RESEARCH ARTICLE



A free-access online key to identify Amazonian ferns

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Abstract

There is urgent need for more data on species distributions in order to improve conservation planning. A crucial but challenging aspect of producing high-quality data is the correct identification of organisms. Traditional printed floras and dichotomous keys are difficult to use for someone not familiar with the technical jargon. In poorly known areas, such as Amazonia, they also become quickly outdated as new species are described or ranges extended. Recently, online tools have allowed developing dynamic, interactive, and accessible keys that make species identification possible for a broader public. In order to facilitate identifying plants collected in field inventories, we developed an internet-based free-access tool to identify Amazonian fern species. We focused on ferns, because they are easy to collect and their edaphic affinities are relatively well known, so they can be used as an indicator group for habitat mapping. Our key includes 302 terrestrial and aquatic entities mainly from lowland Amazonian forests. It is a free-access key, so the user can freely choose which morphological features to use and in which order to assess them. All taxa are richly illustrated, so specimens can be identified by a combination of character choices, visual comparison, and written descriptions. The identification tool was developed in Lucid 3.5 software and it is available at http://keyserver.lucidcentral.org:8080/sandbox/keys.jsp.

Keywords

Amazonia, Ferns, Identification key, Indicator species, Lucid3, Free-access key, Online identification tools, Pteridophyte, Tropical forests

Introduction

"Science is a sequence of generating new ideas, detailed explorations, incorporation of the results into a toolbox for understanding data, and turning them into useful knowledge." Peterson et al. 2010

The first species identification key was published more than 330 years ago (Griffing 2011). It was a dichotomous key, which means that each question about the morphological characters of the specimen has two alternative answers, and the order in which the characters are assessed is pre-determined by the designer of the key (Hagedorn et al. 2010). A multientry identification system based on punch cards was developed already in the 1960's (Hansen and Rahn 1969). Nevertheless, until the bioinformatics revolution in the last 20 years (Peterson et al. 2010), most people wishing to make species identifications were forced to either use dichotomous single-entry keys or solicit personal assistance from experts (Hardisty et al. 2013). For most parts of the world, compiled identification guides have not been available, so the identification keys, species names, and species descriptions have been scattered in the scientific literature (Polaszek 2005). This has caused taxonomic information to only be accessible for a restricted group of specialists at universities and research institutions with extensive library facilities.

The rise of the Internet has been a significant development for systematics (Farr 2006). First, the Internet makes it possible to deliver taxonomic information to endusers directly, immediately and globally, mitigating important aspects of the taxonomic impediment (Ebach et al. 2011). Second, publishing information online makes rapid and frequent updating of taxonomic works feasible; no expensive printing of a new edition is needed. This flexibility is much needed, given that ca. 25,000 new scientific names for organisms are proposed every year (Knapp et al. 2007). Third, the Internet facilitates the development of identification formats other than the dichotomous single-access keys based on verbal descriptions. Currently, three kinds of keys are found online: single-access, free-access, and hybrid (Hagedorn et al. 2010). In freeaccess keys, it is up to the user to decide which characters and in which sequence to use. Often, guidance in character selection is offered, there is tolerance for errors, and the character choices can be changed at any time (Dallwitz et al. 2000). In contrast, single-access keys provide a single path for each result, errors are not tolerated, and if a diagnostic character is missing, identification cannot be continued beyond the point in the key where that character is used (Hagedorn et al. 2010). For these reasons, freeaccess keys are more flexible and have several advantages over single-access keys.

With the rapid progress in online approaches, taxonomy has become more accessible and integrated to the society. Newly developed software like Lucid (http://www.lucidcentral. com/), FRIDA (Martellos 2010), Intkey (Dallwitz et al. 1993) and Xper (Ung et al. 2010) have made it technically feasible for anyone to produce web-based identification keys that are dynamic, interactive, and easily accessible. Moreover, online keys can make almost unlimited use of colorful photographs and other illustrations, which are constrained by printing costs in paper keys, guides and floras (Brach and Song 2006). Inclusion of

illustrations in identification keys is very important. Most people find it more intuitive to identify a specimen by comparing it with illustrations of diagnostic characteristics than by comparing it with verbal descriptions of those characteristics. Therefore, enabling visual assessment can greatly enhance the usability of taxonomic works, and can also be expected to result in more reliable identifications, especially when done by non-experts (Martellos and Nimis 2015). In addition, online tools can take advantage of additional information by incorporating dynamic objects (such as scalable species distribution maps), associated files (such as PDF versions of taxonomic publications) and links to information published elsewhere in the Internet. All of this is unattainable in printed keys.

In the last 20 years, a wide range of tools has been developed to address different societal demands on species identification. Crowd source identification (http://www.inaturalist.org/) and taxonomic platforms (http://scratchpads.eu/), mobile apps (http:// bien.nceas.ucsb.edu/bien/tools/plant-o-matic/, http://leafsnap.com/), and even games (Seidman et al. 2016) are contributing to a quick delivery of taxonomic information to a global audience. Some keys have more applied purposes, like identifying pests and crop diseases (http://www.idtools.org/id/citrus/diseases/key.php; http://idtools.org/id/palms/sap/keys.php) or classifying vegetation (http://www.lacistemataceae.org/NVC-key.html). Identification keys to a great variety of organisms have also been developed, e.g. for plants (https://gobotany.newenglandwild.org/simple/, http://herbaria.plants.ox.ac.uk/bol/caricaceae/Keys), fungi (http://www.mycokey.com/newMycoKeySite/MycoKeyIdentQuick. html), and animals (http://keyserver.lucidcentral.org/key-server/player.jsp?keyId=68, https://www.rspb.org.uk/discoverandenjoynature/discoverandlearn/birdidentifier/).

With all these facilities, the contribution of non-experts to the accumulation of biological data is increasing, and has already been remarkably successful in providing data on species occurrences and distribution (Bonney et al. 2009).

Identifying indicator species for conservation planning

Biodiversity research and conservation actions are heavily dependent on the availability of adequate species identifications, whether the aim is to understand species origins, patterns in spatial distribution, or responses of organisms to human impact (Polaszek 2005; Hardisty et al. 2013). However, there is a general lack of taxonomic knowledge, such that even describing all the species is not a realistic goal at present, and providing identification tools for them is even more difficult (Godfray 2002; Wheeler et al. 2004). In poorly known areas, such as Amazonia, one approach to maximize the amount of spatial information from biological surveys is to focus on indicator species (Cajander 1926; Noss 1990; Ellenberg et al. 1992; Ruokolainen et al. 1997, 2007; Margules et al. 2002; Tuomisto et al. 2003a; Salovaara et al. 2004). This lessens the taxonomic identification burden by reducing the number of species that need to be dealt with. Consequently, more sites can be inventoried with the same effort, which facilitates habitat mapping and conservation planning (Noss 1990; Howard et al. 1998; Higgins and Ruokolainen 2004; Ruokolainen et al. 2007).

It has been suggested that ferns are good indicators of environmental conditions, forest types and general floristic patterns (Ruokolainen et al. 1997, 2007). Indeed, ferns are easy to observe and collect, broadly distributed geographically, and several studies have documented that they have specific edaphic affinities (Tuomisto and Poulsen 1996; Tuomisto et al. 1998, 2002, 2003a, 2003b; Tuomisto 2006; Cárdenas et al. 2007; Higgins et al. 2011; Zuquim et al. 2014). Fern occurrence patterns have also been used to test proposed biogeographical barriers (Higgins et al. 2011; Tuomisto et al. 2016) and to map soil properties in areas where soil data were unavailable (Sirén et al. 2013; Zuquim et al. 2014). Therefore, a deeper understanding of fern species distribution patterns can provide relevant information for habitat mapping and conservation planning (Zuquim et al. 2014).

There is an urgent need for more biological data to improve conservation planning in poorly known areas (Feeley and Silman 2011; Feeley 2015), such as Amazonia. Data on correctly identified and georeferenced specimens form a valuable source of information about species distributions in Amazonia, regardless of the degree of formal training of the collector. Since the number of taxonomists is limited, non-specialists can play an important role in recording species occurrences and thereby improving the understanding on species distributions. Unfortunately, there is no comprehensive literature on Amazonian fern species as yet, so identification keys and species descriptions need to be searched for in several scattered publications. Some examples include chapters in a special issue of the journal Rodriguésia (several authors, 2005; e.g., Prado 2005a, 2005b, with 75 species from one central Amazonian locality) and books (Zuquim et al. 2008, with 120 species from another central Amazonian locality; Smith 1995, with 671 species from Venezuela and Cremers 1997, with 182 species from French Guyana). Moreover, new Amazonian fern species and even genera (Moran et al. 2010; Mynssen et al. 2016) are being described, and taxonomic rearrangements necessitate moving some of the already known species to different genera (Kessler and Mickel 2006; Prado 2006; Prado and Moran 2008; Cárdenas et al. 2016). Consequently, printed identification keys may become outdated within a few years.

We developed a free-access online identification tool for Amazonian ferns in order to provide a relatively easy and up-to-date resource for species identification. Our aim was to specifically address the problems mentioned above, and thereby to stimulate the collection of ferns and accumulation of data about them.

Structuring and implementing the key to Amazonian ferns

We started the key development by compiling a preliminary list of species and the morphological characters we thought are most useful when identifying them. Preference was given to such external characters that can be observed in the field or in the herbarium with the naked eye. The next step was to decide how the characters and their states are communicated to the user. Rather than programming a key platform from scratch, we decided to use the specialized key design program Lucid v. 3.5 for this

purpose. Lucid v. 3.5 treats the taxa as *entities* and morphological characters as *features* that can have two or more *states*. The characters are implemented in Lucid by *scoring*, for each feature, the states that are present in each taxon. For example, the feature "nodes on the petiole" may be scored to have the state "present" for one species (entity) and "absent" for another. A single taxon can have more than one state scored in a feature, so if a species has nodes on some petioles but not all, both states can be scored. Another example from the present key is the feature "leaf architecture" of *Asplenium pearcei* Baker, which is scored for the states "entire", "lobed" and "pinnate" (Figure 1).

Lucid allows scoring features in two different layout modes. The spreadsheet mode shows all features and all entities at the same time, and is useful for mass scoring of features, as well as to get an overview of the data (Figure 2A). The tree view shows all the scores for either one entity or one feature at a time, which is useful when one is adding or checking a single entity or feature (Figure 2B).

There are more than 500 described species in the paraphyletic group known as pteridophytes (ferns and lycophytes) in Brazilian Amazonia (Prado et al. 2015). Our key contains about half of these. We decided to focus on ferns only, because lycophytes are so distinct that they are best treated in a separate key. Within the ferns, we focused on those species that we have learned to know in the field during our long experience in Amazonia. This was necessary both because these are the species we can confidently identify, and because most of the herbarium material used when scoring the features was deposited at TUR. Some species not present at TUR but included in the Uatumá checklist (Zuquim et al. 2009) were also included in the key. All of the species that are included in the key occur in lowland Amazonia. The vast majority can be found in non-inundated (terra firme) forests, but many species typical of white-sand forests and seasonally inundated forests are also included, as well as some species of disturbed areas and a few aquatic species.

The list of taxa in the key is not complete yet, but accommodating more families, genera, and species is straightforward. The features can be applied to any fern species, and more states can be added if the new species are not adequately described by the existing states. For example, at the moment the key contains no species that are hemidimorphic (only a part of the leaf is fertile). When a hemidimorphic species (such as *Anemia*) gets added, a state "leaf divided into a fertile and sterile part" could be added to the feature about the morphology of fertile leaves in relation to sterile ones.

Features that are relevant to a few taxa only were included in separate subkeys. For example, the number of cells in the hairs on the lamina is a diagnostic feature in species of the genus *Triplophyllum* Holttum, but this is a rather difficult character to observe, and in most other genera it is uninformative. Therefore, the feature was only included in the *Triplophyllum* subkey, which is then embedded in the main key. The subkey can be accessed either as link through the main key or independently from the key portal (http://keyserver.lucidcentral.org:8080/sandbox/keys.jsp). More subkeys are under development for other taxa.

Subkeys can also be used more generally to provide modular structure to the key. They can be developed independently for a taxonomic group of interest or for a geo-



Figure 1. Morphological variation in *Asplenium pearcei* Baker. Three fertile individuals of *Asplenium pearcei* Baker showing different patterns in leaf dissection. **A** entire leaves **B** pinnate leaves and **C** lobed leaves. All states were scored in the features table.

graphic region and then linked to the main key. If new geographical areas were to be included in the key, a practical option might be to add region and/or biome as one of the features, so the user can subset the key to the geographic area of interest (for example, Amazonia, Panama, Neotropics, Andes, etc.).

Regardless of the identification key format, some morphological features are only meaningful in the presence of a specific state of another feature. For example, "number of pinna pairs" is a relevant feature only among those ferns that have pinnately divided leaves in the first place. A conditional list of features is implemented in Lucid by ascribing logical relationships among characters (or dependencies, following Lucid's terminology). In other words, Lucid keys can be designed such that the feature "number of pinna pairs" is unavailable (i.e., invisible to the user) until the user scores the state "pinnate" in the feature "leaf architecture". At that moment, features that are relevant for pinnate leaves automatically appear in the features list. This is called positive dependency (a dependent feature appears when a controlling state is selected). Lucid also allows for negative dependency, which means that the dependent feature is initially available, but disappears from view when a controlling state is an important part of key design, because when they are well used, they help to keep the features list concise and the appearance of the key more inviting. Our fern key



Figure 2. Structure of the key under development in LucidBuilder. Screenshots of the key been developed in Lucid Builder V3.5. **A** spreadsheet scoring table view **B** tree view mode of the scores for a single species **C** an example of features positive relationship: the plus sign associated with "number of included free veinlets" and "orientation of included free veinlets" shows that these features are controlled by the highlighted state ("usually present" under "included free veinlets"). All controlling states for some feature in the key are marked in red **D** frequency plot of the number of differences among pairs of entities and a list of those entities that have at most 10 different scores among them.

uses positive dependencies but not negative ones. For example, the feature "number of veinlets" is not visible when the key is launched, but it has a positive dependency with two of the states in the feature "included veinlets", namely "usually present" and "rarely present" (Figure 2C). If the user chooses one of these states, "number of veinlets" automatically appears.



Figure 3. Available contents of the online fern keys. Screenshots of the online key. **A** Entrance page with a list to all available keys **B** an overview of the key after two features have been scored, (C) link to possible identification occurrence map in GBIF **D** description of the entity that possibly matches the plant needing identification.

Each species in our key is scored for a higher taxon membership, i.e. genus and family. This provides a quick possibility to subset the key when the user already knows the genus or family. Scoring one of these taxa for the specimen to be identified causes the key to work as a key to species within that taxon only. Taking into account that the key is still missing many species known to occur in Amazonia, we recommend that the user checks from other sources if it seems that none of the species of the key matches the plant needing identification.

We used a template to transform the scores of each entity into automatically generated text descriptions (Figure 3D). The descriptions allow the user to view taxon scores in a compact format, and also to access all the pictures that are related to the taxon in the key. In addition, links are provided to external data sources, in particular a map of species records available in the Global Biodiversity Information Facility (GBIF; Figure 3C) and species name status according to The Plant List (http://www.theplantlist.org).

We included more than 5,000 illustrations (photos and drawings) in the key to document the morphological variation within each species and to illustrate the features and their states that were used in the character list. Some of the photos are associated with voucher specimens deposited in TUR, INPA, or SP Herbaria. Species names were updated according to PPG I (2016).

We tried to avoid botanical jargon, but sometimes the precise scientific terms were needed. Then we illustrated them and/or defined them in a glossary that can be accessed in the following link: http://www.utu.fi/en/sites/amazon/publications/Pages/ Glossary.aspx

General aspects of the online key

Lucid identification keys are structured in four tiles containing (a) available features, (b) chosen features, (c) entities remaining, and (d) entities discarded (Figure 3B). Our key includes 302 entities, of which 253 are different species and 59 are separate entries for the juveniles of species that change considerably in appearance during their ontogeny. For example, *Salpichalena volubilis* has three entries, because its small juveniles have entire leaves, intermediate juveniles have once-pinnate leaves, and adult plants have 2-pinnate twining leaves. The list of features contains a total of 54 morphological characters. Of these, 19 are immediately available and the remaining ones have positive dependencies, so they become visible only if they are needed.

Of the 45,451 possible combinations of pairs of taxa, 99.3% pairs were differentiated from each other by more than 10 features (not counting "Family" and "Genus") (Figure 2D). The pairs of species that no differentiating features were included in the key were *Sticherus bifidus* (Willd.) Ching and *S. longipinnatus* (Hook.) Ching; *Vandenboschia collariata* (Bosch) Ebihara & K. Iwats. and *V. radicans* (Sw.) Copel.; and the juveniles of *Lindsaea bolivarensis* V. Marcano and *L. coarctata* K.U. Kramer.

Since ours is a free-access key, the user can select and score the features in any order, and not all features need to be scored. This represents an important improvement in relation to the usual fern identification keys, which have a single-entry structure. Having to identify a specimen using a pre-determined sequence of features is problematic especially because most keys start with reproductive characters, which are absent in many (if not most) of the individuals one is likely to encounter in the field. The present Amazonian fern key includes features related to both fertile and sterile leaves independently, which makes it possible to identify individuals regardless of its reproductive state. The key can be used to identify fertile fern individuals of any size, but sterile individuals only if they have leaves longer than 10 cm. Although it is possible to identify even smaller juveniles than this, their features are difficult to express, and adding them could make the key confusing. One possible disadvantage of free-access keys is that the high number of choices can confuse the user (Martellos and Nimis 2015). Moreover, some features can be redundant or uninformative in certain subsets of species. To overcome these problems, Lucid keys present an efficient solution that can be applied via buttons in the toolbar. For example, the "find best" and "next best" buttons calculate and highlight the feature that is most informative to distinguish between the taxa that remain in the list of possible matches. The most informative character of the Amazonian fern key is "indusia". The most common indusial score reduces the number of potential identifications from 302 to 116 species, which means that one single scored feature reduces the number of possible identification to less than half. Even better, some of the "indusia" scores reduce the number of possible taxa to only one. The next best (i.e., most informative) feature is "rhizome habit". When scored together, "indusia" and "rhizome habit" reduce the list of remaining entities to 1/6 of the original or fewer. Using these tools, the user can focus on the informative characters and identify a fern individual in a few steps.

A subkey to identify Neotropical Triplophyllum (http://keyserver.lucidcentral. org:8080/sandbox/player.jsp?keyId=5&thumbnails=true&gallery=true) based on (Prado and Moran 2008) was embedded in the main key. All the resulting keys are available in the web (http://keyserver.lucidcentral.org:8080/sandbox/keys.jsp). The main page (Figure 3A) contains links to the free-access key to Amazonian species (http://keyserver.lucidcentral.org:8080/sandbox/player.jsp?keyId=19&thumbnails= true&gallery=true&viewer=fancybox), to the same key but with pictures in reduced resolution (for low speed internet connections) and to the *Triplophyllum* subkey. More subkeys are under development. The webpage of the Amazon Team of the University of Turku (http://www.utu.fi/en/sites/amazon/publications/Pages/online-tools.aspx) provides a centralized access to the keys, related glossary, and further information about the project. The keys are also featured in the Ferns of the World – A Digital Herbarium (http://www.fernsoftheworld.com/keys/) and Lucid Central webpages (http://www.lucidcentral.com/en-us/keys173;/searchforakey.aspx). The key runs using the online key player Lucid Key Server with any modern web browser.

Testing the key: the users' point of view

Preliminary versions of our key were tested both by pteridologists and by non-specialists, and several changes were done based on their feedback. Workshops were carried out in Finland, USA, Peru, and Brazil (https://amazonkey.wordpress.com/). In Peru and Brazil, university students went to the field to collect ferns and the fresh specimens were identified using the key. The workshop in Finland focused on preserved material. In USA, the workshop was arranged during the international conference "Next Generation Pteridology (2015)" and the focus was on discussing the structure of the free-access key with fern specialists.

Some outcomes of the workshops were: 1) Even though the order in which the features are presented is not relevant in a free-access key, users tended to score the features in the order that they were listed. Therefore, features were re-sorted such that they start from the easiest and most informative ones. 2) Among the material collected in the field, juvenile individuals often remained unidentified or were even misidentified. This was especially the case with species whose habit and/or laminar dissection characters are very different in juveniles and adult plants. For example, juvenile *Lomariopsis*, which have simple entire leaves, were sometimes misidentified as *Asplenium serratum* or *Elaphoglossum*. Therefore, we added juveniles as separate entries that can be identified independently of the adult plants. For example, *Lomariopsis prieuriana* Fée now appears twice in the entities list of the key: one entry refers to the pinnate adult form and the other to entire-leaved juveniles.

Conclusions

Ferns can be used as indicators of environmental conditions in Amazonia, so mapping fern species distribution can contribute to producing habitat maps, to describing biogeographical patterns and to conservation planning. For all these purposes, a limiting factor is the poor availability of georeferenced and accurately identified species observations. In order to assist in the species identification problem, we have developed a user-friendly free-access key that is available online and summarises some of the existing taxonomic information about Amazonian ferns.

Online keys can be designed such that they require little taxonomic background knowledge, if they focus on intuitive and/or well-explained morphological characters. Our key is almost entirely based on features that are easily observable with the naked eye, and we avoided jargon as much as possible and clarified the terms when needed. Especially the taxa but also the features are richly illustrated, which allows visual comparison between the specimen and the candidate species. We also tried to keep the appearance of the key simple by taking advantage of tools that allow keeping the visible feature list concise and to quickly subset the features and species lists. More species and features can be added to the key any time. Subkeys to the larger and more difficult families and genera can be embedded, similarly to the current *Triplophyllum* subkey. The structure of the key is dynamic and flexible, so in addition of being immediately useful, there is ample room for further development. The next phases will be to expand the key to cover the rest of Amazonian fern flora, and preferably species of adjacent areas as well and to develop more subkeys. We would like to invite colleagues to collaborate on this endeavor.

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RESEARCH ARTICLE



Neotypification of Clytostoma sciuripabulum Hovel.

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Abstract

A neotype is designated for *Chytostoma sciuripabulum*, the basionym of *Bignonia sciuripabulum*, the presently accepted name of the species.

Keywords

Bignoniaceae, Southamerica

Introduction

Revision of the species of Bignoniaceae for 'Flora Argentina' identified the need for a new typification for the basionym of *Bignonia sciuripabulum*. This is a South American species with a wide distribution, occurring in Colombia, Venezuela, Guyana, Brazil, Ecuador, Peru, Bolivia, Paraguay and Northern Argentina (Lohmann and Taylor 2014: 422).

Material and methods

Specimens (digital images) kept at K and P were examined on JSTOR Global Plants (2016). Some digital images were obtained from the source herbaria: C and S. The specimens were carefully analysed taking into account the protologues. The articles cited through the text follow the International Code of Nomenclature (ICN), Melbourne Code (McNeill et al. 2012). Herbaria acronyms follow Thiers (2017).

Historical remarks and typification

The specific epithet "sciuripabulum" was used for the first time by K. Schumann (1894: 224) who published the binomial Arrabidaea sciuripabulum (Bureau) K.Sch. This name (a nomen nudum, see Art. 38.2 Ex.1) is not valid since it has no diagnosis, it is mentioned in a paragraph about the genus Cydista which states that the species is related to Cydista difficilis.

Two years later, Bureau and K. Schumann (1896: 149) published the name *Clytostoma sciuripabulum* with a detailed diagnosis and description. The only gathering mentioned was *J.C. de Mello 22*, collected in Brasilia, Sao Paulo, prope Campinas. Nine specimens of this collection have been located, kept in C, K, P and S, with different dates or not dated (Table 1). According to Art. 8.2, these specimens are syntypes.

Recently, Lohmann (2008: 272 as 'sciuripabula') transferred *Clytostoma sciuripabulum* to the genus *Bignonia*. Lohmann and Taylor (2014: 422) cited as holotype a specimen non extant at Berlin: Brasil, São Paulo, prope Campinas, 20 IX 1867, *Joaquim Correia de Méllo 22* (B†). The label data provided by Lohmann and Taylor (l.c.) belong to the sample C10021684 (a duplicate donated by S, identified by K.Schumann as *Clytostoma sciuripabulum* Bur.), reproduced in F photo neg. 22132. At S, there are two specimens with the same collection date: S15-37635 with a note written in Portuguese by Correia de Mello, stating that he had coined for this plant the genus *Pithecoxanium*, but it was not used because Miers had previously created the genus *Clytostoma* (Figure 1 – left) and S15-37638.

Ulloa Ulloa (2016) detected that the name *Clytostoma sciuripabulum* had been validly published first by Hovelacque (1888: 214). This author published anatomical research about the vegetative organs of Bignoniaceae and other families. Concerning the Bignoniaceae, only the stems and leaves were studied. There is only a brief morphological description of these organs and Hovelacque stated that he had never observed tendrils in *Clytostoma* (page 284). It seems that Hovelacque was not intending to describe a new species, he was only using a name provided most probably by Bureau. Nevertheless, according to Art. 38.1(a), his publication is valid and IPNI (2016) qualified Bureau and Schumann's name as an isonym; the valid combination cited is: *Bignonia sciuripabulum* (Hovel.) Lohmann (2008: 272).

In the introduction to his work, Hovelacque stated that he used material he cultivated or collected in different regions of France and also material provided largely by the Faculté des Sciences de Lille (LILLE), Institut Botanique de Liége (LG), Bruxelles Botanical Garden (BR) and Muséum de Paris (P).

Information has been requested about *Clytostoma sciuripabulum* from the institutions mentioned by Hovelacque and the response was that there is no material at the herbaria LILLE and LG, while at BR there are only some specimens collected in the 20th century. At the herbarium P, there are 6 specimens collected in the 19th century: P02885030 (Venezuela, Funck & Schlim 962), P02885031 (Paraguay, Hassler 4503), P02885034 (Brazil, Saint Hilaire) and 3 specimens of Mello 22. Since Hovelacque

Herb. – number	Locality	Date	Phenology	Identification	Other
P608077 Barcode	Bras aust, Sao Paulo, Campinas	27-10-1866	Sterile, with tendrils		Memo written in French
P608079 Barcode	Bras aust, Sao Paulo, Campinas	27-10-1866	Fl + 2 seeds, with tendrils	Clytostoma noterophilum ou tres voisin	
P608078	Bras aust, Sao Paulo,	– (same date	Sterile, with		
Barcode	Campinas	in P database)	tendrils		
C10021684 Barcode	Brasil prov Sao Paulo	20-9-1867	Fl, with tendrils	Clytostoma sciuripabulum !Schumann	F photo neg. 22132
S15-37635 Herb. number	Brasil Sao Paulo Campinas in silvis	20-9-1867	Fl, with tendrils	Clytostoma sciuripabulum !Schumann	Memo written in Portuguese
S15-37638 Herb. number	Brasil prov Sao Paulo	20-9-1867	Fl, with tendrils	Clytostoma sciuripabulum	
S15-37634 Herb. number	Bras aust, Sao Paulo, Campinas	10-10-1871	Fl, without tendrils	Clytostoma sciuripabulum	Comments in Portuguese
K449461 Barcode	Bras aust, Sao Paulo, Campinas	Rec. from Herb Hanbury 1/67	Fr, with tendrils	Clytostoma sciuripabulum ex nº	
K449462 Barcode	Bras. merid Campinas	Rec. from Herb Hanbury 3/77	Fl, with tendrils	Clytostoma sciuripabulum	
S-S09-553 Herb. number	Brasil prov Sao Paulo	_	Fruit only	Clytostoma sciuripabulum	

 Table 1. Clytostoma sciuripabulum - Mello 22, list of syntypes.

acknowledged his gratitude to Bureau in the introduction to his work and the only collection cited by Bureau and Schumann (1896) is Mello 22, it is assumed that Bureau (author of the specific epithet) had not studied the other samples.

All things stated, no original material studied by Hovelacque is extant, and there is material available for the lectotypification purpose. As a consequence, a neotype should be selected that matches Hovelacque's description (Art. 9.7). Amongst the specimens of Mello 22 found, listed in Table 1, the only one without tendrils is S15-37634 (Figure 1-right), so it is here designated as the neotype of *Clytostoma sciuripabulum* Hovel.

