Systematics, phylogeny and biogeography of Juncaginaceae

Dissertation
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Dekan:
1. Berichterstatter:
2. Berichterstatter:
Tag der mündlichen Prüfung:

Triglochin maritima L. Saltmarsh in Denmark (Photo: SvM).

> "For there are some plants which cannot live except in wet;
> and again these are distinguished from one another by their fondness for different kinds of wetness;
> so that some grow in marshes, others in lakes, others in rivers, others even in the sea […].
>
> Some are water plants to the extent of being submerged, while some project a little from the water;
> of some again the roots and a small part of the stem are under the water, but the rest of the body is
> altogether above it."

Theophrastus (370-c. 285 B.C.) on aquatic plants in *Enquiry into Plants* (*Historia Plantarum*)

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Publications arising from this thesis

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>
> * SvM conducted morphological studies on herbarium material of *Maundia* and related taxa, gathered characters and literature information for *Maundia* and related taxa, and worked on the manuscript.

Introduction

The monocot order Alismatales is a cosmopolitan and highly diverse group comprising 13 (APG III 2009) or 14 families with about 166 genera and ~4500 species (Stevens 2001+; Table 1 in Chapter 1). It forms a well supported monophyletic clade (APG II 2003, APG III 2009, Soltis et al. 2005 and references therein) and the current circumscription includes well-known groups such as the species-rich and highly diverse Arum family (Araceae; e.g., Cabrera et al. 2008, Cusimano et al. 2011) as well as all marine flowering plants (seagrasses; e.g., den Hartog 1970, Green & Short 2003).

Molecular phylogenetic studies have shown that Araceae are sister to all remaining families of the order, and within the latter group Tofieldiaceae are sister to the so-called 'core Alismatales' (e.g., Les et al. 1997, Chase et al. 2006, Iles et al. 2013, Les & Tippery 2013). 'Core Alismatales' (e.g., Iles et al. 2013), approximately corresponding to the traditional order Helobiae (Engler 1909), is a morphologically highly diverse monophyletic group of aquatic and wetland plants found in all regions of the world. Core Alismatales can be divided into two informal groups based on floral characteristics: a "petaloid" and a "tepaloid" clade (Posluszny & Charlton 1993). While petaloid alismatids comprise the three families Hydrocharitaceae, Butomaceae, and Alismataceae (incl. Limnocharitaceae), tepaloid alismatids comprise the following eight families: Scheuchzeriaceae, Aponogetonaceae, Juncaginaceae, Posidoniaceae, Cymodoceaceae, Ruppiaceae, Potamogetonaceae, and Zosteraceae.

As an early-diverging monocot lineage, Alismatales are one key to the understanding of monocot evolution. Several studies of members of Alismatales have concentrated on comparative flower morphology and development (e.g., Posluszny & Charlton 1993, Posluszny et al. 2000, Remizowa et al. 2012b). However, some taxa have been overlooked by researchers and very little information about their biology is available in the literature. Among those poorly known taxa is the small arrowgrass family Juncaginaceae Rich., a family of annual or perennial grass-like herbs characterised by basal, sheathing leaves and spike-like inflorescences (Fig. 1) and underground storage organs such as bulbs, rhizomes, stolons, or tuberous roots.

The family Juncaginaceae was established at the beginning of the 19th century when Louis Claude Marie Richard used the name "Juncagines" and gave the following short description plus a French translation of it: "Juncagines. Caps 2. Sperma s. Akenium: Sem. erectum. Embryo perispermicus, orthotropus, brachypodus." (Richard 1808). Some years later, Richard listed the following genera comprising "Juncagineae": *Lilaea*, *Cathanthes* [a synonym of *Tetroncium*], *Triglochin*, *Scheuchzeria* (Richard 1815). Juncaginaceae is a conserved name (*nomen conservandum*, *nom. cons.*). Circumscription of the family and delimitation of genera have changed considerably since Richard's times. More details on the history of classification are given in Chapter 1 (see also Table 1 in Chapter 1).

The last comprehensive study of the family dates back to the beginning of the 20th century (Buchenau 1903). Few broader studies focussing on members of the family were published afterwards; publications were mostly restricted to regional studies (e.g., Markgraf 1981, Haynes 2004) or focussing on one or few taxa (e.g., Davy & Bisphop 1991). Notable exceptions are the revision of the tuberous-rooted *Triglochin* species (Aston 1993, 1995) and the recent treatment for the Flora of Australia (Aston 2011) covering a major part of the family.

The number of accepted genera in Juncaginaceae has varied between three and five (e.g., Dahlgren et al. 1985, Takhtajan 1997, Haynes et al. 1998) and species numbers range from 12 to 15 (e.g., Haynes et al. 1998, Stevens 2001+, Mabberley 2008). However, these data are based on limited information and considerably underestimate the real number of species. Recent treatments have shown that the number of recognised species exceeds 30 (e.g., Köcke et al. 2010; Aston 1993, 1995, 2011).

The family Juncaginaceae has an almost cosmopolitan distribution, with a notable gap in large parts of the tropics (for a distribution map see Fig. 1 in Chapter 1). Australia is the centre of species diversity with about 26 species in 3 genera (Aston 2011). Australian taxa include a group of annual *Triglochin* species and the *Triglochin procera* complex [*Cycnogeton*] (Aston 2011). Further species are native to the southern hemisphere: several taxa of the *Triglochin bulbosa* complex are endemic to South Africa (see Chapter 3; Fig. 1), and the monotypic *Tetroncium* is restricted to southern South America (see Chapter 4; Fig. 1). While some species are widespread in temperate or cooler regions of both hemispheres (e.g., *T. palustris*, *T. maritima*) others are restricted to small areas (e.g., *T. gaspensis*, *T. buchenaui*).

Despite its small size, the family shows considerable ecological diversity. Members of Juncaginaceae are wind-pollinated herbs which can be found in freshwater wetlands (slow-flowing rivers, bogs, fens), in brackish water (e.g., estuaries), and in coastal salt marshes. Some taxa inhabit only seasonally wet terrestrial sites such as vernal pools or inland salt pans (e.g., annual species of *Triglochin*, *T. bulbosa* L. subspp.). Species of the *T. procera* complex (*Cycnogeton*) are true aquatics, living in freshwater habitats in Australia (Aston 1993, 1995, 2011). *Triglochin* is probably best known for its coastal salt marsh species found in many parts of the world (e.g., *T. maritima*, *T. striata*). However, even those taxa are not confined to coastal habitats, but also occur in inland habitats. Nevertheless, occurrence is usually linked to (seasonal) wetlands and often to habitats with higher salinity. *Triglochin* species occur in habitats from sea level up to 4,000 metres in the Rocky Mountains (Haynes & Hellquist 2000) and even more than 5,000 metres altitude in the Himalayas (Guo et al. 2010).

At the beginning of this project little was known about phylogenetic relationships within Juncaginaceae. Almost no sequence data were available for members of the family and inter- and intrageneric relationships remained largely unresolved. Despite the interesting distribution pattern, biogeographic aspects had not been studied in detail. In addition, the urgent need for taxonomic revisions of several groups within Juncaginaceae had been emphasised by different authors (e.g., Thieret 1988, H. I. Aston, pers. comm.).

In this thesis I have investigated the systematics, phylogeny and biogeographical history of the family Juncaginaceae based on morphological and molecular data. Molecular markers from all three plant genomes (nuclear, chloroplast and mitochondrial) were used in this first study of Juncaginaceae on a global scale and an extensive species-level phylogeny was generated, with a focus on the extra-Australian taxa (Chapter 1 and 2). In the course of this project, more than 7,500 specimens from about 40 herbaria have been revised to clarify species delimitation and to gain insight into the specific diversity of *Triglochin* and relatives (Chapter 3 and 4). The main questions included: Are Juncaginaceae in the traditional circumscription a monophyletic group? How are the genera related to each other? How old is the group and where did it originate?

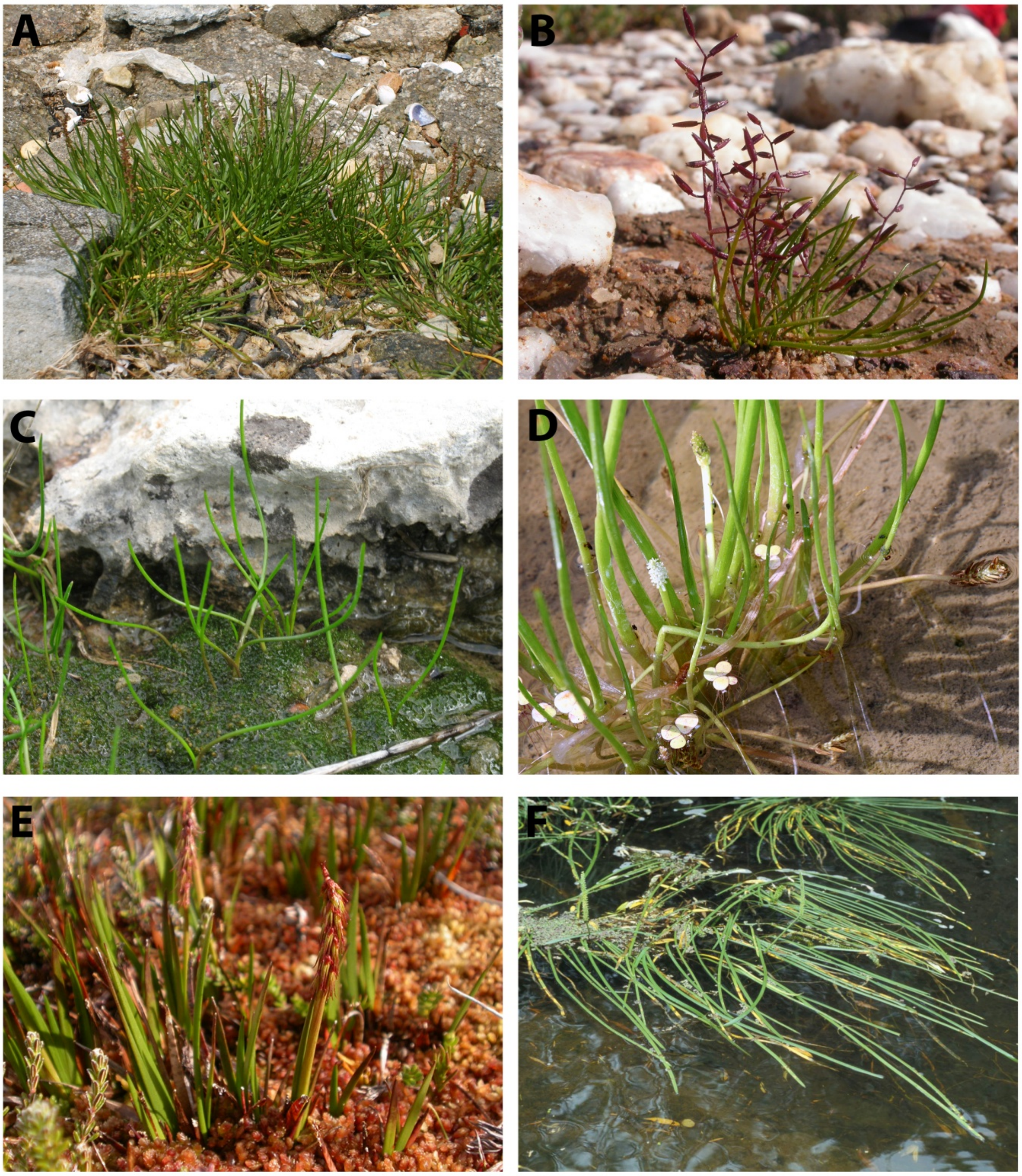

Fig. 1. Juncaginaceae. Representative species of the family showing general habit and habitat diversity. **A.** *Triglochin maritima* (coast, Galicia, Spain), **B.** *T. bulbosa* ssp. *quarcicola* (quartz field, Knersvlakte, South Africa), **C.** *Triglochin laxiflora* (rock pool, Malta), **D.** *Triglochin scilloides (*syn. *Lilaea scilloides*), (pool in Botanic Garden Mainz), **E.** *Tetroncium magellanicum* (*Sphagnum* bog, Argentina), **F.** *Cycnogeton* sp. (freshwater, Australia). Photos: S. v. Mering, U. Schmiedel, S. Mifsud, R. Greissl, R. Douzet, SAJF, A. Schönhofer.

In the five chapters of this thesis I have used a wide range of methods to address these questions and to extend our knowledge of Juncaginaceae, from classical taxonomic revisions to molecular systematic and biogeographic approaches.

In **Chapter 1** 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 accepted genera or those associated with the family in the past were analysed together. Phylogenetic analysis of three molecular markers (*rbc*L, *mat*K, and *atp*A) showed that Juncaginaceae were not monophyletic. As a consequence the family is re-circumscribed to exclude *Maundia* which is proposed to belong to a separate family Maundiaceae. In the new classification the number of accepted genera in Juncaginaceae is reduced to three: *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.str. The enigmatic *Lilaea* is nested within *Triglochin* s. str. and highly supported as sister to the *T. bulbosa* complex. The results of the molecular analysis are discussed in combination with morphological characters. Additionally, a key to the genera of the family is given and several new combinations are made.

In **Chapter 2** the phylogenetic relationships within the largest genus *Triglochin* were investigated. A species-level phylogeny was constructed based on molecular data obtained from nuclear (ITS, internal transcribed spacer) and chloroplast sequence data (*psb*A-*trn*H spacer, *mat*K gene). Based on the phylogeny of the group divergence times were estimated and ancestral distribution areas reconstructed. The monophyly of *Triglochin* is confirmed and relationships between the major lineages of the genus are resolved. A clade comprising the Mediterranean/ African *T. bulbosa* complex and the American *T. scilloides* (formerly *Lilaea s.*) is sister to the rest of the genus which contains two main clades. In the first, the widespread *T. striata* is sister to a clade comprising annual *Triglochin* species from Australia. The second clade comprises *T. palustris* as sister to the *T. maritima* complex, of which the latter is further divided into a Eurasian and an American subclade. Diversification in *Triglochin* began in the Miocene or Oligocene, and most disjunctions in *Triglochin* were dated to the Miocene. Taxonomic diversity in some clades is strongly linked to habitat shifts and can not be observed in old but ecologically invariable lineages such as the non-monophyletic *T. maritima*.

Chapter 3 is an α-taxonomical treatment carried out in collaboration with colleagues. The *Triglochin bulbosa* complex (Juncaginaceae), a monophyletic group from the Mediterranean region and Africa was revised based on the study of a large number of herbarium specimens. One new species, *Triglochin buchenaui*, and two new subspecies, *T. bulbosa* subsp. *calcicola* and subsp. *quarcicola*, are described from South Africa. Furthermore, two taxa are elevated to species rank and two are reinstated. Altogether, seven species and four subspecies are recognised, thus, more than doubling the number of accepted *Triglochin* species for this region. An identification key, detailed descriptions and accounts of the ecology and distribution of the taxa are provided. An IUCN conservation status is proposed for each taxon.

Chapter 4 deals with the monotypic genus *Tetroncium* from southern South America. The remarkable *T. magellanicum* is the only dioecious species of the family. The taxonomic history of the species is described, type material is traced, and a lectotype for the name is designated. Based on an extensive study of herbarium specimens and literature a detailed description of the species and notes on its ecology and conservation status are provided. For the first time, a detailed map showing the complete known distribution area of *T. magellanicum* is presented.

In the final chapter, **Chapter 5**, colleagues and I studied the flower structure of the rare Australian endemic *Maundia triglochinoides* which has recently been shown not to be a member of Juncaginaceae based on molecular data. In the light of this finding re-assessment of views on the morphological evolution and classification of Alismatales is required. As the morphology of *Maundia* is poorly known and some key features are controversially described in the literature, inflorescences, flowers and fruits were studied using serial mictrotome sections and scanning electron microscopy. The isolated phylogenetic placement, affinities to related taxa, and the evolution of certain features are discussed. As *Maundia* exhibits a mosaic of features characteristic of other families of tepaloid core Alismatales its segregation as a separate family is plausible.

Chapter 1

Phylogeny, systematics and recircumscription of Juncaginaceae – a cosmopolitan wetland family

Sabine von Mering & Joachim W. Kadereit

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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*.

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[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 acknowledgement of his support of the project described here.

INTRODUCTION

The order Alismatales (14 families with ca. 4490 species; Stevens 2001+), 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+).

As currently circumscribed, Juncaginaceae comprise four genera, *Triglochin*, *Lilaea*, *Maundia*, and *Tetroncium* (Haynes et al. 1998; Stevens 2001+) 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 et al. 1998; Stevens 2001+; Govaerts 2008; Mabberley 2008). However, recent revisions (Aston 1995; Köcke et al., 2010; H. I. Aston, pers. comm.) have revealed considerably higher numbers of probably more than 30 species.

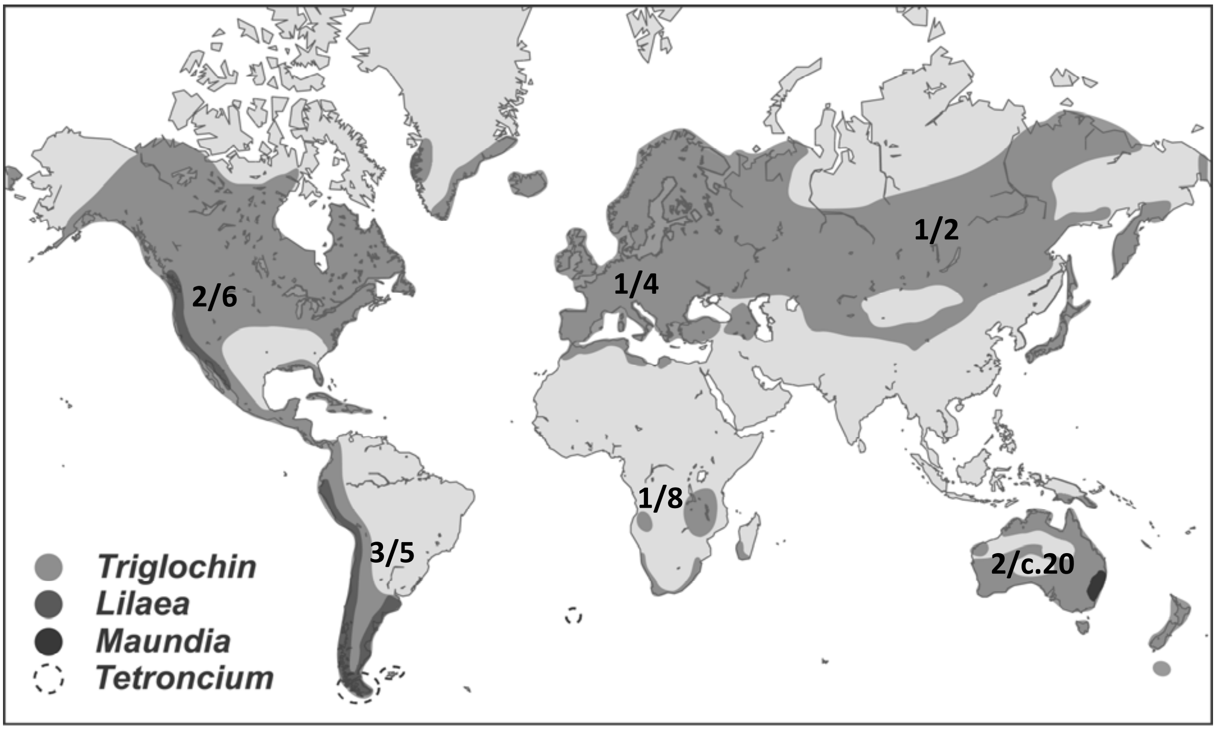

Fig. 1. Geographical distribution of the currently recognized genera of Juncaginaceae. Map prepared using Online Map Creation (Weinelt 1996 onwards).

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+). 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 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).

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.

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*

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^{TM} 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 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 Sequencher 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* sequen-ces 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+), 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.

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

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'
JU*matK* 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'

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 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

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

Data set	*rbc*L	*mat*K	*atp*A	*rbc*L + *mat*K	*rbc*L + *mat*K + *atp*A
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

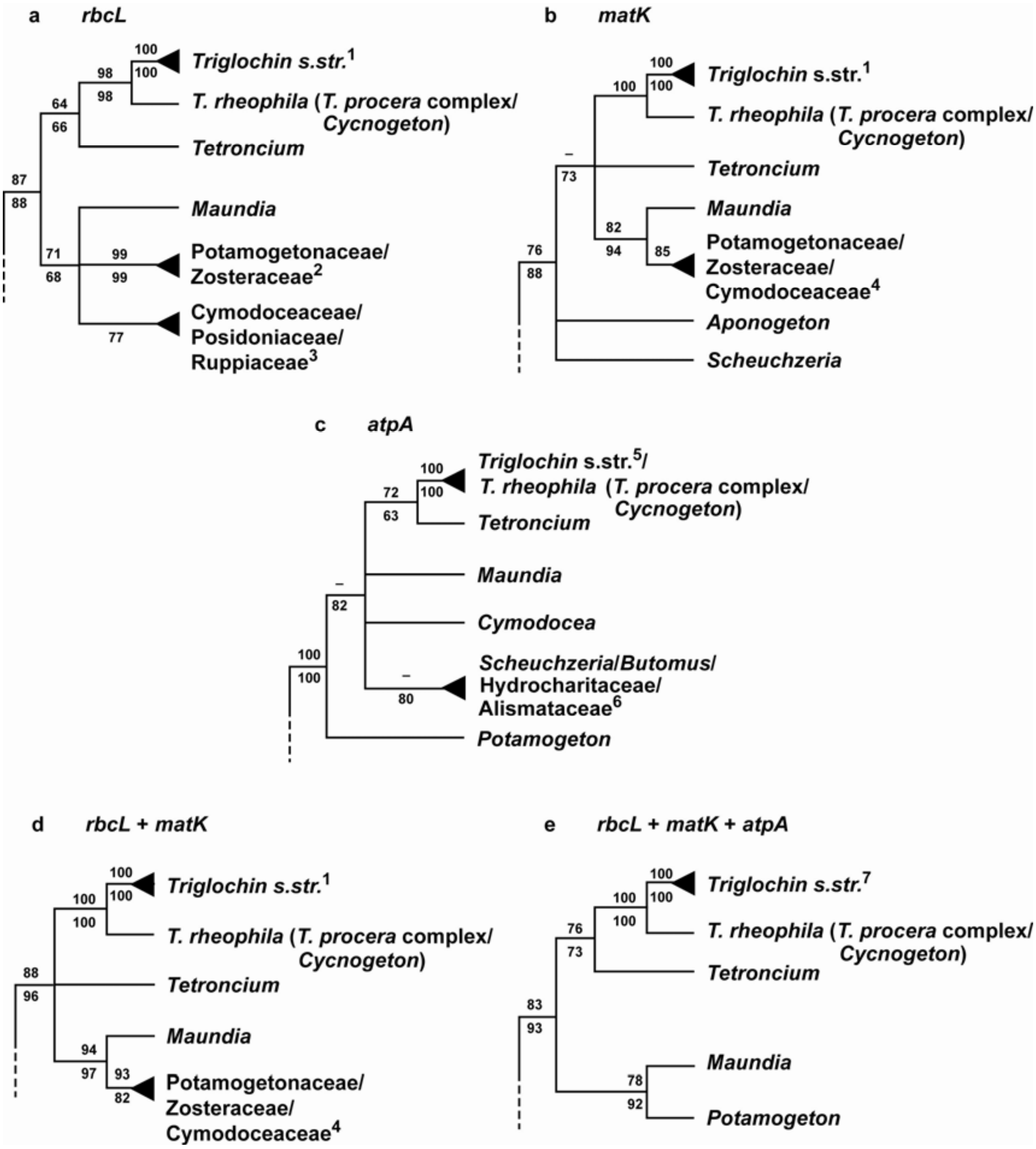

Fig. 2. Phylogenetic analysis of *rbcL*, *matK*, *atpA*, and the combined data sets. MP and ML bootstrap values given above and below the line, respectively. Only bootstrap values higher than 70% included. Dash (-): clade not recovered in MP analysis.

[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] but incl. *T. laxiflora* and excl. *T. palustris*. [6] – *Scheuchzeria palustris*, *Butomus umbellatus*, *Ottelia acuminata*, *Caldesia oligococca*, *Sagittaria latifolia*. [7] – as [1] but 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], *Limoncharis 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].

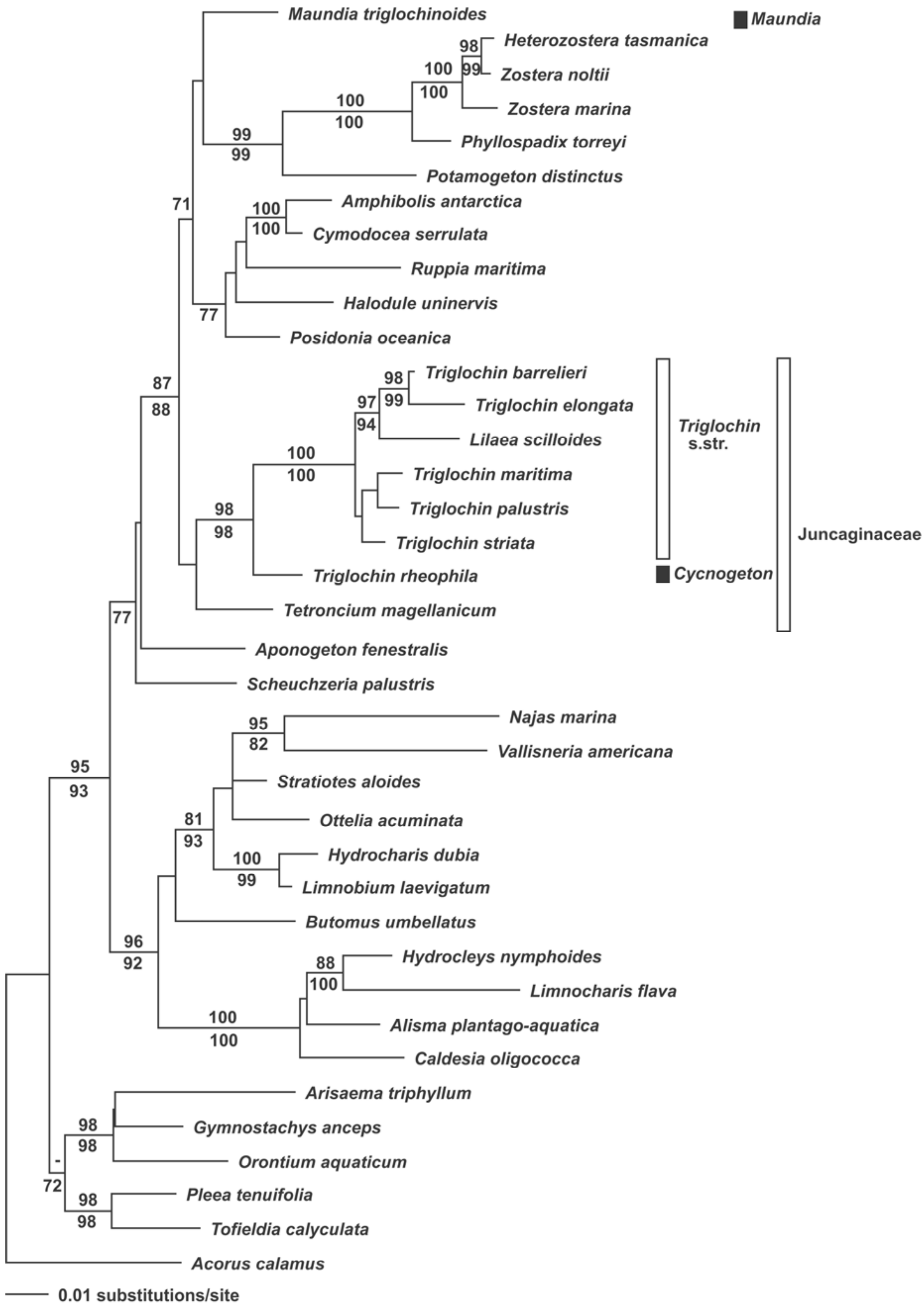

Fig. 3. ML phylogram of *rbcL* data set of Alismatales. MP and ML bootstrap values given above and below the line, respectively. Currently recognized taxa of Juncaginaceae in bold. Dash (-): clade not recovered in MP analysis.

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.

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

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. 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 1 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

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

Character	*Lilaea*	*Triglochin* s.s.	*Triglochin procera* complex (= *Cycnogeton*)
Habitat	seasonal pools	marshes, often saline, brackish water, seasonal pools	freshwater
Cyanogenic	+	+	–
Chromosome no.	$2n = 12$	$2n = 12, 18, 24, 36, 48$, 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	?

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.

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+). 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 comprising 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+) 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+). 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

Chapter 2

Phylogeny, biogeography and evolution of *Triglochin* L. (Juncaginaceae) – morphological diversification is linked to habitat shifts rather than to genetic diversification

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ABSTRACT

A species-level phylogeny is presented for *Triglochin*, the largest genus of Juncaginaceae (Alismatales) comprising about 30 species of annual and perennial herbs. *Triglochin* has an almost cosmopolitan distribution with Australia as centre of species diversity. Trans-Atlantic and trans-African disjunctions exist in the genus. Phylogenetic analyses were conducted based on molecular data obtained from nuclear (ITS, internal transcribed spacer) and chloroplast sequence data (*psb*A-*trn*H spacer, *mat*K gene). Based on the phylogeny of the group divergence times were estimated and ancestral distribution areas reconstructed. Our data confirm the monophyly of *Triglochin* and resolve relationships between the major lineages of the genus. The sister group relationship between the Mediterranean/African *T. bulbosa* complex and the American *T. scilloides* (formerly *Lilaea s.*) is highly supported. This clade is sister to the rest of the genus which contains two main clades. In the first, the widespread *T. striata* is sister to a clade comprising annual *Triglochin* species from Australia. The second clade comprises *T. palustris* as sister to the *T. maritima* complex, of which the latter is further divided into a Eurasian and an American subclade. Diversification in *Triglochin* began in the Miocene or Oligocene, and most disjunctions in *Triglochin* were dated to the Miocene. Taxonomic diversity in some clades is strongly linked to habitat shifts and can not be observed in old but ecologically invariable lineages such as the non-monophyletic *T. maritima*.

Keywords. Alismatales, *Cycnogeton*, disjunctions, dispersal/LDD, *Lilaea*, molecular dating

Introduction

Triglochin L. is an almost cosmopolitan genus with between 25 and 30 currently recognized species (e.g., Aston, 2011; Köcke et al., 2010; Table 1, 2), making it the largest genus in the small monocot family Juncaginaceae (Alismatales) which besides *Triglochin* comprises *Cycnogeton* Endl. and *Tetroncium* Willd. (von Mering and Kadereit, 2010). In the strict sense *Triglochin* includes annual or perennial "grass-like" herbs characterised by sheathing leaves with ligules or auricles and spike-like inflorescences.

Triglochin probably is best known for its coastal salt marsh species found in most temperate regions of the world. However, species of *Triglochin* occur in a wide variety of wet to dry habitats such as estuaries, seasonal wetlands (vernal pools, rock pools), and semi-desert inland habitats from sea level to up to ca. 5,000 m altitude (Guo et al., 2010).

The generic circumscription of *Triglochin* has long been unstable (see Table 1 for details). Following a recent molecular phylogenetic study, the *Triglochin procera* complex (the so-called water ribbons; Aston, 1995, 1993) was segregated from *Triglochin* s. l. and reinstated as *Cycnogeton* Endl. based on a phylogenetic study and morphological, karyological and ecological characters (von Mering and Kadereit, 2010). Furthermore, the monospecific *Lilaea* Bonpl. was found to be nested within *Triglochin* and transferred into the genus as *T. scilloides* (Poir.) Mering & Kadereit. The newly circumscribed *Triglochin* is highly supported as a monophyletic group (Les and Tippery, 2013; von Mering and Kadereit, 2010).

Due to its focus on the delimitation of Juncaginaceae and generic relationships in the family, our previous study (von Mering and Kadereit, 2010) included only a relatively small sample of *Triglochin*. Species of *Triglochin* are found on all continents except Antarctica, and the genus has a distribution in temperate to arctic and subantarctic regions (Fig. 1, Table 2). The few species growing in tropical regions are mostly restricted to high elevations (e.g., *T. scilloides* in the Andes, *T. milnei* Horn in sub-Saharan Africa). While several *Triglochin* species such as *T. maritima* L., *T. palustris* L., and *T. striata* Ruiz & Pav. are very widespread, others are endemics confined to relatively small areas (e.g., *T. gaspensis* Lieth & D.Löve, *T. compacta* Adamson, *T. buchenaui* Köcke, Mering & Kadereit). Australia is the centre of species diversity with about 15 annual species (Aston, 2011; Keighery, 2003). These small, sometimes tiny annual plants are ephemerals inhabiting temporarily wet sites such as clay pans, rock pools or soil-filled depressions and sometimes edges of lakes or swamps (Aston, 2011; Keighery, 1979; Table 2).

Despite its interesting distribution pattern, the biogeographic history of *Triglochin* has not been analysed in any detail. In a study of distribution patterns Camp (1947) only presented a map of the genera of Juncaginaceae and briefly discussed the distribution of *Triglochin* and relatives. Horn af Rantzien (1961) noted that *Triglochin* "is of considerable interest from the view of phytogeography" (p. 82). He suggested an Antarctic origin of the family and its spread from this primary centre of distribution to other regions in the southern hemisphere (Australian temperate region, extratropical South America, South Africa). He further wrote: "Some species apparently migrated northwards from these austral centres to the northern hemisphere, probably along the mountain ranges." (Horn af Rantzien, 1961, p. 83). Based on the southern hemisphere distribution of several taxa related to Juncaginaceae, a Gondwana origin has been proposed for the family by Raven and Axelrod (1974).

Here we investigate the phylogeny, biogeography and evolution of *Triglochin* using sequence data obtained from one nuclear (nrITS, internal transcribed spacer) and two chloroplast DNA regions (*psb*A-*trn*H intergenic spacer, *mat*K gene). The main objectives of this study are: (1) to establish a robust phylogenetic framework for *Triglochin*, (2) to estimate divergence times of its major lineages, (3) to reconstruct its biogeographic history, and (4) to investigate relationships between taxonomic, genetic and ecological diversities in two clades of the genus.

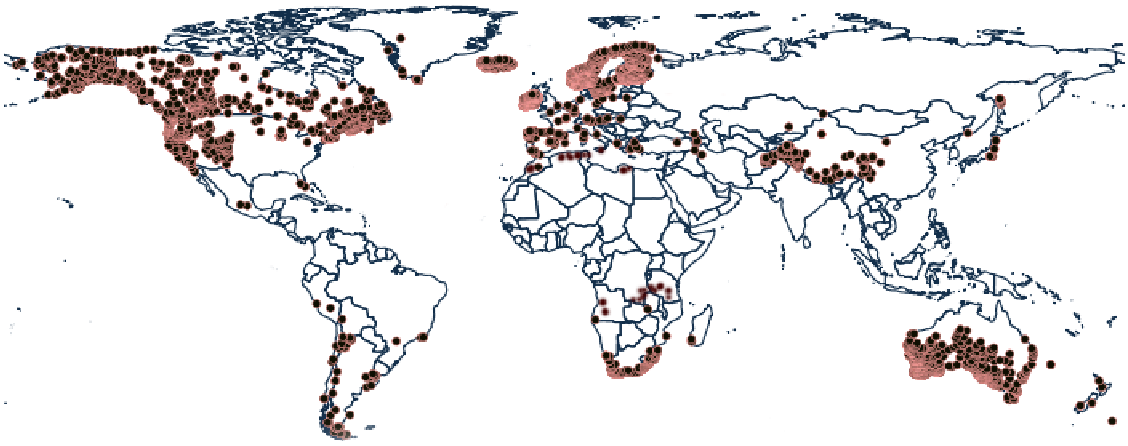

Fig. 1. Distribution of *Triglochin* based on selected specimen data from GBIF (http://data.gbif.org/), complemented by geo-referenced specimen data from a revision (Köcke et al., 2010). Due to the lack of specimen data from some countries (e.g., Russia) in the GBIF portal, parts of the distribution area in eastern Europe and Asia are missing.

Material and Methods

Taxon sampling – To resolve intrageneric phylogenetic relationships, three DNA regions (nrDNA: ITS; cpDNA: *psb*A-*trn*H, *mat*K) were sequenced for this study. We sampled multiple accessions of *Triglochin* representing almost all currently recognised extra-Australian species and subspecies and almost the entire distribution range of the genus (Appendix 2). The recent Flora of Australia treatment of *Triglochin* (Aston, 2011) recognises 14 annual species. Based on the assumption that these species form a monophyletic group (see also 4.1), only four species of this group were included. Within the *T. maritima* complex, we sampled accessions from several parts of Europe, Asia (including *T. maritima* subsp. *asiatica* Kitag. of unclear taxonomic status) and America. In addition to the two species accepted in Flora of North America (*T. maritima* and *T. gaspensis*; Haynes and Hellquist, 2000) we also included two taxa recognised only in regional treatments from western North America (*T. concinna* Burrt Davy vars. *concinna* and *debilis* (M.E.Jones) J.T.Howell, e.g., Keil, 2012). South American material of the *T. maritima* complex is limited to one sample of *T. concinna* from Chile (occurring also in Argentina and Bolivia). Although *T. mexicana* Kunth is accepted by some authors (e.g., Espejo Serena and López-Ferrari, 1996; Novelo and Lot, 2001), its status is unresolved. No material of this taxon was available for our study. For the widespread *T. palustris* and *T. striata* accessions covering the entire distribution area were sampled where possible. The synonymy of several published names with either *T. palustris* or *T. striata* is supported by the study of herbarium material and data from the literature. Our sampling of the *T. bulbosa* complex was exhaustive. We included all taxa accepted in a recent revision (Köcke et al., 2010) except for *T. bulbosa* L. ssp. *tenuifolia* (Adamson) Horn, a rare endemic from the Cape region of South Africa.

