

Oreojuncus, a new genus in the *Juncaceae*

Oreojuncus, nový rod čeledi *Juncaceae*

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Juncus trifidus and *J. monanthos*, two species traditionally included in section *Steirochloa* of the *Juncaceae*, are shown to differ substantially in their morphology from other members of this section. Their relationships with the other groups in this family based on DNA data of selected regions of plastome, chondriome and nrDNA, were examined using phylogenetic analyses of the combined data set of all these DNA regions. The resultant cladograms place *J. trifidus* and *J. monanthos* at a very basal position in the *Juncaceae*. Both the morphology and the phylogenetic analysis support the exclusion of these two species from *Juncus* and placing them in a separate genus. This genus is described under the name *Oreojuncus* Záveská Drábková et Kirschner. A detailed comparison with the other genera of the *Juncaceae* and a key for identifying these genera are provided.

Key words: chondriome, *Juncaceae*, *J. monanthos*, *Juncus trifidus*, nrDNA, phylogeny, plastome, taxonomy

Introduction

Since the early studies on *Juncaceae*, summarized in Buchenau (1890), the supraspecific system of the genus *Juncus* has not undergone substantial changes (if we disregard the newly described taxa and changes in the taxonomic rank of individual groups). It is primarily based on the morphology of the inflorescence and leaf anatomy and morphology. The system is very practical for the elementary classification into species groups, which is one of the reasons for its stability. There are several recent studies that elucidate the generic system of the family (Balslev 1996, 1998) and the supraspecific division of *Juncus* and make necessary nomenclatural adjustments (Novikov 1990, Kirschner et al. 1999, 2002a, b, c). The latter of these studies also provides a complete taxonomic and nomenclatural revision of the *Juncaceae* (see also Table 1). The widely used system of the family can be outlined as given in Table 1; in the literature individual groups may appear as subgenera but their circumscription, in principle, does not vary. There is, however, a group that represents an exception to the generally accepted system of *Juncus*, which consists of *Juncus trifidus* and *J. monanthos*. The evaluation of the aberrant morphological attributes of this group and results of comprehensive molecular analyses of the *Juncaceae* (Drábková et al. 2003, 2004, 2006, Záveská Drábková 2010, Záveská Drábková & Vlček 2009, 2010) showing an unexpected position of *J. trifidus* and *J. monanthos* make it possible to draw the conclusions presented below.

Previous molecular studies of the *Juncaceae* and their limitations

Even the first molecular studies of samples of *Juncaceae* cast doubts upon this well established system of genera and sections. Based on plastome *rbcL* sequences several authors (Chase et al. 1993, Duval et al. 1993, Plunkett et al. 1995, Muasya et al. 1998, 2000) thought that the genus *Oxychloë* did not belong to the same group as the similar Juncaceous genera, *Distichia* and *Patosia*. This, however, was soon reliably disproven by Kristiansen et al. (2005) who analysed in detail both the voucher specimens and the sequences used by the previous authors and show that the previous results were based on contaminated and chimaeric sequences. Starr et al. (2007) arrived at the same conclusions, probably independently, and *Oxychloë* was returned to its original position. In order to summarize why so much effort was spent on the *Oxychloë* problem, we have to emphasize the difficulties associated with removing erroneous data from databases, mostly due to the lack of taxonomic expertise and a non-functional system of data validation or verification.

Later on, the molecular phylogeny of the *Juncaceae* attracted attention of several teams. In the majority of the studies, two main problems emerged: the existence of a so called Southern Hemisphere Clade (SHC) and the position of the group of *Juncus trifidus* L. Drábková et al. (2003) published a *rbcL* phylogeny characterized by substantially more representative sampling and another analysis of plastome (*trnL-trnF* intergenic spacer and the *trnF* intron, Drábková et al. 2004) corroborated the general phylogenetic pattern outlined in the previous work, but not the aberrant position of *J. trifidus*. However, in a comprehensive phylogeny of cyperids based on the *rbcL* of more than 320 taxa, *Juncus trifidus* is a sister taxon to *Luzula* (Záveská Drábková et al., unpubl.).

These studies were followed by that of Roalson (2005), a nrDNA study of a relatively limited sample of species and sections, which did not include three genera; this study again showed the above two problematic groups and in addition highlighted the unstable position of *Juncus capitatus*. It should be pointed out that Roalson (2005) identified *Juncus trifidus* as a sister taxon of *Luzula*. Drábková et al. (2006) summarized the plastome results for an even wider selection of species and corroborated the previous tree topologies that indicated a separate position for the *J. trifidus* group and elucidated the composition of SHC species (the *Juncus* sections *Juncus*, *Graminifolii* and *Caespitosi* were also suspected of belonging to it). Unstable position of *J. capitatus* and *J. filiformis* was accounted for in terms of a Long-Branch Attraction (LBA) phenomenon, an artefact of the MP analyses.

Jones et al. (2007) extended the plastome study by including sequences of the *rps16* intron combined with the *trnL-trnF* data, which placed *Juncus trifidus* in a basal position in the subg. *Agathryon* clade (however, they only present a single tree of the 206 equally parsimonious trees obtained). The low representativeness of the sampling (less than 40 species of *Juncaceae*) and the great gaps in the *rps16* sequences (five of 10 sections of *Juncus* are missing and two of three subgenera and three of seven sections in the subg. *Luzula* not covered) make the results rather unreliable.

The most recent studies (Záveská Drábková & Vlček 2009, 2010 and Záveská Drábková 2010) are characterized by extensive taxon sampling, with *Luzula* almost complete, and the inclusion of sequences of additional DNA regions. A comparison and interpretation of the information content of plastome and chondriome data (Záveská Drábková & Vlček 2009) confirm two remarkable results, i.e. the strange position of the *J. trifidus* group and the existence of the SHC. The last study (Záveská Drábková 2010) repeated

some of the analyses on a wider and corrected selection of taxa and compared the results of the chondriome, plastome and nrDNA studies.

Phylogenetic position of the *Juncus trifidus* group on the basis of previous molecular analyses

In the previous studies on the phylogeny and evolution of the *Juncaceae*, the problematic position of *Juncus trifidus* was first revealed by the analysis of the *rbcL* sequence data (Drábková et al. 2003). The *rbcL* analysis was repeated by Záveská Drábková (2010) using a corrected data set that did not include the wrongly determined GenBank *Oxychloë* sample (see also Kristiansen et al. 2005, Drábková & Vlček 2007). The analysis of the *rbcL* data showed that *J. trifidus* occupies a basal split in the tree for the whole genus *Juncus* and the Southern Hemisphere Clade (Záveská Drábková 2010; simplified tree Fig. 1A). Later on, further DNA regions were analysed by the present authors to ascertain whether there is a support for the unexpected position of the *Juncus trifidus* group.

When more variable non-coding regions of plastome are used (*trnL* intron and *trnL-F* intergenic spacer; Drábková et al. 2006), the *Juncus trifidus* group, together with *J. filiformis* of the sect. *Juncotypus*, form a sister group to the subg. *Juncus* (Fig. 1B). The close relationship between *J. trifidus* and *J. filiformis* seem to be the result of the long-branch attraction (LBA) phenomenon and varied within the topology depending on outgroup selection, as elucidated by Drábková et al. (2006). The maximum likelihood analysis of this data set revealed various positions for *Juncus trifidus*, *J. monanthos*, *J. capitatus* (sect. *Caespitosi*) and *J. filiformis* (for details, see Drábková et al. 2006).

Furthermore, we examined *atp1* and ITS regions in order to determine whether it is possible that the evolution of the plastome, chondriome and nuclear genomes followed divergent pathways. *Juncus trifidus* and *J. monanthos* form an early derived branch within the *Juncaceae* with very high support in the *atp1* tree (Záveská Drábková & Vlček 2009; Fig. 1C). In the nrDNA ITS tree, *J. trifidus* and *J. monanthos* are in an unresolved position with *J. capensis*, *J. fontanesii* and *J. capitatus* as a sister group to *Luzula* (Záveská Drábková 2010; Fig. 1D).

Results of the above analyses are summarized in Fig. 1, which is a slightly modified version of the original sources.

