EVOLUTION AND CLASSIFICATION OF THE CARICEAE-DULICHIEAE-SCIRPEAE CLADE (CYPERACEAE)

by

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A thesis submitted to the Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for a Ph.D. degree
in the Ottawa-Carleton Institute of Biology

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Abstract

For over a century, the origins and mechanisms underlying the diversification of the enormous cosmopolitan genus *Carex* (>2,100 species; Cariceae, Cyperaceae or sedge family) have remained largely speculative. Although its unique morphology (e.g., unisexual flowers, perigynia) clearly indicated it was a natural group, it obscured its relationships to all other Cyperaceae because the morphological gap between it and the rest of the family was so wide. Consequently, no plausible sister group to *Carex* has ever been proposed. Early molecular analyses narrowed the problem by placing *Carex* within a strongly-supported clade with the enigmatic monospecific genus *Khaosokia*, and tribes Dulichieae and Scirpeae (hereafter CDS), a group consisting of 2,250 species, or approximately 41% of all Cyperaceae. However, poor taxonomic sampling and the limited number of molecular markers used in these studies meant that the sister group to *Carex* remained a mystery. The goals of this thesis were to resolve evolutionary relationships within the CDS clade, to identify the sister group to *Carex*, and to develop a new natural tribal classification of CDS that could be used in future biogeographic and comparative analyses of *Carex* and its relatives.

Initial phylogenetic analyses using two plastid markers (*matK*, *ndhF*) identified seven major CDS lineages, and suggested that *Carex* could be nested within a paraphyletic Scirpeae. However, backbone support for these relationships was low due to an ancient rapid radiation (~10 million years) followed by long divergence of the seven major lineages (~40 million years). The addition of conventional sequence-based markers from the plastid genome (*rps16*) and nuclear ribosomal region (ETS-1f, ITS) indicated that a traditional molecular approach would not resolve these key backbone nodes. Consequently, a recently developed flowering-plant-specific anchored enrichment probe kit targeting hundreds of conserved nuclear genes combined with next generation sequencing was used to resolve the CDS backbone.

Although the resulting phylogenomic dataset was able to resolve the CDS backbone with high support, the topology and branch lengths only reaffirmed the isolated position of *Carex*. However, comparative morphological analyses of specimens at key herbaria not only suggested that *Sumatroscirpus*, a rare genus thought to be endemic to Sumatra, could be sister to *Carex*, but they also provided an easily accessible site to collect DNA in Northern Vietnam. Subsequent phylogenetic analyses of plastid (*matK*, *ndhF*, *rps16*) and nuclear ribosomal (ETS-1f, ITS) markers strongly supported *Sumatroscirpus* as the sister to *Carex*, and molecular dating estimates suggested they shared a common ancestor in the late Eocene (~36 million years ago). Comparative studies and ancestral state estimates of key morphological characters were congruent with this hypothesis, suggesting that the perigynium is not unique to *Carex*, but in fact a synapomorphy shared with *Sumatroscirpus*. This means that the initial key innovation in the remarkable diversification of *Carex* is not the perigynium, but could be the release of mechanical constraints that permitted the evolution of the remarkable morphological diversity of *Carex* perigynia seen today.

A taxonomic revision of *Sumatroscirpus* revealed that this purportedly monospecific genus actually consisted of four species, and it extended its range over 2,400 km to the north into Northern Vietnam, Myanmar, and Southwestern China. The phylogenetic framework provided by the previous studies enabled a new tribal and generic classification of CDS to be proposed. Seven monophyletic tribes are recognised including four new tribes (Calliscirpeae, Khaosokieae, Sumatroscirpeae, Trichophoreae), and a new genus (*Rhodoscirpus*). Morphological synapomorphies are identified for all recognized tribes, and a worldwide treatment, including identification keys, is provided for *Sumatroscirpus* species, CDS genera, and Cyperaceae tribes.

Résumé

Depuis plus d'un siècle, les causes et l'origine de la diversification de l'énorme genre *Carex* (>2 100 espèces; tribu des Cariceae, famille des Cypéracées) sont restés énigmatiques. Cela résulte de son incroyable diversité et de sa distribution mondiale, mais surtout de ses caractères morphologiques exceptionnels. Le périgyne, une préfeuille dissimulant une fleur, est apparemment unique à *Carex*, et en fait un groupe clairement naturel. Toutefois, cette curieuse structure a aussi obscurci ses affinités évolutives avec les autres membres des Cypéracées. Les premières analyses phylogénétiques basées sur l'ADN ont placé le genre *Carex* dans un clade avec l'étrange genre monospécifique *Khaosokia*, ainsi que les tribus Dulichieae et Scirpeae (ci-après CDS), un groupe comprenant environ 2 250 espèces, ou près de 41% de toutes les espèces de Cypéracées. Toutefois, dû à l'échantillonnage taxonomique limité et au faible nombre de marqueurs moléculaires utilisés dans ces études, l'identité du groupe sœur de *Carex* est resté un mystère. Les objectifs de cette thèse étaient de résoudre les relations évolutives au sein de CDS, d'identifier le groupe sœur de *Carex*, et de créer une classification naturelle de CDS qui pourra servir dans de futures études comparatives de *Carex* et de ses proches parents.

Nos premières analyses phylogénétiques, basées sur deux marqueurs plastidiques (*matK*, *ndhF*), ont identifié sept lignées importantes au sein de CDS. De plus, elles ont suggéré que *Carex* pourrait être niché au sein de Scirpeae, faisant de cette tribu un groupe paraphylétique. Toutefois, les relations évolutives entre les sept lignées majeures de CDS ont obtenu un faible support statistique, en grande partie à cause de leur apparition soudaine (~10 millions d'années) et ancienne, il y a quelque 50 millions d'années. L'addition de marqueurs conventionnels basés sur des séquences du génome plastidique (*rps16*) et de la région ribosomique nucléaire (ETS-1f, ITS) a confirmé qu'une approche moléculaire traditionnelle ne permettrait pas de résoudre les nœuds les plus importants de l'arbre évolutif de CDS. Par conséquent, une technique récente permettant l'enrichissement de centaines de marqueurs nucléaires à l'aide de sondes ciblant des gènes nucléaires conservés à travers les plantes à fleurs, suivi d'une méthode de séquençage de nouvelle génération, a été utilisée pour clarifier les relations évolutives de CDS.

Bien que les données phylogénomiques résultant de cette nouvelle technique ont résolu toutes les relations profondes de CDS avec un support statistique élevé, la topologie obtenue ne semblait qu'accentuer l'isolation entre *Carex* et les autres membres de CDS. Cependant, des analyses morphologiques comparatives de spécimens d'herbiers finirent par suggérer que *Sumatroscirpus*, un genre très rare et endémique de Sumatra, pourrait être le groupe sœur de *Carex*. Ces spécimens fournirent également un site facilement accessible pour la récolte d'ADN, dans le nord du Vietnam. Les analyses subséquentes basées sur des marqueurs plastidiques (*matK*, *ndhF*, *rps16*) et ribosomiques nucléaires (ETS-1f, ITS) ont confirmées que *Sumatroscirpus* est le groupe soeur de *Carex*, et ont daté leur ancêtre commun le plus récent à l'Éocène supérieur (~36 millions d'années avant aujourd'hui). Des études comparatives et des analyses de reconstruction d'états ancestraux pour certains caractères morphologiques clés supportent cette hypothèse, et ont suggéré que le périgyne n'était pas unique aux *Carex*, mais était en fait une synapomorphie partagée avec *Sumatroscirpus*. Cela signifie que le périgyne n'est pas l'innovation clé qui pourrait expliquer la radiation du genre *Carex*, et que d'autres mécanismes doivent être en cause. Le relâchement de contraintes mécaniques par la perte de l'épillet latéral pourrait avoir joué ce rôle en permettant l'évolution de l'incroyable diversité morphologique de périgynes visible aujourd'hui chez *Carex*.

Une révision taxonomique de *Sumatroscirpus* a révélé que ce genre qu'on croyait monospécifique comprend en fait quatre espèces, et a étendu son aire de répartition de plus de 2 400 km vers le nord, dans le nord du Vietnam et du Myanmar, et dans le sud-ouest de la Chine. Le cadre phylogénétique fourni par ces études a permis d'élaborer une nouvelle classification des genres et des tribus de CDS. Sept tribus monophylétiques sont reconnues, incluant quatre tribus nouvelles pour la science (Calliscirpeae, Khaosokieae, Sumatroscirpeae, Trichophoreae), ainsi qu'un nouveau genre (*Rhodoscirpus*). Des synapomorphies morphologiques sont identifiées pour toutes les tribus de CDS, et un traitement taxonomique mondial, comprenant des clés d'identification, est fourni pour les espèces de *Sumatroscirpus*, les genres de CDS, et les tribus des Cypéracées.

Statement of contributions

The studies contained in this thesis were conceived by Étienne Léveillé-Bourret and Julian R. Starr. Data collection was performed by Étienne Léveillé-Bourret, with contribution of molecular sequence data from Claire N. Gilmour (Chapter 2), Daniel Spalink (Chapter 2), and Sabina Donadío (Chapter 3). Emily Moriarty Lemmon and Alan R. Lemmon designed the probes and the bioinformatic pipeline for assembling and filtering the Anchored Phylogenomics data presented in Chapter 4. Étienne Léveillé-Bourret analyzed the data, prepared the figures, and wrote the manuscripts. Julian R. Starr, Bruce A. Ford, Claire N. Gilmour, Sabina Donadío, Robert F. C. Naczi, Daniel Spalink, Kenneth J. Sytsma, Emily Moriarty Lemmon, and Alan R. Lemmon participated in the revision of the published scientific articles. Authorship on published scientific articles is indicated at the start of each Chapter.

Acknowledgements

I would like to thank my supervisor Julian Starr, for his deep scientific insight, continued support, and unabating faith in the most ambitious of my scientific endeavors. I could not have imagined a better PhD supervisor. Thanks to Bruce Ford, my "academic grandfather", for his thoughtful advice, enthusiasm, and also for his sangfroid in the most unnerving fieldwork situations. I would also like to thank them for a most wonderful fieldwork experience in Northern Vietnam, which drastically changed the course of my PhD, for the better.

Valuable feedback and advice were provided by all members of my thesis advisory committee and examination committee: John Thor Arnason, Tyler Smith, Nicolas Rodrigue, David J. Currie, Jacques Cayouette, Aaron Liston, and Lynn J. Gillespie.

Thanks to Roger Bull, Frankie Janzen, Khaled Himmat, Sarah Chisholm, Elyse Latreille, Jocelyn Pender, and Philip Pelletier for assistance with molecular lab work. Thanks to Paul Hamilton for assistance with optical and scanning electron microscopy at the Canadian Museum of Nature. Alexandre Bergeron (MT), Jacques Cayouette (DAO), Kendra Cippollini, Marie-Ève Garon-Labrecque, Kellina Higgins, Jung Jongduk (AJOU), Roberto Kiesling (SI), Mónica Ponce (SI), and Jack Regalado (MO) helped with fieldwork or provided silica-dried plant material. Atik Retnowati (BO), Laurent Gautier (G), and Armin Löckher (W) were especially helpful in tracing back lost or hard to find specimens and types. We had the pleasure to learn floral ontogenetic work in Leuven due to the hospitality and generosity of Alexander Vrijdaghs. Erik F. Smets (L) made our visit to the Naturalis Biodiversity Center (Leiden) a delight. Mats Hjertson (UPS) generously provided pictures, transcriptions and translations of important pages in Harry Smith's catalogue of plants and 1934 field book. The late Encarnacíon Rosa Guaglianone (1932–2014) was instrumental in the conception of the study presented in Chapter 3. Michelle Kortyna, Alyssa Bigelow, Sean Holland, and Kirby Birch helped with the lab work and bioinformatics for Chapter 4. I am also grateful to Modesto Luceño for the pictures of

Carex he provided for Chapter 5. Bobbi Angell provided line drawings of *Sumatroscirpus* species for Chapter 6. Porter P. Lowry II (MO, P) provided additional information on his *Sumatroscirpus* specimen collected in Vietnam that helped me to find the population in the field. Thanks to Nguyễn Thị Kim Thanh, Vũ Anh Tài, and Scott Ford for their assistance in the field in Northern Vietnam. Thanks to Warren Cardinal-McTeague for valuable feedback and support. Special thanks to Claire Gilmour, whose excellent MSc work provided the foundation for my PhD project.

Many thanks to the herbarium curators and technicians of the numerous collections I visited, requested loans or scans from, or where I obtained permission to sample plant tissue for DNA analysis, anatomical, or embryological work: A, AAH, AJOU, ASU, B, BO, CAN, CAS, CHICO, CHR, CHSC, DAO, DOV, FHO, G, GB, GH, HNU, IBSC, K, L, MICH, MO, MT, NY, OSC, P, PRE, SI, SING, SYS, UBC, UPS, US, W, WIN, and WIS. Thanks especially to Jennifer Doubt, Lyndsey Sharp and Micheline Bouchard, respectively curator, herbarium technician, and ex-herbarium technician of the National herbarium of Canada (CAN), where my numerous specimen loans are housed. I have thoroughly tested their patience with difficult loan and loan extension requests, and I cannot give them enough praise for their professionalism, attention to detail, and hard work.

Merci à ma mère Nicole Léveillé, qui m'a toujours encouragé et soutenu dans tous mes projets. Merci à mon père André Bourret, pour avoir développé chez moi l'amour de la nature et de la science. Merci à ma douce Marie-Ève Garon-Labrecque, tu rends ma vie plus belle. Merci à tous les autres membres de ma famille, notamment à Louise Léveillé pour son support et son intérêt dans mes recherches, à Daniel Fortin pour avoir partagé ses excellentes photos de Cypéracées, ainsi qu'à Lise Garon et à Luc Proulx pour m'avoir accueilli chez eux vers la fin de mes études à l'Université de Montréal. Finalement, je tiens à remercier tout particulièrement mon ami et mentor Jacques Cayouette, pour ses connaissances et son enthousiasme pour la botanique, son expérience du terrain et de la recherche, son sens de l'humour, son incommensurable générosité, et pour avoir été présent pour moi dans les moments plus difficiles.

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CHAPTER 1

INTRODUCTION

With a distribution spanning all continents except Antarctica, the Cyperaceae (sedges) represent one of the most diverse (>100 genera, ~5,500 species; Poales) and floristically important families of flowering plants. Sedges comprise between 2% and 3% of the native floras of the United States and Canada, Europe, and China, dominating vast areas of the northern hemisphere in terms of diversity and biomass (Walters, 1980; Govaerts, 2001; Ball & al., 2002; Dai & al., 2010b; Koopman, 2011; Lu & He, 2017). Moreover, at least 9% of sedges are of either direct (e.g., medicines, crops) or indirect (e.g., weeds) economic importance (Simpson & Inglis, 2001; Bryson & Carter, 2008). Despite the economic and ecological significance of sedges, evolutionary relationships within the Cyperaceae are poorly known due in part to the family's cosmopolitan range and great diversity, but also to its highly reduced floral and vegetative parts. Although the family displays a spectacular diversity of inflorescence morphologies, the main character source for higher-level classifications (Koyama, 1962; Dahlgren & al., 1985; Goetghebeur, 1986; Bruhl, 1995; Goetghebeur, 1998), their grass-like habit and small, typically wind-pollinated flowers arranged in spikelets or spikelet-like structures do not provide many systematically useful characters. Consequently, traditional infrafamilial classifications have often been controversial, with debates between competing systems resting on different interpretations of the family's challenging morphology (Holttum, 1948; Kern, 1962; Koyama, 1962; Dahlgren & al., 1985; Goetghebeur, 1986; Bruhl, 1991, 1995; Goetghebeur, 1998).

Consisting of well over a third of all sedge species, the genus *Carex* L., sole member of tribe Cariceae, stands out due to its diversity in temperate and boreal regions (Ball, 1990; Cayouette, 2008). Since most of its species are found in the northern hemisphere, *Carex* species also represent some of the most familiar and floristically significant taxa for North

American and Eurasian botanists. Commonly dominant in wetlands, they are also present in habitats as diverse as deciduous forests and even deserts. Numerous species are of local economic significance as high-quality natural forage, soil stabilizers, crafting materials, folk medicines, ornamentals, or weeds (Le Cohu, 1967; Simpson & Inglis, 2001; Bryson & Carter, 2008; Barrett, 2013; Mishra & al., 2015; Small & Cayouette, 2016). Moreover, the genus is of interest because its taxa display almost every biogeographic pattern ever recognised (e.g. amphiatlantic, bipolar, Gondwanan; Raymond, 1951; Croizat, 1952), its cytology is unique among living organisms for the length of the aneuploidy series it displays (holocentric chromosomes: n = 6 to 66; (Davies, 1956; Roalson, 2008; Hipp & al., 2009), and the breadth of its ecological range, as noted above, means that interest in using it as a model for exploring all aspects of plant evolution has increased significantly in recent years (~150 papers/year with "Carex" or "Cariceae" as topic between 2013 and 2015 in Web of Science; e.g. Escudero & al., 2010, 2012a, 2012b; Gehrke & Linder, 2011; Spalink & al., 2016b, 2016a). However, the success of *Carex* as a potential model for studies on plant evolution is ultimately hampered by one glaring fact: so little is known about its relationships to other sedges. In other words, confidently polarizing the evolutionary direction of any aspect of its ecology, biogeography, or biology (e.g., chromosome evolution) is currently not possible.

The principal reason why the phylogenetic relationships of *Carex* are so poorly known can largely be explained by its exceptional morphology. *Carex* is morphologically isolated within Cyperaceae by the possession of a combination of highly derived features, such as the absence of a perianth, unisexual flowers, and most strikingly by the presence of a perigynium or utricle, a flask-shaped structure that surrounds and conceals the female flower (Fig. 1.1). The perigynium is a specialized bract inserted on the first node of inflorescence branches (i.e., a prophyll; Holm, 1896; Snell, 1936), and it is unusual because most Cyperaceae prophylls are sterile. The inflorescence of *Carex* is made even more peculiar by the tendency of branches to be truncated after the prophyll node, making the perigynium and associated axis appear like a single terminal flower, when they actually represent a whole lateral inflorescence branch. This "caricoid" inflorescence structure thus

appears very distinct from the more typical "scirpoid" features seen in most other members of Cyperaceae: sterile prophylls, bisexual flowers with a perianth, and branches all terminated by monomorphic spikelets (Fig. 1.2). Ultimately, this thesis aims to resolve the relationships of *Carex* to other sedges and to clarify the homology of caricoid and scirpoid inflorescence structures.

1.1 Theories on the Origins and Evolution of Cariceae

The singular inflorescence morphology of Cariceae has obscured its phylogenetic origin because of the uncertain homology between caricoid and scirpoid inflorescence parts, and the apparent absence of intermediates between these two distinct inflorescence types (Smith, 1967; Le Cohu, 1968; Smith & Faulkner, 1976; Reznicek, 1990; Timonen, 1998; Vegetti, 2002; Vrijdaghs, 2006). Before the advent of molecular phylogenetics, Cariceae had been aligned with a few groups such as tribes Sclerieae and Bisboeckelereae (Holttum, 1948; Kern, 1958; Koyama, 1962a; Schultze-Motel, 1964; Goetghebeur, 1986; Simpson, 1995), and subfamily Mapanioideae (Mattfeld, 1935; Bruhl, 1995; Simpson, 1995), largely based on the traditional subdivision of Cyperaceae into unisexually and bisexually flowered groups (de Jussieu, 1789; Bentham & Hooker, 1883). However, inflorescence architecture (Meert & Goetghebeur, 1979; Goetghebeur, 1986; Richards & al., 2006) and mature embryo morphology (Goetghebeur, 1998) conflicted with proposed relationships, without suggesting any convincing alternatives. This led investigations to focus on evolution within Cariceae, and to neglect enquiries on the origin and evolution of the tribe within the broader context of the family.

As characters could not be polarized by outgroup comparison due to uncertain outgroup relationships, Cariceae classifications were largely constructed on the basis of evolutionary scenarios that focused on a handful of "evolutionary significant" characters, the study of "transitional" taxa, and untested assumptions about the direction of character evolution (e.g. Nelmes, 1951; Savile & Calder, 1953). The large variation in inflorescence complexity exhibited in the tribe, ranging from a unispicate axis containing a few flowers

to highly-compound panicles of hundreds of flowers (e.g. Fig. 1.3, P–W), was central to these theories. Species were arranged into a system of genera and subgenera based on inflorescence structure and perigynium shape, which were in turn ordered into reduction or proliferation series (reviewed in Reznicek, 1990). Whereas most authors hypothesized that ancestrally compound inflorescences evolved by reduction towards a unispicate morphology (Kreczetovicz, 1936; Nelmes, 1951a; Smith & Faulkner, 1976; Reznicek, 1990), others developed more complex scenarios involving an initial reduction of complexity and a loss or fusion of structural elements, followed by increased complexity in more advanced lineages (Kükenthal, 1909; Savile & Calder, 1953). In turn, the presence of many presumably "primitive" species with highly compound inflorescences in Southeastern Asia supported the view that this region was the center of origin for the tribe and genus (Gilly, 1950; Nelmes, 1951b; Raymond, 1955; Koyama, 1957; Raymond, 1959; Egorova, 1999). The largely speculative or conjectural tone of these studies was a direct result of the limited set of morphological characters available, and of their uncertain homology. Molecular phylogenetic studies are now providing new insights into the evolution of Cariceae, because homology assessment is easier at the molecular level, and the number of useable characters much larger (Scotland & al., 2003; Starr & al., 2004).

Phylogenetic studies have revealed that all formerly recognized Cariceae genera are nested within *Carex*, and that almost all *Carex* subgenera are polyphyletic (Starr & Ford, 2009; Waterway & al., 2009; Global Carex Group, 2015; Starr & al., 2015). These studies have also suggested that open perigynia and highly-compound inflorescences could be derived in the tribe, in sharp contrast with previous assumptions and evolutionary scenarios (Starr & Ford, 2009; Waterway & al., 2009). However, they have continued to suggest an Eastern Asian origin for *Carex*, with the discovery of many early-diverged Asian lineages, including the small Siderostictae and Hypolytroides Clades, which are sister to the remainder of *Carex* (Waterway & al., 2009; Starr & al., 2015). Interestingly, all examined Siderostictae Clade species possess a few large chromosomes (n = 6, or 12 in tetraploids; Yano & al., 2014), in contrast to the numerous small chromosomes found in other *Carex* (n = 7–66; Roalson, 2008). This could be in line with the view that low chromosome

numbers are ancestral (Heilborn, 1924), and with the hypothesis that the unusually high chromosomal variation of non-Siderostictae *Carex* was a key innovation in their spectacular radiation (Escudero & al., 2012b). However, the lack of a broader phylogenetic context, and especially of a sister-group for *Carex*, means that the results of these studies remain doubtful. Indeed, the accuracy of any morphological, ecological or geographical character reconstruction is affected by outgroup choice, outgroup relationships, and its effects on ingroup topology, especially near the root (Wheeler, 1990; Lyons-Weiler & al., 1998; Salisbury & Kim, 2001; Graham & al., 2002; Wilberg, 2015).

The earliest of molecular phylogenetic studies in sedges have helped to narrow the possible sister groups to *Carex*, with most familial studies suggesting a relationship with the bisexually-flowered tribes Scirpeae and Dulichieae, and the enigmatic dioecious genus *Khaosokia* D.A.Simpson et al. (clade hereafter known as CDS; Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013). These results were highly unexpected on morphological grounds, rejecting the idea of an association between Cariceae and other unisexually-flowered tribes, and suggesting instead the evolution of caricoid inflorescences by direct reduction from bisexually-flowered scirpoid inflorescences (as previously suggested by Kukkonen & Timonen, 1979; Dahlgren & al., 1985). However, nearly two decades of molecular work have not been able to confidently resolve relationships within the CDS clade, mostly due to limited taxonomic sampling and the use of uninformative or excessively variable markers, meaning that the sister-group to *Carex* is still unknown (Muasya & al., 1998; Simpson & al., 2005, 2007; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013). In-depth studies of the characteristics and relationships of all members of the CDS clade are necessary in order to identify the sister-group to *Carex*. Those groups suggested as possible sister groups to *Carex* are discussed below.

1.2 Dulichieae, Scirpeae and *Khaosokia*

Tribe Dulichieae is a small, mostly Holarctic group of 8–10 species in 4 genera (*Blysmus* Panz ex Schult., *Blysmopsis* Oteng-Yeb., *Dulichium* Pers., and *Sumatroscirpus* Oteng-Yeb.; Oteng-Yeboah, 1977; Goetghebeur, 1998; Govaerts & al., 2007; Fig. 1.3, A–D). The type genus *Dulichium* had previously been included in tribe Cypereae on account of its distichously arranged spikelets and glumes (Holm, 1897), or aligned with tribes Rhynchosporeae and Schoeneae due to winged spikelet glumes (Kükenthal, 1952; Schultze-Motel, 1959), but it differs from these taxa by the lack of sterile spikelet glumes, the presence of perianth bristles, and the possession of a Carex-type embryo (Goetghebeur, 1998). *Blysmus*, *Blysmopsis* and *Sumatroscirpus* were initially included within a broadly circumscribed genus *Scirpus* L., but were later recognized to be more closely related to *Dulichium* due to their possession of a fertile prophyll. This rare characteristic is found in only one other Cyperaceae tribe, Cariceae, which could suggest a close relationship between these tribes.

Dulichieae appears to be well-circumscribed, with previous studies having consistently supported its monophyly, although its phylogenetic position within CDS is unclear (Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013). The enigmatic Eastern Asian *Sumatroscirpus*, which has not yet been included in molecular phylogenetic studies, stands out because of characteristics such as highly compound corymbiform inflorescences (vs spicate/multispicate in other Dulichieae; Fig. 1.3, O), pedicellate spikelets (vs sessile), antrorsely scabrous perianth bristles (vs retrorsely scabrous), and tuberculate fruit apices (vs long beaked). Moreover, its tubular fertile prophylls are sheathing around the flower, which resembles more the utriculiform perigynia of *Carex* than the squamiform spikelet prophylls of other Dulichieae. These characteristics suggest that *Sumatroscirpus* might be more closely related to *Carex* or to other members of CDS, than it is to the genera currently included in Dulichieae.

Tribe Scirpeae contains the majority of CDS species outside Cariceae, with ~77–100 species in 9 genera: *Amphiscirpus* Oteng-Yeb., *Calliscirpus* C.N.Gilmour et al., *Cypringlea* Strong, *Eriophorum* L., *Oreobolopsis* T.Koyama & Guagl., *Phylloscirpus* C.B.Clarke, *Scirpus*, *Trichophorum* Pers., *Zameioscirpus* Dhooge & Goetgh. (Goetghebeur, 1998; Govaerts & al., 2007; Fig. 1.3, F–N). All genera of Scirpeae have at one time or another been included in a broadly circumscribed *Scirpus* s.lat., a taxon whose defining characteristics, such as spirally inserted bisexual flowers and presence of a perianth, are most likely plesiomorphic within Cyperaceae (Koyama, 1958; Goetghebeur, 1998). When thus circumscribed, *Scirpus* comprises a highly heterogeneous collection of more than 200 species, but modern studies based on morphological, anatomical, embryological and molecular data now split this assemblage into more than 20 genera, many of which are placed in distantly-related tribes (Koyama, 1958; Schultze-Motel, 1971; Goetghebeur, 1986, 1998; Gilmour & al., 2013).

Even with this new circumscription that recognizes only ~50 species of *Scirpus*, the limits of the genus are still not entirely resolved. An emerging pattern is the removal of South American and African species of *Scirpus* and *Eriophorum* to other genera. This is clearly demonstrated by a series of new genera segregated from *Scirpus* and *Eriophorum* over just the past 15 years (*Calliscirpus*, *Cypringlea*, *Dracoscirpoides* Muasya, *Zameioscirpus*; Dhooge & al., 2003; Strong, 2003; Muasya & al., 2012; Gilmour & al., 2013). Some of these new genera have been suggested as potential sister-groups to *Carex* (Gilmour & al., 2013), which highlights the need to increase taxonomic sampling in molecular analyses of CDS, with particular emphasis on species considered as atypical for tribe Scirpeae.

Previous systematists have also struggled to draw the line between *Scirpus* and *Eriophorum* due to morphologically-intermediate species that combine the six barbed perianth bristles of *Scirpus* with the black bracts and glumes, and the cottony infructescences of *Eriophorum* (Fernald, 1905; Koyama, 1958; Gilmour & al., 2013). Other Scirpeae genera such as *Trichophorum* and the closely-related *Cypringlea* and

Oreobolopsis are also suspected to be unnatural, although more in-depth studies would definitely be needed before taxonomic changes can be made (Dhooge, 2005; Gilmour & al., 2013).

Beyond problems of generic delimitations, tribe Scirpeae is itself suspected to be artificial, serving as a dumping ground for genera that lack any of the defining characteristics of other better-circumscribed tribes (Goetghebeur, 1998). The tribe also shows unusually high heterogeneity in embryological types, a character of high systematic significance that is usually well-conserved at the generic and tribal level (Van der Veken, 1965; Goetghebeur, 1986, 1998). Some Scirpeae genera possess undifferentiated Carex-type embryos, while others have derived Schoenus-type or Fimbristylis-type embryos with a sublateral or lateral germ pore (Van der Veken, 1965; Goetghebeur, 1986; Dhooge, 2005). This is consistent with the results of previous phylogenetic studies suggesting that Cariceae (Gilmour & al., 2013) or Dulichieae (Muasya & al., 2009) could be nested within a paraphyletic Scirpeae. These results confirm that the circumscription of Scirpeae may need to be revisited, but studies incorporating more comprehensive taxonomic, molecular and morphological sampling are needed to identify well-supported clades that could form the basis of a tribal revision.

Khaosokia is a monospecific genus endemic to limestone cliffs of peninsular Thailand (Fig. 1.3, E). It has seven perianth bristles and narrow elongate spikelets like *Dulichium* (Dulichieae), unisexual flowers and compound inflorescences reminiscent of highly-compound *Carex*, such as *Carex indica* L., and antrorsely-scabrous bristles and sterile prophylls like some genera of Scirpeae (Simpson & al., 2005). Although molecular phylogenetic analyses have aligned it with the CDS clade, branch support has always been weak and its position has varied from sister to Cariceae + Scirpeae, to sister for the whole CDS (Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013). The affinities of this morphologically enigmatic genus remain unresolved, and it is one of the few Cyperaceae genera that has yet to be placed in a tribe.

1.3 Relationships Within CDS, and the Sister Group to *Carex*

Previous phylogenetic results have variously positioned *Carex* as sister to Dulichieae and Scirpeae (Muasya & al., 2009), to a clade comprising *Scirpus* and *Eriophorum* (Jung & Choi, 2012), to *Calliscirpus* (Gilmour & al., 2013), or in a large polytomy including all major lineages of Scirpeae (Hinchliff & Roalson, 2013). These studies have also suggested that Scirpeae was paraphyletic with respect to *Carex*, which is consistent with the hypothesis that Scirpeae is defined by plesiomorphic characters. Difficulties in resolving backbone relationships of CDS are most likely due to a rapid radiation (<15 million years) followed by long divergence (>40 million years) that occurred in CDS (Escudero & Hipp, 2013; Spalink & al., 2016b), combining the problems of substitution saturation and long-branch attraction with low phylogenetic signal of backbone nodes (Whitfield & Lockhart, 2007). This explains why the use of some of the fastest-evolving coding regions of the angiosperm plastid genome (e.g. *matK*, *ndhF*; Moore & al., 2010; Liu & al., 2012) were unable to confidently resolve backbone relationships. Variable noncoding markers such as the nuclear ribosomal ITS and the plastid intergenic spacer *trnL-F* were not more useful, because large numbers of insertion-deletion events makes their alignment (= homology assessment) very difficult across the CDS clade (e.g. Starr & Ford, 2009; Jung & Choi, 2012).

These results parallel those obtained in phylogenetic investigations of the Cyperaceae family as a whole. To date, molecular data has been able to suggest new relationships and to question others, but lack of support for many of the most important nodes have meant that their impact on formal classifications have been limited. This explains why no new Cyperaceae tribe has been named for nearly 30 years (Thomas & Davidse, 1989), despite the widely recognized fact that several large tribes such as Hypolytreae, Schoeneae, Fuireneae and Scirpeae are most likely unnatural (Simpson & al., 2003; Muasya & al., 2009; Hinchliff & Roalson, 2013; Viljoen & al., 2013; Shiels & al., 2014). Resolution of these difficult taxonomic problems depends on the development of new informative and independent phylogenetic markers, especially from the vast and highly complex nuclear

genome, but designing broadly applicable PCR primers for such markers is rarely successful in non-model organisms. This is even true for well-known plant nuclear loci (Hughes & al., 2006) such as the Waxy (granule-bound starch synthase) or LEAFY genes, because gene duplications often necessitate extensive rounds of cloning to differentiate paralogs (e.g., Mason-Gamer & al., 1998; Hoot & Taylor, 2001). Even when primers are successfully designed, they often cannot amplify low-copy loci from degraded tissue samples, such as herbarium specimens, greatly reducing their usefulness in diverse cosmopolitan lineages like the Cyperaceae.

For the first time, the tools necessary to resolve some of the most persistent phylogenetic problems of the Cyperaceae family are available. Recent development of hybridization-based enrichment strategies for next-generation sequencing (Hyb-Seq NGS, e.g. Cronn & al., 2012; Straub & al., 2012; Lemmon & Lemmon, 2013; Weitemier & al., 2014; Harvey & al., 2016) are now making the development of dozens to hundreds of independent nuclear markers possible in a reasonable time, even for large taxonomic groups. Combining these new methodologies with extensive taxonomic sampling of herbarium specimens, and the re-examination of important morphological, anatomical and embryological characters appears to be the best strategy to resolve the difficult phylogenetic and taxonomic problems seen in CDS, and it could provide a model on how to realise a natural tribal and generic classification for the whole family.

1.4 General Aim, Specific Objectives, and Chapter Summaries

The goals of this thesis were to resolve evolutionary relationships within CDS, identify the sister-group to *Carex*, and create a new natural tribal classification for CDS. Such a classification would provide the reliable phylogenetic context that has been lacking in all previous studies aiming to understand the evolution, biogeography and diversification of *Carex* and its relatives.

This thesis is divided into six separate studies (Chapters 2–7) that resolve phylogenetic relationships within the Cariceae-Dulichieae-Scirpeae clade and provide this enormous lineage with a new tribal and generic classification. The implications of these results for our understanding of *Carex* morphology, biogeography and diversification are discussed. Each Chapter is preceded by an introduction that presents the specific objectives and scientific background of the Chapter in more detail. A discussion of the results of each individual study is presented at the end of each Chapter, and general conclusions are given in Chapter 8.

Chapter 2 presents an initial phylogenetic analysis including a comprehensive taxonomic sampling including 114 species (55% of Scirpeae + Dulichieae) covering all CDS genera except *Sumatroscirpus*, and sequence data from two fast-evolving plastid genes (*matK* and *ndhF*). The goal was to establish a broad picture of relationships within CDS and to sort genera into natural groups, which would facilitate the selection of representatives for a future phylogenomic analysis, and enable the identification of areas in need of study. Seven major lineages were identified within CDS, with Dulichieae and *Khaosokia* as successive sisters to Cariceae + Scirpeae. The analyses also indicated that Cariceae could be nested within Scirpeae, that *Scirpus* could be paraphyletic with respect to *Eriophorum*, and that *Trichophorum* could be unnatural, emphasizing the need for a revision of tribal and generic circumscription for Scirpeae. However, all important backbone nodes were unsupported, and the sister-group to *Carex* was still uncertain.

As a first step towards resolving problems of generic circumscription in Scirpeae, **Chapter 3**, presents a study of the taxonomy of *Scirpus asper* J.Presl & C.Presl, a South American species of uncertain affinities. Although this species possesses most of the features used to circumscribe *Scirpus*, its embryo shows affinities with genera of the mostly South American Zameioscirpus Clade, and its presumed allies have already been placed in different genera. This study builds upon Chapter 2 by including DNA sequence data from the nuclear ribosomal ETS-1f region in addition to the plastid genes *matK* and *ndhF*. Phylogenetic analyses demonstrated that *Scirpus asper* is not closely related to *Scirpus*

s.str., but sister to *Phylloscirpus* within the predominantly South American Zameioscirpus Clade (*Amphiscirpus*, *Phylloscirpus* and *Zameioscirpus*). When combined with morphological, anatomical and embryological data, results indicated that *S. asper* was best treated as the sole species of a new monospecific genus, *Rhodoscirpus* Lév.-Bourret, Donadío & J.R.Starr. Phylogenetic results from the nuclear and plastid genomes were congruent with each other and with the results obtained in Chapter 2. These studies supported the existence of seven major lineages within CDS that were moderately to well supported, but relationships between these lineages remained unresolved.

In order to advance towards the ultimate goal of revising the tribal and generic classification of CDS, **Chapter 4** aimed to resolve the difficult backbone branches of the rapid radiation at the base of the CDS clade by using data from flowering plant-specific anchored enrichment probes for hundreds of conserved nuclear genes. By comparing the nuclear matrix of 461 genes obtained with anchored enrichment to a typical Sanger-sequence dataset consisting of plastid and nrDNA markers (from Chapters 2 and 3), I demonstrated that the nuclear phylogenomic dataset was fully compatible with the Sanger dataset and resolved short backbone internodes with high support in both concatenated and coalescence-based analyses. Although the resolution of the CDS backbone clearly indicated that the major lineages identified in Chapters 2, 3 and 4 warranted tribal status, it only confirmed the seemingly remote status of Cariceae from all other sedges. This emphasised the need to sample the only CDS generic lineage that had not yet been included in molecular analyses, the monotypic *Sumatroscirpus*. Although rarely collected and believed to be restricted to only remote parts of northern Sumatra, herbarium studies revealed a new locality in northern Vietnam where DNA could be easily obtained.

Chapter 5 presents the first molecular phylogeny of all the genera of the CDS clade, including *Sumatroscirpus*, using three plastid (*matK*, *ndhF*, *rps16*) and two nuclear ribosomal (ETS-1f, ITS) markers. *Sumatroscirpus* was found to be the sister-group to *Carex*, and to be the sole genus of a new tribe, Sumatroscirpeae, trib. nov. Ancestral state reconstructions of key morphological characters were made, and a time-calibrated tree

estimated. Believed to be unique to *Carex*, the perigynium (prophyllar bract enclosing a flower) was shown to be a synapomorphy shared with *Sumatroscirpus* that appeared 36 million years ago. This meant that the key innovation in the remarkable diversification of *Carex* was not the perigynium, but could be the release of mechanical constraints on perigynia through spikelet truncation, resulting in novel adaptive morphologies. Results of this study open up many avenues for future research, as comparative studies of the tiny tribe Sumatroscirpeae will provide unprecedented insights into the inflorescence homology, evolution, diversification, and biogeographic history of its sister-group *Carex*, one of the world's most diverse plant lineages.

Chapter 6 consists of a taxonomic revision of *Sumatroscirpus*, which will provide the basic taxonomic and geographic information needed for future comparative studies in CDS. *Sumatroscirpus* was previously treated as a monospecific genus endemic to the Indonesian island of Sumatra, based on a species known for over 160 years. The taxonomic revision recognized four species of *Sumatroscirpus* and extended its range into Vietnam, Myanmar and Southwestern China. Identification keys, descriptions, illustrations, distribution maps, and conservation status assessments were provided for all recognized species of *Sumatroscirpus*. A detailed account of the inflorescence morphology of *Sumatroscirpus* was made, with special reference to the perigynium. In light of the results, the importance of herbaria and general collecting for species discovery and conservation was highlighted. The biogeography of *Sumatroscirpus* was also discussed, providing an interesting illustration of a well-known biogeographic link between the Sino-Himalayan and Sundaland mountain floras.

Building upon the phylogenetic results presented in previous chapters, **Chapter 7** presents a new tribal classification for the whole CDS clade, based on a total-evidence phylogenetic analysis combining molecular data with morphological and embryological data. Phylogenetic results were fully consistent with the relationships inferred in the phylogenetic and phylogenomic analyses presented of the previous chapters. Morphological synapomorphies were identified for seven major lineages of CDS, justifying their

recognition as tribes. This new tribal classification placed for the first time all CDS genera into monophyletic tribes, and the seven recognized tribes are all identifiable using morphological characters. Three new tribes were proposed: Calliscirpeae, Khaosokieae and Trichophoreae. Diagnoses for all CDS tribes, and an identification key to all currently recognized Cyperaceae tribes, were provided.

1.5 Figures

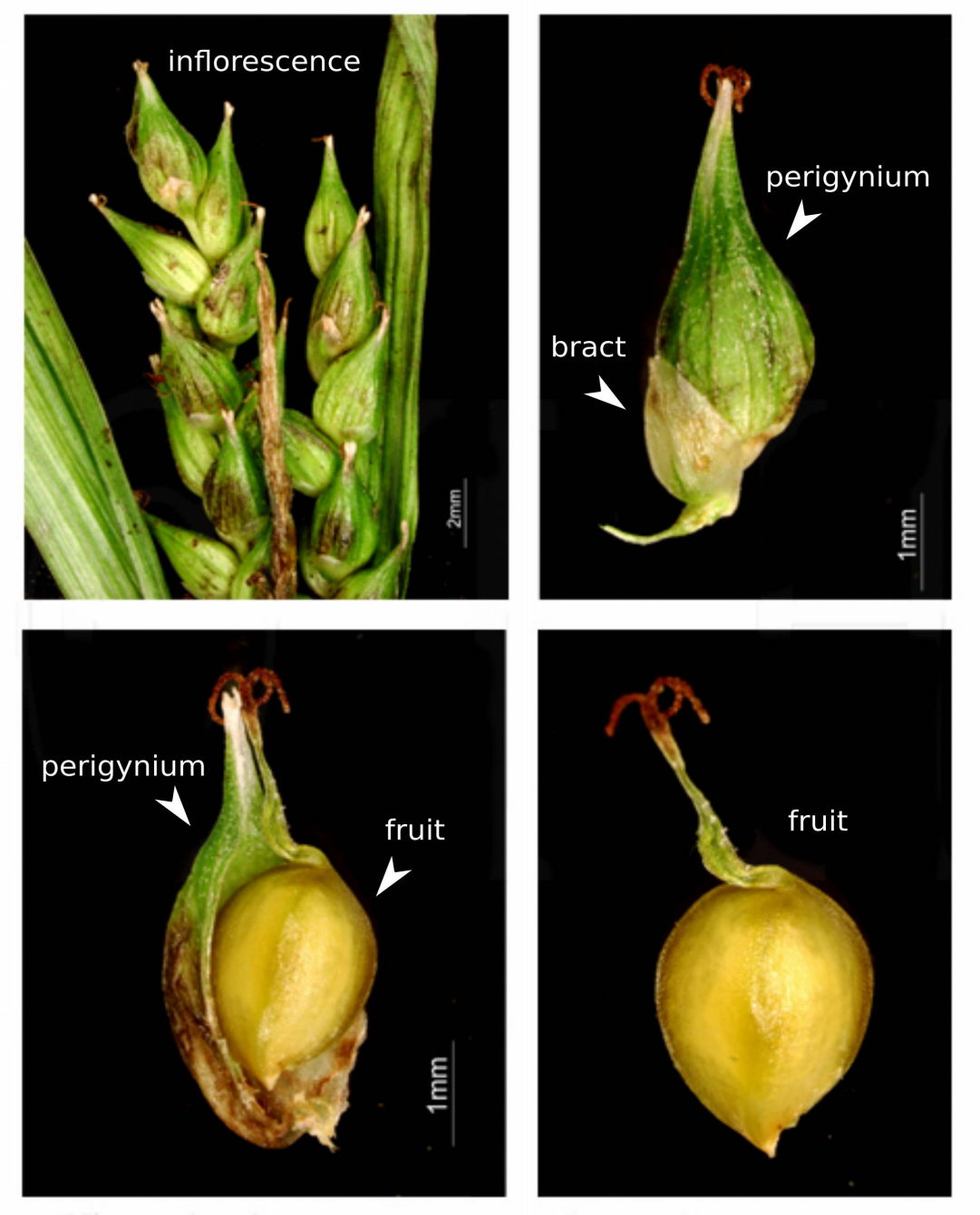


Figure 1.1. Inflorescence, bract, perigynium and fruit of *Carex ischnostachya* Steud. Slightly modified from a figure by Tomomi Masaki, licensed under CC BY-NC 2.0 CA.

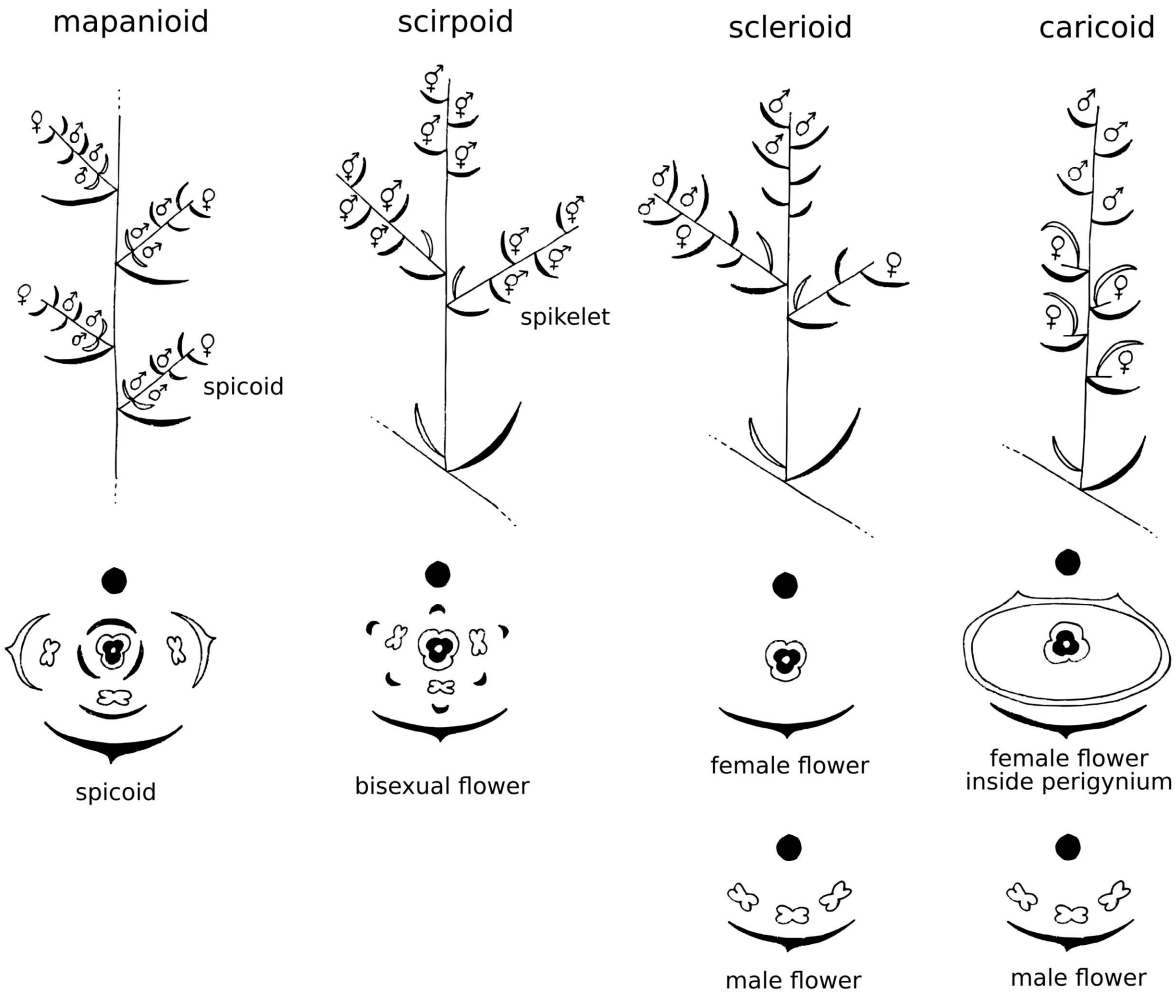


Figure 1.2. A few important inflorescence types seen in the Cyperaceae family. The mapanioid type, seen in subfamily Mapanioideae, features spikes of flower-like units called "spicoids". The scirpoid type, seen in many tribes of subfamily Cyperoideae, features spikelets of bisexual flowers with perianth. The sclerioid type, seen in tribe Sclerieae, features unisexual flowers in androgynous or unisexual spikelets with proximal sterile glumes. The caricoid type, seen in tribe Cariceae, features male flowers in terminal spikelets, and female flowers on short truncated branches, in the axil of a modified prophyll called perigynium. Past hypotheses have suggested evolution of the caricoid type from the mapanioid or sclerioid types, but phylogenetic studies instead suggest a direct derivation from the bisexual scirpoid type. Branch axes, normal bracts, glumes and perianth parts in black, prophylls, stamens and pistils in white.

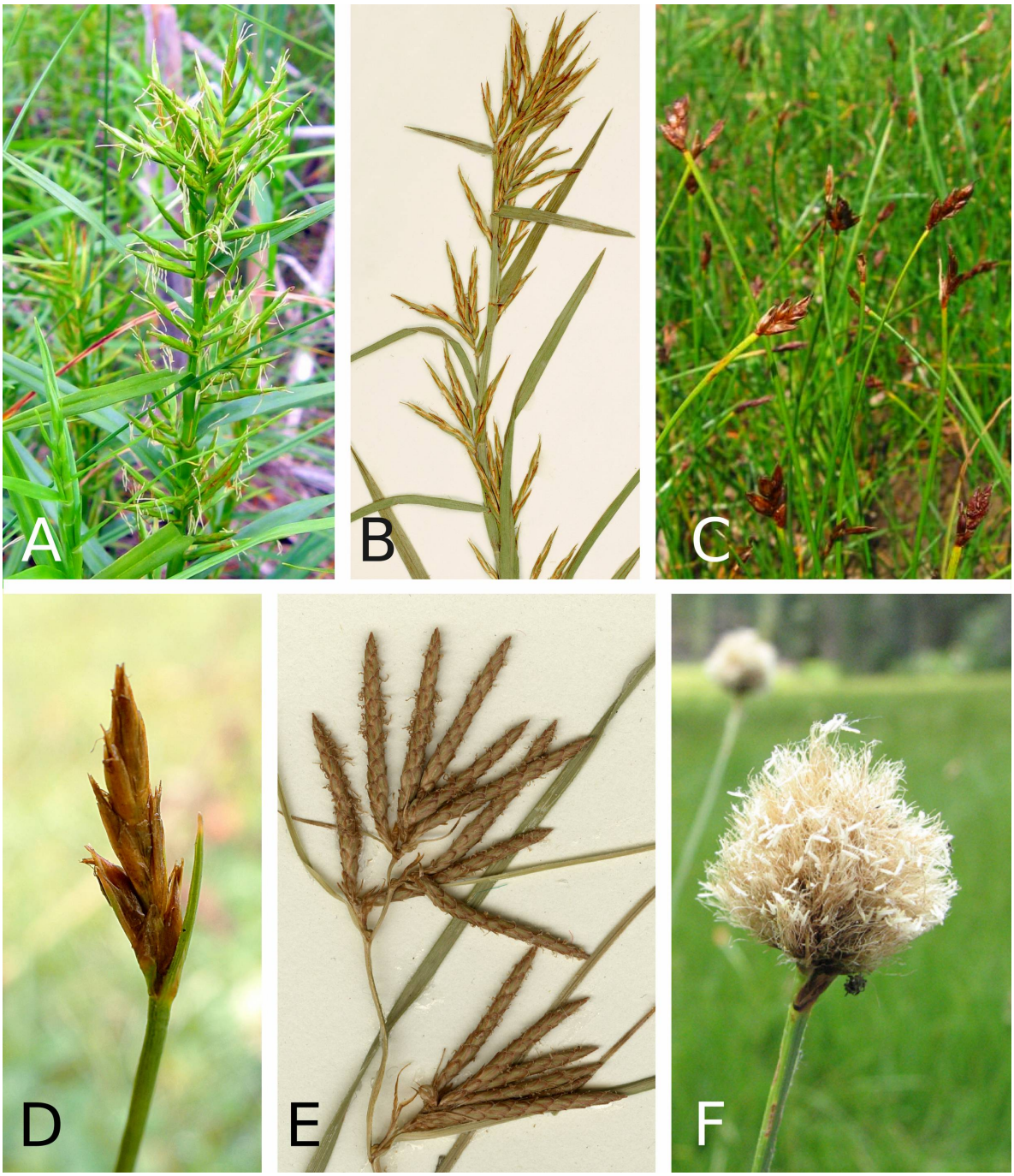


Figure 1.3. Genera of the CDS clade. Dulichieae s.str. A, B: *Dulichium arundincaeum*, Canada. C: *Blysmopsis rufa*, Canada. D: *Blysmus sinocompressus* var. *sinocompressus*, China. Incertae sedis (now Khaosokieae). E: *Khaosokia caricoides*, Thailand. Scirpeae s.lat. (now Calliscirpeae). F: *Calliscirpus brachythrix*, USA. Photographs: A, C: Marie-Ève Garon-Labrecque, B: Bruce A. Ford, D, E: Étienne Léveillé-Bourret, F: Julian R. Starr.

Figure 1.3 [continued]. Genera of the CDS clade. Scirpeae s.str. G: *Amphiscirpus nevadensis*, Canada. H: *Zameioscirpus atacamensis*, Argentina. I: *Phylloscirpus deserticola*, Bolivia. J: *Rhodoscirpus asper*, Chile. K: *Scirpus cyperinus*, Canada. Photographs: G: Julian R. Starr, H, I, J: Étienne Léveillé-Bourret, K: Daniel Fortin.

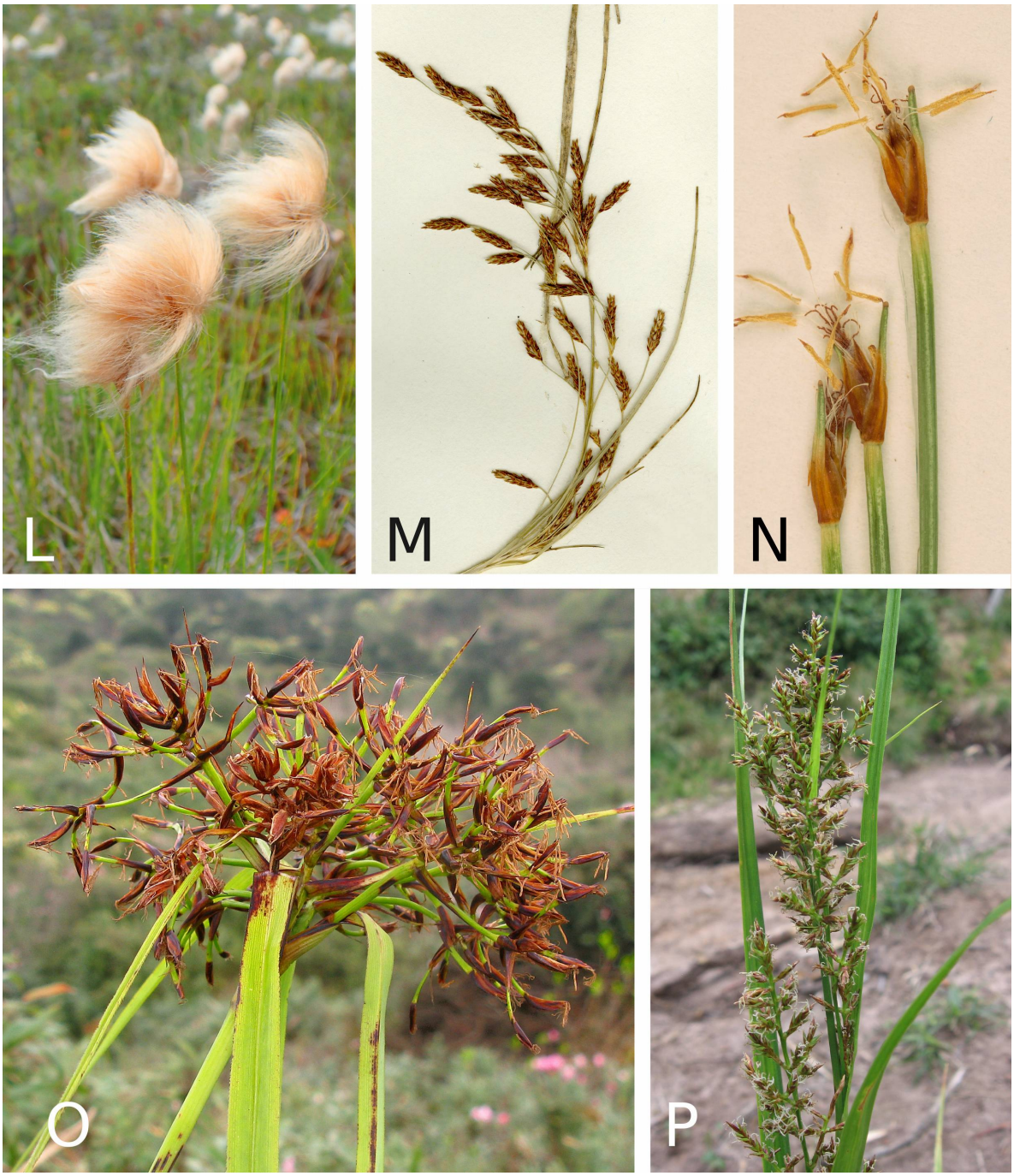


Figure 1.3 [continued]. Genera of the CDS clade. Scirpeae s.str. L: *Eriophorum* ×*medium* subsp. *medium*, Canada. Scirpeae s.lat. (now Trichophoreae). M: *Cypringlea evadens*, Mexico. N: *Trichophorum cespitosum*, Canada. Dulichieae s.lat. (now Sumatroscirpeae). O: *Sumatroscirpus rupestris*, Vietnam. Cariceae. P: *Carex* cf. *filicina*, Vietnam. Photographs: L: Marie-Ève Garon-Labrecque, M, P: Étienne Léveillé-Bourret, N: Bruce A. Ford, O: Julian R. Starr.

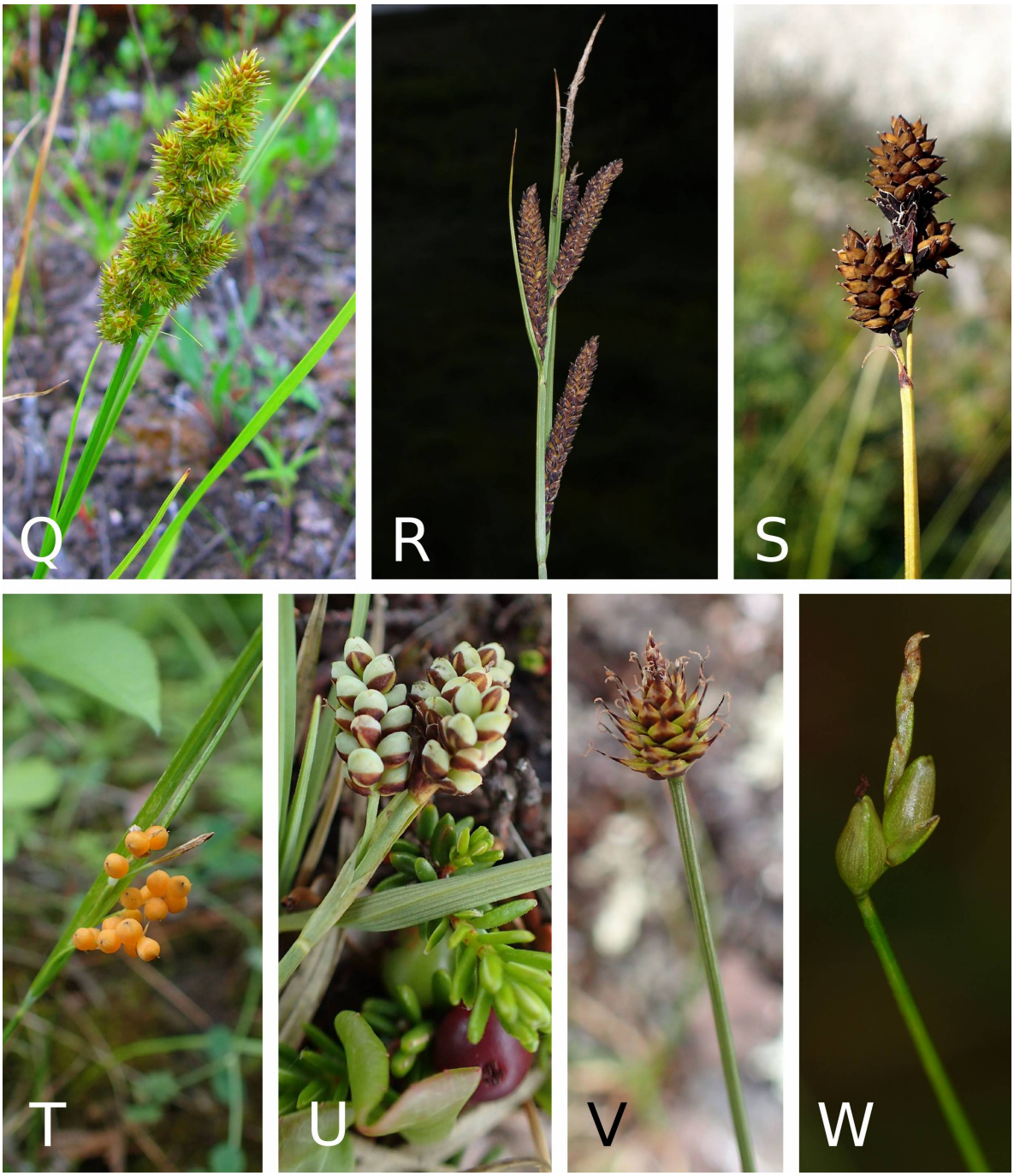


Figure 1.3 [continued]. Genera of the CDS clade. Cariceae. Q: *Carex vulpinoidea*, Canada. R: *Carex nigra*, Canada. S: *Carex norvegica*, Canada. T: *Carex aurea*, Canada. U: *Carex bicolor*, Canada. V: *Carex arctogena*, Canada. W: *Carex leptalea*, Canada. Photographs: Q, S–V: Marie-Ève Garon-Labrecque. R: Daniel Fortin. W: Étienne Léveillé-Bourret.

CHAPTER 2

SEARCHING FOR THE SISTER TO SEDGES (*CAREX*): RESOLVING RELATIONSHIPS WITHIN THE CARICEAE-DULICHIEAE-SCIRPEAE CLADE (CYPERACEAE)

This Chapter is a slightly modified version of an article published in the Botanical Journal of the Linnean Society (http://dx.doi.org/10.1111/boj.12193). Coauthors on the article are: Claire N. Gilmour, Julian R. Starr, Robert F. C. Naczi, Daniel Spalink, and Kenneth J. Sytsma.

2.1 Introduction

Molecular phylogenetic analyses have positioned Cariceae in a strongly supported clade with the genus *Khaosokia*, and tribes *Dulichieae* and *Scirpeae* (Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013). However, the relationships between Cariceae and the genera of tribes Dulichieae and Scirpeae are unresolved. Most notably, the sister group to Cariceae is unknown, relationships of Scirpeae genera are unstable and the position of Dulichieae and *Khaosokia* in the clade is ambiguous (Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013). Previous phylogenetic studies have included no more than 32% of the species of CDS excluding Cariceae in a single analysis (Muasya & al., 2009) and the markers used were either largely uninformative at this level of investigation (e.g. *rbcL*; Simpson & al., 2005; Muasya & al., 2009) or contained such high levels of variability that alignment was difficult (e.g. *trnL-F*, ITS; Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012).

As a first step towards the goal of providing a natural tribal classification of CDS and identifying the sister-group to *Carex*, sequence data was generated from two rapidly evolving plastid genes (*matK* and *ndhF*) and a greatly improved taxonomic sampling of CDS, covering all but one (*Sumatroscirpus* Oteng-Yeb.) of the currently recognised genera

and more than half of the diversity of the clade outside of Cariceae. The implications of the results on the phylogenetic position of *Khaosokia*, the taxonomy of Scirpeae, the monophyly of *Scirpus* and *Trichophorum*, and the quest for the sister-group to Cariceae, will be discussed.

2.2 Materials & Methods

2.2.1 Taxonomic Sampling and Markers

One hundred twelve individuals from 83 taxa were included in this study, covering all currently recognised genera of CDS (Govarts & Simpson, 2007) except for the monospecific *Sumatroscirpus* (Dulichieae; Oteng-Yeboah, 1974) (Appendix 1). Most of the 224 sequences are new, but 37 have already been published by Gilmour & al. (2013). This sampling covers 55% of all species and infraspecific taxa (ca. 114) recognised for this clade outside of Cariceae (Novoselova, 1995; Govaerts & al., 2007). Sampling within Cariceae aimed to represent all the major lineages currently known (Starr & Ford, 2009; Waterway, Hoshino & Masaki, 2009). Outgroup taxa were selected to represent major lineages of the Abildgaardieae-Cypereae-Eleocharideae-Fuireneae clade, which has been shown to be sister to CDS (Muasya & al., 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013). Taxonomy follows Govaerts & al. (2007) except for *Eriophorum*, which follows Novoselova's (1994a, 1994b) revision of the genus.

The plastid genes *matK* and *ndhF* were used because (1) they are easy to amplify even from relatively degraded tissue (herbarium specimens); (2) pilot studies suggested they would have an appropriate level of divergence for assessing tribal and generic level relationships within CDS; and (3) since they are coding sequences, alignment is almost always unambiguous.

2.2.2 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 *matK* and *ndhF* sequences are given in Gilmour *et al*. (2013). PCR amplifications consisted of 1× reaction Buffer (Sigma Aldrich), 2 mM $MgCl_2$ (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 µM of each primer, 1.0 µL Bovine Serum Albumin (BioShop, Canada), 4 U of Hot Start (HS) Taq DNA Polymerase (BioShop, Canada) and 1–3 µL of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. Amplification was done on an Eppendorf EPGradientS Mastercycler with 2 min of initial denaturation followed by 40 cycles of 30 s of 94°C denaturation, 60 s of 47°C primer annealing and 90 s (*matK*) or 120 s (*ndhF*) of 72°C DNA extension, with a final extension step of 8 min. Minor adjustments were made to the recipe or cycling conditions for problematic samples. Successful amplifications were purified using an Exonuclease I – Shrimp Alkaline Phosphatase protocol (MJS Biolynx Inc., Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit version 3.1 (Applied Biosystems; Foster City, CA, USA). 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 Sequencher 4.10 (Gene Codes Corporation, Ann Arbor, MI, USA) and all sequences were submitted to Genbank (Appendix 1).

2.2.3 Phylogenetic Analyses

Sequences of *matK* and *ndhF* were concatenated by individual and the matrix was aligned with the MUSCLE algorithm as implemented in Geneious 4.8.5 (Biomatters). Minor adjustments to the alignment were made by hand using parsimony as an objective criterion (as in Starr & al., 2004). Bases 81–113 (*matK*) were excluded for 13 individuals because of indels that made alignment ambiguous only in these individuals. Excluding this region for all individuals or including the region assuming 2 independent indel events gave

essentially the same parsimony bootstrap values (results not shown). Only results from combined analyses are reported since no well-supported (> 75%) topological incongruence was observed in independent gene analyses (results not shown), and an incongruence length-difference test (Farris & al., 1995) was insignificant ($p = 0.19$, $n = 1,999$). The alignment and all the most parsimonious trees found during searches are available online on TreeBASE (http://treebase.org/treebase-web/).

Heuristic parsimony searches were done in PAUP* 4.0 (Swofford, 2003) using 1,000 random addition sequence replicates, followed by swapping with tree-bisection-reconnection (TBR) and with the MULTREES and COLLAPSE options on. Owing to the length of the analysis and the large number of trees saved per replicate, a limit of 5,000 saved trees and a time limit of 4 minutes was imposed on each replicate. Additionally, 100 parsimony ratchet searches using a random addition sequence were done with TNT 1.1 (Goloboff & al., 2008). Ratchet searches used 2,500 unconstrained and 2,500 constrained iterations on unweighted, 5% upweighted and 5% downweighted matrices, with a maximum of 15 TBR swaps per iteration, and keeping all optimal trees found in each replicate. A strict consensus of all the most parsimonious trees was assembled in PAUP* using the best trees found in the standard and ratchet searches. Branch support was assessed using 10,000 bootstrap replicates in PAUP*, with the MULTREES option off (DeBry & Olmstead, 2000). To determine what would be the next best CDS topology to one that contains a Cariceae + Trichophorum Clade, a search with an inverse constraint was done in PAUP*.

Model-based searches were done using Bayesian Markov chain Monte Carlo (MCMC) in MrBayes 3.2.1 (Ronquist & al., 2012) on the CIPRES server 3.3 (Miller & al., 2010). Two partitions were enforced, the first included 1st and 2nd codon positions and the second included only 3rd codon positions for both genes. This partition scheme was selected in PartionFinder 1.0.1 (Lanfear & al., 2012) with a greedy search using Bayesian information criterion on all possible partition schemes and all implemented models. A GTR+Γ model was used for both partitions (with 6 categories discrete gamma

approximation). Topology and branch lengths were linked between the two partitions with all other model parameters unlinked and allowing for rate variation between partitions. The branch length prior was lowered to Unconstrained:Exponential(10) to decrease the probability of overestimating branch lengths (Marshall, 2010) and proposal parameters were adjusted to get acceptance rates between 10% and 50% (although it was not possible to attain 10% acceptance for the TBR proposals). Two independent chains were run for 20 million generations. Each run was made with one cold and seven heated chains with a temperature parameter of 0.08 to get swap frequencies of 30% to 50% between adjacent chains. Convergence of model parameters was checked with Tracer 1.5.0 (Drummond & al., 2012). Topological convergence was assessed using the average standard deviation of split frequencies reported by MrBayes and by visualising tree samples with multidimensional scaling in TreeSetVis 3.0 (Hillis & al., 2005), a module of Mesquite 2.75 (Maddison & Maddison, 2011).

Parsimony bootstrap support values (BS) were added to the strict consensus with SumTrees 3.3.1 (Sukumaran & Holder, 2010) and posterior probabilities with TreeAnnotator 1.7.5 (Drummond & al., 2012). Unambiguous changes along the branches of the strict consensus were calculated with WinClada 1.00.08 (Nixon, 2002). In the presence of polytomies, the unambiguous changes for the branches of the polytomy were calculated on the corresponding branches of a randomly chosen tree from the parsimony search. Tree figures were produced with TreeGraph 2.0.47 (Stöver & Müller, 2010). 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.

2.2.4 Topological Tests

In order to determine whether the data could exclude important monophyly or sister group hypotheses presented in previous taxonomic or phylogenetic studies, constraint trees were used to find the optimal tree(s) under these alternative hypotheses. A Shimodaira-

Hasegawa (SH) test (Shimodaira & Hasegawa, 1999; Goldman & al., 2000) using the criterion of parsimony was implemented as in Near & al. (2003) to be consistent with our use of the parsimony criterion for tree selection during searches. Likelihood-based SH tests gave similar results (not shown). Each constraint tree was used in a parsimony search in PAUP* using 500 random sequence replicates followed by TBR, with MULTREES off and a maximum of 10 saved trees per replicate. The minimal length of each constrained search (l_x) was compared with the length of the unconstrained search (l_{best}) by computing the length difference ($d_x = l_x - l_{best}$). One thousand bootstrap replicates of the whole matrix were produced with Mesquite. Each bootstrap replicate (i) was used to calculate the parsimony score of a randomly chosen tree from the unconstrained search ($l^{(i)}_{best}$) and a randomly chosen tree from each constrained searches ($l^{(i)}_x$) in PAUP. The mean length of each tree across all replicates ($m_x = n^{-1} \Sigma\, l^{(i)}_x$) was subtracted to the score of all individual bootstrap replicates of the tree for centering ($l'^{(i)}_x = l^{(i)}_x - m_x$). The difference between each centered length and the minimum centered length of each replicate gives a distribution of tree length differences ($d^{(i)}_x$) for each topology. This distribution was used to compute the one-tailed p-values for each length difference (d_x) between constrained and unconstrained trees. Significance was assessed at the $\alpha = 0.05$ level.

2.3 Results

Alignment and character statistics are shown in Table 2.1. Standard parsimony TBR searches found 699 415 trees of 1822 steps (CI = 0.61, RI = 0.84) in 64.5 h. The ratchet searches found 226 trees of the same length in less than 10 h, 223 of which were not found by the standard searches. Despite this, the strict consensus of all the trees, and of separate standard and ratchet analyses produced the same topology (Figs. 2.1, 2.2). Results of the parsimony bootstrap searches are shown on the strict consensus (Figs. 2.1, 2.2). The Bayesian MCMC chains quickly stabilized in model parameters and topology. The first

500,000 generations (2.5%) of each chain were discarded as burn-in, and the remaining 19,500 trees from both chains were used to compute the posterior probabilities of clades (Figs. 2.1, 2.2).

Analyses position the strongly supported tribe Dulichieae (100% BS) and the genus *Khaosokia* as successive sisters (79% and 85% BS) to a strongly supported (85% BS) clade consisting of five major lineages (*Calliscirpus*, *Trichophorum* + *Oreobolopsis* + *Cypringlea* or "Trichophorum Clade", *Scirpus* + *Eriophorum* or "Scirpus Clade", and *Amphiscirpus* + *Phylloscirpus* + *Zameioscirpus* or "Zameioscirpus Clade"), the first four of which receive moderate to strong support (>80% BS; Figs. 2.1, 2.2). Within this clade, *Calliscirpus* (100% BS) is poorly supported as sister to a monophyletic group (42% BS) composed of a Trichophorum Clade + Cariceae (63% BS) and a Zameioscirpus Clade + Scirpus Clade (78% BS). Scirpeae is paraphyletic with respect to Cariceae in the strict consensus, but the monophyly of Scirpeae cannot be rejected by the SH test (Table 2.2). Furthermore, the SH test cannot reject any of the major lineages as a possible sister to Cariceae, except for Dulichieae (Table 2.2). Parsimony searches using an inverse constraint on the Cariceae + Trichophorum Clade found trees 1 step longer than the best topology, with the only major difference being the position of the Trichophorum Clade as sister to a *Calliscirpus* + Cariceae clade (not shown). Additionally, around 79% of bootstrap replicates that did not find the Trichophorum Clade sister to Cariceae instead found *Calliscirpus* as sister to Cariceae. More than 90% of the bootstrap replicates thus had either *Calliscirpus* or the Trichophorum Clade as sister to Cariceae.

Within Dulichieae, *Blysmus* forms a weakly supported monophyletic group sister to *Dulichium*, with *Blysmus rufus* sister to a strongly supported *B*. *compressus* + *B*. *sinocompressus* clade (Fig. 2.1). Within the Trichophorum Clade, a weakly supported *Trichophorum alpinum* + *T*. *subcapitatum* clade is sister to all the other species of *Cypringlea*, *Oreobolopsis* and *Trichophorum*. The relationships within the Trichophorum Clade are not further resolved in the strict consensus. Inside Cariceae, *Carex siderosticta* Hance (*Siderostictae* clade) is strongly supported (100% BS) as sister to four strongly

supported (>95% BS) major clades: (1) Core Care; (2) *Vignea* clade; (3) *Schoenoxiphium* clade, and (4) a Core Unispicate clade (Fig. 2.1). The Core Carex clade is sister to the *Vignea* clade, and the *Schoenoxiphium* clade is sister to the Core Unispicate clade, but both relationships are weakly supported (< 50% BS; Fig. 2.1). Relationships among the genera of the Zameioscirpus Clade are unresolved. *Scirpus divaricatus* Elliott is sister to all other Scirpus Clade species (95% BS), with *S. pendulus* Muhl. sister (47% BS) to a polytomy involving *Scirpus maximowiczii* C.B.Clarke, *S. radicans* Schkuhr, and four major clades: (1) a moderately supported (76% BS) *S. expansus* Fernald + *S. microcarpus* J.Presl & C.Presl clade; (2) a well-supported (89% BS) clade composed of *S. wichurae* Boeckeler–*S. pedicellatus* Fernald; (3) a moderately supported (79% BS) clade composed of *S. ancistrochaetus* Schuyler–*S. hattorianus* Makino, and (4) a well-supported (88% BS) monophyletic *Eriophorum*. Within *Eriophorum*, a strongly supported (98% BS) *Eriophorum virginicum* L.–*E. gracile* Koch in A.W.Roth clade is sister to all other sampled species of *Eriophorum*. Within the bulk of *Eriophorum*, a weakly supported (55% BS) *E. russeolum* Fr. ex Hartm.–*E. brachyantherum* Trautv. & C.A.Mey. clade consists of all sampled unispicate species of the genus (the Unispicate *Eriophorum* clade; Fig. 2.2).

2.4 Discussion

2.4.1 Major Clade Relationships Within CDS

The enhanced taxonomic sampling of this study mostly confirms the relationships inferred by Gilmour & al. (2013), except for the sister position of *Calliscirpus*, which is weakly supported as sister to Cariceae in Gilmour & al. (2013), but is here weakly positioned (42% BS) as sister to a clade comprising Cariceae and other Scirpeae. The results are also comparable to previous phylogenetic studies in that the five major clades that received moderate to strong support in our analyses were also present in other studies that included representatives of those clades (Dhooge, 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al. 2013; Hinchliff & Roalson, 2013, but note *Zameioscirpus*). Although in general the backbone of our tree is weakly supported, the position of

Dulichieae as sister to Cariceae + Scirpeae and the sister relationship of the Zameioscirpus Clade and Scirpus Clade are both congruent with previous molecular phylogenetic analyses, but they receive better parsimony bootstrap support with our dataset (Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013). Our improved taxonomic sampling also gives us better insight into the relationships of *Scirpus* and *Eriophorum*, and of *Trichophorum* and its allied genera.

