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THE JUNCACEAE-CYPERACEAE INTERFACE: A COMBINED PLASTID SEQUENCE ANALYSIS

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ABSTRACT

Phylogenetic relationships of Juncaceae and related families of Cyperales were investigated using DNA sequence data from the plastid *rps16* intron, *trnL* intron, and *trnL*–*F* intergenic spacer. Results using parsimony analysis of the aligned matrices find Juncaceae and Cyperaceae to be monophyletic families, which form a clade sister to a monophyletic Thurniaceae that includes *Prionium* (Prioniaceae), all three clades with 100% bootstrap support. Within Juncaceae, the genus *Luzula* is monophyletic with 100% bootstrap support and sister to the rest of Juncaceae. Further groupings within the family indicate that the genus *Juncus* may be monophyletic only with the inclusion of the single-flowered genera of Juncaceae (*Distichia*, *Oxychloe*, *Marsippospermum*, and *Rostkovia*; sequences of *Patosia* were not included). Major groupings within *Juncus* are supported by the morphological characters of septate or non-septate leaves and the presence or absence of bracts subtending the flowers, which have been used to define subgenera and sections within *Juncus*.

Key words: cpDNA phylogeny, Cyperaceae, Juncaceae, molecular phylogeny, *rps16* intron, Thurniaceae, *trnL*–*F* region.

INTRODUCTION

Juncaceae are a cosmopolitan family of about 440 species and seven genera. However, most of these species are found within just two genera, with 315 species in *Juncus* L. and 115 in *Luzula* DC. (Kirschner 2002a). Both genera have wide distributions, occurring on every continent but Antarctica. The remaining genera in the family (*Distichia* Nees & Meyen, *Marsippospermum* Desv., *Oxychloe* Phil., *Patosia* Buchenau, and *Rostkovia* Desv.) have from one to five species per genus, and a southern hemisphere distribution concentrated in South America (Kirschner 2002a).

Most Juncaceae have a growth form typical of many monocot herbs, with a creeping rhizome from which short-lived culms arise that terminate in an inflorescence, although the rhizome may often be lost or reduced. Several leaves cluster around the base of each culm, and sometimes along it. In a few taxa the leaves are reduced to scale-like structures on the rhizome. *Oxychloe*, *Distichia*, and *Patosia* are the most notable exceptions to this growth form, being cushion-plants found at high elevations in South America (Balslev 1996).

The genera *Juncus* and *Luzula* possess inflorescences with many flowers, although in *Juncus* they may rarely be reduced to few or single flowers. All other Juncaceae genera have a single flower per inflorescence. *Luzula* is identified by the presence of closed-leaf sheaths and multicellular hairs on the leaves, and by the number of ovules and type of placentation. *Luzula* has three basal ovules, while all the other genera have many ovules with axile or parietal placentation.

Among the five single-flowered genera, *Marsippospermum* and *Rostkovia* have a growth habit similar to *Juncus*, but the cushion-plants, *Oxychloe*, *Patosia*, and *Distichia*, differ from other Juncaceae genera strikingly. They are found in damp areas at high altitudes along the line of the Andes,

often with limited distributions but frequently forming a significant component of the vegetation (Balslev 1996). The stems branch dichotomously, die back at the base, and have short, stiff cylindrical leaves arranged close together and at regular intervals along the stems, so that the tips of the younger leaves form the surface of the cushions.

Within Juncaceae there is much variation in the leaves, but the greatest variation is seen within *Juncus*, which may have bifacial or unifacial leaves, or with varying degrees of reduction of the adaxial surface (Cutler 1969). Leaf cross sections vary in shape and reveal that they may sometimes be hollow or filled with pith. Some species with hollow leaves possess septae that cross the hollow channels. Such characters have been important in the infrageneric classification of the genus (Buchenau 1906; Snogerup 1993; Balslev 1996; Kirschner et al. 2002a, b, c). Also considered important at the infrageneric level in *Juncus* is the presence or absence of small, membranous bracts subtending the flowers (Buchenau 1906; Snogerup 1993; Balslev 1996; Kirschner et al. 2002b, c). These bracts are typically present in Juncaceae, but are absent in the genus *Rostkovia* and in certain species of *Juncus*.

