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Phylogeny, Systematics, and Recircumscription of Juncaginaceae – A Cosmopolitan Wetland Family

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Abstract—Juncaginaceae are a small monocot family of mostly coastal and wetland herbs of almost cosmopolitan distribution. A phylogenetic analysis of the family and members of Alismatales was conducted to clarify the circumscription of Juncaginaceae and to understand intrafamilial relationships. For the first time, all genera associated with the family in the past were analysed together. Two plastid (*rbcL* and *matK*) and one mitochondrial gene (*atpA*) were sequenced. The separate and combined analysis of the three markers showed that Juncaginaceae are not monophyletic in their current circumscription. The family is re-circumscribed to exclude *Maundia* which is proposed to belong to a separate family Maundiaceae. In the new classification Juncaginaceae comprise three genera: *Tetroncium*, *Cycnogeton*, and *Triglochin*. *Tetroncium* is weakly supported as sister to the rest of the family. The reinstated *Cycnogeton* (formerly included in *Triglochin*) is highly supported as sister to *Triglochin* s.s. The enigmatic *Lilaea* is nested within *Triglochin* s.s. and highly supported as sister to the *T. bulbosa* complex. The results of the molecular analysis are discussed in combination with morphological characters. A key to the genera of the family is presented, and six new combinations are proposed: **Cycnogeton alcockiae**, **Cycnogeton dubium**, **Cycnogeton microtuberosum**, **Cycnogeton multifructum**, **Cycnogeton rheophilum**, and **Triglochin scilloides**.[1]

Keywords—Alismatales, *Cycnogeton*, *Lilaea*, *Maundia*, *Tetroncium*, *Triglochin*.

1 This article is dedicated to the memory of Dr. Surrey W. L. Jacobs (1946-2009) in appreciation of his outstanding contributions to the knowledge of Australian water plants and in grateful acknowledgment of his support of the project described here.

The order Alismatales (14 families with ca. 4490 species; Stevens 2001 onwards), one of the earliest-diverging lineages of monocotyledons (Janssen and Bremer 2004), comprises mainly aquatic and wetland plants. While several groups of Alismatales have received considerable attention (e.g., seagrasses: e.g., den Hartog 1970; Les et al. 1997; Araceae: e.g., Mayo et al. 1997; Cabrera et al. 2008), some smaller families of the order have not been studied in detail. One of these are Juncaginaceae (Arrow-grass family), part of the so-called aquatic clade (Judd et al. 2007) or core Alismatales (Stevens 2001 onwards).

As currently circumscribed, Juncaginaceae comprise four genera, *Triglochin*, *Lilaea*, *Maundia*, and *Tetroncium*. (Haynes et al. 1998; Stevens 2001 onwards) with together approximately 25-35 annual or perennial species. Despite its small size, the family shows considerable ecological diversity. Members of Juncaginaceae are wind-pollinated, grass-like herbs which can be found in freshwater (slow-flowing rivers, bogs, fens), in brackish water (e.g., estuaries), and in salt marshes, but also in only seasonally wet terrestrial sites (e.g., annual species of *Triglochin*, *T. bulbosa* L. subspp.). The family has an almost cosmopolitan distribution with Australia as centre of specific diversity (Fig. 1).

Triglochin is the largest genus of the family and is distributed almost worldwide. Widely circumscribed (in the following called *Triglochin* s.l.) it comprises the mostly halophytic arrow-grasses (*Triglochin* s.s.) and the water-ribbons (*T. procera* R.Br. and related species of the *T. procera* complex) which are important components of Australian freshwater communities. The latter complex is sometimes segregated from *Triglochin* as *Cycnogeton*. The number of recognized species in *Triglochin* varies greatly in the literature, ranging from 12 to 24 (e.g., Haynes

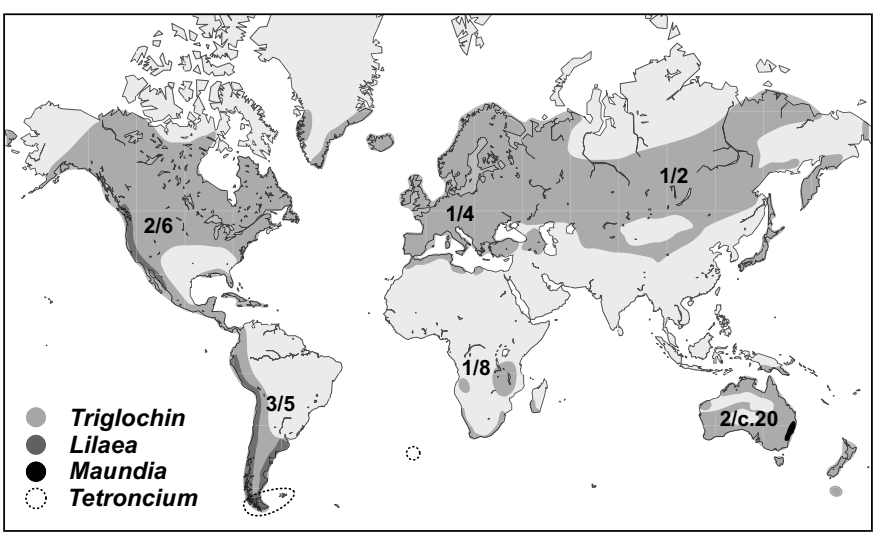

Fig. 1. Geographical distribution of the currently recognized genera of Juncaginaceae. Map prepared using Online Map Creation (Weinelt 1996 onwards).

et al. 1998; Stevens 2001 onwards; Govaerts 2008; Mabberley 2008). However, recent revisions (Aston 1995; Köcke et al., in press; H. I. Aston, pers. comm.) have revealed considerably higher numbers of probably more than 30 species.

While *Triglochin* is distributed almost worldwide, the monotypic genera *Tetroncium* and *Maundia* are restricted to relatively small areas in southern South America and eastern Australia, respectively (Fig. 1). *Tetroncium magellanicum* is a typical component of *Sphagnum* L. bogs in Patagonia and Tierra del Fuego, and on some subantarctic islands (Falkland Islands, Gough Island). *Maundia triglochinoides* is found in swamps, creeks, or shallow freshwater in coastal Queensland and New South Wales. *Lilaea*, the third monotypic genus, is distributed from southern South America to southern Canada (Fig. 1). *Lilaea scilloides* grows emergent or submerged in shallow water of seasonal pools or neighbouring mud flats. This species is also naturalised in Australia (Aston 1967, 1977; Australian Plant Census 2009; H. I. Aston, pers. comm.) and the Iberian Peninsula (González 1968; Nava et al. 2000; Romero Buján 2007).

The circumscription of Juncaginaceae has changed throughout history. The most important historical classifications are summarized in Table 1. In older classifications the genus *Scheuchzeria* L. was included in the family. Morphological (e.g., Tomlinson 1982; Posluszny 1983) and more recently molecular data (Les et al. 1997) have shown the distinctness of *Scheuchzeria*, which is now commonly placed in its own monotypic family Scheuchzeriaceae. The correct placement of *Lilaea* has also been problematic. This genus has often been treated as the only member of Lilaeaceae Dumortier because of its divergent floral morphology (e.g., Taylor 1909; Tomlinson 1982). Results of karyological (Larsen 1966), embryological (Agrawal 1952; Yamashita 1970), and molecular studies (Les et al. 1997) have, however, shown the close relationship between *Lilaea* and Juncaginaceae, resulting in the inclusion of *Lilaea* in Juncaginaceae by most modern authors (e.g., Haynes et al. 1998; Stevens 2001 onwards). Some authors (e.g., Novelo and Lot 2001; Novelo 2003), however, retain Lilaeaceae as a separate family. Finally, the monotypic *Maundia* has been treated also as a separate family, Maundiaceae Nakai (1943). Subsequently, this family was accepted only by Takhtajan (1997).