Taxonomic treatment

Bignonia sciuripabulum (Hovel.) L.G. Lohmann (2008: 272 as 'sciuripabula'). *Arrabidaea sciuripabulum* (Bur.) K. Schumann (1894: 224), nomen nudum. Clytostoma sciuripabulum Bur. & K. Schum. (1896: 149). Basionym: Clytostoma sciuripabulum Hovelacque (1888: 111, 284). **Type:** BRASILIA aust., Sao Paulo, prope Campinas, 10-10-1871 (neotype, designated here, S15-37634, image!).



Figure 1. *Clytostoma sciuripabulum* Hovel. S15-37635, and S15-37634 (Neotype). Copyright of images: Naturhistoriska riksmuseet, Stockholm.

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RESEARCH ARTICLE



A taxonomic revision of three Chinese spurless species of genus *Epimedium* L. (Berberidaceae)

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Abstract

Due to some common or similar features (e.g., small leaf, spurless, yellow flower), three Chinese species of the genus *Epimedium* (Berberidaceae), *E. ecalcaratum*, *E. platypetalum*, and *E. campanulatum*, are controversial based on morphological characteristics. In the present study, the descriptions of morphological characteristics for the three species were revised based on extensive studies and observations both in field and in herbaria. In general, *E. ecalcaratum* has long creeping rhizomes 1–3 mm in diameter, two alternate or opposite trifoliolate leaves, 7–14 flowers, and petals obovate and apex subacute. *Epimedium platypetalum* has short or long-creeping rhizomes 1–3 mm in diameter, one trifoliolate leaf, 2–6 flowers, and petals oblong and apex rounded. *Epimedium campanulatum* has compact rhizomes 4–6 mm in diameter, two alternate or opposite trifoliolate leaves, 15–43 flowers, and petals obovate and apex rounded. Through comparison, we found that despite the close affinity of these three species, they can be distinguished by rhizome differences, stem-leaves, the morphology of flower (e.g., petals), and the number of per inflorenscence.

Keywords

Epimedium, spurless, taxonomy, revision

Introduction

As a traditional Chinese herbal medicine, *Epimedium* has been widely used for "strengthening the kidney" and "reinforcing bone" for thousands of years (Jiang et al. 2016). Forty-nine species have been reported in China (Ogisu and Rix 2011, Xu et al. 2014, Zhang et al. 2014, Zhang et al. 2015, Zhang et al. 2016, Wei et al. 2017). Stearn (2002) divided the Chinese *Epimedium* into four series: *Campanulatae*, *Davidianae*, *Dolichocerae*, and *Brachycerae*. For a long time, *E. ecalcaratum* G. Y. Zhong, *E. platypetalum* K. I. Meyer, *E. campanulatum* Ogisu and *E. shuichengense* S. Z. He were recognized in the series *Campanulatae* (Stearn 2002). Among them, *E. shuichengense* was once thought to be a very special species with spurless petals, representing a transition stage of floral evolution from series *Campanulatae* (spurless small-flowered species) to series *Davidianae* (long-spurred species) (Stearn 2002). However, according to field investigations in the type locality, Zhang et al. (2015a) confirmed that *E. shuichengense* belongs to series *Davidianae* while *E. reticulatum* C. Y. Wu ex S. Y. Bao truly belongs to series *Campanulatae*. Therefore, four species, namely, *E. reticulatum*, *E. ecalcaratum*, *E. platypetalum*, and *E. campanulatum* were included in the series *Campanulatae* in China.

However, *E. reticulatum* is distinctive and can be easily distinguished from other spurless species. The petals of *E. reticulatum* are flat with a slightly cucullate base, the flower size is obviously smaller (about 7 mm) than other spurless species (about 10 mm), and its leaflets are thickly leathery with conspicuous reticulate veins on both sides (Bao 1987; Zhang et al. 2015a). Therefore, the present study focuses on the remaining three species in series *Companulatae* in China that can easily be confused.

On the other hand, numerous *Epimedium* species have been described without extensive morphological observation. There is also a lack of both field investigations and other studies. *Epimedium ecalcaratum* was described as having compact rhizomes based on very limited samples (Zhong 1991), yet this character was adopted by the *Flora Reipublicae Popularis Sinicae* (Ying 2001). Since *E. platypetalum* was described in 1922, there has been very little research concerning morphological observations for *E. platypetalum*. According to the description based on several individuals grown at Blackthorn Nursery Kilmeston, *E. campanulatum* is morphologically similar to *E. ecalcaratum* and *E. platypetalum* (Ogisu 1996) and it was later treated as a insufficiently known species by Ying et al. (2011). In general, very little is known about the range of variation of characters, variation patterns, and the taxonomic value of these allied species.

Based on extensive studies of the three spurless *Epimedium* species, both in field investigations (during flowering seasons) and in herbaria, the aim of this study was to 1) revise and complete morphological descriptions, and 2) compare the morphological differences among the three similar species.

Materials and methods

Field investigation

Field investigations on the germplasm resource and morphological observations have been conducted from 2012 to 2016. Field work was done in Hubei, Shanxi, Chongqing and Sichuan Province, China. A total of 120 individuals (30 individuals per population) from four populations of three spurless species, *E. ecalcaratum* (two populations),

Species	Population code	Location (China)	Elevation (m)	Latitude (N)	Longitude (E)	Collect date
El.	SCLD	Longdong, Baoxing, Sichuan	1641	102°44'	30°26'	2015.4.12
E. ecaicaratum	SCBX	Muping, Baoxing, Sichuan	1426	102°50'	30°22'	2015.4.14
E. platypetalum	SXLP	Yuanba, Nanzhen, Shanxi	1263	106°36'	32°51'	2016.4.26
E. campanulatum	SCLC	Longchi, Dujiangyan, Sichuan	1937	103°35'	31°09'	2016.5.6

Table 1. Location and habitat characters of populations of *E. ecalcaratum*, *E. platypetalum*, and *E. cam-panulatum*.

E. platypetalum and *E. campanulatum*, were collected from Sichuan and Shanxi Provinces (Table 1). All populations were investigated and collected during the flowering, as the floral properties are significant for the taxonomy of *Epimedium* species. To capture variation within populations, 30 individuals per population were observed and sampled. Quantitative measurements on rhizome diameter, height of flowering stem, length of inflorescence, number of flowers, and length and width of the middle leaflet were recorded for each individual. The average data were processed using SPSS 19.0 software. Concurrently, the folloeing discrete morphological characters were observed: the rhizome; pedicel, petiole, underside of leaflet hair characteristics; shape and number of leaflets; number of stem-leaves; inflorescence; leaflets and flowers; shape and color of inner sepals; and shape of petals.

Specimen examination

All 120 individuals of the three species were transplanted at the Jiangxi University of Traditional Chinese Medicine, China. Herbarium specimens were examined from the following herbaria: Chinese Academy of Medical Sciences, Peking Union Medical College Institute of Medicinal Plant Development (IMD); Institute of Botany, Chinese Academy of Sciences (PE); Chongqing Academy of Chinese Materia Medica (SM); Virtual Museum System (HX); Nanjing University (N); and Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (NAS).

Results

Geographical distribution

Based on field investigations and herbarium specimens, *E. ecalcaratum*, *E. platypetalum* and *E. campanulatum* were stenochoric species (Fig. 1). *Epimedium platypetalum* has previously been collected in Sichuan; however, it was not observed during our field investigations, likely due to habitat destruction. Therefore, the populations that can be collected were very limited.



Figure 1. Geographic distribution (black symbols) and sampling populations (white symbols) of *E. ecalcaratum* (square), *E. platypetalum* (circle), and *E. campanulatum* (triangle).

Quantitative characters analysis

Quantitative morphological data from *E. ecalcaratum*, *E. platypetalum*, and *E. campanulatum* are presented in Table 2. Among the three close allies, *E. campanulatum* was easily identified by its long flowering stem and inflorescence, the largest number of flowers, and the stoutest rhizome. *Epimedium ecalcaratum* and *E. platypetalum* were very similar in terms of quantitative traits, but *E. platypetalum* has fewer flowers (2–8 flowers per individual; population mean of four flowers per individual). The main quantitative characters of the two populations of *E. ecalcaratum* were similar, only the length of the flowering stem was found having a slight difference.

Species	E. ecalcaratum		E. platypetalum	E. campanulatum	
Population code	SCLD	SCBX	SXLP	SCLC	
Height of flowering stem (cm)	25.17±5.43	34.53±6.83	29.73±5.84	42.30±7.70	
Length of inflorescence (cm)	12.93±3.40	12.40±2.74	9.46±2.51	17.12±5.84	
Number of flowers	9±3	10±2	4±1	25±11	
The length of middle leaflet (cm)	3.01±0.58	3.32±0.54	4.35±0.48	5.26±0.71	
The width of middle leaflet (cm)	2.30±0.37	2.48±0.44	4.02±0.49	3.37±0.83	
The length/ width of middle leaflet	1.31±0.15	1.35±0.12	1.08±0.07	1.62±0.28	
Rhizome diameter (mm)	1.71±0.73	1.69±0.33	1.97±0.56	5.12±1.28	

Table 2. Quantitative traits (mean ± SD) comparative analysis of populations of *E. ecalcaratum*, *E. platy-petalum*, and *E. campanulatum*.

Table 3. The main discrete morphological characters comparative analysis of populations of *E. ecalcaratum*, *E. platypetalum*, and *E. campanulatum*.

Species	E. ecalcaratum	E. platypetalum	E. campanulatum
Shape of petals	Obviate, apex subacute	Oblong, apex rounded	Obviate, apex rounded
Shape of inner sepals	Elliptic	Ovate	Ovate
Colour of inner sepals	Purple-red	Purple-red	Red-tinged
Pedicel indumentum	Glandular hairs	Glandular hairs	Glandular hairs
Inflorescence	Raceme	Raceme	Panicle
Inflorescence indumentum	Glandular hairs	Almost glabrous	Almost glabrous
Shape of leaves	Ovate	Subrounded	Ovate
Blade back indumentum	Pilose	Sparingly pilose	Pilose, vein evident
Petiolule indumentum	Pilose	Glabrous	Almost glabrous
Stem-leaves	Two alternate or opposite trifoliolate leaves	One trifoliolate leaves, sometimes two opposite trifoliolate leaves	Two alternate or opposite trifoliolate/5-foliolate leaves, sometimes three alternate trifoliolate leaves
Rhizome	Long creeping	Thin, short or long- creeping	Compact

Discrete morphological characters

The main discrete morphological characters of *E. ecalcaratum*, *E. platypetalum* and *E. campanulatum* are presented in Table 3. The three species all had glandular hairy inflorescence and pedicels; flat, spurless, yellow, pendulous flowers; and obovate petals. Although having much in common with its close allies, *E. campanulatum* differed by having compound inflorescences and cup-shaped flowers, while *E. ecalcaratum* differed because of the slightly saccate base of the petals, creating a slightly shouldered flower base (Fig. 2). Among the three species, the most diverse characters are the number of leaflets, the number of stem-leaves, and the arrangement of leaves on the stem (Table 3). The rhizome also presented a clear differentiation among the three species.

Taxonomic treatment

Epimedium ecalcaratum G. Y. Zhong

Figs 2A-D, 3A-G

Epimedium ecalcaratum G. Y. Zhong, Acta. Phytotax. Sin., 29: 89. 1991. Type: China. Sichuan: Baoxing, alt. 1100 m, 30 May 1987, *G. Y. Zhong 87-02* (Holotype, SM).

Description. Flowering stem 20–40 cm long. Rhizome long creeping, 1–3 mm in diameter. Leaves basal and cauline, trifoliolate, 5-foliolate, sometimes 7-foliolate; leaflets ovate, $2.5-4 \times 2-3$ cm, apex acuminate, base deeply cordate with regularly rounded lobes, terminal leaflet with equal rounded lobes, lateral leaflets oblique with outer lobe large and rounded, inner lobe smaller and rounded, margin spinose-serrate, abaxially long-pilose. Flowering stem usually with two alternate or opposite trifoliolate leaves, or sometimes three alternate trifoliolate leaves, occasionally two opposite 5-foliolate leaves, rarely three or four whorled leaves with unifoliolate, trifoliolate and/or 5-foliolate. Inflorescence raceme, 7–14-flowered, 10–16 cm long, glandular hairs. Flowers ca. 10 mm in diam., yellow, pedicels 1–2 cm long, glandular hairy. Outer sepals 4, pale purple, broadly ovate, ca. 4×1.5 mm. Inner sepals 4, purple-red, elliptic, ca. 5×1.5 mm. Petals 4, yellow, or sometimes purple-red spotted at base, spurless, ca. $6-8 \times 4-5$ mm, obovate, apex subacute. Stamens ca. 4.5 mm; anthers yellow, ca. 1.5 mm.

Distribution and habitat. *Epimedium ecalcaratum* occurs in Baoxing, Luding, Shimian and Pengxian, Sichuan, often on mountain slopes and grassland with elevations ranging from 1100 m to 2100 m.

Phenology. *Epimedium ecalcaratum* flowers from April to May, and fruits from May to August.

IUCN Red List category. *Epimedium ecalcaratum* was designated as endangered (EN) according to the International Union for Conservation of Nature (IUCN) Red List criteria (IUCN 2015), because of exploitation for medicinal use.

Specimens examined. China. Sichuan: Baoxing, *B.L. Guo 0539* (IMD), *D.J. Yu* 1892 (PE), *G.Y. Zhong Z-8901* (SM), *Y.Q. Xu & S.X. Liu 2015019* (JXCM), 2016022 (JXCM). Luding, *B.L. Guo 0611* (IMD). Shimian, *Shimian Econ. Pl. Exped. 78-0222* (SM). Pengxian, *Z.B. Feng, D.H. Zhu & X.J. Li, 20070428* (HX).

Epimedium platypetalum K. I. Meyer

Figs 2E-H, 3H-L

Epimedium platypetalum K. I. Meyer, Repert. Spec. Nov. Regni Veg. Beih., 12: 380. 1922. Type: China. Sichuan: Wenchuan, alt. 1600 m, *Limpricht 1386* (Syntypes, WRSLE, WU).

Description. Flowering stem 25–35 cm long. Rhizome thin, short or long-creeping, 1–3 mm in diameter. Leaves basal and cauline, trifoliolate; leaflets subrounded, ca.



Figure 2. Photos of *E. ecalcaratum*, *E. platypetalum*, and *E. campanulatum*. **A–B** Flower of *E. ecalcaratum* **C** Rhizome of *E. ecalcaratum* **D** Morphology of *E. ecalcaratum* **E–F** Flower of *E. platypetalum* **G** Rhizome of *E. platypetalum* **H** Morphology of *E. platypetalum* **I–J** Flower of *E. campanulatum* **K** Rhizome of *E. campanulatum* **L** Morphology of *E. campanulatum*.

 4.5×4 cm, apex rounded, base deeply cordate with regularly rounded lobes, terminal leaflet with equal rounded lobes, lateral leaflets oblique with outer lobe large and rounded, inner lobe smaller and rounded, margin spinose-serrate, abaxially pilose. Flowering stem with 1 trifoliolate (rarely 5-foliolate) leaves, sometimes 2 opposite trifoliolate leaves, occasionally 2 opposite unifoliolate leaves. Inflorescence raceme, 2–6-flowered, 7–12 cm long, almost glabrous. Flowers ca. 10 mm in diameter, yellow, pedicels 0.5–1 cm long, glandular hairs. Outer sepals 4, green, triangular-lanceolate, ca. 2 ×1 mm. Inner sepals 4, purple-red, ovate, ca. 4 × 1.5 mm. Petals 4, yellow, spurless, ca. 6–8 × 4–5 mm, oblong, apex rounded. Stamens ca. 3 mm; anthers yellow, ca. 2 mm.

Distribution and habitat. *Epimedium platypetalum* occurs in Sichuan and Shanxi, often on mountain slopes with elevations ranging from 1300 m to 2800 m.

Phenology. *Epimedium platypetalum* flowers from April to May, and fruits from May to June.

IUCN Red List category. *Epimedium platypetalum* should be designated as Vulnerable (VU) according to the International Union for Conservation of Nature (IUCN) Red List criteria (IUCN 2015), because of habitat destruction.

Specimens examined. China. Sichuan: Maoxian, *S. Y. Chen 5210* (NAS). Nanjiang, *B. L. Guo A56* (IMD). **Shanxi:** Nanzheng, *J.X. Lv, Y.P. Lv & X.F. Zhuo E84009* (PE), *E84010* (PE), *E84052* (PE), *S.X. Liu, J.X. Zhu & L.J. Liu 2016020* (JXCM).

Epimedium campanulatum Ogisu

Figs 2I–L, 3M–Q

Epimedium campanulatum Ogisu, Kew Bull., 51: 401. 1996. Type: China. Sichuan: Dujiangyan, alt. 2000 m, 15 May 1994, *Ogisu 94305* (Holotype, K).

Description. Flowering stem 35–50 cm long. Rhizome compact, 4–6 mm in diameter. Leaves basal and cauline, trifoliolate; leaflets ovate, $4.5-6 \times 2.5-4$ cm, vein evident, apex rounded, base deeply cordate with regularly rounded lobes, terminal leaflet with equal rounded lobes, lateral leaflets oblique with outer lobe large and rounded, inner lobe smaller and rounded, margin spinose-serrate, abaxially pilose. Flowering stem usually with 2 alternate (mostly 2 trifoliolate and occasional 1 trifoliolate leaves also occasionally observed. Inflorescence panicle, 15–43-flowered, 11–23 cm long, almost glabrous. Flowers ca. 10 mm in diam., yellow, pedicels 1.2–1.8 cm long, glandular hairs. Outer sepals 4, green, broadly ovate, ca. 2 ×2.5 mm. Inner sepals 4, red-tinged, ovate, ca. 2.5–3 × 1–1.5 mm. Petals 4, yellow, spurless, ca. 6–8 × 5–7 mm, obovate, apex rounded. Stamens ca. 2.5 mm; anthers yellow, ca. 1 mm.

Distribution and habitat. *Epimedium campanulatum* occurs in Dujiangyan, Sichuan, often on mountain slopes; 2000 m.

Phenology. *Epimedium campanulatum* flowers from May to June, and fruits from June to July.

IUCN Red List category. Only known from Dujiangyan, Sichuan, *Epimedium campanulatum* was designated as endangered (EN) according to the International Union for Conservation of Nature (IUCN) Red List criteria (IUCN 2015), because of environment deterioration.

Specimens examined. China. Sichuan: Dujiangyan, *Q. Wang, K. Yao, 9015* (PE), *Z.B. Feng 960035* (HX), *C. Zhang, Z.B. Feng 960055* (HX), *C. Zhang, G.J. Wu 990210* (HX), *S.X. Liu & L.J. Liu 2016028* (JXCM), *T.T. Yu 801* (N).

Discussion

Key to species of ser. Campanulatae

1	Flower ca. 7 mm; petals are flat with slightly cucullate base; leaflets are thickly
	leathery E. reticulatum C. Y. Wu ex S. Y. Bao
_	Flower ca. 10 mm; petals are flat; leaflets are membranaceous.
2	Rhizome compact, diameter 4–6 mm; inflorescence panicle
	<i>E. campanulatum</i> Ogisu
_	Rhizome long creeping, diameter 1–3 mm; inflorescence raceme.
3	Inflorescence usually 7-14-Flowered, two alternate or opposite trifoliolate
	leaves E. ecalcaratum G. Y. Zhong
_	Inflorescence usually 2-6-Flowered, one trifoliolate leaf, sometimes two op-
	posite trifoliolate leaves

The protologue (Zhong 1991) and the subsequent description in Flora of China (Ying 2001) both described the compact rhizome of *E. ecalcaratum*. We re-examined the holotype and conducted fieldwork in its type locality. However, individuals of *E*. ecalcaratum in the field all had long creeping rhizomes, slender nodes with numerous fibrous roots, 1-3 mm in diameter, and internodes sometimes to 30 cm. In the genus Epimedium, this situation may not be rare. The form of the rhizome, specifically the degree of elongation and thickness, and also the average size of the terminal winter-bud, is constant for each species, and sometimes offers contrasts of taxonomic value (Stearn 2002). But Stearn (1997) pointed out that the different rhizome forms among some Epimedium species can sometimes be very evident, sometimes more subtle. For example, the major difference between E. leptorrhizum Stearn and E. brachyrrhizum Stearn was that the former had a very slender elongated rhizome while the latter bore a more compact clump-forming rhizome. However, examination of a series of E. leptorrhizum specimens showed that its rhizome was often slender and long-creeping but occasionally thicker and compact (Zhang et al. 2015b). In addition, the protologue of E. lishihchenii Stearn differs from E. franchetii Stearn was in having a long-creeping rhizome (Stearn 1997). However, our field observation based on a population found that *E. lishihchenii* 20% of individuals had compact rhizomes (Liu et al. 2016).

Due to the slender elongated rhizome and small broadly ovate or almost orbicular leaflets, Guo et al. (1993) described a variety, *E. platypetalum* var. *tenuis* B. L. Guo et P. G. Hsiao. Stearn (1995) assessed *E. platypetalum* var. *tenuis* and found it differed from *E. platypetalum* bacause of its long-spurred flowers. Then, he treated *E. platypetalum* var. *tenuis* as a synonym of *E. pauciflorum* K. C. Yen, a new species that was published after the description of *E. platypetalum* var. *tenuis* (Yen 1994; Stearn 1995). In a more recent study, Ying et al. (2011) still treated *E. platypetalum* var. *tenuis* as a synonym of *E. platypetalum* var. *tenuis* as a synonym of *E. platypetalum* var. *tenuis* (Yen 1994; Stearn 1995). In a more recent study, Ying et al. (2011) still treated *E. platypetalum* var. *tenuis* as a synonym of *E. platypetalum* var. *tenuis* and found the taxon had obviously long-spurred petals (1.7 cm) and long inner sepals (1.4 cm) that were completely different with *E. platypetalum*. In addition, *E. platypetalum* var. *tenuis* has sympatric distribution with *E. pauciflorum*. Therefore, we agree with Stearn (1995) that *E. platypetalum* var. *tenuis* should be revised as a synonym of *E. pauciflorum*.

The number of stem-leaves was believed to be stable within a species and important for taxonomy, and three informal groups have been divided by the normal number of stem-leaves (Stearn 2002). Stem-leaves, however, are not so unvarying as initially supposed. Stearn (2002), Zhang et al. (2015), and our field observation have recognized and recorded some variation on the number of leaves. For example, usually two opposite or occasionally three whorled leaves were observed in *E. sagittatum* (Sieb. et Zucc.) Maxim., *E. acuminatum* Franch., *E. myrianthum* Stearn and *E. franchetii*, and one leaf or two leaves in *E. epsteinii* Stearn, *E. flavum* Stearn, *E. leptorrhizum* and *E. pauciflorum*.

It is significant that the comparatively unstable species occur in western China, where the genus is best represented and where its evolution may still be proceeding (Stearn 2002). Population is the basic unit of evolution (Chen 2016) and the most important unit to study the formation of species (Chen and Wang 1986; Nooteboom 1992; Hong 2016). Thus, morphological differences recorded among individuals in a population should not be ignored and may be more obvious than populations in sometimes (Yang 1991; Jonas et al. 1999). Previous studies on the taxonomy of the genus *Epimedium* were almost always based on limited samples, or several individuals cultivated abroad (Ying 2001). Our investigations based on populations in their native habitat found that the number of leaves and the habit of the flowering stem presented abundant variation (Fig. 3).

The protologue for *E. ecalcaratum* described that its stem-leaves are usually opposite with two trifoliolate leaves, occasionally alternate with two trifoliolate leaves or three trifoliolate leaves (Zhong 1991). Our investigation showed that it usually had two alternate or opposite trifoliolate leaves, sometimes three alternate trifoliolate leaves, occasionally two opposite 5-foliolate leaves, or rarely three or four whorled unifoliolate, trifoliolate and/or 5-foliolate leaves (Fig. 3A–G). In the protologue of *E. campanulatum* (Ogisu 1996), the species was described with one leaf or two usually alternate, rarely opposite leaves, but our investigation showed that it usually had two alternate (mostly two trifoliolate, and occasionally one trifoliolate and one simple) or opposite leaves, sometimes three alternate leaves, and one trifoliolate leaf was also occasionally observed (Fig. 3M–Q). Our study clearly shows that the number and insertion



Figure 3. Population variation of leaves morphology in *E. ecalcaratum, E. platypetalum*, and *E. campanulatum*. **A–G** *E. ecalcaratum* **H–L** *E. platypetalum* **M–Q** *E. campanulatum*.

of the leaves and the number of leaflets varies in this species. Upon extensive specimen examination, Zhang et al. (2011, 2015b) observed that the leaves of *E. simplicifolium* T. S. Ying were mainly unifoliolate, occasionally trifoliolate, and the leaves of *E. acuminatum* Franch. may be unifoliolate. Subsequently, *E. simplicifolium* was synonymized with *E. acuminatum* (Zhang et al. 2011). Hence, as a taxonomist, it is important to study as many collections as possible (Xu 1998).

Although having much in common with *E. platypetalum* and *E. campanulatum*, *E. ecalcaratum* differs in having slightly a saccate petal base, creating a slightly shouldered base to the flower (Stearn 2002), which is in agreement with our field observations (fig. 2A, B, D). According to Stearn's research, the character could be regarded as

<u> </u>		F 1	
Species E. ecalcaratum		E. platypetalum	E. campanulatum
Shape of petals	Obovate, apex subacute	Oblong, apex rounded	Obovate, apex rounded
Inner sepals	Purple-red, elliptic	Purple-red, ovate	Red-tinged, ovate
Number of flowers	7–14	2–6	15-43
Inflorescence	Raceme, 10–16 cm	Raceme, 7–12 cm	Panicle, 11–23 cm
Leaves	Ovate, 2.5–4 × 2–3 cm	Subrounded, ca. 4.5 × 4 cm	Ovate, 4.5–6 × 2.5–4 cm
Stem-leaves	Two alternate or opposite trifoliolate	One trifoliolate leaves, sometimes two opposite	Two alternate or opposite trifoliolate/5- foliolate leaves, sometimes three
	leaves	trifoliolate leaves	alternate trifoliolate leaves
Rhizome	Long creeping, 1–3 mm	Thin, short or long- creeping, 1–3 mm	Compact, 4–6 mm

Table 4. Comparison of key characteristics of E. ecalcaratum, E. platypetalum, and E. campanulatum.

moving towards development of a nectar-producing spur, and he published a photo to show the petals are typically without a spur, but may have varying degrees of small spurs (Stearn 2002: 53, fig. 18). This may indicate that *E. ecalcaratum* represented a transitional stage in floral evolution from series *Campanulatae* (spurless) to series *Davidiance* (spur with basal lamina). Spur variations have not been observed in the present study, and are not supported by specimens or other literature.

Conclusions

Despite similarity in leaf size and flat, suprless, yellow flowers, *E. ecalcaratum*, *E. platy-petalum* and *E. campanulatum* could be distinguished by the following characters: rhizome form, number of stem-leaves, leaflets, flowers, inflorescence, and petals and inner sepal shape (Table 4 and Fig. 2).

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RESEARCH ARTICLE

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Scientific user requirements for a herbarium data portal

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Abstract

The digitization of herbaria and their online access will greatly facilitate access to plant collections around the world. This will improve the efficiency of taxonomy and help reduce inequalities between scientists. The Botanic Garden Meise, Belgium, is currently digitizing 1.2 million specimens including label data. In this paper we describe the user requirements analysis conducted for a new herbarium web portal. The aim was to identify the required functionality, but also to assist in the prioritization of software development and data acquisition. The Garden conducted the analysis in cooperation with Clockwork, the digital engagement agency of Ordina. Using a series of interactive interviews, potential users were consulted from universities, research institutions, science-policy initiatives and the Botanic Garden Meise. Although digital herbarium data have many potential stakeholders, we focused on the needs of taxonomists, ecologists and historians, who are currently the primary users of the Meise herbarium data portal. The three categories of user have similar needs, all wanted as much specimen data as possible, and for those data, to be interlinked with other digital resources within and outside the Garden. Many users wanted an interactive system that they could comment on, or correct online, particularly if such corrections and annotations

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could be used to rank the reliability of data. Many requirements depend on the quality of the digitized data associated with each specimen. The essential data fields are the taxonomic name; geographic location; country; collection date; collector name and collection number. Also all researchers valued linkage between biodiversity literature and specimens. Nevertheless, to verify digitized data the researchers still want access to high quality images, even if fully transcribed label information is provided. The only major point of disagreement is the level of access users should have and what they should be allowed to do with the data and images. Not all of the user requirements are feasible given the current technical and regulatory landscape, however, the potential of these suggestions is discussed. Currently, there is no off-the-shelf solution to satisfy all these user requirements, but the intention of this paper is to guide other herbaria who are prioritising their investment in digitization and online web functionality.