For the ITS region, a dataset comprising 78 accessions was generated. This dataset was complemented by reduced datasets for the two plastid markers used. The newly generated *psb*A-*trn*H dataset comprised 55 sequences. The *mat*K dataset with eight sequences combined data from our previous study (von Mering and Kadereit, 2010) with newly generated sequences of Australian annuals and *Cycnogeton*. Due to difficulties with amplification and sequencing, some sequences were not available for all taxa. Since *Cycnogeton* is highly supported as sister group to *Triglochin* (Les and Tippery, 2013; von Mering and Kadereit, 2010), representative species of this genus were included as outgroup in the cpDNA datasets. ITS sequences of *Cycnogeton* could not be aligned with those of *Triglochin*.

DNA was extracted from herbarium specimens or from silica-dried leaf samples. Voucher information and GenBank accession numbers are compiled in Appendix 2.

Table 1. Circumscription of *Triglochin* from 1881 to present. [1] - only *Triglochin procera*, [2] - only *Maundia triglochinoides*, [3] - excl. *Maundia*, [4] - excl. *Cycnogeton*, [5] - incl. *Cycnogeton*, [6] - incl. *Lilaea*, n.s. - not specified.

Author / Source		Number of species
Micheli 1881	*Triglochin* sect. *Eutriglochin* sect. *Cycnogeton*[1] sect. *Pseudotriglochin*[2]	9
Buchenau & Hieronymus 1889	*Triglochin* subg. *Eutriglochin* subg. *Cycnogeton*[1] subg. *Pseudotriglochin*[2]	12
Buchenau 1903	*Triglochin*[3] subg. *Eutriglochin* subg. *Cycnogeton*	13
Hutchinson 1934, 1959	*Triglochin*[3,4]	n.s.
Dahlgren et al. 1985	*Triglochin*[3,4]	c. 16
Takhtajan 1997	*Triglochin*[3,4]	13
Haynes et al. 1998	*Triglochin*[3,5]	12
Aston 2011	*Triglochin*[3,5]	24 (Australia)
von Mering & Kadereit 2010, this study	*Triglochin*[3,4,6]	c. 25-30

DNA extraction, amplification, and sequencing – The molecular work followed standard protocols. Total genomic DNA was extracted using commercial plant DNA extraction kits (NucleoSpin, Macherey-Nagel, Düren, Germany or DNeasy plant extraction kit, Qiagen, Hilden, Germany) according to the manufacturers' protocols with minor modifications. Details of PCR amplification of all DNA regions are summarised in Appendix 3. PCR products were purified with a standard clean-up kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with minor modifications. Cycle sequencing was carried out with BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA, USA) using the same primers as for PCR amplification (Appendix 3). The fluorescently labelled samples were sequenced at GENterprise-Genomics GmbH or StarSEQ GmbH (both Mainz, Germany). Some sequences were run on an ABI 3130xl Genetic Analyzer or ABI 3730 automated sequencer at the Botanical Museum Copenhagen, Denmark.

Sequence alignment and phylogenetic analyses – Forward and reverse strands were manually edited and assembled using Sequencer 4.1.2 or 4.10.1 (Gene Codes Corp., Ann Arbor, MI, USA). Consensus sequences were then aligned manually using Maclade 4.1 (Maddison and Maddison, 2000).

Sequence divergence in the ITS region was too high for the unambiguous alignment of ITS sequences of *Triglochin* and the outgroup *Cycnogeton*, and therefore midpoint rooting had to be chosen for this dataset. The root chosen this way was identical to the root suggested by the cpDNA analysis rooted with *Cycnogeton*. In the combined ITS/*psb*A-*trn*H dataset we added the 5.8 S region of the ITS sequence of *Cycnogeton rheophilum* to the alignment (in addition to the *psb*A-*trn*H sequence) in order to reduce the amount of missing data for this taxon.

Sequence analysis revealed that the following accessions had identical ITS sequences: (1) *T. maritima* from Europe incl. Russia (11 accessions), (2) *T. maritima* from the Caucasus region (5 accessions), (3) *T. buchenaui* and *T. elongata* from South Africa. These were represented in the phylogenetic analyses by a single terminal taxon and are marked on the trees by origin/numbers behind the taxon name. The same applies to identical *psb*A-*trn*H sequences generated for eight accessions.

Phylogenetic analyses were conducted (1) separately for each dataset, i.e. ITS with a global sample of *Triglochin* species, *psb*A-*trn*H with a reduced and *mat*K with a small sample, (2) for the combined data matrix of ITS and *psb*A-*trn*H, and (3) for a combined dataset of all three markers.
Separate and combined datasets were analysed using both Maximum Likelihood (ML) and Bayesian Inference (BI). The best-fit models of nucleotide substitution for each separate and combined dataset were determined using jModelTest v2.1.1 (Posada, 2008) and applying the Akaike Information Criterion (AIC).

Maximum Likelihood (ML) analyses were performed with RAxML (Stamatakis et al., 2008) using the GTR+G model. Statistical support was assessed with 100 bootstrap replicates. In this paper, bootstrap support of 75-84% is considered moderate and 85-100% strong.

The Bayesian Inference topology (BI) of the datasets was found using BEAST v1.7.4 (Drummond et al., 2012). The Bayesian analysis was carried out with a Markov Chain Monte Carlo (MCMC) algorithm of 10 million generations, sampling every 1000 generations. A Yule tree prior was employed in all runs.

For all BEAST analyses, convergence of resulting posterior distributions for parameter estimates was examined using Tracer v1.5 (Rambaut and Drummond, 2007) to ensure that all parameters had an effective sampling size (ESS) over 200. After discarding the first 10% as burn-in, maximum clade credibility trees were calculated using TreeAnnotator v1.7.4 (Rambaut and

Drummond, 2009). Trees were visualised and edited using FigTree v1.3.1 (Rambaut, 2006). For conversion of file formats for use in different analyses the programme ALTER (Glez-Peña et al., 2010) was used.

In order to compare levels of genetic differentiation between two clades (the *T. bulbosa* complex and the *T. maritima* complex, see 3.1), pairwise genetic distances (uncorrected *p*-distance and Kimura 2-parameter, K2P) were calculated in PAUP* (Swofford, 2002) using alignments of complete ITS sequences.

Molecular dating – Divergence times were estimated using BEAST v1.7.4 (Drummond et al., 2012) which employs a Bayesian MCMC approach to simultaneously estimate tree topology, substitution rates and node ages. No fossils of Juncaginaceae are known. We therefore used a secondary calibration with an age for the split between Araceae and the rest of Alismatales obtained in an angiosperm-wide dating analysis using fossil calibration (Bell et al., 2010). This split was fixed at 122 million years ago (Ma) with a standard deviation (SD) of 6 Ma. Age estimates for Juncaginaceae from a monocot-wide, fossil calibrated analysis (Janssen and Bremer, 2004; 82 and 52 Ma for stem and crown group age, respectively) could not be used as the sampling in that study did not include all genera of the family. A dataset used earlier, including members of all genera of Juncaginaceae and further representatives of Alismatales (based on the plastid genes *rbc*L and *mat*K, see von Mering and Kadereit, 2010 for details), was used as input data. Dating in BEAST relied on a Yule process tree prior, and either a relaxed lognormal clock with a normal distribution and the GTR+G+I model or a strict clock was selected. MCMC chains were run for 60 million generations in the relaxed clock and for 10 million generations in the strict clock models, with sampling every 10000th and 1000th generation, respectively.

Because *rbc*L and *mat*K sequences were available only for a limited number of taxa, the age estimate obtained for the split between *Cycnogeton* and *Triglochin* (40 Ma, SD=5) was then used for another dating analysis based on the combined ITS/*psb*A-*trn*H dataset.

Biogeographic analysis – To reconstruct ancestral distribution areas of *Triglochin* (and Juncaginaceae), we conducted a statistical dispersal-vicariance analysis (S-DIVA, based on DIVA, Ronquist, 1997) using the programme RASP v2.1b (Yu et al., 2011; Yu et al., 2010). Because available molecular data either could not be aligned for all three genera of Juncaginaceae (ITS) or the sampling of relevant taxa was limited (*rbc*L, *mat*K, *psb*A-*trn*H), we used one consensus tree of all three genera of Juncaginaceae based on different datasets as input. Only groups which were fully resolved in our phylogeny were included in the topology (and reduced to one representative terminal) to provide the required completely bifurcating tree. Therefore, the S-DIVA analysis could not calculate optimised areas over a set of trees and thus did not take into account topological uncertainty.

The following six geographic areas were defined: (A) Australia and New Zealand, (B) Eurasia (excl. Mediterranean region), (C) Mediterranean region, (D) sub-Saharan Africa, (E) North America and (F) South America. The Australian *Cycnogeton* (with *C. multifructum* and *C. rheophilum*) and the South American *Tetroncium* were included in our analyses and coded accordingly.

The maximum number of possible areas at each node (‘maxareas’) was set to two. This reflects that most species of *Triglochin* occur in only one or two areas with only three species (*T. maritima*, *T. palustris* and *T. striata*) being more widespread. Additional runs were performed to explore the effects of area constraints, one that allowed maximally four areas for the ancestral range of a clade and one that did not constrain the number of areas.

Results

Phylogenetic analyses – The final ITS matrix (after removal of identical sequences) comprised 58 terminals, and the alignment was 668 bp long. Monophyly of most species groups and widespread species is highly supported in our analysis (Fig. 2). A clade comprising *Triglochin scilloides* plus the monophyletic *T. bulbosa* complex is highly supported as sister to the remainder of the genus. Within the latter, two main clades are found: (1) *Triglochin striata* is moderately supported as sister to a clade comprising Australian annual *Triglochin*, and (2) *Triglochin palustris* plus the *T. maritima* complex, the latter consisting of two highly supported clades comprising Eurasian and American accessions, respectively.

The *psb*A-*trn*H dataset (after removal of identical sequences) comprised 45 accessions (plus two outgroup taxa), and the alignment was 488 bp long. Phylogenetic analyses resulted in several well supported clades corresponding to species or species complexes. Relationships among these were essentially unresolved due to low sequence variation (results not shown).

Figure 3 compares trees obtained from a reduced ITS dataset and from a small combined (*mat*K and *psb*A-*trn*H) cp dataset (eight accessions incl. *Cycnogeton rheophilum* as outgroup). The inferred topologies were identical and fully resolved in both datasets, with high support found for all clades.

Equally, results from the ITS and *psb*A-*trn*H analyses did not show any supported topological conflict and were combined. The combined matrix of ITS and *psb*A-*trn*H comprised 35 accessions and had 1216 nucleotide positions. Phylogenetic analyses resulted in the following topology (Fig. 4): a highly supported clade comprising *T. scilloides* and the *T. bulbosa* complex is sister to the rest of the genus which comprises two main clades, (1) a moderately supported clade comprising *T. striata* and Australian annual *Triglochin*, and (2) a moderately supported clade of the *T. maritima* complex and *T. palustris*.

Pairwise genetic distances for the *T. bulbosa* complex are slightly higher compared to the *T. maritima* complex. Values for the Kimura 2-parameter and uncorrected *p*-distances (mean and maximum distances) are summarised in Table 3.

Molecular dating analyses – Divergence time estimates (mean ages with confidence intervals, highest posterior density = HPD) for different biogeographically important nodes are summarised in Table 4 based on relaxed and strict clock models and two different datasets. The relaxed and the strict clock models resulted in considerably different age estimates, with the strict clock model yielding older ages. Age estimates using the ITS/*psb*A-*trn*H dataset resulted in even older ages. Differences in age estimates were so large that inferred ages sometimes lay in different geological time periods. The results of the strict clock are preferred here because only one constraint was used to obtain them. However, other age estimates will be considered in the discussion where necessary.

Using the strict clock model, the split between *Triglochin* and *Cycnogeton* occurred around 53.8 Ma (95% HPD: 45.2-62.4 Ma; Fig. 5), i.e. in the Eocene or Palaeocene. Diversification of *Triglochin* began in the Oligocene (or late Eocene), with most of the extant species diversity dating to the Oligocene or Miocene. A chronogram (Fig. 4) shows the mean ages with 95% HPD.

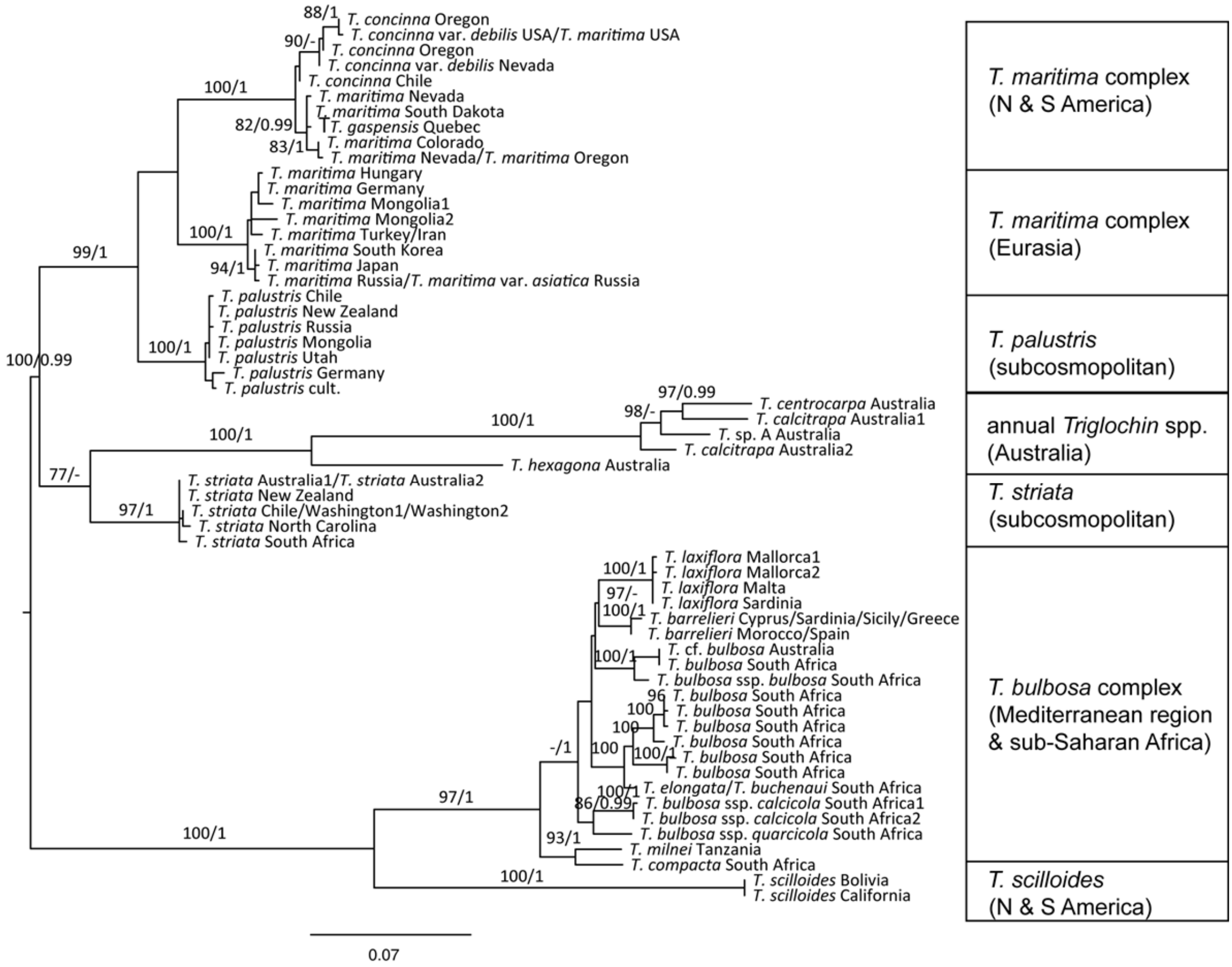

Fig. 2. Phylogeny of *Triglochin* based on ITS sequences. Maximum likelihood bootstrap and Bayesian posterior probability values are shown along the branches.

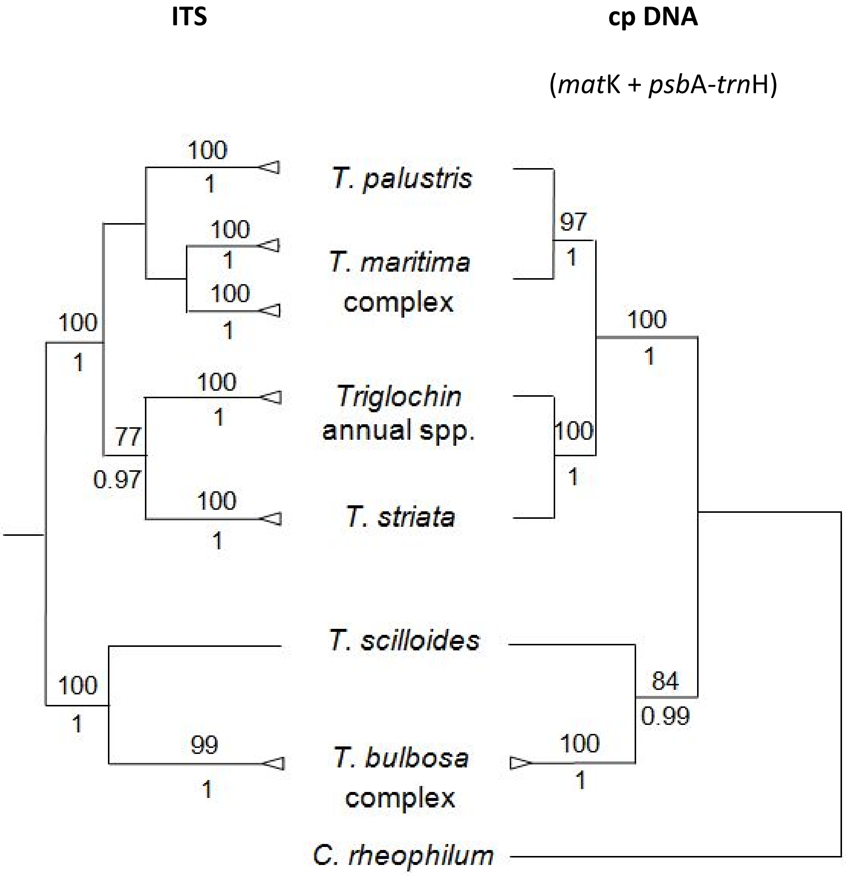

Fig. 3. Phylogeny of *Triglochin.* Topologies of the ITS dataset (left; midpoint rooting) and a combined cp dataset (right) of *mat*K and *psb*A-*trn*H sequences (*Cycnogeton rheophilum* as outgroup). Maximum likelihood bootstrap and Bayesian posterior probability values are shown above and below the branches, respectively.

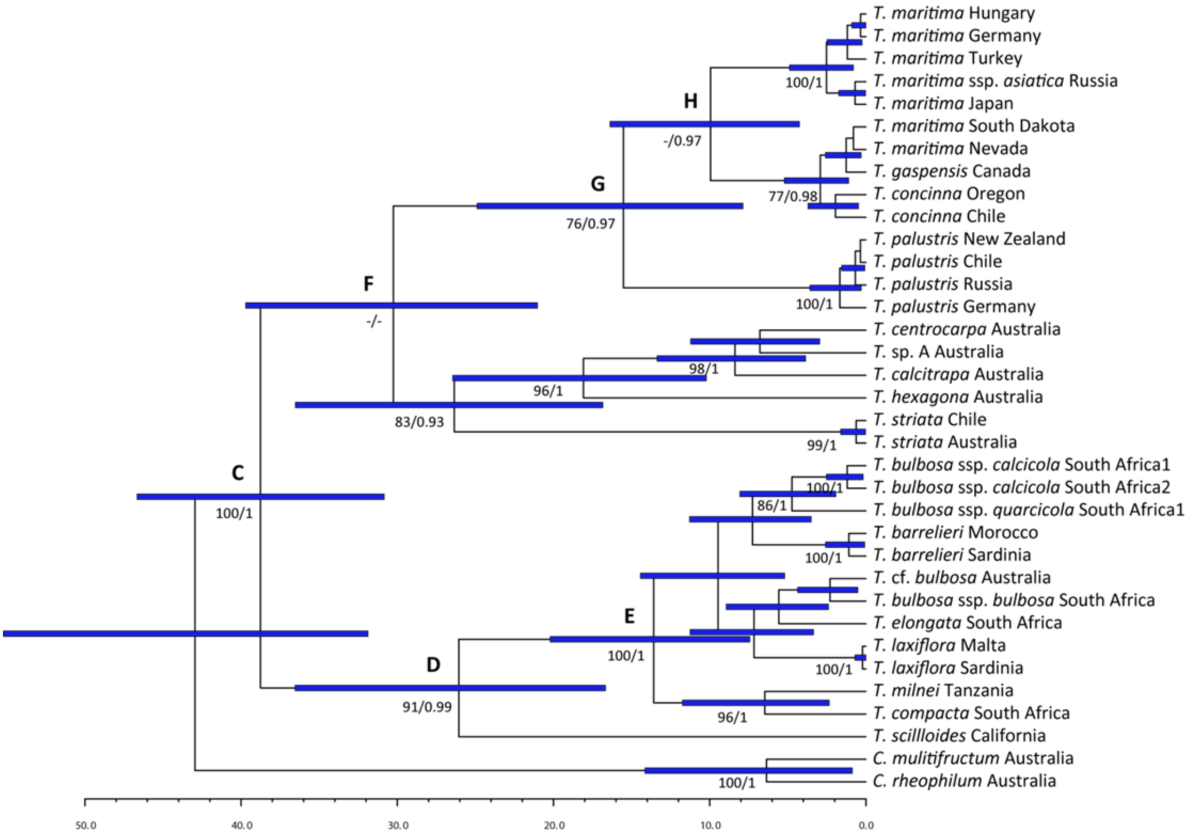

Fig. 4. Phylogeny of *Triglochin*. Chronogram of *Triglochin* obtained from BEAST using the combined ITS and *psb*A-*trn*H dataset. Bootstrap and posterior probability values are shown along the branches. Blue bars show the 95% highest posterior density (HPD) credibility intervals of mean age estimates. The letters indicate nodes as used in Table 3.

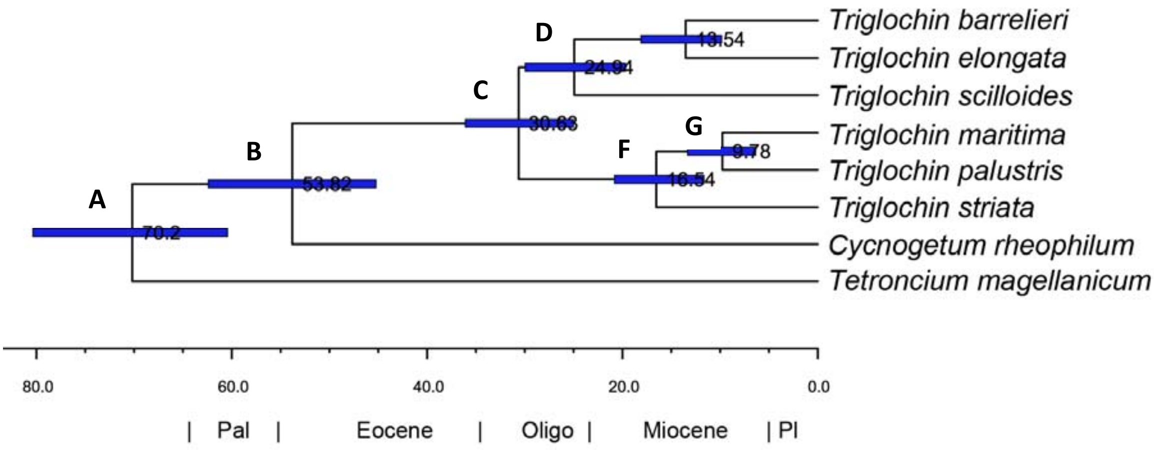

Fig. 5. Chronogram for Juncaginaceae inferred from a Bayesian dating analysis using cpDNA data (*rbc*L and *mat*K) and BEAST modeled under a strict clock model. Mean ages are given in million years (Ma) for selected nodes. Blue bars represent the 95% highest posterior density (HPD) credibility intervals of mean age estimates. Oligo – Oligocene, Pal – Palaeocene, Pl – Pliocene.

Biogeographic analyses – Results of the DIVA analyses are shown in Fig. 6. DIVA analysis with maxarea = 2 inferred that the ancestral distribution area of *Triglochin* is a combined area of Australia and Africa or Australia and (North or South) America. Apart from the inclusion of North America, this suggests an ancestral area in the Southern hemisphere (Fig. 6). The ancestor of *Tetroncium*, *Cycnogeton* and *Triglochin*, i.e. of the entire family, is inferred to have been distributed in Australia or South America. When four areas are allowed at each node (maxareas = 4), Australia, Africa or America were reconstructed as the ancestral area of Juncaginaceae.

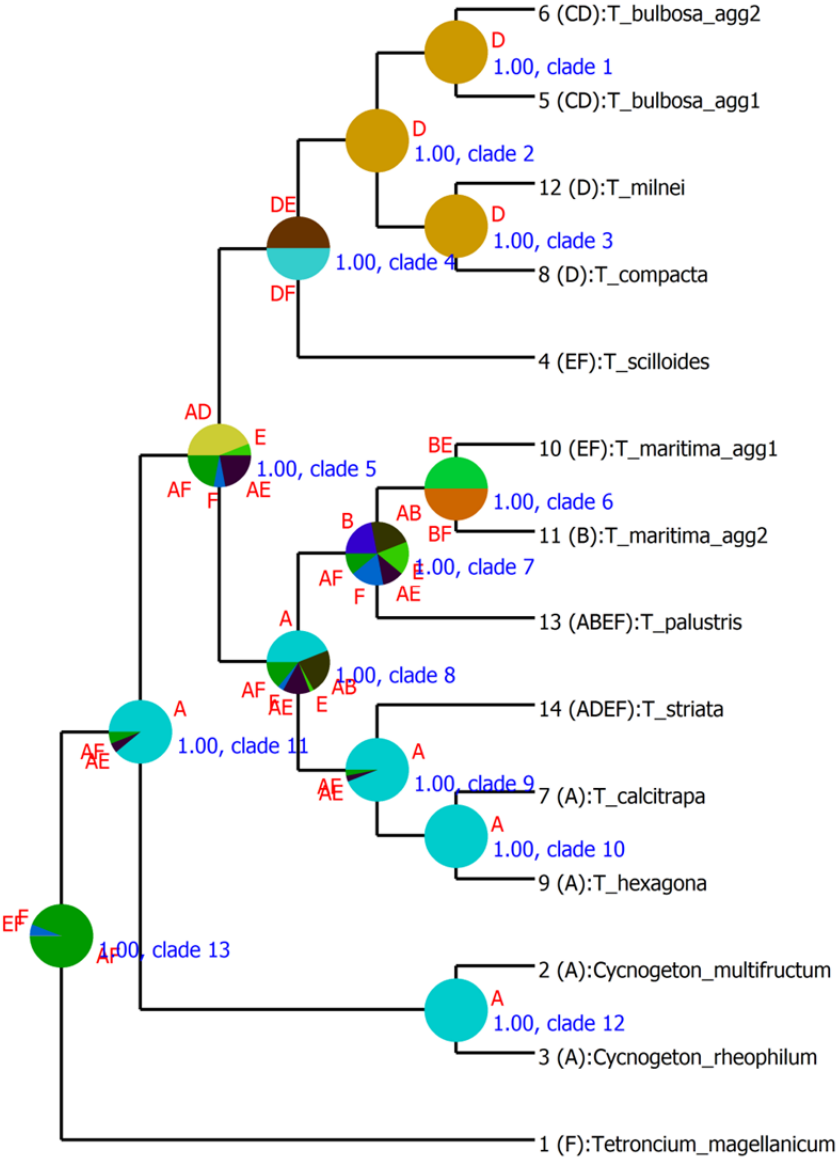

Fig. 6. Dispersal–vicariance scenarios for *Triglochin* reconstructed with RASP. Optimization with the maximum number of area units (‘maxareas’) set to two. A – Australia, B – Eurasia, C – Mediterranean region, D – Africa, E – North America, F – South America.

Discussion

Phylogenetic relationships in Triglochin

Our study provides a robust phylogeny of *Triglochin* (Figs. 2-4) by resolving relationships between all species groups of the genus and most extra-Australian taxa. With regard to the general topology, results from earlier studies based on *rbc*L (Les and Tippery, 2013; von Mering and Kadereit, 2010) were confirmed, and much better support was found for relationships among clades.

***T. scilloides/T. bulbosa* complex** – The inclusion of the formerly separate genus *Lilaea* in *Triglochin* (von Mering and Kadereit, 2010) and its sister group relationship to the *T. bulbosa* complex was confirmed by analyses of all datasets. For a detailed discussion of the inclusion of this species in *Triglochin* see von Mering and Kadereit (2010). No obvious synapomorphies for the clade comprising *T. scilloides* and the *T. bulbosa* complex could be detected. Within the *T. bulbosa* complex, which is characterised by (narrowly) ovoid fruits consisting of three fertile carpels that separate at maturity, a highly supported clade of *T. milnei* and *T. compacta* is sister to the rest of this group (Fig. 2, 4). These two species are morphologically similar to each other (e.g., outline of seed shows through mericarp surface) and share the presence of outgrowths at the base of the carpophores (Köcke et al., 2010). However, they are geographically, ecologically and phenologically clearly differentiated (Köcke et al., 2010). Relationships among the remaining taxa are not fully resolved, and our results suggest that some further adjustments to taxon circumscription in the *T. bulbosa* complex might be necessary (Köcke et al., 2010), especially with regard to *T. bulbosa* which appears to be paraphyletic (Fig. 2, 4). The three sampled subspecies of *T. bulbosa* (material of subsp. *tenuifolia* was not available) do not form a monophyletic group because the two Mediterranean species *T. barrelieri* and *T. laxiflora* are nested in this otherwise South African clade. A sample of naturalised *T. bulbosa* from Western Australia collected close to an Air Force base (Aston, 2011) is highly supported as sister to an accession from South Africa, indicating its African origin. South Africa has been identified as an important source of Australia's alien species (Scott and Panetta, 1993).

T. striata*/Australian annual *Triglochin – *Triglochin striata* is recovered as sister to the lineage of Australian annual species in all datasets, with high support in the combined datasets (Fig. 2, 4). However, support for this sister group relationship is only moderate in the ITS dataset. No obvious synapomorphies for this clade could be detected. An analysis of the relevant literature (e.g., Aston, 2011; Romanowski, 1998; Thieret, 1988) and extensive herbarium material shows that *T. striata* is a widespread and morphologically variable taxon. Numerous forms have been described from different parts of the world which differ in plant size and form or length of leaves (e.g., Gandoger, 1919). Various authors have treated these forms at different taxonomic levels as forms, varieties, subspecies or even species. However, currently all these taxa are regarded as synonyms of *T. striata* (Buchenau, 1882; Goverts, 2013). As long as no global revision of *T. striata* is available, we treat *T. striata* as one polymorphic species. Annual *Triglochin* in Australia is a relatively species-rich group (14 spp. recognized by Aston, 2011, several more expected, e.g., Keighery, 2003). Apart from their annual life form and small size, these species share characters such as filiform, auriculate leaves and fruits with three fertile and three sterile carpels (Aston, 2011; S. von Mering, pers. obs.). Preliminary molecular results for a larger number of species (S. von Mering et al., unpubl. data) suggest that Australian annual *Triglochin* is a monophyletic group.

Table 2. Distribution, habitat, life form, flowering time and chromosome numbers of *Triglochin*, *Cycnogeton* and *Tetroncium* (Juncaginaceae). Compiled using the following main references: Aston, 1993, 1995, 2011; Guo et al., 2010; Haynes and Hellquist, 2000; Goldblatt & Johnson, 1979+; Keil, 2012; Köcke et al., 2010; von Mering and Kadereit, 2010; von Mering, 2013; Tzvelev, 2013.

Taxon	**Distribution**	**Habitat**	**Life form**	**Flowering and fruiting time**	**Chromosome number**
***Triglochin bulbosa* complex**					
Triglochin barrelieri Loisel.	Mediterranean region, reaching the Atlantic coast of NW France and Morocco	coastal salt marshes and along lagoons, less frequently on coastal rocks exposed to salt spray, in depressions in coastal dunes or in cork oak forests	perennial	spring (Feb. - May)	2*n* = 30, 32, 36
Triglochin buchenaui Köcke, Mering & Kadereit	South Africa	restricted to lower (periodically flooded) tidal zone of salt marsh estuaries	perennial	throughout the year	?
Triglochin bulbosa L.	South Africa	variable (see subspecies), ranging from temporary pools to dry (shaded) inland habitats, but never in habitats under direct influence of tidal flooding	perennial	(early) spring (Jul. - Nov.)	?
Triglochin bulbosa ssp. *bulbosa*	South Africa	temporary wetlands of coastal lowlands, e.g., edges of vernal pools, ditches and other depressions; on heavy clayey to lighter clayey-sandy soils; often in clay-filled depressions among stabilised sandy dunes; alt. 0-100 (-500) m		spring (Aug. - Nov.)	?
Triglochin bulbosa ssp. *calcicola* Mering, Köcke & Kadereit	South Africa	on limestone or aeolinite in coastal regions		spring (Aug. - Nov.)	?
Triglochin bulbosa ssp. *quarcicola* Mering, Köcke & Kadereit	South Africa	restricted to saline quartz fields of the Knersvlakte, especially dry slopes of koppies, covered with a layer of white angular quartz gravel		early spring (Jul. - Aug.)	?
Triglochin bulbosa ssp. *tenuifolia* (Adamson) Horn	South Africa	sheltered, shady inland habitats on mountain slopes of the Cape Peninsula and Langebaan Peninsula; restricted to coarse, sandy soils derived from granites; alt. below 400 m		spring (Jul. - Sep.)	?
Triglochin compacta Adamson	South Africa	deep, mainly nutrient-poor sands; inland sand dunes (always outside current direct influence of the sea)	perennial	autumn (mostly Mar. - May)	?

Triglochin elongata Buchenau	South Africa	upper and supratidal zones of estuarine salt marshes and estuarine rivers; coastal habitats (elevated banks of sandy beaches, rocks exposed to salt spray); some inland localities	perennial	throughout the year	?
Triglochin laxiflora Guss.	Mediterranean region (France, Italy, Portugal, Spain, Cyprus, Greece, Malta, Algeria, Morocco)	salt marshes and along lagoons, but also in cork oak or pine forests and in Mediterranean temporal pools, sometimes in limestone areas; alt. 0 -500 (-1100) m	perennial	autumn (mainly Sep. - Nov.)	2*n* = 18
Triglochin milnei Horn	sub-Saharan Africa (Angola, DR Congo, Malawi?, South Africa, Tanzania, Zambia, Zimbabwe)	seasonal (summer-wet) wetlands and seasonally wet, sometimes burnt, grasslands; alt. above (500-)1000 m, in Tanzania up to 1900 m	perennial	spring - summer (Oct. - Feb.)	?
***Triglochin maritima* complex** [1, 2]					
Triglochin maritima L. [3]	widespread in Eurasia and the Americas	coastal salt marshes, inland saline, brackish, alkaline marshes, moist alkaline meadows, seeps, mudflats, stream and lake margins; alt. 0-5200 m	perennial	spring - autumn	2*n* = 12, 24, 36, 48, 96, 120, 144, 156
Triglochin gaspensis Lieth & D.Löve	NE North America	tidal saltwater marshes, regularly submerged	perennial	summer (Jul. - Aug.)	2*n* = 96
Triglochin palustris L.	widespread in Eurasia and the Americas (including Greenland); also in New Zealand	coastal and inland marshes, moist alkaline meadows, wet flats, stream and lake margins; alt. 0-4500 m	perennial	summer - autumn	2*n* = 24
Triglochin scilloides (Poir.) Mering & Kadereit	Canada, USA; Mexico; Argentina, Chile, Ecuador, Peru; introduced in SE Australia, Iberian Peninsula	shallow water and adjacent mud flats, seasonal pools, stream, pond and lake margins; alt. 0-1700 m	annual	spring - summer	2*n* = 12
Triglochin striata Ruiz & Pav.	Australia, New Zealand, French Polynesia; Africa; USA, Mexico, Caribbean; South America; introduced: Iberian Peninsula	coastal alkaline, brackish to freshwater marshes, interdune swamps, salt marshes, tidal estuaries, stream and lake edges, inland springs	perennial	throughout the year (but seasonal in individual regions)	2*n* = 24

Australian annual *Triglochin* spp.						
Triglochin sp. A (Flora of Australia)	Australia		damp sand loam and clay soils of intermittently flooded depressions, swamps, bore overflows and watercourses, including gibber plains and granite rockholes; water fresh to sometimes slightly saline	annual	Jul.-Oct.; Apr.-Jun. (Feb.) [N.T.]	?
Triglochin calcitrapa Hook.	Australia		on damp, winter-wet sandy clay, saline soils of flats, claypans and lake edges	annual	Aug.-Oct.	?
Triglochin centrocarpa Hook.	Australia		saline sands or clay edging salt lakes	annual	late Jul.-Oct.	?
Triglochin hexagona J.M.Black	Australia		in damp to saturated, semi-saline, sand to clay soils of ephemeral lakes and floodplains (rarely edging freshwater swamps)	annual	(Feb.-) Apr.-Sep. [N.T., W.A.], (Sep.-) Oct.-Apr. [other States]	?
Triglochin insingiana (J.M.Black) Aston	Australia		in damp sand to sandy clay soils or shallow water of lake and watercourse edges or ephemerally flooded depressions; also limestone coastal sands and damp shallow soils of rock outcrops; chiefly freshwater situations, sometimes semi-saline	annual	Mar., Jun.-Oct.	?
Triglochin longicarpa (Ostenf.) Aston	Australia		in damp sand, loam and clay saline or subsaline soils, usually edging salt lakes and seasonally flooded claypans; also in shallow soils on granite outcrops	annual	(Jul.-) Aug.-Oct. (-Nov.)	?
Triglochin minutissima F.Muell.	Australia		usually in saline to subsaline sands or sandy clays bordering ephemeral waters of salt pans or lakes, or in subsaline pools in granite rocks, rarely in heathland swamps; also coastal cliffs	annual	Aug.-Oct., Dec.	?
Triglochin mucronata R.Br.	Australia		on damp saline sand or sandy clay soils, occ. loam; usually coastal or around the margins of salt lakes or in shallow seasonally flooded depressions	annual	Jul.-Nov. [W.A.], (Aug.-) Sep. - Nov. (-Dec.) [eastern States]	?