In some studies, Juncaceae have been included in the order Cyperales (e.g., Dahlgren et al. 1985; Givnish et al. 1999), which contains three other families, Cyperaceae, Prioniaceae, and Thurniaceae. Other studies (Angiosperm Phylogeny Group II 2003) have placed all four families in a broadly circumscribed Poales. Prioniaceae and Thurniaceae are small families each containing one small or possibly monospecific genus, *Thurnia* Hook. f. and *Prionium* E. Mey. *Thurnia* is found in Guyana and the Amazon basin, and *Prionium* in the Cape region of South Africa. Both are found in similar habitat types: nutrient-poor sandy soils in sandstone areas, in or at the margins of watercourses. Each genus has a number of autapomorphic characters, in partic-

ular in the leaf anatomy (Cutler 1963, 1969), but shares a basic floral structure with Juncaceae. Typical flowers of Juncaceae, Prioniaceae, and Thurniaceae consist of two whorls of three perianth parts, six stamens (rarely three), and gynoecium of three fused carpels. *Thurnia* was given the status of family by Engler (1907). *Pronium* has often been included within Juncaceae (e.g., Cutler 1969; Dahlgren et al. 1985; Simpson 1995), but was excluded by Munro and Linder (1998) who placed it in a new family Prioniaceae. A recent study (Chase et al. 2000) has assigned it to Thurniaceae. In recent cladistic studies, where both genera are included, *Pronium* and *Thurnia* are always sister to one another, but their position relative to Juncaceae varies. They have been nested within Juncaceae in morphological analyses (Simpson 1995; Stevenson and Loconte 1995), or as sister to Juncaceae and Cyperaceae in molecular analyses (Givnish et al. 1999). However, where only *Pronium* has been included, it is also usually sister to Juncaceae and Cyperaceae (Chase et al. 1993; Duvall et al. 1993; Plunkett et al. 1995; Muasya et al. 1998; Munro and Linder 1998) further supporting its basal position within the order.

Cyperaceae are the largest of the three families and are identified most easily by a floral structure, which is much reduced from that seen in Juncaceae or Thurniaceae. Most notably, the perianth is often absent, and when present, reduced to bristles or scales. The number of stamens is reduced to one to three and the number of ovules to one per ovary. The pollen of Cyperaceae are released as specialized types known as pseudomonads or *Mapania*-type (Simpson et al. 2003), which may be a reduced form of the tetrads in Juncaceae. Silica bodies are present in Cyperaceae but absent in Juncaceae. All these characters suggest that Cyperaceae are specialized with respect to Juncaceae and Thurniaceae.

In both morphological and molecular analyses Cyperaceae are sometimes nested within Juncaceae (Muasya et al. 1998; Munro and Linder 1998; Givnish et al. 1999), and sometimes sister to Juncaceae (Plunkett et al. 1995; Simpson 1995; Drábková et al. 2003), with the exception of *Oxychloe*. *Oxychloe* has usually been placed as sister to Cyperaceae or nested within Cyperaceae in molecular phylogenetic studies (Chase et al. 1993; Plunkett et al. 1995; Muasya et al. 1998; Munro and Linder 1998; Givnish et al. 1999; Drábková et al. 2003). It was suggested by Muasya et al. (1998) that this may be a result of contamination of the sample as, morphologically, the genus bears many similarities to *Patosia* and *Distichia* of Juncaceae. Recent work (Kristiansen et al. 2005) has indicated that *Oxychloe* is nested within Juncaceae. Moreover, Kristiansen et al. (2005) and Starr et al. (2007) presented evidence that one of the *Oxychloe* sequences previously used was a chimera, consisting of both Juncaceae and Cyperaceae DNA.

In contrast to Juncaceae, Cyperaceae have always been shown to be monophyletic, with the exception of the presence of *Oxychloe* sometimes nested within the clade (Duvall et al. 1993; Plunkett et al. 1995).

With the exception of Drábková et al. (2003), in all of these studies the emphasis was on groups other than Juncaceae, and it was usual for few species of Juncaceae to be included, often just a single species per genus. Drábková et al. (2003) does focus on Juncaceae and includes 58 taxa from the family. Some well-supported clades are revealed

within the family, indicating that the genus *Luzula* is monophyletic, and that the genus *Juncus* is not monophyletic, as well as indicating clades within *Juncus*. However, the relationships between many of these clades are unresolved.

