**RESEARCH ARTICLE** 



# Chrysosplenium ramosissimum Y.I.Kim & Y.D.Kim (Saxifragaceae), a new species from Korea

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#### Abstract

This study describes and illustrates *Chrysosplenium ramosissimum*, a new plant species from Mt. Seonjaryeong, located in the central region of the Korean Peninsula. The species is most similar to *C. valdepilosum* but is readily distinguishable by the presence of yellowish-green bracteal leaves during flowering, highly branched sterile branches, shiny silvery dots on sterile branch leaves and larger tubercles on the seed coat.

#### Keywords

Chrysosplenium, endemic species, seed morphology, sterile branch, DNA barcode

# Introduction

*Chrysosplenium* L. is a distinct genus belonging to the family Saxifragaceae, as it possesses tetramerous flowers and petaloid sepals (Bensel and Palser 1975; Soltis 2007; Soltis et al. 2001). This genus is primarily restricted to the northern hemisphere except for two species in Chile, with species occurring in eastern North America (two species), western

North America (four species), Europe (two species) and eastern Asia, where the greatest number of species are present, numbering approximately 50 (Hara 1957; Spongberg 1972). Although estimates of the number of taxa are controversial due to its complex taxonomy, approximately 70 taxa from the genus are recognised worldwide (Maximowicz 1859, 1872, 1877, 1879; Hara 1957; Spongberg 1972; Pan 1986; Ye and Zhang 1994; Wakabayashi and Ohba 1995; Wakabayashi and Takahashi 1999; Han et al. 2011; Bhaumik 2014; Kim 2014; Kim and Kim 2015; Liu et al. 2016; Wakabayashi et al. 2018).

*Chrysosplenium* has been classified into two sections and 17 series (Hara 1957), of which nine species representing two sections and five series are distributed in Korea (Chung and Kim 1988; Kim and Kim 2011, 2105; Han et al. 2011, 2012). The series *Pilosa* Maxim. is known to be endemic to northeast Asia and consists of approximately 20 taxa (Franchet and Savatier 1878; Nakai 1914; Kitagawa 1934; Ohwi 1934; Hara 1957; Pan 1986; Wakabayashi and Takahashi 1999; Han et al. 2011; Kim and Kim 2015). The series is characterised by yellow or white erect sepals, opposite leaves and pilose stems (Hara 1957). Currently, five species of the series are recognised in Korea (Kim 2014): *C. aureobracteatum* Y.I. Kim & Y.D. Kim, *C. barbatum* Nakai, *C. epigealum* J.W. Han & S.H. Kang, *C. flaviflorum* Ohwi and *C. valdepilosum* (Ohwi) S.H. Kang & J.W. Han.

During a floristic survey of Mt. Seonjaryeong, located in Pyeongchang-gun, Gangwon-do, Korea in August of 2014, we collected a species of Chrysosplenium with a distinct stem feature (i.e. highly branched sterile branches). Additional fieldwork was conducted from April through July 2015 to collect flowering individuals and seeds for specimen and morphological examinations. After consulting relevant literature on Chrysosplenium (Franchet and Savatier 1878; Nakai 1914; Kitagawa 1934; Ohwi 1934; Hara 1957; Pan 1986; Han et al. 2011; Kim and Kim 2015) and examining herbarium specimens at HHU, TI, KB, KH, KWNU, KUS, IUI, KYO and PE, as well as images of type specimens available at the Global Plants website at JSTOR (https://plants.jstor. org), we recognised that the taxon belongs to the series Pilosa. Upon further examination, the plant was distinguished from all known species of the series based on morphological characters. The species is most similar to C. valdepilosum, which has been considered a variety of C. pilosum but recently recognised as a distinct species (Kim and Kim 2011, Han et al. 2011). The new species, however, is readily distinguishable by the presence of yellowish-green bracteal leaves during flowering, highly branched sterile branches, shiny silvery dots on sterile branch leaves and larger tubercles on the seed coat. This leads us to the conclusion that it represents an undescribed species. Here, the new species is described and illustrated.

#### Materials and method

#### Morphological observations

Photographs of the habit and macro-morphological characters were taken in the field. Morphological observations and measurements of the new species, based on living and dry plant specimens and preserved materials, were carried out. All morphological characters were observed and photographed with a Zeiss Stemi SV 11 Apo stereoscopic microscope and a Zeiss AxioCam MRc 5 microscope camera. Seed coat characters were revealed by a Hitachi S-3400N scanning electronic microscope.

## **Taxonomic treatment**

*Chrysosplenium ramosissimum* Y.I.Kim & Y.D.Kim, sp. nov. urn:lsid:ipni.org:names:60477346-2 Figs 1, 2, 3A1, A2, 4A 가지털괭이눈(Ga-ji-teol-gwaeng-i-nun)

**Diagnosis.** Chrysosplenium ramosissimum is most similar to the sympatric species C. valdepilosum, but the former is readily distinguishable by the presence of yellowishgreen (vs. bright yellow) bracteal leaves during flowering, highly branched and elongated sterile branches after fruiting (Fig. 4), shiny silvery dots on sterile branch leaves and larger tubercles on the seed coat (Fig. 3).

**Type.** SOUTH KOREA. Gangwon-do: along a stream near a hiking trail to Guksa Seonghwangsa (temple), Mt. Seonjaryeong, Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, 37°41'25.80"N, 128°45'27.22"E, elev. 872 m, 24 Apr. 2015, KYI-2015001 (holotype HHU; isotypes HHU, KB).

Perennial herbs. Small, tender, hermaphroditic. Roots fibrous. Flowering stem erect, 2-6 cm long, pilose, light green or reddish to purple, with 2-5 sterile branches arising from base; sterile branches creeping after fruiting, elongated over 30 cm, 2 or more times branched at axils, densely pilose. Leaves opposite, basal and cauline, simple, estipulate, petiolate. Basal leaves 1 or 2 pairs, withered before flowering. Leaves on flowering stem, 1 pair, attached at 1/2 or below of the stem; petioles 1-5 mm, pilose; blade  $2-5 \times 2-8$  mm, flabelliform, apex subtruncate to rounded, base attenuate, margins obscurely undulate to crenate or distinctly obtusely dentate (3-6 teeth), translucent white or brown ciliate, both surfaces glabrous. Leaves on sterile branches with long internode (to 8 cm at fruiting); petioles 2–12 mm, pilose; blade to  $2 \times 2.5$  cm, suborbicular or widely ovate (upper ones), flabellate (lower ones), apex rounded, base cuneate, margins crenate with 5-10 flat obscure teeth on each side, translucent white or brown ciliate, upper surface glabrous, densely silvery dotted, pale green, lower surface pilose on veins, greenish-grey. Inflorescence 4- to 9-flowered cyme, surrounded by leaf-like bracts; pedicels ca. 1 mm, sparsely pilose. Bracteal leaves yellowish-green during flowering, turning to light green or green after fruiting; petioles 1-3 mm, pilose; blades  $2-6 \times 2-10$  mm, obdeltoid, upper surface glabrous, densely silvery dotted, lower surface glabrous, greenish-grey, margins obscurely undulate to crenate or distinctly obtusely dentate, 2-5 teeth, translucent white or brown ciliate, obtuse to subtruncate at apex, base narrowly cuneate to cuneate. Flowers tetramerous; sepals 4, free, petaloid, 1 pair overlapping the other in bud, erect, yellow, widely ovate to widely subelliptic,



Figure 1. *Chrysosplenium ramosissimum* Y.I.Kim & Y.D.Kim. A Flowering individual B fruiting individual C sterile branch habit after fruiting D inflorescence and bracteal leaves E–F flower G stamen at various stages H flower longitudinal section I infructescence and bracteal leaves J capsule with persistent sepals K capsule, sepals removed L capsule, longitudinal section M capsule, before dehiscence (top view) N capsule, after dehiscence (top view) O node of sterile branch, enlarged P seed, side view (left), top view (right) Q seed coat, enlarged.



**Figure 2.** *Chrysosplenium ramosissimum* Y.I.Kim & Y.D.Kim. **A** Inflorescence with bracteal leaves **B** sterile branches and basal leaves during flowering with withered basal leaves **C** sterile branch leaves with shiny silvery spots during flowering **D** sterile branch after fruiting **E** plant habit during flowering.

ca.  $2.5-3 \times ca. 2$  mm, glabrous, 3-veined, persistent, apex obtuse to truncate, slightly recurved; petals absent; stamens 8, in 2 series, ca. 1.3 mm, shorter than sepal; filaments filiform, 0.8–0.9 mm long; anthers yellow, 2-locular, 0.45–0.5 mm long, longitudinally dehiscent; pistil 2-carpellate, semi-inferior, ovary 1-locular, ovules at 2 parietal placentae, styles 2, free, ca. 1 mm long, stigma round, disc absent. Fruit capsule, pale green, glabrous, ca. 5.5 mm long, 2-lobed (horn shaped), lobes dehiscent along adaxial suture, slightly unequal; seeds numerous, dark brown, ellipsoid, with a carina on one side, thick-walled,  $0.8-1.0 \times 0.65-0.75$  mm, with hemispheroidal tubercles, tubercles ca. 15 µm in diameter, seed surface covered with minute deciduous papillae.



**Figure 3.** Upper surface of sterile branch leaves of *Chrysosplenium ramosissimum* (AI) and *C. valdepilosum* (BI). Scanning electron micrograph of seeds of *C. ramosissimum* (A2) and *C. valdepilosum* (B2).



Figure 4. Sterile branch outline of Chrysosplenium ramosissimum (A) and C. valdepilosum (B) after fruiting.

**Distribution.** *Chrysosplenium ramosissimum* is only known to exist on Mt. Seonjaryeong in Gangwon-do, Korea, at an elevation of 630–910 m. To date, only one population of approximately 2,000 individuals has been discovered, near a small creek. In the absence of additional data, we presently score it as Data Deficient (DD), according to the IUCN Red List criteria (IUCN 2001).

Character	C. ramosissimum	C. valdepilosum
Starila branchas aftar fruiting	branched more than two times,	unbranched or rarely branched,
Sterile branches after fruiting	ca. 30 cm long	> 15 cm long
Size of sterile branch leaf blades after fruiting	up to $2 \times 2.5$ cm	up to 2.5 × 2.6 cm
Upper surface of sterile branch leaves	silvery dotted, glabrous	silvery dots absent, pilose
Bracteal leaves during flowering	Yellowish-green	bright yellow
Seed surfaces	tubercles ca. 15 μm in diam.	tubercles ca. 10 μm in diam.

Table 1. Comparison of the key features of C. ramosissimum and C. valdepilosum.

**Ecology.** Chrysosplenium ramosissimum occurs in deciduous forests of mountain valleys, where it grows in humid and semi-shaded areas near small creeks along with *Quercus mongolica* Fisch. ex Ledeb., *Fraxinus rhynchophylla* Hance and *Acer buergeri-anum* Miq. The flowering period of this species is late March to early May and the fruiting period is late May to early July.

**Etymology.** The specific epithet of the new species refers to the highly branched sterile branches after fruiting.

Additional specimens examined (paratype). SOUTH KOREA. Gangwondo: Mt. Seonjaryeong, Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, 37°41'25.80"N 128°45'27.22"E, elev. 872 m, 24 Apr. 2015, *KYI-2015002* (HHU), *KYI-2015003* (HHU), *KYI-2015004* (HHU), *KYI-2015005* (HHU), *KYI-2015006* (HHU); 37°41'33.65"N 128°45'25.26"E, elev. 872 m, 16 Apr. 2016, *KYI-2016001* (HHU), *KYI-2016002* (HHU), *KYI-2016003* (HHU), *KYI-2016004* (HHU), *KYI-2016005* (HHU), *KYI-2016006* (HHU).

#### Key to taxa of Chrysosplenium series Pilosa modified from Hara (1957)

1	Sepals white. Anthers dark red2
_	Sepals yellow or greenish. Anthers yellow
2	Stamens longer than or equal to sepals. Ovary superior. Seeds tuberculate <i>C. album</i>
_	Stamens shorter than sepals. Ovary subsuperior. Seeds smooth
3	Sterile branches often hypogeous, filiform, with bulbil at top
_	Sterile branches epigeous without bulbil
4	Seeds without tubercules
_	Seeds with tubercules
5	Leaves of sterile branches congested at distal end, with white variegated veins on upper surface
_	Leaves of sterile branches distantly arranged, with silvery dotted upper sur- face
6	Seed tubercles arranged without or on inconspicuous longitudinal ridges7
_	Seed tubercles arranged on prominent longitudinal ridges9

7	Leaves of sterile branches densely ciliate
_	Leaves of sterile branches rarely ciliate
8	Sterile branches highly (more than two times) branched, ca. 30 cm long after
	fruiting. Leaves of sterile branches with silvery dots, upper surface glabrous.
	Bracteal leaves yellowish-green C. ramosissimum
_	Sterile branches unbranched, less than 15 cm long after fruiting. Leaves of
	sterile branches without silvery dots, upper surface pilose. Bracteal leaves
	bright yellow C. valdepilosum
9	Basal leaves persistent after flowering10
_	Basal leaves withered before flowering12
10	Sepals yellow. Stamens shorter than sepals C. sphaerospermum
_	Sepals light green. Stamens equal to or longer than sepals
11	Stamens equal to or slightly longer than sepals. Ovary 1/2 or 1/3 inferior
_	Stamens longer than sepals. Ovary 1/4 inferior or nearly superior
	C. pseudopilosum
12	Leaves of sterile branches distantly arranged after fruiting. Bracteal leaves
	golden yellow, yellowish-green or green at flowering13
_	Leaves of sterile branches congested at distal end after fruiting. Bracteal leaves
	green14
13	Leaves of sterile branches pilose. Bracteal leaves golden yellow at flowering
	C. aureobracteatum
_	Leaves of sterile branches glabrous. Bracteal leaves yellowish-green to green at
	floweringC. pilosum
14	Seeds ca. 720 × 640 $\mu$ m, with ca. 18 ridges, densely papillate <i>C. barbatum</i>
_	Seeds ca. 640 × 510 µm, with ca. 16 ridges, sparsely papillate C. fulvum

#### Notes

It is noteworthy that *C. valdepilosum* and *C. ramosissimum* are sympatric in the type locality. The former species occupies moist soil at the side of a creek, while the latter inhabits damper parts closer to the main stream. The two species exhibit a high degree of morphological similarity upon flowering but can be distinguished by several characters, including the colour of the bracteal leaves at flowering, the vestiture of the leaves of sterile branches and the excrescence of the seeds (Table 1).

It appears that *C. ramosissimum* and *C. valdepilosum* have not been recognised as different lineages until recently due to their sympatric distribution and high morphological affinity. Ignoring the importance of the sterile branch development pattern after fruiting may have been the main cause for the delay of the discovery of the new lineage. Further research on the genetic diversity and discovery of additional populations are necessary for the conservation of *C. ramosissimum*, an endemic species with a very narrow distribution.

*C. ramosissimum* may also be similar to *C. ramosum* due to its highly-branching habit. *C. ramosum* is also distributed in northeast Asia, including Korea, but belongs to the series *Oppositifolia* and differs in its spreading sepals (vs. erect) and smooth seeds (vs. tuberculate).

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**RESEARCH ARTICLE** 



# A new disjunct species of *Eriolaena* (Malvaceae, Dombeyoideae) from Continental Africa

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#### Abstract

*Eriolaena rulkensii* Dorr, **sp. nov**. is described and illustrated. This attractive shrub is endemic to coastal Mozambique. The new species has apically winged seeds, which place it in a group of Malvaceae (Dombey-oideae) that is found in Asia and Madagascar and which had not previously been found in continental Africa.

#### **Keywords**

Dombeyoideae, Eriolaena, Malvaceae, Mozambique

# Introduction

When A.J.H. (Ton) Rulkens encountered a yellow-flowered shrub on the shore of Pemba Bay (Baia de Pemba) near the city of Pemba in north-eastern Mozambique, he could not identify it and sent photographs to John E. Burrows (BRNH) who also was unsure as to its identity. Burrows, in turn, shared photographs of the plant with several botanists including I. Darbyshire (K) who identified it as a species of Dombeyoideae (Malvaceae). Darbyshire noted that it did not match either *Dombeya* Cav. or *Melhania* Forssk., the two genera of Dombeyoideae known from the area (Wild and Gonçalves 1979; Cheek and Dorr 2007), and he observed that it closely resembled *Helmiopsis* H. Perrier, a genus of Dombeyoideae endemic to Madagascar. The lack of scales on the unidentified plant, however, led Darbyshire (personal communication) to doubt whether or not it belonged in that genus. Independently, photographs were sent to one

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of us (LJD) with a simple request for confirmation that the species belonged in the "Sterculiaceae" (i.e. Malvaceae sensu lato). An initial scepticism about this placement very quickly gave way to the realization that the photographs depicted a species that belonged to a genus of Malvaceae (Dombeyoideae) that had not yet been reported from continental Africa. More specifically, the new species appeared to belong to one of two endemic Malagasy genera, either *Helmiopsis* or *Helmiopsiella* Arènes.

One of us (LJD) identified the new species provisionally as a new species of Helmiopsiella, a genus of four species endemic to Madagascar (Barnett 1988a). This determination was based solely on photographs that bore a remarkable but, in retrospect, superficial resemblance to Helmiopsiella poissonii (Arènes) Capuron ex L.C. Barnett. Morphological characters that distinguish *Helmiopsiella* and *Helmiopsis* are weak (see also Skema 2012) and the attempt to place the new species from Mozambique underscored this problem. In any case, the provisional determination was adopted and it is used in Burrows et al. (2018; "Helmiopsiella sp. A"), on a Flickr website maintained by Rulkens (https://www.flickr.com/photos/47108884@N07/; "Helmiopsiella sp. Pemba") and in an on-line version of the flora of Mozambique (Hyde et al. 2018; "Helmiopsiella sp. A"). However, once herbarium material was received and a molecular phylogenetic analysis was initiated (Dorr et al. in prep.), it became apparent that a better generic placement was with the Asian genus Eriolaena DC. The need to have a name for this endangered shrub, which can be used for checklists and conservation reports, leads us to validate *E. rulkensii* in anticipation of publishing a more comprehensive molecular phylogenetic study of the wing-seeded taxa of Dombeyoideae.

## **Taxonomic treatment**

# Eriolaena rulkensii Dorr, sp. nov.

urn:lsid:ipni.org:names:60477347-2 Figures 1, 2

Helmiopsiella sp. A: Burrows et al., Trees shrubs Mozambique: 590. 2018.

**Diagnosis.** Differs from *Eriolaena wallichii* DC. in having entire or sparingly toothed epicalyx bracts (versus laciniate epicalyx bracts) and an androecium of 10–15 anthers alternating with 5 staminodes (versus an androecium comprised of numerous stamens and no staminodes).

**Type.** MOZAMBIQUE. Cabo Delgado: Pemba, close to Pemba Bay, near Chibuaburare, 12°58'26"S, 040°30'10"E, 9 m alt., 23 Feb 2014 (fl, fr), *A.J.H. Rulk-ens 1* (holotype: US-01184177; isotypes: BNRH, K-0001291030, K-0001291031, LMA, US-01184178).

**Description.** *Shrubs* or straggly trees, 2–6 m tall. Bark smooth, mottled grey and brown; young stems with scattered, appressed multi-radiate stellate hairs; older stems



**Figure 1.** *Eriolaena rulkensii.* **A** Habit **B**, **C** Flower buds with 3 epicalyx bracts each **D** Immature flower with petals (yellow), sepals (green) and scar of one dehisced epicalyx bract **E** Detail of sepal **F** Flower at anthesis showing petals, anthers, staminodes and gynoecium **G** Detail of petal **H** Detail of androecium showing androecial tube, anthers in fascicles and staminodes **I** Gynoecium and base of two staminodes **J** Immature capsule showing scars from dehisced calyx lobes and epicalyx bracts. (Source: *A.J.H. Rulkens 1*, US).

lenticellate, ± glabrescent. Leaves simple, alternate, petiolate, stipulate; leaf blades ovate to broadly ovate, 7-12.5(-14) cm long, 5-9(-9.5) cm wide, apices long acuminate, bases cordate to truncate, margin coarsely crenate except base of blade entire, (3-)5-nerved from the base, primary, secondary and tertiary nerves clearly visible below, veinlets visible below with  $10 \times$  magnification,  $\pm$  glabrous above and below except for scattered minute multi-radiate stellate hairs that are more numerous on the primary and secondary nerves and toward leaf base, somewhat lustrous above, matt below; domatia absent; petioles 3-4.5(-7) cm long, sparingly pubescent with scattered minute multi-radiate stellate hairs; stipules long acicular, 9-12(-15) mm long, ca. 1 mm wide at base and tapering to 0.25 mm below apex, sparingly pubescent with scattered minute multi-radiate stellate hairs, caducous. Inflorescence paniculate, axillary and terminal, lax, 20–27 cm long, 20–25 cm wide; pedicels to 9(–10) cm long. Epicalyx bracts 3, acicular,  $7-10 \times 2$  mm, entire or sparingly toothed apically,  $\pm$  evenly spaced around the axis in bud, but clustered on one side at anthesis, caducous. Calyx 5-lobed, valvate, shortly (1.5–2 mm) connate at base, lobes lanceolate, 7–8 × 2 mm, apices acute, somewhat thickened distally, sparingly pubescent externally with appressed, minute multi-radiate stellate hairs, glabrous internally, smooth (i.e. nerves not visible). Petals 5, broadly obovate, 14-16(-22) mm long, 14-15(-20) mm wide,  $\pm$  symmetrical, apices crispate, bases cuneate, bright yellow in vivo, glabrous externally and internally. Androecium of 10–15 anthers alternating with 5 staminodes; anthers in an outer whorl, borne in fascicles of 2(3), common filaments ca. 3-3.25 mm long, glabrous; anther sacs 2-2.25 mm long; staminodes in an inner whorl, ligulate,  $10 \times 1$  mm, glabrous. Style 1, ca. 4 mm tall; stigmas 10, recurved apically, pale yellow to white. Fruit a loculicidally dehiscent capsule, obovoid,  $\pm$  1.5 cm in diameter, 10-ridged, sparingly pubescent with scattered minute multi-radiate stellate hairs, eventually splitting into separate mericarp-like structures. Seeds 1(2) per locule, obovate,  $4 \times 2$  mm, laterally flattened, glabrous, each seed with a narrow, ca. 1 mm wide, hyaline, dorsal and apical wing.

**Etymology.** Named for A.J.H. (Ton) Rulkens, an agronomist who works for OX-FAM Belgium to strengthen small-scale farmer organizations in Cabo Delgado province, Mozambique. Rulkens is also a keen amateur botanist and photographer who has made many interesting plant discoveries in Mozambique, especially amongst representatives of the succulent flora (McCoy et al. 2014, 2017; McCoy and Baptista 2016).

**Distribution and ecology.** Endemic to northern Mozambique where it is known from several localities on the eastern and southern shore of Pemba Bay (Baia de Pemba) near the city of Pemba where it occurs on heavy clay over coral-rag in coastal scrub at the upper margin of mangrove communities (Burrows et al. 2018); ca. 10 m alt. According to Rulkens (personal communication), Ernst Schmidt observed the shrub between Macomia and the coast in 2009 but later discovered the plant had been cut down. Rulkens also observed additional locations with many plants about 10 km from Pemba in small patches of coastal forest on fossil coral substrate.

**Threats.** *Eriolaena rulkensii* is exploited for firewood (fide *Rulkens 1*, in sched.) and, on a different scale, it is threatened because the coastal forests and woodlands of northeast Mozambique are subject to increased development following the instability resulting from the independence and civil wars (Timberlake et al. 2011). *Eriolaena rulkensii* is



**Figure 2.** *Eriolaena rulkensii.* **A** Trunk and bark **B** Infructescence **C** Immature capsules with prominent ridges **D** Habit (shrub in centre with yellow flowers) **E** Flower (foreground) with stamens in fascicles of 2(3), each alternating with a ligulate staminode, and a simple style with 10 stigmas; flower buds (background) with 3 epicalyx lobes subtending each calyx **F** Capsules (mature) with loculicidally dehiscent mericarp-like structures. Photographs: A.J.H. Rulkens.

only one of many new species discoveries and new records from the Cabo Delgado area of Mozambique, others of which are enumerated by Timberlake et al. (2011).

# Discussion

Within the Dombeyoideae, Barnett (1988b) recognized morphological and anatomical similarities that united the Asian *Eriolaena* and Malagasy *Helmiopsis* and *Helmiopsiella* as a group. All three genera have woody and ovoid- or obovoid-conical capsules as well as seeds with apical wings. Skema (2012) subsequently published molecular data that support the distinctive nature of this "winged-seed clade" and demonstrated that the clade was early diverging from *Dombeya* sensu stricto. *Eriolaena rulkensii* represents the first species of this "winged-seed clade" to be found in continental Africa.

*Eriolaena rulkensii* is not the only example of an Asian/Malagasy species that also occurs in Mozambique but is otherwise absent from continental Africa. *Dianella ensifolia* (L.) DC. (Asphodelaceae) is widespread around the Indian Ocean, but confined in continental Africa to the foothills of a few mountain ranges in Mozambique (Hyde et al. 2018). Additionally, there are numerous examples of genera, both plants and animals, found in Asia, Madagascar and continental Africa (Schatz 1996; Renner 2004; Warren et al. 2010).

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**RESEARCH ARTICLE** 



# Two new species of *Raphia* (Palmae/Arecaceae) from Cameroon and Gabon

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#### Abstract

*Raphia* (Arecaceae, Calamoideae) is the most diverse genus of African palms with around 20 species. Two new species from Cameroon and Gabon, *Raphia gabonica* Mogue, Sonké & Couvreur, **sp. nov.** and *Raphia zamiana* Mogue, Sonké & Couvreur, **sp. nov.** are described and illustrated. Their affinities are discussed and the conservation status of each species is assessed. For both species, distribution maps are provided. *Raphia gabonica* is restricted to two small populations from central Gabon, where it occurs on hillsides on *tierra firme* soil, and close to small streams. Its preliminary IUCN status is Endangered, being amongst the five most threatened palm species in Africa. *Raphia gabonica* potentially belongs to the moniliform section. *Raphia zamiana* is largely distributed from south Cameroon to south Gabon and is very common. It is also a multi-used palm, from which wine, grubs and construction material are extracted and sold. It generally occurs in large stands in a wide range of ecosystems such as swamps, coastal forests on partially inundated sandy soils and inundated savannahs. Its large stature, hard to access habitat (swamps) and abundant presence might have discouraged botanists to collect it until now. *Raphia zamiana* belongs to the taxonomically complex raphiate section.

#### **Keywords**

Cameroon, Gabon, Raphia, Arecaceae, Calamoideae, IUCN conservation status, new common species

# Introduction

Raphia (Raphiinae, Calamoideae) is the most species-rich genus of African palms with now 22 species currently recognised to date (Dransfield et al. 2008, Stauffer et al. 2014). In addition, most *Raphia* species are socio-economically important and widely used across tropical Africa (Tuley and Russell 1966, Balick and Beck 1990, Burkill 1997, Obahiagbon 2009). Despite their importance, the taxonomic understanding of this group remains very limited. This is mainly linked of their massive stature and hardto-access wild populations, often thriving in swampy and inundated areas, rendering botanical collections difficult. Based on the shape of the partial inflorescence, five sections were described by Otedoh (1982). Almost all species are restricted to tropical Africa (Stauffer et al. 2014), with a single species occurring in Madagascar (R. farinifera (Gaertn.) Hylander) and one species (Raphia taedigera (Mart.) Mart.) occurring disjunctly in South and Central America (Dransfield et al. 2008). Most species occur in swampy environments forming large dense populations and only one species is so far known from tierra firme in forests (e.g. R. regalis Becc.). Raphia palms are acaulescent or more commonly with an aerial stem which can be solitary or clustered, bearing conspicuous fibres formed through the disintegration of leaf sheath margins (Otedoh 1982, Dransfield et al. 2008). Their leaves are pinnate and very large, up to 25 m in *R. regalis* (Hallé 1977, Dransfield et al. 2008), being amongst the longest in the plant kingdom. The species of *Raphia* are hapaxanthic (they die after flowering), monoecious, with basal female and apical male flowers on the same rachillae. The fruits are covered by imbricate scales typical of the Calamoideae subfamily genera (Dransfield et al. 2008).

The two new species reported here stem from extensive field work in Gabon, Cameroon, Republic of Congo and the Democratic Republic of Congo between September 2015 and February 2018.

#### Material and methods

Herbarium collections were made following methods described in Dransfield (1986). Measurements were taken from fresh and dry plant material collected in the field. Flowers were described following micro-morphology methods. *Raphia* collections from important international herbaria were studied (BR, FI, G, K, LBV, P, WAG, YA) (herbarium acronyms according to Thiers 2018) and online repositories (http://plants. jstor.org). The descriptions are based on herbarium specimens, field notes and spirit material when available. Unless otherwise stated, the dimensions mentioned for the various organs refer to dry material and the colours to fresh material. The conservation status was estimated for each species following the criteria and categories of the IUCN Red List version 3.1 (IUCN 2012). These were based on the geographical range estimated from herbarium specimens (Schatz 2002). The extent of occurrence (EOO) and the area of occupancy (AOO) were estimated using the online tool GeoCAT (Bachman et al. 2011). The minimum AOO was estimated based on a user defined grid cell of 2 km<sup>2</sup>. Phytogeographical considerations follow White (1979, 1983, 1993).

# Taxonomy

## Raphia gabonica Mogue, Sonké & Couvreur, sp. nov.

urn:lsid:ipni.org:names:60477348-2 Figures 1, 2

**Type.** Gabon. Moyen Oogoué: 5 km from Alèmbé on national road in the direction to la Lope, 0.07916S, 11.00836E, 228 m a.s.l., 18 November 2015, *Mogue K.S. 22* (holotype: WAG; isotypes: LBV, YA).

**Diagnosis.** *Raphia gabonica* closely resembles *R. gentiliana* by its solitary stem with curly fibres and the shape of the partial inflorescences. *Raphia gabonica* differs from *R. gentiliana* in being a mainly *tierra firme* species (vs. a swamp species), the shape of its fruits being globose, deltoid or ovoid (vs. ellipsoid) with 11 or 12 rows of scales (vs. 9–11) and a thick mesocarp measuring >8 mm.

Description. Stem 3-7 m tall, 20-30 cm in diameter, solitary; dead leaf sheaths persistent; trunk covered by fibres and old leaf sheaths; fibres formed through disintegration of leaf sheath margins ca. 1-2 mm in diameter, curly, dark brown to grey. Leaves 7 or 8, 8–13 m long in total, horizontal, then arching downwards towards the apex; sheath 80-140 cm long, 15-30 cm wide basally, channelled, smooth, margin fibrous, light brown with black and grey spots; *petiole* 1.7-4 m long, 7-10 cm in diameter basally, channelled, smooth, brown with dark and grey spots, green at younger stages; rachis 5-7 m long, channelled basally and keeled towards the apex, smooth, no spines on keel, light brown to brown, spotted black and grey; *pinnae* 170–195 per side, irregularly arranged in 4 planes, arching downwards towards the apex, extreme basal pinnae 55-70 cm long, 1.2-1.3 wide, filiform, middle pinnae 1.10-1.20 m long, 4.5-5 cm wide, linear, apical pinnae 9-23 cm long, 0.6-2.4 cm wide, linear, midrib prominent adaxially, brown spines along pinnae midrib and margins, older pinnae bearing spines more basally, younger ones throughout the pinnae, pinnae adaxial surface dark green, abaxial surface waxy green. Leaves next to inflorescence reduced with split sheaths. Inflorescences 5, pendulous, 1.2-1.8 m long, 9 - 10 cm (young) to 30-35 cm (mature) in diameter at base (including rachillae); light brown to brown; *prophyll* tubular, bearing 2 keels merging to form a pointed beak; *peduncle* 20-25 cm long, 7-8 cm in diameter, dorsi-ventrally compressed, smooth dark brown abaxially; *penduncular bracts* 8-20, tubular with triangular apices, smooth, dark brown abaxially; rachis 1.40-1.60 m long, bearing numerous bracts rarely empty, 50-70 first order rachillae, moniliform in shape, circular, alternating in 2 rows on each side of the rachis, smooth, prophyllar bract at the base of first order rachillae, tubular, bearing 2 keels at the side, smooth; basal first order rachillae 30-40 m long, 1.5–2 cm in diameter excluding rachillae, 25–30 cm including second order rachillae, bud flattened; prophyllar bract at the base bearing 2 keels on both sides, other bracts bearing flowers, rarely empty; second order rachillae ca. 38 per side, basal second order rachillae 15–19 cm long, 0.3–0.5 cm in diameter, middle second order rachillae 9-12 cm long, 0.4-0.5 cm in diameter, apical second order rachillae 2.5-5 cm long, 0.3-0.4 cm in diameter, circular, alternating in 2 rows on each side of first order rachillae, bud 1.5-1.7 cm long, flattened, smooth; middle first order rachillae 27-30 cm long, 1.5–2 cm in diameter excluding rachillae, 20–22 cm including second order rachillae, bud flattened; prophyllar bract at the base bearing 2 keels on both sides, other bracts bearing flowers, rarely empty; second order rachillae ca. 33 per side, basal second order rachillae 14-15 cm long, ca. 0.5 cm in diameter, middle second order rachillae 8.5–9 cm long, ca. 0.5 cm in diameter, apical second order rachillae 2.5–3 cm long, ca. 0.5 cm in diameter, circular, alternating in 2 rows on each side of first order rachillae, bud flattened, smooth; apical first order rachillae: 17-20 cm long, 1–1.5 cm in diameter excluding rachillae, 7–10 cm including second order rachillae, bud flattened; prophyllar bract at the base bearing 2 keels on both sides, other bracts bearing flowers rarely empty; second order rachillae 20 per side, basal second order rachillae 4-7 cm long, 0.2-0.3 cm in diameter, middle second order rachillae 4-5 cm long, 0.2-0.3 cm in diameter, apical second order rachillae 2.5-3.5 cm long, 0.2-0.3 cm in diameter, circular, alternating in 2 rows on each side of first order rachillae, bud flattened, smooth, light brown. *Flowers*: only very old or very young flowers observed, solitary, exerted, arranged in one row (sometimes two) on each side of second order rachillae, staminate flowers distal, pistillate flowers basal, stamens 6. Fruit: ca. 4 cm long, 3.5 cm in diameter, beak 0.5 cm; globose, deltoid or ovoid; scales arranged in 11 or 12 rows, shallowly furrowed, dark green to brown when young, turning orange-red at maturity; mesocarp ca. 0.8 cm thick, yellow; seeds 1-3, circular covered in a white coating.