Triglochin muelleri Buchenau	Australia	coastal plain in sand, loam or clay of winter-wet flats and swamps, or edging drainage lines and creeks; saline to fresh	annual	Sep.-Nov.	?
Triglochin nana F.Muell.	Australia	mesophytic or ephemerally flooded freshwater sites (claypans, creek edges, swamps, roadside seepages, shallow depressions), sometimes edging salt lakes or subsaline swamps	annual	Aug.-Dec. [se areas], Apr.-Dec. elsewhere	?
Triglochin protuberans Aston	Australia	recorded from a creek edge, an *Acacia* woodland flat, and frequently from soils in ephemeral rock pools of granite outcrops, once from saline clay; soils of red loam and gritty red-brown clay recorded	annual	Aug.-Oct.	?
Triglochin stowardii N.E.Br.	Australia	salt-affected clay flat adjacent to salt lake, salt-impacted *Melaleuca lateriflora* shrubland, a clay flat in *Eucalyptus wandoo* woodland, flats in an open wet reedy area, a roadside ditch, and from the base of a quartzite hill	annual	Sep.-Oct.	?
Triglochin trichophora Nees ex Endl.	Australia	chiefly in sands and calcareous soils of limestone bedrock and coastal dunes, in moist sites incl. mossy seepages and springs	annual	Jul.-Dec.	?
Triglochin turrifera Ewart	Australia	in soakages or damp to wet soils near receding fresh water of swamps, pools, small depressions or temporary streams	annual	Aug.-Nov.	?
***Cycnogeton* Endl.**					
Cycnogeton alcockiae (Aston) Mering & Kadereit	Australia	usually in fresh, still, clear water to 30 cm deep in ephemeral swamps; occasional in small streams, stagnant water or coastal brackish habitats	perennial	Sept. - Mar.	?

Cycnogeton dubium (Aston) Mering & Kadereit	Australia, New Guinea	usually in saturated soils or still ephemeral fresh water to 50 cm deep, in swamps, creeks or floodplains; also in water to 2 m deep or strong-flowing streams in the tropics	perennial	(Sep.-)Nov.- Mar. [N.S.W., Vic.]; throughout the year elsewhere	?
Cycnogeton huegelii Endl.	Australia	fresh, still to flowing water to 1 m deep, edging permanent lakes and streams or sometimes in ephemerally flooded sites	perennial	(Jul.-) Aug. - Dec. (-Jan.)	?
Cycnogeton lineare (Endl.) Sond.	Australia	fresh, usually still and ephemeral water to 40 cm deep, in swamps, ditches and low-lying floodland; occasionally in small streams and creek pools	perennial	(May-) Jul. - Oct. (-Dec.)	?
Cycnogeton microtuberosum (Aston) Mering & Kadereit	Australia	near-coastal regions, in fresh, still or slow-flowing water to 50 (-120) cm deep, in small creeks, swamps and farm dams, on silt, peat, loam or sand soils; stagnant water often highly eutrophic and humic from farmland runoff; alt. c. 3-100 (-400) m	perennial	(Aug.-) Nov. - Apr. (-May)	$2n = 32$
Cycnogeton multifructum (Aston) Mering & Kadereit	Australia	fresh, still to slow-flowing water to 70 cm deep in seasonal to permanent swamps , irrigation channels, ditches, lagoons and creeks, mostly on sandy to heavy grey clays; alt. usually <200 m, rarely to 1050 m	perennial	Aug. - May (-Jun.) [Vic., s. N.S.W.]; Apr. - Sep. [n. tropics]	$2n = 16$
Cycnogeton procerum (R.Br.) Buchenau	Australia	still to slow-flowing fresh water to 2 m deep mostly in permanent swamps, lagoons and streams, but withstands stranding for extensive periods	perennial	through-out the year, mainly Sep. - Mar.	?
Cycnogeton rheophilum (Aston) Mering & Kadereit	Australia	clear flowing fresh water to 1 m deep in permanent, often rocky streams and rivers usually subject to severe flooding; recorded to c. 970 m alt.	perennial	Aug. - Feb.	$2n = 64$

Tetroncium **Willd.**					
Tetroncium magellanicum Willd.	southern South America (Argentina, Chile, Falkland Islands); Gough Island	different peatland types, especially *Sphagnum* and cushion bogs	perennial	summer (mainly Dec. - Jan.)	?

[1] unresolved complex requiring a global revision; [2] taxonomic status of *T. mexicana* Kunth unclear; [3] including ssp. *asiatica* Kitag. and *T. concinna* Burrt Davy vars. *concinna* and *debilis* (M.E.Jones) J.T.Howell; status of these taxa needs re-evaluation. N.S.W. – New South Wales, N.T. – Northern Territory, Vic. – Victoria, W.A. – Western Australia.

***T. palustris*/*T. maritima* complex** – The lineage comprising *T. palustris* and two clades composed of members of the *T. maritima* complex (Eurasian and American material) is highly supported in all our analyses. Relationships among the three highly supported clades are basically unresolved. A sister group relationship of *T. palustris* to the *T. maritima* complex is only weakly supported in the combined ITS/*psb*A-*trn*H dataset. There are good morphological characters clearly distinguishing *T. palustris* from the *T. maritima* complex. All members of the *T. maritima* complex have ovoid fruits composed of six fertile carpels that fully separate at maturity (e.g., Correa, 1969; Haynes and Hellquist, 2000). Presence of six fertile carpels is a diagnostic character not found in any other *Triglochin* species and a synapomorphy for the complex. In contrast, *T. palustris* is characterised by narrowly ovoid, club-shaped fruits which are composed of three fertile and three sterile carpels. The fertile carpels split from the base of the fruit and spread widely, thus resembling the eponymous arrow head (Juncaginaceae = arrow-grass family). In accordance with several other authors (e.g., Jafri, 1973; Keil, 2012; Markgraf, 1981), our observations clearly show that the carpels usually remain distally united with the carpophore. However, carpels may separate completely in the second year (M. Remizowa, pers. comm.). Furthermore, *T. palustris* differs from members of the *T. maritima* complex by having slender, fugacious stolons with terminal bulbs which secure reproduction and hibernation (e.g., Buchenau, 1903; Markgraf, 1981).

Wide distribution, morphological variation and polyploidy in the *T. maritima* complex have resulted in the recognition of several taxa at different taxonomic levels and in changing classifications (e.g., Ford and Ball, 1988; Löve and Löve, 1958). Several authors have stressed the need for a global study of the *T. maritima* complex (Löve and Löve, 1958; Reveal, 1977; Thieret, 1988). A numerical study of morphological variation in the complex (Ford and Ball, 1988) concluded that two species, *T. maritima* and *T. gaspensis*, should be recognised. However, this study was based on specimens from eastern and central North America and Europe and thus did not include western North American material sometimes recognized as a distinct taxon (e.g., Keil, 2012). In the Flora of North America (Haynes and Hellquist, 2000) only *T. maritima* and *T. gaspensis* are accepted, while regional treatments from western North America recognise *T. concinna* with two subspecies (e.g., Keil, 2012). In the Panarctic Flora (Tzvelev, 2013) it is noted that "*Triglochin maritima* s. lat. is an unresolved aggregate of probably several taxa". Our molecular results indicate that the *T. maritima* complex is divided into two highly supported geographical clades, a Eurasian and an American clade, respectively. There are two possible options to deal with the taxonomic implications of this result. Either all taxa of the *T. maritima* complex are included in a polymorphic *T. maritima* with several intraspecific taxa. Alternatively, several distinct species could be recognised.

Table 3. Pairwise genetic distances in the *T. maritima* and *T. bulbosa* complexes. K2P – Kimura 2-parameter distance, *p*-distance – uncorrected *p*-distance or dissimilarity.

Pairwise genetic distance	***T. maritima* complex**	***T. bulbosa* complex**
Mean K2P distance	0.045	0.055
Max. K2P distance	0.079	0.087
Mean *p*-distance	0.043	0.053
Max. *p*-distance	0.075	0.082

Table 4. Estimated divergence times for selected nodes as used in Figs. 4 and 5. Mean ages are in million years (Ma) and values given in brackets are the 95% HPD (Ma). SD – standard deviation.

Node	Description	*rbc*L/*mat*K Relaxed clock	*rbc*L/*mat*K Strict clock	ITS/*psb*A-*trn*H Relaxed clock
A	Juncaginaceae (*Tetroncium* vs. rest)	44.1 (67.4-27.4)	70.2 (80.4-60.4)	n.a.
B	*Cycnogeton* vs. *Triglochin*	39.6 (54.0-19.6)	53.8 (62.4-45.2)	fixed at 40.0 (SD=5/6)
C	*T. scilloides*/*T. bulbosa* complex vs. rest	21.6 (36.3-11.3)	30.6 (36.1-25.0)	38.8 (46.7-30.8)
D	*T. scilloides* vs. *T. bulbosa* complex	16.3 (25.4-5.9)	24.9 (30.0-19.7)	26.1 (36.5-16.7)
E	*T. bulbosa* complex (*T. milnei*/*T. compacta* vs. rest)	n.a.	n.a.	13.6 (20.2-7.4)
F	*T. striata*/Australian annuals vs. *T. palustris*/*T. maritima* complex	18.8 (22.5-3.2)	16.5 (20.8-11.7)	30.3 (39.7-21.0)
G	*T. palustris* vs. *T. maritima* complex	4.1 (13.7-1.2)	9.8 (13.3-6.4)	15.5 (24.9-7.9)
H	*T. maritima* complex (Eurasian vs. American clade)	n.a.	n.a.	10.0 (16.4-4.3)

Biogeography

The ancestral distribution of *Triglochin* was inferred to be Australia and (North or South) America or Australia and Africa. Apart from the inclusion of North America this supports the hypothesis that *Triglochin* originated in the southern hemisphere and spread from there to the north as suggested by earlier authors (e.g., Camp, 1947; Horn af Rantzien, 1961). Ancestral area reconstruction suggested an origin of Juncaginaceae in Australia or South America. The split of *Tetroncium* from *Cycnogeton* plus *Triglochin* in the Late Cretaceous/Paleocene (mean: 70 Ma, 95%

HPD: 80-60 Ma under the strict clock model; mean 45 Ma, 95% HPD: 67–27 Ma under the relaxed clock model; Fig. 5, Table 4), which essentially confirms an estimated divergence time between *Tetroncium* and *Cycnogeton* of 51.7 Ma (based on ITS sequences) by Les et al. (2003), is consistent with a distribution of the ancestor of this lineage from South America to Australia via Antarctica. Connections between those areas existed until the opening of the Drake Passage and the Tasmanian Gateway which separate Antarctica from South America and Australia, respectively. Despite some controversy about the exact timing of the opening of the Drake Passage (estimated to between 42 to 17 Ma; e.g., Lyle et al., 2008; Scher and Martin, 2006; Upchurch, 2008, and references therein), it probably opened near the Eocene/Oligocene boundary. The opening of the Tasmanian Gateway is largely considered to have occurred around 34 Ma (e.g., McLoughlin, 2001; Stickley et al., 2004; Upchurch, 2008) resulting in the isolation of the Australian continent from other landmasses. Thus, land connections between Australia, Antarctica and South America allowing exchange of biota could have existed in the Late Eocene and Early Oligocene and might explain the disjunction between *Tetroncium* (South America) and *Cycnogeton* (Australia).

Assuming a south hemispherical origin of the family, the northern hemisphere was colonised at least four times in the following clades: (1) *T. maritima* complex/*T. palustris*, (2) *T. striata* (apart from its wide distribution in the southern hemisphere this species is also native to the Caribbean and southern and western North America), (3) *T. barrelieri* and *T. laxiflora*, both distributed in the Mediterranean region (these two species may have reached the Mediterranean region independently, see below), and (4) *T. scilloides*, which is distributed in the Americas from Chile to Canada.

Diversification in *Triglochin* apparently started in the Late Eocene/Early Oligocene and intensified in the Miocene (Table 4; Fig. 4, 5). Several disjunctions exist in *Triglochin*, some of which are Trans-Atlantic (*T. scilloides* vs. *T. bulbosa* complex, *T. maritima* Eurasian vs. American clade) and some trans-African (*T. bulbosa* complex). Possible explanations include ancient isolation (vicariance) and more recent events of long-distance dispersal (LLD) across sea barriers.

***T. scilloides*/*T. bulbosa* complex** – *Triglochin scilloides*, a native of western North and South America, diverged from its sister clade, the *T. bulbosa* complex from the Mediterranean region and sub-Saharan Africa, in the Oligocene or Miocene (Fig. 5, Table 4). The split between the two lineages occurred well after the presumed separation of Africa and South America which has been suggested to have taken place between 110-80 Ma in the Cretaceous (e.g., McLoughlin, 2001; Upchurch, 2008). Therefore, Gondwanan vicariance is a highly unlikely explanation for this trans-Atlantic disjunction which probably is the result of LLD, possibly by sea water (e.g., Renner, 2004; Won and Renner, 2006). Relationships between American and African clades have been reviewed by Christenhusz and Chase (2013), emphasizing the importance of dispersal for this type of relationship. Trans-Atlantic sister clades have also been studied by Lavin et al. (2004) and Renner (2004).

Diversification of the *T. bulbosa* complex is likely to have started in the Miocene. The sister group relationship of *T. milnei* and *T. compacta* to the rest of the complex suggests an origin of the group in sub-Saharan Africa, possibly in South Africa. This is consistent with the results of our DIVA analysis. Our data partly suggest two colonisations of the Mediterranean region from South Africa, by the autumn-flowering *T. barrelieri* and the spring-flowering *T. laxiflora*, but relationships are not fully resolved. The disjunction between sub-Saharan Africa and the Mediterranean region could either have been established by long-distance dispersal (e.g., by migratory birds resting in wetlands) or by migration through the so-called arid corridor connecting southwestern and northeastern Africa (e.g., Jürgens, 1997 and references therein) and vicariance following its disruption (e.g., Galley et al., 2007;

Thiv et al., 2011). Different age estimates exist for the disruption of the corridor, some dating it to the Pleistocene, others to the Miocene (e.g., Bobe, 2006; Schuster et al., 2006). *Triglochin* is absent from some regions of the corridor such as northeastern Africa and occurs only further north in the Mediterranean region (*T. barrelieri*, *T. laxiflora*) and further south (*T. milnei* in parts of Central and East Africa). This pattern could be the result of extinction, and *T. milnei* as the only more widespread African species (South Africa to Angola and Tanzania; Köcke et al., 2010) might be a relic of a formerly wider distribution of the group. Our data do not allow us to decide whether this trans-African disjunction is the result of long-distance dispersal or vicariance.

Diversification of the *T. bulbosa* complex in the Miocene coincides with age estimates for other plant groups of (partly) similar distribution, e.g. *Androcymbium* Willd. (Caujapé-Castells et al., 2001; del Hoyo et al., 2009) and *Thamnosma* Torr. & Frém. (Thiv et al., 2011).

T. striata*/Australian annual *Triglochin – The split between the Australian annual lineage and the widespread *T. striata* took place in the Miocene. Apart from its distribution on the southern continents (Australia, Africa, South America), *T. striata* has colonised the Caribbean and North America (Corell and Corell, 1982; Haynes and Hellquist, 2000; Leon, 1946) and has been introduced to the Iberian Peninsula (Talavera, 2010). It also seems to have the ability to establish on isolated oceanic islands, e.g., French Polynesia and some sub-Antarctic islands (S. von Mering, study of herbarium material). Typical (but not exclusive) habitats of *T. striata* are coastal marshes and tidal estuaries (see Table 2). Therefore, and also considering fruit morphology, dispersal by sea currents or birds seems a likely explanation for the wide distribution of *T. striata*.

***T. palustris*/*T. maritima* complex** – Divergence of *T. palustris* from the *T. maritima* complex was dated to the Miocene (Fig. 4, Table 4). An unexpected finding of our analyses is the split between a Eurasian and an American clade in the *T. maritima* complex. This disjunction could be the result of LDD or migration via land bridges that linked the continents on the northern hemisphere in the past. The relevant land bridges are (1) an Eocene North Atlantic Land Bridge, and (2) an Early Miocene Bering Land Bridge (Tiffney and Manchester, 2001 and references therein). Due to the age of the split between the American and Eurasian *T. maritima* clade (mean: 30.3 Ma, 95% HPD: 21.0-39.7 Ma), the North Atlantic Land Bridge is a rather unlikely explanation for this disjunction. The Bering Land Bridge, however, could have served as a migratory route for the group. Considering the high dispersability of *T. maritima* by sea currents and probably also by birds (Davy and Bishop, 1991; Koutstal et al., 1987), the current distribution of the *T. maritima* complex could also be the result of long-distance dispersal.

Dispersal of fruits or mericarps – For some species of *Triglochin* it is known that fruits have the ability to float for long times, e.g. *T. maritima* (Markgraf, 1981), for others this has been strongly suspected, e.g. *T. striata* (see above). Mericarps of *T. maritima* can float for more than six months and retain viability for up to five months (Koutstal et al., 1987). Flotation of *Triglochin* fruits is facilitated by the possession of air filled cavities (aerenchyma; e.g., Markgraf, 1981). The importance of water for dispersal of *Cycnogeton* species has also been reported (e.g., Aston, 2011; Keighery, 1975). Besides dispersal by flotation, birds have been suggested as a dispersal vector for species of Juncaginaceae, at least over short distances (Haynes et al., 1998). Endozoochorous dispersal by waterfowl is very likely, but feeding experiments are needed to verify that fruits of *Triglochin* survive gut passage and germinate afterwards (pers. comm., J. Figuerola). Epizoochory in mud on bird feet has been suggested repeatedly (e.g., Pojar, 1974). Fruits or mericarps with mucronate, spurred or even hooked apices and/or bases are found in several species of *Triglochin* (e.g., *T. palustris*, several Australian annuals such as *T. calcitrapa* Hook., *T. mucronata* R.Br.), making epizoochory by adherence to feathers plausible. However, this possibility has been questioned for the Australian annual species (Keighery, 1975).

Taxonomic, genetic and ecological diversity

Among perennial *Triglochin*, our analysis identified two clades of roughly comparable crown group age and genetic diversity, i.e., the *T. maritima* complex (mean: 10 Ma, 95% HPD: 4.25-16.4 Ma; mean K2P distance: 0.045, max. *p*-distance: 0.075) and the *T. bulbosa* complex (mean: 13.6 Ma, 95% HPD: 7.4-20.2 Ma; mean K2P distance: 0.055, max. *p*-distance: 0.082). These two groups, however, differ strongly in taxonomic diversity with only two or three species (with two varieties) in the *T. maritima* complex but seven species (with three subspecies) in the *T. bulbosa* complex. This difference appears to be correlated with ecological diversification.

When a broad concept of *T. maritima* is applied as, e.g., in Flora of North America (Haynes and Hellquist, 2000), the *T. maritima* complex comprises the very widespread *T. maritima* and the narrow endemic *T. gaspensis*. While *T. maritima* is distributed along the coasts of temperate regions of the world but also widely in saline inland localities, *T. gaspensis* is ecologically more specialised and restricted to the lower tidal zone of salt marshes where plants are subject to daily flooding (Haynes and Hellquist, 2000; Löve and Lieth, 1961). *Triglochin gaspensis* is ecologically, morphologically and karyologically differentiated from *T. maritima* (Löve and Lieth, 1961) but the ITS sequences of the two species are almost identical. In addition to these two species, some authors recognise *T. concinna* with two varieties as distinct from *T. maritima* (e.g., Keil, 2012; Munz and Keck, 1968; Reveal, 1977). Whereas the type variety of *T. concinna* is typically found in coastal salt marshes (e.g., Brayshaw, 2000; Keil, 2012), *T. concinna* var. *debilis* is found in inland habitats such as on sinter and travertine deposits, at the edges of hot spring pools as well as alkali or salt lakes at higher elevations in western North America (ca. 400-2500 m, Great Basin region; Keil, 2012; S. v. Mering, pers. obs. on herbarium material). *Triglochin mexicana* has been reported to occur in brackish or saline sites along lakes or in swamps (Espejo Serena and López-Ferrari, 1996; Novelo and Lot, 2001). However, this taxon needs to be re-evaluated to clarify its status.

Triglochin maritima occupies a very similar niche in the Americas and Eurasia, i.e., mainly coastal salt marshes and inland saline habitats (salt or alkali lakes, salt pans, salt marshes, etc.). The species is relatively tolerant to physical stress and changing conditions (tolerating saline and alkaline conditions, water-logging etc.). It is a facultative halophyte and apparently less strictly halophytic at high latitudes and altitudes (Davy and Bishop, 1991). In summary, the *T. maritima* complex essentially comprises one species (*T. maritima*) which is ancient, very widespread and ecologically rather invariable, and one (*T. gaspensis*) or two (*T. concinna*) more narrowly distributed species of specialized ecology.

In comparison to the *T. maritima* complex, ecological diversity, including flowering time diversification, is considerably higher in the taxonomically more diverse *T. bulbosa* complex (Table 2). While some species occur in coastal habitats such as salt marshes or tidal estuaries (e.g., *T. barrelieri*, *T. elongata*, *T. buchenaui*), others are never found in habitats under the direct influence of tidal flooding (e.g., *T. bulbosa;* see Table 2, Köcke et al., 2010). Edaphic differentiation is especially pronounced in the South African *T. bulbosa* (Köcke et al., 2010). While the widely distributed subsp. *bulbosa* is water-bound and occurs in temporarily wet coastal and inland habitats, the other three subspecies grow in fully terrestrial inland habitats characterised by granite (subsp. *tenuifolia*), limestone (subsp. *calcicola* Mering, Köcke & Kadereit) and quartz (subsp. *quarcicola* Mering, Köcke & Kadereit).

Interesting similarities can be found when comparing *T. elongata*/*T. buchenaui* (*T. bulbosa* complex) and *T. maritima*/*T. gaspensis* (*T. maritima* complex). These two species pairs comprise a widespread and a regionally endemic species, of which the regional endemic in both cases grows in regularly flooded habitats (*T. buchenaui*, Köcke et al., 2010; *T. gaspensis*, Lieth and Löve, 1961). ITS sequences within these two species pairs are identical or almost identical.

Considering that crown group age and genetic diversity (as measured with the DNA regions used in our phylogenetic analyses) are similar in the *T. maritima* and *T. bulbosa* complexes, but taxonomic diversity (reflecting morphological diversity) is clearly higher in the latter, we conclude that taxonomic/morphological diversity in these two clades of *Triglochin* is linked to ecological diversity, which would illustrate the prominent role of ecology in speciation (Schluter, 2009; Sobel et al., 2010). It seems plausible that habitat shifts triggered taxonomic/morphological diversification.
Probably our argument can be extended to most other clades of the genus. Thus, habitat diversity is low in *T. scilloides* (annual), *T. striata* and *T. palustris*. Of these, *T. scilloides* is usually found in shallow water and adjacent mud flats of seasonal pools or other temporary wetlands (e.g., Haynes and Hellquist, 2000; Keil, 2012), *T. striata* is common in coastal salt marshes and estuaries but also found in saline inland habitats (e.g., Haynes and Hellquist, 2000; Lisowski et al., 1982; Moore, 1970), and *T. palustris* inhabits saline as well as freshwater marshes or wet meadows (Table 2). Australian annual *Triglochin* mostly inhabit ephemeral wetlands or winter-wet habitats (Aston, 2011; Keighery, 1979; Pignatti and Pignatti, 2005). On the basis of the ecological knowledge available for these species (Table 2), we can not decide whether the species richness of this clade also is the result of ecological diversification or of other causes.

Chapter 3

Revision of the Mediterranean and southern African *Triglochin bulbosa* complex (Juncaginaceae)

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ABSTRACT

The *Triglochin bulbosa* complex (Juncaginaceae) from the Mediterranean region and Africa is revised. One new species, *Triglochin buchenaui*, and two new subspecies, *T. bulbosa* subsp. *calcicola* and subsp. *quarcicola*, are described from South Africa. The only two Mediterranean taxa in the complex (*Triglochin barrelieri*, *T. laxiflora*) are elevated to species rank. Altogether seven species and four subspecies are recognized: *Triglochin barrelieri*, *T. buchenaui*, *T. bulbosa* subsp. *bulbosa, T. bulbosa* subsp. *calcicola*, *T. bulbosa* subsp. *quarcicola*, *T. bulbosa* subsp. *tenuifolia, T. compacta*, *T. elongata*, *T. laxiflora* and *T. milnei*. An identification key, detailed descriptions and accounts of the ecology and distribution of the taxa are provided. An IUCN conservation status is proposed for each taxon.

Keywords. *Triglochin*, Juncaginaceae, revision, taxonomy, morphology, typification, ecology, Mediterranean Floristic Region, South Africa, Cape Peninsula, Cape Floristic Region, IUCN conservation assessment.

INTRODUCTION

Triglochin L. is the largest of the currently recognized four (Haynes *et al.*, 1998; Stevens, 2001+) or five (Dahlgren *et al.*, 1985) genera of the small monocotyledon family Juncaginaceae, and contains 25-35 annual and perennial species. The genus has an almost cosmopolitan distribution with Australia as centre of specific diversity. Common habitats of *Triglochin* are coastal salt marshes, tidal mud-flats, freshwater wetlands, seasonal pools and inland saltpans.

In the Mediterranean region and southern Africa *Triglochin* is represented by a group of widely distributed taxa with ovoid to very narrowly ovoid fruits consisting of three fertile carpels that separate at maturity. A carpophore may be present or absent. Since all taxa belonging to this group at some point have been included in *Triglochin bulbosa* L., the group is here called the *T. bulbosa* complex. In a molecular phylogenetic study of *Triglochin* (von Mering & Kadereit, unpubl. data), this complex forms a well-supported monophyletic clade. In addition to the *Triglochin bulbosa* complex, *T. maritima* L., *T. palustris* L. and *T. striata* Ruiz & Pav. can be found in the Mediterranean region, and *T. striata* in southern Africa. These three latter species can easily be distinguished from the *Triglochin bulbosa* complex by fruit characters. The fruits of *Triglochin maritima* are composed of six fertile carpels, *T. palustris* has fruits with only three carpels that do not separate at maturity, and *T. striata* is easily recognizable by roundish fruits consisting of three fertile and three sterile carpels.

The *Triglochin bulbosa* complex is a systematically difficult group as evident from its complex taxonomic history. In South Africa, five species of the *Triglochin bulbosa* complex have been recognized in the past, but currently only a broadly circumscribed *T. bulbosa* is accepted within the complex (e.g., Obermeyer, 1966; Goldblatt & Manning, 2000; Glen & Cook, 2003; Trinder-Smith, 2003). In this broad circumscription, *Triglochin bulbosa* shows substantial morphological (e.g., in leaf and fruit characters and underground parts) and ecological variation (Fig. 1). In the Mediterranean area, *Triglochin barrelieri* and *T. laxiflora* have been variously treated as separate species (e.g., Talavera, 1987; S. Talavera, ms. Flora Iberica) or as subspecies of the South African *T. bulbosa* (e.g., Bolòs & Vigo, 2001).

No revision of the entire complex has ever been attempted. This paper reports the findings of an analysis of extensively researched herbarium material and our own collections from the Mediterranean area and South Africa. Based on morphology, phenology, ecology and geographical distribution, seven species and four subspecies are recognized. These are *Triglochin barrelieri* Loisel., *T. buchenaui* Köcke, Mering & Kadereit, sp. nov., *T. bulbosa* L. (with subspp. *bulbosa*, *tenuifolia* (Adamson) Horn, *calcicola* Mering, Köcke & Kadereit, subsp. nov., *quarcicola* Mering, Köcke & Kadereit, subsp. nov.), *T. compacta* Adamson, *T. elongata* Buchenau, *T. laxiflora* Guss and *T. milnei* Horn.

TAXONOMIC HISTORY

Triglochin bulbosa L. was described by Linnaeus in 1771 based on collections by Thunberg from the Cape of Good Hope (Buchenau, 1896). However, Barrelier's account of "Juncago maritima" (1714) from the western Mediterranean is the first description of a member of the *Triglochin bulbosa* complex. Despite its morphological distinctness stressed by Barrelier (1714), Linnaeus did not recognize Barrelier's taxon as a separate species but included it as var. *β* in *Triglochin palustris* L. (Linnaeus, 1762). Loiseleur-Deslongchamps (1806) at first included this taxon as *Triglochin palustris* L. var. *β* in his Flora Gallica, but in the amendments to this Flora (Loiseleur-Deslongchamps, 1807) recognized it as a distinct new species, *T. barrelieri* Loisel. An affinity to or a possible synonymy with

Triglochin bulbosa was suggested ("An *Triglochin bulbosum.* Lin. Mant. 226 ?", Loiseleur-Deslongchamps, 1807). The second Mediterranean taxon, *Triglochin laxiflora* Guss., was described by Gussone (1825) from Sicily and Corsica.

Subsequently, *Triglochin barrelieri* and *T. laxiflora* were both recognized as distinct species (e.g., Loiseleur-Deslongchamps, 1828; Battandier & Trabut, 1884; Bonnet, 1896; Jahandiez & Maire, 1931). Other authors accepted only *Triglochin laxiflora* as a species while *T. barrelieri* was treated as a synonym (e.g., Micheli, 1881; Buchenau, 1903) or as a subspecies of the South African *T. bulbosa* (e.g., Hayek, 1933; Maire, 1952). Rouy (1912) was the first to reduce the two Mediterranean taxa to subspecies of the South African *Triglochin bulbosa* (*T. bulbosa* subsp. *barrelieri* (Loisel.) Rouy, and subsp. *laxiflora* (Guss.) Rouy). Some recent Floras treat them as distinct species, *Triglochin barrelieri* and *T. laxiflora* (Talavera, 1987; S. Talavera, ms. Flora Iberica), and others as subspecies of *T. bulbosa* L. (e.g., de Bolòs & Vigo, 2001).

Based mainly on differences in fruit size and pedicel length, Pau (1914) described *Triglochin barrelieri* var. *maura* Pau, a variety endemic to Morocco, which was later also treated as *T. bulbosa* var. *maura* (Pau) Maire (Maire, 1952) or as a synonym of *T. bulbosa* subsp. *barrelieri* (Govaerts, 2008).

Buchenau (1903) newly described *Triglochin elongata* Buchenau from South Africa and recognized *T. bulbosa* (syn. *T. barrelieri*) and *T. laxiflora*. The occurrence of the latter in South Africa was recorded with reservations because only one specimen could be assigned to *Triglochin laxiflora* by Buchenau (1903). Bennett (1897) also believed *Triglochin laxiflora* to occur in South Africa, while Horn af Rantzien (1961) excluded this species from the South African flora.

Adamson (1939), who contributed considerably to the compilation of the "Flora of the Cape Peninsula", studied the South African species of *Triglochin* in more detail and also published notes on habitat preferences. He provided detailed descriptions and diagnostic characters for the South African taxa of the *Triglochin bulbosa* complex known to him (Adamson, 1939). Adamson described two new *Triglochin* species, *T. tenuifolia* Adamson (1939) and *T. compacta* Adamson (1943), and accepted *T. elongata*. Thus, Adamson (1939, 1943) recognized the following four species of the *Triglochin bulbosa* complex in South Africa: *T. bulbosa*, *T. elongata*, *T. tenuifolia* and *T. compacta*. We follow Adamson in the recognition of his newly described *T. compacta* and *T. tenuifolia*, but treat the latter as a subspecies of *T. bulbosa*.

The last treatment of the African species of *Triglochin* was carried out by Horn af Rantzien (1961). This author did not regard his publication as a full revision since it was based on herbarium material from Kew and Stockholm only. He provided an identification key to the species and subspecies of *Triglochin* in Africa which was mainly based on differences in fruit characters and underground organs. In his study only taxa with bulbs were investigated in great detail, especially with respect to flower and fruit morphology. As a result of this he described a new species, *Triglochin milnei* Horn, from southern Central Africa (Tanzania, Angola, Zambia, eastern South Africa), recognizable by its conspicuously larger flowers and fruits. Apparently Horn af Rantzien (1961) was not aware of the existence of *Triglochin compacta* and did not include this taxon in his study. He did, however, include *Triglochin tenuifolia* in *T. bulbosa* as subsp. *tenuifolia* (Adamson) Horn, and also treated the other taxa at intraspecific rank. This resulted in the recognition of five subspecies of *Triglochin bulbosa*: subsp. *bulbosa* (South Africa), subsp. *tenuifolia* (South Africa), subsp. *barrelieri* (Mediterranean), subsp. *laxiflora* (Mediterranean) and subsp. *maura* (Pau) Horn (Morocco). Such treatment seemed justified by great similarities within the complex and the occurrence of transitional forms. Like Adamson (1939), Horn af Rantzien (1961) also accepted *Triglochin elongata* as a separate species. However, this taxon was overlooked by later authors.

Fig. 1. *Triglochin bulbosa* L. subsp. *calcicola* Mering, Köcke & Kadereit subsp. nov. A, habit, B, habitat (De Hoop); *T. bulbosa* L. subsp. *quarcicola* Mering, Köcke & Kadereit subsp. nov. C, habitat (Moedverloren), D, habit; *T. compacta* Adamson. E, bulbs, F, habitat (Goukamma), G, infructescence; *T. elongata* Buchenau. H, habit (with rhizomes), L, habitat (Overberg); *T. buchenaui* Köcke, Mering & Kadereit sp. nov. I, habitat (Knysna), K, habit. Photos A,B,F,H-L: L. Mucina; C-D: U. Schmiedel; E,G: J. Naudé.

Most recent Floras and checklists of Central and southern Africa accept *Triglochin bulbosa* as the only species (plus the distinct *T. striata*, which does not fall into the *T. bulbosa* complex; e.g., Obermeyer, 1966; Goldblatt & Manning, 2000; Glen & Cook, 2003; Trinder-Smith, 2003) and treat *T. milnei* as a synonym (e.g., Obermeyer, 1966; Lisowski *et al.*, 1982). Only Cook (2004) recognized *T. milnei* as a distinct species. The World Checklist of Monocotyledons (Govaerts, 2008) currently recognizes only *Triglochin bulbosa* with a broadly circumscribed subsp. *bulbosa* (Tanzania to South Africa), and subspp. *barrelieri* and *laxiflora* (both in the Mediterranean).

MATERIAL AND METHODS

This revision is based mainly on the study of ca. 800 specimens from AV, B, BC, BM, BOL, EA, FB, HBG, JE, K, M, MSB, MJG, MO, NAP, NBG, NBG-SAM, NBG-STE, NY, P, POZG, RNG, RO, S, STEU, WHB, Z and ZT (abbreviations according to Holmgren & Holmgren, 1998). Names were typified as far as possible, and most type material cited has been seen, in some cases as digital images (indicated by "!" and "photo!", respectively).

The distribution maps are based on information from herbarium labels and unambiguous records in the literature. Coordinates of older collections were taken mainly from the NGA GEOnet Names Server (GNS) (National Geospatial-Intelligence Agency, http://earth-info.nga.mil/gns/html/index.html) and the Gazetteer of the Cape Region (Institute of Systematic Botany, University Zurich, http://www.systbot.uzh.ch/Bestimmungsschluessel/GazeteerRSA.html). In addition, the Google Earth web-based satellite imagery database (<earth.google.com>) was used. All maps were generated using the programme ArcGIS 9.0 (ESRI, 2006). The locations of new collections made between 2000 and 2008 were ascertained by using a GPS device. Some information on collector names and/or localities in Africa was verified using Aluka (Ithaka Harbors, Inc., www.aluka.org). Measurements were made on herbarium material and may deviate from fresh material. Longer field trips were made in 2006 (March–May) in South Africa, in October 2006 in Sardinia (Italy), and in 2007 (March-April) in France and Spain. The conservation status of each taxon was assessed using the IUCN Red List categories and criteria (IUCN, 2001).