The strongly supported monophyly of Dulichieae in our plastid dataset (Fig. 2.1) is consistent with previous studies (Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013) and supports the distichous arrangement of spikelets and the fertile prophylls as two good morphological synapomorphies for the tribe (Goetghebeur, 1998). The position of Dulichieae as sister to Cariceae and Scirpeae is also well supported and congruent with previous results (Dhooge, 2005; Gilmour & al., 2013; Hinchliff & Roalson, 2013), but the position of *Khaosokia* in relation to these tribes is not clear.

Previous studies have found *Khaosokia* either as sister to the remainder of CDS (Muasya & al., 2009; Jung & Choi, 2012) or in a polytomy with Cariceae and Scirpeae (Hinchliff & Roalson, 2013). Using the same chloroplast markers as this study, Gilmour & al. (2013) found a strongly supported *Khaosokia* + Cariceae + Scirpeae clade. Our significantly increased taxonomic sampling appears to reduce the support for this relationship, highlighting the fact that the interpretation of phylogenetic results and their taxonomic significance must take limited sampling into account (Hedtke & al., 2006). Like *Dulichium arundinaceum* (L.) Britton, *Khaosokia caricoides* D.A.Simpson et al. has more than six bristles per flower (Simpson & al., 2005) and long spikelets in an elongate raceme of spikes. Although *matK* and *ndhF* sequences alone cannot exclude the possibility of a sister relationship between *Khaosokia* and Dulichieae (Table 2.2), including *Khaosokia* in this tribe would have to allow for *Khaosokia*'s antrorsely scabrous perianth bristles, spirally inserted spikelets, and sterile prophylls (Simpson & al., 2005). This would make Dulichieae morphologically heterogeneous as currently defined. Constraining Scirpeae to be monophyletic with *Khaosokia* included resulted in a tree 10 steps longer than the most

parsimonious trees, but SH tests could not reject this topology (Table 2.2). Our current data therefore suggest that *Khaosokia* could be treated either in Scirpeae or as a separate tribe, although only additional data will resolve this problem.

The tribe Scirpeae is characterised by what appear to be morphological plesiomorphies, and it is often considered a dumping ground for genera that do not fit easily in other Cyperoideae tribes (Goetghebeur, 1998). This is clearly reflected by the continuing trend of gradually segregating *Scirpus* species in other genera (*Calliscirpus*, *Fuirena* Rottb., *Isolepis* R.Br., *Schoenoplectus* (Rchb.) Palla, *Trichophorum*) and transferring traditional Scirpeae genera to other distantly related tribes (e.g. Cypereae, Eleocharideae, Fuireneae; Koyama, 1958; Schultze-Motel, 1971; Goetghebeur, 1986; Gilmour & al., 2013). It therefore comes as no surprise that Scirpeae is paraphyletic in our strict consensus tree (Fig. 2.1), although SH tests could not reject the possibility of a monophyletic Scirpeae including or excluding *Khaosokia* (Table 2.2).

Scirpeae forms three groups that appear natural based on morphological and embryological characters. The Trichophorum Clade is strongly supported and contains all Scirpeae genera that possess a Carex-type embryo except *Calliscirpus*: *Trichophorum*, the closely allied *Oreobolopsis*, and *Cypringlea* (Fig. 2.1) (Dhooge, 2005). This clade has also been found in most previous studies (Dhooge, 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013) and only conflicts in a minor way with the supertree approach of Hinchliff & Roalson (2013; see discussion on *Zameioscirpus* below). Despite being consistently monophyletic in other studies, the position of the Trichophorum Clade has varied from sister to the remainder of Scirpeae + Dulichieae (Muasya & al., 2009), sister to Dulichieae or the Scirpus Clade + Cariceae (Jung & Choi, 2012), sister to a Scirpus Clade + Zameioscirpus Clade (Hinchliff & Roalson, 2013), to sister to a *Calliscirpus* + Cariceae clade (Gilmour & al., 2013). It therefore appears that with the addition of our results, the Trichophorum Clade has been associated with almost all other major lineages of the CDS.

The Zameioscirpus Clade is an almost entirely South American group consisting of species with capitate to unispicate inflorescences of sessile spikelets, Schoenus-type embryos, and distally ascending rhizomes (Oteng-Yeboah, 1974; Dhooge & al., 2003; Dhooge & Goetghebeur, 2004; Dhooge, 2005). *Amphiscirpus* has been treated as synonymous with *Phylloscirpus* on the basis of their minutely alveolate fruit epidermis and Schoenus-type embryo (Goetghebeur, 1986). However, *Phylloscirpus* and *Zameioscirpus* share a series of characters such as a loosely tufted habit (colonial in *Amphiscirpus*), short arched rhizomes (long mostly horizontal rhizomes in *Amphiscirpus*), spreading leaves in a basal rosette (stiffly erect in *Amphiscirpus*), and clearly terminal inflorescences (pseudo-lateral in *Amphiscirpus*) that suggest *Phylloscirpus* is not only distinct from *Amphiscirpus* (Goetghebeur & Simpson, 1991; Dhooge, 2005), but may be closer to *Zameioscirpus*. Unfortunately, the current analysis does not resolve the position of *Amphiscirpus* due to a lack of statistical support for the Zameioscirpus Clade and poor resolution within this group. The Zameioscirpus Clade had already been found on some occasions (Dhooge, 2005; Gilmour & al., 2013), but the position of *Amphiscirpus* is highly unstable, having been recovered as sister to the Scirpus Clade (Dhooge, 2005), or in a polytomy with the Scirpus Clade and the Zameioscirpus Clade p.p. (Muasya & al., 2009). The genus *Zameioscirpus* has also been found within the Trichophorum Clade with the supermatrix approach of Hinchliff and Roalson (2013), but this position conflicts with our analysis, embryological data, and all previous analyses (Dhooge, 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013). This incongruity might be due to the fact that the only locus sequenced across most members of the Zameioscirpus Clade and Trichophorum Clades was the largely uninformative *rbcL*, suggesting that this topology may be due to noise rather than phylogenetic signal.

The Scirpus Clade contains the type genus of Scirpeae and it is characterized by often pedicellate spikelets in open anthelae, leafy culms with long internodes, and Fimbristylis-type embryos (Schuyler, 1967; Ball & Wujek, 2002; Van der Veken, 1965). It has consistently been seen in previous studies, although sampling within or support for the clade was generally poor (Dhooge, 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour

& al., 2013; Hinchliff & Roalson, 2013). Our analyses place it as sister to the Zameioscirpus Clade, a relationship that has been found in most previous molecular phylogenetic studies (Dhooge, 2005; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013), although it has never received more than about 65% parsimony bootstrap support. Although molecular support is weak, these two clades are united by morphologically similar embryos of the Schoenus-type and Fimbristylis-type, with the root cap (sub-)laterally displaced (Van der Veken, 1965; Goetghebeur, 1986). On the contrary, species of *Calliscirpus*, Cariceae, Dulichieae and the Trichophorum Clade possess embryos of the Carex-type (Van der Veken, 1965; Goetghebeur, 1986; Gilmour & al., 2013), a state that is probably ancestral for CDS, if not for the Cyperaceae as a whole (Goetghebeur, 1986).

Cariceae is nested within a paraphyletic Scirpeae and sister to the Trichophorum Clade in the strict consensus (Fig. 2.1). This relationship is novel, with previous authors having variably found Cariceae to be sister to a monophyletic Scirpeae (Muasya & al., 2009), to a Scirpus Clade + Zameioscirpus Clade (Jung & Choi, 2012) or to *Calliscirpus* (Gilmour & al., 2013). An association between Cariceae and *Trichophorum* was originally proposed by Kukkonen and Timonen (1979) based on the infection of *Trichophorum cespitosum* (L.) Hartm. by a species of *Anthracoidea* Brefeld, a genus of smut fungi once thought to be an exclusive parasite on Cariceae, but now known to infect several distantly related sedge genera such as *Carpha* Banks & Sol. ex R.Br., *Fuirena* and *Schoenus* L. (Fuireneae and Schoeneae; Vánky, 2002). A sister relationship of the Trichophorum Clade and Cariceae would be interesting, since the Trichophorum Clade contains mostly unispicate species (Crins, 2002; Liang & Tucker, 2010c), whereas Starr & Ford (2009) have found that multispicate inflorescences are probably ancestral to Cariceae. However, the sister relationship of the Trichophorum Clade and Cariceae receives low support and only Dulichieae could be excluded as sister to Cariceae in the topological test (Table 2.2). Based on our parsimony bootstrap results and constrained searches, the most probable sister group to Cariceae appears to either be *Calliscirpus* or the Trichophorum Clade.

2.4.2 Dulichieae

Within Dulichieae, *Blysmus rufus* is weakly supported as sister to the rest of the genus. Its branch is very long (26 unambiguous changes) as is the branch leading to *Blysmus compressus* and *B. sinocompressus* (11 unambiguous changes), and yet, as a genus, *Blysmus* is supported by only two unambiguous changes. On account of its channeled, subterete leaves (flat in other *Blysmus* spp.), obscure antrorse barbs on whitish caducous bristles (retrorsely barbed, yellowish persistent bristles in other *Blysmus* spp.), its smooth anther crest (scabrous in other *Blysmus* spp.) as well as anatomical differences, such as the absence of adaxial bulliform cells in the leaf (present in other *Blysmus* spp.) and the presence of large air spaces in the stem (absent in other *Blysmus* spp.), Oteng-Yeboah (1974) erected the monospecific genus *Blysmopsis* Oteng-Yeb. The high molecular divergence between *Blysmus rufus* and its congeners would appear to support the recognition of *Blysmopsis*. Our analysis also appears to support the recognition of *Blysmus sinocompressus*, a species recently segregated from *B. compressus* mostly based on perianth bristle and anther length (Liang & Tucker, 2010a).

2.4.3 The Trichophorum Clade

Until the inclusion of *Cypringlea* M.T.Strong (Gilmour & al., 2013), with its simple or compound anthelae and well developed leaves (Strong, 2003; Reznicek & al., 2008), the Trichophorum Clade could be characterized by spikelets solitary or in paucispicate racemes and by the frequent reduction of leaves to mucronate sheaths (Beetle, 1946; Crins, 2002; Dhooge & Goetghebeur, 2002). *Cypringlea* M.T.Strong was segregated from *Scirpus* largely on the basis of its Carex-type embryo and aligned with *Trichophorum* for this very reason (Strong, 2003). It can be further linked to *Trichophorum* by its mostly basal leaves and patent bristle barbs (Strong, 2003), whereas the leaves are cauline and the barbs most often retrorse in *Scirpus* (Schuyler, 1967). Despite its distinct morphology within the Trichophorum Clade, it is not clear whether *Cypringlea* is nested within *Trichophorum* or if both genera are reciprocally monophyletic (Fig. 2.1). Although the position of *Cypringlea* within a clade of species with reduced vegetative and reproductive features may seem

incongruent, close relationships among multispicate, paucispicate and unispicate species are seen in all the major CDS clades (save *Calliscirpus*, 2 spp.), suggesting that reduction and proliferation are common throughout CDS. The genus *Oreobolopsis* differs from *Trichophorum* mostly on the basis of its tepaloid perianth, which is bristle-like or absent in *Trichophorum* (Koyama & Guaglione, 1987; Dhooge & Goetghebeur, 2002). However, the taxonomic value of this perianth character is not clear, since some *Oreobolopsis* species appear to be most closely related to *Trichophorum* species (e.g. *Oreobolopsis tepalifera* T.Koyama & Guagl. and *Trichophorum rigidum* (Steud.) Goetgh., Muasya & D. A. Simpson; see Dhooge & Goetghebeur, 2002). A tepaloid perianth has also been observed in a specimen of *Trichophorum subcapitatum* (Thwaites & Hook.) D.A.Simpson, a species that normally possesses long bristles (noted by T. Koyama and confirmed by ÉLB on a 1972 collection from China by Shiu Ying Hu, no. 11812, MICH). Pending more studies, *Oreobolopsis* may need to be synonymized with *Trichophorum* as previously noted by Dhooge (2005).

2.4.4 The Scirpus Clade

Within the Scirpus Clade, *Scirpus divaricatus* Elliott is sister to all other sampled species and is unique due to features such as spikelets in open terminal anthelae, glumes with broad green midribs and concavely trigonous nutlets (Schuyler, 1967). It also has the lowest chromosome number known for this clade (n = 14; Schuyler, 1967). Although the backbone relationships within *Scirpus* are largely unresolved, there is a series of clades that appears natural on the basis of morphological and molecular characters. Within the bulk of *Scirpus* Tourn. ex L., a moderately supported *Scirpus expansus–S. microcarpus* clade is characterized by culms growing singly from rhizomes, red-colored base of leaf sheaths, spikelets in dense glomerules and short stout bristles with sharp retrorse barbs (Schuyler, 1967). Two representatives of this clade were also monophyletic in the study of Jung & Choi (2012), and Hinchliff & Roalson (2013) found weak support for a monophyletic *Scirpus microcarpus* + *S. sylvaticus* L. clade, but *S. expansus* was in an unresolved *Scirpus* polytomy. The well-supported *Scirpus wichurae –S. pedicellatus* clade is characterized by

caespitose growth (except for *Scirpus longii* Fernald) and by bristles that are smooth or antrorsely barbed at the tip and many times longer than the glumes (Koyama, 1958; Schuyler, 1967; Liang & Tucker, 2010b). Representatives of this clade have appeared as a monophyletic group in several previous analyses (Muasya & al., 2009; Jung & Choi, 2012); however, in the analysis Hinchliff & Roalson (2013), they form separate East Asian and North American clades that are part of a large *Scirpus* polytomy. *Scirpus pendulus* Muhl. appears transitional in its possession of glumes with conspicuous green midribs like *S. divaricatus*, but with smooth and contorted bristles as in the *S. wichurae–S. pedicellatus* clade, whilst its bristle length is intermediate between the two (Schuyler, 1967; Whittemore & Schuyler, 2002). This morphological situation is congruent with its phylogenetic placement in our analyses (Fig. 2.2). A moderately supported *Scirpus ancistrochaetus* Schuyler–*S. hattorianus* Makino clade is morphologically characterised by a caespitose habit, spikelets in dense glomerules and retrorse barbs (Schuyler, 1963). This clade is also supported by a 12 bp deletion in *matK* (see matrix in TreeBase), and it is monophyletic but weakly supported in all previous analyses incorporating representatives of the clade (Muasya & al.,, 2009; Hinchliff & Roalson, 2013). Within it, a strongly supported *Scirpus flaccidifolius* (Fernald) Schuyler–*S. hattorianus* subclade can be characterized by weak and blunt bristle barbs (Schuyler, 1963). Finally, the genus *Eriophorum* forms a well-supported clade within the Scirpus Clade that is natural based on inflorescences reduced to a simple anthela or a single spikelet, very large spikelets and a high number (> 10) of long, smooth and contorted perianth bristles (Koyama, 1958) that emerge from a ring primordium (Mora-Osejo, 1987; Vrijdaghs & al., 2005). The genus has also been found to be monophyletic with weak support in Jung & Choi (2012) and strong support in Hinchliff & Roalson (2013) and Gilmour & al. (2013), although sampling in all these studies was limited to no more than four species.

Inside *Eriophorum*, the resolution is surprisingly good with many morphologically recognisable clades. The *Eriophorum virginicum* L.–*E. gracile* Koch in A.W.Roth clade is sister to the rest of *Eriophorum* and it can be morphologically characterised by obtuse glumes with many prominent nerves and a rhizomatous habit (Novoselova, 1994a, 1994b;

Ball & Wujek, 2002). The bulk of *Eriophorum* comprises both rhizomatous and caespitose taxa, and both multispicate and/or unispicate species, with all of them possessing a single prominent midnerve on glumes (Novoselova, 1994a, 1994b; Ball & Wujek, 2002). Within a weakly supported Unispicate clade (Fig. 2.2), the *Eriophorum russeolum* Fr. ex Hartm.–*E. scheuchzeri* Hoppe complex is strongly supported as monophyletic and characterized by a rhizomatous habit, 1–7 sterile proximal glumes and by glumes with well-defined hyaline margins (Novoselova, 1994a, 1994b). The sister clade to this complex comprises species with a caespitose habit and more than 12 sterile glumes that generally lack clear hyaline margins (Novoselova, 1994a, 1994b). Overall, there appears to be a reductive trend in inflorescence complexity within the Scirpus Clade, with *Scirpus* species possessing compound anthelae, followed by a reduction to a simple anthela in multispicate *Eriophorum* and to a solitary terminal spikelet in the nested unispicate *Eriophorum* clade. Another trend is that of ascending chromosome counts, with *Scirpus divaricatus* having $n = 14$, *S. pendulus* Muhl. $n = 20$, the bulk of *Scirpus* Tourn. ex L. $n = 25–34$, and *Eriophorum* L. $n = 29–30$ meiotic units (Schuyler, 1963; Ball & Wujek, 2002). Such a trend appears to further support the strict consensus tree, although a more resolved topology would be necessary to objectively study chromosome evolution.

2.4.5 Eriophorum Nested Within Scirpus

Our analysis supports the position of *Eriophorum* as nested within a grade of *Scirpus* species in the Scirpus Clade. The evolution of *Eriophorum* from within *Scirpus* has already been hypothesised, mostly on the basis of transitional species like *Scirpus maximowczii* (Koyama, 1958; Gilmour & al., 2013). Nonetheless, only Koyama (1958) has gone so far as to include all *Eriophorum* species within *Scirpus*, although he also included many other species now known to belong to very distant lineages (e.g. *Fuirena*, *Isolepis*, *Schoenoplectus*; Muasya & al., 2009). Previous molecular phylogenetic analyses have not been able to discriminate between the possibility of reciprocally monophyletic genera or a nested *Eriophorum* (Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013) due to insufficient taxonomic sampling and low branch

support. Our analysis, which includes over half of *Eriophorum* and over 40% of *Scirpus* species, uncovers two levels of branching before the *Scirpus* + *Eriophorum* polytomy, making *Scirpus* clearly paraphyletic (Fig. 2.2). Assuming our relationships hold, Koyama's lumping of *Eriophorum* within *Scirpus* will be necessary if paraphyletic genera are to be avoided. However, the SH test could not reject the hypothesis of a monophyletic *Scirpus* (Table 2.2), indicating that more data are needed before taxonomic changes should be considered.

2.4.6 Cariceae

All five major clades previously discovered in Cariceae wide analyses (Starr & Ford, 2009; Waterway & al., 2009) are also present in our tree, but our analysis generally differs in the level of clade support. Unlike previous analyses, all clades receive strong bootstrap support including the *Schoenoxiphium* and Core Unispicate clades which have never received strong support in the same analysis (Waterway & Starr, 2007; Starr & Ford, 2009; Gehrke & al., 2010) with the exception of Hinchliff & Roalson (2013) who used a 23 loci supermatrix of 16,016 aligned base pairs. Comparable support was achieved with just *matK* and *ndhF*, highlighting the phylogenetic utility of these two genes for exploring relationships above and below the tribal level.

Among the major CDS clades, Cariceae has by far the greatest number of unambiguous molecular synapomorphies, with 11 more changes than are seen in the next longest branch to a major clade (Cariceae 28 vs. Dulichieae 17; Fig. 2.1). This long branch parallels the morphological distinctiveness of Cariceae, whose inflorescence morphology is so derived that it is difficult to determine the homology of its unusual structures with other Cyperaceae. This is also confounded by the fact that some of the most important Cariceae characters appear to have been independently derived in other lineages; for example, fertile prophylls are also found in Dulichieae and unisexual flowers in *Khaosokia* and tribes Cryptangiae, Trilepidae, Sclerieae and Bisboeckelereae (Simpson & al., 2005; Goetghebeur, 1998). The origins and homologies of other highly derived groups like the Podostemaceae (Gustafsson & al., 2002), Ceratophyllaceae (Les, 1988; Soltis & al., 2011)

and aquatic genera like *Callitriche* L. and *Hippuris* L. (Olmstead & Reeves, 1995) have been equally difficult to resolve on the basis of morphology, but their relationships are now being successfully addressed using plastid markers. However, the origin of Cariceae may prove harder to determine, given the fact that *matK* and *ndhF* are among the fastest evolving genes in the angiosperm plastome (Moore & al., 2010; Liu & al., 2012), and yet the backbone of our tree consists of short and very poorly supported branches. The use of more rapidly evolving non-coding regions (e.g. ITS, *trnL-F*) is problematic at this level largely due to numerous insertion-deletion events amongst taxa, which can make alignment (= homology assessment) ambiguous. This may explain the high levels of homoplasy seen in some studies employing such markers at this taxonomic level and above (e.g. CI = 0.34, Starr & Ford 2009; CI = 0.27, Jung & Choi, 2012). This suggests that further investigations on the origins of Cariceae should focus on the development of new, rapidly evolving coding markers in order to avoid alignment ambiguities.

2.5 Tables and Figures

Table 2.1. Sequence statistics for the separate and combined *matK* and *ndhF* data sets used in phylogenetic analyses. The sequence length range includes incompletely sequenced taxa.

	matK	***ndhF***	**Combined**
Sequence length range (bp)			
Ingroup only	697–1 295	656–1 209	1 828–2 498
Ingroup and outgroup	697–1 295	656–1 209	1 828–2 498
Aligned length			
Ingroup only	1 324	1 229	2 553
Ingroup and outgroup	1 330	1 229	2 559
Number of indels			
Ingroup only	6	6	12
Ingroup and outgroup	7	9	16
Gaps and missing data (%)			
Ingroup only	2.6	2.7	3.8
Ingroup and outgroup	3.1	2.7	3.9
GC content (%)			
Ingroup only	28.8	29.1	27.9
Ingroup and outgroup	28.2	29.0	29.0
Variable sites			
Ingroup only	362 (27.3%)	319 (26.0%)	681 (26.6%)
Ingroup and outgroup	484 (36.4%)	402 (32.7%)	886 (34.6%)
Potentially informative sites			
Ingroup only	218 (16.5%)	208 (16.9%)	426 (16.6%)
Ingroup and outgroup	304 (22.8%)	273 (22.2%)	557 (21.8%)

Table 2.2. Parsimony based Shimodaira-Hasegawa test results for different topological hypotheses. Legend: * significant at α = 0.05.

Topology	**Length**	**Length difference**	**Parsimony p-value**
Best tree (Figs. 2.1, 2.2)	**1822**	**best**	
Scirpeae monophyletic (excluding *Khaosokia*)	1826	4	0.520
Scirpeae monophyletic (including *Khaosokia*)	1832	10	0.085
Scirpus monophyletic	1829	7	0.171
Cariceae and *Calliscirpus* monophyletic	1823	1	0.866
Cariceae and Dulichieae monophyletic	**1836**	***14**	***0.017**
Cariceae and *Khaosokia* monophyletic	1832	10	0.066
Cariceae and the Scirpus Clade + Zameioscirpus Clade monophyletic	1824	2	0.760
Khaosokia sister to monophyletic CDS	1827	4	0.312
Khaosokia and Dulichieae monophyletic	1827	4	0.347

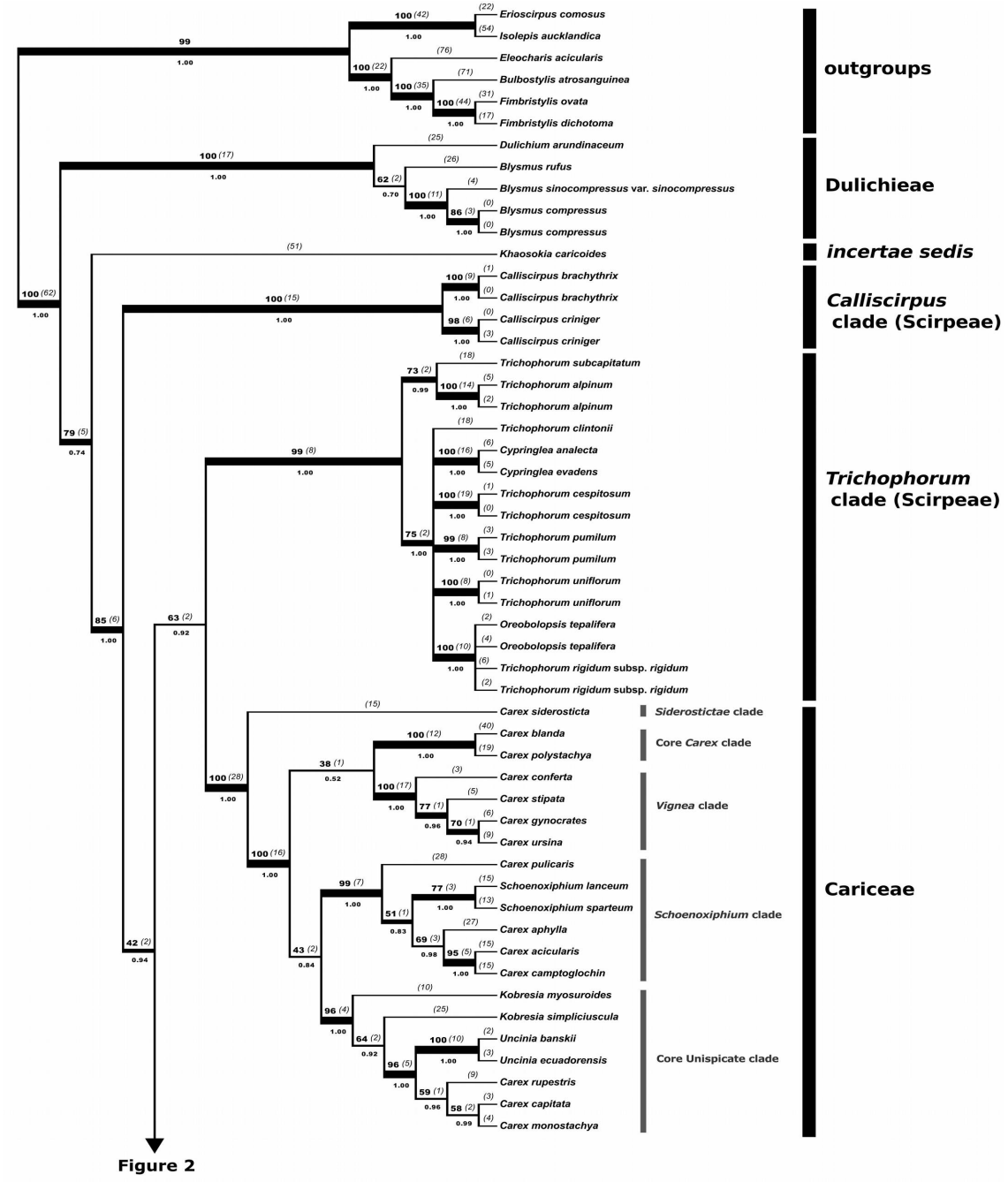


Figure 2.1. Strict consensus tree of parsimony searches, with parsimony bootstrap support (bold) and unambiguous branch lengths (italics, in parentheses) indicated over branches, and Bayesian posterior probabilities of clades under branches. Tribes and major clades are indicated on the right. [Continued in Fig. 2.2]

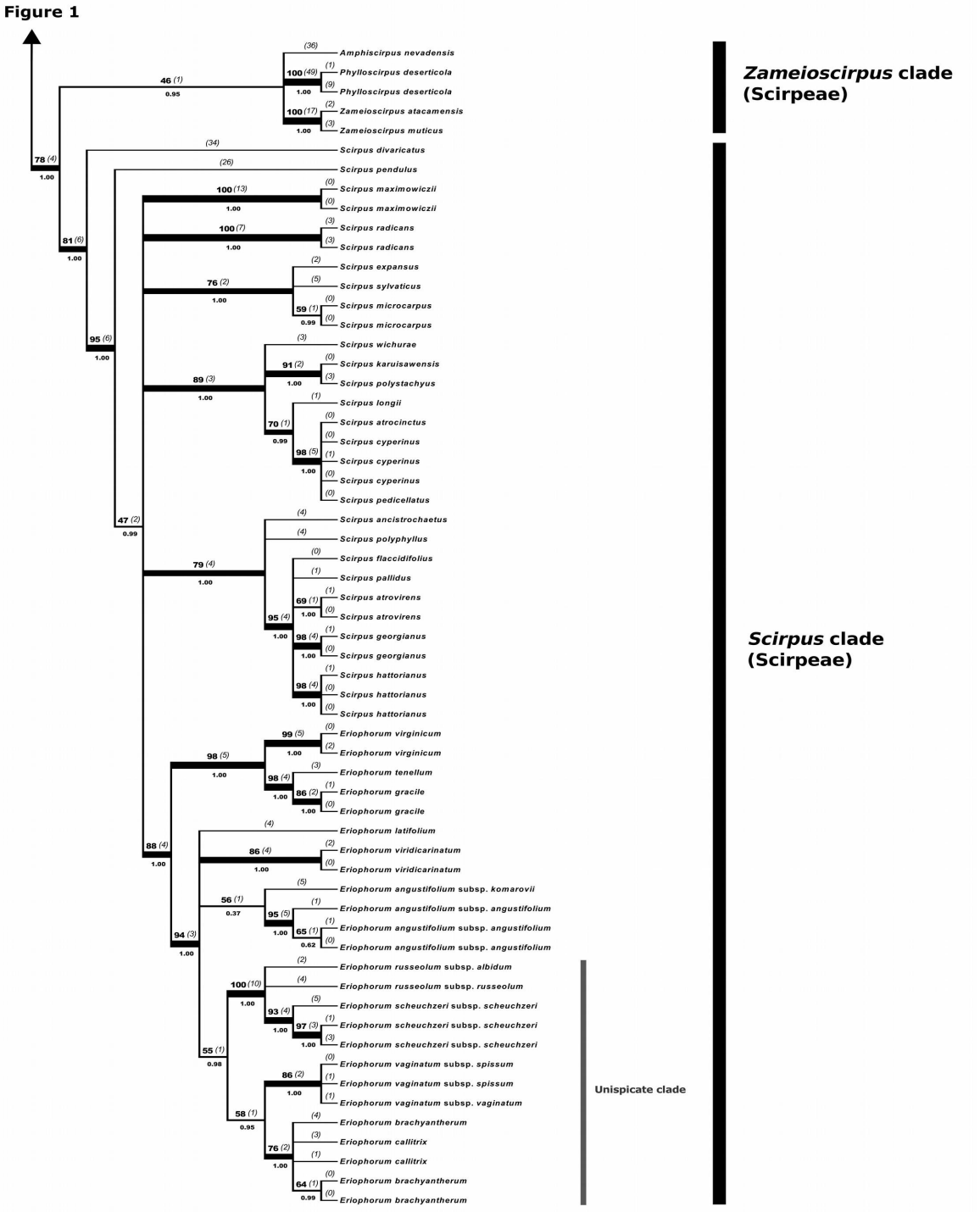


Figure 2.2. Strict consensus tree of parsimony searches, with parsimony bootstrap support (bold) and unambiguous branch lengths (italics, in parentheses) indicated over branches, and Bayesian posterior probabilities of clades under branches. Tribes and major clades are indicated on the right. [Continued from Fig. 2.1]

CHAPTER 3

RHODOSCIRPUS (SCIRPEAE, CYPERACEAE), A NEW SOUTH AMERICAN SEDGE GENUS SUPPORTED BY MOLECULAR, MORPHOLOGICAL, ANATOMICAL AND EMBRYOLOGICAL DATA

This Chapter is a slightly modified version of an article published in the journal Taxon (http://dx.doi.org/10.12705/645.4). Coauthors on the article are: Sabina Donadío, Claire N. Gilmour, and Julian R. Starr. Disclaimer: new names presented here are not intended to be effectively published for nomenclatural purposes.

3.1 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, 1974b; 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*, Gilmour & al., 2013; *Cypringlea*, Strong, 2003; *Zameioscirpus*, Dhooge & al., 2003) and even in the distantly related Cypereae (*Dracoscirpoides*, 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). 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; Dhooge & al., 2003; Dhooge & Goetghebeur, 2004; Dhooge, 2005). One notable exception is *Scirpus 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, 1944b, 1946; Koyama, 1958) have suggested affinities between *Scirpus asper* and a series of species which share little except that they all appear morphologically aberrant compared to the bulk of *Scirpus* (e.g. *Scirpus petelotii* Raymond) or have already been transferred to other genera and tribes of Cyperaceae (e.g. *Scirpus analecti* Beetle ≡ *Cypringlea analecta* (Beetle) M.T.Strong, Strong, 2003; *Scirpus giganteus* Kunth ≡ *Androtrichum giganteum* (Spreng.) H.Pfeiff., Pfeiffer, 1937). Despite this and the availability of a decent number of specimens in major herbaria, *Scirpus 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 (Léveillé-Bourret & al., 2014). Molecular, morphological, anatomical and embryological evidence indicate that *Scirpus 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* (a single North and South American species) than it is to *Scirpus* s.str. In consequence, a new South American genus is here described, *Rhodoscirpus* Lév.-Bourret, Donadío & J.R.Starr, to accommodate a single morphologically distinctive species, *Rhodoscirpus asper* (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr, comb. nov.

3.2 Materials & Methods

3.2.1 Taxon Sampling for Phylogenetic Analyses

Sampling aimed to represent all major clades of CDS as identified in Chapter 2 (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 2). 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 level 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 region 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 2). Outgroup taxa were selected to represent the major lineages of the Abildgaardieae-Cypereae-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, 1994b) revision of the genus.

3.2.2 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× reaction buffer (Bioline, United Kingdom), 2 mM $MgCl_2$ (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 µM of each primer, 1.1 µL Bovine Serum Albumin (BioShop, Canada), 0.6 U of Biotaq DNA Polymerase (Bioline) and 1–3 µL (~20–30 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. 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× reaction Buffer (Bioline, United Kingdom), 2.5 mM $MgCl_2$ (Sigma Aldrich), 1 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.4 µM of each primer, 1 M Betaine (Sigma Aldrich), 0.6 U of Biotaq DNA Polymerase (Bioline) and 2–4 µL (~25–35 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. 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 Inc., Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit version 3.1

(Applied Biosystems; Foster City, CA, USA). 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 v.4.8.5 (Biomatters) and all sequences were submitted to Genbank (Appendix 2).

3.2.3 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 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* 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 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 v8.1.2 (Stamatakis, 2014). Model and partitioning scheme were selected with PartitionFinder v1.1.0 (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 1,000 (standard) bootstrap replicates.

Maximum likelihood BS values were placed on the highest scoring ML tree with SumTrees 3.3.1 (Sukumaran & Holder, 2010) and parsimony BS and Bremer support values were added by hand. Tree figures were produced with TreeGraph 2.0.50 (Stöver & Müller, 2010) and Inkscape 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.

3.2.4 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 graticules. 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×. 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 *Scirpus 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.

3.3 Results

3.3.1 Phylogenetic Results

The *matK*, *ndhF* and ETS-1f alignments were respectively 1,319 bp, 1,215 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 3,355 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.

The parsimony searches found 189 trees of 2,143 steps with consistency and retention indices of 0.66 and 0.73. The best topology found in ML searches (Fig. 3.1) had a log-likelihood of -15843.81 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* C.N.Gilmour, J.R.Starr & Naczi as sister to the clade comprising the Zameioscirpus Clade + Scirpus Clade, and the position of *Carex acicularis* Boott in J.D.Hooker as sister to *Carex 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. 3.1). 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* as successive sisters (56% and 84% BS) to a moderately supported (84% BS) clade consisting of five major lineages (*Calliscirpus*, Cariceae, *Trichophorum* Pers., *Scirpus* + *Eriophorum* or "Scirpus Clade", and *Amphiscirpus* Oteng-Yeb. + *Phylloscirpus* + *Zameioscirpus* Dhooge & Goetgh. or "Zameioscirpus Clade"), all of which receive good support (>90% BS; Fig. 3.1). 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.

3.3.2 Comparative Morphology

The general vegetative and reproductive morphology of *Rhodoscirpus* is similar to that found in species of *Scirpus* s.str. (Fig. 3.2). 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. *Scirpus microcarpus* J.Presl & C.Presl) or sparsely ciliate (e.g. *Scirpus 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. *Scirpus radicans* Schkuhr). With respect to these characters, *Rhodoscirpus* is more similar to *Amphiscirpus*, 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. 3.3, F, G), while *Scirpus* spp. with retrorsely barbed bristles have the barbs mostly spirally, or sometimes pseudo-distichously, arranged near the base of the bristle (Fig. 3.3, H), 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 Clade + Scirpus Clade in having no developed perianth. *Rhodoscirpus* has brown to grey-brown mature nutlets with a broadly obovoid to suborbicular body (including stipe, but not beak) 1–1.3 times as long as wide (Fig. 3.3, A, D), while *Scirpus* spp. have generally pale brownish or yellowish nutlets with an ellipsoid to slightly obovoid body (1.4)1.5–1.7(2) times as long as wide (Fig. 3.3, C), with the exception of *Scirpus polystachyus*, *Scirpus ternatanus* Rein. ex Miq.

and allies (*Scirpus chunianus* Tang & F.T.Wang, *Scirpus rosthornii* Diels), which have almost orbicular bodies. For these characters, *Rhodoscirpus* is closer to *Amphiscirpus*, which has dark brown, broadly obovoid nutlet bodies 1–1.5 times as long as wide, while *Phylloscirpus* has brown obovoid to almost ellipsoid nutlet bodies 1.2–2.2 times as long as wide (Fig. 3.3, B, E) and *Zameioscirpus* has brown obovoid to narrowly obovoid 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.

3.3.2 Comparative Culm and Blade Anatomy

The culm and leaf blade anatomy of *Rhodoscirpus asper* is similar to that of *Scirpus pendulus* and *Scirpus microcarpus* in most aspects. All examined species have culms with scattered tannin idioblasts, 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. 3.4, D), 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. 3.4, F). 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. 3.4, A–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 of large thin-walled cells (Fig. 3.4, C). *Rhodoscirpus asper* and *Scirpus pendulus* have abaxial and adaxial girders (sometimes partial) on each secondary bundle, whereas *Scirpus 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.

3.4 Discussion

3.4.1 Rhodoscirpus, a New Genus for Tribe Scirpeae

The genera of the Zameioscirpus Clade and Scirpus Clade form a well-supported clade in our combined analyses (Fig. 3.1). This Zameioscirpus Clade + 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).

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 *Scirpus 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*.

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*, *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. 3.3, C), 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.

3.4.2 Morphology, Anatomy, Ecology and Biogeography

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. 3.3, F, G). In addition, its brown, broadly obovoid nutlets (Fig. 3.3, A, 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. 3.4, E) 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. *Scirpus petelotii*, *Scirpus ternatanus*; Van der Veken, 1965). However, the cauline leaves, large size and open anthelate inflorescence (Fig. 3.2) 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, 1994b; Ball & Wujek, 2002). There remains no accepted African species of *Scirpus* with the recent removal of *Dracoscirpoides* (Cypereae; Muasya & al., 2012), except for *Scirpus pinguiculus* which is probably an *Isolepis* R.Br. given its unispicate inflorescence, lack of perianth and presumed close relationship to *Isolepis cernua* (Vahl) Roem. & Schult. (Cypereae; Clarke, 1898: 222). Likewise, an examination of the protologues and pictures of type specimens for all remaining South American *Scirpus* spp. suggest that they all have affinities with other Cyperaceae genera, often in distantly related tribes (Dhooge, 2005; ÉLB, pers. obs.). This leaves the Australian *Scirpus 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).

3.5 Taxonomic Treatment

3.5.1 Revised Identification Key to Scirpeae s.lat. Genera

1a. Inflorescence a white to red cottony mass at maturity because of the exserted flat and silky perianth bristles.....**2**

2a. Bristles >> 10, devoid of barbs except at the very apex; cauline leaves generally present; spikelets large, 8–50 mm long in fruit.....***Eriophorum***

2b. Bristles 6–7, antrorsely barbed or smooth; leaves all basal; spikelets small, 5–15 mm long in fruit.....**3**

3a. Inflorescence a dense congested head of many spikelets; bristles antrorsely barbed almost to the base; ligules ciliate; leaf blades flat and elongate.....***Calliscirpus***

3b. Inflorescence a single terminal spikelet; bristles smooth; ligules entire; leaf blades reduced to short mucros.....***Trichophorum*** (in part)

1b. Inflorescence not appearing as a cottony mass; bristles included to shortly longer than glumes, not flat and silky.....**4**

4a. 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**

5a. 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 obovoid to suborbicular body (incl. stipe) 1.0–1.3 times as long as wide.....***Rhodoscirpus*** gen. nov.

5b. 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***

4b. Leaves all basal, node of the distalmost leaf hidden in the sheath of the leaf below; inflorescence various, but rarely anthelate.....**6**

6a. 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***

6b. 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**

7a. Inflorescence a dense head of many spikelets; perianth bristles retrorsely barbed.....**8**

8a. Leaves ligulate; inflorescence pseudo-lateral; glumes ciliate.....***Amphiscirpus***

8a. Leaves eligulate; inflorescence terminal; glumes entire .....***Phylloscirpus*** (in part)

7a. Inflorescence unispicate, rarely a paucispicate raceme; perianth bristles various, sometimes absent, but never retrorsely barbed.....**10**

10a. Leaves eligulate.....***Phylloscirpus*** (in part)

10b. Leaves ligulate.....**11**

11a. All spikelet scales similar, without excurrent awn or mucro.....***Zameioscirpus***

11b. Proximal scale of spikelet sterile or differentiated, often awned.....12

12a. Perianth of scale-like tepals.....***Oreobolopsis***

12b. Perianth of bristle-like tepals or absent.....***Trichophorum*** (in part)

3.5.2 Description of the New Genus

Rhodoscirpus Lév.-Bourret, Donadío & J.R. Starr, **gen. nov.** – Type: *Rhodoscirpus asper* (J. Presl & C. Presl) Lév.-Bourret, Donadío & 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 obovoid 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 monospecific pending detailed revisionary studies (see taxonomic notes below).

1. ***Rhodoscirpus asper*** (J. Presl & C. Presl) Lév.-Bourret, Donadío & J.R. Starr, **comb. nov.**
≡ *Scirpus asper* J. Presl & C. Presl, Reliq. Haenk. 1: 194. 1828 – Type: CHILE. Mountains, s.d., *Haenke s.n.* (holotype HAL 0109717 [photo!], PRC 452287 [photo!]).

= *Scirpus glaucus* Nees & Meyen ex Kunth, Enum. Pl. 2: 169. 1837, nom. illeg. ≡ *Scirpus subasper* Beetle in J.F.Macbr., Revista Univ. (Cuzco) 33(87): 139. 1945 – Type: CHILE. Chile austral, Santa Rosa de los Andes, in fossis, 1827? [according to Turrill, 1920], *Poeppig 509* (holotype W [photo!] [destroyed?]).

= *Scirpus trachycaulos* Phil., Anales Univ. Chile 93: 482. 1896 – Type: CHILE. Zanjon, in Valle Carrizal, Sep 1885, *F. Philippi s.n.* (holotype SGO 037805 [photo!]; isotype: SGO 046304 [photo!]).

= *Scirpus asper* var. *polystachyus* C.B. Clarke in Bot. Jahrb. Syst. 30(68): 36. 1901 ≡ *Scirpus subasper* var. *polystachyus* (C.B.Clarke) Beetle in J.F.Macbr., Revista Univ. Cuzco 33(87): 139. 1945 – Type: CHILE. Atacama desert, Feb 1888, *R.A. Philippi s.n.* (holotype K 000632420 [photo!]; isotypes: SGO 037806, 046268, 075159, 075739 [photos!]).

= *Scirpus subasper* var. *diffusus* Beetle, Amer. J. Bot. 33(8): 661. 1946 ≡ *Scirpus asper* var. *diffusus* (Beetle) Beetle, Bol. Soc. Argent. Bot. 5(1–2): 82. 1953 – Type: 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* (holotype RM 0000128 [photo!]; isotype: SI 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 ovoid, 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 obovoid 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*, which was described by Böckeler (1858) as a species closely related to *Scirpus asper* J.Presl & C.Presl, 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* L. Moreover, these specimens were also examined by Kükenthal, who put the name in synonymy of *Cyperus 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. 3.2, C), 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* Beetle, and the type of *Scirpus asper* J.Presl & C.Presl 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. 3.2, B), 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 *Scirpus asper* var. *polystachyus* C.B.Clarke and perhaps also the type of *Scirpus trachycaulos* Phil. correspond to a third, intermediate group, where the spikelets are in 1(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 February 1987 (NY, SI), prov. San Juan, dpto. Angaco, Sa. Pie de Palo, Mogote Corralitos, 3160 m; *Kiesling, R. 6711*, 5 February 1987 (NY), prov. San Juan, dpto. Jáchal, El Salto, 2100–2200 m; *Kiesling, R. 10341*, 7 March 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 March 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; *Schveiter 4545*, January 1926 (MT 00048707), prov. Jujuy, dpto. Tilcara, El Chorro, 2600 m; *Unknown coll. 25*, s.d. (MT 00050042), prov. Cortoba. **BOLIVIA**. *Bang, M. 765*, March 1890 (MICH); *Beck, St. G. 19844*, 2 march 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 January 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 November 1981 (MICH), prov. Valparaiso, 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 (cacti, *Calandrina*) and sclerophyllous shrubs (*Myrceugenia*, *Cryptocarya*, *Lithraea*) dominate; *Wall, E. & Sparre, B. s.n.*, 7 January 1947 (MT 00018980), prov. Santiago, Corral Quemado, about 25 km north-east of Santiago City, 1200 m; *Werdermann, E. 82*, November 1923 (CAS 104513), prov. Coquimbo, Rivadavia, 800 m; *West, J. 5100*, 6 January 1936 (A), prov. Concepcion, near falls of Rio Laja, rock crevices near water of falls, 200 m. **PERU**. *López M., A. 1490*, 28 June 1958 (US 2341140), prov. Huamachuco, dpto. La Libertad, Hacienda Yanazara, 2400 m; *Mostacero L., J. & al. 0984*, 30 July 1985 (US 3458491), prov. Celedín, dpto. Cajamarca, Pumarrume, ladera, 2750 m; *Sagástegui A., A. 14035*, 5 August 1935 (F 2011685, NY), prov. Chota, dpto. Cajamarca, alrededores de Lajas, pastizal, 2200 m; *Soukup, J. 5289*, 8 August 1964

(US 2471595), Quebrada Verrugas (Sa. Bartolomé), 2000 m; *Veja, S. 1965*, 3 April 1977 (F 2216024), prov. Cajamarca, dpto. Cajamarca, alrededores de San Juan, ruta a Pacasmayo, ladera y terrenos de cultivo, 2400 m.

3.5 Figures

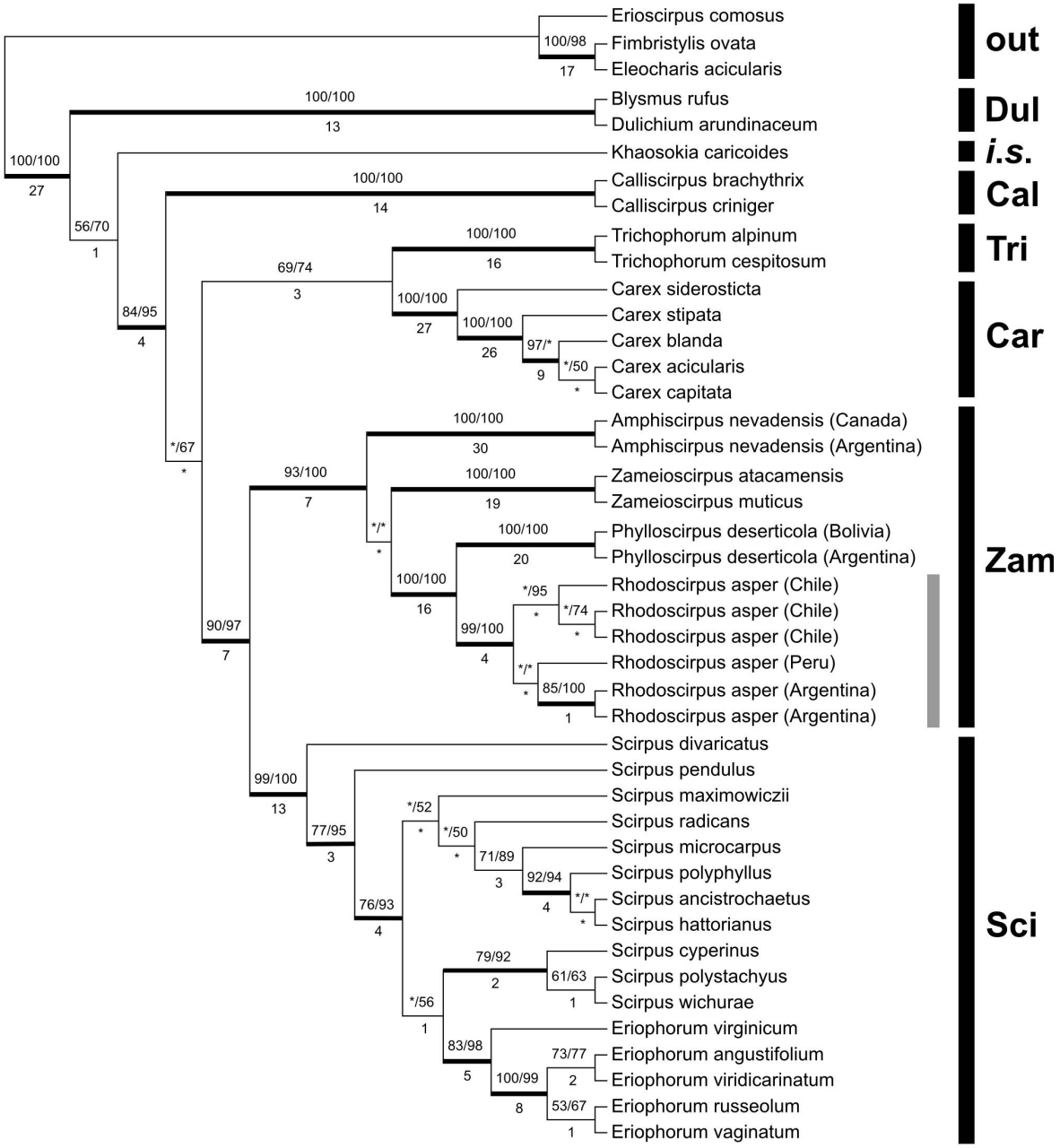

Erioscirpus comosus
Fimbristylis ovata
Eleocharis acicularis
Blysmus rufus
Dulichium arundinaceum
Khaosokia caricoides
Calliscirpus brachythrix
Calliscirpus criniger
Trichophorum alpinum
Trichophorum cespitosum
Carex siderosticta
Carex stipata
Carex blanda
Carex acicularis
Carex capitata
Amphiscirpus nevadensis (Canada)
Amphiscirpus nevadensis (Argentina)
Zameioscirpus atacamensis
Zameioscirpus muticus
Phylloscirpus deserticola (Bolivia)
Phylloscirpus deserticola (Argentina)
Rhodoscirpus asper (Chile)
Rhodoscirpus asper (Chile)
Rhodoscirpus asper (Chile)
Rhodoscirpus asper (Peru)
Rhodoscirpus asper (Argentina)
Rhodoscirpus asper (Argentina)
Scirpus divaricatus
Scirpus pendulus
Scirpus maximowiczii
Scirpus radicans
Scirpus microcarpus
Scirpus polyphyllus
Scirpus ancistrochaetus
Scirpus hattorianus
Scirpus cyperinus
Scirpus polystachyus
Scirpus wichurae
Eriophorum virginicum
Eriophorum angustifolium
Eriophorum viridicarinatum
Eriophorum russeolum
Eriophorum vaginatum
out
Dul
i.s.
Cal
Tri
Car
Zam
Sci

Figure 3.1 [on previous page]. Tree based on *matK* + *ndhF* + ETS-1f data with the highest likelihood found in RAxML searches, with parsimony/likelihood bootstrap percentages above branches and Bremer support below branches. Branches with >80% parsimony BS 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, Zam: Zameioscirpus Clade (Scirpeae), Sci: Scirpus Clade (Scirpeae), Tri: Trichophorum Clade (Scirpeae).

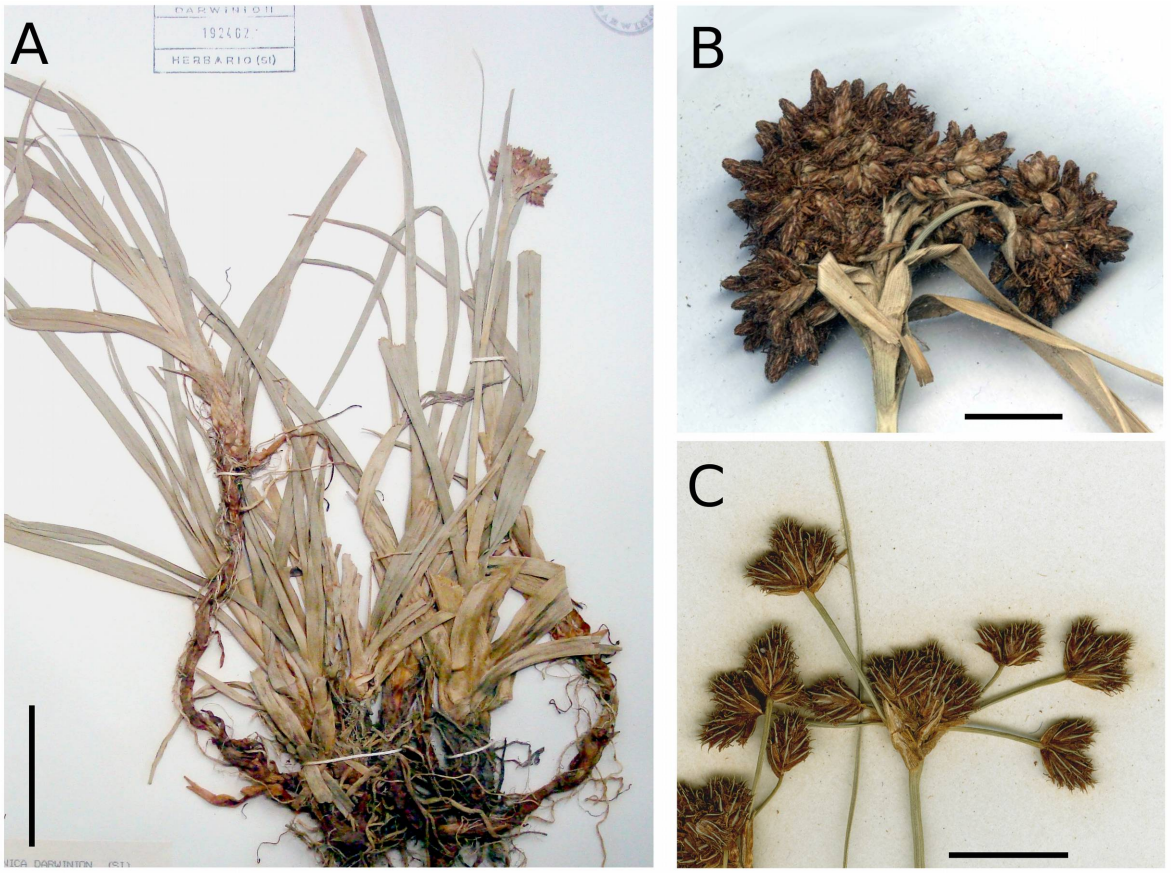


Figure 3.2. General morphology of *Rhodoscirpus asper*. **A**, whole plant showing the cespitose habit and long rhizomes; **B**, dense inflorescence typical of non-Chilean specimens; **C**, part of an open inflorescence with long-pedicellate clusters of 1–3 spikelets typical of Chilean specimens. – Scale bars: **A** = 5 cm; **B–C** = 1 cm.

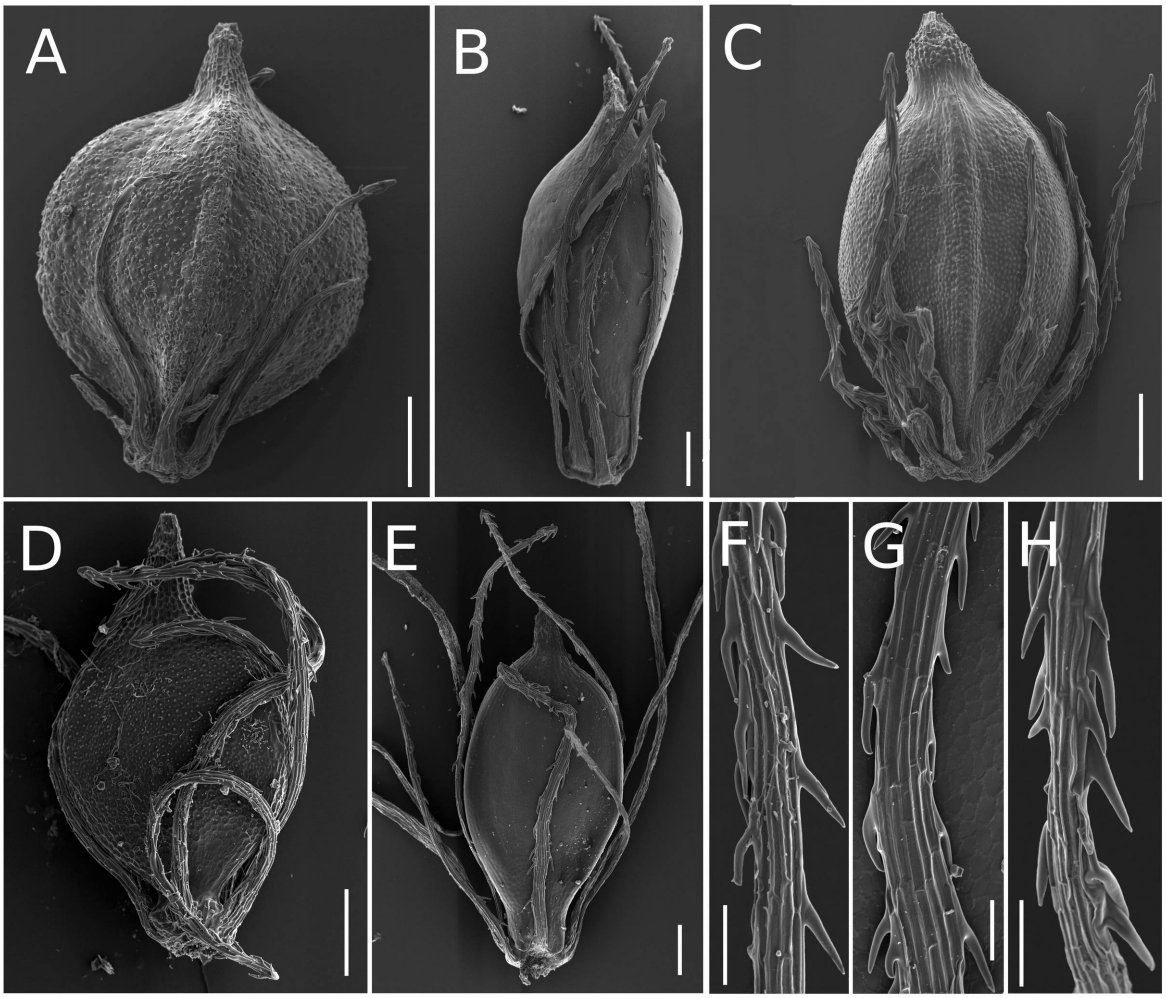


Figure 3.3. Morphology of nutlets (**A–E**) and proximal portion of perianth bristles (**F–H**) of *Rhodoscirpus asper* (**A**, **D**, **F**), *Phylloscirpus deserticola* (**B**, **E**, **G**) and *Scirpus sylvaticus* (**C**, **H**). **A–C**, abaxial view; **D–E**, adaxial view. – Scale bars: **A–E** = 200 μm; **F–H** = 50 μm.

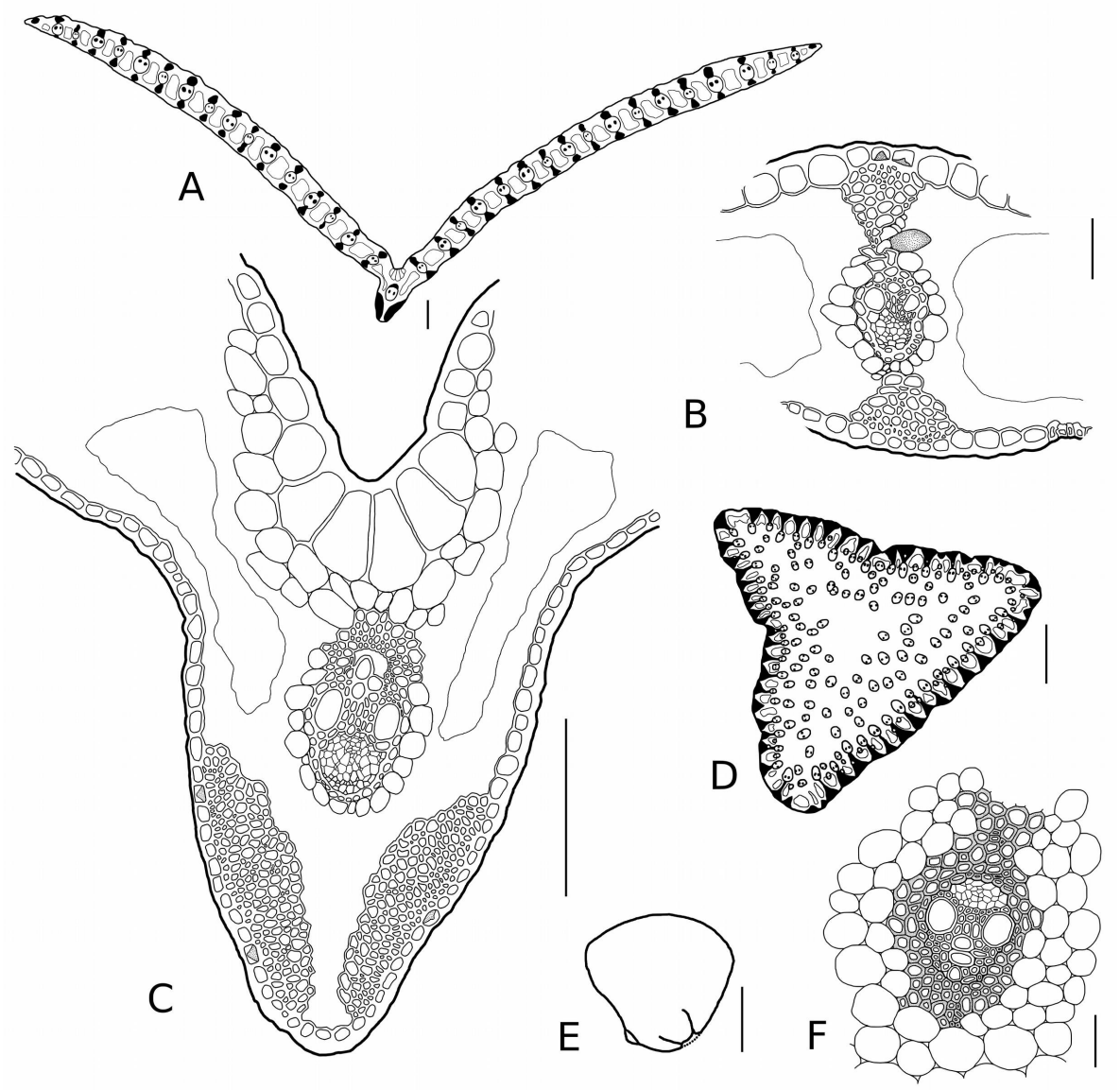


Figure 3.4. Anatomy and embryology of *Rhodoscirpus asper*. **A**, leaf cross-section; **B**, leaf lateral nerve; **C**, leaf midnerve; **D**, culm cross-section; **E**, sagittal view of embryo (redrawn from Van der Veken, 1965); **F**, culm major vascular bundle. Scale bars: A = 500 μm, B = 50 μm, C = 300 μm, D = 1000 μm, E = 100 μm, F = 50 μm.

CHAPTER 4

RESOLVING RAPID RADIATIONS WITHIN ANGIOSPERM FAMILIES USING ANCHORED PHYLOGENOMICS: TRIBAL RELATIONSHIPS WITHIN THE CARICEAE-DULICHIEAE-SCIRPEAE CLADE

This Chapter is a slightly modified version of an article published in the journal Systematic Biology (https://doi.org/10.1093/sysbio/syx050). Coauthors on the article are: Julian R. Starr, Bruce A. Ford, Emily Moriarty Lemmon, and Alan R. Lemmon.

4.1 Introduction

One of the strongest arguments for the use of molecular data in phylogenetic reconstruction was that it provided a seemingly unlimited source of independent characters (Hillis 1987; Hillis and Wiens 2000; Scotland & al., 2003). Although increasing accessibility of next-generation sequencing (NGS) technologies are changing the methodological landscape of plant systematics, most infrafamilial plant phylogenetic studies remain limited to just two regions: the plastid genome and the nuclear ribosomal DNA (nrDNA; Hughes & al., 2006). These regions are still widely used because they are comparable across broad taxonomic groups and easily amplified due to high copy numbers (Álvarez & Wendel 2003) and the availability of universal PCR primers for many of their loci (e.g. White & al., 1990; Taberlet & al., 1991; Baldwin 1992). They remain attractive for phylogenetic research because they consist of coding and non-coding loci which evolve at different rates (White & al., 1990; Wicke & Schneeweiss 2015), and they can be used in combination to study processes such as hybridization (Rieseberg & al., 1990; Feliner & Rosselló 2007).

Until recently, accessing other sources of molecular characters in plants has not been easy. Although the mitochondrial genome should be a prime source of characters due to high copy numbers, low sequence variation, major structural rearrangements and frequent

lateral gene transfer has rendered its sequences impractical for most applications (Palmer & Herbon 1988; Knoop 2004; Bergthorsson & al., 2003; Richardson & Palmer 2006). The plant nuclear genome has been equally problematic. Its vast size (63.4–700,000 Mbp; Greilhuber & al., 2006; Pellicer & al., 2010), independent genealogical histories and biparental inheritance are favourable characteristics; however, its generally higher evolutionary rates, low copy numbers and the scarcity of complete model genomes means that designing broadly applicable PCR primers in non-model organisms is rarely successful. This is true even for well-known plant nuclear loci (Hughes & al., 2006) such as the *Waxy* (granule-bound starch synthase) or *LEAFY* genes, and other challenges, such as gene duplication, often necessitate extensive rounds of cloning to differentiate paralogs (e.g., Mason-Gamer & al., 1998; Hoot & Taylor 2001). Even when primers are successfully designed, they often cannot amplify low copy loci from degraded tissue samples, such as herbarium specimens, which means that their usefulness for studies on species-diverse or geographically widespread groups is limited. Consequently, most specimen-based plant molecular phylogenetic studies still consist of only a handful of linked loci from the plastid genome (e.g., *matK*, *ndhF*, *trnL-F*) and nrDNA cistron (ITS region, ETS; Hughes & al., 2006) and are rarely comprised of combined analyses of more than five sequenced regions from these two character sources.

Next-generation sequencing (NGS) technologies are directly and indirectly facilitating the exploration of new sources of molecular characters in non-model plants. Although full genome sequencing remains beyond the reach of most systematists, NGS promotes the development of genomic resources for new model organisms, which in turn provides data useful for the design of new Sanger-based markers such as low copy nuclear genes (Blischak & al., 2014; Chamala & al., 2015) or microsatellites (Gardner & al., 2011). In addition, the development of efficient multiplexing and enrichment methods are making NGS increasingly accessible as a method to directly gather data for larger species samples in non-model organisms (Cronn & al., 2012; Lemmon & Lemmon 2013). Low-coverage shotgun sequencing (genome skimming) and organellar genome enrichment permit rapid and efficient sequencing of large phylogenomic matrices from the high-copy regions of

genomes (organelles and nrDNA; Straub & al., 2012). However, these approaches are limited by the finite size and generally linked nature of the targeted regions, as they were prior to the invention of NGS. Moreover, they have been unable to completely resolve several important plant radiations (Xi & al., 2012; Barrett & al., 2013, 2014; Ma & al., 2014; Straub & al., 2014). While cost-efficient alternatives, including RADseq (Baird & al., 2008) and transcriptome sequencing (e.g., Wen & al., 2013), can provide data from thousands of unlinked nuclear loci, they both have limitations for phylogenetic analysis. Indeed, RADseq datasets are characterized by short loci of uncertain homology and high amounts of missing data (Rubin & al., 2012; Huang & Knowles 2016), and although it has been used in phylogenetic studies of radiations at least as old as 60 Ma (Gonen & al., 2015; Eaton & al., 2017), the existence of many different protocols and the anonymous nature of RADseq loci (lacking a reference genome) does not facilitate data sharing and reuse across study groups (Harvey & al., 2016). On the other hand, transcriptome sequencing has the potential to be useful at any taxonomic level, but important drawbacks include the complexity of working with RNA (Johnson & al., 2012), especially when living material is not available, and the computational burden of gene assembly and orthology inference in plant genomes where gene families, paralogs, and splice variants are common (Cronn & al., 2012).

A more flexible and promising approach is hybrid enrichment, a method that reduces the bioinformatics and laboratory complexity of transcriptome sequencing by using probes designed from existing genomic or transcriptomic sequences to enrich a fixed set of molecular targets (Lemmon & Lemmon 2013). Low-copy nuclear gene enrichment probes have already been designed to work across vertebrates (Faircloth & al., 2012; Lemmon & al., 2012), and they have been used in the phylogenetic analysis of birds (Prum & al., 2015), snakes (Pyron & al., 2014; Ruane & al., 2015), lizards (Leaché & al., 2014; Brandley & al., 2015; Pyron & al., 2016), frogs (Peloso & al., 2016) and fishes (Eytan & al., 2015) amongst others. In plants, hybrid enrichment probes have been designed for several genera (e.g., de Sousa & al., 2014; Weitemier & al., 2014; Nicholls & al., 2015; Schmickl & al., 2016; Stephens & al., 2015; Heyduk & al., 2016; Johnson & al., 2016), a

subfamily of palms (Arecoideae, Arecaceae; Comer & al., 2016), a subfamily of grasses (Chloridoideae, Poaceae; Fisher & al., 2016), and for the sunflower family (Asteraceae; Mandel & al., 2014). Although this taxon-specific approach in plants has been successful, it requires new probes to be designed for every group, and the data generated from such studies has limited potential to be reused because the targeted regions are group-specific. On the contrary, if conserved targets are selected, hybrid enrichment probes can be designed to work on broad taxonomic scales. This method, known as "anchored phylogenomics" (Lemmon & al., 2012), has the potential to provide parallel datasets for a fixed set of loci across large taxonomic groups, like flowering plants. In other words, anchored phylogenomics has the potential to become the modern NGS equivalent of the "universal" PCR primer papers that resulted in an explosion of phylogenetic studies in non-model organisms during the past decades (White & al., 1990; Taberlet & al., 1991; Baldwin, 1992).

The success of hybrid enrichment in several isolated plant groups has motivated the design of a new set of flowering plant-specific probes that can enrich nuclear genes across all flowering plants. In another study, 517 target loci were identified using 25 angiosperm genomes, and their universality and broad utility in flowering plants was demonstrated (Buddenhagen, 2016; Buddenhagen & al., 2016). This new resource has the potential to greatly simplify and accelerate plant phylogenomic research by reducing the burden of marker choice and probe design, and by promoting the accumulation of parallel data from a standard set of nuclear genes shared by all plant families. Moreover, it would be especially useful if it was able to resolve relationships at both higher and lower taxonomic levels. In fact, the angiosperm probe kit is already being widely adopted through numerous ongoing collaborations to collect data from nearly 90 angiosperm families for more than 2000 samples (to date) at the Center for Anchored Phylogenomics (e.g. Mitchell & al., 2017; Fragoso-Martínez & al., in press; www.anchoredphylogeny.com). Building upon Buddenhagen & al. (2016) where the relationships of major angiosperm lineages was

examined, this contribution demonstrates the utility of the method to resolve difficult branches in a rapid radiation of tribes and genera (Cyperaceae, Cariceae-Dulichieae-Scirpeae).

Phylogenetic analyses presented in Chapters 2 and 3 have identified seven major lineages in CDS using the traditional combination of plastid and nrDNA markers. However, like in many plant groups, the backbone of the tree remained unresolved possibly due to a relatively old crown age (>40 Ma) and an early radiation that occurred over just 10 million years (Escudero & Hipp, 2013; Spalink & al., 2016b). As a result, the most rapidly-evolving plastid genes contain few, if any, characters to support the backbone topology, whereas non-coding plastid and nrDNA regions have diverged so much that they cannot be confidently aligned across the whole group. These factors suggest that large numbers of nuclear genes are needed to resolve the backbone phylogeny of CDS, and universal anchored phylogenomics probes could provide the quick and efficient means to obtain them.

The aims of this study were twofold to: 1) test the utility of anchored phylogenomics in closely related genera of flowering plants showing evidence of rapid diversification; and 2) to resolve long-standing taxonomic problems in CDS by estimating a robust phylogeny of the major lineages of the clade. Using the first set of universal probes available for nuclear gene enrichment in flowering plants, data was collected from hundreds of loci in 34 species selected to represent all major lineages of the CDS clade, as identified in Chapters 2 and 3. The outcome of the new analyses are compared in terms of congruence, resolution and levels of support to a phylogeny estimated from a typical plastid plus nrDNA Sanger-derived dataset that is still commonly generated by many researchers today (i.e., a plastid and nrDNA analysis). The value of anchored phylogenomics for resolving rapid radiations in flowering plants, and the implications of the results on the taxonomy and evolution of the Cariceae-Dulichieae-Scirpeae clade are discussed.

4.2 Materials & Methods

4.2.1 Taxon Sampling and DNA Extraction

A total of 32 ingroup taxa were included to represent all major clades of the CDS clade as based on a previous phylogenetic study with extensive taxonomic sampling of the clade (Léveillé-Bourret & al., 2014). This includes *Dulichium arundinaceum* (Dulichieae; comprising ca. 7 spp.), *Khaosokia caricoides* (incertae sedis), both *Calliscirpus* species (Calliscirpus Clade; 2 spp.), *Amphiscirpus nevadensis* (Zameioscirpus Clade; comprising ca. 8 spp.), 5 *Scirpus* and 3 *Eriophorum* species (Scirpus Clade; comprising ca. 48 spp.), 2 *Trichophorum* species (Trichophorum Clade; comprising ca. 18 spp.), and 18 *Carex* species (Cariceae; comprising ca. 2,150 spp.) representing all major Cariceae lineages identified by Starr & al. (2015). Two outgroup taxa (*Eleocharis obtusa* (Willd.) Schult.; Eleocharideae and *Erioscirpus comosus* (Wall.) Palla; Cypereae) were selected from the CDS sister group, the Abildgaardieae-Eleocharideae-Cypereae-Fuireneae clade (Muasya & al., 2009). Two accessions of *Scirpus atrovirens* were included to test the repeatability of the hybrid-enrichment methodology. Leaves collected fresh in the field and dried immediately in silica gel were used in whole genomic DNA extractions using the silica-column based protocol of Alexander & al. (2007) as modified by Starr & al. (2009). However, increased quantities of leaf tissue (80–100 mg instead of 20 mg) and reagents were used to account for the greater mass of DNA required for NGS protocols. We aimed for 1-3 µg of DNA for hybrid enrichment, although some samples with as little as 0.15 µg of DNA worked very well. Voucher information is available in Appendix 3.

4.2.2 Hybrid Enrichment Data Collection

Data were collected following the general methodology of Lemmon & al. (2012) through the Center for Anchored Phylogenomics at Florida State University (http://anchoredphylogeny.com/). After extraction, genomic DNA was sonicated to a fragment size of ~300-800 bp using a Covaris E220 Focused-ultrasonicator with Covaris microTUBES. Subsequently, library preparation and indexing were performed on a

Beckman-Coulter Biomek FXp liquid-handling robot following a protocol modified from Meyer & Kircher (2010). Briefly, sonication is followed by blunt-end repair using T4 DNA polymerase, two different adapters are ligated to both ends of the DNA molecules using T4 DNA ligase, and indexes and full length adapter sequences are added by amplification with 5'-tailed primers. An important modification of this protocol is the addition of a size-selection step after blunt-end repair using SPRI select beads (Beckman-Coulter Inc.; 0.9× ratio of bead to sample volume). Indexed samples were then pooled at equal molarities (typically 16-18 samples per pool), and then each pool was enriched using the Angiosperm v.1 kit (Agilent Technologies Custom SureSelect XT kit), which contained probes for 517 flowering plant exons (average: 287 bp, median: 225 bp) as described by Buddenhagen & al. (2016). Briefly, the probes were designed by selecting genes that are putatively single copy in *Arabidopsis*, poplar (*Populus*), grape (*Vitis*) and rice (*Oryza*), filtering out exons below the minimum size necessary for enrichment, and then narrowing down on the exons that had ≥55% similarity between *Arabidopsis* and rice. Using these two taxa as reference, orthologous regions from 33 complete flowering plant genomes were identified, and the 517 exons that had an average copy number ≤1.2 per genome were selected for probe design. More details, including the probe sequences, can be found in Buddenhagen & al. (2016). After enrichment, 3–4 enrichment reactions were pooled in equal quantities for each sequencing lane and sequenced on paired-end 150-bp Illumina HiSeq 2500 lanes at the Translational Science Laboratory in the College of Medicine at Florida State University.

4.2.3 Assembly

Reads passing quality filtering were checked for overlap and merged following Rokyta & al. (2012). Reads that could not be merged were treated as unpaired during assembly. Reads were mapped on the flowering plant anchored enrichment references following Buddenhagen & al. (2016) and contigs were extended into flanking regions using a *de novo* assembler. Briefly, preliminary matches between each read and the reference sequences were called if 17 bases matched a library of spaced 20-mers derived from the references. Reads were then considered mapped if 55 matches were found over 100 consecutive bases

in the reference sequences (all possible gap-free alignments between the read and the reference were considered). The approximate alignment position of mapped reads were estimated using the position of the spaced 20-mer, and all 60-mers existing in the read were stored in a hash table used by the *de novo* assembler. Then, the *de novo* assembler maps additional reads by identifying exact matches between a read and one of the 60-mers in the hash table. Simultaneously using the two levels of assembly described above, the reference sequences were traversed repeatedly until a pass produced no additional mapped reads, enabling extension of assemblies into variable flanking regions. Contigs were estimated from 60-mer clusters. For each locus, a list of all 60-mers found in the mapped reads was compiled, and the 60-mers were clustered if found together in at least two reads. Each cluster of 60-mers was then used to separate the reads into contigs. Relative alignment positions of reads within each contig were then refined in order to increase the agreement across the reads. Up to one gap was also inserted per read if needed to improve the alignment. In the absence of contamination, low coverage or gene duplication, each locus should produce one assembly cluster. Consensus bases were called from assembly clusters as ambiguous base calls (IUPAC ambiguity codes) only when polymorphisms could not be explained as sequencing error (assuming a 0.1 probability of error and alpha equal to 0.05, Buddenhagen & al., 2016). Called bases were soft-masked (made lowercase) for sites with coverage lower than 5. Assembly contigs derived from less than 10 reads were removed in order to reduce the effects of cross contamination and rare sequencing errors in index reads.

