

Rhodoscirpus (Cyperaceae: Scirpeae), a new South American sedge genus supported by molecular, morphological, anatomical and embryological data

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Abstract In its broadest sense, *Scirpus* consists of a heterogeneous assemblage of up to 250 species, but modern circumscriptions suggest that only 40–50 species are part of the genus. Despite a narrower definition of the genus, atypical species continue to be segregated from *Scirpus* with a common pattern being the removal of Southern Hemisphere taxa to other genera and tribes. In South America, the morphology of remaining *Scirpus* species also suggests that they are not closely related to *Scirpus* s.str., but most of these taxa are only known from their types, making a detailed analysis of their generic affinities difficult. One notable exception is *Scirpus asper*, a species that is relatively common in the mountains and adjacent lowlands of Peru south to Argentina. Although this species possesses features used in the circumscription of *Scirpus*, such as cauline leaves, flat leaf blades and anthelate inflorescences, it is known to differ from *Scirpus* s.str. by its *Schoenus*-type embryo, and most of its presumed allies are now placed in different genera (e.g., *Scirpus analecti* ≡ *Cypringlea analecta*; *Scirpus giganteus* ≡ *Androtrichum giganteum*). In this study, we use DNA sequence data from the plastid (*matK*, *ndhF*) and nuclear (ETS-1f) genomes to demonstrate that *Scirpus asper* is not closely related to *Scirpus* s.str., but sister to *Phylloscirpus* within the predominantly South American Zameioscirpus clade (*Amphiscirpus*, *Phylloscirpus*, *Zameioscirpus*). When combined with morphological, anatomical and embryological data, results indicate that *S. asper* is best treated as the sole species of a new monotypic genus, *Rhodoscirpus*. The implications of these results on the taxonomy of tribe Scirpeae are discussed.

Keywords *Amphiscirpus*; Andes; *Phylloscirpus*; phylogeny; *Scirpus asper*; *Scirpus trachycaulos*; taxonomy; *Zameioscirpus*

Supplementary Material DNA sequence alignment is available in the Supplementary Data section of the online version of this article at <http://www.ingentaconnect.com/content/iapt/tax>

■ INTRODUCTION

Linnaeus (1753, 1754) originally defined *Scirpus* L. to include all Cyperaceae species with many spirally inserted glumes, flowers with less than seven perianth bristles and three anthers per flower. This broad generic concept brought together a heterogeneous assemblage of species whose most inclusive treatment comprised as many as 250 species (Koyama, 1958). Subsequent morphological, anatomical and embryological studies have prompted authors to split *Scirpus* s.l. into more than 50 genera, many of which are now placed in distantly related tribes (Abildgaardieae, Cypereae, Dulichieae, Eleocharideae; Van der Veken, 1965; Schuyler, 1971; Oteng-Yeboah, 1972, 1974a, b; Goetghebeur, 1986, 1998). Although these major rearrangements have left only 40–50 species in *Scirpus* (Wilson, 1981; Govaerts & al., 2007), the circumscription of the genus is still unsettled as indicated by the recent recognition of many new segregate genera in tribe Scirpeae: *Calliscirpus* C.N.Gilmour & al. (Gilmour & al., 2013), *Cypringlea* M.T.Strong (Strong,

2003), *Zameioscirpus* Dhooge & Goetgh. (Dhooge & al., 2003) and even in the distantly related Cyperaceae: *Dracoscirpoides* Muasya (Muasya & al., 2012).

With the exception of the Australian endemic *Scirpus polystachyus* F.Muell., recent work has made it increasingly clear that *Scirpus* is a circumboreal genus with some north-temperate elements whose centre of diversity is in eastern Asia and North America. Indeed, a common pattern seen in most recent taxonomic changes in *Scirpus* is the removal of Southern Hemisphere species to other genera. All African *Scirpus* have now been removed, mostly to genera in tribes Cypereae and Fuireneae (Govaerts & al., 2007; Muasya & al., 2012), and in South America, a revision of Andean species placed them in three different Scirpeae genera (Dhooge, 2005). This leaves the relationships of only a handful of South American *Scirpus* species unresolved, almost all of them known only from type specimens and whose morphology suggests they are probably not closely related to *Scirpus* s.str. (Dhooge & Goetghebeur, 2002, 2004; Dhooge & al., 2003; Dhooge, 2005). One

notable exception is *S. asper* J.Presl & C.Presl, an Andean species known from northern Peru down to central Argentina that appears to be at least locally common in dry sandy or rocky herb and shrub communities at low to mid-altitude (200–3500 m; Guaglianone & al., 2000).

Scirpus asper possesses all the morphological characteristics used in the modern circumscription of *Scirpus* (Wilson, 1981): cauline leaves, flat leaf blades, an open anthelate inflorescence (compound anthelas of spikelets), relatively small spikelets and nutlets, and a (sub-)lateral root cap in the embryo (Van der Veken, 1965). Previous authors (Beetle, 1944a, c, 1946; Koyama, 1958) have suggested affinities between *S. asper* and a series of species which share little except that they all appear morphologically aberrant compared to the bulk of *Scirpus* (e.g., *S. petelotii* Raymond) or have already been transferred to other genera and tribes of Cyperaceae (e.g., *S. analecti* Beetle \equiv *Cypringlea analecta* (Beetle) M.T.Strong, Strong, 2003; *S. giganteus* Kunth \equiv *Androtrichum giganteum* (Spreng.) H.Pfeiff., Pfeiffer, 1937). Despite this and the availability of a decent number of specimens in major herbaria, *S. asper* has not been included in the most recent revisions of South American “*Scirpus*” (Oteng-Yeboah, 1972; Dhooge, 2005) and its true affinities remain unclear.

This study aims to resolve the phylogenetic position of *Scirpus asper* in order to clarify its taxonomy and its evolutionary and biogeographical significance within the wider Cariceae-Dulichieae-Scirpeae clade (henceforth CDS; Léveillé-Bourret & al., 2014). We present molecular, morphological, anatomical and embryological evidence that *S. asper* is more closely related to the South American *Phylloscirpus* C.B.Clarke (three South American species) and its allied genera *Zameioscirpus* (three South American species) and *Amphiscirpus* Oteng-Yeb. (a single North and South American species) than it is to *Scirpus* s.str. In consequence, we here describe the new South American genus *Rhodoscirpus* Léveillé-Bourret & al. gen. nov., to accommodate a single morphologically distinctive species, *Rhodoscirpus asper* comb. nov.

■ MATERIALS & METHODS

Taxon sampling for phylogenetic analyses. — Sampling aimed to represent all major clades of CDS (Léveillé-Bourret & al., 2014) with an emphasis on diversity within *Scirpus* and allied genera of the *Zameioscirpus* clade (see Results section for clade definitions). A total of 56 individuals from 36 species were included in phylogenetic analyses (Appendix 1). Six individuals of *Rhodoscirpus asper* were sampled for DNA analyses to adequately represent the geographical and morphological diversity of the species. The plastid genes *matK* and *ndhF* were used because (1) they are easy to amplify even from relatively degraded herbarium material, (2) they are easy to align across CDS, and (3) they have the right level of variability for addressing phylogenetic relationships at the generic and tribal levels in Cyperaceae (Gilmour & al., 2013; Léveillé-Bourret & al., 2014). In addition, the nuclear ribosomal ETS-1f region, which is one of the most

easily amplified and informative regions available in sedges (Starr & al., 2003), was included to confirm phylogenetic congruence across genomes. A majority of the 114 sequences used here come from previous phylogenetic studies on CDS (Gilmour & al., 2013; Léveillé-Bourret & al., 2014), but most ETS-1f sequences (26) and a few plastid sequences (9) were newly submitted (Appendix 1). Outgroup taxa were selected to represent the major lineages of the Abildgaardieae-Cyperaceae-Eleocharideae-Fuireneae clade, which is sister to CDS (Muasya & al., 2009). Taxonomy follows Govaerts & al. (2007) except for *Eriophorum* L., which follows Novoselova’s (1994a, b) revision of the genus.

Molecular methods. — Whole genomic DNA was extracted from herbarium specimens or from field samples dried in silica gel using the silica-column protocol of Alexander & al. (2007) as modified by Starr & al. (2009). Primers for the amplification of the plastid *matK* and *ndhF* sequences are given in Gilmour & al. (2013), while primers for the nuclear ribosomal ETS-1f region come from Starr & al. (2003). For plastid genes, PCR amplifications consisted of 1 \times reaction buffer (Bioline, London, U.K.), 2 mM MgCl₂ (Sigma Aldrich, Oakville, Ontario, Canada), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, dGTP), 0.25 μ M of each primer, 1.1 μ l Bovine Serum Albumin (BioShop, Burlington, Ontario, Canada), 0.6 U of Biotaq DNA Polymerase (Bioline) and 1–3 μ l (~20–30 ng) of genomic DNA extract, adjusted to an end volume of 15 μ l using nuclease-free ddH₂O. Amplifications were done on an Eppendorf Mastercycler pro S thermocycler with 120 s of initial denaturation followed by 40 cycles of 30 s of 94°C denaturation, 60 s of 47°C primer annealing and 90–120 s of 72°C DNA extension, with a final extension step of 7–8 min.