TAXONOMIC TREATMENT

Triglochin L., Sp. Pl.: 338 (1753). – Type species: *T. palustris* L., "Habitat in Europae inundatis uliginosis" (lecto LINN 466.1 photo!).
Juncago Ség., Pl. Veron. 3: 90 (1754).
Juncago Tourn. ex Moench, Methodus: 644 (1794).
Tristemon Raf., Amer. Monthly Mag. & Crit. Rev. 4(3): 192 (1819).
Abbotia Raf., New Fl. (Rafinesque) 1: 36 (1838).
Hexaglochin Nieuwl., Amer. Midl. Naturalist 3: 10 (1913).

Note. None of the above synonyms has been used in the recent literature. We have not seen any type material for these names.

Description of the *Triglochin bulbosa* complex

Erect perennial herbs, 5-90 cm high, with scapose inflorescences and rhizomes or (often aggregated) bulbs. Base of plants covered with fibrous remains of old leaves. *Leaves* basal, distichous, ± succulent, linear, ± terete (semi-terete), ligulate, sheathing at the base. *Inflorescence* a spike. *Flowers* hermaphrodite, 3-merous, ebracteate, proterogynous. Perianth of six green or sometimes reddish tepals; tepals conchiform, deciduous. Stamens six or three, sessile or subsessile; anthers dorsifixed, dithecous, dehiscing longitudinally, extrorse. *Infructescence* a raceme. *Fruits* ovoid to very narrowly ovoid (Figs 2, 6). Fertile carpels three, connate, sessile, with one ovule and sessile stigma each, usually separating into one-seeded mericarps at maturity. Sometimes an outer whorl of three vestigial carpels present (*T. milnei* and *T. compacta*). *Carpophore* mostly present, sometimes carpophore very thin or lacking and then mericarps not always separating. *Seeds* erect, embryo straight.

Notes. Flowers of *Triglochin* usually have six stamens. However, this number may be reduced to three as reported for *Triglochin milnei* (Horn af Rantzien, 1961). Such reduction was also noted in this study in *Triglochin bulbosa* subsp. *calcicola*. The gynoecium of *Triglochin* is variable. In principle, carpels are arranged in two whorls of three carpels each (e.g., Tomlinson, 1982). Whereas in some species (e.g., *Triglochin maritima*) all six carpels are fertile, in others (e.g., *Triglochin striata*) the three carpels of the outer whorl are sterile (e.g., Tomlinson, 1982; pers. obs.). In the *Triglochin bulbosa* complex only three fertile carpels are present. However, in some species (especially *Triglochin compacta*, *T. milnei*) membranous outgrowths are present at the base of the carpophore, which have been interpreted as remains of the second whorl of carpels. Size and form of these outgrowths are variable. Sometimes they are extremely small, but in *Triglochin milnei* these structures are often very distinctive. Horn af Rantzien (1961) considered them sterile carpels and accordingly described *Triglochin milnei* as having six carpels, of which three are sterile. However, not all specimens studied have outgrowths that can be identified as sterile carpels. Fruits in the complex mostly separate from bottom to top. In some species this character seems to be variable, i.e., carpels separate from bottom to top or from top to bottom. Currently we are not able to give accurate information on the mode of carpel separation for all taxa and accordingly do not know whether this character can be used for the distinction of taxa. This character should be further studied on fresh material.

Distribution. The *Triglochin bulbosa* complex is native to the Mediterranean region, extending along the Atlantic coast to NW France and Morocco (Fig. 3), and to southern Central Africa extending to South Africa (Figs 7, 11, 12). A subspecies of *Triglochin bulbosa* has been reported to be naturalized in Western Australia (Western Australian Herbarium, 1998 and onwards; Australian Plant Census, 2008; see also under *T. bulbosa* subsp. *bulbosa*).

Habitat and Ecology. Plants of this group are typically found in hygro-halophytic habitats such as coastal salt marshes, tidal mud-flats and along lagoons. They also occur on coastal rocks exposed to salt spray or in temporary pools, in inland saltpans, in dry, non-saline habitats on mountain slopes or in seasonally wet grassland and along river banks. Some collections were made at altitudes above 1000 m.

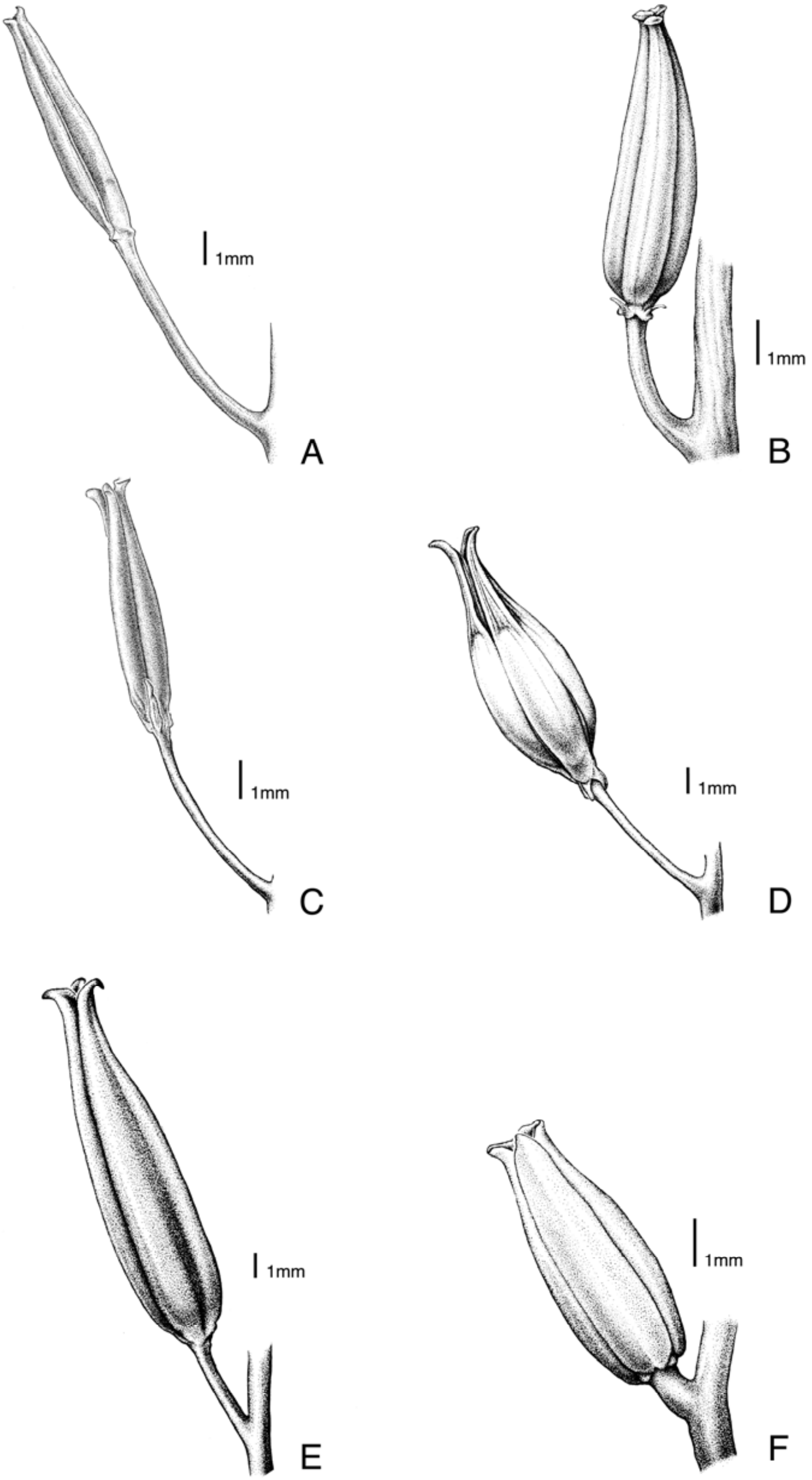

Fig. 2. Fruits of A, *Triglochin barrelieri* Loisel.; B, *T. laxiflora* Guss.; C, *T. compacta* Adamson; D, *T. milnei* Horn; E, *T. elongata* Buchenau; F, *T. buchenaui* Köcke, Mering & Kadereit. Drawings by L. Klöckner (Mainz).

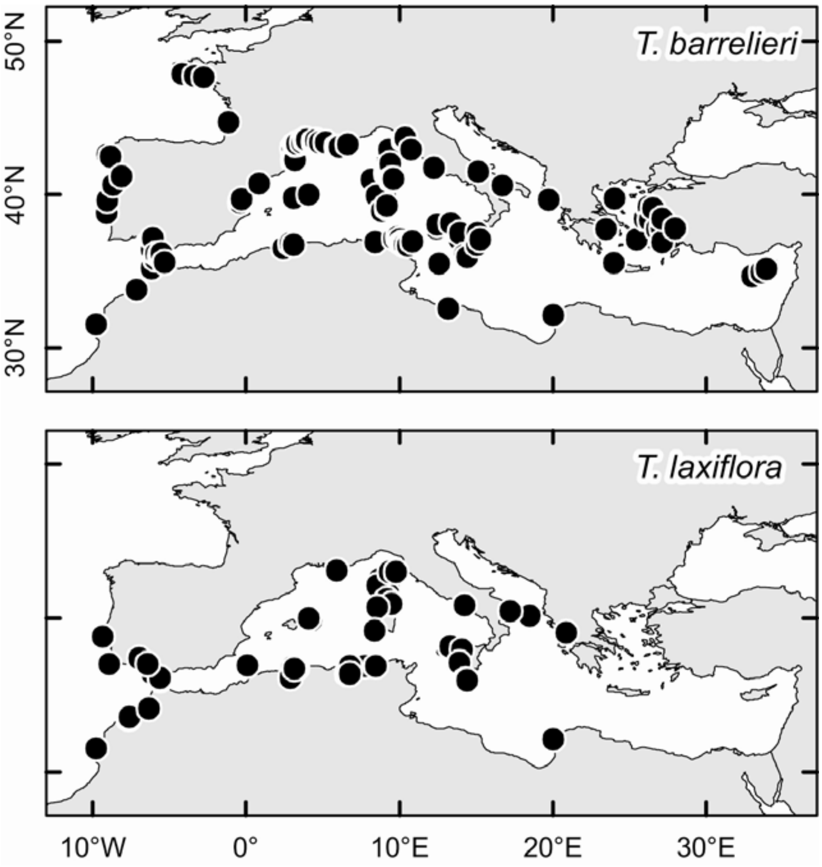

Fig. 3. Geographical distribution of *Triglochin barrelieri* Loisel. and *T. laxiflora* Guss.

Chromosomes. In *Triglochin* chromosome numbers of up to $2n = 144$ are known (e.g., Löve & Löve, 1958; Robb & Ladiges, 1981). No chromosome counts are available for the southern African species of the complex, and only few for the Mediterranean species. For *Triglochin laxiflora* $2n = 18$ (Gardé & Malheiros-Gardé, 1953; Bartolo *et al.*, 1977; Talavera *et al.*, 1995; Castro *et al.*, 2007), and for *T. barrelieri* $2n = 30$ (Castro & Carvalho Fontes, 1946), 32 (Dahlgren *et al.*, 1971; Bartolo *et al.*, 1977) and 36 (Castro *et al.*, 2007) have been reported.

Nomenclatural notes. Although Linneaus (1753) treated *Triglochin* as neuter in his original publication of the name, the feminine gender has to be used (ICBN Vienna Code, 2006, Art. 62.2b and Ex. 5).

Key to the species of the Triglochin bulbosa *complex*

The following key is based on herbarium material. Characters such as size of flowers, fruits or pedicels often are not sufficient to identify species because of the existence of (in this respect) intermediate forms. Underground parts are sometimes lacking in herbarium specimens but are important for the identification of species. When the geographical origin of the material and/or its phenology are unknown, special attention must be paid to the presence of either bulbs or rhizomes. Notes on habitat and distribution should also facilitate the identification of species. As already stated by Horn af Rantzien (1961, p. 102): "In identifications statements on locality, habitat, and time of anthesis are of more use than those on morphological characters." This implies that the combination of morphological attributes, ecology and geographical distribution defines species best.

1a. Plants from the Mediterranean region or the Atlantic coast of western Europe
and North Africa 2
1b. Plants from Central or southern Africa 3
2a. Leaves present at flowering time, mostly as long as or longer than plant height;
pedicels spreading (Fig. 2A); plants flowering in spring (ii-v) .... **1. T. barrelieri**
2b. Leaves formed after flowering, usually much shorter than plant height;
pedicels ascending, ± appressed to infructescence axis (Fig. 2B);
plants flowering in autumn (ix-xii) .... **2. T. laxiflora**
3a. Plants with rhizomes, of salt marsh estuaries or estuarine rivers 4
3b. Plants with bulbs, of inland habitats, never under direct influence of tidal flooding 5
4a. Inflorescences dense, with 10-100 flowers; infructescences (6)10-25 cm long; fruits with
carpophore; plants growing in the upper zone of salt marsh estuaries
and estuarine rivers .... **6. T. elongata**
4b. Inflorescences lax, with 3-17 flowers; infructescences 2-7 cm long, fruits without
carpophore; plants restricted to the lower tidal zone of salt marsh estuaries
.... **7. T. buchenaui**
5a. Flowers usually > 3 mm long; fruits (8)10-14 mm long and ±3 mm wide (Fig. 2D);
plants flowering and fruiting in summer (x-ii/iii); plants from Central
and southern Africa (incl. eastern South Africa) .... **5. T. milnei**
5b. Flowers usually <3 mm long; fruits 4-10 mm long and 1-2 mm wide;
plants flowering and fruiting in spring (vii-xi) or autumn (ii/iii-v);
plants from South Africa 6
6a. Leaves present at flowering time, mostly as long as or longer than plant height;
plants flowering and fruiting in spring (vii-xi) .... **3. T. bulbosa**
6b. Leaves formed after flowering, mostly only half as long as plant height,
plants flowering and fruiting in autumn (ii/iii-v) .... **4. T. compacta**

1. Triglochin barrelieri Loisel., Fl. Gall. ed. 1. 725 (1807), ed. 2. 1: 264 (1828). – *Triglochin bulbosa* subsp. *barrelieri* (Loisel.) Rouy, Fl. France (Rouy) 13: 271 (1912). – Type: France, Arles (Arelatem), "habitat Arelatem ad Rhodanu", *Artaud* s.n. (holo AV photo!; Fig. 4). **Figs 2A, 3.**

Triglochin palustre L. var. *β*, Sp. Pl. ed. 2. 1: 483 (1762).

Triglochin atlantica Willd. herb., teste Kunth, Enum. Pl. (Kunth) 3: 143 (1841). – Type: not in B-W.

Juncago chabraei Bubani, Fl. Pyren. (Bubani) 4: 8 (1901).

Triglochin barrelieri var. *maura* Pau, Bol. Real Soc. Esp. Hist. Nat. 14: 425 (1914). – Type: Morocco, ad ripas fl. Lukos, juxta El Araix, 1 iii 1930, *Font Quer* s.n. (syn MPU-Maire photo!), inter Ceuta et Tetauen, l. d. Rincón de Medik, 13 iii 1930, *Font Quer* s.n. (syn MPU-Maire photo!). – *Triglochin bulbosa* var. *maura* (Pau) Maire, Fl. Afrique N. 1: 213 (1952).

Triglochin bulbosa var. *genuina* Maire, Jahandiez & Maire, Cat. Maroc 859 (1934).

Plants 10-45 cm high, with fusiform *bulbs*; bulbs covered by dry, brown, stiff, sometimes spiky fibres, these up to 4 cm long. Plants without rhizomes. *Leaves* usually many, present at flowering time, mostly as long as or longer than plant height, uniform. *Inflorescences* rather dense, with 10-25(-30) flowers. *Flowers* (2.5-)3.0-4.5 mm long. *Pedicels* elongated at fruit maturity, up to 2-7 mm long, rarely longer, spreading at angles of 45° to 90° from the inflorescence axis, slightly curved inwards towards the apex. *Infructescences* 8- 16 cm long. *Fruits* narrowly ovoid, 5-10 mm long, rarely longer, 1-2 mm wide (Fig. 2A), sometimes turning dark or black at fruit maturity. *Mericarps* linear, connate at the *carpophore*; tips only slightly curved outwards.

Distribution. The species is widely distributed in the Mediterranean region (with a noteworthy gap in Egypt) reaching the Atlantic coast of NW France and Morocco (Fig. 3). It is known from all large Mediterranean islands.

Habitat and ecology. *Triglochin barrerlieri* is commonly found in coastal wetlands, e.g., in salt marshes and along lagoons, and less frequently on coastal rocks exposed to salt spray, in depressions in coastal dunes or in cork oak forests (e.g., in Andalusia, Spain). In North Africa it typically occurs in sebkhas (sabkhas: rarely flooded salt marshes of desert coasts). The species usually grows in very open vegetation and is often associated with *Sarcocornia fruticosa* and *Juncus acutus*.

Phenology. Flowering in spring (ii-v), leaves present at flowering time.

Chromosome number. *2n* = 30 (Castro & Carvalho Fontes, 1946), 32 (Dahlgren *et al.*, 1971, Bartolo *et al.*, 1977), 36 (Castro *et al.*, 2007).

Notes. This species is very variable in overall size and fruit and pedicel length. *Triglochin barrelieri* var. *maura* has been described from Morocco as a taxon of larger size and with longer pedicels. However, such large forms are also found in other parts of the species' distribution range and are not limited to Morocco. Presence of *Triglochin barrelieri* in Israel seems possible because the description of *T. palustris* for this area by Post (1933) partly matches *T. barrelieri*. We have, however, not seen any material of the species from Israel where it may be extinct.

Taxonomic remarks. *Triglochin barrelieri* is clearly distinct from the only other Mediterranean species of the complex, *T. laxiflora*, by flowering in spring (vs. autumn), the presence of leaves at flowering time (vs. leaf formation after flowering), and by having spreading pedicels (vs. appressed to infructescence axis).
The distinction between *Triglochin barrelieri* and *T. bulbosa* subsp. *bulbosa* is difficult, and these two species are morphologically very similar to each other. They can be distinguished by geographical origin and ecology, and results of our molecular studies (von Mering, Köcke & Kadereit, unpubl. data) support their recognition as separate species because they are not closest relative to each other.

Nomenclatural notes. In the protologue of *Triglochin barrelieri* the following localities are given: "Habitat in maritimis Neutstriae, Occitaniae, Provinciae; ad ripas Rhodani prope Arelatem collegit *D. ARTAUD.*" (Loiseleur-Deslongchamps, 1807). The herbarium of Loiseleur-Deslongchamps in Avignon (AV) contains several specimens bearing the name *Triglochin barrelieri*. Only the specimen cited above corresponds to the protologue (location and collector), was annotated by Loiseleur-Deslongchamps himself (P. Moulet, pers. comm.) and thus probably represents the holotype (Fig. 4).

Proposed IUCN conservation status. Least Concern (LC). The species used to be widely distributed in the Mediterranean region and is probably not under immediate threat. However, coastal wetlands (where the species is typically found) are among the most vulnerable habitats in the Mediterranean region. Large areas are already lost, degraded or under severe threat due to drainage, construction, urbanisation and tourism (e.g., Finlayson & Moser, 1991; MedWet, 1996). Only few data are available on the current area of occupancy due to the very low number of recent collections. Several collecting localities of older specimens have been destroyed by settlements. Other populations might also be destroyed or threatened by habitat destruction. Therefore, the area of occupancy might be declining, and the global conservation status of this species may be prone to change. Further conservation assessments are also necessary at the national level. *Triglochin barrelieri* is already protected in Aquitaine, France (Muséum National d'Histoire Naturelle, 2003-2006) and in the Italian regions of Molise, Basilicata, Calabria and Sicily (Conti *et al.*, 1997).

Selected specimens examined. **Europe.** FRANCE. **Aquitaine. Dép. Gironde:** Bordeaux, Bassin d'Arcachon, vi 1831, *Endress* s.n. (B, BM, HBG, JE, M, P). **Bretagne. Dép. Finistère:** Loctudy and Combrit, without date, *Bonnemaison* s.n. (AV). **Dép. Morbihan:** Gâvres, 15 vii 1847, *Debrez* s.n. (ZT); Gâvres, 1847, *Pontarlier* s.n. (P); Gâvres, 1847, *Taslé* s.n. (P); Gâvres, 3 vi 1851 / 4 vii 1847, *Toussaints* s.n. (P); Port-Louis, vi 1847, *Pontarlier* s.n. (ZT), 1849, *Grenier* s.n. (ZT); Port-Louis, 30 vi 1877, *Lloyd* s.n. (BM); Port-Louis, 1891, *Renou* s.n. (P); Port-Louis, 27 v 1912, *Charrier* s.n. (BM). **Corsica. Dép. Haute-Corse:** Ghisonaccia, 30 v 1900, *Rotgès* 339 (P); Saint-Florent, river l'Aliso, 20 v 1973, *Auquier* 3037, *Baguette & Lambinon* (M). **Dép. Corse-du-Sud:** Bonifacio, 29 iii 1849, *Kralik* 787 (P); Porto-Vecchio, 19 iv 1868, *Mabille* s.n. (BM, P, K); Bonifacio, Santa-Manza, 12 iv 1876, *Revelière* s.n. (BM); Bonifacio, 12 v 1880, *Reverchon* s.n. (BM); Bonifacio, Santa-Manza, 12 v 1880, *Reverchon* 246 (JE, P); Bonifacio, Santa-Manza, 2 vi 1894, *Stefani* s.n. (P); Bonifacio, Piantarella, 5 iv 1895, *Stefani* s.n. (BC-SENNEN, P), 9 iv 1895, *Stefani* s.n. (M, ZT); Porto-Vecchio, iv 1911, *Stefani* s.n. (P); iv 1912, *Roux* s.n. (P); Porto-Vecchio, E side of Stabiacco estuary, 13 iv 1939, *Brenan* C.408 (K). **Languedoc-Roussillon. Dép. Aude:** Leucate, 30 v 1887, *Chevallier* s.n. (M); La Nouvelle, iv 1899, *Sennen* s.n. (BC-SENNEN), 10 vi 1904, *Sennen* s.n. (P); **Dép. Hérault:** Maguelonne, 1 vi 1821, *Cufino* s.n. (HBG, RO); Sète (formerly Cette): 1834, *Gath* s.n. (BM), 3 v 1882, *Neyra* s.n. (JE), 4 v 1882, *Neyra* s.n. (B, JE, RO), 9 v 1891, *Dirmède* s.n. (K), v 1905, *Bee* s.n. (P); Roquehaute, near Béziers, v 1855, *Personnat* s.n. (P); Montpellier, v 1857, *Cramer* s.n. (HBG); Maguelonne, 13 vi 1857, *Cosson* s.n. (P); Palavas, 27 v 1899, *Morot* s.n. (P); Palavas, 20 v 1908, *Vichet* s.n. (P); Lattes, Montpellier, 23 iv 1914, *Sudi* s.n (ZT); E of Palavas, 10 v 1914, *Noaik* s.n. (Z); Pérols, 1 vi 1915, *Braun* s.n. (ZT); St. Marcel, Pérols, 1 vi 1915, *Braun-Chur* s.n. (Z); Montpellier, v 1921, *Jenny* s.n. (ZT); Etang de Pérols, Palavas, 12 v 1934, *Zobrist* s.n (ZT); **Dép. Pyrénées-Orientales:** Vendres, 28 iv 1891, *Sennen* s.n. (M), 7 vii 1891, *Sennen* s.n. (BC-SENNEN). **Provence-Alpes-Côte d'Azur. Dép. Bouches-du-Rhône:** Étang de Marignane, 7 vi 1894, *Rouy & Blaise* s.n. (P); Saint-Louis-du-Rhône, v 1906, *Thiébaut* s.n. (P, ZT); without locality, v 1909, *Thiebaut* s.n. (M); Les Saintes-Maries-de-la-Mer, 12 iv 1957, *H. & E. Walter* 68 (B); Ile des Rièges, 24 iv 1967, *Lüpnitz* F 233, F 234, F 235 (MJG)**;** Etang de Vaccarés, 11 iv 1969, *Hecker* F 320 (MJG). **Dép. Var:** Hyères, v 1839, *Kugel* s.n. (BM, M); Toulon, Les Sablettes, 16 v 1848, *Bourgeau* 361 (M, P); Hyères, 1853, *Jordan* s.n. (HBG); Hyères, v 1863, *Huet & Jacquin* s.n (B); Hyères, 29 iv 1869, *Shuttleworth* s.n. (P); Toulon, Les Sablettes, 9 v 1870, *Antheman* s.n (B); Hyères, iv 1873, *Allard* s.n (P, Z); Toulon, 23 vi 1887, *Chevalier* s.n. (P); Hyères, 14 iv 1898, *Mouillefarine* s.n. (P); Hyères, 30 iv 1903, *Raine* s.n. (P); Saint Tropez, Les Salins, iii 1910, *A. & E.G. Camus* s.n. (P), iv 1910, *A. & E.G. Camus* s.n. (P), 22 iii 1911, *A. & E.G. Camus* s.n. (P); Hyères, 25 iii 1913, *Beger* s.n. (B); Hyères, 25 iii 1913, *Noaik* s.n. (Z); Hyères, 3 iv 1913, *Thompson* s.n. (BM); Saint Tropez, Les Salins, 31 iii 1919, *Hibon* s.n. (P); Hyères, Ceinturon, 14 iv 1925, *Ruppert* s.n. (M); Hyères, La Londe, river Pansard, 30 iv 1929, *Ruppert* s.n. (M); Saint Tropez, Les Salins, 5 iv 1931, *Paul* s.n. (M); Hyères, 30 iii 1932, *Oberneder & Oberneder* 457 (BM); Hyères, 5 iv 1955, *Schumacher* s.n. (HBG). ITALY. **Apulia. Prov. Brindisi:** Saline di Punta della Contessa, 23 v 2007, *di Pietro & Wagensommer* s.n. (MJG), 09 iv 2008, *di Pietro* s.n. (MJG). **Prov. Foggia:** Monte Gargano, Manfredonia, iv 1876, *Comes, Freda, Pedicion* s.n. (RO); Mte. Gargano, mouth of river Cervaro, 14 km S of Manfredonia, road to Bari, 20 iv 1964, *Dietrich* s.n. (M), *Hertel* 3575 (M), *Podlech* 9554 (MSB); between Sciale Borgia and the mouth of river Cervaro, 12-14 km S of Manfredonia, 22 v 1972, *Hein* s.n. (B). **Prov. Taranto:** Taranto-Ginosa, 18 iv 1879, *Pasquale* s.n. (RO). **Lazio. Prov. Rome:** Ostia, near Rome, v 1925, *Meebold* s.n. (K). **Sardinia. Prov. Cagliari:** Cagliari, vi 1854, *Huet du Pavillon* s.n. (P); Cagliari, La Madeleine, 3 iv 1958, *Titden* 128 (K); Cagliari, near Maddalena, 6 iv 1858, *Schweinfurth* s.n. (HBG); Quartu Sant Elena, Stagno di Simbirizzi, ca. 20 m, 18 iv 1975, *Urmi* s.n. (Z); Domus de Maria, SW of Stagnioni de su Sali, 4 iv 1992, *Stud. biol. Acad. Turic. in itinere* 92-12 (Z). **Prov. Olbia-Tempio:** Terranova, 11 iii 1885, *Forsyth Major* s.n. (HBG); Golfo Aranci, 1 iv 1909, *Poeverlein* 25009 (B); near Olbia, 8 iv 1962, *Podlech* 7872 (MSB); Porto di Cugnana, iv 1966, *Merxmüller* 20931 *& Oberwinkler*, *Merxmüller* 20932 *& Oberwinkler* (M); 3 km S of Palau, 19 iv 1973, *Humphries & Richardson* 378 (RNG). **Prov. Oristano:** Oristano, prope locum dictum Torre Grande, 19 iii 1912, *Fiori* s.n. (BC, BM, RO, Z). **Prov. Sassari:** Stintino Peninsula, Capo Falcone, 3 iv 1968, *Hecker* I 27, I 28 (MJG); Capo Falcone, ca. 10 m, 13 iv 1971, *Hecker* I 500 (Hec.691) (MJG); Capo Falcone, 0-15 m, 9 iv 1973, *Humphries & Richardson* 167 (BM); Capo Falcone, 4 x 2006, *von Mering* s.n. (MJG). **Sicily. Prov. Agrigent:** Licata, iv 1902, *Ross* 376 (B, HBG, M, ZT); Isola di Lampedusa, 10 iii 1907, *Martarana* s.n. (RO). **Prov. Caltanisetta:** San Cataldo, 1847, *Rabenhorst* s.n. (BM); San Cataldo, 26 v 1875, *Porta & Rigo* 299 (JE, P). **Prov. Catania:** Catania, 26 iii 1856, *E. & A. Huet du Pavillon* s.n. (BM, M, P). **Prov. Siracusa:** Siracusa, 26 iv 1898, *Rigo* 161 (HBG, M, P); near Siracusa, iv 1898, *Rigo* s.n. (B, BM, M, P, Z, ZT); Pachino, 31 iii 1952, *Harries & Doppelbaur* 343 (M); near Siracusa, iii 2005, *Uhink* s.n. (MJG). **Prov. Trapani:** Marsala, v (no year given), *Todaro* s.n. (BM, RO); Trapani, 9 iv 1966, *H. & H. Doppelbaur* 14565 (M). **Tuscany. Prov. Grosseto:** Follonica, 27 v 1911, *Fiori* s.n. (BC-SENNEN). **Prov. Livorno:** Livorno, Paduletta, 19 v 1861, *Savi* s.n. (B, P). **Prov. Pisa:** near Pisa, 1868, *van Heurck* s.n. (BC). SPAIN. **Andalusia. Prov. Cádiz:** Bonanza, 17 iv 1951, *Alston* 10267 (BM); entre San Fernando y Chiclana, 28 v 1951, *de Bolos* s.n. (BC); between Tarifa and Puerto Real, 8 iv 1953, *Merxmüller & Wiedmann* 648/53 (M); near Los Barrios, ca. 150 m, 14 v 1967, *Brinton-Lee* 1306 (BM); Tarifa, Rio de la Jara, iii 1973, *Deil* s.n. (FB); Tarifa, 19 iv 1974, *Carr* 1636 (RNG); Puerto de Santa María, 13 iv 1978, *Luque & Valdés* s.n. (B, BC, HBG, M, RNG); entre San Fernando y Chiclana, 4 v 1979, *Cabezudo, Gallego & Talavera* s.n. (RNG); Tarifa, mouth of Rio Jara, 20 vi 1990, *Deil* 5967 (FB); Tarifa, Zahara de los Atunes, 16 v 1992, *Deil* 6329 (FB). **Prov. Sevilla:** Isla Menor, Rio Guadalquivir, 17 iv 1972, *Heywood, Moore, Bramwell,* et al. 218 (RNG). **Balearic Islands. Mallorca:** near Porrata, 5 iv 1921, *Welti-Hug* s.n. (Z); SE of Albufera, v 1985, *Beckett* s.n. (RNG). **Catalonia. Prov. Girona:** Castelló d'Empúries, 27 iv 1908, *Sennen* 613 (BM); Castelló d'Empúries, vii 1916, *Pascual* s.n. (BC-SENNEN); Rosas, v 1943, *Bolos* s.n. (BC); Aiguamolls de Castelló d'Empúries, 14 v 1983, *Blanché & Vallès* s.n. (M). **Prov. Tarragona:** Delta de l'Ebre, Sant Carles de La Ràpita, 2 iv 1934, *Font Quer* s.n. (BC); Alfaques peninsula, near Sant Carles de la Ràpita, 2 iv 1934, *Koch* 34/50 (ZT); Delta de l'Ebre, Sales, 16 ii 1975, *Balada* s.n. (BC). **Galicia. Prov. A Coruña:** Olveira, 24 iv 1981, *Amich, Rico, Sánchez & Giraldez* s.n. (BC); Olveira, Santa Uxía de Ribeira, 4 v 1985, *Valdés-Bermejo* 10148 *& Grupo Botanico Gallego* (BC, M, Z). **Valenciana. Prov. Valencia:** Valencia, iv 1892, *Reverchon* s.n. (M); Sagunt(o) (French: Sagonte), iv 1893, *Reverchon* 834 (B, BM, HBG, M, P, Z). PORTUGAL. **Dist. Aveiro**: Aveiro, Mina, 20 v 1954, *J. Matos, A. Matos & Marques* 4752 (P); Ílhavo, Gafanha da Boavista, 20 v 1954, *J. Matos, A. Matos & Marques* 4768 (P); Ílhavo, between Forte da Barra and Ponte Nova (Costa Nova), 30 v 1979, *Marques* 1260 (B). **Dist. Coimbra:** Figueira da Foz, Gala, 11 v 1950, *J. Matos & A. Matos* s.n. (BM). **Dist. Faro (Algarve):** between Vila Real de San António and Castro Marim, 22 iv 1956, *Malato-Beliz* et al. 2890 (RO); Martinhal near Sagres, 1 iv 1971, *Davis* 50919 (RNG); Alvor, iii 1990, Univ. of Oxford Plant Sciences undergraduate filed course (RNG). **Dist. Leiria:** S of Nazaré, 7 iv 1961, *C. & J. Poelt* 207, 227 (M). **Dist. Lissabon:** Sacavém, 31 iii 1943, *Carvalho Fontés* 8909 (BC). **Dist. Porto:** Porto, Boucas, Mattosinhos, v 1895, *Sampaio* s.n. (M, P). **Dist. Sétubal:** Bank of river Tejo ("ad Tagum"), Coina, iv 1840, *Welwitsch* 237 (P); "ad Tagum", 1848-1850, *Welwitsch* 993 (BM); Transtagana (SE Portugal, later Prov. Alemtejo), near Fornos d'El Rei, vi 1851, *Welwitsch* 333 (P); Trafaria, iii 1888, *Daveau* 348 (P). CYPRUS. **Dist. Famagusta:** near Paralimni village, 10 iv 1953, *Kennedy* 1782 (D 3755) (K). **Dist. Limassol:** Akrotiri, W side of Limassol salt lake. 15 iv 1992, *Lambinon* 92/Cy/359 *& van den Sande* (MSB). UK. **Gibraltar.** Palmones, 7 ii 1913, *Wolley-Dod* 1573 (K), 11 iii 1922, *Wolley-Dod* 349 (BM). CROATIA. **Dalmatia**. Pag (island), near Vlasici, 13 v 2005, *Bernhardt* s.n. (WHB); near Sibenik, 8 vi 2006, *Bernhardt & Hermanowski* s.n. (WHB). GREECE. near Athens, 1820, *Sartori* s.n. (M); Faliro (also Faliron/Phaliron, latin Phalerum), iv 1849, *Orphanides* s.n. (P); Eleusine, 19 iii 1876, *Heldreich* s.n. (HBG); v 1876, Pichler s.n. (P); Faliro, iv 1886, *Heldreich* s.n. (B), 8 iv 1897, *Heldreich* s.n. (Z); Athens, Loutsa, 15 ii 1931, *Guiol* 1714 (BM). **Peloponnese.** Ahaia, near Kalogria, 33 km W of Patra, 2 v 1995, *Turland* 849 (BM); between Brinia and Manolada (Ilia), 17 v 1996, *Raabe* s.n. (B). **Messeni:** Ep. Pilia: Voidhokoilia Bay between Petrohori and Paleokastro Navarinou, 0-5 m, 17 iv 1979, *Greuter & Merxmüller* 17215 (B, M); Methoni, iv 1981, Bowen 2051 (RNG). **Lakonia:** Ep. Epidhavros Limiras, opposite island Elafonisos, 10 iv 1979, *Greuter & Merxmüller* 17076 (B, M, P); mouth of river Evrotas, near Elos, iv 1979, *Bowen* 1307 (RNG); Vinglafia, opposite Elafonisos, 21 ii 1991, *Jagel* s.n. (B); Argolis, Ep. Ermionidhos, 3.3 km ENE Portoheli, 0-5m, 14 iv 1996, *Eisenblätter & Willing* 43.814, 43.815 (B); near Kalogria SW of Patras, 25 iv 1991, *Raabe* s.n. (B); Evvia: ep. Karistias, W of Karistos, 0-10 m, 4 v 1996, *S. & B. Snogerup* 12651 (B); Loutra Kounoupelli, road to Kalogria/Metohi, 7 v 1996, *Raabe* s.n. (B). **Chios.** c. 1930, *Guiol* s.n. (BM). **Corfu.** Kastrades, am Hylläischen Hafen, 3 m, 22 iv 1896, *Baenitz* s.n. (B, HBG, M, P, ZT); Potami, 21 v 1887, name illegible (ZT); Alikes, iv 1980, *Bowen* 1897 (RNG). **Crete**. Near Suda (also Souda), iii 1846, *Heldreich* s.n. (BM, P); location illegible, iii 1846, *Heldreich* s.n. (BM, K, P). **Kos.** N coast, near Marmari, 0-3 m, 12 iv 1974, *Buttler* 18237 (M). **Naxos.** Chora, 15 v 1992, *Böhling* 1491 (B); Alluvai, 17 iv 1993, *Böhling* 2312 (B). **Samos.** Tigani, 2 iv 1934, *K.H. & F. Rechinger* 3642 (BM, K). TURKEY. **Prov. Izmir.** Smyrna (=Izmir), 29 iii 1854, *Balansa* s.n. (P, ZT); Smyrna, near Halcar-Bunar (Halkar Bunar), 12 v 1906, *Bornmüller* 10018 (B, BM, HBG, JE, P, ZT); Cesme Peninsula, end of Gülbahce Bay, 25 ii 1966, *Bocquet* 1133 (ZT); between Karsyaka and Izmir near Bairakli, 5 iv 1966, *Bocquet* 1887 (ZT).

