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Revision of Juncaceae and Cyperaceae phylogeny based on cpDNA and nDNA

Master thesis

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Annotation

The phylogeny of Juncaceae and Cyperaceae is still not fully understood. The morphology of this group is intricate and without clear homology, and molecular relationships are mostly studied on smaller parts of this large group of Monocots. Proper study is therefore needed. To obtain the most objective view on the phylogeny of these two families, we gathered data on 1174 taxa of *rbc*L, *trn*L-*trn*F, and ITS and analysed them by maximum parsimony, maximum likelihood, and Bayesian inference. Markers of cpDNA appeared much more useful than nDNA (ITS) due to the high rate of mutations in ITS which led to homoplasy and unsure alignment. By considering taxonomical impact of our study, the monophyly of the families and main inner topology of Juncaceae and several tribes of Cyperaceae (Abildgaardieae, Bisboeckelereae, Cariceae, Cypereae, Cryptangieae, Eleocharideae, Rhynchosporeae, Sclerieae, and Trilepideae) were confirmed; some changes in taxonomy were suggested (uniting of Chrysitricheae and Hypolytreae; division of Fuireneae; uniting of Cariceae, Dulicheae, Scirpeae, and *Khaosokia caricoides* into one tribe, or division of Scirpeae; and separation of Cladieae); changes in the classification of certain taxa were also suggested (the transfer of *Distichia, Marsippospermim, Oxychloë, Patosia*, and *Rostkovia* into *Juncus,* or division of *Juncus* into more genera; the transfer of *Nemum spadiceum* into *Bulbostylis; Schoenoplectus corymbosus, S. gemmifer, S. hondoensis*, and *S. multisetus* into the genus *Schoenoplectiella;* and *Oreobolopsis* into *Trichophorum*); and the identification of some taxa which possess special combinations of molecular and morphological features and should be studied further was made (*Juncus capitatus, J. dregeanus, Bulbostylis juncoides, Crosslandia setifolia, Schoenoplectus litoralis, S. americanus, Cyperus iria,* and *Amphiscirpus nevadensis*).

Key words: phylogeny, Cyperaceae, Juncaceae, cpDNA, nDNA

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Contents

Introduction

Insight into phylogeny of Juncaceae and Cyperaceae

Juncaceae and Cyperaceae are traditionally recognised families within the order Poales, which are mainly distinguished by the shape of culm, the presence of silica bodies, and the morphologies of flowers and the inflorescence.

Since the time that molecular analyses have become an important support for phylogeny based on morphology, views on these two families have started changing. Originally (i.e. based on morphology, anatomy, karyology, and chemical data only), the *Thurnia* and *Prionium* genera belonged to the Juncaceae family (Rudall et al., 1999, Simpson, 1995). The view on this classification changed when molecular data were first combined with morphological information (Munro and Linder, 1998); *Prionium* was settled on basis of Juncaceae and Cyperaceae, but monophyly of the two families was not statistically supported. Strong support was provided purely from molecular analyses, which suggested the position of *Thurnia* and *Prionium* (i.e. recent family Thurniaceae) on the basis of the monophyletic sister clades of Juncaceae and Cyperaceae (Bouchenak-Khelladi et al., Chase et al, 2000, Davis et al., 2004, 2014, Givnish et al., 1999, Plunkett et al., 1995). Nowadays, it is known that the group consisting of Thurniaceae, Juncaceae and Cyperaceae is strongly monophyletic and it definitely belongs to the order Poales; its placement within the order is, however, unclear (Bouchenak-Khelladi et al., 2014, Byng et al., 2016, Davis et al., 2004, Givnish et al., 2006, Janssen and Bremer, 2004).

Although the monophyly of these families is virtually doubtless, the use of different markers, different taxa, and different approaches clearly showed that the view on phylogeny within this group might vary and that molecular analyses can reveal relationships hidden behind unclear homology of morphology characteristics (Givnish et al., 1999, Plunkett et al., 1995, Simpson, 1995). This is clearly seen from a variety of studies concerning Juncaceae and Cyperaceae. Some authors studied the relationships within both Juncaceae and Cyperaceae on only a narrow sample of taxa (10 – 22 taxa) and mostly by rather unvariable markers (Simpson, 1995, Plunkett et al., 1995, Givnish et al., 1999). Other studies, despite larger sampling or better markers, focused only on the topology at the family-level and did not discus connections within families (Chase et al, 2000, Davis et al., 2004, Janssen and Bremer, 2004, Bouchenak-Khelladi et al., 2014, Byng et al., 2016). Inner relationships were discussed in studies specialising on only one or the other family and never on both families together. No study, however, focused on the detailed view of the relationships between the two families together with an inside look into the families. Confirmation (or refutation) of the current view on complex phylogeny of Juncaceae and Cyperaceae is therefore needed, and as a large amount of molecular data are available on GeneBank, this can easily be performed by compiling this.

Juncaceae Juss.

Juncaceae is a family traditionally defined by a rounded stem; culm bundles in rings; lack of SiO_2 bodies (just SiO_2 sand in *Juncus*); terete-unifacial or isobifacial leaves; pollen grains central in loculus; shortly stipitate G; basal ovule; often bistomal micropyle; seed with exotesta and endotegmen; and helobial endosperm (Kirschner, 2002a).

As previously described, the family Juncaceae is now believed to be monophyletic. Several authors opposed this view through their molecular studies which suggested the exclusion of *Oxychloë andina* and its placement inside Cyperaceae (Duvall et al., 1993, Plunkett et al., 1995), or as a sister clade to Cyperaceae (Muasya et al., 1998, 2000). These new positions of *Oxychloë*, which were inconsistent with the morphology of the species, were later rejected by new findings showing that these sequences were wrong (Drábková and Vlček, 2007, Kristiansen et al., 2005).

In summary, currently Juncaceae is composed of 8 genera and 464 species (Christenhusz and Byng, 2016). The largest genera (*Juncus* and *Luzula*) are virtually cosmopolitan; just in the tropics they are restricted to the alpine zone. *Luzula* is thought to be monophyletic (Drábková et al., 2003, 2004, 2006, Jones et al., 2007, Roalson, 2005, Záveská Drábková and Vlček, 2009), while *Juncus* is clearly paraphyletic, since its clade also contains a branch of five small southern-hemisphere genera: *Rostkovia*, *Marsippospermum*, *Oxychloë*, *Distichia*, and *Patosia* (Drábková et al., 2003, 2004, 2006, Drábková and Vlček, 2007, Záveská Drábková and Vlček, 2009). All of the five genera are found in South America, *Marsippospermum* and *Rostkovia* also grow in New Zealand, with the latter also found on Tristan da Cunha and the South Pacific Islands. The newly-described genus *Oreojuncus* (previously belonging to the genus *Juncus*, subg. *Agathryon*, sect. *Steirochloa*) is, on the contrary, strictly bound to the northern hemisphere (Kirschner, 2002b, Záveská Drábková and Kirschner, 2013).

Considering the two larger genera in detail, *Luzula* is morphologically defined as a rhizomatous plant with sparsely to densely ciliate leaves; the inflorescence is usually many-flowered, either cymose with flowers borne singly and pseudo-paniculate, or racemose

(Kirschner, 2002a). Based on further morphological characteristics, *Luzula* divides into three subgenera – *Marlenia, Luzula* and *Pterodes*. *Marlenia* is a monotypic group; subgenus *Luzula,* on the other hand, divides into seven sections (*Anthelaea, Atlanticeae, Nodulosae, Diprophyllateae, Alpinae, Thyrsanochlamydeae,* and *Luzula*; Kirschner, 2002a). Nevertheless, molecular analyses showed that traditional subgenera and sections cannot be confirmed. Species of subgenus *Pterodes* were found dispersed in the subgenus *Luzula* (Drábková et al., 2004, 2006, Záveská Drábková and Vlček, 2009, 2010), or it creates one monophyletic clade within the subgenus *Luzula* (except *L. pilosa*; Záveská Drábková and Vlček, 2010). Annual species *Luzula elegans,* belonging to the monotypic subgenus *Marlenia,* was found on the base of genus *Luzula* in most analyses (Drábková et al., 2004, 2006, Záveská Drábková, 2010, Záveská Drábková and Vlček, 2010).

Genus *Juncus* is traditionally divided into two subgenera (*Juncus* and *Agathryon*) which both are divided into a few sections. Subgenus *Juncus* contains sections *Juncus, Graminifolii, Caespitosi, Stygiopsis, Iridifolii,* and *Ozophyllum*. Subgenus *Agathryon* has four sections – *Tenageia, Steirochloa, Juncotypus,* and *Forskalina* (Kirschner, 2002b). Molecular studies, however, showed that this division is unsupported. In the study by Drábková et al. (2003) based on the cpDNA marker *rbc*L, only subgenus *Agathryon* seemed to be monophyletic, but all later studies, based on cpDNA, mDNA and nDNA, show non-monophyly of the genus *Juncus* and both subgenera (Drábková et al., 2006, Záveská Drábková, 2010, Záveská Drábková and Vlček, 2009).

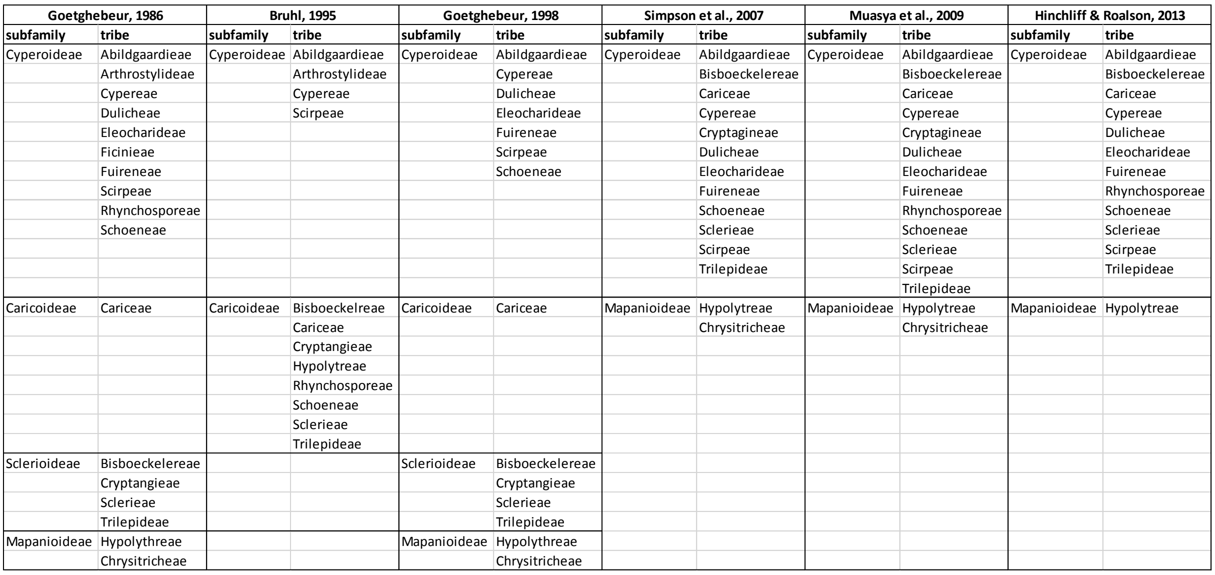
Finally, the genus *Oreojuncus* is thought to be monophyletic and most likely placed on the basis of Juncaceae (Záveská Drábková and Kirschner, 2013). This result was supported by morphology (the lacerate-fimbriate auricles, the serrulate leaf margin, and the mucronate connectives), sequence data of nDNA, cDNA and mDNA (*rbc*L, *trn*L, *trn*L-F, ITS, *rps*16, and *atp*1), chromosome data, and also by host-pathogen interactions (Záveská Drábková and Kirschner, 2013).

Cyperaceae Juss.

The larger family Cyperaceae is defined by angled stems; leaves have a closed sheath; inflorescence units are spikelets or heads, axis terminating in a spikelet; pollen as pseudomonads (Selling, 1947); gynoecium initiated as an annular primordium; ovule one/flower, basal; fruit an achene; exotesta with SiO_2 bodies; and endosperm with micropylar and chalazal haustoria (Goetghebeur, 1998). Cyperaceae is almost cosmopolitan and contains 92 genera and around 5500 species (Christenhusz and Byng, 2016). More than a half of the genera are monotypic or have at most ten species. The largest genera of the family are *Cyperus* with 780 species, and *Carex* with 2100 species (Goetghebeur, 1998).

As described above, Cyperaceae are treated as a monophyletic family, sister to Juncaceae. Inner relationships were based on morphological and molecular data, but results differ among authors. Bruhl (1995) divided Cyperaceae into two subfamilies, whereas Goetghebeur (1998) recognised four subfamilies (Tab. 1). The latest studies of the whole family split Cyperaceae into two subfamilies – Mapanioideae and Cyperoideae (Muasya et al., 2009a, Simpson et al., 2003, 2007). This division was also suggested by some previous studies (Bruhl, 1995, Muasya et al., 1998, 2000), and is supported by the unique floral anatomy of Mapanioideae.

Tab. 1: Review of phylogenetic classification of Cyperaceae according to different authors.



Goetghebeur, 1986		Bruhl, 1995		Goetghebeur, 1998		Simpson et al., 2007		Muasya et al., 2009		Hinchliff & Roalson, 2013	
subfamily	**tribe**	**subfamily**	**tribe**	**subfamily**	**tribe**	**subfamily**	**tribe**	**subfamily**	**tribe**	**subfamily**	**tribe**
Cyperoideae	Abildgaardieae	Cyperoideae	Abildgaardieae	Cyperoideae	Abildgaardieae	Cyperoideae	Abildgaardieae	Cyperoideae	Abildgaardieae	Cyperoideae	Abildgaardieae
	Arthrostylideae		Arthrostylideae		Cypereae		Bisboeckelereae		Bisboeckelereae		Bisboeckelereae
	Cypereae		Cypereae		Dulicheae		Cariceae		Cariceae		Cariceae
	Dulicheae		Scirpeae		Eleocharideae		Cypereae		Cypereae		Cypereae
	Eleocharideae				Fuireneae		Cryptagineae		Cryptagineae		Dulicheae
	Ficinieae				Scirpeae		Dulicheae		Dulicheae		Eleocharideae
	Fuireneae				Schoeneae		Eleocharideae		Eleocharideae		Fuireneae
	Scirpeae						Fuireneae		Fuireneae		Rhynchosporeae
	Rhynchosporeae						Schoeneae		Rhynchosporeae		Schoeneae
	Schoeneae						Sclerieae		Schoeneae		Sclerieae
							Scirpeae		Sclerieae		Scirpeae
							Trilepideae		Scirpeae		Trilepideae
									Trilepideae		
Caricoideae	Cariceae	Caricoideae	Bisboeckelreae	Caricoideae	Cariceae	Mapanioideae	Hypolytreae	Mapanioideae	Hypolytreae	Mapanioideae	Hypolytreae
			Cariceae				Chrysitricheae		Chrysitricheae		
			Cryptangieae								
			Hypolytreae								
			Rhynchosporeae								
			Schoeneae								
			Sclerieae								
			Trilepideae								
Sclerioideae	Bisboeckelereae			Sclerioideae	Bisboeckelereae						
	Cryptangieae				Cryptangieae						
	Sclerieae				Sclerieae						
	Trilepideae				Trilepideae						
Mapanioideae	Hypolythreae			Mapanioideae	Hypolythreae						
	Chrysitricheae				Chrysitricheae						

Finer segmentation into tribes is even more complicated and still ongoing (Tab. 1). Goethebeur (1998), for example, extended Scirpeae (recognised by Bruhl, 1995) into 5 new tribes and newly-defined Chrysitricheae. Simpson et al. (2007), on the other hand, erased two formerly recognised tribes, and similar changes were made by Muasya et al. (2009) and Hinchliff and Roalson (2013) (Tab. 1).

Mapanioideae

Mapanioideae, which is the smaller of the two subfamilies of Cyperaceae, is a group of tropical and southern-hemisphere taxa. The subfamily is defined by the lateral bisexual flowers provided with a lateral pair of larger, keeled, and laminar hypogynous scales.

Mapanioideae is treated undivided with only one tribe (Hypolytreae; Bruhl, 1995, Hinchliff and Roalson, 2013), or it is divided into two tribes (Hypolytreae and Chrysitricheae; Goetghebeur, 1998, Simpson et al., 2007). This division into two tribes depends mainly on morphology and geographical distribution; Hypolytreae are found in the tropics and are defined by a many-spikelet inflorescence, a poorly differentiated embryo, and special *Mapania*-type pollen, while Chrysitricheae has a relict Gondwanan distribution, reduced vegetative apparatus and inflorescence, a highly differentiated embryo, and pseudomonad pollen (Goetghebeur, 1998, Simpson et al., 2003).

Although circumscription of the tribes is clear on the basis of morphology, molecular data suggested other evolutional pathways in the subfamily and do not support the existence of two tribes (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007). Despite this unstable division, the composition of genera in Mapanioideae is relatively stable; only the genus *Hellmuthia* (Chrysitricheae), traditionaly belonging to this clade, is definitely placed outside Mapanioideae (e.g., Bruhl, 1995, Goetghebeur, 1998, Haines and Lye, 1976, Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 1998, 2000, 2001, Simpson et al., 2003). The suggestion is that it belongs to Cypereae (Simpson et al., 2007).

Cyperoideae

Cyperoideae, in contrast to Mapanioideae, is a large group containing 12 (or 13, depending on the particular author, see Tab. 1) tribes. Species belonging to the group do not have the mapanioid hypogynous scales, but no other characteristics can be defined for this highly diverse group. Actually, even morphological delimitation of the tribes is complicated due to high diversity and uncertain homology; embryological characteristics, however, can be effectively used for such division (Goetghebeur, 1998).

