

# A new species of *Cyanea* Gaud. (Lobelioideae, Campanulaceae) from Maui, Hawai`i

Hank Oppenheimer<sup>1</sup>

<sup>1</sup> Plant Extinction Prevention Program, Pacific Cooperative Studies Unit, University of Hawai`i, PO Box 909, Makawao, HI 96768 USA

Corresponding author: Hank Oppenheimer ([henryo@hawaii.edu](mailto:henryo@hawaii.edu))

---

Academic editor: C. Morden | Received 4 June 2020 | Accepted 3 September 2020 | Published 20 November 2020

**Citation:** Oppenheimer H (2020) A new species of *Cyanea* Gaud. (Lobelioideae, Campanulaceae) from Maui, Hawai`i. *PhytoKeys* 167: 1–11. <https://doi.org/10.3897/phytokeys.167.55107>

---

## Abstract

*Cyanea heluensis* H. Oppenheimer, **sp. nov.**, a new, narrowly distributed endemic species, is herein described and illustrated with line drawings and digital field photos. It is currently known from a single mature plant and is restricted to the upper Helu planeze on leeward Mauna Kahalawai, West Maui, Hawaiian Islands. It differs from all other species of *Cyanea* Gaudich. with its narrow, shallowly lobed leaves, gently curved muricate corollas, and undulate sepals caducous in fruit. A key to the new species and its congeners on Mauna Kahalawai is provided. Its conservation status and efforts to propagate the species are discussed.

## Keywords

conservation, Hawaiian Islands, IUCN Red List, Plant Extinction Prevention Program

## Introduction

The Hawaiian lobelioids are the largest plant clade restricted to any archipelago, with *Cyanea* being the most species-rich genus within that clade. It is also the largest genus in Hawai`i, and originated from a single introduction of 8–10 Mya (Givnish et al. 2008). As currently circumscribed, the woody lobelioid genus *Cyanea* Gaudich. (including *Rollandia* Gaudich.) comprises 80 species (Oppenheimer and Lorence 2012; Spork-Koehler et al. 2015) all endemic to the Hawaiian Islands where they occur in wet and mesic forests. Most have a very narrow distribution, and are single-island endemics, or restricted to a single volcano. *Cyanea* was first described by Gaudichaud-Beaupré (1829)

based on the type species *C. grimesiana* Gaudich. The genus was later treated in Rock's (1919) monographic study of the Hawaiian Lobelioideae in which he recognized 52 species in 5 sections. Wimmer (1943) later recognized only 3 sections in his monograph of Campanulaceae. Lammers (1990) revised the Hawaiian members and also recognized 52 species, but stated relationships within *Cyanea* remained poorly understood and consequently did not recognize any formal sections. Givnish et al. (1995) recognized an orange fruited clade and a purple fruited clade. Recent exploration and collecting efforts in poorly explored, often rugged or remote regions in the Hawaiian Islands, continue to yield undescribed species of *Cyanea* (Lammers and Lorence 1993; Lammers 2004; Oppenheimer and Lorence 2012; Spork-Koehler et al. 2015).

In June of 2010, while near the summit of Helu, Mauna Kahalawai (aka West Maui), the author and Jennifer Higashino spotted through binoculars an unusual *Cyanea*. Upon carefully negotiating down the steep, slippery slope and arriving at the plant, it was immediately recognized as distinct from all the other known taxa on Maui by its habit (Fig. 1), and narrow, shallowly lobed and undulate leaf margins. Flower buds were just beginning to emerge in some of the leaf axils (Fig. 1). Return visits to monitor this individual, and to search for others, found the flowers closer to being at full anthesis in September, when the holotype was collected (Fig. 2). Study of this material revealed it to be distinct from all other known species of the genus, herein described.

## Taxonomic treatment

### *Cyanea heluensis* H.Oppenh., sp. nov.

urn:lsid:ipni.org:names:77212950-1

**Diagnosis.** Species allied to *C. asplenifolia* (H. Mann) Hillebr., but differs in its very shallowly lobed leaves (vs. deeply lobed to pinnate-pinnatifid), longer and wider corolla, and larger, undulate sepals (Fig. 3).

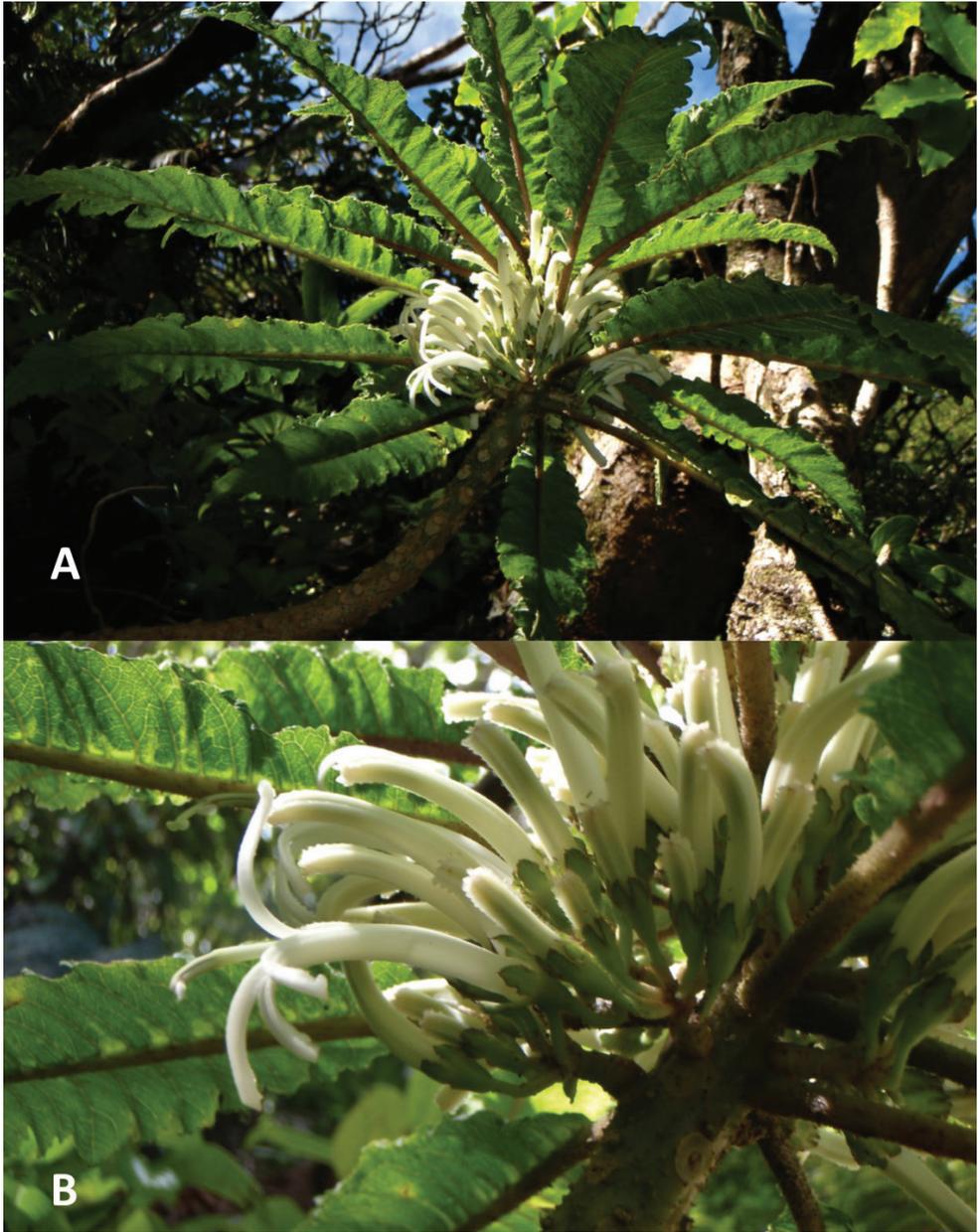
**Type.** USA, Hawaiian Islands, west Maui, Lahaina District, slopes of Helu, north side, 4160 ft. (1268 m), *H. Oppenheimer, S. Perlman & J.Q.C. Lau #H91007*, 8 Sep 2010 (BISH) (Fig. 4).

**Description.** Many-branched, sprawling, decumbent to ascending shrub to 3 m long, stems leaning or tangled in adjacent vegetation, occasionally rooting when in contact with wet soil, very sparingly muricate on the lower trunk, denser on younger stems, leaf scars prominent, nearly orbicular, latex white. Leaves clustered at the ends of the branches, petiolate, chartaceous, dark green above, paler on abaxial surface, midrib usually purple on abaxial surface (live material), blade elliptic to oblanceolate, 26–29 cm long x 4–6 cm wide, apex acuminate, base narrowly cuneate, upper surface mostly glabrous, abaxial surface sparsely pubescent, more so on midrib, margins shallowly and irregularly lobed, the lobes 2–6 mm deep, crenate, undulate, petioles 3–4 cm long, sparsely muricate, pubescent. *Juvenile leaves* weakly (Fig. 4) dimorphic, the lobes more distinct than in adult leaves but cut less than ½ way to midrib, leaf apex rounded.



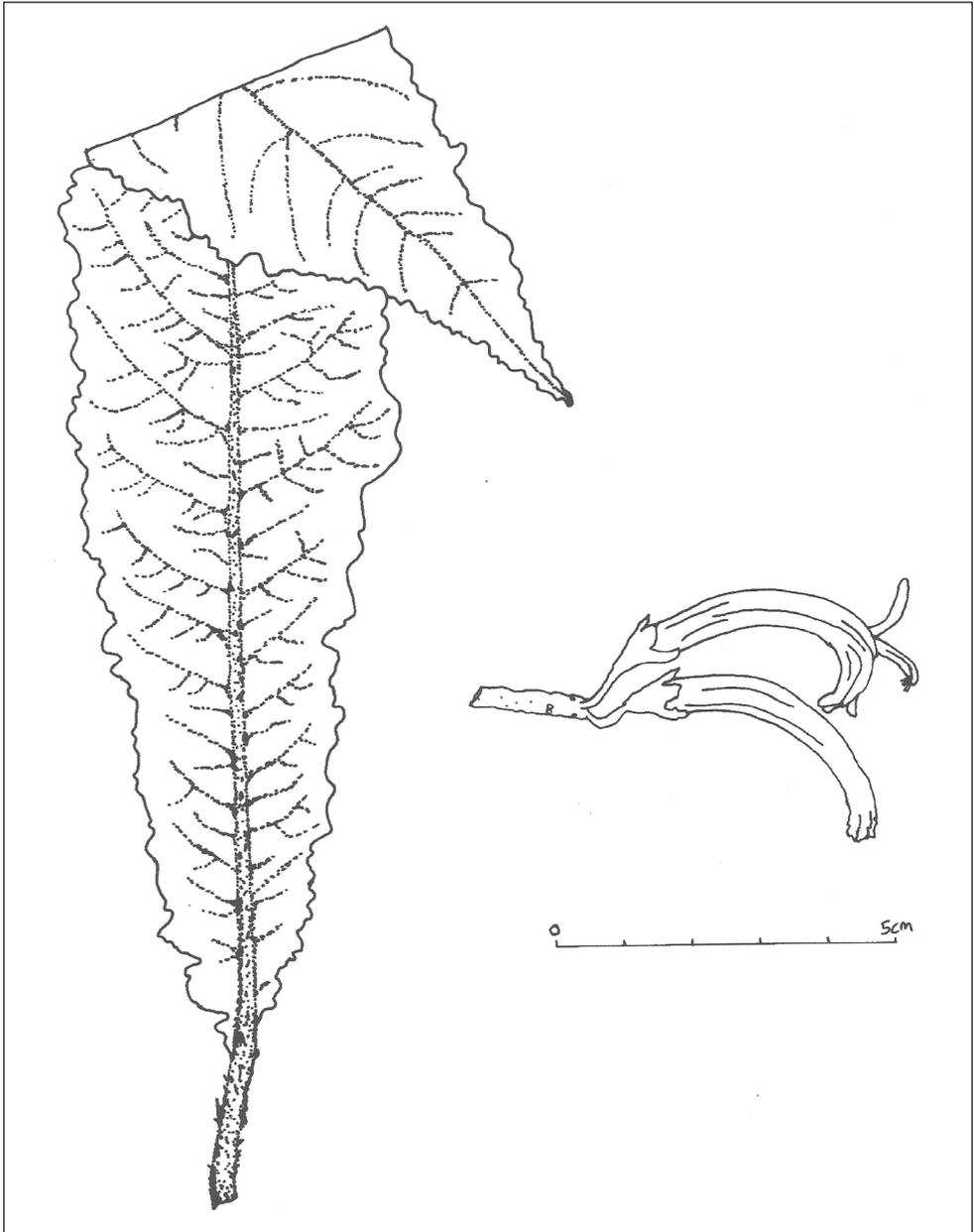
**Figure 1.** *Cyanea heluensis* **A** habit **B** close up of flower buds prior to anthesis. Note mucronations on petioles and corolla. Photos by H. Oppenheimer.

*Inflorescence* axillary, among the lower leaves, peduncles 6–15 mm long, 5–10 flowered, glabrous to sparsely pubescent, bracts caducous (not seen), bracteoles caducous, 1 mm, lanceolate to elliptic, acute, sparsely pubescent. *Pedicels* pubescent, 5–9 mm long, bracteoles usually 1–2 per pedicel, persistent, sparsely pubescent, occurring on basal  $\frac{1}{4}$ – $\frac{1}{2}$  of pedicel. *Calyx* green, the lobes deltate, 4–6 mm long x 2–3 mm wide, margins crenate, undulate, glabrous to sparsely pubescent, purple (most apparent in live material),



**Figure 2.** *Cyanea heluensis* **A** flowering stem **B** close-up of flowers. Photos by H. Oppenheimer.

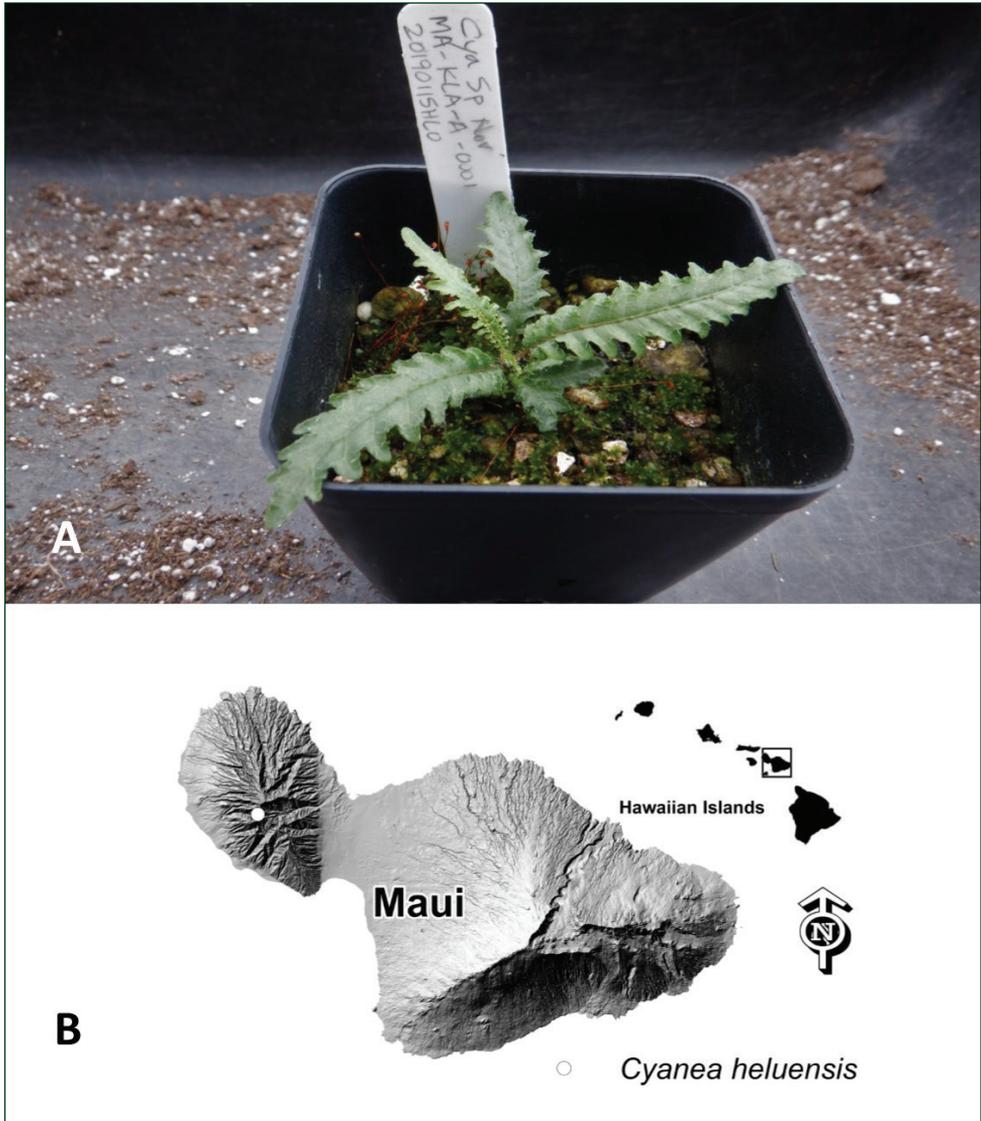
apex acute to short acuminate. *Corolla* white, muricate on corolla lobes especially in bud and distally prior to anthesis, gently curved, 45–55 mm long x 4–5 mm wide, longitudinally pubescent along corolla tube, lobes approximately 1/3 the length of the tube, reflexed, hypanthium 4–5 mm long x 4–5 mm wide, sparsely pubescent. *Berries* small, 5–10 mm, globose, sepals caducous, orange at maturity; *seeds* brown, smooth, round to ellipsoid, < 1 mm.



**Figure 3.** *Cyanea heluensis*. Leaf (left); flowers (right). Illustration by Anna Palomino. Drawn from H. Oppenheimer et al. #H91007 (BISH) and field images taken by H. Oppenheimer.

**Specimens examined.** USA, Hawaiian Islands. West Maui, Lahaina District, slopes of Helu, south, upper slope of Kaua`ula Valley, *H. Oppenheimer & J. Higashino* #H61004, 3 Jun 2010 (BISH); loc. cit. 18 Oct 2018, *H. Oppenheimer* #H101814 (PTBG).

**Affinities.** Several attempts have been made to divide *Cyanea* into sections (Hillebrand 1888; Rock 1919; Wimmer 1943; Lammers 1990), but none successfully



**Figure 4.** *Cyanea heluensis* **A** seedling at Olinda Rare Plant Facility. Plant is approximately 1 year old **B** distribution *Cyanea heluensis* of on Maui, Hawai`i. Photo by H. Oppenheimer.

arranges the genus into clear-cut phylogenetic entities. Givnish et al. (1995) divides the genus into two distinct clades: one with purple fruits and another with orange fruits. *Cyanea heluensis* belongs to a lineage that includes *C. asplenifolia* (H. Mann) Hillebr., its apparent nearest congener and also endemic to Maui, based on their narrow, gently curved tubular, white corollas with murications, and leaf margins that are shallowly lobed to pinnately divided. In contrast to *C. asplenifolia* with its pinnately divided leaves, *C. heluensis* has narrower leaves very shallowly lobed, longer and wider corollas, and larger, undulate sepals. Although Lammers (1990 p. 445) states

the berries of *C. asplenifolia* are unknown, this species is now known to have bright orange fruits (pers. obs.). A preliminary phylogenomic analysis by Steve Hunter (Univ. Wisconsin, Madison pers. comm.) using whole chloroplast genome sequences (not nuclear sequences) supports *C. heluensis* as being sister to the small clade formed by *C. asplenifolia* and *C. duvalliorum* Lammers & H. Oppenh.

Based partly on Lammers (1990, 2004) and Givnish et al. (1995), the following key will distinguish *Cyanea heluensis* from its congeners on Mauna Kahalawai (West Maui).

- 1 Fruit purple ..... 2
- Fruit orange ..... 4
- 2(1) Corolla pubescent ..... *C. obtusa*
- Corolla glabrous ..... 3
- 3(2) Inflorescence 6–25 flowered; peduncles 15–240 mm long ..... *C. angustifolia*
- Inflorescence 6–14 flowered; peduncles 10–50 mm long ..... *C. elliptica*
- 4(1) All 5 anthers with apical tufts of white hairs ..... 5
- Ventral 2 anthers with apical tufts of white hairs ..... 7
- 5(4) Leaves lobed ..... *C. lobata* subsp. *lobata*
- Leaves pinnately divided ..... 6
- 6(5) Hypanthium campanulate; dorsal anthers 15–16 mm long ..... *C. mauiensis*
- Hypanthium obconic; dorsal anthers 9.5–12 mm long ..... *C. magnicalyx*
- 7(4) Corolla laterally compressed ..... *C. scabra*
- Corolla tubular, round in cross-section ..... 8
- 8(7) Corolla blackish purple externally, 60–80 mm long, 6–11 mm wide ..... *C. macrostegia*
- Corolla white or white striped with lilac longitudinal lines, 35–55 mm long, 3–5 mm wide ..... 9
- 9(8) Corolla externally glabrous ..... *C. kauaulaensis*
- Corolla externally pubescent, sometimes only on longitudinal lines ..... 10
- 10(9) Plants unbranched or sparingly branched from base, 0.5–1.5 m tall; corolla pubescent, without murications ..... *C. kunthiana*
- Plants branched above base, 1.5–3 m tall; corolla pubescent along longitudinal lines, muricate ..... 11
- 11(10) Leaves pinnately divided, cut  $\frac{3}{4}$  to  $\frac{7}{8}$  the distance to the midrib ..... *C. asplenifolia*
- Leaves shallowly lobed, cut less than  $\frac{1}{4}$  the distance to the midrib ..... *C. heluensis*

**Phenology.** *Cyanea heluensis* has been observed beginning to flower from mid-summer through October, followed by immature, green fruit observed October to December, maturing in early January.

**Etymology.** The specific name honors Helu, a peak on Mauna Kahalawai (aka West Maui Mountains) *Lit.* scratch or count (Pukui et al. 1966); + Latin suffix *-ensis*, indicating a place of origin or growth.

**Habitat & ecology.** *Cyanea heluensis* occurs in *Metrosideros* Banks ex Gaertn. Lowland Wet Forest (Wagner et al. 1990). The common associated woody elements are

species of *Cheirodendron* Nutt. ex Seem., *Clermontia* Gaud., *Coprosma* J.R. Forst. & G. Forst., *Cyrtandra* J.R. Forst. & G. Forst., *Dubautia* Gaud., *Hydrangea* L., *Ilex* L., *Kadua* Cham. & Schltdl., *Myrsine* L., *Perrottetia* Kunth, *Pipturus* Wedd., *Psychotria* L., and *Urera* Gaud. These taxa form a nearly closed canopy with a well-developed understory. Ferns such as species of *Asplenium* L., *Cibotium* Kaulf., *Cyclosorus* Link, *Deparia* Hook. & Grev., *Diplazium* Sw., *Dryopteris* Adans., *Elaphoglossum* Schott ex J. Sm., *Microlepia* C. Presl, *Pteris* L., *Sadleria* Kaulf., *Sticherus* C. Presl, *Tectaria* Cav., and *Vandenboschia* Copel. are prevalent and form a dense ground cover. *Freycinetia arborea* Gaud. is a widespread liana, and several herbaceous species of *Peperomia* Ruiz & Pav. are also present. The sedges *Machaerina angustifolia* (Gaudich.) T. Koyama and *Rhynchospora sclerioides* Hook. & Arnott are also frequent. Herbaceous *Hillebrandia sandwicensis* Oliv. and *Gunnera petaloidea* Gaudich. are distinctive elements of this plant community.

*Cyanea macrostegia* Hillebr. has been observed infrequently in the general area. *Cyanea elliptica* (Rock) Lammers, *C. kauaulaensis* H. Oppenh. & Lorence, and *C. scabra* Hillebr. occur to the north, along Kaua`ula Stream. These species are nearly 1500 m away and 500 m or more lower in elevation than *C. heluensis* which is located near the ridgetop. Based on genetic analysis and morphology none of these other taxa appear to be potential hybrid parents. The known populations on Mauna Kahalawai of *Cyanea asplenifolia*, one of its two closest relatives, are 3,700 m and over 10,000 m away. Populations on Haleakala, East Maui in Makawao Forest Reserve and Haleakala National Park are 40 kilometers and 70 kilometers to the east. Extant populations of *Cyanea duvalliorum* Lammers & H. Oppenh., the other member of this clade, occur nearly 50 kilometers away on Haleakala. Since these species are also known to be ornithophilus, and populations and densities of native birds have dramatically declined, it is unlikely *C. heluensis* is of hybrid origin.

Soil is of typical basaltic origin derived from the original shield-building Wailuku Volcanic Series (Stearns and MacDonald 1942) with average annual rainfall approximately 3000 mm. (Giambelluca et al. 1986).

The only known plant occurs in deep shade on the upper rim of the steep south side of the large amphitheater-headed Kaua`ula Valley, with a windward aspect. Recently, several other previously undescribed taxa have been discovered in the area, including *Cyanea kauaulaensis* H. Oppenheimer & Lorence (Oppenheimer and Lorence 2012), *Hibiscadelphus stellatus* H. Oppenheimer, Bustamente & Perlman (Malvaceae) (Oppenheimer et al. 2014), and *Stenogyne kauaulaensis* K.R. Wood & H. Oppenheimer (Lamiaceae) (Wood and Oppenheimer 2008). Kaua`ula Valley is an important site not only botanically, but economically (as a water source) as well as culturally and spiritually for Native Hawaiians.

**Conservation status.** *Cyanea kauaulaensis* should be considered Critically Endangered due to its limited range, low population numbers, lack of population structure with no seedling recruitment, probable loss and decline of most or all of its avian pollinators and dispersal agents, threats such as landslides and treefall, herbivory by alien slugs and rats, and competition with alien plants such as *Ageratina adenophora* (Sprengel) R.M. King & H. Robinson, *Buddleia asiatica* Loureiro, *Erigeron karvinskianus* DC, *Melinis minutiflora* P. Beauv., *Rubus rosifolius* Smith, and *Tibouchina herbacea* (DC)

Cogn. When evaluated using the World Conservation Union (IUCN) criteria for endangerment (IUCN 2001), *Cyanea heluensis* easily falls into the Critically Endangered (CR) category, which designates species facing a very high risk of extinction in the wild. The CR designation is met when any of the criteria A to E are met. Both Criterion B1 (Extent of Occupancy or EOO) and B2 (Area of Occupancy or AOO) are met with an EOO of less than 100 km<sup>2</sup> and an AOO of less than 10 km<sup>2</sup>. Criterion D, population size estimated to be fewer than 50 wild individuals is easily met since there is only a single known individual. Criteria A & C address decline in population for which there is no data, although with only a single individual it could reasonably be inferred that this species is in decline. No quantitative analysis predicting the likelihood of extinction (Criterion E) was conducted. The alphanumeric formula CR B1a(I,ii,iv,v)+B2a,b(i,ii,iv,v) represents the current status under IUCN guidelines. Furthermore, *Cyanea heluensis* should be considered by the US Fish & Wildlife Service as a Candidate for listing as Endangered under the Endangered Species Act of 1973, and a Recovery Plan written, funded, and implemented.

**Conservation efforts.** Despite several attempts to locate other populations or individuals elsewhere on Helu and adjacent Kaua'ula and Launiupoko Valleys, including the use of ropes and technical gear, only one single plant has ever been observed. Efforts were made shortly after its discovery to collect mature fruit, including covering flowers with protective nylon mesh bags. These efforts failed due to the predation of the exposed peduncles by non-native slugs (e.g. *Derocerus laevis*). A few short lower branches were collected but only a single one was successfully rooted at the Olinda Rare Plant Facility on Maui, but later died. In 2013, the health and vigor of the plant had declined significantly, but in 2016 it was recovering with new growth and a few new shoots initiated along the main stems. Poor weather prevented helicopter access in late 2017 and early 2018 in attempts to obtain mature fruit. In July of 2018, the plant was showing signs of increased vigor with three ramettes beginning to flower, and several smaller side shoots. Trapping for rats is ongoing, likewise the manual control of weeds. The last flowers of the season and very immature fruit were observed in October of 2018 and October of 2019. A hormone paste was successfully applied in 2018 to the stems to induce branching; three lateral shoots were collected in October of 2019 and sent to the Olinda Rare Plant Facility and Lyon Arboretum Micropropagation Lab on O'ahu. One of these has successfully rooted at Olinda Rare Plant Facility, but the Lyon Arboretum material failed (C. Yamamoto pers. comm.). In January of 2019, a single mature fruit was collected, so resolving which of Givnish's two clades this new species belongs to. Only a single seed germinated, but it is healthy and continues to grow at the Olinda Rare Plant Facility (Fig. 4) along with the rooted cutting. The Maui Invasive Species Committee (MISC) has been working to control the *Cortaderia jubata* (Lem.) Stapf infestation in adjacent Kaua'ula Valley, and on the surrounding vertical cliffs. The region has for the most part escaped the ravages of introduced feral ungulates due to the extremely rugged topography. However, there has been a small incursion of feral goats and feral pigs approximately 1.5 km to the west and northwest at lower elevation. The Mauna Kahalawai Watershed Partnership (formerly West Maui Mountains Watershed Partnership) has been working to mitigate this incipient yet potentially severe threat.

This new species is a target of the Plant Extinction Prevention Program (PEPP), easily meeting the threshold of 50 wild individuals or less for inclusion. The Program strives to collect seeds or cuttings from every individual plant on the list, with propagation of nursery stock, restoration outplantings into appropriate habitat, and *ex situ* seed storage and living collections being the main goals.

## Acknowledgements

The author extends his deepest gratitude to Makila Land Co. and the Hawai'i Dept. of Land & Natural Resources Division of Forestry & Wildlife for permission to access the study area; Mauna Kahalawai Watershed Partnership staff Palani Wright, Kyle Alreck, and Marcus Richter for field assistance; Jennifer Higashino (US Fish & Wildlife Service) for field assistance; everyone at Windward Aviation, Inc. for helicopter services allowing us to access the area; horticulturist Anna Palomino at the Olinda Rare Plant Facility; Nellie Sugii and Cindy Yamamoto at Lyon Arboretum; and especially PEP Program staff Steve Perlman, Joel Q. C. Lau, Keahi Bustamente and Matt Padgett. Anna Palomino generously produced the botanical illustration. Much appreciation is extended to Steve Hunter and Tom Givnish at Univ. Wisconsin, Madison for sharing the genetic analysis. Finally, the author is indebted to Barbara Kennedy, the collections manager at the Bernice P. Bishop Museum *Herbarium Pacificum* (BISH) herbarium for access to specimens for study, and to Tim Flynn, collections manager at the National Tropical Botanical Garden (PTBG) herbarium for the handling, curation, accession and repository of specimens. This manuscript was greatly improved by review by Dr. David Lorence at National Tropical Botanical Garden and two anonymous reviewers. Dr. Clifford Morden at the University of Hawai'i also reviewed the manuscript and suggested changes that have been incorporated. The Plant Extinction Prevention Program is funded by the U.S. Fish & Wildlife Service, and the State of Hawai'i Dept. of Land & Natural Resources, Division of Forestry and Wildlife.