4.2.4 Orthology, Filtering and Alignment

Orthology was determined for genes with multiple copies following Prum & al. (2015). Briefly, for each locus, the distance between each pair of contig sequences was computed as the proportion of shared 20-mers. The list of 20-mers was constructed from both consecutive bases and spaced bases (every third base). For each locus, contig sequences were then clustered with neighbor-joining (NJ) using this alignment-free distance measure, allowing at most one sequence per species in each NJ cluster. This results in multiple clusters per locus, each containing at most one contig sequence per species.

Contig sequences within a cluster are then treated as orthologs, and sequences in different clusters as paralogs. Gene copies were efficiently sorted using their variable flanking regions recovered during extension assembly. Clusters containing fewer than 50% of the species were removed from downstream processing. Finally, alignments of the remaining orthologous sequence clusters were performed with MAFFT v. 7.023b (Katoh & Standley 2013), with the --genafpair and --maxiterate 1000 flags utilized, and alignments were trimmed/masked using the steps from Prum & al. (2015) and Buddenhagen & al. (2016). Briefly, a sliding window of 20 bp was used to mask regions where <10 sites had the most common character present in at least 40% of the sequences. Sites with fewer than 12 unmasked bases were also removed from the alignments. Because of the relatively deep phylogenetic timescale of this study, many variable sites in the regions flanking the conserved exonic core of the probes were masked because they were too variable to align across all taxa.

After the initial automatic alignment in MAFFT, there remained several obviously misaligned regions that were not removed by the previous filtering step. We initially tried using Gblocks 0.91b (Castresana, 2000) to exclude poorly aligned or highly divergent regions, but no parameter combinations could remove some clearly misaligned regions, whereas apparently well-aligned and informative regions were often excluded. This was probably due to the fact that most ambiguous stretches consisted of a few completely misaligned sequences within well-conserved blocks, a situation which is known to confound Gblocks (Castresana, 2000). In consequence, all nuclear gene alignments were visually examined and sites containing misaligned bases, diagnosed by long (>3 bp) stretches of disagreements to the consensus sequence in one or a few taxa, were excluded. All the separate alignments were combined in a single concatenated alignment for concatenated analyses or kept separate for analyses based on gene trees.

Final alignments are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.55h30. Raw sequence reads are deposited in the NCBI SRA BioProject ID PRJNA376770, SRA study SRP101440.

4.2.5 Phylogenetic Analyses

Parsimony analyses.—Heuristic maximum parsimony (MP) searches on the concatenated alignment were performed in PAUP* v4.0 (Swofford, 2003) using 1,000 random addition sequence (RAS) replicates, tree-bisection-reconnection branch swapping, holding 5 trees at each step and with the STEEPEST option ON. To prevent undersampling-within-replicate and frequency-within-replicate artefacts, support was assessed with 1,000 jackknife 50% replicates using 10 RAS replicates, saving a maximum of 10 trees per RAS, and using the strict-consensus jackknife (GRPFREQ=NO) following the recommendations of Simmons and Freudenstein (2011). Single-locus parsimony analyses and partitioned, hidden and total Bremer support values were calculated in PAUP with the help of ASAP, a perl script provided by Sarkar & al. (2008). Concatenated MP analyses excluding outgroups and/or Cariceae were made to determine whether they could be causing long-branch attraction problems affecting ingroup topology.

Concatenated maximum likelihood analyses.—Concatenated maximum likelihood (ML) analyses were performed in RAxML 8.1.11 (Stamatakis, 2014) on the CIPRES Science Gateway v3.3 (Miller & al. 2010). The partitioning scheme was selected among all locus subsets with PartitionFinder v1.1.1 (Lanfear & al., 2012) using the relaxed hierarchical clustering algorithm (Lanfear & al., 2014) based only on subset similarity (--weights 1,0,0,0), with --rclust-percent settings of 1%, 2%, 4% and 10% and using the Bayesian information criterion (BIC), with GTR+G as the only allowed model. The best scoring scheme (BIC = 3,277,453.37809) was found at a --rclust-percent setting of 4% and comprised 19 partitions with 2–127 loci and 395–31,991 distinct alignment patterns. RAxML searches were made with 100 randomized maximum parsimony starting trees and the new rapid hill-climbing algorithm (Stamatakis & al., 2007). Branch support was assessed with 100 (standard) bootstrap replicates (Felsenstein, 1985). Single-locus ML searches were done using the rapid hill-climbing algorithm and 200 rapid bootstrap replicates (option -f a) using a python script to input the parameters to RAxML 8.1.21. Internode and tree certainty values were calculated in RAxML 8.2.4 (Salichos & al., 2014).

Species tree analyses.—Our phylogenetic problem is characterized by very short backbone branches susceptible to gene tree incongruence (Degnan & Rosenberg, 2006). However, fully parametric coalescent-based species tree estimation such as *BEAST is too computationally demanding to be used with hundreds of loci and 34 taxa (Ogilvie & al., 2016). A species tree was therefore estimated with ASTRAL-II v4.10.12 (hereafter ASTRAL), a "summary" species tree method that has been shown to be more accurate and less sensitive to gene tree estimation error than alternatives (e.g. MP-EST) in simulation studies (Mirarab & al., 2014; Chou & al., 2015; Mirarab & Warnow 2015). Trees from the single-locus RAxML searches were used as input in ASTRAL, and branch support was assessed with local posterior probabilities (Sayyari & Mirarab, 2016) and with 100 gene and site bootstrap replicates (option -g -r). Internode certainty of the branches of this tree were calculated in RAxML 8.2.4 based on all bipartitions of each quartet, and using the lossless adjustment scheme to correct for incomplete gene trees (Salichos & al., 2014; Kobert & al., 2016).

Substitution saturation.— Site-specific substitution rates were estimated in IQ-TREE 1.5.0 (Nguyen & al., 2015) by re-optimizing a single GTR+G (16 rate categories) model to the whole concatenated alignment using the ML topology found by RAxML, and using the "-wsr" option. Using these site-specific rates, the ⅓ fastest-evolving sites were extracted and tested for substitution saturation. These sites should be dominated by 3rd codon positions and non-coding bases. Substitution saturation was assessed by plotting raw number of transversions and transitions against GTR distances and noting whether a plateau is attained. Additionally, the statistical test of substitution saturation of Xia & al. (2003) was made in DAMBE (Xia & Lemey, 2009; Xia, 2013) with 1,000 jackknife replicates on subsets of 4, 8, 16 and 32 taxa. Phylogenetic analyses including only the ⅓ fastest-evolving sites or excluding them gave comparable results to analyses of the full dataset, and are therefore not reported.

Sources of incongruence.— Multiple factors such as lateral gene transfer, gene duplication, hybridization, incomplete lineage sorting and gene tree estimation error cause incongruence between gene trees and their associated species tree. Different types of exploratory data analyses were therefore done to pinpoint the sources of incongruence in the phylogenomic dataset, and especially to determine whether the observed incongruence between gene trees is caused by biological factors (hard incongruence) or is simply due to gene tree estimation error (soft incongruence). Robinson-Foulds distances between ML-estimated gene trees and ASTRAL-estimated species trees were calculated with the ape package (Paradis & al., 2004) in R (R Core Team, 2016). A linear regression was calculated for gene tree to species tree distances against the average gene tree bootstrap support. In addition, bootstrap support of gene tree branches present or absent in the species tree were compared with the help of histograms. Several reduced concatenated MP analyses were made including only loci conflicting with selected backbone branches (Fig. 4.1) to determine whether combined analyses would give similarly conflicting results, which would be expected in the case of hard incongruence, or whether combined analysis would negate conflict, which should happen if incongruence was simply due to gene tree estimation error. Scatter plots, histograms and linear regression coefficients were drawn and calculated with R.

Reduced analyses.—The influence of the number of loci and analysis method on the reconstructed phylogeny was assessed with reduced analyses. In each analysis, a number of loci were randomly selected (without replacement) and analyzed either by concatenation in RAxML or with ASTRAL. Then, Robinson-Foulds distance between the resulting tree and the best estimate of the species tree (ASTRAL) was calculated. This procedure was repeated 200 times for 5, 10, 20, 50, 100, 200 and 400 loci in ASTRAL, and 50 times for the same number of loci in RAxML. To determine if the information content of the selected loci has an effect on phylogenetic analyses, loci were ranked based on number of informative characters and repeated the ASTRAL reduced analyses with the 33% highest ranking loci and the 33% lowest ranking loci, making 200 replicate analyses with 5, 10, 20, 50 and 100 loci. Boxplots were drawn with R (R Core Team, 2016).

4.2.6 Comparative Sanger Matrix

To compare phylogenetic results obtained with hybrid enrichment, data from the plastid genes *matK* and *ndhF* (Gilmour & al., 2013), as well as the nrDNA region ETS-1f (Starr & al., 2003), were obtained from Genbank for the same species as those used in phylogenomics analysis (but replacing the outgroup *Eleocharis obtusa* with *E. acicularis* and including only one *Scirpus atrovirens* accession). Five sequences were newly obtained by PCR and Sanger-sequencing using standard procotols detailed in Léveillé-Bourret & al. (2015). Genbank accession numbers and voucher information are available in Appendix 4.

The sequences were concatenated by species, aligned with the MAFFT v7.017b (Katoh & Standley, 2013) plugin in Geneious 8.1.7 (http://www.geneious.com; Kearse & al., 2012), and the resulting alignments were corrected by hand. Concatenated maximum likelihood (ML) analyses were done in RAxML 8.2.4 (Stamatakis, 2014). The partitioning scheme was selected among all codon and locus subsets with PartitionFinder v1.1.1 (Lanfear & al. 2012) using an exhaustive search (Lanfear & al., 2014) and using the Bayesian information criterion (BIC), with GTR+G as the only allowed model. The best scoring scheme (BIC = 31,271.0585328) comprised three partitions: codons 1 and 2 of *matK* and *ndhF*, codon 3 of *matK* and *ndhF*, and ETS-1f. RAxML searches were done using the rapid hill-climbing algorithm and support estimated with 500 rapid bootstrap replicates (option -f a). Support in parsimony was assessed with 500 bootstrap replicates in PAUP* v4.0, using 10 RAS replicates, saving a maximum of 20 trees per RAS, and using the strict-consensus bootstrap (GRPFREQ=NO).

4.3 Results

4.3.1 Sequence Characteristics

A total of 462 loci were recovered from the Illumina reads, including 456 targets in single-copy, three targets duplicated into two paralogs each, and only 59 targets not recovered (11.4% of the 517). A single locus possessed no informative variation and was

excluded. The average base coverage of loci before trimming was 0–5,576 (mean = 234) across terminals, and maximum number of distinct copies per locus (before orthology filtering) ranged from 1 to 4 (mean = 1.4). After alignment and removal of misaligned bases, loci were 219–1,875 bp long (mean = 649), with 1–80% of their length consisting of flanking sequence (mean = 56%), and each with 26–371 parsimony informative characters (mean = 120). Two hundred seventy-nine loci were missing some terminals, but 99% of all loci had at least 29 (83%) terminals. Each terminal had data for 425–462 loci, averaging 457 loci per terminal (98.9% of all loci). The combined dataset was 299,241 bp long after trimming and exclusion of 6,649 misaligned sites. This included 55,417 (18.5%) parsimony informative characters, 4.7% missing, 0.4% ambiguous bases and a GC-content of 40%. Sequence statistics for each locus are found online (Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.55h30). The two *Scirpus atrovirens* accessions had 99.3% identical sites and almost identical coverage, with only 5,865 sites (ca. 1% of total aligned length) present in one accession but absent in the other. No evidence of strong substitution saturation was found in the ⅓ fastest-evolving sites. A non-linear trend was visible in the GTR vs transversions and the GTR vs transition plots (Fig. 4.2), but the Iss values (0.364–0.424) were significantly smaller ($p < 0.001$) than critical thresholds (0.603–0.860) for all subset sizes and for symmetrical and asymmetrical topologies, which suggest little saturation (Xia & al. 2003).

4.3.2 Phylogenetic Results

Concatenated parsimony searches on the phylogenomic matrix found a single shortest tree of 219,733 steps (consistency index = 0.71, retention index = 0.74; Fig. 4.3). The best tree found by concatenated ML searches had a log-likelihood of -1,636,930.180799 as calculated by RAxML (Fig. 4.4). The best MP and ML trees were almost identical to the ASTRAL species tree (Fig. 4.1), except for an unsupported sister-relationship between *Carex canescens* (representing the Vignea Clade) and the Core Carex Clade in MP, and a highly supported (100% BS) sister-relationship between *Scirpus cyperinus* and *S. atrovirens* in MP and ML. Relationships between major lineages of the CDS clade, the

focus of this study, were identical in all analyses, and their relative position remained stable when outgroups and/or Cariceae were excluded from MP analyses. The MP and ML trees obtained with the comparative Sanger matrix (*matK* + *ndhF* + ETS-1f) were completely congruent with the phylogenomics results, except that most backbone branches had low support in the Sanger matrix, but very high support in the phylogenomic matrix (Fig. 4.1).

Phylogenetic analyses position Dulichieae and *Khaosokia* as successive sisters to a highly supported Cariceae + Scirpeae clade. Within this clade, Scirpeae forms four major lineages in three monophyletic groups: a Calliscirpus Clade (*Calliscirpus*) sister to everything else, *Amphiscirpus* (representing the Zameioscirpus Clade) sister to a Scirpus Clade (*Scirpus* + *Eriophorum*), and a Trichophorum Clade (*Trichophorum*) sister to Cariceae. These backbone relationships are highly supported by all analyses except for the position of *Calliscirpus*, which is highly supported in MP and ML, but is supported in only 68% of ASTRAL bootstrap replicates, with the most frequent conflicting BS replicate trees (31%) putting *Calliscirpus* sister to the clade comprising the Zameioscirpus Clade + Scirpus Clade.

4.3.3 Incongruence Between Gene Trees

Incongruence between ML estimated gene trees was high in the short backbone branches of the phylogeny. This is reflected by small internode certainty values and relatively high numbers of loci with negative partitioned Bremer support (supporting conflicting clades in parsimony) for these short branches identified by letters A to E in Figure 3.1 (Table 4.1). However, support and resolution of backbone branches was low in most estimated gene trees, and there was a clear negative relationship between average ML bootstrap (across all branches) of a gene tree and its distance to the ASTRAL species tree (Fig. 4.5, $R^2 = 0.24$, slope = -0.0063, slope p-value < 10^{-15}). In addition, the average bootstrap support for branches present in the ML gene trees, but absent in the species tree, was considerably lower than support for branches present in both (Fig. 4.6). Combined analyses identified extensive emergent support for the backbone branches of the estimated species tree even in loci that are apparently conflicting in single-locus analyses, as shown

by the high proportion of hidden Bremer support for backbone branches (Table 4.1). Likewise, concatenated MP analyses of all loci conflicting with selected backbone branches (identified with letters in Fig. 4.1) always gave highly supported trees completely congruent with the backbone of the estimated species tree, consistent with soft incongruence due to gene tree estimation error rather than hard incongruence due to biological factors.

4.3.4 Reduced Analyses

The 33% highest and 33% lowest ranking loci in terms of number of parsimony-informative characters had an average of 161.2 (sd = 34.4) and 81.6 (sd = 16.7) informative characters, respectively. Results obtained in reduced analyses by using all loci in ASTRAL, RAxML, or with only the highest or lowest ranking loci in ASTRAL, were all broadly similar. Distance between trees estimated in reduced analyses and the best species tree diminished with increasing number of loci per jackknife replicate, with the majority of replicates having less than 10% conflicting bipartitions with 100 loci or more (Fig. 4.7). With ASTRAL and 200 loci or more, all replicates had a backbone identical to the best species tree, whereas the position of *Calliscirpus* was inconsistent in a minority of replicates with 100 loci. Results were similar when using the highest and lowest ranking loci in ASTRAL. With RAxML, 100 loci were sufficient to get a backbone identical to the species tree in all replicates.

4.4 Discussion

4.4.1 Targeted NGS of Conserved Nuclear Genes in Phylogenetic Inference

Using the first set of universal probes available for nuclear gene enrichment in flowering plants, data from hundreds of loci were collected from 34 taxa representing a typical flowering plant radiation encompassing >40 million years of evolution (Escudero & al., 2013; Spalink & al., 2016b). Despite short backbone internodes connected to long branches, typical of ancient rapid radiations (Whitfield & Lockhart, 2007), the inferred

backbone relationships were well supported in both concatenation and coalescence-based analyses. These results illustrate the great promise of anchored phylogenomics for the resolution of rapid ancient radiations of non-model organisms.

Important amounts of incongruence between estimated gene trees was found in the phylogenomic dataset. The observation that gene tree incongruence was inversely proportional to the amount of phylogenetic information content (as measured by average gene tree ML bootstrap) indicates that at least part of the incongruence must be due to gene tree estimation error. This is corroborated by the lower bootstrap support of gene tree branches absent in the species tree, and the high amount of hidden Bremer support in the shortest branches of the backbone. This also explains why ASTRAL analyses necessitate more loci (ca. 200) than ML analyses (ca. 100) to get consistent results on the backbone relationships of CDS: the estimated gene trees that ASTRAL takes as input are highly affected by estimation error, whereas concatenation presumably amplifies the phylogenetic signal common to all loci, thus reducing the relative influence of noise on the results (Townsend & al., 2012; Bayzid & al., 2015; Warnow, 2015; Meiklejohn & al., 2016). The same effect is seen in several simulation studies that have shown higher efficiency of concatenation relative to summary coalescence methods when incomplete lineage sorting is low (e.g. Bayzid & Warnow, 2013; Chou & al., 2015; Mirarab & al., 2016). Because the probes used for enrichment were designed to be universal for flowering plants, and since many sites in the variable flanking regions were filtered out because of the phylogenetic depth of the study, the anchored loci tend to be slow-evolving in this study. This resulted in modest numbers of informative characters per locus and low levels of support for individual gene phylogenies. However, it should be noted that the faster evolving flanking regions of the probes could be retained in studies focusing on shallower divergences where additional sequence variation is needed.

It has been argued that small numbers of highly-informative loci are preferable to larger numbers of more slowly-evolving loci when attempting to resolve phylogenies with short branches (Salichos & Rokas, 2013). However, the matter is certainly more complex,

because more variable loci are often noisier due to multiple substitutions (Townsend & al., 2012) and they have a higher susceptibility to long-branch attraction (Felsenstein, 1978; Bergsten, 2005). In the case of rapid ancient divergences, difficulties arise because of multiple factors: short backbone branches offer poor phylogenetic signal and increase the probability of deep coalescences, whereas long terminal branches are susceptible to problems of substitution saturation and long-branch attraction (Whitfield & Lockhart, 2007). This creates an apparent trade-off, since fast-evolving loci have a higher probability of containing variation informative for short backbone branches, but are also more susceptible to substitution saturation and long-branch attraction. Indeed, selection of loci should not aim for enormous amounts of variation dominated by noise, but rather for sufficient variation with a high signal/noise ratio and good taxonomic coverage (Philippe & al., 2011; Betancur-R. & al., 2014; Hedtke & al., 2006). For this reason, slowly-evolving, homoplasy-free markers have been suggested to be optimal for the resolution of ancient rapid radiations (Whitfield & Lockhart, 2007). Empirical results and simulation studies also indicate that unresolved gene trees are less problematic in a species tree framework than gene trees potentially biased by substitution saturation or long-branch attraction (Chiari & al., 2012; Xi & al., 2015). All these considerations seem to indicate that large numbers of conserved loci could be preferable to similar numbers of more variable loci to resolve ancient rapid radiations. This idea is clearly supported by the success of anchored phylogenomics in resolving the polytomy at the base of the CDS clade with hundreds of conserved nuclear genes, despite low levels of support for individual gene trees. The reduced analyses likewise indicate that using loci with higher or lower numbers of informative characters has almost no effect on the results, whereas the number of loci had a very significant effect on precision of species tree and concatenation analyses. This suggests that future phylogenomic studies based on conserved nuclear loci could profit more from large numbers of loci than from sampling more characters per locus, despite the likelihood that longer loci could decrease gene tree estimation error. However, Meiklejohn & al. (2016) found that species tree estimation methods gave inconsistent results when using gene trees with very low signal (<25 informative characters), whereas more

informative loci gave more consistent results. The lack of relationship between gene tree information content and species tree accuracy could be explained by the fact that all loci contained at least 26 informative characters in the present analysis, which suggests that there could exist a threshold below which methods based on estimated gene trees might lose their accuracy. This subject should be explored further to determine whether such a threshold exists.

This test case suggests that the efficiency of anchored phylogenomics to enrich specific and universal flowering plant loci is highly promising, and the method could thus simplify next-generation sequencing data collection and sharing across diverse flowering plant groups. Of the 517 anchored phylogenomics loci targeted in this study, approximately 90% produced useable data. In addition, different accessions of the same species provided almost identical sequence and coverage, suggesting that the methodology is reproducible and will provide data that can be re-used and combined in future phylogenetic studies. Other next-generation sequencing approaches in plants have up to now focused on target-enrichment of lineage-specific nuclear loci (e.g., Mandel & al., 2014; de Sousa & al., 2014; Weitemier & al., 2014; Nicholls & al., 2015; Schmickl & al., 2016; Stephens & al., 2015; Comer & al., 2016; Heyduk & al., 2016; Johnson & al., 2016) or on the anonymous and very short markers provided by RADseq (e.g. Eaton & Ree 2013; Escudero & al., 2014; Hipp & al., 2014; Gonzalez, 2014; Massatti & al., 2016). Lineage-specific target-enrichment approaches have the advantage of being tailored for the group of interest, and are thus expected to perform better on average. However, this is counter-balanced by the additional cost and time needed to design new probes for every taxonomic group, and the limitations that lineage-specific markers impose on data sharing and reuse across taxonomic groups. RADseq, on the other hand, enables rapid and cost-efficient production of tens to hundreds of thousands of loci in large numbers of individuals without the need for genomic references. The short length of RADseq loci (mostly limited by read-length) makes determination of homology difficult, for instance creating a trade-off between the number of putative loci retained for analysis and the proportion of loci which are truely orthologous (Rubin & al., 2012; Harvey & al., 2016). High levels of missing data due to

uneven coverage, mutation-induced locus-dropout or other causes (ca. 30-80% in published analyses; Mastretta-Yanes & al., 2015; Eaton & al., 2017) creates a similar trade-off where excluding loci with missing data also significantly reduces the total number of informative characters in the dataset. Despite this, several studies have now demonstrated that radiations at least as old as 60 Ma can be successfully resolved using lax similarity cutoffs during assembly and inclusion of all loci with at least 4 terminals, which suggests that paralogy and missing data may not be problematic for RADseq in most applications (Gonen & al., 2015; Eaton & al., 2017; Huang & Knowles, 2016). One advantage of RADseq compared to Anchored Phylogenomics is the ability to tailor the number of loci to the phylogenetic question and resources available, while there is a hard limit to the number of loci in hybridization-based approaches (517 loci in the present case). On the other hand, data sharing and reuse remains an issue with RADseq because of the use of different enzymes and library preparation methods, the difficulty of assessing orthology at deeper evolutionary timescales and the anonymity of RADseq loci when lacking reference genomes (Ree & Hipp, 2015; Harvey & al., 2016). Compared to both lineage-specific approaches and RADseq, the universality and easy comparability of anchored phylogenomics results in significant savings in cost and time whilst simplifying data analysis and sharing.

4.4.2 Phylogenetic and Taxonomic Implications

Continued work towards inclusion of more informative molecular regions or increasing taxonomic sampling has resulted in good support for seven major lineages within a clade consisting of tribes Cariceae, Dulichieae and Scirpeae (CDS), but relationships between these major lineages, and the identity of Cariceae's sister group has remained elusive (Léveillé-Bourret & al., 2014). Previous studies have placed Cariceae sister to a monophyletic Scirpeae (Muasya & al., 2009) or nested within a paraphyletic Scirpeae and sister to either a Trichophorum Clade (Léveillé-Bourret & al., 2014), the genus *Calliscirpus* (Gilmour & al., 2013) or a clade consisting of the Scirpus Clade + Zameioscirpus Clade (Jung & Choi, 2012). The only consistency has been the poor support

for all backbone relationships, a consequence of a rapid radiation (ca. 10 My) followed by long divergence (30–40 My) between major CDS lineages (Escudero & al., 2013; Spalink & al., 2016b). The highly supported results of anchored phylogenomics, based on data from hundreds of nuclear genes encompassing hundreds of thousands of base pairs, identify the Trichophorum Clade as sister to Cariceae and are in complete agreement with the results of the most inclusive plastid phylogeny of CDS (Léveillé-Bourret & al., 2014). Such a high congruence between phylogenetic estimates based on the nuclear and plastid genomes gives us confidence in the robustness of the results and confirms the usefulness of targeted-enrichment of conserved nuclear genes for phylogenetic analysis at the tribal level and above in sedges (Cyperaceae). Moreover, since the enrichment probes used are universal and are flanked by regions of variable evolutionary rates, they could be equally effective for low-level phylogenetic investigation of flowering plants in general.

4.5 Table and Figures

Table 4.1. Comparison of branch support measures for selected backbone branches (A–E; see Fig. 4.1). Branch length (expected changes per site in ML), internode certainty (ICA) based on estimated ML gene trees, ASTRAL bootstrap (BS), number of unambiguous synapomorphies, number of loci with positive and negative partitioned Bremer support, total Bremer support and proportion of hidden Bremer support.

Branch	Branch length	ICA	BS	Unambiguous synapomorphies	Loci positive	Loci negative	Total Bremer	Proportion hidden Bremer
A	0.0047	0.180	100	1404	296	86	790	(43%)
B	0.0023	0.066	99	646	209	90	327	(66%)
C	0.0014	0.006	68	304	195	203	95	(43%)
D	0.0058	0.380	100	1082	299	48	921	(57%)
E	0.0017	0.024	100	364	195	202	137	(42%)

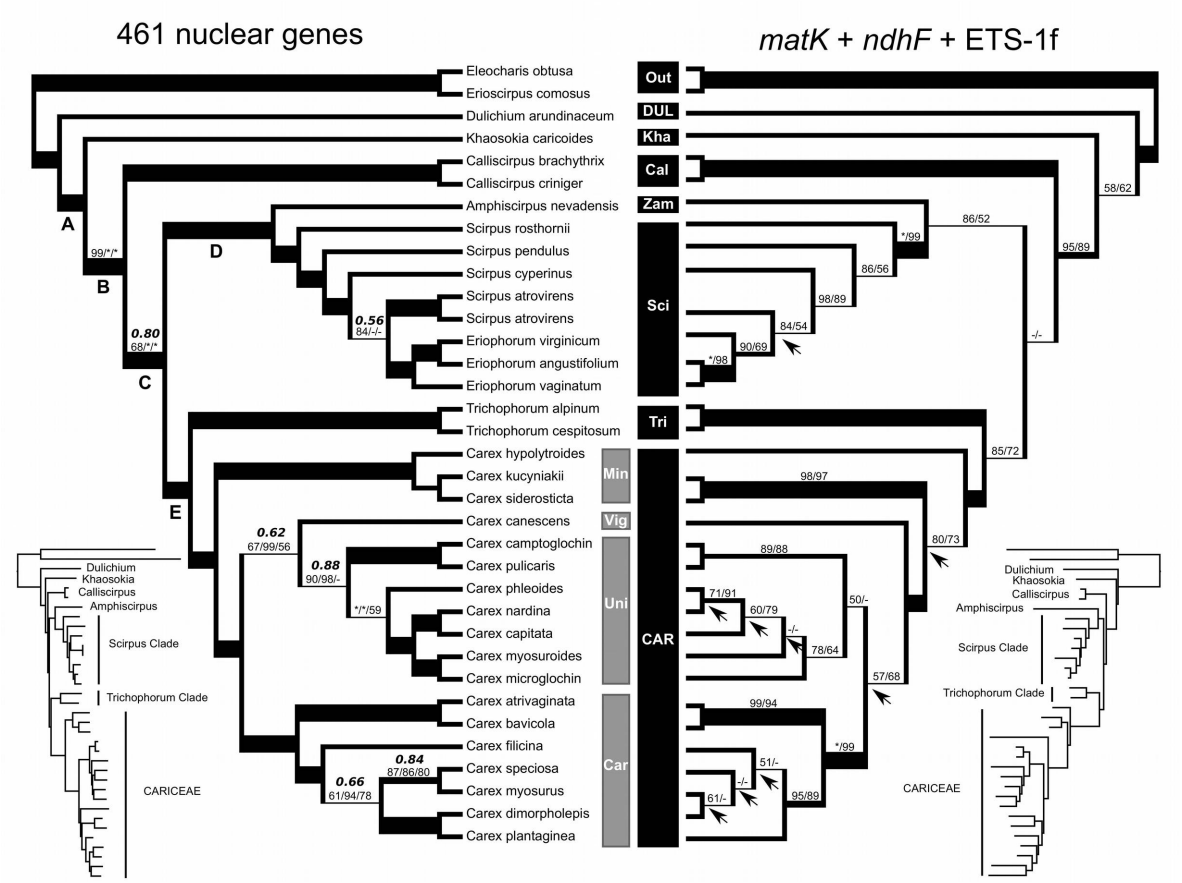


Figure 4.1. Best phylogenetic hypotheses for the CDS Clade, with the species tree estimated by ASTRAL using 461 NGS nuclear loci on the left, and the ML tree estimated using *matK* + *ndhF* + ETS-1f on the right (arrows indicate topological differences with the ASTRAL species tree). The smaller trees on either side represent relative ML branch lengths for each dataset. Branches without values have 100% support for all measures used. When at least one measure was <100%, the support values are reported above branches as follows: ASTRAL tree (bold italics: local posterior probability, normal text: ASTRAL multilocus bootstrap/ML bootstrap/MP jackknife) and ML tree (ML bootstrap/MP bootstrap). An asterisk (*) indicates 100% bootstrap support, and a dash (-) indicates less than 50% bootstrap support. Branch width is a function of support in parsimony. Letters under branches refer to clades in Table 4.1. Legend; Out: outgroups, DUL: Dulichieae, Kha: *Khaosokia*, Cal: Calliscirpus Clade, Zam: Zameioscirpus Clade, Sci: Scirpus Clade, Tri, Trichophorum Clade, CAR: Cariceae, Eri: Eriophorum Clade, Min: Minor Carex Alliance, Vig: *Carex* subg. *Vignea*, Uni: Caricoid (Unispicate) Carex Clade, Car: Core Carex Clade. Scirpeae = Cal + Zam + Sci + Tri.

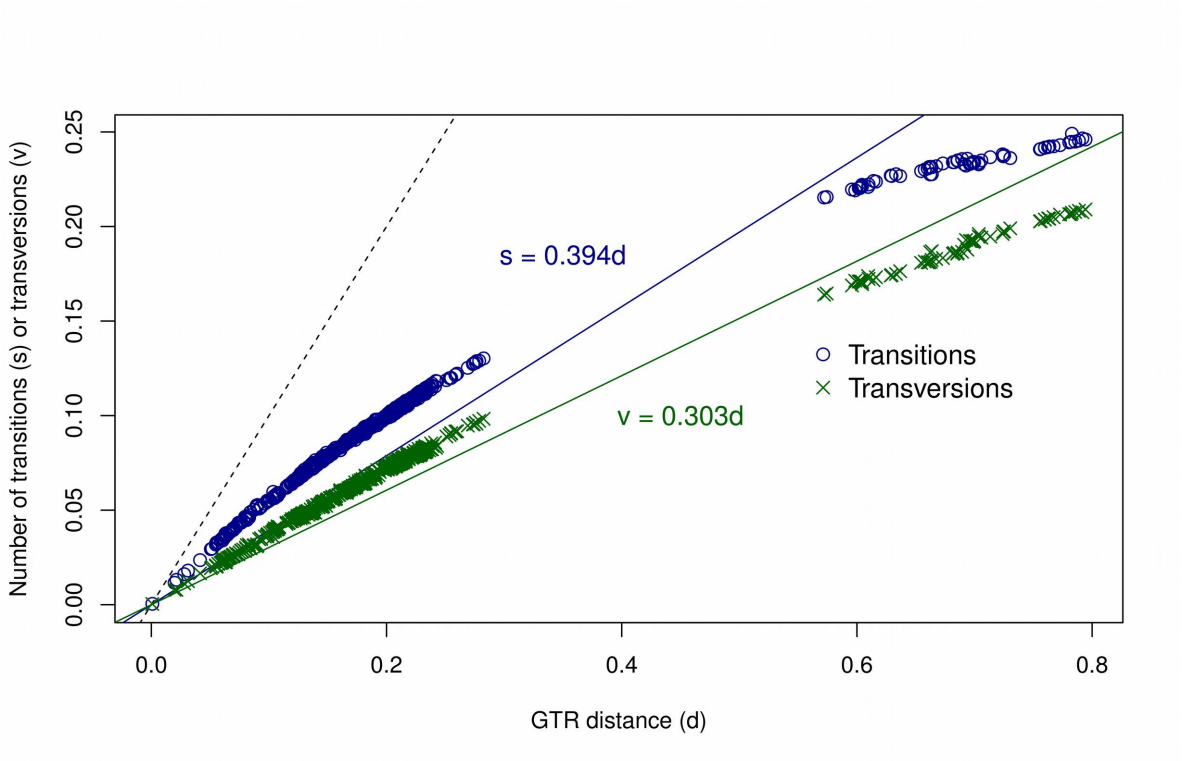


Figure 4.2. Saturation plot as calculated in DAMBE, showing GTR distance estimated for the ⅓ fastest-evolving sites in relation to the number of transitions and transversions at these sites. Regression lines (forced through the origin) and slope coefficients are shown. Dashed line indicates slope of 1. Slope of the regression through the origin of transitions (s) and transversions (v) indicated on the figure.

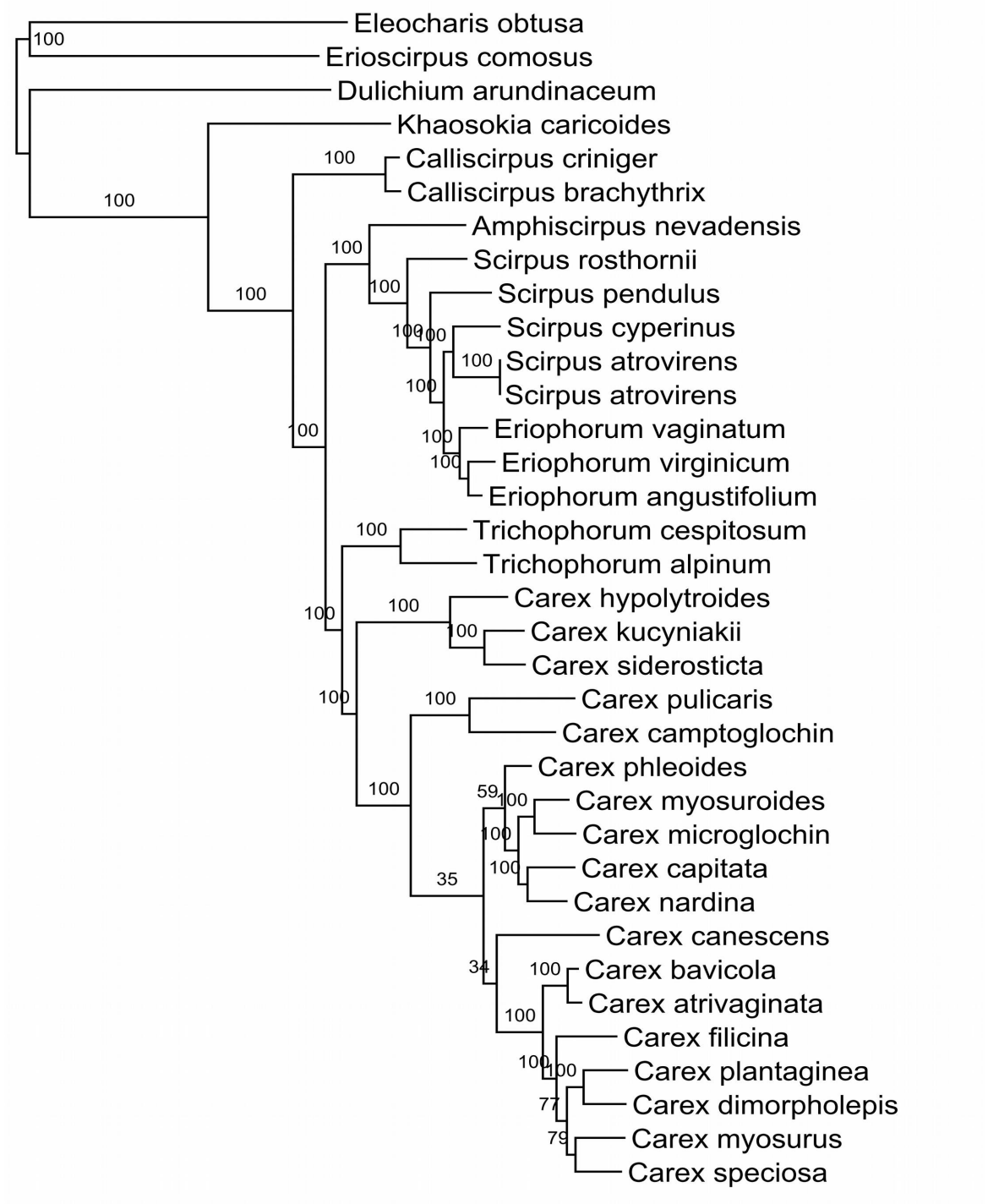


Figure 4.3. Single shortest tree found in PAUP* maximum parsimony searches. Support as MP jackknife, and ACCTRAN branch lengths.

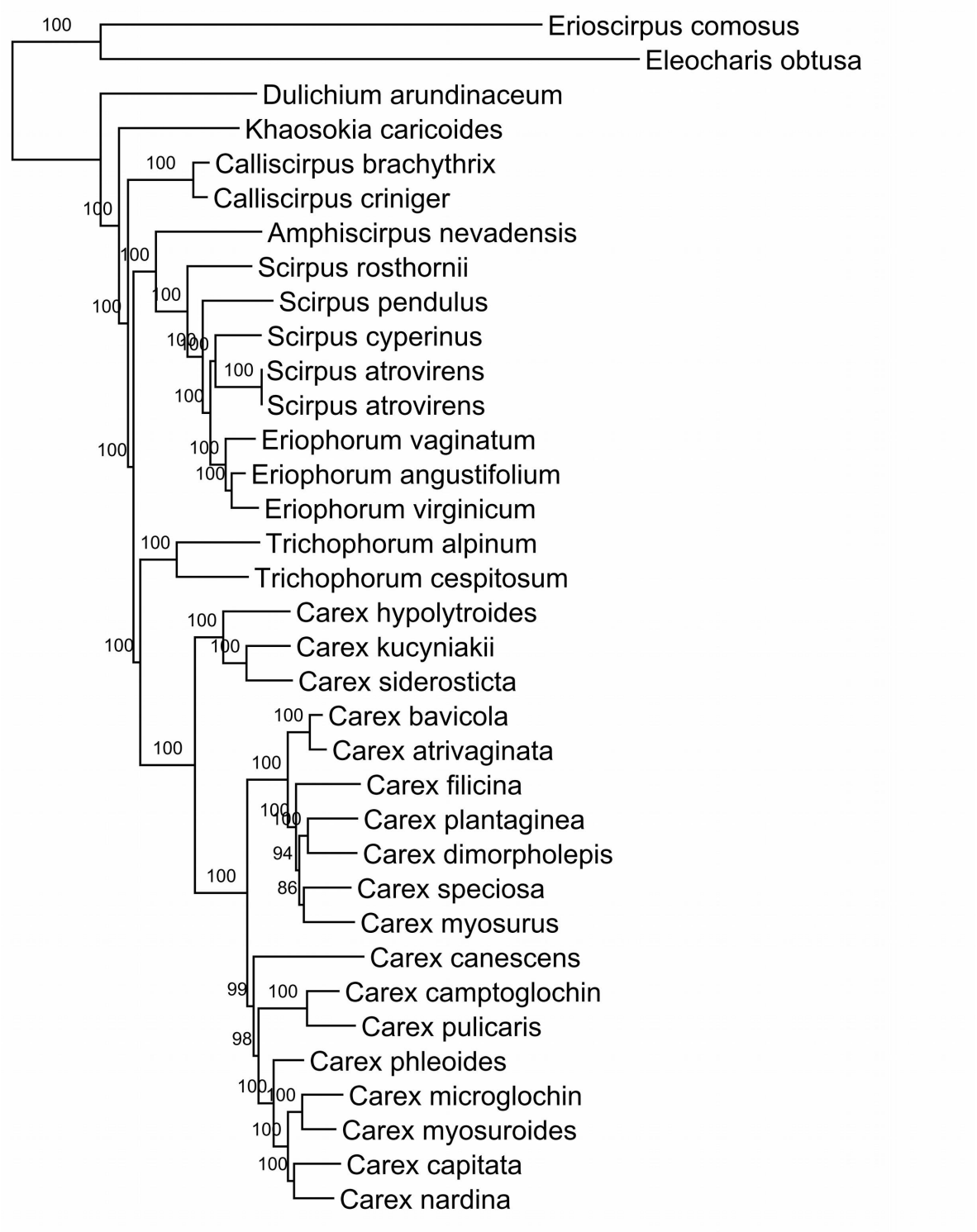


Figure 4.4. Maximum likelihood tree in RAxML searches. Support as ML bootstrap.

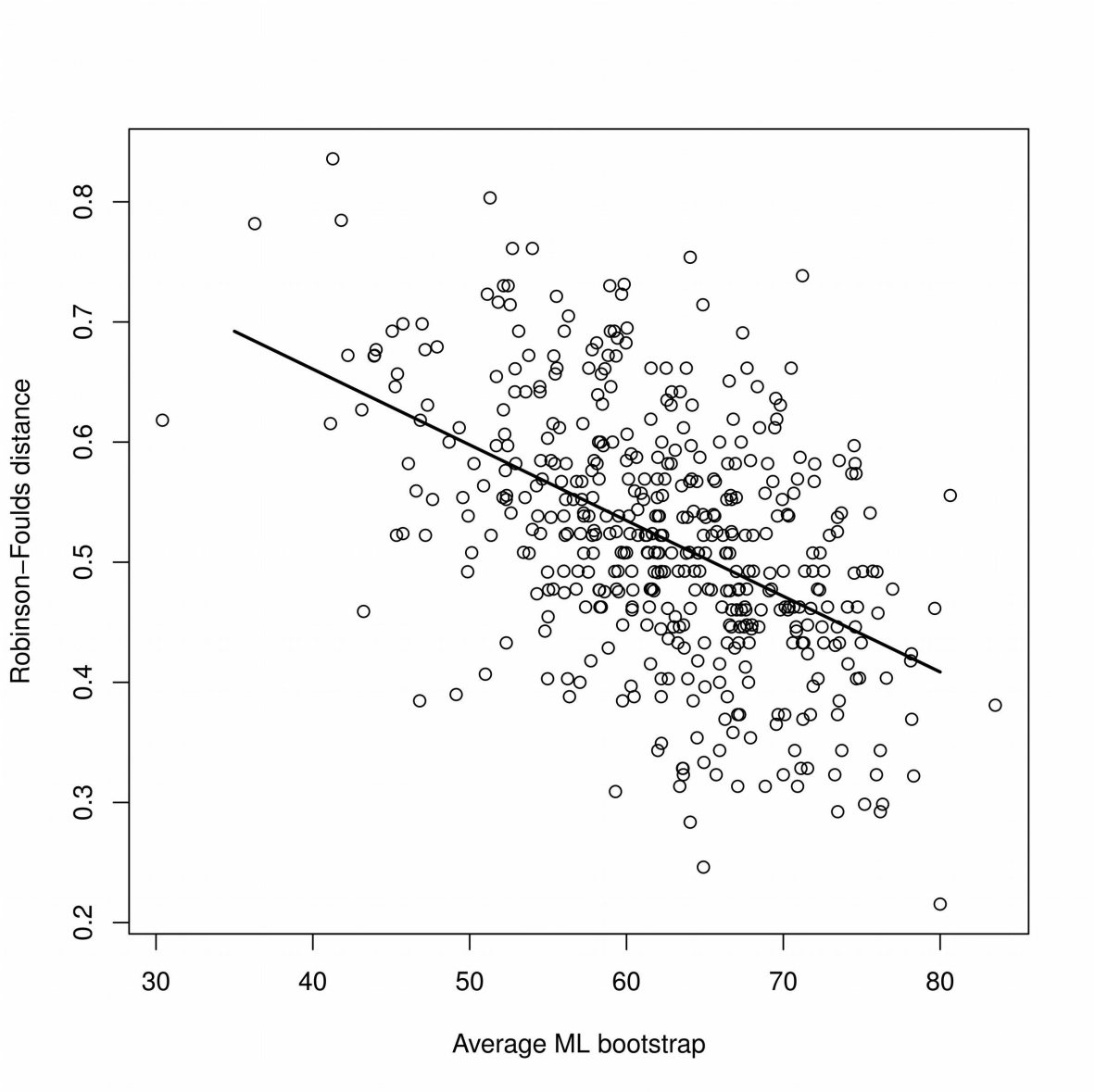


Figure 4.5. Negative relationship between average ML bootstrap of estimated gene trees and Robinson-Foulds distance between that gene tree and the ASTRAL species tree. Regression line estimated by standard linear regression. Regression line: $R^2 = 0.24$, slope = -0.0063, slope p-value < 10^{-15}.

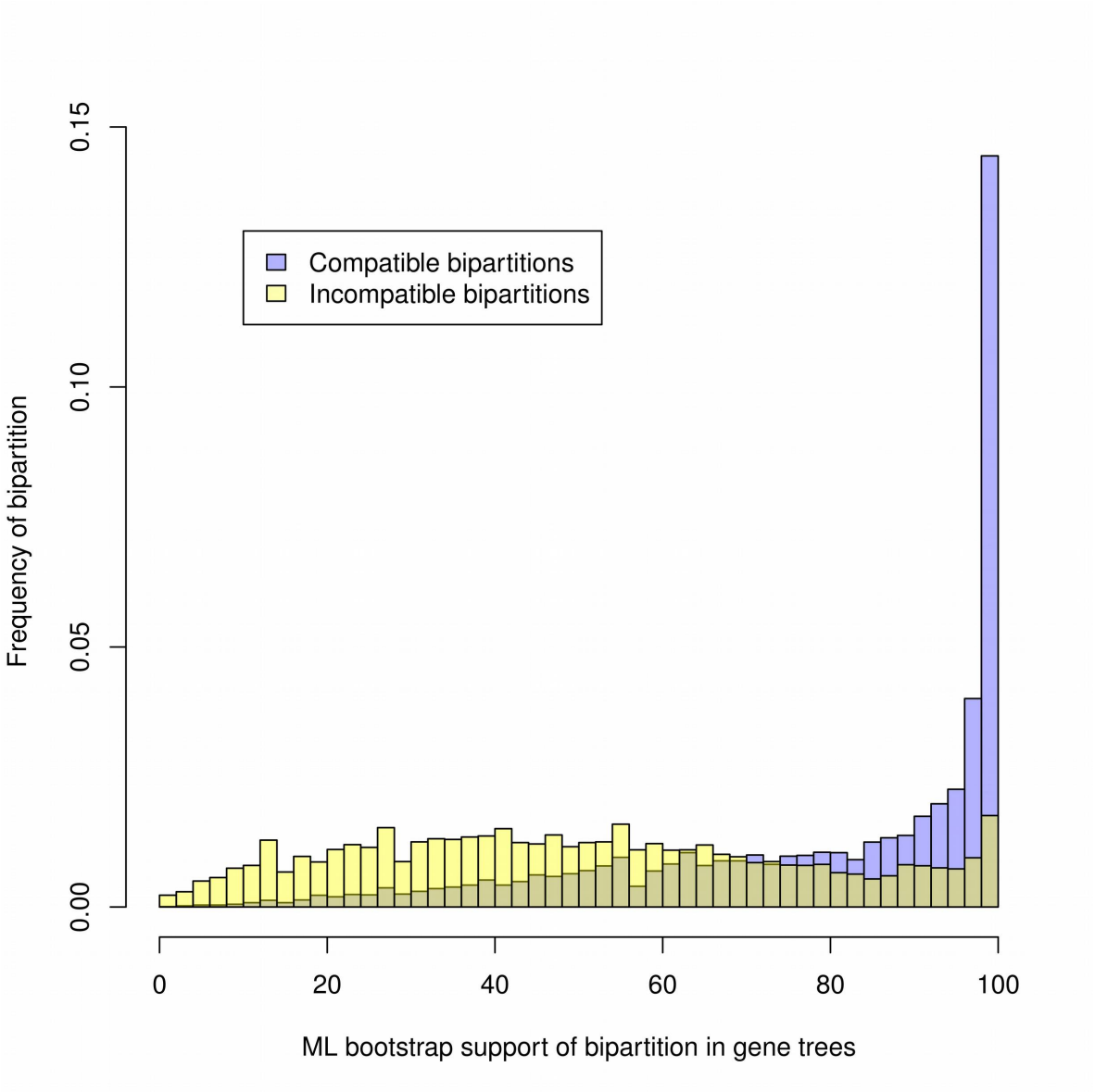


Figure 4.6. Relative frequency of bipartitions of estimated gene trees as a function of their ML bootstrap support. Bipartitions compatible with the ASTRAL species tree in blue, incompatible in yellow, showing that most gene tree bipartitions are compatible with the species tree beyond about 80% BS.

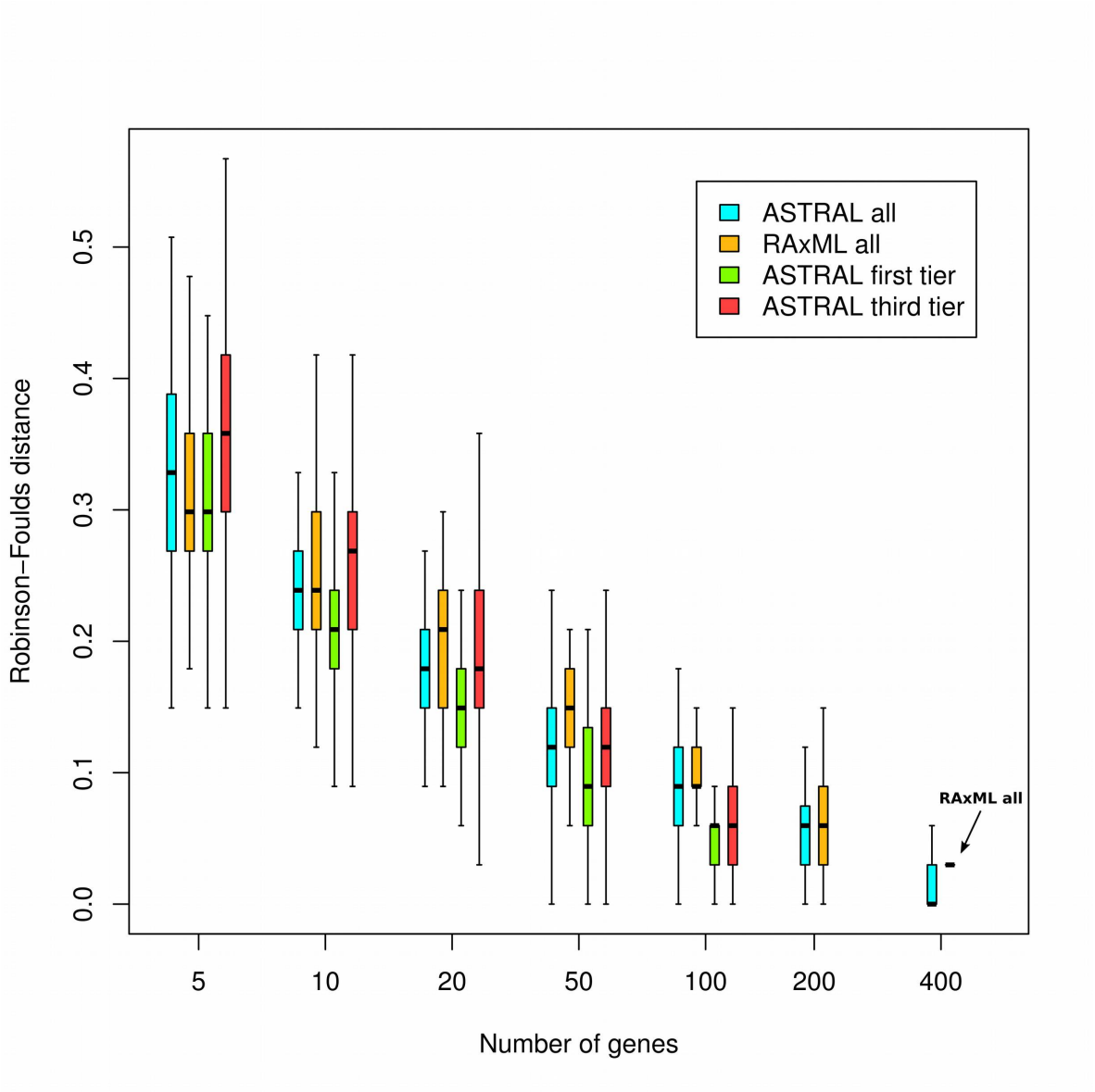


Figure 4.7. Results of reduced analyses, showing the distribution of Robinson-Foulds distance between reduced analyses tree and the best ASTRAL species tree, as a function of analytic method and number of loci. Note that since all trees were bifurcating, the Robinson-Foulds distance is equivalent to the proportion of conflicting nodes.

CHAPTER 5

WHY ARE THERE SO MANY SEDGES? SUMATROSCIRPEAE, A MISSING PIECE IN THE EVOLUTIONARY PUZZLE OF THE GIANT GENUS *CAREX* (CYPERACEAE)

This Chapter is a slightly modified version of an article published in the journal Molecular Phylogenetics & Evolution (http://dx.doi.org/10.1016/j.ympev.2017.10.025). Coauthors on the article are: Julian R. Starr and Bruce A. Ford. Disclaimer: new names presented here are not intended to be effectively published for nomenclatural purposes.

5.1 Introduction

The origins of easily recognized groups are often the most perplexing. Many ecologically important and diverse cosmopolitan lineages such as turtles and palms have been recognized for centuries (Batsch, 1788; de Jussieu, 1789), but the same characteristics that make them so distinctive and are possibly at the origin of their evolutionary success, also obscure their relationships to other taxa. Indeed, the sister-group of such lineages has often been difficult to identify, sometimes remaining contentious to this day (Chiari & al., 2012; Barrett & al., 2013). This has unfortunate consequences for the study of these fascinating groups because knowledge of their sister group is crucial for homology assessment, polarizing character states and biogeographic inferences.

The genus *Carex* L., sole member of tribe Cariceae (sedges, Cyperaceae; Global Carex Group 2015), represents just such an intriguingly singular group. With more than 2,100 species and a worldwide distribution (Global Carex Group, 2015), *Carex* is amongst the five largest plant genera (Frodin, 2004) and is more diverse than 92% of all vascular plant families (Christenhusz and Byng, 2016). Its varied ecology (e.g., deserts, tropical forests, tundra), intriguing cytology (agmatoploidy, n=6 to 66; Davies, 1956; Hipp & al., 2009), and astounding diversity in temperate and boreal latitudes (Ball, 1990; Cayouette,

2008) have made *Carex* a popular model for studying speciation, ecological diversification and biogeography (e.g. Escudero & al., 2010, 2012a, 2012b; Gehrke & Linder, 2011; Elliott & al., 2016; Spalink & al., 2016a, 2016b). However, its exceptional morphology, characterized by unisexual flowers lacking a perianth, but most strikingly by the presence of a perigynium, have obscured its relationships with other Cyperaceae. Consequently, no plausible relatives for *Carex* have ever been proposed despite intensive study.

The few taxa aligned with *Carex* in the past, tribes Sclerieae and Bisboeckelereae (Holttum, 1948; Kern, 1958; Koyama, 1962a; Schultze-Motel, 1964; Goetghebeur, 1986) and subfamily Mapanioideae (Mattfeld, 1935; Bruhl, 1995), correspond to the traditional division of the family into unisexually and bisexually flowered groups (de Jussieu, 1791; Bentham & Hooker, 1883), a convenient but superficial resemblance unsupported by other characters (Meert & Goetghebeur, 1979; Goetghebeur, 1986; Richards & al., 2006). As a result, the evolutionary and biogeographical origins of the genus have remained largely speculative (Starr & al., 2004). Phylogenetic results presented in Chapters 2 and 3 have confirmed its position within a strongly supported clade comprising tribes Dulichieae and Scirpeae, and the unplaced genus *Khaosokia* (Muasya & al., 1998; Simpson & al., 2007; Muasya & al., 2009). However, relationships within this CDS clade have been more difficult to resolve.

Nuclear phylogenomic results presented in Chapter 4 provided convincing evidence for a strongly-supported sister-relationship with the Trichophorum Clade, but this candidate remains so morphologically and genetically remote from *Carex* that it only reaffirms the isolated position of *Carex* within CDS. Indeed, bisexual flowers with perianth bristles are the norm in the Trichophorum Clade as in other Scirpeae s.lat., and while *Carex* shares unisexual flowers with *Khaosokia* and fertile prophylls with Dulichieae, both options are rejected by plastid phylogenetic (Chapters 2 and 3; Léveillé-Bourret & al., 2014) and nuclear phylogenomic analyses (Chapter 4; Léveillé-Bourret & al., 2018b). Considering current phylogenetic results, the peculiar inflorescence structures of *Carex* would thus appear to have no equivalent in any of its closest relatives.

The key to resolving the mystery of the origin of *Carex* might lie in the single CDS genus not included in any molecular phylogenetic studies to date: the small (~4 species) and extremely rare Eastern Asian *Sumatroscirpus* Oteng-Yeb. This genus possesses many characteristics such as highly-compound inflorescences, pedicellate spikelets, antrorsely scabrous bristles and tuberculate fruits that are inconsistent with its current placement in Dulichieae. More importantly, *Sumatroscirpus* is the only Cyperaceae genus besides *Carex* to possess sheathing fertile prophylls that isolate prophyllar flowers from those found in spikelets, a potential link to the perigynium of *Carex*. Combined with a long-held belief that "primitive" *Carex* had highly-compound inflorescences (Kreczetovicz, 1936; Nelmes, 1951a; Koyama, 1962b; Smith & Faulkner, 1976; Dahlgren & al., 1985; Reznicek, 1990), and a presumed Eastern Asian origin for the genus (Nelmes, 1951b; Raymond, 1955, 1959; Koyama, 1957; Waterway & al., 2009; Starr & al., 2015; Ford & al., 2017), *Sumatroscirpus* appears to have all the characteristics to qualify as the closest relative of *Carex*.

This study presents the first molecular phylogeny incorporating sequences from *Sumatroscirpus* and uses them in conjunction with morphological data to test the following hypotheses: (1) *Sumatroscirpus* is the sister-group to *Carex*, (2) the perigynium is not an autapomorphy for *Carex*, but a synapomorphy supporting a *Sumatroscirpus* + *Carex* clade, and hence (3) the perigynium is not the key innovation responsible for the remarkable radiation of *Carex*. Other evolutionary novelties such as spikelet truncation, reduction of flower complexity and rapid inflorescence development may have played a larger role in *Carex* diversification. The major implications of these results for the taxonomy, biogeography and morphological evolution of *Carex*, its allies and the CDS clade as a whole are discussed.

5.2 Materials & Methods

5.2.1 Taxonomic Sampling

Sampling aimed to represent all major CDS clades (Léveillé-Bourret & al., 2014), emphasising phylogenetic and morphological diversity within *Carex*. A total of 122 taxa representing all CDS genera and ~58% of the species outside Cariceae were included in analyses (Appendix 5). Analyses included one Chinese and three Vietnamese individuals of *Sumatroscirpus* sp. nov., a taxon that will be described in an upcoming taxonomic revision of the genus (Chapter 6; Léveillé-Bourret & al., In press). *Sumatroscirpus* sp. nov. is morphologically similar to the type for the genus, *Sumatroscirpus junghuhnii* (Miq.) Oteng-Yeb., but differs by its narrower leaves with abaxial papillae, pedicellate spikelets and abundance of sharp bristle barbs. Additionally, an ETS-1f sequence was obtained from the Chinese endemic *Scirpus paniculatocorymbosus* Kük., which is recombined below as *Sumatroscirpus paniculatocorymbosus* based on the data presented here and morphological evidence that will be presented in Chapter 6 (Léveillé-Bourret & al., In press). Despite efforts to obtain additional sequences, poor DNA quality prevented amplification of other regions for *S. paniculatocorymbosus*. Outgroup taxa were selected to represent the major lineages of the Abildgaardieae-Cypereae-Eleocharideae-Fuireneae and *Rhynchospora* Vahl clades, which are successive sisters to CDS (Muasya *& al.*, 2009). Taxonomy follows Govaerts *& al.* (2007) except for *Eriophorum*, which follows Novoselova's (1994a, 1994b) revision of the genus, and for the recognition of *Blysmopsis* Oteng-Yeb., *Calliscirpus* C.N.Gilmour et al., and *Rhodoscirpus* Lév.-Bourret et al. Names of major *Carex* clades (Minor Carex Alliance, Caricoid Clade, Schoenoxiphium Clade, Core Unispicate Clade, Vignea Clade, Dissitiflora Lineage, Small Core Carex Clade, Large Core Carex Clade) follow Starr & al. (2015).

5.2.2 Molecular Methods

Whole genomic DNA was extracted from herbarium specimens or field samples dried in silica gel using the silica-column protocol of Alexander & al. (2007) as modified by Starr & al. (2009). The plastid genes *matK* and *ndhF*, the plastid region *rps16*, and the nuclear ribosomal (nrDNA) regions ETS-1f and ITS were used. This marker combination includes easily aligned plastid markers that are informative at the generic and tribal levels, with common, genomically independent nrDNA regions that readily amplify from degraded DNA typical of herbarium specimens. Amplification by PCR and sequencing followed standard protocols. PCR primers for *matK* and *ndhF* are given in Gilmour & al.. (2013), for *rps16* in Peterson & al. (2010), and for ETS-1f in Starr & al. (2003). A new ETS-1f forward primer (ETS-1Fs: 5'-CTGTGGCGTCGYATGAGT-3') was designed for *Sumatroscirpus* sp. nov., which did not amplify using the standard ETS-1F primer due to a mutation at the 3' end of the annealing site. For ITS, the primers ITS-L (Hsiao & al., 1995) and ITS-4 (White & al., 1990) were used, or sometimes replaced ITS-L with AB 101 (=17SE; Sun & al., 1994). For plastid genes, PCR amplifications consisted of 1× reaction buffer (Bioline, United Kingdom), 2–2.5 mM $MgCl_2$ (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 µM of each primer, 1.1 µL Bovine Serum Albumin (BioShop, Canada), 0.6–1.5 U of Biotaq DNA Polymerase (Bioline) and 1–3 µL (~20–30 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. For *matK* and *ndhF*, 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 *rps16*, 180 s of initial denaturation followed by 40 cycles of 30 s of 95°C denaturation, 60 s of 47°C primer annealing and 150 s of 68°C extension, with a final extension step of 5 min. For ETS-1f, PCR amplifications consisted of 1× reaction Buffer (Bioline, United Kingdom), 2.5 mM $MgCl_2$ (Sigma Aldrich), 0.3 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.4 µM of each primer, 1 M Betaine (Sigma Aldrich), 0.6–2 U of Biotaq DNA Polymerase (Bioline) and 2–4 µL (~25–35 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free

ddH_2O. 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 48–52°C primer annealing and 120 s of 72°C DNA extension, with a final extension step of 7 min. For ITS, the same recipe was used except for 2.73 M Betaine. Cycling conditions for the ITS region were 120 s of initial denaturation followed by 40 cycles of 30 s of 94°C denaturation, 60 s of 48–50°C primer annealing and 180 s of 68°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 Inc., Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit version 3.1 (Applied Biosystems; Foster City, CA, USA). Sequencing termination products were purified according to a sodium acetate/alcohol protocol (Applied Biosystems) and sequenced on a 3130x1 Genetic Analyser. A few amplifications were purified and sequenced at Génome Québec, McGill University (Montréal, Québec, Canada). Reads were assembled and corrected with Geneious v.4.8.5 (Biomatters). All sequences were submitted to Genbank (Appendix 5).

5.2.3 Phylogenetic Analyses

Sequences were aligned with the MUSCLE or MAFFT algorithm as implemented in Geneious 8.1.8 (Kearse & al., 2012), and adjusted by hand using parsimony as an objective criterion (Starr & al., 2004). Alignments were concatenated by individual, although unavailability of certain sequences sometimes made it necessary to combine different individuals of the same species. All *Sumatroscirpus* sequences were concatenated by individual. The 5.8S region was excluded from the ITS alignment. Indels were coded with 2matrix 1.0 (Salinas & Little, 2014) using simple gap coding (Simmons & Ochoterena, 2000). The alignment is available online (http://dx.doi.org/10.1016/j.ympev.2017.10.025). Only results from combined analyses (*matK* + *ndhF* + *rps16* + ETS-1f + ITS + indels) are reported, as no supported (> 75% parsimony bootstrap) topological incongruence were

observed in single region analyses (results not shown). Additional analyses excluding indel characters, or using other phylogenetic methods, gave highly congruent results (see online Appendices at http://dx.doi.org/10.1016/j.ympev.2017.10.025).

Heuristic maximum parsimony (MP) searches were undertaken in PAUP* 4.0a150 for Linux (Swofford, 2003) using 10,000 random addition sequence (RAS) replicates, followed by swapping with tree-bisection-reconnection (TBR), with MULTREES on, STEEPEST off, COLLAPSE off, and maximum 20 trees retained per RAS. A strict consensus of all MP trees was assembled. Branch support was assessed using 3,000 bootstrap (BS; Felsenstein, 1985) replicates, with each replicate consisting of 5 RAS retaining 5 trees per RAS and using the strict-consensus BS (GRPFREQ = NO) to prevent undersampling-within-replicate and frequency-within-replicate artefacts (Simmons and Freudenstein, 2011). Decay indices (DI; Bremer, 1988, 1994) were computed using the default bremer.run script in TNT 1.5 (Goloboff & al., 2008).

Model-based searches were done using maximum likelihood (ML) in RAxML v8.2.10 (Stamatakis, 2014) on the Cipres web server (Miller & al., 2010). Partitioning scheme was selected with PartitionFinder v1.1.0 (Lanfear & al., 2012) using the greedy search algorithm and the Bayesian information criterion on all partition schemes and models implemented in RAxML. The best scheme included four partitions: (1) codon positions 1 and 2 of *matK* and *ndhF*, (2) rps16 and codon position 3 of *matK* and *ndhF*, (3) ETS-1f, and (4) ITS1 and ITS2. For RAxML searches, four additional indel partitions were used: (1) *matK* + *ndhF*, (2) *rps16*, (3) ETS-1f and (4) ITS1 and ITS2. A RAxML GTR+CAT (10 rate categories) model was used for the DNA partitions, and the binary+CAT model with ascertainment bias correction (Lewis, 2001) for the indel partitions. Substitution rates of every partitions were unlinked. Searches were made in RAxML using 500 random starting trees and the old, slower-but-more-accurate rapid hill-climbing algorithm. Branch support was assessed by 1,000 (standard) bootstrap replicates.

Maximum likelihood BS values were placed on the highest scoring ML tree with SumTrees 4.1.0 (Sukumaran & Holder, 2010) and parsimony BS values were added by hand. Tree figures were produced with TreeGraph 2.10.1-641 (Stöver & Müller, 2010) and Inkscape 0.91 (available at http://www.inkscape.org/). The alignment and ML topology are available online (http://dx.doi.org/10.1016/j.ympev.2017.10.025). Clade support was characterised subjectively as weak or poor (<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.

5.2.4 Molecular Dating

A sequence matrix was created for divergence time dating, including additional outgroup sequences representing major lineages of Cyperaceae, Poales, Commelinales, Zingiberales and Arecales (commelinids) to provide additional clock calibration points (Table 5.1). The plastid genes *matK* and *ndhF*, which could be reliably aligned across commelinids, were used. In addition, ETS-1f sequences were used only in the ingroup to help resolve closely related species, and because this was the only marker available for *Sumatroscirpus paniculatocorymbosus*. Crown or stem nodes were calibrated based on 16 fossils representing the oldest unambiguous representatives of major Cyperaceae and commelinid lineages (Table 5.1). A uniform age constraint was enforced on calibrated nodes with a minimum equal to the youngest date for each fossil calibration, and a maximum at 125 Mya representing the oldest unambiguous monocot and eudicot pollen fossils (Doyle, 2012; Iles & al., 2015). This strategy was chosen because the ingroup is nested within monocots, and this age is consistent with the maximum dates for commelinids obtained in most recent angiosperm node dating studies (e.g. Smith *& al.*, 2010a; Magallón *& al.*, 2015; Foster *& al.*, 2017).

Divergence time dating was done in BEAST 2.3.2 (Bouckaert & al., 2014) on the Cipres web server (Miller & al., 2010), using a Birth-Death tree prior and 17 uniform age constraints (Table 5.1). Partitioning scheme was selected with PartitionFinder 1.1.0 as

above. The best scheme included four partitions: (1) *matK* codon positions 1 and 2, (2) *ndhF* codon positions 1 and 2, (3) *rps16* and codon position 3 of *matK* and *ndhF*, and (4) ETS-1f. All partitions had linked tree and clock models, and unlinked GTR+Γ (4 rate categories) models with estimated relative rates and base frequencies. An uncorrelated log-normal molecular clock was used with as many categories as branches, and all other prior parameters were left at their default values. The program did not accept calibration on a single leaf, so the *Cladium mariscus* (L.) Pohl sequence was duplicated to use the stem calibration for *Cladium* P.Browne. Two Markov Chain Monte Carlo (MCMC) chains were run for 450 million generations, sampling trees every 25,000th generation. Parameter and topological convergence and stationarity were assessed respectively with Tracer 1.6.0 and the rwty 1.0.1 package in R (Warren & al., 2016). Parameters and topology stabilized and converged after 10 million generations, which were discarded as burn-in. All effective sample size (ESS) values were > 800 in the remaining sample. Posterior clade probabilities and node ages were combined with SumTrees 4.1.0 on the maximum clade credibility tree (MCCT) using the median as a point-estimate for node ages, and reporting the 95% highest posterior probability density (HPD) as a confidence interval. The effective shape of age priors and their influence on posteriors was assessed by running one chain on an empty alignment and comparing the shape of parameter estimates in this empty run with the results of the real runs. The BEAST xml file used for dating is available online (http://dx.doi.org/10.1016/j.ympev.2017.10.025).

5.2.5 Ancestral State Estimation

Twelve inflorescence characters of primary importance in evolutionary discussions on Cariceae were coded based on our own observations and the literature (Kükenthal, 1909; Ball & Reznicek, 2002; Dai & al., 2010a): (1) inflorescence complexity (unbranched, unispicate, 0; one branch order, a single terminal fascicle of spikelets, 1; two branch orders, multispicate, 2; three or more branch orders, highly-compound, 3); (2) presence of inflorescence branches on lower leaf-bearing internodes (never, 0; sometimes present, 1); (3) peduncle (epipodium) length of long inflorescence branches: (reduced, lateral

inflorescence units sessile, 0; elongate, lateral inflorescence units pedunculate, 1); (4) pedicel (epipodium) length of short branches (reduced, lateral spikelets sessile, 0; elongate, lateral spikelets pedicellate, 1); (5) shape of spikelet prophylls (tubular or utriculiform, not open, 0; scale-like or with a very large opening, 1); (6) fertile prophyll presence (absent, 0; present, 1); (7) terminal spikelet truncation of short branches: (not truncated, at least one flower or glume present following the prophyllar node, 0; truncated, with no flower or glume present, but sometimes with elongated sterile "rachilla", 1); (8) prophyll presence on long branches (absent, 0; present, 1); (9) disposition of sexes on branches (*Carex* only; always androgynous, 0; at least sometimes gynecandrous or mesogynous, 1); (10) disposition of sexes between branches (*Carex* only; spikes always bisexual, 0; spikes at least sometimes unisexual, 1); (11) perianth presence (absent, 0; present, 1); (12) flower sexuality (bisexual, 0; unisexual, 1). The inflorescence terminology used here is explained and illustrated in Reznicek (1990), Starr & Ford (2009) and Global Carex Group (2015). The character matrix is available online formatted as a Mesquite Nexus file (http://dx.doi.org/10.1016/j.ympev.2017.10.025).

Ancestral states of these characters were estimated in Mesquite 3.10 (Maddison & Maddison, 2011) on the ML topology using MP and ML. For parsimony reconstructions, character (1) was treated as ordered following Thiele (1993), but unordered reconstructions gave similar results. To account for topological uncertainty, reconstructions were made on 2,000 parsimony bootstrap replicates, randomly resolving polytomies, and summarized by counting trees that possess a state in the optimal set for a given node ("Count All Trees with State" option in Mesquite). States were then compared by dividing the count for one state by the sum of the counts for all states at a given node, thus attributing proportions summing to 1 for every state at each node, and the results were reported as proportion of parsimony replicates possessing state (PR). ML reconstructions were performed under a Markov k-state 1 parameter model (Mk1), treating all characters as unordered, reported as proportional likelihoods (pL) adding up to 1 for interpretability, with a star (*) indicating results differing by more than 2 log-likelihood values, a difference considered "significant" (Pagel, 1999). Reconstructions were then made on 1,000 ML bootstrap replicates, and

reported as the average proportional likelihood across all ML replicates (LR). Support for ancestral character reconstructions were characterised subjectively as weak or poor (<0.75), moderate (0.75–0.84), good or well-supported (0.85–0.94) and strong (0.95–1) based on the ML replicate proportions.

5.3 Results

5.3.1 Phylogenetic Results

The *matK*, *ndhF*, *rps16*, ETS-1f and ITS alignments were respectively 1,321 bp, 1,244 bp, 1,198 bp, 779 bp and 617 bp long, with 7, 15, 23, 5 and 14 unsequenced terminals, and with 8.1%, 15.8%, 41.4%, 29.8% and 35.4% missing or ambiguous bases. These loci had respectively 543 (41.1%), 447 (35.9%), 428 (35.7%), 578 (74.2%) and 394 (63.9%) variable characters, of which 292 (22.1%), 257 (20.7%), 238 (19.9%), 438 (56.2%) and 299 (48.5%) were parsimony-informative. They had respectively 14, 13, 159, 200 and 166 indel characters after simple gap coding. The concatenated alignment, including indel characters, was 5,711 characters long with 125 terminals, 23.4% missing data, 2,937 (51.4%) variable and 1,760 (30.8%) parsimony informative characters. Analyses excluding all terminals with missing sequences gave comparable results to those made including all terminals, thus they are not reported. A total of 166 out of 563 sequences used in this study are newly submitted to Genbank (Appendix 5).

The parsimony searches found 3,837 trees of 9,497 steps with consistency and retention indices of 0.44 and 0.73. The ML topology (Fig. 5.3) had a log-likelihood of -58,028.617670 as calculated by RAxML. The ML topology was very similar to the strict consensus of all MP trees, with the only exceptions being weakly-supported minor changes in the position of a few species deep within *Scirpus* L., the Caricoid Clade, the Vignea Clade and the Major Core Carex Clade. Searches excluding all terminals with missing sequences, as well as searches excluding *Carex* and/or *Sumatroscirpus*, all gave similar

results to searches using the full matrix. Results of the MP and ML bootstrap analyses are also broadly congruent, with the MP values generally slightly more conservative (Fig. 5.3). As a result, only parsimony BS values are cited and discussed.

Phylogenetic analyses position the strongly supported *Blysmopsis* + *Blysmus* Panz. ex Schult. + *Dulichium* Pers. clade (Dulichieae s.str., 100% BS, DI=41) and *Khaosokia* as successive sisters (90% BS, DI=7) to a well-supported (93% BS, DI=6) clade consisting of six major lineages (*Calliscirpus*, *Carex* or Cariceae, *Sumatroscirpus* or Sumatroscirpeae, *Cypringlea* M.T.Strong + *Oreobolopsis* T.Koyama & Guagl. + *Trichophorum* Pers. or "Trichophorum Clade", *Scirpus* + *Eriophorum* L. or "Scirpus Clade", and *Amphiscirpus* Oteng-Yeb. + *Phylloscirpus* C.B.Clarke + *Rhodoscirpus* + *Zameioscirpus* Dhooge & Goetgh. or "Zameioscirpus Clade"), all of which receive good to strong support (≥94% BS, DI≥12; Fig. 5.3). Within this clade, *Calliscirpus* (100% BS, DI=57) is poorly supported (<50% BS, DI<1) as sister to a monophyletic group composed of Trichophorum Clade + *Sumatroscirpus* + *Carex* (68% BS, DI=2) and a Zameioscirpus Clade + Scirpus Clade (100% BS, DI=15). *Sumatroscirpus* is strongly supported (100% BS, DI=18) as sister to *Carex*. Within *Carex*, the Minor Carex Alliance (82% BS, DI=4) is strongly supported (99% BS, DI=9) as sister to all other *Carex* species (Fig. 5.3). *Carex satsumensis* Franch. & Sav. is weakly supported (62% BS, DI=2) as sister to all other Major Carex Alliance species. The Schoenoxiphium, Core Unispicate, Vignea and Core Carex Clades are all well-supported (≥89% BS, DI≥4), but the relationships between these clades receive weak or no support.

