

Taxonomic and floristic novelties for *Echeveria* (Crassulaceae) in Central Michoacan, Mexico

Ignacio García-Ruiz¹, Dagoberto Valentín-Martínez², Pablo Carrillo-Reyes³,
Mihai Costea⁴

1 Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Michoacán, Justo Sierra 28, Apdo. postal 109, C.P. 59510, Jiquilpan, Michoacán, México **2** Escuela de Biología de la Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México **3** Universidad de Guadalajara, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, km. 15.5 carretera a Nogales, Predio Las Agujas, Zapopan, Jalisco 45110, México **4** Wilfrid Laurier University, Waterloo, Canada, 75 University Avenue West, Waterloo, Ontario, N2L3C5, Canada

Corresponding author: Mihai Costea (mcostea@wlu.ca)

Academic editor: Pavel Stoev | Received 12 May 2016 | Accepted 16 September 2016 | Published 25 November 2016

Citation: García-Ruiz I, Valentín-Martínez D, Carrillo-Reyes P, Costea M (2016) Taxonomic and floristic novelties for *Echeveria* (Crassulaceae) in Central Michoacan, Mexico. *PhytoKeys* 75: 1–12. doi: 10.3897/phytokeys.75.9198

Abstract

A new species, *Echeveria coruana*, is described and illustrated from the malpaís near San Andrés Corú, Michoacan, Mexico. The species belongs to series *Gibbiflorae* and the new taxon was compared with *E. purhepecha* and *E. patriotica*, with whom it shares the closest morphological affinities. Additionally, *E. yalmanantlaensis* an endangered species from Sierra of Manantlán Biosphere Reserve, State of Colima, was also discovered near San Andrés Corú and is reported for the first time from the State of Michoacan. The conservation status of both species was (re)evaluated according to the criteria of the International Union for Conservation of Nature.

Keywords

Conservation, *Echeveria*, malpaís, morphology, ser. *Gibbiflorae*, ser. *Valvatae*, taxonomy

Introduction

Echeveria DC. comprises ca. 140 species of which the majority (95%) have evolved in Mexico where the genus is characterized by a high degree of endemism (Uhl 1992, Thiede 1995, Meyrán and López-Chavez 2003, Pérez-Calix and Franco 2004, Vázquez et al. 2013). Among the infrageneric groups of this genus, ser. *Gibbiflorae* (Baker) Berger (sensu Walther 1972) is the third most diverse, being surpassed only by ser. *Racemosae* and ser. *Nudae* (Pilbeam 2008). It is noteworthy mentioning that the majority of new *Echeveria* species discovered in the last decade belong to ser. *Gibbiflorae* (García and Pérez-Calix 2007, Jimeno-Sevilla and Carrillo-Reyes 2010, Reyes and González 2010, Reyes et al. 2011a, 2011b, García and Costea 2014, Nieves-Hernández et al. 2014, Jimeno-Sevilla et al. 2015), which suggests an incomplete knowledge of the species diversity in this group.

The village of San Andrés Corú is located at ca. 12 km NE of the National Park Barranca del Cupatitzio, on the eastern side of the city of Uruapan, in the State of Michoacan (19°27.982'N, 101°56.644'W). This area is a part of the Trans-Mexican Volcanic Belt and has a particularly rich flora and vegetation consisting of a mixture of pine-oak and tropical deciduous forest elements (Rzedowski 1978). During the last years, systematic botanical explorations have been conducted to produce a floristic inventory of the malpaís surrounding San Andrés Corú. The malpaís (“badlands”) is a landform that consists of relict yet recognizable lava fields that exhibit various degrees of erosion and vegetation succession stages depending on their age (Neuendorf et al. 2005). In some of the field trips undertaken NW and W–SW of the village, an unknown *Echeveria* belonging to ser. *Gibbiflorae* was discovered. Also, growing in the same type of ecosystem, at ca. 6 km SE of San Andrés Corú, *E. yalmanantlanensis* A. Vázquez & Cházaro, an endangered species of ser. *Valvatae* Moran previously known only from one population in the State of Colima (Vázquez et al. 2013), was also discovered. Thus, the first objective of this article is to describe the new species, which we named *E. coruana*, and to explore its morphological affinities with other species of ser. *Gibbiflorae*. The second aim is to report *E. yalmanantlanensis* as a new species for Michoacan.

Materials and methods

In addition to herbarium specimens, flowers and leaves of *E. coruana* and *E. yalmanantlanensis* were fixed in FAA (Ruzin 1999) for morphological studies. Several living plants of both species were collected with soil and cultivated in Jiquilpan, Michoacán for further study. We examined the basic morphology of both fresh and fixed flowers under a Nikon SMZ1500 stereomicroscope equipped with a PaxCam Arc digital camera and Pax-it 7.8 software (MIS Inc., Villa Park, Illinois). For scanning electron microscopy (SEM), we used hexamethydisilazane (HMDS) as an alternative for critical dry point (Wright et al. 2011). Fixed flowers were dehydrated using a series of ethanol steps (70%, 80%, 95% and 100%; each step 10 minutes), immersed for 10 minutes

in 1:1 ethanol: HMDS, and passed through three changes, each of 30 minutes in 100% HMDS. Samples were air dried and coated with 20 nm gold using an Emitech K 550 sputter coater. Micromorphological examination, measurements and pictures were taken at 10 kV using a Hitachi SU1510 variable pressure scanning electron microscope. Because only *E. coruana* is described, micromorphological data for pollen and seeds are presented only for this species. Additional images than those provided in the article have been uploaded in Phytoimages (Nickrent et al. 2006 onwards).

Results

The new species, *E. coruana* belongs to ser. *Gibbiflorae*, which as summarized by Kimnach (2003), includes glabrous plants, acaulescent or with monopodial stems; leaves are medium-sized to large, commonly narrowed basally into a pseudo-petiole; inflorescences are paniculiform; bracts are similar to the leaves but smaller; pedicels may reach 10 mm in length; calyx has unequal sepals; corolla is large, up to ca. 13 mm long, pentagonal-conical in bud, cylindrical-urceolate to campanulate at anthesis, \pm glaucous, often with carinate petals exhibiting a basal cavity on the inner side; nectaries are large, fleshy; styles whitish to dark-red or nearly black. *Echeveria coruana* possesses a distinctive characteristic encountered only in four other species of ser. *Gibbiflorae* — *E. dactylifera* E. Walther (Walter 1972), *E. novogaliciana* J. Reyes, Brachet & O. González (Reyes et al. 2011), *E. marianae* I. García & Costea (García and Costea 2014), and *E. rulfiana* Jimeno-Sevilla, Santana Mich. & P. Carrillo (Jimeno-Sevilla et al. 2015): the presence of corolla appendages at the base of antipetalous staminal filaments. However, it markedly differs from these species in having smaller leaf rosettes, a different leaf morphology, shorter inflorescences, cincinni with fewer flowers, and shorter pedicels. Among all these species, *E. coruana* has the shortest and most inconspicuous appendages. A detailed comparison of *E. dactylifera*, *E. novogaliciana* and *E. marianae* was provided by García and Costea (2014), and *E. coruana* can be easily contrasted with these species using the data included in Table 1. The most recently described species with corolla appendages, *E. rulfiana*, differs from *E. coruana* in its evidently caulescent habit and canalculated leaves (Jimeno-Sevilla et al. 2015). Here we compared *E. coruana* with two other species, *E. patriotica* I. García & Pérez-Calix and *E. purhepecha* I. García, which appear morphologically closer even if they do not possess corolla appendages (Table 1).

***Echeveria coruana* I. García, D. Valentín & Costea, sp. nov.**

urn:lsid:ipni.org:names:77158795-1

Figures 1, 2

Diagnosis. *Echeveria coruana* morphologically resembles most *E. patriotica* and *E. purhepecha*, with which it shares a similar flower morphology, but differs from both in having acaulescent or inconspicuous stems, acuminate leaves, and corolla appendages at the base

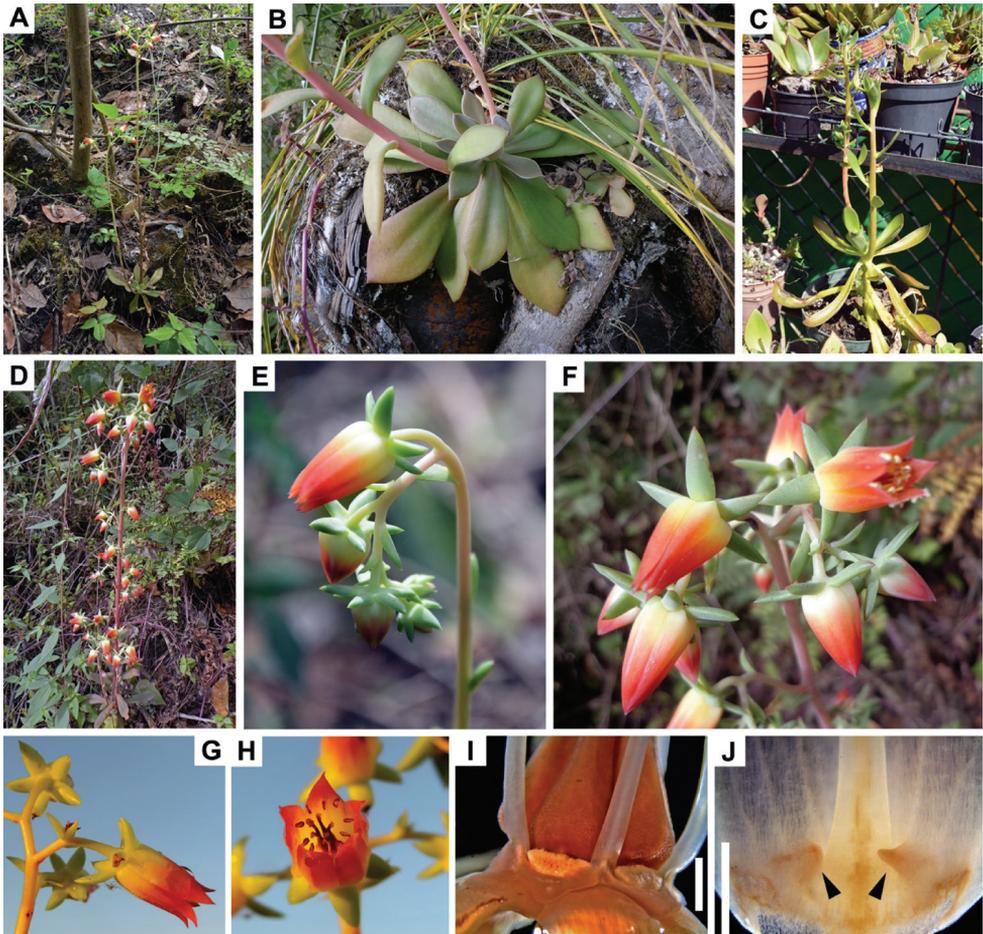


Figure 1. Habitat and general morphology of *Echeveria coruana*. **A** Habitat **B** Leaf rosette of type specimen **C** Developing plant (in cultivation) **D–F** Inflorescence **D** General view **E** Developing cincinnus **F** Terminal cincinni **G–H** Flowers of type specimen viewed in the field from different angles **I–J** Flowers from type specimen fixed in FAA **I** Dissected flower (removed corolla) to show stamen bases and nectaries. **J** Conical appendages at the base of antipetalous staminal filaments (indicated with black arrows). Scale bars 1 mm.

of antipetalous stamen filaments. It differs from *E. patriotica* in having smaller leaf rosettes, 10–15 cm in diameter, smaller inflorescences, 28–65 cm long, longer pedicels, (1.3) 1.6–2.2 mm and a narrower corolla, 7–9 mm in diameter. It can be distinguished from *E. purhepecha* by the larger rosettes with spreading linear-oblancoelate to spatulate leaves, 8–16 cm long, and the larger corolla, 15–20 mm long, light-yellow to orange in the median part and orange-reddish at the tips of corolla lobes.

Type. MÉXICO. Michoacán: Municipio de Ziracuaretiro, lado noroeste de San Andrés Corú; 19°28.116'N, 101°57.410'W; 1730 m; bosque de encino-pino alterado con huertas de aguacate; 27 Nov 2015; *I. García & M. García* 9138 (holotype: CIMI!, isotypes: DAO!, ENCB!, IEB!, MEXU!, MICH!, WLU!).

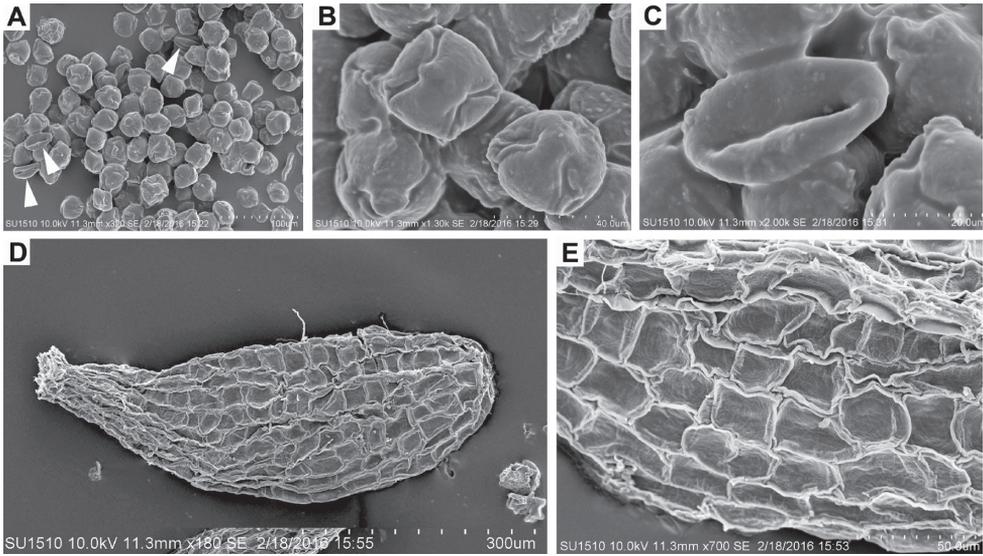


Figure 2. Scanning electron microscopy of *E. coruana*, pollen and seed (*García & García 9138*). **A–C** Heteromorphic pollen grains; arrows indicate a few 3-colpate, prolate pollen grains **B** 4-colpate (left, upper) and 3-colpate pollen (right, down) grains **C** 3-colpate, prolate pollen grain **D–E** Seed morphology.

Description. *Perennial herb*, glabrous, acaulescent or with an inconspicuous caudex, 3–8 cm long and 1–1.3 cm in diameter; *rosette* lax, 10–15 cm in diameter with 15–18 leaves; *leaves* fleshy, light-green to dark green in the median part and apex, leaf blade linear oblanceolate to spatulate, 8–16 × 2–2.5 cm, 0.5–1 cm thick at the base, margin entire, occasionally with a thin, red-colored line, apex acuminate, short mucronate, base narrowed to form a pseudo-petiole, 2–3 cm long, corrugated ventrally; *inflorescence* paniculiform thyrses, 1–3 per rosette, 28–65 cm long and 0.3–0.5 cm wide at the base, with 1–4 secondary axes (cincinni), each with 1–4(5) flowers; bracts spiralled, adpressed, green to yellowish-red, oblanceolate to oblong-lanceolate, 0.8–7.2 × 0.6–3 cm, 1.2–1.5 mm thick in the median part and 7–8.5 mm at the base, base auriculate, soon caducous; pedicels (1.3–) 1.6–2.2 mm long and 2–3 mm thick; *calyx* gamosepalous, star-shaped, the tube 1–1.5 mm long, lobes green, unequal, 6–9 × 3–4 mm, spreading to somewhat reflexed at anthesis, triangular-lanceolate; *corolla* pentagonal-conical in bud, cylindrical-urceolate at anthesis, petals 15–21 × 4–6.5 mm, fused for 1–1.5 mm at the base, lanceolate, crenate, tips mucronate, erect to slightly deflexed, color whitish-yellow at the base, light-yellow to orange in the median part and orange-reddish at the tips; *nectaries* reniform, 1.8–2.2 × 0.8–1 mm, white-yellowish; *stamens* 10, 5 antipetalous, 9–16 mm long (including the anthers), with a pair of conical or dome-like appendages at the base, 0.2–0.3 mm long; episealous stamens 5, 10–16 mm long (including anthers); *pollen* polymorphic, most abundant type is 3-colpate, oblate to oblate-spheroidal in equatorial view and triangular or round in polar view, 30–34 × 17–20 µm, less common grains are 3-colpate, prolate, 34–38 × 18–20 µm

Table 1. Comparative morphology of *Echeveria coruana* with *E. patriotica* (García and Pérez-Calix 2007) and *E. purhepecha* (García 2011); “—” indicates data not available for comparison.

Character	<i>Echeveria coruana</i> sp. nov.	<i>E. patriotica</i>	<i>E. purhepecha</i>
Caudex	Acaulescent or inconspicuous	Evident	Evident
Length × diameter (cm)	3–8 × 1–1.3	20 × 1–2.5	8–11 × 1.2
Rosette			
Diameter (cm)	10–15	10–35	8–10
Position of leaves in rosette	Spreading	Spreading	Ascendant
Leaves			
Color	Light-green to dark green in the median part and apex	Green to reddish in the older leaves	Dark-green
Shape	Linear-oblancoolate to spatulate; apex acuminate, short mucronate	Oblong-obovate to spatulate; apex rounded, short mucronate	Oblong obovate; apex rounded, mucronate
Length (cm)	8–16	6–18	2–6.5
Width (cm)	2–2.5	3.5–11	1–2.5
Inflorescence			
Number of main axes	1–4	1–7	2–3
Length (cm)	28–65	20–100	15–30
Diameter at the base (cm)	0.3–0.5	1	0.3–0.6
Number of flowers/cincinnus	1–4(5)	1–8	1–7
Bracts			
Shape	Oblong-lanceolate	Oblong-lanceolate	Oblong-obovate
Length (cm)	0.8–7.2	1.1–12	0.8–3
Width (cm)	0.6–3	0.6–4	0.3–1.3
Pedicel			
Length (cm)	(1.3) 1.6–2.2	0.7–1	0.3–1.3
Corolla shape at anthesis	Cylindrical-urceolate	Cylindrical-urceolate	Cylindrical-urceolate to campanulate
Length (mm)	15–20	18–20	10–12
Width (mm)	7–9	10–14	ca. 8
Sepals (calyx)	Spreading to ± reflexed at anthesis	Spreading at anthesis	Addressed at anthesis
Shape	Triangular-lanceolate	Triangular-lanceolate	Oblong-elliptic to lanceolate
Length × width (mm)	6–9 × 3–4	9–14 × 3–6	4–9 × 2.5–3.5
Petals			
Shape	Lanceolate	Lanceolate	Oblong-lanceolate
Length × width (mm)	15–21 × 4–6.5	18–20 × 5–7.5	10–11 × 4
(External) color	Whitish-yellow at the base, light-yellow to orange in the median part and orange-reddish at the tips	White to cream white at the base, orange-reddish in the median part and scarlet-red at the tips	Scarlet-red or coral from the base to the tip
Appendages	(1)2 per antipetalous staminal filament; conical or dome-like, 0.2–0.3 mm long	Absent	Absent
Nectaries			
Length × width (mm)	1.8–2.2 × 0.8–1	3 × 1	1.2 × 0.5
Color	White-yellowish	Purple-red	Pale yellow
Follicles	5–6 mm long, erect to somewhat spreading	12–18 mm long, erect	ca. 5 mm long, erect
Flowering	Nov–Jan	Oct–Jan	Sep–Nov
Geographical distribution	Michoacan, Mpio. Ziracuaretiro: Malpaís de San Andrés Corú	Jalisco, Mpio. Mazamitla	Michoacan, Mpio. Nuevo Parangaricutiro
Vegetation type	Mixture of oak-pine and tropical deciduous forest	Oak and oak-pine forest	Oak-pine forest

or 4-lobed, rectangular or spherical, 28–31 × 17–20 mm; in all pollen grains tectum is imperforate, scabrate; pollen grains eventually agglutinate into large masses; ovary with 5 apocarpous carpels, 9–11 × 0.3–0.4 mm; styles (including the stigmas) 4–5 mm long, red-purplish; follicles 5–6 mm long, erect to somewhat spreading; seeds numerous, oblong to obovate, light to dark-brown, reticulate, 0.5–0.65 × 0.2–0.25 mm; reticulum size 15–30 mm.

Discussion. A detailed comparison of *E. coruana* with *E. patriotica* and *E. purhepecha* is presented in Table 1. If these three species are related from an evolutionary point of view, the corolla appendages have evolved at least two times in ser. *Gibbiflorae*. The antipetalous stamen appendages of *E. coruana* are considerably smaller and less complex than those of *E. novogaliciana*, *E. marianae*, *E. dactylifera* and *E. rulfiana*, the other species of ser. *Gibbiflorae* that are known to possess them (García and Costea 2014, Jimeno-Sevilla et al. 2015). García and Costea (2014) indicated that these appendages do not have a secretory function and their role may be to protect the nectar accumulated at the base of petals from pollinators lacking a specialized feeding apparatus. In *E. coruana*, the appendages are too small to cover the corolla cavities in which nectar accumulates. Alternatively, if *E. coruana* is evolutionarily related to these latter four species, the reduction of antipetalous corolla appendages in *E. coruana* likely indicates the loss of this hypothetical nectar defense function. Corolla appendages at the base of stamens have also evolved in *Pachyphytum* (Walther 1972, Thiede and Eggl 2007), a genus that forms a sister clade to the remaining “Echeveria group” (Carrillo-Reyes et al. 2009). A molecular study for ser. *Gibbiflorae* with more extensive sampling than that of Carrillo and et al. (2009) is necessary to understand the evolutionary relationships among the numerous members of this group (Walther 1972), including the several recently described species.

Ecology. The new species grows in the understory of mixed pine-oak and tropical deciduous forest on volcanic basaltic rocky outcrops or small ledges. However, it has also been observed growing epiphytically on *Quercus* sp. The tree layer is dominated by *Quercus magnoliifolia* Née, *Ficus membranacea* C. Wright, *Juglans major* (Torr.) Heller, *Photinia microcarpa* Standl., *Bursera ariensis* (H.B.K.) Mc. Vaugh & Rzed., and *Clusia salvinii* Donn.; the most common shrubs are *Bursera bipinnata* (Sessé & Moc. ex DC.) Engl., *Montanoa bipinnatifida* (Kunth) C. Koch, *Montanoa frutescens* (Maire) ex DC. and *Rhus terebinthifolia* Schlttdl. & Cham. The herbaceous understory vegetation includes among others: *Arenaria lanuginosa* (Michx.) Rohrb., *Bonplandia geminiflora* Cav., *Tripogandra amplexicaulis* (Klotzsch ex C.B. Clarke) Woodson, *Phaseolus acutifolius* var. *latifolius* G.F. Freeman, and *Dryopteris maxonii* Underw. & C. Chr.

Phenology. November to January.

Etymology. The specific epithet derives from San Andrés Corú, the nearest village to the malpaís where the species was discovered. “Corú” in the local Purhépecha language means “a place where the quails sing”.

Conservation status. *Echeveria coruana* is currently known only from three populations located at ca. 1–2 km from one another in the malpaís of San Andrés Corú. Although it is relatively common in the studied sites, it is threatened because of the

increasing demand and exploitation of volcanic rocks in the area. Furthermore, the recent establishment of avocado orchards at elevations of 1670–1750 m has led to significant habitat loss in the area, and this practice is likely to continue in the future. Although it was not possible to use GeoCAT (Bachman et al. 2011) to calculate the extent of occurrence because of the reduced number of localities from which the species is known, we determined the area of occupancy, which was 8 square km (based on 2 km cells). Therefore, using the IUCN (2012) criteria B2 biii, we preliminarily categorize this species as Critically Endangered (CR). More research in the field will be carried out in the future to determine the best strategy to mitigate the above mentioned threats.

Additional specimens examined. MÉXICO. Michoacán: Municipio de Ziracuaretiro, Malpaís de San Andrés Corú, bosque de encino-pino, 1676 m, 1 Dec 2012, *D. Valentín* 502 (CIMI!, EBUM!); Malpaís de San Andrés Corú, lado oeste-suroeste de San Andrés Corú, 1660 m, 29 Apr 2015, *I. García*, *D. Valentín* and *A. Fuentes* 9078 (CIMI!).

***Echeveria yalmanantlanensis* new for the flora of Michoacán**

The exploration of the malpaís located at *ca.* 6 km SE of San Andrés Corú also led to an important floristic discovery: a new record of *E. yalmanantlanensis* (Fig. 3). This species has been considered in danger of extinction and endemic to the Cerro Grande Massif, which is situated in eastern Sierra of Manantlán Biosphere Reserve, Municipality of Comala, State of Colima, where it is known from one single population (Vázquez et al. 2013). More than a decade of concerted explorations conducted by multiple botanists to find additional populations at the type locality, the adjacent volcanic areas, Sierra de Manantlán Central, and Nevado de Colima, have been unsuccessful (reviewed by Vázquez et al. 2013). Under these circumstances, it was totally unexpected to discover it in Michoacan at about 210 km from the type locality. This finding suggests a disjunct distribution of *E. yalmanantlanensis*, which although rare may also be present at other localities in Central Michoacan.

Habitat and phenology of *E. yalmanantlanensis*. In Michoacan, *E. yalmanantlanensis* grows on volcanic rocks and occasionally as epiphyte in shady habitats that maintain sufficient humidity even during the dry season (Fig. 3). The vegetation at the new locality is very similar to that of *E. coruana* (see above), consisting of a mixture of pine-oak and tropical deciduous species. The vegetation at the type locality in Sierra of Manantlán includes some elements of tropical deciduous forest at 1500 m above the sea level; however, the companion species indicated by Vázquez-García et al. (2013)—*Jatropha bartlettii* Wilbur, *Bursera macvaughiana* Cuevas & Rzed., and *Agave attenuata* Salm-Dyck. — have not been observed at the new site in Michoacan. Furthermore, at the original site, oak-pine forest vegetation elements were absent. The substrate at the original site in Colima State is calcareous, while in Michoacan it is volcanic. Also the epiphytic habitat observed in Michoacan (Fig. 3) was not re-

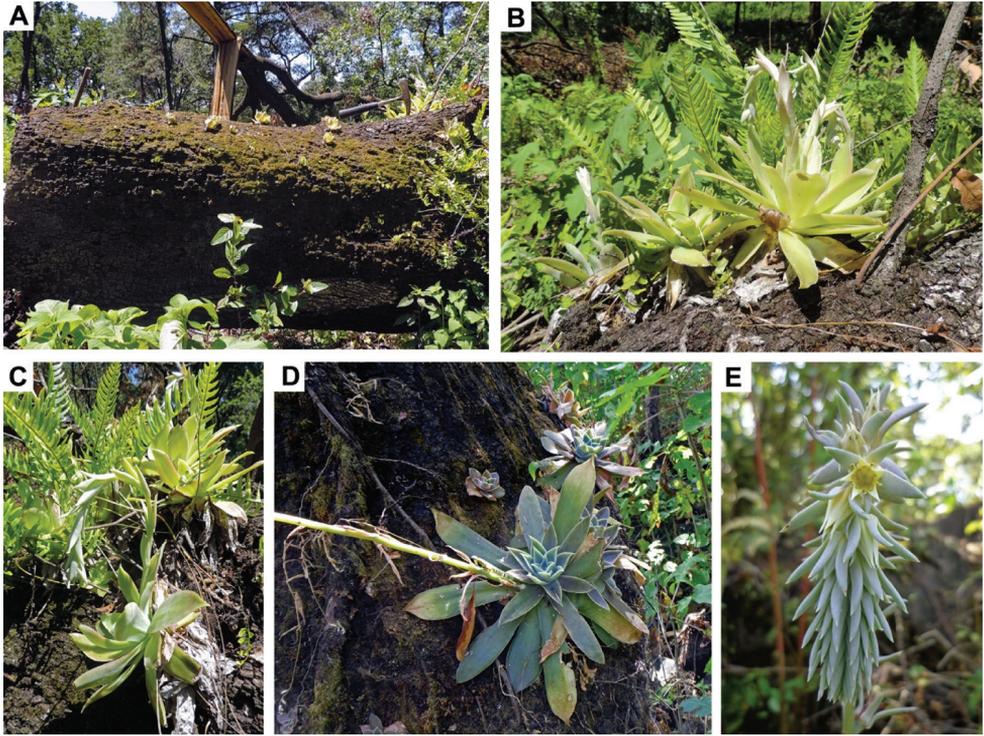


Figure 3. *Echeveria yalmanantlensis* in the new habitat from Michoacan. **A** General habitat view of young epiphytic plants **B–D** Leaf rosettes and developing plants **E** Inflorescence.

ported from the type locality. The phenology is also somewhat different between the two sites: it extends to December at the new locality in Michoacan, while in Sierra of Manantlán plants were noted to flower from the end of July to the beginning of October. Only the climate is more or less similar at both sites, as it belongs to the type (A)C(w)(i')(w₂) (García 1988), semi-warm, sub-humid, with an annual average temperature between 18° and 22°C and characteristics intermediate between warm and temperate climates.

Conservation status of *E. yalmanantlanensis*. Vázquez-García et al. (2013) proposed the inclusion of *E. yalmanantlanensis* in the Mexican Endangered Species Act as an endangered species (Norma Oficial Mexicana, NOM-ECOL-059-2010). In Michoacan, only three populations with very few mature individuals (4–10) were found. An evaluation of the conservation status based on the geographical distribution using GeoCAT (Bachman et al. 2011) revealed that the extent of occurrence and area of occupancy (based on 2 km cells) in Michoacan are 62.01 km² and 8 km², respectively. Including the population from Colima and using the IUCN (2012) criteria, we provisionally propose an endangered status (EN) for this species. Like in the case of *E. coruana*, the habitat of *E. yalmanantlanensis* is threatened by the development of avocado plantations and exploitation of volcanic rock.

Specimens examined. MEXICO. Colima: Municipio de Comala (originally cited as Jalisco-Colima because of the proximity to the border between the two states), camino a Campo Cuatro, Cerro Grande, on a rock of a limestone slope, 1550 m, 26 Sep 2011, *J. Antonio Vázquez-García, M. Cházaro B. & J. Padilla-Lepe* 9175 (holotype: IBUG!; isotype: NY!). Municipios Comala border, camino de Campo Cuatro a La Añilera, Cerro Grande, tropical dry forest, on a rock of a limestone slope, 1500 m, 18 Jul 2004, *Vázquez-García & Contreras* 7830a, 7830b (IBUG!). Michoacán: Municipio de Ziracuaretiro, Malpaís de San Andrés Corú, Bosque de Encino, epífita sobre *Quercus* sp., 1513 m, 22 Dec 2012, *D. Valentín* 537 (CIMI, EBUM!); Malpaís de San Andrés Corú, bosque mixto con encinos, sobre rocosidades, 1510 m, 29 Apr 2015, *I. García & D. Valentín* 9077 (CIMI!); 15 Sep 2015, *I. García & D. Valentín* 9077 (CIMI!, WLU!).

Acknowledgements

We would like to thank the managers/directors of the CIMI, EBUM and IEB herbaria for providing access to collections, and to David Jimeno Sevilla for reviewing the manuscript. We are also grateful to Jesús Contreras León for his help during the field trips, to Patricia Silva Sáenz for her constructive comments, and to Fidel Carrillo for providing the initial information about the location of *E. coruana*. Last but not least, the first author is grateful to COFAA and EDI, and in particular SIP projects 20141105 and 20150493 of the Instituto Politécnico Nacional, Mexico for supporting this research.

References

- Bachman S, Moat J, Hil AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys* 150: 117–126. doi: 10.3897/zookeys.150.2109
- Carrillo-Reyes P, Sosa V, Mort ME (2009) Molecular phylogeny of the Acre clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267–276. doi: 10.1016/j.ympev.2009.05.022
- García E (1988) Modificaciones al sistema de clasificación climática de Köppen. Talleres Larios, S. A. 4th edición, México, 220 pp.
- García RI, Pérez-Calix E (2007) Una especie nueva de *Echeveria* (Crassulaceae) originaria del estado de Jalisco, México. *Acta Botanica Mexicana* 78: 125–132.
- García RI (2011) Nueva especie de *Echeveria* (Crassulaceae) del centro-occidente de Michoacán, México. *Revista Mexicana de Biodiversidad* 1: 63–67.
- García RI, Costea M (2014) *Echeveria marianae* (Crassulaceae), a new species from Jalisco, México. *Phytotaxa* 170(1): 35–040. doi: 10.11646/phytotaxa.170.1.4
- IUCN Standards and Petitions Subcommittee (2016) Guidelines for using the IUCN Red List categories and criteria. Version 12. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [accessed 3.05.2016]

- Jimeno-Sevilla HD, Carrillo-Reyes P (2010) *Echeveria perezcalixii* (Crassulaceae), una especie nueva del occidente de México. *Brittonia* 62: 303–308. doi: 10.1007/s12228-010-9137-1
- Jimeno-Sevilla HD, Santana-Michel FJ, Carrillo-Reyes P (2015) Dos especies nuevas de Crassulaceae del Sur de Jalisco, México. *Acta Botanica Mexicana* 110: 71–88.
- Kimnach M (2003) *Echeveria*. In: Egli U (Ed.) *Illustrated handbook of succulent plants: Crassulaceae*. Springer-Verlag, Berlin, 103–128.
- Meyrán J, López L (2003) *Las Crasuláceas de México*. Sociedad Mexicana de Cactología, A. C. México, D. F., 234 pp.
- Moran R (1974) Division of the genus *Echeveria* into series. In: Jacobsen H (Ed.) *Lexicon of succulent plants*. Blandford Press, London, 184–186.
- Nickrent DL, Costea M, Barcelona JF, Pelsner PB, Nixon K (2006-onwards) *Phytoimages*. <http://www.phytoimages.siu.edu> [accessed 2 Septemeber 2016]
- Neuendorf KKE, Mehl JP Jr, Jackson JA (2005) *Glossary of geology* (5th ed). American Geological Institute, Alexandria, Virginia, 779 pp.
- Nieves-Hernández G, Vázquez-García JA, Muñoz-Castro MA, Cházaro-Basáñez M (2014) *Echeveria cerrograndensis* (Crassulaceae), a new species from Eastern calcareous Sierra de Manantlán, Colima, México. *Phytotaxa* 172: 247–255. doi: 10.11646/phytotaxa.172.3.5
- Norma Oficial Mexicana (NOM-059-ECOL2010) (2010) Protección ambiental. Especies nativas de México de flora y fauna silvestres. Categorías de riesgo y especificaciones para su inclusión, exclusion o cambio. Lista de especies en riesgo. Diario Oficial de la Federación, Instituto Nacional de Ecología, México, 78 pp.
- Pérez-Calix E (2008) Familia Crassulaceae. Flora de Bajío y de Regiones Adyacentes. Fasc. 156. Instituto de Ecología, A. C., Pátzcuaro, Michoacán, 141 pp.
- Pérez-Calix E, Franco IS (2004) Crasuláceas. In: García-Mendoza AJ, Ordoñez MJ, Briones Salas M (Ed.) *Biodiversidad de Oaxaca*. Instituto de Biología, UNAM-Fondo Oaxaqueño para la Conservación de la Naturaleza-World Wildlife Fund, México, 209–217.
- Pilbeam, J (2008) The genus *Echeveria*. *British Cactus and Succulent Society*, Norwich, 333 pp.
- Rzedowski, J. 1978. *Vegetación de México*. Editorial Limusa, México, 432 pp.
- Reyes J, González O (2010) *Echeveria roseiflora* (Crassulaceae), una nueva especie para el estado de Jalisco, México. *Cactáceas y Suculentas Mexicanas* 55: 19–26.
- Reyes J, Brachet C, González O (2011a) *Echeveria guerrerensis* (Crassulaceae), una nueva especie para el estado de Guerrero, México. *Cactáceas y Suculentas Mexicanas* 56: 75–81.
- Reyes J, Brachet C, González O (2011b). *Echeveria novogaliciana*, una nueva especie de la familia Crassulaceae para los estados de Aguascalientes y Jalisco, México. *Cactáceas y Suculentas Mexicanas* 56: 82–95.
- Ruzin SE (1999) *Plant microtechnique and microscopy*. Oxford University Press, Oxford, 336 pp.
- Thiede J (1995) Quantitative phytogeography, species richness, and evolution of American Crassulaceae. In: Hart H, Egli U (Eds) *Evolution and systematics of the Crassulaceae*, Backhuys, Leiden, 89–123.
- Thiede J, Egli U (2007) Crassulaceae. In: Kubitzki K (Ed.) *Flowering Plants. Eudicots*, Vol. 9. Springer, Berlin, Heidelberg, 83–118. doi: 10.1007/978-3-540-32219-1_12
- Uhl CH (1992) Polyploidy, diploidy, and chromosome pairing in *Echeveria* (Crassulaceae) and its hybrids. *American Journal of Botany* 79: 556–566. doi: 10.2307/2444868

- Vázquez-García JA, Jimeno SD, Cuevas GR, Cházaro BM, Muñiz-Castro MA (2013) *Echeveria yalmanantlanensis* (Crassulaceae): A new species from Cerro Grande, Sierra de Manantlán, western Mexico. *Brittonia* 65: 273–279. doi: 10.1007/s12228-012-9274-9
- Vázquez-García JA, Nieves-Hernández G, Padilla-Lepe J, Nuño-Rubio AT, Cházaro-Basáñez M (2014) *Echeveria munizii* (Crassulaceae) a new species of epiphyte from tropical Volcán de Colima, Mexico. *Phytotaxa* 191:165–171. doi: 10.11646/phytotaxa.191.1.11
- Walther E (1972) *Echeveria*. California Academy of Sciences, San Francisco, 426 pp.
- Wright MA, Welsh M, Costea M (2011) Diversity and evolution of the gynoeceium in *Cuscuta* (dodders, Convolvulaceae) in relation to their reproductive biology: two styles are better than one. *Plant Systematics and Evolution* 296: 51–76. doi: 10.1007/s00606-011-0476-5

The identity of *Callicarpa minutiflora* Y.Y. Qian (Lamiaceae) and taxonomic synonym of *C. longifolia* Lamarck

Zhonghui Ma¹, Zhiwei Su²

1 Agricultural College, Guangxi University, Nanning, Guangxi, 530004, China **2** Guangxi Key Laboratory of Marine Environmental Science, Guangxi Academy of Sciences, Nanning, Guangxi, 530007, China

Corresponding author: Zhiwei Su (suzhiwei@gxas.cn)

Academic editor: E. Fischer | Received 2 October 2016 | Accepted 18 November 2016 | Published 29 November 2016

Citation: Ma Z, Su Z (2016) The identity of *Callicarpa minutiflora* Y. Y. Qian (Lamiaceae) and taxonomic synonym of *C. longifolia* Lamarck. *PhytoKeys* 75: 13–18. doi: 10.3897/phytokeys.75.10704

Abstract

Although the specific epithet of *Callicarpa minutiflora* Y. Y. Qian has been revised for many times, during the study of the genus *Callicarpa*, we find that *C. minutiflora* Y. Y. Qian is identical to *C. longifolia* Lamarck by a series of morphologic characters. In order to avoid more confusion, here *C. minutiflora* Y. Y. Qian is reduced as a synonym of *C. longifolia* Lamarck.

Keywords

Callicarpa minutiflora, *Callicarpa longifolia*, Lamiaceae

Introduction

The genus *Callicarpa* L. is currently treated as incertae sedis after being transferred from the family Verbenaceae to Lamiaceae (Harley et al. 2004, Bramley 2009, 2013, Olmstead 2010, 2012, Ma et al. 2015). This genus comprises approximately 140 species mainly distributed in temperate, subtropical and tropical Asia, America, Australia and the Pacific Islands (Harley et al. 2004, Bramley 2009, 2013, Ma et al. 2015), with

48 species and 13 varieties occurring in China (Chen and Gilbert 1994). Although some regional revisions (especially in Southeast Asia) of *Callicarpa* have been completed (Munir 1982, Leeratiwong et al. 2009, Bramley 2009, 2013), this genus is still taxonomically problematic due to lack of field investigations and specimens available for study in some species.

Among the Chinese species with 4-angled branchlets and a conspicuous interpetiolar transverse ridge resembling a stipule scar, *C. acutifolia* Chang, *C. longifolia* Lamarck, *C. longissima* (Hemsley) Merrill and *C. minutiflora* Y. Y. Qian are always troublesome to distinguish between each other. The last species *C. minutiflora* Y. Y. Qian was described based on the type collection (Y.Y. Qian 1800) from Jiangcheng County, Yunnan Province, China and no other collections could be found. However, this specific epithet is a later homonym of earlier *C. minutiflora* Rusby and therefore illegitimate. Although this problem was overlooked during preparation of *Flora of China* (Chen and Gilbert 1994), recently Duan and Zhang (2014) discovered this illegitimate name when they updated the *Flora of China* online version and proposed a new replaced name *Callicarpa tenuiflora* Li Bing Zhang & Yi F. Duan. Unfortunately, this new name is also a later homonym of legitimate earlier *C. tenuiflora* Champ. ex Benth. characterised by very short petiole or subsessile and cordate leaf base which is considered as a synonym of *C. rubella* Lindley (Leeratiwong et al. 2009). Again, Zhang (2014) proposed *C. qianyiyoungii* Li Bing Zhang as a new replaced name for the later homonym *C. tenuiflora* Li Bing Zhang & Yi F. Duan.

In the protologue, Qian (1991) stated that *C. minutiflora* Y. Y. Qian was similar to *C. acutifolia* Chang with 4-angled, grooved branchlets and a transverse scar in the node, but could be distinguished from the latter by its elliptic leaf blade, shorter peduncle compared with petiole, as well as densely tomentellous calyx, corolla and ovary (Figure 1). However, after examination of type specimens and original descriptions, we find that *C. minutiflora* Y. Y. Qian is identical to *C. longifolia* Lamarck by its 4-angled branchlets, conspicuous interpetiolar ridge, elliptic leaf blade and by covering of dense gray pubescent and yellowish sessile glands on branchlets, petioles, both sides of leaf blade (especially dense on the midrib and venation), peduncles, ovary and outer surface of calyx and corolla (Figures 1, 2), which obviously differs from other *Callicarpa* species. Additionally, the plant of *C. minutiflora* Y. Y. Qian is ca. 2 m tall; petiole slender, 1–2.5 cm; leaf blade 10–16 × 4–7 cm, base cuneate, apex acuminate; peduncle 0.6–1.5 cm; calyx 0.7–1 mm, subtruncate; corolla pale pink to lilac, 2–2.5 mm; fruit globose; flowering in Jul.–Aug. and fruiting in Aug.–Oct.; distributed in forests, at an elevation of about 1100 m (Qian 1991, Chen and Gilbert 1994). Although the color of mature fruit is still unknown, these characters above are also perfectly consistent with or in the range of variation of those of *C. longifolia* (Figures 1, 2).

In order to avoid more confusion, it is essential to reduce *C. minutiflora* Y. Y. Qian as a synonym of *C. longifolia* Lamarck.



Figure 1. Type of *Callicarpa minutiflora* Y. Y. Qian and *C. longifolia* Lamarck.

Taxonomic treatment

Callicarpa longifolia Lamarck (1785: 563)

= *C. minutiflora* Y.Y. Qian (1991: 121), *nom. illeg.*, non *C. minutiflora* Rusby (1927: 339); *C. tenuiflora* Li Bing Zhang & Yi F. Duan (2014: 278), *nom. illeg.*, non *C. tenuiflora* Champ. ex Benth. (1853: 135); *C. qianyiyoungii* Li Bing Zhang (2014: 57), **syn. nov.**

Type: China. Yunnan, Jiangcheng, forests, 1050 m, 9 Aug 1988, Y.Y. Qian 1800 (holotype, HITBC!; isotype, SYS!).

Type. Malaysia, Malacca, Sonnerat, s.n. (holotype P-LA); Malaysia, Hooker, J.D., s.n. (syntype K, K000194831, microfiche!).

Distribution. China, Pakistan, India, Bhutan, Bangladesh, South-East Asia through to New Guinea, Australia.

Ecology. In the edge of secondary forest and disturbed areas, such as roadsides, streamside, or open patches in primary forest. Alt. 0–1300 m (Leeratiwong *et al.* 2009, Bramley 2009, 2013, Chang 1951).

Other specimens examined. **China.** Guangdong: Zhaoqing, H.G. Ye 45 (IBSC!); Gaoyao, C. Huang 161917 (IBSC!); Yangshan, L. Deng 1313 (PE!); Luoding, Z.H.

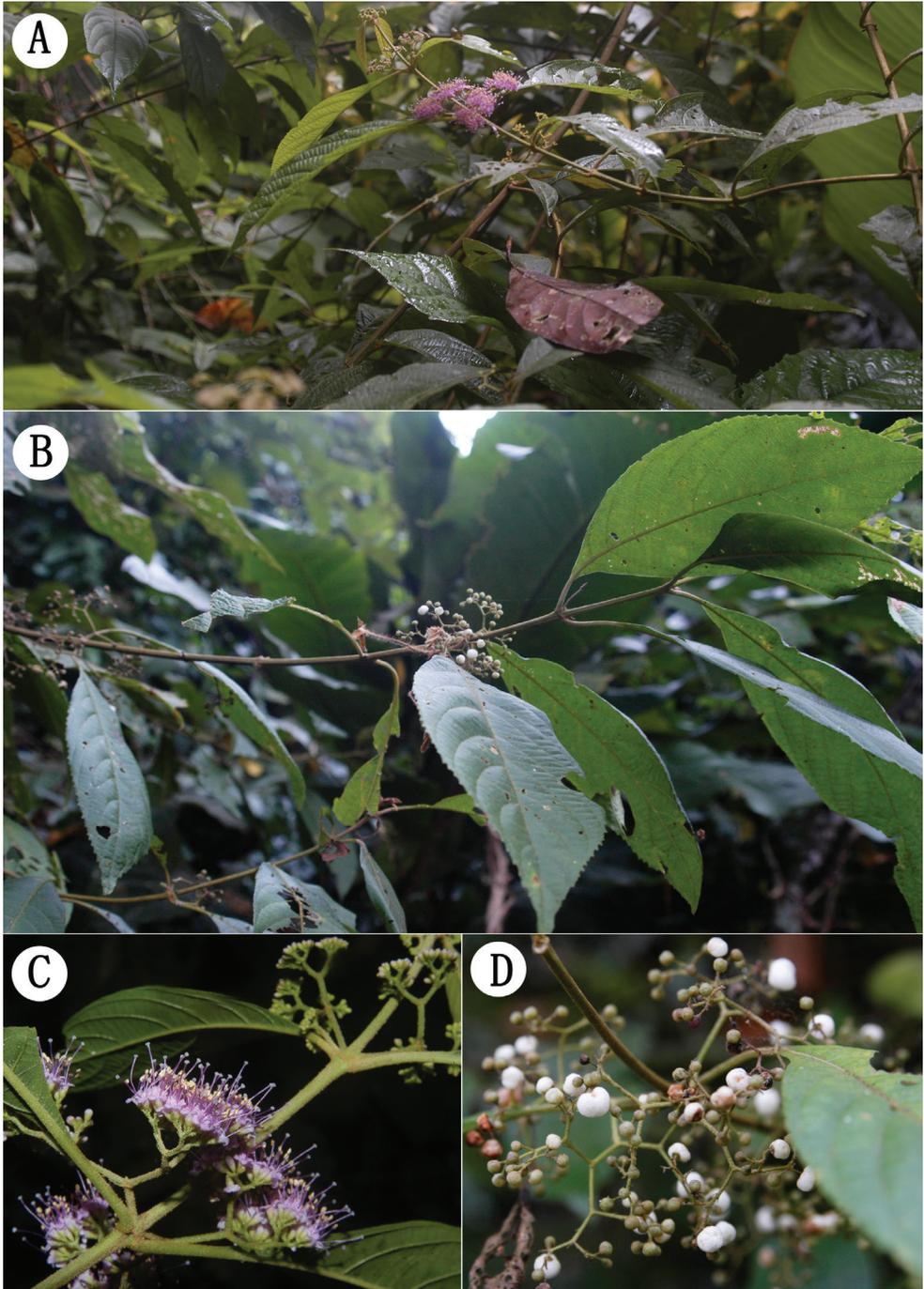


Figure 2. Field images of *Callicarpa longifolia* Lamarck. **A, C** branch with flowers (Z.H. Ma ZHM0154 IBSC) **B, D** branch with white fruits (Z.H. Ma ZHM0117 IBSC).

Ma ZHM003, ZHM 005 (IBSC!). Guangxi: Lingle, Z.Q. Zhang 10547 (IBSC!); Cangwu, S.Q. Chen 9936 (IBSC!); Ningming, Z.H. Ma ZHM0154 (IBSC!). Hainan: Wanning, F.W. Xing 5563 (IBSC!); Z.X. Li 4758 (IBSC!); Baoting, K.Z. Hou 72820 (IBSC!, PE!); Anding, Z. Huang 35683 (IBSC!, PE!); Baisha, X.Q. Liu 25599 (IBSC!, PE!); Ganen, X.Q. Liu 4875 (IBSC!, SYS!); Sanya, Z.X. Li 2611 (IBSC!); Ledong, X.Q. Liu 27118 (IBSC!); Dongfang, S.Q. Chen 11225 (IBSC!). Jiangxi: Jinggangshan, J. Xiong 3181 (PE!). Yunnan: Pingbian, H.T. Tsai 61314 (IBSC!), 61385 (PE!), P.Y. Mao 3033 (IBSC!); Mengla, C.W. Wang 80038 (PE!); Hekou, Anonymous 1741 (PE!); Jinghong, G.D. Tao 17611 (PE!), P.W. Xie 10-145 (IBSC!), Z.H. Ma ZHM0117 (IBSC!); Cangyuan, Y.H. Li 012620 (SYS!). Hongkong: S.Y. Hu 10193 (PE!); Tsiang Ying 609 (SYS!). **Vietnam.** China-Vietnam Expedition 1211 (IBSC!). **India.** U. Singh 81 (IBSC!). **Indonesia.** Johns, R.J. 9851 (K!), J.A. Lorzing 13463 (K!). **Australia.** Drinkel, C. 2 (K). **Papua.** L.J. Brass 1013 (K!), S. Isles & A. Vinas 34486 (K!). **Malaysia.** R. Schlechter 13818 (K!), M. Jacobs 8477 (K!). **Sumatra.** P. Buwalda 6661 (K!), N. Walter & M.B. Catherine 640, 987 (K!), R.J. Morley & M. K. Kardin Morley306 (K!).

Acknowledgements

The authors are indebted to Mr. Bo Pan, Mr. Longyuan Wang and the keepers of herbaria K, SYS, IBSC and HITBC for providing images of herbarium specimens. This work is funded by National Natural Science Foundation of China (Grant No. 31400178) and Natural Science Foundation of Guangxi Province (Grant No. 609187322005).

References

- Bramley GLC (2009) The genus *Callicarpa* (Lamiaceae) on Borneo. *Botanical Journal of Linnean Society* 159: 416–455. doi: 10.1111/j.1095-8339.2009.00907.x
- Bramley GLC (2013) The genus *Callicarpa* (Lamiaceae) in the Philippines. *Kew Bulletin* 68: 1–50. doi: 10.1007/s12225-013-9456-y
- Champion MJG (1853) *Florula Hongkongensis: an enumeration of the plants collected in the island of Hongkong.* *Hooker's Journal of Botany and Kew Garden Miscellany* 5: 135.
- Chang HT (1951) A review of the Chinese species of *Callicarpa*. *Acta Phytotaxonomica Sinica* 1: 269–312.
- Chen SL, Gilbert MG (1994) *Callicarpa*. In: Wu CY, Raven PH (Eds) *Flora of China* 17. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis, 3–16.
- Duan YF, Zhang LB (2014) Eight new names and lectotypification of six names in Lamianae (asterids I) for the Flora of China. *Phytotaxa* 170(4): 278–282. doi: 10.11646/phytotaxa.170.4.5
- Harley RM, Atkins S, Budantsey AL, Cantino PD, Conn BJ, Grayer R, Harley MM, Kok R de Krestovskaja T, Morales R, Paton AJ, Ryding O, Upson T (2004) Labiatae. In: Kubi-

- tzki K, Kadereit JW (Eds) Families and Genera of Vascular Plants – Flowering Plants – Dicotyledons-Lamiales (except Acanthaceae including Avicenniaceae) 7. Springer, Berlin, 167–275.
- Lamarck JBAPM (1785) Encyclopédie Méthodique, Botanique (vol. 1). Chez Panckoucke, Libraire, Hotel de Thou, rue des Poitevins, Paris, 563 pp.
- Leeratiwong C, Chantaranothai P, Paton AJ (2009) A synopsis of the genus *Callicarpa* L. (Lamiaceae) in Thailand. *Thai Forest Bulletin (Botany)* 37: 36–58.
- Ma ZH, Bramley GLC, Zhang DX (2015) Pollen morphology of *Callicarpa* L. (Lamiaceae) from China and its systematic implications. *Plant Systematics and Evolution* 302: 67–88. doi: 10.1007/s00606-015-1244-8
- Ma ZH, Huang RS, Su ZW (2015) Lectotypification of *Callicarpa integerrima* (Lamiaceae). *Phytotaxa* 234(3): 299–300. doi: 10.1007/s00606-015-1244-8
- Munir AA (1982) A taxonomic revision of the genus *Callicarpa* L. (Verbenaceae) in Australia. *Journal of the Adelaide Botanic Gardens* 6: 5–39. <http://www.jstor.org/stable/23873875>
- Olmstead RG (2010) A Synoptical Classification of the Lamiales (Version 2.2). <https://my-plant.org/sites/default/files>
- Olmstead RG (2012) A Synoptical Classification of the Lamiales (Version 2.4). <http://depts.washington.edu/phylo/Classification.pdf>
- Qian YY (1991) Three new species from Yunnan. *Guihaia* 11(2): 121–126.
- Rusby HH (1927) Descriptions of new genera and species of plants collected on the Mulford Biological Exploration of the Amazon Valley, 1921–1922. *Memoirs of The New York Botanical Garden* 7: 205–387.
- Zhang LB (2014) *Callicarpa qianiyongii* (Lamiaceae): A new name for the Flora of China. *Phytotaxa* 178(1): 57–58. doi: 10.11646/phytotaxa.178.1.8

Galium shinasii (Rubiaceae): a new species of *Galium* L. from Eastern Turkey

Levent Şık¹, Hasan Yıldırım², Ademi Fahri Pirhan²,
Yusuf Altıoğlu², Meliha Gemicı²

1 Department of Biology, Faculty of Science and Arts, Celal Bayar University, 45030 Manisa, Turkey **2** Ege University, Faculty of Science, Department of Biology, 35100, Bornova-Izmir, Turkey

Corresponding author: Hasan Yıldırım (hasanyldrm@gmail.com)

Academic editor: S. Knapp | Received 20 August 2016 | Accepted 21 November 2016 | Published 29 November 2016

Citation: Şık L, Yıldırım H, Pirhan AF, Altıoğlu Y, Gemicı M (2016) *Galium shinasii* (Rubiaceae): a new species of *Galium* L. from Eastern Turkey. *PhytoKeys* 75: 19–29. doi: 10.3897/phytokeys.75.10244

Abstract

Galium shinasii Yıldırım (Rubiaceae), is described as a new species from Malatya Province in eastern Anatolia, Turkey. The new species is morphologically related to *Galium cornigerum* Boiss. & Hausskn. *G. lasiocarpum* and *G. sorgereae* Ehrend. and Schönbn. but clearly differs from them based on the morphological differences presented in the species description. In addition, the conservation status, the distribution map, and notes on the biogeography and ecology of the new species are given.