Despite traditional morphological delimitation of the tribes, molecular analyses are often used as the final main marker. The trajectory of evolution of the classification of Cyperaceae is illustrated in Tab. 1 where first three classifications are based on morphology and last three are based on molecular data. The recent view on the topology of Cyperaceae, according to molecular data, is as follows: monophyletic Trilepideae stands on the basis of Cyperoideae (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007); Sclerieae and Bisboeckelerieae are collapsed into one group as sister tribes (Muasya et al., 2009a, Simpson et al., 2007), or Sclerieae is inserted inside Bisboeckelereae (Hinchliff and Roalson, 2013). The position of Cryptangieae is unclear as it stays on the basis of Schoeneae (Muasya et al., 2009a), or inside Schoeneae (Hinchliff and Roalson, 2013, Simpson et al., 2007). Schoeneae is, on the other hand, paraphyletic in all publications (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007).

The next compact branch is composed of the genus *Khaosokia*, the monophyletic Cariceae, the polyphyletic Scirpeae, and the monophyletic Dulicheae (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007).

The last branch is composed of monophyletic Eleocharideae and Abildgaardieae, and an inconsistent part of Fuireneae, and the clade is terminated by a monophyletic large group of Cypereae. Although the tribe Rhynchosporeae is not accepted by all authors, the clade of Rhynchosporeae is always monophyletic and separate (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Simpson et al., 2007).

To sum this up, molecular phylogeny does not completely copy the morphological division of the tribes. Consistently monophyletic tribes are Trilepideae, Rhynchosporeae, and Cypereae. Some tribes are monophyletic, but they are nested inside other tribes – these are Abildgaardieae, Cryptangieae, Sclerieae, Bisboeckelereae, Cariceae, Dulicheae, and Eleocharideae. There are also some genera which create their own monophyletic clades – *Cladium* and *Khaosokia*. Finally, some tribes are clearly non-monophyletic – Schoeneae, Scirpeae, and Fuireneae (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Simpson et al., 2007).

Tribes of Cyperaceae

These few studies focused on the phylogeny of the whole family, but did not distinguish finer relationships inside tribes and between genera. These relationships were studied in only a few larger tribes and are described below.

Abildgaardieae

The Abildgaardieae tribe has only 8 genera, but with ca. 420 species is one of the larger ones. The taxa have clearly differentiated and an often thickened style base. Distichous glumes and moniliform stigmatic hairs can be found in part of the species; three types (*Abildgaardia*-type, *Bulbostylis*-type, and *Fimbristylis*-type) of embryos are present.

The tribe, as defined by morphological characteristics, was slightly enlarged on the basis of molecular data; Muasya et al. (2009a) and Simpson et al. (2007) added a few species of Schoeneae to Abildgaardieae to keep the monophyly of the clade. In all following studies, Abildgaardieae is monophyletic (Hinchliff and Roalson, 2013, Jung and Choi, 2013); it is placed on the basis of a large clade comprising Abildgaardieae + Fuireneae + Eleocharideae +

Cypereae (Simpson et al., 2007), or it clusters with Eleocharideae on the basis of the clade (Hinchliff and Roalson, 2013), or it is inserted inside the clade (Muasya et al., 2009a, Jung and Choi, 2013).

Topology within this tribe was reconstructed by Ghamkhar et al. (2007) who definitely placed Arthrostylideae (formerly recognised tribe of *Arthrostylis* and *Actinoschoenus;* Goetghebeur, 1986, Bruhl, 1995) on the base of Abildgaardieae, and indicated *Bulbostylis* as the only monophyletic genus in the tribe; genus *Abildgaardia* is paraphyletic due to *A. vaginata* which clusters with *Crosslandia* and supports paraphyly of the genus *Fimbristilis* (Ghamkhar et al., 2007). The final adjoining of *Abildgaardia* to the genus *Fimbristylis* was suggested by Govaerts et al. (2007), and it was supported by the morphology of the styles (Reutemann et al., 2012).

Cariceae

The Cariceae group is the largest Cyperaceae tribe – it contains five genera with ca. 2,200 species. It is characterised by unisexual flowers, female flowers completely enclosed by a fertile urticuliform spikelet prophyll, the absence of perianth, and the *Carex*-type embryo (rarely the *Schoenus*-type embryo; Goetghebeur, 1995). The tribe has been recognised for a long time; Goetghebeur (1986, 1995) even placed Cariceae into a separate subfamily. Molecular data also clearly support monophyly, but it is not always separate as per the study by Muasya et al. (2009). In the studies by Simpson et al. (2005, 2007), Yano et al. (2012), Gilmour et al. (2013), Hinchliff and Roalson (2013), Jung and Choi (2013), and Leveille-Bourret et al. (2014), the clade is placed inside branches of Scirpeae and Dulicheae.

As the tribe was studied properly, we can look deeper on the level of genera. The genus *Schoenoxiphium* creates a monophyletic clade on the basis of Cariceae (Yen and Olmstead, 2000), or is placed inside the genus *Carex* nearby unispicate species of *Carex* subg. *Primocarex* (Leveille-Bourret et al., 2014, Roalson et al., 2001, Starr and Ford, 2009, Starr et al., 2004, 2008, Waterway and Starr, 2007). The genera *Kobresia*, *Uncinia,* and *Cymophyllus* are placed in the same clade of unispicate species of *Carex*; genus *Kobresia* is probably paraphyletic (Leveille-Bourret et al., 2014, Starr and Ford, 2009, Starr et al., 2004, Starr et al., 2008, Waterway and Starr, 2007, Yen and Olmstead, 2000); only in study by Roalson et al. (2001) does it seems to be monophyletic. As *Schoenoxiphium* and *Kobresia* are morphologically close, some authors suggest merging these two genera (Kern, 1958, Koyama, 1961, Starr et al., 2004, Yen and Olmstead, 2000). However, molecular analyses show that these two genera should not be merged by virtue of their unnaturality (Leveille-Bourret et al., 2014, Starr and Ford, 2009, Starr et al., 2008, Waterway and Starr, 2007). The genus *Uncinia* is, on the other hand, clearly monophyletic (Leveille-Bourret et al., 2014, Roalson et al., 2001, Starr and Ford, 2009, Starr et al., 2004, Starr et al., 2008, Waterway and Starr, 2007, Yen and Olmstead, 2000). The monotypic genus *Cymophyllus* is placed inside this clade of unispicate species (Roalson et al., 2001, Starr and Ford, 2009, Starr et al., 2004, 2008, Waterway and Starr, 2007, Yen and Olmstead, 2000) which led some authors to suggest its shift into the genus *Carex* (Starr and Ford, 2009).

Concerning the inner relationships, genus *Carex* has four subgenera: *Primocarex* (or *Psyllophora*), *Indocarex* (or *Vigneastra*), *Carex* and *Vignea*. In the light of recent studies, it seems that subgenera *Primocarex*, *Indocarex* and *Carex* are completely unnatural. The most reduced subgenus (*Primocarex*) is spread through the whole Cariceae, and the groups *Indocarex* and *Carex* are situated in one homogenous clade. The subgenus *Vignea* has its own clade with some added species from *Primocarex* (Leveille-Bourret et al., 2014, Roalson et al., 2001, Starr and Ford, 2009, Starr et al., 2004, 2008, Waterway and Starr, 2007, Yen and Olmstead, 2000).

Cypereae

The second biggest tribe of Cyperaceae – Cypereae – is recently composed of 9 genera and ca. 900 species and is defined by the style base which is not distinct and not thickened, but sometimes is persistent as a narrow beak. Other common characteristics are distichous glumes and in some genera reduction of the spikelet. The perianth is reduced completely, and the taxa have *Cyperus*- or *Ficinia*-type embryos. (Goetghebeur, 1998).

Molecular data supported monophyly of this tribe (Glon et al., 2017, Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007) after *Hellmuthia* (Chrysitricheae) and two species of *Scirpus* (recently *Dracoscirpoides*; Scirpeae) were placed in Cypereae (Muasya et al., 2009a). The only exception from monophyly appeared in studies by Jung and Choi (2013) and Yano et al. (2012) due to genus *Eriophorum comosum* and *E. microstachyum* placed on the basis of *Ficinia* clade. Yano et al. (2012), therefore, suggested replacement of this taxa into Cypereae, *Ficinia* clade (as a separate genus *Erioscirpus;* Scirpeae). Also, further inner relationships are complicated. Firstly, Muasya et al. (2009b) described two clades: *Cyperus* Clade (*Alinula, Androtrichum, Ascolepis, Courtoisina, Cyperus, Kyllinga, Kyllingiella, Lipocarpha, Oxycaryum, Pycreus, Queenslandiella, Remirea, Rikliella, Sphaerocyperus*, and *Volkiella*) and *Ficinia* Clade (*Dracoscirpoides*, *Hellmuthia*, *Isolepis*, *Ficinia*, and *Scirpoides*).

Classification of genera of the *Cyperus* Clade was studied Muasya et al. (2001, 2002, 2014), Larridon et al. (2013, 2014), and Beuters et al. (2014); they transferred certain genera to the genus *Cyperus,* and reclassified individual clades into sections of the genus Cyperus. Nomenclature is summarized by Huygh et al. (2010) Larridon et al. (2011, 2014), and Reynders et al. (2011). Interestingly, in the Cypereae, C4 photosynthesis arose once, followed by relatively quick speciation (Bauters et al., 2014, Larridon et al., 2013, Muasya et al., 2002, 2014). This division of the clade define two subgenera of *Cyperus*: *Anosporum* (C3) and *Cyperus* (C4).

Resolution of the relationships in the *Ficinia* clade was studied by Muasya; the first analyses showed only that the genera *Ficinia* and *Isolepis* are nonmonophyletic (Muasya and de Lange, 2010, Muasya et al., 2001, 2009a), but the last analysis showed monophyletic *Scirpoides* in the basal position of the clade, followed by monophyletic *Dracoscirpoides* and *Hellmuthia*. *Isolepis*, then, is divided into more clades and monophyletic *Ficinia* terminates the clade (Muasya et al., 2014). The monotypic genus *Desmoschoenus* was placed inside *Ficinia* (Muasya and de Lange, 2010).

Dulicheae

One of the small tribes, Dulicheae, is delimitated by the presence of a fertile spikelet prophyll, bearing a bisexual flower, and by *Carex*-type embryo. Since the group is very small containing just three small genera (*Dulichum*, *Blysmus,* and *Blysmopsis*), the inner topology is not substantial and the main focus is on the position of the whole tribe within Cyperaceae. Molecular data suggest, that besides its unstable position in the clade of Dulicheae+Scirpeae+Cariceae, the tribe is monophyletic (Gilmour et al., 2013, Hinchliff and Roalson, 2013, Jung and Choi, 2013, Leveille-Bourret et al., 2014, Muasya et al., 1998, 2009a, Simpson et al., 2005, 2007, Yano et al., 2012).

Eleocharideae

The morphological keystone of the Eleocharideae (ca. 200 species) lies in the reduction of the vegetative apparatus, the fixed unispiculate inflorescence, the unique embryo morphology, and specific helio- and helophylous ecology. Monophyly of this tribe was confirmed by molecular data without any doubt (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Simpson et al., 2007), and it is most probable that it is very closely related to Abildgaardieae (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a). Originally, the tribe contained three genera (*Chillania*, *Eleocharis*, and

Websteria); the two smaller ones (i.e. *Chillania* and *Websteria*) were transferred to the large genus *Eleocharis* on basis of molecular data (Hinchliff et al., 2010). Intrageneric relations studied Hinchliff et al. (2010) who confirmed subg. *Limnochloa* as a basal group to all other *Eleocharis* (subg. *Eleocharis*).

Fuireneae

Fuireneae, as defined by Goetghebeur (1998), is highly similar to the tribe Scirpeae (it means that it generally has spikelets with spirally-arranged glumes and flowers with hypogynous scales), but individual genera possess specialized structures; from Scirpeae are, however, clearly differenciated by *Schoenoplectus*- and *Bolboschoenus*-type embryos. The tribe Fuireneae is so unspecific that it is not even recognised by some authors – Bruhl (1995) followed by Muasya et al. (1998) transfer Fuireneae into Scirpeae. Other authors who recognise Fuireneae, however, agree with the opinion that Fuireneae are non-monophyletic. Fuireneae are mixed with monophyletic Eleocharideae (Simpson et al., 2007), or with Eleocharideae and Abildgaardieae (Muasya et al., 2009a), and creates few clades on the basis of Cypereae (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007). In the study by Glon et al. (2017) even Cypereae was inserted inside Fuireneae.

One member of Cypereae, *Isolepis humillima*, repeatedly appeared among species of Fuireneae (Muasya et al., 2001, 2002, 2009a, Simpson et al., 2007), and in few recent studies, it was definitely transferred to Fuireneae (Hinchliff and Roalson, 2013, Shiels et al., 2014) and finally placed in the genus *Schoenoplectiella* (*Schoenoplectiella humillima* (Benth.) Shiels, Glon, & Monfils, comb. nov.). Relations between genera of Fuireneae (*Bolboschoenus, Pseudoschoenus, Actinoscirpus, Fuirena, Schoenoplectus,* and *Schoenoplectiella*) were for long time rather unclear. Shiels et al. (2014) shed light on part of this problematics: They found that monophyletic *Bolboschoenus* creates its own clade, and that *Schoenoplectiella* and *Schoenoplectus* are natural, non-sister genera, both of them consisting of two monophyletic sections. *Actinoscirpus* is most probably related with *Schoenoplectus*, while *Pseudoschoenus* is closest to *Schoenoplectiella* (Shiels et al., 2014). This result was supported in the recent study by Glon et al. (2017); this study, moreover, support separate position of *Fuirena*.

Rhynchosporeae

The tribe Rhynchosporeae was originally distinguished on the basis of a distinct style base (Goetghebeur, 1986, Bruhl, 1995, Muasya et al., 2009). Through time, however, was the tribe rejected (Goetghebeur, 1998, Simpson et al., 2007) and reinstated again (Muasya et al., 2009, Hinchliff and Roalson, 2013). To complicate the classification of the tribe, the delimitation of genera within Rhynchosporeae is inconsistent – Grisebach (1864) recognised only the genus *Rhynchospora*, while Nees von Esenbeck (1835) distinguished 13 small genera. The recent view on the tribe varies between one genus (*Rhynchospora*) or two genera (*Pleurostachys* and *Rhynchospora*), in total ca. 300 species (Goetghebeur, 1998). As for the finer division of the tribe, Kükenthal (1949a, b) divided *Rhynchospora* into two subgenera (subg. *Diplostyleae* and subg. *Haplostyleae*) and 29 sections, whereas *Pleurostachys* was divided into seven sections.

According to molecular data, the tribe Rhynchosporeae seems to be monophyletic (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Simpson et al., 2007), but division into the subgenera suggested by Kükenthal was not confirmed because the two main branches only roughly copy the subgenera. Neither sections appeared monophyletic. As *Pleurostachys* is placed deep inside the genus *Rhynchospora*, Thomas et al. (2009) suggested that the two genera be merged into *Rhynchospora*, otherwise the genus *Rhynchospora* must be divided into more genera.

Schoeneae

Traditional Schoeneae, one of the largest tribes of Cyperaceae (ca. 700 species; Bruhl, 1995, Goetghebeur, 1998) is defined by a low number of bisexual flowers per spikelet, an often well-developed perianth, and more types of embryo (*Carex*-type, *Schoenus*-type, *Helothrix*-type, *Carpha*-type, and *Juncus*-type; Goetghebeur, 1998). As this group is very diverse and contains a high number of genera, Goetghebeur (1998) proposed its finer division, which is an idea supported by molecular data. These suggested that Schoeneae is paraphyletic due to separate clade of monophyletic genus *Cladium*, separate *Gymnoschoenus* (but with low support), and a few species recently belonging to Abildgaardieae (Muasya et al., 2009a, Simpson et al., 2007). Exclusion of *Cladium* and transfer of the problematic species to Abildgaardieae resulted in a monophyletic Schoeneae, as introduced by Hinchliff and Roalson (2013). *Gymnoschoenus* is still a problematic taxon mostly appearing separately (Verboom, 2006, Zhang et al., 2004), sometimes inside Schoeneae (Zhang et al., 2004) but always with low support.

Inner relationships between the rest of the monophyletic Schoeneae are also complicated. Zhang et al. (2004, 2007) clearly showed that monophyletic *Carpha* and *Trianoptiles* are sisters, *Gahnia* is probably sister to *Ptilothrix* + *Cyathochaeta*, *Schoenoides* probably belongs to *Oreobolus*. Relations among all other genera were, however, unclear. Verboom (2006) confirmed the relationship between *Carpha* and *Trianoptiles* and suggested a definition of some mostly consistent clades within Schoeneae, but the structuring was still not well-resolved and it was ambiguous. Only the paraphyly of the genera *Tetraria* and *Costularia* is quite definite (Zhang et al., 2004, Verboom, 2006). All in all, the problematic of Schoeneae is complicated and not yet well understood.

Scirpeae

Scirpeae contains seven genera and 200 species; morphologically, the group is defined by spikelets with spirally arranged glumes and flowers with hypogynous scales. Embryology is variable, as three types of embryo are present: *Carex*-type, *Schoenus*-type, and *Fimbristylis*-type (Goetghebeur, 1998).