Generic limits within Juncaginaceae also have been assessed differently by different authors (Table 1). The status of *Cycnogeton* and *Maundia* has changed several times through history. Both taxa were originally described as monotypic genera, but later treated either as sections (Micheli 1881) or subgenera of a broadly circumscribed *Triglochin* (subg. *Cycnogeton* (Endl.) Buchenau and subg. *Pseudotriglochin* (Micheli) Buchenau, respectively; Buchenau and Hieronymus 1889). While *Cycnogeton* in subsequent treatments usually was included in *Triglochin* without recognition as subgenus (e.g., Aston 1977, 1995; Haynes et al. 1998), *Maundia* was generally accepted as a separate genus (Aston 1977; Haynes et al. 1998).

No molecular study of all genera of Juncaginaceae has yet been published. Molecular studies of monocots or Alismatales included only very few representatives

Micheli 1881	Buchenau & Hieronymus 1889	Buchenau 1903	Hutchinson 1934, 1959	Dahlgren et al. 1985	Takhtajan 1997	Haynes et al. 1998	this study
Juncagineae	**Juncaginaceae**	**Scheuchzeriaceae**	**Juncaginaceae**	**Juncaginaceae**	**Juncaginaceae**	**Juncaginaceae**	**Juncaginaceae**
(11-12 spp.)	(15 spp.)	(17 spp.)	(n.s.)	(c. 20 spp.)	(15 spp.)	(c. 15 spp.)	(c. 30 spp.)
Lilaea[1]	*Lilaea*	*Lilaea*	*Cycnogeton*	*Cycnogeton*	*Cycnogeton*	*Lilaea*	*Triglochin*[4]
Scheuchzeria	*Scheuchzeria*	*Maundia*	*Maundia*	*Lilaea*	*Tetroncium*	*Maundia*	*Cycnogeton*
Tetroncium	*Tetroncium*	*Scheuchzeria*	*Tetroncium*	*Maundia*	*Triglochin*	*Tetroncium*	*Tetroncium*
Triglochin	*Triglochin*	*Tetroncium*	*Triglochin*	*Tetroncium*		*Triglochin*	
sect. *Eutriglochin*	subg. *Eutriglochin*	*Triglochin*		*Triglochin*			
sect. *Cycnogeton*[3]	subg. *Cycnogeton*[3]	subg. *Eutriglochin*					
sect. *Pseudotriglochin*[2]	subg. *Pseudotriglochin*[2]	subg. *Cycnogeton*[3]					
			Lilaeaceae		**Lilaeaceae**		
			(1 sp.)		(1 sp.)		
			Lilaea		*Lilaea*		
					Maundiaceae		**Maundiaceae**
					(1 sp.)		(1 sp.)
					Maundia		*Maundia*
			Scheuchzeriaceae	**Scheuchzeriaceae**	**Scheuchzeriaceae**	**Scheuchzeriaceae**	**Scheuchzeriaceae**
			(1 sp.)	(1 sp.)	(1 sp.)	(1 sp.)	(1 sp.)
			Scheuchzeria	*Scheuchzeria*	*Scheuchzeria*	*Scheuchzeria*	*Scheuchzeria*

Table 1. Classification of genera associated with Juncaginaceae from 1881 to present. [1] – "Genus omnino anomalum: Juncagineis propriis vix affine.", [2] – only *Maundia triglochinoides*, [3] – only *Triglochin procera*, [4] – incl. *Lilaea*, n.s. – not specified.

of the family and sometimes none at all. An analysis of Alismatales based on *rbcL* sequence data (Les et al. 1997) contained only three species (*Triglochin maritima* L., *Cycnogeton procerum*, and *Lilaea scilloides* which formed a strongly supported clade with *Cycnogeton* as sister to a strongly supported *Lilaea*/*Triglochin* clade. In a biogeographical study of hydrophytes (Les et al. 2003), ITS sequences of *Tetroncium* and *Cycnogeton* were used to estimate divergence times, but not to reconstruct the phylogeny of these taxa. Thus, although the position of Juncaginaceae within Alismatales is relatively unambiguous, phylogenetic relationships among genera of Juncaginaceae are still unclear mainly because *Maundia* and *Tetroncium* have never been included in molecular phylogenetic analyses and because the sample of *Triglochin* s.l. has been too small. Even though some anatomical information is available for *Maundia* (Tomlinson 1982) the knowledge of this latter genus and *Tetroncium* is fragmentary and mainly limited to Flora treatments (e.g., Thompson 1961; Correa 1969; Aston 1977; Broughton and McAdam 2005).

The objective of this study is to provide a comprehensive phylogenetic analysis of Juncaginaceae based on molecular data. This phylogeny will be interpreted on the background of the morphology of the family as far as known. To obtain a better understanding of relationships among genera presently assigned to Juncaginaceae, and to evaluate the delimitation of the family, we used the chloroplast (cp) genes *rbcL* and *matK* as well as the mitochondrial (mt) gene *atpA*. In this study we will (1) investigate the monophyly of the family Juncaginaceae in its current circumscription, (2) clarify relationships among the genera of the family, and (3) propose a revised classification of Juncaginaceae where clear evidence from molecular and other data is available.

Materials and Methods

Taxon Sampling—Altogether, nine species currently recognized as members of Juncaginaceae, the monotypic *Lilaea*, *Maundia*, *Tetroncium*, and six species considered representative of *Triglochin,* plus *Scheuchzeria palustris* L. (Scheuchzeriaceae) were included in our analysis. Our molecular studies (S. von Mering and J. W. Kadereit, unpubl. data) have shown that several members of the morphologically well-defined Australian *Triglochin procera* complex (water-ribbons) form a monophyletic group. Difficulties in obtaining high quality DNA prevented the inclusion of an annual species of *Triglochin* in this study. However, species of this group formed a monophyletic clade within *Triglochin* s.s. (S. von Mering and J. W. Kadereit, unpubl. data). Sequences of members of all other families of Alismatales were downloaded from GenBank. Voucher information and GenBank accession numbers are given in Appendix 1.

Molecular Marker—The plastid *rbcL* gene was chosen in this study because *rbcL* sequences are available for members of all other families of Alismatales, providing a rich source for outgroups. Additionally, *matK* was used to improve resolution and/or support, and the *atpA* gene has been used in the analysis

of monocot and Alismatales relationships (e.g., Davis et al. 1998, 2004, 2006; Petersen et al. 2006).

DNA Extraction, Amplification, and Sequencing—Total genomic DNAs were extracted either from silica-dried leaves, from material preserved in saturated NaCl-CTAB solution supplemented with 200 mM sodium ascorbate (Thomson 2002), or from herbarium material using NucleoSpin® plant DNA extraction kits (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. The standard 25 µl PCR reaction mix consisted of the following: 2.5 mM MgCl2, 200 µM dNTPs, 1 pM per primer, 0.025 U/µl *Taq* polymerase, 1-2 µl of DNA extract in the reaction buffer provided by the manufacturer of the polymerase, and 1% BSA. PCR reactions were carried out in a Biometra® T3 or a PTC 100™ thermocycler (MJ Research, Inc., MA, U.S.A.) using the programme: 60 sec at 94°C, followed by 35 cycles of 18 sec at 94°C, 30 sec at 52°C, 60 sec at 72°C and a post-treatment of 8 min at 72°C for *rbcL*. The programme used for the amplification of the *matK* and *atpA* sequences differed in the higher annealing temperature (55°C) and a longer elongation time (90 sec).

