P. Beauv., Balkwill 4206 (MO), South Africa, KX584887, KX584984. *Allophylus arboreus* Choux, Wohlhauser & Stiefle 60072 (MO), Madagascar, KX584888, KX584985. *Allophylus bicruris* Radlk., Barthelat 828 (MO), Mayotte, KX584889, KX584986. *Allophylus bojerianus* (Cambess.) Blume, Ratovoson 961 (MO), Madagascar, KX584890, KX584987. *Allophylus chaunostachys* Gilg, Mwangoko 729 (MO), Tanzania, KX584891, KX584988. *Allophylus chirindensis* Baker f., Hizza 26 (MO), Tanzania, KX584892, KX584989. *Allophylus cominia* Sw., Acevedo-Rodríguez 12216 (US), Mexico, KX584893, KX584990. *Allophylus crassinervis* Radlk., Acevedo-Rodríguez s.n. (no voucher), Puerto Rico, KX584894, KX584991. *Allophylus decipiens* (E. Mey.) Radlk., Phillipson 4194 (MO), South Africa, KX584895, KX584992. *Allophylus gardineri* Summerh., Pignal 1834 (MO), Mayotte, KX584897, KX584994. *Allophylus hirtellus* (Hook. f.) Radlk., Cheek 5059 (?), KX584898, KX584995. *Allophylus pervillei* Blume, Hoffmann 399 (MO), Mayotte, KX584899, KX584996. *Allophylus poungouensis* Pellegr., McPherson 16109 (MO), Gabon, KX584900, KX584997. *Allophylus puberulus* (Cambess.) Radlk., Somner 1069 (US), Brazil, KX584901, KX584998. *Allophylus racemosus* Sw., Acevedo-Rodríguez 12180 (US), Mexico, KX584902, KX584999. *Allophylus rubifolius* (A. Rich.) Engl., Kuchar 23357 (MO), Tanzania, KX584903, KX585000. *Allophylus* sp., Acevedo-Rodríguez 14847 (NY), Brazil, KX584904, KX585001. *Athyana weinmanniifolia* (Griseb.) Radlk., Acevedo-Rodríguez 11166 (US), Bolivia, KX584906, KX585003. *Balsas guerrerensis* Cruz Durán & K. Vega, Vega Flores 1318 (US), Mexico, KX584908, KX585005. *Bridgesia incisifolia* Cambess., Landrum 9824 (NY), Chile, KX584909, KX585006. *Cardiospermum corindum* L., Harder & Bringham 3495 (MO), Zambia, KX584912, KX585007. *Cardiospermum cuchujaquense* Ferrucci & Acev.-Rodr., Van Devender 92-1012 (ARIZ), Mexico, KX584914, KX585008. *Cardiospermum grandiflorum* Sw., ATBP 603 (MO), Uganda, KX584915, KX585009. *Cardiospermum grandiflorum* Sw., Gildenhuys H1 (?), Hawaii, KM062277, KM062362. *Cardiospermum heringeri* Ferrucci, Urdampilleta 437 (US), Brazil, KX584917, KX585010. *Cardiospermum urvilleoides* (Radlk.) Ferrucci, Urdampilleta 425 (US), Brazil, KX584922, KX585013. *Chimborazoa lachnocarpa* (Radlk.) H.T. Beck, Wiggens 11060 (US), Ecuador, KX584923, KX585014. *Diatenopteryx sorbifolia* Radlk., Zardini 43371 (MO), Paraguay, EU720534, EU721303. *Dictyoneura obtusa* Blume, Edwards KE142 (JCT), Australia, EU720428, EU721187. *Diploglottis campbellii* Cheel, Chase 2048 (K), Australia, EU720457, EU721224. *Guindilia dissecta* (Covas & Burkart) Hunz., Ferrucci 2928 (CTES), Argentina, KX584926, KX585017. *Guioa villosa* Radlk., McPherson