Although the presented diagnostic characters are thought to be clearly plesiomorphic (Goetghebeur, 1998), the tribe Scirpeae is clearly paraphyletic according to molecular studies; the only study which presented Scirpeae as monophyletic is Hinchliff and Roalson (2013). Topology of Scirpeae is complicated and weakly supported, and the species are scattered across many clades (Gilmour et al., 2013, Jung and Choi, 2013, Leveille-Bourret et al., 2014, Muasya et al., 1998, 2009a, Simpson et al., 2005, 2007, Yano et al., 2012,). Simpson et al. (2005, 2007) divided the tribe into two clades due to closely-related Dulicheae and Cariceae; moreover, two species of *Trichophorum* were placed separately in this study. Similar result appeared in the study by Muasya et al. (2009a) where Scirpeae are divided into 2 clades, because the tribe Dulicheae is inserted inside the Scirpeae. Yano et al. (2012), Gilmour et al. (2013), and Jung and Choi (2013) also show strong relation between Dulicheae, Scirpeae and Cariceae which is inserted inside Scirpeae. A more detailed study was provided by Leveille-Bourret et al. (2014) who found that Dulicheae stays on the basis of the whole clade, but Scirpeae are divided because of the inserted monophyletic clade of Cariceae.

When considering the composition of genera, Yano et al. (2012) placed two species of *Eriophorum* outside this clade, and Jung and Choi (2013) found *Eriophorum comosum* (now *Erioscirpus comosus*) in the Cypereae. Leveille-Bourret et al. (2014) revealed that the only monophyletic genera of the tribe are *Calliscirpus* and *Eriophorum*; genus *Scirpus* is divided into several clades, and *Zameioscirpus*, *Amphiscirpus* (which create one monophyletic clade), *Trichophorum*, *Cypringlea*, and *Oreobolopsis* are not natural genera as well.

Sclerieae

Although the only genus in this monotypic tribe is *Scleria* with ca. 250 species, the tribe Sclerieae is one of the bigger ones (Bauters et al., 2016). In terms of morphology the group is defined by a paniculate inflorescence, bisexual or unisexual spikelets, achene surrounded at the base by hypogynium and a cupula, and *Fimbristylis*-type embryo. All molecular studies agree with the monophyly of this group and with its close placement to Bisboeckelereae (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Simpson et al., 2007). The genus is divided into four subgenera and fifteen monophyletic sections. The subgenus *Browniae* stays on the basis, the subg. *Hypoporum* separated next and sister clades of the subg. *Trachylomia* and subg. *Scleria* are placed terminal (Bauters et al., 2016).

Main goals of the study

In summary, the phylogeny of Juncaceae and Cyperaceae is still not fully understood. The morphology of this group is complicated and without clear homology, and molecular relationships are mostly studied on smaller parts of this large group of Monocots; a proper study of these two families is therefore needed. As the volume of freely accessible molecular data is still increasing, analysis of these data is easy obtainable and is promising to resolve relationships intra- and interfamilies.

The main goal of this study was to collect accessible molecular data of the most used molecular markers and analyse them by MP, ML and BI to revise monophyly of families; to revise inner topology of the families on the levels of tribes and genera (on the level of genera only in cases where we have enough data and good statistical support); and to suggest changes in taxonomy where the data are congruent.

Material and Methods

For the phylogenetical analyses, sequences of three commonly used markers were employed: chloroplast gene *rbc*L (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene), chloroplast intergenic spacer *trn*L-*trn*F, and nuclear internal transcribed spacer 1 and 2 (ITS1 and ITS2) including 5.8S ribosomal RNA gene. Data for these three markers were obtained from GenBank (mainly for Cyperaceae), or they were sequenced for our previous studies of Juncaceae (Drábková et al., 2006, Záveská Drábková, 2010, Záveská Drábková and Vlček, 2009), or they were prepared from fresh or herbarium material from different collections of L. Záveská Drábková (for summary see Do and Záveská Drábková, 2018) and from the Herbarium at the Royal Botanic Gardens Kew (K) (see Suppl. 1 for the list of used sequences).

Extraction

DNA from fresh leaves was extracted following the protocol by Doyle and Doyle (1987). DNA isolation of the samples from the herbarium of L. Záveská Drábková was done by modified CTAB extractions and the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to Drábková et al. (2002). In this method, DNA was extracted from at least 0.1 g of dried or 1 g of fresh samples.

The herbarium samples from Kew were extracted following a modified protocol of CTAB isolation (Saghaimaroof et al., 1984), commonly used for such extractions in the Herbarium in Kew; these extractions were made directly in a specialized laboratory in Kew by their staff according RBG rules.

PCR and Sequencing

Four sets of primers were used for amplifications of all three regions (ITS4i + ITS5i, trnL5 + trnLR, trnL + trnF, RH1S + J1352R; see Tab. 2). We used various conditions of PCR due to low quality of herbarium specimens and other complicated material. Conditions for PCR were in following intervals: Initial denaturation at 94–95°C for 15 min, 35–45 cycles of 94–95°C for 1 min, 48–53°C for 1 min and 72°C for 3 min, and final extension of 72°C for 10 min. The PCR products were sequenced by Macrogen (Macrogen Inc., Seoul, South Korea) and edited in GeneStudio ver. 2.2.0.0 (Genestudio, Inc., Suwanee, GA, USA).

Tab. 2: Table of the used primers.

primer	sequence	author
ITS4i	GGT AGT CCC GCC TGA CCT GG	Roalson et al., 2001
ITS5i	AGG TGA CCT GCG GAA GGA TCA TT	Roalson et al., 2001
trnL5	CGA AAT CGG TAG ACG CTA CG	Taberlet et al., 1991
trnLR	GGG GAT AGA GGG ACT TGA AC	Taberlet et al., 1991
trnL	GGT TCA AGT CCC TCT ATC CC	Taberlet et al., 1991
trnF	ATT TGA ACT GGT GAC ACG AG	Taberlet et al., 1991
RH1S	ATG TCA CCA CAA ACA GAA ACT	Drábková et al., 2002
J1352R	GCA GCA GCT AGT TCA GCA CTC C	Drábková et al., 2002

Phylogeny analyses

The alignment of whole matrices was assembled in online MAFFT ver. 7 (Katoh and Standley, 2013) under following conditions: for the conserved region *rbc*L the L-INS-I algorithm was used, for intergenic spacer *trn*L-*trn*F the algorithm E-INS-Iwas used , and for alignment of matrix of all accesible ITS sequences (nearly 2000 sequences) a progressive FFT-NS-1 method was used. Settings connected with gap scoring were kept at the default setup (200PAM/k=2, gap opening penalty=1.53, offset value=0.0), because alignment of *rbc*L is so compact and with clear homology that different gap scoring would not influence the result; and, on the contrary, *trn*L-*trn*F and ITS are so divergent that we were concerned only with the most homologous regions and long gaps were not so problematic as an alignment without homology. The matrices were further manually adjusted in BioEdit (Hall, 1999).

Two alignments were prepared for cpDNA matrices: a matrix of 291 accessions and of 1240 bp for *rbc*L (Suppl. 2), and a matrix of 318 accessions and 3294 bp for *trn*L-*trn*F (Suppl. 3). For nuclear ITS, 6 matrices were prepared: a set of matrices of all obtainable taxa for complete ITS (1778 accessions, 1150 bp; Suppl. 4), for ITS1 (1802 accessions, 540 bp; Suppl. 5) and for ITS2 (1805 accessions, 495 bp, Suppl. 6), and a set of matrices reduced in the overrepresented genera (i.e. *Carex* and *Eleocharis*) for ITS (424 accessions; Suppl. 7), ITS1 (426 accessions; Suppl. 8), and ITS2 (427 accessions; Suppl. 9). Sequences which were too short were removed from all matrices, so the sequence length for the matrices are 269–678 bp for the complete ITS, 68–263 bp for ITS1, 120–316 bp for ITS2, 59–175 for 5.8S, 769–1240 bp for *rbc*L, and 573–1172 bp for *trn*L-*trn*F.

Finally, two complete matrices for all three data sets (*rbc*L+*trn*L-*trn*F+ITS) were prepared; the first with all taxa (considering the taxa from reduced ITS matrices) containing 592 sequences in all, the second with only these taxa for which all three markers are available (134 taxa).

For all matrices (except the extended ITS, ITS1, and ITS2) MP, ML and BI were performed. For the three extended ITS matrices, only ML was done (and only as informative analyses to reveal if the topology change dramatically if we extend the matrix).

MP analyses were rooted by *Prionium* for its closest relationship with the families Juncaceae and Cyperaceae. ML and BI analyses were performed without rooting; rooting of the two families (by Thurniaceae) was additionally manually done. No other outgroups were used because of ambiguous relationships with other families.

Initially, phylogenetic analyses were performed under the maximum parsimony (MP) criterion. To increase the likelihood of exploring all possible islands of the shortest trees, the program NONA (Goloboff, 1998) was used under the shell of WinClada 1.00.08 (Nixon, 2002). The parsimony ratchet procedure was used to search tree space by reweighing some iterations of a search (Nixon, 1999). It was performed by running 1000 replicates holding 50 trees in each replicate, and sampling 75 characters. The ambiguity setting was amb=. A strict consensus tree was constructed. The resulting cladograms were then submitted to the commands “hard collapse unsupported nodes in all trees” and “keep best only”. No *a priori* weighting was applied to the characters. The stability of the clades on the parsimony trees was established using bootstrapping (Felsenstein, 1985) with 1000 pseudoreplicates of heuristic searches with 100 interactions of random addition of taxa, and jackknifing. The relative level of homoplasy in all the data sets was assessed using the consistency index (CI; Kluge and Farris, 1969) and the retention index (RI; Farris, 1989).

Secondly, maximum likelihood (ML) analyses of the matrices were performed in RAxML 8.2.4. (Stamatakis, 2014) to examine differences in optimality between alternative topologies. Using the Akaike information criterion as implemented in Modeltest 3.8 (Posada and Crandall, 1998), GTR+I+Γ model was chosen as the best fitting model of DNA evolution for all three markers. 1000 replications were run for bootstrap values.

Thirdly, Bayesian statistical inference (BI) was performed using MrBayes software, version 3.2.6 (Ronquist et al., 2012). A GTR+I+Γ model (invgamma rates) was set. For the BI of the separate matrices, 8 Markov chains were conducted for 10,000,000 generations with a sample frequency of 100 (heating 0.1). When analysing the compound matrices, the genes were unlinked across partitions; two independent runs with 4 Markov chains were performed for 10,000,000 generations with a sample frequency of 100 (heating 0.2).

Results

Sequencing

In total, we managed to obtain 69 readable sequences for 57 taxa; 9 sequences, however, were not used because of contaminations. In fact, all extractions sent from Kew Botanical Garden were in most cases aligned close to *Betula* spp. and *Lactuca* spp. (using online NCBI BLAST server; Johnson et al., 2008). This is a general problem of DNA extractions from herbarium samples: DNA is, on one hand, damaged and broken into pieces, so the amplification is problematic and sometimes impossible; on the other hand, herbarium leaves are very sensitive to contamination, so even when the amplification is possible, the amplified DNA might not come from the studied taxon (details of the taxa in Tab. 3).

Tab. 3: Data of herbarium vouchers from Kew.

species	**name of colletor**	**code**	**date of collection**	**country**	**elevantion (m)**	**coordinates**	**det.**	**BLAST**
Capitularina involucrata	Utteridge, TMA	296	5.4.2000	Papua New Guinea	230	4°22°53°S, 136°56°9°E		*Mapania*
Coleochloa abyssinica	Cheek, M.	7688	7.11.1995	Cameroon	1100	4°47,4°N, 9°41,4°E		*Hypolytrum*
Diplacrum africanum	Bidgood, S.	6851	16.5.2008	Tanzania	1000	6°40°S, 32°06°E		*Betula*
Lepironia articulata	Bruhl, JJ	2638	25.2.2007	NSWales	9	28°19°52°S, 153°33°41°E		*Betula*
Mapania cuspidata	Imin, K.	FRI 71660	14.7.2010	Malaya	60	4°39,9°N, 103°06,87°E		*Lactuca*
Microdracoides squamosus	Bos, J.J.	4360	19.4.1969	Cameroon	1000	3°52°N, 11°26°E		*Betula*
Prionium serratum	Muasya, AM	sn	14.11.1997	Cape penninsula				*Lactuca*
Thurnia sphaerocephala longirostrata	Mutchnick, P.	94	19.10.1994	Guyana	625	5°6°0°N, 59°58°0°W	Strong, MT	*Betula*
Trilepis lhotzchiana	Fraga, C.N.	3148	20.10.2010	Brasil	40	43°18°10°W, 23°00°22°S		*Betula*

*rbc*L

In total, we obtained 291 *rbc*L sequences for 278 taxa. The length of the matrix was 1240bp and it contained 487 variable positions; 366 positions were parsimonious informative.

The strict consensus of the most parsimonious tree made by Winclada was constructed from 22,982 equally parsimonious trees of length 2177 (CI=30, RI=85). Bootstrap and jackknife values are shown above and below individual branches (Suppl. 10). When rooted by Thurniaceae, Juncaceae and Cyperaceae are strongly supported monophyletic groups. Within Juncaceae, three sister clades are found: genus *Oreojuncus*, genus *Luzula*, and paraphyletic genus *Juncus* together with SHC (within SHC are also two southern-hemisphere *Juncus* spp.). Considering *Luzula*, vast polytomies disable finer resolution within the genus; the only annual species of the genus, *L. elegans*, was solely placed on the basis of the whole clade. Within *Juncus*, three remarkable clades are derived (unfortunately, neither one of the two subgenera is monophyletic). Considering the family Cyperaceae, the subfamilies Mapanioideae and Cyperoideae are strongly supported as monophyletic. However, tribes are not monophyletic even within the small group Mapanioideae, where Chrysitricheae are collapsed in a strongly supported clade, but Hypolytreae are non-monophyletic. The rest of the Cyperoideae are derived in three clades: monophyletic Trilepideae, paraphyletic Bisboeckelereae with Sclerieae placed inside, and the rest of the family. Division inside Sclerieae corresponds with the classification *sensu* Bauters et al. (2016) – on the basis stays a taxon of subg. *Hypoporum* (*Scleria distans*), terminate taxa of subg. *Scleria*. On the basis of the rest stays *Cladium*, and then follows polytomy of Cryptangieae, a separated clade of the genus *Carpha* (Schoeneae), the rest of Schoeneae, and the rest of the Cyperaceae. Inside Schoeneae are some good supported clades (*Costularia natalensis* and *Tetraria capillaris*), but the resolution of rest of the tribe is not very proper. After them separates the monophyletic Rhynchosporeae, and follows the very well-supported separation of *Trichophorum cespitosum* (Y12969.1; Scirpeae). The tree is terminated by two large clades. The first is made by three branches – *Khaosokia caricoides*, Dulicheae and the first part of Scirpeae (*Trichophorum, Oreobolopsis, Phylloscirpus,* and *Zameioscirpus*), and Cariceae and the second part of Scirpeae (*Eriophorum*, and *Scirpus*). Considering Cariceae, within *Carex* spp., *Schoenoxiphium* and *Uncinia* create a well-supported monophyletic clade. The last clade is composed of monophyletic Abildgaardieae, clade of Eleocharideae and part of Fuireneae (*Fuirena*), and clade of Cypereae and the second part of Fuireneae. Cypereae, terminating the whole phylogeny, are paraphyletic due to *Schoenoplectus americanus* and *Bolboschoenus* spp. (Fuireneae).

The ML tree (log likelihood -14340.330908) is much more resolved than the MP tree (Suppl. 11). Nevertheless, supporting values of the branches defined in ML are low, and the essential construction is mostly the same as in the MP tree. Some dissimilarities might be found within larger groups only. In Juncaceae, for example, only two sister branches were resolved: monophyletic *Luzula*, and the rest of Juncaceae with monophyletic *Oreojuncus* on the basis. The inner topology of clades of Cyperoideae is slightly different only in low-supported positions. Surprisingly, *Trichophorum cespitosum* (Y12969.1) found its position close to other *Trichophorum* spp. within one of the clade of Scirpeae (but this position is very poorly supported).

The result based on BI (Suppl. 12) is also very similar to both previous topologies. Juncaceae have the same topology as in the ML tree, and also the topology of Cyperaceae corresponds with ML.

***trn*L-*trn*F**

The matrix of the *trn*L-*trn*F region contains 318 sequences for 305 taxa. The length of the matrix is 3294bp; it contains 1965 variable positions and 1347 parsimonious informative positions.

The final strict consensus of the MP was constructed from 7480 equally parsimonious trees of length 8951 (CI=39, RI=84). Bootstrap and jackknife values are shown above and below individual branches (Suppl. 13). The topology based on this second chloroplast region is similar to the previous one, but some important differences can still be found. The tree was rooted by Thurniaceae; Juncaceae and Cyperaceae appeared monophyletic. Considering Juncaceae, *Juncus capitatus* (annual plant) together with *Oreojuncus* stays on the basis of the family, rather than in the separate three clades of *Juncus* (the middle clade contains SHC clade), and the *Luzula* clade is monophyletic and is a sister to the last *Juncus* clade. Within *Luzula, L. bomiensis* is basal followed by *L. elegans*, the next resolution is slightly better than in *rbc*L, but still not significant enough. Within *Juncus*, SHC is clustered in a mid-supported clade with the two *Junsuc* spp. as in *rbc*L trees. Considering Cyperaceae, the family does not divide into the traditional subfamilies Mapanioideae and Cyperoideae. A clade composed of monophyletic sister branches of Bisboeckelereae and Sclerieae (with *S. distans* on the basis) has basal position followed by a small group of Schoeneae (namely the genus *Carpha* and *Trianoptiles capensis*). The next large clade contains 1) the second clade of Schoeneae with a close relative Cryptangieae, 2) a clade containing monophyletic *Cladium* and Hypolytreae which are not monophyletic due to Chrysitricheae nested inside it, and 3) the biggest part of Schoeneae. Relations inside the rest of the Schoeneae differ from these in *rbc*L, but are well supported. The next strong monophyletic clade belongs to Rhynchosporeae, and terminally are placed two large groups. *Khaosokia* stays on the basis of the first, and further separates the monophyletic branch of Dulicheae; Scirpeae is divided into two separate clades, and the second of them (*Trichophorum* + *Oreobolopsis*) is sister to the monophyletic Cariceae clade. Within Cariceae, the only monophyletic genus is *Schoenoxiphium.* The second terminal clade of Cyperaceae is composed of two bigger branches: the first contains monophyletic Eleocharideae and monophyletic Abildgaardieae; the second contains three separate branches of Fuireneae (including a monophyletic genus *Bolboschoenus*) and a monophyletic clade of Cypereae.