The primers used for amplification of the three markers are summarized in Table 2. *RbcL* sequences were amplified in three overlapping fragments with slightly modified standard *rbcL* primers: 1F and 579R, 507F and 994R, and 955F and 1460R. To amplify and sequence the *matK* region one primer was modified after Müller and Borsch (2005): JU*matK* 480F, and the standard primer *trnK* 2R was used as reverse primer (Johnson and Soltis 1994). The following primers were used for amplification of *atpA*: *atpA* F-A1 and *atpA* B-A1 (Davis et al. 2004). PCR products were checked on 0.8% agarose gels and purified directly using a PCR purification kit (QIAGEN GmbH, Hilden, Germany). Purified PCR products

Primer name	Sequence
rbcL 1F	5'-ATG TCA CCA CAA ACA GAA ACT AAA GCA-3'
rbcL 579R	5'-AAA TCA AGT CCA CCR CG-3'
rbcL 507F*	5'-TAT TGG GAT GTA CTA TTA AAC-3'
rbcL 994R*	5'-CCT TCY AGT TTA CCT AC-3'
rbcL 955F*	5'-CGY ATG TCT GGT GGA GAT C-3'
rbcL 1460R	5'-CCT TTA GTA AAA GAT TGG GCC GAG-3'
JUmatK 480F	5'-CAT CTY GAA ATH TTG GTT C-3'
trnK 2R	5'-AAC TAG TCG GAT GGA GTA G-3'
atpA F-A1	5'-CAG TTG GAG ATG GGA TTG CAC G-3'
atpA B-A1	5'-GGC AGT GGT TCA TAT TGT GGT TG-3'

Table 2. Primers used in PCR and sequencing. * Slightly modified standard primer.

were cycle-sequenced with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (BD 3.0 in 10 µl reactions) by Perkin Elmer using the PCR primers listed above and following the manufacturer's protocol. Products were purified and analysed by GENterprise (Mainz, Germany). Forward and reverse sequences were manually edited and merged into consensus sequences using Sequencer 4.1.2 (GeneCodes Corp., Ann Arbor, Michigan), and aligned manually in MacClade 4.1 (Maddison and Maddison 2000). Alignment of *rbcL* and *atpA* was straightforward. The *matK* sequences downloaded from GenBank were pre-aligned automatically using the programme ClustalX (Thompson et al. 1997) and afterwards adjusted manually. Due to difficulties with amplification and sequencing some sequences are not available for all taxa and only partial sequences could be generated for *matK* and *atpA*.

Morphological Data—Morphological characters of Juncaginaceae and related families of Alismatales were compiled from numerous sources, mainly from Buchenau (1903), Aston (1977, 1993, 1995), Tomlinson (1982), Dahlgren et al. (1985), Kubitzki (1998), Igersheim et al. (2001), Stevens (2001 onwards), and Mabberley (2008). These were supplemented by own observations of living and/or preserved material of some genera of Juncaginaceae. We have not coded the morphological data for phylogenetic analysis because the available information is fragmentary for some taxa and coding would have resulted in an incomplete data matrix.

Phylogenetic Analysis—All datasets were separately analysed using Maximum Parsimony (MP) and Maximum Likelihood (ML) implemented in PAUP* 4.10b (Swofford 2003). The *atpA* data set showed little sequence variation and resulted in a poorly resolved tree. The same applies to the *matK* data set with slightly higher resolution. Therefore, a Partition Homogeneity Test (Farris et al. 1994; implemented in PAUP*) with 100 homogeneity replicates, 10 random addition sequences, tree-bisection-reconnection (TBR) branch swapping on, best only and MULTREES on was performed to test whether the three data sets (*rbcL*, *matK*, *atpA*) could be combined. No significant incongruence was detected between the data sets (pairwise ILD test: $p = 0.85$ for *rbcL* and *matK*, $p = 0.13$ for *rbcL* and *atpA*, $p = 0.04$ for *matK* and *atpA*). In consequence, we also analysed a combined data matrix of the cpDNA data and all three genes. All phylogenetic data sets were deposited in TreeBASE (study accession number S2667).

Maximum Parsimony (MP) analyses were performed using PAUP* with 1000 replicated heuristic searches using the same heuristic search settings as described above for the Partition Homogeneity Test. Gaps were treated as missing and gaps were not coded. Branch support was assessed with 100 bootstrap (BS) replicates with 10 random taxon additions each and TBR and MULTREES on.

For Maximum Likelihood (ML), the appropriate model of DNA substitution for the inference of phylogenetic relationships under ML was estimated using Modeltest 3.06 (Posada and Crandall 1998). Best-fit models were selected by the Akaike Information Criterion (Posada and Buckley 2004) and implemented in the corresponding data matrices (see Results). ML heuristic searches and

bootstrap branch support (BS) were performed in PAUP* with 100 replicated heuristic searches and the same settings as in the MP analysis.

Following Chase et al. (2000) in presenting and discussing the results, bootstrap support of 50%-74% is considered low, 75%-84% moderate, and > 85% high.

Results

Molecular Data – Phylogenetic Analysis—Relationships within Juncaginaceae and the delimitation of the family within Alismatales were reconstructed based on variation in the plastid genes *rbcL* and *matK*, the mitochondrial gene *atpA*, and a combination of these. Statistics for all analyses are summarised in Table 3. An overview of all results is given in Fig. 2, where simplified trees including MP and ML bootstrap values are provided.

cpDNA data—The *rbcL* data set comprised 38 species of all families of Alismatales plus *Acorus* (Acoraceae) as outgroup. Of the 1177 nucleotide positions included in the alignment, 358 were variable and 235 parsimony informative. The MP analysis resulted in six shortest trees (consistency index, CI = 0.476; retention index, RI = 0.649) with a length of 1033 steps (Table 3). The ML analysis (GTR+I+G, γ-shape parameter = 0.5070, base frequencies 0.2850 0.1876 0.2184, rate matrix 1.0354 3.9222 0.5658 1.0758 5.3542) yielded one best tree. No major incongruencies were found when comparing the topologies of the MP strict consensus tree and the ML tree when only clades with good support were considered. The ML tree is illustrated in Fig. 3 and described here.

In this tree (Fig. 3), members of the families Araceae and Tofieldiaceae are sister to all other Alismatales (core Alismatales). These strongly supported core Alismatales (ML BS 93%, MP BS 95%) comprise two subclades: 1) members of the families Alismataceae (incl. Limnocharitaceae), Butomaceae, and Hydrocharitaceae form a highly supported clade (ML BS 92%, MP BS 96%), and 2) a large clade in which *Scheuchzeria* and *Aponogeton* L.f. are moderately (to weakly) supported (ML BS 77%, MP BS 57%) as sister groups to a clade comprising Juncaginaceae and several aquatic families (Potamogetonaceae, Zosteraceae, Cymodoceaceae, Posidoniaceae, Ruppiaceae). The latter clade plus *Maundia* is sister to all other Juncaginaceae. This clade, in the following called *Maundia*/*Potamogeton* clade, is weakly supported in this data set (ML BS 68%, MP BS 71%) and not well-resolved. *Tetroncium* is weakly supported (ML BS 66%, MP BS 64%) as sister to the remaining Juncaginaceae. The latter clade, here called *Triglochin* s.l., is highly supported (ML BS 98%, MP BS 98%) and can be divided into *T. rheophila* (*T. procera* complex) and a clade comprising several other *Triglochin* spp. (*Triglochin* s.s.). *Lilaea* is nested within *Triglochin* s.s. (ML BS 100%, MP BS 100%), and is highly supported as sister to members of the *T. bulbosa* complex (ML BS 94%, MP BS 97%).

