

# Simple but long-lasting: A specimen imaging method applicable for small- and medium-sized herbaria

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## Abstract

Major international herbaria, natural history museums and universities have recently begun to digitise their collections to facilitate studies and improve access to collections. In Japan, more than 10 million herbarium specimens are housed in various universities/museums; however, only 1% of these have been digitised. In this paper, we describe a new method for imaging herbarium specimens that is applicable to local/small herbaria. It is safe, fast, simple and inexpensive, but also satisfies usage guidelines for minimum image quality and can produce digital files suitable for long-term storage and future post production. During an eight-month trial at the Museum of Nature and Human Activities, Hyogo, with three part-time workers using a custom-made copy stand and a mirrorless interchangeable lens camera with a large LED light bank system, we were able to image 73,180 herbarium specimens (571 per day on average), obtaining two RAW and two JPEG files for each specimen.

## Keywords

Digitisation, Herbarium specimen, Imaging

## Introduction

The digitisation of museum collections has recently become a major topic of discussion (e.g. Thiers et al. 2016, Le Bras et al. 2017, Barber et al. 2018, Sweeney et al. 2018). The Naturalis Biodiversity Center is in the process of digitising 37 million objects (<https://science.naturalis.nl/en/collection/digitization/digitization/>). The Finnish Museum of Natural History (Tabelberg et al. 2012) is running mass digitisation programmes for their entire collections. Furthermore, major herbaria around the world – such as Harvard University (A, GH, NEBC), the Royal Botanic Garden, Edinburgh (E), the Royal Botanic Gardens, Kew (K), the French Muséum national d'histoire naturelle vascular plant herbarium (P, Le Bras et al. 2017) and the Meise Botanic Garden (BR, De Smedt et al. 2018) – have also conducted digitisation. China (the Chinese Virtual herbarium [<http://www.cvh.ac.cn/>]) and the United States (Integrated Digitized Biocollections [iDigBio]; <https://www.idigbio.org/>) promote specimen digitisation as national projects. A list of the major digital herbaria in the world was compiled and is shown in Table 1.

Compared to the aforementioned countries, Japan is rather behind (Ogawa 2008, Moriguchi et al. 2012, Akihiro et al. 2018). About 10 million herbarium specimens are housed in universities and museums in Japan (The Unions of the Japanese Societies for Systematic Biology 2015) and half of these are deposited at the three largest herbaria: The University of Tokyo (TI) with ca. 1.8 million specimens, the National Museum of Science and Nature (TNS) with ca. 1.7 million specimens and the Kyoto University Museum (KYO) with ca. 1.2 million, while the rest are dispersed in smaller, local herbaria. At present, 20% (ca. 2 million) of the specimen data in Japan have been deposited in the Global Biodiversity Information Facility (G-BIF) (<https://www.gbif.org/>) via J-BIF, the Japan node of G-BIF (<http://www.gbif.jp/v2/>). This facility provides text without images. Some herbaria in Japan – such as TI ([http://umdb.um.u-tokyo.ac.jp/DShokubu/herbarium/en\\_ver2/index.php](http://umdb.um.u-tokyo.ac.jp/DShokubu/herbarium/en_ver2/index.php)), TNS (<http://www.type.kahaku.go.jp/TypeDB/>) and the Metropolitan University of Tokyo (MAK) ([http://ameba.i.hosei.ac.jp/BIDP/MakinoCD/makino/html\\_e/index0.html](http://ameba.i.hosei.ac.jp/BIDP/MakinoCD/makino/html_e/index0.html)) – have published databases of their herbarium collections with specimen images. However, these are limited to specimen type collections or specimens from certain areas (e.g. Nepal and oceanic Islands). The largest herbarium database in Japan with specimen images is the Shimane University virtual herbarium (<http://tayousei.life.shimane-u.ac.jp/herbarium/>) (Akihiro et al. 2018). Currently, ca. 100,000 specimen images from the Tottori Prefecture Museum (TRPM), the Shimane Nature Museum of Mt. Sanbe Sahimel, the Rikuzentakata City Museum and the Herbarium of Faculty of Symbiotic Systems Science at Fukushima University (FKSE) are available online.

Nelson et al. (2015) described three widely used imaging station alternatives for herbarium digitisation: (1) A copy stand with fluorescent lighting, (2) a light box with internal lighting and (3) an inverted flatbed scanner. In the present study, we explored another method and developed a custom-made digitisation system for herbarium specimens using a mirrorless interchangeable-lens camera (MILC) and a large bank light system, even though inverted flatbed scanners are most commonly used in Japan (e.g.

**Table 1.** A list of the major virtual herbarium in the world.

Location	Herbarium	URL
<b>North America</b>		
U.S.A	Atrium Biodiversity Information System	<a href="http://atrium.andamazon.org/digital_herbarium.php">http://atrium.andamazon.org/digital_herbarium.php</a>
	Harvard University Herbaria	<a href="http://kiki.huh.harvard.edu/databases/specimen_index.html">http://kiki.huh.harvard.edu/databases/specimen_index.html</a>
	Missouri Botanical Garden	<a href="http://www.tropicos.org/">http://www.tropicos.org/</a>
	Field Museum, Chicago	<a href="http://emuweb.fieldmuseum.org/botany/detailed.php">http://emuweb.fieldmuseum.org/botany/detailed.php</a>
	Smithsonian Institution National Museum of Natural History	<a href="https://collections.nmnh.si.edu/search/botany/?ti=3">https://collections.nmnh.si.edu/search/botany/?ti=3</a>
	New York Botanical Garden	<a href="http://sweetgum.nybg.org/science/vh/">http://sweetgum.nybg.org/science/vh/</a>
	University of Florida Herbarium	<a href="http://www.flmnh.ufl.edu/herbarium/cat/imagesearch.asp">http://www.flmnh.ufl.edu/herbarium/cat/imagesearch.asp</a>
<b>South America</b>		
Colombia	Universidad Nacional de Colombia	<a href="http://www.biovirtual.unal.edu.co/es/">http://www.biovirtual.unal.edu.co/es/</a>
Brasil	Centro de Referência em Informação Ambiental CRIA	<a href="http://splink.cria.org.br/">http://splink.cria.org.br/</a>
<b>Europe</b>		
U.K.	Natural History Museum, London	<a href="http://data.nhm.ac.uk/dataset/collection-specimens">http://data.nhm.ac.uk/dataset/collection-specimens</a>
	Royal Botanic Gardens, Kew	<a href="http://apps.kew.org/herbcat/navigator.do">http://apps.kew.org/herbcat/navigator.do</a>
	Royal Botanic Gardens, Edinburgh	<a href="http://data.rbge.org.uk/search/herbarium/">http://data.rbge.org.uk/search/herbarium/</a>
	Botanical Society of the British Isles	<a href="http://herbariaunited.org">http://herbariaunited.org</a>
Finland	Finnish museum of natural History	<a href="https://www.luomus.fi/en/botanical-and-mycological-collections">https://www.luomus.fi/en/botanical-and-mycological-collections</a>
Bergium	Botanic Garden Meise	<a href="http://www.br.fgov.be/RESEARCH/COLLECTIONS/HERBARIUM/advancedsearch.php">http://www.br.fgov.be/RESEARCH/COLLECTIONS/HERBARIUM/advancedsearch.php</a>
France	Museum National D'histoire Naturelle	<a href="https://science.mnhn.fr/institution/mnhn/collection/p/item/search/form?lang=en_US">https://science.mnhn.fr/institution/mnhn/collection/p/item/search/form?lang=en_US</a>
Denmark	Aarhus University	<a href="http://www.aubot.dk/search_form.php">http://www.aubot.dk/search_form.php</a>
Austria	Herbarium WU, University of Vienna	<a href="https://herbarium.univie.ac.at/database/search.php">https://herbarium.univie.ac.at/database/search.php</a>
Switzerland	Zürcher Herbarien, University of Zurich	<a href="https://www.herbarien.uzh.ch/en.html">https://www.herbarien.uzh.ch/en.html</a>
Germany	Botanischer Garten und Botanisches Museum Berlin	<a href="http://ww2.bgbm.org/herbarium/default.cfm">http://ww2.bgbm.org/herbarium/default.cfm</a>
Croatia	University of Zagreb	<a href="http://herbarium.agr.hr/search.html">http://herbarium.agr.hr/search.html</a>
<b>Oceania</b>		
	The Australasian Virtual Herbarium	<a href="http://avh.chah.org.au/">http://avh.chah.org.au/</a>
New Zealand	New Zealand national herbarium network	<a href="http://www.virtualherbarium.org.nz">http://www.virtualherbarium.org.nz</a>
<b>Asia</b>		
China	Chinese Virtual Herbarium	<a href="http://www.cvh.org.cn/">http://www.cvh.org.cn/</a>
	Chinese Academy of Sciences Inst. Botany -Beijing	<a href="http://pe.ibcas.ac.cn/herbs/herbariumsearch.aspx">http://pe.ibcas.ac.cn/herbs/herbariumsearch.aspx</a>
Thailand	Thai Forest Herbarium type specimen database	<a href="http://www.dnp.go.th/botany/bkfmmain.aspx">http://www.dnp.go.th/botany/bkfmmain.aspx</a>
Japan	National Museum of Nature and Science(TNS) Type specimen Database	<a href="http://www.type.kahaku.go.jp/TypeDB/">http://www.type.kahaku.go.jp/TypeDB/</a>
	Makino Herbarium Type Specimen Image Database	<a href="http://ameba.i.hosei.ac.jp/BIDP/MakinoCD/makino/html_j/index0.html">http://ameba.i.hosei.ac.jp/BIDP/MakinoCD/makino/html_j/index0.html</a>
	Shimane University ditital herbarium	<a href="http://tayousei.life.shimane-u.ac.jp/herbarium/">http://tayousei.life.shimane-u.ac.jp/herbarium/</a>
	The University of Tokyo, type collection database	<a href="http://umdb.um.u-tokyo.ac.jp/DShokubu/herbarium/en_ver2/index.php">http://umdb.um.u-tokyo.ac.jp/DShokubu/herbarium/en_ver2/index.php</a>

Suh et al. 2006, Ogawa 2008, Moriguchi et al. 2012, Akihiro et al. 2018). There were two reasons for using a digital camera: firstly, it is easier to take multiple images of one specimen in various forms using a camera. For security in the long-term storage of specimen images, it is better to have files of multiple kinds. The long term preservation of digital images is discussed in terms of file formats (Digital Preservation Handbook, 2<sup>nd</sup> edition 2018). Well-known formats are PNG, TIFF, JPEG and JPEG 2000, whose specifications are freely available to users. Although JPEG is the common file format amongst these, it involves using non-invertible compression of the image. Due to this, TIFF is preferred in digital archiving projects, but there are currently no reasonably priced digital cameras able to save TIFF directly. Therefore, we acquired lossless RAW images, which can be converted to TIFF images in parallel with JPEG images. In contrast, flatbed scanners can obtain only one image file per scan and one scan takes up to several minutes to obtain sufficient image quality. Therefore, if we want to obtain multiple images in multiple formats per specimen using a scanner, the time taken will increase accordingly. Secondly, the thickness of specimens does not affect digital camera images. Bulky specimens (e.g. conifer cones and aroid bulbs) are difficult to scan but pose no problem for cameras.

The system we have developed is simple and inexpensive, requires minimal space, could be managed by part-time workers and makes it possible to easily obtain multiple standardised digital files of several kinds. Using a digital camera has often been avoided because it is thought to be extremely difficult to provide sufficient lighting for quality specimen imaging (Suh et al. 2006). We overcame this problem by adopting a light-bank system using LED light. This system is suitable for small and medium-sized herbaria where staff, space and budget are limited, as well as for larger herbaria with larger numbers of staff members and stations. It may also be applied when digitising other kinds of collections, including entomological, mineralogical and fossil collections.

## **Materials and methods**

### **Target collections**

As our target for digitisation, we chose the Shoei Junior College (Higashinada-ward, Kobe City, Hyogo, Japan) herbarium collection, which was previously known as SHO and is currently one of the collections at the Museum of Nature and Human Activities, Hyogo (HYO). The collection consists of ca. 250,000 specimens of vascular plants. SHO has a history of more than 80 years and, along with KYO, it is the most well-known herbarium in western Japan. Many taxonomists have visited SHO, examined the specimens and cited the SHO collection in their papers. Upon the retirement of two taxonomists from the college in 2012, the collection was donated to HYO. Due to current storage space limitations, 95% of the SHO herbarium sheets are kept separate from the main HYO collection in boxes. We set out to achieve the digitalisation of the collection by 2020 by first creating a searchable database to enhance the accessibility of the collection.

## Imaging policies

To complete digitisation within a limited time and budget, we decided to use the minimum acceptable quality point (MAQP) for the obtained images (Fujimoto and Horiuchi 2016, Fujimoto 2017) before developing equipment. Our terms of reference were as follows:

- 1 Images should be usable and suitable for long-term storage. It should be noted that capturing and preserving high-quality specimen images offers opportunities to take advantage of future improvements in image analysis (La Salle et al. 2009), optical character recognition (OCR) (Haston et al. 2012), natural language processing, handwriting analysis and data-mining technologies (Nelson et al. 2012).
- 2 Images should have enough resolution to withstand expansion up to 150% on a display monitor and be capable of withstanding life-size, high-definition printing.
- 3 Images should have applicable OCR for data transcription from the specimen label. To increase the accuracy of the OCR output, images obtained should have sharp margins and be flat with minimal distortion.
- 4 Imaging should be finished within two to three years using the same hardware (camera and lens) to keep the quality of all images consistent. Hardware lifespan is generally in the range of three to five years; that of a digital camera may be shorter.

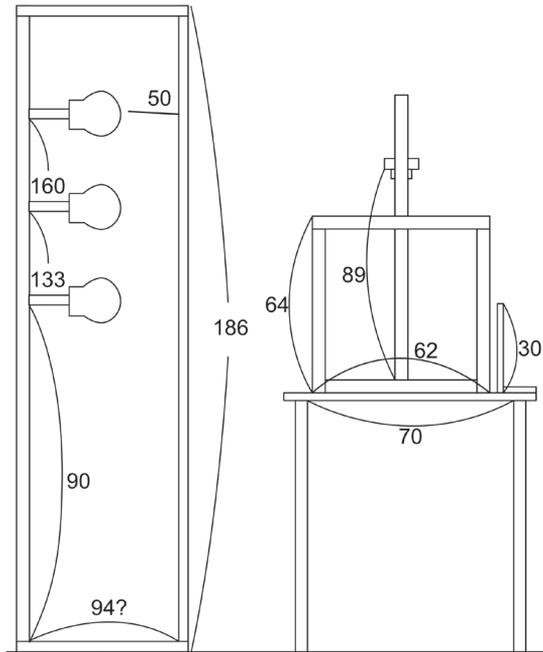
## Component selection for the imaging station

### Digital camera and lens choice

An MILC was selected for our imaging system. They are smaller, lighter and experience fewer vibrations from camera shake and shutter shock than digital single-lens reflex (DSLR) cameras. The lighter body made it easier for us to design a custom-made copy stand and the decreased susceptibility to lens aberrations is better for future OCR image use. After some trial and error, we chose the SONY  $\alpha$ 6300 (ILCE6300), APS-C sensor, digital e-mount camera and an FE 35 mm F.2.8 ultra-compact wide-angle lens for the Sony e-mount full frame (Samyang Optics SYIO35AF-E 35 mm F/2.8). Specimen images obtained using this combination of camera and lens are 5100×3500 pixels or ca. 25 MB in size.

### Custom-made copy stand with LED lighting system

LED light is the only light source that does not generate heat and that offers efficient electric lighting with control over the intensity and wavelength, allowing for the reduction of UV light. LED light is thus beneficial for both specimens and workers' health. To record the colour of herbarium specimens precisely at reasonable cost, LED light with a high colour rendering property was selected (039 SH50 Pro-S LED Lamp,



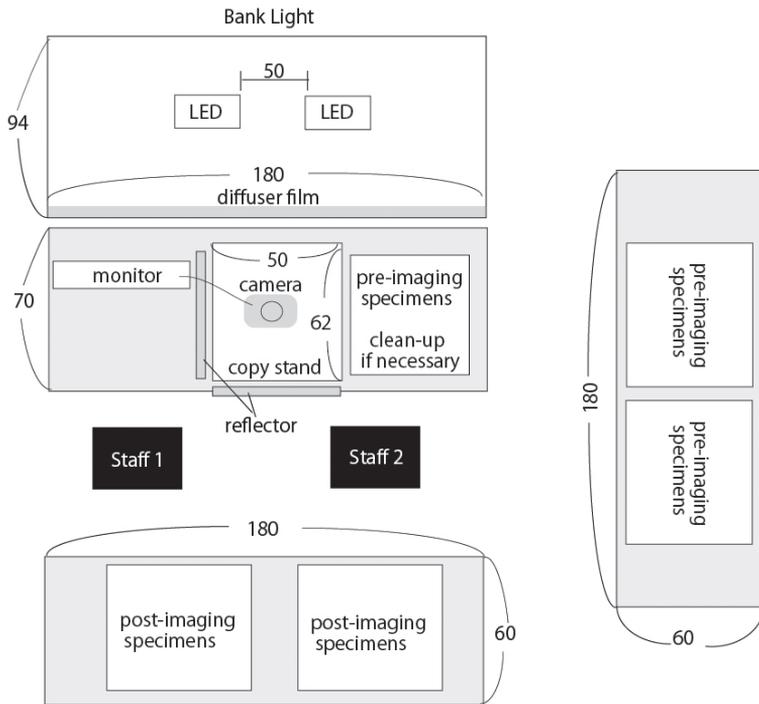
**Figure 1.** Side view of the imaging equipment we developed. The numbers show the size of each part (cm).

China) and diffuser film was also chosen to be durable enough for frequent changes over a long period (Savage Translum<sup>TM</sup>, U.S.A). A large light bank system was designed to apply sufficient light above the specimen in a manner similar to a skylight (Figs 1–3). This involved using two light stands, each bearing three of the LED lights mentioned above, located 50 cm apart from each other and 50 cm behind the diffuser film, to provide sufficient light intensity and to obtain a sharp and clear image of the plants on the sheet (Figs 1–3).

We constructed our own copy stand for imaging specimens because ready-made copy stands are too large, heavy, complicated and expensive. As we chose a lighter MILC, the stand did not have to be as robust to avoid camera shake. The design drawing is provided in Fig. 1. We adapted a lightweight aluminium frame structure with excellent durability and practical use strength for reducing camera shake (Yuki corp., Aichi Japan). On the copy stand, we put a mark to indicate where to place herbarium sheets and the GIN-ICHI Silk Gray Card.

### Space required

For the work production line, we needed space for the light bank, pre-imaging specimens, copy stand and post-imaging specimens and a monitor to check imaging (Fig.3). A total of ca. 27 m<sup>2</sup> was required for this imaging system. Additional space for specimen repair, a laptop and an NAS server for saving images were also needed.



**Figure 2.** Birds-eye-view of imaging equipment. Numbers indicate size (cm).

### Staff training

Three digitisers were hired through public advertisement. None of them was a photographer and two had no experience with handling herbarium specimens. We trained them on how to treat specimens and the specimen imaging workflow in one day. After that, they worked in alternating pairs. One of them treated (if necessary) and moved the target specimen from the pre-position to the copy stand and the other operated the remote-control shutter and checked the image in the monitor (Fig. 3). Protocols are detailed below.

### Imaging protocol

#### *Pre-imaging*

- 1) Take target specimens from storage
- 2) Remove specimen from the genus cover
- 3) Clean-up and/or repair specimens (if necessary)
- 4) Apply a barcode to the specimen sheet
- 5) Place it on the copy stand in accordance with the guide



**Figure 3.** Employees working on the imaging system.

### *Imaging*

After confirmation that all objects are clean and in accordance with the pre-imaging settings above, the shutter is released to capture the image(s) with the following conditions.

- 1) Fixed camera working distance (ca. 89 cm)
- 2) Shutter speed 1/50-60, ISO 100
- 3) White balance measurement using a GIN-ICHI Silk Gray Card

We took multiple photos to obtain two 48-bit RAW and two JPEG files for each specimen.

### *Post-imaging*

- 1) Check image quality (focus, exposure etc.)
- 2) Remove specimen from the copy stand
- 3) Clean-up dust on copy stand (if necessary)
- 4) Clean-up and/or repair specimens (if necessary)
- 5) Return specimen to the original cover
- 6) Apply mark on the genus cover to indicate completion of imaging
- 7) Place them back into storage after cold fumigation ( $-90^{\circ}\text{C}$ , 10 h)

***Save files to the NAS server (at the end of the day)***

- 1) Create a new folder named according to the date in the server
- 2) Put all images collected on that day into the named folder
- 3) Copy these files to another external HDD

**Results**

Specimen images, obtained using our method, are of a quality suitable for OCR output (Fig. 4). All procedures, from pre-digitisation curation to storage and archiving of images, were performed by two part-time workers between 9 am and 5 pm each day. The speed of imaging depended on how many specimens needed conservation or clean-up before and/or after imaging. Most herbaceous specimens, especially Poaceae, Cyperaceae and Saxifragaceae, contained substantial amounts of sand or dry mud amongst their roots and took time to clean-up before imaging. Therefore, we sometimes obtained only 1,200 images (300 specimens) per day. In contrast, for woody or large herbaceous specimens without roots (and associated soil), imaging ran smoothly and we could obtain up to 4,000 images (1,000 specimens) per day. From the start of guidance and training on 10 Nov 2017 to 4 July 2018, a total of 73,180 specimens were imaged and stored as RAW and JPEG files.



**Figure 4.** An image of herbarium specimens taken using imaging method described in this study (*Cedrus deodara* (Roxb.) D. Don, HYO ID: C2-018127).

## Discussion

Our imaging system minimises both initial costs and space requirements using a custom-made copy stand alongside an MILC and other ordinary devices. The imaging system described here costs ca. 314,000 JPY in total (US \$2,778), including 170,000 JPY for the MILC camera, lens, adaptor and monitor, 90,000 for the bank light system (incl. LED lights, ramp folders, diffusion film etc.) and 54,000 for the camera stand. The development of new technologies has afforded us the use of LED or OLED lighting at a low cost. Using a large LED bank light system, we were able to obtain images that were rich in contrast and gradation without high resolution. In addition, the working space was minimised by putting a copy stand and before and after imaging of specimens in line on the same desk, making the workflow compact and smooth. This system is easy to operate, making it simple to train workers. It provides the ability to obtain multiple 48-bit RAW and JPEG files for each specimen. Obtaining multiple files and multiple kinds of digital files is important for long-term storage.

## Additional costs

After three months, during which 40,000 specimens were imaged, a digital camera broke. Repair was possible and free under warranty, but image processing was stopped during the repair period. A remote-control shutter also broke twice during the trial period. Therefore, it would be advantageous to have spare equipment, if possible.

## Future tasks

Images were stored under a default name on the NAS server day by day. We applied a barcode before imaging that bore a unique ID at our museum and we needed to rename the image files to this unique ID number using a barcode reader. Developing and running simple programmes to rename images will be our next goal. Furthermore, to publish searchable specimen images on the web, transcription of label data (Scientific name, Loc. Date etc.) is necessary. OCR has begun to be used to transcribe data from specimen labels over the last ten years (Moen et al. 2008, Heidorn and Wei 2008). There are some OCR-based semi-automatic label information extraction systems, including SALIX (Barber et al. 2013) and HERBIS (Beaman et al. 2006). We are also working to develop a semi-automatic label information extraction programme (Aoki et al. in prep.) that uses Dlib [1] (King 2009) to detect the label area and annotation card area for cropping; these are then used to run the Tesseract open source OCR engine ver. 3.0.4. (Smith 2007). Using this system, label information has been successfully extracted from 1584 of 1970 specimen images (80%).

Digitisation of herbarium specimens benefits both curators and stakeholders: for stakeholders, it becomes possible to access a digital voucher for each specimen remote-

ly via the internet and, for curators, it reduces the need for specimen handling and makes semi-automated label data extraction by OCR possible. Further, crowdsourcing the manual data entry of specimen labels can be considered, given remote access to specimen images. Specimen processing, from mounting until manual data entry, can be facilitated, updated and automated wherever possible as technology develops. The imaging of herbarium specimens is the first important step in this process.

## Conclusion

We developed a new digital imaging system for herbaria that takes up little space, minimises costs, is simple to use and quickly creates data that can be archived long-term and we provide here a step-by-step guide to create the system. We hope our imaging system will facilitate the digitization of small- and medium- sized herbaria where investment possibilities are limited.

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# *Solanum medusae* (Solanaceae), a new wolf-fruit from Brazil, and a key to the extra-Amazonian Brazilian Androceras/Crinitum Clade species

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## Abstract

*Solanum medusae* **sp. nov.** is described from the Cerrado biome in the Serra da Canastra region, south-western Minas Gerais State, Brazil. The new species is morphologically similar to the common *S. lycocarpum* A.St.-Hil. (known as lobeira or wolf-fruit), but differs from it in habit and pubescence characters. We here describe this new taxon and discuss its morphology, some aspects of its ecology, affinities and distribution. Full specimen citations are provided, as well as illustrations, distribution map and a preliminary conservation assessment of the species. A key to all of the known extra-Amazonian Brazilian species of the Androceras/Crinitum clade is also provided to aid in their identification.

## Keywords

Brazil, Cerrado, new species, wolf-fruit, identification key, prickly *Solanum*, Solanaceae

## Resumo

*Solanum medusae* **sp. nov.** é descrita para o Cerrado da região da Serra da Canastra, sudoeste do estado de Minas Gerais, Brasil. A nova espécie é morfológicamente semelhante à comum *S. lycocarpum* A.St.-Hil. (conhecida como lobeira ou fruta-do-lobo), da qual pode ser diferenciada por características do hábito e do indumento. O presente trabalho descreve este novo táxon, discute sua morfologia, alguns aspectos da sua ecologia, suas afinidades e distribuição. Citações completas dos espécimes são fornecidas, assim como ilustrações, mapa de distribuição e uma avaliação preliminar do estado de conservação da espécie. Uma

chave de identificação para todas as espécies conhecidas do clado *Androceras/Crinitum* ocorrentes no Brasil que possuem distribuição extra-amazônica também é fornecida.

### Palavras-chave

Brasil, Cerrado, espécie nova, lobeira, chave de identificação, *Solanum* aculeado, Solanaceae

## Introduction

*Solanum* L. (Solanaceae) is the largest genus of Solanaceae, with some 1,400 species, and one of the biggest angiosperm genera (Frodin 2004). Occurring on all temperate and tropical continents, *Solanum* has its highest diversity of both clades and species in tropical South America, the region considered to be ancestral for the family and major clades within it (Dupin et al. 2017). The last complete monograph of the genus was De Candolle's "*Prodromus*" (Dunal 1852), which included 901 species (with an additional 19 incompletely known). *Solanum* taxonomy has proceeded in a piecemeal fashion until relatively recently and the genus had acquired a reputation of being intractable, but recent monographic work has begun to remedy this situation.

The largest monophyletic group of *Solanum*, known as the *Leptostemonum* clade or *Solanum* subgenus *Leptostemonum* Bitter (Bohs 2005; Weese and Bohs 2007), includes prickly plants with stellate indumentum (the "spiny" solanums) and comprises approximately half the species diversity of the genus. It is composed of a large lineage of approximately 240 species confined to the Old World (see Aubriot et al. 2016), with the remainder primarily New World in distribution. Brazil is a center of diversity for all groups of *Solanum* (see Knapp et al. 2015), including the spiny solanums. Recent intensive work on the Brazilian flora for the Flora do Brasil project (BFG 2015, 2018) has resulted in better understanding of species diversity and distribution in *Solanum*, but numerous new taxa continue to be discovered in the country (e.g., Giacomini and Stehmann 2014; Agra and Stehmann 2016; Gouvêa and Stehmann 2016; Stehmann and Moreira 2016), even in southeastern Brazil, considered to be the best-explored region of the country (Sobral and Stehmann 2009; Forzza et al. 2012; Sousa-Baena et al. 2014).

The recent discovery of *Solanum* species from places close to urban centers where the flora would be expected to be well-known (e.g., Agra and Stehmann 2016; Stehmann and Moreira 2016; Gouvêa and Stehmann 2016; Gouvêa et al. 2018) coupled with threats to tropical vegetation in general draws attention to the continued urgency and relevance of efforts aimed at describing and preserving the still insufficiently known diversity of the Brazilian flora.

Here we describe a new species related to *S. lycocarpum* A.St.-Hil. (*Androceras/Crinitum* clade sensu Levin et al. 2006; Stern et al. 2011), an iconic species from Brazilian Cerrado (savannah-like vegetation), discuss its morphology, conservation status, distribution and affinities, and present a key to the extra-Amazonian Brazilian species of the group.

## Materials and methods

Following discovery of the new species two expeditions were carried out to the Serra da Canastra (Apr 2017 and May 2018) in order to increase our sampling and to ascertain the distribution of *S. medusae*. Specimens with coordinates were mapped directly, and all specimens are cited in the Specimens examined portion of the text. Descriptions are based on field work of YFG and examination of herbarium specimens. Specimens were examined from ALCB, BHCB, CEPEC, HUEFS, HUFU, R, RB, UEC, and UB (acronyms follow Index Herbariorum; <http://sweetgum.nybg.org/science/ih/>); online specimens from HUFU were also examined (Reflora - Herbário Virtual: <http://reflora.jbrj.gov.br/reflora/herbarioVirtual/>). Measurements of reproductive characters were taken from both fresh and dried material. Trichome types follow the terminology proposed in Roe (1972) and Mentz et al. (2000), while that of the general morphology is mainly based on Radford et al. (1976).

Extent of Occurrence (EOO) and Area of Occupancy (AOO) were calculated using GeoCat ([www.geocat.kew.org](http://www.geocat.kew.org)) with a 2 km cell width for AOO calculation. The preliminary conservation status was assessed using the IUCN (2017) criteria based on the GeoCat analyses (Bachman et al. 2011) combined with field knowledge. All specimens examined are cited in the text. Our delimitation of *S. medusae* as it is here presented was based on the “morphological species concept” (Davis and Heywood 1963; Mallet 1995).

## Taxonomic treatment

### *Solanum medusae* Gouvêa, sp. nov.

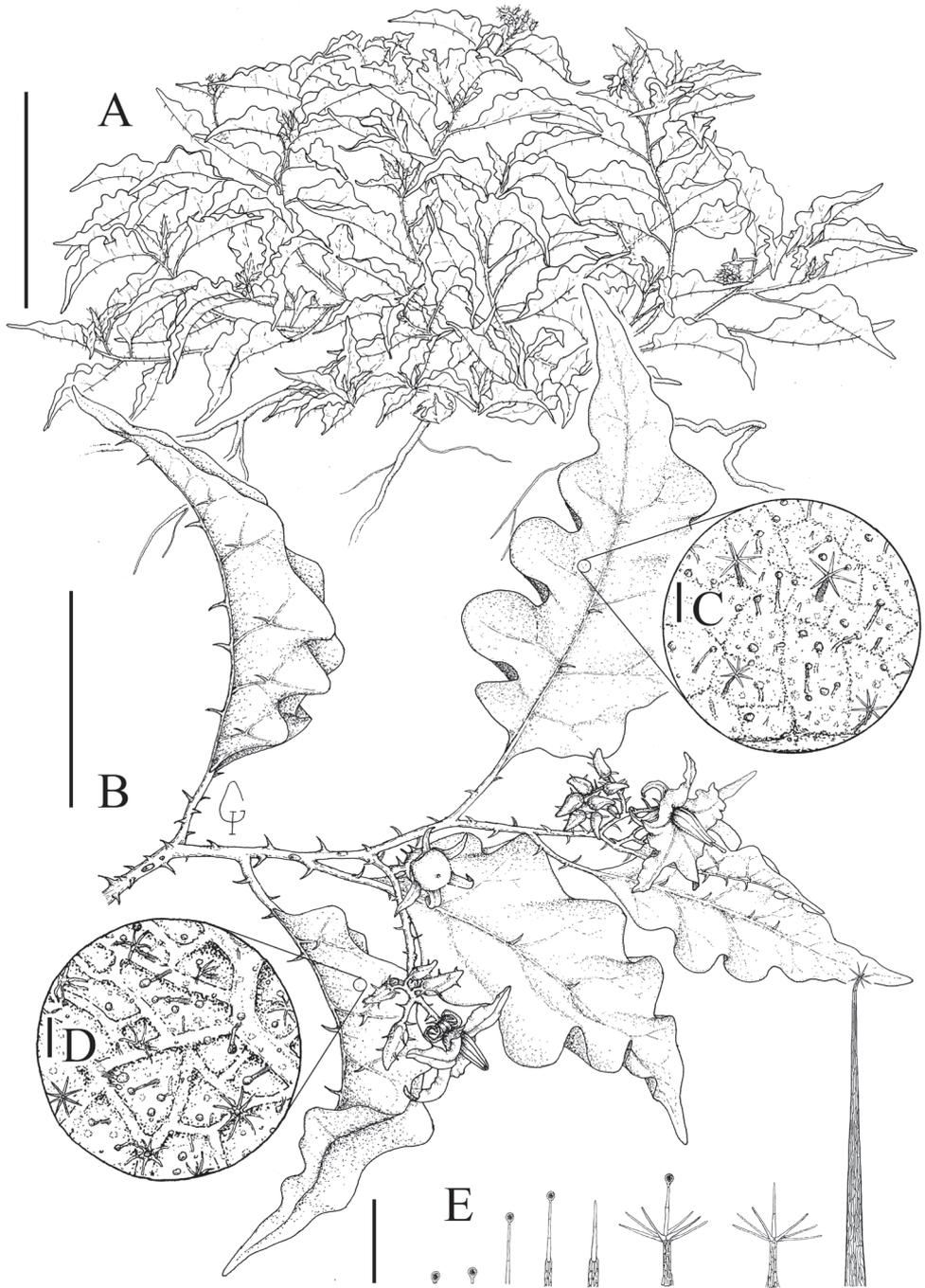
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Figures 1–3

**Diagnosis.** Like *Solanum lycocarpum* A.St.-Hil., but differing in its decumbent habit and densely glandular pubescence of stems and leaves.

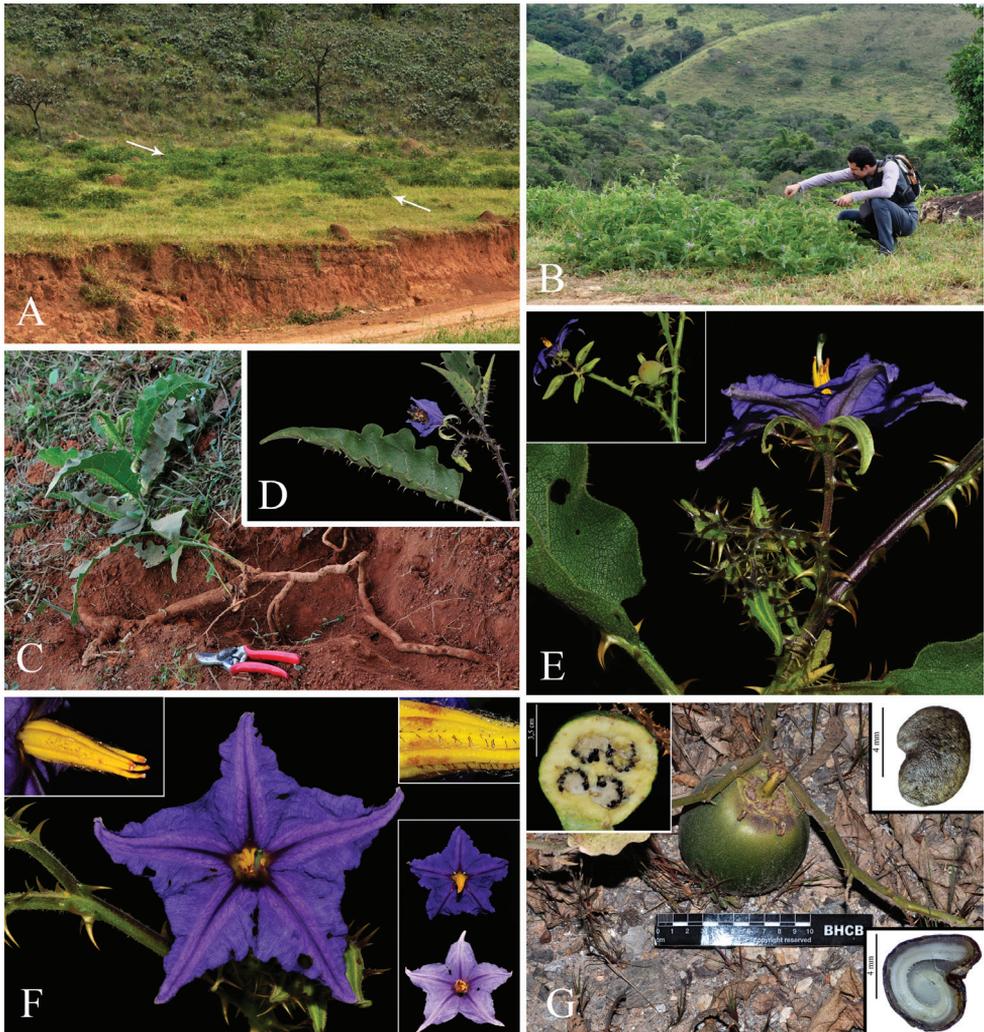
**Type.** BRAZIL. Minas Gerais: Distrito de São Roque de Minas, Parque Nacional da Serra da Canastra, principal estrada de terra que leva de São Roque de Minas à portaria do PN Serra da Canastra (passando por Capão Forro), 20°15'35"S, 46°24'36"W, 1212 m, 5 Apr 2017, Y.F. Gouvêa, T.E. Almeida, A. Salino & I.O. Moura 230 (holotype (2 sheets): BHCB [BHCB188229 (fl), BHCB188229\_2 (fr)]; isotypes: HUFU, RB, UB).