Supraspecific division of *Juncus*: an introductory overview

In principle, the current morphological system of *Juncus* includes two groups, subg. *Juncus* (or “*eprophyllati*” of Buchenau 1906) and subg. *Agathryon* (or “*prophyllati*” of Buchenau, l.c.), each with a series of sections (subgenera in the works of Buchenau). Within the latter subgenus, four sections are recognized (Table 1). The sect. *Tenageia* is comprised of tiny annuals sharing many features of general habit, the sect. *Juncotypus* is easily characterized by leafless stems and the lowest bract forming an apparent prolongation of the stem (the inflorescence therefore is pseudolateral), the monotypic sect. *Forskalina* has a thick creeping rhizome and leafy stem with terete leaf blades; all three sections are relatively homogeneous. On the other hand, the section *Steirochloa* in the traditional system consists of taxa of a very varied appearance, having stem leaves or only

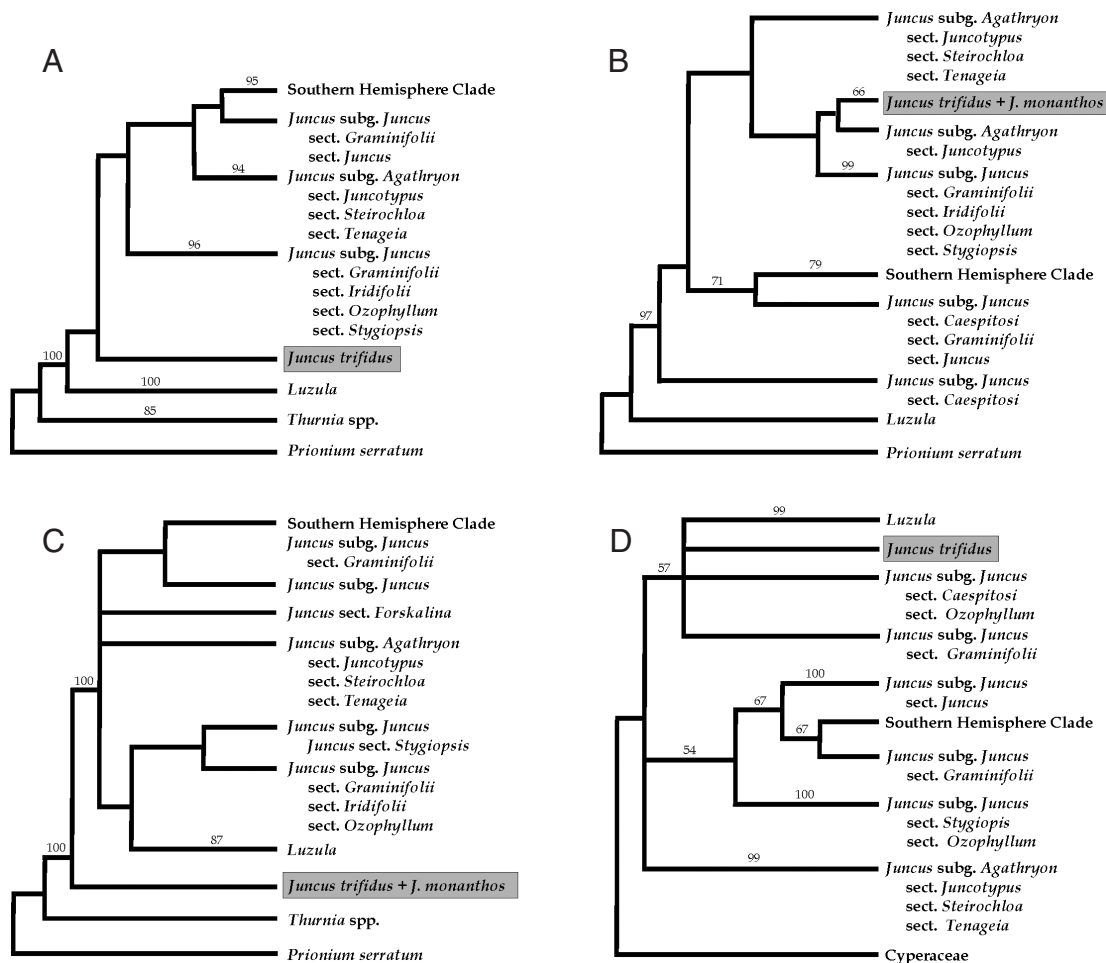


Fig. 1. – Summary of previous studies showing relationships among major clades within the *Juncaceae* with the position of *Juncus trifidus* and *J. monanthos* marked by a shaded box. A simplified strict consensus maximum parsimony (MP) tree based on (A) *rbcL* sequence data. Length 659, CI = 0.55, RI = 0.84, total number of characters TNC = 1,428, total number of parsimony informative characters PIC = 209, modified from Závěská Drábková (2010), (B) *trnL-F* sequence data. Length 2,664, CI = 59, RI = 79, TNC = 2,377, PIC = 612, modified from Drábková et al. (2006), (C) *atp1* sequence data. Length 374, CI = 86, RI = 90, TNC = 1,264, PIC = 169, modified from Závěská Drábková and Vlček (2009), (D) ITS1-5.8S-ITS2 sequence data. Length 1,941, CI = 0.55, RI = 0.90, TNC = 743, PIC = 404, modified from Závěská Drábková (2010). Numbers above branches indicate jackknife support.

basal ones, flowers solitary on pedicells or sessile or in distinct clusters, leaves flat or almost round. The most aberrant group usually included in the sect. *Steirochloa* is comprised of *Juncus trifidus* L. and *J. monanthos* Jacq. It is characterized by a number of attributes otherwise not found in this section or even absent from, or rare in, the genus *Juncus*. These differences (Table 2) are conspicuous; two authors who accorded this group the rank of section, sect. *Trifidi* Rouy (Rouy 1912, Novikov 1990) used some of them, i. e. general habit characters and the lacerate auricles, to support the exclusion of the *J. trifidus* group from the sect. *Steirochloa*.

Table 1. – The established traditional system of the *Juncaceae* (Kirschner et al. 2002a, b, c).

Genus	Subgenus	Section
<i>Juncus</i> L. [type: <i>J. acutus</i> L.]	<i>Juncus</i>	<i>Juncus</i> <i>Graminifolii</i> Engelm. <i>Caespitosi</i> Cout. <i>Stygiopsis</i> Gand. ex Kuntze <i>Iridifolii</i> Snogerup et Kirschner <i>Ozophyllum</i> Dumort. <i>Tenageia</i> Dumort. <i>Steirochloa</i> Griseb. <i>Juncotypus</i> Dumort. <i>Forskalina</i> Kuntze
<i>Luzula</i> DC. [type: <i>L. campestris</i> (L.) DC.]	<i>Marlenia</i> Ebinger <i>Luzula</i>	<i>Anthelaea</i> Griseb. <i>Atlanticae</i> Kirschner <i>Nodulosae</i> Chrtek et Křisa <i>Diprophyllatae</i> Satake <i>Alpinae</i> Chrtek et Křisa <i>Thysanochlamydeae</i> Satake <i>Luzula</i>
<i>Marsippospermum</i> Desv. <i>Oxychloë</i> Philippi <i>Patosia</i> Buchenau <i>Rostkovia</i> Desv. <i>Distichia</i> Nees et Meyen	<i>Pterodes</i> (Griseb.) Buchenau	

Table 2. – Diagnostic morphological features distinguishing *Juncus trifidus*, *J. monanthos* and the sect. *Steirochloa* (data modified from Kirschner et al. 2002c).

Feature	<i>Juncus trifidus</i> L.	<i>J. monanthos</i> Jacq.	sect. <i>Steirochloa</i>
Auricles	lacerate-fimbriate	lacerate-fimbriate	entire
Leaf margin	serrulate	serrulate	smooth, entire
Leaf margin sclerenchyma	not or weakly developed	not or weakly developed	well developed
Enlarged cells in adaxial leaf epidermis	weakly developed	weakly developed	usually well developed
Inflorescence	(1–)2–3(–4) flowered	1–(2–3) flowered	more than 3-flowered, usually more than 10-fl.
Tepals	2.0–4.2 mm	4.0–5.2 mm	2–6 mm
Cataphyll blade	up to 1 cm	6–10 cm	1.5–6.0 cm
Seeds	seed coat loose, appendages 0.2–0.3 mm long	seed coat loose, appendages 0.4–0.5 mm long	seed coat tight, appendages ± absent ¹

¹In *J. vaseyi* appendages are present, 0.2–0.5 mm long but have a different nature from those of *J. trifidus* and *J. monanthos*: they are smooth, thin, tail-like in the former while in the latter two taxa, they are thick and wrinkled.

Material and methods

Plant samples

A list of taxa and specimens used in the phylogenetic analyses, including the GenBank sequence accessions, are given in Drábková et al. (2003, 2004, 2006) and Závěská Drábková & Vlček (2009, 2010), see also Appendix 1. The plant material was collected with permission in AAU, BM, C, K, NY, PRA, RSA-POM, and the specimens newly collected by the authors are deposited in PRA (all the abbreviations follow the Index Herbariorum, see <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Data for the morphological characters were compiled from the literature (Kirschner et al. 2002a, b, c), supplemented and specified by herbarium studies, preferably in PRA, but also in AAU, B, BM, BP, C, E, GOET, K, KRA, KRAM, L, LI, NY, RSA-POM, etc. Names of taxa and authors follow the monographic treatment of the family by Kirschner et al. (2002a, b, c).