The ML tree of *trn*L-*trn*F (Suppl. 14; log likelihood -48592.242327) differs much more from the MP tree. Considering Juncaceae, *Juncus capitatus* (annual plant) stays on the basis of the family, clade of *Luzula* is monophyletic (*L. elegans* highly supported on the basis) and sister to the clade of the rest of Juncaceae with paraphyletic clade of *Juncus* (+ SHC) and *Oreojuncus* placed on the basis of the clade of *Juncus*. Considering Cyperaceae, the family divides into subfamilies Mapanioideae and Cyperoideae. As already shown by *rbc*L analyses, Hypolytreae are not monophyletic due to Chrysitricheae nested inside it. Within Cyperoideae, a clade composed of monophyletic sister branches of Bisboeckelereae and Sclerieae has a basal position, followed by the monophyletic clade of *Cladium*. The next separate clade contains Cryptangieae and part of Schoeneae (specifically genus *Carpha* and *Trianoptiles capensis*). The positions of Rhynchosporeae, *Khaosokia*, and Dulicheae correspond with the MP tree. Scirpeae is not divided into two separate clades, but the two clades are joined in one monophyletic branch. The tribe is a sister to the monophyletic Cariceae clade. Within Cariceae, the only monophyletic genera are *Uncinia* and *Shoenoxiphium*. The second terminal clade of Cyperoideae is composed of two bigger branches: the first contains monophyletic Eleocharideae, monophyletic Abildgaardieae, and part of Fuireneae (including a monophyletic genus *Bolboschoenus*); the second contains two separate branches of Fuireneae, and a monophyletic clade of Cypereae.

The topology of the BI tree (Suppl. 15) is similar to the ML tree, but remarkable differences can still be found. *Oreojuncus* clustered with *J. capitatus* on the basis of Juncaceae (but without support), and *Luzula* and *Juncus* (+ SHC) are monophyletic and sister clades. Within Cyperaceae, the topology corresponded with the ML tree nearly completely; only inner relationships of individual clades differ. One of notable changes in topology occurred in Scirpeae where the two clades separate as in MP tree.

ITS

The matrix of complete ITS region contains 424 sequences for 414 taxa. The length of the matrix is 1150 bp; it contains 789 variable positions and 699 parsimonious informative positions.

The strict consensus MP tree (Suppl. 16) of this dataset (no. of trees=4209, L=8334, CI=21, RI=85) rooted by *Prionium* has monophyletic families Juncaceae and Cyperaceae. Within Juncaceae, *J. dregeanus* stays as a sister clade to monophyletic *Luzula* (with *L. elegans* on the basis) and to paraphyletic *Juncus* with SHC; SHC is monophyletic. On the basis of Cyperaceae is a clade of *Bolboscheonus* and *Schoenoplectus litoralis* (Fuireneae), then derives a clade of *Fuirena* (Fuireneae) in a sister position to monophyletic tribes Abildgaardieae and Eleochartideae. The next big clade is rooted by *Dulichum arundinaceum*

(Dulicheae) and it contains two separate clades of Scirpeae (monophyletic *Trichophorum*, and a clade of *Scirpus* and *Eriophorum*) and a clade of Cariceae (with inserted *Amphiscirpus nevadensis*; Scirpeae). The following branch contains a paraphyletic clade of Rhynchosporeae with *Carpha* and *Trianoptiles capensis* (Schoeneae); a clade of monophyletic *Cladium*, Cryptangieae, Bisboeckelereae, Sclerieae, and a strong part of Schoeneae; and a clade of the rest of Schoeneae with *Hypolytrum* inside. The last clade of the tree is composed of two clades of Fuireneae and monophyletic Cypereae.

The ML tree (Suppl. 17; log likelihood -38990.769599) of the dataset of whole ITS sequences rooted by *Prionium* does not show monophyletic families Cyperaceae and Juncaceae, because Juncaceae creates a terminal clade of Cyperaceae in this analysis. The tree starts by polyphyletic *Fuirena* (Fuireneae), followed by a strongly-supported clade of the sister monophyletic Abildgaardieae and Eleocharideae, and then by a strong clade of two clades of Fuireneae and a monophyletic clade of Cypereae. After this bigger clade is one derived of *Bolboschoenus* and *Schoenoplectus litoralis* (Fuireneae); and a clade of polyphyletic Scirpeae divided to three branches, and monophyletic Cariceae. The last part of Cyperaceae contains monophyletic Rhynchosporeae, *Cladium*, Bisboeckelereae, Cryptangieae, and Sclerieae; paraphyletic Schoeneae is divided into two clades and *Hypolytrum* is inserted as sister to *Ptilothrix deusta*. The tree is terminated by Juncaceae: on the basis of the branch *J. dregeanus* remains, then derives *Oreojuncus* with *J. capitatus*. *Luzula* is monophyletic (with *L. elegans* on the basis), and the clades of *Juncus* and SHC paraphyletic.

The BI tree (Suppl. 18) is very similar to the ML tree; only slight differences in topology between low-supported clades occurred.

ITS1

The matrix of complete ITS1 region contains 426 sequences for 415 taxa. The length of the matrix is 540 bp; it containes 361 variable positions and 330 parsimonious informative positions.

The strict consensus MP tree (Suppl. 19) of this dataset (no. of trees=16691, L=4118, CI=20, RI=84) rooted by *Prionium* has monophyletic family Juncaceae pasted in paraphyletic Cyperaceae. On the basis of the tree, *Bolboschoenus* (+ *Schoenoplectus littoralis*; Fuireneae) remains followed by a clade composed of sister monophyletic tribes Abildgaardieae and Eleocharideae. The next clade contains two separate clades of Fuireneae, and monophyletic clade of Cypereae. A clade of polyphyltetic Scirpeae and monophyletic Cariceae follows. The topology of the rest of the tree is without support and rather unsure. Basally, Dulicheae are separated, and *Amphicirpus nevadensis* (Scirpeae) is derived next. A clade of paraphyletic Rhynchosporeae and Scheneae, and monophyletic Sclerieae and Bisboeckelereae create a complex clade. Within Schoeneae, a clade of *Carpha* + *Trianoptiles* is a sister to majority of Rhynchosporeae and *Ptilothrix* is a sister to *Hypolytrum* (Hypolytreae). After this large clade is separated the monophyletic *Cladium* and Cryptangieae, and as a sister to monophyletic Juncaceae remains the last part of Schoeaneae (*Oreobolus* and relatives). Within Juncaceae, *Luzula* is monophyletic (*L. elegans* on the basis) with *J. dregeanus* in a sister position. Juncus (+ SHC) is not monophyletic due to *J. dregeanus*, and *J. capitatus*, which clusters in a clade of *Oreojuncus*.

ML analysis of ITS1 (log likelihood -19117.067928) does not correspond with the MP analysis in many aspects (Suppl. 20). The family Cyperaceae are not monophyletic too, but the tree starts with polyphyletic Cariceae followed by two clades of paraphyletic Scirpeae with incorporated *Dulichum arundinaceum* (Dulicheae). Within Scirpeae, the first clade is placed as a sister clade to *Dulichum*, and the second clade has high support. The first of the last two big clades of Cyperaceae contains a separate strong monophyletic clade of *Fuirena* (Fuireneae); a strongly-supported separate clade of *Bolboschoenus* and *Schoenoplectus litoralis* (Fuireneae) as sister to a clade composed of monophyletic Eleocharideae and monophyletic Abildgaardieae; a clade of all the rest of Fuireneae; and finally, the monophyletic Cypereae clade. *Amphiscirpus nevadensis* (Scirpeae) has its own clade derived after the large clade of Fuireneae+Abildgaardieae+Cypereae. The second clade contains paraphyletic Schoeneae with pasted monophyletic Hypolyrteae as a sister to the monophyletic Sclerieae, Bisboeckelereae, Cryptangieae, and polyphyletic Rhynchosporeae. The tree is terminated by the monophyletic Juncaceae clade with the monophyletic genus *Luzula* (*L. elegans* on the basis), and the paraphyletic rest of the family where on the basis stays *J. dreganus*, then separates the monophyletic *Oreojuncus* with *J. capitatus* as the closest relative.

Interestingly, the results of ML and BI (Suppl. 21) differ in an important topology change; in the BI tree, Juncaceae is nested inside the basal polyphyly of Cariceae. The tree differs also in some other less-important changes against the ML tree which is based mostly on polytomies originated due to the collapsed topology of low-supported clades.

ITS2

The matrix of complete ITS2 region contains 427 sequences for 414 taxa. The length of the matrix is 495 bp; it containes 391 variable positions and 345 parsimonious informative positions.

The MP tree (Suppl. 22; no. of trees=1686, L=4172, CI=21, RI=85) contains monophyletic Juncaceae and paraphyletic Cyperaceae, as was previously seen in ITS analyses many times. On the basis of the tree, the *Fuirena* (Fuireneae) clade is separated, then the sister monophyletic Abildgaardieae and Eleocharideae clades, followed by a clade where genus Bulbostylis (+ *Schoenoplectus litoralis*) is sister to all Juncaceae. Juncaceae are divided into three clades; in the basal positions are placed the most derived taxa as *Luzula elegans, Juncus dregeanus, J. capitatus*, and the genus *Oreojuncus*. The two other sister clades contain 1) paraphyletic *Juncus* and SHC, and 2) monophyletic *Luzula* (without *L. elegans*). Then follows a clade composed of two larger clades of Cyperaceae – the first includes *Lagenocarpus* (Cryptangieae), Sclerieae and a part of Fuireneae, the second contains the second part of Fuireneae and Cypereae. The tree continues with a clade of polyphyletic Schoeneae, Rhynchosporeae and *Dulichum* (Dulicheae). The tree is terminated by paraphyletic Scirpeae (creating polytomies) and monophyletic Cariceae.

The ML tree (log likelihood -18997.333696) does not support monophyly of the families, Juncaceae are nested in Cyperaceae (Suppl. 23). The tree begins (similarly to the complete ITS tree) by polyphyletic genus *Fuirena* (Fuireneae), then follows a branch containing monophyletic clades of Eleocharideae and Abildgaardieae. In the next derived branch are two separate clades of Fuireneae and monophyletic Cypereae. Then follows independent branch of the rest of Fuireneae. The next clade is based by *Dulichum arundinaceum* (Dulicheae), and contains two separates branches of Scirpeae and a clade of Cariceae mixed with *Amphiscirpus nevadensis*. In the basis of the last clade of the tree (also just poorly supported) is polyphyletic Rhynchosporeae, followed by paraphyletic Schoeneae. Schoeneae is divided into three clades with *Hypolytrum* on the basis. Then, separated *Lagenocarpus albo-niger* (Cryptangieae) appears in the last clade with a clade of monophyletic Bisboeckelereae, then monophyletic Sclerieae, and finally Juncaceae.

The BI tree (Suppl. 24) is basically the same as the ML tree; only polytomies and poorer resolution of topologies among and inside clades can be mentioned.

Extended trees of ITS, ITS1, and ITS2

As much more data for Cariceae and Eleocharideae are obtainable from GeneBank, we have also done ML analyses for these complete data to compare the results. The matrix of ITS / ITS1 / ITS2 region contains 1778 / 1802 / 1805 sequences for 1019 / 1031 / 1032 taxa. The length of matrix is 1150 / 540 / 495 bp; it containes 855 / 375 / 411 variable positions and 751 / 347 / 368 parsimonious informative positions.

Firstly, the extended ML analysis of ITS2 (Suppl. 23; log likelihood -34497.838316) has just minimal changes in topology inside clades against the complete tree. The only remarkable differences are the division of Schoeneae into just two clades, and the position of Hypolytreae as a sister to Bisboeckelereae. However, none of these two topological changes is supported significantly.

The extended ITS1 tree (Suppl. 20; log likelihood -35036.160902) differs slightly more from the reduced analysis than it was in ITS2 analyses. Scirpeae are collapsed into one clade (incl. Dulicheae), and *Amphiscirpus nevadensis* is derived right after Scirpeae. Nevertheless, these changes in topology are poorly supported.

Interestingly, the tree of extended ITS (Suppl. 17; log likelihood -74499.528024) does not resemble the ITS2 tree (as it was in reduced matrices), but its topology is close to the topology of ITS1. The extended ITS differ from reduced ITS1 only in few topology details. *Amphiscirpus nevadensis* stays inside Cariceae; Dulicheae has its separate place after Scirpeae; Fuireneae is divided into four clades; Rhynchosporeae is monophyletic; Schoeneae are divided into two clades by the monophyletic separate clade of Bisboeckelereae; and Hypolytreae is non-monophyletic. All these substitutions are, however, low-supported and insignificant.

Total evidence tree from all three markers

Two matrices were constructed: with all used taxa, and with taxa where all three markers were available. The first and the second matrices contain 595 / 133 sequences for 542 / 133 taxa. The length of both matrices is 5684 bp; each contains 3234 /2682 variable positions and 2409 / 1799 parsimonious informative positions.

MP analysis of the bigger dataset (Suppl. 25; no. of trees=30, L=19,789, CI=30, RI=84) was influenced mostly by the cpDNA markers, because the topology is closer to the topology resulting from cpDNA data than that from nDNA data. Juncaceae and Cyperaceae are monophyletic. Juncaceae are divided into three clades: the first containing *Juncus* *capitatus*, *J.dregeanus* and *Oreojuncus*; the second containing monophyletic *Luzula*; the third containing rest of *Juncus* and SHC. In Cyperaceae, monophyly of the subfamilies is supported. Hypolytreae are paraphyletic due to the inserted monophyletic Chrysitricheae. Trilepideae is monophyletic, while Sclerieae are pasted inside Bisboeckelereae. *Cladium* and Cryptangieae are derived separately. Schoeneae are paraphyletic and the clade of *Carpha* and *Trianoptiles* derived on the basis of the rest of the Cyperaceae. Rhynchosporeae has its own separate clade. On the top of the tree are derived two large sister clades. On the basis of the first is placed *Khaosokia caricoides*, followed by monophyletic Dulicheae, polyphyletic Scirpeae, and finally monophyletic Cariceae (on the basis stays *C. siderostica*). The second large clade contains the sister monophyletic clades of Abildgaardieae and Eleocharideae, three branches of polyphyletic Fuireneae, and the tree is closed by monophyletic Cypereae.

In ML analysis (Suppl. 26; likelihood log -107808.264345), the families Juncaceae and Cyperaceae are resolved as monophyletic. Within Juncaceae, on the basis stays one of two samples of *J. capitatus*. *J. dregeanus* stays as a sister taxon to the entire genus *Luzula*. Inside *Luzula*, *L. elegans* is the most basal. The monophyletic genus *Oreojuncus* is positioned as a sister clade to paraphyletic Juncus with SHC. Concerning Cyperaceae, the topology agrees with MP analysis, except for the tribe Schoeneae which appears monophyletic (excluding *Cladium* in separate clade).

The BI analysis (Suppl. 27), although the main topology agrees with the previous ones, appeared much less differentiated with many polytomies. In contrast to the ML analysis, it possesses a branch of the sister *J. capitatus* and *J. dregeanus*; this branch and the next three branches of Juncaceae have uncertain relationships between them. *Carpha* and *Trianoptiles* are separated as in MP analysis. Unresolved positions have *Amphiscirpus nevadensis* (Scirpeae) placed in the polytomy of Scirpeae, Dulicheae, and *Khaosokia*; and *Trachystylis stradbrokensis* (Abildgaardieae) placed in the polytomy of Eleocharideae and Abildgaardieae.

When we analysed taxa where we had data from all three markers, the MP (Suppl. 28; no. of trees=8, L=11,949, CI=38, RI=76) appeared slightly better resolved. The families and most of the recently-recognised tribes are monophyletic. Within Juncaceae are two clades; the first contains the monophyletic genus *Luzula* (with *L. elegans* on the basis), and the second clade contains the monophyletic *Oreojuncus* in a sister position to the paraphyletic *Juncus* (incl. SHC). Concerning the Cyperaceae, Mapanioideae and Cyperoideae are well resolved. Within Mapanioideae, Hypolytreae is paraphyletic with nested Chrysitricheae. A further derived clade contains monophyletic Trilepideae, and a clade of paraphyletic Bisboeckelereae terminated by monophyletic Sclerieae. The next separate clade belongs to *Cladium*; then follows the separate monophyletic Cryptangieae. The following Schoeneae are divided into two clades: the genus *Carpha*, and the rest of the species. The last small separate clade belongs to the monophyletic Rhynchosporeae, and at the terminus of the tree stays two large branches. The first contains three sister clades: separate *Khaosokia caricoides*, a clade of monophyletic Dulicheae and part of Scirpeae, and a clade of monophyletic Cariceae and the rest of Scirpeae. The second large branch is composed of a clade of sister monophyletic Eleocharideae and the genus *Fuirena* (Fuireneae), monophyletic Abildgaardieae, two separate branches of Fuireneae, and terminaly is situated paraphyletic Cypereae (with inserted *Bolboschoenus* spp. and *Schoenoplectus americanus*, Fuireneae). The ML tree (Suppl. 29; log likelihood -65182.646414) differs just slightly from the MP tree: *Juncus acutus* was inserted inside SHC, and *Carpha* is connected to Schoeneae. The analysis of BI (Suppl. 30) does not differ from the ML tree.