For ETS-1f, PCR amplifications consisted of 1 \times reaction Buffer (Bioline), 2.5 mM MgCl₂ (Sigma Aldrich), 1 mM of each deoxynucleotide (dATP, dCTP, dTTP, dGTP), 0.4 μ M of each primer, 1 M Betaine (Sigma Aldrich), 0.6 U of Biotaq DNA Polymerase (Bioline) and 2–4 μ l (~25–35 ng) of genomic DNA extract, adjusted to an end volume of 15 μ l using nuclease-free ddH₂O. Cycling conditions for the ETS-1f region were 60 s of initial denaturation followed by 40 cycles of 60 s of 94°C denaturation, 60 s of 52°C primer annealing and 120 s of 72°C DNA extension, with a final extension step of 7 min. Minor adjustments were made to PCR protocols for the amplification of problematic samples. Successful amplifications were purified using an Exonuclease I–Shrimp Alkaline Phosphatase protocol (MJS Biolynx, Brockville, Ontario, Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit version 3.1 (Applied Biosystems, Foster City, California, U.S.A.). Sequencing termination products were purified according to a sodium acetate/alcohol protocol (Applied Biosystems) and sequenced on a 3130x1 Genetic Analyser. Reads were corrected and assembled with Geneious v4.8.5 (Biomatters, San Francisco, California, U.S.A.) and all sequences were submitted to GenBank (Appendix 1).

Alignment and phylogenetic analyses. — Sequences were concatenated by species, with most terminals represented by sequences of a single individual, although the unavailability of certain sequences (mostly ETS-1f) sometimes made it

necessary to concatenate sequences from two different individuals of the same species. *Rhodoscirpus asper* sequences were all concatenated by individual. Owing to high levels of variability in ETS-1f, sequences from the outgroup were excluded from phylogenetic analyses and rooted using Dulichieae as a functional outgroup (Watrous & Wheeler, 1981; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014). The matrix was aligned with the MUSCLE algorithm as implemented in Geneious v.4.8.5. Minor adjustments to the alignment were made by hand using parsimony as an objective criterion (as in Starr & al., 2004). Potentially informative, unambiguously aligned gaps (41) were coded by hand using simple indel coding (Simmons & Ochoterena, 2000). Only results from combined analyses (*matK*+*ndhF*+ETS-1f+gaps) are reported as no well-supported (>75%) topological incongruence was observed in single-region analyses (results not shown).

Heuristic maximum parsimony (MP) searches were done in PAUP* v.4.0 (Swofford, 2003) using 10,000 random addition sequence (RAS) replicates, followed by swapping with tree-bisection-reconnection (TBR), with the MULTREES and STEEPEST options on, and the COLLAPSE option off. A strict consensus of all most parsimonious trees was assembled in PAUP*. Branch support was assessed using 10,000 bootstrap (BS; Felsenstein, 1985) replicates in PAUP* with each replicate search consisting of 10 RAS holding 100 trees per RAS and using the strict-consensus BS (GRPFREQ = NO) to prevent undersampling-within-replicate and frequency-within-replicate artefacts (Simmons & Freudenstein, 2011). Bremer support values (decay index; Bremer, 1988, 1994) were computed using TreeRot v.3 (Sorenson & Franzosa, 2007) batch files in PAUP*. Independent searches were also made excluding all terminals with missing sequences to ensure that missing data had no effect on phylogenetic results.

Model-based searches were done using maximum likelihood (ML) in RAxML v.8.1.2 (Stamatakis, 2014). Model and partitioning scheme were selected with PartitionFinder v.1.1.0 (Lanfear & al., 2012) using the greedy search algorithm and the Bayesian information criterion on all possible partition schemes and all models implemented in RAxML. The best scheme included three partitions; (1) codon positions 1 and 2 of *matK* and *ndhF*, (2) codon position 3 of *matK* and *ndhF*, (3) the ETS-1f region. For RAxML searches, two additional partitions were used for the informative indels in *matK* and those of ETS-1f (no potentially informative gaps were found in *ndhF*). A GTR+ Γ model was used for the three DNA partitions, and the binary+ Γ model with ascertainment bias correction (Lewis, 2001) was used for the two indel partitions. Searches were made in RAxML using 100 random starting trees and the old slower-but-more-accurate rapid hill-climbing algorithm (Stamatakis & al., 2007). Branch support was assessed by 1000 (standard) bootstrap replicates.

Maximum likelihood BS values were placed on the highest scoring ML tree with SumTrees v.3.3.1 (Sukumaran & Holder, 2010) and parsimony BS and Bremer support values were added by hand. Tree figures were produced with TreeGraph v.2.0.50 (Stöver & Müller, 2010) and Inkscape v.0.48.4 (available at <http://www.inkscape.org/>). The alignment, strict consensus

of all most parsimonious trees and the best ML topology are available online at TreeBASE (<http://treebase.org/treebase-web/>). Clade support was characterised subjectively as weak (<75% BS), moderate (75%–84% BS), good or well supported (85%–95% BS) and strong (95%–100% BS). When two species are named to circumscribe a clade in the Results and Discussion, it refers to the smallest monophyletic group comprising both species.

Morphology and anatomy. — Representative specimens from the whole range of *Rhodoscirpus asper* were examined from the following herbaria: A, CAS, F, MICH, MT, NY and US. Additional specimens and types were examined from high-resolution pictures of vouchers deposited in ASU, HAL, K, MA, P, PRC, SGO, RM, SI and US. Morphological studies of vegetative and reproductive characters were based on dried and rehydrated herbarium material using dissecting and compound microscopes. Measurements were made using a 0.5 mm ruler and a dissection microscope with calibrated eye-piece grati-cules. Careful comparative studies were made using a representative sample of related species in the CDS clade, including all species of *Phylloscirpus* and *Zameioscirpus*, as well as a large number of *Scirpus* s.str.

Material for the anatomical studies was obtained from well-preserved herbarium vouchers. Samples were taken near the base of the largest leaves and stems. They were briefly rehydrated in boiling water, with a small amount of ethanol as a wetting agent. Cross-sections were made by hand, mounted in water and observed under the compound microscope at 40–200 \times . Polarizing filters were used to highlight birefringent features such as lignified cell walls, waxes and silica deposits. Anatomical sections of *Scirpus pendulus* Muhl. ex Willd. and *S. microcarpus* J.Presl & C.Presl were also examined to make comparative observations. For micromorphological observations, mature nutlets of *Phylloscirpus deserticola* (Phil.) Dhooge & Goetgh., *Rhodoscirpus asper* and *Scirpus sylvaticus* L. were mounted on aluminium stubs, coated with gold using a Desk II Denton Vacuum sputter-coater and examined under high-vacuum in a Philips XL30 ESEM scanning electron microscope.

■ RESULTS

Phylogenetic results. — The *matK*, *ndhF* and ETS-1f alignments were respectively 1319 bp, 1215 bp and 746 bp long, with 3, 2 and 10 unsequenced terminals, and with 11%, 7.6% and 39% missing or ambiguous bases (including unsequenced portions). They had respectively 388 (29%), 320 (26%) and 376 (50%) variable characters, of which 191 (14%), 184 (15%) and 243 (33%) were potentially parsimony informative. There were four potentially informative gaps in *matK*, none in *ndhF* and 37 in ETS-1f. The concatenated alignment, including gap characters, was 3355 characters long with 43 terminals and 17.8% missing data. Analyses made on a matrix excluding all terminals with missing sequences gave comparable results to those made including all terminals, and therefore only results obtained with all terminals are reported.