This paper also focuses on Juncaceae, in order to investigate the nature of the relationships among Juncaceae, Cyperaceae, *Pronium*, and *Thurnia*, and among genera of Juncaceae. It also investigates the placement of *Oxychloe* in relation to genera of Cyperaceae and Juncaceae, as two new sequences of *Oxychloe andina* and one of *O. bisexualis* were included. We chose to sequence the plastid *rps16* intron, *trnL* intron, and *trnL*-F intergenic spacer (hereafter *trnL*-F) because they have proven to be useful for studies at similar taxonomic levels in other related groups (Hodkinson et al. 2002a, b; Simpson et al. 2003). The results demonstrate the phylogenetic utility of the DNA regions studied, and we are currently sequencing more species for future analyses.

MATERIALS AND METHODS

Specimens

Specimens were collected from Ecuador, Guyana, Ireland, and the United Kingdom, and from the living collections at the Royal Botanic Gardens, Kew, UK. Where living material was not available leaf samples were removed from herbarium specimens. Voucher specimens are listed in Table 1.

DNA Extraction

DNA was extracted from ca. 0.1 g of either herbarium material or freshly collected samples dried in silica gel (Table 1) using a modified 2× CTAB procedure (Doyle and Doyle 1987). The DNA was precipitated using isopropanol at -20°C for 2 wk, pelleted, and washed with 70% ethanol. The material was stored in TE buffer at -20°C, and cleaned using Concert PCR purification columns (Life Technologies, Paisley, Scotland).

PCR and Sequencing

The *trnL*-F region was amplified in either one part using primers "c" and "f" or in two parts using primers "c," "d," "e," and "f" described in Taberlet et al. (1991). The *rps16* region was amplified by the F and 2R primers (Oxelman et al. 1997). Thermal cycling (9700 thermal cycler, Applied Biosystems, Warrington, Cheshire, UK) comprised 30 or 35 cycles, with 1 min denaturation at 97°C, 1 min annealing at 50°C, an extension of 3 min at 72°C, and a final extension of 7 min at 72°C. Amplified DNA fragments were purified using Concert PCR purification columns (Life Technologies) following the protocols of the manufacturer. Sequencing was carried out using Big Dye Terminator Cycle Sequencing kits of Applied Biosystems with the same primers as the initial amplification. The reactions were run on an Applied Biosystems 310 Genetic Analyzer or 377 Automated DNA sequencer. Sequence editing and assembly was carried out using Autoassembler 2.1 software (Applied Biosystems).

Data Analysis

The DNA sequences were aligned by eye in PAUP* vers. 4.0b10 (Swofford 2002). Gaps were coded as missing data.

Table 1. Specimens used for molecular analysis and sequences obtained.