**Description.** Decumbent, spreading shrub to 1 m tall and 3 m in diameter, strongly armed. Young stems terete, green to deep purple, the epidermis sometimes with a varnished appearance, nearly glabrous to pubescent with porrect short- to long-stalked stellate trichomes, the stalks up to 2 mm long, multiseriate, the rays 6–8(–11), 0.2–0.5 mm long, the midpoints shorter than or equal in length to the rays, glandular or eglandular; the stem surface more densely covered with variously sized simple glandular trichomes; smaller papillae-like glandular trichomes 0.1–0.2 mm



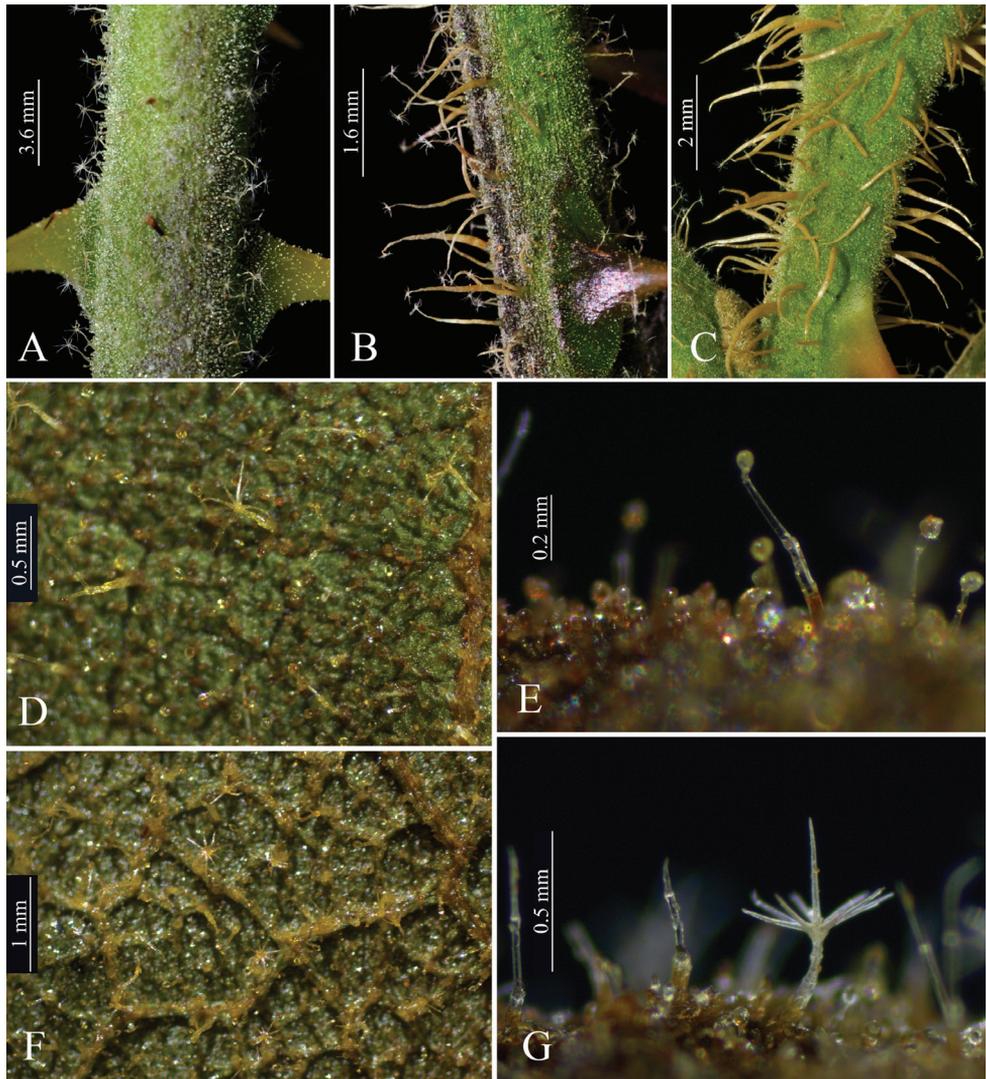
**Figure 1.** *Solanum medusae*. **A** Habit **B** Flowering branch with an immature fruit **C** Detail of the adaxial leaf surface indumentum **D** Detail of the abaxial leaf surface indumentum **E** Trichome types from stems and leaves (Y.F. Gouvêa et al. 230, BHCB). Scale bars: 30 cm (**A**), 8 cm (**B**), 0.5 mm (**C–E**). Drawings by Iago F. Gouvêa.

long, 1–4-celled, uniseriate, the gland single-celled; and longer glandular trichomes to 0.5(–1.8) mm long, multiseriate at the base with single-celled apical glands; prickles (0.2–)0.5–0.7(–0.9) cm long, orange-yellow, broad-based and strongly curved, the base 1–5 mm wide; new growth densely tomentose to pubescent, prickly, pale beige in color in dried plants; stellate trichomes with multiseriate stalks 0.5–1 mm long, the rays 6–10, ca. 0.5 mm long, the glandular or eglandular midpoint shorter than the rays; simple glandular trichomes denser than the stellate trichomes, the shorter papillae-like ones uniseriate, to 0.2 mm long, and the longer ones to 1.5 mm long, multiseriate at the base; prickles 1–5 mm long, strongly curved, yellow, usually tipped with stellate trichomes and sparsely to densely pubescent on the surface with short- to long-stalked stellate trichomes and simple glandular trichomes; bark of older stems reddish purple in live plants and shiny dark reddish brown in herbarium specimens. Sympodial units difoliate, the leaves not geminate. Leaves simple, shallowly lobed, the blades 9–22 cm long, 3–9.3 cm wide, narrowly ovate or trowel-shaped, widest in the lower third, chartaceous, concolorous, armed on both surfaces with curved yellow prickles 0.1–1 cm long, these denser abaxially; adaxial surface epidermis always visible, usually shiny with a varnished appearance, uniformly and sparsely to moderately pubescent with correct stellate short- to long-stalked trichomes, the stalk 0.2–0.5 mm long, multiseriate, the rays 6–8(10), 0.2–0.5 mm long, the midpoint shorter than the rays and occasionally glandular, these sometimes more densely distributed near the margins, more densely pubescent with simple uniseriate papillae-like glandular trichomes to 0.2 mm long, and 2–3 celled gland-tipped simple trichomes from a multiseriate base; abaxial surface with the epidermis always visible, usually shiny with a varnished appearance, moderately to densely pubescent with stellate and simple trichomes like those of the adaxial surfaces, but the simple glandular trichomes and papillae denser on the lamina; principal veins 4–8 pairs, the finer venation prominent, pale yellow and visible as a complex net on the abaxial surfaces, prickly with curved yellow prickles; base attenuate to abruptly truncate, obtuse or rounded, if attenuate then decurrent onto the petiole, asymmetric or not; margins shallowly lobed, the lobes (1)3–4 on each side of the midvein, rounded and semi-circular in outline, the sinuses less than 1/3 of the distance to the midvein; apex long acuminate, the ultimate tip somewhat rounded; petiole 0.5–5.5 cm long, pubescent like the stems, armed with prickles like those of the stems. Inflorescences 4.5–12 cm long, internodal, usually unbranched, less frequently furcate, with 4–15 flowers, sparsely to densely stellate-pubescent and densely glandular pubescent with trichomes like the stems, densely and irregularly prickly along the entire axis with curved yellow prickles 0.1–0.7 cm long, peduncle 1–2 cm long, prickly and pubescent; pedicels 0.6–2 cm long, ca. 1–1.5 mm in diameter at base and apex, spreading, sparsely to densely prickly, the prickles ca. 5 mm long, straight, usually denser on the basal flower, but in more pubescent individuals all pedicels prickly, articulated at the base; pedicel scars widely spaced 1–2 cm apart near the base of the inflorescence, more closely spaced distally. Buds long-fusiform and tapering, the corolla included in the fused calyx lobes until just before anthesis. Flowers 5-merous (occasionally 4-merous some flowers), slightly zygomorphic (see dis-



**Figure 2.** *Solanum medusae*. **A** Habitat **B** Habit; note the distinctive decumbent posture **C** Roots; note the horizontal growth **D** Branch apex; note the deep purple coloration and leaf shape **E** Inflorescence; note that the first flower is always long-styled (upper left corner: a more developed inflorescence with an immature fruit being formed from its first flower, and short-styled flowers distally, some of which have already fallen) **F** Long-styled flower (upper right corner: detail of the slightly unequal anthers with stellate-pubescent connectives; bottom right corner: color difference between the purple post-anthesis corollas and the lilac senescent ones) **G** Fruit (upper left corner: half of a transversally dissected fruit; upper right corner: seed; bottom right corner: dissected embryo). Photographs **A**, **C–G** by Y.F. Gouvêa **B** by Philippe S. Saviott.

cussion), heteromorphic, 1(–3) long-styled hermaphroditic flowers at the base of the inflorescence, more distal flowers short-styled and functionally staminate, the plants andromonoecious. Calyx with the tube ca. 3 mm long, obconical to cupuliform, pubescent like the rest of the inflorescence, densely prickly with straight yellow prickles;



**Figure 3.** Indumentum of *Solanum medusae*. **A–C** Variation in young stem indumentum (A: Y.F. Gouvêa 230; B: Y.F. Gouvêa 264; C: Y.F. Gouvêa 262, BHCB) **D** Adaxial leaf surface epidermis and indumentum **E** Detail of the simple glandular trichomes of the adaxial surface **F** Abaxial leaf surface epidermis and indumentum **G** Detail of the abaxial surface trichome types (**D–G** Y.F. Gouvêa 230, BHCB). Photographs by Y.F. Gouvêa.

the lobes 1.2–2 cm long, foliose, lanceolate to long-triangular, strongly reflexed at anthesis, abaxially pubescent and prickly like the rest of the inflorescence, adaxially pubescent with minute sessile or short-stalked porrect-stellate trichomes to 0.2 mm long, the basal hermaphroditic flower more densely prickly and more distal flower calyces often lacking prickles. Corolla 3–6.5 cm in diameter, deep purple in younger flowers, becoming lilac with flower age, the color deeper adaxially, stellate, lobed ca. halfway to

the base, the lobes 0.9–2.5 cm long, 1–2 cm wide, spreading, slightly to strongly reflexed at anthesis, abaxially densely stellate-pubescent where exposed in bud, the interpetalar tissue glabrous, adaxially densely papillate with minute stellate trichomes along the midvein, the tips acuminate, the acumens 3–4 mm long, cucullate and densely stellate-pubescent abaxially. Stamens slightly unequal, the upper 2 slightly shorter than the other 3; filament tube 0.8–1.5 mm long, glabrous; free portion of the filaments 1.4–2.5 mm long, glabrous; anthers 12.5–18.5 mm long, 1.7–2.6 mm wide at the base, strongly tapering, the 3 lower longer anthers more or less curved upward in their distal portion, yellow, poricidal at the tips, the pores distally directed, the connective abaxially pubescent with weak-walled white to deep purple stellate trichomes along the entire length. Ovary globose, densely stellate-pubescent with hyaline eglandular many-rayed trichomes, the rays and midpoints equal and not easily distinguishable; style 15–19 mm long in long-styled flowers, curved upwards, glabrous to moderately brown-stalked stellate-pubescent in the basal half, densely glandular papillate near the apex; stigma capitate to strongly bi-lobed (or sometimes with several irregular lobes), green in live plants, the surface densely papillate. Fruit a globose or depressed-globose berry, 7–15 cm in diameter, green becoming yellowish green and sweetly fragrant when ripe, the pericarp smooth, sparsely pubescent with minute stellate trichomes, especially near the pedicel, the mesocarp spongy, pale cream; fruiting pedicels 1.8–2.5 cm long, 1.1–1.3 cm in diameter at the base, 6.5–8.5 mm in diameter at the apex, fleshy in live plants, woody in dry specimens, strongly deflexed downwards so some fruits rest on the soil; fruiting calyx lobes ca. 2 cm long, persistent, prickly or not. Seeds > 100 per berry, 6–7 mm long, 5–6.2 mm wide, flattened reniform, dark brown to blackish brown, drying gray to dark gray, the surfaces minutely pitted, the testal cells sinuate in outline. Chromosome number not known.

**Distribution** (Figure 4). *Solanum medusae* is only known from the region of the Serra da Canastra in southwestern Minas Gerais state, Brazil. It has been collected from six municipalities located northeast (Campinópolis, Piumhi, São José do Barreiro and São Roque de Minas), north (São João Batista da Serra) and west (Sacramento) of the Serra da Canastra.

**Ecology.** *Solanum medusae* grows in open areas along roads, pastures and clearings in Cerrado, above 700 m elevation (Figure 2A). Populations have been found in areas originally dominated by Cerrado *stricto sensu* (lower areas), grasslands (higher areas) and seasonal semi-deciduous tropical forests (mountain slopes).

The poricidal anthers of *S. medusae* (similar to the vast majority of *Solanum* species; Figure 2F) narrow down the spectrum of pollinators to female bees able to collect pollen by vibrating their indirect flight muscles (buzz-pollination; Michener 1962, Buchmann 1983). We observed medium- to large-sized bees (e.g., genera *Priloglossa* and *Bombus*; Figure 2D) frequently visiting flowers.

The berries have a suite of characters associated with frugivory and dispersal by terrestrial mammals (Van der Pijl 1972): large size, green to greenish yellow coloration, strong sweet scent released at maturity and close proximity to the soil (Figure 2G). The berries of *S. medusae* are similar to those of *S. lycocarpum* (see Discussion), which



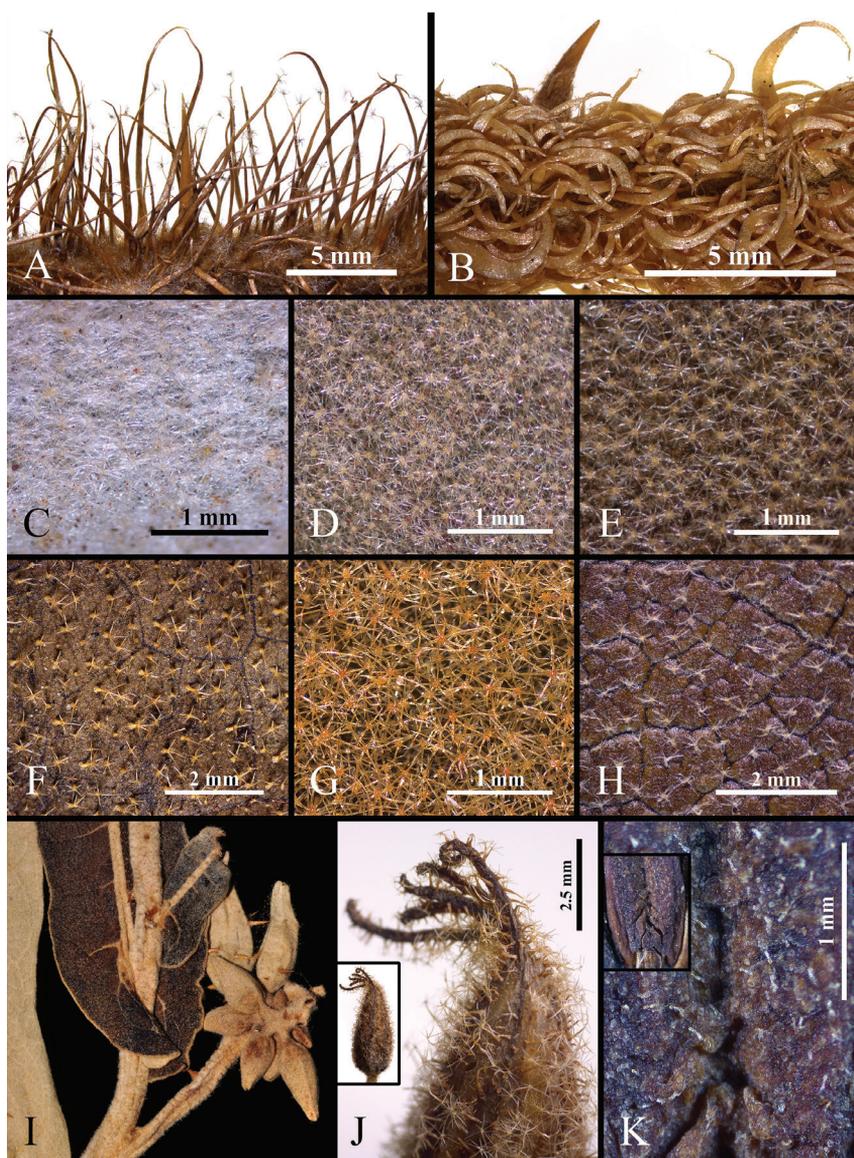
tures. The high frequency of non-natural fires in areas of native vegetation also poses a considerable risk. The presence of *S. medusae* in some areas of the Parque Nacional da Serra da Canastra is encouraging, but not enough to eliminate important risks, such as the loss of genetic diversity.

**Discussion.** *Solanum medusae* belongs to the large monophyletic group commonly known as the spiny solanums (Leptostemonum Clade, sensu Bohs 2005) and is morphologically a member of the “*S. crinitum* group” (sensu Whalen 1984; section *Crinitum* (Whalen) Child). This group is part of the molecularly defined *Androceras/Crinitum* clade (sensu Levin et al. 2006; Stern et al. 2011), and includes prickly herbs of the Mexican deserts with dry fruits such as *S. rostratum* Dunal (section *Androceras* (Nutt.) Whalen, see Whalen 1979), Amazonian vines (i.e. *S. coriaceum* Dunal and *S. sendtnerianum* Van Heurck & Müll.Arg.) and large woody shrubs to trees with relatively large, showy, lilac to deep bluish-purple flowers mostly found in South America (*S. crinitum* group sensu Whalen 1984; Nee 1999). This latter group includes about 15 species (see Whalen 1984; Nee 1999; Farruggia and Bohs 2010; Farruggia et al. 2010), of which at least 10 occur in Brazil; four of these are exclusively Amazonian (i.e., *S. acanthodes* Hook.f., *S. altissimum* Benítez, *S. orientale* Benítez and *S. tricuspdatum* Dunal), and six have strictly or essentially extra-Amazonian distributions (*S. crinitum* Lam., *S. falciforme* Farruggia, *S. gomphodes* Dunal, *S. lycocarpum*, *S. medusae* and *S. quaesitum* C.V.Morton). We present a key for the extra-Amazonian Brazilian species below.

*Solanum medusae* is most similar to *S. lycocarpum*, the wolf-fruit, in its large berries that are yellowish green at maturity (Figure 2G), anthers with abaxially pubescent connectives (Figure 2F), curved yellow prickles, and overlap in range with *S. lycocarpum* (Fig. 4). It differs from that species in its unusual decumbent, spreading habit (Figure 2B), in the dense glandular pubescence composed of simple, uni- to multiseriate trichomes that when dry give the plant a varnished appearance (Figure 3A–G), and in its rigid, easily-broken subterranean system that grows shallow and horizontally in the soil (Figure 2C), from which new stems can emerge in some points along its length (that in some cases can be mistaken as another individual). *Solanum lycocarpum* (popularly called “lobeira”) is an iconic element of Cerrado vegetation and ecologically important for populations of the maned wolf (Portuguese: “lobo-guará”; Guarani “aguará guazú”), and is a common small tree occurring in many habitats, especially open and/or disturbed areas of the Cerrado and Caatinga biomes, and seasonally dry environments within the Atlantic forest domain in Brazil. The leaf pubescence of *S. lycocarpum* is composed of dense eglandular stellate trichomes (Figure 5D) such that the leaves usually appear densely felty and grayish green in live plants and grayish brown in herbarium specimens.

The bristly long-stalked trichomes on the young stems of some *S. medusae* (Figure 3B, C) specimens may resemble those of *S. crinitum* Lam., another widespread species of the *Crinitum* group in Brazil whose distribution is centered in the Amazon basin, but the presence of glandular trichomes throughout the *S. medusae* epidermis easily distinguishes it from *S. crinitum*.

*Solanum medusae* is strongly andromonoecious, with a single (to three) hermaphroditic flower at the base of the inflorescence and the more distal flowers all short-



**Figure 5.** Distinctive characters of extra-Amazonian species of the Androceras/Crinitum clade. **A** Stem indumentum of *S. crinitum*; note the straight bristly stellate trichomes (Y.F. Gouvêa et al. 196, BHCB) **B** Stem indumentum of *S. falciforme*; note the falcate stellate trichomes (L.F. Souza 481, BHCB) **C** Stem indumentum of *S. lycocarpum* (Y.F. Gouvêa 268, BHCB) **D** Adaxial leaf surface indumentum of *S. lycocarpum* (Y.F. Gouvêa 268, BHCB) **E** Adaxial leaf surface indumentum of *S. falciforme* (L.F. Souza 481, BHCB) **F** Adaxial leaf surface indumentum of *S. quaesitum* (U.M. Resende & V.F. Kinupp 1817, BHCB) **G** Adaxial leaf surface indumentum of *S. crinitum* (Y.F. Gouvêa et al. 196, BHCB) **H** Adaxial leaf surface indumentum of *S. gomphodes* (L.L. Giacomini et al. 1274, BHCB) **I** Sessile sagittate leaf bases of *S. gomphodes* (L.L. Giacomini et al. 1274, BHCB) **J** Apiculate calyx lobe apices in *S. quaesitum*; note the extended midribs (U.M. Resende & V.F. Kinupp 1817, BHCB) **K** Abaxial anther surface of *S. quaesitum*; note the papillose epidermis sparsely covered by simple glandular trichomes (upper left side: detail of the distinctly swollen epidermis along the connective region; U.M. Resende & V.F. Kinupp 1817, BHCB). Photographs by Y.F. Gouvêa.

styled and functionally male (Figure 2E). Derived sexual systems like andromonoecy have arisen many times in *Solanum*, and are particularly common in the Leptostemonum Clade (Whalen and Costich 1986; Vorontsova et al. 2013). The flowers of *S. medusae* are somewhat zygomorphic with the ventral corolla lobes slightly longer than the dorsal ones and the anthers and style curved (Figure 2F); this floral form is known from species we postulate as related (Bohs et al. 2007). The stellate-pubescent abaxial connectives of *S. medusae* (Figure 2F) are also shared with *S. crinitum*, *S. falciforme* and *S. gomphodes*, which are all Brazilian endemics except for *S. crinitum*. It has been suggested (G. Davis, pers. comm.) that these act to facilitate a grip for bees buzzing the flowers, but field observations have not been undertaken to confirm this. *Solanum quaesitum*, in contrast, has the abaxial anther surface somewhat swollen (especially at the base) and papillose with sparsely distributed simple glandular trichomes (see Figure 5K); this can be used to distinguish it from other extra-Amazonian Androceras/Crinitum clade species (also see key below).

Intraspecific morphological variation (both individual and populational) of certain characters is particularly notable in spiny *Solanum* species (Roe 1966, Vorontsova and Knapp 2016, Knapp et al. 2017). In *S. medusae*, it is especially evident in indumentum, color, and prickle density of young stems. Individuals of some populations have the stem indumentum completely lacking bristly stellate trichomes (Figure 3A), whereas in specimens of other populations it is present in variable densities (see Fig. 3B, C). Young stem color ranges from completely green or partly to completely deep purple (Fig. 2D, E, 3A–C), as has been found in other *Solanum* species (e.g., *S. asterophorum* Mart., Gouvêa and Stehmann in press.). This distinct coloration can be confined to juvenile plants (as in *S. asterophorum*) or continue to be present on growing stems of reproductive individuals (as is observed in *S. medusae* and *S. kollastrum* Gouvêa & Giacomini, Gouvêa et al. 2018).

The Serra da Canastra lies in the watershed between the Paraná and São Francisco rivers. The protection of the headwaters of the São Francisco, one of the country's most important rivers, was one of the main reasons for the establishment of the Parque Nacional da Serra da Canastra in 1972. The National Park covers about 200,000 hectares of the Cerrado biome in a landscape composed of large quartzite plateaus with areas reaching up to about 1,500 m of altitude separated by lower elevation valleys. The vegetation of the plateau highlands is formed by extensive grasslands along the flatter areas, and campos rupestres in rocky sloping areas, which especially in the Park's northern portion gradually changes to a typical Cerrado vegetation towards valley bottoms. The region of the Serra da Canastra has a relatively long history of farming and mining, and as a result large tracts of native vegetation have been replaced by agriculture, and very few preserved areas remain outside the Park. Across Brazil, the Cerrado has one of the highest rates of deforestation, twice as fast as that of the Amazon (Klink and Machado 2005; Strassburg et al. 2017), putting species endemic to this habitat severely at risk.

We mapped the range of *S. medusae* in order to identify its limits and examine areas where it co-occurs with *S. lycocarpum* (Figure 4). Coming from the municipality of Belo Horizonte, Minas Gerais State (by the roads MG-262, MG-050, and MG-341

respectively), populations of *S. medusae* start to be found just before passing by the city of Piumhi towards São Roque de Minas (northeastern portion of Serra da Canastra), where it seems to completely replace *S. lycocarpum*. In this relatively low region where the native vegetation has been almost replaced by extensive agriculture or pastures, *S. medusae* grows on roadsides and in lesser used areas of these fields; it is not associated with the somewhat more preserved vegetation of narrow riparian forest strips. Within the Park, even with the diversity of somewhat conserved environments, *S. medusae* is mainly found in similar disturbed sites, and is less frequent as the elevation increases (from 700–800 to 1,300–1,400 m) and the vegetation changes from typical Cerrado to high elevation grasslands. In these grasslands *S. medusae* occurs as scattered individuals at road (i.e., MG-341) margins. Diverging from the MG-341 to the district of São João Batista da Serra (northern portion of Serra da Canastra), as the elevation decreases, and the vegetation gradually acquires a more typical Cerrado appearance, here *S. medusae* becomes more frequent and occurs in sympatry with *S. lycocarpum*. The two species co-occur towards the northwestern portion of the Serra da Canastra, in Cerrado areas ranging from 700 to 1,200 m elevation. In the lower (600–900 m) and relatively flat areas in the western and southeastern parts of the Serra da Canastra (i.e. municipalities of Cássia, Delfinópolis, Passos, São João Batista do Glória and São José da Barra) only *S. lycocarpum* was found.

**Additional specimens examined (paratypes). Brazil.** Minas Gerais: Mun. Campinópolis, rodovia MG-341, beira de estrada, 20°21'45"S, 46°13'17"W, 729 m, May 2018 (fl), *Gouvêa* 260 (BHCB [BHCB190630]); 20°22'28"S, 46°16'10"W, 807 m, May 2018 (fl), *Gouvêa* 261 (BHCB [BHCB190631]). Mun. Piumhi, rodovia MG-341, beira de estrada, 20°26'19"S, 46°00'59"W, 785 m, May 2018 (fl), *Gouvêa* 259 (BHCB [BHCB190629]). Mun. Sacramento, povoado de Desemboque, beira da estrada de terra que leva à MG-341, 20°02'30"S, 47°01'38"W, 1046 m, May 2018 (fl), *Gouvêa* 267 (BHCB [BHCB190637]). Mun. São João Batista da Serra, saída da cidade, beira da estrada que leva de São João Batista da Serra a Tapira, 20°08'25"S, 46°39'40"W, 1150 m, May 2018 (fl), *Gouvêa* 266 (BHCB [BHCB190636]). Mun. São José do Barreiro, estrada não pavimentada que leva à Cachoeira Casca d'Anta, 20°20'12"S, 46°28'24"W, 846 m, May 2018 (fl), *Gouvêa* 272 (BHCB [BHCB190642]); estrada não pavimentada que leva à Cachoeira Casca d'Anta, 20°18'56"S, 46°31'50"W, 857 m, May 2018 (fl), *Gouvêa* 273 (BHCB [BHCB190643]). Mun. São Roque de Minas, Parque Nacional da Serra da Canastra, primeiros trechos da principal estrada de terra que corta o PN da Serra da Canastra, 20°15'29"S, 46°24'58"W, 1283 m, 5 Apr 2017 (fl), *Gouvêa et al.* 231, 232, 233 (BHCB [BHCB188230, BHCB188231, BHCB188232]); sentido P.N. da Serra da Canastra, estrada de terra que leva à "Fazenda do Chico Chagas" divergindo da estrada principal que leva à portaria 1, 20°15'16"S, 46°23'31"W, 783 m, May 2018 (fl), *Gouvêa* 262 (BHCB [BHCB190632]); estrada de terra que leva ao P.N. da Serra da Canastra, beira de estrada, 20°15'36"S, 46°24'05"W, 1034, May 2018 (fl), *Gouvêa* 263 (BHCB [BHCB190633]); estrada de terra que leva ao P.N. da Serra da Canastra, beira de estrada, 20°15'36"S, 46°24'36"W, 1424 m, May 2018 (fl), *Gouvêa* 264 (BHCB [BHCB190634]).

## Artificial key to the Brazilian extra-Amazonian species of the *Androceras/Crinitum* clade (sensu Stern et al. 2011)

Morphological traits used to distinguish the species in the key are illustrated in Figure 5. State distributions of these species can be found on the Flora do Brasil 2020 website (<http://floradobrasil.jbrj.gov.br/reflora/listaBrasil>) by searching for the individual species by name.

- 1 Decumbent shrubs; indumentum of the upper leaf surfaces composed of two layers, the longer of short- to long-stalked stellate trichomes with glandular midpoints or not, and beneath them more abundant variously sized simple glandular trichomes ..... *Solanum medusae*
- Erect shrubs to small trees; indumentum of the upper leaf surfaces composed of a single layer of sessile to long-stalked stellate trichomes (which may seem simple because of the lack of rays, but can be recognized by their multiseriate stalks and uniseriate midpoints); simple glandular trichomes absent ..... 2
- 2 Leaves sessile with auriculate bases ..... *Solanum gomphodes*
- Leaves petiolate with cuneate to slightly cordate bases ..... 3
- 3 Calyx lobe apices markedly apiculate with the midrib notably extended beyond the lobe tissue ..... *Solanum quaesitum*
- Calyx lobe apices acute to acuminate with midrib not extending beyond the lobe tips ..... 4
- 4 Indumentum of young stems of a single layer of stramineous sessile to long-stalked stellate trichomes with slender stalks (2–5 cells wide) ..... *Solanum lycocarpum*
- Indumentum of young stems of two layers, the shorter sessile to long-stalked (2–5 cells wide) stellate trichomes, and the longer layer straight or falcate long-stalked bristly stellate trichomes with notably thick stalks (many cells wide) ..... 5
- 5 Longer trichomes of young stems with straight stalks ..... *Solanum crinitum*
- Longer trichomes of young stems with falcate stalks ..... *Solanum falciforme*

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# The taxonomic relevance of flower colour for *Epimedium* (Berberidaceae), with morphological and nomenclatural notes for five species from China

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## Abstract

Morphological variations, particularly flower colour, could be considered as an evolutionarily and ornamentally significant taxonomic criterion for *Epimedium*. Our extensive field investigation based on population studies revealed abundant intraspecific variations in flower colour. Five species, (i.e., *E. acuminatum* Franch., *E. leptorrhizum* Stearn, *E. pauciflorum* K.C.Yen, *E. mikinorii* Stearn, and *E. glandulosopilosum* H.R.Liang) were found to possess polymorphic flower colour, which is first described and illustrated here. Moreover, all these species were found to be polymorphic in other diagnostic characters, such as the type of rhizome, the number and arrangement of stem-leaves, and/or their indumentum, which have not been adequately described in previous studies. Therefore, their morphological descriptions have been complemented and/or revised. We also provide notes on the morphology and nomenclature for each species. Additionally, a key to the species in China has been provided. The present study could serve as a basis for understanding their taxonomy and helping their utilisation as an ornamental plant.

## Keywords

intraspecific, morphology, perianth, polymorphism, population, Ranunculales

## Introduction

*Epimedium* L. is the largest herbaceous genus of Berberidaceae, comprising ca. 60 species distributed in the temperate mountain regions from eastern Asia to northwestern Africa, with enormous distributional gaps within these regions (Stearn 2002; Xu et al. 2014a; Zhang et al. 2015). China represents the diversity centre for the genus, with ca. 50 endemic species (except *E. koreanum* Nakai) (Stearn 2002; Ying 2002). *Epimedium* is an attractive genus, both horticulturally and botanically, and has received increased attention in past decades (Avent 2010; Ward 2004; Ying 1975, 2001, 2002; Stearn 2002; Zhang et al. 2011, 2014, 2015; Liu et al. 2016, 2017). Since the 1980's, further botanical exploration and collections in China have dramatically increased the number of *Epimedium* species in this country (Ying 1975; Ying et al. 2011; Stearn 2002; Xu et al. 2014a). However, many *Epimedium* species have been described for China based on a single locality and/or the descriptions were based on limited specimens or collections (Stearn 1997; Zhang et al. 2011, 2014; Xu et al. 2014a). Due to the lack of additional field investigation and observation of morphological characters, the morphological variation amongst many *Epimedium* species is not yet well understood (Xu et al. 2014a, 2014b; Zhang et al. 2015; Liu et al. 2016, 2017). From 1990 to 1998, Stearn used a small collection of *Epimedium* to describe 16 species, which were based on a limited number of cultivated specimens (Stearn 1990, 1993a, 1993b, 1995, 1996, 1997, 1998), and thus, the morphological descriptions of some species were incomplete and/or inaccurate (Buck 2003; Xu et al. 2014a; Zhang et al. 2015; Liu et al. 2016). Our recent studies have focused on the standardisation of morphological descriptions in the genus (e.g., Liu et al 2016, 2017).

The divergence of floral traits is a striking phenomenon in flowering plants, which plays an important role in ecology, plant systematics and conservation (Vaidya et al. 2018). Specifically, flower colour has been demonstrated to be an important feature for identifying and classifying species (Ying 2001; Stearn 2002; Ying et al. 2011). *Epimedium* has the greatest range of flower colour than any other genus of Berberidaceae, varying from white (e.g., *E. latisepalum* Stearn) and yellow (e.g., *E. ecalcaratum* G.Y.Zhong, *E. fangii* Stearn, *E. flavum* Stearn, and *E. franchetii* Stearn) to pink (e.g., *E. leptorrhizum* Stearn), and to purple (e.g., *E. epsteinii* Stearn, *E. pseudowushanense* B.L.Guo, and *E. zhushanense* K.F.Wu & S.X.Qian) (Stearn 2002; Ying et al. 2011). The flower colour variation could be evolutionarily significant for *Epimedium*. However, compared to interspecific variation, intraspecific variation in flower colour is relatively uncommon (Matsumura et al. 2006). Usually, the colour of flowers is constant at the species level, but *E. grandiflorum* C.Morren and *E. acuminatum* Franch. are believed to be heterochromic (Stearn 2002; Ying et al. 2011). *Epimedium grandiflorum* varies greatly in flower colour from white to pale yellow or light purple to reddish-purple to deep pink or violet (Stearn 2002). For *E. acuminatum*, intraspecific colour variation from white to yellow to pale violet or purple has been reported sporadically (Ying et al. 2011; Zhang et al. 2011, 2015). However, systematic investigations of flower colour variation are scarce for *Epimedium*, with the taxonomic significance or mechanism

of the variation currently unknown. The flower colour diversity may be a signal of plant speciation, which also may be one of the reasons for the difficulty in assessing it for taxonomy (Bradshaw et al. 1995; Matsumura et al. 2006; Hopkins and Rausher 2011). Therefore, the study of the flower colour variation is of great significance to the taxonomy ranking of *Epimedium*, as well as for its evolution (Wang et al. 2017).

As part of our ongoing efforts to study the *Epimedium* diversity in China, we studied the relevance of flower colour variation for the taxonomy of *Epimedium* based on extensive field studies for five species of Chinese *Epimedium*, with additional cultivation and herbaria studies. Moreover, we present a comprehensive revision on their taxonomic description, including the variation on rhizome morphology, the number and arrangement of stem-leaves, and indumentum type. Additionally, we provide an identification key for the species of *Epimedium* recognised in China. The results provide abundant and important information for the taxonomy of *Epimedium*, and subsidies in its exploration and utilisation, for example, as ornamental plants.

## Materials and methods

In *Epimedium*, some diagnostic features, especially those of flowers and inflorescences, are obscured or not visible in the herbarium material. Moreover, the flowers are of frail texture and deciduous. Therefore, it is difficult to infer the exact colouration of *in vivo* flowers by examining dried specimens. Therefore, the vegetative as well as reproductive characters were examined mainly in their natural habitats, while the herbarium specimens were used as aids. Herbarium specimens were examined from the following herbaria: CDBI, E, GXMI, HGAS, HIB, IBK, IBSC, IMD, JXCM, K, KUN, N, NAS, P, PE, and SM (acronyms according to Thiers, continuously updated).

All field investigations and observations were conducted in full bloom from 2012 to 2017. To investigate the morphological variation, we carried out field surveys throughout the Chinese distribution range of *Epimedium*, especially in Sichuan, Guizhou, Guangxi, Hubei, Hunan, Anhui and Jiangxi provinces and Chongqing municipality. In each province or municipality, a survey, as wide and thorough as possible, was conducted. The species that exhibited variations in flower colour and other characters were then observed and sampled (Table 1). Herbarium specimens collected during our fieldwork were deposited in the herbarium of Jiangxi University of Traditional Chinese Medicine (JXCM). The ecological information of the relevant populations was recorded. With the aim of confirming the identifications and better understanding the difference between individuals, as well as populations, 20–30 individuals were collected from each population and posteriorly transplanted to the Jiangxi University of Traditional Chinese Medicine for further observations and studies. Quantitative measurements of rhizome diameter, the number of stem-leaves, height of the flowering stem, length of inflorescence, and the number of flowers were recorded for each specimen. Simultaneously, the following discrete morphological characters were also observed: type of rhizome; indumentum; arrangement of stem-leaves; inflorescence

**Table 1.** Location and habitat characters of five *Epimedium* species.

Species	Population code	Location (China)	Elevation (m)	Latitude (N)	Longitude (E)
<i>E. acuminatum</i>	CQNC*	Tianxing, Nanchuan, Chongqing	899	29°03'	107°07'
	SCEM*	Emei Mountain, Leshan, Sichuan	1049	29°34'	103°25'
	SCMP*	Muping, Baoxing, Sichuan	1248	30°20'	102°48'
	SCSL*	Shangli, Yaan, Sichuan	862	30°11'	103°04'
	SCSS*	Shuangshi, Lushan, Sichuan	928	30°15'	102°55'
	SCYJ*	Siping, Yingjing, Sichuan	1448	29°43'	102°37'
	GZNL	Nanlong, Kaiyang, Guizhou	986	27°05'	107°05'
	GZYH	Hekan, Yanhe, Guizhou	637	29°02'	108°09'
	GZZA	Fengyi, Zheng'an, Guizhou	792	28°43'	107°51'
	CQFL	Mawu, Fuling, Chongqing	935	29°31'	107°19'
	CQYC	Chashanzhuhai, Yongchuan, Chongqing	762	29°28'	105°58'
	SCHJ	Fubao, Hejiang, Sichuan	723	29°28'	105°59'
	SCMB	Dazhubao, Mabian, Sichuan	1147	29°00'	103°30'
	SCXH	Xiaohe, Lushan, Sichuan	1192	30°29'	103°07'
	SCCM	Caomigang, Lushan, Sichuan	1534	30°26'	103°05'
	SCQL	Youzha, Qionglai, Sichuan	1067	30°26'	103°14'
	SCMT	Naobao, Qionglai, Sichuan	1468	30°25'	103°07'
SCLG	Lingguan, Baoxing, Sichuan	1068	30°18'	102°48'	
SCWN	Wanniansi, Emei Mountain, Sichuan	1110	28°43'	107°51'	
<i>E. leptorrhizum</i>	GZGY*	Shuikousi, Guiyang, Guizhou	1145	26°34'	106°44'
	GZST*	Ganlong, Songtao, Guizhou	907	28°19'	108°41'
	HNBJ*	Zhuping, Baojing, Hunan	978	28°36'	109°12'
	HBCP	Gongjiaping, Enshi, Hubei	1523	30°10'	109°44'
	HBMF	Mufu, Enshi, Hubei	1080	30°17'	108°56'
	HBLC	Fubaoshan, Lichuan, Hubei	1386	30°12'	108°43'
	HBTB	Tuanbao, Lichuan, Hubei	1200	30°22'	109°07'
	GZLC	Longchang, Kaili, Guizhou	796	26°39'	107°56'
	GZLS	Lingshan, Guiyang, Guizhou	1207	26°36'	106°42'
	GZMH	Muhuang, Yinjiang, Guizhou	1300	28°02'	108°42'
	ZJLQ	Cukeng, Longquan, Zhejiang	1210	27°55'	119°10'
	GDNL	Ruyuan, Shaoguan, Guangdong	670	24°55'	113°03'
<i>E. pauciflorum</i>	SCWC*	Yanmen, Wenchuan, Sichuan	1817	31°27'	103°34'
	SCMS*	Miansi, Wenchuan, Sichuan	1780	31°35'	103°49'
	SCJZ*	Jingzhou, Maoxian, Sichuan	1800	31°42'	103°53'
<i>E. mikinorii</i>	HBES*	Baiguo, Enshi, Hubei	754	30°14'	109°22'
	HBXT*	Xintang, Enshi, Hubei	1370	30°13'	109°41'
<i>E. glandulosopilosum</i>	CQWX*	Tongcheng, Wuxi, Chongqing	1161	31°23'	109°46'

\*: the population with flower colour variation

morphology; variation of flowers (i.e., shape, colour and proportion); and the colour of pollen *in vivo*. In addition, particular attention was paid to preparing herbarium specimens, deposited at JXCM herbarium, and photographing plants and also their floral parts in their natural habitat.

The Flora of China (Ying et al. 2011) and the monograph of the genus *Epimedium* (Stearn 2002) were followed to describe the vegetative and reproductive characters of the studied species.

## Results

*Epimedium acuminatum* Franch., *E. leptorrhizum* Stearn, *E. pauciflorum* K.C.Yen, *E. mikinorii* Stearn and *E. glandulosopilosum* H.R.Liang, were found with intraspecific flower colour variation. Moreover, all these species were also morphologically variable in the number and arrangement of stem-leaves, the type of rhizomes and/or indumentum. Location, code, latitude, longitude, and elevation of the referred populations are presented in Table 1. The taxonomic descriptions, related illustrations, and information on the five species were updated below.

### 1. *Epimedium acuminatum* Franch., Bull. Soc. Bot. France 33: 109. 1886

Fig. 1

*Epimedium komarovii* H.Léveillé, Fedde Repert. Spec. Nov. Regni Veg. 7: 259. 1909.

Type: CHINA. Guizhou: Pin-Fa, 1908, *Cavalerie 954* (holotype: E00270388!).

*Epimedium simplicifolium* T.S.Ying, Acta Phytotaxon. Sin. 13: 51. 1975. Type: CHINA. Guizhou: Wuchuan, 9 May 1928, *P. C. Tsoong 606* (holotype: PE01432137!; isotype: PE01432138!).

*Epimedium chlorandrum* Stearn, Kew Bull. 52: 660. 1997. Type: CHINA. Sichuan: Baoxing, cultivated in England, Hampshire, Kilmeston, Blackthorn Nursery, April 1996, *Ogisu 94003* (holotype: K000340098!).