18040 (MO), New Caledonia, EU720544, EU721314. *Haplocoelum inoploeum* Radlk., Lap 117 (?), FJ514259, FJ514265. *Houssayanthus biternatus* (Weath) Rzed. & Calderón, Catalán & Terán 837 (MO), Mexico, KX584927, KX585018. *Houssayanthus incanus* (Radlk.) Ferrucci, Ferrucci 2710 (CTES), Argentina, KX584928, KX585019. *Jagera javanica* (Blume) Kalkman, Chase 2130 (K), Bogor, EU721236, EU720468. *Lepisanthes senegalensis* (Poir.) Leenh., Callmander 627 (MO), Madagascar, EU720492, U72126. *Lophostigma plumosum* Radlk., Acevedo-Rodríguez 6554 (US), Bolivia, KX584929, KX585020. *Macphersonia gracilis* O. Hoffm., Rabenantoandro 1081 (MO), Madagascar, EU720550, EU721320. *Matayba guianensis* Aubl., Acevedo-Rodríguez 12342 (US), French Guiana, EU720527, EU721294. *Melicoccus lepidopetalus* Radlk., Acevedo-Rodríguez 11128 (US), Bolivia, EU720443, EU721206. *Paullinia clathrata* Radlk., Acevedo-Rodríguez 14305 (US), Peru, KX584930, KX585021. *Paullinia coriacea* Casar., Somner 1070 (RBR), Brazil, KX584931, KX585022. *Paullinia cuneata* Radlk., Acevedo-Rodríguez 14255 (US), Peru, KX584932, KX585023. *Paullinia elegans* Cambess., Acevedo-Rodríguez 14976 (US), Brazil, KX584933, KX585024. *Paullinia hystrix* Radlk., Acevedo-Rodríguez 14417 (US), Peru, KX584934, KX585025. *Paullinia imberbis* Radlk., Schunke Vigo 14928 (US), Peru, KX584935, KX585026. *Paullinia olivacea* Radlk., Schunke Vigo 16002 (US), Peru, KX584936, KX585027. *Paullinia pinnata* L., Acevedo-Rodríguez 11088 (US), French Guiana, KX584937, KX585028. *Paullinia prevostiana* Acev.-Rodr., Acevedo-Rodríguez 11113 (US), French Guiana, KX584938, KX585029. *Paullinia rubiginosa* Cambess., Thomas 12995 (US), Brazil, KX584939, KX585030. *Paullinia spicata* Benth., Acevedo-Rodríguez 12344 (US), French Guiana, KX584941, KX585032. *Paullinia stellata* Radlk., Acevedo-Rodríguez 14958 (US), Brazil, KX584942, KX585033. *Paullinia xestophylla* Radlk., Hoffman 5955 (US), Suriname, KX584943, KX585034. *Plagioscyphus unijugatus* Capuron, Buerki 145 (NEU), Madagascar, EU720475, EU721245. *Sapindus oligophyllus* Merr. & Chun, How 70627 (US), China, KX584944, KX585035. *Serjania altissima* (Poepp.) Radlk., Acevedo-Rodríguez 14953 (US), Brazil, KX584945, KX585036. *Serjania ampelopsis* Planch. & Lind., Acevedo-Rodríguez 11181 (US), Bolivia, KX584946, KX585037. *Serjania caracasana* (Jacq.) Willd., Acevedo-Rodríguez 15107 (US), Mexico, KX584947, KX585038. *Serjania* cf. *caracasana* (Jacq.) Willd., Acevedo-Rodríguez 3483 (US), Guyana, KX584948, KX585039. *Serjania clematidifolia* Cambess., Somner 1078 (RBR), Brazil, KX584949, KX585040. *Serjania communis* Cambess., Somner 1334 (US), Brazil, KX584950, KX585041. *Serjania cuspidata* Cambess., Somner 1400 (US), Brazil, KX584951, KX585042. *Serjania emarginata* Kunth, Acevedo-Rodríguez 15135 (US), Mexico, KX584954, KX585043. *Serjania erythrocaulis* Acev.