The *matK* data set comprised 31 taxa and 911 characters of which 478 were variable and 309 parsimony informative (Table 3). The MP analysis resulted in

Data set	*rbcL*	*matK*	*atpA*	*rbcL* + *matK*	*rbcL* + *matK* + *atpA*
No. taxa	38	31	23	31	19
No. characters / alignment length (bp)	1177	911	903	2088	2985
No. (%) variable characters	358 (30.4)	478 (52.5)	209 (23.1)	804 (38.5)	900 (30.2)
No. (%) informative characters [MP]	235 (20.0)	309 (33.9)	141 (15.6)	527 (25.2)	514 (17.2)
No. trees [MP]	6	2	10	1	1
tree length [MP]	1033	1190	346	2075	1783
CI/RI [MP]	0.476/0.649	0.576/0.701	0.760/0.869	0.543/0.672	0.651/0.685
No. trees [ML]	1	1	1	1	1
Model of sequence evolution [ML]	GTR+I+G	TVM+G	TVM+G	GTR+I+G	GTR+I+G

Table 3. Summary of sequence characteristics and tree statistics for the individual genes and for the combined analyses. bp – base pairs.

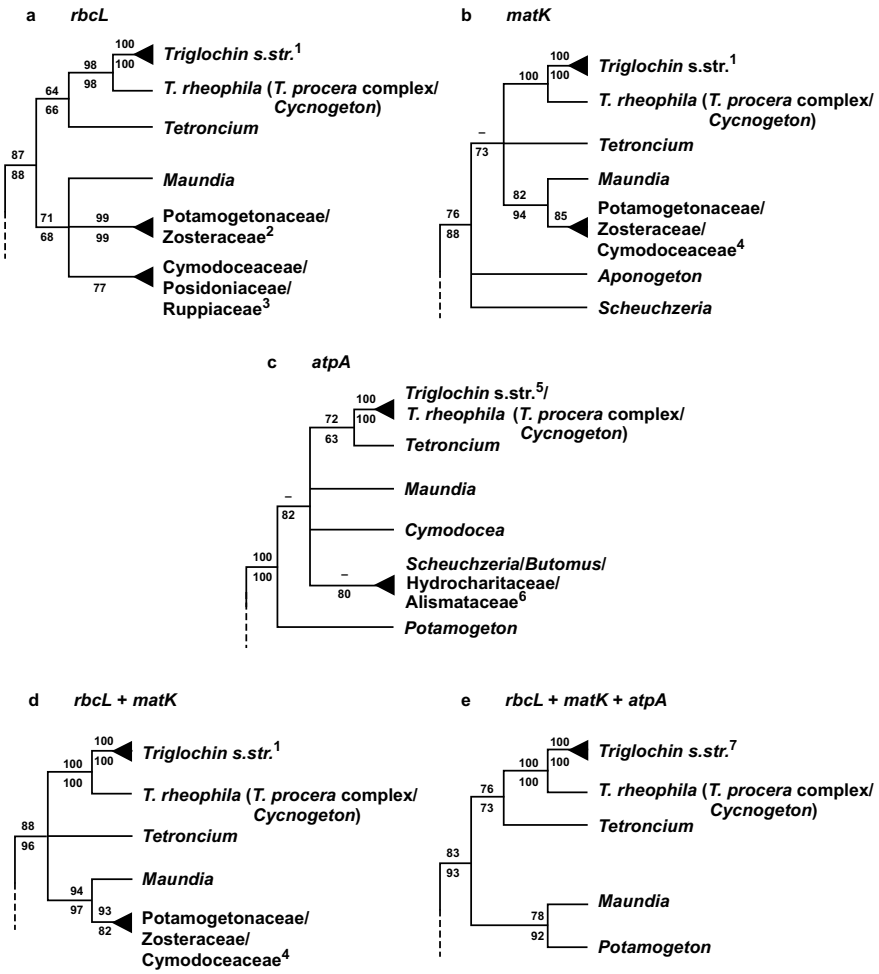

Fig. 2. Phylogenetic analyses of *rbcL*, *matK*, *atpA*, and the combined data sets. MP and ML bootstrap values given above and below branches, respectively. Only bootstrap values higher than 70% indicated. Dash (-): clade not recovered in MP analysis. [1] – *Triglochin barrelieri*, *T. elongata*, *Lilaea scilloides*, *T. maritima*, *T. palustris*, *T. striata*. [2] – *Potamogeton distinctus*, *Heterozostera tasmanica*, *Zostera noltii*, *Z. marina*, *Phyllospadix torreyi*. [3] – *Amphibolis antarctica*, *Cymodocea serrulata*, *Ruppia maritima*, *Halodule uninervis*, *Posidonia oceanica*. [4] – *Halodule uninervis*, *Potamogeton distinctus*, *Heterozostera tasmanica*, *Zostera noltii*, *Phyllospadix torreyi/iwatensis*. [5] – as [1] incl. *T. laxiflora* and excl. *T. palustris*. [6] – *Scheuchzeria palustris*, *Butomus umbellatus*, *Ottelia acuminata*, *Caldesia oligococca*, *Sagittaria latifolia*. [7] – as [1] excl. *T. palustris*. Missing parts of the tree (dashed line): *Aponogeton fenestralis*[a,d], *Scheuchzeria palustris*[a,d,e], *Hydrocharis dubia*[a,b,d], *Limnobium laevigata* [a,b,d], *Najas marina*[a], *Vallisneria americana*[a], *Ottelia acuminata*[a,b,d,e], *Stratiotes aloides*[a,b,d], *Butomus umbellatus*[a,b,d,e], *Hydrocleys nymphoides*[a,b,d], *Limnocharis flava*[a,b,d], *Alisma plantago-aquatica* [a,b,d], *Caldesia oligococca*[a,b,d,e], *Arisaema triphyllum/tortuosum*[a,b,c,d,e], *Gymnostachys anceps*[a,b,c,d,e], *Orontium aquaticum*[a,b,c,d,e], *Pleea tenuifolia*[a,b,c,d,e], *Tofieldia calyculata*[a,b,c,d,e], *Acorus calamus*[a,b,c,d,e].