**Ecology.** *Raphia gabonica* occurs in lowland rain forests, growing on hill sides on *tierra firme* and also near streams. Seen occurring together with *Sclerosperma mannii* H.Wendl. and *Elaeis guineensis* A.Chev.

**Distribution.** Lower Guinea subcentre of endemism (White 1979). *Raphia gabonica* is restricted to the northern part of the Ngounié region in Gabon occurring in very small populations in forests. Altitude 76–228 m (Fig. 3).

**Preliminary conservation status. Endangered: EN B2ab(ii)**: *Raphia gabonica* is a rare species collected from two close locations in central Gabon (Fig. 3). Its minimal area of occupancy (AOO) is estimated to be 8 km<sup>2</sup> (within the limits for Critically Endangered status under criterion B2). To date, two locations are known within the limits of the Endangered status. Both collections were made recently (2015) along important road axes (e.g. Nationale 1) and in unprotected areas (Fig. 3). For each location, several individuals were seen indicating no immediate threat. However, we project that the ongoing loss of its natural habitat linked to increased human activity will induce an important decline of its EOO and AOO. *Raphia gabonica* is therefore assigned a preliminary status of EN B2ab(ii), joining the almost 5% of continental African palms under this category (Cosiaux et al. 2018)

Uses. No uses have been reported for this species.

**Notes.** *Raphia gabonica* appears to belong to the moniliform section of Otedoh (1982) linked to the shape of its partial inflorescences. However, the solitary trunk with curly fibres could also suggest a resemblance to species within the temulentae section (e.g. *Raphia hookeri* G.Mann & H.Wendl. or *R. sese* De Wild.).



**Figure 1.** *Raphia gabonica*, illustrations. **A** Habit (bar = 1 m) **B** Details of trunk - notice curly fibres **C** Detail of full inflorescence **D** Detail of base of inflorescence **E** Penduncular bracts **F** Partial inflorescence with old flowers **G** Detail of basal part of rachillae with old female flower **H** Detail of apical part of rachillae with old male flowers (Scale bar: 1 cm) **I** Partial inflorescence with fruits **J** Fruit **K** Longitudinal section of fruit. Drawings based on **A** from Mogue 22 **B–J** Mogue 23. Drawings by Hans de Vries.



**Figure 2.** *Raphia gabonica* in natural habitat (Alèmbé, Gabon). Notice dry land habitat, not growing in colonies, single stem with curly fibres and long pendulous inflorescences. Photo: Thomas L.P. Couvreur.



Figure 3. Distribution of Raphia gabonica and R. zamiana.

Additional specimen examined. GABON. Ngounié: Zamata village along national road N1, 1.03044S, 10.51881E, 76 m a.s.l., 19 Nov 2015, *Mogue K.S. 23* (LBV, WAG,).

#### Raphia zamiana Mogue, Sonké & Couvreur, sp. nov.

urn:lsid:ipni.org:names:60477349-2 Figures 4, 5

**Type.** Cameroon: South Region: Vallée du Ntem, Nseng avion forest, Fondation agricole Samuel Menye, 1.5 km from Ma'an in the direction of the Ntem river, 2.34805N, 10.63054E, 513 m a.s.l., 25 February 2018, *Mogue K. S. 44* (holotype: WAG; isotypes: K, YA).

**Diagnosis.** *Raphia zamiana* is morphologically most similar to *R. monbuttorum* in their clustering habitat and straight fibres covering the trunk. *Raphia zamiana* differs from *R. monbuttorum* by the size of its stout and stiff rachillae with apical second order rachillae measuring 1 cm in diameter versus less than 1 cm in *R. monbuttorum*. The inflorescences of *R. zamiana* are pendulous even from the early stage of development as opposed to the semi obtuse erect position in *R. monbuttorum*.

**Description.** *Stem* 3–8 m tall, 30–40 cm in diameter, clustering; dead leaf sheaths persistent, trunk hidden in dead leaves and fibres; *fibres* formed through disintegration of the leaf sheath, ca. 1 cm in diameter, straight with pointed tips, brown to black. *Leaves* 10–12, 12–21 m long in total, horizontal and then arched downwards towards apex; *sheath* 90–150 cm long, channelled, smooth, margin fibrous, orange-yellow, spotted with black, white and or grey dots; *petiole* 4–11 m long, 5–35 cm in diameter towards the base, channelled basally and elliptic apically, smooth, green, spotted with

dark and grey; *rachis* 7–13 m long, 10–11 cm in diameter, elliptic basally and keeled towards the apex, smooth abaxially, spiny adaxially (spines on keel), light green to green; *pinnae* 147–268 per side, irregularly arranged in 4 planes, arching downwards towards the apex, pinnae adaxial surface green, abaxial surface waxy green; extreme basal pinnae 0.80–1.26 m long, 7–30 mm wide, filiform, middle pinnae 1.50–1.90 m long, 5–9 cm wide, linear, apical pinnae 20–72 cm long, 1.5–3.6 cm wide, linear, midrib prominent adaxially, spines along pinnae midrib and margins, brown to black. Leaves subtending inflorescence reduced (1.20–1.40 m long).

Inflorescences 3 or 4, pendulous, 1.55–2.80 m long in total, 17–44 cm (mature) in diameter at base (including rachillae); young inflorescences light green to purple green, older ones light brown to grey-brown. prophyll 18-20 cm long, 13.7 cm diameter, tubular, bearing 2 keels merging to form a pointed beak; peduncle 26-30 cm long, 10-13.5 cm diameter, dorsi-ventrally compressed, smooth; penduncular bracts several, tubular, with triangular apices, smooth, dark brown abaxially; rachis 1.25-2.60 m long, bearing numerous bracts rarely empty, 50–70 first order rachillae, raphiate shape, dorsi-ventrally compressed, alternating in 2 rows on each side of the rachis, smooth; prophyllar bract found at the base of first order rachillae, tubular, bearing 2 keels at the sides, smooth; *basal first order rachillae* 0.54-1.05 m long, 4.5-7 cm in diameter excluding rachillae, ca. 14 cm in diameter including rachillae, bud flattened; prophyllar bract bearing 2 keels on both sides, subsequent bracts bearing flowers, rarely empty; second order rachillae 48-65, basal second order rachillae 23-35 cm long, 2-2.5 cm in diameter; middle second order rachillae 15-27 cm long, 2 cm in diameter; apical second order rachillae 9.5–15 cm long, 1.5–1.6 cm in diameter, dorsi-ventrally compressed, alternating in 2 rows on each side of first order rachillae, bud flattened, smooth; *middle first order rachillae* 39-45 cm long, 3-4 cm in diameter excluding rachillae, 12 cm in diameter including second order rachillae, bud flattened; prophyllar bract at the base bearing 2 keels on both sides, subsequent bracts bearing flowers rarely empty; second order rachillae 32-50, basal second order rachillae 16 cm long, 1.5 cm in diameter, middle second order rachillae 13 cm long, 1.2 cm in diameter; apical second order rachillae ca. 10 cm long, ca. 1 cm in diameter, dorsi-ventrally compressed, alternating in 2 rows on each side of first order rachillae, bud flattened, smooth; apical first order rachillae 25–27 cm long, ca. 2.5 cm in diameter excluding rachillae, 12 cm in diameter including second order rachillae, bud flattened; prophyllar bract at the base bearing 2 keels on both sides; second order rachillae 12–30, basal second order rachillae ca. 10 cm long, ca. 1 cm in diameter; middle second order rachillae ca. 8 cm long, 1 cm in diameter; apical second order rachillae ca. 6 cm long, ca. 1 cm in diameter, dorsiventrally compressed, alternating in 2 rows on each side of first order rachillae, bud flattened, smooth; second order rachillae sometimes three times the usual size (more than 25 cm long, 2.5 cm wide at the apex). Inflorescence bud ca. 5 cm long, ca. 1.2 cm wide, buds of basal and medial first order rachillae sometimes elongated. *Flowers* solitary, exerted, inserted in two rows on each side of second order rachillae, staminate flowers distal, pistillate flowers basal. Staminate flower 13-18.5 mm long, 7.5-11.5 mm wide, stalk ca. 1 mm long; subtending bracteole 4.5-13.5 mm long, 7-11.5 mm wide,



**Figure 4.** *Raphia zamiana*, illustrations. **A** Habit in savannah **B** Habitat in forest, trunk not visible **C** Detail of the trunk, with inflorescences and straight fibres **D** Inflorescence **E** Partial inflorescence, young **F** Detail of rachillae **G** Detail of basal part of 2<sup>nd</sup> order rachillae, showing small rachillae bracts encircling young flowers **H** Detail of partial inflorescence (×5) **I** Female flower ×5 **J** Female inner bract ×5 **K** Female calyx ×5 **L** Female corolla ×5 **M** Detail of staminodial ring and staminodes **N** Male flower ×5 **O** Male calyx ×5 **P** Male corolla ×5 **Q** male stamens ×6 **R** Detail of stame of male flower ×12 **S** Influctescence **T** Fruit, longitudinal section. Drawings based on: **A** Couvreur 1122 **B–D, S,T** Mogue; 17 **E–R** Mogue 44. Drawings by Hans de Vries.

tubular, bicarinate, margins entire, smooth, with a conspicuous wide apical slit on one side, displaying conspicuous longitudinal veins on the outer side, bracteole completely covering the calvx; *calvx* 5-11.5 mm long, 5-7 mm wide, fused >2/3 of its length, tubular, bearing 2 or 3 shallow lobes, margins entire to slightly rough, smooth; conspicuous longitudinal veins on outer side; corolla 3, 8.5-15(-20) mm long, 4-6 mm wide, basally connate for 1/3 of their length, oblong, apex slightly blunt to acuminate, margins entire, smooth, stiff, displaying a conspicuous longitudinal venation on the inner side; stamens 11-18, filaments 1-4(-6) mm long, 1-1.7 mm wide, free, basally adnate to the petals for 1–2 mm, cream white to pale pink; anthers 4–8.8 mm long, 1–1.5 mm wide, sagittate-elongate, medifixed, pale yellow; pistillode absent. Pistillate flowers 15-25 mm long, 10-13 mm wide; outer subtending bracteole 12-19 mm long, 10-13 mm wide, tubular, bicarinate, margins entire, with one wide apical slit, smooth; inner subtending bracteole 5-9 mm long, tubular, margins entire, one side longer, smooth, sometimes tearing; calyx 8.5-16 mm long, fused >2/3 of its length, tubular, 3 shallow lobes or the latter rarely absent, margins entire, smooth, with longitudinal veins conspicuous on both sides; corolla 5-8 mm long, fused, 3 lobes with margins serrated, lobes sometimes slightly acuminate, margins entire, smooth, conspicuous longitudinal veins; staminodial ring with 17-19 fused staminodes, 2-5 mm long, adnate to petals for 1-4 mm; anthers sagittate, 0.5-1 mm long; gynoecium 11-18 mm long, 5-6 mm wide, ovary 9-12 mm, 5-6 mm wide, ovate to oblong, completely covered with scales, developing at <sup>3</sup>/<sub>4</sub> height of the gynoecium, larger scales at mid portion to base; style absent or very short; stigma ca. 1 mm long, papillae not observed but hairlike prolongations present on stigma. Fruits 4-8.7 cm long, 3.5-4.7 cm wide, beak 0.5–0.9 cm long; oblong, scales arranged in 11 or 12 rows, length of scale 16–20 mm, width of scale 15-20 mm, diamond shaped, apex texture rough, shallowly furrowed, green, beak pointed, inflated in the middle; mesocarp yellow when young, orange yellow when mature; seed 1, oblong, with ruminations.

**Ecology.** *Raphia zamiana* is an Atlantic central African rain forest species, endemic to lower Guinea. It appears to have a wide ecological amplitude, growing in rain forest swamps on periodically inundated soils, in coastal forests on inundated sandy soils or in open vegetation like savannahs associated with inundated soils (e.g. Lope National Park, Gabon, Figs 3, 5C). It is generally abundant forming large dense almost monodominant stands. It has been recorded growing sympatrically with *R. hookeri* and *S. mannii*.

**Distribution.** Lower Guinea subcentre of endemism (White 1979). *Raphia zamiana* occurs in southern Cameroon (Central and South regions) and western Gabon. It is probably also common in Equatorial Guinea although no collections have been made yet. Altitude: 0–700 m (Fig. 3).

**Preliminary conservation status. Least Concern.** The extent of occurrence of *Raphia zamiana* is estimated to be 128,243,063 km<sup>2</sup> (far exceeding the 20,000 km<sup>2</sup> upper limit for Vulnerable status under criterion B1) and the area of occupancy is estimated to be 36,000 km<sup>2</sup> (far exceeding the 2,000 km<sup>2</sup> upper limit for Vulnerable status under criterion B2). Moreover, this species is known from 9 locations, the upper limit for sub criterion a- of criterion B2 for the Vulnerable status. However, *Raphia zamiana* 



Figure 5. *Raphia zamiana*. A Habitat along the road, with Raoul Niangadouma for scale (Oyem, Gabon)B Close-up of pendulous and "raphiate" type partial inflorescence (coastal forests near Kribi, Cameroon)C Habitat in the savannahs of Lopé National Park, Gabon D Habitat in swamp (Ma'an, Cameroon).Photos: Thomas L.P. Couvreur.

is a widespread and common species in its area of occurrence. It is very common along road sides and is abundant growing in dense colonies. To date, it has not been collected within a protected area, but populations were seen in Campo National Park (Cameroon). Incredibly, its first collection dates to 2012. Botanists might have confused it with the well-known and widespread species *Raphia hookeri* (although both species are very different in their morphology). Given that collecting *Raphias* is a hard task because of their massive stature (Dransfield 1986), it was simply omitted. Populations are affected by road building and the drying of swamps for bridges, but these only impact a small number of individuals. *Raphia zamiana* is therefore assigned a preliminary status of LC, joining most continental African palm species (Cosiaux et al. 2018).

**Etymology.** The name of this species is derived from its vernacular name in Beti (south Cameroon, north Gabon): Zam.

**Uses.** This palm is massive thus providing large amounts of thatching material. Its petiole and rachis commonly referred to as 'bamboo' are used for house construction, beds, chairs, baskets and mats. These are generally sold along the roads in south Cameroon. In Gabon, its fruits are sold in markets. These are boiled and said to cure hypertension and diabetes. Finally, this species is also used for wine tapping and as a source of grubs.

#### Vernacular names. Zam (Beti).

**Notes.** *Raphia zamiana* belongs to the raphiate section (Otedoh 1982). It closely resembles *R. monbuttorum* Drude and *R. laurentii* De Wild. in the morphology of their trunks being clustered and covered with straight fibres. However, it is very distinct by having a pendulous inflorescence bearing stout, stiff and straight rachillae. The inflorescences of *R. monbuttorum* and *R. laurentii* usually hang at an obtuse angle especially during the young stages of development, becoming pendulous only when brought down by the weight of fruits. Young, recently developing inflorescences are grey-blue turning green later on.

*Raphia zamiana* is a very conspicuous palm in southern Cameroon and eastern Gabon, being common along roads and in swamps. In addition, we report several important uses, being one of the most useful *Raphias* (Mogue, personal observation). However, up to now, it remained uncollected, stressing once again that new species well known to local people have yet to be scientifically discovered and described. A similar situation was recorded for the Vietnamese endemic palm *Licuala centralis* (Henderson et al. 2008). This palm was well known and used to make local hats, but was only scientifically described in 2008.

Additional specimen examined. CAMEROON. Central Region. near Ebolbom village, 3 km east of Ngoumou, 2 km northwest of Otélé, 3.599720N, 11.287700E, 700 m a.s.l., 2 May 2013, *Couvreur T.L.P. 427* (WAG, YA). South Region: Mvila, Biyeyem, 2.514020N, 11.081930E, 573 m a.s.l., 19 Sep 2015, *Mogue K.S. 15*, (WAG, YA); Mvila, Biyeyem, 2.514020N, 11.081930E, 573 m a.s.l., 19 Sep 2015, *Mogue K.S. 16* (WAG, YA); mountain chain Ngovoyang, 1.5 km in forest from Bikiliki village situated between Bipindi and Lolodorf, 3.181570N, 10.536960E, 460 m a.s.l., 17 Feb 2012, *Couvreur, T.L.P.392* (WAG, YA); About 20 km N from Kribi, 3 km N

of Longji, N7 road towards Edea, 3.146810N, 9.959510E, 0 m a.s.l., 27 Feb 2018, *Mogue K.S. 45* (WAG, YA).

GABON. Woleu-Ntem: Oyem, 2–3 km from main road in the direction of Konosoville, 01.59849N, 011.62298E, 651 m a.s.l., 12 Nov 2015, *Mogue K.S. 17* (LBV, WAG, YA); Ogooué-Ivindo: 180 km on main road from Lastoursville to Lopé, 0.147300S, 11.726011E, 280 m a.s.l., 9 Jun 2016, *Couvreur T.L.P. 1122* (LBV, WAG, YA). Ogooué-Maritime: Lagune de Fernan Vaz. Koundakoua, 1.4487220S, 9.2066110E, 3 m a.s.l., 20 Nov 2016, *Bidault E. 2722* (BR, BRLU, LBV, MO, P, WAG). Ngounié: Mouila, 19 km from national road, 2.254280S, 11.142840E, 133 m a.s.l., 20 Nov 2015, *Mogue K.S. 24* (LBV, WAG, YA).

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**RESEARCH ARTICLE** 



# Splitting Echinocactus: morphological and molecular evidence support the recognition of Homalocephala as a distinct genus in the Cacteae

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#### Abstract

Molecular phylogenetic studies of the six currently accepted species in the genus *Echinocactus* have partially clarified certain aspects of its phylogeny. Most of the studies lack a complete sampling of *Echinocactus* and are based only in one source of data. Phylogenetic uncertainties in *Echinocactus*, such as the recognition of *Homalocephala* as a different genus from *Echinocactus*, the exclusion of *E. grusonii* or the affinities of *E. polycephalus*, are here resolved. Phylogenetic relationships of *Echinocactus* were reconstructed with a maximum parsimony, a maximum likelihood and a Bayesian approach including 42 morphological characters, four chloroplast markers (*atpB-rbcL*, *trnH-psbA*, *trnL-trnF* and *trnK/matK*) and two nuclear genes. The utility of these two nuclear regions related to the betalain cycles (*DODA* and *5GT*) are explored and discussed in relation to their potential as phylogenetic markers. Concatenated analyses with morphological and molecular data sets, plus 13 indels (2847 characters and 26 taxa), show general agreement with previous independent phylogenetic proposals but with strong support in order to propose the recognition of a reduced *Echinocactus* and the recognition of *Homalocephala* at the generic level. These

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results recovered a polyphyletic *Echinocactus* as currently defined. The here-named HEA clade, recovers the species of *Homalocephala, Echinocacuts* and *Astrophytum* as a monophyletic group with strong internal support. The *Homalocephala* (*H. texensis, H. parryi* and *H. polycephala*), was recovered as sister to the *Echinocactus* clade (*E. platyacanthus* and *E. horizonthalonius*), plus the *Astrophytum* clade. Consequently, we propose here to recognise a monophyletic *Echinocactus* and a monophyletic *Homalocephala* as two distinct genera with their own molecular and morphological synapomorphies. The evolution of some morphological characters supporting these clades are discussed, the necessary new taxonomic combinations for *Homalocephala* are proposed and an identification key for the genera, the species and the subspecies of the HEA clade are presented.

#### **Keywords**

Cactaceae, Cactoideae, HEA clade, morphological character evolution, North American Deserts

# Introduction

Current taxonomic delimitation of *Echinocactus* comprises six species of North American cacti (Barthlott and Hunt 1993; Anderson 2001, Hunt et al. 2006, Hunt 2016), *Echinocactus platyacanthus, E. horizonthalonius, E. texensis, E. parryi, E. polycephalus* and *E. grusonii*. Four intraspecific taxa are also recognised, *E. polycephalus* subsp. *xeranthemoides, E. polycephalus* subsp. *polycephalus, E. horizonthalonius* subsp. *nicholii* L.D. Benson and *E. horizonthalonius* subsp. *horizonthalonius* (Coulter 1896, Benson 1969). Species of *Echinocactus* are distributed in all the North American desert regions, from the Tehuacán-Cuicatlán Valley in Puebla and Oaxaca, through the Chihuahuan, Sonoran and Mohave deserts, as well as into southern edges of the Great Basin Desert (Hernández and Gómez-Hinostrosa 2011). The species are characterised by having discoidal, globose to shortly columnar and strongly ribbed stems, large and elongated areoles that are divergent or confluent, a stem apex with a broad woolly crown, stout spines, short funnelform to campanulate apical flowers, flower and fruit areoles with pointed scales and covered with dense wool and large brown to black-brown seeds that vary from broadly oval to almost circular.

Britton and Rose (1922) based on differences in growth form and fruit morphology, segregated *E. texensis* Hopffer from *Echinocactus* proposing the monotypic genus *Homalocephala* Britton & Rose. Berger (1929) changed the rank of *Homalocephala* to a subgenus of *Echinocactus* in which he included only *E. texensis*. Bravo-Hollis and Sánchez-Mejorada (1991), based on the colour of the flower circumscribed the subgenus *Homalocephala* to include *E. texensis* and *E. horizonthalonius* Lem. Ferguson (1992) distinguished *Homalocephala* from *Echinocactus* by the stems which were rarely larger than 35 cm in diameter with sharp-edged ribs and discrete areoles, pericarpel and fruit with thick walls, pointed scales and trichomes scattered over the entire fruit wall, large keeled seeds with an indented hilum and testa cells surrounded by channelled anticlinal cell-boundaries. Ferguson believed that the unique morphological characters of *E. horizonthalonius* could support the creation of a new monotypic genus to include this species. Ferguson (1992) accepted in his study two subgenera, *Homalocephala*, including *E. texensis, E. parryi* Engelm., *E. polycephalus* Engelm. & J.M. Bigelow subsp. *polycephalus* and *E. polycephalus* subsp. *xeranthemoides* J.M. Coult. and subgenus *Echinocactus* including *E. platyacanthus* Link & Otto and *E. grusonii* Hildm.

The earlier restriction site variation study of Wallace (1995) was the only molecular phylogenetic analysis that included all six species of Echinocactus, accepted in contemporary taxonomic treatments. Wallace did not recover a monophyletic genus, since E. grusonii was recovered as sister to the Astrophytum-Echinocactus clade. The other five species were recovered in a single clade with two subclades, one including the type species, E. platyacanthus plus E. horizonthalonius and the second including E. texensis, E. parryi, E. polycephalus subsp. polycephalus and E. polycephalus subsp. xeranthemoides. Subsequent molecular studies (Butterworth et al. 2002, Bárcenas et al. 2011, Hernández-Hernández et al. 2011) have included only some species of Echinocactus and have also recovered a non-monophyletic genus, with E. grusonii more closely related to Ferocactus. The study of Bárcenas et al. (2011), based on the plastid trnK/matK, did not include E. parryi and recovered three of the five species within a clade and E. grusonii and E. polycephalus nested amongst other species of the Cacteae. The recovery of E. grusonii outside of core Echinocactus led to the proposal of a new monotypic genus named Kroenleinia Lodé to include E. grusonii as Kroenleinia grusonii (Hildm.) Lodé (Lodé 2014). Nevertheless, the new genus Kroenleinia was not recognised in the most recent checklist of the Cactaceae and both E. grusonii and E. polycephalus were retained in the genus, plus no distinction between subgenus Echinocactus and Homalocephala was mentioned (Hunt 2016).

Most molecular systematic studies that have included species of *Echinocactus* have been limited to phylogenetic analyses based only on plastid datasets. Several studies have shown that DNA information, contained in some nuclear genes, can improve phylogenetic resolution at lower taxonomic levels due to higher nucleotide variability than that found in the cpDNA markers (Sang 2002). DODA and 5GT are two nuclear genes that codify for key enzymes implicated in betalain synthesis in most families of Caryophyllales, including Cactaceae (Gandía-Herrero and García-Carmona 2013). Nucleotide sequences of these genes could be useful to generate a phylogenetic hypothesis comparable to those obtained with chloroplast markers and to perform combined analyses that could improve the phylogenetic signal. Likewise, the use of combined molecular and morphological datasets has shown its utility in detecting phylogenetic relationships not recovered in separated analyses (Nixon and Carpenter 1996). Recently, Sánchez et al. (2017), performing a combined analyses of morphological and molecular data resolved the phylogenetic relationships of some taxa in Echinocereus Engelm. (Cactaceae) that were not resolved in previous phylogenetic analyses conducted only with molecular evidence (Sánchez et al. 2014).

In this study, the phylogenetic relationships of all species and subspecies of *Echinocactus* are reconstructed based on DNA sequences of four chloroplast markers (*atpB-rbcL*, *trnH-psbA*, *trnL-trnF* and *trnK/matK*) and 42 morphological characters. In addition, the phylogenetic utility of two nuclear genes (*DODA* and 5GT) in *Echinocactus* and outgroup taxa was explored. The topology obtained in a concatenated analysis, including molecular and morphological datasets, was used to

trace the history of morphological characters. The goals of this study are to provide a phylogenetic hypothesis to clarify the position of *E. grusonii* and *E. polycephalus* by testing the monophyly of the currently accepted delimitation of *Echinocactus* and to test the taxonomic status of *Homalocephala*.

# Methods

## Taxon sampling

A total of 26 taxa, including all six currently accepted species of *Echinocactus*, following Anderson (2001) and Hunt et al. (2006), (Fig. 1) are included in this study. DNA sequences from other genera such as Astrophytum, Aztekium Boed., Geohintonia Glass & W.A. Fitz-Maur. and Ferocactus from Cacteae and Carnegiea gigantea (Engelm.) Britton & Rose, from Phyllocacteae were generated and used as outgroups. When possible, three terminals for each Echinocactus species, representing individuals from different populations or in some cases subspecies, were included (Appendix 1). Samples were collected from wild populations under Mexican and Arizonian permits, from herbarium specimens at ASU, DES, QMEX and MEXU and from the living collection of the Desert Botanical Garden, which also originated from wild collected specimens (Appendix 1). Additionally, 41 nucleotide sequences deposited in GenBank (www. ncbi.nlm.nih.gov/Genbank) were downloaded and included in the analyses. Opuntia ficus-indica (L.) Mill. from subfamily Opuntioideae was used as a functional outgroup for concatenated analyses and for most of the non-concatenated analyses, except for those conducted with the nuclear genes, in which, in order to have a wider sampling, we included Amaranthus tricolor L. and Beta vulgaris L. of Amaranthaceae. For nuclear DNA analyses, A. tricolor was used as the functional outgroup.

#### DNA isolation, PCR and sequencing

Total genomic DNA was extracted from silica gel dried stem tissues or from herbarium samples. Approximately 20 to 30 mg of tissue was ground, from which DNA was extracted with a CTAB 2% protocol described in Doyle and Doyle (1987) and as modified by Bárcenas (2015). We also used previously extracted DNA preserved in TE 10 mM, pH 8.0, deposited in the collection of the Laboratorio de Genética Molecular y Ecología Evolutiva of the Universidad Autónoma de Querétaro, México. The *atpB-rbcL* intergenic spacer was amplified with the primers of Demesure et al. (1995). The amplification parameters were: 96 °C for 5 min; 35 cycles of 94 °C for 50 s, 47 °C for 1 min, 72 °C for 1.5 min and a final extension of 72 °C for 10 min. Primers "E" and "F" designed by Taberlet et al. (1991) were used for the amplification of the *trnL-trnF* intergenic spacer. PCR parameters to amplify the region were: 96 °C for 5 min; 35 cycles of 94 °C for 1 min, 72 °C



**Figure 1.** *Echinocactus* species. **A** *E. platyacanthus* from Querétaro **B** *E. horizonthalonius* from Chihuahua **C** *H. texensis* from Chihuahua **D** *H. parryi* from Chihuahua **E** *H. polycephala* subsp. *polycephala* from Sonora **F** *K. grusonii* from Querétaro. Line bar in fruit photographs is 1 mm.

for 1.5 min and a final extension of 72 °C for 5 min. The primers designed by Hamilton (1999) were used to amplify the *psbA-trn*H intergenic spacer. PCR parameters for the *psbA-trn*H were: 96 °C for 4 min; 35 cycles of 94 °C for 1 min, 48 °C for 1 min, 72 °C for 1.5 min and a final extension of 72 °C for 5 min. Primers used to amplify the two nuclear genes were designed by Felker et al. (2008). The best PCR conditions for *DODA* were 96 °C for 5 min; 35 cycles of 94 °C for 45 s, 54 °C for 1 min, 72 °C for 1.5 min and a final extension of 72 °C for 5 min. The most successful conditions for *5GT* were 96 °C for 5 min; 35 cycles of 94 °C for 45 s, 52 °C for 1.5 min, 72 °C for 1.5 min and a final extension of 72 °C for 4 min. PCR products were commercially purified and sequenced in both directions using the same primers in Macrogen Inc., Seoul, South Korea or at the ASU DNA Laboratory, Tempe, Arizona. The 22 sequences of *trnK/matK*, included in the analyses, were downloaded from GenBank (Appendix 1).

#### Morphological characters

A set of 42 morphological characters were coded (Appendix 2) by observations during field work and by examination of herbarium specimens generated in this study and specimens already deposited in herbaria (ASU, DES, MEXU and QMEX). Primary homology statements (Hawkins et al. 1997) were postulated following the criteria of conjunction and similarity of Patterson (1982) and De Pinna (1991). To code different states of plane figures and three-dimensional shapes, we adopted the terminology published by Radford (1974) and Eggli (1993) and constructed a binary and multistate morphological character matrix in Mesquite 3.02 (Maddison and Maddison 2015). Six characters were coded from seedling stage or during the first year of development. The seedlings were cultivated from seeds collected during fieldwork or commercially bought from nurseries. All seedlings were grown under the same environmental conditions on campus without tracking temperature, light or humidity. Certain morphological characters such as shape of ribs and shape of flowers were also obtained from bibliographic sources (Bravo-Hollis and Sánchez-Mejorada 1991, Ferguson 1992, Chamberland 1997, Barthlott and Hunt 2000, Doweld 2000, Hunt et al. 2006) only for some of the outgroup taxa in Ferocactus. The seed size categories proposed in Barthlott and Hunt (2000) were used to assign character states. To ensure that we assigned the correct category, we measured ten random seeds per species and the average was used as the seed size indicator for each of the categories. Character state delimitations for the number of spines per areole were based on the counts of 25 areoles per species from five different plants and five different areoles of at least two different populations for the majority of the species except for Opuntia ficus-indica (n=1 areole, one plant, one population), Carnegiea gigantea (n=5 areoles, one plant, one population), Echinocactus horizonthalonius (n=13 areoles, three plants, two populations) and Astrophytum asterias (n=15 areoles, three plants, one population). The age of the plants varied from 1 to 12 months and no change in the number of spines per areole was detected in that period of time, that is, plants of one month had the same number of spines that plants of 3, 4 or 12 months old. No plants older than 12 months were included in this delimitation. The average of spine number per seedling was calculated in order to reduce the effect of pseudoreplication (counting different areoles of the same seedling) and, from 421 counts, only 88 observations were included in the statistical analysis as unique events. The hypothesis that three groups existed and were statistically different was confirmed with a Kruskal and Wallis test and a post-hoc test for pairwise multiple comparison (Nemeyi's test) in R with the library PMCMR (Pohlert, 2014). The groups were then defined as state (0) from 1 to 4 spines per areole; state (1) from 5 to 8 spines per areole and state (2) from 9 to 12 spines per areole as defined in Appendix 2.