Keywords

Botanic garden, collections, database, data sharing, digitization, science infrastructure

Introduction

A quiet revolution is happening in the way herbarium specimens are being accessed and used. Botanic gardens, museums and universities, all over the world, are digitally imaging herbarium specimens, transcribing their details while geolocating their origin (Baird 2010, Blagoderov et al. 2012, Van Oever and Gofferjé 2012, Tegelberg et al. 2012, Heerlien et al. 2015, Thiers et al. 2016). These activities will radically improve access to herbarium specimens and will result in many benefits for science. For example, it will empower botanists from southern countries by giving them access to historical herbaria in the north; it will provide the data held on these specimens for research and conservation and it will improve the efficiency of plant taxonomy. By giving a herbarium a virtual existence, we can radically change the way herbarium collections are used, increasing the versatility of herbarium specimen data, and even open them up to an audience who may previously been unaware of their existence.

Improving access to biological collections is a policy goal of many governments and organizations. For example, Article 17 of the Convention on Biodiversity focuses on the exchange of information, and target 19 of the Aichi Biodiversity Targets relates to biodiversity knowledge exchange (Convention on Biological Diversity 2010). Many institutions and individuals support these changes and some have ratified this by signing the Bouchout Declaration (pro-iBiosphere Consortium 2014). This declaration promotes open access to biodiversity data through the use of information technology infrastructure, standards and protocols.

In addition to their traditional role in plant taxonomy, improved access promotes new lines of research and applications for herbarium specimens. Such data can be used to monitor environmental changes, such as changes of plant phenology that result from climate warming (Vellend 2013, Rawal et al. 2015). These data are also essential to the rapidly growing field of species distribution modelling (ecological niche modelling). The data can also be used to study phytogeography, biological invasions and range shifts (Barney 2006, Lavoie 2013, Groom 2015). In regions where the available botanical knowledge is poorly documented, herbarium data can represent a valuable source of information on alien species (Fuentes et al. 2013). In community ecology, they can even be used to identify changes in community composition (Cocquyt et al. 2008). They can also be used in historical research, for example by recreating the prosopographical networks of botanical exchange (Groom et al. 2014). This has recently been demonstrated by a paper on the Belgian botanist Ernest Sonnet (1840-1901) based upon digitalized herbarium sheets kept in the Herbarium of Rio de Janeiro (Hanquart et al. 2016).

The Botanic Garden Meise holds around 3.5 million herbarium specimens from around the world, with important historical collections, and a clear focus on Central Africa and Latin America, as well as additional significant collections from Belgium and Europe. In 2002, the Garden started imaging and cataloguing its collections with two small pilot studies within the EU funded framework of the European Network for Biodiversity Information: namely The Albertine Rift Project (Stoffelen et al. 2005b) and the Martius' Flora Brasiliensis project (Stoffelen et al. 2005a). The digitization effort was intensified by projects such as the African Plants Initiative (Smith 2004), the Latin American Plants Initiative and the Global Plants Initiative, all funded by the Mellon Foundation (Ryan 2013), and by other digitalisation projects funded by the Belgian Science Policy Office through the Belgian Biodiversity Platform (Stoffelen et al. 2009), the European Commission and the Belgian Science Policy Office (Cocquyt et al. 2007, Botanic Garden Meise 2014). Progress has been slow due to limited resources and conflicting priorities. Previous scanning projects have focussed on the type specimens (ca. 55,000 specimens) and selected historical collections. Digitization of the whole herbarium was not deemed feasible in the foreseen future. However, in 2015 the Flemish Community funded a project to digitize all of the Garden's African and Belgian collections and improve the infrastructure for photography and microscopy. This project is called *Digitale Ontsluiting Erfgoedcollecties* (DOE!), which translates to Digital Unlocking of Heritage Collections.

For the Garden this project presents many opportunities. It will update and extend the current digitalisation infrastructure, image storage and web portal. In addition, the project will raise the profile of the Garden; demonstrate the importance of the collections and make the general public more aware of the collection's existence. It will also increase awareness of the Garden's research initiatives and its relevance to conservation, science and society.

As part of the DOE! project, the Garden will update its herbarium portal. However, before redesigning the portal and making key decisions on data management, the Garden decided to conduct a user requirements analysis to establish the needs of scientists and other user groups and help prioritise investment. This prioritisation is necessary as funding is limited and we wish to fulfil the demands of the diverse users. Many different people and organizations interact with the Botanic Garden Meise and may access its data portal for different reasons (Fig. 1). Teachers and schools may use the herbarium portal to demonstrate the diversity of plant life and the role of specimens in research. Citizen scientists may use the portal for their personal interests and



Figure 1. Stakeholders interacting with the Botanic Garden Meise and potentially using its data portal. The stakeholders prefixed by the words 'internal' refer to those that work at the Botanic Garden, whereas those referred to as 'external' refer to researchers in other institutions.

as an element of volunteer work. Also, decision makers may use the summarized data to guide management priorities and decisions. Nevertheless, traditionally the main users of herbarium specimens and their associated data are researchers. Therefore, we have concentrated our user requirements exercise on researchers who we considered to be the primary users, but we consider that improving usability and functionality for this group is likely to benefit all stakeholders. In the long term it is conceivable that different types of users could be directed to different views of the data tailored to their needs.

In parallel with the user requirements analysis, the Garden has also developed a data management plan to clarify its position on data management and access towards digital herbarium data. This plan highlights system requirements for the herbarium data portal, which may not be directly visible to the users, but are nevertheless important to support the accessibility and citability of data. The plan will also clarify issues of licensing and data embargoing that are needed to make it clear what the limitations are on the use of data.

Having completed the process of user requirement analyses, we considered that the insights gained should be shared with other herbaria and museums. To this end, we present here the process and outcomes of this user requirements analysis. We identify similarities and differences in user expectations. We also identify requirements that users considered to be important, and suggested they be prioritised.

The mass digitization of herbaria around the world presents enormous challenges and opportunities for science. Ultimately, its success will be judged on the impact these efforts have on scientific progress and on society in general. An important part of this effort is to empower users with the tools and data they need to make an impact.

Methods

The Botanic Garden Meise contracted Clockwork, the digital engagement agency of Ordina, to conduct the user requirements analysis. Clockwork has extensive knowledge in the field of user experience and digital design and their lack of knowledge of botanical research was considered an asset as it helped to provide a fresh perspective on the user requirements for the herbarium portal.

Preparatory phase

Before consulting stakeholders a small team, comprising staff of the Garden, met to identify potential stakeholders and decided on those to be consulted in a requirements analysis as discussed in the introduction (Fig. 1). In this phase, researchers were divided into three groups based upon their domain of expertise: taxonomists, ecologists and historians, though, it is appreciated that other researchers, including sociologists and geneticists, may also use herbarium data on occasion.

The second step of the preparatory phase was a "market analysis" where Clockwork and a core team from the Garden conducted a survey of the online landscape of herbarium tools and resources. The information gathered at this phase was used to inform ourselves of the current state of the art, so that questions could be framed and the opinions of the stakeholders could be contextualized.

Recruitment

Participants were recruited from scientists and historians of the Botanic Garden as well as from Belgian universities and scientific institutions. Externally, the recruitment was made by invitation in order to have some control on the representation of participants. Within the Garden, participants were recruited from a mixture of invited staff and self-selected volunteers. An effort was made to recruit from a diverse range of participants where scientific discipline, gender, language and origin were considered. Scientists were broadly recruited from within their fields, including those interested in tropical *versus* temperate regions to those studying vascular plants *versus* cryptogams. In total, 23 participants were recruited; 12 taxonomists; 7 ecologists and 4 historians. These included 10 women and 8 external researchers.

Interview format

The approach was for pairs of participants with similar jobs to be interviewed together. Each pair was presented with different task-scenarios related to their daily work and for which they would apply information from the herbarium. These tasks were selected to be representative of the tasks of each type of scientist identified during the market analysis workshop (Table 1). Participants were paired based on their similar scientific backgrounds and interests, to avoid discussions arising from differing perspectives. Each pair had to choose one of the three task scenarios. They were then asked to create a task breakdown of how they would fulfil the task. The goal was to understand the processes that these scientists would routinely perform. The participants wrote each step of their task on individual colour-coded sticky notes and arranged them out in chronological order. The aim of the next step was to create a list of types of data or information that scientists would be looking for when being faced with the specific scenario. The participants were asked to list what types of data or information they would be looking for at each step of their task breakdown. Every type of data or information was written down on an individual sticky note and placed beneath the related step of the task breakdown. Once the lists of data and information had been created, the next step was to investigate what sources and platforms they would use to find these data and information. The participants were asked to list all sources and platforms that they would use to gather the previously listed data and information at each step of their process. They were explicitly triggered to think both in terms of digital and nondigital sources and platforms. All listed platforms and sources were written down on individual sticky notes and placed beneath the related data type.

After listing the platforms and sources that participants would use, we focused on the reasons why they choose these over other sources and platforms. They received a template for each source or platform they listed, up to a maximum of three. On this template, participants were asked to describe their experience of the source or platform; they listed its key functionalities, its strengths, its weaknesses and if there was something that could be improved or is missing.

Then, the focus moved to the current virtual herbarium of the Botanic Garden Meise in order to get an overview of its strengths and weaknesses according to the participants. Table 1. Task scenarios presented to participants in the user requirements interviews.

Historical based scientists:

- 1. Write a biography on a collector called Joseph Bequaert, on his voyages, taxonomic interests and the people he worked with.
- 2. Contrast the traditional uses of the genus Solanum in Africa and South America, then comment on the impact of modern Solanaceous introductions to traditional agriculture.
- 3. The garden has received a large collection of photographs on glass plates. There are some limited details that come with the collection, but you would like to improve the metadata associated with each image.

Ecological based scientists:

- 4. You need to start gathering data for a species distribution model of *Agrimonia eupatoria*. Where would you get the data and ensure that they are free from errors?
- 5. You need to create a red-list for Belgium. Look for the necessary data and determine the status.
- 6. An alien species is gradually moving northward in Europe. Before it has naturalized in Belgium you need to write an impact assessment for decision makers.

Taxonomic based scientists:

- 7. You find a specimen in another collection's herbarium. You can't read the signature but have the locality and the date. How would you figure out who the collector was?
- 8. You think you have discovered a new species in the herbarium collection. How would you verify that it has not already been described?
- 9. You are writing a revision of a large genus. You need to create a distribution map of each species.

Before the participants were able to access and explore the current virtual herbarium, they were asked three questions:

Did you know that you can access a part of the Garden's herbarium collection through the virtual herbarium on the website?

Have you ever accessed or used the virtual herbarium before? If so, what was your reason for using the virtual herbarium?

Did it meet your expectations? If not, why not?

During the exploration of the current virtual herbarium, they were also asked:

When you look back at the task that we worked on during this interview, do you think you could have used this virtual herbarium to fulfil one of your steps? Why, or why not? What is missing in order to help you fulfil your task?

The final step of the interview focused on consolidating the input that was given by the participants during the previous steps. The goal of this step was to stimulate the participants to convert their feedback on external platforms as well as the Garden's virtual herbarium into concrete requirements for the future virtual herbarium portal.

The key question that was asked to the participants at the end of the interview was "*My ideal virtual herbarium should...*". Participants wrote down elements, functionalities, integrations, data-links, etc., they deemed to be crucial for the future virtual herbarium.

Analysis of the outcome of the interviews

The interviews were conducted by two user experience researchers both with backgrounds in qualitative data analysis. One of the user experience researchers moderated the interview, while the other noted the feedback provided by each participant.

The interview notes were then enriched and digitized. The feedback of the participants was initially analysed by reading each comment and interpreting the underlying meaning. Affinity diagramming was used, as a qualitative content analysis method, over two iterations to establish core themes and then establish sub-themes (Kawakita 1991). These themes were linked together and structured on the level of each type of researcher. After creating these structured lists, the insights and needs were mapped using of colour-coded sticky notes that distinguished requirements that were related to data and requirements related to functionality (Fig. 2).

The final step in the analysis approach consisted of manual clustering of closely related needs and insights. These clusters helped to identify overlap among the requirements that were proposed by the different types of researcher. These overlaps served as the basis for defining common user requirements.



Figure 2. A user experience researcher using affinity diagramming to cluster user requirements from the results of the interviews.

Results

A detailed list of all user requirements is provided in the supplementary data, but have been summarized below. An important distinction was between the requirements for data and functionality. This is pertinent because user requirements for functionality can be addressed in portal development, but data requirements need to be address in long term digitization strategy.

Data requirements

The majority of researchers that participated in the interviews explicitly mentioned the need for as much data from a specimen as possible. They felt that the quality of their analysis was strongly dependent on the amount of data that might be interrelated and analysed. The most important source of these data is the specimen itself. Taxonomists, historians and ecologists highly valued the ability to consult the physical specimen in the herbarium or failing that, a high quality zoomable image. The information that can be derived from these specimens goes beyond the data that is written on the labels. Taxonomists for example study the physical specimen anatomically, microscopically and genetically, while historians derive information from details such as the layout of the label, the handwriting, and even the type of paper used to display the specimen and the label. Finally, ecologists are interested in field notes that the collector added to the specimen, as these often contain information about the habitat, substrate and other environmental data. The detailed information on a herbarium specimen includes its label information, annotations and even the way it is mounted. Even when scientists are provided with a virtual herbarium they will often need to consult the physical specimen for further details.

The common data elements identified across the different researcher categories are listed in Table 2 and subdivided by their importance from the researchers' perspective. Also summarized are the shared data elements that different researcher types mentioned (Fig. 3). There is a clear set of common data elements shared by all research groups. An even greater overlap exists between data elements of ecologists and taxonomists. Among the data elements mentioned uniquely, historians mentioned those related to the human creators and curators of specimens, such as details on specimen exchanges. Taxonomists have a specific interest in elements related to botanical nomenclature (type material), while ecologists feature data related to populations and statistics. Nevertheless, it is important to highlight the common elements related to the provenance, date, citation and geographic coordinates. Also, all groups wanted access to the original image.

Linking databases

Throughout the interviews, it became clear that the requirements for data go beyond the data that can be accessed directly *via* the herbarium. When the participants were

to their work.	
Key data elements	'nice to have'
Current name and classification of the specimen	Abiotic factors related to the specimen
The location where it was collected (ideally coordinates)	Information about the habitat of the collected specimen
Country	Ecological information on the location where it was collected
Date of collecting	Information on meteorology
Name of the collector	Description of characteristics on both macro- and micro level
Collection number given by collector	Being able to measure the length of leafs, flowers, on the

high resolution image via an intuitive tool that makes it able

Table 2. Common data elements identified across the different researcher categories as being important to their work.





Figure 3. A summary of the data elements mentioned by the different researcher types, showing which data elements researchers had in common and which were unique. This does not mean that any particular data element is not of interest to another group, only that it did not arise in the series of interviews. Details of these data elements can be found in the supplementary information. The full list of common data elements is listed in Table 2.

asked to describe their ideal virtual herbarium, all three types of researcher repeatedly mentioned the value of creating links between data in different databases within as well as outside the Botanic Garden. The heterogeneity of data sources increases the effort required to find relevant data and also risks data being overlooked.

Internal databases

The Garden's internal databases include those of the library, preserved plant collections, seed bank, living plant collection and photograph collections. Connections between these databases would facilitate research and simplify access to resources. For example, historians strive to reconstruct the sources of collected knowledge and data by looking for links between people, specimens, locations and collections. They would like links between herbarium data, gazetteers, biographies of collectors and the library catalogue. Taxonomists would like data and pictures of the Garden's living collections alongside the dried specimens from the herbarium. Ecologists would like links to field notes of the collector to provide a deeper understanding of the habitat, plant stage, and other factors related to the specimen.

External databases

The main reason to link to external databases and platforms is to facilitate finding relevant literature and additional data. Suggestions for useful linkages included: the Biodiversity Heritage Library (www.biodiversitylibrary.org) for literature; JSTOR (www.jstor.org) for type specimens; the Global Biodiversity Information Facility (www.gbif.org) (GBIF) for plant distribution; and, nomenclatural information from the African Plant Database (www.ville-ge.ch/musinfo/bd/cjb/africa); The Plant List (www.theplantlist.org) and IPNI (www.ipni.org/index.html). Other sorts of data and information can be provided through links to botanical illustrations, photographs and maps (historic and modern). Links to other herbaria, particularly to duplicate specimens, were considered important. This would assist curation and verification of material through taxonomic revisions in other herbaria. Scientists ideally would like a single shared and interactive portal for all herbarium specimen information.

Interlinking names

Taxonomists attach importance to the correct identification and correct names. However, not the only scientists stressed this importance. It was suggested that the integration of a simple nomenclatural overview for each specimen would be valuable where the current name, related synonyms and the common names are mentioned.

This list could be used to search for and collect relevant data even if the accepted name is different in other databases. Using a smart search engine that shows all relevant data, material and literature for a particular taxon would be very valuable, by reducing the risk of overlooking data due to synonymy.

Finally, all three types of researcher expressed value in being able to track name changes on a specimen.

From a complex query to a usable search function

Many researchers within the Garden mentioned that they had to request complex data extracts from the curator. Improving querying and extraction of data would save curatorial staff and scientists' time. Participants were enthusiastic about the idea of accessing the current virtual herbarium *via* a user-friendly online interface. Even though the current web portal lacks some functionality, all of the participants appreciated its speed and liked being able to search for data with a few clicks instead of typing complex search queries.

A specific functionality requested by the taxonomists was the ability to define a bounding box or polygon to select specimens from an area. This would help them plan field trips, but also could help them judge the ecological conditions of the area. It might also be useful for creating simple summaries such as a checklist of trees or endangered species of an area.

All of the participants wanted to be able to filter and sort the results of their queries. After which they should be able to download the dataset in a usable, spreadsheet compatible format. They felt that herbaria are public sources of data and that the virtual herbarium should support them to retrieve the right data. However, the actual analysis of these datasets should be conducted by the scientists outside of the virtual herbarium environment.

Data centralization

The scientists that participated in the interviews were, in principle, open to the idea of moving their personal datasets to a central database. The main reason why they create local datasets is to be able to work with their data within a comparatively simple environment, while the central database is often too rigid.

The idea of using the online portal as a tool to insert data centrally was received quite positively by the different types of researcher. *"We have to digitize our data somewhere, so we might as well do it directly in the central database and get the opportunity to relate our data with other data and extract it in a usable format for analysis".*

Furthermore, it would be convenient to link materials, photographs, data, etc. stored in a central database. Firstly, this database would enable the scientists to access

their data and other material remotely. Secondly, a centrally managed database would lower the risk of catastrophic loss. Finally, this database could take care of standardization of their data, including taxon names, collector names, country names, etc.

Validation process for added or modified data

Researchers pointed out that editing data directly could add errors if editing is unrestricted. Opening up the system to uncontrolled data input could reduce the quality of information, potentially harming the data significantly. To balance the reliability of the herbarium data, openness should be met by a need for transparency of how the data are derived.

In the researchers' opinion data editing rights should only be granted to approved users through username and password control. But, even then, such edits should always go through a review process before overwriting the existing data. Edits would be sent to a validator or data manager, who reviews them. In the meantime, pending adjustments could already be made visible to other users while they are still under consideration. As such, visitors can already benefit from the new data, knowing that it is provisional and not yet validated.

In order to streamline the process of data validation, researchers should be able to take on the role of the validator. As a validator they could subscribe to updates about changes to specific parts of the database linked to their field of expertise. This would enable them to keep track of what happens to data connected to their own work, and also bring their expertise into the validation process.

The need for transparency also reflects on what researchers expect to see after the validation process. Based on the interviews the outcome of this validation process should be made visible via a data history feature. Users should be able to track back what happened to specific data in the past. Incorporating a history of changes would help researchers understand the evolution of data, which in turn could lead to more informed decisions on future modifications. A number of taxonomists and historians mentioned that this history of data could even serve as a starting point for future research projects. The history of data described above would provide transparency on the origin of data, which in turn provides an indication of its reliability. Several participants also suggested adding a clearly marked reliability factor to validated data.

Data access

There was a remarkable difference of opinion among the scientists on whether data should be open or partially closed to external users. One of the main reasons for closing data was the fear that external scientists would "*steal*" the data, ideas and expertise and publish on it first. This concern is particularly present in the case of new specimens, collected during recent expeditions, or for specimens currently being used in research projects. The idea of locking away certain specimens was mentioned a few times. Some scientists want to be able to embargo specimen data for a period or the duration of a project. Others believe it would suffice to make some data hidden from external users. In this case, users with an internal account would still be able to see all data. Data could be hidden by simply marking information 'internal only'.

In contrast, there are researchers who believed hiding specimen data goes against the Garden's role as a public institution. They felt strongly that data should be shared with all those working on biodiversity, regardless of whether the person works inside or outside the Garden. Only for newly collected data do they agree the need for a temporary embargo.

The opinion was also divided about being able to download data sets. Some researchers were opposed to making this option easy for external users. For them, it should be mandatory for external people to identify themselves before being able to download data sets from the platform.

Discussion

There are several methods to produce user requirements, including prototyping, observing users, analysing pre-existing systems, focus groups and surveys. We engaged an external agency to leverage their expertise in creating user requirements with our botanical expertise. In selecting a suitable subcontractor the methodology was an important criterion as we wanted a consultative approach so that the stakeholders at the Botanic Garden were engaged with the process. Nevertheless, owing to the time and costs of such an approach we did have to limit our investigation to researchers living in Belgium. A useful follow-up to this study would to be to repeat this exercise in a tropical country where the benefits of data repatriation could be analysed.

The user requirements exercise demonstrated, to our own surprise, that researchers of different disciplines had similar needs. Both in terms of their data requirements and the functionality for a web portal. Furthermore, delivering all these requirements would be a significant challenge, even for large institutions with sufficient IT resources. It is clear that development has to be prioritised and requirements need to be rated on their cost-effectiveness.

Transcription of label information is one of the most time consuming aspects to digitization. Furthermore, geolocating specimens considerably increases the skill and effort required. Ideally all specimens would be transcribed, catalogued, photographed and geolocated, but decisions need to be made on the best way to achieve this, both from the perspective of cost and user needs. Is it better to have a little data from every specimen or all the data from some of the specimens? Users had broad data requirements, wanting as much of the label information as possible. So for most users it is better to transcribe the whole label of fewer specimens. This also makes the transcribed data more useful for a wide variety of research topics. However, if minimal data were recorded that enable users to find specimens comparatively easily, the image could be consulted directly to gain the additional information. So having an image available, even without much of its metadata, will support full transcription in the future and is a cost effective way to disseminate information. Users anticipate consulting individual specimens even where the same digitized data are available online. Given the limitations on the rate of transcription, the most appropriate strategy would be to consult researchers as to which specimens to prioritise for transcription, but then completely transcribe the label information on those specimens.

The importance of linking herbarium data to internal and external databases was a requirement of all users. For example, in the case of ecology, linking taxonomy to trait data can be used to assign taxa to functional groups and facilitate modelling of ecosystems based upon these functions (Pendry et al. 2007). Such analyses can be used to model the geographic distributions of functional groups, but also predict the impact of environmental changes on these groups and the ecosystem services they provide (Engemann et al. 2016).

A technical requirement related to linking is the need to ensure persistence of these links. Herbarium portals therefore need to provide a permanent URI to a specimen (Hyam et al. 2012, Hagedorn et al. 2013). Such stable identifiers also provide a method to cite specimens from a publication. This is also an important aspect in the FAIR Guiding Principles for scientific data management and stewardship, which aim to ensure data are findable, accessible, interoperable and reusable (Wilkinson et al. 2016). These principles ensure that data are human and machine readable, but also that provenance is tracked to support scholarly citation. These are issues that can be addressed by an institutional data management plan.

Although it was not mentioned by the scientists, interlinking may reduce redundancy between databases and therefore reduce the curatorial effort of maintaining data such as taxonomic names and citations. Linking databases would make it possible to automate the process of updating names when a duplicate receives a new name in another herbarium. These links could also facilitate the exchange of georeferencing information and other details of the specimen. It will be necessary to make the origin of these data clear online, both to credit the sources and to give an indication of its reliability.

Linking of databases potentially brings together a large amount of complicated data that needs to be summarized succinctly. This need for data consolidation was a general requirement of participants. Currently, they often start from sources that consolidate data at the level of species and genus in a handy overview. Two good examples of platforms that provide such summaries are Tropicos (www.tropicos.org) and the GBIF (www.gbif.org). The types of data that are consolidated by these platforms consist of: an overview of names and their related literature; a hierarchical classification; the distribution of the taxon shown on a map; descriptions of the organism; links to other sources that contain related data; links to publications and images of preserved and living specimens.

From the requirements it is clear that it is impossible to separate the user requirements for an internal collections database and an online portal. For example, an online commenting system would either need a workflow to integrate these comments back into the main database, or the web portal would be just one view of the institution's main database, with all the security and capacity implications that architecture would have. There are various competing systems for herbarium database systems including several bespoke solutions. Examples include BG-BASE, DaRWIN, DINA-Web, BRAHMS, JACQ and Specify. It is safe to say that none of these solutions provides all the user and system requirements detailed here. Certainly, the Botanic Garden Meise is not alone in the struggle to maintain legacy systems and create modern interfaces with obsolete technology. The lack of suitable alternatives eliminates the possibility of providing many user requirements with one simple software change. Rather it seems we must aim for incremental change, whilst trying to ensure these investments are at the same time future-proof for when new solutions become available. The best way to do this is to ensure that the data are maintained in standard formats and conform to standard controlled vocabularies.

One of the greatest concerns within the current information technology landscape in biology are orphaned data (Gibney and Van Noorden 2013). Scientists and institutions such as the Botanic Garden Meise house an enormous amount of data. However, much of these data are inaccessible due to scientists creating their own datasets and storing these locally on their computers instead of sharing them centrally. Scientists appreciate the advantages of moving their data sets and material to a centrally managed database. The main barrier to import these data sets into a central one is that scientists perceive the current database as too complex and rigid. Scientists prefer to use their own formats to structure data. They expect the platform to offer the ability to add data regardless of the rigid structure of the central database. One should question whether this is a valid requirement to support. There are numerous advantages for downstream users if data are conformant to community agreed standards and described clearly with metadata. If researchers were able to deposit non-standard data in repositories in whichever format, this may save them time, but would result in unusable data, not because they are lost, but because they are incomprehensible. A solution would be to facilitate scientists by providing software tools that maintain data simply, but also reward and mandate data archiving.

All researchers thought that feedback systems for data would be a valuable addition to an online portal. A good example of where feedback is used to effect is on the Encyclopedia of Life website (http://eol.org). Here, changes and comments are displayed on taxon pages. Such a system would satisfy the researchers' requirement to annotate records with their comments. However, translating these annotations into corrections that can be applied to the master database is an administration challenge, due to difficulties of contentious decisions where it is difficult to judge the authority and priority of edits. A compromise could be to implement a review system, whereby users can rate entries in addition to commenting. In this way potentially problematic entries can be flagged for review. Yet, these problems only need to be resolved when a user wishes to use a datum.

The most contentious subject among scientists is whether and how data are shared. This is a subject of much debate within the research community (Reichman 2011, Groom et al. 2013, Ferguson 2014). In a survey of natural history collections, data sharing was the greatest barrier to digitization that was not related to funding and resources (Vollmar et al. 2010). Many authors have promoted the concept of open data and its advantages for science and society (Molloy 2011, Gibney and Van Noorden 2013, Van Noorden 2013, Groom et al. 2015). In Belgium, both the Federal and Flemish governments have open data policies for public data and open data is considered their default position (Vlaamse Regering 2011, Delafortrie and Springael 2015). These policies follow the European Union's directive on the re-use of public sector information (Cox and Alemanno 2003). Furthermore, other public Flemish scientific institutions have adopted open data policies (Desmet et al. 2014, VLIZ 2016). Nevertheless, there is an inherent conflict of interests between the Garden's role as a guardian of knowledge and as a research facility where the balance between these roles is still being determined. While the pros and cons of data openness will not be debated here, this argument underlines the importance of having a data management plan, as some of the user requirements can only be achieved using open data.