Keywords

Rubiaceae, *Galium*, ecology, taxonomy, Turkey

Introduction

The family Rubiaceae is the fourth-biggest angiosperm family with about 660 genera and 11.500 species (Robbrecht and Manen 2006, Soza and Olmstead 2010). A large number of species of Rubiaceae are herbaceous and many are adapted to xeric habitats. (Robbrecht 1988, Jansen et al. 2000). *Galium* L. is one of the largest genera of the

Rubiaceae, with about 650 species and approximately 780 taxa placed in 16 sections, including perennial and annual herbs that are distributed in temperate and tropical regions of worldwide (Willis 1985, Mabberley 1987, Goavert 2015).

A total of 121 *Galium* taxa (104 species) are found in Turkey and 60 taxa (endemism rate of 50%) are endemic to this country (Karabacak 2012).

Malatya is located in the eastern part of Turkey, one of the richest centres of species endemism in Turkey (Yıldız et al. 2004) and in recent years several new species have been described from this area and still continue to be discovered in Malatya (Yıldırım et al. 2010, Mutlu and Karakuş 2012, Tan et al. 2012, Koç and Aksoy 2013, Yıldırım and Erol 2013, Yıldırım and Şenol 2014a, 2014b, 2014c, Mutlu and Karakuş 2015a, 2015b, Yıldırım 2015a, 2015b).

Levent Canyon is one of the famous areas among biologists because of the high endemism in Malatya province. Recently, several new plant species were described in this area. Levent Canyon is characterized by marlstone, a soft, finely fissured sedimentary rock (Schnurrenberger et al. 2003, Yıldırım and Şenol 2014c) which hosts many chasmophytes. The Levent Canyon is also a centre of diversity and endemism for several chasmophyte species. For example, *Reseda malatyana* Yıldırım (Resedaceae) and Şenol, *Alkanna malatyana* Şenol and Yıldırım (Boraginaceae), *Campanula alisan-kilincii* Yıldırım and Şenol (Campanulaceae), *Galium scopulorum* Schönb.-Tem. (Rubiaceae), *Pimpinella paucidentata* V.A.Matthews (Apiaceae), *Parietaria semispeluncaria* Yıldırım (Urticaceae) and *Galium cornigerum* Boiss. and Hausskn (Rubiaceae).

In June 2011, the second author collected an unusual and distinct specimen of *Galium* on marlstone-calcareous rocky cliffs in the Levent Canyon which authors believe to be of a new species for science.

Materials and methods

Specimens of the putative new species were compared with herbarium specimens at ANK, EGE, E, G, GAZI, HUB, ISTE, K, W and WU. In addition, the relevant literature (Halácsy 1901, Ehrendorfer and Krendl 1976, Ehrendorfer and Schönbeck-Temesy 1982, Davis et al. 1988, Ehrendorfer and Schönbeck-Temesy 2005, Govaerts 2016) was reviewed. The new species was examined by stereo-binocular microscope for morphological characterisation. At least 20 mature seeds and 30 pollen grains were measured using a light microscopy. For scanning electron microscopy (SEM), the selected seed and pollen grains were placed on aluminum stubs using double-sided adhesive tape, sputter coated with gold using an Emiteck K550, and then examined using the FEI Quanta250 FEG SEM. Photographs of living material were taken with a Nikon D300 digital camera. The conservation status of new species was evaluated based on the field observations in accordance with IUCN guidelines (2012). Geographical positions were recorded using a Magellan explorer 500 GPS.

Results

Galium shinasii Yıldırım, sp. nov.

urn:lsid:ipni.org:names:77158796-1

Figure 1–3

Type. TURKEY: B7 Malatya: Akçadağ district, Levent Canyon, on marlstone rocky cliffs 1390 m, 26.06.2011, *H. Yıldırım 2128* (holotype: EGE42431!, isotypes: EGE42432!, NGBB!, ANK!).

Paratype. TURKEY: Malatya: Akçadağ district, Levent Canyon, on marlstone rocky cliffs 1390 m, 29.06.2015, H.Yıldırım 3358 (EGE!) (Figure 2A); loc., ibid., 11.09.2015, H.Yıldırım 3713(EGE!); Doğanşehir, Eskiköy, Meletbaşı mezarısı karşısı kalker kayalıklar, 1630–1800 m., 12.07.2014, H.Yıldırım 3033 (EGE!) (Figure 2B). Erzincan: Sivas-Refahiye yolu, Refahiye'ye 1–2 km kala, kalker kaya üzeri, 1528 m, 09.09.2015, H.Yıldırım 3694 (EGE!) (Figure 2C).

Diagnosis. *Galium shinasii* is related to *Galium cornigerum*, *G. lasiocarpum* and *G. sorgereae* but it differs from them in having very reduced flowers (not flowers relatively larger), 1.2–1.8 mm corolla diam (not 2–5 mm); yellowish-green to reddish-green and 0.5–1 mm long tepals (not white or pink and not at least 2 mm); dorsal and ventral surface of with densely transparent tubercles and lateral surface 0.2–0.4 mm spreading to patent hairy fruits (not tubercles absent and fruits wholly villous, hirsute or subtomentose).

Description. Dwarf, caespitose perennial plant with many headed rootstock, suffruticose at base. Stem 1.5–6 cm long, fragile, prostrate-ascending to erect, many branched at base, glabrous to slightly puberulent, sometimes slightly winged on nerves, upper internodes elongate to 5 mm. Leaves in whorls of mostly 4, rarely 6, linear-lanceolate to narrow elliptic, 2–8 × 0.6–1.3 mm, 1 veined, glabrous to slightly puberulent, revolute at margin. Inflorescence dicashium, mostly terminal and also axillary, 8 to 75 flowered per stem; bracteoles absent. Pedicel glabrous, 1.5–2.5 mm in flowers, 2–5 mm in fruit. Calyx absent. Corolla 4 merious, yellowish-green to reddish-green, 1.2–1.8 mm diam; usually conical or campanulate, rarely infundibular; tube very reduced; lobes 0.5–1 × 0.4–0.7 mm, glabrous, triangular to lanceolate, mucronate at apex and apex incurved on petal inner surface. Stamen 0.4–0.6 mm long; anther yellow. Ovary 0.4–0.5 mm diam, dorsal and ventral surface of with densely transparent tubercles, lateral surface 0.2–0.4 mm spreading to patent hairy. Fruit depressed subglobose in fleshy, 0.5–0.75 mm, dorsal and ventral surface of with densely transparent tubercles, lateral surface 0.2–0.4 mm spreading to patent hairy.

Etymology. This species is named in honour of retired Prof. Dr. Şinasi Yıldırım (Biology Dep. Hacettepe University, Turkey), who is an expert in Plant systematics and taxonomy. He described more than 100 new plant species for science in Turkey. The Turkish name of this species is given as “Levent İplikçığı”, according to the guidelines of Menemen et al. (2013).

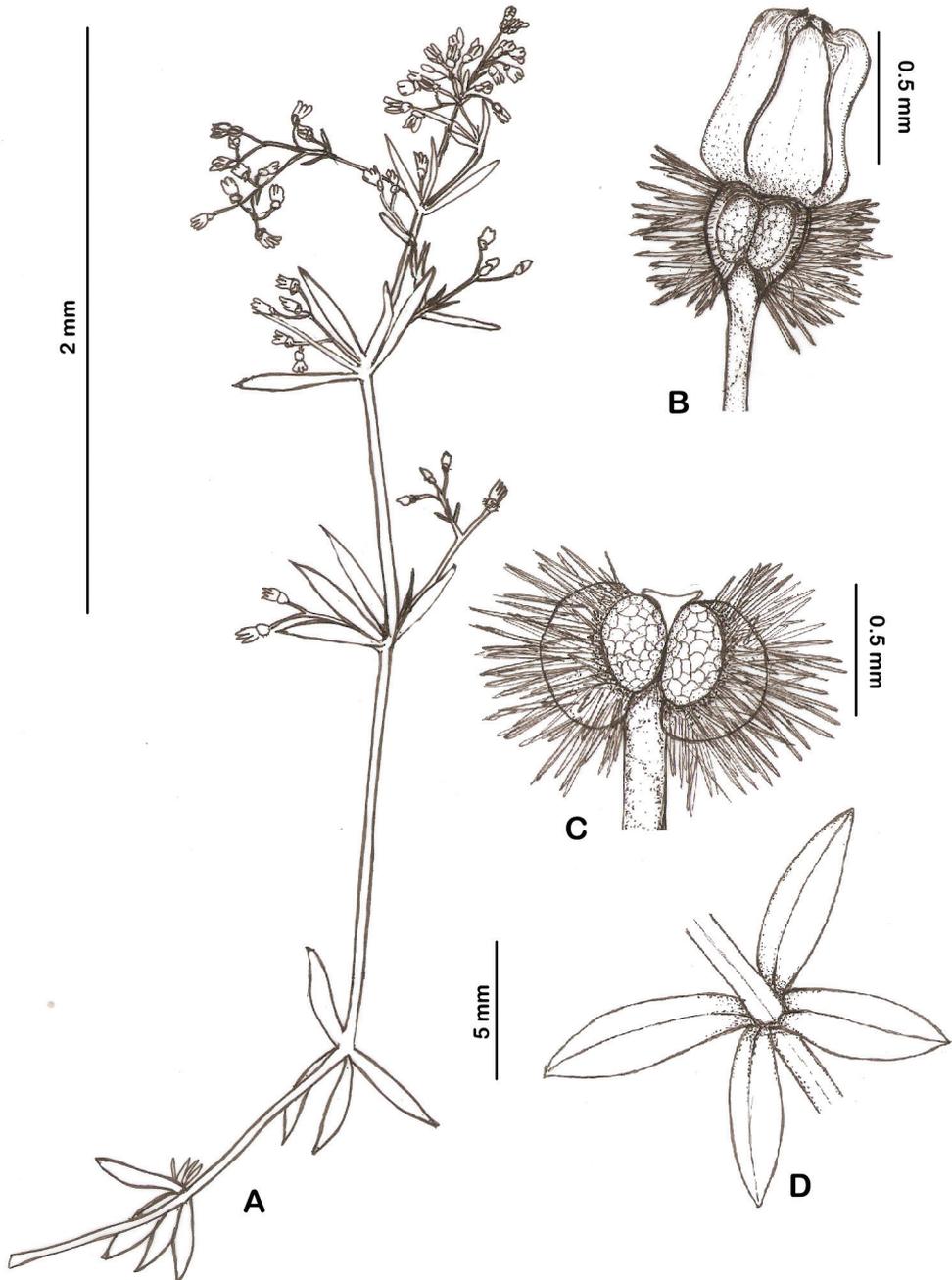


Figure 1. *Galium shinasi*: **A** habit **B** flower **C** fruit **D** leaves.

Additional specimens examined. -*G. cornigerum*: -Turkey: Malatya: Levent Kanyonu inişi, kalker kayalıklar, 30.05.2012, H.Yıldırım 2386 (EGE!); Darende, Engüzek yaylası, Akbabaçalı Dağı zirve, 2100 m, 30.05.2012. H.Yıldırım 2396 (EGE!) (Figure 4D, E).

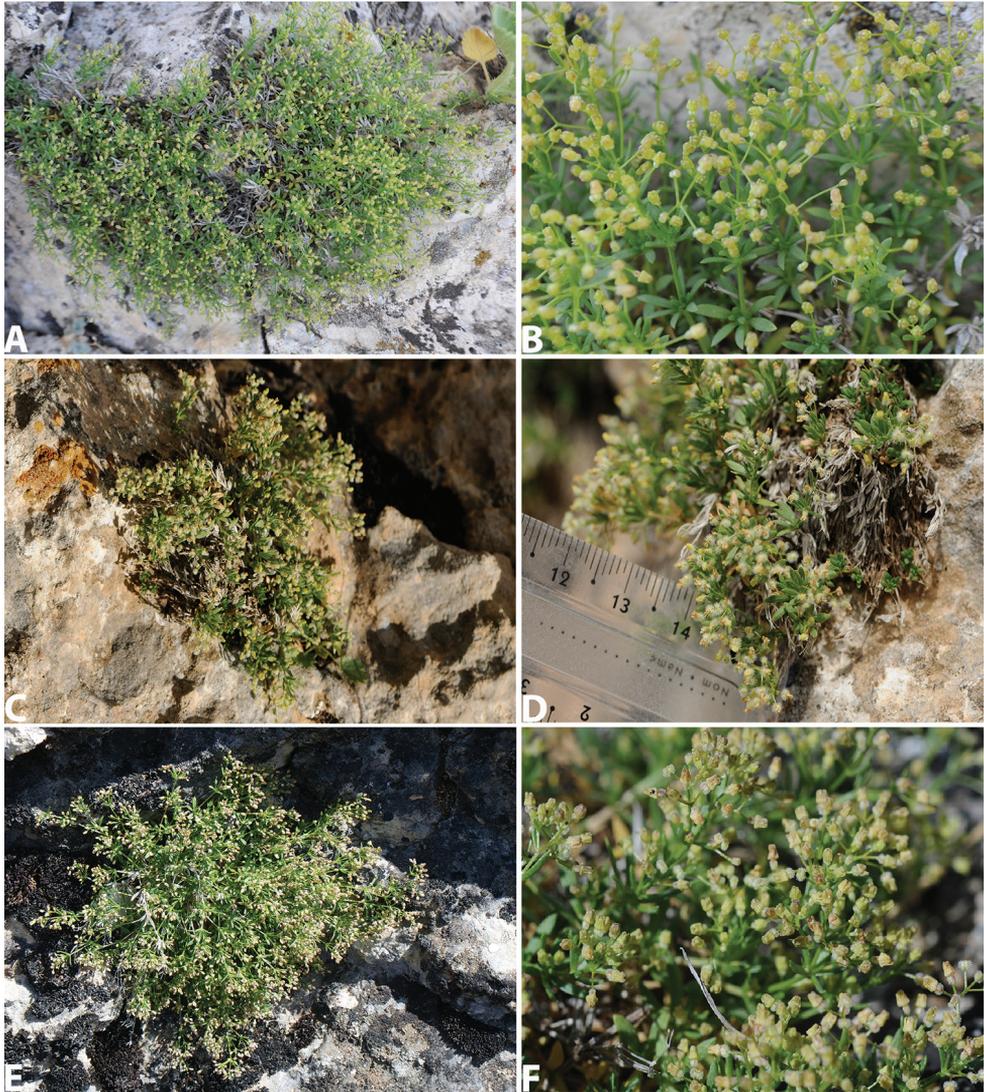


Figure 2. *Galium shinasii* habits: **A–B** from type locality, Levent Canyon, Malatya (H.Yıldırım 3358) **C–D** from Refahiye, Erzincan (H.Yıldırım 3694) **E–F** from Doğanşehir, Malatya (H.Yıldırım 3033).

–*G. incanum* subsp. *pseudocornigerum*: –Turkey: Sivas: Gürün–Pınarbaşı arası, Pınarbaşı’na 68 km kala, yol kenarı kayalık alan, 1662 m, 29.06.2016, H.Yıldırım 3948 (EGE!) (Figure 4B, C).

–*G. lasiocarpum*: –Turkey: Elazığ: in Cappadocia, Aucher 694 (holotype G!).

G. sorgerae: –Turkey: Isparta: Dedegöl Da., 2200 m, 1 vii 1965, Sorger 65-42- 70 (isotype WU!).

Distribution and ecology. *Galium shinasii* is a endemic for Eastern Anatolia. It’s known that is from Levent Canyon (Figure 5) in Akçadağ district, and Eskiköy in

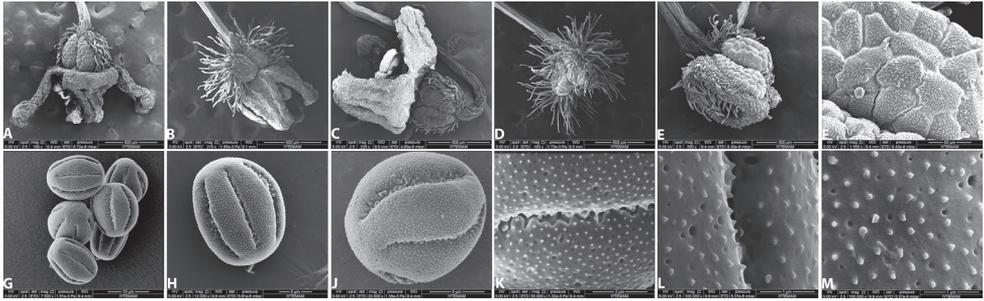


Figure 3. *Galium shinasii*: **A–C** flowers **D–E** fruits **F** fruit tubercles **G–J** pollen grains **K–M** detail of pollen grain surface.

Doğanşehir district in Malatya province and near Refahiye district in Erzincan (Figure 5). It is an element belonging to the Irano-Turanian floristic region and colonizes only marlstone-calcareous cliffs, usually those with an eastern and south-eastern orientation, at an elevation of 1200–1800 m. It is an obligate chasmophyte.

Suggested conservational status. The new species found in 3 populations. Two populations have been discovered in Malatya province, the other has been discovered in Erzincan province. Although the distribution area of *Galium shinasii* seems relatively wide, the populations of the area are very restricted. The total population area of *G. shinasii* was calculated as 0.2 km² and approximately 500 individuals were observed in total. Probably it has still several undiscovered populations. No anthropogenic or grazing effects were observed on the population. According to the present data, following the criteria laid out by the IUCN (2012), the plant is categorized as ‘Vulnerable’ (VU) D1 + 2, on account of its restricted distribution.

Discussion and conclusions

Galium shinasii is a member of *Galium* Sect. *Orientigalium* Ehrend. It is characterized by chasmophyte, dwarf caespitose habit, very fragile 2–6 cm long stems, mostly 4 rarely 6 leaves in a whorl; 1.5–6.5 cm long leaves; flowers diam 1.2–1.8 mm; corolla yellowish-green to reddish-green; usually corolla lobes formed in a conical or campanulate corolla shape, very rarely lobes wholly opens and formed a infundibular corolla shape; fruit dorsal and ventral surface with densely transparent tubercles, lateral surface 0.2–0.4 mm spreading to patent hairy.

Although *Galium shinasii* shows some morphological similarities with *G. lasiocarpum* Boiss., *G. sorgerae* Ehrend. and Schönbn., *G. cornigerum* Boiss. and Hausk. in sect. *Orientigalium*, it is easily distinguished from these by relatively smaller flowers; yellowish-green to reddish-green and very reduced tepals; fruit surface is not only hairy on lateral surface, and also dorsal and ventral surface with densely transparent tubercles. Also it shows slight morphological similarities to *G. incanum* Sm. subsp. *pseudocornigerum* Ehrend. with dwarf caespitose habit, smaller leaves and in having fruits

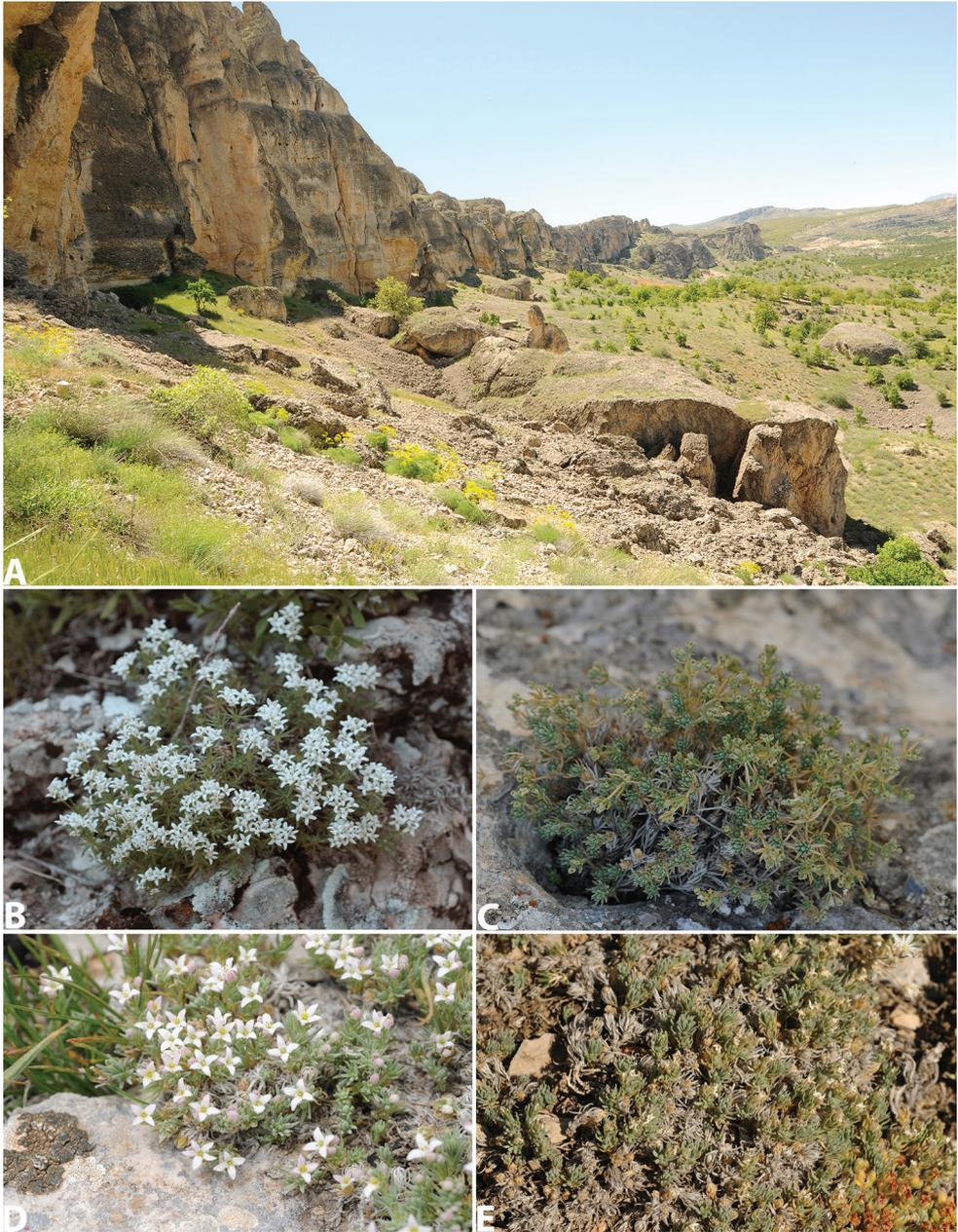


Figure 4. **A** type locality of *Galium shinasii*, Marlstone rocky cliffs in Levent Canyon **B–C** *G. incanum* subsp. *pseudocornigerum* **D–F** *G. cornigerum*

lacking a calyx but it is easily distinguished from *G. incanum*. subsp. *pseudocornigerum* by its especially more reduced and different coloured flowers, a greater numbers flower number per stem; smaller, depressed subglobose and long-hairy fruits.

Table 1. Main differential characters among *Galium shinastii* and close related species *G. sorgerae*, *G. cornigerum* and *G. lasiocarpum*.

Species Characters	<i>Galium shinastii</i>	<i>G. sorgerae</i>	<i>G. cornigerum</i>	<i>G. lasiocarpum</i>
Stem	1.5–6 cm, prostrate-ascending to erect, glabrous to slightly puberulent; sometimes slightly winged on nerves,	3–4 cm, prostrate-ascending, densely hirsute	to 5 cm, prostrate-ascending, with very short, subvelutinous hairy	to 5 cm, erect to ascending, covered with straight spreading hair
Leaves	2–8 × 0.6–1.3 mm, linear-lanceolate to narrow elliptic; in whorls mostly 4, rarely 6 leaved	4–6 × 0.7–1 mm, linear-ob lanceolate or narrowly elliptic; in whorls 6 leaved;	5–9 × 0.4–0.8 mm, linear, linear elliptic to lanceolate; in whorls 6 leaved	6–10 × 1–1.5 mm, linear-elliptic; in whorls 6 leaved
Inflorescence	very reduced, dichasium, mostly terminal and also axillar, 8 to 75 flowered per stem, never hidden by uppermost leaves	very reduced, few-flowered	mainly terminal reduced, corymbiform cymes, few-flowered, often ± hidden by uppermost leaves	very reduced, subumbellate-capitate, 3–8 flowered, hidden by uppermost leaves
Pedicel	1.5–2.5 mm, glabrous, 2–5 mm in fruiting time	2–3 mm, hirsute	0–4 mm, subvelutinous hairy	1–3 mm, hairy
Calyx	absent	–	2–4, subulate, persistent in fruit.	0–2
Corolla	yellowish-green to reddish-green; 1.2–1.8 mm diam;	whitish when dry, infundibular, 2–2.5 mm diam	white, 4–5 mm diam	white or pink, 3.5–4 mm diam
Fruit	dorsal and ventral surface with densely transparent tubercles, lateral surface 0.2–0.4 mm spreading to patent hairy.	hirsute	subtomentose	villous

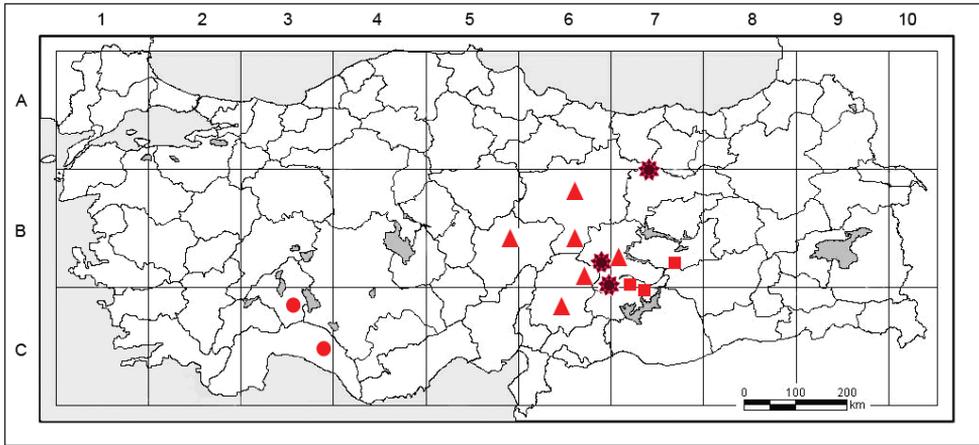


Figure 5. Known distribution of: *Galium shinasii* (✱), *G. cornigerum* (▲), *G. sorgerae* (●), *G. lasiocarpum* (■).

The detailed of the morphological differences between *Galium shinasii* and related *Gallium* species are summarized in the Table 1.

After adding this new species in science literature, the total number of *Gallium* taxa were raised to 121 (105 species) in Turkey and 61 taxa are endemic for Turkey.

Acknowledgements

We are grateful to the curators of the following herbaria for allowing us to access to their *Gallium* material for study: ANK, EGE, E, G, GAZI, HUB, ISTE,K, W and WU. The authors are indebted to The Scientific and Technological Research Council of Turkey (TUBITAK), under Project number: 133Z072, for the financial support.

References

- Davis PH, Mill RR, Tan K (1988) Flora of Turkey and the East Aegean Islands (Vol. 10, Suppl.). Edinburgh University Press, Edinburgh, 216 pp.
- Ehrendorfer F, Krendl F (1976) *Galium* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH (Eds) Flora Europaea, Vol. 4: Plantaginaceae to Compositae (and Rubiaceae). Cambridge University Press, Cambridge, 14–36.
- Ehrendorfer F, Schönbeck-Temesy E (1982) *Galium* L. In: Davis PH (Eds) Flora of Turkey, Volume 7: Flora of Turkey and the East Aegean Islands, Vol. 7. Edinburgh University Press, Edinburgh, 767–849.
- Ehrendorfer F, Schönbeck-Temesy E (2005) *Galium* L. In: Ehrendorfer F, Schonbeck-Temesy E, Puff C, Rechinger W (Eds) Flora Iranica (Volume 176). Verlag des naturhistorischen Museum Wien, Wien, 161–258.

- Halácsy EV (1901) *Conspectus Florae Graecae* (Vol. 1). *Sumptribus Guilelmi Engelmann, Lipsiae* (Leipzig), 720–721.
- Govaerts R (2016) World Checklist of Asparagaceae. Facilitated by the Royal Botanic Gardens, Kew. <http://apps.kew.org/wcsp> [retrieved 15/08/2016]
- Jansen S, Dessein S, Piesschaert F, Robbrecht E, Smets E (2000) Aluminum accumulation in leaves of Rubiaceae: Systematic and phylogenetic implications. *Annals of Botany* 85: 91–101. doi: 10.1006/anbo.1999.1000
- Karabacak E (2012) *Galium* 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, 821–831.
- Koç M, Aksoy A (2013) *Minuartia hamzaoglu* (Caryophyllaceae), a new species from Turkey. *Turkish Journal of Botany* 37: 428–433.
- Mabberley DJ (1987) *The Plant-Book: A Portable Dictionary of the Higher Plants*. Cambridge University Press, Cambridge, 720 pp.
- Menemen Y, Aytaç Z, Kandemir A (2013) *Türkçe Bilimsel Bitki Adları Yönergesi, Bağbahçe Bilim Dergisi* 47: 28–31.
- Mutlu B, Karakuş Ş (2012) A new species of *Ornithogalum* (Hyacinthaceae) from East Anatolia, Turkey. *Turkish Journal of Botany* 36: 125–133.
- Mutlu B, Karakuş Ş (2015a) A new species of *Campanula* (Campanulaceae) from Turkey. *Phytotaxa* 234(3): 287–293.
- Mutlu B, Karakuş Ş (2015b) A new species of *Sisymbrium* (Brassicaceae) from Turkey: Morphological and molecular evidences. *Turkish Journal of Botany* 39: 325–333
- Robbrecht E (1988) Tropical Woody Rubiaceae – Characteristic Features and Progressions – Contributions to a New Subfamilial Classification. Volume 1, *Opera Botanica Belgica*, 271 pp.
- Robbrecht E, Manen JF (2006) The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on rbcL, rps16, trnLtrnF and atpB-rbcL data. A new classification in two subfamilies Cinchonoideae and Rubioideae. *Systematics and Geography of Plants* 76: 85–146.
- Schnurrenberger DW, Russell JM, Kelts KR (2003) Classification of lacustrine sediments based on sedimentary components. *Journal of Paleolimnology* 29: 141–154. doi: 10.1023/A:1023270324800
- Soza VL, Olmstead RG (2010) Evolution breeding systems and fruits in New World *Galium* and relatives (Rubiaceae). *American Journal of Botany* 97: 1630–1646. doi: 10.3732/ajb.1000130
- Tan K, Yıldırım H, Zielinski J (2012) Establishment of *Rosa* sect. *Caninae* subsect. *Orientalis* (Rosaceae) and the recognition of an unusual variety of *Rosa vanheurckiana* from eastern Anatolia, Turkey. *Phytotaxa* 54: 26–36. doi: 10.11646/phytotaxa.54.1.3
- Willis JC (1985) *A Dictionary of the Flowering Plants and Ferns*. Cambridge University Press, Cambridge, 1294 pp.
- Yıldırım H, Tan K, Şenol SG, Pirhan AF (2010) *Chaenorhinum semispeluncarum* sp. nov. and *C. yildirimlii* sp. nov. (Scrophulariaceae) from east Anatolia, Turkey. *Nordic Journal of Botany* 28: 457–464. doi: 10.1111/j.1756-1051.2010.00790.x

- Yıldırım H, Erol O (2013) *Crocus yakarianus* sp. nov. from eastern Turkey. *Nordic Journal of Botany* 31: 426–429. doi: 10.1111/j.1756-1051.2012.01786.x
- Yıldırım H, Şenol SG (2014a) *Campanula alisan-kilincii* (Campanulaceae), a new species from eastern Anatolia, Turkey. *Turkish Journal of Botany* 38: 22–30.
- Yıldırım H, Şenol SG (2014b) *Alkanna malatyana* (Boraginaceae), a new species from East Anatolia, Turkey. *Phytotaxa* 164(2): 124–132.
- Yıldırım H, Şenol SG (2014c) *Reseda malatyana* (Resedaceae), a new chasmophytic species from eastern Anatolia, Turkey. *Turkish Journal of Botany* 38: 1013–1021.
- Yıldırım H (2015a) *Muscari atillae* (Asparagaceae): a new species from Eastern Anatolia, Turkey. *Phytotaxa* 213(3): 291–295.
- Yıldırım H (2015b) *Parietaria semispeluncaria* (Urticaceae): a new species from Eastern Anatolia, Turkey. *Phytotaxa* 226(3): 281–287.
- Yıldız B, Bahçecioglu Z, Arabacı T (2004) Floristic characteristics of Beydağı (Malatya). *Turkish Journal of Botany* 28: 391–419.

***Borneocola* (Zingiberaceae), a new genus from Borneo**

Yen Yen Sam¹, Atsuko Takano², Halijah Ibrahim³, Eliška Závěská⁴, Fazimah Aziz⁵

1 Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia **2** Museum of Nature and Human Activities, Hyogo 6 chome, Yayoigaoka, Sanda, Hyogo 669-1546, Japan **3** Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia **4** Institute of Botany, University of Innsbruck, Austria **5** Department of Aquatic Science, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

Corresponding author: Yen Yen Sam (samyen@frim.gov.my)

Academic editor: Pavel Stoev | Received 12 July 2016 | Accepted 9 November 2016 | Published 29 November 2016

Citation: Sam YY, Takano A, Ibrahim H, Závěská E, Aziz F (2016) *Borneocola* (Zingiberaceae), a new genus from Borneo. *PhytoKeys* 75: 31–55. doi: 10.3897/phytokeys.75.9837

Abstract

A new genus from Borneo, *Borneocola* Y.Y.Sam, is described here. The genus currently contains eight species previously classified as members of the *Scaphochlamys* Baker. The finding is based on the results of the morphological and molecular studies of *Scaphochlamys* throughout its geographical range and its closely allied sister groups, *Distichochlamys* M.F.Newman and *Myxochlamys* A.Takano & Nagam. *Borneocola* is nested within the tribe Zingibereae and its monophyly is strongly supported by both ITS and matK sequence data. The genus is characterised by several thin, translucent and marcescent floral bracts, absence of coloured streaks on the labellum and capitate stigma with two dorsal knobs. The genus is distributed in northwest Borneo and all species are very rare and highly endemic.

Keywords

Distichochlamys, *Myxochlamys*, *Scaphochlamys*, morphology, phylogeny, taxonomy

Introduction

Southeast Asia is the centre of diversity for the family Zingiberaceae. Here, new taxa are continuously being discovered and named, both at the generic and specific levels. Several of the recent discoveries were further supported by the phylogenetic analyses which give a better understanding of the evolutionary relationships within the family (Kress and Larsen 2001; Kress et al. 2010; Leong-Škorničková et al. 2011). During the revision of the genus *Scaphochlamys* throughout its entire geographical range by the first author, some distinctive morphological traits were observed in several Bornean species, suggesting they might represent a separate group from the Peninsular Malaysian taxa. This hypothesis was confirmed by the phylogenetic analyses which are presented here and the eight species previously included in the genus *Scaphochlamys* are recircumscribed in this paper as a new genus, *Borneocola* Y.Y.Sam.

The genus *Scaphochlamys* was described by Baker (1892) in the Flora of British India with *Scaphochlamys malaccana* Baker from Mt. Ophir (now known as Gunung Ledang), Peninsular Malaysia, chosen as the type species. Holttum (1950) carried out the first comprehensive revision of the genus in which he recognised 19 species, all of which were recorded in the peninsula. When Smith (1987) reviewed the tribe Hedychieae in Borneo, she applied the generic delimitation defined by Holttum and recognised five *Scaphochlamys* species in Borneo. Out of the five, *S. polyphylla* and *S. petiolata* were formerly placed in the genus *Haplochorema* K.Schum. Sakai and Nagamasu (2006) discovered that *H. gracilipes* K.Schum. also have the characteristics of *Scaphochlamys* and effected the transfer. Recent years have seen a surge in the new species discovered from Borneo bringing the total number of Bornean *Scaphochlamys* to 14 (Poulsen and Searle 2005, Meekiong et al. 2011, Ooi and Wong 2014; Meekiong 2015).

Distichochlamys M.F.Newman and *Myxochlamys* A.Takano & Nagam. are sister genera to *Scaphochlamys* with several unique characteristics clearly separating them from *Scaphochlamys* (Newman 1995, Searle and Hedderson 2000, Kress et al. 2002, Ngamriabsakul et al. 2004, Takano and Nagamasu 2007). However, the distinction, based on morphological characters, became ambiguous as several taxa described recently exhibit exceptions to the usual generic characters. For example, *S. calcicola* A.D.Poulsen & R.J.Searle, a species named in 2005 from Sarawak, has a distichous inflorescence, a distinguishing character for the genus *Distichochlamys* M.F.Newman. Larsen and Newman (2001) also reported another *Scaphochlamys* species with a distichous inflorescence from north Peninsular Malaysia. A current study on the morphology of *Scaphochlamys* also revealed that some species display the characteristics of *Distichochlamys* and *Myxochlamys*. To test the validity of the current generic concept of *Scaphochlamys* and closely related genera *Distichochlamys* and *Myxochlamys*, we have examined their relationship by utilising ITS and matK markers together with the analysis of the morphology across these genera.

Materials and methods

Morphological study

The morphological study was based on living plants in the forest, cultivated plants in the nursery of the Forest Research Institute Malaysia and specimens in the herbaria of AAU, BKF, C, FI, E, K, KEP, KLU, PSU, SAN, SAR and SING. A total of 372 herbarium specimens were examined in this study which includes 29 *Scaphochlamys* species and four *Borneocola* species (the types of another four *Borneocola* species were not yet deposited in the herbaria). The morphological characters examined in the study were habit; position of the rhizome, thickness and colour; height of leafy stem, its base (whether swollen to form a bulbous base); distance between leafy stems; characters of bladeless sheath such as colour, indumentum, number and length; ligule length, indumentum and shape; petiole length, indumentum, whether channelled or rounded in cross section; number of leaves per leafy stem; lamina colour on both surfaces, size, shape, venation, texture, indumentum, apex and base; length of the inflorescence and infructescence, arrangement of the floral bracts on the rachis, characters of floral bracts and bracteoles (colour, indumentum, texture, shape); size, colour and shape of calyx, floral tube, corolla lobes, labellum, staminodes, stamen, ovary.

DNA extraction, amplification and sequencing

Fresh leaves from the cultivated plants or silica-dried materials from plants collected in the field were used for genomic DNA extraction.

For the ITS, the genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. Two primers, ITS 5P (5'-GGAAGGAGAAGTCGTAACAAGG-3') and ITS 8P (5'-CACGCTTCTCCAGACTACA3') (Moller and Cronk 1997) were used to amplify the ITS region during the polymerase chain reaction (PCR). The thermal cycle of PCR for the amplification of the ITS sequences is initial denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, primers annealing at 48°C for 2 minutes, an extension at 72°C for 45 seconds and final extension at 72°C for 7 minutes. The PCR products were then purified using MinElute Gel Extraction Kit (Qiagen, Valencia, California, USA).

For the matK, the protocols for DNA extraction, condition, purification and DNA sequencing were described previously by Takano and Nagamasu (2007). The PCR and sequencing primers for matK (cpDNA) were TA-240f (5'-GGGAAA GGATGGGGTCTCCCG-3'), TA-150r (5'-CTCAAGGAGTTTTGTGGTTC-3'), TA-470F (5'-CCCTCTCCCGTCCATATGGA-3') (all three were designed in the present study), matK8 (Steele and Vilgalys 1994), m5r (Kress et al. 2002), matK8r (Ooi et al. 1995), trnK2621 (Liston and Kadereit 1995), TA-10F, TA-05R, TA-02F and TA-02R (all from Takano and Okada 2002).

Sequence alignment and phylogenetic analysis

Raw sequence data were assembled and edited manually using BioEdit software ver. 7.2.5 (Hall 1999). DNA sequences were aligned with the CLUSTALW 1.83 software package, with default settings and multiple alignments (Thompson et al. 1994). Alignments of the matK sequences of cpDNA and the ITS sequences of nrDNA were combined. Gaps were deleted.

A total of 100 individuals including 54 taxa of *Scaphochlamys* and allied species were used. The three *Siphonochilus* species were used as an outgroup (Kress et al. 2002). Materials, accession numbers for the sequences, vouchers and references to the literature are presented in Table 1 at the end of this paper. Three datasets which comprise ITS, matK and ITS+matK combined, each containing 82, 78, and 61 taxa, were constructed. These three datasets were analysed using three methods: maximum parsimony, maximum likelihood and Bayesian analysis. A maximum parsimony (MP) analysis was performed with MEGA 6 (Tamura et al. 2013). Heuristic searches were conducted with RANDOM addition, SPR branch swapping and MULPARS options. Support for each branch was estimated with a bootstrap analysis, with 1000 replications (Felsenstein 1985), in a heuristic search with RANDOM addition and TBR branch swapping. The maximum likelihood (ML), based on the Tamura-Nei model (Tamura and Nei 1993), was also determined with MEGA 6 (Tamura et al. 2013). Neighbor-Join and BioNJ algorithms were applied to a matrix of pairwise distances estimated with the maximum composite likelihood approach; then, the topology that had the best log likelihood value was selected. Bootstrap analysis under the MP criterion was conducted with “fast” stepwise, addition searches, with 1000 replicates. In addition, a Bayesian analysis was carried out with MrBayes software ver. 3.1.2 (Huelsenbeck and Rohnquist 2001; Rohnquist and Huelsenbeck 2003). The best fitting substitution model (the GTR+G model for nrDNA datasets, the GTR+G model for cpDNA datasets and the GTR+I+G model for cpDNA+nrDNA datasets) was selected for Bayesian analysis based on a series of hierarchical likelihood ratio tests, implemented in MrModeltest software ver. 2.3 (Nylander 2004). The analysis was performed with the selected model and two simultaneous runs of two million generations with four chains, sampling every 100 generations. Each analysis reached stationarity (i.e. when the average standard deviation of split frequencies between runs was ≤ 0.01) well before the end of the run. Burn-in trees were discarded and the remaining trees and their parameters were saved. A 50 % majority rule consensus tree was constructed. The results of the Bayesian analysis were reported as the posterior probability (PP; Huelsenbeck and Rohnquist 2001), which is equal to the percentage of phylogenetic trees sampled when a given clade was resolved. Only PP scores above 50 % are shown.

Table 1. List of accession details, vouchers and references used in the phylogenetic analyses.

No	Subfamily	Tribe	Species	ITS	matK	References/ Voucher
1	Alpinioideae Link	Alpinieae A.Rich.	<i>Alpinia blepharocalyx</i> K.Schum.	AF478709	AF478809	Kress et al. 2002
2			<i>Alpinia elegans</i> K.Schum.	AF478713	AF478813	Kress et al. 2002
3			<i>Amomum villosum</i> Lour.	–	AF478824	Harris et al. 2000 (ITS), Kress et al. 2002 (matK)
4			<i>Amomum yunnanense</i> S.Q.Tong	AY352012	–	Xia et al. 2004
5			<i>Elettariopsis kerbyi</i> R.M.Sm.	AF414496	AF478845	Pedersen 2004 (ITS)/Kress et al. 2002 (matK)
6			<i>Reenealmia battenbergiana</i> Cummins ex Baker	AF478779	AF478880	Kress et al. 2002
7	Siphonochileae W.J.Kress	Siphonochileae W.J.Kress	<i>Siphonochilus aethiopicus</i> (Schweinf.) B.L.Burtt	AF478792	AF478893	Kress et al. 2002
8			<i>Siphonochilus decorus</i> (Drueten) Lock	AF478793	AF478894	Kress et al. 2002
9			<i>Siphonochilus kirkii</i> (Hook.) B.L.Burtt	AF478794	AF478895	Kress et al. 2002
10	Tamijioideae W.J.Kress	Tamijieae W.J.Kress	<i>Tamijia flagellaris</i> S.Sakai & Nagam.	AF478797	AF478898	Kress et al. 2002
11	Zingiberoideae Haask.	Globbeae Meisn.	<i>Gagnepainia thoreliana</i> K.Schum.	AF478752	AF478851	Kress et al. 2002
12			<i>Hemiorchis rhodorrhachis</i> K.Schum.	AY339706	AY341090	Williams et al. 2004
13			<i>Mantisia wengeri</i> C.E.C.Fischer	–	AF478871	Kress et al. 2002
14	Zingiberoideae Haask.	Zingibereae Meisn.	<i>Boesenbergia pulcherrima</i> Kuntze	AF478725	AF478825	Kress et al. 2002
15			<i>Boesenbergia rotunda</i> (L.) Mansf.	AF478727	AF478826	Kress et al. 2002
16			<i>Borneocola biru</i> (Meekiong) Y.Y.Sam	–	LC148403	FRI 50290 (KEP)
17			<i>Borneocola calcicola</i> (A.D.Poulsen & R.J.Searle) Y.Y. Sam	LC148062	LC148380	FRI 50290 (KEP)
18			<i>Borneocola</i> sp. FRI 50295	LC148085	LC148404	FRI 50295 (KEP)
19			<i>Borneocola</i> sp. S 99106	LC148086	LC148405	S 99106 (SAR)
20			<i>Borneocola stenophyllus</i> (Ooi & S.Y.Wong) Y.Y.Sam	LC148084	LC148400	FRI 50288 (KEP)
21			<i>Borneocola petiolatus</i> (K.Schum.) Y.Y.Sam	LC148075	LC148395	FRI 50291 (KEP)
22			<i>Borneocola reticosus</i> (Ridl.) Y.Y.Sam	LC148078	LC148398	FRI 50294 (KEP)
23			<i>Camptandra parvula</i> Ridl.	AF478730	AF478830	Kress et al. 2002
24			<i>Caulokaempferia saxicola</i> K.Larsen	AY478732	AF478831	Kress et al. 2002
25			<i>Cautleya gracilis</i> (Sm.) Dandy	AF478734	AF478833	Kress et al. 2002
26			<i>Cautleya spicata</i> Baker	AF478735	AF478834	Kress et al. 2002
27			<i>Cornukaempferia aurantiflora</i> J.Mood & K.Larsen	AF478736	AF478835	Kress et al. 2002
28			<i>Curcuma bicolor</i> J.Mood & K.Larsen	AF478737	AF478837	Kress et al. 2002
29			<i>Curcuma roscoeana</i> Wall.	AF478739	AB047741	Kress et al. 2002 (ITS)/Cao et al. unpublished (matK)
30			<i>Distichochlamys citrea</i> M. F. Newman	AY424757	–	Ngamriabsakul et al. 2004
31			<i>Distichochlamys citrea</i> M. F. Newman 2	AB552946	AB552951	Ngamriabsakul 24 (E)

No	Subfamily	Tribes	Species	ITS	matK	References/ Voucher
32	Zingiberoideae Haask.	Zingibereae Meisn.	<i>Distichochlamys</i> sp. AS18	AB552947	AB553309	Adele Smith 18 (E)
33			<i>Distichochlamys</i> sp. Kress01-6848	AF478745	AF478844	Kress et al. 2002
34			<i>Haniffia albiflora</i> K.Larsen & J.Mood	AF478756	AF478855	Kress et al. 2002
35			<i>Hedychium longicornutum</i> Griff. ex Baker	AF478761	AF478860	Kress et al. 2002
36			<i>Hedychium villosum</i> Wall.	AF478762	AF478861	Kress et al. 2002
37			<i>Hitchenia glauca</i> Wall.	AF478765	AF478864	Kress et al. 2002
38			<i>Kaempferia parviflora</i> Wall.	–	AB232052	Searle and Hedderson 2000
39			<i>Kaempferia rotunda</i> L.	AF478767	AF478868	Kress et al. 2002
40			<i>Kaempferia</i> sp. Kress98-6289	AF478768	AF478869	Kress et al. 2002
41			<i>Myxochlamys mullerensis</i> A.Takano & Nagam.	AB245522	AB269791	Takano and Nagamasu 2007
42			<i>Myxochlamys nobilis</i> Nagam. ined.	AB552948	AB553310	Nagamasu 8274 (BO, KYO)
43			<i>Pommereschea lackneri</i> Wittm.	–	AF478877	Kress et al. 2002
44			<i>Pyrogophyllum yunnanense</i> (Gagnep.) T.L.Wu & Z.Y.Chen	AF478777	AF478878	Kress et al. 2002
45			<i>Rhynchanthus beesianus</i> W.W.Sm.	AF478784	AF478885	Kress et al. 2002
46			<i>Roscoea cauleoides</i> Gagnep.	AF478736	AF478887	Kress et al. 2002
47			<i>Roscoea purpurea</i> Sm.	AF478787	AF478888	Kress et al. 2002
48			<i>Scaphochlamys abduallahii</i> Y.Y.Sam & Saw	LC148054	–	FRI 44375 (KEP)
49			<i>Scaphochlamys abduallahii</i> Y.Y.Sam & Saw	LC148055	LC148374	FRI 50198 (KEP)
50			<i>Scaphochlamys atroviridis</i> Holttum	LC148056	–	FRI 68924 (KEP)
51			<i>Scaphochlamys baukensis</i> Y.Y.Sam	LC148057	–	FRI 68955 (KEP)
52			<i>Scaphochlamys biloba</i> (Ridl.) Holttum	LC148059	–	FRI 46606 (KEP)
53			<i>Scaphochlamys biloba</i> (Ridl.) Holttum	LC148081	–	FRI 50224 (KEP)
54			<i>Scaphochlamys biloba</i> (Ridl.) Holttum	LC148083	–	FRI 66331 (KEP)
55			<i>Scaphochlamys biloba</i> (Ridl.) Holttum 1	AF478788	AY478889	Kress et al. 2002
56			<i>Scaphochlamys biloba</i> (Ridl.) Holttum 2	AF202416	–	Wood et al. 2000
57			<i>Scaphochlamys breviscapa</i> Holttum	–	LC148377	FRI 50269 (KEP)
58			<i>Scaphochlamys breviscapa</i> Holttum	LC148060	LC148376	FRI 44984 (KEP)
59			<i>Scaphochlamys burkillii</i> Holttum	–	LC148379	FRI 68928 (KEP)
60			<i>Scaphochlamys burkillii</i> Holttum	LC148061	–	FRI 46504 (KEP)
61			<i>Scaphochlamys concinna</i> (Baker) Holttum	AJ388283	–	Searle and Hedderson 2000
62			<i>Scaphochlamys concinna</i> (Baker) Holttum	LC148063	LC148381	FRI 50351 (KEP)
63			<i>Scaphochlamys cordata</i> Y.Y.Sam & Saw	LC148064	–	FRI 44306 (KEP)
64	<i>Scaphochlamys endauensis</i> Y.Y.Sam & Ibrahim	–	LC148383	FRI 50243 (KEP)		
65	<i>Scaphochlamys endauensis</i> Y.Y.Sam & Ibrahim	LC148080	–	FRI 50218 (KEP)		
66	<i>Scaphochlamys erecta</i> Holttum	LC148065	–	FRI 44987 (KEP)		
67	<i>Scaphochlamys grandis</i> Holttum	–	LC148384	FRI47184 (KEP)		
68	<i>Scaphochlamys grandis</i> Holttum	LC148066	LC148385	FRI 50171 (KEP)		

No	Subfamily	Tribe	Species	ITS	matK	References/ Voucher
69			<i>Scaphochlamys johorensis</i> Y.Y.Sam	LC148082	–	FRI 66566 (KEP)
70			<i>Scaphochlamys klossii</i> (Ridl.) Holttum	LC148067	LC148387	FRI 50238 (KEP)
71			<i>Scaphochlamys kunstleri</i> (Baker) Holttum	AF478789	AY478890	Kress et al. 2002
72			<i>Scaphochlamys kunstleri</i> (Baker) Holttum var. <i>rubra</i> C.K.Lim	AB552950	AB553312	Anon C 8003 & C. Ngamriabsakul 25 (E)
73			<i>Scaphochlamys kunstleri</i> (Baker) Holttum var. <i>kunstleri</i>	–	LC148388	FRI 68926 (KEP)
74			<i>Scaphochlamys kunstleri</i> (Baker) Holttum var. <i>kunstleri</i>	LC148068	–	FRI 68936 (KEP)
75			<i>Scaphochlamys kunstleri</i> var. <i>speciosa</i> C.K.Lim	–	LC148389	FRI 68936 (KEP)
76			<i>Scaphochlamys lanceolata</i> (Ridl.) Holttum	LC148069	LC148390	FRI 50130 (KEP)
77			<i>Scaphochlamys laxa</i> Y.Y.Sam & Saw	–	LC148391	FRI 68961 (KEP)
78			<i>Scaphochlamys longifolia</i> (Ridl.) Holttum	LC148070	LC148392	FRI 47065 (KEP)
79			<i>Scaphochlamys malaccana</i> Baker	–	LC148393	FRI 50203 (KEP)
80			<i>Scaphochlamys malaccana</i> Baker	LC148071	–	FRI 50208 (KEP)
81			<i>Scaphochlamys minutiflora</i> Jenjitt.& K.Larsen	–	LC148394	3175
82			<i>Scaphochlamys obcordata</i> P.Siriruga & K.Larsen	AJ388286	–	Searle and Hedderson 2000
83			<i>Scaphochlamys oculata</i> (Ridl.) Holttum	LC148072	LC148396	FRI 50262 (KEP)
84	Zingiberoideae	Zingibereae	<i>Scaphochlamys pennipicta</i> Holttum	LC148073	–	FRI 50261 (KEP)
85	Haask.	Meisn.	<i>Scaphochlamys perakensis</i> Holttum	LC148074	–	FRI 50214 (KEP)
86			<i>Scaphochlamys polyphylla</i> (K.Schum.) B.L.Burt & R.M.Sm.	LC148076	LC148397	FRI 50289 (KEP)
87			<i>Scaphochlamys pusilla</i> Y.Y.Sam	LC148077	–	FRI 50260 (KEP)
88			<i>Scaphochlamys rubromaculata</i> Holttum	–	LC148399	FRI 50178 (KEP)
89			<i>Scaphochlamys rubromaculata</i> Holttum	LC148079	LC148378	FRI 50172 (KEP)
90			<i>Scaphochlamys samunsamensis</i> Meekiong & Hidir	–	LC148401	MK 2344 (HUMS)
91			<i>Scaphochlamys</i> sp.nov.	–	LC148402	FRI 68983 (KEP)
92			<i>Scaphochlamys sub-biloba</i> (Burkill ex Ridl.) Holttum	–	LC148375	FRI 75334 (KEP)
93			<i>Scaphochlamys sylvestris</i> (Ridl.)Holttum	LC148087	–	FRI 50197 (KEP)
94			<i>Scaphochlamys tenuis</i> Holttum	LC148088	–	FRI 47233 (KEP)
95			<i>Scaphochlamys</i> cf. <i>gracilipes</i> (K.Schum.) S.Sakai & Nagam.	–	LC148386	K.Meekiong (HUMS)
96			<i>Smithatris supranea</i> W.J.Kress & K.Larsen	AF478795	AF478896	Kress et al. 2002
97			<i>Stahlianthus involucratus</i> (King ex Baker) R.M.Sm.	AF478796	AF478897	Kress et al. 2002
98			<i>Zingiber gramineum</i> Noronha	AF478800	AF478902	Kress et al. 2002
99			<i>Zingiber sulphureum</i> Burkill ex I.Theilade	AF478801	AF478904	Kress et al. 2002
100			<i>Zingiber wrayii</i> Prain ex Ridl.	AF478802	AF478905	Kress et al. 2002

Results

Phylogenetic analyses

The ITS datasets for 82 individuals with 29 taxa of *Scaphochlamys* and 6 taxa of *Borneocola* contained 786 characters after alignment, which decreased to 769 after gaps were deleted; 319 of these were parsimony-informative. Likelihood analysis resulted in a ML tree with $-\ln L = 10438.212$. Parsimony analysis produced three parsimonious trees with 1865 steps, a consistency index (CI) of 0.391 and retention index (RI) of 0.609. The ML, MP and Bayesian trees had similar topology; the ML tree is shown with bootstrap (BS) and MP-BS, and Bayesian Posterior Probability (PP) support in Figure 1 below.