#### Alignments and phylogenetic analyses

In order to generate consensus sequences, both forward and reverse sequences were assembled manually in PHYDE (Müller et al. 2007) and checked against each of the
chromatograms to resolve potential ambiguities. Newly generated sequences were blasted in GenBank to ensure that they corresponded to their respective regions, thus avoiding the use of contaminated sequences. Based on the micro-architectural characterisation of the *psbA-trn*H intergenic region (IGR) for the Cactaceae proposed by Hernández-Ledesma and Bárcenas (2016), the intergenic spacer (IGS) was excluded since it is a highly variable mutational hot-spot making alignment highly subjective. The *DODA* forward and reverse sequences were impossible to assemble and thus precluded the generation of consensus sequences. This probably was due to the presence of an intron located at position 199 to 960 as annotated in the sequences of *Opuntia-ficus-indica* (GenBank accessions EU089741 and EU089742). Thus the aligned matrix of *DODA* was constructed by simple concatenation of the forward and reverse sequences for each of the terminals.

Consensus sequences were automatically aligned with MUSCLE (Edgar 2004), as implemented in MEGA 7 (Kumar et al. 2015) and then manually corrected in PHYDE, reducing the number of gaps and increasing the match amongst nucleotides. Three concatenated matrices were constructed in WINCLADA 1.00.08 (Nixon 2002), one included only the four chloroplast markers (here named the cpDNA matrix); the second included the cpDNA matrix plus the 42 morphological characters and 13 indels (cpDNA plus morphology matrix); the third matrix included the cpDNA plus the morphological matrix and the sequences of the two nuclear genes (total evidence matrix). The lack of several sequences for DODA precluded the analysis of the complete dataset for both of the nuclear genes and only the tree for 5GT is shown in Figure 5. The 13 indels were coded from the *atpB-rbcL* marker and were considered as additional presence/absence characters.

For each individual dataset and for the cpDNA concatenated matrix, maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in MEGA 7, while Bayesian analyses (BA) were carried out in MRBAYES 3.2.6 (Huelsenbeck and Ronquist 2001). The cpDNA plus morphology and the total evidence matrices were analysed only by MP and Bayesian methods, since no partition for molecular and morphological characters could be set for the ML analysis. The MP analyses for the cpDNA plus morphology and total evidence matrices were done in PAUP 4.0 (Swofford 2002). The best-fit evolutionary models for all matrices were selected according to the Bayesian Information Criterion (BIC) implemented in MEGA 7. ML analysis for the cpDNA matrix was run with the GTR+G evolutionary model. BA analysis of the cpDNA matrix was run setting the following partition and evolutionary models: *atp*BrbcL (GTR+G), psbA-trnH and trnK/matK (HKY+G) and trnL-trnF (HKY). The BA analyses of cpDNA plus morphology and the total evidence matrices were performed with the same settings as the cpDNA matrix, but for the total evidence matrix the models selected for the DODA and 5GT nuclear genes were K2 and K2+G, respectively. Morphological characters and indels in BA analyses were run under the Mky model and coded as variable. For cpDNA concatenated analyses, we tried when possible to sample the same three individuals for the ingroup; nevertheless, some terminals were assembled by combining sequences from different individuals, as extracted DNA was exhausted and no more tissue existed for other extractions.

MP analyses were run with heuristic searches, TBR, random addition of sequences and 1000 replicates. A 50% majority rule consensus tree was calculated from the most parsimonious trees. ML analyses were run with an initial neighbour-joining tree with the bionNJ algorithms for the heuristic search and then by selecting the tree with the best log likelihood value. Support of nodes for the MP and ML consensus tree was estimated from 1000 non-parametric bootstrap replicates and by setting the same heuristic search parameters as implemented in MEGA 7 and PAUP 4.0. The BA analyses were performed with two runs of four chains and 10,000,000 iterations, sampling every 5000 generations. The value of the average standard deviation of split frequencies (Lakner et al. 2008) was used as an indicator of convergence when it reached 0.002 for the cpDNA, 0.001 for the cpDNA plus morphology and 0.002 for the total evidence analyses. A 25% burnin fraction of trees was set and the remaining trees were summarised in a majority rule consensus tree, in which the posterior probabilities were calculated and used as indicators of support for the clades.

## Character optimisation and character history

To identify putative synapomorphies and homoplasies for each of the recovered clades, an unambiguous character optimisation on an MP strict consensus tree created with the total evidence matrix was conducted in WINCLADA 1.00.08. Acctran and deltran optimisations were explored in WINCLADA to identify additional synapomorphic characters. Since the optimisation analyses identify several characters as homoplastic, they create a very large tree which could not be edited for publishing purposes, thus we only show the synapomorphic characters. A character history was inferred using the resulting topology of the BA analysis of the total evidence matrix, in which ancestral character states for nodes were estimated using the parsimony approach implemented in MESQUITE 3.02.

## Results

## Sequences and character matrices

In this study, 34 new sequences from the *atpB-rbcL* were generated and deposited in GenBank (accessions MH129808–MH129841), 32 of the *psbA-trn*H (accessions MH129842–MH129873), 10 from *trnL-trn*F (accessions MH138282–MH138291), 33 from *5GT* (accessions MG149503–MG149535) and 17 from *DODA* (accessions MG149536–MG149552). Due to the presence of several indels, the newly generated *atpB-rbcL* sequences varied considerably in length, from 385 to 782 nucleotides. New sequences of the other molecular markers were less variable in size and no considerable indels were detected. The sequences of the *psbA-trn*H (including the IGS) ranged from 241 to 236 nucleotides. The length variation of the *trnL-trn*F sequences was from 801 to 826. The newly generated partial *DODA* sequences ranged from 290 to 330 nucleotides long and the 33 sequences of the *5GT* ranged from 469 to 597. Aligned sequences for the three concatenated datasets were 1855 nucleotides long for the cp-DNA matrix, 1910 characters for the cpDNA plus morphology matrix and 2847 for the total evidence matrix. Table 1 shows a statistical summary for each of the individual and for the concatenated matrices. Alignments and trees are available in TreeBASE (https://www.treebase.org/) submission 23037.

#### Phylogenetic analyses

**Polyphyletic** *Echinocactus*. Results obtained with the three concatenated matrices recovered a polyphyletic *Echinocactus*. *E. grusonii* was recovered as sister to *Ferocactus* (hereafter the *K. grusonii-Ferocactus* clade) with strong support in all three concatenated analyses and also in the *5GT* nuclear analyses (Fig. 5). Support values for this clade in the cpDNA analysis were 99% for maximum parsimony bootstrap (mpb), 95% for maximum likelihood bootstrap (mlb) and 1.0 of posterior probability (pp) as shown in Figure 2. The cpDNA plus morphology and the total evidence analyses also recovered the *K. grusonii-Ferocactus* clade with strong support, (90% mpb and 1.0 pp) and (97% mpb and 1.0 pp), respectively (Figs 3, 4).

The cpDNA plus morphology and the total evidence analyses recovered *Echinocactus* in two strongly supported clades. One clade included *E. texensis, E. parryi* and *E. polycephalus* (here after the *Homalocephala* clade) while the other clade included *E. platyacanthus* and *E. horizonthalonius* (here after the *Echinocactus* clade). These two clades, together with the *Astrophytum* clade, were recovered with high support as a monophyletic group (here after the HEA clade), (Figs 2, 3). The HEA clade was previously recovered in several phylogenetic studies (Wallace 1995, Butterworth et al. 2002, Vázquez-Lobo et al. 2015) and also has been recognised as a taxonomic group by some authors (Berger 1929, Benson 1982).

**HEA Clade.** This clade was recovered with the highest support by the cpDNA and morphology and the total evidence analyses (Figure 3, 4), respectively. This clade is

	Total length (nucleotides)	Informative characters	Terminals	Taxa
atpB-rbcL	913	76 (8.3%)	48	28
psbA-trnH	319	48 (15.04%)	47	24
trnL-trnF	1082	62 (5.7%)	48	21
matK	1292	43 (3.3%)	30	24
DODA	333	73 (22%)	23	16
5GT	610	231 (38%)	37	21
cpDNA	1855	103 (5.5%)	35	26
cpDNA + morphology	1910	134 (7.0%)	26	26
Total evidence	2847	207 (7.3%)	26	26

Table 1. Summary statistics of the datasets analyzed.



**Figure 2.** Phylogenetic relationship of *Echinocactus* inferred with the cpDNA matrix. Maximum parsimony 50% majority rule consensus tree of 4 most parsimonious trees of 347 steps with a consistency index of 0.681 and retention index of 0.848. Values in nodes correspond (from left to right) to maximum parsimony bootstrap (mpb), maximum likelihood bootstrap (mlb) and posterior probabilities (pp). The black star indicates the HEA clade with three subclades: *Astrophytum, Echinocactus* and *Homalocephala* highlighted.

also supported by two deletions found in the *atpB-rbcL* dataset, the first deletion being located at position 390 to 394 and the second at position 449 to 457 of the alignment.

All concatenated analyses recovered three major clades within the HEA clade. One clade included the clade of *E. texensis, E. parryi* and *E. polycephalus, (Homalocephala* clade), the second clade included *E. platyacanthus* and *E. horizonthalonius (Echino-cactus* clade), which was recovered with high support and as sister to the third clade, the *Astrophytum* clade (Figs 2, 3). However, the ML and BA analyses of the cpDNA matrix did not recover a monophyletic *Echinocactus* clade, since *E. platyacanthus* and *E. horizonthalonius* were unresolved in a trichotomy along with the *Astrophytum* clade.



**Figure 3.** Phylogenetic relationships of *Echinocactus* inferred with the total evidence matrix. Maximum parsimony strict consensus tree of 2 most parsimonious trees of 863 steps with a consistency index of 0.790 and a retention index of 0.770. Values above/below nodes indicate maximum parsimony bootstrap (mpb) and posterior probabilities (pp), respectively. Black circles indicate synapomorphic characters identified by the character optimisation analyses. Numbers above/below circles indicate characters and character states, respectively (see Appendix 2). The HEA clade is labelled showing the three genera: *Astrophytum, Echinocactus* and *Homalocephala*. Line drawings illustrate the morphological synapomorphies for respective clades.

Homalocephala clade. This clade was recovered as monophyletic in all three concatenated analyses. Support values for this clade ranged from 90% mpb, 73% mlb and 1.0 pp in the cpDNA (Fig. 2), to 95% mpb and 0.99 pp and 99% mpb and 1.0 pp in the cpDNA plus morphology and the total evidence analyses, respectively (Fig. 3, 4). The 5GT nuclear gene analyses also recovered the Homalocephala clade, although support values were lower (56% mlb and 0.72 pp) (Fig. 5). The MP analysis with 5GTdid not recover a monophyletic Homalocephala, since Echinocactus parryi was recovered as sister to the *Echinocactus* clade, but with no support. Internal relationships in the Homalocephala clade were unresolved in the ML and BA analyses of the cpDNA matrix recovering a polytomy of three strongly supported clades, each one integrated by three terminals of the same species (Fig. 2). The MP analysis of the cpDNA matrix recovered E. parryi as sister to a weakly supported clade (58% mpb) including E. polycephalus and E. texensis (Fig. 2). The sister relationship of E. texensis and E. polycephalus was weakly to highly supported in the MP (51%) and the BA (0.90 pp) analyses of the total evidence matrix and by the 5GT nuclear analyses (73% mpb, 56 mlb and 0.99 pp) (Fig. 5). Nevertheless, the cpDNA plus morphology BA results recovered a clade including *E. parryi* and *E. polycephalus* but with low support (0.50 pp). Infraspecific resolution in the Homalocephala clade had low support only in the MP analysis of the cpDNA matrix, in which E. texensis 1 was sister to E. texensis 2, E. polycephalus subsp. xeranthemoides 1 was sister to E. polycephalus subsp. xeranthemoides 2 and E. parryi 1 was recovered as sister to E. parryi 2 (Fig. 2).

*Echinocactus* clade. This clade was recovered with high support in the cpDNA plus morphology (Fig. 4) and the total evidence analyses (Fig. 3). Support values for the *Echinocactus* clade were high in the cpDNA plus morphology (87% mpb and 0.98 pp), as well as in the total evidence analyses (99% mpb and 1.0 pp). Nuclear genes analyses also recovered the *Echinocactus* clade with very high support (Fig. 5). Nevertheless, the cpDNA ML and BA results recovered a low to highly supported clade composed of *E. platyacanthus*, *E. horizonthalonius* and *Astrophytum*, although relationships were unresolved amongst all three taxa (Fig. 2). Internal resolution for *E. platyacanthus* was achieved in the cpDNA analyses, where terminal 3 was recovered as sister to a highly supported clade (97% mpb, 85% mlb and 1.0 pp) including terminals 1 and 2 (Fig. 2). Additionally, the cpDNA analyses provided infraspecific resolution for *E. horizonthalonius*, in which terminal 1 is sister to the strongly supported clade (95% mpb, 85% mlb and 0.89 pp) including terminal 2 and *E. horizonthalonius* subsp. *nicholii* (Fig. 2).

## Discussion

**DODA** and 5GT as molecular markers in the HEA clade. PCR amplifications for both nuclear genes were challenging, as standardisation of PCR conditions were hard to achieve due to lack of consistency even with the same protocols, reagents, concentrations, times and temperatures. It could be that the primers designed by Felker et al. (2008) have low affinity and/or that the *DODA* and 5GT are low copy genes. To



**Figure 4.** Phylogenetic relationship of *Echinocactus* inferred with cpDNA plus morphology. Bayesian 50% majority rule consensus tree. Values in nodes correspond (left to right) to bootstrap values from maximum parsimony (mpb) and posterior probabilities (pp). The black star indicates the HEA clade and its three subclades *Astrophytum, Echinocactus* and *Homalocephala* highlighted.

assess the putative orthology of these two nuclear genes, we applied the procedure of Babineau et al. (2013) ensuring that only one clear PCR band of the same length was amplified for each of the terminals and inspecting chromatograms to make sure that no double-picked nucleotides were present. Furthermore, there were no problems aligning the sequences of either of these two genes, suggesting that the analysed sequences were single copy. Other results reinforced this assumption in that endemic species with restricted distribution, such as *E. parryi*, did not show nucleotide divergence in *5GT*  and *DODA* from individuals of different populations (Fig. 5) and that, even in different species of the clade, HEA sequence divergence was low.

Brockington et al. (2015) discovered two isoforms for *DODA*, the  $\alpha$  and the  $\beta$ , which differ in two diagnostic amino acids at positions 183 and 227. Unfortunately, our new partial sequences were short and determination to which of the isoforms they belong was not possible. However, BLAST searches confirmed that all of our sequences are more similar to the  $\alpha$  isoform found in a clade integrated by two species of Cactaceae, *Lophophora williamsii* (Lem. ex Salm-Dyck) J.M. Coult.) and *Pereskia aculeata* Mill. and members of other families of the Caryophyllales such as Portulacaceae, Basellaceae and Talinaceae (Brockington et al. 2015). Our new sequences are more similar (97%) to the  $\alpha$  isoform of *DODA* found in *Lophophora williamsii* (GenBank accession KR376285) than to the  $\beta$  isoform (70% similarity).

The phylogenetic signal of 5GT and DODA sequences showed that these markers are useful for reconstructing evolutionary relationships in the HEA clade. Topologies obtained with the nuclear markers have good resolution and were highly congruent with those obtained with the cpDNA and cpDNA plus morphology matrices. In these regards, the topologies obtained with cpDNA plus morphology are exactly the same as those resulting from total evidence analyses (which include the nuclear dataset). The only differences between these two analyses are that, in the total evidence analyses, support for several nodes was higher. The low variability found in DODA sequences could be useful to evaluate deep phylogenetic relationships such as tribe assessments, contrary to faster evolving sequences of 5GT that, as shown here, could also be employed for phylogeographic purposes (Fig. 5). All this allows the nuclear genome to be studied for genes related to an important and characteristic pigment pathway, such as the betalains in the Caryophyllales.

**Polyphyletic** *Echinocactus.* As well as in previous studies (Wallace 1995, Butterworth et al. 2002, Bárcenas et al. 2011), all of our analyses recovered *E. grusonii* with very high support within the *Ferocactus* clade and outside of the HEA *clade*. Nevertheless, recognition of the monotypic genus *Kroenleinia* (Lodé 2014) to include *E. grusonii* has to be evaluated more deeply, since the phylogenetic relationships in the *E. grusonii Ferocactus* clade are still obscure (Bárcenas et al. 2011). Furthermore, several morphological characters such as epicotyl type, acute cotyledons, number of spines per areole in seedlings and seed size, are shared amongst *E. grusonii* and other members of *Ferocactus* such as *F. glaucescens*, obscuring the separation between *Kroenleinia* and *Ferocactus*.

Although the unambiguous optimisation of characters could not detect any synapomorphic characters for the *E. grusonii-Ferocactus* clade, deltran optimisation showed that small size seeds are a synapomorphic character for the clade. Acctran optimisation showed that a trullate shape of the floral tube scales, as in *E. grusonii*, is plesiomorphic for the *E. grusonii-Ferocactus* clade switching to ovate scales in *Ferocactus* (Fig. 3). The same pattern was found for the shape of the internal segments of the perianth where a rhombic shape, as in *E. grusonii*, was reconstructed as plesiomorphic and then they changed to a lanceolate shape in *Ferocactus* (Fig. 3). Although the tuberculate epicotyl is homoplastic, most of the species of this clade shared this state, except *F. histrix*,



**Figure 5.** Bayesian 50% majority rule consensus tree showing phylogenetic relationship of *Echinocactus* inferred with 5*GT*. Values in tree nodes correspond (left to right) to bootstrap values from maximum parsimony (mpb), maximum likelihood (mlb) and posterior probabilities (pp). The clades highlighted in dark and light grey correspond to the *Echinocactus, Homalocephala* and *K. grusonii-Ferocactus* clades.

which has a ribbed epicotyl comparable to those in the HEA clade. The same pattern is found in the number of spines per areole at the seedling stage and with the shape of the apex of cotyledons, where species of *E. grusonii-Ferocactus* clade (except *F. histrix*) have areoles that bear 9 to 12 spines and acute cotyledons.

**HEA clade.** The close relationship between *Astrophytum* and *Echinocactus* has been well documented in previous molecular analyses (Wallace 1995, Butterworth et al. 2002, Bárcenas et al. 2011, Hernández-Hernández et al. 2011, Vázquez-Lobo et al. 2015), as well as in some morphological studies (Berger 1929, Benson 1982). This study recovered a strongly supported clade with three internal clades, *Astrophytum*, *Echinocactus* and *Homalocephala*. These results suggest that the HEA clade could be formally recognised as a clear-cut phylogenetic and taxonomic entity of North American cacti. Species of the HEA clade are characterised primarily by their pericarpel and fruit wall with narrowly triangular "pointed scales" and by the presence of trichomes, large seeds (character 34 state 3) and a disjunct configuration of the hilum-micropilar region (character 41 state 0), which the unambiguous optimisation recovered as morphological synapomorphies. As molecular synapomorphies, the analysis recovered two indels, indel 390–394 (character 51 state 0) and indel 448–456 (character 53 state 0) for the *atpB-rbcL* intergenic spacer (Fig. 3). Deltran optimisation showed that character

3, number of spines per areole, state 0, (1 to 4 spines per areole at the seedling stage) is synapomorphic for the clade consisting of the *Echinocactus* and *Astrophytum* clades, but not for the *Homalocephala* clade, in which seedlings bear 5 to 8 spines (state 1) in most of taxa, except *E. polycephalus* subsp. *xeranthemoides*, which bears 9 to 12 spines per areole (Fig. 3).

So far, *K. grusonii* is the only species that has consistently been recovered outside the HEA clade having pointed scales in flowers and fruits, as well as trichomes in the axils of the scales (Wallace 1995, Butterworth et al. 2002, Bárcenas et al. 2011). Nevertheless, *K. grusonii* lacks the putative diagnostic indels for this clade found in the *atpB-rbcL*. Unfortunately, the parsimony reconstruction of ancestral states was ambiguous regarding the presence of pointed scales, so it is unclear whether this character is plesiomorphic or apomorphic in our phylogeny.

Homalocephala clade. Phylogenetic studies conducted here strongly support the results of Wallace (1995) and the morphological study of Ferguson (1992), recognising two clades for *Echinocactus*, the *Echinocactus* and the *Homalocephala* clades. Our results disagree with those of Bárcenas et al. (2011), in which the only terminal of *Echinocactus polycephalus* they sampled was recovered outside of the HEA clade. Here all the terminals of *E. polycephalus* subsp. *polycephalus* and *E. polycephalus* subsp. *xeranthemoides* were recovered within the *Homalocephala* clade, supporting the inclusion of these two subspecies within the genus as Wallace (1995) and Ferguson (1992) proposed.

Within the Homalocephala clade, the MP analysis with the cpDNA matrix, as well as the 5GT and total evidence analyses, recovered E. parryi as sister to a clade with moderate support that included *E. polycephalus* and *E. texensis*. However, the ML and BA analyses of the cpDNA recovered a strongly supported polytomy for these three species (Fig. 2). Thus, more evidence is needed to fully resolve the internal relationships in the Homalocephala clade since our results only weekly support the sister relationship between E. texensis and E. polycephalus. Some taxonomists have elevated the rank of E. polycephalus subsp. xeranthemoides to the level of species (Rydberg 1917, Salywon and Hodgson 2012). Salywon and Hodgson (2012) proposed the recognition of E. xeranthemoides as a distinct species from E. polycephalus based on several morphological characters, such as the colour and number of spines per areole, length, colour and orientation of the fruit scales, seed length and testa ornamentation, that can easily distinguish one taxa from the other. Our hypotheses weakly supports this proposal, because these two taxa were always recovered as sisters, except in the MP analysis of the cpDNA plus morphology matrix when they were unresolved in a polytomy with the other two species of Homalocephala (Fig. 4). Furthermore, divergence in DNA sequences between the subspecies of E. polycephalus was almost null, with only three single nucleotide polymorphisms (SNP) in all the matrices analysed. However, two of these SNPs support the distinction of the two subspecies.

The only synapomorphic character detected by the unambiguous optimisation analysis for the *Homalocephala* clade was the pubescence of the abaxial face of the pericarpelar scales, which apparently was later lost in *Echinocactus polycephalus* subsp. *xeranthemoides* (Fig. 3). However, other morphological characters support the monophyly

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of *Homalocephala* as compared with their most closely related genera, *Astrophytum* and *Echinocactus*. These characters are the campylotropous embryos, the cotyledons with acute apices, seedling areoles with 5 to 8 spines, acute ribs in the juvenile and the adult stages, the pubescence of the spine epidermis (except in *E. polycephalus* subsp. *xeranthemoides*), the slightly tubular flower shape and the testa cells with a channelled boundary relief.

Echinocactus clade. The Echinocactus clade has been consistently recovered with medium to strong support in all previous phylogenetic studies (Wallace 1995, Butterworth et al. 2002, Bárcenas et al. 2011, Hernández-Hernández et al. 2011, Vázquez-Lobo et al. 2015). In our cpDNA plus morphology and total evidence analyses, this clade was recovered with high support (Fig 3, 4). Although, the MP analysis of the cpDNA matrix also recovered a monophyletic *Echinocactus*, support values were much lower and the ML and BA analyses with this matrix instead recovered E. platyacanthus and E. horizonthalonius as unresolved in a strongly supported trichotomy including Astrophytum (Fig. 2). This latter result supports the ideas of Ferguson (1992) and Doweld (2000) who proposed the exclusion of E. horizonthalonius from the genus based on morphology alone. Furthermore, the optimisation analyses identified that an apically tuberculated epicotyl and a black-greyish testa are autapomorphic characters for *E. hori*zonthalonius (Fig. 3). However, due to the recovering of a sister relationship between E. platyacanthus and E. horizonthalonius in most of the analyses developed, as well as in all previous studies, it is here preferred to maintain E. horizonthalonius in the genus Echino*cactus*. The unambiguous optimisation shows that the rounded apices of the cotyledons (character 2 state 2) are a synapomorphic character for the *Echinocactus* clade (Fig. 3).

The surprisingly closer phylogenetic relationship of *Echinocactus* to *Astrophytum*, rather than to *Homalocephala*, recovered in this study, is morphologically supported by two synapomorphies: the rectangular epicotyls (character 6 state 2) and a reduction from 1 to 4 spines per areole (character 3 state 0) in seedlings (Fig. 3). Acctran optimisation showed that a rectangular epicotyl is synapomorphic to this clade, which then evolved to a globose shape in *E. horizonthalonius* and *A. asterias* (Zucc.) Lem. and to a cylindrical shape in *A. caput-medusae* (Velazco & Nevárez) D.R. Hunt (Fig. 3). Deltran optimisation found that a reduction of 1 to 4 spines per areole at seedling stage and one year of age individuals is also a synapomorphic character. Thus, it seems that the *Homalocephala* clade has possibly retained plesiomorphic characters, such as campylotropous embryos, acute cotyledons and more spines per areole in seedling stages, as compared to *Astrophytum* and *Echinocactus*. These characters were modified in *Echinocactus* and *Astrophytum*, where the embryos are anatropous or orthotropous, the cotyledons rounded or obtuse and the areoles bear less spines.

Interestingly, the phylogenetic analyses here conducted recovered with medium to high support an infraspecific structure for the two widely distributed species of *Echinocactus*. It was here found that there is molecular evidence to recognise *E. horizonthaloni-us* subsp. *nicholii* by the presence of various SNPs in the cpDNA alignment. There are at least two genetically well delimited populations or megapopulations of *E. platyacanthus* as Figueroa (2015) previously described (Fig. 2). This study recovered the northern pop-

ulations (Tamaulipas, Coahuila and San Luis Potosí) as sister to a well-supported clade including terminals from central Hidalgo and southern Puebla (Fig. 2). Furthermore, the distinction between these two megapopulations is also supported by a deletion of 60 nucleotides in the *atpB-rbcL* IGS in individuals from the northern populations.

Taxonomic implications. The recovering of Homalocephala as sister to the Echinocactus and Astrophytum clade suggests that changes have to be made to the current status in order to establish a phylogenetically based classification. *Echinocactus*, as currently accepted, cannot be here recognised because this would imply the acceptance of a non-monophyletic genus since E. grusonii was recovered as sister to Ferocactus and Homalocephala as sister to the Astrophytum and Echinocactus clade. One possibility is to recognise the genus *Echinocactus* with three subgenera or sections (*Homalocephala*, Echinocactus and Astrophytum) similar to the proposal of Benson (1982). Nevertheless, the morphological diversity suggests that these clades could be easily recognised as three different genera with a balanced partition of the molecular and morphological diversities. Astrophytum is a morphologically well supported genus (Bravo-Hollis and Sánchez-Mejorada 1991; Anderson 2001; Vázquez-Lobo et al. 2015), with two synapomorphic characters identified in this study, pubescent epidermis (character 7, state 1) and orthotropous embryos (character 1, state 0). Although in the Homalocephala clade, the pubescence of the pericarpel and floral tube are the only synapomorphic characters, there are some other shared characters that support its distinction from its closest relatives of the Astrophytum and Echinocactus clade. The presence of acute cotyledons, 5 to 12 spines per areole at seedling stage, campilotropous embryos and pubescent spine epidermis in most of the species, support the recognition of Homalocephala. Echinocactus is supported by the presence of rounded cotyledons, which is a synapomorphic character for the genus. Other characters that support its distinction from its closest relatives are the campanulate flowers and the presence of a dense apical region covered with abundant trichomes. Thus, the HEA clade is here recognised as circumscribed by three clades, each one representing a genus: Homalocephala, Echinocactus and Astrophytum. The HEA clade could be recognised as a subtribe in the Cacteae, maybe as the Echinocactinae Buxb. (Buxbaum 1958), in which Echinocactus and Homalocephala are included. If this is the case, only the genus Astrophytum would have to be included to delimit a monophyletic subtribe.

## Conclusions

This is the first phylogenetic study that has evaluated and combined molecular data from chloroplast and nuclear genomes with morphology to test the monophyly of all species and subspecies of *Echinocactus* currently accepted. Here we reinforce the proposal of excluding *Echinocactus grusonii* from the genus. Nevertheless, the recognition of *Kroenleinia grusonii* must be deeply evaluated since phylogenetic relationships of the *Ferocactus* clade (including *K. grusonii*, *Leuchtenbergia*, *Stenocactus*, *Thelocactus* and *Glandulicactus*) are still unresolved. The well-known HEA clade was recovered

as monophyletic with strong support. This clade is morphologically and molecularly well defined, suggesting its taxonomic recognition. Our results also support the proposal that *Echinocactus*, as currently accepted (excluding *K. grusonii*), should be considered as two independent lineages, the *Homalocephala* and the *Echinocactus* clades, each one with its own molecular and morphological diagnostic characters, and each one representing different genera. In this study, all of the analyses recovered *E. polycephalus* within the *Homalocephala* clade, supporting its inclusion in this taxon. Here we present the new taxonomic combinations for the species of *Homalocephala* and an identification key for the genera of the HEA clade and for all of their species and subspecies.

## **Taxonomic treatment**

*Homalocephala parryi* (Engelm.) Vargas & Bárcenas, comb. nov. urn:lsid:ipni.org:names:60477372-2

Echinocactus parryi Engelm., Proc. Amer. Acad. Arts 3: 276. 1857.

**Type.** [Mexico], near Lake Santa Maria, Chihuahua, a bunch of spines collected by Dr. Parryi, s/n, apparently lost (**lectotype,** plate 32, figs 6–7 in Engelm., Rep. U.S. Mex. Bound., Bot. [Emory], designated by Chamberland, Syst. Bot. 22: 310. 1997).

*Homalocephala polycephala* (Engelm. & J.M. Bigelow) Vargas & Bárcenas, comb. nov. urn:lsid:ipni.org:names:60477373-2

Echinocactus polycephalus Engelm. & J.M. Bigelow, Proc. Amer. Acad. Arts 3: 276. 1857.

**Type.** U.S.A., California, Mohave Valley, Bigelow s/n., 8 Mar 1854 (**lectotype**, designated by Chamberland, Syst. Bot. 22: 311. 1997: MO 2017480).

# Homalocephala polycephala subsp. xeranthemoides (J.M. Coult.) Vargas & Bárcenas, comb. nov.

urn:lsid:ipni.org:names:77191938-1

*Echinocactus polycephalus* Engelm. & J.M. Bigelow var. *xeranthemoides* J.M. Coult. in Contr. U.S. Natl. Herb. 3(7): 358. 1896 [1 Apr 1896].

**Type.** U.S.A., Arizona, near the Rio Colorado, Siler s/n., Nov., 1881 (**lectotype**, designated by Benson, Cacti U. S. Canada, 951. 1982: MO 106798 [excluding packet labeled "Siler, 1882"]).

## Key to genera of HEA clade

1	Stem epidermis glabrous; ovoid to almost circular seeds; campilotropous or
	anatropous embryos and rounded or acute cotyledons2
_	Stem epidermis pubescent; navicular seeds; orthotropous embryos and ob-
	tuse cotyledons
2	Stem apex with scarce trichomes; acute ribs; slightly tubular flowers; campi-
	lotropous embryos and acute cotyledons
_	Stem apex with a dense cover of trichomes; obtuse ribs; campanulate flowers,
	anatropous embryos and rounded cotyledons Echinocactus

## Key to species of Astrophytum

1	Areoles in mature plants with conspicuous spines
_	Areoles in mature plants with inconspicuous spines
2	Stiff straight spines and completely yellow flowers
_	Flexible curved spines and yellow flowers with a reddish baseA. capricorn
3	Stems ribbed
_	Stems tuberculated
4	Stems globose to cylindrical, commonly with five acute ribs A. myriostigma
_	Stems depressed, commonly with eight obtuse ribs A. asteria

## Key to species of Homalocephala

1	Stems rarely to frequently caespitose; yellow flowers and dry fruits2
_	Stems Simple; pink flowers and juicy fruits
2	Stems rarely caespitose; yellow flowers with a clear reddish base H. parryi
_	Stems commonly caespitose, forming large clumps; yellow flowers without a
	reddish base
3	Spine epidermis and pericarpel scales pubescent; testa cells with periclinal
	walls with convex relief (papillated) H.polycephala subsp. polycephala
_	Spine epidermis and pericarpel scales glabrous; testa cells with periclinal walls
	with inconspicuous relief (flat) H. polycephala subsp. xeranthemoides

## Key to species of Echinocactus

1	Mature plants with stems large globose to barrel shaped with up to 60 ribs;
	yellow flowers; testa cells with inconspicuous relief E. platyacanthus
_	Mature plants with stems depressed, globose to rarely short cylindrical fre-
	quently with eight ribs; pink to crimson flowers, seeds with testa cells with a
	convex relief

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## Appendix I

1.1 Scientific names, voucher information and Genbank accession numbers for *atpB-rbcL*, *psbA-trnH*, *trnL-trnF*, *matK*, *DODA* and *5GT* sequences used in the concatenated analyses. \*indicates terminals with previously extracted DNA.

