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DATA PAPER

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Togo National Herbarium database

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Abstract

This article describes the herbarium database of the University of Lomé. The database provides a good representation of the current knowledge of the flora of Togo. The herbarium of University of Lomé, known also as *Herbarium togoense* is the national herbarium and is registered in Index Herbariorum with the abbreviation TOGO. It contains 15,000 specimens of vascular plants coming mostly from all Togo's ecofloristic regions. Less than one percent of the specimens are from neighbouring countries such as Ghana, Benin and Burkina Faso. Collecting site details are specified in more that 97% of the sheet labels, but only about 50% contain geographic coordinates. Besides being a research resource, the herbarium constitutes an educational collection. The dataset described in this paper is registered with GBIF and accessible at https://www.gbif.org/dataset/b05dd467-aaf8-4c67-843c-27f049057b78. It was developed with the RIHA software (Réseau Informatique des Herbiers d'Afrique). The RIHA system (Chevillotte and Florence 2006, Radji et al. 2009) allows the capture of label data and associated information such as synonyms, vernacular names, taxonomic hierarchy and references.

Keywords

herbarium collection, herbarium sheets, Liliopsida, Magnoliopsida, Occurrence, Plants, RIHA, Togo, University of Lomé, West Africa

Context

Botanical collections are an indispensable tool in the field of biodiversity conservation. Indeed, they provide basic data for the evaluation of species conservation status, a task that presents a significant lack in Africa, especially in Togo (Radji et al. 2010; Billand 2010).

In West Africa as in Central Africa, the level of digital accessible information (DAK) on vascular plants – though far from satisfactory – has been raised by efforts in neighbouring countries (Dauby et al. 2016, Sosef et al. 2017). Remarkable in this regard is the onlined dataset published by Benin (e.g. The census of national forest of Benin (Gaston 2016) and the University of Ghana Herbarium database (Asase 2015). Those contributions spotlighted the lack of information available on plants from Togo, a fact that has been limiting the feasibility of sound floristic and ecological studies in the region until now.

The dataset, here described, narrows this knowledge gap and should be a key resource in the making of a national plant checklist for Togo, monographs, floras and other research crucial to addressing challenges of knowledge, pedagogy, sustainable development and decision-making about natural resources and environments (Chapman 2005; Sousa-Baena et al. 2013).

In a wider context, this dataset contributes to the correction of a global lack of tropical biodiversity data availability (Collen et al. 2008, Feeley and Silman 2011) and to the GBIF Content Mobilization Priorities set in 2017 (https://www.gbif.org/mobilization-priorities-2017).

Data capture of the University of Lomé herbarium collections started in 2003 as part of the RIHA initiative led by the IRD (Institut de Recherche pour le Développement) team at the MNHN Paris. In 2008, this work underwent considerable growth with the Sud Expert Plantes project (http://www.sud-expert-plantes.ird.fr/), which enabled the capture of the "Letouzey" database (Radji et al. 2009) of more than 12,500 specimens. Also in 2008, as part of the "African Plants Initiative" (API) project, http://apps.kew.org/herbcat/gotoApi.do, 8,000 images from TOGO Herbarium specimens where captured and published.

DAK on the plants of Togo is relatively large, based on nearly 85% of primary biodiversity data records derived from specimens in the National Herbarium, plus data from other institutions (Ministry of Environment and NGOs etc.) served through biodiversity informatics initiatives such as GBIF. Data on plants of Togo have not yet been integrated and assessed to establish how complete the site inventories are across the country, so that appropriate levels of confidence can be applied; these gaps in knowledge directly affect the fitness-for-use of the data (Otegui et al. 2013).

Project

The database of the National Herbarium of Togo is the result of several digitisation programmes:

- 2003–2006 RIHA Project: initiated by IRD and funded by MAEE France, allows Togo's Herbarium, to learn and become familiar with the RIHA platform. https://www.ird.fr/les-partenariats/renforcement-des-capacites/des-programmes-specifiques/bourses-d-echanges-scientifiques-et-technologi ques-best

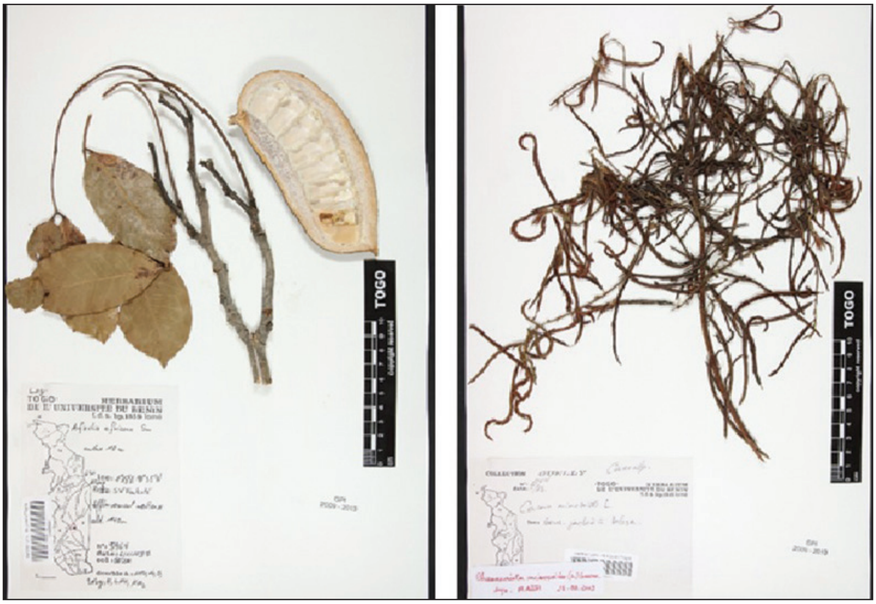

Figure 1. Pictures from the GPI project gallery showing the stamping and the project period.

- 2008–2011 SEP (Sud Expert Plantes programme (http://www.sud-expert-plantes.ird.fr/ projets/Herbiers_et_J_Botaniques funded by MAEE France (SEP Project N°206) (http://www.sud-expert-plantes.ird.fr/ projets/dossier_206) makes it possible for the University of Lomé to start modernising its herbarium by assembling herbarium specimens to international standards and to capture labels' data into the RIHA database.
- 2008–2010 API African Plants Initiative Projects, funded by Mellon Foundation through JsTor https://plants.jstor.org/, allows the publishing of the first 8,000 specimens digitalised (Figure 1).
- 2016–2018 BID Project (BID-AF2015-0004-NAC), led by GBIF secretariat and funded by the European Union https://www.gbif.org/project/82693/ strengthening-the-biodiversity-stakeholders-network-in-togo)

Personnel

Sodjinou Komlan Edjèdu, Allassani Stephane, Tchani Watchiou, Abotsi Eli, Dogbé Yawa and Koda Donko.

Data published through

https://www.gbif.org/dataset/3294d36a-987c-4dcb-8ecf-bd2082796f08#

Taxonomic coverage

Most specimens in Togo Herbarium belong to class Magnoliopsida (2,101 specimens) and Liliopsida (8,508 specimens). These classes are followed by Filicopsida (505 specimens), Lycopsida (138 specimens), Coniferopsida (104 specimens), Equisetopsida (24 specimens), Ophioglossopsida (14 specimens), Gnetopsida (6 specimens), Taxopsida (4 specimens), Cycadopsida and Ginkgopsida (both with 2 specimens) and Psilotopsida (1 specimen) (Figure 2).

Togo herbarium contains specimens belonging to 194 families, of which 13.25% of the specimens belong to Leguminosae-Papilionoideae, followed by Graminae (7.73%), Rubiaceae (7.37%), Euphorbiaceae (6.69%), Asteraceae (5%), Cyperaceae (4.50%), Gleicheniaceae (2.66%), Leguminosae-Caesalpinioideae (2.24%), Combretaceae (2.12%), Commelinaceae (1.90%), Moraceae (1.74%), Scrophulariaceae (1.72%), Ulmaceae (1.66%), Leguminosae-Mimosoideae (1.63%), Asclepiadaceae and Apocynaceae (1.57%).

The herbarium includes 198 genera, significant ones amongst them being *Phyllanthus* (344 specimens), *Ficus* (207 specimens), *Indigofera* (193 specimens), *Combretum* (165 specimens), *Crotalaria* (148 specimens), *Vernonia* (119 specimens), *Euphorbia* (92 specimens), *Trema* (92 specimens), *Cyperus* (168 specimens), *Eriosema* (82 specimens), *Fimbristylis* (82 specimens) and *Uvaria* (45 specimens). Although the herbarium contains specimens from all major botanical groups, Phanerogams (angiosperms-gymnosperms), Pteridophyta (ferns), Bryophyta and Thallophyta (algae-lichens-fungi), the dataset used for this paper covers exclusively Angiosperms (Dicotyledonous and Monocotyledonous) (Figure 3).

The collection includes 508 singletons from 117 different families that represent 4% of the total record. Important families concerned are Graminae (43 specimens), Leguminosae-Papilionoideae 35, Orchidaceae 26, Rubiaceae 25, Euphorbiaceae 22 and Cyperaceae 21 species. Major genera are *Ficus* and *Indigofera* (9 specimens), *Dioscorea* (8 specimens), *Eulophia* (7 specimens), *Crotalaria* and *Phyllanthus* (6 specimens) and *Ceropegia* (5 specimens).

Taxonomic ranks

Kingdom: Plantae
Division: Magnoliophyta (Cronquist, Takht. & W. Zimm., 1996)
Phylum: *Spermaphyta*
Class: *Magnoliopsida, Liliopsida*
Family: Agavaceae, Alismataceae, Amaryllidaceae, Anthericaceae, Araceae, Asparagaceae, Aspidiaceae, Cannaceae, Commelinaceae, Costaceae, Cyperaceae, Dioscoreaceae, Dracaenaceae, Droseraceae, Eriocaulaceae, Erythroxylaceae, Gramineae, Hyacinthaceae, Hydrocharitaceae, Hydrophyllaceae, Hypoxidaceae, Iridaceae, Lemnaceae, Liliaceae, Linaceae, Marantaceae, Musaceae, Najadaceae, Opiliaceae,

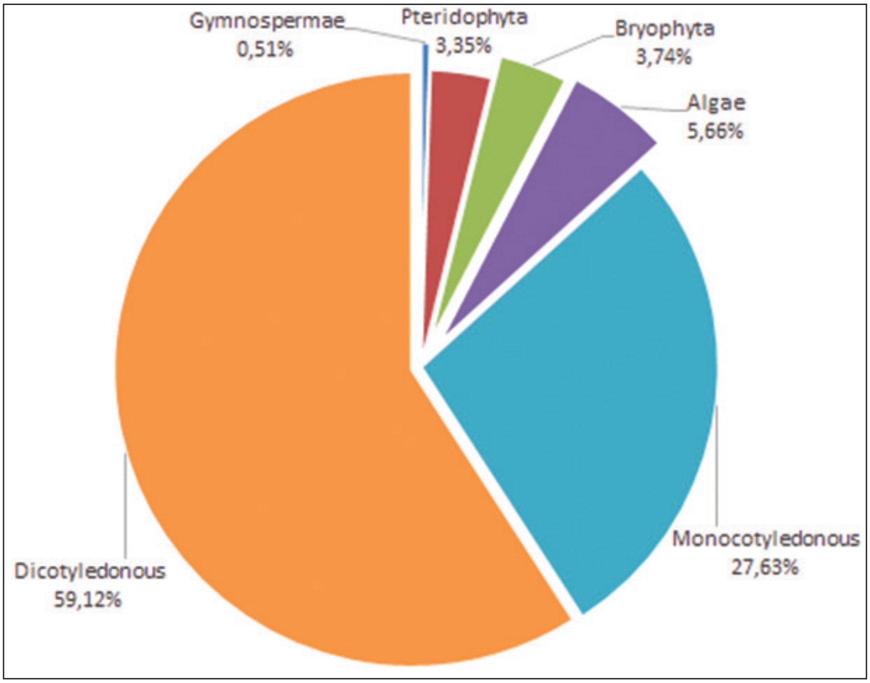

Figure 2. Taxonomic coverage of TOGO dataset in term of specimens.

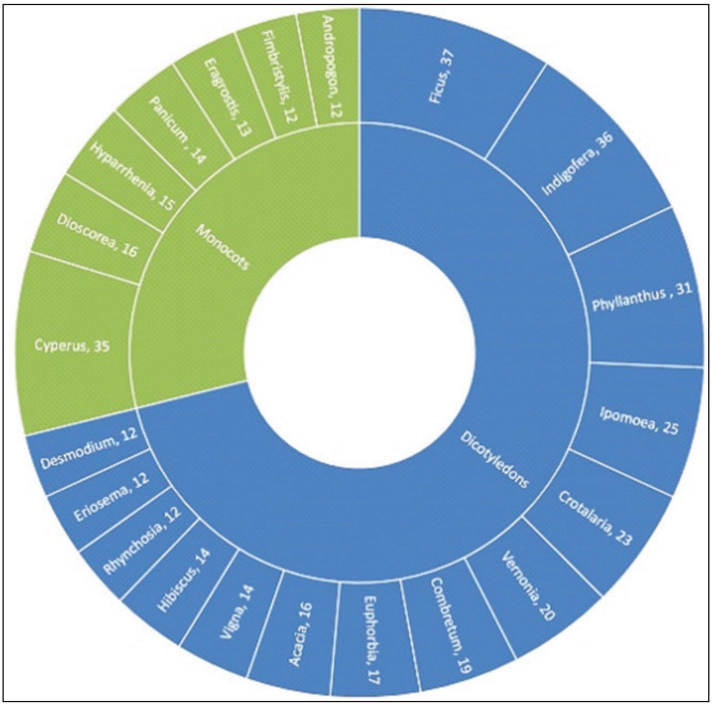

Figure 3. Number of species above 10 per genus in major taxonomic groups.

Orchidaceae, Palmae, Pandanaceae, Pontederiaceae, Smilacaceae, Taccaceae, Typhaceae, Xyridaceae, Zingiberaceae.

Acanthaceae, Aizoaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apocynaceae, Araliaceae, Aristolochiaceae, Asclepiadaceae, Avicenniaceae, Azollaceae, Balanitaceae, Balanophoraceae, Balsaminaceae, Basellaceae, Begoniaceae, Bignoniaceae, Bixaceae, Bombacaceae, Boraginaceae, Burseraceae, Cactaceae, Campanulaceae, Capparaceae, Caricaceae, Caryophyllaceae, Casuarinaceae, Cecropiaceae, Celastraceae, Ceratophyllaceae, Chenopodiaceae, Chrysobalanaceae, Cochlospermaceae, Colchicaceae, Combretaceae, Compositae, Connaraceae, Convolvulaceae, Crassulaceae, Cruciferae, Cucurbitaceae, Dichapetalaceae, Dilleniaceae, Dipterocarpaceae, Ebenaceae, Euphorbiaceae, Flacourtiaceae, Flagellariaceae, Gentianaceae, Gesneriaceae, Goodeniaceae, Guttiferae, Haloragaceae, Hernandiaceae, Hippocrateaceae, Icacinaceae, Irvingiaceae, Labiatae, Lauraceae, Lecythidaceae, Leeaceae, Leguminosae, Leguminosae-Caesalpinioideae, Leguminosae-Mimosoideae, Leguminosae-Papilionoideae, Lentibulariaceae, Loganiaceae, Loranthaceae, Lycopodiaceae, Lythraceae, Malpighiaceae, Malvaceae, Melastomataceae, Meliaceae, Menispermaceae, Molluginaceae, Moraceae, Moringaceae, Myristicaceae, Myrsinaceae, Myrtaceae, Nyctaginaceae, Nymphaeaceae, Ochnaceae, Olacaceae, Oleaceae, Onagraceae, Opiliaceae, Oxalidaceae, Pandaceae, Papaveraceae, Passifloraceae, Pedaliaceae, Phytolaccaceae, Piperaceae, Pittosporaceae, Plumbaginaceae, Podostemaceae, Polygalaceae, Polygonaceae, Portulacaceae, Proteaceae, Punicaceae, Ranunculaceae, Rhamnaceae, Rhizophoraceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae, Santalaceae, Sapindaceae, Sapotaceae, Saxifragaceae, Scrophulariaceae, Simaroubaceae, Solanaceae, Sphenocleaceae, Sterculiaceae, Thymelaeaceae, Tiliaceae, Turneraceae, Ulmaceae, Umbelliferae, Urticaceae, Verbenaceae, Violaceae, Vitaceae, Zygophyllaceae.

Spatial coverage

The described dataset collections come from all over Togo. Togo is located in West Africa which has an area of 56,600 km^2. It stretches for 600 km from North to South and East to West between 50 and 150 km wide (Figure 4).

Togo Herbarium specimens were collected through the 5 Togo ecological zones (ZE) (Figure 9) recognised by Ern (1979). ZE.I refers to the Northern Plains Savannah, with Sudan savannah as the predominant vegetation with few islands of dry forests and gallery forests. ZE.II is covered with a mosaic of dry forests of mountain and forest galleries and climate is Sudano-Guinean. ZE.III corresponds to the Guinean savannahs of central area plains enjoying a tropical climate with one rainy season. Semi-deciduous forests are noticed in the southern part and dry forests in the northern part. ZE.IV covers the southern part of the Togo Mountains and has a sub-equatorial transition climate. The vegetation is constituted of rainforests, on

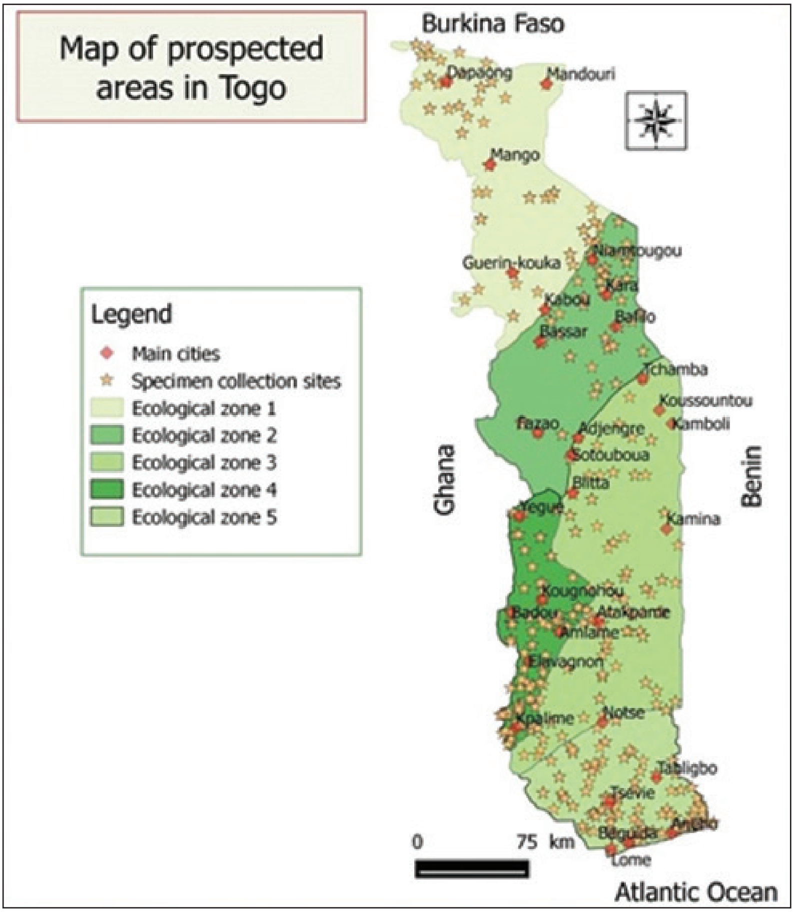

Figure 4. Map of prospected areas in the whole country.

deep red lateritic soils. This ZE is the domain of dense semi-deciduous forests. The last one, ZE.V is a coastal plain of southern Togo with a subequatorial climate marked by a deficit in rainfall. It is characterised by a climate with two rainy seasons and the vegetation is set up by a mosaic of savannah, farmland and dry forests (Kokou and Caballé 2000).

Coordinates

Togo is located between 6° and 11° latitude and 1° East longitude (Figure 4). Data in Togo National Herbarium are referenced between 11.05 latitude and 0.2 and 1.85 longitude. Data are also from "Mont Agou" that is the highest peak in Togo, at an altitude of 986 m and located between Amoussoukopé and Kpalimé in ecological zone IV.

Temporal coverage

With respect to the temporal coverage of the specimens, 5,895 (47.16%) consist of specimens collected from 1970 to 1984, most of them used for the botanical species description and the drafting of the Togo's Flora (Brunel 1975, 1987; Brunel et al. 1984). Newer specimens came from the fieldwork of local researchers (Kokou and Caballé 2000, Akpagana and Guelly 1994, Akpagana 1992b), teaching trips from teachers with students in Plant Biology and the Environment Department from the University of Benin (University of Lomé from 2001 onwards). These collections represent 60% of the current holdings (Figures 5, 6).

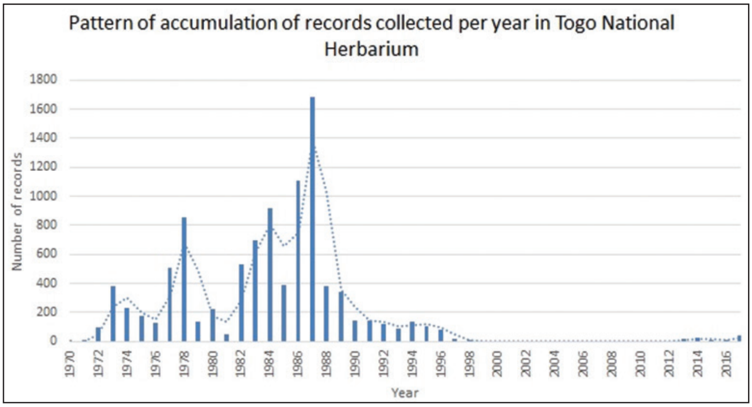

Figure 5. Pattern of accumulation of records collected per year in Togo National Herbarium.

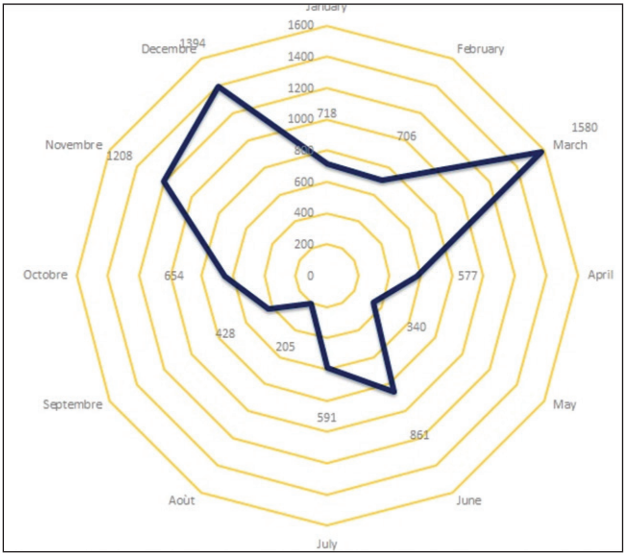

Figure 6. Pattern of record accumulation of Togolese plants collected per month.

Natural collections description

The herbarium specimens were mainly collected in Togo (99%). The remaining 1% come from neighbouring countries such as Ghana, Benin and Côte d'Ivoire collected by Togolese researchers on research trips. These specimens date from the second half of the 20th century. Today, the Herbarium occupies an area of approximately 100 m^2 and is located on the ground floor of the building that houses the Dean's Office of the Faculty of Sciences of the University of Lomé. Thanks to SEP Project N°206 --funded in 2008 by the French Ministry of Foreign Affairs, the storage for 12,572 specimens was upgraded to international standards by use of new closed door wooden cabinets. The collection is arranged in alphabetical order by Family, Genus and Species names. There are no type specimens kept in the Herbarium but the only recognised endemic species is well-represented by 16 specimens. The main collectors are: Brunel (6227 specimens, Figures 7 and 8), Akpagana (2023), Schäfer (911), Guelly (654), Kokou (508), Koumantega (276) and Ern (200) (Figure 8). While most of the samples have little useful information, those of Schäfer PA are well georeferenced and contain an unsuspected amount of information about traditional knowledge as well as the vernacular names of the samples in its collection (Figure 8).

Collection name

University of Lomé herbarium: *Herbarium togoense*; Index Herbariorum Acronym: TOGO.

Methods

The compilation of this dataset was based on digitised data and experiences gained from the RIHA and SEP projects, as well as an updated inventory of existing resources (collections and contacts). In particular, it relied on the establishment of databases in Herbaria (Gaston 2000; Bebber et al. 2010), the standardisation and homogenisation of data through the reference systems (names of plants, localities and people). Consultation of the renowned botanical databases has been carried out (http://www.eol.org; https://www.biodiversitylibrary.org). Linkage with forest and ecological databases (inventories) has been undertaken, as well as on-line consultation of data via relevant international portals such as GBIF (http://www.gbif.org). During the fieldwork, the species were recorded as presence / absence before being sampled for the herbarium - both in surveys of inventories (transects), forest ecology (setting up plots) and ethnobotany. Species identification was made with Togo's flora (Brunel et al. 1984), Senegal's flora (Berhaut 1967), Senegal Illustrated Flora (Berhaut 1971–1988), Descriptive Flora of Côte d'Ivoire (Ake Assi 1984) and Forest Flora of Côte d'Ivoire (Aubreville 1959). Further information was collected from Flora of West Tropical Africa (Hutchinson

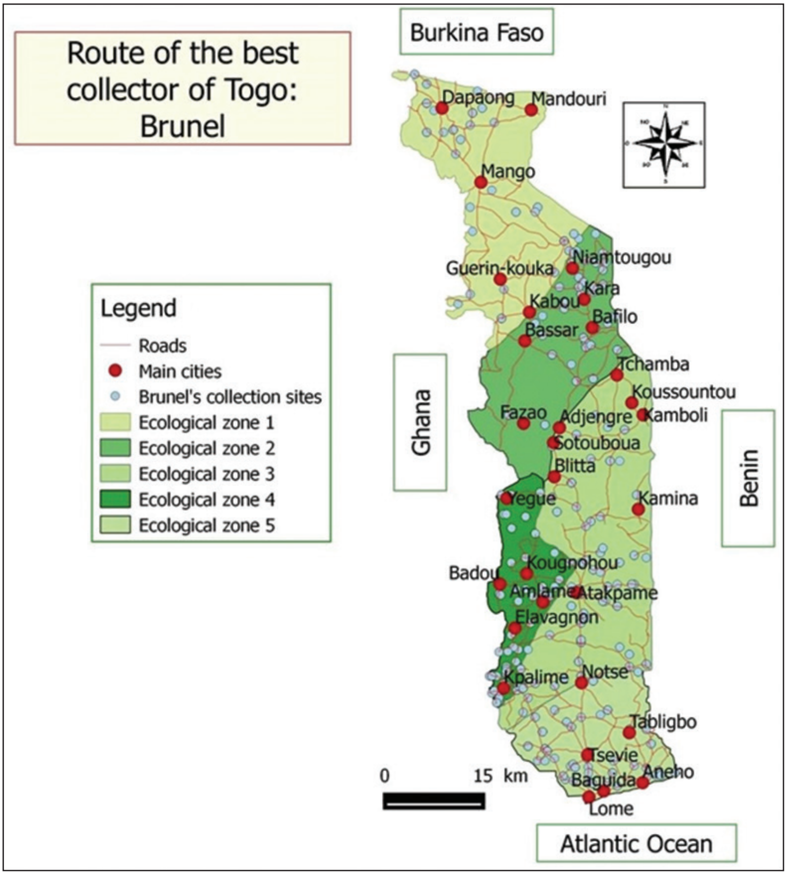

Figure 7. Route of the best collector in Togo: Brunel.

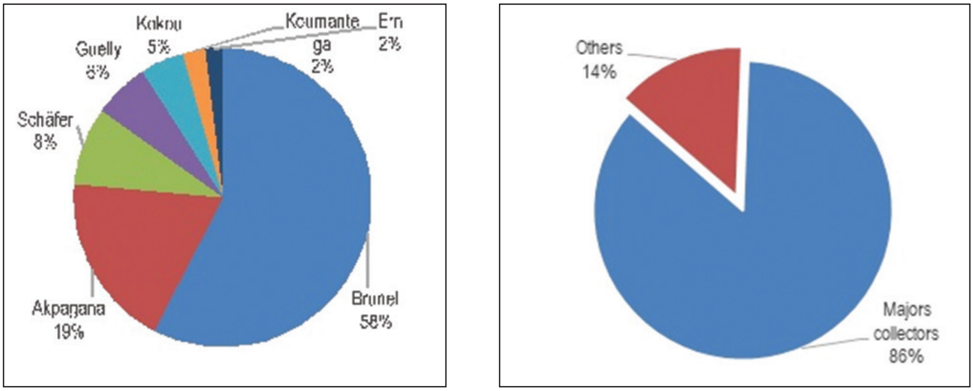

Figure 8. Major collectors who collected around Togo's ecological area from 1971 to 2017 and comparison to the rest of the collection.

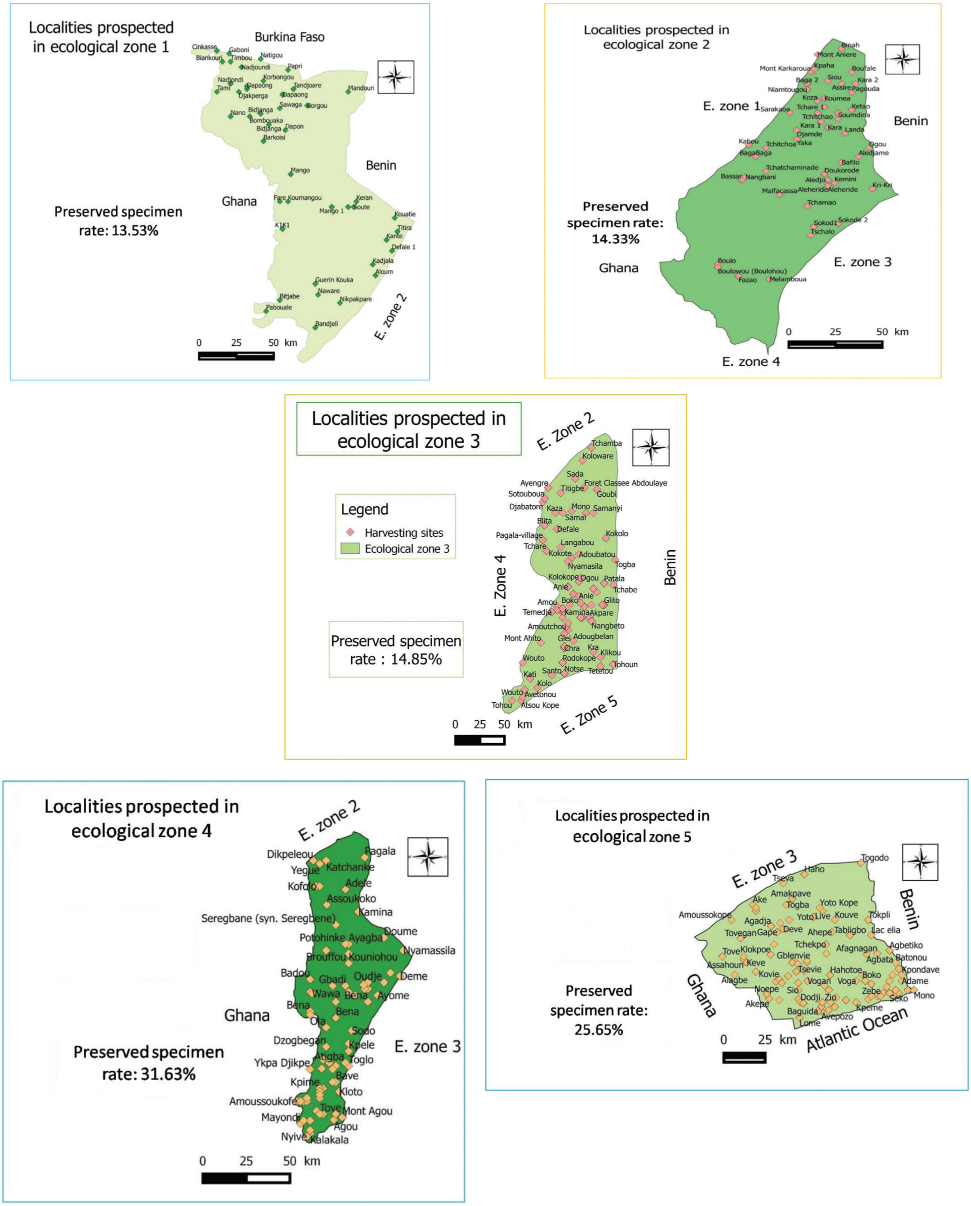

Figure 9. Localities botanically prospected by ecological zone in Togo.

and Dalziel 1954–1972) and from the Enumeration of Tropical African Flowering Plants (Lebrun and Stork (1991–1992, 2006, 2008, 2018). The nomenclature used is that of the original authors and classification in the database released in this paper follows Hutchison and Dalziel (1953–1967) as currently maintained in the Togo National Herbarium. The International Plant Names Index (http://www.ipni.org) and

Botanist's Index (http://kiki.huh.harvard.edu/databases/botanist_index.html) have been used. The list of synonyms followed The Plant List www.theplantlist.org. For Lycophytes and Ferns, the classification followed that established by Christenhusz et al. (2011), while that of flowering plants followed Cronquist (1981).

Method step description

The fieldwork and herbarium specimens study followed traditional procedures (Mori et al. 2011).

Data processing. Details -measures, label facts, nomenclature etc. from all studied specimens were entered into a MS-Access database (RIHA database) designed and built for that purpose. Additional tables and queries were created to record specimen measures as well as bibliographic, taxonomic and nomenclatural information - these are not included in this dataset, but nomenclature information is available through 'The Plant List' (2013) and 'African Plant Database (2018). Specific guidelines regarding data entry and quality control procedures followed Pando et al. (1999). The dataset was published in a standardised format using the Integrated Publishing Toolkit (Robertson et al. 2014) hosted by GBIF France. The standardised format is Darwin Core Archive (DwC-A, GBIF 2010), which is a biodiversity data standard that makes use of Darwin Core terms (Wieczorek et al. 2012). A Darwin Core Archive is a zip file containing a data file in tab delimited text (.txt) format, an xml file describing the data file, the relationships between the archive's data files when there is more than one (meta.xml) and a machine readable dataset metadata in XML format (eml.xml), complying with GBIF Metadata Profile (GBIF 2011), based on EML.

Study extent description: The analysis of this dataset reveals that eco-floristic zone IV is the most significant and prospected with 31.63% of the herbarium specimens whereas ecological zone II remains the least prospected with 11.33% of the collected specimens.

Although quite rich, Togolese spontaneous flora remains incompletely known (Akpagana 1992b, Radji 1997, Kokou 1998).

Figure 9: Localities botanically prospected by ecological zone in Togo

Sampling description: The specimens deposited in the TOGO Herbarium come from several research projects mainly involving first lecturers in Botany in the university followed by the first PhD students studying in French universities whose fieldwork was carried out in Togo. As a result, specimens were collected under a variety of methods and diverse objectives. The situation is the same with materials coming from donations or purchases (e.g. herbarium of Ern). When possible, duplicates of specimens have been sent to renowned herbaria. Some duplicates of herbarium specimens (5,403, 36.02% of the collection) from Togo were deposited in other herbaria committed to long-term maintenance. These where: B, BR, IFAN, K, LMU, MO, MPU, P, STR, UK, US and WAG (http://sweetgum.nybg.org/science/ih/.

During the SEP project (2008–2012), 6 students studying for Master's degrees in Plant Biology were recruited to assemble herbarium specimens deposited by collectors in presses and sometimes in Canson papers. Most specimens have original collectors' labels. Trained in the use of RIHA database (under Microsoft Access), the students digitised the data as and when the specimens were mounted. Data were exported to Excel for publication..

Quality control description: The present dataset was updated to match the Cronquist classification for the orders and families of flowering plants and all species names were checked for validity (spelling, synomyms and authorship) against online databases: http://www.ipni.org/ipni/plantnamesearchpage.do; http://www.theplantlist.org/; http://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php.

Datasets

Dataset description

Object name: Togo National Herbarium database
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution: http://ipt-togo.gbif.fr/resource?r=herbarium_database&v=1.5
Publication date of data: 2018-01-05
Language: French
Licences of use: This database "Togo National Herbarium database" is made available under licence Creative Commons Attribution (CC-BY) 4.0 License
Metadata language: English
Date of metadata creation: 2018-01-05
Hierarchy level: Dataset

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RESEARCH ARTICLE

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Eutrema nanum (Brassicaceae), a new species from Chola Shan, Southwest China

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Abstract

Eutrema nanum, a new high-elevation (4500–4600 m) species from Chola Shan, Sichuan (Southwest China), is described and illustrated. It is similar morphologically to *E. nepalense* but is readily distinguished by having oblong to elliptic or obovate to spatulate (vs. suborbicular to broadly ovate) leaves, glabrous (vs. puberulent) sepals and ovate to oblong fruit 4–7 × 2–3 mm with flattened valves (vs. ovoid to subglobose fruit 2–3 × 1.8–2 mm with rounded valves). The genetic differences amongst *E. nanum*, *E. nepalense* and other close relatives are further confirmed by phylogenetic analyses using ITS and cpDNA sequence variations. The new combination *E. sinense* is proposed.