**Type.** CHINA. Guizhou: 1858, *Perny* s.n. (holotype: P, barcode P02327614!; isolecotype: P, barcode P02327612!).

**Description.** Herbs 20–80(–110) cm tall. Rhizome compact, sometimes long creeping, 2–8 mm in diam. Leaves basal and cauline, usually trifoliolate or occasionally unifoliolate; leaflets of trifoliolate leaves narrowly ovate or lanceolate, 3–19.8 × 1.5–8.9 cm, apex long acuminate, base cordate, lobes rounded or acute, those of the lateral leaflets very unequal; unifoliolate leaves ovate or broadly ovate, 8.7–20 × 6.8–11.5 cm, apex acuminate, base cordate, lobes equal, rounded or rarely acute; leaves leathery when mature, margin spinous-serrate, spines 1–2 mm long, adaxially deep green, glossy, abaxially glaucous, papillose, with dense or sparse shortly appressed stout bristle-like hairs and sometimes densely sericeous. Flowering stem with 2 trifoliolate opposite leaves, sometimes 3-whorled trifoliolate leaves or 2 opposite unifoliolate, rarely with 2 opposite leaves with one trifoliolate and the other unifoliolate, 3 whorled leaves with one trifoliolate and two unifoliolate or 3 whorled unifoliolate leaves. Panicle 6–70(–108)-flowered, 6–33 cm long, with lower peduncles loosely 2–5-flowered, glabrous or occasionally glandular-hairy. Pedicel 1–5 cm long. Flowers large, 3–5 cm in diam. Outer sepals 4, blunt, outer pair ovate-oblong, ca. 3 × 2 mm, inner pair broadly obovate, ca. 4.5 × 4 mm; inner sepals ovate-elliptic, 6–21 × 3–9 mm, apically acute, white, yellowish, pale rose or rose, petals pale yellow, yellow, pale violet, reddish-purple, purple-yellow, pale purple or purple. Petals curving outward, horn-shaped, much

longer than inner sepals, 15–25 mm, tamping from the swollen but lamina-less base. Stamens 3–4 mm long; anthers yellow or green, ca. 2.5 mm long. Follicles oblong, ca. 20 mm long, style rostriform. Seeds numerous.

**Distribution and habitat.** *Epimedium acuminatum* is one of the most widespread species in the genus, distributed in Sichuan, Guizhou, Chongqing, and northern Yunnan. Its large distribution area is predominantly characterised by mountain land. *Epimedium acuminatum* is often found on mountain slopes, forest edges or weedy slopes with elevations ranging from 270 m to 2400 m (Fig. 2).

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

**Taxonomical remarks.** Before this study, flower colour variation in *E. acuminatum* had already been recognised. Overall, yellow (B. Y. Peng 47073, F. T. Wang 23329, D. Y. Peng 47070, W. P. Fang 802, Anon. 86, Z. X. Qu 1305, Q. H. Chen et al. 9411) or pale yellow (*Sanxia Exped.* 0821, X. B. Zhang 19), purple (G. H. Yang 54343, *Sichuan Econ. Pl. Exped.* 0030, K. J. Guan et al. 0273, Xiong & Z. L. Zhou 91045, Z. Y. Liu 15500, T. H. Tu 3116, S. Z. He 96410, S. W. Teng 0008, P. Zhao 807, Y. Tsiang 4994) or pale purple (P. Zhao 762, *Jinfoshan Exped.* 0330, *Jinfoshan Exped.* 0202, G. F. Li 60324), purple-whitish (T. T. Yu 312, Z. Z. Guo 403, J. M. Yuan 003 and J. M. Yuan 005, S. W. Tfng 90093, Z. S. Zhang et al. 401131), and white (K. J. Guan et al. 477, C. H. Li 97-301, K. Y. Lang 3002, K. J. Guan et al. 165) or whitish (Z. X. Qu 1057, *Sanxia Exped.* 0729 and *Sanxia Exped.* 0909) were the most frequently recorded flower colours. However, specific colours, for example, reddish (S. P. Pong 6108), yellowish purple (X. Y. He 4050; T. C. Pan & G. F. Wu 105), and pale purple-green (Z. Y. Wu 60) have also been examined from specimens. Colour differences among individuals of the same location have been slightly recorded, but both yellow and purple flowers were recorded in *Sichuan Econ. Pl. Exped.* 0013. Moreover, continuous variations from yellow to white and from yellow to pale reddish-purple were remarked by T. H. Tu 2763 and B. L. Guo 0608, respectively.

Two synonyms are included in *E. acuminatum*, namely *E. simplicifolium* and *E. chlorandrum*. There were only two specimens of *E. simplicifolium* for reference. The holotype (P. C. Tsoong 606) recorded yellow flowers with purplish red petals. Since these descriptions were based on flowers that are not fully open – the outer sepals soon falling – the outermost is formed of inner sepals. Therefore, the “yellow flower” actually is “yellow or yellowish inner sepals”. Another specimen (S. Z. He 96410) recorded a purplish red flower, and the inner sepals spotted with purplish red. *Epimedium chlorandrum* has six specimens. Greenish inner sepals and pale yellow petals have been described in the holotype (Ogisu 94003). Pale yellow inner sepals and petals were recorded in B. L. Guo 0540 and B. L. Guo 0607 while B. L. Guo 0606 described the colour variation of inner sepals as anything from pale yellow to pale purplish red. And B. L. Guo 0608 also described both inner sepals and petals from pale yellow to pale purplish red.

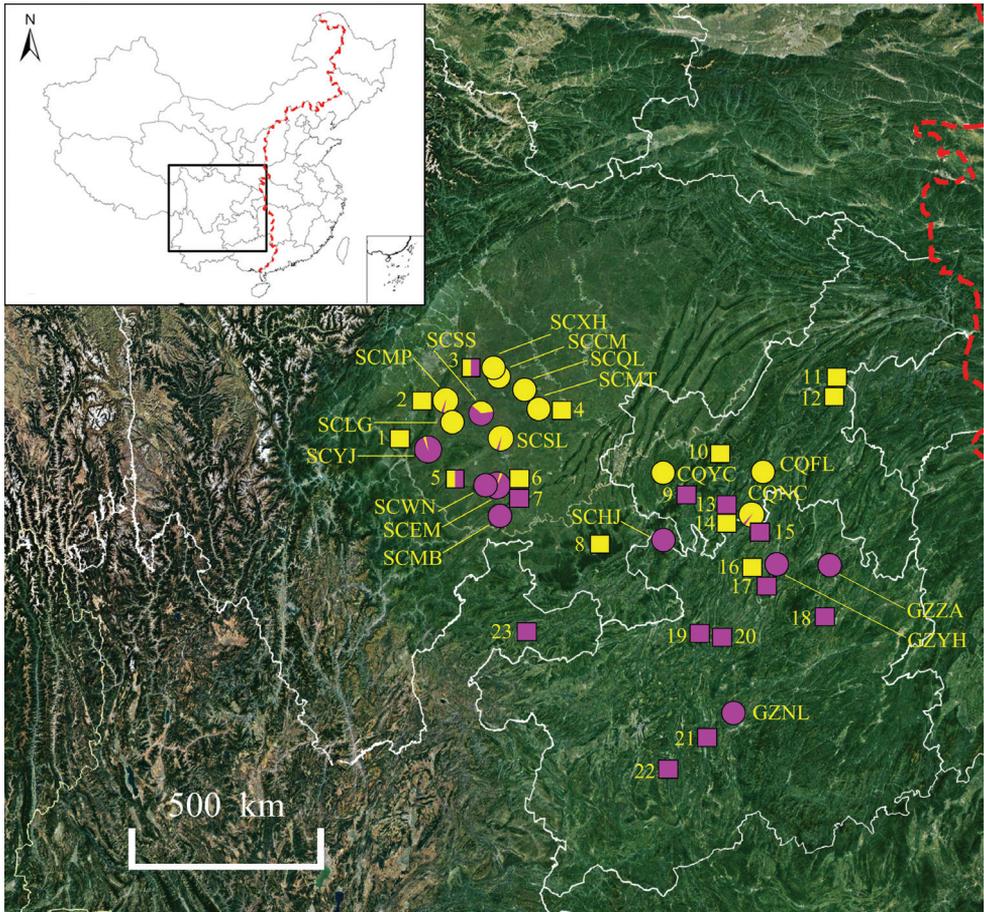
Based on a field survey at the population level, we observed more extensive and continuous colour variation from pale yellow to dark purple (Fig. 1). Pure white flow-



**Figure 1.** The flower colour variations of different populations of *E. acuminatum*. **A** SCMP, mainly yellow **B** SCSL, mainly yellow, occasionally purple-yellow at the base of petals **C** CQNC, mainly yellow, occasionally rose-purple **D** SCSS, from yellow to purple **E** SCEM, mainly purple, occasionally purple-yellow **F** SCYJ, mainly purple, occasionally purple-yellow **G** Individual with yellow flowers **H** Individual with purple-yellow flowers **I** Individual with purple flowers.

ers have not been observed, but the pale yellow and whitish are very close to white. We therefore speculate that the white flowers described in the specimens might represent pale yellow or whitish flowers instead. Regarding the yellow and purple flowers, there was abundant colour variation among populations as well as among individuals. For example, yellow ranged from pale yellow to yellow, while purple ranged from pale violet or reddish-purple to purple and dark purple. Moreover, there were also many transitions between yellow and purple.

For *E. acuminatum*, the populations that showed uniform colour (yellow or purple) were excluded from the illustration in this study. We mainly focused on the populations that presented colour variation (Fig. 1). For example, SCMP showed mainly



**Figure 2.** Geographical variation in flower colour patterns within *E. acuminatum*. The circles and boxes represent wild populations and herbarium specimens, respectively. The colour of the circles and boxes represent flower colour. The 19 population codes are shown in Table 1. The left area of the red dotted line is western China. The information of 23 representative herbarium specimens is as follow: 1. B. Y. Peng 47070; 2. B. L. Guo 0540; 3. B. L. Guo 0608; 4. B. L. Guo 0607; 5. Sichuan Econ. Pl. Exped. 0013; 6. F. T. Wang 23329; 7. G. H. Yang 54343; 8. K. Y. Lang 3002; 9. Sichuan Econ. Pl. Exped. 0030; 10. T. C. Pan & G. F. Wu 105; 11. Sanxia Exped. 0729; 12. Sanxia Exped. 0821; 13. K. J. Guan et al. 0273; 14. X. B. Zhang 19; 15. Jinfoshan Exped. 0202; 16. Q. H. Chen et al. 9411; 17. J. M. Yuan 003; 18. Z. S. Zhang et al. 401131; 19. Y. Tsiang 4994; 20. S. Z. He 96410; 21. P. Zhao 807; 22. S. W. Teng 0008; 23. Z. Y. Wu 60.

yellow flowers; only one out of the 30 individuals presented diverse colour, with a yellow spur tinged with a ray of rose inside the base of the petals. SCSL mainly had yellow flowers; parts of individual flowers showed pale yellow, and one individual presented purple-yellow at the base of the petals. CQNC primarily displayed yellow flowers; several individuals revealed a rose margin at the base of the petals, and one individual revealed reddish-purple flowers. SCSS was the most special population with 20 individuals had purple flowers while the rest (10 individuals) had yellow flowers. Moreover,

SCSS was the only population for which the colour showed a continuous variation from yellow to purple. The flowers of SCEM and SCYJ were mainly with purple flowers, and purple-yellow occasionally (Fig. 1). In the above populations, SCMP and SCSS were *E. chlorandrum*.

Combing the geographical distribution of specimens (both field and herbarium specimens) and their flower colour variation of *E. acuminatum*, we found an interesting result (Fig. 2). Geographic variation in flower colour pattern within *E. acuminatum* showed a north-south geographic trend. The specimens from the northern area of its distribution mainly have yellow flowers, while the southern ones usually have purple flowers. The polymorphism of flower colour is mainly concentrated in the northwest of its distribution area.

In addition, the number and arrangement of the stem-leaves were significantly diverse in *E. acuminatum*. It commonly showed 2 trifoliolate opposite leaves, sometimes 3-whorled trifoliolate leaves (*G. F Li 60514*, *Y. Tsiang 4994*) or 2 opposite unifoliolate (*S. Z. He 96410*, *P. C. Tsong 606*), rarely with 2 opposite leaves with one trifoliolate and the other unifoliolate (*J. H. Xiong 30469*), 3 whorled leaves with one trifoliolate and two unifoliolate (*J. H. Xiong 30469*) or 3 whorled unifoliolate leaves.

**Additional specimens examined.** CHINA. **Sichuan:** Chengxiang, Tianquan, 1100–1200 m, 24 March 1983, *B. Y. Peng 47070* and *B. Y. Peng 47073* (CDBI, yellow flower); Jiulaodong, Mt. Emei, 1800 m, 02 May 1957, *G. H. Yang 54343* (PE, KUN, HIB, NAS, purple flower); Lianhuashi, Mt. Emei, 1950 m, 11 May 1964, *K. J. Guan et al. 477* (PE, white flower); Mt. Emei, 1100 m, 11 June 1933, *S. P. Pong 6108* (PE, reddish flower); Mt. Emei, 1050 m, 13 March 1997, *C. H. Li 97–301* (PE, white flower); Mt. Emei, 900 m, 16 April 1932, *T. T. Yu 312* (N, purplish white flower); Mt. Emei, 2000 m, 15 July 1931, *F. T. Wang 23329* (PE, yellow flower); Mt. Emei, 900 m, 16 April, *T. T. Yu 312* (PE, purplish white flower); Mt. Emei, 1700 m, 27 May 1959, *Z. Z. Guo 403* (PE, purplish white flower); Mt. Emei, 1952, *J. H. Xiong 30469* (PE, 3 whorled leaves with one trifoliolate and two unifoliolate; IBSC, 2 opposite leaves with one trifoliolate and the other unifoliolate); Mt. Emei, 1049 m, 4 April 2015, *Y. Q Xu & S. X. Liu 2015029* (JXCM, mainly purple flower); Mt. Emei, 1110 m, 10 April 2017, *Y. M. He et al. 2017006* (JXCM, purple flower); Biexiandong, Jiang'an, 270 m, 5 April 1964, *K. Y. Lang 3002* (PE, white flower); Wuzhuang, Hongya, 1150 m, 20 April 1959, *Sichuan Econ. Pl. Exped. 0013* (SM, yellow and purple flower); Muping, Baoxing, 13 May 2005, *B. L. Guo 0540* (IMD, pale yellow flower); Dachuan, Lushan, 1484 m, 4 May 2006, *B. L. Guo 0606* (IMD, yellow flower); Youzha, Qionglai, 4 May 2006, *B. L. Guo 0607* (IMD, pale yellow flower); Dachuan, Lushan, 1484 m, 4 May 2006, *B. L. Guo 0608* (IMD, from yellow to pale reddish-purple); Shangli, Ya'an, 862 m, 7 April 2015, *Y. Q Xu & S. X. Liu 2015027* (JXCM, yellow flower); Siping, Yingjing, 1448 m, 8 April 2015, *Y. Q Xu & S. X. Liu 2015026* (JXCM, mainly purple flower); Fubao, Hejiang, 723 m, 10 April 2016, *S. X. Liu & J. X Zhu 2016007* (JXCM, purple flower); Dazhubao, Mabian, 1147 m, 13 April 2016, *S. X. Liu & J. X Zhu 2016008* (JXCM, purple flower); Dachuan, Lushan, 1192 m, 15 April 2016, *S. X. Liu & J. X Zhu 2016009* (JXCM, yellow flower); Dachuan, Lushan, 1534 m, 16 April 2016, *S. X. Liu*

*♂* J. X Zhu 2016010 (JXCM, yellow flower); Youzha, Qionglai, 1067 m, 16 April 2016, S. X. Liu & J. X Zhu 2016011 (JXCM, yellow flower); Nanbao, Qionglai, 1468 m, 16 April 2016, S. X. Liu & J. X Zhu 2016012 (JXCM, yellow flower); Lingguan, Baoxing, 1068 m, 18 April 2016, S. X. Liu & J. X Zhu 2016014 (JXCM, yellow flower); Shuangshi, Lushan, 928 m, 11 April 2015, Y. Q. Xu & S. X. Liu 2015024 (JXCM, purple flower and yellow flower); Baoxing, cultivated at ENGLAND. Hampshire: Kilmeston, Blackthorn Nursery, April 1996, *Ogisu 94003* (holotype, K). **Chongqing:** Baiwuping, Nanchuan, 850 m, 16 April 1957, *G. F. Li 60514* (IBSC, KUN, NAS, 3-whorled trifoliolate leaves, reddish-purple flower); Tianxing, Nanchuan, 899 m, 27 March 2015, *Y. Q. Xu & S. X. Liu 2015035* (JXCM, yellow flower); Nanchuan, 1500–1800 m, 16 May 1928, *W. P. Fang 802* (PE, IBSC, N, yellow flower); Xiaohe, Nanchuan, 1000 m, 10 May 1985, *Z. Luo 0033* (CDBI); Mt. Jinfo (Jinfoshan), Nanchuan, 1700–1900 m, 13 April 1964, *K. J. Guan et al. 0273* (CDBI, PE, purple flower); Mt. Jinfo (Jinfoshan), Nanchuan, 1550 m, 28 May 1957, *J. H. Xiong & Z. L. Zhou 91045* (KUN, purple flower); Mt. Jinfo (Jinfoshan), Nanchuan, 880 m, 25 May 1935, *X. Y. He 4050* (NAS, yellowish purple flower); Hetaoping, Mt. Jinfo (Jinfoshan), Nanchuan, 28 May 1935, *X. Y. He 4148* (NAS, whitish flower); Hetaoping, Mt. Jinfo (Jinfoshan), Nanchuan, 28 April 1935, *X. B. Zhang 19* (NAS, pale yellow flower); Mt. Jinfo (Jinfoshan), Nanchuan, 3 June 1935, *Anon. 86* (NAS, yellow flower); Yangyuping, Mt. Jinfo (Jinfoshan), Nanchuan, 1800 m, 14 May 1986, *Jinfoshan Exped. 0330* (PE, pale purple flower); Mt. Jinfo (Jinfoshan), Nanchuan, 720 m, 2 April 1996, *Z. Y. Liu 15500* (PE, purple flower); Mt. Jinfo (Jinfoshan), Nanchuan, 1200 m, 2 June 1935, *Z. X. Qu 1057* (PE, IBSC, whitish flower); Daheba, Mt. Jinfo (Jinfoshan), Nanchuan, 1020 m, 8 April 1964, *K. J. Guan et al. 165* (PE, white flower); Fenghuangsi, Nanchuan, 2050 m, 12 June 1935, *Z. X. Qu 1305* (PE, yellow flower); Delong, Nanchuan, 1500 m, 12 May 1986, *Jinfoshan Exped. 0202* (PE, pale purple flower); Nanchuan, 4 April 1933, *T. H. Tu 2763* (PE, yellow-white flower); Nanchuan, 1900 m, 22 May 1933, *T. H. Tu 3116* (PE, purple flower); Fangheba, Nanchuan, 550 m, 5 April 1957, *G. F. Li 60324* (PE, pale purple flower); Mt. Jinyun (Jinyunshan), Beibei, 700 m, 4 April 1963, *T. C. Pan & G. F. Wu 105* (PE, yellowish purple flower); Wangerbao Nature Reserve, Wanzhou, 977–1221 m, 25 April 2008, *Sanxia Exped. 0729* and *Sanxia Exped. 0909* (PE, whitish flower); Longju, Wanzhou, 981–1143 m, 27 April 2008, *Sanxia Exped. 0821* (PE, pale yellow flower); Simian, Jiangjin, 1200 m, 16 April 1959, *Sichuan Econ. Pl. Exped. 0030* (KUN, NAS, purple flower); Mawu, Fuling, 935 m, 7 April 2016, *S. X. Liu & J. X Zhu 2016005* (JXCM, yellow flower); Chashanzhuhai, Yongchuan, 762 m, 9 April 2016, *S. X. Liu & J. X Zhu 2016006* (JXCM, yellow flower). **Guizhou:** Fengyi, Zhenggan, 700–1000 m, May 1992, *Q. H. Chen et al. 9411* (HGAS, yellow flower); Fengyi, Zhenggan, 700 m, 1 June 1977, *J. M. Yuan 003* and *J. M. Yuan 005* (HGAS, purple-whitish flower); Huajiang, 1100 m, 1 April 1987, *P. Zhao 762* (HGAS, pale purple flower); Anshun, 25 May 1935, *S. W. Teng 0008* (IBSC, purple flower); Liuchongguan, Guiyang, 1300 m, 21 April 1987, *P. Zhao 807* (HGAS, purple flower); Maoshajing, Guiyang, 16 April 1936, *S. W. Teng 90093* (IBSC, purple-whitish flower); Tongzi, 20 May 1930, *Y. Tsiang 4994* (PE, purple flower, 3-whorled trifoliolate leaves); Tuanlong, Yinjiang, 1090

m, 10 April 1964, Z. S. Zhang *et al.* 401131 (PE, purple-whitish flower); Shuikousi, Guiyang, 1400 m, 16 March 1959, *Qiannan Exped.* 42 (PE, KUN, reddish-purple flower); Suiyang, 1100 m, 10 April 1994, S. Z. He 96410 (PE, 2 opposite unifoliolate leaves, purple flower); Longdong, Wuchuan, 9 May 1928, P. C. Tsoong 606 (PE, KUN, IBSC, 2 opposite unifoliolate leaves); Nanlong, Kaiyang, 986 m, 28 March 2016, S. X. Liu & J. X. Zhu 2016002 (JXCM, purple flower); Hekan, Yanhe, 637 m, 1 April 2016, S. X. Liu & J. X. Zhu 2016003 (JXCM, purple flower); Fengyi, Zhengan, 792 m, 4 April 2016, S. X. Liu & J. X. Zhu 2016004 (JXCM, purple flower). **Yunnan:** Shuanghe, Weixin, 1480 m, 3 June 1960, P. Di 1076 (KUN); Chaotianma, Yiliang, 1973, Z. Y. Wu 60 (KUN, pale purple-green flower).

## 2. *Epimedium leptorrhizum* Stearn, J. Bot. 71: 343. 1933

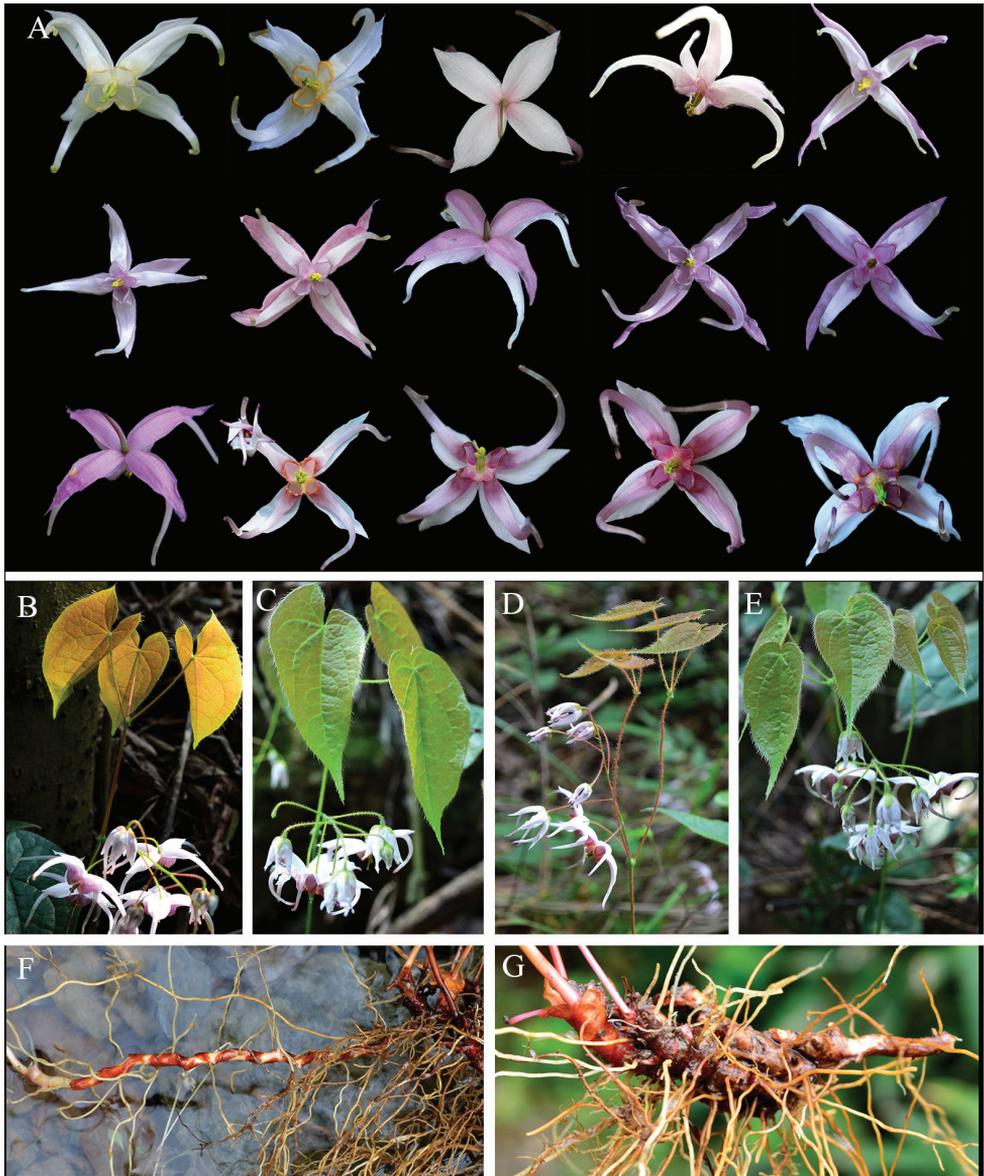
Fig. 3

*Epimedium brachyrrhizum* Stearn, Kew Bull. 52: 659. 1997. Type: CHINA. Guizhou: Mt. Fanjingshan, cultivated in the USA, Massachusetts, Hubbardston, Darrell Probst CPC940495 (holotype: K000340100!).

**Type.** CHINA. Guizhou: Guiyang, *Bodinier* 2184 (holotype: P; barcode P00568282!; isotypes: P; barcodes P00572792!, P00572793!, P00572800!, P00572801!, P00572802!).

**Description.** Herbs 12–50 cm tall. Rhizome long creeping, or compact, 1–5(–8) mm in diam.; internodes sometimes to 20 cm. Leaves basal and cauline, trifoliolate or occasionally unifoliolate. Leaflets of trifoliolate leaves narrowly ovate or ovate, 3–16 × 2–8.6 cm, apex long acuminate, base deeply cordate with usually rounded lobes nearly touching, those of the lateral leaflets very unequal; unifoliolate leaves ovate or broadly ovate, 8–13.7 × 5–11 cm, apex acuminate, base cordate with lobes equal, rounded and rarely acute; leaves leathery, margin spinous-serrate, adaxially deep green, glossy, abaxially glaucous, papillose, and pubescent along veins, especially dense at the insertion of petioles and petiolules. Flowering stem with 1 trifoliolate leaf or 2 opposite leaves. Inflorescence simple, racemose, 3–14-flowered, 12–25 cm long, glandular. Pedicel 1–2.5 cm long, glandular. Flowers large, ca. 4 cm in diam. Outer sepals green or purplish, outer pair ovate-oblong, 3–5 × 2 mm, apex obtuse, inner pair broadly ovate, 4–5.5 × 3–4.5 mm, apex acuminate; inner sepals white, white tinged with rose, or rose, narrowly elliptic, 11–20 × 4–7 mm, apex acute. Petals subequal to or longer than the inner sepals, white or pale yellow with base yellow or orange, white with base rose or deep rose, rose, deep rose or pale purple, horn-shaped, 15–26 mm, tapering from a swollen but lamina-less base. Stamens ca. 4 mm long; anthers, yellow or green, ca. 3 mm long. Follicles oblong, 8–18 mm long; style rostriform. Seeds numerous.

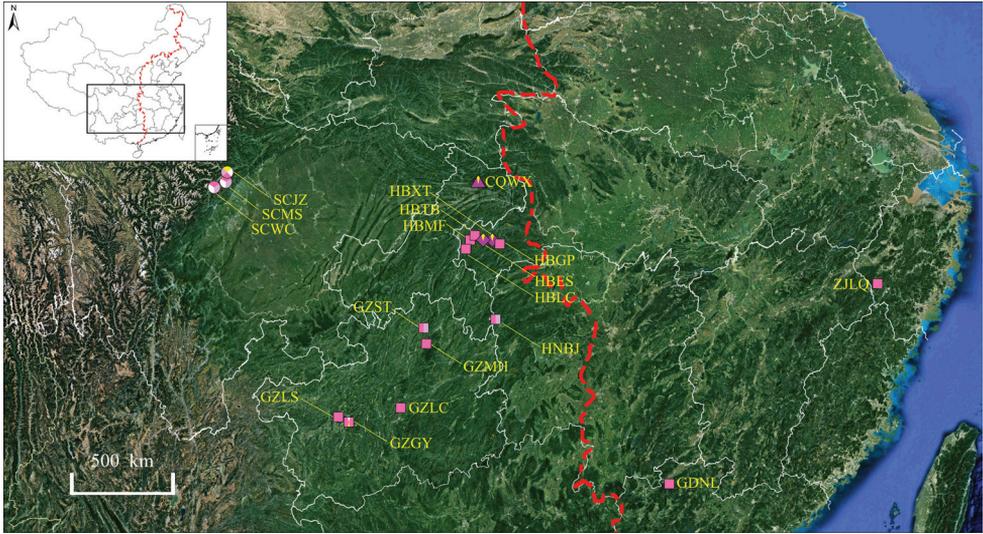
**Distribution and habitat.** *Epimedium leptorrhizum* is distributed in the montane forests or thickets in Guizhou, Hubei, Hunan and Chongqing, in elevations ranging from 350 m to 2100 m (Fig. 4).



**Figure 3.** The variations and morphological characters of *E. leptorrhizum*. **A** the colour variations of inner sepals and petals **B–E** variations of the number and arrangement of stem-leaves **F** long creeping rhizome **G** stout and compact rhizome.

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

**Taxonomical remarks.** Abundant and continuous flower colour variation has been observed and illustrated for this species. The inner sepals presented continuous variation from white, white tinged with rose, through to completely rose. Petals varied



**Figure 4.** Geographical variation in flower colour patterns within *E. leptorrhizum* (boxes), *E. pauciflorum* (circles), *E. mikinorii* (diamonds) and *E. glandulosopilosum* (triangle). The colour of the boxes, circles, diamonds and triangle represent flower colour. The population codes are shown in Table 1. The left area of the red dotted line is western China.

from white or pale yellow with base yellow or orange, white with base rose or deep rose to completely rose, deep rose or pale purple (Fig. 3). Among them, white and pale yellow were only observed in one individual of the GZGY population. However, it is very common that the colour ranged from pale rose or rose to deep rose or pale purple among and within populations. The synonym, *E. brachyrrhizum* (GZLC) didn't show any variation of flower colour, with rose inner sepals and pale rose petals.

In addition, we showed that the rhizome of *E. leptorrhizum* is usually long-creeping, occasionally compact. The flowering stem is usually with 1 trifoliolate leaf or 2 opposite leaves.

**Additional specimens examined.** CHINA. **Guizhou:** Mt. Qianling, Guiyang, 10 March 1957, *Anon.* 0123-635 (IMD); Mt. Qianling, Guiyang, 28 August 1977, *Anon.* 5507 (IMD); Qianling Park, Guiyang, 1400 m, 19 March 1959, *Qiannan Exped.* 0077 (PE); Guiyang, 5 June 1994, *B. L. Guo* 94018 (IMD); Shuikousi, Guiyang, 1145 m, 2 April 2012, *Y. Q. Xu & Y. Xu* 2012001 (JXCM); Minzhu, Longli, 1407 m, 1 April 2004, *B. L. Guo* A38 (IMD); Longli, 1268 m, 15 April 2003, *B. L. Guo* A81 (IMD); Ganlong, Songtao, 775 m, 19 April 2003, *B. L. Guo* A87 (IMD); Songtao, 19 April 2003, *B. L. Guo* A98 (IMD); Huguo Temple, Yinjiang, 1100 m, 8 April 1964, *Z. S. Zhang & T. L. Min* 401219 (PE, HGAS); Pingba, Anshun, 1500 m, 24 September 1959, *Anshun Exped.* 1548 (PE); Ganwangou, Jiangkou, 350 m, 16 April 1990, *S. Z. He* 90005 (HGAS); Wenang, Libo, 850 m, 29 April 1984, *Q. H. Chen* 2377 (HGAS); Fuli, Bijie, 2100 m, 12 July 1959, *Bijie Exped.* 266 (HGAS); Zhennan, Wuchuan, 580 m, 26 May 1977, *J. H. Yuan* 001 (HGAS);

Zhuoshui, Wuchuan, 580 m, 27 May 1977, *J. H. Yuan 002* (HGAS); Mt. Fanjing (Fanjingshan), Yinjaing, 1060 m–1200 m, 18 June 1963, *Anon. 680* (HGAS); Ganlong, Songtao, 907 m, 10 April 2013, *Y. Q. Xu & K. N. Zhang 2012010* (JXCM); Muluang, Yinjiang, 1300 m, 1 April 2017, *Y. M. He & L. J. Liu 2017002* (JXCM). **Hubei:** Maoping, Jianshi, Enshi, 1019 m, 9 April 2008, *B. L. Guo & J. J. Liu 0804* (IMD); Maotian, Jianshi, Enshi, 983 m, 9 April 2008, *B. L. Guo & J. J. Liu 0805* (IMD); Banqiao, Enshi, 1258 m, 12 April 2008, *B. L. Guo & J. J. Liu 0808* (IMD); Daijing, Lichuan, Enshi, 1100 m, 25 April 1989, *B. L. Guo & X. Z. Luo 89005* (IMD); Mt. Fubao (Fubaoshan), Lichuan, Enshi, 1460 m, 29 April 1989, *B. L. Guo & X. Z. Luo 89009* (IMD); Mt. Fubao (Fubaoshan), Lichuan, Enshi, 1293 m, 8 April 2004, *B. L. Guo A54* (IMD); Lichuan, Enshi, 1141 m, 8 April 2004, *B. L. Guo A47* and *B. L. Guo A52* (IMD); Enshi, November 1958, *H. J. Li 8738* (PE); Qianping, Xintang, Enshi, 1523 m, 24 April 2012, *Y. Q. Xu 2012004* (JXCM); Mt. Fubao (Fubaoshan), Lichuan, Enshi, 1386 m, 2 April 2012, *Y. Q. Xu & Y. Xu 2012006* (JXCM). **Hunan:** Jishou University, Jishou (cultivated, from Longshan), 17 April 2003, *B. L. Guo A99* (IMD); Mt. Tianping (Tianpingshan), Sangzhi, 441 m, 5 April 2004, *B. L. Guo A32* (IMD); Mt. Tianping (Tianpingshan), Sangzhi, 26 August 1988, *H. N. Xun 3967* (PE). **Chongqing:** Xinglong, Youyang, 1000 m, 4 May 1959, *Anon. 02265* (PE); Guanyang, Wushan, 1056–1124 m, 9 May 2008, *Sanxia Exped. 1305* (PE). **Guangxi:** Dongshan, Quaxian, 840 m, 17 July 1958, *Y. C. Chen 00102* (IBK). **Zhejiang:** Datianping, Longquan, 1175 m, 9 April 2005, *B. L. Guo 0516* (IMD); Datianping, Longquan, 1210 m, 27 April 2018, *Y. Q. Xu & H. Huang 2018005* (JXCM).

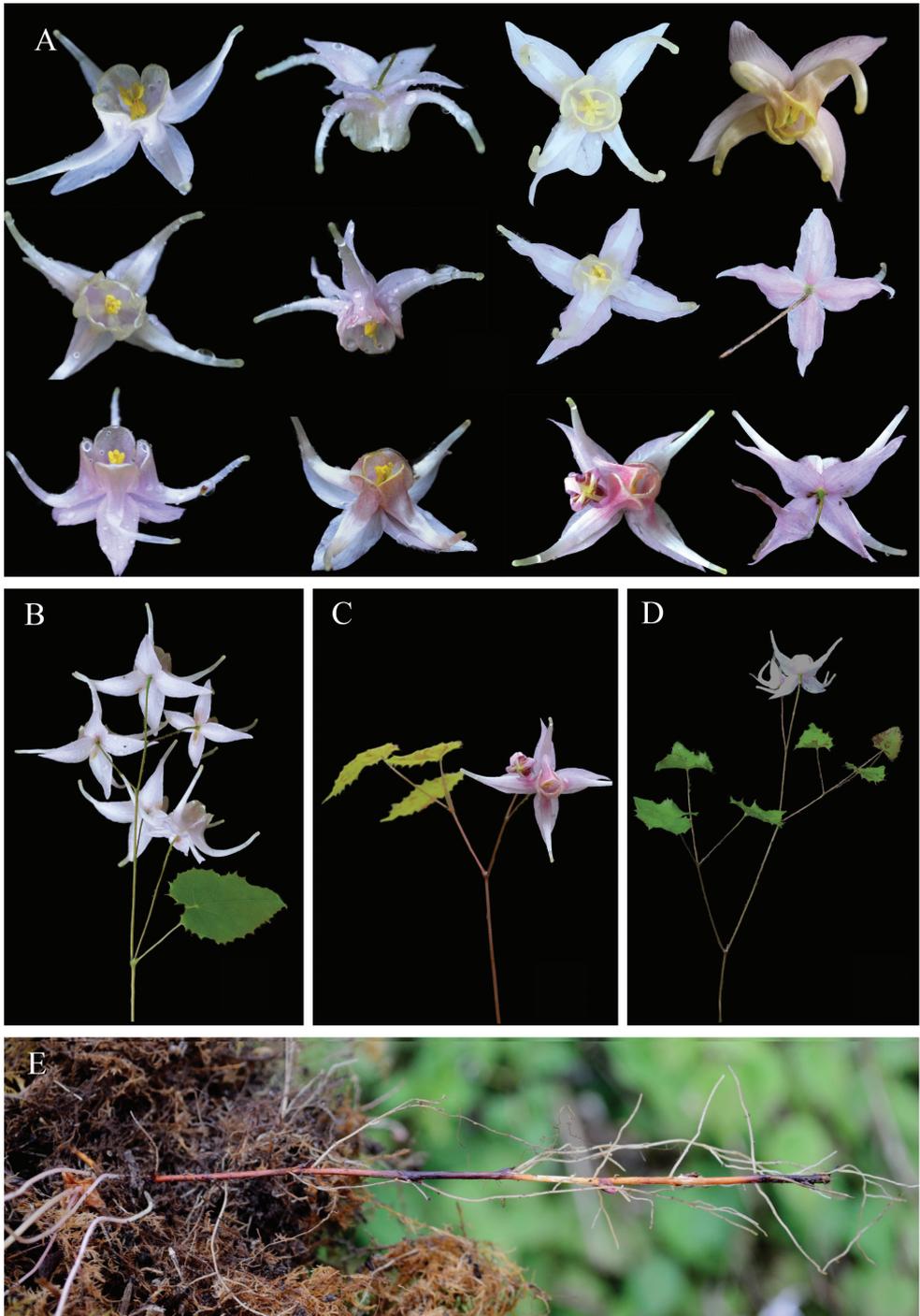
### 3. *Epimedium pauciflorum* K.C.Yen, *Guihaia* 14: 124. 1994

Fig. 5

*Epimedium platypetalum* var. *tenuis* B.L.Guo & P.G.Hsiao, *Acta Phytotaxon. Sin.* 31: 195. 1993. Type: CHINA. Sichuan: Shuixicun, Mao Xian, 1990 m, *H. R. Xie 89023*, (holotype: IMD! without accession number or barcode).

**Type.** CHINA. Sichuan: Dagou, Maowen, 1700 m, *K. C. Yen & S. L. Shao 66535* (holotype: GXMI, barcode GXMI004519!; isotype: PE, barcode PE01432167!).

**Description.** Herbs 6–30 cm tall. Rhizome long creeping, 1–3 mm in diam.; sometimes creeping to 20–25 cm. Leaves basal and cauline, trifoliolate; leaflets ovate or suborbicular, 1.3–4.5 × 1.2–3.5 cm, abaxially sparsely or occasionally densely puberulent, adaxially glabrous when mature, base deeply cordate with rounded lobes nearly touching, those of lateral leaflets conspicuously unequal, margin coarsely serrate, apex acute or shortly acuminate. Flowering stem with 1 trifoliolate, occasionally 1 unifoliolate leaf or 2 alternate trifoliolate leaves. Inflorescence simple, racemose, few-flowered (ca. 2–6 flowers), 3–13.5 cm long; inflorescence axis and pedicels glandular or pubescent. Pedicel 1–2.3 cm long; bracts ovate, 0.8–1.4 mm. Flowers ca.



**Figure 5.** The variations and morphological characters of *E. pauciflorum*. **A** colour variations of inner sepals and petals **B–D** variations of the number and arrangement of stem-leaves **E** long creeping and thread-like rhizome.

2–2.5 cm in diam. Outer sepals fall sooner, greenish, caduceus, narrowly obovate, ca. 4 × 3 mm; inner sepals white, faintly rose-tinged or pale rose, broadly lanceolate, ca. 12 × 5 mm, apex obtuse. Petals declined, white, white tinged with pale rose, rose, pale yellow, pale rose with the base purple yellow, horn-shaped, longer than inner sepals, blunt spurs ca. 15 mm long, expanded at base into a lamina 6 mm high. Stamens ca. 4 mm long; anthers yellow, ca. 2.5 mm long. Follicles oblong, 10–15 cm long; style rostriform. Seeds 4–6.

**Distribution and habitat.** *Epimedium pauciflorum* is only known from the mountains of Maowen (the holotype locality, once and now divided into Maoxian, Wenchuan and Lixian) in Sichuan province, usually occurring in forest edges and weedy slopes, at high elevations approximately 1700–2600 m (Fig. 4).