Ingroup: Echinocactus horizonthalonius 1, Vargas-Luna 74, QMEX, atpB-rbcL (MH129821), psbA-trnH (MH129853), 5GT (MG149506); trnL-trnF Genbank accession: HM041258. Echinocactus horizonthalonius 2, Vargas-Luna 75, QMEX, atpB-rbcL (MH129820), psbA-trnH (MH129854), DODA (MG149542), 5GT (MG149507); R. Bárcenas 1698\*, QMEX, trnL-trnF (MH138285); matK Genbank accession: FN997104. Echinocactus horizonthalonius subsp. nicholii, Ecker 111, living collection of the Desert Botanical Garden, atpB-rbcL (MH129822), psbA-trnH (MH129855), trnL-trnF (MH138286), 5GT (MG149508). Echinocactus platyacanthus 1, M. Figueroa C26M6\*, QMEX, atpB-rbcL (MH129817), DODA (MG149541), 5GT (MG149505); M. Figueroa C16M1\*, QMEX, psbAtrnH (MH129850), trnL-trnF (MH138282). Echinocactus platyacanthus 2, M. Figueroa C9M1\*, OMEX, atpB-rbcL (MH129818), trnL-trnF (MH138283); M. Figueroa C9M3\*, QMEX, psbA-trnH (MH129851). Echinocactus platyacanthus 3, M. Figueroa C13M1\*, QMEX, psbA-trnH (MH129852); M. Figueroa C18M15\*, QMEX, atpB-rbcL (MH129819), trnL-trnF (MH138284). Homalocephala parryi 1, Vargas-Luna 28, QMEX, atpB-rbcL (MH129826), psbA-trnH (MH129859), DODA (MG149544), 5GT (MG149513); matK Genbank accession: KC776965. Homalocephala parryi 2, Vargas-Luna 30, QMEX, psbA-trnH (MH129860); Vargas-Luna 38, QMEX, DODA (MG149545); Vargas-Luna 41, atpB-rbcL (MH129827), 5GT (MG149514). Homalocephala parryi 3, Vargas-Luna 61, QMEX, atpBrbcL (MH129828), 5GT (MG149515); Vargas-Luna 133, QMEX, psbA-trnH (MH129861). Homalocephala polycephala subsp. polycephala, M. Chamberland 51, Living collection of the Desert Botanical Garden, atpB-rbcL (MH129829), psbAtrnH (MH129862), trnL-trnF (MH138289), 5GT (MG149519); Vargas-Luna 83, QMEX, DODA (MG149546); matK Genbank accession number: FN997389. Homalocephala polycephala subsp. xeranthemoides 1, Vargas-Luna 180, DES, atpB-rbcL (MH129830), psbA-trnH (MH129863), trnL-trnF (MH138290), 5GT (MG149520). Homalocephala polycephala subsp. xeranthemoides 2, W. Hodgson 9727, DES, atpB-rbcL (MH129831), psbA-trnH (MH129864); W. Hodgson 25463, DES, trnL-trnF (MH138291), 5GT (MG149521). Homalocephala texensis 1, Vargas-Luna 127, QMEX, atpB-rbcL (MH129823), psbA-trnH (MH129856), DODA (MG149543); Carter without number, living collection of the Desert Botanical Garden, trnL-trnF (MH138288), 5GT (MG149512); matK Genbank accession: FN997302. Homalocephala texensis 2, M. Baker 14286, ASU, atpB-rbcL (MH129824), psbA-trnH (MH129857), 5GT (MG149510). Homalocephala texensis 3, M. Baker 16609, ASU, atpB-rbcL (MH129825), psbA-trnH (MH129858), trnL-trnF (MH138287), 5GT (MG149511). Kroenleinia grusonii 1, Vargas-Luna 92, QMEX, atpB-rbcL (MH129832), psbA-trnH (MH129865); CHB50\*, living collection of Jardín Botánico Charco del Ingenio, *DODA* (MG149547), *5GT* (MG149523); *trnL-trn*F Genbank accession: HM041257; *mat*K Genbank accession: FN997149. *Kroenleinia grusonii* 2, R. Bárcenas 1584\*, QMEX, *atpB-rbcL* (MH129833); R. Bárcenas 1588\*, QMEX, *psbA-trn*H (MH129866); *trnL-trn*F Genbank accession: HM041257.

**Outgroup:** *Astrophytum asterias*, nursey acquired plant, *atp*B-*rbc*L (MH129812), psbA-trnH (MH129845); trnL-trnF Genbank accession: KC776941; matK Genbank accession: FN997357. Astrophytum capricorne, H. Hernández 2320\*, MEXU, atpB-rbcL (MH129816), psbA-trnH (MH129846), trnL-trnF Genbank accession: KC776959, matK Genbank accession: FN997272 Astrophytum caput-medusae, nursery acquired plant\*, atpB-rbcL (MH129813), psbA-trnH (MH129847); trnL-trnF Genbank accession: KC776998; matK Genbank accession: FN997077. Astrophytum myriostigma, Vargas-Luna 116, QMEX, atpB-rbcL (MH129814), psbA-trnH (MH129848), DODA (MG149538); trnL-trnF Genbank accession: KC776951; matK Genbank accession: FN997366. Astrophytum ornatum, Vargas-Luna 95, QMEX, atpB-rbcL (MH129815), psbA-trnH (MH129849), DODA (MG149540); H. Hernández 3703\*, MEXU, 5GT (MG149504); trnL-trnF Genbank accession: KC776947; matK Genbank accession: FN997293. Aztekium hintonii, ZSS991236\*, Living collection Zurich Botanical Garden, atpB-rbcL (MH129810), psbA-trnH (MH129843), DODA (MG149537); matK Genbank accession: FN997040. Aztekium ritteri, nursery acquired plant, atpB-rbcL (MH129811), psbA-trnH (MH129844); matK Genbank accession: AY015290. Carnegiea gigantea, Vargas-Luna 86, QMEX, atpB-rbcL (MH129808), DODA (MG149536), 5GT (MG149503); trnL-trnF Genbank accession: HM041236; matK Genbank accession: KT164775. Ferocactus echidne, R. Bárcenas 49\*, MEXU, atpB-rbcL (MH129835), psbA-trnH (MH129868), 5GT (MG149530); trnL-trnF Genbank accession: HM041276; matK Genbank accession: FN997544. Ferocactus glaucescens, H. Hernández 3701\*, MEXU, atpB-rbcL (MH129834), 5GT (MG149524); H. Hernández 2425\*, MEXU, psbAtrnH (MH129867); matK (FN997274). Ferocactus hamatacanthus, H. Hernández 2096\*, MEXU, atpB-rbcL (MH129836), 5GT (MG149531); H. Hernández 1467\*, MEXU, psbA-trnH (MH129870); matK Genbank accession: FN997248. Ferocactus histrix, J. Álvarez SLP1\*, QMEX, atpB-rbcL (MH129837), DODA (MG149549), 5GT (MG149526); R. Bárcenas 557\*, MEXU, psbA-trnH (MH129869); matK Genbank accession: FN997287. Ferocactus latispinus, Vargas-Luna 90, QMEX, atpBrbcL (MH129838); J. Álvarez without number\*, QMEX, psbA-trnH (MH129871); R. Bárcenas 1620\*, QMEX, 5GT (MG149532); trnL-trnF Genbank accession: HM041278; matK Genbank accession: FN997246. Ferocactus lindsayi, CHB10\*, living collection of Jardín Botánico Charco del Ingenio, atpB-rbcL (MH129839), 5GT (MG149533); matK Genbank accession: FN997152. Ferocactus pilosus, C. Gómez-Hinostrosa 739\*, MEXU, atpB-rbcL (MH129840), psbA-trnH (MH129872), 5GT (MG149534); matK Genbank accession: FN997133. Ferocactus wislizeni, R. Bárcenas 1565\*, QMEX, psbA-trnH (MH129873), 5GT (MG149535); R. Bárcenas

1569\*, QMEX, *atpB-rbcL* (MH129841); *mat*K Genbank accession: FN997459. *Geohintonia mexicana*, Reyes J. 3226\*, MEXU, *atpB-rbcL* (MH129809), *psbA-trn*H (MH129842), *mat*K GenBank accession: FN997326. *Opuntia ficus-indica*, Genbank accessions: *atpB-rbcL* JF787222, *psbA-trn*H FJ026612, *trnL-trn*F KJ735936, *mat*K FN997314, *DODA* EU0899741, *5GT* EU089744.

1.2 Scientific names, voucher information and Genbank accession numbers for the *5GT* nuclear gene analyses. \* indicates terminals with previously extracted DNA.

Ingroup: Echinocactus horizonthalonius 1, Vargas-Luna 74, QMEX, (MG149506). Echinocactus horizonthalonius 2, Vargas-Luna 75, QMEX, (MG149507). Echinocactus horizonthalonius subsp. nicholii, Ecker 111, living collection of the Desert Botanical Garden, (MG149508). Echinocactus horizonthalonius x platyacanthus, W. Hodgson without number, ASU, (MG149509). Echinocactus platyacanthus, M. Figueroa C26M6\*, QMEX, (MG149505). Homalocephala parryi 1, Vargas-Luna 28, QMEX, (MG149513). Homalocephala parryi 3, Vargas-Luna 41, QMEX, (MG149514). Homalocephala parryi 4, Vargas-Luna 61, QMEX, (MG149515). Homalocephala parryi 5, Vargas-Luna 64, QMEX, (MG149516). Homalocephala parryi 6, Vargas-Luna 72, QMEX, (MG149517). Homalocephala polycephala subsp. polycephala 1, E. Anderson 6191, living collection of the Desert Botanical Garden, (MG149518). Homalocephala polycephala subsp. polycephala 2, M. Chamberland 51, Living collection of the Desert Botanical Garden, (MG149519). Homalocephala polycephala subsp. xeranthemoides 1, W. Hodgson 30366, DES, (MG149522). Homalocephala polycephala subsp. xeranthemoides 2, W. Hodgson 25463, DES, (MG149521). Homalocephala polycephala subsp. xeranthemoides 3, Vargas-Luna 180, DES, (MG149520). Homalocephala texensis 1, M. Baker 14286, ASU, (MG149510). Echinocactus texensis 2, M. Baker 16609, ASU, (MG149511). Homalocephala texensis 3, Carter without number, living collection of the Desert Botanical Garden, (MG149512). Kroenleinia grusonii, CHB50\*, living collection of Jardín Botánico Charco del Ingenio, (MG149523).

**Outgroup:** Amaranthus tricolor, Genbank accession: KP174810. Astrophytum ornatum 1, H. Hernández 3703\*, MEXU, (MG149504). Beta vulgaris, Genbank accession: AY526080. Carnegiea gigantea, Vargas-Luna 86, QMEX, (MG149503). Ferocactus echidne, R. Bárcenas 49\*, MEXU, (MG149530). Ferocactus glaucescens, H. Hernández 3701\*, MEXU, (MG149524). Ferocactus hamatacanthus, H. Hernández 2096\*, MEXU, (MG149531). Ferocactus histrix 1, J. Álvarez B1\*, QMEX, (MG149529). Ferocactus histrix 2, J. Álvarez B2\*, QMEX. Ferocactus histrix 3, J. Álvarez SLP1\*, QMEX, (MG149526). Ferocactus histrix 4, J. Álvarez SLP3\*, QMEX, (MG149527). Ferocactus histrix 5, J. Álvarez SLP6\*, QMEX, (MG149528). Ferocactus histrix 6, J. Álvarez T8\*, QMEX. Ferocactus histrix 7, J. Álvarez T10\*, QMEX, (MG149525). Ferocactus latispinus, R. Bárcenas 1620\*, QMEX, (MG149532). Ferocactus lindsayi, CHB10\*, living collection of Jardín Botánico Charco del Ingenio, (MG149533). Ferocactus pilosus, C. Gómez-Hinostrosa 739\*, MEXU, (MG149534). Ferocactus wislizeni, R. Bárcenas 1565\*, QMEX, (MG149535). Opuntia ficus-indica 3, Genbank accession: EU089744, Opuntia ficus-indica 4, Genbank accession: EU089743.

## Appendix 2

**Table A1.** Morphological characters, character states, and development stages at which the characters were coded. **A**. Embryo, **B**. One week to one month seedlings, **C**. One year individuals, **D**. Juvenile, **E**. Adult, **F**. Adult flower, **G**. Adult fruit, and **H**. Seed. Numbers in parenthesis of indels are the positions at which they are located in the total alignment.

Characters and states	Development stages
1) Embryo type, 0 = orthotropous; 1 = anatropous; 2 = campylotropous	A
2) Cotyledon apex, 0 = acute; 1 = obtuse; 2 = rounded	В
<b>3) Number of spines per areole</b> , 0 = 1 to 4; 1 = 5 to 8; 2 = 9 to 12	В
4) Presence of cotyledons, 0 = inconspicuous; 1 = conspicuous	С
<b>5) Epicotyl type</b> , 0 = totally tuberculate; 1 = ribbed; 2 = apically tuberculate	С
6) Epicotyl shape, 0 = globose; 1 = cylindrical; 2 = rectangular	С
7) Epidermis, 0 = glabrous; 1 = pubescent	С
8) Growth form, 0 = simple; 1 = cespitose	E
9) Stem colour, 0 = light green; 1; dark green	Е
<b>10) Epidermis</b> , 0 = glabrous; 1 = pubescent	E
<b>11)</b> Stem apex, 0 = glabrous; 1 = slightly cover with wool; 2 = densely cover with wool	E
<b>12) Rib shape</b> , 0 = acute; 1 = obtuse	D, E
13) Distal portion of the ribs, 0 = undulated; 1 = straight	E
14) Spine epidermis, 0 = glabrous; 1 = pubescent	E
<b>15)</b> Trichomes length in reproductive areoles, 0 = small; 1 = large	E
<b>16) Flower shape</b> , 0 = campanulate; 1 = slightly tubular	F
17) Pericarpel wall, 0 = naked; 1 = with accessory appendages	F
<b>18)</b> Type of accessory appendages of the pericarpel, 0 = scale; 1 = spine	F
<b>19)</b> Presence of trichomes in flower areoles, 0 = absent; 1 = present	F
<b>20) colour of the pericarpel in anthesis</b> , 0 = green; 1 = light green; 2 = whitish with pink; 3 = pink to purple; 4 = brownish	F
<b>21) Pericarpel scale shape</b> , 0 = semicircular; 1 = narrowly triangular "pointed scales"	F
22) Pericarpel scale margin, 0 = entire; 1 = Serrate	F
23) Pericarpel scale apex, 0 = acuminate; 1 = mucronate	F
<b>24)</b> Abaxial surface of the pericarpel scales, 0 = glabrous; 1 = pubescent	F
<b>25)</b> Floral tube scale shape, 0 = triangular; 1 = ensiform; 2 = trullate; 3 = ovate	F
<b>26)</b> Perianth external segments shape, 0 = obovate; 1 = oblanceolate; 2 = elliptic; 3 = oblong; 4 = lanceolate	F
<ul> <li>27) Perianth internal segments shape, 0 = cordate; 1 = obovate; 2 = oblanceolate; 3 = elliptic;</li> <li>4 = rhombic; 5 = lanceolate</li> </ul>	F
<b>28)</b> Fruit type, 0 = dried; 1 = fleshy juicy	G
<b>29)</b> Fruit wall, 0 = naked; 1 = with accessory appendages	G
<b>30)</b> Spine presence in fruit, 0 = absent; 1 = present	G
<b>31)</b> Trichomes presence in fruit, 0 = absent; 1 = present	G
<b>32) Scale presence in fruit</b> , 0 = absent; 1 = presence	G
<b>33)</b> Scale shape of fruit, 0 = ovate; 1 = narrowly triangular "pointed scales"	G
<b>34)</b> Seed size, 0 = very small; 1 = small; 2 = medium; 3 = large; 4 = very large; 5 = extremely large	Н
<b>35)</b> Position of the hilum with respect to the long axis, 1 = perpendicular; 2 = oblique; 3 = parallel	Н
<b>36)</b> Colour of the testa, 0 = black; 1 = black-brown; 2 = reddish; 3 = black-grey	Н
<b>37) Keel presence in seed</b> , 0 = absent; 1 = presence	Н

<b>38)</b> Boundary relief in testa cells, 0 = inconspicuos; 1 = channeled; 2 = raised	Н				
<b>39)</b> Relief of the periclinal walls of the testa cells, 0 = none (flat); 1 = convex; 2 = concave;					
3 = partially convex; 4 = partially concave	Н				
<b>40)</b> Microrelief of the testa cells, 0 = glabrous; 1 = verrucose; 2 = striate					
<b>41) Hilum-micropyle configuration</b> , 0 = disjunct; 1= conjunct but separated by sclerified tissue; 2 = fused					
<b>42)</b> Presence of appendages, 0 = absent; 1 = present	Н				
<b>43) Indel</b> , (54-55)	-				
<b>44) Indel</b> , (58-59)	-				
<b>45) Indel</b> , (82-85)	—				
<b>46) Indel</b> , (122)	-				
<b>47) Indel</b> , (161-169)	-				
<b>48) Indel</b> , (186-191)	-				
<b>49) Indel</b> , (381)	-				
<b>50) Indel</b> , (388-389)	-				
<b>51) Indel</b> , (390-394)	-				
<b>52) Indel</b> , (419-423)	-				
<b>53) Indel</b> , (448-456)	_				
<b>54) Indel</b> , (517-541)	_				
<b>55) Indel</b> , (572-576)	_				

**RESEARCH ARTICLE** 



# Bulbophyllum chrysolabium (Orchidaceae, Epidendroideae, Malaxideae), a new species from Yunnan, China

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#### Abstract

*Bulbophyllum chrysolabium*, a new species belonging to section *Racemosae* from Yunnan, China is described and illustrated. The species is related to *B. orientale* and *B. morphologorum*, but differs by having the following set of characters: obliquely broadly-based triangular petals with a long filiform apex; lip densely glandular papillose and conspicuously ciliolate along margins; lip auricles well developed, narrow-ly falcate, tapering to a long sharp point at the apex; stelidia subulate and twisted inwards, slightly exceeding operculum. The conservation status of *B. chrysolabium* is assessed and taxonomic notes are provided.

#### Keywords

Menglian County, new taxa, section Racemosae, taxonomy

## Introduction

Recently, based on phylogenetic studies, all genera previously proposed in subtribe Bulbophyllinae were formally transferred to *Bulbophyllum* Thou. (Vermeulen et al. 2014). As the largest orchid genus, *Bulbophyllum* Thou. (1822: tab. 93) is pantropical and widespread, with approximately 2200 species described worldwide (Sieder et al. 2007; Vermeulen 2014). However, it is believed that there are still numerous species awaiting discovery and formal description.

The high number of species in the genus Bulbophyllum makes systematic study not easy due to their morphological similarities and difficulties encountered in identifying them in their vegetative states. Bulbophyllum sect. Racemosae Benth. & Hk.f. (1883: 502) includes such species. According to the revised circumscription for section Racemosae (Seidenfaden 1979, 1992; Vermeulen and O'Byrne 2011; Vermeulen 2014), members of this section are characterized by distinct or well-developed pseudobulbs bearing a single leaf; inflorescence with many densely arranged flowers in a cylindrical raceme; mobile labellum usually with proximal auricles; distinct stelidia; basal node of pedicel approximately level with floral bract attachment. During our recent botanical survey in Menglian County of south-western Yunnan, China, an unusual species was discovered and collected. After further studies, the species was confidently classified as a Bulbophyllum species of section Racemosae, with approximately 38 species mainly distributed in tropical and subtropical Asia. There are seven species recorded in China, all of which occur in Yunnan Province (Seidenfaden 1979, 1992; Tsi 1999; Li 2003; Chen and Vermeulen 2009; Ye and Li 2012; Averyanov et al. 2016). A thorough literature and herbarium specimen review indicated that it is clearly different from any other previously known taxon of the section.

### Materials and method

Morphological comparisons and assessments of *Bulbophyllum chrysolabium* and its allies in the section *Racemosae* were performed based on specimens from herbaria BM, E, IBSC, K, KUN, PE (acronyms according to Thiers 2018), as well as consideration of the taxonomic literature. In addition, living plants were collected and transplanted to the nursery of the South China Botanical Garden, Chinese Academy of Sciences (SCBG) for further investigation. Measurements and photographs on the fresh material were made under a stereomicroscope (Olympus MD-90). The conservation status of the putative new species was evaluated following the guidelines of the International Union for Conservation of Nature (IUCN 2017).

#### **Taxonomic description**

*Bulbophyllum chrysolabium* L. Li & D.P. Ye, sp. nov. urn:lsid:ipni.org:names:77191936-1 Figures 1, 2

**Diagnosis.** *Bulbophyllum chrysolabium* is distinguished from all known congeners by having the following unique combination of features: obliquely broadly-based triangular petals with a long filiform apex; lip densely glandular papillose on both sides



**Figure I.** *Bulbophyllum chrysolabium.* **A** Habit **B** Flower, lateral view **C** Flower, frontal view **D** Dorsal sepal, petals and lateral sepals, adaxial view **E** Lip, lateral view **F** Lip, ventral view **G** Pollinia **H** Operculum, ventral view **I** Column, ventral view **J** Column and lip, lateral view. Scale bars: 2 cm (**A**), 2 mm (**B–D**), 1 mm (**E–F, I–J**), 0.2 mm (**G–H**). Drawn by Yun-Xiao Liu.

and conspicuously ciliolate along margins; lip auricles well developed, narrowly falcate, tapering to a long sharp point at the apex; stelidia subulate and twisted inwards, slightly exceeding operculum.

**Taxonomic notes.** Bulbophyllum chrysolabium appears to be related to B. orientale Seidenf. (Seidenfaden 1979: 138), especially in narrowly falcate lip auricles and twisted stelidia, but differs in distinctly longer floral bracts (almost twice as long as the pedicel and ovary); petals with long filiform apices, a rather smaller lip (ca. 2.8 mm long), significantly glandular-papillose and ciliolate at margins; stelidia slightly exceeding operculum and distinctly longer than column. With respect to filiform petals, B. chrysolabium is also superficially similar to B. morphologorum Kräenzl. (1908: 89), however, the latter have a fat, conical protuberance or callus on the front of the column near its base and scape much longer than rachis. In addition, it has subulate, not twisted stelidia, considerably longer than operculum; lip auricles not falcate, but rather obtuse at the apex. A detailed morphological comparison between B. chrysolabium and its allied species is presented in Table 1.

**Type.** CHINA. Yunnan Province. Menglian Dai, Lahu and Va Autonomous County (referred to as Menglian County), 1400–1600 m alt., 23 May 2014, L. Li 1209, (holotype, IBSC! isotype, IBK!).

Description. Epiphytic herbs. Rhizome rigid, creeping, 2-3 mm thick. Pseudobulbs borne on rhizome at an interval of 3-5 cm, ovoid to narrowly ovoid, 2.2-3.5 cm high, base often covered with remnant fibrous sheaths. Leaf apical, single, coriaceous, oblong to linear-oblong,  $12-14 \text{ cm} \times 1.8-2.2 \text{ cm}$ , subsessile, apex retuse or minutely notched. Inflorescences developing from base of pseudobulb, with pendulous densely many-flowered raceme; scape 5 to 6 cm long, nearly completely enveloped by 4 or 5 sterile, broadly ovate sheaths; sheath 1.5-1.8 cm long, scaled, light brownish; rachis about 6.5 cm long, with many flowers spirally arranged into a compact spike-like cylindrical raceme, opening simultaneously; floral bracts light brownish, dry papyraceous, oblong-lanceolate, ca. 6.5 mm long, almost twice as long as the pedicel and ovary, apex apiculate; pedicel and ovary ca. 3.5 mm, longitudinally slightly grooved. Flowers resupinate, not much opening, with unpleasant fishy smell. Sepals 3-6-veined, greenishyellow, without spots or stripes. Dorsal sepal oblong-ovate or narrowly ovate, 4.5-5.0  $mm \times 1.0-1.5 mm$ , 3-veined, abaxially sparsely papillate, apex shortly aristate or apiculate, often recurved. Lateral sepals obliquely oblong-ovate, 5.5-6 mm × 2.0-2.5 mm, 5–6-veined, abaxially sparsely papillate, base adnate to column foot, margins strongly involute, with the apex shortly caudate, loosely adhering along the lower edges, forming an ovoid sac. Petals pale greenish-yellow, obliquely and broadly-based triangular, apex long acuminate, tapering into a long filiform or threadlike tail, usually contorted to spirally twisted,  $2.0-2.2 \text{ mm} \times 0.8-1.0 \text{ mm}$  near the base, with a tail 2.8-3.0 mmlong, margins somewhat irregularly denticulate or erose. Lip yellowish-orange or deep golden yellow, fleshy, oblong narrowly ovoid, 2.5–2.8 mm × 1.3–1.5 mm, mobile on a thin ligament, recurved near the base, conspicuously auriculate at the base; disc with a longitudinal central groove, apex rounded or obtuse; auricles narrowly falcate, arching forward and gradually tapering to a long sharp point at the apex; densely glandular pap-

Character	B. chrysolabium	B. morphologorum	B. orientale				
Floral bract	6–6.5 mm, distinctly longer than pedicel and ovary.	4.5–5 mm, distinctly longer than pedicel and ovary.	5–6 mm, nearly as long as or slightly longer than pedicel and ovary.				
Scape	5–6 cm, rachis about 6.5 cm, longer than scape	10–13 cm, much longer than rachis	4–8 cm, rachis about the same length				
Dorsal sepal	oblong-ovate or narrowly ovate, apex shortly aristate or apiculate, 4.5–5 mm × 1.0–1.5 mm	ovate, apex acute, 6.5–7 mm × 2.5–3 mm	broadly ovate, apex acute, ca. 7.0 × 3.5 mm,				
Lateral sepals	obliquely oblong-ovate, margins strongly involute, with the apex shortly caudate, 5.5–6 mm × 2.0–2.5 mm	obliquely ovate-lanceolate, margins not involute, apex acute, 7.5–8.0 × 3.5 mm	obliquely ovate-lanceolate, margins not involute, apex acute, ca. 8.0 × 4.5 mm				
Petals	apex with long filiform apices, ca. 3.0 mm long, usually contorted to spirally twisted	apex with filiform apices, ca. 1.8 mm long, not twisted	apex with cuspidate apices, ca. 0.8 mm				
Lip	ca. 2.8 mm long, significantly glandular-papillose and ciliolate at margins	ca. 5 mm long, not papillate or papillose at margins	ca. 6 mm long, somewhat papillate at margins, not ciliolate				
Lip auricles	narrow falcate, long acuminate with a sharp point	not falcate, rather obtuse at apex	narrow falcate, apex apiculate and unequally 2- or 3-toothed				
Column callus	absent	present, conical	absent				
Stelidia	subulate and twisted, exceeding operculum	subulate but not twisted, exceeding operculum	subulate and twisted, as long as or shorter than operculum				

Table 1. Diagnostic morphological characters distinguishing B. chrysolabium from its two allied species.

illose on both sides and conspicuously ciliolate along margins, whitish. Column greenish-yellow, subcylindric, 2.0–2.2 mm tall; foot ca. 1.5 mm; stelidia well-developed and subulate, ca. 1.2 mm, often twisted inwards, equalling or slightly exceeding operculum, with a sharp point along the upper margin. Anther abaxially with a rounded crest, golden-yellow. Pollinia 4, the inner ones more than half as long as the outer ones.

**Distribution and habitat.** So far known only from Menglian County in southwest Yunnan Province, China, growing as an epiphyte amongst mosses on the tree trunk near the edge of river in rather exposed circumstances in subtropical evergreen broad-leaved forest.

**Etymology.** The specific epithet comes from the Ancient Greek word *chryso*-"golden" and the Latin derived *labium* "labellum", referring to the golden-yellow lip of the type.

Phenology. Flowering observed in December.

**Conservation status.** *Bulbophyllum chrysolabium* is known only from its type locality, where it is represented by a single population of about 30–50 individual plants or clumps. The extent of occurrence (EOO) of the species cannot be estimated and its area of occupancy (AOO) is estimated to be 4 km<sup>2</sup>, which falls within the thresholds for Critically Endangered under criterion B2. Additional individuals of this species may yet be found in adjacent areas as the investigation continues. The location is relatively inaccessible to casual hikers and the potential for habitat destruction is limited, but it is not in a national reserve or other kinds of protected areas. Fortunately, a few



**Figure 2.** *Bulbophyllum chrysolabium.* **A** Habitat **B** Inflorescences **C** Close-up of inflorescence **D** Flower, lateral view showing floral bract **E** Flower, frontal view **F** Dorsal sepal, petal and lateral sepal, abaxial view **G** Lip, ventral view **H** Column and lip, lateral view. Scale bars, 1 mm (**G**), 2 mm (**D–F, H**).

individuals were brought into cultivation at SCBG. Although there is limited data to determine its actual distribution range, biology and threats, the current available information (one location in a non-protected area and AOO less than 10 km<sup>2</sup>) led us to assign a preliminary status of Critically Endangered [CR B2ab(iii)] according to the IUCN Red List Categories and Criteria (IUCN 2017).

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**RESEARCH ARTICLE** 



# Phylogeny and taxonomic synopsis of Poa subgenus Pseudopoa (including Eremopoa and Lindbergella) (Poaceae, Poeae, Poinae)

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#### Abstract

*Eremopoa* is a small genus of annual grasses distributed from Egypt to western China. Phylogenetic analyses of plastid and nuclear ribosomal DNA show that *Eremopoa* species, together with the monotypic genus *Lindbergella* and a single species of *Poa* (*P. speluncarum*), are nested within the genus *Poa*, in a clade that we accept as *Poa* subg. *Pseudopoa*. Here we accept seven species, four subspecies and four varieties in *Poa* subg. *Pseudopoa*. Five new combinations are made: *Poa attalica, P. diaphora* var. *alpina, P. diaphora* var. *songarica, P. nephelochloides* and *P. persica* subsp. *multiradiata; P. millii* is proposed as a replacement name for *E. capillaris*; and *Poa* sections *Lindbergella* and *Speluncarae* are proposed. We provide a diagnosis for *Poa* subg. *Pseudopoa*, synonymy for and a key to the taxa. Eight lectotypes are designated: *Eragrostis barbeyi* Post, *Eremopoa nephelochloides* Roshev, *Glyceria taurica* Steud., *Nephelochloa tripolitana* Boiss. & Blanche, *Poa cilicensis* Hance, *Poa paradoxa* Kar. & Kir., *Poa persica* var. *alpina* Boiss and *Poa persica* subsp. *cypria* Sam. *Eremopoa medica* is re-identified as a species of *Puccinellia*.

#### Keywords

Annuals, classification, DNA, Eremopoa, grasses, Lindbergella, phylogeny, Poa, Poaceae, taxonomy

## Introduction

*Eremopod* Roshey, is a small, primarily west and central Asian genus of annual grasses. Roshevitz (1934) named the genus *Eremopoa* (Greek: *eremos* = desert, *poa* = fodder / > bluegrass) and included six species of annuals for the former U.S.S.R. Up to that time, one or more of the taxa had been described or treated in Aira L. (Trinius 1835), Eragrostis Wolf (Post and Autran 1897), Festuca L. (Koch 1848), Glyceria R. Br. (Fischer and Meyer 1841, Steudel 1854), Nephelochloa Boiss. (Grisebach 1852, Boissier and Blanche 1859) and Poa L. (Trinius 1830, 1836, Steudel 1854, Boissier 1884, Hackel 1887, Stapf 1897, Ascherson and Graebner 1900). Poa persica Trin. is the type species of Eremopoa, Festuca sect. Pseudopoa K. Koch, Poa subgen. Pseudopoa (K. Koch) Stapf and P. sect. Pseudopoa (K. Koch) Hack. After Eremopoa was described, most authors accepted the genus (Grossheim 1939, Köie 1945, Bor 1960, 1968a, 1970, Pavlov and Gamajunova 1964, Tzvelev 1966, 1976, 1989, Scholz 1980, 1981, Tutin 1980, Czerepanov 1981, 1995, Cope 1982, Mill 1985, Clayton and Renvoize 1986, Watson and Dallwitz 1992, Soreng 2003, Valdés and Scholz 2006, Darbyshire 2007, Cabi and Doğan 2012, Nikiforova et al. 2012). Few taxonomists continued to refer the species to Poa (Samuelsson 1950, Kovalevskaja 1968). No revision of the genus as a whole exists.