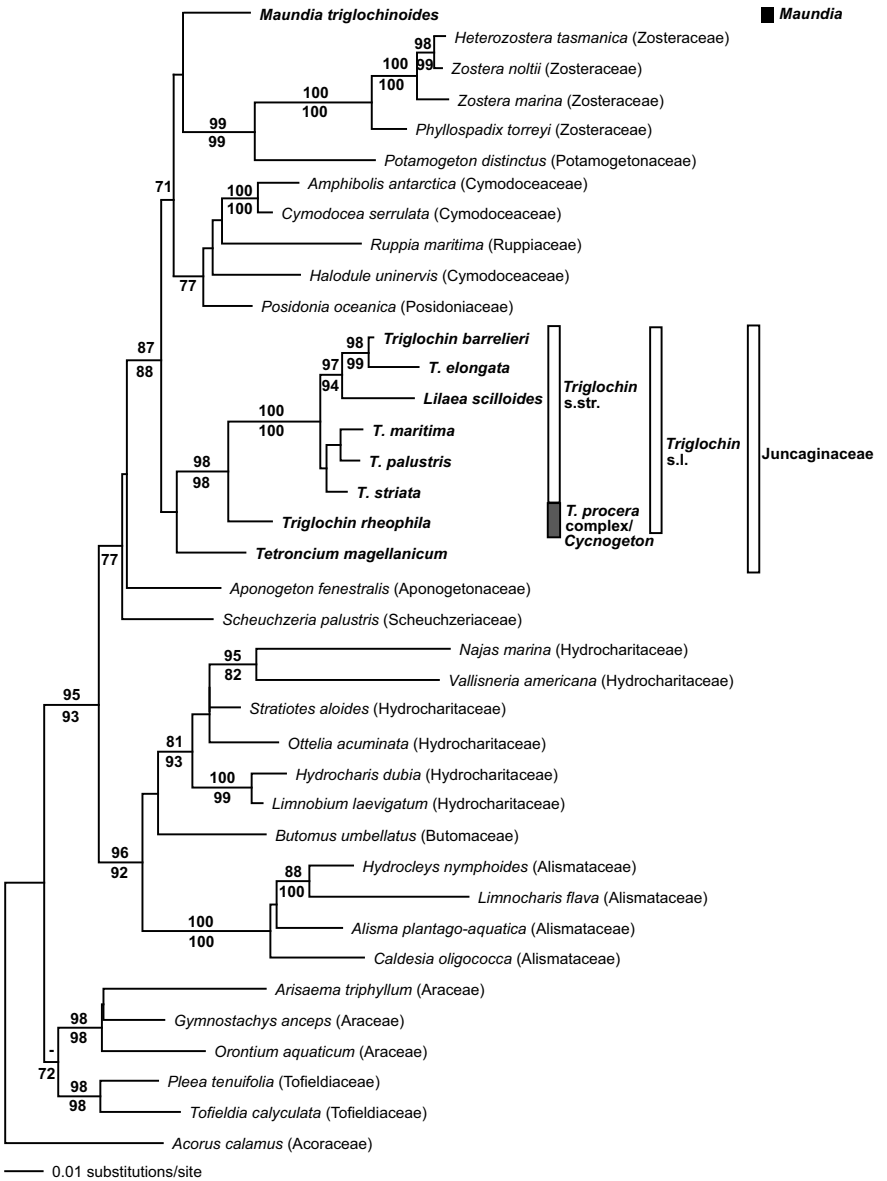

Fig. 3. ML phylogram of the *rbcL* data set of Alismatales. MP and ML bootstrap values given above and below branches, respectively. Only bootstrap values higher than 70% indicated. Currently recognized taxa of Juncaginaceae in bold. Dash (-): clade not recovered in MP analysis.

two shortest trees (CI = 0.576, RI = 0.701) of 1190 steps. The ML reconstruction (TVM+G, γ-shape parameter = 0.6700, base frequencies 0.3160 0.1483 0.1578, rate matrix 1.4885 2.9422 0.1615 1.2022 2.9422) resulted in one tree. This and the MP strict consensus tree are congruent, but differ in resolution. The ML tree resolves a trichotomy of *Triglochin* s.l., *Tetroncium,* and the *Maundia*/*Potamogeton* clade (ML BS 73%), whereas the MP tree essentially resulted in a polytomy of *Triglochin* s.l., *Tetroncium*, the *Maundia*/*Potamogeton* clade, *Aponogeton,* and *Scheuchzeria* (Fig. 2).

MTDNA DATA—The *atpA* data set comprised 23 taxa and 903 characters of which 209 were variable and 141 parsimony informative (Table 3). The MP analysis resulted in ten shortest trees (CI = 0.760, RI = 0.869) of 346 steps. The ML reconstruction (TVM+G, γ-shape parameter = 0.7648, base frequencies 0.2934 0.2118 0.2387, rate matrix 1.7514 2.0460 0.7166 0.4594 2.0460) resulted in one tree.

Resolution in the *atpA* data set was poor. *Tetroncium* is weakly supported as sister to a highly supported *Triglochin* s.l. (ML BS 100%, MP BS 100%). This group is part of a large polytomy including members of most families of core Alismatales (Fig. 2). The ML tree and the MP strict consensus tree are similar except for the position of *Potamogeton* L. In the ML tree this genus is sister to all other core Alismatales, while it is part of the polytomy in the MP analysis.

COMBINED MOLECULAR DATA—The combined cp data set (*rbcL* and *matK*) comprised 31 taxa and 2088 characters of which 804 were variable and 527 parsimony informative (Table 3). The MP analysis resulted in one shortest tree (CI = 0.543, RI = 0.672) of 2075 steps. The ML reconstruction (GTR+I+G, γ-shape parameter = 0.8140, base frequencies 0.2953 0.1713 0.1908, rate matrix 1.4691 3.3168 0.3026 1.0491 3.7757) resulted in one tree. The three gene data set (*rbcL*, *matK*, and *atpA*) comprised 19 taxa and 2985 characters of which 900 were variable and 514 parsimony informative (Table 3). The MP analysis resulted in one shortest tree (CI = 0.651, RI = 0.685) of 1783 steps. The ML reconstruction (GTR+I+G, γ-shape parameter = 0.7257, base frequencies 0.2890 0.1898 0.2121, rate matrix 1.6790 3.2857 0.3953 0.8581 3.8972) resulted in one tree.

In the two combined data sets, the topologies of the MP and ML trees are identical (Fig. 2) and differ only in branch support. As in the *rbcL* data set, *Scheuchzeria* and *Aponogeton* are the first diverging lineages in the larger subclade of the core Alismatales. Reconstruction of the two gene and the three gene data set differ mainly in the position of *Tetroncium*. Whereas in the less resolved cpDNA data set *Tetroncium* forms a trichotomy with *Triglochin* s.l. and the *Maundia*/*Potamogeton* clade, the three gene data set moderately supports *Tetroncium* as sister to *Triglochin* s.l. (ML BS 73%, MP BS 76%). In both data sets, *Triglochin* s.l. can be divided into *T. rheophila* and a highly supported clade comprising several species of *Triglochin* s.s. and *Lilaea* (ML BS 100%, MP BS 100%). This sister group relationship is highly supported (ML BS 100%, MP BS 100%). The position of *Maundia* as sister to a clade containing members of the aquatic families is better supported in the cpDNA.

Taxon	Genera/ Species	Habitat	Underground organs	Leaves	Flowers	No. carpels	Carpel fusion	Placentation	Ovules	Endosperm formation
Scheuchzeriaceae	1/1	*Sphagnum* bogs, marshes	rhizomes, stolons, turions	ligulate	3-merous	3(-6)	free	basal-axile	anatropous, erect	helobial
Aponogetonaceae	1/43	aquatic, freshwater	rhizomes, corms	eligulate	variable	3-6	free	basal or marginal	anatropous	helobial
Juncaginaceae *(Tetroncium)*	1/1	*Sphagnum* bogs	rhizomes	eligulate	2-merous	4	partly fused	basal	anatropous, erect	?
Juncaginaceae *(Triglochin s.s.)*	1/ca. 20	fresh to brackish water, marshes	rhizomes, stolons, bulbs	ligulate, auriculate	3-merous	3-6	fused	basal	anatropous, erect	nuclear
Juncaginaceae *(Lilaea)*	1/1	aquatic, seasonal pools	rootstocks	ligulate	1-merous	1	n/a	basal	anatropous, erect	nuclear
Juncaginaceae *(Cycnogeton)*	1/ca. 8	aquatic, freshwater	rhizomes, root-tubers	eligulate	3-merous	3-6	free	basal	anatropous, erect	?
Juncaginaceae *(Maundia)*	1/1	aquatic, freshwater	rhizomes	eligulate	perianthless or 2-merous	(2-)4	partly fused	apical	orthotropous, pendulous	?
Posidoniaceae	1/9	marine	rhizomes	ligulate	perianthless	1	n/a	apical	orthotropous, pendulous	helobial
Ruppiaceae	1/1-10	fresh to brackish water, not marine	rhizomes	eligulate, ± auriculate	perianthless	(2-)4(-16)	free	apical	campylotropous, ± pendulous	? (helobial and nuclear reported)
Cymodoceaceae	5/16	marine	rhizomes	ligulate	perianthless	2	free	apical	orthotropous, pendulous	nuclear
Zosteraceae	2/14	marine, rarely brackish water	rhizomes	ligulate	perianthless	1	n/a	apical	orthotropous, pendulous	? (helobial and nuclear reported)
Potamogetonaceae	4/102	aquatic, freshwater, rarely brackish water	rhizomes, stolons, turions	mostly ligulate	generally 4-merous	(1-)4(-7)	free	± apical	orthotropous (later campylotropous), pendulous	helobial

Table 4. Species no., habitat, and morphology of Juncaginaceae and related families. Compiled from various sources (see text for details). n/a – not applicable, ? - unknown.