Species	Voucher	Collection location and date	Sequences	
			<i>trnL</i> -F	<i>rps16</i>
<i>Carex sylvatica</i> Huds.	Simpson 2667 (K)	Ireland 1999	Yes	Yes
<i>Chorizandra cymbaria</i> R. Br.	Wilson 9738 (NSW)	Australia 1997	Yes	Yes
<i>Cladium mariscus</i> (L.) Pohl	Simpson 2669 (TCD)	Ireland 1999	Yes	Yes
<i>Distichia acicularis</i> Balslev & Laegaard	Laegaard, Dhooge & Jones 21503 (TCD)	Ecuador 2001	Yes	Yes
<i>Distichia muscoides</i> Nees & Meyen	Laegaard, Dhooge & Jones 21476 (TCD)	Ecuador 2001	Yes	Yes
<i>Eriophorum angustifolium</i> Honck.	Simpson 2672 (TCD)	Ireland 1999	Yes	Yes
<i>Hypolytrum testui</i> Cherm.	Apemu 163 (K)		Yes	Yes
<i>Juncus acutus</i> L.	Jones 47 (TCD)	Ireland 2002	Yes	No
<i>Juncus arcticus</i> Willd.	Laegaard, Dhooge & Jones 21518 (TCD)	Ecuador 2001	Yes	Yes
<i>Juncus bufonius</i> L.	Hodkins 2 (TCD)	Ireland (cult.) 1999	Yes	Yes
<i>Juncus bulbosus</i> L.	Simpson s. n. 2002 (K)	Ireland 2002	Yes	No
<i>Juncus capillaceus</i> Lam.	Villagrán & Meza 1062 (SGO)	Chile 1981	Yes	No
<i>Juncus compressus</i> Jacq.	Hayden 1 (TCD)	Ireland 2003	Yes	No
<i>Juncus ecuadoriensis</i> Balslev	Laegaard, Dhooge & Jones 21498 (TCD)	Ecuador 2001	Yes	Yes
<i>Juncus effusus</i> L.	Simpson 2665 (TCD)	Ireland 1999	Yes	Yes
<i>Juncus ensifolius</i> Wikstr.	Jones 49 (TCD)	England 2003	Yes	No
<i>Juncus gerardii</i> Loisel.	Simpson 2668 (TCD)	Ireland 1999	Yes	Yes
<i>Juncus inflexus</i> L.	Hodkinson 13 (TCD)	Ireland (cult.) 1999	Yes	Yes
<i>Juncus maritimus</i> Lam.	Jones 45 (TCD)	Ireland 2002	Yes	No
<i>Juncus oxycarpus</i> E. Mey. ex Kunth	Muasya 2690 (K)	Kenya 1996	Yes	No
<i>Juncus planifolius</i> R. Br.	Jones 46 (TCD)	Ireland 2002	Yes	Yes
<i>Juncus sheuchzerioides</i> Gaudich.	McAdam s. n. 2002 (TCD)	Falkland Islands 2002	Yes	No
<i>Juncus squarrosus</i> L.	Jones 34 (TCD)	Scotland 2002	Yes	Yes
<i>Juncus stygius</i> L.	Chase 14363 (K)		Yes	No
<i>Juncus subulitepalus</i> Balslev	Laegaard, Dhooge & Jones 21499 (TCD)	Ecuador 2001	Yes	Yes
<i>Juncus trifidus</i> L.	Jones 40 (TCD)	Scotland 2002	Yes	Yes
<i>Luzula arcuata</i> Sw.	Jones 37 (TCD)	Scotland 2002	Yes	Yes
<i>Luzula campestris</i> (L.) DC.	Jones 35 (TCD)	Scotland 2002	Yes	Yes
<i>Luzula johnstonii</i> Buchenau	Muasya 2682 (K)	Kenya 1996	Yes	No
<i>Luzula luzuloides</i> (Lam.) Dandy & Wilmott	Hodkinson 11 (TCD)	Ireland (cult. 1999)	Yes	Yes
<i>Luzula multiflora</i> (Ehrh.) Lej.	Hodkinson 12 (TCD)	Ireland (cult.) 199	Yes	Yes
<i>Luzula nodulosa</i> E. Mey.	Bowen 3607 (RNG)	Turkey 1985	Yes	No
<i>Luzula spicata</i> (L.) DC.	Jones 36 (TCD)	Scotland 2002	Yes	Yes
<i>Luzula sylvatica</i> (Huds.) Gaudin	Davis 21564 (BM)	Turkey 1954	Yes	No
<i>Mapania cuspidata</i> (Miq.) Uittien	Marsh 4 (K)	England (cult.) 1996	Yes	Yes
<i>Mapania lorea</i> Uittien	Simpson 2663 (K)	Malaysia 1998	Yes	Yes
<i>Mapania mediterranea</i> D. A. Simpson	Simpson 2515 (K)	Malaysia 1998	Yes	Yes
<i>Mapania tenuiscapa</i> C. B. Clarke	Simpson 2661 (K)	Malaysia	Yes	Yes
<i>Marsippospermum grandiflorum</i> (L. f.) Hook. f. 1	Pisano 5951 (RNG)	Patagonia 1985	Yes	Yes
<i>Marsippospermum grandiflorum</i> 2	McAdam 4 (TCD)	Falkland Islands 2002	Yes	Yes
<i>Oxychloe andina</i> Phil. 1	Billiet & Jadin 5429 (BR)	Chile 1991	Yes	No
<i>Oxychloe andina</i> 2	Moreira & Muñoz 272 (SGO)	Chile 1996	Yes	No
<i>Oxychloe bisexualis</i> Kuntze	Muñoz et al. 3558 (SGO)	Chile 1995	Yes	No
<i>Prionium serratum</i> (L. f.) Drège	Muasya s. n. 1997 (K)	South Africa 1997	Yes	Yes
<i>Rhynchospora alba</i> (L.) Vahl	Simpson 2671 (TCD)	Ireland 2003	Yes	Yes
<i>Rostkovia magellanica</i> (Lam.) Hook. f. 1	Pisano 6048 (RNG)	Argentina 1985	Yes	Yes
<i>Rostkovia magellanica</i> 2	Laegaard, Dhooge & Jones 21516 (TCD)	Ecuador 2001	Yes	Yes
<i>Rostkovia magellanica</i> 3	Laegaard, Dhooge & Jones 21586 (TCD)	Ecuador 2001	Yes	Yes
<i>Scirpodendron bogneri</i> S. S. Hooper	Simpson 2560 (K)	Malaysia 1996	Yes	Yes
<i>Scirpodendron ghaeri</i> Merr.	Lye 34 (K)	Australia 1998	Yes	Yes
<i>Scirpoides holoschoenus</i> (L.) Soják	Parnell s. n. 1999 (TCD)	Hungary 1999	Yes	Yes
<i>Thurnia sphaerocephala</i> Hook. f.	Jones 2 (TCD)	Guyana 2001	Yes	Yes
<i>Typha minima</i> Funk ex Hoppe	Hodkinson 5 (TCD)	Ireland (cult.) 1999	Yes	Yes