Most, if not all, of these user requirements will be familiar to curators, taxonomists and others who regularly work with herbarium data. Nevertheless, it is valuable to record these requirements for several reasons. We need to deliver as many of the requirements as possible, but also keep a record of our progress. Prioritisation is also critically important to make effective use of the available budget. Furthermore, it is useful to communicate our needs to other institutions because fulfilment of some of the user requirements requires cooperation and adoption of common standards by many institutions.

Conclusion

Researchers have high expectations of biodiversity informatics, both for the software and the data that have been digitized. User requirements are similar for different types of researcher and we should prioritize access to core data fields in an easily searchable and useable format. Nevertheless, the most useful way to prioritize the transcription of label information is to work on data that is required immediately for research, but always transcribe the whole label data. Furthermore, though researchers appreciate simple access to digital images and data, they still value access to the original specimens.

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Supplementary material I

Gathered needs per type of researcher

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RESEARCH ARTICLE



Synopsis of Commelina L. (Commelinaceae) in the state of Rio de Janeiro, reveals a new white-flowered species endemic to Brazil

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Abstract

A synopsis for the genus *Commelina* in the state of Rio de Janeiro, Brazil, is presented here, including a new species, ten new synonyms, five designated lectotypes, two designated epitypes and an excluded name. *Commelina huntii*, a new species, is remarkable due to the combination of rusty to rusty-brown hairs at the margin of its leaf-sheaths, connate spathes, white flowers with auriculate medial petal, ovaries with sparse black papillae and dehiscent fruits. Additionally, we provide an identification key, illustrations, and conservation status for the species of *Commelina* recorded in the state of Rio de Janeiro.

Keywords

Atlantic Forest, Commelineae, Commelinopsis, Neotropical flora, Phaeosphaerion

Introduction

Commelina L. is the largest genus of Commelinaceae, comprising between ca. 170 species (Faden 1998) and 205–215 species (Govaerts and Faden 2009; The Plant List 2013, respectively). It is one of the six genera of Commelinaceae (out of 42) to have a cosmopolitan distribution (Faden 1998), and one of the most complicated taxonomically. *Commelina* is easily differentiated from the remaining genera in the tribe by its inflorescences which are subtended by spathaceous basal bracts and reduced to (1–)2

fasciculate cincinni, zygomorphic flowers, petals clawed, unequal and mostly blue (but sometimes white or lilac, rarely yellow, apricot or orange), three posterior staminodes with 6-lobed cruciform antherodes, three anterior stamens, and 2-locular or unequally 3-locular and 2-valved capsules (Faden 1998).

The state of Rio de Janeiro is entirely placed within the Atlantic Forest domain (IBGE 2012), being one of the four diversity centers of the family, and possessing 67 of the 96 accepted Commelinaceae species for Brazil (BFG 2015). With 1,109,546 ha of continuous forests, which represent 7% of what remains of the Atlantic Forest, Rio de Janeiro is also the state with the greatest amount of preserved forest remnants from this biome (Ribeiro et al. 2009). Nevertheless, the most recent published state flora is nearly two centuries old (*i.e.* Vellozo 1829) and no satisfactory taxonomic treatment for the Commelinaceae has been done since. As a first attempt to clarify the taxonomy of *Commelina* in the state of Rio de Janeiro, we describe a new species of *Commelina*, together with a synopsis for the genus in the state. This work includes an identification key, illustrations and an overview of some overlooked Brazilian *Commelina* names.

Methods

The descriptions and phenology of the species were based on herbaria, spirit, fresh material, field data, and literature. All species were studied in the field and had their descriptions complemented with field notes, photographs, and cultivated specimens, gathered between the years of 2010-2016. Live specimens collected by the authors were kept in cultivation at the greenhouse of the Jardim Botânico do Rio de Janeiro, in order to better observe, photograph and analyze fresh flowers, fruits and seeds, as well as other phenological data. Specimens from the following herbaria were also analyzed: ALCB, BHCB, BHZB, BM, BOTU, CEPEC, CESJ, CGE, CNMT, CVRD, ESA, FCAB, FLOR, FURB, GUA, HAMAB, HAS, HB, HBR, HRB, HSTM, HUEFS, HURB, IAC, ICN, JOI, K, MBM, MBML, PMSP, R, RB, RFA, RFFP, SP, SPF, UEC, UPCB, and US (herbaria acronyms according to Thiers, continuously updated). While specimens of the following herbaria were analyzed using high-resolution images available on-line: B, BRIT, C, CAL, F, INPA, L, MG, MO, MY, NY, P, U, and WAG. The classification of the vegetation patterns follows IBGE (2012). The indumenta and shapes terminology follows Radford et al. (1974); the inflorescence terminology and morphology follows Weberling (1965, 1989) and Panigo et al. (2011); the fruit terminology follows Spjut (1994) and Joseph & Nampy (2012); and seeds terminology follows Faden (1991) and Joseph & Nampy (2012). The conservation statuses were proposed following the recommendations of IUCN Red List Categories and Criteria, Version 3.1 (IUCN 2001). GeoCAT (Bachman et al., 2011) was used for calculating the Extent of Occurrence (EOO) and the Area of Occurrence (AOO). The typification of Vellozo's names for *Commelina* followed the same methodology used by Pellegrini (2015), Pellegrini et al. (2015) and Pellegrini & Carvalho (2016).

Results

Key to the species of Commelina in Rio de Janeiro state, Brazil

1 Inflorescences predominantly axillary and leaf-opposed, long pedunculate (peduncle the same length or longer than 1/2 length of the spathe); medial Inflorescences terminal or apparently so, short-pedunculate to sessile (peduncle shorter than $\frac{1}{2}$ length of the spathe); medial petal sessile2 2 Spathe base free, in vivo much lighter than the leaves; capsules indehiscent, not constricted between the seeds, crustaceous, pearly-white to silvery; all Spathe base connate, in vivo the same color as the leaves; capsules dehiscent, constricted between the seeds, green to light brown; dorsal seeds adhered to the capsule wall, ventral seeds free from each other, dispersed separately.....4 Leaf-sheaths hirsute throughout, hairs rusty to rusty-brown, blades lanceo-3 late to elliptic-lanceolate, hispid on both sides, hairs hyaline, sparsely hirsute along the midvein and near base, hairs rusty to rusty-brown, base cuneate, apex acute Commelina rufipes Seub. var. rufipes Leaf-sheaths glabrous, margin glabrous to setose, hairs rusty to rusty brown, blades ovate-elliptic to ovate, glabrous, base round to obtuse, apex acuminate to caudate Commelina rufipes var. glabrate (D.R.Hunt) Faden & D.R.Hunt 4 Leaf-sheaths with auriculate margin; upper cincinnus aborted, included; medial petal hyaline; capsules 3-seeded, dorsal locule commonly verrucous, rarely smooth; testa smooth Commelina erecta L. Leaf-sheaths with patent and erect margin; upper cincinnus present, exerted; medial petal slightly paler to concolorous with the paired petals; capsules 5-seeded, dorsal locule smooth; testa ornate......5 5 Leaves subpetiolate; spathe transversally rhomboid; cleistogamous subterraneous flowers present, medial petal trullate, ovary minutely pilose, stigma capitate; capsules ellipsoid; seeds black, testa shallowly reticulate Leaves sessile; spathe depressed ovate to subcordate; cleistogamous subterraneous flowers absent, medial petal obovate to oblong obovate, ovary glabrous, stigma trilobate; capsules obovoid; seeds dark-brown, testa shallowly 6 Leaf-sheath margin densely bearded with rusty to rusty brown hairs; petals white, paired petals broadly rhomboid to rhomboid-reniform, medial petal cucullate, biauriculate; ovary and capsules with black papillae, 1-2 capsules per spathe; seeds with peach-colored farina, dorsal locule seed testa shallowly Leaf-sheath margin with light to dark brown to atro-vinaceous hairs; petals blue to light-blue to lilac to pale lilac, paired petals broadly ovate to broadly

1. Commelina benghalensis L., Sp. Pl. 1: 41. 1753, nom. cons. Fig. 1A

Neotype (conserved and designated by Faden 1992). INDIA. Habitat in Benghala, s.dat., s.leg., Herb. Linn. 65.16 (LINN!).

Selected specimens seen. BRAZIL. Rio de Janeiro: Angra dos Reis, Ilha Grande, matas da praia de Abraão, 18 Apr 1987, L.C. Giordano 277 (RB). Armação de Búzios, 19 Aug 1998, D. Fernandes 19 (R). Cabo Frio, 22 Oct 2013, H.F. Uller s.n. (RB 612224). Campos dos Goytacazes, Morro do Itaoca, Pedra Negra ponto 1, 13 Oct 2009, L.P. Mauad & I.O.R. Areias 20 (RB). Casimiro de Abreu, Praião, Avenida Oceânica, 24 Jun 2012, A.J. Castelo 39 (RB). Iguaba Grande, Km 94, 1981, H. Barreto s.n. (RB 275353). Macaé, Córrego de Ouro, Fazenda Vitória, Morro do Oratório, 2 May 1971, J.P.P. Carauta 1364 (RB, U n.v.). Niterói, Itaipuaçu, próximo ao Canal da Costa, 18 Sep 2004, T.T. Carrijo 143 (RB). Resende, margem da rodovia Dutra, Km 302 sentido RJ, ao lado da Light, próximo ao Rio Paraíba do Sul, 9 Jun 2012, M.O.O. Pellegrini et al. 233 (RB). Rio de Janeiro, Morro do Rangel, Recreio dos Bandeirantes, 31 May 1973, D. Sucre 10005 (RB, US). São José de Ubá, 14 May 2014, T.M. Scarponi s.n. (RB 612228). Saquarema, Tingui em Sampaio Correia, 14 Apr 1995, J.A. Lira Neto 56 (RB). Silva Jardim, Próximo a sede da REBIO, 29 Oct 1997, J.A. Lira Neto 719 (RB).

Distribution and habitat. Tropical and subtropical regions of the world. In the state of Rio de Janeiro it is especially common in disturbed areas of drier regions inland or near the coast, being common in *restingas* (*i.e.* sandbank vegetation), and as a weed in agricultural fields (Fig. 2).

Phenology. Throughout the year, but especially in the rainy season.

Conservation status. It is a weed of worldwide distribution being very common in sunny disturbed areas and in agricultural fields. Following the IUCN recommendations (IUCN 2001), it should be considered as Least Concern (LC) in the state of Rio de Janeiro and worldwide.

Morphological and ecological notes. The underground cleistogamous flowers and fruits seem to be produced only in areas where the soil is soft. The flower morphology differs from the aerial chasmogamous in pigmentation (being paler), while the fruits are sub-globose (due to larger seeds).

2. *Commelina diffusa* Burm.f., Fl. Indica 18: pl. 7, f. 2. 1768. Fig. 1B

Commelina communis Vell., Fl. Flumin.: 30, 1829, nom. illeg. non *C. communis* L., Sp. Pl. 1: 40, 1753. Lectotype (designated here). [illustration] Original parchment



Figure 1. Commelina in the state of Rio de Janeiro. A C. benghalensis L. B C. diffusa Burm.f. C-D C. erecta L.: C detail of the inflorescence showing aborted upper cincinnus D flower E-F C. obliqua Vahl:
E detail of the inflorescence showing the two developed cincinni F flower G C. rufipes var. glabrata (D.R.Hunt) Faden & D.R.Hunt H-I C. rufipes var. rufipes Seub.: H detail of the leaf-sheaths, showing the hirsute rusty hairs I mature fruits. G by Flora Virtual Estación Biológica El Verde group, remaining field photos by M.O.O. Pellegrini.



Figure 2. Distribution map of *Commelina* in the state of Rio de Janeiro. Crosses *C. benghalensis* green dots *C. diffusa* var. *diffusa*; blue dots *C. erecta*; stars *C. obliqua*; empty square *C. rufipes* var. *glabrata*; full squares *C. rufipes* var. *rufipes*.

plate of *Flora fluminensis* in the Manuscript Section of the Biblioteca Nacional do Rio de Janeiro [mss1095062_079] and later published in Vellozo, Fl. flumin. Icon. 1: t. 75. 1831. **Syn. nov.**

Holotype. INDIA. Coromandel, s.dat., D. Outgaerden s.n. (G barcode G00360106!).

Selected specimens seen. BRAZIL. Rio de Janeiro: Arraial do Cabo, 24 Jul 1953, F. Segadas Vianna 1157 (R, US). Casimiro de Abreu, Barra de São João, 27 May 1953, F. Segadas Vianna 347 (R, US). Guapimirim, trilha das andorinhas, 20 Dec 1995, L.A. Lira Neto 161 (RB). Itaboraí, entre os rios Caceribu e Macacu, 10 Aug 2007, A. Rodarte 4Cf (RB). Itatiaia, Parque Nacional do Itatiaia, Hotel Repouso, 14 Dec 1997, J.M.A. Braga 4539 (RB). Mangaratiba, Reserva Ecológica Rio das Pedras, trilha para o mirante, 12 Jul 1997, J.A. Lira Neto 603 (RB). Niterói, Jurujuba, 16 Jan 1959, A. Castellanos 22336 (R). Petrópolis, Serra da Estrela, meio da Serra, antigo leito da estrada de ferro, próximo ao Poço do Cipó, 9 Mar 1978, P.P. Jouvin 121 (RB). Resende, margem da rodovia Dutra, Km 302 sentido RJ, ao lado da Light, próximo ao Rio Paraíba do Sul, 9 Jun 2012, M.O.O. Pellegrini et al. 232 (RB). Rio de Janeiro, Urca, 10 Jan2012, M.O.O. Pellegrini et al. 182 (RB). São João da Barra, 30 May 1953, F. Segadas Vianna 428 (R, US). Silva Jardim, próximo à sede da REBIO, 29 Oct 1997, J.A. Lira Neto 714 (RB). Teresópolis, Laje, estrada para Campo Limpo, Granja Mafra, 28 May 1977, L.D.A. Freire de Carvalho 600 (RB).

Distribution and habitat. Tropical and subtropical regions of the world, being very common in shady disturbed areas such as road sides, gardens and forest margins, and in agricultural fields. It is also found growing on the edge of wet paddy fields, ponds, ditches and stream sides (Fig. 2).

Phenology. Throughout the year, but especially in the rainy season.

Conservation status. Following the IUCN recommendations (IUCN 2001), it should be considered as Least Concern (LC) in the state of Rio de Janeiro and worldwide.

Morphological notes. The specimens from the state of Rio de Janeiro usually show a staminode malformation, *i.e.* the central antherode is lacking in most of the herbaria and living specimens examined.

Nomenclatural and taxonomical notes. *Commelina communis* Vell. is a later homonym of *C. communis* L. (the genus' type species), thus, rendering it illegitimate. Vellozo's description (1929) is little informative, especially for *Commelina*, lacking all the characters evidenced by Faden (2008) as important to delimitate species in the genus. Despite this, the spathe and flower details (Vellozo 1831: t. 75), along with the leaf shape and stem diagnosis (Vellozo 1929), make it possible to associate *C. communis* Vell. to *C. diffusa*, rather than to *C. deficiens* Herb. (= *C. erecta*), as pointed out in the *Index Methodicus* of *Flora fluminensis* (Vellozo 1831, v. 1).

3. Commelina erecta L., Sp. Pl. 1: 41. 1753.

Fig. 1C–D

Commelina erecta var. *angustifolia* (Michx.) Fern., Rhodora 42(503): 439.1940. **Syn. nov.** *Commelina virginica* var. *angustifolia* (Michx.) C.B.Clarke, in De Candolle ALPP & De Candolle ACP Monogr. Phan. 3: 183. 1881. **Syn. nov.**

Commelina angustifolia Michx., Fl. Bor.-Amer. 1: 24. 1803. Holotype. USA. Sabulosis in Carolinae, s.dat., A. Michaux 100 (P barcode P00680427!). Syn. nov.

Eudipetala deficiens (Herb.) Raf., Fl. Tellur. 3: 68. 1837. Syn. nov.

- *Commelina deficiens* Herb., Bot. Mag. 53: t. 2644. 1826. Lectotype (designated here). [illustration] Original parchment plate of "Curtis's Botanical Magazine" at the Library of the Royal Horticultural Society, published in Hooker, Curtis's Bot. Mag. 53: t. 2644. 1826. **Syn. nov.**
- Commelina erecta f. villosa (C.B.Clarke) Stand. & Steyerm., Publ. Field Mus. Nat. Hist., Bot. Ser. 23(2): 33. 1944. Syn. nov.
- Commelina villosa (C.B.Clarke) Chodat & Hassl., Bull. Herb. Boissier, sér. 2, 1: 438. 1901. Syn. nov.
- *Commelina virginica* var. *villosa* C.B.Clarke, Monogr. Phan. 3: 183. 1881. Lectotype (designated here). BRAZIL. Rio Grande do Sul: "provincia de Rio Grande do Sul", 1816–1821, A. St.-Hilaire 2598 (P barcode P01742038!; isolectotype: P barcode P01742041!). **Syn. nov.**

Lectotype (designated by Clarke 1881). "*Commelina erecta, ampliore subcaeruleo flore*" in Dillenius, Hort. Eltham. 1: 91, t. 77, f. 88. 1732.

Selected specimens seen. BRAZIL. Rio de Janeiro: Araruama, 20 Apr 2008, A.C.S. Cavalcanti 139 (SPF). Armação de Búzios, loteamento de João Fernandes, quadra A, rua I, lote 10, 27 Jul 2013, M. Furtado 28 (RB). Arraial do Cabo, Praia do Pontal, 31 Jul 1953, F. Segadas Vianna s.n. (US barcode US 2283943.2455262). Cabo Frio, Peró, Sítio Guriri, 21 Jul 2003, G.S.Z. Rezende 191 (RB). Campos dos Goytacazes, Feb 1918, A.J. Sampaio 2813 (R). Carapebus, 23 Mar 1996, V. Esteves 947 (R). Macaé, Parque Nacional da Restinga de Jurubatiba, margem da estrada principal, entre a praia e as moitas, próximo a Lagoa Cabiúnas, 23 Jun 2013, L.S.B. Calazans 219 (RB). Mangaratiba, Ilha da Marambaia, Praia Grande, 15 Jan 1986, E.M. Occhioni 484 (RB). Maricá, 16 Feb 2005, A.T.A. Rodarte 195 (RB). Niterói, Parque Estadual da Serra da Tiririca, Pedra de Itacoatiara, 16 Feb 2000, M.C.F. Santos 496 (RB, RFFP). Paraty, Parati Mirim, Fazenda Parati-Mirim, propriedade da Flumitur, s.dat., C. Almeida 1931 (RB). Rio de Janeiro, Urca, 10 Jan 2012, M.O.O. Pellegrini et al. 181 (RB). Santo Antônio de Pádua, Monte Alegre, Mar 1927, J. Vidal s.n. (R 205994). São Gonçalo, Paraíso, Faculdade Formação de Professores da Universidade do Estado do Rio de Janeiro, 20 Oct 2006, N. Coqueiro 297 (RB, RFFP). São João da Barra, Restinga de Iquipari, 11 Dec 2002, M.C. Gaglione 8 (RB). Saquarema, 21 Feb 1996, A.Q. Lobão 76 (RB).

Distribution and habitat. Tropical and subtropical regions of the world, being common in disturbed areas of drier regions inland or near the coast, commonly found in *restingas* or in urban areas (Fig. 2).

Phenology. Throughout the year, but especially in the rainy season.

Conservation status. Following the IUCN recommendations (IUCN 2001), it should be considered as Least Concern (LC) in the state of the Rio de Janeiro and worldwide.

Nomenclatural and taxonomical notes. Clarke (1881), in his revision of Commelinaceae, erroneously considered *C. erecta* as a synonym of *C. virginica* L., a species endemic to the USA (Faden 2000). Thus, some names currently placed under the synonymy of *C. erecta* were originally described under *C. virginica*, or transferred to it at some point. According to Faden (1993, 2000), *Commelina erecta* can be differentiated by its leaf-sheaths with auriculate margins, medial petal linear and hyaline, and all locules 1-seeded (*vs.* leaf-sheaths not auriculate, medial petal blue and conspicuous, and dorsal locule 1-seeded and ventral locules 2-seeded, in *C. virginica*).

There seems to be some doubt regarding *C. deficiens* Herb. synonymy. According to Tropicos.org (2015), this species is considered a synonym of *C. erecta*. Nevertheless, eMonocot.org (2010) and The Plant List (2013) treat *C. deficiens* under the synonymy of *C. virginica*. As abovementioned, there is an historical confusion regarding *C. erecta* and *C. virginica*. If we exclusively take into account that *C. deficiens* was described by Herbert (1826) from the surrounding areas of Rio de Janeiro, it is impossible for *C. deficiens* to be conspecific to *C. virginica*. Added to that, the watercolour presented by the author perfectly illustrates the habit, flower morphology and the inflorescence characteristic of *C. erecta* (with the aborted upper cincinnus). Thus, there is little doubt that *C. deficiens* is a specific to *C. virginica*.

deficiens is a synonym of the latter. According to Stafleu and Cowan (1979), Herbert's type specimens were deposited at K, but no specimen corresponding to *C. deficiens* was found. Thus, in accordance to the *Code* (McNeill et al. 2012, Art. 9.12), in the absence of herbarium specimens, we designate this illustration as the lectotype for *C. deficiens*.

Commelina villosa (C.B.Clarke) Chodat & Hassl. has long been a name of dubious application. Clarke (1881) had already noticed that its morphological concept overlapped with the one of the highly variable C. erecta, and that the difference between them relied solely on the plant's indumenta. The observation of a great number of natural populations and specimens kept in greenhouses showed that most of the morphological variation known for C. erecta has an environmental background. Large flowered specimens developed into small flowered specimens after being transplanted from sunny to shady areas. The same thing happened to narrow-leafed and erect plants (which would represent C. erecta var. angustifolia), developing into creeping and small to wide-leafed plants. The indumenta also varied when specimens were transplanted from the field to the greenhouse. Regarding growth form and position of the stem of C. erecta, the plants can vary from creeping to sub-scandent (i.e. stems leaning generally on bushes or any other kind of support) to partially or completely erect. The only morphological characters, constant in all areas and environmental conditions were: the auriculate leaf-sheath margins; terminal to apparently terminal inflorescences (1-3 per stem), broadly sagittate to subcordate spathes with connate margin, aborted upper cincinnus (generally completely absent, but sometimes only vestigial); hyaline, linear and involute medial petal (almost invisible at blind sight); capsules with 1-seeded locules; and smooth seed testa.

After analyzing the original descriptions and the type specimens, it became clear that *C. villosa* and *C. erecta* var. *angustifolia* are conspecific to *C. erecta*. Thus, no varieties or subspecies are accepted in Brazil for *C. erecta*.

4. *Commelina huntii* M.Pell., sp. nov. urn:lsid:ipni.org:names:60474145-2

Figs 3-5

Diagnosis. Commelina rufipes Seub. affinis, sed ab ea spathis depressum-ovatum vel subcordato, basi adnata, petalo inferioris auriculata, ovarium sparse nigro-papillose, capsulae ellipsoide dehicens, parda, seminibus libera differt.

Holotype. BRAZIL. Rio de Janeiro: Itatiaia, Parque Nacional do Itatiaia, subida para o brejo da Lapa, beira de estrada, fl., fr., 24 January 2012, M.O.O. Pellegrini & L.S. Sylvestre 191 (RB!; isotypes: SPF!, US!).

Description. *Herbs* 15–35 cm tall, perennial, terrestrial. *Roots* thin, fibrous, cream colored to light yellow, glabrous or minutely pubescent with absorbent hairs. *Stems* decumbent, apex ascending, becoming trailing or straggling, rooting only near the base; internodes 2.2–11.1 cm long, green, minutely velutine to minutely pilose, with line of uniseriate hairs opposite to the leaves, hairs hyaline. *Leaves* distichously-alter-



Figure 3. *Commelina huntii* M.Pell. **A** habit **B** open spathe, showing eventual rusty cilia and villous margin **C** male flower **D** medial petal, showing auricles **E** staminode **F** lateral stamen and medial stamen **G** gynoecium, showing papillate ovary and trilobate stigma **H** capsule, showing the black papillae. Line drawings by M.O.O. Pellegrini, based on the holotype.

nate, distributed along the stem, sessile; sheaths 1.4-2.6 cm long, pilose, hairs hyaline, margins densely setose, with a line of setose hairs opposite to the leaves, hairs rusty to rusty-brown; blades 3.3-9.1 × (0.9-)1.6-2.3(-3.3) cm, chartaceous, adaxially dark-green to green, abaxially light-green to light-green tinted vinaceous to completely vinaceous, drying olive-green on both sides, lanceolate to ovate lanceolate, rarely ovate, adaxially scabrous, abaxially minutely villous, pilose along the midvein, hairs hyaline, base obtuse, rarely cuneate, margins green, scabrous, apex acuminate; midvein conspicuous, impressed adaxially, prominently obtuse abaxially, secondary veins (2–)4–6 pairs, adaxially conspicuous, abaxially inconspicuous. *Inflorescences* 1–4, terminal or apparently so, peduncles 1.3-5.5 mm, rarely inconspicuous, puberulous with hook hairs throughout, hairs hyaline; spathes $0.7-2 \times 1.4-3.2$ cm, depressed ovate to subcordate, usually slightly falcate, base connate for 3-6 mm, cordate to truncate, margin green to vinaceous, minutely pilose along the edge, hairs hyaline, sometimes also ciliate, cilia rusty to rusty-brown, apex acute, internally light-green, glabrous, veins inconspicuous, externally green, minutely villous with eventual cilia, hairs hyaline, cilia rusty to rusty-brown, veins inconspicuous, becoming conspicuous when dry; upper cincinnus 2–5-flowered, flowers male, very rarely bisexual, peduncle (0.7-)1.7-2.4 cm long, exserted from the spathe, commonly arcuate at post-anthesis, sparsely to densely puberulous with hook hairs, sometimes of 2 heights, hairs hyaline; lower cincinnus 2-4-flowered, flowers mainly bisexual, sometimes male, peduncle 0.5-1 cm, glabrous or sparsely puberulous with minute hook hairs. *Flowers* bisexual or male, zygomorphic, 6.5–9 mm diam.; pedicel 1–4 mm long, light green, glabrous, reflexed and slightly elongate in fruit; sepals hyaline white to hyaline light-green, glabrous, persistent in fruit, upper sepal $3,4-4,2 \times 1,1-1,4$ mm, elliptic, cucullate, round apex, lower sepals 4.1-5.3 × 2.2-2.6 mm, obovate, cucullate, connate, round apex; paired petals 6.2-6.9 × 4.9-5.4 mm, clawed, limb broadly rhomboid to rhomboidreniform, 4.7–5.3 × 4.9–5.4 mm, white, apex rounded, base cordate, claw 1.6–2 mm, white to tinted vinaceous, medial petal $3.1-4 \times 1-1.4$ mm, sessile, obovate to oblongobovate, with 2 auricles near the middle, cucullate, concolorous with or slightly paler than the paired petals; staminodes 3, subequal, filaments 3-3.6 mm long, tinted vinaceous, antherodes 6-lobed, $1-1.2 \times 1.2-1.4$ mm, yellow with tiny light-yellow pollen sacs; lateral stamen filaments gently sigmoid, geniculate distal to the middle, 5.6-6.6 mm long, white, anthers elliptic to oblong-elliptic, $1.2-1.4 \times 0.9-1.2$ mm, yellowishorange to cream-orange with margins tinted purple, pollen yellowish-orange to creamorange; medial stamen filament straight or arcuate-decurved, decurved at the apex, 2.2–2.8 mm long, white to tinted vinaceous, anther $1.5-2.2 \times 1-1.8$ mm, broadly elliptic to broadly oblong-elliptic, strongly curved, held near the antherodes, yelloworange to cream-orange, connective purple to dark-purple, pollen yellowish-orange to cream-orange; ovary oblong-ellipsoid, ca. $1-1.3 \times 0.6-0.7$ mm, 5-ovulate, glabrous, sparsely papillose, papillae black, style exceeding or equaling the stamens, sigmoid, strongly recurved apically, 8-11.3 mm, white, stigma trilobate, white. Capsules 1-2 per spathe, $5.5-8.1 \times 3.8-5$ mm, obovoid, constricted between the seeds, brown to light brown, glabrous, sparsely papillose, papillae black, 3-locular, 2-valved, dorsal



Figure 4. *Commelina huntii* M.Pell. **A** apex of the stem, showing terminal inflorescence **B** detail of the densely setose leaf sheath margins, with rusty hairs **C** detail of the inflorescence, showing the fused spathe and developed upper and lower cincinni **D** detail of a male flower **E** detail of the medial petal, showing the two auricles **F** detail of the androecium **G** dorsal and ventral view of the seed of the dorsal locule **H** dorsal and ventral view of one of the seeds of the ventral locules. **A** by L.S.B. Calazans, **B**, **C**, **E**, **G**, **H** by M.O.O. Pellegrini, **D** by M.S. Wängler and **F** by R.S. Couto.

locule 1-seeded, indehiscent, ventral locules 2-seeded. *Seeds* slightly dimorphic, dark brown with orange-brown verrucae, farinose, farina peach-colored; dorsal locule seed strongly adhered to the capsule wall, ellipsoid, strongly dorsiventrally compressed, ventrally flattened, not cleft towards the embryotega, $3.4-4.2 \times 2.8-3.3$ mm, testa

shallowly foveolate, embryotega semilateral, relatively inconspicuous, without a prominent apicule, hilum linear, $\frac{1}{2}$ the length of the seed; ventral locule seeds free from the capsule wall, ellipsoid, truncate at one end, ventrally flattened, not cleft towards the embryotega, $2.7-3.4 \times 2-2.4$ mm, testa foveolate, embryotega semilateral, relatively inconspicuous, without a prominent apicule, hilum linear, $\frac{1}{2}$ the length of the seed.