Discussion

How deep can see individual molecular markers

It is known that individual molecular markers would be able to distinguish different levels of the phylogeny. The marker *rbc*L, as a conservative gene, is supposed to be a good marker for higher taxonomical levels, while *trn*L-*trn*F might not be so secure at higher levels, but might be useful on smaller scales (Gielly & Taberlet, 1994). ITS is also used as a marker for the resolution of finer relationships (Feliner & Rosselló, 2007); moreover, as ITS belongs to the nucleus, and *rbc*L and *trn*L-*trn*F are carried by chloroplast, they might evolve by different pathways and show different results of evolutionary relationships (Soltis & Kuzoff, 1995).

These presumptions were globally confirmed; *rbc*L was able to provide good support for genera, but lower levels were mainly unsupported or even unresolved in polytomies. Only rare cases supported resolution inside genera (e.g. in *Juncus* or *Rhynchospora*).

Resolution of the trees based on *trn*L-*trn*F is much better; well-supported clades can be found much deeper inside genera than in *rbc*L trees. However, as the marker is more heterogenous than *rbc*L, it is inclined towards changes in global topology between MP vs. ML and BI trees. These changes occur mostly in unsupported positions (for example different spreading of individual clades of Fuireneae within Cyperaceae), or they are based on LBA (placement of Mapanioideae inside Cypereae in MP tree).

As was mentioned, ITS belongs to diverse markers which might not be able to resolve relationships on higher phylogenetic levels. Indeed, nearly no support was found on the level of tribes and higher; good support was observed only on level of genera; lower relations often ended in polytomies. The poor resolution of phylogenetic relationships by ITS is really not unpredictable – ITS was identified as an inappropriate marker for resolution of both higher and lower taxonomic levels of plants (Alvarez and Wendel, 2003). The complications of interpretation of ITS evolution is caused mainly by hybridization, which might lead to incorrect topology due to incomplete lineage sorting, polyploidization which produces more copies of ITS, degradation of some of the copies into pseudogenes, and homogenization of the copies (that might be incomplete in individual species). The incomplete homogenization is a problem mostly of the lower taxonomic levels; resolution of the higher taxonomic levels suffers mostly from the origin of pseudogenes and the relatively high rate of mutations and, thus, a high rate of homoplasy (Alvarez and Wendel, 2003). Despite this rather unpredictable behaviour of ITS evolution, many molecular analyses are based on this marker; in fact, it is one of the most popular DNA markers. Therefore, large amount of ITS sequences can be found in GenBank which was the reason to involve ITS inour study and compare results based on ITS and cpDNA markers. Although ITS does not provide reasonable resolution of higher and lower levels of phylogeny, some clades are well-supported and comparison of nDNA and cpDNA is, therefore, available on these positions.

Considering classification of the families

Unfortunately, our dataset includes only a limited number of taxa with all three markers present (specifically 134 taxa). These taxa were analysed, resulting in an analysis with very strongly-supported branches. However, as the number of taxa is still very low and the tree calculated on the basis of such a narrow selection of species might give biased (or over-optimistic) results, we also performed an analysis based on all the data available to us (even though the matrix was full of missing data). Comparision of the results of these two datasets showed that global topology remains stabile for both datasets across the methods used; therefore, the following discussion will be based on the analyses of the larger dataset (because it is only here that we have taxa from nearly all tribes of Cyperaceae) putting great emphasis on the analyses of the smaller dataset, and slightly taking account of the partial analyses.

As in the Results Section where we were described the topologies of the higher taxonomical level, the Discussion will describe the relationships more deep within tribes, and the possible solutions of the taxonomical problems will be discussed in following chapters.

Juncacaea vs. Cyperaceae

One of the main goals of this study was to resolve relationship of Juncaceae and Cyperaceae, which was satisfactorily fulfilled. The total evidence trees unambiguously separated the families into two sister monophyletic clades (Fig. 1). Just ML and BI trees based on nDNA did not support monophyly of the families – in these analyses, the strongly supported clade of Juncaceae was inserted inside Cyperaceae which has an unclear and unsupported topology of its inner clades. This was, obviously, caused by the already discussed limits of ITS in terms of the resolution of higher phylogenetic levels.

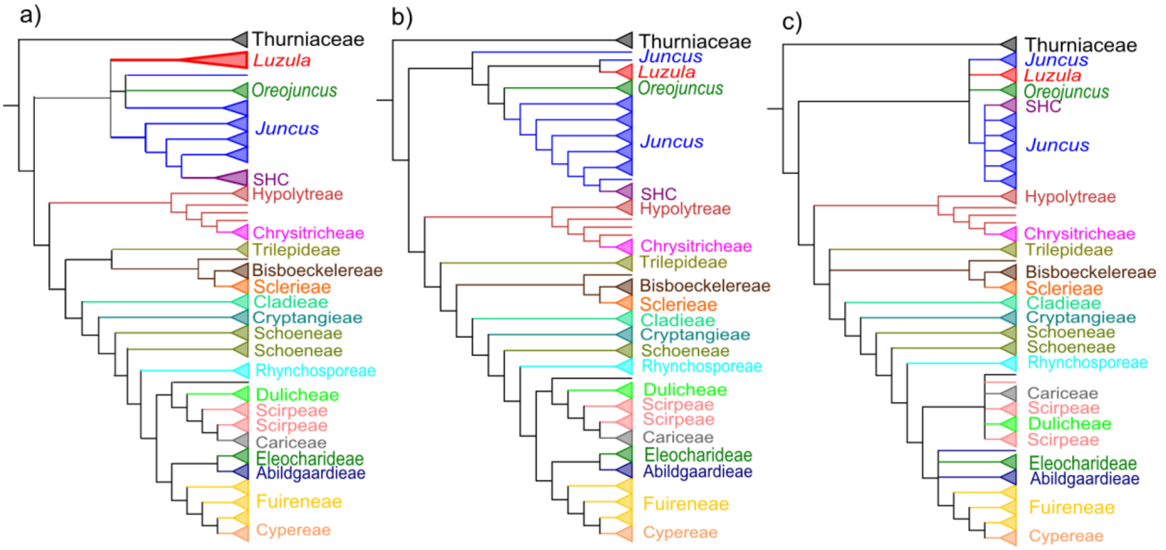


Fig. 1: Topology of Juncaceae and Cyperaceae based on total-evidence analyses; ; a) MP, b) ML, c) BI.

Juncaceae Juss.

Strong monophyly of Juncaceae was supported by all analyses, and the structure of inner clades is also stable (Fig. 1). The always strongly monophyletic *Oreojuncus* is placed in a sister position to the *Juncus* clade. The strongly supported separation of *Oreojuncus* from the genus *Juncus* is in agreement with the study which defined this new genus on the basis of much narrower molecular sampling than ours. The separation was also supported also by a characteristic combination of morphological features and especially by lacerate-fimbriate auricles which are unique to the family (Záveská Drábková and Kirschner, 2013).

Considering the much larger and more complicated genus *Luzula*, this appeared to be strongly supported as a monophyletic clade. *L. elegans* certainly has its position at basis of the whole genus, which only reflects the molecular and evolutional distinctiveness of this annual taxon. Considering the further topology of *Luzula*, subg. *Pterodes* is supported as a monophyletic clade nested within paraphyletic subg. *Luzula*; the other topology of *Luzula* is, however, so weakly supported that no definite solution of this paraphyly can be suggested.

Within Juncaceae, the most problematic is the paraphyletic clade *Juncus* (+SHC; Fig. 2). The clade is, according to the total evidence trees, most probably paraphyletic. This non-monophyly causes *Juncus capitatus* and *J. dregeanus* which are placed on the base of Juncaceae, or in a close relationship to *Oreojuncus*. The special position of *J. capitatus* was already discovered by Drábková et al. (2006), Roalson (2005), Záveská Drábková and Vlček (2009), and Záveská Drábková and Kirschner (2013) who ascribed this topology to LBA of

MP. In our analysis, however, the described position of *J. capitatus* appeared in all total evidence analyses. Looking into the separate analyses of individual markers, despite belonging to the subg. *Juncus*, the taxon was placed in a close relationship with *J. bufonius* and *J. tenuis* (subg. *Agathryon*) by *rbc*L analyses (affinity to *J. tenuis* was described by Drábková et al., 2006, Záveská Drábková and Kirschner, 2013). Besides the molecular-based topology, *J. capitatus* has also some special characteristics within subg. *Juncus*, sect. *Caespitosi* (where is traditionally placed). Analogous to *L. elegans*, plants of sect. *Caespitosi* are annual, and *J. capitatus*, moreover, has a cosmopolitan distribution in contrast to other taxa of the section which have much narrower areas. We do not have many possibilities to compare this position with other taxa from sect. *Caespitosi*; the only another species is *J. tiehmii* (we have only *trn*L-*trn*F data) which is placed near SHC, far from *J. capitatus*.

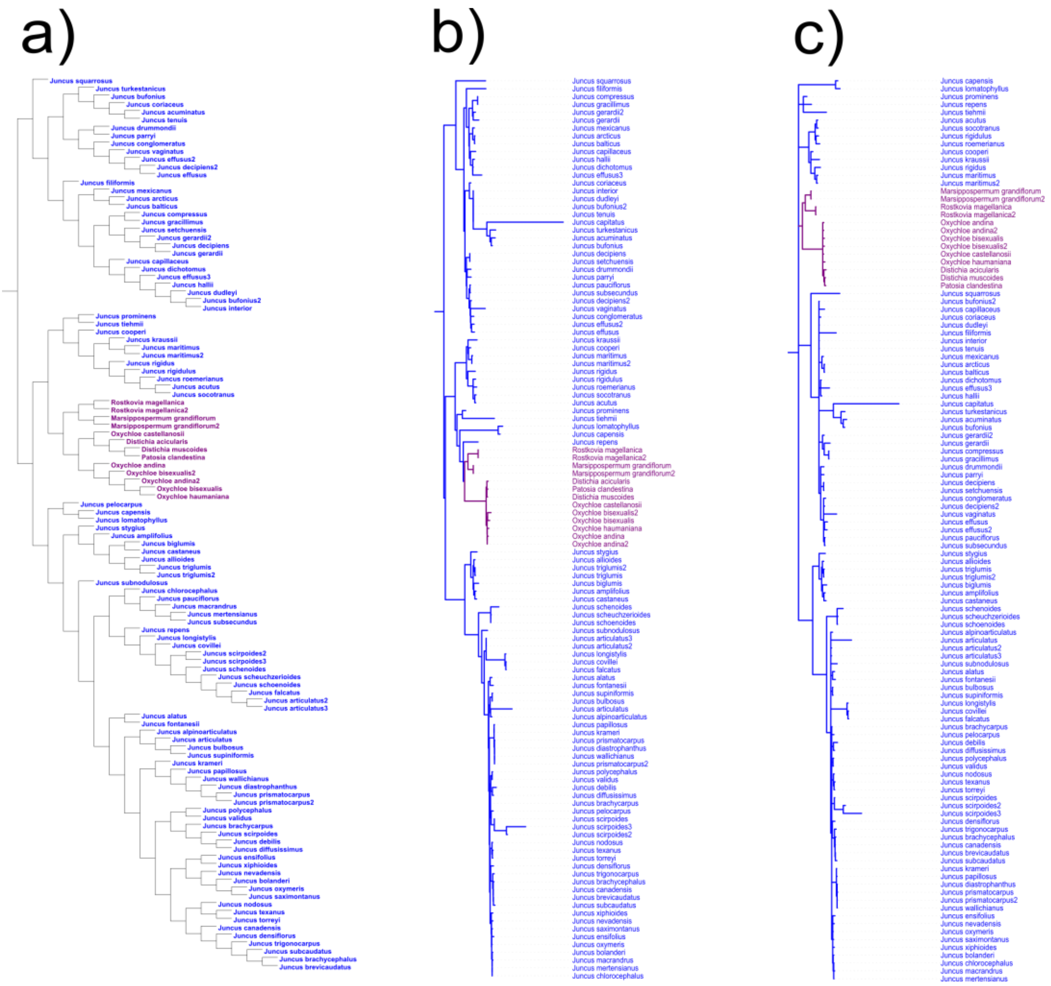


Fig. 2: Phylogeny of Juncus + SHC based on total-evidence analyses; a) MP, b) ML, c) BI.

J. dregeanus, similar to *J. capensis* and *J. lomatophyllos* which are sometimes shifted to SHC, belongs traditionally to subg. *Juncus*, sect. *Graminifolii*. As we only obtained sequences for ITS and the position of the species had never been discussed before, we cannot compare its unique problem with other data (Starr et al., 2004). Moreover, the taxa of sect. *Graminifolii* are gathered mainly on the basis of their similar distribution, and their non-monophyly is proposed. Therefore, this taxon might be studied more properly on the basis of both molecular data and morphology.

Finally, the inner relationships of the paraphyletic *Juncus* (+ SHC) can be generally described. The traditional subgenera of *Juncus* were not supported: subg. *Agathryon* and subg. *Juncus* are paraphyleric, and SHC is also paraphyletic (incl. *J. capensis* and *J. lomatophyllus*). Inside subg. *Agathryon*, *J. acuminatus* and *J. capitatus* (subg. *Juncus*) was found. *J. capitatus* was discussed already. *J. acuminatus* was sequenced for a study of pseudogene in *trn*L-*trn*F of Juncaceae which is in preparation, and the sequence has not yet been published. As we have only one sequence of this taxon, we cannot be sure of its placement in the phylogeny of Juncaceae, but we cannot confirm monophyly of subg. *Agathryon* neither, even though it is monophyletic according to the previous studies (Drábková et al., 2006, Roalson, 2005, Záveská Drábková and Kirschner, 2013) and morphological characteristics, which distinguished the subgenus by floral bracteoles and a cymose inflorescence (Buchenau, 1890).

The SHC clade is paraphyletic unless *J. capensis* and *J. lomatophyllus* are accepted as a part of it. After this acceptance, the only problem bound with the SHC remains in its nesting inside the genus *Juncus*, which can be solved by two different ways – enlarging *Juncus* for the southern-hemisphere genera, or division of *Juncus* into more genera.

Firstly, despite the distributional and morphological specifics of *Distichia, Marsippospermum, Oxychloë, Patosia,* and *Rostkovia*, their shift into genus *Juncus* can be considered. Morphologically, the connection of the five southern hemisphere genera to *Juncus* might be supported only by glabrous stems and leaves and many seeds in a fruit (while *Luzula* has ciliate leaves and only 3 seeds in a fruit; Kirschner, 2002a, b).

Secondly, as the small southern hemisphere genera are still clearly morphologically distant from *Juncus*, the other possibility is to divide *Juncus* into smaller separate genera. The easiest way how to do this dissection is to establish a genus from subg. *Agathryon* (incl. *J. acuminatus*), another genus woud be based on a part of subg. *Juncus*, and the taxa clustering in a clade with SHC would have to be divided into more genera as they are not in a monophyletic clade. This division would not be, however, without problems; some genera (specifically *J. lomatophyllus, J. capensis,* and *J. covillei*) are placed close to SHC by cpDNA and appear in subg. *Juncus* in the analyses based on nDNA. The solution of this problem of SHC would need much more proper analysis.

Cyperaceae Juss.

As the family Cyperaceae is much larger than Juncaceae with only 8 genera, discussion is divided into smaller parts considering compact clades of the phylogeny. In the first chapter "Mapanioideae", phylogeny of the whole subfamily and inner tribes is discussed (Fig. 1); in the following chapters, important and complicated tribes of Cyperoideae are considered (for the summary of tribes and genera see Tab. 4).