5.3.2 Node Dating

The Bayesian topology estimate is compatible with MP and ML estimates of the phylogeny, and support values are broadly similar (Fig. 5.4). The effective shapes of the node age priors were non-uniform due to the problem of prior interaction in Bayesian node dating (Warnock & al., 2014). However, posterior ages appeared relatively unaffected by prior shapes within Cyperaceae, with most age posterior distributions departing strongly from the effective priors (Fig. 5.4). The root age (commelinids) is estimated to be 124 Mya

(HPD: 120–125 Mya; early Cretaceous). The crown node of Cyperaceae is dated at 85 Mya (HPD: 74–95 Mya; late Cretaceous); the CDS crown at 50 Mya (HPD: 43–57 Mya; early Eocene); the *Sumatroscirpus*-*Carex* divide at 36 Mya (HPD: 34–41 Mya; late Eocene); the *Sumatroscirpus* crown at 13 Mya (HPD: 4–25 Mya; middle Miocene); the *Carex* crown at 31 Mya (HPD: 27–36 Mya; early Oligocene); and all major *Carex* clades (Core Unispicate, Schoenoxiphium, Vignea, Minor Core Carex and Major Core Carex Clades) by 19 Mya (HPD: 14–23 Mya; early Miocene).

5.3.3 Ancestral Character States

Ancestral state reconstructions support the CDS ancestor as having had a "multispicate" (pL = 0.35*, LR = 0.54, PR = 0.56) or highly-compound (pL = 0.59*, LR = 0.45, PR = 0.44) inflorescence, with open (pL = 0.98*, LR = 1.00, PR = 1.00) sterile spikelet prophylls (pL = 1.00*, LR = 0.99, PR = 1.00), and bisexual flowers (pL = 1.00*, LR = 1.00, PR = 1.00) with a developed perianth (pL = 1.00*, LR = 1.00, PR = 1.00). Highly-compound inflorescences are supported for most of the backbone, including the branch leading to *Sumatroscirpus* + *Carex* (pL = 0.98*, LR = 0.99, PR = 0.76), the one leading to *Carex* (pL = 0.99*, LR = 0.96, PR = 0.76), the Minor Carex Alliance (pL = 0.99*, LR = 0.96, PR = 0.78) and the Major Carex Alliance (pL = 0.94*, LR = 0.76, PR = 0.62), with multiple reductions of inflorescence complexity within *Carex*. Androgynous spikes are strongly supported as ancestral in *Carex* (pL = 1.00*, LR = 1.00, PR = 1.00), with gynecandry/mesogyny appearing multiple times deeply nested within the Vignea Clade and Core Carex Clade, and with a moderately supported transition to unisexual spikes happening on the branch leading to the *Carex myosurus* Nees–*Carex aquatilis* Wahlenb. clade (pL = 0.89*, LR = 0.76, PR = 0.80).

Fertile prophylls appear twice; in Dulichieae s.str. (pL = 1.00*, LR = 0.99, PR = 1.00), and on the branch leading to *Sumatroscirpus* + *Carex* (pL = 0.96*, LR = 1.00, PR = 1.00). The prophylls of Dulichieae s.str. are ancestrally open (pL = 1.00*, LR = 0.99, PR = 1.00), while they are closed in the most recent common ancestor of *Sumatroscirpus* + *Carex* (pL = 0.83, LR = 0.97, PR = 1.00), with several reversals to the open state within

Carex. Fertile sheathing prophylls (perigynia) are thus a synapomorphy for the *Sumatroscirpus* + *Carex* clade. Additional details on the ancestral state estimation analyses and figures showing the maximum likelihood estimates can be found online (http://dx.doi.org/10.1016/j.ympev.2017.10.025).

5.3.4 Taxonomic results

Sumatroscirpeae Lév.-Bourret & J.R.Starr, **trib. nov.** – Type: *Sumatroscirpus* Oteng-Yeboah.

Diagnosis: Differs from all other Cyperaceae tribes by this unique combination of characters: prophylls of the inflorescence fertile and sheathing, spikelets spirally inserted on rachis, all glumes fertile, flowers bisexual, perianth present, style base inflated.

A single genus: *Sumatroscirpus* Oteng-Yeboah.

Sumatroscirpus paniculatocorymbosus (Kük.) Lév.-Bourret & J.R.Starr, **comb. nov.** ≡ *Scirpus paniculatocorymbosus* Kük., Acta Horti Gothob. 5: 35. 1930 – TYPE: CHINA. Sichuan: Prov. Sze-ch'uan, reg. austr.: Ta-hsiang-ling. Ad rivulum in prato. 2800 m. May 28, 1922, Harry Smith 2092 (holotype: UPS [V-142813]!; isotypes: B [10 0525489]!, GB [GB-004 7626]!, PE [00026382] [photo!]).

5.4 Discussion

5.4.1 Sumatroscirpeae and Cariceae: Two Monogeneric Sister Tribes of Tertiary Age

The phylogenetic analyses place *Sumatroscirpus* as sister to *Carex* (Cariceae) with strong support and provide the first robust phylogenetic hypothesis to include all CDS genera in a single analysis, an essential framework for future studies of the spectacular radiation of *Carex*, one of the world's most diverse plant genera (Frodin, 2004). We can now be confident that *Sumatroscirpus* is the sister-group to *Carex* because all other Cyperaceae genera not yet included in molecular studies lack fertile prophylls and possess

morphological or embryological features not found in the CDS clade (Goetghebeur, 1998). Furthermore, the few morphologically unusual species remaining in *Eriophorum* and *Scirpus* (Scirpeae) possess characters such as pseudolateral inflorescences, sterile basal glumes, or distichous flowers, indicating affinities with unrelated CDS lineages, or distantly-related tribes such as Abildgaardieae, Fuireneae, and Cypereae.

The backbone topology is also fully consistent with the findings of Chapters 2 and 3 (Léveillé-Bourret & al. 2014, 2015), supporting the Trichophorum Clade as the next closest relative to *Carex* after *Sumatroscirpus*, and confirming the isolated position of *Khaosokia*. This contrasts with earlier studies that included a more limited taxonomic sampling and no representatives of *Sumatroscirpus*, and which variously placed a monophyletic Scirpeae (Muasya & al., 2009), *Calliscirpus* (Gilmour & al., 2013), or a clade consisting of the Scirpus + Zameioscirpus Clades (Jung & Choi, 2012) as sister to *Carex*. The accuracy of the phylogenetic estimate presented here is supported by the congruent results obtained in a recent phylogenomics study based on hundreds of single copy nuclear genes presented in Chapter 4 (Léveillé-Bourret & al., 2018b). The paraphyly of Scirpeae and polyphyly of Dulichieae s.l. is therefore well demonstrated and highlight the need for a complete revision of tribal circumscriptions in CDS. As a first step in this direction, the new tribe Sumatroscirpeae is recognized here to highlight the significance of the 36 million years of unique evolutionary history experienced by the genus *Sumatroscirpus*, and to restore the monophyly of Dulichieae.

The close relationship between *Carex* and *Sumatroscirpus* has major implications for future phylogenetic studies, because it will reduce potential long-branch attraction problems and facilitate the use and alignment of more informative, rapidly-evolving markers. Backbone relationships within *Carex* remain an outstanding question in Cyperaceae systematics, with previous studies showing variable and unsupported relationships between major *Carex* clades (Starr & Ford, 2009; Starr & al., 2015 and references therein). A bottleneck to resolving this question is the low information content of currently used molecular markers (nrDNA, plastid genes), which are unable to support the

very short backbone branches of the *Carex* radiation. Next-generation sequencing methodologies such as RADseq (Eaton & al., 2017) or taxon-specific hybrid-enrichment (Harvey & al., 2016) offer the hope of resolving the *Carex* backbone, but the number and informativeness of markers retained in such analyses depend in large part on the ability to align divergent sequences between ingroups and outgroups. Thus, using the most closely-related outgroups available will reduce the chance for long-branch attraction problems to happen, and will also facilitate alignment of more rapidly evolving markers between *Carex* ingroups and the selected outgroups. The more recent divergence between *Carex* and *Sumatroscirpus* (ca. 36 Mya), as compared to other potential outgroups (e.g. *Scirpus*, ca. 43 Mya), would appear to make *Sumatroscirpus* the obvious choice in future studies.

5.4.2 The Perigynium: A Synapomorphy for Carex and Sumatroscirpus and its Role in Diversification

The sister-group relationship between *Carex* and *Sumatroscirpus* has major implications for understanding the perigynium (= utricle), one of the most distinctive and enigmatic structures found in plants. Although highly variable in size, color and texture, the perigynium is easily recognizable due to its flask-like shape and the presence of an enclosed female flower. Because of its peculiar shape and position, few plant organs have perplexed plant morphologists more than the perigynium, with discussions on the homology of the structure dating back more than 200 years (Holm, 1896; Jiménez-Mejías & al., 2016a, and references therein). Morphological, anatomical and developmental studies have demonstrated that the perigynium is a prophyll (Smith & Faulkner, 1976; Vrijdaghs & al., 2010; Jiménez-Mejías & al., 2016a), but the evolution of the organ has remained puzzling because of the lack of comparable structures in other Cyperaceae, until the present discovery of a relationship with *Sumatroscirpus*.

A prophyll is the first leaf (or pair/whorl) of a branch in flowering plants, recognized by a tendency to be modified compared to succeeding leaves (e.g. bracteole, palea, perigynium). In Cyperaceae, as in many other monocotyledons (Arber, 1925), prophylls are inserted close to the bract subtending the branch, addorsed to the main axis, and often

consist of a bladeless sheath. Contrary to previous reports (Koyama, 1962a; Dahlgren & Clifford, 1985), differentiated prophylls are present on all Cyperaceae branches, with few exceptions (Haines, 1966; Goetghebeur, 1998; pers. obs.). Typical Cyperaceae prophylls are sterile and associated either with a compound branch (cladoprophylls) or a lateral spikelet (spikelet prophylls). Cladoprophylls are usually tubular, while spikelet prophylls are variously tubular to scale-like, depending mostly on the length of the internode above them (epipodium). In the inflorescence of *Carex*, cladoprophylls are mostly tubular and sterile, while spikelet prophylls, called perigynia, are fertile (Fig. 5.2).

Fertile prophylls are not unique to *Carex*; they are also seen in tribe Dulichieae and in *Sumatroscirpus*. In Dulichieae, fertile prophylls look like normal flower scales except for the presence of two midribs. In *Sumatroscirpus* and *Carex*, they are generally sheathing around their flower, with their margins fused, and are morphologically distinct from bracts and flower scales (Fig. 5.2). These differences are consistent with the ancestral state reconstructions estimating two independent origins for fertile prophylls: (1) open scale-like fertile prophylls in Dulichieae, and (2) sheathing fertile prophylls in the most recent common ancestor of *Carex* and *Sumatroscirpus*. Therefore, the fertile spikelet prophylls of *Carex* and *Sumatroscirpus* not only share basic structural and positional similarity, they also share a common evolutionary origin, strongly suggesting that both structures should be called by the same name. Homology between the perigynium of *Carex* and the spikelet prophyll of *Sumatroscirpus* (sensu Patterson, 1982; de Pinna, 1991) means that the perigynium is not an autapomorphy for *Carex*, but a synapomorphy for the *Sumatroscirpus* + *Carex* clade. In other words, *Sumatroscirpus* possesses perigynia.

The tubular perigynia of *Sumatroscirpus* are most similar to the tubular, sterile cladoprophylls at the base of main branches in many *Carex*. However, *Sumatroscirpus* perigynia have a small inflated "pulvinus" at their bases, opening up the inflorescence branch in the same way as the pulvini of the sterile "inflorescence prophylls" (secondary cladoprophylls) of *Carex* subgen. *Vigneastra* and the pulvinate cladoprophylls seen in many genera like *Hypolytrum* Pers., *Rhynchospora*, *Khaosokia* or *Cyperus* L., to name but a few

(Holm, 1903; Goetghebeur, 1998; Starr & al., 2015; pers. obs.). This demonstrates the high morphological lability of perigynia and the fact that utriculiform and tubular perigynia represent only extremes of a morphological continuum, even within *Carex* (Le Cohu, 1968). Morphological differences between *Sumatroscirpus* and *Carex* perigynia also highlight the more important role of congruence over similarity (Patterson, 1982, 1988; de Pinna, 1991), and of position over special quality (Vrijdaghs & al., 2010) in demonstrating homology in plants.

The exceptional diversity of perigynium morphology seen in *Carex* might result from reduction and truncation of the perigynium axis ("rachilla"), thereby releasing mechanical constraints imposed by a large protruding axis on perigynium shape. Indeed, in *Sumatroscirpus* as in other Cyperaceae, prophylls appear constrained to a tubular sheath, sometimes pulvinate, surrounding peduncles and pedicels, or forced to an open scale-like shape when contiguous with sessile spikelets (pers. obs.; Timonen, 1985). These prophylls closely fill the available space, leaving little room for further shape variation. In *Carex*, however, reduction or truncation of the axis beyond the perigynium node and flower reduction (perianth loss, unisexuality) has freed space around and inside the perigynium, perhaps enabling the evolution of the diverse array of perigynium morphologies seen today. Examples include balloon-shaped perigynia rolling on sand in the desert-dwelling *Carex physodes* M.Bieb. (Egorova, 1999), inflated perigynia floating on water in numerous wetland "bladder sedges", winged samara-like perigynia (*C.* sect. *Cyperoideae* G.Don), retrorse ballistochorous perigynia (*C. pauciflora* Lightf., *C. microglochin* Wahlenb.), elaiosomes for ant dispersal (*C. digitata* L., *C. pedunculata* Muhl. ex Willd.), retrorse teeth adapted to epizootic dispersal (*C. collinsii* Nutt., *C. comosa* Boott), and brightly-colored perigynia (*C. aurea* Nutt., *C. baccans* Nees) encouraging endozootic bird dispersal (Savile & Calder, 1953; Kern, 1974; Handel, 1976; Hutton, 1976; Egorova, 1999; Leck & Schütz, 2005). Reduction of the spikelet axis has thus resulted in a new dispersive and protective unit for each fruit, and the reduced axis itself sometimes becomes secondarily adapted for dispersal, such as the hook-shaped "rachilla" of *Carex* species previously placed in the genus *Uncinia* (Reznicek, 1990). These novel adaptive morphologies may have in turn

driven an increase in diversification rate or facilitated the maintenance of high species diversity generated by other mechanisms by facilitating colonization of new habitats and niches (Spalink & al., 2016a). Hence, we hypothesise that the initial key innovation eventually leading to the diversification of *Carex* was not the evolution of the perigynium (in agreement with the view of Escudero & al., 2012b), but those changes which permitted selection to act on perigynia. In other words, the perigynium is a single step in a series of evolutionary innovations, culminating in a combination of traits that only taken together might explain the diversification of *Carex*. This is similar to many other important plant radiations that appear to have been caused by a series of small changes occuring over many successive branches of the phylogeny, rather than by a "key innovation" occuring on any single branch (Donoghue, 2005).

Characteristics such as the separation of female and male flowers might also have been important to reduce selfing by moving anthers out of the confines of the closed perigynium, and secondarily enabled selection to act on flower sex ratios. Other innovations such as the initiation of flowers at the end of the previous growth season and simultaneous development of all flowers (Smith, 1966; pers. obs.) may also explain the extraordinary temperate radiation of *Carex* by compressing flowering phenology, and thus facilitating phenological isolation between sympatric populations. The unusually high rate of chromosomal evolution by fission and fragmentation seen in *Carex* has also been invoked to explain its radiation (Escudero & al., 2012b, 2016). Future studies should test for potential links between diversification rates and inflorescence complexity, perigynium morphological disparity, phenology, chromosome numbers, and ecological niche. These studies will need to include comparative data on a more comprehensive taxonomic sample of *Carex*, *Sumatroscirpus*, and their relatives.

5.4.3 Sumatroscirpus Clarifies Inflorescence Evolution and Male Flower Homology in Carex

Evolution of *Carex* inflorescences has been the subject of intensive studies due to their high structural diversity and the simplified morphology of their flowers. Early hypotheses proposed highly-compound inflorescences as ancestral in Cariceae, with reduction viewed as the most important trend in *Carex* inflorescence evolution (Kreczetovicz, 1936; Nelmes, 1951a, 1951b; Koyama, 1962; Smith & Faulkner, 1976; Dahlgren & al., 1985; Reznicek, 1990). Recent phylogenetic analyses have contested these hypotheses because of the nested position of the highly-compound *Carex* species formerly included in the genera *Schoenoxiphium* and *Kobresia*, and in the *Carex* subgenera *Vigneastra* and *Vignea* p.p. (Ford & al., 2006; Global Carex Group, 2015 and references therein). Previous ancestral state reconstructions thus supported a multispicate inflorescence as ancestral in *Carex* (Starr & Ford, 2009). However, the present analyses strongly support a highly-compound inflorescence as ancestral, with repeated subsequent reductions and proliferations, illustrating the importance of outgroup relationships in determining character polarity (Watrous & Wheeler, 1981; Wheeler, 1990; Lyons-Weiler & al., 1998; Graham & al., 2002; Wilberg, 2015). This result is also in line with the recent recognition of many early-diverged lineages with highly-compound inflorescences throughout the *Carex* phylogeny (e.g. sect. *Hypolytroides* Nelmes, sect. *Hemiscaposae C.B.Clarke*, sect. *Surculosae* Raymond, *Carex satsumensis*, Small Core Carex Clade; Starr & al., 2015).

Another feature that has caused much debate is the homology of male "flowers" in *Carex*. This is mostly due to the views of Martens (1939), Smith (1966), Smith & Faulkner (1976), and more recently Timonen (1998) and Vegetti (2002), who suggested that *Carex* male "flowers" were highly reduced spikelets. The spikelets of perfect flowers found at the tip of every axis in *Sumatroscirpus* support the opposite view. These spikelets can be most parsimoniously interpreted as homologous with the terminal male portion of the ancestral androgynous spikes of *Carex* (Figs. 5.1, 5.2), suggesting the latter are spikelets of true male flowers. Although gynecandrous and mesogynous spikes break up the whole concept of the

spikelet (Timonen, 1998), these states are secondarily derived in *Carex*. The interpretation of male flowers as true flowers also finds support from teratology (Gehrke & al., 2012) and floral ontogeny (Vrijdaghs & al., 2009, 2010). It is worth noting that at early developmental stages, plant primordia are free to follow several different developmental courses, and the capacity of young *Carex* primordia to develop into either male flowers or a short branch (Martens, 1939; Smith, 1967; Smith & Faulkner, 1976) is probably only a reflection of this developmental flexibility (see Vrijdaghs & al., 2010 for further discussion).

The inflorescence structure of *Sumatroscirpus* also contradicts common ideas on the homology of inflorescence parts of Cyperaceae: that prophylls are "modified glumes", that prophylls are part of spikelets, or that *Carex* can have "bisexual spikelets" (e.g. Reznicek, 1990; Goetghebeur, 1998; Vegetti, 2002; Global Carex Group, 2015; Jiménez-Mejías & al., 2016a). Fertile prophylls are present at the base of every branch in *Sumatroscirpus*, and are similar in morphology throughout the inflorescence. Long branches consist of a fertile prophyll, followed by one or several bracteate branch-bearing nodes, ending with a terminal spikelet. On these branches, the prophyll is thus separated from the spikelet by multiple non-spikelet nodes (as in *Carex*, see Timonen 1985, 1989), and to consider the prophyll as part of a spikelet would necessitate spikelets to be made of unconnected, widely distant parts. This shows that prophylls are not modified glumes or part of spikelets, but are distinct leafy structures of the same rank as bracts and glumes. A corollary is that the internode following the prophyll node (epipodium), commonly called "rachilla" when it follows a *Carex* perigynium, is also not part of a spikelet; it is a spikelet pedicel, and thus not a "rachilla" in the usual sense (i.e. axis of a spikelet). *Carex* spikelets are thus never "bisexual"; they are always unisexual and male. Female flowers are found only at the prophyll node, which is not part of the spikelet.

5.4.4 Southeast Asia: Cradle or Museum for Sumatroscirpus and Carex?

Southeast Asia has been suggested as the center of origin of *Carex*, a hypothesis initially proposed on the basis of the high number of morphologically unusual endemic *Carex* lineages in the region (Nelmes, 1951b; Raymond, 1955, 1959; Koyama, 1957). This

hypothesis would be in line with the sister position of the Southeast Asian *Sumatroscirpus* and the high number of early-diverged, Asian-endemic lineages in *Carex* (already noted by Waterway & al., 2009; Starr & Ford, 2009; Starr & al., 2015) and CDS as a whole (Simpson & al., 2005; Léveillé-Bourret & al., 2014, 2015). However, Southeast Asia is known to possess many relictual plant lineages due to its climatic stability and topographic diversity throughout the Tertiary and Quaternary (Morley, 1998; Thorne, 1999; Milne & Abbott, 2002; Manchester & al., 2009), a fact that should at least suggest the possibility that the region acted more as a museum than a cradle for *Sumatroscirpus* and *Carex* (López-Pujol *& al.*, 2011a, 2011b). That extinction played an important role in the current biogeographic patterns of the *Sumatroscirpus* + *Carex* clade is also suggested by the early Tertiary origin and the highly unbalanced partitioning of diversity throughout the clade, with early-diverged, species-poor Asian lineages almost always sister to diverse cosmopolitan lineages (as discussed in Starr & al., 2015).

The alternative hypothesis of Eastern Asia acting as a refugium for early-diverged CDS lineages requires further investigation using a taxonomic sampling representative of both the phylogenetic and biogeographical diversity of the entire clade. Methodologies able to cope with the effects of unequal extinction rates between geographic areas are needed (e.g. GeoSSE; Goldberg *& al.*, 2011), since this phenomenon has been shown to bias ancestral area estimation when ignored (Iles & al., 2014; Sanmartín and Meseguer, 2016; Pinzón, 2016), and was significant in the northern hemisphere during the late Tertiary and Quaternary coolings (Milne and Abbott, 2002; Manchester & al., 2009). Nonetheless, our results highlight the need to include *Sumatroscirpus* in any future studies aimed at resolving the historical biogeography of *Carex* and allies.

5.5 Table and Figures

Table 5.1. List of fossils and priors utilized in BEAST analysis. The constrained node, prior distribution range in million years before present (Mya), fossil name and organ are provided for each prior. References provide fossil descriptions and justifications for placement and minimum age.

Constrained node	Prior (Mya)	Fossil name	Affinity	Organ	Reference
Maximum constraint	125	*Liliacidites* "sp. A" Doyle & Hickey, 1976	Monocot indet.	pollen	Iles & al., 2015
Arecaceae crown	84–125	*Sabalites carolinensis* E.W.Berry	Coryphoideae	leaf	Iles & al., 2015
Musaceae + Zingiberaceae crown	72–125	*Spirematospermum chandlerae* E.M.Friis	Zingiberaceae	seed	Iles & al., 2015
Typhaceae crown	52–125	*Typha* L.	*Typha*	inflorescence	Iles & al., 2015
Anarthriaceae + Restionaceae crown	30–125	*Restiocarpum latericum* M.E.Dettman & Clifford	Restionaceae + Centrolepidaceae	seed	Iles & al., 2015
Bambusoideae + Oryzoideae + Pooideae crown	66–125	*Changii indicum* V.Prasad & al.	Oryzeae	epidermis	Iles & al., 2015
Juncaceae crown	34–125	*Juncus vectensis* Collinson	*Juncus*	seed	Smith & al., 2010b
Cyperaceae crown	47–125	*Volkeria messelensis* S.Y.Sm. & al.	Mapanioideae	infrutescence	Iles & al., 2015
Sclerieae crown	34–125	*Scleria* P.J.Bergius	*Scleria*	fruit	Smith & al., 2010b
Cladium stem	23–125	*Cladium* P.Browne	*Cladium*	fruit	Smith & al., 2010b
Eleocharideae + Fimbristylideae crown	23–125	*Fimbristylis* Vahl	*Fimbristylis*	fruit	Smith & al., 2010b
Cypereae crown	23–125	*Cyperus* L.	*Cyperus*	fruit	Smith & al., 2010b
Dulichieae crown	23–125	*Dulichium* Pers.	*Dulichium*	fruit	Smith & al., 2010b
Scirpus stem	28–125	*Scirpus* L.	*Scirpus*	fruit	Smith & al., 2010b

Constrained node	Prior (Mya)	Fossil name	Affinity	Organ	Reference
Carex stem	34–125	*Carex colwellensis* M.Chandler	*Carex*	fruit	Jiménez-Mejías & al., 2016b
Core *Carex* Clade stem	23–125	*Carex hartauensis* Mai	*Carex* subgen. *Carex*	fruit & perigynium	Jiménez-Mejías & al., 2016b
Carex subgen. *Vignea* stem	16–125	*Carex* spp.	*Carex* subgen. *Vignea*	fruit	Jiménez-Mejías & al., 2016b

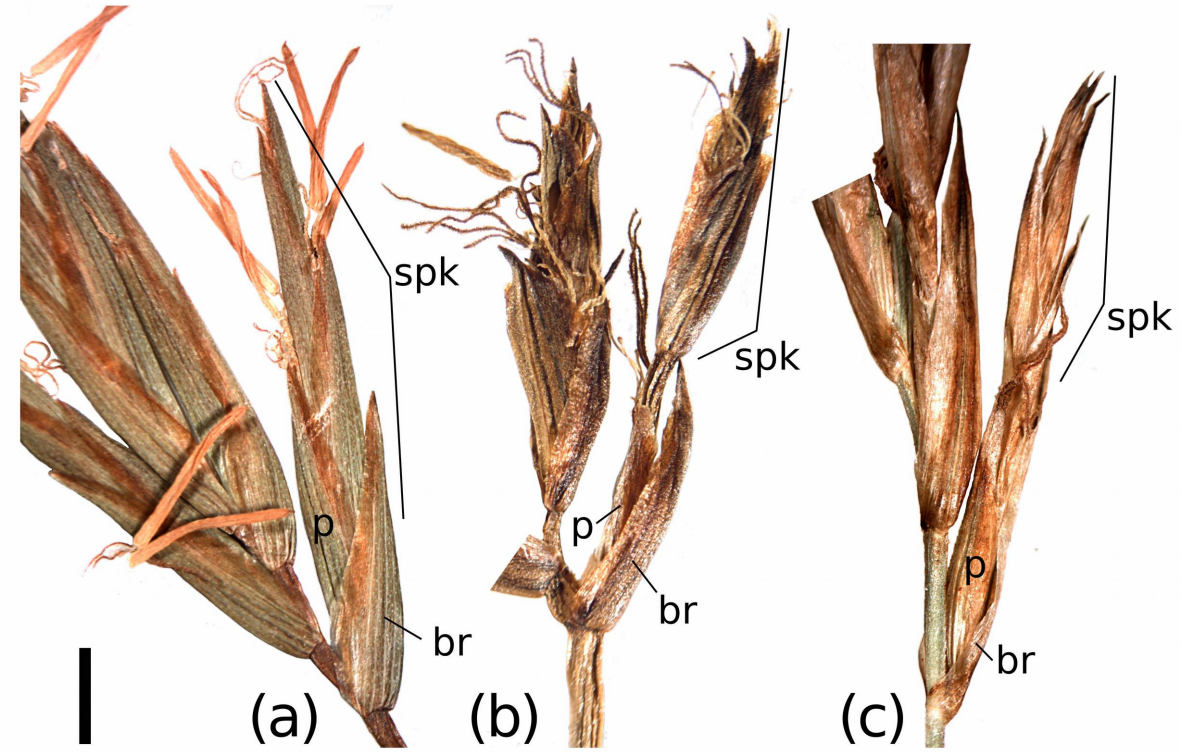


Figure 5.1. Short branch morphology in (a) *Dulichium arundinaceum* (Dulichieae); (b) *Sumatroscirpus junghuhnii* var. *minor* (Sumatroscirpeae); (c) *Carex lancea* (=*Schoenoxiphium lanceum*, Cariceae). Opposite the bract (br) subtending the short branch, the perigynia (p) of *Carex* and *Sumatroscirpus* can be seen to be sheathing around the prophyllar flower and separated by an elongated internode from their spikelet (spk). On the other hand, the fertile spikelet prophyll (p) of *Dulichium* is adjacent and almost identical to the glumes of its spikelet (spk). Scale: 2 mm.

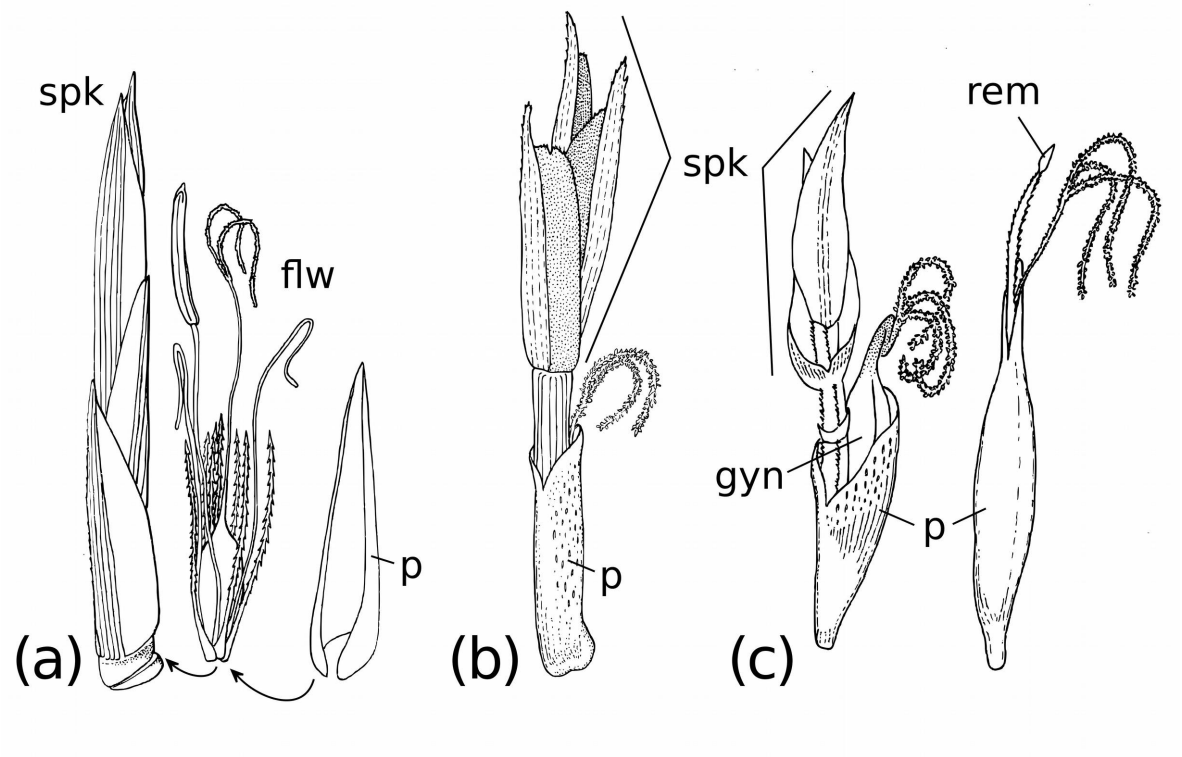


Figure 5.2. Short branch morphology, schematic drawing. (a) *Dulichium arundinaceum* (Dulichieae) showing spikelet (spk), prophyllar flower (flw), and scale-like spikelet prophyll; (b) *Sumatroscirpus junghuhnii* var. *minor* (Sumatroscirpeae), with tubular perigynium; (c) *Carex lancea* (Cariceae) showing two types of perigynia (p): "open", with the gynoecium (gyn) easily visible and a 3-flowered male spikelet (spk) at the tip of the "rachilla" on the left, and "closed", flask-like with a reduced spikelet (rem) on the right. All intermediates between the "open" and "closed" perigynium morphologies can be seen in a single inflorescence of *Carex lancea*.

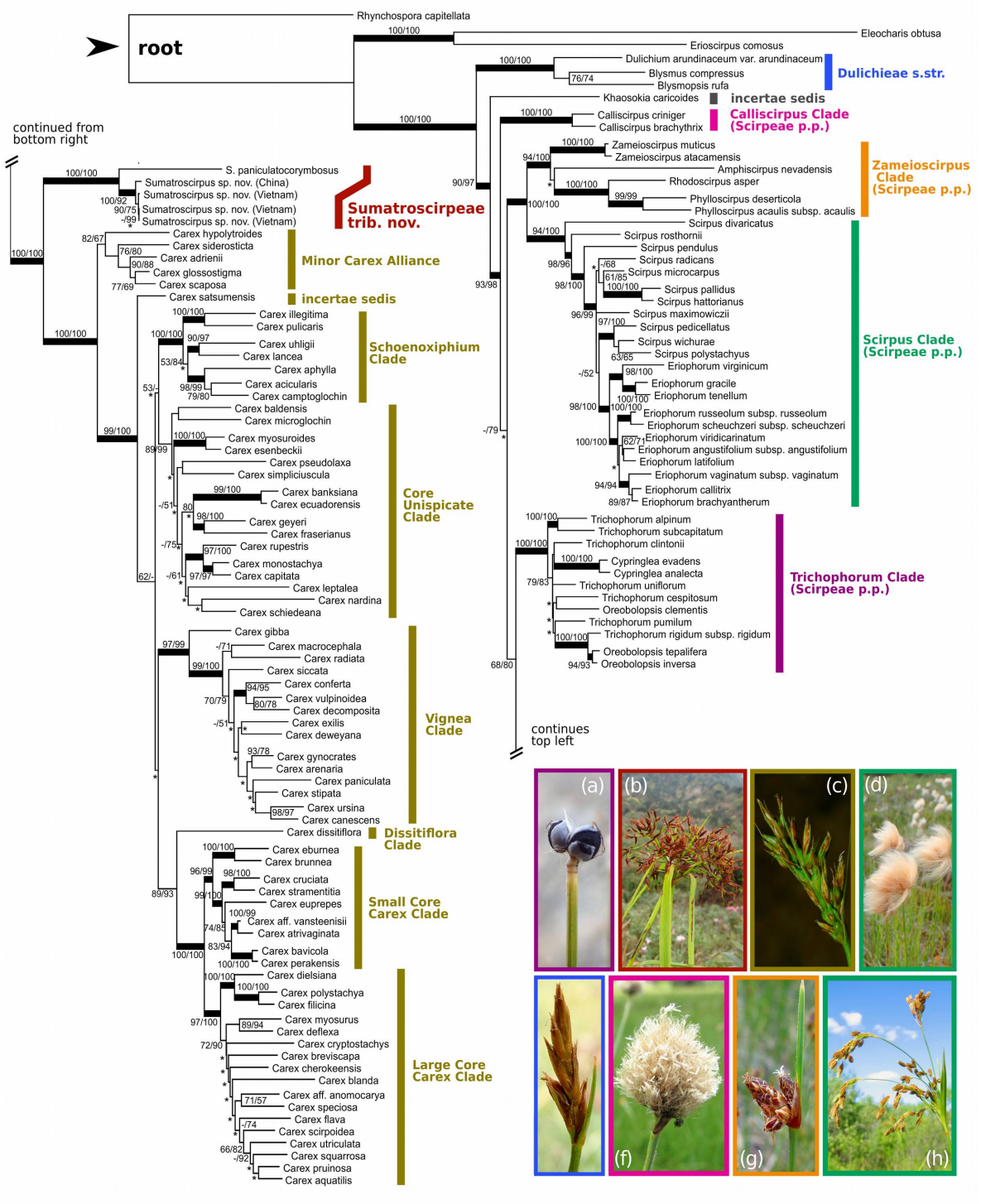

root
Rhynchospora capitellata
Eleocharis obtusa
Erioscirpus comosus
Dulichium arundinaceum var. arundinaceum
Blysmus compressus
Blysmopsis rufa
Dulichieae s.str.
Khaosokia caricoides
incertae sedis
Calliscirpus criniger
Calliscirpus brachythrix
Calliscirpus Clade (Scirpeae p.p.)
continued from bottom right
S. paniculatocorymbosus
Sumatroscirpus sp. nov. (China)
Sumatroscirpus sp. nov. (Vietnam)
Sumatroscirpus sp. nov. (Vietnam)
Sumatroscirpus sp. nov. (Vietnam)
Sumatroscirpeae trib. nov.
Carex hypolytroides
Carex sideroslicta
Carex adrienii
Carex glossostigma
Carex scaposa
Minor Carex Alliance
Carex satsumensis
incertae sedis
Carex illegitima
Carex pulicaris
Carex uhligii
Carex lancea
Carex aphylla
Carex acicularis
Carex camptoglochin
Schoenoxiphium Clade
Carex baldensis
Carex microglochin
Carex myosuroides
Carex esenbeckii
Carex pseudolaxa
Carex simpliciuscula
Carex banksiana
Carex ecuadorensis
Carex geyeri
Carex fraserianus
Carex rupestris
Carex monostachya
Carex capitata
Carex leptalea
Carex nardina
Carex schiedeana
Core Unispicate Clade
Carex gibba
Carex macrocephala
Carex radiata
Carex siccata
Carex conferta
Carex vulpinoidea
Carex decomposita
Carex exilis
Carex deweyana
Carex gynocrates
Carex arenaria
Carex paniculata
Carex stipata
Carex ursina
Carex canescens
Vignea Clade
Carex dissitiflora
Dissitiflora Clade
Carex eburnea
Carex brunnea
Carex cruciata
Carex stramentitia
Carex euprepes
Carex aff. vansteenisii
Carex atrivaginata
Carex bavicola
Carex perakensis
Small Core Carex Clade
Carex dielsiana
Carex polystachya
Carex filicina
Carex myosurus
Carex deflexa
Carex cryptostachys
Carex breviscapa
Carex cherokeensis
Carex blanda
Carex aff. anomocarya
Carex speciosa
Carex flava
Carex scirpoidea
Carex utriculata
Carex squarrosa
Carex pruinosa
Carex aquatilis
Large Core Carex Clade
Zameioscirpus muticus
Zameioscirpus atacamensis
Amphiscirpus nevadensis
Rhodoscirpus asper
Phylloscirpus deserticola
Phylloscirpus acaulis subsp. acaulis
Zameioscirpus Clade (Scirpeae p.p.)
Scirpus divaricatus
Scirpus rosthornii
Scirpus pendulus
Scirpus radicans
Scirpus microcarpus
Scirpus pallidus
Scirpus hattorianus
Scirpus maximowiczii
Scirpus pedicellatus
Scirpus wichurae
Scirpus polystachyus
Eriophorum virginicum
Eriophorum gracile
Eriophorum tenellum
Eriophorum russeolum subsp. russeolum
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum viridicarinatum
Eriophorum angustifolium subsp. angustifolium
Eriophorum latifolium
Eriophorum vaginatum subsp. vaginatum
Eriophorum callitrix
Eriophorum brachyantherum
Scirpus Clade (Scirpeae p.p.)
Trichophorum alpinum
Trichophorum subcapitatum
Trichophorum clintonii
Cypringlea evadens
Cypringlea analecta
Trichophorum uniflorum
Trichophorum cespitosum
Oreobolopsis clementis
Trichophorum pumilum
Trichophorum rigidum subsp. rigidum
Oreobolopsis tepalifera
Oreobolopsis inversa
Trichophorum Clade (Scirpeae p.p.)
continues top left
(a)
(b)
(c)
(d)
(f)
(g)
(h)

Figure 5.3 [on previous page]. Maximum likelihood (ML) tree based on the full concatenated dataset, with parsimony/likelihood bootstrap values. Branches not present in the MP strict consensus identified with an asterisk (*), and bootstrap values <50% indicated with a dash (-). Branches without support values are unsupported (<50% BS) by both ML and MP analyses. Inset: exemplars of CDS clade species, with color matching major clade names in the phylogenetic tree. (a) *Trichophorum cespitosum* (Trichophorum Clade) parasitized by *Anthracoidea* Bref., a genus of smut fungi otherwise largely restricted to Cariceae; (b) *Sumatroscirpus* sp. nov. (Sumatroscirpeae); (c) *Carex badilloi* Luceño and Márquez-Corro (Cariceae); (d) *Eriophorum* ×*medium* Andersson subsp. *medium* (Scirpus Clade); (e) *Blysmus sinocompressus* var. *sinocompressus* (Dulichieae s.str.); (f) *Calliscirpus brachythrix* (Calliscirpus Clade); (g) *Amphiscirpus nevadensis* (Zameioscirpus Clade); (h) *Scirpus pendulus* (Scirpus Clade). Credits: (a, e, h) É. Léveillé-Bourret, (b, f, g) J. Starr, (c) M. Luceño, (d) M.-È. Garon-Labrecque.

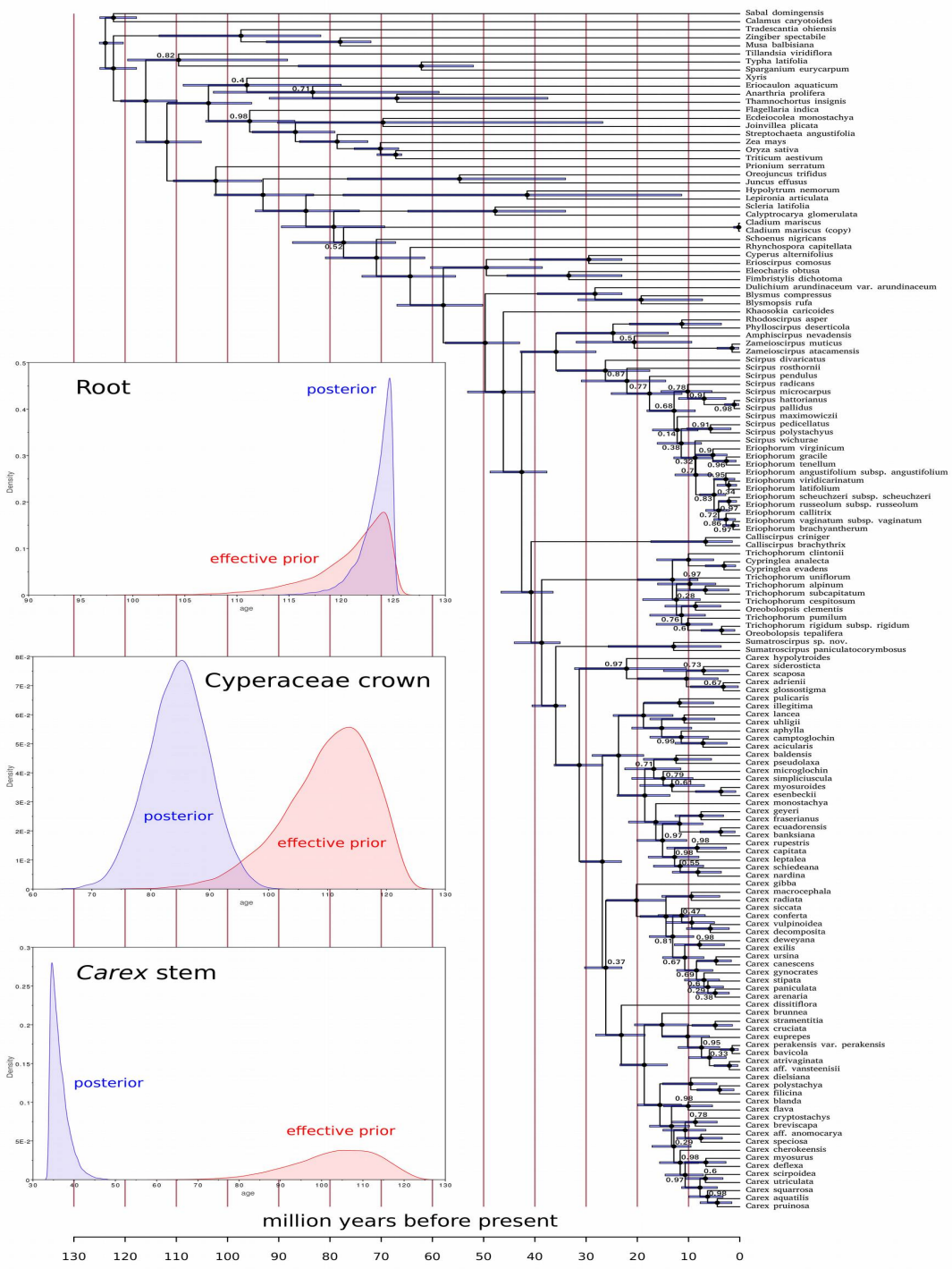


Figure 5.4. Chronogram obtained in the BEAST analysis, showing the 95% highest probability density of clade ages as blue bars, and clade posterior probabilities when they are less than 1. On the left, the effective prior and posterior densities of node age are shown for selected clades.

CHAPTER 6

A REVISION OF *SUMATROSCIRPUS* (SUMATROSCIRPEAE) WITH DISCUSSIONS ON SOUTHEAST ASIAN BIOGEOGRAPHY, GENERAL COLLECTING, AND HOMOLOGUES WITH *CAREX* (CARICEAE, CYPERACEAE)

This Chapter is a slightly modified version of an article in press in the journal Systematic Botany. Coauthors on the article are: Julian R. Starr and Bruce A. Ford. Disclaimer: new names presented here are not intended to be effectively published for nomenclatural purposes.

6.1 Introduction

Sumatroscirpus Oteng-Yeb. is a Cyperaceae (sedge) genus currently treated as a monospecific endemic to the Indonesian island of Sumatra (Govaerts & al., 2007; Fig. 6.1,A). The genus was first described by Oteng-Yeboah (1974) for *Scirpus junghuhnii* Miq., a distinctive Sumatran species that differed from other *Scirpus* by numerous characters such as compressed spikelets, decurrent glumes, papillose leaf surfaces, and a peculiar anatomy (e.g., no foliar air cavities). However, one character in particular made it stand out: the presence of fertile, sheathing prophylls in the inflorescence (first leaf of a branch). This is a rare characteristic in Cyperaceae that was known from only two tribes: Cariceae Kunth ex Dumort and Dulichieae Reichenb. ex Schultze-Motel (Bruhl, 1995; Goetghebeur, 1998).

Tribe Cariceae includes a single genus, the cosmopolitan and exceptionally diverse *Carex* L. (>2000 species; Global Carex Group, 2015), which possesses unusual features like unisexual flowers lacking a perianth and distinctive flask-shaped spikelet prophylls surrounding female flowers that are called “perigynia” or “utricles”. Because of this distinctive morphology, few hypotheses of relationship have ever been made for Cariceae, and they have mostly been made on the basis of the traditional division of the family into unisexually- and bisexually-flowered groups (de Jussieu, 1789; Bentham & Hooker, 1883). Cariceae has thus been linked to other unisexually-flowered tribes such as the Sclerieae and Bisboeckelereae (Holttum, 1948; Kern, 1958; Koyama, 1962a; Schultze-Motel, 1964; Goetghebeur, 1986; Bruhl, 1995) or the Hypolytreae and Chrysitricheae (Mattfeld, 1935) despite a

substantial morphological gap between it and these other tribal groups (Meert & Goetghebeur, 1979; Goetghebeur, 1986; Richards & al., 2006; Nagels & al., 2009). By comparison, Dulichieae (sensu Goetghebeur 1998) is a small tribe (~4 species) characterised by fertile prophylls with margins unfused to the base (scale-like, open), bisexual flowers with a perianth, and laterally compressed spikelets.

Goetghebeur (1998) recognised that with its bisexual flowers and long perianth bristles, *Sumatroscirpus* could not fit in Cariceae, and consequently placed it in Dulichieae. Although *Sumatroscirpus* shares compressed spikelets with other Dulichieae genera, atypical characteristics such as filaments elongating after flowering (Goetghebeur, 1986), upwardly-curved inflorescence branches and spikelets, minutely ciliate ligules, sheathing spikelet prophylls, and enlarged style bases forming a small tubercle on the fruits (pers. obs.) suggested its alliances might lie elsewhere. Phylogenetic analyses presented in Chapters 2, 3 and 4 resolved most of the key nodes of CDS, but failed to provide a credible sister-group to *Carex* (Muasya & al., 2009; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014; Léveillé-Bourret & al., 2015, 2018b), and the question as to whether *Sumatroscirpus* was closer to Dulichieae or Cariceae could not be determined owing to the lack of DNA sequences for the genus. When DNA sequences were finally obtained, *Sumatroscirpus* was not only strongly supported as sister to Cariceae, but there was ample morphological evidence for the recognition of a new tribe, Sumatroscirpeae Lév.-Bourret & J.R.Starr (Fig. 6.2; Chapter 5). This sister-group relationship has enormous implications for the study of *Carex* because it will have a strong impact on all future character polarizations, homology assessments and biogeographic inferences within the genus. In other words, all aspects of the biology of *Sumatroscirpus* have the potential to provide insights into the evolutionary mechanisms and biogeographic history involved in the diversification of *Carex*, one of the world's most diverse plant lineages (Frodin, 2004; Global Carex Group, 2015). However, we are lacking even the most basic taxonomic, geographic and ecological information on *Sumatroscirpus*, as result of its apparent rarity and restricted distribution. A taxonomic revision of the genus would provide this basic information that will provide the foundation for future comparative studies.

The most recent taxonomic treatment of *Sumatroscirpus* was published as part of the treatment for *Scirpus* in the Flora Malesiana (Kern, 1974). In this study, Kern (1974) recognized a single species, *Scirpus junghuhnii* Miq. (=*Sumatroscirpus junghuhnii* (Miq.) Oteng-Yeb.), and he placed a variant

described by Kükenthal (1940: 301), *Scirpus junghuhnii* var. *minor* Kük., in its synonymy as "merely a less robust form of very high altitudes, without systematic value" (Kern, 1974). Our re-examination of the Sumatran material suggests that this variant is morphologically distinct and should be recognized as a distinct species. Furthermore, recent work on Eastern Asian Cyperaceae has revealed that the Chinese endemic *Scirpus paniculatocorymbosus* Kük. is a species of *Sumatroscirpus*, and it has also uncovered several new records of an undescribed species in northern Vietnam, northern Myanmar, and southwestern China (Léveillé-Bourret & al., 2018a) that is formally described in this paper.

The aim of this study is to provide a comprehensive taxonomic revision of *Sumatroscirpus*, including keys, detailed descriptions, illustrations, distribution maps, and conservation status assessments for all its species. The inflorescence morphology of the genus is also discussed, and its perigynia compared to those of *Carex*. Notes on the importance of herbaria and general collecting, and a discussion of the biogeographical implications of the new continental records are presented here.

6.2 Materials & Methods

6.2.1 Material Examined

A total of 19 gatherings (types included) comprising 47 sheets of *Sumatroscirpus* from herbaria A, B, BO, GH, IBSC, K, L, MO, SING, and UPS were examined. Herbarium acronyms follow Index Herbarium (Thiers, 2017+). Digital images of material deposited at PE were obtained online (http://pe.ibcas.ac.cn/en/). Most specimens documented here were found by sifting through herbarium folders containing undetermined Cyperaceae, *Carex*, *Fimbristylis* Vahl, and *Rhynchospora* Vahl, or in folders of *Rhynchospora corymbosa* (L.) Britton. Additional specimens might be found in other herbaria by following the same procedure. Fresh material of *Sumatroscirpus rupestris* (described in this paper) was collected during fieldwork in Northern Vietnam in April 2015, by returning to the locality of a specimen collected by P. P. Lowry II and E. J. Sterling in 1997 (*Lowry II & Sterling 4892*, MO). The first author was also able to examine live individuals of *Sumatroscirpus paniculatocorymbosus* in the field in Western Sichuan in July-August 2017. As collecting was not permitted, a voucher was not obtained, but pictures were taken.

6.2.2 Morphological Data

Morphological descriptions and measurements are based on dry material and were made using a dissecting microscope with a calibrated eye-piece graticule, or using a ruler with 0.5 mm graduations. All available specimens were measured. Stem length measurements exclude the inflorescence. Spikelet bracts are the bracts subtending lateral spikelets (with their perigynia and pedicel). Lateral spikelets were considered to be "pedicelled" when the lower spikelets of a fascicle possessed long pedicels. Sometimes, the lower lateral spikelets of a fascicle are long-pedicelled while the higher lateral spikelets appear almost sessile. In this case, lateral spikelets are said to be "pedicelled", even though it would be more accurate to state that "lower lateral spikelets are pedicelled". Spikelets are considered "sessile" in the descriptions and keys only when they are all sessile. A bivariate plot was made for the two quantitative characters best separating *Sumatroscirpus minor* from *S. rupestris* in R v.3.4.1 (R Core Team, 2017). For micromorphological observations, dry leaves of *Sumatroscirpus minor* and *S. rupestris* were mounted on aluminium stubs, coated with gold for 40 seconds using a Desk II Denton Vacuum sputter-coater and examined under high-vacuum at 15 kV in a Philips XL30 ESEM scanning electron microscope.

6.2.3 Species Concept

Species are defined here as the smallest groups of individuals that differ consistently in at least two morphological characters from all other such groups. Assuming that the studied characters are heritable and genetically uncorrelated, species thus delimited should fit the cohesion species concept (i.e. the most inclusive group of individuals having the potential for phenotypic cohesion through intrinsic means; Templeton, 1989).

6.2.4 Inflorescence Morphology and Terminology

The inflorescences of *Sumatroscirpus* consist of spikelets arranged in compound corymbs (Fig. 6.3). Inflorescence branches are subtended by a bract (Fig. 6.4, b), have a prophyll (Fig. 6.4, cp and pg) on their first node, and end in a spikelet (Fig. 6.4, s). Proximal bracts are leaf-like, with a quick transition distally to scale-like bracts with a scabrous awn. The prophyll is the first leaf of a branch, which is inserted adaxially, close to the subtending bract, and forms a sheath around the branch axis.

While usually sterile in other Cyperaceae, all prophylls of the inflorescence are fertile in *Sumatroscirpus*: they each subtend a bisexual flower that is often slightly larger than flowers subtended by glumes. Spikelets consist of a short axis (rachilla), with several spirally-inserted glumes each subtending a single bisexual flower.

Inflorescence branches can be organized into two categories: (1) long branches, possessing additional branch-bearing nodes separating the prophyll from the spikelet, and (2) short branches (Fig. 6.4, sBr), possessing only a prophyll and a spikelet (lateral spikelet). Long branches are present below short branches, such that branch complexity decreases distally on an axis. Spikelets are organized in small clusters called "fascicles" (also "cymules" or "umbellets" in the literature), due to the short branches (lateral spikelets) being always produced in groups and in close proximity of a terminal spikelet. Prophylls of long branches are called "cladoprophylls" and are separated from the terminal spikelet of their branch by several intervening branch nodes. Prophylls of short branches are called "spikelet prophylls" because they are adjacent to their spikelet. In *Carex*, the sister-group of *Sumatroscirpus*, fertile spikelet prophylls have been called "perigynia" or "utricles" because they are flask-shaped and surround a female flower. However, the definition of the term "perigynium" is extended to include the spikelet prophylls of *Sumatroscirpus* (see Discussion and Table 6.1).

In the descriptions, typological terminology such as "short paracladia", "long paracladia" and "epipodia" (Weberling, 1965; Vegetti 2002, 2003; Global Carex Group, 2015) was avoided because these terms tend to be confusing to those unfamiliar with inflorescence typology. For clarity, such technical terms can be easily replaced by an equivalent terminology that uses customary botanical words that are equally precise within the current context, such as "short branch", "long branch", and "spikelet pedicel"/"branch peduncle" (Table 6.1).

6.2.5 Geographic Analyses

Most locality names in Sumatra were found by correlating Junghuhn's (1847) and de Wilde and Duyjfes' (1994) maps with satellite images. One specimen label indicates "Gunung Malintang" (*Bünnemeijer 4203*, B, BO), but there are two mountains with this name in Sumatra. The elevation on the label (2260 m) is too high for the smaller (1994 m) of the two, and we thus believe that the

collection locality is the one now better known as Gunung Sago (2271 m). By comparing elevations and names of nearby North Burmese peaks in Kingdon-Ward (1954) with those of a contemporaneous map (US Army Map Service, 1954), the only Myanmar specimen of *Sumatroscirpus rupestris* (*Kingdon-Ward 21063*, A) could be unambiguously located, despite the fact that the name used for the mountain by the collector (Tama Bum) is different from the one on the map (Hkangri Bum).

The type locality of *Sumatroscirpus paniculatocorymbosus* (*Smith 2092*, B, GB, PE), Ta-Hsiang-Ling pass, was located by correlating locality names in Alexander Hosie's (1897: 97–99) description of his expedition through the pass with a map using the old Wade-Giles Romanization system (US Army Map Service 1957). More recent maps were less helpful because of name changes and because of the use of the more recent pinyin Romanization system, which made old locality names difficult to recognize. This approach permitted us to get more accurate coordinates for the Ta-Hsiang-Ling pass than those given in Herner (1988). In some instances, older maps had to be georeferenced using the georeferencer plugin in Quantum GIS 2.18 (qgis.org). In these cases, control points were selected at sharp turns in the course of major rivers, junctions of major rivers, extremities of small islands, tips of pointed capes and bays, and other easily identifiable topographical features, and maps were fitted to satellite images using thin plate spline. Georeferenced records were then imported into GeoCAT (Bachman et al. 2011) in order to estimate the extent of occurrence (EOO) and area of occupancy (AOO) of each species for preliminary IUCN conservation status assessment (IUCN 2012). For the AOO, a grid cell width of 2 km was used following the recommendations of IUCN (2017). Additionally, a modified AOO (sliding-scale) was also calculated using a grid size of 1/10th the maximum distance between two occurrences, following the recommendations of Rivers et al. (2010), but allowing a maximum grid cell width of 50 km. Occurrences on mountain peaks separated by at least 10 km were treated as different "subpopulations," defined as geographically distinct groups between which there is little genetic exchange (IUCN 2012).

6.2.6 Embryology

Most nutlets were too immature to give representative embryos, but one nutlet of *Sumatroscirpus junghuhnii* (*Bünnemeijer 4203*, B barcode 10 0676589) included a slightly immature, but well-formed embryo that showed some taxonomically important features, such as the root cap and germ pore

positions. After rewetting with dilute ethanol and distilled water, the embryo was mounted in chloral-lactophenol on a slide with a coverslip elevated by sticking bands of coverslips on either side of the embryo (Van der Veken, 1965; Dhooge, 2005). This prevents crushing or squeezing, and the orientation of the embryo can be changed by gently moving the coverslip. However, bleaching was not done because previous trials did not show it improved the clarity of embryographic features. Examination and pictures were made at 100× on a Nikon Microphot-FX microscope equipped with a PixeLINK PL-B686CU camera. Embryo types are named according to the typology of Goetghebeur (1986).

6.3 Results

6.3.1 Taxonomic Results

Sumatroscirpus, sole member of tribe Sumatroscirpeae, possesses a combination of morphological characteristics that is unique in Cyperaceae. It shares sheathing fertile prophylls with Cariceae, but differs by the possession of bisexual flowers with a perianth (reduced in *S. paniculatocorymbosus*). In these characters, it is closer to Dulichieae and Scirpeae, but differs from both by its upwardly-curved inflorescence branches and enlarged style base, characteristics that are unique in the Cariceae-Dulichieae-Scirpeae clade (CDS; Table 6.2). The embryo of *Sumatroscirpus junghuhnii* possesses a basal root cap and lateral germ pore (Fig. 6.5), similar to the embryos of Dulichieae, Cariceae, *Calliscirpus* C.N.Gilmour et al. and the Trichophorum Clade (Scirpeae p.p.: *Cypringlea* M.T.Strong, *Oreobolopsis* T.Koyama & Guagl., *Trichophorum* Pers.). It differs from the embryos of the Scirpus Clade (Scirpeae p.p.: *Eriophorum* L., *Scirpus*) and Zameioscirpus Clade (Scirpeae p.p.: *Amphiscirpus* Oteng-Yeb., *Phylloscirpus* C.B.Clarke, *Rhodoscirpus* Lév.-Bourret et al., *Zameioscirpus* Dhooge & Goetgh.), which possess a (sub-)lateral root cap and (sub-)basal germ pore. Full clade descriptions are found in Chapters 2 and 5 (Léveillé-Bourret & al., 2014, 2018a; Fig. 6.2).

Four phenotypic clusters, here recognized as species, can be consistently identified within *Sumatroscirpus* (Table 6.3). *Sumatroscirpus junghuhnii* (Miq.) Oteng-Yeb. is the only taxon to possess sessile lateral spikelets, wide leaves (19–25 mm) that are smooth on both surfaces, and perianth bristles with few barbs and numerous blunt warts (Fig. 6.6G–L). *Sumatroscirpus minor* (Kük.) Lév.-Bourret & J.R.Starr, comb. nov. and *S. rupestris* Lév.-Bourret & J.R.Starr, sp. nov. are morphologically similar in

their possession of narrower leaves (7–16 mm) that are papillose at least abaxially, pedicellate spikelets, and perianth bristles with numerous sharp barbs and few warts. *Sumatroscirpus rupestris* differs from *S. minor* in its dense abaxial papillae on leaves (scattered in *S. minor*, Figs. 6.1, C–D, 6.6, M–S, 6.7), absence of adaxial papillae (present in *S. minor*), shorter spikelets with fewer glumes, and shorter glumes (Fig. 6.8). *Sumatroscirpus paniculatocorymbosus* (Kük.) Lév.-Bourret & J.R.Starr is unique in its possession of open terminal inflorescences with few spikelets (5–17), proximal bracts that are shorter than the inflorescence, red leaf sheaths, reduced perianth bristles, and rhizomatous habit (Fig. 6.1E, Fig. 6.6A–F). In contrast, the three other *Sumatroscirpus* species have dense inflorescences with many spikelets (70–hundreds), proximal bracts usually longer than the inflorescence, green leaf sheaths, perianth bristles that are longer than the nutlets, and cespitose habit.

6.3.2 Geography and Ecology

Sumatroscirpus junghuhnii is known from only three volcanic peaks of the Barisan Mountain Range, in the Sumatran provinces of North Sumatra and West Sumatra, Indonesia (Fig. 6.9), at elevations of 1860–2400 m. The species has an EOO of 5584 km^2, an AOO of 12 km^2, an AOO (sliding-scale) of 5308 km^2, and three known subpopulations represented by collections > 95 years old. Two subpopulations occur in protected areas: the Kerinci Seblat National Park, and the Gunung Sago Malintang Karas Protection Forest. No data on habitat or population size are available from any of the subpopulations.

Sumatroscirpus minor has a more restricted distribution, being found only in the subalpine plateaus (2500–3400 m) of the non-volcanic mountains of the Gunung Leuser area, Aceh Province, Sumatra, Indonesia (Fig. 6.9). Herbarium labels indicate that it is restricted to wet mountain blang, an open subalpine vegetation type dominated by low shrubs, sedges and moss on impermeable kaolinitic clay (de Wilde and Duyfjes 1996). Its EOO is of 2777 km^2, its AOO of 36 km^2, its AOO (sliding-scale) of 472 km^2, and it has only seven known subpopulations, two of which occur in the Gunung Leuser National Park. No data on population size are available.

Sumatroscirpus rupestris is known from only three localities, with occurrences on both sides of the border between the Kachin State of Myanmar and northwest Yunnan, China, and a disjunct subpopulation on mount Phan Xi Pang, in northern Vietnam (Fig. 6.9). In 2015, we found thousands of fertile tufts at elevations of 2800–2950 m on steep slopes (50–90˚) of its type locality, mount Phan Xi Pang (Vietnam), and on Đỉnh Sét Đánh (Thundering Summit), a subpeak located approximately 800 m to the northwest. The plants grew on rock cliffs and in bamboo-*Rhododendron* L. thicket (Fig. 6.1B). The plants we collected (*Ford et al. 15081*, DAO, GH, K, L, MT, MICH, MO, NY, HNU, WIN) were rooted in black wet organic soil, with a pH of about 4.8 and with a cover of dead bamboo leaves, *Sphagnum* L. and brown pleurocarpous mosses. The specimen from China was also collected in a bamboo-*Rhododendron* thicket at similar elevation (2999 m), while the Myanmar specimen was collected at higher elevation (3230 m) along the joints of a large granite slab. *Sumatroscirpus rupestris* has an EOO of 23,518 km^2, an AOO of 12 km^2, an AOO (sliding-scale) of 7500 km^2, and three known subpopulations. Two subpopulations occur in protected areas: in the Hoàng Liên National Park, Vietnam, and in the Gaoligongshan National Nature Reserve, China.

Sumatroscirpus paniculatocorymbosus has an extremely restricted range on the eastern edge of the Daxue Mountain Range, in the area between Luding, Shimian, and Hanyuan, Western Sichuan, China (Fig. 6.9). On the 31st of July 2017, the first author found a small population of this species at the foot of Gongga Shan, Hailuogou Scenic Area, Western Sichuan China (Fig. 6.1E). It probably corresponds to the same population that was sampled by Lang, Liang and Fei in 1982 (*Lang et al. 401*, PE). The plants were found in a mixed montane forest, in the mist zone, along the trail, at 2800 m elevation, and were in early-fruiting stage. Detailed localization information and GPS coordinates can be provided on request. *Sumatroscirpus paniculatocorymbosus* has also been found in alpine meadows and in the understory of conifer forests, at elevations of 2600–2800 m. Its EOO is approximately 1446 km^2, its AOO of 12 km^2, and its AOO (sliding-scale) of 113 km^2, with only three verified subpopulations, and no information on population sizes. However, one additional voucher is listed in the PE herbarium virtual database (http://pe.ibcas.ac.cn/en/): *Liu 518* (PE barcodes 00026379, 00026380). This specimen was collected in the same general area as the examined specimens, but its identity could not be verified because pictures were unavailable, and loans were not possible. If this unverified voucher is included in the GeoCAT analyses, the AOO increases to 16 km^2.

6.4 Discussion

6.4.1 Unrecognized Taxonomic Diversity Within Sumatroscirpus

The genus *Sumatroscirpus* is recognized as the sole member of tribe Sumatroscirpeae, based on the morphological differences given here (see taxonomic treatment and Table 6.1) and the molecular phylogenetic results presented in a Chapter 5 (Fig. 6.2; Léveillé-Bourret & al., 2018a). The embryo of *Sumatroscirpus junghuhnii* could correspond to either a Carex-type or Juncus-type embryo (sensu Goetghebeur, 1986). This is congruent with phylogenetic results demonstrating no close relationship between *Sumatroscirpus* and the Scirpus and Zameioscirpus Clades (Chapter 5; Léveillé-Bourret & al., 2018a). New observations on a fully mature embryo would be necessary to determine the exact embryological type.

The results of this study support the existence of four well-defined species within *Sumatroscirpus*, a genus that was previously thought to be monospecific (Govaerts & al., 2007). The species here recognized are: *Sumatroscirpus junghuhnii* (Miq.) Oteng-Yeb., *S. minor* (Kük.) Lév.-Bourret & J.R.Starr, comb. nov., *S. rupestris* Lév.-Bourret & J.R.Starr, sp. nov., and *S. paniculatocorymbosus* (Kük.) Lév.-Bourret & J.R.Starr (see Taxonomic Treatment below). *Sumatroscirpus junghuhnii* is restricted to volcanic peaks in West Sumatra and North Sumatra, while collections on non-volcanic peaks of the Aceh Province of Sumatra correspond to *Sumatroscirpus minor* (≡*Scirpus junghuhnii* var. *minor* Kük.). A lectotype is designated for the basionym of the latter (see below). *Sumatroscirpus rupestris* is a new species found in Northern Vietnam, Northern Myanmar, and Southwestern China that is most similar to the Sumatran *S. minor*, but differs in its very dense abaxial leaf papillae, and its smaller spikelets and glumes. *Sumatroscirpus paniculatocorymbosus*, a recently made combination for *Scirpus paniculatocorymbosus* Kük. (Léveillé-Bourret & al., 2018a), is an endemic to Western Sichuan, China. It is a very distinct species due to its small inflorescence, reduced perianth bristles, and rhizomatous habit, and is placed it in a separate section, *Sumatroscirpus* sect. *Paniculatocorymbosi* (Kük.) Lév.-Bourret & J.R.Starr, a new combination for *Scirpus* sect. *Paniculatocorymbosi* Kük.

6.4.2 Diversity, Herbaria, and General Plant Collecting

The specimens that formed the basis of this revision have almost all been collected by field botanists during general collecting trips that were not limited in scope to a single family or genus. Most of these vouchers remained without a name for years before being recognized as new to science by taxonomic specialists working in herbaria. Taxonomic work on such a rare and broadly distributed genus as *Sumatroscirpus* would not have been possible without experienced field botanists and the institutions that preserved their specimens, highlighting the importance of these undervalued people and institutions in the discovery and conservation of biodiversity (Bebber & al., 2010; Gross, 2011; Bebber & al., 2012; Fontaine & al., 2012). Although most collections of *Sumatroscirpus* were made in the 19th and early 20th centuries, general collecting still plays a key role today. The single specimen of *Sumatroscirpus rupestris* collected by P. P. Lowry II and E. J. Sterling during an expedition to Vietnam in 1997 (*Lowry II & Sterling 4892*, MO) provided us with the only precise and accessible locality where we could collect plants for DNA analysis and the subsequent naming of the first new sedge tribe in 29 years, Sumatroscirpeae (Thomas & Davidse, 1989; Léveillé-Bourret & al., 2018a). Another example is the Gaoligonshan Biodiversity Survey of 2004, which provided the only confirmed Chinese specimen of *Sumatroscirpus rupestris* (*Gaoligong Shan Biodiversity Survey 20184*, GH).

The continued discovery of new taxa during field inventories at heavily-surveyed biodiversity hotspots, like the type locality for *Sumatroscirpus rupestris* sp. nov. on Mount Phan Xi Pang (Vietnam; Thin & Harder, 1996; Xiang, 1997; Thin, 1998; Vũ, 2006; Nguyễn Nghĩa, 2008; Shaw, 2011; Vu & al., 2011; Vũ, 2013; Ford & al., 2015; He & Nguyen, 2016), demonstrates the continued relevance of field studies to contemporary biodiversity science. A lack of support for local taxonomic research and collecting at such biodiversity hotspots represents a major bottleneck for completing the planet's biodiversity inventory (Prather & al., 2004; Whitfield, 2012; Paknia & al., 2015) and it has important consequences for ecological, biogeographical and conservation research (Lavoie & al., 2012; Lavoie, 2013; Engemann & al., 2015; Stropp & al., 2016; Nualart & al., 2017).

6.4.3 Sumatroscirpus Shares the Perigynium With its Sister Carex

Although traditionally thought to be unique to *Carex*, the perigynium is a structure that *Carex* shares with its sister group *Sumatroscirpus*. The perigynium is a specialized spikelet prophyll that contains a flower. The structure is most often described as "flask-shaped" or "sac-shaped", but it may vary in appearance from utriculiform to squamiform (Jiménez-Mejías & al., 2016a). Consequently, it is best defined by its position as a fertile prophyll at the base of short branches, with position being a better indicator of homology than external appearance (Vrijdaghs & al., 2010). Position can thus consistently distinguish perigynia from cladoprophylls, even when cladoprophylls are described as "perigynium-like" (e.g. "inflorescence prophylls" of Reznicek, 1990 and references cited therein), or when they are fertile (see below), because cladoprophylls are found at the base of long branches.

The inflorescence structure of *Carex* and *Sumatroscirpus* can superficially appear to be quite different because the vast majority of *Carex* species lack fertile cladoprophylls, and their inflorescences have undergone frequent truncation and homogenization, characteristics not directly related to the presence of perigynia (see Bender & al., 2016 and below). In *Sumatroscirpus*, all prophylls of the inflorescence are fertile, perigynia and cladoprophylls alike, but in *Carex* species, cladoprophylls are sterile with a few notable exceptions (Fig. 6.3). Fertile cladoprophylls are present in a small number of species from *Carex* sections *Mundae* and *Japonicae* (~4 spp.), and in several highly compound species recently transferred from the genera *Kobresia* Wild. p.p. and *Schoenoxiphium* Nees (Timonen, 1985, 1989; Jin & al., 2005; Dai & al., 2010a). Likewise, the non-homogenized compound corymbs of *Sumatroscirpus* species may appear different from *Carex* because the vast majority of *Carex* species possess highly homogenized inflorescences where short branches are truncated beyond the prophyll node (Vegetti, 2002). This gives typical *Carex* their distinctive morphology within Cyperaceae because the axis on which the perigynium is inserted is often truncated beyond the first node, leaving only the perigynium and associated flower visible to the naked eye. The homology of *Carex* perigynia is therefore difficult to understand because it is a spikelet prophyll, but its female flower is not a part of the spikelet, and the spikelet is almost always missing due to branch truncation (multispicate and "unispicate" *Carex* in Fig. 6.3). However, the correspondence of *Carex* and *Sumatroscirpus* perigynia can be easily demonstrated in the small number of *Carex* species that have non-truncated short

branches. In such species, a male spikelet protrudes from a perigynium as seen in some *Carex* subgen. *Vigneastra* (Tuck.) Kük., species formerly included in *Kobresia* p.p. and *Schoenoxiphium*, and in teratological specimens of numerous other *Carex* species (Smith, 1967; Le Cohu, 1968; Smith & Faulkner, 1976; Waterway & al., 2009; highly compound *Carex* in Fig. 6.3). These non-truncated short branches are structurally identical in *Carex* and *Sumatroscirpus*: a sheathing fertile prophyll is followed by an elongated internode and a terminal spikelet. In fact, the only universal differences in inflorescence structure between *Sumatroscirpus* and *Carex* are that the flowers are bisexual with perianth bristles in *Sumatroscirpus*, but unisexual and naked in *Carex*.

The hypothesis that the shared structural and positional characteristics of *Carex* and *Sumatroscirpus* inflorescences are due to common ancestry is strongly supported by molecular phylogenetic analyses because *Carex* and *Sumatroscirpus* form a monophyletic group (Chapter 5; Léveillé-Bourret & al., 2018a). Accordingly, we conclude that the fertile spikelet prophylls at the base of the short branches of *Carex* and *Sumatroscirpus* are the same homologous structure. In other words, their specialized, fertile spikelet prophyll is a synapomorphy (homology sensu Patterson, 1982; de Pinna, 1991) supporting a *Carex* + *Sumatroscirpus* clade. Consequently, to restrict the use of the word "perigynium" to *Carex*, when the same structure occurs in *Sumatroscirpus*, would be illogical. The definition of the perigynium is thus extended to include the fertile spikelet prophyll of *Sumatroscirpus* (Fig. 6.3, 6.4, Table 6.1). The fertile spikelet prophylls of the unrelated tribe Dulichieae are convergent, and not included in the definition of perigynium (Fig. 6.2; Léveillé-Bourret & al. 2018a). They differ from perigynia in being almost indistinguishable from normal glumes, except for the presence of two major ribs.

The shared presence of perigynia and common inflorescence structure of *Carex* and *Sumatroscirpus* raises some issues regarding the clarity of the terminology applied to both. The recent proposal to restrict the use of "utricle" to closed perigynia (fused to apex; Jiménez-Mejiás & al., 2016a) is problematic for three reasons: (1) the terms "utricle" and "perigynium" are used as synonyms in most of the literature and a departure from this convention could create confusion; (2) it gives a special name to the plesiomorphic state of perigynia (closed perigynia = utricles), but leaves the derived state (Starr & al., 2004; Starr & Ford, 2009; Léveillé-Bourret & al., 2018a) without a name (open perigynia = ?),

and (3) for highly compound *Sumatroscirpus* and *Carex* species formerly placed in the genera *Kobresia* and *Schoenoxiphium* (Nelmes 1951a), a single inflorescence may contain a gradation of morphologies ranging from completely "open" to completely "closed" perigynia depending on their position within the inflorescence (see Kukkonen, 1983; Timonen, 1985; pers. obs.). We thus follow the opinion of the Global Carex Group (2015) that "utricle" and "perigynium" should be treated as synonyms. However, we recommend the use of "perigynium" over "utricle" for two reasons. First, the modern botanical usage of "perigynium" has been restricted to this specialised prophyll in angiosperms; and secondly, the literal meaning of the word in Greek points to its position, a better indicator of homology than shape, which is often deceiving (i.e. analogy). The word "utricle" is a case in point. It is derived from the Latin for a leather bag or bottle (utriculus). It is thus unsurprising that "utricle" is analogously applied to many different bladdery or inflated structures across angiosperms such as the fruits of certain Amaranthaceae, Betulaceae, and Caryophyllaceae (Kubitzki, 1993; Kühn & al., 1993; Townsend, 1993; Rabeler & Hartman, 2005), the basally inflated portion of the calyx of *Aristolochia* L. (Barringer & Whittemore, 1997), and the traps of *Utricularia* L. (Lentibulariaceae; Lloyd, 1942). Differences in perigynium morphology are thus best expressed by adjectives such as "tubular", "utriculiform" and "squamiform", which can be equally applied to perigynia and cladoprophylls, rather than by redefining well-known terms. Such adjectives are less ambiguous, and they remove the need to propose definitions remote from current and historical usage.

6.4.4 Distribution, Ecology, and Conservation of Sumatroscirpus Species

The discovery of *Sumatroscirpus rupestris* and the addition of *S. paniculatocorymbosus* extend the range of this genus by more than 2700 km to the north, into the Sikang-Yunnan floristic Province of the Eastern Asiatic Region (Takhtajan, 1986). The distribution of *Sumatroscirpus* thus spans from Southern China to Sumatra, an area that has been hypothesized to be the center of origin of its sister-group *Carex* (Nelmes, 1951b; Raymond 1955, 1959; Koyama, 1957; Waterway & al., 2009; Starr & Ford, 2009; Starr & al., 2015). However, it differs from most early-diverging *Carex* lineages in its habitat, occurring mostly in the subalpine and alpine zones (1860–3400 m), whereas the former are more characteristic of the temperate (montane) zones of tropical mountains (Starr & al., 2015). The occurrence of *Sumatroscirpus* in the highest altitudinal zones of Southeast Asian mountains probably

explains in large part its apparent rarity, only a handful of specimens being available from the most important herbaria for Southeast Asia and Indonesia. Whether the small number of specimens truly indicates rarity in nature, or whether it is a symptom of its occurrence in remote and inaccessible habitats, remains to be determined.