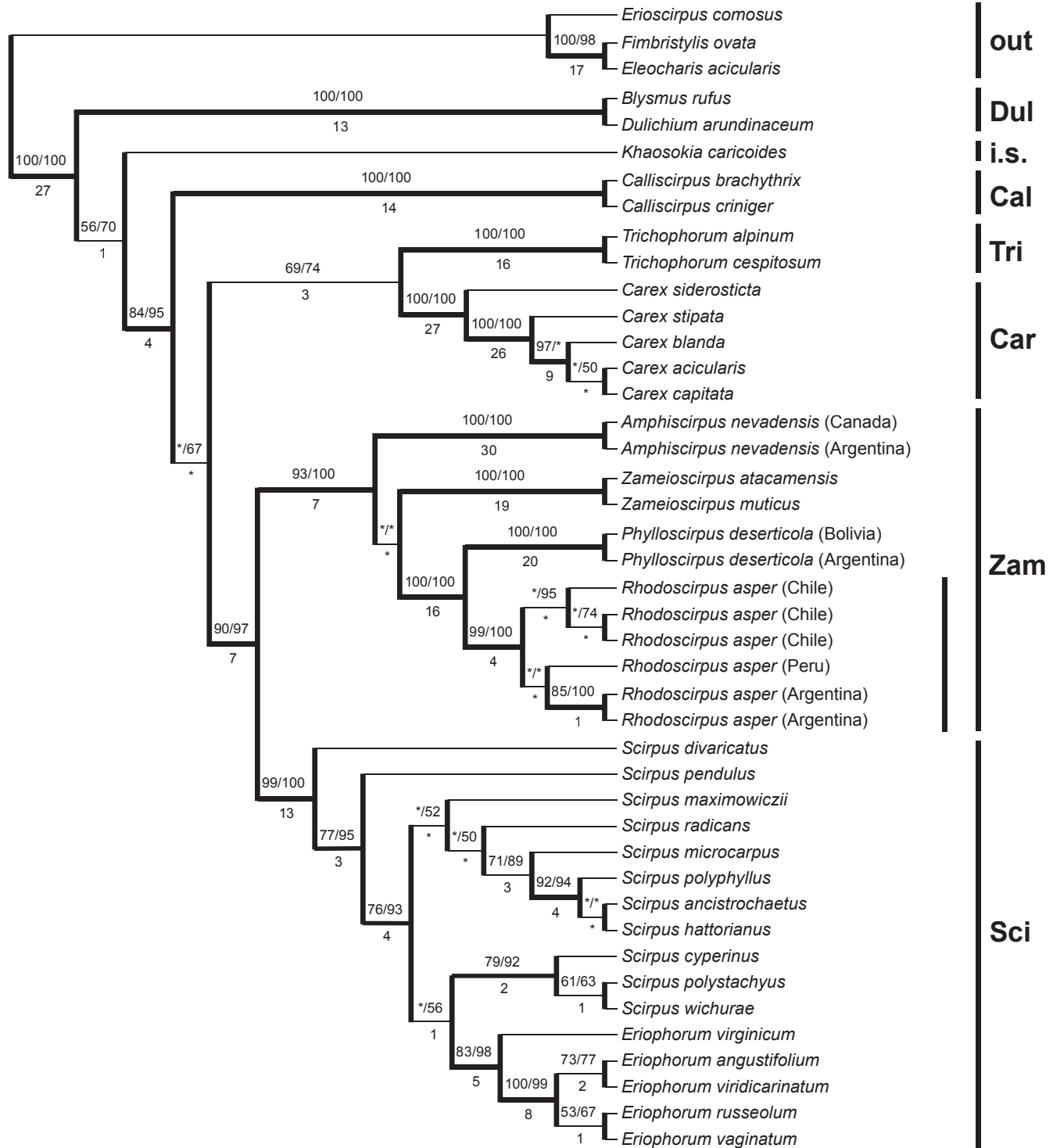


Fig. 1. Tree based on *matK*+*ndhF*+ETS-If data with the highest likelihood found in RAxML searches, with parsimony/likelihood bootstrap percentages above branches and Bremer support below branches. Branches with >75% parsimony BS are highlighted with bold lines. An asterisk (*) indicates either <50% BS support or the absence of a clade in the MP strict-consensus. Cal, Calliscirpus clade (Scirpeae); Car, Cariceae; Dul, Dulichieae; i.s., incertae sedis; out, outgroups; Sci, Scirpus clade (Scirpeae); Tri, Trichophorum clade (Scirpeae); Zam, Zameioscirpus clade (Scirpeae).

The parsimony searches found 189 trees of 2143 steps with consistency and retention indices of 0.66 and 0.73. The best topology found in ML searches (Fig. 1) had a log-likelihood of $-15,843.81$ as calculated by RAxML. The ML topology was nearly completely compatible with the strict consensus of all most parsimonious trees, with the only exceptions being the weakly-supported position of *Calliscirpus* as sister to the clade comprising the Zameioscirpus+Scirpus clade, and the position of *Carex acicularis* Boott as sister to *C. blanda* Dewey in the MP strict consensus. Results of the MP and ML bootstrap analyses are also broadly congruent, with the MP values slightly more conservative (Fig. 1). In consequence, only parsimony BS values are cited and discussed.

Results of the phylogenetic analyses position the strongly supported tribe Dulichieae (100% BS) and *Khaosokia* D.A.Simpson as successive sisters (56% BS) to a moderately supported (84% BS) clade consisting of five major lineages (*Calliscirpus* or “Calliscirpus clade”, Cariceae, *Trichophorum* Pers. or “Trichophorum clade”, *Scirpus*+*Eriophorum* or “Scirpus clade”, and *Amphiscirpus*+*Phylloscirpus*+*Zameioscirpus*

or “Zameioscirpus clade”), all of which receive good support ($>90\%$ BS; Fig. 1). Within this clade, *Calliscirpus* (100% BS) is poorly supported ($<50\%$ BS) as sister to a monophyletic group composed of a *Trichophorum* clade+Cariceae (69% BS) and a Zameioscirpus clade+Scirpus clade (90% BS). Within the Zameioscirpus clade, *Rhodoscirpus asper* is strongly supported as sister to *Phylloscirpus* (100% BS). Within *Rhodoscirpus*, the Chilean accessions form an unsupported clade sister to the Peruvian and Argentinian accessions.

Comparative morphology. — The general vegetative and reproductive morphology of *Rhodoscirpus* is similar to that found in species of *Scirpus* s.str. (Fig. 2). However, it differs by its densely ciliate ligules with hairs 0.1–0.4 mm long, while *Scirpus* spp. have entire, or rarely very minutely toothed (e.g., *S. microcarpus*) or sparsely ciliate (e.g., *S. radicans* Schkuhr) ligules with hairs up to 0.1 mm long. The glumes of *Rhodoscirpus* are also ciliate, while *Scirpus* s.str. generally have sub-entire glumes, sometimes with a few distal teeth, although there are exceptions (e.g., *S. radicans*). With respect to these characters, *Rhodoscirpus* is more similar to *Amphiscirpus*,

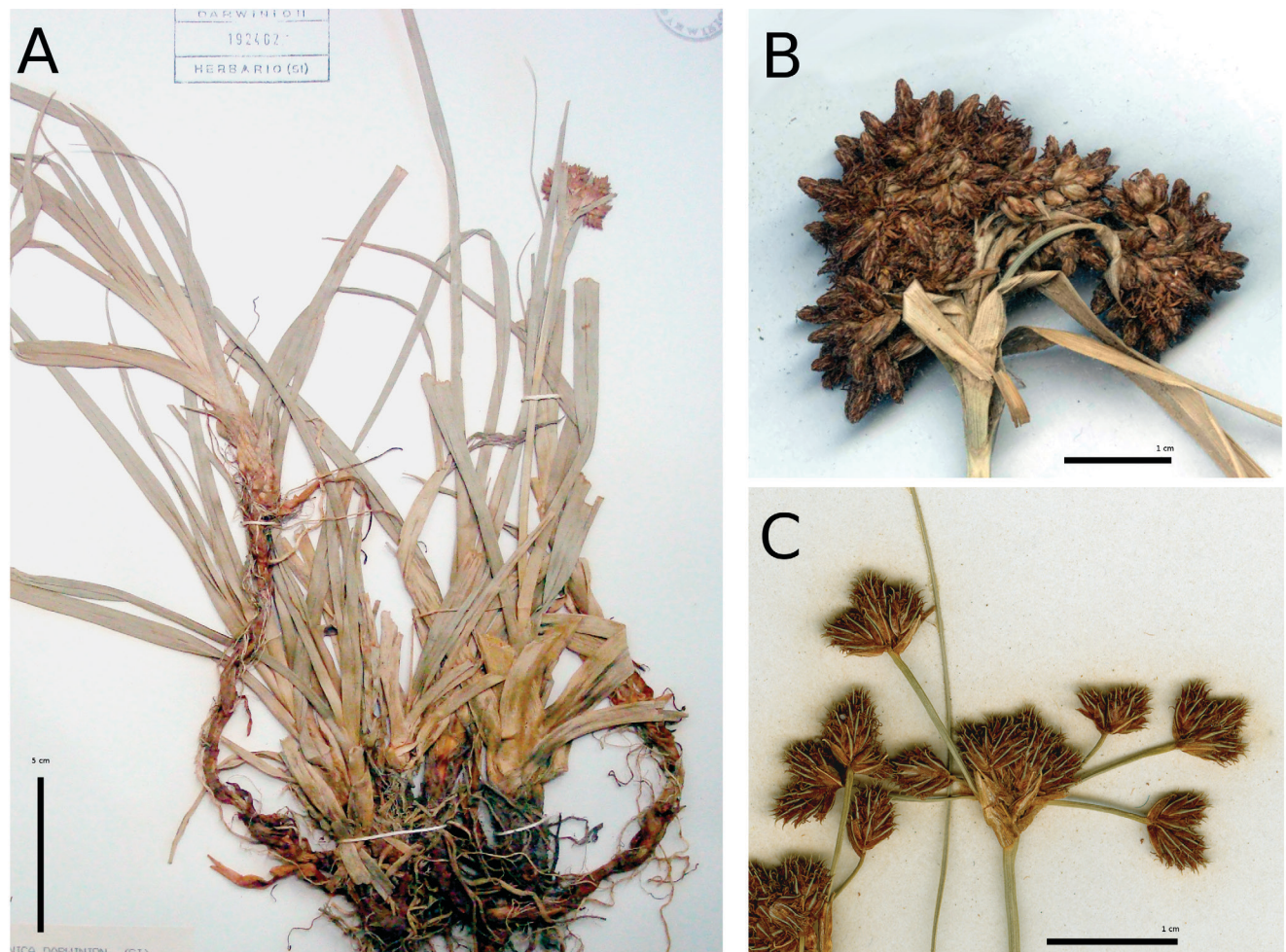


Fig. 2. General morphology of *Rhodoscirpus asper*. **A**, Whole plant showing the cespitose habit and long rhizomes; **B**, dense inflorescence typical of non-Chilean specimens; **C**, part of an open inflorescence with long-pedicellate clusters of 1–3 spikelets typical of Chilean specimens. — Scale bars: A = 5 cm; B & C = 1 cm.

which also possesses ciliate ligules and glumes. *Rhodoscirpus* differs from *Phylloscirpus* with its eligulate leaves and entire glumes, and *Zameioscirpus* with its entire to very minutely toothed glumes and ligules. *Amphiscirpus*, *Rhodoscirpus* and *Phylloscirpus* all differ from *Scirpus* s.str. by having perianth bristles with hyaline retrorse barbs proximally arranged in two rows along the margins of the thick flattened brownish-orange body (Fig. 3F, G), while *Scirpus* spp. with retrorsely barbed bristles have the barbs mostly spirally, or sometimes pseudodistichously, arranged near the base of the bristle (Fig. 3H), but in more than two rows, with the bodies often terete and generally white to pale yellow or brown. *Zameioscirpus* differs from all above-mentioned members of the Zameioscirpus-*Scirpus* clade in having no developed perianth. *Rhodoscirpus* has brown to grey-brown mature nutlets with a broadly obovate to suborbicular body (including stipe, but not beak) 1–1.3 times as long as wide (Fig. 3A, D), while *Scirpus* spp. have generally pale brownish or yellowish nutlets with an elliptic to slightly obovate body (1.4)1.5–1.7(2) times as long as wide (Fig. 3C), with the exception of *S. polystachyus*, *S. ternatanus* Rein. ex

Miq. and allies (*S. chunianus* Tang & F.T.Wang, *S. rosthornii* Diels), which have almost orbicular bodies. For these characters, *Rhodoscirpus* is closer to *Amphiscirpus*, which has dark brown, broadly obovate nutlet bodies 1–1.5 times as long as wide, while *Phylloscirpus* has brown obovate to almost elliptic nutlet bodies 1.2–2.2 times as long as wide (Fig. 3B, E) and *Zameioscirpus* has brown obovate to narrowly obovate nutlets with bodies 1.5–2.6 times as long as wide. A detailed morphological description of *Rhodoscirpus asper* is found in the taxonomic treatment below.