Scaphochlamys formed a well supported clade (ML-BS/MP-BS/BA-PP support, 84/87/1.00). Each *Myxochlamys* and *Borneocola* consisted of a well supported subclade and became sisters to each other and they also became sister to the *Scaphochlamys* clade. *Distichochlamys* species formed a well supported subclade and became sister to the *Myxochlamys* + *Scaphochlamys* + *Borneocola* clade (ML-BS/MP-BS/BA-PP 99/100/1.00).

The matK datasets for 78 individuals including 25 taxa of *Scaphochlamys* and 7 taxa of *Borneocola* contained 1,599 characters after alignment; 182 of these were parsimony-informative. Likelihood analysis resulted in a ML tree with $-\ln L = 5952.438$. Parsimony analysis produced ten parsimonious trees with 557 steps, a consistency index (CI) of 0.613 and retention index (RI) of 0.080. The ML, MP and Bayesian trees had similar topology; the ML tree is shown with BS and MP-BS, PP support in Figure 2 below.

Each of the two *Myxochlamys* species and seven *Borneocola* species formed a strongly supported subclade and became sisters to each other. *Scaphochlamys* became sister to them, but bootstrap or probability support was weak. The *Distichochlamys* species formed a well supported subclade, but all the genera that belong to subfamily Zingiberoideae became sisters to *Scaphochlamys* + *Myxochlamys* + *Borneocola* clade and not only to *Distichochlamys*.

The combined ITS and matK datasets for 61 individuals including 13 taxa of *Scaphochlamys* and 6 taxa of *Borneocola*, resulted in 2,336 characters, 488 of these were parsimony-informative (Figure 3 below). Likelihood analysis resulted in a ML tree with $-\ln L = 16671.531$. Parsimony analysis produced the most parsimonious trees with 2247 steps, a CI of 0.440 and a RI of 0.635. The ML, MP strict consensus and Bayesian trees had almost the same topology; the ML tree is shown with MP-BS, ML-BS and BA/PP support in Figure 3.

Two *Myxochlamys* species and six *Borneocola* species formed a strongly supported subclade each and became sisters to each other. *Scaphochlamys* became sister to them and the bootstrap or posterior probability support was moderate. *Distichochlamys* species formed a well supported subclade and became sister to *Scaphochlamys* + *Myxochlamys* + *Borneocola* clade.

Morphology

The *Borneocola* and *Scaphochlamys* species look similar in their vegetative morphologies. They are mostly small-sized ginger without the conspicuous pseudostem, with one to several leaves arranged spirally and tightly on a very short stem at the base. So far, all the *Borneocola* species examined are unifoliate. Similarly, most of the *Scaphochlamys* species also bear one leaf except for several species which have leafy shoots composed of multiple leaves, for example, *S. grandis*, *S. lanceolata*, *S. kunstleri*, *S. malaccana* and *S. minutiflora*. The basal part of the leaves is covered with a few bladeless sheaths which are rather different for both groups in terms of their texture and colour. For *Scaphochlamys*, the sheaths are coriaceous, green, green with a red tinge or red and mostly persistent until the end of flowering (Figure 4A, B). On the other hand, the sheaths of *Borneocola* are thinner in texture with a lighter shade of green or brown. The thin sheaths normally dry up early (Figure 4C) and sometimes they are completely shredded during the time of flowering.

The inflorescences of *Borneocola* and *Scaphochlamys* are terminal, stalked and consisted of few to many floral bracts. The differences lie in the characteristics of the floral bracts and flowers. *Borneocola* species have thin, translucent, early decaying and marcescent floral bracts. The colours of the bracts can be pink, pale brown, pale or light green (Figure 5A). On the contrary, the bracts of *Scaphochlamys* are coriaceous and sometimes hard in texture. They are usually green, green tinged red, red or reddish brown and remain fresh throughout the flowering (Figure 5B, C).

Besides the characteristics of the floral bracts, the variegation on the labellum can give a quick guide to the two genera. Most *Scaphochlamys* have white flowers with a yellow median band and lilac, purple, red streaks or patches flanking the band on the labellum (Figure 5D, E). However, there is no such variegation on the labellum of *Borneocola* (Figure 5F). The whole labellum of *Borneocola* is pale pink, lilac, violet or white with a light yellow or greenish yellow median band.

Both *Borneocola* and *Scaphochlamys* have a long slender floral tube which is mostly puberulent externally in *Borneocola* (except for *B. calcicola*) but glabrous for *Scaphochlamys*. Another marked difference observed is in the stigma shape. *Scaphochlamys* has a funnel-shaped or beak-like stigma (Figure 6A, B) while it is almost oblate with two dorsal knobs in *Borneocola* (Figure 6C).

Taxonomic treatment

Borneocola Y.Y.Sam, gen. nov.

urn:lsid:ipni.org:names:77158811-1

Diagnosis. Similar to *Scaphochlamys* and *Myxochlamys*. *Borneocola* has thin, translucent and marcescent floral bracts, absence of coloured streaks on labellum and two dorsal knobs on the stigma versus the coriaceous and persistent floral bracts, coloured streaks

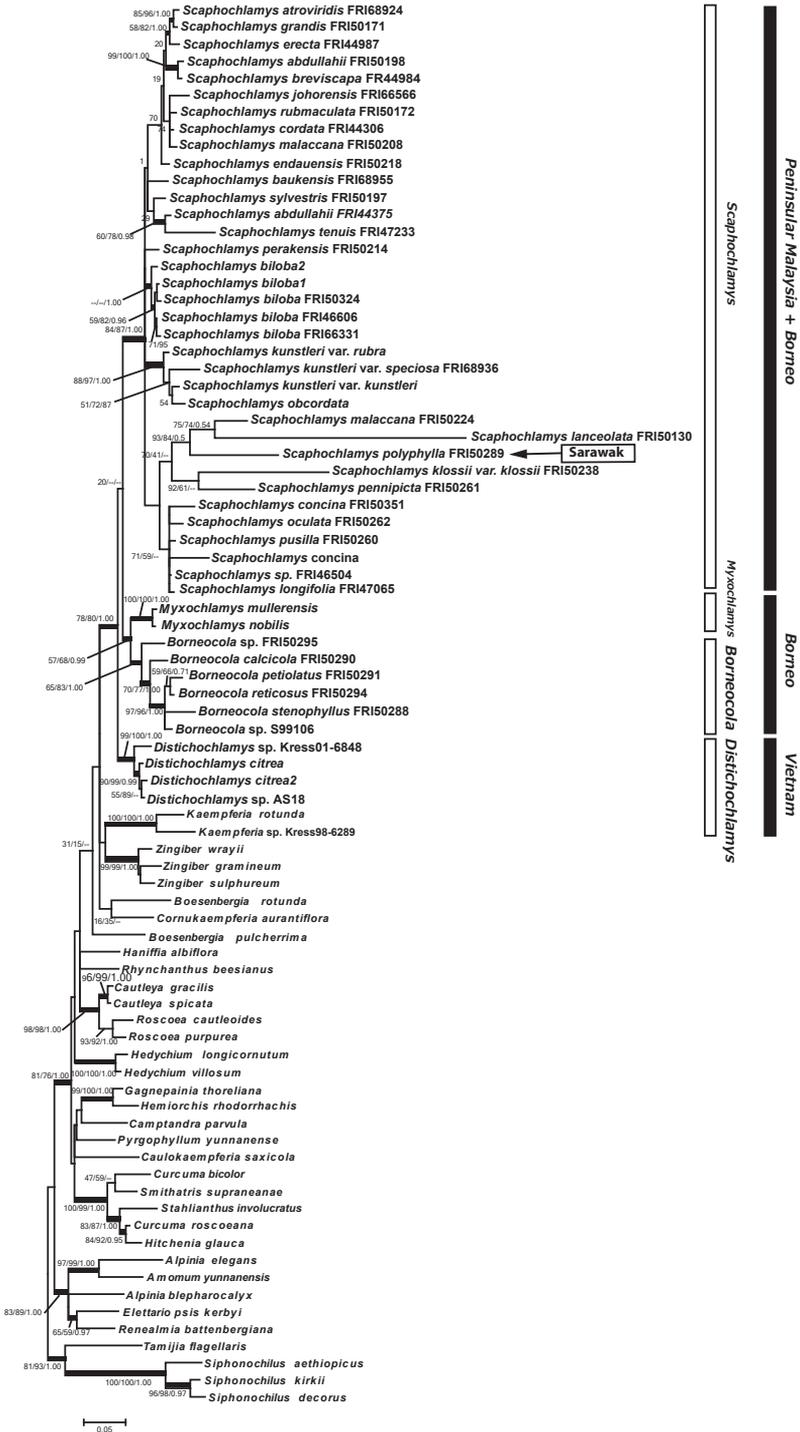


Figure 1. Molecular phylogenetic analysis of the ITS sequence data by the Maximum Likelihood method. Numbers above branches indicate bootstrap values of ML and MP and posterior probability of Bayesian Analysis.

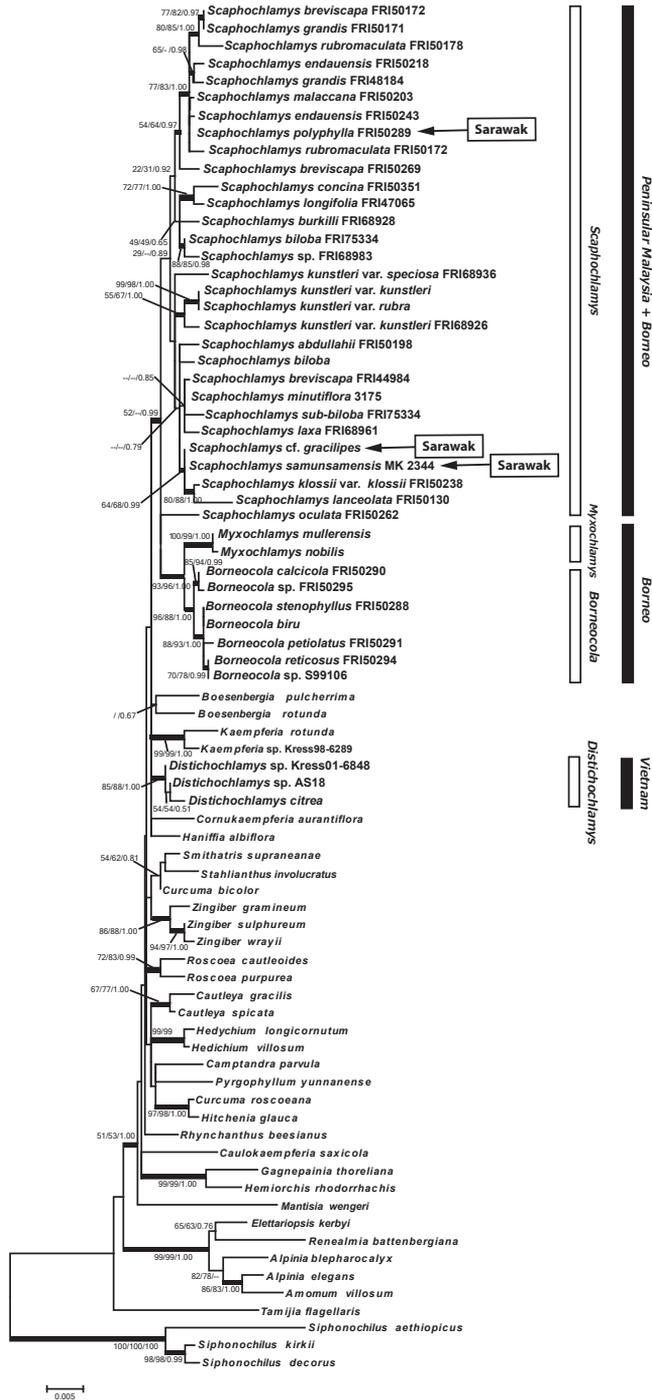


Figure 2. Molecular phylogenetic analysis of the matK sequence data by the Maximum Likelihood method. Numbers above branches indicate bootstrap values of ML and MP and posterior probability of Bayesian Analysis.

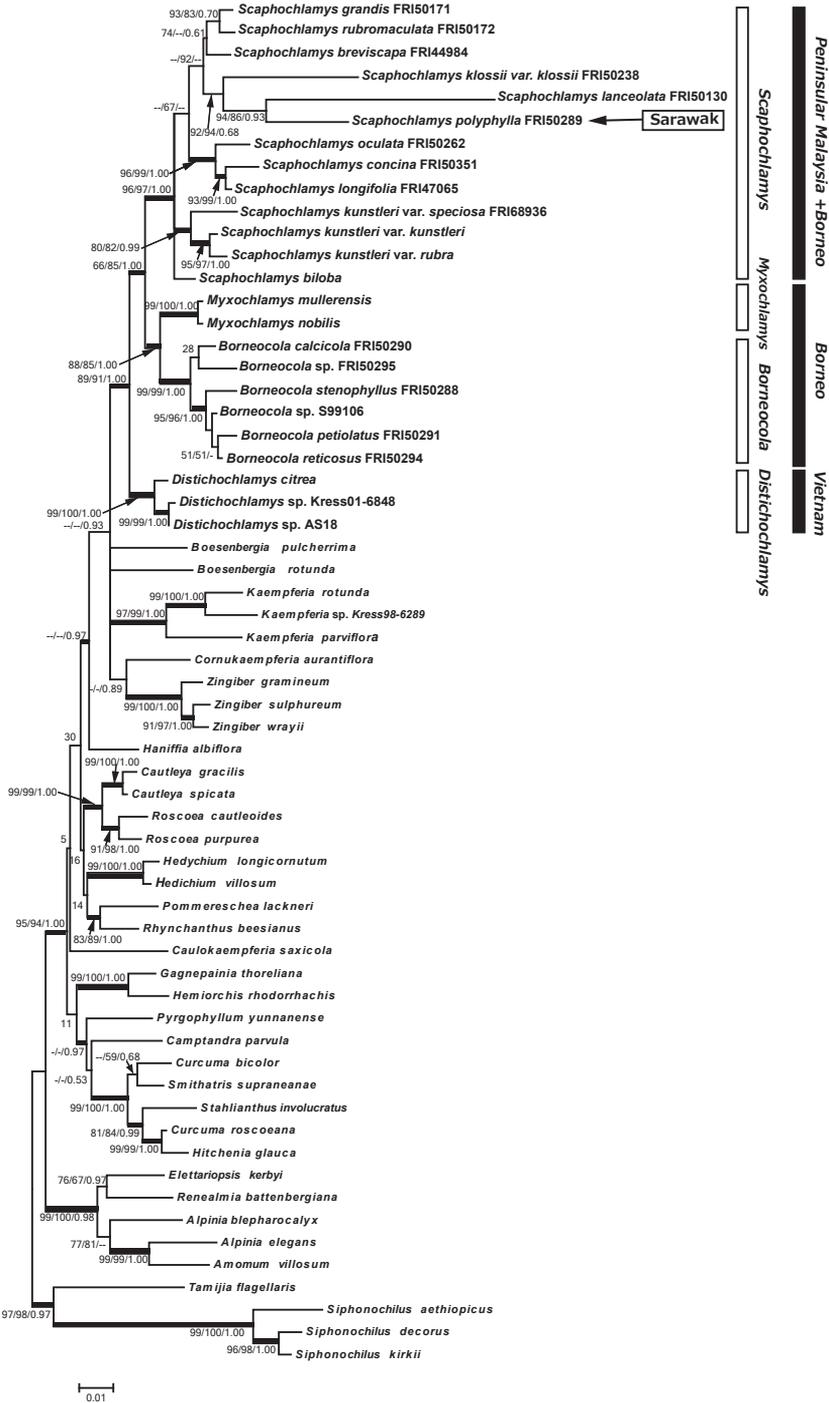


Figure 3. Molecular phylogenetic analysis of the ITS+matK sequence data by the Maximum Likelihood method. Numbers above branches indicate bootstrap values of ML and MP and posterior probability of Bayesian Analysis.

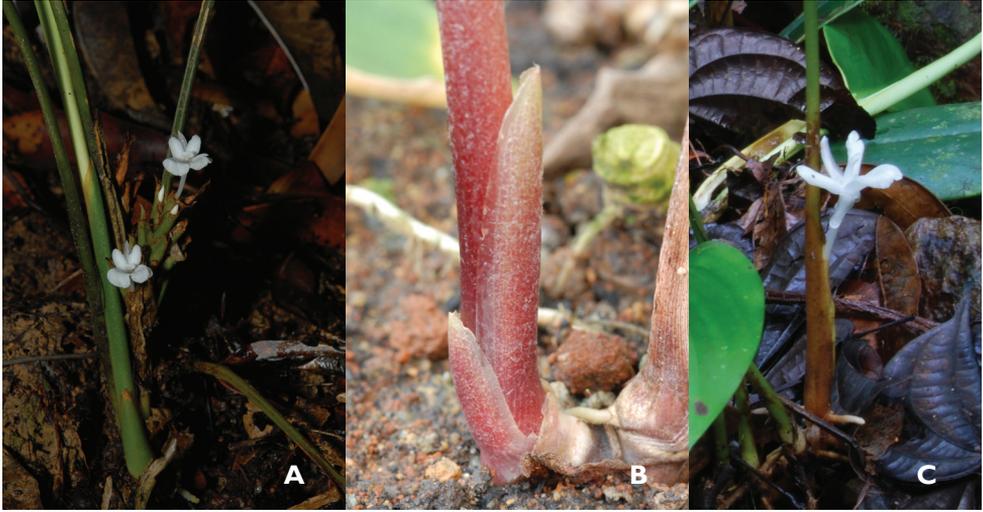


Figure 4. Bladeless sheaths **A** Green and coriaceous in *Scaphochlamys klossii* (Peninsular Malaysia) **B** Red and coriaceous in *Scaphochlamys abdullahii* (Peninsular Malaysia) **C** Papery and marcescent in *Borneocola calcicola* (Sarawak). (Photographs by Y.Y. Sam).

on labellum and absence of dorsal knobs on the stigma in *Scaphochlamys*. The mucilage on the floral bracts and the versatile anther of *Myxochlamys* are absent in *Borneocola*.

Type species. *Borneocola reticosus* (Ridl.) Y.Y.Sam, comb. nov. *Gastrochilus reticosus* Ridl., J. Straits Branch Roy. Asiatic Soc. 44: 195 (1905).

Description. Terrestrial rhizomatous herb, evergreen, rarely exceeding 50 cm in height. Rhizome creeping on the ground, terminal decumbent, rhizome elements short or long; roots fine, extensive, not tuberous. Leafy stem unifoliate, enclosed by a few bladeless sheaths at base, bladeless sheaths linear, papery, glabrous to hairy, light green or light brown, decaying early, leaf sheath glabrous or hairy, base swollen, margin thin and narrow; ligule membranous, inconspicuous, decaying early; petiole channelled in cross section, glabrous, lamina narrowly ovate to elliptic, rarely oblong, asymmetric, margin entire, smooth.

Inflorescence flowering from base to apex; peduncle short, usually hidden within leaf sheath; spike composed of compact rachis and 2–5 (–13) fertile bracts, bracts spirally and closely overlapping (rarely distichous), boat-shaped, 2-keeled, pink, pale brown, pale or light green, thin, translucent, glabrous or hairy, decaying early, marcescent, amplexicaul at the base of the bract, cincinni compact, 2–3 flowers in each cincinnus. First bracteole directly opposite floral bract and enclosing all the flowers and subsequent bracteoles, linear-shaped, 2-keeled, shorter than bracts, rarely same length. Flowers thin, delicate, ephemeral. Calyx tubular, splitting unilaterally on one side, floral tube long slender, usually puberulent externally, inner surface with a groove enveloping the style, corolla lobes 3, triangular ovate, translucent, glabrous, dorsal lobe apex hooded, lateral lobes 2, narrower than dorsal lobe. Staminodes elliptic to

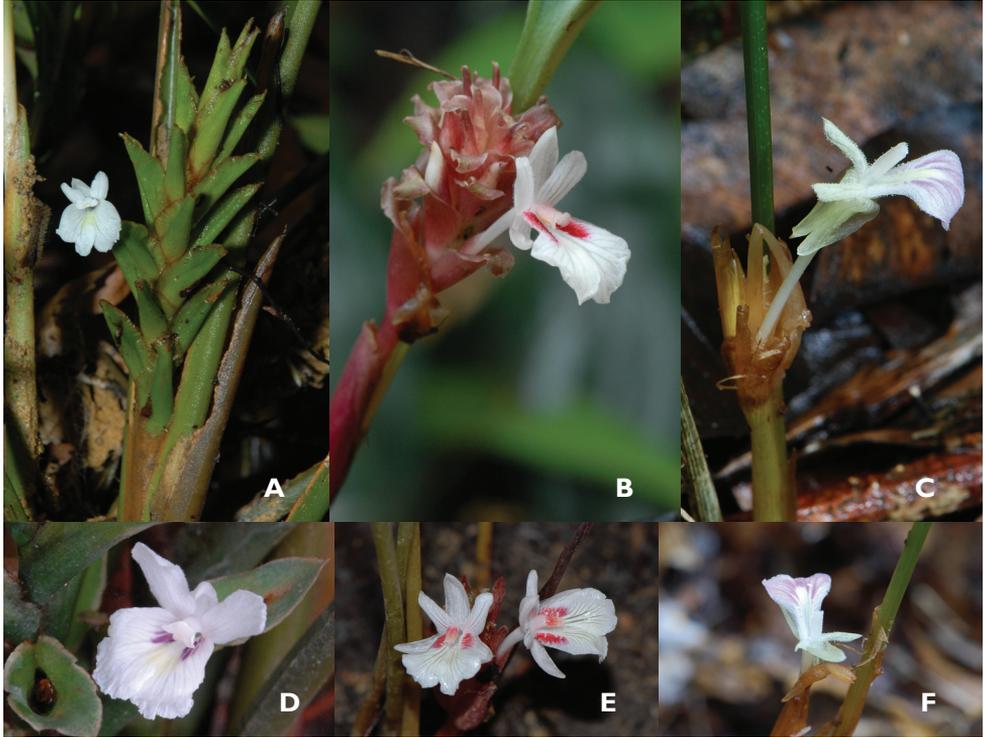


Figure 5. A–C Floral bracts **A** Green and coriaceous in *Scaphochlamys klossii* **B** Red and coriaceous in *Scaphochlamys pusilla* **C** Scarios and marcescent in *Borneocola petiolatus* **D–F** Variegation on labellum **D** White labellum with purple lines beside the median band in *Scaphochlamys malaccana* **E** White labellum with red streaks beside the band in *Scaphochlamys concinna* **F** Lilac labellum without coloured streaks beside the band in *Borneocola petiolatus*. (Photographs by Y.Y. Sam)

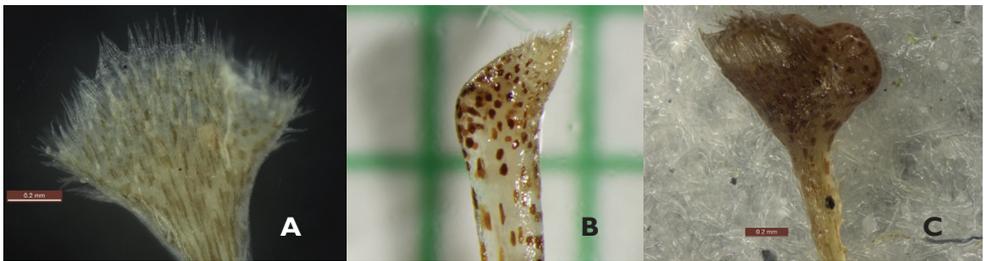


Figure 6. Stigma **A** Funnel-shaped in *Scaphochlamys endauensis* **B** Beak-like in *Scaphochlamys biloba* **C** Capitata in *Borneocola petiolatus*. (Photographs by **A** & **C** N.M. Aidil, **B** Y.Y. Sam)

narrowly obovate, white, light yellow or green, spreading laterally, lined with translucent veins from base to apex, covered with glandular hairs on adaxial surface. Labellum obovate, flat, bilobed distally, rarely entire, translucent veins spread from base to

apical part, pale white, pink, lilac or violet, median band light yellow or greenish yellow, without coloured streaks or patches beside the band, adaxial surface covered with glandular hairs. Stamen bends forward over labellum, usually white and covered with glandular hairs on abaxial surface, filament grooved, short, anther thecae 2, linear, dehiscing along entire length, basal ending with or without short spur, anther crest short, not recurved. Ovary ellipsoid, glabrous or sparsely hairy, unilocular, with basal placentation, less than 10 locules. Stigma small, held at apex of thecae, near oblate, 2 dorsal knobs, ostiole forward facing, ciliate. Epigynous glands 2, filiform, yellow. Fruit a capsule, ellipsoid or ovoid. Seeds not seen.

Etymology. This new genus is named after the island of Borneo and *-cola* (Latin) means dweller or inhabitant. This is to recognise the extremely rich and unique biodiversity that is found in Borneo.

Distribution. Borneo. The genus is currently known to occur only in the north-west and possibly central Borneo. Eight species are recorded from Sarawak, Malaysia and many more are undescribed.

Key to *Borneocola* species (modified from Ooi and Wong 2014)

- 1 Adaxial lamina with distinctly raised tessellate venation **6 *Borneocola reticosus***
- Adaxial lamina without distinctly raised tessellate venation **2**
- 2 Lamina broadly oblanceolate and elliptic to oblong, lateral veins conspicuously raised on adaxial surface..... **1 *Borneocola argenteus***
- Lamina linear, lanceolate to broadly ovate and elliptic, lateral veins not conspicuously raised on adaxial surface..... **3**
- 3 Lamina linear to very narrowly lanceolate, < 3 cm wide **8 *Borneocola stenophyllus***
- Lamina lanceolate, ovate to elliptic, > 3 cm wide **4**
- 4 Leaf sheath plus petiole < 10 cm long; lamina apex broadly acute to rounded, base cuneate **4 *Borneocola iporii***
- Leaf sheath plus petiole > 10 cm long; lamina apex acute to acuminate, base attenuate, cordate, rounded or truncated **5**
- 5 Lamina lanceolate to ovate, < 7 cm wide **6**
- Lamina elliptic to broadly elliptic and ovate, > 7 cm wide..... **7**
- 6 Basal lamina rounded to truncated; inflorescence stalk 3–9 cm long; labellum pale lilac..... **5 *Borneocola petiolatus***
- Basal lamina attenuate; inflorescence stalk 1–2 cm long; labellum purplish blue **2 *Borneocola biru***
- 7 Inflorescence about 3 cm long; labellum pale pink.....
- **7 *Borneocola salahuddinianus***
- Inflorescence > 7 cm long; labellum white **3 *Borneocola calcicola***

1. *Borneocola argenteus* (R.M.Sm.) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158824-1

Scaphochlamys argentea R.M.Sm., Notes Roy. Bot. Gard. Edinburgh 44: 209 (1987).
Scaphochlamys depressa Mas Izzaty, A.Ampeng & K.Meekiong, Folia Malaysiana 14(2): 19 (2013).

Type. MALAYSIA. Sarawak, First Division, Lundu, near foot of Gunung Perigi, 6 Aug 1962, Burt B2700 (holotype: E!).

Notes. It is one of the most distinctive species, either in the field or herbarium sheet. This dainty plant has a long creeping rhizome and well spaced leafy shoots, prominently stiff lateral veins raised on its adaxial lamina, both on living plants and dried specimens.

Scaphochlamys depressa Mas Izzaty, Ampeng & Meekiong is unmistakably the same as *S. argentea* with its prominent raised lateral veins, broadly elliptic lamina and well spaced leafy shoots. Meekiong (2015) explained that the inflorescence of *S. depressa* which exerted from the petiole is different from *S. argentea* where the inflorescence emerges from the base of the petiole. This observation is incorrect as all gingers have terminal inflorescences.

2. *Borneocola biru* (Meekiong) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158812-1

Scaphochlamys biru Meekiong, Folia Malaysiana 16(1): 37 (2015).

Type. MALAYSIA. Sarawak, Kuching Division, Matang Wildlife Centre, 21 May 2014 Meekiong et al. s.n. (holotype: SAR; isotype: Herbarium, Universiti Malaysia Sarawak. Types not yet deposited as of 5 May 2016).

Notes. *Borneocola biru* is the most recent species described from Sarawak. It has a deep purplish blue labellum, different from all other *Borneocola* species which are white or in lighter shades.

3. *Borneocola calcicola* (A.D.Poulsen & R.J.Searle) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158813-1

Scaphochlamys calcicola A.D.Poulsen & R.J.Searle, Gard. Bull. Singapore 57: 29 (2005).

Type. MALAYSIA. Sarawak, Kuching Division, Bau area, Gunung Tai Ton, 1°24'N, 110°8'E, 20 June 2003, Poulsen, Jugah & Clausager 2022 (holotype: SAR!; isotypes: AAU, E!, K!, L).

Notes. *Borneocola calcicola* is the largest amongst the *Borneocola* species. Poulsen and Searle (2005) observed that the distichous inflorescence is one of the character-

istics of the plant. However, a recent collection of *B. calcicola*, Sam FRI 50290, from Seromah, Bau, showed spirally arranged floral bracts. There was a mixture of spirally and distichously arranged floral bracts in its population in Bau, Sarawak.

4. *Borneocola iporii* (Meekiong & A.Ampeng) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158814-1

Scaphochlamys iporii Meekiong & A.Ampeng, Folia Malaysiana 12(1): 19 (2011).

Type. MALAYSIA. Sarawak, Kapit, Lanjak Entimau Wildlife Sanctuary, Bukit Menyarin, 3 April 2008, Meekiong MK1839 (holotype: SAR; isotype Herbarium, Universiti Malaysia Sarawak. Types not yet deposited as of 5 May 2016).

Notes. *Borneocola iporii* is a small ginger creeping on the humus rich forest floor. It is most similar to *B. argenteus* with both having a unifoliate shoot, leafy shoots far apart, broad lamina, short inflorescence and compact rachis. However, the conspicuously raised lateral veins of *B. argenteus* can readily distinguish it from *B. iporii*.

5. *Borneocola petiolatus* (K.Schum.) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158815-1

Haplochorema petiolatum K.Schum. in Engler, Pflanzenr. IV, 46 (Heft 20): 90 (1904).

Scaphochlamys petiolata (K.Schum.) R.M.Sm., Notes Roy. Bot. Gard. Edinburgh 44: 210 (1987).

Type. MALAYSIA. Sarawak, First Division, Mt. Singhi (= Gunung Singai), Dec 1892, Haviland 2026 (lectotype: K! designated by Searle 2010; isolectotype: E!, SAR!).

Notes. *Borneocola petiolatus* is distinguished by its long petiole and narrow leaves from the other species. Its lamina length is almost 3 times the width (12–21.5 × 3.1–7.1 cm). Smith (1987) found that *B. petiolatus* has small inflorescences as in *B. argenteus*. However, both can be easily separated by their leaf characters. *Borneocola petiolatus* has much longer petioles compared to *B. argentea* (12.7–31.5 cm versus 3–6 cm). *Borneocola argenteus* also has prominently raised lateral veins on the adaxial surface of lamina, more conspicuous on dried specimens than fresh materials. This character is lacking in *B. petiolatus*.

6. *Borneocola reticosus* (Ridl.) Y.Y.Sam, comb. nov.

urn:lsid:ipni.org:names:77158816-1

Gastrochilus reticosus Ridl., J. Straits Branch Roy. Asiat. Soc. 44: 195 (1905). *Boesenbergia reticosus* (Ridl.) Merr., Bibl. Enum. Born. Pl. 122 (1921). *Scaphochlamys reticosus* (Ridl.) R.M.Sm., Notes Roy. Bot. Gard. Edinburgh 44: 209 (1987).

Type. Cultivated in Singapore Botanic Gardens, originally from Borneo, Sarawak, First Division, Bidi, 22 Nov 1904, Ridley s.n. (holotype: SING!).

Notes. *Borneocola reticosus* is chosen as the type species as it is the easiest to recognise in the genus. Its reticulate lamina readily distinguishes it from other *Borneocola* species.

7. *Borneocola salahuddinianus* (Meekiong, A.Ampeng & Ipor) Y.Y.Sam, comb. nov.
urn:lsid:ipni.org:names:77158817-1

Scaphochlamys salahuddiniana Meekiong, A.Ampeng & Ipor, *Folia Malaysiana* 12(1): 22 (2011).

Type. MALAYSIA. Sarawak, Kapit, Ulu Katibas, Lanjak Entimau Wildlife Sanctuary, Bukit Sepali, 30 April 2008, Meekiong MK1856 (holotype SAR; isotype Herbarium, Universiti Malaysia Sarawak.. Types not yet deposited as of 5 May 2016).

Note. *Borneocola salahuddinianus* is unique amongst the Bornean species with its broadly elliptic or ovate lamina held by a long slender petiole. It is doubtful that *B. salahuddinianus* is a lithophyte as observed by Meekiong et al. (2011). The plants are more of an opportunist growing on humus-rich substrate accumulated on the rocks.

8. *Borneocola stenophyllus* (I.H.Ooi & S.Y.Wong) Y.Y.Sam., comb. nov.
urn:lsid:ipni.org:names:77158818-1

Scaphochlamys stenophylla I.H.Ooi & S.Y.Wong, *Willdenowia* 44(2): 241-245 (2014).

Type. MALAYSIA. Sarawak, Kuching Division, Bau, Gunung Buan, 1°33'28.9"N, 10°08'35.2"E, 92 m, 21 Nov 2013, Ooi Im Hin & Jepom ak Tisai OIH74 (holotype: SAR. Type not yet deposited as of 5 May 2016).

Note. *Borneocola stenophyllus* is another new species recently discovered from Sarawak. Its grass-like leaves instantly separate it from other species in the genus.

Incompletely known species

Scaphochlamys anomala (Hallier f.) R.J.Searle, *Edinburgh J. Bot.* 67: 85 (2010).

Kaempferia anomala Hallier f., *Bull. Herb. Boissier* 6: 357 (1898). *Gastrochilus anomalum* (Hallier f.) K.Schum. in Engler, *Pflanzenr.* IV, 46 (Heft 20): 92 (1904). *Boesenbergia anomala* (Hallier f.) Schltr., *Repert. Spec. Nov. Regni Veg.* 12: 315 (1913).

Gastrochilus hallieri (Hallier f.) Ridl., *J. Straits Branch Roy. Asiat. Soc.* 32: 109 (1899), nom. illegit.

Type. INDONESIA. Cultivated in Bogor, originally from Liang Gagang, Kalimantan Borneo, Hallier s.n. (original material: BO, specimen lost; lectotype (designated by Searle, 2010) Figure drawn from original Hallier's material and published as t. IX, fig. 3, Bull. Herb. Boissier 6: 357 (1898).

Notes. The type, the only specimen ever collected, was lost. However, Hallier (1898) gave a very detailed description and drawing of the plant and this has convinced Searle (2010) to place it in the genus *Scaphochlamys*. The drawing, which is based on the type specimen and designated by Searle as the lectotype, is the only material that gives a glimpse of the appearance of the species. In the drawing, the flower and spirally arranged floral bracts are typical of both *Scaphochlamys* and *Borneocola*. Until another specimen is collected and is available for close examination, we prefer to retain this imperfectly known species in *Scaphochlamys*.

Discussion

The phylogenetic analyses confirm the distinctive character of *Borneocola* and *Scaphochlamys* and their placement in the tribe Zingibereae (Figures 1, 2, 3). The *Borneocola* species form a monophyletic group which is sister to *Myxochlamys*. It is surprising to find *Borneocola* having a closer affinity to *Myxochlamys* than to *Scaphochlamys*, considering it shares more morphological similarities with *Scaphochlamys* than with *Myxochlamys*.

Morphologically, *Myxochlamys* is very different from *Borneocola*. There are two *Myxochlamys* species named so far: *M. amplioxa* and *M. mullerensis* (Takano and Nagamasu 2007; Searle and Newman 2010) and a third undescribed species, also from Borneo. All three *Myxochlamys* species are very robust plants that can attain a height of 70 cm. Most *Borneocola* species examined so far are small-sized (not more than 50 cm tall), except for *B. calcicola* which can grow to 60 cm tall. *Myxochlamys* has 3–10 large leaves (50–60 cm long) in each shoot whereas *Borneocola* are unifoliate and the leaves are small (less than 20 cm long except for *B. calcicola*). The leaves of *Myxochlamys* are sessile compared to the conspicuously stalked leaves in *Borneocola*. The most marked difference is in the inflorescence structure. *Borneocola* has small inflorescences consisting of less than 15 fertile bracts but *Myxochlamys* has large torch-like inflorescences with easily more than 40 bracts. The bracts of *Borneocola* are membranous and marcescent, often measuring less than 2 cm long (except for *B. calcicola* measuring 2.5–3.2 cm long). By contrast, the floral bracts of *Myxochlamys* are coriaceous, persistent, measuring 2.5–5 cm long and most notably are covered with transparent slimy mucilage. In addition, the unique versatile anthers of *Myxochlamys*, a rare feature in the Zingiberaceae, are distinct from the adnate anthers in *Borneocola* and also from all its sister genera. Based on morphological features, *Myxochlamys* is more similar to *Scaphochlamys*, the closest being *S. grandis*. Both have large sessile leaves and decurrent lamina base, large, coriaceous

and persistent floral bracts, their bracts being concave with reflexed and spreading apices.

Based on morphology, *Borneocola* is also similar to *Distichochlamys*. However, *Distichochlamys* is distinguished from *Borneocola*, *Myxochlamys* and *Scaphochlamys* by its unique tubular bracteoles, floral tube without a groove on the inner surface and trilobular ovary (Newman 1995). Other characteristics such as distichous floral bracts, 2-keeled bracteoles, thecae without basal spurs have been observed in the three closely allied sister genera in this study (Table 2).

Haplochorema K.Schum. is another small-sized genus endemic to Borneo, which can be mistaken for *Borneocola*. It has short and few-flowered inflorescences as in *Borneocola* but its flowers appear somewhat quadrate with the labellum and lateral staminodes held flat, more resembling *Kaempferia* L. *Haplochorema* has distichous floral bracts, single-flowered cincinni and the flowering proceeds from apex to base, to name some of the characters which distinguish it from *Borneocola*. In fact, the genus is more allied to *Boesenbergia* Kuntze than *Borneocola*.

Borneocola is morphologically most similar to *Scaphochlamys* but both can be distinguished by the texture of the bladeless sheath and floral bracts, variegation on the labellum, indumentum on the floral tube and the stigma shape. The current study recognises eight *Borneocola* species while *S. gracilipes*, *S. polyphylla* B.L.Burt & R.Sm., *S. limiana* Meekiong & K.Yazid and *S. samunsamensis* Meekiong & Hidir from Borneo remain in the genus *Scaphochlamys*. There are no recent collections of *S. gracilipes* but the lax inflorescence and persistent floral bracts in the type specimens clearly distinguish it from the *Borneocola* species. *Scaphochlamys polyphylla*, *S. limiana* and *S. samunsamensis* can be readily distinguished from the *Borneocola* species by their papery bladeless sheath and large, green or green tinged red, coriaceous floral bracts. This shows that the distinct morphologies that separate *Borneocola* and *Scaphochlamys* are significant and are also supported by the phylogenetic analyses (Figures 1, 2 and 3). An anatomical study on the leaves also discovered some characteristics that separate *Borneocola* from *Scaphochlamys* (Norhati, pers. comm).

The morphology of *Borneocola* is very similar to *Scaphochlamys* but, combining both, necessitates synonymising *Myxochlamys* and possibly *Distichochlamys* and this will result in a very heterogenous genus. A similar situation is observed in the naming of *Newmania* N.S. Lý & Škorníček, a genus very similar in morphology to *Haniffia* Holttum but appears as its sister group in the molecular phylogenetic analyses. The authors decided against placing *Newmania* under *Haniffia* which would create a heterogenous group. The current description of *Borneocola* is further supported by the chromosome number with $2n=10$ (Šída et al., unpublished data), different from *Distichochlamys* ($2n=26$) and *Scaphochlamys* ($2n=28$). Such significant differences in molecular data and chromosome number have conclusively supported the circumscription of the new genus *Borneocola*.

Table 2. Comparison between the morphological characters of *Borneocola*, *Distichoclamys*, *Myxoclamys* and *Scaphochlamys*.

Morphology	<i>Borneocola</i>	<i>Distichoclamys</i>	<i>Myxoclamys</i>	<i>Scaphochlamys</i>
Plant height	to 50(–60) cm	to 60 cm	70 cm	to 100 cm
Number of leaf in each leafy shoot	1	1–3	3–10	1–7
Bladeless sheath	Papery, drying fast	Papery, decaying fast	Not mentioned	Coriaceous, persistent
Leaf (cm)	6–37 × 1–18; petiolate	15–28 × 8.3–14.5; petiolate	50–65 × 7–17; sessile	9–50 × 3–24; petiolate or sessile
Inflorescence height (cm)	3–11.5	to 15.5	6.5–18	4–28
Number of floral bracts	3–13	7–13	c. 40	4–44
Arrangement of floral bracts	Spiral, rarely distichous	Distichous	Spiral	Spiral, rarely distichous
Floral bracts	Thin, translucent, without mucilage; drying fast	Without mucilage; persistent	Coriaceous, with mucilage; persistent	Coriaceous, without mucilage; persistent
Flowers	In cincinni	In cincinni	Solitary	In cincinni
First bracteole	Open to base, 2-keeled	Tubular, 2-keeled	Open to base, 2-keeled	Open to base, 2-keeled
Floral tube	With a groove in inner surface, glabrous to puberulent externally	Without a groove in inner surface, glabrous externally	With a groove in inner surface, glabrous externally	With a groove in inner surface, glabrous externally
Labellum	Bilobed, rarely entire, not concave; without coloured streaks beside median band	Bilobed, not concave; without coloured streaks beside median band	Not bilobed, entire, concave; without coloured streaks beside median band	Bilobed, rarely entire, not concave; with coloured streaks beside median band
Thecae	Spurs absent or with short free basal spurs	Spurs absent	Spurs present and long	Spurs absent or with short free basal spurs
Anther	Adnate	Adnate	Versatile	Adnate
Ovary	Unilocular with basal placentation	Trilocular with axile placentation	Unilocular with basal placentation	Unilocular with basal placentation
Chromosome number	2 <i>n</i> =10 (Šída et al., unpublished data)	2 <i>n</i> =26	—	2 <i>n</i> =28
Geographical distribution	Borneo	Vietnam	Borneo	Southern Thailand, Peninsular Malaysia, Sumatra, Borneo

Key to the genera of the Zingibereae tribe in Borneo

- 1 Inflorescence arising directly from the rhizome on a leafless shoot.....2
 – Inflorescence emerging at the terminal of the leafy shoot3
 2 Distinct swelling at the base of the petiole; anther with long extended crest wrapped around the style *Zingiber*
 – No swelling at the base of the petiole; anther crest short, not long extended and not wrapped around the style..... *Haniffia*
 3 Flowers with versatile anther4
 – Flowers with adnate anther5
 4 Inflorescence with few to many floral bracts, bracts mucilage....*Myxochlamys*
 – Inflorescence with one single large floral bract, bracts not mucilage
 *Camptandra*
 5 Flowers opening from top to bottom of inflorescence6
 – Flowers opening from bottom to top of inflorescence7
 6 Flowers appearing quadrate with the two petaloid staminodes....*Haplochorema*
 – Flowers no quadrate appearance, staminodes not petaloid.....*Boesenbergia*
 7 Flowers with long narrow corolla lobes and long exerted stamens
 *Hedychium*
 – Flowers without such features8
 8 Floral bracts coriaceous and persistent, labellum with coloured streaks on both sides of the median band*Scaphochlamys*
 – Floral bracts thin, translucent and marcescent, labellum without coloured streaks on both sides of the median band.....*Borneocola*

Acknowledgements

The authors wish to thank the Forestry Department of Peninsular Malaysia and its State Forestry Departments, Sarawak Forestry Department, Department of Wildlife and National Parks and Johor Parks Corporation for permission to conduct research in their forests; herbaria of AAU, BKF, C, FI, E, K, KEP, KLU, PSU, SAN, SAR and SING for the loan of specimens; Lucy Chong and Julia Sang from the Sarawak Forestry Corporation, Wong Sin Yeng and Peter C. Boyce from Universiti Malaysia Sarawak, Sarawak for their hospitality and logistics help in Sarawak. We are also grateful to Kalu Meekiong from the Universiti Malaysia Sarawak, Sarawak and John Mood for the leaf samples for the molecular study; J.F. Veldkamp for advice on the genus and epithet names; Jana Leong-Škorničková, Richard C.K. Chung, Leng Guan Saw, Ruth Kiew and the reviewers for their critical comments and suggestions to improve the manuscript. This work is supported by the Malaysian Ministry of Higher Education through the Fundamental Research Grant Scheme (FRGS FP 075/2007C) and Special Research University Grant (FR 150/2007A), University of Malaya, the Ministry of Science, Technology and Innovation (Project No. 01-04-01-0000 Khas2), the Ministry of Natural Resources and

Environment under the 10th and 11th Malaysian Plans (SPPII No. P23085100010021 & P23085100018003 respectively) and Grants-inAid (KAKENHI) for Scientific Research (No.26440227) from the Japan Society for the Promotion of Science.

References

- Baker JG (1892) Scitaminae. In: Hooker JD (Ed.) Flora of British India 6. Reeve & Co., London, 225–264.
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. doi: 10.2307/2408678
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hallier H (1898) Neue und Bemerkenswerte Pflanzen. *Bulletin de l'Herbier Boissier*, 6, 348.
- Harris DJ, Poulsen AD, Fridodt-Moller C, Preston J, Cronk QCB (2000) Rapid radiation in *Aframomum* (Zingiberaceae): evidence from nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. *Edinburgh Journal of Botany* 57(3): 377–395. doi: 10.1017/S0960428600000378
- Holtum RE (1950) The Zingiberaceae of the Malay Peninsula. *Gardens' Bulletin Singapore* 13: 1–249.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. doi: 10.1093/bioinformatics/17.8.754
- Kress WJ, Larsen K (2001) *Smithatris*, a new genus of Zingiberaceae from Southeast Asia. *Systematic Botany* 26(2): 226–30. doi: 10.1043/0363-6445-26.2.226
- Kress WJ, Mood J, Sabu M, Prince L, Dey S, Sanoj E (2010) *Larsenianthus*, a new Asian genus of Gingers (Zingiberaceae) with four species. *PhytoKeys* 11: 15–32. doi: 10.3897/phytokeys.1.658
- 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* 89(10): 1682–1696. doi: 10.3732/ajb.89.10.1682
- Larsen K, Newman M (2001) A new species of *Distichochlamys* from Vietnam and some observations on generic limit in Hedychieae (Zingiberaceae). *Natural History Bulletin of Siam Society* 49: 77–80. doi: 10.1007/BF00984630
- Leong-Škorničková J, Lý NS, Poulsen AD, Tosh J, Forrest A (2011) *Newmania*: A new ginger genus from central Vietnam. *Taxon* 65(5): 1386–1396.
- Liston A, Kadereit J W (1995) Chloroplast DNA evidence for introgression and long distance dispersal in the desert annual *Senecio flavus* (Asteraceae). *Plant Systematics and Evolution* 197(1–4): 33–41.
- Meekiong (2015) Three new *Scaphochlamys* species from Sarawak, Malaysia. *Folia Malaysiana* 16(1): 31–44.
- Meekiong K, Ipor I, Tawan CS, Ibrahim H, Norhati MR, Lim CK, Ampeng A (2011) Five new ginger species (Zingiberaceae) from the eastern part of Lanjak Entimau Wildlife Sanctuary, Sarawak, Borneo. *Folia Malaysiana* 12(1): 9–26.

- Moller M, Cronk Q (1997) Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany* 84(7): 956–965. doi: 10.2307/2446286
- Newman MF (1995) *Distichochlamys*, a new genus from Vietnam. *Edinburgh Journal of Botany* 52: 65–69. doi: 10.1017/S096042860000192X
- Ngamriabsakul C, Newman MF, Cronk QCB (2004) The phylogeny of tribe Zingibereae (Zingiberaceae) based on ITS (nrDNA) and trnL-F (cpDNA) sequences. *Edinburgh Journal of Botany* 60(3): 483–507.
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author, Evolutionary Biology Center, Uppsala University.
- Ooi IH, Wong SY (2014) *Scaphochlamys stenophylla* (Zingiberaceae): a new species from Sarawak, Malaysian Borneo. *Willdenowia* 44(2): 241–245. doi: 10.3372/wi.44.44205
- Ooi K, Endo Y, Yokoyama J, Murakami N (1995) Useful primer designs to amplify DNA fragments of the plastid gene matK from angiosperm plants. *Journal of Japanese Botany* 70(6): 328–331.
- Pedersen LB (2004) Phylogenetic analysis of the subfamily Alpinioideae (Zingiberaceae), particularly *Etilingera* Giseke, based on nuclear and plastid DNA. *Plant Systematics and Evolution* 245: 239–258. doi: 10.1007/s00606-004-0126-2
- Poulsen AD, Searle RJ (2005) *Scaphochlamys calcicola* (Zingiberaceae): a new and unusual species from Borneo. *The Gardens' Bulletin Singapore* 57: 29–35.
- Rohnquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. doi: 10.1093/bioinformatics/btg180
- Sakai S, Nagamasu H (2006) Systematic studies of Bornean Zingiberaceae V. Zingiberoideae of Lambir Hills, Sarawak. *Blumea* 52: 95–115. doi: 10.3767/000651906X622364
- Searle RJ (2010) The genus *Scaphochlamys* (Zingiberaceae - Zingibereae): A field compendium for the field worker. *Edinburgh Journal of Botany* 67(1): 75–121. doi: 10.1017/S0960428609990254
- Searle RJ, Hedderson TAJ (2000) A preliminary phylogeny of the *Hedychieae* tribe (Zingiberaceae) based on ITS sequences of the nuclear rRNA cistron. In: Wilson KL, Morrison DA (Eds) *Monocots: Systematics and Evolution*. CSIRO, Collingwood, 710–718.
- Searle RJ, Newman M (2010) *Myxochlamys amphiloza* (Zingiberaceae): A new species from Central Kalimantan, Indonesia. *Edinburgh Journal of Botany* 67(2): 347–352. doi: 10.1017/S0960428610000053
- Smith RM (1987) A review of Bornean Zingiberaceae: III (Hedychieae). *Notes of the Royal Botanic Gardens Edinburgh* 44: 203–232.
- Steele KP, Vilgalys R (1994) Phylogenetic analysis of Polemoniaceae using nucleotide sequences of the plastid gene matK. *Systematic Botany* 19: 126–142. doi: 10.2307/2419717
- Takano A, Nagamasu H (2007) *Myxochlamys* (Zingiberaceae), a new genus from Borneo. *Acta Phytotaxonomica et Geobotanica* 58: 19–32.
- Takano A, Okada H (2002) Multiple occurrences of triploid formation in *Globba* (Zingiberaceae) from molecular evidence. *Plant Systematics and Evolution* 230: 143–159. doi: 10.1007/s006060200001

- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. doi: 10.1093/molbev/mst197
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. doi: 10.1093/nar/22.22.4673
- 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. doi: 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. doi: 10.1017/S0960428600000196
- Xia YM, Kress WJ, Prince LM (2004) Phylogenetic analyses of *Amomum* (Alpinioideae: Zingiberaceae) using ITS and matK DNA sequence data. *Systematic Botany* 29(2): 334–344. doi: 10.1600/036364404774195520

Arabis watsonii (P.H.Davis) F.K.Mey.: An overlooked cruciferous species from eastern Anatolia and its phylogenetic position

Barış Özüdoğru¹, Mehmet Fırat²

1 Department of Biology, Faculty of Science, Hacettepe University, Ankara Turkey **2** Department of Biology, Faculty of Education, Yüzüncü Yıl University, Van, Turkey

Corresponding author: Barış Özüdoğru (barisoz@hacettepe.edu.tr)

Academic editor: K. Marhold | Received 20 September 2016 | Accepted 19 November 2016 | Published 1 December 2016

Citation: Özüdoğru B, Fırat M (2016) *Arabis watsonii* (P.H.Davis) F.K.Mey.: An overlooked cruciferous species from eastern Anatolia and its phylogenetic position. *PhytoKeys* 75: 57–68. doi: 10.3897/phytokeys.75.10568

Abstract

Arabis watsonii (P.H.Davis) F.K.Mey. was initially reported as *Thlaspi watsonii* P.H.Davis in Flora of Turkey. Although F.K.Meyer transferred this species to *Arabis* L., this species has been overlooked and treated as *Thlaspi* L. in relevant literature for Flora of Turkey. In this study this species was evaluated using molecular (nuclear ITS and plastidic trnL-F sequences) and morphological data. Results clearly show that *A. watsonii* is sister to the *A. hirsuta* aggregate and its relatives. In conclusion, our results increased the number of known *Arabis* species in Turkey to 23. Furthermore, detailed description and distribution of the species are given and a new IUCN threat category for *A. watsonii* is proposed.

Keywords

Arabis watsonii, *A. hirsuta* aggregate, *Arabideae*, Brassicaceae, phylogeny

Introduction

The genus *Arabis* L. is represented by ca. 60 species and distributed in temperate regions of the northern hemisphere (Koch et al. 2010, Al-Shehbaz 2012). Although Al-Shehbaz (1988) earlier delimited the genus with ca. 180 species, molecular phylogenetical studies of *Arabis* and its relatives clearly show that *Arabis* s.l. is polyphyletic

(Koch et al. 1999, 2000, 2001, 2010, Al-Shehbaz et al. 2011) and subsequently some of the highly supported phylogenetic lineages were described as separate genera, e.g. *Scapiarabis* M.A. Koch, R. Karl, D. German & Al-Shehbaz, *Acirostrum* Y.Z.Zhao, and *Sinoarabis* R. Karl, D. German, M.A. Koch & Al-Shehbaz (Karl et al. 2012). Nevertheless, several highly supported *Arabis* clades (including main *Arabis* clade, *A. alpina* L. clade, *A. aucheri* Boiss. clade etc.) were also described (Karl and Koch 2013).

After the first revision of J. Cullen (1965) who reported 17 *Arabis* species in Flora of Turkey, five new species (*A. lycia* Parolly & P.Hein, *A. alanyensis* H.Duman, *A. davisii* H.Duman & A.Duran, *A. erikii* Mutlu and *A. kaynakiae* Daşkın) and two new records for Turkey (*A. allionii* DC. and *A. mollis* Steven) were added (Davis et al. 1988, Parolly and Hein 2000, Duman 2001, Duman and Duran 2001, Mutlu and Dönmez 2003, Mutlu 2004, Daşkın 2013). In addition, *A. graellsiiiformis* Hedge was treated as a subspecies of *A. mollis* Steven (Mutlu and Erik 2012) and *Arabis turrita* L. transferred to *Pseudoturritis* Al-Shehbaz (Al-Shehbaz 2005). Finally, the genus *Arabis* is currently represented by 22 species (24 taxa) in Turkey (Mutlu and Erik 2015).