North Africa. ALGERIA. La Calle (=El Kala), 11 v 1841, *Durieu de Maisonneure* s.n. (P); Algiers, Marais ou Baba Alg., iv 1850, *Jamin* s.n. (P); Algiers, Maison-Carrée, iii 1856, *Wolfe* s.n. (K); Algiers, Maison-Carrée, 8 iv 1894, *Chevalier* s.n. (P); Marais de la Meteija, iv 1851, *Jamin* s.n. (P); Salines d'Argess, 6 v 1875, *Cosson* s.n. (P); La Calle, 18 v 1918, *Clavé* s.n. (P); Algiers, marais de la Raasaura près Fort-de-l'Eau, 28 ii - 10 iv 1960, *d'Alleizette* s.n. (M); near Garaetz el Mkhada, 23 iii 1968, *Leippert* 7107 (B); La Calle, 5-10 m, 11 v 1971, *Davis* 52174 (RNG, BM). LIBYA. Benghazi, 9 iv 1883, *Ruhmer* 315 (JE, HBG, P); Benghazi, 1884, *Petrovich* s.n. (Z); Tadjoura, near Tripoli, 8 iv 1886, *Letourneux* s.n. (P); Tripoli, Mlacha, 22 iii 1887, *Taubert* 87 (JE); Cyrenaica, without locality, 13 v 1887, *Taubert* 87 (P); road between Benghazi and Tocra, 19 iii 1929, *Rikli* s.n. (ZT); El-Hania, Sebka Chascem el-Chelb, 8 Mag.(v) 1934, *Pampanini & Pichi-Sermolli* 165 (K); near Tripoli, 29 iii 1938, *Kracht* 38/001, 38/002 (B); El Magazin, near Bezenicem, 20 iv 1938, *Maire & Weiller* 1461 (P); Barsis (also Bersis), 11 iii 1958, *Guichard* CYR/96/58 (BM); 4-5 km N of Benghazi, 3 iv 1970, *Davis* 50488 (K, RNG). MOROCCO. Tanger, Cap Spartel, 20 iv 1911, *Pitard* s.n. (P); Bouznika, 23 iv 1979, 60 m, *Lewalle* 9020 (P); Oulad Amara, N de Bouznika, 40 km SW of Rabat, 23 iv 1979, *Damblon* 79/63 (B, RNG); Tanger, Cap Spartel, 10 m, 30 iv 1986, *Deil* 1321 (FB); Oued Laou, ca. 8 km E of Tetouan on road to Martil, 25 m, 25, ii 1994, *Jury* 13327 *& Taleb, Upson, Walters* (RNG); Larache, bridge over river Loukkos, 17 iii 1995, 3 m, *Mateos, Reina, Sangalli, Sardón & Valdés* 4824/95 (RNG); Tahaddart, Sansouire, 17 v 2003, *Deil & Hamman* 34 (FB); Larache, 15 v 2003, *Deil & Hamman* 5 (FB). TUNISIA. Bône (=Annaba), 1834, *Steinheil* s.n. (P); Tunis, 4 iii 1874, *Doûmet-Adanson* s.n. (P); Tunis, Goletta, 9 iii 1880, *Armitage* s.n. (RO)***; Tunis, La Goulette,*** 2 v 1883, *Cosson, Letourneux, Reboud, Barratte, Bonnel* s.n. (P); Hammam-el-Lif, 8 v 1883, *Cosson, Letourneux, Reboud, Barratte & Bonnel* s.n. (P); Zaouiet-el-Mgaiz, 18 v 1883, *Cosson, Letourneux, Reboud, Barratte & Bonnel* s.n. (P); Cap Bon, between Kurba and Menzel, Temim, v 1883, *Cosson, Letourneux, Reboud, Barratte & Bonnel* s.n. (P); Annaba, near Boudjima, 28 iii 1884, *Letourneux* s.n. (P); Ruins Utique, 21 vi 1887, *Letourneux* s.n. (P); between Dindja and lake Ichkeul, 23 vi 1887, *Letourneux* s.n. (P); between Djebel Ichkeul and Mateur, 26 vi 1887, *Letourneux* s.n. (P); Utique, 4 v 1888, *Barratte* s.n. (P); Menzel, Djemil, 11 v 1888, *Barratte* s.n. (P); Tunis, La Goulette, 19 v 1888, *Barratte* s.n. (P); Hammam-el-Lif, 20 v 1888, *Barratte* s.n. (P); Menzel, Djemil, 2 vi 1888, *Cosson, Barratte & Duval* s.n. (P); Hammam-el-Lif, 6 v 1893, *Chevalier* s.n. (P); Djebel Djeloud, iv 1909, *Pitard* s.n. (ZT); near Protville, between Tunis and Bizerta, 16 iv 1938, *Simpson* 38298 (BM); Carthage-Tunis road, 28 iv 1939, *Sandwith* 2845 *& Simpson* 39779 (BM); ca. 7 km SW of Hammamet, 14 iv 1968, *Wagenitz* 1177 (B); Tunis, 12 iv 1971, *Bolay & Kramer* s.n. (B); Kelibia, E side of Cap Bon peninsula, 28 iv 1975, *Davis* 56931 *& Lamond* (BM, RNG).

2. Triglochin laxiflora Guss., Index Seminum Horto Boccad. (PAL) 1825: s.p. (1825); Fl. Sic. Prod. 1: 451 (1827). – *T. bulbosa* subsp. *laxiflora* (Guss.) Rouy, Fl. France (Rouy) 13: 272 (1912). – Type: Italy, Sicily, Palermo, "Palermo a Monte Peregrino" [= Monte Pellegrino], *Gussone* s.n. (lecto NAP photo!, designated here; Fig. 5). **Figs 2B, 3.**

Triglochin palustre Desf., Fl. Atlant. 1: 322 (1798), non L. (nom. illeg.).

Plants 10-25(-35) cm high, with fusiform *bulbs*; bulbs covered by dry, brown fibres which are usually stiff, sometimes soft or spiky; fibres up to 5 cm long. Plants without rhizomes. *Leaves* few (mostly 2-4 per bulb), formed after flowering, usually much shorter than plant height (mostly reaching only half of the height of plants). Outer leaves shorter and wider than inner leaves, up to 6 cm long, mucronate, inner leaves abruptly narrowing above the ligule, less than 1.5 mm wide. *Inflorescences* lax, with 4-15(-25) flowers. *Flowers* 2.0-3.0 mm long. *Pedicels* 2-3 mm long, rarely longer, usually much shorter than fruit, ascending, ± appressed to infructescence axis, diverging from it at angles of up to 45°, slightly curved inwards towards the apex. *Infructescences* 3-10 cm long. *Fruits* narrowly ovoid, 5-10 mm long, rarely longer, 1-2 mm wide (Fig. 2B). *Mericarps* linear, connate at the *carpophore*; tips curved outwards.

Distribution. Widely distributed in the Mediterranean region, largely sympatric with *Triglochin barrelieri* but absent from the Atlantic coast of France and from parts of the eastern Mediterranean (Fig. 3).

Habitat and ecology. This species is typically found in salt marshes and along lagoons, but also in cork oak or pine forests (e.g., Morocco, Andalusia) and in Mediterranean temporal pools (e.g., Corsica, Malta). *Triglochin laxiflora* is sometimes found in limestone areas (e.g., Malta, Sicily). Growing from 0-500 m (e.g., Monte Pellegrino, Sicily), in Algeria reaching ca. 1100 m (e.g., Djebel Ouach).

Phenology. The species flowers and fruits in autumn (ix - xii) and leaves are formed after flowering.

Chromosome number. $2n = 18$ (Gardé & Malheiros-Gardé, 1953 ; Bartolo *et al.*, 1977 ; Talavera *et al.*, 1995 ; Castro *et al.*, 2007).

Notes. The distributional gaps in many parts of the eastern Mediterranean might be a collecting artefact resulting from the autumnal flowering time of the species. Dandy (1980) indicated Albania, Crete, Greece and ex-Jugoslavia as part of the distribution area in the eastern Mediterranean. *Triglochin laxiflora* is not known from Turkey (Uotila, 1984).
Although reported from South Africa by some authors (Bennett, 1897; Buchenau, 1903), these records are due to misidentification and refer, at least in part, to *Triglochin elongata* or *T. compacta*. *Triglochin laxiflora* does not occur there and has to be excluded from the South African flora (Adamson, 1939; Horn af Rantzien, 1961).

Taxonomic remarks. See *Triglochin barrelieri* for a short comparison with the only other Mediterranean species of the complex.

Nomenclatural notes. In the protologue of *Triglochin laxiflora* Gussone (1825) does not cite any specimens or localities, but in his Florae Siculae Prodromus (Gussone, 1827) the following localities are given: "Palermo a Monte Peregrino a Monte Castellana sotto Baida Mondello Sferracavallo, Catania, Agosta, Siracusa". Several *Triglochin* specimens from Gussone's collections in the Herbarium Neapolitanum (NAP) were studied. Four specimens of *Triglochin laxiflora* are labelled with localities that were cited in Gussone (1827). The specimen from the Monte Pellegrino in Palermo was here chosen as lectotype (Fig. 5) because it contains several fruiting and some flowering plants in good condition.

Variation. One herbarium sheet (BC 76587) is a plant grown from bulbs in the Botanic Garden of Barcelona. This specimen has exceptionally large fruits which are 10-12 mm long.

Proposed IUCN conservation status. Least Concern (LC). The species used to be widely distributed in the Mediterranean region and probably is not under immediate threat. However, the habitat degradation problems described for *Triglochin barrelieri* also apply here. Mediterranean temporary pools have also been destroyed in considerable numbers by human activities (e.g., Grillas *et al.*, 2004; Ruiz, 2008). Only few data are available on the current area of occupancy. In contrast to the many collections of the species from the 19th century only a small number of recently collected specimens has been seen. Several collecting localities of older specimens have been destroyed by settlements. Other populations might also be destroyed or threatened by habitat destruction. This might imply that the species has become rare, and the global conservation status of this species may be prone to change. Further conservation assessments are also necessary at the national level. *Triglochin laxiflora* is a protected species in France (Muséum National d'Histoire Naturelle, 2003-2006) and in Lazio and Sicily, Italy (Conti *et al.*, 1997).

Specimens examined. **Europe.** FRANCE. **Corsica**. Bonifacio, 11 x 1856, *Revelière* 494 (BM, P); Porto-Vecchio, 16 x 1858, *Revelière* s.n. (P); Ajaccio, 20 ix 1868, *Mabille* s.n. (JE, K, P); Bonifacio, 26 x 1880, *Reverchon* 393 (BM, JE, P); Ajaccio, 1881, *Reverchon* s.n. (ZT); Bonifacio, x 1885, *Reverchon* 393 (B); Ajaccio, Parata, 16 x 1888, *Le Grand* s.n. (P, RO); Bonifacio, 9 x 1901, *Stefani* s.n. (BM, WTU), 6 x 1903, *Stefani* s.n. (ZT), 14 x 1910, *Stefani* s.n. (P); Ajaccio, Parata, 1 x 1916, *Forsyth-Major* 292 (K); Cargese, x 1923, *Wyatt* 63 (K); Calvi, golfe de la Revellata, plage de l'Alga, 14 x 1979, *Lambinon* 79/1043, *Bellotte*, *Dellens & Monfort* (BC, MSB, RNG); Bonifacio, Tonnara-plage, 3 x 1993, *Dutartre* s.n. (B, M, MSB, RNG). ITALY. **Sardinia. Prov. Carbonia-Iglesias:** Portoscuso, 9 x 1912, *Bonomi* s.n. (BM). **Prov. Olbia-Tempio:** Santa Teresa Gallura, Tempio, Bancamino, 20 ix 1881, *Reverchon* 194 (B, HBG, JE, K, M, RO, ZT); Terrnanova (=Olbia), 2 x 1922, *Rikli* s.n. (ZT); Terranova, x 1922, *Schinz* s.n. (ZT); road between Olbia and San Teodoro, km 310, Le Vecchie Saline, close to lagoons (stagno), 6 x 2006, *von Mering* s.n. (MJG). **Prov. Sassari:** Sassari, ix 1895, *Nicotra* s.n. (RO), ix 1899, *Nicotra* s.n. (B, M); Isola di Reulino, 23 x 1988, *Bocchieri* s.n. (B). **Sicily. Prov. Agrigent:** Licata, x 1911, *Ross* s.n. (ZT). **Prov. Palermo:** Palermo, Mte. Pellegrino, ix 1855, *E. & A. Huet du Pavillon* s.n. (BM, P, ZT); Mondello, x 1879, *Lojacono* s.n. (K, P); Castelbuono, x 1885, *Lojacono* s.n. (P); Palermo, x 1885, *Todaro* s.n. (ZT), 1886, *Lojacono* s.n. (K), x 1892, *Todaro* s.n. (RO); Palermo, Aquasanta, 19 x 1897, *Fisch* s.n. (ZT); Palermo, x 1901, *Ross* 377 (B, BC, HBG, JE, M, ZT); Palermo, Mte. Pellegrino, 300 m, 25 x 1903, *Spencer* s.n. (M); Palermo, x 1903, *Ross* s.n. (HBG, M), x 1906, *Ross* s.n. (BC); Palermo, Mte. Pellegrino, 430 m, 30 x 1935, *Lusirne* s.n. (RO); Palermo, Mte. Pellegrino, 400 m, 7 ix 1964, *Davis* 40243 (RNG). **Puglia. Prov. Taranto:** Taranto, Leucaspide, 23 x 1905, *Lacaita* 4808 (BM), 2 xi 1909, *Lacaita* 11461 (BM, P). **Tuscany. Prov. Livorno:** Isola di Capraia, 7 i 1898, *Bèguinot* s.n. (RO). MALTA. Mtahleb, W of Rabat, near coastal cliffs (limestone), ca. 600m, 28 x 1974, *Westra & Rooden* 252 (Z); Mosta, Wied il-Ghasel, 22 xi 2007, *Mifsud* s.n. (MJG). SPAIN. **Prov. Cádiz:** Cádiz, Laguna de la Paja, Chiclana, 20m, 29 x 1925, *Gros* s.n. (M, RNG), ix 1929, *Font Quer* s.n. (RNG); entre Los Barrios y Casas del Castaño, Puerto de la Cebada, 1 xi 1967, *Galiano & Valdés* 128/67 (RNG); Cádiz, Laguna de la Janda, 3 xi 1978, *Galiano, Rivera & Valdés* 7097/78 (RNG). **Prov. Islas Baleares:** Menorca. Marina de Benisarmeña y San Antonio, ix/x sin anno, *Tremols* s.n. (BC); without locality, 13 x 1865, *Rodriguez* s.n. (P); near Mahón, 21 x 1872, *Rodriguez* s.n. (P); near Mahón, 21 x 1872, *Rodriguez* s.n. (P); S. Antonio, x 1872, *Rodriguez* s.n. (P).

North Africa. ALGERIA. Algiers, Pointe Pescade, ix (without year), *Jamin* 208 (P); La Calle (=El Kala), 17 xi 1840, *Durieu de Maisonneure* s.n. (P); Mostaganem, 14 ix 1848, *Barlanez* (partly illegible) s.n. (P); Algiers, Cap Caxine, x 1850, *Jamin* s.n. (P); Algiers, Cap Caxine, xii 1850, *Jamin* s.n. (P); Algiers, x 1851, *Jamin* s.n. (P); Bône (=Annaba), 1860, *Letourneux* s.n. (B, K, P); Annaba, Oued-Fourcha, 25 x 1865, *Tribout* s.n. (BM, P); Constantina, mountain Djebel Ouach, ca. 1100m, 10 xi 1868, *Paris* s.n. (BM, P); Algiers, 1869, *Durando* s.n. (P); Algiers, La Reghaia, xi 1880, *Battandier & Trabut* 986 (P, RO); Berrouaghia, road to Médéa, 1100 m, 6 x 1886, *Luizet* s.n. (P); Maison-Carrée, x 1886, *Luizet* s.n. (P); Annaba, Saint-Clous, 6 xi 1890, *Luitfroy* 337 (P); Bône, 29 x 1891, *Luitfroy* 370 (P); Bône, 1906/1907, *Gandoger* s.n. (BM). MOROCCO. Lukos, El Araix, 4 xii 1929, *Font Quer* s.n. (BM); Chaouia, Bouskoura, 190 m, x 1932, *Gattefossé* s.n. (M, P); 20 xii 1936, *Gattefossé* s.n. (B, JE, K, P); Tanger, between Cap Spartel and the Airport, 30 x 1993, 75 m, *García Murillo, Gibb & Talavera* ST 40/93 (RNG).

3. Triglochin bulbosa L., Mant. Pl.: 226 (1771). – Type: South Africa, Cape of Good Hope, (neo LINN 466.3 photo!, BOL photo!); Malmesbury Dist., at turn out to Gansekraal, 15 ix 1940, *Compton* 9451 (epi NBG!, designated here, Fig. 8). **Figs 6, 7.**

Triglochin bulbosa β *robustior* Roem. & Schult., Syst. Veg., ed. 15 bis [Roemer & Schultes], 7(2): 1585 (1830) (teste Kunth, Enum. Pl. (Kunth) 3: 144 (1841).

Triglochin patens Steud., Nomencl. Bot., ed. 2, 2: 709 (1841) nom illeg. = *T. bulbosa* L. B *robustior* (teste Kunth, Enum. Pl. (Kunth) 3: 144, 1841).

Plants 5-50 cm high, with fusiform *bulbs* and without rhizomes, bulbs covered by dry, brown or black, soft or stiff fibres, these 0.5-3.0 cm long. *Leaves* present when flowering, uniform, as long as or longer than plant height. *Inflorescences* lax or dense, with 4-50 flowers. *Flowers* 1.5-3.0 mm long. *Pedicels* elongated at fruiting time, 2-10 mm long, diverging at angles of 45° or 45°-90° from the infructescence axis, not or only slightly curved inwards towards the apex. *Infructescences* 2-20 cm long. *Fruits* narrowly to very narrowly ovoid, 4-10(-12) mm long, 0.5-2.0 mm wide (Fig. 6), turning dark or black at fruit maturity. *Mericarps* linear, connate at the *carpophore*; tips not or only slightly curved outwards.

Distribution. Endemic to South Africa, where it is restricted to the Western Cape Province (mainly on Cape Peninsula, West Coast and in Overberg) and the Hantam region of the Northern Cape Province (Fig. 7).

Habitat and ecology. Variable (see subspecies), ranging from temporary pools to dry (shaded) inland habitats, but never in habitats under direct influence of tidal flooding.

Phenology. Flowering in spring (vii-xi).

Chromosome number. Unknown.

Nomenclatural notes. The neotype (LINN 466.3, photo!) designated by Obermeyer (1966) is ambiguous. Most importantly, its fruits are not mature which makes identification difficult. Additionally no data on locality or collecting date are given. Thus, an additional epitype (*Compton* 9451, NBG; Fig. 8) was designated here which should be used in conjunction with the neotype.

Taxonomic remarks. We characterize *Triglochin bulbosa* as a bulbous species with narrowly to very narrowly ovoid fruits, the outline of seeds not visible through the pericarp, and pedicels spreading at an angle of 45-90° from the inflorescence axis. The fruits of *T. bulbosa* often turn dark or black at maturity unlike those of the other South African species of the complex. The species differs from *T. compacta*, which has a similar distribution area, in flowering time (spring vs. late summer/autumn) and the presence of leaves at flowering time (vs. leaf formation after flowering). Additionally, the fruits of *T. bulbosa* have no basal outgrowths and the outline of the seeds is not visible on the mericarp surface (as in *T. compacta*).

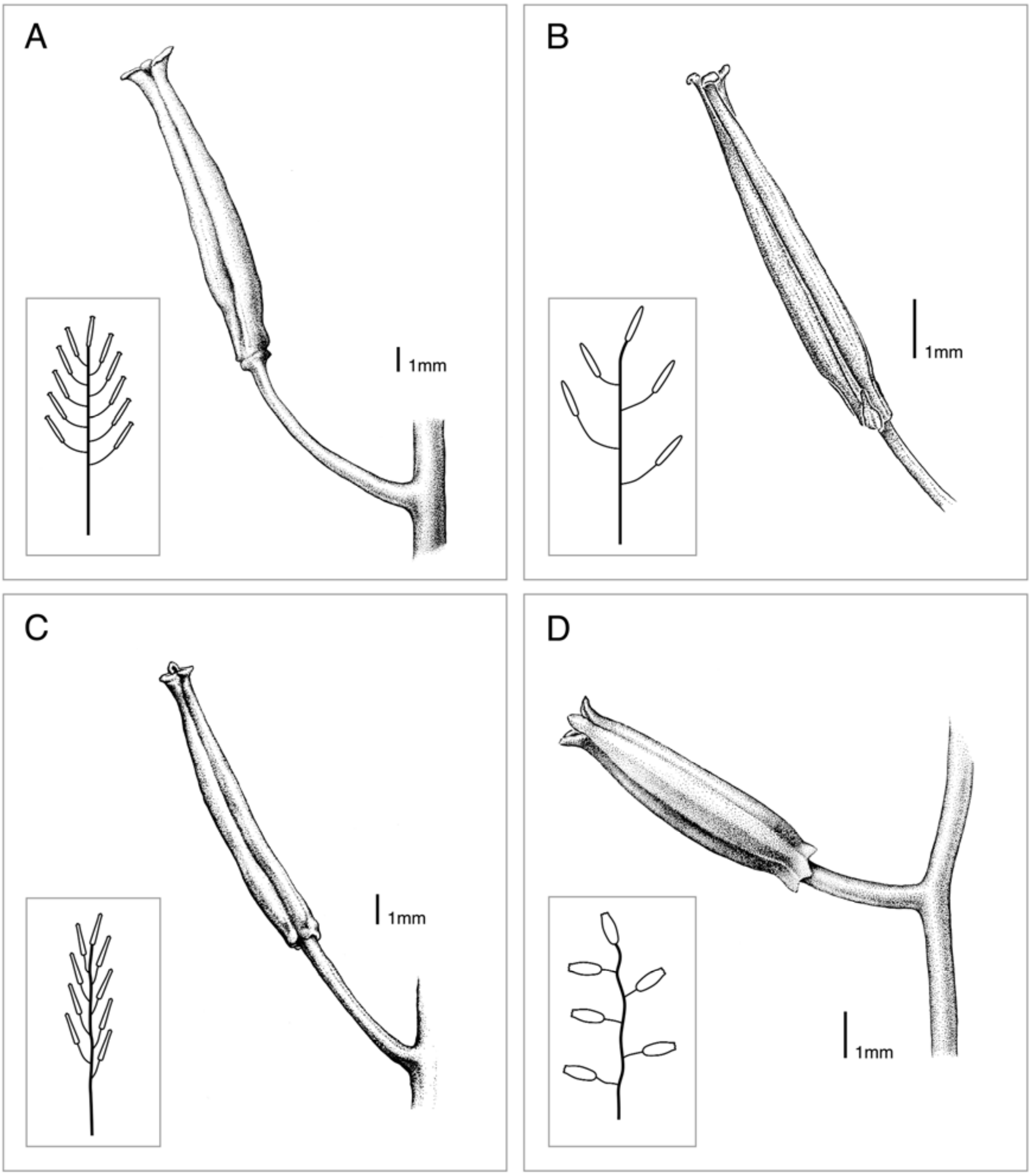

Fig. 4. Fruits and infructescence structure of *Triglochin bulbosa* L. A, *T. bulbosa* subsp. *bulbosa*; B, *T. bulbosa* subsp. *tenuifolia* (Adamson) Horn; C, *T. bulbosa* subsp. *Calcicola* Mering, Köcke & Kadereit subsp. nov.; D, *T. bulbosa* subsp. *quarcicola* Mering, Köcke & Kadereit subsp. nov. Drawings by L. Klöckner (Mainz).

Adamson (1939) newly described *Triglochin tenuifolia* on the basis of narrow leaves, few-flowered spikes, and its characteristic habitat. However, this species was later reduced to a subspecies of *Triglochin bulbosa* by Horn af Rantzien (1961). The newly circumscribed *Triglochin bulbosa* shows substantial variation in width of leaves as well as size and number of flowers and fruits. Several forms are geographically and ecologically well-differentiated, but transitions in morphological characters are found. Thus, we recognize four subspecies in *Triglochin bulbosa*: subsp. *bulbosa*, subsp. *tenuifolia*, subsp. *calcicola* and subsp. *quarcicola*.

Key to the subspecies

1a. Plants (8-)20-50 cm high, robust; leaves 1-3 mm wide; fruits 1-2 mm wide;
widely distributed in the Western Cape and southern regions of the Northern Cape
……………………………………………………………………………………………… **3A.** subsp. **bulbosa**
1b. Plants (5-)8-25(-35) cm high, delicate; leaves less than 1 mm wide; fruits less
than 1 mm wide; distributed in the Western Cape ……………………………………… **2**
2a. Inflorescences lax with 4-15(-25) flowers, pedicels diverging at 45-90° or 90°
from the inflorescence axis, plants growing on granite soils or in quartz fields
……………………………………………………………………………………………… **3**
2b. Inflorescences dense with (4-)10-30 flowers, pedicels diverging at 45°
from the inflorescence axis, plants growing on limestone or aeolinite in
coastal regions of the Western Cape
……………………………………………………………………………………………… **3C.** subsp. **calcicola**
3a. Pedicels diverging at angles of 45-90° from the inflorescence axis, plants
growing on granite soils on mountain slopes of the Cape Peninsula and
Langebaan Peninsula
……………………………………………………………………………………………… **3B.** subsp. **tenuifolia**
3b. Pedicels diverging at angles of mostly 90° from the inflorescence axis,
plants growing in quartz fields of the Knersvlakte
……………………………………………………………………………………………… **3D.** subsp. **quarcicola**

3A. subsp. **bulbosa Figs 6A, 7.**

Plants (8-)20-50 cm high, with fusiform *bulbs*, bulbs covered by dry, rather stiff, brown or black fibres, these up to 3 cm long, usually separate, sometimes fused into layers. *Leaves* as long as or longer than plant height, 1-3 mm wide. *Inflorescences* dense to lax, with (8-)15-50 flowers. *Flowers* 1.5-3.0 mm long, rarely longer. *Pedicels* 2-10 mm long, diverging at angles of 45° to 90° from the inflorescence axis, not or only slightly curved inwards towards the apex, but often fruits bent further upwards. *Infructescences* 6-20 cm long. *Fruits* narrowly ovoid, 5-10(-12) mm long, 1-2 mm wide (Fig. 6A). *Mericarp* connate at the *carpophore*; tips slightly curved outwards.

Distribution. Endemic to South Africa, restricted to the Western Cape Province and southern regions (Hantam) of the Northern Cape Province (Fig. 7).

Habitat and ecology. Found in temporary wetlands, such as edges of vernal pools, in ditches and in other depressions, mainly in vegetation types classified as Cape Vernal Pools and Cape Inland Saltpans, and rarely also Swartland Granite Renosterveld (Mucina & Rutherford, 2006). The habitats are characterised by heavy clayey to lighter clayey-sandy soils, derived from nutrient-rich substrates such as shale, granite or geologically young sandy sediments of marine origin covering coastal lowlands; here it is often found in clay-filled depressions among stabilised sandy dunes. Water-logging seems to be a major habitat-structuring factor. The taxon was found at altitudes of up to 500 m (Cederberg Mountains), but most localities are limited to altitudes below 100 m a.s.l.

Phenology. Flowering and fruiting in spring (viii-xi).

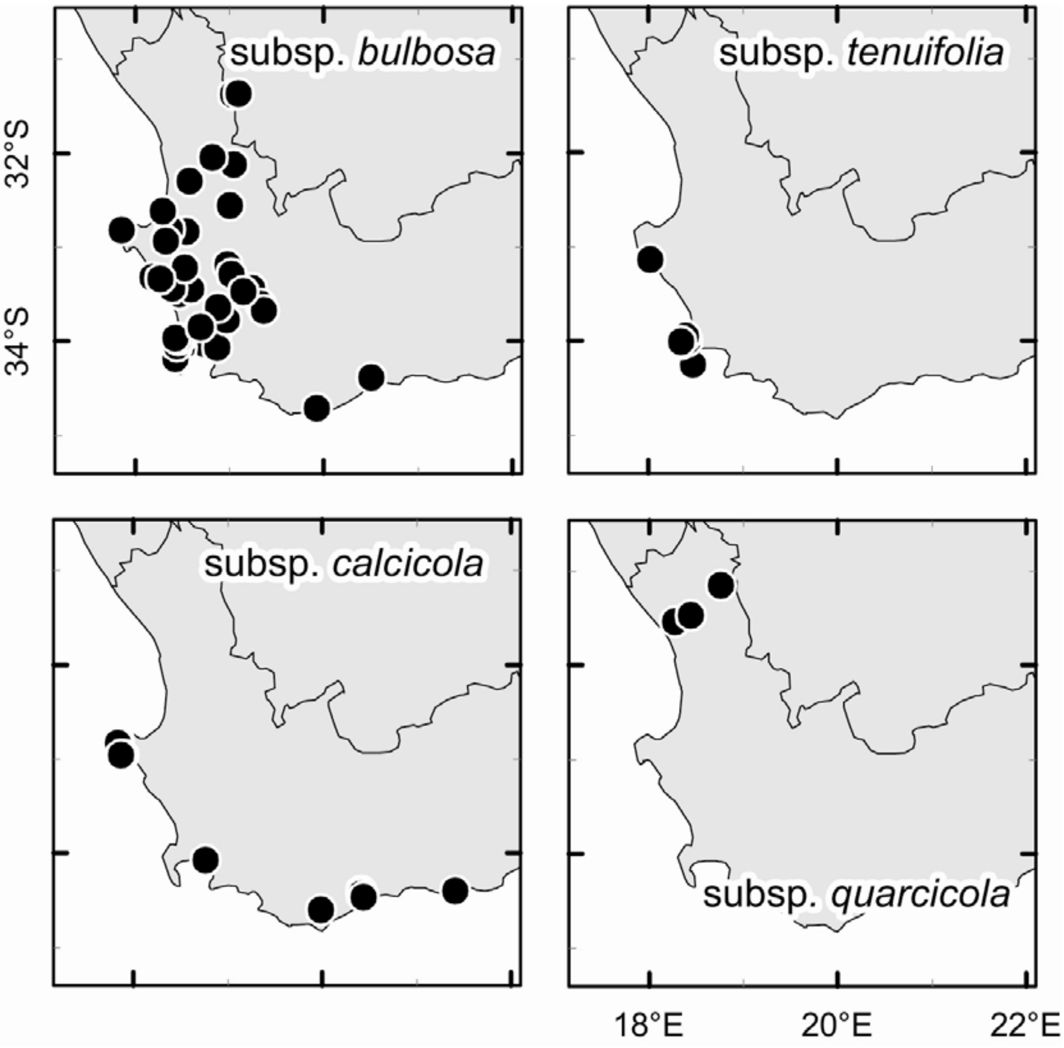

Fig. 5. Geographical distribution of *Triglochin bulbosa* L. subsp. *bulbosa*, *T. bulbosa* subsp. *tenuifolia* (Adamson) Horn, *T. bulbosa* subsp. *calcicola* Mering, Köcke & Kadereit subsp. nov., and *T. bulbosa* subsp. *quarcicola* Mering, Köcke & Kadereit subsp. nov.

Notes. One specimen (*Wörz* 04.10.06.01, PERTH *n.v.*, STU!) which is morphologically very similar to *Triglochin bulbosa* ssp. *bulbosa* was collected in 1994 in Australia (Western Australia, Swan-Valley near Perth), where the taxon has become established as a recent introduction (H. Aston, pers. comm.; see notes for *T. bulbosa* complex).

Taxonomic remarks. Subspecies *bulbosa* differs from the other subspecies in having wider leaves (1-3 vs. < 1 mm wide) and wider fruits (1-2 vs. < 1 mm wide). The plants are more robust and often larger than those of the other subspecies.

Proposed IUCN conservation status. Least concern (LC). The subspecies is widely distributed in the Western Cape Province, with some localities found in southern regions of the neighbouring Northern Cape Province. It does not appear to be immediately threatened. However, as seasonal pools (as all lowland wetlands in general) are becoming rare due to cultivation of land and construction work (Mucina & Rutherford, 2006), the conservation status should be re-assessed in the near future.