Comparative culm and leaf blade anatomy. — The culm and leaf blade anatomy of *Rhodoscirpus asper* is similar to that of *Scirpus pendulus* and *S. microcarpus* in most aspects. All examined species have culms with scattered tannin idiosperms, an outer ring of major bundles with sclerenchyma girders alternating with air cavities, scattered major bundles in the ground tissue, a ring of minor bundles external to the outer ring of major bundles, and undefined bundle sheaths made up of birefringent (presumably sclerified) cells. *Rhodoscirpus* differs from *Scirpus* in that no central cavity is formed in old culms

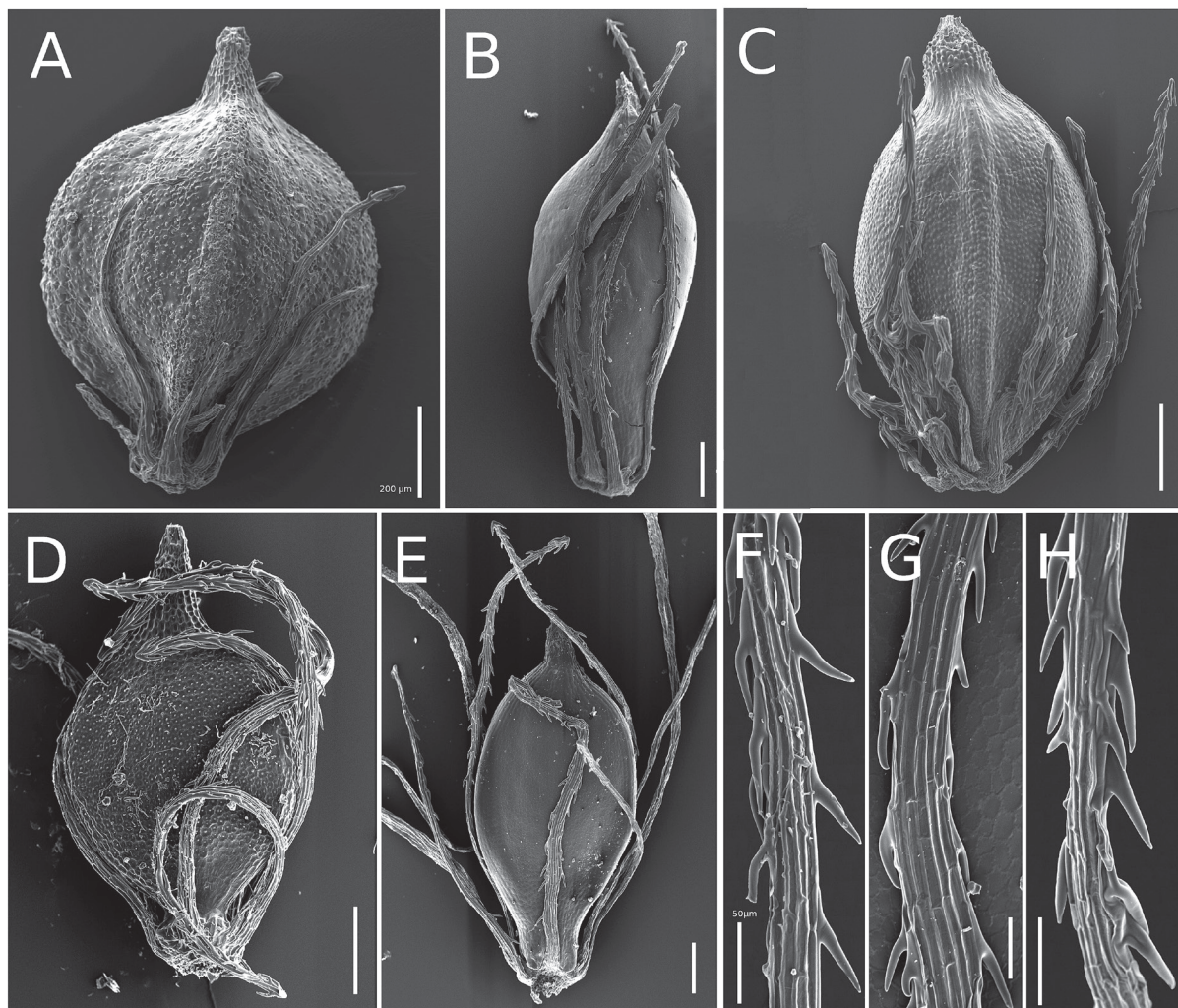


Fig. 3. Morphology of nutlets (A–E) and proximal portion of perianth bristles (F–H) of *Rhodoscirpus asper* (A, D, F), *Phylloscirpus deserticola* (B, E, G) and *Scirpus sylvaticus* (C, H). A–C, abaxial view; D–E, adaxial view. — Scale bars: A–E = 200 μm; F–H = 50 μm.

(Fig. 4D), whereas there is a large central cavity in *Scirpus*. It also differs by the strong development of the sclerenchymatous bundle sheaths, with sometimes up to seven layers of birefringent cells surrounding the bundles (Fig. 4F). The culm of *Scirpus microcarpus* is peculiar in the net-like arrangement of cells of the ground tissue which make up two distinct layers: an outer layer of small thin-walled cells with scattered small schizogenous cavities and an inner layer with large schizogenous cavities. The ground tissue in *Scirpus pendulus* and *Rhodoscirpus asper* is of relatively large, thin-walled cells that are closely imbricate with no hint of a net-like arrangement.

The anatomy of the leaf blades (Fig. 4A–C) of all examined species is also similar, with a thickly V-shaped outline and abaxially keeled midrib. All species possess an epidermis with adaxial cells larger than abaxial cells and with smaller cells over girders, and bulliform cells prominent over midvein and accompanied by 1–2 subepidermal layers of large hyaline cells. The mesophyll consists of tightly imbricate chlorenchymatous cells surrounding large rectangular air cavities that alternate with vascular bundles. The inner bundle sheath layer is formed of sclerified cells with U-shaped thickenings most prominent at the phloem pole, and the outer bundle sheath is

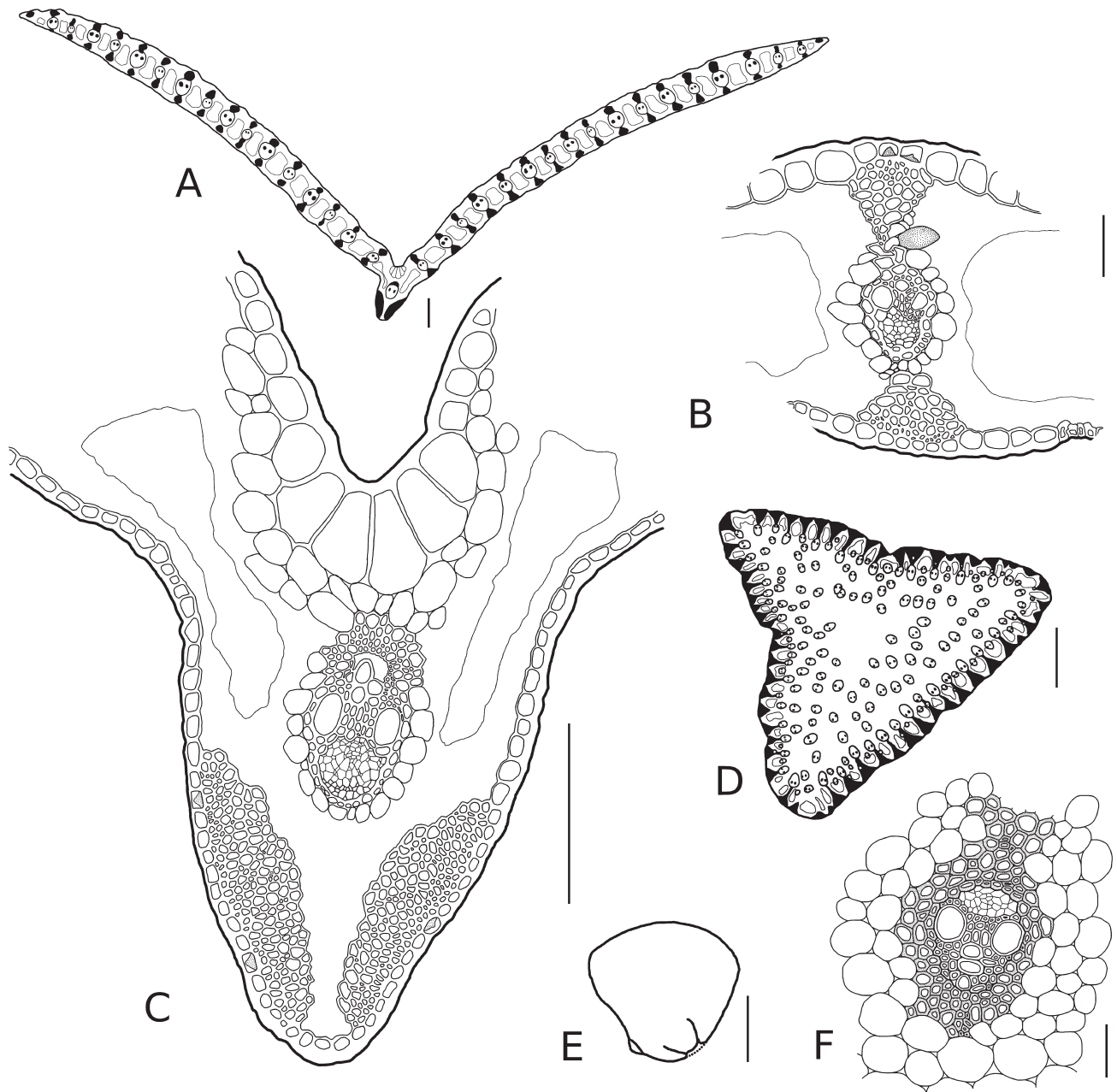


Fig. 4. Anatomy and embryology of *Rhodoscirpus asper*. **A**, Leaf cross-section; **B**, leaf lateral nerve; **C**, leaf midrib; **D**, culm cross-section; **E**, sagittal view of embryo (redrawn from Van der Veken, 1965); **F**, culm major vascular bundle. — Scale bars: A = 500 μm ; B = 50 μm ; C = 300 μm ; D = 1000 μm ; E = 100 μm ; F = 50 μm .