Electron microscopy

SEM examinations of seeds were made on at least five seeds per taxon under a scanning electron microscope FEI Quanta 200 in the high vacuum mode (HV), low vacuum mode (LV) and Environmental Scanning Electron Microscope mode (ESEM). Upon comparison, the HV mode proved to be the best. Samples were used without any treatment as it was not necessary to remove the outer membranous layer of the seeds (Ertert 1983).

Molecular data analysis

DNA sequences were assembled in GeneSkipper (EMBL Heidelberg). Alignment of sequences was done manually using the sequence alignment editor BioEdit (Hall 1999) and insertions or deletions were detected. The nucleotide sequences used in this paper are deposited in GenBank (Appendix 1), and sequence alignments stored in TreeBase.

Phylogenetic analyses were performed on the combined data sets and separately on subsets of *trnL* intron plus *trnL*-F intergenic spacer, *rbcL*, *atpA* and ITS. In total, the matrix comprised ca 5.7 kb per taxon. Phylogenetic trees were constructed using *Pronium serratum* (*Prioniaceae*), *Thurnia polycephala* and *T. sphaerocephala* (*Thurniaceae*) as outgroups based on previous analyses (e.g. Plunkett et al. 1995, Drábková et al. 2003, 2006, Závěská Drábková & Vlček 2009, Závěská Drábková 2010). In order to reduce the potential effect of distant outgroups (from the *Cyperaceae*) on inference of relationships within the *Juncaceae*, these outgroups were excluded from the first analyses, although the inferred topologies from these different analyses are very similar. The ingroup taxa contained representatives of all currently recognized subgenera and sections of *Juncus* and *Luzula*. The gaps in the data matrix were treated as missing data. We included all codon positions based on presupposition that the third position in plastid data contains most of the phylogenetic information (Rydin et al. 2002). Two methods, maximum parsimony (MP) and maximum likelihood (ML) were used to avoid errors where high support values are found for groups not supported by the original data (Goloboff et al. 2003) and also to test the effect of long and extremely short branches on the tree topology.

Initially, phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). Due to the size of the matrix only heuristic search could be used with

1000 random input orders saving 100 trees per replicate, tree bisection and reconnection branch swapping (TBR), holding five trees per step and using steepest descent. A strict consensus tree was constructed.

In the second analysis, WinClada version 1.00.08 (Nixon 2002) running NONA version 2.0 (Goloboff 1999) as a daughter process, was used for the parsimony ratchet procedure to search treespace by re-weighting alternating iterations of a search (Nixon 1999). We performed the ratchet procedure (Nixon 1999) running 1000 replicates holding 25 trees at each replicate and sampling 45 characters. A strict consensus tree was constructed. The resultant cladograms were then submitted to commands “hard collapse unsupported nodes in all trees” and “keep best trees only”. Nodal support was determined by jackknifing where proportions were calculated in NONA using 1000 replicates, TBR branch swapping, holding five trees per replication.

Maximum likelihood analysis of the complete *Juncaceae* matrix was performed in GARLI version 0.96b8 (Zwickl 2006) to identify and avoid influence of the long-branch attraction to the tree. The best fitting model of DNA GTR+ Γ +I evolution was chosen using AIC criterion as implemented in Modeltest 3.7 (Posada & Crandall 1998). Multiple runs were performed to ensure that results are consistent as the algorithm is stochastic (Zwickl 2006). The log likelihood values of each run were retained in order to compare the individual runs. Branch support was determined by 1000 ML bootstrap iterations in GARLI.

Results

Separate status of Juncus trifidus and J. monanthos: evidence from morphology

With the exception of the lacerate-fimbriate auricles, the peculiar characters of these two species remained unnoticed. Buchenau (1890, 1906) mentioned only the unusual character of auricles, and from his drawing (Buchenau 1906: 110, fig. 60) it is obvious that he also recorded the absence of marginal sclerenchyma strands in transverse sections of its leaves, a character mentioned by S. Snogerup (Nilsson & Snogerup 1972). The latter author also summarized the other important characters of *J. trifidus*: mucronate connective, serrulate leaf margins, etc., which had not attracted the attention of Buchenau (*opera varia*). Not even the authors who accepted a separate section for the two species (Rouy 1912, Novikov 1990) mention any of the important distinguishing features summarized in Table 2 (except for lacerate auricles).

The lacerate-fimbriate auricles of the group of *J. trifidus* and *J. monanthos* are unique in this family but the other remarkable characters, when considered separately, are not so diagnostic or conspicuous. The marginal sclerenchyma strands $\pm$ missing from the leaves of this group and consistently present in the leaves of the rest of the sect. *Steirochloa*, for instance, are not developed in the closely related sect. *Tenageia* Dumort. The mucronate connective can be found also in two species of the subg. *Juncus*, *J. (Ozophyllum) scheuchzerioides* Gaudich. and *J. (Stygiopsis) castaneus* Sm. Also the loose seed coat is very occasionally found in other species of *Juncus* (Figs 2 and 3).

Only when all these characters are simultaneously compared with those of *Juncus* and other genera of *Juncaceae*, is it obvious that, morphologically speaking, the *J. trifidus* group is quite distinct (Table 3).

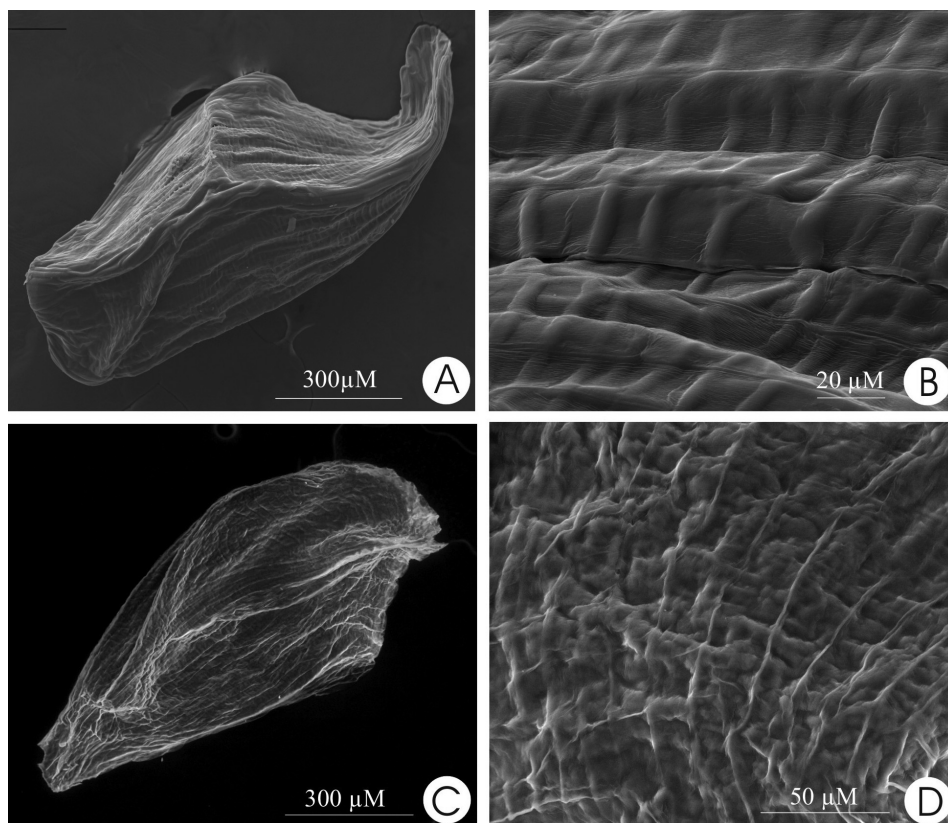


Fig. 2. – Seeds (left) and seed surface (right) of *Juncus trifidus* (A, B), *J. monanthos* (C, D). SEM pictures, scale bars on the photographs.

Separate status of the Juncus trifidus and J. monanthos group: evidence from karyology

Karyology traditionally is a source of important data for inferring evolutionary trends in the *Juncaceae*. As a consequence of chromosome fragmentation (agmatoploidy), it should be emphasized that chromosome number cannot be used for determining ploidy level, at least in *Luzula*. Most of the *Juncaceae* proved to be a rather difficult material for karyological studies and many chromosome counts are thought to be inaccurate (although often close to the correct number). There was a recent attempt to summarize chromosome evolution in the *Juncaceae* (Roalson 2005) in which conclusions were drawn about its phylogenetic importance. We shall try to rectify some of the most serious mistakes in the latter work. The chromosome counts cited below are referred to in the revision of this family by Kirschner et al. (2002a, b, c) and in Závěská Drábková (2013).