## References

- Gaudichaud-Beaupré C (1829) Voyage autour de monde, enterpris par ordre du roi., exécuté sur les corvettes de S. M. l'Uranie et la Physicienne, pendant les années 1817, 1818, 1819 et 1820; publiés ...par M. Louis de Freycinet. Botanique. Part 11. Pillet-ainé, Pairs, 433–464.
- Giambelluca TW, Nuller D, Schroeder TA (1986) Rainfall atlas of Hawai'i. Dept. of Land and Natural Resources, Honolulu.
- Givnish TJ, Millam KC, Theim TT, Mast AR, Patterson TB, Hipp AL, Henss JM, Smith JF, Wood KR, Sytsma KJ (2008) Origin, adaptive radiation, and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society of London, Series B* 276: 407–416. <https://doi.org/10.1098/rspb.2008.1204>

- Givnish TJ, Sytsma KJ, Smith JF, Hahn WJ (1995) Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). In: Wagner WL, Funk VA (Eds) Hawaiian biogeography, evolution on a hot spot archipelago. Smithsonian Institution Press, Washington & London, 288–337.
- Hillebrand W (1888) Flora of the Hawaiian Islands. 1965 reprint, Hafner Publishing Co., New York & London.
- IUCN (2001) IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland and Cambridge, 30 pp.
- Lammers TG (1990) Campanulaceae. In: Wagner WL, Herbst DR, Sohmer SH (Eds) Manual of the Flowering Plants of Hawai'i. University of Hawai'i Press & Bishop Museum Press, Honolulu, 420–489.
- Lammers TG (2004) Five new species of the endemic Hawaiian genus *Cyanea* (Campanulaceae: Lobelioideae). *Novon* 14: 84–101.
- Lammers TG, Lorence DH (1993) A new species of *Cyanea* (Campanulaceae: Lobelioideae) from Kaua'i, and the resurrection of *C. remyi*. *Novon* 3(4): 431–436. <https://doi.org/10.2307/3391390>
- Oppenheimer HL, Bustamente KM, Perlman SP (2014) A new species of *Hibiscadelphus* Rock (Malvaceae, Hibisceae) from Maui, Hawaiian Islands. *PhytoKeys* 39: 65–75. <https://doi.org/10.3897/phytokeys.39.7371>
- Oppenheimer H, Lorence DH (2012) A new species of *Cyanea* (Campanulaceae: Lobelioideae) from Maui, Hawaiian Islands. *PhytoKeys* 13: 15–23. <https://doi.org/10.3897/phytokeys.13.3447>
- Pukui MK, Elbert SH, Mookini ET (1966) Place names of Hawai'i. University of Hawai'i Press, Honolulu.
- Rock JF (1919) A monographic study of the Hawaiian species of the tribe Lobelioideae, Family Campanulaceae. Bishop Museum Press, Honolulu, 1977 Krauss Reprint Co., Millwood, New York.
- Spork-Koehler MJ, Koehler TB, Marquez SN, Waite M, Williams AM (2015) A new species of *Cyanea* (Campanulaceae, Lobelioideae), from the Ko'olau Mountains of O'ahu, Hawaiian Islands. *PhytoKeys* 46: 45–60. <https://doi.org/10.3897/phytokeys.46.8694>
- Stearns HT, MacDonald GA (1942) Geology and ground-water resources of the island of Maui, Hawaii. U.S. Geological Survey Bulletin 7, Advertiser Publishing Co. Ltd., Honolulu.
- Wagner WL, Herbst DR, Sohmer SH (1990) Manual of the flowering plants of Hawai'i. 2 vols. University of Hawai'i Press & Bishop Museum Press, Honolulu.
- Wimmer FE (1943) Campanulaceae-Lobelioideae. I. *Pflanzenreich* IV. 276b (Heft 106): 1–260.
- Wood KR, Oppenheimer H (2008) *Stenogyne kauaualaensis* (Lamiaceae), a new species from Maui, Hawaiian Islands. *Novon* 18(4): 544–549. <https://doi.org/10.3417/2008053>



# An amazing new *Capsicum* (Solanaceae) species from the Andean-Amazonian Piedmont

Gloria E. Barboza<sup>1</sup>, Carolina Carrizo García<sup>2</sup>, Marisel Scaldaferrro<sup>3</sup>, Lynn Bohs<sup>4</sup>

**1** Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Córdoba, Argentina & Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina **2** Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Córdoba, Argentina & Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria **3** Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Córdoba, Argentina & Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina **4** School of Biological Sciences, University of Utah, Salt Lake City, UT, USA

Corresponding author: Gloria E. Barboza ([gbarboza@imbiv.unc.edu.ar](mailto:gbarboza@imbiv.unc.edu.ar))

---

Academic editor: S. Knapp | Received 19 August 2020 | Accepted 25 October 2020 | Published 20 November 2020

**Citation:** Barboza GE, Carrizo García C, Scaldaferrro M, Bohs L (2020) An amazing new *Capsicum* (Solanaceae) species from the Andean-Amazonian Piedmont. *PhytoKeys* 167: 13–29. <https://doi.org/10.3897/phytokeys.167.57751>

---

## Abstract

*Capsicum regale* Barboza & Bohs, **sp. nov.**, a new species from the tropical wet forests of the eastern Andean slopes (Colombia, Ecuador, and Peru) is described and illustrated. This new species belongs to the Andean clade (all species  $2n = 26$ ) of *Capsicum* and is similar to *C. longifolium* Barboza & S.Leiva in its glabrescence, calyx morphology, and corolla and seed color but differs in its membranous and elliptic leaves, fleshy calyces, deeper stellate corollas, longer filaments, longer and purple fruiting pedicels, purple berries, and larger seeds. Its chromosome number was counted ( $2n = 26$ ), a preliminary assessment of conservation status is given and discussed, and an updated identification key to the species of the Andean clade is provided.

## Keywords

Andean clade, *Capsicum*, chromosomes, phylogeny, South America, taxonomy

## Introduction

*Capsicum* L. (Capsiceae, Solanaceae), the chili pepper genus, consists of approximately 42 species distributed in temperate and tropical Central and South America, Mexico and the West Indies (Barboza et al. 2020). It includes five species cultivated worldwide

as vegetables, spices, and medicines (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L. and *C. pubescens* Ruiz & Pav.). *Capsicum* peppers are major crops worldwide, and along with potato, tomato, and eggplant in the genus *Solanum* L., are amongst the most economically important members of the Solanaceae (Samuels 2015).

The Andes are one of the main centers of diversity for *Capsicum*, where new species continue to be discovered (Nee et al. 2006; Barboza et al. 2019). Approximately 50% of the species (ca. 20 species) occur in tropical Andean forests or in dry inter-Andean valleys (Barboza et al. in prep.). The tropical montane forest ecoregion is located on the slopes of the Andes extending north to south from southern Colombia, through Ecuador, and into northern Peru (WWF 2020). This region is characterized by a lush vegetation with evergreen seasonal broad-leaved forests and a rich fauna (Stewart et al. 2020; WWF 2020). It is one of the most biologically diverse ecosystems in the world (Gentry 1992; Bruijnzeel et al. 2010; Tapia-Armijos et al. 2015) with a high level of species endemism (Myers et al. 2000). Khoury et al. (2020) have demonstrated that the highlands of Colombia, Ecuador, Peru, and Venezuela represent one of the hotspots for *Capsicum* that need further investigation in terms of collecting taxa for *ex situ* conservation of the wild species.

During recent field explorations in the Colombian Cordillera Oriental (Dept. Caquetá), an atypical species of Solanaceae was collected. Despite the presence of several Solanaceae experts in the group, no one was sure what genus it belonged to. Its deeply stellate yellowish corollas, long-exserted stamens, and purple fruits and fruiting pedicels were striking and called to mind some characters of the poorly known genus *Cuatresia* Hunz., whereas its thick, triangular-compressed, and reflexed calyx appendages resembled those of some *Lycianthes* (Dunal) Hassler taxa, whose species are not well understood in Colombia. Puzzled, we provisionally named it “*Cuatresianthes*” and placed some bets on its eventual generic identity. DNA was extracted and sequenced in the Bohs lab from leaf material collected on these field trips. BLAST results indicated that the species belonged not to *Cuatresia* or *Lycianthes*, but to *Capsicum*. A preliminary molecular study placed the collection unequivocally in the Andean clade of *Capsicum*, but it did not belong to any known species. Through an exhaustive search amongst unidentified *Cuatresia* collections in herbaria, we found other specimens from Ecuador and Peru that matched our Colombian gatherings. Here, we describe this species as new to science and provide information on its morphology, distribution, karyology and phylogenetic position in the genus *Capsicum*.

## Materials and methods

Two field trips were made in Colombia (Dept. Caquetá) during 2016 and 2019. Fresh material was preserved in 70% alcohol to perform measurements of reproductive organs using a Zeiss Stemi 2000-C stereomicroscope at 6.5–50× magnification. Descriptions were based on living plants observed during field work and examination of digital images of herbarium specimens housed at the following seven herbaria: BM, COAH, COL, F, MO, QCNE, US. Seeds were also examined using scanning electron microscopy (SEM); they were prepared using enzyme etching (Lester and Durrands 1984) to

dissolve outer cell walls, affixed to aluminum stubs with double-sided adhesive tape, coated with gold, and examined using a FE-SEM Sigma (LAMARX, National University of Córdoba, Argentina) microscope.

Information about flower, fruit, and seed color was taken mainly from our own observations in the field and photographs sent by some collectors; we tested pungency in the field on immature and mature fruits.

The distribution map was produced using QGIS 3.8 (QGIS Development Team 2019) and was based on georeferenced data of all the collections analyzed. Conservation status was assessed using IUCN criteria B, geographic range in the form of B1 (EEO: extent of occurrence) and B2 (AOO; area of occupancy) (IUCN 2019). The extent of occurrence and area of occupancy were calculated using the Geospatial Conservation Assessment Tool GeoCAT (Bachman et al. 2011; GeoCAT 2020).

Somatic metaphases were examined in root tip squashes obtained from germinated seeds. The root apices were fixed in 3:1 ethanol: acetic acid mixture for 12 hr after a pretreatment in 2 mM 8-hydroxyquinoline solution for two hr at room temperature and two hr at 4 °C. The material was kept at -20 °C until examination. The root tips were macerated in pectinase-cellulase solution (Moscone et al. 1993), and chromosomes were stained with 4'-6-diamidino-2-phenylindole (DAPI) (Schweizer and Ambros 1994). Metaphase chromosomes were observed and photographed with epifluorescence using an Olympus BX61 microscope equipped with the appropriate filter sets (Olympus, Shinjuku-ku, Tokyo, Japan) and a JAI CV-M4 + CL monochromatic digital camera (JAI, Barrington, N.J., USA). Three individual seeds from the collection *Orejuela et al. 3034* were germinated and grown until root tips were produced, and 10 cells from each seedling were studied in metaphases.

Phylogenetic affinities were explored using DNA sequences from four markers, namely: the intergenic spacers *psbA-trnH*, *ndhF-rpl32* and *trnL-trnF* from the plastid genome, and the single-copy nuclear gene *waxy* (GBSSI, granule-bound starch synthase, exons 2 to 10). Representatives of different clades recognized within *Capsicum* and several outgroup species were included. Genomic DNA of *C. regale* was extracted from silica-gel dried leaves using the Qiagen DNeasy Plant mini kit (Qiagen Inc., Valencia, California, EUA) and a modified CTAB protocol. Most sequences included in this study were used in previously published analyses and therefore were retrieved from GenBank, except for a few sequences from outgroup species (see Suppl. material 1: Table S1), for which DNA extracts were already available. Amplification and sequencing protocols for the markers used were as in Carrizo García et al. (2016, 2020) and Barboza et al. (2019). PCR amplicons were sequenced on an automated capillary sequencer [University of Vienna (Vienna, Austria), and the University of Utah HSC Core Research Facility (Salt Lake City, Utah, USA)]. A single concatenated dataset was assembled in MEGA 7 (Kumar et al. 2016). Phylogenetic reconstructions were done using maximum parsimony [MP, in PAUP\* 4.0b10 (Swofford 2003)], maximum likelihood [ML, in RAxML v8.2.10 (Stamatakis 2014)] and Bayesian inference [BI, in MrBayes 3.2.2 (Ronquist et al. 2012)] approaches as in Carrizo García et al. (2016, 2020). The GTR+R nucleotide substitution model was selected a priori following the Akaike Information Criteria in jModelTest 2.1.3 (Darriba et al. 2012) for ML and BI analyses.

## Taxonomic treatment

### *Capsicum regale* Barboza & Bohs, sp. nov.

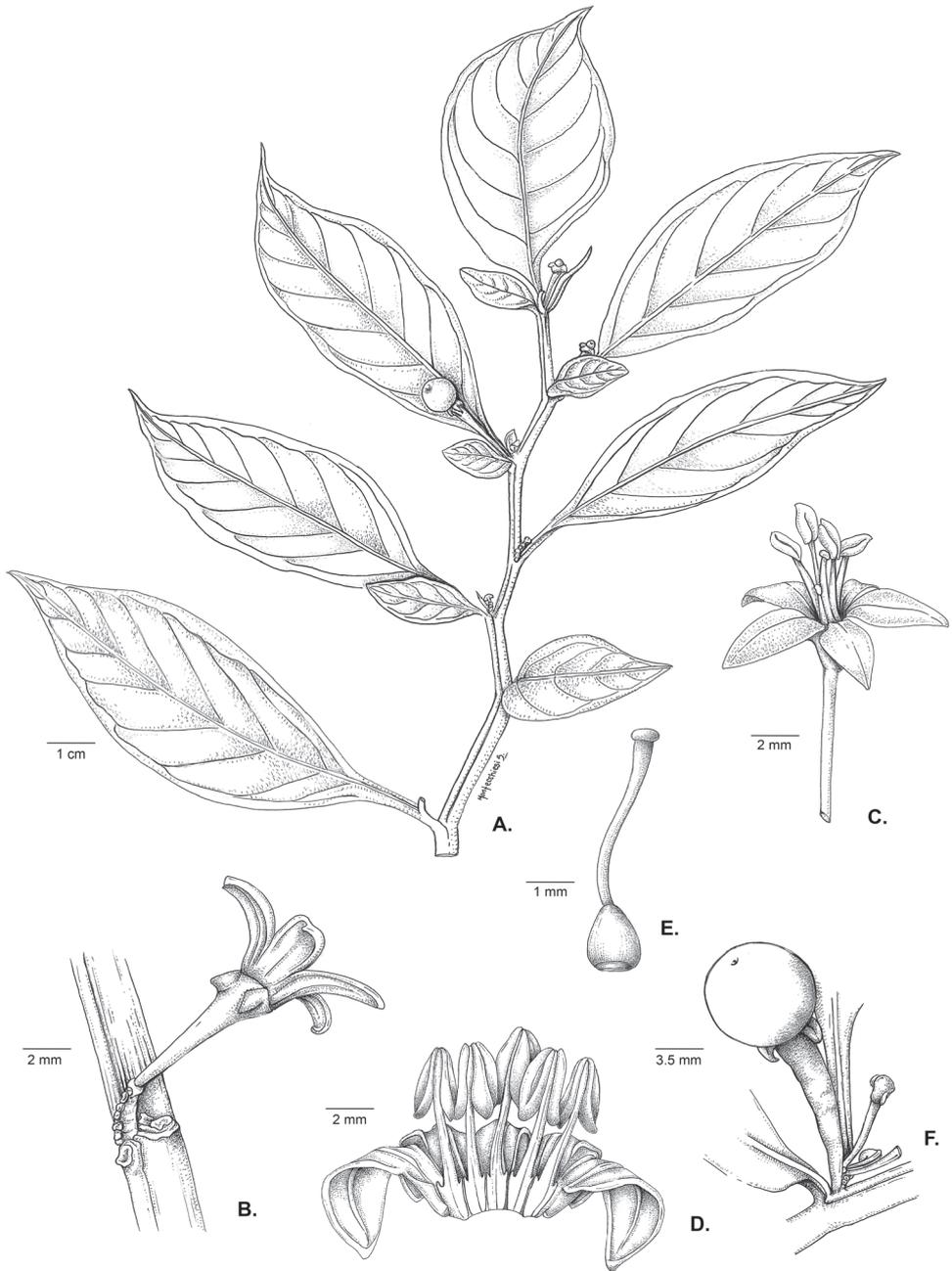
urn:lsid:ipni.org:names:77212951-1

Figs 1–3

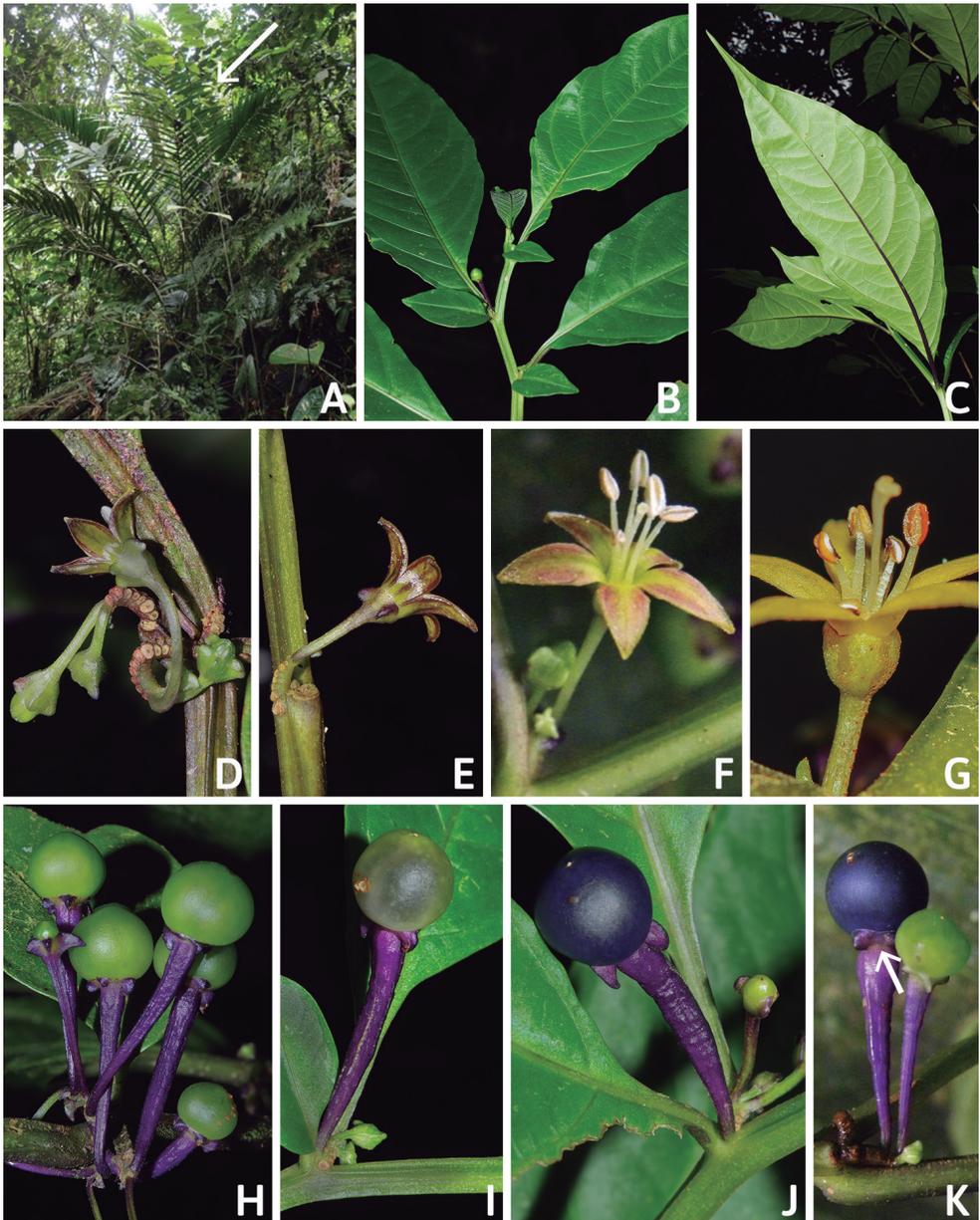
**Diagnosis.** *Capsicum regale* is morphologically most similar to *C. longifolium* Barboza & S.Leiva, but the former differs in having membranous and elliptic leaves, fleshy calyces, more deeply stellate corollas, longer filaments, longer and purple fruiting pedicels, dark blue to purple berries, larger seeds, smooth seed coats, and spine-like projections along the seed margins.

**Type.** COLOMBIA. Caquetá: Mun. Florencia, Corregimiento El Caraño, Finca de Don Isauro, camino al río, en interior de bosque fuertemente inclinado, 01°44'10.6"N, 75°40'78.3"W, 1004 m, 22 Aug 2019 (fl, fr), A. Orejuela, L. Bohs, G.E. Barboza, P. González, R. Deanna, J. Urdampilleta, J. Valencia & G. Sierra 3034 (holotype: COL; isotypes: COAH, CORD, HUAZ [to be distributed]).

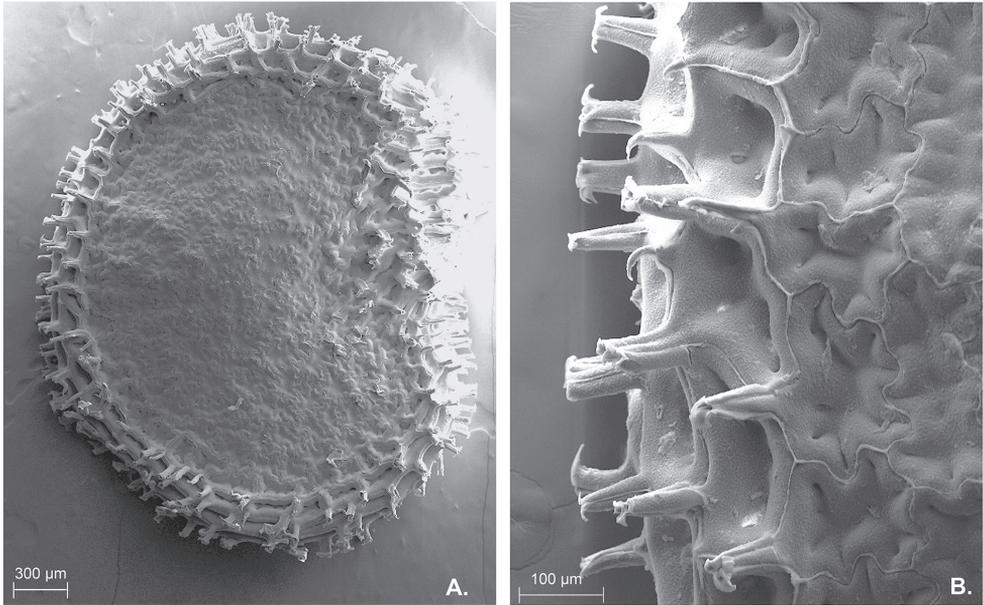
**Description.** Slender shrubs (1–) 1.8–2.5 (–3) m tall, with the main stem somewhat thick, ca. 0.8 cm in diameter at base, sparsely branched toward apex, the branches dichotomous, weak, spreading horizontally. Stems solid and terete at base, the young stems pale green, glossy, striate, glabrous, the nodes green; bark of older stems dark brown, glabrous; lenticels present. Sympodial units difoliate, geminate, the leaf pairs markedly differing in size. Leaves simple, membranous, slightly discoloured, green adaxially, pale green with the midvein prominent and purple and the secondary veins lilac or green abaxially; adaxial and abaxial surfaces glabrous; major leaves with blades 17–20 (–24) cm long, 4.7–8 (–9.2) cm wide, elliptic, the major veins 6–8 on each side of midvein, the base unequal and attenuate, the margins entire and glabrous, the apex apiculate to long-apiculate; petioles (0.8–) 1.5–2.3 cm long, green adaxially and purple abaxially, glabrous; the minor leaves 2–5 cm long, 1–3 cm wide, ovate, the major veins 3–5 on each side of midvein, the base unequal, the margins entire, glabrous, the apex obtuse; petioles 0–0.4 cm long, green, glabrous. Inflorescence ca. 10 mm long, unbranched or rarely shortly forked, with 5–13 flowers, the axes glabrous; peduncle 0–5.5 mm; rachis 4.5–6 mm long; pedicels 1.2–1.4 cm long, thin, 2–3-edged, erect to spreading, straight, purple to green, glabrous, nearly contiguous, articulated at the base, leaving conspicuous scars. Buds ellipsoid, green. Flowers 5-merous, all perfect. Calyx 2–3 mm long, ca. 2 mm wide, cup-shaped, fleshy, green or greenish purple, the margin truncate, circular in outline, glabrous, the appendages (0–) 4–5, 1–1.8 mm long, 0.8–1.1 mm wide, purple, thick, triangular-compressed, reflexed, inserted very close to the margin. Corolla 7–8 mm long, ca. 10 mm in diameter, deeply stellate, thick, with narrow interpetalar tissue, pure yellow or yellow with maroon pigmentation abaxially and greenish yellow with lobes marginally maroon adaxially, glabrous, the tube 2–2.5 mm long, the lobes 5–5.5 mm long, ca. 2 mm wide, triangular, the tips papillose,



**Figure 1.** *Capsicum regale* Barboza & Bohs. **A** fruiting apical branch **B** unbranched inflorescence **C** flower, in lateral view **D** opened corolla **E** gynoecium **F** fruit. From Orejuela *et al.* 3034. Drawn by S. Montecchiesi.



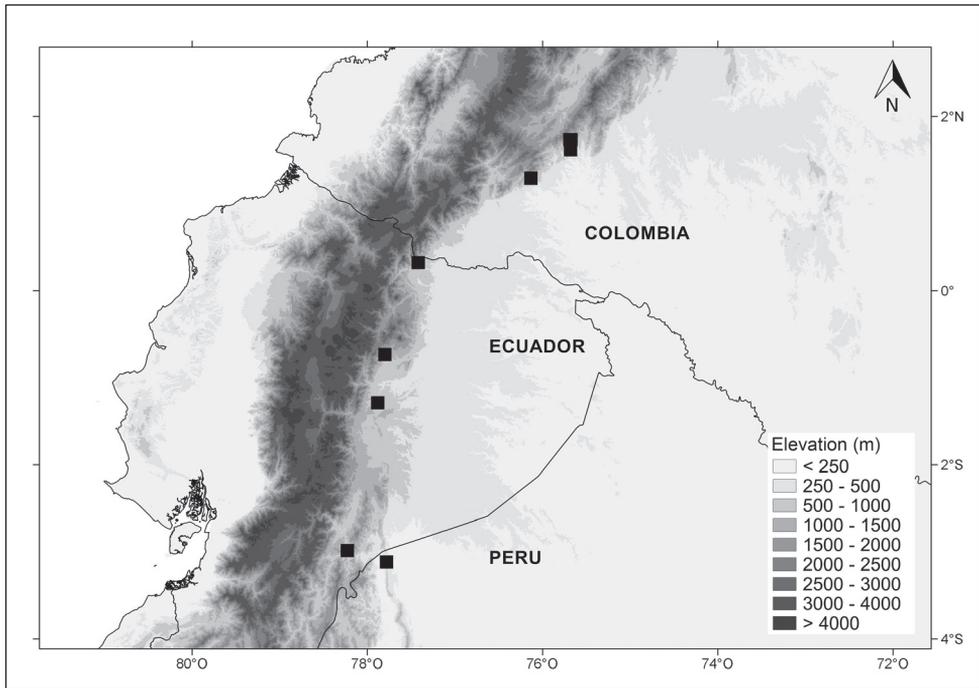
**Figure 2.** *Capsicum regale* Barboza & Bohs. **A** habitat **B** apical branch, showing anisophyllous leaf pairs **C** abaxial surface of leaf with purple main vein **D** forked inflorescence; note the scars of the deciduous flowers **E** flower, in lateral view, on a unbranched elongate inflorescence **F, G** Flowers with and without pigmentation respectively **H–K** various stages of fruit maturity, in **K** mature fruit showing the constriction between the pedicel and the berry (arrow) **A–F, H–K** from Orejuela et al. 3034 (photos by A. Orejuela, P. Gonzáles, and G. Barboza) **G** from Hoyos 127 (photo by L. Coca).



**Figure 3.** Seed of *C. regale* Barboza & Bohs viewed under SEM. A Seed B Seed coat sculpture. From Orejuela et al. 3034.

the margins with short eglandular trichomes. Stamens subequal, one filament longer than the others; long filament 3.5–4.3 mm long, shorter filaments (2) 3–3.2 mm long, white, glabrous, inserted on the corolla ca. 1 mm from the base, with inconspicuous auricles; anthers ca. 2 mm long, elliptic, not connivent, the thecae lilac or pale bluish, opening into longitudinal slits. Ovary ca. 1.3 mm long, ca. 1 mm in diameter, light green, ovoid, glabrous; nectary ca. 0.4 mm high, paler than the ovary, conspicuous; style 4.3–4.5 mm long, white, clavate, glabrous; stigma ca. 0.1 mm long, ca. 0.8 mm wide, light green, globose or somewhat discoid. Fruit a berry, globose, 6–9 mm in diameter, green when immature, turning nearly white and translucent during transition to maturity, then becoming dark blue to purple when mature, glabrous, non-pungent, the pericarp opaque, without giant cells, the endocarp smooth; stone cells absent; fruiting pedicels ca. 1.8 cm long, 1.8–2 mm in diameter proximally, 2.5–2.6 mm in diameter distally, brilliant dark purple, erect, fleshy, slightly angled and strongly thickened distally; fruiting calyx 3.75–4.25 mm in diameter, persistent, not accrescent, discoid, brilliant purple, with a conspicuous annular constriction at the junction with the swollen pedicel, the appendages reflexed, brilliant purple, fleshy and laterally compressed. Seeds 7–17 per fruit, 2.7–3.4 mm long, 2.2–2.7 mm wide, flattened, C-shaped, black, the seed coat smooth except for small spine-like projections on the seed margin, the cells irregular in shape to polygonal at seed margins, the lateral walls sinuate to straight.

**Distribution.** *Capsicum regale* occurs in southern Colombia, eastern Ecuador, and northern Peru, known mainly on the eastern slopes of the Andes (the Andean-Amazonian Piedmont), between 700–1900 m elevation (Fig. 4).



**Figure 4.** Distribution of *Capsicum regale* Barboza & Bohs.

**Ecology.** The small populations inhabit the understory of the premontane or montane humid tropical forests of the Amazonian slopes of the Andes.

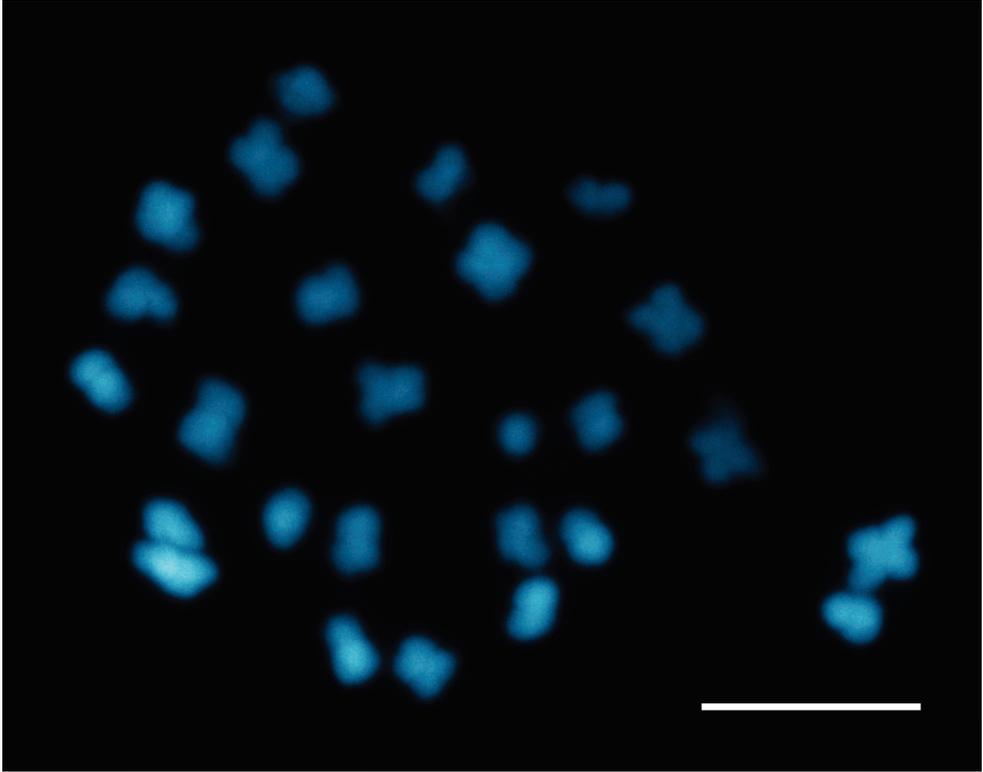
**Phenology.** The species has been collected in flower and fruit in April and from August to December.

**Etymology.** The specific epithet comes from the Latin *regalis*, royal or regal, referring to the regal, princely, or magnificent appearance of this special plant and also making reference to the royal purple color that suffuses the leaves, fruits, and fruiting pedicels.

**Preliminary assessment of conservation status.** Assessment using the IUCN Red List Criteria (IUCN 2019) suggests a status of Endangered (EN) B2ab(iii) for *C. regale*. Although this species has an extent of occurrence (EOO) of 47,806.378 km<sup>2</sup>, its area of occupancy (AOO) is calculated to be 32 km<sup>2</sup> (criterion B2 < 500 km<sup>2</sup>), and the habitat quality has experienced a continuing decline, especially associated with fragmentation and deforestation.

**Chromosome number.** The somatic chromosome number found in *C. regale* is  $2n = 2x = 26$  (Fig. 5), as for all of the species of the Andean clade (Scaldeferro and Moscone 2019; Barboza et al. 2019).