Roshevitz (1934) differentiated the genus *Eremopoa* from *Poa* as: always annuals with long panicle branches arranged in half-whorls; glumes unequal, inferior 1-veined, superior 3-veined; lemmas with obscure keel and lateral veins, apex acuminate or briefly aristate; and callus without lanate hairs. Tzvelev (1976) added the following characteristics: lower glumes 2/7-2/3 the first lemma in length; lemmas somewhat keeled with 5 veins, apex gradually tapering, sometimes with a short cusp, somewhat scabrous due to very short spinules and often pilose in the lower part along the keel and marginal veins; callus obtuse, glabrous or almost glabrous; leaf sheaths closed only at the base and leaf blades flat or loosely folded. The genus is relatively easy to recognise as a set of annuals, whereas Poa has few annuals and those are distinct from species included in *Eremopoa*. However, none of the characters by themselves actually differentiates Eremopoa from Poa. In Poa, glumes can also be short, the lower one is commonly 1-veined, the upper one normally 3-veined. Lemmas in Poa are usually distinctly keeled, with soft hairs at least on the keel and with an obtuse, acute or acuminate apex. They are rarely weakly keeled (e.g. in sect. Secundae), sometimes glabrous (ca. 15% of spp.) and rarely produce a minute cusp (a cusp occurs more often than acknowledged in the literature, but is usually irregularly expressed). In Poa, a dorsal tuft of hairs on the callus is present in 2/3 of the species. In the other species, the callus is sometimes glabrous or has a minute or more developed crown of hairs around the base of the lemma. In addition, Poa leaf sheaths are only infrequently closed at the base, most being closed more than 1/10 the length, and leaf blade form runs the gamut from flat and thin to tough and involute. Panicle branches in Poa are infrequently whorled with 6 or up to 9 branches per lower node, the normal range is 1 to 5. Although panicle branches are commonly numerous (ranging up to 27) in *Eremopoa*, with most taxa usually having over 5, E. altaica (Trin.) Roshev. has 1-5(-7) and E. songarica (Schrenk ex Fisch. & C.A. Mey.) Roshev. varies widely with (1-)3-8(-12).

*Eremopoa* species are annual with some extreme features usually not found in *Poa*, but, other than abundantly branching panicles, those characteristics are broached in all cases. No one has doubted that *Eremopoa* was closely related to *Poa*.

The taxa placed in *Eremopoa* range from Egypt (Sinai and north coast) across the northern Middle East (Israel, Lebanon, Syria, Iraq, Turkey [Anatolia], Iran), to Afghanistan, Pakistan, northwest India (Himachal Pradesh, Kashmir), western China (Tibet and Xinjiang), north through Transcaucasia into the Caucasus mountains of Russia and across central Asia in Turkmenistan, Uzbekistan, Tajikistan, Kyrgyz Republic and Kazakhstan. Two taxa have been observed elsewhere as waifs: *E. persica* in western Europe (France, Norway) and *E. altaica* (Trin.) Roshev. in Canada (see references in Taxonomy section). The geographic region with the most diversity of *Eremopoa* taxa is clearly Asia Minor; nearly all of the accepted species occur in Turkey.

There have been many differences of opinion on the species and infraspecific ranks to accept in *Eremopoa* (Table 1). Roshevitz (1934) treated six species in his new genus in the former U.S.S.R (*E. altaica, E. bellula* (Regel) Roshev., *E. oxyglumis* (Boiss.) Roshev., *E. multiradiata* (Trautv.) Roshev., *E. persica* and *E. songarica*). Tzvelev (1976) reduced these six species to two species, *E. persica* and *E. altaica*, with two and three subspecies, respectively, all of which were accepted as species by Czerepanov (1981, 1995). Scholz (1980, 1981) described two new species, *E. attalica* H. Scholz from Turkey and *E. medica* H. Scholz from Azerbaijan. The type of *E. medica* (holotype at W, isotype at B) was determined to be a species of *Puccinellia* Parl. (Soreng pers. obs. 2015). Mill (1985) treated six species in Turkey, including two new species, *E. capillaris* R.R. Mill and *E. mardinensis* R.R. Mill. Rahmanian et al. (2014) accepted four species in Iran, including *E. medica* and *E. persica* with three varieties.

Bor's genus *Lindbergella* (Bor 1968b, 1969) comprises a single annual species that is morphologically similar to *Eremopoa*. It differs from *Eremopoa* only in having firmer lemmas that are 3-veined and obscurely apiculate and panicles with 1–5 branches that are smooth. *Lindbergella sintenisii* (H. Lindb.) Bor was originally published as *Poa sintenisii* by Lindberg (1942) and also as *P. persica* var. *cypria* by Samuelsson (1950), the type of which is a syntype of *P. persica* var. *alpina* Boissier (1884). The species is endemic to Cyprus.

The first molecular data on *Eremopoa*, generated by our lab in 2004/2005, indicated that *E. songarica* was nested within *Poa*. That data was first published by Gillespie et al. (2007) using chloroplast DNA sequences from the *trnT-trnL-trnF* region. Based on this same data, inclusion of *Eremopoa* in *Poa* was already applied in the Flora of China account (Zhu et al. 2006, as *P.* subg. *Pseudopoa* (K. Koch) Stapf) and was continued in Gillespie et al. (2008, 2010), Soreng (2004+) and Soreng et al. (2010, 2015a, 2017a). Although nested within *Poa*, *Eremopoa* was positioned on a long branch separate from other *Poa* clades, justifying its recognition as a distinct subgenus, *P.* subg. *Pseudopoa* (Gillespie et al. 2007).

We published our initial DNA results for only one species of *Eremopoa* (*E. songarica*) based on *trnT-trnL-trnF* and, subsequently, nuclear ribosomal (nrDNA) ITS and ETS sequence data (Gillespie et al. 2007, 2008, 2010, Soreng et al. 2010). We subsequently sequenced two additional plastid regions (*matK* and *rpoB-trnC*) and added

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Here	whole range	Poa persica	subsp. persica	I	ıbsp. multiradiata	P. diaphora	subsp. diaphora	var. diaphora	var. songarica	(= var. <i>alpina?</i> )	var. alpina	subsp. <i>oxyglumis</i>	P. attalica	P. millii	(= <i>P. persica</i> subsp. <i>multivadiata</i> )	P. nephelochloides	(= Puccinellia sp.)	P. sintenisii	P. speluncarum
Euro+Med (on- line)	Europe, Transcaucasia, Turkey, Levant, North Africa	E. persica	1	1	E. multiradiata s1	E. altaica	subsp. altaica		subsp. songarica	1	I	subsp. oxygłumis	E. attalica	E. capillaris	E. mardinensis	1	I	I	I
Rhamanian et al. (2014)	Iran	E. persica	var. p <i>ersica</i>	I	(= <i>persica</i> var. <i>persica</i> )	I	I		var. songarica	E. bellula	I	E. persica var. oxyglumis	I	I	I	E. nephelochloides	E. medica	I	I
Gabrieljan and Oganesian (2010)	Armenia	E. persica	I	I	E. multiradiata	I	I		E. songarica	I	I	E. oxyglumis	I	I	I	I	I	I	I
Zhu et al. (2006)	China (Xinjiang, Xizang)	I	I	I	I	P. diaphora	subsp. diaphora		I	I	I	subsp. oxyglumis	I	I	I	I	I	I	I
Czerepanov (1995)	USSR	E. persica	I	I	E. multiradiata	E. altaica	I		E. songarica	(pp. = altaica, pp = songarica)	I	E. oxyglumis	I	I	I	I	I	I	I
Mill (1985)	Turkey	E. persica	I	I	E. multivadiata	I	1		E. songarica	(indirectly referenced, not accepted)	(indirectly referenced, not accepted)	(=E. songarica)	E. attalica	E. capillaris	E. mardinensis	E. nephelochloides (Iran)	I	I	I
Cope (1982)	Pakistan	E. persica	subsp. persica	I	subsp. multiradiata	E. altaica	subsp. altaica		subsp. songarica	(= altaica s.l.)	I	subsp. oxyglumis	I	I	I	I	I	I	I
Tzvelev (1976, 1983)	USSR	E. persica	subsp. persica	I	subsp. multiradiata	E. altaica	subsp. altaica		subsp. songarica	(pp. = altaica, pp = songarica)	I	subsp. oxyglumis	I	I	I	I	I	I	I
Bor (1970)	Iran, Afghanistan, w. Pakistan, n .w. Iraq, s. Turkmenistan, s.e. Azerbaijan	E. persica	var. <i>persica</i>	I	(= var. <i>songarica</i> )	I	I		var. <i>songarica</i>	E. bellula	I	(= var. songarica)	I	I	I	I	I	I	I
Roshevits (in Köie 1945)	SW Iran	E. persica	I	var. major	I	I			I	I		E. oxyglumis	I	I	I	E. nephelochloides	I	I	I
Roshevits (1934)	USSR	E. persica	1	1	E. multiradiata	E. altaica	I		E. songarica	E. bellula	P. persica var. alpina (under oxyglumis)	E. oxygłumis	I	I	1	I	I	I	I
data for *Eremopoa persica* (Cabi et al. 2017, as *Poa persica*). A DNA analysis of ITS sequence data by Hoffmann et al. (2013) showed *Lindbergella sintenisii* was also nested within *Poa* near *Eremopoa*. Since then, we have accumulated nrDNA and plastid sequence data for most of the *Eremopoa* taxa and *L. sintenisii* and sampled many more species of *Poa* from Turkey and around the world. Analysis of our accumulated phylogenetic data on *Eremopoa* is presented here. All *Eremopoa* taxa were nested well within *Poa*, and *P. speluncarum* J.R. Edm. and *L. sintenisii* were found to be nested within or sister to the set of *Eremopoa* species. Here we place these taxa in *Poa* subg. *Pseudopoa* and present a taxonomic synopsis of all the species and infraspecies, as well as a key to the taxa we currently accept. Further study is needed before a comprehensive revision of the subgenus can be produced.

### Methods

Collections of *Eremopoa* at E and G (except those not available for loan), several from P and two type specimens from BM and B were loaned to RJS at US. Other material was examined by RJS at B, K, LE, P, US and herbaria in Turkey (ANK, ISTE, NKU). Fieldwork in which 38 specimens of *Eremopoa* were collected by us was conducted in Kyrgyz Republic (RJS 2006) and Turkey (RJS and associates 1994, 2013, 2014, 2015; LJG & RJS and associates 2011; EC was a co-collector on the 2011 to 2015 expeditions). Additional material was obtained from R. Hand (*Lindbergella sintenisii*) and M. Assadi and M. Amini-Rad (Iranian *Eremopoa*).

The molecular phylogenetic analysis included 77 samples: 15 *Eremopoa*, 56 *Poa*, 1 *Lindbergella* and 5 outgroup samples (Appendix 1). A diverse set of *Poa* species was chosen to represent the majority of sections, including all sections in southwest Asia. Outgroup taxa were chosen to include representatives of the two taxa (*Phleum* L. and *Milium* L.) and one clade considered most closely related to *Poa* (Gillespie et al. 2010, Soreng et al. 2015b). Sequences of *Lindbergella* and the majority of *Eremopoa* samples, plus many *matK* and *rpoB* sequences, are new to this study (Appendix 1). For simplicity, due to the confusing taxonomy and nomenclature, we refer to *Eremopoa* taxa using names at the species level in the Results, trees and Appendix 1 (see Table 1 for their corresponding names in *Poa*). The collection *TARI 135082* was previously identified as *E. medica* (Rahmanian et al. 2014), but was re-determined by RJS as *P. persica* subsp. *persica*.

DNA was extracted from silica gel dried or herbarium leaf material as described in Gillespie et al. (2008). Three plastid markers (*matK*, *rpoB-trnC* and *trnT-trnL-trnF* [TLF]) and two nuclear ribosomal DNA (nrDNA) markers (internal transcribed spacer [ITS] and external transcribed spacer [ETS]) were sequenced. Amplification and sequencing protocols, including primers used, were described in our previous studies, as follows: ITS and TLF (Gillespie et al. 2008); ETS (Gillespie et al. 2009, 2010); *matK* and *rpoB-trnC* (Soreng et al. 2015b). Sequences were assembled, edited, aligned and concatenated using Geneious ver. 6.1.5 (http://www.geneious.com). The MAFFT ver. 7.017 plugin (Katoh and Standley 2013) was used for alignment, followed by manual adjustment. All samples are complete for all markers, except for several samples with missing ends. The molecular study was conducted at the Canadian Museum of Nature; sequencing was mostly performed by NA, analyses by LJG.

Maximum parsimony (MP) analyses were performed in PAUP\* 4.0b10 (Swofford 2002) using the heuristic search command with default settings, including tree bisection-reconnection (TBR) swapping, saving all multiple shortest trees (Multrees) with a maximum number set to 100,000. Branch support was assessed using MP bootstrap analyses performed in PAUP\* with heuristic search strategy, 10,000 bootstrap replicates, each with ten random addition sequence replicates, saving ten trees per replicate.

Bayesian Markov chain Monte Carlo analyses were conducted in MrBayes (Ronquist et al. 2011). Optimal models of molecular evolution for individual markers were first determined using the Akaike information criterion (AIC; Akaike 1974) conducted through likelihood searches in jModeltest with default settings (Darriba et al. 2012). Models were set at GTR +  $\Gamma$  for ITS, ETS and *rpoB-trnC* partitions and GTR + I +  $\Gamma$ for *matK* and TLF partitions based on the AIC scores and the models allowed in Mr-Bayes. Two independent runs of four chained searches were performed for either two or three million generations (analyses were stopped when split frequency of 0.005 was reached or closely approached), sampling every 500 generations, with default parameters. A 25% burn-in was implemented prior to summarising a 50% majority rule consensus tree and calculating Bayesian posterior probabilities (pp).

MP heuristic searches and bootstrap analyses were performed initially on the separate marker alignments. Strict consensus trees were examined for conflicting topologies with incongruence identified by branch conflicts with  $\geq$ 75% bootstrap support (BS). No supported incongruence was found between ITS and ETS trees, nor amongst the three plastid trees. Further MP and Bayesian analyses were performed on the separate concatenated nrDNA (77 samples, 1251 aligned characters) and plastid (77 samples, 4465 characters) alignments. Since supported incongruence was detected between the nrDNA and plastid strict consensus trees, species and clades determined to be incongruent were removed prior to performing analyses on the concatenated combined nrDNA and plastid alignment (68 samples, 5599 aligned characters). Trees were viewed in FigTree v1.4.0 (Rambaut 2006+). Clade designations follow Soreng et al. (2010) with modifications as in Cabi et al. (2017) and Soreng et al. (2017b), wherein well-supported major clades are assigned letters.

### Results

Plastid and nrDNA Bayesian trees are given in Fig. 1 with summary statistics in Suppl. material 1. There are 100 new sequences reported in GenBank and these are given in Appendix 1. MP trees (bootstrap values shown below branches in Fig. 1) were very similar to the Bayesian trees with a few minor unsupported differences. Major clades (shown by letter and colour in Fig. 1) are identical in both nrDNA and plastid trees,

with two exceptions: *Poa arctica* R. Br. and *P. sect. Secundae* members (*P. curtifolia* Scribn., *P. secunda* J. Presl and *P. stenantha* Trin.), each belonging to different major clades in the two trees. The position of three major clades differs significantly between the nrDNA and plastid trees: J clade (sect. *Jubatae*: *P. jubata* A. Kern.), S clade (sects. *Stenopoa* and *Abbreviatae*) and V clade (sect. *Pandemos*: *P. trivialis* L.). *Poa* major clades have been described elsewhere (Gillespie et al. 2007, 2008, 2009, Soreng et al. 2010, 2017b, Cabi et al. 2017); here we focus on the position of *Eremopoa*.

Eremopoa species, together with Lindbergella sintenisii and Poa speluncarum, form a clade (E clade) in both nrDNA and plastid trees, but are strongly supported only in the plastid analysis (pp = 1, BS = 99%). All E. multiradiata, E. oxyglumis, E. persica and E. songarica accessions form a strongly supported clade (core Eremopoa clade) in both trees (pp = 1, BS = 100%). In the plastid analysis *E. attalica*, *L. sintenisii* and *P. speluncarum* form a strongly supported clade (pp = 1, BS = 100%), with *L. sintenisii* sister to *E. attalica* (pp = 1, BS = 96%). In the nrDNA tree, *E. attalica* and *P. speluncarum* are sister taxa (pp = 0.99, BS = 77%) and *Lindbergella* is weakly supported as sister to this clade plus the core *Eremopoa* clade (pp = 0.97, BS = 59%). Within the core *Eremopoa* clade, all E. oxyglumis and E. songarica samples form a strongly supported clade in the nrDNA analysis (pp = 1, BS = 100%), whereas in the plastid analysis, these samples are divided between two strongly supported clades corresponding to *E. oxyglumis* plus one E. songarica sample (IRAN 20357, identification needs confirmation) (pp = 1, BS = 89%) and all remaining samples of *E. songarica* (pp = 1, BS = 100%). *Eremopoa* multiradiata and E. persica samples do not form a clade in either analysis, although all except one (E. persica, Soreng 9215) are strongly supported as a clade (pp = 1, BS = 95%) in the plastid tree.

The combined nrDNA and plastid Bayesian tree with proportional branch lengths is shown in Fig. 2. Prior to analysis, species and clades with positions incongruent (branch conflicts with  $\ge 75\%$  BS) between the nrDNA and plastid trees were removed, including *Lindbergella sintenisii*, *P. arctica*, *P.* sect. *Secundae* species and the **J**, **S**, and **V** clades. The **E** clade is strongly supported, as are its two subclades, *E. attalica-P. speluncarum* and the core *Eremopoa* clade (all pp = 1, BS = 100%). Both subclades are on long branches and separated by considerable genetic distance. The core *Eremopoa* clade is subdivided into two strongly supported clades: *E. multiradiata-E. persica* (pp = 0.99, BS = 96%) and *E. oxyglumis-E. songarica* (pp = 1, BS = 94%). *Eremopoa oxyglumis* and three of four accessions of *E. songarica* each form moderately or strongly supported clades (pp = 1, BS = 86%; pp = 1, BS = 100%), respectively).

In the combined nrDNA and plastid tree (Fig. 2), the **E** clade is strongly supported as sister (pp = 1, BS = 100%) to a clade comprising *Poa* supersects. *Homalopoa* (**H** clade) and *Poa* (**P** clade) and the **N** clade (*P* sect. *Nanopoa* plus unassigned species). In the nrDNA analysis, the **E** clade is strongly supported as sister to clades **P+H** (not differentiated), **N**, and **X** (represented here by *P. arctica*) (Fig. 1). In the plastid analysis, the **E** clade is sister to a larger clade comprising clades **H**, **N**, and **P**, plus **J**, **S** and **V** (Fig. 1).



**Figure 1.** *Poa* nrDNA and plastid Baysian analyses showing placement of *Eremopoa* and *Lindbergella*. Bayesian 50% majority rule consensus trees of nrDNA ITS and ETS (left) and plastid data (*trnT-trnL-trnF, matK* and *rpoB-trnC*) (right). Bayesian posterior probabilities are shown above branches, MP boot-strap values below branches. Outgroups are not shown. Major clades are indicated by colour and capital letter. Taxa shown in black belong to different major clades in plastid and nrDNA trees.

### Discussion

Our molecular analyses of plastid and nuclear ribosomal DNA strongly support the position of *Eremopoa* and *Lindbergella* within the genus *Poa*. *Eremopoa* and *Lindbergella* were united in a clade along with *Poa speluncarum* with strong support in the plastid and combined trees (weak support in the nuclear tree). We call this set the **E** clade



**Figure 2.** *Poa* combined nrDNA and plastid Baysian analysis showing placement of *Eremopoa*. Bayesian 50% majority rule consensus tree of combined nrDNA (ITS and ETS) and plastid data (*trnT-trnL-trnF*, *matK and rpoB-trnC*). Bayesian posterior probabilities are shown above branches, MP bootstrap values below branches. Major clades are indicated by colour and capital letter; outgroups are shown in black.

(Soreng et al. 2010, Cabi et al. 2017) and accept it as *Poa* subg *Pseudopoa*. In its recent usage, this subgenus was initially considered to include only *Eremopoa* (Zhu et al. 2006, Gillespie et al. 2007); here it is expanded to include *Lindbergella* and *P. speluncarum*.

Within the E clade, three taxa of southwest Turkey and Cyprus, *E. attalica, P. speluncarum* and *Lindbergella sintenisii*, are phylogenetically isolated from all the other species of *Eremopoa* sampled (the core *Eremopoa* clade). All three taxa formed a strongly supported clade in the plastid tree, while in the nuclear tree only the first two species form a clade and *L. sintenisii* is sister to this clade plus the core *Eremopoa* clade. The position of *L. sintenisii* is moderately supported as incongruent between the nuclear and plastid trees suggesting that the genus may be of hybrid origin; however, further studies are needed to confirm incongruence over lack of support.

All *Eremopoa* taxa sampled, excluding *E. attalica*, form a strongly supported clade in all trees, called here the core *Eremopoa* clade. This clade includes two strongly supported subclades in the combined nuclear-plastid tree, corresponding to *E. persica* s.l. and *E. altaica* s.l. In the first subclade, *E. multiradiata* is nested amongst *E. persica* samples, as is the sample originally determined as *E. medica* (*TARI 35082*). The *E. multiradiata* sample (*Soreng 9240*) comes from the type locality of *E. mardinensis* in SW Turkey and is a good match for that species, but we believe that *E. mardinensis* should be treated as a synonym of *E. multiradiata*. The *E. altaica* s.l. subclade in the combined tree includes a strongly supported and divergent clade of three *E. songarica* samples and a clade of *E. oxyglumis* plus one sample of *E. songarica* (identification needs confirmation). The position of *E. songarica* (tetraploid) with *E. oxyglumis* (diploid and hexaploid) is strongly supported in the combined and nuclear trees, but is weakly supported with *E. persica* (diploid) in the plastid tree. This, together with ploidy level, is suggestive of a possible hybrid origin for *E. songarica*, but this hypothesis needs to be further explored.

As noted in the introduction and Table 1, there has been no consensus on the taxonomy of *Eremopoa* species. Bor (1970, p. 49) wrote "As far as the genus *Eremopoa* Roshev. is concerned I am prepared to accept two species only: *Eremopoa persica* (Trin.) Roshev. and *E. bellula* (Regel) Roshev." He considered *E. songarica, multiradiata* and *oxyglumis* "only worthy of varietal rank" as the single taxon, *E. persica* var. *songarica*. Tzvelev (1976), Cope (1982) and Mill (1985) dismissed the *E. bellula* form as indistinct, yet it was maintained as a species by Bor (1970) and Rahmanian et al. (2014). As such, the array of taxa has been treated as a series of species, subspecies or varieties. The taxonomy proposed by Tzvelev (1976) seems the most useful for treating *E. persica* s.l. and *E. altaica* s.l.; each is treated as a separate species with subspecies. His classification, supported by molecular data, is adopted here with some minor modifications.

Here, we present a synopsis of *P*. subg. *Pseudopoa* based on our current understanding. Further herbarium and molecular study is needed before a more comprehensive revision of the subgenus can be produced. We treat all *Eremopoa* species, *Lindbergella sintenisii* and *P. speluncarum* in *P.* subg. *Pseudopoa*. We merge all *Eremopoa* taxa and *L. sintenisii* into *Poa* and treat the *Eremopoa* taxa as five species. *Poa diaphora* Trin. is the correct name for *E. altaica* within *Poa*. Two subspecies, subsp. *diaphora* and *oxyglumis* (Boiss.) Soreng & G.H. Zhu, are recognised in *P. diaphora* based in part on their mostly clear separation in the plastid analyses and morphological distinctions. Subspecies *diaphora* includes three difficult to distinguish varieties: var. *diaphora* (formerly *E.*  *altaica*), var. *alpina* and var. *songarica* (formerly *E. songarica*). *Poa persica* includes two subspecies and is clearly separated from both *P. diaphora* subspecies in the analyses. Most *Eremopoa* taxa already have names in *Poa* or the epithets used in *Eremopoa* are available in *Poa* (with one exception).

#### Taxonomy

### *Poa* subg. *Pseudopoa* (K. Koch) Stapf in J. D. Hooker, Fl. Brit. India 7(22): 337. 1897 [1896].

Festuca [unranked] Pseudopoa K. Koch, Linnaea 21(1[4]): 409. 1848. Poa sect. Pseudopoa (K. Koch) Hack., Nat. Pflanzenfam. 2(2): 73. 1887. Eremopoa Roshev., Fl. URSS 2: 429, 756. 1934. Type. Poa persica Trin. ≡ Festuca persica (Trin.) K. Koch. Lindbergia Bor, Svensk Bot. Tidskr. 62: 467, 1968 (nom. illeg. hom., non Kindb., 1897). Lindbergella Bor, Svensk Bot. Tidskr. 63: 368. 1969. Type. Poa sintenisii H. Lindb. ≡ Lindbergella sintenisii (H. Lindb.) Bor.

**Emended diagnosis.** Like species of other *Poa* subgenera, but annual (*P. speluncarum* a weak stooling perennial) and differing from other annual species of *Poa* by combination of sheath margins fused only near the base (basal sheaths fused to 16%, top sheath 4-12% [to 50% in *P. speluncarum*]), panicle branches scabrous along angles (*P. sintenisii* smooth), arranged in whorl-like groups of 5 to 27 per node (sometimes fewer in *P. diaphora* and *P. sintenisii*), sometimes the lower whorls of branches naked or with only a few sterile spikelets, flowers bisexual, glumes short (lower glume 2/7-2/3 (-3/4) the first lemma in length), 1-veined (3-veined in *P. sintenisii*), apex sharply pointed, sometimes apiculate, rachilla internodes exposed, scaberulous, callus glabrous (or with a short crown of hairs in *P. sintenisii*), lemmas membranous to subchartaceous (*P. sintenisii* chartaceous), 3-5 veined, the intermediate veins faint when present, laterally compressed, but the keel not pronounced, glabrous or keel and marginal veins short sericeous (also sericeous between the veins in *P. sintenisii*), but keel scabrous distal to the hairs.

**Distribution.** Southwest Asia from Israel, Lebanon, Cyprus and Turkey eastwards through Transcaucasia, Iran, central Asia to western China and northwest India. Sporadic elsewhere, possibly adventive on Egypt's North African coast but native east of the Red Sea, adventive in Europe and Canada.

**Notes.** A subgenus of seven species with several infraspecies, distributed mainly in semi-arid midlands to uplands (usually 300 m plus) to alpine, with winter spring / summer drought precipitation pattern, often along trails and roads, cultivated fields and pastures, around puddles, shallow springs, swales and vernal pools, snow beds, in pine/ oak forests to open grasslands and deserts, also in shallow caves, in shallow sandy or stony soils or screes of igneous or metamorphic rocks of igneous or sedimentary origin, including pumice, lava, serpentine, shale, sandstone, limestone and marble.

Key to *Poa* subgen. *Pseudopoa* taxa and other annual species of *Poa* in the coincident geographic region

Plants annual (infrequently perennial or perenniating); anthers mostly 0.2–1 mm (to 1.7 mm in the weak stemmed, stooling perennial *P. speluncarum*, to 2.8 mm in the annual species *Poa persica*).

- 1 Palea keels soft hairy, never scabrous; callus glabrous (*Poa* sect. *Micrantherae*)... 2 Palea keels scabrous at least in part (if hairy in part, then distally scabrous); callus 2 Anthers 0.2–0.5 mm long; panicle branches ascending, spikelets congested along the branches; plants light green ...... Poa infirma Kunth Anthers 0.5–1 mm long; panicle branches spreading to ascending, spikelets mod-3 Spikelets ovate; lemma keels densely villous medially, many hairs over 0.5 mm long; callus with a plicate web; anthers 0.4-0.8 mm long; panicles short (to 5 cm long), branches terete, smooth or sparsely scabrid, with 1-2 branches per node; upper culm sheath margins fused 25-35(-50)% their length; plants of vernal swales, Albania, Croatia, Greece, Bulgaria and European part of Turkey (Poa sect. Jubatae)......Poa jubata Spikelets generally lanceolate; lemma keels glabrous or sericeous, hairs less than 0.3(-0.5) mm long; callus glabrous or with a short crown of hairs; anthers 0.2-2.8 mm long; panicles short or long, branches angled, smooth or scabrous, mostly with 2 to 27 branches per node, commonly appearing whorled; upper culm sheath margins fused 4-12% their length (40-50% in P. speluncarum); plants of Cyprus, Anatolian Turkey, southwards and eastwards across Asia into China (Poa 4 Uppermost culm sheath margins fused 40-50% their length; spikelets mostly 1-flowered; lemmas glabrous; callus glabrous; anthers 1.1–1.7 long; plants feeble, stooling perennials of caves and shady cool moist places in the Taurus Mts. of Turkey (rare) (*Poa* sect. *Speluncarae*)......*Poa speluncarum* Uppermost culm sheath margins fused 4-12% their length; spikelets (1-)2 to 10-flowered; lemmas glabrous or pubescent; callus glabrous or with a minute Lemmas 3-veined, apex slightly apiculate, lemmas and paleas subcoriaceous, 5 sericeous along the keel(s) and marginal veins and between the veins; panicle branches smooth, mostly 1-5 at lower nodes; callus glabrous or with a short crown of hairs; plants endemic to Cyprus (usually on serpentine substrates) (Poa sect. Lindbergella)......Poa sintenisii

6	Panicles with 1 to 3 lower whorls of 7 or more sterile/naked or mostly sterile branches; panicles 7–20 cm long, effusely branched; lemmas 2–2.5 mm long, sericeous along the keel and marginal veins; spikelets $1-4(-6)$ -flowered
	long, effusely to sparsely branched; lemmas 1.8–4.5 mm long, glabrous or seri- ceous along the keel and marginal veins; spikelets 1–12-flowered
7	Anthers 1.1–1.5 mm long; ligules 1.5–2.5 mm long; branches 7–20 per lower whorl; spikelets 1–4(–6)-flowered; plants of Zagros Mts., Iran
	P. nephelochloides
_	Anthers 0.8–1 mm long; ligules 1–1.5 mm long; branches 7–15 per lower whorl;
	spikelets 1-3-flowered; plants of Taurus Mts., Turkey P. attalica
8	Anthers $(1.2-)1.4-2.8$ mm long; lemma apex blunt or obtuse to acutely pointed, with a broad membraneous margin ( <i>P</i> pareiag s 1)
	Anthers 0.2.1.3 mm long: lemma anex acute or parrowly acute to acuminately
_	pointed, with a narrow membranous margin (blunt or slightly pointed in <i>P. millii</i>
	but then with 13–27 branches at lower panicle nodes) 10
9	Lemmas all glabrous or rarely with a few hairs near the base of the keel or mar-
	ginal veins; spikelets $(4-)5-10(-12)$ -flowered; panicles usually $\frac{1}{4}-\frac{1}{2}$ the plant
	height; anthers 1.5–2.8 mm long P. persica subsp. multiradiata
_	Lemmas (at least of the lowest flower in a spikelet) minutely sericeous along
	the keel and marginal veins for $\frac{1}{4}-\frac{2}{3}$ the length; spikelets (2–)3–7(–9)-flowered;
	panicles usually $\frac{4}{5}-\frac{4}{3}$ the plant height; anthers (1.2–)1.4–1.8 mm long
10	And an analysis of the second
10	Anthers mostly 0.2–0.0 mm long; lemmas 1.8–4.3 mm long, apex snarply point-
	few soft hairs scattered near the base; spikelets $(1-)2-3(-5)$ -flowered; plants 2-40
	cm tall
_	Anthers 0.6–1.3 mm long; lemmas 2.3–3 mm long, apex acute and sharply point-
	ed to obtuse and blunt, at least the lowest lemma in a spikelet evenly sericeous
	(hairs ca. 0.1–0.3(–3.5) mm long, stiff, appressed) along the keel in the proximal
	$\frac{1}{4}-\frac{1}{2}$ and along the marginal veins near the base; spikelets 3-5(-9)-flowered;
	plants mostly 15–60 cm tall
11	Lemmas 3.5–4.5 mm long; panicles $(2-)3-8(-9)$ cm long, branches $1-5(-7)$ at
	lower nodes, divaricately rebranched and relatively stout, spikelets usually sparse
	and few; plants mostly 5–25(–30) cm tall
_	Lemmas $1.8-3.5(-3.8 \text{ in large specimens with many spikelets})  mm long; pani-$
	cles $2-15(-20)$ cm long, branches $(1-)3-8(-12)$ at lower nodes, divaricately re-
	branched or not, capillary to somewhat stout, spikelets sparse to crowded, few to
12	Plants low growing with dance fascicles of rahranching culms, culms 2, 6 are tall
12	with lateral inflorescences from lower culm leaves: panicles contracted to open

1.5-4 cm long, included in tuft of basal leaves or slightly exerted; lemmas 3-3.5 mm long; plants alpine...... P. diaphora subsp. diaphora var. alpina Plants low growing or taller, without fascicles of rebranching culms; culms solitary to several, mostly 10-40 cm tall, without lateral inflorescences; panicles effuse, usually more than 5 cm long, usually exerted; lemmas 1.8–3.5 mm long; plants of various habitats...... P. diaphora subsp. diaphora var. songarica 13 Spikelet pedicels mostly 2-5 mm long; panicle branches 5-18 at lower nodes, stiffly spreading, lower whorls never naked or with rudimentary spikelets; lemma apices acutely pointed; anthers 0.6–1.0(1.1) mm long ..... Spikelet pedicels mostly 5-10 mm long: panicle branches (9-)13-27 at lower nodes, slender, slightly flexuous, lower whorls sometimes with a few branches that are naked or with some rudimentary spikelets in addition to normal spikelets; lemma apices obtuse to acute, blunt or slightly pointed; anthers 0.8–1.3 mm 

## *Poa* subg. *Pseudopoa* sect. *Pseudopoa* (K. Koch) Hack., Nat. Pflanzenfam. 2(2): 73. 1887.

**Emended description.** Tufted annuals. Leaf sheaths keeled, margins fused for 4-12% their length; blades flat to convolute, surfaces scabrous. Panicles open, with (1-)3-27 branches at lower nodes, lower whorls sometimes sterile; branches ascending to widely spreading, scabrous angled, with pedicels mostly equalling or up to  $4\times$  longer than their spikelets. Spikelets 1-10-flowered; glumes unequal,  $1^{st}$  glume 1-veined,  $2^{nd}$  glume 3-veined, usually reaching to less than  $\frac{2}{3}$  the adjacent lemma; rachilla internodes terete, scabrous; callus smooth, glabrous, with a round disarticulation scar; lemmas laterally compressed, weakly keeled, glabrous or short sericeous in lower half of the keel and also along the marginal veins, between veins smooth or scabrous, glabrous (rarely sericeous), 5-veined, intermediate veins obscure to distinct, margins narrowly to broadly scarious, apex obtuse to acuminate, sometimes briefly muticus. Flowers perfect, ovaries glabrous, anthers 0.2-2.8 mm long; caryopsis 1.5-2.5 mm long, narrowly elliptical, laterally compressed, fused to the palea, solid, hilum  $\frac{1}{8}-\frac{1}{6}$  the grain in length.