Although there is no habitat information on any of the *Sumatroscirpus junghuhnii* herbarium sheets examined, it seems probable that the species is found either in the understory of forests or in small openings of closed vegetation types, given the localities and elevations recorded on herbarium labels. The type (*Junghuhn 477*, L) was collected on the top of Mount Lubukraya (elevation 1862 m) which is said to be covered with moss forest shrouded in eternal fog, the canopy reaching 15–18 m even on its highest peak (Junghuhn, 1847:109–114). On Gunung Kerinci, *Sumatroscirpus junghuhnii* was collected around 2400 m (*Bünnemeijer 10468*, BO), while shrubby subalpine vegetation only begins at 2900 m on this mountain according to Ohsawa & al. (1985). The same authors report at 2400 m a peculiar *Gleichenia* Sm. fern belt reaching a height of 2–4 m marking the transition between the lower *Lithocarpus* Blume-*Eugenia* L.-*Haemocharis* Salisb. ex Mart. moss forest and the higher *Symplocos* Jacq.-*Rapanea* Aubl. subalpine forest. All the examined collections are in the montane altitudinal zone of van Steenis (1984), dominated by closed forests, and well below the forest limit that is usually around 3600–3700 m in the Malay Archipelago. The limited AOO (12 km^2) of *Sumatroscirpus junghuhnii* would qualify this species as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected.

Sumatroscirpus minor is restricted to the Gunung Leuser area of the Aceh Province of Sumatra. The mountains of this region, called “Gayo Mountains”, are distinct from the other mountains of the Barisan Range based on their older age and non-volcanic origin. The peculiar flora of the Gunung Leuser area has been hypothesised to have become distinct due to the mega-eruption that gave rise to Lake Toba, ca. 75000 years ago, destroying adjoining vegetation and isolating the mountain flora of Aceh Province from the rest of the Barisan Range (de Wilde & Duyfjes, 2001). This biogeographical break is supported by the distribution of *Sumatroscirpus minor* north of Lake Toba, and *S. junghuhnii*

to the south. These species also appear to differ in their ecology, with *S. minor* being apparently restricted to herbaceous or shrubby vegetation on flat high-altitude terrain, whereas *S. junghuhnii* is likely found in forest understory or clearings. The restricted AOO (36 km^2) of *S. minor* would qualify it as Vulnerable (VU) under IUCN (2012) criterion D2 if a plausible future threat was identified. It would also qualify as Endangered (EN) under criteria B1 and B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected. However, this species occurs in remote subalpine areas unlikely to be susceptible to anthropogenic disturbances, and appears to have remained relatively common in its habitat in the Gunung Leuser area, with three different expeditions between 1937 and 1975 having produced several specimens. Field studies would be necessary to better assess its conservation status.

Sumatroscirpus rupestris has been found throughout its range in subalpine bamboo-*Rhododendron* thickets or on bare rocks, which distinguishes it from the morphologically similar *S. minor*. It also differs from the latter in its occurrence in the Sikang-Yunnan floristic Province, more than 2,000 km to the north, and separated by the Malay Peninsula and Strait of Malacca. The specimen from the Shan-ngaw Range of Myanmar (*Kingdon-Ward 21063*, A) would be placed in the Northern Burmese Province of Takhtajan (1986), but Kingdon-Ward (1939, 1954, 1957) remarked that northern Myanmar is characterized by a mix of Eastern-Himalayan and Sikang-Yunnanese elements in the high montane zone, Indomalesian elements in the lowlands, with and admixture in the intermediate temperate zone, and is thus not a very distinct floristic unit. The known distribution of *Sumatroscirpus rupestris* features an important disjunction, between the subpopulations in China and Myanmar, and the subpopulation in northern Vietnam. However, the occurrence of a large number of high mountain peaks with presumably similar vegetation types in the Hengduan Mountain Range that follows the southwestern border of China makes it probable that additional subpopulations exist at other localities. Its small AOO (12 km^2) would qualify it as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected. Two of the three subpopulations occur in protected areas. However, the construction of a cable car to the summit of Mount Phan Xi Pang in 2015 impacted a major portion of the summit, and the consequent increase in tourism could cause pressure to

this geographically-isolated subpopulation. Given the high endemism of the flora of Phan Xi Pang (Thin and Harder 1996; Thin 1998), it would be prudent to give the Vietnamese subpopulation of *Sumatroscirpus rupestris* a special status to guarantee its conservation.

Sumatroscirpus paniculatocorymbosus is the most northern species in the genus, occurring at high altitudes in alpine prairies and coniferous or mixed forest understories, in a narrow area in Western Sichuan, China. Because of its small AOO (12 km^2), it would qualify as Vulnerable (VU) under IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected. More information on its habitat, distribution, and ecology are needed to properly assess its conservation status.

6.4.5 Sumatroscirpus Provides a Biogeographical Link Between the Sino-Himalayan and Sumatran Mountain Floras

Previously thought to be monospecific and endemic to Sumatra, the genus *Sumatroscirpus* now includes four species and a range that extends more than 2700 km north of this Indonesian island, and into Northern Vietnam, Myanmar, and Southwestern China (Fig. 6.9). Such a distribution portrays a long-recognized link between the mountain floras of Eastern Asia and Sundaland, the region encompassing the Malay Peninsula and the islands of Sumatra, Borneo, and Java (Stapf, 1894; van Steenis, 1934, 1935, 1936, 1964). Distributions encompassing continental Eastern Asia and Sumatra are notably frequent in *Sumatroscirpus*' sister group *Carex*, with >15 examples given by Raymond (1959). In most cases, montane taxa shared by these regions have their centre of diversity in continental Eurasia, suggesting migration into Sundaland during cold periods of the Tertiary or Quaternary (Wallace, 1880; van Steenis, 1934, 1936). Phylogeographic analyses of diverse plant and animal lineages support this hypothesis by inferring frequent Tertiary and Quaternary dispersals from Indochina into Sumatra, Java or Borneo, especially during the Plio-Pleistocene (1–5 Ma; Bruyn & al., 2014).

The connection between the montane floras of Southeast Asia and Sundaland was already well established in the pollen record during the Oligocene and earliest Miocene (34–21 Ma), after which pollen of Asian species gradually declined in Sundaland, with occasional returns in small pulses especially in the Plio-Pleistocene (1–5 Ma; Morley 1998). Periods of colder and drier climate during the mid- to late-Tertiary and Quaternary glacials probably played a strong role in connecting these montane floras by lowering altitudinal zones, and thus providing important stepping stones between the Eastern Asian highland and insular mountains of Sundaland. For instance, Indonesian mountain zones have been estimated to have been 350–500 m lower during the last Pleistocene glaciations (Whitten & al., 1987), and even more drastic changes in the highland environment can be inferred from the 1000 m depression of the snowline in the Central Range of New Guinea and 1700 m drop of the vegetation belts in some montane palynological sites (Verstappen, 1980; Heaney, 1991). Even assuming a 1000 m drop in vegetation zones in Southeast Asia, long-distance dispersal in the range of at least 400–600 km would have been necessary to connect peaks of suitable habitat between the Sino-Himalayan and Sundaland montane floras, unless a more continuous highland corridor existed in the past, as some interpretations of Oligocene pollen fossils suggest (Morley, 1998).

Dispersal between distant mountain peaks might have been possible by attachment of fruits to the fur, hooves or toes of large mammals, such as tigers, rhinoceroses, elephants and deer, which are known to ascend high in Indonesian mountains (Junghuhn, 1847; van Steenis, 1935; de Schauensee & Ripley, 1939) and migrate over long distances (Corlett, 2009). Such epizootic dispersal by large mammals has been well documented in Europe and is favored by characteristics such as small propagule size, presence of bristles and occurrence in open areas (Heinken & Raudnitschka, 2002; Albert & al., 2015). *Sumatroscirpus* species possess small nutlets, elongated barbed perianth bristles (except *S. paniculatocorymbosus*), and curved spikelets and bracts with scabrous awns, characteristics that appear favorable to epizootic dispersal by large mammals. Dispersal by mammals between Malay islands and the Asian mainland would have been possible through the Sunda shelf during Tertiary or Quaternary episodes of low ocean level (Bird & al., 2005; Hall, 2009), and cooling would have promoted southward migration of northern large mammal faunas (Tougard, 2001). Epizootic dispersal by birds is thought to be very rare (Nogales & al., 2012), but the hard fruits of *Sumatroscirpus* could have been dispersed internally through ingestion as grit replacement (Beer & Tidyman, 1942; Peres &

van Roosmalen, 1996), a phenomenon that has been observed in ducks and waders ingesting seeds of *Bolboschoenus* (Asch.) Palla, *Carex*, *Cyperus* L. and *Eleocharis* R.Br. (Cyperaceae; DeVlaming & Proctor, 1968; Alexander & al., 1996). Few migratory birds are known from the high mountains of Sundaland (van Steenis, 1935), but they do exist. For instance, *Gallinago stenura* (Bonaparte), a small wader that migrates from Siberia to Southeast Asia and Indonesia, has been seen in subalpine habitats on Gunung Leuser (Aceh, Sumatra; de Schauensee & Ripley, 1939; Lepage & al., 2014).

Sumatroscirpus rupestris, although found in China, Myanmar and Vietnam, is morphologically most similar to the Sumatran *S. minor*, and very distinct from the Chinese *S. paniculatocorymbosus* (*S.* sect. *Paniculatocorymbosi*), perhaps indicating a weaker biogeographical relationship between the Indo-Chinese Region (incl. Myanmar, sensu Takhtajan 1986) and China than between the Indo-Chinese region and the more remote Sumatra. A similar pattern is seen in the early-diverged *Carex* sect. *Hypolytroides* Nelmes, with *Carex hypolytroides* Ridl. distributed in Northern Indochina and Sumatra, while its sister *C. moupinensis* Franch. is endemic to Southwestern China (Raymond, 1959; Koyama, 1979; Dai & al., 2010a; Starr & al., 2015). In both cases, the major biogeographical break is at the Hengduan Mountain Range, on the eastern edge of the Tibetan plateau.

The divergence between *Sumatroscirpus paniculatocorymbosus* and *S. rupestris* has been estimated to be in the range of 4–25 Ma (million years ago; Chapter 5; Léveillé-Bourret & al. 2018a), coinciding with intense uplift of the Hengduan Mountain Range, which attained high elevations 3–10 Ma (Sun & al., 2011; Favre & al., 2015). Presumably, Indo-Chinese and Sumatran populations would have remained connected until more recent times by Quaternary glaciations that offered periodic stepping stones or corridors for dispersal across the Malay Peninsula and Strait of Malacca. The similarity between *S. rupestris* and *S. minor* might thus be explained by a relatively recent Quaternary divergence.

6.5 Taxonomic Treatment

6.5.1 Key to Cyperoideae Tribes With Fertile Prophylls

1a. Fertile spikelet prophyll squamiform, not differentiated from glumes except for the presence of 2 major ribs; spikelets generally distichous on rachis.....**Dulichieae**

1b. Fertile spikelet prophylls sheathing, very rarely squamiform, but always sharply differentiated from glumes; spikelets spirally-inserted on rachis.....**2**

2a. Flower bisexual; perianth bristles present; spikelet prophyll not inflated; style base enlarged and persistent as differentiated tubercle on fruit; inflorescence corymbiform.....**Sumatroscirpeae**

2b. Flower unisexual, male in spikelet and female in prophyll; perianth bristles absent; spikelet prophyll generally conspicuously inflated (utriculiform); style base enlarged or not, but generally not persistent as differentiated tubercle on fruit; inflorescence usually spicate to multispicate or paniculiform, rarely corymbiform.....**Cariceae**

6.5.2 Description of the Genus Sumatroscirpus

SUMATROSCIRPEAE Lév.-Bourret & J.R.Starr, Molecular Phylogenetics and Evolution 119: 100. 2018. TYPE: *Sumatroscirpus* Oteng-Yeb.

Diagnosis—Differs from all other Cyperaceae tribes by this unique combination of characters: cladoprophylls and spikelet prophylls all fertile and sheathing, spikelets with many fertile glumes, flowers bisexual, perianth present, style base enlarged and forming a tubercle on fruit, and embryo (immature) with basal root cap and lateral germ pore.

A single genus: *Sumatroscirpus* Oteng-Yeb.

SUMATROSCIRPUS Oteng-Yeb., Notes Roy. Bot. Gard. Edinburgh 33(2): 307. 1974.—TYPE: *Sumatroscirpus junghuhnii* (Miq.) Oteng-Yeb. ≡ (*Scirpus junghuhnii* Miq.).

Diagnosis—Similar to *Scirpus* L., but differs by flowers present in the prophylls of the inflorescence, style base enlarged and forming a tubercle on the fruits, and embryo (immature) with basal root cap and lateral germ pore.

Perennial herb 0.1-1.5 m tall or more, densely cespitose or loosely tufted. **Fertile culms** 1–2 per tuft, phyllopodic (with remnants of previous years' leaves at the base), rounded to obtusely trigonous in section, smooth, scabrous, or papillose. **Vegetative pseudoculms** several per tuft, forming rosettes of 4–7 leaves. **Leaves** basal and cauline; cauline leaves 1–6 per culm. Leaf sheaths same colour as leaves and culm or red, smooth or papillose; inner bands present to almost absent, hyaline to translucent yellow-brown with red dots, or uniformly dark red to brownish-red; cauline sheaths terminated in an

inverted U-shape projection, or shallowly to deeply U- or V-shaped at apex; ligule ca. 1–11 mm long, present in basal leaves, reduced or absent in cauline leaves, with a membranous projection fringed with dense to sparse, short (0.06–0.45 mm), red brown to hyaline hairs, or hairs absent. **Leaf blades** 23–120 cm long, the widest 3.5–25 mm wide, W-shaped when unfolding and becoming flat at maturity, thin or thick, with raised nerves on abaxial surface, smooth or papillose. Leaf midvein prominent, forming a low to high keel on the abaxial surface, with or without sharp antrorse barbs. Leaf margins smooth or antrorsely scabrous. **Inflorescence** a terminal simple or compound corymb of 5–300 spikelets, and sometimes also 1–4 lateral inflorescences on peduncles 22–230 mm long from proximal cauline nodes. **Basal bracts** of inflorescence leaf-like or glume-like, proximal 2–10 bracts longer than inflorescence, or all shorter than inflorescence, following bracts becoming glume-like, but with long, green, antrorsely scabrous or sometimes smooth awns. Main inflorescence rachis 6–125 mm long. Primary inflorescence branches 0–15 (excluding lateral spikelets), with upward-arching peduncles, the longest 28–125 mm long, round to sharply trigonous or crescent shaped in section, smooth or scabrous on angles. **Cladoprophylls** all fertile, tubular to glume-like, 5.5–35 × 0.7–2.7 mm, with small adaxial swelling near base acting as a pulvinus, coriaceous, green to dark red brown adaxially, smooth or papillose, with 9–30 raised nerves, with hyaline, red dotted or uniformly red membranous nerveless zone near apex and on inner band, margin smooth or with scattered hyaline hairs 0.05–0.3 mm long at apex. **Fascicles** of 2–9 sessile or pedicellate spikelets terminating branches. Fascicle rachis smooth or papillose, sharply winged-trigonous, often antrorsely scabrous. **Perigynia (spikelet prophylls)** all fertile, similar to cladoprophylls, but smaller. **Spikelets** ellipsoid to falciform, 6–17 × 1.3–4.5 mm, appearing flattened or terete, sessile or on upward-arching pedicels up to 1.5-21 mm long that are smooth or slight scabrous, terete to crescent-shaped in section. **Glumes** ca. 4–20 per spikelet, all fertile, dark red to brownish red, membranous, smooth or papillose, laterally compressed or not; proximal glumes with bodies lanceolate to narrowly ovate, 4–8.7 × 1.3–3.9 mm, with a terminal to subterminal awn continuous with midrib; awns flat, to 0.3–3 mm long, straight to slightly recurved, smooth or with marginal antrorse barbs; midrib green, 0.2–0.6 glume width, with 1(–5) prominent nerves. **Flowers** bisexual, spirally inserted. **Bristles** 6, slightly flattened, straight and 1.2–2.3 times longer than nutlet, or reduced, contorted, and much smaller than nutlet, dark red, with antrorse hyaline barbs, warts, or smooth. **Stamens** 3, filament 2.7–8.2 mm long, narrower than anthers, anthers 1.2–4.9

× 0.2–0.4 mm, with obtuse to acute red apiculum 0.1–0.3(–0.5) mm long, narrower than thecae, sometimes with a few hyaline barbs at apex. **Style** 3.1–13 mm long, mostly 3-fid, rarely a few 2-fid, branches densely papillose with papillae longer than wide, papillae absent below branches; style base enlarged, forming a tubercle on fruit. **Nutlet** body obovoid to narrowly ellipsoid or oblong, rounded triangular to biconvex in section, 1–2.7 × 0.7–1.1 mm, 1.2–3.7 times as long as wide, 0.5–0.7 mm thick, 0.55–0.9 times as thick as wide, dark yellow to very dark reddish brown, often with grey luster, very shortly stipitate; tubercle blackish, oblong to triangular, terete to trigonous, 0.15–0.7 × 0.1–0.3 mm, 0.8–3 times as long as wide, on a short neck ca. 0.05–0.3 × 0.1–0.3 mm.

6.5.3 Key to the Species of Sumatroscirpus

1a. Terminal inflorescence open, with only 5–17 spikelets; bracts shorter than inflorescences; leaf sheaths red; widest leaf blades 3.5–5.5 mm wide; bristles reduced, much shorter than nutlet; nutlet 1–1.4 mm long; forming loose clumps on elongate rhizomes (2. sect. *Paniculatocorymbosi*) .....2.1. ***S. paniculatocorymbosus***

1b. Terminal inflorescence dense, generally with ≥70 spikelets; proximal bracts 1.8–4.6 times longer than inflorescence; leaf sheaths green; widest leaf blades 7–25 mm wide; bristles longer than nutlet; nutlet 1.7–2.7 mm long; densely cespitose (1. sect. *Sumatroscirpus*).....2

2a. Widest leaves 19–25 mm wide, smooth on both surfaces (at 20×); lateral spikelets all (sub-)sessile (pedicels rarely to 2.5 mm long); perianth bristles with many small rounded warts and only a few (< 20) large sharp barbs.....1.1. ***S. junghuhnii***

2b. Widest leaves 7–16 mm wide, with numerous papillae at least on abaxial surface (at 20×); lateral spikelets pedicellate, with longest pedicels 5–20 mm long; perianth bristles with very few warts and many (25–50) large sharp barbs.....**3**

3a. Leaves loosely papillose on both surfaces, with the leaf surface visible between the scattered papillae (at 20×); longest spikelets 9.5–17 mm long, with 5–12 glumes; longest proximal glumes 5.9–8.7 mm long.....1.2. ***S. minor***

3b. Leaves densely papillose on abaxial surface only, with the leaf surface hidden beneath the closely packed papillae (at 20×); longest spikelets 7–9.5 mm long, with 3–8 glumes; longest proximal glumes 5.7–7.1 mm long.....1.3. ***S. rupestris***

6.5.4 Description of Sumatroscirpus Sections and Species

1. SUMATROSCIRPUS SECT. SUMATROSCIRPUS

Perennial herb densely cespitose. **Leaf sheaths** concolorous with blades. **Terminal inflorescence** dense, with many spikelets. **Proximal bract** generally longer than inflorescence. **Perianth bristles** 6, slightly flattened, 2.8-4.9 mm long, longer than nutlet, dark red, with antrorse hyaline barbs and warts.

1.1. SUMATROSCIRPUS JUNGHUHNII (Miq.) Oteng-Yeb., Notes Roy. Bot. Gard. Edinburgh 33(2): 307. 1974. ≡ *Scirpus junghuhnii* Miq., Fl. Ned. Ind. 3: 307. 1856. TYPE: INDONESIA. Sumatra. Padang Lamas. Lubu Radja. In silvis communis superis. 5–10' alt. 1840 Novb., *Junghuhnn 477* [= Lubuk Raya, ca. 1.478° N 99.21° E ±1.5 km, collected between the 3rd and 6th of November 1840 based on Junghuhn 1847] (holotype: L barcode L.0042802!) .

Perennial herb to at least 156 cm tall (incl. inflorescence), cespitose(?). **Roots** dark reddish brown, to at least 1.5 mm wide. **Elongate rhizomes** absent. **Aerial vegetative parts** yellow green when dry. **Culms** 1 per tuft (excluding vegetative pseudoculms), to at least 138 cm long, phyllopodic (with remnants of previous years' leaves at the base), obtusely trigonous in section, smooth, 15–17 mm wide near base, 2.3–3.5 mm wide near apex, sheath-clad base ca. 33–40 mm wide; vegetative pseudoculms not seen. **Leaves** basal and cauline, longest basal sheath on fertile culm ca. 18 cm long, ca. 0.13 times culm length, cauline leaves 3–6. Cauline leaf sheaths 6–16 mm wide, distalmost 27–120 mm long, loosely sheathing, same colour as leaves and culms, smooth; inner bands absent except for a short dark red zone 0.7–3.5 mm long at the apex on adaxial side; proximal cauline sheaths terminated in an inverted U-shape projection 7.5–8.5 mm long on adaxial side, distally becoming shallowly U-shaped at apex; ligule ca. 2.5–11 mm long, reduced or sometimes absent on cauline leaves, with a very short thick membranous projection 0.8–1.2 mm long, margin of membranous projection fringed with dense red brown hairs to ca. 0.2 mm long. **Leaf blades** to at least 80–102 cm long, the widest 19–25 mm wide, W-shaped when unfolding and becoming flat at maturity, thick, with conspicuous raised nerves especially on abaxial surface, smooth on both surfaces. Leaf midvein prominent, forming a low to high keel on the abaxial surface, with sharp antrorse barbs mostly near apex or sometimes from tip to base. Leaf margins almost smooth to antrorsely scabrous with sharp teeth to ca. 0.2–0.4 mm long. **Inflorescence** of several compound corymbs, the terminal open, 12–18.5 × 9.5–15 cm, totalling ca. 70–300 spikelets, and 1–4 lateral inflorescences on peduncles 115–230 mm

long from distal cauline nodes. **Basal bracts** of inflorescence leaf-like, proximal 2–3 bracts longer than inflorescence, proximalmost bract blade 26–47 cm long and 9.5–12 mm wide, 1.8–2.8 times as long as inflorescence, following bracts becoming glume-like, but papillose and with long, green, antrorsely scabrous or sometimes smooth awns. Main inflorescence rachis 45–60 mm long. Primary inflorescence branches 11–15 (excluding lateral spikelets), with upward-arching peduncles, the longest 60–117 mm long, 0.5–1.9 mm wide, round to obtusely trigonous in section, smooth. **Cladoprophylls** all fertile, tubular with large oblique opening at the apex, 5.5–35 × 0.7–2.7 mm, with a very small adaxial swelling near base acting as a pulvinus, dark red brown, coriaceous, smooth or papillose, with 10–30 raised nerves, mouth with red membranaceous nerveless zone 1.5–3.3 mm long, without a differentiated abaxial inner band or forming a yellowish brown translucent inner band ca. 0.9–1.2 mm wide abaxially, margin of mouth generally smooth, or with a few scattered hairs to ca. 0.05 mm long, often retuse. **Fascicles** of 3–6 (sub-)sessile spikelets terminating branches. Fascicle rachis 2.3–8.1 mm long and 0.8–1.2 mm wide, green with numerous small red spots and lines, smooth or papillose, sharply winged-trigonous, wings with dense sharp antrorse barbs to ca. 0.4 mm long. **Perigynia (spikelet prophylls)** all fertile, similar to cladoprophylls, but more often partly open, almost glume-like, 3.8–5.8 × 1.1–1.5 mm, with a small pulvinus, with 10–17 raised nerves, often 2 lateral nerves prominent, nerveless mouth 1.4–1.8 mm long and inner band 0.7–1 mm wide. **Longest lateral spikelet pedicels** 1.5–2.6 mm long, 0.2–0.55 times as long as the spikelet bract and 0.2–0.55 times as long as the perigynium, 0.4–0.9 mm wide, round in section, smooth. **Spikelets** fusiform to falciform, 7–20 × 1.3–3 mm, longest 13–20 mm long, often slightly flattened laterally, with spreading glumes at maturity. **Glumes** ca. 7–20 per spikelet, all fertile, brownish red, membranous, smooth or papillose, sometimes with several long antrorse barbs distally on both sides of midrib, slightly laterally compressed, straight to recurved, with a narrow pale margin, sometimes with a few to several short marginal hairs; proximal glumes lanceolate to narrowly ovate, 4–7.6 × 1.5–2.5 mm, with a terminal to subterminal awn continuous with midrib, becoming shorter and relatively narrower distally; awns flat, to 0.1–1.4 mm long, longer proximally, straight to slightly recurved, smooth; midrib green with red dots, 0.5–0.9 mm wide, 0.26–0.41 whole glume width, with 1(–5) prominent nerves, flanked with a diffuse region slightly paler than the rest of the body. **Flowers** bisexual, spirally inserted. **Bristles** 6, slightly flattened, 3.9–4.9 mm long, very unequal with shortest bristle ca. 0.5–0.8 length of longest, longest 1.7–2.3 times

longer than nutlet, dark red, throughout with few (ca. 7–23) long sharp antrorse hyaline barbs ca. 0.05–0.15 mm long, and numerous (ca. 8–34) wart-like reduced rounded barbs. **Stamens** 3, filament ca. 4.9–8 mm long, narrower than anthers, orange brown, anthers 2.6–3.4 × 0.25–0.4 mm, with low, obtuse red apiculum narrower to as wide as thecae, ca. 0.15–0.25 mm long. **Style** ca. 4.2–7.2 mm long, 3-fid or rarely a few 2-fid, with branches ca. 2–4.3 mm long, branches densely papillose with papillae longer than wide, papillae also present up to ca. 1.3 mm below branches; style base enlarged, forming a tubercle on fruit. **Nutlet** body ellipsoid, rounded triangular to biconvex in section, 1.7–2.3 × 0.9–1.1 mm, 1.7–2.4 times as long as wide, widest width at ca. 0.48–0.57 body length, 0.55–0.7 mm thick, ca. 0.6–0.8 times as thick as wide, very dark reddish brown with a grey luster, very shortly stipitate; tubercle reddish black, conic to cylindric, 0.3–0.5 × 0.15–0.25 mm, 1.2–3 times as long as wide, terminated in a reddish black style remnant ca. 0.15–0.15 mm long, on a long neck ca. 0.2–0.3 × 0.15–0.3 mm.

***Etymology*—**The specific epithet honours Franz Wilhelm Junghuhn (1809–1864), who collected the type during his exploration of West Sumatra in 1840–1841.

***Distribution*—**On volcanic peaks of the Barisan Mountain Range, South of Lake Toba, North Sumatra and West Sumatra provinces, Sumatra, Indonesia.

***Habitat and Ecology*—**Montane zone of volcanic peaks, at 1,860–2,400 m elevation.

***Phenology*—**Flowering and fruiting at least from May to July, with fruits still present in November. Possibly flowers year-round.

Conservation Status*—Sumatroscirpus junghuhnii* would qualify as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected.

***Notes*—**The new circumscription of this taxon deviates significantly from Kükenthal (1940).

***Additional Material Examined*—Sumatra**: G Malintang, Sum. W. K. [= Gunung Sago, ca. 0.33000° S 100.67000° E ±2 km], Standplaats: Kreupehant, 2260 m, 1918.viii.1, *Bünnemeijer 4203* (B barcode 10 0676589, BO 1585389, BO 1587917). Gg. Koernitji, Sum. W. K. [= Gunung Kerinci, ca. 1.69° S 101.27° E ±3 km], Standplaats: Burch, 2400 m, 1920.v.11, *Bünnemeijer 10468* (BO 1587915, BO 1587916).

1.2. ***Sumatroscirpus minor*** (Kük.) Lév.-Bourret & J.R.Starr, comb. nov. ≡ *Scirpus junghuhnii* var. *minor* Kük., Bull. Jard. Bot. Buitenzorg III. 16: 301. 1940. TYPE**:** INDONESIA. Sumatra. Atjeh Gajolanden, Potjoek Angasan, bivak 1 naar 2, offene Gebirgsheiden, rehr gemein. 2000–3400 m alt. 28-1-1937, *van Steenis 8397* [Gunung Pucuk Angkasan, ca. 3.9347° N 97.2160° E ±5 km] (lectotype designated here: B barcode 10 0676592!; isolectotypes: BO? [not found], K!, L barcode L.1399061!, SING barcode 0217616!). Other specimens in the original syntype series (lectoparatypes): INDONESIA. Sumatra. Atjeh, Gajolanden: Top Goh Lemboeh, offene Gebirgsheiden, gemein. 3000 m alt. 21-22/2-1937, *van Steenis 9084* [Gunung Gohlembuh, ca. 4.236° N 97.423° E ±1.5 km] (B barcode 10 0676591!, BO 1585391!). Sumatra. Atjeh Gajolanden, Top plateau G.Kemiri. Standplaats: Gebirgswiese, feucht. rehr gemein, niemals. Frequentie: betandbildend, da. Bijzonderheden: die Pflanzen heine blatter bilden. 3150–3314 m alt. 8-9/3-1937, *van Steenis 9671* [Gunung Kemiri, ca. 3.761° N 97.478° E ±3 km] (BO 1587918!; L barcode L.1399062!).

Perennial herb 51–112 cm tall (incl. inflorescence), densely cespitose. **Roots** dark reddish brown, densely felted with hyaline to very pale orange brown hairs, to 2.1 mm wide, with a central white strand surrounded by a reddish brown ring, free from the dark reddish brown rind. **Elongate rhizomes** absent. **Aerial vegetative parts** yellow green to olive green when dry. **Culms** 1–2 per tuft (excluding vegetative pseudoculms), 40–97 cm long, phyllopodic (with remnants of previous years' leaves at the base), obtusely trigonous in section, with numerous scattered papillae throughout, 2.5–5 mm wide near base, 1.5–3.2 mm wide near apex, sheath-clad base ca. 10–19 mm wide; vegetative pseudoculms with 4–6 leaves and 7.5–31.5 cm high. **Leaves** basal and cauline, longest basal sheath on fertile culm 8.5–28 cm long, 0.13–0.38 times culm length, cauline leaves 1–3. Cauline leaf sheaths 3–9 mm wide, distalmost 45–100 mm long, loosely sheathing, same colour as leaves and culms, papillose; inner bands of basal and cauline leaves similar, short and narrow, ca. 6.5–16 × 2–6.5 mm,

uniformly brownish red, faintly nerved, smooth to papillose, bordered by numerous dark red dots; cauline sheaths deeply U-shaped at apex; ligule ca. 1–2.5 mm long, reduced or sometimes absent on cauline leaves, with a very short thick membranous projection 0.2–1.6 mm long, margin of membranous projection fringed with dense red brown hairs to ca. 0.15–0.45 mm long. **Leaf blades** to at least 47–120 cm long, the widest 7–16 mm wide, W-shaped when unfolding and becoming flat at maturity, or sometimes with margins strongly recurved to revolute (at least upon drying), thick, with conspicuous raised nerves especially on abaxial surface, papillose on both surfaces or rarely only on abaxial surface, with the leaf surface visible between the scattered papillae (at 10×), ca. 300–800 papillae/mm^2, denser near apex, papillae longer than wide on abaxial surface, generally less dense and smaller on adaxial surface. Leaf midvein prominent, forming a high keel on the abaxial surface, with sharp antrorse barbs almost to base. Leaf margins antrorsely scabrous with sharp teeth to ca. 0.2–0.4 mm long. **Inflorescence** a terminal compound corymb, open, 8–17.5 × 8–15 cm, totalling ca. 70–230 spikelets, with sometimes 1 lateral inflorescence on a peduncle 22–213 mm long from the distalmost cauline node. **Basal bracts** of inflorescence leaf-like, proximal 2–4 bracts longer than inflorescence, proximalmost bract blade 16–41 cm long and 4–9 mm wide, 1.5–3.4 times as long as inflorescence, following bracts becoming glume-like, but with long, green, antrorsely scabrous awns. Main inflorescence rachis 35–125 mm long. Primary inflorescence branches 5–14 (excluding lateral spikelets), with upward-arching peduncles, the longest 33–125 mm long, 0.7–1.4 mm wide, round to obtusely trigonous in section, smooth to papillose, rarely with one or two lines of antrorse barbs. **Cladoprophylls** all fertile, tubular with large oblique opening at the apex, 5.5–13 × 0.8–1.7 mm, with a very small adaxial swelling near base acting as a pulvinus, adaxially green, coriaceous, smooth, with 9–14 raised nerves, mouth with red membranaceous nerveless zone 0.6–1.8 mm long, forming an inner band 0.8–1.2 mm wide abaxially, margin of mouth generally smooth, or with a few scattered hairs to ca. 0.1–0.3 mm long. **Fascicles** of 2–7 pedicellate spikelets terminating branches. Fascicle rachis 3.5–8.2 mm long and 0.5–1 mm wide, green with numerous small red spots and lines, smooth, sharply winged-trigonous, wings with scattered to dense sharp antrorse barbs to ca. 0.3 mm long. **Perigynia (spikelet prophylls)** all fertile, similar to cladoprophylls, but sometimes glume-like when associated with subsessile distal spikelets, 4–6.5 × 0.7–1.2 mm, with a small pulvinus, adaxially green with numerous red dots, with 5–14 raised nerves, often 2 lateral nerves more prominent, nerveless mouth

0.6–1.1 mm long and inner band 0.4–0.7 mm wide. **Longest lateral spikelet pedicels** 5–19.5 mm long, 0.6–1.6 times as long as the spikelet bract and 0.9–2.7 times as long as the perigynium, 0.5–0.9 mm wide, elliptic to trigonous or crescent-shaped in section, upward-arching, completely smooth or very rarely with a few antrorse barbs. **Spikelets** fusiform to falciform, 7.5–17 × 1–4 mm, longest 9.5–17 mm long, often slightly flattened laterally, with spreading glumes at maturity. **Glumes** ca. 5–12 per spikelet, all fertile, brownish red, membranous, papillose or sometimes smooth, laterally compressed and recurved, without a differentiated margin, sometimes with a few short marginal hairs; proximal glumes lanceolate to narrowly ovate, 5.4–8.7 × 2.1–3.9 mm, with a terminal to subterminal awn continuous with midrib, becoming shorter and relatively narrower distally; awns flat, to 0.3–3 mm long, longer proximally, straight to slightly recurved, with a few small teeth at tip and often several large marginal antrorse barbs; midrib green, 0.5–0.9 mm wide, 0.22–0.38 whole glume width, with 1(–3) prominent nerves, flanked with a diffuse region slighly paler than the rest of the body. **Flowers** bisexual, spirally inserted. **Bristles** 6, slightly flattened, 2.8–4.4 mm long, or up to 6.9 mm in prophyll flowers, very unequal with shortest bristle ca. 0.5–0.9 length of longest, longest 1.2–1.4 times longer than nutlet, dark red, throughout with numerous (ca. 25–73) long sharp antrorse hyaline barbs ca. 0.05–0.2 mm long, and very few (ca. 0–12) wart-like reduced rounded barbs. **Stamens** 3, filament ca. 6–8.2 mm long, narrower than anthers, cream to brownish orange coloured, anthers 2.5–4.4 × 0.3–0.4 mm, with obtuse to acute red apiculum narrower than thecae, sometimes with a few hyaline barbs at apex, ca. 0.1–0.3(–0.5) mm long (including barbs if present). **Style** ca. 7.8–13 mm long, 3-fid or often a few to several 2-fid, with branches ca. 2.9–5 mm long, branches densely papillose with papillae longer than wide, papillae also present up to ca. 1 mm below branches; style base enlarged, forming a tubercle on fruit. **Nutlet** body narrowly ellipsoid to oblong, rounded triangular in section, 2.6–2.7 × 0.7–0.8 mm, 3.3–3.7 times as long as wide, 0.6–0.7 mm thick, ca. 0.7–0.9 times as thick as wide, reddish black with a grey luster, very shortly stipitate; tubercle pale orange brown to greyish brown, with red spots, triangular to oblong, trigonous, 0.35–0.7 × 0.2–0.3 mm, 1.3–2.4 times as long as wide, terminated in a reddish black style remnant ca. 0.1–0.3 mm long, on a short neck ca. 0.1–0.15 × 0.2–0.3 mm.

***Etymology*—**The specific epithet refers to the smaller stature of the plants compared to *Sumatroscirpus junghuhnii*.

***Distribution*—**Restricted to the subalpine plateaus of the non-volcanic mountains of the Gunung Leuser area, Aceh Province, Sumatra, Indonesia.

***Habitat and Ecology*—**In wet blang, an open subalpine vegetation type dominated by low shrubs, sedges and moss on impermeable kaolinitic clay, 2,500–3,400 m elevation.

***Phenology*—**Flowering and fruiting at least from January to August, possibly year-round.

Conservation Status*—Sumatroscirpus minor* would qualify as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criteria B1 and B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected.

***Notes*—**Herbarium labels of the van Steenis 1937 expedition often indicate very broad altitudinal ranges, sometimes as low as 2,000 m, which is due to the fact they include the full altitudinal range of a day's travel, rather than the actual elevation of every collection. However, *Sumatroscirpus minor* appears strictly associated with mountain blang, a vegetation type found mostly above 2,600 m in the Gunung Leuser Reserve (de Wilde & Duyfjes, 1996). The new circumscription of this taxon deviates significantly from Kükenthal (1940). This species is morphologically similar to the continental *Sumatroscirpus rupestris* (see notes under this species). A picture of this species in its open habitat is found in Kern (1974: 500).

***Additional Material Examined*—Sumatra**: Atjeh, Gajolanden, Losir massief, bivak 3 naar 4, groote waterscheiding [ca. 3.87000° N 97.13600° E], Standplaats: offene Gebirgsheiden, Frequentie: rehr gemein, an einigen, 2250–2750 m, 1937.i.30, *van Steenis 8484* (B barcode 10 0676590, BO 1585399, K). Atjeh, higher elevation of Gunung Kemiri [ca. 3.7822° N 97.4850° E], In Ericoid forest at mountain ridge, 2600–2900 m, 1971.viii.4, *Iwatsuki, Murata, Dransfield & Saerudin 1167* (BO 1585390). Climbing Gunung Bandahara; ca. 10 km NE of kampung Seldok (Alas Valley), ca. 25 km N of Kutatjane, Gunung Leuser Nature Reserve, Atjeh [ca. 3.7150° N 97.8100° E ±1 km], Wet blang. Locally on half-shaded places, Plants growing in loose groups, 2500–2600 m, 1975.ii.23, *de Wilde & de Wilde-Duyfjes 15236* (A barcode 00914679, L barcode L.1399057, MO 2418688). Gunung Leuser Nature Reserve, Atjeh, North Sumatra, Camp 6, summit area, Climbing Gunung Bandahara, ca. 12 km

NE of kampung Seldok (Alas Valley), ca. 25 km N of Kutatjane [ca. 3.7230° N 97.7890° E ±0.5 km], Montane scrub/blang vegetation, locally, a few tussocks, 2800–3000 m, 1975.ii.27, *de Wilde & de Wilde-Duyfjes 15272* (K, L barcode L.1399059). Gunung Leuser Nature Reserve, Atjeh, North Sumatra, Climbing Gunung Leuser West top, from Penosan via Putjuk Angasan, ca. 25 km SW of Blang Kedjeren, Camp 9, Leuser W. top and vicinity [ca. 3.7720° N 97.19000° E ±2.5 km], Scrub edge, 3100–3420 m, 1975.iv.9, *de Wilde & de Wilde-Duyfjes 16227* (L barcode L.1399060). Gunung Leuser Nature Reserve, Atjeh, North Sumatra, Mt. Mamas, Climbing Gunung Mamas, c. 24 km SW from the mouth of Lau Ketambe, c. 30 km NW of Kutatjane [ca. 3.5750° N 97.5740° E ±2 km], Open marshy place, Mossy forest / montane scrub, 2650–2700 m, 1975.v.13, *de Wilde & de Wilde-Duyfjes 16874* (K, L barcode L.1399058).

1.3. ***Sumatroscirpus rupestris*** Lév.-Bourret & J.R.Starr, sp. nov. TYPE: VIETNAM. Lào Cai Province, Hoàng Lien National Park, Sapa District, San Sa Ho Commune, Trạm Tôn Trail (main trail) to summit of Mount Phan Si Pang (Fan Si Pan), 100 m from its junction with the Sin Chai trail, 22˚18’26.5”N, 103˚46’33.6”E [WGS 84], 2958 m (GPS), 2820 m (altimeter). NE facing 50˚–90˚slope dominated by *Chimonobambusa* sp., *Rhododendron* spp., and other Ericaceae. Wet organic soil (pH 4.7–4.8) with dense cover of bamboo leaves, *Sphagnum*, and brown pleurocarpous Bryopsida. Hundreds of individuals within 100 m of this lat./long. Population is close to cable car corridor. April 21, 2015. *Bruce A. Ford, Julian R. Starr, Étienne Léveillé-Bourret, Nguyễn Thị Kim Thanh, Vũ Anh Tài, & Scott Ford 15081* (holotype: WIN!; isotypes: DAO!, GH!, K!, L!, MT!, MICH!, MO!, NY!, HNU!, WIN!).

Perennial herb 27–80 cm tall (incl. inflorescence), densely cespitose. **Roots** dark reddish brown, smooth to densely felted with hyaline to very pale orange brown hairs, to 2.3 mm wide, with a central white strand surrounded by a reddish brown ring, free from the dark reddish brown rind. **Elongate rhizomes** absent. **Aerial vegetative parts** light green to yellow green when dry. **Culms** 1 per tuft (excluding vegetative pseudoculms), 22–73 cm long, phyllopodic (with remnants of previous years’ leaves at the base), obtusely trigonous in section, smooth, but with scattered papillae near apex, 2.3–3.5 mm wide near base, 0.8–2.3 mm wide near apex, sheath-clad base ca. 12–23 mm wide; vegetative pseudoculms with 5–8 leaves and 12–34 cm high. **Leaves** basal and cauline, longest basal sheath on

fertile culm 12.5–21 cm long, 0.17–0.59 times culm length, cauline leaves 2–5. Cauline leaf sheaths 2–7 mm wide, distalmost 16–73 mm long, loosely sheathing, same colour as leaves and culms, papillose; inner bands of basal and cauline leaves similar, narrow, ca. 8–16 × 2–6.5 mm, uniformly brownish red or hyaline with red dots, faintly nerved, smooth to papillose, bordered or not by numerous dark red dots; cauline sheaths deeply U- to V- shaped at apex; ligule ca. 3–8.5 mm long, reduced or sometimes absent on cauline leaves, with a very short thick membranous projection 0.8–2 mm long, margin of membranous projection fringed with dense red brown hairs to ca. 0.15–0.3 mm long. **Leaf blades** to at least 50–60 cm long, the widest 10–13 mm wide, W-shaped when unfolding and becoming flat at maturity, thick, with conspicuous raised nerves especially on abaxial surface, papillose only on abaxial surface, with the leaf surface not visible between the dense papillae (at 10×), ca. 900–2000 papillae/mm^2, denser near apex, papillae low, not much longer than wide. Leaf midvein prominent, forming a high keel on the abaxial surface, with sharp antrorse barbs almost to base. Leaf margins antrorsely scabrous with sharp teeth to ca. 0.2–0.4 mm long. **Inflorescence** a terminal compound corymb, open, 5–7 × 5.5–11.5 cm, totalling ca. (30–)80–160 spikelets. **Basal bracts** of inflorescence leaf-like, proximal 1–10 bracts longer than inflorescence, proximalmost bract blade (5–)18.5–23 cm long and 2–7 mm wide, (1–)2.7–4.6 times as long as inflorescence, following bracts becoming glume-like, but with long, green, antrorsely scabrous awns. Main inflorescence rachis 20–46 mm long. Primary inflorescence branches 4–17 (excluding lateral spikelets), with upward-arching peduncles, the longest 28–45 mm long, 0.3–0.9 mm wide, round to obtusely trigonous in section, upward-arching, smooth or with a few scattered antrorse barbs in lines. **Cladoprophylls** all fertile, tubular with large oblique opening at the apex, 5.7–17 × 0.7–1.5 mm, with a very small adaxial swelling near base acting as a pulvinus, adaxially green, coriaceous, smooth, with 10–14 raised nerves, mouth with red membranaceous nerveless zone 0.9–2.2 mm long, forming an inner band 0.8–2 mm wide abaxially, red to hyaline with red lines, margin of mouth generally smooth, or with a few scattered hairs to ca. 0.1–0.15 mm long. **Fascicles** of 2–9 pedicellate spikelets terminating branches. **Fascicle** rachis 2.3–6.7 mm long and 0.4–0.8 mm wide, green with numerous small red spots and lines, smooth, sharply winged-trigonous, wings with scattered to dense sharp antrorse barbs to ca. 0.2 mm long. **Perigynia (spikelet prophylls)** all fertile, similar to cladoprophylls, but sometimes glume-like when associated with subsessile distal spikelets, 4.3–7.5 × 1.1–1.5 mm, with a small pulvinus, adaxially green with numerous

red dots, with 4–8 raised nerves, often 2 lateral nerves more prominent, nerveless mouth 1.7–3.5 mm long and inner band 0.7–1.4 mm wide. **Longest lateral spikelet pedicels** 5.5–12 mm long, 0.6–1.3 times as long as the spikelet bract and 1–2 times as long as the perigynium, 0.5–0.9 mm wide, trigonous or crescent-shaped in section, smooth. **Spikelets** fusiform to falciform, 5.5–9.5 × 1.3–2.6 mm, longest 7–9.5 mm long, often slightly flattened laterally, with spreading glumes at maturity. **Glumes** ca. 3–8 per spikelet, all fertile, brownish red, membranous, smooth, laterally compressed and recurved, without a differentiated margin, with short marginal hairs to ca. 0.1 mm long; proximal glumes narrowly ovate to narrowly obovate, 5–7.1 × 1.8–3 mm, with a terminal to subterminal awn continuous with midrib, becoming shorter and relatively narrower distally; awns flat, to 0.4–1.4 mm long, longer proximally, straight to slightly recurved, with a few small teeth at tip and sometimes several large marginal antrorse barbs; midrib green with red dots, 0.4–0.9 mm wide, 0.17–0.36 whole glume width, with 1(–3) prominent nerves, flanked with a diffuse region slighly paler than the rest of the body. **Flowers** bisexual, spirally inserted. **Bristles** 6, slightly flattened, 2.8–4.5 mm long, unequal with shortest bristle ca. 0.6–0.9 length of longest, longest 1.6–1.8 times longer than nutlet, dark red, throughout with numerous (ca. 23–50) long sharp antrorse hyaline barbs ca. 0.05–0.2 mm long, and very few (ca. 0–11) wart-like reduced rounded barbs. **Stamens** 3, filament ca. 5–6.7 mm long, narrower than anthers, cream coloured, anthers 3.2–4.9 × 0.3–0.4 mm, with obtuse to acute red apiculum narrower than thecae, sometimes with a few hyaline barbs at apex, ca. 0.15–0.35 mm long (including barbs if present). **Style** ca. 6–9.2 mm long, 3-fid, or very rarely 2-fid, with branches ca. 2.1–5.4 mm long, branches densely papillose with papillae longer than wide, papillae also present up to ca. 0.8 mm below branches; style base enlarged, forming a tubercle on fruit. **Nutlet** (immature) body narrowly ellipsoid to oblong, rounded triangular in section, ca. 2–2.3 × 0.8–0.9 mm, ca. 2.6–2.9 times as long as wide, ca. 0.5–0.6 mm thick, ca. 0.6–0.8 times as thick as wide, very shortly stipitate; tubercle ca. 0.4 × 0.1–0.2 mm, ca. 3 times as long as wide, on a short neck ca. 0.15 × 0.25 mm.

***Etymology*—**The specific epithet refers to its occurence on rock cliffs and steep slopes.

***Distribution*—**Known from the Gaoligong Shan, Hengduan Mountains, Yunnan, China and the Shan-ngaw Range just across the border in Kachin State, Myanmar, in addition to a disjunct population on Mount Fan Si Pan, North Vietnam.

***Habitat and Ecology*—**In low subalpine bamboo-*Rhododendron* thickets on steep slopes and hanging down rock cliffs, 2,800–3,200 m elevation.

***Phenology*—**Flowering late April to late July.

Conservation Status*—Sumatroscirpus rupestris* would qualify as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected.

***Notes*—**This species is morphologically similar to the Sumatran *Sumatroscirpus minor*, but is readily distinguished by the very dense papillae under its leaves, shorter spikelets (Fig. 6.8), and occurence in the Sikang-Yunnan floristic Province, more than 2,000 km across the Malay Peninsula and Strait of Malacca.

***Additional Material Examined*—China**: Fugong Xian, Yaping Xiang. Between Shibali logging station and Yaping pass, ca. 7.2 km W of Shibali, on the road from the Nujiang to Yaping pass, E side of Gaoligong Shan. Yunnan, 27° 10’ 45’’ N 98° 43’ 44’’ E, Bamboo-*Rhododendron* thicket with scattered *Abies-Tsuga* forest, Growing along rock at bank of road, 2999 m, 2004.v.2, *Heng, Zhiling, Yunheng, Fritsch, Lihua & Armstrong, Gaoligong Shan Biodiversity Survey 20184* (GH barcode 00308189). **Myanmar**: North Triangle (Tama Bum) [Hkangri Bum on recent maps, ca. 26.7958° N 98.2673° E ±100 km], Open places on a big granite slab, along the joints, 3230 m, 1953.vi.27, *Kingdon-Ward 21063* (A barcode 00914680). **Vietnam**: West of Sa Pa, along trail to summit of Fan Si Pan Mountain from village of Xin Chai, Lao Cai, 22° 18’ 52’’ N 103° 46’ 34’’ E, Forest on wet slope, 2800 m, 1997.iv.20, *Lowry II & Sterling 4892* (MO 05080625).

2. **Sumatroscirpus sect. Paniculatocorymbosi** (Kük.) Lév.-Bourret & J.R.Starr, **comb. nov.** ≡ *Scirpus* sect. *Paniculatocorymbosi* Kük., Acta Horti Gothob. 5: 36. 1930. TYPE: *Sumatroscirpus paniculatocorymbosus* (Kük.) Lév.-Bourret & J.R.Starr. ≡ (*Scirpus paniculatocorymbosus* Kük.).

Perennial herb forming loose clumps on elongate rhizomes. **Leaf sheath** pale red. **Terminal inflorescence** open, with a few spikelets. **Proximal bract** shorter than inflorescence. **Perianth bristles** 6, reduced, contorted, slightly flattened, 0.3–0.7 mm long, very unequal, shorter than nutlet, dark red, smooth or with a few antrorse barbs at apex.

2.1. SUMATROSCIRPUS PANICULATOCORYMBOSUS (Kük.) Lév.-Bourret & J.R.Starr, Molecular Phylogenetics and Evolution 119: 100. 2018 ≡ *Scirpus paniculatocorymbosus* Kük., Acta Horti Gothob. 5: 35. 1930. TYPE: CHINA. Sichuan: Prov. Sze-ch'uan, reg. austr.: Ta-hsiang-ling. Ad rivulum in prato. 2800 m. May 28, 1922, *Smith 2092* ["Great Elephant Pass", ca. 29.626° N 102.648° E ±1 km] (holotype: UPS V-142813!; isotypes: B barcode 10 0525489!, GB barcode GB-004 7626!, PE barcode 00026382 [photo!]).

Perennial herb 11.5–43 cm tall (incl. inflorescence), loosely tufted. **Roots** pale pinkish brown to straw-coloured, smooth to sparsely villose with hyaline hairs, to 0.8 mm wide, with a central white strand surrounded by a brown ring, free from the pale pinkish brown to straw-coloured rind. **Elongate rhizomes** creeping, 1.5–8.5 cm long, 1.1–1.8 mm wide, dark reddish-brown, terminating in loose tufts of 1–3 shoots (including vegetative pseudoculms); rhizome completely ensheathed by long cataphylls; cataphylls 6–20 mm long, oblong with rounded tip and very short obtuse mucro, with many prominent veins. **Aerial vegetative parts** pale green to pale grey-green when dry. **Culms** solitary (excluding vegetative pseudoculms), 21.5–33 cm long, phyllopodic (with remnants of previous years' leaves at the base), round to rounded trigonous in section, smooth, rarely slightly scabrous in lines, 1.3–2 mm wide near base, 0.5–1.1 mm wide near apex, sheath-clad base ca. 3.5–8 mm wide; vegetative pseudoculms with 4–6 leaves and 10–34 cm high. **Leaves** basal and cauline, longest basal sheath on fertile culm 3.5–7 cm long, 0.13–0.26 times culm length, cauline leaves 2–3. Cauline leaf sheaths 1.3–4.5 mm wide, distalmost 18–40 mm long, loosely sheathing, pale red, smooth; inner bands of basal leaf sheaths long, to 2.5 mm wide, yellow translucent with red dots; inner bands of cauline leaves short and narrow, ca. 2–7.7 × 0.8–1.9 mm, uniformly dark red except for a very short yellow translucent nerveless zone at apex, sometimes with red dots or lines, faintly nerved, smooth, sometimes bordered by red dots; cauline sheaths deeply U- to V-shaped at apex; ligule ca. 1–2 mm long, reduced or often absent on cauline leaves, with a very short thick membranous projection to ca. 0.3–0.7 mm long, margin smooth

or irregular, sometimes with hyaline hairs to ca. 0.06 mm long. **Leaf blades** to at least 23–40 cm long, the widest 3.5–5.5 mm wide, W-shaped when unfolding and becoming flat at maturity, thin, with raised nerves, smooth on both surfaces. Leaf midvein prominent, forming a low keel on the abaxial surface, mostly smooth or sometimes with a few antrorse barbs especially near apex. Leaf margins antrorsely scabrous with sharp or blunt teeth to ca. 0.1–0.15 mm long. **Inflorescence** of several simple or compound corymb, the terminal open, 3–7.5 × 2–6.5 cm, totalling 5–17 spikelets, and 1–3 lateral corymbs on peduncles 65–140 mm long from distal cauline nodes. **Basal bracts** of inflorescence leaf-like or sometimes glume-like, all shorter than inflorescence, proximalmost bract blade 2.5–6 cm long and 0.7–1.9 mm wide, 0.7–0.9 times as long as inflorescence, following bracts becoming glume-like, but with long, green, antrorsely scabrous awns. Main inflorescence rachis 4–16 mm long. Primary inflorescence branches 0–4 (excluding lateral spikelets), with upward-arching peduncles to 31–64 mm long and 0.5–0.8 mm wide, sharply trigonous to crescent-shaped in section, antrorsely scabrous on angles. **Cladoprophylls** all fertile, glume-like or rarely tubular with large oblique opening, 6.2–8 mm long, 1.1–1.6 mm wide in natural position (sheathing flower), with a very small adaxial swelling near base acting as a pulvinus, adaxially green, coriaceous, smooth, with 14–16 raised nerves, bordered with red membranous zone 0.8–1.7 mm long at apex, when tubular with yellow translucent inner band with red dots ca. 0.5–0.6 mm wide, margin at apex smooth or with scattered to numerous hyaline hairs to ca. 0.05–0.1 mm long. **Fascicles** of 2–6 pedicellate spikelets terminating branches. Fascicle rachis 3–6.7 mm long and 0.4–1.2 mm wide, green with a few small red spots and lines, smooth, sharply winged-trigonous, wings sometimes with a few sharp antrorse barbs. **Perigynia (spikelet prophylls)** all fertile, similar to cladoprophylls, but sometimes paler with red dots, 3–5.7 mm long, 0.7–1.5 mm wide in natural position (sheathing flower), without adaxial swelling, with green midrib containing 5–8 raised nerves, inner band ca. 0.1–0.2 mm wide if present, red membranous nerveless zone 0.6–1.3 mm long at apex, margin at apex smooth to irregular or with a few hyaline hairs. **Longest lateral spikelet pedicels** 11–21 mm long, 0.8–3.2 times as long as the spikelet bract and 2–3.8 times as long as the perigynium, 0.3–0.6 mm wide, trigonous to crescent-shaped in section, upward-arching, often with several antrorse barbs in lines, especially on the angles. **Spikelets** ellipsoid to fusiform, 6–13 × 1.5–4.5 mm, longest 9.5–13 mm long, terete, with spreading glumes at maturity. **Glumes** ca. 4–12 per spikelet, all fertile, dark red to brownish red, membranous, smooth, not laterally compressed, with a very narrow

pale margin; proximal glumes with bodies lanceolate, 4–6.5 × 1.3–1.6 mm, with a terminal to subterminal awn continuous with midrib, becoming shorter and relatively narrower distally; awns flat, to 0.5–3 mm long, longer proximally, straight to slightly recurved, smooth or with marginal antrorse barbs; midrib green, 0.6–0.8 mm wide, 0.40–0.54 times glume width, with 1(–3) prominent nerves, with very narrow differentiated paler flanking region or without differentiated flanks. **Flowers** bisexual, spirally inserted. **Bristles** 6, reduced, contorted, slightly flattened, 0.3–0.7 mm long, very unequal with shortest bristle ca. 0.4–0.9 length of longest, longest 0.3–0.5 times longer than nutlet, dark red, smooth or with a few antrorse barbs at apex. **Stamens** 3, filament ca. 2.7–6.2 mm long, narrower than anthers, dark red passing to light yellow or white near apex, anthers 1.2–2.1 × 0.2–0.3 mm, with low, obtuse red apiculum narrower than thecae, ca. 0.1–0.2 mm long. **Style** ca. 3.1–4.9 mm long, 3-fid or rarely with a few 2-fid, with branches ca. 1.7–2.8 mm long, branches densely papillose with papillae longer than wide, papillae absent below branches; style base enlarged, forming a tubercle on fruit. **Nutlet** body obovoid, rounded triangular in section, 1–1.4 × 0.7–0.95 mm, 1.2–1.6 times as long as wide, widest width at ca. 0.58–0.76 body length, 0.5–0.6 mm thick, ca. 0.55–0.7 times as thick as wide, dark yellow to orange brown, with a light grey luster, shortly stipitate; tubercle reddish black to pure black, papillose, very widely ovoid, conical, 0.15–0.3 × 0.2–0.25 mm, 0.8–1.2 times as long as wide, terminated in a black style remnant ca. 0.05–0.1 mm long, on a short neck ca. 0.05 × 0.1–0.15 mm.

***Etymology*—**The epithet refers to the shape of the inflorescence of this species.

***Distribution*—**Known only in the alpine zone on the eastern edge of the Daxue Mountain Range in the area between Luding, Shimian and Hanyuan, Western Sichuan.

***Habitat and Ecology*—**Alpine meadows and understory of conifer forests, 2,600–2,800 m elevation.

***Phenology*—**Flowering and fruiting late May to early July.

Conservation Status*—Sumatroscirpus paniculatocorymbosus* would qualify as Vulnerable (VU) according to IUCN (2012) criterion D2 if a plausible future threat was identified. It would qualify as Endangered (EN) under criterion B2 if a continuing decline or extreme fluctuations in its distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected.

***Notes*—**The small inflorescences, rhizomatous habit, red leaf sheaths and reduced bristles of this species makes it highly distinctive and justify its placement in a distinct section. One additional voucher is listed in the PE herbarium virtual database (http://pe.ibcas.ac.cn/en/): *Liu 518* (PE barcodes 00026379, 00026380). This specimen was collected in the same general area as the examined specimens, but its identity could not be verified because pictures were unavailable, and loans were not possible.

***Additional Material Examined*—China**: Szechuan, reg. Occ.: Tahsiangling ["Great Elephant Pass", ca. 29.626° N 102.648° E ±1 km], in prato herboso-graminoso, 2800 m, 1934.vi.26, *Smith 10172* (GB, MO 4391069, PE barcode 01550172 [photo!], S 06-2030, UPS V-158616, UPS V-671350). Shimian county, Sichuan [ca. 29.23°N 102.36°W ±30 km], 1955, *Xie 40354* (IBSC barcode 0661196, PE barcode 00026381 [not seen]). Mt. Gongga, Dongpo, Yanzigou, Luding County, Sichuan [ca. 29.6974° N 102.0222° E ±2 km], in understory of *Picea* + *Tsuga*, 2600 m, 1982.vii.1, *Lang, Liang & Fei 401* (PE barcode 01191270 [photo!], PE barcode 01191271 [photo!]).

6.6 Tables and Figures

Table 6.1. Definitions of key terms used to describe inflorescence structures in *Sumatroscirpus* and *Carex*.

Accepted term	Definition	Synonyms
Bract	A foliar organ subtending an inflorescence branch, excluding prophylls.	
Cladoprophyll	Prophyll of a long branch.	
Open perigynium	Perigynium with margins unfused, or only fused near base, often scale-like.	
Glume	Scale-like leaf of a spikelet, generally subtending a flower. Note: glume here is not homologous to the glumes of Poaceae.	**Floral bract**
Closed perigynium	Perigynium with margins fused for all or most of their length, often tubular or utriculiform.	
Inflorescence branch peduncle	Internode of a long branch separating the cladoprophyll from the node above.	**Long paracladium epipodium**
Perigynium	Spikelet prophyll subtending a flower in *Carex* and *Sumatroscirpus*.	**Utricle**
Prophyll	First leaf of an axis, which in most monocots is singular, two-keeled, and adaxial, as in most Cyperaceae (but not reproductive units of Mapanioideae).	
Lateral spikelet	Spikelet of a short branch.	
Long branch	Inflorescence branch that is branched again, compound.	**Long paracladium**
Short branch	Inflorescence branch that is unbranched, possessing at most a prophyll and a spikelet.	**Short paracladium**
Spikelet fascicle	Group of spikelets including the terminal spikelet of an axis and all contiguous lateral spikelets.	
Spikelet pedicel	Internode of a short branch separating the spikelet prophyll from its spikelet.	**Short paracladium epipodium**
Spikelet prophyll	Prophyll of a short branch.	
Rachis	Axis with branch-bearing nodes.	
Rachilla	Axis of a spikelet, generally with flower-bearing nodes.	

Table 6.2. Diagnostic morphological features of the four currently recognized tribes of the Cariceae-Dulichieae-Scirpeae clade, which includes the only three Cyperoideae tribes with fertile prophylls.

	Scirpeae T. Lestib.	**Dulichieae** Reichenb. ex Schultze-Motel	**Sumatroscirpeae** Lév.-Bourret & J. R. Starr, trib. nov.	**Cariceae** Dumort.
Inflorescence morphology	various	spike or raceme of spikes	raceme of compound corymbs	various
Fertile spikelet prophyll	absent	present	present	present
Spikelet prophyll morphology	tubular to squamiform	squamiform	tubular	utriculiform, rarely squamiform
Spikelet disposition	spiral	distichous	spiral	spiral
Sterile proximal glumes	present or absent	absent	absent	absent
Perianth presence	present, rarely absent	present	present	absent
Flower sexuality	bisexual, or functionally unisexual with rudiment of opposite sex	bisexual	bisexual	unisexual
Enlarged, distinct, persistent style base	absent	absent	present	generally absent
Embryo germ pore position	basal to lateral	basal	basal	basal, rarely subbasal
Embryo type	Carex-, Fimbristylis- or Schoenus-type	Carex-type	Carex-type	Carex-type, rarely almost Schoenus-type

Table 6.3. Diagnostic morphological features of *Sumatroscirpus* species.

	S. junghuhnii (Miq.) Oteng-Yeb.	***S. minor*** (Kük.) Lév.-Bourret & J.R.Starr	***S. rupestris*** Lév.-Bourret & J.R.Starr	***S. paniculatocorymbosus*** (Kük.) Lév.-Bourret & J.R.Starr
Habit	cespitose	cespitose	cespitose	**rhizomatous**
Leaf sheath color	green	green	green	**red**
Widest leaf width	**19–25 mm**	7–16 mm	10–13 mm	**3.5–5.5 mm**
Leaf papillae	absent	**adaxially present, abaxially sparse**	**adaxially absent, abaxially dense**	absent
Proximal bract length	longer than inflorescence	longer than inflorescence	longer than inflorescence	**shorter or equal to inflorescence**
Inflorescence size	≥70 spikelets	≥70 spikelets	≥70 spikelets	**5–17 spikelets**
Lateral spikelets	**sessile**	pedicellate	pedicellate	pedicellate
Longest spikelet length	13–20 mm	**9.5–17 mm**	**5.5–9.5 mm**	6–13 mm
Number of glumes per spikelet	ca. 7–20	ca. 5–12	ca. 3–8	ca. 4–12
Longest proximal glume length	4–7.6 mm	5.9–8.7 mm	5.7–7.1 mm	4–6.5 mm
Perianth bristles	longer than nutlet	longer than nutlet	longer than nutlet	**reduced, shorter than nutlet**
Perianth bristle barbs	many small rounded warts, **few large sharp barbs**	few small rounded warts, many large sharp barbs	few small rounded warts, many large sharp barbs	**inconspicuous**
Nutlet length	1.7–2.3 mm	ca. 2.6–2.7 mm	ca. 2–2.3 mm	**1–1.4 mm**
Distribution	North and Western Sumatra	Aceh Province, Sumatra	Northern Vietnam, Northern Myanmar, Southwestern China	Western China

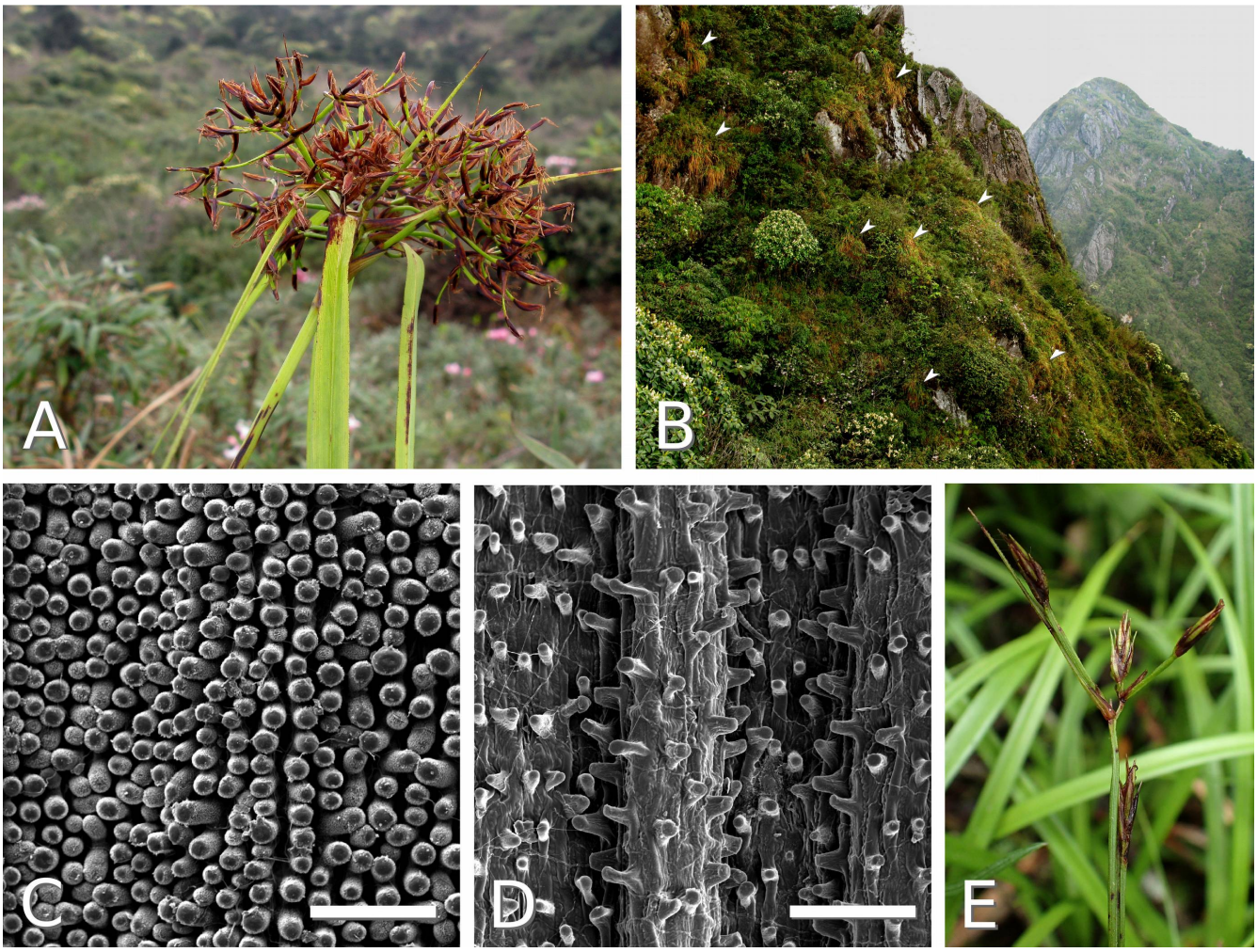


Figure 6.1. Inflorescence, habitat, and abaxial leaf surface of some species of *Sumatroscirpus*. *Sumatroscirpus rupestris*. A. Live inflorescence. B. Habitat on mount Phan Xi Pang, ca. 2,950 m elevation, with tufts of *S. rupestris* indicated by white arrows. The peak in the background on the right is a subpeak located approximated 800 m to the northwest, Đỉnh Sét Đánh (Thundering Summit). C. Abaxial leaf surface with very dense papillae. *Sumatroscirpus minor*. D. Abaxial leaf surface with scattered papillae. *Sumatroscirpus paniculatocorymbosus*. E. Live inflorescence. Scale: C–D = 0.1 mm. Photographs: A–B = J.R. Starr; C–E = É. Léveillé-Bourret. Vouchers: A–C = *Ford et al. 15081* (WIN), D = *de Wilde & de Wilde-Duyfjes 15236* (MO), E = no voucher.

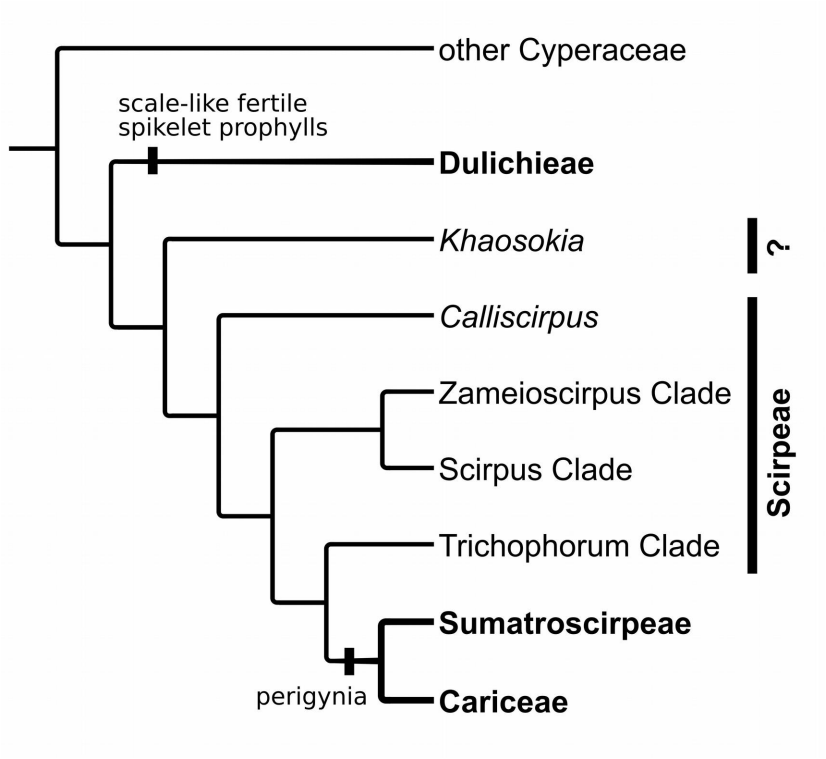
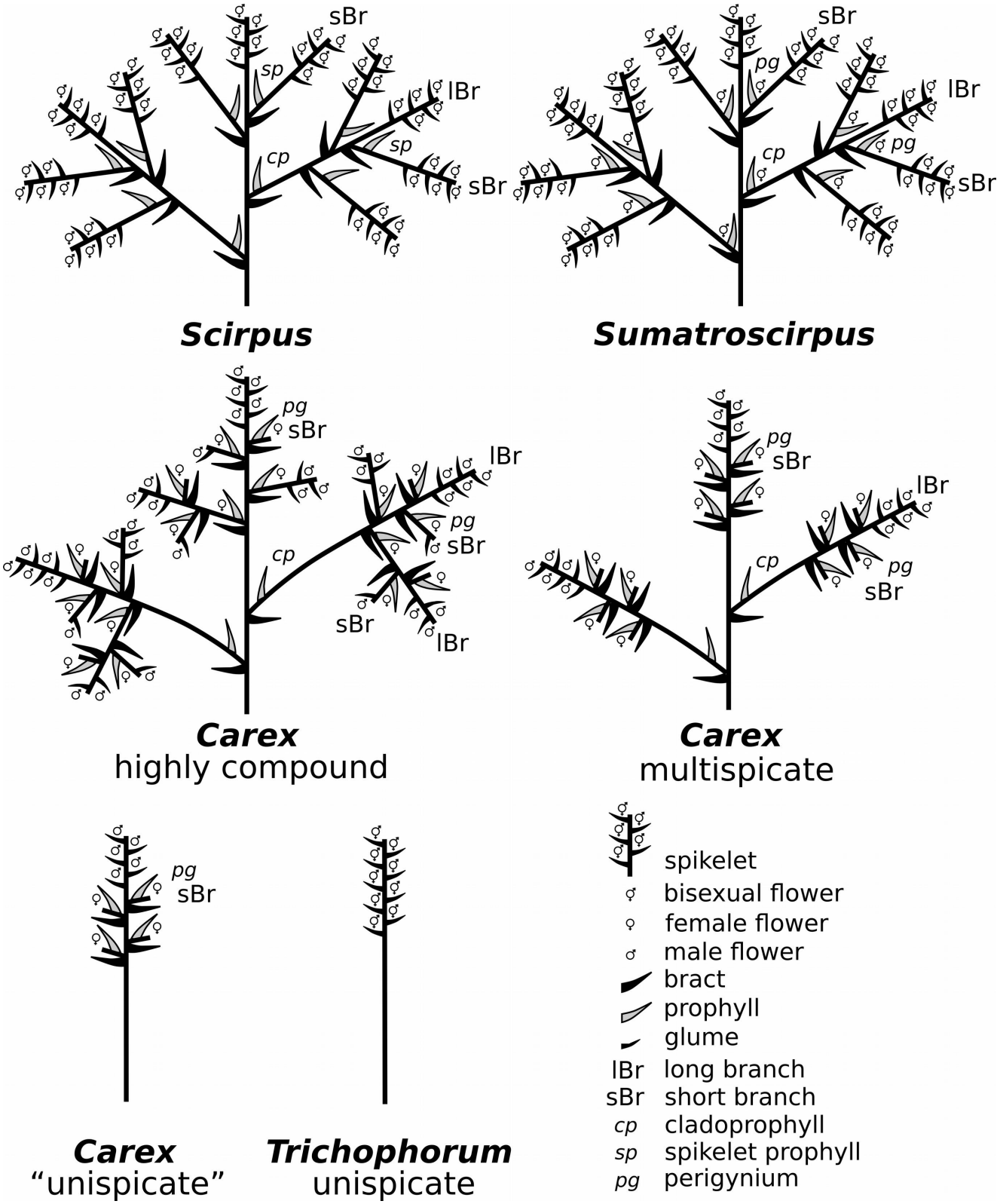


Figure 6.2. Phylogenetic relationships in the Cariceae-Dulichieae-Scirpeae clade (CDS), showing the independent evolution of scale-like (open) fertile spikelet prophylls in Dulichieae, and of (ancestrally closed) perigynia in the most recent common ancestor of Sumatroscirpeae and Cariceae. Tree based on genetic data from 3 plastid and 2 nuclear ribosomal DNA regions (Léveillé-Bourret & al. 2018a).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Carex
multispicate
pg
sBr
Carex
"unispicate"
Trichophorum
unispicate
spikelet
bisexual flower
female flower
male flower
bract
prophyll
glume
lBr long branch
sBr short branch
cp cladoprophyll
sp spikelet prophyll
pg perigynium

Figure 6.3 [on previous page]. Comparative inflorescence structure of *Sumatroscirpus* and related genera. *Scirpus* possesses corymbs of bisexual spikelets and sterile cladoprophylls and spikelet prophylls. *Sumatroscirpus* is similar, but also has fertile cladoprophylls and perigynia. *Carex* shows a variety of different inflorescence shapes, from highly compound panicles to apparently unispicate inflorescences composed of a terminal male spikelet and truncated short branches. *Carex* has male flowers in spikelets, and female flowers in the axil of perigynia, and rarely also of some cladoprophylls. *Trichophorum* typically possesses truely unispicate inflorescences, composed of a single terminal bisexual spikelet.