Keywords

Cruciferae, *Eutrema nanum*, molecular phylogeny, Sichuan, *Eutrema sinense*

Introduction

The boundaries of *Eutrema* R.Br. (Brassicaceae or Cruciferae) have recently been expanded to include 38 species, several of which were previously placed in the six genera *Taphrospermum* C.A.Mey., *Thellungiella* O.E.Schulz, *Neomartinella* Pilg., *Platycraspedum* O.E.Schulz, *Chalcanthus* Boiss. and *Pegaeophyton* Hayek & Hand.-Mazz. (Al-Shehbaz and Warwick 2005; Hao et al. 2017a). The taxonomic knowledge of this genus is still incomplete because numerous collections from the high-elevation regions in Southwest China were often overlooked and many areas remain poorly explored. We reported two new species during recent field investigations and molecular analyses (Hao et al. 2016, 2017b). Here we report the third one, *Eutrema nanum*, found in Chola Shan at a high elevation of 4500–4600 m in Sichuan Province, Southwest China. This new species is morphlogically similar to *E. nepalense* (Al-Shehbaz, Kats Arai & H.Ohba) Al-Shehbaz, G.Q.Hao & J.Quan Liu but, as shown below, it is readily distinguished by several aspects of leaves and fruit. The phylogenetic studies on both species and their other relatives were also conducted herein and the results support the recognition of this novelty. In addition, one of six species which were used to determine the systematic position of *E. nanum* was found to need a taxonomic combination and a new name *Eutrema sinense* (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz is therefore proposed herein.

Material and methods

We examined morphological traits of *Eutrema nanum* and several relative species. We followed Hu et al. (2015) and Hao et al. (2017a) in examining the genetic differences between this novelty (two accessions) and the morphologically similar *E. nepalense* (one accession). In order to determine the systematic position of *E. nanum*, we futher included six species (*E. scapiflorum* (Hook.f. & Thomson) Al-Shehbaz, G.Q.Hao & J.Quan Liu, *E. sinense* (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz, *E. hookeri* Al-Shehbaz & Warwick, *E. fontanum* (Maxim.) Al-Shehbaz & Warwick, *E. verticillatum* (Jeffrey & W.W.Sm.) Al-Shehbaz & Warwick and *E. deltoideum* (Hook.f. & Thomson) O.E.Schulz) in our analyses. All six species were shown to be close relatives to *E. nepalense* in our previous study (Hao et al. 2017a) and two (*E. scapiflorum* and *E. sinense*) were previously placed in the genus *Pegaeophyton*. The related *E. integrifolium* Bunge (see Hao et al. 2017a) was selected as the outgroup. The collection information of the sampled species is listed in Table 1 and Figure 3 and the voucher specimens were deposited in the Sichuan University Herbarium (SZ).

We extracted the total DNA and amplified and sequenced four DNA markers, the nuclear internal transcribed spacer (ITS) and three chloroplast DNA (cpDNA) regions (*psb*A-*trn*H, *rbc*L, *mat*K), following Hu et al. (2015) and Hao et al. (2017a). The sequences firstly reported here were placed in GenBank under the accession numbers (MH702367, MH793597, MH793598, MH793599). We aligned all sequences using Clustal X (Thompson et al. 1997) and refined them manually. We concatenated three cpDNA sequences into a single matrix for Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses. We coded indels using the simple code method by Gap-Coder (Young and Healy 2003). We constructed phylogenetic relationships based on two datasets (ITS and cpDNAs) using MP analyses by PAUP* 4.10b (Swofford 2003) and ML analyses using RAxML 7.2.6 (Stamatakis 2006). MP analyses employed a heuristic search with 10,000 replicates and TBR branch swapping and bootstrap values (Felsenstein 1985) were estimated with 1000 replicates and 100 random-addition-

Table 1. The sources of materials used for molecular analyses of Himalayan *Eutrema* (all vouchers at SZ).

Taxon	Voucher	Source	Coordinate
E. nanum	Liu & Hao 14091	Chola Shan, Sichuan, China	31°55'N, 98°54'E
E. nanum	Liu 17124	Chola Shan, Sichuan, China	31°55'N, 98°54E
E. nepalense	Long et al. 605	Sikkim, India	27°36'N, 88°12'E
E. sinense	Liu 13114	Biluo Snow Mountain, Yunnan, China	27°59'N, 98°47'E
E. scapiflorum	Liu & Hao 13074	Yarla Shampo Mountain, Tibet, China	28°51'N, 91°59E
E. fontanum	Liu & Hao 13144	Zhuodala Mountain, Sichuan, China	31°24'N, 99°56'E
E. hookeri	Liu 17108a	Mila Mountain, Tibet, China	29°49'N, 92°90'E
E. verticillatum	Liu & Hao 14094	Maila Mountain, Sichuan, China	30°58'N, 98°58'E
E. deltoideum	Liu 13024	Lasa, Tibet, China	29°42'N, 91°09'E
E. integrifolium	Liu & Hao 13049	Tianshan Mountain, Xinjiang, China	43°12'N, 84°49'E

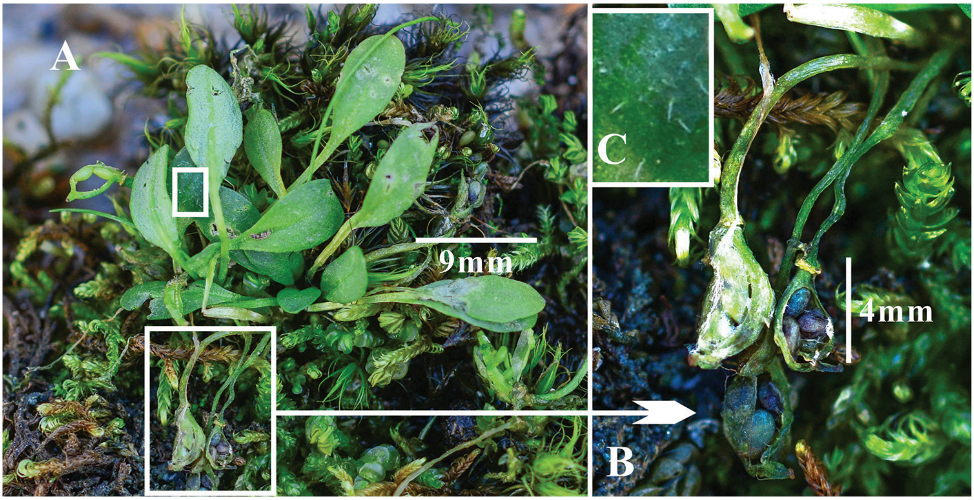


Figure 1. *Eutrema nanum.* G.Q. Hao, J.Quan. Liu & Al-Shehbaz. **A** Plant **B** Fruit **C** Leaf trichomes.

sequence replicates per bootstrap replicate. ML analyses were performed with raxml-HPC -f a -s sequence. phy -n boot2 -m GTRGAMMA -x 1234 -# 1000 -n outname. The most suitable GTRGAMMA models were used and bootstrap analyses were estimated with 1000 replicates.

Taxonomy

Eutrema nanum G.Q.Hao, J.Quan Liu & Al-Shehbaz, sp. nov.

urn:lsid:ipni.org:names:60477015-2
小山嵛菜

Type. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., 16 August 2014, *Liu & Hao 14091* (Holotype, SZ). Figures 1, 2.

标本采集记录
Collect Record of Herbarium

采集人 Collector(s) 郝国谦、张磊
采集号 No. LiuJQ-Hao2014091
日期 Date 2014.09.01
科名 Family 十字花科
俗名 Local Name
学名 Species Eutrema nanum
产地 Location 中国四川省甘孜州德格县雀儿山
经度 Lon. E98° 54′ 35″
纬度 Lat. N31° 55′ 32″
丰富度 Frequency 海拔 Alt（m）4500m
生境 Habitat
基质 Substrate
性状 Habit 生活型 Life f.
根 root 茎 Stem
叶 Leaf 花 Flower
果实 Fruit 种子 Seed

矮山嵛菜 (Holotype)
Eutrema nanum G.Q.Hao, J.Quan Liu & Al-Shehbaz.
鉴定人: ihsan 2018 年 5 月 7 日
四川大学生物系植物标本室

Figure 2. Holotype of *Eutrema nanum*.

Description. Herbs perennial, 3–6 cm tall, glabrous or puberulent; caudex slender, ca. 3–5 mm long. Leaves basal, rosulate, 20–25 per caudex; petiole 13–20 mm long, slender at base, glabrous or with few trichomes; blade oblong, elliptic,

Figure 3. Geographical distribution of the sampled populations of *Eutrema nanum* and related species.

obovate, spatulate, 6–10 × 3–4 mm, fleshy, glabrous or abaxially pubescent with trichomes, 0.3–0.6 mm long, base subattenuate, to cuneate, margin entire, apex obtuse to subrounded. Pedicels slender, 18–23 mm long at anthesis, not elongated in fruit, not persistent. Flowers 5–8 per plant; sepals ovate to oblong, 1–1.5 mm long; petals white, broadly obovate to spatulate, blade 2–3 ×1–2 mm, persistent to fruit maturity, claw-like base 0.5–1 mm long. Ovules 2–4 per ovary. Fruit latiseptate, dehiscent, ovate to oblong, somewhat curved, 4–7 × 2–3 mm; valves nearly flat, extending along part of fruit length; gynophore 0.1–0.3 mm long; replum 0.3–0.4 mm wide; style 0.6–1 mm long. Seeds broadly ovate, brown, plump, 2–4 per fruit, 1.4–2 × 0.6–1 mm.

Eutrema nanum is morpholgically most similar to *E. nepalense*, from which it is readily distinguished by having oblong, elliptic, obovate to spatulate leaves, glabrous sepals and ovate to oblong larger fruit 4–7 × 2–3 mm with flattened, glabrous valves. In contrast, *E. nepalense* (https://www.gbif.org) has suborbicular to broadly ovate leaves, puberulent sepals and ovoid to subglobose smaller fruit 2–3 × 1.8–2 mm with rounded, puberulent valves. *Eutrema nanum* was only found with around 100 individuals along a stream in a valley about 2 kilometres from the Chola Shan peak, whereas *E. nepalense* occurs across Himalyas Mountains in Bhtan, China, Nepal and India.

Phenology. Flowering: June–August. Fruiting: August–September.

Distribution and habitat. *Eutrema nanum* is currently known only from Chola Shan, part of Hengduan Mountains in West Sichuan, China (Fig. 3). It grows under rocks by streams close to glaciers, damp or gravelly scree, wet sand at a very high elevation of 4500–4600 m.

Paratype. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., *Liu 17124* (SZ).

Table 2. Diagnosing sites of the aligned ITS and three cpDNA sequences between *Eutrema nanum* and *E. nepalense*.

Species	ITS	*rbc*L		*mat*K										
	508	82	337	165	276	333	342	391	449	483	495	497	549	
Eutrema nanum	C	C	T	C	T	T	T	T	T	T	C	T	T	
Eutrema nepalense	T	A	C	T	C	G	C	A	G	C	T	C	A	
	*mat*K					*psb*A-*trn*H								
	601	603	633	638	657	28	40	48	92	114	115	138-	212	228	235
Eutrema nanum	C	C	T	T	G	T	C	G	-	G	C	-	2 nt	C	C
Eutrema nepalense	T	T	C	A	A	A	G	A	6 nt	T	A	74 nt	-	T	A

nr=nucleotide.

Eutrema sinense (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz, comb. nov. Based on _Braya sinensis_ Hemsl., J. Linn. Soc., Bot. 30: 303. 1892.
urn:lsid:ipni.org:names:77190124-1

***Syn.*:** *Eutrema robustum (O.E.Schulz) Al-Shehbaz, G.Q.Hao & J.Quan Liu, Bot.* J. Linn. Soc. 184: 219. 2017. Basionym: *Pegaeophyton sinense* var. *robustum* O.E.Schulz, Notizbl. Bot. Gart. Gerlin-Dahlem 9: 477. 1926.

The earliest available epithet of this taxon at the species rank is "*sinensis*" and it should have been been transferred to *Eutrema* by Hao et al. (2017a) instead of using the varietal epithet "*robustum*."

Genetic differences between *Eutrema nanum*, *E. nepalense* and other relatives

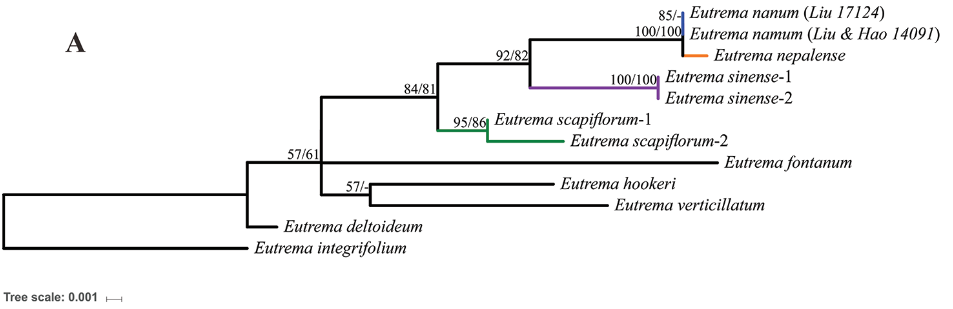
Sequence data from *Eutrema nanum* and *E. nepalense* reveals that one nucleotide substitution in ITS, two in *rbc*L, 18 in *mat*K and eight substitutions and three indels in *psb*A-*trn*H distinguish them very well (Table 2).

Based on sequence variations of ITS and cpDNAs (Table 3), phylogenetic analyses suggested that *Eutrema nanum* is mostly related to *E. nepalense*, *E. sinense* and *E. scapiflorum*. However, phylogenetic relationships of these four species are incongurent between ITS and plastid DNA tree (Fig. 4). In the ML analyses of ITS sequence data, *E. nanum* and *E. nepalense* formed a single cluster sister to *E. sinense* and together are sister to *E. scapiflorum* with high support values (>80%) (Fig. 4A). By contrast, in the ML analyses of cpDNAs sequences, the phylogenetic relationships were maintined between *E. nanum* and sister *E. sinense* and together as sister to *E. scapiflorum*, but *E. nepalense* fell outside that relationship and was separated from them by *E. hookeri* with medium support (>50%) (Fig. 4B). MP analyses produced almost the same tree topologies with similar bootstrap support values.

Table 3. Tree statistics for analyses of the datasets.

Data set	ITS*	*psb*A-*trn*H	*rbc*L	*mat*K	Combined cpDNA*
No. of sequences	30	30	30	30	30
Aligned length used in analyses	698	455	506	779	1786
No. of variable characters	141	58	17	66	169
No. of parsimony-informative characters	56	15	7	22	47
Tree length (steps)	78	70	10	71	195
Consistency (CI)	0.833333	0.900000	1.000000	0.873239	0.892308
Retention index (RI)	0.803030	0.708333	1.000000	0.790698	0.764045
Rescaled consistency index (RC)	0.669192	0.637500	1.000000	0.690468	0.681763

*gaps were coded and included.



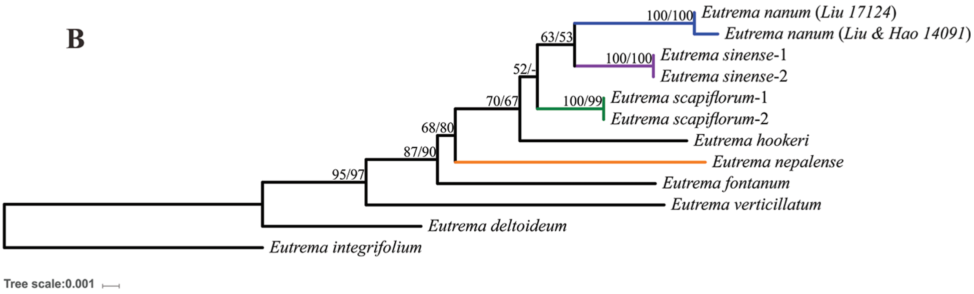


Figure 4. The Maximum Likelihood tree based on analysis of ITS (**A**) and Three cpDNA regions (**B**). Numbers above branches are maximum likelihood bootstrap support values and maximum parsimony bootstrap support values. '-' represents <50%.

Discussion

Both *Eutrema nanum* and *E. nepalense* are small plants similar in flower traits and seed size. However, as discussed above, they are quite different morphologically. In addtion, phylogenetic analyses of cpDNAs variations suggested these two species did not comprise a monophyletic clade. Furthermore, the Himalayan *E. nepalense* is disjunctly separated by a distance of at least 1200 air kilometres from the Chola Shan (Sichuan, SW China), where *E. nanum* is endemic (Fig. 3). *Eutrema nanum* is also closely related to *E. sinense* in the phylogenetic analyses of the cpDNA sequence variations, but both are easily distinguished from each other. *Eutrema nanum* is a small and weak herb with entire leaves (0.5–1.5 cm long) and small flowers (petals 2–3 mm long), whereas *E. sinense* is obviously stout with entire or toothed leaves (1.5–8 cm long) and distinctly larger flowers (petals 8–15 mm long) and fruit (10–20 mm long).

Pylogenetic relationships amongst *E. nanum*, *E. nepalense* and *E. sinense* are incongruent between ITS and cpDNA trees. This incongruence may suggest possible hybridisations or incomplete lineage sorting during the rapid and recent species diversifications (Soltis and Soltis 2000, 2009). However, it is not possible at present to determine which of these two factors had caused the incongruent phylogenies observed here. More analyses and molecular data, especially based on more individuals and genomic evidence, are needed to solve these phylogenetic inconsistences.

Acknowledgements

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RESEARCH ARTICLE

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Phryna hamzaoglui Koç & Budak (Caryophyllaceae), a new species from Central Anatolia, Turkey

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Abstract

A new species *Phryna hamzaoglui* was discovered in Hekimhan (Turkey, Malatya province) where it grows on hillsides. The *P. hamzaoglui* and *P. ortegioides* were compared with each other in terms of their general morphology and seed micromorphology. Description, distribution, illustration and conservation status of the new species are given. Seed lateral and surface, cells, anticlinal cell walls, periclinal cell walls structures were examined by scanning electron microscopy. The images were captured with the 500×, 100×, and 40× objective lens for the details.

Keywords

Phryna, Malatya, taxonomy, new species

Introduction

The family Caryophyllaceae Juss. includes approximately 88 genera and 3000 species that are almost entirely distributed throughout the northern hemisphere and occasionally throughout the southern hemisphere. The main distribution area of this family is the mediterranean phytogeographical region (Lawrence 1951, Rabeler and Hartman 2005). This family is split into three subfamilies, namely Alsinoideae (DC.) Fenzl, Caryophylloideae and Paronychioideae (A.L.Juss.) Meisn (Bittrich 1993). The Caryophylloideae subfamily is divided into the tribes *Caryophylleae, Drypideae* Fenzl and *Sileneae* DC. (Bittrich 1993).

The genus *Phryna* (Boiss.) Pax & K.Hoffm. belongs to the tribe Caryophylleae that exist in subfamily Caryophylloideae of the family Caryophyllaceae. A specimen of this genus collected from Turkey was last revised by Huber-Morath in his work entitled "Flora of Turkey and the Aegean Islands" which was published on 1967. In this revision, the existence of a species belonging to this genus was reported (Huber-Morath 1967), the genus being similar to *Ankyropetalum* Fenzl, *Acanthopyllum* C.A.Mey., *Allochrusa* Bunge, *Gypsophila* L. and *Bolanthus* (Ser.) Rchb. (Huber-Morath 1967, Barkoudah 1962).

Materials and methods

Some interesting specimens belonging to the genus *Phryna*, in which their main distribution area is the Irano–Turanian phytogeographical region, were collected from Malatya province. As a result of examining the literature (Barkoudah 1962, Huber-Morath 1967) and herbarium specimens, it was found that the collected specimens resemble *Phryna ortegioides* but differ from this species in terms of calyx length, calyx teeth length, petal colour, petal calyx ratio and capsule calyx ratio. Flower, petal and capsule pictures were taken with an OLYMPUS SZX-16 Stereomicroscope, DP 72 digital camera and seed surface images were taken by a Quanta Feg 450 scanning electron microscope (SEM) at Bozok University Research and Application Centre. The vegetative parts were measured with a ruler with 0.5 mm accuracy and the floral characteristics were studied using an ocular micrometer.

Taxonomic treatment

Phryna hamzaoglui Koç & Budak, sp. nov.

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Figs 1, 2, 3

Diagnosis. *Phryna hamzaoglui* is related to *P. ortegioides*. It differs from *P. ortegioides* mainly because it has a calyx 4–5.5 mm long, teeth 2–2.5 mm long (not 3–4 mm long, teeth 1–2 mm long); petals completely white not purple veins, as long as calyx (not white with purple veins, 1.5–2 times calyx); capsule including calyx (not longer than calyx); seeds ventral and dorsal cells margine S-undulate (not V-Undulate) (Table 1).

Type. TURKEY. B6 Malatya: Between Hekimhan and Hasançelebi town, 3 km from Hekimhan, 1100 m a.s.l., hillside, 10 October 2015, Koç 2353, Hamzaoğlu & Budak (holotype ANK; isotypes ANK, GAZI, Bozok Univ. Biology Dept. Herbarium).

Description. Perennial herb. Stems woody caudex, dichotomously forked stems, 30–35 cm, base 1–1.2 mm diam., entirely mixed eglandular and with glandular hairs; internodes 13–20 mm, with glandular hairy. Leaves linear, 6–15 × 0.9–1.1 mm, with glandular hairs, 3–nerved, greenish, solid; acute at apex; sheath membranous, 0.2–0.3 mm long, having glandular hairy. Inflorescence mostly solitary, forming lax terminal

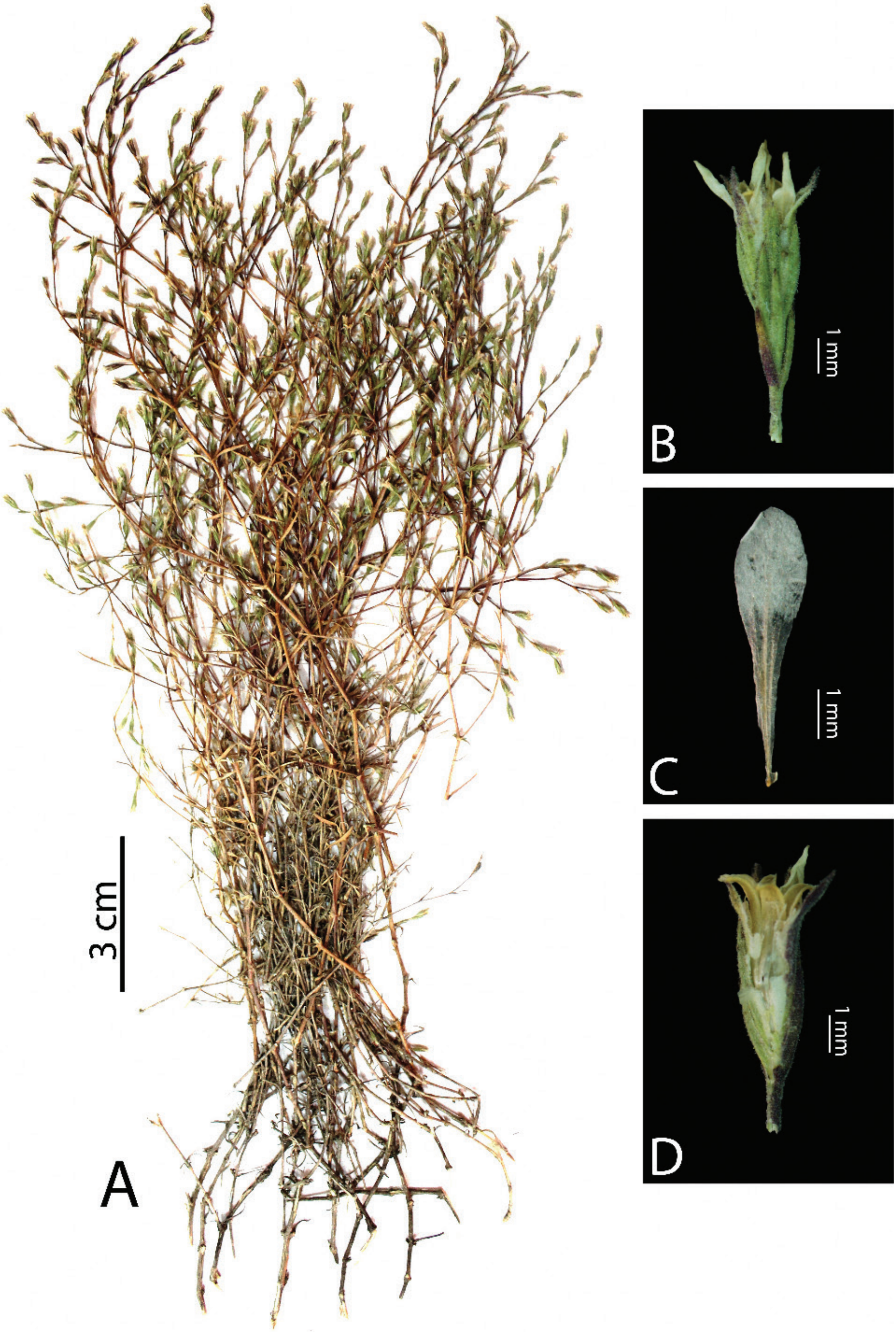

Figure 1. *Phryna hamzaoglui*. **A** habit **B** flower **C** petal **D** capsule.

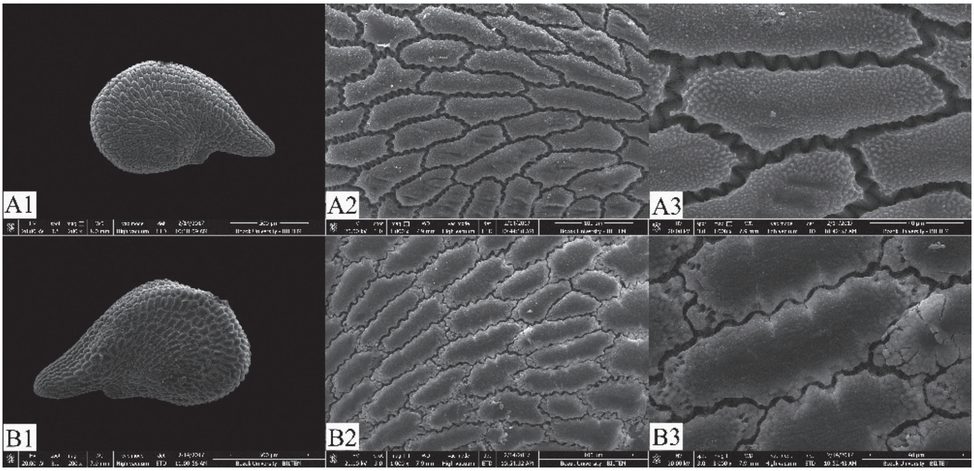

Figure 2. SEM photographs of the seed coat. **A** *Phryna ortegioides* **B** *P. hamzaoglui*. Scale bars: 500 µm (**A1, B1**); 100 µm (**A2, B2**); 40 µm (**A3, B3**).

racemes. Flowers sessile or subsessile. Bracts lanceolate, 3–4 × 0.8–1 mm; acute at apex. Bracteoles 2–3 pairs; acute at apex. Calyx narrowly campanulate, having densely glandular hairy, 4–5.5 × 1–1.2 mm, with 5 projecting ribs; teeth lanceolate, 2–2.5 mm long; acuminate at apex. Petals oblanceolate, 4–6 × 0.7–0.9 mm, as long as calyx, entirely white not purple veins; rounded at apex; base cuneate. Stamens 4; filaments 3–4 mm long. Styles 2, 3–4 mm long. Capsule oblong, 3.5–5 × 1–1.2 mm, 2–seeded, including calyx.

Seed micromorphology. Seeds of *Phryna hamzaoglui* are comma-shaped with prominent radicle, 1–1.4 × 0.8–1 mm; blackish; lateral and dorsal surfaces tuberculate; cells subrectangle, regular; anticlinal cell walls certainly S-undulate; periclinal cell walls convex, wrinkled, upper smooth (Figure 2).

Etymology. The species is named in honour of the eminent Turkish botanist Prof. Dr. Ergin Hamzaoğlu (Gazi University, Ankara).

Distribution and habitat. *Phryna hamzaoglui* is an endemic species known only from the type gathering in Central Anatolia (Hekimhan, Malatya) and is an Irano–Turanian element. *Phryna hamzaoglui* prefers hillsides at an altitude of 1100 m in Malatya province.

Phenology. Flowering from August to September; fruiting from October.

Conservation status. According to current data, *Phryna hamzaoglui* grows in an area of approximately 1000 km^2 covering the Hekimhan districts of Malatya province. The species, which has a discontinuous distribution, has been reported in only one locality in Malatya. Due to overgrazing, the habitat of this species is under threat and destruction of the species is leading to the reduction in the number of plants. For this reason, it is proposed that the species should be classified as Endangered [EN (B1a)] according to the International Union for Conservation of Nature (IUCN 2014).

Other specimens examined. *Phryna ortegioides.* TURKEY. Kayseri: Kıranadardı waterfall, 1710 m, 20 July 2013, Hamzaoğlu 6889 & Koç (Bozok Univ. Biology Dept.

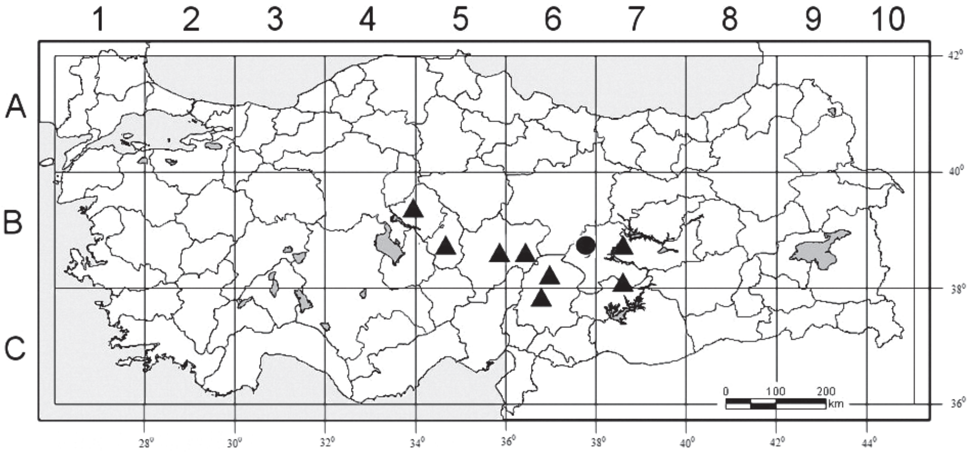

Figure 3. Distribution map of *Phryna ortegioides* (▲) and *P. hamzaoglui* (●) in Turkey.

Herbarium); Kayseri: Sarız, Büyüksöbeçimen town, 15 July 2008, Hamzaoğlu 5229 (Bozok Univ. Biology Dept. Herbarium); Adıyaman: Nemrut mountain, 2000–2200 m, 18 June 1997, Vural & Adıgüzel (GAZI, No: 8222); Elazığ: Keban Altınayva town, 1300 m, 11 August 1995, Ali.A.Dönmez 4899 & Aslantaş (GAZI); Kahramanmaraş: Elbistan, Şardağı, 1600 m, 21 August 1989, Aytaç (GAZI, No: 3012); Kayseri: Erciyes mountain, 2250–2350 m, 21 September 1993, Vural 7028, Duman, Adıgüzel & Erik (GAZI); Nevşehir: Zelve, Akdağ, 1250 m, 08 August 1989, Vural & Eyüboğlu (GAZI, No: 5487); Kırşehir: Kaman, 1200 m, 05 September 1995, Aytaç 7329 & Adıgüzel (GAZI); Kahramanmaraş: Göksu, Çardak, Binboğa mountain, Tülüce hill, 1450–1850 m, 08 August 1988, Aytaç (GAZI, No: 2537).

Results

The *Phryna* is an endemic genus for Turkey. This genus is similar to *Petrorhagia*, *Ankyropetalum*, *Acanthopyllum*, *Allochrusa*, *Gypsophila* and *Bolanthus*. All of these genera have calyx with membranous hyaline intervals between the nerves (Barkoudah 1962, Huber-Morath 1967). *Petrorhagia* can separate from the above genera by seeds with the presence of peltate with facial hilum. While *Ankyropetalum*, *Acanthopyllum*, *Allochrusa* and *Gypsophila* have reniform seeds, *Bolanthus* and *Phryna* genera have comma seeds. In addition to these characters, the genus Phryna is distinguished from all the above-mentioned genera by having 1-4 pairs of bracteoles on the base of calyx (Barkoudah 1962).

While *Phryna ortegioides* species has a wide distribution in Turkey, the distribution of P. *hamzaoglui* is known only around Hekimhan (Malatya). The specimens of Hekimhan (Malatya) show similarity to *Phryna ortegioides* due to their having stems covered with glandular-puberulent hairy, woody caudex and flowers sessile, axillary and terminal, mostly solitary, seeds comma-shaped with prominent radicle, blackish;

Table 1. Diagnostic characters between *Phryna hamzaoglui* and *P. ortegioides*.

	P. hamzaoglui	***P. ortegioides***
Calyx	4–5.5 mm long, teeth 2–2.5 mm long	3–4 mm long, teeth 1–2 mm long
Petal	Completely white not purple veins, as long as calyx	White with purple veins, 1.5–2 times calyx
Capsule	Including calyx	Longer than calyx
Seeds	Ventral and dorsal cells margine S-undulate	Ventral and dorsal cells margine V-undulate

lateral and dorsal surfaces tuberculate; cells subrectangle, regular; periclinal cell walls convex, wrinkled, upper smooth. However, they differ from this taxon in terms of having calyx length of 4–5.5 mm, petals entirely white not purple veins and as long as calyx, capsule including calyx, seeds ventral and dorsal cells margine S-undulate (Table 1). As a result, all the obtained morphological and micromorphological data showed that the species distributed in Malatya province are new to science. The genus is still endemic for Turkey and but will be regarded as a polytipic genus.

Key to the species of Genus *Phryna* in Turkey

1 Calyx 4–5.5 mm long, teeth 2–2.5 mm long; petals completely white not purple veins, as long as calyx; capsule including calyx .... ***P. hamzaoglui***

– Calyx 3–4 mm long, teeth 1–2 mm long; petals white with purple veins, 1.5–2 times calyx; capsule longer than calyx .... ***P. ortegioides***

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A series of unfortunate events: the forgotten botanist and the misattribution of a type collection

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Abstract

In 1777, Spain sent The Royal Expedition to Peru and Chile, commonly referred to as the "Ruiz and Pavón Expedition", to document the flora of this richly diverse part of its colonies. The Expedition was active in the New World for 38 years and was led by Hipólito Ruiz and José Pavón, who collected in Peru and Chile for the first 11 years before returning to Spain to work on publication of the *Flora Peruviana, et Chilensis*. Work on the ground for the reminder of the project in Peru and later Ecuador, was continued by Juan Tafalla and Juan Manzanilla. With few exceptions, all collections from the Expedition including types, have conventionally been attributed to Ruiz and Pavón despite the fact that Tafalla and Manzanilla were responsible for a large part of the collections. The purpose of this note is to bring to light the important but often overlooked figure of Juan Tafalla, to highlight his accomplishments as a collector and to increase precision in botanical nomenclature by giving him due credit by correctly attributing collections.

Resumen

En 1777, España mandó la Real Expedición Botánica al Virreinato del Perú, más conocida como la "expedición de Ruiz y Pavón," con el fin de documentar la flora de esta parte de sus colonias notable por su diversidad. La expedición continuó en el nuevo mundo por 38 años y fue dirigida por Hipólito Ruiz y José Pavón durante los primeros 11 años, quienes recolectaron en Perú y Chile antes de volver a España para trabajar en la publicación de la *Flora Peruviana, et Chilensis*. El proyecto fue continuado en Perú, y luego Ecuador, por Juan Tafalla y Juan Manzanilla. Con pocas excepciones, todas las colecciones de la expedición, incluyendo tipos, han sido atribuidos a Ruiz y Pavón a pesar de que Tafalla y Manzanilla son responsables de una gran cantidad de las colecciones. El propósito de esta nota es traer a la luz la figura importante pero frecuentemente ignorada de Juan Tafalla, destacar sus logros como colector, y mejorar la precisión en la nomenclatura botánica.