**Phenology.** *Epimedium pauciflorum* flowers from April to May, and fruits also from April to May.

**Taxonomical remarks.** *Epimedium pauciflorum* is a low-growing species with a few-flowered inflorescence. Some information about the colour variation was recorded in part of the specimens. The colours of three specimens (*Z. L. Kun 89022*, *B. L. Guo & W. K. Bao 97040*, *B. L. Guo 88182*) were described as white, while the other two (*B. L. Guo & W. K. Bao 97031* and *97032*) were described as yellow. We observed a variety of colour variations; the inner sepals were white, faintly purple-tinged or pale rose, whereas the petals were white, white tinged with pale rose, rose, pale yellow, pale rose with the base purple yellow (Fig. 5). Among them, the pale-yellow flowers were only observed in SCJZ population. The specimens (*B. L. Guo & W. K. Bao 97031* and *97032*) that were with yellow flowers and the SCJZ population in this study were sampled from the same location.

All the examined specimens were with 1 trifoliolate leaf on the flowering stem uniformly. But 1 unifoliolate leaf (only in SCWC population) or 2 alternate trifoliolate leaves (only in SCJZ population) (Fig. 5) were also observed in our field investigation.

**Additional specimens examined.** CHINA. **Sichuan:** Jingzhou, Maoxian, 1700 m, 16 April 1987, *K. C. Yen & S. L. Shao 66535* (PE, GXMI); Jingzhou, Maoxian, 1770 m, 19 April 1997, *B. L. Guo & W. K. Bao 97031* and *B. L. Guo & W. K. Bao 97032* (IMD); Jingzhou, Maoxian, 1770 m, 19 April 1997, *B. L. Guo & W. K. Bao 97040* (IMD); Jingzhou, Maoxian, 1800 m, 2 May 1989, *Z. L. Kun 89022* (IMD); Shuishi, Maoxian, 2000 m, 19 May 1989, *H. R. Xie 89023* (IMD); Jingzhou, Maoxian, 1800 m, 3 September 1988, *B. L. Guo 88182* (IMD); Daheba, Maoxian, 1800 m, 6 September 1988, *B. L. Guo 88195* (IMD); Mt. Bianshi (Bianshishan), Maowen, 2600 m, 22 August 1985, *F. Li 822-1* (IMD); Mt. Bianshi (Bianshishan), Maowen, 2600 m, 23 August 1985, *F. Li 823-1* (IMD); Weizhou, Wenchuan, 2 May 1959, *Maowen Exped. 2046* (PE, CDBI); Miansi, Wenchuan, 1780 m, 12 April 2017, *Y. M. He et al. 2017007* (JXCM); Fengyi, Maoxian, 1800 m, 13 April 2017, *Y. M. He et al. 2017009* (JXCM). ENGLAND: cultivated at Blackthorn Nursery (from Maoxian, *Ogisu 92020*), *W. T. Stearn* (PE).

#### 4. *Epimedium mikinorii* Stearn, Kew Bull. 53: 214. 1998

Fig. 6

**Type.** CHINA. Hubei: Enshi, 670 m, April 1995, *Ogisu 95039*, cultivated at Blackthorn Nursery, Kilmeston, Hampshire; collected by W.T.Stearn, 5 April 1997 (holotype: K, not seen).

**Description.** Herbs 26–94 cm tall. Rhizome compact. Leaves basal and cauline, trifoliolate; leaflets adaxially dark green, lanceolate, 8–17.4 × 3–8.6 cm, leathery, abaxially glaucous, glabrous or with appressed hairs, base cordate with equal lobes rounded, those of lateral leaflets oblique with outer lobe large and acute, inner lobe smaller and rounded, margin closely spinose-serrulate, apex long acuminate. Flowering stem with 2 opposite or occasionally 3-whorled trifoliolate leaves. Inflorescence compound, loose, 8–50(–85)-flowered, ca. 7–30 cm long, glabrous, with lower peduncles 3–5-flowered. Pedicel 1.0–1.5 cm long, glabrous. Flowers ca. 2.5–3.5 cm in diam. Outer sepals oblong, 4–6 × 2–4 mm; inner sepals yellowish, white, rose-tinged or rose, elliptic, 11–16 × 7–12 mm. Petals longer and much narrower than inner sepals, yellow, orange, purple or purple with yellow-edged lamina ca. 3.5 mm high; spur slightly curved or almost straight, subulate, elongated, 15–20 mm long. Stamens ca. 4 mm long; anthers yellow or green, ca. 3 mm long. Follicles oblong, 8–15 mm long; style rostriform. Seeds numerous.

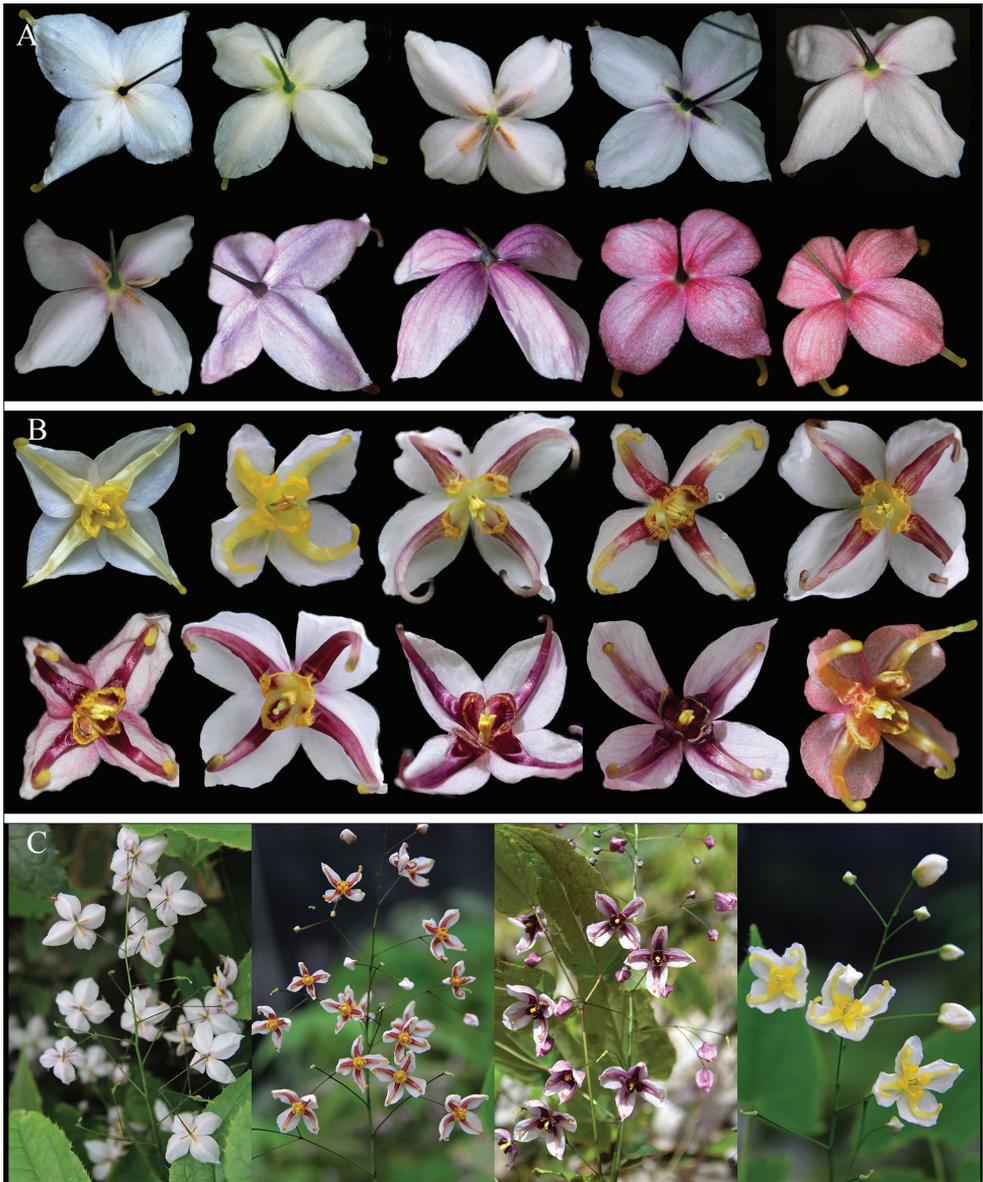
**Distribution and habitat.** *Epimedium mikinorii* is restricted to the mountains of Hubei (Enshi), Western of China, usually occurring at elevations ranging from 500 m to 1700 m (Fig. 4).

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

**Taxonomical remarks.** The field investigation found extensive colour variations. The inner sepals have different colours, ranging from white, rose-tinged, purple-tinged, through to rose and pale purple. Petals also exhibited abundant colour variation, such as yellow, orange, purple, purple but laminae yellow-edged or yellow at both ends while purple in the middle. Although the specimens of *E. mikinorii* that can be referred to are very limited, the characters and the descriptions of some herbarium specimens were consistent with our observations. Some specimens from Enshi had yellow flowers (*B. L. Guo & X. Z. Luo 89012*) or the flower colour varied from pale yellow to pale purple and purple (*B. L. Guo A29*, *B. L. Guo A50*) or from yellow to orange and pale purple (*B. L. Guo & J. J. Liu 0810*).

According to our field investigation and the common garden experiment on two populations, parts of the spurs were almost straight, while most were slightly curved (Fig. 6). The petals, in fact, presented a continuous variation from much longer to slightly longer than the inner sepals (Fig. 6).

As far as the indumentum of leaves concerned, we observed that approximately 40% of the individuals from HBES (near its type locality) and some individuals from HBXT were with white appressed hairs abaxially. Moreover, all the previous descrip-



**Figure 6.** The variations and morphological characters of *E. mikinorii*. **A** colour variations of inner sepals **B** colour variations of petals **C** individuals that with different flower colour.

tions and specimens on the flowering stem of *E. mikinorii* were with 2 opposite trifoliolate leaves. However, 3-whorled trifoliolate was also observed in the HBES population (S. X. Liu et al. 2016017).

**Additional specimens examined.** CHINA. **Hubei:** Baiguo, Enshi, 754 m, 21 April 2016, S. X. Liu et al. 2016017 (JXCM); Xiashuba, Baiguo, Enshi, 500 m, 4 May 1989, B. L. Guo & X. Z. Luo 89012 (IMD); Baiguo, Enshi, 852 m, 8 April 2004, B.

*L. Guo A29* (IMD); Jinlong, Dengta, Enshi, 1100 m, 22 April 1974, *X. S. Zou 74001* (HIB); Dongliushui, Dengta, Enshi, 900 m, 25 April 1974, *X. S. Zou 74006* (HIB); Xintang, Enshi, 1100 m, 5 December 2004, *Y. J. Zhang & X. D. Li 011* (HIB); Baiguoping, Enshi, 970 m, 5 December 2004, *Y. J. Zhang & X. D. Li 017* (HIB); Xintang, Enshi, 985 m, 6 December 2004, *Y. J. Zhang & X. D. Li 019* (HIB); Xintang, Enshi, 2 June 1974, *Y. J. Ma 277* (HIB); Huangjindong, Xianfeng, 829 m, 7 April 2004, *B. L. Guo A48* (IMD); Longfeng, Enshi, 533 m, 8 April 2004, *B. L. Guo A50* (IMD); Changlinggang, Shaunghe, Enshi, 1700 m, 20 August 2005, *B. L. Guo 05118* (IMD); Luzhuba, Baiyangping, Enshi, 635 m, 9 April 2008, *B. L. Guo & J. J. Liu 0807* (IMD); Shaziba, Sancha, Enshi, 944 m, 12 April 2008, *B. L. Guo & J. J. Liu 0809* (IMD); Changlinggang, Enshi, 1677 m, 13 April 2008, *B. L. Guo & J. J. Liu 0810* (IMD).

**5. *Epimedium glandulosopilosum* H.R.Liang, Acta Phytotaxon. Sin. 28: 323. 1990**

Fig. 7

**Type.** CHINA. Chongqing: Wushan, 850 m, 25 April 1987, *H. R. Liang 144* (holotype: BCM, lost); Chongqing: Wushan, 1000 m, 19 April 1989, *B. L. Guo 89003* (neotype, designated by Zhang et al. 2011: IMD! without accession number or barcode).

**Description.** Herbs 16–80 cm tall. Rhizome long creeping or compact, 1–5 mm in diam., internodes can be more than 10 cm. Leaves basal and cauline, usually trifoliolate or occasionally unifoliolate; leaflets of trifoliolate leaves narrowly ovate or lanceolate, 4.6–15.3 × 2.4–7.6 cm, apex acuminate, base deeply or shallowly cordate with a narrow sinus, terminal leaflet with equal and obtuse lobes, lateral leaflets conspicuously oblique with inner lobe small and obtuse, outer lobe larger and obtuse, acute or acuminate. Unifoliolate leaves ovate, broadly ovate or lanceolate, 5.0–13.0 × 2.5–6.5 cm, apex acuminate, base deeply cordate with lobes equal and obtuse or acute. Leaves coriaceous, margin spinous-serrate, adaxially deep green, obtuse, abaxially covered with villi. Petiolule, petiole and flowering stem with multicellular glandular hairs and villi, which are densest at nodes. Flowering stems usually have 2 opposite trifoliolate leaves, sometimes with 3 whorled trifoliolate leaves, 1 unifoliolate or trifoliolate leaf, rarely with 2 opposite unifoliolate or 2 leaves (alternate or opposite) with one trifoliolate and the other unifoliolate. Inflorescence racemose or compound with 8–24(–36)-flowered, 9.6–16 cm long; inflorescence axis and pedicels glandular pubescent. Pedicel 1–3 cm long. Flowers, ca. 3 cm, pale yellow, pale purple or purple. Sepals 8 in 2 whorls; outer sepals ovate, ca. 3.5 × 2 mm, red-purple; inner sepals narrowly ovate, 8–10 × 4–6 mm, white to faintly pink. Petals spurred without lamina, pale yellow, pale purple or purple, horn-shaped, 13–20 mm long. Stamens ca. 4 mm long; anthers yellow or green, ca. 3 mm long. Follicles oblong, 12–19 mm long; style rostriform. Seeds numerous.

**Distribution and habitat.** Endemic to Chongqing, Western of China, usually occurs in forests or thickets. The elevations ranged from 850 m to 1160 m (Fig. 4).

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



**Figure 7.** The variations and morphological characters of *E. glandulosopilosum*. **A** variations of flower colour **B–F** variations of the number and arrangement of stem-leaves **G** creeping and slender rhizome.

**Taxonomical remarks.** The observations of the present study showed that only several individuals had yellowish flowers, while the rest had pale purple or purple flowers (Fig. 7). Only two specimens of *E. glandulosopilosum* are available for reference, but the specimens showed similar results. *B. L. Guo & X. Z. Luo 89003* described the flower colour as whitish to pale purple, rarely yellow, while *B. L. Guo A15* described it as pale purple or pale yellow.

The protologue and the *Flora of China* described *E. glandulosopilosum* as “outer sepals narrowly ovate, 8–9 × 4–5 mm, inner sepals similar to outer sepals in size and shape” (Liang et al. 1990; Ying et al. 2011). However, according to our observation in the field and cultivation, the outer sepals (ovate, 3.5 × 2 mm) were obviously narrower and shorter than inner sepals (narrowly ovate, 8–10 × 4–6 mm).

In the present study, all individuals from the population CQWX (Wuxi) were with long creeping and slender rhizomes (1–3 mm in diam.) (Fig. 7; S. X. Liu *et al.* 2016018). The herbarium specimens from Wushan (B. L. Guo & X. Z. Luo 89003) and Wuxi (B. L. Guo A15) also presented creeping rhizome. The rhizome was therefore revised as long creeping or compact.

The leaves of some individuals were densely covered with golden-yellow villi on the abaxial side. And the petiolule, petiole and flowering stem were covered with multicellular glandular-hairs and golden-yellow villous, which are densest at the nodes. However, these indumentum characters were not stable and exhibited great variation in colour and thickness. Depending on the individuals and/or developmental stage (young or old), the indumentum of the abaxial villi varied continuously from dense to sparse, and the colour was also not always typical golden-yellow, ranging from white to yellow.

Our field investigation showed that the flowering stem primarily had 2 opposite trifoliolate leaves. In addition, abundant variations, such as 1 unifoliolate or trifoliolate leaf, 2 opposite unifoliolate leaves or 2 alternate leaves with one trifoliolate and the other unifoliolate, were also observed. And all these styles were presented in two specimens (Wushan: B. L. Guo & X. Z. Luo 89003 and Wuxi: B. L. Guo A15).

**Additional specimens examined.** CHINA. **Chongqing:** Tongcheng, Wuxi, 1159 m, 14 April 2004, B. L. Guo A15 (IMD); Tongcheng, Wuxi, 1161 m, 31°23', 109°46', 23 April 2016, S. X. Liu *et al.* 2016018 (JXCM); Guandu, Wushan, 1000 m, 19 April 1989, B. L. Guo & X. Z. Luo 89003 (IMD).

Key to the species of *Epimedium* in China

In total, 57 species and 6 varieties have been described from China, although 16 of these were designated as synonyms. *Epimedium platypetalum* var. *tenue* B.L.Guo & P.K.Hsiao was treated as synonym of *E. pauciflorum* (Stearn 2002). *Epimedium simplicifolium* T.S.Ying and *E. chlorandrum* Stearn were treated as synonyms of *E. acuminatum* (Zhang *et al.* 2011; Zhang *et al.* 2015). *Epimedium sagittatum* var. *guizhouense* S.Z.He & B.L.Guo and *E. pudingense* S.Z.He, Y.Y.Wang & B.L.Guo were treated as a synonym of *E. sagittatum* (Sieb. & Zucc.) Maxim. and *E. sagittatum* var. *glabratum* T.S.Ying, respectively (Xu *et al.* 2014b). *Epimedium coactum* H.R.Liang *et al.* W.M.Yan, *E. coactum* var. *longtoubum* H.R.Liang, *E. myrianthum* var. *jianheense* S.Z.He *et al.* B.L.Guo, *E. multiflorum* Ying and *E. jingzhouense* G.H.Xia & G.Y.Li were integrated into *E. myrianthum* Stearn (Xu *et al.* 2014b). *Epimedium rhizomatosum* Stearn, *E. brachyrrhizum* Stearn, *E. dewuense* S.Z.He, Probst *et al.* W.F.Xu, *E. sagittatum* var. *oblongifoliolatum* were treated as synonyms of *E. membranaceum* Stearn, *E. leptorrhizum*, *E. dolihostemon* Stearn and *E. borealiguizhouense* S.Z.He & Y.K.Yang, respectively (Zhang *et al.* 2015). *Epimedium lishihchenii* Stearn and *E. baojingense* Q.L.Chen & B.M.Yang were treated as subspecies and variety of *E. franchetii* Stearn, respectively (Liu *et al.* 2016). Additionally, *Epimedium tianmenshanense* T.Deng, D.G.Zhang & H.Sun is an insufficiently known taxon, due to the petals being found to be highly variable in morphology (both shape and size) (our field observation). Therefore, 45 species, 1 subspecies (*E. franchetii* ssp. *lishihchenii*) and 2 varieties (*E. sagittatum* var. *glabratum* T.S.Ying, *E. franchetii* var. *baojingense*) are recognised.

Key to the species of *Epimedium* in China

- 1 Leaves always unifoliolate..... **2**  
 – Leaves usually trifoliolate, leaflets 3 or 9, sometimes 7 or 5, occasionally unifoliolate ..... **3**
- 2 Rhizome long creeping, 1.5–3 mm diam.; flowering stem usually with 1 leaf, occasionally with 2 leaves; leaves cordate, 2.5–4 × 3–4 cm, abaxially sparsely pubescent; inflorescence raceme or panicle; inner sepal light pink, 4.6–6 × 1.8–3.2 mm; spurs orange red, saccate, 1.5–2 mm long..... ***E. elachyphyllum* Stearn**  
 – Rhizome compact, 2–5 mm diam.; flowering stem usually with 2 opposite leaves; leaves broadly ovate, 5–7 × 3–5 cm, glabrous on both surfaces; inflorescence panicle; inner sepal whitish, 4.5 × 2 mm; spurs yellowish brown, 2 mm long ..... ***E. mubuangense* S.Z.He & Y.Y.Wang**
- 3 Petals flat or flat with slightly cucullate base; flowers campanulate, spurless, yellow..... **4**  
 – Petals saccate, slipper-shaped, or with spurs; flowers not campanulate, white, pink, purple or yellow ..... **7**
- 4 30–50 cm tall; rhizome compact; leaflets coriaceous; inflorescence panicle, 30–80-flowered, 30–35 cm long, glandular; flower ca. 7 mm in diam.; petals flat with slightly cucullate base ..... ***E. reticulatum* C.Y.Wu ex S.Y.Bao**  
 – 20–50 cm tall; rhizome compact or long creeping; leaflets membranous; inflorescence raceme or panicle, 2–43-flowered, 7–23 cm long, glabrous or glandular; flower ca. 10 mm in diam.; petals flat..... **5**
- 5 35–50 cm tall; rhizome always compact, 4–6 mm in diam.; leaflets ovate, 4.5–6 × 2.5–4 cm; inflorescence panicle, 15–43-flowered, 11–23 cm long, glabrous; inner sepals red-tinged; petals spurless, obovate, 6–8 × 5–7 mm ..... ***E. campanulatum* Ogisu**  
 – 20–40 cm tall; rhizome long creeping or compact, 1–3 mm in diam.; leaflets subrounded or ovate, 2.5–4.5 × 2–4 cm; inflorescence raceme, 2–14-flowered, 7–16 cm long, glandular; inner sepals purple-red; petals spurless, oblong or obovate, 6–8 × 4–5 mm ..... **6**
- 6 20–40 cm tall; rhizome always long creeping; leaves trifoliolate, 5-foliolate, sometimes 7-foliolate, leaflets ovate, 2.5–4 × 2–3 cm; inflorescence usually 7–14-flowered, 10–16 cm long, glandular; pedicels 1–2 cm long, glandular; out sepals pale purple, broadly ovate, 4 × 1.5 mm; inner sepals elliptic, 5 × 1.5 mm ..... ***E. ecalcaratum* G.Y.Zhong**  
 – 25–35 cm tall; rhizome compact or long creeping; leaves always trifoliolate, leaflets subrounded, 4.5 × 4 cm; inflorescence usually 2–6-flowered, 7–12 cm long, pedicels 0.5–1 cm long, out sepals green, triangular-lanceolate, 2 × 1 mm; inner sepals ovate, 4 × 1.5 mm ..... ***E. platypetalum* K.Meyer**
- 7 Petals with elongated curved spurs and basal laminae ..... **8**  
 – Petals with long slender spurs but without lamina, or petals very short ..... **23**
- 8 Leaves on flower stem biternate (leaflets 9), or 7-, 5-, 3-foliolate ..... **9**  
 – Leaves on flower stem always 3-foliolate, occasionally unifoliolate..... **12**

- 9 15–40 cm tall; rhizome long creeping; flowering stem always with 1 biternate leaf, leaflets ovate, 3–13 × 2–8 cm; inflorescence raceme, usually 3–8-flowered, 6–12 cm long, glabrous; pedicels 1–2 cm long, glabrous; flower yellowish or white, 2.5–3 cm in diam.; inner sepals narrowly ovate to lanceolate, 8–10 × 3–4 mm; spurs 10–15 mm; laminae ca. 6 mm high ..... ***E. koreanum* Nakai**
- 25–60 cm tall; rhizome compact; flowering stem usually with 2–4 leaves or 1 leaf (5, 3-foliolate); inflorescence raceme or panicle with 2–5-flowered peduncles below, usually 4–60-flowered, 6–32 cm long, glandular; pedicels 1.5–3 cm long, glandular; flower pale yellow or pale sulphur yellow, 2–4 cm in diam.; spurs 10–19 mm; laminae 6–13 mm high..... **10**
- 10 25–44 cm tall; flowering stem usually with 1 leaf or 2–3 leaves (5-, 3-foliolate); inflorescence raceme; usually 4–18-flowered, 6–17 cm long; inner sepals yellow, 11 × 4 mm; spur 10–14 mm ..... ***E. flavum* Stearn**
- 33–60 cm tall; flowering stem with 2–4 leaves (biternate or 7-, 5-, 3-foliolate); inflorescence panicle with 2–5-flowered peduncles below; usually 11–60-flowered, 14–32 cm long; inner sepals purplish red, 4–6.5 × 2–4 mm; spur 10–19 mm ..... **11**
- 11 50–60 cm tall; flowering stem usually with 3 leaves, biternate, or leaflets 7, 5, 3; inflorescence 20–60-flowered, 20–30 cm long; petal spurs horizontally spreading, spurs 17–19 mm; laminae 6–7 mm high ..... ***E. xichangense* Y.J.Zhang**
- 33–60 cm tall; flowering stem usually with 2 leaves, leaflets 5 or 3; inflorescence 11–44-flowered, 14–32 cm long; petal spurs downward-curved, spurs 10–15 mm; laminae 7–13 mm high..... ***E. davidii* Franch.**
- 12 Rhizome long creeping or compact; inflorescences usually raceme, occasionally compound below ..... **13**
- Rhizome always compact; inflorescences always panicle ..... **20**
- 13 Petals slightly shorter or nearly as long as inner sepals ..... **14**
- Petals longer than inner sepals..... **15**
- 14 25–35 cm tall; rhizome long creeping, 1 mm in diam.; leaflet ovate or narrowly ovate, 3–6 × 1–3 cm, almost glabrous or with scattered hairs; inflorescence 3–12-flowered, 12–14 cm long; pedicels 2–3 cm long, glabrous; flower 2.5 cm in diam., inner sepals white, spurs white, 15–18 mm, nearly as long as inner sepals; laminae 7–8 mm high ..... ***E. ogisui* Stearn**
- 18 cm (or longer) tall; rhizome compact, 4.5–8 mm in diam.; leaflet narrowly ovate, 6.5–11.2 × 3.7–6.1 cm, with scattered hairs; inflorescence 8–22-flowered, 10–17 cm long; pedicels 2–2.7 cm long, glabrous or sometime glandular; flower 3–3.8 cm in diam., inner sepals white, spurs deep purple, 15–17 mm, shorter or nearly as long as inner sepals; laminae 5–6 mm high.....
- ..... ***E. shennongjiaensis* Y.J.Zhang & J.Q.Li**
- 15 Flowers large, ca. 4–5 cm in diam ..... **16**
- Flowers less than 4 cm in diam..... **17**
- 16 30 cm tall; leaflet narrowly ovate, 6–9 × 2.5–4 cm, with scattered, short, erect hairs; inflorescence raceme, 8-flowered, ca. 20 cm long; pedicels 2.5–5 cm long, glabrous; flower 4–5 cm in diam., inner sepals white, elliptic, 16 × 8–9 mm; spurs

- white, slight yellowish or purple-tinged at base, 25 mm, elongated; laminae 7 mm high ..... *E. latisepalum* Stearn
- 25 cm tall; leaflet narrowly ovate, 4–8 × 2.5–5.5 cm, almost glabrous, or with scattered, appressed hairs; inflorescence raceme, 6–10-flowered, ca. 13 cm long; pedicels 2 cm long, glabrous; flower 4.5 cm in diam., inner sepals reddish, cymbiform, 6 × 2.5 mm; spurs pale yellow, 22 mm, elongated; laminae 10 mm high ...  
..... *E. fangii* Stearn
- 17 Flowering stem usually with 1 trifoliolate, occasionally 1 unifoliolate leaf or 2 alternate trifoliolate leaves; 3–6-flowered..... *E. pauciflorum* K.C.Yen.
- Flowering stem with 2 opposite trifoliolate leaves, occasionally 1 trifoliolate leaves or 3 whorled leaves; 6–18-flowered ..... **18**
- 18 Rhizome always long creeping, 1.5–2.5 mm in diam.; leaflet narrowly ovate, 4–5.5 × 2–2.5 cm, abaxially with appressed hairs; inflorescence always raceme, 6–9-flowered, 6–7 cm long; pedicels 1–1.5 cm long, glabrous; flower 1.5–2 cm in diam., inner sepals red, ovate-oblong, 5 × 2–3 mm; petals yellow, spur subulate, obviously curved, 12 mm long, much longer inner sepals; laminae 7 mm high ...  
..... *E. shuichengense* S.Z.He
- Rhizome compact or long creeping, 2.1–5.6 mm in diam.; leaflet narrowly ovate, 6.8–19 × 2.5–6.3 cm, almost glabrous; inflorescence raceme or sometimes compound with 3-flowered peduncles below, 6–18-flowered, 8–15 cm long; pedicels 1.5–3 cm long, glabrous or glandular; flower 3–3.5 cm in diam., inner sepals white to pinkish, ovate, 11–14 × 5–10 mm; spur slightly curved, 15–16 mm long, a little longer inner sepals; laminae 5–7 mm high ..... **19**
- 19 12–68 cm tall; rhizome long creeping or compact; leaflet narrowly ovate, 6.8–13.5 × 2.9–6.3 cm, almost glabrous or with minute hairs; flower stem with 2 opposite trifoliolate leaves or 1 trifoliolate leaf; flower ca. 3 cm in diam., inner sepals white to pale pink; petals purple, laminae 5 mm high ..... *E. epsteinii* Stearn
- 15–25 cm tall; rhizome long always compact; leaflet narrowly ovate, 7–19 × 2.5–5.5 cm, glabrous; flower stem with 2 opposite trifoliolate leaves; flower 3–3.5 cm in diam., inner sepals pinkish-lilac; petals chestnut-brown, spur pale greenish, laminae 7 mm high ..... *E. stearnii* Ogisu & Rix
- 20 Flower yellow or pale yellow, 3.5–4 cm in diam., petals with obvious basal laminae 7–8 mm high..... **21**
- Flower usually purple, occasionally yellow, 2.5–3 cm in diam., petals with slight basal laminae 2–3.5 mm high ..... **22**
- 21 Leaflet oblong-elliptic or narrowly ovate, 8.1–15.5 × 3.6–6.3 cm, almost glabrous or sparingly pubescent; inflorescence compound with 2–3-flowered peduncles below, 7–38-flowered, 9–24 cm long, almost glabrous; pedicels 1–2 cm, glandular; flower 3.5 cm in diam., inner sepals broadly elliptic, 5–6 × 3–4 mm, purplish red; petals yellow, spur 15–18 mm long, much longer inner sepals; laminae 8 mm high ..... *E. hunanense* (Hand.-Mazz.) Hand.-Mazz.
- Leaflet lanceolate or narrowly lanceolate, 6.4–23 × 1.8–6.3 cm, abaxially pubescent; inflorescence panicle, 9–70(–100)-flowered, 13–30 cm long, sparsely glan-

- dular or glabrous; pedicels 0.7–1.5 cm, almost glabrous; flower ca. 4 cm in diam., inner sepals ovate, 12 × 6–8 mm, milky white; petals pale yellow, spur 20 mm long, much longer inner sepals; laminae 7 mm high ..... ***E. wushanense* T.S.Ying**
- 22 27–54 cm tall; leaflet narrowly ovate to narrowly lanceolate, 6–14.2 × 2–6.1 cm, abaxially sparingly pubescent; inflorescence panicle, 11–45-flowered, 13–22 cm long, glabrous; inner sepals ovate or broadly ovate, 8–13 × 4–8 mm; spurs of petals slightly longer than inner sepals, 10–15 mm long, laminae 2–3 mm high .....  
..... ***E. pseudowushanense* B.L.Guo**
- 26–94 cm tall; leaflet lanceolate, 8–17.4 × 3–8.6 cm, abaxially glabrous or sometimes sparingly pubescent; inflorescence panicle, 8–50(–85)-flowered, 7–30 cm long, glabrous; inner sepals elliptic, 11–16 × 7–12 mm; spurs of petals obviously longer than inner sepals, 15–20 mm long, laminae ca. 3.5 mm high .....  
..... ***E. mikinorii* Stearn**
- 23 Petals very short, 2–8 mm, and much shorter than the inner sepals..... **24**
- Petals with long slender spurs, 12–26 mm, without lamina..... **33**
- 24 Rhizome always compact; stamens 7–10 mm long, conspicuous, protruding, filament much longer than anther..... **25**
- Rhizome long creeping or compact; stamens 2–6 mm long, filament equalling or much shorter than anther..... **27**
- 25 Inner sepals always spreading, narrowly elliptic, 8–10 × 3–4.5 cm; petals cucullate, obviously incurved; filament yellowish, 4.5–5 mm long; anthers green, 2.5 mm long ..... ***E. dolichostemon* Stearn**
- Inner sepals reflexing or slightly reflexing, ovate or narrowly lanceolate, 11–18 × 2.5–5 cm; petals almost straight; filament purple or white, 4–5 mm long; anthers green or yellow, 2–4 mm long ..... **26**
- 26 Leaflets narrowly ovate, 6.5–13.5 × 2.7–5.7 cm, abaxially glabrous; inflorescences panicle, 19–69-flowered, 10–28 cm long, glabrous; inner sepals ovate, 11 × 5 mm; petals purple, straight, 4 mm long; filaments purple; anthers green.....  
..... ***E. qingchengshan* G.Y.Zhong & B.L.Guo**
- Leaflets narrowly ovate, 7.5–14.4 × 2.3–6.4 cm, abaxially glabrous or sparsely pubescent; inflorescences panicle, 9–48-flowered, 9.2–28.6 cm long, glandular; inner sepals narrowly lanceolate, 15–18 × 2.5–3.5 mm; petals purple, with whitish tip, straight, 6.5–9.5 mm long; filaments purple or white; anthers green or yellow ..... ***E. fargesii* Franch.**
- 27 Flower stem with 1 biternate leaf, 9 leaflets ..... ***E. brevicornu* Maxim.**
- Flower stem with 2 (occasionally 3) trifoliate leaves..... **28**
- 28 Plant glabrous throughout; the two lateral leaflets not cordate at base but obliquely truncate..... ***E. truncatum* H.R.Liang**
- Plant always with hairs; the leaflets all cordate at base ..... **29**
- 29 Inner sepals 5–12 mm long and flowers more than 10 mm in diam.; inflorescences glandular ..... **30**
- Inner sepals 3–5 mm mm long and flowers 6–8(–10) mm in diam.; inflorescences usually glabrous..... **31**

- 30 Rhizome always compact; leaflets ovate, 8–9 × 4–7 cm; inflorescences 20–40-flowered; inner sepals lanceolate, 12 × 3–3.5 mm; petals with slight laminae and blunt spur..... *E. stellulatum* Stearn
- Rhizome compact, sometimes elongated; leaflets ovate, narrowly ovate or lanceolate, 3–15 × 2–8 cm; inflorescences many-flowered, usually more than 50 flowers; inner sepals narrowly lanceolate, 5–10 × 1.5–2.5 mm; petals with blunt spur, almost straight, saccate..... *E. pubescens* Maxim.
- 31 Inflorescences narrow and straight, 4–5 cm broad, 21–153-flowered; petals saccate, blunt, 2–4 mm long..... *E. sagittatum* (Sieb. & Zucc.) Maxim.
- Inflorescences loose, 7–15 cm broad, 54–1140-flowered; petals slipper-shaped, 2–3 mm long..... 32
- 32 Leaflets narrowly ovate, 9.1–16.4 × 4.2–10.3 cm; inflorescences 7–9 cm broad, 54–572-flowered; outer sepals black or dark purple, petals orange-yellow and red ..... *E. myrianthum* Stearn
- Leaflets lanceolate or narrowly lanceolate, 13–18 × 2.5–4 cm; inflorescences usually 12–18 cm broad, 61–1142-flowered; outer sepals purplish, petals yellow ..... *E. borealiquizhouense* S.Z.He & Y.K.Yang
- 33 Leaves on flower stem biternate (9 leaflets)..... *E. elongatum* Komarov
- Leaves on flower stem with 3 leaflets, occasionally 1 leaflet ..... 34
- 34 Inflorescences usually raceme ..... 35
- Inflorescences usually panicle ..... 40
- 35 Leaflet 3.2–9.5 × 2.2–6 cm; flower 2–3 cm in diam.; inner sepals ovate, 5–7 × 2–4 mm..... 36
- Leaflet 3–16 × 2–8.6 cm; flower 3–4.5 cm in diam.; inner sepals narrowly ovate, narrowly lanceolate or narrowly elliptic 8–20 × 3–7 mm..... 37
- 36 Rhizome long creeping; leaflets ovate-deltoid to lanceolate, 3.7–8.2 × 2.2–3.4 cm; raceme with 8–15 flowers, inner sepals reddish, ovate, 5–7 × 2–4 mm, 1/3 to 1/4 length of petals, petals yellow, horn shaped, 15–22 mm.... *E. zhaotongense* G.W.Hu
- Rhizome compact; leaflets elliptic or broadly ovate, 3.2–9.5 × 2.5–6 cm; raceme with 11–20 flowers, inner sepals pale yellow, 6–7 × 3.2–3.7 mm, more than 1/2 length of petals, petals pale yellow, nearly straight, 7–12 mm..... *E. ensbiense* B.L.Guo
- 37 Rhizome always compact; flower stem usually with 2 opposite leaves; inflorescences glabrous; flowers ca. 4.5 cm in diam.; inner sepals pale yellow, ca. 2/5 length of petals, petals sulphur-yellow, ca. 20 mm..... *E. franchetii* Stearn
- Rhizome long-creeping, sometimes compact; flower stem with 2 opposite leaves or 1 leaf; inflorescences glandular; flowers 3–4 cm in diam.; inner sepals whitish, pale pink or rose, 2/3 to 3/4 length of petals, petals pale purplish red, rose, pale yellow or whitish, 13–26 mm ..... 38
- 38 16–80 cm tall; inflorescences raceme, sometimes panicle, 8–24 (–36) flowers, ca. 3 cm in diam.; inner sepals narrowly ovate, 8–10 × 4–6 mm; petals horn-shaped, 13–20 mm..... *E. glandulosopilosum* H.R.Liang
- 12–50 cm tall; inflorescences always raceme, 3–14 flowers, ca. 3–4 cm in diam.; inner sepals narrowly lanceolate or narrow elliptic, 11–20 × 3–7 mm; petals horn-shaped, 15–26 mm ..... 39

- 39 Rhizome always long-creeping, 1–3 mm in diam.; leaflets ovate or narrowly ovate, 3–11 × 2.3–5.7 mm, almost glabrous with only a few scattered hairs; inner sepals narrowly lanceolate, 15–17 × 3–4 mm; petals purplish red, 15–20 mm .....  
..... *E. sutchuense* Franchet
- Rhizome long-creeping or compact, 1–5(–8) mm in diam.; leaflets narrowly ovate or ovate, 3–16 × 2–8.6 cm; inner sepals narrowly elliptic, 11–20 × 4–7 mm; petals rose, pale purple, occasionally yellowish, 15–26 mm.....  
..... *E. leptorrhizum* Stearn
- 40 Leaflets broadly ovate or ovate, 4–6 × 2–3 mm; flower stem with 2 opposite or alternate leaves; inner sepals red, 1/5 to 1/4 length of the petals .....  
..... *E. membranaceum* K.Mey.
- Leaflets narrowly lanceolate, lanceolate, narrowly ovate, or ovate; flower stem with 2 opposite or occasionally 3 whorled leaves; inner sepals white, pale yellow, purple or reddish-purple, 1/4 to 3/4 length of the petals ..... 41
- 41 Leaflets abaxially densely and fully covered with long and tangled white hairs, thick as a blanket; inner sepals 3/4 length of the petals; petals deep purple; spur 2.3–3 cm ..... *E. zhushanense* K.F.Wu & S.X.Qian
- Leaflets abaxially glabrous or pubescent; inner sepals 1/4 to 2/3 length of the petals; petals white, pale yellow, yellow or purple; spur 13–25 mm ..... 42
- 42 Flower purple, purplish or yellowish; pedicels glabrous or occasionally glandular, 1–5 cm; inner sepals 1/2 to 2/3 length of the petals ..... 43
- Flower always yellow; pedicels glandular, 1.5–2 cm; inner sepals 1/4 to 1/3 length of the petals..... 44
- 43 40–60 cm tall; rhizome always compact; leaflets thick, fleshy, ovate to narrowly ovate, 4.1–5.6 × 2.1–2.7 mm, abaxially glabrous; flower 2–4 cm in diam.; inner sepals margin corrugated, ovate-elliptic, 6–21 × 3–9 mm; petals a little longer than inner sepals, 1.3–2.0 cm; chromatids  $4n=24$  .....  
..... *E. yingjiangense* M.Y.Sheng & X.J.Tian
- 20–80 cm tall; rhizome compact, sometimes long creeping; leaflets leathery, narrowly ovate to lanceolate, 3–19.8 × 1.5–8.9 mm, abaxially with dense or sparse hairs or glabrous; flower 3–5 cm in diam.; inner sepals with smooth margin, ovate-elliptic, 6–21 × 3–9 mm; petals much longer than inner sepals, 15–25 mm; chromatids  $2n=12$ ..... *E. acuminatum* Franch.
- 44 40–86 cm (usually 70–80 cm) tall; leaflet lanceolate to narrowly lanceolate, 11.5–17.6 × 2.7–4.1 cm, abaxially glabrous or sparingly pubescent; inflorescence panicle, 20–100-flowered, 20–40 cm long, glandular; inner sepals ovate, 5–6 × 3 mm, inner sepals 1/4 length of the petals; spur horn-shaped, 15–25 mm.....  
..... *E. jingchengshanense* Y.J.Zhang & J.Q.Li
- 28–59 cm tall; leaflet lanceolate, 8–12 × 1.2–4.1 cm, abaxially sparingly or densely pubescent; inflorescence panicle, 25–32-flowered, 12–23 cm long, glandular; inner sepals elliptic or narrowly ovate, 10–12 × 5–6 mm, inner sepals 1/2 to 1/3 length of the petals; spur horn-shaped, 20 mm ..... *E. ilicifolium* Stearn

## Discussion

Although the genus *Epimedium* is colourful in flower, from white, through yellow, to rose and purple, intraspecific flower colour variation is relatively uncommon. Before this study, only the polymorphism of *E. grandiflorum* and *E. acuminatum* has been described in the monograph or in the *Flora of China* (Stearn 2002; Ying et al. 2011). In addition, the colour variation of *E. glandulosopilosum* (yellow or pale purple) (Zhang et al. 2011) and *E. pseudowushanense* B.L.Guo (pale purple rose or purple, occasionally pale yellow) (Guo et al. 2007) have been mentioned. Since 2012, systematic studies and illustrations on morphology variation of *Epimedium* have been conducted by our group and we found five species with abundant intraspecific variations in flower colour.