Character	*Lilaea*	*Triglochin* s.str.	*Triglochin procera* complex (= *Cycnogeton*)
Habitat	seasonal pools	marshes, often saline, brackish water, seasonal pools	freshwater
Cyanogenic	+	+	–
Chromosome number	$2n = 12$	$2n = 12$, 18, 24, 36, 48, etc. (up to 144)	$2n = 16$, 32, 64
Habit	annual	annual or perennial	perennial
Leaves	ligulate	ligulate, auriculate	eligulate
Carpels	1	3-6, fused	3-6, free (to fused)
Carpophore	n/a	mostly present	absent
Endosperm formation	nuclear	nuclear	?

Table 5. Comparison of *Lilaea*, *Triglochin* s.s. and the *T. procera* complex (= *Cycnogeton*). ? – unknown.

Morphological Data—Selected morphological characters for Juncaginaceae and related families are compiled in Table 4, and for *Lilaea*, *Triglochin* s.s., and the *Triglochin procera* complex (*Cycnogeton*) in Table 5.

Discussion

Circumscription of Juncaginaceae and Intrafamilial Relationships—The circumscription of Juncaginaceae has changed through time particularly with respect to the inclusion or exclusion of *Scheuchzeria*, *Lilaea*, and *Maundia* (Table 1).

SCHEUCHZERIA—*Scheuchzeria palustris* is a rare species that is native to cool temperate regions of the Northern hemisphere, where it grows in wet *Sphagnum* bogs. It shares this habitat with *Tetroncium*, which has a similar ecology, but is confined to the Southern hemisphere. Even though the genus has been included in Juncaginaceae in earlier classifications, it is now generally acknowledged (and also supported by our data) that *Scheuchzeria* belongs to a separate family.

Recently the family Juncaginaceae has been considered to consist of four genera, *Triglochin*, *Tetroncium*, *Maundia*, and *Lilaea* (e.g. Haynes et al. 1998; Stevens 2001 onwards). In this circumscription, it is difficult to detect any convincing morphological synapomorphies for the family.

In this study, for the first time, all genera at some point affiliated with Juncaginaceae were analysed together. Our results clearly show that Juncaginaceae as currently circumscribed are not monophyletic (Figs. 2, 3).

MAUNDIA—*Maundia* does not group with the remaining taxa of the family in any of our analyses. The genus is moderately supported as part of a clade com-

prising members of Posidoniaceae, Ruppiaceae, Cymodoceaceae, Zosteraceae, and Potamogetonaceae (here called *Potamogeton* clade). Some morphological characters support the close relationship between *Maundia* and this clade of aquatic families (*Maundia*/*Potamogeton* clade). *Maundia* as well as most members of the *Potamogeton* clade show apical placentation and one pendulous, orthotropous ovule per carpel (e.g. Buchenau 1903; Aston 1977; Dahlgren et al. 1985; Kubitzki 1998; Table 4). These characters and the aquatic habitat are listed by Stevens (2001 onwards) as potential synapomorphies of the *Potamogeton* clade. In contrast to this, all other Juncaginaceae have one basal anatropous ovule per carpel (e.g., Buchenau 1903; Tomlinson 1982). The flower structure of *Maundia* is peculiar and has led to different interpretations. Flowers of *Maundia* have either been interpreted to have two (to four) perianth segments (Mueller 1858; Bentham 1878; Buchenau 1903; Nakai 1943) or these organs have been regarded as two bracts (Jacobs 2009; H. I. Aston, pers. comm.). When interpreted as bracts, the then perianthless flowers would constitute another similarity to several members of the *Potamogeton* clade. The stamens of *Maundia* have been interpreted as either (four to) six sessile, bilocular (tetrasporangiate) anthers (Bentham 1878; Buchenau 1903; H. I. Aston, pers. comm.) or as (eight to) 12 unilocular (bisporangiate) anthers (Mueller 1858). Thecae are almost separate (probably the reason for Mueller's interpretation as unilocular anthers), but adnate in pairs to a common connective (Bentham 1878; H. I. Aston, pers. comm.). This character is also found in members of the *Potamogeton* clade (e.g., Posidoniaceae and Zosteraceae; Tomlinson 1982; Dahlgren et al. 1985; Stevens 2001 onwards). We favour the interpretation of *Maundia* flowers as lacking a perianth and possessing six bilocular anthers, but developmental studies are needed to fully clarify the floral structure. Unlike most other Juncaginaceae (except *Tetroncium*, see below) which possess carpels that are free or fused but separate at maturity, the carpels of *Maundia* are fused (almost to the apex) and remain united at maturity.

The combined molecular and morphological evidence thus indicates that *Maundia* cannot be regarded as closely related to the remaining genera of Juncaginaceae. Consequently, this genus should be excluded from the family. Several potential synapomorphies uniting *Maundia* with the families of the *Potamogeton* clade can be identified (apical placentation, one orthotropous, pendulous ovule, and perianthless flowers). However, the exact relationships of the genus in the *Potamogeton* clade cannot be determined with our data. Taxon sampling in the *Maundia*/*Potamogeton* clade is low in our combined analyses. In the three-gene data set only one member of the different families of this clade is included (*Potamogeton*). Therefore, better sampling within this clade is necessary to resolve the relationships of *Maundia*. The lack of more detailed information about *Maundia* (e.g., karyological, palynological, and embryological data) and the uncertainty regarding the interpretation of the flower structure does not allow a more specific placement in the *Potamogeton* clade either. No clear affinities to one of the other families were found and *Maundia* might also form a separate lineage

within this order which includes several monotypic or monogeneric lineages (e.g., Scheuchzeriaceae, Butomaceae, and Aponogetonaceae). This would support a treatment of *Maundia* as the only genus of Maundiaceae as proposed by Nakai (1943) and accepted by Takhtajan (1997). Based on the currently available knowledge, this classification is adopted here.

TETRONCIUM—The monotypic *Tetroncium* is weakly supported as sister to *Triglochin* s.l. in the *rbcL* and the three-gene data set. However, this sister group relationship is not recovered in all analyses. Although *Tetroncium* and *Triglochin* s.l. have several characters in common (Table 4), none of these can be interpreted as synapomorphic. The two genera are clearly different in flower morphology and other characters. Thus, in contrast to *Triglochin* with bisexual, trimerous flowers, *Tetroncium* is dioecious and has dimerous flowers. Carpels of *Tetroncium* are fused (basally to lower half) and do not separate at maturity, whereas carpels of *Triglochin* s.l. are either fused (*Triglochin* s.s.) or free (*T. procera* complex) and mostly separate at maturity. Fruits of *Tetroncium* are reflexed (similar to *Carex pulicaris* L.), a character not known from *Triglochin* s.l. The two genera also differ in their seeds. While in *Triglochin* the endosperm is lacking (used up) in the mature seeds (as in most core Alismatales, e.g., Dahlgren et al. 1985), seeds of *Tetroncium* are endospermic (Hooker 1844; Buchenau 1903). Pollen data do not contradict a close relationship between *Tetroncium* and *Triglochin* s.l. (Grayum 1992). However, the "genera [*Lilaea*, *Tetroncium*, *Triglochin*] are quite uniform palynologically, and hardly to be distinguished on this basis from *Potamogeton*" (Grayum 1992). The stiff sword-shaped leaves of *Tetroncium* lack a ligule (Buchenau 1903; own obs.), while leaves of *Triglochin* s.s. (incl. *Lilaea*) are ligulate or auriculate, but eligulate in the *T. procera* complex. This latter character thus does not contradict the placement of *Tetroncium* as sister to *Triglochin* s.l. Provided such relationship would be correct, it would imply that ligulate leaves originated within *Triglochin* s.l.