Keywords

Ecuador, Juan Tafalla, Peru, Real Expedición, Hipólito Ruiz and José Pavón, Scientific expeditions

Introduction

The following is a correction of a previously published revision of *Solanum* sect. *Herpystichum* (Tepe and Bohs 2011) and a historical note intended to help future authors avoid making the same mistake. In that work, I repeated Michel Felix Dunal's attribution of the type collection of *Solanum trifolium* Dunal, a species endemic to the central Andes of Ecuador, to Ruiz and Pavón (Tepe and Bohs 2011). Ruiz and Pavón, however, never collected in Ecuador and, in fact, by the time that collection was made in August 1804, the pair had already been back in Spain for over six years (Figure 1). The type specimen of this species was actually collected by a little-known botanist and collaborator of Ruiz and Pavón, Juan José Tafalla y Navascués (sometimes spelled Nabasques) and his assistant, Juan Manzanilla (Estrella 1989; Goicoechea Marcaida 1989; Adanero Oslé 2011).

This error stems from several interrelated causes. Spain organized an effort in the late 18th century to document the flora of Peru and, although a number of people would conduct the project on the ground in the New World, the entire enterprise is frequently referred to as "The Ruiz and Pavón Expedition." A second reason lies with the ownership and control that Ruiz and Pavón exerted over the entire enterprise, even after their return to Spain. And thirdly, confusion over the collectors of specimens was further confounded by the manner in which specimens collected during the Expedition were initially labeled, later intermixed and, eventually, disseminated. All of this has contributed to largely pushing Juan Tafalla, one of the most prolific early collectors of Neotropical flora, into obscurity.

The story of Tafalla, the botanist, begins with the establishment of the *Real Expedición Botánica al Virreinato del Perú* (1777–1816), more commonly known, as mentioned above, as "The Ruiz and Pavón Expedition" (Steele 1964). In 1777, the Spanish Crown sent Hipólito Ruiz López, José Antonio Pavón Jiménez, French botanist Joseph Dombey, along with artists José Brunete and Isidro Gálvez, to the Viceroyalty of Peru, on what was initially planned to be a four-year effort to produce a comprehensive and beautifully-illustrated flora of the territory. The Viceroyalty, during the time of the Expedition ("*Peruvia*" on labels and in subsequent works), was much larger than the current Peruvian territory and encompassed modern day Peru, along with parts of Bolivia, Brazil, Chile and Ecuador. The job turned out to be much larger than expected – not surprising for what we now recognize as one of Earth's biodiversity hotspots (Myers et al. 2010; Ulloa Ulloa et al. 2017) – and, instead of four years, the venture was active in the New World for 38 years (Steele 1964).

Having already invested more time than anticipated in the New World, the ageing polymath Dombey retired from the Expedition and departed from Peru for Europe in 1784 (Steele 1964). As a replacement for Dombey, and planning for long-term productivity, Spain appointed Juan Tafalla to apprentice under Ruiz and Pavón and another artist, Francisco Pulgar, to work under Brunete and Gálvez. These newcomers were initially not well received by the original members of the Expedition and, throughout much of Ruiz's journals, they are referred to as the "agregados," or attachés

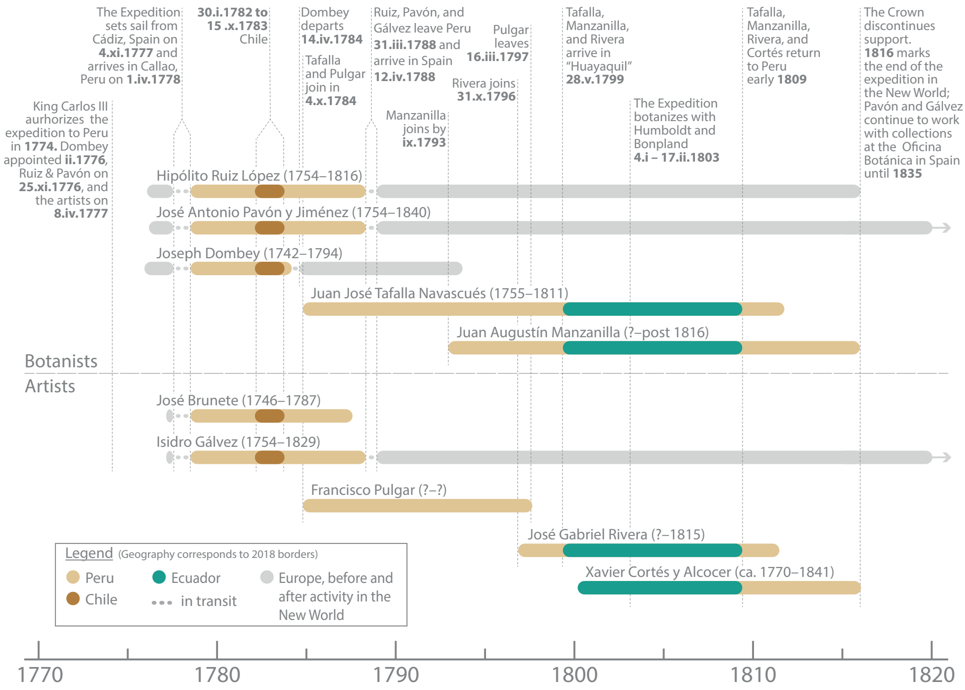

Figure 1. Timeline of participants during the New World portion of the *Real Expedición Botánica al Virreinato del Perú* (1778–1816). Dates are day, month in Roman numerals and year. The years following participants' names are birth–death. Bars in the timeline are limited to individuals' active participation in the project. Dates from Steele (1964); Estrella (1989); Adanero Oslé (2011); Pupulin (2012a); A. Wulf (pers. comm.).

(Steele 1964). This lack of recognition by name would last for years and Ruiz even requested a replacement for Tafalla (Estrella 1989; Ruiz 2007). Ruiz, however, ultimately came around and acknowledged Tafalla's careful work and dedication to the Expedition (Estrella 1989; Ruiz 2007). Eventually, Ruiz honored Tafalla with the dedication of the genus *Tafalla* Ruiz & Pav. (now a synonym of *Hedyosmum* Sw., Chloranthaceae) stating that the genus was "…dedicated to our esteemed disciple…" (Ruiz and Pavón 1794; author's translation).

Tafalla was appointed as head of the New World portion of the Expedition when Ruiz and Pavón received orders on 12 Sept 1787 to return to Spain and begin working towards publishing the Flora (Steele 1964; Ruiz 2007). Although he was now in charge, Tafalla never gained independence from Ruiz and Pavón, who considered him their subordinate throughout the project and to whom they frequently sent requests for additional details to refine their work. Tafalla was productive, despite limited financial support and, ultimately, the Crown provided the Expedition with another botanist, Juan Augustín Manzanilla, in 1793; other new additions to the project came in 1796 with the artist José Gabriel Rivera and, in 1800, the artist Xavier Cortés y Alcocer (Steele 1964; Adanero Oslé 2011; Pupulin 2012a). Tafalla went on to lead the Expedition in Peru for another eleven years until, upon receiving orders from the Crown, moving on to Ecuador (at the time, the *Real Audiencia de Quito*). The team collected in the coastal plains and foothills of the Andes from 1799 to 1803 before switching their focus to the southern Andes of Ecuador and to the investigation of *Cinchona* L. (Rubiaceae), the source of the antimalarial quinine (Steele 1964). While in Ecuador, members of the Expedition met and botanized with Alexander von Humboldt and Aimé Bonpland from 4 January to 17 February 1803 (A. Wulf, pers. comm.), and Humboldt wrote favorably about Tafalla and his team (Estrella 1989; Adanero Oslé 2011).

Meanwhile, shortly after their return to Spain, Ruiz and Pavón had established the *Oficina Botánica* or *Oficina de la Flora Americana* in Madrid, where they studied specimens and prepared their works for publication (Steele 1964; Rodríguez Nozal 1995). During this time, Tafalla continued to collect and to send a large number of specimens, seeds, illustrations, descriptions of new species and other items of interest to Ruiz and Pavón. Between 1788 and 1815, Tafalla sent no fewer than 101 shipments which, upon arrival, were mixed with previous collections and published in the "Flora Peruviana" and other works (Ruiz and Pavón 1794, 1798a, 1798b, 1799, 1802). Tafalla received acknowledgement for is contribution to these works, but not co-authorship (Adanero Oslé 2011). In 1799, shortly after arriving in Ecuador, Tafalla changed his labeling of plants from "*F. P.*" for *Flora Peruviana* to "*F. H.*" for *Flora Huayaquilensis* (Adanero Oslé 2011). Even though Ruiz and Pavón ignored this new labeling system and continued to group Tafalla's collections with the rest, this change suggests that Tafalla saw this work as an independent project. During his time in Ecuador, Tafalla produced a nearly complete manuscript, which remained unpublished and was only rediscovered by the medical doctor and science historian Eduardo Estrella in 1985, who published both the descriptions and the illustrations (Tafalla Navascués 1989, 1991). Several authors suggest that the independent publication of Tafalla's *Flora Huayaquilensis* during his lifetime may have been suppressed (Herrera 1937; Estrella 1989). The fact that his work remained unpublished for centuries is not only a historic oversight, but also had the tragic consequence that numerous Ecuadorian species, although Tafalla was likely the first European to see most of them, were ultimately described by botanists from Germany, France and England etc. (e.g. Bonpland et al. 1815; Don 1830) – some even based on Tafalla's collections – while Tafalla authored none.

The specimen labels from the Expedition are typical of the era and, when not missing, bear minimal information. A subset of specimens has labels with locality information or collection dates, but, with the exception of some of Dombey's labels, very few identify the collector. To make matters worse, the identity of the collector is further obscured by the gradual and questionable way in which specimens were disseminated. Following Ruiz's death in 1811, Pavón began selling specimens to collectors throughout Europe, using the profits to maintain operations of the *Oficina Botánica* (Steele 1964; Miller 1970; Rodríguez Nozal 1994, 1995). For example, Pavón sold at least 15,894 specimens to the renowned British collector and prominent member of the Linnean Society of London, Aylmer Bourke Lambert (many of these specimens are now in the collections of B, BM, FL, G, K, L, P, US and other herbaria.; Rodríguez Nozal 1994). These specimens, having come from Pavón, were frequently attributed to him, regardless of where and by whom they had been collected. Many species were described from these purchased specimens and most of the type collections were attributed to Ruiz and Pavón or merely to Pavón. The rare exceptions were Ecuadorian species described by David Don who attributed two types to Tafalla (Don 1830). In addition to these, Tafalla has also been recognized as the type collector of several additional species in more recent revisions and floristic studies (e.g. Harling 1995; Delprete 1999; Pupulin 2012a, b; Morales and Zamora 2017). On the other hand, authors who are aware of Tafalla and his collections, often assume that all of his collections are Ecuadorian in origin (e.g. O'Leary 2015), even when that would place a specimen well outside a species' normal range. Of the 27 years that Tafalla spent collecting in South America, 17 were spent collecting in Peru, so a great many of his collections are certainly from Peru. Unfortunately, because of the minimalistic nature of the labels, it is often impossible to know whether the Peruvian specimens were collected by Ruiz and Pavón or by Tafalla, and it is likely that many of the Expedition's collections from Peru that are attributed to Ruiz and Pavón were, in fact, collected by Tafalla or Tafalla and Manzanilla.

Juan Tafalla deserves recognition for having explored Ecuador and Peru more extensively and more deeply than any other Spanish botanist of his time (Adanero Oslé, 2011), as well as being one of the most prolific collectors of his time and a major contributor to the Ruiz and Pavón Expedition. A small step toward making amends for this historic oversight is to accurately acknowledge Tafalla for his collections. In that spirit, the following is a correction to the type information of *Solanum trifolium* published by Tepe and Bohs (2011), along with the addition of a recently discovered isotype at OXF.

Solanum trifolium Dunal, DC. Prodr. 13(1): 68. 1852.

Type. Ecuador. "In Peruviæ provinciâ Huyaquil" Aug 1804 (fl), J. Tafalla & J. Manzanilla s.n. (holotype: G [G00080145!]; photos of holotype [F neg. 8591]: F [F–651206!], MO [MO–1691471!], NY!; isotypes: MA [MA–747193!], OXF [OXF–00006573!]; possible isotype: G-DC [G00144594!]).

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RESEARCH ARTICLE

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Thesium nautimontanum, a new species of Thesiaceae (Santalales) from South Africa

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Abstract

Thesium nautimontanum M.A. García, Nickrent & Mucina, a new species from the Matroosberg Mt. of Western Cape Province of South Africa, is described and illustrated. This species shows several morphological features unusual for the genus including stem sympodial branching, indeterminate spicate inflorescences subtended by numerous bracts and fleshy, non-trichome tissue lining the inside of the corolla lobes. Molecular phylogenetic analyses using nuclear ribosomal internal transcribed spacer sequences place this taxon as sister to all African, Madagascan and South American *Thesium* species. Given that only two proximal populations are known, this species is of conservation concern.

Keywords

Capensis, endemic plant, Matroosberg mountain, parasitic plant, Santalaceae

Introduction

Thesium L. is the most speciose genus in the sandalwood order (Santalales) with over 350 species (Nickrent and García 2015). The genus has traditionally been placed in Santalaceae s. lat., however, the Nickrent et al. (2010) classification places *Thesium* in Thesiaceae. Recent taxonomic changes have expanded the genus by including previously described genera such as *Kunkeliella* Stearn and *Thesidium* Sond. (Forest and Manning 2013). Although *Thesium* can be found in Europe, Asia, Australia, South America and North America (by human introduction), the vast majority of species are African with the greatest diversity being found in the Cape region. Indeed, the fact that *Lacomucinaea* Nickrent & M.A. García and *Osyridicarpos* A. DC. are sister to *Thesium* (Nickrent and García 2015) and are both restricted to southern Africa indicates this is the likely region where the genus originated.

The Fynbos Biome, embedded within the South African Cape Floristic Region, sometimes referred to as a Floristic Kingdom (Takhtajan 1986), is one of the most floristically diverse regions in the world (Mucina and Rutherford 2006). Indeed, of the ca. 170 named species of *Thesium* from South Africa, over 40 of these are found only in the Cape region. High diversity complicates identification of all angiosperms but this is particularly true in poorly documented genera such as *Thesium*. The last major taxonomic works dealing with *Thesium* in South Africa were by Hill (1915, 1925), but since then a number of new species have been named. The keys presented in Hill's works are today not particularly useful in identifying South African *Thesium* because they do not include more recently named species, e.g. those from Brown (1932) or Compton (1931). Moreover, the characters used to distinguish species are not effective when a broad spectrum of samples is examined. This is exacerbated by phenotypic plasticity seen in some species across developmental stages and under different ecological conditions (fire, grazing, elevation, substrate etc.).

This paper describes a new species of *Thesium* that is exceptional from several perspectives. In terms of morphology, it shows several features that are unique in the genus. From the molecular phylogeny perspective, this species holds a special place in that it is sister to the entire clade of African *Thesium* (250 species).

Materials and methods

The morphology of this new species was studied from herbarium samples collected by J. Šibík and I. Šibíková in October 2007 and Mr. Eugene Pienaar in December of the same year. Material from the herbarium samples was rehydrated and examined with a stereomicroscope. DNA extraction, PCR and sequencing of nuclear internal transcribed spacer of ribosomal DNA (ITS) was conducted as specified in Nickrent and García (2015). The new ITS sequence generated for this study is available in GenBank with accession number MH807489.

The ITS sequence from the new species was added to the alignment from Nickrent and García (2015). This alignment has been submitted to TreeBase with study reference number S23256. Phylogenetic analyses were conducted as detailed in Nickrent and García (2015) and included maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses.

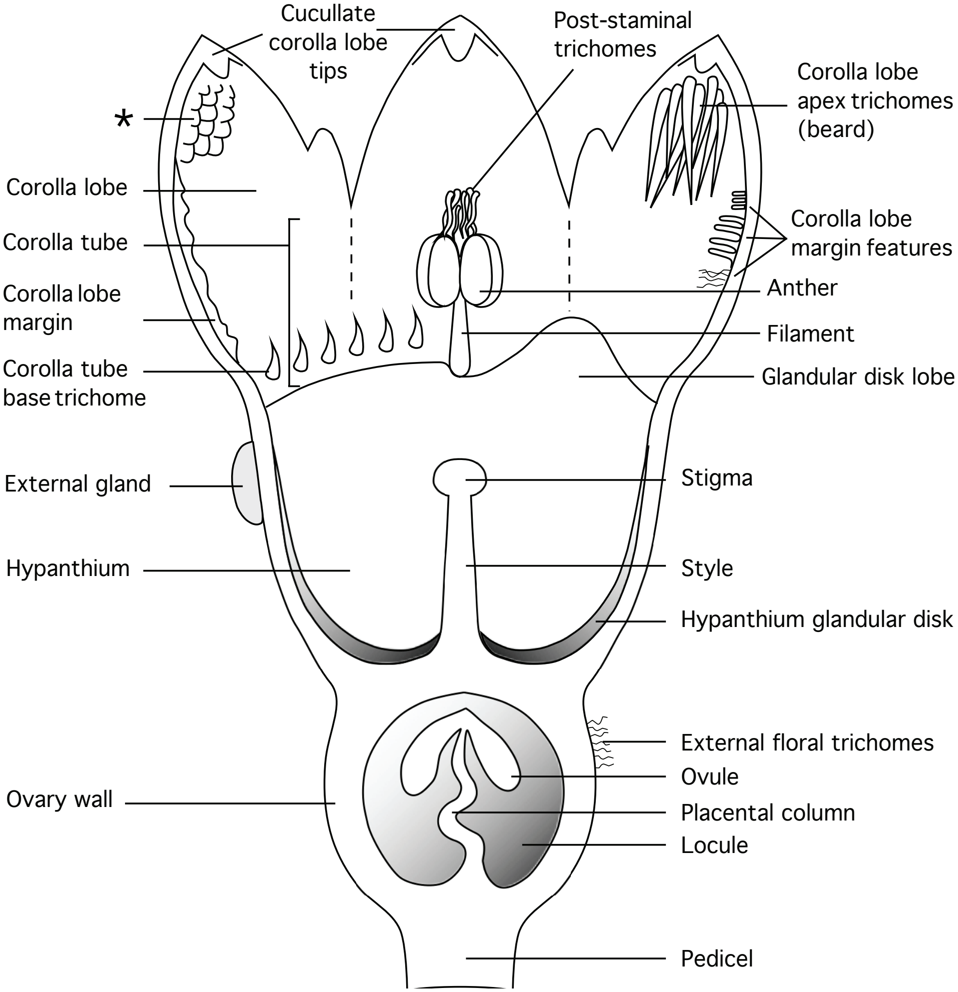

Figure 1. Diagramatic longitudinal section through a generalised *Thesium* flower. The figure was composited from numerous species, hence not all features occur in one species. Nearly all characters shown are variable within the genus. The asterisk (*) indicates the unique fleshy tissue seen lining the corolla lobes in *T. nautimontanum*.

To aid interpretation of the flower description, a generalised *Thesium* flower longitudinal section is shown in Fig. 1.

The extent of occurrence (EOO) and area of occupancy (AOO) were determined using the Geospatial Conservation Assessment Tool with a default cell width of 2 km (GeoCAT, Bachman et al. 2011). The conservation status of the species was determined by consulting the Guidelines for Using the IUCN (International Union for Conservation of Nature) Red List Categories and Criteria (IUCN 2014).

Taxonomy

***Thesium nautimontanum* M.A. García, Nickrent & Mucina, sp. nov.**
urn:lsid:ipni.org:names:77190706-1
Figs 2, 3

Type. SOUTH AFRICA. Western Cape Province, Cape Winelands District, Matroosberg, southern slope, 33°23'49.3"S, 19°40'29.5"E, elev. 1016 m; 6 Oct 2007, *J. Šibík & I. Šibíková MS463* (holotype: MA-877227).

Diagnosis. Squamate, glabrous, perennial herb 30–35 cm tall with erect, terete to ribbed sympodial branches; leaves reduced to triangular, acute scales up to 1.7 mm long, increasing in size and density apically, grading into inflorescence bracts. Inflorescences terminal, oblong, crowded spikes bearing up to 50 bracteate flowers, ending in a group of sterile bracts. Flower ca. 2.0 mm long, greenish outside, whitish inside, with triangular external glands between the corolla lobes, internal surface of lobes bearing thick, irregular, spongy and somewhat papillose tissue. Fruit wall marked with conspicuous, strongly raised reticulations.

Description. Perennial herb, squamate, all parts glabrous; rootstock slender, bearing one or few erect flowering stems 30–35 cm tall with sympodial branching that occurs mostly at the base of inflorescences, branches erect, terete or shallowly ribbed. Leaves all reduced to minute scales, the lower ones 0.5–1.0 × 0.8–1.7 mm, broadly triangular, acute, with darkened tips, erect and more or less appressed to the stem, increasing in size upwards and transitioning into bracts, 1.1–1.9 × 0.8–1.1 mm, ovate-triangular to ovate, acute to acuminate, with entire margins, incurved to naviculate but lacking a marked midrib and bearing in their axils undeveloped buds, progressively more crowded towards the base of the inflorescence, greenish to straw-coloured. Inflorescences terminal (on long and short shoots), oblong, spikes 10.5 × 18.5 mm, bearing (15-)24–50 crowded flowers, ending in a group of about 7–14 sterile bracts. Flowers ca. 2.0 mm long, solitary and sessile in the axil of each bract or with a short (up to 0.8 mm long) pedicel. Flower bracts 1.5–2.2 × 1.0–1.4 mm, similar in shape to the bracts at the base of the inflorescence; bracteoles 2, similar to bracts but smaller. Corolla greenish outside, whitish inside; external glands between the corolla lobes inconspicuous, triangular-ovate, pale orange in colour; hypanthium shallow, 0.2 mm long, internal surface glandular; corolla tube ca. 0.4 mm long; corolla lobes 0.7–0.8 × 0.6–0.7 mm, ovate or ovate-triangular, ± obtuse to slightly hooded and with inflexed tips on fruit, erect, margins entire, lacking hairs inside but with a thick, irregular, fleshy and somewhat papillose tissue. Staminal filaments inserted at junction of hypanthium and corolla tube, very short and inconspicuous, stamen not reaching the corolla lobe sinuses, anthers rounded ca. 0.3 mm in diameter, attached to the corolla tube by a small tuft of hairs. Style very short (ca. 0.1 mm) or lacking, thus stigma sessile, lobes not apparent, reaching the base of the anthers. Placenta twisted, ovules 3. Fruits ca. 3.0 mm long, ovary portion subglobose, 1.5–2.1 × 1.6–2.0 mm, greenish, with slightly marked ribs but reticulations strongly raised, crowned with the 1.0 mm long corolla remnant, pedicel not enlarging into an elaiosome.

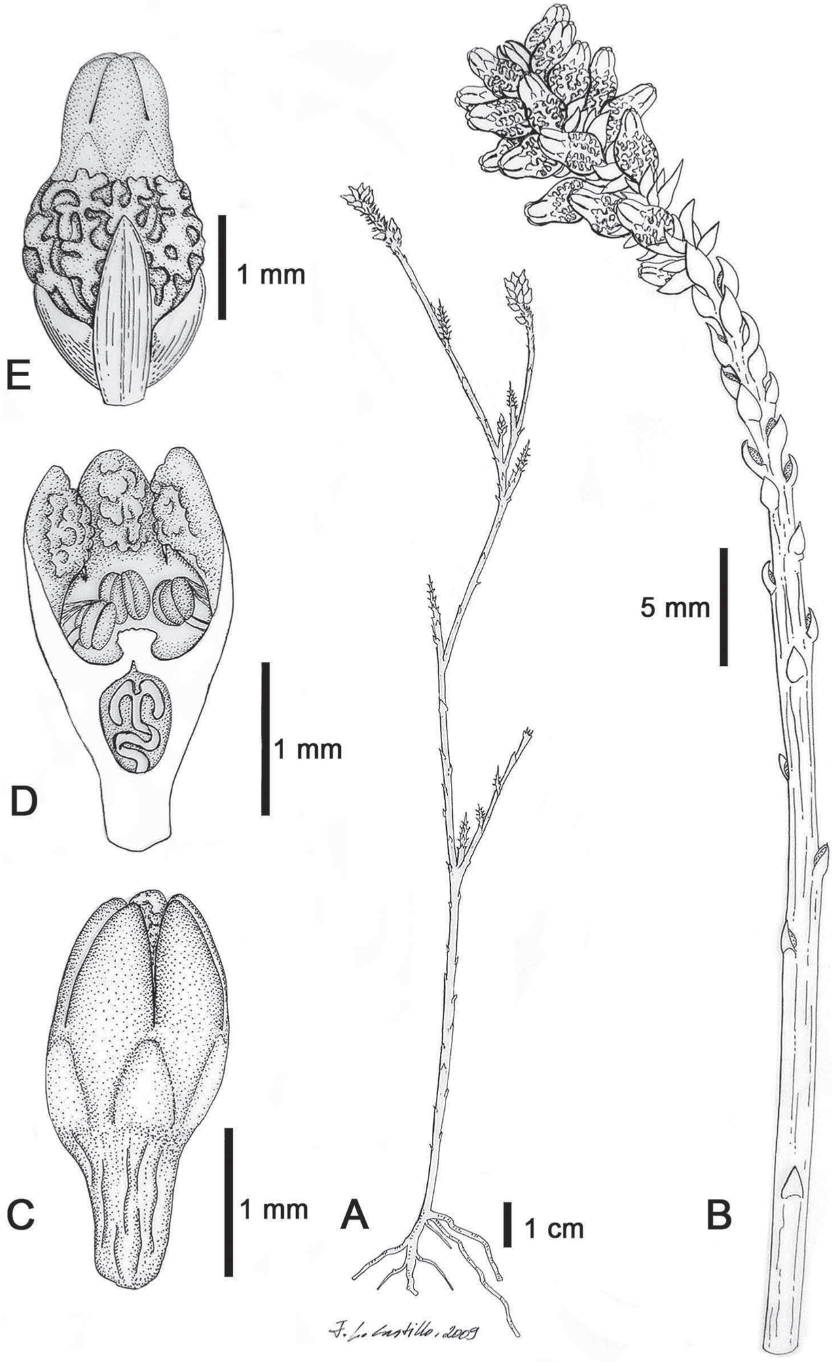

Figure 2. *Thesium nautimontanum* M.A. García, Nickrent & Mucina, sp. nov. **A** habit of plant showing sympodial branching **B** flowering shoot showing squamate condition, vegetative bracts grading into floral bracts and terminal spicate inflorescence **C** flower, lateral view **D** flower longitudinal section **E** young fruit subtended by bract and two bracteoles. Drawing by J. L. Castillo.

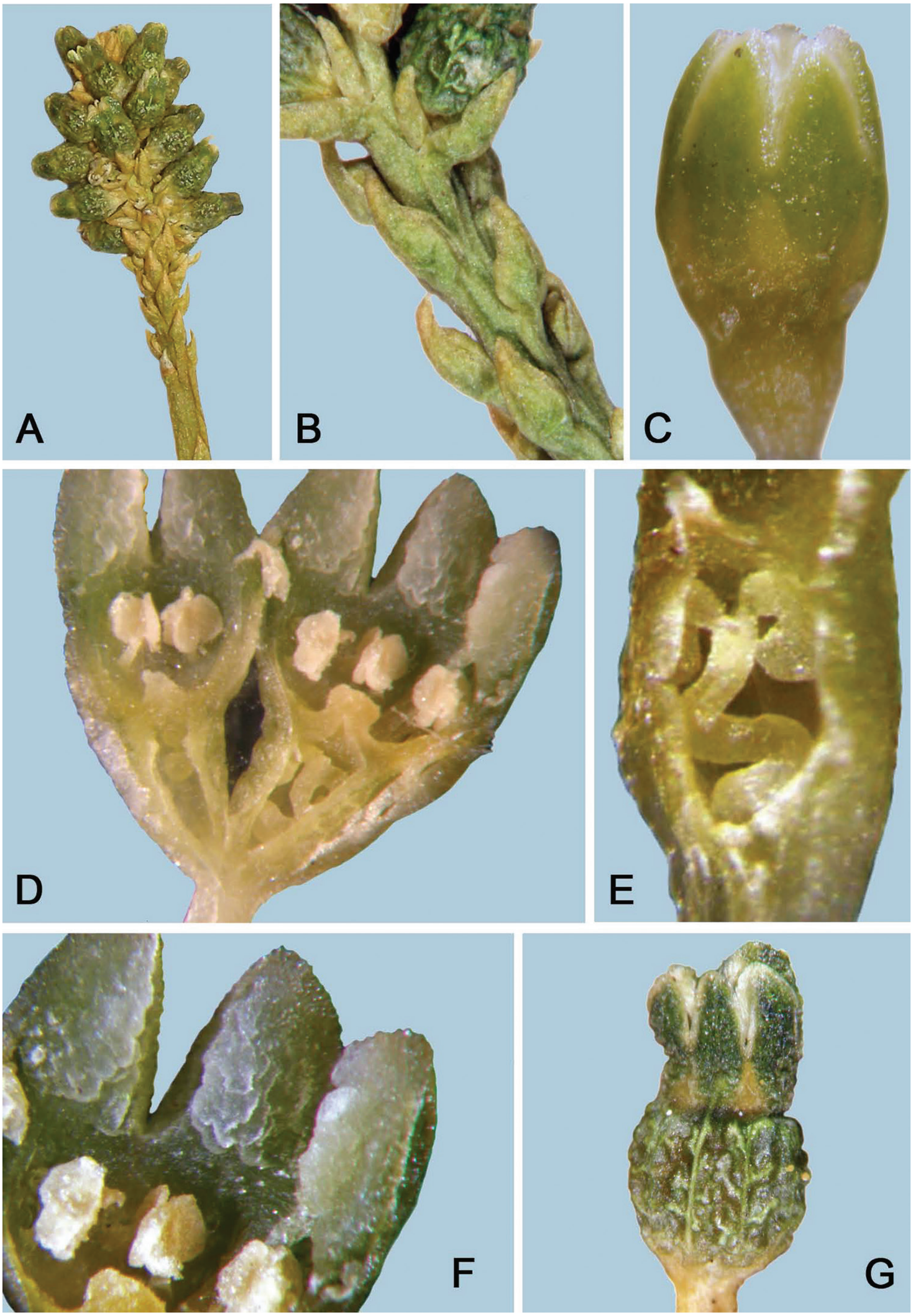

Figure 3. *Thesium nautimontanum* from the type herbarium specimen. For dimensions, see Figure 2. **A** spicate inflorescence subtended by bracts **B** close-up of inflorescence bracts **C** flower, lateral view, showing triangular external glands in corolla lobe sinuses **D** flower longitudinal section **E** ovary longitudinal section showing twisted placental column and ovules (one ovule removed to visualise the placentation) **F** close-up view of interior surface of corolla lobes showing unusual fleshy tissue on inner surface of corolla lobes **G** young fruit showing persistent corolla and reticulate outer wall of ovary.

Distribution and ecology. Both collections of this species were made on the Matroosberg Mt. in the Southern Hex River mountain range, Western Cape Province, Cape Winelands District, northwest of the De Doorns settlement.

The holotype collection, from 1016 m elevation, occurred on the southern slopes of the Matroosberg Mt. in the FFs8 South Hex Sandstone Fynbos vegetation according to Rebelo et al. (2006). The soils are acidic lithosols derived from Ordovician sandstone of the Table Mountain Group. This vegetation is, on the type locality, characterised by sclerophyllous, microphyllous fynbos scrub dominated by proteaceous, ericaceous and asteraceous shrubs, with rich graminoid understory containing numerous Cyperaceae and Restionaceae. The area of the South Hex Sandstone Fynbos receives, on average ca. 950 mm of rain annually, peaking from May to August. Summer weather may include heavy mist precipitation at higher altitudes. The mean daily temperatures are 26°C and 2.7°C for February and July, respectively.

The second population was discovered at an elevation of 1920 m, close to the summit of Matroosberg (2249 m). Here *Thesium nautimontanum* was found in a slight depression supporting a dense restioid-ericoid fynbos wetland (dominated by graminoids of the family Restionaceae and shrubs of *Erica* L.), surrounded by dry fynbos scrub of the FFs30 Western Altimontane Sandstone Fynbos. The soils here are nutrient-poor and sandy, overlying Table Mountain Sandstone. In contrast to the South Hex Sandstone Fynbos, the altimontane fynbos unit receives higher mean annual rainfall (1380 mm on average), peaking from May to August; frequent fog occurrence (due to summer tradewinds) may carry additional precipitation, mitigating the effect of summer drought at high elevations – a phenomenon common to many Cape Fold Mountains (see Rebelo et al. 2006, p. 69). The mean annual temperature is lower, as it ranges between 22.9°C (February) and 0.1°C (July). The summit regions of Matroosberg regularly receive snow during austral winter.

Phenology. The specimens examined had spikes containing both flowers and maturing fruits and were thus at similar developmental stages. As the lower elevation collection was flowering in October and the higher elevation one in December, one can extrapolate that the flowering time ranges from ca. September to January.

Etymology. The specific name stems from the Matroosberg Mt. where the species was first discovered. In Afrikaans, “matroos” means “sailor” and “berg” means mountain. As the Code of Botanical Nomenclature suggests, epithets formed from a geographic name should be in adjectival rather than substantive form, “nauta” (sailor) and “mons” (mountain) become *nautimontanum*.

Conservation status. According to the World Database of Key Biodiversity Areas (http://www.keybiodiversityareas.org/home), the Hex River Mountains are surrounded by globally and regionally significant areas (UNESCO World Heritage Site ‘Cape Floral Region’), but are themselves not currently included. Due to rugged terrain, nutrient-poor soils not viable for large-scale agricultural activities and relative low-key tourism interest (more of importance on the northern flank of the Matroosberg Mt.), the biota of the Hex River Mts. is not under direct, unmitigated threat. However, fynbos scrub is a naturally fire-prone ecosystem, yet too frequent (or too hot) fires might have negative impacts on the natural post-fire regeneration (e.g. Le Maitre and Midgley

(1992), Bond and van Wilgen (1996)). The morphology of Pienaar's collections, having a loose bunch of thin roots, suggests the species is a seeder, hence regenerates after fire from the seed bank.

A section of the Hex River Mts. is under management of CapeNature (the Western Cape provincial nature conservancy authority) and is formally protected in Bokkeriviere Nature Reserve. The coordinates of E. Pienaar M1249 are at 1920 m a.s.l., which place this higher elevation population just south of the boundary of the Reserve. This may well indicate that the species might also occur within the boundaries of the Reserve.

The limited geographic distribution of *Thesium nautimontanum* is, however, of concern. Using GeoCAT for the two populations, the extent of occurrence was 0.0 and the area of occupancy was estimated at 8.0 km^2 which translates to an IUCN Red List category of Critically Endangered, criteria B2 (IUCN 2014).

Further exploration of these regions should be conducted to determine whether additional populations exist. Even if more populations are encountered, most likely it will stay in the category of Critically Endangered because it is an endemic species restricted to the Matroosberg Mt.

Additional specimens examined. SOUTH AFRICA. Western Cape Province, Cape Winelands District, Matroosberg, southern slope, 33°23'43.48"S, 19°40'19.46"E, alt. 1920 m, 7 Dec 2007, *E. Pienaar M1249* (MA-877228, NBG).

Phylogenetic analyses. The molecular phylogenetic analyses of the ITS data using the three inference methods (ML, MP and BI) produced results generally comparable to those reported in Nickrent and García (2015), but in addition strongly supported *Thesium nautimontanum* as sister to the South African and Tropical African clades (Fig. 4).