### *Poa attalica* (H. Scholz) Soreng, Cabi & L.J. Gillespie, comb. nov. urn:lsid:ipni.org:names:77191831-1

Eremopoa attalica H. Scholz, Willdenowia 10(1): 33, f. 1. 1980.

**Type.** TURKEY. Antalya, "nordwestl. Antalya bei Termessos, ausgetrockneter Gebirgsbach", 300 m, 23 Jul 1979, *Kehl s.n.* (holotype: B! [B-100272775])

Distribution. Turkey (western Taurus Mts.).

**Notes.** We provisionally retain this species in sect. *Pseudopoa*, despite its divergent phylogenetic placement. The species is morphologically similar to other members of the section. As noted by Mill (1985), it is most like *Poa nephelochloides* Roshev., but the anthers are smaller. Some populations of *P. millii* approach *P. attalica* and are problematical to separate (see under *P. millii*). Further molecular study is needed to determine if the three species are closely related and if a new section is warranted.

### *Poa diaphora* Trin., Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 4,2(1): 69–70. 1836.

 Aira altaica Trin., Mém. Acad. Imp. Sci. St.-Pétersbourg Divers Savans 2: 526. 1835.
 Nephelochloa altaica (Trin.) Griseb., Fl. Ross. 4(13): 367. 1852. Poa diaphana Boiss., Fl. Orient. 5: 611. 1884, nom. inval. Eremopoa altaica (Trin.) Roshev., Fl. URSS 2: 431. 1934.

**Type.** "Sterilissimus salsuginosis deserti editi Tschujae", [1800–3000 m], July 1832, *A. Bunge* (lectotype, designated by Tzvelev 1976, pg. 480, and marked in herbarium: LE! [Trinius herbarium microform image 424-A4! p.p. Bunge 1832]; isolectotypes: LE [3 specimens: TRIN-2620.01! with original description (Trinius herbarium microform 312-A1), Trinius herbarium microform images 424-A3!, 424-A5!], K [K000789849 image!; specimen labelled "Aira altaica Trin. Altai", "Acad. St. Petrop, mis. 8br 1835" is a good match for LE type material]). See Soreng et al. (1995) for explanation of Trinius herbarium citations.

Distribution. Egypt (Sinai Peninsula) to China (Xinjiang, Xizang).

**Notes.** Separating the four forms of *Poa diaphora* s.l. treated here is often difficult. Here we choose to recognise two subspecies as divided in the molecular plastid analysis. Subspecies *diaphora* and *oxyglumis* are most easily separated by the minute anthers (0.2–0.6 mm) combined with glabrous or nearly glabrous lemmas in the former and slightly longer anthers (0.6–1.1 mm) combined with hairy lemma keels and marginal veins in the latter. The other forms, *diaphora* s.s., *songarica* and *alpina* are essentially intergrading and are here treated as varieties in subsp. *diaphora*.

The specimen K000789848 (image!) ("Al. Bunge" ex hrbr. Alexandri Lehmann, Reliquiae botanicae, original det "*Poa diaphora* Tr.") might be original material of *Aira altaica*, but RJS doubts it as it is not a good match for LE types; it is a taller plant more like K00789847 (also Reliquiae Lehmannianae), which is *Bunge* material collected 20 May 1842, in Karakum desert.

### Poa diaphora subsp. diaphora var. diaphora

Fig. 3A

Poa persica var. diaphora (Trin.) Asch. & Graebn., Syn. Mitteleur. Fl. 2: 437. 1900. Eremopoa altaica (Trin.) Roshev. subsp. altaica.



Figure 3. Poa subgenus Pseudopoa sect. Pseudopoa. A P. diaphora subsp. diaphora var. diaphora, Chu, Kyrgyz Republic (Soreng et al. 7537) B, C P. persica subsp. persica, Adiyaman, Turkey (Soreng et al. 9215) B habit
C closeup of base of plant showing keeled leaf sheaths and caniculate blades D, E P. persica subsp. multiradia-ta, Mardin, Turkey (Soreng et al. 9240) D habit E spikelet showing glabrous lemmas. Photos by R.J. Soreng.

**Distribution.** China (Xinjiang, Xizang), Kazakhstan, Kyrgyz Republic, Pakistan, Russia (Altai Mts.), Tajikistan, Turkey.

**Notes.** A single specimen recorded from Turkey (Kars Prov., *Litvinov 4790* US ex K) evidently belongs to this variety and was also cited by Mill (1985) under *E. songarica*.

*Poa diaphora* subsp. *diaphora var. alpina* (Boiss.) Soreng, Cabi & L.J. Gillespie, comb. nov. urn:lsid:ipni.org:names:77191833-1

. .

Poa persica var. alpina Boiss., Fl. Orient. 5: 610. 1884.

**Type.** TURKEY. Plantae Lyciae, ad fonts reginis alpinae montis Elmalu, 25 Jun 1860, *E. Bourgeau 271* (lectotype, **here designated**: G [G00330280 image!]; isolectotypes: G [G00380172 image!, p.p. central and right top two samples], G [G0038173 image!], K [K-000789856 image!]).

**Distribution.** Armenia, Azerbaijan, Afghanistan, Georgia, Iran, Kyrgyz Republic, Pakistan, Turkey and Turkmenistan(?).

**Notes.** This taxon, accepted as *Eremopoa bellula* by several authors (see Names of Uncertain Application below), was first recognised infraspecifically by Boissier (1884) as *Poa persica* var. *alpina*. The variety is common in the highest elevations at which the genus occurs, in the alpine of Turkey, Iran and Afghanistan to the Pamir mountains, reaching 4000 m. Further study is needed to clarify the distinction of var. *alpina* from var. *diaphora* and these from *Eremopoa bellula*, as the material placed here appears heterogenous.

Of the six syntypes of var. *alpina* cited by Boissier (*Bourgeau 271*, hab. in alpinis, montes supra Elmali Lyciae [G00380172, G0038173, G00330280, K000789856]; *Kotschy 12*, Tarus Cilicicus, 5–6000'; Prairies humides de la region alpine du Taurus, au Boulgarmden [as *12d*: G00330281, K000789851 image!]; *Balansa s.n.*, Jul-Aug 1855 [K000789857, P02358251 p.p. bottom right]; *Blanche s.n.*, Libani cacuminal; *Kotschy 477*, mons Kuh Delu Persiae australis, 10 Jun 1842 [BM000959359 image!, E!, G00308632 image!, P02358251! p.p. "fo. pygmaea" bottom left]), we select *Bourgeau 271* as the lectotype as it is typical of the form. As noted by Samuelsson (1950), the *Sintenis* syntype (mons Troodos, Cypri) represents a separate form that is treated here as *Poa sintenisii. Poa persica* var. "*minor*" Boiss. (cited by Mill, in Fl. Turkey 9: 492. 1985) is a nomen nudum since it is a herbarium name on *Bourgeau 271*, syntype of var. *alpina* Boiss.; this name is also inscribed on *Kotschy 12d* (p.p. G00308174), but the latter is original material, not a syntype, mentioned by Boissier (1884).

### Poa diaphora subsp. diaphora var. songarica (Schrenk ex Fisch. & C.A. Mey.) Soreng, Cabi & L.J. Gillespie, comb. nov.

urn:lsid:ipni.org:names:77191834-1

Glyceria songarica Schrenk ex Fisch. & C.A. Mey., Enum. Pl. Nov. 1: 1–2. 1841. Nephelochloa songarica (Schrenk ex Fisch. & C.A. Mey.) Griseb., Fl. Ross. 4(13): 367. 1852. Nephelochloa persica var. songarica (Schrenk ex Fisch. & C.A. Mey.) Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 7: 603. 1881. Poa songarica (Schrenk ex Fisch. & C.A. Mey.) Boiss., Fl. Orient. 5: 611. 1884. Poa persica var. songarica (Schrenk ex Fisch. & C.A. Mey.) Stapf, Fl. Brit. India 7(22): 337. 1897 [1896]. *Eremopoa songarica* (Schrenk ex Fisch. & C.A. Mey.) Roshev., Fl. URSS 2: 431, pl. 32, f. 11. 1934. *Eremopoa persica* var. *songarica* (Schrenk ex Fisch. & C.A. Mey.) Bor, Grass. Burma, Ceylon, India & Pakistan 532. 1960. *Eremopoa altaica* subsp. *songarica* (Schrenk ex Fisch. & C.A. Mey.) Tzvelev, Bot. Zhurn. (Moscow & Leningrad) 51(8): 1104. 1966. Poa diaphora subsp. *songarica* (Schrenk ex Fisch. & C.A. Mey.) Soreng & G.H. Zhu, Fl. China vol. 22: 266. 2006. Poa songarica var. *argaea* Hausskn. & Bornm. ex R.R. Mill, Fl. Turkey & E. Aegean Isl. 9: 491. 1985, nom. inval., as syn. of *Eremopoa songarica*.

Poa paradoxa Kar. & Kir., Bull. Soc. Imp. Naturalistes Moscou 864. 1841, nom. illeg. hom. Poa subtilis Kar. & Kir., Bull. Soc. Imp. Naturalistes Moscou 15(3): 532. 1842. nom. nov. (cited Poa paradaxa Kar. & Kir., 1941 [entry no.] 926). Type protologue. Hab. in herbosis ad rivulum Ai deserti Soongoro-Kirghisici, Jun, Karelin & Kiriloff. Type: Hab. in herbosis ad rivulum Ai deserti Soongoro-Kirghisici, Jun 1840, Karelin & Kiriloff (Herb. Fischer no. 504) (lectotype, here designated: LE!; isotypes: P [P02663383!], W [W0028251 image!]).

**Type.** Ad fl. Karatal versus montes Karatau, 13 June 1840, *H. Schrenk* s.n. (holotype: LE; isotype: LE).

**Distribution.** Afghanistan, Armenia, Azerbaijan, China (Xizang), Georgia, Iran, Israel, Kazakhstan, Kyrgyz Republic, Tajikistan, Turkey, Turkmenistan and Uzbekistan.

**Notes.** *Poa diaphora* var. *songarica* was recently recorded (as *Eremopoa songarica*; determination verified here) from one locality in northernmost Israel (Danin and Fragman-Sapir 2016+). It was collected as a waif in Canada (Manitoba) in the 1950s (Stevenson 1965, as *E. persica*; Darbyshire 2007, as *E. altaica*: re-identified here), but is apparently not persistent (Darbyshire 2007, B.A. Ford, pers. comm, 2018).

Tzvelev (1976, pg. 480) cited "In herbidis Songaria ad rivulum Tschulak [Jun 1841], *Karelin & Kiriloff 2123*" (LE!) as type of *P. subtilis* (duplicates at BM000959360 image!, K000789846 image!, BR0000006600860 image!, P02663388!, P02663405!), but the type is the one [1840] collection cited by Karelin and Kiriloff (1841) distributed as Herb. Fischer no. *504*.

#### Poa diaphora subsp. oxyglumis (Boiss.) Soreng & G.H. Zhu, Fl. China 22: 266. 2006.

Poa persica var. oxyglumis Boiss., Fl. Orient. 5: 610. 1884. Eremopoa oxyglumis (Boiss.) Roshev., Fl. URSS 2: 430, 756, pl. 32, f. 9–10. 1934. Eremopoa persica var. oxyglumis (Boiss.) Grossh., Fl. Kavkaza (ed. 2) 1: 268. 1939. Eremopoa altaica subsp. oxyglumis (Boiss.) Tzvelev, Bot. Zhurn. (Moscow & Leningrad) 51(8): 1104. 1966. Eremopoa persica var. oxyglumis (Boiss.) Rahmanian, Iran. J. Bot. 21(11): 214. 2014. nom. inval. isonym. **Type.** TURKEY. In collibus prope Baibout, 17 Jul 1963, *E. Bourgeau* (lectotype, designated by Tzvelev 1976, pg. 479: LE! [LE00009676]; isolectotypes: LE [LE00009678 image!], P [P02358146! pp a, P03142400!]).

**Distribution.** Armenia, Azerbaijan, Georgia, China (Xizang), Kyrgyz Republic, Pakistan, Tajikistan, Turkey, Turkmenistan and Uzbekistan.

**Notes.** Most accounts have recognised this taxon at one rank or another, except Mill (1985) who treated it as a synonym of *E. songarica*. Several collections were cited in the original protologue: *Tchihatcheff*, Hab. in Ponto; *Balansa*, Ponto Lazico ad Djimil [*Balansa 1549* G00308631, E, LE!, P02014318 (= subsp. oxyglumis), P02014317 (= *P. persica* subsp. *multiradiata*), US!]; *Huet*, Erzurum [G00330279, G00308633]; *E. Bourgeau*, Armenia, in collibus et agris in cultis Armeniae Turcicae ad Gumuchkhane.

### Poa millii Soreng, Cabi & L.J. Gillespie, nom. nov.

urn:lsid:ipni.org:names:60477374-2

Eremopoa capillaris R.R. Mill, Fl. Turkey & E. Aegean Isl. 9: 624, 490. 1985 (non Poa capillaris L. 1753). Eremopoa persica var. ramosissima Azn. ex R.R. Mill, Fl. Turkey & E. Aegean Isl. 9: 490. 1985, nom. inval.

**Type.** TURKEY. Adana, distr. Feke, Sencan Dere nr Gurumze, 1300 m, 30 May 1952, *P.H. Davis, Dodds & Cetic 19681* (holotype: E! [E00196495]; isotypes: BM! [BM000959355], K! [K000789852]).

Distribution. Turkey (central and eastern Taurus Mts. and adjacent ranges).

**Notes.** Morphologically *Poa millii* is intermediate between *P. persica* subsp. *persica* and *P. attalica*. However, we are not sure which of these it is actually related to or if it is a hybrid between them. The type approaches *P. persica* in having anthers 1.2–1.3 mm long and *P. attalica* in having abundant branching and sometimes having some sterile branches amongst the lower branch whorls. Much of the material of *P. millii* from further west than the type location from the Taurus Mts. has smaller anthers and is problematical to separate from *P. attalica*.

*Poa nephelochloides* (Roshev.) Soreng, Cabi & L.J. Gillespie, comb. nov. urn:lsid:ipni.org:names:60477375-2

Eremopoa nephelochloides Roshev., in Köie, M., Beitr. Fl. Sudwest Iran I. Danish Sci. Invest. Iran In K. Jessen & R. Sparck. (Eds) Danish Sci. Invest. Iran, pt. 4: 50. 1945. Eremopoa persica var. nephelochloides Roshev., nom. inval. as syn. of *E. nephelochloides*.

**Type.** IRAN. 60 km north of Dizful, 3 May 1937, *M. Köie* 475 (lectotype, here designated: C [C10016935 image!]; isolectotype: LE).

**Distribution.** Iran (Zagros Mts.).

Notes. Due to its sterile whorls of branches, this species seems very close to Poa millii and P. attalica, but may be a derivative of P. persica since it has longer anthers than the previous taxa. Roshevits cited two gatherings of Köie: "Kechwar, 700 m (3 May 1937; no. 475). Chah-Bazan, 500 m" (Kechvar is about 60 km north of Dizful). The specimen at C has the same date and collection number as Roshevits cited and was annotated by Roshevits as this taxon; we select it as the lectotype. The anthers are ca. 1.1-1.2 mm as measured from the C photo and other characters seem to match P. attalica. The anther length is given as 1.5 mm in Roshevits' diagnosis. The specimen clearly has the hyaline lemma apices of *P. persica* s.l. (in contrast to *P. diaphora*). However, these features are also present in the type of *E. capillaris* (=*P. millii*). *Poa attalica* has shorter anthers, ca. 0.8 to 1 mm, on the type (anthers not described by Scholz 1980 or Mill 1985). Poa nephelochloides and P. attalica may represent the same species, diagnosed as different from P. persica by sterile branches and from Nephelochloa orientalis Boiss. by glabrous lemmas (P. nephelochloides has pubescent lemmas). However, Poa nephelochloides and P. attalica are geographically isolated by over 1500 km and have different anther lengths.

### *Poa persica* Trin., Mém. Acad. Imp. Sci. St.-Pétersbourg, Sér. 6, Sci. Math. 1(4): 373. 1830.

Festuca persica (Trin.) K. Koch, Linnaea 21(1[4]): 410. 1848. Nephelochloa persica (Trin.) Griseb., Fl. Ross. 4(13): 366. 1852. Poa pamphylica Boiss., Diagn. Pl. Orient., ser. 1, 13: 58. 1854[1853?], nom. inval. as syn. of Poa persica. Eremopoa persica (Trin.) Roshev., Fl. URSS 2: 430, pl. 32, f. 8. 1934.

**Type.** IRAN: in collibus ad Akar-Tschai prob. Karabagh, 1400–1900 m, 27 May 1829, *Szowits 246* (lectotype, designated by Tzvelev 1976, pg. 479: LE! [photo E000327964!, TRIN-microform 434-B4!]; isolectotypes: LE [TRIN-2666.02!, TRIN-microform 434A8!, 434-B1!, 434-B2!, 434-B3!]).

**Notes.** Other original material includes: Iran, Prov. Aderbeidschan. distr. Khoi, ad Seidchadzi, 18 May 1828, *Szovits 258* (LE!, LE0009678 [image!], LE0009679, LE0009680 [image!], LE0009681 [image!], W0028250 [image!]; In apricis prov. Aderbeidschan e Karabahg, *Fischer* [herb. Fischer] (K000789867 [image!]). *Poa persica* has two major variations: subsp. *persica* with pubescent lemmas and relatively narrower panicle length to plant height ratio; and subsp. *multiflora* with glabrous lemmas and relatively greater panicle length to plant height ratio, and often more flowers per spikelet.

#### Poa persica subsp. persica

Fig. 3B, C

- *Eremopoa persica* var. *typica* Grossh., Trudy. Bot. Inst. Azerbaidzh. Fil. Akad. Nauk. S.S.S.R. 8: 268. 1939, nom. inval. *Eremopoa persica* var. *persica*. 1960.
- Poa cilicensis Hance, Ann. Sci. Nat., Bot., sér. 4, 18: 234. 1862. Type protologue. In Tauro cilicio, *Kotschy 529*. Type. In monte Tauro, aestate 1836, *Kotschy 529*, this from hb. H.F. Hance [via Reed 1887] no. 7498 (lectotype, here designated: BM! [BM000551484, right hand plant (2 left hand specimens are Poa diaphora var. songarica and are clearly excluded from Hance's description written on the sheet)]; isolectotype: P! [P02642319]).
- Glyceria taurica Steud., Syn. Pl. Glumac. 1: 286. 1854 (non *Poa taurica* E. Pojarkova, 1965, *Poa × taurica* H.N. Pojark., 1963). Type protologue. In monte Tauro, 1836, *Kotschy* (Kotschy hrbr.). Type. In monte Tauro, Aestate, 1836, *Kotschy 529* (lectotype, here designated: P! [P02642319]; isolectotype: BM [BM000551484 image!]).

**Distribution.** Armenia, Azerbaijan, Georgia, Egypt (north coast, possibly adventive), Iran, Iraq, Lebanon, Pakistan, Syria, Turkey; waif in France (introduced in wool, Marseille, *H. Roux*, P06768417!, P03370109!; RJS determination, 2015) and Norway (Greuter et al. 1984+).

**Notes.** Although Kotschy's herbarium is mainly at W, a search of the W herbarium website did not turn up *Kotschy 529* except as the genus *Arenaria* from Tauro cilicio or a *Scrophularia* from Persia. *Kotschy 528* at W is a *Poa* of the *P. bulbosa* complex from "In monte Tauro" in 1836. Presumably the earlier 1836 set was broken up and *529* ended up at BM and P. The anthers in the *G. taurica* lectotype are 1.8 mm long and the lemmas are pubescent along the keel and marginal veins.

### *Poa persica* subsp. *multiradiata* (Trautv.) Soreng, Cabi & L.J. Gillespie, comb. nov. urn:lsid:ipni.org:names:60477377-2 Fig. 3D, E

- Poa palustris var. multiradiata Trautv., Trudy Imp. S.-Peterburgsk. Bot. Sada 4: 406.
  1876. Poa multiradiata (Trautv.) Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 7: 620. 1880. Eremopoa multiradiata (Trautv.) Roshev., Fl. URSS 2: 430, t. 32. 1934.
  Eremopoa persica subsp. multiradiata (Trautv.) Tzvelev, Zlaki SSSR 479. 1976.
- Nephelochloa tripolitana Boiss. & Blanche, Diagn. Pl. Orient., ser. 2, 4: 133–134. 1859.
   Poa persica var. major Boiss., Fl. Orient. 5: 610–611. 1884. Type protologue. Hab. ad margines semitarum inter hortos ad Tripolium Syriae (*Blanche*), circa Byrouth in Libano (*Gaillardot*). Type. LEBANON. S. Tripoli, dans les bords des chemins, 16

May 1854, *Blanche* 1267 (lectotype, **here designated**: JE [JE00005064 ex herb. Gaillardot, image!]). Note. Two of the original specimens turned up in our search, *Blanche* 1267 (JE00005064 ex herb. Gaillardot) and *Gaillardot* s.n. (JE00005065 ex herb Gaillardot no. 2323 [image!]). *Blanche* in 1869 (P02530724) might also be original material, with a distribution date rather than a collection date.

- Eragrostis barbeyi Post, Bull. Herb. Boissier 5: 760–761. 1897. Type protologue. Habitat in collibus prope Midyat (Mardin), no. 38. Type. TURKEY. Midyat, Hillsides, May 1895, 38 Barbey (lectotype, here designated by Nada Sinno Saoud & RJS: BEI! (image seen by RJS!)). Note. The BEI sheet has "No. 55 38 Barbey, 1895" (55 was originally written as 54 but the 4 written over by 5).
- Eremopoa mardinensis R.R. Mill, Fl. Turkey & E. Aegean Isl. 9: 624, 488. 1985. Type. Turkey. Mardin, Mardin to Nusaybin, 8 km from Mardin, 850 m alt., shallow limestone gully, 22 May 1957, P. H. Davis & D. Hedge 28491 (holotype: E! [E00196494]).

**Type.** Armenia rossica, prope monasterium Kiptschach, 1875, *G. Raddi*. Type: Armenia rossica: prope monasterium Kiptschach in monte Alagos, Jun 1875, *G. Radde 124* (holotype: LE! [photo E00326521!]; isotypes: LE, LE, W [W19160014191 image!]).

Distribution. Armenia, Georgia, Iran, Lebanon, Pakistan, Syria and Turkey.

**Notes.** The presence of hairs on the lemmas in material treated as "*multiradiata*" is confused in the literature. Mill (1985) indicates that *E. multiradiata* and *E. persica* s.s. have lemma keels hairy in the lower  $\frac{1}{3}-\frac{1}{2}$ . We concur with Tzvelev (1976), who keyed *E. persica* subsp. *persica* as lemmas short pilose along the base of keel and marginal veins and subsp. *multiradiata* as lemmas glabrous or with a few solitary hairs.

Mill (1985) distinguished his new species *Eremopoa mardinensis* from *E. multiradiata* based on its glabrous lemmas, 8–12-flowered spikelets and florets strongly divergent from the rachilla. However, subsp. *multiradiata* also has glabrous lemmas (as noted above) and divergent florets (when spikelets are in flower) and its (4)5–9(10)-flowered spikelets overlap in number; therefore, we treat *E. mardinensis* as a synonym of *E. multiradiata*. The type material of *Eragrostis barbeyi* is from the same place as *E. mardinensis* and is clearly the same form (spikelets many-flowered); *Nephelochloa tripolitana*, with ca. 12–14-flowered spikelets, also appears to belong to this form. If *E. mardinensis* were accepted as a species, the basionym names *Eragrostis barbeyi* or *Nephelochloa tripolitana* would have priority.

### *Poa* subg. *Pseudopoa* sect. *Speluncarae* Soreng, Cabi & L.J. Gillespie, sect. nov. urn:lsid:ipni.org:names:60477378-2

#### **Type.** *Poa speluncarum* J.R. Edm.

**Diagnosis.** Differing from *Poa* sect. *Pseudopoa* in being perennial and stooling, with top culm sheath margins fused 40–50% their length and from almost all *Poa* in proximal spikelets being 1-flowered.

#### Poa speluncarum J.R. Edm., Fl. Turkey & E. Aegean Isl. 9: 623. 473. 1985.

**Type.** TURKEY. C4, Konya, distr. Ermenek, Kamis Dere between Ermenek and Oyuklu Dag., floor of caverns, 1400–1500 m, 14 Aug 1949, *P. H. Davis 16180* (holotype: K! [K000641325]; isotype: E! [E00367874]).

Distribution. Turkey (central Taurus Mts.).

**Notes.** *Poa speluncarum* was described by Edmondson (1985) as an annual species of *Poa* sect. *Ochlopoa* Asch. & Graebn ( $\equiv$  *Poa* sect. *Micrantherae* Stapf. Type: *Poa annua*). Our investigation found it to be a feeble, stooling perennial with sparsely scabrous panicle branches, uppermost sheaths closed up to half their length, spikelets sparsely scaberulous, mostly 1-flowered, the distal-most ones frequently 2(–3) flowered, anthers 1.1–1.7 mm, caryopsis 1.7–1.8 mm long, hilum 0.3 mm long and grain adherent to the palea. DNA data have clearly placed it in the *Poa* clade that includes *Eremopoa* species (E clade), either as sister to *P. attalica* (nuclear data) or as sister to *P. attalica* + *P. sintenisii* (plastid data). The species is odd in subgenus *Pseudopoa* for its perennial habit (albeit weak) and more closed sheaths, and in *Poa* generally by its mostly uniflorous spikelets. It is a very rare species that lives in the backs of shallow, moist, cool caves in the Taurus Mts., along with other cave endemics.

*Poa* subg. *Pseudopoa* sect. *Lindbergella* (Bor) Soreng, Cabi & L.J. Gillespie, sect. nov. urn:lsid:ipni.org:names:60477379-2

*Lindbergia* Bor, Svensk Bot. Tidskr. 62: 467, 1968 (nom. illeg. hom., non Kindb., 1897). *Lindbergella* Bor, Svensk Bot. Tidskr. 63: 368. 1969.

**Type.** *Poa sintenisii* H. Lindb. ≡ *Lindbergella sintenisii* (H. Lindb.) Bor.

**Diagnosis.** Differing from *Poa* sect. *Pseudopoa* in: panicle branches smooth; lower glume 3-veined, up to 3/4 as long as the lower lemma; lemmas 3-veined, relatively firm, sericeous on keel marginal veins and sides; callus with short crown of hairs, the hairs 0.2 mm long; and palea keels sericeous in part.

# *Poa sintenisii* H. Lindb., Årsbok-Vuosik. Soc. Sci. Fenn. 20 B (7): 5. 1942 (emend. Lindberg 1946).

- Lindbergia sintenisii (H. Lindb.) Bor, Svensk Bot. Tidskr. 62: 467. 1968. Lindbergella sintenisii (H. Lindb.) Bor, Fl. Cyprus 63: 368. 1969.
- Poa persica subsp. cypria Sam., Ark. Bot., n.s. 1(9): 417. 1950 [1951]. Type. CYPRUS. auf dem Troodos, 20 Jun 1880, *P. Sintenis 881* (lectotype, here designated: S; isolectotypes: B [B 10 0365891!], LD [LD1808162 image!, LD1808226 image!], G?, K [K000789835 image!, K000789836 image!, K000789837 image!], W [W0012225 image!, W0033518 image!, W00096518 image!, W0019026 image!]).

Type protologue. CYPRUS. In pineto (*P. pallasiana*) in m. Troodos lecta est. 1939.
Type. CYPRUS. Troodos in pineto juxta via huad procul ab "Olympus Camp Hotel", 22 Jun 1939, *H. Lindberg s.n.* (holotype: S [S-11-34137 image!]; isotypes: S [S-G-4941 image!], K [K000789839 image!], LD [LD1807330 image!], W [image!]).
Distribution. Cyprus (Mt. Troodos, endemic to serpentine rocks).

Names of uncertain application within Poa subgen. Pseudopoa

### Festuca bellula Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada 7: 594. 1881. Eremopoa bellula (Regel) Roshev., Fl. URSS 2: 431, pl. 32, f. 12. 1934.

**Type protologue.** Ad fontes calidos Araschan Bulak in Turkestania occidentali, *Krause* s.n. **Type**: Taschkenter Alatau, Araschan Bulak, 11 Jun 1871, (*Hieronymous*) *Krause* s.n. (holotype: LE [only one collection cited]).

**Notes.** Eremopoa bellula was applied by several authors to small densely tufted alpine annual plants of south-central and southwest Asia, which we recognise as *P. diaphora* var. *alpina* (based on *Poa persica* var. *alpina* Boissier [1884]). Tzvelev (1976, pg. 480) noted that the holotype collection of *E. bellula* appeared to be a mix of *altaica* (*diaphora*) and *songarica* forms ("p.p. max" = *E. altaica* subsp. *songarica*, somewhat intermediate between this subsp. and subsp. *altaica*, and "p.p. minor" = *E. altaica* subsp. *altaica*); he considered *E. bellula* to be a synonym of *E. altaica* subsp. *songarica*. Further study is needed to clarify the placement of *Eremopoa bellula* and determine if it is synonymous with *P. diaphora* var. *alpina*.

### *Eremopoa glareosa* Gamajun., Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Kazahsk. SSR 2: 2. 1964.

**Type protologue.** Usbekistanica, Tian Schan Occid., Bostandyk, fonts Aksar-sai, 28 Jul 1949, *N. V. Pavlov* s.n. (holotype: AA).

**Notes.** Tzvelev (1976, pg. 480) included *E. glareosa* as a synonym under *E. altaica* subsp. *songarica*, but noted that it is somewhat intermediate between this taxon and *E. altaica* subsp. *altaica*. As the protologue indicates the plants are 10–28 cm tall, with 3 to 4 florests per spikelet, spikelets 4–7 mm long and anthers 2.5 mm long, this is more likely to be *Poa persica*, perhaps subsp. *multiradiata*, since no pubescence is indicated.

### *Festuca heptantha* K. Koch, Linnaea 21(3): 410. 1848. *Poa heptantha* (K. Koch) Steud., Syn. Pl. Glumac. 1: 255. 1854.

**Type protologue.** Im Hochgebirge, auf sumpfigen Wiesen, auf Urgestein, 5500 ft, *C. Koch* s.n. (holotype: B, probably destroyed).

Note. There is no location in the species protologue beyond the article title "Beitrage zu einer Flora des Orients". Tzvelev (1976) indicated this name and the next, *Festuca polygama*, probably apply to *Eremopoa persica* and that the types of these were in Berlin (B). Clayton et al. 2002+ (GrassBase) reflect the same information. RJS was unable to locate type material of either of these two names at B, P or via internet searches.

### Festuca polygama K. Koch, Linnaea 21: 409. 1848. Poa polygama (K. Koch) Steud., Syn. Pl. Glumac. 1: 255. 1854.

**Type protologue.** "Aus dem Wilhelm'schen Herbr als *Poa persica.*" **Type**: *Wilhelms* (holotype: B, probably destroyed).

**Notes.** Tzvelev (1976) indicates "Caucasus?", but there is no location in the species protologue beyond the article title "Beitrage zu einer Flora des Orients".

### Excluded names

### Eremopoa medica H. Scholz, Willdenowia 11(1): 96. 1981.

Type. Persia, Prov. Azerbaijan occid.: In pratis paludosis SE Shahpur versus lacum Rezaiyeh (Urmia), 1300 m; 12 Jun 1971, *Rechinger 41820* (holotype: W [W1972-0000975 image!; isotypes: B! [B 10\_0272774], GZU [GZU000201751 image!], WU [WU0033125 image!]).