Fig. 3. – Seeds (left) and seed surface (right) of *Juncus gerardii* (section *Steirochloa*, A, B) and *J. squarrosus* (sect. *Steirochloa* (C, D), *J. filiformis* (sect. *Juncotypus*, E, F) and *Luzula confusa* (*Luzula* sect. *Thysanochlamydeae*, G, H). SEM pictures, scale bars on the photographs. ►

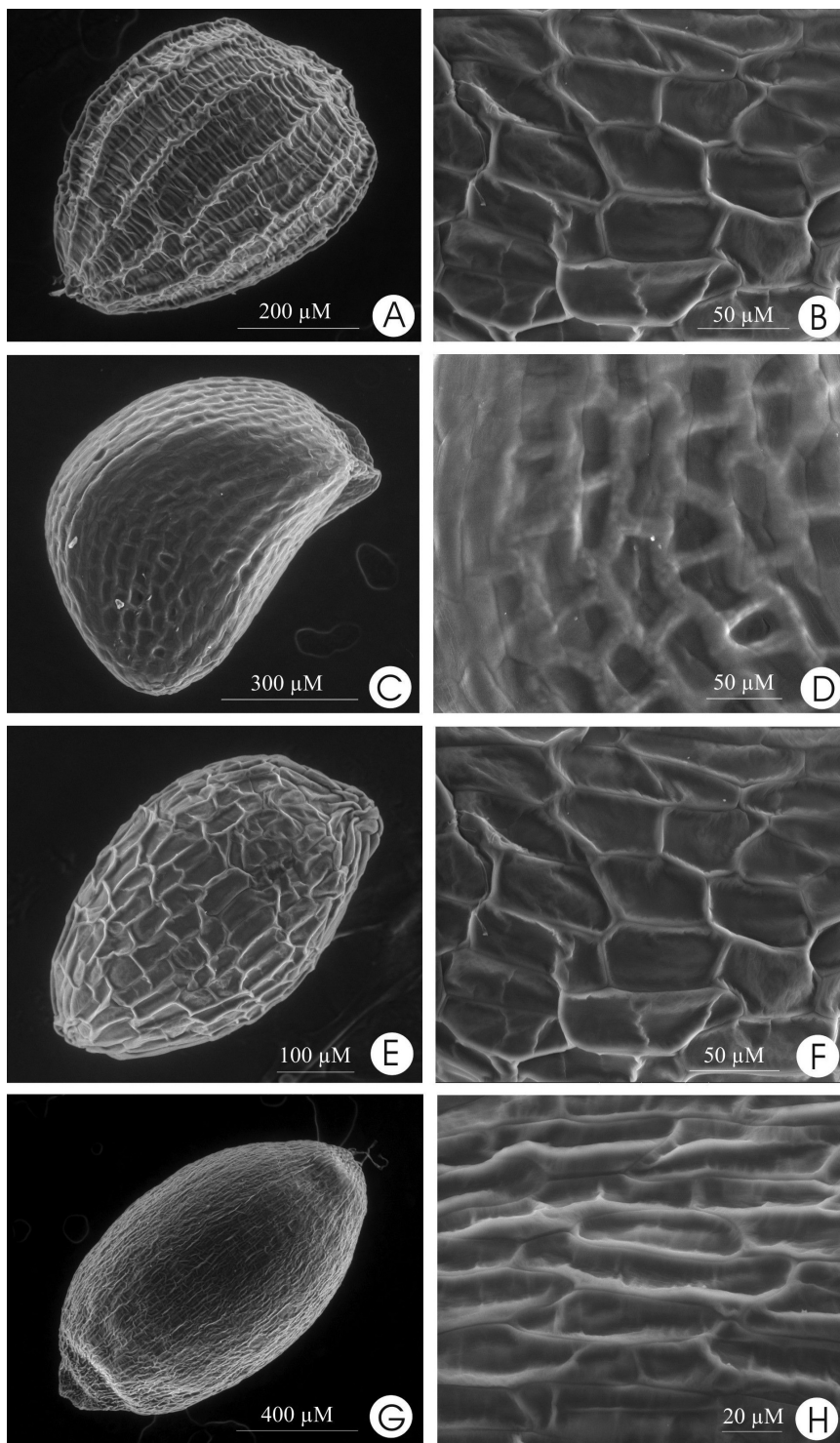


Table 3. – A morphological comparison of the *Juncus trifidus* group with the other genera of the *Juncaceae*.

	<i>Juncus trifidus</i> group	<i>Juncus</i> the rest of	<i>Luzula</i>	<i>Distichia</i>	<i>Patosia</i>	<i>Oxychloë</i>	<i>Rostkovia</i>	<i>Marsippospermum</i>
Auricles	lacerate-fimbriate	absent or entire	absent	entire	entire	entire	entire	entire
Anther connectives	mucronate	without mucro ¹	without mucro	mucronate	mucronate	mucronate	mucronate	mucronate ²
Leaf margin	serrulate	smooth	serrulate	smooth	serrulate	smooth	smooth	smooth
Seed coat ⁶	loose	tight or loose	tight	loose	loose	loose	tight	tight
Leaf indumentum	absent	absent	present	absent	absent	absent	absent	absent
seeds in capsule	many	many	three	many	many	many	many	many
Leaf sheath	open	open or closed ³	closed	open	open	open	open	open
Cushion growth	absent	absent	absent ⁴	present	present	present	absent	absent
Leaves arrangement	spirally	spirally ⁵	spirally	distichous	spirally	spirally	spirally	spirally
Gynophore	absent	absent	absent	developed	absent	absent	absent	absent

¹In *J. castaneus* Sm. and *J. scheuchzerioides* Gaudich., connectives may bear a short mucro; its presence is variable within species

²In *M. grandiflorum* (L. fil.) Hook., the connective is shorter than the pollen sacs and the tips of pollen sacs are horn-like.

³In *J. engleri* Buchenau, *J. tomatophyllus* Spreng., *J. dregeanus* Kunth and *J. capensis* Thunb., all belonging to the so called Southern Hemisphere Clade, leaf sheaths are initially closed and split later.

⁴A group of New Zealand species, *L. pumila* Hook. fil., *L. crenulata* Buchenau and *L. colensoi* Hook. fil. form small dense cushions.

⁵An aberrant form of the group of *J. capensis*, originally described as *J. singularis* Steud., is also characterized by distichously arranged leaves. This form requires further study (it was found only once in the early 19th century).

⁶See also Figs 2 and 3.

The genus *Luzula* has been studied extensively by several authors, most so by Nordenskiöld (opera varia, for summary see Kirschner et al. 2002a), and its karyotype evolution may be summarized as follows:

In *Luzula*, for which there are counts for about 70% of the species, invariably $x = 6$ and four basic karyotype evolution phenomena are known, i.e. (i) true polyploidy (up to octoploid level), (ii) agmatoploidy (in *Luzula*, it is simultaneous chromosome fragmentation reaching and in the subg. *Pterodes* exceeding the number $2n = 48$ of twice fragmented chromosomes), (iii) chromosome fusion (resulting in $2n = 6$ in *L. elegans*, subg. *Marlenia*) and (iv) mixed allopolyploidy with euploid and agmatoploid genome donors (or resulting from the combination of fragmented and unfragmented chromosomes, e.g. in some hybrids, or variously fragmented chromosomes, mainly subg. *Pterodes*).