Tab. 4: Table of the classification of Cyperaceae as recognised in this study. The suggested changes of classification are described in it.

subfamilies	tribes	morphological delimitation of the tribes	genera	no. of species	suggested changes in classification
Cyperoideae	Abildgaardieae	clearly differenciated and thickened style base, distichous glumes moniliform stigmatic hairs, *Abildgaardia* -type, *Bulbostylis* -type, and *Fimbristylis* -type embryos	*Abildgaardia*	10	
			Actinoschoenus	3	
			Arthrostylis	1	
			Bulbostylis	100	
			Crosslandia	1	
			Fimbristylis	300	
			Nelmesia	1	
			Nemum	10	
	Bisboeckelereae	empty glumes surrounding the apparently terminal female flower, one stamen per male spikelet, *Carex* -type, *Schoenus* -type, and *Fimbristylis* -type embryos	*Becquerelia*	5	
			Bisboeckelera	4	
			Calyptrocarya	8	
			Diplacrum	7	
	Cariceae	unisexual flowers, female flowers completely enclosed by a fertile urticuliform spikelet prophyll, absence of perianth, *Carex* -type embryo	*Carex*	2100	
			Cymophyllus	1	
			Kobresia	50	
			Schoenoxiphium	12	
			Uncinia	60	
	Cypereae	style base is not distinct and thickened, but sometimes persistent as a narrow beak, distichous glumes, reduction of the spikelet, perianth is reduced completely, *Cyperus* - or *Ficinia* -type embryos	*Androtrichum*	2	
			Ascopholis	1	
			Cyperus	780	
			Dracoscirpoides	2	
			Erioscirpus	2	
			Ficinia	60	
			Hellmuthia	1	
			Isolepis	60	
			Scirpoides	5	
	Cryptagineae	unisexual spikelets, spirally aranged glumes, perianth formed by 3 fimbriate scales opposite the flat sides of the achene, probably *Juncus* -type embryo	*Cephalocarpus*	3	
			Didymiandrum	1	
			Everardia	12	
			Exochogyne	1	
			Lagenocarpus	30	
	Dulicheae	fertile spikelet prophyll, bisexual flower, *Carex* -type embryo	*Blysmopsis*	1	
			Blysmus	3	
			Dulichum	1	
	Eleocharideae	reduction of vegetative apparatus, fixed unispiculate inflorescence, unique embryo morphology, bristlelike perianth, differenciated and thickened style base, moniliform stigmatic hairs, specific helio- and helophylous ecology	*Eleocharis*	200	
	Fuireneae	spirally arranged glumes, flowers with hypogynous scales, *Schoenoplectus* - and *Bolboschoenus* -type embryos	*Bolboschoenus*	15	Fuireneae
			Fuirena	58	
			Pseudoschoenus	1	Pseudoschoeneae
			Schoenoplectiella	51	
			Actinoscirpus	1	Actinoscirpeae
			Schoenoplectus	29	
	Rhynchosporeae	distinct style base, Kranz syndrome, *Carex* -type embryo	*Pleurostachys*	30	
			Rhynchospora	250	
	Sclerieae	paniculate inflorescence, bisexual or unisexual spikelets, achene surrounded at the base by hypogynium and a cupula, *Fimbristylis* -type embryo	*Scleria*	250	
Cyperoideae	Scirpeae	spirally arranged glumes, flowers with hypogynous scales, *Carex* -type, *Schoenus* -type, and *Fimbristylis* -type embryos	*Amphiscirpus*	1	Scirpeae
			Eriophorum	20	
			Phylloscirpus	5	
			Scirpus	160	
			Zameioscirpus	3	
			Oreobolopsis	2	Trichophoreae
			Trichophorum	10	
	Schoeneae	low number of bisexual flowers per spikelet, often well-developed perianth, inclusion of the flowers by thy wings of the next glume, *Carex* -type, *Schoenus* -type, *Helothrix* -type, *Carpha* -type, and *Juncus* -type embryos	*Carpha*	15	
			Caustis	6	
			Costularia	20	
			Cyathochaeta	4	
			Cyathocoma	3	
			Epischoenus	8	
			Evandra	2	
			Gahnia	30	
			Gymnoschoenus	2	
			Lepidosperma	55	
			Machaerina	50	
			Mesomelanea	5	
			Morelotia	2	
			Neesenbeckia	1	
			Oreobolus	15	
			Ptilothrix	1	
			Reedia	1	
			Schoenus	100	
			Tetraria	50	
			Trachystilis	1	
			Trianoptiles	3	
			Trichoschoenus	1	
			Tricostularia	6	
			Cladium	5	Cladieae
			Rhynchocladium	1	
	Sumatroscirpeae	highly-compound inflorescences, pedicellate spikelets, antrorsely scabrous bristles, sheathing fertile prophylls, tuberculate fruits	*Sumatroscirpus*	4	
	Trilepideae	many dense spikes of many tiny spikelets with few distichous glumes, perianth formed by 3 fimbriate scales opposite the flat sides of the achene, *Trilepis* -type	*Afrotrilepis*	2	
			Coleochloa	7	
			Microdracoides	1	
			Trilepis	3	
Mapanioideae	Hypolytreae	many-spikelet inflorescence, poorly differenciated embryo, special *Mapania* -type pollen, tropical distribution	*Capitularina*	1	Hypolytreae
			Diplasia	1	
			Exocarya	1	
			Hypolytrum	40	
			Mapania	70	
			Paramapania	7	
			Principiana	1	
			Scirpodendron	2	
	Chrysitricheae	reduced vegetative apparatus and inflorescence, highly differenciated embryo, pseudomonads, Gondwanan distribution	*Chrysitrix*	4	
			Chorizandra	7	
			Lepironia	1	

Mapanioideae

The subfamily Mapanioideae appeared monophyletic with a high level of support, and also well-supported is its sister position to Cyperoideae.

Considering the inner relationships of the subfamily in the Goetghebeur (1998) sense (i.e. *Chrysitrix*, *Chorizandra*, and *Lepironia* in Chrysitricheae; *Capitularina*, *Diplasia*, *Exocarya*, *Hypolytrum*, *Mapania*, and *Scirpodendron* in Hypolytreae), Hypolythreae are paraphyletic, while Chrysitricheae are strongly supported monophyletic clade nested inside Hypolytreae (Fig. 3). Reclassification of the group was suggested by Simpson et al. (2003), who replaced *Capitularina* and *Exocarya* into Chrysitricheae on the grounds of the morphology of pollen and on molecular evidence. Our data, however, placed also *Diplasia* on the basis of Chrysitricheae; therefore, the monophyly of Hypolytreae cannot be confirmed. It seems, in light of this result, that *Mapania*-type pollen, which is characteristic for Hypolytreae *sensu* Simpson et al (2003), evolved once from ancestral pseudomonads (Selling, 1947), and it was also once lost in a clade of Chrysitricheae. Nevertheless, the tribes are unnatural and should not be treated this way; Mapanioideae should be recognised as the only tribe due to unstable relationships between taxa and non-monophyly of individual inner clades. Moreover, it seems that the genera *Hypolytrum*, *Mapania*, and *Scirpodendron* might not be natural, but larger sampling and deep insight into morphology, which is highly problematic because of the lack of proper morphological characters (Bruhl, 1995, Goetghebeur, 1998, Simpson, 1992, Simpson et al., 2003), might give more suitable data to understand this taxonomic group.

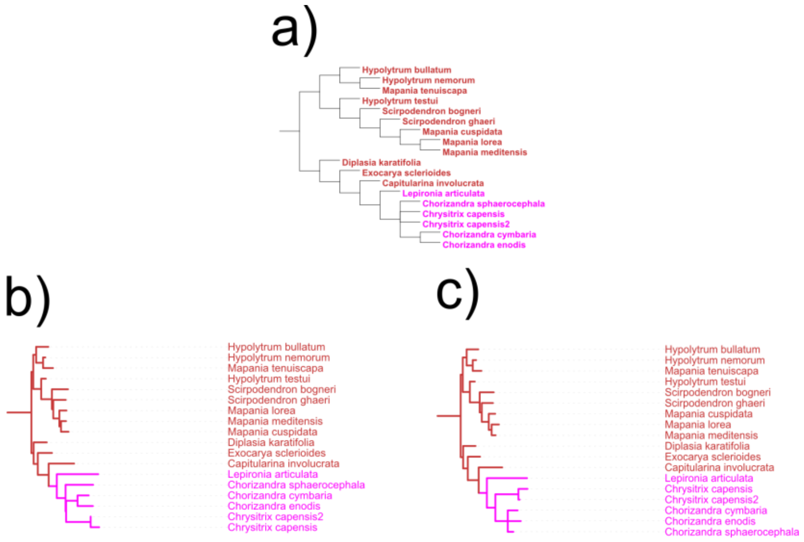


Fig. 3: Phylogeny of Mapanioideae based on total-evidence analyses; a) MP, b) ML, c) BI.

Abildgaardieae

Abildgaardieae *sensu* Govaerts et al. (2007) appeared monophyletic in all of our analyses (Fig. 4). Considering inner relationships, our results are not in complete agreement with previous publications, as *Bulbostylis* is not monophyletic (as appeared in Ghamkhar et al., 2007) due to *B. juncoides* that is placed outside the *Bulbostylis* spp., and due to *Nemum spadiceum* placed, on the contrary, inside the *Bulbostylis* Clade.

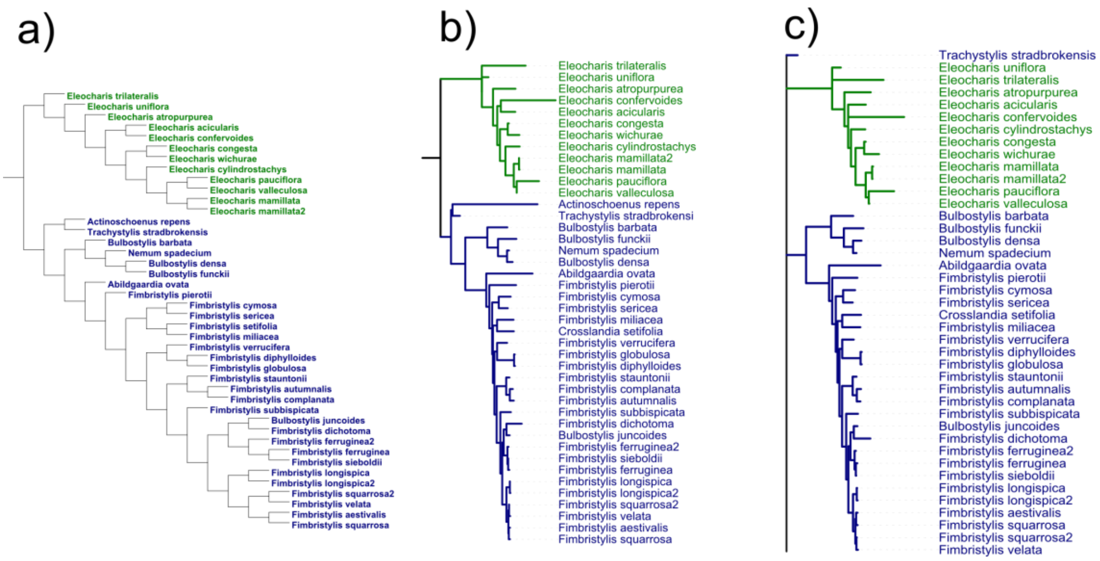


Fig. 4: Phylogeny of Abildgaardieae and Eleocharideae based on total-evidence analyses; a) MP, b) ML, c) BI.

Firstly, *B. juncoides* was not involved in the previous studies, so we cannot compare our results with theirs. Nevertheless, the morphology of inflorescence of this taxon supports its placement in *Bulbostylis*. One possible explanation is that the voucher (from which came both sequences for ITS and *trn*L-*trn*F) was incorrectly determined, or the DNA was contaminated, and therefore the position is biased. Resampling of this taxon might shed light on this problem.

Secondly, *Nemum spadiceum* is strongly bound with *B. densa* in the *Bulbostylis* Clade. Placing this genus inside *Bulbostylis* appeared also in previous studies; always with strong support (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007). The morphology of *Nemum* does not place the genus definitely closer to *Bulbostylis* or *Fimbristylis* – it has a *Bulbostylis*-type embryo (Van der Veken, 1965), a *Fimbristylis*-type inflorescence (Goetghebeur, 1986), and a fimbristyloid leaf with C4 photosynthesis (Bruhl & Wilson, 2007, Raynal, 1973). Therefore, *Nemum spadiceum* might be merged with *Bulbostylis* on the basis of molecular data, or it might be resampled from different material (because the same sequence of *Nemum spadiceum* was used in all analyses) and used in a narrowly-focused analysis.

Finally, let´s focus on the second clade of Abildgaardieae – the *Fimbristylis* Clade. *Crosslandia setifolia*, which is a taxon placed inside *Fimbristylis* spp., was already suggested for transfer into the genus *Fimbristylis* by Ghamkhar et al. (2007), and we also provided strong support for this incorporation. Morphology places *Crosslandia setifolia* into a separate genus due to unisexual flowers and amphicarpy which are unique features in Abildgaardieae (Bruhl, 1994, Goetghebeur, 1998). On the other hand, the *Fimbristylis*-type embryo (Goetghebeur, 1986) shows a close evolutionary relationship between these two genera.

Cariceae

Cariceae is a separate and a well-supported clade (Fig. 5). The only problematic taxon is *Amphiscirpus nevadensis* (Scirpeae) which is discussed in the chapter Scirpeae. The inner topology is basically in agreement with the recent conception of the tribe: *Siderostictae* clade on the basis (in our analyses, only *C. siderosticta* is present), the *Vignea* Clade (subg. *Vignea* and *Primocarex*), the Core *Carex* Clade (mixed subg. *Carex*, *Indocarex* and *Primocarex*), and the Caricoid Clade (subg. *Carex*, *Primocarex* and genera *Cymophyllum, Kobresia, Schoenoxiphium* and *Uncinia*) (Jung and Choi, 2013, Leveille-Bourret et al., 2014, Starr and Ford, 2009, Waterway and Starr, 2007). The last named clade is recently divided into two clades: the *Schoenoxiphium* Clade, and the Core Unispicate Clade (Leveille-Bourret et al., 2014, Starr and Ford, 2009, Waterway and Starr, 2007). In all our analyses (including the extended ITS analysis, see Fig. 6), the *Vignea* clade appeared monophyletic (*C. dioica*, subg. *Primocarex*, is present similarly to Starr and Ford, 2009), and the Core *Carex* Clade also corresponds with other publications. The previously-stated topology of the Caricoid Clade was also presented in most of our analyses; the extended ITS dataset, however, does not support the resolution of the two basic clades. Nevertheless, all the topologies of Cariceae are with no or very low support, so no definite conclusion can be proposed.

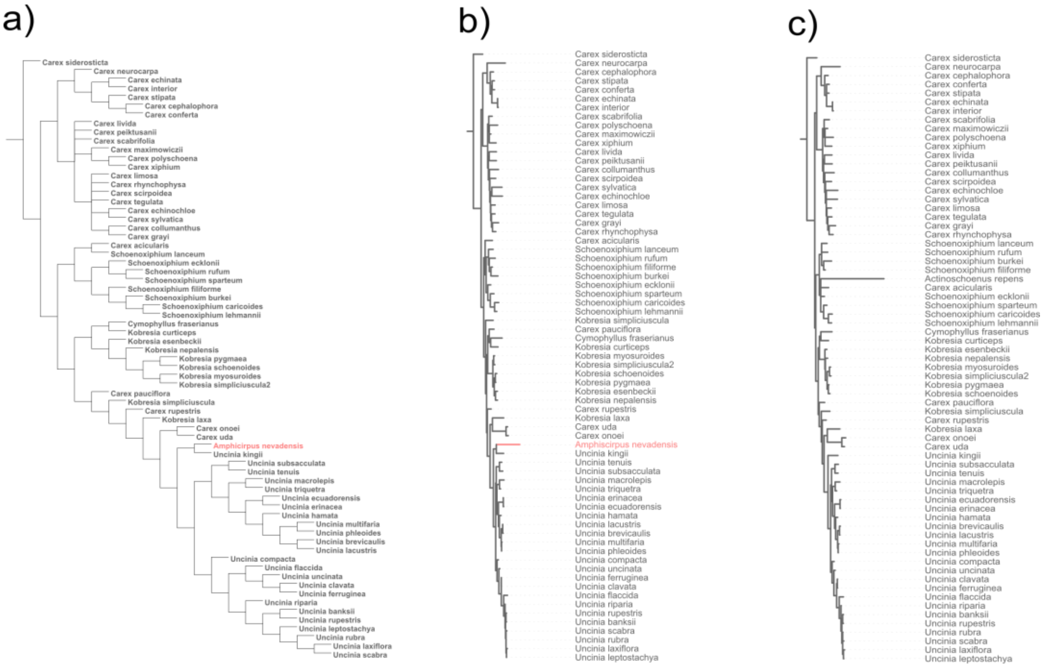


Fig. 5: Phylogeny of Cariceae based on total-evidence analyses; a) MP, b) ML, c) BI.

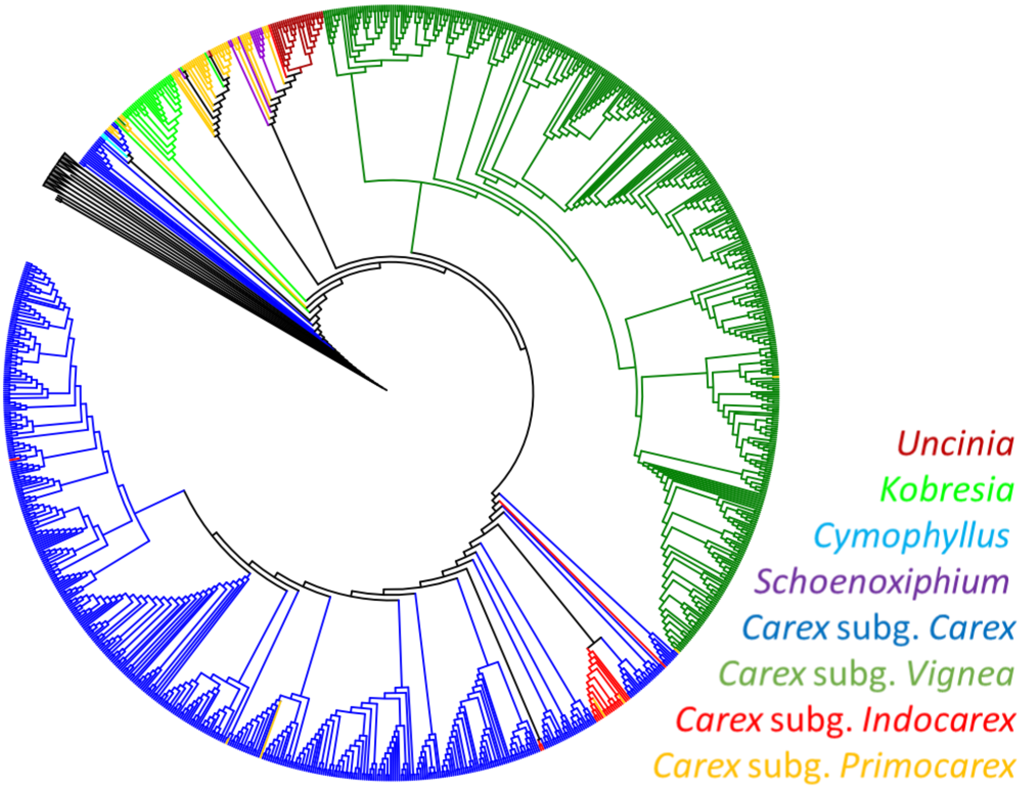


Fig. 6: Phylogeny of Cariceae based on the ML analysis of ITS2.