of large thin-walled cells (Fig. 4C). *Rhodoscirpus asper* and *Scirpus pendulus* have abaxial and adaxial girders (sometimes partial) on each secondary bundle, whereas *S. microcarpus* differs by having abaxial and adaxial subepidermal strands of sclerenchyma connected to the bundles by unicellular rows of large hyaline thin-walled cells. *Scirpus microcarpus* also differs by the extreme size of the air cavities, leaving only very thin strips of chlorenchyma around the bundles and epidermis. *Rhodoscirpus* has tannin idioblasts mostly restricted to the area around the base of the adaxial girders, whereas examined *Scirpus* spp. have tannin idioblasts scattered throughout the mesophyll. A detailed description of the culm and leaf blade anatomy of *Rhodoscirpus asper* is found in the taxonomic treatment below.

■ DISCUSSION

***Rhodoscirpus*, a new genus for tribe Scirpeae.** — Molecular and morphological data are consistent in indicating that *Scirpus asper* is more closely related to *Phylloscirpus* and allied South American genera of tribe Scirpeae than it is to *Scirpus* s.str. This close relationship demonstrates that *S. asper* should be recognized as a separate generic lineage, *Rhodoscirpus* gen. nov., to preserve the monophyly of *Scirpus* s.str. while maintaining the morphologically distinctive genera *Amphiscirpus*, *Phylloscirpus* and *Zameioscirpus*.

The phylogenetic position of *Rhodoscirpus* as sister to *Phylloscirpus* in the Zameioscirpus clade is not surprising when the morphological, embryological, biogeographical and ecological data are critically examined. Although ligules are lacking in *Phylloscirpus*, the ciliate ligules of *Rhodoscirpus* are found in *Amphiscirpus* and to a certain degree in some specimens of *Zameioscirpus* (Dhooge & al., 2003). *Rhodoscirpus* also shares ciliate glume margins with *Amphiscirpus* (Smith, 2002; Dhooge, 2005), and its peculiar reddish bristles with distichously arranged retrorse barbs are shared with *Amphiscirpus* and *Phylloscirpus* (Fig. 3F, G). In addition, its brown, broadly obovate nutlets (Fig. 3A, D) are most similar in shape to those of *Amphiscirpus* and to a certain extent to those of *Phylloscirpus deserticola*, although fruit shape is variable within the Zameioscirpus clade. Finally, Van der Veken (1965) presents an embryo of *Rhodoscirpus asper* (Fig. 4E) with a broadly turbinate outline and a sub-lateral root cap positioned more basally than the sub-basal germ pore, characters that correspond most closely to the *Schoenus*-type embryo as defined by Goetghebeur (1986). All other members of the Zameioscirpus clade also possess *Schoenus*-type embryos, whereas *Scirpus* spp. normally have *Fimbristylis*-type embryos which have an oblong to narrowly turbinate shape, a lateral root cap and a (sub-)basal germ pore, with rare exceptions known from species of uncertain affinities (e.g., *S. petelotii*, *S. ternatanus*; Van der Veken, 1965). However, the cauline leaves, large size and open anthelate inflorescence (Fig. 2) make *Rhodoscirpus* strikingly different from all other genera of the Zameioscirpus clade, which are generally small and reduced in stature, with basal leaves and capitate to unispicate inflorescences (Dhooge, 2005).

Biogeography and ecology also support the close relationship of *Rhodoscirpus* with the other genera of the Zameioscirpus clade. *Scirpus* species are mostly found in Northern Hemisphere boreal to temperate wetlands, whereas the closely related *Eriophorum* is characteristic of northern boreal to arctic wetlands and peatlands (Novoselova, 1994a, b; Ball & Wujek, 2002). There remains no accepted African species of *Scirpus* with the recent removal of *Dracoscirpoides* (Cyperaceae; Muasya & al., 2012), except for *S. pinguiculus* C.B. Clarke which is probably an *Isolepis* R.Br. given its unispicate inflorescence, lack of perianth and presumed close relationship to *I. cernua* (Vahl) Roem. & Schult. (Cyperaceae; Clarke, 1898: 222). Likewise, an examination of the protologues and pictures of type specimens for all remaining South American *Scirpus* spp. suggests that they all have affinities with other Cyperaceae genera, often in distantly related tribes (Dhooge, 2005; ÉLB, pers. obs.). This leaves the Australian *S. polystachyus* as the only representative of the genus in the Southern Hemisphere. In contrast, *Rhodoscirpus* and all other members of the Zameioscirpus clade are endemic to the South American Andes and adjacent lowland regions, except for *Amphiscirpus* which is also represented by disjunct populations in western North America (Smith, 2002; Dhooge, 2005).

Ecologically, *Rhodoscirpus* is found in wet to dryish low- to mid-elevation environments (200–3500 m), and it has even been reported to grow on sandy beaches close to streams alongside cacti and other succulents (*Landrum 3834*, NY) or in moderately saline water (*Taylor 10753*, ASU). In these characteristics, it is most similar to *Amphiscirpus*, which is also often found in saline, low- to high-elevation (400–3950 m) marshes, whereas *Phylloscirpus* and *Zameioscirpus* are cushion forming plants of high-elevation (3130–4840 m) páramo or puna vegetation (Dhooge, 2005). These habitat differences may in fact explain the strikingly different gross morphology of *Rhodoscirpus* and *Phylloscirpus*, the latter being reduced in almost all vegetative and reproductive characters probably as a result of its adaptation to climatically harsh, high-elevation grasslands (Hedberg & Hedberg, 1979).

Although *Rhodoscirpus* possesses many characteristics used in the modern circumscription of *Scirpus*, such as cauline leaves, flat leaf blades with large air spaces, open anthelate inflorescences, small spikelets and nutlets, and six perianth bristles, many combinations of these characters are found in other unrelated CDS genera (e.g., *Calliscirpus*, *Cypringlea*, *Dulichium* Pers., *Khaosokia*), indicating that they are probably plesiomorphies (Strong, 2003; Simpson & al., 2005; Gilmour & al., 2013; Léveillé-Bourret & al., 2014). However, most *Scirpus* spp. possess other less common characters, absent in *Rhodoscirpus*, which are most likely derived and would be better to circumscribe monophyletic units for classificatory purposes. For instance, *Scirpus* spp. are generally characterized by pale, sometimes almost white, nutlets, with bodies generally at least 1.5 times as long as wide (Fig. 3C), and black-tinted glumes, whereas *Rhodoscirpus* has brown to grey-brown nutlets with broad bodies and reddish glumes with no hint of black color. In summary, there appears to be no clearly derived character in support of a close relationship between *Rhodoscirpus* and *Scirpus* s.str.

Implications for the circumscription of *Scirpus*. — The genera of the Zameioscirpus clade and *Scirpus* clade form a well-supported clade in our combined analyses (Fig. 1). This Zameioscirpus-*Scirpus* clade has also been found in many previous molecular phylogenetic studies, although it has never received such a strong parsimony BS support before (Muasya & al., 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014). In addition to the molecular data, the clade appears to be supported by the presence of tannin cells in leaves and culms of all examined members (Dhooge, 2005; this study) and the displacement of the root cap to a (sub-)lateral position in the embryo (Van der Veken, 1965).

The position of *Rhodoscirpus* as sister to *Phylloscirpus* (Fig. 1) indicates that most morphological and anatomical characters used in the circumscription of *Scirpus* are probably ancestral for the whole Zameioscirpus-*Scirpus* clade. This is also supported by the fact that *Eriophorum* appears to be derived from within *Scirpus* s.str., a result which is common to all recent phylogenetic analyses of tribe Scirpeae (Jung & Choi, 2012; Gilmour & al., 2013; Léveillé-Bourret & al., 2014). The situation is similar to that seen in tribe Cypereae, where many morphologically distinctive genera (e.g., *Ascolepis* Nees ex Steud., *Lipocarpa* R.Br., *Oxycaryum* Nees, *Remirea* Aubl., etc.) are nested within a large paraphyletic *Cyperus* L. (Larri-don & al., 2011a, b, 2013, 2014; Bauters & al., 2014). In the case of the Zameioscirpus-*Scirpus* clade, all genera have probably diverged from the presumed ancestral scirpoid morphology because of high selective pressure on certain morphological characteristics. For instance, the long bristles of *Eriophorum* are probably an adaption to dispersal by wind (Goetghebeur, 1998), the gross vegetative morphology of *Amphiscirpus* is strongly reminiscent of that of species of *Schoenoplectus* (Rchb.) Palla (Fuireneae) found in similar saline marshes (Beetle, 1943; Hammer & Heseltine, 1988; Smith, 2002), and the low cushion-forming habit and congested, reduced inflorescences of *Phylloscirpus* and *Zameioscirpus* are common adaptations found in many páramo and puna plants (Hedberg & Hedberg, 1979).