*Epimedium acuminatum* was the species with the best-known colour variation. The flower colour of *E. acuminatum* was described as “white, yellow, rose-purple or pale violet” in *Flora of China* (Ying et al. 2011). And variations ranging from whitish, pale yellow, yellow, pale purple, purple-whitish to purple or reddish-purple have been noted in the specimens. Additionally, two species, *E. simplicifolium* and *E. chlorandrum*, akin to *E. acuminatum* have been described. The flower colour of *E. simplicifolium* was originally described as yellow (Ying 1975). It was subsequently revised to be purple or yellow (Ying 2001), and then revised again to reddish purple by the same author (Ying et al. 2011). *Epimedium chlorandrum* is notable for its anthers being green and the flowers being very pale primrose yellow (Stearn 1997). But yellow flower and polymorphism from yellow to pale reddish-purple have been described in its specimens *B. L. Guo 0606* and *B. L. Guo 0608*, respectively. In 2015, Zhang et al. found that the diagnostic characters of *E. simplicifolium* (with unifoliolate leaves) and *E. chlorandrum* (with green anthers and pollen) were within the range of morphological variations of *E. acuminatum* and finally were treated as synonyms of *E. acuminatum* (Zhang et al. 2011, 2015). Now, all of these descriptions and revisions can be well explained. *Epimedium acuminatum* is one of the most widespread species in the genus, and exhibits much variation in flower colour and other morphological characters (Zhang et al. 2015). And the polymorphism of flower colour exists both among populations and among individuals within populations.

It is very interesting and unexpected that we observed abundant intraspecific flower colour diversity. Our study suggests that the flower colour is not as stable as previously imagined. Therefore, flower colour seems to be a feature liable to great variation within some species and its taxonomic value should be discounted. From the geographical distribution of the five species, the results are consistent with the viewpoint that the comparatively unstable species often occur in western China where the genus is best represented and its evolution is still ongoing (Stearn 2002).

Although abundant flower colour variation has been observed in the present study, the reason for, or mechanism of, the colour variation is still unclear. The natural variation in flower colour may occur via the deposition of various anthocyanin pigments (Yang et al. 2010). Substantial variation in flower colour can be due to the differences in the presence, amount, or type of the carotenoid pigments (Wessinger 2015). In

general, flower colour polymorphisms appeared to be a natural starting point. Flower colour polymorphisms are widely used as model traits from genetics to ecology (Alan 2007; Takahashi et al. 2013; Wang et al. 2017; Vaidya et al. 2018). In the present study, geographic variation in flower colour pattern within *E. acuminatum* showed a north-south geographic trend. The specimens with yellow flowers are mainly from the northern region of its distribution, while those with purple flowers are usually from the southern. The specimens with mixed colours occurred in the northwest of its distribution area (Fig. 2). The geographic pattern suggests that the variation in colour may be influenced by climate and ecology. Additionally, variation in flower colour has commonly been interpreted as adaptive. The differentiation in flower colour therefore was considered an important factor in promoting plant speciation (Bradshaw et al. 1995; Matsumura et al. 2006; Hopkins and Rausher 2011) or promoting adaptive, resulting from the disruptive selection by different pollinators (Mascó et al. 2004; Irwin and Strauss 2005; Veiga et al. 2015). On the other hand, the reproductive system may influence the patterns of variation in some taxa, and might account for the morphological complexity. Some colours, especially the continuous colour variation or the transition colours, may be generated by hybridisation, including hybrid speciation, historical hybridisation and ongoing speciation. Strong evidence for an outbreeding system and no internal barrier to hybridisation (high incompatibility and cross-ability) in *Epimedium* species has been proved (Suzuki 1983, 1984; Sheng et al. 2011). *Epimedium* are promiscuous, with bees creating garden hybrids and natural hybrids in the wild (Stearn 2002; Avent 2010; Horie et al. 2012). So far, more than 20 hybrids have been found in field or in garden cultivation (Stearn 2002; Avent 2010). For example, in the wild, on Emei Mountains, *E. acuminatum* was hybridised with *E. fangii* and produced a hybrid swarm (named *E. × omeiense*) (Stearn 1995). Hybrids tend to have different colours which enriches the colour variation (Stearn 2002; Avent 2010; Horie et al. 2012). Hybridisation may be one of the mechanisms of colour variation in *Epimedium*.

From another perspective, our results could have important implications for the utilisation of germplasm. Bearing lovely foliage and graceful flowers, *Epimedium* plants were previously mainly introduced as garden plants in Europe and America (Stearn 2002; Ward 2004). The species from China and their hybrids are about to set the gardening world on fire (Probst 1998). With great commercial prospects, *Epimedium* had received increased attention from cultivators. The abundant flower colour variations of intraspecific are of great significance in promoting their ornamental value and in creating many possibilities (Avent 2010).

## Conclusion

In this study, based on the extensive field investigation of populations during flowering seasons, comprehensive descriptions and illustrations for five *Epimedium* species were established. The flower colour used to be an important character in delimiting

species in *Epimedium* (Stearn, 2002; Ying et al. 2011), but according to our results, flower colour might be too variable in the genus to be used in species delimitation. The flower colour and other characteristic variations in *Epimedium* are more extensive and complex than previously recognised. Therefore, it is not surprising that the *Epimedium* is taxonomically difficult and bewildering. The present study suggests that the flower colour is not constant at least in some species of *Epimedium*, which could be important to further taxonomic and evolutionary study.

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# *Microchirita hairulii* (Gesneriaceae), a new species from Perlis, Peninsular Malaysia

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## Abstract

A new species, *Microchirita hairulii* Rafidah (Gesneriaceae) from limestone hills in Perlis, Peninsular Malaysia, is described and illustrated. Diagnostic characters, description, detailed illustrations, geographical distribution, regional provisional conservation status assessment (Endangered) and ecological observations of the new taxon, as well as an updated key to *Microchirita* species in Peninsular Malaysia, are provided.

## Keywords

conservation, flora, limestone, Malaysia, taxonomy

## Introduction

In Peninsular Malaysia, *Microchirita* (C.B. Clarke) Yin Z. Wang comprises six species [viz., *M. involucrata* (Craib) Yin Z. Wang, *M. rupestris* (Ridl.) A. Weber & Rafidah and *M. viola* (Ridl.) A. Weber & Rafidah] with three endemics, *M. caliginosa* (C.B. Clarke) Yin Z. Wang, *M. ruthiae* Rafidah and *M. sericea* (Ridl.) A. Weber & Rafidah (Rafidah 2017). *Microchirita* grows exclusively in limestone habitats and its species are also found in India, Myanmar, southern China, Thailand, Vietnam, Laos, Cambodia, Sumatra, Java and Borneo, with Thailand as the centre of biodiversity with 28 species (Puglisi and Middleton 2017). Whilst conducting botanical exploration of limestone hills in Perlis under the limestone flora project for Peninsular Malaysia, I discovered

an interesting *Microchirita* species on several limestone karst hills. The new species shows affinities with *M. caliginosa*, *M. sericea* in having a branched stem, pale purple corolla and hairy capsule. The new species also shows some similarities with *M. viola*. However, it is significantly different in the leaf shape, floral characters (flower size, indumentum of anthers) and seed characters (Table 1). To confirm that this taxon is indeed a new species, herbarium specimens and spirit material were taken back to the Forest Research Institute Malaysia herbarium (KEP), and living materials collected and grown in the nursery of FRIM were examined.

### Updated key to *Microchirita* species of Peninsular Malaysia

- 1 Lamina narrowly elliptic or elliptic or narrowly ovate (sometimes in *M. sericea*) with widest point at the middle of the lamina ..... **2**
- Lamina ovate, obovate or lanceolate with widest point below or above the middle of the lamina..... **4**
- 2 Margin serrate. Corolla white, without conspicuous stripes..... ***M. ruthiae***
- Margin serrulate. Corolla pale purple, with conspicuous stripes ..... **3**
- 3 Stem erect; lower leaf surface pale green to yellowish green, sometimes reddish green, base narrowly cuneate ..... ***M. caliginosa***
- Stem creeping; lower leaf surface very pale green or whitish green, base attenuate or sometimes cordate ..... ***M. sericea***
- 4 Inflorescences pedunculate, bracts present..... **5**
- Inflorescences epiphyllous and crested, bracts absent (or minute)..... **6**
- 5 Bracts connate-perfoliate into a cup-like arrangement..... ***M. rupestris***
- Bracts not fused at base, leaf-like..... ***M. involucrata***
- 6 Calyx lobes 7–10 mm long, narrowly ovate. Corolla tube pale violet, lobes with conspicuous dark purple stripes; glandular hairs golden yellow, apically swollen in a cluster above the anther ..... ***M. viola***
- Calyx lobes 3–4 mm long, narrowly lanceolate. Corolla tube white or cream, pale yellow or pale purple, lobes very faintly striped or plain; with glandular hairs above the anthers, translucent pale brown ..... ***M. hairulii***

### Taxonomy

#### ***Microchirita hairulii* Rafidah, sp. nov.**

urn:lsid:ipni.org:names:77195469-1

Figures 1–3, Table 1

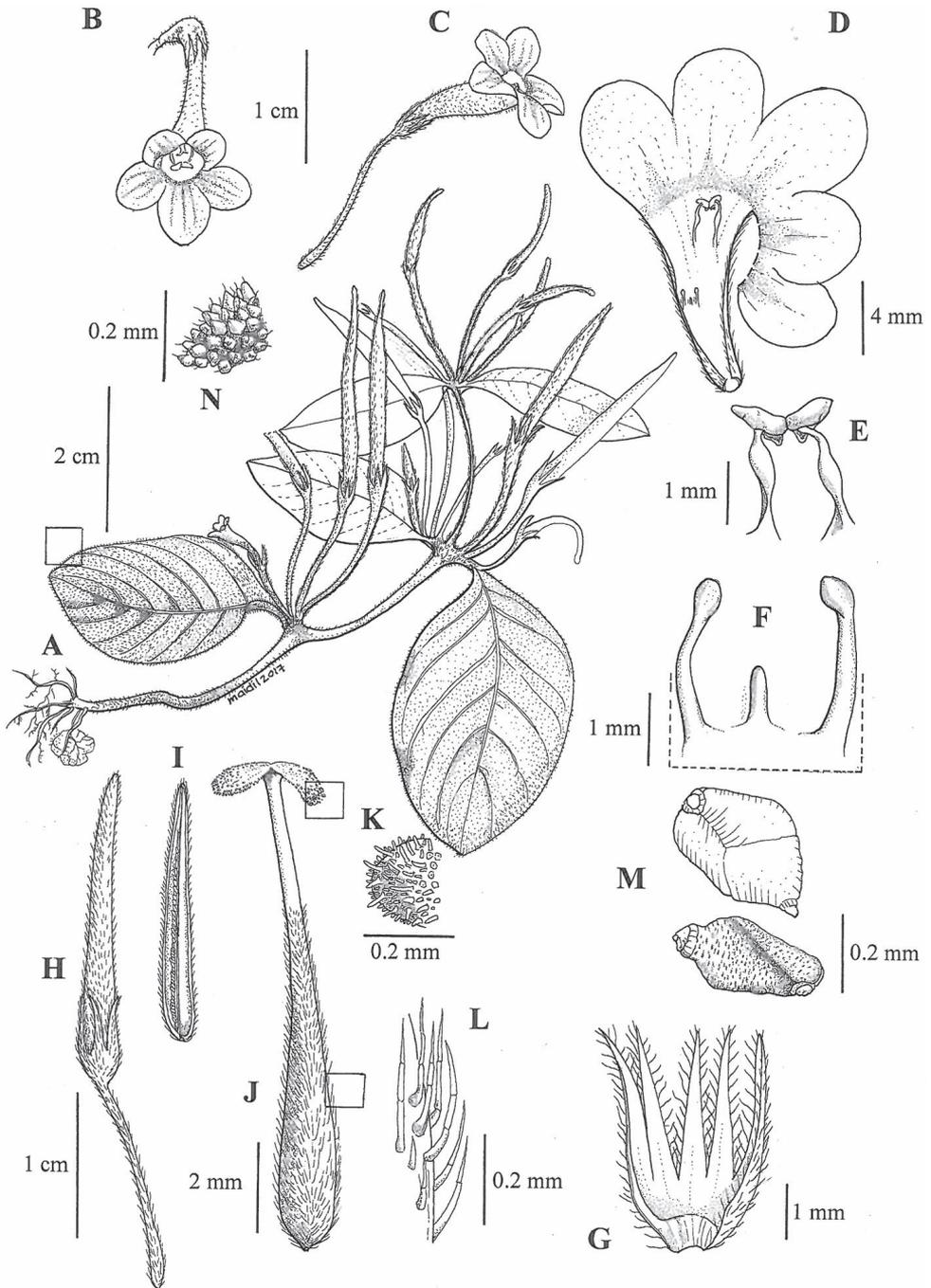
**Type.** Peninsular Malaysia, Perlis, Bukit Manik, 9 February 2017, *Rafidah FRI* 86669 (holotype: KEP).

**Table 1.** Comparison of *Microchirita hairulii* with *M. caliginosa*, *M. sericea* and *M. viola*.

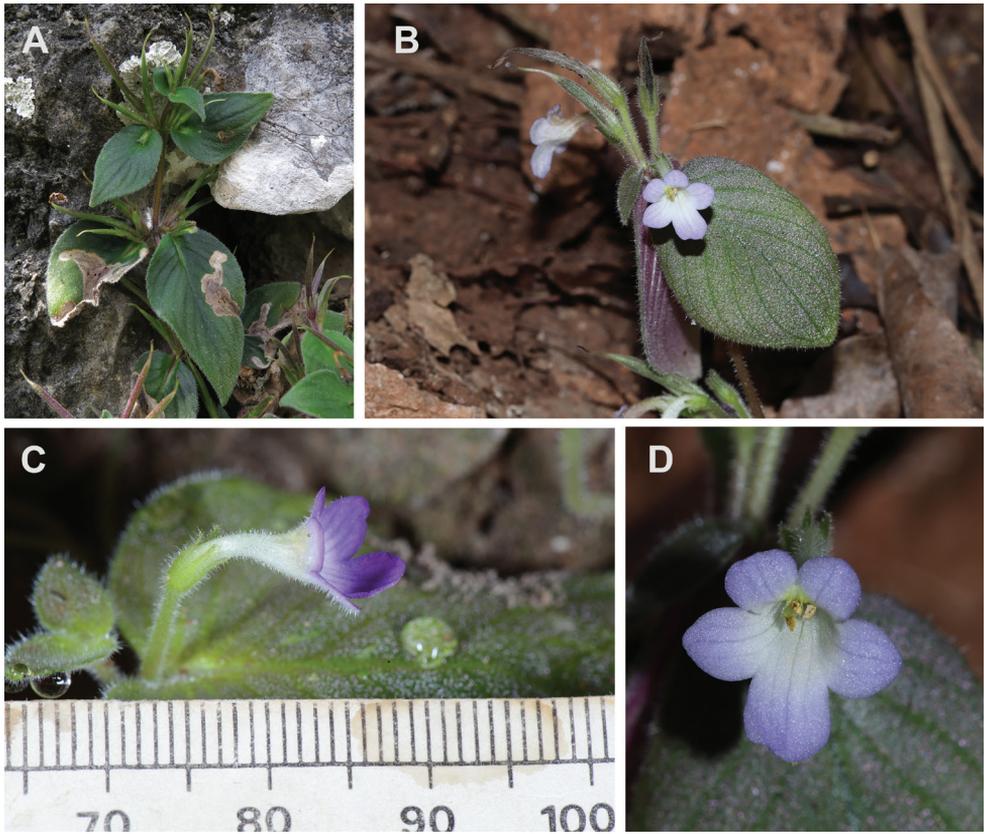
Characters	<i>M. hairulii</i>	<i>M. caliginosa</i>	<i>M. sericea</i>	<i>M. viola</i>
Stem	branched	branched	branched	branched
Lamina				
Arrangement	lowermost solitary opposite decussate	lowermost solitary opposite decussate	opposite	lowermost solitary opposite decussate
Shape	ovate	narrowly elliptic or elliptic	narrowly elliptic or narrowly ovate	ovate, sometimes orbicular
length (cm)	3.5–5.5(9)	6–15.5	4–11	3–8.5
width (cm)	2–2.2(6.2)	2.5–7	4–4.5	2–5.5
Base	cordate, cuneate, sometimes unequal	narrowly cuneate	attenuate, cordate	slightly cordate or rounded
Margin	serrate	serrulate	serrulate	serrate
Lateral vein pairs	5–6	5–10	5–10	6–16
Inflorescences	1–4-flowered	1–6-flowered	1–6-flowered	1–6-flowered
Corolla				
lobe colour	pale purple	pale purple	pale purple, lilac	violet
lobes stripes	faint	faint	conspicuous	conspicuous
tube length (mm)	5–10	24–55	10–26	20–22
Filaments	straight	slightly curved	slightly geniculate proximally	slightly geniculate
Anthers	free or connate	connate	connate	connate
Indumentum	glabrous	hairy	hairy	glabrous
Capsule				
length (mm)	15–18	20–80	15–70	c. 50
width (mm)	1–2	1.3–1.8	1.8–3	1–2
Indumentum	densely hairy	sparsely hairy	densely hairy	sparsely hairy
Seed				
surface	not papillate or canaliculate	papillate or canaliculate	papillate or canaliculate	rounded papillate

**Diagnosis.** *Microchirita hairulii* most closely resembles *M. caliginosa* and *M. sericea* in having a branched stem, pale purple corolla and hairy capsule. This new species differs in having ovate leaves (vs elliptic to narrowly elliptic in *M. caliginosa* and *M. sericea* or sometimes narrowly ovate in *M. sericea*), serrate leaf margin (vs serrulate in *M. caliginosa* and *M. sericea*), 5–10 mm long corolla tube (vs 24–55 mm long in *M. caliginosa* and 10–26 mm long in *M. sericea*), glabrous anthers (vs hairy in *M. caliginosa* and *M. sericea*) and the seed without papillate surfaces (papillate or canaliculate in *M. caliginosa* and *M. sericea*).

*Microchirita hairulii* is distinct from *M. viola* in the length of calyx lobes, 3–4 mm long, narrowly lanceolate (7–10 mm long, narrowly ovate in *M. viola*), corolla lobes very faintly striped or plain (conspicuous dark purple stripes in *M. viola*), having glandular hairs above the anthers, translucent pale brown (glandular hairs golden yellow, apically swollen in a cluster above the anther in *M. viola*)

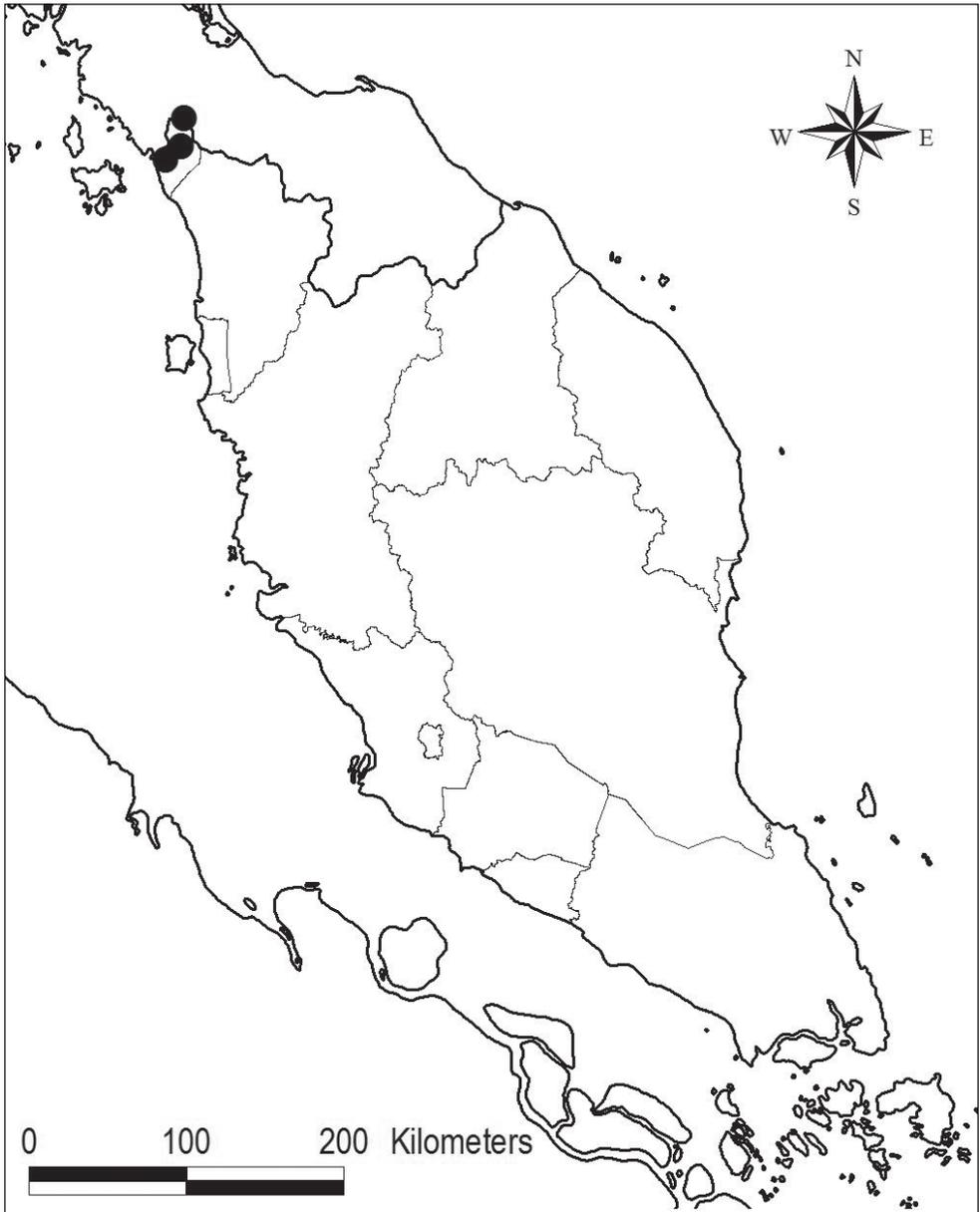


**Figure 1.** *Microchirita hairulii* Rafidah. **A** Habit **B** mature flower, front view **C** flower, side view **D** dissected corolla tube showing five lobes and a pair of stamens and staminodes **E** stamens **F** staminodes **G** calyx **H** fruit **I** LS section of fruit **J** pistil **K** indumentum of stigma **L** indumentum of ovary **M** seeds, upper and lower view **N** leaf epidermis with indumentum (Rafidah FRI86669). Drawn by Mohamad Aidil Noordin.



**Figure 2.** *Microchirita hairulii* Rafidah. **A** Habit **B** flowering and fruiting plant **C** flower, side view **D** flower, front view. Photographs **A**, **C**, **D** by Ong Poh Teck. Scale bar: 5 mm.

**Description.** Branched herb. **Stems** pale green or maroon green (in life), erect or sub-erect, if with a single leaf the stem elongated, flowering at *c.* 10 cm tall, shortly hairy, internodes 3.5–6.5 cm long. **Leaves** opposite, decussate, lowermost solitary; petiole pale green, 0.5–1.5 cm long, densely and shortly hairy; lamina ovate, 3.5–5.5(–9) × 2–2.5(–6.2) cm, yellowish green to dark green above, pale green to maroon beneath, thinly leathery (when fresh) or chartaceous (when dried), shortly hairy, base slightly cordate to cuneate, sometimes unequal, margin serrate, apex acute; midrib sunken above, prominent beneath, lateral veins 5–6 pairs, sparsely hairy, intercostal veins reticulate. **Inflorescence** epiphyllous, crested, 1–4-flowered, flowering from petiole base; bracts absent; pedicels green to maroon, to *c.* 1.2 cm long, glandular hairy. **Flowers:** calyx pale green, lobes almost divided to the base, 3–4 × 1–2 mm, acute, narrowly lanceolate, hairy outside, glabrous inside, margin entire; corolla tube very sparsely hairy outside, cream, 5–10 mm long, slightly curved, to *c.* 10 mm wide at the mouth, lobes very faintly striped, spreading, purple, throat cream, glandular hairs above the anthers, translucent pale brown, glistening; stamens 2, filaments whitish, inserted 6–8 mm



**Figure 3.** Distribution of *Microchirita hairulii* in Peninsular Malaysia.

from the base of the corolla, *c.* 4 mm long, straight, glabrous; anthers yellow, free or connate, without hairs, anther-thecae divergent, 1–2 mm long, staminodes 3, pale green, inserted *c.* 6 mm from the base of the corolla tube, 1.5–2 mm long; nectary pale yellow or cream, forming a complete ring, less than 1 mm high; pistil pale green, *c.* 1 cm long, ovary 2–4 mm long, *c.* 1.5 mm wide at the base narrowing to 0.5 mm be-

low the stigma, shortly hairy, style *c.* 4 mm long, stigma deeply 2-lobed, *c.* 1 mm long, *c.* 2 mm wide, inserted between the anthers, with fine dense papillose hairs towards the tip; ovules cream, less than 1 mm long. **Capsules** green to maroon, 1.5–1.8 cm long, *c.* 2 mm wide, slender, densely hairy; calyx persistent, pale green or sometimes maroon, hairy. **Seeds** yellowish cream, many in one row, broadly ovate or elliptic, slightly elongated or rounded, surface without knobs or papillae.

**Etymology.** The specific epithet honours Mohd. Hairul bin Mohd. Amin, a dedicated field collector who collected the species in the field.

**Geographic distribution and ecology.** Endemic in Perlis, Peninsular Malaysia (Fig. 3). The species is restricted to karst limestone, where it grows on cliffs in crevices or soil pockets, or on a very thin soil layer at cave mouths, below the canopy or sometimes directly exposed to sunlight. It is found in very small populations.

**Provisional regional conservation status.** Provisionally, the species is assessed as Endangered (EN B1ab(iii)). This endemic species occurs in five localities on the limestone hills. None of the hills lies in Totally Protected Areas, so the hills are potentially vulnerable to be exploited for other commercial uses (IUCN, 2012). Most of the hills are surrounded by paddy fields, rubber plantations and quarrying activities.

**Specimens examined. *Microchirita hairulii* - PENINSULAR MALAYSIA: Perlis.** Bkt. Jernih, February 2017, *Rafidah et al. FRI 86671* (KEP); Bkt. Keteri, November 2013, *Rafidah FRI 75880* (KEP); Bkt. Mata Ayer, February 2018, *Rafidah et al. FRI 90347* (KEP); Kg. Ujong Bukit, May 2017, *Rafidah et al. FRI 85902* (KEP).

***Microchirita caliginosa* - PENINSULAR MALAYSIA: Kedah:** Kodiang, Bkt. Kaplu, 6 November 2009, *Rafidah FRI 64417* (KEP); *Ibid.*, 27 May 2010, *Rafidah FRI 64545* (KEP). **Pahang:** Bkt. Charas, 15 October 1931, *Henderson 25233* (SING); *Ibid.*, 26 November 1984, *Kiew RK 1557* (KEP); *Ibid.*, 1 April 2008, *Rafidah FRI 55717* (KEP); Bkt. Chintamani, 4 October 1931, *Henderson SFN 25033* (SING, BK); Gn. Jebak Puyuh, 10 February 1986, *Kiew RK 2158* (KEP); *Ibid.*, 10 February 1986, *Kiew RK s.n.* (KEP); Gn. Senyum, 30 July 1929, *Henderson s.n.* (SING); *Ibid.*, 28 November 1984, *Kiew RK 1587* (KEP); *Ibid.*, 2 April 2008, *Rafidah FRI 55721* (KEP); Gua Bama, 3 April 2008, *Rafidah FRI 55726* (KEP); Gua Cermin R.F., 31 March 2008, *Rafidah FRI 55713* (KEP); *Ibid.*, 31 March 2008, *Rafidah FRI 55714* (KEP); Gua Kechil, 30 July 2009, *Rafidah FRI 64379* (KEP); Kota Glanggi, 4 August 1929, *Henderson SFN 22419* (SING); *Ibid.*, 2 April 2008, *Rafidah FRI 55724* (KEP); Panching, 26 November 1984, *Kiew RK 1571* (KEP); Panching F.R., 15 October 1931, *Henderson SFN 25223* (SING); Taman Negara, Batu Subuh, 5 October 1984, *Dawn RK 1470* (KEP); Taman Negara, Kuala Keniyam, 29 September 1982, *Kiew RK 1202* (KEP). **Perak:** Batu Kurau, December 1884, *Scortechini 1582* (SING); Ipoh, 17 July 1917, *Burkill 2558* (SING); *Ibid.*, s.d., *Gordon GS 435* (KLU); *Ibid.*, February 1904, *Ridley s.n.* (SING); Gopeng, 8 March 1993, *Davison GD 4* (KEP); Gn. Lanno, 16 April 1925, *Mills 15061* (SING); Gn. Mesah, 20 April 1962, *Burt B1665* (SING); *Ibid.*, May 1902, *Curtis s.n.* (SING); Gn. Pipit, 23 April 1987, *Kiew RK 2524* (KEP); Gn. Rapat, 21 July 2009, *Rafidah FRI 64347* (KEP); *Ibid.*, 9 March 1931, *Samsuri SA 560* (KLU, SING); Kinta, August 1898, *Curtis 3109* (SING); *Ibid.*, January 1885,

*King's collector* 7028 (SING); *Ibid.*, 1885, *King's collector* 937 (SING); Kuala Dipang F.R., February 1890, *Curtis* 2359 (SING); *Ibid.*, 1898, *Ridley s.n.* (SING); Lenggong, Gua Badak, 28 October 2008, *Imin FRI* 63212 (KEP); *Ibid.*, July 1909, *Ridley s.n.* (SING); Sg. Siput, 21 May 1985, *Anthony'samy SA* 842 (KEP); Sg. Siput Utara, 7 January 2015, *Rafidah FRI* 82007 (KEP). **Selangor:** Batu Caves, 18 October 1983, *Anthony'samy SA* 379 (KEP); *Ibid.*, 19 November 1916, *Burkill* 2253 (SING); *Ibid.*, s.d., *Chung* 331 (KLU), s.d., *Chung* 390 (KLU); *Ibid.*, February 1890, *Curtis* 2359 (SING); *Ibid.*, May 1902, *Curtis s.n.* (SING); *Ibid.*, s.d., *Ding Hou* 715 (KEP); *Ibid.*, 23 January 1966, *Hardial* 477 (SING); *Ibid.*, 1889, *Kelsall s.n.* (SING); *Ibid.*, 1 May 1981, *Kiew RK* 1023 (KEP); *Ibid.*, *Mohd. Kasim* 391 (KLU); *Ibid.*, 14 October 1966, *Ng FRI* 1629 (KEP, SING); *Ibid.*, 23 June 1889, *Ridley s.n.* (SING); *Ibid.*, 4 November 1953, *Sinclair SFN* 40066 (KEP, SING); *Ibid.*, 29 November 1959, *Smith* KEP 85205 (KEP); *Ibid.*, s.d., *Yap SK* 26 (KLU); Bkt. Anak Takun, 27 April 2006, *Phoon FRI* 51570 (KEP, SING); *Ibid.*, 3 May 2005, *Sam FRI* 50118 (KEP); Kanching F.R., 10 July 1995, *Julia JS* 26 (KEP); Bkt. Takun, 3 November 1937, *Mohd. Nur* 34389 (SING); *Ibid.*, 9 March 1988, *Saw FRI* 36215 (SING); *Ibid.*, 20 November 1962, *Sinclair* 10732 (SING); *Ibid.*, November 1969, *Stone* 8934 (KLU); *Ibid.*, 21 September 1969, *Stone* 8794 (SING); *Ibid.*, 24 June 1933, *Symington FMS* 30796 (KEP); *Ibid.*, 8 May 1935, *Symington* 39598 (KEP); Kanching, 16 March 1935, *Symington FMS* 37431 (KEP). **Terengganu:** Taman Negara, Batu Biwa, 25 October 1986, *Kiew RK* 2339 (KEP, SING); *Ibid.*, 22 October 1986, *Kiew RK* 2284 (SING).

***Microchirita sericea* - PENINSULAR MALAYSIA:** **Kedah:** Gn. Baling, 25 November 1941, *Corner s.n.* (SING). **Perak:** Gn. Rapat, 21 July 2009, *Rafidah FRI* 64347 (KEP); *Ibid.*, 26 May 2010, *Rafidah FRI* 64544 (KEP); Gn. Tasek, Perak Tong Temple, 21 July 2009, *Rafidah FRI* 64348 (KEP); *Ibid.*, 23 October 1958, *Sinclair* 9844 (SING); Ipoh, 4 July 1917, *Burkill* 2552 (SING); *Ibid.*, August 1898, *Corner s.n.* (KEP); *Ibid.*, February 1904, *Ridley* 11952 (SING); *Ibid.*, February 1904, *Ridley s.n.* (KEP); *Ibid.*, 18 August 1986, *Weber UPM* 4167 (KEP); *Ibid.*, 18 August 1986, *Weber s.n.* (KEP); Kuala Dipang F.R., 1898, *Ridley s.n.* (SING); Tambun, 10 September 1920, *Burkill* 6284 (SING); Sg. Siput Utara, 29 January 2015, *Rafidah FRI* 82017 (KEP).

***Microchirita viola* - PENINSULAR MALAYSIA:** **Kedah:** Gn. Keriang, February 1890, *Allen s.n.* (SING); Langkawi, 20 November 1941, *Corner s.n.* (SING); Langkawi, Ayer Hangat, *Chung* 505 (KLU); Langkawi, Bkt. Malut F.R., 4 November 1968, *Keng* 80 (SING); Langkawi, Batu Puteh, August 1941, *Nauen* 38120 (SING); Langkawi, Kuah, 8 November 1968, *Chung RC* 7 (KEP); *Ibid.*, 5 November 2009, *Rafidah FRI* 64407 (KEP); *Ibid.*, 1979, *Stone* 14349 (KLU); Langkawi, P. Langgun, 4 November 2009, *Rafidah FRI* 64398 (KEP); Langkawi, P. Timun, 1926, *Holtum* 17433 (SING); Langkawi, Selat Panchor F.R., 19 November 1941, *Corner* 37832 (SING); *Ibid.*, 21 November 1934, *Henderson SFN* 28931 (SING); *Ibid.*, November 1934, *Henderson SFN* 29185 (SING); *Ibid.*, 3 November 2009, *Rafidah FRI* 64388 (KEP); Langkawi, Tg. Rhu, 21 November 1993, *Anthony'samy SA* 1144 (KEP, SING); Langkawi, Tg. Sawah, 22 November 1941, *Corner s.n.* (SING); Langkawi, Tg. Terai, 13 November 1941, *Corner s.n.* (SING).

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# *Selaginella dianzhongensis* (Selaginellaceae), a new spikemoss from China

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## Abstract

A new species of spikemoss from Yunnan Province of China, *Selaginella dianzhongensis*, is described and illustrated based on evidence from gross morphology, micromorphology and molecular phylogeny. *S. dianzhongensis* is most similar to *S. amblyphylla* in its habit of creeping stem, leaf size, and obviously dimorphic sporophylls, but is distinct by its ventral leaves ovate-oblong, subcordate at base, basicopic base entire, axillary leaves ovate and decurrent at base. Molecular phylogeny analysis of three chloroplast gene regions (*rbcL*, *atpI*, *psbA*) shows that *S. dianzhongensis* forms an independent branch with strong support which is distantly related to *S. amblyphylla* and *S. kurzii*, but sister to *S. bodinieri* which is quite different in habitat of erect or ascending stem and rhizophores restricted to the lower part, and slightly dimorphic sporophyllus.

## Keywords

Lycophytes, *Selaginella amblyphylla*, taxonomy, Yunnan, *rbcL*, *atpI*, *psbA*

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\*Authors contributed equally.

## Introduction

The initial critical taxonomic revision of Chinese *Selaginella* was published by Alston (1934), who recognized 41 species from China. In the Flora Reipublicae Popularis Sinicae and Flora of China (Zhang 2004, Zhang et al. 2013) the numbers of recognized species increased to 64 and 72, respectively. Recently, several new species and new records have been reported from China (Zhou et al. 2015a, Zhang and Sun 2015, Wu et al. 2017). Yunnan province is one of the species diversity centers of plants in China, with more than 53 species of *Selaginella* (Chu, 2006).

During field trips in Yunnan, we collected an unknown *Selaginella* species from Yimen County in central Yunnan. Morphology characters show it is similar to *S. amblyphylla* Alston, but phylogenetic analysis based on three chloroplast gene regions show it is close to *S. bodinieri* Hieron. Both molecular and morphology data support the taxon as a new species, which is described and illustrated here.

The new species belongs to subgenus *Heterostachys* in the classification system of Jermy (1986), and sect. *Heterostachys* of subgenus *Heterostachys* in the molecular-phylogeny classification proposed by Zhou and Zhang (2015), but this was rejected in the more robust molecular phylogenetic analysis based on chloroplast and nuclear genes by Weststrand and Korall (2016) that only recognized a broad subgenus *Stachygynandrum*.

## Material and methods

Herbarium specimens, silica gel and living materials were collected from Longquan Forest Park of Yimen (Yunnan province), in evergreen forest at 24°40'86"N, 102°08'27.86"E. Herbarium specimens are preserved in PE, and compared with similar species. The morpho-photographs of the plants were taken with a Nikon DXM 1200F camera connected to a stereomicroscope (Nikon SMZ 1000) and computer, measurements were done by D 3.10 (<http://www.nikoninstruments.com>). The application ImageJ (<https://imagej.nih.gov/ij/>) was used to measure morphological characteristics (such as axillary, dorsal, ventral leaves, stems and strobili).

For study of spore morphology, scanning electron microscopy (SEM) was used. The spores were taken from mature sporangia and fixed on double line tape, and then covered with gold-palladium mixture. Spores were photographed and measured under different magnifications using a Hitachi S-4800 at 10–20 kV.

In this study, we sampled 28 taxa, representing almost all Chinese species with dimorphic strobili. Total genomic DNA was isolated from silica-dried material using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocols. For each species, we attempted to amplify three chloroplast gene regions (*rbcL*, *atpI*, *psbA*) for the possible new taxa and its putative closely related taxa. These three regions were amplified with newly designed primers *rbcL* 192F (5'CACGTGGACTACCGTTTGG3') and 1324R (TACCCTCAAGAGCGG-GATCA3'), *atpI* 119F (5'CYCAGGTTTCATGGACAAGTAC3') and *atpI* 540R

(5'GRGTATYGGGGTTGGTTG3'), *psbA* 169F (5'CACGTGGACTACCGTTTGA3') and *psbA* 1026R (5'ATCTRGWGGGAAGTTGTGAGC3'), respectively. According to recent classification of *Selaginella* (Zhou and Zhang 2015; Weststrand and Korall 2016), we download 10 *rbcL* sequences of *S. sect. Homostachys* (*S. laxistrobila*, *S. helvetica*, *S. nipponica*), *S. sect. Auriculate* (*S. remotifolia*, *S. kraussiana*), *S. sect. Oligomacrosporangiatae* (*S. braunii*, *S. uncinata*, *S. delicatula*), and *S. subg. Selaginoides* (*S. deflexa*, *S. selaginoides*) from Genbank as outgroups. The 25  $\mu$ L volume polymerase chain reactions (PCRs) contained 1  $\mu$ L of plant DNA, 2.5  $\mu$ L dNTPs, 1  $\mu$ L each primer, 0.15  $\mu$ L Taq polymerase (Takara Biotechnology Co., Dalian, China), 16.85  $\mu$ L ddH<sub>2</sub>O. The PCR amplification profiles were identical for the three fragments: one cycle at 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 52 °C for 1 min, 72 °C for 1.5 min; and one cycle at 72 °C for 10 min. All PCR products were directly sequenced using ABI 3730XL analyzer (Applied Biosystems, Foster City, California, USA). Newly obtained sequences were assembled with ContigExpress and then aligned with the downloaded sequences using Clustal X v.1.83 (Thompson et al. 1997) followed by manual adjustment in BioEdit v.7.1.11 (Hall 1999). We have deposited all sequences into GenBank.

