

Home at last III: Transferring *Uechtritzia* and Asian *Gerbera* species into *Oreoseris* (Compositae, Mutisieae)

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Abstract

Recently the Asian *Gerbera* species were shown to form a clade that was not the sister group of the African *Gerbera*. In this study, the position of the Asian *Gerbera* species was further assessed based on morphology and molecular phylogenetic analyses that included six Asian *Gerbera* and 26 other species from the *Gerbera*-complex. Morphological results showed that the six Asian *Gerbera* species, which were sampled, bear leaves with the adaxial epidermal surface lacking stomates, possess bracteate scapes and lack inner ray florets. These characters suggest that the Asian *Gerbera* species are most closely related to the species of *Uechtritzia*, which also share similar pollen grain size and shape with the Asian *Gerbera*, rather than to the African *Gerbera*. Furthermore, the phylogenetic results based on two nuclear (ITS and ETS) and three chloroplast (*trnL-trnE*, *trnL-rpl32* and *trnC-petN*) sequences strongly support the Asian *Gerbera* and *Uechtritzia* forming a clade, with the latter nested within the Asian *Gerbera* species. Both morphological and molecular phylogenetic data thus confirmed the taxonomic identity of the Asian *Gerbera* and *Uechtritzia*. The authors herein formally treat the nine species of the Asian *Gerbera* and the three species of *Uechtritzia* as members of the genus *Oreoseris*, which is the earliest generic name of this lineage and has the nomenclatural priority.

Keywords

Compositae, *Gerbera*-complex, *Oreoseris*, *Uechtritzia*, SEM, stomata, pollen, South America, Africa, Asia

Introduction

The *Gerbera*-complex (Compositae: Mutisieae) contains eight genera: *Gerbera* L., *Leibnitzia* Cass., *Uechtrizia* Freyn, *Amblysperma* Benth., *Chaptalia* Vent., *Trichocline* Cass., *Perdicium* L. and *Lulia* Zardini. *Gerbera* currently contains about 31 species, which belong to six sections: the five African sections: sect. *Gerbera* (8 species), sect. *Parva* H.V.Hansen (1 species), sect. *Lasiopus* (Cass.) Sch.Bip. (6 species), sect. *Pseudoseris* (Baill.) C.Jeffrey (8 species, distributed in Madagascar) and sect. *Piloselloides* Less. (2 species, one of which is widespread in Asia and Africa) and the Asian sect. *Isanthus* (Less.) Jeffrey (6 species; Hansen 1985a, 1985b, 1988, Johnson et al. 2014, Funk et al. 2016). One South American species *G. hieracioides* (Kunth) Zardini was not included in any of the above-mentioned sections of *Gerbera* (Zardini 1974) and the authors have recently transferred it to *Chaptalia* based on both morphological and molecular data (Xu et al. 2018).

The Asian *Gerbera* section *Isanthus* is characterised mainly by campanulate involucre, naked receptacles and rostrate achenes (Hansen 1988). A recent molecular phylogenetic analysis showed that the Asian *Gerbera* species did not form a clade with the African species (Pasini et al. 2016): the Asian *Gerbera* + *Uechtrizia* formed a clade and the African *Gerbera* and *Amblysperma* constituted another clade. Some earlier workers also suggested treating the Asian section as an entity separate from the African *Gerbera* (Candolle 1838, Jeffrey 1967). Hansen (1990), however, argued that, while the Asian *Gerbera* sect. *Isanthus* differed somewhat from the African *Gerbera*, it shared four apomorphies as well as 11 plesiomorphies with *Uechtrizia* and the three entities could not be discerned from one another.

Species of *Uechtrizia* have hemispherical involucre, fimbriate receptacles and slightly rostrate achenes (Hansen 1988). This genus contains three species, namely *U. armena* Freyn endemic to Turkey (Doganet al. 2016) and Armenia, *U. kokanica* (Regel et Schmalh.) Pobed. from Central Asia (Kazakhstan, Uzbekistan, Tajikistan, Kyrgyzstan, Turkmenistan to Afghanistan) and *U. lacei* (G.Watt) C.Jeffrey of the Himalayan region (Hansen 1988).

Pasini et al. (2016) included one *Uechtrizia* species, *U. kokanica* and showed that the species was nested within the two sampled Asian *Gerbera* species based on nuclear (ITS) and chloroplast (*trnL-trnF*) sequence data. This result indicated the possibility that the Asian *Gerbera* may belong to the genus *Uechtrizia*. However, the phylogenetic position and the taxonomic identity of the Asian *Gerbera* need to be tested with an expanded taxon sampling by adding more Asian and African species of *Gerbera* and *Uechtrizia* before any taxonomic decisions can be made.

In this study, the phylogenetic position of the Asian *Gerbera* was tested by expanding the taxon sampling of the Asian and African *Gerbera* and the *Uechtrizia* species and using both molecular (two nuclear markers: ITS and ETS and three chloroplast markers: *trnL-trnF*, *trnL-rpl32* and *trnC-petN*) and morphological data (leaf adaxial surface, pollen, scape and floral morphology).

Materials and methods

A total of 32 species from eight genera of the *Gerbera*-complex and *Adenocaulon chilense* (outgroup) were sampled for this study (Tables 1, 2). The morphological data were taken from specimens at the United States National Herbarium (US) and included characters of the leaf epidermis, pollen, flowers and scapes.

Adaxial leaf epidermal and pollen morphology. A small area of the leaf lamina (about 0.5–1.0 cm²) was placed with the adaxial side exposed, on carbon tape over stubs for the scanning electron microscopy (SEM). For the pollen analysis, samples were dehydrated and were then placed on aluminium stubs using double-sided adhesive tape following Wen and Nowicke (1999). The stubs bearing the leaf sample and pollen were treated with gold-palladium to 16.6 µm thickness and were examined under a Philips XL-30 scanning electron microscope at the SEM Lab of the National Museum of Natural History (NMNH), Smithsonian Institution. The 22 samples were subsequently observed and photographed under the SEM using the proprietary software associated with the Philips SEM. Images of at least 15 different areas of the adaxial leaf surface were captured for each sample, as well as 20 pollen grains. The polar and equatorial axes of pollens were measured by ImageJ 1.8.0.

DNA extraction, amplification and sequencing. The DNA molecular work was undertaken in the Laboratory of Analytical Biology (LAB) of NMNH. DNA from 16 samples (15 species) was extracted through AutoGen (AutoGen Inc., Holliston, Massachusetts, USA) or the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Leaf tissue samples, along with 1.0 and 2.3 mm diameter beads, were dipped in liquid nitrogen then immediately shaken for 60 seconds at 1800 rpm by TissueLyser. About 500 µl of the CTAB extraction buffer was added to the tubes, vortexed and incubated overnight (500 rpm at 55 °C). Then 300 µl of the supernatant was transferred to an AutoGen plate. The AutoGen was run according to the manufacturer's default settings.

Five markers including two nuclear ribosomal ITS and ETS and three chloroplast *trnL-trnF*, *trnL-rpl32* and *trnC-petN* intergenic spacers were amplified. The ITS primers were designed by Downie and Katz-Downie (1996) and White et al. (1990), ETS primers by Baldwin and Markos (1998); *trnL-trnF* primers by Taberlet et al. (1991), *trnL-rpl32* spacer primers by Timme et al. (2007) and *trnC-petN* spacer primers by Lee and Wen (2004) (Table 3). The PCR reaction mixture had a total of 25 µl volume, comprising 14.05 µl nuclease free water, 2.5 µl 10x buffer, 2 µl dNTPs, 1.25 µl MgCl₂, 1 µl of both forward and reverse primers, 0.5 µl BSA, 0.2 µl Taq DNA polymerase and 2.5 µl of template DNA. The PCR reactions were performed in a Veriti PCR Thermal Cycler. The amplification protocols for all markers are summarised in Table 3. The amplified products were purified with ExoSapIT enzyme with activation at 37 °C and deactivation at 95 °C. 4 µl of the purified product and same primers (1 µl, 1 µM) were cycle-sequenced in a mixture containing 0.8 µl Big Dye (Applied Biosystems, Foster City, California, USA) and 2.0 µl 5x Big Dye buffer and 4.2 µl water.

Table 1. Voucher information and morphological characters of *Gerbera* and related species.

| Species | Section | Locality | Voucher information | Adaxial leaf stomata | Bracts on scape | Inner rays | Pollens | |
|--|----------------------|--------------|-------------------------------------|----------------------|-----------------|------------|-----------------|-----------|
| | | | | | | | Polar axis (µm) | P/E ratio |
| <i>Gerbera viridifolia</i> (DC.) Sch.Bip. | <i>Lasiopus</i> | Kenya | <i>T.H. Trinder-Smith s.n.</i> (US) | + | – | + | 44.12 | 1.21 |
| <i>G. jamesonii</i> Adlam | <i>Lasiopus</i> | Cultivar | <i>T. Derby s.n.</i> (US) | + | – | + | 45.77 | 1.29 |
| <i>G. aurantiaca</i> Sch. Bip. | <i>Lasiopus</i> | South Africa | <i>Bayliss 2505</i> (US) | + | – | + | 43.48 | 1.20 |
| <i>G. ambigua</i> Sch. Bip. | <i>Lasiopus</i> | South Africa | <i>M. Koekemoer 2097</i> (US) | + | – | + | 44.98 | 1.38 |
| <i>G. piloselloides</i> Cass. | <i>Piloselloides</i> | Swaziland | <i>M. Koekemoer 2590</i> (US) | + | – | + | 42.09 | 1.28 |
| <i>G. cordata</i> Less. | <i>Piloselloides</i> | Madagascar | <i>T.B. Croat 29083</i> (MO) | + | – | + | 43.19 | 1.27 |
| <i>G. perrieri</i> Humbert | <i>Pseudoseris</i> | Madagascar | <i>L. Gautier 3110</i> (MO) | + | – | + | 44.04 | 1.29 |
| <i>G. diversifolia</i> Humbert | <i>Pseudoseris</i> | Madagascar | <i>B. Lewis 1201</i> (MO) | + | – | + | 45.31 | 1.20 |
| <i>G. crocea</i> Kuntze | <i>Gerbera</i> | South Africa | <i>M. Koekemoer 2029</i> (US) | + | + | – | 53.83 | 1.39 |
| <i>G. linnaei</i> Cass. | <i>Gerbera</i> | South Africa | <i>E. Werdermann 749</i> (US) | + | + | – | 47.01 | 1.25 |
| <i>G. tomentosa</i> DC. | <i>Gerbera</i> | South Africa | <i>P. Bond 745</i> (US) | + | + | – | 50.43 | 1.26 |
| <i>G. wrightii</i> Harv. | <i>Gerbera</i> | South Africa | <i>P. Goldblatt 5287</i> (US) | + | + | – | N | N |
| <i>G. serrata</i> Druce | <i>Gerbera</i> | South Africa | <i>M. Koekemoer 2001</i> (PRE) | + | + | – | N | N |
| <i>G. gossypina</i> Beauverd | <i>Isanthus</i> | India | <i>W.N. Koelz 4828</i> (US) | – | + | – | 50.05 | 1.40 |
| <i>G. maxima</i> Beauverd | <i>Isanthus</i> | India | <i>D.H. Nicolson 2755</i> (US) | – | + | – | 50.41 | 1.26 |
| <i>G. delavayi</i> Franch. | <i>Isanthus</i> | China | <i>X. Xu 1102</i> (KMUST) | – | + | – | 51.90 | 1.27 |
| <i>G. nivea</i> Sch.Bip. | <i>Isanthus</i> | China | <i>J.F. Rock 6430</i> (US) | – | + | – | 50.30 | 1.39 |
| <i>G. raphanifolia</i> Franch. | <i>Isanthus</i> | China | <i>J.F. Rock 10504</i> (US) | – | + | – | 51.74 | 1.28 |
| <i>G. henryi</i> Dunn | <i>Isanthus</i> | China | <i>W.B. Hemsley 1903</i> (US) | – | + | – | 51.91 | 1.33 |
| <i>Uechritzia armena</i> Freyn | N | Turkey | <i>A. Kaya 1835</i> (EU) | N | + | – | N | N |
| <i>U. lacei</i> (G.Watt) C.Jeffrey | N | India | <i>W. Koelz 8710</i> (NA) | – | + | – | 50.86 | 1.36 |
| <i>U. kokanica</i> (Regel et Schmalh.) Pobed. | N | Tajikistan | <i>FL. Zaprjagaev 4682</i> (US) | – | + | – | 55.80 | 1.31 |
| <i>Leibnitzia anandria</i> (L.) Nakai | N | China | <i>I. Thomas 8183</i> (US) | + | + | – | 34.45 | 1.10 |
| <i>L. nepalensis</i> (Kunze) Kitam. | N | China | <i>J. Wen 542</i> (US) | + | + | – | 32.16 | 1.20 |
| <i>L. occimadrensis</i> G.L.Nesom | N | Mexico | <i>H.S. Gentry 7189</i> (US) | + | + | – | 37.33 | 1.16 |
| <i>Amblyserma scapigera</i> Benth. | N | Australia | <i>A. Morrison s.n.</i> (US) | + | + | – | 51.60 | 1.17 |
| <i>A. spathulata</i> (A.Cunn. ex DC.) D.J.N.Hind | N | Australia | <i>R.A. Davis 8267</i> (US) | + | + | – | 55.10 | 1.23 |

Notes: + designates those mentioned present; – designates those mentioned absent; N represents data not available.

Table 2. Voucher information and GenBank accessions of *Gerbera* and the related species.

| Species | Locality | Voucher information | ITS | ETS | trnL-trmF | trnL-rpl32 | trnC-petN |
|--|--------------|---|-----------|-----------|-----------|------------|-----------|
| <i>Gerbera viridifolia</i> (DC.) Sch.Bip. | South Africa | <i>T.H. Trinder-Smith s.n.</i> (US) | MG661696* | MG661588* | MG661639* | MG661670* | MG661628* |
| <i>G. crocea</i> Kunze | South Africa | <i>M. Koekemoer 2029</i> (US) | MG661709* | MG661606* | MG661645* | MG661683* | MG661618* |
| <i>G. delavayi</i> Franch. | China | <i>X. Xu 1102</i> (KMUST) | MG661708* | MG661605* | MG661659* | MG661682* | MG661619* |
| <i>G. henryi</i> Dunn | China | <i>X. Xu 1103</i> (KMUST) | MG661706* | MG661602* | MG661655* | MG661681* | MG661621* |
| <i>G. nivea</i> Sch.Bip. | China | <i>Y.S. Chen 2674</i> (PE) | MG661703* | MG661598* | MG661648* | MG661678* | N |
| <i>G. amantiaea</i> Sch.Bip. | South Africa | <i>Boylis 2505</i> (US) | MG661711* | MG661610* | MG661637* | MG661687* | MG661615* |
| <i>G. ambigua</i> Sch.Bip. | South Africa | <i>M. Koekemoer 2097</i> (US) | MG661712* | MG661611* | MG661636* | MG661688* | N |
| <i>G. jamesonii</i> Adlam | Cultivar | <i>T. Derby s.n.</i> (US) | MG661704* | MG661599* | MG661638* | MG661679* | MG661624* |
| <i>G. cordata</i> Less. | South Africa | <i>J. Wen 10067</i> (US) | N | MG661608* | MG661661* | MG661685* | MG661617* |
| <i>G. piloselloides</i> Cass. | Swaziland | <i>M. Koekemoer 1972</i> (US) | MG661701* | MG661592* | MG661650* | MG661675* | MG661625* |
| <i>G. wrightii</i> Harv. | South Africa | <i>P. Goldblatt 5287</i> (US) | MG661695* | MG661587* | MG661642* | N | N |
| <i>G. serotata</i> Druce | South Africa | <i>M. Koekemoer 2001</i> (PRE) | MG661697* | MG661590* | MG661656* | MG661671* | N |
| <i>G. diversifolia</i> Humbert | Madagascar | <i>B. Lewis 1201</i> (MO) | N | MG661604* | MG661640* | N | N |
| <i>G. raphanifolia</i> Franch. | China | <i>Rock JF 10504</i> (US) | N | N | MG661658* | N | MG661626* |
| <i>G. gossypina</i> Beauverd | India | <i>W.N. Koelz 4824</i> (US) | MG661707* | MG661603* | MG661646* | N | MG661620* |
| <i>G. maxima</i> Beauverd | India | <i>F. Kingdon-Ward 18199</i> (NY) | KX349402 | N | KX349371 | N | N |
| <i>Uechtrizia laei</i> (G. Wirt) C. Jeffrey | India | <i>W. Koelz 8710</i> (NA) | N | N | MG661644* | N | N |
| <i>U. kokanica</i> (Regel & Schmalh.) Pobed. | Tajikistan | <i>F.L. Zapryagaev 4682</i> (US) | N | MG661580* | MG661643* | N | MG661635* |
| <i>U. kokanica</i> (Regel & Schmalh.) Pobed. | Tajikistan | <i>Zapryagaev s.n.</i> (NY) | KX349400 | N | KX349401 | N | N |
| <i>Amblyserma scopigena</i> Benth. | Australia | <i>A. Morrison s.n.</i> (US) | MG661713* | MG661612* | N | MG661689* | N |
| <i>A. spathulata</i> (A.Cunn. ex DC.) D.J.N.Hind | Australia | <i>Cranfield 16197</i> (CANB) | JX564767 | N | KF989620 | N | N |
| <i>Adenocaulon chilense</i> Less. | Chile | <i>G.L. Sobel 2558</i> (US) | MG661714* | N | N | MG661690* | N |
| <i>Chaptalia pringlei</i> Greene | Mexico | <i>G. Nesom 4405</i> (US) | GU126773 | N | N | N | N |
| <i>C. hircinioides</i> (Kunth) X.-D.Xu & W.Zheng | Ecuador | <i>P.M. Peterson 9287</i> (US) | MG661705* | MG661601* | MG661657* | MG661680* | N |
| <i>Tricholine reptans</i> (Wedd.) Hieron | Argentina | <i>E. Pasini & F. Torcheisen 1025</i> (ICN) | KX349398 | N | KX349399 | KX349410 | N |
| <i>Leibnitzia anandria</i> (L.) Nakai | China | <i>I. Thomas 8183</i> (US) | MG661694* | MG661585* | MG661662* | MG661668* | MG661629* |
| <i>L. anandria</i> (L.) Nakai | Japan | <i>Z.Y. Wu 8985</i> (KUN) | MG661692* | MG661584* | MG661664* | MG661667* | MG661631* |
| <i>L. occimadrensis</i> G.L.Nesom | Mexico | <i>H.S. Gentry 7189</i> (US) | GU126784 | MG661583* | N | MG661666* | MG661632* |
| <i>L. nepalensis</i> (Kunze) Kitam. | China | <i>J. Wen 542</i> (US) | KX349373 | MG661582* | KX349374 | GU126759 | MG661633* |
| <i>L. lyrata</i> (Sch.Bip.) G.L.Nesom | USA | <i>G. Nesom 24778</i> (ARIZ) | GU126779 | N | N | GU126757 | N |

Notes: * designates the new sequences from this study; N represents data not available.

Table 3. Amplification protocols for all markers.

| Marker | Primers and sequences 5'–3' | PCR protocol: initial pre-heating; DNA denaturation; primer annealing; DNA extension; final extension |
|-------------------|--|---|
| ITS | ITS5A: GGAAGGAGAAGTCGTAACAAGG ITS4: TCCTCCGCTTATTGATATGC | 95 °C 1 min; 54 °C 1 min; 72 °C 1 min; 72 °C 10 min; 35 cycles |
| ETS | 18s-ETS: ACTTACACATGCATGGCTTAATCT ETS-HeI-1: GCTCTTTGCTTGCGCAACAACCT | 94 °C 0:30 min; 60 °C 0:40 min; 72 °C 1:20 min; 72 °C 5 min; 30 cycles |
| <i>trnL-trnF</i> | <i>trnL</i> -Fc: CGAAATCGGTAGACGCTACG <i>trnL</i> -Ff: ATTTGAACTGGTGACACGAG | 94 °C 1 min; 53 °C 1 min; 72 °C 2 min; 72 °C 10 min; 35 cycles |
| <i>trnL-rpl32</i> | <i>trnL</i> : TACCGATTTCACCATAGCGG <i>rpl32</i> : AGGAAAGGATATTGGGCGG | 95 °C 3 min; 51 °C 40 s; 72 °C 1:20 min; 72 °C 5 min; 35 cycles |
| <i>trnC-petN</i> | <i>trnC</i> : CCAGTCAAATCTGGGTGTC <i>petN</i> : GGATATAGTAAGTCTTGCTTGGG | 95 °C 3 min; 54 °C 45 s; 72 °C 1:20 min; 72 °C 8 min; 35 cycles |

The cycle sequencing programme was 30 cycles of 95 °C for 30 s, 50 °C for 30 s and 60 °C for 4 min. The resultant product was sephadex-filtered and sequenced through an ABI 3730 automated sequencer (Applied Biosystems, Foster City, USA). Sequences were aligned by using MAFFT (Katoh and Standley 2013) in Geneious 10.0.9. (Bio-matters Ltd., Auckland, New Zealand) and checked manually. A total of 90 newly generated sequences from the 23 samples were deposited in GenBank (Table 2).

A total of 16 sequences of eight species were retrieved from NCBI for the related taxa within the tribe Mutisieae (Table 2). Phylogenetic relationships were inferred based on the concatenated ITS+ETS+*trnL-rpl32*+*trnL-trnF*+*trnC-petN* data with MrBayes v. 3.2.2 (Ronquist et al. 2012) by using the substitution model of GTR based on the best-fitting model determined by jModelTest 2.1.6 (Posada 2008), the chain length of 10,000,000, rate variation of gamma, gamma categories of 4, heated chains of 4, heated chain temp of 0.2, subsampling freq. of 200 and burn-in length of 100,000. Tracer v. 1.5 (Rambaut and Drummond 2009) was used to confirm that the effective sample size (ESS) for all relevant parameters was > 200. After discarding the trees as burn-in, a 50 % majority-rule consensus tree and posterior probabilities (PP) for node support were calculated using the remaining trees.

Results

Adaxial leaf epidermal morphology. The results of the SEM work (Table 1) showed that the six tested Asian *Gerbera* species have no stomates on the adaxial leaf surface (Figure 2A, B, C, D). This adaxial leaf morphological trait differs from that of the African *Gerbera* species: (1) Three East African *Gerbera* sections sampled [sect. *Lasiopus* (4 species), sect. *Piloselloides* (2 species) and sect. *Pseudoseris* (2 species)] have stomates and stiff, straight, upright trichomes on the adaxial surface. Figure 1 has representative images for each of the above sections: *G. ambigua* (Fig. 1A), *G. piloselloides* (Fig. 1B) and *G. perrieri* (Fig. 1C), respectively. (2) Members of the South African sect. *Gerbera* have stomates.

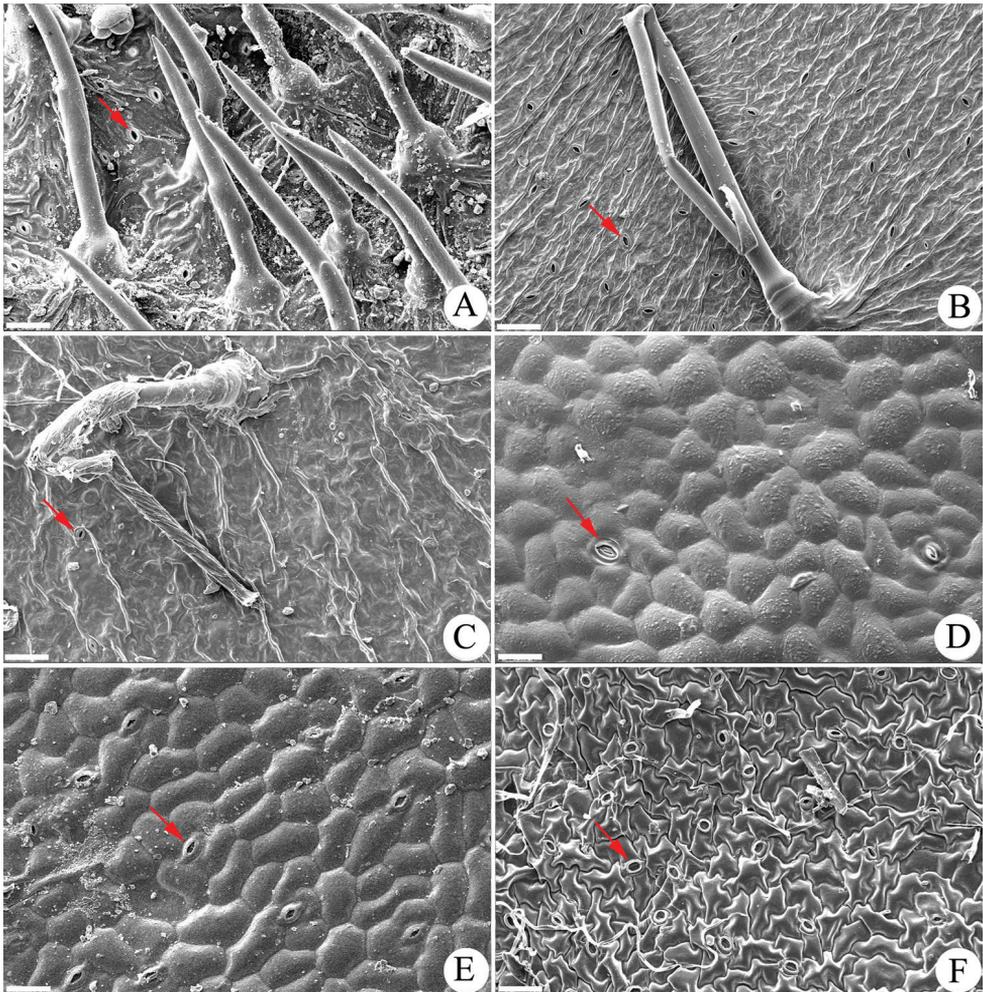


Figure 1. Adaxial leaf epidermal surface morphology of African *Gerbera* and Asian *Leibnitzia*. **A** *G. ambigua* (sect. *Lasiopus*) **B** *G. piloselloides* (sect. *Piloselloides*) **C** *G. perrieri* (sect. *Pseudoseris*) **D** *G. serrata* (sect. *Gerbera*) **E** *G. crocea* (sect. *Gerbera*) **F** *L. nepalensis*. Bar=50 μ m.

Five species were examined and the epidermal characters are represented by *G. serrata* (Fig. 1D) and *G. crocea* (Fig. 1E). Furthermore, the adaxial leaf morphological traits of the Asian *Gerbera* species also deviate from two Asian-American disjunct *Leibnitzia* species, which have stomates on the adaxial leaf epidermal, as represented by *L. nepalensis* (Fig. 1F). Nevertheless, the Asian *Gerbera* samples share similar adaxial leaf epidermal characters of lacking stomates with the two examined *Uechtritzia* species, *U. kokanica* (Fig. 2E) and *U. lacei* (Fig. 2F). Based on the adaxial leaf epidermal morphology, the Asian *Gerbera* is most closely related to *Uechtritzia* rather than to the African *Gerbera*.

Pollen morphology. The pollen grains of the examined species of the *Gerbera*-complex are very similar to one another, differing only in the size of the grains as well

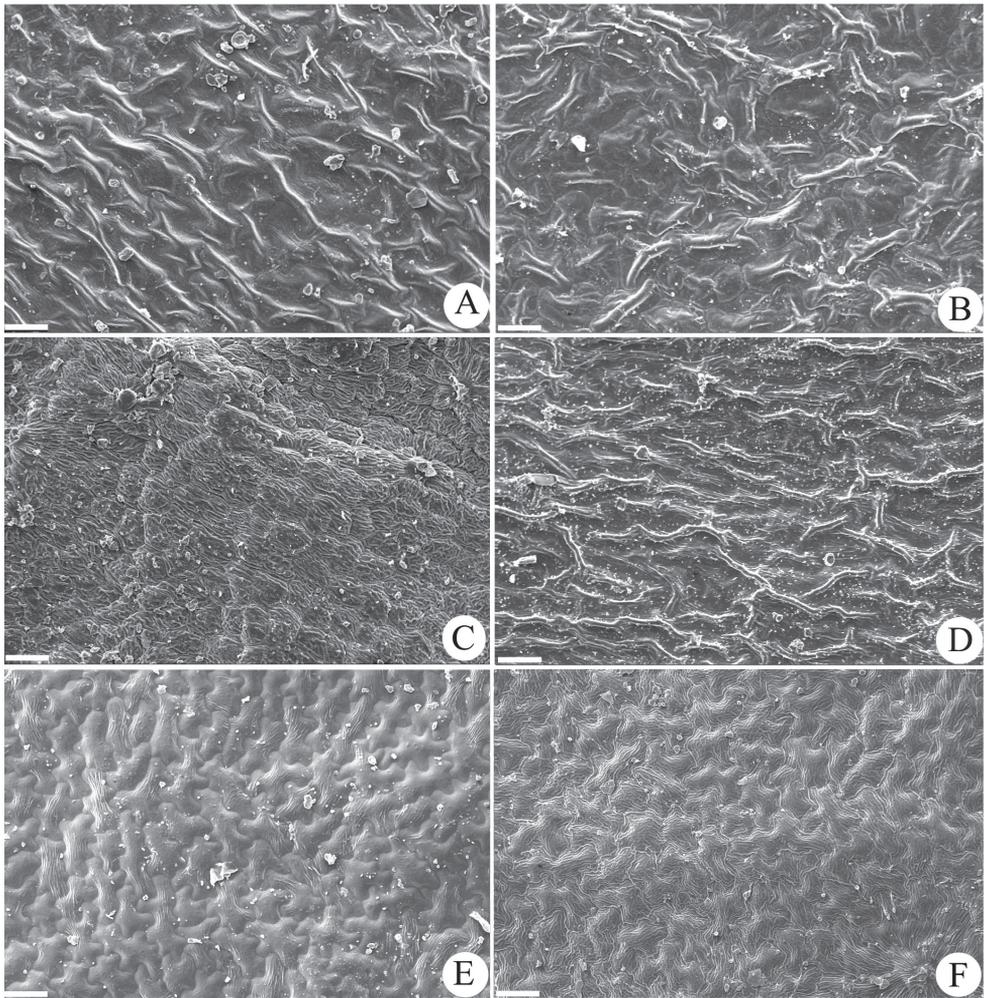


Figure 2. Adaxial leaf epidermal surface morphology of Asian *Gerbera* and *Uechtrizia*. **A** *G. maxima* **B** *G. delavayi* **C** *G. gossypina* **D** *G. nivea* **E** *U. kokanica* **F** *U. lacei*. Bar=50 μm .

as the granules on the surfaces (Figs 3 and 4). They are tricolporate, have a granule exine and are prolate and subprolate in shape. The ratios of the polar axis and equatorial axis (P/E) are given in Table 1. For *Gerbera* and *Uechtrizia*, the P/E ratios are between 1.2–1.4. The average polar axis of the Asian *Gerbera* and *Uechtrizia* pollen grains is 50.05–55.80 μm . For the African *Gerbera*, however, the average polar axis of pollen grains is 42.09–45.77 μm in sects. *Lasiopus*, *Piloselloides* and *Pseudoseris* and 47.01–53.83 μm in sect. *Gerbera*. The P/E ratio of the pollen grains of the Asian *Gerbera* and *Uechtrizia* (Table 1) differs from that of the East Asian–North American *Leibnitzia* and the Australian *Amblyserma*, which fall between 1.10–1.20 (Fig. 5). Furthermore, the average polar axis of the Asian *Gerbera* and *Uechtrizia* pollen grains is higher than that of *Leibnitzia* species, which has the range of 32.16–37.33 μm .

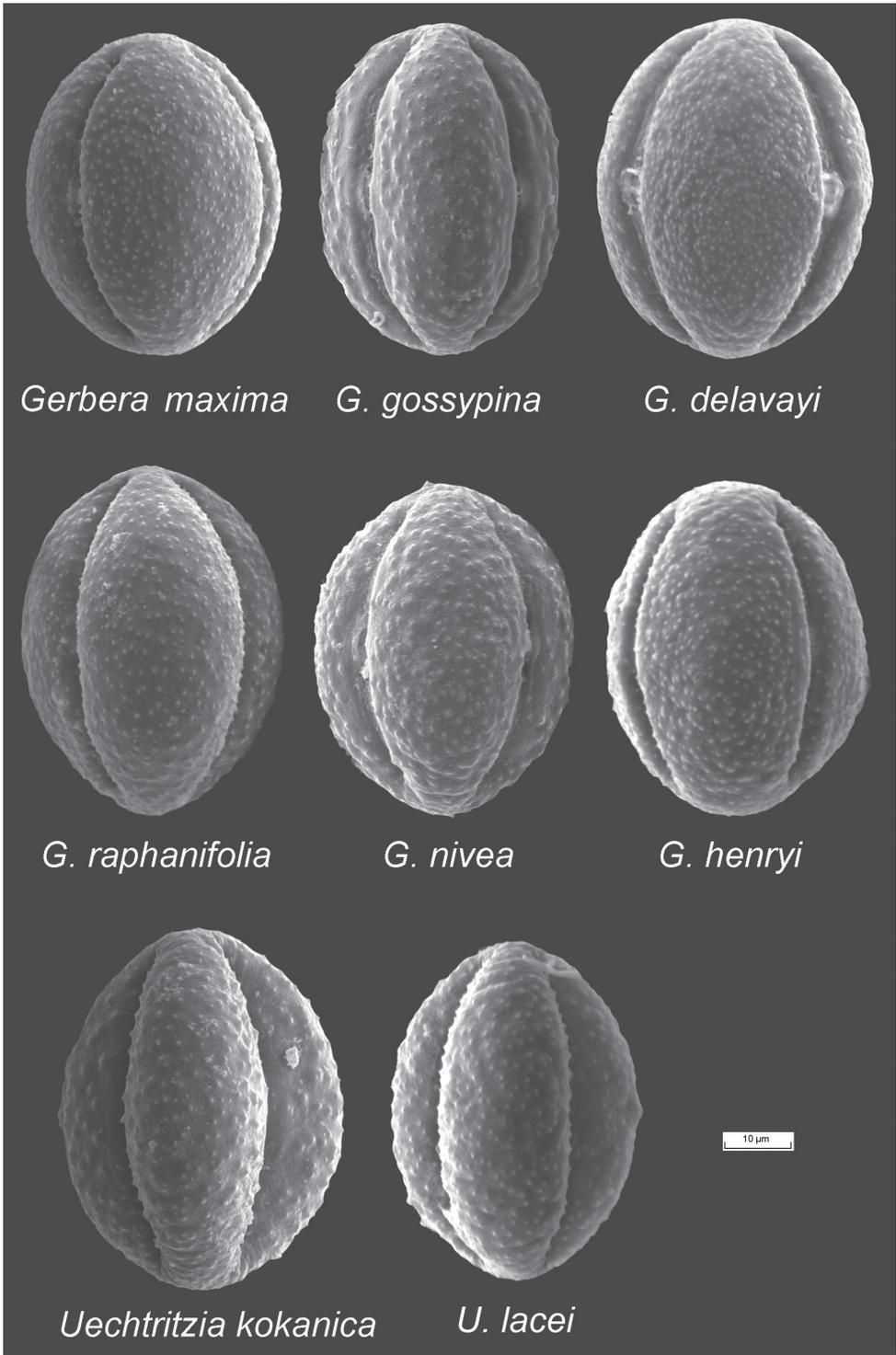


Figure 3. Pollen morphology of Asian *Gerbera* and *Uechtritzia*.

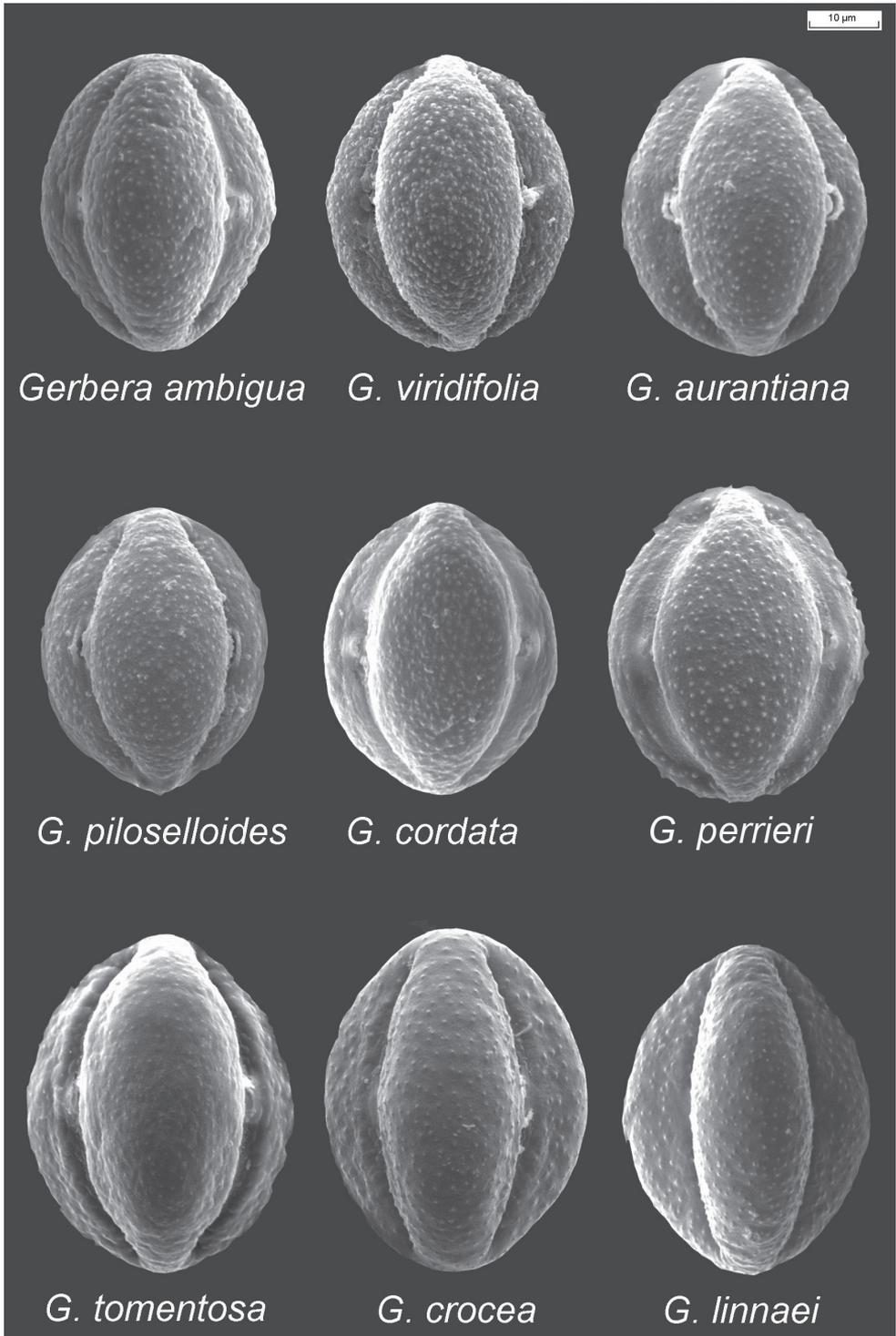


Figure 4. Pollen morphology of African *Gerbera* species.

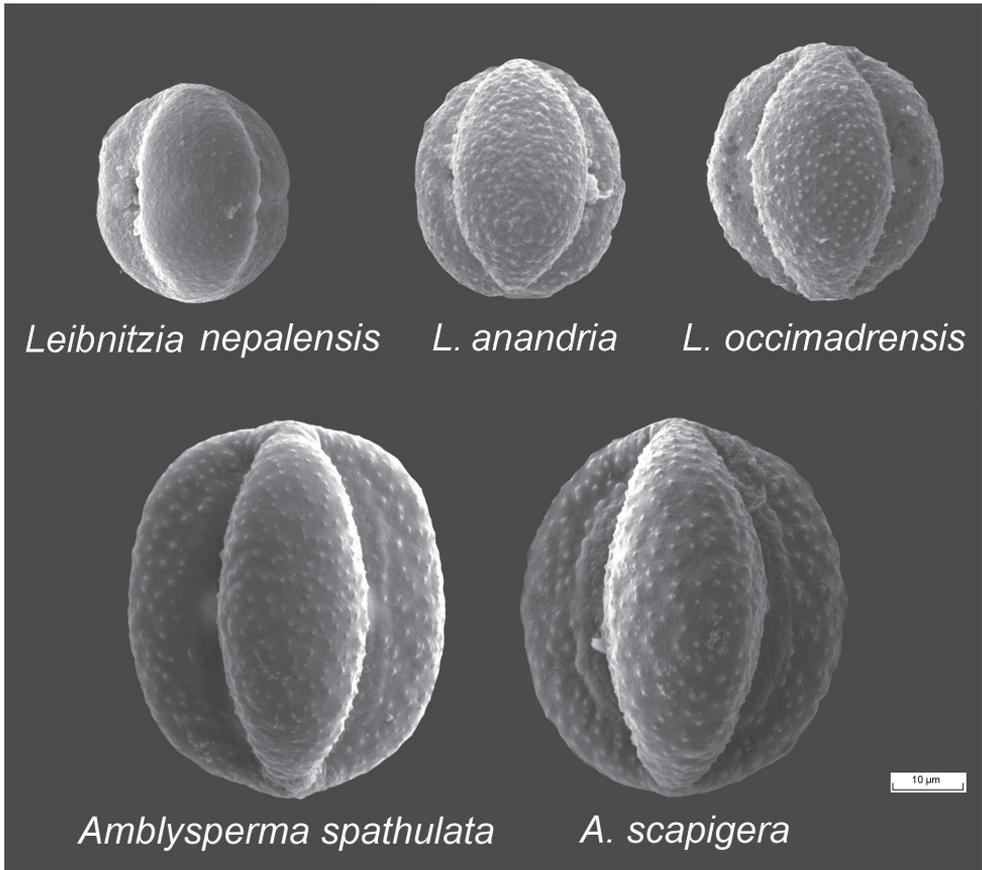


Figure 5. Pollen morphology of *Leibnitzia* and *Amblysperma* species.

Phylogenetic analysis. The Bayesian analysis of the combined nuclear markers and three plastid genes showed six clades of the sampled species of the *Gerbera*-complex, all showing a strong geographic signal (Fig. 6): (1) the Asian *Gerbera* and the *Uechtritzia* species, (2) the East Asian and North American *Leibnitzia* species, (3) the New World genus *Chaptalia*, (4) the African *Gerbera* species, (5) the Australian genus *Amblysperma* and (6) the South American genus *Trichocline*. The three samples of *Uechtritzia* (two species of *U. kokanica* and *U. lacei*) were clearly nested within the Asian *Gerbera* clade (Fig. 6).

Discussion

Based on this study, the Asian *Gerbera* and the *Uechtritzia* species share several morphological characters, including bracteate scapes, absence of inner ray florets, no stomates on the adaxial leaf surface and similar pollen size and shape (Table 1). Hansen (1990) also commented that the Asian *Gerbera* (i.e. sect. *Isanthus*) is morphologically

lar receptacles that are fimbriate-ciliate; margins of involucre bracts (at least the upper part) often with reddish hairs; achenes that are slightly or indistinctly tapering with hairs that are long-villose, ca. 1 mm long (Katinas 2004) and sericeous (Hansen 1988). *Gerbera* sect. *Isanthus* has heads campanulate; a receptacle that is alveolate and naked; the margins of involucre bracts are without reddish hairs; the achenes are tapering and pilose glabrous, with hairs that are shorter, tapered and not sericeous (Hansen 1988). The heads of *Uechtritzia* were reported as hemispherical, in contrast to the heads of the Asian *Gerbera* sect. *Isanthus* which are campanulate. However, the species of *U. armena* (the type species of the genus) from Turkey showed the heads as campanulate in the fresh plants (Dogan et al. 2016), which is the same as the Asian *Gerbera* sect. *Isanthus* (e.g. *G. delavayi*; Zheng et al. 2017).