Arabis watsonii (P.H.Davis) F.K.Mey. (Fig. 1) was initially published as *Thlaspi watsonii* P. H. Davis in Flora of Turkey by P. H. Davis. Davis argued that this taxon is closely related to the pink flowered *Thlaspi lilacinum* Boiss & Huet (*Callothlaspi lilacinum* (Boiss & Huet) F.K.Mey or *Noccaea lilacina* (Boiss. & A. Huet) Al-Shehbaz, depending on the authors and treatments). F.K. Meyer investigated type specimen of *T. watsonii* while working on his *Callothlaspi* account (Meyer 2006), and he transferred this species to *Arabis* since due to the presence of siliques (rather than silicles which are typical for *Thlaspi*) and leaves with bifid hairs. Ever since this taxon either has been overlooked (Al-Shehbaz et al. 2007, Özhatay et al. 2009, 2011, Mutlu and Erik 2015) or still treated as *Thlaspi* L. (Güner et al. 2014).

To verify Meyer's treatment of *Arabis watsonii*, plant material was collected from type locality and nearby areas. Collected specimens were evaluated morphologically and molecularly to analyse 1) is *A. watsonii* a member of *Arabis* and 2), which main clade does it belong? We carried out morphological and molecular phylogenetic studies of *A. watsonii* plus representatives of *Arabis* and other Arabideae and combined this data with climatic and biogeographic data.

Methods

Sampling

The present study includes for the first time sequences of nuclear ribosomal ITS1, ITS2 and 5.8 S rRNA (hereafter ITS) and trnL(UAA) intron/trnL-trnF intergenic spacer sequence data (hereafter trnL-F) for *Arabis watsonii* (Voucher: M. Firat 32513 at HUB). All other sequences of the 88 *Arabis* and other Arabideae species were taken from ITS and trnL-F data sets of Karl and Koch (2013). In addition, sequences of the related *A. hirsuta* aggregate (*A. stelleri* DC. and *A. takesimana* Nakai) were taken from

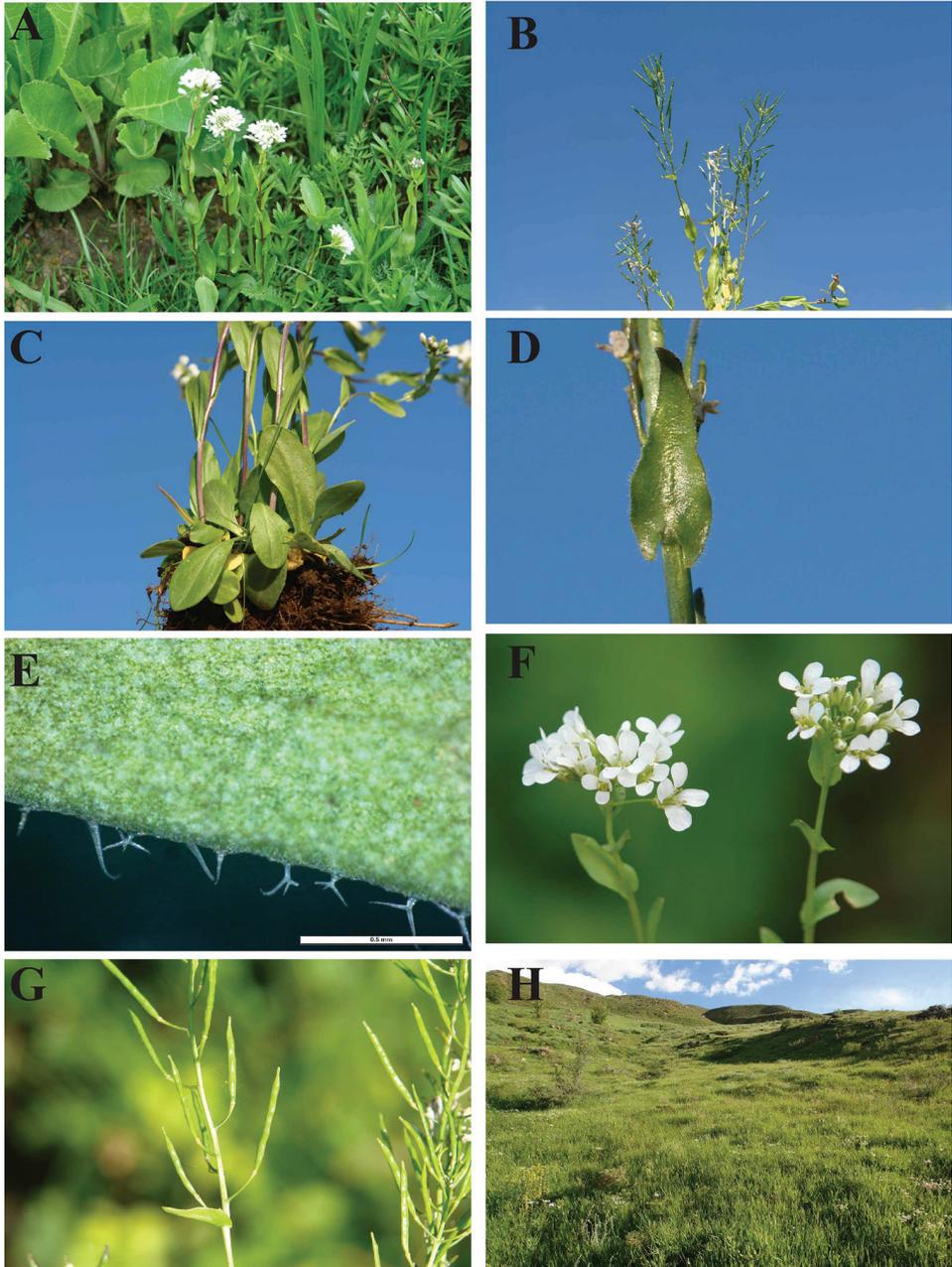


Figure 1. Photographs of *Arabis watsonii*, **A** habit (in flower) **B** habit (in fruit), **C** basal leaves **D** stem leaves **E** trichomes on leaves margins **F** flowers **G** fruits **H** habitat of Bahçesaray population.

GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) and incorporated into the above mentioned data sets. To determine the phylogenetic placement of *A. watsonii*, we reduced the above mentioned data as follows: All *Arabis* species were added along with

two species from *Aubrietia* Adans., two *Draba* L. species and one species each from other small genera including *Sinoarabis*, *Arcyosperma* O.E. Schulz, *Baimashania* Al-Shehbaz, *Acirostrum*, *Botschantzevia* Nabiev, *Dendroarabis* (C.A. Mey.) D. German & Al-Shehbaz, *Pachyneurum* Bunge, *Pseudodraba* Al-Shehbaz, D. German & M. Koch, *Scapiarabis*, *Tomostima* Raf. *Pseudoturritis turrita* (L.) Al-Shehbaz was used as the out-group. Genbank accessions of species included in this study are at terminal nodes of phylogenetic trees (Figs 3, 4).

50 specimens belonging to five populations were used for extending description of *A. watsonii*. The vouchers were deposited at Hacettepe University Herbarium (HUB) and private herbarium of M. Fırat.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. ITS and trnL-F regions were amplified using primers ITS1 and ITS 4 (White et al. 1990) and C and F of Taberlet et al. (1991), respectively. Amplification of ITS and trnL-F followed the protocol in Warwick et al. (2004) and Ansell et al. (2007), respectively. Purification and sequencing were performed by BIOEKSEN (İstanbul, Turkey).

Data analysis

Firstly, to determine whether *A. watsonii* belongs to tribe Arabideae, the phylogenetic tool in Brassibase (Koch et al. 2012; Kiefer et al. 2013) was used. ITS and trnL-F sequences data were edited with Codon Code Aligner (CodonCode Corporation) and directly incorporate into the alignment files of Karl and Koch (2013). Both data sets were analysed using a Bayesian approach as implemented in the software BEAST ver. 1.8 (Drummond et al. 2012).

Sequence evolution models were selected by the Akaike information criterion (AIC) implemented in MEGA v.6 (Tamura et al. 2013).

The GTR + G + I model was selected for ITS and GTR + I for trnL-F and a Yule process of speciation was used as the tree prior. Two independent Markov Chain Monte Carlo (MCMC) runs for each data set were conducted with 10 million generations and sampled every 1000 generations. Each run was checked using TRACER v1.6 (<http://beast.bio.ed.ac.uk/Tracer>) and then log and trees files were combined in LOGCOMBINER (Drummond et al. 2012).

A total of 20000 trees were obtained and 10% (2000) of these were discarded as burn-in. 18000 post-burn-in trees were used in the program TREEANNOTATER v.1.7.5 to obtain a single posterior probability and maximum clade credibility tree as visualized using FIGTREE v1.3.1.

Morphology, distribution and conservation

To redescribe morphological features of *A. watsonii* ca. 50 individuals from five populations were investigated. In order to evaluate the IUCN threat category of *A. watsonii*, occurrence data were obtained from both field and Yüzüncü Yıl University, Science faculty herbarium (VANF) and then total distribution area was calculated using DIVA-GIS (version 7.5.0, <http://www.diva-gis.org>). Some specimens that had no GPS coordinates were georeferenced using Google Earth 7.1. (<http://www.google.com/earth>) according to common names. To evaluate climatic requirements of *A. watsonii* and closely related taxa, bioclimatic data were taken from the WorldClim–Global Climate Database (<http://www.worldclim.org>) at a spatial resolution of 30 s.

Results

Morphology, distribution and conservation

Arabis watsonii (P.H.Davis) F.K.Mey (in Meyer 2006 p.187)

Basionym: *Thlaspi watsonii* P.H.Davis (in Davis et al. 1988 p. 235)

Type. Turkey B9 Van: Çuh pass, Halanduran Da. and Güzeldere Tepe, dry stony slopes, locally common, 2800 m, flowers white, vi 1966, Albury, Cheese & Watson 1438 (holo. K photo !).

Slender perennial herb. Stem erect, nearly glabrose, 13–30 cm high. Basal leaves up to 30 mm, oblong–obovate, petiolate, leaves on sterile shoots completely covered by branched trichomes, fertile shoot with branched trichomes on the leaves margin. Stem leaves narrowly oblong, very shortly auriculate, tapering to subacute apex, 5–32 × 2.5–10 mm with marginal trichomes. Sepals white-purplish, inner sepals saccate, 3–3.2 × 1–1.8 mm, outer sepals smooth, 2.7–3 × 1–1.5 mm. Petals white, 5.5–7 mm long, 1.5 mm broad above, tapering below into 1.5–2 mm claw. Stamens 6, long filaments 4, 3–4 mm, short filaments 2, 2.5–3 mm long, anther yellow, 0.8–1 × 0.3–0.4 mm. Pedicel up to 7–8 mm in fruit. Fruit ± constricted between seeds, 4–14 × 0.8–1.2 mm, with 6–8 seeds, style ca. 1 mm. Seeds brown, ovate-oblong, 0.9–1.1 × 0.5–0.7 mm.

Fl. and fr : 4–6. Alpine damp places, dry stony slopes, steppe. 1980–2800 m.

Distribution: Endemic. Irano–Turanian element (Fig. 2)

Specimens examined. TURKEY. B9 Van: Gürpınar district, from Güzeldere pass to Çuğ pass, damp places, 2495 m, 38°09'57"N, 43°57'47"E, 19 May 2015, M. Fırat 32513 (herb. M. Fırat); ibid M. Fırat 32572 (HUB, herb. M. Fırat) 11 June 2015; Bahçesaray district, from Ulubeyli village to Hizan, damp steppe, 2265 m, 38°07'46"N, 42°40'53"E, 19 May 2014, M. Fırat 30870 (HUB, herb. M. Fırat); ibid M. Fırat 30989 (HUB, herb. M. Fırat) 21 June 2014; Bahçesaray, between Liçan Village and

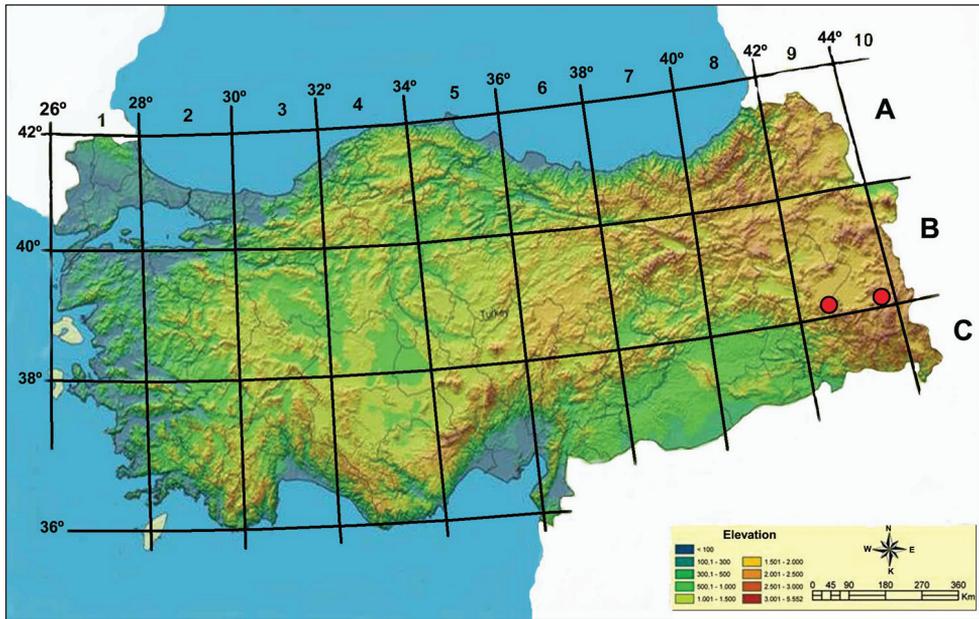


Figure 2. Distribution of *Arabis watsonii* (red dots). Map was taken from Fırat (2016).

Kavuşşahap Mountains (Deve Mountain), humid steppe, 1980 m, 04.04.1999. M. Fırat 1077 (herb. M. Fırat); Bahçesaray, Altındere Village Kavuşşahap Mountains, damp places, 2300 m, 17.05.2000, M. Fırat 2122 (herb. M. Fırat); Bahçesaray, between Cevzilibelen Village and Arnos Mountain, humid steppe, 2400 m, 23.06.2000, M. Fırat 2729 (herb. M. Fırat); between Güzelsu (Hoşap) and Başkale, Güzeldere pass, meadows, 2550–2650 m, 10.06.2001, M. Armağan 1073 (VANF); between Güzelsu (Hoşap) - Başkale, Güzeldere Pass, Güzeldere Hill, moist meadows, slopes, steppe, 2700–2800 m, 07.06.2002, M. Armağan 2423 (VANF); between Güzelsu (Hoşap) and Başkale, Güzeldere pass, from Güzeldere gendarmerie station to Başkale, slopes, steppe and moist meadows, 2600–2730 m, 19.05.2001, M. Armağan 1130 (VANF).

Vernacular name. In Van province, indigenous people use name ‘Nançük’ for *Arabis* species (Fırat 2013).

Field observations and records taken from relevant herbaria indicate that *A. watsonii* has two distinct populations (Fig. 2). A large population growing around Bahçesaray district and a second population occurring in the Gürpınar district especially around the Güzeldere pass. Therefore total distribution areas for these populations were estimated separately. The area around Bahçesaray was calculated as 108.99 km² and the second area around Gürpınaras 2.69 km². In summary, to propose IUCN threat categories of *A. watsonii*, these two population groups and calculated areas were considered. The occupancy area (AOO) of *A. watsonii* was calculated as 111.68 km² in which about 1000 individuals in each population were estimated to occur. Overgrazing and reaping activities by the local people were observed in field studies. Therefore, in accordance with the

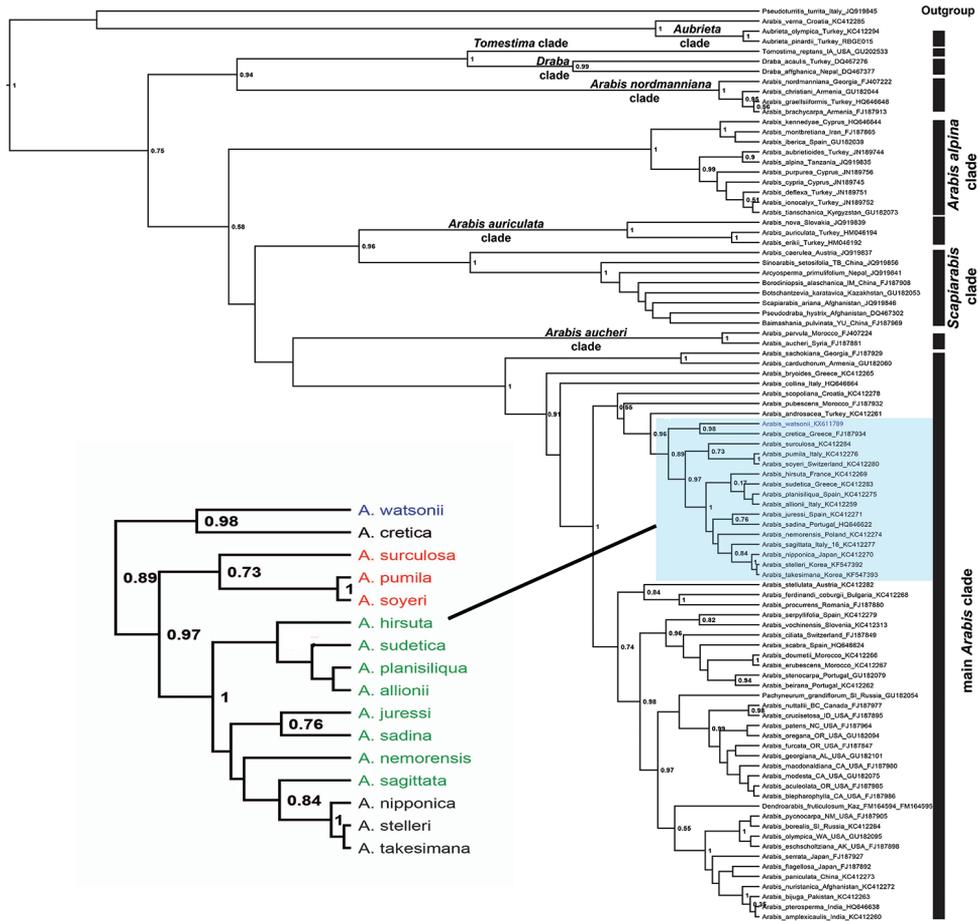


Figure 3. ITS-based phylogenetic backbone of Arabideae that focuses on the placement of *Arabis watsonii*. Shown is the Bayesian maximum clade credibility tree with posterior probability values > 0.5. Highlighted part of the tree, (magnified on the left), is the *Arabis hirsuta* aggregate and its relatives. Color codes: Green = *A. hirsuta* aggregate, red=the closest relatives of the *A. hirsuta* aggregate, blue = *A. watsonii*. Clade names follow Karl and Koch (2013).

criteria) of the IUCN (2016), *A. watsonii* is assessed here as “Vulnerable” (VU) (B2a, C2a(i)), because distribution area of the species is severely fragmented and the species is currently known from no more than ten localities occupying less than 2,000 km², (although it was considered “Endangered” (EN) according to Ekim et al. (2000)).

The basic climatic requirements of *A. watsonii*, annual main temperature and annual precipitation were calculated as 5.7 °C and 583 ml respectively.

Phylogeny. The aligned ITS and trnL-F data matrices included 91 species. The ITS data set was 642 bp, of which 236 were variable and 168 parsimony informative, whereas the trnL-F data set incorporates 855 bp, of which 181 were variable and 108 parsimony informative.

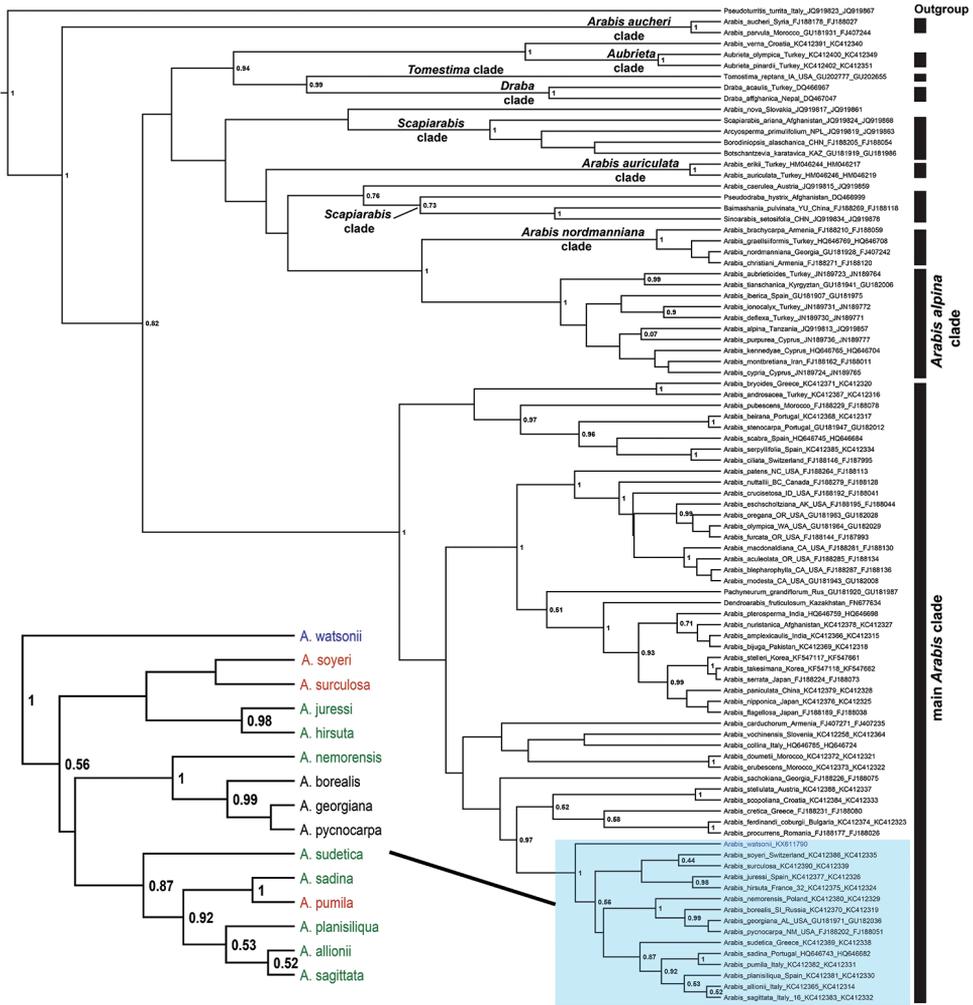


Figure 4. trnL-F-based phylogenetic backbone of Arabideae that focuses on the placement of *Arabis watsonii*. Shown is the Bayesian maximum clade credibility tree with posterior probability values > 0.5. Highlighted part of the tree, (magnified on the left), is the *Arabis hirsuta* aggregate and its relatives. Color codes: Green = *A. hirsuta* aggregate, red=the closest relatives of the *A. hirsuta* aggregate, blue = *A. watsonii*. Clade names follow Karl and Koch (2013).

The query of ITS sequences of *A. watsonii* in BrassiBase (version 1.1) supported its phylogenetic placement within tribe Arabideae and clearly matching *Arabis*. The outcome of Bayesian phylogenetic analyses using ITS and trnL-F data sets (Figs 3, 4) were congruent with each other in regard to the placement of *A. watsonii*. In both analyses *A. watsonii* falls into the main *Arabis* clade as sister to *A. hirsuta* (L.) Scop. aggregate and its relatives outlined in Karl and Koch (2014). Whereas *A. watsonii* forms a monophyletic lineage with *A. cretica* (Bayesian posterior probability (pp) = 0.98) in

ITS analysis (Fig. 3), this sister relationship was not supported by trnL-F analysis (Fig. 4) and chloroplast data shows that *A. watsonii*, the *A. hirsuta* aggregate, its relatives plus non-European *Arabis* species are linked to this aggregate (including *A. georgiana* R.M.Harper, *A. pycnocarpa* M.Hopkins and *A. borealis* DC.) forming a monophyletic clade (pp=1.00).

Discussion

In this study, we used evidence from nuclear ITS and plastidic trnL-F sequences to determine the phylogenetic and taxonomic position of the overlooked Anatolian endemic *A. watsonii*. In addition, morphological and climatic data were used to better understand ecological and evolutionary relationships of *A. watsonii* with representatives of the well-defined *A. hirsuta* aggregate and its relatives.

The differences in the phylogenetic placement of *A. watsonii* in relation to its sister position to *A. cretica*, according to ITS and trnL-F analyses, indicates possible chromosome capture /ancient hybridization. These processes are well known in the *A. hirsuta* aggregate and its relatives (Koch et al. 2010, Karl and Koch 2014).

A recent study of *A. hirsuta* aggregate recognised eight European species including *A. hirsuta* (L.) Scop., *A. sagittata* (Bertol.) DC., *A. planisiliqua* (Pers.) Rchb., *A. nemorensis* (Wolf ex Hoffm.) W.D.J. Koch, *A. allionii* DC., *A. sudetica* Tausch, *A. sadina* (Samp.) Coutinho, and *A. jurensis* Rothm. The historical definition and circumscription of such an aggregate depends on different authors (see Karl and Koch (2014)) and because relationships between the *A. hirsuta* aggregate and its European/non-European relatives have already been discussed in detail before, we will not repeat this discussion here.

As indicated above, the ITS phylogeny supports a clear monophyly between *A. watsonii* and Greece endemic *A. cretica* Boiss. & Heldr. Whereas this relationship does not supported by trnL-F, both species seems to be at a basal position for *A. hirsuta* aggregate and its relatives. This results is somewhat expected because the Western Irano-Turanian and the East Mediterranean regions have already been suggested as diversity centres for almost all Arabideae clades (Jordon-Thaden et al. 2010, Karl and Koch 2013).

Morphologically *A. watsonii* is similar to *A. hirsuta* and *A. sagittata*, therefore specimens were treated under these names in some herbaria. However *A. watsonii* differs from *A. hirsuta* and *A. sagittata* in having glabrous stems and relatively large petals. Meyer (2006) argued that *A. watsonii* is related to *A. abietina* Bornm. from the Ilgaz Mountain (Turkey) since this species is also characterized by glabrous stems and a similar petal length. The latter species has been treated as a synonym of *A. suedica* in Karl and Koch (2014) based on Jalas and Souminen (1994). Despite the relatively large geographic gap between *A. abietina* and *A. suedica*, ITS sequences of the taxa were identical and trnL-F sequences differ only in one single nucleotide position. Thus, *A. abietina* was not included in the current study, although it is treated as a valid species according to the Flora of Turkey (Cullen 1965) and the actual check-list (Güner et al. 2012). Apart from geographic isolation, branched trichomes on leaf margins and rela-

tively small fruits are the main diagnostic characters distinguishing *A. watsonii* from both *A. abietina* and *A. allionii*, which are also members of the *A. hirsuta* aggregate. In summary, more comprehensive studies are needed to clarify the validity of *A. abietina*. With the proper assignment of *A. watsonii* in the current study, the total number of the Turkish *Arabis* species increased from 22 to 23 (25 taxa)

Finally, distribution in the alpine zone and perennial life cycle of *A. watsonii* is concordant with general trends of the tribe Arabideae (Karl and Koch 2013). Estimated climatic conditions for *A. watsonii*, including annual mean temperature, annual precipitation and other bioclimatic variable (not provided here) reflect a continental climate also described for other members of the *A. hirsuta* aggregate including *A. sudei-ca*, *A. hirsuta* etc. In conclusion, all environmental parameters and life cycle strategies of *A. watsonii* are in agreement with the genetic affiliation to the *A. hirsuta* aggregate and its relatives within the main *Arabis* clade.

Acknowledgement

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (Projects no. 014D01601015-489). We thank Çağaçan Karacaoğlu for helping us to use DIVAGIS and İsmail Kudret Sağlam for correcting style and grammar.

References

- Al-Shehbaz IA (1988) The genera of Arabideae (Cruciferae; Brassicaceae) in the south eastern United States. *Journal of the Arnold Arboretum* 69: 85–166. doi: 10.5962/bhl.part.2391
- Al-Shehbaz IA (2005) Nomenclatural notes on Eurasian *Arabis* (Brassicaceae). *Novon* 15: 519–524.
- Al-Shehbaz IA, Mutlu B, Dönmez A (2007) The Brassicaceae of Turkey, updated. *Turkish Journal of Botany* 31: 327–336.
- Al-Shehbaz IA, German DA, Karl R, Jordon-Thaden I, Koch MA (2011) Nomenclatural adjustments in the tribe Arabideae (Brassicaceae). *Plant Diversity and Evolution* 129(1): 71–76. doi: 10.1127/1869-6155/2011/0129-0044
- Al-Shehbaz IA (2012) A generic and tribal synopsis of the Brassicaceae (Cruciferae). *Taxon* 61: 931–954.
- Ansell SW, Schneider H, Pedersen N, Grundmann M, Russell SJ, Vogel JC (2007) Recombination diversifies chloroplast *trnF* pseudogenes in *Arabidopsis lyrata*. *Journal of Evolutionary Biology* 29: 2400–2411. doi: 10.1111/j.1420-9101.2007.01397.x
- Cullen J (1965) *Arabis* L. In: Davis PH (Ed.) *Flora of Turkey and the East Aegean Islands* (Vol. 1). Edinburgh University Press, Edinburgh, 422–429.
- Davis PH, Mill RR, Tan K (1988) *Arabis* L. In: Davis PH, Mill RR, Tan K (Eds) *Flora of Turkey and the East Aegean Islands* (Suppl. 1). Vol. 10, Edinburgh University Press, Edinburgh, 49.

- Daşkın R (2013) *Arabis kaynakiae* (Brassicaceae), a new species from south Anatolia, Turkey. *Phytotaxa* 126: 43–48. doi: 10.11646/phytotaxa.126.1.5
- Duman H (2001) A new species of *Arabis* L. (Brassicaceae) from south Anatolia. *Botanical Journal of Linnean Society* 137: 87–90. doi: 10.1111/j.1095-8339.2001.tb01109.x
- Duman H, Duran A (2001) A new species of *Arabis* L. (Brassicaceae) from south Anatolia. *Israel Journal of Plant Science* 49: 237–240.
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973. doi: 10.1093/molbev/mss075
- Güner A, Aslan S, Ekim T, Vural M, Babaç MT (2012) Türkiye Bitkileri Listesi (Damarlı Bitkiler). *Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayını*, İstanbul.
- Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adigüzel N (2000) *Red Data Book of Turkish Plants (Pteridophyta and Spermatophyta)*. Turkish Association for the Conservation of Nature and Natural Resources, Ankara.
- Fırat M (2013) *Ferhenga Navên Riwekên Bi Kurdî/Kürtçe Bitki Adları Sözlüğü/ Dictionary of Plant Names in Kurdish*, Kalkan Ofset, Ankara, 652 pp.
- Fırat M (2016) *Kemulariella tahirelcii* (Asteraceae; Astereae), a new species from Şırnak, Turkey. *Phytotaxa* 253(1): 90–96. doi: 10.11646/phytotaxa.253.1.7
- IUCN (2016) Standards and Petitions Subcommittee. Guidelines for Using the IUCN Red List Categories and Criteria. Version 12. Prepared by the Standards and Petitions Subcommittee.
- Jalas J, Souminen J (1994) *Atlas Florae europaeae*, vol. 10. The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki, Finland, 187–211.
- Jordon-Thaden IE, Hase I, Al-Shehbaz IA, Koch M (2010) Molecular phylogeny and systematics of the genus *Draba* s.l (Brassicaceae) and identification of its closest related genera. *Molecular Phylogenetics and Evolution* 55: 524–540. doi: 10.1016/j.ympev.2010.02.012
- Karl R, Kiefer C, Ansell S, Koch MA (2012) Systematics and evolution of arctic-alpine *Arabis alpina* L. (Brassicaceae) and its closest relatives in the eastern Mediterranean. *American Journal of Botany* 99: 778–794. doi: 10.3732/ajb.1100447
- Karl R, Koch MA (2013) A world-wide perspective on crucifer speciation and evolution: Phylogenetics, biogeography and trait evolution in tribe Arabideae. *Annals of Botany (Oxford)* 112: 983–1001. doi: 10.1093/aob/mct165
- Karl R, Koch MA (2014) Phylogenetic signatures of adaptation: the *Arabis hirsuta* species aggregate (Brassicaceae) revisited. *Perspectives in Plant Ecology Evolution and Systematics* 16: 247–264. doi: 10.1016/j.ppees.2014.06.001
- Kiefer M, Schmickl R, German D, Lysak M, Al-Shehbaz IA, Franzke A, Mummenhoff K, Stamatakis A, Koch MA (2014) BrassiBase: introduction to a novel knowledge database on Brassicaceae evolution. *Plant Cell Physiology* 55: e3. doi: 10.1093/pcp/pct158
- Koch M, Bishop J, Mitchell-Olds T (1999) Molecular systematics and evolution of *Arabidopsis* and *Arabis*. *Plant Biology* 1: 529–537. doi: 10.1111/j.1438-8677.1999.tb00779.x
- Koch M, Haubold B, Mitchell-Olds T (2000) Comparative analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis* and related genera (Brassicaceae). *Molecular Biology and Evolution* 17: 1483–1498. doi: 10.1093/oxfordjournals.molbev.a026248

- Koch M, Haubold B, Mitchell-Olds T (2001) Molecular systematics of the Cruciferae: Evidence from coding plastome matK and nuclear Chs sequences. *American Journal of Botany* 88: 534–544. doi: 10.2307/2657117
- Koch MA, Karl R, Kiefer C, Al-Shehbaz IA (2010) Colonizing the American continent: systematics of the genus *Arabis* in North America (Brassicaceae). *American Journal of Botany* 97: 1040–1057. doi: 10.3732/ajb.0900366
- Koch MA, Kiefer M, German D, Al-Shehbaz IA, Franzke A, Mummenhoff M, Schmickl R (2012) BrassiBase: tools and biological resources to study characters and traits in the Brassicaceae – version 1.1. *Taxon* 61: 1001–1009.
- Meyer FK (2006) Kritische revision der “*Thlaspi*”-Arten Europas, Afrikas und Vorderasiens, Spezieller Tiel, VII. *Callothlaspi* F.K. Mey. *Haussknechtia* 11: 175–194.
- Mutlu B (2004) A new species of *Arabis* L. (Brassicaceae) from inner Anatolia, *Botanical Journal of Linnean Society* 145: 251–256. doi: 10.1111/j.1095-8339.2004.00275.x
- Mutlu B, Dönmez AA (2003) *Arabis mollis* Steven (Brassicaceae): A new record for Turkey. *Turkish Journal of Botany* 27: 235–238.
- Mutlu B, Erik S (2012) The Taxonomical Position of *Arabis graellsiiiformis* Hedge (Brassicaceae) that it’s known as an endemic species of Turkey. *Hacettepe Journal of Biology and Chemistry* 40: 69–74.
- Mutlu B, Erik S (2015) Distribution maps and New IUCN threat categories for the genus of *Arabis*, *Pseudoturritis* and *Turritis* (Brassicaceae) in Turkey. *Hacettepe Journal of Biology and Chemistry* 43(2): 133–143.
- Özhatay N, Kültür Ş, Aslan S (2009) Check list of additional taxa to the supplement flora of Turkey 4. *Turkish Journal of Botany* 33: 191–226. doi: 10.3906/bot-1101-20
- Özhatay N, Kültür Ş, Gürdal MB (2011) Check-list of additional taxa to the supplement Flora of Turkey 5. *Turkish Journal of Botany* 35: 589–624. doi: 10.3906/bot-1101-20
- Parolly G, Hein P (2000) *Arabis lycia* (Cruciferae), a new chasmophyte from Taurus Mts, Turkey, and notes on related species. *Willdenowia* 30: 293–304. doi: 10.3372/wi.30.30208
- 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. doi: 10.1007/BF00037152
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. doi: 10.1093/molbev/mst197
- Warwick SI, Al-Shehbaz IA, Sauder CA, Murray DF, Mummenhoff K (2004) Phylogeny of *Smelowskia* and related genera (Brassicaceae) based on nuclear ITS DNA and chloroplast *trnL* intron DNA sequences. *Annals of Missouri Botanical Garden* 91: 99–23.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) *PCR protocols: A guide to methods and applications*. Academic Press, New York, 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1

František Nábělek's *Iter Turcico-Persicum* 1909–1910 – database and digitized herbarium collection

Matuš Kempa¹, John Edmondson², Hans Walter Lack³,
Janka Smatanová¹, Karol Marhold^{1,4}

1 Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 23 Bratislava, Slovak Republic

2 Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, U.K. **3** Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Strasse 6-8, D-14195 Berlin, Germany

4 Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Praha 2, Czech Republic

Corresponding author: *Matuš Kempa* (matus.kempa@savba.sk)

Academic editor: S. Knapp | Received 7 July 2016 | Accepted 21 November 2016 | Published 1 December 2016

Citation: Kempa M, Edmondson J, Lack HW, Smatanová J, Marhold K (2016) František Nábělek's *Iter Turcico-Persicum* 1909–1910 – database and digitized herbarium collection. *PhytoKeys* 75: 69–79. doi: 10.3897/phytokeys.75.9780

Abstract

The Czech botanist František Nábělek (1884–1965) explored the Middle East in 1909–1910, visiting what are now Israel, Palestine, Jordan, Syria, Lebanon, Iraq, Bahrain, Iran and Turkey. He described four new genera, 78 species, 69 varieties and 38 forms of vascular plants, most of these in his work *Iter Turcico-Persicum* (1923–1929). The main herbarium collection of *Iter Turcico-Persicum* comprises 4163 collection numbers (some with duplicates), altogether 6465 specimens. It is currently deposited in the herbarium SAV. In addition, some fragments and duplicates are found in B, E, W and WU. The whole collection at SAV was recently digitized and both images and metadata are available via web portal www.nabelek.sav.sk, and through JSTOR Global Plants and the Biological Collection Access Service. Most localities were georeferenced and the web portal provides a mapping facility. Annotation of specimens is available via the AnnoSys facility. For each specimen a CETAF stable identifier is provided enabling the correct reference to the image and metadata.

Keywords

Database, Herbarium, Near East, František Nábělek, Vascular Plants

Introduction

František Nábělek was born on 3 May 1884 in Kroměříž. From 1902 to 1907 he studied at Vienna University (Fig. 1, for further biographic details on František Nábělek see Table 1). With the financial support of the Imperial Royal Ministry of Culture and Education, the Imperial Academy of Sciences (both Vienna), the Svatobor Foundation (Prague) and the archbishop of Olomouc František Saleský Bauer in 1909–1910 Nábělek visited the region comprising the current Israel, Palestine, Jordan, Syria, Lebanon, Iraq, Bahrain, Iran and Turkey (Fig. 2). His plant collecting activities were among the most productive to have taken place in this region in the first decade of the 20th century, and his subsequent publications (*Iter Turcico-Persicum*, Partes I–V, Nábělek 1923–1929, Nábělek 1922, 1924) included descriptions of four new genera, 78 species, 69 varieties and 38 forms of vascular plants. When working on plant identifications and descriptions of new taxa he extensively consulted other herbarium collections, particularly those of Pierre Edmond Boissier and Augustin Pyramus de Candolle in Geneva, Switzerland (G) and of Herbarium Haussknecht at that time held in

Table 1. Biographic data of František Nábělek (more details in Hrabětová 1954, 1964, Hrabětová-Uhrová 1964, Májovský 1966, 1967, Michalko 1966, Stafleu and Cowan 1981: 678–679, Marhold and Feráková 1993, Vacek and Bureš 2001, and Hrabovec 2010).

3 May 1884	Born (http://actapublica.eu/matriky/brno/prohlizec/8997/?strana=129) in Kroměříž, at that time in Austro-Hungarian Empire, now the Czech Republic
1902–1907	Studies at Vienna University under Professor Richard von Wettstein, who held a post at the University of Prague prior to being appointed as Director of the Botanical Gardens and Botanical Institute of the University of Vienna, now Austria
1907–1921	Secondary school (gymnasium) teacher in Lipník nad Bečvou, now Czech Republic
1909–1910	<i>Iter Turcico-Persicum</i> (for details see in the Introduction)
1921–1939	Secondary school (gymnasium) teacher in Brno, now Czech Republic
1925–1934	Private Associated Professor of Botany at the Masaryk University in Brno (now Czech Republic)
1934–1939	Extraordinary Professor of Botany at the Masaryk University in Brno (now Czech Republic)
1939	Lecturer at the Faculty of Philosophy of the Slovak University (before 1939 and after 1954 Comenius University), Bratislava, now Slovak Republic
1940	Professor of Botany and a chair at the Institute of Botany of the newly established Faculty of Science of the Slovak University, Bratislava, now Slovak Republic
1942–1950	Director of the University Botanical Garden of the Slovak University, Bratislava, now Slovak Republic
1945–1947	Dean of the Faculty of Science of the Slovak University, Bratislava, now Slovak Republic
1947–1948	Rector of the Slovak University, Bratislava, now Slovak Republic
1950	Forced retirement from the Slovak University, Bratislava, now Slovak Republic
1950–1953	Institute of Food Industry, Bratislava, now Slovak Republic
1953–1960	Researcher at Arboretum in Mlyňany, now Slovak Republic. The Arboretum Mlyňany (Benčať et al., 1956), was originally a private estate established in 1892 by Štefan (István) Ambrózy-Migazzi (1869, Nice, France – 1933, Tana, now Tanakajd, Hungary), later becoming a state-funded scientific institute of the Slovak University, before its final transfer to the Slovak Academy of Sciences in 1953.
10 June 1965	Died in Uherské Hradiště (now Czech Republic), buried in Bratislava at Slávičie údolie Cemetery



Figure 1. František Nábělek during his travels in 1909–1910 (photo courtesy of family Nábělek).

Weimar, Grand Duchy of Saxe-Weimar-Eisenach, German Empire (now deposited in JE). He was also in contact with numerous specialists working on the flora of the Near East (such as Joseph Bornmüller, Weimar and Heinrich Handel-Mazzetti, Vienna). On the study of material of the genus *Onobrychis* Mill. (Fabaceae) he cooperated with

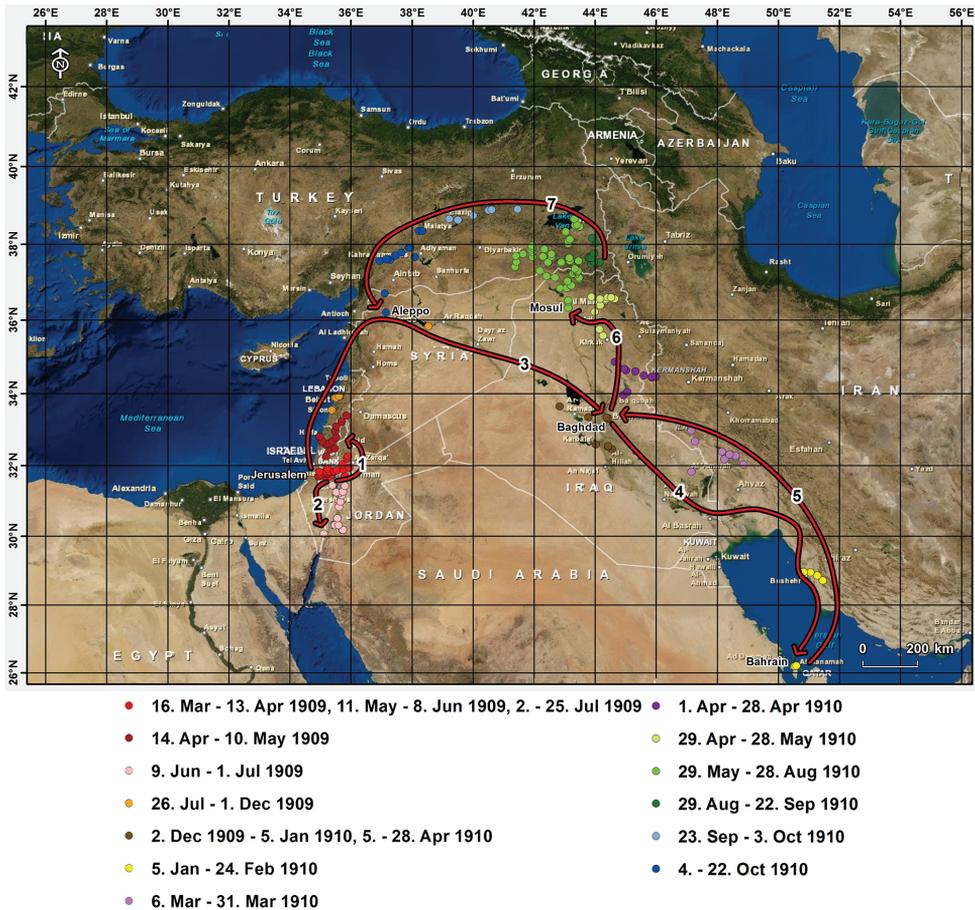


Figure 2. Schematic presentation of Nábělek's Iter Turcico-Persicum.

Grigorij Ivanovič Širjaev of Masaryk University, Brno. There is extensive correspondence he sent to his family during his 1909-1910 journey with a number of details on his travels (in Czech) in the archives of the Institute of Botany, Slovak Academy of Sciences, Bratislava. All these letters have been recently digitized. Some notes relevant to his travels are also kept at the Central Archive of the Slovak Academy of Sciences. They are, however, not easy to read as they are mostly written in stenographic (shorthand) writing. After holding the position at the Masaryk University in Brno (now Czech Republic) in 1939 Nábělek moved to Bratislava (now in the Slovak Republic), where he organized the Institute of Botany at the newly established Faculty of Science. Since 1950, as Czechoslovakia had fallen under the Soviet sphere of influence, he was for political reasons no longer *persona grata* at the University and in order not to have any influence on students, he was forced to retire. Nevertheless, later he continued to work in the Arboretum in Mlyňany, where he established a herbarium collection of mostly cultivated exotic trees (MLY, in 2006 transferred to SAV as SAV-MLY) and prepared a complete botanical inventory of the Arboretum (Nábělek 1958). František Nábělek

died on 10 June 1965 in Uherské Hradiště (now Czech Republic). His successor at Comenius University in Bratislava, Jozef Májovský, characterized him as "... an excellent teacher, organiser, musician and a good man. The Slovakian botanists will forever be grateful to him ..." (Májovský 1966). More detailed biographical data on František Nábělek can be found in Hrabětová (1954, 1966), Hrabětová-Uhrová (1964), Májovský (1966, 1967), Michalko (1966), Stafleu and Cowan (1981: 678–679), Marhold and Feráková (1993) and Hrabovec (2010).

Here we present detailed data on Nábělek's *Iter Turcico-Persicum*, together with an on-line database with digitized and georeferenced specimens and their metadata.

The itinerary

The primary source of information for the itinerary of *Iter Turcico-Persicum* is Nábělek's own published itinerary (Nábělek 1923: 3–12). However, due to the considerable problems of recording and transliterating place-names from a plethora of languages, further complicated by the "ethnic cleansing" of indigenous place-names in certain areas and their replacement by made-up modern names in the national languages, botanists wishing to trace the localities visited by Nábělek and determine their location and modern equivalents have encountered considerable obstacles. For a detailed discussion of these problems, the reader is referred to a useful publication of the United States Board on Geographic Names – Foreign Names Committee (2015). Although this report covers Iran, it does embrace most of the local languages encountered by Nábělek with the possible exception of Armenian. A further source for ethnic minority place names for localities in Turkey is represented by *Index Anatolicus* (Nişanyan 2014).

The primary method adopted in tracing localities has been to "fly" Nábělek's route using GoogleEarth, first pinpointing known locations and then examining possible routes to see whether intermediate place-names could be matched with the spellings adopted by Nábělek. In the case of certain mountains, altitudes cited by Nábělek have provided further evidence.

In many instances, botanists citing specimens collected by Nábělek have already determined the modern equivalents of his collecting sites, and this information has been incorporated into our gazetteer. For example, on 17 June 1910 he visited Hašitha in southernmost Turkey; this is a Kurdish place-name (now written Aşutka) which on modern maps has been replaced with the Turkish name Çiğli. A Persian locality, Nábělek's Chôšab (now written Hoşap) which he visited on 29 August 1910 has been re-named Güzelsu in Turkish (which signifies 'beautiful water' in both languages).

In the earlier part of his travels, Nábělek seems to have chosen a route that would allow him to visit famous (e.g., Petra) and not-so-famous (e.g., Ain ez-Zarra, the ancient Callirhoe hot springs on the eastern bank of the Dead Sea) historic sites. Later, when travelling in northern Iraq, he seems to have sought out Nestorian Christian monasteries. Because those establishments were named using ancient languages such as Syriac, they are often unrecognisable on modern maps.

His journey was interrupted by two lengthy spells of illness, the first from August to November 1909 in Damascus and Beirut (recuperating in the hilly suburb of Brumana) and the second in August 1910 which he spent in the eastern Anatolian town of Van. Despite these setbacks he managed to explore large areas of mountainous terrain in northeastern Iraq and the remotest parts of southeastern Turkey, areas previously only poorly explored and with a high level of floristic endemism, providing him with a rich haul of new species.

During his travels Nábělek must have encountered some interesting companions. Perhaps the most famous of these was the German Vice-Consul in Bushehr, Wilhelm Wassmuss, “the German Lawrence” (von Mikusch 1937 [other references at https://de.wikipedia.org/wiki/Wilhelm_Wassmuss]), with whom he ascended “Kuh Chormûğ” (Kuh-e Khormuj, c. 1870 m), a prominent hill in Iranian Khuzestan. This expedition took six days, suggesting that it was achieved on foot. Wassmuss was only four years older than Nábělek, who had celebrated his 26th birthday three months after the start of the excursion on 15 February, and since Wassmuss returned to Iraq later that same year it was highly fortuitous that they met during this brief interval in Iran. Later in his travels, on 10 March 1910, Nábělek visited Shushtar, a town only some 40 km from Masjed Soleiman, where oil had first been discovered just a year earlier.

Collection

The main herbarium collection of František Nábělek’s *Iter Turcico-Persicum* currently comprises 4163 collection numbers; altogether 6465 specimens (including duplicates). Only a few specimens have been lost during the many transfers between herbaria and loans in the past. The collection was first deposited in the herbarium of the Masaryk University in Brno (currently Czech Republic, BRNU) at the time of Nábělek’s professorship at this University. Then it was moved to University in Bratislava, Slovakia (currently herbarium SLO). After Nábělek left the Slovak (now Comenius) University, the herbarium was first kept in Arboretum Mlyňany, Slovakia (MLY), then at the Institute of Botany of the Slovak Academy of Sciences, Bratislava (acronym originally BAV, now SAV) and for some time also in the Slovak National Museum in Bratislava (BRA). The final place of deposit of this collection is the herbarium SAV. As a result, Nábělek’s type specimens are often referred to in the literature as deposited in BRNU (e.g., Stafleu and Cowan 1981), BRA, SLO or BAV, although they are now all held in SAV. Only some fragments and duplicates are found in B, E, W and WU. Indeed, in some cases they represent original material and may serve as types.

Digitization

With the financial support of the Andrew W. Mellon Foundation, the whole of Nábělek’s *Iter Turcico-Persicum* collection at SAV was recently digitized. The process

of digitization was performed in compliance with the JSTOR Plant Science Handbook (<http://about.jstor.org/content/jstor-plant-science-handbook-english>). All specimens were barcoded with a unique barcode within the institution before the scanning of the herbarium sheets. The barcode consists of letters 'SAV' followed by 7 digits. An Epson Expression Model 10000XL scanner was used for scanning, which was placed on a custom metal frame manufactured by HerbScan Engineering (London, U.K.). A scanned image is required to have, in addition to a barcode, a scale with the herbarium abbreviation and standardized colour chart placed visibly in the area of the sheet. Each specimen was digitized according to the following specifications: resolution: 600 pixels per inch (ppi); colour space: Adobe RGB (1998); colour depth: 24-bit; file format: uncompressed TIFF files; layout: portrait. Data about the specimens have been stored into the DATAflos (<http://dataflos.sav.sk>) database via internal software developed to manage Nábělek's database. The stored data include specimen name, barcode, type status, collectors, collection number, locality description, locality altitude, and name revision history.

Web portal

Digitized images of Nábělek's *Iter Turcico-Persicum* collections at SAV are available via JSTOR Global Plants (<https://plants.jstor.org/>) and Biological Collection Access Service (<http://www.biocase.org/>). Nevertheless, in order to enhance the use of the digitized collection, we created a special web portal that not only presents the specimens, but also digitized publications by Nábělek, and last, but not least, enables annotations of specimens (the portal is available at www.nabelek.sav.sk).

The metadata on specimens are stored in a PostgreSQL (<https://www.postgresql.org>) database. The portal is a web application created in PHP (<http://php.net>) scripting language. It utilizes CakePHP 2.6.5 framework (<http://api.cakephp.org/2.6/>) to manage database connection, requests, their correct execution, and presentation of the results. It runs on Apache HTTP server version 2.4.7 (<http://httpd.apache.org/docs/2.4/>) on Ubuntu 14.04 (<http://releases.ubuntu.com/14.04/>). The presentation part uses HTML5 (<https://www.w3.org/TR/html5/>), CSS (<https://www.w3.org/Style/CSS/>), JavaScript's jQuery library (<http://jquery.com/>), design and mobile friendliness is provided by the Bootstrap framework (<http://getbootstrap.com/>).

To search for a record, the user is presented with a quick search field offering a few choices to aim the search term - taxon name, taxon authors, collection number, specimen barcode, or any text (e.g. a word contained in locality description). In addition, a link 'Search for specimens' in the main menu leads to a page with search fields for the genus, species (both original determination and revisions), collection number, locality, and geographic coordinates with the possibility of specifying a range.

The detail page of a record displays all available information from the database, images associated with the record, and buttons for viewing or downloading the image. Detailed information on specimens includes the name of the plant according to the most recent identification, barcode number, collector name (mostly F. Nábělek), collection

number of Iter Turcico-Persicum, herbarium in which specimen is deposited (SAV in all cases here, but in the future and with permission duplicates deposited in other herbaria will be added). In addition to this information, a CETAF stable identifier for each specimen (2016) [HTTP-URI-based persistent and stable identifiers for physical collection objects, <http://cetaf.org/cetaf-stable-identifiers>; Güntsch and Hagedorn 2013] is presented, to enable subsequent correct reference to the image or data. Locality data includes classification into the Level 1–4 of the World Geographical Scheme for Recording Plant Distributions (Brummitt 2001), and the verbatim detailed description as given on the specimen and in Nábělek's publications. This information is mostly identical on labels and in publications. Locality data was completed by adding geographical coordinates. The revision history of the specimen includes original identification as published in Nábělek's papers and subsequent revisions as found on annotation labels on specimens.

The detail page of a record includes also an interactive image viewer that allows user to zoom-in, zoom-out, or view image in its full resolution without needing to download the whole image. It is provided by IIPImage (About. <http://iipimage.sourceforge.net>). Providing the record has geographic coordinates, the locality is also shown on the minimap included on the detail page of each record. A possibility to post an annotation or updated determination to a record is provided to users. The process of annotation is managed by the AnnoSys facility (<https://annosys.bgbm.fu-berlin.de/>; Tschöpe et al. 2013).

The page “Map of all localities” presents a Google Map populated with all localities where the specimens were collected for which we have exact geographical coordinates. Each locality can be a place of collection of several specimens. Clicking the marker show the list of records. Overall, there are 2454 records that have been georeferenced.

On the page “Nábělek's papers” preliminary digital versions of Nábělek's papers are available for download. As soon as final digital copies are available in the Biodiversity Heritage Library (BHL; where their digitisation is currently ongoing), they will be replaced by the links to the material in BHL.

It is possible for visitor to comment on functionality and to suggest improvements via the project's bugzilla page at Bugzilla: Nabelek herbarium (<http://dataflos.sav.sk:8080/bugzilla/describecomponents.cgi?product=Nabelek%20herbarium>).

The Nábělek website will be improved by introducing additional functionalities in future. All capabilities of the web interface as described here are present and functional in its version 1.0.