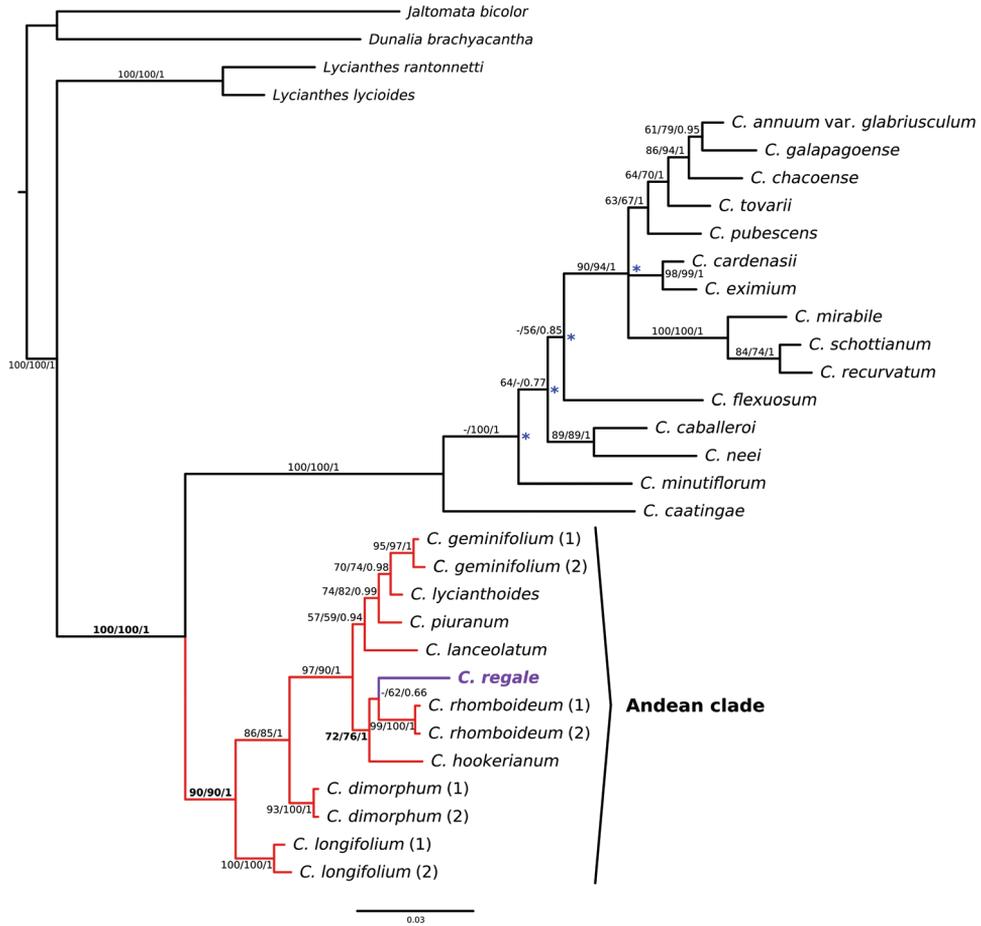
**Phylogenetic affinities.** *Capsicum regale* is strongly resolved within the Andean clade of *Capsicum* in all analyses. Within the Andean clade, *C. regale* is moderately supported in a clade with *C. rhomboideum* and *C. hookerianum*. Within this clade, it is weakly supported as sister to *C. rhomboideum* (Fig. 6).



**Figure 5.** Mitotic metaphase chromosomes of *Capsicum regale* Barboza & Bohs,  $2n = 26$ . Scale bar: 10  $\mu\text{m}$

**Specimens examined.** COLOMBIA. Caquetá: Mun. Florencia, Corregimiento El Caraño, Km 20, finca Las Brisas, propiedad de Isauro Trujillo,  $01^{\circ}44'11.80''\text{N}$ ,  $75^{\circ}40'37.8''\text{W}$ , 1002 m, 7 Oct 2017 (fl, fr), *D. Hoyos, E. Trujillo & J. Sánchez* 118 (COAH, COL); same locality, 9 Dec 2017 (fl, fr), *D. Hoyos, M. Cuellar & F. Vallejo* 146 (COL); Finca de don Isauro, camino al río, en interior de bosque fuertemente inclinado,  $01^{\circ}44'01.4''\text{N}$ ,  $75^{\circ}40'35.4''\text{W}$ , 1000 m, 16 Apr 2016 (fl, fr), *A. Orejuela, L. Bohs, G.E. Barboza, E. Trujillo, J. D. Tovar & J. Castillo* 2640 (COL); same locality,  $01^{\circ}44'09.1''\text{N}$ ,  $75^{\circ}40'40.3''\text{W}$ , 932 m, 22 Aug 2019 (fl, fr), *A. Orejuela, L. Bohs, G.E. Barboza, P. González, R. Deanna, J. Urdampilleta, J. Valencia & G. Sierra* 3035 (COL); finca Las Brisas, debajo de la casa, vereda La Cascada,  $01^{\circ}37'5''\text{N}$ ,  $75^{\circ}40'50''\text{W}$ , 1000 m, 7 Nov 2015 (fl, fr), *D. Sanín* 6236 (COL); Mun. San José del Fragua, vereda La Peneya-camino hacia El Jardín, zona amortiguadora PNN Alto Fragua Indi Wasi,  $01^{\circ}17'31''\text{N}$ ,  $76^{\circ}08'0.64''\text{W}$ , 700–850 m, 23 Oct 2017 (fl, fr), *D. Hoyos et al.* 127 (COAH, COL).

ECUADOR. Morona-Santiago: along new road Mendez-Morona, km 30–35, 800 m, 18 Aug 1989 (fl, fr), *H. van der Werff & E. Gudiño* 11196 (BM, MO, QCNE). Napo: Archidona Cantón, Reserva Ecológica Antisana, Comunidad Shamato, entrada por km 21-Shamato,  $00^{\circ}44'\text{S}$ ,  $77^{\circ}48'\text{W}$ , 1700 m, 27 Apr 1998 (fl), *J. L. Clark et*



**Figure 6.** Bayesian majority-rule consensus tree of *Capsicum* showing the placement of *C. regale* Barboza & Bohs. The Andean clade is highlighted in colored branches. Support values are indicated by each branch (bootstrap support maximum parsimony/ bootstrap support maximum likelihood/posterior probabilities; dashes indicate support values < 50%). Key support values that indicate the position of *C. regale* are shown in bold. Asterisks indicate different resolutions using maximum parsimony.

*al.* 5337 (BM, MO); Parroquia Ahuano, Estación Biológica Jatun Sacha, 8 km E of Misahuallí, Finca Acaro, 01°17'17"S, 77°52'54"W, 910 m, 17 Aug 2005 (fl, fr), *J. L. Clark et al.* 9403 (BM, US). Sucumbíos: Río Bermejo to Cerro Sur Pax, Cofan community of Alto Bermejo, NW between Lumbaqui and Cascales, vicinity of Oso Ridge Camp, 00°19'17.7"N, 77°25'10"W, 1700–1920 m, 2 Aug 2001 (fr), *R. Aguinda et al.* 1537 (F).

PERU. Loreto: Datem del Marañón, Morona District, Pongo Chinim, valley between the eastern and western ridges of the Kampankis range, ca.14 km south of the Peru-Ecuador border, 3 Aug 2011 (fl, fr), *I. Huamantupa 15251* (V0387079F color photo, F).

## Discussion

*Capsicum regale* belongs to the Andean clade of *Capsicum* (Carrizo García et al. 2016; see below). It is a very striking species due to its unbranched (Figs 1B, 2E, J) or forked inflorescence (Fig. 2D) with 5–13 deciduous flowers on an elongate rachis (Fig. 2D), fleshy and laterally compressed calyx appendages (Fig. 2D, E), deeply stellate corollas (Fig. 2F, G), strongly thickened and brilliant purple fruiting pedicels (Fig. 2H–K), dark blue to purple fruits (Fig. 2J, K), and flattened black seeds with spine-like projections at the margins (Fig. 3). This species is morphologically most similar to *C. longifolium* (Barboza et al. 2019) with which it shares lack of pubescence, multi-flowered inflorescences, yellow corollas, laterally compressed calyx appendages, and black seeds (see contrasting characters in the key below).

*Capsicum regale* possesses unusual characters of the genus. Normally, *Capsicum* species have unbranched inflorescences lacking peduncles, with the flowers solitary or congested on a very short axis. Flowers can be arranged on a short or relatively elongated rachis in a few species, e.g., *C. rhomboideum* (Dunal) Kuntze, *C. coccineum* (Rusby) Hunz., *C. lycianthoides* Bitter (Barboza pers. obs.), *C. longifolium* (Barboza et al. 2019), and *C. regale*, but none of them have short peduncles or forked inflorescences as occurs occasionally in *C. regale*. In most *Capsicum* species the calyx appendages, when present, are usually cylindrical or subulate, and green-colored. It is very rare to find laterally compressed calyx appendages that appear as wing-like structures, as occur in *C. longifolium* (Barboza et al. 2019), in some plants of *C. dimorphum* (Miers) Kuntze (Barboza, pers. obs.), and in *C. regale*. Stellate corollas lobed about halfway to the base are common in the genus; exceptions to this are found in *C. benoistii* Barboza (Barboza et al. 2019) and *C. regale*, both of which have deeply stellate corollas lobed more than halfway to the base. In most *Capsicum* species, the fruiting pedicels and fruiting calyx are generally green or green with purple tones or lines; only *C. caatingae* Barboza & Agra (Carrizo García et al. 2016) and sometimes *C. dimorphum* and *C. geminifolium* (Dammer) Hunz. (Jarret et al. 2019) have pedicels and calyces uniformly violet-colored, while those of *C. regale* are uniformly purple-colored. An unusual constriction at the junction of the thickened fruiting pedicels with the fruiting calyx is clearly evident in *C. regale* (Fig. 2K), a character also present in some other species, i.e., *C. chinense* Jacq. (Baral and Bosland 2004), *C. caatingae* (Carrizo García et al. 2013), *C. minutiflorum* (Rusby) Hunz. (Carrizo García et al. 2016), and *C. lanceolatum* (Greenm.) C.V. Morton & Standl. (Barboza pers. obs.). The dark blue to purple fruits are unique to *C. regale* among the wild *Capsicum* species, which have red, orange-red, or greenish-golden yellow fruits at maturity (Hunziker 2001; Carrizo García et al. 2016).

Carrizo García et al. (2016) were the first to provide an extensive phylogenetic analysis of *Capsicum* using broad sampling of 34 of the approximately 35 species of the genus known at the time. They identified and named 11 well supported clades within *Capsicum*. One of these is the Andean clade, which includes species native to Central America and the Andes in northwestern South America. Morphological characters of the Andean clade species include leaves borne in anisophyllous pairs, flowering pedicels straight (not geniculate), corollas mainly yellow, fruits red to orange-red and non-pungent with the pericarp lacking giant cells, seeds black or blackish-brown,

and chromosome base numbers of  $x = 13$  (Jarret et al. 2019; Scaldaferrero and Moscone 2019). *Capsicum regale* exhibits all of these characters except for its dark blue or purple fruits and the occasional forked inflorescences, which are not known in any other wild *Capsicum* species. Molecular data from both chloroplast and nuclear regions place *Capsicum regale* within the Andean clade (Fig. 6). Although *C. regale* is morphologically most similar to *C. longifolium*, the combined molecular data places it in a clade with *C. rhomboideum* and *C. hookerianum* with moderate support. Nevertheless, its closest specific affinities need to be further studied using additional data.

*Capsicum regale* inhabits the Andean-Amazonian Piedmont, encompassing the eastern slopes of the Cordillera Oriental from southern Colombia to the Cerros de Kampanquis, the easternmost branch of the Andes in northern Peru. This area is home to a transitional ecosystem with a distinctive vegetation and biodiversity due, in part, to the juxtaposition between the Amazon basin and the Andean forests (Gentry 1992; Pitman et al. 2002); this unique biodiversity is rapidly disappearing due to intense deforestation, clearing, and fragmentation (Pitman et al. 2002; Mulligan 2010; Tapia-Armijos et al. 2015; Alvarez-B et al. 2019). Some localities where *C. regale* has been collected are protected areas (Parque Nacional Natural Alto Fragua Indi Wasi, Colombia; Reserva Ecológica Antisana, Ecuador; Estación Biológica Jatun Sacha, Ecuador), and it is expected that in these reserves it is not under serious threat. Other sites in which it occurs are susceptible to human disturbance such as crop planting and high levels of deforestation; these locations include Correg. Caraño (Caquetá, Colombia, Alvarez-B et al. 2019, our observations), Río Bermejo to Cerro Sur Pax (Sucumbíos, Ecuador, Pitman et al. 2002), and Cuenca del Río Morona, Pongo Chinim (Loreto, Peru, Pitman et al. 2012). In these areas, *C. regale* is considerably threatened, and a conservation strategy is urgently needed to protect these species-rich ecoregions.

### Artificial key to the species of Andean clade of *Capsicum*

- 1 Flowers solitary, rarely paired; pedicels (15–) 25–43 mm long; calyx with 5 subequal reflexed appendages; corolla white or yellowish-white lined with purple; Mesoamerica..... ***C. lanceolatum* (Greenm.) C.V.Morton & Standl.**
- Flowers 2–10 (–13), rarely solitary; pedicels 3–28 mm long; calyx lacking appendages or with up to 10 subequal or unequal, recurved, spreading or erect appendages; corolla pure yellow or yellowish with maroon or purple pigmentation; South America (*C. rhomboideum* also in Mesoamerica) ..... **2**
- 2 Calyx appendages absent, or appearing as 1–3 small 0.5–1.8 mm long mucronate protuberances below the margin, or well-developed, 2–5, triangular-compressed and wing-like, 2–2.5 mm long..... **3**
- Calyx appendages (2–) 5–10, subulate or linear-subulate, (0.9–) 2–7 mm long.... **5**
- 3 Plants usually pubescent, rarely glabrescent; flowers up to 5, axillary, the rachis very reduced or lacking; calyx with 0–3 small mucronate appendages 0.5–1.5 mm long ..... ***C. dimorphum* (Miers) Kuntze**
- Plants completely glabrous; flowers 3–13, on a developed rachis; calyx with 2–5 thick triangular-compressed wing-like appendages 1–2.5 mm long..... **4**

- 4 Leaves coriaceous; major leaves narrowly elliptic (ratio length/width 6–10.8); corolla stellate-campanulate, lobed about halfway to base; calyx tube membranous; stamens equal, 2–2.6 mm long; fruits 8–13 mm in diameter, orange at maturity; fruiting pedicels 1–1.6 cm long, green, pendent; fruiting calyx green-purple or green; seeds 1.7–2.3 mm long, 1.7–2.2 mm wide, not flattened, tear drop-shaped, the surface reticulate..... ***C. longifolium* Barboza & S.Leiva**
- Leaves membranous; major leaves elliptic (ratio length/width 2.5–4); corolla deeply stellate, lobed more than halfway to base; calyx tube fleshy; stamens subequal (one longer), (2–) 3–4.3 mm long; fruits 6–9 mm in diameter, dark blue to purple at maturity; fruiting pedicels ca. 1.8 cm long, brilliant dark purple, erect; fruiting calyx entirely brilliant purple; seeds 2.75–3.40 mm long, 2.25–2.70 mm wide, flattened, C-shaped, the surface smooth with small spine-like projections..  
..... ***C. regale* Barboza & Bohs**
- 5 Calyx with 8–10 unequal appendages, the longer 4–6 (–7) mm long, the shorter 1.3–4 mm long..... ***C. hookerianum* (Miers) Kuntze**
- Calyx with 2–5 equal or subequal appendages 0.9–6.5 mm long ..... **6**
- 6 Flowers 1–3, axillary; corolla long tubular-campanulate, 14.5–17 mm long, the tube 11–12 mm long, the lobes broadly ovate, 3.5–5 mm long, 4.5–5 mm wide; stone cells 2..... ***C. piuranum* Barboza & S.Leiva**
- Flowers (2–) 3–10 (–13), axillary or on a short rachis; corolla deeply stellate or campanulate to broadly campanulate, (6–) 7–15 mm long, the tube 3–12 (–15) mm long, the lobes absent or incipient to well developed, narrowly triangular or ovate to broadly ovate, (3–) 5–9 mm long, 2–5.5 mm wide; stone cells absent or 5–6 (fruits unknown in *C. benoistii*)..... **7**
- 7 Corolla deeply stellate, 12–13 mm long, the lobes narrowly triangular.....  
..... ***C. benoistii* Barboza**
- Corolla nearly entire, campanulate to broadly campanulate, (6–) 7–15 mm long, the lobes absent or incipient, ovate to broadly ovate..... **8**
- 8 Corolla campanulate, stellate in outline, with a thin interpetalar membrane connecting the lobes in the proximal half ..... ***C. geminifolium* (Dammer) Hunz.**
- Corolla broadly campanulate, pentagonal in outline, with a wide interpetalar membrane connecting the lobes up to the distal end..... **9**
- 9 Inflorescence up to 13-flowered; major leaves membranous, (4–) 4.8–12 cm long, 2–5 cm wide, ovate, elliptic, or rhomboid-ovate; corolla 6–9.5 mm long, 8–12 mm in diameter; fruits up to 0.9 cm in diameter, dark red at maturity; stone cells absent; trees or erect shrubs; trichomes simple, branched, and dendritic on the same plant..... ***C. rhomboideum* (Dunal) Kuntze**
- Inflorescences (2–) 3–8 (–10)-flowered; major leaves coriaceous, (10–) 11–22.5 cm long, (3–) 4–8.5 cm wide, ovate to broadly ovate; corolla 8–15 mm long, 15–18 mm in diameter; fruits up to 1.2 cm in diameter, bright orange or red at maturity; stone cells 0–6; scandent or slender shrub or subshrub; mostly glabrous or sparse, simple trichomes present on young stems only .....  
..... ***C. lycianthoides* Bitter**

## Acknowledgements

We are indebted to the curators and assistants of the herbaria cited who provided digital images of their collections, to E. Trujillo, A. Orejuela and C. I. Orozco for their assistance in field explorations, to S. Montecchiesi and G. Aburrá for preparing the illustrations, to V. Palchetti for her assistance with the map, to L. Coca, D. Hoyos, D. Sanín, P. Gonzáles, and A. Orejuela for providing photographs or specimen vouchers, to the reviewers for their helpful suggestions and critical reading of the manuscript, and to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 0100147 and joint project 2014-0401 PCB-FWF AI2119), the Secretaría de Ciencia y Técnica (SECyT-UNC), and the U.S. National Science Foundation ARTS program (DEB 1457366) for funding.

## References

- Alvarez-B C, Castaño D, Hoyos D, Velasco G, Peña JL, Sanín D (2019) Angiospermas no arbóreas de un bosque húmedo tropical en el piedemonte andino-amazónico colombiano. *Boletín Científico Museo de Historia Natural Universidad de Caldas* 23(2): 62–94.
- Bachman S, Moat J, Hill AW, de Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Baral JB, Bosland PW (2004) Unravelling the species dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): A multiple evidence approach using morphology, molecular analysis, and sexual compatibility. *Journal of the American Society for Horticultural Science* 129(6): 826–832. <https://doi.org/10.21273/JASHS.129.6.0826>
- Barboza GE, Carrizo García C, Leiva González S, Scaldaferrero M, Reyes X (2019) Four new species of *Capsicum* (Solanaceae) from the tropical Andes and an update on the phylogeny of the genus. *PLoS One* 14(1): e0209792. <https://doi.org/10.1371/journal.pone.0209792>
- Barboza GE, de Bem Bianchetti L, Stehmann JR (2020) *Capsicum carassense* (Solanaceae), a new species from the Brazilian Atlantic Forest. *PhytoKeys* 140: 125–138. <https://doi.org/10.3897/phytokeys.140.47071>
- Bruijnzeel LA, Kappelle M, Mulligan M, Scatena FN (2010) Tropical montane cloud forests: state of knowledge and sustainability perspectives in a changing world. In: Bruijnzeel LA, Scatena FN, Hamilton LS (Eds) *Tropical Montane Cloud Forests. Science for Conservation and Management*. Cambridge University Press, New York, 691–740. <https://doi.org/10.1017/CBO9780511778384.074>
- Carrizo García C, Sterpetti M, Volpi P, Ummarino M, Saccardo F (2013) Wild capsicums: identification and in situ analysis of Brazilian species. In: Lanteri S, Rotino GL (Eds) *Breakthroughs in the genetics and breeding of Capsicum and eggplant*. Eucarpia, Turin, 205–213.
- Carrizo García C, Sehr EM, Barfuss MHJ, Barboza GE, Samuel R, Moscone EA, Ehrendorfer F (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). *Annals of Botany* 118(1): 35–51. <https://doi.org/10.1093/aob/mcw079>

- Carrizo García C, Fernández L, Kapetanovic V, Reyes X (2020) Rare Bolivian wild chile *Capsicum esbbaughii* (Solanaceae) located again: Open ending on its identity and conservation. *Plant Systematics and Evolution* 306(5): e85. <https://doi.org/10.1007/s00606-020-01712-5>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): 772. <https://doi.org/10.1038/nmeth.2109>
- Gentry A (1992) Diversity and floristic composition of the Andean forest of Peru and adjacent countries: Implications for their conservation. *Memorias del Museo de Historia Natural* 21: 11–31. [Biogeografía, Ecología y conservación del bosque Montano en el Perú. Universidad Nacional Mayor de San Marcos, Lima]
- GeoCAT (2020) Geospatial Conservation Assessment Tool. <https://www.kew.org/science/projects/geocat-geospatial-conservation-assessment-tool> [accessed: 16 March 2020]
- Hunziker AT (2001) Genera Solanacearum. The genera of Solanaceae illustrated, arranged according to a new system. A.R.G. Gantner Verlag K.-G., Ruggell, 500 pp.
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [accessed: 16 March 2020]
- Jarret RL, Barboza GE, Da Costa Batista FR, Berke T, Chou Y-Y, Hulse-Kemp A, Ochoa-Alejo N, Tripodi P, Veres A, Carrizo García C, Csillery G, Huang Y-K, Kiss E, Kovacs Z, Kondrak M, Arce-Rodriguez ML, Scaldaferrero MA, Szoke A (2019) *Capsicum*. An Abbreviated Compendium. *Journal of the American Society for Horticultural Science* 144(1): 3–22. <https://doi.org/10.21273/JASHS04446-18>
- Khoury CK, Carver D, Barchenger DW, Barboza GE, van Zonneveld M, Jarret B, Bohs L, Kantar M, Uchanski M, Mercer K, Nabhan GP, Bosland PW, Greene SL (2020) Modelled distributions and conservation status of the wild relatives of chile peppers (*Capsicum* L.). *Diversity & Distributions* 26(2): 209–225. <https://doi.org/10.1111/ddi.13008>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lester RN, Durrands P (1984) Enzyme treatment as an aid in the study of seed surface structures of *Solanum* species. *Annals of Botany* 53(1): 129–132. <https://doi.org/10.1093/oxfordjournals.aob.a086662>
- Moscone EA, Lambrou M, Hunziker AT, Ehrendorfer F (1993) Giemsa C-banded karyotypes in *Capsicum* (Solanaceae). *Plant Systematics and Evolution* 186(3–4): 213–229. <https://doi.org/10.1007/BF00940799>
- Mulligan M (2010) Modeling the tropics-wide extent and distribution of cloud forest and cloud forest loss, with implications for conservation priority. In: Bruijnzeel LA, Scatena FN, Hamilton LS (Eds) *Tropical Montane Cloud Forests. Science for Conservation and Management*. Cambridge University Press, New York, 14–38. <https://doi.org/10.1017/CBO9780511778384.004>
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403(6772): 853–858. <https://doi.org/10.1038/35002501>

- Nee M, Bohs L, Knapp S (2006) New species of *Solanum* and *Capsicum* (Solanaceae) from Bolivia, with clarification of nomenclature in some Bolivian *Solanum*. *Brittonia* 58(4): 322–356. [https://doi.org/10.1663/0007-196X\(2006\)58\[322:NSOSAC\]2.0.CO;2](https://doi.org/10.1663/0007-196X(2006)58[322:NSOSAC]2.0.CO;2)
- Pitman N, Moskovits DK, Alverson WS, Borman AR (2002) Ecuador: Serranías Cofán-Bermejo, Sinangoe. The Field Museum, Chicago. Rapid Biological Inventories Report 3: 1–225.
- Pitman N, Ruelas IE, Alvira D, Vriesendorp C, Moskovits DK, del Campo Á, Wachter T, Stotz DF, Noningo SS, Tuesta CE, Smith RC (2012) Perú: Cerros de Kampankis. The Field Museum, Chicago. Rapid Biological and Social Inventories Report 24: 1–452.
- QGIS Development Team (2019) QGIS Version 3.8 Geographic Information System User Guide. Open Source Geospatial Foundation. <http://download.osgeo.org/qgis/doc/manual/&lt;DOCUMENT> [accessed: 18 March 2020]
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Samuels J (2015) Biodiversity of food species of the Solanaceae family: A Preliminary taxonomic inventory of Subfamily Solanoideae. *Resources* 4: 277–322. <https://doi.org/10.3390/resources4020277>
- Scaldaferro MA, Moscone EA (2019) Cytology and DNA content variation of *Capsicum* genomes. In: Ramchiary N, Kole C (Eds) *Compendium of Plant Genomes. The Capsicum Genome*. Springer Nature Switzerland AG, Cham, 57–84. [https://doi.org/10.1007/978-3-319-97217-6\\_4](https://doi.org/10.1007/978-3-319-97217-6_4)
- Schweizer D, Ambros PF (1994) Chromosome banding. Stain combinations for specific regions. In: Gosden JR (Ed.) *Methods in molecular biology* 29. Chromosome analysis protocols. Humana Press, Totowa, 97–112. <https://doi.org/10.1385/0-89603-289-2:97>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stewart NR, Denevan WM, Velásquez MT (2020) Andean Mountains. <https://www.britannica.com/place/Andes-Mountains> [accessed: 16 March 2020]
- Swofford DL (2003) PAUP\*: phylogenetic analysis using parsimony (\*and other methods). Version 4.0b10. Sinauer Associates, Sunderland.
- Tapia-Armijos MF, Homeier J, Espinosa CI, Leuschner C, de la Cruz M (2015) Deforestation and forest fragmentation in South Ecuador since the 1970s – Losing a hotspot of biodiversity. *PLoS One* 10(9): e0133701. <https://doi.org/10.1371/journal.pone.0133701>
- WWF (2020) Eastern South America: Ecuador into Colombia and Peru. <https://www.worldwildlife.org/ecoregions/nt0121> [accessed: 16 March 2020]

## Supplementary material I

### Table S1. Taxa and materials analyzed in the phylogenetic analyses

Authors: Gloria E. Barboza, Carolina Carrizo García, Marisel Scaldaferrro, Lynn Bohs  
Data type: Excel file

Explanation note: Taxa and materials analyzed in the phylogenetic analyses. Position within *Capsicum* (clade) or as outgroup, provenance, voucher specimens, ID in trees, and GenBank accession numbers for each marker analyzed are provided. Sequences retrieved from GenBank are marked with an asterisk. Abbreviations. CGN = Centre for Genetic Resources, Wageningen University, NL. NMCA = College of Agriculture, New Mexico State University, USA. Cult. = cultivated. Authors: Gloria E. Barboza, Carolina Carrizo García, Marisel Scaldaferrro & Lynn Bohs.

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

Link: <https://doi.org/10.3897/phytokeys.167.57751.suppl1>



# A new species of *Eriotheca* (Malvaceae, Bombacoideae) from coastal areas in northeastern Brazil

Jefferson Carvalho-Sobrinho<sup>1,2</sup>, Aline C. da Mota<sup>3</sup>, Laurence J. Dorr<sup>4</sup>

**1** Universidade Federal do Vale do São Francisco – UNIVASF, Colegiado de Ciências Biológicas, Petrolina, Pernambuco, 56300-990, Brazil **2** Universidade Federal Rural de Pernambuco – UFRPE, Departamento de Ciências Florestais, Recife, Pernambuco, 52171-900, Brazil **3** Universidade de Pernambuco – UPE, Instituto de Ciências Biológicas, Recife, Pernambuco, 50100-130, Brazil **4** Department of Botany, MRC-166, Smithsonian Institution, P.O. Box 37012, Washington, D.C. 20013-7012, USA

Corresponding author: Jefferson Carvalho-Sobrinho ([jef.sobrinho@gmail.com](mailto:jef.sobrinho@gmail.com))

---

Academic editor: C. Morden | Received 20 August 2020 | Accepted 6 November 2020 | Published 20 November 2020

**Citation:** Carvalho-Sobrinho J, da Mota AC, Dorr LJ (2020) A new species of *Eriotheca* (Malvaceae, Bombacoideae) from coastal areas in northeastern Brazil. *PhytoKeys* 167: 31–43. <https://doi.org/10.3897/phytokeys.167.57840>

---

## Abstract

A new species of *Eriotheca* (Malvaceae, Bombacoideae) from coastal areas in the northeastern Brazilian states of Alagoas and Bahia is described and illustrated. *Eriotheca alversonii* inhabits Atlantic coastal forest and is found principally on sandy soils in restinga vegetation. It is most similar morphologically to *E. parvifolia*. Both species have 3-foliolate leaves and short petioles on fertile branches, but the new species has smaller flowers, truncate to crenulate calyces, and smaller globose to subglobose capsules. The affinities of *E. alversonii* to morphologically similar species and its phenology are discussed. A distribution map and preliminary assessment of its conservation status are provided.

## Keywords

‘Bombacaceae’, ‘embiruçú’, endemism, plant taxonomy, restinga, sandy soils

## Introduction

*Eriotheca* Schott & Endl. is one of 17 genera in the Bombacoideae (Malvaceae), a pantropical subfamily that includes ca. 160 species (Carvalho-Sobrinho et al. 2016). *Eriotheca* is restricted to South America (Robyns 1963, 1968, 1979; Robyns and Nilsson 1975, 1981; Fernández-Alonso 1999, 2003) and where it occurs, it is an important ele-

ment in the physiognomy and community structure of both seasonally dry tropical and moist forests (e.g., Macbride 1956; Ferreyra 1986; Linares-Palomino and Ponce Alvarez 2005; Thomas et al. 2008; Pennington et al. 2009). It includes ca. 25 species of which 19 occur in Brazil mainly in cerrado and Atlantic coastal forest (Duarte and Esteves 2011; Carvalho-Sobrinho 2013; Carvalho-Sobrinho et al. 2015; Macedo et al. 2018).

Molecular phylogenetic analyses place *Eriotheca* and *Pachira* Aubl. in a clade characterized by striate seeds (Duarte et al. 2011; Carvalho-Sobrinho et al. 2016), alternate eophylls, lack of prickles on trunks and branches, leaflets with brochidodromous venation (Carvalho-Sobrinho et al. 2016), and only two rows of ovules in the ovary (Franca et al. 2018). These analyses, however, also suggest that *Eriotheca* and *Pachira* as currently circumscribed are not monophyletic, and further molecular and taxonomic sampling is necessary to resolve the relationships between these two genera (Carvalho-Sobrinho et al. 2016). Until such sampling is completed, it would be premature to place *Eriotheca* in synonymy with *Pachira* and as a consequence new species have been described in both genera while maintaining their traditional circumscriptions (Duarte and Esteves 2011; Carvalho-Sobrinho 2013; Carvalho-Sobrinho et al. 2014b, 2015; Macedo et al. 2018).

*Eriotheca* differs in a number of characters from *Pachira*. In addition to the smaller flowers in *Eriotheca* (up to 55 mm long), it also has filaments freely originating from a staminal tube (phalanges are absent), a single whorl in the androecium (Robyns 1963; Carvalho-Sobrinho 2013; Carvalho-Sobrinho et al. 2015), often unilaterally apiculate petals (Robyns 1963), reniform anthers (Carvalho-Sobrinho 2013), a different indumentum on the external surface of the petals, and a glabrous tube (Carvalho-Sobrinho, pers. obs.).

*Eriotheca* is characterized by mostly medium to emergent trees, leaves that are palmately compound with leaflets articulate at the petiole apex, flowers with a persistent calyx that is accrescent in fruit, a receptacle often with external nectaries, an androecium with 18 to 170 stamens and dorsifixed anthers, capsules with copious brown kapok, and numerous, striate seeds usually up to 1 cm in diameter (Robyns 1963). An underground xylopodium-like structure was reported for *E. saxicola* Carv.-Sobr. (Carvalho-Sobrinho 2013). *Eriotheca* flowers are pollinated by bees, bats or hawkmoths (Oliveira et al. 1992; Sazima et al. 1999; MacFarlane et al. 2003).

The taxonomy of *Eriotheca* is challenging because type specimens are often phenologically incomplete (cf. Robyns 1963) and affect species circumscriptions because it is difficult to match leaves, flowers, and fruit characters from different specimens (Carvalho-Sobrinho and Queiroz 2008, 2010; Carvalho-Sobrinho et al. 2013a, b, 2014a, b). Identification of *Eriotheca* specimens traditionally has relied largely on floral characters, especially the length of pedicels, flower bud shape, calyx shape and indumentum, petal shape, and staminal tube shape (Robyns 1963). More recently, micromorphological characters from leaves have been used to circumscribe and diagnose species (Duarte and Esteves 2011; Carvalho-Sobrinho et al. 2015) and to elaborate identification keys (Duarte and Esteves 2011), although this innovation has limited applicability in fieldwork or herbarium research.