Even with the removal of *Rhodoscirpus*, the circumscription of *Scirpus* remains problematical because of the distinctive and widely known genus *Eriophorum*, which appears to be nested within *Scirpus* as currently defined (Jung & Choi, 2012; Gilmour & al., 2013; Léveillé-Bourret & al., 2014; Fig. 1). A future dilemma for Scirpeae taxonomy will be to decide whether *Eriophorum* should be treated as a infrageneric taxon in *Scirpus*, or whether *Scirpus* should be further divided into a series of six to eight new genera that would best represent the morphological diversity of the *Scirpus* clade. It is also worth noting that a few species that have not been sampled in molecular analyses, but are currently placed in *Scirpus* and *Eriophorum*, possess characteristics that suggest that they may not clearly fall within the *Scirpus* or even Zameioscirpus clades.

For instance, *Scirpus petelotii* (= ?*S. hainanensis* S.M. Huang) possesses curious terminal hairy appendages on its perianth bristles (ÉLB, pers. obs.), and both *S. petelotii* and *S. ternatanus* have embryos with sub-basal root caps suggestive of *Schoenus*-type embryos (Van der Veken, 1965). As

for *Eriophorum*, its circumscription appears natural with the recent removal of two species to *Erioscirpus* (Cypereae; Yano & al., 2012). However, some rare species endemic to Eastern Asia (*Eriophorum scabriculum* (Beetle) Raymond, *E. transiens* Raymond) appear to combine characteristics which would exclude a close affinity to *Eriophorum* and even to the Zameioscirpus-*Scirpus* clade as a whole (Beetle, 1946; Raymond, 1957, 1959). The recognition of *Rhodoscirpus* is thus only one small step towards the ultimate goal of a satisfactory generic and suprageneric classification of Scirpeae. Future effort should be focused on broadening the taxonomic sampling of molecular phylogenetic studies to include morphologically and biogeographically aberrant species, and on a re-examination of the morphology and embryology of those species in the light of our current knowledge of relationships within the Cariceae-Dulichieae-Scirpeae clade.

■ TAXONOMIC TREATMENT

Revised identification key to Scirpeae genera

- 1 Inflorescence a white to red cottony mass at maturity because of the exerted flat and silky perianth bristles.. **2**
- 1 Inflorescence not appearing as a cottony mass; bristles included to shortly longer than glumes, not flat and silky **4**
- 2 Bristles >>10, devoid of barbs except at the very apex; cauline leaves generally present; spikelets large, 8–50 mm long in fruit *Eriophorum*
- 2 Bristles 6–7, antrorsely barbed or smooth; leaves all basal; spikelets small, 5–15 mm long in fruit..... **3**
- 3 Inflorescence a dense congested head of many spikelets; bristles antrorsely barbed almost to the base; ligules ciliate; leaf blades flat and elongate..... *Calliscirpus*
- 3 Inflorescence a single terminal spikelet; bristles smooth; ligules entire; leaf blades reduced to short mucros *Trichophorum* (in part)
- 4 Cauline leaves present, node of the distalmost leaf clearly visible above the sheath of the leaf below; inflorescence anthelate, usually compound, sometimes contracted in a head **5**
- 4 Leaves all basal, node of the distalmost leaf hidden in the sheath of the leaf below; inflorescence various, but rarely anthelate **6**
- 5 Ligule a densely ciliate rim with hairs 0.1–0.4 mm long; glumes red to brown-red with no hint of black, margins ciliate; perianth bristles sharply retrorsely barbed; nutlet grey-brown to brown, with the broadly obovate to suborbicular body (incl. stipe) 1.0–1.3 times as long as wide.... *Rhodoscirpus* gen. nov.
- 5 Ligule entire or with scarce teeth or hairs ≤0.1 mm long; glumes often black-tinted, often scarcely and minutely toothed, margins rarely short-ciliate; perianth bristles variously antrorsely to retrorsely scabrous or smooth; nutlet often pale yellowish to almost white, rarely brown, the body (incl. stipe) generally >1.5 times as long as wide, rarely almost orbicular *Scirpus*

- 6 Inflorescence open, anthelate; perianth bristles <0.5 times length of nutlet, with reduced barbs; nutlets with very short beak up to 0.4 mm long *Cypringlea*
- 6 Inflorescence a single spikelet, a dense head or a paucispicate raceme; perianth bristles absent to longer than nutlet, barbed or not; nutlets with or without long beak 7
- 7 Inflorescence a dense head of many spikelets; perianth bristles retrorsely barbed 8
- 7 Inflorescence unispicate, rarely a paucispicate raceme; perianth bristles various, sometimes absent, but never retrorsely barbed 9
- 8 Leaves ligulate; inflorescence pseudo-lateral; glumes ciliate *Amphiscirpus*
- 8 Leaves eligulate; inflorescence terminal; glumes entire *Phylloscirpus* (in part)
- 9 Leaves eligulate *Phylloscirpus* (in part)
- 9 Leaves ligulate 10
- 10 All spikelet scales similar, without excurrent awn or mucro *Zameioscirpus*
- 10 Proximal scale of spikelet differentiated and sterile, often awned 11
- 11 Perianth of scale-like tepals *Oreobolopsis*
- 11 Perianth of bristle-like tepals or absent *Trichophorum* (in part)

Description of the new genus

Rhodoscirpus Léveillé-Bourret, Donadio & J.R.Starr, **gen. nov.** – Type: *Rhodoscirpus asper* (J.Presl & C.Presl) Léveillé-Bourret, Donadio & J.R.Starr ≡ (*Scirpus asper* J.Presl & C.Presl).

Diagnosis. – Similar to *Scirpus* L., but differing by its ciliate ligules with hairs >0.1 mm long, reddish glumes with no hint of black, reddish perianth bristles with sharp retrorse barbs distichously arranged near the base, brown to grey-brown nutlets with broadly obovate bodies 1.0–1.3 times as long as wide (incl. stipe), culms lacking a central cavity, and turbinate embryo with sub-basal root cap lower than the germ pore (*Schoenus*-type).

Etymology. – The greek prefix *Rhodo-* means “rose-like” and was chosen in honor of the late Prof. Encarnación Rosa Guaglianone (1932–2014), affectionately known as Rosa, who was a great and dedicated cyperologist at the Darwinion Institute (Buenos Aires, Argentina), but who was also loved for her exceptional kindness and generosity.

Note. – The genus is endemic to South America and is treated as monotypic pending detailed revisionary studies (see taxonomic notes below).

1. ***Rhodoscirpus asper*** (J.Presl & C.Presl) Léveillé-Bourret, Donadio & J.R.Starr, **comb. nov.** ≡ *Scirpus asper* J.Presl & C.Presl, Reliq. Haenk. 1: 194. 1828 – Holotype: CHILE. Mountains, s.d., *Haenke s.n.* (HAL No. 0109717 [photo!]; PRC barcode 452287 [photo!]).
- = *Scirpus glaucus* Nees & Meyen ex Kunth, Enum. Pl. 2: 169. 1837, nom. illeg. ≡ *Scirpus subasper* Beetle in Revista Univ. (Cuzco) 33(87): 139. 1944 – Holotype: CHILE. Chile austral,

Santa Rosa de los Andes, in fossis, 1827? [according to Turrill, 1920], *Poeppig 509* (W [photo!] [destroyed?]).

= *Scirpus trachycaulos* Phil. in Anales Univ. Chile 93: 482. 1896 – Holotype: CHILE. Zanjón, in Valle Carrizal, Sep 1885, *F. Philippi s.n.* (SGO No. 037805 [photo!]; isotype: SGO No. 046304 [photo!]).

= *Scirpus asper* var. *polystachyus* C.B.Clarke in Bot. Jahrb. Syst. 30(2, Beibl. 68): 36. 1901 ≡ *Scirpus subasper* var. *polystachyus* (C.B.Clarke) Beetle in Revista Univ. Cuzco 33(87): 139. 1944 – Holotype: CHILE. Atacama desert, Feb 1888, *R.A. Philippi s.n.* (K barcode K000632420 [photo!]; isotypes: SGO Nos. 037806, 046268, 075159 & 075739 [photos!]).

= *Scirpus subasper* var. *diffusus* Beetle in Amer. J. Bot. 33: 661. 1946 ≡ *Scirpus asper* var. *diffusus* (Beetle) Beetle in Bol. Soc. Argent. Bot. 5: 82. 1953 – Holotype: CHILE. Quebrada los Bruites, tributary of Illapel River, ca. 2 km from houses, Dept. Illapel, Prov. Coquimbo, along irrigation ditch in shade of shrubs, 14 Nov 1938, *Worth & Morrison 16491* (RM barcode RM0000128 [photo!]; isotype: SI barcode 000525 [photo!]).