These phenomena are not randomly distributed in the genus and the following list shows the differences among subgenera and sections:

subg. *Marlenia* ($2n = 6$, chromosome fusion)

subg. *Luzula*

sect. *Anthelaea* (only eudiploids, $2n = 12$)

sect. *Diprophyllatae* (eudiploids and agmatoploids, $2n = 12$ AL¹, 24 BL)

sect. *Alpinae* (eudiploids and agmatoploids, some agmatoploids combining AL and BL chromosomes, e.g. *L. spicata* subsp. *italica* in the Caucasus with $2n = 16 = 8AL + 8BL$)

sect. *Thyrsanochlamydeae* (polyploid group of hybrid origin)

sect. *Luzula* (diploids, polyploids, agmatoploids, mixed polyploids, rarely also with partially fragmented sets of chromosomes)

subg. *Pterodes* (only agmatoploids, sometimes mixed agmatoploids, with $2n = 24BL$ to $2n = 66 CL$ or $CL + DL$)

As regards the genera confined to the southern hemisphere, we conclude that virtually nothing is known about their chromosome evolution. There is a single reliable chromosome count for these five genera of $2n = 56$ for *Rostkovia magellanica*, which is either a highly derived polyploid or agmatoploid number (disregarded by Roalson 2005). The repeatedly cited chromosome number ($n = 8$) for *Oxychloë andina* (Sasaki 1937, see also Kirschner et al. 2002a) was not critically examined, also because of the relative unavailability of Sasaki's paper. It was not until we studied the electronic version of the paper that we realized that the number was probably false. Sasaki (1937) published not only the count of $n = 8$ for *O. andina* but also the chromosome numbers of *Juncus bufonius*, *J. compressus*, *J. filiformis*, *J. lamprocarpus* and *J. squarrosus*, all invariably $n = 8-10$, *J. maritimus* ($n = 20$) and *L. campestris*, *L. multiflora* and *L. nivea*, all $n = 9$. In view of the obviously wrong numbers given for nine species, the *Oxychloë andina* count is at least very doubtful. The speculation of Roalson (2005) that "the low chromosome number ($2n = 16$) of *Oxychloë andina* appears likely to be an agmatoploid reduction from a higher chromosome number, as all of the related *Juncus* species that have been counted range from $2n = 36$ to 48 ", is therefore superfluous.

¹ The terminology of agmatoploid chromosomes in *Luzula* recognizes AL chromosomes (euploid, unfragmented), BL chromosomes (simultaneously fragmented once) and CL chromosomes (fragmented twice).

There are relatively reliable chromosome counts for 93 species of *Juncus*, less than one third of its species (the counts are cited in Kirschner et al. 2002a, b, c). However, the counts cover all sections of the genus and the coverage is reasonably representative. The following conclusions can be made when the chromosome numbers are generalized:

(i) All the species counted, with one notable exception, are highly derived polyploids.

(ii) Agmatoploidy has not been documented in *Juncus*; the most probable polyploid series group to be considered as agmatoploids, the *J. bufonius* agg., was recently studied in detail and found to be eupolyploid (Rooks et al. 2011).

(iii) The basic chromosome numbers that can be derived from the known counts vary between $x = 9$ and $x = 12$ in most cases, although the majority of existing gametic numbers vary between $n = 19$ and $n = 24$ (the most frequent being $n = 20$) and reflect the probable paleopolyploid history of most of the genus.

(iv) The only diploid with a gametic number corresponding to the above conclusion is *J. capitatus* with $2n = 18$ ($x = 9$). The idea of Roalson (2005) that “diploid chromosome numbers based on multiples of 6 or 12 appear to provide a synapomorphy for the *Luzula* + *J. capitatus* clade”, is false.

The following account includes the gametic numbers recorded for the different sections of the genus (the less common numbers are in brackets):

subg. *Juncus*

sect. <i>Juncus</i>	$n = (23) 24$
sect. <i>Graminifolii</i>	$n = (18, 19) 20$
sect. <i>Caespitosi</i>	$n = 16–19$ [excluding <i>J. caespitosus</i>]
<i>Juncus caespitosus</i>	$n = 9$
sect. <i>Stygiopsis</i>	very high polyploids with multiples of 20 (often $2n = 60$ or ca. 120)
sect. <i>Iridifolii</i>	$n = 20$
sect. <i>Ozophyllum</i>	$n = (22) 20$ (and polyploids with $2n = 80$)

subg. *Agathryon*

sect. <i>Tenageia</i>	$n = 13–18$ (and high polyploids with $2n$ around 72 and 108)
sect. <i>Steiroidia</i>	$n = 20–22$ (and corresponding polyploids)
<i>Juncus trifidus</i>	$n = 15$
<i>Juncus monanthos</i>	$n = 15$
sect. <i>Juncotypus</i>	$n = 20, 21$ (and polyploids)
sect. <i>Forskalina</i>	$n = 21$

As regards the chromosome number $2n = 30$, recorded for both *J. trifidus* and *J. monanthos*, it clearly deviates from the numbers recorded for the rest of the sect. *Steiroidia*. A comparison with the other sections shows that similar chromosome numbers are found in the sections *Tenageia* and *Caespitosi* (for details, see discussion).

Evidence from the phylogenetic analysis of combined molecular data

One of the key new results of the previous analyses is the separate position of *Juncus trifidus* and *J. monanthos* outside the section *Steiroidia* (Fig. 1). This result is apparent from all the phylogenetic analyses of *Juncaceae* based on all available molecular data

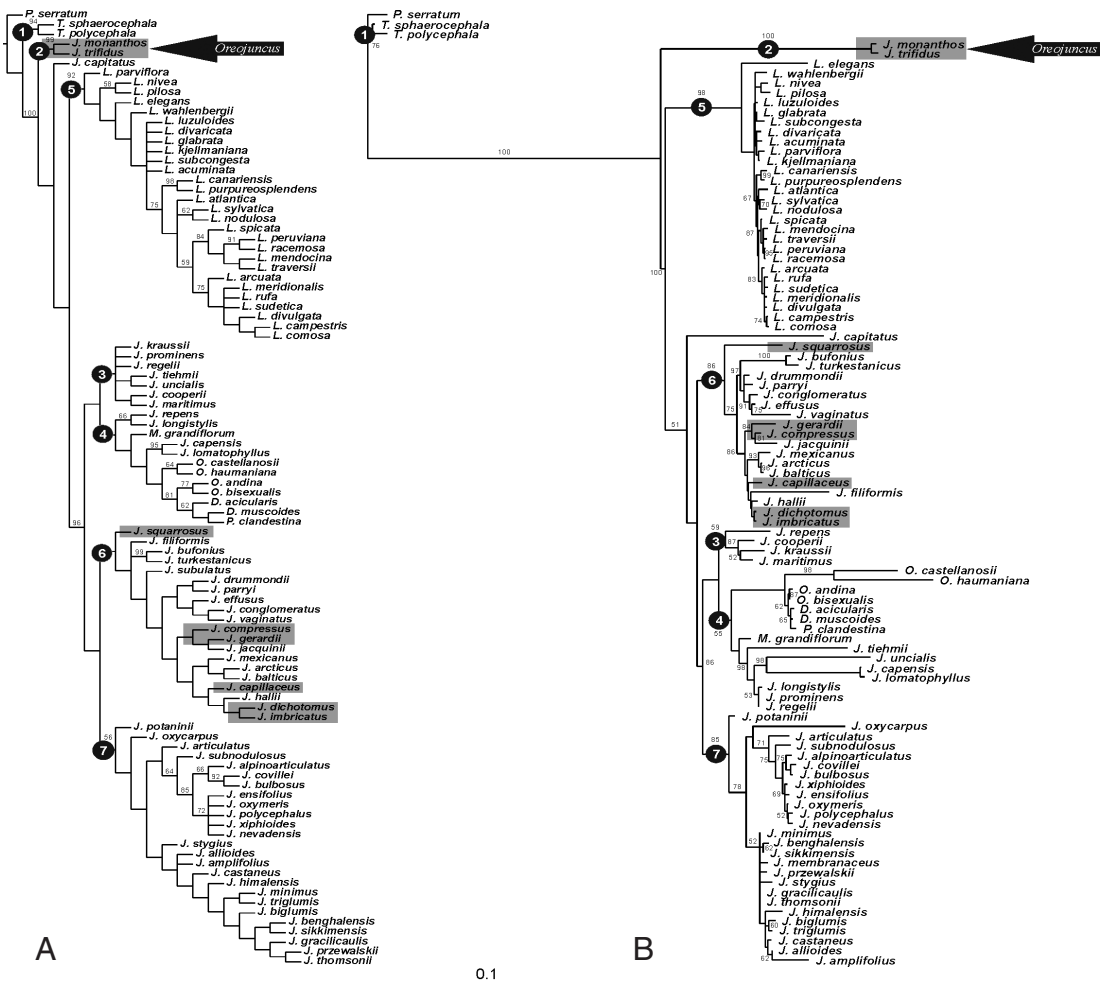


Fig. 4. – Molecular phylogenetic tree of the *Juncaceae* based on data from five regions of all three genomes, *rbcL*, *trnL*, *trnL-F*, *atp1* and ITS1-5.8S-ITS2. (A) Strict consensus maximum parsimony (MP) tree from 1292 most parsimonious trees. Length 5279, CI = 0.59, RI = 0.80, TNC = 5674, PIC = 1310. (B) Best tree from maximum likelihood (ML) analysis under the evolution model GTR+I+I (-lnL = 31471.6336). Numbers above branches indicate jackknife support values above 50%. Representatives of *Juncus* sect. *Steirochloa* are demarcated by a box. 1 *Thurnia*, 2 *Oreojuncus*, 3 *Juncus* subg. *Juncus* I., 4 Southern Hemisphere Clade + sect. *Graminifolii*, 5 *Luzula*, 6 *Juncus* subg. *Agathryon*, 7 *Juncus* subg. *Juncus* II.

sources. However, *Juncus trifidus* (and *J. monanthos*) are either a sister clade to the rest of the genus *Juncus* (*rbcL*, Fig. 1A) or majority of *Juncus* subg. *Juncus* (*trnL-F*; Fig. 1B), or a separate clade close to the root of the *Juncaceae* tree (*atp1*, Fig. 1C) or a sister group to the genus *Luzula* (ITS1-5.8S-ITS2, Fig. 1D).