Specimens examined. SOUTH AFRICA. **Northern Cape.** Nieuwoudtville, Cloudskraal Farm, 7 xi 1962, *Barker* 9799 (NBG); Nieuwoudtville, 3 miles S of Nieuwoudtville, 7 ix 1963, *Nordenstam* 3032 (S). **Western Cape.** Stellenbosch, Hottentotts Holland, sine dato, *Zeyher & Ecklon* s.n. (P); Paarl, Klein Draakensteen, 10 ix 1827, *Drège* 8798 (P); Cape Town, Simon's Bay, Constantia, 1853-1856, *Wright* s.n. (NY); Cape Town, Raapenburg Vlei, viii 1882, *Guthrie* 1242 (BOL); Caledon, near village, vii 1892, *Guthrie* 2526 (NBG); Cape Town, Kenilworth, anno 1892, *Bolus* 7926 (BOL); Ceres, Mostertsberg, 1200 feet, x 1894, *MacOwan* 1991 (BM, P, NBG-SAM, Z); Cape Town, Kenilworth Race Course, 5 ix 1897, *Wolley-Dod* 2858 (NBG); Cape Town, near Maitland Cemetery, 2 x 1897, *Wolley Dod* 3221 (BOL); Cape Town, Vaarsche Vley, 17 x 1897, *Wolley Dod* 3139 (BOL); Cape Town, Simonstown, Constantia, Bergvliet Farm, 11 ix 1916, *Purcell* 66 (NBG-SAM); Cape Town, Simonstown, Constantia, Bergvliet Farm, E of lake, 11 ix 1917, *Purcell* 3 (NBG-SAM); Stellenbosch, behind Boys High School, 8 xi 1918, *Garside* 1139 (K); Worcester, Goudini Road, x 1921, *Michell* 2833 (BOL); Clanwilliam, Zeekoe Vlei, 1500 feet, ix 1925, *Levyns* 1192 (BOL); Cape Town, Somerset West, ix 1931, *Schmidt* 437 (M); Bredasdorp, between Bredasdorp and Elim, damp places near salt vlei, ix 1933, *Levyns* 4542 (BOL); Bredasdorp, The Poort, sides of temporary pool, 100 feet, ix 1933, *Levyns* 4460 (NBG); Stellenbosch, Faure, 9 ix 1934, *Garside* 4649 (K); Cape Town, Rondebosch Common, 15 vi 1936, *Adamson* 940 (BOL); Cape Town, Kenilworth Race Course, 6 x 1936, *Adamson* 1200 (BOL); Tulbagh, Saron Flats, viii 1937, *Compton & party* 1992/36 (BOL); Clanwilliam, Alpha, 28 vi 1938, *Martin* NBG 1258/37 (NBG); Olifantsrivier Valley, 11 miles N of Clanwilliam, 2 ix 1938, *Salter* 7529 (BOL); Napier, 24 viii 1940, *Bond* 470 (NBG); Darling, 15 ix 1940, *Esterhysen* 3864 (BOL); [Yzerfontein], at turn out to Gansekraal, 15 ix 1940, *Compton* 9451 (epi NBG); Piketberg, Berg River, 21 ix 1940, *Compton* 9474 (NBG); Clanwilliam, Pakhuis, 29 ix 1940, *Compton* 9553 (NBG); Clanwilliam, Pakhuis, x 1940, *Esterhuysen* 3170 (BOL); Cape Town, Hout Bay, 4 ix 1941, *Compton* 11294 (NBG); Malmesbury Dist., Mamre Hills, 7 ix 1941, *Compton* 11608 (NBG); Strand, 8 viii 1942, *Parker* 3708 (BOL, NBG); Cape Town, Milnerton, 31 viii 1942, *Compton* 13424 (NBG); Cape Town, Firgrove, 3 ix 1942, *Compton* 13458 (BOL); Malmesbury Dist., Mamre Hills, 12 ix 1942, *Barker* 1596 (NBG); Stellenbosch, Stellenbosch Vlakte, 8 iv 1943, *Jordaan* 18 (NBG); Malmesbury Dist., foot of Mamre Hills, 22 ix 1943, *Henderson* 1858 (NBG); Malmesbury Dist., Kalabas Kraal, 28 ix 1943, *Barker* 3546 (NBG); Piketberg, Papkuils Vlei, 30 ix 1943, *Leighton* 119 (BOL); Piketberg, Sout Kloof, between Sauer and Berg River, 1 x 1943, *Compton* 15122 (NBG); Piketberg, Zoutkloof, 1 x 1943, *Leighton* 397 (BOL); Bredasdorp, The Poort, 2 xi 1943, *Barker* 2491 (NBG); Cape Town, Melkbosch Road, swamp, 31 viii 1944, *Compton* 15865 (NBG); Stellenbosch, Faure, swamp, 16 xi 1944, *Compton* 15984 (NBG); Citrusdal, 1 ix 1945, *Leighton* 1389 (BOL); Ceres, Cold Bokkeveld, near Elandskloof, 9 ix 1945, *Leighton* 1270 (BOL); Ysterfontein [Yzerfontein], 12 ix 1945, *Compton* 17379 (NBG); Darling, ix 1945, *Stokoe* 59918 (NBG-SAM); Cape Town, Kirstenbosch, ix 1945, *Esterhuysen* 11856 (BOL, NBG); Citrusdal, Citrusdal Vlei, 1 xi 1945, *Compton* 17106 (NBG); Paarl, flats N of Paarl, 30 viii 1946, *Leighton* 1961 (BOL, NY); Cape Town, Kenilworth Race Course, ix 1946, *Lewis* 1900 (NBG-SAM); Piketberg, Berg Valley, ix 1947, *Lewis* 2380 (NBG-SAM); Clanwilliam, Nardouw Kloof, ix 1947, *Stokoe* 61194 (NBG-SAM); Cape Town, Kraaifontein, 4 x 1947, *Compton* 20091 (NBG); Stellenbosch, Golfplatz, 6 viii 1948, *Rehm* 1950 (M); Stellenbosch, Stellenbosch Vlakte, ix 1948, *Malherbe* s.n. (NBG); Cape Town, Claremont, 19 ix 1951, *Salter* 9302 (BM); Cape Town, between Fernwood and Liesbeek River, 24 ix 1951, *Salter* 9045 (BOL); Darling, Darling Flora Reserve, 25 vii 1956, *Rycroft* 1973 (NBG); Darling, Darling Flora Reserve, 4 x 1956, *Lewis* 5087 (NBG); Cape Town, Rondebosch Common, 15 x 1962, *Nordenstam* 1564 (S); Cape Town, Durbanville, Peaslake, 15 viii 1963, *Taylor* 5009 (NBG); Cape Town, Rondebosch Common, 7 ix 1963, *Stauffer* 5136 (NBG, P, Z); Darling, 16 ix 1963, *H. & E. Walter* s.n. (B); Stellenbosch, 3 miles from Simondium to Stellenbosch, 16 ix 1968, *Marsh* 653 (NBG-STE); Caledon, Happy Valley Farm, High Noon Estates, on road from Caledon to Frensch Hoek Pass, below Ezeljacht, 30 ix 1971, *Barker* 10834 (NBG); Worcester, Bokkekraal, NW side of Brandvlei Dam, 18 ix 1974, *Mauve & Oliver* 254 (K); Cape Town, Rondebosch Common, 21 ix 1974, *Nordenstam & Lundgren* 1973 (S); Betty's Bay, Groot Vleie, moist peaty area to south of vleis, 13 xi 1974, *Boucher* 2657 (NBG-STE); Darling, Platteklip, ix 1976, *Liebenberg* 8301 (K); Clanwilliam, outside Sandberg on road to Witels Kloof, 13 x 1976, *Hugo* 671 (NBG-STE); Cape Town, Isoetes Vlei, anno 1977, *Gubb* 50 (NBG); Malgas, De Hoop-Potberg Nat. Res., Potberg, flats near Melkbosheuwel, moist sandy flats, 16 ix 1979, *Burgers* 2252 (STE); Tulbagh, foothills of Witzenberg Mountains, 7 ix 1980, *Schonken* 314 (NBG-STE); Malmesbury, Klipfonein, 16 ix 1982, *van Zyl* 3247 (NBG); Cape Town, Rondebosch Common, 15 ix 1983, *Koutnik* 1262 (BOL); Worcester, Worcester Commonage, 26 viii 1985, *Bayer* 4837 (NBG); Cape Town, Somerset West, public open space at Westridge, 5 ix 1993, *Runnalls*

574 (NBG-STE); Cape Town, Kraaifontein, Scottsdene, between Scottsdene Library and Wolwefontein Rd., 29 ix 1997, *Cupido* 48 (NBG); Malgas, De Hoop Nat. Res., Potberg section, valley of Potbergsrivier, 3 iv 2006, *Köcke & Mucina* 030406/22 (MJG); Darling, Tienie Versveld Flower Reserve, at the entrance to the reserve, 14 iv 2006, *Köcke & Mucina* 140406/01 (MJG), Yzerfontein, E of Salt Pan, 14 iv 2006, *Köcke & Mucina* 140406/33 (MJG), 140406/34 (MJG); Stellenbosch, Klipheuwel, S of road crossing, 19 iv 2006, *Köcke & Steffen* 190406/02 (MJG); Velddrif, road Velddrif to Piketberg, Tasaars Kuil Farm 253, at turnoff to Aurora, 19 iv 2006, *Köcke & Steffen* 190406/27 (MJG); Wellington, road between Perderberg and Wellington, Boland Agricultural College, 30 vii 2006, *Mucina* 300706/21 (MJG); Darling, road between Malmesbury and Darling, Langekloof Farm, 4 viii 2006, *Mucina & Jakubowsky* 040806/02 (MJG); Darling, road between Malmesbury and Darling, near Waylands, 4 viii 2006, *Mucina & Jakubowsky* 040806/05 (MJG); Velddrif, road Velddrif to Piketberg, Tasaars Kuil Farm 253, at turnoff to Aurora, 4 viii 2006, *Mucina & Jakubowsky* 040806/28 (MJG); Elim, road Bosheuwel and Wiesdrif, 14 x 2006, *Mucina* 141006/20 (MJG); Paternoster, Tietiesbaai, coastal rocks at the entrance to Cape Columbine Nature Reserve, 25 x 2008, *Mucina* 251008/05 (MJG), 251008/06 (MJG); Darling, Tienie Versveld Flower Reserve, at the entrance to the reserve, 26 x 2008, *Mucina* 261008/04 (NBG).

3B. subsp. **tenuifolia** (Adamson) Horn, Svensk Bot. Tidskr. 55: 85 (1961). – *Triglochin tenuifolia* Adamson, J. S. Afr. Bot. 5: 30-31 (1939). – Type: South Africa, Table Mountain West side, Pipe Track, sheltered slopes, 5 ix 1936, *Adamson* 986 (holo *T. tenuifolia* BOL!). **Figs 6B, 7.**

Plants 8-25(-35) cm high, delicate, slender, with fusiform *bulbs*, bulbs covered by dry, rather stiff or soft, brown fibres, these < 1 cm long. *Leaves* as long as or much longer than plant height, very narrow, about 0.5-1 mm wide. *Inflorescences* lax, with 4-15 flowers. *Flowers* 1.5-2.5 mm long. *Pedicels* as long as or longer than fruit at fruiting time, 5-10 mm long, diverging at angles of 45° to 90° from the inflorescence axis, slightly curved inwards towards the apex. *Infructescences* 2-5 cm long. *Fruits* very narrowly ovoid, 5-8 mm long, 0.5-1.0 mm wide (Fig. 6B). *Mericarp* connate at the *carpophore*; tips slightly curved outwards.

Distribution. This subspecies is (as far as known) restricted to the lower slopes of Table Mountain on the Cape Peninsula and to the Postberg (near Langebaan, West Coast National Park) (Fig. 7).

Habitats and ecology. The subspecies is found in sheltered, shady inland habitats on mountain slopes at altitudes below 400 m. It is restricted to coarse, sandy soils derived from granites (Archaean Cape Granite Suite). The vegetation types supporting this taxon are classified as Cape Peninsula Granite Renosterveld and Saldanha Granite Strandveld (Mucina & Rutherford, 2006).

Phenology. Flowering and fruiting vii-ix, one specimen (with flowers and fruits) was collected in v.

Notes. The preference of this taxon for granite soils was already noted by Adamson (1939).

Taxonomic remarks. Subspecies *tenuifolia* differs from the other subspecies in having lax inflorescences with 4-15 flowers and pedicels diverging at angles of 45-90° from the inflorescence axis. This subspecies is restricted to granite soils on mountain slopes of the Cape Peninsula and Langebaan Peninsula.

Nomenclatural notes. At least one paratype of *Triglochin tenuifolia* (*Dümmer* 1050, SAM) cited by Adamson (1939) represents a misidentified specimen of *T. compacta*. Not all material cited by Adamson has been seen by us.

Proposed IUCN conservation status. Near Threatened (NT): The taxon meets the area requirements under criterion B for threatened (EOO<20,000 km^2 and/or AOO<2,000 km^2) and is declining, but the population is not severely fragmented, and the taxon occurs at 12 locations.
Judging from the available herbarium material, the subspecies has a very restricted distribution area. The extent of occurrence (EOO) is estimated to be only several square kilometres. It is known from parts of the Cape Peninsula and one locality at the Postberg (Langebaan Peninsula). All of the historical localities are today protected within the Table Mountain National Park and the private Postberg Nature Reserve (contractual portion of the West Coast National Park). No data are available on the current area of occupancy (AOO) of *Triglochin bulbosa* subsp. *tenuifolia* as all herbarium specimens seen are at least 60 years old, apart from one collection from Postberg dating from 1966. Several collecting sites are located within the borders of Cape Town and might have been destroyed long ago. Field work is needed to assess the current range and the conservation status of this taxon.

Specimens examined. SOUTH AFRICA. **Western Cape.** Cape Town, Stinkwater, anno 1875-1880, *Rehmann* 1194 (BM, Z); Cape Town, locality illegible, 14 vii 1883, *Wilms* 36451 (BM, Z); Cape Town, top ridge over Klassenbosch, 15 viii 1897, *Wolley Dod* 2918 (BOL); Cape Town, Lion's Head, lower west slopes, 29 viii 1897, *Wolley-Dod* 2915 (para BOL); Cape Town, Signal Hill, ix 1897, *Thode* 6089 (NBG-STE); Cape Town, Table Mountain, Pipe Track, above Camp Bay, viii 1920, *Michell* s.n. (BOL); Cape Town, Simonstown, Baviaans Kloof, 4 v 1927, *Salter* 344/10 (para BM); Cape Town, Hout Bay Nek, 23 viii 1928, *Hutchinson & Pillans* 104 (BOL); Cape Town, Kloof Nek, 27 vii 1929, *Scott* s.n. (para BM); Cape Town, Table Mountain W side, Pipe Track, 5 ix 1936, *Adamson* 986 (holo *T. tenuifolia*, BOL); Cape Town, Table Mountain, W side at 1000 feet, 11 viii 1940, *Adamson* 2899 (BM); Cape Town, Table Mountain, west slopes, 5 ix 1943, *Esterhuysen* 27104 (BOL); Cape Town, Table Mountain, Spring Buttress, ix 1945, *Stokoe* 59916 (NBG-SAM); Cape Town, Table Mountain, Llandudno end, 1000 feet, 19 x 1958, *Esterhuysen* 27923 (BOL); Langebaan, E slopes of Postberg, 9 ix 1966, *Barker* 10471 (NBG).

3C. subsp. **calcicola** Mering, Köcke & Kadereit **subsp. nov.** – Type: South Africa, Western Cape, De Hoop-Potberg Nature Reserve, Dronkvlei, “along track to Koppie Alleen, shallow sandy soils over limestone, low restiad [restioid] vegetation, frequent in damp places and hollows”, 11 ix 1979, *Burgers* 2172 (holo NBG-STE!; Fig. 9). **Figs 1A-B, 6C, 7.**

Differt a subspecie typica habitu minore et graciliore, foliis perangustis, infructescentia densa; solo in substrato calcareo.

Plants (5-)10-25 cm high, stiffly erect, with small, fusiform *bulbs*, bulbs covered by thin soft or thicker stiff, brown fibres, these ± 1 cm long, fibres sometimes not separate but fused into layers. *Leaves* as long as infructescence, very narrow, 0.5(-1.0) mm wide. *Inflorescences* dense, with (4-)10-30 flowers. *Flowers* 1.5-2.5 mm long, number of stamens sometimes reduced to three. *Pedicels* 2-4 mm long, diverging at angles of approximately 45° from the inflorescence axis, slightly curved inwards towards the apex. *Infructescences* 2-8 cm long. *Fruits* very narrowly ovoid, 4-7 mm long, rarely longer, mostly < 1 mm wide, very regularly spaced (Fig. 6C). *Mericarp* connate; tips short and slightly curved outwards. *Carpophore* only very thin and short or lacking.

Fig. 6. Holotype of *Triglochin bulbosa* subsp. *calcicola* Mering, Köcke & Kadereit subsp. nov. (NBG-STE).

Distribution. This taxon is limited to parts of the West Coast (Langebaan Peninsula), the coast of False Bay (Swartklip and Macassar) and to the South Coast (De Hoop and Stillbaai regions) (Fig. 7).

Habitat and ecology. Subspecies *calcicola* is a strict endemic of Tertiary calcareous bedrocks such as lime-rich aeolinites (False Bay) and limestones of the Bredasdorp Group (De Hoop and Stillbaai regions, Fig. 1B) and of the Sandveld Group (Langebaan and Saldanha regions). The sparse populations of this subspecies here occur in shallow loam-filled depressions (filled with fine loam and lime-rich sand) in limestone pavements; these depressions are wet in winter and spring and dry out completely in summer. The vegetation types housing this taxon are De Hoop Limestone Fynbos, Canca Limestone Fynbos, and Saldanha Limestone Strandveld (Mucina & Rutherford, 2006).

Phenology. Flowering and fruiting in spring from viii-xi.

Etymology. The epithet *calcicola* refers to the typical substrate of the species (hard lime-rich substrates such as limestones and aeolinites).

Notes. This subspecies is not well studied. Its delimitation from the broadly sympatric (all co-occuring in the West Coast region) subsp. *bulbosa* and subsp. *tenuifolia* requires further research.

Taxonomic remarks. Subspecies *calcicola* differs from the other subspecies in having dense inflorescences with (4-)10-30 flowers and pedicels diverging at angles of approximately 45° from the inflorescence axis. This subspecies is restricted to limestone or aeolinite in coastal regions of the Western Cape.

Proposed IUCN conservation status. Vulnerable (VU B2ab(iii)). This subspecies is ecologically specialised and occurs only on limestone substrates, which are generally rare in the Western Cape. Its populations on the South Coast (Overberg and Canca) and part of the False Bay coast do not experience direct threats because of current effective conservation (De Hoop Nat. Res., Pauline Bohnen Nat. Res., Wolvengat Nat. Res.). However, the populations on the West Coast (Langebaan and Saldanha) are found outside formally protected areas; limestone mining as well as development of coastal settlements may pose serious threats to these populations. Based on the limited area of occupancy (AOO, estimated to be less than 2000 km^2) and severely fragmented distribution area, plus the threat to the habitat (at least in some areas) this subspecies is here classified as Vulnerable.

Specimens examined. SOUTH AFRICA. **Western Cape.** Bredasdorp, The Poort, limestone ridge, in depressions in rock on top, 400 feet, ix 1933, *Levyns* 4491 (BOL); Strand, Macassar Downs, between St. Joseph's Tomb and sea, 2 ix 1934, *Garside* 4630 (B, K); Strand, 8 viii 1942, *Parker* 3707 (BOL, NBG); Bredasdorp, The Poort, 0.5 miles E of the road Bredasdorp-Elim, on rocks below limestone hill, 19 ix 1962, *Nordenstam* 1503 (S); Cape Town, Swartklip, 6 ix 1972, *Taylor* 8169 (NBG-STE); Stillbaai, ridge below reservoir, southerly aspect, gentle slope, limestone, 60 m, 24 viii 1979, *Bohnen* 6191 (NBG-STE); Malgas, De Hoop-Potberg Nat. Res., Dronkvlei, along track to Koppie Alleen, 11 ix 1979, *Burgers* 2172 (holo NBG-STE); Malgas, De Hoop, 4 km from Moerasfontein turnoff on Bredasdorp-Skipskop road, 8 viii 1984, *Fellingham 732* (NBG-STE); Malgas, De Hoop, Buffelsfontein, 9 viii 1984, *van Wyk 1773* (NBG-STE); Vredenburg, lower NW slopes of limestone koppie at Paternoster, 28 vii 1998, *Goldblatt & Manning* 10928 (MO, NBG); Malgas, De Hoop Nat. Res., plains below the slope at road from entrance of reserve to the information center, limestone pavement, 28 ix 2008, *Mucina & Meruňková* 280908/04 (MJG); Malgas, De Hoop Nat. Res., 28 ix 2008, *Meruňková* KM03/27 (NBG), 14 x 2008 KM27/24 (NBG), KM28/51 (NBG); Langebaan Peninsula, Jacobsbaai, 26 x 2008, *Meruňková* KM44/44 (NBG), KM45/41 (NBG), 30 x 2008, *Meruňková* KM52/28 (NBG), KM53/33 (NBG); Langebaan Peninsula, Paternoster, Cape Columbine, 29 x 2008, *Meruňková* KM50/34 (NBG), KM51/44 (NBG).

3D. subsp. **quarcicola** Mering, Köcke & Kadereit **subsp. nov.** – Type: South Africa, Western Cape, Vanrhynsdorp Div., farm Moedverloren, Knersvlakte Conservation Area, on quartz fields, BIOTA Biodiversity Observatory No 28 (Moedverloren), ha. 78, 31°28' 3.7"S, 018° 26' 52.8"E, 1 ix 2008, *Schmiedel* 124811 (holo MJG; iso NBG; Fig. 10). **Figs 6D, 7.**

Differt a subspecie typica habitu minore et graciliore, foliis perangustis, pedicellis divergens sub angulo circa 90°; solo in substrato quarcitico.

Plants 5-20 cm high, delicate, slender, with small, fusiform *bulbs*, bulbs covered by dry, soft or stiff brown fibres, these 1-2 cm long. Plants without rhizomes. *Leaves* uniform, as long as or shorter than infructescence, very narrow, about 0.5-1 mm wide. *Inflorescences* lax, with 4-15(-25) flowers. *Flowers* (1.5-)2.0-3.0 mm long. *Pedicels* shorter than fruit at fruiting time, 2-5 mm long, held at angles of almost 90° from the inflorescence axis, usually not curved inwards towards the apex. *Infructescences* lax, axis sometimes flexuose. *Infructescences* 3-8 cm long. *Fruits* very narrowly ovoid (to almost ellipsoid), 5-8 mm long, 0.5-1.0 mm wide (Fig. 6D). *Mericarps* linear, sometimes with short basal spurs, connate; tips not curved outwards. *Carpophore* very thin or lacking.

Distribution. *Triglochin bulbosa* subsp. *quarcicola* is only found in the Western Cape Province of South Africa and appears to be endemic to the Knersvlakte (a major centre of plant endemism of the country; van Wyk & Smith 2001; Fig. 7), a slightly undulating, low-lying basin in southern Namaqualand (near the town of Vanrhynsdorp). Subspecies *quarcicola* is the only subspecies of *Triglochin bulbosa* found outside the Fynbos Biome. The farm Moedverloren is known to harbour a high number of very restricted local endemics (U. Schmiedel, pers. comm.; B. Nordenstam, pers. comm.).

Ecology and habitats. The subspecies is restricted to saline quartz fields (Fig. 1C), especially the dry slopes of koppies, covered with a layer of white, angular quartz gravel (Schmiedel & Jürgens 1999). The vegetation housing populations of subsp. *quarcicola* is a dwarf succulent shrubland, rich in succulent shrubs and “stone plants” (Knersvlakte Quartz Vygieveld according to Mucina & Rutherford, 2006). The climate of the Knersvlakte is typical for winter-rainfall semi-desert, with mean annual precipitation of around 120 mm and mean annual temperature of about 18° C (Mucina & Rutherford, 2006).

Phenology. Flowering and fruiting in early spring (vii-viii). The subspecies seems to grow only following sufficient rainfall (U. Schmiedel, pers. comm.). According to notes on one specimen (*Le Roux* 2205, STE), the leaves are deciduous.

Notes. According to notes on two specimens, flowers are brownish-purple or purple (*Nordenstam* 587, S; *Le Roux* 2205, STE).

Taxonomic remarks. Subspecies *quarcicola* differs from the other subspecies in having lax inflorescences with 4-15(-25) flowers and pedicels diverging at angles of mostly 90° from the inflorescence axis. This subspecies is restricted to quartz fields of the Knersvlakte.

Proposed IUCN conservation status. Vulnerable (VU D2). *Triglochin bulbosa* subsp. *quarcicola* shows a very restricted area of occupancy (AOO, estimated to be less than 100 km^2) and is currently only

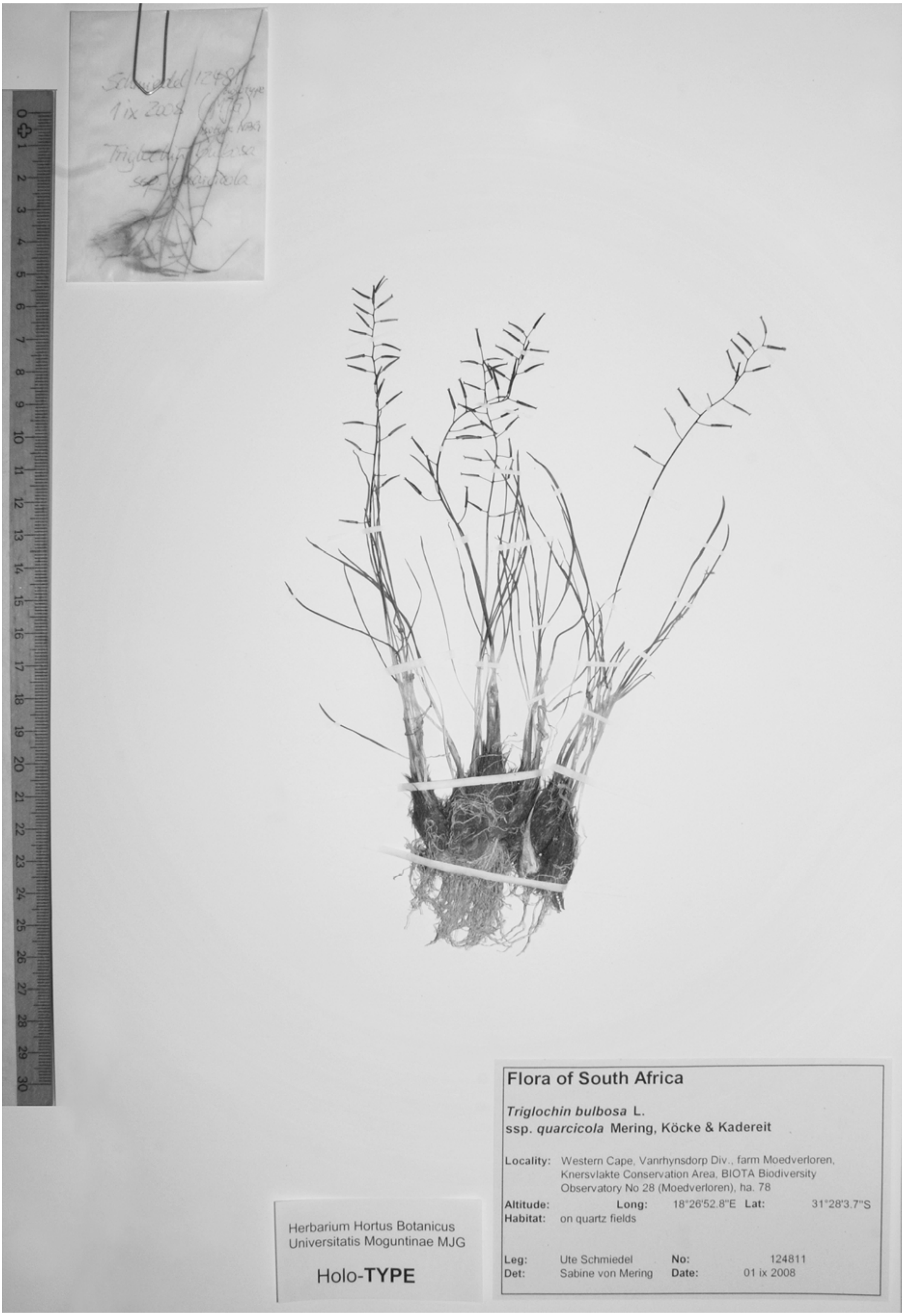

Fig. 7. Holotype of *Triglochin bulbosa* subsp. *quarcicola* Mering, Köcke & Kadereit subsp. nov. (MJG).

known from three locations (Fig. 7), where it was found only in small populations (B. Nordenstam, pers. comm.; U. Schmiedel, pers. comm.; see also a remark on the label of one of the specimen (*Le Roux* 2205, STE).

The farm Moedverloren (or Moedverloor on some maps) is currently managed by CapeNature, the provincial nature conservation authority, as part of the "Knersvlakte Protected Area" (U. Schmiedel, pers. comm.). Some of the populations are found, however, outside the borders of formally protected areas, and the survival of this taxon depends on the owners of these neighbouring farms. Further field studies are required to estimate more precisely the area of occupancy and extent of occurrence.

Specimens examined. SOUTH AFRICA. **Western Cape.** Knersvlakte, Koekenaap, 15 vii 1962, *Nordenstam* 587 (S); 20 vii 1962, *Hall* 2397 (NBG-SAM); Knersvlakte, Vanrhynsdorp, Moedverloor ["Moedverloer" in Hall 4124], 28 vii 1962, *Nordenstam* 790 (S); 10 viii 1962, *Nordenstam* 898 (S); 23 viii 1971, *Hall* 4124 (NBG); 5 viii 1981, *Hall* 5078 (NBG); Knersvlakte, Lutzville, 7 km N of Hol River railway station on the farm Klipdrift, 10 viii 1977, *Le Roux* 2205 (NBG-STE); Knersvlakte, Vanrhynsdorp, farm Moedverloren, Knersvlakte Conservation Area (BIOTA Biodiversity Observatory No 28), quartz fields, 1 ix 2008, *Schmiedel* 124811 (holo MJG, iso NBG).

4. Triglochin compacta Adamson, J. S. African Bot. 9: 152 (1943). – Type: South Africa, Karbonkelberg, 1000 ft, 28 ii 1943, *Compton* 14436 (holo NBG!). **Figs 1E-G, 2C, 11.**

Plants (10-)15-50 cm high, with fusiform *bulbs* and without rhizomes; bulbs covered by dry, rather soft, brown fibres, these up to 5 cm long. *Leaves* few, formed after flowering, mostly reaching only half of the height of plants. Outer leaves strap-shaped, shorter and wider than inner leaves, up to 4 cm long, mucronate, inner leaves abruptly narrowing above the ligule, less than 0.5 mm wide, distinctly longer than outer leaves, leaves generally larger and more numerous after fruit maturity. *Inflorescences* dense, with (6-)10-50 flowers. *Flowers* 1.5-3.0 mm long. *Pedicels* elongated at fruiting time, up to 2-7 mm long, mostly curved inwards towards the apex. *Infructescences* 5-20 cm long. *Fruits* narrowly ovoid, 6-10 mm long, 1-2 mm wide (Fig.1G, 2C). *Mericarps* dorsally curved upwards, connate at the *carpophore*; tips curved outwards. *Carpophore* often with three basal membranous outgrowths.

Distribution. Endemic to South Africa: restricted mainly to the Western Cape Province where it occurs in the Cape Peninsula as far east as Goukamma on the Garden Route and in Seweweekspoort in Klein Swartberg Mountains, the Cederberg and in a handful of localities in the Northern Cape Province in the surroundings of Nieuwoudtville and near Kommagas (Namaqualand) (Fig. 11). The Kommagas collection is an unsual outlier and requires further attention. Unfortunately newly dated material from this locality was not available.

Habitat and ecology. *Triglochin compacta* is invariably found on deep (mainly nutrient-poor) sands. When of marine orgin, these sands form stabilised (and highly leached) inland sand dunes, always outside the current direct influence of the sea. In some places at higher altitudes these deep sandy sediments are derived *in situ* from arenites such as Table Mountain and Nardouw sandstones. The vegetation types characteristically supporting this taxon are Hangklip Sand Fynbos, Cape Flats Sand Fynbos, Albertinia Sand Fynbos, Knysna Sand Fynbos, Cederberg Sandstone Fynbos and Bokkveled Sandstone Fynbos (*sensu* Mucina & Rutherford, 2006).

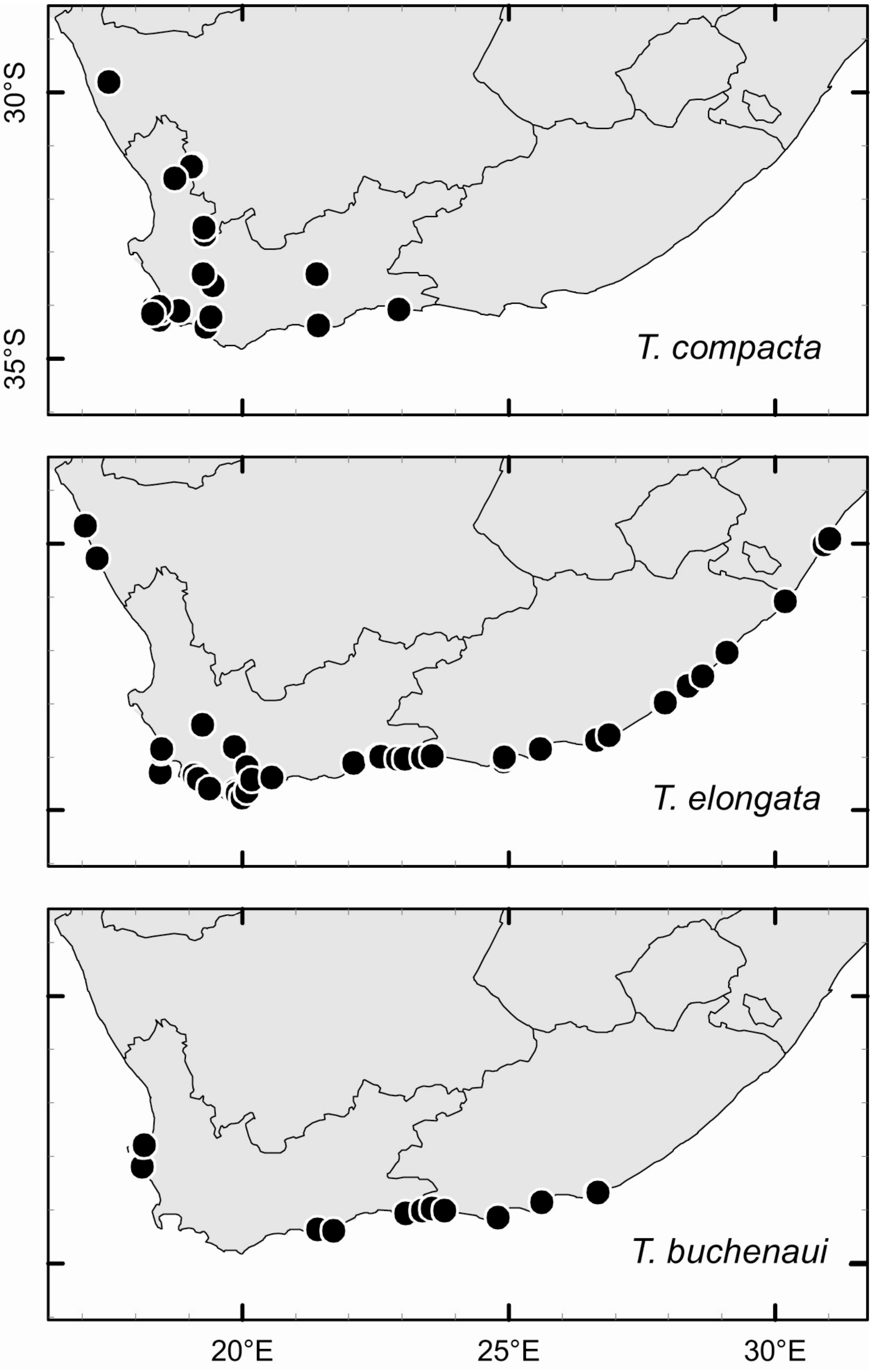

Fig. 8. Geographical distribution of *Triglochin compacta* Adamson, *T. elongata* Buchenau, and *T. buchenaui* Köcke, Mering & Kadereit sp. nov.

Phenology. Flowering mostly in autumn from (ii)iii-v, some fruiting material was collected in vi.

Chromosome number. Unknown.

Taxonomic remarks. Ever since its description by Adamson (1943), this species has been incorrectly classified as being synonymous with *Triglochin bulbosa*. However, the two species can be easily distinguished by phenology as well as morphology (see *T. bulbosa* for a comparison between these two sympatric species). The affinities of *Triglochin compacta* to *T. milnei* will be discussed under the latter species.

Proposed IUCN conservation status. Data Deficient (DD). The low number of collections of this taxon can be a result of poor sampling (failure to distinguish this species in the field from the much more common *Triglochin bulbosa* subsp. *bulbosa*), habitat loss, natural rarity, or a combination of these factors. Some of the sandveld areas (system of lowland stabilised dune-fields) have been suffering from pressure of urban sprawl through both the spread of formal housing development and the explosive spread of informal settlements on the Cape Flats (now part of the Cape Town metropolis). The conservation status of this taxon can only be judged once an intensive search for extant localities has been conducted. We therefore suggest classifying *Triglochin compacta* as Data Deficient.

Specimens examined. SOUTH AFRICA. **Northern Cape.** Niewoudtville, sine dato, *Leipoldt* 808 (BOL); Niewoudtville, 19 ii 1932, *Maughan-Brown* s.n. (BOL); [Namaqualand], Komaggas ("Bethel, Kammaggas"), 24 iii 1952, *van Niekerk* 3833 (BOL); Nieuwoudtville, Farm Arendskraal, 30 iii 1982, *Snijman* 597 (NBG). **Western Cape.** Cape of Good Hope, anno ca. 1770, *Auge* or *Oldenburg* s.n. (BM); iii or iv 1771, *Banks & Solander* s.n. (BM); Cape Town, Wynberg, 23 iii 1892, *Schlechter* 607 (Z); Hermanus, Vogelgat, 11 iv 1897, *Schlechter* 10424 (BM, PRE, Z); Cape Town, Muizenberg, iii 1908, *Dümmer* 1050 (NBG-SAM); Caledon, 21 ix 1916, *Purcell* 104 (NBG-SAM); Cape Town, Constantia, Bergvliet Farm, Peter's Hill, 26 iii 1917, *Purcell* 12 (NBG-SAM); Cape Town, Ladies Mile Hill, 12 iv 1918, *Purcell* 65 (NBG-SAM); Knysna, Goukamma, West Hill, iv 1928, *Fourcade* 3925 (BOL, NBG-STE); Strand, on sand dunes near the sea, 22 vi 1940, *Parker* 3515 (BOL, NBG); Cape Town, Slangkop, 9 v 1942, *Bond* 1498 (NBG); Cape Town, Karbonkelberg, 25 ii 1943, *Isaac* 10 (BOL); 28 ii 1943, *Compton* 14436 (holo NBG); 4 iii 1943, *Leighton* 934 (NBG); 30 iv 1944, *Compton* 15657 (NBG); 2 iv 1945, *Levyns* s.n. (*Adamson* 3615) (BOL); Cape Peninsula, Buffels Bay, 27 v 1945, *Compton* 17061 (NBG); Cape Town, slopes above Rheboksdam Bay, 27 v 1945, *Leighton* 970 (BOL); Cederberg, Sandfontein Peak, 5 iv 1947, *Esterhuysen* 13870 (BOL); Cape Town, hillside above Kommetjie, vi 1947, *Lewis* 2379 (NBG-SAM); Clanwilliam, Krom River, 1 iv 1956, *Esterhuysen* 25489 (BOL); Cape Town, Kalk Bay Mountain, slope below Boyes Drive, 12 v 1974, *Goldblatt* 1792 (NBG); Stillbaai, Panorama Circle, 10 iii 1979, *Bohnen* 5112 (NBG, PRE); Stellenbosch, Bo-Onderpapagaaiberg, 20 v 1988, *Becker* 0001 (NBG); Sedgefield, Buffelsbaai, Goukamma River estuary, camping site, 6 v 2006, *Köcke & Mucina* 060506/02 (MJG); Stillbaai, Pauline Bohnen's ex-garden on Panoramasingel, 18 iii 2008, *Naudé et al.* s.n. (NBG).

5. Triglochin milnei Horn, Svensk Bot. Tidskr. 55: 85 (1961). – Type: ZAMBIA, Mwinilunga Dist., ½ mile S of Matonchi farm, ca. 1350 m, 30 x 1937, *Milne-Redhead* 3012 (holo K photo!, BOL photo!; iso S photo!). **Figs 2D, 12.**

Plants (15-)20-60 cm high, with *bulbs*, bulbs covered by dry, rather soft, brown fibres, these up to 5 cm long. Plants without rhizomes. *Leaves* few at flowering time, much shorter than, and mostly reaching only half of the height of plants, becoming larger and numerous after fruit maturity. Outer leaves strap-shaped, shorter and wider than inner leaves, up to 4 cm long, with mucronate tips, inner leaves abruptly narrowing above the ligule, >1-2 mm wide, distinctly longer than outer leaves.