The combined matrices were analyzed by maximum parsimony using heuristic search options with 1000 replicates of random stepwise addition, with tree-bisection-reconnection (TBR) swapping, saving no more than 50 trees per replicate. The decision to combine data from separate gene regions

was based on the knowledge that the plastid genome is uniparentally inherited and not recombining. Furthermore, no major conflict was seen between analyses based on the single gene regions. Branch support was assessed using 1000 bootstrap replicates (Felsenstein 1985) with simple addition se-

Fig. 1.—Parsimony tree of *trnL*–*F* and *rps16* sequence data for Cyperales, showing only Cyperaceae, Thurniaceae, and outgroup. One of 206 equally most-parsimonious trees of length 2537, CI = 0.64, RI = 0.92. Values above branches are number of steps. Bootstrap percentages ($\geq 50\%$) are below branches. No branches collapse in the strict consensus tree.

quence, saving no more than 50 trees per replicate, and TBR swapping. Groups with frequencies greater than 50% were retained in the final consensus tree. The trees were rooted on *Typha minima* as this has been shown to be closely related to Cyperales (Chase et al. 1993, Plunkett et al. 1995, Munro and Linder 1998, Givnish et al. 1999).

RESULTS

Analysis of Combined Data Sets

The combined *trnL*–*F* and *rps16* matrix was 2640 base pairs (bp) long, 336 bp of which was excluded due to difficulty in aligning highly variable regions. Of the included characters, 1143 were variable and 751 of these were potentially parsimony informative. Analysis produced four trees of length 2537, with a consistency index (CI) of 0.64, and a retention index (RI) of 0.82 (Fig. 1, 2).

A clade consisting of *Prionium* and *Thurnia*, with 100% bootstrap support (100 BP), was found to be sister to a monophyletic Juncaceae and Cyperaceae group (100 BP). Juncaceae and Cyperaceae were both found to be monophyletic (100 BP).

Within Juncaceae (Fig. 2), four main clades were identified. *Luzula* was monophyletic (100 BP) and sister to all other Juncaceae genera. Two separate clades consisting of species of *Juncus* were resolved (100 and 61 BP), labeled A and B in Fig. 2, and a fourth clade, C, consisting of all other genera of Juncaceae, which were included in this analysis (*Distichia*, *Marsippospermum*, *Oxychloe*, and *Rostkovia*), along with *Juncus acutus*, *J. maritimus*, and *J. planifolius* (77 BP). The relationships among these three clades are not clear, as the node placing clade A sister to clades B and C has no bootstrap support.

Within clade C, the position of *Juncus planifolius* as sister

Figure 1

Fig. 2.—Same tree as in Fig. 1 showing only Juncaceae. Numbers on branches as shown in Fig. 1. Arrows mark branches that collapse in the strict consensus.

to the two cushion-plant genera, *Oxychloe* and *Distichia*, was well supported (93 BP), as was the position of *Rostkovia* as sister to these (83 BP).

DISCUSSION

The trees clearly support the monophyly of Juncaceae including the genus *Oxychloe*, and the sister group relationship of Juncaceae with Cyperaceae (both 100 BP). This supports the findings of Kristiansen et al. (2005) who also demonstrated the occurrence of *Oxychloe* within Juncaceae using a different sample from ours, and contrasts with other previous molecular studies (Chase et al. 1993; Muasya et al. 1998; Munro and Linder 1998; Givnish et al. 1999), in which Juncaceae resolves as paraphyletic with respect to Cyperaceae. The monophyly of Cyperaceae is in agreement with previous studies (Chase et al. 1993; Plunkett et al. 1995; Simpson 1995; Muasya et al. 1998; Munro and Linder 1998; Givnish et al. 1999), and is supported by its specialized morphology. The nature of the relationship of *Prionium* and *Thurnia* with Cyperaceae and Juncaceae is also in agreement with previous studies based on molecular data (Chase et al. 1993; Duvall et al. 1993; Plunkett et al. 1995; Muasya et al. 1998; Munro and Linder 1998; Givnish et al. 1999), and it supports the inclusion of *Prionium* within Thurniaceae. Within Juncaceae, *Luzula* is monophyletic, and this is also well supported by morphological characters, as *Luzula* is distinct according to the presence of closed-leaf sheaths and multicellular hairs on the leaves.