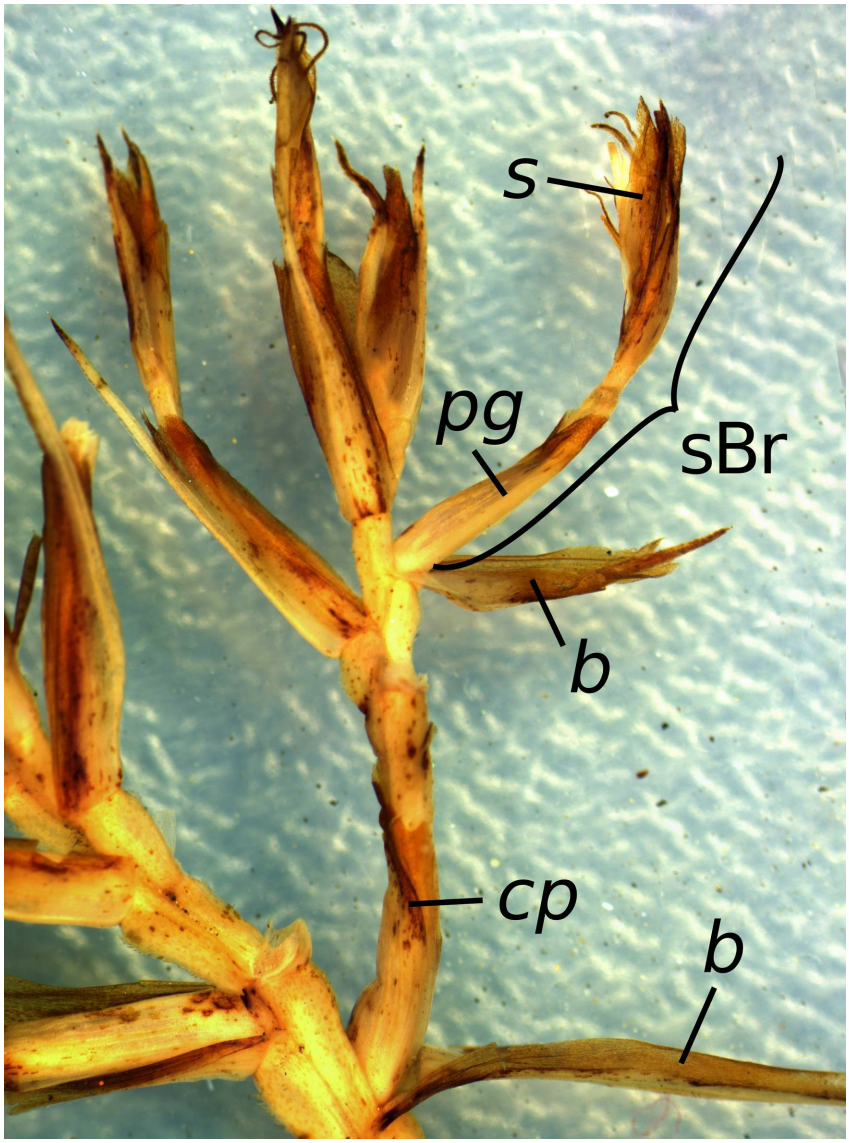


Figure 6.4. Inflorescence morphology of *Sumatroscirpus rupestris*. Legend: *b* = bract, *cp* = cladoprophyll, *pg* = perigynium, *s* = spikelet, sBr = short branch (short paracladium). Voucher: *Ford et al. 15081* (WIN).

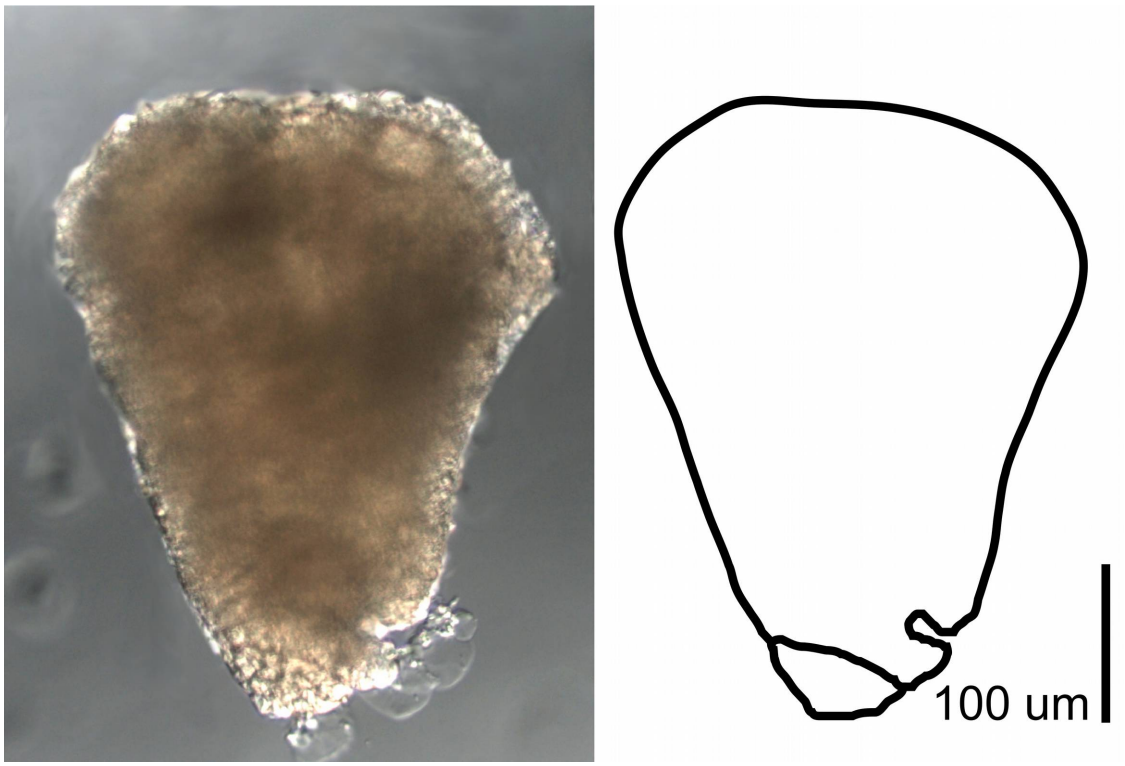
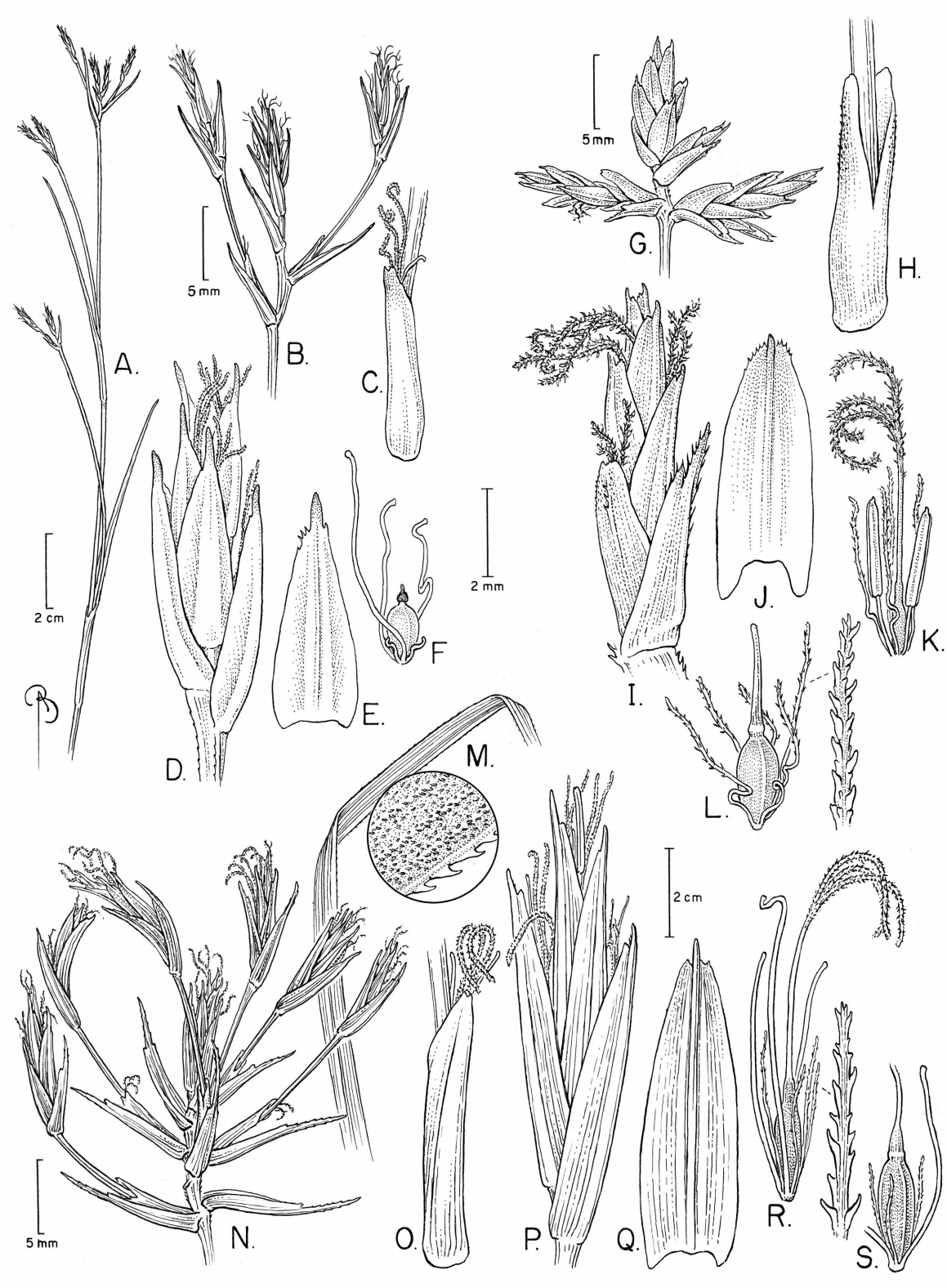


Figure 6.5. Slightly immature embryo of *Sumatroscirpus junghuhnii*. Debris was removed from the picture electronically. Voucher: *Bünnemeijer 4203* (B barcode 10 0676589).


A.
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R.
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2 cm
5 mm
2 mm
5 mm
2 cm

Figure 6.6 [on previous page]. *Sumatroscirpus paniculatocorymbosus*. A. Inflorescence. B. Fascicle of spikelets. C. Perigynium with protruding style branches of the enclosed pistil. D. Spikelet. E. Glume. F. Fruit. *Sumatroscirpus junghuhnii*. G. Fascicle of spikelets. H. Perigynium. I. Spikelet. J. Glume. K. Flower. L. Fruit and bristle closeup. *Sumatroscirpus minor*. M. Leaf (inset showing sparsely papillose abaxial surface and antrorsely scabrous margin). N. Fascicle of spikelets. O. Perigynium with protruding style branches of the enclosed pistil. P. Spikelet. Q. Glume. R. Flower. S. Fruit and bristle closeup. Based on: A–F = *Smith 10172* (MO 4391069), G–L = *Bünnemeijer 10468* (BO 1587916), M–S = *de Wilde & de Wilde-Duyfjes 15236* (MO 2418688). Illustrations by Bobbi Angell.


A.
B.
C.
D.
E.
F.
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H.
I.
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2 cm
5 mm
5 mm
2 mm
2 mm

Figure 6.7 [on previous page]. *Sumatroscirpus rupestris*. A. Habit. B. Inflorescence (inset showing bract with densely papillose abaxial surface and antrorsely scabrous margin). C. Main inflorescence rachis with cladoprophylls. D. Fascicle of spikelets with perigynia. E. Spikelet. F. Bract of lateral spikelet, side view. G. Perigynium with protruding style branches of the enclosed pistil. H. Glume in abaxial and lateral view. I. Flower and bristle closeup. J. Pistil (immature fruit) and pistil in longitudinal section. Based on: *Ford et al. 15081* (WIN). Illustrations by Bobbi Angell.

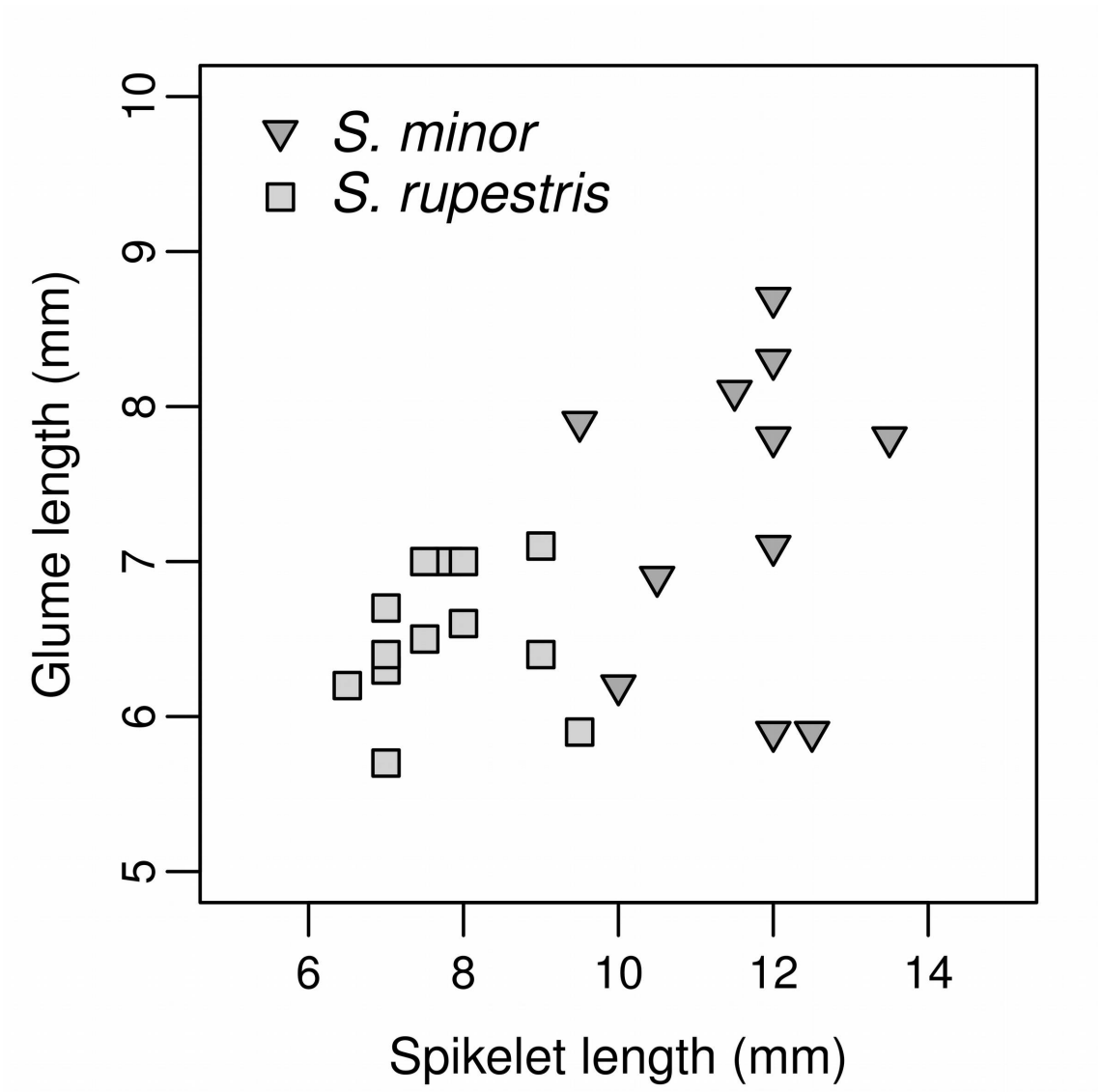


Figure 6.8. Scatter plot of longest spikelet length against longest glume length for *Sumatroscirpus minor* and *S. rupestris*.

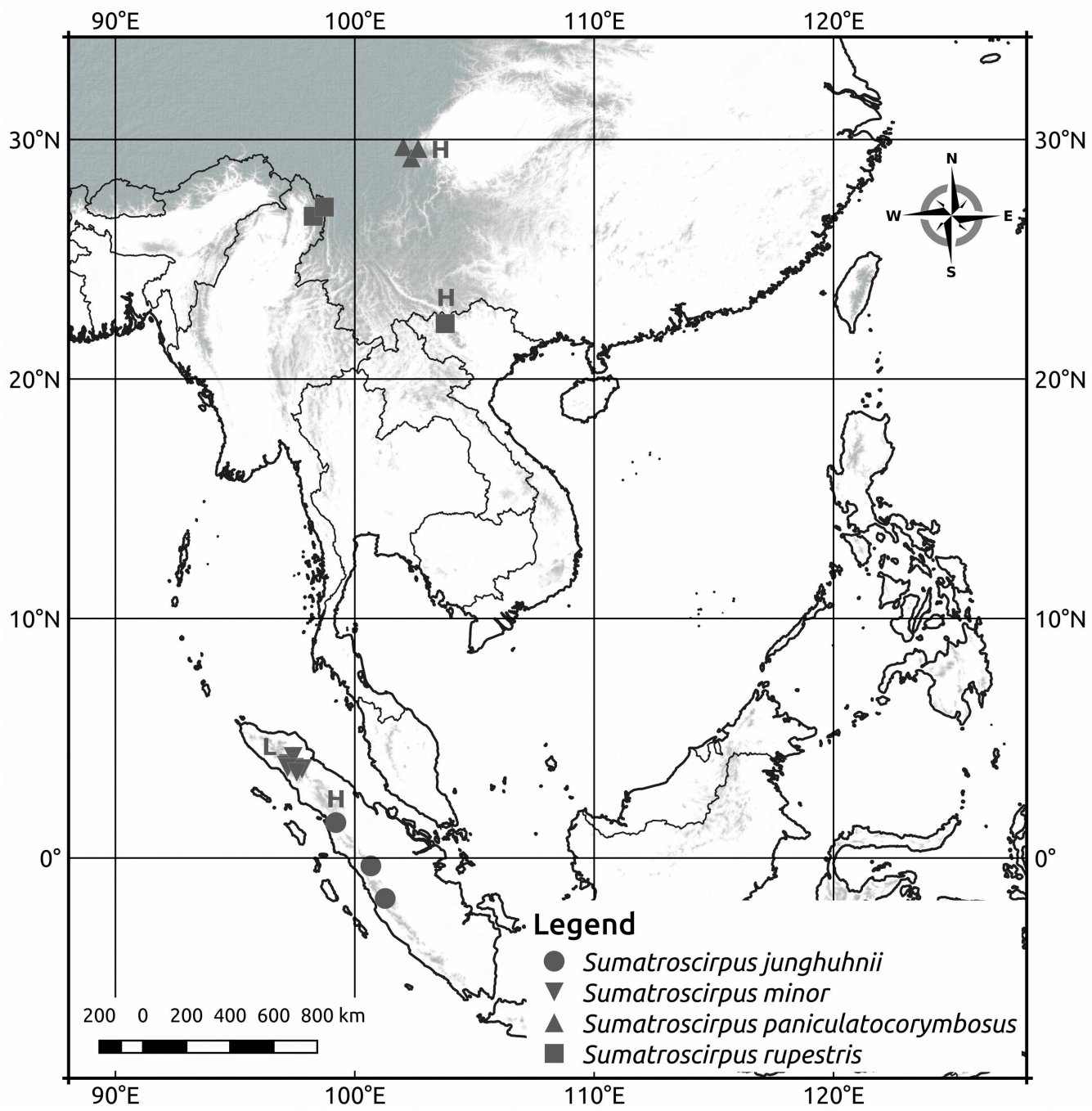


Figure 6.9. Distribution of *Sumatroscirpus* species. Grey zones indicate elevations of 1500 m or more. Holotypes and lectotypes are indicated respectively by an "H" and an "L", respectively, next to their occurence point.

CHAPTER 7

A RECLASSIFICATION OF THE CARICEAE-DULICHIEAE-SCIRPEAE CLADE WITH THREE NEW TRIBES (CYPEROIDEAE, CYPERACEAE)

This Chapter is a preliminary version of a manuscript to be published in a scientific journal at a later date. Co-author of the manuscript: Julian R. Starr. Disclaimer: new names presented here are not intended to be effectively published for nomenclatural purposes.

7.1 Introduction

The Cyperaceae are a diverse (>100 genera, ~5,500 species) and cosmopolitan family of mostly wind-pollinated, grass-like herbs that are common throughout the humid tropics, but also very well represented in the temperate and boreal regions of the world (Goetghebeur, 1998; Govaerts & al., 2007). For instance, tribe Scirpeae consists of 10 genera and approximately 80 species mostly distributed in the temperate, boreal, alpine and arctic regions of the northern hemisphere and South America (Govaerts & al., 2007; Léveillé-Bourret & al., 2015). The tribe also contains several genera that are important components of wet tundra, acid bogs and other wetlands of North America and Eurasia, where they notably are a major source of food for caribou and muskoxen, wild forage for domestic ungulates, and a food and cover resource for many birds and small mammals (e.g. *Scirpus* L., *Eriophorum* L., *Trichophorum* Pers.; Bergerud, 1972; Wein, 1973; Johnstone & al., 2002; Small & Cayouette, 2016). Despite its ecological significance, the tribe is plagued with problems of generic circumscription (Strong, 2003; Dhooge, 2005; Gilmour & al., 2013; Léveillé-Bourret & al., 2014, 2015), and its status as a natural tribe has been questioned for decades (Goetghebeur, 1986; Bruhl, 1995; Goetghebeur, 1998).

Early circumscriptions of tribe Scirpeae included all Cyperaceae species with bisexual flowers, many-flowered spikelets, and spirally inserted glumes (e.g. Clarke, 1908; Koyama, 1962a, 1969, 1971; Schultze-Motel, 1964). These characters represented what was thought to be the most "unspecialized" reproductive morphology in the Cyperoideae subfamily (Koyama, 1958, 1961a; Haines, 1966; Koyama, 1969; Raynal, 1973; Dahlgren & al., 1985). In the 1960s and 1970s, studies on flower

structure, inflorescence architecture, anatomy, and embryology made it increasingly apparent that the disparate assemblage of genera brought together under this definition of Scirpeae was unnatural (Van der Veken, 1965; Haines & Lye, 1971; Schuyler, 1971a; Raynal, 1973; Oteng-Yeboah, 1974a, 1974b). However, many authors initially continued to promote a broad circumscription for the tribe, mainly out of convenience (Koyama, 1958; Schultze-Motel, 1971; Schuyler, 1971b; Bruhl, 1995). Splitting the tribe along natural lines was also difficult because of the similarly unwieldy circumscription used at the time for its type genus *Scirpus* L., whose most inclusive treatment included as many as 250 species (Koyama, 1958; Schuyler, 1971b). The accumulation of morphological, embryological and molecular evidence eventually resulted in the transfer of the most morphologically atypical *Scirpus* species to other genera and tribes, with modern classifications now splitting Scirpeae s.lat. into 5 tribes, and leaving only ~50 species in *Scirpus* (Wilson, 1981; Goetghebeur, 1998; Govaerts & al., 2007; Muasya & al., 2009; Léveillé-Bourret & al., 2014). Nonetheless, the circumscription of tribe Scirpeae remains unsatisfactory because its remaining genera are still united by the same plesiomorphic characters that have been used since the earliest of classifications (Goetghebeur, 1998).

Molecular phylogenetic analyses have shown that Scirpeae forms a strongly supported clade with Cariceae, Dulichieae, the recently discovered Sumatroscirpeae, and the enigmatic monospecific genus *Khaosokia* (Simpson & al., 2005; Muasya & al., 2009; Jung & Choi, 2012; Léveillé-Bourret & al., 2018a). The monogeneric and cosmopolitan tribe Cariceae stands out in this clade due to its amazing diversity (~2,150 species), but also because of its unusual inflorescence structure, characterized by unisexual flowers lacking a perianth, and utriculiform prophylls subtending female flowers, called perigynia (Global Carex Group, 2015). The monogeneric Sumatroscirpeae (4 species) shares perigynia with Cariceae, but differs by the possession of bisexual flowers and perianth bristles (Léveillé-Bourret & al., In press). The small tribe Dulichieae (3 genera, 4–6 species) possesses fertile prophylls like Cariceae and Dulichieae, but they are open and squamiform, and the tribe is further characterized by its distichous spikelets and flowers (Léveillé-Bourret & al., In press). *Khaosokia* is a recently discovered monospecific genus of unclear affinity that is endemic to limestone cliffs of peninsular Thailand (Simpson & al., 2005). It shares unisexual flowers with Cariceae, seven perianth bristles and narrow elongate spikelets reminiscent of *Dulichium* (Dulichieae), but spirally-inserted flowers and sterile prophylls like Scirpeae (Simpson & al., 2005).

The studies presented in Chapters 2 and 3 have shown Dulichieae and *Khaosokia* to form two isolated, early-diverging lineages, and Scirpeae to be composed of four distinct clades forming a grade relative to the monophyletic tribes Cariceae and Sumatroscirpeae (Simpson & al., 2005, 2007; Jung & Choi, 2012; Léveillé-Bourret & al., 2014, 2015). However, relationships between these lineages were poorly supported due to the short branches forming the backbone of the CDS phylogeny, representing a rapid radiation that occurred in less than 15 million years (Escudero & Hipp, 2013; Spalink & al., 2016b; Léveillé-Bourret & al., 2018a). In Chapter 4, phylogenomic analysis incorporating data from hundreds of nuclear markers, resolved the backbone relationships of CDS with strong support, confirming the paraphyly of Scirpeae and the isolated position of *Khaosokia*, and thus establishing the phylogenetic framework needed for a full revision of tribal limits for the clade (Léveillé-Bourret & al., 2018b).

The objectives of the current study are to resolve the paraphyly of Scirpeae and the position of *Khaosokia* by proposing a new tribal classification that accurately reflects phylogenetic relationships within CDS. This new classification will be based on a total evidence phylogenetic analysis combining molecular and morphological data that will enable morphological synapomorphies to be identified for CDS tribes and genera. Descriptions of three new tribes, and an identification key to all currently recognized Cyperaceae tribes will be provided. Ongoing problems of generic circumscription will be discussed.

7.2 Materials & Methods

7.2.1 Taxonomic Sampling

Species were chosen to represent all major clades of CDS, as identified in previous Chapters (Léveillé-Bourret & al., 2014, 2018a), and to include the most extensive sample of Scirpeae species possible. A total of 137 ingroup taxa representing all CDS genera and more than 60% of the species of Scirpeae, Dulichieae and Sumatroscirpeae combined (Appendix 6). Thirteen outgroup taxa were selected to represent all major lineages of the Abildgaardieae-Cypereae-Eleocharideae-Fuireneae and *Rhynchospora* Vahl clades, which are successive sisters to CDS (Muasya & al., 2009). Taxonomy follows Govaerts & al. (2007) except for *Eriophorum*, which follows Novoselova's (1994a, 1994b)

revision of the genus, and for the recognition of *Blysmopsis* Oteng-Yeb., *Calliscirpus* C.N.Gilmour et al., and *Rhodoscirpus* Lév.-Bourret et al. Names of major *Carex* clades (Minor Carex Alliance, Caricoid Clade, Schoenoxiphium Clade, Core Unispicate Clade, Vignea Clade, Dissitiflora Lineage, Small Core Carex Clade, Large Core Carex Clade) follow Starr & al. (2015).

7.2.2 Molecular Methods

Whole genomic DNA was extracted from herbarium specimens or field samples dried in silica gel using the silica-column protocol of (Alexander & al., 2007) as modified by Starr & al. (2009). The plastid genes *matK* and *ndhF*, the plastid region *rps16*, and the nuclear ribosomal (nrDNA) regions ETS-1f and ITS were used. This marker combination includes easily aligned plastid markers that are informative at the generic and tribal levels, with common, genomically independent nrDNA regions that readily amplify from degraded DNA typical of herbarium specimens. Amplification by PCR and sequencing followed standard protocols. PCR primers for *matK* and *ndhF* are given in Gilmour & al. (2013), for *rps16* in (Peterson & al., 2010), and for ETS-1f in (Starr & al., 2003). For ITS, the primers ITS-L (Hsiao & al., 1995) and ITS-4 (White & al., 1990) were used, or sometimes replaced ITS-L with AB 101 (=17SE; (Sun & al., 1994). For plastid genes, PCR amplifications consisted of 1× reaction buffer (Bioline, United Kingdom), 2–2.5 mM $MgCl_2$ (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 µM of each primer, 1.1 µL Bovine Serum Albumin (BioShop, Canada), 0.6–1.5 U of Biotaq DNA Polymerase (Bioline) and 1–3 µL (~20–30 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. For *matK* and *ndhF*, 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 *rps16*, 180 s of initial denaturation followed by 40 cycles of 30 s of 95°C denaturation, 60 s of 47°C primer annealing and 150 s of 68°C extension, with a final extension step of 5 min. For ETS-1f, PCR amplifications consisted of 1× reaction Buffer (Bioline, United Kingdom), 2.5 mM $MgCl_2$ (Sigma Aldrich), 0.3 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.4 µM of each primer, 1 M Betaine (Sigma Aldrich), 0.6–2 U of Biotaq DNA Polymerase (Bioline) and 2–4 µL (~25–35 ng) of genomic DNA extract, adjusted to an end volume of 15 µL using nuclease-free ddH_2O. 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 48–52°C primer annealing and 120 s of 72°C DNA extension, with a final extension step of 7 min. For ITS, the same recipe was used except for 2.73 M Betaine. Cycling conditions for the ITS region were 120 s of initial denaturation followed by 40 cycles of 30 s of 94°C denaturation, 60 s of 48–50°C primer annealing and 180 s of 68°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 Inc., Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit version 3.1 (Applied Biosystems; Foster City, CA, USA). Sequencing termination products were purified according to a sodium acetate/alcohol protocol (Applied Biosystems) and sequenced on a 3130x1 Genetic Analyser. A few amplifications were purified and sequenced at Génome Québec, McGill University (Montréal, Québec, Canada). Reads were assembled and corrected with Geneious v.8.1.9 (Biomatters). All sequences were submitted to Genbank (Appendix 6).

7.2.3 Morphological Dataset

To identify morphological synapomorphies for tribes and genera of CDS, a total of 64 binary and multistate morphological characters were scored for all species included in this study (Table 7.1). This included 11 vegetative, 48 reproductive, 4 embryological, and 1 micromorphological character. These characters were traditionally used in the recognition of CDS tribes and genera, or appeared to show informative variation at this taxonomic level. Most species were scored by direct examination of herbarium specimens, often including types, from the following herbaria: A, AAH, ASU, B, BO, CAN, CAS, CHICO, CHR, CHSC, DAO, DOV, FHO, G, GB, GH, HNU, IBSC, K, L, MICH, MO, MT, NY, OSC, P, PRE, SI, SING, SYS, UBC, UPS, US, W, WIN, and WIS. Data were also obtained from personal field observations in eastern Canada, western USA, South America, and northern Vietnam, as well as from original descriptions, floras, revisions, and other literature sources (e.g. Kükenthal, 1909; Kukkonen, 1983; Ball & Reznicek, 2002; Whittemore & Schuyler, 2002; Strong, 2003; Dhooge, 2005; Reznicek & González Elizondo, 2008; Dai & al., 2010a; Liang & Tucker, 2010a, 2010b, 2010c; Zhang & Noltie, 2010; Gilmour & al., 2013; Léveillé-Bourret & al., 2015, 2018a). Embryological characters were scored exclusively from the literature (Van der Veken, 1965; Goetghebeur & Coudijzer, 1984;

Egorova, 1999; Strong, 2003; Dhooge, 2005; Gilmour & al., 2013; Semmouri, 2016; Léveillé-Bourret & al., In press; Semmouri & al., In Review). Micromorphological data on fruit anticlinal cell walls were also obtained from the literature (e.g. (Schuyler, 1971a; Tucker & Miller, 1990; Waterway, 1990; Menapace, 1991; Zhang, 2004; Sawtell, 2012; Villaverde Hidalgo, 2012). Embryological and micromorphological characters of outgroups were coded using data of congeneric species when data were not available for the particular species included in the present phylogenetic analysis. The morphological character matrix is available in Appendix 7.

7.2.4 Phylogenetic Analyses

Sequences were aligned with the MAFFT algorithm as implemented in Geneious 8.1.9 (Kearse & al., 2012), and adjusted by hand using parsimony as an objective criterion (Starr & al., 2004). Alignments were concatenated by species, although in most instances all sequences came from a single individual. Indels were coded with 2matrix 1.0 (Salinas & Little, 2014) using simple indel coding (Simmons & Ochoterena, 2000).

Heuristic maximum parsimony (MP) searches were undertaken in PAUP* 4.0a159 for Linux (Swofford, 2003) using 10,000 random addition sequence (RAS) replicates, followed by swapping with tree-bisection-reconnection (TBR), with MULTREES on, STEEPEST off, COLLAPSE off, and maximum 20 trees retained per RAS. A strict consensus of all MP trees was assembled. Branch support was assessed using 5,000 bootstrap (BS; (Felsenstein, 1985) replicates, with each replicate consisting of 5 RAS retaining 5 trees per RAS and using the strict-consensus BS (GRPFREQ = NO) to prevent undersampling-within-replicate and frequency-within-replicate artefacts (Simmons & Freudenstein, 2011). Parsimony searches were conducted on the full concatenated dataset (*matK* + *ndhF* + *rps16* + ETS-1f + ITS + indels + morphology). Separate searches were also conducted on the plastid (*matK* + *ndhF* + *rps16* + plastid indels), nuclear (ETS-1f + ITS + nuclear indels), and morphological partitions to test for incongruence.

Model-based searches were done using maximum likelihood (ML) in RAxML v8.2.10 (Stamatakis, 2014) on the Cipres web server (Miller & al., 2010) on the full concatenated dataset. The partitioning scheme was selected with PartitionFinder v2.1.1 (Lanfear & al., 2016) using the greedy

search algorithm and the Bayesian information criterion to select among all region and codon partition schemes, and between the GTR and GTR+Gamma model for each partition. PartitionFinder was also used to select among all region partition schemes for the indel dataset, and between the Binary and Binary+Gamma models for each indel partition. The scheme used included nine partitions: (1) codon positions 1 and 2 of *matK* and *ndhF*, (2) codon position 3 of *ndhF*, (3) *rps16* and codon position 3 of *matK* (4) ETS-1f, (5) ITS, (6) indels of *matK* and *ndhF*, (7) indels of *rps16* and ITS, (8) indels of ETS-1f, and (9) morphological characters. A RAxML GTR+Gamma model was used for DNA partitions, the Binary+Gamma model with ascertainment bias correction for indel partitions, and the Mk+Gamma model with ascertainment bias correction for the morphological character partition (Lewis, 2001). Substitution rates of each partitions were unlinked. Searches were made in RAxML using 500 random starting trees and the old, slower but more accurate rapid hill-climbing algorithm. Branch support was assessed by 2,000 (standard) bootstrap replicates.

Maximum likelihood BS values were placed on the highest scoring ML tree with the SumTrees 4.0.0 function of the DendroPy 4.0.3 library (Sukumaran & Holder, 2010), and parsimony BS values were added by hand. Unambiguous morphological synapomorphies were drawn on the highest scoring ML tree using Winclada v.1.00.08 (Nixon, 2002). Figures were produced with TreeGraph 2.10.1-641 (Stöver & Müller, 2010) and Inkscape 0.91 (available at http://www.inkscape.org/). Clade support was characterised subjectively as weak or poor (<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.

7.3 Results

7.3.1 Phylogenetic Results

Statistics of the molecular markers, indels and morphology datasets used in phylogenetic analyses are found in Table 7.2. A total of 61 out of 486 sequences used in this study are newly submitted to Genbank (Appendix 6). Only results from combined analyses (*matK* + *ndhF* + *rps16* + ETS-1f + ITS + indels + morphology) are discussed, as no supported (> 75% parsimony bootstrap) topological incongruence were observed in separate analyses (Figs. 7.1, 7.2, 7.3). The parsimony searches on the

full concatenated dataset found 119,260 trees of 13,019 steps with consistency and retention indices of 0.399 and 0.718. The ML topology (Fig. 7.4) had a log-likelihood of -75295.764324 as calculated by RAxML. The ML topology was very similar to the strict consensus of all MP trees, with the only exceptions being weakly-supported minor changes in the position of a few species deep within *Scirpus* and *Carex*, and in the weakly-supported position of *Calliscirpus*. Searches excluding all terminals with missing sequences gave similar results to searches using the full matrix. Results of the MP and ML bootstrap analyses are also broadly congruent, with the MP values generally slightly more conservative (Fig. 7.4). Consequently, only parsimony BS values are cited and discussed. Unless otherwise stated, tribe names refer to the updated tribal nomenclature presented here (see Taxonomic Treatment).

Phylogenetic analyses identify seven major clades within CDS, which are here recognized as tribes: Dulichieae, Khaosokieae trib. nov., Calliscirpeae trib. nov., Trichophoreae trib. nov., Scirpeae s.str., Sumatroscirpeae, and Cariceae. The strongly supported Dulichieae (*Blysmopsis*, *Blysmus* and *Dulichium*; 100% BS) and the monospecific Khaosokieae (*Khaosokia caricoides*) are successive sisters (98% BS) to a well-supported (86%) clade consisting of all other CDS tribes (Fig. 7.4). The monogeneric Calliscirpeae (*Calliscirpus*; 100% BS) is sister to Scirpeae s.str., Trichophoreae, Sumatroscirpeae and Cariceae, but with only weak ML support (78% ML BS) and no MP support. A strongly supported tribe Scirpeae s.str. (95% BS) is composed of two subclades: a strongly supported South American subclade (*Amphiscirpus*, *Phylloscirpus* and *Zameioscirpus*; 97% BS), and a strongly supported (98%) circumboreal subclade consisting of the genus *Eriophorum* nested deeply within the paraphyletic genus *Scirpus*. The strongly supported tribe Trichophoreae (*Cypringlea*, *Oreobolopsis* and *Trichophorum*; 100% BS) is weakly supported (69% BS) as sister to the strongly supported sister (100% BS) tribes Sumatroscirpeae (100% BS) and Cariceae (100% BS).

7.3.2 Morphological Synapomorphies

Ancestral state reconstruction on the ML tree from the combined analysis identifies unambiguous morphological synapomorphies for all tribes recognized here (Fig. 7.5). Monophyly of Dulichieae is supported by five synapomorphies: a colonial habit, a paniculiform inflorescence, fertile prophylls, distichous spikelets, and anthers with an elongate apiculum. The tribe is further characterized by fruits with an elongate and narrow beak, a unique feature in the CDS clade, but it is unclear whether this

character is apomorphic or plesiomorphic. Khaosokieae is distinguished by four autapomorphies: a triquetrous stem, basal glumes smaller than other glumes, unisexual flowers, and a perianth of 7 bristles. Its sterile proximal glumes are also distinctive, although this character is shared with Trichophoreae, and with unispicate species of *Eriophorum* in Scirpeae s.str. Calliscirpeae are united by five synapomorphies: a colonial habit, a ciliate ligule, a capitate inflorescence, perianth bristles projecting beyond the glumes, and white anthers. Scirpeae are united by two synapomorphies: an embryo with a lateral germ pore, and sinuate anticlinal walls of the fruit, although there is a reversal to straight anticlinal walls in *Eriophorum*. Tribe Trichophoreae is supported by six synapomorphies: leaf blades shorter than sheaths, leaf blades canaliculate or crescent-shaped in section, a unispicate inflorescence, sterile proximal glumes, barbs present throughout the length of perianth bristles, and blunt perianth bristle barbs. However, none of these characters is present in all members of Trichophoreae. The most consistent character is the presence of sterile proximal glumes, which are absent in a single species (*Trichophorum cespitosum* (L.) Hartm.), and which combined with bisexual flowers distinguish Trichophoreae from all other CDS tribes. Tribes Sumatroscirpeae and Cariceae are united by the presence of fertile and sheathing spikelet prophylls, called perigynia. Pedicellate spikelets are identified as a synapomorphy for Sumatroscirpeae, although they are sessile in one unsampled species of *Sumatroscirpus* (the type *S. junghuhnii* (Miq.) Oteng-Yeb.). Cariceae are supported by three synapomorphies: truncated lateral spikelets, unisexual flowers, and absent perianth.

7.4 Discussion

7.4.1 General Discussion

This study presents the most comprehensive phylogenetic analysis of the CDS clade, including all genera and more than half of the species of CDS outside *Carex*, and is the first to combine molecular sequences with morphological data compiled at the species level. The results obtained here are consistent with previous studies (e.g. Gilmour & al., 2013; Léveillé-Bourret & al., 2014, 2018a 2018b) that have suggested an isolated position for the genus *Khaosokia*, and the paraphyly of tribe Scirpeae as circumscribed by (Goetghebeur, 1998) and (Muasya & al., 2009). Although backbone relationships are poorly supported in the present analyses, the same topology was obtained with very

strong support in a recent phylogenomic study incorporating data from hundreds of nuclear regions (Léveillé-Bourret & al., 2018b). Our analyses identify morphological synapomorphies for all major CDS clades, supporting the recognition of seven tribes, including a narrowly circumscribed Scirpeae s.str. and three new tribes: Khaosokieae, Calliscirpeae and Trichophoreae. These taxonomic changes place for the first time all CDS genera into strongly supported monophyletic tribes.

7.4.2 Dulichieae

Tribe Dulichieae is monophyletic in our analyses, consistent with previous molecular phylogenetic studies (Muasya & al., 2009; Léveillé-Bourret & al., 2014, 2018a), and supported by several morphological synapomorphies including distichous spikelets and fertile spikelet prophylls. However, fertile prophylls are not unique to Dulichieae, as they are also present in Cariceae and Sumatroscirpeae. Although the position of Dulichieae within the CDS clade has varied in previous studies (Simpson & al., 2007; Muasya & al., 2009; Jung & Choi, 2012), most recent analyses have shown it to be sister to all other CDS lineages, as in the present study (Dhooge, 2005; Gilmour & al., 2013; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014, 2015, 2018a, 2018b). This means that fertile prophylls have evolved twice in CDS: once in Dulichieae, where they look like glumes except for the presence of two midribs, and a second time in the most recent common ancestor of Cariceae and Sumatroscirpeae, where they are usually tubular or utriculiform.

All three small genera of Dulichieae are reciprocally monophyletic, with *Blysmopsis* sister to *Blysmus*, albeit with low support. The former contains a single circumboreal species, *Blysmopsis rufa*, that is very similar in external appearance to *Blysmus*, in which it is sometimes included, but differs in its possession of channeled leaves (flat in *Blysmus*), blunt caducous bristles with obscure antrorse barbs (long, persistent with prominent sharp retrorse barbs in *Blysmus*), smooth anther crests (barbed in *Blysmus*), and anatomical differences in the leaf and fruit (Oteng-Yeboah, 1974a, 1977). The molecular divergence between *Blysmopsis rufa* and *Blysmus* is similar to the divergence between *Dulichium* and *Blysmus*, and the sister relationship between *Blysmus* and *Blysmopsis* is only moderately supported (Fig. 7.4). This means that placing *Blysmopsis* in the synonymy of *Blysmus* would not promote taxonomic stability, as future analyses could very well demonstrate a sister relationship between *Blysmopsis* and *Dulichium*. The best option is to recognize the monospecific genus *Blysmopsis*.

7.4.3 Khaosokieae

The Southeastern Asian genus *Khaosokia* possesses a combination of unusual characters that show affinity with several different lineages of the CDS clade. It has seven perianth bristles and narrow elongate spikelets like *Dulichium* (Dulichieae), unisexual flowers and compound inflorescences reminiscent of highly-compound *Carex* like *Carex indica* L. (Cariceae; Simpson & al., 2005), and sterile prophylls, empty basal glumes and antrorse bristle barbs showing affinity with certain species of *Trichophorum* or *Cypringlea* (Trichophoreae). These characters, combined with the variable and often unsupported relationships inferred in phylogenetic analyses, made it impossible to assign *Khaosokia* to any existing tribe in previous studies (Simpson & al., 2005, 2007; Muasya & al., 2009; Jung & Choi, 2012; Gilmour & al., 2013; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014). The position of the genus as an isolated lineage sister to all CDS tribes except the early-diverging Dulichieae was strongly supported in (Léveillé-Bourret & al., 2018b), supporting our decision to recognize tribe Khaosokieae for this singular genus.

7.4.4 Scirpeae

Tribe Scirpeae has traditionally been circumscribed using morphological plesiomorphies, including the possession of spirally-inserted bisexual flowers, the presence of perianth bristles, and embryos with a perpendicular germ pore (Goetghebeur, 1998). It therefore came as no surprise when molecular phylogenetic analyses suggested Scirpeae to be paraphyletic (Muasya & al., 2009; Gilmour & al., 2013; Léveillé-Bourret & al., 2014, 2015, 2018a, 2018b). To restore the monophyly of the tribe, we here restrict Scirpeae to the genera possessing a lateral or sublateral root cap (Fimbristylis- or Schoenus-type embryo sensu (Goetghebeur, 1986), the only unambiguous morphological synapomorphy identified in our analyses. This includes the four genera that have been included in the mostly South American Zameioscirpus Clade (sensu (Léveillé-Bourret & al., 2014), *Amphiscirpus*, *Phylloscirpus*, *Rhodoscirpus* and *Zameioscirpus*, in addition to the circumboreal *Eriophorum* and *Scirpus*. The genus *Calliscirpus*, and the genera previously assigned to the Trichophorum Clade (sensu (Léveillé-Bourret & al., 2014), possess Carex-type embryos with basal root caps, and are here assigned respectively to Calliscirpeae and Trichophoreae, two new tribes that can be differentiated from Scirpeae s.str. using macroscopic characters.

The genera *Eriophorum* and *Scirpus* still contain several Asian, African and South American species that have affinities with genera of other unrelated Cyperaceae tribes. The diagnosis and key given here does not apply to these species, although their taxonomy cannot be revised pending more studies. In addition, the circumscription of *Scirpus* and *Eriophorum* is problematical because the latter is consistently found to be deeply nested in the former in molecular phylogenetic analyses (Jung & Choi, 2012; Gilmour & al., 2013; Léveillé-Bourret & al., 2014, 2015, 2018a, 2018b). Whether *Eriophorum* should be treated as an infrageneric taxon of *Scirpus*, as (Koyama, 1958) had already proposed, or whether *Scirpus* should be further divided in a series of six to eight new genera that would permit the preservation of *Eriophorum*, is currently under study.

7.4.5 Calliscirpeae

The genus *Calliscirpus* contains only two species endemic to California and Oregon (Gilmour & al., 2013). Its type species, *Calliscirpus criniger*, was previously thought to be transitional between *Eriophorum* and *Scirpus* due to a combination of characteristics including its small spikelets and 6–12 perianth bristles forming a white cottony mass at maturity (Beetle, 1942; Gilmour & al., 2013). However, *Calliscirpus* differs from both previously mentioned genera by its ciliate ligules, Carex-type embryo, and white anthers. This last characteristic could suggest insect pollination in *Calliscirpus* given that white anthers appear to be correlated with insect visitation or pollination in *Carex*, such as in *C. baldensis*, *C. continua*, and *C. scaposa*, and also in other genera such as *Cyperus*, *Eleocharis* and *Rhynchospora* (Hesse, 1980; Thomas, 1984; Yano & al., 2015; Costa & al., 2017). Nevertheless, white anthers are restricted to *Calliscirpus* and a few *Carex* species in CDS, and are thus a good taxonomic character to segregate Calliscirpeae from other former members of Scirpeae s.lat. Although Schuyler (1971b) proposed that *Calliscirpus* could be separated from typical *Scirpus* species by its coarser styles with denser and shorter papillae, similar styles are seen in some species of *Scirpus* (e.g. *Scirpus maximowiczii*, *S. microcarpus*; pers. obs.). Style micromorphology, and especially shape and density of stigmatic papillae, may be of some taxonomic significance in CDS, as is the case with the annulate stigmatic papillae of Abildgaardieae and Eleocharideae (Raynal, 1973; Bruhl, 1995). However, detailed

studies are required to determine the extent and significance of the variability of style morphology. It is probable that the morphology of the stigmatic surface is driven to some extent by pollination vector(s), as with anther colour.

7.4.6 Trichophoreae

The genera *Cypringlea*, *Oreobolopsis* and *Trichophorum* form a strongly-supported group that is here recognized as tribe Trichophoreae. Whereas *Oreobolopsis* and *Trichophorum* both possess unispicate or paucispicate inflorescences and reduced leaves, the genus *Cypringlea* differs markedly in general habit, with numerous spikelets in compound anthelae and well-developed leaf blades (Strong, 2003; Reznicek & González Elizondo, 2008). However, these three genera share one important derived characteristic: sterile proximal glumes. In *Trichophorum*, the first 1–2 glumes are sterile, and often possess an enlarged mucro or awn. In *Oreobolopsis*, the first few glumes are sterile, but often similar to the following fertile glumes (except for *Oreobolopsis clementis*). *Cypringlea* possesses 1-several sterile glumes, but they are generally much smaller than the following glumes. A single species of *Trichophorum* has a spikelet with all glumes fertile: *Trichophorum cespitosum*. However, this species possesses the typical habit of *Trichophorum*, with leaf blades reduced to short mucros, a unispicate inflorescence, and an awned first glume. Members of Trichophoreae also differ from Scirpeae s.str. in their Carex-type embryo, and bristle barbs that are blunt and antrorse to variously-oriented, when present.

All previous phylogenetic studies agree with our results in suggesting that *Cypringlea* and *Oreobolopsis* are nested within *Trichophorum*, but branch support has been consistently low, and topologies unstable (Gilmour & al., 2013; Léveillé-Bourret & al., 2014, 2015). The morphological and anatomical similarity between *Oreobolopsis* and *Trichophorum* has been highlighted, and there has already been suggestions to merge the former with the latter (Dhooge, 2005; Léveillé-Bourret & al., 2014). However, a better argument needs to be made for the inclusion of the highly-compound *Cypringlea* within the mostly unispicate *Trichophorum*, especially given the low nodal support obtained for relationships between Trichophoreae genera. Generic circumscriptions of Trichophoreae need to be revised, but such a revision depends on a well-supported and robust phylogenetic hypothesis, which does not appear attainable using traditional Sanger markers. In addition, several

Eastern Asian species currently placed in other genera, but that also possess sterile proximal glumes, are likely to be additional members of this tribe; e.g. *Eriophorum scabriculme*, *Scirpus filipes*, and *S. huae* (Beetle, 1946; Raymond, 1957; Koyama, 1961; Liang & Tucker, 2010b). Work is currently under way to clarify the taxonomy of these unusual species, and to resolve evolutionary relationships and generic limits within Trichophoreae using next-generation sequencing techniques.

In addition to *Cypringlea*, *Oreobolopsis* and *Trichophorum*, the genus *Neoscirpus* would also be included in this tribe. *Neoscirpus* was proposed by Lee & Oh (2006) to account for a species that possesses functionally unisexual flowers (Lee & Oh, 2007), but that would otherwise fit perfectly in *Trichophorum*. Other CDS species are known to produce functionally unisexual flowers in CDS, for instance in *Trichophorum distigmaticum* (Liang & Tucker, 2010c), dioiecious populations reported for *Trichophorum cespitosum* (Hegi, 1909; Swan, 1999), and gynodioecious populations for *Eriophorum vaginatum* (Stevens & Blackstock, 1993). Jung & Choi (2010) correctly interpreted *Neoscirpus* to be a synonym of *Trichophorum*, but wrongly thought that the former was invalidly published. In consequence, they published a new name based on a new type for *Neoscirpus dioicus* Y.N.Lee & Y.C.Oh, which is unwarranted. We here prefer to interpret (Jung & Choi, 2010) name as a recombination, *Trichophorum dioicum* (Y.N.Lee & Y.C.Oh) J.Jung & H.K.Choi. The complex nomenclature of this species, and its relationship to *Trichophorum schansiense* Hand.-Mazz., with which *Trichophorum dioicum* may be allied, will be treated in a future study.

7.5 Taxonomic treatment

7.5.1 Identification Key to Cyperaceae Tribes

1a. Inflorescence composed of flower-like units[1] with a basal pair of lateral keeled scales, sometimes fused, or with 10–100+ stamens each subtended by a linear scale in *Chrysitrix*; embryo Juncus- or Carex-type (subfam. Mapanioideae).....2

2a. Pollen in monads.....**Hypolytreae**

2b. Pollen in pseudomonads.....**Chrysitricheae**

1. These units are probably homologous to the spikelets of the Cyperoideae family, but they are superficially similar to Cyperoideae flowers, and generally subtended by a large scale-like bract arranged on a spike that looks very much like a Cyperoideae spikelet.

1b. Inflorescence composed of true flowers without a basal pair of lateral keeled scales (except in *Hellmuthia*, Cypereae[2]), the prophyll of lateral spikelets single and adaxial (except in *Oreobolus oligocephalus*, Schoeneae[3]); embryo various (subfam. Cyperoideae).....3

3a. Pistil contained in an utriculiform, or rarely squamiform, prophyll, always lateral; flower unisexual; perianth absent; embryo Carex- or Schoenus-type.....**Cariceae**

3b. At least some pistils subtended by a scale-like glume (in Cryptangieae rarely contained in an utriculiform foliar organ but then terminal on an axis bearing a few male or neuter spikelets below); flower bisexual or unisexual; perianth present or absent; embryo various.....4

4a. Flower unisexual and pistils 1–2 per female-fertile spikelet.....5

5a. Pistillate flowers with perianth scales or bristles.....6

6a. Pistillate flower with 2–5 perianth bristles, at least 2 opposite the edges of the fruit..... .....**Koyamaeae**

6b. Pistillate flower with 3 perianth scales, opposite the faces of the fruit.....7

7a. Spikelet glumes distichously inserted; embryo with scutellum transversally widened, Trilepis-type.....**Trilepideae**

7b. Spikelet glumes spirally inserted; embryo with scutellum not transversally widened, Carex-, Schoenus- or Fimbristylis- type.....**Cryptangieae** (in part)

5b. Pistillate flower without perianth scales or bristles, sometimes with a 3-lobed cupule.....8

8a. Pistil apparently terminal on spike axis, surrounded by a few glumes or an utriculiform foliar organ; male flower with 1 stamen.....**Bisboeckelereae**

8b. Pistil lateral in a spikelet, subtended by a glume and with 1-several male or empty glumes above; male flower with 2–3(–6?) stamens.....9

9a. Ovary surrounded by hypogynous cupule, often 3-lobed; style 3-fid, fruit trigonous; male flower with 3 stamens.....**Sclerieae**

9b. Ovary without hypogynous cupule; style 2-fid, fruit laterally compressed; male flower with 2(–6?) stamens.....**Cryptangieae** (in part, *Exochogyne* C.B. Clarke)

4b. Flower perfect, rarely functionally unisexual but with rudiment of the other sex, or unisexual but pistils >2 per female-fertile spikelet.....10

10a. Most prophylls containing a flower.....11

11a. Inflorescence spicate or multispicate; spikelet prophyll squamiform, scarcely differentiated from following glumes; spikelets distichous on rachis; style base linear, forming a long narrow beak on fruit.....**Dulichieae**

11b. Inflorescence anthelate; spikelet prophylls tubular; spikelets spirally-inserted on rachis; style base enlarged, persistent as a small tubercle on fruit.....**Sumatroscirpeae**

2. *Hellmuthia* possesses some flowers with 2 lateral keeled perianth parts that are partly fused at the base, but also possesses three stamens, one of which is not subtended by a scale. This combination of characteristic distinguishes it from all Mapanioideae genera.

3. *Oreobolus oligocephalus* possesses a lateral spikelet with a pair of lateral keeled prophylls. These prophylls subtend a spikelet with several distichous glumes, usually two of which subtend a complete flower with six tepaloid perianth parts, three stamens, and one pistil. This combination of characteristic distinguishes it from all Mapanioideae genera.

10b. All prophylls sterile.....12

12a. Pistils 1–2 per spikelet, rarely more but then glume wings enveloping the flower of the node below; embryo with (sub-)basal root cap and perpendicular germ pore.....13

13a. Glumes of spikelet all deciduous together as a unit, but leaving the rachilla intact; perianth absent.....**Abildgaardieae** (in part, former "Arthrostylideae")

13b. Glumes persistent or deciduous individually; perianth generally present.....14

14a. Style 2-fid, fruit dorsiventrally flattened, straight; style base enlarged, persistent as a tubercle on fruit.....**Rhynchosporeae**

14b. Style 3(–9)-fid, fruit trigonous to terete, or rarely style 2-fid but then dorsiventrally flattened fruit conspicuously curved and style base not persistent as a tubercle on fruit 15

15a. Anthers greenish-yellow; embryo with invagination under root cap, Carpha-type .....**Carpheae**

15b. Anthers not greenish-yellow; embryo without invagination under root cap16

16a. Fruit drupe-like, with thick corky beak undifferentiated from fruit body, seated on a broad disc leaving a scar on the fruit; bristles absent; stamens 2(–3); leaf with inverted vascular bundles (with adaxial phloem).....**Cladieae**

16b. Fruit never simultaneously drupe-like and seated on a broad disc leaving scar on fruit; bristles present or absent; stamens 1–6; leaves without inverted vascular bundles.....**Schoeneae**

12b. Pistils >2 per spikelet, rarely less but then glume wings not enveloping the flower of the node below; embryo various.....17

17a. Plant dioecious; flower with 7 antrorsely scabrous perianth bristles; spikelet with 7–9 sterile proximal glumes and >7 upper fertile glumes.....**Khaosokieae** trib. nov.

17b. Flower bisexual, or if unisexual then never with the above combination of characters 18

18a. Style base enlarged, differentiated.....19

19a. Perianth absent; inflorescence generally corymbose or anthelate; leaf blade generally present; embryo not mushroom-shaped, Abildgaardia-, Bulbostylis- or Fimbristylis-type.....**Abildgaardieae** (in part)

19b. Perianth generally present; inflorescence unispicate; leaf bladeless sheaths, or blade sometimes represented by a tiny mucro; embryo mushroom-shaped, Eleocharis- or Webesteria-type.....**Eleocharideae**

18b. Style base not or only slightly enlarged, not differentiated.....20

20a. Embryo with basal or lateral root cap, and perpendicular germ pore.....21

21a. Infrutescence a white cottony mass because of the exserted flat and silky perianth bristles; perianth bristles 6–10, antrorsely scabrous their whole length; ligule and glume ciliate; all glumes fertile; anthers white.....**Calliscirpeae** trib. nov.

21b. Not this combination of characters.....22

22a. Spikelet with 1–5 sterile proximal glumes, rarely all fertile but then basal glume with longer mucro; spikelet 1.5–4 mm wide; perianth parts 0–6; perianth barbs antrorse or divaricate when present; cauline leaves absent; embryo with basal root cap, Carex-type.....**Trichophoreae** trib. nov.

22b. Spikelet with all glumes fertile, or rarely with sterile proximal glumes but then basal glume not with longer mucro, spikelet 6–15+ mm wide, and perianth bristles >10 (*Eriophorum* p.p.); perianth bristle barbs generally retrorse when present; cauline leaves present or absent; embryo with lateral root cap, Schoenus- or Fimbristylis-type.....**Scirpeae** s.str.

20b. Embryo with a lateral root cap and parallel germ pore.....23

23a. Perianth generally present; embryo mushroom-shaped, Bolboschoenus- or Schoenoplectus-type.....**Fuireneae**

23b. Perianth generally absent; embryo not mushroom-shaped, Cyperus-type .....**Cypereae**

7.5.2 Tribal Diagnoses for the CDS Clade

Dulichieae Reichenb. ex W.Schultze-Motel, in Willdenowia 2: 173. 1959. – Type: *Dulichium* Pers.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: flower bisexual, ligule glabrous, spikelet prophyll fertile, squamiform, spikelets distichous on rachis, glume disposition usually distichous at least on terminal spikelet of main stem, all glumes of spikelet fertile, glume wings sometimes partially enveloping the flower of the node below, flowers 3–7 per spikelet, perianth setiform, style base continuous in texture with fruit, leaving a long narrow beak of variable length on fruit, embryo Carex-type.

Accepted genera. — *Blysmopsis* Oteng-Yeb., *Blysmus* Panz. ex Schult., *Dulichium* Pers.

Key to Dulichieae genera:

1a. Spikelets in pedunculate spikes scattered throughout the upper part of the culm, in the axil of normal leaves; perianth of 6–9 (usually 7) bristles.....***Dulichium***

1b. Spikelets in a single terminal spike, rarely with an additional lateral spike; perianth of 0–6 bristles.. 2

2a. Culm 3-angled; leaf flat; fruit ~2 mm long; perianth bristles as long or longer than fruit, with sharp retrorse barbs, persistent; staminal crest barbed.....***Blysmus***

2b. Culm terete; leaf terete to canaliculate; fruit 3.5--4 mm long; perianth bristles shorter than fruit, with obscure antrorse barbs, deciduous; staminal crest smooth.....***Blysmopsis***

Khaosokieae Lév.-Bourret & J. R. Starr, **trib. nov.** – Type: *Khaosokia* D. A. Simpson, Chayam. & J. Parn.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: plant dioecious, ligule glabrous, prophyll sterile, spikelets spirally inserted on rachis, glume disposition spiral, proximal glumes of spikelet sterile, glume wings not enveloping the flower of the node below, flowers ≥10 per spikelet, perianth of 7 antrorsely scabrous bristles, anthers yellowish-white, style base not enlarged, not differentiated. Embryo not available.

Accepted genus. — Khaosokia D. A. Simpson, Chayam. & J. Parn.

Calliscirpeae Lév.-Bourret & J. R. Starr, **trib. nov.** – Type: *Calliscirpus* C. N. Gilmour, J. R. Starr & Naczi.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: flower bisexual, ligule ciliate, prophyll sterile, spikelets spirally inserted on rachis, glume disposition spiral, all glumes of spikelet fertile, glume wings not enveloping the flower of the node below, flowers ≥10 per spikelet, perianth of 6–7(–12) long silky antrorsely scabrous bristles forming a cottony mass at maturity, anthers white, style base not enlarged, not differentiated, embryo Carex-type.

Accepted genus. — Calliscirpus C. N. Gilmour, J. R. Starr & Naczi.

Scirpeae Kunth ex Dumort., emend, in Fl. Belg. 143. 1827. – Type: *Scirpus* L. nom. cons.

Diagnosis. — With the new circumscription proposed here, the tribe differs from all other Cyperaceae tribes by this unique combination of characters: flower bisexual or rarely functionally unisexual with remnant of opposite sex, ligule glabrous or ciliate, prophyll sterile, spikelets spirally inserted on rachis, glume disposition spiral, all glumes of spikelet fertile, or rarely 1–12 proximal glumes sterile, glume wings not enveloping the flower of the node below, flowers (3–)10+ per spikelet, perianth setiform or absent, anthers yellow, style base not enlarged, not differentiated, embryo Schoenus-type or Fimbristylis-type.

Accepted genera. — *Amphiscirpus* Oteng.-Yeb., *Phylloscirpus* C. B. Clarke, *Rhodoscirpus* Lév.-Bourret, Donadío & J. R. Starr, *Scirpus* L., *Zameioscirpus* Dhooge & Goetgh.

1a. Cauline leaves present, node of the distalmost leaf visible above the sheath of the leaf below.....**2**

2a. Inflorescence a white to red cottony mass at maturity because of the exserted flat and silky perianth bristles > 10 in number per flower; spikelet 8–50 mm long in fruit.....***Eriophorum***

2b. Inflorescence not appearing as a cottony mass; perianth bristles 0–6, barbed or smooth; spikelets small, 2–15 mm long in fruit.....**3**

3a. 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 obovoid to suborbicular body (incl. stipe) 1.0–1.3 times as long as wide..... .....***Rhodoscirpus***

3b. 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***

1b. Leaves all basal, node of the distalmost leaf hidden in the sheath of the leaf below; inflorescence various, but rarely anthelate.....**4**

4a. 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***

4b. 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.....**5**

5a. Inflorescence a dense head of many spikelets, rarely unispicate; perianth of retrorsely barbed bristles.....**6**

6a. Leaves ligulate; inflorescence pseudo-lateral; glumes ciliate.....***Amphiscirpus***

6b. Leaves eligulate; inflorescence terminal; glumes entire .....***Phylloscirpus*** (in part)

5b. Inflorescence unispicate; perianth absent.....**7**

7a. Leaves ligulate.....***Zameioscirpus***

7b. Leaves eligulate.....***Phylloscirpus*** (in part)

Trichophoreae Lév.-Bourret & J. R. Starr, **trib. nov. –** Type: *Trichophorum* (L.) Pers.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: flower bisexual, or rarely functionally unisexual with remnant of opposite sex, ligule glabrous, prophyll sterile, spikelets spirally inserted on rachis, glume disposition spiral, basal 1–9 glumes of spikelet sterile, glume wings not enveloping the flower of the node below, flowers 1–10+ per spikelet, perianth setiform, squamiform, or absent, anthers yellow, style base not enlarged, not differentiated, embryo Carex-type.

Accepted genera. — Cypringlea M. T. Strong, *Oreobolopsis* T. Koyama & Guagl., *Trichophorum* (L.) Pers.

Key to Trichophoreae genera:

1a. Inflorescence open, corymbiform, of dozens of spikelets; leaf blades long, flat; proximal glumes not mucronate, shorter than other glumes; perianth bristles shorter than fruit, barbs obscure.....***Cypringlea***

1b. Inflorescence unispicate, rarely a spike or corymb of 2–6 spikelets; leaf blades usually reduced to a short mucro, rarely long and flat; proximalmost glume usually mucronate and longer than other glumes (when including mucro); perianth various.....2

2a. Perianth of 6 bristles, or completely absent; sterile glumes (0–)1–2.....***Trichophorum***

2b. Perianth of 6 flat tepals; sterile glumes 2–5.....***Oreobolopsis***

Sumatroscirpeae Lév.-Bourret & J.R.Starr, in Mol. Phylogenet. Evol. 119: 100. 2018. – Type: *Sumatroscirpus* Oteng-Yeb.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: flower bisexual, ligule ciliate or glabrous, spikelet prophyll (perigynium) fertile, tubular, spikelets spirally inserted on rachis, glume disposition spiral, sometimes pseudodistichous, all glumes of spikelet fertile, glume wings sometimes partially enveloping the flower of the node below, flowers 7–10+ per spikelet, perianth setiform, anthers yellow, style base enlarged, differentiated and persistent on fruit, embryo with basal root cap and lateral germ pore (embryo type undetermined).

Accepted genus. — *Sumatroscirpus* Oteng-Yeb.

Cariceae Kunth ex Dumort., in Fl. Belg. 144. 1827. – Type: *Carex* L.

Diagnosis. — Differs from all other Cyperaceae tribes by this unique combination of characters: flower unisexual, male flowers arranged in spikelet and female flower at the prophyll node, ligule glabrous, spikelet prophyll (perigynium) fertile, usually utriculiform, rarely tubular or squamiform, lateral spikelets usually truncated or highly reduced, glume disposition spiral, all glumes of spikelet fertile, glume wings not enveloping the flower of the node below, male flowers usually 10+ per spikelet, rarely 1–3 in lateral spikelets, perianth absent, anthers yellow, rarely white, style base enlarged and differentiated or not, and persistent or not on fruit, embryo Carex-type, or rarely almost Schoenus-type.

Accepted genus. — *Carex* L.

7.6 Tables and Figures

Table 7.1. Morphological characters scored for phylogenetic analysis and delimitation of CDS tribes.

Character	Character states
0. Rhizome type[1]	(0) short creeping, plant cespitose; (1) long creeping, plant colonial; (2) ascending, plant cespitose or mat-forming
1. Stem cross-section shape[2]	(0) circular to obtusely trigonous; (1) triquetrous to crescent-shaped
2. Stem widest width	(0) 0–2 mm; (1) >2 mm
3. Distal leaf sheath shape	(0) tubular; (1) wider at apex
4. Basal leaf sheaths coloration	(0) green; (1) red tinged
5. Basal bract sheaths coloration	(0) green; (1) dark brown to black
6. Ligule presence	(0) absent; (1) present
7. Ligule ciliae presence	(0) absent; (1) present
8. Leaf blade length relative to sheath	(0) blade often much longer than sheath; (1) blade always shorter or equal to sheath
9. Leaf blade cross-section shape	(0) flat; (1) canaliculate, crescent-shaped or rounded
10. Cauline leaves presence	(0) absent; (1) present
11. Inflorescence complexity	(0) unispicate; (1) a single terminal fascicle; (2) multispicate; (3) highly compound
12. Highly-compound inflorescence shape[3]	(0) corymbiform to antheliform; (1) racemiform to paniculiform; (2) capitate
13. Basal bract morphology	(0) leaf-like; (1) scale-like
14. Basal bract sheath length	(0) <4 mm; (1) ≥4 mm
15. Main inflorescence branches	(0) pedunculate; (1) sessile
16. Inflorescence branches firmness	(0) stiff, ascending to spreading; (1) lax, pendant
17. Distal branches scabrosity	(0) smooth or scabrous only at apex; (1) scabrous ≥½ length
18. Number of spikelets per fascicle[4]	(0) mostly 2–9; (1) mostly >10
19. Spikelet width	(0) <5 mm wide; (1) >5 mm wide
20. Lateral spikelets[5]	(0) all sessile; (1) mostly pedicelled
21. Spikelet prophyll fusion	(0) margins fused, prophyll tubular to utriculiform; (1) margins unfused, prophyll squamiform
22. Spikelet prophyll fertility	(0) sterile; (1) fertile
23. Spikelet insertion	(0) spiral; (1) distichous
24. Lateral spikelet truncation	(0) not truncated; (1) truncated or highly reduced
25. Pseudospike sexuality[6]	(0) all bisexual; (1) mostly unisexual
26. Bisexual pseudospikes sex position[6]	(0) androgynous; (1) gynecandrous or mesogynous

Character	Character states
27. Perigynium abaxial suture appearance[6]	(0) not visible; (1) a short line near beak; (2) short apical hyaline zone; (3) broad hyaline band from apex to base
28. Perigynium beak length[6]	(0) ≤0.6 mm; (1) >0.6 mm
29. Perigynium beak teeth presence[6]	(0) absent or obscure; (1) conspicuous
30. Perigynium "rachilla" morphology[6]	(0) reduced to a minute knob; (1) present as terete sterile axis; (2) present as a flattened scabrous axis with 0–3 male flowers; (3) present as a smooth axis with hooked-shaped tip
31. Sterile proximal glumes presence	(0) absent; (1) present
32. Number of sterile proximal glumes	(0) 1–2; (1) 2–9; (2) >9
33. Basal glume size	(0) similar to other glumes; (1) conspicuously smaller
34. Basal glume awn differentiation	(0) undifferentiated; (1) much larger than on other glumes
35. Glume width	(0) >½ as wide as spikelet; (1) <½ as wide as spikelet
36. Glume margin ornamentation	(0) entire; (1) scabrous or ciliate
37. Glume scar shape[7]	(0) V-shaped; (1) M-shaped; (2) straight
38. Glume midrib differentiation	(0) differentiated; (1) undifferentiated, midnerve sometimes visible, but no prominent band
39. Glume ribs number	(0) single prominent midrib, sometimes a midnerve accompanied by two secondary nerves; (1) 2–9 equally prominent ribs
40. Glume black pigments presence[8]	(0) without black pigmentation; (1) black pigmentation present
41. Flower sexuality	(0) bisexual, sometimes functionally unisexual, but with remnant of nonfunctional sex; (1) unisexual
42. Perianth presence	(0) absent; (1) present
43. Perianth parts shape	(0) setiform; (1) tepaliform
44. Perianth parts number	(0) <6; (1) 6; (2) 7–9; (3) 10–20+
45. Perianth parts length	(0) <½ nutlet length; (1) ≥ nutlet length, but included in glume; (2) projecting beyond glume
46. Perianth bristles cross-section shape	(0) (sub-)terete; (1) conspicuously flattened
47. Perianth bristle barbs presence	(0) absent; (1) present
48. Perianth bristle barbs position	(0) present from apex to base of bristle; (1) restricted to a small zone near apex of bristle
49. Perianth bristle barbs orientation	(0) retrorse; (1) antrorse; (2) variously oriented
50. Perianth bristle barbs sharpness	(0) sharp; (1) blunt
51. Number of stamens per flower	(0) 3; (1) 2; (2) 1
52. Anther color	(0) yellow to reddish; (1) white
53. Anther apiculum shape	(0) as long as wide; (1) longer than wide
54. Anther apiculum ornamentation	(0) smooth; (1) barbed; (2) papillose
55. Style base shape	(0) linear; (1) enlarged

Character	Character states
56. Style branches number	(0) 3; (1) 2
57. Fruit color	(0) dark greyish to reddish brown; (1) pale yellow to yellowish white, often almost translucent
58. Fruit beak length[9]	(0) short apiculum longer than wide; (1) absent, or short and wider than long; (2) very long narrow beak of variable length, formed by style base continuous in texture with fruit body
59. Embryo sagittal outline shape	(0) obovate to oblong; (1) mushroom-shaped
60. Embryo plumule position	(0) lateral; (1) displaced to (sub-)basal position
61. Embryo root cap position relative to (sub-)basal plumule[10]	(0) lower; (1) same height; (2) higher
62. Embryo germ pore orientation relative to first leaf	(0) perpendicular; (1) parallel
63. Fruit surface anticlinal cell walls shape[11]	(0) straight; (1) sinuate

[1] Rhizome is coded "long" only when stems can be separated by more than a few centimeters on the rhizome, and the length between each stem is variable.

[2] The middle of the stem was examined to score its cross-sectional shape.

[3] Only coded when inflorescence is highly compound.

[4] When consisting of >10 spikelets, spikelet fascicles often appear spherical.

[5] For a spikelet to be considered pedicelled, its pedicel needs to be longer than the subtending bract.

[6] Character only applies to *Carex*.

[7] Glumes that leave an M-shaped or straight scar on the rachilla typically have wings that very slightly cover the flower below.

[8] Black pigmentation can be accompanied by red to reddish-brown pigments, giving an overall brownish or reddish look to glumes. However, pure black pigments are visible at 30× magnification.

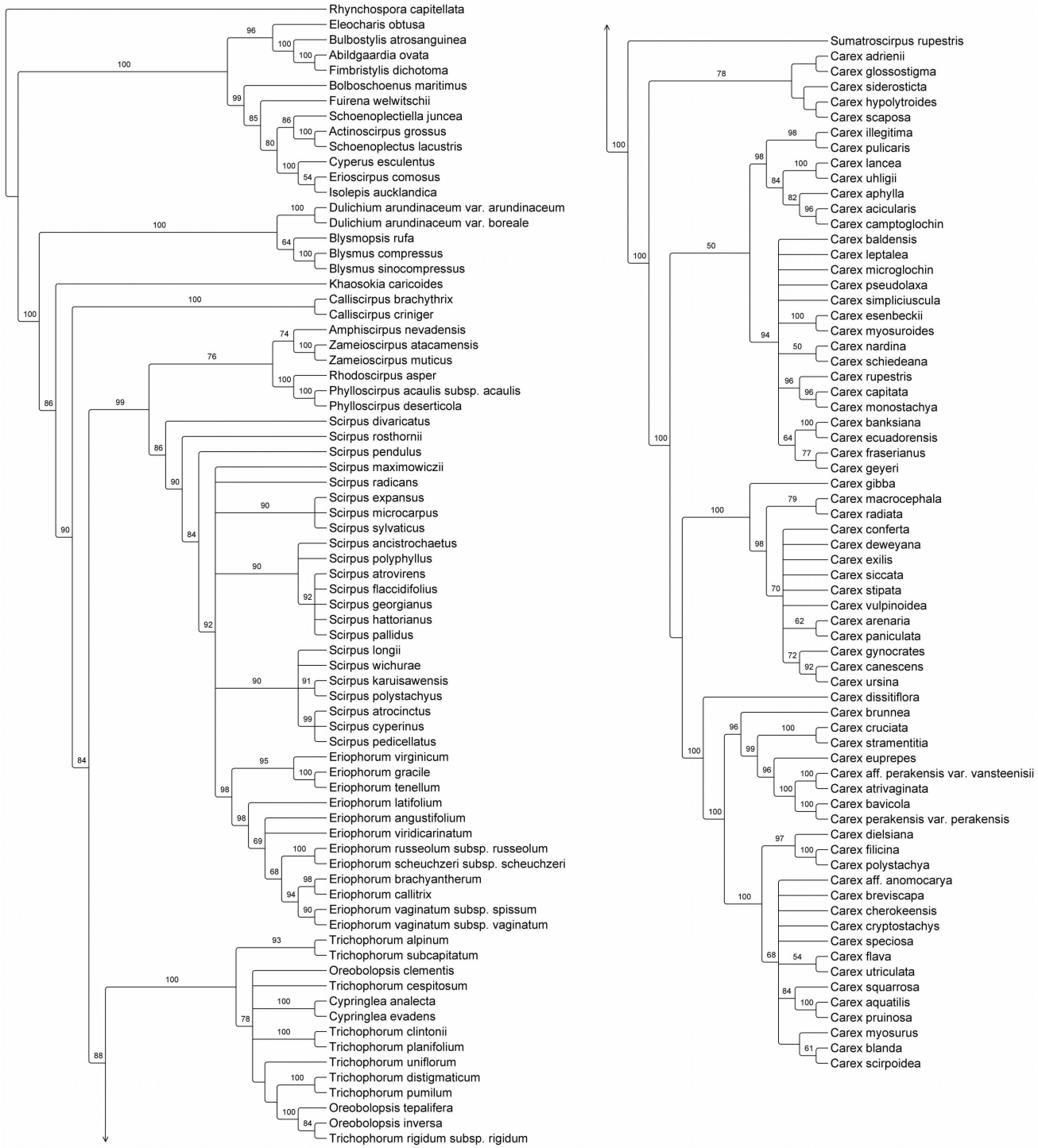
[9] Excluding persistent differentiated style base, if present.

[10] Only coded for embryos with a plumule displaced to a (sub-)basal position. The height of the root cap relative to the plumule is of systematic importance in these embryos.

[11] Only sinuosity of amplitude >2× wall width are considered.

Table 7.2. Statistics for individual and combined datasets used in phylogenetic analyses, including aligned length (number of characters), number and percentage of missing terminals, percentage of gaps, missing and ambiguous characters, number and percentage of variable characters, number and percentage of parsimony informative characters, as well as consistency and retention indices on the shortest tree for that dataset.