As each of the DNA regions used place the *Juncus trifidus* group in a different (though remarkable) position, each potentially leading to a different interpretation, the four individual data partitions were combined (Fig. 4A) using the maximum parsimony criterion in terms of maximizing the explanatory power and informativeness of all the data.

Simultaneous molecular analyses of combined chloroplast, mitochondrial and nuclear regions (*rbcL*, *trnL-F*, *atp1* and ITS1-5.8S-ITS2, 5684 characters in total) under the maximum parsimony criterion generated 1,292 most parsimonious trees (MPT) of length 5,279, CI = 0.59 and RI = 0.80 (Fig. 4A). Total number of parsimony informative characters (PICs) was 1310. We then used maximum likelihood analysis to determine the occurrence of long branches within the tree (Fig. 4B). The ML analysis using the evolution model GTR+G+I (-lnL = 31471.6336) yielded the same topology for relationships among the major clades that are depicted in Fig. 4B. One of the longest branches showed *Juncus trifidus*–*J. monanthos* supported by 100% JS.

These analyses show seven main groups within the *Juncaceae*: *Juncus* subg. *Agathryon* I. & II., *Juncus* subg. *Juncus* I. & II., the genus *Luzula*, the so called Southern Hemisphere Clade (SHC) and *J. trifidus* and *J. monanthos*. The majority of members of subgenera *Agathryon* and *Juncus* form well-defined clades generally corresponding to the accepted taxonomic idea based on Buchenau (1890). They are characterized by a pair of floral bracteoles and a cymose inflorescence or absence of bracteoles and racemose inflorescence, respectively. However, two traditional representatives of the sect. *Steirochloa*, *Juncus trifidus* and *J. monanthos*, occupy the first derived lineage in the *Juncaceae* tree as a sister clade to *Juncus*, *Luzula* and the Southern Hemisphere Clade. *Atp1* was revealed as the most diagnostic region within the whole matrix, where *Juncus trifidus* and *J. monanthos* appeared exactly in the same position in the basal-most split (Fig. 1C).

When the molecular evidence gained from the combined data sets (Fig. 4) is compared with the established traditional system (Table 1), a few conspicuous features can be emphasized:

- (i) The monophyletic status of the genus *Luzula* and paraphyletic status of *Juncus* and the other genera.
- (ii) Subgenus *Agathryon* partially retained in the traditional circumscription (but see the case of *J. trifidus*) with little support for sectional subdivision.
- (iii) The southern hemisphere genera (*Distichia*, *Marsippospermum*, *Oxychloë*, *Patosia* and *Rostkovia*), together with a few S. African members of the sect. *Graminifolii* (and with some species of other *Graminifolii* and with all the members of the sect. *Juncus* studied), form a clade embedded in the *Juncus* clade, which is supported also by all separate data sets.
- (iv) The rest of subg. *Juncus* form a separate clade (but there is an unclear, variable and not very well supported position of *J. capitatus* of the sect. *Caespitosi*).
- (v) *Juncus trifidus* and *J. monanthos* form a sister clade to the rest of the family.

Evidence from the host-pathogen compatibility in the Juncaceae as an indicator for generic taxonomy

There is an interesting link in the field of compatibility between plants and pathogenic fungi. The ascomycetous fungus, *Septoria chanousiana* Ferraris, which was previously thought to infect only species of *Luzula*, was recently found also in leaves of *Juncus trifidus* (Suková & Chlebicki 2004). Another similar example is the ascomycetous filamentous fungus, *Arthrinium juncoideum* (J. G. Hall) Sacc. that occurs in the leaves of *Juncus* spp., but not those of *Juncus trifidus*, which are infected with *Arthrinium luzulae* M. B. Ellis, a typical pathogen of the genus *Luzula* (Suková & Chlebicki 2004). These findings support the hypothesis that

Juncus trifidus (fungi in *J. monanthos* have not been studied) occupies a unique position. The above facts are even more significant when we take into account the specificity of fungi found infesting representatives of the *Juncaceae*. For instance, *Ustilago vuijckii* Oudem. et Beijer is known to be widely distributed and to infest various species of *Luzula* in North America, Australia and Eurasia (Hämet-Ahti 1972, 1979, 1982) while in the genus *Juncus* it is *Ustilago abstrusa* Malen., with the exception of South Africa, where it is *Ustilago capensis* Reess (Hämet-Ahti & Piispala 1971).

Discussion

In the present study we analysed the evidence from multiple sources in order to elucidate the character divergence and phylogenetic position of *Juncus trifidus* and *J. monanthos*. These two species form a sister clade to the rest of the *Juncaceae*, and evidence from morphology, chromosome numbers and host-pathogen compatibility strongly support the separate status of this group. We therefore treat these two species as members of a separate genus described here, *Oreojuncus*.

Molecular studies based on different molecular markers (*rbcL*, *trnL*, *trnL-F*, *atp1*, ITS and *rps16*) place *J. trifidus* and *J. monanthos* in different separate positions within the *Juncaceae* tree topology (Drábková et al. 2003, 2004, 2006, Drábková & Vlček 2007, Záveská Drábková & Vlček 2009, 2010, Záveská Drábková 2010) even though a limited number of taxa from crucial groups were used in several of the studies (Roalson 2005, Jones et al. 2007).

All the principal morphological characters usually used in the delimitation of genera in the *Juncaceae* are displayed in Table 3. It is evident, that the traditional *Juncaceae* system using natural and evolutionary principles and taking character divergence into account is primarily based on autapomorphies or combinations of rare character states. As regards *Juncus trifidus* and *J. monanthos*, our attention to this species pair was attracted by the results of molecular analyses showing their position outside the traditional system (sect. *Steirochloa* or even outside *Juncus*). When the same morphological criteria as those applied to the genera of *Juncaceae* are used for the *J. trifidus* group, the most appropriate solution is its classification as a separate genus. The *Juncus trifidus* group is easily distinguished from the rest of *Juncus* on the basis of distinct characters – the lacerate-fimbriate auricles, the serrulate leaf margin and the mucronate connectives, and the differences between the *J. trifidus* group and the other *Juncaceae* genera are of equal magnitude (see Table 3 and the identification key to genera below).

However, the position of the *J. trifidus* group is not the only fundamentally new phenomenon revealed in the *Juncaceae*. Within *Juncus*, a distinct and well supported clade exists including all the genera confined to the southern hemisphere (*Marsippospermum*, *Rostkovia*, *Distichia*, *Patosia* and *Oxychloë*, the latter having been safely returned to the *Juncaceae* after some adventures caused by sample confusion, see the chapter dealing with previous molecular studies of this family) and probably some or perhaps all the species of several sections of *Juncus* (sect. *Juncus*, *Graminifolii* and *Caespitosi*). The paraphyly of *Juncus* (as a result of the existence of the Southern Hemisphere Clade, SHC) is not dealt with in detail in the present paper. This is because of incomplete sampling of the SHC, particularly the section *Graminifolii* (in the two main regions of its occurrence,

southern and south-eastern Africa and western North America, six of 22 species sampled). The African and western North American members of the sect. *Caespitosi* might also belong to SHC but they have been sampled inadequately for drawing such conclusion (three out of 17 species, and only two from the U.S., none of the at least six African taxa). The same is to be concluded about the section *Juncus*, with three out of nine species covered by the present study. We therefore refrain from making conclusions about the generic delimitation in *Juncus* (excluding *Oreojuncus*, see below), and a new, more detailed study of the SHC is needed. On the other hand, we have to point out that we would anyway refrain from sinking the SHC genera into the broad *Juncus* merely on the basis of departures from holophyly (cf. Hörandl & Stuessy 2010) when it is in conflict with the character evolution in the family.