With the exception of three species (*Juncus acutus*, *J. maritimus*, and *J. planifolius*), *Juncus* appears to be divided into two clades, A and B (Fig. 2). Clade A is strongly supported (100 BP). Buchenau (1906) placed the species of clade A in subgen. *Septati* Buchenau, although most subsequent authors have split this group further, in particular separating *J. ensifolius*, which has laterally compressed (ensiform) leaves. In the most recent revision, Kirschner et al. (2002b, c) placed these taxa in subgen. *Juncus* sects. *Iridifolii* S. Snogerup & Kirschner, *Ozyphyllum* Dumort., and *Stygiopsis* Gand. ex Kuntze. Clade A can be identified by the anatomy of the leaves, which are hollow and divided by septae. The arrangement of the septae differs in *J. ensifolius*, but septae of any form are absent from all other Juncaceae in this analysis. A similar clade to this one is resolved in Drábková et al. (2003), but which also contains a single non-septate species (*Juncus covillei* Piper), not included in this study.

Clade B represents *Juncus* subgen. *Agathyron* Raf. (Kirschner et al. 2002c), but it is not well supported (61 BP). However, a similar well-supported clade is resolved in Drábková et al. (2003).

The two *Juncus* clades A and B can also be distinguished from one another by the presence or absence of membranous bracts immediately subtending the flowers. This character has been used to divide *Juncus* into two broad groups by Buchenau (1906), and has also been used in subsequent classifications (Snogerup 1993; Balslev 1996; Kirschner et al. 2002b). The septate-leaved genera, clade A, are among those species that lack bracts subtending the inflorescence, while all the species in clade B possess them. With the exception

of some species of *Juncus*, and *Rostkovia*, this character is present throughout the family.

The genera *Distichia*, *Marsippospermum*, *Oxychloe*, and *Rostkovia* are all distinguished by having a single flower per culm, and these associate into clade C. The position of the three *Juncus* species also within this clade is surprising, as their floral morphology is closer to *Juncus* than to these genera. These three species do, however, differ from the *Juncus* clades A and B in some respects. Most notably, they lack bracts subtending the flowers, and they lack septae in the leaves. This combination of characters associates them morphologically with neither of the two *Juncus* clades, as clade A possesses septate leaves, and clade B possesses bracts subtending the flowers. In Kirschner's (2002b) classification these three species have been placed in *Juncus* subgen. *Juncus*; *J. acutus*, and *J. maritimus* in sect. *Juncus*, and *J. planifolius* in sect. *Graminifolii* Engelm.

Drábková et al. (2003) also recognized a clade consisting of all single-flowered Juncaceae genera, with the exception of *Oxychloe*. However, the sequences of *Oxychloe* were those used in previous studies, and may be contaminated (see Introduction). As was found here, the clade also contained some species of *Juncus* sect. *Graminifolii*, but the position of *Juncus* sect. *Juncus*, also placed in clade C in this study, was not resolved in Drábková et al. (2003).

In conclusion, the results support the placement of *Prionium* and *Thurnia* within a single family, separate from a Cyperaceae and Juncaceae clade, as well as the monophyly of Cyperaceae and Juncaceae. Within Juncaceae, this study is in broad agreement with the results of Drábková et al. (2003). The monophyly of *Luzula* is supported, but it appears that *Juncus* may be paraphyletic with respect to the single-flowered genera of Juncaceae. However, the exact status of these genera is not clear due to poor support of some of the deeper branches within Juncaceae. The association of *Juncus acutus*, *J. maritimus*, and *J. planifolius* with the single-flowered genera will be further investigated by inclusion of more species of *Juncus*. However, the morphology of the genus does show a degree of consistency with the molecular data, supporting in particular divisions within *Juncus* based on the presence/absence of septate leaves, and the presence/absence of bracts subtending the flowers.

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