Dataset	Aligned length	Terminals missing	Gaps, missing & ambiguous	Variable	Parsimony informative	CI	RI
matK	1339	6 (4%)	7.8%	632 (47.2%)	369 (27.5%)	0.556	0.820
ndhF	1245	17 (11.3%)	12.4%	522 (41.9%)	321 (25.8%)	0.499	0.798
rps16	1224	34 (22.7%)	45.3%	463 (37.8%)	262 (21.4%)	0.602	0.834
ETS-1f	881	19 (12.7%)	42.8%	642 (72.9%)	500 (56.8%)	0.341	0.694
ITS	816	13 (8.7%)	30.9%	500 (61.3%)	379 (46.5%)	0.296	0.607
Combined sequences	**5505**	**none**	**26.2%**	**2759 (50.1%)**	**1831 (33.3%)**	**0.394**	**0.713**
matK indels	17	6 (4%)	20.7%	all	8 (47%)	0.895	0.938
ndhF indels	14	17 (11.3%)	13.7%	all	4 (28.6%)	1	1
rps16 indels	189	34 (30%)	42.3%	all	94 (49.7%)	0.656	0.866
ETS-1f indels	256	19 (12.7%)	33.8%	all	124 (48.4%)	0.656	0.850
ITS indels	261	13 (8.7%)	32.5%	all	127 (48.7%)	0.584	0.823
Combined indels	**737**	**none**	**34.8%**	**all**	**357 (48.4%)**	**0.596**	**0.819**
Morphology	**64**	**none**	**26.8%**	**all**	**64 (100%)**	**0.182**	**0.767**
Combined sequences + indels + morphology	**6306**	**none**	**27.2%**	**3560 (56.5%)**	**2252 (35.7%)**	**0.399**	**0.718**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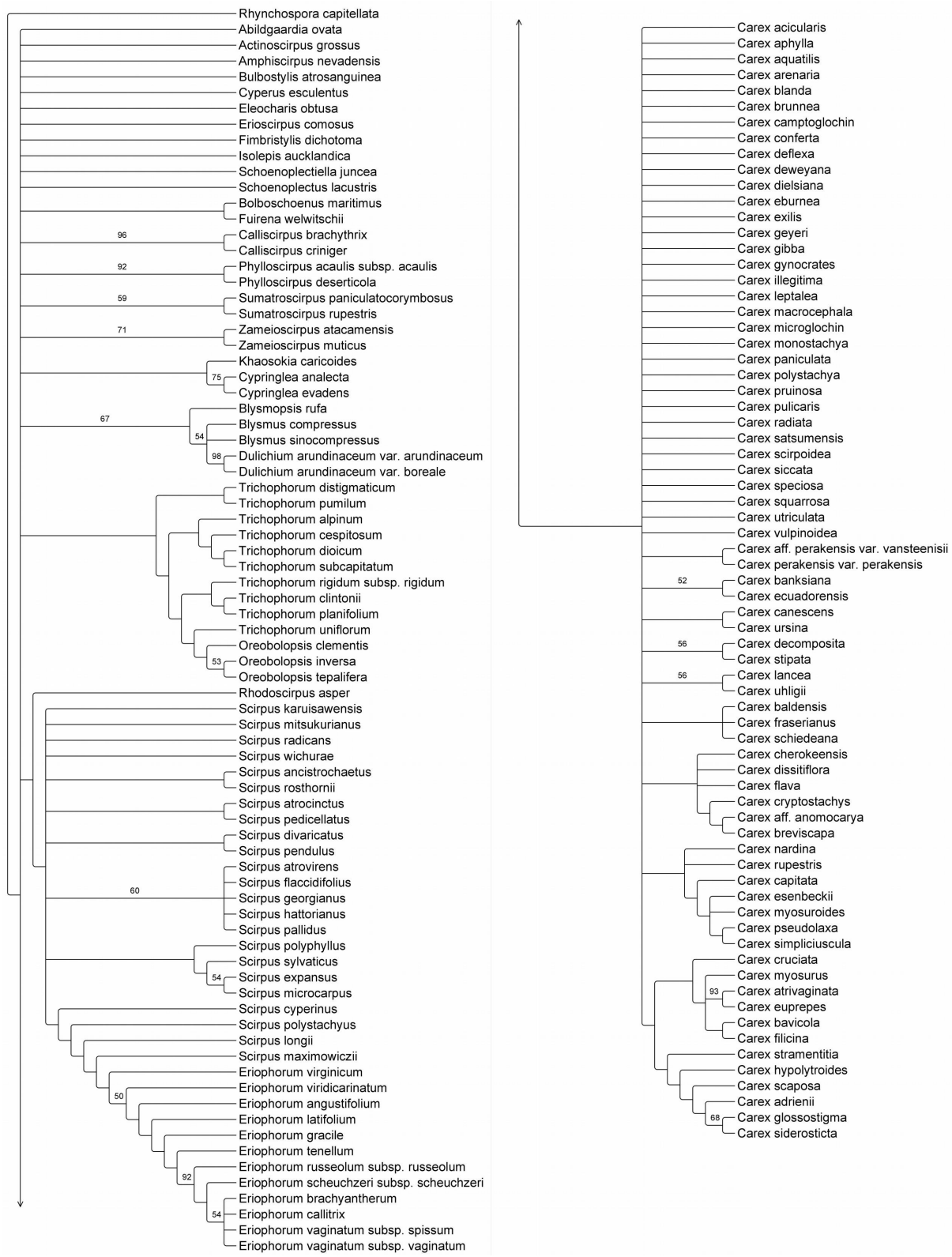
Eleocharis obtusa
Bulbostylis atrosanguinea
Abildgaardia ovata
Fimbristylis dichotoma
Bolboschoenus maritimus
Fuirena welwitschii
Schoenoplectiella juncea
Actinoscirpus grossus
Schoenoplectus lacustris
Cyperus esculentus
Erioscirpus comosus
Isolepis aucklandica
Dulichium arundinaceum var. arundinaceum
Dulichium arundinaceum var. boreale
Blysmopsis rufa
Blysmus compressus
Blysmus sinocompressus
Khaosokia caricoides
Calliscirpus brachythrix
Calliscirpus criniger
Amphiscirpus nevadensis
Zameioscirpus atacamensis
Zameioscirpus muticus
Rhodoscirpus asper
Phylloscirpus acaulis subsp. acaulis
Phylloscirpus deserticola
Scirpus divaricatus
Scirpus rosthornii
Scirpus pendulus
Scirpus maximowiczii
Scirpus radicans
Scirpus expansus
Scirpus microcarpus
Scirpus sylvaticus
Scirpus ancistrochaetus
Scirpus polyphyllus
Scirpus atrovirens
Scirpus flaccidifolius
Scirpus georgianus
Scirpus hattorianus
Scirpus pallidus
Scirpus longii
Scirpus wichurae
Scirpus karuisawensis
Scirpus polystachyus
Scirpus atrocinctus
Scirpus cyperinus
Scirpus pedicellatus
Eriophorum virginicum
Eriophorum gracile
Eriophorum tenellum
Eriophorum latifolium
Eriophorum angustifolium
Eriophorum viridicarinatum
Eriophorum russeolum subsp. russeolum
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum brachyantherum
Eriophorum callitrix
Eriophorum vaginatum subsp. spissum
Eriophorum vaginatum subsp. vaginatum
Trichophorum alpinum
Trichophorum subcapitatum
Oreobolopsis clementis
Trichophorum cespitosum
Cypringlea analecta
Cypringlea evadens
Trichophorum clintonii
Trichophorum planifolium
Trichophorum uniflorum
Trichophorum distigmaticum
Trichophorum pumilum
Oreobolopsis tepalifera
Oreobolopsis inversa
Trichophorum rigidum subsp. rigidum
Sumatroscirpus rupestris
Carex adrienii
Carex glossostigma
Carex siderosticta
Carex hypolytroides
Carex scaposa
Carex illegitima
Carex pulicaris
Carex lancea
Carex uhligii
Carex aphylla
Carex acicularis
Carex camptoglochin
Carex baldensis
Carex leptalea
Carex microglochin
Carex pseudolaxa
Carex simpliciuscula
Carex esenbeckii
Carex myosuroides
Carex nardina
Carex schiedeana
Carex rupestris
Carex capitata
Carex monostachya
Carex banksiana
Carex ecuadorensis
Carex fraserianus
Carex geyeri
Carex gibba
Carex macrocephala
Carex radiata
Carex conferta
Carex deweyana
Carex exilis
Carex siccata
Carex stipata
Carex vulpinoidea
Carex arenaria
Carex paniculata
Carex gynocrates
Carex canescens
Carex ursina
Carex dissitiflora
Carex brunnea
Carex cruciata
Carex strammentitia
Carex euprepes
Carex aff. perakensis var. vansteenisii
Carex atrivaginata
Carex bavicola
Carex perakensis var. perakensis
Carex dielsiana
Carex filicina
Carex polystachya
Carex aff. anomocarya
Carex breviscapa
Carex cherokeensis
Carex cryptostachys
Carex speciosa
Carex flava
Carex utriculata
Carex squarrosa
Carex aquatilis
Carex pruinosa
Carex myosurus
Carex blanda
Carex scirpoidea

Figure 7.1 [on previous page]. Strict consensus of all maximum parsimony trees based on concatenated analysis of plastid sequences and indels (*matK* + *ndhF* + *rps16* + plastid indels). Parsimony bootstrap percentage above branches.


Rhynchospora capitellata
Eleocharis obtusa
Abildgaardia ovata
Fimbristylis dichotoma
Cyperus esculentus
Erioscirpus comosus
Isolepis aucklandica
Fuirena welwitschii
Schoenoplectiella juncea
Bolboschoenus maritimus
Actinoscirpus grossus
Schoenoplectus lacustris
Dulichium arundinaceum var. arundinaceum
Dulichium arundinaceum var. boreale
Blysmopsis rufa
Blysmus compressus
Blysmus sinocompressus
Khaosokia caricoides
Calliscirpus brachythrix
Calliscirpus criniger
Zameioscirpus atacamensis
Zameioscirpus muticus
Amphiscirpus nevadensis
Rhodoscirpus asper
Phylloscirpus acaulis subsp. acaulis
Phylloscirpus deserticola
Scirpus divaricatus
Scirpus pendulus
Scirpus rosthornii
Scirpus maximowiczii
Scirpus radicans
Scirpus expansus
Scirpus microcarpus
Scirpus sylvaticus
Scirpus georgianus
Scirpus hattorianus
Scirpus pallidus
Scirpus pedicellatus
Scirpus polystachyus
Scirpus wichurae
Scirpus karuisawensis
Scirpus mitsukurianus
Eriophorum virginicum
Eriophorum gracile
Eriophorum tenellum
Eriophorum viridicarinatum
Eriophorum angustifolium
Eriophorum latifolium
Eriophorum russeolum subsp. russeolum
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum callitrix
Eriophorum brachyantherum
Eriophorum vaginatum subsp. spissum
Eriophorum vaginatum subsp. vaginatum
Trichophorum dioicum
Trichophorum uniflorum
Trichophorum alpinum
Trichophorum subcapitatum
Trichophorum distigmaticum
Trichophorum pumilum
Oreobolopsis inversa
Oreobolopsis tepalifera
Trichophorum rigidum subsp. rigidum
Oreobolopsis clementis
Trichophorum cespitosum
Cypringlea analecta
Cypringlea evadens
Trichophorum clintonii
Trichophorum planifolium
Sumatroscirpus paniculatocorymbosus
Sumatroscirpus rupestris
Carex hypolytroides
Carex sierosticta
Carex adrienii
Carex glossostigma
Carex scaposa
Carex satsumensis
Carex gibba
Carex siccata
Carex macrocephala
Carex radiata
Carex conferta
Carex decomposita
Carex vulpinoidea
Carex deweyana
Carex exilis
Carex paniculata
Carex stipata
Carex arenaria
Carex gynocrates
Carex canescens
Carex ursina
Carex dissitiflora
Carex fraserianus
Carex geyeri
Carex baldensis
Carex microglochin
Carex schiedeana
Carex simpliciuscula
Carex esenbeckii
Carex myosuroides
Carex rupestris
Carex capitata
Carex monostachya
Carex pseudolaxa
Carex banksiana
Carex ecuadorensis
Carex leptalea
Carex nardina
Carex illegitima
Carex pulicaris
Carex lancea
Carex uhligii
Carex acicularis
Carex aphylla
Carex camptoglochin
Carex bavicola
Carex perakensis var. perakensis
Carex brunnea
Carex eburnea
Carex euprepes
Carex aff. perakensis var. vansteenisii
Carex atrivaginata
Carex cruciata
Carex strammentitia
Carex breviscapa
Carex cryptostachys
Carex dielsiana
Carex filicina
Carex polystachya
Carex deflexa
Carex myosurus
Carex cherokeensis
Carex aff. anomocarya
Carex speciosa
Carex blanda
Carex flava
Carex scirpoidea
Carex aquatilis
Carex squarrosa
Carex pruinosa
Carex utriculata

Figure 7.2 [on previous page]. Strict consensus of all maximum parsimony trees based on concatenated analysis of nuclear sequences and indels (ETS-1f + ITS + nuclear indels). Parsimony bootstrap percentage above branches.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Actinoscirpus grossus
Amphiscirpus nevadensis
Bulbostylis atrosanguinea
Cyperus esculentus
Eleocharis obtusa
Erioscirpus comosus
Fimbristylis dichotoma
Isolepis aucklandica
Schoenoplectiella juncea
Schoenoplectus lacustris
Bolboschoenus maritimus
Fuirena welwitschii
96
Calliscirpus brachythrix
Calliscirpus criniger
92
Phylloscirpus acaulis subsp. acaulis
Phylloscirpus deserticola
59
Sumatroscirpus paniculatocorymbosus
Sumatroscirpus rupestris
71
Zameioscirpus atacamensis
Zameioscirpus muticus
Khaosokia caricoides
75
Cypringlea analecta
Cypringlea evadens
Blysmopsis rufa
67
Blysmus compressus
54
Blysmus sinocompressus
98
Dulichium arundinaceum var. arundinaceum
Dulichium arundinaceum var. boreale
Trichophorum distigmaticum
Trichophorum pumilum
Trichophorum alpinum
Trichophorum cespitosum
Trichophorum dioicum
Trichophorum subcapitatum
Trichophorum rigidum subsp. rigidum
Trichophorum clintonii
Trichophorum planifolium
Trichophorum uniflorum
Oreobolopsis clementis
53
Oreobolopsis inversa
Oreobolopsis tepalifera
Rhodoscirpus asper
Scirpus karuisawensis
Scirpus mitsukurianus
Scirpus radicans
Scirpus wichurae
Scirpus ancistrochaetus
Scirpus rosthornii
Scirpus atrocinctus
Scirpus pedicellatus
Scirpus divaricatus
Scirpus pendulus
Scirpus atrovirens
Scirpus flaccidifolius
60
Scirpus georgianus
Scirpus hattorianus
Scirpus pallidus
Scirpus polyphyllus
Scirpus sylvaticus
54
Scirpus expansus
Scirpus microcarpus
Scirpus cyperinus
Scirpus polystachyus
Scirpus longii
Scirpus maximowiczii
Eriophorum virginicum
Eriophorum viridicarinatum
50
Eriophorum angustifolium
Eriophorum latifolium
Eriophorum gracile
Eriophorum tenellum
Eriophorum russeolum subsp. russeolum
92
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum brachyantherum
54
Eriophorum callitrix
Eriophorum vaginatum subsp. spissum
Eriophorum vaginatum subsp. vaginatum
Carex acicularis
Carex aphylla
Carex aquatilis
Carex arenaria
Carex blanda
Carex brunnea
Carex camptoglochin
Carex conferta
Carex deflexa
Carex deweyana
Carex dielsiana
Carex eburnea
Carex exilis
Carex geyeri
Carex gibba
Carex gynocrates
Carex illegitima
Carex leptalea
Carex macrocephala
Carex microglochin
Carex monostachya
Carex paniculata
Carex polystachya
Carex pruinosa
Carex pulicaris
Carex radiata
Carex satsumensis
Carex scirpoidea
Carex siccata
Carex speciosa
Carex squarrosa
Carex utriculata
Carex vulpinoidea
Carex aff. perakensis var. vansteenisii
Carex perakensis var. perakensis
52
Carex banksiana
Carex ecuadorensis
Carex canescens
Carex ursina
56
Carex decomposita
Carex stipata
56
Carex lancea
Carex uhligii
Carex baldensis
Carex fraserianus
Carex schiedeana
Carex cherokeensis
Carex dissitiflora
Carex flava
Carex cryptostachys
Carex aff. anomocarya
Carex breviscapa
Carex nardina
Carex rupestris
Carex capitata
Carex esenbeckii
Carex myosuroides
Carex pseudolaxa
Carex simpliciuscula
Carex cruciata
Carex myosurus
93
Carex atrivaginata
Carex euprepes
Carex bavicola
Carex filicina
Carex strammentitia
Carex hypolytroides
Carex scaposa
Carex adrienii
68
Carex glossostigma
Carex siderosticta

Figure 7.3 [on previous page]. Strict consensus of all maximum parsimony trees based on analysis of morphological data. Parsimony bootstrap percentage above branches.


Rhynchospora capitellata
Eleocharis obtusa
Bulbostylis atrosanguinea
Fimbristylis dichotoma
Abildgaardia ovata
Bolboschoenus maritimus
Fuirena welwitschii
Schoenoplectiella juncea
Actinoscirpus grossus
Schoenoplectus lacustris
Cyperus esculentus
Isolepis aucklandica
Erioscirpus comosus
Outgroups
Dulichium arundinaceum var. arundinaceum
Dulichium arundinaceum var. boreale
Blysmopsis rufa
Blysmus compressus
Blysmus sinocompressus
Dulichieae
Khaosokia caricoides
Khaosokieae
Calliscirpus brachythrix
Calliscirpus criniger
Calliscirpeae
Amphiscirpus nevadensis
Zameioscirpus atacamensis
Zameioscirpus muticus
Rhodoscirpus asper
Phylloscirpus acaulis subsp. acaulis
Phylloscirpus deserticola
Scirpus divaricatus
Scirpus rosthornii
Scirpus pendulus
Scirpus radicans
Scirpus sylvaticus
Scirpus expansus
Scirpus microcarpus
Scirpus polyphyllus
Scirpus ancistrochaetus
Scirpus hattorianus
Scirpus flaccidifolius
Scirpus atrovirens
Scirpus georgianus
Scirpus pallidus
Scirpus polystachyus
Scirpus wichurae
Scirpus karuisawensis
Scirpus mitsukurianus
Scirpus longii
Scirpus cyperinus
Scirpus pedicellatus
Scirpus atrocinctus
Scirpus maximowiczii
Eriophorum virginicum
Eriophorum gracile
Eriophorum tenellum
Eriophorum viridicarinatum
Eriophorum angustifolium
Eriophorum latifolium
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum russeolum subsp. russeolum
Eriophorum vaginatum subsp. vaginatum
Eriophorum vaginatum subsp. spissum
Eriophorum brachyantherum
Eriophorum callitrix
Scirpeae s.str.
Trichophorum dioicum
Trichophorum subcapitatum
Trichophorum alpinum
Trichophorum pumilum
Trichophorum distigmaticum
Trichophorum cespitosum
Trichophorum planifolium
Trichophorum clintonii
Trichophorum uniflorum
Cypringlea analecta
Cypringlea evadens
Oreobolopsis clementis
Trichophorum rigidum subsp. rigidum
Oreobolopsis inversa
Oreobolopsis tepalifera
Trichophoreae
Part 2

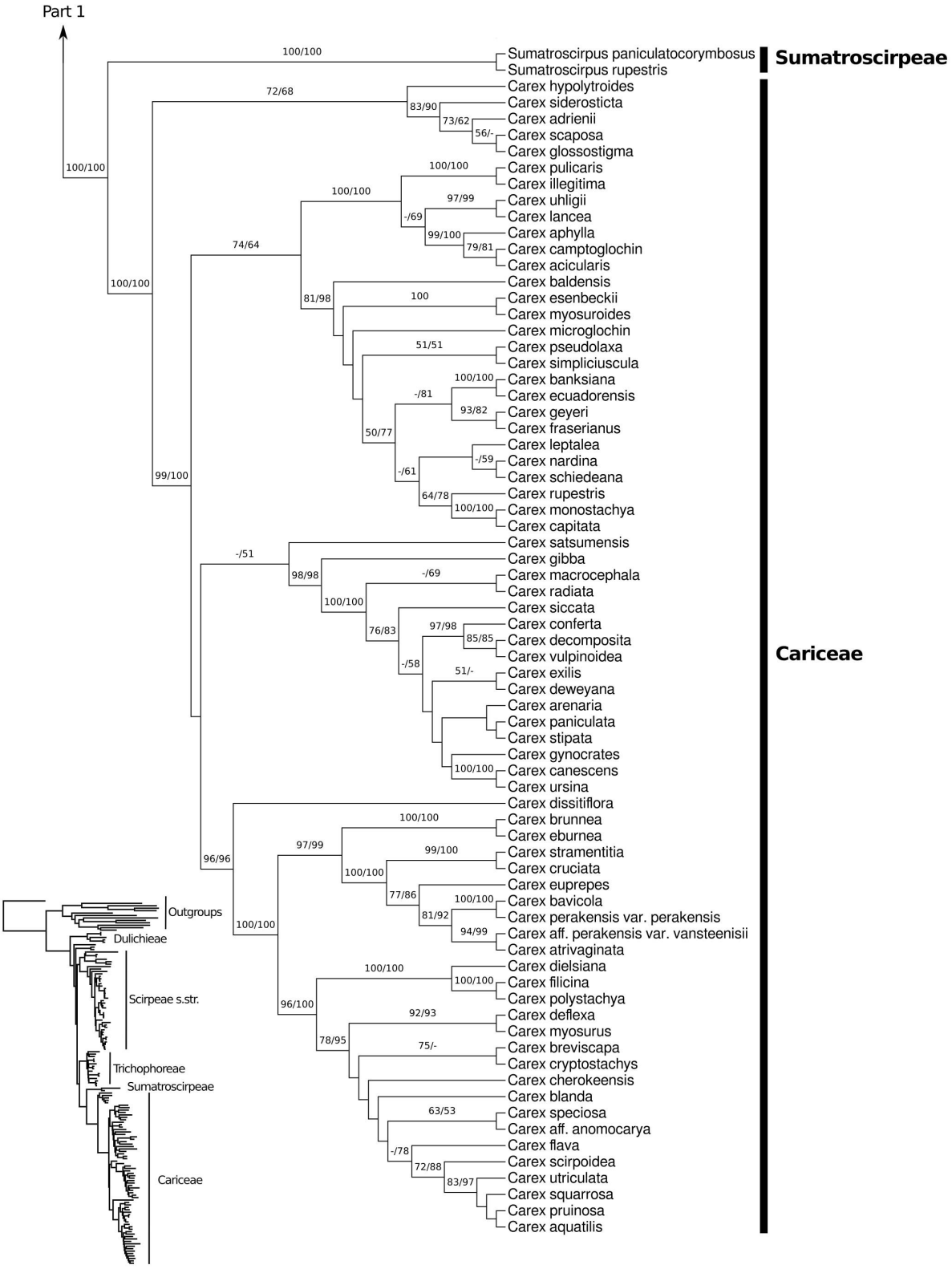
Part 1
Sumatroscirpus paniculatocorymbosus
Sumatroscirpus rupestris
Sumatroscirpeae
Carex hypolytroides
Carex siderosticta
Carex adrienii
Carex scaposa
Carex glossostigma
Carex pulicaris
Carex illegitima
Carex uhligii
Carex lancea
Carex aphylla
Carex camptoglochin
Carex acicularis
Carex baldensis
Carex esenbeckii
Carex myosuroides
Carex microglochin
Carex pseudolaxa
Carex simpliciuscula
Carex banksiana
Carex ecuadorensis
Carex geyeri
Carex fraserianus
Carex leptalea
Carex nardina
Carex schiedeana
Carex rupestris
Carex monostachya
Carex capitata
Carex satsumensis
Carex gibba
Carex macrocephala
Carex radiata
Carex siccata
Carex conferta
Carex decomposita
Carex vulpinoidea
Carex exilis
Carex deweyana
Carex arenaria
Carex paniculata
Carex stipata
Carex gynocrates
Carex canescens
Carex ursina
Carex dissitiflora
Carex brunnea
Carex eburnea
Carex stramentitia
Carex cruciata
Carex euprepes
Carex bavicola
Carex perakensis var. perakensis
Carex aff. perakensis var. vansteenisii
Carex atrivaginata
Carex dielsiana
Carex filicina
Carex polystachya
Carex deflexa
Carex myosurus
Carex breviscapa
Carex cryptostachys
Carex cherokeensis
Carex blanda
Carex speciosa
Carex aff. anomocarya
Carex flava
Carex scirpoidea
Carex utriculata
Carex squarrosa
Carex pruinosa
Carex aquatilis
Cariceae
Outgroups
Dulichieae
Scirpeae s.str.
Trichophoreae
Sumatroscirpeae
Cariceae

Figure 7.4 [on previous two pages]. Maximum likelihood topology based on concatenated analysis of *matK* + *ndhF* + *rps16* + ETS-1f + ITS + indels + morphology. Parsimony/likelihood bootstrap percentages above branches. Branches with >85% parsimony bootstrap support are emphasized with bold lines. An asterisk (*) indicates the absence of a clade in the MP strict-consensus. Inset shows branch lengths. Tribes marked with a grey vertical line are segregated from the former paraphyletic Scirpeae s.lat.

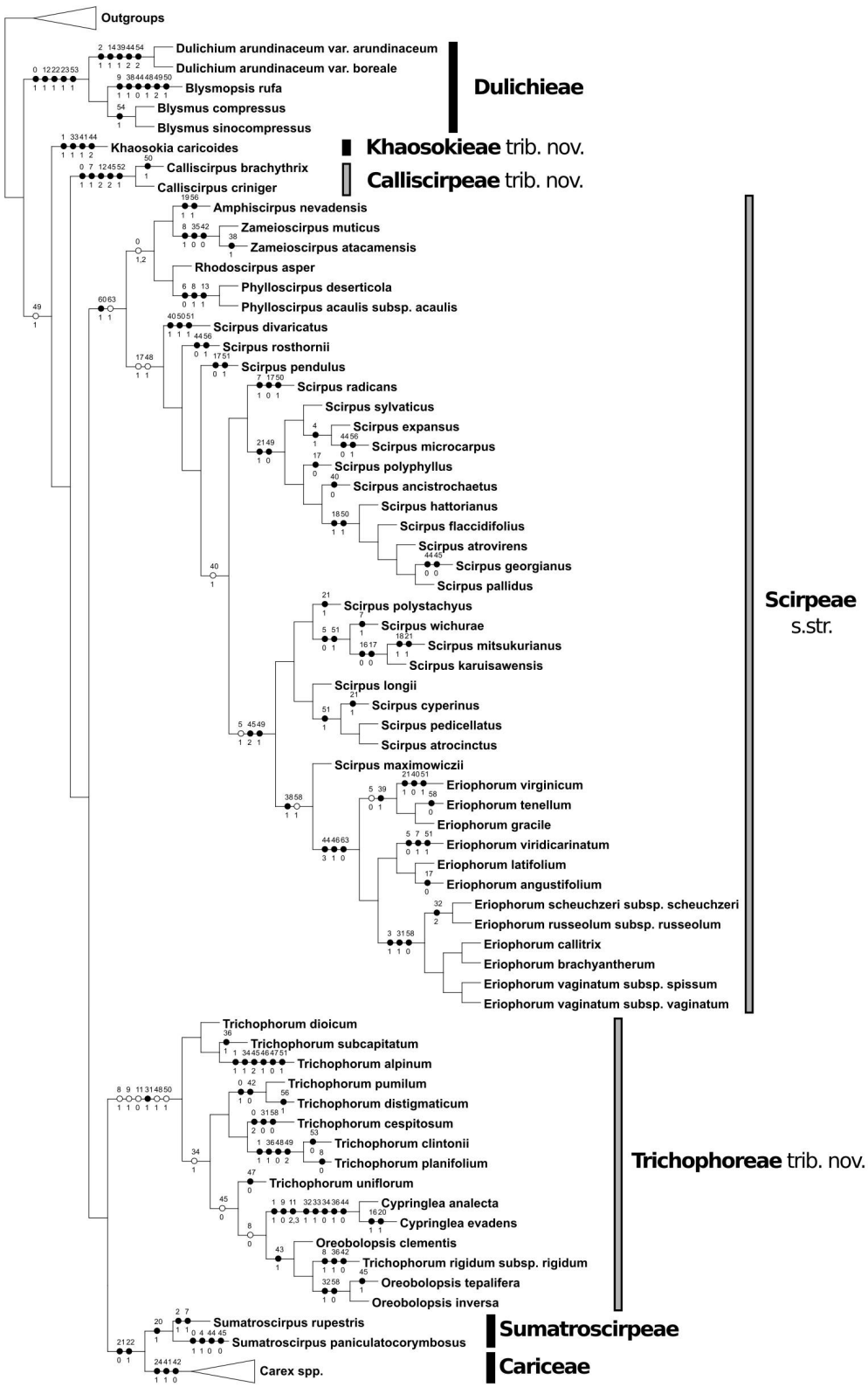
Outgroups
Dulichium arundinaceum var. arundinaceum
Dulichium arundinaceum var. boreale
Blysmopsis rufa
Blysmus compressus
Blysmus sinocompressus
Dulichieae
Khaosokia caricoides
Khaosokieae trib. nov.
Calliscirpus brachythrix
Calliscirpus criniger
Calliscirpeae trib. nov.
Amphiscirpus nevadensis
Zameioscirpus muticus
Zameioscirpus atacamensis
Rhodoscirpus asper
Phylloscirpus deserticola
Phylloscirpus acaulis subsp. acaulis
Scirpus divaricatus
Scirpus rosthornii
Scirpus pendulus
Scirpus radicans
Scirpus sylvaticus
Scirpus expansus
Scirpus microcarpus
Scirpus polyphyllus
Scirpus ancistrochaetus
Scirpus hattorianus
Scirpus flaccidifolius
Scirpus atrovirens
Scirpus georgianus
Scirpus pallidus
Scirpus polystachyus
Scirpus wichurae
Scirpus mitsukurianus
Scirpus karuisawensis
Scirpus longii
Scirpus cyperinus
Scirpus pedicellatus
Scirpus atrocinctus
Scirpus maximowiczii
Eriophorum virginicum
Eriophorum tenellum
Eriophorum gracile
Eriophorum viridicarinatum
Eriophorum latifolium
Eriophorum angustifolium
Eriophorum scheuchzeri subsp. scheuchzeri
Eriophorum russeolum subsp. russeolum
Eriophorum callitrix
Eriophorum brachyantherum
Eriophorum vaginatum subsp. spissum
Eriophorum vaginatum subsp. vaginatum
Scirpeae s.str.
Trichophorum dioicum
Trichophorum subcapitatum
Trichophorum alpinum
Trichophorum pumilum
Trichophorum distigmaticum
Trichophorum cespitosum
Trichophorum clintonii
Trichophorum planifolium
Trichophorum uniflorum
Cypringlea analecta
Cypringlea evadens
Oreobolopsis clementis
Trichophorum rigidum subsp. rigidum
Oreobolopsis tepalifera
Oreobolopsis inversa
Trichophoreae trib. nov.
Sumatroscirpus rupestris
Sumatroscirpus paniculatocorymbosus
Sumatroscirpeae
Carex spp.
Cariceae

Figure 7.5 [on previous page]. Maximum likelihood topology from Figure 7.4, with unambiguous morphological synapomorphies indicated on branches. Numbers above branches indicate character number, and below branch character state, as found in Table 7.1. Tribes marked with a grey vertical line are segregated from the former paraphyletic Scirpeae s.lat.

CHAPTER 8

CONCLUSIONS

8.1 Relationships Within CDS

The goals of this thesis were to resolve evolutionary relationships within CDS, identify the sister-group to *Carex*, and create a new natural tribal classification for CDS, a clade of more than 2,250 species that is larger than 92% of all plant families. The goal of the first study presented in **Chapter 2** was to identify the major lineages within CDS and to provide the general phylogenetic framework within which hypotheses of relationship and homology across the clade would be assessed in later studies. Using the most comprehensive taxonomic sampling of CDS to date and a two marker plastid dataset, this study identified seven major lineages with tribe Dulichieae and the genus *Khaosokia* as successive sisters to a clade composed of four strongly supported major lineages (Dulichieae, Calliscirpus, Trichophorum and Cariceae clades), and good support for a Scirpus Clade sister to a weakly supported Zameioscirpus Clade. It also suggested that tribe Scirpeae was paraphyletic with respect to Cariceae and thus might consist of multiple tribal lineages, that *Eriophorum* could be nested within *Scirpus*, and that the circumscription of *Trichophorum* might need to be revised. However, key relationships within major lineages were unresolved, and the backbone for the phylogeny remained poorly supported. This suggested that additional coding markers from the nuclear genome would be necessary to resolve deep and shallow relationships within CDS if the goal of reclassifying the clade to the tribal and generic level was going to be achieved.

As a first step towards resolving problems of generic circumscription in the genus *Scirpus*, **Chapter 3** presented a taxonomic study of the unusual South American "*Scirpus asper*". This species was shown to correspond to a new genus, *Rhodoscirpus*, which is most closely related to the South American genus *Phylloscirpus*. The position of *Rhodoscirpus* as sister to *Phylloscirpus* indicated that most morphological and anatomical characters used in the circumscription of *Scirpus* were probably ancestral for the whole Zameioscirpus Clade + Scirpus Clade. This was also supported by the fact that *Eriophorum* appeared to be derived from within *Scirpus* s.str. The situation is similar to that seen in

tribe Cypereae, where many morphologically distinctive genera (e.g. *Ascolepis*, *Lipocarpha*, *Oxycaryum*, *Remirea*, etc.) are nested within a large paraphyletic *Cyperus* (Larridon & al., 2011a, 2011b, 2013, 2014; Bauters & al., 2014). In the case of the Zameioscirpus Clade + Scirpus Clade, all genera have probably diverged from the presumed ancestral *Scirpus*-like 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* (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). These observations strengthen the need for new classifications based on the results of molecular phylogenetic studies, as they suggest high levels of homoplasy for morphological characters.

In **Chapter 4**, data from 461 unique nuclear markers were obtained from 34 taxa representing the phylogenetic breadth of CDS, using next-generation sequencing of whole genomic DNA enriched using a set of flowering plant-specific hybrid-enrichment probes. Incongruence between the estimated gene-trees was inversely proportional to the phylogenetic information content of gene-trees, and large amounts of hidden support were present especially for the shortest backbone branches. This suggested that gene-tree incongruence in this dataset was largely caused by gene-tree estimation error due to the low information content of individual markers. Concatenation and species-tree analyses recovered highly-supported backbone relationships within CDS, despite the short radiation (~10 million years) followed by long divergence (~40 million years) that is characteristic of this clade. The relationships inferred with Anchored Phylogenomics perfectly corroborated those estimated using the plastid dataset of Chapter 2.

Anchored phylogenomics thus fully supported the paraphyly of tribe Scirpeae, a long-expected result given the likely plesiomorphic nature of its defining characteristics (Goetghebeur 1998). Moreover, the isolated phylogenetic position of *Khaosokia* definitely excluded it from any Cyperaceae tribe recognized at the time. These phylogenetic results were congruent with previously identified morphological and embryological variation (Chapter 2), but a lack of support in previous phylogenetic

analyses had prevented taxonomic changes from being made. The robust phylogenetic estimates obtained in Chapter 4 provided for the first time the solid foundation needed for a complete revision of the tribal taxonomy of the CDS clade. It became clear that preservation of the highly distinctive Cariceae within a natural and inclusive tribal classification would necessitate the naming of at least three new tribes. Such strongly supported results would probably never have been achieved without genome-scale phylogenetic analyses, which clearly demonstrates the importance of new data acquisition and analysis methodologies in the progress of systematics and taxonomy.

Studies presented in Chapters 2, 3 and 4 placed all sampled Cariceae-Dulichieae-Scirpeae (CDS) genera into seven lineages, and resolved relationships between these lineages with strong support. They also identified the Trichophorum Clade as the most likely sister-group to *Carex*. The genus *Trichophorum* has been suggested as closely related to *Carex* in only one previous study based on the fact that one of its species, the circumboreal *Trichophorum cespitosum,* was infected by a species of *Anthracoidea*, a genus of parasitic smut fungi common on *Carex* species (Kukkonen & Timonen, 1979). The significance of this observation is limited given that *Anthracoidea* is also known to infect other unrelated sedge genera, such as *Carpha*, *Fuirena*, and *Schoenus* (Vánky, 2002, 2012), and the presence of *Anthracoidea* on *T. cespitosum* could also be explained by its close association with various *Carex* species in subarctic bogs and wet tundra, which could promote a jump from a *Carex* host to *T. cespitosum* (Savile, 1979). Moreover, members of the Trichophorum Clade are very morphologically and genetically distant from *Carex*, and the proposed sister-group relationship only appears to accentuate the isolated position and unusual morphology of *Carex*. The peculiar inflorescence structure of *Carex*, with its fertile prophylls and truncated lateral spikelets, would thus find no equivalent in any of its closest relatives. However, comparative morphological analyses of all CDS genera eventually suggested that the only CDS genus not yet sampled in molecular analyses, the rare monospecific Sumatran endemic *Sumatroscirpus,* which possessed fertile prophylls like *Carex*, could be of critical importance to understanding the evolution of *Carex* and the CDS clade as a whole. After herbarium work discovered a previously unknown and easily accessible locality in Northern Vietnam, it became possible to include this genus in a molecular analysis of CDS.

8.2 The Sister Relationship Between *Carex* and *Sumatroscirpus*

The genus *Sumatroscirpus* was described by Oteng-Yeboah (1974) for *Scirpus junghuhnii* Miq., a distinctive Sumatran species known since 1856 that differed from *Scirpus* in numerous morphological and anatomical characters, such as in its compressed spikelets, slightly decurrent glumes, papillose leaf surface and lack of foliar leaf cavities. However, its most distinctive character is the presence of fertile prophylls, a character it shares with both Cariceae and Dulichieae. Although *Sumatroscirpus* was initially placed in Dulichieae due to its compressed spikelets and bisexual flowers, its corymbiform inflorescences with upwardly-curved branches, filaments elongating after anthesis (Goetghebeur, 1986), minutely ciliate ligules, and enlarged style base argued against a close affinity with other Dulichieae genera. In addition, *Sumatroscirpus* is the only Cyperaceae genus besides *Carex* to possess sheathing fertile prophylls, a clear link to the perigynium of *Carex*. Its highly-compound inflorescence and Southeast Asian distribution also fit with some commonly-hypothesized primitive characteristics for *Carex* (Kreczetovicz, 1936; Gilly, 1950; Nelmes, 1951a, 1951b; Raymond, 1955, 1959; Koyama, 1957, 1961; Smith and Faulkner, 1976; Dahlgren & al., 1985; Reznicek, 1990; Waterway & al., 2009; Starr & al., 2015; Ford & al., 2017).

In **Chapter 5**, results of the first molecular phylogenetic analysis to include samples of *Sumatroscirpus* confirmed it to be the closest living relative to *Carex*, with no close affinity with Dulichieae s.str., necessitating the naming of a new Cyperaceae tribe, Sumatroscirpeae. These results have important implications for understanding the morphology and homology of *Carex* inflorescence features. The most striking morphological characteristic of *Carex* is the perigynium. Its peculiar shape, position and homology have been a source of discussion for plant morphologists and systematists for more than 200 years (Holm, 1896; Jiménez-Mejías & al., 2016a and references therein). The similarity of perigynia to the palea of grasses (Poaceae), and their apparent absence in other Cyperaceae, has even been used to justify placing *Carex* in its own family, Kobresiaceae (Gilly, 1950), although such a proposition is inconsistent with other morphological and embryological evidence, and is rejected by molecular and morphological phylogenetic results (Plunkett & al., 1995; Simpson, 1995). We know that *Carex* perigynia are a type of spikelet prophyll, and that prophylls are usually sterile in Cyperaceae (Goetghebeur, 1998). Fertile prophylls are also found in tribe Dulichieae and in *Sumatroscirpus*, but

those in Dulichieae are squamiform with unfused margins, and phylogenetic analyses clearly demonstrate that they have evolved independently from those in *Carex*. In contrast, the fertile prophylls of *Sumatroscirpus* are sheathing around their flowers and are thus similar in shape to the perigynia of *Carex*. The sister-relationship of *Carex* and *Sumatroscirpus* and ancestral state reconstructions presented in Chapter 5 indicate a common evolutionary origin of their fertile prophylls. The fertile spikelet prophylls of *Sumatroscirpus* are thus perigynia. The perigynium was inherited from the most recent common ancestor of *Carex* and *Sumatroscirpus*; it is a synapomorphy for the clade (an homology sensu Patterson, 1982 and de Pinna, 1991). This also means that the perigynium cannot be invoked as the initial key innovation leading to the radiation of *Carex*, but is more likely a single step in a series of innovations that only taken together might explain the diversification of *Carex*. Other characteristics such as the truncation of lateral spikelets in *Carex* may have enabled perigynia to diversify in shape due to the release of mechanical constraints, potentially leading to ecological diversification.

Southeast Asia has been suggested to be the center of origin of *Carex*, an hypothesis initially proposed on the basis of the occurrence of many unusual and putatively "primitive" lineages of *Carex* in that region (Gilly, 1950; Nelmes, 1951b; Raymond, 1955, 1959; Koyama, 1957). This hypothesis would be consistent with the Eastern Asian distribution of Sumatroscirpeae. However, it is also plausible that Eastern Asia acted as a museum that preserved old lineages from extinction due to its topographic diversity and relative climatic stability during the Tertiary and Quaternary coolings (Morley, 1998; Thorne, 1999; Milne & Abbott, 2002; Manchester & al., 2009; López- Pujol & al., 2011a, 2011b). This would be consistent with the Late Eocene divergence of *Sumatroscirpus* and *Carex* (34–41 million years ago), and highly unbalanced partitioning of the diversity in these clades that suggest high extinction rates. Although additional studies are needed before conclusions can be drawn on the biogeographical origin and history of *Carex*, *Sumatroscirpus* reinforces the idea that Southeast Asia played a key role in the evolution of *Carex*.

Although *Sumatroscirpus* was believed to consist of a single species endemic to the Indonesian island of Sumatra, the taxonomic revision presented in **Chapter 6** revealed that the genus actually comprises four species and ranges from Sumatra north to Vietnam, Myanmar, and Southwestern China.

This is not only a significant increase in the species diversity in the genus, but also a 2,700 km range extension of its distribution to the north, portraying a long-recognized link between the mountain floras of Eastern Asia and Sundaland (Stapf, 1894; van Steenis, 1934, 1935, 1936, 1964). All four species have a restricted range and number of occurences which would make them qualify for a IUCN (2012) status of Vulnerable or Endangered, if a plausible future threat was identified or if a continuing decline or extreme fluctuations in their distribution, number of subpopulations, or number of mature individuals could be demonstrated or was suspected. Additional fieldwork research would be needed to arrive at accurate estimates of the number of occurrences, population sizes and viability. This taxonomic revision provides the basic taxonomic and geographic information that will facilitate future studies on the biology of *Sumatroscirpus*.

The implications of the sister relationship between *Sumatroscirpus* (Sumatroscirpeae) and *Carex* (Cariceae) on Cyperaceae tribal taxonomy, future phylogenetic studies of *Carex*, the homology of the perigynium, inflorescence evolution, and biogeography have already been discussed. The 36 million years of unique evolutionary history represented by Sumatroscirpeae is of major conservation significance, especially given the key role of this tiny Eastern Asian tribe in our comprehension of the evolution of *Carex*. *Sumatroscirpus* also demonstrates that the *Carex* radiation is not due to any single character such as the perigynium, just as a feather alone does not make a bird (Hu & al., 2009). Future comparative studies focusing on other aspects of the biology of *Sumatroscirpus*, such as chromosome numbers, genome size, and inflorescence development, will provide unprecedented insights into the many traits that accumulated over the course of evolutionary history and ultimately led to the extraordinary diversification of *Carex*.

8.3 A New Tribal Classification for the Cariceae-Dulichieae-Scirpeae Clade

Studies presented in Chapters 2, 3 and 5 of this thesis have provided a strongly supported and robust phylogenetic hypothesis for the evolution of all major lineages of the CDS clade, based on a two-step approach involving extensive taxonomic sampling of a few plastid and nuclear ribosomal markers, followed by nuclear phylogenomics of selected representatives. In Chapter 5, *Sumatroscirpus* was found to be unrelated to Dulichieae s.str. and closer to Cariceae, and a new tribe, Sumatroscirpeae,

was erected to restore the monophyly of Dulichieae. However, results presented in these chapters also indicated a nested position of Cariceae and Sumatroscirpeae within a paraphyletic Scirpeae s.lat., and an isolated position for *Khaosokia*, indicating the need for a complete revision of tribal circumscriptions of the CDS clade.

In **Chapter 7**, molecular phylogenetic results were combined with morphological and embryological data in a total-evidence analysis that supported the recognition of seven CDS tribes. The paraphyletic tribe Scirpeae s.lat. was split into three monophyletic and morphologically-distinct tribes: Calliscirpeae, Scirpeae s.str., and Trichophoreae. The genus *Khaosokia*, which had never been placed in a Cyperaceae tribe due to its mix of morphological characters showing affinities with Dulichieae, Cariceae, and Scirpeae (Simpson & al., 2005; Chapter 7), was placed in its own monogeneric tribe due to its isolated phylogenetic position. The new tribal classification presented in Chapter 7 thus places for the first time all CDS genera into monophyletic tribes, and it resolves one of the most difficult problems in the higher-level classification of Cyperaceae (Koyama, 1958; Schultze-Motel, 1971; Schuyler, 1971b; Bruhl, 1995; Goetghebeur, 1998; Hinchliff & Roalson, 2013; Léveillé-Bourret & al., 2014). Moreover, it demonstrates that by following the methods used in this thesis, a truly natural evolutionary classification of sedge tribes and genera supported by morphology is entirely possible and could soon be realised. For at least 41% of sedge species, this has now been achieved.

Morphological synapomorphies were identified for all newly-circumscribed CDS tribes. The distichous spikelets and fertile squamiform prophylls that serve as synapomorphies for Dulichieae s.str. are well-known, defining characters for the tribe (Goetghebeur, 1986, 1998). Cariceae and Sumatroscirpeae are united by sheathing fertile spikelet prophylls (perigynia), but the former differs from the latter due to its autapomorphic unisexual flowers, absent perianth and truncated or reduced lateral spikelets. Sumatroscirpeae is thus characterised by the possession of one derived "caricoid" inflorescence feature, the perigynium, combined with many plesiomorphic "scirpoid" inflorescence characteristics such as bisexual flowers with a perianth, and monomorphic, many-flowered spikelets. Khaosokieae is supported by several autapomorphies including sterile proximal glumes reduced in size compared to fertile glumes, unisexual flowers, and a perianth of 7 bristles. The members of Scirpeae s.str. are united by the (sub-)basal germ pores of their embryos, corresponding to the Schoenus-type or

Fimbristylis-type embryos of Goetghebeur (1986). They are otherwise very variable in habit, vegetative and reproductive morphology, but they can be distinguished from the two other tribes segregated from Scirpeae s.lat. by their lack of sterile or differentiated proximal glumes, and yellow anthers (at least in species where anther color is known). Calliscirpeae is a new monogeneric tribe that includes two species possessing distinctive white cottony infructescences due to their numerous elongate bristles. They are similar in these characters to the Scirpeae s.str. genus *Eriophorum*, but differ by their possession of white anthers, ciliate ligules, and Carex-type embryos with lateral germ pore. The new tribe Trichophoreae comprises species with variable habit and reproductive morphology, with the genera *Oreobolopsis* and *Trichophorum* mostly represented by small, bladeless, unispicate species, and the genus *Cypringlea* by taller species with wide leaf blades and compound corymbiform inflorescences. However, members of Trichophoreae possess 1–several sterile glumes at the base of every spikelet, with *Trichophorum cespitosum* being the single exception, and are thus easily distinguished from Calliscirpeae and Scirpeae s.str.

Although morphological characters are useful for the delimitation and identification of natural CDS tribes, they show high levels of homoplasy in the clade (CI = 0.182). Previous studies have likewise shown high homoplasy in morphological characters studied across the whole Cyperaceae family (Bruhl, 1995; Simpson, 1995; Muasya & al., 2000). These results are probably caused in part by the relatively reduced morphology of Cyperaceae, which diminishes the potential number of characters, and complicates homology assessment (Starr & al., 2004; Naczi, 2009). Morphological characters sometimes appear more informative at lower taxonomic levels (e.g. Muasya, 2002; Naczi, 2009), although success varies on a case-by-case basis (Starr, 2001). Although morphological synapomorphies could be identified for all CDS tribes recognized here, most of these synapomorphies are homoplasious at the family level. For instance, fertile prophylls have evolved independently in Dulichieae and in the Cariceae + Sumatroscirpeae clade, and may also be present in Mapanioideae if spicoids are homologous to lateral spikelets in Cyperoideae (Goetghebeur, 1998; Prychid & Bruhl, 2013). Likewise, unisexual flowers, characteristic of Cariceae and Khaosokieae in CDS, are seen in several early-diverged Cyperaceae tribes including Bisboeckelereae, Cryptangieae, Koyameaea, Sclerieae, and Trilepideae (Goetghebeur, 1998). The Schoenus- or Fimbristylis-type embryos that appear to be the only identifiable morphological synapomorphy for Scirpeae s.str. are seen in various other non-CDS

tribes, as in Abildgaardieae and Schoeneae (Goetghebeur, 1998). Sterile proximal glumes, which are unique to Trichophoreae, Khaosokieae, and *Eriophorum* p.p. in CDS, is a prominent feature of most early-diverged Cyperoideae tribes, and of many members of Abildgaardieae and Fuireneae (Goetghebeur, 1986; Muasya & al., 2009), suggesting that they might even be plesiomorphic within Cyperoideae. One potentially important source of homoplasy might be constraints of design. Developmental constraints, combined with the morphological simplicity of Cyperaceae, means that only limited options exist for the expression of morphological diversity in the family (Alberch, 1989; Donoghue & Ree, 2000), as in other morphologically-simplified groups like plethodontid salamanders (Wake, 1991), leafy liverworts (Yu & al., 2013) and rust fungi (Savile, 1954). These observations suggest that in Cyperaceae, morphological characters may hold limited phylogenetic potential at higher taxonomic levels when analyzed alone (Scotland & al., 2003; Starr & al., 2004; Muasya & al., 2000), but they can still play an important role when combined with molecular data. The noise introduced by morphological homoplasy is balanced by the signal of molecular sequences in combined analyses, which permits the identification of morphological synapomorphies at lower taxonomic levels. These synapomorphies can facilitate the identification of extinct and extant species lacking molecular data, the identification of morphologically diagnosable clades that can be used in formal classifications, and also provide an independent source of characters for strengthening hypotheses of relationships.

8.4 Remaining Problems in the Circumscription of CDS Genera

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). A future dilemma for Scirpeae taxonomy will be to decide whether *Eriophorum* should be treated as an 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 Clade or even Zameioscirpus Clade.

For instance, *Scirpus petelotii* (=? *Scirpus hainanensis* S.M.Huang) possesses curious terminal hairy appendages on its perianth bristles, and a sub-basal root cap suggesting a Schoenus-type embryo, usually seen in the South American Zameioscirpus Clade (Van der Veken, 1965; Liang & Tucker, 2010b). 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, e.g. *Eriophorum scabriculme* and *Eriophorum transiens*, appear to combine characteristics which would exclude a close affinity to *Eriophorum* and even to the Zameioscirpus Clade + Scirpus Clade as a whole (Beetle, 1946; Raymond, 1957, 1959). Most of these oddities show strong affinity to other currently-recognized Cyperaceae genera, and are unlikely to correspond to new generic lineages. In consequence, they are minor problems that should not affect the new tribal circumscription given in this thesis, beyond minor adjustments. 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.

Problems of generic circumscriptions are not limited to Scirpeae s.str., as phylogenetic studies presented in Chapters 2, 3, 5 and 7 have consistently suggested *Trichophorum* to be paraphyletic relative to *Oreobolopsis* and *Cypringlea*. While the reduced vegetative and reproductive morphology of *Oreobolopsis* would make it easy to transfer its species to *Trichophorum* (as already suggested by Dhooge, 2005), the highly compound corymbiform inflorescences and flat leaf blades of *Cypringlea* are quite unlike those seen in *Trichophorum*. Furthermore, species currently placed in *Trichophorum* show significant variation in several important taxonomic characters, as most conspicuously demonstrated by *Trichophorum alpinum*, which possesses long white perianth bristles and fruit silica bodies very similar to those found in the distantly related *Eriophorum* (Schuyler, 1971; Tucker & Miller, 1980). This variation was the basis for three generic names, *Leucocoma* Ehrh. ex Rydb., *Eriophorella* Holub. and *Kreczetoczia* Tzvelev, that are now put in the synonymy of *Trichophorum* (Rydberg, 1917: 108; Holub, 1984; Tzvelev, 1999). This is reflected in part in the most frequent topologies of phylogenetic analyses placing *Trichophorum alpinum*, often in a clade with the Southeast Asian *T. subcapitatum* s.lat., as sister to the remainder of Trichophoreae, suggesting that it might be possible to split *Trichophorum* in a morphologically-sensible way in order to conserve *Cypringlea*.

However, poor support across the phylogeny of Trichophoreae and uncertain patterns of morphological variation prevent taxonomic changes to be made. In addition, several species still placed in *Scirpus* or *Eriophorum*, e.g. *Scirpus filipes*, the possibly related *S. huae*, and *Eriophorum scabriculme*, possess sterile proximal glumes and other characters suggesting an affinity with Trichophoreae (Beetle, 1946; Raymond, 1957; Koyama, 1961; Liang & Tucker, 2010b). Work is currently under way to clarify the taxonomy of these unusual species, and to resolve evolutionary relationships and generic limits within Trichophoreae using next-generation sequencing techniques.

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APPENDICES

Appendix 1. Voucher information and Genbank accession numbers of samples included in the molecular study of Chapter 2.

Herbarium acronyms follow Index Herbarium, except for Wright State University Herbarium (Wright). Legend: ** sequence already published in Gilmour *et al*. (2013).

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
Bulbostylis atrosanguinea (Boeckeler) C.B.Clarke	Muasya	1037	K	Kenya	KJ513580	KJ513485
Eleocharis acicularis (L.) Roem. & Schult.	Fields	2583	WIS	United States, Wisconsin	KJ513595	KJ513502
Erioscirpus comosus (Wall.) Palla	Hing & al.	22413	A	China	KJ513619	KJ513526
Fimbristylis dichotoma (L.) Vahl	Muasya	1006	K	Kenya	KJ513620	KJ513527
Fimbristylis ovata (Burm.f.) J.Kern	Muasya & al.	684	K	Kenya	**JX065086	**JX074642
Isolepis aucklandica Hook.f.	McIntosh	12-II-1977	CAN	New Zealand	KJ513621	KJ513528
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	Hudson	5177	CAN	Canada, Saskatchewan	**JX065075	**JX074631
Blysmus compressus (L.) Panz. ex Link	Kotowicz	871	CAN	Poland	KJ513577	KJ513482
Blysmus compressus (L.) Panz. ex Link	Shtamm	15-VIII-1962	CAN	Russia	KJ513578	KJ513483
Blysmus rufus (Huds.) Link	Jokela	9-VIII-1958	CAN	Finland	**JX065076	**JX074632
Blysmus sinocompressus Tang & F.T.Wang var. *sinocompressus*	Stangokovich	30-VII-1955	CAN	Tajikistan	KJ513579	KJ513484
Calliscirpus brachythrix C.N.Gilmour, J.R.Starr & Naczi	Janeway	6344	CHS	United States, California	**JX074667	KJ513486
Calliscirpus brachythrix C.N.Gilmour, J.R.Starr & Naczi	Ahart & Oswald	5099	CHS	United States, California	**JX065078	**JX074634
Calliscirpus criniger (A. Gray) C.N.Gilmour, J.R.Starr & Naczi	Tracy	9380	DAO	United States, California	**JX074654	KJ513487
Calliscirpus criniger (A. Gray) C.N.Gilmour, J.R.Starr & Naczi	Chambers	2973	DAO	United States, Oregon	**JX074655	KJ513488
Carex acicularis Boott in J.D.Hooker	Ford	29/94	CHR	New Zealand	KJ513581	KJ513489
Carex aphylla Kunth	Starr & Villaverde	P20-2	CAN	Argentina	KJ513582	KJ513490
Carex blanda Dewey	Bakowski	97-176	WIN	Canada, Ontario	KJ513583	KJ513491
Carex camptoglochin V.I.Krecz.	Molau & al.	2329	GB	Ecuador	KJ513584	KJ513492
Carex capitata Sol.	Starr &	6016	CAN	United States,	KJ513585	KJ513493

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
	Thibeault			California		
Carex conferta Hochst. ex A.Rich.	Muasya	1055	K	Kenya	KJ513586	KJ513494
Carex gynocrates Wormsk.	Ford & al.	02283	WIN	Canada, Manitoba	KJ513587	KJ513495
Carex monostachya A.Rich.	Muasya	1052	K	Kenya	KJ513588	KJ513496
Carex polystachya Sw. ex Wahlenb.	Jones & Wipff	1127	MICH	Belize	KJ513589	KJ513497
Carex pulicaris L.	Starr & Scott	98001	FHO	United Kingdom	KJ513590	KJ513576
Carex rupestris All.	Starr	10S-029 P29-10	CAN	United States, Colorado	KJ513591	KJ513498
Carex siderosticta Hance	Léveillé-Bourret	545	CAN	Garden	KJ513592	KJ513499
Carex stipata Muhl. ex Willd.	Dugal & Camfield	3728	CAN	United States, Ontario	KJ513593	KJ513500
Carex ursina Dewey	Porsild	8828	CAN	Greenland	**JX065081	**JX074637
Cypringlea analecta (Beetle) M.T.Strong	Reznicek & al.	11094	MICH	Mexico	KJ513594	KJ513501
Cypringlea evadens (C.D.Adams) Reznicek & S.González	Rawlins & Sholes	2830	MICH	Mexico	**JX065082	**JX074638
Dulichium arundinaceum (L.) Britton	Ford & Punter	94233	FHO	Canada, Manitoba	**JX065083	**JX074639
Eriophorum angustifolium Honck. subsp. *angustifolium*	Scoggan	10947	CAN	Canada, Manitoba	KJ513597	KJ513504
Eriophorum angustifolium Honck. subsp. *angustifolium*	Starr & al.	10S-011	CAN	United States, New Mexico	KJ513598	KJ513505
Eriophorum angustifolium Honck. subsp. *angustifolium*	Judziewicz	11218	WIS	United States, Wisconsin	KJ513596	KJ513503
Eriophorum angustifolium Honck. subsp. *komarovii* (V.N.Vassil.) Vorosch. in A.K.Skvortsov (ed.)	Given & Soper	73466	CAN	Canada, British Columbia	KJ513599	KJ513506
Eriophorum brachyantherum Trautv. & C.A.Mey.	Gillett & Boudreau	17512	CAN	Canada, British Columbia	KJ513600	KJ513507
Eriophorum brachyantherum Trautv. & C.A.Mey.	Schofield & al.	7645	CAN	Canada, Yukon	KJ513601	KJ513508
Eriophorum brachyantherum Trautv. & C.A.Mey.	Roivainen	15-VII-1958	CAN	Finland	KJ513602	KJ513509
Eriophorum callitrix Cham. ex C.A.Mey.	Malte	126887	CAN	Canada, Nunavut	KJ513603	KJ513510
Eriophorum callitrix Cham. ex C.A.Mey.	Porsild & Porsild	4753	CAN	Canada, Northwest Territories	**JX074653	**JX074641

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
Eriophorum gracile Koch in A.W.Roth	Talbot	6237-4	CAN	Canada, Northwest Territories	KJ513604	KJ513511
Eriophorum gracile Koch in A.W.Roth	Starr & Thibeault	6014	CAN	United States, California	KJ513605	KJ513512
Eriophorum latifolium Hoppe	Jokela	20-VII-1965	OSC	Finland	KJ513606	KJ513513
Eriophorum russeolum Fr. ex Hartm. subsp. *albidum* F.Nyl.	Pegg	19-VI-1957	CAN	Canada, Alberta	KJ513607	KJ513514
Eriophorum russeolum Fr. ex Hartm. subsp. *russeolum*	Gauthier	75-208	CAN	Canada, Québec	KJ513608	KJ513515
Eriophorum scheuchzeri Hoppe subsp. *scheuchzeri*	Pearson	67-80	CAN	Canada, Yukon	KJ513609	KJ513516
Eriophorum scheuchzeri Hoppe subsp. *scheuchzeri*	Jorgensen & Larsson	66-1555	CAN	Greenland	KJ513610	KJ513517
Eriophorum scheuchzeri Hoppe subsp. *scheuchzeri*	Argus & Chunys	5813	CAN	United States, Alaska	KJ513611	KJ513518
Eriophorum tenellum Nutt.	Dugal & Shchepanek	6354	CAN	Canada, Nova Scotia	KJ513612	KJ513519
Eriophorum vaginatum L. subsp. *spissum* (Fernald) Hultén	Porsild	12	CAN	Canada, Labrador	KJ513614	KJ513521
Eriophorum vaginatum L. subsp. spissum (Fernald) Hultén	Spalink	160	WIS	United States, Wisconsin	KJ513613	KJ513520
Eriophorum vaginatum L. subsp. *vaginatum*	Starr & Scott	98007	FHO	United Kingdom	KJ513615	KJ513522
Eriophorum virginicum L.	Shchepanek	1415	CAN	Canada, Québec	KJ513616	KJ513523
Eriophorum virginicum L.	Dickson & Brunton	3214	CAN	Canada, Newfoundland	KJ513617	KJ513524
Eriophorum viridicarinatum (Engelm.) Fernald	Darbyshire	2532	CAN	Canada, Ontario	KJ513618	KJ513525
Eriophorum viridicarinatum (Engelm.) Fernald	Shea	11351	CAN	Canada, Ontario	**JX074652	**JX074640
Khaosokia caricoides D.A.Simpson, Chayam. & J.Parn.	Middleton & al.	4071	MICH	Thailand	**JX065087	**JX074643
Kobresia myosuroides (Vill.) Fiori in A.Fiori & al.	Jones	146	UBC	Canada, British Columbia	KJ513622	KJ513529
Kobresia simpliciuscula (Wahlenb.) Mack.	Porsild	1825	CAN	Canada, Yukon	**JX065088	**JX074644
Oreobolopsis tepalifera T.Koyama & Guagl.	Salvador & al.	749	MICH	Peru	KJ513623	KJ513530
Oreobolopsis tepalifera T.Koyama & Guagl.	Wood	1046	NY	Bolivia	**JX065089	**JX074645

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	Solomon	15819	CAS	Bolivia	KJ541072	KJ541073
Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	Ru	9797	US	Argentina	**JX065090	**JX074646
Schoenoxiphium lanceum (Thunb.) Kük.	Dahlstrand	1302	PRE	South Africa	KJ513625	KJ513532
Schoenoxiphium sparteum (Wahlenb.) C.B.Clarke	Smook	6625	PRE	South Africa	KJ513626	KJ513533
Scirpus ancistrochaetus Schuyler	Cippolini	SA-13	Wright	United States, Pennsylvania	KJ513627	KJ513534
Scirpus atrocinctus Fernald	Spalink	283	WIS	United States, Massachusetts	KJ513628	KJ513535
Scirpus atrovirens Willd.	Spalink	180	WIS	United States, Wisconsin	KJ513629	KJ513536
Scirpus atrovirens Willd.	Spalink	186	WIS	United States, Ohio	KJ513630	KJ513537
Scirpus cyperinus (L.) Kunth	Lindsay	1025	CAN	Canada, Ontario	**JX065092	**JX074648
Scirpus cyperinus (L.) Kunth	Spalink	164	WIS	United States, Wisconsin	KJ513631	KJ513538
Scirpus cyperinus (L.) Kunth	Spalink	188	WIS	United States, Ohio	KJ513632	KJ513539
Scirpus divaricatus Elliott	Spalink	124	WIS	United States, Alabama	KJ513633	KJ513540
Scirpus expansus Fernald	Spalink	158	WIS	United States, Michigan	KJ513634	KJ513541
Scirpus flaccidifolius (Fernald) Schuyler	Spalink	193	WIS	United States, Virginia	KJ513635	KJ513542
Scirpus georgianus R.M.Harper	Hudson	409	CAN	United States, Missouri	KJ513637	KJ513544
Scirpus georgianus R.M.Harper	Spalink	121	WIS	United States, Alabama	KJ513636	KJ513543
Scirpus hattorianus Makino	Baldwin & Breitung	4196	CAN	Canada, Québec	KJ513638	KJ513545
Scirpus hattorianus Makino	Shchepanek & Dugal	5974	CAN	Canada, New Brunswick	KJ513639	KJ513546
Scirpus hattorianus Makino	Bergeron & al.	81-111	CAN	Canada, Québec	KJ513640	KJ513547
Scirpus karuisawensis Makino	Jung	807017	AJOU	South Korea	KJ513641	KJ513548
Scirpus longii Fernald	Spalink	251	WIS	United States, New Jersey	KJ513642	KJ513549
Scirpus maximowiczii C.B.Clarke	Petrochenko & al.	5613	CAN	Russia	KJ513643	KJ513550
Scirpus maximowiczii C.B.Clarke	Petrochenko	357	CAN	Russia	KJ513644	KJ513551

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
Scirpus microcarpus J.Presl & C.Presl	Dugal & Camfield	3770	CAN	Canada, Ontario	KJ513646	KJ513553
Scirpus microcarpus J.Presl & C.Presl	Spalink	284	WIS	United States, Massachusetts	KJ513645	KJ513552
Scirpus pallidus (Britton) Fernald	Hudson	5079	CAN	Canada, Saskatchewan	KJ513647	KJ513554
Scirpus pedicellatus Fernald	Houle	76-1185	CAN	Canada, Québec	KJ513648	KJ513555
Scirpus pendulus Muhl.	Cruise	1388	CAN	Canada, Ontario	KJ513649	KJ513556
Scirpus polyphyllus Vahl	Spalink	246	WIS	United States, Virginia	KJ513650	KJ513557
Scirpus polystachyus F.Muell.	Pullen	4091	A	Australia	KJ513651	KJ513558
Scirpus radicans Schkuhr	Samuelsson	296	CAN	Sweden	KJ513653	KJ513560
Scirpus radicans Schkuhr	Jung	80632	AJOU	South Korea	KJ513652	KJ513559
Scirpus sylvaticus L.	Jung	806038	AJOU	South Korea	KJ513654	KJ513561
Scirpus wichurae Boeckeler	Jung	808322	AJOU	South Korea	KJ513655	KJ513562
Trichophorum alpinum (L.) Pers.	Spetzman	4941	CAN	United States, Alaska	**JX065093	**JX074649
Trichophorum alpinum (L.) Pers.	Cayouette & al.	J75-78	CAN	Canada, Québec	KJ513656	KJ513563
Trichophorum cespitosum (L.) Hartm.	Saarela & Percy	1219	CAN	Canada, British Columbia	**JX065094	**JX074650
Trichophorum cespitosum (L.) Hartm.	Aiken & Iles	02-048	CAN	Canada, Nunavut	KJ513657	KJ513564
Trichophorum clintonii (A.Gray) S.G.Sm.	Pratt	128	CAN	Canada, Ontario	KJ513658	KJ513565
Trichophorum pumilum (Vahl) Schinz & Thell.	Bennett & al.	06-097	CAN	Canada, Yukon	KJ513659	KJ513566
Trichophorum pumilum (Vahl) Schinz & Thell.	Mejland	5-VII-1963	CAN	Norway	KJ513660	KJ513567
Trichophorum rigidum (Steud.) Goetgh., Muasya & D. A. Simpson subsp. *rigidum*	Ritter & Wood	2832	A	Bolivia	KJ513662	KJ513569
Trichophorum rigidum (Steud.) Goetgh., Muasya & D.A.Simpson subsp. *rigidum*	Unknown collector	1102	NY	Bolivia	KJ513661	KJ513568
Trichophorum subcapitatum (Thwaites & Hook.) D.A.Simpson	Luo	1903	CAS	China	KJ513663	KJ513570
Trichophorum uniflorum (Trautv.) Malyschev & Lukitsch.	Malishev	27-VII-1950	CAN	Russia	KJ513664	KJ513571
Trichophorum uniflorum (Trautv.) Malyschev & Lukitsch.	Ivanova & Moskvin	756	CAN	Russia	KJ513665	KJ513572

Taxonomic name	Collector(s)	Coll. no.	Herbarium	Origin	*matK*	*ndhF*
Uncinia banksii Boott in J.D.Hooker	Ogle	303	CHR	New Zealand	KJ513666	KJ513573
Uncinia ecuadorensis G.A.Wheeler & Goetgh.	Starr & Amigo	99020	FHO	Ecuador	KJ513667	KJ513574
Zameioscirpus atacamensis (Phil.) Dhooge & Goetgh.	Ru	9884	US	Argentina	**JX065095	**JX074651
Zameioscirpus muticus Dhooge & Goetgh.	Salvador & al.	881	MICH	Mexico	KJ513668	KJ513575

Appendix 2. Samples used in phylogenetic analyses of Chapter 3, 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.
Eleocharis acicularis (L.) Roem. & Schult.	Fields	2583	WIS	United States	*KJ513595	*KJ513502	-
Erioscirpus comosus (Wall.) Palla	Hing & al.	22413	A	China	*KJ513619	*KJ513526	-
Fimbristylis dichotoma (L.) Vahl	Muasya	1006	K	Kenya	*KJ513620	*KJ513527	-
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	Hudson	5177	CAN	Canada	*JX065075	*JX074631	KP705256
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	Ruthsatz	170/1	SI	Argentina	KP165400	KP212420	-
Blysmus rufus (Huds.) Link	Jokela	9-VIII-1958	CAN	Finland	*JX065076	*JX074632	KP705280
Calliscirpus brachythrix C.N.Gilmour, J.R.Starr & Naczi	Ahart & Oswald	5099	CHS	United States	*JX065078	*JX074634	*JX065112
Calliscirpus criniger (A. Gray) C.N.Gilmour, J.R.Starr & Naczi	Chambers	2973	DAO	United States	*JX074655	*KJ513488	*JX065099
Carex acicularis Boott in J.D.Hooker	Ford	29/94	CHR	New Zealand	*KJ513581	*KJ513489	-
Carex acicularis Boott in J.D.Hooker	Ford	113/98	FHO	New Zealand	-	-	*AY242012 S2
Carex blanda Dewey	Bakowski	97-176	WIN	Canada	*KJ513583	*KJ513491	*AY241983
Carex capitata Sol.	Starr & Thibeault	6016	CAN	United States	*KJ513585	*KJ513493	-
Carex capitata Sol.	Ford	02379	WIN	Canada	-	-	*DQ115119
Carex siderosticta Hance	Léveillé-Bourret	545	CAN	Garden	*KJ513592	*KJ513499	-
Carex siderosticta Hance	Waterway	2004.268	MTMG	Japan	-	-	*DQ998892
Carex stipata Muhl. ex Willd.	Dugal & Camfield	3728	CAN	United States	*KJ513593	*KJ513500	-
Carex stipata Muhl. ex Willd.	Waterway	99.072	MTMG	United States	-	-	*AY757375
Dulichium arundinaceum (L.) Britton	Ford & Punter	94233	FHO	Canada	*JX065083	*JX074639	-
Dulichium arundinaceum (L.) Britton	Bergeron	81-113	CAN	Canada	-	-	KP705281

Taxonomic name	Collector(s)	Coll. no.	Herb.	Origin	*matK* access.	*ndhF* access.	ETS-1f access.
Eriophorum angustifolium Honck. subsp. *angustifolium*	Scoggan	10947	CAN	Canada	*KJ513597	*KJ513504	-
Eriophorum angustifolium Honck. subsp. *angustifolium*	Keleher	755	CAN	Canada	-	-	KP705276
Eriophorum russeolum Fr. ex Hartm. subsp. *russeolum*	Gauthier	75-208	CAN	Canada	*KJ513608	*KJ513515	-
Eriophorum russeolum Fr. ex Hartm. subsp. *russeolum*	Clément & al.	30	CAN	Canada	-	-	KP705279
Eriophorum vaginatum L. subsp. *spissum* (Fernald) Hultén	Porsild	12	CAN	Canada	*KJ513614	*KJ513521	-
Eriophorum vaginatum L. subsp. *spissum* (Fernald) Hultén	Léveillé-Bourret	632	DAO	Canada	-	-	KP705278
Eriophorum virginicum L.	Dickson & Brunton	3214	CAN	Canada	KJ513617	KJ513524	-
Eriophorum virginicum L.	Léveillé-Bourret	633	DAO	Canada	-	-	KP705269
Eriophorum viridicarinatum (Engelm.) Fernald	Darbyshire	2532	CAN	Canada, Ontario	*KJ513618	*KJ513525	KP705277
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
Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	Ru	9797	US	Argentina	*JX065090	*JX074646	KP705260
Scirpus ancistrochaetus Schuyler	Cippolini	SA-13	Wright	United States	*KJ513627	*KJ513534	-
Scirpus divaricatus Elliott	Spalink	124	WIS	United States	*KJ513633	*KJ513540	-
Scirpus divaricatus Elliott	Anderson	10630	MO	United States	-	-	KP705268
Scirpus hattorianus Makino	Bergeron & al.	81-111	CAN	Canada	*KJ513640	*KJ513547	-
Scirpus hattorianus Makino	Léveillé-Bourret	621A	DAO	Canada	-	-	KP705271
Scirpus maximowiczii C.B.Clarke	Petrochenko & al.	5613	CAN	Russia	*KJ513643	*KJ513550	KP705275
Scirpus microcarpus J.Presl & C.Presl	Dugal & Camfield	3770	CAN	Canada	*KJ513646	*KJ513553	-
Scirpus microcarpus J.Presl & C.Presl	Léveillé-Bourret	608	DAO	Canada	-	-	KP705274
Scirpus pendulus Muhl.	Cruise	1388	CAN	Canada	*KJ513649	*KJ513556	-

Taxonomic name	Collector(s)	Coll. no.	Herb.	Origin	*matK* access.	*ndhF* access.	ETS-1f access.
Scirpus pendulus Muhl.	Léveillé-Bourret	611	DAO	Canada	-	-	KP705270
Scirpus polyphyllus Vahl	Spalink	246	WIS	United States	*KJ513650	*KJ513557	-
Scirpus polystachyus F.Muell.	Pullen	4091	A	Australia	*KJ513651	*KJ513558	KP705272
Scirpus radicans Schkuhr	Samuelsson	296	CAN	Sweden	*KJ513653	*KJ513560	KP705273
Scirpus wichurae Boeckeler	Jung	808322	AJOU	South Korea	*KJ513655	*KJ513562	-
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	Kiesling	10341	SI	Argentina	KP165402	KP212422	KP705261
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	Ponce	114	SI	Argentina	KP165403	KP212423	KP705262
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	Landrum	3834	MICH	Chile	KP165401	KP212421	KP705263
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	Werdermann	82	CAS	Chile	-	-	KP705264
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	West	5100	A	Chile	-	-	KP705265
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret, Donadío & J.R.Starr	Vega	1965	F	Peru	-	KP212424	-
Trichophorum alpinum (L.) Pers.	Spetzman	4941	CAN	United States, Alaska	*JX065093	*JX074649	KP705266
Trichophorum cespitosum (L.) Hartm.	Aiken & Iles	02-048	CAN	Canada, Nunavut	*KJ513657	*KJ513564	KP705267
Zameioscirpus atacamensis (Phil.) Dhooge & Goetgh.	Ru	9884	US	Argentina	*JX065095	*JX074651	KP705257
Zameioscirpus muticus Dhooge & Goetgh.	Salvador & al.	881	MICH	Mexico	*KJ513668	*KJ513575	KP705258

APPENDIX 3. Samples used for anchored phylogenomics analysis of Chapter 4.