Specimens seen (paratypes). BRAZIL. Minas Gerais: Araponga, Parque Estadual Serra do Brigadeiro, Fazenda Neblina, 17 February 2005, L.S. Leoni et al. 6112 (RB, UEC); Camanducaia, Monte Verde, Serra da Mantiqueira, 11 December 2001, L.D. Meireles & R. Belinello 775 (HURB, UEC); Delfim Moreira, Fazenda da Onça, trilha de subida para o Pico do Carrasco, 17 March 2011, L.L. Giacomin et al. 1429 (BHCB, RB); Lima Duarte, Parque Estadual do Ibitipoca, Conceição do Ibitipoca, gruta da Cruz, 30 November 2004, E.V.S. Medeiros et al. 364 (RB); loc. cit., gruta do Cruzeiro, 20 January 2005, L.G. Temponi et al. 407 (RB, UEC); loc. cit., gruta do Pião, 18 January 2005, R.C. Forzza et al. 3926 (RB, UEC); loc. cit., gruta do Cruzeiro, 26 January 2010, J.C. Lopes et al. 76 (RB, SPF); Poços de Caldas, Fazenda Campo da Cachoeira, área destinada para a instalação do Jardim Botânico de Poços de Caldas, 12 December 2001, C.N. Fraga & F.M. Fernandez 864 (RB); loc. cit., 12 December 2001, F.M. Fernandez 151 (BHZB, RB). Rio de Janeiro: Nova Friburgo, Morro da Caledônia, 8 June 1977, G. Martinelli 2469 (BA, RB); loc. cit., Reserva Macaé de Cima, cerca de 900 m do Hotel São João, 19 January 1999, L. Anderson et al. 99/15 (UEC); loc. cit., Reserva Macaé de Cima, cerca de 900 m do Hotel São João, 19 January 1999, L. Anderson et al. 99/20 (UEC); loc. cit., Parque Estadual dos Três Picos, Vale dos Deuses, 28 January 2015, M.S. Wängler et al. 1565 (RB); Resende, Parque Nacional do Itatiaia, estrada BR-485, próximo ao km 02, 22 February 2014, L.S.B. Calazans et al. 242 (RB). São Paulo: Itararé, divisa entre as Fazendas Santa Andreia e Prieto, 14 May 1989, C.A.M. Scaramuzza & V.C. Souza 259 (ESA); Ribeirão Grande, Parque Estadual Intervales, 15 April 2003, R.A.G. Viani et al. 79 (ESA).

Etymology. This species is named after the British botanist Dr. David R. Hunt, in honor of his extensive contribution to Commelinaceae systematics worldwide, especially for his contributions to Tradescantieae and the "*Phaeosphaerion* group" of *Commelina*.

Distribution and habitat. *Commelina huntii* was collected in moist and shaded nebular forests, generally near water bodies, in the states of Minas Gerais, Rio de Janeiro, and São Paulo, in elevations from 800 to 1,700 m above sea level (Fig. 5). In very rare cases it can also be found in open sometimes disturbed areas.

Phenology. It was found in bloom from November to June and in fruit from December to March, rarely in June.

Conservation status. Despite the wide EOO (112,904.528 km²), the AOO (40.000 km²) is considerably small, since all known populations are significantly small and fragmented. Following the IUCN recommendations (IUCN 2001), *C. huntii* should be considered as Endangered [EN, B2b(ii, iii)c(iv)+C2a(i)] in its overall distribution.

Affinities. *Commelina huntii* can be recognized by its white flowers with auriculate medial petal and sparsely papillose ovary and capsule. It is similar to *C. rufipes* Seub.



Figure 5. Distribution map of Commelina huntii M.Pell.

due to its white flowers and rusty hairs on the leaf-sheaths, but it can be readily distinguished from the latter by its connate spathe base (*vs.* free base); auriculate medial petal without medial constriction (*vs.* entire medial petal with medial constriction); light-brown, ellipsoid, dehiscent capsules (*vs.* pearly-white to silvery, globose, crustaceous capsules); and by its free, ornamented seeds (*vs.* seeds fused to the capsule septa, forming a dispersal unit, with smooth testa). The gross floral morphology of *C. rufipes* is much more similar to the *C. benghalensis* than the one of *C. huntii*, possessing only the white petals in common.

Commelina huntii is most similar to *C. obliqua* Vahl due to its oblique leaf blades, connate spathe base, dehiscent capsules, and ventral seeds free with foveolate testa. Despite this, *C. huntii* can be distinguished from *C. obliqua* by its densely setose leaf-sheath margins with rusty to rusty brown hairs (*vs.* leaf-sheath margins long-ciliate with light to medium to dark brown to atro-vinaceous hairs); petals white (*vs.* blue to light blue to lilac to pale lilac), paired petals broadly rhomboid to rhomboid reniform (*vs.* broadly ovate to broadly ovate reniform), medial petal cucullate and biauriculate (*vs.* involute and entire); anthers of the lateral stamens light-yellow to cream with margins tinted vinaceous (*vs.* completely orange); ovary and capsules sparsely black papillate (*vs.* smooth); 1–2 capsules per spathe (*vs.* 5–7); seeds with peach-colored farina (*vs.* seeds white farinose), and dorsal locule seeds with shallowly foveolate testa (*vs.* rugose foveolate testa).
5. Commelina obliqua Vahl, Enum. Pl. 2: 172. 1806. Fig. 1E-G

Lectotype (designated by Hunt 1994). s.loc., cultivated in France, ex horto Celsii, Ventenat s.n. (C barcode C10009563!).

Selected specimens seen. BRAZIL. Rio de Janeiro: Guapimirim, trilha das Andorinhas, 20 Dec 1995, J.A. Lira Neto 145 (RB). Itatiaia, Parque Nacional do Itatiaia, parte baixa, atrás da casa do pesquisador, 21 Jan 2012, M.O.O. Pellegrini & L.S. Sylvestre 188 (RB). Nova Friburgo, Macaé de Cima, Reserva Ecológica Municipal de Macaé de Cima, estrada de terra próximo ao Hotel São João, 19 Jan 1999, J. Anderson et al. 9912 (UEC). Nova Iguaçu, Serra do Tinguá, Reserva Biológica do Tinguá, 13 May 1943, Guerra s.n. (NY 498159). Paraty, Fazenda São Lourenço, 17 Nov 1993, E. Martins s.n. (UEC 29410). Petrópolis, Quitandinha, Pedra do Quitandinha, 2 May 2010, M.O.O. Pellegrini 2 (RFA). Resende, margem da rodovia Dutra, Km 302 sentido RJ, ao lado da Light, próximo ao Rio Paraíba do Sul, 9 Jun 2012, M.O.O. Pellegrini et al. 231 (RB). Silva Jardim, 31 Jan 2015, L.S.B. Calazans 485 (RB). Teresópolis, Serra dos Órgãos, 26 Oct 1949, E. Pereira 635 (RB, US).

Distribution and habitat. Mexico to Argentina being very common in shady disturbed areas such as road sides, gardens and forest margins, and in agricultural fields. It is less commonly found growing in drier regions and rocky outcrops (Fig. 2).

Phenology. Throughout the year, but especially in the rainy season.

Conservation status. Following the IUCN recommendations (IUCN 2001), as currently circumscribed, C. obliqua should be considered as Least Concern (LC) in the state of Rio de Janeiro and worldwide.

Morphological notes. A great deal of morphological variation can be observed in C. obliqua and in its current circumscription. It comprises plants from small to large stature (sometimes more than a 1.5 m high); stems from creeping with ascending apex to erect to sub-scandent, and thin and fibrous to robust and somewhat succulent stems. The leaves can vary from 4-20 cm long, from glabrous to scabrous to pilose, and from green to dark green to vinaceous abaxially. Flower size and color also vary, which as in C. erecta seems to be environmentally influenced, probably by differences in soil pH and light intensity (Pellegrini, pers. obs.). The petals of C. obliqua can range from intense blue to light blue, sometimes varying from lilac to pale lilac. Commelina obliqua likely represents a species complex and biosystematic studies are necessary in order to better understand and elucidate its boundaries. Until this is addressed we believe that a wide circumscription, as presented here, is currently the best way to deal with this taxon.

6. Commelina rufipes Seub., in Martius Fl. Bras. 3(1): 265. 1855. Figs 1H-J

Distribution and habitat. Mexico to Southeastern Brazil, being found in the understory of preserved rainforests, in the Amazon and Atlantic domains, as well as in gallery forests in the Cerrado biome. It is a rare species in the Atlantic Forest and Cerrado biomes, with most of its collections being from the Amazon Forest.

Conservation status. As abovementioned, *C. rufipes* is locally rare in the state of Rio de Janeiro and not as frequent in the field as the blue flowered species of the genus. Despite its wide distribution, it seems to occur only in preserved rainforests, forming dense but isolated populations. Data regarding its reproductive cycle would be of great value for this species' conservation. Following the IUCN recommendations (IUCN 2001), *C. rufipes* as currently circumscribed should be considered as Least Concern (LC) in its worldwide distribution.

Taxonomical notes. We currently accept two varieties within this species (sensu Faden & Hunt 1987). The floral morphology of both varieties of *C. rufipes* is poorly understood as little reproductive material exists. However there seems to be no morphological overlap in vegetative characters and very little overlap in their distributions. Further biosystematic study, focusing especially on floral morphology, would be most useful in evaluating their boundaries and taxonomic status.

6a. Commelina rufipes var. glabrata (D.R.Hunt) Faden & D.R.Hunt, Ann. Missouri Bot. Gard. 74(1): 122. 1987. Fig. 1H

Commelinopsis glabrata D.R.Hunt, (1981: 195). Holotype. TRINIDAD. Irois Forest district, 25 January 1928, Broadway 6716 (K barcode K 000363259!).

Specimens seen. BRAZIL. Rio de Janeiro: Cardoso Moreira, Fazenda Santa Rosa, 11 Jan 2014, I.G. Costa 319 (RB). Santa Maria Madalena, Parque Estadual do Desengano, Serra da Agulha, Fazenda Agulha do Imbé, between Santa Maria Madalena and Santo Antônio do Imbé, 23 February 1983, T.C. Plowman & H.C. Lima 12933 (US).

Taxonomical notes. Few collections of this variety are known for the Southeastern region of Brazil, with several specimens previously identified as *C. rufipes* var. *glabrata* actually representing the herein described *C. huntii*.

6b. *Commelina rufipes* Seub. var. *rufipes*, in Martius Fl. Bras. 3(1): 265. 1855. Fig. 1I–J

Lectotype (designated here). BRAZIL. São Paulo: s.loc., 1817, C.F.P. Martius 76 (M barcode M0210921!). Epitype (designated here). BRAZIL. São Paulo: Bertioga, estrada Bertioga/São Sebastião, bairro São Rafael, 25 Oct 2007, R.C. Forzza et al. 4823 (RB barcode RB00515585!)

Selected specimens seen. BRAZIL. Rio de Janeiro: Duque de Caxias, Reserva da Petrobrás, trilha para a barragem, 28 August 1997, J.A. Lira Neto et al. 696 (RB). Magé, 1 November 1983, R.R. Guedes et al. 537 (RB). Sapucaia, estrada que liga

Sapucaia das Terras Frias até o Rio Vermelho, 13 March 1981, M.G.A. Lobo 223 (RB). Silva Jardim, Reserva Biológica de Poço das Antas, Juturnaíba, trilha Rodolfo Norte, caminho para a Pelonha, 18 August 1995, J.M.A. Braga et al. 2735 (RB). Teresópolis, Parque Nacional da Serra dos Órgãos, trilha para a Pedra do Sino, da entrada até a primeira cachoeira, 14 Jul 2011, J.A. Lombardi 8616 (HRCB, UPCB)

Nomenclatural notes. When describing *C. rufipes*, Seubert (1855) only mentions that his new species was based on a Martius specimen, at M. After searching the M collection, we found just two specimens from this collector — *Martius 76* (M0210921) and *Martius 77* (M0210920). Since the specimen *Martius 76* was clearly annotated in Seubert's handwriting it is the obvious choice for a lectotype. Nonetheless, Seubert's original description makes it clear that all available specimens had few if any flowers, which matches the specimens found by us at M. This has caused great taxonomic problems over the years, with this name being ascribed to a number of different genera (*i.e. Athyrocarpus* Schltdl., *Commelina, Commelinopsis* Pichon, and *Phaeosphaerion* Hassk.), and as either accepted or as a synonym by different authors (Faden & Hunt 1987). Thus, in accordance to the *Code* (McNeill et al., 2012, Art. 9.8), we also designate a well-preserved flowering specimen as an epitype, to avoid further taxonomic and nomenclatural problems.

Taxonomical notes. Apart from the obvious difference in indumenta, the leaves of *C. rufipes* var. *rufipes* tend to be thinner (lanceolate to elliptic-lanceolate), with a cuneate base and acute apex, while the leaves of *C. rufipes* var. *glabrata* tend to be wider (ovate-elliptic to ovate), with a round to obtuse base and acuminate apex.

Excluded name

After a thorough analysis of Vellozo's description and original illustration for *C. singularis* Vell., it became clear that this species does not belong in the genus *Commelina*. This name is better placed under the synonymy of *Tripogandra diuretica* (Mart.) Handlos (**Syn. nov.**), and the necessary taxonomic and nomenclatural comments and typifications are made below.

Commelina singularis Vell., Fl. Flumin.: 31. 1829. Fig. 6

Lectotype (designated here). [illustration] Original parchment plate of Flora fluminensis in the Manuscript Section of the Biblioteca Nacional do Rio de Janeiro [mss1095062_080] and later published in Vellozo, Fl. flumin. Icon. 1: t. 76, pro parte, flowers and inflorescence only. Epitype (designated here). BRAZIL. Rio de Janeiro: Rio de Janeiro, Área do Jardim Botânico do Rio de Janeiro, fl., fr., 21 Dec 1995, J.A. Lira Neto 194 (RB 2ex, barcode RB00685321!).

Accepted name. Tripogandra diuretica (Mart.) Handlos.



Figure 6. *Commelina singularis* Vell. **A–B** Original plate of Vellozo's *C. singularis*: **A** line drawings of habit, inflorescence and floral characters **B** detail of floral characters. **C,** photos of a natural population of *Tripogandra diuretica* from the Jardim Botânico Rio de Janeiro, RJ: detail of the inflorescence, showing flowers with white corolla **D–E** photos of *T. diuretica* from the municipality of Petrópolis, RJ: **D** detail of floral characteristic of the species) **SS** sterile stamens; **FS** fertile stamens; **Gy** gynoecium. Photo of *C. singularis* plate from Biodiversity Heritage Library; All field photos by M.O.O. Pellegrini.

Taxonomical notes. Vellozo's plate for *C. singularis* (1831: t. 76) shows a creeping plant with eudicot-like leaves (net-veined and a single apparently trifoliate leaf), not identifiable as any known species of Commelinaceae. Nevertheless, the inflorescence type (Fig. 6A, C), details of the androecium (Fig. 6B, D), and the morphological description of six dimorphic stamens, three of which are staminodial — "*Stamina sex, quorum tria nectaria mentiuntur*" — (Vellozo 1829), consistently allows this name to be associated to the genus *Tripogandra* Raf. Another remarkable feature of Vellozo's plate is the gynoecium, which is illustrated with a very short and slightly curved style (Fig. 6B). This feature distances *C. singulars* from the genus *Commelina* where the style is long and sigmoid, bringing it closer to *Tripogandra*. The leaves illustrated by Vellozo belong to the genus *Polygonum* L. (Polygonaceae), which usually possesses white to pink to lilac flowers, and occurs in the same marshes as *T. diuretica*. This confusion is apparently common in Brazilian herbaria, where *Polygonum* specimens are commonly misidentified as commelinaceous taxa (Pellegrini pers. obs.).

Vellozo (1829: 31) also mentions that *C. singularis* is found growing in slow-water environments — "*Aquis stagnantibus, et confluentibus habitat*" —. Only *T. diuretica* and *T. warmingiana* (Seub.) Handlos occur in the state of Rio de Janeiro; the first being very common, extremely variable in size and flower morphology, and normally occurring in marshy areas; the second being very rare, uniformly small in size and flower morphology, and occurring in drier areas (Pellegrini et al. 2013). Thus, *C. singularis* is here regarded as a synonym of *T. diuretica*. In accordance to the *Code* (McNeill et al., 2012, Art. 9.8), in order to avoid future confusions and to fix the application of this name, we herein designate an epitype for *C. singularis*.

Discussion

Our work has reaffirmed the importance of thorough descriptions, fieldwork, photographs, spirit collections, and cultivation of specimens to better understand the taxonomy and systematics of intricate genera such as Commelina. This genus in particular poses problems as floral characters are difficult to observe in dried specimens (e.g. Faden 1993, 2008, 2012; Nampy et al. 2013), which calls for particular attention to be paid to adequate description of these in any new species (Faden 2008). Historically there are examples where either floral, fruit, or seed characters are only incompletely described, or even omitted. In some cases, the available specimens possess such strikingly different habit or vegetative characters, that the name can be easily applied (e.g. Faden et al. 2009). Nevertheless, in most cases, the lack of appropriately detailed description may cause confusion or prevent identification and the application of a correct name. Capturing the range of a species' phenotype is also important and population studies have shown to be of great use, especially in the Neotropical species, allowing us to record and compare wide ranges of morphology. The description of new taxa, based on few and odd specimens needs to be carefully considered, and is a strategy that tends to inflate the description of unnecessary or invalid new species and infraspecific taxa.

Characters such as inflorescence position and morphology, spathe shape and conation, petal and fruit morphology, and seed ornamentation play important roles in species distinction and delimitation, in Commelina. Nevertheless, characters once thought to be useful in species delimitation such as indumenta and leaf shape have shown to be highly variable within the same species and thus not completely reliable. This is easily observed in all Neotropical species, and most of the wide-spread species (e.g. C. benghalensis, C. diffusa, and C. erecta). Growth-form and subterraneous system morphology are also potentially interesting for the taxonomy of *Commelina* worldwide. On the other hand, most of the morphological characters pointed out by previous authors (e.g. Faden 1993, 2008; Nampy et al. 2013) as key to the taxonomy of the genus are mostly difficult to observe in herbaria specimens. Thus, work to expand and refine the morphological tools available to workers in this group should be ongoing. It is also apparent that some species still need further systematic study in order to clarify their boundaries. The Pantropical C. diffusa complex is poorly understood in the Neotropical region and is probable that more than one species, being treated under the widely polymorphic C. diffusa subsp. diffusa. The C. obliqua and C. rufipes complexes also need critical attention. The C. rufipes complex seems to be exclusively Neotropical, while the *C. obliqua* complex is here confirmed to be Pantropical, reaching Asia. These two species groups are historically problematic, and many names have been accommodated under one concept or another, depending on the author. It is very likely that both complexes will need to be studied concomitantly in order to fully understand their phylogenetic history, taxonomy and nomenclature.

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RESEARCH ARTICLE



Phylogenetic analyses place the monotypic Dryopolystichum within Lomariopsidaceae

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Abstract

The monotypic fern genus Dryopolystichum Copel. combines a unique assortment of characters that obscures its relationship to other ferns. Its thin-walled sporangium with a vertical and interrupted annulus, round sorus with peltate indusium, and petiole with several vascular bundles place it in suborder Polypodiineae, but more precise placement has eluded previous authors. Here we investigate its phylogenetic position using three plastid DNA markers, *rbcL*, *rps4-trnS*, and *trnL-F*, and a broad sampling of Polypodiineae. We also provide new data on Dryopolystichum including spore number counts, reproductive mode, spore SEM images, and chromosome counts. Our maximum-likelihood and Bayesian-inference phylogenetic analyses unambiguously place Dryopolystichum within Lomariopsidaceae, a position not previously suggested. Dryopolystichum was resolved as sister to a clade comprising Dracoglossum and Lomariopsis, with Cyclopeltis as sister to these, but clade support is not robust. All examined sporangia of Dryopolystichum produced 32 spores, and the chromosome number of sporophyte somatic cells is ca. 164. Flow cytometric results indicated that the genome size in the spore nuclei is approximately half the size of those from sporophyte leaf tissues, suggesting that Dryopolystichum reproduces sexually. Our findings render Lomariopsidaceae as one of the most morphologically heterogeneous fern families. A recircumscription is provided for both Lomariopsidaceae and Dryopolystichum, and selected characters are briefly discussed considering the newly generated data.

* Equal contribution

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Keywords

Fern, morphology, Papua New Guinea, phylogeny, recircumscription, taxonomy, the Solomon Islands

Introduction

Dryopolystichum Copel., with its single species *D. phaeostigma* (Ces.) Copel., is distributed along streams in lowland forests in New Guinea, the Bismarck Archipelago, and the Solomon Islands (Copeland 1947; Fig. 1A). Christensen (1937) was the first to point out that *D. phaeostigma* had been independently described under three different genera or subgenera. All told, generic placements has included *Aspidium* (\equiv *Tectaria*) (Cesati 1877, Baker 1891), *Dryopteris* (Christensen 1906, Alderwerelt van Rosenburgh 1908, Copeland 1911, Brause 1920, Alderwerelt van Rosenburgh 1924), and *Polystichum* (Rosenstock 1911). Copeland (1947) inaugurated the new monotypic genus *Dryopolystichum* in his *Genera Filicum*, and argued that it was closest to *Ctenitis*. Pichi Sermolli (1977) agreed, citing the ctenitoid rachis, free venation, and peltate indusium as critical characters. Holttum included the genus in his "Tectarioid Group" in his list of Malaysian pteridophytes (Holttum 1959), but then omitted it in his 1991 treatment of that group.

Although Copeland did not provide an etymological explanation, the name *Dry-opolystichum* presumably reflects the combination of peltate indusium (which is similar to those of polystichoid ferns) and pinnate-pinnatifid lamina division (which is similar to that of most *Dryopteris*). Such a combination of characters resulted in taxonomic confusion giving that peltate indusia are never found in *Dryopteris*, and the laminae of *Dryopolystichum* do not include prominulous segment apices, the hallmark of polystichoid ferns (Little and Barrington 2003). A peltate indusium is diagnostic of polystichoid ferns, including *Phanerophlebia* and *Polystichum*, but also found in a few distantly related genera in Polypodiineae such as *Cyclodium*, *Cyclopeltis*, *Rumohra*, *Megalastrum*, and *Tectaria* (Kramer and Green 1990).

Despite recent advances in fern phylogenetics and classification, the position of *Dryopolystichum* remains unclear. The thin-walled sporangium with a vertical and interrupted annulus, round sorus, and petiole with several vascular bundles suggest that this genus belongs to suborder Polypodiineae (= eupolypods I) (Sundue and Rothfels 2014, PPG I 2016). However, the remaining prominent features including pinnate-pinnatifid leaf dissection (Fig. 1B), peltate indusium (Fig. 1C), catadromous free veins (Fig. 1D), and sulcate rachis-costa architecture (Fig. 1E), do not clearly place it within any Polypodiinea family (Christensen 1937, Copeland 1947).

One other conspicuous character of *Dryopolystichum* not emphasized by previous authors is that the distal pinnae are decurrent onto the rachis, and the basal pinnules of its distal pinnae are served by veins that emerge from the rachis, rather than the pinna costa (Fig. 1D). This character is relatively uncommon in the Polypodiineae. It can be found in Dryopteridaceae, mostly in *Megalastrum*, and less commonly in *Stigmatopteris*, *Ctenitis*, and *Pleocnemia* (Moran et al. 2014, Moran and Labiak 2016).



Figure 1. Dryopolystichum phaeostigma (based on SITW10443). A Habitat B Plants C Peltate indusiaD Venation E Sulcate rachis-costa architecture F Longitudinal section of the rhizome.

It can also be found in some Tectariaceae such as *Pteridrys* and *Tectaria* (Ding et al. 2014). Among these genera, *Pleocnemia* seems morphologically the most similar to *Dryopolystichum* because its rachises are adaxially sulcate and narrowly winged laterally. *Pleocnemia*, however, lacks a peltate indusium (Holttum 1974).

Subsequent to its establishment as a new genus in *Genera Filicum* (Copeland 1947), and Sermolli's (1977) contribution, no other substantial argument was made for generic placement of *Dryopolystichum*. More recent studies maintained *Dryopolystichum* as a distinct genus, placing it under Dryopteridaceae (Kramer and Green 1990, Smith et al. 2006, Christenhusz et al. 2011). The recently published community-derived classification for extant lycophytes and ferns also places *Dryopolystichum* in the Dryopteridaceae but without assigning it to subfamily (PPG I 2016).

To resolve the phylogenetic placement of *Dryopolystichum*, we employ a molecular phylogenetic approach using three chloroplast DNA regions, *rbcL*, *rps4-trnS*, and *trnL-F*. Based on our observations, we further provide new data on *Dryopolystichum* including spore counts, reproductive mode, spore SEM images, and a chromosome count. Finally, we discuss its diagnostic characters in the light of the inferred phylogeny.

Materials and methods

We examined the morphology of *Dryopolystichum phaeostigma* using material collected from the Solomon Islands (*Braithwaite R.S.S.* 4557, SING; *SITW10443*, BSIP, TAIF, TNM) and Papua New Guinea (*James & Sundue 1688*, BISH, LAE, VT).

Living plants of *SITW10443* were transplanted to the Dr. Cecilia Koo Botanic Conservation Center in Taiwan (KBCC). The collection of *SITW10443* was made under the "Census and Classification of Plant Resources in the Solomon Islands" project (http://siflora.nmns.edu.tw/). Mitotic chromosomes were counted from these cultivated plants following the protocol of Chen et al. (2014).

Fertile pinnae of *SITW10443* were air-dried in an envelope for one day to release the spores. The spores were observed and measured by a tabletop scanning electron microscope (TM-3000 Hitachi, Ibaraki, Japan). The sizes (the length of equatorial axes including the perine ornamentation) of 35 randomly selected spores were measured. Five intact sporangia were observed under a stereo microscope (Leica MZ6, Wetzlar, Germany) to count the number of spores per sporangium.

The genome sizes of spore and leaf nuclei of *SITW10443* were examined by flow cytometry in order to infer the reproductive mode (Kuo et al. 2017). The genome size of spore nuclei should be half the genome size of leaf nuclei in the case of sexual and the same size in the case of apomictic reproduction (Kuo et al. 2017). We followed Kuo et al. (2017) for the extraction of leaf nuclei. For extraction of spore nuclei, we used an optimized bead-vortexing treatment with vertex duration of 1 minute and vertex speed of 1,900 rpm, as described by Kuo et al. (2017). An external standard was not necessary since we only need to compare the two phases of the life-cycle to each other.