Inflorescences lax, with 5-30 flowers. *Flowers* 2-3 mm long, usually > 3.0 mm long. *Pedicels* elongated at fruit maturity, then 3-15 mm long, diverging at angles of 45° and curved inwards towards the apex. *Infructescences* 6-14 cm long. *Fruits* narrowly ovoid to ovoid, (8-)10-14 mm long, ± 3 mm wide (Fig. 2D). *Mericarps* connate at the *carpophore*. *Carpophore* with three basal membranous outgrowths.

Distribution. Angola, Democratic Republic of Congo (DRC), Tanzania, Zambia, Zimbabwe; in South Africa in KwaZulu-Natal, southern Mpumalanga and Zuurberg, Griqualand East, Eastern Cape (Fig. 12). Usually found at altitudes above (500-)1000 m, in Tanzania at up to 1900 m. According to Govaerts (2008), *Triglochin milnei* is also found in Malawi, but no herbarium material from there has been seen.

Habitat and ecology. *Triglochin milnei* is found in seasonal (summer-wet) wetlands and seasonally wet, sometimes burnt, grasslands, often on grey or black clayey soils. At least some of these grasslands of the mid- and high-altitudes in Tanzania and Zimbabwe (and possibly also in Malawi) would qualify as “afromontane”. At low altitudes in the DRC and Zambia this taxon is found in so-called dambos - seasonally waterlogged, predominantly grass-covered depressions bordering headwater drainage lines (Mackel, 1985). On the Mpumalanga Highveld and in the KwaZulu-Natal Midlands (South Africa), this taxon appears to be limited to azonal wetlands embedded within the Grassland Biome.

Phenology. Flowering and fruiting from x-ii(iii).

Chromosome number. Unknown.

Nomenclatural notes. The holotype (*Milne-Redhead* 3012, K) does not have fruits and should be used together with fruiting material.

Taxonomic remarks. Triglochin milnei is morphologically similar to *T. compacta* by having outgrowths at the base of the carpophore and narrowly ovoid to ovoid fruits, in which the outline of the seeds is often visible on the mericarp surface. However, the fruits differ in width (±3 mm vs. 1-2 mm) and in most cases in length ((8)10-14 mm vs. 6-10 mm). Furthermore, the two species are geographically, ecologically and phenologically clearly differentiated.

Most floras covering the distribution area of the species do not recognize *Triglochin milnei*, but treat it as a synonym of *T. bulbosa* (e.g., Bennett, 1902; Obermeyer, 1966; Lisowski *et al.*, 1982). Napper (1971) accepted *Triglochin milnei* (p. 3: "The circumscription of *T. bulbosa* in F.S.A. 1: 93 (1966), which includes *T. milnei*, is less satisfactory.") but doubted the sole record of this species in South Africa. However, the study of extensive *Triglochin* material from South Africa and comparison with material of *T. milnei* from other parts of Africa revealed that the species occurs in eastern South Africa. Nevertheless, only limited material is available for South Africa and more collecting is needed.

Proposed IUCN conservation status. Data Deficient (DD). *Triglochin milnei* seems to be widely distributed in Central and southeastern Africa and is probably not under immediate threat. However, little is known about the current area of occupancy. The species is underrepresented in herbarium collections and few other data are available. In economically more developed African countries, such as South Africa, subtropical and warm-temperate grasslands are under pressure from intensive stock grazing associated with frequent (often too frequent) burning. Sparse and vulnerable wetlands within

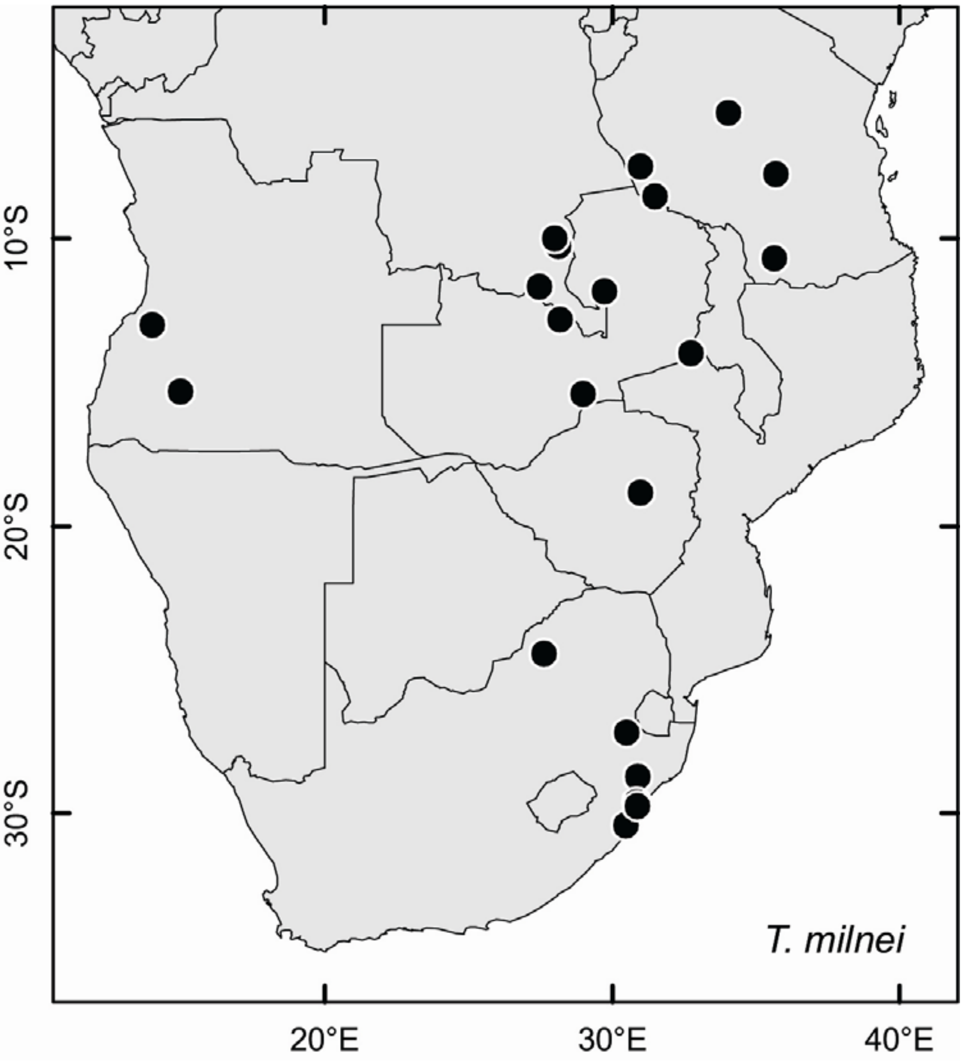

Fig. 9. Geographical distribution of *Triglochin milnei* Horn.

these grasslands also suffer from over-utilization by high grazing stock concentrations. Threats and current conservation status at the national level are likely to differ among countries, and therefore differential assessments are needed. In South Africa the species is only known from six, mostly very old, collections.

Specimens examined. ANGOLA. Dist. Huilla, Alto plana, ad ripas Rivi de Lopollo, prope Ohai (Háy), 5000 feet, xi/xii 1859, *Welwitsch* 3017 (para BM, P); Benguela [formerly Benguella], country of the Ganguellas and Ambuellas, anno 1910, *Gossweiler* s.n. (para K). DEMOCRATIC REPUBLIC OF CONGO. 1.5 km E of Kabiasha, Katanga, 1020 m, 7 xii 1967, *Malaisse* 6189 (POZG); Haut-Shaba, close to Lubumbashi (formerly Elisabethville), 8 km W of Kasapa University, 25 xii 1968, *Lisowski* 84458 (POZG); Kinshasa, Haut-Katanga, close to Lubumbashi, on the fringes of Natwebo, 1220 m, 22 x 1969, *Lisowski* 731 & 732 (POZG), 27 xi 1970, *Lisowski* 733 (POZG); Plateau de Kundelungu, Katanga, bank of Kalundariver, 1590 m, 9 i 1971, *Lisowski*, *Malaisse & Symoens* 12645 & 12698 (POZG); Plateau des Kundelungu, 3 km N of source of river Lutshipuka, 9 i 1971, *Lisowski, Malaisse & Symoens* 12644 (BOL).TANZANIA. Uyansi, Lake Chaya, ca. 1240 m, 4 i 1926, *Peter* 45784 (B); Sumbawanga, 6200 feet, 30 i 1950, *Bullock* 2364 (para K, S photo!); 12 km E of Songea by Nonganonga stream, 1050 m, 28 xii 1955, *Milne-Redhead & Taylor* 7934 (para B, EA, P, S photo!); Iringa Dist., 4 miles N of Iringa, Great North Road, 5150 feet, 5 ii 1962, *Polhill & Paulo* 1362 (B, EA, P); 7 miles from Iringa on Dabaga road, 26 xii 1965, *Harris* 10256

(EA); Sumbawanga, Tatanda Mission, 1700 m, *Bidgood, Mbago & Vollesen* 2409 (K); Nkansi Distr., 5 km on Namanyere-Karonga road, 1500 m, 4 iii 1994, *Bidgood, Mbago & Vollesen* 2603 (P). SOUTH AFRICA. **KwaZulu-Natal.** Inanda, i 1800, *Medley Wood* 997 (BM); Griqualand East, Zuurberg Mountains, x 1883, *Tyson* 1866 (BOL); Alexandra Dist., Station Dumisa, Farm Friedenau, Umgaye, 600 m, 15 x 1908, *Rudatis* 440 (para BM, P); Krantzkop ("Kranskop"), 4500 feet, xii 1910, *Thode* 3870 (NBG-STE); Krantzkloof, Gillitto Kloof, 1800 feet, x 1921, *Haygarth* s.n. (STE 260, 261). **Mpumalanga.** Wakkerstroom Dist., N of Dirkiesdorp, SE of main road, on the farm Roodekraal, 1340 m, 13 xii 1995, *Balkwill* 9389 (PRE). ZAMBIA. Mwinilunga Dist., ½ mile S of Matonchi farm, 16 xii 1937, *Milne-Redhead* 3693 (para S photo!); 5 miles E of Lusaka, 4200 feet, 10 i 1958, *King* 403 (para K); Chadiza, 850 m, 25 xi 1958, *Robson* 686 (BM); Lusaka, 4 xii 1964, *Robinson* 6266 (M); Kitwe, 9 ii 1969, *Fanshawe* 10515 (K). ZIMBABWE. Charter Dist., Charter, 4000 feet, 27 xii 1926, *Eyles* 4587 (para K).

6. Triglochin elongata Buchenau, in A. Engler, Das Pflanzenreich 4. 14: 10, fig. 3 (1903). –

Type: South Africa, Div. Malmesbury, Umgegend von Hopefield, *Bachmann* 1662, 1693 (syn B†); South Africa, Pondoland, *Bachmann* 309 (syn B†); South Africa, Div. Malmesbury, “Umgegend von Hopefield, Weg nach Coeuratenberg”, xi 1886, *Bachmann* 1693 (lecto., designated here, Z!; Fig. 13); drawing of fruit and habit (from *Bachmann* 1693, B) in Buchenau (1903), fig. 3, p. 11 (epi, designated here; Fig. 14). **Figs 1H, 1L, 2E, 11.**

Plants 15-90 cm high, with *rhizomes* (Fig. 1H). Rhizomes whitish to beige with bracts. *Leaves* uniform, thickened at the base but not forming a bulb, as long as or longer than plant height, 1-2 mm wide. Leaf bases covered by soft, whitish to brown fibres, fibres mostly 3-6 cm, rarely up to 15 cm long. *Inflorescences* dense, with 10-100 flowers. *Flowers* 1.5-2.5 mm long. *Pedicels* elongated at fruiting time, then 2-6 mm long. *Infructescences* (6)10-25 cm long. *Fruits* narrowly ovoid to ovoid, often secund, 5-9 mm long and 1.0-2.5 mm wide (Fig. 2E). *Mericarps* connate at the *carpophore*; tips mostly curved outwards.

Distribution. Endemic to South Africa: distributed mainly in coastal regions of the KwaZulu-Natal, Eastern, Western and Northern Cape Provinces (Fig. 11). In the latter Province there are two outlying localities – always located near the mouths of intermittent rivers draining the coastal plains of Namaqualand. We have failed to detect any morphological uniqueness of these Namaqualand populations. They are exceptional in terms of their geographical distribution and deserve further study. Many isolated inland localities are presumably of relictual character as they are found abundantly in regions frequently flooded by the sea during the altitermal marine transgressions (Overberg, parts of West Coast).

Habitat and ecology. *Triglochin elongata* commonly grows in the upper tidal and supratidal zones of estuarine salt marshes (Fig. 1L), usually on heavy clayey (partly also clayey-sandy) saline soils. In coastal habitats it is found on elevated banks of sandy beaches, edges of estuarine rivers as well as on rocks exposed to salt spray. Along some estuarine rivers it penetrates deeply inland (Bushmans River in the Eastern Cape), and in some regions (West Coast, Breede River, Overberg) it is found in (obviously relictual) inland localities, on banks of intermitted rivers (in Namaqualand) and on the edges of saline and brackish temporary pans, here often intermingled with dense *Juncus* stands. According to Mucina & Rutherford (2006) these habitats belong to the following vegetation units: Arid Estuarine Salt Marshes, Cape Estuarine Salt Marshes, Cape Inland Saltpans, Cape Lowland Freshwater Wetlands and Cape Seashore Vegetation.

Phenology. Flowering throughout the year as long as water is available.

Chromosome number. Unknown.

Nomenclatural notes. In the protologue of *Triglochin elongata*, Buchenau (1903) cites three specimens which probably were destroyed in B during World War II. A duplicate of one of these syntypes was discovered (*Bachmann* 1693, Z; Fig. 13) and is designated as lectotype here. This specimen, however, has only flowers but no fruits. The fruit (and habit) of one of the original syntypes (*Bachmann* 1693, B) is illustrated in Buchenau (1903, fig. 3, p. 11; Fig. 14). This drawing should be used as an epitype (designated here) in conjunction with the lectotype.

Taxonomic remarks. *Triglochin elongata* is similar to *T. buchenaui* in having rhizomes instead of bulbs, but differs in having dense inflorescences with 10-100 flowers (vs. lax inflorescences with 3-17 flowers), (6)10-25 cm long infructescences (vs. 2-7 cm long), and fruits with a carpophore. Unlike *T. buchenaui*, which is restricted to the lower tidal zone of salt marsh estuaries, *T. elongata* is ecologically more variable and grows in the upper zone of salt marsh estuaries and estuarine rivers.

Variation. Several exceptionally large individuals (up to 90 cm) with long infructescences and numerous fruits have been seen.

Proposed IUCN conservation status. Least Concern (LC). *Triglochin elongata* is a widespread species recorded from many riversides near the sea and almost all major estuaries in South Africa (Fig. 11). However, some of these estuaries are under serious pressure by silt deposition or reduced freshwater flow (Turpie *et al.*, 2002; Turpie, 2004).

Specimens examined. SOUTH AFRICA. **Eastern Cape.** Uitenhage Div. [probably Swartkops Estuary near Port Elizabeth], xii sine anno, *Ecklon & Zeyher* 620 (BOL); Port Elizabeth, Zwartkopsrivier, sine dato, *Zeyher* 4326 (NBG-SAM); East London, Kwenxura (= Kwenqura) River mouth, 23 xii 1900, *Galpin* 5815 (NBG); [Transkei], Kentani, coast, 100 feet, anno 1903, *Pegler* 335 (BOL); [Transkei], Kentani, 4 xii 1905, *Pegler* 335 (SAM, Z); [Transkei], Kei River Bridge, 19 x 1931, *Taylor* 3669 (NBG); Tsitsikamma, Nature's Valley, Groot River lagoon, ii 1932, *J. & B. Rennie* 545 (BOL); [Transkei], near Kei River mouth, 25 xi 1945, *Compton* 17675 (NBG); [Transkei], Coffee Bay, iii 1947, *Lewis* 2374 (NBG-SAM); East London, 27 x 1962, *Batten* 1.Pl.115. (NBG); East London, Nahoon River, 26 vi 1963, *Tölken* 402 (NBG-STE); Port Alfred, Kowie River estuary, 28 i 1964, *Mauve & Wells* 7 (NBG); East London, Bridle Drift, 2 iv 1966, *Pamphlett* 5 (NBG); Humansdorp, on water edge, 21 xi 1972, *Montgomery* 182 (NBG-STE); Tsitsikamma, Nature's Valley, Groot River estuary, 6 v 2006, *Köcke & Mucina* 060506/29 (MJG, NBG); Jefferey's Bay, Kabeljous River estuary, 7 v 2006, *Köcke & Steffen* 070506/01 (MJG); Port Alfred, Kowie River estuary, 9 v 2006, *Köcke & Steffen* 090506/22 (MJG). **KwaZulu-Natal.** [Herbarium Natal, locality burnt, possibly Durban], xi 1882, *Medley Wood* 396 (BM); Durban, shores at bay, 20 xi 1884, *Medley Wood* 396 (BOL); Durban, xi sine anno, *Medley Wood* 925 (BM); Durban, Congella, sine dato, *Medley Wood* 11985 (BOL); Durban, x 1888, *Wilms* 2278 (BM); Durban, Levy, iii 1894, *Kuntze* s.n. (NY); Durban, Beach Terminus, vi 1913, *Thode* 4901 (NBG-STE); Durban, Isipingo, 22 iv 1921, *Forbes* STE 12526 (NBG-STE); 1 xi 1926, *Schröder* s.n. (ZT); Durban, Fusel, ix 1933, *Meebold* 12994 (M); Durban, Isipingo Beach, 13 viii 1949, *Ward* 917 (K); Port Edward, Palm Beach, 21 iii 1992, *Weigend* 2335 (M); Port Edward, Umtamvuna River mouth, 9 iv 2006, *Pienaar, Jakubowsky & Swelankomo* 090406/1 (MJG, NBG). **Northern Cape.** Namaqualand, Hondeklipbaai, Spoeg River mouth, 5 m, 18 x 1980, *Le Roux & Parsons* 59 (NBG-STE); Namaqualand, Kleinzee, 5 ix 2005, *Mucina* 050905/04 (MJG). **Western Cape.** Cape Town, in humidasis in littore maris False Bay prope Muizenberg, iii 1892, *Schlechter* 7184 (BOL); Bonnievalle, swamp near Breede River, iii sine anno, *Marloth* 11592 (NBG-STE); Cape Peninsula, Muizenberg Vley, 11 iii 1896, *Wolley-Dod* 969 (BOL); 14 xi 1897, *Wolley-Dod* 3658 (BOL); Cape Town, Paarden Island, ii 1908, *Dümmer* 1171 (NBG-SAM); Cape Town, Lakeside, i 1918, *Michell* s.n. (BOL); West Coast, Graafwater, ix 1923, *Adamson* 39042 (NBG-SAM); Cape Peninsula, E of

Paulsberg, 3 v 1929, *Salter* 344/9 (K); Robertson, Breede River, 25 ix 1935, *Lewis* s.n. (BOL); Cape Town, Paarden Island, 24 iii 1936, *Adamson* 843 (BOL); Cape Town, Lakeside Vlei, 17 x 1936, *Adamson* 1235 (BOL); 17 xi 1936, *Adamson* 1370 (BOL); 17 i 1937, *Adamson* 1614 (BOL); Cape Town, Sand Vlei, 17 xi 1936, *Adamson* 1373 (K); Cape Town, Sand Vlei, E side, 17 xi 1936, *Adamson* 1370 (NBG-SAM); Cape Town, Muizenberg, Sand Vlei, 20 iii 1938, A*damson* 1689 (BM); West Coast, between Bokbaai and Darling, 15 ix 1940, *Esterhuysen* 3866 (BOL); Caledon, at streamside, xi 1940, *Esterhuysen* 3865 (BOL); Hermanus, Onrust, beside the lagoon, 22 iii 1944, *Leighton* 413a (BOL), *Leighton* 413 (NBG); Gansbaai, Frikkiesbaai [Uilkraalsmond], 21 iv 1946, *Leighton* 1669 (BOL); Gansbaai, Franskraal, 21 iv 1946, *Leighton* 1669 (NY); George, Kaaimansgat, Kaaimans River, xi 1947, *Wilman* s.n. (BOL, K); Plettenberg Bay, 24 ix 1967, *Thompson* 588 (NBG-STE); Cape Town, Cape Flats, Isoetesvlei, 9 iii 1970, *Strauss* 10 (NBG); Pearly Beach, Klein Hagelkraal, 50 feet, 10 iii 1979, *Thompson* 3898 (M, NBG); Stillbaai, Kransfontein Farm, humus rich river bank in forest, 7 x 1980, *Bohnen* 7731 (NBG-STE); Gansbaai, Franskraal, N bank of Uilkraal River, 2 m, 3 xi 1987, *O'Callaghan* 3/11/2 (NBG-STE); Struisbaai, at entrance to Corona Farm, 28 xii 2003, *Mucina* 281203/05 (MJG); Cape Town, Zandvlei, near station and boat club area, 20 viii 2004, *Walton* 341 (MJG); Gansbaai, Franskraal, Uilkraalsmond Estuary, N of the road bridge, 8 v 2005, *Mucina* 080505/05 (MJG); Malgas, De Hoop Nat. Res., Potberg section, Cupido's Kraal, 3 iv 2006, *Köcke & Mucina* 030406/14 (MJG); Struisbaai, Vogelzang Farm, 4 iv 2006, *Köcke & Mucina* 040406/22, 040406/24 (MJG, NBG); Gansbaai, Franskraal, Uilkraalsmond Estuary, N of the road bridge, 5 iv 2006, *Köcke & Mucina* 050406/03 (MJG), 050406/07 (MJG), 050406/09 (MJG); Hermanus, Fisherhaven, near jetty at the Botrivier Lagoon, 5 iv 2006, *Köcke & Mucina* 050406/11 (MJG, NBG); Bredasdorp, Patryskraal on road between Oupos and Bredasdorp, 5 iv 2006, *Köcke & Mucina* 050406/18 (MJG, NBG); Gansbaai, Franskraal, Uilkraalsmond estuary, 5 iv 2006, *Köcke & Mucina* 050406/19 (MJG, NBG); Struisbaai, W of Zoetendalsvlei, at road Struisbaai to Elim, km 59.9, 5 iv 2006, *Köcke & Mucina* 050406/24 (MJG, NBG); Struisbaai, at entrance to Corona Farm, 5 iv 2006, *Köcke & Mucina* 050406/27 (MJG); Cape Town, Milnerton, Rietvlei Nat. Res., close to parking area, 20 iv 2006, *Köcke & Steffen* 200406/01, 200406/03 (MJG); on the way to bird hide, 20 iv 2006, *Köcke & Steffen* 200406/10 (MJG); at bird hide, 20 iv 2006, *Köcke & Steffen* 200406/17 (MJG); Hermanus, Voëklip, 23 iv 2006, *Köcke & Mucina* 230406/18 (MJG); Mosselbaai, Hartenbos, near the bridge over river, 4 v 2006, *Köcke & Mucina* 040506/24 (MJG, NBG); [Cape Town], Faure, Vergenoeg Farm, 4 x 2006, *Boucher* 7412 (NBG); Elim, near homestead of Heuningrug Farm, 14 x 2006, *Mucina* 141006/23 (MJG); Malgas, De Hoop Nature Reserve, Potberg section, near Bultfontein, 21 x 2006, *Mucina* 211006/17 (MJG); Struisbaai, Vogelzang Farm, N of Heuningnes River estuary, 17 iii 2007, *Mucina* 170307/03 (MJG), 170307/04 (MJG); Struisbaai, De Mond Nature Reserve, Heuningnes River estuary, left bank, 17 iii 2007, *Mucina* 170307/13A (MJG).

7. Triglochin buchenaui Köcke, Mering & Kadereit, **sp. nov.** – Type: South Africa, Western Cape, West Coast National Park, E of Seeberg Bird Hide, intertidal salt marsh flats, 14 iv 2006, *Köcke & Mucina* 140406/06 (holo MJG; iso NBG; Fig. 15). **Figs 1I-K, 2F, 11.**

Herba scaposa, rhizomatosa, non bulbosa, ad 30 cm alta. Differt a *Triglochin elongata* inflorescentia laxa cum 3-17 flores, fructibus angustus ovoideus usque fere globosus, carpophoris deficiens.

Plants 9-30 cm high, with *rhizomes*. Rhizomes whitish to beige with bracts. *Leaves* uniform, thickened at base but not forming a bulb, as long as or longer than infructescence, leaf bases covered by usually (very) few, soft, whitish to beige fibres which can be more than half as long as height of plant. *Inflorescences* lax, with 3-17 flowers. *Flowers* ±2 mm long. *Pedicels* up to 2 mm long at fruiting time, usually shorter. *Infructescences* 2-7 cm long, fruits distant. *Fruits* narrowly ovoid to ovoid, 4-8 mm long and 1 to >2 mm wide (Fig. 2F). *Mericarps* dorsally curved upwards, filled with air, tips not curved or weakly curved outwards, *carpophore* absent.

Herbarium Hortus Botanicus
Universitatis Moguntinae MJG

Holo-**TYPE**

Flora of South Africa
140406/06 **Date:** 14-04-2006

Triglochin buchenaui Köcke, Mering & Kadereit

Locality: Western Cape, West Coast National Park, E of Seeberg Bird Hide
Altitude: 0 m **Long:** 18-03-36.3 **Lat:** 33-07-40.6
Habitat: intertidal salt-marsh flats; seed sandy soil with slick on surface

VegMap: Cape Estuarine Salt Marshes
Leg: V. Köcke & L. Mucina
Det: V. Köcke
Notes: sample collected for DNA analyses

DNA Accession No.:

Fig. 10. Holotype of *Triglochin buchenaui* Köcke, Mering & Kadereit sp. nov. (MJG).

Distribution. Endemic to South Africa, restricted to major estuaries of the West and South Coast, spanning Groot Berg River estuary near Velddrif (Western Cape Province) and Kariega estuary near Kenton-on-Sea (Eastern Cape Province) (Fig. 11).

Habitat and ecology. Triglochin buchenaui is a typical species of periodically flooded lower salt marsh estuaries (Fig. 1I). The soils in these habitats are sandy to sandy-clayey. *Triglochin buchenaui* usually occurs together with *Sarcocornia tegetaria*, *T. striata*, *Cotula coronopifolia*, *Poecilolepis ficoidea, Chenolea diffusa* and various *Limonium* species. The vegetation in these habitats is classified exclusively as Cape Estuarine Salt Marshes (Mucina & Rutherford, 2006).

Phenology. Flowering throughout the year.

Chromosome number. Unknown.

Taxonomic remarks. See *Triglochin elongata* for a comparison of these two rhizomatous species.

Etymology. The species is named after Franz Georg Philipp Buchenau (1831-1906), a German botanist who contributed significantly to the knowledge of *Triglochin* and Juncaginaceae.

Proposed IUCN conservation status. Least Concern (LC). *Triglochin buchenaui* is a widespread species recorded from a number of large lagoons and estuaries in South Africa (Fig. 11) and therefore is not immediately threatened. The Berg River and Swartkops River estuaries support large populations of *Triglochin buchenaui* and are also among the highest ranked South African estuaries in terms of botanical and conservation importance (Turpie *et al.*, 2002). The estuaries of the Langebaan and Knysna Lagoons as well as estuaries of Kromme and Swartkops enjoy formal protection either as part of national parks or local authority nature reserves.

Specimens examined. SOUTH AFRICA. **Eastern Cape.** Port Elizabeth, Redhouse, i 1915, *Paterson* 288 (BOL); Boesmansriviermond, Bushmans River mouth, 14 i 1936, *Dyer* 3365 (PRE); Port Elizabeth, Swartkops River mouth, 4 ii 1964, *Mauve & Wells* 38 (NBG-STE); Tsitsikamma, Blaubaai by Robbehoek, 8 iii 1979, *Taylor* 9954 (NBG-STE); Kenton-on-Sea, Ghio Wetland, below the bridge over Bushmans River, 26 xi 2005, *Mucina* 261105/05 (MJG); St. Francis Bay, Kromme River estuary, at the bridge, 7 v 2006, *Köcke & Steffen* 070506/07 (MJG); Port Elizabeth, Swartkops River estuary, 8 v 2006, *Köcke & Steffen* 080506/06 (MJG); Kenton-on-Sea, Ghio Wetland, at bridge over Bushmans River, 9 v 2006, *Köcke & Steffen* 090506/04 (MJG); 9 v 2006, *Köcke & Steffen* 090506/08 (MJG); Kenton-on-Sea, Kariega River estuary, 9 v 2006, *Köcke & Steffen* 090506/13 (MJG). **Western Cape.** Knysna, Woodbourne, edge of lagoon, 31 i 1924, *Duthie* 876 (NBG-STE); Knysna, Belvidere, edge of lagoon, 22 i 1925, *Duthie* 876 (BOL); Langebaan, wet places, ix 1925, *Leipoldt* 27103 (BOL); Knysna, Knysna Lagoon, 6 ii 1964, *Mauve* 36 (PRE), *Wells* 37 (PRE); Langebaan Lagoon, near Oesterwal, 4 v 1967, *Simons* 1991 (BOL); Knysna, Nature's Valley, Groot (Wes) River mouth, 8 iv 1981, *Parsons* 161 (NBG-STE); Velddrif, Port Owen, Berg River, 13 ii 1981, *Le Roux* 2855 (NBG-STE); 16 ii 1986, *Boucher* 5123 (NBG-STE); 22 ii 1988, *Becker* 0002 (NBG-STE); Velddrif, salt marsh opposite Berg River Mouth, 14 x 1986, *O'Callaghan* 157 (NBG); Velddrif, N bank of Berg River, Wreck, 4 ii 1987, *O'Callaghan* 1468 (NBG-STE); Velddrif, Berg River, W of Port Owen, 8 ix 1987, *O'Callaghan* 8/13 (NBG-STE); Langebaan, West Coast Nat. Park, Churchhaven, 21 viii 1997, *Mucina* 6435/11 (PRE); Langebaan, West Coast National Park, Geelbek at Bird Hide, 14 iv 2006, *Köcke & Mucina* 140406/27 (MJG); Velddrif, Berg River estuary, at Carinus Bridge, 19 iv 2006, *Köcke & Steffen* 190406/15 (MJG, NBG), 190406/19 (MJG); Stillbaai, Goekoe River estuary, E bank of the river, 4 v 2006, *Köcke & Mucina* 040506/10 (MJG); Knysna, Knysna Lagoon, turnoff to Leisure Island, 5 v 2006, *Köcke & Mucina* 050506/05 (MJG); Plettenberg Bay, Bitou River estuary, 6 v 2006, *Köcke & Steffen* 060506/25 (MJG), 060506/27 (MJG).

DISCUSSION

The seven species of the *Triglochin bulbosa* complex recognized here are not only morphologically distinct but also differentiated in terms of geographical distribution, ecology, phenology and, where known, chromosome number.

In the Mediterranean region, *Triglochin laxiflora* is autumn-flowering whereas *T. barrelieri* is spring-flowering. The two Mediterranean species also have different ploidy levels, with *Triglochin laxiflora* having $2n = 18$ and *T. barrelieri* having $2n = 30$, 32 or 36 chromosomes. In Africa, *Triglochin milnei* is the only species of the complex found in several countries of Central and southern Africa. The distribution of *Triglochin milnei* does not overlap with the other four species in South Africa. In eastern South Africa it is the only species to be found in inland localities and at altitudes above 500 metres. Both rhizomatous species, *Triglochin elongata* and *T. buchenaui*, grow sympatrically (in a broad sense) along the South and West coasts of South Africa. However, they differ in ecology by *Triglochin buchenaui* being limited to lower tidal habitats, while *T. elongata* is found in upper tidal or semi-terrestrial inland habitats such as edges of water courses and pans. Where the two bulbous species, *Triglochin bulbosa* and *T. compacta* occur in the same area (e.g., Cape Peninsula), they differ both in phenology and ecology. *Triglochin bulbosa* flowers between July and November, while the flowering time of *T. compacta* is between February/March and May. *Triglochin bulbosa* subsp. *bulbosa* occurs on Cape Peninsula on Archaean granites, while *T. compacta* is found on quartzite sands derived from Ordovician Table Mountain sandstone.

The importance of edaphic factors (geology, soils and associated pedo-hydrological conditions) in diversification of the Cape flora has been widely suspected (Marloth, 1908) and in many instances also well-documented (Rourke, 1972; Goldblatt, 1979; Williams, 1982; Linder & Ellis, 1990; Kurzweil *et al.*, 1991; see also Linder, 2003 and van der Niet *et al.*, 2006). *Triglochin bulbosa* may be a good candidate for the study of adaptive radiation of a non-insect pollinated taxon. The four subspecies now distinguished within *Triglochin bulbosa* are clearly differentiated along pedo-hydrological habitat axes. The only water-bound subspecies of *Triglochin bulbosa* is the widely distributed subsp. *bulbosa* occurring in temporarily wet (flooded) both coastal and inland habitats, while the other three subspecies radiated into fully terrestrial inland habitats characterised by contrasting geologies, such as granite (subsp. *tenuifolia*), limestone (subsp. *calcicola*) and quartz fields (subsp. *quarcicola*).

Morphology of underground parts appears to be correlated with water availability. Whereas the species with rhizomes (*Triglochin elongata*, *T. buchenaui*) are limited to habitats with constantly available water, the bulbous species (*Triglochin bulbosa*, *T. compacta*, *T. milnei*, *T. barrelieri*, *T. laxiflora*) grow in places where at least abundant water is available only for a short period of the year.

The similarities in differentiation between *Triglochin* species from the Mediterranean Floristic Region and the Cape Floristic Region, respectively, are striking. Thus, morphologically similar species from the two regions are flowering either in spring (Mediterranean: *Triglochin barrelieri*, South Africa: *T. bulbosa*) or in autumn (Mediterranean: *T. laxiflora*, South Africa: *T. compacta*). This divergence of flowering time was already noted by Buchenau (1896) and Rainha (1944) for the Mediterranean region and by Adamson (1943) for South Africa. The species of the complex thus are a nice example for parallel ecological differentiation under similar climatic conditions in mediterranean-type ecosystems.

Several species of the *Triglochin bulbosa* complex are of conservation concern. Even though only *Triglochin bulbosa* subspp. *calcicola* and *quarcicola* are threatened according to the IUCN Red

List categories (IUCN, 2001), the habitats of many taxa (e.g., seasonal pools, estuaries) are under manifold pressure from human activities.

Chapter 4

Tetroncium and its only species *T. magellanicum* (*Juncaginaceae*): distribution, ecology and lectotypification

Sabine von Mering

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ABSTRACT

Tetroncium magellanicum (*Juncaginaceae*) was described by Willdenow in 1808, based on material collected by Commerson at the Strait of Magellan during Bougainville's voyage around the world. Type material of this species was traced and a lectotype for the name is designated. A description of the species and notes on its ecology and conservation status are provided. For the first time, a detailed map showing the known distribution area of *T. magellanicum* is presented.

Additional key words: Gough Island, Herbarium Willdenow, lectotype, peatlands, southern South America, typification

Introduction

The monotypic genus *Tetroncium* Willd. belongs to the small monocot family *Juncaginaceae* (Alismatales) and is sister to the other two genera of the family, *Cycnogeton* R.Br. and *Triglochin* L. (von Mering & Kadereit 2010). *Tetroncium magellanicum* Willd. is the only dioecious species in the family (Fig. 1B, 6). It is a perennial herb growing mainly in the peatlands of southern South America and some neighbouring islands (Fig. 1A, 4).

Tetroncium magellanicum was described by Carl Ludwig Willdenow in 1808. In the protologue, Willdenow stated that the new genus and species are based on material collected by Commerson at the Strait of Magellan (Willdenow 1808). Philibert Commerson (sometimes spelled Commerçon) was a French naturalist who accompanied Louis Antoine de Bougainville during his voyage around the world from 1766 to 1769 (Bougainville 1772). The botanical collections of this voyage reached Paris in 1774, and duplicates were subsequently distributed to several botanists and botanical institutions in Europe (Stafleu & Cowan 1976-1988).

Tetroncium magellanicum was mentioned in several publications on the early botanical exploration of southern South America (e.g., Hooker 1847, Gay 1849, Alboff 1896, Reiche 1907) and also in later works covering the flora of the region (e.g., Correa 1969, Moore 1968, 1983, Marticorena & Quezada 1985, Zuloaga et al. 2008). Franz Buchenau, who contributed significantly to our knowledge of *Juncaginaceae*, also added to our knowledge of *Tetroncium* (e.g., Buchenau 1868, 1882, 1903). However, the species has never been studied in full detail and Tomlinson (1982) correctly stated that little is known about it. For example, embryological and karyological data are lacking, and details of flower and fruit development as well as the mode of fruit dispersal are only incompletely known.

In the course of taxonomic studies in *Juncaginaceae* I noted that the name *Tetroncium magellanicum* has not been typified. Apart from typifying the species, I will also provide a description, information on its ecology, an assessment of its conservation status (according to the IUCN threat categories, IUCN 2001), and a distribution map based on data obtained from revised herbarium specimens and reliable literature sources.