Some previous workers argued that the species of Asian *Gerbera* (sect. *Isanthus*) should be treated as an entity, separate from African *Gerbera* (Candolle 1838, Jeffrey 1967, Pasini et al. 2016). The results presented here show that the Asian *Gerbera* sect. *Isanthus* differs from the African *Gerbera* sect. *Lasiopus*, sect. *Piloselloides* and sect. *Pseudoseris* in the ebracteate scapes, presence of inner ray florets, stomates on the adaxial leaf surface and smaller pollen size of the African *Gerbera* compared with the Asian *Gerbera*. Although the Asian *Gerbera* sect. *Isanthus* shares the traits of bracteate scapes, absence of inner ray florets and similar pollen size with *Gerbera* sect. *Gerbera*, the Asian species have no stomates on the adaxial leaf surface. Hansen (1990) stated that the Asian *Gerbera* sect. *Isanthus* shows style-arms laterally dilated and truncate achenes; in contrast, the African *Gerbera* sect. *Gerbera* has the style-arm slender and achenes tapering or beaked. Additionally, most species of the African *Gerbera* sect. *Gerbera* grow in open areas, have leathery leaves and flower only in the spring and summer (Manning et al. 2016), whereas the Asian species of *Gerbera* sect. *Isanthus* often grow in forest habitats, have herbaceous leaves and flower in the winter (Gao et al. 2011).

The two *Uechtritzia* species sampled in the molecular phylogeny (Fig. 6) were nested within the Asian *Gerbera* species based on two nuclear markers (ITS and ETS) and three chloroplast markers (*trnL-trnF*, *trnL-rpl32* and *trnC-petN*). This result, based on the authors' expanded taxon and character sampling, is consistent with the findings of Pasini et al. (2016). This study included two of the three species of *Uechtritzia* (Hansen, 1988) and six of the nine Asian *Gerbera* sect. *Isanthus* taxa (Gao et al. 2011). The phylogenetic analysis clearly supports the species of *Uechtritzia* as nested within the Asian *Gerbera* and this clade is the sister group of *Leibnitzia* with strong support (PP=0.99) (Fig. 6).

Leibnitzia is a genus containing about six species with a disjunct distribution: four species in Asia (Gao et al. 2011) and two species in Mexico (Baird et al. 2010). It shows the same characters of bracteate scapes and no inner ray florets as the Asian *Gerbera* + *Uechtritzia*. It differs from the latter by the presence of stomates on the adaxial leaf surface and smaller pollen size (polar axis of 32.16–37.33 μm) compared with Asian *Gerbera* + *Uechtritzia* (polar axis of 50.05–55.80 μm). Furthermore, *Leibnitzia* has two generations of heads (a vernal generation with chasmogamous capitula and an aestival generation with cleistogamous capitula), subseriate involucre bracts, slender style-arm, anthers of the ray flowers reduced to threads or wanting

and achenes that are tapering or beaked. The Asian *Gerbera* + *Uechtrizia*, on the other hand, have one generation of heads, imbricate involucre bracts, laterally dilated style-arms, a fully developed apex (and base) on the anthers in the ray flowers and truncate achenes (Hansen, 1990).

Based on the molecular phylogenetic results, the Asian *Gerbera* species are closest to *Uechtrizia*, with the latter nested within the Asian *Gerbera* species. *Leibnitzia* shows significant morphological differences to the Asian *Gerbera* + *Uechtrizia*. The taxonomic identity of *Uechtrizia* and the Asian *Gerbera* is strongly supported by the morphology of inflorescences, scapes, capitula, pollen and the lack of stomates on the adaxial leaf surface. Therefore, the authors herein include the nine Asian *Gerbera* species and the three *Uechtrizia* species in *Oreoseris* DC. which is the earliest available name for the expanded Eurasian genus.

Taxonomic synopsis with nomenclatural changes

In trying to determine the correct genus name for the Eurasian clade, it is necessary to investigate three relevant generic names. *Gerbera* L. was described in 1758; *Arnica gerbera* L. is the basionym of the African species *G. linnaei* Cass., the conserved type of *Gerbera* L. (lectotype designated by Hansen 1985a). *Gerbera* was named after Traugott Gerber, a German naturalist who died in 1743. *Oreoseris* DC. was described in 1838 and its type species is *O. nivea* DC. which was designated by Hansen in 1988. While de Candolle (1838) did not say why he named the genus, *Oreo* is from the Greek *oreos* for mountain and, in his description, de Candolle says that the genus is a “... perennial herb from the mountains of eastern India (translated).” *Uechtrizia* Freyn was described in 1892; the type species is *U. armena* Freyn (lectotype designated by Pobedimova, 1963). The genus was named in honour of Rudolf Karl Friedrich von Uechtriz (1838–1886), a botanist from Wrocław, Poland (ex-Breslau) (Freyn 1892).

When *Oreoseris nivea* DC. was absorbed into *Gerbera*, the priority was given to *Gerbera* because the latter was the older generic name and, as long as this species stayed in *Gerbera*, the name *Oreoseris* was not available. *Uechtrizia* was described later in 1892; and, as long as *O. nivea* remained in *Gerbera*, then *Oreoseris* continued to be unavailable.

However, as soon as *Gerbera nivea* from Asia was removed from *Gerbera* and a separate genus was formed from the Asian species of *Gerbera* + *Uechtrizia*, then the name *Oreoseris* became available and it is the oldest available name. Hence, these species have been transferred into *Oreoseris*.

***Oreoseris* DC., Prodr. 7(1): 17. 1838.**

Onoseris Willd. sect. *Isanthus* Less., *Linnaea* 5: 338. 1830. *Onoseris* Willd. subgen. *Isanthus* (Less.) Less., *Syn. Comp.*: 119. 1832. *Gerbera* L. sect. *Isanthus* (Less.) C. Jeffrey, *Kew Bull.* 21: 213. 1967.

Gerbera L. sect. *Oreoseris* (DC.) Sch.Bip., Flora 27: 780. 1844.

Uechtritzia Freyn, Oesterr. Bot. Z. 42(7): 240. 1892. *Gerbera* sect. *Uechtritzia* (Freyn) Beauverd, Bull. Soc. Bot. Genève Ser. 2, 2: 43. 1910.

Type species. *Oreoseris nivea* DC., designated by Hansen (1988).

Oreoseris has the following 12 species from Eurasia.

1. *Oreoseris armena* (Freyn et Sint.) V.A.Funk & J.Wen, comb. nov.

urn:lsid:ipni.org:names:77176439-1

Uechtritzia armena Freyn et Sint., Oesterr. Bot. Z. 42(7): 241. 1892. *Gerbera armena* Beauverd, Bull. Soc. Bot. Genève, ser. 2, 2: 43. 1910.

Distribution. Armenia and Turkey.

2. *Oreoseris delavayi* (Franch.) X.D.Xu & W.Zheng, comb. nov.

urn:lsid:ipni.org:names:77176440-1

Gerbera delavayi Franch., J. Bot. (Morot). 2: 68. 1888.

Distribution. China (Guizhou, Sichuan, Yunnan) and N Vietnam.

3. *Oreoseris gossypina* (Royle) X.D.Xu & V.A.Funk, comb. nov.

urn:lsid:ipni.org:names:77176441-1

Chaptalia gossypina Royle, Ill Bot. Himal. 251. T. 59. F. 2. 1835. *Gerbera gossypina* (Royle) Beauverd, Bull. Soc. Bot. Genève Ser. 2, 2: 40. 1910.

Oreoseris lanuginosa DC., Prodr. 7(1): 17. 1838. *Gerbera lanuginosa* (DC.) Sch.Bip., Flora 27: 780. 1844.

Distribution. Karakoram, N and C Himalaya.

4. *Oreoseris henryi* (Dunn) W.Zheng & J.Wen, comb. nov.

urn:lsid:ipni.org:names:77176442-1

Gerbera henryi Dunn, J. Linn. Soc., Bot. 35: 511. 1903. *Gerbera delavayi* var. *henryi* (Dunn) C.Y.Wu et H.Peng, Acta Bot. Yunnan. 24: 143. 2002.

Distribution. China (Yunnan).

5. *Oreoseris kokanica* (Regel et Schmalh.) J.Wen & W.Zheng, comb. nov.

urn:lsid:ipni.org:names:77176443-1

Gerbera kokanica Regel et Schmalh., Descr. Pl. Nov. Rar. Fedtsch. 53. 1882 (published as Izv. Imp. Obsc. Ljubit. Estesv. Moskovsk. Univ. 34(2): 53. 1882). *Uechtrizia kokanica* (Regel et Schmalh.) Pobed., Fl. URSS 28: 597. 1963.

Distribution. Pamir-Altai and Tian-Shan regions of C Asia, south to Afghanistan and Kashmir.

6. *Oreoseris lacei* (G.Watt) V.A.Funk & W.Zheng, comb. nov.

urn:lsid:ipni.org:names:77176444-1

Gerbera lacei G.Watt Bull. Misc. Inform. Kew 1911(6): 272. 1911. *Uechtrizia lacei* (G.Watt) C.Jeffrey, Kew Bull. 21(2): 213. 1967.

Distribution. N India (Himachal Pradesh), S Jammu and Kashmir (Nachar, Baspa, E and NE of Simla, Chamba and Kisthwar).

7. *Oreoseris latiligulata* (Y.C.Tseng) W.Zheng & J.Wen, comb. nov.

urn:lsid:ipni.org:names:77176445-1

Gerbera latiligulata Y.C.Tseng, Acta Bot. Austro-Sin. 3: 11. 1986.

Distribution. China (in Qiaojia county of Yunnan).

8. *Oreoseris maxima* (D.Don) X.D.Xu & W.Zheng, comb. nov.

urn:lsid:ipni.org:names:77176446-1

Chaptalia maxima D.Don, Prodr. Fl. Nepal. 166. 1825. *Gerbera maxima* (D.Don) Beauverd, Bull. Soc. Bot. Genève Ser. 2, 2: 44. 1910.

Distribution. China (Xizang), Bhutan, India, Nepal, Pakistan and Thailand.

9. *Oreoseris nivea* DC., Prodr. 7: 18. 1838.

Gerbera nivea (DC.) Sch.Bip., Flora 27: 780. 1844.

Distribution. China (W Sichuan, S Xizang, NW Yunnan), Bhutan, India and Nepal.

10. *Oreoseris raphanifolia* (Franch.) V.A.Funk & J.Wen, comb. nov.

urn:lsid:ipni.org:names:77176447-1

Gerbera raphanifolia Franch., J. Bot. (Morot). 2: 67. 1888.

Distribution. China (NW Yunnan).

11. *Oreoseris rupicola* (T.G.Gao & D.J.N.Hind) X.D.Xu & V.A.Funk, comb. nov.

urn:lsid:ipni.org:names:77176448-1

Gerbera rupicola T.G.Gao et D.J.N.Hind, Fl. China 20–21: 14. 2011.

Gerbera macrocephala Y.C.Tseng, Acta Bot. Austro Sin. 3: 12. 1986, not *Gerbera macrocephala* Less., Linnaea 5: 295. 1830.

Distribution. China (NW Yunnan).

12. *Oreoseris tanantii* (Franch.) W.Zheng & X.D.Xu, comb. nov.

urn:lsid:ipni.org:names:77176449-1

Gerbera tanantii Franch., J. Bot. (Morot). 7: 155. 1893.

Distribution. China (Yunnan).

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References

- Baird KE, Funk VA, Wen J, Weeks A (2010) Molecular phylogenetic analysis of *Leibnitzia* Cass. (Asteraceae: Mutisieae: *Gerbera*-complex): An Asian-North American disjunct genus. *Journal of Systematics and Evolution* 48: 161–174. <https://doi.org/10.1111/j.1759-6831.2010.00077.x>
- Baldwin BG, Markos S (1998) Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463. <https://doi.org/10.1006/mpev.1998.0545>
- Candolle AP de (1838) *Prodromus systematis naturalis regni vegetabilis*, vol. 7(1). Treuttel & Würtz, Paris. <https://doi.org/10.5962/bhl.title.286>
- Dogan YN, Kandemir A, Osmalı E (2016) Genetic diversity and variability among populations and ecological characteristics of the *Uechtrizia armena* Freyn (Asteraceae) endemic to Turkey. *Research & Reviews: Research Journal of Biology* 4: 20–27.
- Downie SR, Katz-Downie DS (1996) A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 83: 234–251. <https://doi.org/10.1002/j.1537-2197.1996.tb12701.x>
- Freyn J (1892) *Plantae novae Orientales*. *Österreichische Botanische Zeitschrift* 42: 235–242. <https://doi.org/10.1007/BF01791047>
- Funk VA, Pasini E, Bonifacino JM, Katinas L (2016) Home at last: The enigmatic genera *Eriachaenium* and *Adenocaulon* (Compositae, Mutisioideae, Mutisieae, Adenocaulinae). *PhytoKeys* 60: 1–19. <https://doi.org/10.3897/phytokeys.60.6795>
- Gao TG, Chen YS, Hind DJN, Freire SE (2011) Mutisieae. In: Wu ZY, Raven PH, Hong DY (Eds) *Flora of China*, vol 20–21 (Asteraceae). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis.
- Hansen HV (1985a) A taxonomic revision of the genus *Gerbera* (Compositae, Mutisieae) sections *Gerbera*, *Parva*, *Piloselloides* (in Africa) and *Lasiopus*. *Opera Botanica* 78: 5–36.
- Hansen HV (1985b) Notes on *Gerbera* sect. *Pseudoseris* (Compositae, Mutisieae). *Nordic Journal of Botany* 5: 451–453. <https://doi.org/10.1111/j.1756-1051.1985.tb01675.x>
- Hansen HV (1988) A taxonomic revision of the genera *Gerbera* sect. *Isanthus*, *Leibnitzia* (in Asia), and *Uechtrizia* (Compositae, Mutisieae). *Nordic Journal of Botany* 8: 61–76. <https://doi.org/10.1111/j.1756-1051.1988.tb01707.x>
- Hansen HV (1990) Phylogenetic studies in the *Gerbera*-complex (Compositae, tribe Mutisieae, subtribe Mutisiinae). *Nordic Journal of Botany* 9: 469–485. <https://doi.org/10.1111/j.1756-1051.1990.tb00537.x>
- Jeffrey C (1967) Notes on Compositae, III. The Cynareae in east tropical Africa. *Kew Bulletin* 22: 107–140. [Jstor.org/stable/4107829](https://www.jstor.org/stable/4107829)
- Johnson IM, Crouch NR, Edwards TJ (2014) *Gerbera sylvicola* (Asteraceae: Mutisieae), a new forest species from KwaZulu-Natal, South Africa. *Phytotaxa* 186: 229–235. <https://doi.org/10.11646/phytotaxa.186.4.7>
- Katinas L (2004) The *Gerbera*-complex (Asteraceae, Mutisieae): To split or not to split. *SIDA, Contributions to Botany*, 935–940. [Jstor.org/stable/41968349](https://www.jstor.org/stable/41968349)

- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Lee C, Wen J (2004) Phylogeny of *Panax* using chloroplast *trnC–trnD* intergenic region and the utility of *trnC–trnD* in interspecific studies of plants. *Molecular Phylogenetics and Evolution* 31: 894–903. <https://doi.org/10.1016/j.ympev.2003.10.009>
- Manning JC, Simka B, Boatwright JS, Magee AR (2016) A revised taxonomy of *Gerbera* sect. *Gerbera* (Asteraceae: Mutisieae). *South African Journal of Botany* 104: 142–57. <https://doi.org/10.1016/j.sajb.2015.10.002>
- Pasini E, Funk VA, de Souza-Chies TT, Miotto STS (2016) New insights into the phylogeny and biogeography of the *Gerbera*-complex (Asteraceae: Mutisieae). *Taxon* 65: 547–562. <https://doi.org/10.12705/653.7>
- Pobedimova YG (1963) Mutisieae. In: Brobov EG, Cherepanov SK, Komarov VL (Eds) *Flora USSR* 28: 588–598.
- Posada D (2008) jModelTest, phylogenetic model averaging. *Molecular Biology and Evolution* 257: 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut A, Drummond AJ (2009) Tracer v1.5. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. <https://doi.org/10.1007/BF00037152>
- Timme R, Kuehl EJ, Boore JL, Jansen RK (2007) A comparative analysis of the *Lactuca* and *Helianthus* (Asteraceae) plastid genomes: Identification of divergent regions and categorization of shared repeats. *American Journal of Botany* 94: 302–313. <https://doi.org/10.3732/ajb.94.3.302>
- Wen J, Nowicke JW (1999) Pollen ultrastructure of *Panax* (the ginseng genus, Araliaceae), an eastern Asian and eastern North American disjunct genus. *American Journal of Botany* 86: 1624–1636. <https://doi.org/10.2307/2656799>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (Eds) *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Xu X, Zheng W, Funk VA, Wen J (2018) Home at Last II: *Gerbera hieracioides* (Kunth) Zardini (Mutisieae, Asteraceae) is really a *Chaptalia*. *PhytoKeys* 95: 93–106. <https://doi.org/10.3897/phytokeys.95.22916>
- Zardini EM (1974) About the presence of the genus *Gerbera* in America. *Boletín de la Sociedad Argentina de Botánica* 16: 103–108.
- Zheng W, Xu X, Wen J (2017) The ethnic textile use of natural fibers from fireweed (*Gerbera de-lavayi*) in Southwest China. *Economic Botany*. <https://doi.org/10.1007/s12231-017-9394-y>

Describing terminologies and discussing records: More discoveries of facultative vivipary in the genus *Hedychium* J.Koenig (Zingiberaceae) from Northeast India

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Abstract

The authors introduce the term facultative vivipary for the first time in gingers and elaborate on this reproductive strategy. Four new observations of facultative vivipary are reported in the genus *Hedychium* which were discovered during botanical explorations by the authors in Northeast India (NE India) over the past three years. The viviparous taxa are *H. marginatum* C.B.Clarke, *H. speciosum* var. *gardnerianum* (Ker Gawl.) Sanoj & M.Sabu (previously, *H. gardnerianum* Sheppard ex Ker Gawl.), *H. thyrsoforme* Buch.-Ham. ex Sm. and *H. urophyllum* G.Lodd. The authors also attempt to summarise the occurrence of vivipary in the family Zingiberaceae from published reports and to clarify a taxonomic misidentification in a previously known report of vivipary in *Hedychium elatum*.

Keywords

Facultative vivipary, gingers, Meghalaya, Nagaland, phenology, pseudovivipary, recalcitrant seeds

Introduction

Vivipary in plants is a heterogeneous term that describes a unique and rare reproductive strategy where seedlings are precociously produced while still on the maternal parent (Goebel 1905). With the discovery of several examples of germination on the

parent plant in various angiosperm families, vivipary has become a biologically complex term because it now accommodates both sexual (true vivipary and cryptovivipary, see Elmqvist and Cox 1996) as well as asexual reproductive (pseudovivipary) strategies in plants (Farnsworth 2000).

True vivipary refers to vivipary *sensu stricto* and it is defined as penetration of a sexually reproduced embryo through the fruit pericarp and the resultant dispersal of this seedling (Cota-Sánchez 2004). Contrastingly, cryptovivipary is defined as vivipary wherein the seedling does not penetrate the fruit pericarp (Cota-Sánchez and Abreu 2007), while pseudovivipary is defined as production of apomictic or asexual propagules (like bulbils) on the parent plant (Law et al. 1983, Ofir and Kigel 2014). Thus sexually reproduced progeny (seed) is an integral part of the definition of vivipary and should not be confused with pseudovivipary which refers to production of apomictic or asexual plantlets or bulbils (Poulsen and Nordal 2005).

Within the family Zingiberaceae, vivipary is rarely discussed as an important reproductive strategy, except in *Hedychium elatum* R.Br. by Bhadra et al. (2013) (taxonomic misidentification discussed later). However, vivipary has been recorded in taxonomic descriptions such as in *Camptandra latifolia* Ridl. (Ridley 1899), in natural history observations (*Hedychium gardnerianum* Sheppard ex Ker Gawl.; Djeddour et al. 2012) and in pollination studies (*Alpinia mutica* Roxb.; Aswani and Sabu 2015). In contrast, pseudovivipary is very common in the form of bulbils and it has been recorded in at least six genera within Zingiberaceae: *Alpinia* Roxb., *Boesenbergia* Kuntze, *Globba* L., *Hedychium* J.Koenig, *Larsenianthus* W.J.Kress & Mood and *Zingiber* Mill. (Table 1). Interestingly, pseudovivipary (bulbil formation) was one of the key characters used to distinguish section *Globba* into two series by Schumann (1904) during the revision of Zingiberaceae, although recent molecular studies have not addressed the role of bulbil as an important character defining clades (see Williams et al. 2004).

The genus *Hedychium* (more than 80 species, see Sanoj 2011) is native to the Indian subcontinent, China, Southeast Asia (mainland and maritime) and Madagascar (Kress et al. 2002, Newman et al. 2004, Newman and Pullan 2007, Sanoj et al. 2013, WCSP 2018). The highly speciose regions are identified to be NE India (Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Sikkim), Southwest China (Yunnan), Myanmar, Thailand, Borneo and Java (Holtum 1950, Sirirugsa and Larsen 1995, Gao et al. 2008, Sarangthem et al. 2013). The plant habit ranges from terrestrial to epiphytic as well as lithophytic and each plant has a perennial rhizome which bears new ramets annually. Each ramet gives rise to a terminal inflorescence (known as thyrses) which consists of primary inflorescence bracts that hold flowers in cincinni (Kirchoff 1997). Two main types of inflorescence bracts are identified in *Hedychium*- imbricate (where bracts are broad and overlapping, hiding the rachis) and folded (where bracts are narrow and fold partially or completely to enclose the flowers, leaving rachis visible, Holtum 1950). The flowers are hermaphroditic, characterised by reduced petals and petaloid staminodes. The fruit is a septifragal capsule, light to dark green when young and changes to yellow with age (Fig. 1A). It splits open into three fleshy lobes, deep yellow or orange internally, with seeds arranged as in axile placentation. Mature seed is brown

Table 1. Summary of published and personal records where vivipary *sensu lato* were identified within Zingiberaceae. All *Globba* synonyms following Williams et al. (2004), Newman and Pullan (2007), WCSP (2018).

| Vivipary recorded in Zingiberaceae | Type of vivipary | Reference(s) |
|--|------------------|--|
| <i>Alpinia</i> Roxb. | | |
| <i>A. mutica</i> Roxb. | Vivipary* | Aswani and Sabu 2015 |
| <i>A. purpurata</i> (Vieill.) K.Schum. | Pseudovivipary | Dekkers et al. 1991 |
| <i>Boesenbergia</i> Kuntze | | |
| <i>B. parvula</i> (Wall. ex Baker) Kuntze | Pseudovivipary | Mood et al. 2016 |
| <i>B. pulcherrima</i> (Wall.) Kuntze | Pseudovivipary | Aishwarya and Sabu 2015 |
| <i>Camptandra</i> Ridl. | | |
| <i>C. latifolia</i> Ridl. | Vivipary* | Ridley 1899 |
| <i>Curcuma</i> L. | | |
| <i>C. coriacea</i> Mangaly & M.Sabu | Vivipary* | Leong-Škorničková 2007 |
| <i>Globba</i> L. | | |
| <i>G. aurantiaca</i> Miq. | Pseudovivipary | Ridley 1899 |
| <i>G. bicolor</i> Gagnep. | Pseudovivipary | Gagnepain 1901 |
| <i>G. bulbifera</i> Roxb. | Pseudovivipary | Horaninow 1862, Baker 1894 |
| <i>G. cambodgensis</i> Gagnep. | Pseudovivipary | Gagnepain 1901 |
| <i>G. cernua</i> Baker Synonym: <i>G. brachycarpa</i> Baker or <i>G. trachycarpa</i> Baker | Pseudovivipary | Schumann 1904, Box and Rudall 2006 |
| <i>G. chinensis</i> K.Schum. | Pseudovivipary | Schumann 1904 |
| <i>G. colpicola</i> K.Schum. | Pseudovivipary | Schumann 1904 |
| <i>G. lancangensis</i> Y.Y.Qian | Pseudovivipary | Zhou et al. 2007 |
| <i>G. leucantha</i> Miq. Synonym: <i>G. pallidiflora</i> Baker ex Ridl. | Pseudovivipary | Ridley 1899 |
| <i>G. marantina</i> L. Synonyms: <i>G. barthei</i> Gagnep., <i>G. ectobolos</i> K.Schum., <i>G. heterobracteata</i> K.Schum., <i>G. strobilifera</i> Zoll. & Moritzi | Pseudovivipary | Horaninow 1862, Gagnepain 1901, Liu et al. 2004, Williams et al. 2004, Box and Rudall 2006 |
| <i>G. multiflora</i> Wall. Synonym: <i>G. rubromaculata</i> J.Lal & D.M.Verma | Pseudovivipary | Baker 1894, Schumann 1904, This paper |
| <i>G. parva</i> Gagnep. | Pseudovivipary | Gagnepain 1901, Schumann 1904 |
| <i>G. pendula</i> Roxb. Synonyms: <i>G. calophylla</i> Ridl., <i>G. kingii</i> Baker, <i>G. panicoides</i> Miq., <i>G. stenothyrsa</i> Baker, <i>G. wallichii</i> Baker | Pseudovivipary | Ridley 1899 |
| <i>G. platystachya</i> Baker | Pseudovivipary | Baker 1894 |
| <i>G. racemosa</i> Sm. Synonyms: <i>G. clarkei</i> Baker, <i>G. hookeri</i> C.B.Clarke ex Baker | Pseudovivipary | Baker 1894, Schumann 1904 |
| <i>G. ranongensis</i> Picheans. & Tiyawora. | Pseudovivipary | Picheansoonthon and Tiyaworanant 2010 |
| <i>G. schomburgkii</i> Hook.f. Synonym: <i>G. globulifera</i> Gagnep. | Pseudovivipary | Gagnepain 1901, Schumann 1904 |
| <i>G. sessiliflora</i> Sims Synonyms: <i>G. canarensis</i> Baker, <i>G. careyana</i> Roxb., <i>G. ophioglossa</i> Wight | Pseudovivipary | Horaninow 1862, Baker 1894, Schumann 1904 |
| <i>G. substrigosa</i> Synonym: <i>G. aphanantha</i> K.Larsen | Pseudovivipary | Larsen et al. 1998 |
| <i>G. unifolia</i> Ridl. | Pseudovivipary | Holttum 1950 |
| <i>G. ustulata</i> Gagnep. | Pseudovivipary | Gagnepain 1901 |

| Vivipary recorded in Zingiberaceae | Type of vivipary | Reference(s) |
|---|----------------------|--|
| <i>Hedychium</i> J.Koenig | | |
| <i>H. greenii</i> W.W.Sm. | Pseudovivipary | Smith 1911 |
| <i>H. marginatum</i> C.B.Clarke | Facultative vivipary | This paper |
| <i>H. speciosum</i> var. <i>gardnerianum</i> (Ker Gawl.) Sanoj & M.Sabu | Facultative vivipary | Djeddour et al. 2012, Bhadra et al. 2013, This paper |
| Synonym: <i>H. gardnerianum</i> Sheppard ex Ker Gawl. | | |
| <i>H. thyriforme</i> Buch.-Ham. ex Sm. | Facultative vivipary | This paper |
| <i>H. urophyllum</i> G.Lodd. | Facultative vivipary | This paper |
| <i>Hornstedtia</i> Retz. | | |
| <i>H. scyphifera</i> J.Koenig ex Steud. | Vivipary* | Leong-Škorničková (pers. comm.) |
| <i>Larsenianthus</i> W.J.Kress & Mood | | |
| <i>L. careyanus</i> (Benth. & Hook.f.) W.J.Kress & Mood | Pseudovivipary | Mibang and Das 2017, Poulsen (pers. comm.), This paper |
| <i>Zingiber</i> Mill. | | |
| <i>Z. puberulum</i> Ridl. | Pseudovivipary | Leong-Škorničková (pers. comm.) |
| <i>Z. singaporense</i> Škorničk. | Pseudovivipary | Leong-Škorničková et al. 2014 |

*Possibly, Facultative vivipary

or black, covered by an aril which is either deep red or bluish-violet (in *H. hookeri* Baker, Srivastava 1984; fig. 1B). Frugivorous birds (Orchard 1973, Larsen et al. 1998) and rodents (Ridley 1899, Shiels 2011) are major dispersers of *Hedychium* seeds.

Although the name *Hedychium* is more than 230 years old (Koenig 1783), the report of vivipary in this genus is very recent (Djeddour et al. 2012, Bhadra et al. 2013). The first vivipary in *Hedychium* was detailed by Bhadra et al. (2013) in *H. elatum* from Kalimpong, on the way to Darjeeling (West Bengal, India) although it had already been reported in Djeddour et al. (2012). Here, four new observations are reported of facultative vivipary in the genus *Hedychium*: *H. marginatum*, *H. speciosum* var. *gardnerianum*, *H. thyriforme* and *H. urophyllum*. The taxonomic misidentification in Bhadra et al. 2013 is also reported and it is suggested that vivipary was not observed by them in *H. elatum* but the plant observed by them could likely be *H. speciosum* var. *gardnerianum*. Since voucher information is not available from Bhadra et al. (2013), their published images were used to assign an identity to the plant identified by them as *H. elatum*. Based on a published image (figure 1A in Bhadra et al. 2013) and the characters listed here in Table 2, it is clear that the species they reported cannot be *H. elatum*. Since voucher specimens or high resolution images are critical in species-level identification in *Hedychium*, it is suggested that the two likely species illustrated by Bhadra et al. in their figure 1A are either *H. gracile* Roxb. or *H. griffithianum* Wall. (Wallich 1853) based on the inflorescence shape and delicate nature of the inflorescence bracts and flowers, which are characteristics to these species (Table 2). Further, the plant shown in figures 1B–H of Bhadra et al. is identified as *H. speciosum* var. *gardnerianum* (Ker Gawl.) Sanoj & M.Sabu. Therefore, the plant image shown with vivipary (figures 1B–H in Bhadra et al. 2013) is identified as *H. speciosum* var. *gardnerianum* because of the taxonomic description given by Bhadra et al. as “leaves white pulverulent beneath” which is characteristic of *H. speciosum* var. *gardnerianum* (Baker 1894).



Figure 1. Septifragal capsule of *Hedychium*. **A** Unripe fruit of *H. marginatum* **B** Ripe fruit of *H. spicatum* (seeds can be seen covered by aril). Photographed by A. Ashokan.

Table 2. List of characters used to resolve the taxonomic identifications of plants in Figures 1A–H in Bhadra et al. 2013. Characters in bold were used by them to describe *H. elatum*, which are characteristics of either *H. gracile* or *H. griffithianum* or *H. speciosum* var. *gardnerianum*.

| Taxon | Nature of lamina (abaxial) | Midrib type (adaxial) | Inflorescence height (cm) | Inflorescence density | Colour (labellum and filament) | Length of corolla segments (cm) |
|--|----------------------------|-----------------------|---------------------------|--------------------------------|---|---------------------------------|
| <i>H. elatum</i> | not pulverulent | faintly grooved | >30 | dense to moderately dense | labellum pinkish-white with red centre; filament reddish-pink | 3.5 |
| <i>H. gracile</i> | not pulverulent | faintly grooved | 5–10 | lax to moderately dense | labellum creamy white with pale red base; filament bright red | 2.5 |
| <i>H. griffithianum</i> | not pulverulent | faintly grooved | 15–22 | moderately dense | labellum creamy white with pale red base; filament bright red | 2.5 |
| <i>H. speciosum</i> var. <i>gardnerianum</i> | pulverulent | deeply grooved | 30–45 | dense to moderately dense | labellum lemon yellow; filament bright red | 2.5–3.6 |

For taxonomic clarity, the key morphological and ecological characters of the (*Hedychium* taxa) are listed below where facultative vivipary has been observed. Voucher information is provided below as: collector name, voucher number, herbarium deposited.

1. *Hedychium marginatum* C.B. Clarke (Figs 2A–B, 3A; V. Gowda, VG-NL1899, BHPL)

Plant terrestrial, up to 1.5 m tall. Inflorescence conical; bracts imbricate; cincinni 3–6 flowers. Flowers orange-yellow, fragrant. Capsule elliptic, green and minutely hairy. Seeds many; aril red. The viviparous individual (bearing at least 10 seedlings on its dried infructescence) was observed along the Mokokchung-Tuensang Road, Mokokchung, Nagaland (26°19'59"N; 94°32'50"E) in August 2017 (Fig. 4).

It is very common in the Indian states of Manipur and Nagaland (Clarke 1890, Baker 1894, Sanoj 2011).

Flowering period: August to October; fruiting from September to November.

2. *Hedychium speciosum* var. *gardnerianum* (Ker Gawl.) Sanoj & M.Sabu (Figs. 2C, D, 3B; A. Ashokan, VG-ML1660, BHPL)

Plant terrestrial, up to 2.5 m tall. Inflorescence cylindrical; bracts folded, folding suprevolute; cincinni 2 flowers. Flowers lemon-yellow with bright red stamen, highly fragrant. Capsule oblong, green and glabrous. Seeds many; aril red. The viviparous individuals were found growing along the Shillong-Dawki Road in the East Khasi Hills district, Meghalaya (25°22'25"N; 91°52'37"E) in August 2016 and 2017 (Fig. 4).

It has a wide distribution range covering Eastern Himalaya and NE India, especially the Indian states of Arunachal Pradesh and Meghalaya (Edwards 1823, Roscoe 1828).

Flowering period: July to October; fruiting from September to November.

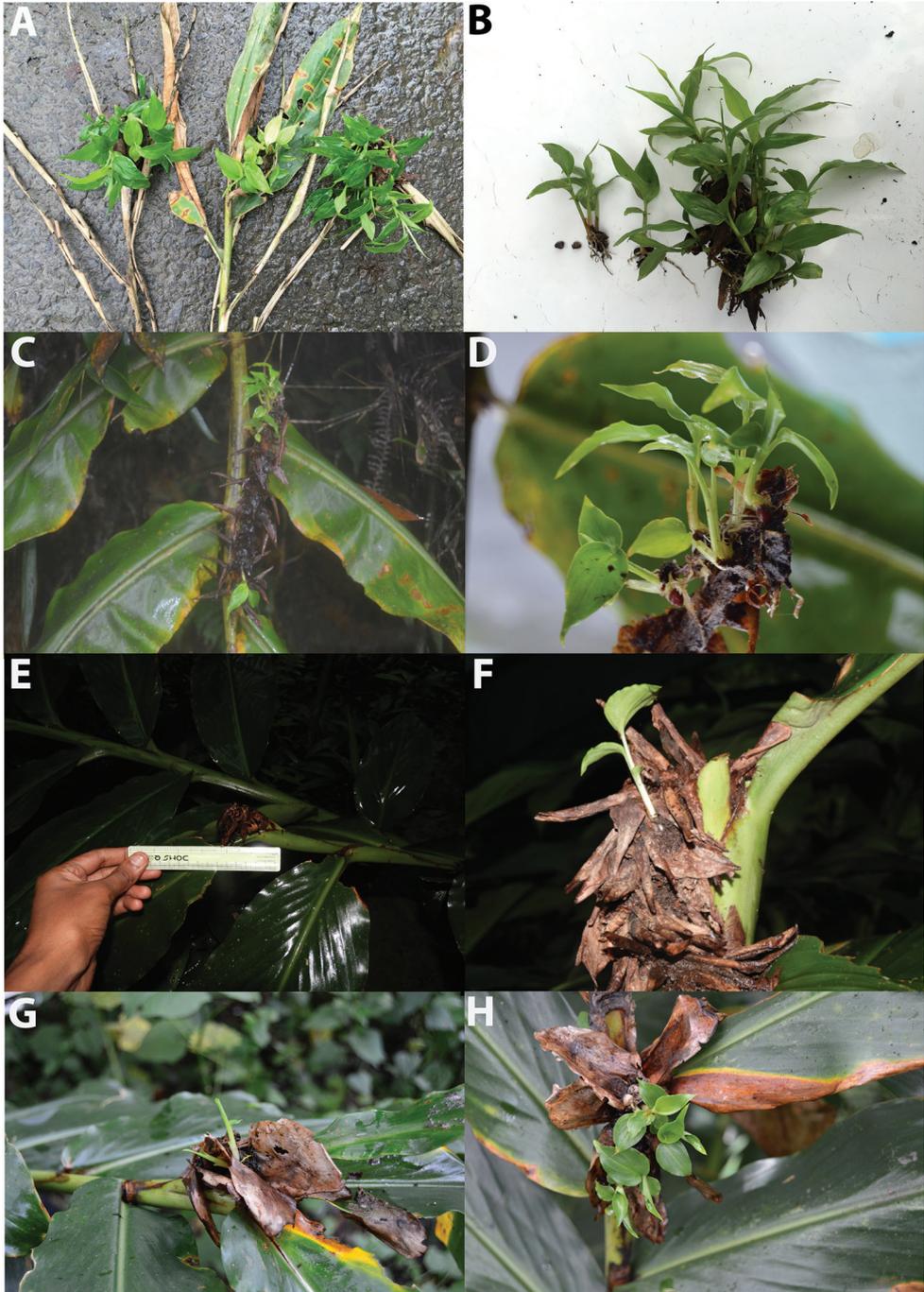


Figure 2. Facultative vivipary in *Hedychium*. **A–B** *Hedychium marginatum* **C–D** *H. speciosum* var. *gardnerianum*, **E&F** *H. thyriforme*, **G&H** *H. urophyllum*. Photographed by A. Ashokan & N.S. Prasanna (E&F).

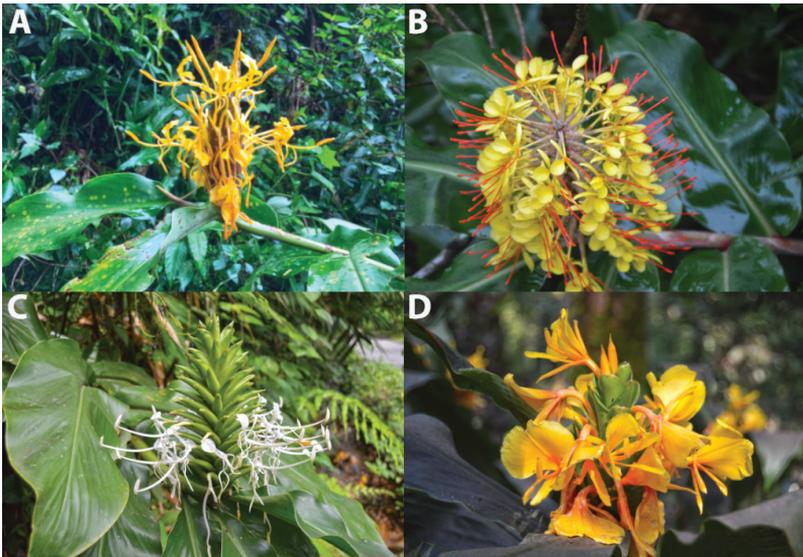


Figure 3. Inflorescence. **A** *Hedychium marginatum* **B** *H. speciosum* var. *gardnerianum* **C** *H. thyriforme* **D** *H. urophyllum*. Photographed by A. Ashokan.

3. *Hedychium thyriforme* Buch.-Ham. ex Sm. (Figs 2E–F, 3C; N.S.Prasanna, VG-MZ2201, BHPL)

Plant terrestrial, up to 1.5 m tall. Inflorescence pyramidal; bracts folded, folding supervolute; cincinni 3–8 flowers. Flowers white, mildly fragrant. Capsule oblong, green and glabrous. Seeds many; aril red. The viviparous individual (bearing one seedling on its dried infructescence) was observed in Aizawl, Mizoram (23°43'48"N; 92°43'52"E) in July 2017 (Fig. 4).

It grows very commonly in the Indian state of Mizoram. It was originally described from Nepal (Smith 1811).

Flowering period: August to October; fruiting from September to November.

4. *Hedychium urophyllum* G.Lodd. (Figs 2G–H, 3D; A. Ashokan, VG-ML1612 & 1725, BHPL)

Plant terrestrial, up to 2.2 m tall. Inflorescence conical; bracts imbricate; bracts are found to retain water; cincinni 2–4 flowers. Flowers bright yellow, highly fragrant. Capsule elliptic, dark green and glabrous. Seeds many; aril red. The viviparous *H. urophyllum* was growing in a mixed pine forest at Sanmer along the Shillong–Elephant Falls Road, East Khasi Hills district, Meghalaya (25°32'47"N; 91°50'16"E) in June 2015 and August 2016 (Fig. 4).

It is endemic to Meghalaya (Loddiges 1831, Sanoj 2011).

Flowering period: July to September; fruiting from September to November.

Discussion

During the three year study in NE India, four different *Hedychium* taxa showing vivipary have been identified: *H. marginatum*, *H. speciosum* var. *gardnerianum*, *H. thyriforme*

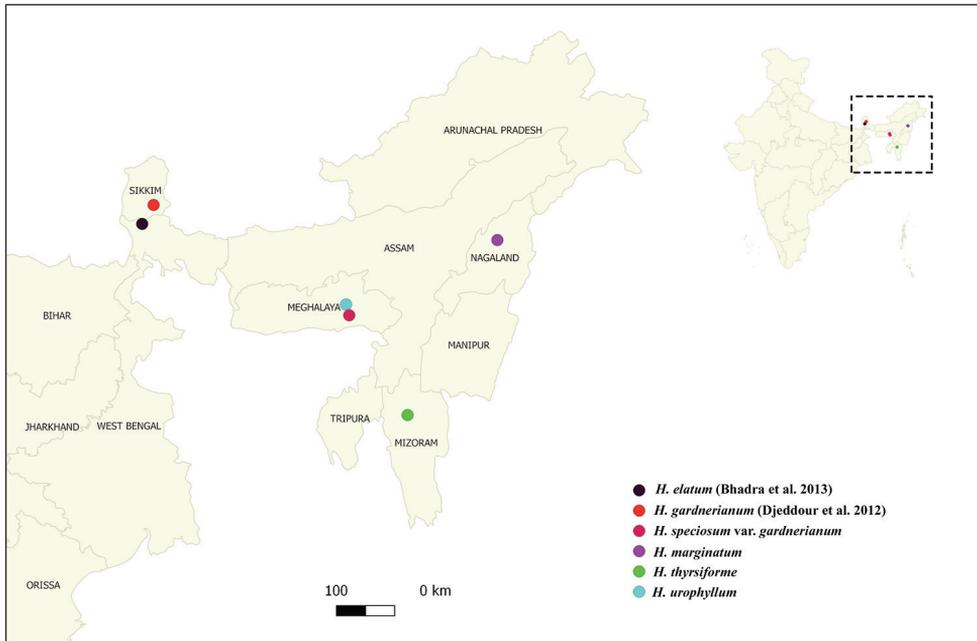


Figure 4. Distribution records for facultative vivipary in *Hedychium* in NE India.

and *H. urophyllum*. However, current observations of vivipary in *Hedychium* cannot be strictly identified as true vivipary or cryptovivipary (Elmqvist and Cox 1996, Cota-Sánchez 2004) since the fruit of *Hedychium* (septifragal capsule) dehisces before seed maturation while true vivipary requires the embryo to penetrate the fruit pericarp during germination. This makes the viviparous seed germination in *Hedychium* or gingers a case of “facultative vivipary”, which is introduced for the first time in gingers. The term “facultative vivipary” is proposed for the reproductive strategy observed in *Hedychium* since it represents the facultative nature of this reproductive strategy (occurs only when seeds are retained by bracts) as well as the viviparous nature of the germinating seed (seedling borne on the parent plant).

The factors leading to the incidence of facultative vivipary can be identified as a combination of climatic, ecological and physiological variables (Lee and Harmer 1980, Cox and Humphries 1993). In most *Hedychium*, flowering and fruiting coincide with the rainy season (an ecological trait shared amongst almost all *Hedychium*). The morphology of bracts in many *Hedychium* is such that the bracts can hold water. Thus the bracts provide a platform for the germination of the recalcitrant seeds by holding water which favours their germination. Further, these bracts are retained even after maturity of seeds thus facilitating their continued growth as seedlings. Thus, facultative vivipary may be a result of ‘failure to disperse’ either due to the structural hindrance imparted by the bracts or due to the absence of a disperser. The importance of facultative vivipary was not discounted as an important reproductive strategy that may facilitate seed germination under unpredictable climatic conditions, but this remains to be tested.