Discussion

As far as known, *Thesium nautimontanum* is restricted to the Matroosberg Mt., the highest mountain of the Hex River mountain range. This geomorphologically diverse landscape supports many local and regional endemic plants (see descriptions of the relevant vegetation types in Rebelo et al. 2006). Indeed, two other species of *Thesium*, *T. annulatum* A.W. Hill and *T. microcephalum* A.W. Hill, are endemic to the mountain range as well. Discovering a new species of *Thesium* in South Africa is not in itself remarkable, however, *T. nautimontanum* has particular importance for several reasons. According to our review of morphological characters from descriptions, type material and herbarium and living specimens from species across the entire area of distribution of the genus, it has a combination of features not seen in any other *Thesium* species. The inside surfaces of the corolla lobes are covered with spongy, irregular lumps of tissue that defy description. This feature is neither the hairs seen in Sect. *Barbata* A.W. Hill, nor the papillae or short hairs seen along the corolla lobe margins in other species (e.g. Subsect. *Fimbriata* A.W. Hill). The manner of inflorescence and branch development is also unique in *Thesium*. The bracts at the base of the spikes bear undeveloped

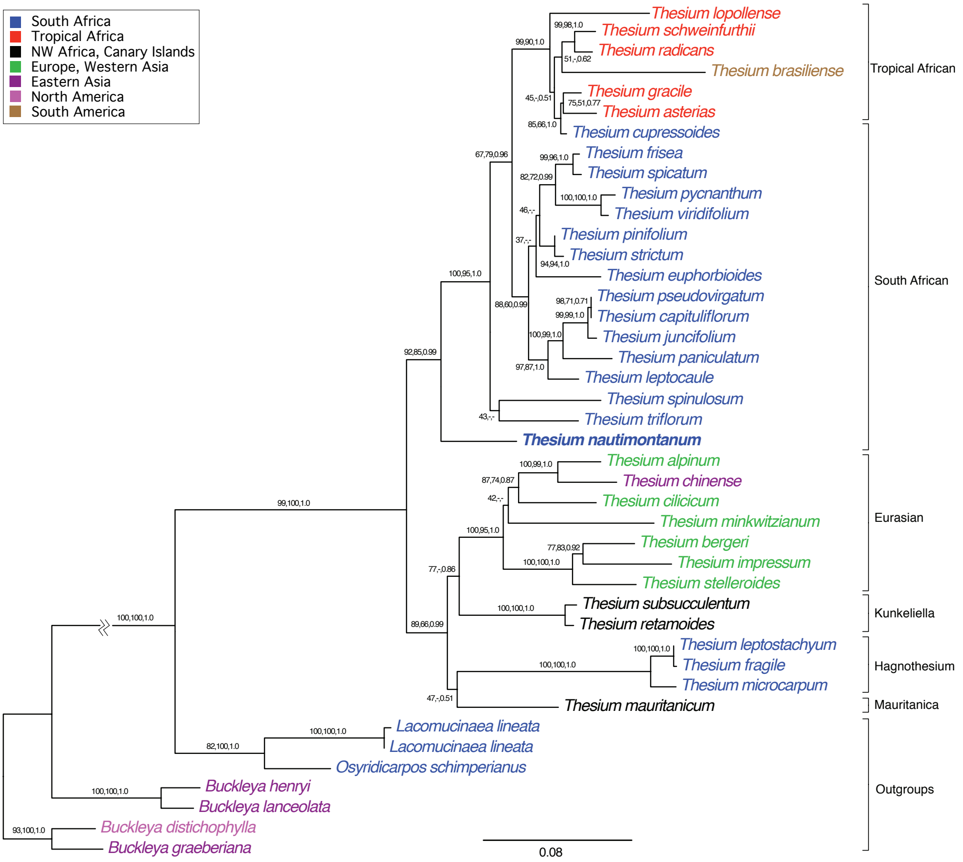

Figure 4. Phylogram for Thesiaceae that includes *Thesium nautimontanum* sp. nov. (bold). Values at the nodes indicate maximum likelihood bootstrap, maximum parsimony bootstrap and Bayesian posterior probabilities. The scale bar represents number of substitutions per site. Branch lengths are proportional to the amount of substitutional change, except for the branch connecting the outgroup to the ingroup (here shorted for graphical purposes). Group (clade) names are from Nickrent and García (2015). The colour coding for the taxa indicates the general geographic distribution for the species as shown in the inset.

buds that start forming some flowers when the upper ones have developed fruits. Each bud forms a shoot that will generate another inflorescence and so on, thus resulting in the characteristic sympodial branching pattern of this species. Another uncommon feature in the inflorescence is the presence of sterile bracts at the apex. The inflorescence appears to be truly indeterminate because there is not a terminal flower. In many species of *Thesium* (e.g. *T. aggregatum* A.W. Hill, *T. capitatum* L., *T. diversifolium* Sond., *T. scabrum* L. and *T. spicatum* L.), the inflorescence appears to be a spike, but upon further inspection, it can be seen that it is cymose (monotelic) and determinate with the main and lateral axes terminating in flowers. A few species of *Thesium*, like *T. impeditum* A.W. Hill or *T. gnidiaceum* A. DC., have indeterminate vegetative growth from the apical part of the inflorescence. This can later generate another inflorescence, having two (or more) inflorescences on the same axis separated by leafy segments. This feature is lacking in *T. nautimontanum*. Finally, the reticulations present on the fruit walls are also unusual. The 5 main longitudinal ribs are scarcely marked but the reticulations are strongly raised and swollen, giving a characteristic tuberculate surface.

The present molecular phylogenetic analyses are in agreement with another study with greater taxon sampling (390 accessions representing over 200 taxa) that is currently being prepared for publication (D. Nickrent et al., in prep.). Interestingly, using chloroplast spacer sequences, this species is sister to most African *Thesium*, however, the equivalent basalmost position is occupied by two clades, one containing *Thesium spinosum* L.f. and *T. spinulosum* A. DC. and the next one composed of seven species that often bear simple or compound dichasial inflorescences that mostly occur in the South African Karoo. Incongruences between the nuclear and chloroplast gene trees are currently being investigated and preliminary data indicate chloroplast capture may be occurring, thus obscuring species relationships when using the plastid data.

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Phlegmariurus vanuatuensis (Huperzioideae, Lycopodiaceae) a new species from Vanuatu, re-circumscription of *P. nummulariifolius* and new combinations in *Phlegmariurus*

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Abstract

Phlegmariurus vanuatuensis A.R.Field is described as a new species for plants endemic to the islands of Vanuatu that were previously identified with *P. nummulariifolius* (Blume) Ching. The Vanuatuan species differs from the widespread Asian-Oceanian species in several characteristics, most notably its acutely divergent leaf arrangement and thicker less branched fertile spikes. *Phlegmariurus nummulariifolius* is here re-circumscribed as plants occurring in Asia and into Oceania as far east as the Solomon Islands, being replaced eastwards by *P. vanuatuensis*. In addition, new nomenclatural combinations are made for *Phlegmariurus australis*, a species from Polynesia and for *Phlegmariurus copelandianus*, a species from Malesia.

Keywords

Phlegmariurus, Lycopodiaceae, Vanuatu, Oceania

Introduction

Phlegmariurus Holub is a genus including epiphytic, epilithic and terrestrial plants that inhabit perhumid forests and montane regions throughout the tropics and subtropics globally. The global diversity of *Phlegmariurus* is grouped into a predominantly Neotropical clade which has dispersed several times into Africa, Madagascar and Hawaii and a predominantly Palaeotropical clade with a single dispersal into the Neotropics (Field et al. 2016; Bauret et al. 2018; Testo et al. 2018a, b). In Asia and Oceania, almost half the species diversity belong in as-yet unresolved clades related to *P. phlegmaria* (L.) Holub, *P. phlegmarioides* (Gaudich.) A.R.Field & Bostock and *P. carinatus* (Desv.) Ching or *P. squarrosus* (G.Forst.) Á.Löve & D.Löve and *P. myrtifolius* (G.Forst.) A.R.Field & Bostock (Field et al. 2016; Bauret et al. 2018; Testo et al. 2018a, b). Within Oceania, the diversity of *Phlegmariurus* has been taxonomically reviewed only in New Guinea, the Solomon Islands, New Caledonia, Fiji and Australia (Herter 1909; Herter 1916; Alderwerelt van Rosenburgh 1917; Compton 1922; Copeland 1929a, b, 1931, 1936; Brownlie 1977; Jones and Gray 1985; Chinnock 1998; Field and Bostock 2008); but Vanuatu has been relatively neglected. Only one species, *P. oceanianus* (Herter) A.R.Field & Bostock has previously been described coming from Vanuatu, even though many other Vanuatuan specimens fit poorly into preexisting taxa from elsewhere. This paper examines the systematic placement of plants hitherto regarded as *Phlegmariurus nummulariifolius* (Blume) Ching occurring in Vanuatu and this species is herein described as new.

Methods

Herbarium materials of *Phlegmariurus* from across Asia and Oceania were examined in AAU, B, BONN-Nessel, BR, BRI, CANB, CNS, KLU, P, PR, PRC, QRS, UC and US. Living materials were examined both in the field in Australia and Asia as well as in cultivation at James Cook University, Australia. Measurements were taken both from herbarium specimens and living specimens, and where this differs it is noted. Herbarium acronyms follow Index Herbariorum [http://sweetgum.nybg.org/science/ih/] and standard forms of author and publication citations follow International Plant Name Index [https://www.ipni.org/]. Specimens annotated '!' have been physically seen, and specimens annotated '*' have been seen as photographs or scans.

A proposed Conservation status was assessed against the IUCN Red List Categories and Criteria (IUCN 2012). Area of Occupancy (AOO) and Extent of Occurrence (EOO) where calculated using the GeoCAT tool (http://geocat.kew.org/ Bachman et al. 2011) based upon map-assigned latitudes and longitudes extrapolated from herbarium labels and a grid cell of 10 km^2. The centre of the island from which a specimen was selected in instances where no finer detail localities were recorded on herbarium labels.

Taxonomy

Phlegmariurus vanuatuensis **A.R.Field, sp. nov.**

urn:lsid:ipni.org:names:60477108-2
Figure 1 A–C, Figure 2 D–F

Diagnosis. *Phlegmariurus vanuatuensis* is similar to *Phlegmariurus nummulariifolius* but differs in having acutely spreading non-flattened sterile leaves (compared with adpressed and imbricate leaves flattened in on plane in *P. nummulariifolius*), thicker pale green-brown stem bases 3.5–5.5 mm diameter in *P. vanuatuensis* (compared to thinner dark black-brown stem bases 1.5–3.5 mm diameter in *P. nummulariifolius*) and a gradual transition to thicker less ramified fertile spikes 2–5.5 mm diameter in *P. vanuatuensis* (compared to an abrupt transition to slender ramified fertile spikes 1–2.5 mm in *P. nummulariifolius*).

Type. Nouvelle-Hebrides: Erromango, forêt dense au N du camp du km 17, alt. 300 m, 4 Aug 1971, *J. Raynal RSNH 16213* (Holotype: P01221002!; isotype: P01219313!).

Description. Sporophytes herbaceous, epiphytic, with tufted isodichotomous arching to pendulous shoots and with dichotomous roots emerging from the base of the tuft. Shoots abruptly to gradually heterophyllous; sterile sections leafy, 12–18 mm in diameter and usually 20–50 cm long, evenly branched 1–4 times; fertile sections filiform-funiform, 2–4.5 mm in diameter and up to 300 mm long, branched 0–3 times, usually unbranched at base. Stems fleshy, 2.5–5.5 mm in diameter in basal module without the leaves, pale green or light stramineous brown, and bearing indistinct longitudinal grooves between the rows of leaves. Leaves sessile, supine, decurrent, firm, orthostichous in four strict rows comprised of 2 alternating sub whorls of 2, acutely spreading, ovate to ovate-oval, 6–14.5 mm long × 6–9 mm wide, with a broad rounded base and rounded, obtusely pointed or acutely pointed apex, leaves flat to twisted with entire margins, mid glossy green to light yellow green. Leaves in the basal modules more crowded, ovate with an acute apex and with a narrowed sub-petiolate base, in median modules more widely spaced and diverging, sessile with an almost amplexicaule base, and in the distal modules transitioning in shape to sporophylls. Sporophylls gradually to sharply differentiated from sterile leaves, sessile, supine, orthostichous in two alternating pairs of two, acutely divergent to adpressed, scale like, ovate-rhomboid with a cuneate to rounded amplexicaule base and an acute apex, 1.5–5.2 mm long × 1.5–2.8 mm wide, overlapping. Sporangia borne on the upper surface in the axils of sporangia, reniform, 1–1.2 mm in diameter, mostly covered by the sporophyll. Spores isotetrahedral, 30–40 µm in diameter, with convex lateral margins, smooth proximal surfaces and moderately foveolate distal surface. Gametophytes holomycoheterotrophic, dorsiventral with paraphyses among the gametangia on the upper surface. *Vanuatu flat tassel-fern.*

Additional specimens examined. VANUATU: Nouvelles Hebrides, [n.d.], *M. MacDonald 59* (P01219315). Ile de Pentecôte, zone axiale: Lalak (cote 520), 15 Sep 1936, *E. Aubert de la Rüe s.n.* (P01219311). Vate, 500–600 m, May 1965, *M. Schmid*

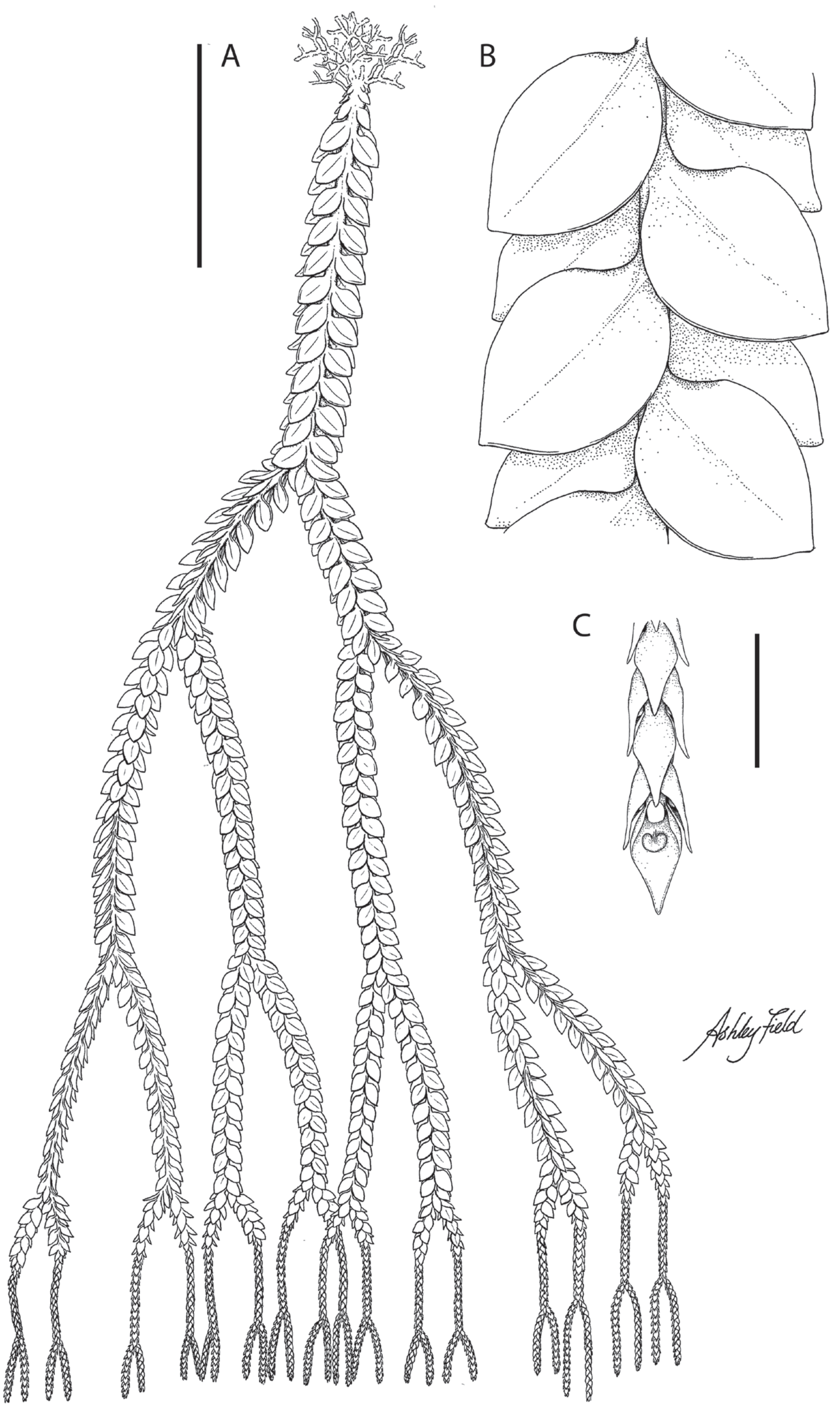

Figure 1. *Phlegmariurus vanuatuensis* ARF1140: **A** habit, leaf arrangement and fertile spikes **B** close up of sterile leaf arrangement showing subopposite decussate leaf arrangement and ovate-oval leaves **C** close up of fertile spike showing scale like sporophylls and a sporangium. Scale bar: 10 cm (**A**); 1 cm (**B, C**). Illustration by A.R.Field.

188 (P01222833). Iles Banks, Vanua-Lava, epiphyte en forêt, 600 m, 14 Jun 1983, *P. Morat 7472* (P01238484). NW of Efate, unnamed hill N of Trig Point above Narabut camp, occasional epiphyte in forest on summit of hill, 1600 ft [488 m], 5 Jul 1971, *A.F. Braithwaite RSNH 2040* (P01219314). S. Erromanga, high epiphyte in forest, 300 ft [91 m], 6 Aug 1971, *A.F. Braithwaite RSNH 2275* (P01219309). N. Espiritu Santo, Apouna Valley, epiphytic in forest, 3000 ft [914 m], 1 Sep 1971, *A.F. Braithwaite RSNH 2355A* (P01219310). N. Espiritu Santo S.W. Bay, occasional epiphyte in ridge top forest, 1300 ft [396 m], 11 Oct 1971, *A.F. Braithwaite RSNH 2601*(P01219312). N. Espiritu Santo S.W. Bay, occasional epiphyte in ridge top forest, 1300 ft [396 m], 11 Oct 1971, *J. Raynal RSNH 16213* (P01221002). Erromango epiphyte 300 m, 4 Aug 1971, *J. Raynal RSNH 16213* (P01219313). Iles Banks, Vanua-Lava, crête vers, 500 m, 13 Jun 1983, *J.M. Veillon 5542* (P01253017). Pentecôte, Enkul, 550 m, 28 Sep 1984, *P. Cabalion 2568* (P01236864). Mallicolo, SW Bay, entre Lenbongbong et Lendemboi, 25 Sep 1986, *G. Vourdy 823* (P01238278). Efate, *M.A. Clements 5640* (CBG8916282). Cultivated James Cook University ex Efate, 30 Aug 2006, *A.R. Field 1140* (BRI, CNS).

Distribution, habitat and ecology. Endemic to Vanuatu where it occurs as an uncommon epiphyte on the bark of tree trunks and branches in the canopy to subcanopy of mature trees in lowland to montane primary tropical rainforest.

Conservation status. Vulnerable. *Phlegmariurus vanuatuensis* is considered eligible for IUCN listing as Vulnerable (IUCN 2012) on the basis of its Area of Occupancy being less than 20,000 km^2. Its EOO was calculated at EOO 34,668 km^2 and the AOO at 800 km^2 using the GeoCAT tool. In addition, it meets criteria A.1.c. as it has experienced a catastrophic reduction in numbers and in particular loss of its intact rainforest canopy habitat following Tropical Cyclone Pam in 2015. It is also considered eligible for listing as Vulnerable on the basis of criteria B.1.a. as it has severely fragmented population scattered over disjunct islands, and is estimated to occur at fewer than 10 locations. In addition, it meets criterion B.1.b. (v & iv) as it is inferred to have experienced, and continue to experience an ongoing decline in the extent and quality of habitat and number of locations or subpopulations and number of mature individuals owing to land clearing of its habitat, and in particular owing to destruction of its habitat by severe tropical cyclones. In addition, the uniqueness of this species may make it a target for rare plant collectors.

Etymology. Named for the origin of this species in Vanuatu. Vanuatu is a composition of the Austronesian words '*Vanua*' meaning home or land and '*Tu*' meaning stand.

Note. *Phlegmariurus vanuatuensis* is closely related to *P. nummulariifolius* and has been hitherto included in that species. It appears to be the easternmost allopatric species of the *P. nummulariifolius* group, differing in several stable morphological characters. Living plants of both species are more distinct than pressed material (Figure 2), as the characteristic divergent layered leaf architecture of *P. vanuatuensis* is flattened during pressing, the thick fleshy stems collapse and colour is lost during drying (Figure 2). The lower stems of *P. vanuatuensis* are thicker, fleshier and light green or pale stramineous brown compared to *P. nummulariifolius* which has stems that are more slender, dark purplish brown and more lignified. The sterile leaves of *P. vanuatuensis* are acutely spreading,

Figure 2. *Phlegmariurus nummulariifolius* ARF0002: **A** basal region of shoot showing branching pattern and leaf arrangement **B** medial region of shoot showing adpressed leaf arrangement in facial and lateral view **C** distal region of shoot showing abrupt transition to sporophylls and filiform fertile spikes. *Phlegmariurus vanuatuensis* ARF1140 **D** basal region of shoot showing branching pattern and leaf arrangement **E** medial region of shoot showing divergent leaf arrangement in facial and lateral view **F** distal region of shoot showing gradual transition to sporophylls and funiform-filiform fertile spikes. Photos by A.R.Field

twisting slightly and being almost amplexicaule at their base compared with the tightly adpressed and uniformly planar flat leaves of *P. nummulariifolius*. The leaf colour of *P. vanuatuensis* is usually a bright green whereas in *P. nummulariifolius* it is usually a dark green. The fertile spikes of *P. vanuatuensis* are funiform and relatively unbranched compared with being filiform and multibranched in *P. nummulariifolius*. The transition from sterile to fertile spikes is more gradual than for *P. nummulariifolius.* Young plants of both species are very similar but can usually be differentiated on the basis of leaf divergence.

The diagnostic traits of *Phlegmariurus vanuatuensis* appear to be consistent across its population whereas the traits of *Phlegmariurus nummulariifolius* appear to be consistent throughout its Malesian, New Guinean and Solomon Island range with plants as close as the Santa Cruz Islands typical of *P. nummulariifolius*. It is expected that *P. vanuatuensis* has dispersed eastward from *P. nummulariifolius*, or a shared ancestor, but has become isolated in the islands Vanuatu leading to allopatric divergence there. Alternatively, it could have colonized Vanuatu and hybridized with *P. phlegmarioides*, as it also shares some traits with that species such as the divergent leaf planes, layered arching branches and the thicker stems and fertile spikes.

Phlegmariurus vanuatuensis can be readily differentiated from *P. delbrueckii* (Herter) A.R.Field & Bostock by its larger size, and by having terete fertile spikes rather than the quadrangular fertile spikes found in *P. delbrueckii*. It can be readily distinguished from *P. phlegmarioides* by its leaves being supine and somewhat more flattened in one plane, rather than radiating in four planar ranks as found in *P. phlegmarioides*. Another species occurring further east in Polynesia, *P. ribourtii* (Herter) A.R.Field & Bostock has thicker and distinctly quadrangular fertile spikes and more lingulate-oval leaves with a different arrangement.

Phlegmariurus vanuatuensis is remarkably convergent with the unrelated broad leafed form of *P. obtusifolius* (P.Beav.) A.R.Field & Bostock *s.l.* [=*P. pachyphyllus* (Kuhn ex Herter) A.R.Field & Testo *s.s.*] which occurs in a similar habitat in Madagascar and islands of the West Indian Ocean. Together, these species repeat a general trend in which several unrelated species of *Phlegmariurus* occurring epiphytically in lower altitudes of offshore oceanic islands have broader leaves with rounded apices.

***Phlegmariurus nummulariifolius* (Blume) Ching, Acta Bot. Yunnan. 4(2): 125 (1982)**

≡*Lycopodium nummulariifolium* Blume, Enum. Pl. Javae 2: 263 (1828). *Urostachys nummulariifolius* (Blume) Herter, Philipp. Sci. 22: 182 (1923). *Huperzia nummulariifolia* (Blume) Chambers, Jermy & Crabbe, Brit. Fern Gaz. 10: 176 (1971). **Type citation.** 'Crescit Javae montosis ex arboribus pendulum'. **Type.** Sallak [Java], *C.L.Blume s.n.* (lectotype, designated here: L0057363* [L 908.342-266]; isolectotypes/syntypes: L 908.326-17 *n.v.*, NY00127360*). **Type note.** Øllgaard (1989 p. 59) lists two syntypes in L, and a third duplicate of a Blume gathering is known in NY. The most complete specimen in L is selected as the lectotype, the other having not been relocated. **Etymology.** Named in reference to the round coin like leaves.

=*Lycopodium rotundifolium* Roxb., Calcutta J. Nat. Hist. 4: 473 (1844). **Type citation**. '*Wallich* cat. 65, no. 2183' and '*Nat.* of *Sumatra*, and a most beautiful species it is.'. **Type.** [s.l., n.d., s.c. s.n. (plant labelled in Roxburgh's hand] (? holotype [see Morton 1973 p. 328–329]: E00429111* [E-Greville]). **Etymology.** Named in reference to the round leaves.

Description. Sporophytes herbaceous, epiphytic, with tufted isodichotomous pendulous shoots and with dichotomous roots emerging from the base of the tuft. Shoots abruptly heterophyllous; sterile sections leafy, 5–12 mm in diameter and usually 20–250 cm long, branched 1–6 times being relatively unbranched basally and more frequently ramified distally; fertile sections filiform, 1–2.2 mm in diameter and up to 400 mm long, multibranched 1–6 times. Stems slender and woody, 1.5–3.5 mm in diameter in basal module without the leaves, light green to dark purplish-brown, and bearing indistinct longitudinal grooves between the rows of leaves. Leaves sessile, supine, decurrent, firm, orthostichous in four strict rows comprised of 2 alternating sub whorls of 2, adpressed and imbricate, ovate-oval, 6–14 mm long × 4–7.5 mm wide, with a rounded base and similar rounded apex, leaves flat with entire margins, dark glossy green to light yellow green. Basal leaves sometimes more scale like and leaves in basal modules sometimes lanceolate-ovate and more widely spaced out on elongated naked stems, leaves in median and distal regions ovate-oval. Sporophylls markedly differentiated from sterile leaves, sessile, supine, orthostichous in two alternating pairs of two, scale like, adpressed throughout, ovate-rhomboid with a cuneate base and acute apex, 2.1–3.5 mm long × 1.4–2.2 mm wide, overlapping or occasionally spaced apart with stem visible between subwhorls of sporophylls. Sporangia borne on the upper surface in the axils of sporangia, reniform, 0.8–1 mm in diameter, mostly covered by the sporophyll. Spores isotetrahedral, 30–40 μm in diameter, with convex lateral margins, smooth proximal surfaces and moderately foveolate distal surface. Gametophytes holomycoheterotrophic, dorsiventral with paraphyses among the gametangia on the upper surface. *Flat tassel-fern* [Australia], *Kied Hoy* [Thailand], *Pum-borey* [Bougainville Island], *Yúlín* [Singapore]. Figure 2A–C.

Specimens examined. ASIA. VIETNAM. Tonkin: Saigon, [n.d.], *Faurie 182* (BONN-Nessel 646b). INDONESIA. Java: [s.l.], 1869, [s.c.] *13* (P1238007). Enviriv du Jardin Botanique du Buitenzorg, Jan-Feb 1877, *H.de Poli [s.n.]* (P1596127). Java, 1854, *M.Göring 210* (P1238008; P1238006). Java, [n.d.], *Kollmann [s.n.]* (P1238019). Gedeh Gebirge, 1910, *Königsberger [s.n.]* (BONN-Nessel 642b). [s.l.], [n.d.], *Schneider [s.n.]* (P1220849). Java, [n.d.], *C.Treub [s.n.]* (P1238013). [s.l.], [n.d.], *Zollinger [s.n.]* (BONN-Nessel 642a). Java, [n.d.], *Zollinger [s.n.]* (P1238004). [s.l.], [n.d.], *Zollinger [s.n.]* (P1238014). Java, [n.d.], *Zollinger 175-Z* (P01238005; P01238012; P01238025). Kalimantan: W. KokLai [illeg.], 800 m, 12 Sep 1925, *F.H. Enderl 3804* (BONN-Nessel 646a). Gunung Samarinda, Ost-Borneo, Aug 1910, *E.Rŭdel [s.n.]* (BONN-Nessel 643). Sumatra: Island of Siberut, 8 Sep 1924, *C. Boden-Kloss 10581* (US1346685). Siberut, 8 Sep 1924, *C. Boden-Kloss 10581* (US1346686). Enggano, Boea-boea, 100 m, 29 May 1936, *W.J. Lutjeharms 3874* (US2139904). East Coast, Vicinity of Aek

Moente, 500 m, 15 Jun-9 Jul 1936, *Rahmat Si Boeea 9168* (US2717673). Province indet: Archipel. Ind. ?, [n.d.], *[s.c.] 14* (P1220851). Archipel. Ind., [n.d.], *[s.c.] 219* (P1220850). Ostindien, [n.d.], *Schneider [s.n.]* (P1220852). Boesoe Nal' Besie, May 1921, *Toxopeus 181* (P1220854). MALAYSIA. Johore: Gunung Janing Plateau, 430 m, 23 Oct 1985, *K.M. Wong FRI 30916* (QRS113912). Kedah: B. Blukang Parang, G. Bintang, 16 Apr 1928, *Haniff Singapore Field # 21065* (UC346255; US1704974). Kelantan: Gua Panjang, Gua Nimik, 305 m, 21 Oct 1927, *M.R. Henderson 19534* (US1274282). Suga Keteh, 16 Feb 1924, *Nur 12108* (UC234463). Negri Sembilan: Ulu Langat, Stream side vegetation along Sungei Kenaboi, about 2 miles downstream of Sungei Damar junction. Kenaboi Forest Reserve, 610 m, 20 May 1976, *D.W. Lee UL-61** (P1266534; US2920343). Pahang: S.Sat., Pahang, 29 Aug 1929, *M.R. Henderson 22091* (UC462868). Malay Peninsula, S. Sat, 19 Jul 1929, *M.R. Henderson Singapore Field # 20091* (US1526658). Malay Peninsula. Kuala Teku, 152 m, 21 Jul 1936, *Kiah Singapore Field # 31750* (US1703674). Malaya, Near Tunnel Rd., Penang Hill, 610 m, 4 Mar 1956, *B.E.G. Molesworth-Allen 2765* (US2255844). Penang, [n.d.], *H. Norris [s.n.]* (P1220855). Perak: Perak, [n.d.], *Scortechini [s.n.]* (BONN-Nessel 644). Sabah: Mt Kalawat, Oct-Dec 1915, *M.S. Clemens 11144* (UC211777). cultivated ex Sabah, Malaysia, 25 Apr 2018, *A.R. Field 4670* (CNS). G. Lumaku, 300 m, 7 Mar 1969, *H.P. Nooteboom 1212* (US2951438). Sarawak: [s.l.], Jul 1865, *O. Beccari [s.n.]* (US1918110). Gilam Bakun, 23 Aug 1954, *W.M. Brooke 9077* (US2292818). Bakelalan, 914 m, 23 Aug 1955, *Collector illegible 10486* (US2290680). Foot of Mt Santubong, 16 Mar 1914, *collectuer indigene 97* (P1238015; P1238016). cultivated ex Sarawak, Malaysia, 25 Sep 2016, *A.R. Field 2785* (CNS). Borneo, 17 Jun 1894, *E. Langlassé 57* (P1238009; P1238010; P1238017). Borneo, [n.d.], *Native collector 58* (US1173888). Sarawak, [n.d.], *Native collector 774* (P1238018; US1174046). Sarawak, [n.d.], *Native collector 1547* (UC218747; US1174144). Sarawak, Feb-Jun 1914, *Native collector 2143* (UC218752). Terengannu: Malay Penins.: Bukit Besar, 914 m, May 1899, *D.T. Gwynne-Vaughan 425* (US1506242). Ind. Or., [n.d.], *[s.c.] [s.n.]* (P1220856). OCEANIA. INDONESIA. West Papua: 6 km SW of Bernhard Camp Idenburg River, 1200 m, Feb 1939, *L.J. Brass 12837* (BRI327894). Mt Nettoti, Tamrau Range, 1460 m, 28 Oct 1954, *P. van Royen 3899* (P1238493; UC40507). Bernhard Camp Idenburg River, 50 m, Apr 1939, *L.J. Brass 14079* (BRI327897). PAPUA NEW GUINEA. Central Province: cultivated ex Brown River, Papua New Guinea, 3 Feb 2001, *A.R. Field 2* (CNS). Madang Province: Bundi Mission, 1300 m, 19 Jul 1992, *P.I. Forster PIF10979* (BRI549514). Manus Province: Admiralty Islands, Tingau #1, Number One Road, Manus Island, 10–12 Jul 1946, D.F. Grether 4522 (UC729030; US1918392). Morobe Province: New Guinea, Umi River, Markham Valley, 480 m, 17 Nov 1959, *L.J. Brass 32571* (US2358020). Nordöstliches New-Guinea, Morobe-Distrikt, Sattleberg, 3300 ft, 1 Feb 1936, *[M.S.] Clemens 1840* (P1242270). Charles Luis Gebirge, 1911, *Harking [s.n.]* (BONN-Nessel 655b). Waria River below Garaina, 3300 ft, 20 Jun 1962, *T.G. Hartley TGH10376* (BRI149761). Sattleberg, 600 m, Jan 1899, *E. Nyman [s.n.]* (BONN-Nessel 655a). Kaiser-Wilhelmsland, 2 Aug 1907, *R. Schlechter 16352* (P1238020; P1238022; US2990287). New Guinea. Tiaure

Village, 5 miles S. of Garaina, Lae sub-district, 488 m, 22 Jul 1970, *H. Streimann NGF 45048* (US2613636). Nawata Banda, 720 m, 30 Jun 1966, *H. Streimann NGF27818* (BRI149765). Between Kaisenik and Wuri-Wuri, 1140 m, 18 Oct 1968, *H. Streimann NGF39129* (BRI149995). Tiaure Village 5 M S of Garaina, 480 m, 22 Jul 1970, *H. Streimann NGF45048* (BRI365353). Lae, FRI Botanical Gardens, 10 m, 5 Jul 1993, *W. Takeuchi 9005* (US3633126). Kikiepa Village near Wantoat, 1500 m, 5 Jun 1960, *J.S. Womersley NGF12728* (BRI149760). New Ireland Province: Namatanai sub-province; Hans Meyer Range, 625 m, 9 Oct 1975, *M.J. Sands 2169* (US3685033). North Solomons Province: Pavairi, 810 m, 20 Jan 1967, *C.E. Ridsdale NGF31021* (BRI149971). North Solomons Province: Kupei Gold Field., 1000 m, 24 Apr 1930, *S.F. Kajewski 1777* (P1219318). Bougainville Island, Kugumaru, Buin, 150 m, 18 Jul 1930, *S.F. Kajewski 1952* (P1219317; UC542111). Territory of New Guinea, Near Korpei village, 11 miles southwest of Keita, 670 m, 2 Nov 1961, *D.H. Nicolson 1533* (US2416100). Near Korpei village, 11 miles southwest of Keita, 670 m, 2 Nov 1961, *D.H. Nicolson 1544* (P1243720). Vicinity of Aku village, c. 9 miles west of Buin Station, 30 m, 23 Sep 1964, *R. Schodde 4116* (US2577448 A). Oro Province: Kokoda, 360 m, 29 Jul 1964, *A.N. Millar NGF 23563* (BRI149759). Province indet: Loganeng, [n.d.], *M.G. Balmer 54* (P1238011; P1238023; UC391741). Nouvelle-Guinee allemande, Loganeng, auf Baumen, [n.d.], *M.G. Bamler 54* (US3030998; US3031008). Bella Vista, Dieni [illeg.], 1933, *L.J. Brass 3792* (BONN-Nessel 642c). SOLOMON ISLANDS. Kolambangara: New Georgia Group, Kolombangara Island. West coast, inland from Iri iri Village (Merusu Cove), 610 m, 28 Sep 1963, T.C. Whitmore BSIP 2148 (US2577636 A). South Kolambangara, 4000 ft, 2 August 1965, *A.F. Braithwaite 411* (P1219316). Sancristobal: E. Sancristobal. Confluence of Warahite and Pagato Rivers, 150 ft, 25 Jul 1965, *A.F. Braithwaite 4175* (P1219319). Santa Cruz Islands: Vanikoro Island, 100 m, 13 Nov 1928, *S.F. Kajewski 621* (UC422664; US1758589). Ngarabu camp to end road then follow ridge, Vanikoro island, Santa Cruz province, 120–600 m, 17 Jun 2016, *C.-W. Chen SITW10583* (CNS). Santa Ysabel: Dedeu R. S.W. Santa Ysabel, 43 m, 21 Apr 1966, *Beer's collectors BSIP 6686* (US2691129). Santa Ysabel: nr. Maringe Lagoon, Mt. Sasari, 853 m, 26 Oct 1963, *T.C. Whitmore BSIP 2429* (US2577645 A). Guadalcanal: Mt. Gallego, Guadalcanal Island, 400–1000 m, 30 Jun 2014, *C.-W. Chen SITW5240* (CNS). Hut to Mt. Tepalamenggutu, 600–1500 m, 15 Oct 2012, *T.-Y. Yang SITW1018* (CNS).

Distribution, habitat and ecology. Widespread in Malesia and western Oceania where it occurs in New Guinea and the Solomon Islands as far east as the Santa Cruz Islands. A canopy and subcanopy epiphyte in lowland to montane tropical rainforest. This species often grows from decaying nests of other epiphytes, rotting logs and tree hollows but also will grow on bark on tree branches, especially where humus has accumulated.

Notes. *Phlegmariurus nummulariifolius* is here circumscribed as plants occurring throughout Malesia, New Guinea and the Solomon Islands that have uniformly flattened shoots comprised of decurrent imbricate leaves, excluding plants previously identified with *P. nummulariifolius* that occur in Vanuatu which have divergent leaves and are described below as a new species. *Phlegmariurus nummulariifolius* is a widespread and remarkably uniform species throughout its range that is readily identified by its unique leaf shape and arrangement and flattened shoots on very long slender stems.