Phylogenetic tree of combined dataset (*rbcL+atpI+psbA*) was constructed using maximum likelihood (ML) and Bayesian inferences (BI). jModelTest 0.1.1 (Posada 2008) was used to select the appropriate substitution model for ML and BI analyses. The ML analysis was performed on the XSEDE online computing cluster accessed via the CIPRES Science Gateway (<http://www.phylo.org>) using RAxML-HPC2 v.8.2.8 (Stamatakis 2014), with 1000 bootstrap replicates under the GTRGAT model. Bayesian analyses and posterior probability (PP<sub>BI</sub>) calculation were conducted in Mr-Bayes 3.2.6 (Ronquist et al. 2012) implemented on the CIPRES Science Gateway Portal (Miller et al. 2010). We ran four chains of the Markov chain Monte Carlo (MCMC), sampling one tree every 100 generations for 1,000,000, starting with a random tree. Bayesian posterior probabilities (PP) were calculated as the majority consensus of all sampled trees with the first 25% discarded as burn-in.

## Results

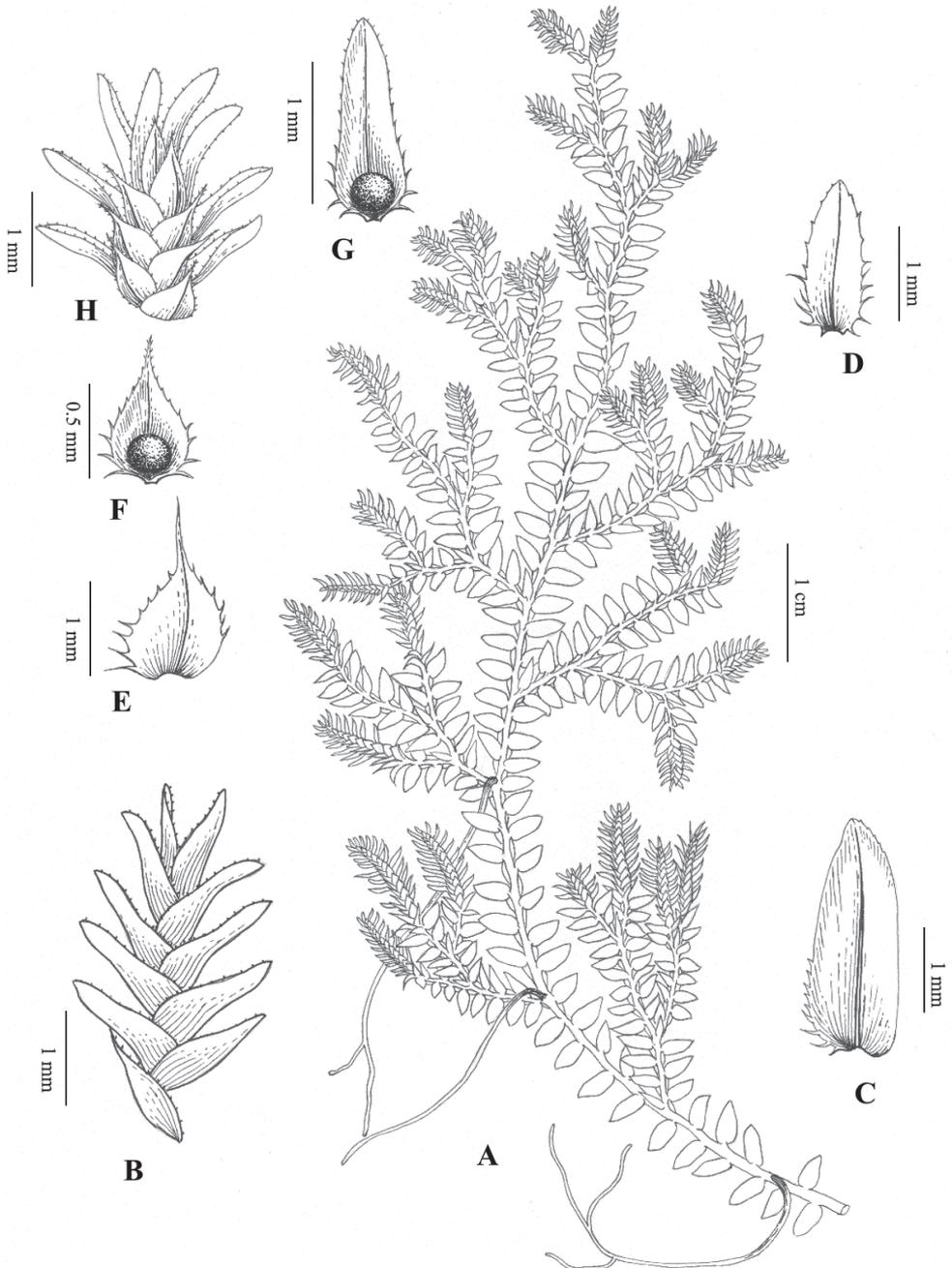
### Taxonomic treatment

#### *Selaginella dianzhongensis* X.C.Zhang, sp. nov.

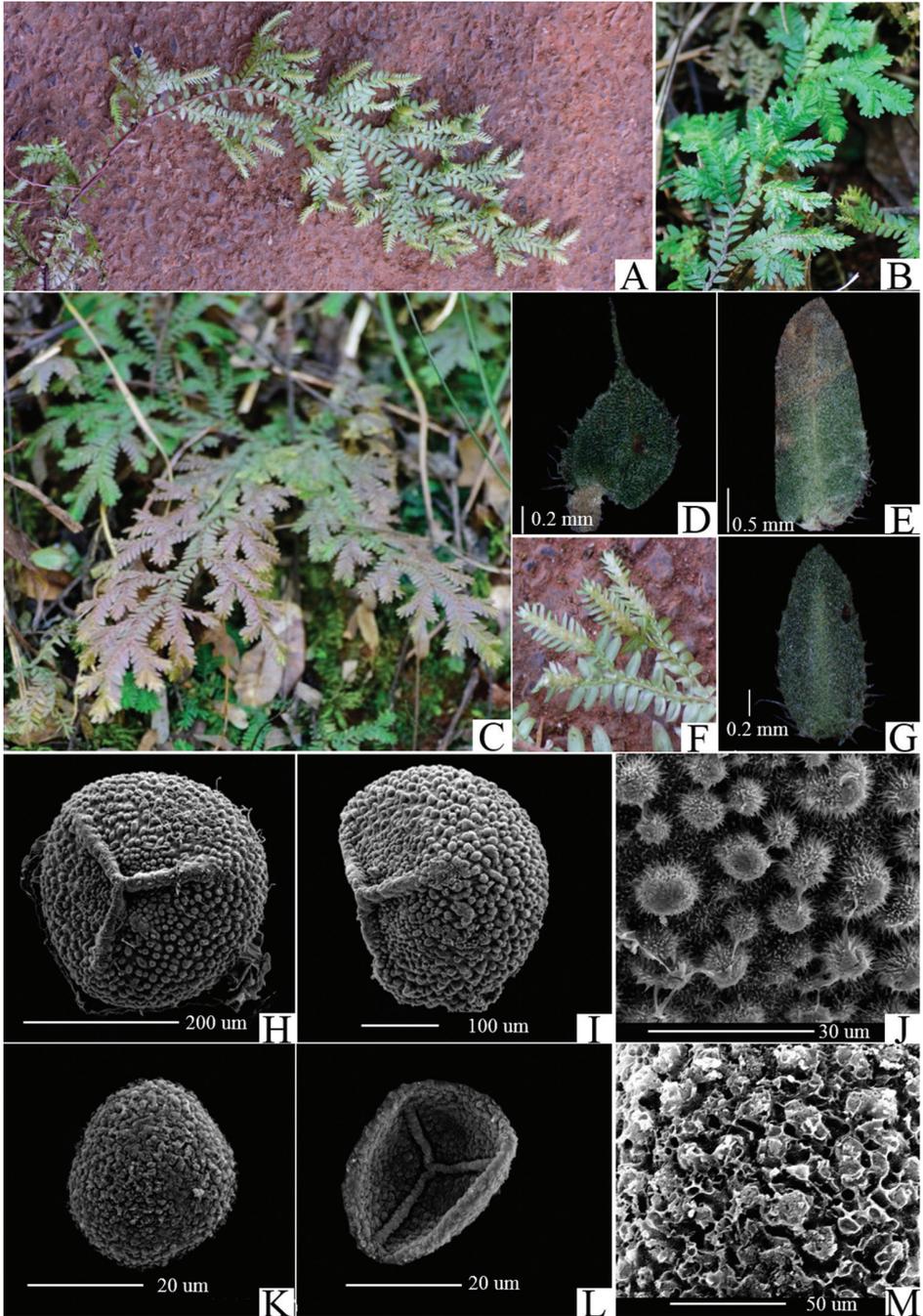
urn:lsid:ipni.org:names:60478269-2

Figures 1, 2

**Diagnosis.** The new species resembles *Selaginella amblyphylla* in habit and gross morphology, but it is different in stems and branches reddish (vs. stramineous in *S. amblyphylla*), ventral leaves ovate-oblong, 1.1–2.2  $\times$  0.4–0.8 mm (vs. oblong, 2–3  $\times$  0.6–1.2 mm), base subcordate, basispic margin not ciliolate (vs. rounded and margin sparsely ciliolate); dorsal leaves oblique subcordate or cordate at base (vs. obliquely cordate),



**Figure 1.** *Selaginella dianzhongensis* X.C.Zhang, sp. nov. **A** habit **B** adaxial view of strobilus **C** ventral leaf **D** axillary leaf **E** dorsal leaf **F** adaxial view of lower sporophyll **G** adaxial view of upper sporophyll **H** abaxial view of strobilus (Illustration made by Huixia Dong).



**Figure 2.** *Selaginella dianzhongensis* X.C.Zhang, sp. nov. **A** individual **B** portion of plant **C** habit **D** dorsal leaf **E** ventral leaf **F** strobili **G** axillary leaf **H** proximal surface of megaspore **I** distal surface of megaspores **J** portion of megaspore surface enlarged to show infrastructural detail **K** distal surface of microspore **L** proximal surface of microspore **M** portion of microspore surface enlarged to show infrastructural detail surface (Taken from Yan-Mei Zhu 8158 (PE)).

margin with rather long cilia (vs. denticulate or ciliolate); axillary leaves ovate and decurrent at base (vs. ovate or triangular and obtuse to decurrent at base); strobili 3.2–4.0 × 2.3–3.5 mm (vs. 3.5–10 × 3.2–4.4 mm), ventral sporophylls margin ciliolate, dorsal sporophylls margin denticulate (vs. both sporophylls margin ciliolate).

**Type.** CHINA, Yunnan Province, Yimen County, Longquan Forest Park, 9 Feb 2017, *Yan-Mei Zhu* 8158 (Holotype, PE!; isotype PE).

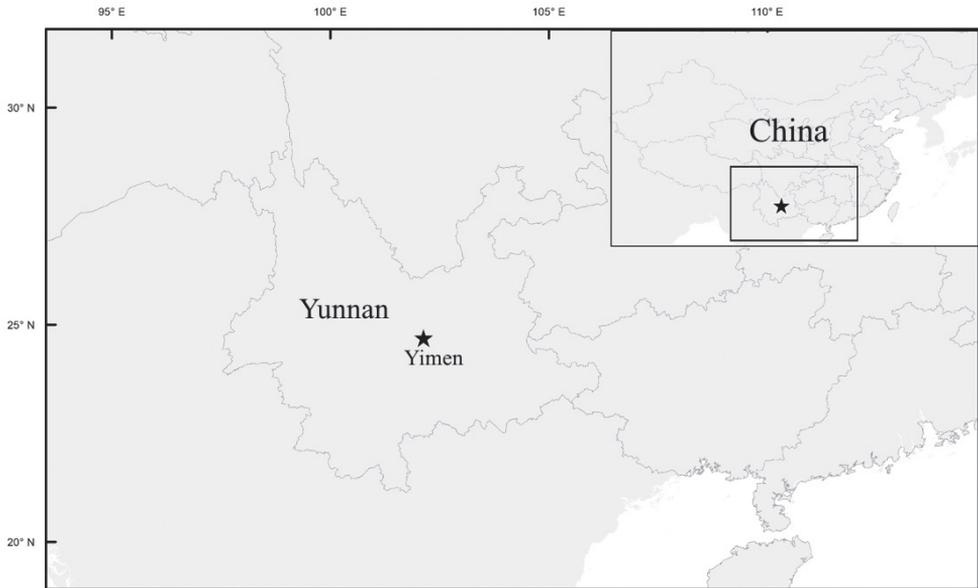
**Description.** Plants terrestrial, evergreen. Main stems reddish, creeping or suberect, stems 15–25 cm long, 0.6–1.0 mm in diam., branches stramineous. *Rhizophores* restricted in basal part of main stems or at intervals throughout length of creeping stem and branches, borne on ventral side in axils of branches. *Main stems* branched from near base, slightly sulcate, primary branches 0.7–1.5 cm apart, secondary branches once or twice pinnately branched, leafy portion of main stem including leaves 6–8 mm wide at middle, ultimate branches 4–7 mm wide including leaves. *Axillary leaves* on main stems and branches symmetrical, ovate, 1.1–2.2 × 0.4–0.8 mm, at base decurrent, margin sparsely long ciliate at base, ciliolate or denticulate to the apex, apex acute. *Dorsal leaves* ± symmetrical, on main stems distantly, on branch imbricate, on main stem larger than on branches, ovate to broadly ovate, 1.2–1.9 × 0.5–1.0 mm, slightly carinate on abaxial surface, in base oblique subcordate or cordate, not peltate, margin ciliolate at base, apex long aristate, arista 0.3–0.6 mm long. *Ventral leaves* asymmetrical, slightly distant, on main stem bit larger than those on branches, ovate-oblong, 1.7–3.9 × 0.7–1.6 mm, in base subcordate; *acroscopic base* slightly overlapping stems and branch, margin long ciliolate at base, denticulate to the apex, *basiscopic bases* free from stems, margin entire, apex acute. *Strobili* compact, solitary, terminal on branch tips, dorsiventrally complanate, 3.2–4.0 × 2.3–3.5 mm. *Sporophylls* strongly dimorphic: dorsal sporophylls ovate-lanceolate, margin denticulate, apex acuminate, with sporophyll-ptyx slightly incomplete, margin denticulate, ventral sporophylls ovate-lanceolate, ascending, carinate, margin ciliolate, apex cuspidate. Megaspores white-yellow; proximal and distal surfaces verrucate, micro-sculpture densely echinate. Microspores yellowish, proximal and distal surfaces irregularly verrucate, micro-sculpture with interconnected and blunt spinules.

**Etymology.** Dianzhong means central Yunnan in Chinese: the type locality (Yimen) is in the central Yunnan area which is centered on the Provincial capital city Kunming.

**Distribution and habitat.** *Selaginella dianzhongensis* is known only from Yimen county, Yunnan, growing on mossy soils in a mixed evergreen forest, at ca. 1576 m a.s.l. (Fig. 3).

**Conservation status (VU).** *Selaginella dianzhongensis* is known only from one locality inside the Longquan Forest Park in Yimen county, with more than 300 individuals. This park has a heavy recreational load and human pressure, and there are no specific measures to protect the habitats. Considering the restricted distribution and plausible threats, we tentatively assessed *Selaginella dianzhongensis* as vulnerable (VU) according to the IUCN (2018) categories and criteria.

**Specimens examined.** *Selaginella amblyphylla* Alston: **THAILAND:** *Payar*, Doi Angka, *H. M. Smith* 357 – BM [BM000779901, image online!] (holotype), – US [US00134348, image online!], MICH [MICH1191432, image online!], GH

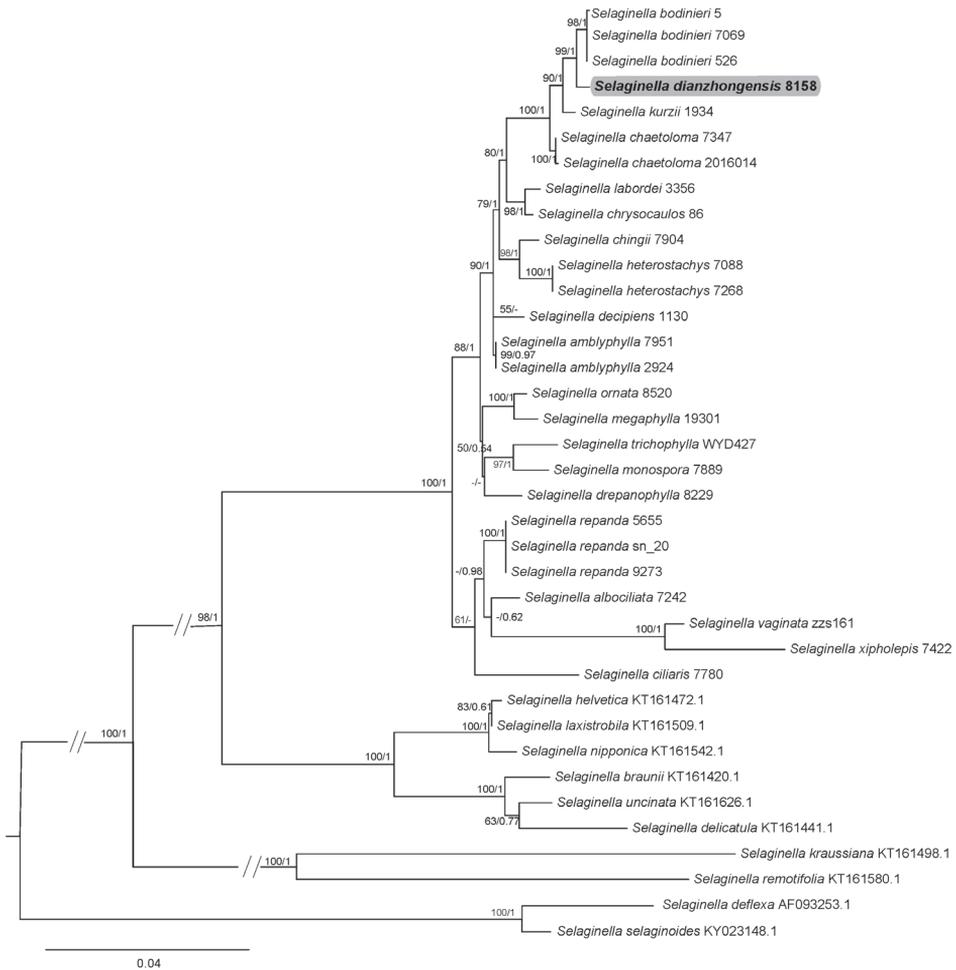


**Figure 3.** Distribution of *Selaginella dianzhongensis* X.C.Zhang, sp. nov.

[GH00022032, image online!] (isotypes), Doi Cheng Dao, 4800 ft., on rock, Oct–Nov 1922, *E. Smith* 1262 – SING [!, image] (paratype); Udawn, 900–1400 m, *Tagawa c.s. T-1816* [PE01622140]; **CHINA:** Yunnan, Mengla County, *B. G. Li* 48926 [PE00405395]; Zhenkang *W. M. Chu et al. 15204* [PE00405401]; Yunnan, Gengma, *W. M. Chu et al. 15279* [PE00405408]; Guangxi, Lingui, *J. X. Zhong* 808194 [PE01593730]; Yunnan, Simao (Szemao), ravine, 4000 ft., *A. Henry* 13529 – NY [NY00127369, image online!] (paratype). *Selaginella bodinieri* Hieron. Guizhou, Bijie, *X. C. Zhang et al.* 6842 [PE 01962745]; Guangxi, Fengshan, Alt. 750 m, *X. C. Zhang* 1272 [PE 00405447]; *Selaginella kurzii* Baker, Yunnan, Cangyuan, *J. C. Zhao* 2000-13 [PE 00405796]; Luquan, *W. M. Chu* 1649 [PE 00405795]; Mengla (Cha-li-Hsien), alt. 950 m, *C. W. Wang* 77750 [PE 01634093].

### Phylogenetic Analysis

The combined data matrix included up to 2045 nucleotides for each of the 37 taxa with 374 parsimony informative sites ( $374/2045 = 18.29\%$ ), consistency index (CI) = 0.66, retention index (RI) = 0.80, when the gaps were treated as missing data. The tree recovered from maximum likelihood (ML) and Bayesian inferences (BI), with bootstrap values (BS) of ML and Bayesian posterior probabilities (PP) for each clade is shown in Fig. 4. The new species sampled from Yimen clustered with *Selaginella bodinieri* with strong support ( $BS_{ML} = 99$ ;  $PP_{BI} = 1.0$ ), but the new species is quite similar to the *S. amblyphylla* rather than *S. bodinieri* in morphological characters.



**Figure 4.** The 50% majority rule consensus tree derived from maximum likelihood showing the position of *Selaginella dianzhongensis*. Support values (BSML/PP<sub>BI</sub>) are shown above the main braches; the dash (–) indicates BS < 50%. The new species is shown in bold.

## Discussion

Morphologically, the shape and margin of ventral and dorsal leaves of *Selaginella dianzhongensis* is most similar to *S. amblyphylla*. But the axillary leaves of the former are ovate, 1.1–2.2 × 0.4–0.8 mm, margin with a few long cilia (vs. ovate or triangular, 2–3 × 0.6–1.2 mm, and denticulate at margin in *S. amblyphylla*). Ventral leaves of the former are ovate-oblong, apex acute (vs. oblong and obtuse or subacute at apex in *S. amblyphylla*), basiscopic margin entire and not ciliolate (vs. basiscopic margin sparsely ciliolate at base in *S. amblyphylla*), acroscopic base of ventral leaf long ciliolate (vs. shortly ciliolate in *S. amblyphylla*).

Molecular data showed that *S. dianzhongensis* clustered with two other species: *S. kurzii* Baker and *S. bodinieri*.

*S. dianzhongensis* is indeed similar to *S. kurzii*, but fertile branches are not erect (vs. erect in *S. kurzii*), dorsal leaves are ovate to broadly ovate with arista at apex (vs. ovate or ovate-elliptic, acuminate or aristate at apex), and ventral leaves are ovate-oblong, basiscopic margin entire and not ciliolate (vs. ovate-triangular, basiscopic margin entire or with 1 or 2 cilia at base).

*Selaginella bodinieri* is widely distributed in the limestone areas from central to southwestern China: main differences between this species (and the other ones mentioned above) and *S. dianzhongensis* are reported in the key below, and in Table 1.

Finally, mega- and microspores of *S. dianzhongensis* are morphologically different from the spores of similar species studied by Zhou et al. (2015b). Megaspores of *S. dianzhongensis* have verrucae on proximal and distal side; micro-sculptures of megaspores are densely echinate on both sides (Fig. 2 H–J). Microspores of *S. dianzhongensis* are verrucate, with blunt spinules (Fig. 2 K–M). Morphological comparison of mega- and microspores between *S. dianzhongensis* and closely related species is presented in Table 2.

**Table 1.** Morphological characters of *Selaginella amblyphylla*, *S. bodinieri*, *S. dianzhongensis* and *S. kurzii*.

Characters	<i>S. amblyphylla</i>	<i>S. bodinieri</i>	<i>S. dianzhongensis</i>	<i>S. kurzii</i>
Main stems	creeping, up to 35 cm	erect or ascending, (15–)30–40(–50) cm	creeping, 15–25 cm	erect or ascending, 10–20 cm
Axillary leaves	ovate or triangular, 2–3 × 0.6–1.2 mm	ovate or triangular, 2–3.2 × 0.9–1.6 mm	ovate, 1.1–2.2 × 0.4–0.8 mm	ovate or ovate-lanceolate, 1–2.5 × 0.6–1.6 mm
Base of axillary leaf	denticulate	denticulate or ciliolate	with a few long cilia	long ciliolate
Dorsal leaves	ovate-lanceolate or ovate, 1.4–2.2 × 0.4–0.8 mm	obliquely ovate, 2.4–3.4 × 1.2–1.8 mm	ovate to broadly ovate, 1.2–1.9 × 0.5–1.0 mm	ovate or ovate-elliptic, 1–1.2 × 0.4–0.8 mm
Base of dorsal leaf	obliquely cordate, denticulate to ciliolate	obliquely cordate, denticulate or ciliolate	obliquely subcordate or cordate, ciliolate	subcordate or obtuse, ciliolate,
Apex of dorsal leaf	aristate, arista ca. 1 mm long	acuminate, aristate, or cuspidate	aristate, arista 0.3–0.6 mm long	acuminate or aristate, arista 0.3–0.6 mm long
Ventral leaves	oblong, 2.2–3.5 × 1.6–2 mm, apex obtuse or subacute	oblong-ovate or oblong, 3.4–4.4 × 1.6–2.2 mm, acute or obtuse	ovate-oblong, 1.7–3.9 × 0.7–1.6, apex acute	ovate-triangular, 1.6–3.8 × 0.6–1.6 mm, apex acute or acuminate
Acroscopic base of ventral leaf	shortly ciliolate in basal portion, elsewhere entire	denticulate or ciliolate	long ciliolate	rather long ciliolate at base, subentire upward
Basiscopic base of ventral leaf	sparsely ciliolate at base	entire	slightly auriculate in base, margin entire	entire or with 1 or 2 cilia at base
Strobili	3.5–10 × 3.2–4.4 mm	4–16 × 1.4–2.4 mm	3.2–4.0 × 2.3–3.5 mm	6–8 × 2–3 mm

**Table 2.** Morphological characters of mega- and microspores of *Selaginella amblyphylla*, *S. bodinieri*, *S. dianzhongensis* and *S. kurzii*.

Characters	<i>S. amblyphylla</i>	<i>S. bodinieri</i>	<i>S. dianzhongensis</i>	<i>S. kurzii</i>
<b>Megaspores</b>				
Megaspores: proximal and distal surfaces	verrucae	verrucae	verrucae	verrucae
Megaspores: micro-sculptures	vermiculate	spinulose	densely echinate	vermiculate
<b>Microspores</b>				
Microspores: proximal and distal surfaces	irregularly sized and spaced verrucae	irregularly sized and spaced verrucae	irregular size verrucae	irregularly sized and spaced verrucae
Microspores: micro-sculptures	dense spinules	not present	blunt spinules	dense spinules

### Key to the *S. dianzhongensis* and related species in Yunnan

- 1 Main stems creeping or suberect, fertile stems not erect ..... **2**
- Main stems creeping, fertile stems erect ..... ***S. kurzii***
- 2 Ventral leaves strongly overlapping stems and branches, basiscopic base exauriculate and margins ciliolate..... ***S. amblyphylla***
- Ventral leaves not overlapping stems and branches, basiscopic base slightly auriculate and margins entire or ciliolate..... **3**
- 3 Plants 40–50 cm long, main stems unbranched in lower to middle part, with stolons at bases, basiscopic base of ventral leaves slightly auriculate, acrosopic base denticulate or ciliolate at margins ..... ***S. bodinieri***
- Plants about 15–25 cm long, main stems branched from near base, rhizophores restricted to lower part of stem, basiscopic base ventral leaves entire, acrosopic base rather long ciliolate at margins ..... ***S. dianzhongensis***

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## Appendix

Information of the plant materials used in this study is presented in the following order: taxon name, locality (if available), collection number (if available), *rbcL* GenBank accession number, *atpI* GenBank accession number, *psbA* GenBank accession number references (if available). –, sequences not available. \*, sequences downloaded from NCBI.

*Selaginella albociliata* P.S. Wang, Guizhou, China, Zhang X.-C. 7242.(PE), MH814882, MH814826, MH814854. *Selaginella amblyphylla* Alston, Yunnan, China, Zhang X.-C. 2924.(PE), MH814883, MH814827, MH814855. *Selaginella amblyphylla* Alston, Yunnan, China, Zhang X.-C. 7951.(PE), MH814884, MH814828, MH814856. *Selaginella bodinieri* Hieron., Chongqing, China, Zhang X.-C. 5.(PE), MH814885, MH814829, MH814857. *Selaginella bodinieri* Hieron., Sichuan, China, Zhang X.-C. 526.(PE), MH814886, MH814830, MH814858. *Selaginella bodinieri* Hieron., Guizhou, China, Zhang X.-C. 7069.(PE), MH814887, MH814831, MH814859. *Selaginella chaetoloma* Alston, Guizhou, China, Guo Z.-Y. 2016014.(PE), MH814888, MH814832, MH814860. *Selaginella chaetoloma* Alston, Guizhou, China, Zhang X.-C. 7347.(PE), MH814889, MH814833, MH814861. *Selaginella chingii* Alston, Guangxi, China, Zhang X.-C. 7904.(PE), MH814890, MH814834, MH814862. *Selaginella chrysocaulos* (Hook. & Grev.) Spring, Sichuan, China, Zhang X.-C. 86.(PE), MH814891, MH814835, MH814863. *Selaginella ciliaris* (Retz.) Spring, Yunnan, China, Zhang X.-C. 86.(PE), MH814892, MH814836, MH814864. *Selaginella decipiens* Warb., Guangxi, China, Zhang X.-C. 7253.(PE), MH814893, MH814837, MH814865. *Selaginella dianzhongensis* X.C.Zhang, Yunnan, China, Zhu Y.-M. 8158.(PE), MH814909, MH814853, MH814881. *Selaginella drepanophylla* Alston, Yunnan, China, Zhang X.-C. 8229.(PE), MH814894, MH814838, MH814866. *Selaginella trichophylla* K. H. Shing, Guizhou, China, Wu Y.-D. 427.(PE), MH814895, MH814839, MH814867. *Selaginella heterostachys* Baker, Guizhou, China, Zhang X.-C. 7088.(PE), MH814896, MH814840, MH814868. *Selaginella heterostachys* Baker, Guizhou, China, Zhang X.-C. 7268.(PE), MH814897, MH814841, MH814869. *Selaginella kurzii* Baker, Yunnan, China, Zhang X.-C. 1934.(PE), MH814898, MH814842, MH814870. *Selaginella labordei* Hieron. ex Christ, Hubei, China, Zhang X.-C. 3356.(PE), MH814899, MH814843, MH814871. *Selaginella megaphylla* Baker, Tibet, China, Jin X.-H. 19301.(PE), MH814901, MH814845, MH814873. *Selaginella monospora* Spring, Guangxi, China, Zhang X.-C. 7889.(PE), MH814902, MH814846, MH814874. *Selaginella ornata* (Hook. & Grev.) Spring, Yunnan, China, Zhang X.-C. 8520.(PE), MH814903, MH814847, MH814875. *Selaginella repanda* (Desv. ex Poir.) Spring, Yunnan, China, Zhang X.-C. 5655.(PE), MH814904, MH814848, MH814876. *Selaginella repanda* (Desv. ex Poir.) Spring, Yunnan, China, Zhang X.-C. 9273.(PE), MH814905, MH814849, MH814877. *Selaginella repanda* (Desv. ex Poir.) Spring, Yunnan, China, Li B.-G. sn\_20.(PE), MH814906, MH814850, MH814878. *Selaginella vaginata* Spring, Shaanxi, China, Zhang Z.-S. 161.(PE), MH814907, MH814851, MH814879. *Selaginella xipholepis* Baker, Guizhou,

China, Zhang X.-C. 7422.(PE), MH814908, MH814852, MH814880. *Selaginella braunii* Baker, voucher Zhang 1332 (PYU, CDBI), KT161420.1\*, -, -. *Selaginella delicatula* (Desv. ex Poir.) Alston, voucher Gao & al. HGX10734 (CDBI), KT161441.1\*, -, -. *Selaginella helvetica* (L.) Link, voucher Zhou 093 (CDBI), KT161472.1\*, -, -. *Selaginella kraussiana* (Kunze) A. Braun, voucher Zhou 062 (CDBI), KT161498.1\*, -, -. *Selaginella laxistrobila* K.H. Shing, voucher Chu & al. 24449 (PYU), KT161509.1\*, -, -. *Selaginella nipponica* Franch. & Sav., voucher Zhou & al. DJY07479 (CDBI), KT161542.1\*, -, -. *Selaginella remotifolia* Spring, voucher Zhou 005 (PYU, CDBI), KT161580.1\*, -, -. *Selaginella uncinata* (Desv. ex Poir.) Spring, voucher Zhang & Zhou DJY04101 (CDBI), KT161626.1\*, -, -. *Selaginella deflexa* Brack., AF093253.1\*, -, -. *Selaginella selaginoides* (L.) P. Beauv. ex Schrank & Mart., voucher S. Weststrand 104 (UPS), KY023148.1\*, -, -.



# ***Nothodissotis* (Melastomataceae), a new genus from Atlantic Central Africa, including the new species *N. alenensis* from Equatorial Guinea**

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## **Abstract**

Based on morphological and phylogenetic evidence, a new genus of Melastomataceae (Melastomataceae), *Nothodissotis* Veranso-Libalah & G.Kadereit, **gen. nov.**, is described from Atlantic Central Africa. *Nothodissotis* is distinguished from other African Melastomataceae genera by its calyx-lobes that are notched at apex and asymmetrical (vs. entire and symmetrical). *Nothodissotis* includes two species: the type species *N. barteri* (Hook.f.) Veranso-Libalah & G.Kadereit, **comb. nov.** (syn. *Dissotis barteri* Hook.f.), and the new species *N. alenensis* Veranso-Libalah & O. Lachenaud, **sp. nov.**, described and illustrated here. Both species are restricted to open vegetation on rock outcrops within the forested region of Atlantic Central Africa. *Nothodissotis barteri* has a scattered distribution in Cameroon, Equatorial Guinea, Gabon and Príncipe Island, while *N. alenensis* is endemic to the Monte Alén massif in Equatorial Guinea, an area where *N. barteri* does not occur. *Nothodissotis alenensis* differs from *N. barteri* by its hypanthium bearing sessile appendages with penicillate hairs (vs. stalked stellate appendages) and its staminal appendages that are much smaller in antepetalous than in antesealous stamens (vs. subequal in all stamens). The conservation status of both *N. barteri* and *N. alenensis* is assessed as Vulnerable in accordance with IUCN criteria.

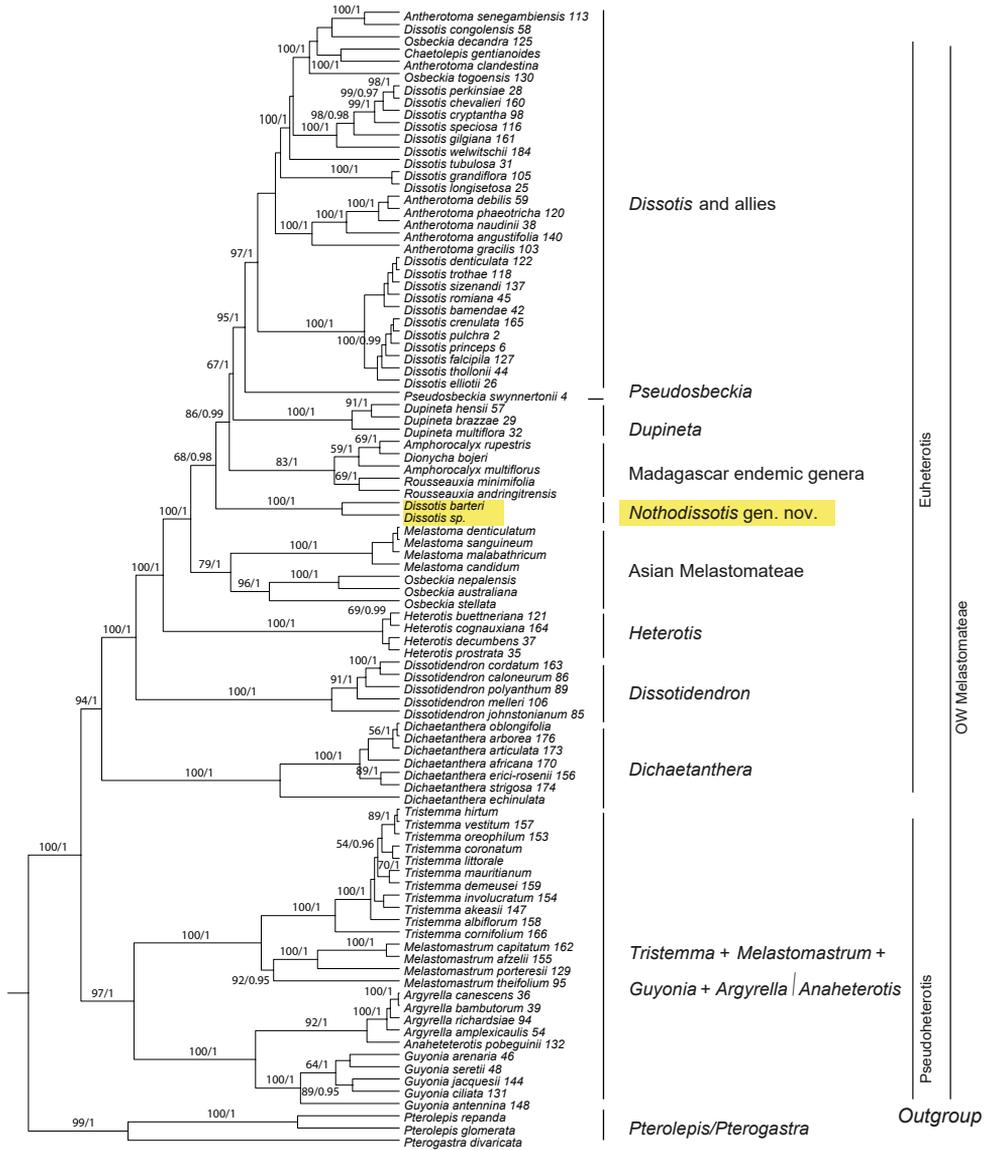
## Keywords

Africa, morphology, *Dissotis*, Equatorial Guinea, Melastomataceae, new species, *Nothodissotis*, phylogeny, plant conservation, vulnerable species

## Introduction

Melastomataceae are a large pantropical family with about 4700 species in 170 genera (Clausing and Renner 2001). The majority of their species (c. 3000) occur in the Neotropics, with an important secondary centre of diversity in tropical Asia (c. 1000 species). Continental Africa is relatively poor with c. 330 species, while Madagascar has about the same number (Renner 1993). Most African representatives of the family belong to the pantropical tribe Melastomateae (excluding *Marcetia* DC. and allies now treated in Marcetieae), which includes about 650 species in 32 genera (Michelangeli et al. 2013; Veranso-Libalah et al. 2017a; Rocha et al. 2018). In continental Africa, around 186 species in 13 genera of Melastomateae are currently recognised (Veranso-Libalah et al. 2017a, b). *Dissotis* Benth. has long been regarded as the largest African genus of the tribe, with about 120 species on the continent (Renner 1993) and a single species in Madagascar (Jacques-Félix 1995). Its delimitation, however, has been problematic (Fernandes and Fernandes 1969; Jacques-Félix 1981, 1995), and phylogenetic study has shown the genus to be polyphyletic (Veranso-Libalah et al. 2017a). As a result, the genera *Dissotidendron* (A.Fern. & R.Fern.) Veranso-Libalah & G. Kadereit, with 11 species, and *Dupineta* Raf., with five species, both previously regarded as subgenera of *Dissotis*, have been segregated from the latter. The rest of the *Dissotis* species form a clade together with *Antherotoma* Naudin, *Chaetolepis gentianoides* (Naudin) Jacq.-Fél. (formerly treated in *Nerophila* Naudin) and African species of *Osbeckia* L. (sensu Jacques-Félix 1995), and are paraphyletic with respect to these three genera; the phylogenetic relationships and revised taxonomy of this group (hereafter referred to as ‘*Dissotis* and allies’) are the subject of a forthcoming paper (Veranso-Libalah et al. in prep.).

The affinities of the little-known Central African species *Dissotis barteri* Hook.f. were not investigated by Veranso-Libalah et al. (2017a). However, this species was included in a later phylogenetic and biogeographical study of the group (Veranso-Libalah et al. 2018) using three plastid (*accD-psaI*, *ndhF* and *psbK-psbL*) and two nuclear markers (nrETS and nrITS). In that study, *D. barteri*, together with an undescribed species from Equatorial Guinea, were recovered in a monophyletic clade separate from *Dissotis* and allies (see Fig. 1). Jacques-Félix (1981, 1983a) had previously treated *D. barteri* in *D.* sect. *Macrocarpae* A.Fern. & R.Fern., but this is not supported by its morphology or by our molecular phylogenetic results (Veranso-Libalah et al. 2018). Both *D. barteri* and the new species from Equatorial Guinea differ from the members of *D.* sect. *Macrocarpae* (and indeed from the rest of the genus) by being deciduous (vs. evergreen) shrubs, and by their calyx lobes that are notched at apex and asymmetrical (vs. entire and symmetrical). Therefore, both molecular and morphological evidence support their exclusion from *Dissotis*.