Description. – Perennial herb, 30–160 cm tall, forming tufts or mats. *Roots* minutely papillose, faded yellow-brown to grey-brown, the central white strand surrounded by a thin ring of brown tissue and free from the rind. *Rhizomes* short creeping, distally shortly ascending, completely sheathed with overlapping reddish-brown cataphylls with acute tips, to ca. 4 mm wide including cataphylls. *Aerial vegetative parts* light yellow-green to light glaucous-green upon drying. *Culms* solitary or loosely clumped, erect, obtusely trigonous proximally, often becoming triquetrous distally, sharply antrorsely scabrous at the angles but becoming smooth proximally, 1.5–6 mm wide near base, the sheath-clade bases 5–11 mm wide including sheaths. *Leaves* basal and cauline, basal numerous, cauline (0)1–3. Leaf sheaths loose, dark to pale red-brown basally, distally concolorous with blades; inner bands narrowly obtriangular, straight to shallowly U-shaped at the apex, white-hyaline to dark red-brown, white margined, generally abundantly dotted with red tannin cells and sometimes with prominent dark veins, sometimes papillose, the distalmost sheath 3–10 cm long; ligule wider than long, rounded to cordate, often asymmetrically so, margined with a thin densely ciliate membrane with hyaline hairs 0.1–0.4 mm long. Leaf blades about as long as mature culms, widest 3–9 mm wide, often conduplicate basally, but generally flat distally, abaxially keeled and antrorsely scabrous on the midnerve, antrorsely scabrous on the margins especially distally, but sometimes with a few retrorse barbs near the proximal sheaths. *Inflorescence* a terminal compound anthelodium, congested to open at maturity, to about 12 × 10 cm, with spikelets arranged in dense glomerules of up to 20+ spikelets, or in visibly stipitate fascicles of 1–3(4) spikelets. Basal bracts sheathless, leaf-like to linear setaceous, the first 1–4 with ascending blades longer than inflorescence, antrorsely scabrous on the midnerve abaxially and on the margins. *Spikelets* ovoid when immature, becoming narrowly ovoid to ellipsoid, the appressed glumes often spreading at fructification, 4–16 × 2–4 mm; spikelet prophyll empty, broadly ovate, obtuse at apex, completely encircling

the pedicel at base but not sheathing, ca. 0.5 times as long as proximal glumes, covered with red dots, with two nerves visible as more profusely red-dotted lines. *Glumes* ca. 15–80+, all fertile, deciduous, ovate to oblong, 1.6–3 × 0.9–1.3 mm red to brownish-red with abundant red lines, proximally paler orange to yellow, membranous to subchartaceous, margin undifferentiated; midrib green, with a central prominent nerve, not reaching the apex or excurrent in a recurved, papillose to antrorsely scabrous awn 0.1–0.6 mm long; margins ciliate with hairs to 0.1 mm long. *Flowers* bisexual, spirally inserted; perianth bristles (5)6, reddish, 0.22–1.46 mm long, the longest 0.9–1.5 times as long as mature nutlet, retrorsely barbed 0%–60% of their length from the apex, the barbs divaricate, hyaline and sharp; stamens 3, all displaced to an abaxial position, the largest mature anthers 1.1–2 mm long, with very short acute to dome-shaped red apiculum; style red, 3-branched, the branches with large erect papillae longer than wide. *Nutlets* 0.9–1.1 mm long, brown to grey-brown, surface with an areolate cell pattern visible especially when young, becoming papillose from the silica-body projections at maturity; body obovate to suborbicular, 0.70–0.90 × 0.66–0.74 mm, 1–1.3 times as long as wide, ca. 0.4 mm thick, compressed-triangular in section with a thickness/width ratio of ca. 0.6, basally constricted in a short “stipe” 0.06–0.14 mm long; beak clearly defined, 0.14–0.20 mm long including dark style remnant. *Embryo* broadly turbinate in outline, with sub-lateral root cap and sub-basal germ pore (*Schoenus*-type).

Culm anatomy. – Culm transverse section obtusely trigonous. *Epidermis* of rectangular cells with thickened outer periclinal walls, of equal heights, smaller than the ground tissue cells. *Ground tissue* parenchymatous, not breaking down in a central cavity, of large round thin-walled cells of unequal size, with small air spaces at junction of each triplet of touching cells, with many scattered large tannin idioblasts. *Aerenchyma* present as an external ring of elongate-rectangular to inverse-U shaped cavities alternating with the external ring of major vascular bundles, apparently lysigenous. *Vascular bundles* collateral with internal xylem and external phloem, of two sizes; major bundles forming an outer ring alternating with aerenchyma and capped by sclerenchyma girders, and also scattered throughout the stem except for a small central area; minor bundles half the size of major bundles, forming a single ring external to the outer ring of major bundles, scattered just below the aerenchyma cavities or in the sclerenchyma girdles. *Bundle sheath* not clearly defined, of 2–7(?) disorganized layers of small sclerified cells intergrading with the ground tissue. *Sclerenchyma* in elongate obtriangular girders connected to the outer ring of major bundles and alternating with the aerenchyma cavities, often interrupted by minor bundles.

Leaf blade anatomy. – Leaf blade transverse section thickly V-shaped near base, with abaxially keeled midnerve. *Epidermis* of rectangular cells with outer periclinal wall thickened, adaxial cells larger than abaxial but becoming subequal near tips, cells over sclerenchyma girders much smaller and bearing conical silica deposits, leaf apparently amphistomatic, the stomata mostly adjacent to air cavities. *Bulliform cells* very prominent adaxially over midvein, sometimes accompanied by

1–2 subepidermal layers of large round thin-walled empty cells. *Mesophyll* chlorenchymatous throughout except for bundle sheaths, of closely imbricate cells, with tannin idioblasts mostly distributed near the base of the adaxial girders. *Aerenchyma* present as large rectangular cavities between each vascular bundle, apparently lysigenous. *Vascular bundles* ca. 16–34, collateral with adaxial xylem and abaxial phloem, the main bundle larger than the laterals, but similar in all other aspects. *Bundle sheaths* of two layers; inner layer of rectangular birefringent thick-walled cells with U-shaped thickenings, the thickenings much more prominent at the phloem pole and sometimes almost absent at the xylem pole, sometimes apparently interrupted by the adaxial girder; outer layer of large round thin-walled empty cells, often interrupted by adaxial, and sometimes also abaxial, girders. *Sclerenchyma* present as triangular adaxial and abaxial girders, rarely incomplete (forming strands), and as two elongate dome-shaped strands under the main bundle, on both sides of the abaxial keel.

Distribution and ecology. – Mountains and Pacific shore of Peru, Bolivia, Chile and Argentina. Dry sandy or rocky valleys and slopes, beaches, pastures, cultivated areas, often in or near flowing water. Sometimes associated with succulents and sclerophyllous shrubs. Altitude 200–3500 m.

Taxonomic notes. – *Scirpus leptopus* Boeck., which was described by Böckeler (1858) as a species closely related to *S. asper*, was put in synonymy of the latter by Govaerts & al. (2007). We disagree with that decision since we have seen pictures of two syntypes deposited at E and they are clearly distinct and appear most similar to *Cyperus*. Moreover, these specimens were also examined by Kükenthal, who put the name in the synonymy of *C. xanthostachyus* Steud. (Kükenthal, 1936).

Rhodoscirpus asper is here delimited in the widest sense to include what might be a complex of many distinct entities, the number and boundaries of which are unclear. The plants are especially variable in stature, degree of scabrosity, length of ligule hairs, inflorescence structure and glume awn length. One of the most distinct part of the complex comprises all examined Chilean specimens, which are distinct in having an open inflorescence with spikelets solitary or in long-pedunculate glomerules of 2–3 spikelets (Fig. 2C), glumes with long awns 0.4–0.7 mm long, anthers 1.7–2.0 mm long, and with leaf sheaths, blades and glume midnerves often distinctly glaucous when dry and abundantly papillose. These characteristics are clearly seen in the type of *Scirpus subasper* var. *diffusus*, and the type of *S. asper* might also represent a young individual of the “Chilean morphology”. Specimens from Argentina, Bolivia and Peru have a congested or semi-open inflorescence with short-pedunculate, dense glomerules of often more than 20 spikelets (Fig. 2B), glume awns absent or rarely up to 0.2 mm long, generally shorter anthers to 1.2–1.8 mm long, and dried yellowish-green leaf sheaths, blades and glume midnerves that are glabrous or somewhat papillose. The types of *S. asper* var. *polystachyus* and perhaps also the type of *S. trachycaulos* correspond to a third, intermediate group, where the spikelets are in l(2) dense (sub)sessile glomerules, but the glumes are long-awned and the leaves all basal. Additional variability is found

in bristle length and scabrosity with some specimens bearing subequal bristles much longer than the mature nutlet that are sharply retrorsely scabrous to about the middle, while others have very unequal reduced bristles shorter than the mature nutlet and with only a few weak barbs clustered near their tips. The genus is clearly in need of a thorough taxonomic revision.