Cypereae

The monophyly of this tribe was confirmed by all total-evidence analyses, and monophyly of the two recognised clades of Cypereae (i.e. *Cyperus* and *Ficinia* Clades) was confirmed as well (Fig. 7). The previously-stated topology of *Cyperus* Clade, where C3 plants of subgenus *Anosporum* stay on the basis in polyphyly and C4 plants evolved once clustering in a monophyletic clade, was confirmed with only one exception – a C4 plant *Cyperus iria* (sampled in ITS datasets) was placed inside subg. *Anosporum*. This placement of a C4 plant inside the C3 plants might have two possible explanations: either the sequence was provided for a suprageneric study with a low sampling in Cypereae (Hirahara et al., 2007) where the wrong determination of *Cyperus* could not be found out, or the uncertainty of the ITS marker in such low-level phylogeny was expressed.

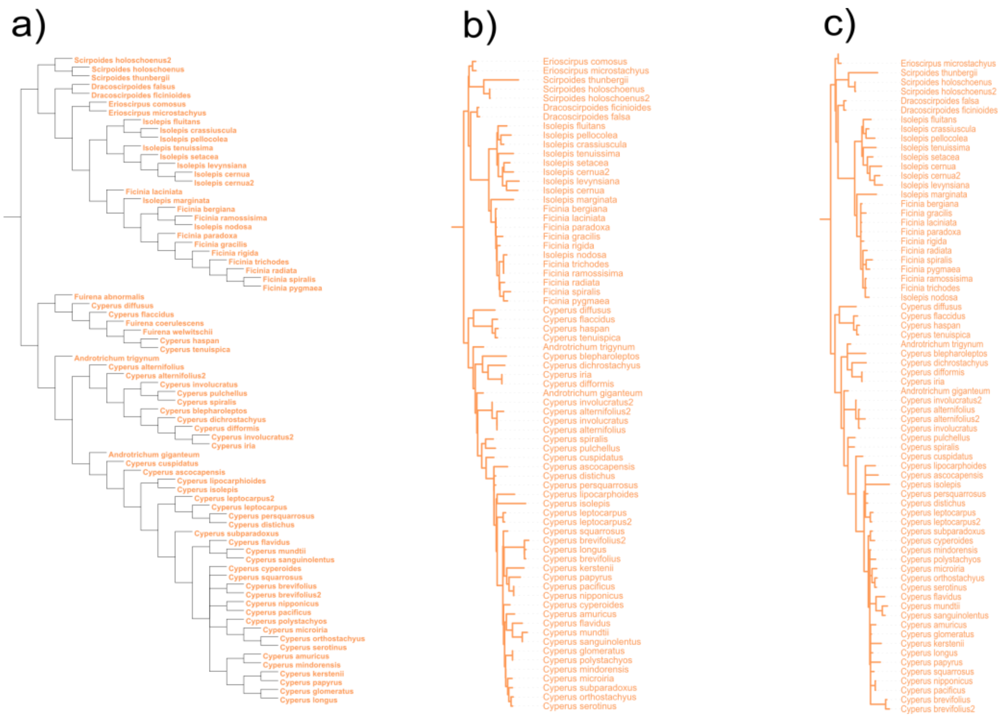


Fig. 7: Phylogeny of Cypereae based on total-evidence analyses; a) MP, b) ML, c) BI.

The position of the genus *Androtrichum* is slightly unsure, as it was placed by previous studies on the basis of the *Cyperus* Clade (Muasya et al., 2009a, 2014), but our total-evidence analyses placed it inside subg. *Androtrichum*. Unfortunately, we obtained sequences only for *rbc*L gene; analysing *rbc*L independently, *Androtrichum* remains on the basis of *Cyperus*, so the positioning in total-evidence trees might be caused by missing data from the other two markers. *Androtrichum*, therefore, most probably stays on basis of *Cyperus* Clade with the other C3 plants (Bruhl and Wilson, 2007).

When considering the *Ficinia* Clade of Cypereae, some unclear cases appeared. *Isolepis marginata* and *I. nodosa* was placed among *Ficinia* spp. This paraphyly of *Ficinia* and *Isolepis* is in agreement with latest studies of the group (Hinchliff and Roalson, 2013, Muasya et al., 2009a, 2014, Simpson et al., 2007). Moreover, the diagnostic morphological characteristic for *Ficinia* vs. *Cyperus* does not work without exceptions (Muasya et al., 2001, 2006, 2007, 2009a, 2009b, Larridon et al., 2011); therefore, detailed study and reclasification of these two genera is needed. The other genera of the *Ficinia* Clade (namely *Dracoscirpoides*, *Erioscirpus*, and *Scirpoides*) separated mostly in monophyletic clades.

Eleocharideae

This tribe appeared as monophyletic in all of our analyses as well as in previous studies (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Simpson et al., 2007). Its close relationship with Abildgaardieae was already stated, but the specific closeness of the relationship was not clear so far. Muasya et al. (2009) suggested a mixed clade of Eleocharideae, Abildgaardieae and some parts of Fuireneae; Simpson et al. (2007) did not join the two tribes into one clade, and placed a part of Fuireneae as a sister to Eleocharideae; only Hinchliff and Roalson (2013) and Hinchliff et al. (2010) separated clade of Eleocharideae and sister Abildgaardieae. Our data strongly support the latter-mentioned topology (Fig. 4). Therefore, the monophyly of a clade of Abildgaardieae + Eleocharideae (and their sister position) can be stated.

The inner relationships can also be viewed in detail. Support for involvement of *Chillania* and *Websteria* into the genus *Eleocharis* (Hinchliff et al., 2010) was found in our analyses, and even the structure of inner topology of *Eleocharis* was outlined. Specifically, the analyses of the complete dataset of *Eleocharis* in ITS (Fig. 8) suggested monophyly of subgenera *Limnochloa* and *Scirpidium*; *Zinserlingia* might be monophyletic if we admit *E. tortilis* (subg. *Eleocharis*) to be part of this subgenus even despite the morphological and distributional dissimilarities (as already suggested by Roalson et al., 2010).

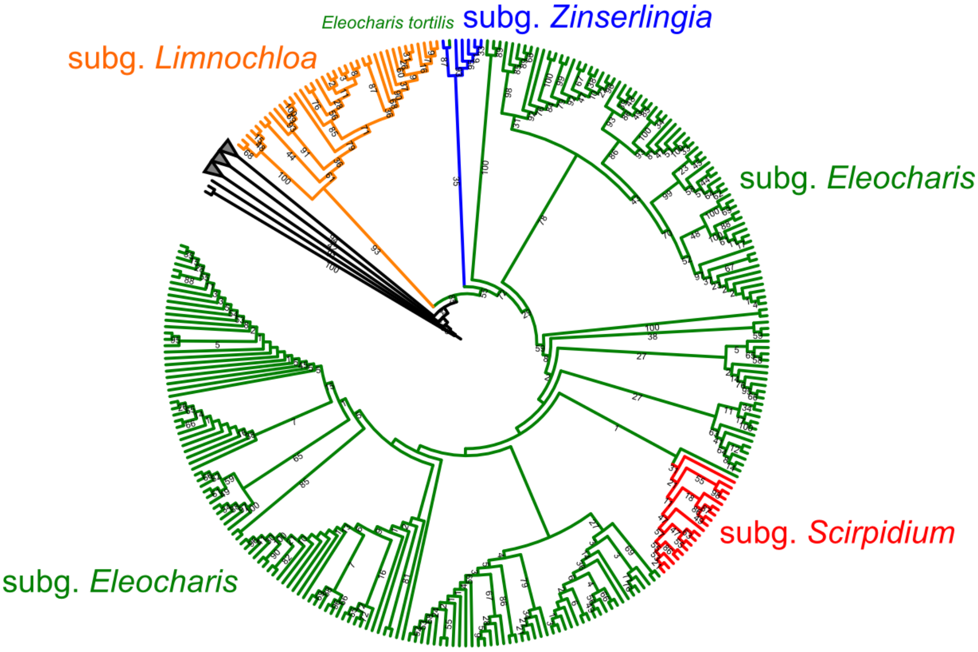


Fig. 8: Phylogeny of Eleocharideae based on the ML analysis of ITS2.

Fuireneae

Fuireneae is one of the clearly polyphyletic and unnatural tribes (Hinchliff and Roalson, 2013, Muasya et al., 2009a, Shiels et al., 2014, Simpson et al., 2007) and this was also documented by our results, which divided the tribe into three clearly separate groups (Fig. 9). The inner relationships are, however, not so clear and have to be discussed more completely.

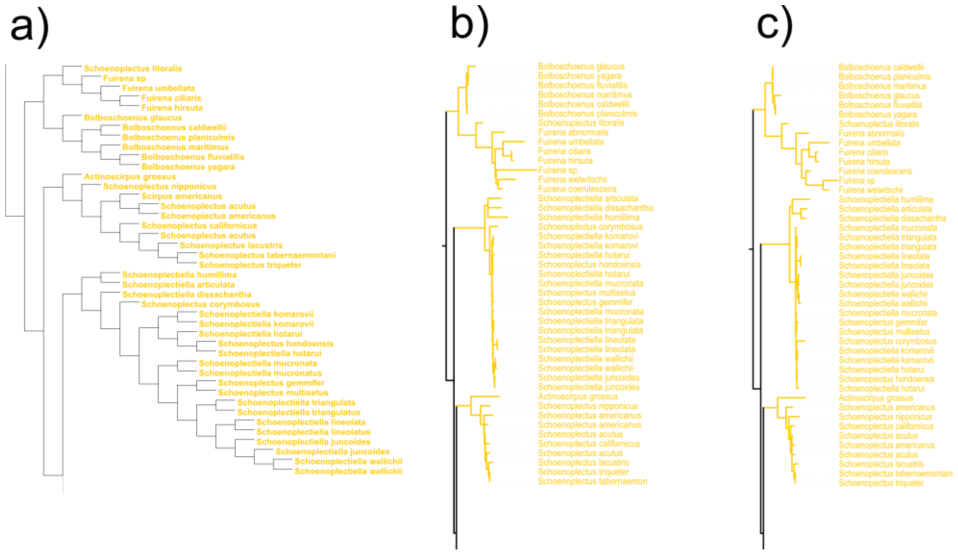


Fig. 9: Phylogeny of Fuireneae based on total-evidence analyses; a) MP, b) ML, c) BI.

Firstly, a very curious case is *Schoenoplectus litoralis*, which appeared in a clade of *Fuirena* in the total-evidence trees. Considering analyses of individual markers, nDNA analyses placed it in a clade of *Bolboschoenus*, *rbc*L analyses among other *Schoenoplctus* spp., and in *trn*L-*trn*F (similarly as in the total evidence analyses) together with *Fuirena* spp. This rather enigmatic position of the taxon might most probably be caused by an uncertain homology in alignment and, thus, unsteady position in the trees. Contamination or incorrect determination could be the second possible explanation in case of ITS and *trn*L-*trn*F, because data for these loci were sequenced as an outgroup (Ghamkhar et al., 2007), and a mistake of this type could not be identified then. In the case that these sequences are wrong, genus *Fuirena* is monophyletic and placed in a separate clade, sister to the monophyletic genus *Bolboschoenus*. As these genera are strongly supported as sister clades, they might belong to one separate tribe – Fuireneae. Nevertheless, this conclusion will not be definite until an investigation of the *Schoenoplectus litoralis* is done.

In spite of the resolution of *Schoenoplectus* and *Schoenoplectiella* as monophyletic genera in a previous study (Shiels et al., 2014), our data do not support this result, as four species of *Schoenoplectus* (namely S. *corymbosus, S, gemmifer, S. hondoensis*, and *S. multisetus*) appeared in the clade of *Schoenoplectiella*. Morphologically, *Schoenoplectiella* differs from *Schoenoplectus* by an unbranched inflorescence and culm-like primary bracts (Jung and Choi, 2011, Lye, 2003, Muasya et al., 2009a). Considering these two identification marks, *S. corymbosus* might be a morphologically transitional taxon with mediate-long culm-like primary bracts, but a rather branched inflorescence (Jimenez-Mejias et al., 2007); molecular data, however, have already clearly shown its affinity to *Schoenoplectiella* (Muasya et al., 2014). *S. gemmifer* is morphologically very close to *Schoenoplectiella mucronata* and *S. triangulata* (Maeda and Uchino, 2004, Maeda et al., 2004); and *S. hondoensis* and *S. multisetus* belong to the former section *Actaeogeton* (determined by the rugulose and blackish achenes when ripe and by the entire floral scale apices) as well as the three before-mentioned species (Yano and Hoshino, 2005). Therefore, all four *Schoenoplectus* spp. might be transferred to the genus *Schoenoplectiella* to keep both genera monophyletic in both aspects – morphological and molecular.

Similar to the clade of *Fuirena* and *Bolboschoenus*, *Schoenoplectus* (together with *Actinoscirpus* which was placed by our results, by Hinchliff and Roalson, 2013, and by Shiels et al., 2014, at the basis of *Schoenoplectus* clade) and *Schoenoplectiella* also keep strongly supported monophyletic clades in all analyses. Since both genera keep appearing separately, each of them should be placed in an individual tribe – *Schoenoplectiella* and *Pseudoschoenus* in the tribe Pseudoschoeneae, *Schoenoplectus* and *Actinoscirpus* in the tribe Actinoscirpeae.

Schoeneae

As was previously proposed on the basis of morphology and molecular data, Schoeneae is most probably a non-monophyletic tribe. The most outstanding result in our data is for *Cladium* which was placed separately and should be treated in a separate tribe Cladieae (probably together with its relative *Rhynchocladium* – not involved in our study). Morphologically, Cladieae has a unique inflorescence organisation where partial inflorescences are anthelate, corymbose, or capitately contracted (only one other genus of Schoeneae, *Machaerina,* has also partial inflorescences capitately contracted sometimes), and it has 2 (rarely 3) stamens (only *Carpha* and *Cyathochaeta* dispose with 2 stamens). The species of Cladieae are rhizomatous and stoloniferous perennials; leaves are elingulate with flat or revoluted blades; primary bracts are long, leaf-like and sheathing; the lower flower in the spikelet is male, upper flowers bisexual, or contrariwise; bristles are 4(–6) or absent; styles are 2(–3)-fid, style base is not distinct, is thickened and persistent; and achene is ovoid or oblong (Goetghebeur, 1998).

Monophyly of the remaining Schoeneae is also doubtful (Fig. 10). In many analyses there appeared a strongly-supported separate clade including *Carpha* and *Trianoptiles*; the rest of Schoeneae settled in another monophyletic clade. The close relationship of *Carpha* and *Trianoptiles* was already suggested by Verboom (2006) and Zhang et al. (2004, 2007); the exclusion of the genera from Schoeneae, however, is not supported strongly enough by our data.

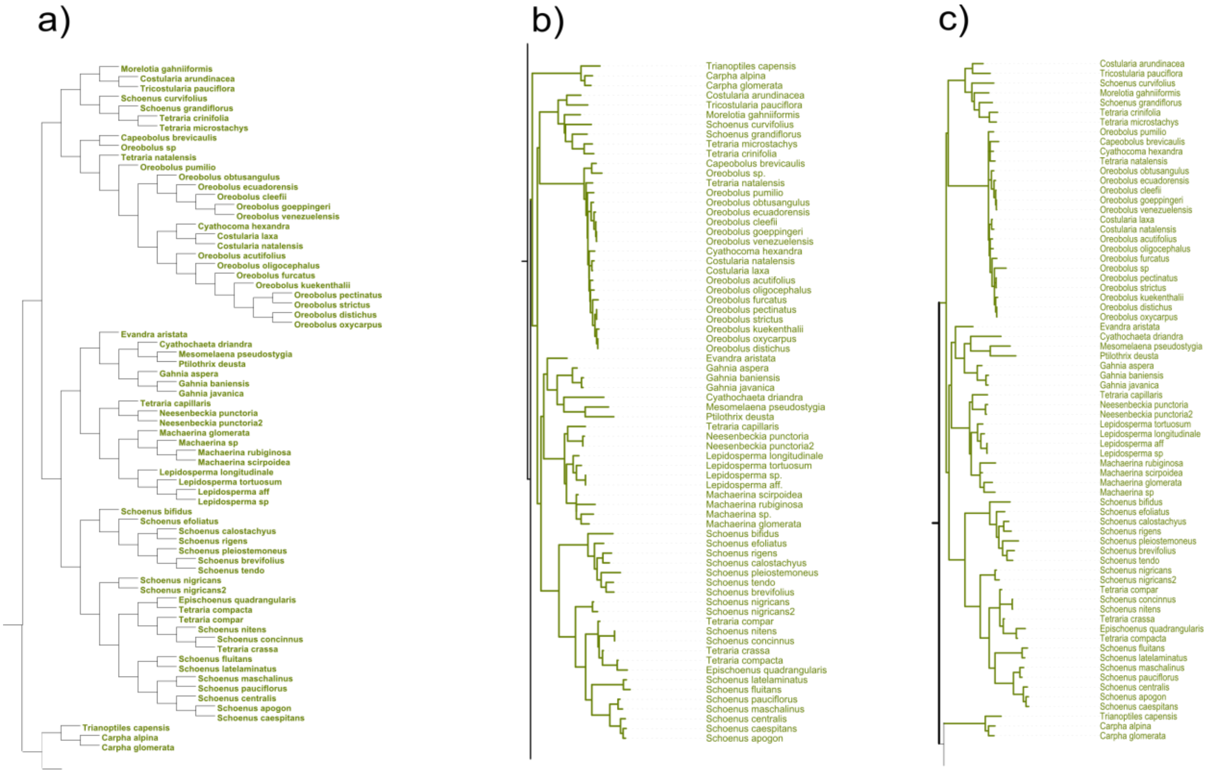


Fig. 10: Phylogeny of Schoeneae based on total-evidence analyses; a) MP, b) ML, c) BI.