Examination of G.H.Brownlie determinations on herbarium specimens indicates that reports of *P. nummulariifolius* in Fiji (Brownlie, 1977) are based on misidentifications of *P. delbrueckii* (Herter) A.R.Field & Bostock [e.g. *Brownlie 1835* (AAU! & CHR341130] and juvenile specimens of the Fijian population of *P. phlegmarioides* (Gaudich.) A.R.Field & Bostock *s.l.* [=*P. pseudophlegmaria* (Kuhn) A.R.Field & Testo *s.s.*], both of which also have rounded leaf apices. I have not encountered any material of *P. nummulariifolius s.s.* south or east of the Sant Cruz Islands, Solomon Islands.

The determination '*Lycopodium kempterianum Schlecht. n. sp.* Schlechter' appears on the gathering 'Neuguinea: Kaiser-Wihlems-Land, 2 Aug 1907, *R. Schlechter 16352* (BONN-Nessel 646a!, P01238020!, US2990287!), and a citation in Nessel (1939 p. 254). Schlechter's specimen is typical of *P. nummulariifolius* and it is presumed that he did not proceed with publication of a new taxon.

New combinations in *Phlegmariurus*

Recent combinations and descriptions of *Phlegmariurus* bring the number of combinations in the genus globally to 288 (Hsieh et al. 2012; Øllgaard 2012a, b; Field and Bostock 2013; Øllgaard 2015; Øllgaard 2016; Arana 2016; Field et al. 2016; Duy et al. 2016; Testo 2017; Kiew and Kamin 2018; Bauret et al. 2018; Øllgaard 2016). Although the *Phlegmariurus* species diversity of the Neotropics have been comprehensively reviewed by Øllgaard (1992, 2012a, b, 2015, 2016), the species occurring in the Palaeotropics have not been as intensively reviewed. Many Palaeotropical taxa are poorly defined, being either mixed assemblages of convergent species (e.g. species in the polyphyletic *P. phlegmaria* group, Field et al. 2016) or multiple taxa described for extremes of an apparently continuously variable species (e.g. species in the *P. macgregorii* group). The two following combinations are for more clearly definable species.

Phlegmariurus australis (Willd.) A.R.Field, comb. nov.

urn:lsid:ipni.org:names:77190776-1

≡*Lycopodium australe* Willd., Sp. pl., Ed. 4 [Willdenow] 5: 11 (1810). *Lycopodium phlegmaria* var. *australe* (Willd.) Domin, Biblioth. Bot. 89(4) (1928). *Urostachys australis* (Willd.) Herter ex Nessel, Bärlappgewächse 224 (1939). *Huperzia australis* (Willd.) Holub, Folia Geobot. Phytotax. 20: 70. (1985). **Type citation.** 'Habitat in societas insulus (v.s.)'. **Type.** societas insulis [Tuha'a Pae or Austral Islands, Society Islands, French Polynesia, Aug1773, *J.R.Foster & G.A.Foster s.n.*] (lectotype, designated by Øllgaard 1989 p. 33: B-W 19341-01!; isolectotypes/syntypes: GOET01283*, GOET012844*, LE00008976*). **Etymology.** Named for the southern origin of the type gathering.

=*Lycopodium subtrifoliatum* Brownlie, Nova Hedwigia Beih., 55 (Pterid. Fl. Fiji) 24 (1977). *Huperzia subtrifoliata* (Brownlie) Holub, Folia Geobot. Phytotax. 26(1): 93 (1991). *Phlegmariurus subtrifoliatus* (Brownlie) A.R.Field & Bostock, PhytoKeys 20: 48 (2013). **Type.** Mt. Korobaba, Viti-Levu, Fiji, 20 Jun 1971, *G.H.Brownlie 1287* (holotype: CHR20341227*). **Etymology.** Named for its smaller size compared to *L. trifoliatum* Copel.

Note. *Phlegmariurus australis* (Willd.) A.R.Field is an earlier name for the Polynesian black-stem tassel-fern recognised by Field and Bostock (2013) as *P. subtrifoliatus* (Brownlie) A.R.Field & Bostock. It occurs in Polynesia, where it is the sole, and easternmost, vicariant of the *P. phlegmaria* group. It also occurs in eastern Melanesia and in sympatry with *Phlegmariurus trifoliatus* (Copel.) A.R.Field & Bostock in Fiji. *Phlegmariurus australis* can be distinguished by its shorter ovate leaves and smaller shoot diameter compared with the longer lanceolate leaves and larger overall size of *P. trifoliatus.*

Phlegmariurus copelandianus (R. C.-Y. Chou & Bartlett) A.R.Field, comb. nov.

urn:lsid:ipni.org:names:77190774-1

≡*Lycopodium copelandianum* R. C.-Y. Chou & Bartlett, Bull. Torrey Bot. Club 74: 369 (1947). *Huperzia copelandiana* (R.C.-Y. Chou & Bartlett) Holub, Folia Geobot. Phytotax. 26(1): 92 (1991). *Lycopodium petiolatum* Copel., Univ. Calif. Publ. Bot. 14: 377 (1929) [non Herter 1909]. **Type.** Berastagi Karoland, Sumatra, 1–5 Feb 1926, *H.H.Bartlett 6575* (holotype: UC349249!; isotype: MICH1287188*). **Etymology.** Named in honour of American botanist Edwin Bingham Copeland (1873–1964).

Note. *Phlegmariurus copelandianus* is a rare species that is endemic to Indonesia where it is an epiphyte in montane rainforest. It was placed in the group of the New Guinea endemic *P. macgregorii* (Copel.) A.R.Field & Bostock by Øllgaard (1989), but does not appear to have a close relative and has not yet been placed in molecular phylogenetic analyses. It is unique among *Phlegmariurus* in having minute, distinctly petiolate, almost spoon like leaves with a cupped apex and reflexed lateral margins.

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A new genus of temperate woody bamboos (Poaceae, Bambusoideae, Arundinarieae) from a limestone montane area of China

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Abstract

Ampelocalamus calcareus is a climbing and slender bamboo, known from south Guizhou, China. This species grows in broadleaved forests of limestone montane areas. Recent molecular phylogenetic analyses demonstrated that *A. calcareus* was sister to all other lineages of the tribe Arundinarieae rather than a member of *Ampelocalamus*. The morphological features and habitats of *A. calcareus* and related genera including *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* were compared and discussed. The characteristics of the branch complements, nodes and foliage leaves distinguish *A. calcareus* from morphologically similar taxa. On the basis of molecular and morphological evidence, we propose to establish a new genus, *Hsuehochloa*, to accommodate *A. calcareus* and to honour the late Chinese bamboo taxonomist Chi-Ju Hsueh (Ji-Ru Xue). In addition, we describe the inflorescence of *Hsuehochloa* for the first time.

Keywords

Ampelocalamus, climbing bamboos, *Hsuehochloa*, new genus

Introduction

Temperate woody bamboos or the tribe Arundinarieae (Bambusoideae, Poaceae) comprise approximately 550 species in 31 genera (BPG 2012, Clark et al. 2015). They are mainly distributed in temperate to subtropical montane areas of East Asia (Ohrnberger

1999) with China as the centre of species diversity (Li et al. 2006), but also in Southeast Asia, south India, Sri Lanka, North America and Africa.

The recent plastid molecular phylogenetic results indicated that there were 12 major lineages in temperate woody bamboos, i.e. I. *Bergbambos*, II. *Oldeania*, III. *Chimonocalamus*, IV. *Shibataea* clade, V. *Phyllostachys* clade, VI. *Arundinaria* clade, VII. *Thamnocalamus*, VIII. *Indocalamus wilsonii*, IX. *Gaoligongshania*, X. *Indocalamus sinicus*, XI. *Ampelocalamus calcareus* and XII. *Kuruna*. However, relationships amongst them remain largely uncertain (Triplett and Clark 2010, Zeng et al. 2010, Yang et al. 2013, Attigala et al. 2014, 2016, Ma et al. 2014, Zhang et al. 2016, Zhang et al. 2017). Those lineages are strongly inconsistent with the morphological classification at the generic and subtribal levels (Keng and Wang 1996, Li 1997, 1999, Ohrnberger 1999). Most species and genera were nested within lineages IV, V and VI, while some lineages included only one species (lineages I, VIII, IX, X, XI). Lineages I and IX consisted of *Bergbambos tessellata* (Nees) Stapleton and *Gaoligongshania megalothyrsa* (Handel-Mazzetti) D. Z. Li, Hsueh & N. H. Xia, respectively and *Bergbambos* Stapleton and *Gaoligongshania* D. Z. Li, Hsueh & N. H. Xia are both monotypic (Li et al. 1995, Stapleton 2013). Lineages VIII and X were formed by *Indocalamus wilsonii* (Rendle) C. S. Chao & C. D. Chu and *I. sinicus* (Hance) Nakai, respectively, with *I. sinicus* as the lectotype of the genus *Indocalamus* Nakai. *Ampelocalamus calcareus* C. D. Chu & C. S. Chao (lineage XI) was recovered as the sister taxon to all the other temperate woody bamboos (Yang et al. 2013, Ma et al. 2014). The phylogenetic positions of the above-mentioned five monotypic lineages have also obtained some support from nuclear gene trees (Zhang et al. 2012, Yang et al. 2013).

Molecular phylogenetic results provide fresh perspectives for taxonomy, especially for lineages VIII, X and XI with only one species. Continuing to include these bamboos in the present genera renders these genera polyphyletic and causes problems when describing or citing them. In this paper, we propose to establish a new genus for *Ampelocalamus calcareus* based on morphological characters and previous molecular results. For the other two monotypic lineages (VIII and X), taxonomic revisions will be made in a separate paper.

Materials and methods

Drepanostachyum P. C. Keng and *Himalayacalamus* P. C. Keng are morphologically close to *Ampelocalamus* S. L. Chen, T. H. Wen & G. Y. Sheng (Li et al. 1996). These three genera all have pachymorph rhizomes, prominent or conspicuous nodal sheath scars and pendulous culms. Sometimes it is difficult to see the difference when only the vegetative features are available. Some species of *Ampelocalamus* were transferred from the genus *Drepanostachyum* (Keng and Wang 1996, Stapleton et al. 2005, Li et al. 2006) and several taxa of *Drepanostachyum* were combined into *Himalayacalamus* (Stapleton 1994). It is necessary to compare characters of *Ampelocalamus calcareus* with those two genera in order to clarify their morphological similarities and differences.

Specimen examination

The type specimen of *Ampelocalamus calcareus* was examined at the herbarium of Nanjing Forestry University (NF). We also examined specimens of *A. calcareus*, other species of *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* at herbaria of Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Nanjing University (N), Institute of Botany, Chinese Academy of Sciences (PE) and Sichuan Agricultural University, Dujiangyan Campus (SIFS) (specimens of N and PE were checked through the website http://www.cvh.ac.cn/).

Living plant observation

In 2010, one clump of *Ampelocalamus calcareus* was introduced by P. F. Ma and Z. M. Cai from Libo, Guizhou and cultivated at the greenhouse of the Germplasm Bank of Wild Species (GBOWS), Kunming Institute of Botany, Chinese Academy of Sciences, Kunming. This introduced clump flowered between 2012 and 2013. Unfortunately, the clump did not grow well and only a small piece of inflorescence was collected before it died. After fieldwork in early 2015, it was confirmed that the wild populations also flowered and died during the same period. More seedlings were introduced into the greenhouse of GBOWS at Kunming by C. Guo and Y. Guo in March 2015. They grew well in the greenhouse and became mature individuals after three years. Vegetative features including culms, culm sheaths, branch complements and foliage leaves were observed based on those individuals.

Morphological comparison

Four reproductive characters and 15 vegetative characters were selected and compared across *Ampelocalamus calcareus*, *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus.* For *A. calcareus*, the morphological data were observed and obtained based on herbarium specimens, living plants and literature. We observed and measured the structure of the inflorescence of *A. calcareus* by hand lens (30×) and stereomicroscope (Leica M166FC) without dissecting the spikelet due to the scarcity of materials. For the other genera, the morphological data were obtained from specimens and literature. The habitats of *Ampelocalamus calcareus*, *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* were also compared based on literature.

Results

Morphological characteristics and habitat data are summarised in Table 1. *Ampelocalamus calcareus* and species of *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* are all unicaespitose. Culms of *A. calcareus* are procumbent or scrambling, while culms

Table 1. Comparison of morphological characters and habitats of *Ampelocalamus calcareus* (= *Hsuehochloa*), *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus*.

	Hsuehochloa	***Ampelocalamus***	***Drepanostachyum***	***Himalayacalamus***
Clump form	Unicaespitose, drooping, procumbent or scrambling	Unicaespitose, pendulous or scrambling	Unicaespitose, pendulous	Unicaespitose, nodding to pendulous
Culm height (length)	4–6 m	3–10 m (usually 3–5 m)	1.5–5.4 m (usually 2–3 m)	2–9 m
Culm diameter	4–5 mm	5–15 (40) mm	7–25 mm	10–35 mm
Internode	Terete, densely white pubescent and white powdery, later subglabrous	Terete, finely ridged, usually glabrous	Terete, glabrous	Terete, glabrous
Branch complement	Solitary at the base, 3–7 at the middle and upper, subequal	Many (less than 20), geniculate, central often dominant	Numerous (15 to 80), verticillate, subequal, slender	Many (15 to 30), subequal, slender
Nodal sheath scar	inconspicuous	prominent, often with corky collar	prominent	prominent
Culm sheath	1/2 as long as the internode, persistent, densely white pubescent abaxially, glabrescent	Shorter than the internode, deciduous, often sparsely setose abaxially	Longer or shorter than the internode, deciduous or persistent, glabrous or sparsely setose abaxially, adaxially scabrous apically	Longer or shorter than the internode, deciduous, glabrous or seldom setose abaxially
Culm sheath auricle	Falcate, amplexicaul	Absent or minute (*A. actinotrichus* with prominent auricles)	Absent	Absent
Culm sheath oral setae	Several, radiate	Absent except *A. actinotrichus*	Absent	Absent
Culm sheath blade	Ovate-lanceolate, reflexed	Linear, linear-lanceolate, lanceolate, ovate-lanceolate, erect or reflexed	Subulate or linear, erect or reflexed	Subulate or linear, erect or reflexed, readily deciduous
Leaf number of the ultimate branch	2–5	3–11	3–5	3–7
Leaf sheath	Glabrous	Glabrous or pubescent	Glabrous	Glabrous
Leaf auricle	Falcate	Absent or present	Absent or minute	Absent or minute
Leaf oral setae	Several, radiate	Radiate when present	Absent or present	Absent or present
Leaf blade	Leathery, glabrous	Papery, glabrous or pubescent	Papery, glabrous	Papery, glabrous or abaxial midrib hairy proximally
Inflorescence	Racemes	Panicles	Panicles	Racemes
No. of florets per spikelet	5	2–7	2–6	1 or 2
Stamen	3, anthers purple	3, anthers yellow	3, anthers yellow	3, anthers yellow
Stigma	2, plumose	2, plumose	2, plumose	2, plumose
Habitat	Limestone montane areas, alt. 500–950 m	Broad-leaved forests, stony slopes (limestone, granite or basalt), riverside slopes, alt. 200–1800 m	Slopes, coniferous and broadleaf mixed forests, 1300–3200 m	Temperate forests, 1200–3000 m

of *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* are pendulous or seldom scrambling. The characteristics of culm sheaths, internodes, branch complements, nodal sheath scars and foliage leaves are variable across *A. calcareus*, *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus*.

The inflorescence of *Ampelocalamus calcareus* is semelauctant and racemose. The spikelet has five florets and the floret possesses a purple-green lemma (ca. 1 cm long), palea shorter than the lemma (ca. 0.8 cm long), three purple stamens (4 mm long) and two plumose stigmas. The inflorescence of *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* has been described in detail in other literature (e.g. Stapleton 1994, Li et al. 2006), therefore, we only list some key features in Table 1.

Analysis of the habitat data demonstrates that *Ampelocalamus calcareus* mainly occurs under broadleaved forests of limestone areas below 1000 m; other *Ampelocalamus* species grow under broadleaved forests, on stony slopes (limestone, granite or basalt) and riverside slopes usually from 200 m to 1800 m alt.; taxa of *Drepanostachyum* are usually distributed under coniferous and broadleaved mixed forests from 1300 m to 3200 m alt.; species of *Himalayacalamus* occur under temperate forests from 1200 m to 3000 m alt. (Table 1).

Discussion

Ampelocalamus calcareus was described by Chao and Chu (1983) based on vegetative specimens. This species has pachymorph rhizomes with short necks and apically drooping culms (Fig. 1) that are similar to other species of the genus *Ampelocalamus*, especially to the type species *A. actinotrichus* (Merrill & Chun) S. L. Chen, T. H. Wen & G. Y. Sheng. Moreover, the conspicuous auricles and radiate oral setae on the culm sheath and leaf sheath are similar to *A. actinotrichus* as well. However, characteristics of nodes, branch complements and leaf blades are quite different from *Ampelocalamus*. *Ampelocalamus calcareus* has inconspicuous nodal sheath scars, a solitary branch at the base and 3–7 subequal branches at the middle and upper parts of the culm and leathery leaf blades. Other taxa in *Ampelocalamus* usually possess prominent nodal sheath scars with a corky collar, many branches with a central dominant one that may replace the culm and papery leaf blades. Branches at the nodes of *A. calcareus* are long (50–100 cm), pendulous and nearly as thick as the culm, which makes culms scrambling or procumbent. There are also some other vegetative features that can distinguish *A. calcareus* from typical *Ampelocalamus* species, as summarised in Table 1.

Culms of *Drepanostachyum* and *Himalayacalamus* are distally pendulous, but not scrambling, which is different from *Ampelocalamus calcareus*. Branches on mid-culms of *Drepanostachyum* and *Himalayacalamus* are usually more than 15 in number and subequal without a central dominant one, while *A. calcareus* has no more than 10 subequal branches. Culm sheaths of *Drepanostachyum* and *Himalayacalamus* are usually deciduous and glabrous abaxially, whereas culm sheaths of *A. calcareus* are persistent and densely white pubescent abaxially. *Ampelocalamus calcareus* has conspicuous auri-

Figure 1. *Hsuehochloa calcarea*. **A, B** Habit and habitat **C** Clump **D** Young culm with white pubescence **E, F** Branch complement **G** Culm sheath **H** Leaves **I** Inflorescence **J** Floret (**A–D, G** from *P. F. Ma & Z. M. Cai 10050* **E, F, H** from seedlings introduced from Libo, Guizhou, China **I, J** from *P. F. Ma s.n*). Scale bars: 5 cm (**A–C**); 0.5 cm (**D, G**); 2 cm (**E, F**); 1 cm (**H, I**); 1mm (**J**).

cles and oral setae on culm sheaths and leaf sheaths and ovate-lanceolate culm blades, while auricles and oral setae are often absent and culm blades are subulate or linear in *Drepanostachyum* and *Himalayacalamus*.

Due to the incomplete nature of the flowering material (Fig. 1), the description and comparison provided in Table 1 may not be fully accurate for healthy individuals flowering in the wild. The type of inflorescence of *A. calcareus* is similar to *Himalayacalamus* (racemose); the number of florets per spikelet is similar to *Ampelocalamus* and *Drepanostachyum* (5 vs. 2–7); they all have three stamens and two plumose stigmas, but the anther colour of *A. calcareus* is purple while anthers are yellow in *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus*.

Through comparison of morphological characters, we conclude that *Ampelocalamus calcareus* morphologically resembles species of *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* in its pachymorph rhizomes and is especially similar to *Ampelocalamus* in its climbing habit. However, the branch complements and the characteristics of its nodes, culm sheaths and foliage leaves can distinguish this species from all taxa in these three genera. The inflorescence of *A. calcareus* is also similar to these three genera (on the basis of our incomplete material) in its semelauctant structure, the presence of three stamens and two stigmas.

Molecular phylogenetic studies indicated that *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* had a close relationship in nuclear gene based phylogenies, although only limited taxa of those genera were sampled (Yang et al. 2013). Nonetheless, *Ampelocalamus calcareus* was sister to all the other taxa of the tribe Arundinarieae in plastid and nuclear gene trees (Yang et al. 2013, Ma et al. 2014, Attigala et al. 2016, Zhang et al. 2016). The morphological similarity between the distantly related *A. calcareus* and those three genera (*Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus*) demonstrated that morphological characters had undergone complex evolutionary trajectories in those taxa and also in the whole tribe and some important features in bamboo taxonomy were homoplastic or convergent that was illustrated in other studies of Arundinarieae and some tropical woody bamboos (Yang et al. 2008, Tyrrell et al. 2012, Attigala et al. 2016).

The habitat and altitude of *A. calcareus* are more similar to other typical species of *Ampelocalamus* than they are to *Drepanostachyum* and *Himalayacalamus* (Table 1).

Based on the above analysis of morphology, molecular phylogenetic relationships and habitat, we propose to establish a new genus to accommodate *Ampelocalamus calcareus*.

Taxonomic treatment

***Hsuehochloa* D. Z. Li & Y. X. Zhang, gen. nov.**
urn:lsid:ipni.org:names:77190833-1

Diagnosis. *Hsuehochloa* resembles genera *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus*, but differs from those genera by its thin culms (4–5 mm), fewer branches in each branch complement (1, 3–7), inconspicuous nodal sheath scar, falcate auricles and leathery foliage leaves.

Type. *Hsuehochloa calcarea* (C. D. Chu & C. S. Chao) D. Z. Li & Y. X. Zhang, comb. nov. (77190834-1)

Basionym. *Ampelocalamus calcareus* C. D. Chu & C. S. Chao, Acta Phytotax. Sin. 21: 204–206. 1983. Type: CHINA, Guizhou, Libo, 500 m, *C. D. Chu, C. S. Chao, J. Q. Zhang & K. M. Lan 81018* (holotype, NF!; isotype, PE!)

Description. Rhizomes pachymorph. Culms caespitose, apically drooping, procumbent or scrambling, 4–6 m long, 4–5 mm in diameter, internodes terete, 8–18 cm long, densely white pubescent initially at the upper part, later subglabrous; nodes and sheath scars inconspicuous. Branch complements with one branch proximally and 3–7 branches apically, branches 0.5–1 m long, slender, subequal. Culm sheaths persistent, 1/2 as long as internodes, densely white pubescent, glabrescent, margins densely white ciliate; auricles falcate, amplexicaul; oral setae many, radiate, ca. 1 cm; ligule short, apex densely white fimbriate; blade reflexed, green, ovate-lanceolate. Foliage leaves 2–5 per ultimate branch; sheaths glabrous, glossy, margins ciliate; auricles present; oral setae deciduous, radiate, 5–7 mm; ligule short, apex long, white ciliate; blade 7–20 × 1.2–3 cm, thinly leathery, abaxially slightly glaucous, glabrous on both surfaces, secondary veins indistinct, 4–7 pairs. Inflorescence imperfectly known, semelauctant, racemose possibly with 1 or few spikelets; glumes not seen; florets 5; lemma ca. 1 cm long, purple green; palea ca. 0.8 cm long; lodicules not seen; stamens 3, anthers purple, 4 mm long; ovary and style not seen; stigmas 2, plumose.

Etymology. *Hsuehochloa* was named in honour of the late Prof. Chi-Ju Hsueh (Ji-Ru Xue in *Pinyin* transliteration) (1921–1999), a pioneer Chinese botanist on bamboos of SW China and mentor of the senior author in 1983–1986. *Hsueh* stands for his family name and *chloa* means grass.

Distribution and habitat. Endemic to south Guizhou, China, under broadleaved forests in a limestone montane area at 500–950 m altitude.

Additional specimens examined. CHINA. Guizhou: Libo, 950 m alt., May 1982, *X. H. Song 919* (NF), *J. P. Ruan 90041* (N), 600–700 m alt., November 6 2006, *T. P. Yi 06093 & 06094* (SIFS), 679 m alt., 25°26.691'N, 107°56.823'E, 14 April 2010, *P. F. Ma & Z. M. Cai 10050* (KUN), 653 m alt., 25°25.783'N, 107°56.533'E, 28 March 2015, *C. Guo* & *Y. Guo GC 82* (KUN), 667 m alt., 25°25.7'N, 107°56.25'E, 16 May 2015, *X. Y. Ye & M. Y. Zhou YXY190* (KUN).Yunnan (Kunming): cultivated in the greenhouse of GBOWS, Kunming, 1900 m alt., January 2013, *P. F. Ma s.n.* (KUN).

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RESEARCH ARTICLE

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Synthetic cartography for mapping biodiversity in the Mediterranean region: Sicily as a case study

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Abstract

This paper proposes a new hierarchical land classification system for the mapping of species distribution at national or regional scales. Our integrative framework incorporates two hierarchical levels inferred from historical, climatic, geomorphological and geological attributes. The feasibility of this proposal is based on the use of historical collections and literature data, as well as on its ability to combine old low-precision data with more recent records of higher resolution. The system is set up for vascular plants, but it can also be used for other taxonomic groups. Furthermore, it has the potential for application to the whole Mediterranean region because it is based on information that is generally available in all Mediterranean countries. This model is tested with the distribution of *loci classici* of the Italian endemic plants occurring in Sicily.

Keywords

plants, distribution, biological collections, spatial analysis, data analysis, GIS software

Introduction

Biodiversity mapping is widely considered as the basis for effective territorial and conservation planning. In the last 20 years, thanks in part to the development of GIS tools, the accuracy, quality and speed of realisation of such mapping has increased significantly (Lahti and Lampinen 1999). The first well known example in Europe of an atlas covering an entire regional flora was carried out for the British Isles (Perring and Walters 1962). The two main approaches adopted for mapping biological distribution data are, according to Pedrotti (2013), maps of dots and maps of polygons. Polygons can be regular or irregular in size and either freely arranged or according to a fixed grid. Dots in a fixed grid system can be assimilated to form regular polygons. Freely distributed dot mapping allows the identification of the geographical position of a taxon on a cartographic support with the highest possible precision (e.g. Barina 2017), but the output is not suitable for further statistical elaboration with commensurable units of investigation. Dot maps can be used when highly detailed distribution data are available, but for large-scale analysis, their conversion in a matrix is advisable. Maps comprising irregular polygons use portions of territory of different sizes and shapes as survey units, such as political-administrative borders or the limits of protected areas. For example, a large part of biodiversity data collection and processing activities in France is based on the 96 departments into which its territory is subdivided (e.g. Prelli and Boudrie 1992).

Although this approach has the advantage of ease of data collection regarding the areas investigated, subsequent statistical processing of the results may be difficult due to the irregular size of the geographic units. On the other hand, whereas the use of regular grids facilitates statistical analysis, e.g. the identification of grid cells of higher biodiversity (Raimondo 2000, Finnie et al. 2007, Carta et al. 2018), a large quantity of highly detailed data is required in order to provide reliable results. Grids can be arranged according to the U.T.M. (Universal Transverse Mercator) projection that subdivides Europe into units of 100 km^2, with this main grid then further subdivided down to the desired level of detail (Raimondo and Venturella 1991, Domina et al. 2018). The first floristic maps of the entire European territory, based on its subdivision into survey units of 50 km^2, were proposed in the Atlas Florae Europeae (Jalas and Suominen 1972); this system was later adapted to the whole Mediterranean region (Uotila et al. 2003).

Although subdivision into kilometric squares is currently little used, it does allow reference to areas of equal size. The Floristic Cartography Project of Central Europe was based on the geographical division of the territory (Ehrendorfer and Hammann 1965); thus, although its Operational Geographical Units (OGU) vary in size according to latitude, data can be located rapidly.

As a result of the increasing use of GPSs and easy access to geo-referenced maps (e.g. Google Earth), an almost global transition in the survey of primary biodiversity data has taken place from OGU-based systems to point systems (e.g. Bartolucci et al. 2016, Bedini et al. 2016).

Nevertheless, herbaria and other historical collections remain one of the main sources of primary biodiversity data that should not be neglected. This is true both for historical data from countries that are also covered by updated surveys, for the study of trends, but also for those countries for which data sets more than 50 years old remain the most abundant. Therefore, a problem has arisen concerning the use of low resolution data either on their own or together with more detailed datasets.

Brundu et al. (2017), in the framework of the project for the identification of the *loci classici* of the flora endemic to Italy (Domina et al. 2012, Peruzzi et al. 2015), highlighted the problem of managing historical herbarium data, but ultimately limited their considerations only to data that could be projected on to a GIS system as reliable points. Within this context, this paper presents a hierarchical approach specifically designed for the mapping and comparison of plan distribution data at different scales, on behalf of environmental analysis. The results are a synthetic environmental cartography, with the purpose of delimiting of spatial sets, which are groupings of unitary areas of analysis characterised by homogeneous variables. The system proposed here integrates floristic data with existing information from various environmental disciplines, including geology, bioclimatology, vegetation science and soil science (Blasi 1995).

A proposal for classifying and mapping floristic data

The key problem in organising a classification system based on biologically homogeneous areas is the development of the criteria used to identify homogeneity at different spatial scales (disregarding time). In fact, different natural processes occur on different temporal scales (Klijn and de Haes 1994) and thus the use of fixed study areas also allows such a comparison of these phenomena.

Typically, the land attributes used to classify homogeneous areas include flora, climate, lithology, geomorphology, human activities, soil, vegetation and fauna (Forman and Godron 1986, Zonneveld 1995). As the weight selected for each of these different attributes determines the delimitation of the homogeneous areas (Bunce et al. 1996), arbitrary elements may be introduced even in objective approaches. The system proposed here is thus based largely on an intuitive, divisive approach, using generally available data with superimposed maps.

Materials and methods

Study area

Sicily is located at the centre of the Mediterranean Basin and is considered one of its most important biodiversity hotspots (Medail and Quezel 1997). The total area of Sicily is about 25,700 km^2, of which approximately 61.4% is hilly, 24.5% is mountainous and the remaining 14.1% consists of alluvial plains.

Sicily is surrounded by more than 300 smaller islands and islets, some of which are only rocks isolated from the mainland, on which plants permanently occur. According to Basilone (2018), eight main geological complexes can be distinguished in Sicily: Continental deposition clastic, Volcanic, Clayey-marly, Evaporitic, Sandstone-clayey-calcareous, Carbonate, Phyllitic and shale-crystalline. Bazan et al.

Table 1. Subdivision of the Sicilian territory into groups, units and subunits.

Groups	Units	Subunits
1. Islands	1.1 Egadi	1.1.1 Egadi
	1.2 Ustica	1.2.1 Ustica
	1.3 Eolie	1.3.1 Eolie
	1.4 Pelagie	1.4.1 Lampedusa & Lampione 1.4.2 Linosa
	1.5 Pantelleria	1.5.1 Pantelleria
2. Coasts	2.1 Northern coast	2.1.1 Northern coast
	2.2 Eastern coast	2.2.1 Eastern coast
	2.3 Southern and Western coast	2.3.1 Southern and Western coast
3. Hills and plains	3.1 Western Sicily and inland Palermo	3.1.1 Western Sicily 3.1.2 Inland Palermo
	3.2 Hilly inland	3.2.1 Sulphur serie 3.2.2 Upper Himera basin 3.2.3 Central Clayey 3.2.4 Plain of Catania 3.2.5 Sandy plain of Gela and Caltagirone
4. Mountain Systems	4.1 Mts of Trapani	4.1.1 Mts of Trapani
	4.2 Mts of Palermo	4.2.1 Mts of Palermo
	4.3 Sicani Mts	4.3.1 Sicani Mts
	4.4 Madonie Mts	4.4.1 Upper Madonie 4.4.2 Lower Madonie
	4.5 Erei Mts	4.5.1 Erei Mts
	4.6 Nebrodi Mts	4.6.1 Upper Nebrodi 4.6.2 Lower Nebrodi
	4.7 Peloritani Mts	4.7.1 Peloritani Mts
	4.8 Etna Mt.	4.8.1 Upper Etna Mt. 4.8.2 Lower Etna Mt.
	4.9 Iblei and Siracusa Mts	4.9.1 Iblei Mts 4.9.2 Lower Iblei and Siracusa Mts

(2015) recognise 11 different bioclimatic belts, ranging from Upper lnframediterranean to Upper Cryomediterranean. This variation in geology, morphology, climate and land use is largely responsible for Sicily's extremely rich biological diversity. Indeed, its currently known vascular flora, 3224 specific and subspecific taxa, include more than 13% of the Italian endemic taxa (Bartolucci et al. 2018, Galasso et al. 2018).

Spatial subunits Attributes and variables	1.1.1 Egadi	1.2.1 Ustica	1.3.1 Eolie	1.4.1 Lampedusa & Lampione	1.4.2 Linosa	1.5.1 Pantelleria	2.1.1 Northern coast	2.2.1 Eastern coast	2.3.1 Southern and Western coast	3.1.1 Western Sicily	3.1.2 Inland Palermo	3.2.1 Sulphur serie	3.2.2 Upper Himera basin	3.2.3 Central Clayey	3.2.4 Plain of Catania	3.2.5 Sandy plain of Gela and Caltagirone	4.1.1 Mts of Trapani	4.2.1 Mts of Palermo	4.3.1 Sicani Mts	4.4.1 Upper Madonie	4.4.2 Lower Madonie	4.5.1 Erei Mts	4.6.1 Upper Nebrodi	4.6.2 Lower Nebrodi	4.7.1 Peloritani Mts	4.8.1 Upper Etna Mt.	4.8.2 Lower Etna Mt.	4.9.1 Iblei Mts	4.9.2 Lower Iblei and Siracusa Mts
Altitude < 100 m a.s.l.	3	3	1	3	3	1	3	3	4	1	1	1	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	3
Altitude 100-300 m a.s.l.	1	1	2	1	1	2	1	1	0	1	1	1	1	1	0	2	1	1	1	0	1	1	0	1	1	0	1	1	1
Altitude 300-600 m a.s.l.	0	0	1	0	0	1	0	0	0	1	1	1	2	2	0	1	2	1	1	0	1	1	0	1	1	0	1	2	0
Altitude 600-1000 m a.s.l.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1	0	1	2	0	1	1	0	1	1	0
Altitude 1000-2000 m a.s.l.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	1	0	4	1	1	2	1	0	0
Altitude > 2000 m a.s.l.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Annual rainfall < 400 mm	0	4	0	4	4	2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Annual rainfall 400-600 mm	4	0	2	0	0	2	1	1	3	2	2	4	4	4	4	3	4	3	4	0	1	4	0	0	0	0	1	0	4
Annual rainfall 600-800 mm	0	0	2	0	0	0	3	1	0	2	2	0	0	0	0	0	0	1	0	0	3	0	0	1	1	0	1	2	0
Annual rainfall 800-1000 mm	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	3	3	0	2	2	0
Annual rainfall > 1000 mm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	0	0	4	0	0	0
Avg. Annual Temperature <13 °C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	0	0	4	0	1	4	0	0	0
Avg. Annual Temperature 13-16 °C	0	0	0	0	0	0	0	0	0	0	3	1	3	1	0	0	1	2	3	0	3	2	0	3	1	0	2	2	0
Avg. Annual Temperature 16-18 °C	1	0	2	0	0	2	1	0	0	3	1	3	1	3	1	3	3	1	0	0	1	2	0	1	2	0	2	2	2
Avg. AnnualTemperature 18-19 °C	3	4	2	0	0	2	3	4	4	1	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	2
Avg. Annual Temperature >19 °C	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bioclimate: Lower Thermomediterranean & Inframediterranean	2	2	1	4	4	1	3	2	3	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Bioclimate: Upper Thermomediterranean	2	2	2	0	0	3	1	2	1	3	1	2	1	2	4	3	2	1	0	0	1	1	0	1	1	0	1	0	2
Bioclimate: Lower Mesomediterranean	0	0	1	0	0	0	0	0	0	0	2	2	2	2	0	1	1	2	2	0	1	2	0	1	2	0	2	3	0
Bioclimate: Upper Mesomediterranean	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	0	2	1	0	2	1	0	1	1	0
Bioclimate: Supramediterranean & Cryomediterranean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	4	0	0	0
Substrate: alluvial and coastal deposits	0	0	0	0	0	0	1	2	2	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Substrate: volcanites	0	4	4	0	4	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	1	0
Substrate: carbonatic	4	0	0	4	0	0	2	1	1	1	1	0	0	0	0	0	4	4	3	2	0	0	0	1	0	0	0	3	3
Substrate: clayey & sandy formations	0	0	0	0	0	0	1	0	1	2	3	2	4	4	0	4	0	0	1	2	4	4	4	3	0	0	0	0	1
Substrate: "gessoso solfifera" formation	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Substrate: metamorphic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Land use: artificial sufaces	0	0	0	0	0	0	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Land use: agricultural areas	2	3	1	2	2	2	2	1	2	4	4	3	4	3	4	4	1	1	2	0	2	3	0	1	1	0	2	2	4
Land use: forest and Semi-natural areas	2	1	3	2	2	2	0	0	0	0	0	1	0	1	0	0	3	3	2	4	2	1	4	3	3	4	1	2	0
Floristic knowledge: poor	0	0	0	0	0	0	0	0	0	2	0	3	1	4	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Floristic knowledge: fair	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4	0	4	2	0	0	0	0
Floristic knowledge: good	0	0	0	0	0	0	0	1	4	2	4	1	3	0	3	1	0	0	4	0	0	0	4	0	2	0	4	0	4
Floristic knowledge: very good	4	4	4	4	4	4	4	3	0	0	0	0	0	0	0	1	2	4	0	4	4	0	0	0	0	4	0	4	0

Figure 1. Matrix in numerical and graphical form with the data attributes and variables relative to the elementary spatial subunits.