Importance of the collection for local floras

Local and regional floras benefited from Nábělek's collections, because at the time of his expedition there were no up-to-date accounts of the flora of the regions he visited. Boissier's *Flora Orientalis* was already becoming out of date, having been published some 35 years earlier, and there were no contemporary Floras of Turkey, Palestine, Iraq or Iran. Moreover, the large number of new species that he had discovered, especially from the border areas of Iraq, Iran and Turkey, meant that his catalogue remained of

major importance to workers in the region for the next fifty or so years. In particular, Peter H. Davis's *Flora of Turkey and the East Aegean Islands* (Davis 1965–1985, Davis et al. 1988) was heavily dependent on Nábělek's records from C9 Hakkari, as Peter Davis himself was the only other collector to have explored the flora of the Cilo Dağ region. Following Nábělek's visit, the next significant botanical work in the area was carried out during the peregrination of Russian and Soviet botanists into the easternmost provinces of the former Ottoman Empire. Some of their records were mapped in Grossheim's *Flora Kavkaza* (1928–1934, 1939–1967).

Acknowledgements

The authors and the Institute of Botany Slovak Academy of Sciences acknowledge the generous support of the Andrew W. Mellon Foundation towards digitization of the F. Nábělek's herbarium collection from the Iter Turcico-Persicum. John Edmondson wishes to acknowledge the support of the Linnean Society of London's Appleyard Fund for his work on the *Flora of Iraq* project.

References

- AnnoSys (2016) About the Project. <https://annosys.bgbm.fu-berlin.de/> [accessed 30.6.2016]
- Apache (2016) HTTP: Apache HTTP Server Version 2.4 Documentation. <http://httpd.apache.org/docs/2.4/> [accessed 30.6.2016]
- Benčať F, Nábělek F, Steihübel G (1956) Arboretum Mlyňany vždzyelený park. Osveta, Martin, 70 pp.
- Biological Collection Access Service (2016) Biological Collection Access Service. <http://www.biocase.org/> [accessed 30.6.2016]
- Bootstrap (2016) Bootstrap. <http://getbootstrap.com/> [accessed 30.6.2016]
- Brummitt RK (2001) World geographical scheme for recording plant distributions (2nd edn). Hunt Institute for Botanical Documentation Carnegie Mellon University, Pittsburgh, 137 pp. <https://github.com/tdwg/prior-standards/tree/master/world-geographical-scheme-for-recording-plant-distributions/109-488-1-ED/2nd%20Edition>
- Bugzilla (2016) Bugzilla: Nabelek herbarium. <http://dataflos.sav.sk:8080/bugzilla/describe-components.cgi?product=Nabelek%20herbarium> [accessed 30.6.2016]
- CakePHP (2016) CakePHP 2.6 API. <http://api.cakephp.org/2.6/> [accessed 30.6.2016]
- CETAF (2016) CETAF Stable Identifiers. <http://cetaf.org/cetaf-stable-identifiers> [accessed 30.6.2016]
- CSS (2016) W3C Cascading Style Sheets home page. <https://www.w3.org/Style/CSS/> [accessed 30.6.2016]
- DATAflos (2016) Databáza flóry Slovenska. <http://dataflos.sav.sk> [accessed 30.6.2016]
- Davis PH (Ed.) (1965–1985) *Flora of Turkey and the East Aegean Islands*, vols 1–9. University Press, Edinburgh.

- Davis PH, Mill RR, Kit Tan (Eds) (1988) *Flora of Turkey and the East Aegean Islands* (Supplement), vol 10. University Press, Edinburgh.
- Grossheim AA (1928–1934) *Flora Kavkaza* (vols 1–4). Nar. komissariat zemledeliya SSR Armenii, Tiflis.
- Grossheim AA (1939–1967) *Flora Kavkaza* (vols 1–6). Akademiya Nauk SSSR, Azerbaidzhansii filial, Baku.
- Güntsch A, Hagedorn G (2013) Stable identifiers for specimens – A CETAF ISTC initiative supported by pro-iBiosphere. http://www.pro-ibiosphere.eu/news/4296_stable-identifiers-for-specimens-a-cetaf-istc-initiative-supported-by-pro-ibiosphere [accessed 1 July 2016]
- Hrabětová A (1954) PhDr. František Nábělek, sedmdesátník. *Preslia* 26: 329–330.
- Hrabětová A (1966) František Nábělek (1884–1965). *Preslia* 38: 218.
- Hrabětová-Uhrová A (1964) PhDr. František Nábělek – 80 let. *Preslia* 36: 311–312.
- Hrabovec I (2010) Nábělek František. In: Vozárová M, Šípošová H (Eds) *Osobnosti botaniky na Slovensku*. VEDA, vydavateľstvo SAV, Bratislava, 364–366.
- HTML5 (2016) A vocabulary and associated APIs for HTML and XHTML, W3C Recommendation 28 October 2014. <https://www.w3.org/TR/html5/> [accessed 30.6.2016]
- IIPImage (2016) About. <http://iipimage.sourceforge.net> [accessed 30.6.2016]
- jQuery (2016) jQuery. <http://jquery.com/> [accessed 30.6.2016]
- JSTOR (2016) Global Plants. <https://plants.jstor.org/> [accessed 30.6.2016]
- JSTOR (2016) Plant Science Handbook – English. <http://about.jstor.org/content/jstor-plant-science-handbook-english> [accessed 30.6.2016]
- Májovský J (1966) F. Nabelek (1884–1965). *Taxon* 15: 82.
- Májovský J (1967) Univ. Prof. Dr. phil. František Nábělek (1884–1965). *Acta Facultatis Rerum Naturalium Universitatis Comenianae, Botanica* 14: 3–5.
- Marhold K, Feráková V (1993) Brief history of Slovak herbaria. *Webbia* 48: 247–254.
- Michalko J (1966) Spomenka na Univ. Prof. Dr. Františka Nábělka. *Biológia, Bratislava* 21: 387–388.
- Mikusch von D (1937) *Waßmuß, der deutsche Lawrence. Auf Grund der Tagebücher und Aufzeichnungen des verstorbenen Konsuls, deutscher und englischer Quellen und des unter gleichem Titel erschienenen Buches von Christopher Sykes*. Paul List Verlag, Leipzig, 1–350. doi: 10.2307/1569237
- Nábělek F (1922) *Papaver curviscapum* sp. nova orientalis e sectione *Miltantha* Bernh. *Acta Botanica Bohemica* 1: 56–57.
- Nábělek F (1923) *Iter Turcico-Persicum, Pars I, Plantarum collectarum enumeratio (Ranulaceae-Dipsacaceae)*. *Spisy Vydávané Přírodovědeckou Fakultou Masarykovy University* 35: 1–144.
- Nábělek F (1924) *Cruciferarum Orientalium genus novum*. *Acta Botanica Bohemica* 3: 32–34.
- Nábělek F (1925) *Iter Turcico-Persicum, Pars II, Plantarum collectarum enumeratio (Compositae)*. *Spisy Vydávané Přírodovědeckou Fakultou Masarykovy University* 52: 1–58.
- Nábělek F (1926) *Iter Turcico-Persicum, Pars III, Plantarum collectarum enumeratio*. *Spisy Vydávané Přírodovědeckou Fakultou Masarykovy University* 70: 1–75.

- Nábělek F (1929a) *Iter Turcico-Persicum, Pars IV, Plantarum collectarum enumeratio (Plumbagineae-Cyperaceae)*. Spisy Vydávané Přírodovědeckou Fakultou Masarykovy University 105: 1–48.
- Nábělek F (1929b) *Iter Turcico-Persicum, Pars V, Plantarum collectarum enumeratio (Gramineae-Cryptogamae)*. Spisy Vydávané Přírodovědeckou Fakultou Masarykovy University 111: 1–42.
- Nábělek F (1958) Květena Arboreta Mlýňany. In: Benčat F (Ed.) *Přírodní podmínky Arboreta Mlýňany, Sborník prac Arboreta Mlýňany I. Biologické Práce SAV* 4/12: 9–78, 170, 177–178, 185.
- Nişanyan S (2014) *Index Anatolicus, Türkiye yerleşim birimleri envateri*. <http://www.nisan-yanmap.com/>
- PHP (2016) PHP. <http://php.net> [accessed 30.6.2016]
- PostgreSQL (2016) <https://www.postgresql.org> [accessed 30.6.2016]
- Staffeu FA, Cowan RS (1981) *Taxonomic literature* (Vol. 3): Lh–O. *Regnum Vegetabile* 105, 980 pp. <http://www.biodiversitylibrary.org/creator/35945#/titles>
- Tschöpe O, Macklin JA, Morris RA, Suhrbier L, Berendsohn WG (2013) Annotating biodiversity data via the Internet. *Taxon* 62: 1248–1258. doi: 10.12705/626.4
- Ubuntu (2016) Ubuntu 14.04.4 LTS (Trusty Tahr). <http://releases.ubuntu.com/14.04/> [accessed 30.6.2016]
- United States Board on Geographic Names – Foreign Names Committee (2015) *Geographic Names Standardization Policy for Iran*. Available online at http://geonames.nga.mil/gns/html/Policies/Iran_Country_Policy_webversion_Dec2012_Rev_2015.pdf
- Vacek V, Bureš P (2001) *Botanika: Dějiny oboru na Masarykově univerzitě v Brně*. Masarykova Univerzita, Brno, *Folia Historica* 70: 1–100.

A remarkable new species of *Brunfelsia* (Solanaceae) from the eastern Andes of Central Peru

James G. Graham^{1,2}, John P. Janovec²

1 Botany Department, Field Museum, 1400 S. Lake Shore Drive, Chicago, IL USA **2** Herbario Forestal MOL, Facultad de Ciencias Forestales, Universidad Nacional Agraria La Molina, Lima, Peru

Corresponding author: James G. Graham (jgraham@fieldmuseum.org)

Academic editor: Eric Tepe | Received 10 October 2016 | Accepted 27 November 2016 | Published 1 December 2016

Citation: Graham JG, Janovec JP (2016) A remarkable new species of *Brunfelsia* (Solanaceae) from the eastern Andes of Central Peru. *PhytoKeys* 75: 81–91. <https://doi.org/10.3897/phytokeys.75.10759>

Abstract

Brunfelsia cabiesesiana J. G. Graham, **sp. nov.** (Solanaceae), a new species from montane cloud forests of Ucayali and Pasco Departments, Peru, is described and illustrated. The new species differs from all other members of the genus *Brunfelsia* by its cauline inflorescences. A key to the Peruvian species of *Brunfelsia* is presented.

Resumen

Brunfelsia cabiesesiana J. G. Graham, **sp. nov.** (Solanaceae), descrita e ilustrada aquí, constituye una nueva especie de los bosques nublados de los departamentos de Ucayali y Pasco, Perú. La nueva especie difiere de todas las demás especies de *Brunfelsia* por sus inflorescencias caulinas. Se presenta aquí también una clave para las especies peruanas de *Brunfelsia*.

Keywords

Brunfelsia, Solanaceae, Peru, Cordillera El Sira, Cordillera Yanachaga

Plabras clave

Brunfelsia, Solanaceae, Cordillera El Sira, Cordillera Yanachaga

Introduction

During the course of botanical exploration in the Cordillera El Sira (see Figure 1), we encountered an interesting species of *Brunfelsia* with a unique combination of features differing from all other members of the genus. After reviewing specimens of *Brunfelsia* deposited in herbaria at F, HOXA and MOL (Thiers 2016), and digitized specimens available at JSTOR Global Plants (<http://plants.jstor.org/>), we describe a unique new species. Illustrations, photographs, a discussion of affinities, and a key to Peruvian species of *Brunfelsia* is presented.

The Solanaceae (nightshade family) are widely distributed across the globe, including ca. 96 genera and approximately 2800 species of herbs, shrubs, trees, vines, lianas and epiphytes, with its greatest concentration of species found in the New World. The most recent taxonomic classification by Barbosa et al. (2016) recognizes five subfamilies and 14 tribes.

Brunfelsia is a neotropical genus known from the Caribbean and South America as far north as Panama. Its taxonomic history has been well documented by Plowman (1974, 1998), beginning with a short description, diagnosis and illustration by Plumier (1703), named (and misspelled) *Brunsfelsia* in honor of German herbalist Otto Brunfels. Linnaeus included *Brunfelsia* in the second edition of *Genera Plantarum* (1742), based on Plumier's description; the valid publication of the genus dates from 1753, when he published *Brunfelsia americana* in *Species Plantarum*. Tribal placement of *Brunfelsia* has fluctuated. Beginning with Bentham's (1835) assignment of the genus to tribe Salpiglossidae, reassignment to tribe Francisceae (Don 1837), back to Salpiglossidae again (Endlicher 1839, Bentham 1846), until Miers (1849) placed the genus in tribe Brunfelsiae. Bentham and Hooker (1873) moved it back to Salpiglossidae, where it was conserved by Baillon (1888), van Wettstein (1895), Baehni (1946), and Plowman (1974). Hunziker (2001) placed *Brunfelsia* as the sole genus in Tribe Francisceae, and Olmstead et al. (2008), considering chloroplast DNA sequences, placed the genus in tribe Petuniae. The most recent taxonomic treatment of Solanaceae by Barbosa et al. (2016) places the genus with those taxa lacking clear relationships, i.e. *Incertae sedis*. It is interesting to note that the Petuniae of Olmstead et al. (2008), and Plowman's Salpiglossidae share nearly half of their genera (four of nine- *Brunfelsia* L., *Hunzikeria* D'Arcy, *Leptoglossis* Benth. and *Plowmania* Hunz. & Subils).

Plowman (1974, 1998) considered *Brunfelsia* to be a distinct genus, not readily confused with other genera. Its closest relatives were considered to be the genera *Browallia* and *Streptosolon*; these are easily distinguished because the woody habit and indehiscent capsules of *Brunfelsia* are not present in *Browallia*, nor is the twisted corolla tube and bright red-orange limb of *Streptosolon* found in *Brunfelsia*.

Plowman (1978, 1998) recognized three subgeneric sections, *Brunfelsia* sect. *Guianensis* Plowman; *B.* sect. *Francisceae* (Pohl) Griseb., and *B.* sect. *Brunfelsia* L., based on differences in floral morphology and distinct and mostly allopatric areas of distribution. This classification has been tested by recent molecular work of Filipowicz and Renner (2012). Their sequence data supported two clades, an Antillean clade consist-

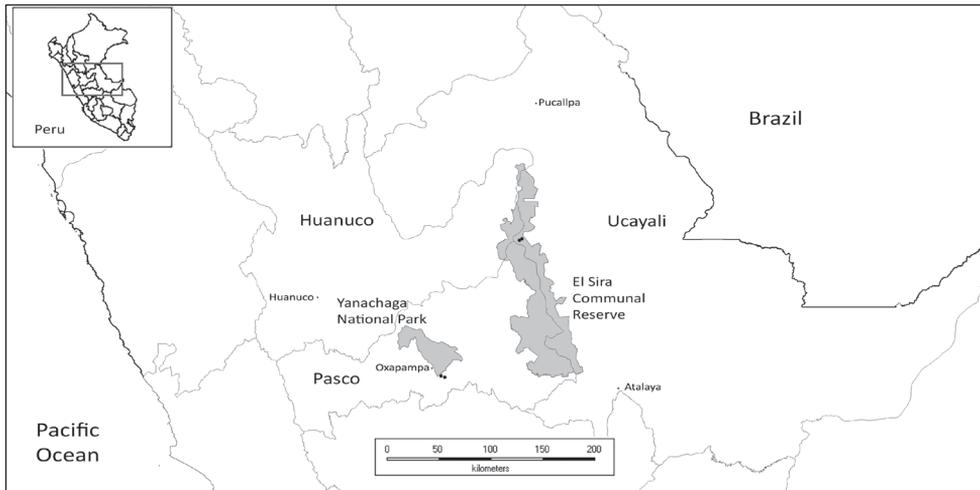


Figure 1. Location of known specimens of *Brunfelsia cabiesesiana*.

ing of all *Brunfelsia* sect. *Brunfelsia* and a second clade consisting of *Brunfelsia* sect. *Guianensis* and Plowman's *B.* sect. *Francisceae*. Their molecular work resulted in the description of a new species (*B. plowmaniana* N. Filipowicz & M. Nee) (Filipowicz et al. 2012), recovered from the Bolivian/Argentine members of *B. uniflora* (Pohl) D. Don.

All *Brunfelsia* species reported from Peru (i.e., *B. chircaspi* Plowman, *B. grandiflora* D. Don, and *B. mire* Monach.) have showy violet flowers that fade in color as they mature, each with a distinctive white eye at the throat. No fewer than five species are recorded from Andean regions of South America and ours is readily distinguishable from all members of the genus, including its nearest neighbors, by the presence of cauline inflorescences.

Taxonomic treatment

This new species is distinguished by its unique cauline inflorescence not encountered in any other member of the genus. A key to distinguish other known Peruvian species is provided.

Key to the Peruvian species of *Brunfelsia* (adapted from Plowman 1998)

- 1 Inflorescences terminal, subterminal or axillary 2
- Inflorescences cauline (borne along the main trunk or on tertiary vertical branchlets) *B. cabiesesiana*
- 2 Corolla lobes spreading at anthesis; leaves less than 8 cm wide 3
- Corolla lobes deflexed at anthesis; leaves greater than 8 cm wide ... *B. chircaspi*

- 3 Leaves more or less two ranked, scattered along branchlets, with 5–9 lateral nerves; inflorescences lax, often short-branched; corolla tube 15–40 mm long *B. grandiflora*
- Leaves crowded towards apex of stem, subverticillate, to about six per whorl, with 8–13 lateral nerves; inflorescences dense, capituliform; corolla tube 25–38 mm long..... *B. mire*

***Brunfelsia cabiesesiana* J.G.Graham, sp. nov.**

urn:lsid:ipni.org:names:77158825-1

Figures 2–6

Type. PERU. Ucayali: Prov. Coronel Portillo, Dist. Iparia, Reserva Comunal El Sira, 1500 m, 9°27.8'S, 74°33.5'W, 24 Oct 2007, *J. G. Graham 5970* (holotype: MOL; isotypes: F, NY).

Diagnosis. *Brunfelsia cabiesesiana* distinguitur ab omnibus aliis speciebus *Brunfelsia* possidendo caulifloris inflorescentiis.

Description. Pachycaulescent shrub to few-branched, sprawling small tree to 3 m. *Trunk* solitary, terete, to 5 cm in diameter near base. *Bark* brownish-gray and rough at stem base, becoming dark green and smooth on upper stem; glabrous. *Branches* lacking, or, if present, terete, to 2 cm diameter, tending to arch over with age, with vertically ascending branchlets. *Leaves* crowded toward apex of stem, subverticillate, up to seven per whorl, simple, often in terminal whorls on single stem, occasionally in multiple whorls 20–30 cm apart; petioles sub-terete, often canaliculate above, up to 1 cm long, 3–5 mm wide, brownish when dry, blades elliptic to broadly obovate, 15–35 cm long, 6–15 cm wide, glabrous, dull, dark green above, pale green beneath, young leaves purplish, smooth, subcoriaceous, glabrous, the base narrowly decurrent, the apex cuspidate to lightly acuminate, the margins entire; the midvein prominent below, the secondary veins 6–8-nerved, spaced up to 2 cm apart, arcuate-ascending, with light collective vein on margin, the tertiary venation reticulate. *Inflorescences* cauline, corymbiform, flowering branches stunted, woody at base, persistent, leafless, densely bracteate, to 3 cm long, with 1–7 branchlets up to 5 mm long, few flowered, usually only 1 flower per branchlet. *Bracts* spirally arranged, lanceolate, lightly keeled below, 0.7–1.3 mm long, tan to brown, lightly pubescent at base and along margins. *Flowers* showy violet fading with age, with 5-angled white spot at mouth. *Pedicels* 4–10 mm long, slender, 1.5–2 mm in diameter, glabrous. *Calyx* tubular-campanulate, weakly inflated, 2–2.3 cm long, 6–8 mm in diameter, ellipsoid to ovoid in bud, yellow-green to green, lightly punctate, firmly membranaceous, connate at base, 5-lobed at apex, the lobes subequal, ovate-lanceolate, 4–8 mm long, acute to acuminate and glandular at apex; calyx to 2.2 cm in fruit, coriaceous, smooth, partially enclosing the fruit, calyx and pedicel often with raised lenticels at maturity. *Corolla tube* terete, curved and inflated slightly at apex, then constricted at throat, gradually widening from base, 2.5–4 cm long, 2 mm diameter at base, to 5 mm diameter at apex, glabrous; estivation

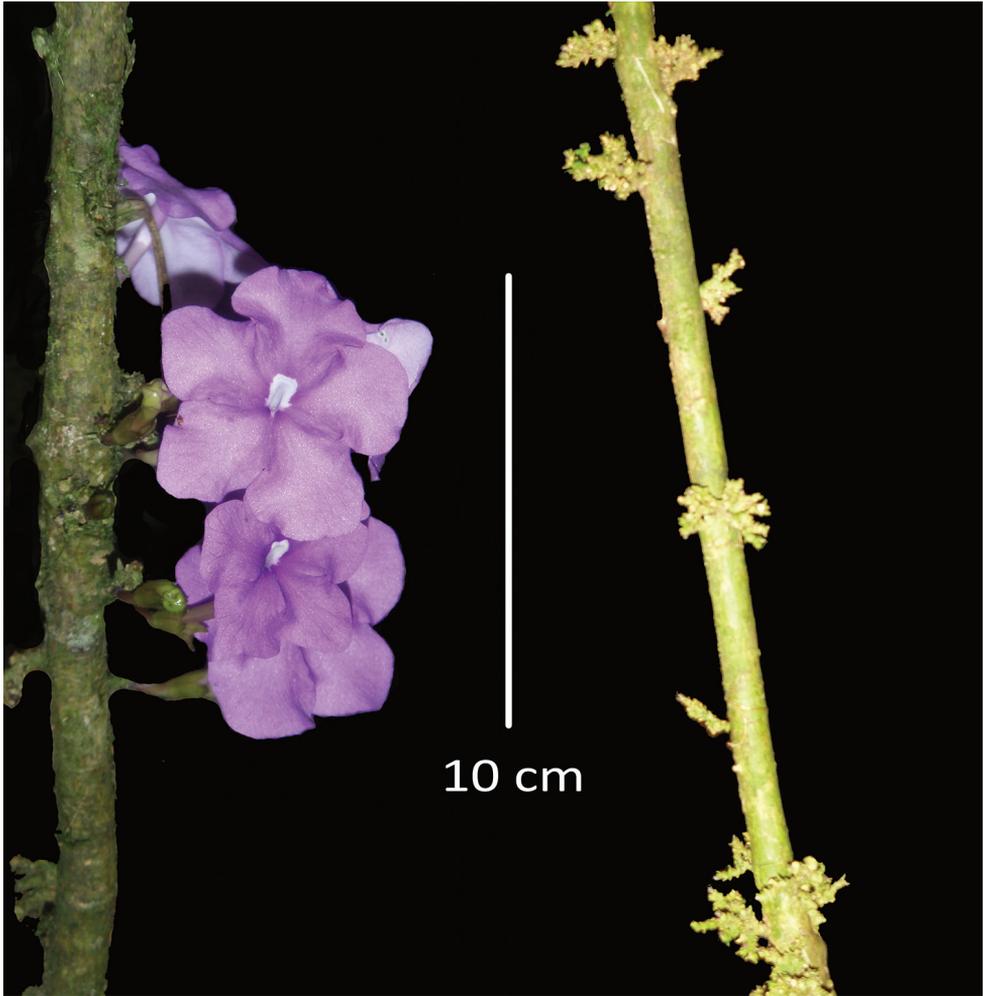


Figure 2. Inflorescences of *Brunfelsia cabiesesiana*. Left, cauline corymbiform inflorescences showing limb of corolla at anthesis. Right, stem with bracteate inflorescence branches.

quincuncial and imbricate, the limb spreading to 5.5 cm diameter, the lobes rounded, subequal, uppermost slightly larger, subtruncate to rounded at apex, overlapping at sides, narrowing lightly at base. *Stamens* four, in two pairs, included in upper portion of corolla tube; filaments ligulate, curved at apex, 2–3 mm long; anthers reniform, to 2 mm long. Ovary ovoid-conical, 2–3 mm long, glandular at base; style slender, curved and thickened at apex, 3–3.5 cm long; stigma weakly/briefly bifid, lobes equal, oval, 1–2 mm long, 8–10 mm in diameter. *Fruit* a capsule, globose to ovoid, 1.5–2 cm long, 1.2–1.8 cm wide, partially enclosed by accrescent calyx, slightly acute at apex with conspicuous scar where corolla tube was attached, medial septicidal suture present, not dehiscing along suture, dark green when fresh, light brown and lightly veined when dry, smooth, glabrous, pericarp thin walled, 1–2 mm thick, drying crustaceous,



Figure 3. Fruits of *Brunfelsia cabiesesiana*. Below, dried, dehiscent capsules with seeds. Above left, developing fruits.

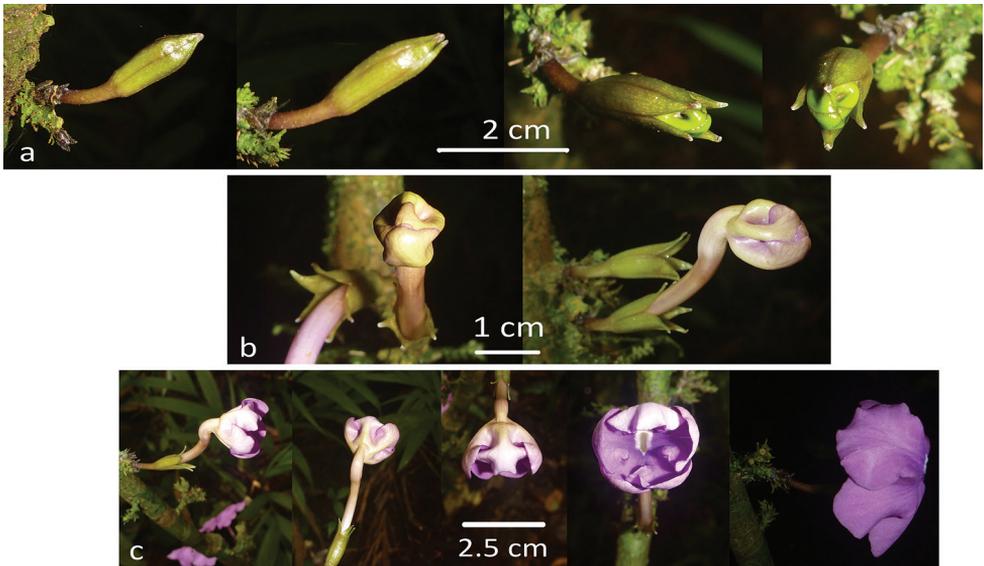


Figure 4. Estivation of *Brunfelsia cabiesesiana*. **A** calyces in bud. (day 1–3) **B** emerging flower. (day 4–5) **C** petals unfold (day 6–7).

exocarp coriaceous, lenticelate. *Seeds* 10–20 per fruit, oblong-ellipsoid, 5–7 mm long, ca. 3 mm in diameter, dark reddish-brown with brilliant prismatic reflection, reticulate pitted, glabrous.

Habitat and ecology. Known from central Peru in the Departments of Ucayali and Pasco where it is of extremely limited distribution but locally abundant at the type locality in the El Sira Communal Reserve. This understory species inhabits rocky slopes and ridge tops in cloud forests on the eastern slopes of the Cordillera El Sira, between 1100–1600 m, and has been found as a rare element in cloud forests on the northwestern slopes of the Cordillera Yanachaga, at ca. 2300 m.

Phenology. Flowering in *B. cabiesesiana* appears to be photomorphogenic in nature, associated with highest annual light intensities. Flowering observed in the El Sira populations is closely associated with the dry season, from August to October. Fruits appear to mature relatively slowly and are persistent, having been found green on the plant two or more months following anthesis.

Etymology. The species epithet honors Dr. Fernando Cabieses Molina, noted neurosurgeon, ethno-pharmacologist, author and educator. Dr. Cabieses was cofounder of the *Museo de la Nación* of Peru and founding rector of the Universidad Científica del Sur. He served as director of the Peruvian Museum of Health Sciences and the Peruvian National Institute of Traditional Medicine. Dr. Cabieses had profound interest in tropical biodiversity -both its history and utilization- and he was a tireless supporter of biodiversity conservation efforts in Peru.

Conservation status. This species is of extremely limited distribution (see Figure 1), although it appears to be locally abundant as evidenced by preliminary density studies along an elevational transect near the type locality, where 18 individuals were recorded in 2000 sq. m area. Three of these had reached maturity, as evidenced by stunted, persistent inflorescence branches (see Figure 2). This species appears to be extremely rare in the Cordillera Yanachaga, ca. 125 km SW from the type location and nearly 800 m higher in elevation.

Given the extremely limited known area of occupancy of *Brunfelsia cabiesesiana*, and the fragmented nature of the occurrence of the two known subpopulations in Pasco and Ucayali, we estimate the Pasco subpopulation to be critically endangered and the Ucayali population to be endangered, using International Union for the Conservation of Nature Red Book guidelines (IUCN, 2012). Both of the Pasco collections were located in fragmented forests near to roads. Given that anthropogenic activity in this region continues to expand, and that only two collections have ever been made, it is considered to face an extremely high risk of extinction in the wild. The Ucayali subpopulation, with the benefit of larger species densities, as well as a more favorable location inside a reserved zone, faces less threat of extinction.

Specimens examined. PERU. Ucayali: Dist. Iparia, Reserva Comunal El Sira, 9°28'S, 74°34'W, 1550 m, 24 Oct 2007, *J. G. Graham 4968* (F, MOL, NY); **Pasco:** Dist. Oxapampa, 10°37'S, 75°20'W, 2100 m, Villa Rica - Oxapampa, 4 Jan 1984, *R. Foster et al. 7788* (F); Dist. Oxapampa, 10°30'S, 75°20'W, 4 Aug 2009, forest remnant at the edge of a road, *R. Vasquez et al. 36203* (HOXA).



Figure 5. Profile of a flower of *Brunfelsia cabiesesiana* at anthesis.

Discussion

Brunfelsia cabiesesiana sp. nov. has a combination of characters that clearly separate it from other species of *Brunfelsia*: its strictly cauliflorous habit distinguish it from all other members of the genus, including the verticillate-leaved (*Brunfelsia mire* Monachino, *Brunfelsia hydrangeiformis* (Pohl) Benth.) members of the genus, as well as those with one central trunk (*Brunfelsia densifolia* Krug & Urb., *Brunfelsia mire*, *Brunfelsia chiricaspi* Plowman).

There is a clear divergence in elevational range between the Yanachaga and El Sira populations of *B. cabiesesiana*. A similar elevational-displacement phenomenon has been recorded for other organisms in the Cordillera El Sira. Terborgh and Weske (1975) noted a downward-displacement in elevation for species ranges of birds in the

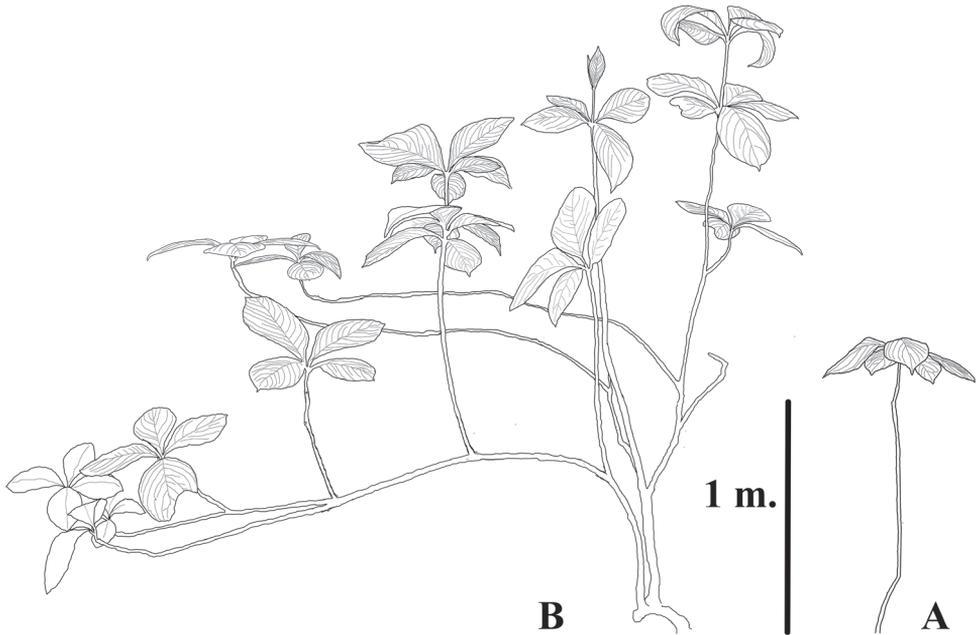


Figure 6. Two habit forms of *Brunfelsia cabiesesiana* found in the El Sira Mountains (drawing by JGG).

Cordillera El Sira, compared with the Cordillera Vilcabamba, with displacements of approximately 800 m. A similar pattern of elevational range displacement between the Cordilleras Yanachaga and El Sira, in both vascular plants and bryophytes, has been observed during our own field research.

Acknowledgements

Our sincere gratitude is extended to Carlos Reynel at Herbario Forestal, La Molina, Lima, Peru (MOL) for his enthusiastic support over the years. Rodolfo Vasquez and Robin Foster provided helpful discussions regarding their Yanachaga collections of *B. cabiesesiana*. Mike Nee offered useful advice on our *Brunfelsia* collections. Michael O. Dillon provided valuable comments on the manuscript, his efforts are highly appreciated. We also wish to thank the Jefatura of the El Sira Communal Reserve, including (in chronological order) Hector Sueyo, Rosa Mishari, Luis Saavedra and Alfredo Neyra. Furthermore, we extend our warmest regards to the indigenous communities of the buffer zone of the Communal Reserve, including Sharara, Puerto Nuevo, Dos de Mayo, Juancito de Sipiria, Amaquiria and Selva. Many thanks for the support of Field Museum colleagues, especially Botany Department staff including Chris Niezgoda, Anna Balla and Darlene Dowdy. The meticulous work of Tim Plowman on *Brunfelsia* including the ample collections compiled and annotated by him during his tenure at the Field Museum has been an invaluable resource.

References

- Baehni C (1946) L'Ouverture du Bouton chez les Fleurs de Solanées. *Candollea* 10: 399–492.
- Baillon H (1888) Histoire des plantes. Librairie Hachette & Co. Paris, 10: 360–364, 413–417.
- Barbosa GE, Hunziker AT, Bernardello G, Coducci AA, Moscone AE, Carrizo Garcia C, Fuentes V, Dillon MO, Bittrich V, Cose MT, Subils R, Romanutti A, Arroyo S, Anton A (2016) In: Kadereit JW, Bittrich V (Eds) Flowering Plants. Eudicots. The Families and Genera of Flowering Plants 14. Springer International Publishing, Switzerland.
- Bentham G (1835) Scrophularineae in Edward's Botanical Register, n. s., 8: sub pi. 1770.
- Bentham G (1846) Scrophulariaceae: Brunfelsia. In: de Candolle A (Ed.) *Prodromus Systematis Naturalis Regni Vegetabilis*, Vol. 10, 198–201, 590–591.
- Bentham G, Hooker J (1873) *Genera Plantarum*, vol. 2. Reeve and Company, London, England, 882–913.
- Don D (1837) *A General System of Gardening and Botany*, vol. 4. Rivington et al., London, England, 476–477.
- Endlicher S (1839) *Genera Plantarum*. Fr. Beck, Vienna, Austria, 676 pp.
- Filipowicz N, Renner SS (2012) Brunfelsia (Solanaceae): A genus evenly divided between South America and radiations on Cuba and other Antillean islands. *Molecular Phylogenetics and Evolution* 64(1): 1–11. <https://doi.org/10.1016/j.ympev.2012.02.026>
- Filipowicz N, Nee MH, Renner SS (2012) Description and molecular diagnosis of a new species of *Brunfelsia* (Solanaceae) from the Bolivian and Argentinian Andes. *Phytokeys* 10: 83–94. <https://doi.org/10.3897/phytokeys.10.2558>
- Hunziker A (2001) *Genera Solanacearum: The Genera of Solanaceae Illustrated, Arranged According to a New System*. A.R.G. Gantner; Königstein, Germany.
- IUCN (2012) IUCN red list categories and criteria, Version 3.1, 2nd edition. Gland, Switzerland.
- Linnaeus C (1742) *Genera Plantarum*. Ed. 2. Leiden, Netherlands.
- Linnaeus C (1753) *Species Plantarum*. Stockholm, Sweden.
- Miers J (1849) Observations upon several genera hitherto placed in Solanaceae, and upon others intermediate between that family and the Scrophulariaceae. *Annals and Magazine of Natural History, Series 2*, 3: 161–179.
- Olmstead RG, Bohs L, Abdel Migid H, Santiago-Valentin E, Garcia VF, Collier SM (2008) A molecular phylogeny of the Solanaceae. *Taxon* 57(4): 1159–1181.
- Plowman T (1974) The South American species of *Brunfelsia*. Doctoral Thesis, Department of Biology, Harvard University, Cambridge, MA.
- Plowman T (1978) A new section of *Brunfelsia*: section *Guianensis*. In: Hawkes JG (Ed.) *Systematic notes on the Solanaceae*. *Botanical Journal of the Linnean Society* 76: 294–295.
- Plowman T (1979) The genus *Brunfelsia*: a conspectus of the taxonomy and biogeography. In: Hawkes JG, Lester, Skelding (Eds) *The Biology and Taxonomy of the Solanaceae*. Academic Press, London.
- Plowman T (1998) A Revision of the South American species of *Brunfelsia*. In: Knapp S, Press J (Eds) *Fieldiana Botany New Series No. 39*.
- Plumier C (1703) *Catalogus Plantarum Americanarum*, p. 12, t. 22. In *Nova Plantarum Americanarum Genera*. J. Boudot, Paris, France.

- Terborgh J, Weske J (1975) The role of competition in the distribution of Andean birds. *Ecology* 56: 562–576. <https://doi.org/10.2307/1935491>
- Thiers B (2016) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>
- van Wettstein R (1895) Solanaceae. In: Engler A, Prantl K (Eds) *Die Natürlichen Pflanzenfamilien* 4(3B): 4–39.

Taxonomy and nomenclature of the polymorphic European high mountain species *Androsace vitaliana* (L.) Lapeyr. (Primulaceae)

Christopher J. Dixon¹, Walter Gutermann¹,
Peter Schönswetter², Gerald M. Schneeweiss¹

1 Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Wien, Austria

2 Institute of Botany, University of Innsbruck, Sternwartstrasse 15, A-6020 Innsbruck, Austria

Corresponding author: Gerald M. Schneeweiss (gerald.schneeweiss@univie.ac.at)

Academic editor: P. Stoev | Received 6 October 2016 | Accepted 28 November 2016 | Published 6 December 2016

Citation: Dixon CJ, Gutermann W, Schönswetter P, Schneeweiss GM (2016) Taxonomy and nomenclature of the polymorphic European high mountain species *Androsace vitaliana* (L.) Lapeyr. (Primulaceae). *PhytoKeys* 75: 93–106. <https://doi.org/10.3897/phytokeys.75.10731>

Abstract

Androsace vitaliana (syn. *Vitaliana primuliflora*; Primulaceae) has been subject to several taxonomic treatments, whose conclusions ranged from a single species with numerous infraspecific taxa to several species usually without infraspecific taxa. Here, following molecular investigation, several taxonomic changes are made. A single species with the following infraspecific taxa is recognized: subsp. *vitaliana* (Pyrenees), subsp. *cinerea* (south-western Alps), subsp. *lepontina* (Pennine Alps), subsp. *sesleri* (south-eastern Alps), subsp. *praetutiana* (Apennines) and subsp. *assoana* (Iberian Peninsula excluding the Pyrenees), the last of which is divided into the four allopatrically distributed varieties *assoana*, *centriberica*, *flosjugorum* and *nevadensis*. Contrary to some previous assertions, all taxa are allopatric and, especially for subspp. *vitaliana*, *cinerea* and *lepontina*, where clear diagnostic characters are lacking, they can best be determined by their geographic origin.

Keywords

Androsace vitaliana, Bayesian species delimitation, European mountain ranges, nomenclature, typification, *Vitaliana primuliflora*

Introduction

The infraspecific taxonomy of *Androsace vitaliana* (L.) Lapeyr. has been studied by several authors (Lüdi 1927, Chiarugi 1930, Schwarz 1963, Kress 1967, 1997a, 1999), who have reached different conclusions, based on morphological differences, geographical distributions and a certain amount of intuition. Recent genetic studies of *A. vitaliana* by Vargas (2003) and Dixon et al. (2009) offered new and unexpected insights into the species' phylogeny. Based on the results of these works and new analyses presented here, we now present a new taxonomy of *A. vitaliana*.

Taxonomic history

The first infraspecific taxa to be distinguished in *A. vitaliana* (under the name *Gregoria Vitaliana*—for the nomenclatural evaluation of the names that have been used and for full nomenclatural authorities, see “Nomenclature”) were the three varieties named by Sündermann (1916), although he ascribed two of them to Robert Buser. He defined var. *praetutiana* from the central Apennines by its relatively short leaves with rounded tips and by the density of the indumentum, and var. *cinerea* from the south-western Alps by the size and colour of the flowers, the shape of the leaves, and the ash-grey tomentum. Sündermann characterised the south-east Alpine var. *sesleri* by its slightly broader and greener leaves, but did not consider it to be very different from the “typical” form supposed to occur in the western Alps. Lüdi (1927) followed Sündermann's classification, but reduced the varieties to the rank of forma.

Chiarugi (1930) divided the species (as *Vitaliana primuliflora*) into three varieties: plants from the Sierra Nevada (var. *nevadensis*) form dense cushions and have persistent, distinctly keeled leaves tightly arranged on columnar stems; plants from the Apennines (var. *praetutiana*) form loose cushions or mats and have deciduous, broader leaves with rounded leaf tips; plants from the Pyrenees and the Alps (var. *alpina*) also form loose cushions or mats, but their leaves are narrower and have acute tips. Due to a lack of material, Chiarugi did not assign plants from the Sierra de Javalambre (eastern Spain) to any of his infraspecific taxa. Within the Pyrenean–Alpine taxon, he distinguished the eastern f. *orientalis* (Pennine Alps to southeastern Alps), with hairs either only on the leaf margins or on both the leaf margins and the median nerve on the lower leaf surface, from the western f. *occidentalis* (Pyrenees and south-western Alps), with hairs dispersed over at least the lower but commonly both leaf surfaces. Chiarugi further divided these formae into two subformae each, but the morphological differences were only reported to coincide with a geographical separation in f. *orientalis*, where the south-eastern Alpine taxon (subf. *tridentina*) had the least hairy leaves, while the taxon in the Pennine Alps (subf. *lepontina*) had hairier leaves. In contrast, for the two taxa within f. *occidentalis*, subf. *genuina* with longer corolla tubes and subf. *cinerea* with shorter corolla tubes, Chiarugi himself was unable to determine the exact distributional limits, and he only noted that both were present in both the eastern Pyrenees and the

southwestern Alps. Later authors (e.g., Schwarz 1963, Kress 1997a, b) have been more specific about the distributions in the Pyrenees, limiting one taxon to the central parts of the range and the other to the eastern parts.

In his taxonomic revision, Schwarz (1963) divided *Androsace vitaliana* (as the genus *Vitaliana*) into five species. While the distinctness of the Apennine taxon (*V. obtusifolia*) and the taxon from the Sierra Nevada (*V. congesta*) remained undisputed, populations from the Sierra de Javalambre were separated as a new species, *V. intermedia*, based on characters such as keeled leaves with obtuse tips and the shape of the calyx lobes, thus extending Chiarugi's observation of keeled leaves to cover the Sierra de Javalambre as well as the Sierra Nevada. Schwarz distinguished those plants that have leaves that are hairy on both sides (as *V. chionotricha*) from those with leaves that are glabrous on at least the upper surface (as *V. primuliflora*), dividing the latter into two subspecies, one with hairy lower leaf surfaces (subsp. *canescens*) and the other with both leaf surfaces glabrous (subsp. *primuliflora*). Although *V. chionotricha* and *V. primuliflora* subsp. *canescens* were considered to occur in both the Pyrenees and the western Alps (Schwarz' subsp. *primuliflora* is the taxon from the south-eastern Alps), Schwarz suggested that these are not sympatric, with *V. chionotricha* confined to the eastern Pyrenees and the south-western Alps, and *V. primuliflora* subsp. *canescens* occurring in the central Pyrenees and the Pennine and Lepontine Alps (see Fig. 6 in Schwarz 1963: p. 28). The Alpine distribution area of subsp. *canescens* is thus congruent with that of Chiarugi's f. *orientalis* subf. *lepontina* (see above). However, although Schwarz' map shows a clear geographic division between the two taxa, Kress (1967) asserts that the distributions are not as clearly separated as shown on Schwarz' map.

Kress (1967), as well as taking a different view about the type material, which affects the use of the epithets *vitaliana* and *sesleri* (see "Nomenclature"), rejected the division of *Vitaliana* into five species. Instead, he considered the taxon to be a single species and returned it to the genus *Androsace*. Since this treatment was done for Hegi's "Illustrierte Flora von Mitteleuropa", Kress only commented on the Alpine taxa, listing them as subsp. *cinerea* (eastern Pyrenees and western Alps), subsp. *sesleri* (south-eastern Alps) and subsp. *vitaliana* (Pyrenees, western Alps). While providing a diagnostic key (based on Schwarz 1963), Kress commented that the values for the number of palisade layers in the upper leaf surface and the size of the flowers, characters used by previous authors (Sündermann 1916, Chiarugi 1930, Schwarz 1963), are not always correct. In a later publication, Kress (1997a) also stated that the shape of the leaves is highly dependent on the environmental conditions under which the plant grows, with drier environments giving rise to longer, proportionally narrower leaves, making this another unreliable character. Kress (1967) followed Schwarz' view of the indumentum, albeit with some reservation about the uniformity of the character within and between populations. In contrast, Heß et al. (1970) dismissed the indumentum characters entirely, having observed that glabrous individuals could be found even in the southwestern Alps, where, according to the traditional viewpoint, plants should have hairs on at least the lower leaf surface.

Laínz (1964) combined specimens from the Cordillera Cantábrica and the Sierra de Javalambre as subsp. *assoana*. Since then, the major changes have been in the recognition of an increasing number of taxa on the Iberian Peninsula, and at increasing rank. Kress (1997a) described a new subspecies for plants from the Cordillera Cantábrica (subsp. *flosjugorum*, which forms loose cushions or mats and has a fraction of leaves that are distally hairy on the upper surface) as well as a variety in the Sistema Central (var. *centriberica*, which forms dense cushions with spreading dead leaves and juvenile leaves that have obtuse tips), which Luceño (1998) raised to the subspecific rank as subsp. *aurelii*. Kress (1999) raised the rank further, creating the species *A. centriberica*, with allopatric subspecies in the Sistema Central (subsp. *centriberica*), Sierra de Javalambre (subsp. *assoana*) and Montes de León (subsp. *maragatorum*, which differs from the previous two subspecies by more strongly hairy upper leaf surfaces and acute leaf tips), but excluding the Cordillera Cantábrica. At the same time, he also resurrected Schwarz' species from the Sierra Nevada as *A. congesta*. This last work by Kress contradicts earlier works by the same author, introducing new taxa based on characters that he had previously deemed unreliable.

Previous molecular findings

In the first molecular investigation of the species, using sequences from the nuclear ribosomal ITS region, Vargas (2003) was not able to resolve all the relationships within *A. vitaliana*, but did detect three distinct lineages: one in the Apennines, one in the Alps, and one on the Iberian Peninsula. Dixon et al. (2009), using additionally plastid sequences and AFLPs, identified six major groups, corresponding closely with the geographical distribution of the species. All Spanish accessions from outside the Pyrenees formed a single group, with only weak differentiation between the different mountain ranges. Kress' (1999) opinion that these populations belong to three different species (*A. congesta*, *A. centriberica*, and *A. vitaliana*) found no support. No material from the Montes de León was included in the study of Dixon et al. (2009), but in Vargas' (2003) study, the Cordillera Cantábrica and Montes de León grouped together in agreement with their geographic proximity. Populations from the Pyrenees constituted a single group (ribotype sharing with Spanish populations outside the Pyrenees was interpreted as the result of ITS convergence after gene flow into the Pyrenees: Dixon et al. 2009). In the Alps, three groups were identified: in addition to populations from the southwestern Alps (subsp. *cinerea*) and from the southeastern Alps (subsp. *sesleri*) populations from the Pennine Alps were found to be as distinct as the previous two or as subsp. *praetutiana* from the Apennines, supporting Chiarugi's (1930) assessment that the populations from the Pennine Alps formed a distinct taxon. This disagrees with most recent treatments, in which plants from the Pennine Alps have been grouped with those from the central Pyrenees.

Bayesian species delimitation

To further test the distinctness of the six major groups identified by Dixon et al. (2009), their AFLP data set was re-analysed via Bayesian multispecies coalescent species delimitation using genome-wide biallelic markers (Leaché et al. 2014). The applicability of this method may be compromised in case of substantial gene flow between diverging entities (populations, subspecies, species); high bootstrap support obtained from a Neighbour-Joining analysis for all major lineages except the one from the southwestern Alps (Fig. 3 in Dixon et al. 2009: p. 585), however, suggests that the evolution of this group can be reasonably well described by a tree. Applying this method allows competing species delimitation concepts to be compared using Bayes Factors (Kass and Raftery 1995). To decrease the computational burden, the original AFLP data set was pruned to include only two randomly chosen individuals per population, retaining 60 of the 143 individuals in the data set of Dixon et al. (2009). Analyses were conducted using the SNAPP 1.2.5 package (implementing the method of Bryant et al. 2012) in BEAST 2.1–2.3 (Bouckaert et al. 2014). Priors for forward and backward mutation rates, u and v , were fixed on 1.017056 and 0.983506, calculated from the state frequencies and applying the constraint that the mutation rate is 1. Priors for other parameters (λ for the Yule prior on species trees, α and β for the gamma distribution priors on the θ values of the ancestral population sizes) were fixed at their default values. Bayes Factors were calculated from marginal likelihoods obtained via path sampling (Baele et al. 2012) with 60 steps (each with 10^6 MCMC steps and 50,000 pre-burnin steps). Tested species delimitation scenarios were: (i) all six major groups constitute distinct lineages; (ii) groups from the southwestern and Pennine Alps constitute a single lineage, while the remaining four are distinct; (iii) the three groups from the Alps constitute a single lineage, while the remaining three are distinct; (iv) the three groups from the Alps plus the Apennines constitute a single lineage, while the remaining two are distinct. Although the estimated marginal likelihoods need to be viewed with some caution (up to seven consecutive steps between step 20 and 30 of the path sampling had effective sampling size [ESS] values below 100), large positive Bayes Factors (i.e., twice the difference in marginal likelihoods between model 1 and model 2) provided strong support for the first scenario, i.e., recognition of all six major groups identified by Dixon et al. (2009) as distinct lineages (Table 1).

Table 1. Results for Bayesian lineage delimitation in *Androscae vitaliana*.

Model	Marginal likelihood	Bayes Factor
(i) Six lineages (Iberian Peninsula, Pyrenees, southwestern Alps, Pennine Alps, southeastern Alps, Apennines)	-2580.65	-
(ii) Five lineages (Iberian Peninsula, Pyrenees, southwestern plus Pennine Alps, southeastern Alps, Apennines)	-2745.68	330.05
(iii) Four lineages (Iberian Peninsula, Pyrenees, Alps, Apennines)	-2761.82	362.33
(iv) Three lineages (Iberian Peninsula, Pyrenees, Alps plus Apennines)	-2835.78	510.27

Classification

Previous taxonomic treatments range from a very elaborate infraspecific classification down to the level of subformae (Chiarugi 1930) to ones using a very narrow species concept, which distinguish up to five different species (Schwarz 1963, Kress 1999). The prevailing view lies between these two extremes and treats *A. vitaliana* as a single species with several subspecies (Kress 1967, 1997b, Ferguson 1972, Pignatti 1982), a scheme we follow here for several reasons. Firstly, there is evidence for (ancient) gene flow between the different mountain ranges (Dixon et al. 2009); secondly, there is a lack of reliable diagnostic features that could be used for species discrimination, especially between taxa from the Pyrenees and the western Alps. Finally, this is the choice of rank that requires the fewest changes to the established names, thus helping to ensure nomenclatural stability.

Three subspecies possess distinctive morphological features that allow them to be distinguished, at least to some extent, from other taxa: subsp. *praetutiana* (Apennines) has rounded leaf tips (also found in some populations of subsp. *assoana*, but these form dense cushions, and rarely in subsp. *vitaliana*: Kress 1997a); subsp. *assoana* (Iberian Peninsula outside the Pyrenees) has keeled leaves (only weakly so in populations from the Cordillera Cantábrica), which tend not to fall off after dying away and give rise to thick columnar stems (in contrast to the slender stems seen in other taxa, but also in the populations of subsp. *assoana* from the Cordillera Cantábrica: Kress 1997a); subsp. *sesleri* (south-eastern Alps) has the least hairy leaves that are either glabrous or only sparsely hairy along the leaf margin (Schwarz 1963, Kress 1967). The remaining three subspecies have chiefly been defined in terms of their indumentum, but despite the presence of some morphological tendencies (subsp. *cinerea* is generally the most densely hairy taxon with the longest leaves, for instance), these characters are variable both within and between populations, and do not correlate with genetic relatedness. The easiest way to distinguish these subspecies remains, therefore, knowledge of their geographic origins: subsp. *vitaliana* occurs in the Pyrenees, subsp. *cinerea* in the south-western Alps, and subsp. *lepontina* in the Pennine Alps. We acknowledge that it is a disadvantage that these taxa cannot be confidently diagnosed from their morphology, but we consider it necessary to reflect our current understanding of their phylogenetic relationships and distinctness as far as possible in the taxonomy. The taxa from the Pyrenees and the western Alps are genetically as distinct as the other taxa that are better defined morphologically (Dixon et al. 2009). Although only the geographic location can currently be used to identify these taxa accurately, it is possible that suitable diagnostic characters exist, but have as yet been overlooked.

This problem also applies to the varieties within subsp. *assoana*, which are difficult to distinguish from herbarium material without knowledge of the geographic origin as acknowledged by Kress in his latest contribution (Kress 1999), ironically the very study where he uses the highest taxonomic ranks. However, given that diagnostic tendencies have been recognised by previous authors (Kress 1997a,b, 1999), we have chosen to preserve these varieties, in order to retain as much as possible of the existing nomen-

clature while also reflecting the genetic relationships discovered by Vargas (2003) and Dixon et al. (2009). Specifically, var. *flosjugorum* is distinguished by growing in loose cushions or mats in contrast to the other three varieties that form dense cushions; var. *centriberica* has slightly shorter and broader leaves than the other varieties, with a tendency to be rounded at the tip, while the leaves of var. *assoana* are narrower and have acute tips; var. *nevadensis* reportedly forms smaller cushions than the other varieties of subsp. *assoana* and has imbricate leaves that are only occasionally rounded.