This multi-year observation of facultative vivipary in four *Hedychium* taxa suggests that facultative vivipary may be a more common reproductive strategy than that which is known so far. The immediate germination of seeds on the parent plant in at least four taxa also implies that recalcitrance of seeds (i.e. absence of dormancy) may be common in *Hedychium* (also true for other gingers, Leong-Škorničková, pers. com.). Recalcitrance has been suggested to be a common characteristic of plants growing in wet habitats (Farnsworth 2000), which is true for most *Hedychium*. Further, morphologically, it has been shown that *Hedychium* and other gingers have a thin seed coat (Benedict et al. 2015), which may also facilitate recalcitrance. Thus, it is predicted that facultative vivipary may be more common in *Hedychium* because of the physiology of the seed, i.e. its recalcitrant nature.



Figure 5. Pseudovivipary (Bulbil formation). **A** *Hedychium greenii* (the inset in the upper right is an inflorescence) **B** *Alpinia purpurata* **C** *Globba multiflora* **D** *Larsenianthus careyanus*. Photographed by A. Ashokan & Preeti (A).

The authors' efforts to summarise vivipary *sensu lato* across Zingiberaceae from published literature showed that vivipary *sensu stricto* is absent and that all reported cases of vivipary in other gingers can also only be identified as "facultative vivipary" or pseudovivipary, as in the case of *Hedychium*. Other genera that display facultative vivipary (except in *A. mutica*) also show similar characters as observed in *Hedychium* that favour this reproductive strategy, such as persistent bracts, bract structure that may retain the seeds, and recalcitrant seeds. It was found that both facultative vivipary and pseudovivipary are common amongst gingers with pseudovivipary being more frequent only in *Globba* (Table 1). Finally, in the genus *Hedychium*, pseudovivipary is only reported in *H. greenii* (Fig. 5).

To understand if vivipary *sensu lato* is a convergent character in *Hedychium*, different types of vivipary were mapped on a preliminary molecular phylogenetic tree (modified from nrDNA ITS tree of Wood et al. 2000, with the addition of *H. marginatum* and *H. speciosum* var. *gardnerianum*). It was found that both facultative vivipary and pseudovivipary are currently restricted only to a few members within the Clade IV (as defined in Wood et al. 2000). To understand the relevance of facultative vivipary and pseudovivipary as important reproductive strategies in gingers, it is suggested that future studies should treat different types of vivipary as an important morphological character along with seed characteristics (i.e. presence or absence of dormancy).

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References

- Aishwarya K, Sabu M (2015) *Boesenbergia pulcherrima* and *B. tiliifolia* (Zingiberaceae) in India: notes on the identity, variability and typification. *Rheedea* 25(1): 59–68.
- Aswani K, Sabu M (2015) Reproductive biology of *Alpinia mutica* Roxb. (Zingiberaceae) with special reference to flexistylly pollination mechanism. *The International Journal of Plant Reproductive Biology* 7(1): 48–58.

- Baker JG (1894) Scitamineae. In: Hooker JD (Ed.) *Flora of British India*. L. Reeve & Co., London, 6, 198–264.
- Benedict JC, Smith SY, Collinson ME, Leong-Škorničková J, Specht CD, Marone F, Xiao X, Parkinson DY (2015) Seed morphology and anatomy and its utility in recognizing sub-families and tribes of Zingiberaceae. *American Journal of Botany* 102(11): 1814–1841. <https://doi.org/10.3732/ajb.1500300>
- Bhadra S, Ghosh M, Mukherjee A, Bandyopadhyay M (2013) Vivipary in *Hedychium elatum* (Zingiberaceae). *Phytotaxa* 130(1): 55–59. <https://doi.org/10.11646/phytotaxa.130.1.7>
- Box MS, Rudall PJ (2006) Floral structure and ontogeny in *Globba* (Zingiberaceae). *Plant Systematics and Evolution* 258(1): 107–122. <https://doi.org/10.1007/s00606-005-0395-4>
- Clarke CB (1890) Plants of Kohima and Moneypore. *Journal of Linnean Society (Botany)* 25: 75.
- Cota-Sánchez JH (2004) Vivipary in the Cactaceae: its taxonomic occurrence and biological significance. *Flora-Morphology, Distribution, Functional Ecology of Plants* 199(6): 481–490. <https://doi.org/10.1078/0367-2530-00175>
- Cota-Sánchez JH, Abreu DD (2007) Vivipary and offspring survival in the epiphytic cactus *Epiphyllum phyllanthus* (Cactaceae). *Journal of Experimental Botany* 58(14): 3865–3873. <https://doi.org/10.1093/jxb/erm232>
- Cox PA, Humphries CJ (1993) Hydrophilous pollination and breeding system evolution in sea-grasses: a phylogenetic approach to the evolutionary ecology of the Cymodoceaceae. *Botanical Journal of the Linnean Society* 113: 217–226. <https://doi.org/10.1111/j.1095-8339.1993.tb00338.x>
- Dekkers AJ, Rao AN, Goh CJ (1991) In vitro storage of multiple shoot cultures of ginger at ambient temperatures of 24–29°C. *Scientia Horticulturae* 47(1/2):157–167. [https://doi.org/10.1016/0304-4238\(91\)90037-Y](https://doi.org/10.1016/0304-4238(91)90037-Y)
- Djeddour DH, Pratt C, Shaw RH (2012) The potential for the biological control of *Hedychium gardnerianum*. A report of the 4th Phase Research on the Biological Control of *Hedychium gardnerianum*. Produced for Landcare Research, New Zealand and The Nature Conservancy, Hawai'i, 16–17.
- Edwards S (1823) *The Botanical Register* 9: 774.
- Elmqvist T, Cox PA (1996) The evolution of vivipary in flowering plants. *Oikos* 77(1): 3–9. <https://doi.org/10.2307/3545579>
- Farnsworth E (2000) The ecology and physiology of viviparous and recalcitrant seeds. *Annual Review of Ecology and Systematics* 31(1): 107–138. <https://doi.org/10.1146/annurev.ecolsys.31.1.107>
- Gagnepain MF (1901) Révision des genres *Mantisia* et *Globba* (Zingibérées) de l'herbier du muséum. *Bulletin de la Société Botanique de France* 48(3): 201–216. <https://doi.org/10.1080/00378941.1901.10831839>
- Gao L, Liu N, Huang B, Hu X (2008) Phylogenetic analysis and genetic mapping of Chinese *Hedychium* using SRAP markers. *Scientia Horticulturae* 117(4): 369–377. <https://doi.org/10.1016/j.scienta.2008.05.016>
- Goebel KE (1905) *Organography of Plants*. Hafner, New York.
- Holtttum RE (1950) The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin Singapore* 13: 1–249.
- Horaninow PF (1862) *Prodromus Monographiae Scitaminarum*. St. Petersburg.

- Kirchoff BK (1997) Inflorescence and flower development in the *Hedychieae* (Zingiberaceae): *Hedychium*. Canadian Journal of Botany 75(4): 581–594. <https://doi.org/10.1139/b97-065>
- Koenig JG (1783) Descriptions Monandrarum pro annis. In: Retzius AJ (Ed.) Observationes Botanicae. Lipsiae, 3, 45–76.
- Kress WJ, Prince LM, Williams KJ (2002) The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. American Journal of Botany 9(10): 1682–1696. <https://doi.org/10.3732/ajb.89.10.1682>
- Larsen K, Lock JM, Maas H, Maas PJ (1998) Zingiberaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants IV. Flowering Plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae). Springer, Berlin, Heidelberg, 474–495. https://doi.org/10.1007/978-3-662-03531-3_49
- Law R, Cook RE, Manlove RJ (1983) The ecology of flower and bulbil production in *Polygonum viviparum*. Nordic Journal of Botany 3(5): 559–566. <https://doi.org/10.1111/j.1756-1051.1983.tb01468.x>
- Lee JA, Harmer R (1980) Vivipary, a reproductive strategy in response to environmental stress? Oikos 35(2): 254–265. <https://doi.org/10.2307/3544433>
- Leong-Škorničková J (2007) Taxonomic Studies in Indian *Curcuma* L. PhD Thesis, Charles University, Prague.
- Leong-Škorničková J, Thame A, Chew PT (2014) Notes on Singapore native Zingiberales I: A new species of *Zingiber* and notes on the identities of two further *Zingiber* taxa. Gardens' Bulletin Singapore 66: 153–167.
- Liu Z, Chen J, Bai Z (2004) Comparative studies on reproductive mechanisms of three species in *Globba* (Zingiberaceae). Wuhan Botanical Research 22(2): 145–152.
- Loddiges CL (1831) No. 1785. *Hedychium urophyllum*. The Botanical Cabinet: consisting of coloured delineations of plants, from all countries, with a short account of each, directions for management.
- Mibang T, Das AK (2017) Taxonomic Investigation on Genus *Larsenianthus* (Zingiberaceae) of Siang Valley, Arunachal Pradesh. Bulletin of Arunachal Forest Research 32(1–2): 41–48.
- Mood JD, Tanaka N, Aung MM, Murata J (2016) The genus *Boesenbergia* (Zingiberaceae) in Myanmar with two new records. Gardens' Bulletin Singapore 68(2): 299–318. <https://doi.org/10.3850/S2382581216000235>
- Newman M, Lhuillier-Chaigneau A, Poulsen AD (2004) Checklist of the Zingiberaceae of Malesia. Blumea Suppl. 16: 1–166.
- Newman M, Pullan M (2007) Zingiberaceae Resource Centre. Edinburgh: Royal Botanic Garden. <http://rbg-web2.rbge.org.uk/ZRC/home.html> [accessed 18 February 2018]
- Ofir M, Kigel J (2014) Temporal and intracloonal variation of flowering and pseudovivipary in *Poa bulbosa*. Annals of Botany 113(7): 1249–1256. <https://doi.org/10.1093/aob/mcu037>
- Orchard AE (1973) Zingiberaceae in New Zealand. Records of the Auckland Institute and Museum 10: 109–117.
- Picheansoonthon C, Tiyaworanant S (2010) A New Species of *Globba* (Zingiberaceae) from Southern Thailand. Journal of Japanese Botany 85(1): 25–29.

- Poulsen AD, Nordal I (2005) A phenetic analysis and revision of Guineo-Congolese rainforest taxa of *Chlorophytum* (Anthericaceae). *Botanical Journal of the Linnean Society* 148(1): 1–20. <https://doi.org/10.1111/j.1095-8339.2005.00386.x>
- Ridley HN (1899) The Scitamineae of the Malay Peninsula. *Journal of the Straits Branch of the Royal Asiatic Society* 32: 85–184.
- Roscoe W (1828) *Monandrian Plants of the Order Scitamineae*. Liverpool.
- Sanoj E (2011) Taxonomic Revision of the Genus *Hedychium* J. Koenig (Zingiberaceae) in India. PhD Thesis, University of Calicut, Kerala.
- Sanoj E, Sabu M, Pradeep AK (2013) Circumscription and lectotypification of *Hedychium villosum* and its variety *H. villosum* var. *tenuiflorum* (Zingiberaceae). *PhytoKeys* 25: 75–85. <https://doi.org/10.3897/phytokeys.25.4113>
- Sarangthem N, Talukdar NC, Thongam B (2013) Collection and evaluation of *Hedychium* species of Manipur, Northeast India. *Genetic resources and crop evolution* 60(1):13–21. <https://doi.org/10.1007/s10722-012-9810-1>
- Schumann K (1904) Zingiberaceae, IV, 46. In: Engler A (Ed.) *Das Pflanzenreich*, Heft 20. Leipzig, Berlin, 1–458.
- Shiels AB (2011) Frugivory by introduced black rats (*Rattus rattus*) promotes dispersal of invasive plant seeds. *Biological Invasions* 13: 781–792. <https://doi.org/10.1007/s10530-010-9868-7>
- Siriruga P, Larsen K (1995) The genus *Hedychium* (Zingiberaceae) in Thailand. *Nordic Journal of Botany* 15(3): 301–304. <https://doi.org/10.1111/j.1756-1051.1995.tb00156.x>
- Smith JE (1811) *Hedychium*. In: Rees A (Ed.) *Cyclopaedia*. Vol. 17. London.
- Smith WW (1911) Some additions to the Flora of Eastern Himalaya. *Records of the Botanical Survey of India* 4: 261–272.
- Srivastava SC (1984) A Taxonomic Study of Genus *Hedychium* Koen. (Zingiberaceae) in India and its Vicinity. PhD Thesis, University of Calcutta, West Bengal.
- Wallich N (1853) Initiatory attempt to define the species of *Hedychium*, and settle their synonymy. *Hooker's Journal of Botany and Kew Garden Miscellany* 5: 367–377.
- WCSP (2018) World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. <http://wcsp.science.kew.org/> [Retrieved 18 February 2018]
- Williams KJ, Kress WJ, Manos PS (2004) The phylogeny, evolution, and classification of the genus *Globba* and tribe Globbeae (Zingiberaceae): Appendages do matter. *American Journal of Botany* 91: 100–114. <https://doi.org/10.3732/ajb.91.1.100>
- Wood TH, Whitten WM, Williams NH (2000) Phylogeny of *Hedychium* and related genera (Zingiberaceae) based on ITS sequence data. *Edinburgh Journal of Botany* 57(2): 261–270. <https://doi.org/10.1017/S0960428600000196>
- Zhou H, Chen J, Chen F (2007) Ant-mediated seed dispersal contributes to the local spatial pattern and genetic structure of *Globba lancangensis* (Zingiberaceae). *Journal of Heredity* 98(4): 317–324. <https://doi.org/10.1093/jhered/esm032>

The identity of *Hypolepis robusta*, as a new synonym of *Hypolepis alpina* (Dennstaedtiaceae), based on morphology and DNA barcoding and the new distribution

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Abstract

Based on field observations and examinations of herbarium specimens (including type material), consulting the original literature and molecular phylogenetic analysis of the *rbcl* and *trnL-F* sequences, it is concluded that *Hypolepis robusta* is conspecific with *Hypolepis alpina* and is here formally treated as a synonym of it. Additionally *H. alpina* is reported with new distribution records in Guangdong, Guangxi and the Hainan Island of China, respectively.

Keywords

Hypolepis alpina, molecular phylogenetic, synonym, taxonomy, Type material

Introduction

Hypolepis Bernh. (1805) is one of the largest genera in the family Dennstaedtiaceae, with approximately 80 species (PPG I 2016) widespread in tropical and southern temperate parts the world, mainly in tropical Asia and tropical America, but the

exact number of species in China is still unclear (Brownsey 1987, Ching 1959, Xing et al. 2013). Amongst them, *Hypolepis alpina* (Blume) Hook. was initially described as *Cheilanthes alpina* Blume from Java in the first publication relating to the ferns of Malaya (Blume 1828). It was later transferred to *Hypolepis* by Hooker (1858) in the last comprehensive treatment of the genus (Brownsey 1987). Afterwards, one endemic species in the Taiwan province of China, *Hypolepis alte-gracillima* Hayata (1915), was reduced to a synonymy of *H. alpina*, according to the *Flora of Taiwan* (Shieh 1975). In addition to Taiwan, *H. alpina* is also distributed in Indonesia, Japan, Malaysia, Papua New Guinea and Philippines (Brownsey 1987, Fig. 1). Subsequently, the species (as *H. alte-gracillima*) was found in Gongshan County, in the Yunnan Province of China and recorded in *Flora Yunnanica* (Chu et al. 2006) as having a Yunnan-Taiwan discontinuous distribution. Another endemic species, *H. robusta* W. M. Chu was described for Yunnan (Chu et al. 2006). This name was treated as a synonymy of *H. polypodioides* (Blume) Hook. (Fraser-Jenkins 2008). Xing et al. (2013) cited a null name, (“*H. robusta* Hayata”) as a synonym of *H. polypodioides* in *Flora of China*, but Hayata’s name has not nomenclatural bearings nor taxonomic implications for Chu’s name. However, even Chu’s *H. robusta* is easily distinguishable from *H. polypodioides* in morphology as an obviously different species. *Hypolepis robusta* has densely multicellular brown glandular hairs and sori protected by well-developed reflexed adaxial indusium, whereas *H. polypodioides* has abundantly colourless non-glandular hairs and sori unprotected or occasionally protected by slightly reflexed green lamina segments. In June 2017, as part of the floristic inventory of Yunnan, *H. robusta* was collected at its type locality, Fugong County and *H. alpina* was collected at its recorded locality in Gongshan County. In addition, during the field work from 2013 to 2017, several specimens of *H. alpina* were collected from Taiwan as well as several others that were initially identified as *H. robusta* in Guangdong, Guangxi, Hainan Island and other locations of Yunnan. After conducting field observations, examinations of the herbarium specimens (including both types studied) and consulting the original literature (Hooker 1858, Chu 1992), it was suspected that *H. robusta* is conspecific with *H. alpina*. Therefore, the identity of *H. robusta* was determined by a more detailed examination of the morphology and molecular phylogenetic analysis.

Materials and methods

Morphological studies

For morphological comparisons, herbarium specimens or high-resolution images of specimens in CSH, K, KUN, L, P, PE, PYU, TAI, TAIF and US were critically checked. Field observations and collections were made in Guangdong, Guangxi, Hainan Island, Taiwan and Yunnan of China (Suppl. material 1: Table S1).

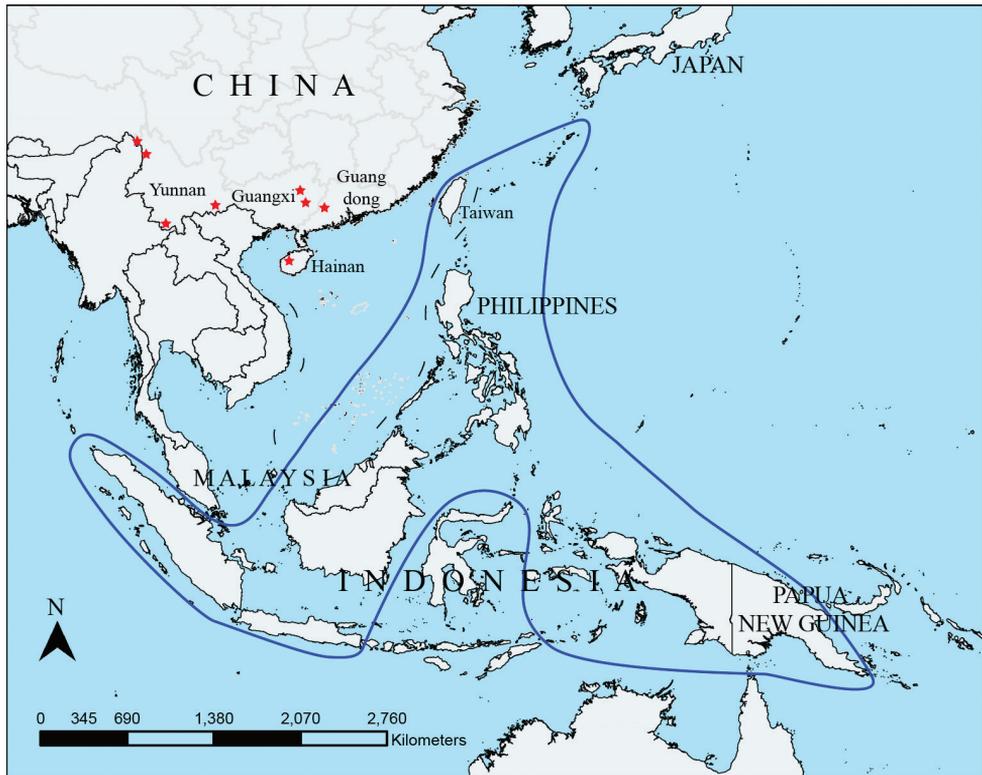


Figure 1. The distributions of *Hypolepis alpina* noted by Brownsey (1987, blue line) and new record localities since then (red stars), using a map available from <http://219.238.166.215/mcp/index.asp>.

Molecular phylogenetic studies

Nineteen specimens were sampled, including the outgroup taxa *Blotiella stipitata* (Alston) Faden and *Histiopteris incisa* (Thunb.) J. Sm., *Pteridium aquilinum* subsp. *wightianum* (J. Agardh) W.C. Shieh. Total genomic DNA was extracted from silica gel-dried leaves by using a DNA secure Plant Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocols. The PCR reactions were performed in a Veriti 96-Well Thermal Cycler. Two plastid markers were amplified, the *rbcL* gene and the *trnL-trnF* intergenic spacer. Primers used for amplification and sequencing were: *rbcL* primers 1379R and 1F (Little and Barrington 2003) and *trnL-F* primers trn-F and trn-r1 (Taberlet et al. 1991, Li et al. 2011). The amplification profiles were: initial denaturation (94 °C, 3 min) followed by 29 cycles of amplification, hybridisation and extension (94 °C, 45 s; 52 °C, 30 s; 72 °C, 1.5 min) and 10 min of final extension at 72 °C for *rbcL*, initial denaturation (95 °C, 3 min) followed by 35 cycles of amplification, hybridisation and extension (95 °C, 30 s; 52 °C, 30 s; 72 °C, 1 min) and 10 min of final extension at 72 °C for *trnL-trnF*. Sequencing was conducted using an ABI 3730xl DNA analyser (Applied Biosystems, Invitrogen, Foster City, CA, USA).

Phylogenetic analyses

Sequences were assembled and edited with SeqMan (DNA STAR package; DNA Star Inc., Madison, WI, USA), aligned by Bio Edit (Hall 1999) and adjusted manually where necessary. All sequences are available from GenBank (Table 1).

For phylogeny reconstructions, two methods were used, maximum likelihood (ML) and Bayesian Inference (BI). The ML analyses were conducted with RAxML-HPc BlackBox8.2.10 (Stamatakis 2014). For the Bayesian analyses, the best-fitting models (HKY+G) were selected using jModeltest2 web server under the Bayesian Information Criterion (BIC) (Darriba et al. 2012). Four chains were used with random initial trees as BI settings. Trees were generated for 1,000,000 generations and sampling was conducted every 100 generations. Before stationarity was conducted, the first 2,500 trees were discarded as burn-in trees and the remaining trees were used to construct the majority-rule consensus trees. The remaining trees were used to construct a consensus tree. ML bootstrap values and BI posterior probabilities were labelled on the tree branches.

DNA barcoding analyses

For species delimitation between *H. alpina* and the other species of *Hypolepis*, the DNA barcoding gap method, based on the Kimura two parameter (K2P) distance, was used. Intra- and inter-taxa genetic distances were evaluated using MEGA 5.0 (Tamura et al. 2011).

Results

A total of 19 new sequences amongst the total of 19 specimens were generated in the cpDNA matrix of *rbcL* and *trnL-F* containing 2,166 bp characters with 374 variable sites and 149 parsimony-informative sites. The optimal ML tree showed a negative log-likelihood score (-lnL) of 5577.824547 and the Bayesian tree was consistent with the ML tree. The statistical support is shown along the branches (ML/BI). Individuals of *H. alpina* and *H. robusta* formed a highly supported monophyletic group with an MLBS of 100 as sister clades of *H. tenuifolia*. Moreover, all *rbcL* and *trnL-F* sequences of the *H. robusta*, from type locality, were identical to those of *H. alpina* from Taiwan. The sequences of *H. robusta* from Guangdong, Guangxi and from Hainan Island were also clustered in the *H. alpina* clade, which had an MLBS of 100 (Fig. 2).

No differences were observed in the *rbcL* and *trnL-F* barcoding sequences of both *H. alpina* and *H. robusta*, except that two specimens have two base differences respectively. The genetic distance between *H. robusta* and *H. alpina* ranges from zero to 0.002. Their inter-taxon distances were significantly larger than their intra-taxon distances compared with the other species of *Hypolepis* and the ratio between the minimum inter-taxon distance and the maximum intra-taxon distance is 11 (Fig. 3).

Table 1. Plant materials, voucher information, and GenBank accession numbers of the samples used in the phylogenetic analyses.^a

| Taxon | Voucher | Locality | Geographic coordinates | GenBank accession number | |
|---|-----------|---|-----------------------------------|--------------------------|---------------|
| | | | | <i>rbcL</i> | <i>trnL-F</i> |
| <i>Hypolepis glandulifera</i> Brownsey & Chinnoek | BLD01 | Bali, Indonesia | NA | MG944782 | MG944788 |
| <i>Hypolepis robusta</i> W.M. Chu | DRS005 | Darong Mountain, Guangxi, China | NA | MG944773 | MG944789 |
| <i>Hypolepis punctata</i> (Thunb.) Mett. ex Kuhn | FLX6 | Hunan, China | NA | MG944784 | MG944790 |
| <i>Hypolepis tenuifolia</i> (G. Forst.) Bernh. | HN31 | Wuzhishan Mountain, Hainan, China | 18°55'1"N, 109°42'13"E | MG944786 | MG944791 |
| <i>Hypolepis robusta</i> W.M. Chu | HND6 | Bawang Mountain, Hainan, China | 19°07'26"N, 109°04'46"E | MG944774 | MG944792 |
| <i>Hypolepis alpina</i> (Blume) Hook. | Knapp4486 | Yilan County, Taiwan, China | 24°49'N, 121°41'E | MG944769 | MG944794 |
| <i>Hypolepis robusta</i> W.M. Chu | SG958 | Shengtang Mountain, Guangxi, China | NA | MG944777 | MG944801 |
| <i>Blotiella stipitata</i> (Alston) Faden | SG1185 | Kenya | NA | MG944780 | MG944795 |
| <i>Peridium aquilinum</i> subsp. <i>wightianum</i> (J. Agardh) W.C. Shieh | SG1760 | Yunnan, China | NA | MG944787 | MG944796 |
| <i>Hypolepis robusta</i> W.M. Chu | SG1812 | Ada Village, Fugong County, Yunnan, China | 26°49'5.6964"N, 98°53'36.715"E | MG944776 | MG944797 |
| <i>Hypolepis alpina</i> (Blume) Hook. | SG1838 | Dulongjiang Village, Gongshan County, Yunnan, China | 27°41'11.004"N, 98°16'54.340"E | MG944771 | MG944798 |
| <i>Hypolepis alpina</i> (Blume) Hook. | SG1871 | Dulongjiang Village, Gongshan County, Yunnan, China | 27°54'49.306"N, 98°20'37.03"E | MG944772 | MG944799 |
| <i>Hypolepis resistens</i> (Kunze) Hook. | SG2900 | Bawangling Mountain, Hainan, China | 19°5'28"N, 109°10'59"E | MG944785 | MG944800 |
| <i>Hypolepis polypodioides</i> (Blume) Hook. | SIWS28 | Sulawesi, Indonesia | NA | MG944783 | MG944802 |
| <i>Histiopteris incisa</i> (Thunb.) J. Sm. | WYD016 | Guangdong, China | NA | MG944781 | MG944804 |
| <i>Hypolepis robusta</i> W.M. Chu | WYD574 | Dawu Mountain, Guangdong, China | NA | MG944778 | MG944805 |
| <i>Hypolepis alpina</i> (Blume) Hook. | YYH11628 | Xitou Village, Nantou County, Taiwan, China | NA | MG944770 | MG944803 |
| <i>Hypolepis robusta</i> W.M. Chu | YYH12064 | Mengsong Village, Jinghong City, Yunnan, China | NA | MG944775 | MG944793 |
| <i>Hypolepis robusta</i> W.M. Chu | ZXC8465 | Gulingqing Village, Maguan County, Yunnan, China | 22°51'43.64"N, 104°0'15.59"E | MG944779 | MG944806 |

Note: NA = not available.

a: Specimens are deposited at the Shanghai Chenshan Botanical Garden Herbarium (CSH), except for voucher Knapp 4486, which is deposited at the Muséum National d'Histoire Naturelle (P).

Discussion

Hypolepis robusta was first reported by Chu (1992), being endemic to the Yunnan Province (Chu et al. 2006). After carefully comparing the type (including holotype and lectotype) of *H. robusta* and *H. alpina*, it was found that their morphological characteristics, e.g. the adventitious bud at stipe base, frond size, indusium and others (lamina, stipe, hair), are basically the same.

One of the main differences of *H. robusta* and *H. alpina* (*H. alte-gracillima*), mentioned in the key in *Flora Yunnanica*, is that the former has a few adventitious buds

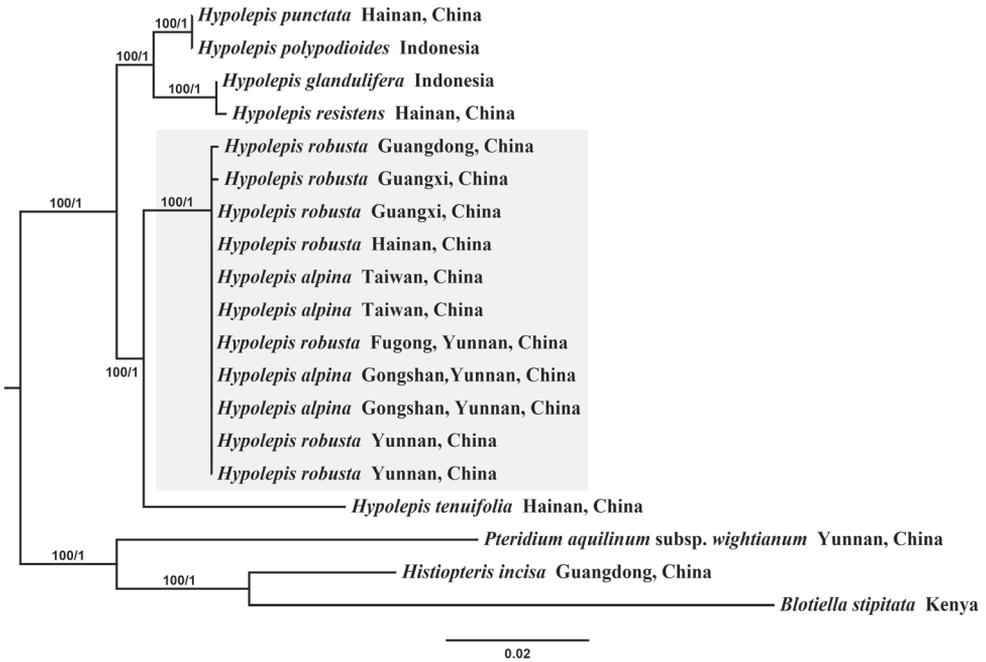


Figure 2. Phylogeny of 16 *Hypolepis* samples and *Blotiella stipitata*, *Histiopteris incisa*, and *Pteridium aquilinum* subsp. *wightianum* based on *rbcL* and *trnL-F*. Bootstrap values and Bayesian posterior probabilities are shown along branches (ML/B1).

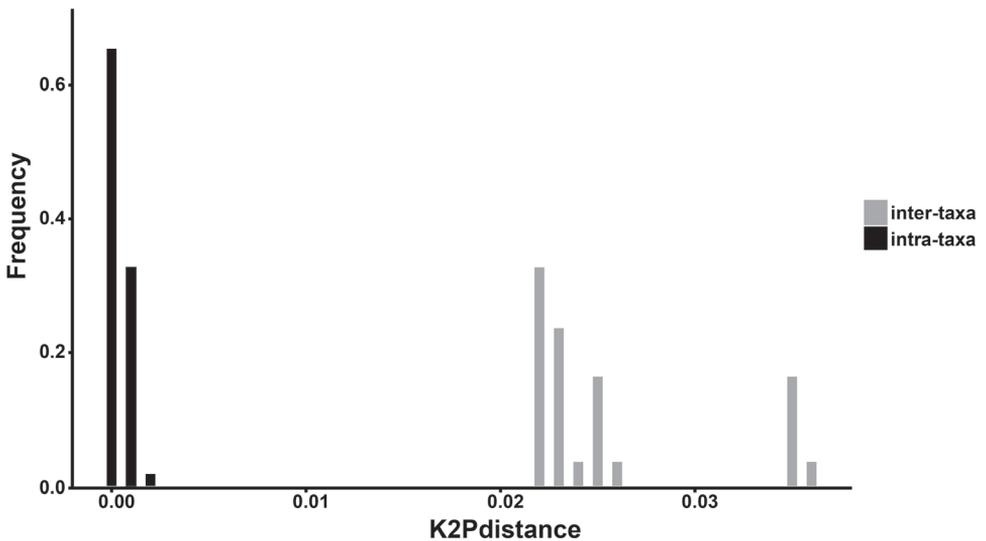


Figure 3. Distribution of intra-taxa (black) and inter-taxa (grey) Kimura two parameter (K2P) distances based on *rbcL* and *trnL-F* sequences as barcode. *Hypolepis alpina* and *Hypolepis robusta* versus the other species of *Hypolepis*.



Figure 4. *Hypolepis alpina*. **A** Frond size (photographed by H. Shang in Fugong) **B** Lamina (photographed by R. Knapp in Nantou) **C** Hair (photographed by R. Knapp in Nantou) **D** The adventitious bud at stipe base (photographed by H. Shang in Fugong) **E** Indusium (photographed by R. Knapp in Nantou).

growing on both sides of the stipe base (Chu et al. 2006). However, when several specimens were examined in the herbarium and those from the authors' own collection, it was found that *H. alpina* also has this feature (Fig. 4D). Therefore, it is concluded that the character used in the description is not relevant for distinguishing between *H. robusta* and *H. alpina*. Moreover, other Asian species of *Hypolepis* also develop adventitious buds, such as *H. pallida* (Blume) Hook. and *H. tenuifolia* (G. Forster) Bernhardt.

Another character used to support *H. robusta* as a new species was its larger size than *H. alpina*. The latter was reported at higher altitudes in the Malaysian region, from about 1,500–3,500 m and also as low as 1,100 m on Mt Kinabalu in Borneo (Brownsey 1987). However, there is considerable variation between plants from the highest elevations in New Guinea, which have rather smaller fronds and a dense covering of chestnut-brown non-glandular hairs, to those at lower altitudes in the northern part of its range (notably Taiwan), which have large fronds and very few chestnut hairs (Brownsey 1987). According to the description in *Flora Yunnanica*, *H. robusta* has a little larger frond than *H. alpina* (*H. alte-gracillima*). The field observation showed that *H. robusta* always occurs at altitudes about 1,000 m or even lower (Fig. 4A) and this is in accordance with the correlation between the altitudes and frond sizes mentioned in previous literature.

The characters of the indusium have been widely used in fern taxonomy. According to the previous literature of *H. alpina* and *H. robusta* (Brownsey 1987, Chu et al. 2006), they could be distinguished morphologically as follows: *H. robusta* has white indusium with marginal laceration, but *H. alpina* has a reflexed broad green lamina flap. Based on careful observations of all available material, it was found that their indusia are both half membranaceous at the margins and still green at the base (Fig. 4E). However, when the sori mature, the membranaceous margin becomes lacerated or exfoliated and the base can lose its chlorophyll, thus turning white. This difference may therefore be due to the fact that the descriptions have been made at different periods for the same species, a fact which had been previously ignored.

In addition to the morphological identification, a molecular phylogenetic analysis was also undertaken. The phylogenetic analysis of the *rbcL* and *trnL-F* sequences strongly supported the monophyly of *H. alpina* and *H. robusta* as a phylogenetic species with a wide distribution and distantly related to *H. polypodioides* (Fig. 2). The DNA barcoding analysis based on the K2P model revealed a significant gap between the inter-taxon and intra-taxon genetic distances, the distance in the *H. robusta* and *H. alpina* clade range from zero to 0.002, which is much lower than the inter-taxon distance and, in particular, the genetic distance between the *H. alpina* from Taiwan and the *H. robusta* from its type locality in Yunnan is zero (Fig. 3).

To sum up, not only does the morphological comparison identify *H. robusta* and *H. alpina* as conspecies, but also the phylogeny analysis identifies these as conspecies. Therefore, *H. robusta* is here reduced to a synonym of *H. alpina*. Consequently, *H. alpina* has three new distribution records in Guangdong, Guangxi and Hainan Island of China (Fig. 1). The new distribution records of *H. alpina* fill in gaps of the disjunct distribution defined in previous studies.

Taxonomic treatment

***Hypolepis alpina* (Blume) Hook. (1852: 63)**

Hypolepis alpina (Blume) Hook. (1852: 63). *Cheilanthes alpina* Blume (1828: 138). *Cheilanthes dissecta* Hook. & Arn. (1841: 75). *Hypolepis dissecta* (Hook. & Arn.) Brack. (1854: 89–90). *Hypolepis alte-gracillima* Hayata (1915: 295–297).

Type: Indonesia. Java: Jawa Barat, Gede, *Blume C. L.* (Lectotype: L-0051753!, L-0051754!).

= *Hypolepis robusta* W. M. Chu (1992: 36), **syn. nov.**

Type. China. Yunnan: Fugong County, 1980, *W. M. Chu* (Holotype: PYU-01017821!, PYU-01017822!, PYU-01017823!, PYU-01017824!).

Fronds up to 1.7 m high. Rhizome long-creeping, 2–10 mm diameter, densely covered in red-brown hairs up to 3 mm long. Stipes reddish-brown, 12–70 cm long, 1.5–13 mm diameter, grooved adaxially, covered in red-brown non-glandular hairs

up to 2 mm long and shorter glandular hairs, few adventitious buds at both sides of the stipe base; lamina ovate in outline, 3- or 4-pinnate, 20–80 (–130) cm × 10–90 cm, rachis red-brown or chestnut-brown at base, becoming chestnut-brown or yellow-brown at apex, densely covered in red-brown or chestnut-brown glandular hairs up to 0.5 mm long with occasional much longer non-glandular hairs; primary pinnae 15–30 pairs, opposite or sub-opposite, the largest at or near base, ovate to narrowly triangular, 10–52 cm × 3–28 cm; secondary pinnules narrowly ovate to ovate, 2–14 cm × 0.8–5 cm; ultimate pinnules to 10 mm × 5 mm. Sori circular or ovate, originating away from margins, without hairs between sporangia, protected by reflexed adaxial indusium, green at base and half membranaceous at margin, when the sori turn mature, the membranaceous margin becomes lacerated or exfoliated and the base part may turn white. Spores very pale under light microscope, perispores with interconnecting flattened projections, (32–) 34–37 (–40) μm × (20–) 21–25 (–28) μm.

Distribution. China (Guangdong, Guangxi, Hainan, Taiwan, Yunnan), Indonesia, Japan, Malaysia, Papua New Guinea, Philippines.

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References

- Bernhardi JJ (1805) Dritter Versuch einer Anordnung der Farrnkräuter. Neues Journal für die Botanik 1(2): 1–50.
- Blume CL (1828) Enumeratio plantarum Javae et insularum adjacentium: minus cognitarum vel novarum ex herbariis Reinwardtii, Kohlii, Hasseltii et Blumii, vol. 2. Lugduni Batavorum, Apud J.W. van Leeuwen, 138. <https://doi.org/10.5962/bhl.title.44901>
- Brownsey PJ (1983) Polyploidy and aneuploidy in *Hypolepis*, and the evolution of the Dennstaedtiaceae. American fern journal 73(4): 97–108. <https://doi.org/10.2307/1546960>
- Brownsey PJ (1987) A review of the fern genus *Hypolepis* (Dennstaedtiaceae) in the Malesian and Pacific regions. Blumea-Biodiversity, Evolution and Biogeography of Plants 32(2): 227–276.
- Ching RC (1959) Flora Reipublicae Popularis Sinica, vol. 2. Science Press, Beijing, 246.

- Chu WM (1992) Taxonomic notes on some pteridophytes from Yunnan (mainly Dulongjiang and neighboring regions). *Acta Botanica Yunnanica* 5: 36.
- Chu WM, He ZR, Zhang GF, Lu SG (2006) Hypolepidaceae. In: Kunming Institute of Botany, Chinese Academy of Sciences (Eds) *Flora Yunnanica*, vol. 20. Science Press, Beijing, 236–238.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772–772. <https://doi.org/10.1038/nmeth.2109>
- Fraser-Jenkins CR (2008) Taxonomic revision of three hundred Indian subcontinental pteridophytes: with a revised census list; a new picture of fern-taxonomy and nomenclature in the Indian subcontinent. Bishen Singh Mahendra Pal Singh, 85.
- Hall TA (1999) Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series* 41: 95–98.
- Hayata B (1915) *Icones plantarum formosandarum nec non et contributiones ad floram formosanam*, vol. 5. Government of Formosa, Taihoku, 295–297.
- Hooker WJ (1858) *Species Filicum* volume 2. *Adiantum-Ceratopteris*. William Pamplin, London, 63. doi.org/10.5962/bhl.title.32186
- Li FW, Kuo LY, Rothfels CJ, Ebihara A, Chiou WL, Windham MD, Pryer KM (2011) *rbcL* and *matK* earn two thumbs up as the core DNA barcode for ferns. *PLoS One* 6(10): e26597. <https://doi.org/10.1371/journal.pone.0026597>
- Little DP, Barrington DS (2003) Major evolutionary events in the origin and diversification of the fern genus *Polystichum* (Dryopteridaceae). *American Journal of Botany* 90: 508–514. <https://doi.org/10.3732/ajb.90.3.508>
- PPG I [The Pteridophyte Phylogeny Group] (2016) A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54(6): 563–603. <https://doi.org/10.1111/jse.12229>
- Shang H, Wang Y, Zhu XF, Zhao GH, Wang FH, Lu JM, Yan YH (2016) Likely allopatric origins of *Adiantum* × *meishanianum* (Pteridaceae) through multiple hybridizations. *Journal of Systematics and Evolution* 54(5): 528–534. <https://doi.org/10.1111/jse.12205>
- Shieh WC (1975) Dennstaedtiaceae. In: Li HL, Liu TS, Huang TC, Koyama T, CeVol CE (Eds) *Flora of Taiwan*, 1st edition, vol. 1. Epoch Publishing Co., Ltd, Taiwan, 243–247.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant molecular biology* 17(5): 1105–1109. <https://doi.org/10.1007/BF00037152>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Xing FW, Wang FG, Funston M, Gilbert MG (2013) *Hypolepis*. In: Wu CY, Raven PH, Hong DY (Eds) *Flora of China*, vol 2-3. Science Press Missouri Botanical Garden Press, Beijing, New York.

Supplementary material I

Table S1

Authors: Morigengaowa, Jun-Jie Luo, Ralf Knapp, Hong-Jin Wei, Bao-Dong Liu, Yue-Hong Yan, Hui Shang

Data type: (measurement/occurrence/multimedia/etc.)

Explanation note: Herbarium specimens information of *Hypolepis alpina* and *Hypolepis robusta* samples checked in this study.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/phytokeys.96.23470.suppl1>

Floristic affinities of the lowland savannahs of Belize and southern Mexico

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Abstract

Environmental heterogeneity of Belize and southern Mexico savannahs as well as their geographical location suggest that these plant communities share floristic elements, making them conducive to a phytogeographical analysis. The aim of this study was to analyse the floristic affinities of nine savannahs of Belize and southern Mexico and to explain the similarities and differences amongst them.

A binary data matrix containing 915 species was built based on the authors' own collections and on nine floristic lists already published. A second data matrix, consisting of 113 species representing trees, was also used since most literature on neotropical savannahs has focused on this life form. In addition, the ten most species-rich families as well as the characteristic species present in more than five savannahs were analysed. Floristic similarities were calculated using the Jaccard index. Dendrograms obtained in both types of analysis showed clusters with low similarity values, corresponding to geographic locations formed by the savannahs of Belize-Tabasco and the Yucatan Peninsula.

The floristic affinities of the savannahs may be explained in terms of heterogeneity in climate and physiography. The Yucatan Peninsula and Belize-Tabasco groups have differences in climate type and the amount of rainfall. In addition, the Yucatan Peninsula savannahs are established at the bottom of karstic valleys, while the Belize and Tabasco savannahs develop on extensive flatlands. The savannahs of Oaxaca have the same climate type and amount of rainfall as those of the Yucatan Peninsula but they are distributed along peaks and the slopes of shale hills. Fabaceae and Poaceae mainly dominated the local floras with 121 and 116 species each; remarkably, Melastomataceae was absent in the Yucatan Peninsula and Oaxaca. Nine species occurred in five to seven savannahs, confirming that they are widespread in both Belize and southern Mexico, and the Neotropics. Geographic location and floristic affinities of the nine savannahs support, to some extent, three different biogeographic provinces.