Species	DNA number	NGS number	Collectors	Collection number	Herb.	Origin
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	STA2640	I8317	Starr, Julian R.	1301-08	CAN	Canada, British Columbia
Calliscirpus brachythrix C.N.Gilmour et al.	STA2625	I8333	Starr, Julian R.	07-037	CAN	United-States, California
Calliscirpus criniger (A.Gray) C.N.Gilmour et al.	STA2629	I8311	Starr, Julian R.	10S-055 (P55-10)	CAN	United-States, California
Carex atrivaginata Nelmes	STA2647	I8330	Ford & al.	1241A	WIN	Vietnam, Lao Cai
Carex bavicola Raymond	STA2644	I8327	Ford & al.	1220	WIN	Vietnam, Hanoi
Carex camptoglochin V.I.Krecz.	STA2656	I8334	Starr & al.	10-001	CAN	Argentina, Tierra del Fuego
Carex canescens L.	STA2654	I8332	Starr	10S-005 (P5-19)	CAN	United States, New Mexico
Carex capitata Sol.	STA2653	I8323	Starr & Villaverde	10-023 (P18-27)	CAN	Argentina, Santa Cruz
Carex dimorpholepis Steud.	STA2645	I8328	Ford & al.	1240A	WIN	Vietnam, Lao Cai
Carex filicina Nees	STA2651	I8321	Ford & al.	1229	WIN	Vietnam, Lao Cai
Carex hypolytroides Ridl.	STA2442	I8306	Ford & al.	1255A	WIN	Vietnam, Lao Cai
Carex kucyniakii Raymond	STA2639	I8316	Ford & al.	1258A	WIN	Vietnam, Lao Cai
Carex microglochin Wahlenb.	STA2658	I8336	Starr	10S-035 (P35-1 & P35-4)	CAN	United States, Colorado
Carex myosuroides Vill.	STA2630	I8312	Starr	10S-012 (P12-9)	CAN	United States, New Mexico
Carex myosurus Nees	STA2636	I8325	Ford & al.	1224	WIN	Vietnam, Lao Cai
Carex nardina (Hornem.) Fr.	STA2657	I8335	Starr	10S-051 (P51-20)	CAN	United States, Utah
Carex phleoides Cav.	STA2652	I8322	Starr & Villaverde Hidalgo	10-026	CAN	Argentina, Neuquén
Carex plantaginea Lam.	STA0178	I8304	Bakowski	96-174	WIN	Canada, Ontario
Carex pulicaris L.	STA0105	I8303	Starr & Scott	98001	FHO	England, North Yorkshire
Carex siderosticta Hance	STA2681	I8337	Léveillé-Bourret	545	CAN	Garden
Carex speciosa Kunth	STA2646	I8329	Ford & al.	1236A	WIN	Vietnam, Lao Cai
Khaosokia caricoides D.A.Simpson et al.	STA0387 B	I8305	Middleton & al.	4071	MIC H	Thailand, Surat Thani
Dulichium arundinaceum Pers. **var.** ***arundinaceum***	STA2682	I8338	Starr	16-001	OTT	Canada, Québec
Eleocharis obtusa (Willd.) Schult.	STA2632	I8314	Bergeron	12-272	MT	Canada, Québec

Erioscirpus comosus (Wall.) Palla	STA2648	I8331	Ford & al.	1269C	WIN	Vietnam, Ha Giang
Eriophorum angustifolium Honck.	STA2631	I8313	Starr	10S-011 (P11-2V)	CAN	United States, New Mexico
Eriophorum vaginatum **subsp. *spissum*** (Fern.) Hultén	STA2634	I8324	Léveillé-Bourret	632	DAO	Canada, Ontario
Eriophorum virginicum L.	STA2608	I8308	Léveillé-Bourret	633	DAO	Canada, Ontario
Scirpus atrovirens Willd.	STA2567	I8307	Léveillé-Bourret	610	DAO	Canada, Québec
Scirpus atrovirens Willd.	STA2638	I8326	Léveillé-Bourret	609	DAO	Canada, Québec
Scirpus cyperinus (L.) Kunth	STA2609	I8309	Léveillé-Bourret	634	DAO	Canada, Ontario
Scirpus pendulus Muhl.	STA2643	I8319	Léveillé-Bourret	611	DAO	Canada, Québec
Scirpus rosthornii Diels	STA2650	I8320	Ford & al.	1223A	WIN	Vietnam, Lao Cai
Trichophorum alpinum (L.) Pers.	STA2633	I8315	Garon-Labrecque	129	MT	Canada, Northwest Territories
Trichophorum cespitosum (L.) Schur	STA2628	I8310	Garon-Labrecque	130	MT	Canada, Northwest Territories

APPENDIX 4. Samples and Genbank accession numbers used in the comparative Sanger-based analyses of Chapter 4.

Species	DNA number	Collectors	Collection number	Herb.	Origin	*matK*	*ndhF*	ETS-1f
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	STA2141	Hudson	5177	CAN	Canada, Saskatchewan	JX065075	JX074631	KP705256
Calliscirpus brachythrix C.N.Gilmour et al.	STA2625	Ahart & Oswald	5099	CHS	United States, California	JX065078	JX074634	JX065112
Calliscirpus criniger (A.Gray) C.N.Gilmour et al.	STA2629	Chambers	2973	DAO	United States	JX074655	KJ513488	JX065099
Carex atrivaginata Nelmes	STA2419	Ford & al.	1230A	WIN	Vietnam, Lao Cai	KY652730	KY652731	KY652729
Carex bavicola Raymond	STA2389	Ford & al.	1215B	WIN	Vietnam, Hanoi	KP273672	KP273726	KP273600
Carex camptoglochin V.I.Krecz.	STA0027	Molau & al.	2329	GB	Ecuador, Chimborazo	KJ513584	KJ513492	AY244520
Carex canescens L.	-	Kaantonen	156/94	H	Finland	KP980061	-	-
Carex canescens L.	-	Bond	s.n.	MTMG	Canada, Québec	-	-	AY757384
Carex capitata Sol.	STA2653	Starr & Thibeault	6016	CAN	United States	KJ513585	KJ513493	-
Carex capitata Sol.	STA1411	Ford	02379	WIN	Canada	-	-	DQ115119
Carex dimorpholepis Steud.	STA2645	[N/A]	MAK accession no. 99052601	MAK	Japan, Nara Pref.	AB079435	AB079422	-
Carex filicina Nees	STA2459	Ford & al.	1247A	WIN	Vietnam, Lao Cai	KP273682	KP273736	KP273608
Carex hypolytroides Ridl.	STA2442	Ford & al.	1255A	WIN	Vietnam, Lao Cai	KP273688	KP273742	KP273610
Carex kucyniakii Raymond	STA2351	Ford & al.	1261A	WIN	Vietnam, Lao Cai	KP273693	KP273747	KP273615
Carex microglochin Wahlenb.	STA2658	Starr & al.	10-008 (P5-9)	CAN	Argentina, Tierra del Fuego	KP273698	KP273752	-
Carex microglochin Wahlenb.	STA0106	Starr & Scott	98017	FHO	Scotland, County of Perth	-	-	AY244518
Carex myosuroides Vill.	B1518	Jones	146	UBC	Canada, British	KJ513622	KJ513529	-

					Columbia			
Carex myosuroides Vill.	STA0101	Playford & al.	9084	FHO	France, Hautes-Alpes/Savoie	-	-	AH012966
Carex myosurus Nees	STA2456	Ford & al.	1246A	WIN	Vietnam, Lao Cai	KP273700	KP273754	KP273620
Carex nardina (Hornem.) Fr.	-	Aiken & al.	86-091	CAN	Canada, Nunavut	FJ548120	-	-
Carex nardina (Hornem.) Fr.	STA0839	Ford & al.	02230	WIN	Canada, Manitoba	-	-	DQ115221
Carex phleoides Cav.	-	Danton	G-(1377)-1142	[N/A]	[N/A]	-	AM999972	-
Carex phleoides Cav.	STA0034	Vann	s.n.	FHO	Chile, Chiloé	-	-	AH010381
Carex plantaginea Lam.	STA0178	Waterway	2000.002	MTMG	Canada, Québec	-	-	AY757674
Carex pulicaris L.	STA0105	Starr & Scott	98001	FHO	England, North Yorkshire	KJ513590	KJ513576	AY242019
Carex siderosticta Hance	STA0733	Léveillé-Bourret	545	CAN	Garden	KJ513592	KJ513499	-
Carex siderosticta Hance	-	Waterway	2004.268	MTMG	MTMG	-	-	DQ998892
Carex speciosa Kunth	STA2417	Ford & al.	1236A	WIN	Vietnam, Lao Cai	KP273706	KP273760	KP273625
Khaosokia caricoides D.A.Simpson et al.	STA0387 B	Middleton & al.	4071	MICH	Thailand, Surat Thani	JX065087	JX074643	-
Dulichium arundinaceum Pers. **var.** ***arundinaceum***	STA0154	Ford & Punter	94233	FHO	Canada, Manitoba	JX065083	JX074639	-
Dulichium arundinaceum Pers. **var.** ***arundinaceum***	STA2469	Bergeron & al.	81113	CAN	Canada, Québec	-	-	KP705281
Eleocharis acicularis (Willd.) Schult.	d484	Fields	2583	WIS	United States, Wisconsin	KJ513595	KJ513502	-
Erioscirpus comosus (Wall.) Palla	STA2092	Hing & al.	22413	A	China, Yunnan	KJ513619	KJ513526	-
Erioscirpus comosus (Wall.) Palla	-	Ikeda & al.	4032	TI	Nepal	-	-	KM462231
Eriophorum angustifolium Honck.	STA1777	Scoggan	10947	CAN	Canada	KJ513597	KJ513504	

Eriophorum angustifolium Honck.	STA2547	Keleher	755	CAN	Canada	-	-	KP705276
Eriophorum vaginatum* subsp. *spissum (Fern.) Hultén	STA1804	Porsild	12	CAN	Canada	KJ513614	KJ513521	-
Eriophorum vaginatum* subsp. *spissum (Fern.) Hultén	STA2607	Léveillé-Bourret	632	DAO	Canada	-	-	KP705278
Eriophorum virginicum L.	STA1807	Dickson & Brunton	3214	CAN	Canada	KJ513617	KJ513524	-
Eriophorum virginicum L.	STA2608	Léveillé-Bourret	633	DAO	Canada	-	-	KP705269
Scirpus atrovirens Willd.	dS043	Spalink	186	WIS	United States, Ohio	KJ513630	KJ513537	-
Scirpus cyperinus (L.) Kunth	STA1777	Lindsey	1025	CAN	Canada, Ontario	JX065092	JX074648	-
Scirpus pendulus Muhl.	STA1878	Cruise	1388	CAN	Canada	KJ513649	KJ513556	-
Scirpus pendulus Muhl.	STA2569	Léveillé-Bourret	611	DAO	Canada	-	-	KP705270
Scirpus rosthornii Diels	STA2437	Ford & al.	1260A	WIN	Vietnam, Lao Cai	KY652733	-	KY652732
Trichophorum alpinum (L.) Pers.	STA1815	Spetzman	4941	CAN	United States, Alaska	JX065093	JX074649	KP705266
Trichophorum cespitosum (L.) Schur	STA1819	Aiken & Iles	02-048	CAN	Canada, Nunavut	KJ513657	KJ513564	KP705267

Appendix 5. Specimens used in Chapter 5, including authorities, localities, herbarium voucher information and GenBank accession numbers for all sequences.

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
CDS Clade										
Amphiscirpus nevadensis (S.Watson) Oteng-Yeb.	STA2141	Hudson	5177	CAN	Canada, Saskatchewan	JX065075	JX074631	MF669267	KP705256	MF669209
Blysmopsis rufa (Huds.) Oteng-Yeb.	STA1913	Jokela	s.n. (9-VIII-1958)	CAN	Finland	JX065076	JX074632	-	KP705280	MF669186
Blysmus compressus (L.) Panz. ex Link	STA1903	Shtamm	s.n. (15-VIII-1962)	CAN	Russia	KJ513578	KJ513483	MF669247	MF669139	MF669185
Calliscirpus brachythrix C.N.Gilmour et al.	STA2134	Ahart & Oswald	5099	CHSC	USA, California	JX065078	JX074634	MF669266	JX065112	MF669208
Calliscirpus criniger (A. Gray) C.N.Gilmour et al.	STA1899	Chambers	2973	DAO	USA, California	JX074655	KJ513488	MF669246	JX065099	MF669184
Carex acicularis Boott	STA84	Ford	29/94	CHR	New Zealand	KJ513581	KJ513489	KP273770	-	-
"	STA85	Ford	113/98	FHO	New Zealand	-	-	-	AH012954	AH012954
Carex adrienii E.G.Camus	STA2380	Ford & al.	1203	WIN	Vietnam	KP273663	KP273717	KP273771	KP273594	KP273628
Carex aff. *anomocarya* Nelmes	STA2392	Ford & al.	1218	WIN	Vietnam	KP273664	KP273718	KP273772	KP273595	KP273629
Carex aphylla Kunth	STA25	Laegaard	13496	AAU	Argentina	-	-	-	AY242015	AY242014
"	STA1278	Starr & Villaverde	10025	CAN	Argentina	KJ513582	KJ513490	KP273774	-	-
Carex aquatilis Wahlenb.	B1343	Bouchard & al.	92251	CAN	Canada, Newfoundland	KP273666	KP273720	KP273775	-	-
"	-	Bérubé	99.009	MTMG	Canada, Québec	-	-	-	AY757651	AY757590
Carex arenaria L.	STA108	Starr & Scott	98-020	FHO	UK, Scotland	KP273667	KP273721	KP273776	AY242004	AY242003
Carex atrivaginata Nelmes	STA2419	Ford & al.	1230A	WIN	Vietnam	KY652730	KY652731	MF669269	KY652729	-
Carex baldensis L.	STA276	Reznicek	8250	MICH	Switzerland	KP273671	KP273725	KP273780	EF363121	EF363120
Carex banksiana K.A.Ford	STA68	Ogle	3003	CHR	New Zealand	KJ513666	KJ513573	-	AH010369	-
Carex bavicola Raymond	STA2389	Ford & al.	1215B	WIN	Vietnam	KP273672	KP273726	KP273781	KP273600	KP273634
Carex blanda Dewey	STA183	Bakowsky	96-176	WIN	Canada, Ontario	KJ513583	KJ513491	KP273782	AY241983	AF027445, AF027484
Carex breviscapa C.B.Clarke	STA2382	Ford & al.	1206	WIN	Vietnam	KP273673	KP273727	KP273783	KP273601	KP273635
Carex brunnea Thunb.	STA653	Bartholomew & Boufford	1574	TRTE	China	KP273674	KP273728	KP273784	KP273602	KP273636
Carex camptoglochin (Boott) V.I.Krecz.	STA27	Molau & al.	2329	GB	Ecuador	KJ513584	KJ513492	KP273785	AY244520	AY244519
Carex canescens L.	B11	Kaantonen	156/94	H	Finland	KP980061	-	-	-	-
"	-	Pusças	s.n.	private	Romania	-	-	KR827139	KR827051	KR827094

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
			(2010-VII-20)							
Carex capitata L.	STA395	Starr & Thibeault	06-016	CAN	USA, California	KJ513585	KJ513493	MF669280	MF669170	-
Carex cherokeensis Schwein.	B211	Reznicek & Naczi	10044	DOV	USA, Tennessee	KP273675	KP273729	KP273786	-	-
"	-	Waterway	2000.044	MTMG	USA, Florida	-	-	-	AY757680	AY757619
Carex conferta Hochst. ex A. Rich.	STA341	Musya	1055	K	Kenya	KJ513586	KJ513494	MF669277	MF669168	MF669217
Carex cruciata Wahlenb.	STA2393	Ford & al.	1214A	WIN	Vietnam	KP273676	KP273730	KP273787	KP273603	KP273637
Carex cryptostachys Brongn.	STA2377	Ford & al.	1202	WIN	Vietnam	KP273677	KP273731	KP273788	KP273604	KP273638
Carex decomposita Muhl.	STA799	Naczi et al.	9313	DOV	USA, Delaware	-	-	-	DQ115141	DQ115140
Carex deflexa Hornem.	-	Waterway	2001.109	MTMG	Canada, Québec	KR902909	-	-	KR902928	KR902922
Carex deweyana Schwein.	STA186	Starr	96-007	WIN	Canada, Alberta	KP273678	KP273732	KP273789	AY242007	AF027437, AF027476
Carex dielsiana Kük.	STA2453	Ford & al.	1248A	WIN	Vietnam	KP273679	KP273733	KP273790	KP273605	KP273639
Carex dissitiflora Franch.	STA673	Bartholomew & Boufford	250	MO	Japan	KP273680	KP273734	KP273791	KP273606	KP273640
Carex eburnea Boott	-	Waterway	s.n. (date?)	MTMG	Canada, Québec	-	-	-	DQ998859	DQ998912
Carex ecuadorensis (G.A.Wheeler & Goetgh.) J.R.Starr	STA148	Starr & Amigo	99-020	FHO	Ecuador	KJ513667	KJ513574	MF669232	AY012662	AY012661
Carex esenbeckii Kunth	STA5	Long & al.	ESIK #335	E	India	KP273712	KP273766	KP273824	AY242033	AY242032
Carex euprepes Nelmes	STA2349	Ford & al.	1262A	WIN	Vietnam	KP273681	KP273735	KP273792	KP273607	KP273641
Carex exilis Dewey	-	Waterway	2001.103	MTMG	Canada, Québec	KR902912	-	-	-	-
"	-	Reznicek	9150	MICH	USA, Maine	-	-	-	DQ115169	DQ115168
Carex filicina Nees	STA2411	Ford & al.	1229	WIN	Vietnam	KP273683	KP273737	KP273794	KP273609	KP273643
Carex flava L.	-	Luceño & Guzman	403	UPOS	Norway	KU939681	-	-	KU939525	-
"	-	Luceño	4305ML	?	?	-	-	JN627777	-	JN634689
Carex fraseriana Ker Gawl	STA41	Starr	s.n. (date?)	K?	Cultivated	-	-	-	AY241970	AY241969
Carex geyeri Boott	STA185	Starr	96-039	WIN	USA, Montana	KP273684	KP273738	KP273795	AY244527	AF027434, AF027474
Carex gibba Wahlenb.	STA678	Zhu & al.	2776	MO	China	KP273685	KP273739	KP273796	-	-
"	STA816	Liu	6741	MO	China	-	-	-	DQ115175	DQ115174
Carex glossostigma Hand.-Mazz.	STA680	Lai & Shan	5682	MO	China	KP273686	KP273740	-	-	KP273644
Carex gynocrates Wormsk. ex Drejer	STA817	Ford & al.	02-283	WIN	Canada, Manitoba	KJ513587	KJ513495	KP273797	DQ115177	DQ115176
Carex hypolytroides Nelmes	STA679	Averyanov & al.	VH107	MO	Vietnam	KP273690	KP273744	KP273800	KP273612	KP273647

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Carex illegitima Ces.	STA615	Alanko	92256	TRTE	Greece	KP273691	KP273745	KP273801	KP273613	-
Carex lancea Dewey	STA710	Dahlstrand & McDonald	1302	PRE	South Africa	KJ513625	KJ513532	KP273829	-	-
"	STA48	McDonald	829	PRE	South Africa	-	-	-	AY242029	AY242028
Carex leptalea Wahlenb.	-	Waterway	2001.09 9	MTMG	USA, Maine	KR902913	-	-	AY757690	AY757630
"	-	?	?	?	-	-	AF163449	-	-	-
Carex macrocephala Willd. ex Spreng.	B831	Stensvold	8154	DOV	USA, Alaska	KP273697	KP273751	KP273807	-	-
"	STA834	Ford & al.	9715	WIN	Canada, British Columbia	-	-	-	DQ115211	DQ115210
Carex microglochin Wahlenb.	STA1203	Starr & Villaverde	10-008	CAN	Argentina	KP273698	KP273752	KP273808	-	-
"	STA106	Starr & Scott	98-017	FHO	UK, Scotland	-	-	-	AY244518	AY244517
Carex monostachya A.Rich.	STA37	Muasya	1052	K	Kenya	KJ513588	KJ513496	MF669278	AY241978	AY241977
Carex myosuroides Vill.	B1518	Jones	146	UBC	Canada, British Columbia	KJ513622	KJ513529	KP273826	-	-
"	STA101	Playford & al.	9804	FHO	France	-	-	-	AH012966	AH012966
Carex myosurus Nees	STA2456	Ford & al.	1246A	WI	Vietnam	KP273700	KP273754	KP273810	KP273620	KP273654
Carex nardina Fr.	-	Gillespie & al.	9094	CAN	Canada, Northwest Territories	KC474362	-	-	-	-
"	-	Ford & al.	02-230	WIN	Canada, Manitoba	-	-	-	DQ115221	DQ115220
Carex paniculata L.	-	Luceño & al.	0808ML	UPOS	Greece	KP980057	-	KR827140	KR827052	KR827095
Carex perakensis C.B.Clarke var. *perakensis*	STA2372	Ford & al.	1211A	WIN	Vietnam	KP273668	KP273722	KP273777	KP273597	KP273631
Carex aff. *perakensis* var. *vansteenisii* (Kük.) Noot.	STA2426	Ford & al.	1235A	WIN	Vietnam	KP273665	KP273719	KP273773	KP273596	KP273630
Carex polystachya Sw. ex Wahlenb.	STA210	Jones & Wipff	11275	MICH	Belize	KJ513589	KJ513497	KP273811	AY241998	AF027448, AF027487
Carex pruinosa Boott	STA2452	Ford & al.	1245A	WIN	Vietnam	KP273701	KP273755	KP273812	KP273621	KP273655
Carex pseudolaxa (C.B.Clarke) O.Yano & S.R.Zhang	STA20	Long & Noltie	EENS #211	E	India	KP273713	KP273767	KP273825	AY241976	AY241975
Carex pulicaris L.	STA105	Starr & Scott	98-001	FHO	UK, Scotland	KJ513590	KJ513576	KP273813	AY242019	AY242018
Carex radiata (Wahlenb.) Small	-	?	?	?	USA, Virginia	KP642808	-	-	-	-
"	-	Hipp	162	WIS	USA, Wisconsin	-	-	-	DQ461025	DQ461147
Carex rupestris All.	STA1581	Starr	10S-029	CAN	USA, Colorado	KJ513591	KJ513498	MF669233	MF669125	MF669171
Carex satsumensis Franch. & Sav.	AB72572 8	Hoshino & al.	17917	OKAY	Japan	-	-	-	-	AB725728
Carex scaposa C.B.Clarke	STA613	Bartholome w & Boufford	2160	PPI	China	KP273702	KP273756	-	KP273622	KP273656

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Carex schiedeana Kunze	STA722	Jones & Manrique	5588	MO	Mexico	KP273703	KP273757	KP273814	KP273623	KP273657
Carex scirpoidea Michx.	STA180	Bayer & al.	AB-96010	WIN	Canada, Alberta	-	-	-	AY241991	AF027447, AF027486
"	B641	Reznicek	11727	DOV	USA, Alaska	KP273704	KP273758	KP273815	-	-
Carex siccata Dewey	B623	Lea	3453	DOV	USA, Colorado	KP273705	KP273759	KP273816	-	-
"	STA866	Naczi & Ford	9862	DOV	Canada, Manitoba	-	-	-	DQ115275	DQ115274
Carex siderosticta Hance	STA733	Léveillé-Bourret	545	CAN	Cultivated	KJ513592	KJ513499	KP273817	KP273624	KP273658
Carex simpliciuscula Wahlenb.	STA1801	Porsild	1825	CAN	Canada, Yukon	JX065088	JX074644	KP273828	-	-
"	STA30	Ford	9710	FHO	Canada, British Columbia	-	-	-	AY241972	AY241971
Carex speciosa Kunth	STA2417	Ford & al.	1236A	WIN	Vietnam	KP273706	KP273760	KP273818	KP273625	KP273659
Carex squarrosa L.	B674	Naczi	9159	DOV	USA, Georgia	KP273707	KP273761	KP273819	-	-
"	-	Waterway	98.020	MTMG	USA, Illinois	-	-	-	AY757648	AY757587
Carex stipata Muhl. ex Willd.	STA1808	Dugal & Camfield	3728	CAN	Canada, Ontario	KJ513593	KJ513500	MF669240	MF669133	MF669178
Carex stramentitia Bott ex Boeckeler	STA2352	Ford & Regalado	1271	WIN	Vietnam	KP273708	KP273762	KP273820	KP273626	KP273660
Carex uhligii K.Schum. ex C.B.Clarke	STA54	Williams	1007	PRE	South Africa	KP273715	KP273769	MF669281	AY242027	AY242026
Carex ursina Dewey	STA1810	Porsild	8828	CAN	Greenland	JX065081	JX074637	MF669241	MF669134	MF669179
Carex utriculata Boott	-	Elven & Guldager	13678	ALA	USA, Alaska	HG915884	-	HG915793	-	-
"	-	Mercure	ALDU1	MTMG	Canada, Québec	-	-	-	KR902933	KR902918
Carex vulpinoidea Michx.	B743	Reznicek	11687	DOV	USA, Maine	KP273710	KP273764	KP273822	-	-
"	STA883	Ford & Naczi	9872	WIN	USA, Kentucky	-	-	-	DQ115309	DQ115308
Cypringlea analecta (Beetle) M.T.Strong	STA2052	Reznicek & al.	11094	MICH	Mexico	KJ513594	KJ513501	MF669258	MF669151	MF669197
Cypringlea evadens (C.D.Adams) Reznicek & S.González	STA2053	Rawlins & Sholes	2830	MICH	Mexico	JX065082	JX074638	MF669259	MF669152	MF669198
Dulichium arundinaceum (L.) Britton var. *arundinaceum*	STA2469	Bergeron & al.	81113	CAN	Canada, Québec	MF669224	MF669285	MF669270	KP705281	-
Eriophorum angustifolium Honck.	STA1777	Scoggan	10947	CAN	Canada, Manitoba	KJ513597	KJ513504	MF669235	MF669127	MF669173
Eriophorum brachyantherum Trautv. & C.A.Mey.	STA1910	Roivainen	s.n. (15-VII-1958)	CAN	Finland	KJ513602	KJ513509	-	MF669140	-
Eriophorum callitrix Cham.	STA1783	Malte	126887	CAN	Canada, Nunavut	KJ513603	KJ513510	-	MF669128	MF669175
Eriophorum gracile W.D.J.Koch ex Roth	STA1792	Talbot	6237-4	CAN	Canada, Northwest	KJ513604	KJ513511	MF669237	MF669129	MF669176

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
					Territories					
Eriophorum latifolium Hoppe	STA2051	Jokela & Paavo	s.n. (20-VII-1965)	OSC	Finland	KJ513606	KJ513513	MF669257	MF669150	MF669196
Eriophorum russeolum Fr. subsp. *russeolum*	STA1793	Gauthier	75-208	CAN	Canada, Québec	KJ513608	KJ513515	-	MF669130	-
Eriophorum scheuchzeri Hoppe	STA1798	Argus & Chunys	5813	CAN	USA, Alaska	KJ513611	KJ513518	MF669238	MF669131	-
Eriophorum tenellum Nutt.	STA1928	Dugal & Shchepanek	6354	CAN	Canada, Ontario	KJ513612	KJ513519	MF669250	MF669143	MF669190
Eriophorum vaginatum L.	STA112	Starr & Scott	98-007	FHO	UK, England	KJ513615	KJ513522	KP273830	AY242009	AY242008
Eriophorum virginicum L.	STA1802	Shchepanek	1415	CAN	Canada, Québec	KJ513616	KJ513523	MF669239	MF669132	MF669177
Eriophorum viridicarinatum (Engelm.) Fernald	STA1780	Shea	11351	CAN	Canada, Ontario	JX074652	JX074640	MF669236	JX065096	MF669174
Khaosokia caricoides D.A.Simpson et al.	STA387	Middleton & al.	4071	MICH	Thailand	JX065087	JX074643	MF669279	MF669169	MF669218
Oreobolopsis clementis (M.E.Jones) Dhooge & Goetgh.	STA2037	Howell & True	4430	NY	USA, California	MF669220	MF669283	MF669255	MF669148	MF669194
Oreobolopsis inversa Dhooge & Goetgh.	STA2108	Laegaard	22246	US	Peru	-	-	MF669265	MF669158	MF669207
Oreobolopsis tepalifera T.Koyama & Guagl.	STA2067	Salvador & al.	749	MICH	Peru	KJ513623	KJ513530	-	MF669153	MF669201
Phylloscirpus acaulis (Phil.) Goetgh. & D.A.Simpson subsp. *acaulis*	STA2076	Seijo	1711	A	Argentina	-	-	-	MF669154	-
Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	STA2101	Solomon	15819	CAS	Bolivia	KJ541072	KJ541073	MF669263	KP705259	MF669204
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret et al.	STA2065	Landrum	3834	MICH	Chile	KP165401	KP212421	MF669261	KP705263	MF669200
Scirpus divaricatus Elliott	STA2703	Anderson	10630	MO	USA, Florida	MF669227	MF669288	-	KP705268	MF669212
Scirpus hattorianus Makino	STA1982	Shchepanek & Dugal	5974	CAN	Canada, New Brunswick	KJ513639	KJ513546	MF669253	MF669146	MF669192
Scirpus maximowiczii C.B.Clarke	STA1920	Petrochenko	357	CAN	Russia	KJ513644	KJ513551	MF669249	MF669142	MF669189
Scirpus microcarpus J.Presl & C.Presl	STA1976	Dugal & Camfield	3770	CAN	Canada, Ontario	KJ513646	KJ513553	MF669251	MF669144	MF669191
Scirpus pallidus (Britton) Fernald	STA1983	Hudson	5079	CAN	Canada, Saskatchewan	KJ513647	KJ513554	MF669254	MF669147	MF669193
Scirpus pedicellatus Fernald	STA1775	Houle	76-1185	CAN	Canada, Québec	KJ513648	KJ513555	MF669234	MF669126	MF669172
Scirpus pendulus Muhl.	STA1978	Cruise	1388	CAN	Canada, Ontario	KJ513649	KJ513556	MF669252	MF669145	-
Scirpus polystachyus F.Muell.	STA38	Wilson	s.n. (date?)	K	Australia	KJ513651	KJ513558	-	AY242011	AY242010

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Scirpus radicans Schkuhr	STA1915	Samuelsson	295	CAN	Sweden	KJ513653	KJ513560	MF669248	KP705273	MF669187
Scirpus rosthornii Diels	STA2407	Ford & al.	1224A	WIN	Vietnam	MF669222	-	MF669268	MF669159	-
Scirpus wichurae Boeckeler	STA2087	Tsugaru & Sawada	34375	A	Japan	-	MF669284	-	-	MF669202
Sumatroscirpus paniculatocorymbosus (Kük.) Lév.Bourret & J.R.Starr comb. nov.	STA2625	Harry Smith	10172	MO	China	-	-	-	MF669162	-
Sumatroscirpus sp. nov.	STA2777	Heng & al.	20184	GH	China	MF669231	MF669292	MF669276	MF669167	MF669216
Sumatroscirpus sp. nov.	STA2769	Ford & al.	15081	WIN	Vietnam	MF669228	MF669289	MF669273	MF669164	MF669213
Sumatroscirpus sp. nov.	STA2772	Ford & al.	15081B	WIN	Vietnam	MF669229	MF669290	MF669274	MF669165	MF669214
Sumatroscirpus sp. nov.	STA2773	Ford & al.	15081C	WIN	Vietnam	MF669230	MF669291	MF669275	MF669166	MF669215
Trichophorum alpinum (L.) Pers.	STA1816	Porsild	861	CAN	Canada, Yukon	MF669219	MF669282	MF669242	MF669135	MF669180
Trichophorum cespitosum (L.) Hartm. subsp. *cespitosum*	STA1817	Saarela & Percy	1219	CAN	Canada, British Columbia	JX065094	JX074650	MF669243	MF669136	MF669181
Trichophorum clintonii (A.Gray) S.G.Sm.	STA1822	Pratt	128	CAN	Canada, Ontario	KJ513658	KJ513565	MF669245	MF669138	MF669183
Trichophorum pumilum (Vahl) Schinz & Thell.	STA1820	Bennett & al.	06-097	CAN	Canada, Yukon	KJ513659	KJ513566	MF669244	MF669137	MF669182
Trichophorum rigidum (Boeckeler) Goetgh. et al. subsp. *rigidum*	STA2043	Unknown	1102	NY	Bolivia	KJ513661	KJ513568	MF669256	MF669149	MF669195
Trichophorum subcapitatum (Thwaites & Hook.) D.A.Simpson	STA2102	Tucker	15100	US	China	KX588069	KX588074	MF669264	MF669156	MF669205
Trichophorum uniflorum (Trautv.) Malyschev & Lukitsch.	STA1917	Malishev	s.n. (27-VII-1950)	CAN	Russia	KJ513664	KJ513571	-	MF669141	MF669188
Zameioscirpus atacamensis (Phil.) Dhooge & Goetgh.	STA2105 STA2143	Ru	9884	US	Argentina	JX065095	JX074651	-	KP705257	MF669206
Zameioscirpus muticus Dhooge & Goetgh.	STA2062	Salvador & al.	881	MICH	Peru	KJ513668	KJ513575	MF669260	KP705258	MF669199
Outgroups for main analysis										
Eleocharis obtusa (Willd.) Schult.	STA2632	Bergeron	12-272	MT	Canada, Québec	MF669226	MF669287	MF669272	MF669163	MF669211
Erioscirpus comosus (Nees) Palla	STA2092	Hing & al.	22413	A	China	KJ513619	KJ513526	MF669262	MF669155	MF669203
Rhynchospora capitellata (Michx.) Vahl	STA2611	Léveillé-Bourret	648	DAO	Canada, Québec	MF669225	MF669286	MF669271	MF669161	MF669210

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Additional sequences for node dating										
Other Cyperaceae										
Calyptrocarya glomerulata (Brongn.) Urb.	-	Hernandez	BioBot05960	?	Costa Rica	JQ587342	-	-	-	-
"	-	Redden	4032	WS	?	-	GU075474	-	-	-
Cladium mariscus (L.) Pohl	-	Crawley	MJC292	K?	?	KC123397	-	-	-	-
"	-	?	?	?	France	-	FM160525	-	-	-
Cyperus alternifolius L.	-	Leebens-Mack	1002-2010	GA	-	HQ180861	HQ181110	-	-	-
"	-	Goetghebeur	11516	GENT	Cultivated	-	-	-	HQ705948	-
Fimbristylis dichotoma (L.) Vahl	-	Muasya	1006	K	Kenya	KJ513620	KJ513527	-	-	-
"	-	Katayama	17519	OKAY	Japan	-	-	-	AB250649	-
Hypolytrum nemorum (Vahl) Spreng.	-	Simpson	1379	K	Malaysia	FR832783	GU075501	-	-	-
Lepironia articulata (Retz.) Domin	-	Simpson	1236	K	Malaysia	FR832787	-	-	-	-
"	-	Uchiyama	21017	OKAY	Vietnam	-	AB373104	-	-	-
Schoenus nigricans L.	-	?	?	?	UK, Wales	JN896217	-	-	-	-
"	-	Besnard	31-2007	?	?	-	AM999963	-	-	-
Scleria latifolia Sw.	-	Hernandez	BioBot05933	?	Costa Rica	JQ587379	-	-	-	-
"	-	Redden	4152	US	?	-	GU075510	-	-	-
Anarthriaceae										
Anarthria prolifera R.Br.	-	Meney	acc. num. 415191	NSW	Australia	DQ257499	EU832882	-	-	-
Arecaceae										
Calamus caryotoides A.Cunn. ex Mart.	-	Perry	s.n. (1997-VII-14)	FTG	?	JX088663	JX088663	-	-	-
Sabal domingensis Becc.	-	?	acc. no. 95371B	FTBG	?	KF928963	KF928963	-	-	-
Bromeliaceae										
Tillandsia viridiflora (Beer) Baker var. *viridiflora*	-	?	garden no. B87/80	WU	Cultivated	AY614066	HQ895768	-	-	-
Commelinaceae										
Tradescantia ohiensis Raf.	-	Moore	337	?	?	HQ180889	HQ181138	-	-	-
Ecdeiocoleaceae										
Ecdeiocolea monostachya F.Muell.	-	?	acc. no. 364828	NSW	?	DQ257528	-	-	-	-
Eriocaulaceae										
Eriocaulon aquaticum (Hill) Druce	-	?	?	?	?	AY952430	-	-	-	-
"		?	?	?	?	-	DQ188990	-	-	-

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Flagellariaceae										
Flagellaria indica L.	-	?	77-394	BH	?	HQ180865	HQ181113	-	-	-
Joinvilleaceae										
Joinvillea plicata (Hook.f.) Newell & B.C.Stone	-	Briggs	612730	NSW	New Caledonia	DQ257535	-	-	-	-
Juncaceae										
Juncus effusus L.	-	McKain	113	?	?	HQ180871	-	-	-	-
"	-	Rai	1004	ALTA	?	-	EU832892	-	-	-
Oreojuncus trifidus (L.) Záv.Drábk. & Kirschner	-	Drábková	86	PRA	Norway	AY973526	-	-	-	-
Musaceae										
Musa balbisiana Colla	-	?	?	?	Malaysia	NC_028439	NC_028439	-	-	-
Poaceae										
Oryza sativa L.	-	?	?	?	?	KM088017	KM088017	-	-	-
Streptochaeta angustifolia Soderstr.	-	Davis	757	?	?	HQ180887	HQ181135	-	-	-
Triticum aestivum L.	-	?	?	?	?	AB042240	AB042240	-	-	-
Zea mays L.	-	?	?	?	?	KF241980	KF241980			
Ŗestionaceae										
Thamnochortus insignis Mast.	-	Linder & al.	7394	Z	?	AY690736	-	-	-	-
"	-	Givnish	UW-8-2009-3	?	?	-	HQ181136	-	-	-
Thurniaceae										
Prionium serratum (L.f.) Drège ex E.Mey.	-	Fay	acc. no. 15139	K	?	KP083053	-	-	-	-
"	-	?	garden no. 19880003	BR	?	-	EU832896	-	-	-
Typhaceae										
Sparganium eurycarpum Engelm.	-	Ames	10/21/2009	?	?	HQ180886	HQ181134	-	-	-
Typha latifolia L.	-	?	?	?	?	GU195652	GU195652	-	-	-
Xyridaceae										
Xyris sp.	-	Chase	s.n.	NCU	?	KP083059	-	-	-	-
"	-	?	?	?	?	-	DQ188987	-	-	-
Zingiberaceae										
Zingiber spectabile Griff.	-	Zomlefer & al.	2298	FTG	?	NC_020363	NC_020363	-	-	-

Appendix 6. Specimens included in molecular phylogenetic analyses of Chapter 7, including authorities, localities, herbarium voucher information and GenBank accession numbers for all sequences.

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Outgroups										
Abildgaardia ovata (Burm. f.) Kral	STA328	Muasya & al.	684	K	Kenya	JX065086	JX074642	–	To be submitted	To be submitted
Actinoscirpus grossus (L. f.) Goetgh. & D.A. Simpson	STA367	Simpson	2660	K	Malaysia	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Bolboschoenus maritimus (L.) Palla	–	Smith	2452	K	Botswana	KX036935	–	–	–	–
"	–	Muasya	2169	EA	?	–	FM160515	–	–	–
"	–	Jung	806008	AJOU	South Korea	–	–	–	–	JF313179
Bulbostylis atrosanguinea (Boeckeler) C.B. Clarke	STA321	Muasya	1037	K	Kenya	KJ513580	KJ513485	–	–	–
Cyperus esculentus L.	–	Gonalez	8129	LSU	USA	KX369454	KX405870	–	–	–
"	–	?	?	?	?	–	–	LK029864	LK029870	–
"	–	Reid	7814	LSU	USA, Louisiana	–	–	–	–	KF150553
Eleocharis obtusa (Willd.) Schult.	STA2632	Bergeron	12-272	MT	Canada, Québec	MF669226	MF669287	MF669272	MF669163	MF669211
Erioscirpus comosus (Nees) Palla	STA2092	Hing & al.	22413	A	China	KJ513619	KJ513526	MF669262	MF669155	MF669203
Fimbristylis dichotoma (L.) Vahl	STA320	Muasya	1006	K	Kenya	KJ513620	KJ513527	–	–	To be submitted
"	–	Katayama	17519	OKAY	Japan	–	–	–	AB250649	–
Fuirena welwitschii Ridl.	STA	Muasya	1024	K	Kenya	To be submitted	To be submitted	–	–	To be submitted
Isolepis aucklandica Hook. f.	STA1906	McIntosh	s.n. (12-II-1977)	CAN	New Zealand	KJ513621	KJ513528	To be submitted	–	To be submitted
Schoenoplectiella juncea (Willd.) Lye	STA359	Muasya & al.	775	K	Kenya	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Schoenoplectus lacustris (L.) Palla		Hutchinson & Pryce	44	NMW	United Kingdom	JN895489	–	–	–	–
"	–	?	s.n. (Kew 5656)	K	United Kingdom	–	KC678042	–	–	KC677958
"	–	Muasya	1043	K	United Kingdom	–	–	AF449554	–	–
Rhynchospora capitellata (Michx.) Vahl	STA2611	Léveillé-Bourret	648	DAO	Canada, Québec	MF669225	MF669286	MF669271	MF669161	MF669210
CDS Clade										
Amphiscirpus nevadensis (S.Watson) Oteng-	STA2141	Hudson	5177	CAN	Canada, Saskatchewan	JX065075	JX074631	MF669267	KP705256	MF669209

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Yeb.										
Blysmopsis rufa (Huds.) Oteng-Yeb.	STA1913	Jokela	s.n. (9-VIII-1958)	CAN	Finland	JX065076	JX074632	–	KP705280	MF669186
Blysmus compressus (L.) Panz. ex Link	STA1903	Shtamm	s.n. (15-VIII-1962)	CAN	Russia	KJ513578	KJ513483	MF669247	MF669139	MF669185
Blysmus sinocompressus Tang & F.T.Wang	STA1904	Stangokovich	s.n. (30-VII-1955)	CAN	Tajikistan	KJ513579	KJ513484	To be submitted	To be submitted	To be submitted
Calliscirpus brachythrix C.N.Gilmour et al.	STA2134	Ahart & Oswald	5099	CHSC	USA, California	JX065078	JX074634	MF669266	JX065112	MF669208
Calliscirpus criniger (A. Gray) C.N.Gilmour et al.	STA1899	Chambers	2973	DAO	USA, California	JX074655	KJ513488	MF669246	JX065099	MF669184
Carex acicularis Boott	STA84	Ford	29/94	CHR	New Zealand	KJ513581	KJ513489	KP273770	–	–
“	STA85	Ford	113/98	FHO	New Zealand	–	–	–	AH012954	AH012954
Carex adrienii E.G.Camus	STA2380	Ford & al.	1203	WIN	Vietnam	KP273663	KP273717	KP273771	KP273594	KP273628
Carex aff. *anomocarya* Nelmes	STA2392	Ford & al.	1218	WIN	Vietnam	KP273664	KP273718	KP273772	KP273595	KP273629
Carex aphylla Kunth	STA25	Laegaard	13496	AAU	Argentina	–	–	–	AY242015	AY242014
“	STA1278	Starr & Villaverde	10025	CAN	Argentina	KJ513582	KJ513490	KP273774	–	–
Carex aquatilis Wahlenb.	B1343	Bouchard & al.	92251	CAN	Canada, Newfoundland	KP273666	KP273720	KP273775	–	–
“	–	Bérubé	99.009	MTMG	Canada, Québec	–	–	–	AY757651	AY757590
Carex arenaria L.	STA108	Starr & Scott	98-020	FHO	UK, Scotland	KP273667	KP273721	KP273776	AY242004	AY242003
Carex atrivaginata Nelmes	STA2419	Ford & al.	1230A	WIN	Vietnam	KY652730	KY652731	MF669269	KY652729	–
Carex baldensis L.	STA276	Reznicek	8250	MICH	Switzerland	KP273671	KP273725	KP273780	EF363121	EF363120
Carex banksiana K.A.Ford	STA68	Ogle	3003	CHR	New Zealand	KJ513666	KJ513573	–	AH010369	–
Carex bavicola Raymond	STA2389	Ford & al.	1215B	WIN	Vietnam	KP273672	KP273726	KP273781	KP273600	KP273634
Carex blanda Dewey	STA183	Bakowsky	96-176	WIN	Canada, Ontario	KJ513583	KJ513491	KP273782	AY241983	AF027445, AF027484
Carex breviscapa C.B.Clarke	STA2382	Ford & al.	1206	WIN	Vietnam	KP273673	KP273727	KP273783	KP273601	KP273635
Carex brunnea Thunb.	STA653	Bartholomew & Boufford	1574	TRTE	China	KP273674	KP273728	KP273784	KP273602	KP273636
Carex camptoglochin (Boott) V.I.Krecz.	STA27	Molau & al.	2329	GB	Ecuador	KJ513584	KJ513492	KP273785	AY244520	AY244519
Carex canescens L.	B11	Kaantonen	156/94	H	Finland	KP980061	–	–	–	–
“	–	Pusças	s.n. (2010-VII-20)	private	Romania	–	–	KR827139	KR827051	KR827094
Carex capitata L.	STA395	Starr & Thibeault	06-016	CAN	USA, California	KJ513585	KJ513493	MF669280	MF669170	–

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
"		DeDecker	4899	RSA	USA, California	–	–	–	–	AF285044
Carex cherokeensis Schwein.	B211	Reznicek & Naczi	10044	DOV	USA, Tennessee	KP273675	KP273729	KP273786	–	–
"	–	Waterway	2000.044	MTMG	USA, Florida	–	–	–	AY757680	AY757619
Carex conferta Hochst. ex A. Rich.	STA341	Musya	1055	K	Kenya	KJ513586	KJ513494	MF669277	MF669168	MF669217
Carex cruciata Wahlenb.	STA2393	Ford & al.	1214A	WIN	Vietnam	KP273676	KP273730	KP273787	KP273603	KP273637
Carex cryptostachys Brongn.	STA2377	Ford & al.	1202	WIN	Vietnam	KP273677	KP273731	KP273788	KP273604	KP273638
Carex decomposita Muhl.	STA799	Naczi et al.	9313	DOV	USA, Delaware	–	–	–	DQ115141	DQ115140
Carex deflexa Hornem.	–	Waterway	2001.109	MTMG	Canada, Québec	KR902909	–	–	KR902928	KR902922
Carex deweyana Schwein.	STA186	Starr	96-007	WIN	Canada, Alberta	KP273678	KP273732	KP273789	AY242007	AF027437, AF027476
Carex dielsiana Kük.	STA2453	Ford & al.	1248A	WIN	Vietnam	KP273679	KP273733	KP273790	KP273605	KP273639
Carex dissitiflora Franch.	STA673	Bartholomew & Boufford	250	MO	Japan	KP273680	KP273734	KP273791	KP273606	KP273640
Carex eburnea Boott	–	Waterway	s.n. (date?)	MTMG	Canada, Québec	–	–	–	DQ998859	DQ998912
Carex ecuadorensis (G.A.Wheeler & Goetgh.) J.R.Starr	STA148	Starr & Amigo	99-020	FHO	Ecuador	KJ513667	KJ513574	MF669232	AY012662	AY012661
Carex esenbeckii Kunth	STA5	Long & al.	ESIK #335	E	India	KP273712	KP273766	KP273824	AY242033	AY242032
Carex euprepes Nelmes	STA2349	Ford & al.	1262A	WIN	Vietnam	KP273681	KP273735	KP273792	KP273607	KP273641
Carex exilis Dewey	–	Waterway	2001.103	MTMG	Canada, Québec	KR902912	–	–	–	–
"	–	Reznicek	9150	MICH	USA, Maine	–	–	–	DQ115169	DQ115168
Carex filicina Nees	STA2411	Ford & al.	1229	WIN	Vietnam	KP273683	KP273737	KP273794	KP273609	KP273643
Carex flava L.	–	Luceño & Guzman	403	UPOS	Norway	KU939681	–	–	KU939525	–
"	–	Luceño	4305ML	?	?	–	–	JN627777	–	JN634689
Carex fraseriana Ker Gawl	STA41	Starr	98024	K	Cultivated	KP273711	KP273765	KP273823	AY241970	AY241969
Carex geyeri Boott	STA185	Starr	96-039	WIN	USA, Montana	KP273684	KP273738	KP273795	AY244527	AF027434, AF027474
Carex gibba Wahlenb.	STA678	Zhu & al.	2776	MO	China	KP273685	KP273739	KP273796	–	–
"	STA816	Liu	6741	MO	China	–	–	–	DQ115175	DQ115174
Carex glossostigma Hand.-Mazz.	STA680	Lai & Shan	5682	MO	China	KP273686	KP273740	–	–	KP273644
Carex gynocrates Wormsk. ex Drejer	STA817	Ford & al.	02-283	WIN	Canada, Manitoba	KJ513587	KJ513495	KP273797	DQ115177	DQ115176
Carex hypolytroides Nelmes	STA679	Averyanov & al.	VH107	MO	Vietnam	KP273690	KP273744	KP273800	KP273612	KP273647
Carex illegitima Ces.	STA615	Alanko	92256	TRTE	Greece	KP273691	KP273745	KP273801	KP273613	–

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Carex lancea Dewey	STA710	Dahlstrand & McDonald	1302	PRE	South Africa	KJ513625	KJ513532	KP273829	–	–
“	STA48	McDonald	829	PRE	South Africa	–	–	–	AY242029	AY242028
Carex leptalea Wahlenb.	–	Waterway	2001.09 9	MTM G	USA, Maine	KR902913	–	–	AY757690	AY757630
“	–	?	?	?	–	–	AF163449	–	–	–
Carex macrocephala Willd. ex Spreng.	B831	Stensvold	8154	DOV	USA, Alaska	KP273697	KP273751	KP273807	–	–
“	STA834	Ford & al.	9715	WIN	Canada, British Columbia	–	–	–	DQ115211	DQ115210
Carex microglochin Wahlenb.	STA1203	Starr & Villaverde	10-008	CAN	Argentina	KP273698	KP273752	KP273808	–	–
“	STA106	Starr & Scott	98-017	FHO	UK, Scotland	–	–	–	AY244518	AY244517
Carex monostachya A.Rich.	STA37	Muasya	1052	K	Kenya	KJ513588	KJ513496	MF669278	AY241978	AY241977
Carex myosuroides Vill.	B1518	Jones	146	UBC	Canada, British Columbia	KJ513622	KJ513529	KP273826	–	–
“	STA101	Playford & al.	9804	FHO	France	–	–	–	AH012966	AH012966
Carex myosurus Nees	STA2456	Ford & al.	1246A	WI	Vietnam	KP273700	KP273754	KP273810	KP273620	KP273654
Carex nardina Fr.	–	Gillespie & al.	9094	CAN	Canada, Northwest Territories	KC474362	–	–	–	–
“	–	Ford & al.	02-230	WIN	Canada, Manitoba	–	–	–	DQ115221	DQ115220
Carex paniculata L.	–	Luceño & al.	0808ML	UPO S	Greece	KP980057	–	KR827140	KR827052	KR827095
Carex perakensis C.B.Clarke var. *perakensis*	STA2372	Ford & al.	1211A	WIN	Vietnam	KP273668	KP273722	KP273777	KP273597	KP273631
Carex aff. *perakensis* var. *vansteenisii* (Kük.) Noot.	STA2426	Ford & al.	1235A	WIN	Vietnam	KP273665	KP273719	KP273773	KP273596	KP273630
Carex polystachya Sw. ex Wahlenb.	STA210	Jones & Wipff	11275	MIC H	Belize	KJ513589	KJ513497	KP273811	AY241998	AF027448, AF027487
Carex pruinosa Boott	STA2452	Ford & al.	1245A	WIN	Vietnam	KP273701	KP273755	KP273812	KP273621	KP273655
Carex pseudolaxa (C.B.Clarke) O.Yano & S.R.Zhang	STA20	Long & Noltie	EENS #211	E	India	KP273713	KP273767	KP273825	AY241976	AY241975
Carex pulicaris L.	STA105	Starr & Scott	98-001	FHO	UK, Scotland	KJ513590	KJ513576	KP273813	AY242019	AY242018
Carex radiata (Wahlenb.) Small	–	?	?	?	USA, Virginia	KP642808	–	–	–	–
“	–	Hipp	162	WIS	USA, Wisconsin	–	–	–	DQ461025	DQ461147
Carex rupestris All.	STA1581	Starr	10S-029	CAN	USA, Colorado	KJ513591	KJ513498	MF669233	MF669125	MF669171
Carex satsumensis Franch. & Sav.	AB72572 8	Hoshino & al.	17917	OKA Y	Japan	–	–	–	–	AB725728
Carex scaposa C.B.Clarke	STA613	Bartholomew & Boufford	2160	PPI	China	KP273702	KP273756	–	KP273622	KP273656
Carex schiedeana Kunze	STA722	Jones & Manrique	5588	MO	Mexico	KP273703	KP273757	KP273814	KP273623	KP273657

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Carex scirpoidea Michx.	B641	Reznicek	11727	DOV	USA, Alaska	KP273704	KP273758	KP273815	–	–
"	STA180	Bayer & al.	AB-96010	WIN	Canada, Alberta	–	–	–	AY241991	AF027447, AF027486
Carex siccata Dewey	B623	Lea	3453	DOV	USA, Colorado	KP273705	KP273759	KP273816	–	–
"	STA866	Naczi & Ford	9862	DOV	Canada, Manitoba	–	–	–	DQ115275	DQ115274
Carex siderosticta Hance	STA733	Léveillé-Bourret	545	CAN	Cultivated	KJ513592	KJ513499	KP273817	KP273624	KP273658
Carex simpliciuscula Wahlenb.	STA1801	Porsild	1825	CAN	Canada, Yukon	JX065088	JX074644	KP273828	–	–
"	STA30	Ford	9710	FHO	Canada, British Columbia	–	–	–	AY241972	AY241971
Carex speciosa Kunth	STA2417	Ford & al.	1236A	WIN	Vietnam	KP273706	KP273760	KP273818	KP273625	KP273659
Carex squarrosa L.	B674	Naczi	9159	DOV	USA, Georgia	KP273707	KP273761	KP273819	–	–
"	–	Waterway	98.020	MTMG	USA, Illinois	–	–	–	AY757648	AY757587
Carex stipata Muhl. ex Willd.	STA1808	Dugal & Camfield	3728	CAN	USA, Ontario	KJ513593	KJ513500	MF669240	MF669133	MF669178
Carex stramentitia Bott ex Boeckeler	STA2352	Ford & Regalado	1271	WIN	Vietnam	KP273708	KP273762	KP273820	KP273626	KP273660
Carex uhligii K.Schum. ex C.B.Clarke	STA54	Williams	1007	PRE	South Africa	KP273715	KP273769	MF669281	AY242027	AY242026
Carex ursina Dewey	STA1810	Porsild	8828	CAN	Greenland	JX065081	JX074637	MF669241	MF669134	MF669179
Carex utriculata Boott	–	Elven & Guldager	13678	ALA	USA, Alaska	HG915884	–	HG915793	–	–
"	–	Mercure	ALDU1	MTMG	Canada, Québec	–	–	–	KR902933	KR902918
Carex vulpinoidea Michx.	B743	Reznicek	11687	DOV	USA, Maine	KP273710	KP273764	KP273822	–	–
"	STA883	Ford & Naczi	9872	WIN	USA, Kentucky	–	–	–	DQ115309	DQ115308
Cypringlea analecta (Beetle) M.T.Strong	STA2052	Reznicek & al.	11094	MICH	Mexico	KJ513594	KJ513501	MF669258	MF669151	MF669197
Cypringlea evadens (C.D.Adams) Reznicek & S.González	STA2053	Rawlins & Sholes	2830	MICH	Mexico	JX065082	JX074638	MF669259	MF669152	MF669198
Dulichium arundinaceum (L.) Britton var. *arundinaceum*	STA2469	Bergeron & al.	81113	CAN	Canada, Québec	MF669224	MF669285	MF669270	KP705281	To be submitted
Dulichium arundinaceum var. *boreale* Lepage	STA2471	Cayouette	73-539	CAN	Canada, Québec	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Eriophorum angustifolium Honck.	STA1777	Scoggan	10947	CAN	Canada, Manitoba	KJ513597	KJ513504	MF669235	MF669127	MF669173
Eriophorum brachyantherum Trautv. & C.A.Mey.	STA1910	Roivainen	s.n. (15-VII-1958)	CAN	Finland	KJ513602	KJ513509	–	MF669140	–
Eriophorum callitrix	STA1783	Malte	126887	CAN	Canada,	KJ513603	KJ513510	To be	MF669128	MF669175

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Cham.					Nunavut			submitted		
Eriophorum gracile W.D.J.Koch ex Roth	STA1792	Talbot	6237-4	CAN	Canada, Northwest Territories	KJ513604	KJ513511	MF669237	MF669129	MF669176
Eriophorum latifolium Hoppe	STA2051	Jokela & Paavo	s.n. (20-VII-1965)	OSC	Finland	KJ513606	KJ513513	MF669257	MF669150	MF669196
Eriophorum russeolum Fr. subsp. *russeolum*	STA1793	Gauthier	75-208	CAN	Canada, Québec	KJ513608	KJ513515	–	MF669130	–
Eriophorum russeolum Fr. subsp. *russeolum*	STA2555	Schueler & Reynolds	16228	CAN	USA	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Eriophorum scheuchzeri Hoppe subsp. *scheuchzeri*	STA1796	Jorgensen & Larsson	66-1555	CAN	Greenland	KJ513610	KJ513517	To be submitted	To be submitted	To be submitted
Eriophorum tenellum Nutt.	STA1928	Dugal & Shchepanek	6354	CAN	Canada, Ontario	KJ513612	KJ513519	MF669250	MF669143	MF669190
Eriophorum vaginatum subsp. *spissum* (Fernald) Hultén	STA2616	Garon-Labrecque	160	MT	Canada, Northwest Territories	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Eriophorum vaginatum L. subsp. *vaginatum*	STA112	Starr & Scott	98-007	FHO	UK, England	KJ513615	KJ513522	KP273830	AY242009	AY242008
Eriophorum virginicum L.	STA1802	Shchepanek	1415	CAN	Canada, Québec	KJ513616	KJ513523	MF669239	MF669132	MF669177
Eriophorum viridicarinatum (Engelm.) Fernald	STA1780	Shea	11351	CAN	Canada, Ontario	JX074652	JX074640	MF669236	JX065096	MF669174
Khaosokia caricoides D.A.Simpson et al.	STA387	Middleton & al.	4071	MICH	Thailand	JX065087	JX074643	MF669279	MF669169	MF669218
Oreobolopsis clementis (M.E.Jones) Dhooge & Goetgh.	STA2037	Howell & True	4430	NY	USA, California	MF669220	MF669283	MF669255	MF669148	MF669194
Oreobolopsis inversa Dhooge & Goetgh.	STA2108	Laegaard	22246	US	Peru	–	–	MF669265	MF669158	MF669207
Oreobolopsis tepalifera T.Koyama & Guagl.	STA2067	Salvador & al.	749	MICH	Peru	KJ513623	KJ513530	–	MF669153	MF669201
Oreobolopsis tepalifera T.Koyama & Guagl.	STA2038	Wood	10463	NY	Bolivia	JX065089	JX074645	To be submitted	To be submitted	To be submitted
Phylloscirpus acaulis (Phil.) Goetgh. & D.A.Simpson subsp. *acaulis*	–	Ruthsaz	9341	TRIER	Argentina	KC123417	–	–	–	–
"	STA2076	Seijo	1711	A	Argentina	–	–	–	MF669154	–
Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	STA2101	Solomon	15819	CAS	Bolivia	KJ541072	KJ541073	MF669263	KP705259	MF669204
Rhodoscirpus asper (J.Presl & C.Presl) Lév.-Bourret et al.	STA2065	Landrum	3834	MICH	Chile	KP165401	KP212421	MF669261	KP705263	MF669200
Scirpus ancistrochaetus	–	Cippolini	SA-13	Wright	USA, Pennsylvania	KJ513627	KJ513534	–	–	–

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
Schuyler										
"	–	Naczi	7544	DOV	Cultivated	–	–	EF174395	–	–
Scirpus atrocinctus Fernald	–	Spalink	283	WIS	USA, Massachusetts	KJ513628	KJ513535	–	–	–
Scirpus atrovirens Willd.	–	Spalink	186	WIS	USA, Ohio	KJ513630	KJ513537	–	–	–
Scirpus cyperinus (L.) Kunth	–	Spalink	188	WIS	USA, Ohio	KJ513632	KJ513539	–	–	–
Scirpus divaricatus Elliott	STA2703	Anderson	10630	MO	USA, Florida	MF669227	MF669288	–	KP705268	MF669212
Scirpus expansus Fernald	–	Spalink	158	WIS	USA, Alabama	KJ513634	KJ513541	–	–	–
"	–	Van Neste	604	US	USA, Virginia	–	–	–	–	MF348929
Scirpus flaccidifolius (Fernald) Schuyler	–	Spalink	193	WIS	USA, Virginia	KJ513635	KJ513542	–	–	–
Scirpus georgianus R.M.Harper	STA1909	Hudson	409	CAN	USA, Missouri	KJ513637	KJ513544	–	To be submitted	To be submitted
Scirpus hattorianus Makino	STA1982	Shchepanek & Dugal	5974	CAN	Canada, New Brunswick	KJ513639	KJ513546	MF669253	MF669146	MF669192
Scirpus karuisawensis Makino	–	Jung	807017	AJOU	South Korea	KJ513641	KJ513548	–	–	GQ130353
Scirpus longii Fernald	–	Spalink	251	WIS	USA, New Jersey	KJ513642	KJ513549	–	–	–
Scirpus maximowiczii C.B.Clarke	STA1920	Petrochenko	357	CAN	Russia	KJ513644	KJ513551	MF669249	MF669142	MF669189
Scirpus microcarpus J.Presl & C.Presl	STA1976	Dugal & Camfield	3770	CAN	Canada, Ontario	KJ513646	KJ513553	MF669251	MF669144	MF669191
Scirpus mitsukurianus Makino	–	Jung	808304	AJOU	South Korea	–	–	–	–	GQ130354
Scirpus pallidus (Britton) Fernald	STA1983	Hudson	5079	CAN	Canada, Saskatchewan	KJ513647	KJ513554	MF669254	MF669147	MF669193
Scirpus pedicellatus Fernald	STA1775	Houle	76-1185	CAN	Canada, Québec	KJ513648	KJ513555	MF669234	MF669126	MF669172
Scirpus pendulus Muhl.	STA2643	Léveillé-Bourret	611	DAO	Canada, Québec	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Scirpus polyphyllus Vahl	–	Spalink	246	WIS	USA, Virginia	KJ513650	KJ513557	–	–	–
Scirpus polystachyus F.Muell.	STA38	Wilson	s.n. (date?)	K	Australia	KJ513651	KJ513558	–	AY242011	AY242010
Scirpus radicans Schkuhr	STA1915	Samuelsson	295	CAN	Sweden	KJ513653	KJ513560	MF669248	KP705273	MF669187
Scirpus rosthornii Diels	STA2407	Ford & al.	1224A	WIN	Vietnam	MF669222	–	MF669268	MF669159	–
Scirpus sylvaticus L.	–	Jung	806038	AJOU	South Korea	KJ513654	KJ513561	–	–	GQ130355
"	–				USA, garden	–	–	EF174396	–	–
Scirpus wichurae Boeckeler	STA2087	Tsugaru & Sawada	34375	A	Japan	–	MF669284	–	–	MF669202
Sumatroscirpus paniculatocorymbosu	STA2625	Harry Smith	10172	MO	China	–	–	–	MF669162	–

Species	DNA number	Collectors	Coll. number	Herb.	Origin	*matK*	*ndhF*	*rps16*	ETS-1f	ITS
s (Kük.) Lév.Bourret & J.R.Starr comb. nov.										
Sumatroscirpus rupestris Lév.-Bourret & J.R.Starr	STA2769	Ford & al.	15081	WIN	Vietnam	MF669228	MF669289	MF669273	MF669164	MF669213
Trichophorum alpinum (L.) Pers.	STA1816	Porsild	861	CAN	Canada, Yukon	MF669219	MF669282	MF669242	MF669135	MF669180
Trichophorum cespitosum (L.) Hartm.	STA1817	Saarela & Percy	1219	CAN	Canada, British Columbia	JX065094	JX074650	MF669243	MF669136	MF669181
Trichophorum clintonii (A.Gray) S.G.Sm.	STA1822	Pratt	128	CAN	Canada, Ontario	KJ513658	KJ513565	MF669245	MF669138	MF669183
Trichophorum dioicum (Y.N.Lee & Y.C.Oh) J.Jung & H.K.Choi	–	Jung	804015	AJOU	South Korea	–	–	–	–	FJ797641
Trichophorum distigmaticum (Kük.) T.V.Egorova	STA2572	Chung & Li	24-2	MOR	China	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Trichophorum planifolium (Spreng.) Palla	STA2660	Naczi	11106	DOV	USA, Pennsylvania	To be submitted	To be submitted	To be submitted	To be submitted	To be submitted
Trichophorum pumilum (Vahl) Schinz & Thell.	STA1820	Bennett & al.	06-097	CAN	Canada, Yukon	KJ513659	KJ513566	MF669244	MF669137	MF669182
Trichophorum rigidum (Boeckeler) Goetgh. et al. subsp. *rigidum*	STA2043	Unknown	1102	NY	Bolivia	KJ513661	KJ513568	MF669256	MF669149	MF669195
Trichophorum subcapitatum (Thwaites & Hook.) D.A.Simpson	STA2102	Tucker	15100	US	China	KX588069	KX588074	MF669264	MF669156	MF669205
Trichophorum uniflorum (Trautv.) Malyschev & Lukitsch.	STA1917	Malishev	s.n. (27-VII-1950)	CAN	Russia	KJ513664	KJ513571	–	MF669141	MF669188
Zameioscirpus atacamensis (Phil.) Dhooge & Goetgh.	STA2105 STA2143	Ru	9884	US	Argentina	JX065095	JX074651	–	KP705257	MF669206
Zameioscirpus muticus Dhooge & Goetgh.	STA2062	Salvador & al.	881	MICH	Peru	KJ513668	KJ513575	MF669260	KP705258	MF669199

Appendix 7. Character matrix of morphological, embryological and micromorphological data used in the phylogenetic analyses of Chapter 7. Legend: cannot be coded = -; unknown state = ?; states 0 & 1= A; states 1 & 2 = B; states 2 & 3 = C; states 0 & 1 & 2 = D.

Taxon	Character number:	000000000111111111122222222223333333333444444444455555555556666666 123456789012345678901234567890123456789012345678901234567890123456789012345678901234
Abildgaardia ovata		0000000-110A0--0000001000------111100100000--------D0??000101-00
Actinoscirpus grossus		1110001000030000010010000------0-000110000101101000000000111211
Bolboschoenus maritimus		1100000-0011-00---0101000------0-00110000010A000---00110A0011-10
Bulbostylis atrosanguinea		0?00001?0A01-00---0001000------??0001000000--------000?100101?01
Cyperus esculentus		0110000-000300000001000010------0-0000100000--------000000020121?
Eleocharis obtusa		000000--1-00------00----0------100010000001011010000010110111211
Erioscirpus comosus		0000000-000300000000000000------111000100001032011311011000001210
Fimbristylis dichotoma		0000000-00030000000010000------100001100000--------0000010101B00
Fuirena welwitschii		1100001100131010010001000------0-0011000000--------000?10011121?
Isolepis aucklandica		1000000-010A------0001000------100100100000--------D01200010121?
Rhynchospora capitellata		000000100013001000000000------111000200001011010000A000110100-0A
Schoenoplectiella juncea		000000??1101-00---0001000------0-0001000000--------0???000?1121A
Schoenoplectus lacustris		101000101-030000000100000------0-000010000101101000000110A0011210
Amphiscirpus nevadensis		101000110101-00---0101000------0-001100000101001000001001010110?
Blysmopsis rufa		100000100101-00---0001110------0-000021000100101121001001020 0-0?
Blysmus compressus		10000010000B1000--0001110------0-000020000101101000001101020 0-0?
Blysmus sinocompressus		10A00010000B1000--0001110------0-000020000101101000001101 02?????
Calliscirpus brachythrix		1000001100A32A0---0001000------0-00110000010B2110110100000000-00
Calliscirpus criniger		1000001100A32A0---0001000------0-00110000010B211010010000000 0-0A
Carex acicularis		000000100101-------0-01010001000-0000000010--------0???0001?????
Carex adrienii		10A100100013101001-0-01010031000-0001000010--------0???1001?????
Carex aff. anomocarya		000000100002101000-0-010110?1100-000?000010--------0???1000????0
Carex aff. perakensis var. vansteenisii		000000100003101000-0-01010001100-0000000010--------0?001001?????
Carex aphylla		000000101101-------0-010100?0000-0000000010--------0????0???????
Carex aquatilis		100000100002101000-0-01011000000-0000000010--------00000000?????
Carex arenaria		100000100002110100-0-01010011100-0000000010--------001??10?00-0?
Carex atrivaginata		101001100013101000-0-01010001100-0001000010--------000?1001?????
Carex baldensis		0000001000022001---0-01010000000-0000000010--------0100?0???????
Carex banksiana		000000100101-------0-010100?1030-0000000010--------0????00?????0
Carex bavicola		000000100013200----0-01010001100-0001000010--------01100000?????

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Carex blanda                          010000100012101000-0-01011000000-0000000010--------00000001?????
Carex breviscapa                      000000100002101000-0-01011001100-0001000010--------0???1001?????
Carex brunnea                         000000100003101000-0-010110B1000-0000000010--------0000100100-00
Carex camptoglochin                   100000100101-------0-01010001010-0000000010--------00100001?????
Carex canescens                       010000100002110100-0-01010111100-0000000010--------00000100????0
Carex capitata                        000000100001-------0-01010001000-0000010010--------0012010100-00
Carex cherokeensis                    000000100002101000-0-01011001100-0000000010--------0????0??????0
Carex conferta                        110000100002110100-0-01010011100-0000000010--------001??10??????
Carex cruciata                        001000100013101001-0-01010001000-0000000010--------00021001?????
Carex cryptostachys                   000000100002101000-0-01011021100-0001000010--------0???0000?????
Carex decomposita                     011000100003110100-0-01010011000-0000000010--------0???0101?????
Carex deflexa                         000000100002100000-0-01011021100-0000000010--------00000001?????
Carex deweyana                        000000100002100100-0-01010111100-0000000010--------00021101?????
Carex dielsiana                       000000100013101000-0-010100?1000-0000000010--------0???1001?????
Carex dissitiflora                    00000010000C101000-0-01011001100-0000000010--------0???0000????0
Carex eburnea                         100000100102101000-0-01011000000-0000000010--------00001001?????
Carex ecuadorensis                    000000100101-------0-010100?1030-0001000010--------0????00?????0
Carex esenbeckii                      000000100001-------0-110100-A020-0000000010--------0???0000????0
Carex euprepes                        101001100013101000-0-01010001100-0001000010--------000?1001?????
Carex exilis                          000000100101-------0-0101AA11100-0000000010--------00001101????0
Carex filicina                        0A0000100010001001-0-01010001100-0001000010--------0???1000?????
Carex flava                           000000100002B01000-0-01011001100-0000000010--------0000000000-00
Carex fraserianus                     0A0000--0001-------0-01010000000-0000000010--------0100000100-0?
Carex geyeri                          100000100001-------0-01010000000-0000000010--------001??00??????
Carex gibba                           00000010001C100100-0-01010011100-0001000010--------00000001????0
Carex glossostigma                    100110100012101000-0-01010031000-0001000010--------0???0001?????
Carex gynocrates                      100000100101-------0-0101A000000-0000000010--------00001101?????
Carex hypolytroides                   101000100013001001-0001011030000-0000000010--------00??1001????0
Carex illegitima                      010000110002100000-0-01010100000-0000000010--------00000001?????
Carex lancea                          000000100013100000-0-A1010001020-0000000010--------0???100100-0?
Carex leptalea                        100000100101-------0-01010000000-0000000010--------00100001?????
Carex macrocephala                    100000100002110100-0-01010011100-0000000010--------00000100?????
Carex microglochin                    100000100101-------0-01010001010-0000000010--------001000010A20?
Carex monostachya                     110000100001-------0-010100?1100-0000000010--------0???0101?????
Carex myosuroides                     000000100001-------0-110100---20-0000010010--------0012000000-00
Carex myosurus                        011000100003101001-0-01010001100-000?000010--------0???100??????
Carex nardina                         000000100001-------0-01010021000-0000010010--------0000000100-00
Carex paniculata                      010000100003110100-0-01010011100-0000000010--------001??10?????0
Carex perakensis var. perakensis      000000100003101000-0-01010001100-0000000010--------0?001001?????
Carex polystachya                     000000100013101000-0-01010031000-0000000010--------00010000?????
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Carex pruinosa                              000000100002100000-0-01011000000-000?000010--------0???0100?????
Carex pseudolaxa                            000000100003100000-0-110100-A020-0000000010--------0????00?????0
Carex pulicaris                             000000100101-------0-01010000000-0000000010--------001?010100-00
Carex radiata                               000000100002100100-0-01010011100-0000000010--------00000101?????
Carex rupestris                             000000100001-------0-01010000000-0000010010--------0010000100-0?
Carex satsumensis                           100000100003110100-0-01010031000-0000000010--------0???1001?????
Carex scaposa                               10A100100013101001-0-01010031000-0000000010--------01??1001?????
Carex schiedeana                            0000001000021001---0-01010030000-0000000010--------0???0001?????
Carex scirpoidea                            10001011000B-------0-01011-00000-0001000010--------00A0000000-0?
Carex siccata                               100000100002110100-0-01010011100-0000000010--------00000101?????
Carex siderosticta                          100110100012101000-0-01010030000-0000000010--------00000001????0
Carex simpliciuscula                        00000010000C110000-0-110100---20-0000010010--------0012000000-00
Carex speciosa                              010000100002100000-0-01010001000-0001000010--------0???100100-0?
Carex squarrosa                             010000100002101000-0-01010101100-0000000010--------00000001????0
Carex stipata                               01100010000C110100-0-01010011100-0000000010--------001A0101?????
Carex stramentitia                          100000100013101001-0-01010001000-0000000010--------0???1001?????
Carex uhligii                               000000100013100000-0-A1010001020-0000000010--------0???100100-0?
Carex ursina                                00000010000B110100-0-01010110000-0000000010--------00000101?????
Carex utriculata                            110000100002100000-0-01011001100-0000000010--------00000000?????
Carex vulpinoidea                           010000100003100100-0-01010011100-0000000010--------00121101?????
Cypringlea analecta                         01A00010000C0000000001000------1110A1100001000011110000000100-00
Cypringlea evadens                          01A0001A000C0000100011000------1110A1100001000011110000000?????
Dulichium arundinaceum var. arundinaceum    101000100012101000000l110------0-000020100102101000012010200-0?
Dulichium arundinaceum var. boreale         101000100012101000000l110------0-000020100102101000012010200?????
Eriophorum angustifolium                    101001100011-00-100110000------0-001001010103210---0000000101100
Eriophorum brachyantherum                   000101100110------01----0------11001001010103210---0000000001100
Eriophorum callitrix                        000101100110------01----0------11001001010103210---00000000????0
Eriophorum gracile                          10000A100111-00-110110000------0-001001110103210---0000000101200
Eriophorum latifolium                       001001100011-00-110110000------0-001001010103210---0000000101200
Eriophorum russeolum subsp. russeolum       100101100110------01----0------12001001010103210---0000000001200
Eriophorum scheuchzeri subsp. scheuchzeri   100101100110------01----0------12001001010103210---0000000001100
Eriophorum tenellum                         100000100111-00-1101A0000------0-001001110103210---00000000????0
Eriophorum vaginatum subsp. spissum         000101100110------01----0------11001001010103210---0000000001100
Eriophorum vaginatum subsp. vaginatum       000101100110------01----0------11001001010103210---0000000001100
Eriophorum virginicum                       101000100012200---0001000------0-001101100103210---1000000101100
Eriophorum viridicarinatum                  001000110111-00-110110000------0-001101010103210---10000001????0
Khaosokia caricoides                        010000100002A000000001000------1110011000110211101000000???????
Oreobolopsis clementis                      000000100100------00----0------100100100001110-----0000000100-00
Oreobolopsis inversa                        000000100100------00----0------110100100001110-----0000000000-0?
Oreobolopsis tepalifera                     000000100100------00----0------110100100001111-----0000000000-0?
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Phylloscirpus acaulis subsp. acaulis      2000000-1101-10---0001000------0-00100000010110100000???00000100?
Phylloscirpus deserticola                 2000000-110A-10---0001000------0-00100000010110100000?0000000100?
Rhodoscirpus asper                        10100011001300000000001000------0-00110000010110100000010000001001
Scirpus ancistrochaetus                   00100010001300000010001000------0-00110000010110110???0010????1
Scirpus atrocinctus                       00100110001300000110010000------0-001100010101200---10000010????1
Scirpus atrovirens                        00100010001300000011001000------0-00110001010110110100000001001201
Scirpus cyperinus                         00100110001300001100A1000------0-001100010101200---1000001001201
Scirpus divaricatus                       00100010001300000110010000------0-00100001010110112110000000????1
Scirpus expansus                          10101010001300000010001000------0-00110001010110100010000010????1
Scirpus flaccidifolius                    00100010001300000A1001000------0-00110001010110110100000010????1
Scirpus georgianus                        00100010001300000011001000------0-00110001010000A1010000001001201
Scirpus hattorianus                       00100010001300000011001000------0-00110001010110110100000010????1
Scirpus karuisawensis                     00100010001300000000000000------0-00110000010120011010000010????1
Scirpus longii                            10100110001300000110010000------0-001100010101200---00000000?????
Scirpus maximowiczii                      10000110001200000110000000------0-00110101010120101A0000000101001
Scirpus microcarpus                       10101010001300000010001000------0-00110001010010100010000110????1
Scirpus mitsukurianus                     00100010001300000001001000------0-00110001010120011010000010????1
Scirpus pallidus                          00100010001300000011001000------0-00110001010110110100000010????1
Scirpus pedicellatus                      00100110001300000110010000------0-001100010101200---10000010?????
Scirpus pendulus                          00100010001300000100010000------0-001000000101100---10000000????1
Scirpus polyphyllus                       00100010001300000000001000------0-00110001010110100001000001001101
Scirpus polystachyus                      00100110001300000110001000------0-00110001010120111000000010?????
Scirpus radicans                          10100011001300000000010000------0-00110001010110112100000010????1
Scirpus rosthornii                        00100010001300000010001000------0-00110000010010112A00000110?????
Scirpus sylvaticus                        10100010001300000010001000------0-00110001010110100 0A000001001101
Scirpus wichurae                          00100011001300001100A0000------0-00110000010120011010000001001201
Sumatroscirpus paniculatocorymbosus       10001010001300000000010100--3---0-000010000100001---00001A01?????
Sumatroscirpus rupestris                  00100011001300000000010100--3---0-00001000010110101000001A01?????
Trichophorum alpinum                      010000101100------00----0------10010010000101210---1010000000-00
Trichophorum cespitosum                   200000101100------00----0------0-01001000010110A1110010000000-00
Trichophorum clintonii                    010000101100------00----0------10010110000101101021000000001????0
Trichophorum dioicum                      0000001?1100------00----0------??00001000010110111110?100000?????
Trichophorum distigmaticum                100000101100------00----0------100100100000--------00100101?????
Trichophorum planifolium                  010000100A00------00----0------10010110000101101021001000010 0-00
Trichophorum pumilum                      100000101100------00----0------100100100000--------0010000100-00
Trichophorum rigidum subsp. rigidum       000000101100------00----0------100101100000--------0000000100-0?
Trichophorum subcapitatum                 0000001AA10A-10-0000A1000------10000110000101101 12A0010000A00-0A
Trichophorum uniflorum                    000000101100------00----0------1001001000010A0?0---00000001?????
Zameioscirpus atacamensis                 200000101100------00----0------0-000001?000--------0?1000010100?
Zameioscirpus muticus                     200000101100------00----0------0-000000?000--------0?1000010100?
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