Considering the rest of the Schoeneae, which are definitely clustered in a monophyletic clade, some strongly-supported clades can be distinguished. Even though their intra- and intertopology is uniform in total-evidence analyses, this varies in the separate analyses. Therefore, we cannot state that the topology is definite, only taxa contained in individual clades are definite.

The first of the strongly supported clades, the *Oreobolus* Clade (Verboom, 2006), contains *Capeobolus*, *Costularia* (namely *C. natalensis* and *C. laxa*), *Cyathocoma*, *Oreobolus*, and *Tetraria* (*T. natalensis*). This clade was also found by Hinchliff and Roalson (2013), Jung and Choi (2013), Muasya et al. (2009a), and Verboom (2006), and it is supported morphologically by special cone-like silica bodies associated with the anticlinal walls of the leaf epidermis (Browning and Gordongray, 1995a, Metcalfe, 1971, Seberg, 1988).

The *Lepidosperma* Clade (Verboom, 2006) contains *Lepidosperma*, *Machaerina*, *Neesenbeckia*, and *Tetraria* (namely *T. capillaris*). The clade, which has a relict Gondwanan distribution (Verboom, 2006), was presented also by Hinchliff and Roalson (2013), Jung and Choi (2013), Muasya et al. (2009a), Simpson et al. (2007), and Verboom (2006).

The *Gahnia* Clade (Verboom, 2006) consistently contains *Cyathochaeta*, *Gahnia*, *Mesomelaena*, and *Ptilothrix*. Considering topology based on individual markers, unstable positions are assigned to *Gahnia* (in the *Lepidosperma* Clade, in the *Gahnia* Clade, or solitary near to *Evandra*) and *Ptilothrix* (placed in the *Gahnia* Clade, in the *Schoenus* Clade, or sister to *Hypolythrum* in ITS); but the total-evidence matrix always supports the topology with *Gahnia* and *Ptilothrix* in the *Gahnia* Clade, and *Evandra* might be basal to the *Gahnia* Clade. Affinity within the clade, as described by Hinchliff and Roalson (2013), Jung and Choi (2013), Muasya et al. (2009a), Simpson et al. (2007), Verboom (2006), Zhang et al. (2004), is strongly supported also by morphological analyses performed by Zhang et al. (2007).

The *Tricostularia* Clade (Verboom, 2006) contains *Costularia* (*C. arundinacea*), *Morelotia*, *Schoenus* (*S. curvifolius, S. grandiflorus*), *Tetraria* (*T. crinifolia, T. microstachys*) and *Tricostularia*. No study with all these genera was performed yet, but all molecular studies clustered part of the mentioned genera together (Hinchliff and Roalson, 2013, Jung and Choi, 2013, Muasya et al., 2009a, Verboom, 2006, Zhang et al., 2004). Only the morphological cladistics analysis of Zhang et al. (2007) did not support the affinity of this group of taxa. Despite no proper differentiation of this clade, some specifics can be found. The *Tetraria* spp. of this clade (in contrast to the *Tetraria* spp. of the *Oreobolus* Clade and the *Schoenus* Clade), for example, all have nodes between leaves and spikelets, and a distinctive, reticulate tunic around the culm base (Verboom, 2006). The reticulate sheaths might be a diagnostic marker for the whole clade (Verboom, 2006).

The last, *Schoenus* Clade (Verboom, 2006), contains *Epischoenus*, *Schoenus*, and *Tetraria*. Also this clade was described before by Hinchliff and Roalson (2013), Jung and Choi (2013), Muasya et al. (2009a), and Verboom (2006). The affinity of *Epischoenus* and *Schoenus* is also based on morphological similarity (Browning and Gordongray, 1995b), and the *Tetraria* spp. pasted in this clade, in contrast to *Tricostularia* Clade, have unnoded culms and sheath bases (Verboom, 2006).

According to the molecular data analysed in a composed matrix, *Carpha*, *Gahnia*, *Lepidosperma*, *Machaerina*, and *Neesenbeckia* might be monophyletic; other genera are either paraphyletic, or represented by only one taxon in our analyses.

Scirpeae

Scirpeae is either monophyletic (in the small total-evidence analyses), or divided into two separate (but closely related) clades (by the larger total-evidence analyses; Fig. 11). These clades can be named the *Trichophorum* Clade (containing *Oreobolopsis* and *Trichophorum*) and the *Scirpus* Clade (containing *Amphiscirpus*, *Eriophorum*, *Phylloscirpus*, *Scirpus*, and *Zameioscirpus*). Whne considering the branches, the two clades are strongly supported. The probable paraphyly of the tribes has already been presented many times (Gilmour et al., 2013, Jung and Choi, 2013, Leveille-Bourret et al., 2014, Muasya et al., 2009a, Yano et al., 2012), and our data also agree with the non-monophyly. The monophyletic clade of Scirpeae, shown by the total-evidence trees based on the small dataset, might be over-optimistic due to the low sampling.

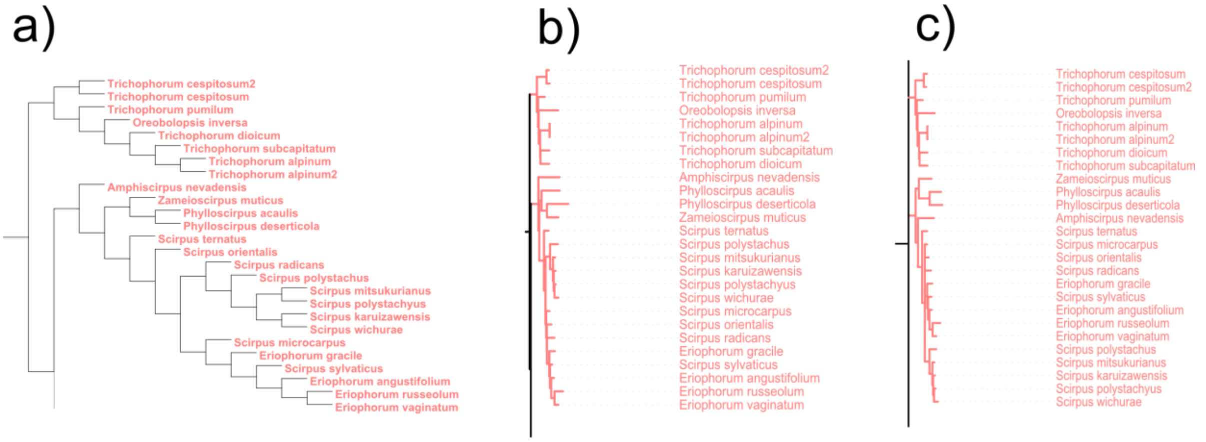


Fig. 11: Phylogeny of Scirpeae based on total-evidence analyses; a) MP, b) ML, c) BI.

Looking on the placement of Scirpeae among the other tribes, previous studies supported its close relationship with the clades of Dulicheae, Cariceae, and *Khaosokia*. Hinchliff and Roalson (2013) suggested resolution of these four mentioned lineages, while Leveille-Bourret et al. (2014) described four clades of Dulicheae, *Calliscirpus*, *Trichophorum*, and Cariceae, and Muasya et al. (2009a) simply pointed out that too many tribes are probably recognised. Therefore, the joining of Cariceae, Scirpeae, Dulicheae and basal *Khaosokia* into one large tribe might be the best solution; another possible treatment is to divide Scirpeae into two tribes (Scirpeae and Trichophoreae), but it will not solve the problem of unstable position among Cariceae, Dulicheae, Scirpeae and *Khaosokia*.

The inner relationships of individual clades of Scirpeae are also complicated. In the *Trichophorum* Clade, *Oreobolopsis* was pasted inside *Trichophorum*. In light of this result, *Oreobolopsis* should be transferred into *Trichophorum*, despite its petaloid perianth (perianth is bristle-like or absent in *Trichophorum*; Dhooge and Goetghebeur, 2002; Koyama and Guaglianone, 1987); this character, nevertheless, is thought to have doubtful value in taxonomic classification (Leveille-Bourret et al., 2014). The join of the two genera was proposed in previous studies (Dhooge, 2005, Leveille-Bourret et al., 2014).

In the *Scirpus* Clade, *Scirpus* and *Eriophorum* are unsupported as monophyletic genera, which was already shown by Gilmour et al. (2013), Hinchliff and Roalson (2013), Jung and Choi (2013), and Leveille-Bourret et al. (2014), who nested *Eriophorum* inside *Scirpus* or placed both genera in unresolved polytomy. The first type of topology is explained by gradual reduction of the compound anthelae of *Scirpus* to simple anthela of multispicate and finally unispicate *Eriophorum* spp. (Leveille-Bourret et al., 2014), and also by increasing chromosome number along this topology (Ball and Wujek, 2002, Shuyler, 1963). Nevertheless, our data possess no statistical support for the topology inside *Scirpus* Clade; therefore, no definite verdict can be stated.

The last two problems that occurred in Scirpeae are sequences of *Amphicirpus nevadensis* and *Trichophorum cespitosum. Amphicirpus nevadensis* (ITS sequence; AF190618.1) is probably a mistaken sequence, which is aligned to Cariceae by NCBI BLAST server (Johnson et al., 2008); and *Trichophorum cespitosum* (*rbc*L sequence; Y12969.1), which was placed outside *Trichophorum* spp. in the separate *rbc*L MP analysis, is probably an LBA artefact of maximum parsimony.

Conclusion

To conclude, we screened the evolutionary relationships of Junaceae and Cyperaceae on the basis of 538 taxa, although data from 1174 taxa were used in the main and the supporting analyses in total. Data from cpDNA and nDNA were analysed by maximum parsimony, maximum likelihood, and Bayesian inference. For phylogeny on this taxonomical level, cpDNA (i.e. *rbc*L and *trn*L-*trn*F) markers appeared much more useful than ITS due to the high rate of mutations in ITS which lead to homoplasy and, hence, unsure homology within alignment. Analyses of ITS are characterized by unsure topology on the level of species, and also on the higher taxonomic level of tribes, subfamilies, and families; only clades on the level of genera were well-supported. Therefore, this marker might not be recommended for phylogenies on the level of tribes, families and higher.

Besides this methodological outcome, main impact of our study lies in the taxonomical resolution of Juncaceae and Cyperaceae. Many previously-stated topologies were confirmed and supported by our large dataset, starting with confirmation of the monophyly of Cyperaceae and Juncaceae, followed by the inner topology of Juncaceae, and the resolution of Cyperaceae into two subfamilies – Mapanioideae and Cyperoideae; also monophyly and main inner topology of tribes Abildgaardieae, Bisboeckelereae, Cariceae, Cypereae, Cryptangieae, Eleocharideae, Rhynchosporeae, Sclerieae, and Trilepideae was confirmed.

Some previously suggested (but poorly supported) topologies were confirmed and taxonomical solutions for them were stated; this was done mostly for Cyperaceae, because Juncaceae were studied in detail over recent decades and inner relationships are known. Within Cyperaceae; the joining of Hypolytreae and Chrysitricheae was strongly supported, the sister position of Abildgaardieae and Eleocharideae was stated, the splitting of Fuireneae into 3 separate clades was suggested, the joining Cariceae, Dulicheae, Scirpeae, and *Khaosokia caricoides* into one tribe (or, on the contrary, splitting of Scirpeae into two tribes), and the separation of *Cladium* and *Rhynchocladium* into the new tribe Cladieae was suggested.

Finally, some reclasification of certain genera were proposed. Within Juncaceae, the small southern hemisphere genera (*Distichia, Marsippospermim, Oxychloë, Patosia*, and *Rostkovia*) should be transferred into the genus *Juncus*, or division of the genus *Juncus* into more genera is the second possible solution to the paraphyly of *Juncus*. Within Cyperaceae, *Nemum spadiceum* might be transferred into *Bulbostylis* (but further study might be required); *Schoenoplectus corymbosus, S. gemmifer, S. hondoensis*, and *S. multisetus* have to be transferred into the genus *Schoenoplectiella* on the basis of these and also formerly published data; and *Oreobolopsis* should be added to the genus *Trichophorum*.

We identified some taxa which possess special combinations of molecular and morphological features and their further study is recommended. These taxa are *Juncus capitatus* and *J. dregeanus* from Juncaeae; *Bulbostylis juncoides, Crosslandia setifolia, Schoenoplectus litoralis, S. americanus, Cyperus iria,* and *Amphiscirpus nevadensis* from Cyperaceae.

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Electronic supplements

Suppl. 1: The list of used sequences. “-” in pink cell indicates missing data for the locus, “#” in orange cell indicates a new sequenced data withou Acc.No.

Suppl. 2: Matrix of *rbc*L; 291 sequences for 278 taxa. The length of the matrix was 1240bp and it contained 487 variable positions; 366 positions were parsimonious informative.

Suppl. 3: Matrix of *trn*L-*trn*F; 318 sequences for 305 taxa. The length of the matrix was 3294bp and it contained 1965 variable positions; 1347 positions were parsimonious informative.

Suppl. 4: Matrix of ITS; 1778 sequences for 1019 taxa. The length of the matrix was 1150bp and it contained 855 variable positions; 751 positions were parsimonious informative.

Suppl. 5: Matrix of ITS1; 1802 sequences for 1031 taxa. The length of the matrix was 540bp and it contained 375 variable positions; 347 positions were parsimonious informative.

Suppl. 6: Matrix of ITS2; 1805 sequences for 1032 taxa. The length of the matrix was 495bp and it contained 411 variable positions; 368 positions were parsimonious informative.

Suppl. 7: Matrix of ITS; 424 sequences for 414 taxa. The length of the matrix was 1150bp and it contained 789 variable positions; 699 positions were parsimonious informative.

Suppl. 8: Matrix of ITS1; 426 sequences for 415 taxa. The length of the matrix was 540bp and it contained 361 variable positions; 330 positions were parsimonious informative.

Suppl. 9: Matrix of ITS2; 427sequences for 414 taxa. The length of the matrix was 495bp and it contained 391 variable positions; 345 positions were parsimonious informative.

Suppl. 10: MP analysis based on *rbc*L sequences. Strict consensus tree calculated from 22,982 equally parsimonious trees of length 2177 (CI=30, RI=85). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 11: Best tree from ML analysis based on *rbc*L under the evolution model GTR + C + Γ (log likelihood -14340.330908); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 12: Bayesian consensus tree of 43,889 trees based on *rbc*L sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 13: MP analysis based on *trn*L-*trn*F sequences. Strict consensus tree calculated from 7480 equally parsimonious trees of length 8951 (CI=39, RI=84). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 14: Best tree from ML analysis based on *trn*L-*trn*F under the evolution model GTR + C + Γ (log likelihood -48592.242327); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 15: Bayesian consensus tree of 140,597 trees based on *trn*L-*trn*F sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 16: MP analysis based on ITS sequences. Strict consensus tree calculated from 4209 equally parsimonious trees of length 8334 (CI=21, RI=85). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 17: Best tree from ML analysis based on ITS under the evolution model GTR + C + Γ (log likelihood -38990.769599); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths, c) the tree of extendet data set with 1778 sequences (log likelihood -74499.528024).

Suppl. 18: Bayesian consensus tree of 149,931 trees based on ITS sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 19: MP analysis based on ITS1 sequences. Strict consensus tree calculated from 16691 equally parsimonious trees of length 4118 (CI=20, RI=84). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 20: Best tree from ML analysis based on ITS1 under the evolution model GTR + C + Γ (log likelihood -19117.067928); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths, c) the tree of extendet data set with 1802 sequences (log likelihood -35036.160902).

Suppl. 21: Bayesian consensus tree of 150,000 trees based on ITS sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 22: MP analysis based on ITS2 sequences. Strict consensus tree calculated from 1686 equally parsimonious trees of length 4172 (CI=21, RI=85). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 23: Best tree from ML analysis based on ITS2 under the evolution model GTR + C + Γ (log likelihood -18997.333696); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths, c) the tree of extendet data set with 1805 sequences (log likelihood -34497.838316).

Suppl. 24: Bayesian consensus tree of 150,000 trees based on ITS sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 25: MP analysis based on the all *rbc*L + *trn*L-*trn*F + ITS sequences. Strict consensus tree calculated from 30 equally parsimonious trees of length 19,789 (CI=30, RI=84). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 26: Best tree from ML analysis based on the all *rbc*L + *trn*L-*trn*F + ITS sequences under the evolution model GTR + C + Γ (log likelihood -107808.264345); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 27: Bayesian consensus tree of 145,184 trees based on the all *rbc*L + *trn*L-*trn*F + ITS sequences; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 28: MP analysis based on the complete *rbc*L + *trn*L-*trn*F + ITS data set. Strict consensus tree calculated from 8 equally parsimonious trees of length 11,949 (CI=38, RI=76). Bootstrap and jackknife values are shown above and below individual branches.

Suppl. 29: Best tree from ML analysis based on the complete *rbc*L + *trn*L-*trn*F + ITS data set under the evolution model GTR + C + Γ (log likelihood -65182.646414); a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.

Suppl. 30: Bayesian consensus tree of 20,387 trees based on the complete *rbc*L + *trn*L-*trn*F + ITS data set; a) the tree without branch-lengths, numbers above branches indicate bootstrap support values, b) the tree with displayed branch-lengths.