Keywords

Belize, biogeography, climate, México, relief, tropical grasslands

Introduction

Savannahs cover approximately 40% of the area of the Neotropics (Furley 1999), occupying just over two million km² (Mistry 2000). In Central America and the Caribbean, the savannah areas are not as extensive as, but they are ecologically similar to those in South America (Beard 1953, Huber 1987) and all neotropical savannahs are defined by their tendency to be on poorer soils and their grass-rich ground layer (Pennington et al. 2006). These plant communities are considered natural, but there also exist similar ones in anthropic areas, considered as secondary savannahs (Miranda and Hernández 1963, Pennington and Sarukhán 1968, 2005). Lowland savannahs generally experience annual events of waterlogging and drought related to the wet and dry seasons, so Sarmiento (1983) established a classification according to ecological criteria (i.e. seasonal and hyperseasonal).

Lowland savannahs of Belize and southern Mexico occur below 800 m and are defined here as any natural or semi-natural, fire-influenced ecosystem with a continuous herbaceous layer dominated by native grasses and sedges (Goodwin et al. 2013). Trees and shrubs may occur to a lesser or greater extent. Where present, *Pinus caribaea* Morelet (pine), *Acoelorrhaphe wrightii* H. Wendl. (palmetto), *Crescentia cujete* L. (calabash), *Byrsonima crassifolia* (L.) Kunth (craboo), *Curatella americana* L. (tachi-con), Melastomataceae spp. and *Quercus oleoides* Schlttdl. & Cham. (oak) are usually amongst the most structurally conspicuous non-herbaceous elements (Miranda and Hernández 1963, Rzedowski 1975, 1978, Bridgewater et al. 2002, Goodwin et al. 2013). Several plant associations (such as grass savannah, transitional savannah forest, gallery forests, oak savannah, palm savannah, pine savannah and wetland) have been described in Tabasco (Puig 1972) and Belize (Bridgewater et al. 2002, Penn et al. 2004, Stuart et al. 2006, Goodwin et al. 2013).

Gradients of floristic variation associated with latitude and longitude in neotropical savannahs have shown the great heterogeneity (Lenthall et al. 1999). In an analysis of the woody elements of neotropical savannahs, Lenthall et al. (1999) identified four phytogeographic zones: central Brazil and Bolivia extending to southern Amazonia; north of Amazonia extending across the isthmus of Central America and including the Caribbean; Belize, Guatemala and southern Mexico and north of the Mexican Plateau.

The savannahs of Belize and southern Mexico are floristically and environmentally similar, but such relationships have not been explored. Heterogeneity in climate and relief suggests gradients of floristic variation. Floristic knowledge, derived from several botanical surveys (Puig 1972, Pérez-García et al. 2001, Bridgewater et al. 2002, Farruggia et al. 2008, Hicks et al. 2011, Vázquez-Vázquez et al. 2012, Ortiz-Díaz et al. 2014), suggests that on a large scale they share floristic elements. However, on a local scale, they may differ.

The lowland savannahs of Belize and southern Mexico form an archipelago-like distribution and are thus discontinuous and embedded within other major vegetation types such as wet and seasonal forests as well as three biogeographic provinces, described by Morrone (2014). This archipelago-like distribution seems to be well suited for a phytogeographic analysis, so the aim of this study is: 1) to analyse the existence of common floristic patterns amongst Belize and southern Mexico lowland savannahs based on the authors' own collections and floristic lists available in literature and 2) to describe the probable effects of physical factors on those floristic patterns.

Methods

Study area. Nine sites of Belize and southern Mexico were selected to represent the lowlands savannahs as they have well documented floristic lists to compare with each other along the latitudinal gradient, as well as showing the heterogeneity in climate and physiography of three biogeographic provinces (Table 1) (Morrone 2014): four in the Yucatan Peninsula, four in Veracruz and one in the Pacific Lowlands. A map with the localities was built in SimpleMappr (Shorthouse 2010), according to the terrestrial ecoregions of the world (Fig. 1) (Olson et al. 2001). Terrestrial ecoregions exhibit a wide range of environmental conditions.

Regarding the climate, these savannah study areas can be grouped under the warm, humid tropical zone delineated by the Köppen system (García 1998) from Aw to Am. These ecoregions have a marked seasonality throughout the year; the dry season occurring from March to May and the rainy season with abundant rainfall during the summer (Table 1).

The geological substrate and geomorphic features along the Belize and southern Mexico region vary from north to south. In the north, the Yucatan Peninsula is formed mainly by a homogeneous limestone substrate with no rivers. In addition to limestone substrates, the middle portion of the study area belonging to Tabasco and Belize is more heterogeneous, being crossed by a complex fluvial system and including the Maya Mountains and the Highlands of Chiapas, which separate the regions from the Pacific Lowlands, where the Nizanda savannah is located (Table 1). The savannahs of the Yucatan Peninsula, Belize and Tabasco are developed at the bottom of karstic valleys (Vázquez-Vázquez et al. 2012, Ortiz-Díaz et al. 2014), open flat areas (Puig 1972, Bridgewater 2002) and on slopes of the Maya Mountains (Penn et al. 2004, Hicks et al. 2011) while the Pacific lowlands savannahs are found close to or at the top of hills (Pérez-García et al. 2001).

Data matrix construction. The database was developed using the floristic lists already published for the study areas (Table 1) and collections made by the authors of this study in the Yucatan Peninsula. Due to some information being older than forty years, old scientific names were updated using TROPICOS (www.tropicos.org) and by following the classification system of the Angiosperm Phylogeny Group (Stevens 2001). All the species included in the published floristic lists were considered.

Table 1. Geographic location and environmental data of the lowland savannahs of Belize and southern Mexico. ¹: Ortiz-Díaz et al. (2014), ²: Vázquez-Vázquez et al. (2012), ³: Hicks et al. (2011), ⁴: Bridgewater et al. (2002), ⁵: Farruggia et al. (2008), ⁶: Puig (1972), ⁷: Pérez-García et al. (2001).

| Biogeographic Province | Site | Climate | Latitude N | Longitude W | Substrate | Altitude m.a.s.l | Mean annual temperature °C | Annual rainfall mm | Source |
|------------------------|----------------|---------|------------|-------------|-------------|------------------|----------------------------|--------------------|--------|
| Yucatan Peninsula | Chacho Lugo | Aw | 19°48' | -89°21' | Clayey | 77 | 26 | 1043 | 1 |
| | Miguel Allende | Aw | 19°44' | -89°05' | Clayey | 90 | 26.3 | 1100 | 1 |
| | Xkahi | Aw | 19°11' | -89°17' | Clayey | 135 | 26.3 | 1100 | 2 |
| | Xpujil | Aw | 19°13' | -89°14' | Clayey | 92 | 26.3 | 1078 | 2 |
| Veracruz | San Pastor | Am | 16°45' | -89°00' | None | 680 | 24.1 | 1500 | 3 |
| | Rio Bravo | Am | 17°41' | -88°53' | None | 50 | 26–32 | 1500 | 4 |
| | Sapodilla | Am | 16°37' | -88°51' | Sand clayey | 120 | 26 | 4526 | 5 |
| | Huimanguillo | Am | 18°06' | -93°23' | Sand clayey | 50 | 26.2 | 2275 | 6 |
| Pacific Lowlands | Nizanda | Aw | 16°39' | -95°48' | Sand | 160 | 25 | 1000 | 7 |

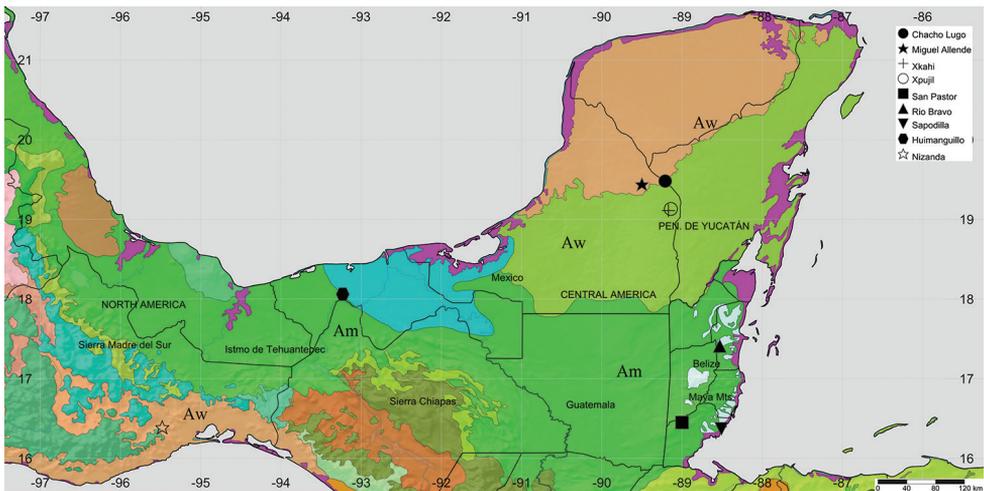


Figure 1. Geographic location of the nine savannahs of Belize and southern Mexico.

Data analysis. The flora compiled from the nine sites were compared using a multivariate method to obtain similarity measurements with the Jaccard coefficient and the UPGMA clustering technique. The matrix consisted of presence-absence data for 915 species. Since trees have been used as a model to assess the phytogeographical patterns in the neotropical savannahs (Lenthall et al. 1999), a data matrix was built with 113 tree species in order to gain more resolution and to explore how clusters change in relation to the full matrix. For both matrices, two analyses were performed for each matrix

using all species and informative species only. The definition of groups was based on bootstrap values above 70 % after 5000 replicates were performed using the software PAST v.3.15 (Hammer et al. 2001). Analysis was also carried out on the number of species of the ten most species-rich families at each site in the form of a quantitative matrix to assess floristic relationships amongst the three biogeographic provinces proposed by Morrone (2014). The characteristic species of the savannah flora were determined by considering at least five sites where they were present.

Results

In general, dendrograms from all the analyses conducted on the two matrices (full and trees only) showed clusters in a geographic arrangement and low similarity values amongst the nine sites (Figs 2, 3). Dendrograms 2A, 2B, using the whole flora and 3A and 3B, using the tree flora, grouped the Belize and Tabasco sites together with bootstrap values above 90 %. When Nizanda is grouped with this cluster, the bootstrap support is lower than 60 %. The four sites of the Yucatan Peninsula appeared clustered only when the whole species are considered but with a low bootstrap support. When the tree matrix is analysed (both considering the whole species and the informative species only), Nizanda's savannah clusters with three sites of the Yucatan Peninsula savannahs, with a very low bootstrap support (Fig. 3A–B).

Fabaceae and Poaceae mainly dominated the flora of the studied sites with 121 and 116 species each. Table 2 shows the number of species for the ten most species-rich families of Belize and southern Mexico. These families sum up to 552 (60.32 %) species. Asteraceae, Euphorbiaceae, Convolvulaceae and Apocynaceae were absent in one site and remarkably, Melastomataceae was absent in five of the nine sites, four in the Yucatan Peninsula and one in Nizanda.

Nine of 915 species were present in five or more sites, but none of the species was present in all sites. *Byrsonima crassifolia*, *Mimosa albida* Humb. & Bonpl. ex Willd. recorded at seven sites; *Piriqueta cistoides* (L.) Griseb., *Setaria parviflora* (Poir.) Kerguelen

Table 2. The ten most species-rich families and number of species for each savannah.

| | Chacho Lugo | Miguel Allende | Xkahi | Xpujil | San Pastor | Rio Bravo | Huimanguillo | Sapodilla | Nizanda | Total |
|-----------------|-------------|----------------|-------|--------|------------|-----------|--------------|-----------|---------|-------|
| Fabaceae | 8 | 12 | 8 | 9 | 13 | 38 | 56 | 14 | 38 | 121 |
| Poaceae | 20 | 19 | 7 | 16 | 3 | 17 | 48 | 34 | 27 | 116 |
| Cyperaceae | 9 | 1 | 5 | 9 | 1 | 9 | 42 | 26 | 11 | 77 |
| Asteraceae | 4 | 0 | 2 | 3 | 9 | 8 | 27 | 2 | 14 | 60 |
| Melastomataceae | 0 | 0 | 0 | 0 | 12 | 7 | 24 | 9 | 0 | 34 |
| Euphorbiaceae | 1 | 1 | 5 | 0 | 4 | 2 | 5 | 17 | 1 | 33 |
| Malvaceae | 5 | 6 | 6 | 3 | 3 | 9 | 15 | 2 | 3 | 32 |
| Rubiaceae | 2 | 2 | 1 | 3 | 11 | 16 | 1 | 12 | 2 | 29 |
| Convolvulaceae | 6 | 5 | 6 | 3 | 0 | 4 | 8 | 2 | 3 | 27 |
| Apocynaceae | 0 | 1 | 1 | 3 | 2 | 6 | 7 | 2 | 5 | 23 |

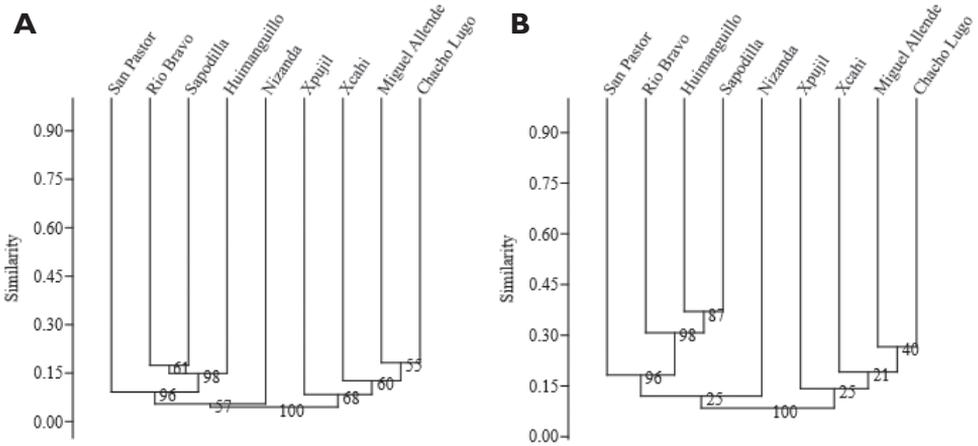


Figure 2. Similarity dendrograms for the nine savannahs of Belize and southern Mexico using 915 species. 2A: Built using all species. 2B: Built taking out single-site species.

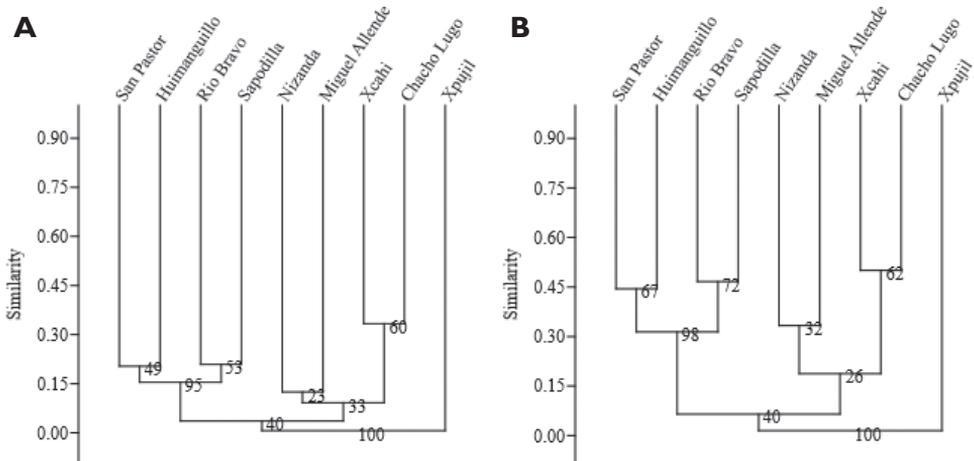


Figure 3. Similarity dendrograms for the nine savannahs of Belize and southern Mexico using 113 species of trees. 3A: Built using all species. 3B: Built taking out single-site species.

recorded at six sites; and *Andropogon virginicus* L., *Crescentia cujete*, *Paspalum plicatum* Michx., *Psidium guineense* Sw. and *Sida linifolia* Juss. ex Cav. recorded at five sites were the widespread species of the lowland savannahs of Belize and southern Mexico.

Discussion

Dendrograms of Belize and southern Mexico savannahs showed arrangements according to their geographical locations and suggested floristic patterns associated with heterogeneity in climate and physiography. Differences in the amount of annual rainfall and geomor-

phological characteristics may be the major determining variables at both continental and regional scales (Bailey 2009). The low floristic similarity amongst all sites (less than 50%) reflects the archipelago effect, since some of the studied sites are separated by extensive seasonal forests locally different in species composition (Pennington et al. 2006), rainforests (Puig 1972, Bridgewater et al. 2002, Pennington et al. 2006, Stuart et al. 2006) and xerophytic vegetation (Pérez-García et al. 2001, Pérez-García and Meave 2006).

The dendrograms resulting from the two analyses were similar in structure except by the position of Nizanda and Xpujil savannahs. Both appeared as the most distant sites in the dendrograms and this may be explained by having more single-site species. Furthermore, the similarity levels increase when only shared species are taken into account, which reinforces the Belize-Tabasco and Yucatan Peninsula groups. The similarities and differences amongst clusters can be interpreted as a response to similar environmental conditions amongst some sites. In terms of climate, the Yucatan Peninsula savannahs are close to each other and share Aw climate, with relative low annual rainfall. Although the Nizanda savannah is distant on the Pacific Coast, it also has Aw climate. The Belize and Tabasco savannahs have Am climate with higher rainfall than the other regions. In terms of physiography, the savannahs of the Yucatan Peninsula are small and established at the bottom of karstic valleys (Vázquez-Vázquez et al. 2012, Ortiz-Díaz et al. 2014), while the Belize and Tabasco savannahs develop on extensive flatlands (Puig 1972, Penn et al. 2004, Bridgewater et al. 2002). The savannahs on the Pacific Coast are distributed along peaks and the slopes of shale hills and this does not occur in other study regions (Rzedowski 1978, Pérez-García et al. 2001).

Plant inventories and differences in area size did not seem to influence the cluster outcome and lack of outstanding geological barriers may facilitate the similarity. In contrast, the archipelago effect may explain the low similarity values obtained at the adjacent sites of the Yucatan Peninsula. There, heterogeneity in physiographical and hydrological dynamics may lead to variation in the floristic composition (Ortiz-Díaz et al. 2014). The four Yucatan sites are categorised into two different geomorphological units; for instance, Miguel Allende is placed in an extensive karstic valley that favours rain overflow in the rainy season, while the other three savannahs are situated at the bottom of a tectonic valley forming part of a major structural valley system on a NW to SE orientation, such that they remain waterlogged during the rainy season (Vázquez-Vázquez et al. 2012, Ortiz-Díaz et al. 2014).

Individual site floras were largely dominated by species of Fabaceae, Poaceae, Cyperaceae, Asteraceae, Melastomataceae, Euphorbiaceae, Malvaceae, Rubiaceae, Convolvulaceae and Apocynaceae. A similar floristic pattern was found in South American savannahs by Huber et al. (2006) in the Llanos of Venezuela and by Ratter et al. (2006) in the Cerrados of Brazil. It is possible that the relative abundance of the Melastomataceae family in the four savannahs of Belize (Penn et al. 2004) and Tabasco (Puig 1972) and its apparent absence in the Yucatan Peninsula and Nizanda is a good example of how the amount of rainfall defines Melastome distribution in Belize and southern Mexico since this family occurs in climates with high rainfall. High richness in Melastomataceae has been reported for other regions of tropical America in mesic,

cool environments and on deep soils rich in nutrients, such as the Llanos of Venezuela (Huber et al. 2006) and the Cerrados of Brazil (Ratter et al. 2006).

The most common species of Belize and southern Mexico savannahs were the trees *Byrsonima crassifolia* and *Crescentia cujete*. Next most common, but only found in two or three localities were *Acoelorrhapha wrightii*, *Curatella americana*, *Pinus caribaea* and *Quercus oleoides*. Along with these tree species, which are widespread in neotropical grasslands, can be added the common shrubs *Mimosa albida*, and *Psidium guineense* and the herbs *Piriqueta cistoides*, *Setaria parviflora*, *Andropogon virginicus*, *Paspalum plicatulum* and *Sida linifolia*, which also shape the so-called basic floristic matrix present in most of neotropical savannahs (Huber 1987, Lenthall et al. 1999, Bridgewater et al. 2002). Floristic affinity of the common species confirms that the geographic origin of savannah flora of the study region is mainly neotropical.

Geographic location and floristic affinities of these nine savannahs support to some extent three different biogeographic provinces, proposed by Morrone (2014) as follows: four savannahs in the Yucatan Peninsula, four in Veracruz and one in the Pacific Lowlands. Moreover, the nine savannahs show the influence of environment associated with heterogeneity in the climate and physiography of these plant communities.

Lastly, it is emphasised that the floristic variation amongst these savannahs and their unique contribution to the flora of Central America give them high conservation importance. Yet they are environmentally undervalued and severely affected by human activities. Conserving Central American savannahs is more of an urgent task for Mexico, since the Belize conservation system cover savannah areas (Bridgewater et al. 2002, Hicks et al. 2011).

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References

- Bailey RG (2009) *Ecosystem Geography: from Ecoregions to Sites*. Springer, New York, 251 pp. <https://doi.org/10.1007/978-0-387-89516-1>
- Beard JS (1953) The savanna vegetation of northern tropical America. *Ecological Monographs* 23(2): 149–215. <https://doi.org/10.2307/1948518>
- Bridgewater S, Ibañez A, Ratter JA, Furlley P (2002) Vegetation classification and floristics of the savannas and associated wetlands of the Rio Bravo conservation and management area, Belize. *Edinburgh Journal of Botany* 59(3): 421–442. doi.org/10.1017/S0960428602000252

- Farruggia FT, Henry M, Stevens H, Vincent MA (2008) A Floristic Description of a neotropical Coastal Savanna in Belize. *Caribbean Journal of Science* 44(1): 53–69. <https://doi.org/10.18475/cjos.v44i1.a7>
- Furley PA (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. *Global Ecology and Biogeography* 8(3–4): 223–241. <https://doi.org/10.1046/j.1466-822X.1999.00142.x>
- García E (1998) Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). <http://www.conabio.gob.mx/informacion/gis/> [accessed 07.06.2017]
- Goodwin ZA, López GN, Stuart N, Bridgewater SGM, Haston EM, Cameron ID, Michelakis D, Ratter JA, Furley PA, Kay E, Whitefoord C, Solomon J, Lloyd AJ, Harris DJ (2013) A checklist of the vascular plants of the lowland savannas of Belize, Central America. *Phytotaxa* 101(1): 1–119. <https://doi.org/10.11646/phytotaxa.101.1.1>
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1): 1–9.
- Hicks J, Godwin ZA, Bridgewater SGM, Harris DJ, Furley PA (2011) A floristic description of the San Pastor Savanna, Belize, Central America. *Edinburgh Journal of Botany* 68(2): 273–296. <https://doi.org/10.1017/S0960428611000102>
- Huber O (1987) Neotropical savannas: their flora and vegetation. *Trends in Ecology and Evolution* 2(3): 67–71. [http://dx.doi.org/10.1016/0169-5347\(87\)90151-0](http://dx.doi.org/10.1016/0169-5347(87)90151-0)
- Huber O, de Stefano RD, Aymard G, Riina R (2006) Flora and Vegetation of the Venezuelan Llanos. In: Pennington RT, Lewis GP, Ratter J (Eds) *Neotropical Savannas and Seasonally Dry Forests* 95–120.
- Lenthall JC, Bridgewater SGM, Furley P (1999) A phytogeographic analysis of the woody elements of the New World savannas. *Edinburgh Journal of Botany* 56(2): 293–305. <https://doi.org/10.1017/S0960428600001153>
- Miranda F, Hernández E (1963) Los tipos de vegetación de México y su clasificación *Boletín de la Sociedad Botánica de México* 28: 1–179.
- Mistry J (2000) *World Savannas: Ecology and Human Use*. Pearson Education, Harlow, 344 pp.
- Morrone JJ (2014) Biogeographical regionalisation of the neotropical region. *Zootaxa* 3782(1): 1–110. <https://doi.org/10.11646/zootaxa.3782.1.1>
- Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001) Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 51(11): 933–938. [https://doi.org/10.1641/0006-3568\(2001\)051\[0933:TEOTWA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2)
- Ortiz-Díaz JJ, Tun-Garrido J, Arnelas-Seco I, García-Gil G (2014) Flora fanerogámica de dos enclaves de sabana de la Península de Yucatán. *Revista Mexicana de Biodiversidad* 85(3): 665–676. <https://doi.org/10.7550/rmb.35642>
- Penn MG, Sutton DA, Monro A (2004) Vegetation of the greater Maya Mountains, Belize. *Systematics and Biodiversity* 2(1): 21–44. <https://doi.org/10.1017/S1477200004001318>
- Pennington TD, Sarukhán J (1968) *Manual para la identificación de campo de los principales arboles tropicales de México*. Instituto nacional de Investigaciones Forestales FAO. Mexico, 1–413.

- Pennington TD, Sarukhán J (2005) *Arboles tropicales de México: Manual para la identificación de las principales especies*. Universidad Nacional Autónoma de México Fondo de Cultura Económica, Mexico, 1–523.
- Pennington RT, Lewis GP, Ratter JA (2006) An overview of the plant diversity, biogeography and conservation of Neotropical savannas and seasonally dry forests. In: Pennington RT, Lewis GP, Ratter J (Eds) *Neotropical Savannas and Seasonally Dry Forests*, 1–29.
- Pérez-García EA, Meave JA, Gallardo C (2001) Vegetación y flora de la región de Nizanda, Istmo de Tehuantepec, Oaxaca, México. *Acta Botanica Mexicana* 56: 19–88. <https://doi.org/10.21829/abm56.2001.879>
- Pérez-García EA, Meave JA (2006) Coexistence and divergence of tropical dry forests and savannas in southern Mexico. *Journal of Biogeography* 33(3): 438–447. <https://doi.org/10.1111/j.1365-2699.2005.01459.x>
- Puig H (1972) La sabana de Huimanguillo, Tabasco, México. In: *Memorias del Primer Congreso Latinoamericano de Botánica*, Mexico, 389–411.
- Ratter JA, Bridgewater SGM, Ribeiro JF (2006) Biodiversity patterns of the woody vegetation of the Brazilian Cerrado. In: Pennington RT, Lewis GP, Ratter JA (Eds) *Neotropical Savannas and Seasonally Dry Forests*, 31–66.
- Rzedowski J (1975) An ecological and phytogeographical analysis of the grasslands of Mexico. *Taxon* 24(1): 67–80. <https://doi.org/10.2307/1219002>
- Rzedowski J (1978) *Vegetación de México*. Limusa, México, 1–432.
- Sarmiento G (1983) The savannas of tropical America. In: Bourliere F (Ed.) *Ecosystems of the World XIII. Tropical Savannas*, Elsevier, Amsterdam, 245–288.
- Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. Retrieved from <http://www.simplemappr.net> [accessed 12.06. 2017].
- Stevens PF (2001 onwards) Angiosperm Phylogeny Website. Version 12, July 2012 <http://www.mobot.org/MOBOT/research/APweb/> [accessed 08.06.2017]
- Stuart NT, Barrat T, Place C (2006) Classifying the neotropical savannas of Belize using remote sensing and ground survey. *Journal of Biogeography* 33: 476–490. <https://doi.org/10.1111/j.1365-2699.2005.01436.x>
- Tropicos.org (2017) Missouri Botanical Garden. <http://www.tropicos.org> [Accessed 08.06.2017]
- Vázquez-Vázquez C, Ortiz-Díaz JJ, Tun-Garrido J, García-Gil G (2012) Flora vascular de las sabanas de Xmabén, Hopelchén, Campeche, México. *Polibotánica* 34: 1–19. <http://www.herbario.encb.ipn.mx/pb/pdf/pb34/hope.pdf>

Taxonomic studies of *Glochidion* (Phyllanthaceae) from the Indo-China Peninsula (I): *G. shanense*, a new species from Myanmar

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Abstract

Based on morphological studies performed on live plants in the field and specimens deposited in herbaria, a new species, *Glochidion shanense* Gang Yao & Shixiao Luo (Phyllanthaceae, Phyllanthaceae), is here described and illustrated. The species is morphologically most similar to *G. ellipticum* Wight, but can be distinguished from the latter by having hairy branchlets, longer pedicels, uniseriate and narrowly triangular sepals of female flowers, 4–5-locular ovaries, stout and cylindrical persistent style on fruits.

Keywords

Phyllanthaceae, *Glochidion*, Morphology, Myanmar

Introduction

Glochidion J.R. et G. Forst. is the second largest genus in the tribe Phyllanthaeae Dumortier, Phyllanthaceae Martynov (Webster 1994). It consists of more than 300 species mainly distributed in the Indo-Pacific, east to southeast Polynesia and south into Australia (Govaerts et al. 2000, Li and Gilbert 2008) and can be distinguished from other members in Phyllanthaeae by the absence of a floral disc, apiculate anthers, usually confluent styles and fleshy seed-coat (Li 1994, Webster 1994).

Recently, the leafflower plant and leafflower moth system, a new mutualism model system that is similar to the fig-fig wasp and yucca-yucca moth pollination systems, was described between Phyllanthaeae plants and *Epicephala* moth (Kato et al. 2003, Kawakita et al 2015, Luo et al. 2017) and it provides a new opportunity to study the mechanisms of biodiversity development and maintenance. *Glochidion* is the largest host plant lineage within this newly described mutualism system, in which at least five host plant lineages were identified (Kawakita and Kato 2009). However, a comprehensive taxonomic study of *Glochidion* is still lacking, especially for relevant species distributed in the Indo-China Peninsula, since the latest comprehensive taxonomic investigation of *Glochidion* from there can be dated back to Beille (1927). Recently, taxonomic studies of *Glochidion* species from Thailand (van Welzen 2007) and Vietnam (Nguyen 2007) have been conducted. In Myanmar, Kurz (1877) accepted 14 *Glochidion* species in Forest Flora of British Burma and Kress et al. (2003) recorded 33 *Glochidion* species in A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar.

During the fieldwork in Shan State, eastern Myanmar, in December 2015, two of the authors (J. Cai and X.F. Yang) collected some Phyllanthaeae specimens and one belongs to *Glochidion*, which superficially differs from congeneric taxa recorded in Myanmar and its adjacent countries. After a detailed morphological investigation and herbaria examination for all the *Glochidion* species recorded in this region, it was confirmed that the species is new to science, thus it is formally described below. The new species belongs to section *Glochidion*, which is characterised by having three stamens in male flowers (Li 1994).

Materials and methods

In addition to fieldwork in mountain areas of the eastern Myanmar, the present study also included analyses of *Glochidion* material from herbaria HITBC, IBSC, K, KUN, P, PE and US, as well as consideration of the taxonomic literature of China (Li 1994, Li and Gilbert 2008), India (Hooker 1887, Balakrishnan and Chakrabarty 2007, Chakrabarty and Balakrishnan 2009), Indo-China Peninsula (Beille 1927), Myanmar (Kurz 1877, Kress et al. 2003), Thailand (van Welzen 2007) and Vietnam (Nguyen 2007). Morphological analyses in the present study were performed on live plants in the field and specimens deposited in herbaria. Herbarium abbreviations follow the Index Herbarium (Thiers 2018+).

Taxonomy

Glochidion shanense Gang Yao & Shixiao Luo, sp. nov.

urn:lsid:ipni.org:names:77177610-1

Figures 1–2

Diagnosis. The species is similar to *G. ellipticum* Wight in morphology, but differs from the latter by its branchlets pubescent, pedicels of female flowers 1.5–2 mm long, sepals of female flowers uniseriate and narrowly triangular, apex of style truncate slightly, persistent style of fruit stout and cylindrical.

Type. MYANMAR. Shan State, Pindaya, near the Htwet Ni village, West of Pindaya town, at an elevation of 1396 m, forest understory, in flowering and fruiting, 25 December 2015, *Jie Cai et al.* 15CS10794 (holotype, KUN!; isotype, KUN!, IBSC!)

Description. Shrubs or treelets, up to 2 m; monoecious; branchlets pubescent. Leaf blade oblong or elliptic, 9–13.5 × 4.5–6.5 cm, papery, slightly leathery, with apex acuminate to round and base broadly cuneate, sparsely pubescent along veins adaxially, pubescent abaxially; midvein and 6–9-paired lateral veins elevated abaxially. Petiole 3–4 mm long, pubescent. Stipules narrowly triangular, 2–4 mm long, pubescent. Male flowers: pedicels 6–10 mm long, densely tomentose; sepals 6, biseriate, oblong or ovate, densely tomentose; stamens 3. Female flowers: in axillary clusters, pedicels 1.5–2 mm long, densely strigose; sepals 6, uniseriate, narrowly triangular, densely strigose; ovary depressed globose, 4–5-locular, densely strigose; style connate into a cylinder, ca. 1 mm long, slightly truncate at apex, apex 4–5-lobed. Capsules depressed globose, 8–9 mm in diameter, ca. 4 mm high, sparsely pubescent, 8–10-grooved; persistent style cylindrical, ca. 1 mm long; fruiting pedicels 4–5 mm long, stout, densely pubescent; seed laterally compressed, orange.

Distribution and habitat. This new species is currently known only from its type locality, Shan State, eastern Myanmar, where it grows in the broadleaved and coniferous dry forest dominated by *Docynia indica* (Wall.) Decne., *Schima wallichii* (DC.) Korth. and *Pinus* species.

Etymology. *Glochidion shanense* is named after its type locality, Shan State in eastern Myanmar.

Taxonomic discussion. The species resembles *Glochidion ellipticum* Wight, a species widely distributed from eastern Himalaya to Taiwan Island, but differs from the latter by its branchlets densely pubescent (Figure 2G), pedicels of male flowers tomentose, pedicels of female flowers densely strigose and up to 1.5–2 mm long (Figures 2A, D), sepals of female flower uniseriate and narrowly triangular in shape (Figures 2A, D), ovaries 4–5-locular, style cylindrical and truncate at apex (Figure 2D), fruits 8–9 mm in diameter and 8–10-grooved (Figure 2G), persistent styles cylindrical and ca. 1 mm long (Figure 2G). In contrast, *G. ellipticum* has the branchlets glabrous (Figures 2B, H), pedicels of male flowers glabrous (Figure 2H), female flowers sessile (Figures 2B, E), sepals of female flowers biseriate and oblong in shape (Figure 2B), ovaries 3–4 (5)-locular, style columnar to columnar-conical (Figures 2B, E), fruits 6–8 (rarely up to 10) mm in diameter and shallowly 6–8 (rarely 10)-grooved (Figure 2H), persistent styles obscure or slightly elevated but far less than 1 mm long (Figure 2H).

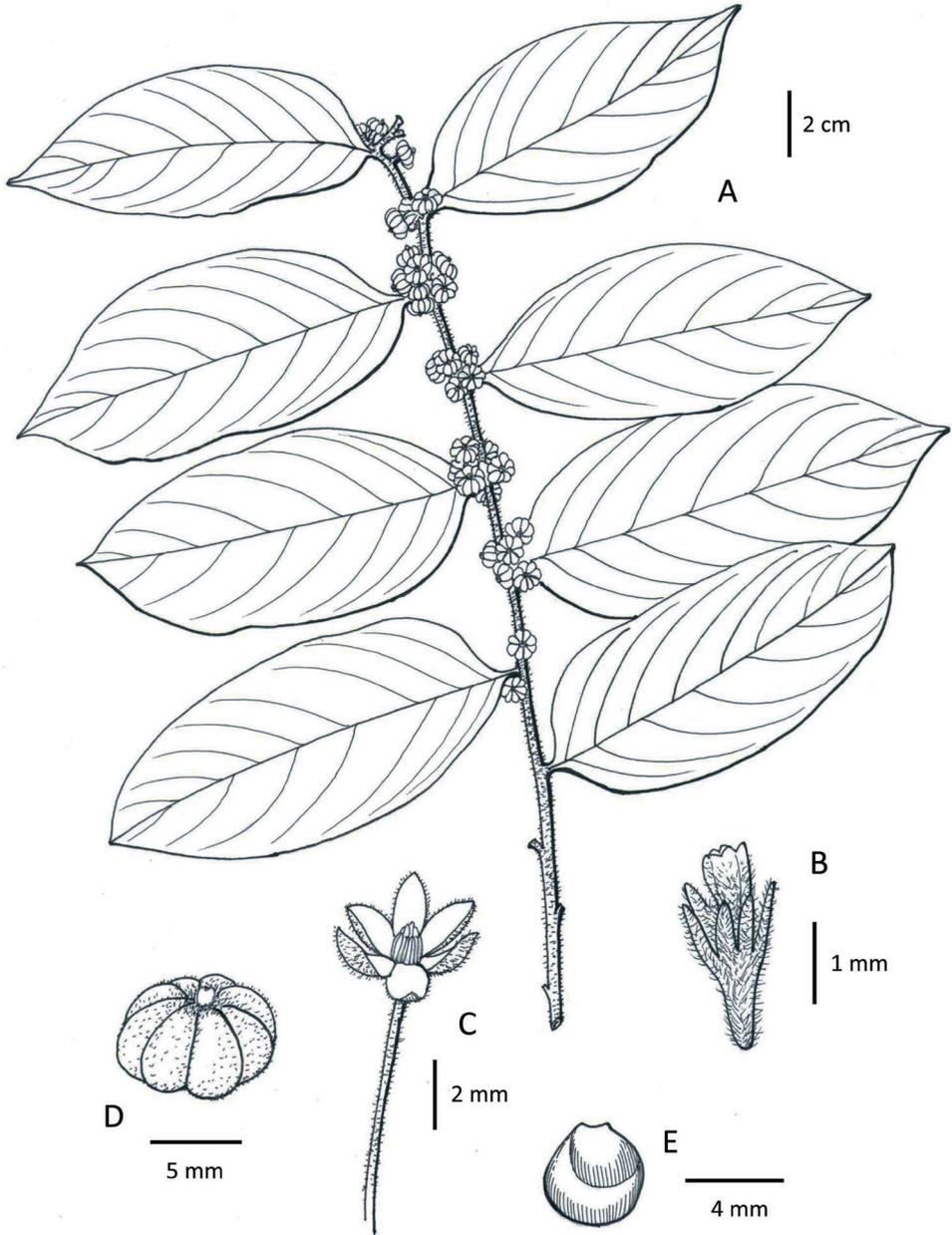


Figure 1. Line drawing of *Glochidion shanense* Gang Yao & Shixiao Luo, sp. nov. **A** Habit **B** Female flower **C** Male flower **D** Fruit **E** Seed. Draw by Ling Wang on *Jie Cai et al. 15CS10794* (KUN).

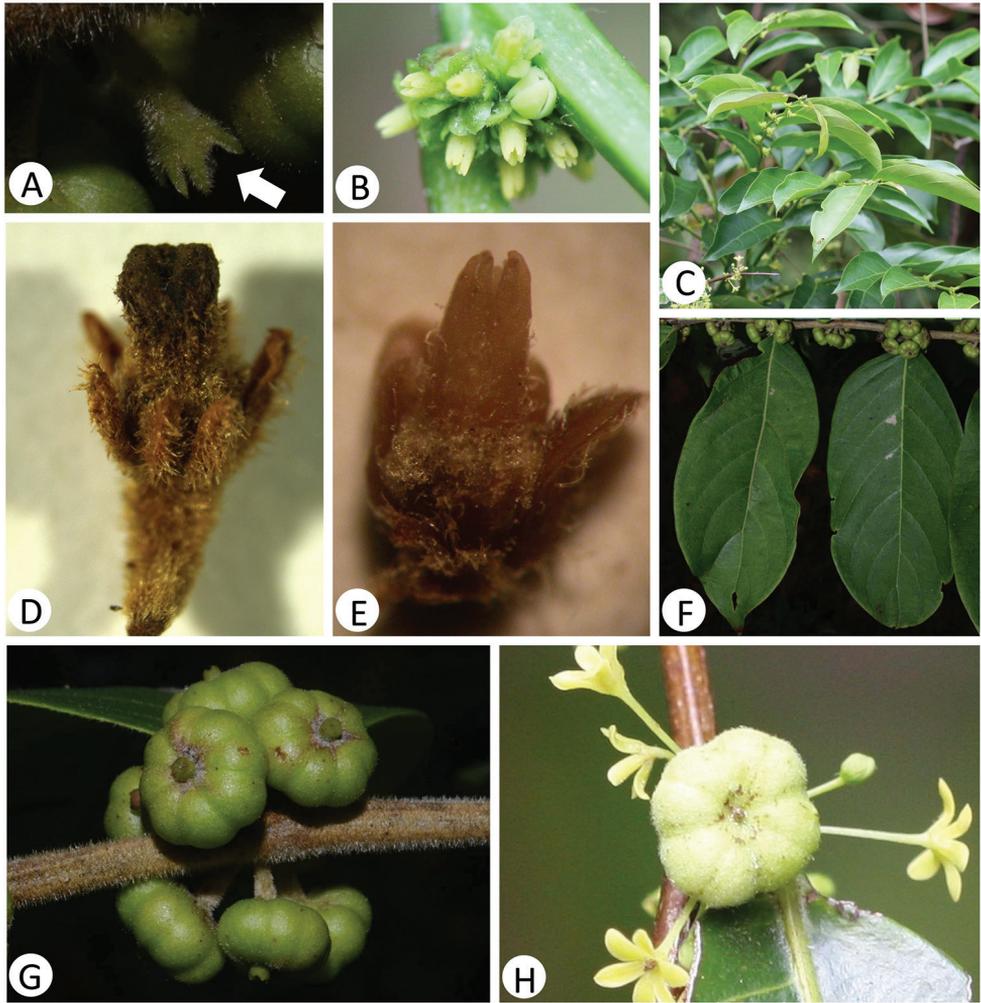


Figure 2. Morphological comparisons between *Glochidion ellipticum* Wight (**B, C, E, H**) and *G. shanense* Gang Yao & Shixiao Luo (**A, D, F, G**). **A, B, D, E** Female flower **C, F** Branchlets **G, H** Fruit.

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References

- Balakrishnan NP, Chakrabarty T (2007) The family Euphorbiaceae in India: A synopsis of its profile, taxonomy and bibliography. Bishen Singh and Mahendra Pal Singh, Dehradun, 500 pp.
- Beille L (1927) *Glochidion*. In: Lecomte H (Ed.) Flore générale de l'Indo-Chine 5. Masson, Paris, 608–628.
- Chakrabarty T, Balakrishnan NP (2009) The family Euphorbiaceae in Sikkim State, India. *Journal of Economic and Taxonomic Botany* 33: 483–539.
- Govaerts R, Frodin DG, Radcliffe-Smith A, Carter S (2000) World checklist and bibliography of Euphorbiaceae (Pandaceae). Royal Botanic Garden, Kew, UK, 1622 pp.
- Hooker JD (1887) The Flora of British India 5. Macmillan, London, 910 pp.
- Li PT (1994) Flora Reipublicae Popularis Sinicae 44. Science Press, Beijing, 217 pp.
- Li PT, Gilbert MG (2008) *Glochidion*. In: Wu ZY, Raven PH (Eds) Flora of China 11. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis, 193–202.
- Luo SX, Yao G, Wang ZW, Zhang DX, Hembry DH (2017) A novel, enigmatic basal leafflower moth lineage pollinating a derived leafflower host illustrates the dynamics of host shifts, partner replacement, and apparent coadaptation in intimate mutualisms. *The American Naturalist* 189: 422–435. <https://doi.org/10.1086/690623>
- Kato M, Takimura A, Kawakita A (2003) An obligate pollination mutualism and reciprocal diversification in the tree genus *Glochidion* (Euphorbiaceae). *Proceedings of the National Academy of Sciences of the United States of America* 100: 5264–5267. <https://doi.org/10.1073/pnas.0837153100>
- Kawakita A, Kato M (2009) Repeated independent evolution of obligate pollination mutualism in the Phyllentheae-*Epicephala* association. *Proceedings of the Royal Society of London B: Biological Sciences* 276: 417–426. <https://doi.org/10.1098/rspb.2008.1226>
- Kawakita A, Mochizuki K, Kato M (2015) Reversal of mutualism in a leafflower-leafflower moth association: the possible driving role of a third-party partner. *Biological Journal of the Linnean Society* 116: 507–518. <https://doi.org/10.1111/bij.12633>
- Kress J, Robert A, DeFilippes E, Kyi YY (2003) A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar. <http://www.botany.si.edu/myanmar> [Accessed 3 February 2018]
- Kurz S (1877) Forest Flora of British Burma 2. Office of the superintendent of government printing, Calcutta, 613 pp.
- Nguyen NT (2007) Taxonomy of Euphorbiaceae in Vietnam. Vietnam National University Publishers, Hanoi, 407 pp.
- Thiers B (2018+) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. The New York Botanical Garden, New York. Available from: <http://sweetgum.nybg.org/ih/> [Accessed: 3 February 2018]
- van Welzen PC (2007) *Glochidion*. In: Chayamarit K, van Welzen PC (Eds) Flora of Thailand 8(2). Royal Forest Department, Bangkok, 308–331.
- Webster GL (1994) Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* 81: 33–144. <http://www.jstor.org/stable/2399909>

Comparative anatomical studies of some *Teucrium* sect. *Teucrium* species: *Teucrium alyssifolium* Stapf, *Teucrium brevifolium* Schreb. and *Teucrium pestalozzae* Boiss. (Lamiaceae)

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Abstract

Teucrium alyssifolium Stapf (endemic), *Teucrium pestalozzae* Boiss. (endemic) and *Teucrium brevifolium* Schreb. are three closely related taxa in *Teucrium* sect. *Teucrium*. The obtained data from the anatomical studies revealed that these three taxa represent the general anatomical characteristics of the Lamiaceae family. Leaves, anatomical features such as thick cuticle, abundant trichomes, rich palisade parenchyma layer in the mesophyll provide evidence that these three species are xeromorphic structures. Leaf and stem anatomy showed that the taxa have generally similar anatomical features. However, cuticle layers, epidermis cells size, indumentum density, mesophyll types, palisade parenchyma occupied in the mesophyll, presence of spherocrystals in leaves and parenchyma, collenchyma and sclerenchyma layers in stems show differences amongst the taxa. Anatomical characters of leaf and stem of these taxa are examined for the first time in this study.