**Figure 1.** Bayesian maximum clade credibility tree of African Melastomateae based on nuclear (nrITS and nrETS) and plastid (*accD-psaI*, *ndhF* and *psbK-psbL*) matrices. Values above branches refer to bootstrap values resulting from the ML analysis (only values  $\geq 50$ ) and posterior probabilities resulting from Bayesian inference (only values  $\geq 0,95$ ). Modified from Veranso-Libalah et al. (2018).

The above-mentioned new species was previously misidentified as *Dissotis thollonii* Cogn., and was cited under this name in Parmentier and Geerinck's (2003) checklist of Equatorial Guinean Melastomataceae. These authors reported 16 genera and 57 species of Melastomataceae from Equatorial Guinea, including five *Dissotis* species: *D. barteri*,

*D. congolensis* (Cogn.) Jacq.-Fél., *D. hensii* Cogn. [≡ *Dupineta hensii* (Cogn.) Veranso-Libalah & G.Kadereit], *D. multiflora* (Sm.) Triana [≡ *Dupineta multiflora* (Sm.) Raf.] and *D. thollonii*. While the first four species were correctly identified, *D. thollonii* does not occur in Equatorial Guinea, and most of the specimens cited under this name in the checklist (*Parmentier & Esono 1530, 2721, 2763 and 3453*) actually represent our new species. As discussed above, this species is very close to *D. barteri*, being a ramose shrub with stems and leaves bearing simple hairs, inflorescences few-flowered, and calyx-lobes asymmetrical, while *D. thollonii* is an unbranched shrub with hairs of the vegetative parts more or less branched, inflorescences many-flowered, and calyx-lobes symmetrical. Parmentier and Geerinck (2003) cited two other specimens under *D. thollonii*, *Lejoly 99/004* and *99/345*, of which the former has not been traced (it is apparently not in BRLU), while the latter is sterile and cannot be identified, but differs from the other four collections in vegetative characters.

In this paper we describe a new genus of African Melastomateae, *Nothodissotis* Veranso-Libalah & G.Kadereit, to accommodate both *Dissotis barteri* and the new species from Equatorial Guinea discussed above. The former species becomes *Nothodissotis barteri* (Hook.f.) Veranso-Libalah & G.Kadereit, while the latter is described as *N. alenensis* Veranso-Libalah & O.Lachenaud. A review of relevant literature (Keay 1954; Fernandes and Fernandes 1969, 1978; Wickens 1975; Jacques-Félix 1983a, 1983b) confirms that *N. alenensis* differs from all taxa of African Melastomateae so far described.

Material from the following herbaria was consulted for this paper: BR, BRLU, C, EA, K, MO, P, UPS and WAG (Thiers 2018). The description of the new species is based on herbarium specimens and data derived from field notes; all measurements (except plant height) thus refer to dry or rehydrated material were made for both species, following the IUCN criteria (IUCN 2012). The extent of occurrence (EOO) and area of occupancy (AOO) were estimated using GeoCAT (Bachman et al. 2011) with a cell width of 2 km. A distribution map is provided for both species of *Nothodissotis*, as well as a key to the species of the genus, and a key to the currently recognized genera of African Melastomateae.

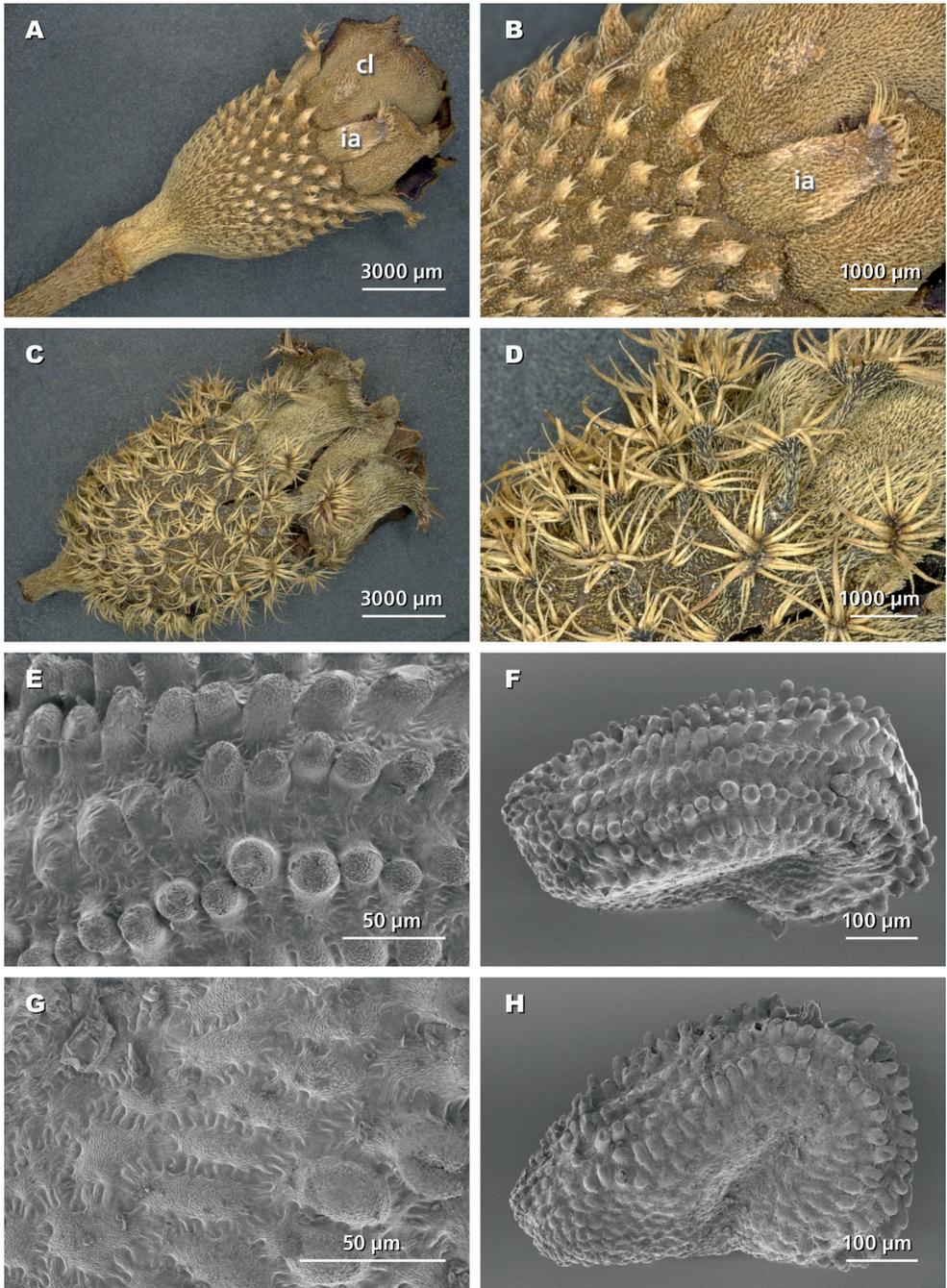
## Taxonomic treatment

### *Nothodissotis* Veranso-Libalah & G.Kadereit, gen. nov.

urn:lsid:ipni.org:names:60478296-2

**Type.** *Nothodissotis barteri* (≡ *Dissotis barteri* Hook. f.)

**Morphological diagnosis.** *Nothodissotis* species resemble *Dissotis* by their 5-merous flowers, calyx with caducous lobes and tube not accrescent on the fruit, presence of intersepal appendages, dimorphic stamens with the connective bearing bipartite ventral appendages and a well-developed pedoconnective, anthers opening by an introrse apical pore, and cochleate seeds. They differ by being deciduous shrubs



**Figure 2.** Digital microscope photographs of the hypanthia of *Nothodissotis* spp. (A–D) and SEM photographs of the seeds of *N. barteri* (E–H). A, B hypanthium of *Nothodissotis alenensis* (Parmentier & Esono 3453); cl = calyx-lobes and ia = intersepal appendages C, D hypanthium of *N. barteri* (Ngok Banak 1196) E, F seeds of *N. barteri* in dorsal view G, H same in lateral view (Parmentier 3544).

(vs. evergreen shrubs and herbs) and having the calyx-lobes notched at apex and asymmetrical (vs. entire and symmetrical); the latter character is unique within African Melastomateae.

**Description.** Deciduous, ramose shrubs, 1.5–4 m tall; stems 4-angular to cylindrical, glabrous or strigillose; internodes short, nodes setulose or strigillose (Figs 2, 3). Leaves elliptic with appressed hairs on both sides; 3–5-nerved from the base, margins entire or minutely serrulate. Inflorescences terminal, with 1–7(–15) flowers, flowers 5-merous, pedicellate, subtended by a pair of caducous short ovate bracts. Hypanthium broadly urceolate, with scattered appendages, these either sessile and penicillate (*N. alenensis*; Fig. 2) or stipitate and bearing a stellate crown of hairs at their apex (*N. barteri*; Figs 2, 3). Intersepalal appendages present, similar to hypanthial appendages but much larger, and caducous (Fig. 2 A–D). Calyx-lobes contorted in aestivation and completely concealing the floral buds, elliptic to obovate, asymmetrically notched and bearing penicillate hairs at apex, uniformly pubescent outside, caducous. Petals mauve, broadly obovate, glabrous except for the ciliate margin. Stamens 10, dimorphic in size but not in colour, pedo-connective well-developed, connective with bipartite ventral appendage, anther falcate, opening by an introrse apical pore. Ovary with a crown of persistent bristles, style simple, linear, glabrous. Fruits capsular, enclosed within the hypanthium, splitting loculicidally in 5 valves, the seeds attached on placentas borne on a central column. Seeds (only known in *N. barteri*; see Fig. 2 E–H) cochleate, exarillate, with parallel rows of tubercles.

**Etymology.** Derived from the Greek word ‘*nothos*’ meaning false, and *Dissotis*, the genus which *Nothodissotis* most closely resembles.

**Distribution and habitat.** *Nothodissotis* includes two species in Atlantic Central Africa, both of which are restricted to rocky outcrops within the equatorial rainforest zone (Fig. 4).

#### Key to the species of *Nothodissotis*

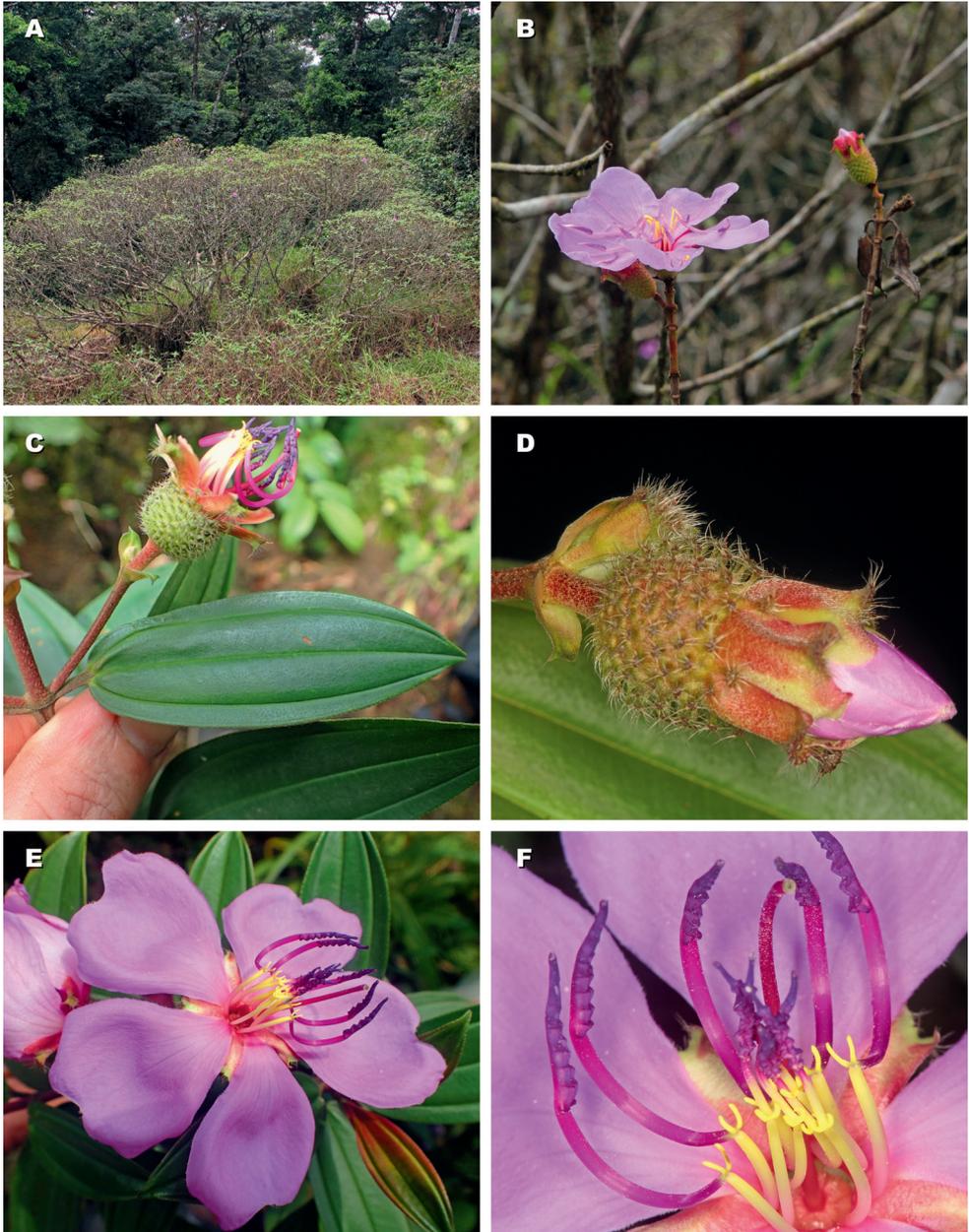
- 1 Hypanthium with stalked stellate appendages; staminal appendages of all stamens  $\pm$  equal in length, but those of antesealous stamens more distinctly curved; Cameroon, Equatorial Guinea (excluding Monte Alén), Gabon, Príncipe island ..... *N. barteri*
- Hypanthium with sessile appendages bearing simple penicillate hairs; staminal appendages much smaller in antepetalous than in antesealous stamens; Equatorial Guinea (Monte Alén) ..... *N. alenensis*

#### *Nothodissotis barteri* (Hook.f.) Veranso-Libalah & G.Kadereit, comb. nov.

urn:lsid:ipni.org:names:60478333-2

Figs 2C–H, 3

$\equiv$  *Dissotis barteri* Hook.f., Fl. Trop. Afr. [Oliver et al.] 2: 454 (1871).



**Figure 3.** *Nothodissotis barteri*. **A** habit **B** branches and inflorescence **C** leaf seen from above, and flower (petals fallen) **D** flower bud **E** blooming flower **F** stamens. From Droissart *et al.* 1668 (**A, B**) and Stévant & Oliveira 5136 (**C–F**).

**Lectotype (designated here).** Principe, 1859, *Barter s.n.* (K ! [K000313101]; isolectotype: K ! [K000313102]).

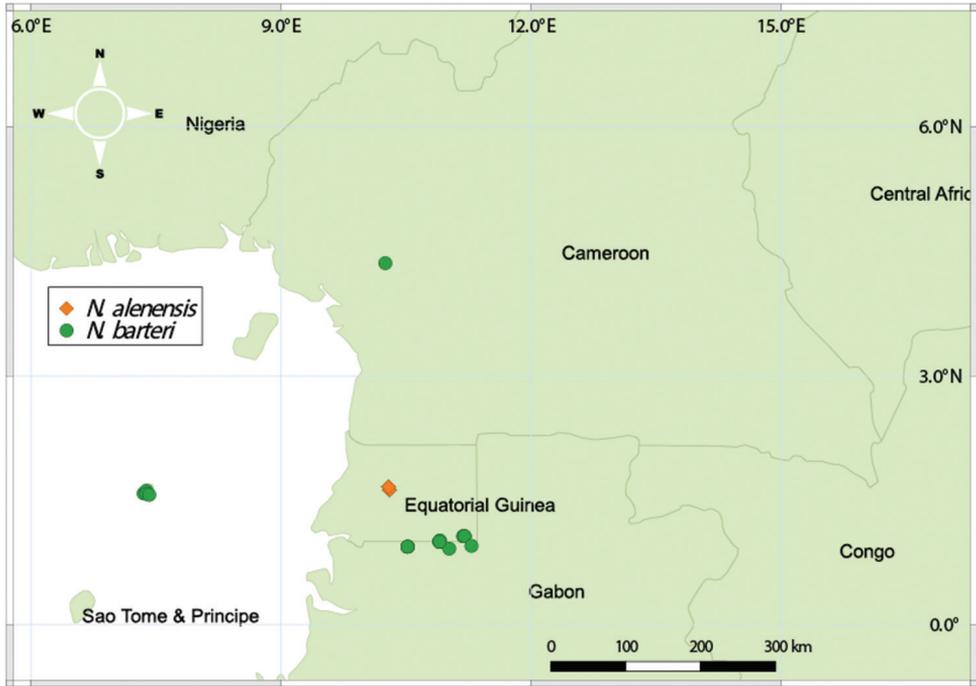
**Additional specimens examined.** CAMEROON. Réserve de Faune d'Ebo, village de Ndokbaguengue, campement de Djouma, sommet après le transect "Gachaka",

4°21.7164'N, 10°14.9694'E, 1003 m, 15 Feb. 2014 (fl.), *Droissart et al. 1668* (BRLU!, MO!); Ebo forest proposed National park, Ebo Forest Research Station, Bekango trail, 13 Dec 2006 (fl.), *Osborne & Emang Abwe 323* (K!). EQUATORIAL GUINEA. Inselberg Acoak Banga près de Ngong Mocomo, 1°04'N, 11°11'E, 8 Aug 1998 (fr.), *Lejoly & Elad 98/77* (BRLU!); inselberg de Acoak Ebanga à 1 h du village de Ngong Mocomo, à 10 km de Nsork, 1°04'N, 11°12'E, 585 m, 31 May 2002 (fr.), *Parmentier & Esono 3495* (BRLU!); *ibid.*, 590 m, 1 Jun 2002 (fr.), *Parmentier & Esono 3544* (BRLU!). GABON. rocher Fané, Efout, E of Médouneu, 5 Feb. 1968 (fl.), *N. Hallé & Villiers 4952* (P05264604!); inselberg Milobo, 0°56.35'N, 10°31.31'E, 750 m, 8 Jul 2001 (st.), *Ngok Banak et al. 39* (BRLU!, WAG!); *ibid.*, 0°56.35'N, 10°30.94'E, 760 m, 26 Nov 2001 (fl. buds), *Ngok Banak et al. 301* (BRLU!, WAG!); *ibid.*, 0°56.29'N, 10°30.87'E, 770 m, 6 Dec 2001 (fl.), *Ngok Banak et al. 357* (BRLU!, WAG!); c. 9 km ESE of Médouneu, Efot, inselberg Voma, 1°00.92'N, 10°54.30'E, 500 m, 24 Dec 2002 (fl.), *Ngok Banak et al. 1196* (BRLU!, MO!, WAG!); *ibid.*, 1°00.19'N, 10°54.08'E, 523 m, 26 Dec 2002 (fl. & fr.), *Ngok Banak et al. 1264* (BRLU!, WAG!); Mont Mengong, inselberg au pied du village de Nzec 1, à 45 km de Mitziç vers Sam, 0°57'N, 11°17'E, 670 m, 6 Jan 2000 (fl.), *Parmentier & Nguema 585* (BRLU!); Mont Fene, inselberg au pied du village d'Efot, 1°00'N, 10°54'E, 15 Jan 2000 (st.), *Parmentier & Nguema 650* (BRLU!); Mont Voma, inselberg au pied du village d'Efot, 1°00'N, 10°54'E, 19 Jan 2000 (fl.), *Parmentier & Nguema 745* (BRLU!, WAG!); c. 28 km ESE of Médouneu, 0°55'N, 11°01'E, 500 m, 3 Feb 1986 (fl.), *J.M. & B. Reitsma 1796* (WAG!). PRÍNCIPE. Infante D. Henrique, c. 215 m (c. 700 ft), 21 Dec. 1932 (fl.), *Exell 652* (BR0000017285346); sommet du Pico, 30 Aug 1999 (fl.), *Joffroy 202* (BRLU!); Pico Mesa, 600 m, 25 Mar. 1998 (fr.), *Oliveira 546* (BRLU!); Morro Fundao, 1°37'N, 7°23'E, 370 m, 8 Oct. 1997 (fr.), *Stévert & Oliveira 259* (BRLU!). Príncipe Island, summit of the Pico de Príncipe, submontane forest with many epiphytes on a ridge, 01°34'48"N, 007°23'01"E, 945 m, 16 Feb. 2018 (fl. & fr.), *Stévert & Oliveira 5136* (BRLU!, MO!).

**Distribution and habitat.** *Nothodissotis barteri* is sparsely distributed in Cameroon (Ebo forest), south-eastern Equatorial Guinea (near Nsork), northern Gabon, and Príncipe Island (Fig. 4). It occurs exclusively on rock outcrops at 370–1000 m elevation, mainly in low shrubby vegetation near the edge of the rocks (“manteau arbustif”) where it is locally dominant, and sometimes also as isolated plants in rocky grassland dominated by *Afrotrilepis pilosa* (Boeck.) J.Raynal (Cyperaceae).

**Phenology.** Flowering recorded mainly from November–February, once in August; fruits in March, May–June, August, October and December.

**Conservation status.** Vulnerable [VU B2ab(iii)]. The EOO of *Nothodissotis barteri* is estimated to be 82,625 km<sup>2</sup> (above the upper limit for Vulnerable status under sub-criterion B1) and its AOO to be 48 km<sup>2</sup> (within the limit for Endangered under sub-criterion B2). The species is sparsely distributed in Cameroon, Equatorial Guinea, northern Gabon and Príncipe island, and is restricted to rocky outcrops where it occurs in low shrubby vegetation or grassland. It is known from 21 collections representing eleven subpopulations, most of which (except three on Príncipe island) lie outside protected areas. In most of its range, bushfires and agriculture (mostly pineapple plantations) represent the main threats to its habitat; planned tourism development in



**Figure 4.** Distribution of *Nothodissotis* species.

Príncipe is another threat. A decline in habitat extent and quality is therefore expected. The eleven subpopulations represent a total of ten locations (sensu IUCN 2012), falling within the limit for Vulnerable status, and the species is therefore assessed as Vulnerable under these conditions B2ab(iii).

**Notes.** This species, originally described from Príncipe Island (Hooker 1871), has since been reported from Gabon (Jacques-Félix 1983b) and Equatorial Guinea (Parmentier and Geerinck 2003). The collections cited above from Cameroon are the first for the country and represent an important range extension northwards.

The seeds of this species have not been described previously (e.g. Jacques-Félix 1983b). They are cochleate, c.  $0.5 \times 0.35$  mm, and bear rounded tubercles arranged in parallel rows (Fig. 2 E–H).

Two specimens, probably from the same field collection by Barter in 1859, are housed in K, with neither of them designated as the holo- or isotype. For this reason, we designate the specimen K000313101 as the lectotype and K000313102 as the isolectotype.

***Nothodissotis alenensis* Veranso-Libalah & O. Lachenaud, sp. nov.**

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Figs 2A, B; 5

**Type.** EQUATORIAL GUINEA. Río Muni: Monte Alén National Park, Engong rock slab, 5 km west of Engong village, 1°37'N, 10°18'E, 1100 m, 11 May 2002 (fl & buds), *Parmentier & Esono 2763* (holotype: BRLU! [BRLU0000194]; isotype: BRLU! [BRLU0000197]).

**Diagnosis.** This new species differs from *N. barteri* by its hypanthial appendages that are sessile with penicillate hairs (not stipitate with a crown of stellate hairs) and its more strongly dimorphic stamens, the staminal appendages being much longer in antesealous stamens than in antepetalous ones (vs. staminal appendages  $\pm$  equal in length in all stamens).

**Description.** Deciduous shrub, 1.5–2.5 m tall; stems glabrous except for strigillose pubescence at the nodes; internodes short, 15–80 mm long (Fig. 5A). Leaves simple, opposite, petiole 8–17 mm long with appressed pubescence; blades elliptic, 50–95  $\times$  15–30 mm, base obtuse, apex acute, margins minutely serrulate, hairs appressed on both sides, longitudinal nerves 3(5) from the base, somewhat prominent adaxially, strongly so abaxially and with simple appressed pubescence (Fig. 5B, C). Inflorescence terminal, 1–4 flowered, the flowers 5-merous; pedicels 2–3.5 mm long with strigillose pubescence (Fig. 5D). Bracts 1 pair per flower, at the base of the hypanthium, pubescent outside, 5–8  $\times$  2–5 mm, caducous. Hypanthium broadly campanulate, 8.5–10 mm long, 7–8.5 mm in diameter in fully opened flowers, with scattered sessile appendages bearing simple penicillate hairs, arranged radially and increasing in size from bottom to top. Intersepalar appendages present, elliptic, c. 2 mm long, with short appressed pubescence and a tuft of long hairs at the apex, caducous. Calyx-lobes uniformly pubescent outside, glabrous inside, asymmetrically obovate and notched at apex, 10–15  $\times$  5–8 mm, contorted in aestivation and completely concealing the floral buds, caducous. Petals 26–30  $\times$  30–40 mm, mauve, broadly obovate, glabrous except for the ciliate margin. Stamens 10, dimorphic in size; antesealous stamens 5, filament 8–12 mm long, pedoconnective 15–18 mm long, curved, ventral appendage bipartite 4–6 mm long, anther falcate, 10–14 mm long; antepetalous stamens 5, filament 7–10 mm long, pedoconnective 3.5–4.5 mm long, ventral appendage bipartite, ca. 1 mm long, anther falcate, 8–13 mm long (Fig. 5E). Ovary with a crown of persistent bristles, style 25–30 mm long, red, glabrous; stigma simple. Fruit and seeds not seen.

**Additional specimens examined.** EQUATORIAL GUINEA. Río Muni: Monte Alén National Park, Monte Alén 2 rock slab, 1°40'N, 10°17'E, 1125 m, 12 Feb 2001 (ster.), *Parmentier & Esono 1530* (BRLU0000197!); Monte Alén National Park, Engong rock slab, 5 km west of Engong village, 1°37'N, 10°18'E, 1100 m, 10 May 2002, (fl. buds), *Parmentier & Esono 2721* (BRLU0000195!); Monte Alén National Park, Monte Alén 2 rock slab, 1°40'N, 10°17'E, 1110 m, 27 May 2002 (fl.), *Parmentier & Esono 3453* (BRLU0000196!).

**Etymology.** The species is named *alenensis* after Monte Alén range and national park in Equatorial Guinea, where it is apparently endemic.

**Distribution and habitat.** *Nothodissotis alenensis* is endemic to Monte Alén National Park in Equatorial Guinea (Río Muni), where it occurs in low shrubby vegetation on rocky outcrops (“manteau arbustif”) at  $\pm$  1100 m a.s.l. (Fig. 4).



**Figure 5.** *Nothodissotis alenensis*, **A** habit **B, B'** leaf adaxial surface **C, C'** leaf abaxial surface **D** floral buds in different developmental stages; cl = calyx-lobes, ia = intersepal appendages, p = petals **E** stamens of the outer (left) and inner (right) stamen whorls (drawn from *Parmentier & Esono 1560, 2721, 2763 and 3453*). Illustration by Doris Franke.

**Phenology.** Flowering in May.

**Conservation status.** Vulnerable [VU D2]. *Nothodissotis alenensis* is endemic to Monte Alén National Park in Equatorial Guinea, where it has been collected four times and is known from two rock outcrops, representing two subpopulations. Its EOO cannot be calculated (since only two sites are known) while its AOO is estimated to be 8 km<sup>2</sup>, within the limit for Critically Endangered status under criterion B2. The species occurs in a remote area within a national park, and there is no evidence of an immediate threat or of a population decline. However, its extremely limited range makes it vulnerable to any threat that might arise in the future, e.g. climatic change or introduction of invasive species; it is therefore assessed as Vulnerable according to criterion D2.

### Key to African Melastomateae genera

- 1 Calyx either truncate or with lobes not contorted, leaving the corolla exposed in bud; trees or shrubs with 4-merous flowers; seeds often provided with dorsal hyaline papillae ..... *Dichaetanthera*
- Calyx-lobes always developed, contorted and concealing the corolla in young bud stage; herbs, or if shrubs then flowers always 5-merous; seeds tuberculate, smooth, ridged or foveolate, without hyaline papillae ..... **2**
- 2 Flowers involucrate, solitary or in heads; calyx-lobes persistent; intersepal appendages absent; fruits capsular or baccate ..... **3**
- Flowers not involucrate (except in *Dissotis speciosa*), solitary, glomerulate, paniced or racemose; calyx-lobes caducous or persistent; intersepal appendages present; fruits capsular or irregularly dehiscent, never baccate ..... **5**
- 3 Shrub with 1-flowered inflorescences; stamens isomorphic, with two ventral and one dorsal appendage, no distinct pedoconnective and erect anthers ..... *Cailliella*
- Herbs, or if shrubs then flowers several per inflorescence; stamens not as above, with two ventral appendages only, and pedoconnective usually well-developed..... **4**
- 4 Fruit fleshy, baccate; stamens isomorphic (except *T. cornifolium*), with erect anthers; hypanthium with hairs often arranged in rings (but sometimes glabrous or hairy all over) ..... *Tristemma*
- Fruit dry, capsular; stamens heteromorphic (except *M. porteresii*), with anthers patent or curved; hypanthium glabrous or with hairs not arranged in rings..... *Melastomastrum*
- 5 Stems, leaves, and inflorescences with stellate hairs, sometimes with simple hairs present as well; leaves sessile, amplexicaul ..... *Argyrella*
- Stems, leaves and inflorescences with simple or dendritic hairs, or sometimes glabrescent; leaves usually petiolate ..... **6**
- 6 Shrubs ..... **7**
- Herbs, sometimes ± woody at base ..... **11**

- 7 Calyx-lobes notched at apex, asymmetrical, caducous; leaves deciduous.....  
 .....***Nothodissotis***
- Calyx-lobes entire,  $\pm$  symmetrical, caducous or persistent (always persistent if  
 leaves deciduous) ..... **8**
- 8 Intersepalal appendages absent or reduced to a bristle; calyx-lobes persistent;  
 hairs on stems and leaves  $\pm$  bulbous at base; leaves deciduous or not ..... **9**
- Intersepalal appendages well-developed; calyx-lobes caducous (sometimes  
 tardily so); hairs on stems and leaves not bulbous at base; leaves never  
 deciduous ..... **10**
- 9 Inflorescence a 1–3(–7) flowered cyme; intersepalal appendages present; sta-  
 mens isomorphic; leaves small, 1.5–2.5  $\times$  1–1.5 cm.....***Dionychastrum***
- Inflorescence a many-flowered panicle; intersepalal appendages absent; sta-  
 mens usually dimorphic; leaves much larger.....***Dissotidendron***
- 10 Anthers isomorphic, opening by an extrorse pore; leaves distinctly bicoloured,  
 dark green above and yellowish-green beneath .....***Pseudosbeckia***
- Anthers usually dimorphic, opening by an introrse pore; leaves not as above  
 ..... ***Dissotis* (*D. sects. Macrocarpae, Squamulosae, Sessilifoliae*)**
- 11 Calyx-tube accrescent in fruit, developing a long neck with longitudinal ribs;  
 intersepalal appendages absent; stamens dimorphic ..... ***Dupineta***
- Calyx tube not accrescent (except *Dissotis tubulosa*) and lacking longitudinal  
 ribs; intersepalal appendages usually present; stamens dimorphic or  
 isomorphic..... **12**
- 12 Seeds longitudinally ridged or foveolate, with a (sometimes very short) basal  
 aril; stems creeping at base; staminal appendages bilobed or bipartite in outer  
 stamens; hypanthium with stalked stellate emergences (except *H. decumbens*  
 with simple hairs) ..... ***Heterotis***
- Seeds tuberculate or smooth, not arillate; stems usually erect, or if creeping  
 (*Guyonia*), then staminal appendages entire in outer stamens; hypanthium  
 usually glabrous or with simple hairs (in *Antherotoma* sometimes with stellate  
 emergences) ..... **13**
- 13 Seeds with parallel rows of tubercles; staminal appendages bilobed or bipartite;  
 flowers 4- or 5-merous; calyx-lobes persistent or caducous; hypanthium with  
 simple eglandular hairs or stellate emergences, rarely glabrous (*Chaetolepis*  
*gentianoides*)..... ***Dissotis* and allies (*Antherotoma, C. gentianoides,***  
***D. sect. Dissotis, D. congolensis, D. tubulosa, African “Osbeckia”*)**
- Seeds smooth, or with tubercles not arranged in parallel rows; staminal ap-  
 pendages entire in the outer whorl at least; flowers usually 5-merous (4-mer-  
 ous in *Guyonia rupicola*) calyx-lobes always persistent; hypanthium glabrous  
 or with glandular hairs, rarely (*Guyonia pygmaea*) with simple eglandular  
 hairs..... **14**
- 14 Stems thick and fleshy, winged, erect; hypanthium with prominent  
 longitudinal nerves; inflorescence an elongate cyme (raceme) with two well-

- developed bracts under each flower; plant almost glabrous except the ciliate leaves and calyx.....*Anaheterotis*
- Stems slender, usually not winged, creeping or more rarely erect; hypanthium with nerves hardly distinct; inflorescence not as above, usually with very small bracts, often 1-flowered; plant glabrous to densely hairy ..... *Guyonia*

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# The Reserva de la Biosfera Barranca de Metztlán (Hidalgo): An illustrated checklist of bromeliads and orchids and their high levels of Mexican endemisms

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## Abstract

This study presents a list of species of the two most important families with epiphytic elements, Bromeliaceae and Orchidaceae, from the Reserva de la Biosfera Barranca de Metztlán (RBBM), the largest Reserve in Hidalgo, Mexico. Thirty-four species are included, 26 corresponding to species in three genera of bromeliads, and eight species in six genera of orchids. The new records represent 26.5% of the total listed in the area; nine of them are new records for the Reserve (RBBM) and one is new for Hidalgo State. This study reveals that endemism for both families is very important in the Reserve (55.88%), since it includes 13 Mexican bromeliads, of which two are endemic to Hidalgo and one to the Reserve, and three orchids, two endemic to Mexico and one to the Reserve. We found species with different types of relative abundance: rare (16) and occasional (7). Additionally, we include information about the category (IUCN, CITES, NOM-059-SEMARNAT) as well as uses reported in the literature for the species in the RBBM. The checklist is strictly based on information obtained from deposited herbarium specimens as well as from those collected during fieldwork. We suggest that a conservation plan (*in situ* and *ex situ*) for the RBBM is important and necessary. The predominant habit for both families is epiphytic (17 species); even though there are terrestrial (7) and saxicolous (2), and the remaining are facultative species (8). Nine species are included in some risk category. The present work is the most complete and updated list of Bromeliaceae and Orchidaceae for this important natural area in the Mexican State of Hidalgo. However, more fieldwork is needed to document the biodiversity of the area in general and its flora in particular, as a way to highlight the importance of protected areas in preserving biodiversity.

**Keywords**

Metztlilán, Bromeliaceae, Orchidaceae, flora, endemism, conservation, rare species

**Introduction**

The Reserva de la Biosfera Barranca de Metztlilán (from here on RBBM) is located in the east-central region of the Mexican State of Hidalgo and is considered to be the most important protected natural area in Hidalgo State (see Figure 1). RBBM is considered a Pleistocene refuge of the Mexican desert biota since it has characteristics that show the strong relation that existed in the past with the Chihuahua and Sonora deserts, and is currently functioning as a biological corridor of arid zones in the central highlands of the country (CONANP 2003).

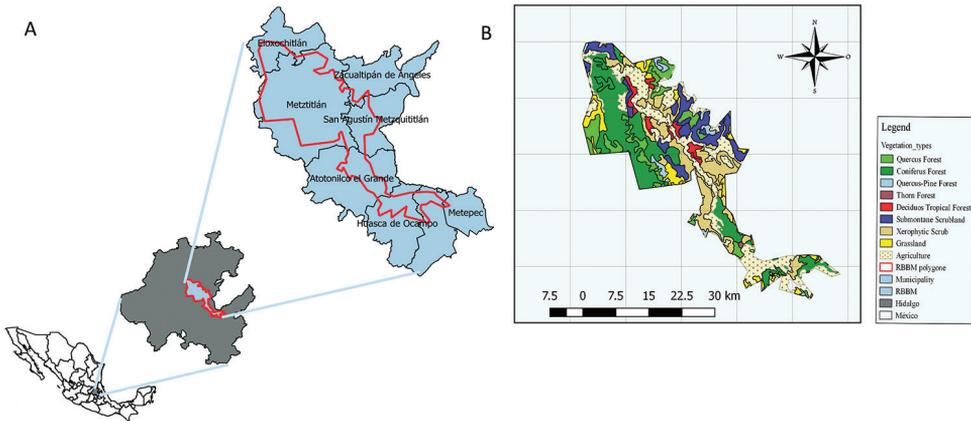
Tolantongo is the only area in Hidalgo State that has a formal floristic inventory (Hiriart and González-Medrano 1983), while RBBM flora is not well known. The management plan for RBBM (CONANP 2003) includes 11 bromeliads and six species of orchids. However, a careful study of this list shows some misidentifications. Moreover, the information used to produce the list was mainly taken from literature, which makes it impossible for specialists to review the species identification since the cited taxa are not supported by specimens housed in herbaria.

A preliminary orchid list of Hidalgo (Chavarria et al. in prep.) positioned this state in an important place (around the 5<sup>th</sup> national position) for orchid species richness in Mexico. However, the RBBM has been very poorly explored and, in our opinion, it is vital to carry out floristic studies that may enhance research in the State and improve management plan strategies. The present study aims to contribute to this goal, providing an updated list of bromeliads and orchids of RBBM.

Some previous publications on the Bromeliaceae of Hidalgo (Pintado 2010, Ceja-Romero et al. 2010, Hornung-Leoni and Pintado 2011, Hornung-Leoni 2017) have added information about the family in the region, but the present approach is focused on the RBBM. Recent studies in Mexican Bromeliads have documented 442 species for the country and 35 for Hidalgo State (Espejo-Serna and López-Ferrari 2018). On the other hand, Hornung-Leoni (2017) in the Hidalgo Bromeliad flora reports 47 species, ranking it in 8<sup>th</sup> position among the richest states for bromeliads species in Mexico.

**Methods****Area of study**

The RBBM includes several biogeographical regions (“Sierra Madre Oriental” and “Eje Neovolcanico Transmexicano”) and presents at least six, mainly dry vegetation types: xerophytic scrub, submontane scrubland, deciduous tropical forest, coniferous forest, grassland and riparian vegetation (Rzedowski 1978, Morrone 2001, CONANP 2003). In order to elaborate the map (Figure 1), we also considered the information about the



**Figure 1.** Map of the study area indicating: **A** Location of the RBBM in Hidalgo State and municipalities, and **B** Vegetation types.

use of soil and nine types of vegetation (e.g. agricultural, *Quercus* forest, among others) provided by INEGI (2017).

The RBBM has an area of 960.42 km<sup>2</sup> and is located in the eastern-central region of Hidalgo State (19°35'52"–21°25'00"N and 97°57'27"–99°51'51"W) (Figure 1). The topography is rugged and the altitudinal gradient ranges from 1100 to 2600 m a.s.l., which determines the presence of different climates and vegetation types. The climate varies from semi-dry to temperate dry, with an annual average temperature of 18–22 °C and average annual rainfall of 400–700 mm (CONANP 2003). This area constitutes a biological corridor between the Nearctic and Neotropical regions; it is located in northern Mexico and includes eight municipalities.

## Fieldwork and herbaria

Fieldwork in bromeliads has been conducted in RBBM since 2008. All collected specimens are housed in the Centro de Investigaciones Biológicas Herbarium (HGOM) of the Universidad Autónoma del Estado de Hidalgo. On the other hand, orchid data from the herbarium HGOM or from fieldwork, started to be collected and data-based in 2010.

For species identifications we used specialized botanical literature: Espejo-Serna et al. (2005, 2010), Pintado (2010), Ceja-Romero et al. (2010) and Hornung-Leoni (2017) for bromeliads and we reviewed Hågsater et al. (2005) and Soto-Arenas et al. (2007) for orchids. Dr. Gerardo Salazar identified the orchid specimens in HGOM. Bromeliads were identified by C. Hornung-Leoni and I. Ramirez-Morillo.