Ongoing studies on the systematics of Neotropical Bombacoideae (Carvalho-Sobrinho and Queiroz 2008, 2010, 2011; Carvalho-Sobrinho et al. 2009, 2012, 2013a, b, 2014a, b, 2015, 2016; Carvalho-Sobrinho and Dorr 2017) have revealed herbarium specimens of *Eriotheca* from the coastal Atlantic states of Alagoas and Bahia in northeastern Brazil that are noteworthy because their leaves and fruits are smaller than others in the genus. Careful study of these specimens has led to the recognition of a new species, which is described and illustrated here. Notes on this species' distribution and phenology, comments on morphologically similar species, and a preliminary assessment of its conservation status are provided.

## Material and methods

This study was based on examination of herbarium collections, field observations, and digital images of specimens. Specimens were studied by visits to or loans from the following herbaria: ALCB, ASE, CEPEC, F, HUEFS, K, MBM, MO, NY, RB, SP, SPF, and US. Images of additional herbarium specimens were studied through the following websites: JSTOR Global Plants (<https://plants.jstor.org/>) and INCT – Herbário Virtual da Flora e dos Fungos (<http://inct.splink.org.br/>). Descriptions and measurements are based on dry herbarium specimens. The distribution map was prepared using QGIS v.3.12.2 (QGIS Development Team 2020). A preliminary extinction risk assessment of the new species was made using the IUCN Red List Categories and Criteria (IUCN 2019). Georeferenced specimen data were imported into GeoCAT (Bachman et al. 2011) to estimate the extent of occurrence (EOO) and the area of occupancy (AOO) using  $2 \times 2$  km grid cells.

## Taxonomic treatment

### *Eriotheca alversonii* Carv.-Sobr. & Dorr, sp. nov.

urn:lsid:ipni.org:names:77212952-1

Figs 1, 2

**Diagnosis.** Similar to *Eriotheca parvifolia* (Mart.) A.Robyns in its 3-foliolate, glabrous leaves, and short petioles on fertile branches, but differing in its linear-oblong (vs. large elliptic) flower buds, smaller ( $3\text{--}4 \times 3\text{--}5$  vs.  $7 \times 8\text{--}11$  mm) cupuliform (vs. campanulate) calyces with apices truncate to crenulate (vs. mostly 3–5-lobed), fewer stamens (ca. 70 vs. ca. 120), and smaller capsules ( $15\text{--}21$  vs.  $30\text{--}35$  mm long).

**Type.** BRAZIL. Bahia: Maraú, entrada à direita ca. 3 km da entrada da cidade, propriedade particular 'Espaço 21',  $14^{\circ}10'27''\text{S}$ ,  $38^{\circ}59'53''\text{W}$ , 7 m a.s.l., 08 Jul 2011 (lf, fl buds, fl), J.G. Carvalho-Sobrinho et al. 3126 (holotype: HUEFS).

**Description.** Treelets or more often trees to 20 m tall; trunks to 50 cm dbh; buttresses  $40 \times 60$  cm; branches often blackish in herbarium specimens. Terminal buds



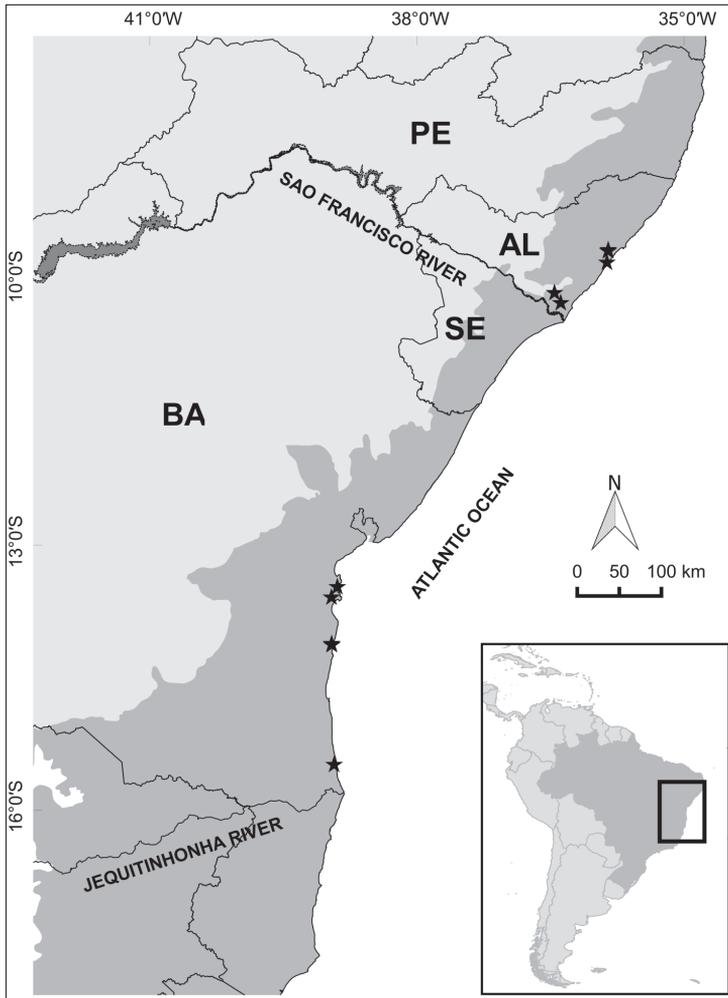
**Figure 1.** *Eriotheca alversonii* **A** flowering branch **B** three-foliolate leaf **C** flower buds and vegetative terminal buds **D** flower bud **E** flower **F, G** petals; adaxial and abaxial views **H** staminal tube **I** ovary **J, K** anthers; undehiscent and dehiscent **L** stigma **M** fruit. All drawn from the holotype, except for fruit (*L.A. Mattos Silva* 1769). Scale bars: 3 cm (**A, B**); 1 cm (**C, M**); 5 mm (**D–I**); 0.5 mm (**J–L**).

often persistent at branch apices, 5–11 mm long, attenuate and falcate apically. Leaves palmately compound; petioles on fertile branches up to 8 mm long (to 20 mm long on vegetative branches); petiolules absent to greatly reduced; leaflets 1–3(–5, in vegetative branches), 15–46(107) × 8–56 mm, coriaceous; proximal leaflets 8–27 mm wide; distal leaflets 8–56 mm wide; leaflet length-to-width ratio (1.5)1.9–2.5(3); leaflets narrowly obovate, elliptic to widely elliptic in fertile branches, rarely obcordate, apices retuse to emarginate, bases cuneate, margin entire, revolute, strongly revolute at base, glabrous on both surfaces, except for sparse microtrichomes on abaxial surface, discolorous, adaxial surface of fresh leaflets dark green and abaxial surface light green, abaxial surface of dry leaflets often reddish-brown, midrib prominent abaxially, secondary veins 7–10, impressed on both surfaces, intersecondary veins impressed on abaxial surface. Inflorescences axillary, 1–6-flowered cymes, borne on younger, terminal often leafy branches; pedicels 10–22 mm long, covered with blackish indumentum; bracteoles caducous. Flowers linear-oblong in bud, ca. 25 mm long; receptacles lacking glands; calyces 3–4 × 3–5 mm, cupuliform, truncate to crenulate, accrescent in fruit, outer surface covered with ferruginous indumentum, blackish when very young; petals 15–23 × 3–6 mm, oblanceolate, unilaterally apiculate, tomentose on both faces, internally with longitudinal lines of longer trichomes (sericeous) on one longitudinal half, whitish when fresh; stamens ca. 70, cream-colored when fresh; staminal tube 5 mm long, oblong, slightly expanded at apex, producing free filaments 11 mm long; ovary subglobose, the style inconspicuously 5-lobed. Capsules 15–21 × 13–20 mm, globose to subglobose, externally glabrous, kapok abundant, brown. Seeds numerous, 5 mm in diam., pyriform, glabrous.

**Phenology.** Flower buds in June and July, open flowers in August and September and mature fruits in October and December to February.

**Distribution and habitat.** *Eriotheca alversonii* is known from coastal vegetation mainly over quaternary white sand (restinga forest) or less frequently on clay-sandy soils in transitional vegetation between restinga forest and wet dense forest (“floresta ombrófila densa”), in the northeastern states of Alagoas and Bahia, Brazil.

**Conservation status.** *Eriotheca alversonii* is known from 19 collections from six different localities (municipalities). The extent of occurrence (EOO) of this species has been calculated to be 18,466 km<sup>2</sup>, which qualifies the species for the Vulnerable (VU) category, and the area of occupancy (AOO) was estimated to be 28 km<sup>2</sup>, which qualifies it for the Endangered (EN) category (Bachman et al. 2011; IUCN 2019). Based on herbarium specimen labels, three collections of *E. alversonii* were made inside one state-level protected Reserve (APA de Santa Rita) as explicitly stated in collectors’ descriptions, and an additional four collections probably were made inside state- (APA Pratigi and APA Marituba do Peixe) or federal-level (Reserva Extrativista de Canavieiras) protected areas; nevertheless, all these protected areas allow sustainable use of natural resources and none of them are of the highest level of protection (level I or II) described by the IUCN (Dudley 2008). Furthermore, restinga habitat currently is being lost at an accelerated rate due to anthropogenic pressures (Rocha et al. 2007; Pergentino and Landim 2014) and most collections of *E. alversonii* were made on



**Figure 2.** Distribution map of *Eriotheca alversonii*. Gray-shaded areas represent the original extent of Atlantic forest. State boundaries are indicated by continuous lines. Abbreviations for Brazilian states: AL: Alagoas; BA: Bahia; PE: Pernambuco; SE: Sergipe.

farms. Therefore, due to the rapid rate of deforestation of the much fragmented restinga vegetation and the small AOO (32 km<sup>2</sup>) of *E. alversonii*, we consider this species to be Endangered (EN category) according to IUCN criteria (IUCN 2019).

**Etymology.** The specific epithet honors the North American botanist Dr. William ('Bil') Surprison Alverson (b. 1953) who has contributed greatly to our understanding of the phylogeny and systematics of Neotropical Bombacoideae.

**Additional specimens examined.** BRAZIL. **Alagoas:** Barra de São Miguel, 9°50'25"S, 35°54'28"W, 28 Aug 1981 (lf, buds), *M.N.R. Staviski et al.* 940 (MAC); *ibidem*, loteamento próximo ao Rio Niquim, 24 Jan 2008 (lf, fr), *L. Omena* 4 (MAC); Marechal Deodoro, APA de Santa Rita, Sítio Campo Grande, vegetação sobre cordões

litorâneos, 25 Sept 1990 (lf, fl, fr), 10°11'4"S, 36°29'50"W, *R.P. Lyra-Lemos 1750* (ALCB, MAC, SP); *ibidem*, APA de Santa Rita, Sítio Campo Grande, 25 Sept 1990 (lf, fl), *R.P. Lyra-Lemos & J.E. de Paula 1762* (MAC, SPF); *ibidem*, APA de Santa Rita, próximo a Campo Grande, 10°11'4"S, 36°29'50"W, 24 Aug 1999 (lf, fl), *R.P. Lyra-Lemos & I.A. Bayma 4207* (ESA, MAC, SP); *ibidem*, Dunas do Cavalo Russo, 04 Feb 2009 (lf), *Chagas-Mota & L.M. Leão 1826* (MAC); *ibidem*, Dunas do Cavalo Russo, 12 Feb 2009 (lf), *Chagas-Mota 1987* (MAC); *ibidem*, Dunas do Cavalo Russo, 9°42'37"S, 35°53'42"W, s.d. (st), *J.C. Lemos 28* (MAC); *ibidem*, Dunas do Cavalo Russo, Povoado Cabreiras, 30 Aug 2008 (lf, fl), *R.P. Lyra-Lemos et al. 11457* (MAC); *ibidem*, encosta de tabuleiro próximo às dunas do Cavalo Russo, 09 Dec 1998 (lf, fr), *R.P. Lyra-Lemos 4086* (MAC, SP); *ibidem*, Mucuri, próximo a Campo Grande, vegetação sobre cordões arenosos, 24 Aug 1999 (lf, fl), *R.P. Lyra-Lemos & I.A. Bayma 4235* (ASE, MAC); *ibidem*, próximo Praia do Francês, 31 Jan 1982 (fr), *D. Araújo s.n.* (RB1382616); *ibidem*, Sítio Bom Retiro, 09°41'52"S, 35°53'36"W, 07 Feb 2007 (lf, fr), *A.I.L. Pinheiro & S. Mendes 327* (MAC). Penedo, Marituba do Peixe, 19 Aug 2006, *M.N. Rodrigues et al. 1983* (lf, fl), 10°17'55"S, 36°25'37"W (MAC). **Bahia:** Cairu, Gamboa, 13 Aug 1993 (lf, imm fr), *M.L. Guedes et al. s.n.* (ALCB 26059); *ibidem*, Fazenda Bela Vista, 14 Sept 1993 (lf, imm fr), *M.L. Guedes et al. s.n.* (RB 426439); Maraú, estrada à direita para uma propriedade particular, a ca. 3 km da entrada da cidade de Maraú, 14°09'32"S, 39°00'1"W, 19 Aug 2008 (lf, fl), *L.P. Queiroz et al. 13018* (HUEFS); *ibidem*, entrada à direita ca. 3 km da entrada da cidade, propriedade particular 'Espaço 21', 14°10'17"S, 38°53'53"W, 20 m a.s.l., 07 Jul 2011 (lf, buds), *J.G. Carvalho-Sobrinho et al. 3125* (HUEFS); Nilo Peçanha, ramal para o povoado de Itiúca, ramal com entrada no km 8 da rodovia Nilo Peçanha/Cairu (BA 250), lado direito, piaçaval em capoeira, solo arenoso, 24 Oct 1984 (lf, fr), *L.A. Mattos Silva & T.S. Santos 1769* (CEPEC).

## Discussion

*Eriotheca alversonii* is characterized by leaves on fertile branches 1–3-foliolate, petioles up to 8 mm long, leaflets coriaceous, elliptic to broadly-elliptic or narrowly obovate, proximal leaflets up to 27 mm wide, flower buds linear-oblong, and small capsules globose to subglobose up to 21 mm long with glabrous valves. On herbarium sheets, specimens are characterized by terminal vegetative buds with attenuate, falcate apices and by leaves on fertile branches often 1–2-foliolate and often reddish-brown on the abaxial surface.

*Eriotheca alversonii* is morphologically similar to *E. parvifolia* – a shrubby species to 2.5 m tall endemic to the arenitic-quartzite rock outcrops in the Espinhaço Mountain Range in the state of Minas Gerais – by the small petioles, the small, narrowly obovate leaflets with retuse to emarginate apices, and the often persistent leaves on fertile branches; furthermore, both species flower from July to September. However, *E. alversonii* differs from *E. parvifolia* in its oblong-linear (vs. large elliptic) flower buds, calyces 3–4 × 3–5 (vs. 7 × 8–11) mm that are cupuliform and truncate to crenu-

late (vs. campanulate and mostly 3–5-lobed), stamens ca. 70 (vs. ca. 120), and capsules 15–21 (vs. 30–35) mm long (Table 1).

*Eriotheca alversonii* emerged as sister to *E. candolleana* (K.Schum.) A.Robyns in a multi-locus DNA sequence-based phylogeny (labeled as ‘*Eriotheca* sp. CS3125’ in Fig. 2B of Carvalho-Sobrinho et al. 2016). It shares with *E. candolleana* the arborescent habit with a regular, well-defined closed crown (“spherical” sensu Ribeiro et al. 1999) and relatively small leaflets and fruit. However, *E. alversonii* can be distinguished readily from *E. candolleana* by 1–3 (vs. 5–9) leaflets that are coriaceous (vs. papyraceous) and glabrous (vs. pubescent with ferruginous trichomes on the veins abaxially), oblong-linear (vs. globose) flower buds, absence (vs. presence) of glands on the receptacle, and calyces 3–4 × 3–5 (vs. 8–11 × 9–12) mm that are cupuliform (vs. campanulate to tubular), truncate to crenulate (vs. mostly 3–5-lobed), and puberulent to glabrescent (vs. covered with dense ferruginous trichomes). Furthermore, *E. alversonii* inhabits coastal forest over predominantly sandy soils (restinga) in Alagoas and southern Bahia (i.e., 14°S to 10°S) while *E. candolleana* inhabits semideciduous forest on the southeastern Atlantic coast from São Paulo to southern Bahia (i.e., 23°S to 17°S) and in the cerrado biome where it reaches 1,200 m in elevation.

*Eriotheca alversonii* has been frequently misidentified as *E. macrophylla* – a tree species inhabiting restinga and semideciduous forest in the Atlantic coast of northeastern Brazil – probably because in both species the calyces are cupuliform and the leaflets of the fertile branches are 1.5–2.3× longer than wide. However, *E. alversonii* can be readily distinguished from *E. macrophylla* by its terminal, falcate vegetative buds with attenuate apices (vs. straight buds with acute apices), linear-oblong (vs. broadly elliptic

**Table 1.** Comparison of *Eriotheca alversonii* to morphologically similar species.

Character	<i>E. alversonii</i>	<i>E. macrophylla</i>	<i>E. parvifolia</i>
Leaves on fertile branches	Present	often absent	present
Petiole length of terminal leaf	up to 8	21–45(–65)	8–26(–37)
Number of leaflets of terminal leaf	1–3	3(–5)	(2–)3(–5)
Proximal leaflets width (mm)	8–27	24–66	8–31
Number of secondary veins	7–10	12–18	14–20
Inflorescence position	younger, terminal, often leafy branches	old branches often leafless and modified brachyblasts	terminal, leafy branches
Number of flowers per cyme	1–5	2–7	1–3
Pedicle length (mm)	10–22	14–25	10–15(–25)
Flower bud shape	linear-oblong	broadly elliptic	broadly elliptic
Calyx dimensions (mm)	3–4 × 3–5	5–6 × 7–9	7 × 8–11
Calyx apex	truncate to crenulate	crenulate	mostly 3–5-lobed
Calyx shape	cupuliform	cupuliform	campanulate
Petal dimensions (mm)	15–23 × 3–6	30 × 11	24–31 × 9–15
Number of stamens	ca. 70	140	125
Staminal tube length (mm)	4–5	4	5
Fruit length (mm)	15–21	38–60	27–60
Fruit shape	globose to subglobose	obovoid	obovoid
Seed diameter (mm)	4	ca. 10	6–7
Flowering period	July to September	October to December	July to September
Fruiting period	August to October and December to February	December to February	October to December

to oblong-obovate) flower buds, smaller calyces (3–4 × 3–5 mm vs. 5–6 × 7–9 mm), smaller petals (19–20 × 4–5 vs. 20–32 × 10–15 mm), fewer stamens (ca. 70 vs. 90–140), and smaller fruit (15–21 mm vs. 38–60 mm long) that are globose to subglobose (vs. obovoid). Moreover, *E. alversonii* is characterized by inflorescences borne on younger terminal and often leafy branches while the inflorescences of *E. macrophylla* are borne on old branches that are often leafless and modified as brachyblasts.

## Acknowledgements

We thank the curators of the herbaria cited in the text for kindly sending material on loan; Natanael Nascimento for the line drawings; Jefferson Rodrigues Maciel for the map; Mário Terra for help during fieldwork; and PPGBot/UEFS for financial support for the fieldwork; Jacquelyn Kallunki and one anonymous reviewer for valuable suggestions on the manuscript.

Fieldwork was supported by the Fundação de Amparo à Pesquisa do Estado da Bahia (process APP0006/2011). JGCS thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (processes 563546/2010-7-REFLORA and 158916/2014-0) for financial support. JGCS also thanks CAPES for a sandwich fellowship through the REFLORA program (process BEX 5415/13-6). The authors have declared that no competing interests exist.

## References

- Bachman S, Moat J, Hill AW, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Carvalho-Sobrinho JG (2013) A new species of *Eriotheca* (Malvaceae: Bombacoideae) from Espírito Santo, eastern Brazil. *Phytotaxa* 108(1): 49–53. <https://doi.org/10.11646/phytotaxa.108.1.3>
- Carvalho-Sobrinho JG, Dorr LJ (2017) A new combination and taxonomic notes in *Pseudobombax* Dugand (Malvaceae). *PhytoKeys* 85: 27–30. <https://doi.org/10.3897/phytokeys.85.13930>
- Carvalho-Sobrinho JG, Queiroz LP (2008) *Ceiba rubriflora* (Malvaceae: Bombacoideae), a new species from Bahia, Brazil. *Kew Bulletin* 63(4): 649–653. <https://doi.org/10.1007/s12225-008-9070-6>
- Carvalho-Sobrinho JG, Queiroz LP (2010) Three new species of *Pseudobombax* (Malvaceae, Bombacoideae) from Brazil. *Novon* 20(1): 13–20. <https://doi.org/10.3417/2007054>
- Carvalho-Sobrinho JG, Queiroz LP (2011) Morphological cladistic analysis of *Pseudobombax* Dugand (Malvaceae, Bombacoideae) and allied genera. *Revista Brasileira de Botânica. Brazilian Journal of Botany* 34(2): 197–209. <https://doi.org/10.1590/S0100-84042011000200007>

- Carvalho-Sobrinho JG, Santos FAR, Queiroz LP (2009) Morfologia dos tricomas das pétalas de espécies de *Pseudobombax* Dugand (Malvaceae, Bombacoideae) e seu significado taxonômico. *Acta Botanica Brasílica* 23(4): 929–934. <https://doi.org/10.1590/S0102-33062009000400003>
- Carvalho-Sobrinho JG, Machado MC, Queiroz LP (2012) *Spirotheca elegans* (Malvaceae: Bombacoideae), a new species from Bahia, Brazil. *Systematic Botany* 37(4): 978–982. <https://doi.org/10.1600/036364412X656554>
- Carvalho-Sobrinho JG, Queiroz LP, Alverson WS (2013a) Reinterpretation of the nomenclatural type of *Pseudobombax heteromorphum* (Malvaceae, Bombacoideae) reveals an overlooked new species from Bolivia. *PhytoKeys* 21(0): 53–61. <https://doi.org/10.3897/phytokeys.21.5213>
- Carvalho-Sobrinho JG, Queiroz LP, Dorr LJ (2013b) Does *Pseudobombax* have prickles? Assessing the enigmatic species *Pseudobombax endecaphyllum* (Malvaceae: Bombacoideae). *Taxon* 62(4): 814–818. <https://doi.org/10.12705/624.30>
- Carvalho-Sobrinho JG, Ramella L, Queiroz LP, Dorr LJ (2014a) Towards a revision of *Pseudobombax* Dugand (Malvaceae-Bombacoideae): Typification of names published by E. Hassler and R. Chodat in the related genus *Bombax* L. *Candollea* 69(1): 93–99. <https://doi.org/10.15553/c2014v691a12>
- Carvalho-Sobrinho JG, Alverson WS, Mota AC, Machado MC, Baum DA (2014b) A new deciduous species of *Pachira* (Malvaceae: Bombacoideae) from a seasonally dry tropical forest in northeastern Brazil. *Systematic Botany* 39(1): 260–267. <https://doi.org/10.1600/036364414X678224>
- Carvalho-Sobrinho JG, Mota AC, Queiroz LP (2015) *Eriotheca estevesiae* (Malvaceae: Bombacoideae): A new species from the cerrado vegetation of Brazil. *Brittonia* 67(1): 29–36. <https://doi.org/10.1007/s12228-014-9350-4>
- Carvalho-Sobrinho JG, Alverson WS, Alcantara S, Queiroz LP, Mota AC, Baum DA (2016) Revisiting the phylogeny of Bombacoideae (Malvaceae): Novel relationships, morphologically cohesive clades, and a new tribal classification based on multilocus phylogenetic analyses. *Molecular Phylogenetics and Evolution* 101: 56–74. <https://doi.org/10.1016/j.ympev.2016.05.006>
- Duarte MC, Esteves GL (2011) A new species of *Eriotheca* (Malvaceae, Bombacoideae) from Bahia, Brazil. *Brittonia* 63(3): 338–342. <https://doi.org/10.1007/s12228-010-9174-9>
- Duarte MC, Esteves GL, Salatino MLF, Walsh KC, Baum DA (2011) Phylogenetic analyses of *Eriotheca* and related genera (Bombacoideae, Malvaceae). *Systematic Botany* 36(3): 690–701. <https://doi.org/10.1600/036364411X583655>
- Dudley N [Ed.] (2008) Guidelines for applying protected area management categories. Gland, Switzerland: IUCN, 86pp. <https://doi.org/10.2305/IUCN.CH.2008.PAPS.2.en>
- Fernández-Alonso JL (1999) Sobre la identidad de *Pseudobombax squamigerum* y de *Eriotheca genryi* (Bombacaceae). *Anales del Jardín Botánico de Madrid* 57(1): 162–164. <http://rjb.revistas.csic.es/index.php/rjb/article/view/198/195>
- Fernández-Alonso JL (2003) Bombacaceae neotropicae novae vel minus cognitae VI. Novedades en los géneros *Cavanillesia*, *Eriotheca*, *Matisia* y *Pachira*. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 27(102): 25–37. <http://www.accefyn.org.co/Publicad/Periodicas/Volumen27/102/25-37.pdf>

- Ferreira R (1986) Flora y vegetación del Perú. In: Águila CP, Miranda MI, Ferreira R, Egg AB, Dourojeanni MJ, Zender J, Vélez MV, Malleux J, Boggio MS, Baca MF (Eds) Gran geografía del Perú: Naturaleza y hombre. Vol. II. Manfer-Juan Mejía Baca, Barcelona, 174 pp.
- Franca R, Carvalho-Sobrinho JG, Marzinek J, Mourão K (2018) Morfoanatomia do ovário em cinco gêneros de Bombacoideae (Malvaceae). Anais do 68° Congresso Nacional de Botânica e XXXVI Jornada Fluminense de Botânica. Rio de Janeiro, 20 a 25 de agosto de 2017. <https://www.botanica.org.br/wp-content/uploads/Anais68CNBot.pdf>
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommittee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf> [accessed 29 April 2020]
- Linares-Palomino RL, Ponce Alvarez SI (2005) Tree community patterns in seasonally dry tropical forests in the Cerros de Amotape Cordillera, Tumbes, Peru. *Forest Ecology and Management* 209(3): 261–272. <https://doi.org/10.1016/j.foreco.2005.02.003>
- Macbride JF (1956) Bombacaceae. *Flora of Peru*. Field Museum of Natural History, Botanical Series 13(3A/2): 593–622.
- Macedo TM, Yoshikawa VN, Duarte MC (2018) A new species of *Eriotheca* (Malvaceae, Bombacoideae) from Espírito Santo state, Brazil. *Systematic Botany* 43(4): 1000–1004. <https://doi.org/10.1600/036364418X697706>
- MacFarlane AT, Mori SA, Purzycki K (2003) Notes on *Eriotheca longitubulosa* (Bombacaceae), a rare, putatively hawkmoth-pollinated species new to the Guianas. *Brittonia* 55(4): 305–316. [https://doi.org/10.1663/0007-196X\(2003\)055\[0305:NOELBA\]2.0.CO;2](https://doi.org/10.1663/0007-196X(2003)055[0305:NOELBA]2.0.CO;2)
- Oliveira PE, Gibbs PE, Barbosa AA, Talavera S (1992) Contrasting breeding system in two *Eriotheca* (Bombacaceae) species of the Brazilian Cerrados. *Plant Systematics and Evolution* 179(3–4): 207–219. <https://doi.org/10.1007/BF00937597>
- Pennington RT, Lavin M, Oliveira-Filho A (2009) Woody plant diversity, evolution, and ecology in the tropics: Perspectives from Seasonally Dry Tropical Forests. *Annual Review of Ecology Evolution and Systematics* 40(1): 437–457. <https://doi.org/10.1146/annurev.ecolsys.110308.120327>
- Pergentino TC, Landim MF (2014) Restingas de Sergipe: contribuição ao conhecimento da sua composição florística e análise sobre o status de conservação atual. In: Landim MF, Guimarães CP (Eds) *Ecologia, uso Potencial e Conservação de Ecossistemas Costeiros Sergipanos*. Editora UFS, São Cristóvão, 103–128.
- QGIS Development Team (2020) QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>
- Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MAD, Martins LHP, Assunção PACL, Pereira EC, Silva CF, Mesquita MR, Procópio LC (1999) *Flora da Reserva Ducke: Guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central*. INPA, Manaus, 816 pp.
- Robyns A (1963) Essai de monographie du genre *Bombax* L. s.l. (Bombacaceae). *Bulletin du Jardin botanique de l'État à Bruxelles* 33: 1–315. <https://doi.org/10.2307/3667210>
- Robyns A (1968) Bombacaceae Neotropicae Novae. New species of *Eriotheca*, *Hampea* and *Quararibea*. *Annals of the Missouri Botanical Garden* 55(1): 51–59. <https://doi.org/10.2307/2394821>

- Robyns A (1979) Bombacaceae Neotropicae Novae VIII. A new species of *Eriotheca* from Brazil. Bulletin du Jardin Botanique National de Belgique 49(3/4): 457–459. <https://doi.org/10.2307/3668098>
- Robyns A, Nilsson S (1975) Bombacaceae Neotropicae Novae V. A new species of *Eriotheca* from Brazil. Bulletin du Jardin Botanique National de Belgique 45(1/2): 155–157. <https://doi.org/10.2307/3667594>
- Robyns A, Nilsson S (1981) Bombacaceae Neotropicae Novae IX. A new species of *Eriotheca* from Colombia. Bulletin du Jardin Botanique National de Belgique 51(1/2): 201–204. <https://doi.org/10.2307/3667742>
- Rocha CFD, Bergallo HG, Van Sluys M, Alves MAS, Jamel CE (2007) The remnants of restinga habitats in the Brazilian Atlantic Forest of Rio de Janeiro state, Brazil: Habitat loss and risk of disappearance. Brazilian Journal of Biology 67(2): 263–273. <https://doi.org/10.1590/S1519-69842007000200011>
- Sazima M, Buzato S, Sazima I (1999) Bat-pollinated flower assemblages and bat visitors at two Atlantic forest sites in Brazil. Annals of Botany 83(6): 705–712. <https://doi.org/10.1006/anbo.1999.0876>
- Thomas WW, de Carvalho AM, Amorim AMA, Garrison J, dos Santos TS (2008) Diversity of woody plants in the Atlantic coastal forest of southern Bahia, Brazil. In: Thomas W (Ed.) The Atlantic Coastal Forest of Northeastern Brazil. Memoirs of the New York Botanical Garden 100: 21–66.

## Appendix I

### Selected herbarium specimens of *Eriotheca macrophylla* and *E. parvifolia* examined for this study.

*Eriotheca macrophylla* (K.Schum.) A.Robyns

BRAZIL. **Alagoas:** São José da Lage, BR 104, 11 Nov 2010, *Chagas-Mota 9270* (MAC). **Bahia:** Ilhéus, on road to Vila Brasil, 10 km West of junction with BA001, the junction ca. 40 km south of Ilhéus, just north of the Rio Acuípe, 15°06'S, 39°04'W, 10 May 1993 (lf, fl), *W. Thomas et al. 9843* (CEPEC, NY, SP). Itacaré, entre a Praia do Farol e a Praia da Ribeira, 14 Dec 1992 (lf, fl), *A. Amorim et al. 951* (CEPEC). Itapebi, Faz. Dois Irmãos, Rodovia para Potiraguá, 10 Nov 1970 (lf, fl), *R.S. Pinheiro & T.S. Santos 419* (CEPEC). Jequié, Fazenda Brejo Novo, a 10,5 km da Av. Otávio Mangabeira entrado pela Exupério Miranda no Bairro do Mandacaru, 13°56'53.6"S, 40°06'42"W, 716 m a.s.l., 08 Dec 2004 (st), *G.E.L. Macedo & J.L. Paixão 1499* (HUEFS). Morro do Chapéu, na rodovia, ca. 20 km antes da cidade, 21 Jan 2012 (lf, fr), *J.G. Carvalho-Sobrinho et al. 3305* (HUEFS). Porto Seguro, parte sul entre os municípios de Ajuda e Porto Seguro, 08 Nov 1963 (lf, buds), *A.P. Duarte 7999* (RB, SP). Santa Cruz de Cabrália, Res. Bio. Pau-Brasil, 11 Dec 1971 (lf, fl), *A. Eupunino 94* (CEPEC). Santa Terezinha, 14,5 km na rodovia Elísio Medrado/Sta. Teresinha, Torre da Embratel, ca. 7 km distante do Distr. de Pedra Branca, Serra da Jiboia, 12°51'13"S,

39°28'33"W, 750 m a.s.l., 24 Feb 2000 (lf, fr), *J.G. Jardim et al.* 2808 (HUEFS, NY, SPF). **Espírito Santo:** Conceição da Barra, Área 157 da Aracruz Celulose S.A., 28 Oct 1993 (lf, buds) *O.J. Pereira et al.* 5163 (VIES). Linhares, Reserva Natural da CVRD, estrada Flamengo, km 08, 03 Dec 2004 (lf, buds), *D.A. Folli* 4999 (CVRD); *ibidem*, Reserva Natural da CVRD, estrada Flamengo, km 07, próximo ao pátio, 14 Jan 1994 (lf, fr), *D.A. Folli* 2170 (CVRD). **Pernambuco:** Brejo da Madre de Deus, Mata do Bituri, Serra do Prata, próximo do mirante, 08°12'27"S, 36°23'32"W, 920–1030 m a.s.l., *L.M. Nascimento s.n.* (HUEFS 57136, PEUFR).