Nomenclature

Androsace vitaliana (L.) Lapeyr., Hist. Pl. Pyrénées: 94. 1813 ≡ *Primula vitaliana* L., Sp. Pl.: 143. 1753 [basionym] ≡ *Aretia vitaliana* (L.) L., Syst. Veg. ed. 13: 162. 1774 ≡ *Androsace lutea* Lam., Fl. franç. 2: 258. 1778, nom. superfl. ≡ *Primula sedifolia* Salisb., Parad. Lond. 2: t. 107. 1807, nom. superfl. ≡ *Androsace rugosa* Clairv., Man. herbor. Suisse: 57. 1811, nom. superfl. ≡ *Gregoria vitaliana* (L.) Duby, Bot. Gall. ed. 2, 1: 383. 1828 ≡ *Vitaliana primuliflora* Bertol., Fl. Ital. 2: 368. 1835 ≡ *Macrotibus luteus* Dulac, Fl. Hautes-Pyrénées: 425. 1867, nom. superfl. ≡ *Vitaliana gregorii* Saccardo, Sto. lett. fl. venet.: 46. 1869, nom. superfl. ≡ *Gregoria lutea* St.-Lager in Ann. Soc. Bot. Lyon, 7: 144. 1880, nom. superfl. ≡ *Douglasia vitaliana* (L.) Pax in Engler & Prantl, Nat. Pflanzenfam. 4(1): 109. 1890. — Lectotype [designated by Kress (1967: 2248d)]: Spain, Pyrenees, “*Sanicula alpina pumila tenuifolia lutea*, non descripta. In Pyrenaeis Hispanicis” in herb. Burser XIII.154 (UPS).

The Linnaean basionym *Primula vitaliana* has been repeatedly typified (Kress 1967, Ferguson 1969). Schwarz (1963) suggested as putative type material a specimen in the Linnaean herbarium in London (not explicitly designated by Schwarz, but identified as LINN 198-15 by Ferguson). However, this is a specimen that represents *Androsace alpina*, not *A. vitaliana*. Additionally, this accession has been acquired later than 1753, which is also the case for another specimen (LINN 196-4, preserved as *Aretia vitaliana*) of true *Androsace vitaliana* (Kress 1970). Kress (1967) identified a specimen held in Burser’s Hortus Siccus from the Spanish Pyrenees for the type specimen. As this specimen lacks flowers, Ferguson (1969) considered Kress’ selection unsatisfactory and instead favoured as lectotype a plate by Sesler, which was based on a specimen from the south-eastern Alps and shows flowers, descriptions of which are included in the protologue. Kress (1970) argues that Sesler’s drawing is chimerical, because it includes parts of *Silene acaulis* (Caryophyllaceae), and continues to favour the specimen held in Burser’s Hortus Siccus (Kress 1970, 1993). We follow Kress’ choice of type, accepting his argument that the current lack of flowers does not necessarily preclude the specimen from having once possessed some and having been used at the time of description. We also concur that the possibility of having at least part of the type material belonging to a different species or even family should be avoided.

One synonym of *A. vitaliana* given by both Chiarugi (1930) and Schwarz (1963) appears to result from a misunderstanding. Chiarugi cites “*Primula lutea* Vill., Cat. du Gard. de Strasbur., 121 (1807).” as a synonym of *A. vitaliana*, referring to Villars’ Catalogue méthodique des plantes du Jardin de l’École de Médecine de Strasbourg, where a footnote concerning “*Primula vitaliana*, Fl. fr. III, 450” appears at the end of the treatment of the genus *Primula*, adjacent to the name “*P. lutea*, Vill.”, which (as is apparent from its typographic position) is cited here as synonym of *P. auricula*. The footnote refers to the critical placement of *P. vitaliana* between *Primula*, *Aretia*, and *Androsace*. Thus, it applies simply to the whole genus and cannot be seen as an indication of synonymy between *P. lutea* and *P. vitaliana*. Schwarz (1963) went further and erroneously interpreted the name *Primula lutea* as a recombination of Lamarck’s *Androsace lutea*, adding Lamarck to the citation as the author of the basionym. The name *Androsace lutea* is itself illegitimate, since Lamarck ought to have adopted the basionymic epithet *vitaliana*, and all recombinations of *A. lutea* are therefore new (and likewise illegitimate) names.

Androsace vitaliana subsp. *vitaliana*

- Vitaliana primuliflora* var. *alpina* f. *occidentalis* subf. *genuina* Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337. 1930, nom. inval. (ICBN 24.3), p. p.
- Vitaliana primuliflora* subsp. *canescens* O. Schwarz in Feddes Repert. 67: 24. 1963, nom. inval. (without type indication), p. p. (Pyrenean plants only)
- Vitaliana chionotricha* O. Schwarz in Feddes Repert. 67: 24. 1963, p. p. (Pyrenean plants only; type excluded)

Distribution. Pyrenees.

Following the findings of Dixon et al. (2009), and contrary to previous hypotheses, only one subspecies of *A. vitaliana* occurs in the Pyrenees. Because the type specimen of the species name is from the Pyrenees, the Pyrenean taxon must bear the autonym.

Androsace vitaliana subsp. *cinerea* (Sündermann) Kress in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 2248d. 1967 ≡ *Gregoria vitaliana* var. *cinerea* Sündermann in Allg. Bot. Z. 22: 59. 1916 [basionym] ≡ *Gregoria vitaliana* subsp. *cinerea* (Sündermann) Bornm. in Mitt. Thür. Bot. Ver. 37: 6, 1927 ≡ *Gregoria vitaliana* f. *cinerea* (Sündermann) Lüdi in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 1789, 1927 ≡ *Vitaliana primuliflora* subf. *cinerea* (Sündermann) Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337, 1930 ≡ *Vitaliana primuliflora* subsp. *cinerea* (Sündermann) I. K. Ferguson in Taxon 18: 303, 1969 ≡ *Vitaliana primuliflora* var. *cinerea* (Sündermann) Vigo in Acta Bot. Barcinonensia 35: 435, 1983 — Type: none designated (described from Mercantour, south-western Alps)

= *Vitaliana chionotricha* O. Schwarz in Feddes Repert. 67: 24, 1963 — Type: In colle “Galibier” montium Delphinientium. Leg. Ozanon. 1858. (holotype: JE; isotypes: W, M).

- Gregoria vitaliana* subsp. *gaudini* Sündermann ex Bornm. in Mitt. Thüring. Bot. Vereins 37: 6. 1927, nom. nud.
- Vitaliana primuliflora* var. *alpina* f. *occidentalis* subf. *genuina* Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337, 1930, nom. inval. p. p. (Alpine plants only)

Distribution. Alps south and west of Mont Blanc.

Sündermann (1916) did not designate a type specimen for his var. *cinerea*, but did give a location: “von den hohen Bergen zwischen Pietraporzio und St. Etienne in den Seealpen” (“high mountains between Pietraporzio and St. Etienne in the Maritime Alps”). Saint-Étienne-de-Tinée (Alpes-Maritimes, France) and Pietraporzio (Cuneo, Italy) lie on either side of the northern part of the Mercantour mountain range. We refrain from selecting a neotype until a collection from the immediate type region (between Col du Fer and Pas de Corborant) is available, preferably investigated by molecular means.

Since no material from the Savoyan or Graian Alps has been included by Dixon et al. (2009), it is difficult to know to which subspecies these populations would belong. Pignatti (1982) had extended the area of his “subsp. *canescens*” (which largely corresponds to our subsp. *lepontina*) to Valle Locana (thus including the Gran Paradiso massif), whereas Chiarugi considered this population to be part of his forma *occidentalis* (i.e., our subsp. *cinerea*). This area is contiguous with the south-west Alpine distribution of *A. vitaliana*, and not with the area in the Pennine Alps (Niklfeld et al., unpubl. data). We therefore assume, in the absence of any genetic data, that these plants belong to subsp. *cinerea*.

Androsace vitaliana subsp. *lepontina* (Chiarugi) Dixon, Gutermann & Schneew. comb. nov. ≡ *Vitaliana primuliflora* var. *alpina* f. *orientalis* subf. *lepontina* Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337, 1930 — Lectotype (here designated): Switzerland, Valais, “Seehorn a. d. Südseite d. Simplon. Gipfelregion”, Gneiss, 2450 m, leg. H. Handel-Mazzetti, 29.7.1906 (WU 044259; <http://herbarium.univie.ac.at/database/detail.php?ID=157653>).

- Vitaliana primuliflora* subsp. *canescens* O. Schwarz in Feddes Repert. 67: 24, 1963. nom. inval. (without type indication: ICBN 40.1), p. p. (plants from “Penninische und Lepontinische Alpen” only)

Distribution. Alps from the Great St. Bernard Pass to the Simplon Pass (Swiss canton of Valais, Italian provinces of Verbano and Valle d’Aosta).

A taxon restricted to the Pennine Alps has previously been recognized only by Chiarugi (1930) as *Vitaliana primuliflora* var. *alpina* f. *orientalis* subf. *lepontina*. Schwarz (1963) described two subspecies within his *Vitaliana primuliflora*, one of which (subsp. *canescens*) explicitly includes Chiarugi’s taxon, but also includes some plants from the Pyrenees. Schwarz (1963), however, failed to indicate a holotype, rendering the name

(and thus also the later combination made by Vigo i Bonada 1983) invalid. The possibility of Schwarz having intended merely to provide a new name for Chiarugi's taxon is ruled out by Schwarz' explicit statement of "ssp. nov." and is also made less likely by the additional Pyrenean area. To find a suitable name for a new combination, we must therefore return to Chiarugi's subf. *lepontina*, which covers the same area as the present taxon, for which several syntypes are listed, and whose epithet *lepontina* is considerably more apt and less ambiguous than Schwarz' "*canescens*". We have chosen one of the syntypes which has detailed location information, which comes from a representative location, and whereon all the parts that are used for diagnosis within the species are preserved. The new lectotype is from the summit region of the Seehorn, south-east of the Simplon Pass in the Swiss canton of Valais.

Androsace vitaliana subsp. *praetutiana* (Buser ex Sündermann) Kress in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 2248d, 1967 ≡ *Gregoria vitaliana* var. *praetutiana* Buser ex Sündermann in Allg. Bot. Z. 22: 59. 1916 [basionym] ≡ *Gregoria vitaliana* subsp. *praetutiana* (Buser ex Sündermann) Bornm. in Mitt. Thür. Bot. Ver. 37: 6. 1927 ≡ *Gregoria vitaliana* f. *praetutiana* (Buser ex Sündermann) Lüdi in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 1789. 1927 ≡ *Vitaliana primuliflora* var. *praetutiana* (Buser ex Sündermann) Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337, 1930 ≡ *Vitaliana primuliflora* subsp. *praetutiana* (Buser ex Sündermann) I. K. Ferguson in Taxon 18: 303, 1969 — Neotype (here designated): Gran Sasso d'Italia. Traversa di Portella, nei pascoli, leg. U. Martinelli 19.8.1893 (FI)

= *Vitaliana obtusifolia* O. Schwarz in Feddes Repert. 67: 26, 1963 — Type: Majella, Mte. Amaro, leg. H. Handel-Mazzetti (W).

Distribution. Mountains of Abruzzo: Corno Grande, Maiella and Sirente-Velino.

Sündermann (1916), in his description, ascribed subsp. *praetutiana* to Buser, although we are unaware of any publication by Buser dealing with *A. vitaliana*. Franz Sündermann was a commercial horticulturist in Lindau (Bavaria, Germany), and is unlikely to have maintained a herbarium himself. Buser's herbarium is held at the Conservatoire et Jardin botaniques de la Ville de Genève (G) and does not include any specimens of *A. vitaliana* (Laurent Gautier, pers. comm.), suggesting that none of the material seen by the author of the protologue survives. For the purposes of typification, the location information is rather vague, being given simply as "in den Abruzzen" (i.e. Abruzzo, Italy), which covers the entire range of the subspecies. Since Chiarugi (1930) was the first to provide information on this taxon beyond the bald description of Sündermann, any neotype should be chosen from among the specimens seen by Chiarugi and identified by him as var. *praetutiana*. We have chosen a specimen that has detailed location information, which comes from a representative location, and whereon all the parts that are used for diagnosis within the species are preserved. Schwarz (1963) created a new epithet (*obtusifolia*) and selected a type specimen which Chiarugi did not investigate.

Androsace vitaliana subsp. *sesleri* (Buser ex Sündermann) Kress in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 2248d, 1967 \equiv *Gregoria vitaliana* var. *sesleri* Buser ex Sündermann in Allg. Bot. Z. 22: 59. 1916 [basonym] \equiv *Gregoria vitaliana* subsp. *sesleri* (Buser ex Sündermann) Bornm. in Mitt. Thür. Bot. Ver. 37: 6. 1927 \equiv *Gregoria vitaliana* f. *sesleri* (Buser ex Sündermann) Lüdi in Hegi, Ill. Fl. Mitt.-Eur. 5 (3): 1789. 1927 \equiv *Vitaliana primuliflora* subsp. *sesleri* (Buser ex Sündermann) Pignatti in Giorn. Bot. Ital. 111(1–2): 48. 1977 (1977) — Neotype (here designated): Italy, Trentino-Alto Adige: Trento, northern surroundings of Passo di San Pellegrino: little south of Pas de le Sele; 2485 m, 46°23'48" N, 11°45'50" E, leg. R. Flatscher & P. Schönswetter, 5.9.2010; cultivated in the Botanical Garden of the University of Innsbruck and prepared as herbarium sheet in spring 2011 (WU).

= *Vitaliana primuliflora* var. *alpina* f. *orientalis* subf. *tridentina* Chiarugi in Nuov. Giorn. Bot. Ital. 37: 337, 1930

—*Vitaliana primuliflora* subsp. *primuliflora* sensu I. K. Ferguson in Taxon 18: 302, 1969.

Distribution. South-eastern Alps from the Bergamo Alps (Italy) to the Carnic Alps (Italy and a small area in Austria), chiefly the Italian Dolomites.

As was the case for subsp. *praetutiana*, Sündermann (1916) ascribed subsp. *sesleri* to Buser and no type material has been identifiable. The location information for this taxon is stated as “die südtiroler und von da weiter östlich vorkommende Form” (“the form occurring in South Tyrol and further to the east”). Since no original material is available, a neotype is necessary, ideally from material collected in the source region of the material used for Sesler’s drawing, which has previously been used for typification of the species (see above). Sesler reports the location as “dans la montagne de S. Pellegrino” (cited in Ferguson 1969), which is a pass between Falcade and Moeno in the southern Dolomites. In the course of a dedicated search, the taxon has been collected in the northern surroundings of the Passo di San Pellegrino, and material from this population is chosen for the neotype.

Androsace vitaliana subsp. *assoana* (Láinz) Kress in Phytion (Horn) 13: 221. 1969 \equiv *Vitaliana primuliflora* subsp. *assoana* Láinz in Bot. Inst. Edt. Astur. Supplem. Cienc. 10: 199, 1964 \equiv *Androsace centribérica* (Kress) Kress in Primulaceen-Studien 15: 2. 1999 — Type: Spain, Teruel, “Sierra de Jabalambre” [*sic*], “in solo petroso cacuminis”, 2020 m, leg. J. Borja 3.8.1960, in herb. Borja (holotype: MAF; isotype: JE).

Distribution. Mountains of the Iberian Peninsula, excluding the Pyrenees.

Androsace vitaliana subsp. *assoana* var. *assoana* (Láinz) Kress in Primulaceen-Studien 13: 10. 1997 \equiv *Vitaliana intermedia* O. Schwarz in Feddes Repert. 67: 40. 1963 \equiv *Vitaliana primuliflora* subsp. *assoana* Láinz in Bot. Inst. Edt. Astur. Supplem.

Cienc. 10: 199. 1964. \equiv *Androsace centriberica* subsp. *assoana* (Lainz) Kress in Primulaceen-Studien 15: 2. 1999 — Type: Spain, Teruel, “Sierra de Jabalambre” [*sic*], “in solo petroso cacuminis”, 2020 m, leg. J. Borja 3.8.1960 (holotype: MAF; isotype: JE).

Distribution. Sierra de Javalambre.

Androsace vitaliana subsp. *assoana* var. *flosjugorum* (Kress) Dixon, Guterm. & Schneew. stat. nov. \equiv *Androsace vitaliana* subsp. *flosjugorum* Kress in Primulaceen-Studien 13: 9. 1997 — Type: Spain, Cantabria, Peña Prieta, “zwischen Alto de Cubil de Can und Alto del Tio Celestino”, 2200–2450 m, 8.6.1967. “In herb. Kress V41+”.

= *Androsace centriberica* subsp. *maragatorum* Kress in Primulaceen-Studien 15: 2. 1999 — Type: Spain, León, Montes de León, “vom Paß im OOSO von Peñalba de Santiago - (14 km sssö Ponferrada) auf der S-Seite - des Baches Santiago ca. 150 m abwärts - 1450–1300 m”, in herbario Kress.

Distribution. Cordillera Cantábrica and Montes de León.

Androsace vitaliana subsp. *assoana* var. *centriberica* Kress in Primulaceen-Studien 13: 10. 1997 \equiv *Androsace vitaliana* subsp. *aurelii* Luceño in Anales Jard. Bot. Madrid 56: 165. 1998 — Type: Spain, Ávila, Sierra de El Barco, cuerda de la Covacha del Losat, 30T TK 8056, 2300 m, en pastizales psicroxerófilos, 27.7.1983. Leg. M. Luceño, 260028 (MA).

\equiv *Androsace centriberica* subsp. *centriberica* [per Kress in Primulaceen-Studien 15: 2. 1999]

Distribution. Sistema Central (Sierra de Gredos and probably also Sierra de Guadarrama).

This variety is found in the Sierra de Gredos and we assume on geographical grounds that this is also the taxon from the Sierra de Guadarrama, from where we have not seen any material.

Androsace vitaliana subsp. *assoana* var. *nevadensis* (Chiarugi) Kress in Primulaceen-Studien 13: 10. 1997 \equiv *Vitaliana primuliflora* var. *nevadensis* Chiarugi in Nuov. Giorn. Bot. Ital. 37: 338, 1930 [basonym] \equiv *Androsace vitaliana* subsp. *nevadensis* (Chiarugi) Luceño in Anales Jard. Bot. Madrid 56: 165. 1998 (1998) — Lectotype (here designated): Spain, Sierra Nevada, “région nivieuse, au *Picacho de Veleta*”, 5.7.1851. J. Gay in E. Bourgeau, *Plantes d’Espagne*. Herbarium Webbianum, 120348 (FI).

= *Vitaliana congesta* O. Schwarz in Feddes Repert. 67: 25, 1963 = *Androsace congesta* (O. Schwarz) Kress in Primulaceen-Studien 15: 2. 1999 — Type: Spain, Sierra Nevada, Cerro de Medio Dia, 2800 m, leg. Dürck (M).

Distribution. Sierra Nevada.

The basionym of this name, Chiarugi's (1930) var. *nevadensis*, has no explicitly designated type specimen; Chiarugi lists six specimens which he consulted, and the lectotype must be chosen from among these six syntypes. We have been able to see detailed photographs of five of the six specimens, and only one of those bears a significant number of flowers. We have therefore chosen this specimen to be the lectotype. Schwarz (1963) used the epithet *congesta* and chose a specimen not investigated by Chiarugi to be the type.

Acknowledgements

We thank Lajos Somlyay and an anonymous reviewer for their helpful comments. We thank the herbaria of the University of Vienna (WU) the Real Jardín Botánico, Madrid (MA), the Museo di Storia Naturale dell'Università di Firenze (FI) and the Conservatoire et Jardin botaniques de la Ville de Genève (G). Financial support was provided by the European Community's programme "Structuring the European Research Area", under SYNTHESYS grant ES-TAF-1632 and by the Austrian Science Fund (FWF) under grant P16104-B03. This article was supported by the Open Access Publishing Fund of the University of Vienna.

References

- Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV (2012) Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution* 29: 2157–2167. <https://doi.org/10.1093/molbev/mss084>
- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10(4): e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- Bryant D, Bouckaert R, Felsenstein J, Rosenberg NA, RoyChoudhury A (2012) Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution* 29: 1917–1932. <https://doi.org/10.1093/molbev/mss086>
- Chiarugi A (1930) *Vitaliana primulaeflora* Bertol., studio carilogico, sistematico e fitogeografico. *Nuovo Giornale Botanico Italiano (Nuova Serie)* 37: 319–368. <https://doi.org/10.1080/11263503009440374>
- Dixon CJ, Schönswetter P, Vargas P, Ertl S, Schneeweiss GM (2009) Bayesian hypothesis testing supports long-distance Pleistocene migrations in a European high mountain plant

- (*Androsace vitaliana*, Primulaceae). *Molecular Phylogenetics and Evolution* 53: 580–591. <https://doi.org/10.1016/j.ympev.2009.07.016>
- Ferguson IK (1969) Notes on the typification of the name *Vitaliana primuliflora* (Primulaceae). *Taxon* 18: 300–303. <https://doi.org/10.2307/1218832>
- Ferguson IK (1972) *Vitaliana* Sosl. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Moore DM (Eds) *Flora Europaea* 3. Cambridge University Press, Cambridge (U. K.), 20.
- Heß HE, Landolt E, Hirzel R (1970) *Flora der Schweiz* 2. Birkhäuser, Basel, 1–956.
- Kass RE, Raftery AE (1995) Bayes factors. *Journal of the American Statistical Association* 90: 773–795. <https://doi.org/10.1080/01621459.1995.10476572>
- Kress A (1967) 104. Familie Primulaceae. Nachträge, Berichtigungen und Ergänzungen. In: Hegi G (Ed.) *Illustrierte Flora von Mitteleuropa* 5. Carl Hanser, München, 2246b–2250b.
- Kress A (1970) Anmerkungen zur erneuten Typisierung von *Androsace vitaliana* (L.) Lap. (Primulaceae). *Taxon* 19: 890–899. <https://doi.org/10.2307/1218304>
- Kress A (1993) Zur Deutung und Typisierung einzelner Namen. *Primulaceen-Studien* 11: 1–26.
- Kress A (1997a) Zur Kenntnis der südwesteuropäischen *Vitaliana*-Sippen [*Androsace vitaliana* (L.) Lapeyr.]. *Primulaceen-Studien* 13: 1–40.
- Kress A (1997b) *Androsace*. In: Castroviejo S, Aedo C, Laínz M, Morales R, Muñoz Garmienda F, Nieto Feliner G, Paiva J (Eds) *Flora Iberica* 5. Real Jardín Botánico, CSIC, Madrid, 22–40. http://www.floraiberica.es/floraiberica/texto/pdfs/05_080_02%20Androsace.pdf
- Kress A (1999) Zur Kenntnis der iberischen *Androsace vitaliana* (L.) Lapeyr. *Primulaceen-Studien* 15: 1–2.
- Laínz M (1964) Aportaciones al conocimiento de la flora Cántabro-Astur, VIII (1). *Boletín del Instituto de Estudios Asturianos, Suplemento de Ciencias* 10: 173–218. https://bibliotecavirtual.asturias.es/i18n/publicaciones/numeros_por_mes.cmd?idPublicacion=24856&anyo=1964
- Leaché A, Fujita MK, Minin VN, Bouckaert RR (2014) Species delimitation using genome-wide SNP data. *Systematic Biology* 63: 534–542. <https://doi.org/10.1093/sysbio/syu018>
- Luceño M (1998) Una combinación y un nombre nuevos en *Androsace* (Primulaceae). *Anales del Jardín Botánico de Madrid* 56: 164–165. <http://rjb.revistas.csic.es/index.php/rjb/article/view/227/223>
- Lüdi W (1927) *Gregoria*. In: Hegi G (Ed.) *Illustrierte Flora von Mitteleuropa* 5. Pichler, Wien, 1787–1789.
- Pignatti S (1982) *Flora d'Italia* 2. Edagricole, Bologna, 1–732.
- Schwarz O (1963) Die Gattung *Vitaliana* Sosl. und ihre Stellung innerhalb der Primulaceen. *Feddes Repertorium* 67: 16–41.
- Sündermann F (1916) Aus verschiedenen Florengeländen. Beiträge, Bemerkungen und Notizen. *Allgemeine Botanische Zeitschrift für Systematik, Floristik, Pflanzengeographie* 22: 57–63. <http://www.biodiversitylibrary.org/item/38751>
- Vargas P (2003) Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon* 52: 463–476. <https://doi.org/10.2307/3647446>
- Vigo i Bonada J (1983) El poblament vegetal de la Vall de Ribes. I. Generalitats Catàleg florístic. *Acta Botanica Barcinonensia* 35: 1–793. <http://www.raco.cat/index.php/ActaBotanica/article/view/59516/86661>

Resurrection of the genus *Aphyllon* for New World broomrapes (*Orobanche* s.l., Orobanchaceae)

Adam C. Schneider¹

¹ Jepson Herbarium and Department of Integrative Biology, 1001 Valley Life Sciences Building, University of California, Berkeley, CA 94720-2465

Corresponding author: Adam C. Schneider (acschneider@berkeley.edu)

Academic editor: E. Fischer | Received 12 September 2016 | Accepted 28 November 2016 | Published 9 December 2016

Citation: Schneider AC (2016) Resurrection of the genus *Aphyllon* for New World broomrapes (*Orobanche* s.l., Orobanchaceae). *PhytoKeys* 75: 107–118. <https://doi.org/10.3897/phytokeys.75.10473>

Abstract

Recent phylogenetic studies support a monophyletic clade of New World broomrapes (*Orobanche* sects. *Gymnocaulis* and *Nothaphyllon*) sister to the Old World genus *Phelipanche*. I place the New World taxa in the genus *Aphyllon*, propose 21 new combinations, and provide a list of currently accepted taxa.

Keywords

Aphyllon, broomrape, *Gymnocaulis*, *Myzorrhiza*, *Nothaphyllon*, nomenclature, *Orobanche*, Orobanchaceae

Introduction

Phylogenetic analysis of broomrapes and related holoparasites using nuclear DNA have found that the small eastern Mediterranean genus *Diphelypaea* Nicolson is nested within *Orobanche* sensu lato (s.l.) as circumscribed by Beck (1890) (Schneeweiss et al. 2004a). Morphological and cytological differences between groups of taxa within *Orobanche* s.l. have led some botanists to adopt a narrower generic circumscription. In this taxonomic concept, *Orobanche* sensu stricto is limited to Old World species that lack bracteoles and have a base chromosome number of $x = 19$, a calyx divided to the base, and generally unbranched stems (Holub 1977, 1990). Other Old World broomrapes are treated as *Phelipanche* Pomel or the monotypic genus *Boulardia* F.W. Schultz

(syn: *O.* sect. *Trionychon* Wallr. and *O. latisquama* (F.W. Schultz) Batt., respectively; Joel 2009; Schneeweiss 2013).

Broomrape species native to the New World constitute two well-supported clades that together form a clade sister to *Phelipanche* (Schneider et al. 2016). Taxonomically, these clades have been recognized as two separate genera *Aphyllon* (= *Orobanche* sect. *Gymnocaulis* Nutt.) and *Myzorrhiza* Phil. (= *O.* sect. *Nothaphyllon* (A. Gray) Heckard) by Holub (1977, 1990) and others (Schneeweiss 2013), or more rarely, together as *Aphyllon* s.l. (Gray 1876). However, neither of these generic taxonomies has been widely adopted among American botanists, in part because of the lack of available names for many taxa. Providing evidence to support the treatment of all New World broomrapes as *Aphyllon* and a providing list of recognized species (with homotypic synonymms) is the purpose of this paper. New combinations are made where appropriate.

Methods

In order to compare molecular branch lengths of major clades of *Orobanche* s.l., a maximum likelihood (ML) phylogram of *Aphyllon* and related holoparasites was inferred from 3 nuclear DNA loci (ITS, phytochrome A, and phytochrome B). All sequences were downloaded from Genbank, aligned, and concatenated into a supermatrix using SUMAC (Freyman 2015). The ML phylogeny was estimated using RAxML (Stamatakis 2014) with a GTR+ Γ nucleotide substitution model and 1000 rapid bootstrapping replicates.

Information about type specimens, basionyms, and synonymy of these new combinations was gathered by examining protologues and images of type specimens using major databases, including Tropicos (<http://www.tropicos.org>), JSTOR Global Plants (<http://plants.jstor.org>), and the International Plant Names Index (<http://www.ipni.org>). Types for all North American taxa and *O. weberbaueri* Mattf. have been designated by previous authors and are presented here. For three of the four South American taxa, typification would require more careful efforts beyond the scope of this article. No repository is given in the protologue for two syntypes of *Orobanche tacnaensis* Mattf. (Woitschach 71 and F. J. F. Meyen s.n.). The current existence of these specimens could not be verified, although a photograph of the Woitschach 71 (possibly from a specimen at B) is available at F. No specimens are cited by Rodolfo Phillipi in the protologues of the two taxa that he described.

Discussion

Molecular phylogenetic analyses have consistently supported a sister-group relationship between two strongly supported two American clades, representing *O.* sect. *Gymnocaulis* and *O.* sect. *Nothaphyllon* (McNeal et al. 2013; Schneider et al. 2016; Fig. 1). This relationship is supported by biogeography and synapomorphies such as a calyx with five fully developed lobes and a base chromosome number of $x = 12$, with polyploidy in most

***Aphyllon fasciculatum* (Nutt.) Torr & A. Gray, Manual (ed. 2) 281. 1848.**

Orobanche fasciculata Nutt., Gen. N. Amer. Pl. 2: 59. 1818.

Phelipaea fasciculata (Nutt.) Spreng., Syst. Veg. [Sprengel] 2: 818. 1825.

Loxanthes fasciculatus (Nutt.) Raf., Neogenyt. 3. 1825.

Anoplon fasciculatum (Nutt.) G. Don., Gen. Hist. 4: 633. 1838.

Anoplanthus fasciculatus (Nutt.) Walp., Repert. Bot. Syst. 3: 480. 1844.

Thalesia fasciculata (Nutt.) Britton, Mem. Torrey Bot. Club 5: 298. 1894.

Type. USA: “Missouri”, ca. 1811, *Nuttal s.n.*, (holotype, PH).

***Aphyllon purpureum* (A. Heller) Holub, Preslia 70: 100. 1998.**

Thalesia purpurea A. Heller, Bull. Torrey Bot. Club 24: 313. 1896.

Orobanche porphyrantha Beck, Pflanzenr. 96[IV,261]: 49. 1930.

Orobanche uniflora var. *purpurea* (A. Heller) Achey, Bull. Torrey Bot. Club 60: 445. 1933.

Type. USA: Idaho: Nez Perce Co.: near mouth of the Potlatch, 20 May 1896, *Heller 3099*. (no holotype designated; isotypes, CAS, DAO, K, MIN, MO, MSC, NDG, PH, US).

***Aphyllon uniflorum* (L.) Torr & A. Gray, Manual (Gray) 290. 1848**

Orobanche uniflora L., Sp. Pl. 2: 633. 1753.

Anoplanthus uniflorus (L.) Endl., Gen. Pl. [Endlicher] 727. 1839.

Thalesia uniflora (L.) Britton, *Mem. Torrey Bot. Club* 5: 298. 1894.

Type locality. USA: Virginia (lectotype, *Clayton 387*, BM).

***Aphyllon* sect. *Nothaphyllon* A. Gray, Bot. California [W.H. Brewer] 1: 584. 1876**

Myzorrhiza Phil., Linnaea 29: 36. 1858. [Type: *Myzorrhiza chilensis* Phil.]

Orobanche sect. *Myzorrhiza* Beck, Bibliotheca Botanica 4(19): 78. 1890.

Orobanche sect. *Nothaphyllon* (A. Gray) Heckard, Madroño 22: 41. 1973.

Type. *Aphyllon californicum* (Cham. & Schltld.) A. Gray, lectotype designated by Heckard, Madroño 22: 41. 1973.

Description. Stems clearly rising above ground. Pedicels equal to or shorter than flower. Bracteoles subtending the calyx 1 or 2.

***Aphyllon arizonicum* (L.T. Collins) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77158997-1

Orobanche arizonica L.T. Collins, *Phytoneuron* 2015–48: 16, f. 1, 2, 4, 5, 6A, 7. 2015.**Type.** USA: Arizona, Coconino Co.: near Tuba City, 1539 m, 27 September 1935, *Kearney & Peebles 12867* (holotype, ARIZ; isotype, US).***Aphyllon californicum* (Cham. & Schltdl.) A. Gray, Bot. California 1: 584. 1876.***Orobanche californica* Cham. & Schltdl., *Linnea* 3: 134–136. 1828.*Phelypaea californica* (Cham. & Schltdl.) G. Don, *Gen. Hist.* 4: 632. 1838.*Myzorrhiza californica* (Cham. & Schltdl.) Rydb., *Bull. Torrey Bot. Club* 36: 696. 1909.**Type.** USA: California: Near Port of San Francisco, Aug 1816, Chamisso s.n (holotype, LE).***Aphyllon californicum* subsp. *condensum* (Heckard) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159010-1

Orobanche californica subsp. *condensa* Heckard, *Madroño* 22: 59–60, f. 1I-L, 5. 1973.**Type.** USA: California: San Luis Obispo Co.: Yaro Creek, 25 May 1955, *Bacigalupi, Ferris & Robbins 5242* (holotype, JEPS; isotypes, NY, RSA, US, WTU).***Aphyllon californicum* subsp. *feudgei* (Munz) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159005-1

Orobanche grayana var. *feudgei* Munz, *Bull. Torrey Bot. Club* 57: 616–617, pl. 38, f. 8. 1930.*Orobanche californica* subsp. *feudgei* (Munz) Heckard, *Madroño* 22: 62. 1973.**Type.** USA: California: San Bernardino Co.: Baldwin Lake, 2 June 1924, *Munz 8177* (holotype, POM).***Aphyllon californicum* subsp. *grande* (Heckard) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159011-1

Orobanche californica subsp. *grandis* Heckard, *Madroño* 22: 60–62, f. 1P-R, 3A, 4E, 5. 1973.

Type. USA: California: Santa Barbara Co.: dunes at Surf, 22 July 1954, *H. M. Pollard* (holotype, UC; isotype, CAS).

***Aphyllon californicum* subsp. *grayanum* (Beck) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159006-1

Orobanche grayana Beck, *Biblioth. Bot.* 4: 79. 1890.

Myzorrhiza grayana (Beck) Rydb., *Bull. Torrey Bot. Club* 36: 695. 1909.

Orobanche californica subsp. *grayana* (Beck) Heckard, *Madroño* 22: 54. 1973.

Type. USA: Oregon: banks of the Columbia River, 1825, *Douglas s.n.* (lectotype, K).

***Aphyllon californicum* subsp. *jepsonii* (Munz) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159007-1

Orobanche grayana var. *jepsonii* Munz, *Bull. Torrey Bot. Club* 57: 617, pl. 38, f. 10. 1930.

Orobanche californica subsp. *jepsonii* (Munz) Heckard, *Madroño* 22: 57. 1973.

Type. USA: California: Colusa Co.: Princeton, October 1905, *H. P. Chandler s.n.* (holotype: POM, isotype: UC).

***Aphyllon chilense* (Phil.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77158998-1

Myzorrhiza chilensis Phil., *Linnaea* 29: 36–37. 1857.

Orobanche chilensis (Phil.) Beck, *Biblioth. Bot.* 4: 82–83. 1890.

***Aphyllon cooperi* A. Gray, Proc. Amer. Acad. Arts 20: 307. 1885.**

Orobanche ludoviciana var. *cooperi* (A. Gray) Beck, *Biblioth. Bot.* 4(Heft 19): 81. 1890.

Orobanche cooperi (A. Gray) A. Heller, *Cat. N. Amer. Pl.* 7. 1898.

Myzorrhiza cooperi (A. Gray) Rydb. *Bull. Torrey Bot. Club* 36: 695. 1909.

Type locality. USA: Arizona: Fort Mojave (lectotype designated by Munz, *Bull. Torrey Bot. Club* 57: 620–21, *Cooper s.n.* in 1860–61, GH).

***Aphyllon cooperi* subsp. *latilobum* (Munz) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159008-1

Orobanche ludoviciana var. *latiloba* Munz, *Bull. Torrey Bot. Club* 57: 621–622, pl. 39, f. 18. 1930.

Orobanche cooperi subsp. *latiloba* (Munz) L.T. Collins, *Phytoneuron* 2015–48: 15. 2015.

Type. USA: California: Riverside Co.: Colorado Desert, 22 April 1922, *Munz & Keck 4960* (holotype: POM, isotype, US).

***Aphyllon cooperi* subsp. *palmeri* (Munz) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159009-1

Orobanche multicaulis var. *palmeri* Munz, *Bull. Torrey Bot. Club* 57: 613, pl. 38, f. 2. 1930.

Orobanche cooperi subsp. *palmeri* (Munz) L.T. Collins, *Phytoneuron* 2015–48: 16. 2015.

Type. Mexico, Durango, April–November 1896, *Palmer 7* (holotype: GH, isotypes, MO, UC).

***Aphyllon corymbosum* (Rydb.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77158999-1

Myzorrhiza corymbosa Rydb., *Bull. Torrey Bot. Club* 36: 696. 1909.

Orobanche corymbosa (Rydb.) Ferris, *Contr. Dudley Herb.* 5: 99. 1958.

Type. USA: Reynold's Creek, 2 July 1892, *Isabel Mulford s.n.* (holotype, NY; isotype, MO).

***Aphyllon corymbosum* subsp. *mutabile* (Heckard) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159012-1

Orobanche corymbosa subsp. *mutabilis* Heckard, *Canad. J. Bot.* 56: 187–188. 1978.

Type. USA: Washington: Grant Co.: O'Sullivan Dam, 11 July 1950, *S. W. Harris 97* (holotype, WS).

***Aphyllon dugesii* S. Watson, *Proc. Amer. Acad. Arts* 18: 132. 1883.**

Orobanche dugesii (S. Watson) Munz, *Bull. Torrey Bot. Club* 57: 613, t. 38, f. 3. 1931.

Type. Mexico: Gueanajatao, *Dugès s.n.* (holotype, GH).

***Aphyllon ludovicianum* (Nutt) A. Gray. Bot. California [W.H.Brewer] 1. 585.**

Orobanche ludoviciana Nutt. Gen. N. Amer. Pl. 2: 58–59. 1818.

Phelypaea ludoviciana (Nutt) Walp. Repert. Bot. Syst. 3: 461. 1844.

Myzorrhiza ludoviciana (Nutt) Rydb. Fl. S.E. U.S 1338. 1903.

Type. USA: Fort Mandan, 1810-1811, *Nuttall s.n.* (holotype, PH).

***Aphyllon multiflorum* (Nutt) A. Gray. Bot. California [W.H.Brewer] 1. 585.**

Orobanche multiflora Nutt., J. Acad. Nat. Sci. Philadelphia, ser. 2 1: 179. 1848.

Type. USA: Rio Grande, 1845, *Gambel s.n.* (neotype designated by White & Holmes, Sida 19: 623, USA: Texas: Jim Wells Co., 19 April 1944, *Lundell & Lundell* 12809, LL; isoneotype, LL).

***Aphyllon parishii* (Jeps.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159001-1

Orobanche californica var. *parishii* Jeps. *Man. Fl. Pl. Calif.* 952. 1925.

Orobanche parishii (Jeps.) Heckard. *Madroño* 22: 66. 1973.

Type. USA: California: San Bernardino Co.: Bear Valley, 1894, *S. B. Parish s.n.* (holotype, JEPS).

***Aphyllon parishii* subsp. *brachylobum* (Heckard) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159013-1

Orobanche parishii subsp. *brachyloba* Heckard, *Madroño* 22: 68–70, 2J, 3N, 5. 1973.

Type. USA: California: Ventura Co.: Dutch Harbor, San Nicolas Island, 23 April 1966, *Raven & Thompson 20794* (holotype, JEPS; isotypes, MO, RSA, SBBG).

***Aphyllon pinorum* (Geyer ex Hook.) A. Gray, Bot. California 1: 585. 1876.**

Orobanche pinorum Geyer ex Hook., *Hooker's J. Bot. Kew Gard.* 3:297–298. 1851.

Type. USA: Idaho/Washington border, *Geyer 445* (holotype, K).

***Aphyllon riparium* (L.T. Collins) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159002-1

Orobanche riparia L.T. Collins, *J. Bot. Res. Inst. Texas* 3: 7–10, f. 1A-B, 2. 2009.**Type.** USA: Indiana, Gibson Co.: Griffin, 16 August 1931, *Deam 50941* (holotype, IND; isotypes, A, F, GH, IND, MINN, WIS).***Aphyllon robbinsii* (Heckard ex Colwell & Yatsk.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159015-1

Orobanche robbinsii Heckard ex Colwell & Yatsk., *Phytoneuron* 2016-58: 2. 2016.**Type.** USA: California: San Francisco Co.: Lands End, 13 August 1956, *Robbins 3707* (holotype, JEPS; isotypes, CAS, GH, NY).***Aphyllon tacnaense* (Mattf.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159016-1

Orobanche tacnaensis Mattf., *Notizbl. Bot Gart. Berlin-Dahlem* 8: 185–186. 1922.**Syntypes.** Peru: Tacna, 1890, *Woitschach 71* (photograph of type: F); Peru: Tacna, 1833, *F. J. F. Meyen s.n.****Aphyllon tarapacatum* (Phil.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159003-1

Orobanche tarapacana Phil., *Anales Mus. Nac. Santiago de Chile* 1891: 69. 1891.***Aphyllon tuberosum* (A. Gray) A. Gray, *Bot. California* 1: 585. 1876.***Phehypaea tuberosa* A. Gray, *Proc. Amer. Acad. Arts* 7: 371. 1868.*Orobanche bulbosa* Beck, *Biblioth. Bot.* 4: 83–84. 1890.**Type.** USA: California: Monterey Co: Gavilan Mountains, 1860–1862, *Brewer 743* (holotype, GH; isotype K).

***Aphyllon validum* (Jeps.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159017-1

Orobanche valida Jeps., *Madroño* 1: 255–256. 1929.*Orobanche ludoviciana* var. *valida* (Jeps.) Munz, *Bull. Torrey Bot. Club* 57: 621. 1930.**Type.** USA: California: Rock Creek, San Gabriel Mountains, 2 June 1923, *F. W. Peirson* 7937 (holotype: JEPS, isotype: RSA).***Aphyllon validum* subsp. *howellii* (Heckard & L.T Collins) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159018-1

Orobanche valida subsp. *howellii* Heckard & L.T Collins, *Madroño* 29: 98–100, f. 1A–E. 1982.**Type.** USA: California: Mendocino Co.: Impassable Rock, 14 July 1951, *Donald V. Hemphill s.n.* (holotype: UC).***Aphyllon vallicolum* (Jeps.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159004-1

Orobanche comosa var. *vallicola* Jeps., *Man. Fl. Pl. Calif.* 952. 1925.*Orobanche vallicola* (Jeps.) Heckard, *Madroño* 22: 64. 1973.**Type.** USA: California: Santa Clara Co.: Coyote, 14 October 1914, *W. L. Jepson* 6196 (holotype: JEPS, isotypes: GH, MO).***Aphyllon weberbaueri* (Mattf.) A.C. Schneid., comb. nov.**

urn:lsid:ipni.org:names:77159019-1

Orobanche weberbaueri Mattf., *Notizbl. Bot Gart. Berlin-Dahlem* 8: 185. 1922.**Type.** Peru: Camaná: Areuipa, Hafen Chala, 26 November 1915, *A. Weberbauer* 7185 (isotypes: GH, US).**Acknowledgments**

I thank Bruce Baldwin, Alison Colwell, Gerald Schneeweiss, John Strother, and George Yatskievych for reviewing earlier versions of this article and for nomenclatorial advice.

References

- Beck G (1890) Monographie der Gattung *Orobanchae*. Bibliotheca Botanica 19. Theoder Fischer, Cassel.
- Freyman WA (2015) SUMAC: software for constructing phylogenetic supermatrices and assessing partially decisive taxon coverage. *Evolutionary Bioinformatics* 11: 263–266. <https://doi.org/10.4137/EBO.S35384>
- Gray A (1848) Manual of the botany of the northern United States. James Munroe and Co, Boston.
- Gray A (1856) Manual of the botany of the northern United States (edn 2). George P. Putnam and Co, New York. <https://doi.org/10.5962/bhl.title.50405>
- Gray A (1876) *Aphyllon*. In: Brewer WH, Watson S (Eds) Geological Survey of California Botany. Volume 1, 584–585.
- Heckard LR, Chuang TI (1975) Chromosome numbers and polyploidy in *Orobanchae* (Orobanchaceae). *Brittonia* 27: 179–186. <https://doi.org/10.2307/2805479>
- Holub J (1977) New names in Phanaerogamae 6. *Folia Geobotanica et Phytotaxonomica* 12: 417–432. <https://doi.org/10.1007/BF02890652>
- Holub J (1990) Some taxonomic and nomenclatural changes within *Orobanchae* s.l. (Orobanchaceae). *Preslia* 62: 193–198.
- Joel DM (2009) The new nomenclature of *Orobanchae* and *Phelipanche*. *Weed Research* 49: 6–7. <https://doi.org/10.1111/j.1365-3180.2009.00748.x>
- McNeal JR, Bennett JR, Wolfe AD, Mathews S (2013) Phylogeny and origins of holoparasitism in Orobanchaceae. *American Journal of Botany* 100: 971–983. <https://doi.org/10.3732/ajb.1200448>
- Mitchell J (1769) *Dissertatio brevis de principiis botanicorum et zoologorum*. W. Schwartzkopf.
- Rydberg PA (1906) Studies on the Rocky Mountain Flora XVI. *Bulletin of the Torrey Botanical Club* 33: 137–162. <https://doi.org/10.2307/2478824>
- Rydberg PA (1909) Studies on the Rocky Mountain Flora XX. *Bulletin of the Torrey Botanical Club* 36: 675–720. <https://doi.org/10.2307/2479009>
- Schneeweiss GM (2013) Phylogenetic relationships and evolutionary trends in Orobanchaceae. In: Joel DM, Gressel J, Mussleman LJ (Eds) *Parasitic Orobanchaceae*. Berlin Heidelberg: Springer, 243–265. https://doi.org/10.1007/978-3-642-38146-1_14
- Schneeweiss GM, Colwell AEL, Park J-M, Jang C-G, Stuessy TF (2004a) Phylogeny of holoparasitic *Orobanchae* (Orobanchaceae) inferred from nuclear ITS sequences. *Molecular Phylogenetics and Evolution* 30: 465–478. [https://doi.org/10.1016/S1055-7903\(03\)00210-0](https://doi.org/10.1016/S1055-7903(03)00210-0)
- Schneeweiss GM, Palomeque T, Colwell AEL, Weiss-Schneeweiss H (2004b) Chromosome numbers and karyotype evolution in holoparasitic *Orobanchae* (Orobanchaceae) and related genera. *American Journal of Botany* 91: 439–448. <https://doi.org/10.3732/ajb.91.3.439>
- Schneider AC, Colwell AEL, Schneeweiss GM, Baldwin BG (2016) Cryptic host-specific diversity among western hemisphere broomrapes (*Orobanchae* s.l., Orobanchaceae). *Annals of Botany* 118: 1101–1111. <https://doi.org/10.1093/aob/mcw158>
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>

Systematics of *Simplicia* Kirk (Poaceae, Agrostidinae) – an endemic, threatened New Zealand grass genus

Peter J. de Lange¹, Rob D. Smissen², Jeremy R. Rolfe³, Colin C. Ogle⁴

1 Terrestrial Ecosystems, Science and Policy Group, Department of Conservation, Private Bag 68908, Auckland 1145, New Zealand **2** Allan Herbarium, Landcare Research, P.O. Box 69, Lincoln 7640, New Zealand **3** Terrestrial Ecosystems, Science and Policy Group, National Office, Department of Conservation, P.O. Box 10420, Wellington 6143, New Zealand **4** 22 Forres Street, Whanganui, New Zealand

Corresponding author: Peter J. de Lange (pdelange@doc.govt.nz)

Academic editor: M. Nobis | Received 30 August 2016 | Accepted 28 November 2016 | Published 9 December 2016

Citation: de Lange PJ, Smissen RD, Rolfe JR, Ogle CC (2016) Systematics of *Simplicia* Kirk (Poaceae, Agrostidinae) – an endemic, threatened New Zealand grass genus. *PhytoKeys* 75: 119–144. <https://doi.org/10.3897/phytokeys.75.10328>

Abstract

A new species of the New Zealand endemic grass *Simplicia*, *S. felix* is described. The new species is segregated from and compared with *S. buchananii* and *S. laxa*. *Simplicia felix* occurs mostly in lightly shaded areas of seasonally dry alluvial forest. A distribution map and an assessment of the conservation status of the new species are presented. Genetic variation in the genus was examined, building on previously published work but including additional sampling. Analysis of nrDNA ITS and ETS and plastid *trnL* intron and *trnL*–F intergenic spacer sequences show *S. felix* to be more closely related to *S. laxa* than to *S. buchananii*. NeighborNet analyses of AFLP profiles for the three species of *Simplicia* show each to consist of distinct clusters of genotypes well separated from each other.

Keywords

Poaceae, *Simplicia*, new species, *S. felix*, *S. buchananii*, *S. laxa*, conservation status, New Zealand flora

Introduction

During 2005 one of us (Colin Ogle), chanced upon an unremarkable grass growing in an alluvial forest remnant on the margin of a grazed pasture in the Kawhatau Valley Mangaweka, in the Rangitikei District, North Island (Ogle 2010) (Fig. 1). When the identity of the grass could not be satisfactorily determined, the specimen was sent to

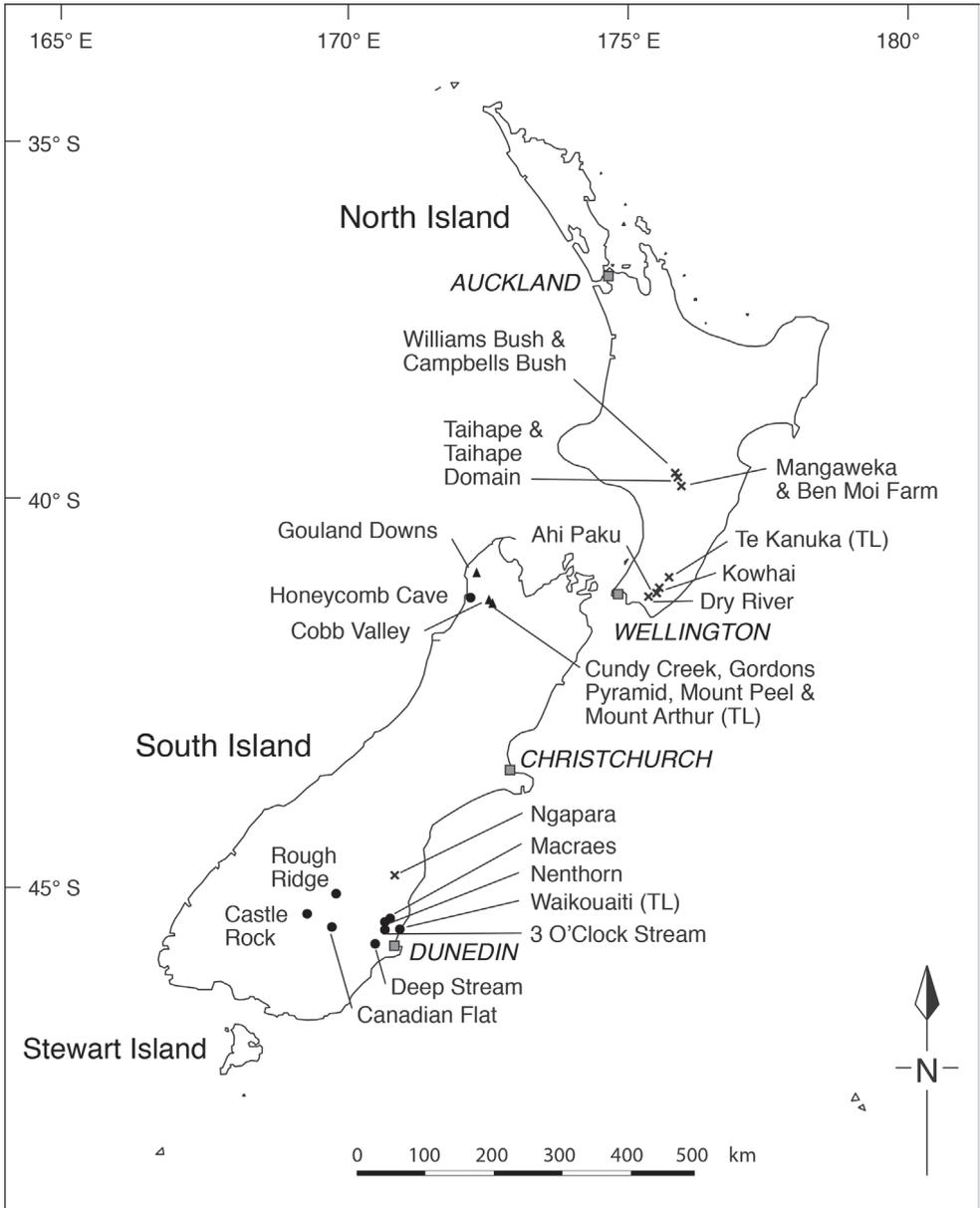


Figure 1. Distribution of *Simplicia* species and showing locations discussed in text.

various colleagues whereupon it was eventually determined as a species of the endemic ditypic New Zealand genus *Simplicia* Kirk. There the matter would have rested if it were not for the fact that there was no consensus on the identity of the species. The *Simplicia* had features in common with both *S. buchananii* (Zotov) Zotov and *S. laxa* Kirk.

To resolve the matter a DNA-based investigation of the genus was initiated to try to determine which species the plant actually was (Smissen et al. 2011). That study, utilising nuclear ribosomal DNA (nrDNA) sequences, plastid DNA sequences, and AFLP profiles, found that, while the North Island *Simplicia* was 'referable to *S. laxa*', those plants were genetically distinct from other South Island *S. laxa* populations (Smissen et al. 2011). A complication was that nrDNA sequence for a *Simplicia* sample from Ngapara (North Otago) that was not included in the AFLP study suggested it was more closely related to the North Island *Simplicia* than to other South Island samples (Fig. 1). Although Smissen et al. (2011) suggested a taxonomic revision of the genus was warranted, they advised that AFLP data needed to be obtained from the North Otago (Ngapara) population to confirm its relationship with North Island samples indicated by nrDNA sequences. They also recommended that a survey for *Simplicia* within the Wairarapa (Fig. 1) of the North Island be carried out. It was in the eastern Wairarapa during 1880, that Thomas Kirk first discovered the genus (Kirk 1897) and, whilst Kirk referred his Wairarapa material to *S. laxa* (which he described from plants collected in 1880 by Donald Petrie from Waikouaiti (Fig. 1) on the north-eastern Otago coastline, South Island), his herbarium material seemed to be closer to Ogle's potentially 'new' *Simplicia* than to *S. laxa* sens. str. When Smissen et al. (2011) concluded their study, 131 years had elapsed since *Simplicia* had been seen in Wairarapa, and Kirk's herbarium material was unlikely to yield suitable DNA for sequencing or AFLP profiling.

During February 2014 *Simplicia* was rediscovered in the Wairarapa (de Lange et al. 2014), from which fresh material and herbarium specimens were obtained. These enabled us to generate new genetic data for these plants and also a plant we received from Ngapara, for which we could now generate AFLP profiles and a full set of DNA sequences. This additional data confirmed that the Wairarapa and Ngapara *Simplicia* were part of the same genetic cluster as those found in the Mangaweka–Taihape area.

That data, along with morphological evidence, suggests that the recognition of a third species of *Simplicia* and a narrower circumscription of *S. laxa* are justified.

Materials and methods

Molecular phylogenetics

Details of samples and GenBank accessions are shown in Table 1.