Tomlinson (1982) correctly states that little is known about *Tetroncium*. Although the currently available data provide no unambiguous support for the relationships of *Tetroncium* and several characters seem autapomorphic, we retain it as a member of Juncaginaceae. The finding of nuclear endosperm formation would provide good support for the continued inclusion of *Tetroncium* in Juncaginaceae.

TRIGLOCHIN S.L.—All data sets revealed a highly supported *Triglochin* s.l. comprising *Triglochin* s.s. with *Lilaea* nested inside and *T. rheophila* of the *T. procera* complex (BS 100%, Fig. 2). This clade (*Triglochin* s.l.) was even recovered with moderate support (MP BS 83%) in a phylogeny obtained from the conserved 5.8S rRNA gene of the ITS region (only 163 bp; S. von Mering and J. W. Kadereit, unpubl. data).

The species of the *T. procera* complex are morphologically (Aston 1993, 1995) and molecularly (this study; S. von Mering and J.W. Kadereit, unpubl. data) clearly differentiated from the remaining *Triglochin* species (Table 5). Potential

synapomorphies of this monophyletic group include the presence of root-tubers, the lack of a ligule (unless the ligule originated within *Triglochin* s.l. as discussed above), the absence of a carpophor (carpels are free), and a chromosome base number of x = 8 (Robb and Ladiges 1981). Also, in contrast to several species of *Triglochin* s.s. and *Lilaea*, *T. procera* is not cyanogenic (Gibbs 1974).

The *T. procera* complex at times has been treated at generic rank as *Cycnogeton*, which was first described in 1838 as a monotypic genus, comprising only *C. huegelii*. Later, two *Triglochin* species (*T. linearis* and *T. procera*) were included in *Cycnogeton* (Sonder 1856; Buchenau 1867). However, *Cycnogeton* later was treated as section or subgenus *Cycnogeton* of *Triglochin* (Micheli 1881; Buchenau and Hieronymus 1889; Buchenau 1903, Table 1). In her revision of the *T. procera* complex Aston (1995, p. 332) wrote that the "tuberous-rooted species form a natural grouping based on their thickened, woody, fibre-covered rhizomes and their conspicuous storage tubers terminal on the roots. These subterranean features are quite unlike those of other species currently placed in *Triglochin* and could possibly be used as a distinguishing character applicable at generic rank. If further studies within the family supported such a generic distinction then the name *Cycnogeton* Endl. should be reinstated.".

Our molecular data in combination with the morphological distinctness of the group (Table 5) in our opinion warrant the segregation of the *T. procera* complex (water-ribbons) as *Cycnogeton* as proposed by Aston (1995).

LILAEA—Surprisingly, *Lilaea scilloides* is nested within *Triglochin* s.s. This position is highly supported in all data sets, with high support for a sister group relationship between *Lilaea* and the *Triglochin bulbosa* complex (highly supported in all analyses except the *atpA* data set). Thus, *Triglochin* s.s. would be paraphyletic if *Lilaea* were not included.

Lilaea has often been placed in its own family Lilaeaceae (e.g., Schumann 1894; Taylor 1909) based on its divergent floral morphology. This enigmatic species has unisexual and bisexual flowers of five different types (see Posluszny et al. 1986 for details). All flowers are monomerous, i.e., have only one carpel in female flowers, one stamen and one perianth segment in male flowers, and a combination of both in bisexual flowers. In contrast to this, flowers of *Triglochin* s.s. are always bisexual and trimerous. In spite of these striking differences in floral morphology, the two taxa share a number of characters. For example, the vegetative habit of *Triglochin* s.s. and *Lilaea* is similar and both taxa have semi-terete leaves with sheath and ligule. Furthermore, nuclear endosperm formation was described for both taxa (Agrawal 1952), and both have the same chromosome base number of x = 6 (Larsen 1966). Table 5 summarizes characters found in *Lilaea* and *Triglochin* s.s.

Although several studies had recognized the close relationship between *Lilaea* and Juncaginaceae (e.g., Markgraf 1936; Larsen 1966; Tomlinson 1982), the recognition of the position of *Lilaea* within *Triglochin* s.s. probably was hampered by the autapomorphic divergence of *Lilaea*.

The morphological characters discussed above and compiled in Tables 4 and 5 largely support the clades recovered in our molecular phylogenetic analyses. Even though large morphological data sets are available for the monocotyledons (e.g., Chase et al. 1995; Stevenson and Loconte 1995) knowledge of some taxa of Juncaginaceae is incomplete. Especially embryological, karyological, and palynological data are lacking, and more work is needed to allow a combined analysis of morphological and molecular data.

With the removal of *Maundia* from Juncaginaceae, a recircumscription of the family is necessary. In its new circumscription Juncaginaceae are characterised by having flowers in spike-like inflorescences, nuclear endosperm formation (unknown for *Tetroncium* and *Cycnogeton*), basal placentation, and one anatropous ovule per carpel. None of these characters can be regarded as synapomorphic. Our results necessitate several new combinations relating to the inclusion of *Lilaea* in *Triglochin* s.s. and the reinstatement of *Cycnogeton*.

Taxonomic Treatment

Proposed New Classification for Juncaginaceae
Juncaginaceae Rich., Démonstr. Bot. 9. Mai 1808 [as "Juncagines"], *nom. cons*.

Annual or perennial herbs with rhizomes or bulbs, sometimes with tuberous roots (*Cycnogeton*), mostly ± scapose. Leaves ± terete or flattened, sheathing, ligulate or eligulate. Inflorescence spike-like. Flowers inconspicuous, trimerous, dimerous or monomerous (*Triglochin scilloides*), bisexual or unisexual, then plants monoecious or dioecious (*Tetroncium*), or with bisexual and some unisexual flowers (*Triglochin scilloides*). Fruits or partial fruits indehiscent.

Three genera with ± 30 species, subcosmopolitan, mostly temperate. Centre of specific diversity in Australia.

Key to the genera of Juncaginaceae

1. Plants monoecious or with bisexual flowers, leaves semi-terete or ± flattened, ligulate or eligulate, flowers usually 3-merous (rarely 1-merous), of almost cosmopolitan distribution 2
1. Plants dioecious, leaves stiff, sword-shaped, eligulate, flowers 2-merous, from *Sphagnum* bogs in southern South America (Patagonia and Tierra del Fuego) and on some subantarctic islands (Falkland Islands, Gough Island) 1. *Tetroncium*

2. Plants with rhizomes and tuberous roots, leaves ± flattened, eligulate, fruits without carpophore, freshwater aquatics from Australasia 2. *Cycnogeton*

2 Plants with bulbs or rhizomes, leaves semi-terete, ligulate or auriculate, fruits mostly with carpophore, plants from most temperate regions of the world 3. *Triglochin*

1. TETRONCIUM Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 2: 17. 1808.

TETRONCIUM MAGELLANICUM Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 2: 17. 1808.