Mapping

A geographical information system was used to collate the information and to draw the maps (QGIS 3.0, https://www.qgis.org). The base maps are, in order: a 20 × 20 m Digital Terrain Model (http://wms.pcn.minambiente.it); a map of regional water bodies (http://www.pcn.minambiente.it/arcgis/services); a structural map of Sicily (Accaino et al. 2011); a lithological map of Sicily (Fierotti 1997); maps of temperature (Drago 2005), rainfall (Drago 2005), bioclimate (Bazan et al. 2015) and land use (Corine Land Cover, level 1, http://www.sitr.regione.sicilia.it/geoportale).

Area boundaries were traced according to level curves, rivers and geological structures. We started tracing the outlines of the areas historically visited by botanists following Raimondo et al. (2005). Subsequently, these and the remaining areas were divided by tracing the boundaries according to altitude, bioclimatology and geology (Table 1). We used a matrix according to Martinelli (2018) for synthetic cartography, which is obtained from an array of spatial data with columns representing the elementary spatial units of analysis and rows their attributes. The ordinate matrix with the attributes and variables extracted from the thematic maps of analysis reports the class; this matrix is transcribed into a graphical form, as a grid, with cells, which are filled by proportional sizes established in five classes (Fig. 1). The classes range from 0 to 4 giving 0 as a null value, 1 for 25%, 2 for 50%, 3 for 75% and 4 for 100% of occupied surface. The identified areas are homogenous concerning the selected attributes.

In order to verify the adequacy and the advantage of the proposed model, we used the dataset of the *loci classici* (localities reported in protologues) of the Italian endemics described from Sicily (Domina et al. 2012, Peruzzi et al. 2015, Brundu et al. 2017) and compared our results with those obtained in Brundu et al. (2017). The endemics, in fact, have been considered the most sensible element of the flora of a region and provide valid indications of the whole (Brundu et al. 2017). We projected the data collected for the project "Italian Loci Classici" on the obtained map. The loci with high geographic detail were projected using their exact coordinates. The loci with low geographic detail were projected using the coordinates of their toponyms. For this project, the geographic locations reported in the protologues or in the types of plants endemic to Italy were mapped reporting their geographical accuracy. In Brundu et al. (2017), all the type localities with low geographic accuracy (>10 km) were discarded. Here we used the complete Sicilian dataset excluding only data generically referred to the whole Sicily.

Results

Area subdivision

Overall, the Sicilian territory can be divided into four main groups: islands, coasts, hills and plains and mountains (Fig. 2). These four groups can be subdivided into

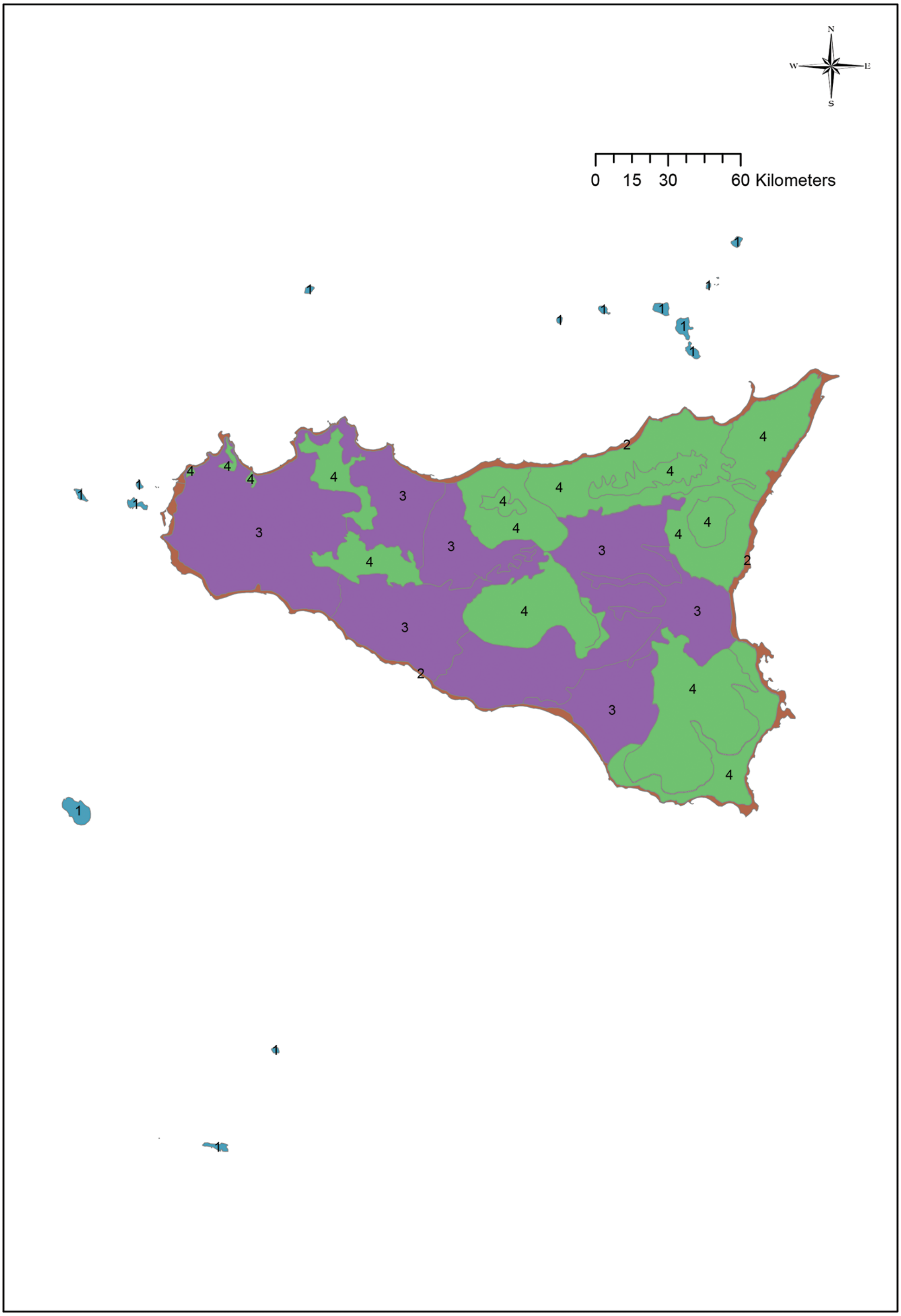

Figure 2. The four groups into which Sicily is divided, as reported in Table 1.

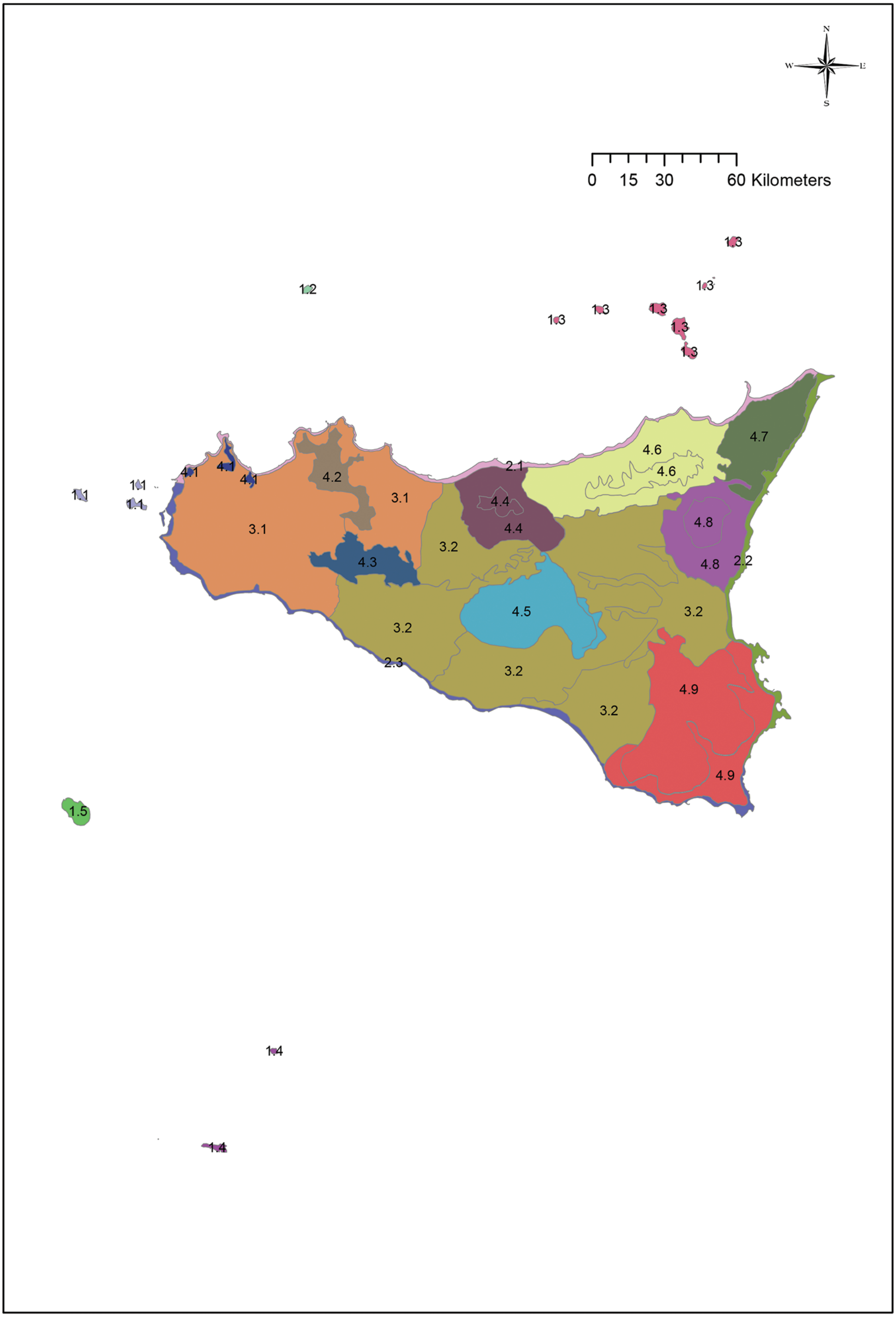

Figure 3. The 19 units into which Sicily is divided, as reported in Table 1.

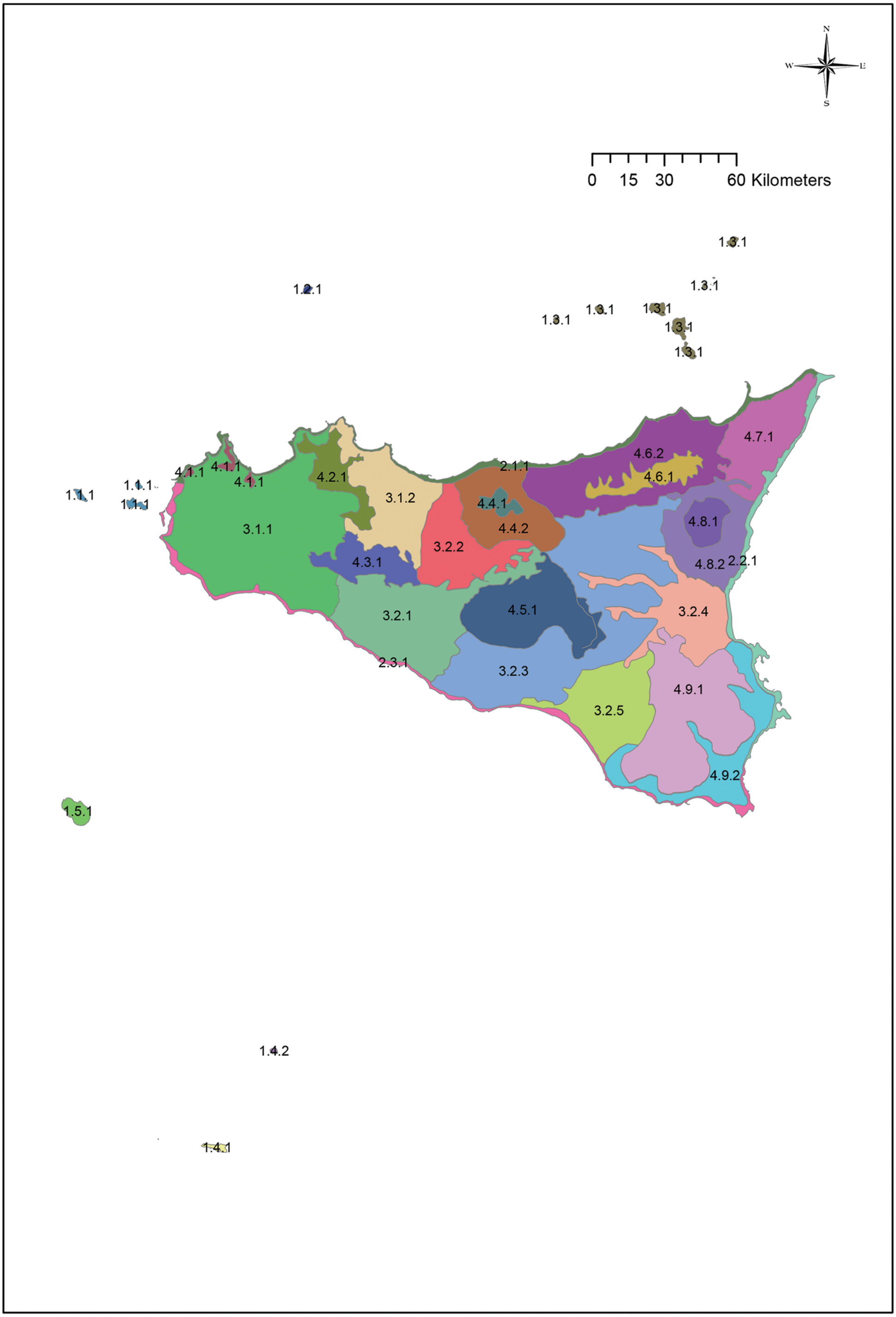

Figure 4. The 29 sub-units into which Sicily is divided, as reported in Table 1.

19 units (Fig. 3) that can in turn be further split into 29 subunits (Fig. 4) (Table 1). A detailed description and presentation of these subunits is beyond the scope of the present paper. Unit delimitation is based on major morphological features, while that of subunits is based mainly on altitude or geological substrata that determine different floristic contingents. The main islands surrounding Sicily include two isolated volcanic islands (Ustica and Pantelleria) and three archipelagoes. Egadi and Eolie archipelagoes consist of islands that are homogeneous in terms of substrate (carbonatic and volcanic, respectively) and thus, despite some of the islands hosting narrow endemic species, these archipelagoes are not subdivided further. In contrast, the Pelagie Archipelago comprises two carbonatic islands and one volcanic island whose variation in substrate has resulted in large differences in their floras; for this reason these islands are divided into two subunits (Lampedusa & Lampione and Linosa).

Coasts are subdivided according to the nature of their substratum and morphology (mainly sandy in the south and rocky in the north). This diversity is reflected in the presence of species exclusive to coastal cliffs (class *Chitmo-Limonietea* Br.-Bl.) or sandy dunes (class *Cakiletea maritimae* Br.-Bl. & Tüxen). The Sicilian hinterland is more homogeneous from a floristic point of view and thus can be considered as a single unit. In contrast, the subdivision of the hilly inland on the basis of substrate allows, for example, to highlight areas containing species restricted to the local gypsum-sulphur formation. Although the plant landscape of the region is largely anthropogenic (Gianguzzi et al. 2016), variation in soil types can also be used to distinguish areas traditionally devoted to agriculture, such as the Plain of Catania, where non-native communities are dominant. The main mountain chains are identified as the following distinct mapping units: Trapani, Palermo, Sicani, Madonie, Nebrodi, Etna, Peloritani and Iblei. The highest mountain ranges are divided into upper and lower subunits on the basis of the lower altitudinal limit of the beech forest (e.g. 1200 m a.s.l. in the Madonie Mts). The upper part of these particular mountains is characterised by unique bioclimatic features and hosts the highest percentage of Italian endemic species on the island (Baiamonte et al. 2015).

Model test

In total, we projected 472 points on the map (Fig. 5), representing a gain of 111 points (31%) over the work of Brundu et al. (2017) where only 361 points were included. All the points have been attributed to single areas without ambiguity. This allows us to distinguish areas richer in *loci classici* (i.e., likely richer in endemics) and, therefore, to make biogeographical speculations.

Overall (Table 2), the mountain areas host more loci than the other areas identified in the region (277 vs. 195). Islands, coasts and hills have a comparable number of loci (71, 59 and 65, respectively). The richest area overall is the upper part of the Madonie mountains (74 loci) followed by the mountains around Palermo (53). The Nebrodi Mountains, siliceous and rounded, are richer on the lower belt (23) than on the summit (9). The Etna Mountain is about equally rich on the lower (16) and the upper

Table 2. Occurrence of *loci classici* of the Italian endemic flora in the recognised groups, units and subunits.

Groups	Loci	Units	Loci	Subunits	Loci
1. Islands	71	1.1 Egadi	17	1.1.1 Egadi	17
		1.2 Ustica	1	1.2.1 Ustica	1
		1.3 Eolie	28	1.3.1 Eolie	28
		1.4 Pelagie	18	1.4.1 Lampedusa & Lampione	16
				1.4.2 Linosa	2
		1.5 Pantelleria	7	1.5.1 Pantelleria	7
2. Coasts	59	2.1 Northern	21	2.1.1 Northern	21
		2.2 Eastern	14	2.2.1 Eastern	14
		2.3 Southern and Western	24	2.3.1 Southern and Western	24
3. Hills and plains	65	3.1 West Sicily and Palermo's inland	31	3.1.1 West Sicily	15
				3.1.2 Palermo's inland	16
		3.2 Hilly inland	34	3.2.1 Sulphur serie	12
				3.2.2 Upper Himera basin	6
				3.2.3 Central Clayey	11
				3.2.4 Plain of Catania	0
				3.2.5 Sandy plain of Gela and Caltagirone	5
4. Mountain Systems	277	4.1 Mts of Trapani	4	4.1.1 Mts of Trapani	4
		4.2 Mts of Palermo	53	4.2.1 Mts of Palermo	53
		4.3 Sicani Mts	6	4.3.1 Sicani Mts	6
		4.4 Madonie Mts	101	4.4.1 Upper Madonie	74
				4.4.2 Lower Madonie	27
		4.5 Erei Mts	9	4.5.1 Erei Mts	9
		4.6 Nebrodi Mts	32	4.6.1 Upper Nebrodi	9
				4.6.2 Lower Nebrodi	23
		4.7 Peloritani Mts	20	4.7.1 Peloritani Mts	20
		4.8 Etna Mt.	34	4.8.1 Upper Etna Mt.	18
				4.8.2 Lower Etna Mt.	16
		4.9 Iblei and Siracusa Mts	18	4.9.1 Iblei Mts	14
				4.9.2 Lower Iblei and Siracusa Mts	4

belt (18). As pointed out by Mazzola et al. (2001), on the basis of the analysis of the biological and chorological spectra of the whole vascular floras, the Aeolian archipelago is the richest archipelago (28 loci) while the poorest island is Ustica (only 1 locus).

Discussion and Conclusion

Practical methods for tracing homogeneous areas are required in all types of studies at various scales. The most topical issue facing regional landscape planning is the identification of homogeneous areas to which individualised management should be applied (Blasi et al. 2000).

Figure 5. Projection on the sub-units of the loci classici of the Italian endemics described from Siciliy (Brundu et al. 2017).

Brullo et al. (1995) proposed a phytogeographic subdivision of Sicily into 2 sectors, 4 sub-sectors and 15 districts based on the presence of groups of endemic plants. This proposal is partially congruent with the 17 units identified here. However, such floristic districts, although functional from a large-scale biogeographical point of view, are less useful on the regional scale due to variations in altitude and geology.

The land classification, here proposed, integrates biotic and abiotic parameters at different scales. Its units and subunits are easily recognisable and comprehensible and can be used effectively at supra-national and regional scales for biodiversity, historical and ecological analysis. This approach also enables easy data presentation and allows the comparison of time series of historical and modern data. In addition, it is a method that facilitates rapid analysis on a regional scale in areas for which no detailed data are available, as is still the case for large portions of North Africa.

Our suggestion is to project all the available data in the GIS system, indicating their accuracy, date and reliability, using the geographical coordinates of the selected points or the toponyms to which they refer. More detailed data can be easily lowered to the accuracy level proposed here. Subsequent extrapolation for spatial or statistical analyses can then be performed on different sub-sets of data or in different layers depending on the required output. Point data collection and analysis in terms of administrative areas are used in the Wikiplantbase project for the mapping of plant species in some regions of Italy (Peruzzi et al. 2017). Our proposal allows the projection of historical data at a low level of detail, making them available for various analyses. Although these data are often overlooked, due to a lack of proper spatialisation, they may in fact be useful for regional floras and national checklists.

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RESEARCH ARTICLE

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Completing the jigsaw: the first record of the female plant of *Daphnopsis filipedunculata* (Thymelaeaceae), an endemic species from the Brazilian Amazon

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Abstract

The results of intensive fieldwork in the National Forest of Carajás (FLONA Carajás) led to the discovery of pistillate plants of *Daphnopsis filipedunculata*, an endemic species from the Serra dos Carajás, previously known only from staminate individuals. These newly discovered populations add valuable missing information related to pistillate buds, mature flowers and fruits.

Keywords

Amazon rainforest, Brazil, dioecy, emendation, endemism, Malvales, neotropics, Pará, taxonomy, Thymelaeoideae.

Introduction

Daphnopsis Mart. comprises 72 species and is the largest American genus of Thymelaeaceae (Rogers 2009–onwards). This Neotropical genus ranges from central Mexico to central Argentina, including the Caribbean (Rogers 2010). The last taxonomic revision of the genus presented an identification key (Nevling 1960); however, as noted by Rogers (2010), both staminate and pistillate flowers were required to key specimens to species. The main difficulty regarding the taxonomy of the genus lies in the fact that its species are dioecious and it often proves difficult to locate specimens of both sexual morphs in the field. More than 20 new species have been described since the last revision almost 60 years ago (Nevling 1960), further complicating identification without complete material.

Daphnopsis filipedunculata Nevling & Barringer is a species that demands special attention. According to its protologue, the systematic position of this species is included in *Daphnopsis* subg. *Neivira*, based on its morphological characters, such as axillary inflorescences, monopodial branching and flowers with a lobed disc (Nevling and Barringer 1993). The phylogeny of *Daphnopsis* has not yet been the subject of published molecular studies. Described as restricted to the Brazilian Amazon, *D. filipedunculata* is only known from the Serra dos Carajás (Nevling and Barringer 1993; Mota and Giulietti 2016). In the protologue, the specimens of *D. filipedunculata* were noted to occur in the ecotone between ombrophilous forest and *canga*, a type of vegetation that grows over iron-rich or ferruginous outcrops on the Serra dos Carajás plateau, which contains a mosaic of mostly open vegetation types (Viana et al. 2016). Considered rare, this species was previously recorded only by staminate individuals. According to Mota and Giulietti (2016), the populations appear to have had a large number of plants. However, on closer examination, these “plants” appear to be subterranean sprouts emanating from a single older and more robust individual. Population genetic studies are under way to investigate this hypothesis. The staminate individuals of *D. filipedunculata* are characterised by elliptic leaves and distally developed axillary, subcapitate inflorescences with long peduncles up to 13 cm long (minimum peduncle length measurement ca. 8 cm at anthesis). Staminate flowers have eight stamens attached to the inside of an obconic hypanthium (Nevling and Barringer 1993; Mota and Giulietti 2016).

In this paper, we provide an emended description for *D. filipedunculata* (including vegetative and pistillate data from female individuals), as well as taxonomic notes, photographs and illustrations.

Methods

Monthly expeditions to the *canga* of the Serra dos Carajás, specifically to the National Forest of Carajás (FLONA Carajás) and surroundings, were carried out over nearly three years, between March 2015 and January 2018, with visits to known localities of *Daphnopsis filipedunculata* and with targeted searches for additional populations and individuals of the species. The main goal of those visits was to inventory plants for the “Flora of the *canga* of the Serra dos Carajás, Pará, Brazil” (Viana et al. 2016).

Resulting voucher specimens of *Daphnopsis filipedunculata*, representing staminate and pistillate plants, found in the FLONA Carajás, Pará state, Brazil, were deposited in the herbarium of the Museum Paraense Emílio Goeldi (MG) and Universidade Federal de Minas Gerais (BHCB) (herbarium acronyms according to Thiers 2018). Available duplicates will be distributed to additional national and international herbaria.

For the morphological analysis, all measurements of vegetative parts were taken from dried herbarium collections and field notes. The description of pistillate parts was based on rehydrated specimens dissected under a stereomicroscope (Zeiss Discovery. V8) and measured using a digital pachometer.

The conservation status was evaluated following the IUCN criteria (IUCN 2012; 2017). The parameters extent of occurrence (EOO) and area of occupancy (AOO) were estimated using the GeoCAT software (Bachman et al. 2011) and maps were made using the QGis 2.18 software.

Results

The protologue description of *Daphnopsis filipedunculata* was based only on male specimens, making it difficult to associate collections of female individuals with this name. Recently, populations of pistillate individuals were collected in four different areas in the FLONA Carajás (plateaus N1, N2, N3 and N5).

An emended taxonomic description of *Daphnopsis filipedunculata*

Daphnopsis filipedunculata Nevling & Barringer (1993: 335)

Type. Brazil. Pará: Serra dos Carajás, 6 km SE of AMZA camp N-1, 6°03'S, 50°16'W, 650 m elev., 19 May 1982, *C.R. Sperling et al. 5734* (holotype: F; isotypes: GH, K, MG, MO, NY).

Emended description. Pistillate individuals: Shrubs to treelets to 3 m tall. Leaves alternate, concentrated distally on the branches; petioles 1–3 mm long, green to brownish, tomentose, trichomes erect; leaf blade elliptic, 6–13.5 × 2.5–5.5 cm, sericeous on both surfaces, denser on the primary veins; base cuneate; apex acute to acuminate; primary and secondary veins prominent on both surfaces. Pistillate inflorescence axillary, producing densely pilose, subcapitate 3–5-flowered racemes; bracts absent; primary peduncle elongating greatly during anthesis, when flowers opened 3–7.4 cm long, 0.2–0.5 mm wide at the midpoint. Pistillate flowers 3–5 mm long; hypanthium urceolate, 2–4 mm long, 2–3 mm wide at opening/constriction, pubescent outside, trichomes erect; calyx lobes 4, triangular to rounded, ca. 1 mm long, ca. 0.5 mm wide, revolute; staminodes absent; disc free, irregularly 6–8–lobed, the lobes acute, ca. 0.5 mm long; gynoecium 3–4 mm long, style 1–2 mm long, subterminal, stigma discoid, ovary ovoid, unilocular, 1–1.2 mm wide, densely pilose. Fruits berry-like, green to yellow initially, turning dark red when ripe, irregularly globose, 5–15 × 4–12 mm, pilose, trichomes white to greyish, the style and hypanthium often persistent, tearing regularly, longitudinally as fruit matures. Seeds pyriform, 2–3 × 4–5 mm. (Figures 1, 2)

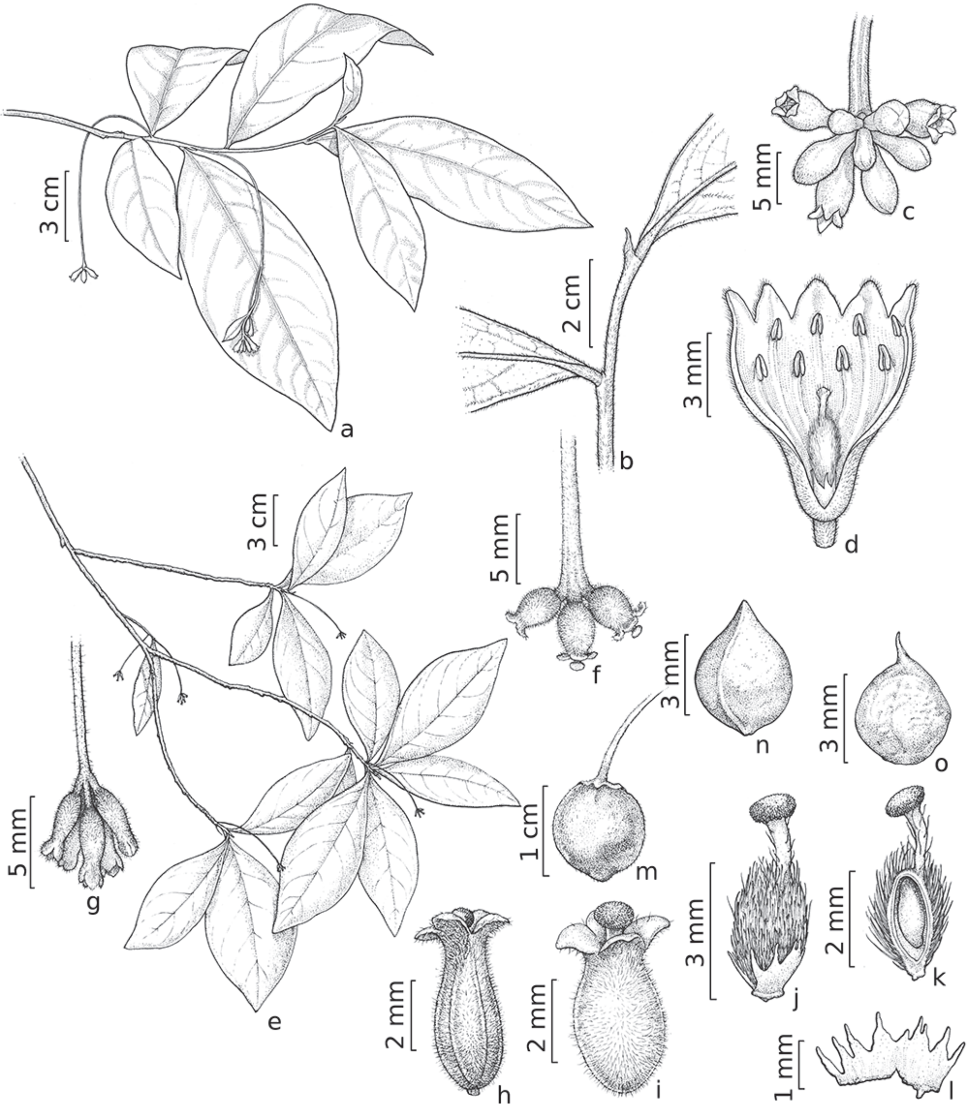

Figure 1. *Daphnopsis filipedunculata* Nevling & Barringer, male individual (**A–D**) and female individual (**E–O**). **A** staminate flowering branch **B** base of leaf blade **C** detail of inflorescence with staminate flowers **D** dissected staminate flower with two whorls of stamens and one pistillode **E** pistillate flowering branch **F** detail of inflorescence with three pistillate flowers (fresh material) **G** detail of inflorescence with five pistillate flowers (dry material) **H** pistillate flower (dry material) **I** pistillate flower (fresh material) **J** Pistil with disc **K** dissected pistil, longitudinal cut of the ovary showing pendulous ovule **L** details of the lobed disc in female flower **M** fruit **N** seed **O** embryo. Drawn by João Silveira (**A−D** modified from Mota and Giulietti 2016) (**E, G−H, J−L, N−O** *Watanabe et al. 485*, **F, I, M** *Santos et al. 395* and *396*).

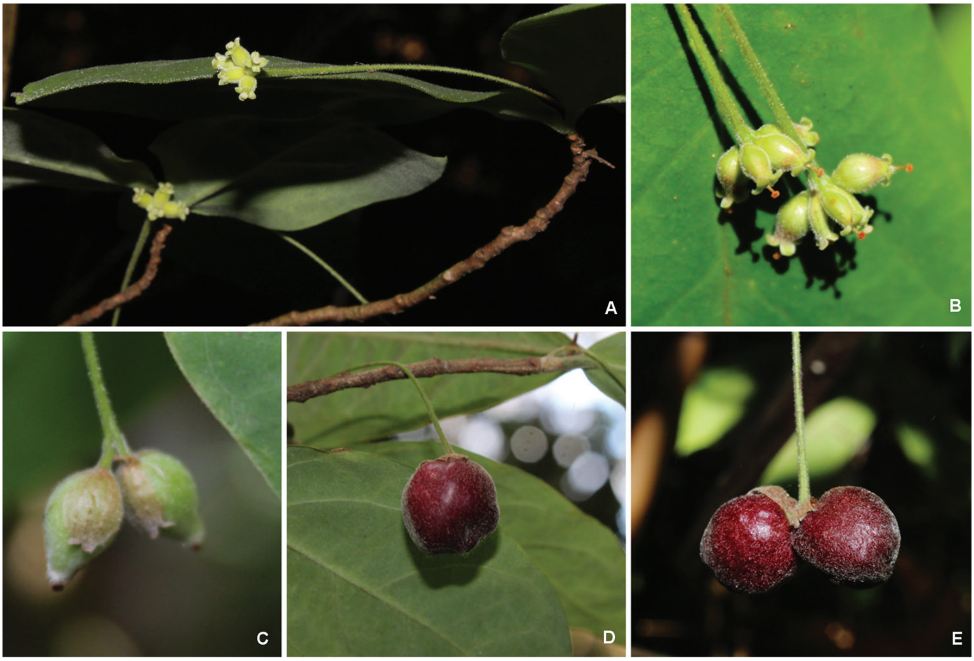

Figure 2. *Daphnopsis filipedunculata* Nevling & Barringer. **A** pistillate flowering branch **B** detail of inflorescence with pistillate flowers **C** immature fruit with persistent hypanthium **D, E** mature fruits. Photographs by Fernando M. Santos.

Material examined. Brazil. Pará: Parauapebas, Floresta Nacional de Carajás–Serra dos Carajás, Serra Norte, Plateau N1, mata baixa, 21 June 2012 [staminate fl.], *L.C.V. Silva et al. 1263* (MG); Plateau N1, 6°02'46"S, 50°16'06"W, 693 m elev., 26 May 2017 [staminate fl.], *F.M.G. Santos et al. 392* (MG); Plateau N1, 06°02'46"S, 50°16'06"W, 693 m elev., 31 May 2017 [pistillate fl.], *F.M.G. Santos et al. 393* (MG); Plateau N1, 06°02'45"S, 50°16'06"W, 679 m elev., 30 May 2017 [pistillate fl.], *F.M.G. Santos et al. 394* (MG); Plateau N1, 06°02'46"S, 50°16'06"W, 693 m elev., 31 May 2017 [pistillate fl.], *F.M.G. Santos et al. 396* (MG); Plateau N1, 6°02'43.2"S, 50°14'44.3"W, 8 Aug. 2017 [pistillate fl.], *M.T.C. Watanabe et al. 485* (BHCB, MG, RB); Plateau N2, 06°03'38"S, 50°14'46"W, 707 m elev., 24 Aug. 2012 [staminate fl.], *A.J. Arruda et al. 1269* (BHCB); Plateau N2, 06°03'09"S, 50°15'21"W, 705 m elev., 26 Jul 2017 [staminate fl.], *F.M.G. Santos et al. 397* (MG); Plateau N2, 06°03'09"S, 50°15'21"W, 697 m elev., 26 July 2017 [pistillate fl.], *F.M.G. Santos et al. 398* (MG); Plateau N3, 21-31 July 2017 [staminate fl.], *L.C.B. Lobato 4298* (MG); Plateau N3, 06°02'44"S, 50°12'35"W, 716 m elev., 30 May 2017 [pistillate fl.], *F.M.G. Santos et al. 395* (MG); Plateau N3, 06°02'45"S, 50°12'29"W, 714 m elev., 30 May 2017 [fruit], *F.M.G. Santos et al. 399* (MG); Plateau N3, 06°02'45"S, 50°12'30"W, 714 m elev., 26 June 2017 [pistillate fl.], *F.M.G. Santos et al. 400* (MG); Plateau N3, 06°02'47"S, 50°12'28"W, 720–869 m elev., 26 Jun 2017 [staminate fl.], *F.M.G. Santos et al. 401* (MG); Plateau N3, Floresta ombrófila aberta, 06°02'45"S, 50°12'30"W, 706 m elev., 22 June 2017 [staminate fl.], *M.V. Waldir 276* (MG); Plateau N4, 06°06'54"S, 50°11'03"W, 678 m elev., 01 June 2017 [staminate fl.], *F.M.G. Santos et al. 402* (MG); Plateau N4, 06°06'55"S, 50°11'02"W, 680 m elev., 1 June 2017 [staminate fl.], *F.M.G. Santos et al. 403* (MG); Plateau N5, 06°06'15"S, 50°08'13"W, 706 m elev., 31 May 2017 [staminate fl.], *F.M.G. Santos et al. 404* (MG); Plateau N5, Lagoa da Mata, 06°02'29"S, 50°05'18"W, 664 m elev., 1 June 2017 [staminate fl.], *F.M.G. Santos et al. 408* (MG); Plateau N5, Lagoa da Mata, 06°02'29"S, 50°05'18"W, 664 m elev., 24 March 2017 [staminate fl.], *F.M.G. Santos et al. 409* (MG); trilha para Lagoa, plateau N5, 06°02'28.5"S, 50°5'15"W, 667 m elev., 21 June 2015 [staminate fl.], *R.M. Harley et al. 57246* (MG); Plateau N5, Aug. 2017 [fr], *M.T.C. Watanabe et al. 482* (MG) Plateau N5, trilha da mata, 06°03'20"S, 50°05'15"W, 682 m elev., 14 Aug. 2016 [staminate fl.], *L.V. Vasconcelos & M.E.L. Lima 933* (MG); Plateau N6, 06°07'33"S, 50°10'44"W, 704 m elev., 1 June 2017 [staminate fl.], *F.M.G. Santos et al. 405* (MG); Plateau N7, 06°09'12"S, 50°10'22"W, 677 m elev., 1 June 2017 [staminate fl.], *F.M.G. Santos et al. 406* (MG); Plateau N7, 6°09'13"S, 50°10'20"W, 697 m elev., 1 June 2017 [staminate fl.], *F.M.G. Santos et al. 407* (MG); Serra dos Carajás, 6 km SE of AMZA camp, 6°03'S, 50°16'W, 650 m elev., 19 May 1982 [staminate fl.], *Sperling et al.* 5734 (F, GH, K, MG, MO, NY).