Keywords

Lamiaceae, *Teucrium*, leaf anatomy, stem anatomy

Introduction

The genus *Teucrium* L. has approximately 300 species all over the world. *Teucrium*'s cosmopolitan distribution is mainly concentrated in Europe, North Africa and in the temperate parts of Asia (Ecevit-Genç et al. 2015, 2017). *Teucrium* is a large and polymorphic genus which is represented by 49 taxa (36 species) in Turkey. There are 18 endemic taxa (Goovaerts 1999, Duman 2000, Dönmez 2006, Parolly and Eren 2007, Dönmez et al. 2010, Dinç et al. 2011a, 2011b, Dirmenci 2012, Özcan et al. 2015a, Vural et al. 2015, Dinç and Doğu 2016). The classification of *Teucrium* is based on sections. The main characters in the separation of sections are the calyx-shape and flower arrangement (Davis 1982; Navarro et al. 2004; Dinç et al. 2008; Özcan et al. 2015a; Vural et al. 2015). Especially, leaf anatomy is important for the classification of the genus (Dinç et al. 2009). Also, absence or presence of trichomes and their types on the nutlets and vegetative parts are very important for classifying the species (Dinç et al. 2011a, Ecevit-Genç et al. 2015, 2017).

Teucrium species have traditionally been used in Turkey for abdominal pain, stomach-ache, common cold, high fever, antipyretic, rheumatic pain and as an antidiabetic (Sezik et al. 2001, Aksoy-Sagirli et al. 2015).

T. alyssifolium is a narrowly distributed endemic species. It is classified as a 'Conservation Dependent (LR/cd)' category of IUCN and it is a source of polyphenols and flavonoids and has confirmed antioxidant activities (Semiz et al. 2016). *T. pestalozzae* is an endemic species and its essential oil is characterised with β -caryophyllene (27.6%) and germacrene D (13.8%) as major constituents (Baser et al. 1997). Spathulenol and δ -cadinene are the main compounds of *T. brevifolium* essential oil and it has shown anti-tumour activities, a selective cytotoxicity on large lung carcinoma (IC₅₀ value of 80.7 μ g/ml) (Menichini et al. 2009).

The chromosome numbers are reported as $2n = 10, 14, 16, 18, 22, 26, 28, 30, 32, 36, 39, 48, 52, 56, 58, 60, 62, 64, 78, 80, 86, 90, 96$ and 104 in the genus *Teucrium* (<http://www.tropicos.org/Project/IPCN>). The chromosome number of *T. brevifolium* examined in this study was determined as $2n = 30$. Another member of the sect. *Teucrium*, *T. sandrasicum*, was studied and it was determined that the chromosome number is the same as *T. brevifolium* (Özcan et al. 2015b).

Pollen morphology supplies useful data at the taxonomic level in *Teucrium* (Navarro et al. 2004, Marzouk et al. 2017). Oybak and İnceoğlu (1988) studied pollen morphology of some Turkish *Teucrium* members. They found out that the species belonging to different sections had different pollen type and pollen shape, while pollen grain size and apocolpia size were the main characters used for distinguishing the species. Especially, *T. alyssifolium* could be easily separated from the other species of the sect. *Teucrium* according to pollen data.

There are several studies on *Teucrium* anatomy (Lakusic et al. 2006, 2010, Dinç et al. 2008, 2009, 2011a, 2011b, Dehshiri and Azadbakht 2012, Dinç and Doğu 2012, Doğu et al. 2013, Özcan 2013, Özcan and Eminagaoglu 2014, Ruiters et al. 2016). However, the anatomy of *T. pestalozzae*, *T. brevifolium* and *T. alyssifolium* has not been investigated. In our previous studies, we investigated the nutlet and leaf micromorphol-

ogy of some species belonging to the sect. *Teucrium* in Turkey (Ecevit-Genç et al. 2015, 2017). In the present study, we report on the anatomical features of the leaves and stems of *T. alyssifolium*, *T. brevifolium* and *T. pestalozzae*. The aim of this paper is to understand the anatomy of these three *Teucrium* species. Also, a better understanding of systematics helps the distinction of morphologically closely related taxa from each other.

Materials and methods

T. pestalozzae samples were collected from Antalya, *T. brevifolium* and *T. alyssifolium* samples were collected from Muğla provinces in Turkey (Figures 1, 3, 5). Voucher specimens are stored in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE). Data about habitats of each investigated species are given in Table 1. Permanent microscopic preparations were made of plant materials fixed in 70% alcohol during the field studies. Cross-sections of the plant leaves and stems were taken manually and stained with Sartur solution (Çelebioğlu and Baytop 1949). Several slides were made and photographed for each species with an Olympus BH-2 and Canon A 640 digital camera.

Results

The anatomy of the collected specimens were assessed by examination of leaf and stem cross sections (Figures 2, 4, 6). This is the first study about the anatomical features of the leaves and stems of *T. alyssifolium*, *T. brevifolium* and *T. pestalozzae*.

T. alyssifolium Stapf

Leaf anatomy

The epidermis at the both surfaces of the leaves is single layered. The epidermis consists of single-layer, ovoid or rectangular cells which are covered by thick cuticula. The upper epidermis cells are larger than the lower ones. Both leaf surfaces are covered by glandular and non-glandular trichomes. Also, the upper epidermis is covered with

Table 1. Collection data of *Teucrium* taxa studied.

| Taxon | Locality, Voucher number (ISTE) |
|------------------------|--|
| <i>T. brevifolium</i> | Muğla: Marmaris-Knidos, Datça peninsula, 30–100 m elev., 16 May 2012, <i>T. Özcan</i> , <i>T. Dirmenci</i> , <i>O. Yıldırım</i> , ISTE 101442. |
| <i>T. pestalozzae</i> | Antalya: Between Antalya and Burdur, Çubuk Beli gateway, 950–1000 m elev., 17 May 2012, <i>T. Özcan</i> , <i>T. Dirmenci</i> , <i>O. Yıldırım</i> , ISTE 101448. |
| <i>T. alyssifolium</i> | Muğla: Fethiye-Çameli road, Tuzla Beli gateway, 1440 m elev., 14 April 2011, <i>T. Özcan</i> , <i>T. Dirmenci</i> , <i>E. Akçiçek</i> , ISTE 101443. |



Figure 1. *T. abyssifolium*. **A** habitus **B** inflorescence **C** flower **D** leaf.

lower-density trichomes than the lower epidermis. The spherocrystals occur in the upper epidermis cells of the leaf in *T. allysifolium*. Leaves are isolateral. The mesophyll is differentiated into 1 layered palisade and 2–3-layered spongy parenchyma. The palisade parenchyma cells are under the upper and lower epidermis.

Their shapes are cylindrical in transverse section. The palisade parenchyma occupies about 60–65% of the mesophyll. The spongy parenchyma cells, ovoid or circular, are located between the palisade tissues. Both parenchyma tissues contain starch grains. The midrib has 3–4 layered collenchyma and 1–2 layered parenchyma below the lower epidermis. The vascular bundle is located in the central part of the midvein. Vascular bundles are collateral. The xylem layer is just below the collenchyma. 1–2 layered parenchyma and 5–6 layered collenchyma are located under the phloem (Figure 2A).

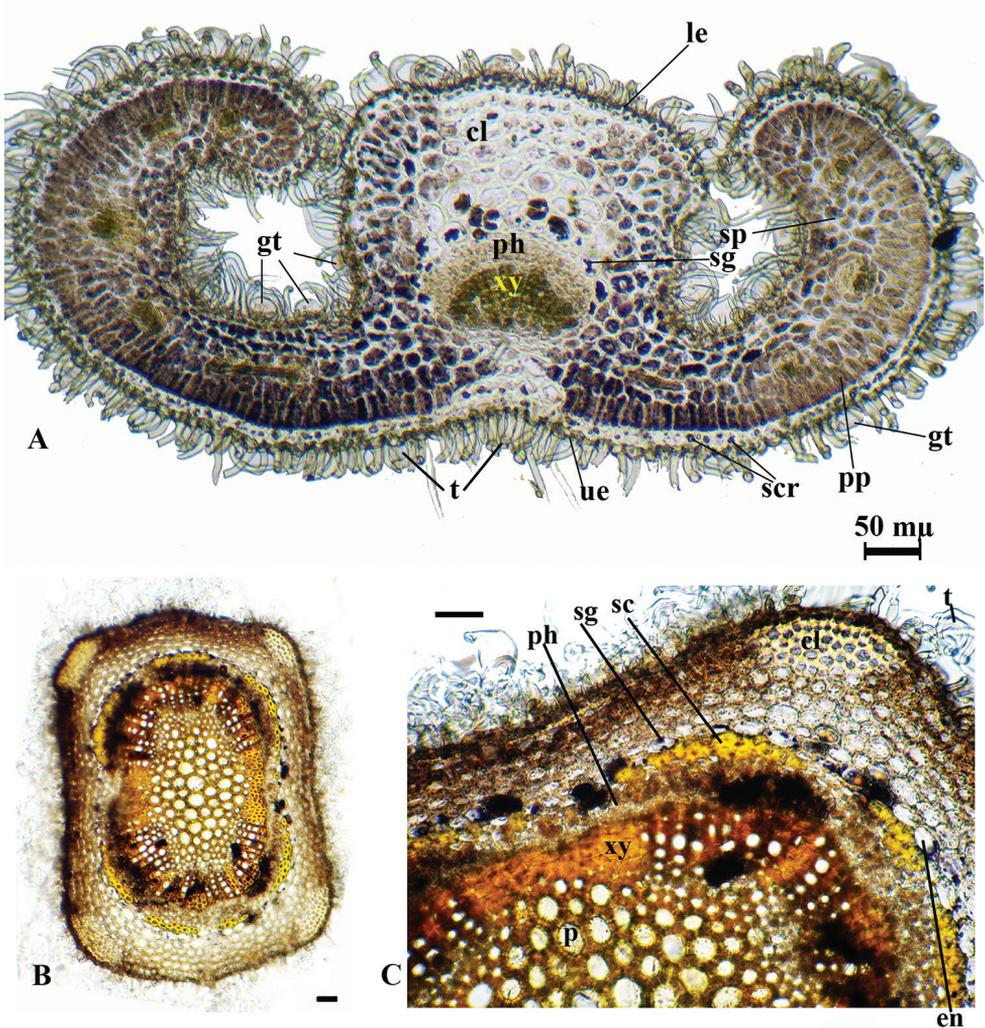


Figure 2. *T. alyssifolium*, cross-section of the leaf (A), stem (B, C); cl: collenchyma; en: endodermis; gt: glandular trichomes; le: lower epidermis; p: parenchyma; ph: phloem; pp: palisade parenchyma; sc: sclerenchyma; scr: sphaerocrystal; sg: starch grains; sp: spongy parenchyma; t: trichome; ue: upper epidermis; xy: xylem; Scale bars: 50 µm.

Stem anatomy

The stem is quadrangular shaped. The epidermis consists of single-layer, ovoid or rectangular cells which are covered by thick cuticula. There are glandular and non-glandular trichomes on the epidermis. Collenchyma with a single layer of cells between the corners but 4–5 layers of collenchyma at the corner of the stem. The cortex, consisting of 3–4 layered ovoidal parenchymatous cells, is located under the collenchyma. The endodermis is conspicuous as a single layer. The vascular tissue is surrounded by 1–2

layers of sclerenchyma fibres. The cambium is indistinguishable. Phloem and xylem members are conspicuous. The pith is present at the middle of the stem and it is completely filled by orbicular parenchymatic cells (Figure 2B, C).

Teucrium brevifolium Schreb.

Leaf anatomy

The epidermis in both surfaces of the leaves is single layered. It consists of single-layered, ovoid or rectangular cells which are covered by cuticula. Both surfaces are covered by a thick cuticula layer, with dense indumentum built of glandular and non-glandular trichomes. The upper epidermal cells are as large as the lower ones. Spherocrystals are observed in both epidermis cells. Leaves are dorsiventral. Palisade parenchyma has two layers and palisade parenchyma cells shapes are cylindrical in transverse section. The palisade parenchyma occupies about 60% of the mesophyll. Spongy parenchyma consists of four or five layers and their cells are ovoid or circular.

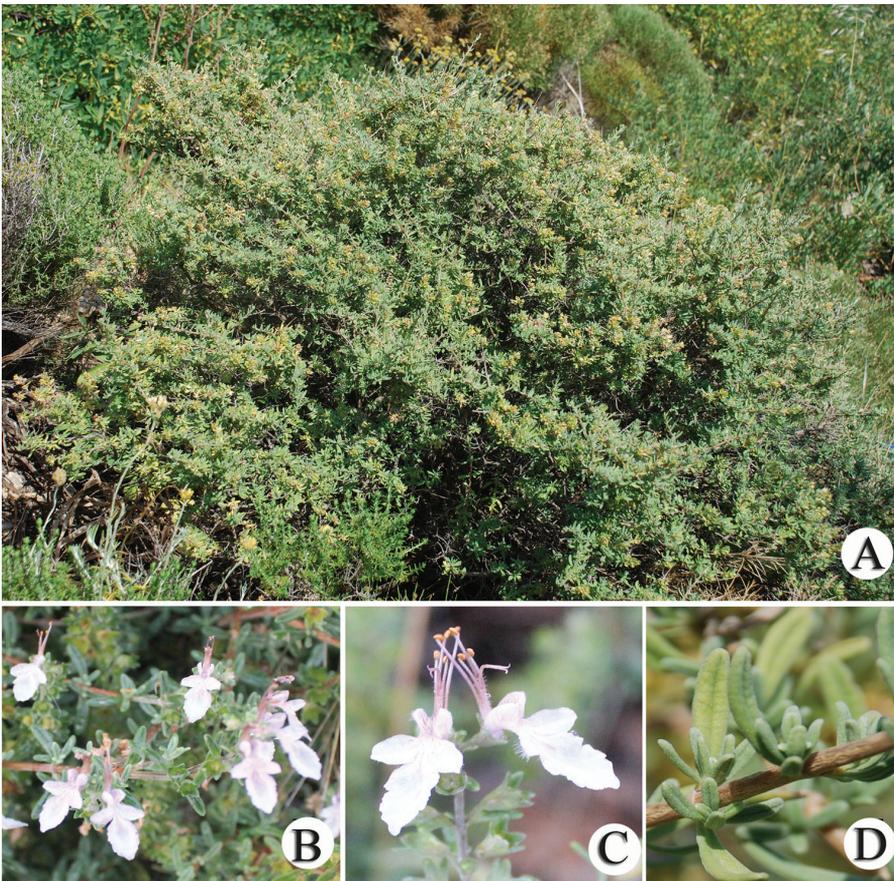


Figure 3. *T. brevifolium*. **A** habitus **B** inflorescence **C** flower **D** leaf.

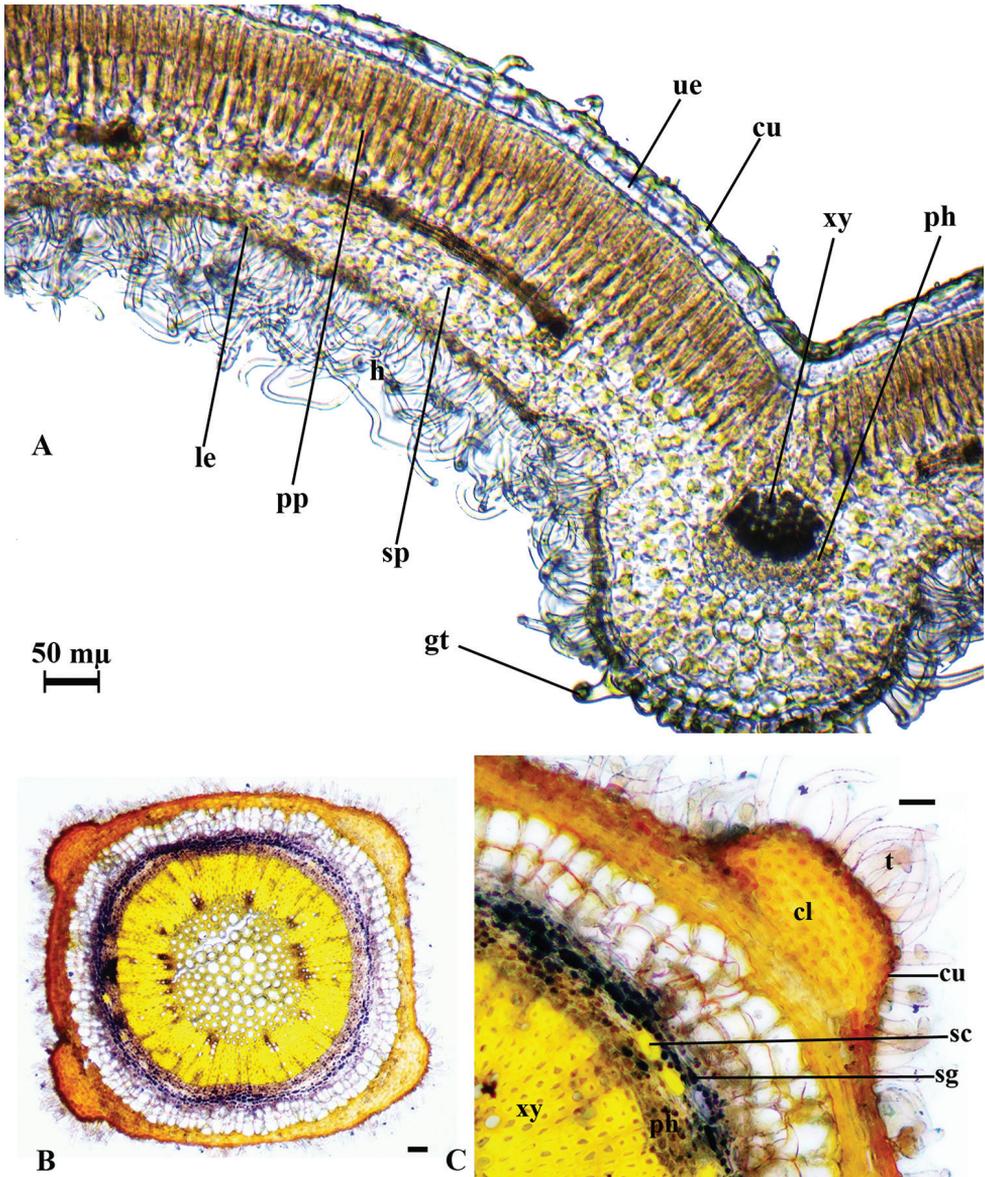


Figure 4. *T. brevifolium*, cross-section of the leaf (**A**), stem (**B, C**); **cl**: collenchyma; **cu**: cuticle; **gt**: glandular trichomes; **le**: lower epidermis; **ph**: phloem; **pp**: palisade parenchyma; **sc**: sclerenchyma; **scr**: sphaerocrystal; **sg**: starch grains; **sp**: spongy parenchyma; **t**: trichome; **ue**: upper epidermis; **xy**: xylem; Scale bars: 50 μm .

Starch accumulated in both spongy and palisade parenchyma. Midrib has 5–6 layered collenchyma and 1–2 layered parenchyma below the lower epidermis. The collateral vascular bundle is located in the central part of the midvein. The xylem layer is found under the collenchyma. 1–2 layered parenchyma and 2–3 layered collenchyma are located under the phloem (Figure 4A).

Stem anatomy

The stem is rectangular shaped. The epidermis consists of single-layer, ovoid or rectangular cells which are covered by thick cuticula. It is covered by glandular and non-glandular trichomes. Underneath the epidermis, 6–7 layers of collenchyma are located at the corners, 3–4 layered collenchyma is located between the corners. Beneath the collenchyma, 5–6 layered rectangle shaped parenchymatous cells are located. Starch grains are also present in the parenchymatous cells. Endodermis and cambium are inconspicuous. 2–3 sclerenchymatic cell clusters are situated at the corners above the phloem. The pith is present in the middle of the stem and is completely filled by orbicular parenchymatic cells (Figure 4B, C).

Teucrium pestalozzae Boiss.

Leaf anatomy

The epidermis in both surfaces of the leaves is single layered. It consists of single-layer, ovoid or rectangular cells which are covered by cuticula. The upper epidermis cells are larger than the lower ones. The upper cuticle layer is slightly thicker than the lower ones.

Both surfaces are covered by glandular and non-glandular trichomes. Also, trichomes are abundant on the lower epidermis of leaves and sparse on the upper epidermis of leaves. Leaves are dorsiventral. The spherocrystals occur in the upper epidermis of the mesophyll. Mesophyll is differentiated into 2-layered palisade and 5–6-layered spongy parenchyma. Palisade parenchyma cells are cylindrical shaped in transverse section. The palisade parenchyma occupies about 50–55% of the mesophyll.

The spongy parenchyma cells are ovoid or circular. Both parenchyma tissues densely contain starch grains. Midrib has 5–6 layered collenchyma and 1 layer of parenchyma below the lower epidermis. The collateral vascular bundle is located in the central part of the midvein. The xylem layer is found under the collenchyma. 1–2 layered parenchyma and 4–5 layered collenchyma are located under the phloem (Figure 6A).

Stem anatomy

The stem is rectangular shaped. The epidermis consists of single-layer, rectangular cells which are covered by cuticula. There are glandular and non-glandular trichomes on the epidermis. Underneath the epidermis, there is collenchyma with 1–2 layers between the corners but 7–8 layers of collenchyma at the corner of the stem. The cortex, consisting of 5–6 layers of ovoid shaped parenchymatous cells, is located under the collenchyma. 1–2 layers of sclerenchyma fibres are located above the phloem. The cambium is indistinguishable. Phloem and xylem members are conspicuous. The pith is present in the middle of the stem and is completely composed of orbicular parenchymatic cells (Figure 6B, C).



Figure 5. *T. pestalozzae*. **A** habitus **B** inflorescence **C** flower **D** leaf.

Discussion

Sect. *Teucrium* is one of the eight *Teucrium* sections distributing in Turkey. The members of this section are perennial and shrubs or subshrubs. Leaves are entire to deeply dissected (in *T. orientale* subspecies, *T. parviflorum*, *T. pruinosum*) and revolute at the lower surface.

Flowers borne in racemes or spreading panicles or axillary in upper leaves. Peduncles/pedicels are 1–3-flowered. Calyx not gibbous, obconical-campanulate, teeth \pm equal and triangular (Ekim 1982).

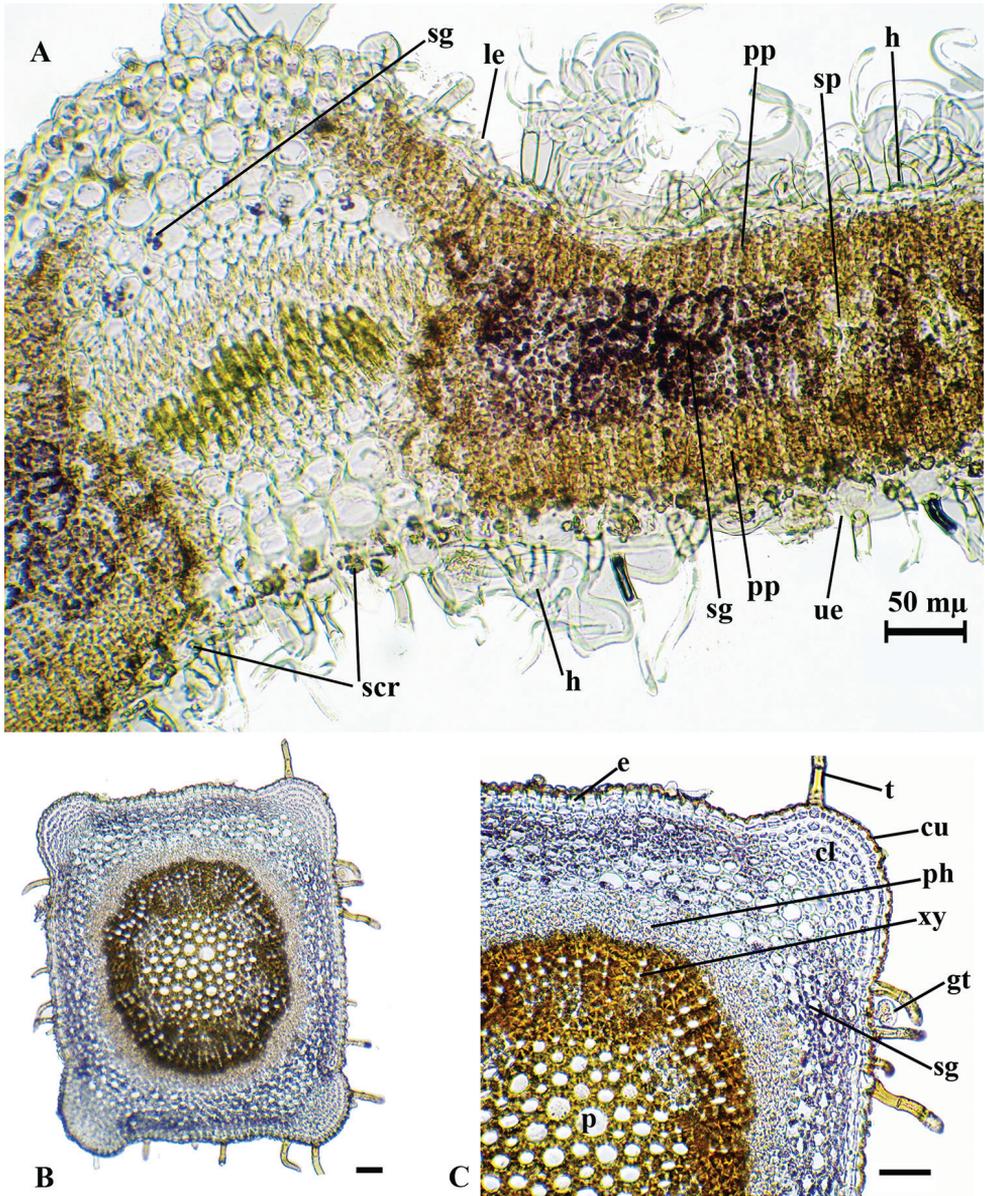


Figure 6. *T. pestalozzae*, cross-section of the leaf (A), stem (B, C); **cl**: collenchyma; **cu**: cuticle; **e**: epidermis; **gt**: glandular trichomes; **le**: lower epidermis; **p**: parenchyma; **ph**: phloem; **pp**: palisade parenchyma; **sc**: sclerenchyma; **scr**: sphaerocrystal, **sg**: starch grains; **sp**: spongy parenchyma; **t**: trichome; **ue**: upper epidermis; **xy**: xylem; Scale bars: 50 μ m.

Three species showing the characteristic features of the section *Teucrium*, investigated in the present study, have some significant distinguishing characters. Especially the size of parts of the flowers are very distinctive. *T. alyssifolium* easily differs with its leaf and bract shape, flower, filament and calyx size from *T. brevifolium* and *T. pestalozzae* in

their natural habitats (Table 2). *T. alyssifolium* is dwarf, suffruticose and *T. brevifolium* and *T. pestalozzae* are shrublet plants. Stamens of *T. alyssifolium* are longer than its lips, stamens subequal to lip in *T. pestalozzae* and slightly shorter than lips in *T. brevifolium*. *T. alyssifolium* has the shortest and *T. brevifolium* has the longest stems.

In this study, morphologically related three taxa belonging to the sect. *Teucrium* have been investigated. Moreover anatomical features of these three species have been reported for the first time. Our results showed the general anatomical characteristics of three *Teucrium* species as well those reported by Metcalfe and Chalk (1950) and Dinç et al. (2008, 2009, 2011a, 2011b), Lakusic et al. (2010), Doğu et al. (2013), Özcan (2013).

The results of the present study revealed that there were differences amongst the leaf anatomy of these three taxa (Table 2). The cuticle layer is on both sides and is of equal thickness to the epidermis for *T. alyssifolium* and *T. brevifolium* leaves. However, the upper cuticle layer of *T. pestalozzae* leaves is slightly thicker than the lower ones. The upper epidermis cells are larger than the lower ones as *T. alyssifolium* and *T. pestalozzae*, but both epidermis cells are the same size as in *T. brevifolium*. *T. alyssifolium* and *T. brevifolium* have a more dense indumentum than *T. pestalozzae*. Also, the indumentum of *T. brevifolium* has the same density on both sides, but the surface of the lower leaves of the other two species is denser than the upper ones. Mesophyll is dorsiventral in *T. brevifolium* and *T. pestalozzae* but isolateral in *T. alyssifolium*. The mesophilic organisation is an important distinguishing character for *T. alyssifolium*. Mesophyll types may be a good distinctive character in different species but sometimes it can be the same in some closer species (Erdoğan et al. 2012).

The palisade parenchyma shows a slight difference in mesophyll amongst the studied taxa. However, these differences can be based on different ecological conditions. Collenchyma layers are different in midrib amongst these studied taxa. The spherocrystals occur in the upper epidermis of the leaf in *T. alyssifolium* and *T. pestalozzae* and both epidermis of the leaf in *T. brevifolium*. According to Metcalfe and Chalk (1950), druse and simple crystals are generally seen in dicotyledon plants. Absence or presence of the crystals and their density are used to distinguish the genera and their species (Salimpour et al. 2009, Güvenç and Kendir 2012). However, spherocrystals and raphides are less common crystal types for dicotyledons. Spherocrystals and raphides have a diagnostic value for dicotyledons (Dinç et al. 2008, 2009, 2013, Ruiters et al. 2016). According to Dinç et al. (2008, 2009) and Ruiters et al. (2016), spherocrystals are an interspecific classification of sect *Teucrium*. Our results supported their observations.

Some characteristics of the leaf anatomy which indicates of xeromorphy have been reported before in previous studies (Metcalfe and Chalk 1983, Lakusic et al. 2010, Dinç et al. 2008, 2009). According to the results of our study, the three of taxa have leaves with xeromorphic features such as cuticula layer thickness, dense trichomes and a high proportion of the palisade parenchyma in the mesophyll.

In conclusion, this study shows that leaf and stem anatomy have a diagnostic value in the distinction of these three closely related *Teucrium* species in sect. *Teucrium*. Anatomical characters contribute to the separation of three species with the morphological characters.

Table 2. Morphological and anatomical comparison of studied taxa.

| | <i>T. abyssifolium</i> | <i>T. brevifolium</i> | <i>T. pestalozzae</i> | |
|---------------------|---|--|--|--|
| Flowers | Pedicellate | Pedicellate | Pedicellate | |
| Pedicels | 2.0–4.0 mm | 2.0–12 mm | 3.0–5.0 mm | |
| Bracts | Orbicular-ovate, oblanceolate | Linear, linear-oblanceolate | Linear-lanceolate | |
| Bract size | 2.0–20 × 1.5–5.0 mm | 3.0–9.0 × 1.0 mm | 10–12 mm | |
| Calyces | Campanulate | Campanulate | Campanulate | |
| Calyx size | 6.0–13 × 4.0–5.0 mm | (3.0-) 4.0–5.0 × 2.0–3.5 mm | 5.0–6.0 × 3.0–4.0 mm | |
| Calyx teeth | 4.0–5.0 mm | 1.0–2.0 × 1.0 mm | 2.5–3.0 mm | |
| Corolla | Lilac, light pinkish | White-pinkish | Bluish | |
| Corolla size | 20–25 mm | 6.5–7.0 mm | 9.0–9.5 mm | |
| Filaments | 18– 22 mm | 8.0–9.0 mm | 7.0–8.0 mm | |
| Style | 20–22 mm | 9.0–10 mm | 8.0–9.0 mm | |
| Leaf | Shape | Orbicular-ovate, oblanceolate | Linear or oblanceolate | Linear, obtuse or oblanceolate |
| | Size | 4.0–28 × 2.5–7.0 mm | 6.0–17 × 1.0–4.0 mm | 11–24 × 2.0–4.0 mm |
| | Leaf apex | Acute or obtuse at apex | Acute or obtuse at apex | Acute or obtuse at apex |
| | Cuticular thickness | Cuticular thickness | Equal | Equal |
| | Epidermal cells | Upper epidermis cells are larger than the lower ones. | Upper epidermal cells are as large as the lower ones | Upper epidermis cells are larger than the lower ones |
| | Indumentum density | Lower surface is denser than upper ones | Same density on both surface | Lower surface is denser than upper ones |
| | Mesophyll type | Isolateral | Dorsiventral | Dorsiventral |
| | Mesophyll | 60–65 % palisade parenchyma | 60% palisade parenchyma | 60–65 % palisade parenchyma |
| | Location of spherocrystals | Upper epidermis | Both epidermis | Upper epidermis |
| | Collenchyma cells layers of midrib | 3–4 layered on the lower surface, 5–6 layered on the upper surface | 5–6 layered on the lower surface, 2–3 layered on the upper surface | 5–6 layered on the lower surface, 4–5 layered on the upper surface |
| Stem | Length | 3.5–9.0 cm | 30–110 cm | 15–18 cm |
| | Collenchyma cells layers | 1 layer between the corners, 4–5 layered at the corners | 3–4 layers between the corners, 6–7 layers at the corners | 1–2 layered between the corners, 7–8 layers at the corners |
| | Cortex parenchyma | 3–4 layered | 5–6 layered | 5–6 layered |
| | Endodermis | Conspicuous | Inconspicuous | Inconspicuous |
| | Sclerenchyma | 1–2 layered | 2–3 cell clusters | 1–2 layered |

The stem is rectangle shaped in all species. In general, the stems of the family Lamiaceae species are rectangular (Metcalf and Chalk 1950, Dinç et al. 2008a, 2009, Kahraman et al. 2009, Çalı 2014) or in some genera not (Khalik 2016). However, the stems of the sect.

Polium species in Turkey are not conspicuously rectangular. Parenchyma, collenchyma and sclerenchyma layers have some differences amongst the stem of studied taxa. Endodermis is conspicuous only in *T. abyssifolium*. Three studied taxa display general characteristics of Lamiaceae anatomy.

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References

- Aksoy-Sagirli P, Ozsoy N, Ecevit-Genc G, Melikoglu G (2015) In vitro antioxidant activity, cyclooxygenase-2, thioredoxin reductase inhibition and DNA protection properties of *Teucrium sandrasicum* L. *Industrial Crops and Products* 74: 545–550. <https://doi.org/10.1016/j.indcrop.2015.05.025>
- Baser KHC, Demirçakmak B, Duman H (1997) Composition of the essential oils of three *Teucrium* species from Turkey. *Journal of Essential Oil Research* 9: 545–549. <https://doi.org/10.1080/10412905.1997.9700774>
- Çalı İÖ (2014) An anatomical study of medicinal species *Ajuga orientalis* L. (Lamiaceae) from Turkey. *Journal of Medicinal Plants Research* 8(6): 331–338.
- Çelebioğlu S, Baytop T (1949) Bitkisel tozların tetkiki için yeni bir reaktif. *Farmakolog* 19: 301.
- Dehshiri MM, Azadbakht M (2012) Anatomy of Iranian species *Teucrium polium* (Lamiaceae) *Journal of Biology and Today's World* 1(2): 48–52. <https://doi.org/10.15412/J.JBTW.01010204>
- Dinç M, Dogu S, Bilgili B, Duran A (2009) Comparative anatomical and micromorphological studies on *Teucrium creticum* and *Teucrium orientale* var. *orientale* (*Teucrium* sect. *Teucrium*, Lamiaceae). *Nordic Journal of Botany* 27: 251–256. <https://doi.org/10.1111/j.1756-1051.2008.00323.x>
- Dinç M, Doğu S (2012) Anatomical and micromorphological studies on *Teucrium* sect. *Isotriodon* (Lamiaceae) in Turkey with a taxonomic note. *Biologia* 67(4): 663–672. <https://doi.org/10.2478/s11756-012-0049-2>
- Dinç M, Doğu S, Doğru KA, Kaya B (2011a) Anatomical and nutlet differentiation between *Teucrium montanum* and *T. polium* from Turkey. *Biologia* 66(3): 448–453. <https://doi.org/10.2478/s11756-011-0035-0>
- Dinç M, Duran A, Pinar M, Ozturk M (2008) Anatomy, palynology and nutlet micromorphology of Turkish endemic *Teucrium sandrasicum* (Lamiaceae). *Biologia* 63(5): 637–641. <https://doi.org/10.2478/s11756-008-0137-5>

- Dinç M, Doğu S, Bağcı Y, (2011b) Taxonomic reinstatement of *Teucrium andrusi* from *T. paederotooides* based on morphological and anatomical evidences. *Nordic Journal of Botany* 29: 148–158. <https://doi.org/10.1111/j.1756-1051.2011.00894.x>
- Dinç M, Doğu S (2016) *Teucrium pruinosum* var. *aksarayense* var. nov. (Lamiaceae) from Central Anatolia, Turkey. *Modern Phytomorphology* (9): 13–17.
- Dirmenci T (2012) *Teucrium* L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT (Eds) *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 595–598.
- Doğu S, Dinç M, Kaya A, Demirci B (2013) Taxonomic status of the subspecies of *Teucrium lamiiifolium* in Turkey: Reevaluation based on macro-and micro-morphology, anatomy and chemistry. *Nord. J. Bot.* 31: 198–207. <https://doi.org/10.1111/j.1756-1051.2012.01452.x>
- Dönmez AA (2006) *Teucrium chasmophyticum* Rech. f. (Lamiaceae): A new record for the flora of Turkey. *Turkish Journal of Botany* 30: 317–320.
- Dönmez AA, Mutlu B, Özçelik AD (2010) *Teucrium melissoides* Boiss. & Hausskn. ex Boiss. (Lamiaceae): A new record for Flora of Turkey. *Hacettepe Journal of Biology and Chemistry* 38: 291–294.
- Duman H (2000) *Teucrium* L. In: Güner A, Özhatay N, Ekim T, Başer KHC (Eds) *Flora of Turkey and East Aegean Islands (Suppl. II)*. Edinburgh University Press, 197–198.
- Ekim T (1982). *Teucrium* L. In: Davis PH (Ed.) *Flora of Turkey and the East Aegean Islands*, Vol. 7. Edinburgh University Press, Edinburgh, 53–75.
- Ecevit-Genç G, Özcan T, Dirmenci T (2017) Nutlet and leaf micromorphology in some Turkish species of *Teucrium* L. (Lamiaceae). *Phytotaxa* 312(1): 71–82. <https://doi.org/10.11646/phytotaxa.312.1.5>
- Ecevit-Genç G, Özcan T, Dirmenci T (2015) Micromorphological characters on nutlet and leaf indumentum of *Teucrium* sect. *Teucrium* (Lamiaceae) in Turkey. *Turkish Journal of Botany* 39: 439–448. <https://doi.org/10.3906/bot-1406-18>
- Erdoğan E, Akçiçek E, Selvi S, Tümen G (2012) Comparative anatomical studies on the two *Stachys* species (sect. *Eriostomum*, subsect. *Germanicae*) growing in Turkey. *African Journal of Pharmacy and Pharmacology* 6(19): 1417–1427. <https://doi.org/10.5897/AJPP12.267>
- Govaerts R (1999) *World Checklist Seed Plants 3*. Continental Publishing, Deurne, 1532.
- Güvenç A, Kendir G (2012) The leaf anatomy of some *Erica* taxa native to Turkey. *Turkish Journal of Botany* 36(3): 253–262.
- Kahraman A, Celep F, Doğan M (2009) Comparative morphology, anatomy and palynology of two *Salvia* L. species (Lamiaceae) and their taxonomic implications. *Bangladesh Journal of Plant Taxonomy* 16(1): 73–82. <https://doi.org/10.3329/bjpt.v16i1.2749>
- Khalik KA (2016) A new species of *Plectranthus* (Lamiaceae) from Saudi Arabia. *Turkish Journal of Botany* 40: 506–513. <https://doi.org/10.3906/bot-1601-8>
- Lakusic B, Lakusic D, Jancic R, Stevanovic B (2006) Morpho-anatomical differentiation of the Balkan populations of the species *Teucrium flavum* L. (Lamiaceae). *Flora* 201: 108–119. <https://doi.org/10.1016/j.flora.2005.05.001>
- Lakusic B, Stevanovic B, Jancic R, Lakusic D (2010) Habitat-related adaptations in morphology and anatomy of *Teucrium* (Lamiaceae) species from Balkan peninsula (Serbia and Montenegro). *Flora* 205: 633–646. <https://doi.org/10.1016/j.flora.2010.04.018>

- Marzouk RI, Salama M, El-Darier M, Askar ABM (2017) Pollen morphology of *Teucrium* L. (Lamiaceae, Ajugoideae) in Libya. *Bangladesh Journal of Plant Taxonomy* 24(2): 219–226. <https://doi.org/10.3329/bjpt.v24i2.35118>
- Menichini F, Conforti F, Rigano D, Formisano C, Piozzi F, Senatore F (2009) Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. *Food Chemistry* 115(2): 15 679–686.
- Metcalf CR, Chalk L (1950) *Anatomy of the Dicotyledons I*. Oxford University Press, London, 1041–1053.
- Navarro T, El Oualidi J, Trigo MM (2004) Pollen morphology of *Teucrium* (Labiatae) and its taxonomic value. *Belgian Journal of Botany* 137(1): 70–84.
- Oybak E, İnceoğlu, Ö (1988) Pollen morphology of some *Teucrium* L. (Labiatae) species. *Communications Faculty of Sciences University of Ankara Series C: Biology* 6: 133–146. https://doi.org/10.1501/Commuc_0000000133
- Özcan M, Eminagaoglu O (2014) Stem and leaf anatomy of three taxa in Lamiaceae. *Pakistan Journal of Botany* 43(3): 345–352.
- Özcan T (2013) Presence of *Teucrium microphyllum* in Turkey: Morpho-anatomical, karyological and ecological studies. *Biodicon* 6: 79–87.
- Özcan T, Dirmenci T, Coskun F, Akcicek E, Güner Ö (2015a) A new species of *Teucrium* sect. *Scordium* (Lamiaceae) from SE of Turkey. *Turkish Journal of Botany* 39: 310–317. <https://doi.org/10.3906/bot-1402-93>
- Özcan T, Dirmenci T, Martin E, Altinordu F (2015b) Cytotaxonomical study in five taxa of the genus *Teucrium* L. (Lamiaceae). *Caryologia* 68(1): 1–8. <https://doi.org/10.1080/00087114.2014.996037>
- Parolly G, Eren Ö (2007) Contributions to the flora of Turkey. 2. *Willdenowia* 37: 245–246. <https://doi.org/10.3372/wi.37.37114>
- Ruiters AK, Tilney PM, Van Vuuren SF, Viljoen AM, Kamatou GPP, VanWyk BE (2016) The anatomy, ethnobotany, antimicrobial activity and essential oil composition of southern African species of *Teucrium* (Lamiaceae). *South African Journal of Botany* 102: 175–185. <https://doi.org/10.1016/j.sajb.2015.06.008>
- Salimpour F, Mazooji A, Onsoni S (2009) Stem and leaf anatomy of ten *Geranium* L. species in Iran. *African Journal of Plant Science* 3(11): 238–244.
- Semiz G, Çelik G, Gönen E, Semiz A (2016) Essential oil composition, antioxidant activity and phenolic content of endemic *Teucrium abyssifolium* Staph. (Lamiaceae). *Natural Product Research* 30(19): 2225–2229. <https://doi.org/10.1080/14786419.2016.1149703>
- Sezik E, Yeşilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T (2001) Traditional medicine in Turkey X. Folk medicine in Central Anatolia. *Journal of Ethnopharmacology* 75: 95–115. [https://doi.org/10.1016/S0378-8741\(00\)00399-8](https://doi.org/10.1016/S0378-8741(00)00399-8)
- Vural M, Duman H, Dirmenci T, Özcan T (2015) A new species of *Teucrium* Sect. *Stachyobotrys* (Lamiaceae) from South of Turkey. *Turkish Journal of Botany* 39: 318–324. <https://doi.org/10.3906/bot-1403-50>

Afropectinariella (Vandaeae, Orchidaceae), a new genus of the *Angraecum* alliance

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Abstract

A recent phylogenetic study showed that species assigned to the newly recognised genus *Pectinariella* Szlach., Mytnik & Grochocka (previously treated as *Angraecum* Bory sect. *Pectinaria* Benth.) are polyphyletic, comprising a clade with species primarily in Madagascar and the Western Indian Ocean islands (including the type) and another non-sister clade whose members occur in continental Africa and the Gulf of Guinea islands. In order to render *Pectinariella* monophyletic, the five continental African species must therefore be removed. A new genus, *Afropectinariella* M.Simo & Stévant, is described and the following combinations are made: *Afropectinariella atlantica* (Stévant & Droissart) M.Simo & Stévant, *Afropectinariella doratophylla* (Summerh.) M.Simo & Stévant, *Afropectinariella gabonensis* (Summerh.) M.Simo & Stévant, *Afropectinariella pungens* (Schltr.) M.Simo & Stévant and *Afropectinariella subulata* (Lindl.) M.Simo & Stévant.