2. CYCNOGETON Endl., Stirp. Herb. Hügel.: 22. 1838 (Ann. Wien. Mus. 2: 210. 1838).

CYCNOGETON HUEGELII Endl., Stirp. Herb. Hügel.: 23. 1838 (Ann. Wien. Mus. 2: 211. 1838). *Triglochin huegelii* (Endl.) Aston, Muelleria 8: 3: 346.

CYCNOGETON LINEARE (Endl.) Sond., Linnaea 28: 225. 1851. *Triglochin linearis* Endl., Pl. Preiss. 2: 54. 1848.

CYCNOGETON PROCERUM (R.Br.) Buchenau, Abh. Naturwiss. Vereine Bremen 1: 224. 1867. *Triglochin procera* R.Br., Prodr. Fl. Nov. Holland.: 343. 1810.

Cycnogeton alcockiae (Aston) Mering & Kadereit, comb. nov. *Triglochin alcockiae* Aston, Muelleria 8: 85. 1993.

Cycnogeton dubium (R.Br.) Mering & Kadereit, comb. nov. *Triglochin dubia* R.Br., Prodr. Fl. Nov. Holland.: 343. 1810.

Cycnogeton microtuberosum (Aston) Mering & Kadereit, comb. nov. *Triglochin microtuberosa* Aston, Muelleria 8: 88. 1993 [*as T. microtuberosum*].

Cycnogeton multifructum (Aston) Mering & Kadereit, comb. nov. *Triglochin multifructa* Aston, Muelleria 8: 90. 1993 [as *T. multifructum*].

Cycnogeton rheophilum (Aston) Mering & Kadereit, comb. nov. *Triglochin rheophila* Aston, Muelleria 8: 94. 1993 [as *T. rheophilum*].

3. TRIGLOCHIN L., Sp. Pl.: 338 (1753).

Triglochin scilloides (Poir.) Mering & Kadereit, comb. nov. *Phalangium scilloides* Poir., Encycl. (Lamarck) 5: 251. 1804. *Lilaea scilloides* (Poir.) Hauman, Publ. Inst. Invest. Geogr. Fac. Filos. Letras Univ. Buenos Aires, A 10: 26. 1925.

Excluded taxa

MAUNDIA F.Muell., Fragm. 1: 22. 1858.

M. TRIGLOCHINOIDES F.Muell., Fragm. 1: 23. 1858. *Triglochin triglochinoides* (F.Muell.) Druce, Bot. Soc. Exch. Club Brit. Isles 4: 651. 1916 (publ. 1917).

Triglochin maundii F.Muell., Fragm. 6: 83. 1867, nom. inval., nom. prov.

Maundiaceae Nakai, Chosakuronbun Mokuroku [Ord. Fam. Trib. Gen. Sect. ... nov. ed.]: 213. 1943.

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Appendix 1

List of Juncaginaceae and other Alismatales species used in this study (families given in bold). Accession information is listed as follows: species name; voucher specimen; and GenBank accession numbers (*rbcL*, *matK*, and *atpA*) [with placeholder taxa in parenthesis]. Voucher specimen information is given only for newly obtained sequences, indicated by bold accession numbers. Herbarium abbreviations are from Holmgren and Holmgren (1998). — indicates missing sequence data.

* * *

Acoraceae: *Acorus calamus* L., AJ879453, AB040154, AF039256. **Alismataceae:** *Alisma plantago-aquatica* L., L08759, AF542573, —. *Caldesia oligococca* (F.Muell.) Buchanan, AY277799, AY952427, AY277800. *Hydrocleys nymphoides* (Humb. & Bonpl. ex Willd.) Buchenau, U80716, AB002580, —. *Limnocharis flava* (L.) Buchenau, U80717, AB088778, —. *Sagittaria latifolia* Willd. —, —, AY299832. **Aponogetonaceae:** *Aponogeton fenestralis* (Pers.) Hook.f., AB088808, AB088779, —. **Araceae:** *Arisaema triphyllum* (L.) Torr., AJ005629, AF3877428 (*A. tortuosum* (Wall.) Schott), AY299717. *Gymnostachys anceps* R.Br., AB088806, AB040177, AF039244. *Orontium aquaticum* L., AJ005632, AF543744, AY299816. **Butomaceae:** *Butomus umbellatus* L., U80685, AY870364, AY299733. **Cymodoceaceae:** *Amphibolis antarctica* (Labill.) Asch., U80686, —, —. *Cymodocea serrulata* (R.Br.) Asch. & Magnus, U80715, —, AY277801. *Halodule uninervis* (Forssk.) Boiss., AY952436, AY952424, —. **Hydrocharitaceae:** *Hydrocharis dubia* (Blume) Backer, AB004892, AB002572, —. *Limnobium laevigatum* (Humb. & Bonpl. ex Willd.) Heine, AB004894, AB002574, —. *Najas marina* L., U80705, —, —. *Ottelia acuminata* (Gagnep.) Dandy, AY952435, AY952432, AY277802. *Stratiotes aloides* L., U80709, AB002576, —. *Vallisneria americana* Michx., U03726, —, —. **Juncaginaceae:** *Lilaea scilloides* (Poir.) Hauman, USA, California, Moore s.n. (MJG), U80715, **GQ452345**, **GQ452348**. *Maundia triglochinoides* F.Muell., Australia, *S. Jacobs 9453* (MJG, NSW), **GQ452330**, **GQ452347**, **GQ452349**. *Tetroncium magellanicum* Willd., Argentina, *A. Vogel s.n.* (MJG), **GQ452337**, **GQ452346**, **GQ452351**. *Triglochin barrelieri* Loisel., Italy, *C. Uhink s.n.* (MJG), **GQ452331**, **GQ452342**, **GQ452352**. *Triglochin elongata* Buchenau, South Africa, *P. Vargas 537PV00* (MJG), **GQ452332**, **GQ452343**, **GQ452353**. *Triglochin laxiflora* Guss., Italy, *S. von Mering s.n.* (MJG), —, —, **GQ452354**. *Triglochin maritima* L., Turkey,

D. Albach & F. Özgokce 912 (MJG), **GQ452333**, **GQ452339**, **GQ452355**. *Triglochin palustris* L., Russia, *P. Schönswetter & A.Tribsch* T145 (WU), **GQ452334**, **GQ452340**, —. *Triglochin rheophila* Aston, Australia, *S. Jacobs 9392* (MJG, NSW), **GQ452335**, **GQ452344**, **GQ452356**. *Triglochin striata* Ruiz & Pav., Australia, *N. Schmalz s.n.* (MJG), **GQ452336**, **GQ452341**, **GQ452357**. **Posidoniaceae:** *Posidonia oceanica* (L.) Delile, U80719, —, —. **Potamogetonaceae:** *Potamogeton distinctus* A.Benn., AB088809, AB088780, AY299829 (*P. natans* L.). **Ruppiaceae:** *Ruppia maritima* L., U03729, —, —. **Scheuchzeriaceae:** *Scheuchzeria palustris* L., Germany, *C. Uhink s.n.* (MJG), U03728, **GQ452338**, **GQ452350**. **Tofieldiaceae:** *Pleea tenuifolia* Michx., AJ131774, AF465301, AY299827. *Tofieldia calyculata* (L.) Wahlenb., AB183410, AB183403, AY299851. **Zosteraceae:** *Heterozostera tasmanica* (M.Martens ex Asch.) Hartog, U80730, AB096171, —. *Phyllospadix torreyi* S.Watson, U80731, AB096172 (*P. iwatensis* Makino), —. *Zostera marina* L., U80734, —, —. *Z. noltii* Hornem., U80733, AB096170, —.