Taxonomic notes. Vegetative parts of pistillate plants of *Daphnopsis filipedunculata* are very similar to male plants. Staminate flowers have pistillodes and a deeply 4–lobed disc, while the pistillate flowers lack staminodes and the disc is irregularly 6–8–lobed. The female inflorescences bears three to five flowers, however only one or two of these usually develop into fruit (Figures 1, 2). Figure 1A (adapted from Mota and Giulietti 2016) represents a staminate individual with a floral bract and branched peduncle; however, through analysis of the above-listed material, it was found that only a single duplicate of *Harley et al. 57246* (unfortunately the one selected to be drawn) shows these characters. No other specimen had bracts, not even bract scars and none had apically branched peduncles. Therefore, we believe that the bract illustrated in Mota and Giulietti (2016) is an unusual feature uncommonly found in this species.

Populations are formed of isolated clumps of individuals separated by small distances (20–50 m), which makes it difficult to define the limits between adjacent individuals. Within a clump, sometimes individuals are found growing very close together and sometimes found suckering, presenting clonal behaviour that presumably also occurs in other groups of Thymelaeaceae, such as the African and Malagasy *Gnidia* L. (Rogers 2009). It is impossible to distinguish morphologically between individuals of different sexes of *D. filipedunculata* unless they are fertile, but during our studies, we observed that pistillate individuals are normally found at lower frequencies than staminate ones. Fertile specimens were collected between May and August, with occasional fertility in March in both sexes.

Distribution and habitat. Endemic from the *canga* of the Serra dos Carajás, this species is restricted to the formation known as Serra Norte, where it grows in low, deciduous forest over ferruginous soil (Mota and Giulietti 2016) in transitional areas between open *canga* and ombrophilous forest. It is restricted to the plateau areas, having never been found in open forests on the slope. Populations with individuals from both sexes were found on all iron rock outcrops plateaus from N1 to N7 and an additional population was found only to contain male individuals on a small *canga* outcrop area known as Lagoa da Mata.

IUCN preliminary conservation assessment. *Daphnopsis filipedunculata* has only been recorded within the FLONA Carajás, at the Serra Norte (N1, N2, N3, N4, N5, N6 and N7). According to the criteria set by IUCN (2012, 2017), the species is assigned a preliminary conservation assessment of Endangered [EN B1ab(i,ii,iii)+2ab(i,ii,iii); D], as it has a restricted distribution with an extent of occurrence (EOO) of 159.28 km^2 and an area of occupancy (AOO) of 44 km^2, based on a cell size of 4 km^2. The total population size is estimated as fewer than 250 mature individuals. In addition to being known from a single general site, the species has an inferred and projected decline in both its EOO and AOO and in its quality of habitat that is located adjacent to the mining area within the FLONA Carajás. Conservation proposals are currently being elaborated for this species in the management plan of this sustainable use protected area.

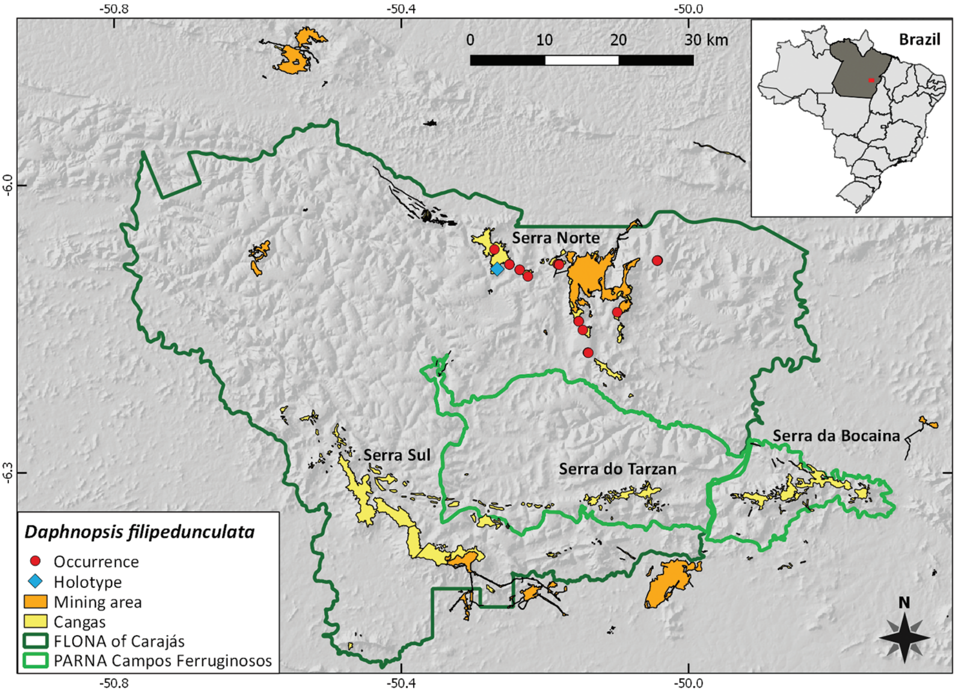

Figure 3. Distribution of *Daphnopsis filipedunculata* Nevling & Barringer along the Serra dos Carajás.

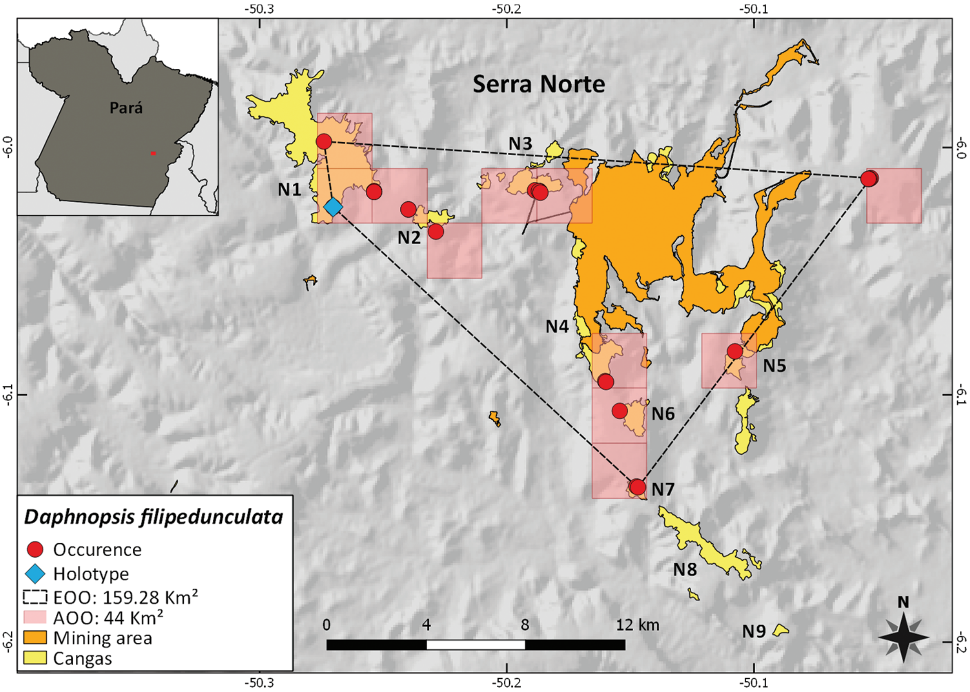

Figure 4. Distribution of *Daphnopsis filipedunculata* Nevling & Barringer along the Serra dos Carajás with convex polygon and grids of 4 km^2.

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Molecular phylogenetic data and seed coat anatomy resolve the generic position of some critical Chenopodioideae (Chenopodiaceae – Amaranthaceae) with reduced perianth segments

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Abstract

The former *Chenopodium* subgen. *Blitum* and the genus *Monolepis* (Chenopodioideae) are characterised in part by a reduced (0–4) number of perianth segments. According to recent molecular phylogenetic studies, these groups belong to the reinstated genera *Blitum* incl. *Monolepis* (tribe Anserineae) and *Oxybasis* (tribe Chenopodieae). However, key taxa such as *C. antarcticum*, *C. exsuccum*, *C. litwinowii*, *C. foliosum* subsp. *montanum* and *Monolepis spathulata* were not included and so their phylogenetic position within the Chenopodioideae remained equivocal. These species and additional samples of *Blitum asiaticum* and *B. nuttallianum* were incorporated into an expanded phylogenetic study based on nrDNA (ITS region) and cpDNA (*trnL-trnF* and *atpB-rbcL* intergenic spacers and *rbcL* gene). Our analyses confirm the placement of *C. exsuccum*, *C. litwinowii* and *C. foliosum* subsp. *montanum* within *Blitum* (currently recognised as *Blitum petiolare*, *B. litwinowii* and *B. virgatum* subsp. *montanum,* respectively); additionally, *C. antarcticum*, currently known as *Oxybasis antarctica*, is also placed within *Blitum* (reinstated here as *B. antarcticum*). Congruent with previous studies, two of the three accepted species of *Monolepis* – the type species *M. trifida* (= *M. nuttalliana*) as well as *M. asiatica* – are included in *Blitum*. The monotypic genus

Carocarpidium described recently with the type *C. californicum* is not accepted as it is placed within *Blitum* (reinstated here as *B. californicum*). To date, few reliable morphological characters have been proposed that consistently distinguish *Blitum* (incl. two *Monolepis* species) from morphologically similar *Oxybasis*; however, two key differences are evident: (1) the presence of long-petiolate rosulate leaves in *Blitum* vs. their absence in *Oxybasis* and (2) a seed coat structure with the outer wall of the testa cells lacking stalactites ('non-stalactite seed coat') but with an obvious protoplast in *Blitum* vs. seed coat with the outer walls of the testa cells having stalactites ('stalactite seed coat') and a reduced protoplast in *Oxybasis*. Surprisingly, the newly sequenced North American *Monolepis spathulata* nested within the tribe Dysphanieae (based on ITS and *trnL-trnF* + *rbcL* + *atpB-rbcL* analyses).The phylogenetic results, as well as presence of the stalactites in the outer cell walls of the testa and lack of the rosulate leaves, confirm the distinctive nature of *Monolepis spathulata* from all *Blitum* and, therefore, the recent combination *Blitum spathulatum* cannot be accepted. Indeed, the morphological and molecular distinctive nature of this species from all Dysphanieae supports its recognition as a new monotypic genus, named herein as *Neomonolepis* (type species: *N. spathulata*). The basionym name *Monolepis spathulata* is also lectotypified on a specimen currently lodged at GH. Finally, while *Micromonolepis pusilla* is confirmed as belonging to the tribe Chenopodieae, its position is not fully resolved. As this monotypic genus is morphologically divergent from *Chenopodium*, it is retained as distinct but it is acknowledged that further work is required to confirm its status.

Keywords

Blitum, Chenopodioideae, *Chenopodium*, *Oxybasis*, new genus, taxonomy

Introduction

The family Chenopodiaceae Vent. comprises ~1500 species distributed worldwide (Sukhorukov 2014). It is divided into several subfamilies and at least one third of them belong to the core subfamily Chenopodioideae in the tribes Axyrideae G.Kadereit & Sukhor. (*Axyris* L., *Ceratocarpus* L., *Krascheninnikovia* Gueldenst.), Chenopodieae incl. Atripliceae Duby (*Archiatriplex* G.L.Chu, *Atriplex* L., *Chenopodiastrum* S.Fuentes, Uotila & Borsch, *Chenopodium* L. s.str., *Exomis* Fenzl ex Moq., *Extriplex* E.H.Zacharias, *Grayia* Hook. & Arn., *Halimione* Aellen, *Holmbergia* Hicken, *Lipandra* Moq., *Manochlamys* Aellen, *Microgynoecium* Hook.f., *Micromonolepis* Ulbrich, *Oxybasis* Kar. & Kir., *Proatriplex* Stutz & G.L.Chu and *Stutzia* E.H.Zacharias), Anserineae (*Blitum* L. incl. *Scleroblitum* Ulbr., *Spinacia* L.) and Dysphanieae (*Cycloloma* Moq., *Dysphania* R.Br., *Suckleya* A.Gray and *Teloxys* Moq.) (Kadereit et al. 2003, 2010; Zacharias and Baldwin 2010; Fuentes-Bazan et al. 2012a, 2012b). While tribal boundaries are becoming well established, the status of a number of genera is far from stabilised, as ongoing molecular phylogenetic analyses continue to highlight new and sometimes unexpected relationships.

Some of the most recent and drastic taxonomic changes have been proposed by Fuentes-Bazan et al. (2012b) following their phylogenetic study of the large genus *Chenopodium* (~200–250 species) (Fuentes-Bazan et al. 2012a) and this classification is currently accepted by many authors (e.g. Iamonico 2011, 2014; Mosyakin 2013; Uotila 2017; Sukhorukov et al. 2013; Sukhorukov and Kushunina 2014, Hernández-Ledesma et al. 2015; Mosyakin and Iamonico 2017). According to the findings by Fuentes-Bazan et al. (2012b), *Chenopodium sensu lato* was shown to be polyphyletic and members previously included in the genus are now placed in tribes Chenopodieae incl. Atripliceae (*Chenopodium* s.str. 100–150 spp., *Oxybasis* ~12 spp., *Chenopodias-*

trum 8–9 spp., *Lipandra* Moq., 1 sp.), Dysphanieae (*Dysphania* >50 spp., *Teloxys* 1 sp.) and Anserineae (*Blitum* ~12 spp.). To accomplish this, they reinstated the genera *Oxybasis* (type species *O. minutiflora* Kar. & Kir. = *O. chenopodioides* (L.) S.Fuentes, Uotila & Borsch) and *Lipandra* (type species *L. polysperma* (L.) Moq. ≡ *Chenopodium polyspermum* L.) and recognised the new genus *Chenopodiastrum* S.Fuentes, Uotila & Borsch. Finally, two of three known species of the genus *Monolepis* Schrad. included in the study (the type species *M. trifida* (Trev.) Schrad. = *M. nuttalliana* (Schult.) Greene) as well as *M. asiatica* Fisch. & C.A.Mey.) were shown to be nested within *Blitum* based on ITS (nrDNA) and *trnF* intergenic spacer with moderate statistical support (Fuentes-Bazan et al. (2012a). As *Blitum* is the oldest available name (Linnaeus 1753), *Monolepis asiatica* was transferred and *M. nuttalliana* was re-instated as *Blitum asiaticum* (Fisch. & C.A.Mey.) Fuentes et al. and *B. nuttallianum* Schult., respectively (Fuentes-Bazan et al. 2012b). The third *Monolepis* species, *M. spathulata* A.Gray, was not sequenced, but also transferred into *Blitum* [as *B. spathulatum* (A.Gray) Fuentes et al.] due to its morphological similarity to both *B. asiaticum* and *B. nuttallianum*.

Further changes were subsequently proposed by Theodorova (2014), provided without a detailed explanation, suggesting that *Blitum* should be expanded to include *Lipandra*, *Oxybasis* and *Chenopodiastrum*, resulting in the proposed new combinations *Blitum urbicum* (L.) T.A.Theodorova (≡ *Oxybasis urbica* (L.) S.Fuentes, Uotila & Borsch), *B. polyspermum* (L.) T.A.Theodorova (≡ *Lipandra polysperma* (L.) S.Fuentes, Uotila & Borsch) and *B. hybridum* (L.) T.A.Theodorova (≡ *Chenopodiastrum hybridum* (L.) S.Fuentes, Uotila & Borsch). Recently, Zhu and Sanderson (2017) described a new monotypic genus *Carocarpidium* S.C.Sanderson et C.L.Chu with the type species *C. californicum* (S.Wats.) S.C.Sanderson & G.L.Chu (≡ *Blitum californicum* S.Wats. ≡ *Chenopodium californicum* (S.Wats.) S.Wats.), based on the fruits having a fleshy pericarp.

The recent split of *Chenopodium sensu lato* into genera belonging to different tribes as suggested by Fuentes-Bazan et al. (2012b) is supported in part by morphological characters. First, all species of *Chenopodium* with obvious glandular hairs, ovoid or roundish, yellow or orange subsessile glands and simple hairs now belong to the tribe Dysphanieae (placed in either *Dysphania* R.Br. or *Teloxys* Moq.), while the remaining former *Chenopodium* (now included in Chenopodieae and Anserineae) have an indumentum of white bladder ("mealy") hairs, sometimes with scattered simple hairs (Reimann and Breckle 1988; Simón 1997; Sukhorukov et al. 2015b). The number of perianth segments was also traditionally thought to be a good diagnostic character, which usually corresponds to the number of stamens. *Chenopodium* s.str., *Lipandra* and *Chenopodiastrum* are characterised by the presence of five perianth segments and five stamens, while various genera across the subfamily are characterised by a lower number (1–4) of perianth segments and stamens, as observed in some *Oxybasis* and *Micromonolepis* (Chenopodieae), *Blitum* incl. *Monolepis* (Anserineae) and many *Dysphania* (Dysphanieae), especially amongst Australian species (e.g. Ulbrich 1934; Wilson 1984; Judd and Ferguson 1999; Holmgren 2003). However, this character may not be consistently informative as species such as *Oxybasis urbica* usually has 5 perianth segments and 5 stamens.

It has become apparent in recent years that fruit and seed characters are also useful in distinguishing members of the former *Chenopodium*, particularly amongst groups that are quite morphologically similar (Sukhorukov 2006, 2014; Sukhorukov and Zhang 2013; Sukhorukov et al. 2015a). A good example is *Chenopodium gubanovii* Sukhor. Originally this species was described as a member of the former *Chenopodium* subgen. *Blitum* sect. *Pseudoblitum* (Sukhorukov 1999). Its generic status was discussed by Fuentes-Bazan et al. (2012b) and finally resolved by Sukhorukov et al. (2013) as being a part of *Oxybasis* [*Oxybasis gubanovii* (Sukhor.) Sukhor. et Uotila] based on molecular phylogenetic data supported by morphological and seed characters. Almost all Chenopodieae (*Archiatriplex*, *Chenopodium*, *Chenopodiastrum*, *Exomis*, *Holmbergia*, *Lipandra*, *Manochlamys*, *Microgynoecium*, *Proatriplex* and all *Atriplex* with red or black seeds) possess a seed-coat testa with thickened outer cell walls impregnated with vertical or oblique stalactites and a reduced protoplast (hereafter 'stalactite seed coat') (Sukhorukov 2006; Kadereit et al. 2010; Sukhorukov and Zhang 2013; Sukhorukov 2014). There are a few exceptions, however, for example the seed coat in *Halimione* and three *Chenopodium* species endemic to Juan Fernández Archipelago (Chile) (*C. nesodendron* Skottsb., *C. sanctae-clarae* Johow, *C. sancti-ambrosii* Skottsb.), does not contain the stalactites in the outer cell walls and possesses a visible protoplast (hereafter 'non-stalactite seed coat') (Sukhorukov 2014). These three geographically isolated Chilean species are closely allied and highly unusual, as they not only possess a non-stalactite seed coat but have a tree-like habit and fruits with an apically swollen pericarp. Of these, only *C. sanctae-clarae* has been included in molecular analyses (Kadereit et al. 2010), which confirmed its phylogenetic position within this genus. The non-stalactite seed coat morphology is also evident in the Dysphanieae, *Chenopodium antarcticum* Hook.f [≡ *Oxybasis antarctica* (Hook.f.) Mosyakin], almost all *Blitum* sensu Fuentes-Bazan et al. (2012b) with the exception of *Blitum spathulatum* (A.Gray) S.Fuentes, Uotila & Borsch, or *Monolepis spathulata* (Sukhorukov 2014).

Amongst the species of the former *Chenopodium* or *Monolepis* investigated carpologically but not included in recent molecular phylogenetic studies, two taxa are of special interest. The first, *Monolepis spathulata*, is endemic to western states of USA and North Mexico and was transferred to *Blitum* (as *B. spathulatum*) due to morphological affinities with other species of the genus. The second taxon, *Chenopodium antarcticum*, is another poorly known taxon endemic to Tierra del Fuego (southernmost parts of Argentina and Chile) that still occupies a pending position within Chenopodioideae. Previously, it was described as *Blitum antarcticum* Hook.f. (Hooker 1847) and later transferred by the same author to *Chenopodium* as *C. antarcticum* (Hook.f.) Hook.f. (Bentham and Hooker 1880). The latter name was widely accepted in subsequent taxonomic treatments (Reiche 1911; Aellen 1929, 1931; Aellen and Just 1943; Moore 1983; Giusti 1984; Zuloaga and Morrone 1999). Recently, *Chenopodium antarcticum* was transferred into *Oxybasis* by Mosyakin [2013, as *O. antarctica* (Hook.f.) Mosyakin] based on its morphological similarity to other *Oxybasis*. However, the stalactite seed coat morphology of *Blitum spathulatum* and non-stalactite seed coat of *Oxybasis antarctica* contrast with those of other members of *Blitum* and *Oxybasis,* respectively (Sukhorukov 2014), which raises the question of their true phylogenetic position.

To resolve this issue, we have included these two species, in addition to several accessions of taxa sampled for the first time [*Chenopodium antarcticum*, *C. exsuccum* (C.Loscos) Uotila, *C. litwinowii* (Paulsen) Uotila, *C. foliosum* (Moench) Asch. subsp.

montanum Uotila and *Monolepis spathulata*], as well as an additional sample of *Blitum asiaticum* (Fisch. & C.A.Mey.) S.Fuentes, Uotila & Borsch. in expanded molecular analyses based on nrDNA (ITS region) and cpDNA (*atpB-rbcL* intergenic spacers + *rbcL* and *trnL-trnF* intergenic spacer + *rbcL*, hereafter as *atpB-rbcL* and *trnL-trnF*, respectively) to determine their phylogenetic position within the Chenopodioideae. Furthermore, we discuss the role of fruit and seed characters for delimitating morphologically similar but phylogenetically distant taxa and conclude with proposed taxonomic changes that reflect our findings.

Methods

Taxon sampling

Several new taxa were included in the phylogenetic analysis for the first time: *Chenopodium antarcticum* (Hook.f.) Hook.f. [≡ *Oxybasis antarctica* (Hook.f.) Mosyakin: Chile, Tierra del Fuego, December 1971, *Moore & Goodall s.n.* (LE)]; *C. exsuccum* (C.Loscos) Uotila: Algeria, Zenina, July 1968, *V.P. Boczantsev 681* (LE); *C. foliosum* (Moench) Asch. subsp. *montanum* Uotila: Iran, prov. Tehran, Elburz, June 1977, *K.-H. Rechinger 57243* (B); *C. litwinowii* (Paulsen) Uotila: Afghanistan, Parwan prov., Salang, 8 August 1969, *J.E. Carter 602* (LE); *Monolepis spathulata* A.Gray: USA, California, Susanville, August 1983, *I.Yu. Koropachinsky & al. 404* as *Monolepis nuttalliana* (MHA). Additionally, we have included a new accession of *Blitum asiaticum* (Fisch. et C.A.Mey.) S.Fuentes, Uotila et Borsch (Russia, Yakutiya, Ust-Yansky distr., August 1976, *E.V. Ter-Grigoryan 1009*, MHA). The taxa included in the molecular analyses and their GenBank accession numbers are given in the Table 1.

DNA extraction

Total genomic DNA was extracted from herbarium samples according to Krinitsina et al. (2015). Following the homogenisation of plant fragments (MiniLys, Bertin Technologies, France), total DNA was extracted using the CTAB-method (Doyle and Doyle 1987) and further purified using AMPure Beads (Beckman Coulter, USA).

PCRs for two chloroplast markers (*atpB-rbcL* and *trnL-trnF*) and nrDNA (ITS region) were carried out in a Thermal Cycler T100 (Bio-Rad, USA) using primers and cycler programmes listed in Table 2. A 10 ng aliquot of DNA was used to make a 25 µl total volume reaction, containing 1 µM of each primer, 200 µM of each dNTP and 0.5 U Encyclo polymerases (Evrogen, Russia). PCR products were checked on 1.2% agarose gels and purified using AMPure Beads (Beckman Coulter, USA) according to the owner's manual. AMPure Beads suspension was mixed with a solution containing PCR-product ratio 1 vol. PCR-mix: 1.2 vol. AMPure Beads for *atpB-rbcL* and ITS primer pairs and 1 vol. PCR-mix: 1.4 vol. AMPure Beads for *rbcL*, Tab C/Tab D and Tab E/Tab F primer pairs.

Table 1. Voucher information and GenBank accession numbers for the species of Chenopodioideae and outgroups included in the phylogenetic analysis (arranged in alphabetical order). The newly sequenced samples are highlighted in bold. Some vouchers in GenBank may be stored under old names.

Species	Old names (if applicable)	GenBank accession number			
		ITS	*rbcL*	*trnL-trnF*	*atpB-rbcL*
Atriplex hortensis	–	HM005854	KX678160	HE577500	–
Atriplex patula	–	HE577358	MG249776	HE577498	HM587650
Atriplex spongiosa	–	–	AY270060	–	HM587661
Atriplex undulata	–	–	AY270061	–	HM587665
Atriplex phyllostegia	–	HM005870	HM587590	–	HM587651
Atriplex peruviana	–	HM005867	–	–	–
Atriplex watsonii	–	HM005871	–	–	–
Atriplex rusbyi	–	HM005865	–	–	–
Atriplex patagonica	–	HM587541	–	–	–
Atriplex lentiformis	–	HM005872	–	–	HM587637
Atriplex cinerea	–	HM587491	–	–	–
Atriplex centralasiatica	–	DQ086481	HM587583	–	HM587621
Atriplex suberecta	–	HM005863	–	–	–
Axyris amaranthoides	–	AM849227	KX678411	HE577510	–
Axyris hybrida	–	HE577371	–	HE577511	–
Blitum antarcticum	*Chenopodium antarcticum* (*Oxybasis antarctica*)	**MH155315**	**MH632743**	**MH632745**	**MH152573**
Blitum asiaticum	*Monolepis asiatica*	**MH150882**	**MH731231**	**MH731229**	–
Blitum bonus-henricus	*Chenopodium bonus-henricus*	HE577372	KF613023	HE577512	HM587670
Blitum californicum	*Chenopodium californicum*	HE577376	MF963177	HE577516	–
Blitum capitatum	*Chenopodium capitatum*	KJ629064	MG249277	HE577513	–
Blitum litwinowii	*Chenopodium litwinowii*	**MH153781**	**MH632744**	**MH632746**	**MH632749**
Blitum nuttallianum	*Monolepis nuttalliana*	HE577375	JX848452	HE577515	HM587702
Blitum petiolare	*Chenopodium exsuccum*	**MH150883**	–	**MH632747**	**MH152574**
Blitum virgatum L.	*Chenopodium foliosum*	JF976147	AY270081	HE577518	HM587673
Blitum virgatum* subsp. *montanum	*Chenopodium foliosum* subsp. *montanum*	**MH155242**	–	–	–
Ceratocarpus arenarius	–	AY556430	HM587594	HE577505	–
Chenopodiastrum coronopus	*Chenopodium coronopus*	HE577403	HM587595	HE577543	HM587671
Chenopodiastrum hybridum	*Chenopodium hybridum*	HE577530	–	HE577530	–
Chenopodiastrum murale	*Chenopodium murale*	HE577392	HM849890	HE577531	HM587675
Chenopodium album	–	JF976146	JF941270	HE577609	MF073794
Chenopodium atrovirens	–	KP226648 /	KX679232	HE577587	–
Chenopodium auricomum	–	KP226671	–	–	–
Chenopodium bengalense	*Chenopodium giganteum*	HE577458	–	–	–
Chenopodium berlandieri var. *boscianum*	–	HE577426	MG249740	HE577564	–
Chenopodium berlandieri var. *zschackei*	–	HE577425	–	–	–
Chenopodium desertorum	–	HE577417	AY270042	HE577555	HM587672
Chenopodium desiccatum	–	HE577412	KX678128	HE577550	–
Chenopodium ficifolium	–	HE577466	KM360714	HE577606	–
Chenopodium fremontii	–	HE577408	KX679065	HE577572	–
Chenopodium hians	–	HE577470	MG248000	HE577610	–
Chenopodium iljinii	–	HE577468	–	–	–
Chenopodium incanum	–	HE577410	MG246401	HE577548	–
Chenopodium leptophyllum	–	HE577428	MG248863	HE577566	–
Chenopodium neomexicanum	–	KJ629054	–	–	–
Chenopodium nevadense	–	HE577411	–	–	–
Chenopodium opulifolium	–	HE577454	MG248036	HE577594	–
Chenopodium pallescens	–	HE577409	–	–	–
Chenopodium pallidicaule	–	KJ629055	–	–	–
Chenopodium nutans	*Einadia nutans*	–	KM896090	–	HM587686
Chenopodium parabolicum	*Rhagodia parabolica*	–	KU564859	–	HM587704
Chenopodium quinoa	–	HE577443	KY419706	–	KY419706

Species	Old names (if applicable)	GenBank accession number			
		ITS	*rbcL*	*trnL-trnF*	*atpB-rbcL*
Chenopodium standleyanum	–	KJ629051	MG249838	HE577560	–
Chenopodium subglabrum	–	HE577465	MG249459	HE577605	–
Chenopodium vulvaria	–	HE577407	JN892907	HE577591	–
Chenopodium watsonii	–	HE577462	MG246238	HE577602	–
Cycloloma atriplicifolium	–	HQ218998	HM587598	–	HM587681
Dysphania ambrosioides	*Chenopodium ambrosioides*	DQ005963	MG249540	HE577493	HM587682
Dysphania botrys	*Chenopodium botrys*	KJ629068	MG247946	DQ499383	HM587683
Dysphania cristata	*Chenopodium cristatum*	KJ629066	AY270046	–	HM587684
Dysphania glomulifera	*Chenopodium glomuliferum*	–	AY270086		HM587685
Dysphania pumilio	*Chenopodium pumilio*	HE577343	MG248652	HE577485	–
Dysphania schraderiana	*Chenopodium schraderianum*	HE577349	–	–	–
Exomis microphylla	–	–	HM587601	–	HM587687
Grayia brandegeei	–	HM005845	HM587604	HE577497	HM587690
Grayia spinosa	–	HM005844	HM587605	HE577496	HM587691
Halimione verrucifera	*Atriplex verrucifera*	HM587575	HM587606	–	HM587695
Halimione pedunculata	*Atriplex pedunculata*	HM587573	AY270093	–	HM587694
Holmbergia tweedii	–	HM005842	AY270100	–	HM587696
Krascheninnikovia ceratoides	–	HE577367	AY270105	HE577507	HM587697
Krascheninnikovia ceratoides subsp. *lanata*	*Krascheninnikovia lanata*	HE577368	MG248963	HE577508	HM587698
Lipandra polysperma	*Chenopodium polyspermum*	KJ629061	KX677934	HE855686	–
Micromonolepis pusilla	–	–	HM587608	–	HM587701
Neomonolepis spathulata	*Monolepis spathulata* (*Blitum spathulatum*)	**MH675518**	**MH731232**	**MH731230**	**MH152575**
Oxybasis glauca	*Chenopodium glaucum*	KJ629060	MG249300	HE577527	MF073807
Oxybasis rubra	*Chenopodium rubrum*	HE577381	MG249329	HE577525	–
Oxybasis urbica	*Chenopodium urbicum*	KJ629057	MG246691	HE577524	HM587678
Oxybasis micrantha	–	KU359325	–	–	–
Spinacia oleracea	–	EU606218	–	AJ400848	–
Suckleya suckleyana	–	HE577347	–	–	–
Teloxys aristata	*Chenopodium aristatum*; *Dysphania aristata*	KJ629070	AY270140	–	HM587708
Outgroups	–				
Bassia laniflora	*Kochia laniflora*	KF785942	–	–	–
Bassia prostrata	*Kochia prostrata*	KF785963	AY270104	HE577478	KF785926
Beta vulgaris	–	AY858597	–	–	DQ074969
Hablitzia tamnoides	–	AY858590	AY270092	HE577475	JQ407841
Polygonum aviculare	–	–	MF158792	HQ843161	JN234937
Polygonum aviculare subsp. *buxiforme*	–	GQ339988	–	–	–

Table 2. Primers and cycler programmes used for the molecular analysis.

Marker	Primer sequences and combination	Reference	Cycler programmer
ITS	ITS5 5'-GGA AGT AAA AGT CGT AAC AAG G-3'	White et al. (1990)	95 °C for 5 min, 33 cycles of amplification (95 °C for 15 s, 55 °C for 30 s, 72 °C for 40 s), 72 °C for 5 min
	ITS4 5'-TCC TCC GCT TAT TGA TAT GC-3'		
rbcL (partial)	rbcLaF 5'- ATG TCA CCA CAA ACA GAG ACT AAA GC-3'	Levin et al. (2003)	95 °C for 5 min, 35 cycles of amplification (95 °C for 10 s, 55 °C for 30 s, 72 °C for 40 s), 72 °C for 5 min
	rbcLaR 5'-GTA AAA TCA AGT CCA CCR CG-3'	Kress et al. (2009)	
atpB-rbcL spacer	atpB-rbcL F 5'-GAA GTA GTA GGA TTG ATT CTC-3'	Golenberg et al. (1993)	95 °C for 5 min, 35 cycles of amplification (95 °C for 20 s, 56 °C for 30 s, 72 °C for 60 s), 95 °C for 20 s, 56 °C for 80 s, 72 °C for 8 min
	atpB-rbcL R 5'-CAA CAC TTG CTT TAG TCT CTG-3'		
trnL-F	Tab C 5'-CGA AAT CGG TAG ACG CTA CG-3' Tab D 5'-GGG GAT AGA GGG ACT TGA AC-3' Tab E 5'- GGT TCA AGT CCC TCT ATC CCC-3' Tab F 5'ATI' TGA ACT GGT GAC ACG AG 3'	Taberlet et al. (1991)	95 °C for 5 min, 35 cycles of amplification (95 C for 1 min, 50 °C – 65 °C (increasing in 0.3 C per cycle) for 1 min, 72 °C for 4 min), 72 °C for 5 min

Sequencing and alignment

Sequencing was performed following Sanger methods on an Applied Biosystems 3730 DNA Analyser using ABI PRISM BigDye Terminator v. 3.1 (Center of Collective Use "Genome", Institute of Molecular Biology, Moscow, Russia). The sequencing primers were the same as the amplification primers.

The raw forward and reverse sequences were checked and combined in BioEdit sequence alignment editor v. 7.0.5.3 (Hall 1999). Sequences were edited and aligned using Muscle 3.6 (Edgar 2004). The obtained alignments were manually edited using PhyDe (version 0.9971: Müller et al. 2010) following the rules outlined in Löhne and Borsch (2005). Mutational hotspots (regions of uncertain homology) were excluded from the analysis (Borsch et al. 2003). Gaps were treated as missing data during the phylogenetic inference.

Phylogenetic inference

To show the relationships between taxa, we reconstructed various phylogenies using Bayesian analysis, maximum likelihood (ML) and maximum parsimony (MP) methods for the ITS and combined *trnL-trnF* + *rbcL* + *atpB-rbcL* datasets. Models of nucleotide substitution were selected using the MrModeltest 2.1.7 (Nylander 2004) via the Akaike information criterion (AIC: Akaike 1974). The substitution model was set to GTR + G + I. For the ML analyses, we employed RAxML Version 8 (Stamatakis 2014). Bootstrap analyses were conducted with 2500 replicates for ML. Parsimony analyses were conducted in PAUP* 4.0a162 (Swofford 2002) with the following settings: all characters have equal weight, MaxTrees set to 1000 (auto increased by 1000), TBR branch swapping and with 20000 jackknife (JK) replicates to calculate node support. Bayesian analyses were conducted in BEAST 2.5.0 (Bouckaert et al. 2014). Four Markov Chain Monte Carlo analyses with four chains were run for 20 million generations for every dataset, sampling every 1000 generations. Burn-in was set to remove 5% of the total trees sampled after assessing likelihood convergence by inspection of the trace plots in the programme Tracer v.1.6 (Rambaut et al. 2014). A birth and death prior was chosen for branch lengths (Gernhard 2008). The maximum clade credibility tree was calculated in the programme TreeAnnotator v1.4.8 (Drummond and Rambaut 2007) with a posterior probability limit of 0.7. Final trees were edited in the programme TreeGraph ver. 2.14.0 (Stöver and Müller 2010).