Résumé

Une analyse phylogénétique récente a montré que les espèces attribuées au nouveau genre *Pectinariella* Szlach., Mytnik & Grochocka (anciennement *Angraecum* Bory sect. *Pectinaria* Benth.) forment un groupe polyphylétique, comprenant un clade avec des espèces présentes principalement à Madagascar et dans les îles de l'ouest de l'Océan Indien (incluant le type) et un autre clade qui ne lui est pas apparenté et dont les espèces sont distribuées en Afrique continentale et dans les îles du Golfe de Guinée. Afin de rétablir la monophylie du genre *Pectinariella*, les cinq espèces d'Afrique continentale et des îles du Golfe de Guinée doivent donc en être exclues. Un nouveau genre, *Afropectinariella* M.Simo & Stévant, est décrit et les combinaisons suivantes sont proposées: *Afropectinariella atlantica* (Stévant & Droissart) M.Simo & Stévant, *Afropectinariella doratophylla* (Summerh.) M.Simo & Stévant, *Afropectinariella gabonensis* (Summerh.) M.Simo & Stévant, *Afropectinariella pungens* (Schltr.) M.Simo & Stévant et *Afropectinariella subulata* (Lindl.) M.Simo & Stévant.

Keywords

Angraecoid orchids, continental Africa, Malagasy and Indian Ocean islands, *Pectinaria*, phylogenetics, taxonomy

Introduction

Since its description, the delimitation of *Angraecum* Bory (1804) has been controversial (Garay 1973). Much importance has been ascribed to the structure of the pollinia within *Angraecum*, which has led to the recognition of spurious genera to encompass closely related species (Schlechter 1913). Based on vegetative and floral features, along with geographic distribution, a number of partial or comprehensive revisions of the genus have been proposed over the decades (see Finet 1907; Garay 1973; Perrier de la Bathie 1941; Schlechter 1918, 1925; Senghas 1986; Stewart et al. 2006; Summerhayes 1958). Garay (1973) was the last to provide a full taxonomic treatment for *Angraecum*. He recognised 19 sections, of which nine are endemic to Madagascar and the Western Indian Ocean islands, one to Madagascar, the Western Indian Ocean islands and Sri Lanka, two to continental Africa and the Gulf of Guinea islands and seven have representatives in both Africa and Madagascar.

With the advent of molecular techniques, relationships within *Angraecum* have been explored in more detail over the last 15 years. The studies of Carlswald et al. (2006) on the phylogenetics of leafless angraecoids and of Micheneau et al. (2008) on the biogeography of Mascarene angraecoid orchids have shown the polyphyly of *Angraecum* and of at least five of the 19 recognised sections. In fact, African species of *Angraecum* belong to a group clearly distinct from that of members of the genus in Madagascar and the Indian Ocean islands (Micheneau et al. 2008). While investigating the diversification of the genus in Madagascar, Andriananjamanantsoa et al. (2016) also confirmed the polyphyly of *Angraecum s.l.* and of all *Angraecum* sections with representatives in Madagascar, with the sole exception of section *Hadrangis* Schltr. Agreeing with Micheneau et al. (2008), Szlachetko et al. (2013) indicated that most of the sectional

arrangements within the genus are unnatural. Based on molecular and morphological data, Szlachetko et al. (2013) raised most of the sections, *sensu* Schlechter (1918) and Garay (1973), to the rank of genus, albeit with a different placement for some species. However, their sampling of DNA material lacked more than 3/4 of the species of *Angraecum* (only 53 of the 221 currently recognised species were included in their phylogeny) and several sections were unrepresented. In all, Szlachetko et al. (2013) recognised 18 genera that included species previously placed within *Angraecum*, 12 of which resulted from raising sections to the generic level, five of which were described as new genera and one of which involved resurrecting a genus previously placed in synonymy.

Szlachetko et al. (2013) established the genus *Pectinariella* Szlach., Mytnik & Grochocka to accommodate the species of *Angraecum* sect. *Pectinaria* Benth. *sensu* Garay (1973) since the sectional name was already occupied at the generic level in another family (*Pectinaria* Haworth, Apocynaceae). They circumscribed *Pectinariella* to include all members previously assigned to sect. *Pectinaria* from continental Africa and the Gulf of Guinea islands as well as those from the western Indian Ocean islands. However, their molecular study lacked material of the type species, *A. pectinatum* Thouars and included only five of the ten species of *Angraecum* sect. *Pectinaria*: four from Africa (*viz.* *A. doratophyllum* Summerh., *A. gabonense* Summerh., *A. pungens* Schltr. and *A. subulatum* Lindl.) and one from Madagascar (*A. dasycarpum* Schltr.).

Cribb (2014) suggested that part of *Angraecum* sect. *Pectinaria* might be separated from *Angraecum sensu stricto*, given that the generitype, *A. eburneum* Bory, was placed in the large Malagasy/Mascarene clade identified by Micheneau et al. (2008). The polyphyly of the section was confirmed by Simo-Droissart et al. (2013) based on sequence data from all five currently recognised species from Africa (the four mentioned above plus *A. atlanticum* Stévant & Droissart), along with three species from Madagascar and the Mascarene Islands. Confirming the results of Micheneau et al. (2008), Simo-Droissart et al. (2013) showed that the African members of *A.* sect. *Pectinaria* formed a well-supported clade that appears to be most closely related to *A.* sect. *Dolabrifolia* Pfitzer and is not sister to the Malagasy/Mascarene clade, which includes the type species of the section.

Based on these results, Simo-Droissart et al. (2013) suggested that it would be necessary to remove the African species from *Angraecum* sect. *Pectinaria* in order to maintain its monophyly. The generic name *Pectinariella* proposed by Szlachetko et al. (2013) cannot, however, be applied to these African species since its type, *P. pectinata* (Thouars) Szlach., Mytnik & Grochocka, belongs to the clade comprising Malagasy/Mascarene taxa. Following the treatments of Szlachetko and Romowicz (2007) and Szlachetko et al. (2013), which treat the taxa generally placed in *A.* sect. *Dolabrifolia* as a genus, *Dolabrifolia* (Pfitzer) Szlach. & Romowicz and, since the African members of their polyphyletic genus *Pectinariella* are sister to those of *Dolabrifolia*, they must also be recognised at the rank of genus.

Here we thus propose a new genus to accommodate the species from continental Africa and the Gulf of Guinea islands of *Pectinariella*.

Taxonomy

Afropectinariella M.Simo & Stévant, gen. nov.

urn:lsid:ipni.org:names:60476170-2

Type. *Afropectinariella doratophylla* (Summerh.) M.Simo & Stévant [= *Angraecum doratophyllum* Summerh.].

Etymology. The name of the genus is based on the geographic distribution of its five species, all of which occur in Africa and the generic name *Pectinariella* in which they were previously placed.

Diagnosis. *Afropectinariella* resembles the related genera *Dolabrifolia* and *Pectinariella* in having a sessile ovary, i.e. without a pedicel and with a very short peduncle that is hardly developed, but differs from *Dolabrifolia* by its elongate leaves that are never compressed laterally (vs. imbricate and laterally compressed) and from *Pectinariella* by its transversely oval lip that is wider than long (vs. the lip longer than wide) and its occurrence in continental Africa and Gulf of Guinea islands (vs. Madagascar and adjacent islands).

Description. Epiphytic herbs. Stem erect to pendent, branched and loosely leafy. Leaves fleshy, alternate and elongate, subulate or linear to oblong-ovate, apex acute to apiculate, petiole twisted. Inflorescences suberect and subsessile, in general 1-flowered, sometimes 2-flowered, borne along the stem or opposite a leaf, sheath brown. Flowers small, nearly sessile in the axils of the leaves, white and often scented. Floral bract one, amplexicaul, broadly ovate. Sepals and petals elliptic to obovate, apex subacute. Lip entire, ovate-triangular, ecallose, apex acute to apiculate. Spur ellipsoid or subcylindric, straight or slightly curved, sometimes hooked, with a wide mouth at the base of the lip, apex acute, often blunt or swollen in the apical half. Peduncle short, hardly developed. Pollinia 2, pyriform, often sessile on one or two viscidia, without a distinct stipe or shortly stipitate, rarely with a well-developed stipe.

Afropectinariella includes five species from continental Africa and the Gulf of Guinea Islands, one of which is endemic to São Tomé and Príncipe Islands. Four of these five species were placed in *Angraecum* sect. *Pectinaria sensu* Garay (1973) and, more recently, in *Pectinariella*, as originally circumscribed by Szlachetko et al. (2013). A detailed taxonomic treatment of these five species is presented in Simo-Droissart et al. (2014).

Afropectinariella atlantica (Stévant & Droissart) M.Simo & Stévant, comb. nov.

urn:lsid:ipni.org:names:60476171-2

Basionym. *Angraecum atlanticum* Stévant et al. (2010: 253). Type: Equatorial Guinea (Rio Muni). Monte Alén National Park: Engong inselberg, 5 km NW from Engong village, 01°37'25.8"N, 10°17'49.2"E, 1,100 m alt., 20 July 2001, Stévant 1020 (holotype: BRLU!; isotypes: MO!, K!, WAG!).

***Afropectinariella doratophylla* (Summerh.) M.Simo & Stévant, comb. nov.**

urn:lsid:ipni.org:names:60476172-2

Basionym. *Angraecum doratophyllum* Summerhayes (1937: 465). Type: São Tomé and Príncipe (São Tomé Island) Vanhulst (Macambrará): virgin forest, 1,050–1,200 m alt., 5 November 1932, *Exell 254* (holotype: BM [BM000539167]! isotype: K [K000306497]!).

Homotypic synonym. *Pectinariella doratophylla* (Summerh.) Szlach., Mytnik & Grochocka

***Afropectinariella gabonensis* (Summerh.) M.Simo & Stévant, comb. nov.**

urn:lsid:ipni.org:names:60476173-2

Basionym. *Angraecum gabonense* Summerhayes (1954: 587). Type: Gabon. Upper Ngounyé River, Nimalaba, N. E. of Les Echiras, 15 February 1927, *Le Testu 6384* (holotype: K [K000306496]! isotypes: BM [BM000539175]! P [P00388248]!).

Homotypic synonym. *Pectinariella gabonense* (Summerh.) Szlach., Mytnik & Grochocka

***Afropectinariella pungens* (Schltr.) M.Simo & Stévant, comb. nov.**

urn:lsid:ipni.org:names:60476174-2

Basionym. *Angraecum pungens* Schlechter (1906: 163). Type: Cameroon. Man O'War Bay, auf Bäumen bei Kriegsschiffhafen, 03°57'N, 09°14'E, 27 September 1905, *Schlechter 15774* (holotype: B, destroyed; lectotype: K [K000107121]!, isolectotypes: BM [BM000539131]!, BR [BR000000642136]!).

Homotypic synonyms. *Angraecopsis pungens* (Schltr.) Rice; *Pectinariella pungens* (Schltr.) Szlach., Mytnik & Grochocka

Heterotypic synonyms. *Mystacydium arthrophyllum* Kraenzl.; *Angraecum arthrophyllum* (Kraenzl.) Schltr.

***Afropectinariella subulata* (Lindl.) M.Simo & Stévant, comb. nov.**

urn:lsid:ipni.org:names:77177891-1

Basionym. *Angraecum subulatum* Lindley (1837: 206). Type: Nigeria. Nun River: s. d., *Barter 20125* (lectotype: K!, isolectotype: K!).

Homotypic synonyms. *Epidorkis subulata* (Lindl.) Kuntze; *Listrostachys subulata* (Lindl.) Rchb.f.; *Pectinariella subulata* (Lindl.) Szlach., Mytnik & Grochocka

Heterotypic synonyms. *Angraecum canaliculatum* De Wild.; *Listrostachys canaliculata* De Wild.

Discussion

Although we have removed species from continental Africa and the Gulf of Guinea islands from *Pectinariella*, its delimitation remains open to further discussion. Garay (1973) included four species from Madagascar and the Mascarenes within *Angraecum* section *Pectinaria* (i.e. *A. dasycarpum*, *A. hermannii* (Cordem.) Schltr., *A. humblotianum* Schltr. and *A. pectinatum*). He regarded *A. pterophyllum* H.Perrier as a synonym of *A. hermannii* and placed *A. panicifolium* H.Perrier in *Angraecum* sect. *Conchoglossum* Schltr. In addition to the four species recognised by Garay (1973) as comprising section *Pectinaria*, Stewart et al. (2006) also included *A. panicifolium*. Micheneau et al. (2008) suggested that *A. hermannii* might best be placed in *A.* sect. *Lemurangis* Garay based on its morphological characters, which was followed by Cribb and Hermans (2009) and Verlynde et al. (2016). Cribb and Hermans (op. cit.) recognised *A. pterophyllum* as a distinct species and thus included five species within the section. As they followed the treatment of Garay (1973), Szlachetko et al. (2013) placed only four species from Madagascar and the Mascarenes within their newly described genus *Pectinariella* (*P. dasycarpa* (Schltr.) Szlach., Mytnik & Grochocka, *P. hermannii* (Cordem.) Szlach., Mytnik & Grochocka, *P. humblotiana* (Schltr.) Szlach., Mytnik & Grochocka and *P. pectinata*). In fact, these authors made no mention of *Angraecum pterophyllum*, but they did propose the combination *Angraecoides panicifolia* (H. Perrier) Szlach., Mytnik & Grochocka. Verlynde et al. (2016) described two new species of *Pectinariella* (*P. edmundi* Bosser ex Verlynde & Ramandimbisoa and *P. scroticalcar* Verlynde & Ramandimbisoa) and made the combination *P. pterophylla* (H.Perrier) Verlynde & Ramandimbisoa, thus recognising six species of *Pectinariella* from Madagascar and the Mascarenes. They did not mention *Angraecum panicifolium* or *Angraecoides panicifolia*, even though Simo-Droissart et al. (2013) placed it close to *Pectinariella pectinata*. *Pectinariella* thus comprises seven species, four of which have been included in published molecular phylogenetic studies. These seven species are found in Madagascar or neighbouring islands, including the type species, which occurs in La Réunion, Mauritius and the Comoros Islands.

In the studies of Simo-Droissart et al. (2013, 2016), species belonging to *Dolabrifolia* and *Afropectinariella* form two distinct, well-supported clades (bootstrap support (BS) = 100% and posterior probability (PP) = 1). The sister relationship of these two groups is also well supported by our Bayesian analysis (PP 1). Species assigned to *Pectinariella* also form a clade well supported in both analyses and are not sister to the clade comprising species of our new genus, *Afropectinariella*. The shared presence of elongate leaves that are never compressed, an ovary lacking a stalk and whose peduncle is scarcely developed appear to represent a convergence in *Pectinariella* and *Afropectinariella*. However, members of *Afropectinariella* possess a transversely oval lip that is wider than long, while those of *Pectinariella* have a lip that is longer than wide. Unpublished molecular data indicate that *P. edmundi* and *P. scroticalcar* are not sister to *P. pectinata*, suggesting that the genus, as circumscribed by Verlynde et al. (2016), may not be monophyletic. The fact that some *Angraecum* species assigned to section *Pseudojumellea* Schltr. are placed with *P. pectinata* in molecular phylogenies (see Micheneau et al. 2008) indicates that further studies will be needed to assess the monophyly of *Pectinariella*.

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References

- Andriananjamanantsoa HN, Engberg S, Louis Jr EE, Brouillet L (2016) Diversification of *Angraecum* (Orchidaceae, Vandaeae) in Madagascar: revised phylogeny reveals species accumulation through time rather than rapid radiation. *PloS ONE* 11: e0163194. <https://doi.org/10.1371/journal.pone.0163194>
- Bory de Saint Vincent JBG (1804) Voyage dans les quatre principales îles des mers d'Afrique. F. Buisson, Paris, 409 pp.
- Carlswald BS, Whitten WM, Williams NH, Bytebier B (2006) Molecular phylogenetics of Vandaeae (Orchidaceae) and the evolution of leaflessness. *American Journal of Botany* 93: 770–786. <https://doi.org/10.3732/ajb.93.5.770>
- Cribb PJ (2014) *Angraecum*. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (Eds) *Genera Orchidacearum Volume 6 Epidendroideae (Part 3)*. Oxford University Press, Oxford, 358–363.
- Cribb PJ, Hermans J (2009) *Field guide to the orchids of Madagascar*. Royal Botanic Gardens, Kew, 440 pp.
- Finet EA (1907) Classification et énumération des Orchidées africaines de la tribu des Sarcantées, d'après les collections du Muséum de Paris. *Bulletin de la Société Botanique de France* 54: 1–65. [Pl 1–12] <https://doi.org/10.1080/00378941.1907.10835937>
- Garay LA (1973) Systematics of the genus *Angraecum* (Orchidaceae). *Kew Bulletin* 28: 495–516. <https://doi.org/10.2307/4108894>
- Lindley J (1837) Notes upon some genera and species of Orchidaceae in the collection formed by Mr. Drège, at the Cape of Good Hope. In: Hooker WJ (Ed.) *Companion to the Botanical Magazine*, vol. 2. James Ridgway, London, 200–210.
- Micheneau C, Carlswald BS, Fay MF, Bytebier B, Pailler T, Chase MW (2008) Phylogenetics and biogeography of Mascarene angraecoid orchids (Vandaeae, Orchidaceae). *Molecular Phylogenetics and Evolution* 46: 908–922. <https://doi.org/10.1016/j.ympev.2007.12.001>
- Perrier de la Bathie H (1941) Orchidées, tome II. In: Humbert H (Ed.) *Flore de Madagascar*, 49 famille. Imprimerie Officielle, Tananarive, 202–321.

- Schlechter R (1906) *Orchidaceae africanae* IV. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 38: 144–165.
- Schlechter R (1913) *Orchidacées de Madagascar*. In: Heckel E (Ed.) *Annales du musée colonial de Marseille*. Musée colonial, Marseille, 148–202.
- Schlechter R (1918) Versuch einer natürlichen Neuordnung der afrikanschen angraekoiden *Orchidaceae*. *Beihefte zum Botanischen Centralblatt* 36: 62–181.
- Schlechter R (1925) *Orchidaceae Perrierianae*. *Fedde, Repertorium specierum novarum regni vegetabilis*, Beihefte 33: 306–360.
- Senghas K (1986) 15. Tribus Vandaeae, 48. Subtribus Angraecinae, 49. Subtribus Aerangidinae. In: Brieger FG, Maatsch R, Senghas K (Eds) *Rudolf Schlechter, Die Orchideen*. Paul Parey, Berlin, 975–1044.
- Simo-Droissart M, Micheneau C, Sonké B, Droissart V, Lowry II PP, Plunkett GM, Hardy OJ, Stévant T (2013) Morphometrics and molecular phylogenetics of the continental African species of *Angraecum* section *Pectinaria* (Orchidaceae). *Plant Ecology and Evolution* 146: 295–309. <https://doi.org/10.5091/plecevo.2013.900>
- Simo-Droissart M, Sonké B, Droissart V, Geerinck D, Micheneau C, Lowry II PP, Plunkett GM, Hardy OJ, Stévant T (2014) Taxonomic revision of the continental African species of *Angraecum* section *Pectinaria* (Orchidaceae). *Systematic Botany* 39: 725–739. <https://doi.org/10.1600/036364414X682184>
- Simo-Droissart M, Sonké B, Droissart V, Micheneau C, Lowry II PP, Hardy OJ, Plunkett GM, Stévant T (2016) Morphometrics and molecular phylogenetics of *Angraecum* section *Dolabrifolia* (Orchidaceae, Angraecinae). *Plant Systematics and Evolution* 302: 1027–1045. <https://doi.org/10.1007/s00606-016-1315-5>
- Stévant T, Cawoy V, Damen T, Droissart V (2010) Taxonomy of Atlantic Central African Orchids 1. A new species of *Angraecum* sect. *Pectinaria* (Benth.) Schltr. (Orchidaceae) from Gabon and Equatorial Guinea. *Systematic Botany* 35: 252–256. <https://doi.org/10.1600/036364410791638298>
- Stewart J, Hermans J, Campbell B (2006) *Angraecoid Orchids: Species from the African Region*. Timber Press, Portland, Oregon, 431 pp.
- Summerhayes VS (1937) African orchids: IX. *Kew Bulletin* 9: 457–476. <https://doi.org/10.2307/4107143>
- Summerhayes VS (1954) African orchids: XXII. *Kew Bulletin* 8: 575–591. <https://doi.org/10.2307/4117387>
- Summerhayes VS (1958) African Orchids: XXVI. *Kew Bulletin* 13: 257–281. <https://doi.org/10.2307/4109530>
- Szlachetko DL, Romowicz A (2007) *Dolabrifolia*, un nouveau genre d'orchidées de l'alliance *Angraecum*. *Richardiana* 7: 53–54.
- Szlachetko DL, Tukałło P, Mytnik-Ejsmont J, Grochocka E (2013) Reclassification of the *Angraecum*-alliance (Orchidaceae, Vandoideae) based on molecular and morphological data. *Biodiversity Research and Conservation* 29: 1–23. <https://doi.org/10.2478/biorc-2013-0004>
- Verlynde S, Ramandimisoa B, Bossier J, Stévant T (2016) Contribution à l'étude des *Orchidaceae* de Madagascar. XXXVIII. Deux nouvelles espèces et une nouvelle combinaison pour le genre *Pectinariella* Szlach., Mytnik & Grochocka à Madagascar. *Adansonia* 38: 219–232. <https://doi.org/10.5252/a2016n2a6>

The hidden *Heuchera*: How science Twitter uncovered a globally imperiled species in Pennsylvania, USA

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Abstract

The genus *Heuchera* is recognized as one of the most diverse endemic radiations of Saxifragaceae in North America, yet species delimitation and geographic distribution within the group remain controversial. Many species remain difficult to identify, including *Heuchera alba*, a narrow Appalachian endemic and globally imperiled (G2) taxon recorded only from West Virginia and Virginia that occurs in sympatry with *H. pubescens* and *H. americana*. A recent survey of the cliffside flora of the Shikellamy Bluffs, PA recorded dozens of *Heuchera* individuals that, through the use of social media, were positively identified as *H. alba*. Aided by examination of historical herbarium records, subsequent searches of similar habitats in Pennsylvania led to the discovery of seven more populations and established a significant range expansion for this rare species. The uncovering of *H. alba* in Pennsylvania is an exciting conservation outcome and an example of what can happen when botanists embrace a combination of modern and classical approaches to discovery and collaboration.

Keywords

Heuchera alba, rare species conservation, Susquehanna River Valley, social media networks, iNaturalist, Twitter

Introduction

The genus *Heuchera* represents one of the most diverse endemic radiations of family Saxifragaceae in North America, with approximately 43 species (~10 in eastern North America). Its species are persistently difficult to identify (Wells and Shipes 2009), not least due to frequent introgressive hybridization throughout its history (reviewed in Folk et al. 2017). Despite substantial past taxonomic efforts, species delimitation and geographic distributions remain controversial and poorly resolved in this group, with the result that six species (14% of the genus) have been described in the last decade, in addition to several dramatic species re-circumscriptions (Folk and Freudenstein 2015, Folk and Alexander 2015).

Heuchera alba Rydb. (white alumroot, Saxifragaceae) was first described in 1926 based on a collection made in 1925 from Snowy Mountain in Pendleton County, West Virginia (Rydberg 1926). Since that time, both major monographs of *Heuchera* covering the eastern United States (Rosendahl et al. 1936; Wells 1984) reduced *H. alba* to synonymy with *H. pubescens* Pursh (1814), the only other large-flowered species known from the northern Appalachians. In the Flora of North America treatment of *Heuchera* (Wells and Shipes 2009), *H. alba* was again recognized at the species level, although no rationale for the shift in taxonomic concept was given. Recent morphological and molecular analyses of *Heuchera* (Folk and Freudenstein 2014, Folk et al. 2017, Folk and Stubbs in review) are consistent with this recent recognition of *H. alba* at the species level and its sister relationship to *H. pubescens*. To date, its documented range is restricted to high elevation acidic sandstone ridges and outcrops in the Ridge-and-Valley province of West Virginia and Virginia. Due to its narrow distribution and low number of populations, *Heuchera alba* is considered a globally imperiled (G2) species with 23 extant population occurrences (i.e. those observed since 1990), consisting of a total of 1500 individual plants throughout its entire range. Critically, fewer than 15 of the populations are considered viable and many of them are under threat from trampling and/or housing developments on high mountain ridges (NatureServe 2017).

In the summer of 2017, a group of scientists and students conducted a survey of the cliffside flora above the bank of the West Branch Susquehanna River in the Shikellamy Overlook area of Shikellamy State Park, just across the river from the town of Northumberland, PA. The primary goal of the survey was to assess the status of the golden corydalis (*Corydalis aurea* subsp. *aurea* Willd., Papaveraceae), a state-endangered species only known in Pennsylvania from this single site, as part of a new episode of the YouTube video series, “Plants are Cool, Too!” (Martine et al. 2018). During the course of the survey, numerous specimens were identified as *Heuchera americana* L. (American alumroot) using the Plants of Pennsylvania flora (Rhoads and Block 2007), a resource recognizing *H. americana* L. and *H. pubescens* as the only *Heuchera* species present in the state. A photo of one specimen was posted to Twitter, initiating an electronic discussion that led to a series of new collecting trips and establishment of the first confirmed state records in Pennsylvania for *Heuchera alba*. Here, we re-evaluate the resurrection of *H. alba* from synonymy and review variation

in *Heuchera* species recorded beyond the borders of Pennsylvania to present conclusive evidence that *H. alba* should be added to the flora of the state. We discuss the extent of *H. alba* in Pennsylvania and the impact of this discovery on both our understanding of the overall distribution of this Appalachian endemic and its current conservation status.

Methods

Survey Sites. Visits to eight known *Heuchera* locations in the Ridge-and-Valley Ecoregion were made to collect fresh specimens for identification and determine population sizes. Based on herbarium label data and the habitat at Shikellamy State Park, a search image of potential suitable habitat was established to guide the surveys. GPS coordinates were recorded and geo-referenced images taken of the plants at each location. Using these data along with GPS data from annotated specimens, a map was generated in ArcGIS® Pro 2.0.1 (ESRI Redlands CA USA 2017) to illustrate the range expansion of *H. alba* into Pennsylvania. A general assessment of the habitat and site condition was performed to determine plant community associates and geological affinities.

Taxonomic Identification. Representative specimens were collected from each of the visited locations and deposited at the Wayne E. Manning Herbarium at Bucknell University (BUPL) and the Carnegie Museum Natural History Herbarium (CM). These specimens were used to make species determinations. Specimens were identified using the Flora of North America treatment for *Heuchera*, the Flora of Virginia, and a taxonomic key for *Heuchera* that is soon to be published in the revision of the Gleason and Cronquist Manual of Vascular Plants of Northeastern United States and Adjacent Canada (Wells and Shipes 2009, Weakley et al. 2012, Folk and Stubbs in review). Consequent examinations of *Heuchera* specimens identified as *H. americana* and *H. pubescens*, housed in the Manning Herbarium, revealed four specimens of *Heuchera alba*, all collected in Union County between 1905 and 1949 from the cliffs at and around Shikellamy State Park.

Conservation Rank Assessment. A standardized rank assessment method used by all heritage programs to assist with determining conservation statuses for species of concern is encapsulated in a rank calculator tool that analyzes populations at regional and global scales (NatureServe 2015). A population is defined as a collection of individuals of a species that is separated from the next collection of individuals by a minimum of 1 kilometer. An exception to this definition is those species that populate river corridors where the minimum separation distance is 10 kilometers. Using this definition, eight extant populations of *Heuchera alba* in Pennsylvania were analyzed to determine the state conservation status. The following parameters were used for the rank calculation. Range Extent: 5,000–20,000 km², Area of Occupancy: 1 (4 km² grid cell), Number of Occurrences: 6–20, Population Size: 250–1,000 individuals, Viability: 4–12 occurrences with good viability, Environmental Specificity: Narrow (specialist with key requirements), Threat Impact: Low.

Results

Pennsylvania populations of *Heuchera alba* grow primarily on exposed rock outcrops on cliffs and in shale woodland and barrens plant communities. Populations are found on four different acidic sandstone geologies, including Burgoon Sandstone, the Catskill and Foreknobs formations, and the Hamilton Group of siltstone (Table 1). With the exception of the Burgoon sandstone, which is Mississippian, the other populations are all found over Devonian geology (Miles and Whitfield 2001). These different sandstones are considered to have acidic pH ranges.

The eight extant populations of *H. alba* total between 250–1,000 individuals and range widely from northeastern Bedford County to southeastern Union County, but occupy <4 km² over that range extent in Pennsylvania (Figure 1). These populations extend the known range for *H. alba* approximately 200km northward in the Appalachian Ridge and Valley Ecoregion (Figure 1) The population at Shikellamy State Park is by far the most robust with between 400–800 individuals over a 0.7 km² area, while the remaining populations have fewer than 50 individuals. The Gundy’s Farm and Woy Bridge populations have fewer than 15 individuals. All factors, including potential threats, together yield a rank of critically imperiled at the state level (S1). However, uncertainty about the number of misidentified specimens in herbaria and the amount of unexplored available habitat in Pennsylvania suggests that a range rank of critically imperiled to imperiled (S1S2) is the more appropriate rank (Table 2).

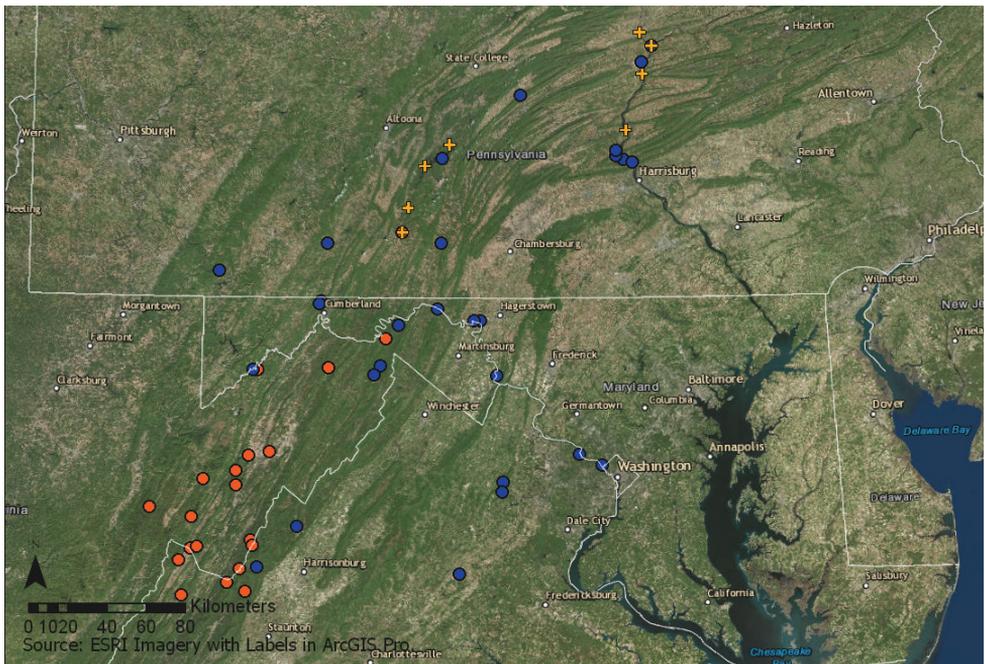


Figure 1. Range extent of *Heuchera alba* and *Heuchera pubescens* in the Appalachian Ridge and Valley. Orange circles indicate range and location of *H. alba* in WV and VA; Blue circles indicate partial range and location of *H. pubescens*; Orange crosses represent new locations of *H. alba* in Pennsylvania.

Table 1. Survey locations with general habitat information for *Heuchera alba* in Pennsylvania.

| County | Locality | Habitat | Geology |
|------------|--|--|--|
| Bedford | Hopewell Outcrops Woy Bridge Barrens | Exposed, south-facing outcrops along road above Yellow Creek Exposed, south-facing outcrops in shale woodland | Burgoon Sandstone (quartzitic sandstone) Catskill Formation (shale, mudstone, sandstone) |
| Huntingdon | Aitch Barrens/Raystown Lake Hawn's Overlook Barrens/ Raystown Lake | Exposed, southwest-facing outcrops in shale woodland Exposed, west-facing outcrops in large shale barren | Foreknobs Formation (sandstone, siltstone, mudstone, shale) Foreknobs Formation (sandstone, siltstone, mudstone, shale) |
| Perry | Cliffs at Montgomery's Ferry | Exposed east/northeast-facing cliff above the Susquehanna River | Hamilton Group (siltstone, claystone, sandstone, shale) |
| Snyder | Cliffs at Dundore | Exposed east-facing cliff above the Susquehanna River | Catskill Formation/ Sherman Creek Member (sandstone, siltstone, claystone) |
| Union | Shikellamy Bluffs/ Shikellamy State Park Cliffs at Gundy's Farm | Exposed east/northeast-facing cliff above West Branch Susquehanna River Exposed east/northeast-facing cliff above West Branch Susquehanna River | Catskill Formation (shale, mudstone, sandstone) Catskill Formation (shale, mudstone, sandstone) |

Table 2. NatureServe Rank Calculator assessment output with comments concerning population number, size, and area of occupancy of *Heuchera alba* in Pennsylvania.

| Rank Calculator Categories and Values for Pennsylvania Populations | | Comments | |
|--|--|---|--|
| RARIETY | Range Extent | E = 5,000 to 20,000 km ² | Relatively wide range extent throughout the Ridge and Valley province in Pennsylvania |
| | Area of Occupancy (AOO): 4 km ² grid cells | A = (1) 4 km ² grid cells | Very small AOO with a total of < 1 km ² throughout the PA range |
| | Number of Occurrences | B = 6 to 20 | Currently 8 confirmed populations |
| | Population Size | C – 250 to 1000 individuals | Total number combined from all occurrences in Pennsylvania |
| | Good Viability/Ecological Integrity: # of occurrences | C = Few (4-12) occurrences with excellent viability or ecological integrity | A small number of occurrences have large population sizes and are in hard to access habitats |
| | Environmental specificity | B = Narrow (Specialist) | Prefers acidic rocky outcrops, cliffs, and exposed rocky woodlands |
| THREATS | Assigned Overall Threat Impact | D = Low | Overall a relatively low threat impact except for the few populations that are along roadsides |
| TRENDS | Short Term Trend | N/A | Not enough data to assign trend |
| | Long Term Trend | N/A | Not enough data to assign trend |
| <p>Calculated Rank: S1 Assigned Rank: S1S2 Assigned Rank Reasons: Given the extensive available potential habitat in Pennsylvania further survey work in addition to in-depth herbarium studies are needed to determine full range extent and area of occupancy necessary for more confident ranking. Until then a range rank of S1S2 is recommended for this newly reported species to Pennsylvania.</p> | | | |

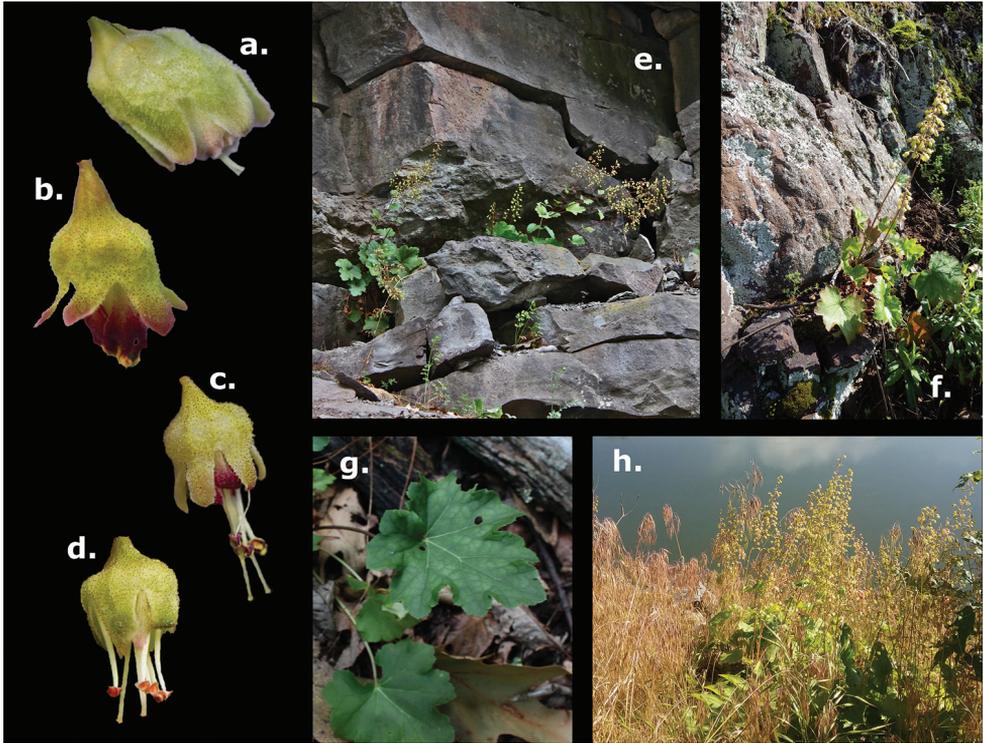


Figure 2. Images **a–d** Flowers of four *Heuchera* taxa overlapping in range in PA, WV, and VA. Flowers are shown at the original angle on the inflorescence; with the exception of the wild accession in (**d**), all flowers were obtained under common greenhouse conditions after at least a year of cultivation **a** *H. alba* (North Fork Mountain, WV; Folk 63 [deposited at OS]) **b** *H. pubescens* (Pilot Mountain, NC; Folk 96 [deposited at OS]) **c** *Heuchera* × *hispida* (= *H. americana* var. *hispida*; near Sandstone Falls, WV; Folk 104 [deposited at OS]) **d** *H. americana* var. *americana* (Blue Ridge Parkway at Twenty Minute Cliff, VA; Folk 102 [deposited at OS]). Images **e–h** *H. alba* plants growing at North Fork Mountain, WV (**e**, **g**) and Shikellamy State Park, PA (**f**, **h**). All photos R. Folk, except (**f**) and (**h**) by C. Martine.

Discussion

The Plants of Pennsylvania flora (Rhoads and Block 2007) currently recognizes two species of *Heuchera*, *H. americana* and *H. pubescens*, as occurring within the state. *Heuchera alba* should now be considered a third member of the genus for Pennsylvania. The species differs from *H. americana* in the length of stamen exertion, length of the free hypanthium, and individual flower shape; and is distinct from *H. pubescens* in flower size, color, and aspect on the inflorescence (see Figs 2, 3). Floral aspect, in particular, is absent from most recent taxonomic treatments and has only recently been recognized as variable and taxonomically informative in *Heuchera* (Folk and Freudenstein 2014); in *H. alba*, flower orientation is subhorizontal, whereas in *H. pubescens* flowers are more or less completely pendent (Folk and Stubbs in review). *Heuchera alba* is distinct from both *H. americana* and *H. pubescens* in having greater floral zygomorphy (Fig. 3).

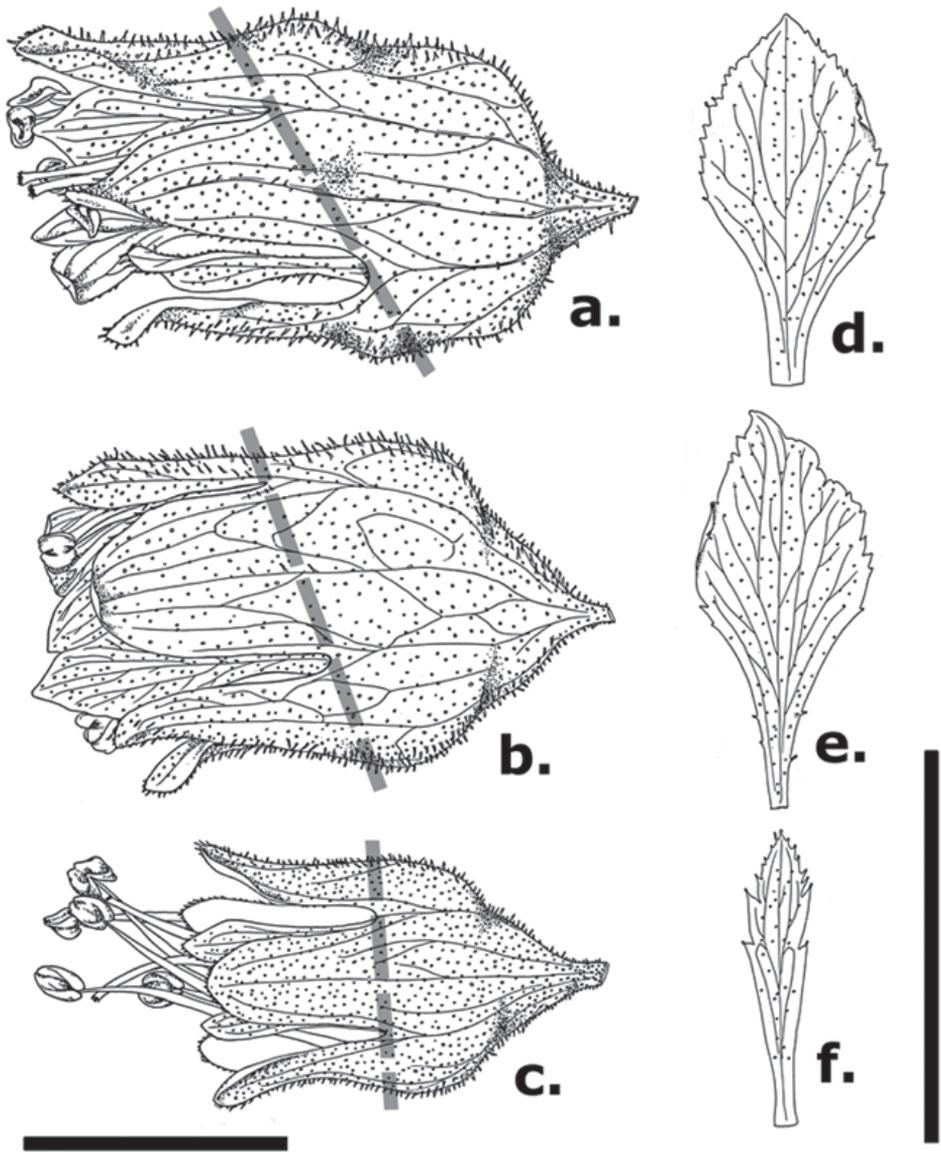


Figure 3. Comparisons of flowers and single petals (adaxial surface) of three *Heuchera* species co-occurring in PA, WV, and VA, based on spirit-preserved materials: *Heuchera alba* (**a, d** North Fork Mountain, WV; Folk 63 [deposited at OS]), *Heuchera pubescens* (**b, e** Rocky Knob Overlook, Blue Ridge Parkway, VA; Folk 100 [deposited at OS]), and unhybridized *H. americana* (i.e. *H. americana* var. *americana* **c, f** Chestoa Overlook, Blue Ridge Parkway; Folk 92 [deposited at OS]). Dotted gray lines show degree of hypanthium zygomorphy. Both scale bars represent 5 mm; the left-hand scale bar applies to **a–c** the right-hand bar is for **d–f**. Illustrations by R. Folk

The “discovery” of *Heuchera alba* in Pennsylvania is especially illustrative as a case study for the potential benefits of merging classical botanical studies with modern modes of information sharing, particularly non-traditional scientific communication through the use of social media networks. The identification of *H. alba* at Shikellamy State Park was only incidentally facilitated because C. Martine (@MartineBotany) posted an image of a specimen on Twitter (Martine 2017) while filming part of the YouTube science outreach program, “Plants are Cool, Too!” (Martine et al. 2018). Within hours of the initial Tweet going live, R. Folk (@ry_folk) had replied to the post, suggesting that what had been identified as *H. americana* was probably *H. alba* – an inference made with confidence stemming, in part, from the fact that S. Schuette (@mossman2000) had recently posted a likely *H. alba* image from another Pennsylvania locality on iNaturalist.org (<https://www.inaturalist.org/observations/2825610>). Unequivocal support for the identification required the collection and examination of specimens, however, including inspection of sheets held at the Wayne E. Manning Herbarium (BUPL) at Bucknell University. BUPL holds a handful of records for *H. alba* that had until now been identified as either *H. americana* or *H. pubescens*, including a specimen collected at the Shikellamy Bluffs by W. Manning in 1946 and another collected by a student ca. 1905 from a site (then known as “Gundy’s Farm”) just about a half-mile from the Bucknell campus. Importantly, the knowledge gleaned from the new and historical collections coupled with statewide bedrock maps (Berg et al. 1980) allowed the authors to make predictions about additional cliffside habitats in the region where *H. alba* might occur, leading to many of the new geographic records shown in Figure 1. Additional herbarium work at CM and PH is necessary to determine the number of misidentified specimens that may point to new locations for this imperiled species in Pennsylvania.