*Eriotheca parvifolia* (Mart.) A.Robyns

BRAZIL. **Minas Gerais:** Grão Mogol, estrada para o Rio Ventania, ca. 16°32'S, 42°49'W, 900 m a.s.l., 05 Sept 1990 (lf, fl), *G.L. Esteves CFCR* 13348 (HUEFS, SPF); *ibidem*, Serra do Espinhaço, área N Diamantina, campo rupestre near Córrego Lajeado and the junction of the side road from Cristais with the Road Diamantina-Biribiri, ca. 9km NNW-N Diamantina, 1080 m a.s.l., 18°10'S, 43°37'W, *G.L. Esteves CFCR* 15511 (NY, SPF); *ibidem*, estrada para Josenópolis, 17 Jul 1998, *G. Hatschbach et al.* 67961 (MBM); *ibidem*, Vale do Rio das Mortes, a oeste da cidade, 24 Jul 1986 (lf, fl), *D.C. Zappi et al.* CFCR 9892 (BHCB, MAC, R, SPF). Monte Azul, estrada de terra para Formosa, 15°12'S, 42°48'W, 1192 m a.s.l., 27 Oct 2010 (lf, fr), *J.G. Carvalho-Sobrinho et al.* 2870 (HUEFS).



# *Hydrostachys flabellifera* (Hydrostachyaceae), a new species from Madagascar

Zhun Xu<sup>1,2,3</sup>, Jing Tian<sup>1,2</sup>, Solo Hery Jean Victor Rapanarivo<sup>4</sup>,  
Rokiman Letsara<sup>4</sup>, Rivontsoa A. Rakotonasolo<sup>3,4,5</sup>,  
Guy E. Onjalalaina<sup>1,2,3</sup>, Guang-Wan Hu<sup>1,2</sup>, Qing-Feng Wang<sup>1,2</sup>

**1** Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China **2** Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan 430074, China **3** University of Chinese Academy of Sciences, Beijing 100049, China **4** Département Flore, Parc Botanique et Zoologique de Tsimbazaza, Antananarivo 101, Madagascar **5** Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Corresponding authors: Guang-Wan Hu ([guangwanhu@wbcas.cn](mailto:guangwanhu@wbcas.cn)); Qing-Feng Wang ([qfwang@wbcas.cn](mailto:qfwang@wbcas.cn))

---

Academic editor: Y. Mutafchiev | Received 10 September 2020 | Accepted 6 November 2020 | Published 20 November 2020

**Citation:** Xu Z, Tian J, Rapanarivo SHJV, Letsara R, Rakotonasolo RA, Onjalalaina GE, Hu G-W, Wang Q-F (2020) *Hydrostachys flabellifera* (Hydrostachyaceae), a new species from Madagascar. *PhytoKeys* 167: 45–56. <https://doi.org/10.3897/phytokeys.167.58538>

---

## Abstract

*Hydrostachys flabellifera*, a new species of Hydrostachyaceae found in a stream in Manandriana, Madagascar, is described and illustrated herein. It is similar to *H. verruculosa* and *H. laciniata* in morphology, but can be distinguished from them by its leaves with sparsely arranged, flabelliform and palmately parted emergences, obvious rachis and the pattern of segments arranged on the male bracts. Molecular phylogenetic analysis of the nuclear ribosomal internal transcribed spacer (ITS) dataset provides a robust support for it as a new species as well.

## Keywords

Aquatic plants, Cornales, endemic, new taxa, taxonomy

## Introduction

*Hydrostachys* Thouars (1806: 2) is the sole genus in the family Hydrostachyaceae (Tul.) Engler (1894: 136) with about 22 known species. Fourteen of them are endemic to Madagascar (Phillipson et al. 2018) and the remaining species are native to southern and tropical Africa. *Hydrostachys* has been used in traditional medicine and probably could be a potential candidate for use in chemotherapy to fight against cancer (Rana-rijaona et al. 2014). The plants of *Hydrostachys*, which are aquatic herbs living in fast-moving streams or rivers, are well adapted to turbulent aquatic environments with their roots and discoidal rhizome adhering to the rocks. *Hydrostachys* are annual or perennial, submerged or partially submerged in the rainy season, flowering in the dry season. Their simple or pinnate leaves emerge from the rhizome, and petiole, rachis and subdivisions are often covered with diverse forms of emergences, including verrucae, scales and lobules (modified leaf blade lobes), which give the plant the appearance of a fern or lycopodium. *Hydrostachys* are dioecious or seldom monoecious, with highly reduced and unisexual flowers borne on the spike, the spikes usually emerging from the rhizome, sepals and petals are absent; the fruit is a capsule with numerous tiny seeds (Perrier 1952; Cusset 1973; Stannard 1997; Verdcourt 1986; Erbar and Leins 2004).

*Hydrostachys* are highly modified aquatic plants and the taxonomic placement of this enigmatic genus has confounded botanists for two hundred years. Due to their similar habitat and highly modified morphological characters, the genus was once placed in Podostemaceae (Tulasne 1849). However, this placement was rejected thanks to evidence from embryology, inflorescence morphology (Jäger-Zürn 1965; Rauh and Jäger-Zürn 1966) and biochemistry (Scogin 1992). Phylogenetic studies showed unstable placements, based on different DNA markers and taxon sampling (Les et al. 1997; Soltis et al. 2000; Burleigh et al. 2009). Currently, Hydrostachyaceae is treated as a distinctive family in Cornales with a phylogenetic long branch in most studies (Albach et al. 2001; Xiang et al. 2002; Fan and Xiang 2003; Xiang et al. 2011; Fu et al. 2019). Despite the uncertain placement at the order level, the inter-species identification is much clearer.

During a field investigation in Madagascar in 2017, a *Hydrostachys* population was found in Manandriana which appeared similar to *H. verruculosa* A. Juss. (1837: tab. 91) and *H. laciniata* Warming (1899: 152). However, after carefully comparing the collection with all available specimens of *Hydrostachys* and consulting relevant literature (Perrier 1952; Cusset 1973), we observed that its leaf structure is different from that of all known species in this genus and that this plant is wholly new to science. Hence, we describe it herein as a new species.

## Materials and methods

The description of the new species is based on field notes and observations of field pictures, dried specimens and FAA-fixed (formalin/acetic acid/alcohol) materials. Specimens were collected from Manandriana, Madagascar (20°14'S, 47°06'E) and deposited at the herbaria of Parc Botanique et Zoologique de Tsimbazaza (TAN) and Wuhan Botanical

**Table 1.** Taxa included in the phylogenetic analysis.

Species	Locality	Voucher	GenBank accession number
<i>Triphyophyllum peltatum</i> (Hutch. & Dalziel) Airy Shaw	–	TR121	HM204913
<i>Nyssa sylvatica</i> Marshall	–	zhangcq0088	JF977171
<i>Nyssa wenshanensis</i> Fang & Soong	China, Yunnan	S2007041304	JQ280761
<i>Nyssa javanica</i> Wangerin	–	S2007040302	JQ280777
<i>Hydrostachys multifida</i> A. Juss.	Madagascar, Boeny, Betsiboka	SAJIT3437	MW233025
<i>Hydrostachys longifida</i> H. Perrier	Madagascar, Analamanga, Antananarivo- Atsimondrano	SAJIT3442	MW233026
<i>Hydrostachys stolonifera</i> Baker	Madagascar, Vakinankaratra, Antanifotsy	SAJIT3446	MW233027
<i>Hydrostachys multifida</i> A. Juss.	Madagascar, Amoron'i Mania, Manandriana	SAJIT3453	MW233028
<i>Hydrostachys flabellifera</i> G.W. Hu, Zhun Xu & Q.F. Wang	Madagascar, Amoron'i Mania, Manandriana	SAJIT3462	MW233029
<i>Hydrostachys imbricata</i> A. Juss.	Madagascar, Vatovavy-Fitovinany, Ifanadiana	SAJIT3473	MW233030
<i>Hydrostachys multifida</i> A. Juss.	Madagascar, Vatovavy-Fitovinany, Ifanadiana	SAJIT3484	MW233031
<i>Hydrostachys distichophylla</i> var. <i>hildebrandtii</i> (Engl.) C. Cusset	Madagascar, Haute-Matsiatra, Iarintsena	SAJIT3490	MW233032
<i>Hydrostachys multifida</i> A. Juss.	Madagascar, Haute-Matsiatra, Ambalavao	SAJIT3498	MW233033

Garden, Chinese Academy of Sciences (HIB). Some leaves and spikes were fixed and conserved in formalin/acetic acid/alcohol (FAA) fixatives. Detailed characteristics of the bracts and emergences were observed and measured on the fixed materials by using a stereomicroscope (Nikon Stereo Microscope SMZ25). Terminology was referenced in several books and literature (Perrier 1952; Cusset 1973; Verdcourt 1986; Simpson 2010; Beentje and Williamson 2016). The herbarium abbreviations follow Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>). Physical specimens of *Hydrostachys*, deposited at BM, E, K and TAN, were examined. High-resolution digital specimen images from B, BNRH, BR, GH, MA, P and US were checked via JSTOR Global Plants (<https://plants.jstor.org>) and GBIF (<https://www.gbif.org>). The distribution map was produced by QGIS3 (available from: <https://qgis.org/>).

The nuclear ribosomal ITS was used as the DNA marker, with 13 samples included in the phylogenetic analysis. All *Hydrostachys* sequences were newly generated, while three taxa from *Nyssa* and one from *Triphyophyllum* were treated as outgroups. GenBank accession numbers are available in Table 1. Genomic DNA was extracted from dry specimens using Mag-MK Plant Genomic DNA extraction kits (Sangon Biotech, Shanghai). Primers for polymerase chain reactions (PCR) were referred to White et al. (1990). PCR products were sequenced by Sangon Biotech using the 3730xl DNA Analyzer and Geneious v.11.1.5 (available from: <http://www.geneious.com/>) was used for DNA assembling and manually editing. The dataset was aligned by MAFFT v.7.294 (Katoh and Standley 2013), then trimmed by trimAl v.1.2 (Capella-Gutierrez et al. 2009). The Maximum Likelihood tree was inferred using IQ-TREE v.2.0.6 (Minh et al. 2020) with default parameters and ultrafast bootstrap approximation was assessed with 1000 replicates. The consensus tree was visualised and annotated by ggtree v.2.2.1 (Yu et al. 2017). Dataset, scripts and command lines in the phylogenetic analysis are available in Github ([https://github.com/xuzhun1008/Hydrostachys\\_flabellifera\\_paper.git](https://github.com/xuzhun1008/Hydrostachys_flabellifera_paper.git)).

## Taxonomy

*Hydrostachys flabellifera* G.W. Hu, Zhun Xu & Q.F. Wang, sp. nov.

urn:lsid:ipni.org:names:77212953-1

Figs 1, 2

**Diagnosis.** *Hydrostachys flabellifera* is similar to *H. verruculosa* and *H. laciniata* in having simple leaves, but it can be easily distinguished from these species by short leaves, 3–12 cm long, the sparsely and spirally-arranged, flabelliform and palmately-parted emergences, the presence of a distinct and thin rachis between emergences and the pattern of segments arranged on the male bracts with acute apex.

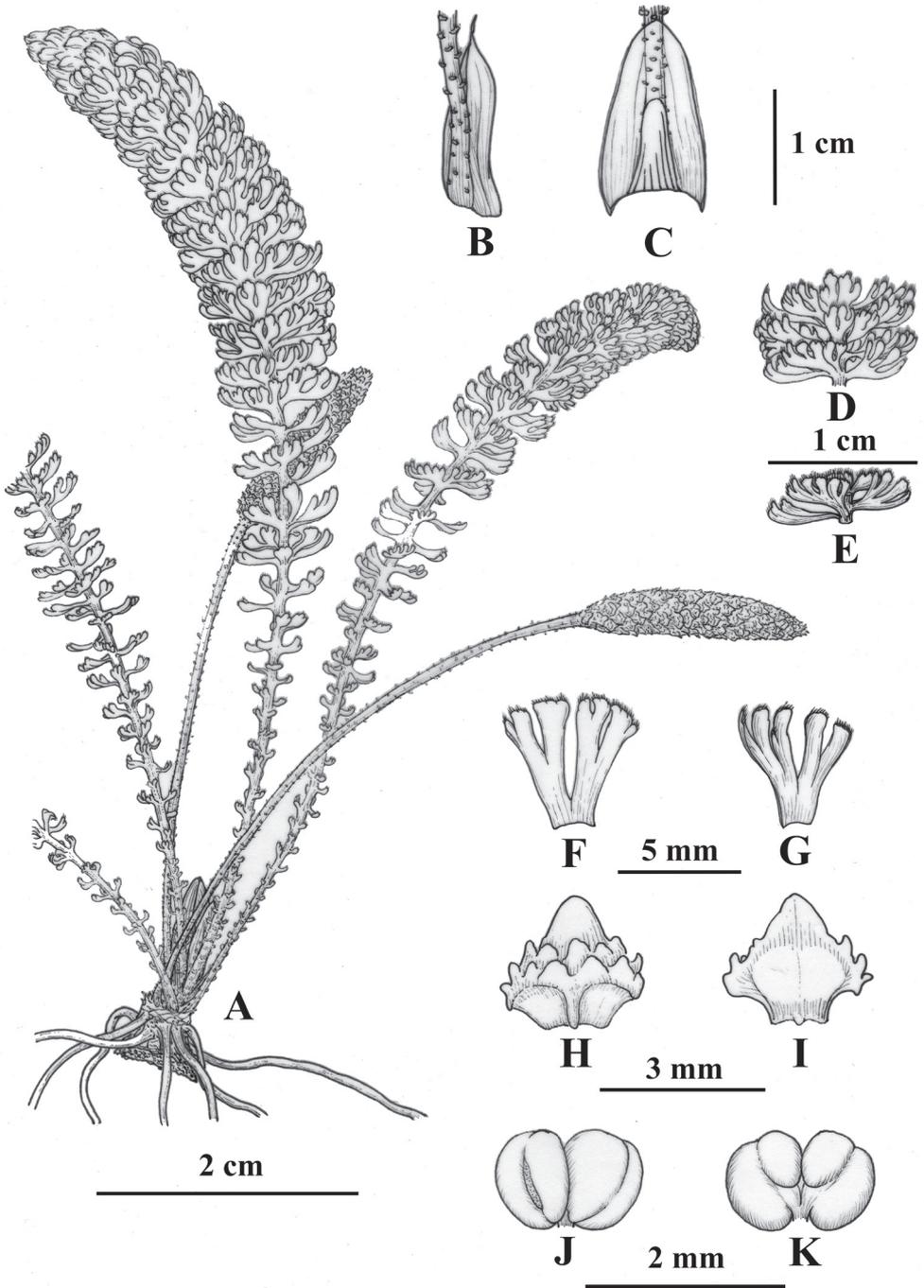
**Type.** MADAGASCAR. Fianarantsoa Province: Amoron'i Mania Region, Manandriana District, elev. 1400 m, 20°14'S, 47°06'E, 20 September 2017, *Sino-Africa Joint Investigation Team (SAJIT)-003462* (holotype, HIB!, isotypes, HIB!, TAN!)

**Description.** A hydrophyte herb. Rhizomes discoid, 3–8 mm in diameter; 7–12 leaves emerging from the rhizome. Leaves simple, 3–12 cm long, the upper part slightly curved when rising from water, the base enlarged with stipule; stipule ovate-elliptical, basal half dorsally attached on petiole, apex sometimes with a tail ca. 1.5 mm; centre bud enclosed by stipules of inner leaves; emergences spirally arranged on rachis and stretching out into loose layers, denser towards the apex of the leaf and gradually reduced to the base. Rachis obvious, 1–2 mm in diameter, white to light green. Petioles indistinct. Emergences (modified leaf blade lobes) 1–6 mm long, flabellate, basal ones reduced into scale-like, upper ones palmately parted, lobes cuneiform, secondly divided into rectangular to linear terminal lobes; the flat of emergences almost perpendicular to the axis; the apex of emergence slightly rolling up, ciliate at the end, cilia gathering into tufts after rising from water; emergences green to mauve at the pinnacle, the rest dark green. Male spikes 5.4–8.0 cm long, peduncles 4.7–6.2 cm long, covered with few small scale-like emergences. Bracts 1–2.2 mm × 1–2.2 mm, rhombic, dark green; segment I (the terminal segment) acute, flanked by 1–2 tiny lobules on each side; segments II (lobules at the dorsal side of bract) acute or obtuse, 2 rows, each row with 3–5 separated lobules, lobules ca. 0.3 mm high. Stamen sessile, anther oblate, with two divergent thecae dehiscing longitudinally. Female spike not found.

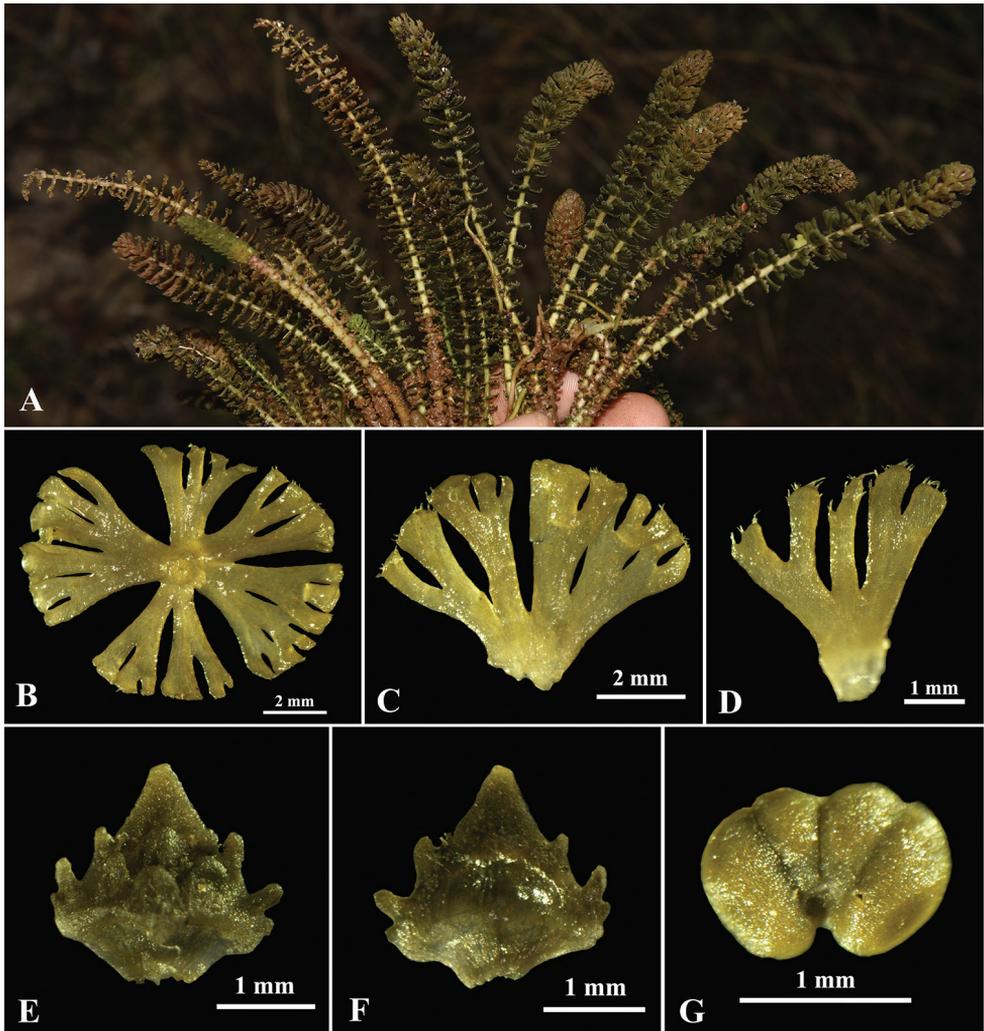
**Etymology.** The epithet refers to the flabellate shape of emergences on leaves.

**Distribution and ecology.** Only one population was found on rocks in a stream in Manandriana, Madagascar (20°14'S, 47°06'E), at an elevation of 1400 m (Fig. 3).

**Conservation status.** *Hydrostachys flabellifera* is currently only known from one location with a very small population. Additionally, all species of *Hydrostachys* are highly dependent on the moving aquatic environment which is threatened by water pollution, natural system modifications, energy production and mining, all of which could drive the taxon to Critically Endangered (CR) or Extinct (EX) in a very short time (IUCN 2020). Following Guidelines for IUCN Red List Categories and Criteria (2020), *H. flabellifera* should be categorised as Vulnerable (VU D2).



**Figure 1.** *Hydrostachys flabellifera* G.W. Hu, Zhun Xu & Q.F. Wang. **A** habit **B** lateral view of stipule and petiole base **C** ventral view of stipule and petiole base **D, E** part of the leaf **F** downside of a leaf emergence **G** upperside of a leaf emergence **H** dorsal view of male bract **I** ventral view of male bract **J, K** stamen. Drawn by Jing Tian.

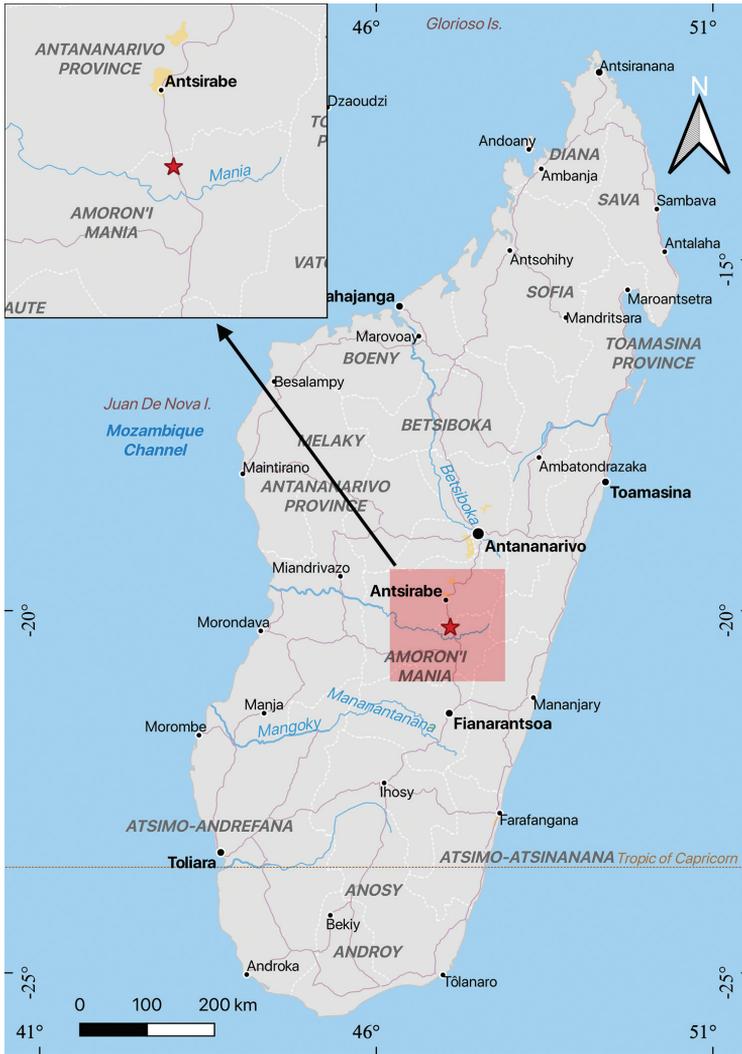


**Figure 2.** *Hydrostachys flabellifera* G.W. Hu, Zhun Xu & Q.F. Wang **A** habit **B** emergences on rachis, cross-section **C**, **D** emergences **E** dorsal view of male bract **F** ventral view of male bract **G** top view of stamen.

**Phylogenetic analysis.** *Hydrostachys flabellifera* was placed in a robust clade together with *H. stolonifera* and *H. imbricata* (Fig. 4) with a high bootstrap support (BS = 96%), while they share limited morphological characteristics. *H. multifida*, considered as a clade in morphology, is not a monophyletic group, although with low bootstrap support.

## Discussion

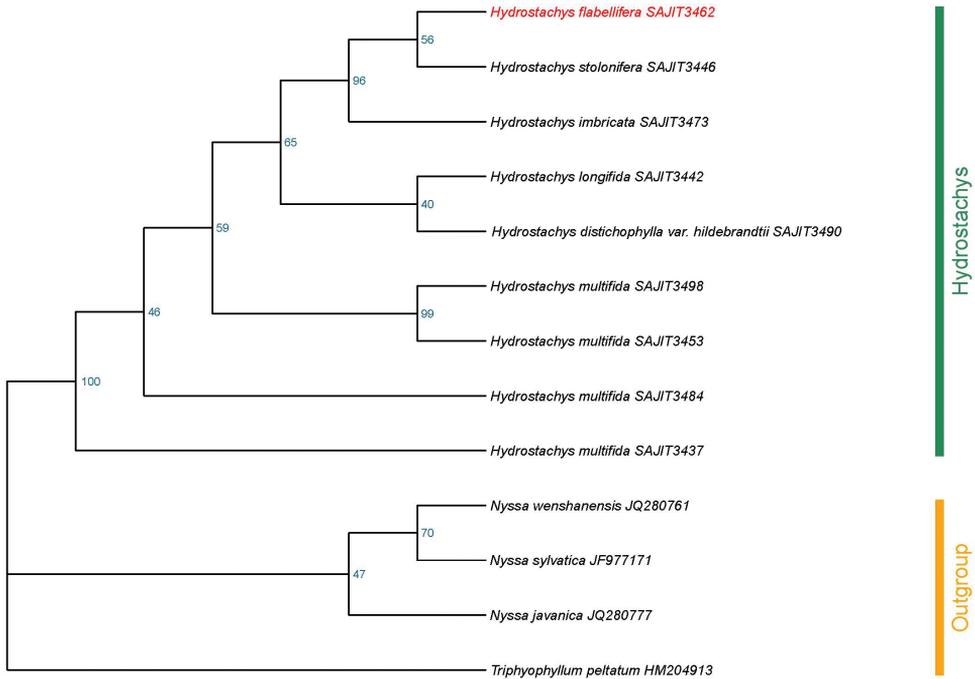
*Hydrostachys* are mostly annual, only a limited number of species with stolons are perennial (Perrier 1952), like *H. monoica* and *H. stolonifera*. In this case, we did not ob-



**Figure 3.** Distribution of *Hydrostachys flabellifera* G.W. Hu, Zhun Xu & Q.F. Wang.

serve any structure, like stolons, that could help *H. flabellifera* live for many more years. Therefore, *H. flabellifera* probably is annual, but continuous observation is needed.

*Hydrostachys flabellifera* closely resembles *H. verruculosa* and *H. laciniata* in having simple leaves, but can be easily recognised by several characters. The leaf emergences of the latter two species are densely arranged and overlapping and they constitute a thick cylindrical leaf with indistinct rachis. In contrast, the rachis of *H. flabellifera* are thin and obvious and the emergences stretch out into layers, sparsely arranged and significantly reduced at the lower leaves. Their segments patterns on male bract are also obviously different. *H. laciniata* was once treated as a form of *H. verruculosa* (Perrier 1952) after it was published as a new species (Warming 1899). Cusset (1973)



**Figure 4.** Maximum Likelihood tree, based on ITS. Bootstrap values are labelled alongside each node.

also recognised it as a distinct species and further presented a diagram to compare it with *H. verruculosa* and that diagram clearly showed the differences in the bracts and the emergences on the leaf. After examining the type specimens of these two species, we accepted the treatment of Cusset (1973) and continued to compare the new species with these two species. Combining with morphology, phylogenetic results also provide solid evidence for the newly-discovered species. *Hydrostachys flabellifera*, *H. stolonifera* and *H. imbricata* form a robust clade, but share limited morphological characters. *H. stolonifera* and *H. imbricata* are much larger than *H. flabellifera* in size and they are definitely different in leaf types. *H. distichophylla* var. *hildebrandtii* is similar to *H. flabellifera* in morphology, but they are located in two distinct clades. The phylogenetic position of *H. flabellifera* would be much clearer when more taxa are included in the analysis. The combined results from phylogenetic analysis and detailed comparisons of morphological characteristics amongst *H. flabellifera*, *H. verruculosa*, *H. laciniata*, *H. distichophylla* var. *distichophylla* and *H. stolonifera* are listed in Table 2.

*Hydrostachys* are adaptable to diverse aquatic environments, from clean mountain streams to muddy rivers. These species can be distinguished by the type of leaves, emergences, spikes, also the bract shape and segments arrangement are valuable identification characteristics. Due to different statuses between fresh plant and pressed specimens, greater attention to detail is needed when comparing and describing these species in different conditions. Based on our empirical research, in some specific cases,

**Table 2.** Comparison of morphological characteristics of *Hydrostachys flabellifera*, *H. verruculosa*, *H. laciniata*, *H. distichophylla* var. *distichophylla*, and *H. stolonifera*.

Characters	<i>Hydrostachys flabellifera</i>	<i>H. verruculosa</i>	<i>H. laciniata</i>	<i>H. distichophylla</i> var. <i>distichophylla</i>	<i>H. stolonifera</i>
Leaf division	Simple	Simple	Simple	Simple	Tripinnatifid
Leaf length	3–12 cm	4–20 cm	10–30 cm	20–40 cm	2–7 cm
Leaf emergences arrangement	Sparsely arranged, not overlapped	Densely arranged, overlapped	Densely arranged, overlapped	Sparsely arranged, not overlapped	Sparsely arranged, not overlapped
Leaf emergence shape	Flabellate, palmately parted	Obovate, margin entire	Irregular shape with laciniate margin	Falcate, margin entire	Often falcate, margin entire
Appendix of leaf emergence	With cilia at the apex	Glabrous or with short cilia or tufts of cilia at the apex	Without cilia	Without cilia	Without cilia
Length of male spike (including peduncle)	5.4–8 cm	4–13 cm	5–10 cm	10–30 cm	1–8 cm
Male bract	Rhombic, 1–2.2 mm × 1–2.2 mm	Rhombic, 3 mm × 3 mm	Sub-rhombic, ca. 3 mm × 3 mm	Rounded, 1.6 mm in diameter	Rhombic, 2–2.5 mm × 2.5–3 mm
Segment I on male bract	Entire, margins sinuous, flanked by 1–2 tiny lobules on each side, apex acute	Entire, margins sinuous, flanked by 1–2 lobules on each side, apex obtuse	3-lobed, the medium lobe larger than the lateral ones, apex of lobes obtuse to rounded	Entire, apex rounded or slightly angular	Generally entire, sometimes lobulated laterally, apex acute, obtuse or rounded
Segments II on male bract	2 rows, each row with 3–5 separated lobules, ca. 0.3 mm high, apex acute or obtuse	2–3 rows, each row with 3–4 lobules, 0.6 mm high, apex angular or acute	2 rows, upper row with one larger lobule, lower row with 4–5 smaller lobules, 0.3–0.6 mm high, apex obtuse or rounded	Without segments II	Generally one row with 3 lobules, 0.7–0.8 mm high, apex rounded

it is tricky to connect the living plant to the corresponding dry specimens. We highly recommend combining field investigations and herbarium examinations to obtain the full knowledge of this aquatic family.

**Key to identification of *Hydrostachys* in Madagascar**

- 1 Leaf simple.....2
- Leaf 1–4-pinnate.....5
- 2 Cylindrical leaf with emergences densely arranged and overlapped.....3
- Emergences sparsely arranged and stretched out.....4
- 3 Dorsal side of the female bract densely covered with emergences....*H. verruculosa*
- Dorsal side of the female bract with bare surface, only few emergences on the top..... *H. laciniata*
- 4 Emergences falcate, margin entire ..... *H. distichophylla* var. *distichophylla*
- Emergences flabellate, palmately parted ..... *H. flabellifera*
- 5 Plants with stolons; leaf in indefinite growth.....6
- Plants without stolon; leaf in definite growth .....7
- 6 Leaf pinnate or bipinnate, yellowish-white or pale green ..... *H. monoica*
- Leaf tripinnate, moss green or dark moss green ..... *H. stolonifera*

- 7 Leaf only once pinnate ..... 8  
 – Leaf more than once pinnate ..... 11  
 8 Pinnules bearing long and capillary emergences ..... *H. longifida*  
 – Pinnules bearing scale-like emergences ..... 9  
 9 Pinnules distantly arranged, terminated with a brush in the rainy season .....  
 ..... *H. distichophylla* var. *hildebrandtii*  
 – Pinnules closely arranged, without brush at the apex ..... 10  
 10 Petiole bare at the base, upper part covered with short emergences .... *H. plumosa*  
 – Petiole completely covered with imbricata emergences ..... *H. imbricata*  
 11 The middle of the leaf wider than the base and the top ..... *H. multifida*  
 – The base of the leaf wider than the upper ..... 12  
 12 Pinnule covered with capillary emergences ..... 13  
 – Pinnule covered with scale-like or irregular emergences ..... 14  
 13 Leaf divided into 3–5 pinnae; petiole covered with small spatulate emergences ...  
 ..... *H. trifaria*  
 – Leaf divided into 5–20 pinnae; petiole without obvious emergences but bristles .  
 ..... *H. decaryi*  
 14 Leaf large, 3–4-pinnate; rhizome fist-sized; petiole 0.5–1 m long ..... *H. maxima*  
 – Leaf short, 1–3-pinnate; rhizome smaller; petiole less than 0.1 m long ..... 15  
 15 Leaf irregularly 1–2-pinnate; petiole and rachis covered with few distant emer-  
 gences ..... *H. perrieri*  
 – Leaf regularly 2–3-pinnate; petiole and rachis densely covered with emergences..  
 ..... *H. fimbriata*

## Acknowledgements

We would like to thank the University of Antananarivo and the Parc Botanique et Zoologique de Tsimbazaza for arranging the field expedition and thank Ministère de l'Environnement et du Développement Durable of Madagascar for issuing the research permit (31/17/MEEF/SG/DGF/DSAP/SCB.Re). This work was financially supported by grants from the National Natural Science Foundation of China (31970211) and Sino-Africa Joint Research Center, CAS (SAJC201614). We appreciate Shuai Peng and Cai-Fei Zhang for revising the manuscript and our guides and team members for the assistance in the fieldwork and also appreciate the constructive comments and suggestions from reviewers and editors.