The other problematic taxa are *J. capitatus*, perhaps in a sister position to the rest of *Juncus* and the SHC, and *J. potaninii*, a slender, morphologically reduced putative member of the sect. *Stygiopsis*, otherwise a distinct, monophyletic group. *J. potaninii* appears in a position sister to the clade formed by the majority of subg. *Juncus* (sections *Ozophyllum*, *Iridifolii* and *Stygiopsis*). This problem cannot be resolved without a new study. It was Roalson (2005) who first discussed the unexpected phylogenetic position of *J. capitatus* and suggested a tentative solution, i.e. that it is a consequence of the Long-Branch Attraction (LBA) phenomenon, an artefact of the MP analyses.

The summary of the karyological data raises a few questions. First, the highly derived chromosome numbers in *Juncus* indicate a high probability of reticulation and horizontal gene transfer in *Juncus*, a factor potentially complicating the phylogenetic inferences using cladistic methods. Another fact to be discussed is the chromosome number in the *Juncus trifidus* group, $n = 15$. There are another two groups where gametic numbers of $n = 13$ to $n = 18$ are known, the sect. *Tenageia* and the Californian members of the sect. *Caespitosi* (nothing is known about chromosome numbers of the South African members). It is hypothesized that these numbers can be derived from $n = 20$, a common number in closely related perennial taxa, and that in both groups, these gametic numbers were reached by gradual reduction of the paleopolyploid genome as is the case in annual, predominantly selfing taxa in other groups (Albach & Greilhuber 2004, Wright et al. 2008). The chromosome number of the *J. trifidus* group might have undergone a similar evolution but there is a hypothetical possibility that the number is derived from a separate basic number of $x = 8$. A cytogenetic study is needed to solve this problem.

Taxonomic treatment

Oreojuncus Závěská Drábková et Kirschner, **genus novum**

Plantae perennes estolonosae, laminis foliorum canaliculatis marginibus minute serrulatis, auriculis lacerato-fimbriatis, inflorescentiis paucifloris, floribus diphyllatis, hexandris, connectivis mucronatis, capsulis trilobularibus rostratis, seminibus numerosis bicaudatis testa laxa.

Plants perennial, without stolons, leaf blade canaliculate with minutely serrulate margin, auricles lacerate-fimbriate, inflorescence few-flowered, flowers with two bracteoles, six anthers, anthers with mucronate connective, capsule trilobular, rostrate, seeds numerous, with loose coat and two appendages.

Type of the generic name, designated here: *Oreojuncus monanthos* (Jacq.) Záveská Drábková et Kirschner
[= *Juncus monanthos* Jacq.]

Members of the genus:

Oreojuncus trifidus (L.) Záveská Drábková et Kirschner, **comb. nova**

Basionym: *Juncus trifidus* L., Sp. Pl. 326 (1753)

Oreojuncus monanthos (Jacq.) Záveská Drábková et Kirschner, **comb. nova**

Basionym: *J. monanthos* Jacq., Enum. Stirp. Vindob. 61, 236 (1762)

Key to the genera of *Juncaceae*

- 1a Auricles lacerate *Oreojuncus*
- 1b Auricles absent or entire 2
- 2a Leaf margin hairy, at least sparsely so near sheath opening; seeds 3 *Luzula*
- 2b Leaf margin not developed (leaves round, glabrous), or glabrous; seeds many 3
- 3a Leaf margin minutely serrulate *Patosia*
- 3b Leaf margin not developed (leaves round) or smooth 4
- 4a Flowers solitary and anthers mucronate (if anthers not mucronate then outer tepals at least 15 mm long) 5
- 4b Flowers in inflorescences; anther connective not mucronate (if flowers occasionally solitary then tepals shorter than 10 mm) *Juncus*
- 5a Plants cushion-forming, upper part of stem densely covered with leaves, flower lateral (subterminal, axillary) 6
- 5b Plants not cushion-forming, upper part of stem leafless, flower terminal 7
- 6a Leaves regularly distichous; gynophore developed, elongating during capsule ripening *Distichia*
- 6b Leaves ± spirally arranged; gynophore absent *Oxychloë*
- 7a Flower bracts 2, the lower one herbaceous, conspicuously longer than perianth, upper bract ± equalling perianth, capsule suborbicular to obovoid, obtuse, to c. 5 mm long, seeds without conspicuous appendages *Rostkovia*
- 7b Flower bracts 1 or 2, membranous, much shorter than perianth, capsule oblong to ellipsoidal, trigonous, acuminate, at least 7 mm long, seeds with two distinct appendages *Marsippospermum*

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Souhrn

Dvojice druhů rodu *Juncus*, *J. trifidus* L. a *J. monanthos* Jacq., byla tradičně řazena do sekce *Steirochloa*, ačkoliv někteří autoři je na základě pozoruhodných morfologických znaků řadili do samostatné sekce *Trifidi* Rouy. Předchozí molekulárně-fylogenetické studie čeledi *Juncaceae* ukázaly, že tyto dva druhy do sekce *Steirochloa* nepatří. Předložená práce shrnuje dostupné údaje morfologické, karyologické, a zejména kombinované výsledky molekulárních analýz s využitím sekvencí kódujících i nekódujících úseků chloroplastové DNA, nukleární ribosomální DNA a mitochondriální DNA. Ukázalo se, že *J. trifidus* a *J. monanthos* z fylogenetického hlediska představují raně odštěpenou vývojovou větev čeledi *Juncaceae*, sesterskou rodu *Luzula*. Rovněž soubor morfologických znaků, zejména třísnitá ouška, prašníky s nasazenou špičkou, semena s volným vnějším osemením a s přívěsky a papílnatě pilovitý okraj listů, ale také rodová specifická houbových patogenů, tyto dva druhy staví mimo sekci *Steirochloa*, ba dokonce mimo rod *Juncus*. Proto jsou tyto dva druhy odděleny do samostatného nového rodu *Oreojuncus* jako *O. trifidus* a *O. monanthos*. Je též uveden klíč k určování rodů čeledi *Juncaceae*.

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Appendix 1. – Species included in the study with GenBank accession numbers. Family, genus, subgenus, section, species: accession numbers of *atp1*, *trnL-F*, *rbcL*, ITS1-5.8S-ITS2.