DNA extraction, amplification and sequencing

Total DNA was extracted using a modified CTAB-Qiagen column protocol (Kuo 2015). Three plastid DNA regions, *rbcL*, *rps4-trnS* (*rps4* gene + *rps4-trnS* intergenic spacer), and *trnL-F* (*trnL* gene + *trnL-trnF* intergenic spacer), were amplified and sequenced using the primers "ESRBCL1F" and "1379R" for *rbcL* (Pryer et al. 2001, Schuettpelz and Pryer 2007), "RPS5F" and "TRNSR" for *rps4-trnS* (Nadot et al. 1995, Smith and Cranfill 2002), and "FernL 1Ir1" and "f" for *trnL-F* (Taberlet et al. 1991, Li et al. 2010).

The PCR amplifications were performed in 16 μ l reactions containing ca. 10 ng template DNA, 1×Taq DNA Polymerase Master Mix RED solution (Ampliqon, Den-

mark), and 1 µl each of 10 µM primers. The PCR reactions were carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, California, USA). Thermocycling conditions were the same for PCRs of these three regions and comprised an initial denaturation of 2 minutes at 94°C followed by a core sequence of 35 repetitions of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute followed by a final extension of 10 minutes at 72°C. Resulting PCR products were sequenced using the same PCR primers with BigDyeTM terminator (Applied Biosystems, Carlsbad, California, USA). The newly generated sequences were deposited in GenBank. GenBank accession numbers and voucher information are provided in Appendix.

DNA alignment and phylogenetic analyses

Initial BLAST against the NCBI nucleotide database (Altschul et al. 1990) based on *rbcL* sequences indicated that *Dryopolystichum phaeostigma* is closely related to the species of Polypodiineae families, including Lomariopsidaceae, Nephrolepidaceae, Tectariaceae, and Dryopteridaceae. Accordingly, we assembled a data matrix including 250 species representing 36 genera from these families (Appendix). Sampling included all the four genera in which *D. phaeostigma* has been placed (i.e., *Dryopteris, Polystichum*, and *Tectaria*).

Sequences were aligned using Geneious v6.1.8 (Drummond et al. 2011) and then manually checked for errors. The three single-region (*rbcL*, *rps4-trnS*, and *trnL-F*) and dataset combining all three were independently subjected to both maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. Data matrices are available in TreeBASE, study number 20506, at https://treebase.org/. ML tree searches were conducted using RAxML (Stamatakis 2006) employing the GTRGAMMA substitution model through the CIPRES portal (Miller et al. 2010). Five independent searches for the 'best tree' and 1,000 bootstrap replicates were performed using a region-partitioned dataset. BI analyses were conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003) employing the same substitution model as in ML analysis. Each analysis consisted of two independent runs with four chains for 10⁶ generations, sampling one tree every 1000 generations. Burn-in was set to 10000 based on our preliminary analysis. The convergences of MCMC runs were checked using Tracer v.1.6 (Rambaut et al. 2014).

We addressed the possibility of phylogenetic bias due to long branches following the recommendation of Siddal and Whiting (1999). Since *Dracoglossum* and *Lomariopsis* were resolved on long branches in preliminary analyses (not shown), we conducted two additional analyses in which each one of the two long-branched genera, *Dracoglossum* and *Lomariopsis*, was excluded to examine whether phylogenetic placement and branch support for *Dryopolystichum*'s placement changed. Since maximum parsimony (MP) phylogeny is considered to be more susceptible to long-branch attraction (Philippe et al. 2005), we analyzed the concatenated dataset under MP in order to compare those results with our ML phylogeny. The MP analyses were conducted using TNT (Goloboff et al. 2008) following the search strategy detailed in Sundue et al. (2014).

Results

Phylogenetic analyses

All phylogenies resolved Dryopolystichum phaeostigma single-region in Lomariopsidaceae, but with two slightly different topologies. The *rbcL* and rps4-trnS phylogenies placed D. phaeostigma sister to a clade of Dracoglossum + *Lomariopsis* with 93% and 72% maximum likelihood bootstrap percentages (BS), respectively (Suppl. materials 2, 3). In comparison, the trnL-F phylogeny placed D. phaeostigma sister to Cyclopeltis (BS = 74%), and Dryopolystichum + Cyclopeltis was sister to Dracoglossum + Lomariopsis (Suppl. material 4). There was no strongly supported conflict between the ML and BI phylogenies (Suppl. materials 1-4). Both the ML and BI phylogenies based on the combined dataset (Fig. 2, Suppl. material 1) reveal the same topology as those based on the *rbcL* and *rps4-trnS* regions. Bootstrap support and posteriori probability (PP) for the above relationships were generally very high except for the branches placing D. phaeostigma, where BS was \leq 70% and PP were \leq 0.9 in all the phylogenies.

Removing *Dracoglossum* from the analysis had little effect on the topology within Lomariopsidaceae, and BS supports for the generic placement of *Dryopolystichum* remained low (\leq 70%, data not shown). In contrast, the removal of *Lomariopsis* resulted in higher BS values for all clades within Lomariopsidaceae (\geq 99%, data not shown). MP analyses also resulted in a clade comprising all the Lomariopsidaceae genera and *Dryopolystichum*, but *Dryopolystichum* was resolved as sister to *Cyclopeltis* (data not shown).

Karyology, reproductive mode, and spore measurements

All examined sporangia (*SITW10443*) produced 32 normal spores, and the mean spore length was 64.1 \pm 4.5 μ m (Fig. 3). The chromosome number of the three sporophyte somatic cells observed was ca. 164 (Fig. 4). Results of flow cytometry revealed that the genome size of spore nuclei is approximately half of those of leaf nuclei (Fig. 5).

Discussion

Phylogenetic placement of Dryopolystichum

The reconstructed maximum likelihood and Bayesian inference phylogenies unambiguously resolved *Dryopolystichum* within Lomariopsidaceae (Fig. 2), a position not previously suggested (Kramer and Green 1990, Smith et al. 2006, Christenhusz et al. 2011, PPG I 2016). This placement is consistent in all our analyses. Nonetheless, the generic position of *Dryopolystichum* within Lomariopsidaceae remains poorly resolved.



Figure 2. Simplified maximum likelihood phylogram of Polypodiineae obtained from the *rbcL* + *rps4-trnS* + *trnL-F* combined dataset. Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) \ge 0.9. Original phylogram with support values for all the nodes is available in Suppl. materials 1. Voucher information and GenBank accession numbers are shown in Appendix.

This uncertainty may be partially explained by the incongruence between *trnL-F* and the other analyzed regions, but our process of removing the long-branched genera showed that low BS was retrieved only when *Dryopolystichum* and *Lomariopsis* were



Figure 3. Spores SEM of *Dryopolystichum phaeostigma*. **A** Lateral view of the spore **B** Detail of surface. Scale bars: $\mathbf{A} = 50 \ \mu\text{m}$, $\mathbf{B} = 10 \ \mu\text{m}$.



Figure 4. Chromosome number of *Dryopolystichum phaeostigma*. **A** Chromosomes at mitosis metaphase, 2n = ca. 164 (*SITW10443*) **B** explanatory illustration of A. Scale bars = 10 μm.

both included in the analysis. These results may also be explained by the large amounts of missing data in *Lomariopsis*; 19 of the 25 species included were represented by trnL-F data alone. We recommend further phylogenetic study using an expanded dataset to resolve the intergeneric relationships within Lomariopsidaceae.



Figure 5. Relative DNA contents of *Dryopolystichum phaeostigma* spore and leaf nuclei inferred by flow cytometry.

Recircumscription of Lomariopsidaceae

Phylogenetic analyses using DNA sequences have served as the basis for redrawing fern classifications in the 21th century (Smith et al. 2006, Christenhusz et al. 2011, PPG I 2016). With respect to family circumscription, one of the most dramatically changed families is Lomariopsidaceae (Tsutsumi and Kato 2006, Schuettpelz and Pryer 2007, Christenhusz et al. 2013). Just prior to the molecular era, Lomariopsidaceae was treated as one of the largest fern families with six genera and over 500 species (e.g., Kramer and Green 1990) and was strongly supported by the following combination of characters: rhizomes with ventral root insertion, dictyosteles with elongate ventral meristeles, and dimorphic leaves where the fertile leaves had acrostichoid sori (Holttum and Hennipman 1959, Kramer and Green 1990).

Subsequent molecular phylogenetic analyses demonstrated that most genera previously treated in Lomariopsidaceae should be transferred to Dryopteridaceae (Tsutsumi and Kato 2006, Schuettpelz and Pryer 2007). The combination of characters uniting the former Lomariopsidaceae are now interpreted to have evolved multiple times, and to be correlated with dorsiventrality of the rhizome (Moran et al. 2010, McKeown et al. 2012). Meanwhile, *Cyclopeltis* was transferred from Dryopteridaceae to Lomariopsidaceae as suggested by molecular phylogeny (Schuettpelz and Pryer 2007), although it has none of the characters formerly used to circumscribe Lomariopsidaceae (Holttum and Hennipman 1959, Kramer and Green 1990).

More recently, the neotropical genus *Dracoglossum* was established (Christenhusz 2007) and later transferred to Lomariopsidaceae from Tectariaceae based on a molecular phylogeny (Christenhusz et al. 2013). This pattern was also unexpected since there are essentially no shared morphological characters by *Dracoglossum* and *Lomariopsis*, except for the ribbon-like gametophyte (R. C. Moran pers. com.). Our finding, that *Dryopolystichum* belongs to Lomariopsidaceae, comes as a further surprise. With these

Genera	Cyclopeltis	Dracoglossum	Dryopolystichum	Lomariopsis	Thysanosoria
Habit	terrestrial	terrestrial	terrestrial	hemiepiphyte	hemiepiphyte
Rhizome	erect	short creeping	erect	climbing	climbing
Frond division*	pinnate	simple	pinnate-pinnatifid	pinnate	pinnate
Pinnae articulation	articulate	_	not articulate	articulate	articulate
Venation	free	reticulate, with included veinlet	free	free	free
Rachis-costa architecture	prominent	prominent	grooved	grooved or flat	grooved
Sporangia	form rounded sori	form rounded sori	form rounded sori	acrostichoid	form rounded sori
Indusia	peltate if present	peltate if present	peltate	absent	absent
Perine ornamentation	broad folds	narrow crests	narrow crests	various	broad folds

Table I. Comparison of morphological characters of the five Lomariopsidaceae genera [based on Holttum and Hennipman (1959), Holttum (1991), Roubik and Moreno (1991), Moran (2000), Christenhusz (2007), Rouhan et al. (2007), and this study].

*matured plant, -not applicable

changes, Lomariopsidaceae is a family of five genera (*Cyclopeltis, Dracoglossum, Dryopol-ystichum, Lomariopsis*, and *Thysanosoria*) and ca. 70 species. As far as we can tell, none of the morphological traits commonly used unify these genera (Table 1). In the following paragraphs, we provide a recircumscription of both Lomariopsidaceae and *Dryopolysti-chum*, and then discuss selected characters in the light of our phylogenetic placement.

Taxonomic treatment

Lomariopsidaceae Alston, Taxon 5(2): 25. 1956.

Type. Lomariopsis Fée, Mém. Foug., 2. Hist. Acrostich.: 10. 1845.

Description. Habit erect, creeping, or climbing; rhizomes dictyostelic, the ventral meristele elongate in transverse section or not; scaly at least when young; scales non-clathrate, basally attached or shallowly peltate, margins entire, toothed, or ciliate; fronds monomorphic or dimorphic; petioles with multiple vascular bundles arranged in a U-shape; laminae simple, pinnate, or pinnate-pinnatifid, provided distally with proliferous buds or not; pinnae articulate to the rachis or not; veins free, ± parallel or pinnate; sori acrostichoid or discrete and then round, with peltate indusia or exindusiate; spores brown, olive or green, chlorophyllous or not, bilateral, monolete, perine loosely attached, variously winged or ornamented.

Five genera and an estimated 70 species. *Thysanosoria* is included based on its morphological similarity to *Lomariopsis* (Holttum and Hennipman 1959), but it has not been, to the present, subject to molecular phylogenetic analysis.

Dryopolystichum Copel., Gen. Fil. 125, t. 4. 1947.

Type. Dryopolystichum phaeostigma (Ces.) Copel., Gen. Fil. 125, t. 4. 1947.

Description. Habit terrestrial, on slopes along streams at lowland forests; rhizome short erect, stout and woody, apex densely scaly, blackish sclerenchyma strands visible in sections; scales dark brown, linear-lanceolate, entire, not clathrate; fronds approximate, stipe not articulate, scaly at base, scales similar to those on rhizome; lamina ovate, pinnate-pinnatifid, catadromous, subleathery, nearly glabrous, only very sparse narrow scales on rachis, costa, and costule; rachis and costa grooved adaxially, not connected to each other; veins free, pinnate, veins of basal pinnules on upper pinnae emerge from the rachis rather than costa, all veins terminating in a prominent hydathode, not reaching frond margin; sori round, dorsally on veinlets near hydathode, indusiate; indusia round, persistent, superior, entire, brownish, thick; sporangia long-stalked, annulus with ca. 14 indurated cells, 32 normal spores in each sporangium; spores monolete, $64.1 \pm 4.5 \mu m$ in lateral view, surface with broadly winged wall; 2n = ca. 164.

Monotypic.

Dryopolystichum phaeostigma (Ces.) Copel., Gen. Fil. 125, t. 4. 1947.

Aspidium phaeostigma Ces., Rend. Ac. Napoli 16: 26, 29. 1877. Type. Papua New Guinea. Andai, Beccari 12533 (FI [FI013622]). Dryopteris phaeostigma (Ces.) C.Chr., Index Filic. 284. 1905 Type. Based on Aspidium phaeostigma Ces. Dryopteris tamatana C.Chr., Index Filic., Suppl. (1906-1912) 40. 1913. Replaced: Dryopteris kingii Copel., Phillipp. J. Sci., C 6: 73. 1911., not Dryopteris kingii (Bedd.) C.Chr., Index Filic. 273. 1905. Type. Papua New Guinea. Tamata, C. King 149 (MICH [MICH1287049]). Polystichum lastreoides Rosenst., Repert. Spec. Nov. Regni Veg. 9: 425. 1911. Type. Papua New Guinea. C. King 194 (MICH [MICH1190927]). Dryopteris ledermannii Brause, Bot. Jahrb. Syst. 56: 90. 1920. Type. Papua New Guinea. Sepik, Ledermann 9619 (B [B_20_005865], L [L0063060], S [S-P-8581]). Dryopteris cyclosorus Alderw., Nova Guinea 14: 21. 1924. Type. Indonesia. Irian Jaya, H. J. Lam 1086 (BO [BO1529719, BO1529720], K [K000666126], L [L0051583], U [U0007385]).

Type. Based on Aspidium phaeostigma Ces.

Description. Equal to the genus.

Distribution. New Guinea, the Bismark archipelago, and the Solomon Islands.

Comparison of selected characters of Dryopolystichum

Perine architecture of *Dryopolystichum* is very similar to that of *Dracoglossum plan-tagineum* (Christenhusz 2007, Fig. 3). They are loosely attached, forming thin crests, and having a spiculate microstructure. Perine of *Cyclopeltis* and *Thysanosoria* are also similar in being loosely attached and having a spiculate microstructure, but they differ by having broader folds (Holttum and Hennipman 1959, Tryon and Lugardon 1991). The perine characters, however, are not shared by all the taxa of Lomariopsidaceae especially considering the variation of ornamentation existing in *Lomariopsis* (Rouhan et al. 2007). Moreover, these perine characters also appear in other Polypodiineae lineages particularly in bolbitidoid ferns (Moran et al. 2010) as well as in various Aspleniineae lineages (Sundue and Rothfels 2014, PPG I 2016).

Blackish sclerenchyma strands are visible in the rhizome sections of *Dryopolystichum* (Fig. 1F). These are also present in *Dracoglossum*, *Cyclopeltis*, and *Lomariopsis*, but similar characters are known from various groups throughout Polypodiineae (Hennipman 1977, Moran 1986, Hovenkamp 1998). Further studies might reveal variation in these strands to be of systematic value.

The rachis-costae architecture of *Dryopolystichum* is characterized by an adaxially sulcate rachis with grooves that do not connect to those of the pinna-costae. The rachis is also narrowly winged laterally. Both characters are seen in *Thysanosoria* and in some species of *Lomariopsis* (Holttum and Hennipman 1959, Moran 2000). In contrast, *Dracoglossum* and *Cyclopeltis* have non-winged and non-sulcate rachises (Holttum 1991, Christenhusz 2007).

The chromosome number in somatic cells of *Dryopolystichum phaeostigma* was ca. 164 (Fig. 4). The base numbers for Lomariopsidaceae genera (*Cyclopeltis, Dracoglossum*, and *Lomariopsis*) are 40 or 41 (Walker 1985, Kato and Nakato 1999, Moran 2000), suggesting that *D. phaeostigma* is a tetraploid.

Our flow cytometry and spore count results indicate that *Dryopolystichum phaeostigma* is sexually reproducing and has 32 spores per sporangium (Fig. 5). In Polypodiales, sporogenesis leading to the formation of 64 spores in a sporangium is by far the most common pattern of sexually reproducing species, e.g., Aspleniaceae (Gabancho et al. 2010), Athyriaceae (Kato et al. 1992, Takamiya et al. 1999), Davalliaceae (Chen et al. 2014), Dryopteridaceae (Lu et al. 2006), Polypodiaceae (Wang et al. 2011), Pteridaceae (Huang et al. 2006), and Thelypteridaceae (Ebihara et al. 2014). Cases of sporogenesis resulting in 32 spores per sporangium are known from a few Polypodiales ferns but all belong to the suborders Lindsaeineae and Pteridineae, i.e., Lindsaeaceae (Lin et al. 1990), Cystodiaceae (Gastony 1981), and *Ceratopteris* (Pteridaceae; Lloyd 1973). Our study provides the first confirmed case of a sexual reproduction with 32 spores per sporangium in the suborder Polypodiineae.

Conclusion

We have shown, based on molecular phylogenetic evidence, the placement of *Dry-opolystichum* within Lomariopsidaceae. A revised description was provided for both Lomariopsidaceae and *Dryopolystichum* resulting from a review of literature and our own observations. Future studies using an expanded dataset are necessary to resolve intergeneric relationships in Lomariopsidaceae.

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Appendix

Individuals sampled in this study. For each individual, the species name and GenBank accession numbers (*rbcL*, *rps4-trnS*, *trnL-F*) are provided. A n-dash (–) indicates unavailable information; new sequences are in bold.

Terror	Genbank accession numbers			
laxon	rbcL	rps4-trnS	trnL-F	
Dryopteridaceae				
Arachniodes aristata (G.Forst.) Tindale	KJ464418	-	KJ464592	
Arachniodes denticulata (Sw.) Ching	KJ464419	-	KJ464593	
Arthrobotrya articulata J.Sm.	-	GU376714	GU376565	
Arthrobotrya wilkesiana Copel.	-	GU376719	GU376569	
Bolbitis acrostichoides (Afzel.) Ching	KJ464420	GU376644	GU376500	
Bolbitis aliena (Sw.) Alston	-	GU376646	GU376502	
Bolbitis angustipinna (Hayata) H.Ito	_	GU376654	GU376509	
Bolbitis appendiculata (Willd.) K.Iwats.	-	GU376647	GU376503	
Bolbitis auriculata (Lam.) Alston	KJ464421	GU376649	GU376505	
Bolbitis bipinnatifida (J.Sm.) K.Iwats.	-	GU376676	GU376530	
Bolbitis fluviatilis (Hook.) Ching	-	GU376656	GU376510	
Bolbitis gemmifera (Hieron.) C.Chr.	-	GU376657	GU376511	
Bolbitis heteroclita (Pr.) Ching	_	GU376659	GU376513	
Bolbitis heudelotii (Bory) Alston	_	GU376662	GU376515	
Bolbitis humblotii (Baker) Ching	KJ464422	GU376663	GU376516	
Bolbitis lonchophora (Kunze) C.Chr.	_	GU376664	GU376517	
Bolbitis major (Bedd.) Hennipman	-	GU376665	GU376518	
Bolbitis portoricensis (Sprengel) Hennipman	_	GU376670	GU376523	
Bolbitis salicina (Hook.) Ching	-	GU376671	GU376525	
Bolbitis semipinnatifida (Fée) Alston	-	GU376672	GU376526	
Bolbitis serratifolia (Mertens) Schott	-	GU376673	GU376527	
Bolbitis sinuata (C.Presl) Hennipman	-	GU376675	GU376529	
Bolbitis tibetica Ching & S.K.Wu	-	GU376677	GU376531	
Ctenitis eatonii (Baker) Ching	KF709483	-	KJ196645	
Ctenitis sinii (Ching) Ohwi	-	-	KJ196643	
Ctenitis subglandulosa (Hance) Ching	-	-	KJ196655	
Ctenitis yunnanensis Ching & Chu H.Wang	-	-	KJ196715	
Cyclodium heterodon (Schrad.) T. Moore var. heterodon	KJ464425	-	KJ464596	
Cyclodium rheophilum A.R.Sm.	KJ464426	-	KJ464597	
Dryopteris apiciflora (Wall. ex Mett.) Kuntze	-	-	KJ196641	
Dryopteris christensenae (Ching) Li Bing Zhang	_	_	KJ196679	
Dryopteris heterolaena C.Chr.	-	-	KJ196623	
Dryopteris integriloba C.Chr.	-	_	KJ196701	
Dryopteris mariformis Rosenst.	-	-	KJ196686	
Dryopteris nidus (Baker) Li Bing Zhang	-	-	KJ196687	
Dryopteris patula (Sw.) Underw.	KJ464427	-	KJ464598	
Dryopteris polita Rosenst.	_	-	KJ196700	
Dryopteris squamiseta (Hook.) Kuntze	_	GU376678	KJ196632	

T		Genbank accession numbers			
laxon	rbcL	rps4-trnS	trnL-F		
Dryopteris wallichiana (Spreng.) Hyl.	KJ464428	GU376680	KJ464599		
Elaphoglossum amygdalifolium (Mett.) Christ	_	GU376681	_		
Elaphoglossum burchellii (Baker) C.Chr.	_	GU376682	GU376533		
Elaphoglossum decoratum (Kunze) T.Moore	KJ464429	GU376683	KJ464600		
Elaphoglossum guentheri Rosenst.	_	GU376684	GU376535		
Elaphoglossum langsdorffii T.Moore	_	GU376685	GU376536		
Elaphoglossum lloense (Hook.) T.Moore	_	GU376686	GU376537		
Elaphoglossum luridum Christ	_	-	GU376538		
Elaphoglossum squamipes (Hook.) T.Moore	_	-	GU376539		
Lastreopsis amplissima (C.Presl) Tindale	KJ464432	_	KJ464604		
Lastreopsis decomposita (R.Br.) Tindale	KJ464439	-	_		
Lastreopsis hispida (Sw.) Tindale	KJ464446	-	KJ464614		
Lastreopsis killipii (C.Chr. & Maxon) Tindale	KJ464448	KF709505	_		
Lastreopsis marginans (F.Muell.) Tindale	KJ464449	GU376691	KJ464616		
Lastreopsis poecilophlebia (Hook.) Labiak, Sundue & R.C.Moran	KJ464423	GU376692	KJ464594		
Lastreopsis tenera (R.Br.) Tindale	KJ464467	GU376699	KJ464636		
Lastreopsis tripinnata (F.Muell. ex Benth.) Labiak, Sundue & R.C.Moran	KJ464491	GU376700	_		
Lastreopsis walleri Tindale	KJ464472	GU376701	-		
Lastreopsis wurunuran (Domin) Tindale	KJ464474	GU376704	-		
Lomagramma brooksii Copel.	_	GU376705	GU376542		
Lomagramma cordipinna Holttum	_	GU376707	GU376543		
Lomagramma lomarioides (Blume) J.Sm.	_	_	GU376550		
Lomagramma matthewii (Ching) Holttum	KJ464476	-	KJ464640		
Lomagramma perakensis Bedd.	_	-	GU376552		
Lomagramma pteroides J.Sm.	_	_	GU376555		
Lomagramma sinuata C.Chr.	-	-	GU376556		
Lomagramma sumatrana Alderw.	-	-	GU376558		
Maxonia apiifolia (Sw.) C.Chr.	KJ464477	GU376709	KJ464641		
Megalastrum abundans (Rosenst.) A.R.Sm. & R.C.Moran	KJ464478	-	KJ464642		
Megalastrum atrogriseum (C.Chr.) A.R.Sm. & R.C.Moran	KJ464479	GU376710	KJ464643		
Megalastrum connexum (Kaulf.) A.R.Sm. & R.C.Moran	KJ464481	-	KJ464645		
Megalastrum lanatum (Fée) Holttum	KJ464483	_	KJ464647		
Megalastrum littorale R.C.Moran, J.Prado & Labiak	_	GU376651	GU376561		
Megalastrum macrotheca (Fée) A.R.Sm. & R.C.Moran	KJ464484	GU376697	KJ464648		
Megalastrum vastum (Kunze) A.R.Sm. & R.C.Moran	KJ464487	GU376658	KJ464651		
Mickelia bernoullii (Kuhn ex Christ) R.C.Moran, Labiak & Sundue	-	GU376666	GU376506		
Mickelia guianensis (Aubl.) R.C.Moran, Labiak & Sundue	-	GU376667	GU376548		
Mickelia hemiotis (Maxon) R.C.Moran, Labiak & Sundue	_	_	GU376512		
Mickelia nicotianifolia (Sw.) R.C.Moran, Labiak & Sundue	_	KF667557	GU376519		
Mickelia oligarchica (Baker) R.C.Moran, Labiak & Sundue	KJ464489	_	GU376520		
Mickelia scandens (Raddi) R.C. Moran, Labiak & Sundue	-	GU376696	GU376547		
Olfersia cervina Kunze	KJ464493	DQ153079	KJ464652		
Parapolystichum acuminatum (Houlston) Labiak, Sundue & R.C.Moran	KJ464430	KC977454	KJ464601		
Parapolystichum boivinii (Baker) Rouhan	KJ464435	_	KJ464607		
Parapolystichum confine (Maxon ex C.Chr.) Labiak, Sundue & R.C.Moran	KJ464438	-	—		

T		Genbank accession numbers		
laxon	rbcL	rps4-trnS	trnL-F	
Parapolystichum effusum (Sw.) Ching	KJ464441	_	_	
Parapolystichum effusum (Sw.) Ching subsp. divergens (Willd. ex Schkuhr) Tindale	KJ464440	_	_	
Parapolystichum excultum (Mett.) Labiak, Sundue & R.C.Moran	_	KF709501	GU376541	
Parapolystichum glabellum (A.Cunn.) Labiak, Sundue & R.C.Moran	KJ464445	KF709503	KJ464613	
Parapolystichum microsorum (Endl.) Labiak, Sundue & R.C.Moran	KJ464451	GU376712	KJ464617	
Parapolystichum perrierianum (C.Chr.) Rouhan	KJ464455	_	KJ464623	
Parapolystichum rufescens (Blume) Labiak, Sundue & R.C.Moran	KJ464461	_	KJ464629	
Parapolystichum vogelii (Hook.) Rouhan	KJ464470			
Parapolystichum windsorensis (D.L.Jones & B.Gray) Labiak, Sundue & R.C.Moran	KJ464473	_	KJ464639	
Pleocnemia conjugata C.Presl	_	GU376713	KF709510	
Pleocnemia cumingiana C.Presl	KJ196828	_	KJ196705	
Pleocnemia dahlii (Hieron.) Holttum	KJ196829	_	KJ196706	
Pleocnemia hemiteliiformis (Racib.) Holttum	KF709482	KF667560	KF709511	
Pleocnemia irregularis (C.Presl) Holttum	KF709491	_	KF709513	
Pleocnemia leuzeana (Gaudich.) C.Presl	KJ196830	_	_	
Pleocnemia olivacea (Copel.) Holttum	KJ464495	_	_	
Pleocnemia presliana Holttum	KJ464496	KF667561	-	
Pleocnemia rufinervis Nakai	JF303976	KF667562	_	
Pleocnemia winitii Holttum	EF460686	_	KF709515	
Polybotrya alfredii Brade	KJ464497	KF667563	KJ464653	
Polybotrya andina C.Chr.	KJ464498	KP271084	KJ464654	
Polybotrya pubens Mart.	KJ464499	KP271085	-	
Polystichum tsus-simense (Hook.) J.Sm. var. mayebarae (Tagawa) Sa.Kurata	AB575224	_	DQ150408	
Pseudotectaria biformis (Mett.) Holttum	-	_	KF897951	
Pseudotectaria decaryana (C.Chr.) Tardieu	-	_	KF897952	
Rumohra adiantiformis (G.Forst.) Ching	KJ464500	_	KJ464655	
Rumohra berteroana (Colla) J.J. Rodr.	KJ464503	_	KJ464657	
Stigmatopteris ichthiosma (Sodiro) C.Chr.	KJ464504	_	KJ464658	
Stigmatopteris killipiana Lellinger	KJ464505	_	KJ464659	
Stigmatopteris lechleri (Mett) C.Chr.	KJ464506	KP271087	KJ464660	
Stigmatopteris sordida (Maxon) C.Chr.	KJ464507	_	KJ464661	
Teratophyllum koordersii Holttum	-	_	GU376566	
Teratophyllum ludens (Fée) Holttum	-	_	GU376567	
Teratophyllum wilkesianum Holttum	KJ464508	_	_	
Nephrolepidaceae				
Nephrolepis abrupta (Bory) Mett.	HM748137	KF667559	_	
Nephrolepis acutifolia (Desv.) Christ.	HM748139	_	_	
Nephrolepis biserrata (Sw.) Schott	AB575227	GU376688	_	
Nephrolepis brownii (Desv.) Hovenkamp & Miyam.	KR816691	-	_	
Nephrolepis cordifolia (L.) C.Presl	AB575228	_		
Nephrolepis davalliae Alderw.	HM748147	_		
Nephrolepis davallioides Kunze	HM748148	GU376690	_	
Nephrolepis exaltata (L.) Schott	HM748149	-	_	