## References

- Albach DC, Soltis DE, Chase MW, Soltis PS (2001) Phylogenetic placement of the enigmatic angiosperm *Hydrostachys*. *Taxon* 50(3): 781–805. <https://doi.org/10.2307/1223707>

- Beentje H, Williamson J (2016) The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms. Royal Botanic Gardens, Kew, London, 164 pp.
- Burleigh JG, Hilu KW, Soltis DE (2009) Inferring phylogenies with incomplete data sets: A 5-gene, 567-taxon analysis of angiosperms. *BMC Evolutionary Biology* 9(1): 61. <https://doi.org/10.1186/1471-2148-9-61>
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics (Oxford, England)* 25(15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Cusset C (1973) Révision des Hydrostachyaceae. *Adansonia* 13(1): 75–119.
- Engler A (1894) Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie. Schweizerbart, Stuttgart, 136–137.
- Erbar C, Leins P (2004) Hydrostachyaceae. In: Kubitzki K (Ed.) Flowering Plants – Dicotyledons. Springer, Berlin, 216–220. [https://doi.org/10.1007/978-3-662-07257-8\\_23](https://doi.org/10.1007/978-3-662-07257-8_23)
- Fan C, Xiang QY (2003) Phylogenetic analyses of Cornales based on 26S rRNA and combined 26S rDNA-*matK-rbcL* sequence data. *American Journal of Botany* 90(9): 1357–1372. <https://doi.org/10.3732/ajb.90.9.1357>
- Fu CN, Mo ZQ, Yang JB, Ge XJ, Li DZ, Xiang QJ, Gao LM (2019) Plastid phylogenomics and biogeographic analysis support a trans-Tethyan origin and rapid early radiation of Cornales in the Mid-Cretaceous. *Molecular Phylogenetics and Evolution* 140: 106601. <https://doi.org/10.1016/j.ympev.2019.106601>
- IUCN (2020) The IUCN Red List of Threatened Species. Version 2020-2. <http://www.iucn-redlist.org> [accessed 25 July 2020]
- Jäger-Zürn I (1965) Zur Frage der systematischen Stellung der Hydrostachyaceae auf Grund ihrer Embryologie, Blüten- und Infloreszenzmorphologie. *Österreichische Botanischeski Zhurnal* 112(4): 621–639. <https://doi.org/10.1007/BF01373191>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Les DH, Philbrick CT, Alejandro Novelo R (1997) The phylogenetic position of river-weeds (Podostemaceae): Insights from *rbcL* sequence data. *Aquatic Botany* 57(1–4): 5–27. [https://doi.org/10.1016/S0304-3770\(96\)01117-5](https://doi.org/10.1016/S0304-3770(96)01117-5)
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Perrier H (1952) 89<sup>e</sup> Famille. – Hydrostachyacées. In: Humbert H (Ed.) Flore de Madagascar et des Comores (plantes vasculaires). Typographie Firmin-Didot, Paris, 1–32.
- Phillipson P, Andriambololona S, Letsara R, Maharombaka C, Ramiandrisoa BA, Manjato N, Ranarijaona HL, Darwall W, Máiz-Tomé L (2018) The status and distribution of aquatic plants. In: Máiz-Tomé L, Sayer C, Darwall W (Eds) The status and distribution of freshwater biodiversity in Madagascar and the Indian Ocean islands hotspot. IUCN Cambridge, UK in collaboration with IUCN Gland, Switzerland, 59–74.

- Ranarijaona HLT, Harilandy E, Ravelontsoa F, Rajaonarison JF, Ramanandraibe V, Tsitomotra A, Andrianasetra GS, Johnson CM, Rabesa ZA (2014) Etude ethnobotanique et screening phytochimique d'*Hydrostachys plumosa* A. Juss ex Tul. (Hydrostachyaceae): espèce aquatique endémique de Mandritsara Madagascar. Paper presented at colloquium: Les Zones Humides de Madagascar, 19–21 June 2014, Antsirabe, Madagascar.
- Rauh W, Jäger-Zürn I (1966) Le problème de la position systématique des Hydrostachyacées. *Adansonia* 6(4): 515–523.
- Scogin R (1992) Phytochemical profile of *Hydrostachys Insignis* (Hydrostachyaceae). *Aliso* 13(3): 471–474. <https://doi.org/10.5642/aliso.19921303.06>
- Simpson MG (2010) Plant morphology. In: Simpson MG (Ed.) *Plant Systematics* (Second Edition). Academic Press, San Diego, 451–513. <https://doi.org/10.1016/B978-0-12-374380-0.50009-9>
- Soltis DE, Soltis PS, Chase MW, Mort ME, Albach DC, Zanis M, Savolainen V, Hahn WH, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS (2000) Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* 133(4): 381–461. <https://doi.org/10.1006/boj.2000.0380>
- Stannard BL (1997) Hydrostachyaceae. *Flora Zambesiaca*. Volume 9. Part 2. Royal Botanical Gardens, Kew.
- Thouars LMA (1806) *Genera Nova Madagascariensia*: 2.
- Tulasne LR (1849) *Podostemacearum Synopsis Monographica*. *Annales des Sciences Naturelles. Botanique* 11: 87–114.
- Verdcourt B (1986) Flora of Tropical East Africa (Hydrostachyaceae). In: Polhill MR (Ed.) *Flora of Tropical East Africa*. Royal Botanical Gardens, Kew, London, 1–7.
- Warming E (1899) Familien Podostemaceae. *Det Kongelige Danske videnskabernes selskabs skrifter Naturvidenskabelig og Mathematisk Afdeling ser. 6*, 9(2): 105–154.
- White TJ, Bruns T, Lee S, Taylor J (1990) 38 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) *PCR Protocols*. Academic Press, San Diego, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Xiang Q-Y, Moody ML, Soltis DE, Fan C, Soltis PS (2002) Relationships within Cornales and circumscription of Cornaceae—*matK* and *rbcL* sequence data and effects of outgroups and long branches. *Molecular Phylogenetics and Evolution* 24(1): 35–57. [https://doi.org/10.1016/S1055-7903\(02\)00267-1](https://doi.org/10.1016/S1055-7903(02)00267-1)
- Xiang QY, Thomas DT, Xiang QP (2011) Resolving and dating the phylogeny of Cornales – Effects of taxon sampling, data partitions, and fossil calibrations. *Molecular Phylogenetics and Evolution* 59(1): 123–138. <https://doi.org/10.1016/j.ympev.2011.01.016>
- Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y (2017) GGTREE: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution* 8(1): 28–36. <https://doi.org/10.1111/2041-210X.12628>

# A phylogeny of species near *Agrostis* supporting the recognition of two new genera, *Agrostula* and *Alpagrostis* (Poaceae, Pooideae, Agrostidinae) from Europe

Paul M. Peterson<sup>1</sup>, Steven P. Sylvester<sup>2</sup>, Konstantin Romaschenko<sup>1</sup>, Robert J. Soreng<sup>1</sup>, Patricia Barberá<sup>3</sup>, Alejandro Quintanar<sup>4</sup>, Carlos Aedo<sup>5</sup>

**1** Department of Botany MRC-166, National Museum of Natural History, Smithsonian Institution, Washington DC 20013-7012, USA **2** College of Biology and the Environment, Nanjing Forestry University, Long Pan Road No. 159, Nanjing, 210037, China **3** Department of Africa and Madagascar, Missouri Botanical Garden, St. Louis, Missouri 63110-2291, USA **4** Herbarium MA, Unidad de Herbarios, Real Jardín Botánico, Consejo Superior de Investigaciones Científicas, 28014, Madrid, Spain **5** Department of Biodiversity and Conservation, Real Jardín Botánico, Consejo Superior de Investigaciones Científicas, 28014, Madrid, Spain

Corresponding author: Paul M. Peterson (peterson@si.edu)

Academic editor: Marcin Nobis | Received 6 June 2020 | Accepted 31 July 2020 | Published 20 November 2020

**Citation:** Peterson PM, Sylvester SP, Romaschenko K, Soreng RJ, Barberá P, Quintanar A, Aedo C (2020) A phylogeny of species near *Agrostis* supporting the recognition of two new genera, *Agrostula* and *Alpagrostis* (Poaceae, Pooideae, Agrostidinae) from Europe. *PhytoKeys* 167: 57–82. <https://doi.org/10.3897/phytokeys.167.55171>

## Abstract

Based on a molecular DNA phylogeny of three plastid (*rpl32-trnK*, *rps16* intron, and *rps16-trnK*) and nuclear ITS regions investigating 32 species of Agrostidinae, we describe two new genera, *Agrostula* **gen. nov.** with a single species and *Alpagrostis* **gen. nov.** with four species; provide support for five species in a monophyletic *Podagrostis*; and include a small sample of 12 species of a monophyletic *Agrostis* s.s. (including the type and most species of *Neoschischkinia*), that separates into two clades corresponding to *A.* subg. *Agrostis* and *A.* subg. *Vilfa*. *Agrostula* differs from *Agrostis* in having leaf blades with pillars of sclerenchyma which are continuous between the adaxial and abaxial surface of the blades, dorsally rounded glumes with blunt to truncate and erose to denticulate apices, florets  $\frac{1}{2}$  the length of the glumes, lemmas equally wide as long, widest at (or near) apex, apices broadly truncate, irregularly 5 to 7 denticulate to erose, awnless, anthers longer than the lemmas, and rugose-papillose caryopses. *Alpagrostis* differs from *Agrostis* in having geniculate basally inserted awns and truncate lemma apices with lateral veins prolonged from the apex in (2)4 setae. The following eight new combinations are made: *Agrostula truncatula*, *Agrostula truncatula* subsp. *durieu*, *Alpagrostis alpina*, *Alpagrostis alpina* var. *flavescens*, *Alpagrostis barceloi*, *Alpagrostis setacea*,

*Alpagrostis setacea* var. *flava*, and *Alpagrostis schleicheri*. In addition, we provide a key separating *Agrostula* and *Alpagrostis* from *Agrostis* s.s. and other genera previously considered as synonyms of *Agrostis*; lectotypify *Agrostis alpina* Scop., *A. schleicheri* Jord. & Verl., *A. truncatula* Parl., and *A. truncatula* var. *durieui* Henriq.; and neotypify *A. setacea* Curtis.

## Resumen

Sobre la base de una filogenia molecular de ADN de tres regiones plastidiales (*rpl32-trnK*, *rps16* intrón y *rps16-trnK*) e ITS nuclear de 32 especies de Agrostidinae, describimos dos nuevos géneros, *Agrostula* **gen. nov.** con una sola especie, y *Alpagrostis* **gen. nov.** con cuatro especies; mostramos el apoyo para las cinco especies dentro de *Podagrostis* monofilético; e incluimos una pequeña muestra de 12 especies de *Agrostis* s.s. (que incluye el tipo y la mayoría de las especies de *Neoschischkinia*), este último dividido en dos subclados que corresponden a *A.* subg. *Agrostis* y *A.* subg. *Vilfa*. *Agrostula* se diferencia de otras especies de *Agrostis* por tener láminas foliares con haces de esclerénquima continuos entre las superficies adaxial y abaxial de los limbos, glumas de dorso redondeado y ápice embotado a truncado y eroso a denticulado, antecios de ½ de la longitud de las glumas, lemas tan anchas como largas, lo más ancho en o cerca del ápice, ápices anchamente truncados, irregularmente 5 a 7 denticulados o erosos, sin arista, anteras más largas que los lemas y cariopsis rugosa-papilosa. *Alpagrostis* se diferencia de otras especies de *Agrostis* por tener aristas geniculadas insertas basalmente y ápices de lema truncados con venas laterales que se prolongan en (2)4 arístulas apicales. Presentamos las siguientes ocho nuevas combinaciones: *Agrostula truncatula*, *Agrostula truncatula* subsp. *durieui*, *Alpagrostis alpina*, *Alpagrostis alpina* var. *flavescens*, *Alpagrostis barceloi*, *Alpagrostis setacea*, *Alpagrostis setacea* var. *flava* y *Alpagrostis schleicheri*. Además, proporcionamos una clave que separa *Agrostula* y *Alpagrostis* de *Agrostis* s.s. y otros géneros previamente considerados como sinónimos de *Agrostis*, lectotipificamos *Agrostis alpina* Scop., *A. schleicheri* Jord. & Verl., *A. truncatula* Parl. y *A. truncatula* var. *durieui* Henriq. y neotipificamos *A. setacea* Curtis.

## Keywords

*Agrostis*, *Agrostula*, *Alpagrostis*, classification, ITS, *Neoschischkinia*, plastid DNA sequences, phylogeny, *Podagrostis*, taxonomy

## Introduction

The genus *Agrostis* L. includes ca. 224 species worldwide and is placed in subtribe Agrostidinae Fr., supersubtribe Agrostidodinae Soreng, tribe Poeae R.Br., and supertribe Poodae L. Liu in subfamily Pooideae Benth. (Soreng et al. 2017). The length of the palea was recognized by Trinius (1820, 1824) as an important character in separating species of *Agrostis* into two groups, those with short paleas in *A.* sect. *Trichodium* (Michx.) Trin. and those with longer paleas in *A.* sect. *Vilfa* (Adans.) Roem. & Schult. The term “Trichodium net”, based on observations of the Swedish scientist T. Vestergrén, to describe the lemma epidermis of *Agrostis* which bear a fine-meshed network when observed under high magnification, is found almost exclusively in those species with short paleas (Björkman 1960).

A detailed review of the infrageneric classification of the *Agrostis* was given by Björkman (1960) and later summarized in Widén (1971), Romero García et al. (1988a), and Saarela et al. (2017). In the former Soviet Union, Tzvelev (1976, 1983)

recognized four sections in the genus: *Agrostis* sect. *Agrostis* (now = *A.* sect. *Vilfa* s.s. due to type conservation of *Agrostis*) containing species with long paleas  $1/2$ – $2/3$  the length of an usually unawned lemma; *A.* sect. *Pentatherum* (Nabel.) Tzvelev with long paleas  $2/3$ – $1$  the length of a dorsally awned lemma; *A.* sect. *Agraulus* (P. Beauv.) Tzvelev with short paleas  $<1/3$  the length of a dorsally awned lemma, and; *A.* sect. *Trichodium* (Michx.) Dumort. with paleas absent or short  $<1/6$  the length of a usually unawned lemma. Romero García et al. (1988a, b) in the Iberian Peninsula divided *Agrostis* into two subgenera: *A.* subg. *Zingrostis* A.T. Romero García, G. Blanca López & C. Morales Torres containing species that have diffuse panicles with widely spreading, capillary and divaricate branches, and paleas  $1/2$ – $1$  the length of an unawned lemma; and *A.* subg. *Agrostis* consisting of three sections, *A.* sect. *Agrostis* [= *A.* sect. *Trichodium*, *A.* sect. *Agraulus* (P. Beauv.) Tzvelev] with paleas  $<1/3$  the length of the lemma; *A.* sect. *Vilfa* with paleas  $1/2$ – $2/3$  the length of a usually unawned lemma; and *A.* sect. *Aperopsis* Asch. & Graeb. [= *Neoschischkinia* Tzvelev] with paleas  $<1/6$  the length of the awned or unawned lemma, and an annual lifecycle.

*Podagrostis* (Griseb.) Scribn. & Merr. was initially described as a section of *Agrostis* (Grisebach 1852) and was recently updated and revised by Sylvester et al. (2019a, b, 2020) to include ten species native to the western hemisphere. Five additional species of *Agrostis* were transferred into *Podagrostis* in Sylvester et al. (2020) of which *P. bacillata* (Hack.) Sylvester & Soreng and *P. rosei* (Scribn. & Merr.) Sylvester & Soreng are newly included in our molecular analysis using nuclear internal transcribed spacer (ITS) and three plastid DNA (*rpl32-trnK*, *rps16* intron, and *rps16-trnK*) markers. Previously, *P. thurberiana* (Hitchc.) Hultén was included in a phylogenetic study based on morphology and three plastid regions, and the taxon was weakly supported as the sister group of a strongly supported *Agrostis* + *Polypogon* Desf. clade (Soreng et al. 2007). A limitation of that analysis was that only a single species was included for each of the three genera. No molecular study has included more than two species to test the monophyly of this putative lineage (Saarela et al. 2017). The salient characters separating *Podagrostis* from *Agrostis* are: a) floret usually equaling or subequaling the glumes, sometimes slightly shorter but reaching past  $3/4$  the length of the glumes, b) palea well-developed, usually reaching from  $(2/3)$   $3/4$  to almost the apex of the lemma, c) presence of a glabrous or distally hairy rachilla extension emerging from under the base of the palea as a slender short stub up to 1.4 mm long (rudimentary in most florets of *P. rosei*), and d) lemmas unawned or with a short straight mucro 0.2–0.6 mm long, inserted medially or in the upper half of the lemma, not surpassing the glumes (awn 1.6–2 mm long, inserted in lower  $1/3$  of lemma, straight or geniculate and usually not surpassing glumes in *P. rosei*) [Sylvester et al. 2020].

Sáez and Rosselló (2000) described *Agrostis barceloi* L. Sáez & Rosselló from the northern mountains of Mallorca (Balearic Islands) placing it in the *Agrostis alpina* Scop. complex along with *A. curtisii* Kerguélen and *A. schleicheri* Jord. & Verl. These four species share the following synapomorphies: geniculate basally inserted awns and truncate lemma apices that bare lateral setae (extension of the lateral veins) [Romero

García et al. 1988a, b; Sáez and Rosselló 2000]. Other species of *Agrostis* with truncate lemma apices include: *A. nebulosa* Boiss. & Reut. [= *Neoschischkinia nebulosa* (Boiss. & Reut.) Tzvelev], *A. reuteri* Boiss., *A. truncatula* Parl. (these three placed in *A.* subg. *Zingrostis*), *A. pourretii* Willd. (placed in *A.* sect. *Aperopsis*), and *A. tenerrima* Trin. (placed in *A.* sect. *Agrostis*) [Romero García et al. 1988a, b].

*Neoschischkinia* Tzvelev (1968) initially included two species [*N. elegans* (Thore) Tzvelev = *Agrostis tenerrima* Trin., and *N. nebulosa* (Boiss. & Reut.) Tzvelev = *Agrostis nebulosa* Boiss. & Reut.] characterized by having diffuse, open panicles with divaricate and capillary branches, trapezoid lemmas with truncate apices, and caryopses with transverse furrows (Tzvelev 1968). Valdés and Scholz (2006) transferred three more species into *Neoschischkinia* [*N. reuteri* (Boiss.) Valdés & H. Scholz = *A. reuteri*, *N. truncatula* (Parl.) Valdés & H. Scholz = *A. truncatula*, and *N. pourretii* (Willd.) Valdés & H. Scholz = *A. pourretii*].

The main goals of this study were to estimate the phylogenetic relationships of species near or sister to *Agrostis* based on ITS and three plastid DNA regions (*rpl32-trnK*, *rps16* intron, and *rps16-trnK*) for species of Agrostidinae and provide names for two clades that align near but not within *Agrostis* s.s. In addition, we provide a key separating *Agrostula*, gen. nov., and *Alpagrostis*, gen. nov., from *Agrostis* s.s. and other genera considered as synonyms of *Agrostis*. We propose lectotypes for *Agrostis alpina*, *A. schleicheri*, *A. truncatula* and *A. truncatula* var. *durieui* Henriq., and a neotype for *A. setacea*.

## Material and methods

### Phylogenetic analyses

Detailed methods for DNA extraction, amplification, and sequencing are given in Romaschenko et al. (2012) and Peterson et al. (2014, 2015a, b, 2016). We used Geneious Prime 2020 (Kearse et al. 2012) for contig assembly of bidirectional sequences of *rpl32-trnL*, *rps16* intron, *rps16-trnK*, and ITS regions, and Muscle (Edgar 2004) to align consensus sequences and adjust the final alignment. We identified models of molecular evolution for the cpDNA and nrDNA regions using jModeltest (Posada 2008) and applied maximum-likelihood (ML) and Bayesian searches to infer overall phylogeny. The combined data sets were partitioned in accordance with the number of markers used. Nucleotide substitution models selected by Akaike's Information Criterion, as implemented in jModelTest v.0.1.1, were specified for each partition (Table 1). The ML analysis was conducted with GARLI 0.951 (Zwickl 2006). The ML bootstrap analysis used 1000 replicates with 10 random addition sequences per replicate. The tree file from the ML result was read into PAUP where the majority-rule consensus tree was constructed. Bayesian posterior probabilities (PP) were estimated using a parallel version of the MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) where the run of eight Markov chain Monte Carlo iterations was split between an equal number of processors. Bayesian analysis

was initiated with random starting trees and was initially run for four million generations, sampling once per 100 generations. The analysis was run until the value of the standard deviation of split sequences dropped below 0.01 and the potential scale reduction factor was close to or equal to 1.0. The fraction of the sampled values discarded as burn in was set at 0.25.

It is critically important to include the type species of genera and other higher taxa when doing molecular studies to know you are using the name correctly as intended by the original author. The following species are the types of their respective genera and are included in our analyses: *Agrostis canina* L. (type conserved), *Calamagrostis canescens* (Weber) Roth, *Chascolytrum subaristatum* (Lam.) Desv., *Gastridium ventricosum* (Gouan) Schinz & Thell., *Neoschischkinia elegans* (= *Agrostis tenerrima*), *Podagrostis aequivalvis* Trin., and *Triplachne nitens* (Guss.) Link.

Our study was designed to test relationships of three of the four species (*A. alpina*, *A. curtisii*, and *A. schleicheri*) of the *Agrostis alpina* group, all five species that have been attributed to *Neoschischkinia* (*N. elegans*, *N. nebulosa*, *N. pourretii*, *N. reuteri*, and *N. truncatula*), *Podagrostis*, *Gastridium* P. Beauv., *Triplachne* Link, and representative samples of *Agrostis*, *Calamagrostis* Adans., and *Chascolytrum* Desv. All of these genera have been found in a clade in previous molecular analyses and in our unpublished trees investigating a large number of species in *Agrostis*, *Calamagrostis*, *Cinnagrostis* Griseb., and *Koeleria* Pers. (Saarela et al. 2017; Barberá et al. 2019a, b; Peterson et al. 2019). Previous analyses of *Polypogon* found members of the genus nested in a grade within *Agrostis* and there was incongruence between the plastid and nuclear signals (Saarela et al. 2017; Romaschenko et al. unpubl.). We do not address this question here (i.e., *Polypogon* is not included in our sampling) since we lack a large sample of species within *Agrostis* and it is beyond the scope of our study. *Echinopogon caespitosus* C.E. Hubb. in subtribe Echinopogoninae Soreng was chosen as the outgroup since it lies outside of the Agrostidinae, but inside supersubtribe Agrostidodinae (Soreng et al. 2017; Tkach et al. 2020).

## Taxonomy

Herbarium acronyms follow Index Herbariorum (Thiers, continuously updated). In this treatment glabrous means without pubescence (in the sense of slender, relatively soft hairs). Smooth indicates no prickle-hairs with broad bases and/or hooked or pointed apices (i.e., pubescence can occur on a smooth surface, and a rough or scabrous surface can be glabrous). Specimens in the United States National Herbarium (US) and the Real Jardín Botánico Herbarium (MA) were reviewed for this study, in addition to Romero Zarco (1987), Romero García et al. (1988a, b), Sáez and Roselló (2000), Clayton et al. (2006), Cope and Gray (2009), and Portal (2009) were consulted during preparation of the descriptions. Beyond types (some only seen in images), only material from herbaria where specimens have been checked and verified by the authors are cited. Parts of the generic key were adapted from Sylvester et al. (2020).

## Results

### Phylogeny

A total of 176 new sequences from 33 species (48 individuals) are reported in GenBank (Table 1). Total aligned characters for individual regions and other parameters are noted in Table 2. The resulting plastid and ITS topologies were inspected for conflicting nodes (see Fig. 1) with  $\geq 80\%$  bootstrap support (BS) and/or posterior probabilities (PP)  $\geq 0.95$ . No supported conflict was found so plastid and ITS sequences were combined.

The ML tree from the combined plastid and ITS regions (Fig. 1) is well resolved (posterior probabilities identified in the Bayesian analysis are included on the ML tree, and most clades include a PP = 1), with strong support (BS  $\geq 96$ –100) for the following clades: two species of *Gastridium*, four accessions of *Triplachne nitens*, an *Agrostis* s.s. clade that includes two subclades A and B, three accessions of *Agrostis truncatula*, and the *Agrostis alpina*–*A. curtisii*–*A. schleicheri* clade; moderate support (BS = 84–86%) for seven species of *Calamagrostis* and two species of *Chascolytrum*; and weak support (BS = 57%) for five species of *Podagrostis*. *Chascolytrum* is basal followed by, in order of divergence, a clade with *Agrostis truncatula* sister to *Calamagrostis*, a clade with *Gastridium* sister to *Triplachne* which is sister to the remaining species in the *Agrostis* s.l. clade (PP = 1, BS = 66). In *Agrostis* s.l., *Podagrostis* is sister to the *Agrostis alpina*–*A. curtisii*–*A. schleicheri* clade and the *Agrostis* s.s. clade.

### Discussion

Our molecular sampling of five species of *Podagrostis* is the largest to date. In an earlier Romaschenko et al. (unpubl.) study of the three species then in the genus, *P. humilis* (Vasey) Björkman exhibited incongruence with the nuclear ITS signal aligning within the *Podagrostis* clade and the plastid signal aligning as sister to *Agrostis* s.s. in a grade with the *Agrostis alpina*–*A. curtisii*–*A. schleicheri* clade at the base. The addition of *P. bacillata* and *P. rosei* in our analysis eliminated this anomaly. In an earlier study primarily using different DNA markers with only *P. aequivalvis* and *P. rosei* (as *Agrostis rosei* Scribn. & Merr.), Saarela et al. (2017) found *P. rosei* to be part of a well-supported clade with four Chinese species of *Deyeuxia* Clarion ex P. Beauv. and *Calamagrostis bolanderi* Thurber + *P. aequivalvis*. Although *C. bolanderi*'s placement in a strongly supported lineage with *P. aequivalvis* provides support for its transfer to *Podagrostis*, we hesitate to include it here because it may represent a separate hybrid between *Podagrostis* and *Calamagrostis* (Sylvester et al. 2020). A robust phylogeny with the inclusion of *P. colombiana* Sylvester & Soreng, *P. exserta* (Swallen) Sylvester & Soreng, *P. liebmannii* (E. Fourn.) Sylvester & Soreng, and *P. trichodes* (Kunth) Sylvester & Soreng is needed, as well as the Asian species of *Deyeuxia* that are allied with the group and are in need of generic realignment.

**Table 1.** Taxon voucher (collector, number, and where the specimen is housed), country of origin, and GenBank accession for DNA sequences of *rps16-trnK*, *rps16 intron*, *rpl32-trnL*, and ITS regions; a dash (-) indicates missing data.