-Rodr. & Somner, Acevedo-Rodríguez 3729 (US), Brazil, KX584955, KX585044. *Serjania eucardia* Radlk., Somner 1072 (RBR), Brazil, KX584956, KX585045. *Serjania fuscifolia* Radlk., Somner 1455 (RBR), Brazil, KX584957, KX585046. *Serjania ichthyoctona* Radlk., Somner 1081 (RBR), Brazil, KX584960, KX585048. *Serjania lethalis* St. Hil., Roque 1860 (ALCB), Brazil, KX584961, KX585049. *Serjania lethalis* St. Hil., Somner 1381 (RBR), Brazil, KX584962, KX585050. *Serjania marginata* Casar., Acevedo-Rodríguez 11131 (US), Bolivia, KX584963, KX585051. *Serjania mexicana* (L.) Willd., Acevedo-

Rodríguez 12014 (US), Jamaica, KX584965, KX585052. *Serjania mexicana* (L.) Willd., Acevedo-Rodríguez 15080 (US), Mexico, KX584966, KX585053. *Serjania paniculata* Kunth, Acevedo-Rodríguez 15143 (US), Mexico, KX584967, KX585054. *Serjania perulacea* Radlk., Acevedo-Rodríguez 11134 (US), Bolivia, KX584968, KX585055. *Serjania unguiculata* Radlk., Acevedo-Rodríguez 15081 (US), Mexico, KX584969, KX585056. *Serjania yucatanensis* Standl., Acevedo-Rodríguez 12183 (US), Mexico, KX584970, KX585057. *Talisia nervosa* Radlk., Pennington 628 (MO), ~, EU720474, EU721244. *Talisia obovata* A.C. Sm., Lombello 13 (MO), Brazil, EU720485, EU721255. *Thinouia cazumbensis* sp. nov., Medeiros 3401 (RB) Brazil, MT853074, MT847016. *Thinouia mucronata* Radlk., Keller 6919 (US), Argentina, KX584971, KX585058. *Thinouia* cf. *mucronata* Radlk., Medeiros 3800 (RB) Brazil, MT853076, MT847018. *Thinouia myriantha* Radlk., Torke 2024 (HSTM), Brazil, MT853071, MT847013. *Thinouia obliqua* Radlk., Medeiros 3793 (RB) Brazil, MT853075, MT847017. *Thinouia* sp., Medeiros 2193 (RB), Brazil, MT853072, MT847014. *Thinouia restingae* Ferrucci & Somner, Somner 1074 (RBR), Brazil, KX584972, KX585060. *Thinouia trifoliata* (Radlk.) Acev.-Rodr. & Ferrucci, Medeiros 3331 (RB), Brazil, MT853073, MT847015. *Thouinia acuminata* S. Watson, Liston 633-2, —, EU720478, EU721249. *Thouinia villosa* DC., Hall 825 (US), Mexico, KX584975, KX585062. *Tristiropsis acutangula* Radlk., Chase 1358 (K), Bogor, EU720453, EU721220. *Urvillea chacoensis* Hunz., Acevedo-Rodríguez 11133 (US), Bolivia, KX584976, KX585063. *Urvillea chacoensis* Hunz., Keller 6834 (US), Argentina, KX584977, KX585064. *Urvillea pterocarpa* (Radlk.) Acev.-Rodr. & Ferrucci, Urdampilleta 321 (US), Brazil, KX585012, KX584921. *Urvillea rufescens* Cambess., Somner 1073 (RBR), Brazil, KX584978, KX585065. *Urvillea ulmacea* Kunth, Acevedo-Rodríguez 15145 (US), Mexico, KX584979, KX585066. *Urvillea ulmacea* Kunth, Reyes-García 5585 (MO), Mexico, KX584980, KX585067. *Vouarana guianensis* Aubl., Acevedo-Rodríguez 5031 (US), French Guiana, KX584981, KX585068.