**Notes.** The type collection of *Eremopoa medica* is clearly a perennial species of *Puccinellia* (possibly *P. gigantea* (Grossh.) Grossh.) with lemmas rounded on the back, a distinct short crown of callus hairs and papillae common on vegetative structures (pedicels and leaves). Material cited as *E. medica* in Rahmanian et al. (2014, fig. 5) appears to us to be *Poa persica* subsp. *persica*; their description and illustration indicate an annual habit, pubescent lemmas and panicles with 10 or more branches per whorl. The single specimen (TARI 35082) cited was included in our molecular analysis and formed a clade with other *P. persica* accessions in all trees.

Invalid names, not vouchered

# *Festuca amherstiana* Nees, Ill. Bot. Himal. Mts. 417. 1839, nom. nud., name in list, no voucher.

**Notes.** Kew GrassBase (Clayton et al. 2002+) indicates it is equal to *E. persica*. The specimen K00078950 (ex P) (image!), *Voyage V. Jacquemont aux Indes orient. no. 1902*, has this name on the label. The specimen is certainly *P. diaphora*, not *P. persica*.

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Appendix I

(nr) and section. Voucher information (herbarium indicated in parentheses) and country of origin are provided; where there is no collector or collector number, the Table A1. Eremopoa, Lindbergella, Poa and outgroup samples used in the phylogenetic analyses. Ingroup samples are arranged by plastid clade (pl), nuclear clade herbarium specimen number is given. GenBank Accession numbers are provided for ITS, ETS, trnT-trnL-trnF, matK and rpoB-trnC sequences for each sample; those in BOLD are new to this study.

rpoB-trnC	KM524001	KY378827	KY378842	KM524014	MH921369	KY378829	MH921370	KY378852	MH921379	MK060117	MH921355	MH921356	MH921358	MH921359	MH921360	MH921357	MH921363	MH921362	KY378845	MH921361	MH921364	MH921366	MH921365	KY378834
matK	KM523888	KY378861	KY378876	KM523901	MH921344	KY378863	MH921345	KY378886	MH921354	MH921342	MH921329	MH921330	MH921332	MH921333	MH921334	MH921331	MH921337	MH921336	KY378879	MH921335	MH921338	MH921340	MH921339	KV378868
TLF	DQ353985.2	GQ324402	GQ324432.2	KM524088	KX118751	AH015557.3	GQ324404	KX118766	KX118768	MH921393	MH921380	MH921381	MH921383	MH921384	MH921385	MH921382	MH921388	MH921387	KY378816	MH921386	MH921389	MH921391	MH921390	DO353988.2
ETS	GQ324287	GQ324295	GQ324346	KM523729	KX118716	GQ324297.2	GQ324298	KX118731	KX118732	MH921310	MH921297	MH921298	MH921300	MH921301	MH921302	MH921299	MH921305	MH921304	KY378823	MH921303	MH921306	MH921308	MH921307	GO324311
STI	GQ324483	GQ324490	GQ324522	KM523802	KX118734	EU792388	GQ324492	KX118748	KX118750	MH921326	MH921313	MH921314	MH921316	MH921317	MH921318	MH921315	MH921321	MH921320	KY378812	MH921319	MH921322	MH921324	MH921323	FI 1792400
Country	USA, Colorado	Bulgaria	Spain	Turkey	Iran	Spain	USA, Nevada (introd.)	Turkey	Greece	Cyprus	Turkey	Turkey	Turkey	Turkey	Turkey	Turkey	Iran	Iran	Turkey	Iran	Iran	Iran	Iran	Turkev
Voucher	Gillespie 6299 (CAN)	Hajkova et al. 2004-12 (US)	(JACA 166095)	Gillespie et al. 10400 (CAN)	Gauba (IRAN 21237)	Catalan 13-2000 (UZ)	Soreng & Soreng 5814 (US)	Soreng & Cabi 9249 (US)	Soreng et al. 7509-1 (US)	Hand 6102 (US)	Gillespie et al. 10612 (CAN)	Soreng & Cabi 9240 (US)	Gillespie & Levin 10313 (CAN)	Gillespie et al. 10578 (CAN)	Gillespie et al. 10584 (CAN)	Soreng & Cabi 8855 (US)	Assadi & Vosoughi (TARI 24939)	Mozaffarian (TARI 53671)	Soreng & Cabi 9215 (US)	Yazdanfard (IRAN 51968)	Mozaffarian & Nowrozi (TARI 35082)	Assadi & Mozaffarian (TARI 36867)	Iranshahr (IRAN 20357)	Sareno & Günev 4165 (UJS)
Taxon	Poa alpina L.	Poa badensis Haenke ex Willd.	Poa ligulata Boiss.	Poa thessala Boiss. & Orph.	<i>Poa bactriana</i> subsp. <i>glabriflora</i> (Roshev.) Tzvelev	Poa bulbosa L.	<i>Poa bulbosa</i> subsp. <i>vivipara</i> (Koeler) Arcang.	Poa sinaica Steud. subsp. sinaica	Poa timoleontis Heldr. ex Boiss.	Lindbergella sintenisii (H. Lindb.) Bor	Eremopoa attalica H. Scholz	Eremopoa multiradiata (Trautv.) Roshev.	Eremopoa oxyglumis (Boiss.) Roshev.	Eremopoa oxyglumis	Eremopoa oxyglumis	Eremopoa oxyglumis	Eremopoa persica (Trin.) Roshev.	Eremopoa persica	Eremopoa persica	Eremopoa persica	Eremopoa persica	<i>Eremopoa songarica</i> (Schrenk ex Fisch. & C.A. Mey.) Roshev.	Eremopoa songarica	Evenapod songarica
Section	Alpinae	Alpinae	Alpinae	Alpinae	Arenariae	Arenariae	Arenariae	Arenariae	Arenariae	Lindbergella	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopoa	Pseudopod
pl nr	AA	AA	AA	AA	AA	A A	A A	AA	AA	ы ы	ы ы	ЕE	ы ы	ы ы	ы ы	ы ы	ы ы	Е	Е	ы ы	Е	Е Е	н Ш	н

pl n	tr Section	Taxon	Voucher	Country	SLI	ETS	TLF	matK	rpoB-trnC
н Ш	Pseudopoa	Eremopoa songarica	Soreng & Cabi 9320 (US)	Turkey	MH921325	MH921309	MH921392	MH921341	MH921367
ΕE	Speluncame	Poa speluncarum J.R. Edm.	Soreng et al. 8202 (US)	Turkey	MH921328	MH921312	MH921395	MH921353	MH921378
H P+	H unclassified	Poa pseudobulbosa Bor	Soreng et al. 8246 (US)	Turkey	KX118747	KX118729	KX118765	MH921352	MH921377
H P+	H Acutifoliae	Poa planifolia Kuntze	Peterson et al. 19233 (US)	Argentina	KM523800	KM523727	KM524087	KM523896	KM524009
H P+	H Brizoides	Poa poiformis (Labill.) Druce	Gillespie et al. 7381 (CAN)	Australia	GQ324534	GQ324361	GQ324445	KM523897	KM524010
H P+	H Homalopoa s.l.	Poa reflexa Vasey & Scribn.	Soreng 7422 (US)	USA Colorado	GQ324543	KX118730	GQ324450	KY378882	KY378848
H P+	H Homalopoa	Poa asiae-minoris H. Scholz & Byfield	Soreng et al. 8100 (US)	Turkey	MH921327	MH921311	MH921394	MH921343	MH921368
H P+	H Homalopoa	Poa chaixii Vill.	Soreng 4677 (US)	Russia	EU792404	GQ324299	EU854590	KM523890	KM524003
H P+	H Homalopoa	Poa chaixii	Soreng 7524 (US)	Germany	GQ324493	GQ324300	GQ324405	MH921346	MH921371
H P+	H Homalopoa	Poa masendarana Freyn & Sint.	Assadi (TARI 73254)	Iran	KX118743	KX118725	KX118761	MH921351	MH921376
H P+	H Homalopoa	Poa occidentalis Vasey	Peterson & Valdes Rena 18918 (US)	Mexico	KU756540	KU763436	KU763514	KY378877	KY378843
H P+	H Homalopoa	<i>Poa remota</i> Forselles	Soreng et al. 7540 (US)	Kyrgyz Republic	GQ324545	GQ324372	GQ324452	KY378883	KY378849
H P+	H Madropoa	Poa fendleriana (Steud.) Vasey	Gillespie 6292 (CAN)	USA, Colorado	EU792403	GQ324319	DQ354027	KY378869	KY378835
H P+	H (supersect. Homalopoa)	Poa calycina (J. Presl) Kunth	Peterson et al. 17923 (US)	Peru	EU792425	KU763395	EU792467	KY378864	KY378830
H P+	H unclassified (supersect. <i>Homalopoa</i> )	Poa marshallii Tovar	Peterson et al. 21546 (US)	Peru	KM523799	KM523726	KM524086	KM523895	KM524008
ĺ	Jubatae	Poa jubata A. Kern.	Soreng et al. 9029-2 (US)	Turkey	KY378810	KY378820	KY378814	KY378873	KY378839
<u> </u>	Jubatae	Poa jubata	Soreng et al. 9266 (US)	Turkey	KY378811	KY378821	KY378815	KY378874	KY378840
M	Micranthera	Poa infirma Kunth	Catalan 3-2000 (UZ)	Spain	GQ324516	GQ324334	GQ324427	KY378871	KY378837
M M	Micranthera	Poa supina Schrad.	Soreng & Cayouette 5950-2 (US)	USA, cult. (from Europe)	EU792387	GQ324383	DQ353984	KY378888	KY378854
Z Z	Nanopoa	Poa trichophylla Heldt. & Sart. ex Boiss.	Soreng et al. 7508 (US)	Greece	GQ324554	GQ324386	GQ324461	KY378889	KY378855
Z Z	unclassified	Poa dolosa Boiss. & Heldr.	Soreng et al. 7495-1 (US)	Greece	GQ324502	GQ324312	GQ324414	KM523891	KM524004.2
Z Z	unclassified	Poa iconia var. pelasgis (H. Scholz) Soreng	Gillespie et al. 10492 (CAN)	Turkey	KX118744	KX118726	KX118762	MH898827	MH898844
Z Z	unclassified	Poa ursina Velen.	Stoneberg SH17 (US)	Bulgaria	GQ324527	GQ324352	GQ324437	KY378892	KY378858
N	Secundae	Poa curtifolia Scribn.	Soreng & Soreng 6347c-1 (US)	USA, Washington	EU792394	KY378819	DQ353994.2	KY378867	KY378833
N S	Secundae	Poa secunda J. Presl. subsp. secunda	Soreng & Soreng 5812 (US)	USA, Nevada	EU792393	KU763450	DQ353991	KY378884	KY378850

pl nr	Section	Taxon	Voucher	Country	STI	ETS	TLF	matK	rpoB-trnC
N S	Secundae	Poa stenantha Trin.	Soreng & Soreng 6068-1 (US)	USA, Alaska	KU756554	KU763455	DQ354057.2	KY378887	KY378853
H+d d	Macropoa	Poa densa Troitsky	Soreng & Cabi 9306 (US)	Turkey	KX118738	KX118720	KX118755	MH921347	MH921372
H+d d	Macropoa	Poa bucharica Roshev.	Soreng et al. 7662 (US)	Kyrgyz Republic	KX118735	KX118717	KX118752	KY378862	KY378828
P+q q	Macropoa	Poa diversifolia (Boiss. & Balansa) Hack. ex Boiss.	Gillespie et al. 10529 (CAN)	Turkey	KX118739	KX118721	KX118756	MH921348	MH921373
P+q d	Macropoa	Poa <i>iberica</i> Fisch. & C.A. Mey.	Soreng et al. 7977 (US)	Russia, Cabardino- Balkaria	KX118741	KX118723	KX118758	MH921349	MH921374
P+A q	Macropoa	Poa longifolia Trin. subsp. longifolia	Soreng et al. 7945 (US)	Russia, Cabardino- Balkaria	KX118742	KX118724	KX118760	MH921350	MH921375
H+d d	Macropoa	Poa sibirica Roshev. subsp. sibirica	Olonova 2003-45 (CAN)	Russia, Khakasia	GQ324547	KY378824	GQ324455	KY378885	KY378851
P+H d	Poa	Poa irkutica Roshev.	Kasanovskiy 2002-7 (CAN)	Russia, Irkutsk	EU792402	GQ324335	DQ354007.2	KY378872	KY378838
H+d d	Poa	Poa pratensis L. subsp. pratensis	Gillespie et al. 10592 (CAN)	Turkey	KX118746	KX118726	KX118764	KY378880	KY378846
P X	Malacanthae	Poa arctica R. Br. subsp. arctica	Gillespie & Aiken 5701 (CAN)	Canada, Nunavut	GQ324487	GQ324291	DQ354009	KY378860	KY378826
R R	Parodiochloa	<i>Poa cookii</i> (Hook.f.) Hook.f.	Hennion Gen1 (P)	Subantarctic Islands, Crozet I.	EU792383	GQ324306	EU792454	KY378866	KY378832
R R	Parodiochloa	Poa flabellata (Lam.) Raspail	Wright 9NSG (not vouchered)	South Georgia Islands	EU792381	GQ324321	EU792453	KM523892	KM524005
s S	Abbreviatae	Poa flexuosa Sm. subsp. flexuosa	Brochmann 2000-3-1 (CAN)	Norway	GQ324520	GQ324342	GQ324418	KY378875	KY378841
s S	Abbreviatae	Poa pseudoabbreviata Roshev.	Soreng & Soreng 6032-1 (US)	USA, Alaska	EU792398	GQ324370	DQ353997	KY378881	KY378847
s s	Stenopoa	Poa biebersteinii H.N. Pojark. (cf)	Gillespie & Cabi 10327 (CAN)	Turkey	KY944706	KY944668	KY987089	KY944622	KY987044
S S	Stenopoa	Poa glauca Vahl	Gillespie 5804 (CAN)	Canada, Nunavut	AY237839	GQ324324	GQ324421	KY378870	KY378836
S S	Stenopoa	Poa palustris L.	Gillespie 6461 (CAN)	Canada, Ontario	EU792396	KY378822	DQ354000	KY378878	KY378844
s s	Tichopoa	Poa compressa L.	Gillespie 6457 (CAN)	Canada, Quebec	EU792395	KY378818	DQ354003	KY378865	KY378831
>	Pandemos	Poa trivialis L. subsp. trivialis	Soreng 4681-1 (US)	USA, Maryland (introd.)	GQ324555	GQ324387	GQ324462	KY378891	KY378857
>	Pandemos	Poa trivialis subsp. sylvicola (Guss.) H. Lindb.	Gillespie et al. 10368 (CAN)	Turkey	KY378813	KY378825	KY378817	KY378890	KY378856
YY	Sylvestres	Poa autumnalis Elliott	Soreng 4680 (US)	USA, Maryland	EU792379	GQ324294	DQ353979	KM523889	KM524002
Y Y	Sylvestres	Poa saltuensis Fernald & Wiegand	Gillespie 7043 (CAN)	Canada, Ontario	EU792378	GQ324374	EU792451	KM523899	KM524012
YY	Sylvestres	Poa wolfii Scribn.	Soreng & Soreng 5800 (US)	USA, Missouri	EU792377	GQ324389.2	AH015556.2	KY378893	KY378859
	outgroup	Arctagrostis latifolia (R. Br.) Griseb.	Gillespie et al. 6586 (CAN)	Canada, Nunavut	EU792351	GQ324245	DQ353969	KM523924	KM523954
	outgroup	Milium effusum L.	Soreng 7771 (US)	Sweden	KM523785	KM523711	KM524072	KM523870	KM523983
	outgroup	<i>Nicoraepoa andina</i> (Trin.) Soreng & L.J. Gillespie	Soreng & Soreng 7182 (US)	Chile	EU792354	GQ324275	DQ353971	KM523874	KM523987
	outgroup	Phleum montanum K. Koch	Gillespie et al. 10614-2 (CAN)	Turkey	KM523793	KM523720	KM524081	KM523883	KM523996
	outgroup	Phleum pratense L.	Soreng 7943 (US)	Russia, Stavropol	KM523796	KM523723	KM524084	KM523886	KM523999

### Supplementary material I

### Table S1. Characteristics of the DNA alignments and data partitions and parameters and summary statistics of the PAUP and Bayesian analyses

Authors: Lynn J. Gillespie, Robert John Soreng, Evren Cabi, Neda Amiri Data type: (measurement/occurence/multimedia/etc.)

- Explanation note: Five DNA sequence alignments for *Poa* were analysed: ETS, ITS, *matK, rpoB-trnC* and *trnT-trnL-trnF* (TLF). For each data partition (five individual markers, plastid, nuclear and combined), the number of samples and the total number of aligned characters are given. For the PAUP analyses, the following statistics are given: the number of parsimony informative (PI) characters, percentage of characters that are parsimonious trees, consistency index excluding uninformative characters (CI) and retention index (RI). Parameters used and statistics of the Bayesian analyses, as determined by the Akaike Information Criterion (AIC) implemented in jModeltest, are given as follows: likelihood score (-InL), number of substitution schemes, substitution rates (rAC, rAG, rAT, rCG, rCT, rGT), character state frequencies (fA, fC, fG, fT), substitution model, proportion of invariable sites and gamma shape parameter.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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**RESEARCH ARTICLE** 



### A sticky and heavily armed new species of Solanum (Solanum subg. Leptostemonum, Solanaceae) from eastern Brazil

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#### Abstract

We describe a new species of spiny *Solanum* (*Solanum* subg. *Leptostemonum*), endemic to the Brazilian Atlantic Forest and associated with granitic outcrops (inselbergs or sugar loaf mountains). *Solanum kollastrum* Gouvêa & Giacomin, **sp. nov.** is morphologically similar to the poorly known *S. sublentum* Hiern, but is a heavily armed, much more robust plant with stellate-glandular indumentum. Together with *S. sublentum*, it is morphologically related to some species of *Solanum* such as *S. hexandrum* Vell., *S. robustum* H.Wendl., and *S. stagnale* Moric. that share strongly accrescent calyces, large leaves with the bases decurrent on to the petiole, pendent simple inflorescences and large, robust flowers. The new species is restricted to a few known populations in southern Bahia and north-eastern Minas Gerais states and conservation efforts are needed.

#### Resumo

Neste trabalho descrevemos uma nova espécie aculeada de *Solanum (Solanum* subg. *Leptostemonum)* endêmica da Floresta Atlântica brasileira, associada a afloramentos graníticos (inselbergs ou páes de açúcar). *Solanum kollastrum* Gouvêa & Giacomin, **sp. nov.** é morfologicamente semelhante a *S. sublentum* Hiern, mas difere desta pelo hábito mais robusto e pelos ramos fortemente armados e recobertos por tricomas estrelados glandulares. Juntamente com *S. sublentum, S. kollastrum* é morfologicamente relacionada com algumas espécies sul-americanas que possuem cálices frutíferos fortemente acrescentes, folhas

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grandes com a base da lâmina decurrente no pecíolo, inflorescências pendentes simples e flores robustas, como *S. hexandrum* Vell., *S. robustum* H.Wendl. e *S. stagnale* Moric.. A espécie possui distribuição restrita ao sul da Bahia e nordeste de Minas Gerais, com poucas populações conhecidas, e necessita de esforços para conservação.

#### **Keywords**

Neotropics, South America, Brazilian flora, spiny Solanum, new species, inselbergs, restinga, taxonomy

#### Palavras-chave

Neotrópico, América do Sul, flora brasileira, Solanum "espinhoso", espécie nova, inselbergs, restinga, taxonomia

### Introduction

*Solanum* L., with about 1,400 species, is the most species-rich genus of the economically important Solanaceae family, in addition to being amongst the largest genera of flowering plants (Frodin 2004; Hawkes 1999). The distribution range of *Solanum* is proportional to its species richness: species belonging to the genus occur in all continents but Antarctica, with the highest diversity being found in tropical and subtropical regions of South America (Knapp 2002; Dupin et al. 2016). Phylogenetic studies have recovered the major lineages within *Solanum*, with the prickly species that possess stellate trichomes composing the largest of them, a monophyletic group known as the Leptostemonum clade or *Solanum* subg. *Leptostemonum* Bitter (Bohs 2005, Levin et al. 2006, Weese and Bohs 2007, Särkinen et al. 2013). The 'spiny solanums', as the group is informally named, comprises two major groups: the Old World lineage, which is particularly diversified in Australia, eastern Africa (incl. Madagascar) and tropical Asia and the highly diverse New World grade, that includes some Torva and Lasiocarpa clade representatives native to both the New and Old Worlds (Stern et al. 2011; Vorontsova et al. 2013; Aubriot et al. 2016).

Brazil, especially the eastern portion of its territory, is one of the primary centres of diversity and endemism for both non-spiny (Knapp 2002) and spiny solanums (Whalen 1984) in the New World. With approximately 110 species of spiny solanums (Agra 2007; BFG 2015) and 10 of the 13 New World lineages recovered in Stern et al. (2011), the Brazilian *Solanum* flora is exceedingly diverse. Ongoing efforts to document and describe the diversity of *Solanum* in the country through modern taxonomy and intensive fieldwork efforts (i.e. Flora do Brasil 2020 project; http://floradobrasil. jbrj.gov.br/, also see BFG 2015) have shed light on the taxonomy of endemic groups (e.g. Asterophorum clade, Gouvêa and Stehmann in press.; Inornatum clade, Giacomin 2015) and led to the discovery of various undescribed species (e.g. Giacomin and Stehmann 2014; Knapp et al. 2015; Gouvêa and Stehmann 2016; Agra and Stehmann 2016). Here we describe a new species of spiny *Solanum* from the states of Bahia and Minas Gerais associated with granitic outcrops (inselbergs), a poorly sampled environment with a high degree of endemism in many plant groups (Martinelli 2007, Porembski 2007, de Paula et al. 2017).

### Material and methods

For the present study, specimens from the following herbaria were examined: ALCB, BHCB, CEPEC, FURB, HUEFS, MBM, MBML, NY, RB, RFFP and UFP (acronyms from Index Herbariorum; http://sweetgum.nybg.org/science/ih). Expeditions to the areas where the new species occurs were carried out in June 2014, September 2015 and June 2018. During these expeditions, in addition to herbarium collections, juvenile plants were also collected in situ and cultivated at the Museu de História Natural e Jardim Botânico da Universidade Federal de Minas Gerais to obtain further information about plant development and morphology. Measurements of reproductive characters were performed in both dry and fresh or fixed (70% alcohol) material. Terminology used to describe the overall morphology and indumentum follows Radford et al. (1976), but trichome typology was based on Roe (1972) and Mentz et al. (2000). Conservation status was assessed using the IUCN Red List Categories and Criteria (IUCN 2017) and for that, estimates of extent of occurrence (EOO) and area of occupancy (AOO) were calculated using the GeoCat tool (www.geocat.kew.org; Bachman et al. 2011) with the cell size of 2 km<sup>2</sup> for AOO. The criteria used for species delimitation is based on the morphological cluster species concept (Mallet 1995).

#### **Taxonomic treatment**

**Solanum kollastrum Gouvêa & Giacomin, sp. nov.** urn:lsid:ipni.org:names:77192011-1 Figures 1–3, 5

**Diagnosis.** Differs from *S. sublentum* Hiern in its tomentose young stems, petioles and inflorescence axis with the indumentum composed of long-stalked (up to 1 cm) stellate-glandular trichomes with all rays glandular (versus pubescent-glandular indumentum composed of persistent simple glandular and persistent to early deciduous sessile to short-stalked stellate trichomes with only the midpoint glandular), in its straight stem prickles up to 17 mm long (versus recurved to oblique stem prickles up to 6 mm long) and in its large mature leaves 20.5–42 cm long and 20–38 cm wide (versus mature leaves 5.7–17 cm long and 3.8–14 cm wide).

**Type.** BRAZIL. Minas Gerais: Ataléia, povoado de Canaã do Brasil, estrada não pavimentada que liga o município de Ouro Verde de Minas ao povoado de Canaã do Brasil, crescendo em área alterada próximo a afloramento rochoso gnáissico (inselberg ou pão de açucar), 18°00'19"S, 41°12'17"W, 313 m elev., June 2018 (fl, fr), *Y.F Gouvêa 280* (holotype: BHCB [BHCB190863]; isotype: RB).

**Description.** Shrubs up to 3.5 m, erect, moderately branched. Young stems terete, densely tomentose with hyaline to ochraceous stellate-glandular trichomes, these sessile to long-stalked with multiseriate stalks up to 1 cm long, multiangulate, the rays

5–20, 2–3-celled, unequal in length, all or almost all with a capitate glandular distal cell, the midpoint 2–3-celled, equal to or twice the length of the longest ray, the distal cell glandular; stems densely armed with prickles up to 17 mm long and to 2.3 mm wide at the base, straight, slightly flattened, stramineous to yellowish at base, becoming ferruginous towards the apex, pubescent with stellate trichomes like those of the stems and some small, stalked, uniseriate glandular trichomes at the base; bark of older stems greyish dark brown. Sympodial units difoliate to plurifoliate, the leaves not geminate, the leaves arranged in a 2/5 phyllotaxic spiral. Leaves simple, lobed, 20.5–42 cm long, 20-38 cm wide, the blade broadly elliptic to broadly ovate, membranous, discolorous, green adaxially and whitish light green abaxially when fresh, becoming dark green adaxially and light green to pale brown abaxially when dried; adaxial surface densely stellateglandular tomentose but always visible, with multiangulate trichomes, these short- to long-stalked, with multiseriate stalks 3-4 cells wide, up to 1 mm long, the rays 4-11, 1-celled, all eglandular or with one or more glandular ones (then 2-3-celled), unequal in length, the midpoints 2-3-celled, usually longer than the rays, mixed with smaller porrect to antrorse, usually eglandular stellate trichomes, these sessile to short-stalked (stalks to 0.1 mm long), the rays 2–5, 1-celled and minute, inconspicuous, unbranched, subsessile uniseriate glandular trichomes; the abaxial surface densely stellate-glandular tomentose, the epidermis barely visible, with trichomes like those of the adaxial surface, but more densely distributed; sparsely to moderately armed along the midrib and the primary veins of both surfaces with straight, laterally compressed prickles reaching up to 10 mm long and to 1.3 mm wide at the base adaxially, up to 17.5 mm long and to 1.8 mm wide at the base abaxially; primary veins 5–7 pairs; base cordate, the two major basal lobes obtuse to rounded, 2.5-7 cm long at the longest point, often overlapping each other over the petiole, not decurrent on to the petiole; margins with the lateral lobes 1.5-4.8 cm long, 4-9 cm wide at base, acute or less often obtuse or rounded at the apex, both basal and lateral lobes sometimes with small secondary lobes; apex acute; petiole 4.5–19.5 cm, densely tomentose with trichomes like those of the stem, armed. Inflorescence a scorpioid cyme, usually unbranched, rarely forked or trifurcate, internodal or subopposite the leaves, the axis densely glandular tomentose with trichomes like those of the stem, but these hyaline to ochraceous, armed; peduncles 2.6-6 cm long, the rachis 4.3-11 cm long, with 11-35 flowers, with up to 3 open at the same time; pedicel insertions generally unequally spaced, adjacent to spaced 2.3 cm apart; pedicels 4.8–18 mm long in open flowers, straight, articulated at base, armed, densely tomentose with trichomes like those of the stem, but with the epidermis and trichomes often purple-coloured. Flowers 5-merous, the plants and romonoecious, producing hermaphroditic flowers (long-styled) and functionally male short-styled flowers, which vary in proportion (number of long- vs short-styled flowers) between inflorescences. Calyx somewhat urceolate, inflated, foliaceous, purple (mainly along the margins and apex of the calyx lobes) to green, armed, densely tomentose with the epidermis barely to not visible basally, becoming gradually more visible towards the apex of the lobes, with trichomes like those of the stem but these sometimes purple and with some eglan-



**Figure 1.** Line drawing of *Solanum kollastrum*. **A** habit with notably dense prickles, leaves lacking secondary lobes and internodal inflorescences **B** detail of the prickly inflorescence with a hermaphrodite flower at anthesis **C** detail of a dissected flower **D** detail of the fruits enclosed by the strongly accrescent fruiting calyces (with the uppermost fruiting calyx opened to expose the fruit) from *Gouvêa 102* (BHCB).



**Figure 2.** *Solanum kollastrum.* **A** plant habit **B** detail of stem prickles **C** young inflorescence (upper right corner: detail of a more developed inflorescence) **D** flowering calyx **E** a short-styled and a long-styled flower displaying the extremes of variation of corolla size and colour found in the species (here exhibited by flowers of the same inflorescence); also note the various degrees of anther curvature and location of the apical pores compared with the images F and G **F** long-styled flower (hermaphroditic) with extrorse pores and slightly outwardly curved apices **G** short-styled flower (functionally male) with extrorse pores and markedly outwardly curved apices **H** infructescence with details of the strongly accrescent fruiting calyces (one of which was dissected to show the fruit) and the mature fruit colour (left side: details of fruit shape; right side: detail of a dissected fruit showing the four locules, placentation, seed colour and shape). Scale bars: 1.2 m (**A**); 3 cm (**B**, **C**, **H**); 1.5 cm (**D**, **F**, **G**); 1.8 cm (**E**). Photographs by Y.F. Gouvêa.

dular rays; base rounded, markedly plicate on the fusion line at the base of the adjacent sepals, these basally concave, the calyx tube 4.5–8.2 mm long, 9.4–15.2 mm in diameter at the point with the largest diameter, the lobes 7.5–15.6 mm long, 6–9 mm wide at the base, triangular, the margins plane to strongly undulate and revolute, the apices acute to caudate. Corolla 2.3–3.9 cm in diameter, purple to lilac or bluish-lilac, stellate, lobed 2/5 to 1/2 of its length, interpetalar tissue absent, the tube 1.1–2.2 cm long, the lobes 10.9–15 mm long, 8.8–13.4 mm wide, deltate to triangular, the margins straight


**Figure 3.** Detail of *Solanum kollastrum* indumentum. **A** trichomes composing the indumentum of the stems, petioles and inflorescence axis **B** indumentum of the adaxial leaf surface **C** Indumentum of the abaxial leaf surface **D** Trichomes composing the indumentum of the abaxial leaf surface. Scale bars: 6 mm (A); 5 mm (B); 3 mm (C); 1.5 mm (D). Photographs by Y.F. Gouvêa

to slightly convex at base, the apex acute, apiculate or not, stellate-glandular tomentose abaxially with trichomes like those of the leaves, almost glabrous adaxially with trichomes sparsely distributed along the veins and near the apex. Stamens equal; filament tube 1–2.1 mm long; free portion of the filaments 1.3–2.9 mm long, glabrous; anthers 7.5-10 mm long, 2.8-4.3 mm wide, 2.4-2.9 mm thick at the widest point, slightly gibbous, broadly lanceolate, narrowed towards the apex, sagittate at base, connivent, with the pores directed to apex and slightly extrorse, the epidermis papillose, slightly swollen dorsally. Ovary conical to somewhat cupuliform, 4-lobed, 4-locular, densely stellate-glandular tomentose at the apex, becoming glabrous with age, the trichomes 2-7-rayed, stellate, sessile, with a 2-4-celled, eglandular or glandular midpoint longer than the 1-celled rays; style 13.7-15.9 mm long in long-styled flowers, 1.2-3.7 mm long in short-styled flowers, cylindrical, glabrous; stigma globose to clavate, up to 1.4 mm long in long-styled flowers, papillose, green when fresh. Infructescence axis up to 29 cm long. Fruit a widely depressed ovoid to obloid berry, 11.4–20 mm long, 12–22.5 mm wide, the pericarp smooth, pale green to white, with scattered stellate trichomes at the apex; fruiting pedicels 1.4-2.2 cm long, armed; fruiting calyx strongly accrescent, completely covering the fruit in all stages of development, the tube 16-20.4 mm long and 19-34 mm in diameter at the widest point, the lobes 11-21.8 long, 13.7-19 mm



Figure 4. Distribution of Solanum kollastrum.

wide at base. Seeds ca. 230 per berry, ca. 2 mm long and 2.4 mm wide, flattened, reniform, dark brown. Chromosome number: not known.

**Distribution.** Endemic to eastern Brazil (Figure 4). The known records of *Solanum kollastrum* are mostly concentrated along the Mucuri River watershed, ranging from the municipality of Ataléia, in northeastern Minas Gerais state, to Mucuri at the southern coast of Bahia. The only exception, so far, is one collection (*J.G. Jardim et al. 3151*; CEPEC, NY) made further north, in Caatiba, a municipality of the southcentral region of Bahia State.