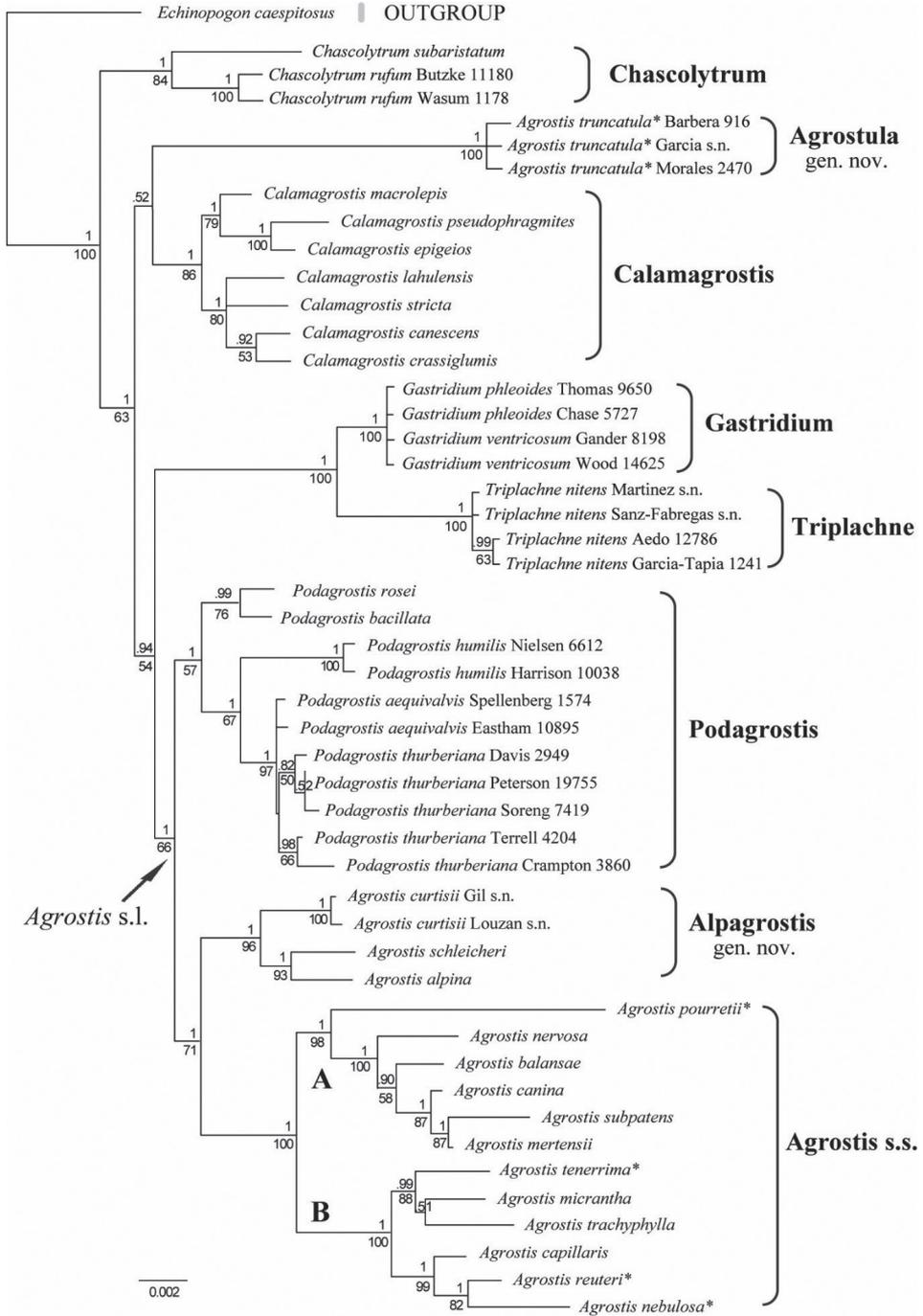
	Taxa	Voucher	Country	rps16-trnK	rps16 intron	rpl32-trnL	ITS
1	<i>Agrostis alpina</i> Scop. [= <i>Alpagrostis alpina</i> (Scop.) P.M. Peterson, Romasch., Soreng & Sylvester]	Soreng 7484, Gillespie & Peterson (US)	Austria, Niederosterrich	MT410018	-	MT409931	MT396529
2	<i>Agrostis bacillata</i> Hack. [= <i>Podagrostis bacillata</i> (Hack.) Sylvester & Soreng]	Evans 145, Lellingier & Bowers (US)	Costa Rica, San Jose	MT410019	MT409978	MT409932	MT396530
3	<i>Agrostis balansae</i> (Boiss.) Tzvelev	Soreng 8967b & Cabi (US)	Turkey, Erzurum	MT410020	MT409979	MT409933	MT396531
4	<i>Agrostis canina</i> L.	Herrero 1874, Aedo, Aizpuru, Alarcón, Aldasoro, Castroviejo, Conti, Estébanez, Güemes, Guillén, Navarro, Pedrol, Prunell, Rico, Rodríguez Gracia & Tinti (MA)	Italy, Abruzzo	MT410021	MT409980	MT409934	MT396532
5	<i>Agrostis capillaris</i> L.	Aedo 19209 (MA)	France, Landes	MT410022	MT409981	MT409935	MT396533
6	<i>Agrostis curtisii</i> Kerguélen [= <i>Alpagrostis setacea</i> (Poir.) P.M. Peterson, Romasch., Soreng & Sylvester]	Gil s.n. (MA)	Spain, Coruña	MT410023	MT409982	MT409936	MT396534
7	<i>Agrostis curtisii</i> Kerguélen [= <i>Alpagrostis setacea</i> (Poir.) P.M. Peterson, Romasch., Soreng & Sylvester]	Louzan s.n. & Rodríguez-Oubiña (MA)	Spain, Coruña	MT410024	MT409983	MT409937	MT396535
8	<i>Agrostis mertensii</i> Trin.	Smith 1288 (US)	Sweden, Härjedalen	MT410025	MT409984	MT409938	MT396536
9	<i>Agrostis micrantha</i> Steud.	Tibet-MacArthur 1516, Wen, Nie, Soreng, Rankin, Yue, Wang & Yue (US)	China, Yunnan	MT410026	MT409985	MT409939	MT396537
10	<i>Agrostis nebulosa</i> Boiss. & Reut.	Serra 8114 (US)	Spain	MT410027	MT409986	MT409940	MT396538
11	<i>Agrostis nervosa</i> Nees ex Trin.	Soreng 5276, Peterson & Sun Hang (US)	China, Yunnan	MT410028	MT409987	MT409941	MT396539
12	<i>Agrostis pourretii</i> Willd.	Carrera s.n. (MA)	Spain	MT410029	MT409988	MT409942	MT396540
13	<i>Agrostis reuteri</i> Boiss.	Escobar-García s.n. (MA)	Spain	MT410030	MT409989	MT409943	MT396541
14	<i>Agrostis rosei</i> Scribn. & Merr. [= <i>Podagrostis rosei</i> (Scribn. & Merr.) Sylvester & Soreng]	Peterson 19053 & Sánchez Alvarado (US)	Mexico, Durango	MT410031	MT409990	MT409944	MT396542
15	<i>Agrostis schleicheri</i> Jord. & Verl. [= <i>Alpagrostis schleicheri</i> (Jord. & Verl.) P.M. Peterson, Romasch., Soreng & Sylvester]	Arán 5627, Patino & Valencia (MA)	Spain, Cantabria	MT410032	MT409991	MT409945	MT396543
16	<i>Agrostis subpatens</i> Hitchc.	Lathrop 5571 (US)	Costa Rica	MT410033	MT409992	MT409946	MT396544

	Taxa	Voucher	Country	rps16-trnK	rps16 intron	rpl32-trnL	ITS
17	<i>Agrostis trachyphylla</i> Pilg.	Peterson 24374, Soreng & Romaschenko (US)	Tanzania, Kilimanjaro	MT410034	MT409993	MT409947	MT396545
18	<i>Agrostis truncatula</i> Parl. [= <i>Agrostula truncatula</i> (Parl.) P.M. Peterson, Romasch., Soreng & Sylvester]	Barberá 916 (MA)	Spain	MT410035	MT409994	MT409948	MT396546
19	<i>Agrostis truncatula</i> Parl. [= <i>Agrostula truncatula</i> (Parl.) P.M. Peterson, Romasch., Soreng & Sylvester]	García Río (MA)	Spain, Ciudad Real	MT410036	MT409995	MT409949	MT396547
20	<i>Agrostis truncatula</i> Parl. [= <i>Agrostula truncatula</i> (Parl.) P.M. Peterson, Romasch., Soreng & Sylvester]	Morales 2470 (MA)	Spain	MT410037	MT409996	MT409950	MT396548
21	<i>Calamagrostis canescens</i> (Weber ex F.H. Wigg.) Roth	Barta 1999-14 (MA)	Austria, Niederösterreich	MT410038	MT409997	MT409951	MT396549
22	<i>Calamagrostis crassiglumis</i> Thurb.	Howell 23214 (US)	USA	MT410039	MT409998	MT409952	MT396550
23	<i>Calamagrostis epigeios</i> (L.) Roth	Calvo 4970 (MA)	Czech Republic, South Bohemian	MT410040	MT409999	MT409953	MT396551
24	<i>Calamagrostis labulensis</i> G. Singh	Tibet-MacArthur 1317 (US)	China	MT410041	MT410000	MT409954	MT396552
25	<i>Calamagrostis macrolepis</i> Litv.	Soreng 7637, Johnson, Shuvalov, Chapurin, Samsaliev & Samsaliev (US)	Kyrgyzstan, Naryn	MT410042	MT410001	MT409955	MT396553
26	<i>Calamagrostis pseudophragmites</i> (Haller fil.) Koeler	Cabezas 688, Aedo, Calvo, Castroviejo, Constantidinis, Gonzalo, Güemes, Herrero, Karidas, Medina, Navarro, Pedrol, Prunell, Quintanar, Rico & Rodríguez Gracia (MA)	Greece, Epiro	MT410043	MT410002	MT409956	MT396554
27	<i>Calamagrostis stricta</i> (Timm) Koeler	Soreng 7722, Johnson, Shuvalov, Chapurin, Samsaliev & Samsaliev (US)	Kyrgyzstan, Chu	MT410044	MT410003	MT409957	MT396555
28	<i>Chascolytrum rufum</i> J. Presl	Butzke 11180 (US)	Brazil	–	–	MT409958	MT396556
29	<i>Chascolytrum rufum</i> J. Presl	Wasum 1178 (US)	Brazil, Rio Grande do Sul	–	–	MT409959	MT396557
30	<i>Chascolytrum subaristatum</i> (Lam.) Desv.	Hale 20420 & Soderstrom (US)	Mexico, Chiapas	MT410045	MT410004	MT409960	MT396558
31	<i>Echinopogon caespitosus</i> C.E. Hubb.	Craven 672 (NSW)	Australia, New South Wales	MT410046	MT410005	MT409961	MT396559
32	<i>Gastridium phleoides</i> (Nees & Meyen) C.E. Hubb.	Thomas 9650 (US)	USA	MT410048	MT410007	MT409963	MT396561
33	<i>Gastridium phleoides</i> (Nees & Meyen) C.E. Hubb.	Chase A. 5727 (US)	USA	MT410047	MT410006	MT409962	MT396560
34	<i>Gastridium phleoides</i> (Nees & Meyen) C.E. Hubb.	Gander 8198 (US)	USA	MT410049	MT410008	MT409964	MT396562
35	<i>Gastridium ventricosum</i> (Gouan) Schinz & Thell.	Wood 14625 (US)	USA Hawaii, Kaua'i	MT410050	MT410009	MT409965	MT396563

	Taxa	Voucher	Country	rps16-trnK	rps16 intron	rpl32-trnL	ITS
36	<i>Podagrostis aequivalvis</i> (Trin.) Scribn. & Merr.	Eastham 10895 (US)	Canada, British Columbia	–	–	MT409966	MT396564
37	<i>Podagrostis aequivalvis</i> (Trin.) Scribn. & Merr.	Spellenberg 1574 & Spellenberg (US)	Canada, Alberni-Clayoquot	–	–	MT409967	MT396565
38	<i>Podagrostis humilis</i> (Vasey) Björkman	Harrison 10038 (US)	USA, Utah	–	–	MT409968	MT396566
39	<i>Podagrostis humilis</i> (Vasey) Björkman	Nielsen 6612 (US)	USA, Utah	–	–	MT409969	MT396567
40	<i>Podagrostis thurberiana</i> (Hitche.) Hultén	Crampton 3860 (US)	USA, California	–	–	–	MT396568
41	<i>Podagrostis thurberiana</i> (Hitche.) Hultén	Davis 2949 (US)	USA, Idaho	MT410051	MT410010	MT409970	MT396569
42	<i>Podagrostis thurberiana</i> (Hitche.) Hultén	Peterson 19755, Saarela & Sears (US)	USA, California	MT410052	MT410011	MT409971	MT396570
43	<i>Podagrostis thurberiana</i> (Hitche.) Hultén	Soreng 7419 & Soreng (US)	USA, California	MT410053	MT410012	MT409972	MT396571
44	<i>Podagrostis thurberiana</i> (Hitche.) Hultén	Terrell 4204 (US)	USA, California	MT410054	MT410013	MT409973	MT396572
45	<i>Triplachne nitens</i> (Guss.) Link	Aedo 12786 (MA)	Spain, Murcia	MT410055	MT410014	MT409974	MT396573
46	<i>Triplachne nitens</i> (Guss.) Link	López Jiménez 1241 & García Tapia (MA)	Morocco, Nador	MT410056	MT410015	MT409975	MT396574
47	<i>Triplachne nitens</i> (Guss.) Link	Rivas Martínez, Costa & Regueiro (MA)	Spain, Islas Baleares	MT410057	MT410016	MT409976	MT396575
48	<i>Triplachne nitens</i> (Guss.) Link	Sanz Fábregas s.n. (MA)	Spain, Almeria	MT410058	MT410017	MT409977	MT396576

Affinities of *Agrostis truncatula* are unclear, given the lack of support for its position (PP = 0.52) in the phylogeny sharing a common ancestor with *Calamagrostis* rather than aligning within *Agrostis* s.l. *Agrostis truncatula* has many unique morphological characteristics and differs from other species of *Agrostis* in having the combination of perennial habit, leaf blades with pillars of sclerenchyma that are continuous between the adaxial and abaxial surface of the blades, dorsally rounded glumes with blunt to truncate and erose to denticulate apices, open and diffuse panicles, florets ½ the length of the glumes, lemmas equally wide as long, widest at (or near) apex, apices broadly truncate, irregularly 5 to 7 denticulate to erose, awnless, anthers longer than the lemmas, and rugose-papillose caryopses. We, thus, describe *Agrostula* gen. nov. below based on the single species, *A. truncatula*, with two subspecies. We find no support for recognizing *Neoschischkinia* (Tzvelev 1968; Valdés and Scholz 2006), since four of the five species attributed to the genus align in the *Agrostis* s.s. clade: *A. nebulosa*, *A. reuteri*, and *A. tenerrima* (type of *Neoschischkinia*) in *Agrostis* subg. *Vilfa* (clade B), and *A. pourretii* in *A.* subg. *Agrostis* (clade A); whereas *A. truncatula* is phylogenetically isolated from *Agrostis* (*Agrostula*). All these species exhibit unusual characteristics not commonly found within *Agrostis*, i.e., diffuse, open panicles with divaricate and capillary branches, trapezoid lemmas with truncate apices, and caryopses with transverse furrows. However, without molecular DNA evidence, earlier systematists could not predict the complicated phylogenetic history of *Agrostula truncatula*.

Our rationale for recognizing the *Agrostis alpina* complex in a new genus, *Alpagrostis* gen. nov., is straightforward. Much like *Podagrostis*, there are salient morphological



**Figure 1.** Maximum-likelihood tree inferred from combined plastid (*rpl32-trnL*, *rps16* intron, *rps16-trnK*) and ITS sequences. Numbers above the branches are posterior probabilities; numbers below the branches are bootstrap values; accessions marked with an asterisk\* were formerly included in *Neoschischkinia*; and letters refer to clade A = *Agrostis* subg. *Agrostis* and clade B = *A.* subg. *Vilfa*. Scale bar: 0.002 substitutions per site.

**Table 2.** Characteristics of *rps16-trnK*, *rps16* intron, *rpl32-trnL*, and ITS, and parameters used in Bayesian analyses indicated by Akaike Information Criterion (AIC).

	<i>rps16-trnK</i>	<i>rps16 intron</i>	<i>rpl32-trnL</i>	Combined plastid data	ITS	Overall
Total aligned characters	738	845	904	2487	712	3199
Number of sequences	41	40	47	128	48	176
Likelihood score (-lnL)	1259.16	1449.21	1888.70		1989.55	
Number of substitution types	6	6	6	–	6	–
Model for among-sites rate variation	gamma	gamma	gamma	–	gamma	–
Substitution rates						
rAC	2.44683	2.33760	1.03926	–	0.78611	–
rAG	2.12801	1.84060	0.64852		2.03233	
rAT	0.11415	0.31850	0.20833		1.27811	
rCG	1.41016	0.78529	0.73967		0.31482	
rCT	2.47892	2.54521	0.97480		5.07499	
rGT	1.00000	1.00000	1.00000		1.00000	
Character state frequencies						
fA	0.28602	0.35597	0.36767	–	0.22141	–
fC	0.16385	0.15120	0.14893		0.29792	
fG	0.16537	0.18750	0.13618		0.29123	
fT	0.38477	0.30534	0.34722		0.18944	
Proportion of invariable sites	0.37013	0.1041	0.36504	–	0.30563	–
Substitution model	TVM+G	GTR+I+G	GTR+G	–	GTR+I+G	–
Gamma shape parameter ( $\alpha$ )	0.90138	0.45913	0.83500	–	0.38018	–

features, i.e., geniculate basally inserted awns and truncate lemma apices with setaceous lateral veins, and there is strong clade support as sister to *Agrostis* s.s. The branch length of the *Alpagrostis* clade is moderately long indicating genetic differentiation shared among its members separating it from other clades. Sáez and Rosselló (2000) suggested that *Agrostis barceloi*, a tetraploid ( $2n = 28$ ), is closely related to *A. schleicheri*, a hexaploid ( $2n = 42$ ), and might have originated by the splitting of the shared ancestral lineage. The morphological features shared by *A. barceloi* and *A. schleicheri* suggest they may be derived from the diploids, *A. alpina* ( $2n = 14$ ) or *A. curtisii* ( $2n = 14$ ) since the former two species are geographically and genetically isolated (Sáez and Rosselló 2000). Massó et al. (2016) surveyed 40 of the 100 known individuals of the extremely narrow endemic, *A. barceloi*, for allozyme diversity, showing all loci to be monomorphic or with fixed heterozygosity consistent with allopolyploid origin (interspecific hybridization process and subsequent chromosome duplication) [Stebbins 1947; Crawford 1989; Soltis and Soltis 2000].

The *Agrostis* s.s. clade is divided into two strongly supported A and B clades that correspond to species that align in the *Agrostis* subg. *Agrostis* (clade A) or *Agrostis* subg. *Vilfa* (Adans.) Rouy (clade B) [ $\equiv$  *A.* sect. *Vilfa* (Adans.) Roem. & Schult.]. As mentioned in the introduction, palea length is an important character used to separate these two subgenera and all species in clade A have paleas  $\leq 1/3$  the length of the lemma as expected, sometimes rudimentary or absent as in e.g. *A. mertensii* Trin., *A. subpatens* Hitchc. However, not all species in clade B have paleas  $1/2$ – $2/3$  the length of the lemma since *A. tenerrima* has paleas  $1/6$  the length of the lemma and only about 0.1 mm long. This is not terribly surprising since hybrids among species of *Agrostis* are often fertile, and inter-subgeneric hybrids include *A. canina*  $\times$  *A. stolonifera* L., a cross between the type of each subgenus of *Agrostis* (Widén 1971; Belanger et al. 2003; Watrud et al. 2004). In the future we intend to publish a large phylogeny of *Agrostis* with a

comprehensive species sampling. In this larger paper we will also address the hybrid origins that complicate species relationships in *Agrostis* s.s. with members of *Polypogon*, *Lachnagrostis* Trin., and *Chaetotropis* Kunth, genera that form a clade sister to or are reticulately intermeshed within *Agrostis* s.s. (Saarela et al. 2017; Soreng et al. 2017: 268).

### Taxonomic treatment

#### *Agrostula* P.M. Peterson, Romasch., Soreng & Sylvester, gen. nov.

urn:lsid:ipni.org:names:77212587-1

**Type.** *Agrostis truncatula* Parl.

**Diagnosis.** The one species of *Agrostula* differs from all other species of *Agrostis* by its glumes being dorsally rounded, not keeled, smooth throughout, and with apices blunt to truncate and erose to denticulate. Further differentiation can be made by the combination of perennial habit, leaf blades with pillars of sclerenchyma that are continuous between the adaxial and abaxial surface of the blades, panicles open and diffuse, florets  $\frac{1}{2}$  the length of the glumes, lemmas equally wide as long, widest at (or near) apex, apices broadly truncate, irregularly 5 to 7 denticulate to erose, awnless, paleas c.  $\frac{1}{2}$  the length of the lemma, anthers longer than the lemma, caryopsis surface rugose-papillose, and its ecology, being found growing in very shallow soils.

**Description.** *Perennials* moderately to densely tufted. *Culms* 10–40 cm tall, erect, arching, or geniculate-ascendant, slender, smooth, usually with 3 or 4 nodes extended above the basal foliage. *Tillers* intravaginal, extravaginal innovations absent. *Leaves* mostly basal, in fascicles of few to many leaves; *sheaths* often as long as or sometimes longer than the internodes, glabrous, smooth; *ligules* 0.5–4 mm long, longer than they are wide in subsp. *truncatula* and shorter than they are wide in subsp. *durieui*, oblong, hyaline, glabrous, smooth, apices truncate to acute, dentate; basal and tiller ligules 0.5–2.5 × 1–2.5 mm; upper culm ligules 3–4 × 1–2.5 mm in subsp. *truncatula*; *blades* flat, conduplicate, or convolute, straight to sometimes recurved after flowering, acute, firm to rigid, glabrous, abaxially scabrous, adaxially scabrous; blades of lower culm and tillers 3–7 cm long, 0.7–2 mm in diameter as flat, folded or rolled; blades of upper culm 1–4 cm long, 0.5–1.2 mm in diameter as flat, folded or rolled. *Inflorescence* c. 2–20 × 2–12 cm, a panicle, diffuse and open, broadly ovoid; *panicle branches* divaricate, capillaceous, with spikelets present only in the distal  $\frac{1}{3}$ – $\frac{1}{2}$ , glabrous, smooth; *pedicels* generally twice as long as the spikelets or longer, thickened, apices clavate, glabrous, smooth. *Spikelets* 1–1.7 mm long, 1-flowered, disarticulating above the glumes, dorsally compressed or very weakly laterally compressed; *glumes* equal or subequal, ovoid-lanceolate, membranous, 1-veined, the vein inconspicuous, dorsally rounded, smooth throughout, apices truncate to blunt and minutely notched, erose to denticulate; *floret* c.  $\frac{1}{2}$  the length of the glumes, sessile; *lemmas* 0.5–0.8 mm long in subsp. *truncatula*

and (0.7–)0.9–1(–1.2) mm long in subsp. *durieui*, broadly ovoid, equally wide as long, widest at (or near) apex, membranous, dorsally rounded, 5-veined, veins usually evident to distinct, with at least the outer veins excurrent, usually glabrous or sometimes pubescent, smooth throughout, apex broadly truncate and denticulate, with the veins terminating in 5 to 7 teeth 1/8–1/5 the length of the lemma, awnless; *paleas* 0.3–0.5 mm long, c. 1/2 the length of the lemma, glabrous, smooth, apices bifid, denticulate; *calluses* rounded, blunt, glabrous or almost so, abaxially smooth; *rachilla* prolongation absent. **Flowers** perfect; *lodicules* 0.1–0.3 mm long, c. 1/2 as long as the palea, 2 in number, acute; *anthers* 0.7–1 mm long, 3 in number; *ovaries* glabrous. **Caryopses** 0.8–1.1 mm long, generally longer than the lemmas, only partially concealed at maturity, ellipsoid, surface rugose-papillose, ventrally sulcate, sulcus distinct, almost without rostellum; hilum narrowly elliptic c. 1/6–1/3 the length of the caryopsis; endosperm liquid.  $2n = 14 + 0-4B$  (Garde 1951; Björkman 1960; Fernandes and Queiros 1969; Queiros 1974, 1979; Romero García and Blanca López 1988).

**Distribution and ecology.** Iberian Peninsula and northern Africa, distributed in France, Spain, Portugal, and Morocco. Found in Mediterranean, Iberian-Atlantic and cold temperate, often high-elevation, environments of the Pyrenees. Forms part of pioneer grassland species assemblages which grow on very shallow and sandy ‘skeleton’ soils, apparently reliant on climatic humidity in addition to precipitation for its water supply. Usually flowering from June to July.

**Notes.** *Agrostula truncatula* also differs in its leaf blade anatomy from most other species of *Agrostis* in having pillars of sclerenchyma which are continuous between the adaxial and abaxial surface of the blades. These continuous pillars of sclerenchyma are exceptionally thick and found only on the margins and central vein in subsp. *truncatula*, while subsp. *durieui* has thinner continuous sclerenchyma packets in the margins, central and primary veins (Romero García and Blanca López 1988: fig. 4C–F). Costal cells and intercostal long cells of the abaxial blade surface are also distinct, with *A. truncatula* differing from species of *Agrostis* in the Iberian Peninsula in having paired  $s_0$ -z cells in the costal zone, and  $l_3$  type long cells in the intercostal zone (Romero García and Blanca López 1988). Stomata are also apparently absent on the abaxial blade surface, a character found in only a few other species in the Iberian Peninsula, i.e., *Agrostis reuteri* and *Alpagrostis setacea* (Romero García & Blanca López, 1988).

***Agrostula truncatula* (Parl.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**  
urn:lsid:ipni.org:names:77212590-1

≡ *Agrostis truncatula* Parl., Fl. Ital. 1: 185. 1848 ≡ *Neoschischkinia truncatula* (Parl.) Valdés & H. Scholz, Willdenowia 36(2): 663. 2006. Type: Spain, Sierra de Guadarrama, Aug 1841, *G. Reuter s.n.* (lectotype, **designated here**: FI-016207 [image!]; isolectotypes, FI-016206 [image!], FI-012389 (Webb herbarium, left hand plant) [image!]).

***Agrostula truncatula* subsp. *durieui* (Boiss. & Reut. ex Willk.) P.M. Peterson, Romasch., Quintanar, Soreng & Sylvester, comb. nov.**

urn:lsid:ipni.org:names:77212591-1

- ≡ *Agrostis durieui* Boiss. & Reut. ex Willk., Suppl. Prodr. Fl. Hisp. 15. 1893 ≡ *Agrostis truncatula* subsp. *durieui* (Boiss. & Reut. ex Willk.) Asch. & Graebn., Syn. Mitteleur. Fl. 2(1): 193. 1899 ≡ *Agrostis delicatula* subsp. *durieui* (Boiss. & Reut. ex Willk.) Rivas Mart., Lazaroa 2: 328. 1980 ≡ *Neoschischkinia truncatula* subsp. *durieui* (Boiss. & Reut. ex Willk.) Valdés and H. Scholz, Willdenowia 36(2): 663. 2006. Type: Spain. Asturias: Peñafior [“Hab. in Asturiis freq., usque ad summa juga occident., Peñafior”], 16 Jun 1835, *M.C. Durieu de Maisonneuve s.n.* [Durieu Plant. Select. Hispano-Lusit sect. 1 Asturicae. Collectae, no. 173] (lectotype, designated by A.T. Romero García and G. Blanca, Taxon 35(4): 695. 1986: P-02219803 [image!]; isolectotypes: P-03487772 [image!], W-18890096450 [image!]).
- = *Agrostis durieui* Boiss. & Reut. ex Gand., Bull. Soc. Bot. France 43: 210. 1896, nom. illeg. hom., non Boiss. and Reut. ex Willk. 1893. Type: Spain. Palencia: m. “Peña Labra, in fissuris, rupium cacuminis, 5700 ft, 26 Jul 1894, *M. Gandoger s.n.*” (lectotype, designated by S. Castroviejo and A. Charpin, Candollea 54(2): 475. 1999: LY [lower specimen]).
- = *Agrostis truncatula* var. *durieui* Henriq., Bol. Soc. Brot. 20: 49. 1903, nom. Illeg. hom., non subsp. *durieui* Asch. & Graebn. Type: Spain. Asturias, 27 May 1864, *Borgeau 2716*. (lectotype, **designated here**: P-03330466 [image!]; isolectotypes: P-02220227 [image!], P-03330465 [image!], P-03487775 [image!]).
- = *Agrostis truncatula* subsp. *commista* Castrov. & Charpin, Candollea 38(2): 676. 1983, nom. illeg. superfl. Type: Spain. Zamora: Lubián, Chanos, proximidades del puerto de Padornelo, 29T PG 7356, 1200 m, 30 Dec [Jul] 1977, *S. Castroviejo 790* (holotype: MA-242072 [image!]; isotype: G-00191448 [image!]).

**Notes.** Romero García et al. (1988a) provide a key to differentiate the two subspecies. The typical subspecies has ligules as long or longer than wide with acute apices, conduplicate leaf blades that recurve at anthesis, and shorter lemmas 0.5–0.8 mm long whereas *Agrostula truncatula* subsp. *durieui* has ligules wider than long with truncate apices, flat, rarely conduplicate leaf blades that do not recurve at anthesis, and longer lemmas (0.7–)0.9–1(–1.2) mm long. Portal (2009) treated subsp. *durieui* as *Agrostis durieui* for France, and did not recognize *A. truncatula* as being in France.

***Alpagrostis* P.M. Peterson, Romasch., Soreng & Sylvester, gen. nov.**

urn:lsid:ipni.org:names:77212592-1

**Type.** *Agrostis alpina* Scop.

**Diagnosis.** The species of *Alpagrostis* differ from *Agrostis* by a combination of characters in having plants densely tufted with only intravaginal innovations, leaves mainly basal, basal leaf blades involute and setaceous or filiform, conduplicate and acute,

0.1–1.2 mm in diameter as folded or rolled, ligules longer than they are wide, spikelets generally > 3 mm long, lemma apices truncate with lateral veins prolonged from the apex in 2 (*A. setacea*) or 4 setae 0.1–0.5 mm long, and, crucially, and lemmas with a well-developed awn, 3–7.4 mm long, inserted basally c. 0.1–0.4 mm from the base of the lemma, conspicuously twisted and geniculate.

**Description.** *Perennials*, densely tufted. *Culms* 4–75 cm tall, erect or slightly geniculate at the base, slender, smooth or scabrous in the upper part, usually with 2–3 nodes extended above the basal foliage. *Tillers* intravaginal, extravaginal innovations absent. *Leaves* mostly basal, in fascicles of few to many leaves; *sheaths* shorter than the internodes, glabrous, smooth or scabrous; *ligules* 0.4–5 mm long, longer than they are wide, oblong, hyaline, glabrous, smooth, apices truncate, subacute, acute, entire to dentate; basal and tiller ligules 0.4–3 × 0.15–1.3 mm; upper culm ligules 1.7–5 × 0.7–1.5 mm; *blades* involute and setaceous or filiform and acute, tender to firm, straight to recurved, glabrous, abaxially smooth to scabrous, adaxially scabrous; blades of the lower culms and tillers 2–25 cm long, 0.1–1.2 mm in diameter as folded or rolled; blades of upper culm 1.5–10 cm long, 0.2–1.5 mm in diameter as folded or rolled, generally wider and shorter than tillers. *Inflorescence* (1.5–)2–15 × 0.5–3.5 cm, a panicle, lax and open to loosely to densely contracted and spikelike; *panicle branches* erect, ascendant or patent, with spikelets present from the base to only in the distal ½, glabrous, densely scabrous (or smooth in *A. barceloi*); *pedicels* as long as the spikelets, cylindrical, apices clavate, glabrous, densely scabrous (or smooth in *A. barceloi*). *Spikelets* (2.7 in *A. barceloi*)–3–5.2(–5.5) mm long, 1-flowered, disarticulating above the glumes, weakly laterally compressed; *glumes* unequal, the lower shorter and thinner than the upper, upper glume longer than the length of the floret by c. 0.8–1.9 mm, lanceolate, membranous, glabrous, keel scabrous throughout or in the distal ½, lateral veins smooth or scabrous distally, surfaces smooth or scabrous distally, apices acute or mucronate; lower glume 1-veined; upper glume (1-veined in *A. barceloi*) 3-veined; *floret* sessile, much shorter than the glumes; *lemmas* (1.8 in *A. barceloi*)–2–3.7, lanceolate, membranous, dorsally rounded, 5-veined, veins usually evident to distinct, with at least the outer veins excurrent, glabrous or thinly pubescent at the base with hairs up to 0.4 mm long, surface smooth to densely scabrous with aculeate (thin short stiff) prickles throughout, apex truncate with lateral veins prolonged from the apex in 2 (*A. setacea*) or 4 setae 0.1–0.5 mm long, awned with awn inserted basally c. 0.1–0.4 mm from the base of the lemma (or sometimes in the lower 1/5–1/4 in *A. barceloi*), awn well-developed, 3–7.4 mm long, surpassing the glumes, geniculate in roughly the middle, distinctly twisted proximally with usually at least 2 full twists below the bend, smooth proximally, scabrous distally or for most of the length; *paleas* 0.4–1 mm long, 1/5–1/3 the length of the lemma, glabrous, smooth, apices bifid, dentate, irregularly dentate or emarginate; *calluses* rounded, blunt, pilose, with hairs 0.3–0.7 mm long inserted all around or in 2 lateral tufts, abaxially smooth; *rachilla* prolongation absent. *Flowers* perfect; *lodicules* 0.4–0.6 mm long, ½–2/3 as long as the palea, 2 in number, acute to lanceolate; *anthers* 0.7–2.3 mm long, 3 in number; *ovaries* glabrous. *Caryopses* 1.7–2 mm long, shorter than the lemmas, concealed at maturity, ellipsoid or fusiform, surface smooth (becoming narrow and shriveled with age), ventrally sulcate, sulcus distinct, almost without rostellum; *hilum* 1/6–1/3 length of the caryopsis,

narrowly elliptic; **endosperm** liquid.  $2n = 14$  (In *A. setacea*, *A. alpina*), 28 (*A. barceloi*), or 42 (*A. schleicheri*) [Frey 1997; Sáez and Rosselló 2000].

**Distribution and ecology.** Europe and Mediterranean. Found in cold temperate, often high-elevation environments, often found growing on nutrient poor soils. Usually flowering from June to August.

**Notes.** All caryopses examined from herbarium specimens had a liquid lipid endosperm or were shriveled with a deep sulcus, implying that fresher specimens likely had a liquid endosperm. *Agrostis* sect. *Bromidium* (Nees & Meyen) E. Desv. shares many characteristics with *Alpagrostis*, such as lemma apices terminating in scabrous setae, well-developed, thickened, twisted and geniculate awns inserted in the lower 1/3 of the lemma, palea < 1/3 the length of the lemma, caryopses with liquid to semi-liquid endosperm. Based on molecular DNA studies, Romaschenko et al. (unpubl.) and Tkach et al. (2020) found *Bromidium* to align within *Agrostis* s.s.

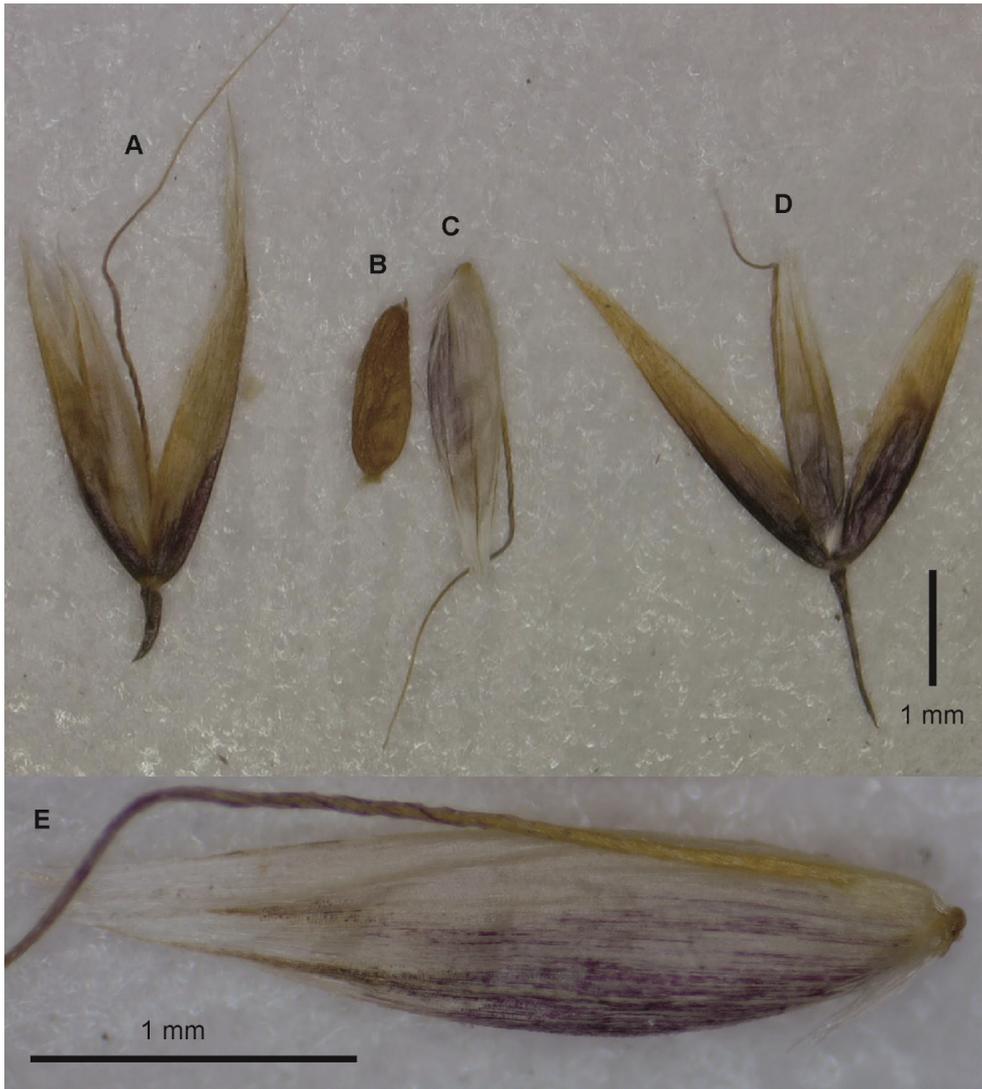
*Alpagrostis barceloi* differs somewhat from the other species in the genus, in terms of the panicle branches and pedicels being smooth, spikelets sometimes being shorter, 1-veined upper glumes, and awn sometimes inserted slightly higher up the lemma.