	Genbank accession numbers			
laxon	rbcL	rps4-trnS	trnL-F	
Nephrolepis falcata (Cav.) C.Chr.	HM748150	_		
Nephrolepis falciformis J.Sm.	AB232404	_	_	
Nephrolepis lauterbachii (Christ) Christ	HM748153	_	_	
Nephrolepis pectinata (Willd.) Schott	HM748155	_		
Nephrolepis pendula (Raddi) J.Sm.	HM748156	_	_	
Nephrolepis radicans (Burm.) Kuhn	HM748157	_	_	
Nephrolepis rivularis (Vahl) Mett.	HM748158	-	_	
Nephrolepis undulata J.Sm.	HM748159	-	_	
Lomariopsidaceae				
Cyclopeltis crenata (Fée) C.Chr.	DQ054517	EF540718	DQ51448	
Cyclopeltis novoguineensis Rosenst.	KY397974	KY397978	KY397970	
Cyclopeltis semicordata (Sw.) J.Sm.	EF463234	KY397977	KY397969	
Dracoglossum plantagineum (Jacq.) Christenh.	KC914564	KY397979	KY397971	
Dracoglossum sinuatum (Fée) Christenh.	-	_	KU605106	
Dryopolystichum phaeostigma (Ces.) Copel.	KY397972	KY397976	KY397968	
Lomariopsis crassifolia Holttum	_	_	DQ396559	
Lomariopsis guineensis (Underw.) Alston	_	KJ628952	DQ396560	
Lomariopsis hederacea Alston	_	_	DQ396561	
Lomariopsis jamaicensis (Underw.) Holttum	_	_	DQ396562	
Lomariopsis japurensis (C.Martius) J.Sm.	-	_	DQ396563	
Lomariopsis kunzeana (Underw.) Holttum	_	_	DQ396569	
Lomariopsis latipinna Stolze	_	_	DQ396571	
Lomariopsis lineata (C.Presl) Holttum	-	-	DQ396572	
Lomariopsis longicaudata (Bonap.) Holttum	_	_	Q396573	
Lomariopsis madagascarica (Bonap.) Alston	_	-	DQ396575	
Lomariopsis mannii (Underw.) Alston	-	_	DQ396577	
Lomariopsis marginata (Schrad.) Kuhn	AY818677	-	DQ396578	
Lomariopsis maxonii (Underw.) Holttum	-	-	DQ396580	
Lomariopsis muriculata Holttum	-	-	DQ396582	
<i>Lomariopsis palustris</i> (Hook.) Mett. ex Kuhn	-	HM748162	DQ396585	
Lomariopsis pervillei Kuhn	-	-	DQ396586	
Lomariopsis pollicina (Willemet) Mett. ex Kuhn	EF463235	_	DQ396588	
Lomariopsis prieuriana Fée	-	-	DQ396590	
Lomariopsis recurvata Fée	-	-	DQ396592	
Lomariopsis rossii Holttum	-	-	DQ396594	
Lomariopsis salicifolia (Kunze) Lellinger	-	-	DQ396595	
Lomariopsis sorbifolia (L.) Fée	EF463236	-	-	
Lomariopsis spectabilis Mett.	AB232401	_	KJ196685	
Lomariopsis vestita E.Fourn.	-	-	DQ396598	
Lomariopsis wrightii Mett.	-	_	DQ396600	
Tectariaceae				
Arthropteris altescandens J.Sm.	KF667636	KF667550	KF667606	
Arthropteris articulata (Brack.) C.Chr.	KC977367	KC977437	KC977411	
Arthropteris beckleri (Hook.) Mett.	U05605	_	KF667607	
Arthropteris cameroonensis Alston	KF667638	_	_	

T	Genbank accession numbers		
laxon	rbcL	rps4-trnS	trnL-F
Arthropteris guinanensis H.G.Zhou & Y.Y.Huang	KC977364	KC977442	KC977404
Arthropteris monocarpa (Cordem.) C.Chr.	HM748132	_	KF897941
Arthropteris orientalis (Gmel.) Posth.	HM748133	KC977435	KC977420
Arthropteris palisotii (Desv.) Alston	AB575230	KC977427	KC977406
Arthropteris parallela (Baker) C.Chr.	EF463266	KC977453	KC977425
Arthropteris paucivenia (C.Chr.) H.M.Liu, Hovenkamp & H.Schneid.	EF463268	_	KC977426
Arthropteris repens (Brack.) C.Chr.	KC977368	KC977438	KC977412
Arthropteris tenella (G.Forst.) J.Sm. ex Hook.f.	KC977363	KF011547	KC977424
Hypoderris brauniana (H.Karst.) F.G.Wang & Christenh.	KF667647	_	KF667618
Hypoderris brownii J.Sm.	KF667642	_	KF667611
Hypoderris nicotianifolia (Baker) R.C.Moran, Labiak & J.Prado	KF667653	_	KF667626
Pteridrys australis Ching	KJ196892	_	KJ196678
Pteridrys cnemidaria (Christ) C.Chr. & Ching	KF709488	_	KF709517
Pteridrys lofouensis (Christ) C.Chr. & Ching	EF460687	KF667566	_
Pteridrys microthecia (Fée) C.Chr. & Ching	KJ196848	_	KF709518
Pteridrys syrmatica (Willd.) C.Chr. & Ching	KJ196875	_	KF709519
Tectaria acerifolia R.C.Moran	KF887170	_	KF897954
Tectaria angulata (Willd.) Copel.	KJ196876	_	KJ196656
Tectaria aurita (Sw.) S.Chandra	KJ196849	_	KJ196631
Tectaria barberi (Hook.) Copel.	KJ196846	_	KJ196628
Tectaria borneensis S.Y.Dong	KJ196854	KF667555	KJ196642
Tectaria cicutaria (L.) Copel.	KF667649	_	KF667620
Tectaria coadunata (J.Sm.) C.Chr.	KJ196851	_	KJ196661
Tectaria crenata Cav.	KF667650	KF667568	KF667621
Tectaria decurrens (C.Presl) Copel.	AB575232	_	DQ514524
Tectaria devexa (Kunze ex Mett.) Copel.	AB575233	KP271088	KF897956
Tectaria dilacerata (Kunze) Maxon	KF887173	_	KF897957
Tectaria fauriei Tagawa	AB575234	_	KJ196658
Tectaria fernandensis C.Chr.	KF887174	_	KF897958
Tectaria gigantea (Blume) Copel.	KJ196853	_	KJ196660
Tectaria griffithii (Baker) Ching	KF667652	_	KF667624
Tectaria grossedentata Ching & Chu H.Wang	KJ196882	KP271089	KJ196667
Tectaria harlandii (Hook.) C.M.Kuo	AB575231	_	KJ196648
Tectaria harlandii (Hook.) C.M.Kuo	KF887178	_	KF897961
Tectaria heracleifolia (Willd.) Underw.	KF887180	_	KF897963
Tectaria herpetocaulos Ching & Chu H. Wang	KJ196884	_	KJ196669
Tectaria heterocarpa C.V.Morton	KF887181	_	KF897964
<i>Tectaria impressa</i> (Fée) Holttum	KJ196841	_	KF897965
Tectaria kusukusensis (Hayata) Lellinger	EF460681	_	KF897968
Tectaria labrusca (Hook.) Copel.	KJ196818	_	KJ196692
Tectaria luchunensis S.K.Wu	KJ196845	KP271090	KJ196627
Tectaria macleanii (Copel.) S.Y.Dong	KJ196810	_	KJ196680
Tectaria melanocaula (Blume) Copel.	KJ196832	_	KJ196709
Tectaria morsei (Baker) P.J.Edwards ex S.Y.Dong	KJ196893	KF667570	KF561675

Tayon		Genbank accession numbers		
laxon	rbcL	rps4-trnS	trnL-F	
<i>Tectaria nayarii</i> Mazumdar	EF463267	_	KJ196699	
Tectaria paradoxa (Fée) Sledge	KF887189	_	KF897971	
Tectaria phaeocaulis (Rosenst.) C.Chr.	AB232397	KF709499	KF897972	
Tectaria pica (L.) C.Chr.	KF887191	GU376715	KF897973	
Tectaria polymorpha (Wall. ex Hook.) Copel.	KJ196888	GU376716	KJ196657	
Tectaria prolifera (Hook.) R.M.Tryon & A.F.Tryon	EF463273	_	KF897974	
Tectaria psomiocarpa S.Y.Dong	KJ196822	KF667572	KJ196698	
Tectaria pubens R.C.Moran	KF887193	KF667573	KF897975	
Tectaria quinquefida (Baker) Ching	KJ196885	_	KJ396622	
Tectaria repanda (Willd.) Holttum	KJ196831	_	KJ196707	
Tectaria sagenioides (Mett.) Christenh.	KF887194	KF667575	KF561672	
Tectaria semipinnata (Roxb.) Morton	KJ196817	KF667577	KJ196691	
Tectaria simonsii (Baker) Ching	AB575236	_	KF897977	
Tectaria singaporiana (Wall. ex Hook. & Grev.) Ching	KF887196	_	KF897978	
Tectaria subglabra (Holttum) S.Y.Dong	_	_	KJ196676	
Tectaria subsageniacea (Christ) Christenh.	KF887197	KF667576	KF561670	
Tectaria subtriphylla (Hook. & Arn.) Copel.	AB575237	_	KF897980	
Tectaria tricuspis (Bedd.) Copel.	KJ196820	_	KJ196694	
Tectaria variolosa (Wall. ex Hook.) C.Chr.	EF460690	_	KF897982	
Tectaria vasta (Blume) Copel.	KF667655	_	KF667628	
Tectaria vivipara Jermy & T.G. Walker	KF887201	_	KF897983	
Tectaria zeilanica (Houtt.) Sledge	AB232395	_	KF709521	
Triplophyllum crassifolium Holttum	KF887203	_	KF897985	
Triplophyllum fraternum (Mett.) Holttum	KF667657	_	KF667630	
Triplophyllum funestum (Kunze) Holttum	EF463276	_	KF667631	
Triplophyllum glabrum J.Prado & R.C.Moran		_	KF897989	
Triplophyllum heudelotii Pic.Serm.		_	KF897990	
Triplophyllum jenseniae (C.Chr.) Holttum	KF667660	_	KF667633	
Triplophyllum pentagonum (Bonap.) Holttum	KF667662	_	KF667635	
Triplophyllum pilosissimum (J.Sm. ex T.Moore) Holttum	_	_	KU605127	
Triplophyllum securidiforme (Hook.) Holttum	_	_	KU605128	
Triplophyllum vogelii (Hook.) Holttum	KF667661	_	KF667634	
Oleandraceae				
Oleandra articulata (Sw.) C.Presl	KF667644	KF709500	KF667613	
Oleandra cumingii I.Sm.	KI196816	_	KI196690	
Oleandra neriiformis Cav.	KI196815	_	KI196689	
Oleandra pilosa Hook.	KF667646	_	KF667615	
Davalliaceae				
Davallodes hirsuta (LSm.) Copel.	AY096196	_	_	
Davallodes vunnanensis (Christ) M.Kato & Tsutsumi	IX103718	KC914565	_	
Polypodiaceae	J			
Campyloneurum minus Fée	KF667665	_	_	
Microgramma lycopodioides (L.) Copel.	KF667664	_	_	
Niphidium longifolium (Cav.) C.V.Morton & Lellinger	KF667663	KF709495	_	

Supplementary material I

Figure S1. Maximum likelihood phylogram of Polypodiineae obtained from the combined (*rbcL* + *rps4-trnS* + *trnL-F*) dataset.

Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang

Data type: statistical data

- Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Supplementary material 2

Figure S2. Maximum likelihood phylogram of Polypodiineae obtained from the *rbcL* dataset.

Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang

Data type: statistical data

- Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.
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Supplementary material 3

Figure S3. Maximum likelihood phylogram of Polypodiineae obtained from the *rps4-trnS* dataset.

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Data type: statistical data

- Explanation note: Maximum likelihood phylogram of Polypodiineae obtained from the *rps4-trnS* dataset. Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.
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Supplementary material 4

Figure S4. Maximum likelihood phylogram of Polypodiineae obtained from the *trnL-F* dataset.

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Data type: statistical data

Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.

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RESEARCH ARTICLE



Adetogramma (Polypodiaceae), a new monotypic fern genus segregated from Polypodium

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Abstract

Polypodiaceae is one of the most diverse and abundant families of ferns in tropical and subtropical forests. Despite multiple studies investigating its phylogeny and taxonomy, several generic boundaries within the family still need clarification. One of the most problematic circumscriptions is that of *Polypodium* L., and one species that still contributes to this uncertainty is *Polypodium chrysolepis* Hook. The main goal of this study was to use molecular and morphological data to clarify the relationships of *P. chrysolepis* inside the polygrammoid clade. Sequences from three plastid regions (cpDNA – *rbc*L, *rps*4 and *rps*4–*trn*S IGS) from fifty species belonging to thirty-two genera of Polypodiaceae were analyzed using maximum likelihood and Bayesian inference. *Polypodium chrysolepis* constitutes an isolated lineage among the neotropical polygrammoid ferns, close to *Serpocaulon* and the grammitids, and is recognized here in a new genus. It can be distinguished by its entire leaves with free veins and peltate, pedicellate, lanceolate paraphyses. A new combination, *Adetogramma chrysolepis*, is proposed and a new taxonomic treatment is presented; its conservation status was assessed using IUCN Red List Categories and Criteria.

Keywords

Andes, Polypodiaceae, phylogeny, Serpocaulon, taxonomy

Introduction

Polypodiaceae is one of the richest fern families, and one of the most diverse and abundant groups of vascular plants in tropical and subtropical forests (Schneider et al. 2004). The current family circumscription (PPG I 2016) is based on many phylogenetic studies (e.g., Schneider et al. 2004, Schuettpelz and Pryer 2007). With this definition, Polypodiaceae includes the large segregate family, Grammitidaceae (sensu Parris 1990), and comprises 65 genera (PPG I 2016). Despite multiple studies investigating the phylogeny and taxonomy of Polypodiaceae, Smith et al. (2008) recognized that certain generic boundaries within the family still need clarification.

One of the most problematic circumscriptions is that of *Polypodium* L. (Smith et al. 2008). As recognized by Hennipman et al. (1990), *Polypodium* is polyphyletic, and several groups historically included in it (Hennipman et al. 1990) have been recognized as other genera [e.g., *Goniophlebium* (Blume) C.Presl, *Microgramma* C.Presl (Salino et al. 2008), *Pleopeltis* Willd., *Phlebodium* (R.Br.) J.Sm. (Otto et al. 2009), *Serpocaulon* A.R.Sm. (Smith et al. 2006a), *Synammia* C.Presl (Schneider et al. 2006)]. Nevertheless, recognition of all the above-cited genera still does not render *Polypodium* monophyletic (Schneider et al. 2004, Otto et al. 2009, Assis et al. 2016). In its latest circumscription (PPG I 2016), the genus is considered presumably monophyletic, but the groups that remain in it [*Polypodium dulce* group, *Polypodium plesiosorum* group, and *Polypodium colpodes* group (Tejero-Díez 2005, Sigel et al. 2014)], still need to be comprehensively included in phylogenetic studies.

One species that still contributes to this uncertainty is *Polypodium chrysolepis* Hook., a species occurring in the Andes from northern Argentina to Ecuador. The generic placement of this species has been controversial: it was described in *Polypodium* by Hooker (1844) in *Icones Plantarum*, tentatively placed in *Lepicystis* [now treated within *Pleopeltis* (Smith and Tejero-Díez 2014)] by Diels (1899), recognized as a distinct entity within *Polypodium* by de la Sota (1960) and placed in *Microgramma* by Crabbe (1967), following notes by A.H.G. Alston. The first molecular phylogenetic placement of *P. chrysolepis* (Schneider et al. 2004) showed that none of the above-mentioned generic positions are acceptable. In Schneider et al. (2004), *P. chrysolepis* was recovered as sister to the *Serpocaulon*+grammitids clade, and it is distantly related to the campyloneuroid clade (which includes *Campyloneurum*, *Microgramma*, and *Niphidium*) and to *Polypodium s.s.* clade. No formal taxonomic changes were proposed for *P. chrysolepis* by Schneider et al. (2004), as its position was ambiguous, and since then no new studies have been conducted on this species.

The main goal of this study was to employ molecular and morphological data to investigate the relationships of *Polypodium chrysolepis* within the polygrammoid clade, and to use available morphological and phylogenetic information to formally propose an adequate generic placement for this species in Polypodiaceae.

Material and methods

Taxon sampling

Fifty species from thirty-two genera (sensu PPG I 2016) of Polypodiaceae were included in our phylogenetic analyses (Appendix). *Davallia solida* (G.Forst.) Sw. (Davalliaceae) and *Oleandra pistillaris* (Sw.) C.Chr. (Oleandraceae) were used as outgroups, following Schneider et al. (2004). All vouchers and GenBank accessions are listed in the Appendix. Aligned data matrix was deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S20420).

Sequence acquisition

Total DNA was extracted from field-acquired silica gel-dried or fresh tissues, using the Qiagen DNeasy Plant mini kit (Qiagen Inc., Valencia, CA, USA). PCR amplifications were performed for two chloroplast genome regions: *rbc*L (coding region; ca. 1,300 bp) and *rps*4 (the coding region *rps*4 and the intergenic spacer *rps*4–*trn*S; ca. 1,100 bp). Amplifications were done in a single reaction with primers 1F and 1365R for the *rbc*L region (Haufler and Ranker 1995) and primers rps5F (Nadot et al. 1995) and trnSR (Smith and Cranfill 2002) for the *rps*4 and *rps*4-*trn*S regions. These regions have shown their utility for inferring phylogenetic relationships in Polypodiaceae, as shown in Janssen and Schneider (2005), Janssen et al. (2007), Kreier and Schneider (2006), Kreier et al. (2007), Kreier et al. (2008), Schneider et al. (2002), Schneider et al. (2004), and Salino et al. (2008).

Polymerase chain reactions were performed in a 20 μ L solution containing 1.0 μ L of non-diluted genomic DNA template, 2.0 μ L of PCR buffer (Qiagen 10 × PCR Buffer), 1.0 μ L of DMSO, 1.0 μ L of BSA (4 mg/mL), 0.8 μ L of dNTPS (10 mM), 0.32 μ L (10 μ M) of each primer, 0.12 units of *Taq* Dna polymerase (Qiagen, 5 units μ L), and 14.44 μ L of ultra-pure water. Thermal cycling conditions were the same for both regions: 3 min at 94°C, 35 cycles of 45 s at 94°C, 60 s at 53°C and 90 s at 72°C, followed by 5 min at 72°C. Amplicons were purified by precipitation with polyethylene glycol (PEG) and sequenced by Macrogen (Seoul, South Korea) in a bidirectional sequencing reaction in an ABI3730XL.

Alignment and phylogenetic analyses

Sequence electropherograms were edited using the STADEN package software (Bonfield et al. 1995). Edited sequences were submitted to automated alignment with MUSCLE (Edgar 2004) and the resulting alignment was checked manually using MEGA 7 (Kumar et al. 2016).

Datasets were analyzed using maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood was performed using IQ-TREE (Nguyen et al. 2015) with partitioned matrix (Chernomor et al. 2016), automatic selection of the bestfit substitution model (using Bayesian Information Criterion, Schwarz 1978), and branch support assessed with 10,000 ultrafast bootstrap replicates (Minh et al. 2013). Best-fit models according to BIC were TIM2e+G4 for rbcL, K3Pu+G4 for rps4 gene and TVM+G4 for rps4-trnS IGS. For BI, a model-based phylogenetic analysis using Markov chain Monte Carlo-based was performed using MrBayes v3.2.2 (Ronquist et al. 2012), treating each DNA region (rbcL, rps4 gene and rps4-trnS IGS) as separate partitions. An evolutionary model for each DNA region was selected in jModelTest 2 (Darriba et al. 2012; Guindon and Gascuel 2003), using the Bayesian Information Criterion (Schwarz 1978, Table 1). For the *rbc*L dataset, the SYM+I+G was selected, and for the rps4 gene and rps4-trnS datasets the GTR+G model was selected. Each analysis consisted of two independent runs with four simultaneous Markov Chains run for 5,000,000 metropolis-coupled generations, starting with random initial trees and sampling one tree every 1000 generations. To improve swapping of chains the temperature parameter for heating the chains was lowered to 0.05. To check the convergence of the runs, ESS (effective sample size) and PSRF (potential scale reduction factor) were examined (Ronquist et al. 2012) using Tracer v.1.6 (Rambaut et al. 2014). Based on the sampled parameter values examined, 2000 trees, including the ones generated during the burn-in phase, were discarded. Remaining trees were used to assess topology and posterior probabilities (PP) in a majority-rule consensus tree. Because PP in Bayesian analysis are not equivalent to bootstrap (BP) (Erixon et al. 2003), we used criteria similar to a standard statistical test, considering groups with PP > 95% as strongly supported, PP 90–95% as moderately supported and PP < 90% as weakly supported. Results were summarized on a majority rule consensus tree.

Taxonomic studies and conservation status

Taxonomic conclusions were based on the study of specimens from the following herbaria: BHCB, BM, BR, G, GH, K, LPB, M, MO, NY, P, Q, QCA, QCNE, QPLS, US, USM, and USZ. Specimens with barcode are cited with herbarium acronym followed by barcode number. Abbreviation of genera and species followed IPNI (ipni. org) and morphological terms follow Lellinger (2002). Scanning electron microscope (SEM) images were made using a FEI Quanta 200 SEM, with an accelerating voltage of 30 kV. Samples were sputter-coated with gold and imaged digitally. Spore terminology follows Tryon and Lugardon (1991). Conservation status was assessed using IUCN Red List Categories and Criteria (IUCN 2016) to calculate the Extent of Occurrence (EOO) and the Area of Occupancy (AOO), using the GeoCAT tool (Bachman et al. 2011). The specimens that did not present coordinates were attributed one, whenever locality information was available. A grid cell of 10 km² was used for AOO estimation.

		Bas	e frequenc	ies					
Region	Selected model	Α	С	G	Т				
rps4 gene	GTR+G	0.3204	0.1924	0.1964	0.2908				
rsp4-trnS IGS	GTR+G	0.3249	0.1545	0.1605	0.3601				
<i>rbc</i> L	SYM+I+G	-	-	-	-				
			Su	bstitution	model (ra	te matrix))		
	A-C	A-G	A-T	C-G	C-T	G-T	Ti/tv	Ι	G
rps4 gene	0.7965	3.5099	0.1724	0.4720	3.0330	1	0	0	0.9640
rsp4-trnS IGS	0.9825	2.7675	0.2147	0.7566	2.9398	1	0	0	0.5615
rbcL	1.9337	8.1010	1.1861	0.8284	11.3400	1	0.5474	0	0

Table 1. Selected models and parameter values for data partitions used in this study.

Results

The final combined dataset presented 2339 bp, 599 from *rps*4 gene, 473 from the *rps*4*trn*S IGS, and 1267 from *rbc*L. All analyses recovered the main polygrammoid clades found by Schneider et al. (2004; see Fig. 1): the loxogrammoid clade (1.00 PP, 100% BS), the drynarioid + selligueoid clade (0.91 PP, 71% BS), the platyceroid-microsoroid clade (1.00 PP, 100% BS), and a clade comprising neotropical representatives (0.62 PP, 84% BS).

Inside the neotropical clade, *Synammia* appears as sister to all the other neotropical clades, a result also obtained by Schneider et al. (2006) (Fig. 1). Sister to *Synammia*, our analyses recovered three main clades: the polypodioid clade including *Pecluma*, *Phlebodium*, *Pleopeltis*, and *Polypodium s.s.* (1.00 PP, 70% BS,), the campyloneuroid clade, containing *Campyloneurum*, *Microgramma*, and *Niphidium* (1.00 PP, 95% BS), and a clade (1.00 PP, 99% BS) that included the grammitid ferns (1.00 PP, 100% BS), *Serpocaulon* (1.00 PP, 100% BS), and *Polypodium chrysolepis* (1.00 PP, 100% BS). Both maximum likelihood and Bayesian inference hypotheses recovered *P. chrysolepis* as sister to *Serpocaulon* (0.91 PP, 88% BS).

Discussion

The polygrammoid topology recovered in our analyses agrees with previous results from several studies (Schneider et al. 2004, Schneider et al. 2006, Smith et al. 2006b, Salino et al. 2008, Otto et al. 2009). The placement of *Polypodium chrysolepis* as an isolated lineage inside neotropical Polypodiaceae and closely related to *Serpocaulon* and the grammitid ferns (1.00 PP, 97% BS) confirms results previously found by Schneider et al. (2004); this species is not part of the campyloneuroid clade, containing *Microgramma*, nor does it belong to the polypodioid clade, containing *Polypodium s.s.* and *Pleopeltis*. The afore-mentioned genera have all been used as a "home" for *Polypodium chrysolepis* by various authors in the past (e.g., Hooker 1844, de la Sota 1960, Crabbe 1967).



Figure 1. Majority rule consensus tree resultant from Bayesian inference analyses from combined *rbcL* and *rps4* datasets. Values above branches nodes correspond to Bayesian posterior probabilities and maximum likelihood bootstrap support, respectively. +/+ indicates with 1.00 PP and 100% BS support. Clades cited in text follow Schneider et al. (2004); gray shade indicates neotropical Polypodiaceae. *Adetogramma chrysolepis* is highlighted in orange.

In our analyses, *Serpocaulon*, a group segregated from *Polypodium* (Smith et al. 2006a), appears as sister to *P. chrysolepis* (0.97 PP, 88% BS), supporting the results of Schneider et al. (2004). Morphologically *Polypodium chrysolepis* can be readily distinguished from *Serpocaulon* by having free veins, non-clathrate scales, peltate scales covering the laminar surfaces, and peltate scales as paraphyses. These characters contrast with features of *Serpocaulon* species: regular anastomosing veins (goniophlebioid venation), clathrate scales, and the absence of scales in veins or laminar surface between veins or paraphyses, or if paraphyses are present, they are 2-3 celled glands (Smith et al. 2006a) (Table 2). Hernández et al. (2014) reported similarities in anatomical features of root cortical cells between *P. chrysolepis* and *Serpocaulon gilliesii* (C.Chr.) A.R.Sm, the only *Serpocaulon* species sampled in their study. Of all *Serpocaulon* species, *S. levigatum* (Cav.) A.R.Sm. is the only one that has entire leaves, and therefore the single species resembling *P. chrysolepis* morphologically. However, in addition to the characteristics mentioned above, *S. levigatum* differs from *P. chrysolepis* by having multiple rows of sori on each side of midvein and by rhizomes be-

ing covered by whitish wax-like deposits, with few roundish scales, not covering the entire surface of the rhizome (Smith et al. 2006a, Labiak and Prado 2008), while in *P. chrysolepis* only one row of sori is found on each side of midvein, and rhizomes lack whitish wax-like deposits and are covered by lanceolate rhizome scales (Figs 2, 3). Smith et al. (2006a) highlighted a possible trend of increasing pinnation in *Serpocaulon*, but the fact their results showed *S. levigatum* to be closely related to species with completely free, non-adnate pinnae would make the entire lamina shared with *P. chrysolepis* a homoplastic feature.