This discovery may also serve as a cautionary tale of relying entirely for plant identification on floras which, through no fault of their own, become incomplete or ‘static’ over time (both relative to taxonomic circumscriptions and also taxon distributions) and have the mixed benefit / danger of including only the species known at their time of writing to be in the geographic area of the flora. While the exclusion of species known from nearby (but outside the area covered) simplifies keys and makes identification easier, it can also decrease the likelihood of the discovery of range extensions.

The likelihood of additional populations of *H. alba* existing in Pennsylvania is quite high, but confusion between this species and the state’s other two might hamper the ease of discovery. Until the new treatment of *Heuchera* is published in the revision of the Gleason and Cronquist Manual of Vascular Plants of Northeastern United States and Adjacent Canada, using the identification key for *Heuchera* in the Flora of Virginia (Weakley et al. 2012) or the FloraQuest app (Weakley and Lee 2018) will facilitate quick determinations of species within the genus. We present a set of illustrations and photographs (Figs 2, 3) comparing *H. alba*, *H. americana*, and *H. pubescens*, plus the occasional hybrid between the latter two species (*Heuchera* × *bispida* Pursh.). Although the distributions of these taxa overlap in Pennsylvania, West Virginia, and Virginia, there are clear differences in substrate specificity between *H. alba* and the other two species. Acidic rock outcrops are the preferred habitat and substrate for *H. alba*, while *H. americana* grows in rich woods over

base-rich granite and gneiss or shallow, rocky soils, and *H. pubescens* grows on circumneutral rock outcrops, ledges, and rock cuts (Wells and Shipes 2009). Local sympatry is rare across the range of these species, yet sympatric populations of *H. alba* and *H. pubescens* at the Shikellamy Bluffs location, together with the weak reproductive barriers present in this group (Wells 1979), raise questions about the potential for hybridization and the subsequent impacts to the population genetics of both species at this key site.

The preponderance of *H. alba* localities now recorded for Pennsylvania underlines the need to continually assess imperiled taxa with integrative field surveys and taxonomic methods, and might further suggest that the species is not quite as globally rare as previously understood. This assumption should be made with caution, however, based on two observations: 1) The Pennsylvania populations are all relatively small (see above) and restricted to specific and uncommon habitat conditions, and 2) Most populations suffer from incursions of exotic invasive species. For example, the “Gundy’s Farm” population mentioned above consists of just 12 individuals on a low cliff face inundated with *Lonicera morrowii* A. Gray, *Alliaria petiolata* (M.Bieb.) Cavara & Grande, *Celastrus orbiculatus* Thunb., and other invaders. While it consists of many more individuals, the Shikellamy Bluffs population also suffers from invasion – largely because mowing and weeding of the parkland above it consistently sends weedy debris and seeds over the edge of the cliff. This latter locality is particularly critical to the status of *H. alba* in Pennsylvania given its size and status as a protected area; and preservation of the population there was made more certain following recent efforts to nearly double the amount of protected bluffs habitat led by the Merrill W. Linn Land and Waterways Conservancy.

Conclusion

The conservation needs for *Heuchera alba* will become more apparent as new population locations are revealed following a thorough review of existing *Heuchera* collections from the Ridge-and-Valley region of Pennsylvania. In the meantime, the Twitter-fueled identification of this species in Pennsylvania is an exciting outcome that provides a model for the sorts of strides we can make when botanists embrace a combination of modern and classical approaches to discovery and collaboration.

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References

- Berg TM, Edmunds WE, Geyer AR (1980) Geologic map of Pennsylvania (2nd ed.) map 1, 3 sheets scale 1:250,000. Pennsylvania Geological Survey, 4th ser.
- Folk RA, Freudenstein JV (2014) Phylogenetic relationships and character evolution in *Heuchera* (Saxifragaceae) on the basis of multiple nuclear loci. *American Journal of Botany* 101(9): 1532–1550. <https://doi.org/10.3732/ajb.1400290>
- Folk RA, Freudenstein JV (2015) “Sky islands” in the eastern U.S.A.? – Strong phylogenetic structure in the *Heuchera parviflora* group (Saxifragaceae). *Taxon* 64(2): 254–271. <https://doi.org/10.12705/642.3>
- Folk RA, Alexander PJ (2015) Two new species, *Heuchera soltisi* and *H. inconstans*, with further taxonomic notes for the western group of *Heuchera* section *Heuchera* (Saxifragaceae). *Systematic Botany* 40(2): 489–500. <https://doi.org/10.1600/036364415X688853>
- Folk RA, Mandel JR, Freudenstein JV (2017) Ancestral gene flow and parallel organellar genome capture result in extreme phylogenomic discord in a lineage of angiosperms. *Systematic Biology* 66(3): 320–337. <https://doi.org/10.1093/sysbio/syw083>
- Folk RA, Stubbs RL (in review) Treatment of Saxifragaceae. In: Naczi RFC (Ed.) Revised Manual of Vascular Plants of the Northeastern United States and Adjacent Canada.
- Martine CT (2017) (@MartineBotany). “*Bombus* on *Heuchera americana*. Shikellamy Bluffs, PA “6 June 2017, 7:13 am. Tweet.
- Martine CT, Frederick P, Kramer T (Producers) (2018) Plants are Cool, Too!: Rappelling Scientists Find Rare Species Hiding for 100+ Years. <https://www.youtube.com/watch?v=SFAPGT8cHcE>
- Miles CE, Whitfield TG (2001) Bedrock geology of Pennsylvania dataset, scale 1:250,000. Pennsylvania Geological Survey, 4th ser.
- NatureServe (2015) NatureServe conservation status assessments: Rank calculator version 3.185. NatureServe, Arlington, VA. <http://www.natureserve.org/conservation-tools/conservation-rank-calculator> [Accessed: 15.12.2017]
- NatureServe (2017) NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. <http://explorer.natureserve.org> [Accessed: 20.09.2017]
- Pursh F (1814) *Flora Americae Septentrionalis*. White, Cochran and co., London. <https://doi.org/10.5962/bhl.title.100>
- Rhoads AR, Block TA (2007) *The Plants of Pennsylvania: An Illustrated Manual* (2nd edn). University of Pennsylvania Press, Philadelphia, Pennsylvania, 498–499.
- Rosendahl CO, Butters FK, Lakela O (1936) A monograph on the genus *Heuchera*. *Minnesota Studies in Plant Science* 2: 1–180. <http://www.jstor.org/stable/10.5749/j.cttttq74>

- Ryberg PA (1926) Two new species from the mountains of West Virginia. *Torreyana* 26: 29–33. <http://www.jstor.org/stable/40596445>
- Weakley AS, Lee MT (2018) FloraQuest 2.0, an app for the identification of the >8000 species of vascular plants in the southeastern United States. Apple Store.
- Weakley AS, Ludwig JC, Townsend JF (2012) *Flora of Virginia*, Bland Crowder, [ed.] Foundation of the Flora of Virginia Project Inc., Richmond. Botanical Research Institute of Texas Press, Fort Worth, Texas, USA.
- Wells E (1979) Interspecific hybridization in eastern North American *Heuchera* (Saxifragaceae). *Systematic Botany* 4(4): 319–338. <https://doi.org/10.2307/2418808>
- Wells EF (1984) A revision of the genus *Heuchera* (Saxifragaceae) in eastern North America. *Systematic Botany Monographs* 3: 45–121. <https://doi.org/10.2307/25027594>
- Wells EF, Shipes BG (2009) *Heuchera*. In: *Flora of North America* Editorial Committee (Eds) *Flora of North America north of Mexico*, vol 8. Oxford University Press, New York, New York, 84–104. http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=115382

Two new *Phlegmariurus* species (Lycopodiaceae) and one new combination from Peninsular Malaysia

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Abstract

Two new species, *Phlegmariurus iminii* Kiew (Lycopodiaceae) from limestone karst and *P. monticola* Kiew from montane habitats, are described from Peninsular Malaysia and a new combination is made for *Phlegmariurus pinifolius* (Trevis.) Kiew. *Phlegmariurus iminii*, known from a single hill threatened by quarrying, is Critically Endangered; while *P. monticola* and *P. pinifolius* that are relatively widespread are of Least Concern.

Keywords

Phlegmariurus, limestone, montane forest, Lycopodiaceae, new species, new combination, Peninsular Malaysia

Introduction

The last published flora account of Lycopodiaceae for Peninsular Malaysian with keys and species descriptions was that of Ridley (1919) who recorded 13 species. The most recent reliable checklist (Parris and Latiff 1997) listed 12 species. At the generic level, molecular studies show that *Lycopodium* L. *s.l.* is paraphyletic (Wikström and Kenrick 2001; Field et al. 2016) and should be divided into 16 monophyletic genera (PPG I 2016) of which *Diphasiastrum* Holub, *Huperzia* Bernh., *Lycopodiastrum* Holub, *Lycopodium* L., *Palhinhaea* Franco & Vasc., *Pseudodiphasium* Holub and *Pseudolycopodiella* Holub are each represented by a single species in Peninsular Malaysia and *Phlegmariurus* Holub, which is more diverse, has 12 species including the two new species described below, bringing the total for the family to 19 species.

Revision of the family currently underway for the Flora of Peninsular Malaysia and the focus of botanical exploration by the Flora of Peninsular Malaysia team (Kiew and Rafidah 2007) have brought to light two new species of *Phlegmariurus*, one an epiphyte on trees growing on limestone; the other an epiphyte on trees in lower to upper montane forest.

Materials and methods

Specimens of all Peninsular Malaysian *Phlegmariurus* species and those of the surrounding region (Thailand, Sumatra and Borneo) in the herbaria at BM, K, KEP, KLU and UKMB (acronyms follow Thiers et al. 2017, continuously updated) were examined. Type material was examined in these herbaria as were type images relevant to this study available on JSTOR Global Plants website (<http://jstor.org>). Literature relevant to the region (West Malesia and Thailand), including protocols, were consulted. Conventional methods employed in herbarium taxonomy were applied in this study. All measurements were taken from dried herbarium specimens. Photographic documentation was taken from living specimens in their natural habitat. Provisional conservation assessments follow the guidelines in IUCN (2012) and Chua (2010).

Taxonomic account

Phlegmariurus iminii Kiew, sp. nov.

urn:lsid:ipni.org:names:77177909-1

Figures 1, 2

Type. Malaysia. Pahang, Merapoh District, Gua Gunting. 30 May 2013, Imin et al. FRI 78296 (holotype KEP! barcode 235330; isotype SING!).

Description. Medium-sized, tufted epiphyte. **Stems** lax, pendulous, ca. 30 cm long, slender, terete, ca. 2.5–3 mm in diameter; branching dichotomously 4 times, branches equal. **Leaves** spaced 3–4 mm apart, arranged in two alternating subspiral whorls of three, ascending at ca. 40° to stem, sessile; lamina dark green, thin and papery, lanceolate, (7–)10 × 1.8–2 mm, base flat, cuneate, margin flat, entire, narrowed to a minutely apiculate apex, glabrous above and beneath; midrib distinct on both surfaces, prominent above, keeled beneath. **Strobilus** slender ca. 1 mm thick, branched once dichotomously near the base with a short stalk 8–14 mm long and branches ca. 20 mm long followed by a short 50–90 mm long section of stem with sterile leaves, terminating in a strobilus (20–)40–70 mm long. **Sporophylls** distinct from leaves, spaced along the axis, sessile, ovate, much smaller than leaves, 1.5–2 × 0.75–1 mm, keeled on outer surface, base rounded and concave around the sporangium, margin entire, apex acute. **Sporangium** broadly reniform, ca. 2 mm long and wide, creamy becoming yellow when mature. **Spores** isotetrahedral with convex margins, polar axis ca. 20 µm, distal surface minutely foveolate.

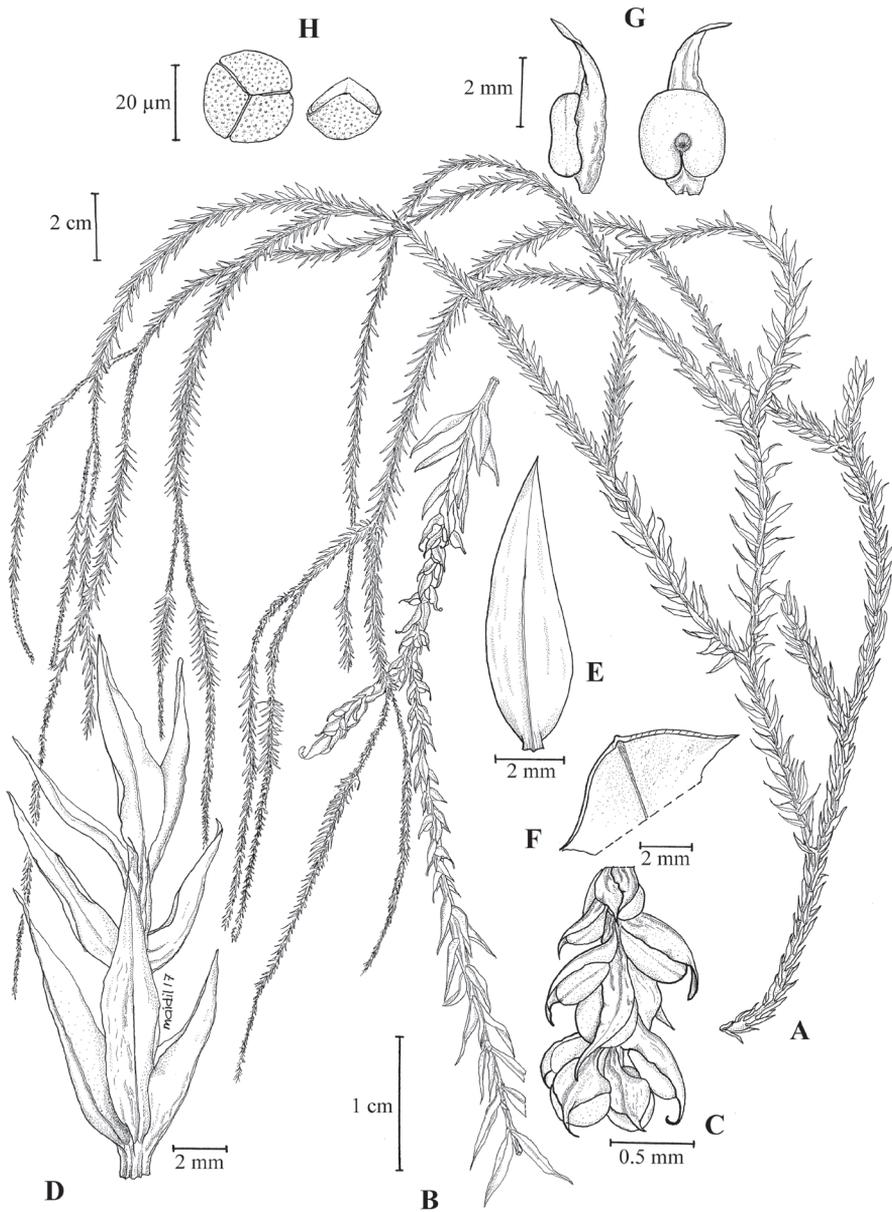


Figure 1. *Phlegmariurus iminii*. **A** habit **B** strobilus **C** tip of strobilus with sporangia and sporophylls **D** portion of leafy stem **E** distinct midrib on upper leaf surface **F** section of leaf to show keel **G** top view of sporangium and sporophyll **H** spores. (Drawing by Mohamad Aidil Noordin from Imin et al. FRI 81470).

Diagnosis. This new species belongs to the *Phlegmariurus phlegmaria* group of species in being a medium-sized epiphyte with pendent stems, flat leaves broadest at base with a distinct midrib and sporophylls much smaller than vegetative leaves. Amongst

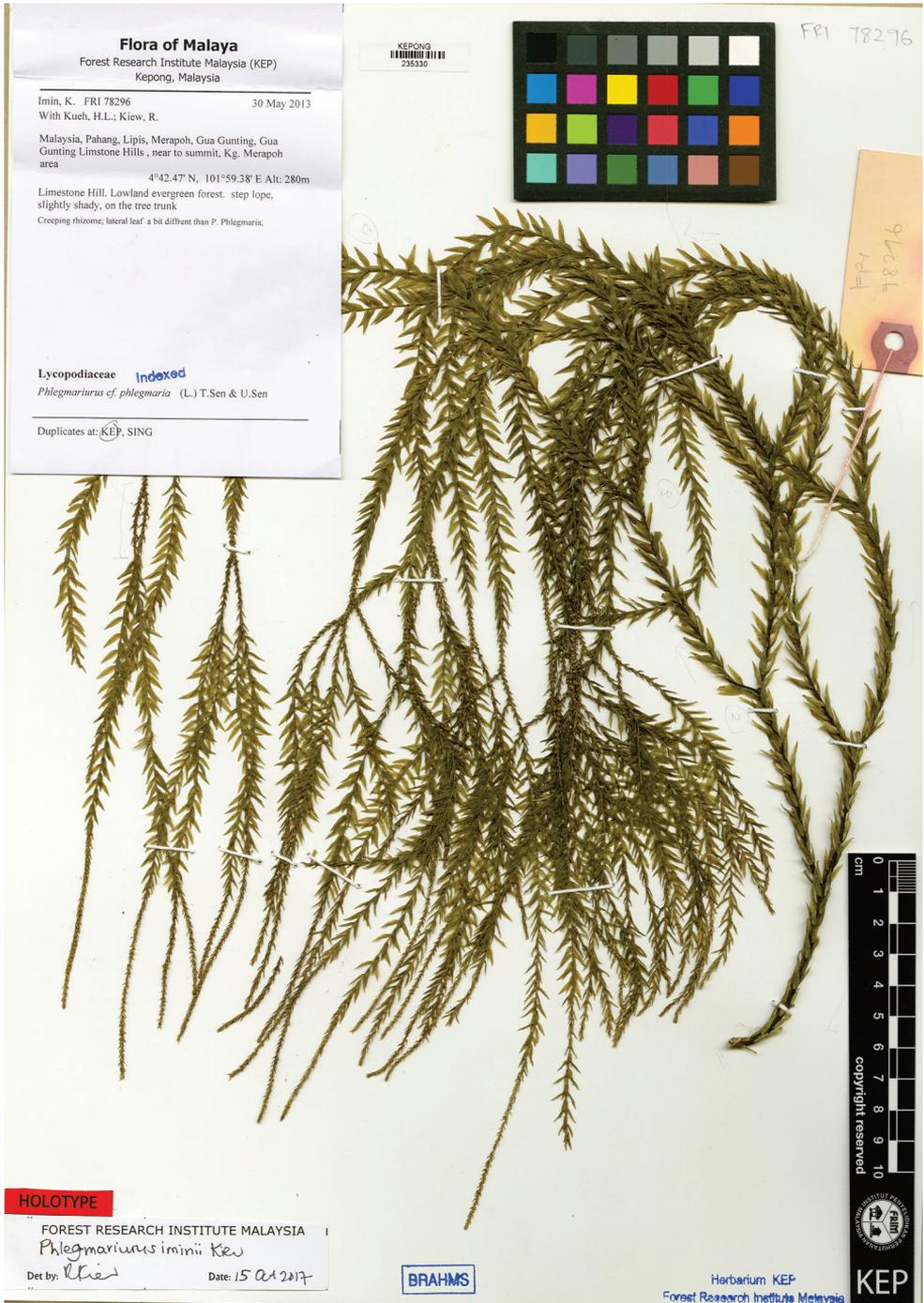


Figure 2. Holotype specimen (Imin et al. FRI 78296) of *Phlegmariurus iminii* Kiew.

Peninsular Malaysian species, it is different from *P. phlegmaria* (L.) T.Sen & U.Sen and *P. salvinoides* in its narrower, sessile leaves, 7–9 × 1.8–2 mm, positioned at 40° to the stem (vs. broader, subpetiolate leaves 5–15 × 2–5.5 mm, perpendicular to the stem) and in its unbranched strobili (vs. much branched). While the distinction between *P. phlegmaria* and *P. salvinoides* (Herter) Ching tends to intergrade, *P. iminii* is distinct without any specimens of these two species resembling it. It is similar to *P. pinifolius* (Trevis.) Kiew in its narrow, sessile leaves but its leaves are less crowded and positioned at 40° to the stem (vs. crowded and perpendicular to the stem) and its strobili are short and unbranched (vs. long and branched from the base). It also superficially resembles *P. banayanicus* (Herter) A.R. Field & Bostok from the Philippines but that species has broader leaves narrowed to the base (usually 8–10 × 2–3 mm) that are perpendicular to the stem and has branched inflorescences. *Phlegmariurus iminii* is distinct from all these species by a combination of its narrow, sessile leaves positioned at 40° to the stem and its straight, short, unbranched strobili with sporophylls that are not strongly appressed to the stem (Table 1).

Distribution. In Peninsular Malaysia, it is at present known only from the type locality, Pahang, Merapoh District, Gua Gunting. Ashley R. Field (pers. comm.) notes that the species is a target for plant collectors and it is in cultivation from parts of Thailand and other parts of Peninsular Malaysia from a variety of habitats, although we have not been able to locate it in commercial nurseries in Malaysia.

Provisional conservation status. Critically Endangered (A1d, B2ab[iii,v]). Its only confirmed locality is a single karst limestone hill that lies outside the network of Totally Protected Areas and has been threatened by quarrying for cement and is surrounded by oil palm plantations that expose it to disturbance from agricultural activities, in particular by the practice of clearing vegetation by burning. The limestone flora occupies only 0.4% of land area but is biodiverse harbouring at least 14% of the Peninsula's vascular flora (Chin 1977), so it is particularly vulnerable to disturbance (Kiew 1997). In addition, many of its species like *Phlegmariurus iminii* are known from less than five limestone hills (Kiew et al. 2017).

Ecology. The type specimen grew on a tree on a steep slope, slightly shaded near the summit of a limestone karst hill.

Etymology. Named after Imin Kamin, Research Assistant In-Charge of the lycophyte and fern collection in the Forest Research Institute Malaysia Herbarium (KEP), who discovered this species.

***Phlegmariurus monticola* Kiew, sp. nov.**

urn:lsid:ipni.org:names:77177910-1

Figures 3, 4A

Type. Malaysia. Pahang, Cameron Highlands, Gunung Beremban, trail from the Parit Falls to MARDI. 24 May 2007, Nor Ezzawanis & Zamri FRI 54517 (holotype KEP! barcode KEP139948; isotype KEP! barcode KEP139947).

Table 1. *Phlegmariurus iminii*, *P. banayanicus*, *P. pinifolius*, *P. phlegmaria* and *P. salvinioides* compared.

| Character | <i>iminii</i> | <i>banayanicus</i> | <i>pinifolius</i> | <i>phlegmaria</i> | <i>salvinioides</i> |
|--------------------|---|--------------------------------------|--|---------------------------------|----------------------------------|
| Leaf shape | lanceolate | lanceolate | elongate-lanceolate to narrowly lanceolate | ovate-lanceolate to lanceolate | lanceolate |
| Leaf base | cuneate | rounded | slightly narrowed | rounded to cordate | rounded to broadly truncate |
| Leaf angle to stem | 45° | 90° | 90° | 90° | 90° |
| Leaf apex | narrowed to attenuate | acuminate | extremely acuminate | acute | acute |
| Leaf size (mm) | 7–9 × 1.8–2 | 8–12 × (1–)2–3 | 6–8 × 1–1.7 | 4–20 × 2.5–6 | 5–9.5 × 2–5 |
| Strobili | dichotomous branch at base, branches and straight | branched 2–3 times, branches curving | 2, 4 or 6 branches, branches straight | much branched, branches curving | much branched, branches straight |
| Sporophylls | not appressed | appressed | appressed | appressed | not appressed |
| Leaf attachment | sessile | subpetiolate | sessile | subpetiolate | subpetiolate |
| Leaf spacing | spaced | spaced | very compact | spaced | spaced |

Description. Small epiphyte, tufted with (1–)6–7 stems. **Stems** pendulous or semi-erect, 11–22(–32.5) cm long, 1–2 mm diam., 1.2–1.7 cm wide across the leaves, green, branched once or sometimes twice towards the apex. **Leaves** crowded, spreading more-or-less at right angles to the stem, more-or-less in 6 rows, mid-green, soft, sessile, narrowly lanceolate to subulate, 8–10 × 0.75–1.5 mm, acutely narrowed to a sharp point at apex, margin entire, minutely revolute, glabrous above and beneath, midrib obscure above, prominent beneath. **Strobilus** green or yellowish-green, in pairs, each branching dichotomously once or twice, sometimes unbranched, (7.5–)11–12(–22) cm long, ca. 3 mm diameter. **Sporophylls** similar in shape to leaves but smaller and more compact, transition to strobilus gradual. Sporophylls sessile, ascending, arranged in 4 rows, narrowly lanceolate, 3.5–5(–7) × 0.5–1 mm at base, abruptly narrowed above the sporangium and 0.25 mm wide, margin entire. Sporangium yellow, broadly reniform, ca. 1–1.5 mm across. **Spores** isotetrahedral with convex margins, polar axis ca. 20 µm, distal surface minutely fossulate-foveolate.

Diagnosis. It belongs to the *Phlegmariurus squarrosus* group in that its sporophylls are similar in shape, although smaller, than the leaves. *Phlegmariurus monticola* is immediately distinct from *P. squarrosus* (G.Forst.) Á.Löve & D.Löve *s.l.* that in Peninsular Malaysia is morphologically relatively uniform by a combination of its tufted, shorter stems 11–32.5 cm long (vs. single-stemmed at the base and (20–)40–55(–200) cm long), leaf midrib obscure above and prominent beneath (not distinct above and faint beneath), strobili more slender ca. 3 mm wide, that are in pairs and usually branch dichotomously once or twice (vs. strobili 4–5 mm wide, single and always unbranched). In addition, their distributions do not overlap; *P. squarrosus* is a lowland species grow-

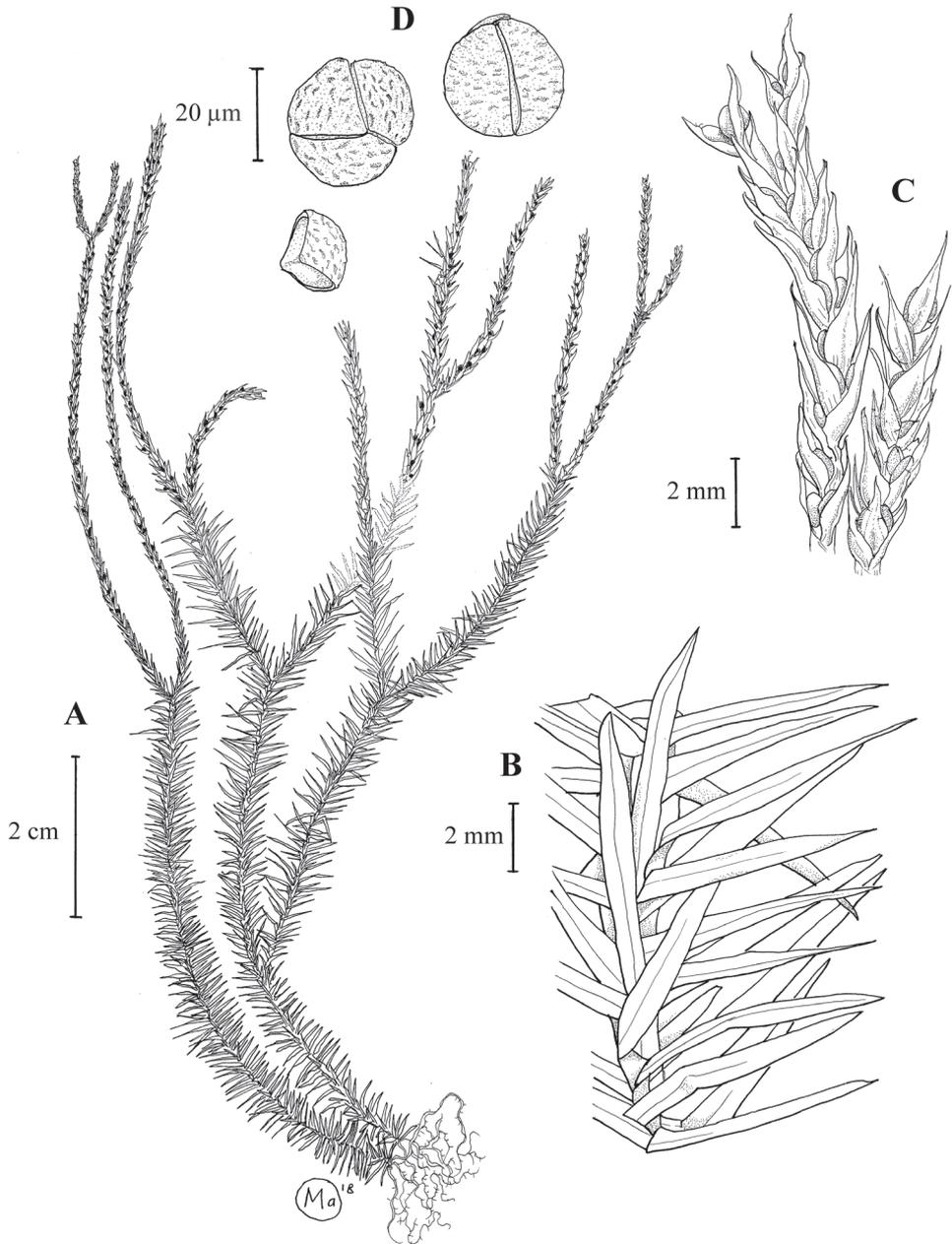


Figure 3. *Phlegmariurus monticola*. **A** habit **B** portion of leafy sterile branch **C** strobilus **D** spores. (Drawing by Mohamad Aidil Noordin from Ezzawanis et al. FRI 54517).

ing on trees usually at less than 300 m elevation, while *P. monticola* is a montane species occurring at 1400–2100 m elevation. It also superficially resembles *P. prolifera* (Blume) A.R. Field & Bostok in its slender strobili ca. 3 mm wide and sporophylls arranged

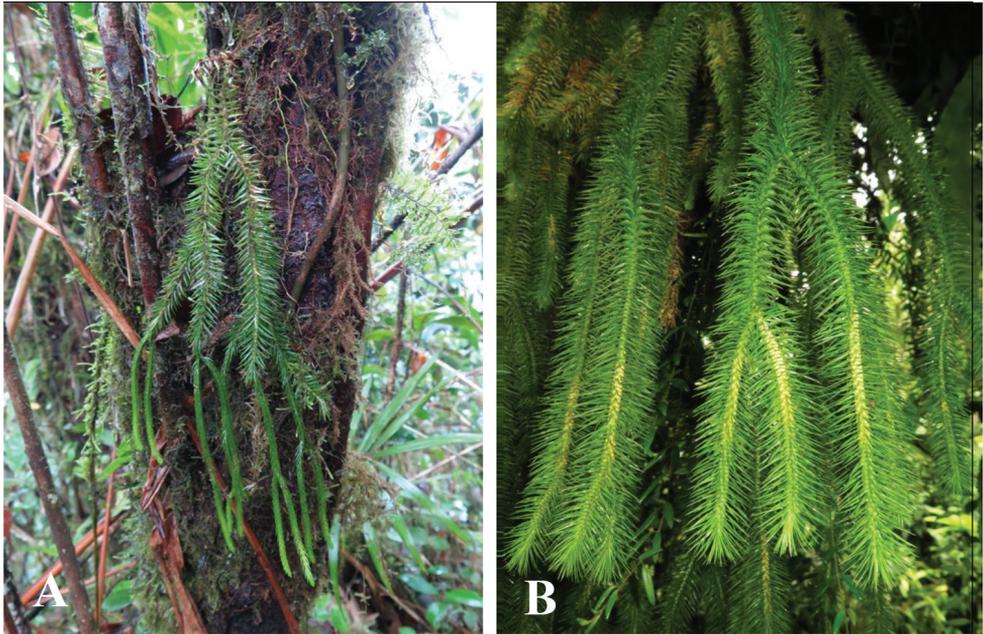


Figure 4. **A** *Phlegmariurus monticola* **B** Peninsular Malaysian form of *P. squarrosus* s.l. (Photographs Imin Kamin).

in four rows, but it differs in its shorter, narrower leaves (8–10 × 0.75–1.5 mm (vs. leaves 10–15 × 1.5–2 mm) and its narrowly lanceolate sporophylls 0.5–1 mm wide (vs. triangular-ovate sporophylls 1.5–2 mm wide).

Distribution. Peninsular Malaysia (Kelantan, Perak and Pahang).

Provisional conservation status. Least Concern. It is found in most accessible montane areas in the Main Range suggesting that it is likely to be more widespread. The montane forest above 1000 m is protected because of the restriction on clearing forest on steep slopes. However, this does not apply to hill resorts where forest is cleared for resort infrastructure and at Cameron Highlands for vegetable and flower farms (Kiew 1997). However, its populations need to be monitored because it is for sale in nurseries in Malaysia, Singapore and Thailand (AR Field, pers. comm.) so collecting of plants from the wild may become a threat.

Ecology. In Peninsular Malaysia, in light shade usually in lower montane forest, sometimes in upper montane forest, at 1400–2100 m elevation.

Etymology. Latin, *monticola* - dweller in mountains.

Notes. In appearance, it is immediately distinct from *P. squarrosus* in being less robust, in being tufted with up to 7 stems, having shorter stems that are only about twice the length of the strobili and leaves with the midrib obscure above and prominent beneath; while Peninsular Malaysian individuals of *P. squarrosus* have single stems (not tufted) that are longer so their strobili are about a fifth or less the length of the stem and the leaf midrib is distinct above and faint beneath.

Specimens examined. Kelantan: Gunung Chamar Imin et al. FRI 71786 (KEP, L); Sungai Kenerong Imin et al. FRI 68171 (KEP), Kueh et al. FRI 58410 (KEP). Pahang: Cameron Highlands Aishah 15 (KLU); Holttum SFN 23443 (SING), Holttum s.n. May 1936 (SING); Imin et al. FRI 68482 (KEP), Imin et al. FRI 71946 (KEP), FRI 74765 (KEP, L), Imin et al. FRI 87114 (KEP), Nor-Ezzawanis et al. FRI 54517 (KEP), Poore 1019 (KLU); Fraser's Hill Henderson SFN 11507 (SING); Genting Highlands Lim et al. GHC 1 (KLU); Aishah 22 (KLU), Stone 15422 (KLU), Tan et al. FRI 77645 (KEP, TAIF). Perak: Birch's Hill Burkill SFN 12739 (SING); Gunung Hijau Julius et al. FRI 53305 (KEP), Sinclair & Kiah SFN 38728 (SING).

New combination

Lycopodium pinifolium Blume (1828: 264) was described and recorded to occur from Malaya to Papua, but it was not until 1984 that this species appeared in Malaysian publications (as *Huperzia pinifolia* Trevis.) when it was recorded from the Gunung Mulu National Park, Sarawak (Parris et al. 1984) where it was keyed out with *Huperzia phlegmaria* and distinguished from that species by its linear-lanceolate leaves at least six times longer than broad with a cuneate base and by its sporophylls with acuminate tips that protrude beyond the sporangia. A detailed account described and illustrated this species (Johns 1991) based on specimens from Gunung Mulu grown in the Royal Botanic Gardens Kew. However, it was not until Parris and Latiff (1997) listed it that it appeared in Peninsular Malaysian publications. However, its identity became confused when subsequent publications illustrated different species under this name. For example, that of Aziz-Bidin (2002, figs. 10, 11) illustrated *P. tetrastichus* and that of Noraini et al. (2010, figs. 47, 48) figured *P. squarrosus*. Names on herbaria specimens were similarly in a muddle. A full description is therefore provided below.

The name *Lycopodium pinifolium* Blume was an illegitimate name because it had earlier been used for an African species by Kaulfuss (1824). In 1874, Trevisan described *Huperzia pinifolia* Trevis. as a new name: in fact he was conscious of the illegitimacy of the name of Blume because of the (older) name of Kaulfuss. In this case (International Code of Nomenclature, art. 7.4 (ICN 2012)) the new name is typified by the type of the replaced synonym. Blume (1828), in describing his species, recognised varieties B, C and D but for varieties C and D he noted '(an species?)' implying that there was doubt that they belonged to this species. Specimens representing these varieties were all annotated by his hand, the labels noting only 'Java' without recording a collector, locality or date. The specimen annotated as var. C (barcode L 0057377) is closely similar to *P. proliferus* (Blume) A.R.Field & Bostock and does not belong to *P. pinifolius* and the specimen representing var. D is a piece too small to identify with certainty. The other two specimens both belong to *P. pinifolius* in their habit (relatively short dichotomously branching stems with dense, narrowly lanceolate leaves perpendicular to the stem) and the abruptly distinct, straight strobili. The typical variety (barcode L 0057375) has strobili that are branched dichotomously while var. B (barcode L0057376) has unbranched

strobili, which represent an earlier stage in the growth of the strobili, which eventually branch. The herbarium sheet with barcode L 0057375 is here selected as the lectotype because it best represents a mature plant of this species.

***Phlegmariurus pinifolius* (Trevis.) Kiew, comb. nov.**

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Basionym. *Huperzia pinifolia* Trevis., Atti Soc. Ital. Sci. Nat. 17: 247. 1874 \equiv *Lycopodium pinifolium* Blume, Enum. Pl. Javae. 2: 264 (1828), non Kaulf. (1824).

Type. Java. Without collector, number, date or precise locality (lectotype here selected: L!, electronic image with barcode L 0057375).

Description. Medium-sized epiphyte, tufted with 2–4(–6) stems. **Stems** pendulous, 10–20(–50) cm long, ca. 1.5 mm diam., branching dichotomously 2–3(–4) times, branches equal. **Leaves** crowded, at right angles to stem, sessile, glossy, mid-green, thinly coriaceous, narrowly lanceolate, 6–8 \times 1–1.7 mm, slightly narrowed at base, margin entire, slightly revolute, apex narrowed to a sharp point, glabrous, midrib obscure above, faint beneath. **Sporophylls** smaller, dissimilar to leaves, transition to strobilus abrupt. **Strobili** light green, 2–9 cm long, slender, 1–1.5 mm diam., branched at base to form a pair of straight branches, sometimes further branched once or twice. Sporophylls crowded, not arranged in 4 rows, sessile, green, base broadly ovate and partially covering the sporangium, narrowed abruptly above the sporangium to a short point, ca. 1.5 \times 0.7 mm, margin entire, keeled outside. **Sporangium** discoid, ca. 1 mm diam.

Distribution. Thailand, Peninsular Malaysia, Java, Borneo (Sabah and Sarawak), the Philippines, Indonesia (Sumatra to New Guinea). Hassler (2018) also records it from Sri Lanka, Vanuatu and the Solomon Islands. In Peninsular Malaysia, collected from Penang, Perak, Kelantan and Pahang.

Provisional conservation status. Least Concern.

Ecology. Not common, in upper hill dipterocarp to lower montane forest, in light shade, on trees on hill sides at 800–1250 m altitude.

Etymology. Latin, *-folius* = leaf; referring to the similarity to leaves of *Pinus*.

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References

- Aziz-Bidin A (2002) The fern allies Lycopodiaceae in Malaysia: variations in surface structure of spores. *Folio malaysiana* 3(1): 49–66.
- Blume CL (1828) *Enumeratio Plantarum Javae et Insularum Adjacentium*, fasciculus II. JW van Leeuwen, Lugduni Batavorum, 274 pp.
- Chin SC (1977) The limestone flora of Malaya. 1. *Gardens' Bulletin* (Singapore) 30: 165–219.
- Chua LSL (2010) Species assessment and conservation in Peninsular Malaysia. *Flora of Peninsular Malaysia. Series I. 1*: 37–44.
- Field AR, Testo W, Bostok PD, Holtum JAM, Waycott M (2016) Molecular phylogeny and the morphology of the Lycopodiaceae subfamily Huperzioidae supports three genera: *Huperzia*, *Phlegmariurus* and *Phylloglossum*. *Molecular Phylogenetics and Evolution* 94: 635–657. <https://doi.org/10.1016/j.ympev.2015.09.024>
- Hassler M (2018) World Ferns: Checklist of Ferns and Lycophytes of the World. In: Roskor et al. (Eds) *Species 2000 & ITIS Catalogue of Life*. [updated 26 Feb 2018]
- IUCN (2012) *IUCN Red list Categories and Criteria: Version 3.1, 2nd ed.* IUCN, Gland, Switzerland and Cambridge, UK.
- Johns RJ (1991) 177. *Huperzia pinifolia* Lycopodiaceae. *Curtis's Botanical Magazine* 8(3): 123–128. <https://doi.org/10.1111/j.1467-8748.1991.tb00373.x>
- Kaulfuss GF (1824) *Enumeratio Filicum. Sumtibus Caroli Cnoeloch, Lipsiae*, 315 pp.
- Kiew R (1997) The Malaysian highlands and limestone hills: threatened ecosystems. In: *State of the Environment in Malaysia*. Consumers Association of Penang, Malaysia, 66–73.
- Kiew R, Rafidah AR (2007) The Flora of Peninsular Malaysia. *Conservation Malaysia* 5: 1–4.
- Kiew R, Rafidah AR, Ong PT, Ummul-Nazrah AR (2017) Limestone treasures: Rare plants in Peninsular Malaysia – what they are, where they grow and how to conserve them. *Malaysian Naturalist* 71(1): 32–41.
- Noraini T, Ruzi AR, Jaman R, Nurnida MK, Farrah-Melissa M (2010) Ferns and Fern-allies. *Taman Paku Pakis UKM* 2: 1–107.
- Parris BS, Jermy AC, Camus JM, Paul AM (1984) The Pteridophyta of Gunung Mulu National Park. In: Jermy AC (Ed.) *Studies on the Flora of Gunung Mulu National Park*, Sarawak Forest Dept., Sarawak, 145–233.
- Parris BS, Latiff A (1997) Towards a pteridophyte flora of Malaysia: A provisional checklist of taxa. *Malayan Nature Journal* 50: 235–280.
- PPG I (2016) A community-derived classification for extant lycophytes and ferns. *Journal Systematics & Evolution* 54: 563–603. <https://doi.org/10.1111/jse.12229>
- Ridley HN (1919) The fern-allies and Characeae of the Malay Peninsula. *Journal Straits Branch Royal Asiatic Society* 80: 139–164.