Representative specimens. – ARGENTINA. *Giraldez s.n.*, s.d. (MT 00048131), prov. San Juan, Bolivar, 1250 m; *Kiesling, R. 6704*, 4 Feb 1987 (NY, SI), prov. San Juan, dpto. Angaco, Sa. Pie de Palo, Mogote Corralitos, 3160 m; *Kiesling, R. 6711*, 5 Feb 1987 (NY), prov. San Juan, dpto. Jáchal, El Salto, 2100–2200 m; *Kiesling, R. 10341*, 7 Mar 2010 (SI 201866), prov. Mendoza, dpto. Luján de Cuyo, entre Vistalba y Dique Chipoleti, fuente a dependencia de Aguas de Mendoza; *Ponce, M.M. 114*, 10 Mar 2010 (SI 201665), prov. San Juan, dpto. Jáchal, ca. 20 km de Bella Vista, El Divisadero, El Salto, 20°07'06" S 68°52'00" W, en el salto de agua, 2100 m; *Schweitzer 4545*, Jan 1926 (MT 00048707), prov. Jujuy, dpto. Tilcara, El Chorro, 2600 m; *Unknown coll. 25*, s.d. (MT 00050042), prov. Cortoba. — BOLIVIA. *Bang, M. 765*, Mar 1890 (MICH); *Beck, St. G. 19844*, 2 Mar 1991 (NY), prov. Loayza, dpto. La Paz, Alrededor del Balneario Termal de Urmiri, al borde del arroyo en quebrada rocosa, 3450 m; *Beck, St.G. 21935*, 26 Jan 1996 (NY), prov. José Román de Loayza, dpto. La Paz, Baños Termales de Urmiri, 17°09' S 68°05' W, matorral, bajo de los valles secos interandinos, 3500 m; *Rusby, H.H. s.n.*, 1885 (MICH), Yungas, 1829 m. — CHILE. *Landrum, L.R. 3834*, 11 Nov 1981 (MICH), prov. Valparaíso, playa Mirasol, about 36 km north of San Antonio, 33°20' S 71°40' W, herb in crack in rocks, along stream, steep 20–30 m high slopes and adjacent beaches, succulents (caeti, *Calandrina*) and sclerophyllous shrubs (*Myrceugenia*, *Cryptocarya*, *Lithraea*) dominate; *Wall, E. & Sparre, B. s.n.*, 7 Jan 1947 (MT 00018980), prov. Santiago, Corral Quemado, about 25 km north-east of Santiago City, 1200 m; *Werdermann, E. 82*, Nov 1923 (CAS 104513), prov. Coquimbo, Rivadavia, 800 m; *West, J. 5100*, 6 Jan 1936 (A), prov. Concepcion, near falls of Rio Laja, rock crevices near water of falls, 200 m. — PERU. *López M., A. 1490*, 28 Jun 1958 (US 2341140), prov. Huamachuco, dpto. La Libertad, Hacienda Yanazara, 2400 m; *Mostacero L., J. & al. 0984*, 30 Jul 1985 (US 3458491), prov. Celedín, dpto. Cajamarca, Pumarrume, ladera, 2750 m; *Sagástegui A., A. 14035*, 5 Aug 1935 (F 2011685, NY), prov. Chota, dpto. Cajamarca, alrededores de Lajas, pastizal, 2200 m; *Soukup, J. 5289*, 8 Aug 1964 (US 2471595), Quebrada Verrugas (Sa. Bartolomé), 2000 m; *Veja, S. 1965*, 3 Apr 1977 (F 2216024), prov. Cajamarca, dpto. Cajamarca, alrededores de San Juan, ruta a Pacasmayo, ladera y terrenos de cultivo, 2400 m.

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Appendix 1. Samples used in phylogenetic analyses, with GenBank accession numbers. Sequences already published (retrieved from GenBank) are indicated by an asterisk (*), missing sequences are indicated by a dash (–).

Taxonomic name, collector(s) coll. no. (herb.), origin, *matK* access., *ndhF* access., ETS-1f access.

Amphiscirpus nevadensis (S.Watson) Oteng-Yeb., *Hudson 5177* (CAN), Canada, *JX065075, *JX074631, KP705256; *A. nevadensis*, *Ruthsatz 170/1* (SI), Argentina, KP165400, KP212420, –; *Blysmus rufus* (Huds.) Link. *Jokela 9-VIII-1958* (CAN), Finland, *JX065076, *JX074632, KP705280; *Calliscirpus brachytrix* C.N.Gilmour, J.R.Starr & Naczi, *Ahart & Oswald 5099* (CHS), U.S.A., *JX065078, *JX074634, *JX065112; *C. criniger* (A.Gray) C.N.Gilmour, J.R.Starr & Naczi, *Chambers 2973* (DAO), U.S.A., *JX074655, *KJ513488, *JX065099; *Carex acicularis* Boott, *Ford 29/94* (CHR), New Zealand, *KJ513581, *KJ513489, –; *C. acicularis*, *Ford 113/98* (FHO), New Zealand, –, *AY242012S2; *C. blanda* Dewey, *Bakowski 97-176* (WIN), Canada, *KJ513583, *KJ513491, *AY241983; *C. capitata* Sol., *Starr & Thibeault 6016* (CAN), U.S.A., *KJ513585, *KJ513493, –; *C. capitata*, *Ford 02379* (WIN), Canada, –, *DQ115119; *C. siderosticta* Hance, *Léveillé-Bourret 545* (CAN), Garden, *KJ513592, *KJ513499, –; *C. siderosticta*, *Waterway 2004.268* (MTMG), Japan, –, *DQ998892; *C. stipata* Muhl. ex Willd., *Dugal & Camfield 3728* (CAN), U.S.A., *KJ513593, *KJ513500, –; *C. stipata*, *Waterway 99.072* (MTMG), U.S.A., –, *AY757375; *Dulichium arundinaceum* (L.) Britton, *Ford & Punter 94233* (FHO), Canada, *JX065083, *JX074639, –; *D. arundinaceum*, *Bergeron 81-113* (CAN), Canada, –, KP705281; *Eleocharis acicularis* (L.) Roem. & Schult., *Fields 2583* (WIS), U.S.A., *KJ513595, *KJ513502, –; *Eriophorum angustifolium* Honck. subsp. *angustifolium*, *Scoggan 10947* (CAN), Canada, *KJ513597, *KJ513504, –; *E. angustifolium* subsp. *angustifolium*, *Keleher 755* (CAN), Canada, –, KP705276; *E. russeolum* Fr. ex Hartm. subsp. *russeolum*, *Gauthier 75-208* (CAN), Canada, *KJ513608, *KJ513515, –; *E. russeolum* subsp. *russeolum*, *Clément & al. 30* (CAN), Canada, –, KP705279; *E. vaginatum* subsp. *spissum* (Fernald) Hultén, *Porsild 12* (CAN), Canada, *KJ513614, *KJ513521, –; *E. vaginatum* subsp. *spissum*, *Léveillé-Bourret 632* (DAO), Canada, –, KP705278; *E. virginicum* L., *Dickson & Brunton 3214* (CAN), Canada, *KJ513617, *KJ513524, –; *E. virginicum*, *Léveillé-Bourret 633* (DAO), Canada, –, KP705269; *E. viridicarinatum* (Engelm.) Fernald, *Darbyshire 2532* (CAN), Canada, Ontario, *KJ513618, *KJ513525, KP705277; *Erioscirpus comosus* (Wall.) Palla, *Hing & al. 22413* (A), China, *KJ513619, *KJ513526, –; *Fimbristylis ovata* (Burm.f.) J.Kern, *Muasya 684* (K), Kenya, *JX065086, *JX074642, –; *Khaosokia caricoides* D.A.Simpson, Chayam. & J.Parn., *Middleton & al. 4071* (MICH), Thailand, *JX065087, *JX074643, –; *Phylloscirpus deserticola* (Phil.) Dhooge & Goetgh., *Solomon 15819* (CAS), Bolivia, *KJ541072, *KJ541073, KP705259; *P. deserticola*, *Ru 9797* (US), Argentina, *JX065090, *JX074646, KP705260; *Rhodoscirpus asper* (J.Presl & C.Presl) Léveillé-Bourret, Donadio & J.R.Starr, *Kiesling 10341* (SI), Argentina, KP165402, KP212422, KP705261; *R. asper*, *Werdermann 82* (CAS), Chile, –, KP705264; *R. asper*, *Landrum 3834* (MICH), Chile, KP165401, KP212421, KP705263; *R. asper*, *Ponce 114* (SI), Argentina, KP165403, KP212423, KP705262; *R. asper*, *West 5100* (A), Chile, –, KP705265; *R. asper*, *Vega 1965* (F), Peru, –, KP212424, –; *Scirpus ancistrochaetus* Schuyler, *Cippolini SA-13* (Wright), U.S.A., *KJ513627, *KJ513534, –; *S. cyperinus* (L.) Kunth, *Lindsay 1025* (CAN), Canada, *JX065092, *JX074648, –; between *KJ513534, –; *S. divaricatus* Elliott, *Spalink 124* (WIS), U.S.A., *KJ513633, *KJ513540, –; *S. divaricatus*, *Anderson 10630* (MO), U.S.A., –, KP705268; *S. hattorianus* Makino, *Bergeron & al. 81-111* (CAN), Canada, *KJ513640, *KJ513547, –; *S. hattorianus*, *Léveillé-Bourret 621A* (DAO), Canada, –, KP705271; *S. maximowiczii* C.B.Clarke, *Petrochenko & al. 5613* (CAN), Russia, *KJ513643, *KJ513550, KP705275; *S. microcarpus* J.Presl & C.Presl, *Dugal & Camfield 3770* (CAN), Canada, *KJ513646, *KJ513553, –; *S. microcarpus*, *Léveillé-Bourret 608* (DAO), Canada, –, KP705274; *S. pendulus* Muhl., *Cruise 1388* (CAN), Canada, *KJ513649, *KJ513556, –; *S. pendulus*, *Léveillé-Bourret 611* (DAO), Canada, –, KP705270; *S. polyphyllus* Vahl, *Spalink 246* (WIS), U.S.A., *KJ513650, *KJ513557, –; *S. polystachyus* F.Muell., *Pullen 4091* (A), Australia, *KJ513651, *KJ513558, KP705272; *S. radicans* Schkuhr, *Samuelsson 296* (CAN), Sweden, *KJ513653, *KJ513560, KP705273; *S. wichurae* Boeckeler, *Jung 808322* (AJOU), South Korea, *KJ513655, *KJ513562, –; *Trichophorum alpinum* (L.) Pers., *Spetzman 4941* (CAN), U.S.A., Alaska, *JX065093, *JX074649, KP705266; *T. cespitosum* (L.) Hartm., *Aiken & Iles 02-048* (CAN), Canada, Nunavut, *KJ513657, *KJ513564, KP705267; *Zameioscirpus atacemensis* (Phil.) Dhooge & Goetgh., *Ru 9884* (US), Argentina, *JX065095, *JX074651, KP705257; *Z. muticus* Dhooge & Goetgh., *Salvador & al. 881* (MICH), Mexico, *KJ513668, *KJ513575, KP705258.