Morphology and anatomy

The carpology of the tribe Chenopodioideae was described in detail in a previous study by Sukhorukov (2014). In this study, we pay particular attention to the fruit and seed of *Chenopodium antarcticum* and to the general structure of the reproductive shoot of *Monolepis spathulata* that were not illustrated in Sukhorukov (2014). The samples were observed using a scanning electron microscope (SEM) JSM–6380 (JEOL Ltd., Japan) at 15 kV after sputter coating with gold-palladium in the laboratory of Electron Microscopy at Lomonosov Moscow State University. Prior to SEM, the fruits were dehydrated in aqueous ethyl alcohol solutions of increasing concentration, followed by alcohol-acetone solutions and pure acetone. No dehydration of the seeds is required prior to SEM observation due to the absence of soft tissues (e.g. papillae or trichomes) on their surface.

The cross-sections of the seeds were prepared using a rotary microtome Microm HM 355S (Thermo Fisher Scientific, USA) and then examined using a Nikon Eclipse Ci (Nikon Corporation, Japan) light microscope and photographed using a Nikon DS-Vi1 camera (Nikon Corporation, Japan) at the Department of Higher Plants, Lomonosov Moscow State University. Before sectioning, the seeds were soaked in water:alcohol:glycerine (1:1:1) solution, dehydrated in ethanol dilution series and embedded in the Technovit 7100 resin (Heraeus Kulzer, Germany).

Results

Phylogenetic analysis

The phylogenetic analysis based on nrDNA (ITS) and combined cpDNA analyses (*trnL-trnF* + *rbcL* + *atpB-rbcL*) revealed that the tribes Axyrideae, Chenopodieae s.str., Anserineae and Dysphanieae are well-supported within Chenopodioideae and congruent with previous molecular analyses by Fuentes-Bazan et al. (2012b) (Figures 1–2). The results outlined below focus on the phylogenetic position of the newly included taxa *Chenopodium antarcticum* [≡ *Oxybasis antarctica*], *C. litwinowii*, *C. exsuccum*, *C. foliosum* subsp. *montanum* and *Monolepis spathulata*.

In the ITS analysis (Figure 1), the tribe Axyrideae is placed sister to the remaining Chenopodioideae. The next diverging lineage is a well-supported Dysphanieae, with *Monolepis spathulata* + *Teloxys* forming a sister lineage to the remaining representatives of the tribe. *Chenopodium antarcticum*, *C. litwinowii*, *C. exsuccum* and *C. foliosum* subsp. *montanum* fall well within *Blitum*, which is sister to a well-supported Chenopodieae. *Blitum californicum* and *B. bonus-henricus* (L.) C.A.Mey. form part of the polytomy with the rest of the genus.

Like the ITS phylogenetic analysis, the combined *trnL-trnF* + *rbcL* + *atpB-rbcL* tree (Figure 2) shows the Axyrideae as an early branching lineage in Chenopodioideae, sister to a polytomy of Dysphanieae, Anserineae and Chenopodieae. Within the Dysphanieae, *Monolepis spathulata* and *Teloxys* form a polytomy with the remaining representatives of the tribe, which includes *Cycloloma* nested within *Dysphania*. *Chenopodium antarcticum*, *C. litwinowii* and *C. exsuccum* are nested within *Blitum* (*C. foliosum* subsp. *montanum* is not included in the combined tree). *Chenopodium antarcticum* is sister to *Chenopodium exsuccum* + *C. litwinowii* – *Blitum virgatum*.

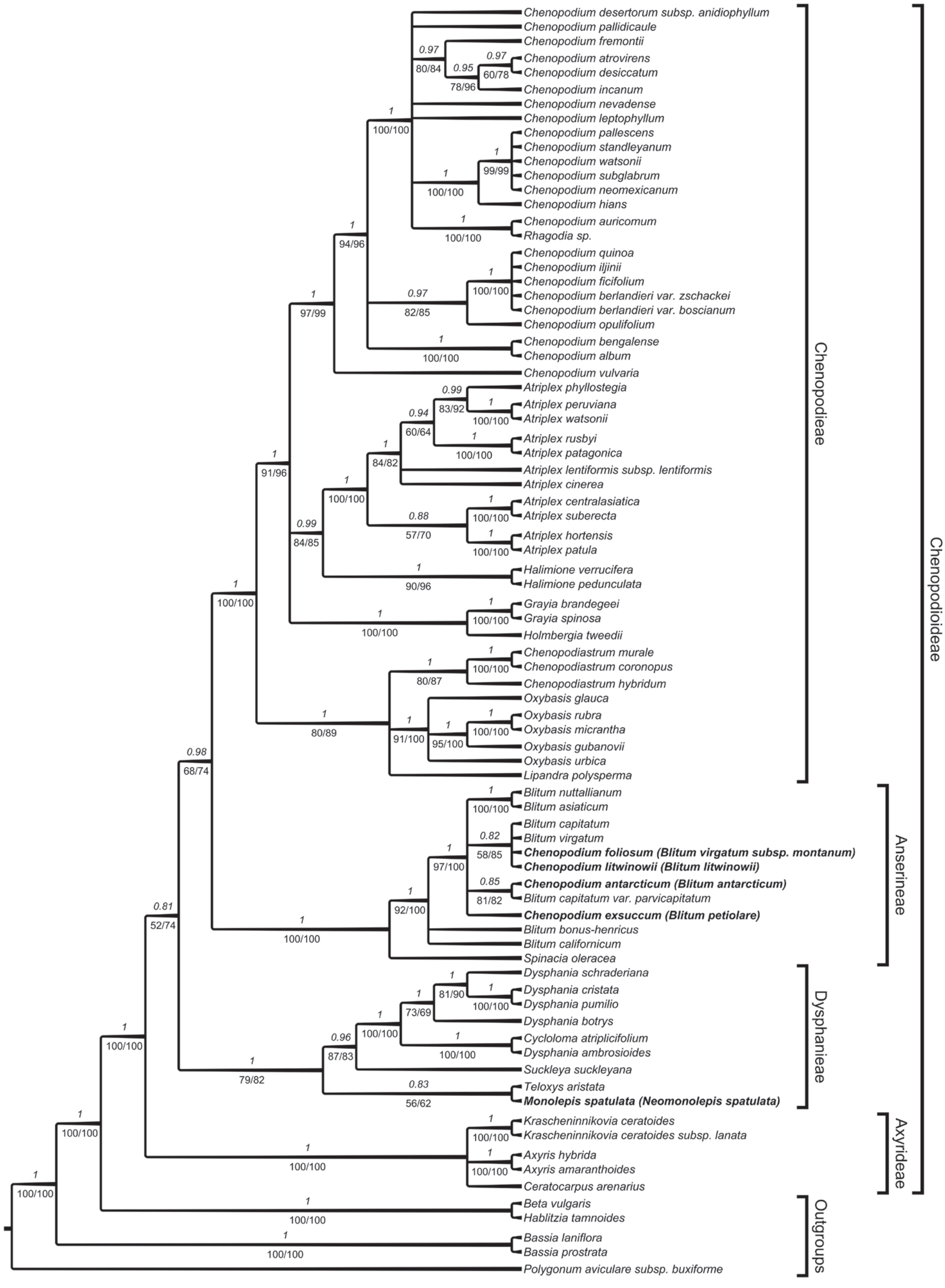

Figure 1. Best tree from the BEAST analysis of the ITS Chenopodioideae dataset. Bayesian posterior probabilities are given above the branches, jackknife values (left) and bootstrap percentages of the maximum likelihood analyses (right) are given below branches.

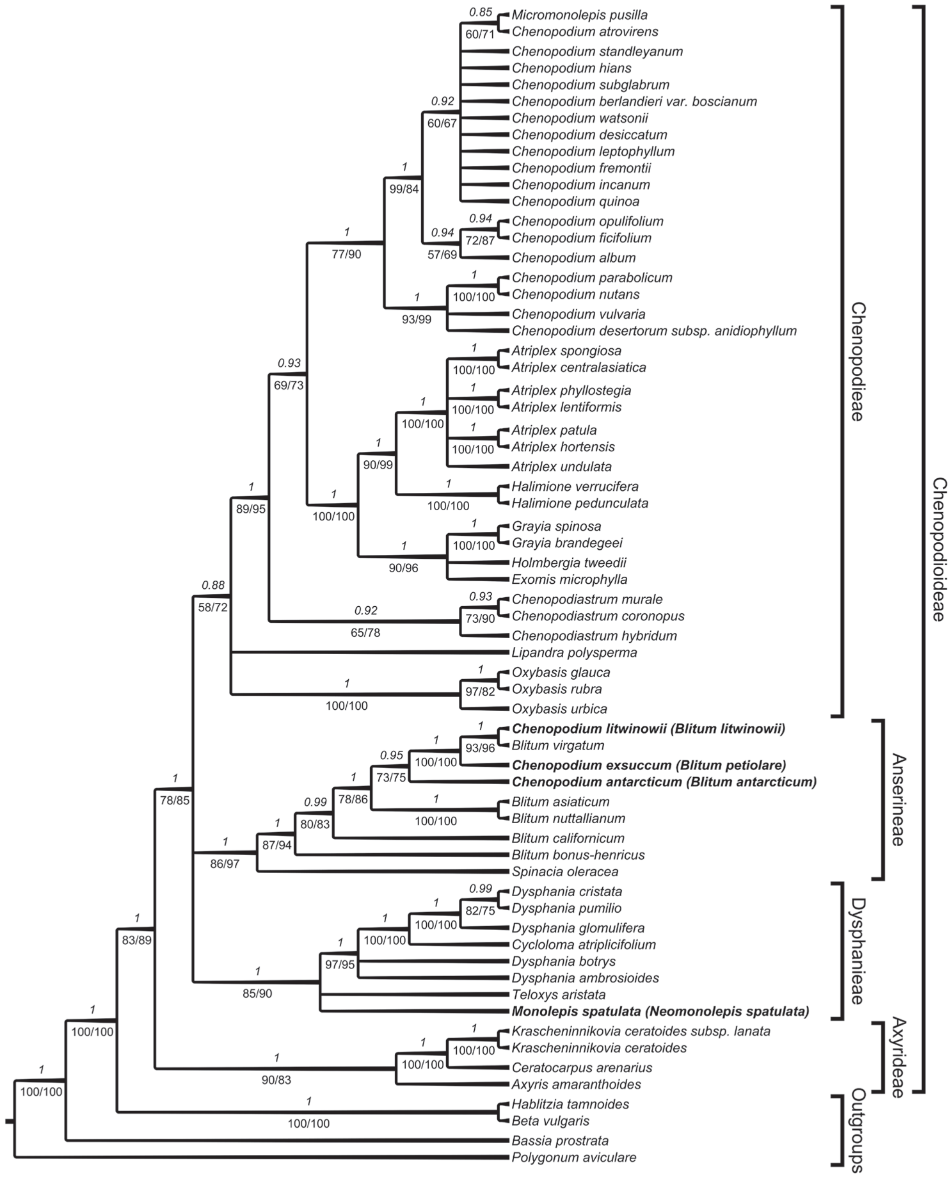

Figure 2. Best tree from the BEAST analysis of the combined *trnL-trnF* + *rbcL* + *atpB-rbcL* Chenopodioideae dataset. Bayesian posterior probabilities are given above the branches, jackknife values (left) and bootstrap percentages of the maximum likelihood analyses (right) are given below branches.

Carpological studies

This study highlighted the fact that these species, with the exception of *Monolepis spathulata*, possess the same fruit and seed anatomy as other *Blitum* species such as a mamillate pericarp (Figure 3) and non-stalactite seed-coat with obvious (visible) protoplast (Table 3; Figure 4). In contrast, the carpology of *Monolepis spathulata* somewhat resembles the morphology observed in species of *Oxybasis* and many other Chenopodieae in having a papillate pericarp and a stalactite seed coat with a highly reduced protoplast (Figure 5). Other important characters such as life history, the degree of fusion of reduced perianth segments, pericarp structure and adherence, the colour, shape and morphology of seeds and an embryo position, are recorded for representative species of each genus, as summarised in Table 3.

Discussion

The phylogenetic position of *Chenopodium foliosum* subsp. *montanum* [≡ *Blitum virgatum* L. subsp. *montanum* (Uotila) S.Fuentes, Uotila et Borsch], *C. exsuccum* [= *Blitum petiolare* Link] and *C. litwinowii* [≡ *B. litwinowii* S.Fuentes, Uotila et Borsch] within *Blitum* as proposed by Fuentes-Bazan et al. (2012b) was supported by the findings of

Figure 3. Pericarp of *Blitum antarcticum*. Scale bar: 200 µm.

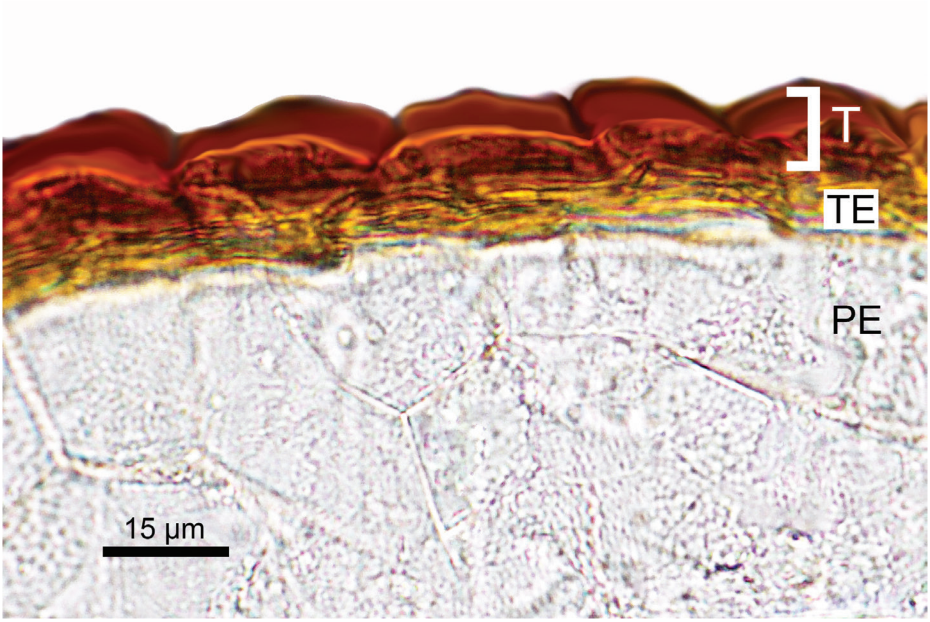

Figure 4. Cross-section of the seed of *Blitum antarcticum*. Abbreviations: T – testa, TE- tegmen, PE – perisperm.

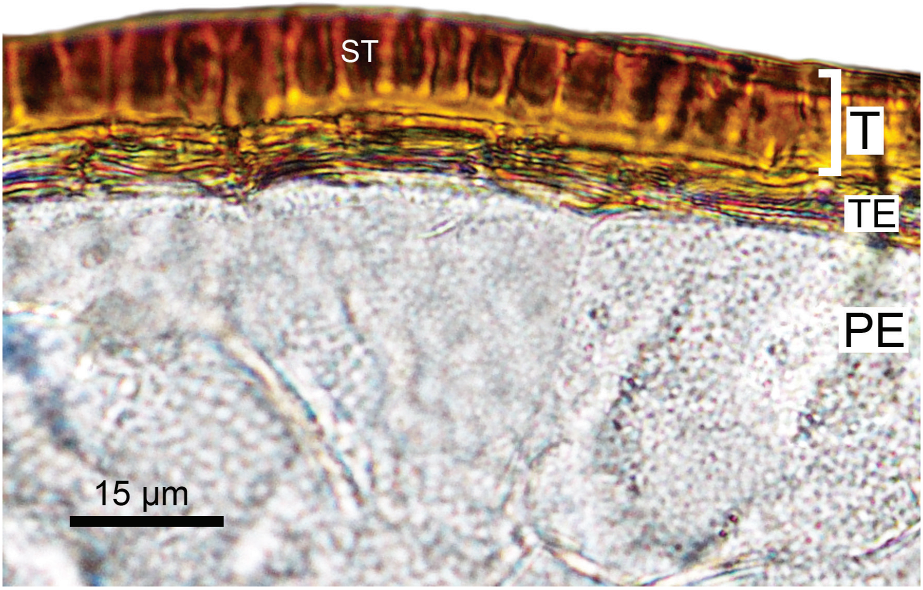

Figure 5. Cross-section of the seed of *Neomonolepis spathulata*. Abbreviations: T – testa, TE – tegmen, PE – perisperm, ST – stalactites in the outer walls of the testa cells.

Table 3. Additional noteworthy characters evolved in *Blitum* and *Oxybasis*. This table summarises life history and carpological data from Sukhorukov and Zhang (2013), Sukhorukov et al. (2013), Sukhorukov (2014), with additional information included for *Blitum virgatum* subsp. *montanum* and *B. korshinskyi*.

Taxon/Character	Life history	Perianth segments	Cells of the outer pericarp layer	Pericarp adherence to the seed coat	Seed shape and colour	Seed surface	Seed keel	Thickness of seed-coat testa (µm)	Acicular outgrowths of the testa cells	Presence of spatial hetero-spermy	Seed embryo position
Blitum antarcticum	short-lived perennial herb	basally connate	spongy	scraped off the seed	roundish, red	alveolate	–	12–20	–	–	vertical
B. asiaticum	annual	free	not spongy	easily ruptured	roundish, red	undulate	+	7–10	–	–	vertical
B. atriplicinum	annual or short-lived perennial herb	basally connate	not spongy	hardly removed	roundish, red	alveolate, with hairy-like outgrowths	–	17–25	+	–	vertical
B. bonus-henricus	perennial herb	basally connate	spongy	scraped off the seed	roundish, red	smooth	–	37–45	–	+	vertical, rarely horizontal
B. californicum	perennial herb	basally connate	spongy	scraped off the seed	roundish, red	alveolate	–	25–30 and 37–45 (heterospermous)	–	+	vertical
B. capitatum	annual or short-lived perennial herb	basally connate	not spongy	hardly removed	ovate, red	undulate	+ (two keels and a groove between them)	12–15	–	+	vertical
B. hastatum	annual or short-lived perennial herb	connate to 1/3	not spongy	hardly removed	ovate, red	undulate	+ (two keels and a groove between them	15–18	–	+	vertical
B. korshinskyi	annual or short-lived perennial herb	almost free	not spongy	hardly removed	ovate, red	undulate	+ (two keels and a groove between them)	10–12	–	–	vertical
B. litwinowii	annual or short-lived perennial herb	basally connate	not spongy	hardly removed	ovate, red	alveolate	+ (two keels and a groove between them)	10–12	–	–	vertical
B. nuttallianum	annual	free, or perianth absent	not spongy	hardly removed	roundish, red	alveolate, with hairy-like outgrowths	–	8–10	+	–	vertical
B. petiolare	annual or short-lived perennial herb	basally connate	not spongy	hardly removed	ovate, red	alveolate	+ (two keels and a groove between them)	15–17	–	–	vertical
B. virgatum	annual or short-lived perennial herb	basally connate	not spongy	hardly removed	ovate, red	undulate	+ (two keels and a groove between them)	10–12	–	+	vertical

Taxon/Character	Life history	Perianth segments	Cells of the outer pericarp layer	Pericarp adherence to the seed coat	Seed shape and colour	Seed surface	Seed keel	Thickness of seed-coat testa (µm)	Acicular outgrowths of the testa cells	Presence of spatial hetero-spermy	Seed embryo position
Oxybasis chenopodioides	annual	fused in almost all flowers, free only in some flowers	not spongy	easily ruptured	roundish, red	minutely pitted	–	10–15	+		vertical and horizontal
O. glauca	annual	basally connate	not spongy	easily ruptured	roundish, red	minutely pitted	–	10–15 and 17–25 (heterospermous)	+	+	vertical and horizontal
O. gubanovii	annual	basally connate	not spongy	hardly removed	roundish, red	smooth (minutely pitted)	+ (one keel)	12–15	+	–	vertical
O. macrosperma	annual	connate to the middle or almost to the top	spongy	scraped off the seed	roundish, red	reticulate with minutely pitted dots	–	12–20	+	–	vertical and horizontal
O. mexicana	annual	basally connate	not spongy	easily ruptured	roundish, red	reticulate with minutely pitted dots	–	20–25	+	+	vertical and horizontal
O. micrantha	annual	basally connate	not spongy	scraped off the seed	roundish, red	minutely pitted	+ (one keel)	12–15	+	–	horizontal, rarely vertical
O. rubra	annual	basally connate	not spongy	easily ruptured	roundish, red	reticulate with minutely pitted dots	–	10–15	+		vertical and horizontal
O. urbica	annual	basally connate	papillate	scraped off the seed	roundish, black	minutely pitted	–	42–50	+	–	horizontal

this study. Indeed, the results were predictable due to the shared morphological and carpological affinities of these species to *B. virgatum*, such as the presence of a leaf rosette, tight adherence of the pericarp to the seed coat and the ovoid and keeled seeds having the same anatomical structure (e.g. Uotila 1993, 1997; Sukhorukov 2014). For this reason, while *Chenopodium korshinskyi* (Litv.) Minkw. has not been included in any molecular phylogenies to date, it should be treated as *Blitum korshinskyi* Litv. (Fuentes-Bazan et al. 2012b) due to the shared presence of these diagnostic traits. It is also evident, based on phylogenetic and carpological data from this study, that *Oxybasis antarctica* (formerly *Chenopodium antarcticum*) must be treated as *Blitum antarcticum* as proposed by Hooker (1847). Moreover, as *Oxybasis antarctica* is the type of *Oxybasis* sect. *Thellungia* (Aellen) Mosyakin [including *Oxybasis antarctica* and *O. erosa* (R.Br.) Mosyakin: Mosyakin 2013], this section may be recognised within *Blitum* but this requires further exploration as the phylogenetic position of *B. antarcticum* remains equivocal.

Diagnostic characters for *Blitum* and *Oxybasis*

The importance of morphological characters used to delineate species within the genus *Chenopodium* that are now considered to belong to either *Blitum* or *Oxybasis* have been discussed by various authors (e.g. Moquin-Tandon 1840, 1849; Aellen and Just 1943; Scott 1978; Fuentes-Bazan et al. 2012b). However, the morphological similarity of some species has led to taxonomic confusion. For example, many macromorphological characters overlap in *Blitum* and *Oxybasis*, including previous diagnostic traits such as: reduced (1–4) number of perianth segments, presence of the vertical seed embryo position and emergence of spatial heterospermy. Such characters are clearly homoplastic in Chenopodieae, Anserineae and some other groups of the Chenopodioideae (Sukhorukov and Zhang 2013). Only one trait visible to the naked eye, the presence of leaf rosette in *Blitum* (Figure 6) and its absence in *Oxybasis*, can be used for the delimitation of both genera (see diagnostic key and generic descriptions in Fuentes-Bazan et al. 2012b). However, it should be noted that the leaf rosette in some *Blitum*, especially in species previously included in *Monolepis* (*B. asiaticum*, *B. nuttallianum*), is reduced to 1–2 leaves that may wither away completely by anthesis. From this study and from previous work (Sukhorukov and Zhang 2013; Sukhorukov et al. 2013; Sukhorukov 2014), it is evident that another character, the structure of the testa cells of the seed coat, is also diagnostic. In *Oxybasis*, as well as almost all other Chenopodieae, the seed testa cells have a reduced protoplast and "stalactites" hanging vertically in the outer wall (stalactite seed coat). In contrast, the presence of non-stalactite seed coat with a highly visible protoplast, unambiguously distinguishes *Blitum*. Other characters, such as reduced perianth segments, mamillate pericarp, red seeds, seed keel, vertical embryo position of note for representative species of each genus, are summarised in Table 3 and they play a role for the diagnostics at the species level or species group (see Sukhorukov 2014 for further detail).

Figure 6. Habit of *Blitum asiaticum* showing the senescing leaf rosette. Photographer: Igor Pospelov (Russia, Krasnoyarsk prov., Taymyr, Khatanga, August 2014).

In the absence of molecular phylogenetic data, it is clear that carpological characters must be taken into consideration when determining the generic placement of taxa in either *Blitum* or *Oxybasis*. Molecular data from this study and previous investigations (Kadereit et al. 2010; Fuentes-Bazan et al. 2012a, 2012b), when examined in conjunction with carpological evidence (Sukhorukov 2014), show that two taxonomic changes recently proposed: (1) the merger of *Oxybasis*, *Lipandra* and *Chenopodiastrum* (Chenopodieae) into an extended *Blitum* (Anserineae) as suggested by Theodorova (2014) and (2) the description of a new monotypic genus *Carocarpidium* S.C.Sanderson et G.L.Chu with the type *C. californicum* (≡*Blitum californicum*) by Zhu and Sanderson (2017), cannot be accepted. Additionally, it should be noted that the pericarp of *B. californicum* is not fleshy as previously described (Zhu and Sanderson 2017), but its outer layer consists of spongy (mamillate) cells that imitate a "fleshy" pericarp. This type of mamillate pericarp is present in some *Blitum* and *Oxybasis* (Figure 3, see also Table 3) and so this character is clearly not unique to *Carocarpidium*.

Micromonolepis pusilla

This species was initially described as *Monolepis pusilla* Torr. ex Watson (Watson 1871) and it is noteworthy to consider its morphology and phylogenetic position in context with

Figure 7. Shoot of *Micromonolepis pusilla* showing the characteristic fleshy leaves. Photographer: Steve Matson (USA, California, Mono County, Long Valley, 2007).

other species previously known as *Monolepis*. It is a small annual herb covered with bladder hairs that has fleshy leaves (Figure 7), unisexual flowers with reduced (1–3) perianth segments and tiny papillate fruits. Due to its unusual habit, *M. pusilla* was transferred into a new monotypic genus *Micromonolepis* (Ulbrich 1934). The species was included in a *atpB-rbcL* molecular analysis, where it was unexpectedly placed within the "Chenopodieae I" clade comprising *Rhagodia*, *Einadia* and a part of *Chenopodium* s.l. (Kadereit et al. 2010). The papillate pericarp and the stalactite seed coat provide a good support for its placement into Chenopodieae, based on cpDNA being a part of *Chenopodium* s.str. (Kadereit et al. 2010, as Chenopodieae I; Figure 2). However, the limited number of taxa used in the *atpB-rbcL* analysis, the lack of additional molecular data and the significant morphological differences evident between *Micromonolepis* and the remaining *Chenopodium* species in this clade, such as the presence of fleshy leaves and reduced perianth segments, precludes the formal transfer of *M. pusilla* to *Chenopodium*. Further work is needed to evaluate the exact position of *Micromonolepis pusilla* within Chenopodieae.

Monolepis spathulata is neither *Monolepis* nor *Blitum*

Recently, *Monolepis spatulata* was transferred to *Blitum* (as *B. spathulatum*) based on its resemblance to other species of the genus due to the presence of a reduced number of perianth segments (Fuentes-Bazan et al. 2012b). It is evident, however, that the reduced number of perianth segments independently evolved in Chenopodieae (e.g. in *Micromonolepis* and some *Oxybasis*), Anserineae and many Dysphanieae (Sukhorukov and Zhang 2013). In light of carpological evidence (Sukhorukov 2014), it seemed doubtful that *M. spathulata* should be included in *Blitum*, as this species possesses a stalactite seed coat with a reduced protoplast. Our phylogenetic results show that *Monolepis spathulata* is not closely related to the other species in *Monolepis* (*M. asiatica*, and *M. nuttalliana*) that are now included in *Blitum* (Anserineae) as *B. asiaticum* and *B. nuttallianum,* respectively. This species falls within Dysphanieae forming a polytomy with *Teloxys* and *Dysphania* + *Cycloloma*. *M. spathulata* is a glabrous annual and differs from all Dysphanieae by the absence of simple hairs and subsessile glands that are diagnostic characters of this tribe. Additionally, *M. spathulata* is found to have the stalactite seed coat, a character missing in all Dysphanieae (Sukhorukov 2014). The close relationship between *M. spathulata* and the Dysphanieae, evidenced by molecular data, is unexpected given the obvious morphological and carpological differences. Indeed, *M. spathulata* is considered so distinct that it warrants recognition at the generic level. As the type for *Monolepis*, *M. trifida* (Trev.) Schrad. [= *M. nuttalliana* (Schult.) Greene], is synonymised within *Blitum* (as *Blitum nuttallianum*), a new name is required for *Monolepis spathulata*. As such, a new monotypic genus named *Neomonolepis* Sukhor., gen. nov. is established here.

Taxonomy

***Neomonolepis* Sukhor., gen. nov.**
urn:lsid:ipni.org:names:77191294-1

Type species. *Neomonolepis spathulata* (A.Gray) Sukhor., comb. nov.

Description. Annual, glabrous, branched or not; lateral branches if present ascending; leaves cauline (rosulate leaves absent), densely located, spatulate-oblong, with a short petiole up to 1 cm or sessile, entire; inflorescence leafy (bracts similar to stem leaves); flowers sessile or shortly pedicellate, unisexual intermixed in small glomerules (Figure 8); male flowers with 2-lobed hyaline perianth, stamens 1–2, anthers 0.10–0.15 mm long; female flowers without perianth, fruits 0.55–0.65 mm in diameter, almost round, with blackish papillate pericarp (when dry) that is easily raptured, styles 2(3); seeds 0.4 × 0.3 mm, reddish, with smooth surface, with small irregular pits (seen at a higher magnification), seed-coat testa with stalactites in the outer cell walls and reduced protoplast; embryo vertical.

***Neomonolepis spathulata* (A.Gray) Sukhor., comb. nov.**
urn:lsid:ipni.org:names:77191295-1

≡ *Monolepis spathulata* A.Gray, Proc. Amer. Acad. Arts 7: 389 (1868). Lectotype (Sukhorukov, designated here): [USA, California, Sierra Nevada], Mono Pass, 1866, *H.N. Bolander 6373* lower right-hand specimen (GH00037208 [image]!,

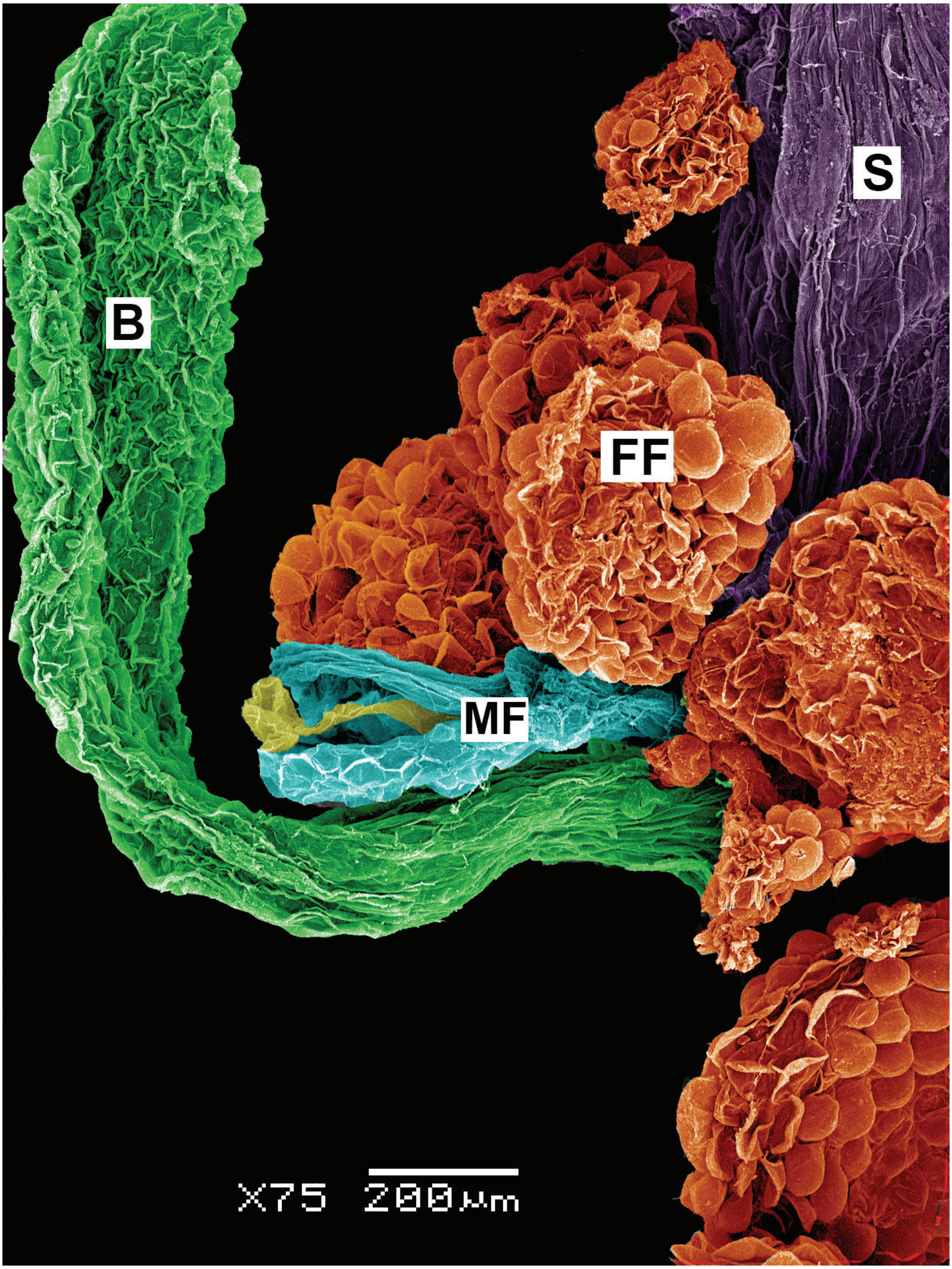

Figure 8. SEM detail of the inflorescence of *Neomonolepis spathulata*. Abbreviations: B – bract (stained in green), FF – female flowers (orange), MF –male flower (perianth stained in blue, stamen in yellow), S – stem.

isolectotypes MO-216255 [image]! NY01085540 [image]! US00921387 [image]! YU064591 [image]!).

≡ *Blitum spathulatum* (A.Gray) S.Fuentes, Uotila et Borsch, Willdenowia 42(1): 17 (2012).

Morphological notes. As *Neomonolepis* is a monotypic genus, the description of *N. spathulata* corresponds to the generic description above. *Neomonolepis spathulata* is morphologically distant from all Dysphanieae (*Teloxys*, *Suckleya* A.Gray, *Dysphania* R.Br. and *Cycloloma* Moq.) in being glabrous in all parts (vs. glandular and/or simple hairs), having unisexual flowers (vs. bisexual or polygamous) and 'stalactite' seed-coat testa (vs. 'non-stalactite'). For this reason, we prefer to refer to the clade with the abovementioned genera as the 'Dysphanieae + Neomonolepis' clade.

Typification. The type specimen lodged at GH contains several plants collected from different areas in California and almost all of them were collected after the description of *Monolepis spathulata* (Gray 1868). The lectotype selected here (lower right-hand specimen on the GH00037208 sheet) is a part of original material cited in the protologue as "Sierra Nevada, at Mono Pass, in loose soil, Bolander" (Gray 1868) and it is chosen in accordance with Art. 9 of ICN (Turland et al. 2018). The description of the species is consistent with the image of the lectotype. Gray (1868) also noted that the seeds of *Monolepis spathulata* are notably smaller than those of *M. chenopodioides* [= *Blitum nuttallianum*]. The small seed dimensions of *Neomonolepis spathulata* (0.4 × 0.3 mm) are similar to those observed in many Australian *Dysphania* (Wilson 1984 sub *Chenopodium*; Sukhorukov 2014).

Distribution. South-western North America (USA, North Mexico).

Etymology. The new generic name is composed by the prefix "neo" (new) and the core name *Monolepis*.

Conclusion

In the Chenopodioideae, some phylogenetically distant taxa often look similar due to convergence of various morphological characters, some of which were previously thought to be diagnostic such as the number of perianth segments. A remarkable example is highlighted by the different phylogenetic positions occupied by members of the former genus *Monolepis*, which are currently included in Anserineae (*M. nuttalliana* ≡ *Blitum nuttallianum*; *M. asiatica* ≡ *B. asiaticum*), Dysphanieae (*Neomonolepis spathulata* ≡ *Monolepis spathulata*) and Chenopodieae (*Monolepis pusilla* ≡ *Micromonolepis pusilla*). This study shows that fruit and seed characters such as seed-coat structure are valuable traits for taxonomic study. These features are particularly useful in distinguishing the morphologically similar but phylogenetically distinct genera *Blitum* and *Oxybasis*.

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