- Thiers B (2017) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>. [Accessed 15 December 2017]
- Wikström N, Kenrick P (2001) Evolution of Lycopodiaceae (Lycopodiopsida): Estimating divergence times from *rbsL* gene sequence by use of nonparametric rate smoothing. *Molecular Phylogenetics and Evolution* 19(2): 177–186. <https://doi.org/10.1006/mpev.2001.0936>

Staying cool: preadaptation to temperate climates required for colonising tropical alpine-like environments

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Abstract

Plant species tend to retain their ancestral ecology, responding to temporal, geographic and climatic changes by tracking suitable habitats rather than adapting to novel conditions. Nevertheless, transitions into different environments or biomes still seem to be common. Especially intriguing are the tropical alpine-like areas found on only the highest mountainous regions surrounded by tropical environments. Tropical mountains are hotspots of biodiversity, often with striking degrees of endemism at higher elevations. On these mountains, steep environmental gradients and high habitat heterogeneity within small spaces coincide with astounding species diversity of great conservation value. The analysis presented here shows that the importance of *in situ* speciation in tropical alpine-like areas has been underestimated. Additionally and contrary to widely held opinion, the impact of dispersal from other regions with alpine-like environments is relatively minor compared to that of immigration from other biomes with a temperate (but not alpine-like) climate. This suggests that establishment in tropical alpine-like regions is favoured by preadaptation to a temperate, especially aseasonal, freezing regime such as the cool temperate climate regions in the Tropics. Furthermore, emigration out of an alpine-like environment is generally rare, suggesting that alpine-like environments – at least tropical ones – are species sinks.

Keywords

Alpine speciation, biome change, island biogeography, niche conservatism

Introduction

Latitudinal and elevational gradients (Pianka 1966; Dowle et al. 2013) of plant distribution and richness are wellstudied patterns and appear to result from the tendency of plants to retain their ancestral ecology (Donoghue 2008 and references therein). These patterns are the basis for the establishment of floristic regions, biomes, ecoregions and floristic elements (Cain 1944; Good 1974; Whittaker 1975; Takhtajan 1986; Olson et al. 2001; Sklenář 2011). However, isolation can occur at any scale where there are discontinuities in realised environmental niches, such as between lakes or ponds, inselbergs or mountain tops. Movement between such habitats can be rare, irrespective of their size, location and geographical proximity (see for example Crisp et al. 2009).

Alpine-like or supra-alpine environments, especially those on isolated mountains, are a notable example of such isolation (Hedberg 1965). The alpine-like flora is typically strikingly different from that of the surrounding lowlands. Trees are absent and, in their place, are species with life histories and growth forms that are adapted to grow under harsh conditions that include frequent and lasting freezing temperatures and short growing seasons (Körner 2003). At low latitudes, the contrast between alpine-like conditions found only on the highest mountains (Fig. 1) and the tropical environments that surround them is particularly stark. Plants adapted to high elevations generally cannot survive at low elevations and vice versa. Alpine-like climate regions in general and those of the Tropics in particular, are therefore considered akin to isolated oceanic islands (Smith and Cleef 1988).

It is well established that recruitment via long-distance dispersal is of paramount importance to the evolution of both oceanic- and mountaintop ‘sky’- islands (Alsos et al. 2007; Weigelt and Kreft 2013) and that recruitment was followed only in some cases by *in situ* radiations (MacArthur and Wilson 1967; Carlquist 1974; Baldwin and Sanderson 1998; Ackerly 2003; Cameron et al. 2013). However, the relative importance of these processes remains poorly understood but is significant, especially for the prediction of changes in species diversity in the light of climate change.

Here, the origin of species is investigated in tropical-alpine floras across the world, comparing South American tropical (Supra-)Páramo, the tropical Afroalpine in Africa, tropical alpine-like Mount Kinabalu in Southeast Asia and high elevation areas on the Pacific Ocean Islands of Hawaii (Fig. 1). The following hypotheses are tested: i) the importance of *in situ* speciation in tropical alpine-like areas has been underestimated, ii) the impact of dispersal from regions with alpine-like environments is large compared to that of immigration from other regions and iii) emigration out of an alpine-like environment is generally rare.

Methods

Area

Tropical alpine-like areas are defined as regions located above the natural high-altitude treeline, that is the upper limit of tall upright, woody life forms, within 23°26'N, 23°26'S

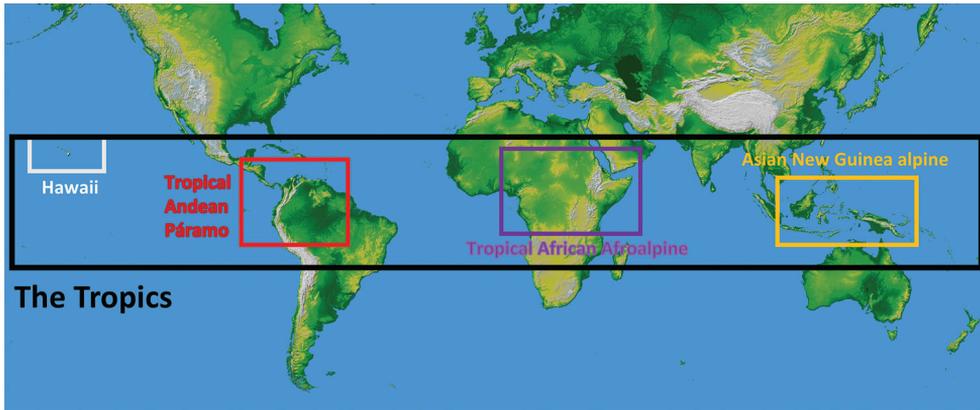


Figure 1. Location of the tropical alpine-like climate regions in the Tropics on a Mercator projection of the world with shaded relief and coloured height based on SRTM data with 1 arc second resolution. Credit: NASA/JPL/NIMA downloaded from <http://photojournal.jpl.nasa.gov/catalog/PIA03395>. Detailed maps for each region are included in the Suppl. material 3 (figures S3–S6).

(Smith and Young 1987; Smith and Cleef 1988; Smith 1994; Rundel et al. 1994; Körner 2003, Nagy and Grabherr 2009). Mountains, especially those in the Tropics, show distinct changes in life conditions with elevation and the rise of isotherms as one moves towards the equator. The location of the natural treeline is considered here to be at about 4000 m, as long as annual precipitation is >250 mm (Körner and Paulsen 2004; Körner 2012). The upper altitudinal limit commonly extends to between 4600 and 5000 m (Smith and Young 1987; Luteyn 1999).

True tropical alpine-like environments are characterised by a mean daytime soil temperature of about 6 °C (Körner 2011) but also by recurring events of frost especially at night with a small amplitude of climatic differences throughout the year. For East Africa, this has been described as “summer every day and winter every night” (Hedberg 1965) which also leads to the absence of a distinct growing or flowering ‘season’ as most plants can photosynthesis and have been reported as flowering throughout the year.

Other authors have used a different definition of the term “tropical alpine” or tropicalpine by only considering the present day upper tree limit as the cut-off line, therefore including areas above ~3500 m (Sklenář et al. 2011; Gehrke and Linder 2014) or even as low as 2000 m in various tropical islands (Leuschner 1996). Some authors tried to avoid the term alpine and referred to these regions using terms such as Páramo and Afro-alpine (see Smith and Young 1987, for a detailed review on terms) or even just summit zone or high elevation areas (Smith 1980, 1994).

Due to the high frequency of plants growing in micro niches along a wide elevational amplitude and the mosaic-like patterns of elevational plant zones, a strict definition of the term ‘alpine-like’ is used and only includes plants in areas above 3800 m with a clear diurnal rather than seasonal pattern of vegetation growth and flowering (Table 1; Fig. 1). Therefore, the tropical alpine-like areas are defined here as follows: they are regions located above the natural high-altitude treeline, within 23°26'N, 23°26'S,

Table 1. Overview of high tropical alpine-like regions as used in this study.

| Region | Andean Páramo | Afroalpine | Malesia and New Guinea | Hawaii |
|--|--------------------------|---------------------------------------|---|--------------------------------|
| Estimated area (km ²) | 23,452 ¹ | 4510 | 2000 | <500? |
| Number of species | 3026 | 521 | 1.118 | 13 |
| Number of genera | 449 | 191 | 226 | 10 |
| Typical alpine species | 998 | 163 | ~400/67 ² | 13 |
| Alpine species used | 668 | 145 | 18 | 12 |
| % endemism | 60 ³ | 67 ⁴ | 60 ⁵ | 100 |
| Species turnover ⁶ | highest | lowest | medium | n.a. |
| Largest genus (no. species in alpine/ region/ genus) | <i>Draba</i> (27/70/370) | <i>Senecio</i> s.str. (32/>130/~1000) | <i>Rhododendron</i> (21/219/>1000) ⁸ | Silversword alliance (3/28/28) |

¹estimated based on “altoandino” (Josse et al. 2009), ²number of species recorded to occur in the alpine zone on Mt.Kinabalu, ³Nagy and Grabherr 2009, ⁴Gehrke and Linder 2014, ⁵estimated here, ⁶beta diversity (Sklenář et al. 2014) based on the analysis of seven mountains in each region, ⁷*Draba* is listed here as largest genus despite 37 *Senecio* species recorded in the high Andean Páramo and 68 in the wider Páramo (Luteyn 1999) because the number of species belonging to a monophyletic *Senecio* s.str. is not clear, ⁸insufficient data.

characterised by a mean daytime soil temperature of about 6 °C (Körner 2011) and by recurring events of frost especially at night with a small amplitude of climatic differences throughout the year. To avoid including too many species that barely reach into the tropical alpine-like regions, a lower limit of 3800 m has been chosen.

Northern (Supra-)Páramo

Following the above definition, the majority of areas with a tropical alpine-like environment are located in the Americas, i.e. in the Andes between Venezuela and Peru and residual Páramo ecosystems in Costa Rica (highest point: 3810 m), with the exclusion of the drier regions at higher altitudes in Mexico (Nagy and Grabherr 2009) and the drier Puna and Jalca regions in the Central and southern Andes (Luteyn 1999). Some authors use the term Supra-Páramo for this (Lauer 1981). These regions are by far the largest in area (probably more than 90% of the total tropical alpine area, Jacobsen 2008; Sklenář et al. 2011, 2014) as well as the most species rich even when correcting for area size (Gehrke and Linder 2014).

Africa

In Africa, most alpine-like environments are located in the eastern mountain ranges of the continent (White 1983; Gehrke and Linder 2014) as well as a smaller area on volcanic Mount Cameroon (4095 m, Letouzey 1985). In the tropical Afroalpine, mountain ranges are well separated from each other, mostly by large tracks of lowlands (Hedberg 1951; Chala et al. 2017).

Malesia and New Guinea

New Guinea harbours the most extensive alpine-like environment in South-east Oceanic Asia in addition to scattered areas on a number of Indonesian islands and in

Malaysia including Mt. Kinabalu on the island of Borneo (Smith 1994; Sklenář et al. 2014). Unfortunately, floristic and phylogenetic data for plants in the New Guinea alpine region are even scarcer than that for plants from the northern Andean Páramo or tropical Afroalpine. Only data on the relatively well-studied plants from Mt. Kinabalu in the northern part of the island of Borneo are therefore included.

Hawaii

Hawaii is the most isolated and most northerly area investigated. The absence of a strong seasonal climate is likely due to the moderating effect of the surrounding ocean. Hawaii is situated close to the northern limits of the Tropics (max. elevation 4100 m) and only harbours a small high alpine-like environment (Leuschner 1996). In general, most recent phylogenetic evidence suggest that tropical-alpine like climate developed only fairly recently around the world in the Plio-Pleistocene (Madrrián et al. 2013; Gehrke et al. 2014).

Character coding

All analyses were undertaken at the species level, ignoring sub-specific taxa even if these were confined to tropical alpine-like areas. The origins of species in the tropical alpine-like environments were coded as *in situ* speciation versus immigration. The former includes all species in the tropical alpine-like that have originated from an ancestor in the tropical alpine of the same area, the latter includes those with a most recent common ancestor inferred to have been elsewhere. For species that originated by immigration, the author inferred whether they originated from areas with an alpine-like climate, a tropical climate or otherwise.

Initially, the author attempted to draw finer distinctions within the non-alpine and non-tropical category, such as distinguishing temperate climate regions located in the Tropics or elsewhere. However, this proved to be impossible due to low resolution, low sampling and inadequate support in most phylogenetic reconstructions. The three categories of alpine-like-, tropical or other climate were therefore used. It proved extremely difficult to discern between biome change and *in situ* speciation for species that have a widespread distribution, as phylogeographic data would be necessary to exactly place their origins and such data are not available.

Only a minority of species, found in the tropical alpine-like are exclusively confined to these regions, even if they predominantly occur there (i.e. they are regarded as typical alpine elements). For example, 60 percent of Afroalpine species (Gehrke and Linder 2014) and 85 percent of those on Mt. Kinabalu also occur elsewhere. Within the Andes, this value seems to vary more widely and it is particularly high on the western slopes of the Peruvian Andes, where alpine species merge gradually with species of montane pasture, savannah or desert (Smith 1994). Only species that grow most frequently above 3800 m and higher were therefore included, i.e. those that either have most of their distribution above 3800 m or that are able to grow above 4200 m. When molecular phylogenetic evidence was unavailable, the most recent taxonomic classification was

used as a hypothesis of evolutionary relationships, in the knowledge of the limitations of this approach, particularly due to morphological convergence. For detailed coding examples and the full list of taxa and their coding see Suppl. material 1: Methods.

Results

The relative contribution of *in situ* speciation versus immigration differs between the different tropical alpine regions (Fig. 2). *In situ* speciation seems to have been rare on Mt. Kinabalu and Hawaii (one to two or no recorded *in situ* speciation events respectively), while it contributed between 199–392 species to the Andean Páramo (30–59%) and 48–61 to the Afroalpine species (29–37%).

In total, colonisations from other environments accounted for 318–428 (36–75%) of all species in the Páramo, 67–74 (51–61%) in the Afroalpine, 8–9 (83–94%) on Mt. Kinabalu and 10 (91–100 %) on Hawaii. Species originating from other alpine-like biomes, i.e. alpine-like regions outside the Tropics, account for 46–102 species in the Páramo (7–15% of all species or 12–27% of colonisation events), 16–25 in the Afroalpine (10–15% total or 15–23%), 7–8 on Mt. Kinabalu (39–44% total or 42–48%), and 1 on Hawaii (see Suppl. material 2: Results). There was no evidence for direct transitions between tropical climate environments.

Despite the lack of phylogenetic evidence for direct dispersals between alpine-like regions, there are 52 genera found in both the Northern Andean Páramo (Luteyn 1999, Sklenář 2011 including 449 genera) and the tropical Afroalpine (*sensu* Gehrke and Linder 2014 including 191 genera). Shared genera are:

- **Holarctic element** (north temperate and Mediterranean distribution, 12 of a total 41 of genera in the Páramo s.l.): *Astragalus*, *Bartsia*, *Cerastium*, *Clinopodium*, *Erigeron*, *Lathyrus*, *Lithospermum*, *Potentilla*, *Salvia*, *Saxifraga*, *Silene*, *Vicia*.
- **Wide temperate element** (temperate and cool regions of both hemispheres, 35 of a total of 70 genera in the Páramo s.l.): *Agrostis*, *Alopecurus*, *Aphanes*, *Bromus*, *Callitriche*, *Cardamine*, *Carex*, *Crassula*, *Cynoglossum*, *Danthonia*, *Deschampsia*, *Epilobium*, *Festuca*, *Galium*, *Geranium*, *Gnaphalium*, *Hypericum*, *Isolepis*, *Juncus*, *Limosella*, *Luzula*, *Myosotis*, *Poa*, *Polypogon*, *Potamogeton*, *Ranunculus*, *Rubus*, *Rumex*, *Senecio*, *Sedum*, *Stellaria*, *Trisetum*, *Valeriana*, *Veronica*, *Viola*.
- **Cosmopolitan** (3 of a total of 17 genera in the Páramo s.l.): *Euphorbia*, *Hydrocotyle*, *Lobelia*.
- **Wide tropical** according to Sklenář et al. 2011: *Conyza*, *Eragrostis*, *Eriocaulon*.

The author did not include either *Lithospermum* or *Euphorbia* in the analyses of origin because neither meet the stricter definition of alpine-like outlined in the methods, i.e. they do not occur in true alpine-like conditions above 3800 m.

In general, the vast majority of plant lineages present in tropical alpine-like environments are also present at lower elevations (see Suppl. material 3, Figures).

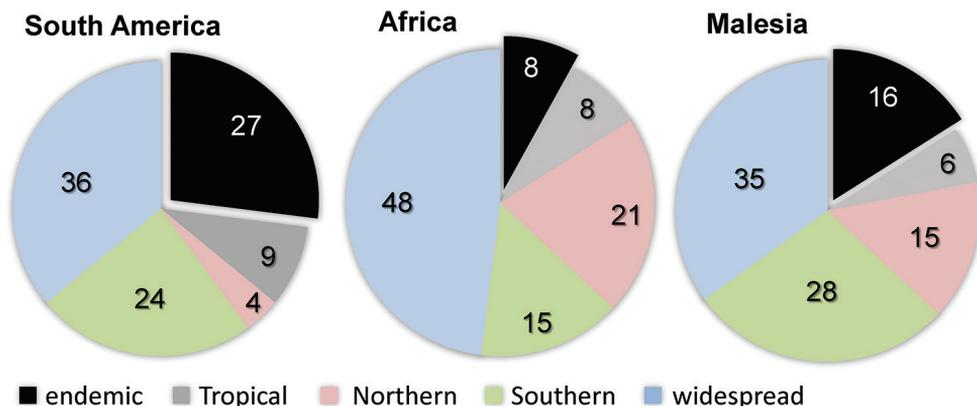


Figure 2. Relative contribution of *in situ* speciation and immigration to species richness in selected tropical alpine regions (pie charts on the left). Blue: *in situ* speciation, green: colonisation, light blue: uncertainty regarding *in situ* speciation, light green: uncertainty about colonisation. In the right pie charts, colonisation is further decoupled into species derived from other regions with alpine-like climate (black) and species that originated by colonisation from a different biome (red). Uncertainty is indicated by grey.

Discussion

Several studies have assessed the origin of plant lineages in one or more tropical alpine-like regions, mostly summarising their data by genus (Engler 1879, 1892; Hedberg 1965; Smith 1975; Smith and Cleef 1988; Beaman and Beaman 1990; Nagy and Grabherr 2009; Sklenář et al. 2011, 2014) and more rarely using phylogenetic evidence (Sklenář et al. 2011). Most studies reflect a strong notion of (broad) climatic niche conservatism within genera. However, genera are a poor proxy for independent evolutionary lineages in the tropical alpine, as shown by the various African taxa derived from multiple independent colonisation events from Eurasia (Assefa et al. 2007; Gehrke and Linder 2009), Southern Africa (Galley et al 2007, Kandziora et al. 2016) or lower elevations (Knox 2004; Galbany-Casals et al. 2014). Prominent examples of these repeated colonisations of the Afroalpine are the giant lobelias and giant senecios, which have both been postulated to have originated by repeated upward adaptation to alpine-like environments (Knox 2004). Similarly, in *Halenia*, the majority of species in the high elevation Páramo are the result of repeated immigrations, in this case from Central America (von Hagen and Kadereit 2003). However, the phenomenon of repeated immigration is better understood for regions elsewhere, for example the alpine-like tundra environment on the isolated island of Svalbard in the Northern Sea that has been colonised by many plant groups several times, even within individual species (Alsos et al. 2007).

It has been suggested that much of the plant diversity in tropical alpine regions originated outside the tropics (Hedberg 1961; Smith 1975; Smith and Cleef 1988, Fig. 3). For example, Sklenář et al. (2011) calculated that around half of all lineages in the Andean Páramo are of temperate origin (in a geographical, rather than climatic, sense). Similar estimates have been made for tropical Afroalpine plants (Hedberg 1961). All this would

suggest a high proportion of long distance dispersal from similar cool climates, a pattern notably lacking in the results reported here (i.e. the small reported proportion of direct alpine or alpine-like to alpine-like dispersals). In fact, alpine to alpine long-distance dispersal seems rare compared to biome shifts, irrespective of the degree of isolation by distance and despite the numerous genera in common (e.g. Páramo and Afroalpine share about 50 of 449 genera in the Páramo and 191 in the Afroalpine, see Suppl. material 2: Results). An intuitive explanation for limited dispersal between tropical alpine habitats would be the extensive intervening distances and the small overall areas involved. The greater importance of alpine-like regions as a source of species on Mt. Kinabalu probably reflects discontinuity within the New Guinea alpine region and it might therefore be better compared to single mountains within other such areas. Hawaii, as the most extreme case of low importance of alpine-like source regions, is geographically isolated, which probably limits colonisation success. However, the low proportion of alpine-like immigration for the Páramo cannot be explained in the same way. The Andes form a more or less continuous chain of high elevation, cool-temperate environments ranging from northern Colombia to southern Chile. Nevertheless, many Andean plant lineages show – even at lower elevations – limited dispersal between the Northern and Southern Andes with independent diversifications in each region (e.g. *Puya*: Jabaily and Systma 2012, *Ourisia*: Meudt and Simpson 2006, *Azorella*: Nicolas et al. 2012). This may suggest that transitions from a seasonal to aseasonal freezing climate (i.e. from more southern Andean temperate climate regions to more northern Andean tropical alpine-like environments) might require more adaptations at different life stages than has previously been recognised (Neuner 2014).

There is a striking lack of evidence for any direct establishment of warm-tropical species in tropical alpine-like climates. This reflects a similar lack of evidence in numerous plant lineages and even families in general for transitions from tropical to temperate climates. As such, freezing temperatures seem to be a major factor limiting species distributions in (sub-) tropical high mountain ecosystems (Neuner 2014). Overall, it seems that most plants that colonise the true tropical alpine-like aseasonal ‘summer every day and winter every night’ climatic regime first establish at lower elevations. Additionally, there is no unequivocal evidence for long-distance dispersal of tropical alpine plants to cool temperate zones elsewhere. This is similar to the pattern revealed in Southern Hemisphere biome transitions (Crisp et al. 2009), in which the alpine biome was the most common destination of biome transitions but rarely the source of them. This might, at least in part, be explained by the young age of alpine-like regions and the relatively small area they occupy.

An important factor in reaching these conclusions is a consistent differentiation between alpine-like climate regions and those at lower elevations, a distinction that is often blurred in literature. For example, a commonly cited Páramo group is the Asteraceae subtribe Espeletiinae, consisting of over 140 species distributed across Venezuela, Colombia and Ecuador in both Páramo and in montane forest (Rauscher 2002, Sklenář et al. 2011). Interestingly, of the 140 species, only 61 are reported to occur in the Páramo (Luteyn 1999), of which only 18 pass the high alpine-like criterion used here. Similarly, Hedberg (1961) states that 81% of Afroalpine species are endemic. Endemic in this context might easily be misinterpreted as endemic to the Afroalpine, however it instead means endemic to mountains of (eastern) Africa and while the

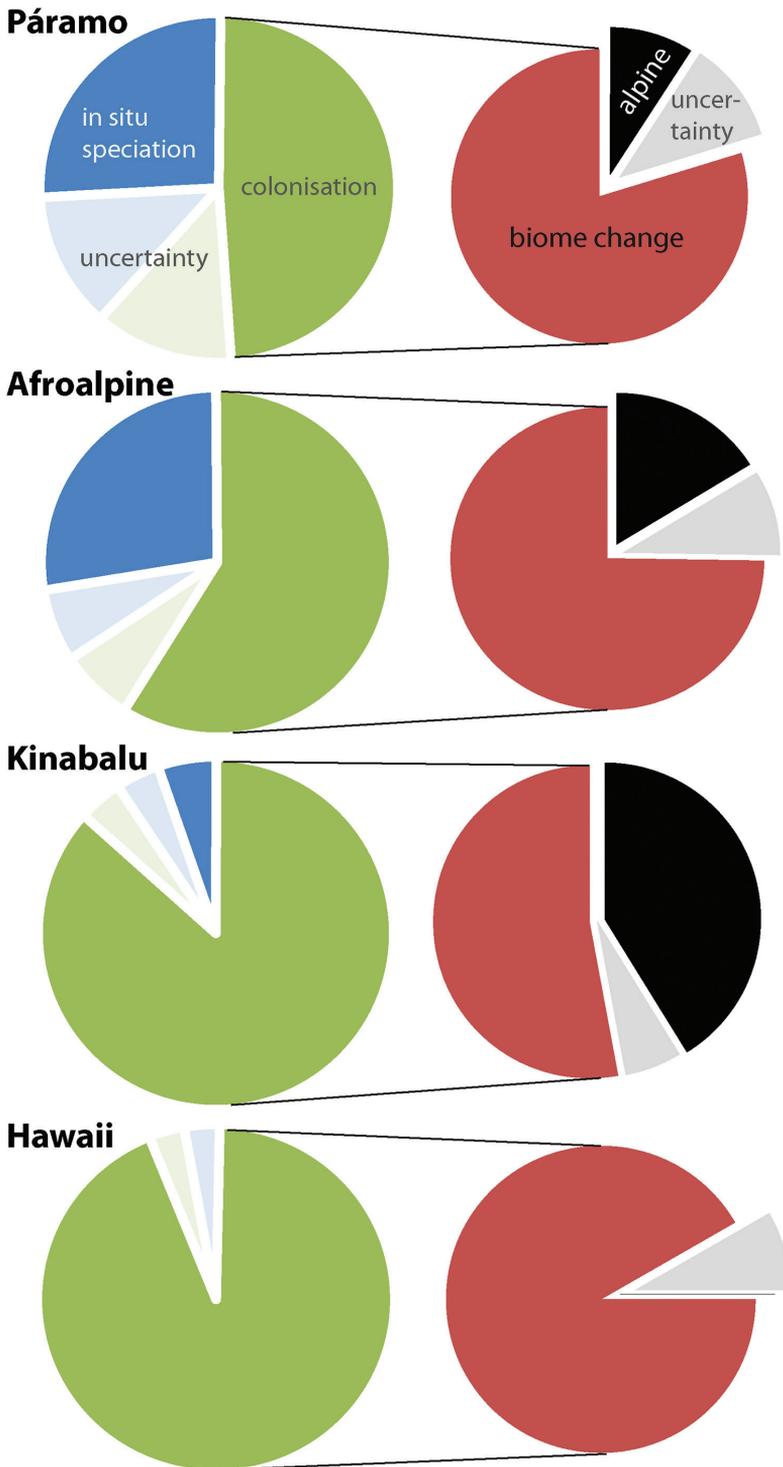


Figure 3. Proportion of plant elements in tropical alpine regions based on generic distribution patterns according to Smith and Cleef (1988).

Afroalpine does harbour a distinct set of species, many of these have a wider altitudinal range. The author would argue that, by distinguishing true alpine-like conditions from those at lower elevations and by identifying clades endemic to each, one can better understand the evolutionary history of true tropical alpine-like plants.

Despite the author's use of the available phylogenetic data, the analysis presented here is still limited by uncertainty in discerning *in situ* alpine speciation and colonisation from lower elevations in the same area. The Andean dataset used for the estimation of species origins is particularly ridden with uncertainty as most published phylogenies of Andean Páramo plants are not well enough sampled or resolved to code species unambiguously. Additionally, the definition of Páramo by many authors is wide, often including all alpine-like or otherwise cool temperate areas without forest as low as 2800 m (Madriñán et al. 2013). It is possible that even more tropical alpine-like plants might have originated at lower elevations than inferred here (see also discussion in Hughes and Eastwood 2006; Sklenář et al. 2011; Madriñán et al. 2013). Many tropical alpine-like species and lineages in general have widespread geographical and altitudinal distributions in their respective areas and, for many of those, there is currently a lack of sufficiently sampled and/or resolved phylogenetic data necessary to code them accurately. Nevertheless, the overall patterns revealed in these analyses are clear and likely to be robust to the future addition of more fine-grained data for individual plant groups.

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References

- Ackerly DD (2003) Evolution of functional traits in plants. Univ. of Chicago Press, Chicago.
- Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY, Taberlet P, Brochmann C (2007) Frequent long-distance plant colonization in the changing Arctic. *Science* 316: 1606–1609. <https://doi.org/10.1126/science.1139178>
- Alves TLS, Chauveau O, Eggers L, de Souza-Chies TT (2014) Species discrimination in *Sisyrinchium* (Iridaceae): assessment of DNA barcodes in a taxonomically challenging genus. *Molecular Ecology Resources* 14: 324–335. <https://doi.org/10.1111/1755-0998.12182>
- Antonelli A, Zizka A, Silvestro D, Scharn R, Cascales-Miñana B, Bacon CD (2015) An engine for global plant diversity: highest evolutionary turnover and emigration in the American tropics. *Frontiers in Genetics* 6: 1–14. <https://doi.org/10.3389/fgene.2015.00130>

- Assefa A, Ehrich D, Taberlet P, Nemomissa S, Brochmann C (2007) Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine *Arabis alpina*. *Heredity* 99: 133–142. <https://doi.org/10.1038/sj.hdy.6800974>
- Baldwin BG, Sanderson MJ (1998) Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences of the United States of America* 95: 9402–9406. <https://doi.org/10.1073/pnas.95.16.9402>
- Baldwin BG, Wessa BL (2000) Origin and relationships of the tarweed-silversword lineage (Compositae-Madiinae). *American Journal of Botany* 87: 1890–1908. <https://doi.org/10.2307/2656841>
- Beaman JH, Beaman RS (1990) Diversity and distribution patterns in the flora of Mount Kinabalu. In: Baas P, Kalkman K, Geesink R (Eds) *The plant diversity of Malesia*. Kluwer, Dordrecht, 147–160. https://doi.org/10.1007/978-94-009-2107-8_14
- Bell CD, Donoghue MJ (2005) Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American valerians. *Organisms Diversity & Evolution* 5: 147–159. <https://doi.org/10.1016/j.ode.2004.10.014>
- Berglund BE, Persson T, Bjorkman L (2008) Late Quaternary landscape and vegetation diversity in a North European perspective. *Quaternary International* 184: 187–194. <https://doi.org/10.1016/j.quaint.2007.09.018>
- Cain SA (1944) *Foundations of plant geography*. Harper, New York.
- Cameron RAD, Triantis KA, Parent CE, Guilhaumon F, Alonso MR, Ibanez M, Martins AMD, Ladle RJ, Whittaker RJ (2013) Snails on oceanic islands: testing the general dynamic model of oceanic island biogeography using linear mixed effect models. *Journal of Biogeography* 40: 117–130. <https://doi.org/10.1111/j.1365-2699.2012.02781.x>
- Carlquist SJ (1974) *Island biology*. Columbia University Press, New York. <https://doi.org/10.5962/bhl.title.63768>
- Chala D, Zimmermann NE, Brochmann C, Bakkestuen V (2017) Migration corridors for alpine plants among the 'sky islands' of eastern Africa: do they, or did they exist? *Alpine Botany*: 1–12. <https://doi.org/10.1007/s00035-017-0184-z>
- Crisp MD, Arroyo MTK, Cook LG, Gandolfo MA, Jordan GJ, McGlone MS, Weston PH, Westoby M, Wilf P, Linder HP (2009) Phylogenetic biome conservatism on a global scale. *Nature* 458: 754–U790. <https://doi.org/10.1038/nature07764>
- Crisp MD, Cook LG (2012) Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? *New Phytologist* 196: 681–694. <https://doi.org/10.1111/j.1469-8137.2012.04298.x>
- Donoghue MJ (2008) A phylogenetic perspective on the distribution of plant diversity. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11549–11555. <https://doi.org/10.1073/pnas.0801962105>
- Dowle EJ, Morgan-Richards M, Treweek SA (2013) Molecular evolution and the latitudinal biodiversity gradient. *Heredity* 110: 501–510. <https://doi.org/10.1038/Hdy.2013.4>
- Engler A (1879) *Versuch einer Entwicklungsgeschichte der Pflanzenwelt insbesondere der Florengebiete seit der Tertiärperiode*. Engelmann, Leipzig.
- Engler A (1892) *Über die Hochgebirgsflora des tropischen Afrika*. Königliche Akademie der Wissenschaften zu Berlin, Berlin.

- Galbany-Casals M, Unwin M, Garcia-Jacas N, Smitsen RD, Susanna A, Bayer RJ (2014) Phylogenetic relationships in *Helichrysum* (Compositae: Gnaphalieae) and related genera: Incongruence between nuclear and plastid phylogenies, biogeographic and morphological patterns, and implications for generic delimitation. *Taxon* 63: 608–624. <https://doi.org/10.12705/633.8>
- Galley C, Bytebier B, Bellstedt DU, Linder HP (2007) The cape element in the Afrotemperate flora: from Cape to Cairo? *Proceedings of the Royal Society B-Biological Sciences* 274: 535–543. <https://doi.org/10.1098/rspb.2006.0046>
- Gehrke B, Linder HP (2009) The scramble for Africa: pan-temperate elements on the African high mountains. *Proceedings of the Royal Society B-Biological Sciences* 276: 2657–2665. <https://doi.org/10.1098/rspb.2009.0334>
- Gehrke B, Linder HP (2014) Species richness, endemism and species composition in the tropical Afroalpine flora. *Alpine Botany* 124: 165–177. <https://doi.org/10.1007/s00035-014-0132-0>
- Goetsch LA, Craven LA, Hall BD (2011) Major speciation accompanied the dispersal of *Vireya* *Rhododendrons* (Ericaceae, *Rhododendron* sect. *Schistanthe*) through the Malayan archipelago: Evidence from nuclear gene sequences. *Taxon* 60: 1015–1028.
- Good R (1974) *The geography of flowering plants*. Longmans, London.
- Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford University Press, Oxford.
- Hedberg O (1951) Vegetation belts of the East African Mountains. *Svensk Bot Tidskr* 45: 140–202.
- Hedberg O (1961) The phytogeographical position of the afroalpine flora. *Recent Advances Bot* 1: 914–919.
- Hedberg O (1965) Afroalpine flora elements. *Webbia* 19: 519–529.
- Hobbs CR, Baldwin BG (2013) Asian origin and upslope migration of Hawaiian *Artemisia* (Compositae-Anthemideae). *Journal of Biogeography* 40: 442–454. <https://doi.org/10.1111/jbi.12046>
- Hughes C, Eastwood R (2006) Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America* 103: 10334–10339. <https://doi.org/10.1073/pnas.0601928103>
- Jabaily RS, Sytsma KJ (2012) Historical biogeography and life-history evolution of Andean *Puya* (Bromeliaceae). *Botanical Journal of the Linnean Society* 171: 201–224. <https://doi.org/10.1111/j.1095-8339.2012.01307.x>
- Jacobsen D (2008) Tropical high-altitude streams. In: Dudgeon D (Ed.) *Tropical stream ecology*. Elsevier Science, London, UK, 219–256. <https://doi.org/10.1016/B978-012088449-0.50010-8>
- Jessen K, Jonassen H (1935) The composition of the forests in northern Europe in Epipalaeolithic time. *Kongelige Danske Videnskabernes Selskabs Skrifter – Naturvidenskabelig og Matematisk Afdeling* 12: 1–64.
- Jordon-Thaden I, Hase I, Al-Shehbaz I, Koch MA (2010) Molecular phylogeny and systematics of the genus *Draba* (Brassicaceae) and identification of its most closely related genera. *Molecular Phylogenetics and Evolution* 55: 524–540. <https://doi.org/10.1016/j.ympev.2010.02.012>
- Josse C, Cuesta F, Navarro G, Barrera V, Cabrera E, Chacón-Moreno E, Ferreira W, Peralvo M, Saito JAT (2009) *Ecosistemas de los Andes del Norte y Centro. Bolivia, Colombia, Ecuador, Perú y Venezuela*. Secretaría General de la Comunidad Andina, Lima, Peru.
- Kandziora M, Kadereit JW, Gehrke B (2016) Frequent colonization and little in situ speciation in *Senecio* in the tropical alpine-like islands of eastern Africa. *American Journal of Botany* 103: 1483–1498. <https://doi.org/10.3732/ajb.1600210>

- Knox EB (2004) Adaptive radiation of African montane plants. In: Dieckmann U, Doebeli M, Metz JAJ, Tautz D (Eds) Adaptive Speciation. Cambridge University Press, Cambridge, 345–361. <https://doi.org/10.1017/CBO9781139342179.020>
- Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin – New York. <https://doi.org/10.1007/978-3-642-18970-8>
- Körner C (2012) Alpine treelines. Springer, New York. <https://doi.org/10.1007/978-3-0348-0396-0>
- Körner C, Paulsen J (2004) A world-wide study of high altitude treeline temperatures. *Journal of Biogeography* 31: 713–723. <https://doi.org/10.1111/j.1365-2699.2003.01043.x>
- Lauer W (1981) Ecoclimatological Conditions of the Paramo Belt in the Tropical High Mountains. *Mountain Research and Development* 1: 209–221.
- Letouzey R (1985) Notice de la carte phytogéographique du Cameroun au 1/500 000. I. Institut de la carte internationale de la végétation, Toulouse, France.
- Leuschner C (1996) Timberline and alpine vegetation on the tropical and warm-temperate oceanic islands of the world: Elevation, structure and floristics. *Vegetatio* 123: 193–206. <https://doi.org/10.1007/BF00118271>
- Luteyn JL (1999) Páramos: A checklist of plant diversity, geographical distribution, and botanical literature. The New York Botanical Garden Press, New York.
- MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton, N.J.
- Madríñán S, Cortés AJ, Richardson JE (2013) Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics* 4: 1–7. <https://doi.org/10.3389/fgene.2013.00192>
- Merckx VSFT, Hendriks KP, Beentjes KK, Mennes CB, Becking LE, Peijnenburg KTCA, Afendy A, Arumugam N, de Boer H, Biun A, Buang MM, Chen PP, Chung AYC, Dow R, Feijen FAA, Feijen H, Soest CFV, Geml J, Geurts R, Gravendeel B, Hovenkamp P, Imbun P, Ipor I, Janssens SB, Jocque M, Kappes H, Khoo E, Koomen P, Lens F, Majapun RJ, Morgado LN, Neupane S, Nieser N, Pereira JT, Rahman H, Sabran S, Sawang A, Schwallier RM, Shim PS, Smit H, Sol N, Spait M, Stech M, Stokvis F, Sugau JB, Suleiman M, Sumail S, Thomas DC, van Tol J, Tuh FYY, Yahya BE, Nais J, Repin R, Lakim M, Schilthuizen M (2015) Evolution of endemism on a young tropical mountain. *Nature* 524: 347–350. <https://doi.org/10.1038/nature14949>
- Meudt HM, Simpson BB (2006) The biogeography of the austral, subalpine genus *Ourisia* (Plantaginaceae) based on molecular phylogenetic evidence: South American origin and dispersal to New Zealand and Tasmania. *Biological Journal of the Linnean Society* 87: 479–513. <https://doi.org/10.1111/j.1095-8312.2006.00584.x>
- Muasya AM, Simpson DA, Chase MW, Culham A (2001) A phylogeny of *Isolepis* (Cyperaceae) inferred using plastid *rbcL* and *trnL-F* sequence data. *Systematic Botany* 26: 342–353.
- Nagy L, Grabherr G (2009) The biology of alpine habitats. Oxford University Press, Oxford.
- Neuner G (2014) Frost resistance in alpine woody plants. *Frontiers in Plant Science* 5. <https://doi.org/10.3389/fpls.2014.00654>
- Nürk NM, Madríñán S, Carine MA, Chase MW, Blattner FR (2013) Molecular phylogenetics and morphological evolution of St. John's wort (*Hypericum*; Hypericaceae). *Molecular Phylogenetics and Evolution* 66: 1–16. <https://doi.org/10.1016/j.ympev.2012.08.022>

- Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, d'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TE, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001) Terrestrial ecoregions of the world: a new map of life on earth *Bioscience* 51: 933–938. [https://doi.org/10.1641/0006-3568\(2001\)051\[0933:TEOTWA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2)
- Pianka ER (1966) Latitudinal gradients in species diversity: a review of concepts. *American Naturalist* 100: 33–46. <https://doi.org/10.1086/282398>
- Rundel PW (1994) Tropical alpine environments plant form and function. Cambridge Univ. Press, Cambridge. <https://doi.org/10.1017/CBO9780511551475>
- Sanchez-Baracaldo P, Thomas GH (2014) Adaptation and convergent evolution within the *Jamesonia-Eriosorus* complex in high-elevation biodiverse Andean hotspots. *Plos One* 9: e110618. <https://doi.org/10.1371/journal.pone.0110618>
- Simpson BB, Todzia CA (1990) Patterns and processes in the development of the high Andean flora. *American Journal of Botany* 77: 1419–1432. <https://doi.org/10.2307/2444752>
- Sklenář P, Dusková E, Balslev H (2011) Tropical and temperate: evolutionary history of Páramo flora. *Botanical Review* 77: 71–108. <https://doi.org/10.1007/s12229-010-9061-9>
- Sklenář P, Hedberg I, Cleef AM (2014) Island biogeography of tropical alpine floras. *Journal of Biogeography* 41: 287–297. <https://doi.org/10.1111/jbi.12212>
- Smith AP (1994) Introduction to tropical alpine vegetation. In: Rundel PW, Smith AP, Meinzer FC (Eds) *Tropical alpine environments: plant form and function*. Cambridge University Press, Cambridge, 1–20. <https://doi.org/10.1017/CBO9780511551475.002>
- Smith AP, Young TP (1987) Tropical alpine plant ecology. *Annual Review of Ecology and Systematics* 18: 137–158. <https://doi.org/10.1146/annurev.es.18.110187.001033>
- Smith JMB (1980) Vegetation of the summit zone of Mount Kinabalu. *New Phytologist* 84: 547–573. <https://doi.org/10.1111/j.1469-8137.1980.tb04562.x>
- Smith JMB, Cleef AM (1988) Composition and origins of the worlds tropical alpine floras. *Journal of Biogeography* 15: 631–645. <https://doi.org/10.2307/2845441>
- Takhtajan AL (1986) *Floristic regions of the world*. University of California Press, Berkeley.
- Webb CO (2000) Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. *American Naturalist* 156: 145–155. <https://doi.org/10.1086/303378>
- Weigelt P, Kreft H (2013) Quantifying island isolation – insights from global patterns of insular plant species richness. *Ecography* 36: 417–429. <https://doi.org/10.1111/j.1600-0587.2012.07669.x>
- White F (1983) *Vegetation of Africa a descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa*. Unesco, Paris.
- Whittaker RH (Ed.) (1975) *Communities and ecosystems*. MacMillan, New York.
- Wiens JJ, Graham CH (2005) Niche conservatism: Integrating evolution, ecology and conservation biology. *Annual Review of Ecology Evolution and Systematics* 36: 519–539. <https://doi.org/10.1146/annurev.ecolsys.36.102803.095431>
- Wölk A, Röser M (2014) Polyploid evolution, intercontinental biogeographical relationships and morphology of the recently described African oat genus *Trisetopsis* (Poaceae). *Taxon* 63: 773–788. <https://doi.org/10.12705/634.1>

Supplementary material 1

Detailed examples on how the coding was done

Author: Gehrke B

Data type: methods

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Supplementary material 2

List of genera investigated for the analysis including information on generic distribution, coding and references to the literature used

Author: Gehrke B

Data type: species data

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Supplementary material 3

Location of the tropical alpine-like climate regions in the Tropics

Author: Gehrke B

Data type: occurrence

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