Sanín (2014, 2015) described a species of *Serpocaulon* with free veins (*S. tayronae* D.Sanín), but no phylogenetic evidence is presented to support its placement in *Serpocaulon*. Sanín also stated that *S. eleuterophlebium* (Fée) A.R.Sm. and *S. patentissimum* (Mett.) A.R.Sm. have free veins, although Hensen (1990) describes their venation as regularly anastomosing in the taxonomic treatment of the group (treated as the *Polypo-dium loriceum*-complex). More evidence is needed to elucidate the generic position of *S. obscurinervium* and *S. tayronae*, as is the case with several other groups morphologically related to *Polypodium* (Tejero-Díez 2005, Assis et al. 2016).

Polypodium chrysolepis was combined by Crabbe (1967) in *Microgramma*, but it exhibits morphological characters distinct from that genus. It has free venation while *Microgramma*, as circumscribed by Almeida et al. (unpublished data) and Salino et al. (2008), has anastomosing veins on the sterile fronds. Moreover, *P. chrysolepis* has lanceolate, peltate, long-stalked paraphyses (Fig. 2E) while in *Microgramma*, paraphyses (if present) are hairlike or lanceolate and sessile, never stalked (Table 2). *Polypodium chrysolepis* resembles some *Pleopeltis* species in having entire laminae, long-creeping rhizomes, and peltate paraphyses. The main difference between *Pleopeltis* and *Polypodium chrysolepis* lies in the shape of the paraphyses – ovate-lanceolate in *P. chrysolepis* vs. roundish in *Pleopeltis*. Furthermore, species of *Pleopeltis* with entire blades always have anastomosing veins (Table 2).

Grammitid ferns, the lineage sister to the *Polypodium chrysolepis+Serpocaulon* clade, are a very distinct group of species inside the polygrammoid ferns. Once recognized as a separated family (Parris 1990) or a subfamily inside Polypodiaceae (Tryon and Tryon 1982), this lineage can be distinguished by usually tetrahedral chlorophyllous spores, sporangia stalk reduced to a single cell wide in the middle, absence of scales in the fronds, and free or occasionally anastomosing without free included veinlets (Parris 1990, Sundue et al. 2010). Although sharing the free veins with *Polypodium chrysolepis*, we can distinguish the latter from grammitids by the bilateral spores, and the presence of scales in the blades (Table 2).

Polypodium s.s., following Tejero-Díez (2005), includes the species groups of *Polypodium plesiosorum*, *Polypodium colpodes*, *Polypodium dulce* (sensu Moran 1995) and *Polypodium vulgare* species group (sensu Haufler et al. 1995). Using this circumscription, *Polypodium s.s.* remains a polyphyletic assemblage of species, with species from the *Polypodium dulce* complex apparently closer to *Pecluma* than to *Polypodium s.s.* (Otto et al. 2009, Assis et al. 2016); in fact, some species in this group were moved to *Pecluma* (Assis et al. 2016). Further studies are needed to define a monophyletic

	Adetnorramma	Serboraulan ¹	Grammitide	Pocluma	Polymodium ²	Pleaneltis	Mirrocedum d ⁴
Rhizome	Long-creeping, branched	Long- to short-creeping, sparingly branched	Short-creeping to erect, usually unbranched	Long- to short-creeping, unbranched	Long- to short-creeping, branched	Long- to short- creeping, branched	Long-creeping, branched
Rhizome scales	Peltate, non- clathrate	Peltate, clathrate	Basifixed, non-clathrate or clathrate, or absent	Basifixed to peltate, non-clathrate	Peltate, non- clathrate	Peltate, non-clathrate, to clathrate at margins	Peltate, non-clathrate
Fronds	Monomorphic	Monomorphic	Monomorphic, or the distal fertile portion modified	Monomorphic	Monomorphic	Monomorphic to dimorphic	Monomorphic to dimorphic
Lamina	Simple	Pinnatifid to pinnate, rarely simple	Simple to 3-pinnate	Pinnatisect to pinnate	Deeply pinnatifid to pinnate	Simple to pinnatifid, rarely pinnate- pinnatifid or more divided	Simple to lobate
Indument on lamina	Scales	Glabrous, trichomes, or scales (confined to costae and rachises)	Trichomes, and sometimes glands	Trichomes	Glabrous or with trichomes	Scales	Glabrous, trichomes and/or scales
Veins	Free	Regularly anastomosing (goniophlebioid), areoles with one included veinlet	Usually free, sometimes anastomosing with or without included free veinlets	Free, rarely anastomosing, but never reticulate	Free to anastomosing, with one single included veinlet	Free to anastomosing, areoles with 1-3 free or netted included veins	Anastomosing, with simple included veinlets
Sori	Round to oblong, 1 row between costa and margins	Round, 1-10 rows between costa and margins	Round to elongate, 1 row between costa and margins, or confluent	Round to oblong, 1 row between costa and margins	Round to oblong, 1-5 rows between costa and margins	Round to oblong, or linear, rarely marginal and coalescing, in 1 row between costa and margins	Round to elongate, 1 row between costa and margins, confluent or forming several irregular rows between costa and margins
Paraphyses	Peltate, pedicellate, scales	Absent or short 2-3-celled glands	Present or absent	Present, glandular trichomes	Absent or if present, filamentous or branched	Absent, or round peltate,	Trichomes or sessile scales, or absent

Table 2. Comparison of character states among Adetogramma and the morphologically or phylogenetically closest genera/groups.

Bilateral, monolete, tuberculate
Bilateral, monolete, shallowly to prominently verrucate
Bilateral, monolete
Bilateral, monolete, smooth to tuberculate
Tetrahedral-globose, trilete
Bilateral, monolete, verrucate, generally tuberculate, occasionally winged
Bilateral, monolete, verrucate
Spores

¹Serpocaulon circumscription follows Smith et al. (2006).

² Polypodium circumscription accepted here includes Polypodium plesiosorum and Polypodium colpodes species groups (sensu Tejero-Diez 2005, Sigel et al. 2014), Polypodium dulce species group (sensu Moran 1995) and Polypodium vulgare species group (sensu Haufler et al. 1995).

³*Pleopeltis* circumscription accepted here follows Smith and Tejero-Díez (2014).

⁴*Microgramma* circumscription follows Salino et al. (2008).

Polypodium. Nevertheless, following this circumscription, *Polypodium s.s.* can be distinguished from *P. chrysolepis* by its deeply-pinnatifid to pinnate leaves with free (in the *Polypodium dulce* complex species) to anastomosing veins, with a single included veinlet in each areole. Indument is also a useful character for separating *P. chrysolepis*: *Polypodium s.s.* shows glabrous to pilose laminar surfaces and the paraphyses, when present, are filamentous or branched, while *P. chrysolepis* has scaly laminae and the paraphyses are pedicellate scales. Table 2 summarizes the features and main differences amongst the related genera.

Our results do not support the inclusion of *Polypodium chrysolepis* in any genus previously recognized, including *Microgramma*, *Pleopeltis*, or *Polypodium s.s.* Therefore, we consider this species as constituting a separated, isolated lineage inside the polygrammoid clade. Because the species also has a morphology distinct from that of all other known genera in Polypodiaceae, we believe it merits recognition as a genus, and is described below.

Taxonomic treatment

Adetogramma T.E.Almeida, gen. nov.

urn:lsid:ipni.org:names:77161864-1

Note. Adetogramma is similar to Microgramma and Pleopeltis in its epiphytic habit, long-creeping rhizomes and in having entire leaves with one row of sori on each side of the midrib, but differs from these genera by having free veins (vs. veins anastomosing in Microgramma and Pleopeltis) and peltate, pedicellate, lanceolate paraphyses (vs. hair-like or lanceolate and sessile paraphyses in Microgramma, and round, peltate, pedicellate paraphyses in Pleopeltis).

Type. *Adetogramma chrysolepis* (Hook.) T.E.Almeida, comb. nov., *Polypodium chrysolepis* Hook., Icon. Pl. 8: t. 721. 1845.

Adetogramma chrysolepis (Hook.) T.E.Almeida, comb. nov.

urn:lsid:ipni.org:names:77161865-1 Figures 2–4

Lepicystis chrysolepis (Hook.) Diels, Nat. Pflanzenfam. 1(4): 322, f. 167A–B. 1899. Type: Based on *Polypodium chrysolepis* Hook.

Microgramma chrysolepis (Hook.) Crabbe, Brit. Fern Gaz. 9: 316. 1967. Type: Based on *Polypodium chrysolepis* Hook.

Polypodium bangii Baker, Bull. Misc. Inform. Kew 1901: 145. 1901.

Type: Bolivia. Yungas, 1890, *A.M. Bang 734* (lectotype, designated here: BM! [BM000936895]; isolectotypes: B! [B200075587], BR!, GH!, K! [K000590773], LE!, MO! [MO5472871)], NY! [NY00144786, NY00144787], US! [US00065725]).

Basionym. Polypodium chrysolepis Hook., Icon. Pl. 8: t. 721. 1845.

Type. Ecuador. Andes de Quito, *W. Jameson 37* (wrongly typed in protologue as "73"; lectotype, designated by Tryon et al., 1993, pg. 151: K! [K000590772]; isolecto-types: BM! [BM000936896], G!, FI [FI004543!]).

Plants epiphytic or epipetric, rarely terrestrial. Rhizomes long-creeping, branched, 0.6–0.9 mm wide, cylindrical, with four vascular bundles; short, perpendicular roots about 5-20 mm long, these regularly spaced, covered with brownish root hairs; rhizome scales 4.3-7.1 mm long, peltate, not clathrate, linear-lanceolate, with elongate cells, the margins entire from the base to the middle and toothed beyond the middle, with 1- or 2-celled marginal teeth, scales concolorous, stramineous and usually darker at the attachment point. Fronds remote, 2.2-4.5 mm apart, articulate, monomorphic. Stipes nearly absent to 55 mm long, 0.4-0.7 mm in diameter, covered with sparse peltate, lanceolate, sessile, non-clathrate, concolorous, stramineous scales, these 1.4-3.5 mm long, darker at the attachment point; phyllopodia darker than stipes. Laminae light green, $5.0-17.0 \times 1.0-2.5$ cm, simple, chartaceous, linear-lanceate to lanceolate, bases acuminate to attenuate, decurrent in the distal third of the stipe, apices acute to obtuse, laminar surfaces squamose on both sides, scales lanceolate, peltate, sessile, non-clathrate, concolorous, translucent, stramineous, darker at attachment point, slightly erose at bases and entire at apices, scales present also on abaxial and adaxial sides of costae and veins, and on the laminar margins, 1.3-2.6 mm long. Veins free, immersed, obscure, 1-2 furcate, not reaching laminar margins, midribs and lateral veins immersed on both sides of the laminae, not evident. Sori superficial, rounded to oblong, $1.6-2.6 \times 1.9-5.0$ mm, terminal to subterminal on veins, receptacles elongate, sporangia long-stalked, paraphyses present, scale-like, similar those of the laminar surfaces, peltate, pedicellate, with pedicels as long as those of the sporangia, paraphyses completely covering immature sori. Spores yellow, with verrucate surfaces.

Distribution and ecology. Restricted to central and southern Andes, with known collections from Ecuador, Peru, Bolivia, and Argentina (Fig. 5). It occurs preferably in high elevation formations, ranging from 1,800 to 4,100 meters above sea level, with most collections between 3,000–4,000 m in the central part of the Andes, but found at lower elevations further south in Southern Bolivia and Argentina. *Adetogramma chrysolepis* is mostly epiphytic or epipetric, rarely terrestrial. Epiphytic specimens usually grow on *Polylepis, Berberis*, or *Buddleja*, inside highland humid montane forests (Yungas). Epipetric ones were recorded as growing on rocks inside Yungas forest or in sub-alpine grasslands with scattered shrubs, normally associated with mosses (de la Sota 1960). According to herbarium sheet labels, the species also occurs in secondary forests and modified areas.

Conservation status. Least Concern (LC - IUCN 2016). *Adetogramma chrysolepis* presents EOO of 1,108,559.773 km² and AOO of 5,300 km², respectively, and its conservation status is considered Least Concern. However, the species occurs in a fragile environment that is undergoing an increasing pressure due to human settlement, and extensive grazing by cattle and camelids. Although it is known from at least 40 localities and occurs in several protected areas (e.g., Parque Nacional Carrasco, Cocha-



Figure 2. *Adetogramma chrysolepis* (Hook.) T.E.Almeida. **A** Habit **B** Rhizome scale **C** Sterile lamina detail showing free venation and laminar scales **D** Fertile lamina detail showing sori, paraphyses, and laminar scales **E** Paraphysis detail. Drawings A–D by H. Fukuda from *Dorr* et al. *6764* (NY); drawing E by Juliana Ventura from *T.E. Almeida & L.L. Giacomin 3121* (BHCB). Scale bars: **A** = 5 cm, **B–E** = 1 mm.



Figure 3. *Adetogramma chrysolepis* (Hook.) T.E.Almeida. **A, B** Scanning electron micrography (SEM); magnification 75× and 1750×, respectively **A** Sori showing sporangia and paraphysis (the latter indicated by an arrow head) **B** Spores. Images from *Almeida & Giacomin 3121* (BHCB). Scale bars: **A** = 500 μm, **B** = 20 μm.

bamba, Bolivia; Reserva Nacional de Flora e Fauna de Tariquía, Tarija, Bolivia; Parque Nacional Huascarán, Ancash, Peru; Santuario Nacional de Ampay, Apurimac, Peru; Parque Nacional del Rio Abiseo, San Martín, Peru) no information on population fluctuations is available. Decline in the quality and area of occupancy in the near future seems feasible and it is advised that the species be monitored.

Etymology. The generic epithet refers to the most distinctive character of the species, the free venation (Fig. 2C); from the Greek adetos-, free and -gramma, line.

Notes. *Adetogramma* is a monotypic genus, and although its sole species, *A. chry-solepis*, varies in laminar size and shape and stipe length, all other characters, such as the rhizome and stipe scales, venation, and paraphyses, are constant. Specimens from the Argentinean Provinces of Tucumán and Salta and Bolivian Provinces of Tarija and Chuquisaca have very long, linear laminae, and longer stipes, but in other characters are congruent with the circumscription here adopted for *A. chrysolepis*. This variation may reflect colonization of drier, seasonal habitats in a subtropical region.

Morphologically, *Adetogramma chrysolepis* shares features with several neotropical genera of Polypodiaceae (Table 2), while presenting unique characteristics within the group. Free veins (Figs 2C, 4C) are shared with the *Polypodium dulce* species group, some *Pecluma* species, a few *Pleopeltis* species, few pinnatisected species of *Serpocaulon, S. tayrona* and most grammitids. Presence of peltate scales on the receptacle is shared between *Adetogramma* and *Pleopeltis* (Figs 2D–E, 3A, 4A–B). In both genera, paraphyses may almost completely cover the sporangia in immature sori, and presumably have a protective function.

Kreier et al. (2008) hypothesized that *Serpocaulon*, sister group to *Adetogramma*, has the Bolivian Andes and adjacent southeastern Brazil as its ancestral area. According to this hypothesis, the Bolivian Andes formed a path for migration and successful colo-

nization of the Northern Andes, with subsequent migration into Central America and Caribbean regions. With *Adetogramma* as sister to *Serpocaulon*, we believe the hypothesis of the Bolivian Andes as putative ancestral area of the *Adetogramma+Serpocaulon* clade common ancestor to be more likely. From Bolivia, *Adetogramma* could have dispersed southwards where colder climate allowed it to colonize lower elevation habitats, and also migrated northwards to Peru and Ecuador. *Adetogramma chrysolepis* may represent a single relictual species from a previously more diverse and geographically widespread group, or a single, undiversified lineage that colonized high elevation environments. More detailed phylogenetic or phylogeographic studies are required to support or refute these hypotheses.

Specimens examined. ARGENTINA. Jujuy: San Antonio, 27 Apr 2015, C. Martín 479 (SI); Valle Grande, 14 Apr 2016, C. Martín 730 (SI). Salta: Salta, 21 Nov 1945, S.A. Pieroffi 1326 (NY!); Santa Victoria, 05 Dec 2015, C. Martín 673 (SI). Tucumán: s.l., 1952, H. Brücher s.n. (LP!); s.l., s.d., L. Castillón 2248 (BM!); s.l., s.d., M. Lillo 11534 (BM!, GH!, K!, NY!); La Ventanita, s.d., M. Lillo 16713 (BM!, GH!, K!); Burruyacu, 20 Jan 1947, Borsini s.n. (LP!); Chicligasta, 11 Dec 1925, S. Venturi 4062 (BM!, GH!); idem, 13 Feb 1924, S. Venturi 3151 (GH!); Tafi del Vale, Jan 1912, L. Castillón 35 (BM!, GH!, K!, LP!, NY!). BOLIVIA. Chuquisaca: Hernando Siles, 09 Nov 2007, J. Villalobos 928 (MO!, UC). Cochabamba: Avopaya, 07 May 1990, E. Hennipman 8148 (LPB!); idem, 28 Oct 2007, J. Terán 1486 (MO!); idem, 30 Oct 2007, J. Terán 1563 (MO!); Carrasco, 20 Mar 1991, I. Hensen 1819 (LPB!); idem, 25 Feb 1996, M. Mercado 501 (MO!); Chapare, 30 Sep 2001, J.R.I. Wood 17271 (LPB!); Cochabamba, 27 Jan 1950, W.M.A. Brooke 6081 (BM!, NY!); Jose Carrasco Torrico, 27 Jun 1996, M. Kessler 6763 (NY!, UC); idem, 02 Jul 1996, M. Kessler 6877 (NY!, UC); Tiraque, 10 May 2005, E. Zurita 390 (MO!). La Paz: Bautista Saavedra, 27 Apr 1993, P. Gutte 559 (LPB!); Franz Tamayo, 24 Feb 2008, A.F. Fuentes Claros 11982 (MO!, UC); idem, 04 Mar 1980, J. Krach 9207 (LPB!); idem, 06 Apr 2009, M.I.L. Rivera 599 (MO!); Inquisivi, 21 Dec 1989, L.J. Dorr 6764 (LPB!, NY!); idem, 18 Feb 1989, M.A. Lewis 35242 (LPB!, MO!, NY!, UC); idem, 09 Mar 1991, M.A. Lewis 38230 (MO!); idem, 04 Sep 1991, M.A. Lewis 39744 (F, GH!, LPB!, MO!); Larecaja, 1818, G. Mandon 1560 (BM!, G!, K!, NY!); Murillo, 16 May 1985, J.C. Solomon 13742 (LP!, LPB!, MO!, NY!); Nor Yungas, XI/1900, O. Buchtien 2751 (P!); idem, 07 Mar 1969, H. Doppelbaur s.n. (MO!); Pedro Domingo Murillo, 17 Mar 2012, T.E. Almeida 3121 (BHCB!, LPB!); Pongo, s.d., W.M.A. Brooke 5456 (BM!, NY!); Unduavi, 1890, A.M. Bang 734 (B!, BR!, GH!, K!, LE!, MO!, US!); idem, 30 Mar 1977, J.D. Boeke 1375 (NY!); idem, Feb 1914, O. Buchtien 420 (B!, BM!, G!, GH!, K!, NY!, P!); idem, 19 Jun 1912, E. Rosenstock 49 (B!, P!). Santa Cruz: Manuel M. Caballero, 11 Apr 2004, R. Nuñez Cabrera 682 (MO!, NY!, UC, USZ!); idem, 08 Mar 2012, T.E. Almeida 3083 (BHCB!, HSTM!, LPB!); s.l., 26 Oct 1928, J. Steinbach 8531 (BM!, GH!, K!, MO!, NY!). Tarija: Aniceto Arce Ruiz, 13 Jun 2004, I. Jimenez 2420 (NY!, UC); Arce, 10 Nov 2004, H. Huaylla 1509 (MO!); idem, 27 Jun 2005, H. Huaylla 1882 (MO!); idem, 12 Nov 2004, M. Serrano 5082 (MO!); idem, 12 Nov 2004, M. Serrano 5136



Figure 4. *Adetogramma chrysolepis* (Hook.) T.E.Almeida. **A** Fertile and sterile fronds **B** Detail of abaxial surface of fertile frond, showing laminar scales and paraphyses **C** Juvenile sterile leaves. (A) and (B) from *C. Martin 730* (SI), (C) from *C. Martin 479* (SI).



Figure 5. Distribution of Adetogramma chrysolepis.

(MO!); idem, 03 Feb 2005, M. Serrano 5945 (MO!); O'Connor, 22 Oct 1983, S.G. Beck 9651 (LPB!); s.l, s.d., M. Cárdenas 1036 (GH!); s.l., 07 Mar 1969, H. Doppelbaur 291 (M!); s.l., 06 May 1928, [illegible] 1569 (B!); s.l., 21 Aug 1926, [illegible] 2559 (B!). ECUADOR. Pichincha: Quito, s.d., W. Jameson 73 (G!, K!). PERU. Amazonas: s.l., 03 Aug 1962, J.J. Wurdack 1592 (GH!). Ancash: Carhuaz, 14 Feb 1985, D.N. Smith 9571 (MO!, USM); idem, 18 Jul 1985, D.N. Smith 11264 (BM!, ENAG, F, GH!, MICH, MO!, NY, UC, USM); Huari, 08 May 1986, D.N. Smith 12415 (BM!, F, LPB!, MO!, NY, UC); idem, 13 Jun 1986, Smith 12648 (MO!); Yungay, 13 Jan 1985, Smith 9150 (GH!, MO!, USM); idem, 17 Apr 1985, D.N. Smith 10326 (MO!, USM); idem, 19 Apr 1985, D.N. Smith 10463 (MO!, USM). Apurimac: Abancay region, Oct 1935, students of Santander C. s.n. (UC); Abancay, Tamburco, 6 Jun 2015, V. Zuñiga 452 (USM). Cusco: s.l., 31 May 2002, W.L. Galiano 4034 (MO!); s.l., 14 Feb 1987, P.Núñez Vargas 6988 (MO!); s.l., 10 Jun 2002, L. Valenzuela Gamarra 238 (NY!, MO!); La Convención, 11 Jul 2003, E. Bonino 832 (MO!); idem, 23 Jul 2003, E. Bonino 909 (MO!, UC); idem, 26 Mar 2004, I. Huamantupa 4428 (MO!); idem, 21 Sep 2005, I. Huamantupa 6844 (MO!, USM!); idem, 30 Mar 1939, C. Vargas 4505 (MO!); Urubamba, 23 Apr 1982, B. Peyton 51 (MO!); idem, 04 Jul 1982, B. Peyton 766 (MO!); idem, 17 Aug 1982, B. Peyton 1053 (GH!, MO!); idem, 05 Nov 1988, A. Tupayachi 758 (GH!, MO!, NY!); idem, 30 Dec 1963, L. Valenzuela 14983 (GH!). Huánuco: Yanano, 13-16 May 1923 J.F. Macbride 3826 (G!). La Libertad: Pataz, 24 Feb 1986, K. Young 2988 (NY); s.l., 23 Jun 1974, A. López M. 8129 (G!, MO!, NY!). Lambayeque: Ferreñafe, 9 Jun 2012, L. García Llatas s.n. (USM). San Martín: Mariscal Cáceres, Huicungo, 15 Jun 2001, B. León 5249 (NY!, USM).

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Appendix

Collection information for voucher specimens and GenBank accession numbers for sequences used in this study. Locality and voucher specimen (herbarium) are given to sequences newly generated in this study. *sequences submitted to GenBank

Taxon	rbcL	rps4-trnS
Adenophorus oahuensis (Copel.) L.E.Bishop	AY057382	AY096236
Adetogramma chrysolepis (Hook.) T.E.Almeida; Bolivia, Almeida 3083 (BHCB)	KY847865*	KY847858*
Adetogramma chrysolepis (Hook.) T.E.Almeida; Bolivia, Almeida 3121 (BHCB)	KY847859*	KY847863*
Aglaomorpha pilosa (J.Sm.) Copel.	AY529156	AY529180
Alansmia cultrata (Willd.) Moguel & M.Kessler	GU376496	JN654943
Arthromeris himalayensis (Hook.) Ching	JQ685378	JQ685442
Campyloneurum gracile A.Rojas; Panama, Salino 15357 (BHCB)	KY847860*	KY847864*
Campyloneurum phyllitidis (L.) C.Presl	KT780752	KT794132
Davallia solida (G.Forst.) Sw.	AY096193	AY096210
Dictymia brownii (Wikstr.) Copel.	DQ227292	DQ227295
Goniophlebium percussum (Cav.) W.H.Wagner & Grether	AY362561	AY362628
Gymnogrammitis dareiformis (Hook.) Tardieu & C.Chr.	AY096201	JQ685456
Lecanopteris carnosa (Reinw.) Blume	AF470322	AY096227
Lellingeria apiculata (Klotzsch) A.R.Sm. & R.C.Moran	GU387021	GU387046
Lellingeria brevistipes (Kuhn) A.R.Sm. & R.C.Moran	GU387030	GU387049
Lemmaphyllum carnosum (Hook.) C.Presl	GU126698	GU126717
Lepidomicrosorium subhemionitideum (Christ) P.S. Wang	GU126693	GU126711
Lepisorus heterolepis (Rosenst.) Ching	GQ256270	GQ256344
Leptochilus digitatus (Baker) Noot.	JX103695	EU363250
Leucotrichum madagascariense Rakotondr. & Rouhan	JN654924	JN654949
Leucotrichum organense (Gardner) Labiak	GU376490	JN654946
Loxogramme salicifolia (Makino) Makino	DQ227294	DQ227297
Melpomene flabelliformis (Poir.) A.R.Sm. & R.C.Moran	GU387028	GU387114
Melpomene melanosticta (Kunze) A.R.Sm. & R.C.Moran	GU387024	GU387115
Microgramma baldwinii Brade; Brazil, Almeida 2631 (BHCB)	KY847861*	KY847866*
Microgramma bifrons (Hook.) Lellinger	AY362582	AY362654
Microgramma mauritiana (Willd.) Tardieu	DQ642148	DQ642185
Microgramma persicariifolia (Schrad.) C.Presl	KT780753	KT794133
Microsorum membranaceum (D.Don) Ching	EU482963	AY725047
Mycopteris attenuatissima (Copel.) Sundue	GU476927	GU387121
Mycopteris subtilis (Klotzsch) Sundue	GU476875	GU387128
Neocheiropteris palmatopedata (Baker) Christ	JX103706	GQ256396
Niphidium crassifolium (L.) Lellingerl; Brazil, Almeida 3247 (BHCB)	KY847862*	KY847867*
Niphidium nidulare (Rosenst.) Lellinger	EF551064	EF551080
Oleandra pistillaris (Sw.) C.Chr	AB232405	AY096209
Pecluma ptilotos (Kunze) M.G.Price	AY362588	AY362661
Phlebodium pseudoaureum (Cav.) Lellinger	AY362589	AY362663
Platycerium andinum Baker	DQ164446	DQ164477
Pleopeltis angusta Willd.	EU650122	EU650161
Pleopeltis desvauxii (Klotzsch) Salino	AY362584	AY362657
Polypodium vulgare L.	JF832081	EF551081

Adetogramma (Polypodiaceae), a new monotypic fern genus segregated from Polypodium 131

Taxon	<i>rbc</i> L	rps4-trnS
Pyrrosia angustata (Sw.) Ching	DQ642165	DQ642204
<i>Selliguea plantaginea</i> Brack.	EF463262	EU128510
Serpocaulon articulatum (C.Presl) Schwartsb. & A.R.Sm.	DQ151910	DQ151935
Serpocaulon dissimile (L.) A.R.Sm.	DQ151908	DQ151933
Serpocaulon levigatum (Cav.) A.R.Sm.	DQ151917	EF551103
Serpocaulon loriceum (L.) A.R.Sm.	EF551074	EF551104
Stenogrammitis hellwigii (Mickel & Beitel) Labiak	GU386990	GU387058
Stenogrammitis hildebrandtii (Hieron.) Labiak	GU386975	GU387059
<i>Synammia feuillei</i> (Bertero) Copel.	AY362597	AY362670