Ecology and habitat. Solanum kollastrum inhabits the edge of small forest fragments, especially those at the base or on granitic outcrops (inselbergs), which are geological formations characterising the peculiar landscape of the type locality. Some populations were also found in disturbed sites near these rock outcrops, such as borders of unpaved roads and pastures. The restinga (herbaceous to arboreal vegetation growing along the Brazilian sandy coastal lowlands; Araújo 1992) is a most distinct environment in which S. kollastrum has been found [S.A. Mori et al. 10459 (CEPEC, NY), Y.F. Gouvêa 283 (BHCB) and Y.F. Gouvêa 284 (BHCB)]. In restinga formations, S. kollastrum was observed in open disturbed areas dominated by grasses and at the edge of forest fragments near the Mucuri River mouth in Bahia State (Fig. 4). The known S. kollastrum habitats vary from environments subject to periods of drought (e.g. the edge of small seasonal semi-deciduous forest fragments or vegetation islands on inselbergs) to constantly wetter environments, at the edge of the aforementioned coastal forests, where the climate is under a strong oceanic influence. Its observed elevational range is from sea level to about 900 m. Field observations as well as its anther morphology (i.e. poricidal dehiscence and the anthers' robustness) suggest that its primary pollinators are medium- to large-sized bees (e.g. genus Ptiloglossa) with buzzing behaviour (Michener 1962, Buchmann 1983). Solanum kollastrum fruits hang outside (below) the foliage on long inflorescence axes and are enclosed until their maturity by an inflated Physalis-like calyx. This, along with the persistent green to white epicarp colour, the fleshy mesocarp, the numerous relatively small seeds and the release of a mild sweetish scent at fruit maturity are characteristics associated with fruits eaten by bats (Van der Pijl 1972, Cooper et al. 1986, Charles-Dominique and Cockle 2001). Actually, several studies have shown the importance of the fruits of Solanum species in bat diets (Marinho-Filho 1991, Passos et al. 2003, Zanon and dos Reis 2007, Mello et al. 2008a) and the role of bats as dispersal agents for Solanum species (Uieda and Vasconcellos-Neto 1985, Iudica and Bonaccorso 1997, Galindo-Gonzáles et al. 2000, Mello et al. 2008b). Many other Solanum species also present such features (e.g. those here considered morphologically related to S. kollastrum; see discussion). However, species-level studies on pollination or fruit dispersal of Brazilian Solanum species are virtually non-existent, although being fundamental to confirm and better understand the interactions between these species and their pollinators and dispersal agents.

**Phenology.** Flowering specimens were found from April to November, when immature fruits were also observed, indicating that *Solanum kollastrum* may bloom throughout most of the year. Specimens with mature fruits were observed at the end of June.



**Figure 5.** Habitats of *Solanum kollastrum.* **A** general view of the small village of Canaã do Brasil illustrating the typical landscapes of the type locality **B** a specimen growing in the soil amongst rocks at the base of an inselberg **C** general view of an area of altered restinga vegetation with the arrow pointing to a *S. kollastrum* specimen (upper right corner: detail of the distinct architecture of the specimens growing in this environment) **D** a specimen growing at the edge of a restinga forest fragment. Photographs by Y.F. Gouvêa

**Etymology.** The epithet *kollastrum* is derived from the Greek words for glue  $(\varkappa \delta \lambda \lambda \alpha)$  and star  $(\dot{\alpha} \sigma \tau_Q \circ \nu)$ , referring to the notable stellate-glandular trichomes observed on the younger stems, petioles and inflorescence axis of this species.

**Preliminary conservation status.** Endangered (EN) B2 a, b (ii, iii, iv); Extent of Occurrence (EOO) 32,626 km<sup>2</sup> (NT); Area of Occupancy (AOO) 20 km<sup>2</sup> (EN). Despite the relatively large EOO (>20,000 km<sup>2</sup>) of *S. kollastrum*, its small AOO (<500 km<sup>2</sup>), the few and disjunct collections, all outside protected areas and the vulnerability of its habitats, lead us to suggest it should be attributed an Endangered status (IUCN 2017).

Additional specimen examined (paratypes). BRAZIL. Bahia: Mun. Mucuri, Rodovia Mucuri/Nova Viçosa (BA-001), crescendo em área de restinga aberta alterada dominada por gramíneas à margem da rodovia, 18°02'08"S, 39°31'10"W, 3 m elev., June 2018 (fl, fr), Y.F. Gouvêa 283 (BHCB); a 4 km a W de Mucuri, Restinga, 13 September 1978 (fl), S.A. Mori et al. 10459 (CEPEC, NY); Mun. Nova Viçosa, Rodovia Mucuri/Nova Vicosa (BA-001), crescendo em borda de fragmento de restinga arbórea à margem da rodovia, 17°56'37"S, 39°26'54"W, 5 m elev., June 2018 (fl, fr), Y.F. Gouvêa 284 (BHCB); Mun. Caatiba, entrada para a cidade ca. 11 km de Itapetinga, rod. para Caatiba 31.2 km da BR-415, 14°59'48"S, 40°23'12"W, 427 m elev., 3 November 2000 (fl, fr), J.G. Jardim et al. 3151 (CEPEC, NY). Minas Gerais: Mun. Ataléia, estrada de terra que leva da BR-418 à comunidade Canaã, 17°56'34"S, 41°10'39"W, 382 m elev., 15 June 2014 (fl, fr), Y.F. Gouvêa et al. 102 (BHCB); Mun. Teófilo Otoni, Rodovia BR-418, crescendo à sombra entre rochas da base de afloramento rochoso gnáissico (inselberg ou pão de acúcar) à margem da rodovia, 17°54'33"S, 41°11'37"W, 225 m elev., June 2018 (fl, fr), Y.F. Gouvêa 281 (BHCB); Pedra da Boca, topo do inselberg, crescendo na borda de capão de mata, 17°55'44.18"S, 41°11'1.36"W, 911 m elev., 20 September 2015 (fl, fr), J.R. Stehmann et al. 6387 (BHCB); Mun. Carlos Chagas, Rodovia BR-418, crescendo em área alterada no entorno de afloramento rochoso gnáissico (inselberg ou pão de açúcar) próximo à margem da rodovia, 17°52'16"S, 41°02'07"W, 280 m elev., June 2018 (fl, fr), Y.F. Gouvêa 282 (BHCB); Rod. BR-418, km 112, base dos paredões rochosos, 11 April 1984 (fl, fr), G. Hatschbach 47806 (CEPEC, NY).

### Discussion

*Solanum kollastrum* is morphologically related to a group of species endemic to the south-eastern Brazilian Atlantic Forest that share strongly accrescent fruiting calyces, large leaves with decurrent bases and large, robust flowers (see Fig. 2). This unnamed group appears to include five known species (i.e. *S. hexandrum* Vell., *S. kollastrum, S. robustum* H.Wendl., *S. stagnale* Moric., and *S. sublentum*). Of these, three species (*S. hexandrum, S. robustum* and *S. stagnale*) were sampled in the molecular phylogeny of Stern et al. (2011), forming a moderately supported clade sister to the clade that includes species traditionally placed in *Solanum* section *Erythrotrichum* A.Child. Although Stern et al. (2011) included both of these groups in the Erythrotrichum clade, the lineage containing *S. kollastrum* and related species may deserve recognition as a separate clade, emphasising its morphological, ecological and geographical distinctive nature.

Amongst the species in this group, only *S. kollastrum* and *S. sublentum* have glandular trichomes on the entire plant (see Fig. 3) and cordate leaf bases. Decurrent leaf bases of *S. kollastrum* are only seen in the first leaves of the seedlings, with the subsequent leaves gradually changing shape to become cordate and non-decurrent. In contrast, the leaf bases in *S. hexandrum*, *S. robustum* and *S. stagnale* remain decurrent throughout the plants' life, varying in shape from attenuate to truncate. *Solanum kollastrum* most closely resembles *S. sublentum*, of which it can be readily distinguished by the robust long-stalked (up to 1 cm) stellate-glandular trichomes with all rays having a glandular distal cell (some rays may lose the glandular cell through breakage or by the disruption of the gland wall) composing the indumentum of its young stems, petioles and inflorescence axis (see Fig. 3A; trichomes in *S. sublentum* are mostly simple). The shape and length of its stem prickles and the robustness of its leaves also are useful for the distinction between these species (see diagnosis for more details). Although easily differentiated, Solanum kollastrum and S. sublentum have very similar floral morphologies, sharing well-developed calyces that are strongly accrescent in fruits, showy purple to lilac corollas and robust anthers (see Fig. 2). Their leaves also resemble each other: both are lobed (with secondary lobes or not), elliptic to ovate (or broadly ovate in S. kollastrum) and have cordate bases (varying from truncate to cordate or sagitate in S. sublentum). In addition, the glandular nature of their trichomes, although they differ in type, is also a common character to both species. In the field, S. kollastrum has notably larger leaves than those of S. sublentum, however, usually only the apices of the branches are collected, with the fully developed leaves not represented in herbarium material, so this character is often not apparent from herbarium specimens. The diameter of the stems at the middle portion of the internode between the two youngest mature leaves is, in most cases, an additional distinguishing character between these species (0.9-3 mm in S. sublentum and 3.8-6.6 mm in S. kollastrum). Despite the fact that S. kollastrum and S. sublentum occur in similar environmental conditions (associated with outcrops or at edges of lowland forests, see Fig. 5), they have not been observed in sympatry.

The size and colour of the *S. kollastrum* corollas, the shape of its anthers and density and shape of its stem prickles are quite variable. The corollas of the examined specimens vary from 2.3 to 3.9 cm in diameter and from purple to bluish-lilac, with flowers exhibiting sometimes the extremes of variation of these characters in the same inflorescence (see Fig. 2E). The anther shape and the position of the apical pores also varies considerably; the anthers of plants from the type locality are straight (typical) with slightly extrorse apical pores, while those of plants from the coastal region have apices with varying degrees of curvature outwards from the cone, with pores markedly extrorse (see Figs 2E–G). Despite the observed variability, apically curved anthers are only found in *S. kollastrum* when compared to morphologically similar species. This distinct anther morphology may reflect differences in the plant-pollinator interaction, being an interesting issue for further investigation. The length of trichome rays and midpoints is also variable; plants collected in Caatiba, Bahia, have stem, petiole and inflorescence axis trichomes with rays and midpoints much longer than those specimens from other localities.

Plants growing in open restinga vegetation sites exhibit distinct architecture. These plants are lower and wider in their overall appearance due to the branching near the base of the major stem, with which the first order branches form angles close to 90° (see Fig. 5C). Plants from other habitats are more erect and become taller, with the first order branches forming angles close to 45° (habit with Y-shaped overall appearance; see Figs 5B, 5D and 2A). Differences in density and shape of the stem prickles between populations from inland and coastal areas is also observed, with the coastal populations possessing moderately distributed stem prickles with slightly broader bases, rather than the densely distributed narrowly based needle-like prickles of inland populations.

The discovery of *S. kollastrum*, a robust and conspicuous plant growing at the roadsides in regions close to large urban centres, highlights how insufficiently known the Brazilian flora is, even at present, and how urgent the need is to describe, study and conserve the country's plant diversity. Thus, we hope that this discovery encourages the study on the most diverse aspects of this species' biology.

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**RESEARCH ARTICLE** 



# Haplophyllum ermenekense (Rutaceae), a new species from Turkey

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### Abstract

A new species of *Haplophyllum*, *Haplophyllum ermenekense* (Rutaceae) is described and illustrated in line drawing. It grows on stony slopes of Ermenek town, Karaman province, in southern Turkey. It is compared with the closely related species *H. myrtifolium*. *H. ermenekense* is distinguished from the morphologically similar *H myrtifolium* chiefly by sepal shape, petal size, capsule size, presence of capsule hair and appendage form. On the other hand, the seed coat and pollen grains surface of *H. ermenekense* and *H. myrtifolium* are demonstrated in SEM photographs. In addition to the detailed description, the illustration, distribution map, conservation status and ecology of the new species are also provided.

### **Keywords**

Endemic, Haplophyllum, Karaman, Rutaceae, taxonomy

## Introduction

With its 69 species, *Haplophyllum* Jussieu is one of the richest genera in the Rutaceae family (Townsend 1986, Navarro et al. 2004, Soltani and Khosravi 2005, Tugay and Ulukuş 2017). The genus is widely distributed in subtropical and tropical regions of the northern hemisphere of the Old World, notably in Iran, Turkey and Central Asia (Townsend 1986).

Some authors have endeavoured to subdivide *Haplophyllum* into different sections by using morphological characters (Spach 1849, Boissier 1867, Engler 1896, Vvedensky 1949, Townsend 1986). The most comprehensive studies conducted with re-

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gard to *Haplophyllum* were published by Vvedensky (1949) and Townsend (1986). In these studies, the genus was divided into four sections by Vvedensky (1949), based on capsule opening, ovule and carpel number. In the last monographic study, Townsend (1986) divided the genus into three sections according to carpel number, capsule opening, petal colour, plant architecture, stamen form and ovary shape.

*Haplophyllum* species are perennial herbs, growing mainly on sandy soil, rocky hills, slopes, stony landscapes or steppes (Townsend 1986). Morphologically, the genus is characterised by the presence of exstipulate, cymose inflorescences with, bracts, lax to dense. Flowers have five petals and five sepals, creamy-white to bright yellow petals, ten stamens with free filaments expanded below and pubescent on the inner surface. Fruits have three to five connate carpels, five-lobed capsules which are dehiscent or indehiscent (Townsend 1986). Pollen grains are tricolporate, radially symmetrical and isopolar (Ulukuş et al. 2016). The equatorial view of pollen of *Haplophyllum* is distinctly rhomboid (Townsend 1986). Tectum ornamentation is commonly striate or striate perforate (Townsend 1986, Ulukuş et al. 2016, Tugay and Ulukuş 2017).

Turkey is one of the most important centres for *Haplophyllum* diversity with three phytogeographical regions; Euro-Siberian, Irano-Turanian and Mediterranean (Ulukuş et al. 2016). Boissier (1867), in his Flora Orientalis, recognised 15 species in Turkey. At a later date, Townsend (1967) recognised 17 taxa in the Flora of Turkey. Recently, one *Haplophyllum* species was published by Tugay and Ulukuş (2017), bringing the genus to 18 taxa. With the new species described in this paper, Turkey harbours 19 *Haplophyllum* taxa, 11 (58%) of which are endemic.

The Irano-Turanian region in SW Asia is one of the richest floristic areas of the Holarctic Kingdom. Most of its species diversity is concentrated in the Anatolian plateau, Iranian plateau and Central Asia (Zohary 1973, Manafzadeh et al. 2014).

*Haplophyllum* has mostly been studied from a morphological point of view by several authors (Jussieu 1825, Spach 1849, Boissier 1867, Engler 1896, Vvedensky 1949, Townsend 1986, Salvo et al. 2011, Ulukuş et al. 2016, Tugay and Ulukuş 2017). Only a few palynological studies have been conducted in the genus *Haplophyllum* (Townsend 1986, Perveen and Qaiser 2005, Akyol et al. 2012, Al-Eisawi and Al-Khatib 2015, Ulukuş et al. 2016, Tugay and Ulukuş 2017). There are several studies about the seeds of some species belonging to the *Haplophyllum* genus (Townsend 1986, Navarro et al. 2004, Tugay and Ulukuş 2017).

From a biogeographical standpoint, Manafzadeh et al. (2014) showed that the clade, formed by the Mediterranean species of *Haplophyllum* and Anatolian *H. tel-ephioides*, diverged from its geographically diverse sister clade in the middle Miocene probably in the Irano-Turanian region and, from there, it quickly invaded the eastern Mediterranean region.

Ermenek, located within the boundaries of Karaman province, in the Mediterranean region of Turkey, is one of the most interesting plant diversity centres in Turkey.

The aim of this study is to describe the new species, *H. ermenekense*, found in Ermenek and to compare it with similar species, especially *H. myrtifolium* Boiss., based on evidence from (micro) morphology and palynology.

### Material and methods

Between 2011 and 2015, during the process of writing a revision of the *Haplophyllum* genus in Turkey, the authors carried out fieldwork around Ermenek and collected samples. All available specimens of *Haplophyllum* harboured in Turkish herbaria (ANK, EGE, GAZI, HUB, ISTE, KNYA), relevant Turkish collections from herbaria out of Turkey (E, K), as well as all specimens collected during recent fieldwork, were examined under dissecting microscopes. Examined specimens were checked and evaluated comprehensively by relevant literature (Boissier 1867, Vvedensky 1949, Townsend 1966, 1967, 1968, 1985, 1986). The Townsend (1967, 1986) terminology was used to describe the new species.

For palynological investigations, the pollen slides were prepared according to Wodehouse's (1935) technique. The pollen micromorphology of *H. ermenekense* and *H. myrtifolium* were examined by using scanning electron microscopy (SEM) techniques. For SEM, pollen grains were first mounted on double-sided carbon tape affixed to aluminium stubs, covered with gold with a Hummle VII sputter coater and photographed at a magnification of  $2000 \times$  to  $7000 \times$  with a JEOL-5600. SEM micrographs were used to determine exine sculpturing of the pollen. For pollen morphology, Punt et al. (2007) terminology was used.

Morphometric measurements of seeds were made under a stereomicroscope (Leica S8AP0) coupled to a Leica DFC 295 digital camera. The seed length and width of (10–) 30–35 seeds per species were measured. Measurements were made using the Image Tool software. Minimum-maximum ranges, mean, standard deviations of seed length and width, as well as length/width ratio, were calculated. SEM micrographs were used to determine seed coat sculpturing of the seeds. The terminology of Stearn (1983) was adopted to describe the SEM aspects of the seed coat.

### Taxonomy

Haplophyllum ermenekense Ulukuş & Tugay, sp. nov. urn:lsid:ipni.org:names:77192112-1

Figs 1–6

**Diagnosis.** *Haplophyllum ermenekense* most resembles the closely related *H. myrtifolium*. It differs from *H. myrtifolium* by its inflorescence usually lax form (versus dense), sepals ovate or ovate-oblong (versus lanceolate or lanceolate-oblong) and deciduous in fruit (versus persistent in fruit), petals  $4-5.5 \times 1.5-2.5$  mm (versus  $6.5-9.5 \times 3.5-4.5$  mm), capsule  $2-2.5 \times 3-4$  mm (versus  $3-3.5 \times 5-6$  mm) and glabrous (in contrast to not glabrous), with a conspicuous usually erect appendage on the outer upper surface (versus incurved appendage on the outer upper portion).

**Type.** TURKEY. C4 Karaman; Ermenek, limestone slopes, steppe, 1200 m alt., 36°37.356'N, 32°51.543'E, 21 June 2014, *O. Tugay* 9641 & *Ulukuş* (holotype: KNYA; isotype: ANK, GAZI)



**Figure 1.** Distribution map of *Haplophyllum ermenekense* ( $\Delta$ ), *Haplophyllum myrtifolium* ( $\Box$ ) in Turkey.

Description. Perennial herbs, 25-45 cm; woody at the base with usually ascending or barely erect flowering stem with sterile shoots; stems simple below the inflorescence, furnished with rather crisped, flexuose hairs or seldom patent hairs, punctate glands. Leaves varying  $8-20 \times 2-8$  mm, usually lanceolate or lanceolate-elliptic, both surfaces ± densely covered with flexuose-appressed to crisped white hairs, densely furnished with small, dark punctate glands; with sterile shoots present in the leafaxils. Inflorescence lax, 4-12 cm in diameter, 10-50 flowered, the branches with flexuose hairs, with numerous punctate glands. Bracts numerous, linear-lanceolate, all ± densely white-pilose. Sepals ovate, ovate-oblong, fused at the extreme base, obtuse, white-lanate,  $1-1.25 \times 0.75-1$  mm, with very small glands, deciduous in fruit. Petals obovate, glabrous,  $4-5.5 \times 1.5-2.5$  mm, white, with numerous very small glands. Filaments free, narrow, somewhat expanded in the lower half, 3.5-4 mm, bearded with long hairs within about the central half, with glands very small; anthers yellow, oblong, 1.5-2 mm, Ovary segments 5, glabrous, with small acute tuberculate glands below, conical apical appendage, loculi biovulate; style glabrous, slender, 3 mm. Capsule  $2-2.5 \times 3-4$  mm, glabrous, with a conspicuous usually erect appendage on the outer upper surface; seeds reniform, grey to black  $1.25-1.5 \times 1-1.15$  mm, with widely spaced transverse ridges.

**Paratypes.** TURKEY. C4 Karaman; Ermenek, Kazancı, limestone slopes, steppe, 1000 m alt., 36°28.872'N, 32°54.433'E, 21 June 2014, *O. Tugay* 9642 & *Ulukuş* (KNYA); 16 July 2012, *O. Tugay* 8116 & *Ulukuş* (KNYA); Kazancı, limestone slopes, 1200 m alt., 36°30.072'N, 32°52.433'E, 10 July 2016, *O. Tugay* 13.175 & *Ertuğrul* (KNYA).

**Ecology.** Haplophyllum ermenekense is endemic to Turkey. It grows at altitudes between 980 and 1200 m on limestone slopes amongst bushes (e.g. Quercus coccifera L., Juniperus oxycedrus L., Pistacia terrebinthus M.Bieb. etc.). Plant diversity in this place is mainly composed of herbaceous and suffruticose plants including Adonis flammea Jacq.,



**Figure 2.** Line drawing of *Haplophyllum ermenekense*. **A** habit **B** petal of *H. ermenekense* **C** petal of *H. myrtifolium* **D** calyx of *H. ermenekense* **E** calyx of *H. myrtifolium* **F** stamen of *H. myrtifolium* **G** stamen *H. ermenekense* **H** capsule of *H. myrtifolium* **I** capsule of *H. ermenekense* **J** flower of *H. ermenekense* (Drawn from the holotype by O.Tugay).



Figure 3. General view of habit and flowers: A, B H. ermenekense C, D H. myrtifolium.

Aegilops cylindrica Host, Aethionema stylosum DC., Capsella bursa-pastoris (L.) Medik., Centaurea virgata Lam., Digitalis cariensis Boiss. ex Jaub. & Spach, Ebenus plumosa Boiss. & Bal. subsp. speciosa Boiss. Bal., Glaucium corniculatum (L.) Rud. subsp. corniculatum, Glaucium leiocarpum Boiss., Hyoscyamus aureus L., Hyoscyamus niger L., Isatis ermenekense Yıld., Micromeria cristata (Hampe) Griseb. subsp. cristata, Salvia albimaculata Hedge & Hub-Mor. and Salvia aucheri Bentham var. canescens Boiss. & Heldr.

**Phenology.** Flowering time was observed at the end of July and August, mature fruits were collected in September.

**Etymology.** The name of Ermenek town where new species found is given to the species epithet.

Proposed Turkish name for the new species. Ermenek sedosu.

**Distribution and conservation status.** *H. ermenekense* is endemic to Karaman province. It is an element belonging to the east Mediterranean phytogeographic region (Fig. 1). The range of this new species is limited to a single locality and its area of occupancy is estimated to be less than 5 km or 5 km<sup>2</sup>. The number of mature individual plants is estimated to be less than 250. As it is perennial, this new species has a crucial advantage for its future as destruction of the bushes by local people, road construction and deterioration of habitats may cause some threats. Thus, according to criterion D, it can be included in the EN (Endangered) category (IUCN 2001; 2016).



Figure 4. Capsules, A H. ermenekense B H. myrtifolium.

## Key to related Haplophyllum species

1	Capsule without apical appendage
_	Capsule with apical appendage
2	Current year's stem and branches and also inflorescence branches, very dense-
	ly furnished with white, crisped hairs
-	Current year's stem and branches and also inflorescence branches, glabrous or sparsely bairy
3	Low shruh: young twigs dark purplish H amoenum
_	Not as above 4
4	Plant with numerous long, stiff, viragete stems, with the upper leaves much
_	Plant without long, stiff, virgate stems; inflorescence a broad, many-flowered
_	corymb
5	Plant vegetative parts and inflorescence branches with stipitate glandular
	hairs
-	Plant not as above
6	Flowers larger, petals 10–14 mm long, upper leaves distinctly rhomboid-lan- ceolate
-	Flowers smaller, petals at most 9.5 mm long, leaves linear-lanceolate to elip-
7	Capsule with a conspicous usually blunt tuberculiform appendage on the outer upper surface, leaves not exceeding 17 mm long
_	Capsule with a conspicous usually incurved appendage or with a conspicous usually erect appendage on the outer upper surface; leaves 8–40 mm long8
8	Petals 6.5–9.0 mm oblong-ovate, sepals persistent in fruit, capsule usually
_	Petals 4–5.5 mm obovate, sepals deciduous in fruit, capsule glabrous



Figure 5. SEM photographs of seeds *Haplophyllum* species. **A**, **B** *H. ermenekense* (*O.Tugay* 8116 & *D.Ulukuş*) **C**, **D** *H. myrtifolium* (*O.Tugay* 8535 & *D.Ulukuş*).

## Seed morphology

The seed features of *H. ermenekense* and its immediate relative *H. myrtifolium* were investigated. It was seen that *H. ermenekense* has a reniform seed type. Seeds are 1.40–1.83 mm × 1.18–1.39 mm and the range of the L/W ratio is  $1.28 \pm 0.09$ . The seeds of *H. ermenekense* have widely spaced transverse ridges and micromorphologically, the sculpturing of the seed coat is not clearly striate. On the other hand, *H. myrtifolium* has narrowly spaced longitudinal ridges and a clear striate sculpturing pattern on the seed coat surface in the detailed view (Fig. 5).

## Pollen morphology

It is found that pollen grains of *H. ermenekense* are tricolporate, radially symmetrical, isopolar and their shape is oblate-spheriodal to suboblate. The measurements of pollen are as follows: polar axis (P)  $40.15 \pm 2.00 \mu m$  (mean  $\pm$  standard deviation), equatorial axis (E)  $44.44 \pm 1.79 \mu m$ ; the exine thickness  $0.88 \pm 0.11 \mu m$  and the intine thickness  $0.83 \pm 0.25 \mu m$ ; and the ratio of P/E of pollen grains is between  $0.80-0.90 \mu m$ . Exine sculpturing pattern is striate, striate-perforate or striate microreticulate (Fig. 6). Pollen features of *H. myrtifolium* have already been defined in Ulukuş et al. (2016).



**Figure 6.** SEM micrographs of the pollen grains. **A, B** equatorial view and exine sculpturing of *H. ermenekense* (*O.Tugay* 9641 & *D.Ulukuş*) **C, D** equatorial view and exine sculpturing of *H. myrtifolium* (*D.Ulukuş* 1467 & *O.Tugay*).

## Discussion

*Haplophyllum ermenekense* is morphologically similar to *H. myrtifolium*, but it differs from *H. myrtifolium* by significant vegetative and reproductive characters (Table 1).

According to Townsend (1986), *H. molle, H. amoenum* and *H. viridulum* have white petals yet *H. canaliculatum* has creamy-white petals. However, these species are basically separated from the other white or creamy-white flowering group without appendage. According to Townsend (1967), *H. vulcanicum* differs from *H. myrtifolium* and *H. megalanthum* by having stipitate glands on stems and inflorescence and *H. megalanthum* differs from *H. myrtifolium* by having larger corollas and rhomboid-lanceolate upper leaves. In addition, Townsend (1967) reported that *H. megalanthum* could be a large flowered variety of the western variant of *H. myrtifolium*. Ulukuş et al (2016) reported that *H. myrtifolium* differs from *H. vulcanicum* by its white to sulphur-yellow petals (versus cream-white), conical and rod-like appendages on the ovary (versus smooth line and acute), patent and crisped hairy indumentum (versus stipitate glands) and it differs from *H. megalanthum* by its lanceolate, lanceolate-elliptic and rarely ovate upper leaves (versus rhomboid-lanceolate), crisped or patent hairy leaf indumentum (versus silky), oblong-ovate (versus lanceolate-elliptic), white to sulphur-yellow (versus creamy-white) and larger petals (in *H. myrtifolium* 6–8.5 × 3–4.5 mm,

Diagnostic morphological characters	H. ermenekense	H. myrtifolium
Leaf shape	usually lanceolate or lanceolate-elliptic	linear-lanceolate or ovate
Leaf size (mm)	8-20 × 2-8	40 × 2.5–12
Inflorescence form in each stem	inflorescence usually lax	inflorescence usually dense
Sepals shape	ovate, ovate-oblong	lanceolate-oblong
Sepals size (mm)	$1-1.25 \times 0.75-1$	$1.5-3 \times 0.75-1.25$
Petal shape	obovate	oblong-ovate
Petals size (mm)	4–5.5 × 1.5–2.5	6.5–9.5 × 3.5–4.5
Petals colour	creamy-yellow	creamy-white
Anther shape	oblong	ovate
Filament form	expanded in the lower half	expanded in the lower third to half
Filaments length (mm)	3.5–4	4.5–5
Capsule size (mm)	2-2.5 × 3-4	3–3.5 × 5–6
Capsule appendage	with a conspicuous usually erect appendage on the outer upper surface	with a conspicuous usually incurved appendage on the outer upper surface
Seed ridges type	transverse ridges	longitudinal ridges

Table 1. Morphological comparison between *H. ermenekense* and *H. myrtifolium*.

in *H. megalanthum*  $7-14 \times 4-5$  mm), conical and rod-like appendages on the ovary (versus incurve corniculus).

Furthermore, *H. ermenekense* is related to *H. fruticulosum* (Labill.) G.Don, (not distributed in Turkey) differing in its hairiness, upper leaf width, capsule appendage and floral features as follows: upper leaf width 2–8 mm (versus 2–3 mm); capsule appendage conspicuous usually erect (versus blunt tuberculiform), capsule glabrous (pubescent).

Salvo et al. (2011) assembled a morphological matrix of 27 characters for 45 *Haplophyllum* species to study the similarities and differences amongst species. According to this study, *H. canaliculatum*, *H. myrtifolium* and *H. viridulum* species are similar to *H. ermenekense* in terms of flower colour character.

*Haplophyllum ermenekense* is related to *H. canaliculatum* (not distributed in Turkey), differing in its apical appendage on ovary, tuberculate glands on the ovaries (versus non-tuberculate glands) and in its linear bracts (versus broad bracts).

Townsend (1986) reported that seeds of *Haplophyllum* commonly have transverse and longitudinal ridges. Tugay and Ulukuş (2017) showed that seeds are significant characters for differentiation between related species. In addition, in this study, the micromorphological study of the seeds showed that there are clear differences between the studied species. However *H. ermenekense* does not have a striate sculpturing pattern on the seed surface and *H. myrtifolium* has a distinctly striate sculpturing pattern in detailed view. On the other hand, *H. ermenekense* has transverse ridges while *H. myrtifolium* has longitudinally ridges (Fig. 5). According to Townsend's (1986) palynologic study on some genera of the Rutaceae, including 14 species of *Haplophyllum*, it was shown that *H. myrtifolium* has striate pollen exine sculpturing. In this study, palynological results showed that there are no clear differences between the studied species. Both species have often striate-perforate and striate-microreticulate exine sculpturing patterns (Fig. 6).

## Conclusion

With the description of this new species, the number of species within *Haplophyllum* has risen to 70. This study provides material and data to aid further research on this important genus of the Rutaceae.

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## Appendix I

Additional examined specimens

Haplophyllum myrtifolium; A5 Ankara: between Polatli-Sivrihisar, Acıkır region, 840 m alt., 11 July 1991, Z.Aytaç & H.Duman 3871 (GAZI!); B2 Uşak: Yaparlar Village, 900 m alt., 22 July 1993 Ö.Seçmen 4230 (EGE!); B3 Afyon: 5 km South of Emirdağ, Bolvadin road, Lime slopes, 1100 m alt., 13 July 1965, M.J.E Coode & B.M.G Jones 2338 (E!); B4 Ankara: Polatli, Acıkır region, conserved steppe, 840–850 m alt., 06 June 1990, Z.Aytaç 3077 (HUB!); Konya: between Cihanbeyli-Yunak, steppe, 1000 m alt., 23 July 2000, E.Hamzaoğlu 2511 (BOZOK!); B5 Adana: Seyhan, Feke, Bakır mountain, 2000 m alt., 30 July 1952, Davis 19394 (E!); B5 Adana: Saimbeyli in mon-

te Kasbel, 15 July 1893, *Manissadjian 851* (K!); C2 Denizli: Pamukkale region, 320 m alt., 19 July 1973, *O. Tugay 9638 & D. Ulukuş* (KNYA); C3 Konya: Beyşehir, Sarıköy, steppe, 1180 m alt., 16 September 2012; C4 Konya: Çumra, Apasaraycık Village, steppe slopes, 1180 m alt., 01 June 2012, *D. Ulukuş 1467 & O. Tugay* (KNYA); 28 July 2013, *O. Tugay 8535 & D. Ulukuş* (KNYA); İçel: Gülnar, Kayrak Village, 1100 m alt., 28 July 1993, *Z.Aytaç 6033* (GAZI!); C5 Adana: In monte Tauro, *Aucher-Eloy 812* (isotip, K!); C6 Kahramanmaraş: Engizek Mountain, Sırasöğüt Plateau environment, stony area, 2100–2200 m alt., 31 May 1988, *H. Duman 3948* (GAZI!); C7 Malatya: Sürgü, around Sürgü Dam, steppe, 1322 m alt., 01 July 2011, *B. Özüdoğru* 3063 (HUB!).