For DNA sequencing methods see Smissen et al. 2011. New sequences for the *trnL* intron, *trnL*–*trnF* intergenic spacer, nuclear ribosomal (nrDNA) ITS and nrDNA ETS regions were generated for three Wairarapa *Simplicia* samples and one from Ngapara (Otago) and aligned with those from our previous study (see table 1). Outgroup sequences were those used in Smissen et al. (2011). Gaps were coded as binary characters using the method of Simmons and Ochoterena (2000) as implemented in Fastgap1.2

(Borchsenius 2009). Congruence between DNA regions was not re-assessed since new sequences were closely similar to those included in Smissen et al. (2010). DNA sequences were analysed under parsimony using PAUP 4.0b10 (Swofford 2003). A heuristic search strategy using default settings was employed. Trees were condensed to collapse branches where minimum branch length was 0. Bootstrap values were calculated from 1000 replicates saving a maximum of 10000 trees for each replicate.

New AFLP profiles were generated from the same three Wairarapa samples plus an additional sample (see Table 1) along with new AFLP profiles for a representative group of existing DNA samples from our previous study. One DNA sample (Ngapara) was subjected to duplicate DNA extraction and digestion/ligation, another (Te Kowhai) to duplicate digestion/ligation. All samples were duplicated for PCR amplification (preamp and selective), capillary separation and scoring. AFLP protocols followed the same method as Smissen et al. (2011) except that selective primers targeting EcoRI restriction sites were labelled with 6-FAM or VIC fluorescent dyes, and fragments were separated by capillary electrophoresis on an ABI3500XL genetic analyser using ROX labelled GS500 size standard instead of polyacrylamide slab gels. The selective primer combinations used were Eco-ACG with Mse-CAG and Eco-AAC with Mse-CTC. Automated scoring was conducted using GeneMapper 4.1 (ABI) starting at 100 bp and finishing at 500bp, using a bin width of 0.5 (see Holland et al. 2008), peak threshold of 30, signal strength among samples normalised using the sum of signalsetting, and max peak width of 1.5. We called alleles in three states based on the maximum peak intensity for each bin such that samples displaying peaks < 10% of the max intensity were scored as absent (0), samples displaying peaks > 10% but < 20% were scored as ambiguous (?) and samples with peaks > 20% were scored as present (1). AFLP profiles were analysed by NeighborNet as implemented in Splitstree 4.10 (Huson and Bryant 2006) using p-distances.

Plant collections and specimens

Descriptions of *S. buchananii*, *S. laxa* sens. str. and the new species were prepared using cultivated plants and herbarium specimens held at AK, CHR and WELT (herbarium acronyms follow Thiers (2016)). Live plants of *S. laxa* sens. str. and the new species were cultivated for this study at Oratia Native Plant Nursery, West Coast Road, Oratia, West Auckland. Measurements of spikelets, florets, fruits and hairs were obtained using a binocular Leica Wild M3C light microscope fitted with a graticule, which had been calibrated by staff at AK. Leaf, culm and inflorescence measurements were obtained using digital calipers (Mitutoyo Digimatic 500-321 CD-6). Measurements are based on a full sampling of the Herbarium specimens available, usually with 5–10 measurements made per specimen. The sole exception are those measurements obtained for the caryopsis of the three *Simplicia*, for which only limited examples were available (*Simplicia buchananii* $n = 2$, *S. laxa* sens. str. $n = 4$, *S. sp. nov.* $n = 5$)

Table 1. Sample details.

Species	Location	Region	Lat/long	vouchers	GenBank ITS	GenBank ETS	GenBank <i>trnL/F</i>
<i>Simplicia buchananii</i>	Cobb Valley	Nelson	41°7'50.96"S, 172°36'31.53"E	No Voucher	HM191441	HM191453	HM191463
<i>Simplicia buchananii</i>	West of Gordon's Pyramid	Nelson	41°11'15.24"S, 172°40'3.32"E	AK 304801	HM191439	HM191451	HM191465
<i>Simplicia buchananii</i>	Cundy Creek	Nelson	41°11'14.32"S, 172°40'02.28"E	AK 304802	HM191440	HM191452	HM191464
<i>Simplicia felix</i>	Williams's Bush	Wellington	39°37' 20.7"S, 175°45'54.02" E	CHR 607323	HM191437	HM191449	HM191461
<i>Simplicia felix</i>	Taihape Reserve	Wellington	39°40'14.29"S, 175°48'18.96"E	CHR 607322	HM191435	HM191447	HM191459
<i>Simplicia felix</i>	Ben Moi Farm	Wellington	39°46'50.25"S, 175°51'59.14"E	CHR 607321	HM191438	HM191450	HM191462
<i>Simplicia felix</i>	Kaumingi 1	Wellington	40°57' 50.18"S, 175°52' 15.83"E	AK 351330	KU724189	KU728132	KU728136
<i>Simplicia felix</i>	Kaumingi 2	Wellington	40°58' 4.56"S, 175°52' 26.49"E	AK 351325	KU724190	KU728131	KU728135
<i>Simplicia felix</i>	Te Kowhai	Wellington	41°10' 57.62"S, 175°40' 59.85"E	AK 351290	KU724191	KU728130	KU728134
<i>Simplicia laxa</i>	Honeycomb Cave	Nelson	41°8'25.79"S, 172°11'42.41"E	AK 288071 CHR 607318	HM191442	HM191454	HM191466
<i>Simplicia felix</i>	Ngapara	Otago		AK285424	KU724188	KU728129	KU728133
				CHR 607324 CHR 607326			
<i>Simplicia laxa</i>	Nenthorn	Otago	45°28'39.6"S, 170°22'47.3"E	CHR 607327 CHR 607328 CHR 607329	HM191445	HM191457	HM191470
<i>Simplicia laxa</i>	Macraes	Otago	45°25'35.95"S, 170°27'13.9"E	CHR 607319 CHR 607320	-	-	-
<i>Simplicia laxa</i>	Castle Rock	Otago	45°17'53.92"S, 169°16'13.87"E	AK 304847 AK 304848	HM191443 HM191446	HM191455 HM191458	HM191467 HM191469

Results

DNA sequences

Little resolution was obtained by phylogenetic analysis of the plastid DNA sequences which included only 12 variable characters (four with outgroups excluded), only five of which were parsimony informative (three with outgroups excluded). In the single most parsimonious tree (length 13) found by heuristic search of these (not shown) the three *S. buchananii* samples are characterised by two synapomorphic substitutions in the region sequenced. Some but not all of the North Island *S. laxa* samples share a single synapomorphic substitution.

More resolution was obtained in analysis of the nrDNA sequences which contained 196 variable characters (25 with outgroups excluded), 53 of which were parsimony informative (20 with outgroups excluded). Three equally parsimonious trees (length 218) were recovered by the heuristic search. One of these (Fig. 2) has the same topology as the strict consensus of most parsimonious trees. In all the most parsimonious nrDNA trees *S. buchananii* and *S. laxa sensu lato* are recovered as reciprocally monophyletic sister groups which received 99% and 95% bootstrap support respectively. Within *S. laxa sensu lato* all the North Island samples together with the Ngapara sample form a clade excluding the other South Island samples (bootstrap support 87%). For ease of readability the new name *Simplicia felix* is used for the North Island/Ngapara clade in the following Results and Discussion, and the name *S. laxa* is used for the South Island clade.

Accounting for scoring error in AFLP

Fragment scoring with the settings described above resulted in data matrices with 475 fragments for Eco-AAC with M-CTC and 559 for Eco-ACG with Mse-CAG. NeighborNet graphs generated for each primer pair showed similar grouping of samples (not shown) and we combined them to form a single data matrix with 1034 fragments. The NeighborNet for the full data matrix is shown in Fig. 3. Samples are grouped into three major groups; all North Island *S. laxa* samples together with the Ngapara sample, the Nelson *S. buchananii* samples, and the South Island *S. laxa* samples (i.e., excluding the Ngapara sample). It is apparent that replicate amplifications of the same DNA sample did not return the same scored genotype. Pairwise differences between replicate amplifications of the same sample ranged from 6.1% to 17.2%. Examination of the chromatograms suggests that at least most of the difference between replicates was due to differences in automated scoring as a result of binning and threshold effects rather than problems with the underlying chemistry producing different sets of fragments. The samples replicated from the DNA extraction or digestion/ligation stages were not notably more different than the remainder which were only duplicated from preamp stage on. We have eliminated this scoring

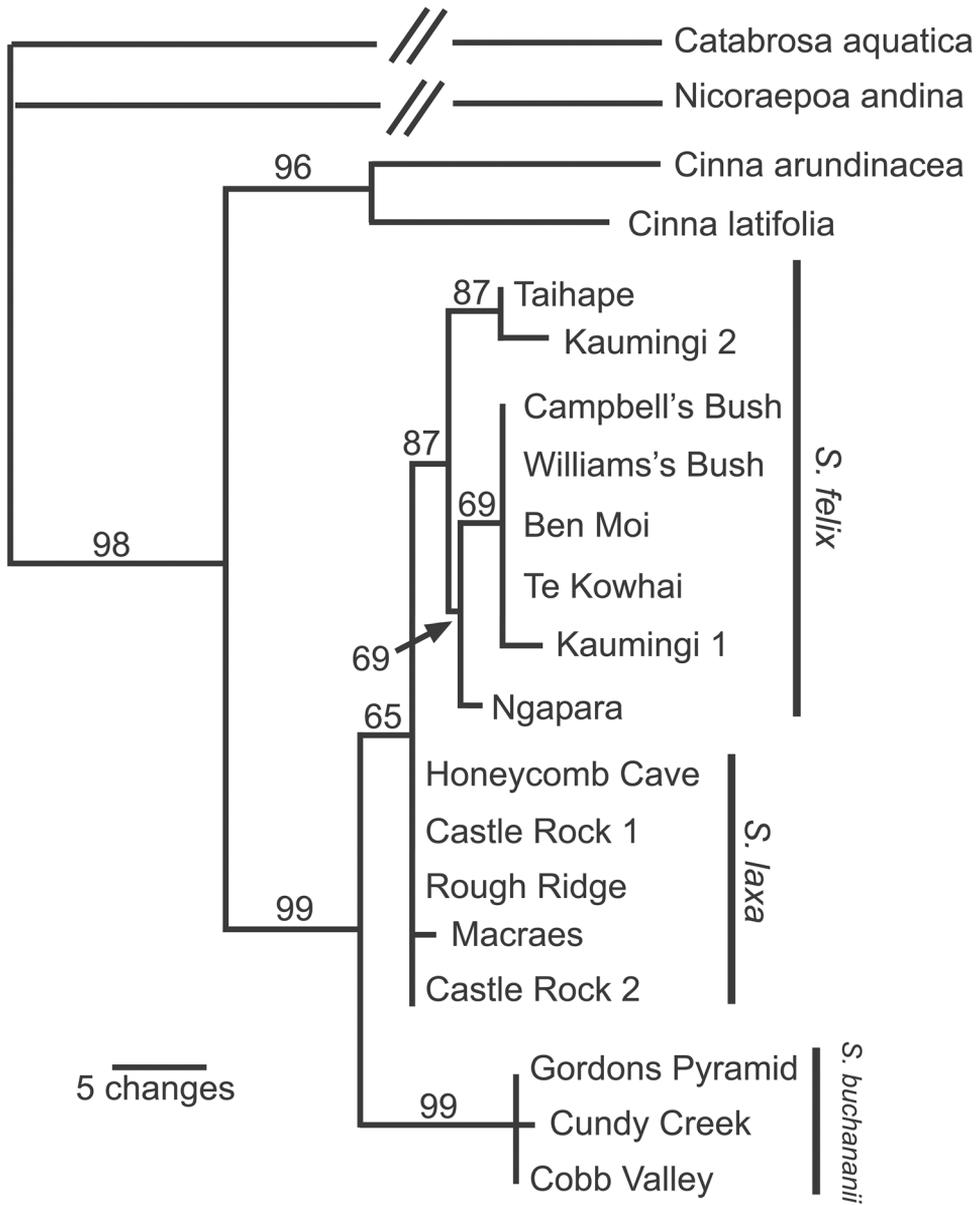


Figure 2. Selected most parsimonious tree for nrDNA sequence data. Numbers above branches are bootstrap percentages. Diagonal lines indicate that the branches leading to the outgroup *Nicoraepoa* and *Catabrosia* sequences are not shown to scale.

error through a two-step process. Firstly, we deleted from the data matrix all those bins where any sample displayed a peak with intensity ranging from 10 to 20% of the highest peak scored in that bin from any sample. We then deleted any remaining bins that were scored as present in one replicate and absent in the other replicate

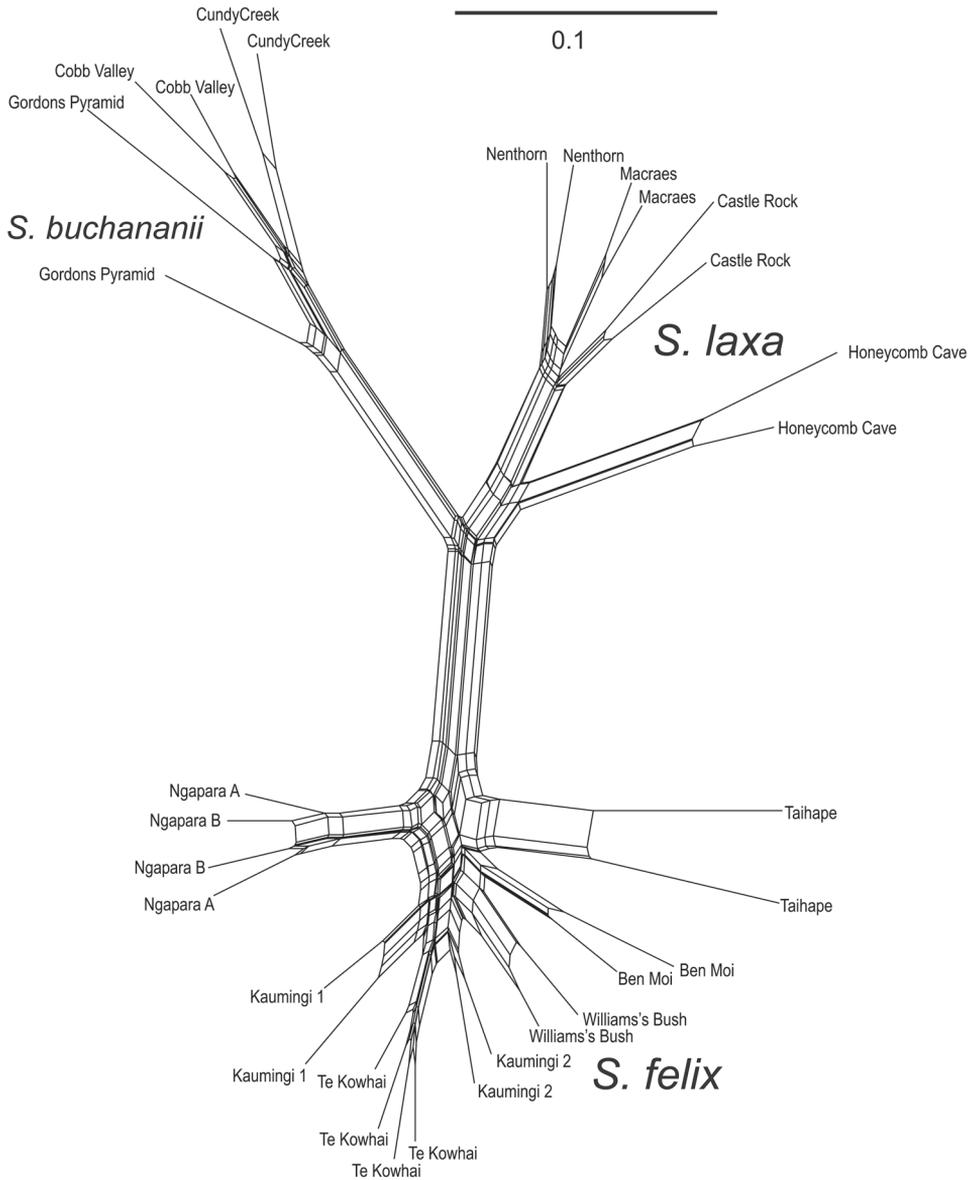


Figure 3. NeighborNet graph for AFLP data with all automatically scored polymorphisms.

for any sample. This left a much reduced matrix of 204 characters that were consistently scored between duplicates. A NeighborNet graph generated for this data set (Fig. 4) recovered the same major groups as the analysis with all automatically scored fragments.

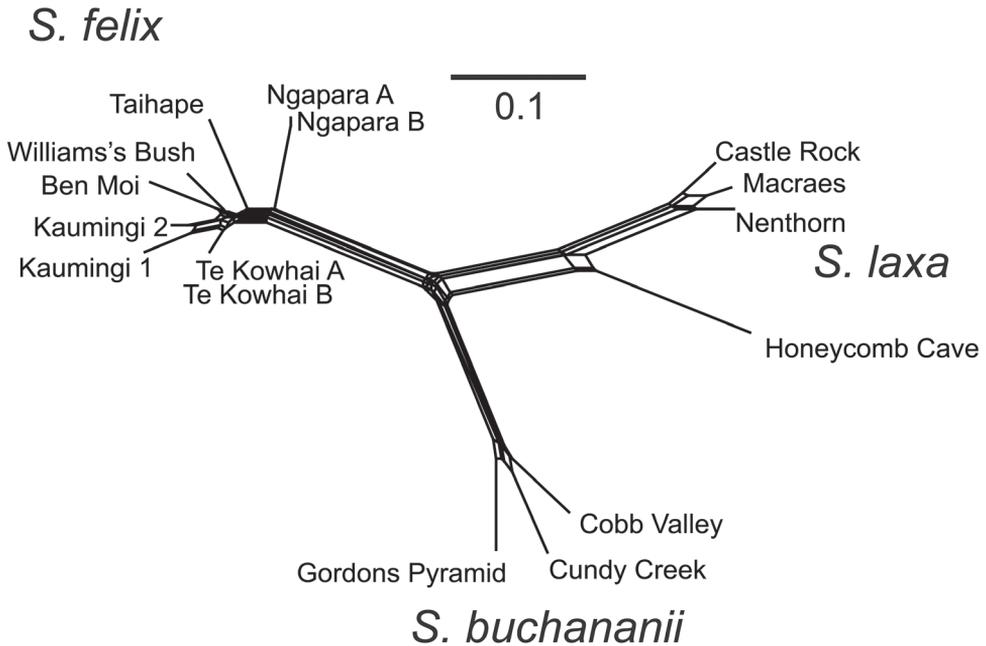


Figure 4. NeighborNet graph for reduced AFLP data with reproduced polymorphisms only.

AFLP groupings

Three major groups are obtained in both NeighborNet analyses of AFLP profiles. One group corresponds with *S. buchananii* including samples from Gordons Pyramid, Cobb Valley, and Cundy Creek (see Fig. 4). Samples of *S. laxa* sens. lat. are split into two groups. The first group, now *S. felix*, includes all the North Island samples and the sample from Ngapara. The second group, *S. laxa*, includes the Honeycomb Cave sample and the remaining Otago samples.

Discussion

Relative to eyeball scoring of silver-stained slab gels, fluorescent labelling and automated scoring AFLP profiles produces many more apparent polymorphisms but with considerably reduced profile reproducibility, at least for *Simplicia* in our hands (see Smissen et al. 2011). At face value, the error rates reported here (6.1% to 17.2%) are high compared to those reported by other studies (see Meudt and Clarke 2007) and could perhaps be lowered by adjusting scoring parameters. Instead, since most of this reduction in reproducibility appears to be the result of threshold and binning effects in automated scoring, much of it can be accounted for by duplicating samples and using a scoring protocol recording peaks of low–intermediate intensity as uncertain. Analyses of our AFLP profiles using all scored polymorphisms or just reproducible polymor-

phisms yield essentially similar results in terms of relationships among plants, but the former approach exaggerates the level of variation within genetic clusters of individuals at the expense of the level of variation among these clusters.

The results reported here are in accord with those obtained by Smissen et al. (2011) that suggested two distinct genetic groups are included within the current circumscription of *S. laxa*. Additionally, we show here that the sampled Wairarapa plants of *Simplicia* are most closely related to Taihape area and Ngapara (Otago) plants (*S. felix* sp. nov.), and less closely related to those from Honeycomb Cave (Nelson) and the remaining plants sampled from Otago (*S. laxa* sens. str.). The presence of these two genetic groups, distinguishable by AFLP profiles and nrDNA sequences, suggests that more than one taxon might be recognised within the current circumscription of *S. laxa*.

Seed set in isolated, glasshouse-grown plants indicate that both *S. buchananii* and *S. laxa* are self-compatible (Zotov 1971; Connor 1988). Moreover, Connor (1988) stated that their flower morphology suggests that self-fertilization is “not-infrequent”. It is possible that outcrossing is naturally rare in *Simplicia* and that genetic races could therefore persist in sympatry through a preponderance of selfing. The relatively low levels of genetic distance within the genetic groups of *S. laxa* compared to the distance between them (Fig. 4) is consistent with a predominantly uniparental reproductive strategy resulting in strong genetic bottlenecks. Therefore, the coexistence in Otago of two genotypes of *S. laxa* sens. lat. does not necessarily imply they should be recognised at species, or indeed any rank. On the other hand, the genetic (and morphological) differences between the two groups appear to be substantial, compared to the variation within each and there are consistent, if subtle, morphological differences between them. Therefore we recognise them here at species rank. Zotov’s (1971) lectotypification of *S. laxa* using Petrie’s Waikouaiti plants means that those plants with hairy culms, leaves and inflorescences, and larger inflorescences encompass *S. laxa* and that the remaining plants require a new name (*S. felix*). The necessary action to formalize this name is taken below.

Systematics

***Simplicia* Kirk, T.P.N.Z.I. 29: 497 (1897)**

Type. *S. laxa* Kirk (fide Zotov 1971)

Key to New Zealand *Simplicia*

- 1 Plants tufted; culms erect, up to 1 m tall (culm nodes not root-forming); inflorescences erect, linear, branches, erect, appressed to rachis, bearing spikelets almost to base ***S. buchananii***
- Plants decumbent; culms weakly ascendant, rooting freely from culm-nodes so forming diffuse interconnected widely sprawling clonal patches 0.6–1.0 m

- diameter; inflorescences linear to pyramidal, binate, basal branch or branches reflexed, devoid of spikelets from lower $\frac{1}{2}$ to $\frac{2}{3}$ **3**
- 2 Mid-stem and upper stem leaf sheaths finely ribbed, copiously hairy (hairs 0.35–0.40 mm long); adaxial leaf-blade ribs hairy; inflorescence branches antrorsely hairy, pedicels 1.00–1.06 mm long; lemma pubescent..... *S. laxa*
- Mid-stem and upper stem leaf sheaths strongly ribbed, \pm glabrous (occasionally bearing minute hairs towards sheath apex); adaxial leaf-blade ribs smooth or finely scabrid; inflorescence branches scabrid, pedicels 0.20–0.30 mm long; lemma minutely scabrid..... *S. felix*

The species

Simplicia buchananii (Zotov) Zotov, *New Zealand J.Bot.* **9**: 542 (1971)

\equiv *Poa uniflora* Buchanan Indig. Grasses N.Z. t49B (1880) non Muhl. (1817)

\equiv *Simplicia laxa* var. *buchananii* Zotov T.R.S.N.Z. 73: 236 (1943)

Holotype. ‘Mt Arthur’ *A. McKay s.n.*, 1874, (WELT SP059605!) (*vide* Zotov 1971).

Etymology. Named by Zotov (1971) for John Buchanan FLS (1819–1898), Scottish born draughtsman, and New Zealand’s first government employed botanist (Taylor 2002). Buchanan first described *Simplicia buchananii* as a species of *Poa*, *P. uniflora* Buchanan but that species name was preoccupied (*P. uniflora* Muhl.) (Zotov 1971).

Description (Fig. 5). Plants gracile, tufted, 0.40–0.60(–1.0) m tall. Culms 0.40–0.80 m long, bright green when fresh, wiry, erect (sometimes with apices weakly pendent), culm internodes 3–5, elongated, glabrous; internodes \pm equal in length to subtending leaf-sheaths. Culm-nodes slightly swollen when fresh, glossy orange-brown to dark red-brown (0.1–)0.3–0.4(–0.5) mm long. Basal leaf-sheaths stramineous or dull brown, membranous, strongly ribbed, usually glabrous, sometimes scabrid on ribs or evenly, finely pubescent; hairs when present retrorse to patent, minute (0.06–0.08 mm long); mid stem and upper leaf-sheaths stramineous to green, membranous, strongly ribbed, glabrous. Ligule 2.0–3.5(–4.0) mm, membranous, lanceolate, apex erose to very deeply lacerate; glabrous. Leaf-blade 100–200 \times (1.8–)3.0(–4.0) mm, green to yellow-green, flat, linear-lanceolate with acuminate apices, finely ribbed; adaxial ribs finely scabrid, abaxial ribs glabrous; margins smooth. Panicle (40–)160(–180) mm long, linear; rachis glabrous, branches (10–)30(–36) mm long, erect, glabrous (sometimes bearing minute antrorse prickle-teeth near pedicels), bearing spikelets almost to base, pedicels 0.6–1.0(–1.2) mm long, \pm glabrous (sometimes bearing sparse, minute, antrorse prickle-teeth), \pm appressed to branchlets. Spikelets 2.8–3.0 mm, 1(–2)-flowered, lanceolate, light green to stramineous. Glumes green (\pm hyaline), glabrous, ovate-lanceolate to ovate, acute to subacute, 1-nerved, lower and upper glume margins entire, ciliate; lower glume 0.3–1.0 mm, upper glume 1.0(–1.6) mm. Lemma 2.6–3.0 mm, light green to grey, scabrid (densely covered in minute prickle-teeth), 3(–5)-nerved,

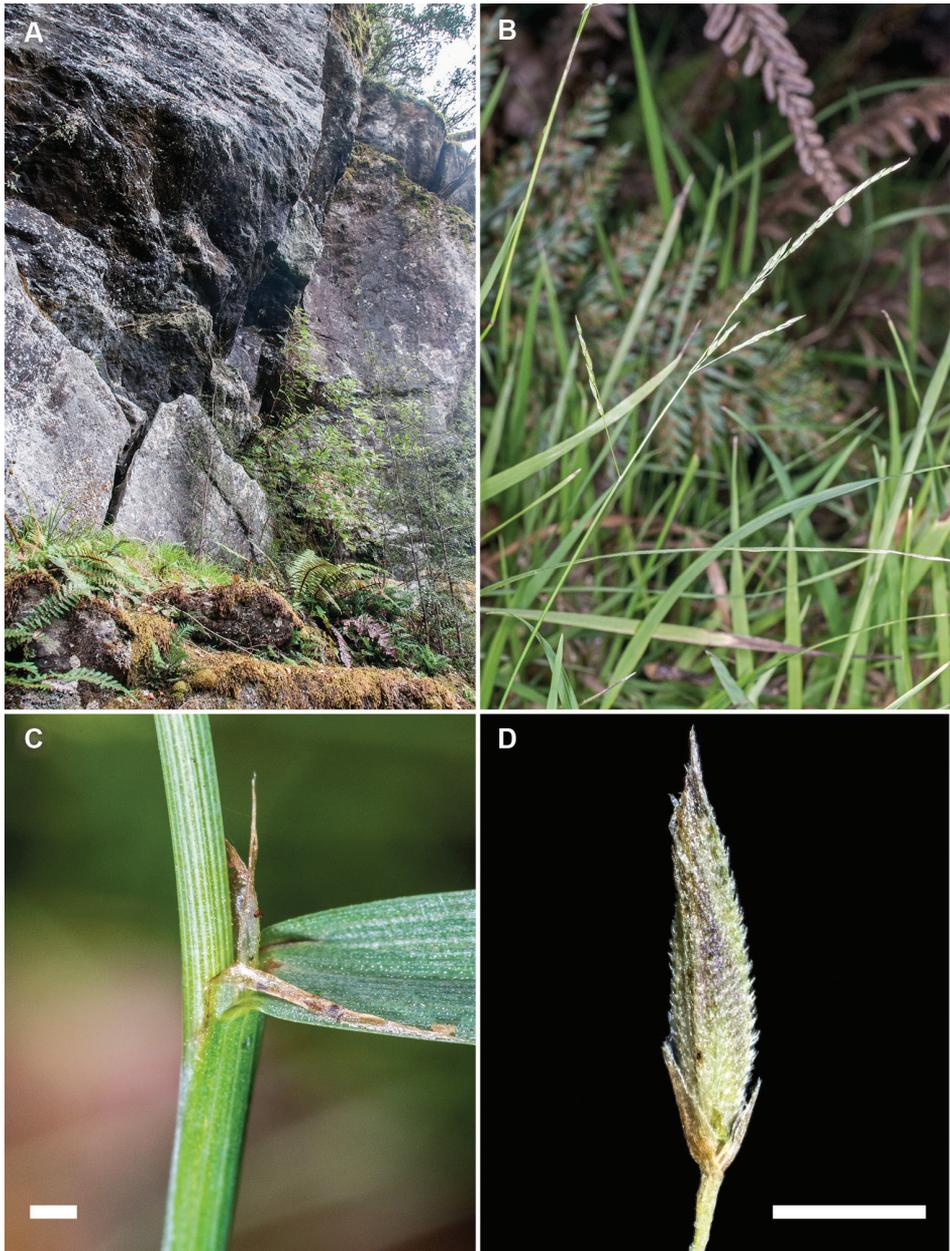


Figure 5. *Simplicia buchananii*. **A** habitat, Kahurangi National Park, North West Nelson, South Island, New Zealand **B** growth habit and inflorescence **C** culm, leaf base, sheath and ligule **D** spikelet showing reduced glumes and lemma (images: J.R. Rolfe).

ovate-lanceolate, acute to mucronate, sometimes with a minute subapical awnlet 0.06–0.08 mm long. Palea 2.4–2.8 mm, scabrid (densely covered in minute prickles), lanceolate, pale green to green, pubescent, 1–2-nerved. Rachilla prolongation 0.3–0.5

mm, filiform, hyaline, glabrous. Stamens 3. Filaments 0.3 mm long, hyaline. Anthers 0.7–1.5 mm, purple or yellow. Ovary narrowly ovoid to weakly trigonous 1.0–1.25 mm long, green, ± glabrous (basal portion sometimes minutely ciliate); styles apical, 0.10–0.25 mm, hyaline; stigmas plumose, white. Caryopsis 1.3–1.5 mm long, laterally compressed, orange when mature. Chromosome number: $2n = 28$ (Zotov 1971).

Specimens seen. North-West Nelson, Gouland Downs, *A.P. Druce s.n.*, Jan 1969, WELT SP069213; North-West Nelson, Cobb Valley, Chaffey's Stream, *A.M. Hamilton s.n.*, 23 Feb 1965, CHR 119546; North-West Nelson, Peel Range, above Lake Henderson, *A.P. Druce s.n.*, Mar 1982, CHR 369973; North-West Nelson, below Balloon Hut, *I.M. Ritchie s.n.*, 29 Mar 1967, CHR 175738; North-West Nelson, Kahurangi National Park, Cundy Creek, *M.J. Thorsen 101/09*, 11 Apr 2009, AK 304802; North-West Nelson, Kahurangi National Park, west of Gordon's Pyramid, *M.J. Thorsen 106/09*, 12 Apr 2009, AK 305801; North-West Nelson, Mt Arthur, ?*A. Mackay s.n.*, c.1879, CHR 13277; North-West Nelson, South Arthur Range, west side of Baton Saddle, *A.P. Druce 478*, Feb 1991, CHR 469403; North-West Nelson, Lockett Range, near Ruby Lake, *A.P. Druce s.n.*, Jan 1982, CHR 387666–387667; North West of Mt FZ (between Glenroy and Sheriff Rivers), *A.P. Druce s.n.*, 13 Mar 1984, CHR 394262.

Distribution (Fig. 1). *Simplicia buchananii* is endemic to North-West Nelson, South Island.

Recognition. *Simplicia buchananii* is distinguished from *S. laxa* and *S. felix* by the tufted growth habit, erect culms, and by the linear inflorescences, whose branches are usually tightly appressed to the rachises (Fig. 5, for other differences see Table 1).

Ecology. *Simplicia buchananii* is a biologically sparse species of calcareous rock habitats (including shaded rock outcrops, boulderfalls, rock overhangs and cave entrances) within montane forest (de Lange et al. 2010).

Conservation status. Using the New Zealand Threat Classification System (Townsend et al. 2008) *Simplicia buchananii* has been assessed as 'Threatened / Nationally Critical' with the qualifiers 'DP' [Data Poor], 'RR' [Range Restricted] and 'Sp' [Sparse] (de Lange et al. 2013). The threats this species faces were summarised by de Lange et al. (2010) and Smissen et al. (2011). Based on our current knowledge of this species the current threat status remains appropriate.

***Simplicia laxa* Kirk, T.N.Z.I. 29: 497, t.44 (1897)**

≡ *Simplicia laxa* Kirk var. *laxa* (autonym, Zotov T.R.S.N.Z. 73: 236 (1943))

Lectotype. 'Waikouaiti, Otago' *D. Petrie s.n.*, n.d. (WELT SP043017!) (*vide* Zotov 1971)

Isolectotype. 'Waikouaiti, Otago, *D. Petrie s.n.*, n.d. (WELT SP043021!) (*vide* Zotov 1971)

Etymology. Kirk (1897) did not explain the meaning of his species epithet '*laxa*' though his intent is clear from his protologue where he describes the new species as

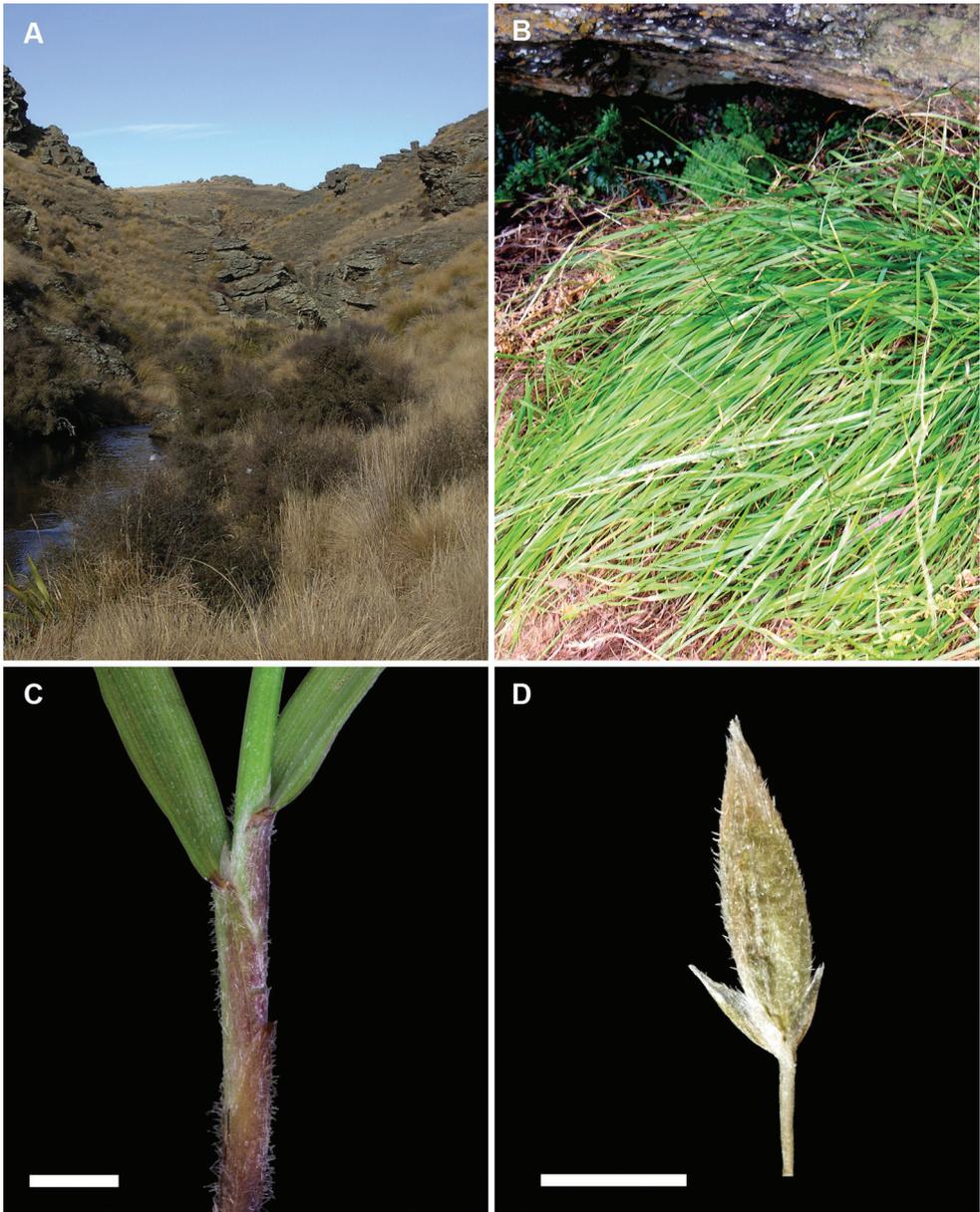


Figure 6. *Simplicia laxa*. **A** habitat, Emerald Stream, McCraes, North Otago, South Island, New Zealand (image D.A. Houston) **B** growth habit and inflorescence **C** culm, leaf base, sheath and ligule **D** spikelet showing reduced glumes and lemma. (Photo credit images **C** and **D**: K. Ford, Allan Herbarium, Landcare Research Manaaki Whenua)

having ‘weak, decumbent, flaccid’ culms. The epithet is derived from Latin ‘*laxus*’ meaning ‘loosely arranged’ as in ‘wide, loose’ structures or growth (Taylor 2002).

Description (Fig. 6). Plants trailing forming thick sprawling mats or diffuse interconnected patches up to 0.6 m across. Culms 0.40–0.80 m long, green to pale-

green when fresh, wiry, decumbent, with the apices weakly erect, culm internodes 4–8, elongated, sparsely (sometimes densely) hairy, or glabrous; hairs weakly flexuous, patent up 0.18 mm long; internodes usually shorter than subtending leaf-sheaths. Culm-nodes conspicuously swollen when fresh, maroon-black to black (0.13–)0.18–0.30 mm long, rooting freely on contact with ground. Basal leaf-sheaths glossy light brown to amber, membranous, ribbed, abaxially (often copiously) pubescent on ribs (and usually on interstices), hairs 0.20–0.25(–0.30) mm long, patent to retrorse; mid stem and upper leaf-sheaths pale-green to green, membranous, ribbed, abaxially pubescent on ribs (and sometimes on interstices), hairs copious, 0.35–0.40 mm long patent, mostly straight, sometimes curved or weakly flexuous. Ligule 2.8–3.5(–10) mm, membranous, lanceolate, apex erose to very deeply lacerate; abaxially sparsely to copiously hairy; hairs 0.20–0.24 mm long. Leaf-blade (100–)160(–200) × (2.8–)3.0(–3.6) mm, green to dark green, flat, linear-lanceolate, finely ribbed; adaxial ribs finely pubescent, abaxially glabrous (sometimes sparsely hairy at leaf base; margins ± smooth, sometimes irregularly finely scabrid and sparsely hairy. Panicle (40–)100(–150) mm long, linear to ± pyramidal, usually with basal branch or branch pair reflexed (often unevenly so); rachis glabrous, branches (20–)40(–60) mm long, finely, antrorsely hairy (hairs 0.20–0.25 mm long), binate, initially contracted but as inflorescences mature, spreading to reflexed, devoid of spikelets in lower half; pedicels appressed to branchlets, 1.00–1.06 mm long, finely pubescent. Spikelets 2.8–3.2 mm, 1-flowered, lanceolate, light green. Glumes pale green (± hyaline), glabrous, broadly ovate-lanceolate to ovate, acute, 1-nerved, nerve extending beyond apex as a minute mucro, lower glume margins entire (sometimes with apex erose), ciliate towards apex, upper glume margins usually erose (sometimes subentire), ciliate; lower glume 0.5–0.8 mm, upper glume 0.75–1.0(–1.2) mm. Lemma 2.8–3.2(–3.4) mm, light green to grey-green (sometimes purple-green), ± evenly, densely pubescent, lanceolate, acute, apex mucronate (mucro 0.10–0.25 mm long), 3(–5)-nerved (nerves obscured by hairs); lemma hairs antrorse appressed, sericeous, 0.12–0.13 mm long. Palea 2.4–2.8 mm, lanceolate, pale green to green, pubescent, 1–2-nerved, (nerves obscured by hairs). Rachilla prolongation 1.25–1.30 mm, narrowly lanceolate, hyaline, margins minutely ciliate. Stamens 3. Filaments 0.20–0.25 mm long, hyaline. Anthers 0.30–0.45 mm, yellow. Ovary narrowly ovoid to weakly trigonous 1.0–1.25 mm long, dark green, ± glabrous (basal portion sometimes minutely ciliate); styles apical, 1.10–1.25 mm, hyaline; stigmas plumose, white. Caryopsis 1.4–1.5 mm long, laterally compressed, orange-brown when mature. Chromosome number: $2n = 28$ (Zotov 1971, *I.M. Ritchie s.n.*, CHR 202752)

Specimens seen. Karamea, Honeycomb Cave, *P. Wardle s.n.*, 22 Jan 1985, CHR 489550; Karamea Ecological District, Karamea, Kahurangi National Park, Honeycomb Cave, *P.J. de Lange 4774*, 6 Dec 2000, AK 252968; Otago, Taieri County, near Deep Stream Hotel, Rock & Pillar Road, *D. Petrie s.n.*, Feb 1877, WELT SP010498, WELT SP043018, WELT SP043020A, WELT SP043020B, WELT SP043020C, WELT SP069211; Old Man Ecological District, Old Man Range, Castle Rock Summit, *P.J. de Lange 7859*, 18 Jan 2008, AK 304848 (Duplicate: US); Old Man Ecological District, Old Man Range, Castle Rock Summit (south side), *P.J. de Lange 7858*, 18 Jan 2008,

AK 304847; Macraes Ecological District, Nenthorn, Deighton Stream tributary, 'John's Cave', *P.J. de Lange 7206 & M.J. Thorsen*, 15 Jan 2008, AK 301580; Macraes Ecological District, Nenthorn, Upper Emerald Stream, *P.J. de Lange 7835 & M.J. Thorsen*, 15 Jan 2008, AK 304808; Macraes Ecological District, Nenthorn, Upper Emerald Stream, *P.J. de Lange 7836 & M.J. Thorsen*, 15 Jan 2008, AK 304809; Macraes Ecological District, Nenthorn, Emerald Stream, 'Old Otagense site', *P.J. de Lange 7856 & M.J. Thorsen*, 15 Jan 2008, AK 304845 (Duplicate: US); Summerhills Station, 3 O'Clock Stream, *M.J. Thorsen 100/07*, 14 May 2007, CHR 591912; Nevis Valley, Barn Creek, *G. Loh s.n.*, 26 Nov 1994, CHR 509148. CULTIVATED: Ex. Cult., Karamea Ecological District, Karamea, Kahurangi National Park, Honeycomb Cave, *P.J. de Lange 6104*, 3 Sep 2004, AK 288071 (Duplicate: HO). Macraes Ecological District, north-east of Nenthorn, Macraes, Emerald Stream, T Whitaker Falcon Site, *M.J. Thorsen s.n.*, 30 Mar 2006, AK 295631; Ex. Cult., Central Otago, Old Man Range, Castle Rock, *I.M. Ritchie s.n.*, 25 Oct 1969 (grown on and harvested by V.D. Zotov on 31 Dec 1970 as G8128).

Distribution (Fig. 1). As recircumscribed here *Simplicia laxa* is now endemic to the South Island. Nevertheless, there are a few historical collections held in world herbaria that suggest that *S. felix* and *S. laxa* once grew sympatrically in the eastern Wairarapa. de Lange (2016) has shown that these herbarium specimens are the result of accidental mixing of unmounted Thomas Kirk, North Island (*S. felix*) and Donald Petrie, South Island (*S. laxa*) specimens, and mislabelling by Thomas Cheeseman and, possibly, Victor Zotov, rather than genuine North Island wild occurrences of *S. laxa*. *Simplicia laxa* is currently known from one site near Karamea, North-West Nelson (Honeycomb Cave) and otherwise from 10 sites in Northern and Central Otago (Smitsen et al. 2011). This disjunct distribution is unlikely to be natural, however, it more likely reflects the loss of interconnecting habitat as well as the difficulty of recognising this species in the field.

Recognition. Smitsen et al. (2011) showed that *Simplicia laxa* is more closely related to *S. felix* than it is to *S. buechananii*. From *Simplicia felix*, *S. laxa* can be distinguished by the culm internodes which are shorter than the subtending leaf sheaths and usually hairy (sometimes glabrous); and by the less strongly ribbed, glossy light brown to amber basal sheaths, whose ribs are pubescent (hairs 0.20–0.30 mm long). The mid-stem and upper-stem leaf sheaths of *S. laxa* are less prominently ribbed than those of *S. felix* and the ribs and, usually the interstices are hairy. The adaxial leaf surface ribs of *S. laxa* are hairy (abaxially glabrous), while the leaf margin is mostly smooth, though sometimes irregularly hairy or finely scabrid. The leaves of *S. laxa* are also wider than those of *S. felix* (2.8–3.6 mm wide cf. 1.0–3.0 mm wide in *S. felix*). However, in cultivation the leaves of both species can get up to 4.0 mm wide. Although the inflorescences of *S. laxa* and *S. felix* are similar, those of *S. laxa* are larger (up to 150 mm rather than 80 mm long), and the branches are antrorsely hairy rather than scabrid. Although the lemma of both species overlap in range, those of *S. laxa* are longer (2.8–3.4 mm long) than those of *S. felix* (2.0–3.0 mm long) and minutely pubescent rather than scabrid. The rachilla prolongation of *S. laxa* is narrowly lanceolate, 1.25–1.30 mm long and with the margins minutely ciliate, while that of *S. felix* is filiform, 0.8 mm long; bearing sparse cilia only near the apex. Other differences are given in Table 2.

Table 2. Distinguishing features of *Simplicia* species based on wild collected material.

	<i>Simplicia buchamanii</i>	<i>Simplicia laxa</i>	<i>Simplicia felix</i>
Growth habit	Tufted, erect. culms up to 1 m tall	Decumbent, culms sprawling, forming mats up to 0.6 m diameter	Decumbent, sprawling, forming mats up to 1 m diameter
Culm internodes	Glabrous, ± equal in length to subtending leaf sheaths	Hairy or glabrous, < subtending leaf-sheaths. Hairs if present up to 0.18 mm long	Glabrous, > subtending leaf-sheaths
Culm nodes	Glossy orange-brown to dark red-brown	Glossy maroon-black to black	Glossy dark brown-green to brown-black
Basal leaf sheaths	Strongly ribbed, stramineous or dull brown, glabrous or with ribs scabrid (very rarely finely pubescent). Hairs (if present) 0.06–0.08 mm long	Finely ('weakly') ribbed, glossy light brown to amber, ribs pubescent. Hairs 0.20–0.30 mm long	Strongly ribbed, dull dark brown, ribs glabrous or pubescent. Hairs (if present) 0.10–0.15 mm long
Mid-stem and upper-stem leaf sheaths	Strongly ribbed. Glabrous	Hairy. Hairs copious, 0.35–0.40 mm long	Strongly ribbed. Usually glabrous, occasionally ribs finely short pubescent towards sheath apex
Ligule	Glabrous	Sparsely to copiously hairy. Hairs 0.20–0.24 mm long	Glabrous or with both surfaces finely hairy. Hairs 0.15–0.18 mm long
Leaf-blade	1.8–4.0 mm wide. Adaxially finely scabrid, abaxially glabrous, margins smooth. Apex acuminate	2.8–3.6 mm wide. Adaxially with hairy ribs, abaxially glabrous; margins ± smooth, sometimes, finely scabrid and/or sparsely hairy. Apex acute	1.0–3.0 mm wide. Ribs (both surfaces) smooth or finely scabrid; margins smooth or finely scabrid. Apex acute
Inflorescence	Paniculate, linear up to 180 mm long. Branches glabrous, erect, appressed to rachis, bearing spikelets almost to base.	Paniculate, linear to ± pyramidal, up to 150 mm long. Branches antrorsely hairy, basal branches (or branch) reflexed, others weakly appressed to rachis, lower half of branch devoid of spikelets	Paniculate, linear to ± pyramidal, up to 80 mm long. Branches scabrid, basal branches (or branch) reflexed, others weakly appressed to rachis, lower ½ to ⅔ or branch devoid of spikelets
Pedicels	Glabrous, 0.60–1.2 mm long	Pubescent, 1.00–1.06 mm long	Pubescent 0.20–0.30 mm long
Glumes	Lower glume 0.3–1.0 mm long, upper glume 1.0–1.6 mm long	Lower glume 0.5–0.8 mm long, upper glume 0.75–1.2 mm long	Lower glume 0.5–0.6 mm long, upper glume 0.75–0.9 mm long
Lemma	2.6–3.0 mm long, scabrid	2.8–3.4 mm long, pubescent	2.0–3.0 mm long, minutely scabrid
Rhacilla prolongation	0.3–0.5 mm long, filiform, glabrous	1.25–1.30 mm long, narrowly lanceolate, margins minutely ciliate	0.8 mm long, filiform, glabrous except for sparse cilia cresting apex
Anther filaments	0.3 mm long,	0.20–0.25 mm long	0.6–0.9 mm long
Anthers	0.7–1.5 mm long,	0.30–0.45 mm long	1.0–1.2 mm long

Because of the lax, decumbent trailing growth habit, and loose linear to pyramidal inflorescences *Simplicia laxa* is easily distinguished in the field from the shortly tufted *S. buchananii* whose inflorescences are erect and whose inflorescence branches are held tightly appressed to the rhacis (Zotov 1971; Edgar and Connor 2010; de Lange et al. 2010). Other differences are provided here in the key to the species and under Table 2.

Ecology. Much of what has been written about *Simplicia laxa* (Johnson 1995; de Lange et al. 2010) we now believe is based on observations made of remnant populations persisting in possibly suboptimal habitats (rock overhangs, rock crevices, river gorges) within locations that had once been forested. Nevertheless, the North-West Nelson, Honeycomb Cave population, which occurs in dense lowland forest, is still confined to a cave entrance and the species has yet to be found within the surrounding forest. It seems likely that *Simplicia laxa* is a species of deeply shaded habitats, which may, like *S. felix* also occur in forested situations. Further survey is needed. In the interim, all of the extant *Simplicia laxa* populations occur on base-rich substrates, chlorite schist and limestone, and in having this in common with the other two species it is unlikely that it will be found on less fertile substrates.

Conservation status. *Simplicia laxa* was assessed as ‘Threatened / Nationally Critical’ qualified ‘CD’ (Conservation Dependent), ‘Sp’ (Sparse) by de Lange et al. (2013). That assessment included plants described here as *Simplicia felix*. With the recircumscription of *S. laxa* the species remains appropriately assessed as ‘Nationally Critical’. Currently there are < 15 populations known, and several of these are in decline, and very few are substantial in size. Many occur on private land without direct conservation management or in places subject to ongoing habitat deterioration through invasive weed pressure and habitat loss. The qualifiers however need adjustment. Because the species is managed in a number of sites it is appropriate to retain the qualifier ‘CD’, as ceasing management would have a serious impact on the survival of the species. It is debatable whether *Simplicia laxa* is truly biologically sparse. It is sparsely distributed but this is more likely an artefact of past habitat loss leaving highly fragmented, disjunct ‘remnant’ populations rather than any natural pattern of distribution or species biology. We recommend that ‘Sp’ be removed from the conservation assessment for this species. The seemingly peculiar North-West Nelson, Honeycomb Cave outlier suggests that *Simplicia laxa* should be looked for throughout the South Island rather than, as it currently has, only in the Central and Eastern Otago Region. Also, it is now evident that we lack trend data for the species, though the overall impression is that many populations are in decline. For these reasons, we recommend that the species be qualified ‘DP’ (Data Poor) be added to the species conservation status.

***Simplicia felix* de Lange, J.R.Rolfe, Smissen & Ogle, sp. nov.**

urn:lsid:ipni.org:names:77159000-1

Diagnosis. Differs from *Simplicia laxa* by dark brown, prominently ribbed leaf sheaths; mostly glabrous, strongly ribbed mid-stem to upper-stem leaf sheaths; longer culm

internodes; narrower, glabrous (sometimes with the adaxial ribs finely scabrid) leaves; shorter panicles (up to 80 mm long) with scabrid branches; minutely scabrid lemma and smaller filiform rachilla prolongation bearing cilia only at the apex.

Holotype (Fig. 7). 'Eastern Wairarapa Ecological Region and District, Te Kanuka Farm Station, Kaumingi Stream' *P.J. de Lange 12167, J.R. Rolfe & T. Silbery, 27 Feb 2014, (AK 351325)* Isotypes. CAN, CHR, F, WAIK, WELT, US

Etymology. The epithet '*felix*' is taken from the Latin for '*lucky*' (N.G. Walsh, MEL pers comm., 14 January 2016) as in '*lucky find*' in reference to the circumstances of this species' discovery; that came about through the desire to get a name on an unremarkable little tuft of grass that was discovered fortuitously near Mangaweka, Central North Island by CCO on 29 January 2005 (Ogle 2010).

Description (Fig. 8). Plants forming flaccid, diffuse, often much interconnected, sprawling patches up to 1 m across. Culms 0.25–0.65 m long, green to dark brown when fresh, wiry, initially decumbent, becoming ascendant with the apices weakly erect, culm internodes 5–8, elongated, glabrous; internodes longer than subtending leaf-sheaths. Culm-nodes conspicuously swollen when fresh, dark green-brown to brown-black 0.15–0.25 mm long, rooting freely on contact with ground. Basal leaf-sheaths dull dark brown, membranous, strongly ribbed, usually abaxially pubescent (sometimes glabrous) on ribs, hairs 0.10–0.15 mm long, patent to retrorse; mid stem and upper leaf-sheaths pale-green to green, membranous, strongly ribbed, glabrous (rarely abaxially ribs finely pubescent toward sheath apex). Ligule 2.0–2.6 mm, membranous, lanceolate, apex entire, or deeply lacerate; glabrous, or with both surfaces hairy; hairs 0.15–0.18 mm long. Leaf-blade (20–)40(–60) × (1.0–)1.2–2.4(–3.0) mm, yellow-green to dark green, flat, narrow linear-lanceolate, finely ribbed, ribs smooth (sometimes minutely scabrid); margins minutely scabrid. Panicle 20–40(–80) mm long, linear to ± pyramidal, usually with basal branch or branch pair reflexed (often unevenly so); rachis glabrous (sometimes bearing a few minute prickle-teeth), branches 20–30 mm long, scabrid, binate, initially contracted but as inflorescences mature, spreading to reflexed, devoid of spikelets in lower half to two-thirds; pedicels appressed to branchlets, 0.20–0.25(0.30) mm long, finely pubescent. Spikelets 2.7–3.0 mm, 1-flowered, lanceolate, light green. Glumes pale green (± hyaline), glabrous, ovate-lanceolate to ovate, acute, 1-nerved, nerve sometimes extending beyond apex as a minute mucro, margins initially entire, becoming erose near apex, very sparsely ciliate in upper third; lower glume 0.5–0.6 mm, upper glume 0.75–0.8(–0.9) mm. Lemma 2.0–2.8 (–3.0) mm, light green to cream, ovate-lanceolate to lanceolate, acute, apex mucronate (mucro 0.1 mm long), 5-nerved, the inner 3 nerves conspicuous, the outer less prominent; nerves bearing evenly spaced minute (0.02–0.03 mm long), antrorse, appressed prickle-teeth, interstices usually densely (sometimes sparsely) covered with minute antrorse prickle-teeth. Palea 2.0–2.8 mm, lanceolate, green to purple-green, 1–2-nerved, nerves bearing evenly spaced minute prickle (0.02–0.03 mm long) teeth, interstices usually glabrous, sometimes sparsely covered with minute prickle-teeth. Rachilla prolongation 0.8 mm, filiform, hyaline, glabrous except for sparse cilia cresting prolongation apex. Stamens 3. Filaments 0.6–0.9 mm long, hyaline. Anthers 1.0–1.2



Figure 7. Holotype of *Simplicia felix* de Lange, J.R.Rolfe, Smissen & Ogle.