All our reports are strictly based on herbarium specimens deposited in several herbaria (AMES, AMO, CHAP, CICY, ENCB, GH, F, IEB, IBUG, MICH, MEXU, MO, UAMIZ, US, SEL, WU). Additionally, we used high resolution images from the

same herbaria as well as online databases to complement some data (e.g. distribution and altitudes) (Table 1).

For authors, we followed Brummitt and Powell ([www.ipni.org](http://www.ipni.org)); for synonymy, we used The Plant List ([www.theplantlist.org](http://www.theplantlist.org)) and for the species names, we consulted Tropicos (<http://tropicos.org/>).

Information about endemism was obtained from previous studies about recently discovered new species (Espejo-Serna et al. 2004, Espejo-Serna 2012, Ramírez et al. 2015, Villaseñor 2016); as for risk categories, we consulted IUCN (2017), NOM-059-SEMARNAT (SEMARNAT 2010) and CITES (2017) ([www.cites.org](http://www.cites.org)). For species with restricted distribution inside the RBBM limits (like *Tillandsia tortilis*, *T. mauryana* and *Sotoa confusa*), GeoCAT tool interface program (Bachman et al. 2011) was employed in order to calculate risk category *sensu* IUCN criteria, considering extent of occurrence (EOO) and area of occupancy (AOO) based on coordinates, for those cases in which we found at least three different collecting points as required by the software.

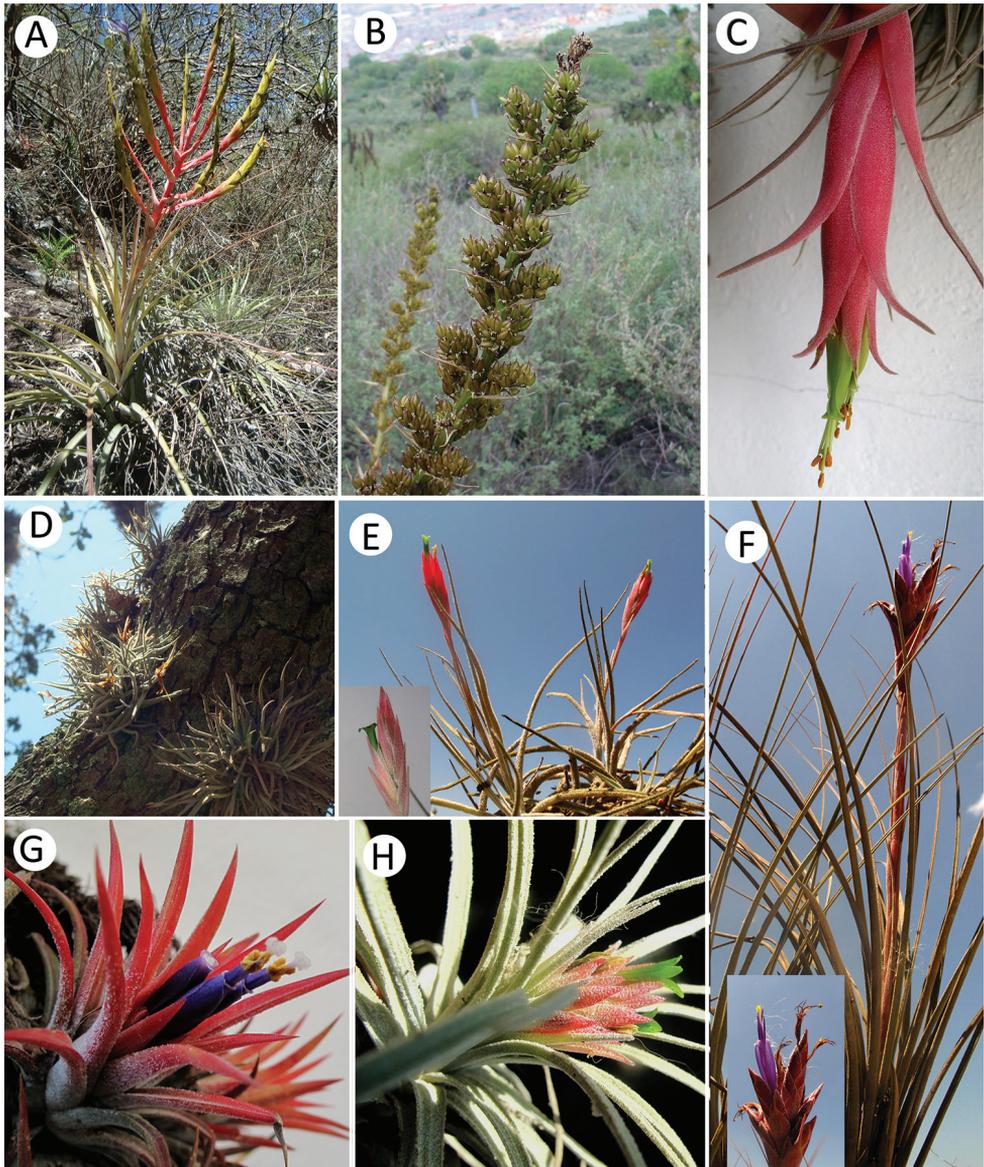
## Results

A total of 34 species from both families (Table 1, Figures 2–4) were found in RBBM. Of these, 26 species belong to Bromeliaceae classified into three genera and eight are orchid species distributed among six genera (Table 1). Three orchids and six bromeliads were added as new records of RBBM. The most representative genus in bromeliads was *Tillandsia* with 21 species, followed by *Hechtia* with five species. *Laelia* is the species-richest genus of orchids.

Namely, from the xerophytic area we are reporting orchids like *Laelia* and *Cyrtopodium* species and bromeliads like *Hechtia* spp., *Tillandsia albida* and *T. inopinata*. In humid environments, orchids like *Aulosepalum*, *Sarcoglottis* and *Malaxis*, and bromeliads like *T. violacea*, *T. imperialis* and *T. deppeana*, are found.

Epiphytic habit is predominant in both families corresponding to 50% of the species (see Figure 5); however, terrestrial and facultative epiphytic species are also important in the RBBM. *Hechtias* grow both as saxicolous and terrestrials. *Aechmea bracteata* was found only as an epiphyte, while *Tillandsia mauryana* prefers the saxicolous habit. As facultative we listed individuals that can be found in two different categories (e.g. saxicolous and epiphytes or saxicolous and terrestrial).

Different levels of endemism are present in the RBBM including both families (Table 1), totaling 55.88% of the recorded species. Thirteen of the bromeliads found in the Reserve are endemic to Mexico (like *Tillandsia tortilis*, *Hechtia lepidophylla*); two are endemic to Hidalgo (*H. deceptrix* and *T. mauryana*) and one bromeliad is endemic to the Reserve (*Hechtia* sp.). For orchids, a total of three species present some level of endemism: two are endemic to Mexico (*Sotoa confusa* and *L. speciosa*), and one (*Laelia gouldiana*) is present only inside the RBBM even though it has been considered *extinct in situ* in the NOM-059-SEMARNAT (SEMARNAT 2010). This species is only



**Figure 2.** Species of bromeliads in Reserva de la Biosfera Barranca de Metztlán (RBBM): **A** *Tillandsia inopinata* **B** *Hechtia podantha* **C** *Tillandsia erubescens* **D** *Tillandsia recurvata* **E** *Tillandsia tortilis* **F** *Tillandsia juncea* **G** *Tillandsia ionantha* **H** *T. mauryana*. Photographs: **A–H** by Hornung-Leoni.

found on “Mesquite” trees (*Prosopis laevigata*, Fabaceae: Mimosoideae) which grow near local people’s houses inside the RBBM (Soto-Arenas et al. 2002).

The categories of threatened taxa (IUCN (2017), CITES (2017), NOM-059-SE-MARNAT (SEMARNAT 2010) found in literature are reported in Table 1.

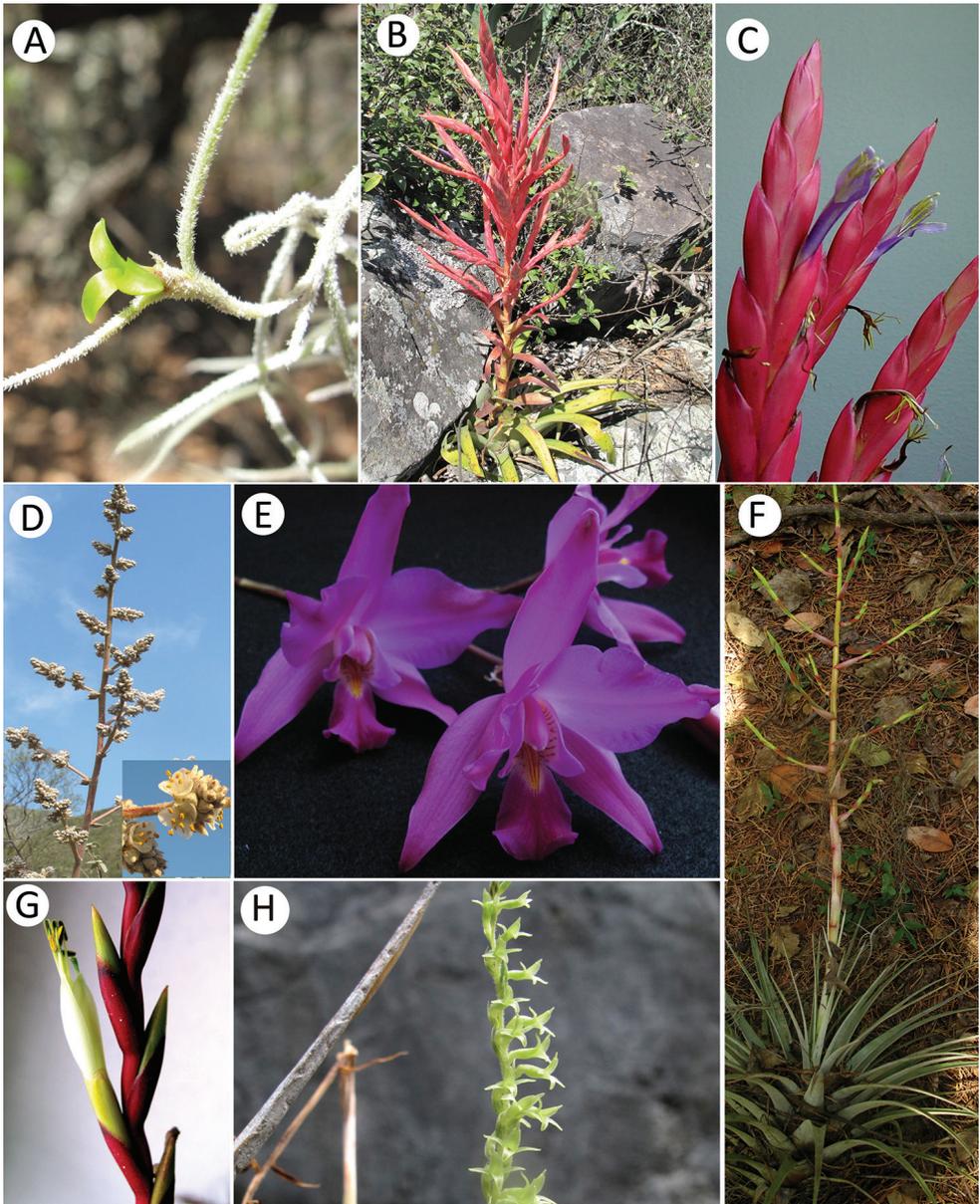
**Table 1.** List of the bromeliad and orchid species recognized in this study at the RBBM. Taxa are arranged alphabetically in families and genera; the collector's name, number and herbaria are indicated. For more details of vouchers and distribution of species, see Suppl. material 1. Species marked with \* indicate a new register of RBBM. Endemism: MEX: endemic to Mexico, HGO: endemic to Hidalgo State, and RBBM: endemic to the Reserve. NOM-059-SEMARNAT Mexican categories: threatened (A), probably extinct in the wild (E), in danger of extinction (P), and subject to special protection (Pr). Habit: E= epiphytic, T=terrestrial, S= saxicolous. See Suppl. material 1 for more details.

Species	Vouchers	Examined Herbariums	Endemism	Habit	Relative species abundance	Threatened taxa
<b>Bromeliaceae</b>						
1. <i>Aechmea bracteata</i> (Sw.) Griseb.	AGPP 114	HGOM		E	rare	
2. <i>Hechtia deceptrix</i> I. Ramírez & C.T. Hornung *	CTHL 1598	HGOM	HGO	T	rare	VU (IUCN)
3. <i>H. glomerata</i> Zucc.	AGPP 178, CTHL 1249, CTHL 1329, CTHL 1330, CTHL 1430, CTHL 1431, CTHL 1432, CTHL 1433, CTHL 1564	HGOM		T/S	abundant	
4. <i>H. lepidophylla</i> I. Ramírez	LGQ 1189	IEB	MEX	T	rare	
5. <i>H. podantha</i> Mez	G 5291, UG 2135,	MEXU	MEX	T	abundant	
6. <i>Hechtia</i> sp. *	AGPP 118	HGOM	RBBM	T	rare	
7. <i>Tillandsia albida</i> Mez & Purpus	AGPP 66, AGPP 122, AGPP 173, ARLF 2132, AES 1782, EM 38486, CTHL 1153, CTHL 1331, CTHL 1435, CTHL 1603,	HGOM, MEXU	MEX	T/S	common	
8. <i>T. atroviridipetala</i> Matuda	ARLF 3279, JR 19504	UAMIZ, MEXU	MEX	E	rare	
9. <i>T. bartramii</i> Elliott	AGPP 68, CTHL 1559, CTHL 1605	HGOM		E	occasional	
10. <i>T. deppeana</i> Steud.	AGPP 121, JLLG 412, CS 53,	HGOM, CHAP, UAMIZ	MEX	E	frequent	
11. <i>T. erubescens</i> Schltdl. *	CTHL 1572	HGOM	MEX	E	occasional	
12. <i>T. festucoides</i> Brongn. ex Mez *	AGPP 124	HGOM		E	rare	Pr category NOM-059
13. <i>T. gymnotrya</i> Baker	AGPP 119, JC 1954, JC 1960, JLLG 494, JLLG 410, JC 1296, JC 1301, CS 59	UAMIZ, MEXU, IEB, CHAP	MEX	E	rare	
14. <i>T. imperialis</i> É. Morren ex Mez	AGPP 120, JC 1969, AES 6661, JC 1960	HGOM, UAMIZ		E	rare	A category NOM-059

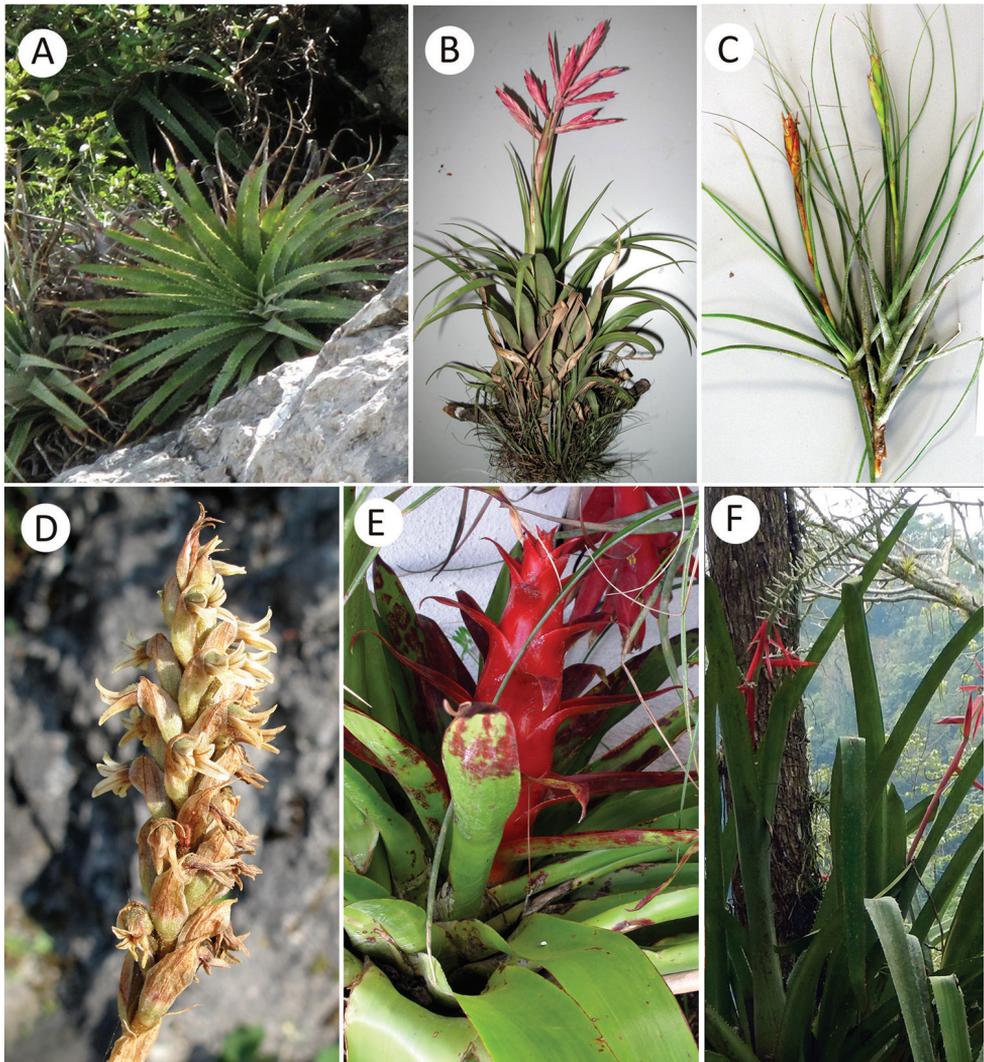
Species	Vouchers	Examined Herbariums	Endemism	Habit	Relative species abundance	Threatened taxa
15. <i>T. inopinata</i> Espejo, López-Ferr. & W. Till	AGPP 63, AGPP 64, AGPP 112, AGPP 113, AGPP 115, AGPP 136, AGPP 141, CTHL 1187, CTHL 1240, 1241, CTHL 1242, CTHL 1541, CTHL 1542, CTHL 1555, ARLF 3275, LGQ 1202, LGQ 2567, ALB 37, PM 5765. SS 76/30, SS 77/7, HEM 2471, JR 19505	HGOM, ENCB, MEXU, GH, WU, US, UAMIZ	MEX	T/S/E	abundant	
16. <i>T. ionantha</i> Planch.	AGPP 130, AGPP 134, AGPP 138, CTHL 1505, CTHL, 1506, CTHL 1508, CTHL 1522, CG 22	HGOM, ENCB		E/S	frequent	LC IUCN
17. <i>T. juncea</i> (Ruiz & Pav.) Poir.	AGPP 110, AGPP 111, AGPP 142, AGPP 174, JC 1966, EG 5131, EG 5276, BL 2645. ARLF 3274, JC 1292, TBC 65815, FG 8402, LG 1203, ARLF 3281, MM 2533, HEM 2469, JR 19503, CTHL 1540, CTHL 1543, CTHL 1544, CTHL1547, CTHL 1604	HGOM, UAMIZ, CHAP, MEXU, ENCB, US		E	abundant	
18. <i>T. lepidosepala</i> L.B.Sm.	HEM 4221, ARLF 2131	MICH, UAMIZ	MEX	E	rare	
19. <i>T. mauriyana</i> L.B.Sm.	CTHL 1328; CTHL 1557, CTHL 1561, CTHL 1570, CTHL 1666, AGPP 140, YJCO 75; DG 2, ARLF 2133, PM 5747, JC 1768, JC1967, DG 2, ALS <i>sn</i> , ARLF 2133, PM 5747, EZ <i>sn</i> .	HGOM, MEXU, SEL UAMIZ, F, GH, WU	HGO	S	occasional	CITES, IUCN: EN (EOO/ AOO)
20. <i>T. parryi</i> Baker	JV 1961, RE 942403, JC 1298, JC 1300, ARLF 3282, JLLG 411	UAMIZ, M, CICY, IBUG, MEXU	MEX	E	rare	
21. <i>T. pringlei</i> S.Watson *	CTHL 1434, CTHL 1560	HGOM	MEX	E	rare	
22. <i>T. recurvata</i> (L.) L.	AGPP 67, AGPP 117, AGPP 125, AGPP 177, AGPP 179, LCR 426, EGN 5122, EGN 5222, EGN 5262, ARLF 3277, JMQ <i>sn</i> , <i>sc sn</i> , ARLF 3353, RR <i>sn</i> , ID 757, MF 167, CTHL 1507	MEXU, CHAP, UAMIZ		E	abundant	

Species	Vouchers	Examined Herbariums	Endemism	Habit	Relative species abundance	Threatened taxa
23. <i>T. schiedeana</i> Steud. *	AGPP 132, AGPP 175	HGOM		E	abundant	
24. <i>T. tortilis</i> Klotzsch ex Baker *	CTHL 1600, CTHL 1601	HGOM	MEX	E	rare	IUCN: EN (AOO)/ NT (EOO)
25. <i>T. usneoides</i> (L.) L.	AGPP 116, AGPP 143, JJC 1956, EG 5262, FGM 7963, FGM 10359, ARLF 3280, ARLF 3283, CTHL 1545	UAMIZ, XAL, MEXU, UAMIZ		E/S	abundant	
26. <i>T. violacea</i> Baker	JLLG 507	MEXU	MEX	E	occasional	
<b>Orchidaceae</b>						
1. <i>Aulosepalum pyramidale</i> (Lindl.) M.A.Dix & M.W.Dix *	AMR 1513, CTHL 1333; CTHL 1334, CTHL 1339	UAMIZ, HGOM		T	rare	
2. <i>Cyrtopodium macrobulbon</i> (La Llave & Lex.) G.A.Romero & Carnevali	AES 2431, AES 2432, AES 243; JAM 6	UAMIZ, HGOM		S	occasional	CITES
3. <i>Laelia anceps</i> Lindl.	FGM 8438, JAM 15	MEXU, HGOM		E/ S	occasional	P category NOM-059 CITES
4. <i>L. gouldiana</i> Rchb.f.	AES 2213, EH 6000, WBT 2500, WBT 6085, GAS 8194	UAMIZ, US, ENCB, AMO, MEXU	RBBM	E	rare	E category NOM-059 CITES
5. <i>L. speciosa</i> (Kunth) Schltr.	RHM 6078, GAS 8194, ARLF 3273	MEXU, UAMIZ, MO	MEX	E/ S	occasional	Pr category NOM-059 CITES
6. <i>Mesadenus polyanthus</i> (Rchb.f.) Schltr. *	CTHL 1552, CTHL 1562	HGOM		T/ S	rare	
7. <i>Sarcoglottis schaffneri</i> (Rchb.f.) Ames *	CTHL 1255, CTHL 1708	HGOM		T	occasional	
8. <i>Sotoa confusa</i> (Garay) Salazar	JG 2194	AMES	MEX	T	rare	CITES, IUCN: LC (EOO) / EN (AOO)

Collector's legend: AGPP: A.G. Pintado Peña; ALB: A. López B.; AMR: A. Mendoza R.; ARLF: A.R. López-Ferrari; CG: C. García; CS: C. Sanchez; JCR: J. Ceja R.; CTHL: C.T. Hornung-Leoni; EG: E. Guízar; EH: E. Hagsater; EM: E. Matuda; FGM: F. González Medrano; FM: F. Miranda; G: Guízar; GAS: G.A. Salazar; HEM: H. E. Moore jr.; ID: I. Díaz; JAM: J. Ángeles Mota; JG: J. González; JLLG: J. L. López G.; JMQ: J. M. Quintanilla; JR: J. Rzedowski; LB: B. Leuenberger; LGQ: L. González Q.; MASA: M.A. Soto Arenas; MF: M. Flores; MM: M. Medina; PG: P. Gold; PM: P. Maury; RE: R. Ehlers; sc: without collector; RHM: R. Hernández Magaña; RR: R. Robledo; SS: S. Schatzl; TBC: T.B. Croat; UG: U. Guzmán Cruz; YJCO: Y.J. Chavarria Olmedo; WBT: W.B. Thurston.



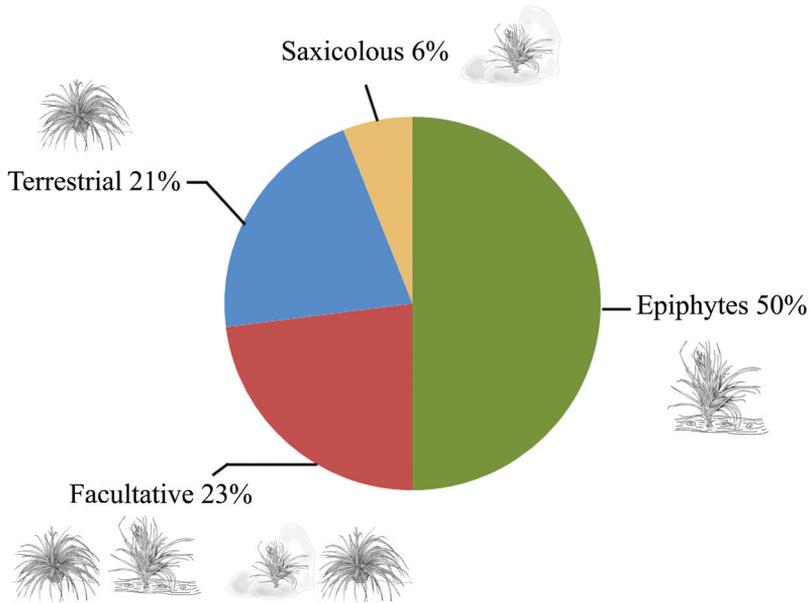
**Figure 3.** Species of Bromeliaceae (*cont.*) (A–D, F, G) and Orchidaceae (E, H) in Reserva de la Biosfera Barranca de Metztitlán (RBBM): Bromeliaceae: **A** *Tillandsia usneoides* **B** *Tillandsia parryi* **C** *Tillandsia deppeana* **D** *Hechtia glomerata* **E** *Laelia gouldiana* **F** *Tillandsia pringlei* **G** *Tillandsia albida* **H** *Mesadenus polyanthus*. Photographs: **A–D** and **F–H** by Hornung-Leoni. Photograph E by Chavarria-Olmedo.



**Figure 4.** Bromeliaceae and Orchidaceae (*cont.*) in Reserva de la Biosfera Barranca de Metztitlán (RBBM) (*cont.*) **A** *Hechtia deceptrix* **B** *Tillandsia gymnobotrya* **C** *Tillandsia schiedeana* **D** *Aulosepalum pyramidale* **E** *Tillandsia imperialis* **F** *Aechmea bracteata*. Photographs: **A–E** by Hornung-Leoni and **F** by I. Ramírez-Morillo.

## Discussion

After revising the available literature for Hidalgo, we found that seven species of bromeliads were mentioned for the RBBM (Espejo-Serna et al. 2004) and 12 species in the management plan (CONANP 2003); eight species were cited by Ceja-Romero et al. (2010) and 24 bromeliads by Pintado (2010). However, it is important to point out that the main goal of these studies is not the bromeliad flora of the RBBM and that, in some cases, it was impossible to establish the existence of some species inside the



**Figure 5.** Habits in species present in RBBM

limits of the RBBM. On the other hand, the taxonomic status of the species included in the management plan (CONANP 2003) needs to be revised, since some of the cited names are synonymous or misplaced species (see details below).

For orchids, although the management plan CONANP (2003) reported six species, Soto-Arenas and Solano-Gómez (2007) as well as Ceja-Romero et al. (2010), cited only three species of *Laelias* (*Laelia autumnalis*, *L. gouldiana*, *L. speciosa*).

In neotropical studies, both families here included are very representative as far as richness is concerned (Rodrigues et al. 2017). Therefore, new studies are required to improve the characterization of the biodiversity of the region and to promote the species protection inside the RBBM.

### Species in RBBM and Endemism

It is important to emphasize that several of the species found by us at RBBM were mentioned before in the literature (CONANP 2003, Vincenzo et al. 2012), but their presence based on previous reports is impossible to be confirmed since many of them lack herbarium specimens. Therefore, ours constitutes the first confirmed and verifiable report, since it is supported by specimens housed in public collections.

In the management plan of the RBBM (CONANP 2003), 12 bromeliads and orchids are cited; however, some nomenclatural problems need to be mentioned. First, among bromeliads they reported *Tradescantia* sp., a member of Commelinaceae, which

needs to be discarded. Secondly, some of the cited species' names are currently treated as synonymous or are invalidly published (*sensu* Plant List and Tropicos): for example, *Tillandsia ehrenbergiana* Hemsl., is a synonym of *T. utriculata* but we have no records of this last species in the study area. *Tillandsia ehrenbergiana* Klotzsch ex Baker is synonymous with *T. tortilis* and for this taxon we have several records. Another case is that of *Tillandsia benthamiana* Klotzsch ex Baker, currently treated as a synonym of *T. erubescens*, a taxon here reported for the RBBM. *Tillandsia benthamiana* var. *andrieuxii* Mez is recognized as *T. andrieuxii*, a species for which we also did not find specimens deposited in herbaria because we did not collect it in the study area. For these reasons, we consider that the correct names for the species previously cited for the area (CONANP 2003) are *T. tortilis* and *T. erubescens*. Moreover, even if they are synonyms, these species have not been found in herbaria until now. So, *T. tortilis* and *T. erubescens* were considered as new records because our vouchers are the first ones collected for both species.

For orchids, in the management plan (CONANP 2003) species like *Cyrtopodium macrobulbon*, *C. punctatum* and *Epidendrum ramosissimum* Ames & C. Schweinf. were reported, even though the last species is only documented for Costa Rica; it is probably a case of misidentification and will not be clarified since there is no specimen or image to verify this report. Other orchid species reported for the RBMM (*sensu* Vincenzo et al. 2012), are *Anathallis minutalis*, *Epidendrum ramosum*, *Laelia autumnalis*, *L. gouldiana*, *L. anceps*, and *L. speciosa*. The management plan reports the presence of *E. ramosissimum* that Vincenzo et al. (2012) recognize as *E. ramosum*, but both are only literature references. For this reason they are not included in the present study. For *Pleurothallis minutalis* (*sensu* CONANP 2003 and Vincenzo et al. 2012), currently treated as *Anathallis minutalis*, its presence has not been collected yet for the orchid flora of Hidalgo State (Chavarria et al. in prep.).

Since RBBM provides many habitats, vegetation types and different substrates, the Reserve is a relevant area for the preservation of the flora in the state and as more collections become available the numbers reported here for endemism may change, since they are affected by the collection effort as well as the growing knowledge about species distribution. The number of species present in the RBBM is considerable, in relation to the area occupied by this reserve within the State. Nevertheless, this fact was not unexpected because it is the largest Reserve of Hidalgo State and includes several vegetation types as well as a variety of climatic and environmental conditions. In spite of this richness, in our opinion the diversity of bromeliads and mainly orchids has been underestimated. This is unsurprising considering that Rzedowski (2015) mentioned that knowledge of Mexican flora is still a work in progress with approximately 30% of species still unknown.

The greatest richness in the RBBM is represented by *Laelia* for orchids and *Tillandsia* for bromeliads, the latter being the most diverse genus of the bromeliads in Hidalgo (Hornung-Leoni 2017). Some of them are still poorly collected. For example, *Tillandsia tortilis*, has been reported before outside the limits of Metztlán (Ceja-Romero et al. 2010), but we found this species inside the RBBM near its borders. We consulted some local people who commented that they do not have any interest in this plant

since they only consider it as a parasite and, in some cases, it is removed from trees. Another species, *T. recurvata*, is very abundant in *Prosopis* spp. trees and other bushes in the Reserve. Sterile individuals of *Hechtia deceptrix*, a recently described species endemic to Hidalgo (Ramírez et al. 2015), were observed inside the RBBM (on the road from Metztitlán to Tolantongo). This finding extends the distribution of the species reported earlier by Ramírez et al. (2015) for the Carso Huasteco within the Sierra Madre Oriental Province (*sensu* Morrone 2014). However, the presence of this species inside the RBBM was expected since it is located in the same biogeographic area. Other species that we expected to find are *Pitcairnia ringens* (Bromeliaceae) and *Dicromanthus cinnabarinus* (Orchidaceae), since both of them have been reported near the limits of the RBBM, despite not being vouchered up to now.

The RBBM can be cited as an example of how a Reserve created for other purposes (e.g. to preserve Cactaceae), can be a refuge for other endemic, rare, and even new species like bromeliads and orchids. After completing floristic inventories, conservational strategies should be developed, counting on people who live inside the Reserve to assist in the species/habitats conservation. In fact, people living inside the reserve are very conscious of the natural resources they have, and may take part to protect the biota. Additional strategies may include ex-situ cultivation of threatened species and the promotion of campaigns to avoid extraction of individuals from their habitat.

### Habit predominance

Even though epiphytic species are dominant in RBBM, some species like *T. juncea* can be facultative (epiphytes and occasionally saxicolous). However, as was expected, the predominance of epiphytes is related to the presence of abundant tree species of the deciduous tropical forest. This means that species from different substrate preferences can coexist in the same habitat and vegetation type. For example, on scrubs (“matorral”) we can find epiphytes like *T. juncea*, and *T. ionantha* on cactus or even on rocks; on the other hand, saxicolous species as *T. inopinata* can also grow as epiphytes in the same area.

Most orchids seem to be facultative; for example, species of *Laelia* generally appear as epiphytes, although it has been reported that *L. speciosa* and *L. anceps* can be saxicolous in other regions (Halbinger and Soto-Arenas 1997, Soto-Arenas and Solano-Gómez 2007). Another orchid that can also be facultative is *Mesadenus polyanthum*; the remaining species here reported are limited to one type of habit (epiphyte, saxicolous, or terrestrial).

Due to the variation in orography, vegetation types and climates found within the RBBM, it is possible that different species can find there an appropriate microclimate/niche to grow. For example, from the species cited in Table 1, orchids like *Laelia* and *Cyrtopodium* species, as well as bromeliads of the genus *Hechtia*, and particularly *Tillandsia albida* and *T. inopinata*, are found in xerophytic areas; while in humid environments one may find orchids like *Aulosepalum* and *Sarcoglottis* and bromeliads as *T. violacea*, *T. imperialis*, and *T. deppeana*.

Some species of bromeliads and orchids grow in drier habitats (Gentry and Dodson 1987) which is related to CAM metabolism and economy of water (Benzing 1990, Fontoura and Reiniert 2009). For example, *T. mauryana* and *T. ionantha* grow in xerophytic areas and are adapted to water stress conditions (Benzing 2000). Both have CAM metabolism that may be used for saving water (Benzing 2000, Matiz et al. 2013).

Orchid diversity is considered low in arid areas because there are limiting factors, like water and type of soil that are essential for mycorrhizae formation and seed germination. Even in rocky sites, organic soil matter is usually low or almost nonexistent and hence a limitation (Hågsater et al. 2005). Nevertheless, most of the species reported here are terrestrial and saxicolous. The epiphytic orchids depend not only on the mycorrhizae, but also on the characteristics of trees' bark and the microclimatic gradients generated by their height (Benzing 1990, Shaw 2004), which could explain the low diversity presented by this habit.

### Species in some risk category

A total of nine endangered or protected species are included in Table 1 (IUCN, CITES, NOM-059-SEMARNAT). The NOM-059-SEMARNAT only proposed *Tillandsia festuoides* and *Laelia speciosa* as species subject to special protection; *T. imperialis* is referred to as a threatened species, and is locally used in beverages (as food or as medicine) by people in Hidalgo (Hornung-Leoni 2011 a,b). *Laelia anceps* is also mentioned as an endangered species and *L. gouldiana* as an extinct one (*in situ*). On the other hand, the IUCN list only considers *T. ionantha* as a Least Concern (LC) category, and CITES (2017) includes species like *T. mauryana*, *L. anceps*, *L. gouldiana* and *L. speciosa* as Endangered and states that for *Laelia*, the main problem is its use as an ornamental plant subjected to extraction of individuals from the wild for the horticultural trade.

*Tillandsia mauryana* is considered at risk in CITES appendices (2017). According to Espejo-Serna et al. (2004), this species is saxicolous and endemic to Mexico, for the states of Hidalgo, Jalisco, Oaxaca, and Zacatecas. More recently, Espejo-Serna and López-Ferrari (2018) considered *T. mauryana* endemic to Hidalgo, a fact that we have corroborated after reviewing specimens collected in the field and in herbaria. We detected some areas in the RBBM where the species covers a rocky wall, while in other areas only a few individuals are present. In general, we observed scanty populations of *T. mauryana* in the Reserve (see IUCN category assigned in Table 1). No particular use of this species in local trade was reported here, but due to the small number of these populations and to their potential use as ornamental plants, some kind of protection is needed (see Table 1 for IUCN status). Furthermore, it needs to be protected due to the slow population growth rate and the frequent extraction of rocks in the Reserve area (Valverde-Valdés 2013). For those species that are not yet used by local people, the principal risk is habitat destruction.

In Orchidaceae, *Laelia* has categories subject to protection in both CITES and NOM-059-SEMARNAT, since species are exploited commercially and for cultural uses during local festivities. Nevertheless, they are not included in any of the IUCN categories.

*Laelia gouldiana* is the most exploited species and although it is reported as extinct *in situ* (NOM-059-SEMARNAT), it is common to find it in local houses' backyards inside RBBM, a preponderance that has probably contributed to perpetuate its existence. For the remaining orchid species reported in the RBBM, there are no known ethnobotanical uses. Terrestrial orchids are recognized as such by very few people and are susceptible to vegetation fires during the dry season and to changes in the use of soil.

### Comparing data. Adding new records

For bromeliads, principally tillandsias were included in the management plan (CONANP 2003), but some of them are synonymous or misidentifications. Three out of six species of the orchids included in the management plan are Laelias [*L. autumnalis*, *L. gouldiana* and *L. speciosa* (Kunt) Schltr]. The last two can be found in NOM-059-SEMARNAT, in the categories of extinct in nature and in special protection, respectively (SEMARNAT 2010).

### Biological and ethnobotanical information about species found in the Reserve

Both families are important because they not only provide biological resources to pollinators, visitors and fauna, which fulfill at least a part of their life cycles in these plants (Benzing 2000, Hornung-Leoni et al. 2013), but because they are also used by local people for ornamental, medicinal and ceremonial purposes (Hornung-Leoni 2011a).

Orchids such as Laelias are considered beautiful, abundant and easy to grow and are commonly used in religious celebrations such as the Día de Muertos (Day of the Dead) (Hágsater et al. 2005). Due to the flowering period of *L. anceps* and *L. gouldiana*, it is common to find them in November as an ornamental element in altars, tombs, and churches. For people who live inside the RBBM, *L. gouldiana* is a well-known and widely cultivated species to be used during November festivities and represents an occasional economic income as well (Soto-Arenas and Solano-Gómez 2007). As for *L. speciosa*, we found only one publication in which food and ornamental uses in Hidalgo are included (Pérez Escandón et al. 2003), even though we could personally verify that it is locally sold at Actopan markets in May. Although García-Peña and Peña (1981) report the use of pseudobulbs of *Cyrtopodium macrobulbon* to prepare cataplasms, it is not known if local people of the RBBM apply the same use to this or other species.

### Suggestions for conservation

Bromeliads and orchids are important in areas that have a conservation focus. It is clear that areas with species under threat like RBBM are important for Mexico if there is, as we think, the intention to create corridors for plants and pollinators.

Summarizing, bromeliads are important components in RBBM, with predominance of *Tillandsia* species, and with a high component of Mexican endemism inside the Reserve. The number of species of orchids has been underestimated and needs to be explored further in the future. Even if the principal habit is epiphytic, both families represent important elements for species diversity in the RBBM, as well as a dominant component in some habitat types. The endemism inside the RBBM highlights the necessity of preserving this variable area and enhancing environmental education and ethnobotanical studies together with local people.

## Conclusions

The Reserve is an important area for flora protection. Even if the RBBM is the most important reserve in the State, it has hitherto not featured in detailed flora inventories and additions to this list are expected in the near future. This study reports 34 species: ten of these are new registers for the Reserve, seven are new records of bromeliads and three are of orchids. Twenty-six bromeliads and eight orchids of the RBBM were recorded and properly documented for the first time. Moreover, the presence of endemic and rare species, as well as of those in risk categories (NOM-059-SEMARNAT, IUCN and CITES), makes it necessary to implement new strategies to maintain the reserve and increase efforts to update its biodiversity data.

Since an important number of endemic taxa (19 species), as well as rare (16 spp.) and occasional species (7) have been found inside the RBBM, the Reserve plays a determinant role in conserving such species. *Ex situ* and *in situ* strategies for such species may integrate a new plan of management focused on the conservation.

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## Supplementary material I

### Information about species distribution present in Meztlán

Authors: Claudia T. Hornung-Leoni, Yesenia J. Chavarria-Olmedo, Ivón M. Ramírez-Morillo

Data type: occurrence

Explanation note: In this table extra information is included with details principally about species distribution.

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