***Alpagrostis alpina* (Scop.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**  
urn:lsid:ipni.org:names:77212593-1

≡ *Agrostis alpina* Scop., Fl. Carniol. ed. 2, 1: 60. 1772 ≡ *Agraulus alpinus* (Scop.) P. Beauv., Ess. Agrostogr.: 5. 1812 ≡ *Agrestis alpina* (Scop.) Bubani, Fl. Pyren. 4: 287. 1901. Type: “Habitat in Alpibus Vochinensibus” and “HALL Hist. n. 1477”, SCHEUCHZ. Gram pag. 140, Prodr. P. 22, tab. 4, fig. 1.”, original material: In siccioribus Alpium Helveticarum & Rhaeticarum pratis, *J. Scheuchzer s.n.* (lectotype, **designated here**: W-18890240472 [image!], fig. 2

***Alpagrostis alpina* var. *flavescens* (Honck.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**  
urn:lsid:ipni.org:names:77212596-1

≡ *Aira flavescens* Honck., Gew.: 212. 1782 ≡ *Avena aurata* All., Fl. Pedem. 2: 255. 1785, nom. nov. (non *Avena flavescens* L.) ≡ *Agrostis aurata* (All.) Suter, Fl. Helv. 1: 61. 1802, nom. superfl. ≡ *Agrostis flavescens* (Honck.) Host, Icon. Descr. Gram. Austriac. 4: 52. 1809 ≡ *Agrostis rupestris* var. *aurata* (All.) Clairv., Man. Herbor. Suisse: 16. 1811 ≡ *Avena rupestris* var. *aurata* (All.) Clairv., Man. Herbor. Suisse: 16. 1811 ≡ *Trichodium flavescens* (Host) Schult., Oestr. Fl., ed. 2, 1: 165. 1814 ≡ *Agraulus flavescens* (Host) Sweet, Hort. Brit., ed. 2: 556. 1830 ≡ *Agrostis alpina* var. *flavescens* (Honck.) Schrad., in Schlechtendal, Linnæa 12: 435. 1838 ≡ *Agrostis alpina* var. *aurata* (All.) Ducommun, Taschenb. Schweiz. Bot.: 852. 1869 ≡ *Agrostis alpina* f. *aurata* (All.) Beldie, Fl. Reipubl. Popularis Sin. 12: 163. 1972. Type: Switzerland. Bagnes A. Haller hist. 1488 [a description] (lectotype needed).



**Figure 2.** *Alpagrostis alpina* **A, D** spikelets **B** caryopsis **C** floret **E** floret, showing dorsal surface. Plant fragments taken from *Sain-Lager 3* (US-1628154).

***Alpagrostis barceloi* (L. Sáez & Rosselló) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**

urn:lsid:ipni.org:names:77212597-1

≡ *Agrostis barceloi* L. Sáez & Rosselló, Bot. J. Linn. Soc. 133: 361–365, f. 1. 2000.

Type: Spain. Insulae Balearicae [Balearic Islands], Majorca, in praeruptis rupium umbrosis calcareis septentrionalibus loco dicto Puig Major de Son Torrella, 1400 m, 31SDE8206, 14 Aug 1998, L. Sáez 5132 (holotype: BC-852322; isotypes: BCC, M, W-20040000640 [image!], herb. L. Sáez).

**Notes.** This species is included in *Alpagrostis* based on its similar morphology, although this needs to be confirmed in molecular analyses. Certain characteristics sometimes differ from the other species in the genus, i.e., spikelets and lemmas sometimes shorter, insertion of the awn sometimes higher on the lemma, panicle branches and pedicels smooth or scaberulous. *Alpagrostis barceloi* shares with other member of the genus, conduplicate leaf blades, truncate lemma apices with setaceous extensions of the lateral veins, and ecologically is a strict orophyte, much like *A. alpina* and *A. schleicheri* (Sáez and Rosselló 2000).

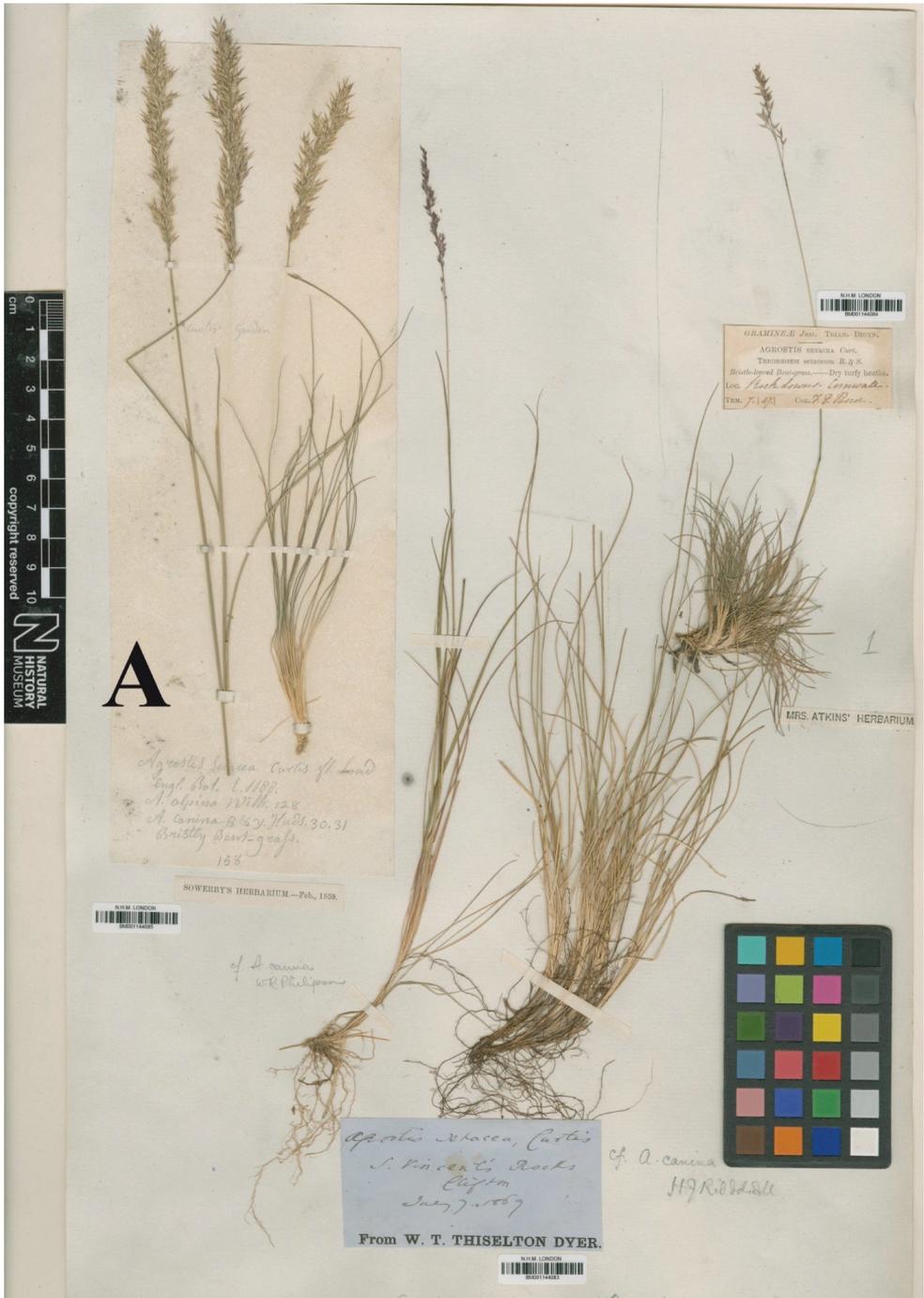
***Alpagrostis setacea* (Poir.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**  
urn:lsid:ipni.org:names:77212601-1

≡ *Agrostis setacea* Curtis, Pract. Obs. Brit. Grasses ed. 1: 35, no. 4. post (Aug) 1787, nom. illeg. hom. (non Villars (Feb) 1787) ≡ *Agrostis setacea* Curtis, Fl. Londin. 6, t. 12. 1798 ≡ *Agrostis rupestris* var. *setacea* Poir. in Lam., Encycl., Suppl. 1: 247. 1810 ≡ *Vilfa setacea* (Poir.) P. Beauv., Ess. Agrostogr.: 16. 148. 1812 ≡ *Trichodium setaceum* (Poir.) Roem. & Schult., Syst. Veg. ed. 15 bis, 2: 280. 1817 ≡ *Agraulus setaceus* (Poir.) Gray, Nat. Arr. Brit. Pl. 2: 149. 1821 [1822] ≡ *Agrestis setacea* (Poir.) Bubani, Fl. Pyren. 4: 286. 1901 ≡ *Agrostis curtisii* Kerguélen, Lejeunia, n.s., 75 (Err. & Corr.): 1. 1975 ≡ *Agrostis curtisii* Kerguélen, Lejeunia, n.s., 75 (Err. & Corr.): 1. 1975. Type: England. Curtis's garden [Sowerby's Herbarium], (neotype, **designated here:** BM-001144085 [image!]). fig. 3A.

**Notes.** Philipson (1937) mentions “No authentic specimens of Curtis have been preserved. There is one specimen in the British Museum Herbarium, originally from “Curtis's garden” (BM-001144085), which may be taken as representative of the species.” Philipson was possibly referring to this specimen. On the neotype there are three different collections on the same sheet. The specimen on the upper left of the sheet is BM-001144085 (Fig. 2A).

***Alpagrostis setacea* var. *flava* (Des Moul.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**  
urn:lsid:ipni.org:names:77212602-1

≡ *Agrostis setacea* var. *flava* Des Moul., Actes Soc. Linn. Bordeaux 11: 320. 1840  
≡ *Agrostis curtisii* var. *flava* (Des Moul.) Portal, *Agrostis* de France: 193. 2009.  
Type: France. Dans les bois découverts, les bruyères et les landes rases, aux environs de Sagonzac (Périgord), 26 May 1838, M.C. Durieu de Maisonneuve #90bis (holotype: not found; isotypes: MPU-027078 [image!], MP-027079 [image!], W-18890240353 [image!], W-18890240354 [image!]).



**Figure 3.** Neotype of *Agrostis setacea* Curtis [= *Alpagrostis setacea* (Poir.) P.M. Peterson, Romasch., Soreng & Sylvester] from Curtis's garden (BM-001144085), upper left hand specimen indicated by **A**.

***Alpagrostis schleicheri* (Jord. & Verl.) P.M. Peterson, Romasch., Soreng & Sylvester, comb. nov.**

urn:lsid:ipni.org:names:77212600-1

≡ *Agrostis schleicheri* Jord. & Verl., Arch. Fl. France Allemagne 1: 347, 346–348. 1855  
 ≡ *Trichodium schleicheri* (Jord. & Verl.) Fourn., Ann. Soc. Linn. Lyon, n.s., 17: 181. 1869  
 ≡ *Agrostis subspicata* Arv.-Touv., Essai Pl. Dauphiné: 67. 1871, nom. illeg. superfl.  
 ≡ *Agrostis alpina* proles *schleicheri* (Jord. & Verl.) Asch. & Graebn., Syn. Mitteleur. Fl. 2(1): 187. 1899  
 ≡ *Agrostis schleicheri* (Jord. & Verl.) Bubani, Fl. Pyren. 4: 288. 1901  
 ≡ *Agrostis alpina* subsp. *schleicheri* (Jord. & Verl.) Rouy, in G. Rouy & J. Foucaud, Fl. France 14: 69. 1913. Type: France. Débris mouvants des rochers calcaires de Mt. St-Nizier près de Grenoble (Isère), 15 Jul 1854, *Jean-Baptiste Verlot 1584* (lectotype, **designated here**: P-03161255 [image!], isolectotypes: BM-001134099 [image!], BM-001134098 [image!], MPU-027081 p.p. *Verlot 1584* [image!], MPU-027082 [no image], P-03656627 [image!]).

**Notes.** Jordon (1855) cited the following five collections: the Jura sur le Reculet (Ain), and Mont Ventoux, in August 1841, *A. Jordan*; Mt. St-Nizier near Grenoble, *Verlot*; Bex (canton of Vaud), *E. Thomas*; Mt. St-Nizier near Grenoble, *Clement*. Also cited is a report of Reuter of his collection from Jura sur le Reculet [P-03161256, image!], and *Agrostis filiformis* sensu *Vill.* We select The *Verlot 1584* specimen as there are several duplicates, and P-03161255 as the lectotype because that sheet is not mounted with any other collection as the MPU and BM sheets seem to be.

Key to differentiate taxa of *Agrostula* and *Alpagrostis* from *Agrostis* and other genera previously considered as synonyms of *Agrostis* by Watson and Dallwitz (1992) and Clayton and Renvoize (1986)

- 1 Spikelets disarticulating below the glumes, the glumes, floret, and part of the pedicel falling together as a unit; glume apices lanceolate or lanceolate-subulate, muticous, mucronate or awned; palea < ½ the length of the lemma ..... ***Polypogon* Desf.**
- Spikelets disarticulating above the glumes, the glumes remaining on the inflorescence after the florets have fallen; glumes acute to acuminate, not awned; palea of varying length, absent or rudimentary to equaling the length of the lemma ..... **2**
- 2 Rachilla extension present (cases where it is sometimes rudimentary key both ways), of varying lengths (sometimes very short, and requiring the base of the palea checked closely to distinguish the structure from hairs), glabrous or pilulose to densely pilose; palea well-developed, generally > 2/3 the length of the lemma ..... **3**
- Rachilla extension absent; palea of varying length ..... **4**

- 3 Lemmas densely pubescent, with rigid and abundant hairs; callus and rachilla notably hairy; lemmas with a well-developed usually geniculate and twisted awn, > 1 mm long, inserted in the lower or upper half of the lemma, clearly exceeding the glumes; taxa from southern Hemisphere (Australia, Malaysia, New Zealand, South Africa and South America) ..... ***Lachnagrostis* Trin.**
- Lemmas glabrous; callus and rachilla glabrous or with short hairs emerging from only the rachilla apex and the basal side-ridges of the callus; lemmas unawned or with a short straight awn, usually < 0.5 mm long, inserted in the upper half of the lemma, not or barely exceeding the glumes (awn well-developed, 1.6–2 mm long, inserted in lower 1/3 of lemma, straight or geniculate and usually not surpassing glumes in *Podagrostis rosei* (Scribn. & Merr.) Sylvester & Soreng, but then callus and rachilla glabrous, rachilla very short, < 0.3 mm long, glabrous, plants from Mexico); taxa from North, Central and South America.....***Podagrostis* (Griseb.) Scribn. & Merr. (in part)**
- 4 Lemma apex terminating in 2 or 4 scabrous setae 0.1–2 mm long; lemma with a well-developed geniculate and twisted awn inserted basally or in the lower 1/3 and surpassing the glumes; paleas < 1/3 the length of the lemma; calluses pilulose or densely tufted; leaf blades often filiform or involute; lemma surfaces pilose (*Bromidium*) or usually glabrous (*Alpagrostis*); caryopses with liquid endosperm becoming narrow and shriveled with age ..... **5**
- Lemma apex entire or finely dentate with short teeth at the end of each lateral vein; lemmas mucicous, with a straight mucron 0.2–1 mm long, or with a long geniculate and twisted awn to 6+ mm long, inserted in the lower, middle or upper 1/3 of the lemma but usually not basally, not surpassing to greatly surpassing the glumes; lemma surface usually glabrous (sometimes pilose e.g. *Agrostis castellana* L.); calluses usually glabrous or with hairs restricted to lateral lines continuous with the basal lemma margins; leaf blades of various forms but less often filiform or involute; caryopsis usually rounded, with hardened endosperm, less often with liquid endosperm ..... **6**
- 5 Anthers 0.2–0.7 mm long; lemma surface often pilose; awn inserted in the lower 1/3 but usually not basally; longest setae of lemma apex 0.4–2 mm long; caryopsis thin or with liquid endosperm; leaf blades filiform or flat, generally 1–4 mm diam.; annuals from southern South America.....  
..... ***Agrostis* sect. *Bromidium* (Nees & Meyen) E. Desv.**
- Anthers 0.7–2.3 mm long; lemma surface usually glabrous or pilulose basally; longest setae of lemma apex 0.1–0.5 mm long; awn inserted basally; leaf blades filiform or involute, 0.1–1.5 mm diam. as folded or rolled; perennials of Europe and NW Africa .....***Alpagrostis* P.M. Peterson, Romasch., Soreng & Sylvester**
- 6 Floret equaling or subequaling the glumes, sometimes slightly shorter but reaching past  $\frac{3}{4}$  the length of the glumes, usually with a short rachilla prolongation emerging behind the palea (sometimes absent in many florets of *P. rosei* and *P. humilis* so check many spikelets); paleas well-developed, usually reaching from (2/3)  $\frac{3}{4}$  to almost

the apex of the lemma; lemmas muticous or with a short straight awn 0.2–0.6 mm long, inserted medially or in the upper half of the lemma, not surpassing the glumes (awn well-developed, 1.6–2 mm long, inserted in lower 1/3 of lemma, straight or geniculate and usually not surpassing glumes in *P. rosei*).....

.....***Podagrostis* (Griseb.) Scribn. & Merr. (in part)**

- Floret notably shorter than the glumes, usually 1/3–3/4 the length of the glumes, rarely longer, without a trace of a rachilla prolongation; paleas well-developed, poorly-developed, or absent, when well-developed reaching from 1/2–3/4 the length of the lemma; lemmas muticous, with a short straight awn 0.2–1 mm long, or with a long geniculate and twisted awn to 6+ mm long, inserted basally, medially or in the upper half of the lemma, not surpassing to greatly surpassing the glumes ..... 7

7 Glumes dorsally rounded, not keeled, smooth throughout, apices blunt to truncate and erose to denticulate; palea c. 1/2 the length of the lemma; panicles open and diffuse; lemmas equally wide as long, widest at (or near) apex, apices broadly truncate, irregularly 5 to 7 denticulate to erose, awnless; anthers longer than the lemma, caryopsis surface rugose-papillose; perennials; growing from very shallow soils; from the Iberian Peninsula and Northern Africa.....

.....***Agrostula* P.M. Peterson, Romasch., Soreng & Sylvester**

- Glumes keeled, usually scabrous (at least in part), rarely upper glume smooth throughout, apices obtuse to acute-acuminate, rarely blunt to truncate, rounded to muticous; palea absent or rudimentary to 3/4 the length of the lemma; panicles open and diffuse to condensed and spikelike; lemmas usually longer than wide (rarely equally wide as long), usually narrowed towards the apex, apices variable, ranging from somewhat broadly to usually narrowly truncate, usually with 2 to 5 dents (sometimes aristulate), to blunt and entire, awnless or with an awn 0.2–6+ mm long; anthers sometimes longer to usually shorter than the lemma; caryopsis surface usually smooth; perennials or annuals; usually growing from well-developed soils, less often from shallow soils, and generally reliant on soil moisture for their water supply; cosmopolitan.....***Agrostis* L.**

**Acknowledgements**

We thank Flora iberica project (CGL2014–52787–C3–1–P, CGL2012–32914, CGL201785204–C3–1–P), and FPI fellowship BES-2012-053754 to P. Barberá; the National Geographic Society Committee for Research and Exploration (Grant No. 8848-10, 8087-06) for field and laboratory support; the Smithsonian Institution’s Restricted Endowments Fund, the Scholarly Studies Program, Research Opportunities, Atherton Seidell Foundation, Biodiversity Surveys and Inventories Program, Small Grants Program, the Laboratory of Analytical Biology, and the United States Department of Agriculture. We thank Jeffery M. Saarela, Kanchi N. Gandhi and Francisco Máquez-García for suggesting changes to the manuscript.

## References

- Barberá P, Soreng RJ, Peterson PM, Romaschenko K, Quintanar A, Aedo C (2019a) Molecular phylogenetic analysis resolves *Trisetum* Pers. (Poaceae: Pooideae: Koeleriinae) polyphyletic: Evidence for a new genus, *Sibirotrisetum* and resurrection of *Acrospelion*. *Journal of Systematics and Evolution* 58(4): 517–526. <https://doi.org/10.1111/jse.12523>
- Barberá P, Quintanar A, Peterson PM, Soreng RJ, Romaschenko K, Aedo C (2019b) New combinations, new names, typifications, and a new section, *Hispanica* in *Koeleria* (Poaceae, Poaceae). *Phytoneuron* 2019-46: 1–13. <http://www.phytoneuron.net/2019Phytoneuron/46PhytoN-KoeleriaNames.pdf>
- Belanger FC, Meagher TR, Day PR, Plumley K, Meyer WA (2003) Interspecific hybridization between *Agrostis stolonifera* and related *Agrostis* species under field conditions. *Crop Science* 43: 240–246. <https://doi.org/10.2135/cropsci2003.0240>
- Björkman SO (1960) Studies in *Agrostis* and related genera. *Symbolae Botanicae Upsalienses* 17(1): 1–112.
- Clayton WD, Renvoize SA (1986) *Genera Graminum: Grasses of the World*. Kew Bulletin, Additional Series 13: 1–389.
- Clayton WD, Vorontsova MS, Harman KT, Williamson H (2006 onwards) GrassBase – The Online World Grass Flora. <http://www.kew.org/data/grasses-db.html> [accessed 28 Apr 2020]
- Cope T, Gray A (2009) *Grasses of the British Isles*. BSBI Handbook 13. Botanical Society of the British Isles with Royal Botanic Gardens, Devon, United Kingdom.
- Crawford DJ (1989) Enzyme electrophoresis and plant systematics. In: Soltis DE, Soltis PS (Eds) *Isozymes in Plant Biology*. Dioscorides Press, Portland, 146–164. [https://doi.org/10.1007/978-94-009-1840-5\\_8](https://doi.org/10.1007/978-94-009-1840-5_8)
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Fernandes A, Queiros M (1969) Contribution á la connaissance cytotoxonomique des Spermatophyta du Portugal 1. Gramineae. *Boletim da Sociedade Borteriana*, ser 2, 43: 20–140.
- Frey L (1997) Distribution of *Agrostis rupestris* and *A. alpina* (Poaceae) and remarks on their taxonomy and karyology. *Fragments Floristica Geobotanica* 41: 25–42.
- Garde A (1951) Breve nota sobre la cariólogía de algunas Gramíneas Portuguesas. *Genetica Iberica* 3: 145–154.
- Grisebach A (1852) *Agrostis* L. In: Ledebour C (Ed.) *Flora rossica* 4(13): 436–442.
- Huelsenbeck JP, Ronquist FR (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Jordon CTA (1855) Notice sur plusieurs plantes nouvelles. *Archives de la Flore de France et d'Allemagne* 2: 140–448.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious-Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28(12): 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>

- Massó S, López-Pujol J, López-Alvarado J, Blanché C, Sáez L (2016) One species, one genotype: No genotypic variability in the extremely narrow endemic tetraploid *Agrostis barceloi* (Gramineae). *Plant Systematics and Evolution* 302(5): 609–615. <https://doi.org/10.1007/s00606-016-1283-9>
- Peterson PM, Romaschenko K, Soreng RJ (2014) A laboratory guide for generating DNA barcodes in grasses: a case study of *Leptochloa* s.l. (Poaceae: Chloridoideae). *Webbia* 69(1): 1–12. <https://doi.org/10.1080/00837792.2014.927555>
- Peterson PM, Romaschenko K, Herrera Arrieta Y (2015a) A molecular phylogeny and classification of the Eleusininae with a new genus, *Micrachme* (Poaceae: Chloridoideae: Cynodonteae). *Taxon* 64(3): 445–467. <https://doi.org/10.12705/643.5>
- Peterson PM, Romaschenko K, Herrera Arrieta Y (2015b) Phylogeny and subgeneric classification of *Bouteloua* with a new species, *B. herrera-arrietae* (Poaceae: Chloridoideae: Cynodonteae: Boutelouinae). *Journal of Systematics and Evolution* 53(4): 351–366. <https://doi.org/10.1111/jse.12159>
- Peterson PM, Romaschenko K, Herrera Arrieta Y (2016) A molecular phylogeny and classification of the Cynodonteae (Poaceae: Chloridoideae) with four new genera: *Orthacanthus*, *Triplasiella*, *Tripogonella*, and *Zaiqiqab*; three new subtribes: Dactylocteniinae, Orininae, and Zaiqiqahinae; and a subgeneric classification of *Distichlis*. *Taxon* 65(6): 1263–1287. <https://doi.org/10.12705/656.4>
- Peterson PM, Soreng RJ, Romaschenko K, Barberá P, Quintanar A, Aedo C (2019) New combinations and new names in American *Cinnagrostis*, *Peyritschia*, and *Deschampsia*, and three new genera: *Greeneochloa*, *Laegaardia*, and *Paramochloa* (Poaceae). *Phytoneuron* 39: 1–23. <http://www.phytoneuron.net/2019Phytoneuron/39PhytoN-CinnagrostisNames.pdf>
- Philipson WR (1937) A revision of the British species of the genus *Agrostis* Linn. *Journal of the Linnean Society of London* 51(338): 73–152. <https://doi.org/10.1111/j.1095-8339.1937.tb01905.x>
- Portal R (2009) *Agrostis* de France. (privately published) Imprimerie Jeanne-d'Arc.
- Posada D (2008) jModelTest model averaging. *Molecular Biology and Evolution* 25(7): 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Queiros M (1974) Contribucao para o conhecimento citotaxonomico das Spermatophyta de Portugal. I, Gramineae.supl.2, Boletim da Sociedade Broteriana, ser 2, 48: 81–98.
- Queiros M (1979) Números cromosómicos para a flora Portuguesa 16–37. Boletim da Sociedade Broteriana, ser 2, 53: 15–28.
- Romaschenko K, Peterson PM, Soreng RJ, Garcia-Jacas N, Futorna O, Susanna A (2012) Systematics and evolution of the needle grasses (Poaceae: Pooideae: Stipeae) based on analysis of multiple chloroplast loci, ITS, and lemma micromorphology. *Taxon* 61(1): 18–44. <https://doi.org/10.1002/tax.611002>
- Romero García AT, Blanca López G (1988) Contribución al estudio cariosistemático del género *Agrostis* L. (Poaceae) en la Península Ibérica. Boletim da Sociedade Broteriana, ser. 2. 61: 81–104.
- Romero García AT, Blanca López G, Morales Torres C (1988a) Revisión del género *Agrostis* L. (Poaceae) en la península Ibérica. *Ruizia* 7: 1–160.
- Romero García AT, Blanca López G, Morales Torres C (1988b) Relaciones filogenéticas entre las especies ibéricas del género *Agrostis* L. (Poaceae). *Lagascalía* 15: 411–415.

- Romero Zarco C (1987) *Agrostis* L. In: Valdés B, Talavera S, Fernández-Galiano E (Eds) Flora vascular de Andalucía Occidental 3: 336–441.
- Ronquist FR, Huelsenbeck JP (2003) Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* (Oxford, England) 19(12): 1575–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Saarela JM, Bull RD, Paradis MJ, Ebata SN, Peterson PM, Soreng RJ, Paszko B (2017) Molecular phylogenetics of cool-season grasses in the subtribes Agrostidinae, Anthoxanthinae, Aveninae, Brizinae, Calothecinae, Koeleriinae and Phalaridinae (Poaceae, Pooideae, Poaceae, Poaceae chloroplast group I). *PhytoKeys* 87: 1–139. <https://doi.org/10.3897/phytokeys.87.12774>
- Sáez L, Rosselló JA (2000) A new species of *Agrostis* (Gramineae) in the *A. alpina* complex. *Botanical Journal of the Linnean Society* 133(3): 359–370. <https://doi.org/10.1111/j.1095-8339.2000.tb01551.x>
- Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences of the United States of America* 97(13): 7051–7057. <https://doi.org/10.1073/pnas.97.13.7051>
- Soreng RJ, Davis JI, Voionmaa MA (2007) A phylogenetic analysis of Poaceae sensu lato based on morphological characters and sequence data from three plastid-encoded genes: Evidence for reticulation, and a new classification for the tribe. *Kew Bulletin* 62: 425–454.
- Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Teisher JK, Clark LG, Barberá P, Gillespie LJ, Zuloaga FO (2017) A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *Journal of Systematics and Evolution* 55(4): 259–290. <https://doi.org/10.1111/jse.12262>
- Stebbins Jr GL (1947) Types of polyploids: Their classification and significance. *Advances in Genetics* 1: 403–429. [https://doi.org/10.1016/S0065-2660\(08\)60490-3](https://doi.org/10.1016/S0065-2660(08)60490-3)
- Sylvester SP, Soreng RJ, Bravo-Pedraza WJ, Cuta-Alarcon LE, Giraldo-Cañas D, Aguilar-Cano J, Peterson PM (2019a) Páramo *Calamagrostis* s.l. (Poaceae): An updated list and key to the species known or likely to occur in páramos of NW South America and southern Central America including two new species, one new variety, and five new records for Colombia. *PhytoKeys* 122: 29–78. <https://doi.org/10.3897/phytokeys.122.33032>
- Sylvester SP, Soreng RJ, Giraldo-Cañas D (2019b) *Podagrostis colombiana* sp. nov. (Poaceae): A new genus record and species for Colombia. *Kew Bulletin* 74: 1–25. <https://doi.org/10.1007/s12225-019-9814-5>
- Sylvester SP, Peterson PM, Romaschenko K, Bravo-Pedraza WJ, Cuta-Alarcon LE, Soreng RJ (2020) New combinations and updated descriptions in *Podagrostis* (Agrostidinae, Poaceae) from the Neotropics and Mexico. *PhytoKeys* 148: 21–50. <https://doi.org/10.3897/phytokeys.148.50042>
- Tkach N, Schneider J, Döring E, Wölk A, Hochbach A, Nissen J, Winterfeld G, Meyer S, Gabriel J, Hoffmann MH, Röser M (2020) Phylogenetic lineages and the role of hybridization as driving force of evolution in grass supertribe Pooideae. *Taxon* 69(2): 234–277. <https://doi.org/10.1002/tax.12204>
- Trinius CB (1820) *Fundamenta Agrostographiae*. J.G. Heubner, Vienna.
- Trinius CB (1824) *De Graminibus Unifloris et Sesquifloris*. Impensis Academiae Imperialis Scientiarum, St. Petersburg.

- Tzvelev NN (1968) Zlaki (Gramineae). *Botanicheskii Zhurnal* (Moscow & Leningrad) 53: 1–309.
- Tzvelev NN (1976) Zlaki SSSR [Grasses of the Soviet Union]. Nauka Publishers USSR, Leningrad.
- Tzvelev NN (1983) Grasses of the Soviet Union [Zlaki SSSR] part 1. Amerind Publishing Co., New Delhi.
- Valdés B, Scholz H (2006) The Euro+Med treatment of Gramineae – a generic synopsis and some new names. *Willdenowia* 36(2): 657–669. <https://doi.org/10.3372/wi.36.36202>
- Villars D (1787) *Histoire des Plantes de Dauphiné* 2: 1–690. [Chez l’auteur, Grenoble, France] <https://doi.org/10.5962/bhl.title.116232>
- Watrud LS, Lee EH, Fairbrother A, Burdick C, Reichman JR, Bollman M, Storm M, King G, Van de Water PK (2004) Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proceedings of the National Academy of Sciences of the United States of America* 101(40): 14533–14538. <https://doi.org/10.1073/pnas.0405154101>
- Watson L, Dallwitz MJ (1992) *The Grass Genera of the World*. CAB International, Wallingford.
- Widén KG (1971) The genus *Agrostis* L. in Eastern Fennoscandia. *Taxonomy and Distribution. Flora Fennica* 5: 1–209.
- Zwickl DJ (2006) *Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion*. University of Texas, Austin.