JUNCACEAE: *Luzula* subg. *Marlenia*, *L. elegans* Lowe: EU523158^D, AY437928^B, AY216648^A, FJ213845^E, AB261692. ***Luzula* subg. *Luzula*, section *Anthelaea*, *L. canariensis* Poir.:** EU523159^D, AY437929^B, AY216655^A, AY973498^C. ***L. luzuloides* (Lam.) Dandy & E. Eillm.:** EU523160^D, –, –, FJ213848^E. ***L. nivea* (Nath.) DC.:** EU523161^D, AY437930^B, AY216650^A, –, *L. purpureosplendens* Seub.: EU523162^D, AY437931^B, AY216654^A, FJ213850^E. ***L. sylvatica* (Huds.) Gaudin:** EU523163^D, AY437932^B, AY216637^A, FJ213851^E. ***Luzula* subg. *Luzula*, section *Atlanticeae*, *L. atlantica* Braun-Blanq.:** –, DQ099455^B, AY216639^A, FJ213852^E. ***Luzula* subg. *Luzula*, section *Nodulosae*, *L. nodulosa* (Bory & Chaub.) E. Mey.:** EU523164^D, AY437933^B, AY216636^A, AY973499^C. ***Luzula* subg. *Luzula*, section *Diprophyllatae*, *L. divaricata* S. Watson:** –, AY437934^B, –, FJ213854^E, AY727771. ***L. glabrata* (Hoppe) Desv.:** EU523165^D, AY437935^B, AY216644^A, FJ213856^E. ***L. parviflora* (Ehrh.) Desv.:** –, –, U49228, –, *L. wahlenbergii* Rupr.: EU523166^D, AY437936^B, AY216649^A, AY973500^C. ***Luzula* subg. *Luzula*, section *Alpinae*, *L. peruviana* Desv.:** –, AY437937^B, –, FJ213863^E. ***L. racemosa* Desv.:** –, AY437938^B, –, FJ213864^E. ***L. spicata* (L.) DC. s. str.:** EU523167^D, AY437939^B, AY216645^A, FJ213865^E, AY727775. ***L. mendocina* Barros:** –, DQ099456^B, –, FJ213866^E. ***L. traversii* (Buchenau) Cheesem. s. str.:** –, AY437940^B, AY216635^A, FJ213867^E. ***Luzula* subg. *Luzula*, section *Thyrssanochlamydeae*, *L. arcuata* (Wahlenb.) Sw.:** –, AY437941^B, AY216651^A, FJ213869^E. ***L. kjellmaniana* Miyabe & Kudo:** –, AY437942^B, AY216633^A, FJ213871^E. ***L. subcongesta* (S. Watson) Jeps.:** –, AY216657^A, –, FJ213873^E. ***Luzula* subg. *Luzula*, section *Luzula*, *L. campestris* (L.) DC. s. str.:** EU523168^D, AY437943^B, AY216652^A, FJ213882^E. ***L. comosa* E. Mey.:** –, DQ099457^B, FJ213887^E, FJ213888^E, AY727776. ***L. divulgata* Kirschner:** EU523169^D, AY437944^B, AY216646^A, FJ213893^E. ***L. meridionalis* H. Nordensk.:** –, –, AY216641^A, –, *L. rufa* Edgar: EU523170^D, AY437945^B, AY216642^A, FJ213903^E. ***L. sudeica* (Willd.) Schult.:** EU523171^D, AY437946^B, AY216647^A, AY973501^C. ***Luzula* subg. *Pterodes*, *L. acuminata* Raf.:** AY124521², AY437947^{1, B}, AY216656^{1, A}, FJ213905^E. ***L. pilosa* (L.) Willd.:** –, AY437948^B, AY216653^A, –, *Juncus* subg. *Juncus*, section *Juncus*, *J. cooperi* Engelm.: EU523172^D, DQ099458^B, –, AY727781. ***J. kraussii* Hochst. in C. Krauss s. str.:** –, –, AY216609^A, –, *J. maritimus* Lam.: EU523173^D, AY437949^B, AY216629^A, –, *Juncus* subg. *Juncus*, section *Caespitosi*, *J. capitatus* Weigel.: EU523174^D, DQ099459^B, –, AY727769. ***J. tiehmii* Ertter:** –, DQ099460^B, –, –, *J. uncialis* Greene: –, DQ099461^B, –, –, *Juncus* subg. *Juncus*, section *Graminifolii*, *J. capensis* Thunb.: EU523175^D, AY437950^B, AY216616^A, AY973502^C, AY277825. ***J. covillei* Piper s. str.:** EU523176^D, AY437951^B, AY216606^A, –, *J. lomatosphyllus* Spreng.: EU523177^D, AY437952^B, AY216617^A, AY973503. ***J. longistylis* Torr.:** EU523186^D, –, –, AY727786. ***J. prominens* (Buchenau) Miyabe & Kudo:** EU523178^D, DQ099465^B, –, –, *J. regelii* Buchenau: EU523179^D, DQ099466^B, –, –, *J. repens* Michx.: –, AY216627^A, –, ITS: AY727785. ***Juncus* subg. *Juncus*, section *Stygiopsis*, *J. allioides* Franch.:** EU523180^D, –, –, AY727809. ***J. amplifolius* A. Camus:** EU523181^D, –, –, AY727807. ***J. bengalensis* Kunth:** EU523182^D, –, –, *J. biglumis* L.: EU523183^D, AY437953^B, –, –, *J. castaneus* Sm.: EU523184^D, AY437954^B, AY216623^A, AY727908. ***J. gracilicaulis* A. Camus:** EU523185^D, –, –, –, *J. himalensis* Klotzsch: –, –, AY216628^A, –, *J. membranaceus* Royle: EU523187^D, –, –, –, *J. minimus* Buchenau: EU523188^D, –, –, –, *J. sikkimensis* Hook. f.: EU523189^D, –, –, –, *J. potaninii* Buchenau: EU523190^D, –, –, –, *J. przewalskii* Buchenau: EU523191^D, –, –, –, *J. stygius* L.: EU523192^D, AY437955^B, AY216610^A, AY973504^C. ***J. thomsonii* Buchenau:** EU523193^D, –, –, –, *J. triglumis* L.: EU523194^D, AY437956^B, AY216605^A, –, *Juncus* subg. *Juncus*, section *Iridifolii*, *J. ensifolius* Wikstr.: EU523195^D, AY437957^B, AY216611^A, –, *J. oxymeris* Engelm.: EU523196^D, AY437958^B, AY216621^A, AY973505^C. ***J. polycephalus* Michx.:** –, –, AY216626^A, ITS: AY727813. ***J. xiphioides* E. Mey.:** EU523197^D, AY437959^B, AY216624^A, –, *Juncus* subg. *Juncus*, section *Ozophyllum*, *J. articulatus* L. s. str.: –, AY437961^B, AY216614^A, AY727819. ***J. bulbosus* L.:** EU523199^D, AY437962^B, AY216622^A, AY973506^C. ***J. nevadensis* S. Watson:** –, AY437963^B, AY216601^A, AY727815. ***J. subnodulosus* Schrank:** EU523200^D, AY437964^B, AY216630^A, –, *J. oxycarpus* E. Mey. ex Kunth: –, –, AY216631^A, –, *Juncus* subg. *Agathryon*, section *Tenageia*, *J. bufonius* L.: EU523201^D, AY437965^B, AY216615^A, AY727889. ***J. turkestanicus* V. Krecz. & Gontsch.:** EU523202^D, AY437966^B, –, *Juncus* subg. *Agathryon*, section *Steirochloa*, *J. capillaceus* Lam.: –, AY437967^B, AY216604^A, AY727796. ***J. compressus* Jacq.:** EU523203^D, AY437968^B, AY216625^A, AY973507^C. ***J. dichotomus* Elliott:** –, DQ099467^B, AY216607^A, AY727800. ***J. gerardii* Loisel.:** –, AY437969^B, AY216613^A, –, *J. imbricatus* Laharpe: EU523204^D, –, AY216602^A, –, *J. monanthos* Jacq.: EU523205^D, DQ099464^B, –, –, *J. squarrosus* L.: EU523206^D, AY437970^B, AY216619^A, –, *J. trifidus* L.: EU523207^D, AY437971^B, AY216618^A, AY973508^C, AY727770. ***Juncus* subg. *Agathryon*, section *Juncotypus*, *J. arcticus* Willd.:** EU523208^D, AY437972^B, –, –, *J. balticus* Willd. s. str.: EU523209^D, AY437973^B, AY216620^A, –, *J. conglomeratus* L.: EU523210^D, AY437974^B, –, –, *J. drummondii* E. Mey.: –, AY437975^B, –, –, *J. effusus* L. s. str.: ¹EU523211^D, ¹AY437976^B, ¹AY216612^A, ¹AY973509^C,

²⁻⁵AY727791-AY727794. *J. filiformis* L.: EU523212^D, AY437977^B, –, AY727790. *J. hallii* Engelm.: EU523213^D, DQ099462^B, AY216603^A, –, *J. jacquinii* L.: EU523214^D–, –, *J. mexicanus* Willd ex Schult. & Schult. f.: EU523215^D, DQ099463^B –, –, *J. parryi* Engelm.: –, AY437978^B, AY216600^A, –, *J. vaginatus* R. Br.: –, –, AY216608^A, –, *Juncus* subg. *Agathryon*, section *Forskalina*, *J. subulatus* Forssk.: EU523216^D, –, –, *Marsippospermum*, *M. grandiflorum* (L. f.) Hook. f.: EU523217^D, AY973535, U49226, AY973515^C. *Distichia*, *D. muscoides* Nees et Meyen: EU523218^D, AY973537, U49227, AY727784. *D. acicularis* Balslev & Laegaard: –, AY973538, AJ419944, AY973513^C. *Patosia*, *P. clandestina* (Phil.) Buchenau: EU523219^D, AY973536, U49225, AY973514^C. *Oxychloë*, *O. andina* Phil.: –, AY437980^B, AY660587, AY727783^C. *O. bisexualis* Kuntze: EU523220^D, AY437981^B, AY660584, AY973510^C. *O. castellanosi* Barros: –, –, AY660585, AY973512^C. *O. haumaniana* (Barros) Barros: –, –, AY660586, AY973511^C. **PRIONIACEAE:** *Pronium*, *P. serratum* (L. f.) Drège ex E. Mey.: AY124527, AY344155, U49223, –. **THURNIACEAE:** *Thurnia*, *T. polycephala* Schnee.: AY124532, –, AY123239, –. *T. sphaerocephala* Hook f.: –, –, AF03688, –. **CYPERACEAE:** *Cyperus*, *C. involucratus* Rottb.: –, –, Y12967.1, AY242052.1. *Gahnia*, *G. deusta* Benth.: –, –, U49231, –. *Eleocharis*, *E. pauciflora* Link.: –, –, U49232.1, –. *Kobresia*, *K. simpliciuscula* Mack.: –, –, U49232.1, AY241971.1. *Rhynchospora*, *R. fascicularis* (Michx.) Vah.: –, –, U49223, –. **Published sequences:** Superscript codes for site of first publication: A) Drábková et al. (2003); B) Drábková et al. (2004 and 2006); C) Drábková & Vlček (2007); D) Záveská Drábková & Vlček (2009); and E) Záveská Drábková & Vlček (2010); accessions without letter codes are from Genbank (without additional voucher information, for most, see Roalson 2005).