Figure 8. *Simplicia felix*. **A** habitat, Te Kanuka Farm Station, Upper Kaumingi Stream east Wairarapa, North Island, New Zealand **B** growth habit and inflorescence **C** culm, leaf base, sheath and ligule **D** spikelet showing reduced glumes and lemma (images: J.R. Rolfe)

mm, yellow. Ovary narrowly ovoid to weakly trigonous 1.0 mm long, pale green, glabrous; styles apical, 1.0–1.2 mm, hyaline; stigmas plumose, white. Caryopsis 1.2–1.4(–1.5) mm long, laterally compressed, pale orange to orange-brown when mature. Chromosome number: $2n = 28$ (Murray et al. 2005, *P.J. de Lange* 5897, AK 285424— as *Simplicia laxa*)

Specimens seen. New Zealand, North Island: Rangitikei Ecological Region and District, North of Taihape, north of Paengaroa Road and east of State Highway One, Ngawaka Stream, ‘Stevies Bush’, *P.J. de Lange* 7834 & *C.C. Ogle*, 29 Feb 2008, AK 304807 (Duplicates: CHR, US); Rangitikei Ecological Region and District, North of Taihape, south of Paengaroa Road and east of State Highway One, *C.C. Ogle* 4955, 17 Feb 2006, AK 295628; Rangitikei Ecological Region and District, North of Taihape, south of Paengaroa Road and east of State Highway One, *C.C. Ogle* 4954, 17 Feb 2006, AK 295627; Rangitikei Ecological Region and District, North of Taihape, south of Paengaroa Road and east of State Highway One, ‘Campbells Bush’ *P.J. de Lange* 7832 & *C.C. Ogle*, 29 Feb 2008, AK 304805; Taihape, Oraukura Stream, *C.C. Ogle* 5625, 6 Apr 2008, AK 306012; Rangitikei Ecological Region and District, Taihape Scenic Reserve, Hautapu, *P.J. de Lange* 7833, *C.C. Ogle* & *V. McGlynn*, 28 Feb 2008, AK 304806; Rangitikei Ecological Region and District, Taihape Scenic Reserve, Hautapu, *C.C. Ogle* 4958 & *V. McGlynn*, 7 Mar 2006, AK 297357; Rangitikei Ecological Region and District, Rangitikei, Kawhatau Valley, Toetoe Road, *C.C. Ogle* 4893, *V. McGlynn* & *G. La Cock*, 13 Dec 2005, AK 297353; Rangitikei Ecological Region and District, Rangitikei, Kawhatau Valley, Toetoe Road, *P.J. de Lange* 7831, *C.C. Ogle* & *V. McGlynn*, 28 Feb 2008, AK 304804; Rangitikei Ecological Region and District, Rangitikei, Kawhatau River, Toetoe Road, ‘Ben Moi’ farm, *C.C. Ogle* 4734, 29 Jan 2005, AK 289755 (Duplicate: CHR); Eastern Wairarapa Ecological Region and District, Te Kanuka Farm Station, Swamp Ridge Covenant, *P.J. de Lange* 12165, *J.R. Rolfe* & *T. Silbery*, 27 Feb 2014, AK 351320 (Duplicates lodged in: CHR, WELT, US); Eastern Wairarapa Ecological Region and District, Te Kanuka Farm Station, Upper Kaumingi Stream, *P.J. de Lange* 12168, *J.R. Rolfe* & *T. Silbery*, 27 Feb 2014, AK 351330 (Duplicates lodged in: CAN, WELT, US); Wairarapa, Ruamahanga, *T. Kirk s.n., n.d.*, WELT SP043016; Ruamahanga Valley, *T. Kirk s.n.*, 26 Jan 1880, WELT SP043022; Eastern Wairarapa Ecological Region and District, Admiral Road, Wainuoru River, Te Kowhai, Moetapu Bush, *P.J. de Lange* 12160, *J.R. Rolfe* & *T. Silbery*, 26 Feb 2014, AK 351290 (Duplicates: WELT, US); Eastern Wairarapa, Longbush, Tawhiriwaimanuka Stream. Ahipaku QE II Covenant, *J. R. Rolfe* 15017, 9 Dec 2015, AK 360429 (Duplicate: WELT). South Island: South-East of Duntroon, Prydes Gully Road (Ngapara, The Knolls), *B.P.J. Molloy s.n.*, 18 Dec 1991, CHR 616708; Duntroon Ecological District, Ngapara, The Knolls, *P.J. de Lange* 1340 & *B.P.J. Molloy*, 7 May 1992, AK 208577 (Duplicates: CHR, WAIK, WELT). Cultivated: Ex. Cult., Rangitikei Ecological Region and District, Rangitikei, Kawhatau Valley, Toetoe Road, *P.J. de Lange* 6791, 30 Nov 2006, AK 297927; Ex. Cult., Rangitikei Ecological Region and District, Rangitikei, Kawhatau Valley, Toetoe Road, *P.J. de Lange* 6824, 23 Dec 2006, AK 298065; Ex. Cult., Duntroon Ecological District, Ngapara, The Knolls,

P.J. de Lange 5897, 23 Feb 2004, AK 285424; Ex. Cult., Duntroon Ecological District, Ngapara, The Knolls, *P.J. de Lange* 6103, 3 Sep 2004, AK 288070.

Distribution (Fig. 1). *Simplicia felix* has so far been collected from the North and South Islands, from the current northern limit at Ngawaka Stream, near Taihape, North Island to Ngapara, North Otago, South Island. In the North Island the species has been found in two broad geographic areas, around Taihape–Mangaweka, and in the eastern Wairarapa. In the South Island, *Simplicia felix* is so far known only from the one location at Ngapara.

Recognition. Genetically and morphologically *Simplicia felix* is more closely related to *S. laxa* than it is to *S. buchananii*. From *Simplicia laxa* it can be distinguished by the culm internodes which are longer than the subtending leaf sheaths and consistently glabrous; and by the strongly ribbed, dull dark brown basal sheaths, whose ribs are glabrous or pubescent (if pubescent then with the hairs 0.10–0.15 mm long). The mid-stem and upper-stem leaf sheaths of *S. felix* are strongly ribbed and usually glabrous (occasionally the ribs are finely pubescent towards the sheath apex). The leaf surfaces and margins of *S. felix* are mostly smooth though the ribs and leaf margins may be minutely scabrid. As a rule, the leaves of *S. felix* are also shorter (up to 60 mm in *S. felix*, 200 mm in *S. laxa*) and narrower than those of *S. laxa* (1.0–3.0 mm long cf. 2.8–3.6 mm long in *S. laxa*). However, in cultivation the leaves of both species can get up to 4.0 mm wide. Although the inflorescences of *S. laxa* and *S. felix* are similar, those of *S. felix* are smaller (up to 80 mm long rather than 150 mm long), and the branches are scabrid rather than antrorsely hairy. Although the lemma of both species overlap in range, those of *S. felix* tend to be shorter (2.0–3.0 mm long) than those of *S. laxa* (2.8–3.4 mm long) and minutely scabrid rather than pubescent. The rachilla prolongation of *S. laxa* is narrowly lanceolate, 1.25–1.30 mm long and with the margins minutely ciliate, while that of *S. felix* is filiform, 0.8 mm long and bearing sparse cilia only near the apex. Other differences are given in Table 2.

Simplicia felix was initially confused with *S. buchananii* (see Ogle 2010; Smissen et al. 2011) mostly because the culm internodes of both species are glabrous; they have superficially similar strongly-ribbed, often glabrous leaf-sheaths, similar smooth or finely scabrid leaves, and minutely scabrid lemma. Despite these similarities, *Simplicia felix* is not closely allied to *S. buchananii* from which it is readily distinguished by the lax, sprawling, rather than erect culms; linear-pyramidal rather than linear inflorescences with binate branching, and by the lower branch or branches usually reflexed rather than appressed to the rachis. The pedicels of *S. felix* are also pubescent rather than glabrous (rarely minutely scabrid).

Ecology. With the exception of the Ngapara population which grows within a limestone overhang, *Simplicia felix* has only been collected from central and eastern North Island lowland to lower montane, often riparian, seasonally dry (drought prone), Podocarp forests overlying base-rich substrates such as limestone, calcareous mudstone and siltstone. In these areas plants have been found only in lightly shaded situations within forest remnants that have been lightly under-grazed by cattle or sheep (Ogle 2010; de Lange et al. 2014). The reduced competition from other grasses and herbs

that grazing causes, seems crucial to this species' survival (de Lange et al. 2014) as, in some locations where *S. felix* had been found, portions of the same forest that had been fenced to exclude livestock did not have *Simplicia*). In the Taihape–Rangitikei area, Ogle (2010) noted a close association with a range of other indigenous grasses and herbs, most notably *Echinopogon ovatus*, *Poa imbecilla*, *P. matthewsii*, *Cardamine debilis* agg., *Oxalis exilis*. In the Eastern Wairarapa the same species along with *Arthropodium candidum*, *Australina pusilla* subsp. *pusilla*, *Stellaria parviflora* and the mosses *Camptochaete angustata*, *Echinodium hispidum* and *Plagiomnium novae-zelandiae* were key associates of *Simplicia felix* (P. J. de Lange unpubl. data). Further survey of similar forest remnants with ground covers dominated by these species is, we believe, likely to locate further populations.

The Ngapara site, as *S. laxa*, was described in some detail by Johnson (1995). By comparison with the North Island sites it is anomalous and it seems likely that *Simplicia* persists there because the rock overhang affords some shade and protection from competing plants. Within the rock overhang *Simplicia felix* grows with *Poa imbecilla*, *P. matthewsii*, *Chenopodium allanii* and an unnamed member of the *Cardamine corymbosa* complex.

Conservation status. *Simplicia felix* occupies a very small area of only a few square metres wherever it occurs. It appears to have quite specific light requirements and tolerates only limited competition from other ground-cover species. The healthiest populations occur at sites where competition is reduced by grazing mammals such as cattle and sheep. This poses a quandary for conservation managers because, whilst grazing apparently benefits *S. felix*, it will ultimately lead to the collapse of the forest canopy that provides the level of shade that is also necessary for *S. felix* to survive. Collectively, the area of the sites where *S. felix* occurs amounts to considerably less than 1 ha. Therefore, *S. felix* meets the criteria to be assessed Threatened—Nationally Critical B2 using the New Zealand Threat Classification System (Townsend et al. 2008). We also recommend that the qualifiers Data Poor ('DP') and Range Restricted ('RR') be appended to the assessment—'DP' because there are no population trend data available and because several areas of possibly suitable habitat in the Rangitikei and Eastern Wairarapa have not been surveyed for the presence of *S. felix*; 'RR' because its habitat requirements are apparently very narrow.

Acknowledgements

The authors would like to thank Dave Houston, Mike Thorsen, Peter Johnson, Tony Silbery, the late Phil Knightbridge, Graeme Wood, John Barkla, Rhys Gardner, Kerry Ford, Robert Soreng, Neville Walsh, Geoff Davidson, Viv McGlynn, Graeme La Cock and Phyllis Leigh for company in the field and sharing their knowledge of *Simplicia*. We thank Ewen Cameron (AK) and Leon Perrie (WELT) for access to specimens held in their care and for facilitating the loan of specimens. The Department of Conservation, Conservation Management Units Fund provided funds for a survey for *Simplicia*

in the Eastern Wairarapa during February 2014. Genotyping and DNA sequencing were conducted at Landcare Research, Auckland by Shelley Myers and Duckchul Park. Rob Smissen's contribution to this work was partially supported by Core funding for Crown Research Institutes from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group. Kerry Ford of the Allan Herbarium, Landcare Research kindly commented on a draft of this paper. We thank Thomas Horn and Robert Soreng for their detailed review of the submitted manuscript.

References

- Borchsenius F (2009) FastGap 1.2. Department of Biological Sciences, University of Aarhus, Denmark. Available from: http://www.aubot.dk/FastGap_home.htm
- Connor HE (1988) Breeding systems in New Zealand grasses. 10, Species at risk for conservation. *New Zealand Journal of Botany* 26: 163–167. <https://doi.org/10.1080/0028825X.1988.10410108>
- de Lange PJ (2016) When labels get mixed – lessons to be learned from a study of the Thomas Kirk 'herbarium' and historical *Simplicia* collections. *Trilepidea* 152: 1–11. <http://www.nzpcn.org.nz/publications/Trilepidea-152-160704.pdf>
- de Lange P, Heenan P, Norton D, Rolfe J, Sawyer J (2010) Threatened plants of New Zealand. Canterbury University Press, Christchurch.
- de Lange PJ, Murray BG (2002) Contributions to a chromosome atlas of the New Zealand flora – 37. Miscellaneous families. *New Zealand Journal of Botany* 40: 1–24. <https://doi.org/10.1080/0028825X.2002.9512767>
- de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2012) Conservation status of New Zealand indigenous vascular plants, 2012. *New Zealand Threat Classification Series* 3. Department of Conservation, Wellington. <http://www.doc.govt.nz/Documents/science-and-technical/nztc3entire.pdf>
- de Lange PJ, Rolfe JR, Silbery T (2014) Seen but unseen—rediscovering *Simplicia laxa* in the southern North Island. *Trilepidea* 124: 5–9. <http://www.nzpcn.org.nz/publications/Trilepidea-124-140326.pdf>
- Edgar E, Connor HE (2010) *Flora of New Zealand*. Vol. V. Manaaki Whenua Press, Lincoln.
- Holland BR, Clarke AC, Meudt HM (2008) Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. *Systematic Biology* 57: 347–366. <https://doi.org/10.1080/10635150802044037>
- Huson DH, Bryant D (2006) Application of Phylogenetic Networks in Evolutionary Studies. *Molecular Biology and Evolution* 23: 254–267. <https://doi.org/10.1093/molbev/msj030>
- Meudt HM, Clarke A (2007) Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science* 12: 106–117. <https://doi.org/10.1016/j.tplants.2007.02.001>
- Johnson PN (1995) The rare grass *Simplicia laxa*: field status ecology and conservation. *Science for Conservation* 15. Department of Conservation, Wellington. <http://www.doc.govt.nz/Documents/science-and-technical/sfc015.pdf>

- Kirk T (1897) Description of a new genus of Gramineae. Transactions and Proceedings of the New Zealand Institute 29: 497. http://rsnz.natlib.govt.nz/volume/rsnz_29/rsnz_29_00_004370.html
- Murray BG, de Lange PJ, Ferguson AR (2005) Nuclear DNA variation, chromosome numbers and polyploidy in the endemic and indigenous grass flora of New Zealand. *Annals of Botany* 96: 1293–1305. <https://doi.org/10.1093/aob/mci281>
- Ogle CC (2010) Rediscovery of a rare species of grass in the genus *Simplicia* in the North Island. *Wellington Botanical Society Bulletin* 52: 38–46. http://bts.nzpcn.org.nz/bts_pdf/WBS52%2D38%2D46%2DOgle.pdf
- Simmons MO, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analysis. *Systematic Biology* 49: 369–381. <https://doi.org/10.1600/036364411X604930>
- Smissen RD, de Lange PJ, Thorsen MJ, Ogle CC (2011) Species delimitation and genetic variation in the rare New Zealand endemic grass *Simplicia*. *New Zealand Journal of Botany* 49: 187–199. <https://doi.org/10.1080/0028825X.2010.525244>
- Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland (MA). <http://paup.csit.fsu.edu/>
- Taylor M (2002) Meanings and origins of Botanical Names of New Zealand Plants. *Auckland Botanical Society Bulletin* 26.
- Thiers B (2016) Index Herbariorum: a global directory of public herbaria and associated staff; [updated continuously; cited 2016 Jan 15]. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>
- Townsend AJ, de Lange PJ, Norton DA, Molloy J, Miskelly C, Duffy C (2008) New Zealand Threat Classification manual. Department of Conservation, Wellington. <http://www.doc.govt.nz/Documents/science-and-technical/sap244.pdf>
- Zotov VD (1971) *Simplicia* T.Kirk (Gramineae). *New Zealand Journal of Botany* 9: 539–544. <http://dx.doi.org/10.1080/0028825X.1971.10430200>

Spathelia belizensis, a new species and first record for the genus in Central America (tribe Spathelieae, Rutaceae)

Pedro Acevedo-Rodríguez¹, Steven W. Brewer²

1 Department of Botany, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012, U.S.A. **2** Copperhead Environmental Consulting, Inc., 11641 Richmond Rd. P.O. Box 73 Paint Lick, KY 40461

Corresponding author: *Pedro Acevedo-Rodríguez* (acevedop@si.edu)

Academic editor: *Pavel Stoev* | Received 27 July 2016 | Accepted 28 November 2016 | Published 14 December 2016

Citation: Acevedo-Rodríguez P, Brewer SW (2016) *Spathelia belizensis*, a new species and first record for the genus in Central America (tribe Spathelieae, Rutaceae). *PhytoKeys* 75: 145–151. <https://doi.org/10.3897/phytokeys.75.10015>

Abstract

Spathelia L. as currently circumscribed is endemic to the western portion of the West Indies, and contains nine species, one endemic to the Bahamas, three endemic to Jamaica and five endemic to Cuba. The discovery of a new species in Belize brings the total number of species in *Spathelia* to ten and expands its known distribution beyond the West Indies. *Spathelia belizensis* **sp. nov.** is herein described, illustrated and contrasted to its most morphologically similar congener. A key to the species of *Spathelia* is provided.

Resumen

El género *Spathelia* es en la actualidad circunscrito como endémico de la porción occidental de las Antillas con un total de nueve especies, una endémica de las Bahamas, tres endémicas de Jamaica y cinco endémicas de Cuba. El descubrimiento de una nueva especie en Belice amplía el número total de especies a diez y expande la distribución del género más allá de las Antillas. En este artículo *Spathelia belizensis* es descrita e ilustrada y se compara con el congénere morfológicamente más similar. También, se proporciona una clave para las especies de *Spathelia*.

Keywords

Belize, Golden Stream Corridor Preserve, identification key, Spathelieae

Introduction

The genus *Spathelia* L. (tribe Spathelieae, Rutaceae) is characterized by palm-like trees with unbranched, slender trunks, and a distal crown of spirally arranged, compound leaves. The trees are reported as monocarpic, i.e., producing a distal massive inflorescence after six to eight years and dying right after fruiting. As currently circumscribed, *Spathelia* is considered endemic to the western portion of the West Indies and closely related to *Dictyoloma* and *Sohnreyia*, two monospecific South American genera (Appelhans et al. 2011, Pirani and Groppo 2015).

While carrying out ecological surveys in the Golden Stream Corridor Preserve in Belize, the junior author discovered a population of trees that refers to an undescribed species of *Spathelia*. This discovery documents for the first time the occurrence of *Spathelia* s.s. outside the West Indies, i.e., the northern, eastern tip of Central America. Previous reports of *Spathelia* (i.e., *S. rhoifolia* DC.) from Mexico are referred to the anacardiaceous genus *Pseudosmodium*.

The study of this novel collection from Belize reveals a new species which seems to be closely related to *S. brittonii* P. Wilson due to morphological similarities. The new species, *Spathelia belizensis* is herein described, illustrated and contrasted to *S. brittonii* its putative closely related species. A key to the species of *Spathelia* is presented to facilitate the identification of the species.

Material and methods

The description of the new species is based on a single collection, field notes and photographs obtained by the junior author. The study is based on a morphological comparative study of the species of *Spathelia* as recognized by Beurton (2008) and indicates that the Belizean collection is an undescribed species in the genus. A key to the species is based on various floristic treatments of the Antilles (Marie-Victorin 1948, Adams 1972, Correll and Correll 1982, Beurton 2008) and the examination of specimens when necessary. The type specimens are composed of two sheets, one bearing leaf material and the other bearing portions of the fruiting inflorescence. The holotype is deposited in US herbarium, and the isotypes were distributed to K and NY herbaria (acronyms follow Index Herbariorum; <http://sweetgum.nybg.org/science/ih/>).

Taxonomic treatment

Key to the species of *Spathelia*

- | | | |
|---|---|----------------------------|
| 1 | Sepals almost as long as the petals | 2 |
| – | Sepals half as long as or shorter than the petals | 8 |
| 2 | Leaflets 140–200 per leaf | <i>S. splendens</i> (Cuba) |
| – | Leaflets 24–92 per leaf | 3 |



Figure 1. *Spathelia belizensis*. **A** Portion of distal branch **B** Leaf **C** Distal portion of branch showing flush of new leaves **D** Flower, frontal view **E** Flower basal view **F** Calyx **G** Fruit **H** Cross section of fruit. By Alice Tangerini based on *Brewer & Stott 7110* (US).

opposite for the most part, alternate towards the distal portion of the leaf, oblong-lanceolate, 4–12 × 1.5–2.5 (gradually smaller toward both ends of the leaf), sessile, with slightly asymmetrical, rounded/cordate base, long acuminate at apex, margins

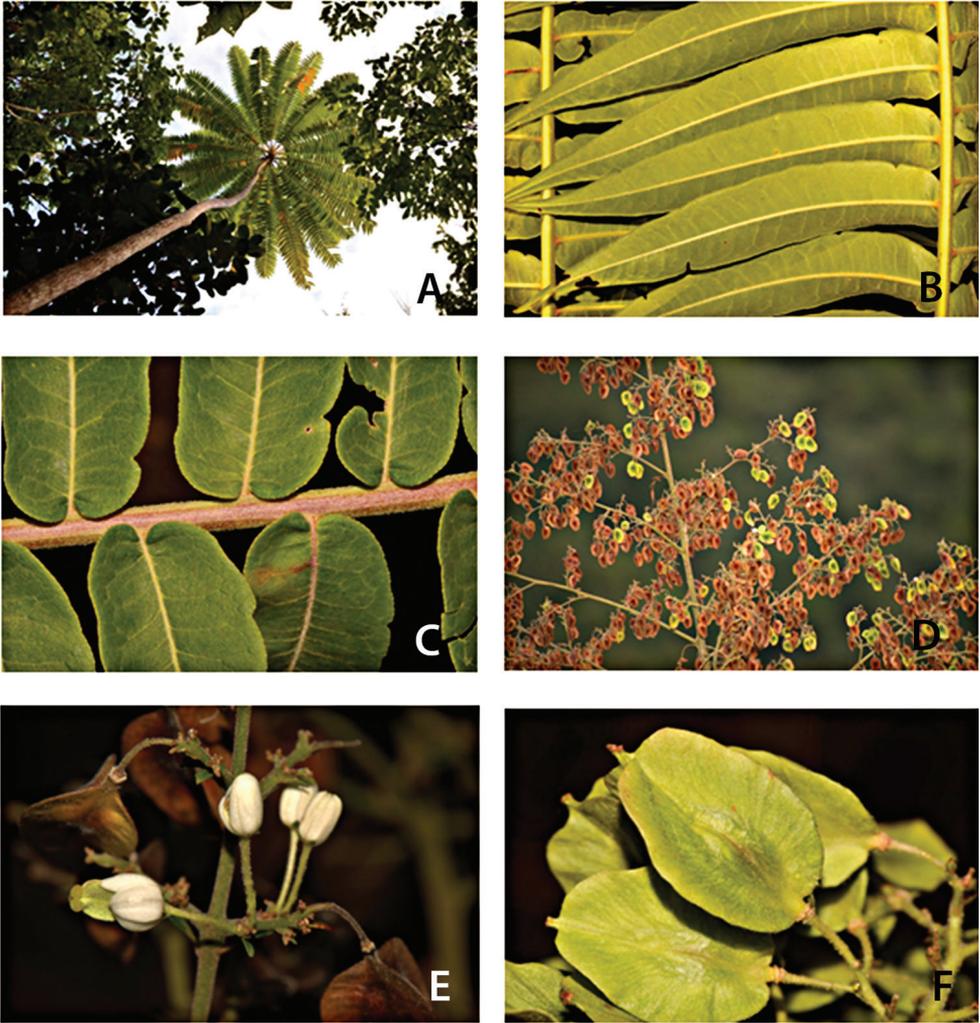


Figure 2. *Spathelia belizensis*. **A** Habit showing crown of leaves **B** Middle portion of leaf showing abaxial surface of leaflets **C** Portion of leaf rachis showing basal portion of leaflets **D** Portion of infructescence **E** Botryoid cyme showing flowers and fruits **F** Young fruits. Pictures by S. Brewer, based on *Brewer & Stott 7110* (US).

revolute, crenate (sinuate in basal leaflets), with numerous oil glands, adaxial surface sparsely stellate except for the densely stellate midvein, abaxial surface sparsely strigose especially along the prominent midvein, and sparsely stellate. Inflorescence a distal frondo-bracteate, terminal, panicle-shaped thyrse, $> 1 \times 1$ m. Flowers in loose botryoid cymes; pedicels strigose, 5–9 mm long; calyx green, slightly fleshy, strigose, sepals unequal, oblong-lanceolate, 1.2–1.5 mm long, with an enlarged apical gland; petals 6, white, oblong-ovate, obtuse at apex with an apical gland; filaments (pistillate flowers) ca. 1.5 mm long, wingless or shortly winged at base, setulose on lower

half; gynoecium oblong, trigonous, ca. 4 mm long, strigose; stigma subglobose, yellowish, nearly sessile. Fruit strigose, trigonous-winged, with ovate outline and obtuse apex, turning from green to reddish brown; endocarp slightly woody, with large cavity on dorsal side.

Distribution and ecology. Known only from the northern portion of the Golden Stream Corridor Preserve and adjacent portions of the Columbia River Forest Reserve in Belize; on steep slopes of Cretaceous limestone hills at elevations of c. 80–250 m.

Specimens examined. Only the type collection was studied.

Etymology. The specific epithet refers to the country where the new species is known to occur.

Conservation status

Spathelia belizensis appears to be limited to the Golden Stream Corridor Preserve in Belize where several individuals have been spotted by the junior author. However, in the absence of precise information about its frequency the species can only be treated as DD (Data deficient) within IUCN guidelines.

Discussion

Spathelia belizensis and *S. brittonii* seem closely related as they share similar heights, large leaves, and indument of stellate and simple hairs. Nevertheless, *Spathelia belizensis* differs from the latter by having leaves with 75–91 leaflets (vs. 42–65), crenate leaflets (vs. crenate-dentate), unequal sepals (vs. equal), petals 6 and white (vs. 5 and bright red to pink), and fruits with ovate outline and obtuse apex (vs. obovate and marginate at apex).

The type specimens of *Spathelia belizensis* contained only 6-merous flowers, a feature that departs from other species of *Spathelia* as they are known to have only 5-merous flowers. Because this collection only had few flowers, it is premature to regard the presence of 6-merous flowers as a distinctive character of the new species. Future collections may show it also to have 5-merous flowers.

Acknowledgements

We thank Judith Knight and Mark T. Strong for proofreading the manuscript and Alice Tangerini for the accurate illustration of the new species. The authors are also indebted to Paul Kessler for his valuable comments upon reviewing the manuscript. Logistical and financial support of fieldwork were provided to S. Brewer and G. Stott by the Ya'axche Conservation Trust (Belize) and by the Global Trees Campaign (a joint initiative between Fauna & Flora International and Botanic Gardens Conservation International dedicated to saving the world's threatened tree species).

References

- Adams CD (1972) Flowering plants of Jamaica. University of the West Indies, Mona, 848 pp.
- Appelhans MS, Smets E, Razafimandimbison G, Haevernsmans T, van Marle EJ, Couloux A, Rabarison R, Randrianarivojosia M, Kessler PJA (2011) Phylogeny, evolutionary trends and classification of the *Spathelia*-*Ptaeroxylon* clade: morphological and molecular insights. *Annals of Botany* 107: 1259–1277. <https://doi.org/10.1093/aob/mcr076>
- Beurton C (2008) Rutaceae. In: Greuter W, Rankin R (Eds) *Flora de la República de Cuba* Fascículo. Volume 14(3): 3–177.
- Correll DS, Correll HB (1982) *Flora of the Bahama archipelago*. Cramer, Vaduz, 1692 pp.
- Marie-Victorin F (1948) Nouvelles études taxonomiques sur la flore de Cuba III. Le genre *Spathelia* (Rutacées), avec description de cinq espèces et d'une variété nouvelles. *Contr. Inst. Bot. Univ. Montréal* 63: 14–48, 76.
- Pirani J, Groppo M (2015) Rutaceae. In: *Lista de Especies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB1010>

The pollen morphology of *Pelargonium endlicherianum* and *Pelargonium quercetorum* (Geraniaceae) in Turkey

Birol Başer¹, Mehmet Fırat², Akın Aziret³

1 Bitlis Eren University, Faculty of Arts and Science, Department of Biology, Bitlis, Turkey **2** Yüzüncü Yıl University, Faculty of Education, Department of Biology, Van, Turkey **3** Fırat University, Keban Vocational School, Department of Environmental Protection, Elazığ, Turkey

Corresponding author: Mehmet Fırat (kuyucak65@yahoo.com)

Academic editor: H. Schaefer | Received 1 November 2016 | Accepted 9 December 2016 | Published 15 December 2016

Citation: Başer B, Fırat M, Aziret A (2016) The pollen morphology of *Pelargonium endlicherianum* and *Pelargonium quercetorum* (Geraniaceae) in Turkey. *PhytoKeys* 75: 153–162. <https://doi.org/10.3897/phytokeys.75.11011>

Abstract

The pollen morphology of *Pelargonium endlicherianum* Fenzl. and *Pelargonium quercetorum* Agnew. from the family Geraniaceae was examined under light microscopy and scanning electron microscopy. Pollen morphologies are eurypalynous. The pollen grains were tricolporate, prolate-spheroidal and large. Aperture was ectoaperture, colpus was short, and the pores were oblate-spheroidal and large. The exine ornamentations were striate-reticulate and the reticula were heterobrachate. The 2 species are invasive exotics in Turkey.

Keywords

Morphology, *Pelargonium*, Pollen, Turkey

Introduction

Geraniaceae is cosmopolitan family of mostly temperate and subtropical annual or perennial herbs and a few small shrubs, comprising about 841 species belonging to 5 genera *Erodium* L'Hérit., *Geranium* L., *Monsonia* L., *Sarcocaulon* (DC) Sweet, and *Pelargonium* L'Hérit. (Hutchinson 1969, The Plant List 2013). In Turkey, it is represented by 4 (*Biebersteinia* Steph., *Geranium* L., *Erodium* L'Hérit, *Pelargonium*

L'Hérit.) genera and 72 taxa in Turkey (Davis 1967). *Pelargonium quercetorum* and *Pelargonium endlicherianum* growing in Turkey are completely natural and uncultivated. These species have a rhizome root structure. The fruit are beaked and they have poured into the surroundings after maturity. The probability of germination is weak. It is usually distributed by rhizomes. It has been reported that *P. quercetorum* shows distribution only in N. Iraq (Agnew 1967). However, it has been determined that *P. quercetorum* grows in a narrow area of Hakkari in Turkey as well. *P. endlicherianum* species show a natural distribution in eastern and inner Anatolia of Turkey. There are no other *Pelargonium* species close to both of these 2 species. For this reason, there are no ancestor species to hybridize. As a result, there are no hybrids of these 2 species.

The genus *Pelargonium* in the family Geraniaceae numbers over 200 species divided into 15 sections in the world (Van der Walt and Vorster 1981, 1988). It is a familiar group of ornamental plants and represents the sole genus in the tribe Pelargonieae, characterized within the Geraniaceae by zygomorphic flowers and the presence of a hypanthium. The largest concentration of the species occurs in the Cape Province of South Africa, but the genus is also represented in southern central, east, and north-east Africa, Madagascar, western Asia, Australia (Stafford and Gibby 1992). *Pelargonium* is a genus predominantly S. African. Several cultivars of the S. African species are grown as ornamentals in the warmer parts of Turkey. These include *P. zonale*, *P. peltatum* and *P. x hybridum* (*P. inquinans* x *P. zonale*). *Pelargonium endlicherianum* and *P. quercetorum* from this genus naturally grow in Turkey and N. Iraq. These species are perennial herbaceous or semi-woody bush, have different colored flowers (red, fire red, orange-red, pink, and white edged), and fruit beaked, splitting from base to apex into 5 mericarps (Davis 1967). In a preliminary study of the family Geraniaceae, Bortenschlager (1967) demonstrated that, in the compound light microscope, pollen morphology among the different genera was heterogeneous. The genus *Pelargonium* was shown to have some affinities with the related genera *Erodium*, *Monsonia*, and *Sarcocaulon*, and from an examination of 63 species representing all 15 sections of *Pelargonium*, he identified 3 different pollen types: *P. hymnifolium*, *P. rapaceum*, and *P. echinatum*. More recently, the general pollen morphology of the family has been studied by Verhoeven and Marais (1990), who demonstrated that pollen morphological characters were useful in delimiting the different sections of *Pelargonium* and also the subsections of Section Polyactium, although they did not include details of the species investigated or descriptions of the types to aid in identification. Hutchinson (1969) divided the family Geraniaceae into 2 tribus: Geranieae (*Geranium* L., *Erodium* L., *Monsonia* L., and *Sarcocole*) and Pelargonieae (*Pelargonium* L. Herit ex Aiton). Oltmann (1967) defined the pollen morphology of Geraniales. Bortenschlager (1967) examined 33 *Erodium* L. species and recognized 2 basic types, namely *Geranium multiflorum* and *Erodium* L., and the tectum in all 3 species can be described as striate-reticulate. El-Oqlah (1983) also identified the 2 basic pollen types in *Erodium* L. The shape and size, and apertural type of the grain do not show much variation throughout the pollen type. Pollen type-II is readily distinguished by a reticulatestriate tectum, which is heavily ornamented

with baccula and gemmae. Verhoeven and Venter (1987) reported a similar pollen type in *Erodium* L. (except *Erodium oxyrrhynchum* ssp. *oxyrrhynchum* striate-reticulate tectum with gemmate and baculate muri). The morphology of the pollen grains of all 3 species corresponds to that of the rest of the genus *Pelargonium*, in that the grains are spherical and tricolporate (Marais 1991). The structure or the wall of the pollen grain is semitectate (Verhoeven and Marais 1990, Marais 1997) *Pelargonium hirtipetalum*, *P. pubipetalum*, and *P. aridicola* were described as new species, and they correspond with regard to the leaf anatomy, the structure of the androecium and pollen morphology (Perveen and Gaiser 1999) The pollen morphology of 13 species belonging to the 3 genera of the family Geraniaceae was investigated with a light microscopy (LM) and scanning electron microscopy (SEM). The pollen morphology of Geraniaceae or some of its representatives have been studied by several researchers (Kuprianova and Alyoshina 1972, Moore and Webb 1978, El-Oqlah 1989, Stafford and Blackmore 1991). Recently, Aedo et al. (2007) and Shehata (2008) have used the palynological data in their taxonomic revision of the genera *Pelargonium* and *Geranium*. Boukhris et al. (2013) performed a study of the essential oil composition, and trichomes distribution, morphology, and anatomy of the aerial organs of *Pelargonium graveolens* L., which is an aromatic and medicinal species originating from Sfax (Tunisia)

The systematic investigation of the pollen morphology of *Pelargonium* in Turkey has not been studied comprehensively. According to Flora of Turkey, *P. quercetorum* differs from *P. endlicherianum* by its larger size, less hairy or glabrescent leaves with lobed and dentate segments, more numerous shorter pedicels, and narrower upper petals (Davis 1967). These 2 species are systematically different from each other. The purpose of this study was to determine pollen morphological differences in *Pelargonium* of Turkey and to take these differences into account.

The terminology of pollen morphology was used based on Erdtman (1952), Kremp (1965), Faegri and Iversen (1964), Walker and Doyle (1976), and Punt et al. (1994).

Materials and methods

While *Pelargonium endlicherianum* was collected from Hakkari in the C9 grid of Flora of Turkey, *Pelargonium quercetorum* was collected from Tunceli in the B7 grid of Flora of Turkey. The pollen characteristics of these 2 species were examined in preparations by the method of Woodhouse (1935) for the LM. The polar and equatorial axis, colpus length and width, and exine and intine thickness of the pollen were measured 30 times. The morphological characteristics under the LM were measured using an Olympus BX41 microscope. Microphotographs were taken from this microscope's camera. During SEM, selected dry samples of pollens were placed on the aluminum stabs with the help of double-sided adhesive tape and coated in gold with a vacuum. The images of the pollen were taken with a Jeol JSM 7001-F SEM in the SEM laboratory of the Department of Biology, Firat University.

Results

The morphological variation of the pollen grains of 2 species of *Pelargonium* was described in terms of the size and shape of the pollen grains, morphology of apertures, and exine ornamentation. Specifically, the surface ornaments were defined in detail using the SEM microphotographs. Tables 1 and 2 show the pollen size variations and measurements. Figs 1–2 illustrate the representative pollen characters.

The morphological characteristics of the pollen

Pelargonium endlicherianum

Pollen shape. Tricolporate

P/E ratio. 0.96

Shape. prolate-spheroidale.

Aperture. Ectoapertur and colpus are short, pore shape oblate-spheroidale and large.

Ornamentation. Striate-reticulate.

LM measurements. Polar axis (P) 73.00–81.53–92.00 μm , equatorial axis (E) 77.00–89.30–109.00 μm , P/E ratio: 0.96, colpus length 44.00–50.37–57.00 μm , Pore length 19.00–23.01–32.00 μm , exine thickness 4.75–5.40–6.00 μm , intine thickness 1.50–1.84–2.25 μm (Table 1, Fig. 1a, b).

SEM measurements. The number of lumina per 10 μm^2 is 5–6, the size of the lumina of the pollen grains is approximately between 2.42 μm to 5.71 μm , and the average thickness of the muri is 0.62 μm to 0.88 μm , polar axis (P): 79 μm , equatorial diameter (E) 85 μm (Fig. 2).

Pelargonium quercetorum

Pollen shape. Tricolporate

P/E ratio. 1.03

Shape. Prolate-spheroidal.

Aperture. Ectoaperture and colpus are short, pore shape prolate-spheroidal and large.

Ornamentation. Striate-reticulate.

LM measurements. Polar axis (P) 73.00–81.53–92.00 μm , equatorial axis (E) 75.00–79.33–87.00 μm , P/E ratio: 1.03, colpus length 40.00–47.60–55.00 μm , Pore length 17.00–21.40–25.00 μm , exine thickness 4.00–4.80–5.50 μm , intine thickness 1.00–1.57–2.00 μm (Table 1, Fig. 1c–d).

SEM measurements. The number of lumina per 10 μm^2 is 4–5, the size of the lumina of the pollen grains is approximately between 2.50 μm to 5.63 μm , and the average thickness of the muri is 1 μm to 1.25 μm , polar axis (P): 69 μm , equatorial diameter (E) 67 μm (Fig 2).

Table 1. The measurements of *P. endlicherianum* ve *P. quercetorum* in ligh microscope.

Taxon	Polar axis min-max (µm)	Equatorial axis min-max (µm)	P/E ratio (pollen shape)	Colpus length min-max (µm)	Colpus width min-max (µm)	Por length min-max (µm)	Por width min-max (µm)	Exine thickness min-max (µm)	Intine thickness min-max (µm)
<i>P. endlicherianum</i>	85.93±6.69 75-100	89.30±7.13 77-109	0.96	50.37±3.47 44-57	24.78±2.89 20-29	23.01±2.89 19-32	21.93±2.27 18-26	5.40±0.44 4.75-6	1.84±0.26 1.5-2.25
<i>P. quercetorum</i>	81.53±4.45 73-92	79.33±2.87 75-87	1.03	47.60±3.93 40-55	16.93±2.60 13-23	21.40±2.37 17-25	17.20±2.02 12-21	4.80±0.57 4-5.50	1.57±0.35 1-2

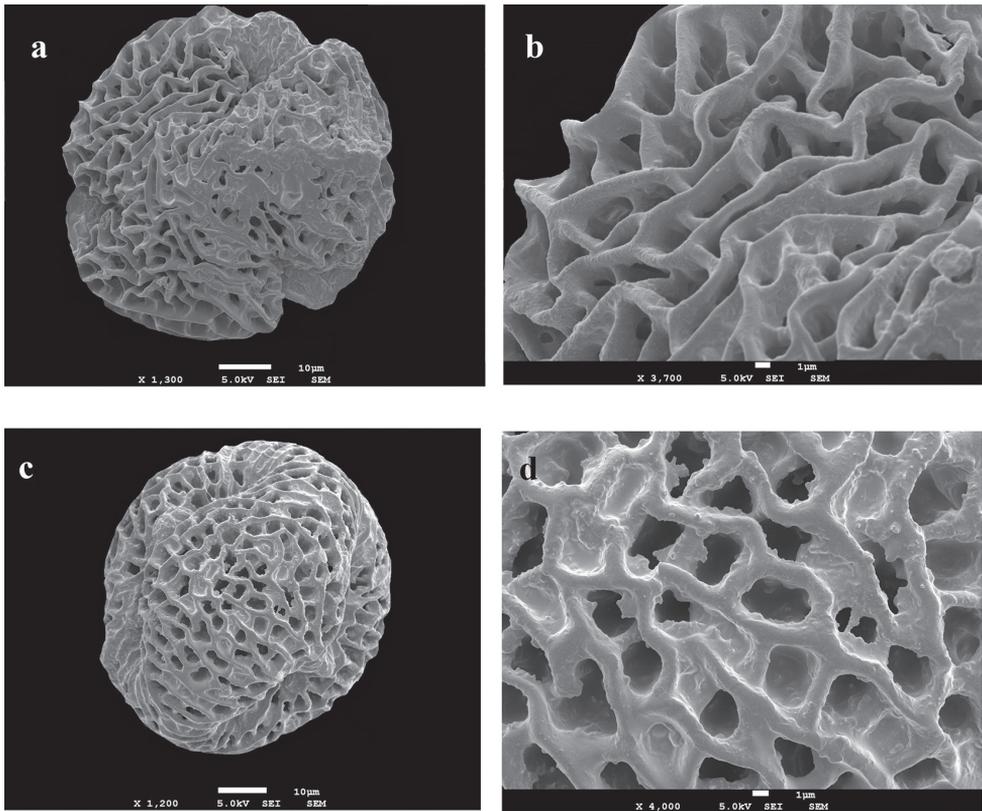


Figure 1. Light microscope microphotography; *Pelargonium endlicherianum*: **a** Polar sight **b** Equatorial sight **c** exine ornamentation, *Pelargonium quercetorum* **d** Polar view **e** Equatorial view **f** exine ornamentations. Scale: **a–f**=20 µm.

Table 2. The comparison of species *P. endlicherianum*, *P. quercetorum*, and *P. grandiflorum*.

Pollen	<i>Pelargonium endlicherianum</i>	<i>P. quercetorum</i>	<i>P. grandiflorum</i> (Shehata A. A,2008)
Polar axis	85.93 (75–100) µm	81.53 (73–92) µm	44 (42–46) µm
Equatorial axis	89.30 (77–109) µm	79.33 (75–87) µm	32 (36–38) µm
Shape	Prolate- spheroidal	Prolate- spheroidal	Prolate
Ornamentations	Striate-reticulate	Striate-reticulate	Reticulate
Aperture	Tricolporate	Tricolporate	Tricolpate

Discussion

The present study aimed to: 1) survey the pollen morphology of 2 taxa belonging to *Pelargonium* growing naturally in Turkey, and 2) to determine pollen morphological differences of this genus. The first comprehensive report on the pollen morphology of the 2 taxa belonging to the genus *Pelargonium* was examined using LM and SEM. The pollen morphology of Geraniaceae is eurypalynosis; The pollen morphology of

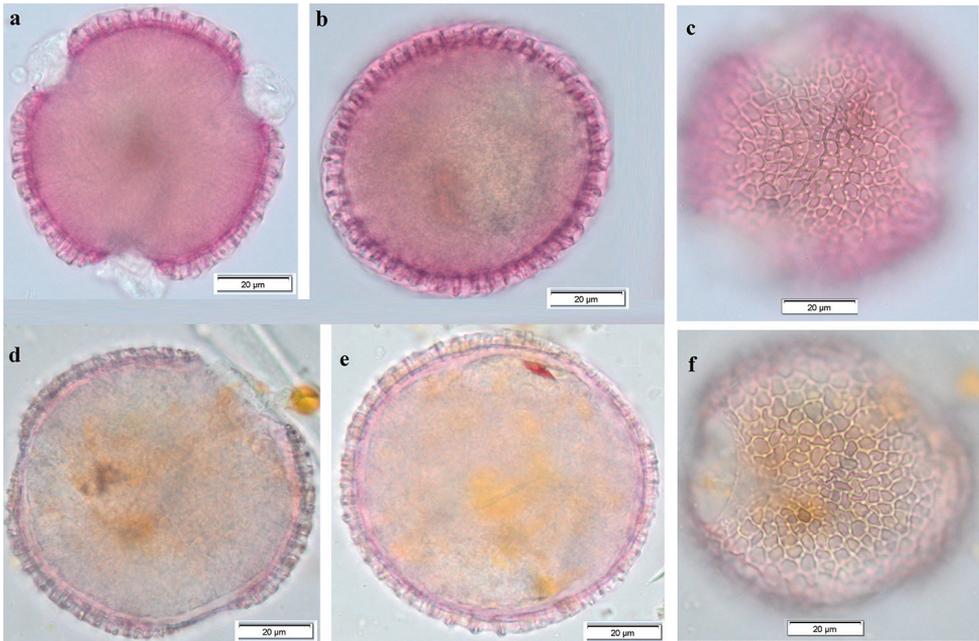


Figure 2. SEM microphotography; *Pelargonium endlicherianum*: **A** Polar view (x1.300) **B** exine surface (x3.700). *Pelargonium quercetorum*: **c** Equatorial view (x1.200) **d** exine surface (x4000).

Geraniaceae is significant in the systematics of the genus. The pollens are usually radial symmetric, isopolar, oblate-spheroidal, rarely sub-oblate, tricolporate, rarely colpate, colpus are short, and the sexine is thicker than the nexine. Tectum dense-reticulate. baculate or gemmate muri or striate (Erdtman 1952, 1966, 1969). The pollen grains of *Pelargonium* are usually trizonocolporate. The size of the grains varies from P 47–120 µm E 47–120 µm. The pollen grains are characterized by a distinctive reticulum, which may be multi-layered with striate elements of various lengths and thicknesses. Ornamentations; reticulate, coarsely-reticulate, striate/reticulate (Stafford and Gibby 1992). The species examined in this study were separated into 4 main pollen types and 5 subgroups, summarized as follows: Type 1: *Pelargonium longifolium*, Type 2: *P. echinatum*, Type 3: *P. hirtum*, and Type 4: *P. rapaceum* type (Subgroup A: *P. tragacanthoides*, Subgroup B: *P. fasciculaceum*, Subgroup C: *P. schlechteri*). In their work, the species *Pelargonium endlicherianum* was measured and recorded as P: 94–(96.6)–100 µm, E: 83–(91)–95 µm and exine: 7–9 µm, pollen shape prolate-spheroidal, ornamentation striate/reticulate and was identified as type 3, *Pelargonium hirtum*. Based on our study, we had the following results, which are consistent with the previous work: *P. endlicherianum* (P: 75–(85.93)–100 µm, E: 77–(89.30)–109 µm, exine: 5.40 µm), striate/reticulate, and pollen shape; prolate-spheroidal. The results of the micromorphical investigations we conducted on the pollens of the other studied species, which were *P. quercetorum* (P: 73.00–81.53–92.00 µm, E: 75.00–79.33–87.00 µm exine: 4.80 µm and pollen shape; prolate-spheroidal), and these

were understood to be in accordance with their evaluations and were accepted as type 3. In their work, species *Pelargonium endlicherianum* were P: 94–(96.6)–100 μm , E: 83–(91)–95 μm and exine: 7–9 μm , pollen shape prolate-spheroidal, ornamentation striate/reticulate and type 3. The classification of *Pelargonium* pollen types in terms of ornamentation and exine structure does not clearly conform to the generic boundaries within the family Geraniaceae or sectional divisions within the genus. The most frequent pollen type in the genus *Pelargonium* is *P. hirtum*, which has been found in species from every section of the genus. In another study on the family Geraniaceae, The studies on pollen morphology and taxonomic importance of the family Geraniaceae in Egypt have shown the existence of 3 main types and 3 subtypes in terms of the aperture type, exine structure, exine surface, and pollen type (P/E ratio): Type I: striate/striate-reticulate, Type II: reticulate/gemmate type, and Type III: reticulate type (Subtype A: *Monsonia heliotropioides*, Subtype B: *Monsonia senegalensis*, and Subtype C: *Pelargonium grandiflorum*). It was determined that there was a distinctive striation on the tricolpate and ornament of the species *P. grandiflorum*, which is a type of *Pelargonium* (Shehata 2008). It was determined in the present study that the pollen sizes, apertures, and ornamentations of the species *Pelargonium endlicherianum* and *P. quercetorum* showed differences compared to the pollens of *P. grandiflorum* studied by Shehata (2008) (Table 2, Fig. 2). The pollen was examined and separated into pollen types representing the lowest recognizable taxonomic units on the basis of the pollen morphological characteristics, principally, the exine structure and ornamentation. The greater majority of species were found to fall into a general striate/reticulate ornamentation type within which it was not possible to distinguish between species, although a number of other pollen types and subgroups could be identified by differences in their reticulum and ornamentation, the palynological results are discussed with respect to current systematic treatments of *Pelargonium* (Stafford and Gibby 1992).

In conclusion, we found a correlation between our results and the classification of the taxa in this genus, that is, pollen features, especially pollen size, exine and ornamentation, proved to be the most useful characters for the systematics of the taxa.

References

- Aedo C, Garcia M, Maria AL, Aldasoro J, Navarro C (2007) Taxonomic revision of *Geranium* Subsect. *Mediterranea* (Geraniaceae). *Systematic Botany* 32(1): 93–128. <https://doi.org/10.1600/036364407780360111>
- Agnew ADQ (1967) Contributions to the Flora of Iraq: IV: Notes on the Geraniaceae of Iraq, with a New Species of *Pelargonium*. *Kew Bulletin* 21(2): 225–227.
- Boukhris M, Nasri-Ayachi MB, Mezghani I, Bouaziz M, Boukhris M, Sayadi S (2013) Trichomes morphology, structure and essential oils of *Pelargonium graveolens* L. (Geraniaceae). *Industrial Crops and Products* 50: 604–610. <https://doi.org/10.1016/j.indcrop.2013.08.029>

- Bortenschlager S (1967) Present information of pollen morphology of family Geraniaceae and its systematic meaning. *Grana Palynologica* 7: 400–468. <https://doi.org/10.1080/00173136709430046>
- Davis PH (1967) *Pelargonium* L. In: Davis PH (Ed.) *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, 451–474.
- El-Oqlah AA (1983) Pollen morphology of the genus *Erodium* L'Hérit. in the Middle East. *Pollen et Spores* 25(3–4): 383–394.
- El-Oqlah E (1989) A revision of the genus *Erodium* L'Hérit. in the Middle East. *Feddes Report* 100: 3–4.
- Erdtman G (1952) *Pollen Morphology and Plant Taxonomy – Angiosperms*. Almquist and Wiksell, Stockholm, 650 pp. <https://doi.org/10.1080/11035895209453507>
- Erdtman G (1966) *Pollen Morphology and Plant Taxonomy – Angiosperm*. Hafner Publishing Co., New York, 560 pp.
- Erdtman G (1969) *Hand Book of Palynology – An Introduction to the Study of Pollen Grains and Spores*. Munksgaard, Copenhagen, 486 pp.
- Faegri K, Iversen J (1964) *Testbook of Pollen Analysis*. Munsgaard, Copenhagen, 237 pp.
- Hutchinson J (1969) *Evolution and Phylogeny of Flowering Plants*. Academic Press, London, 717 pp.
- Kremp GOW (1965) *Morphologic Encyclopedia of Palynology; An International Collection of Definitions and Illustrations of Spores and Pollen*. University of Arizona Press, Tuscon, 185 pp.
- Kuprianova LA, Alyoshina LA (1972) *Pollen and spores of plants from the flora of European part of USSR (Vol. 1)*. The Academy of Sciences of the USSR, Komarov Botanical Institute, 170 pp.
- Marais EM (1991) Four new species of *Pelargonium* (Geraniaceae) from the Western Cape Province. *South African Journal of Botany* 57: 55–66. [https://doi.org/10.1016/S0254-6299\(16\)30985-1](https://doi.org/10.1016/S0254-6299(16)30985-1)
- Marais EM (1997) Three new species of *Pelargonium* (Geraniaceae) from Namaqualand. *South African Journal of Botany* 63(2): 82–89. [https://doi.org/10.1016/S0254-6299\(15\)30707-9](https://doi.org/10.1016/S0254-6299(15)30707-9)
- Moore PD, JA Webb (1978) *An Illustrated Guide to Pollen Analysis*. Hodder and Stoughton, London, 192 pp.
- Oltmann O (1967) *Pollen morphologisch-systematische Urzersuchungen innerhalb der Geraniales*. *Dissert Botany* 11: 1–163.
- Perveen A, Gaiser M (1999) *Pollen Flora of Pakistan-XV Geraniaceae*. *Turkish Journal of Botany* 23: 263–269.
- The Plant List (2013) *The Plant List*. Version 1.1. <http://www.theplantlist.org/Geraniaceae> [accessed: 26 November 2016]
- Punt W, Blackmore S, Nilsson S, Thomas LE (1994) *Glossary of pollen and spore terminology*. *Laboratory of Paleobotany and Palynology* 10: 39–60.
- Shehata AA (2008) *Pollen Morphology of Egyptian Geraniaceae: An Assessment of Taxonomic Value*. *International Journal of Botany* 4(1): 67–76. <https://doi.org/10.3923/ijb.2008.67.76>

- Stafford PJ, Blackmore S (1991) The Northwest European pollen flora – Geraniaceae. Review of Palaeobotany and Palynology 69: 49–78. [https://doi.org/10.1016/0034-6667\(91\)90066-C](https://doi.org/10.1016/0034-6667(91)90066-C)
- Stafford PJ, Gibby M (1992) Pollen morphology of the genus *Pelargonium* (Geraniaceae). Review of Palaeobotany and Palynology 71: 79–109. [https://doi.org/10.1016/0034-6667\(92\)90158-D](https://doi.org/10.1016/0034-6667(92)90158-D)
- Verhoeven RL, Venter HJT (1986) Pollen morphology of *Monsonia* L. South African Journal of Botany 52: 361–368. [https://doi.org/10.1016/S0254-6299\(16\)31535-6](https://doi.org/10.1016/S0254-6299(16)31535-6)
- Van der Walt JJA, Vorster PJ (1981) Pelargoniums of Southern Africa (Vol. 2). Jura, Cape Town, 149 pp.
- Van der Walt JJA, Vorster PJ (1988) Pelargoniums of Southern Africa. National Botanic Gardens, Kirstenbosch, 149 pp.
- Verhoeven RL, Venter HJT (1987) Pollen morphology of *Erodium* in South Africa. South African Journal of Botany 53(4): 279–283. [https://doi.org/10.1016/S0254-6299\(16\)31417-X](https://doi.org/10.1016/S0254-6299(16)31417-X)
- Walker JW, Doyle JA (1976) The basis of Angiosperm phylogeny: Palynology. Annals of the Missouri Botanical Garden 62(3): 64–723.