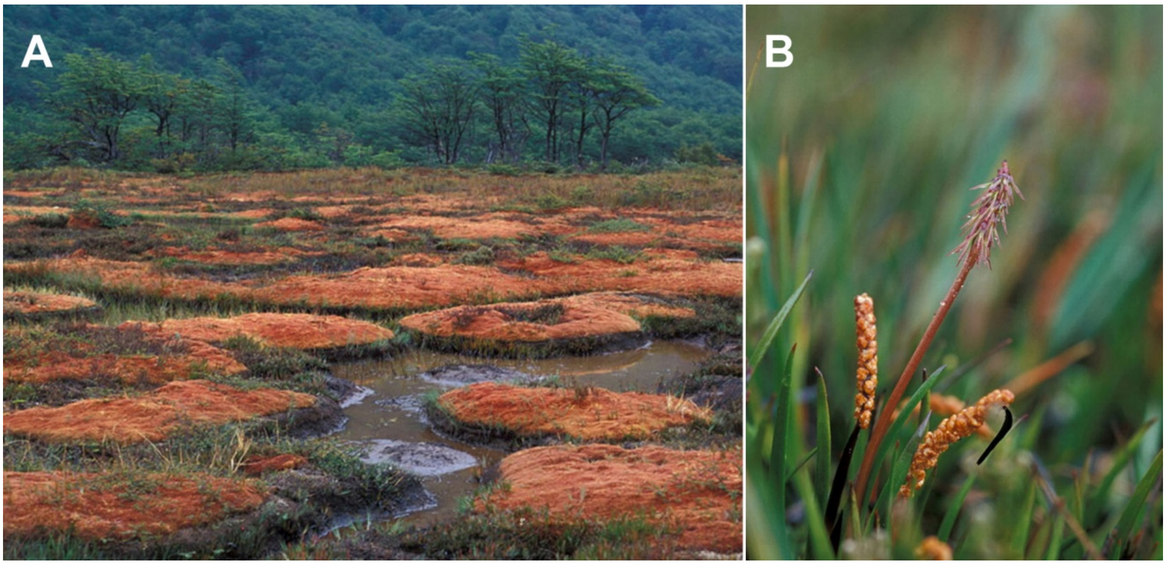


Fig. 1. *Tetroncium magellanicum*. A. Typical habitat, continental bog dominated by *Sphagnum magellanicum*, near Ushuaia, Argentina. B. Female (centre) and two male inflorescences (photographs: R. Douzet, SAJF, University Grenoble).

Material and methods

Collections from Herbarium Willdenow (housed at the Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin as separate collection B-W; e.g., Hiepko 1972) and from several other major herbaria (see Acknowledgements for a full list) were revised to locate type material of the species. Additionally, online databases of herbaria or virtual herbaria were reviewed, as well as the GBIF Data Portal (Global Biodiversity Information Facility 2007+), JSTOR Plant Science (2010+), Flora del Conosur (2009+), Flora Argentina (2012+), British Antarctic Survey (BAS) Higher Plants Herbarium (2009+), and UK Overseas Territories (UKOTs) Online Herbarium (2011).

Altogether, more than 150 specimens of *Tetroncium magellanicum* were studied. Specimens that were only seen as a scan or photograph are marked with “image!” in the list of specimens seen below. A lectotype for the name of this species is designated here according to the International Code of Botanical Nomenclature (McNeill & al. 2006; Fig. 3).

Information given on labels of historic specimens is usually limited, important specifications such as (exact) collection dates or localities are often missing. Primary literature such as original travelogues of expeditions and secondary literature was consulted to decipher, verify and - wherever possible - complement available label information. Godley’s accounts of botanical exploration of the Southern hemisphere (Godley 1965, 1970) were especially valuable in this respect. Furthermore, the following online resources were used: Taxonomic Literature II (TL-2, Stafleu & Cowan 1976-1988 and supplements), JSTOR Plant Science Collection of Plant Collectors (JSTOR Plant Science 2010+) and the HUH Index of Botanists (Harvard University Herbaria 2011+).

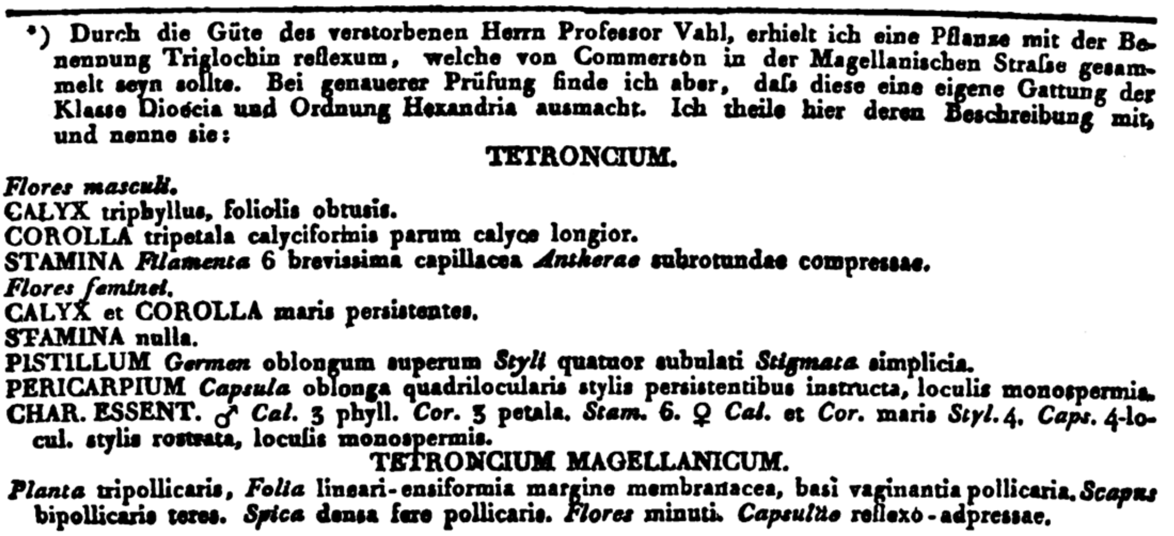
The distribution map was created using georeferenced localities obtained from revised herbarium specimens and reliable literature sources. Georeferencing of localities was facilitated by the use of electronic gazetteers such as GEOnet Names Server (GNS 1994+), GeoNames (2012) or Google Earth (Google 2012), and the map was generated using the EDIT mapViewer (2012).

Name and typification

Tetroncium Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 2: 17. 1808.
= *Cathanthes* Rich., Mém. Mus. Hist. Nat. 1: 365. 1815. [Type: *non designatus*]

Notes. – The material of *Tetroncium* that Willdenow had on hand at the time of writing the description was named ‘*Triglochin reflexum*’ and sent to him by the late Professor Martin Vahl from Copenhagen (Willdenow 1808; Fig. 2). Willdenow noticed its distinctness from *Triglochin* and described the new genus (Willdenow 1808).

Little is known about the identity of *Cathanthes* Rich., a name established only seven years after *Tetroncium* by Richard (1815). Richard’s brief description of *Cathanthes* in his listing of genera of “Juncaginaeae” reads as follows: “[*Lilaea*.] *Cathanthes*, floribus dioici, reclinatis, etc., a sequente diversa. [*Triglochin*. *Scheuchzeria*.]” (Richard 1815). Kunth (1841) and Hooker (1843) cite *Cathanthes* as a synonym of *Tetroncium*. This statement seems well supported by the few characters noted in the protologue as *Tetroncium* is the only dioecious taxon in the group, and posseses reflexed female flowers and fruits. No *Cathanthes* species were described later and no specimens bearing this name were found. Buchenau (1868, 1903) and Micheli (1881) mention ’Catanthes‘ as a synonym of *Tetroncium*. This is most probably the result of a spelling error.



*) Durch die Güte des verstorbenen Herrn Professor Vahl, erhielt ich eine Pflanze mit der Benennung Triglochin reflexum, welche von Commerson in der Magellanischen Straſse gesammelt seyn sollte. Bei genauerer Prüfung finde ich aber, daſs diese eine eigene Gattung der Klasse Dioecia und Ordnung Hexandria ausmacht. Ich theile hier deren Beschreibung mit, und nenne sie:

TETRONCIUM.

Flores masculi.
CALYX triphyllus, foliolis obtusis.
COROLLA tripetala calyciformis parum calyce longior.
STAMINA *Filamenta* 6 brevissima capillacea *Antherae* subrotundae compressae.
Flores feminei.
CALYX et COROLLA maris persistentes.
STAMINA nulla.
PISTILLUM *Germen* oblongum superum *Styli* quatuor subulati *Stigmata* simplicia.
PERICARPIUM *Capsula* oblonga quadrilocularis stylis persistentibus instructa, loculis monospermis.
CHAR. ESSENT. ♂ *Cal.* 3 phyll. *Cor.* 3 petala. *Stam.* 6. ♀ *Cal.* et *Cor.* maris *Styl.* 4. *Caps.* 4-locul. stylis rostrata, loculis monospermis.

TETRONCIUM MAGELLANICUM.

Planta tripollicaris, *Folia* lineari-ensiformia margine membranacea, basi vaginantia pollicaria. *Scapus* bipollicaris teres. *Spica* densa fere pollicaris. *Flores* minuti. *Capsulae* reflexo-adpressae.

Fig. 2. Protologue of *Tetroncium magellanicum* Willd. (Willdenow 1808; from microfiche, library of the BGBM).

Tetroncium magellanicum Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 2: 17. 1808.
− Lectotype (designated here): "Habitat ad Fretum Magellanicum" [Strait of Magellan], [December 1767], *Commerson s.n.* (B -W 17531-02 0!; Fig. 3C); isolectotypes (designated here): B -W 17531-01 0!, BM000820840!, C!, G00098732 [image!], P01759004!, P01759005!).
− *Triglochin reflexa* Vahl ex Kunth, Enum. Pl. 3: 142 (1841), "*magellanicum*", nom. inval. (ICN Art. 36.1(c)).
− *Triglochin magellanica* Vahl ex Kunth, Enum. Pl. 3: 142 (1841), "*reflexum*", nom. inval. (ICN Art. 36.1(c)).

Notes. – Material of *T. magellanicum* located in the Herbarium Willdenow comprises two sheets placed in one of the typical blue folders of this historic collection. They are labelled "*T. magellanicum* 1" and "*T. magellanicum* 2" (B -W 17531-01 0 and B -W 17531-02 0). This material clearly matches the details of the protologue, the label attached to the folder gives the locality as cited above and the designation '*Triglochin reflexum*' written in Willdenow's hand (Fig. 3A). The name "Tetroncium magellanicum" and the literature reference written on this label on the folder was later added by D. F. K. Schlechtendal, as explained by the note "Schlechtendal p.", i.e. Schlechtendal pater (Latin for father), written by his son D. F. L. Schlechtendal. Another smaller label on the folder, reading "Vahl. W.", was also written by D. F. L. Schlechtendal and is indicating that the material originated from Vahl and Willdenow (Fig. 3B).

It seems certain that both specimens were in the hands of Willdenow. Therefore, a lectotype has to be designated. The first of the two sheets in Herbarium Willdenow consists of a female and a male plant. In contrast, only a male plant is mounted on the second sheet. However, the latter bears an additional label reading '*Triglochin magellanicum*' written by Willdenow himself (P. Hiepko, pers. comm.; Fig. 3C) and it is therefore chosen here as lectotype (B -W 17531-02 0). The other specimen is designated as isolectotype.

The lectotype and isolectotype from B are available online as high resolution images through the Digital Herbarium (Röpert 2000+). Duplicates of Commerson's collection of *T. magellanicum* (representing isolectotypes) were found in BM, C, G and P. Most of these isolectotypes (and further

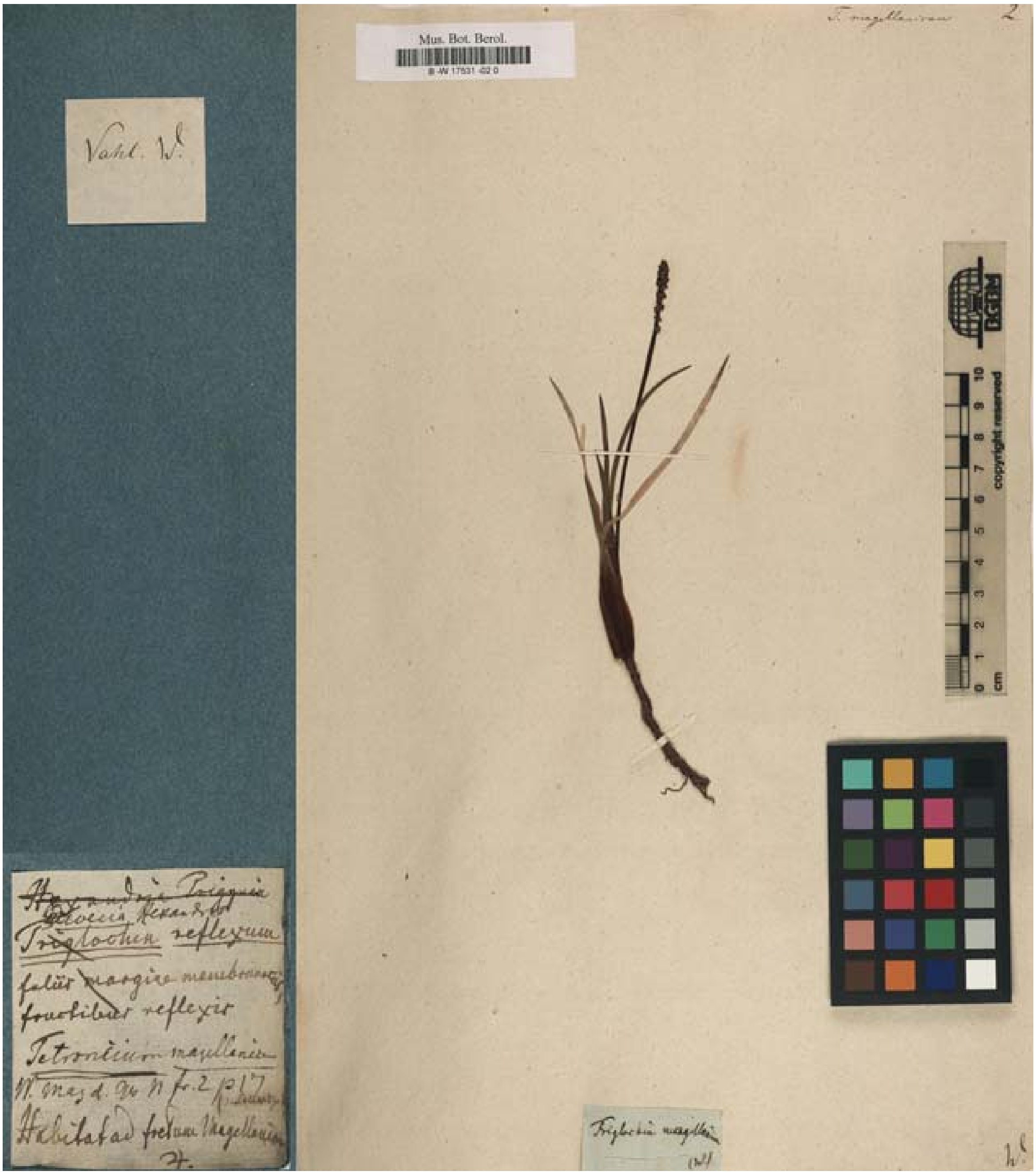


Fig. 3. *Tetroncium magellanicum* in Herbarium Willdenow – A: folder with label; B: additional label; C: specimen designated as lectotype (barcode B-W 17531-02 0 / ImageId: 267191), see text for details. – Photographs: Herbarium, Botanic Garden and Botanical Museum Berlin-Dahlem (Röpert 2000+). – [Published at http://data.bgbm.org/herbarium/BW17531020].

historic material mentioned below) are accessible online via JSTOR Plant Science or the collection databases of the herbaria, respectively.

Label information of the type material in B does not include a collection date or a locality apart from Fretum Magellanicum. However, labels attached to one of the isolectotypes present in Paris (P01759004) give further information on the date and locality where it was collected. The inscription “Xbri 1767” is indicating that the material was collected in December 1767. This is confirmed by the fact that the passage of the Strait of Magellan lasted from December 1767 to January 1768 (Bougainville 1772). Several labels list localities where the species was found in the Strait of Magellan: “Baye francaise, Baye Bougainville, Port Galant & in Sylvis Commersonianis”, “In alpibus Commersonianis, Port Bougainville". However, it remains unclear in which of these precise localities the material was collected.

The label on the isolectotype of *T. magellanicum* in C was written by M. Vahl (information from P. Wagner via O. Seberg/G. Petersen, pers. comm.), the botanist who sent the material to Willdenow. A specimen located in the Herbier Genève (G00098732) and another in the Herbarium HAL (HAL0109729), both annotated as type material (by R. R. Haynes & L. B. Holm-Nielsen in 1985 and by N. Tkach in 2010, respectively) could represent further isolectotypes. However, in both cases the source could not be identified with certainty and the specimens are therefore not listed as isolectotypes until further evidence becomes available. Further sets of material collected during Bougainville's circumnavigation were distributed to several other herbaria (Stafleu & Cowan 1976 - 1988) which accordingly might house further type material of *Tetroncium*.

'Triglochin reflexum' was used as a provisional name by Vahl and not validated by later publication. Willdenow (1808) referred to this name in the protologue of *Tetroncium magellanicum*. The name '*Triglochin magellanicum'* was used by Willdenow on the herbarium sheet (see small label on lectotype, Fig. 3C). Both names were cited by Kunth (1841) as synonyms under *Tetroncium magellanicum*, but this did not result in the valid publication of these names (see Art. 34.1c, McNeill & al. 2006).

Ic.: Hooker 1843, t. 534; Hooker 1847, t. 128 (Fig. 5); Correa 1969, Fig. 15, p. 36.

Description (based on own observations and measurements of herbarium material and partly on Hooker 1847, Buchenau 1903, Moore 1968, 1983; see also Fig. 6)

Perennial, rhizomatous, glabrous herb, 5-25(-35) cm high. ***Rhizome*** ascending, up to 20 cm long, 2-4(-6) mm in diameter, producing aboveground stems towards the apex. ***Stems*** ascending or erect, branching near base, densely covered with brown leaf remains at base and with leaves towards apex. ***Leaves*** distichous, simple, coriaceous, rigid, linear-ensiform (sword-shaped), acute, (15-)20-100(-120) × 1.5-3.0 mm, equitant, with basal sheath but without ligule or auricles. Plant dioecious, scapose; scapes erect, 25-200(-250) mm long. ***Inflorescences*** terminal, dense, c. 10-50 mm long, ebracteate spikes of up to 30 flowers; pedicels very short (c. 0.5 mm) or absent. ***Male flowers:*** tepals 4, yellowish with reddish brown spots, concave or conchiform, broadly ovate, acute or subobtuse, 1.2-2.0 × 0.5-1.5 mm. Stamens 4, opposite tepals and inserted at their base, (sub)sessile; anthers yellowish, extrorse. ***Female flowers:*** tepals 4, as in male flowers but narrower, ovate to ovate-lanceolate. Carpels 4, subulate, fused from base to about half of their length, with one basal anatropous ovule per carpel; styles divergent. ***Fruits*** 4-locular (or possibly unilocular with incomplete septa), dry, indehiscent, reflexed, narrowly conical, with long persistent beaklike styles, 4-8(-10) × c. 1 mm, smooth, reddish brown, usually one-seeded through abortion of three ovules. ***Seeds*** linear-oblong, endospermic. ***Pollen*** inaperturate, subspheroidal, medium-sized, dispersed in monads, exine reticulate (Kupriyanova 1948, Grayum 1992). Chromosome number unknown.

Tetroncium differs from the other genera of *Juncaginaceae* mainly in its dioecy and flower merosity (dimerous vs. trimerous in *Triglochin* and *Cycnogeton*). Further differences include seeds with endosperm, the general habit, as well as leaf form and structure (ensiform, rigid and coriaceous in *Tetroncium* vs. semiterete, ± succulent in *Triglochin* and flattened, strap-shaped, ± spongy in *Cycnogeton*).

Note: Willdenow (1808, Fig. 2) as well as Hooker (1843) erroneously described the flowers apart from the gynoecium as trimerous instead of dimerous. This was clarified by Hooker (1847, see also Fig. 5) and later discussed by Buchenau (1882). Further morphological and developmental studies are needed to examine flower and fruit structure in more detail.

Distribution

The distribution area of *T. magellanicum* comprises mainly southern South America (Patagonia and Tierra del Fuego) northwards to c. 40° S in western Argentina and to c. 37° S in Chile (Fig. 4 & 5). Collections from Parque Nacional Nahuel Huapi in the Andes (Argentina) as well as Parque Nacional Nahuelbuta and Parque Nacional Chiloé in the Chilean Coastal Range represent the northern limits of the distribution of the species. It is also distributed in the Falkland Islands (East and West Falkland) but absent from the Lafonia region of East Falkland probably due to summer drought there (Broughton & McAdams 2005). Furthermore, *Tetroncium* is found on Gough Island in the South Atlantic Ocean (Wace 1961, Groves 1981) but not known from the other islands of the Tristan da Cunha group (N. Gremmen, pers. comm.).

Fig. 4 and 5 give the first detailed map of localities representing the known distribution area of the species. Previously, only a map showing a rough outline of the distribution area (without Gough Island, Camp 1947) and a map limited to Tierra del Fuego (Moore 1983) had been published.

Habitat, ecology and conservation aspects

Tetroncium magellanicum is found in different peatland types, especially *Sphagnum* bogs and cushion bogs. These peatlands are part of characteristic vegetation sometimes termed Magellanic moorland (e.g., Moore 1983, Arroyo & al. 2005 and references within). *Tetroncium* is common in continental bogs dominated by *Sphagnum magellanicum* Brid., where the moss forms orange or reddish carpets or hummocks (Fig. 1A). *Tetroncium magellanicum* predominantly occurs in wetter parts of the peatlands and is frequently found accompanied by e.g. *Carex magellanica* Lam. (e.g., Kleinebecker & al. 2007). In Pacific bogs dominated by cushion-forming vascular plants *Tetroncium* is associated with cushion plants such as *Astelia pumila* (J.R.Forst.) Gaudich. and *Donatia fascicularis* J.R.Forst. & G.Forst. or *Drosera uniflora* Willd. (e.g., Dusén 1900, Moore 1983, Kleinebecker & al. 2007; A. Vogel, pers. comm.; B. Ruthsatz, pers. comm.). In a transition zone both peatland types intermingle or occur mosaic-like side by side (Kleinebecker & al. 2007). *Tetroncium* is also frequent in this *Sphagnum magellanicum* cushion plant mixed type and along a moisture gradient in all three types (Kleinebecker & al. 2007). Highest frequencies were, however, recorded from *Sphagnum cuspidatum* hollows (exclusively found in continental *Sphagnum* bogs and *Sphagnum*-cushion mixed bogs) and wet *S. magellanicum* carpets often surrounding hollows of continental bogs (Kleinebecker & al. 2007).

Habitats belonging to the Magellanic moorland are continuously distributed from the extreme south of the continent to around 43° S in Chile and from sea level to above treeline (Pisano 1983). *Tetroncium magellanicum* is not only known from most parts of this region but also from outlying areas of moorland vegetation occurring further north, e.g. in the Cordillera de Piuchué (Chiloé Island) and the Cordillera de Nahuelbuta, both in the Chilean coastal range (Ruthsatz & Villagrán 1991, Arroyo & al. 2005, see Fig. 4 & 5).

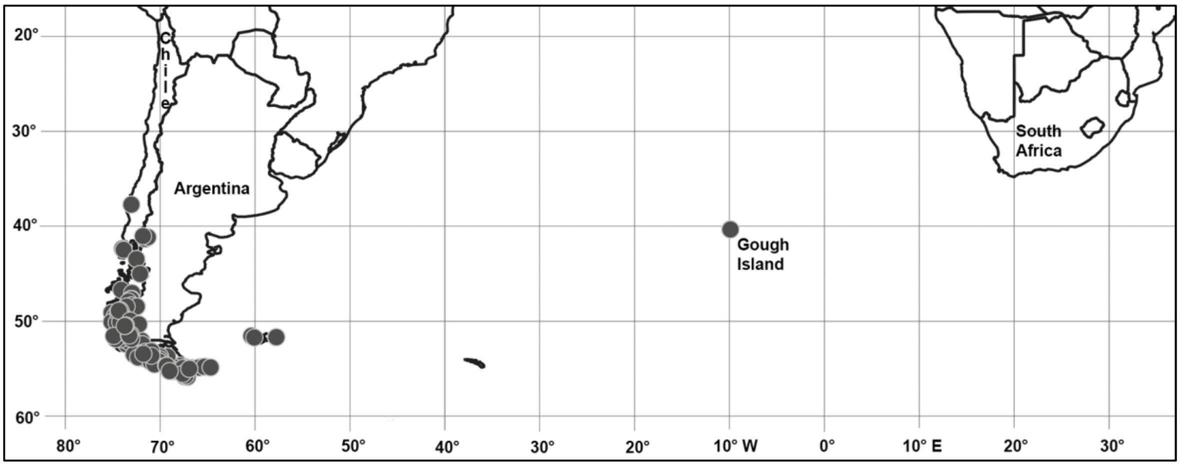


Fig. 4. Distribution of *Tetroncium magellanicum* – southern South America including Falkland Islands and Gough Island in the South Atlantic (ca. 2,800 km from Cape Town, South Africa and 3,300 km from the South American mainland); map created using the online tool EDIT mapViewer (2012).

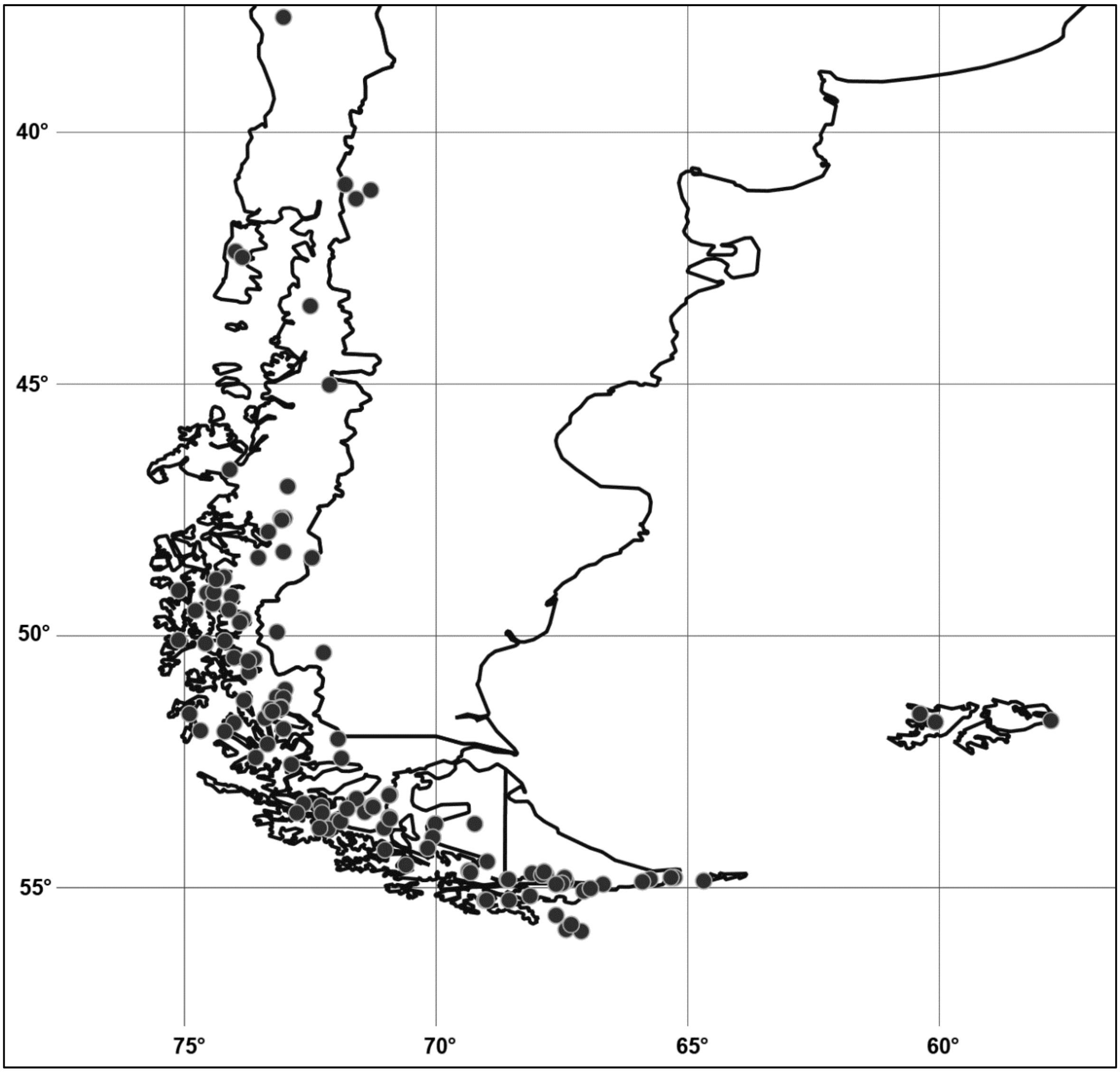


Fig. 5. Distribution of *Tetroncium magellanicum* – Chile and Argentina – map created using the online tool EDIT mapViewer (2012).

In southern Chile, Magellanic moorland occurs together with subantarctic evergreen forest dominated by *Nothofagus betuloides* (Mirb.) Oerst. (e.g., Dusén 1903, Ruthsatz & Villagrán 1991). Further east such peatlands can also be found in the transition zone to deciduous forest dominated by *N. pumilio* (Poepp. & Endl.) Krasser and *N. antarctica* (G. Forst.) Oerst. *Tetroncium* grows in clearings or open parts of these *Nothofagus* woodlands.

Habitats on the Falkland Islands are bogs, dwarf shrub heath and acid grassland (Moore 1968, 1983). On Gough Island *T. magellanicum* is one of the few abundant flowering plants found in high altitude bogs, i.e. *Sphagnum*-dominated peat bogs above 600 m (Wace 1961; N. Gremmen, pers. comm.).

The species is usually found from sea level to about 500 metres. Higher altitudes of up to 1300 metres are only recorded from the northernmost part of the distribution area (e.g., Parque Nacional de Nahuelbuta, *Ricardi 5697 & Marticorena*, CONC; Zuloaga et al. 2008).

Tetroncium is predominantly found in wetter sites and tolerates frequent and prolonged periods of inundation. Sometimes only upper leaves and inflorescences are emerging from the water. Patagonian bog ecosystems are characterised by extreme nutrient-poor conditions and *T. magellanicum* appears to be highly efficient in nutrient acquisition (Schmidt et al. 2010).

Phenology: Flowering and fruiting in summer, mainly from December to January (sometimes to April). The species is probably wind-pollinated as most other species of the family. Fruits of *T. magellanicum* are indehiscent (pers. obs.). Their long, rigid appendages formed by the persistent styles might serve dispersal by animals such as birds. However, pollination as well dispersal mechanisms have not been directly observed in *Tetroncium*. Male and female plants can grow intermingled (see, e.g., specimen *James 1500*, BM, SI) but unisexual populations can be also separated by some distance (R. W. Woods, pers. comm.). This implies that *T. magellanicum* is able to reproduce vegetatively, probably through regeneration from rhizome fragments.

Proposed IUCN conservation status: Least Concern (LC). *Tetroncium magellanicum* is relatively widespread in southern South America, locally abundant, and not under immediate threat. However, as in the Northern hemisphere, peatlands in the Southern hemisphere are increasingly threatened by drainage (e.g., for urban development or road construction) and other land use changes, in particular peat mining. In recent decades, the number of peat extraction sites has risen continuously especially in Tierra del Fuego, and further extraction permits have been issued (e.g., Blanco & de la Balze 2004, Iturraspe & Urciuolo 2004, Grootjans et al. 2010; A. Vogel, pers. comm.).

Tetroncium magellanicum is not confined to continental *Sphagnum* bogs which are most affected by this growing trend in peat extraction but also occurs in oceanic cushion bogs which are protected to a higher degree. Nevertheless, conservation assessments might become more important in the future, especially on a regional scale.

Fortunately, a relatively high proportion of southern South American wetlands are protected as part of National Parks or National Reserves (e.g., Parque Nacional Tierra del Fuego in Argentina, Bernardo O'Higgins National Park and Cabo de Hornos National Park in Chile). However, protection of different peatland types is unbalanced, and *Nothofagus* forests further north in the Coastal range containing disjunct areas of peatlands have been reported to be under threat (Arroyo & al. 2005). Further threats to fragile wetland ecosystems include the introduction of non-native species and tourism.

Fig. 6. *Tetroncium magellanicum* – Plate from Flora Antarctica illustrating male and female plants, flowers and fruits (Hooker 1847). – [Published at http://biodiversitylibrary.org/page/13448638].

Gough Island is recognized as a nature reserve and an ordinance entitled 'Conservation of Native Organisms and Natural Habitats (Tristan da Cunha) Ordinance 2006' which replaced the previous conservation ordinance from 1976 gives protection to all all native plants including *T. magellanicum*. The island was also inscribed to the UNESCO World Heritage List in 1995 (modified in 2004) as part of the Gough and Inaccessible Islands World Heritage Site. Nevertheless, threats to the island's ecosystems are not to be underestimated and include (further) introduction of non-native species and climate change (e.g., Jones & al. 2003, Middleton & Kleinebecker 2012).

Chapter 5

Morphology of *Maundia* supports its isolated phylogenetic position in the early-divergent monocot order Alismatales

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ABSTRACT

According to recent molecular phylogenetic data, the rare Australian endemic *Maundia triglochinoides* does not form a clade with taxa traditionally classified as members of Juncaginaceae. Therefore, views on the morphological evolution and taxonomy of Alismatales require re-assessment. As the morphology of *Maundia* is poorly known and some key features have been controversially described in the literature, the flowers, fruits, inflorescence axes and peduncles were studied using light and scanning electron microscopy. Inflorescences are bractless spikes with flowers arranged in trimerous whorls. Except in the inflorescence tip (where the flower groundplan is variable), flowers possess two tepals in transversal-abaxial positions, six stamens in two trimerous whorls and four carpels in median and transversal positions. Fruits are indehiscent. The shared possession of orthotropous ovules supports the molecular phylogenetic placement of *Maundia* as sister to a large clade including Potamogetonaceae and related families. *Maundia* and *Aponogeton* spp. share the same highly unusual floral groundplan, a homoplastic similarity that can be explained by spatial constraints in developing inflorescences. The nucellar coenocyte of *Maundia* appears to be unique among monocots. As *Maundia* exhibits a mosaic of features characteristic of other families of tepaloid core Alismatales, its segregation as a separate family is plausible.

Key words: anatomy, Aponogetonaceae, bract, flower, fruit, Juncaginaceae, Maundiaceae, nucellus, ovule, Potamogetonaceae, tepal, vasculature

* Material crucial for this study was provided by the late Surrey Jacobs, who sadly passed away before this study could be completed. He contributed to earlier discussions on *Maundia*, provided important additional information and allowed use of photographs taken by him in the field.

Introduction

The 'core Alismatales' (e.g., Iles, Smith & Graham, 2013), a species-poor but morphologically highly diverse monophyletic group of aquatic and wetland plants traditionally known as the order Helobiae (Engler, 1909; Eckardt, 1964), superorder Alismatanae (Takhtajan, 2009) or Alismatiflorae (Dahlgren, Clifford & Yeo, 1985) or subclass Alismatidae (Takhtajan, 1987, 1997; Les & Tippery, 2013), have long been a focal point of discussion regarding the evolutionary history of monocots. The group was often viewed as a basal monocot lineage, probably sister to the rest of the monocotyledons, an opinion supported by the highly unstable floral groundplan, frequent occurrence of apocarpy and some other features considered as potentially primitive (Wettstein, 1924; Takhtajan, 1966, 1987; Cronquist, 1981). Molecular phylogenetic data do not support the hypothesis of the basal-most placement of Helobiae among monocots, but still show that the order Alismatales belongs to a group of early-divergent monocots (Chase *et al.*, 2000, 2006; Davis *et al.*, 2004; Graham *et al.*, 2006; APG III, 2009; Iles, Smith & Graham, 2013). Thus, Helobiae are significant for the understanding of early monocot evolution. Molecular phylogenetic trees suggest that apocarpy and an unstable flower groundplan could be derived rather than ancestral features in core Alismatales (Doyle & Endress, 2000; Chen *et al.*, 2004; Endress & Doyle, 2009; Remizowa, Sokoloff & Rudall, 2010; Sokoloff, Remizowa & Rudall, 2013). The high interest in members of core Alismatales has made this group one of the most extensively studied with respect to comparative flower morphology and development (reviewed by Posluszny & Charlton, 1993; Posluszny, Charlton & Les, 2000; Remizowa *et al.*, 2012b). However, a few key taxa remain poorly known, particularly due to technical problems with obtaining appropriate plant material. In this paper, we present the first detailed data on peduncle, flower and fruit anatomy in *Maundia triglochinoides* F.Muell., a member of core Alismatales whose phylogenetic placement has been re-assessed using molecular phylogenetic data (von Mering & Kadereit, 2010; Iles, Smith & Graham, 2013; Les & Tippery, 2013). The new phylogeny requires an updated comparative analysis of morphological characters in *Maundia* F.Muell., many of which are currently either poorly known or for which existing interpretations are controversial.

The Australian endemic *M. triglochinoides* is an erect rhizomatous perennial herb restricted to freshwater swamps and streams in coastal New South Wales, extending into southern Queensland (Aston, 2011). Due to habitat loss and fragmentation, the range of the species has been much reduced (Sainty & Jacobs, 2003) and it is listed as "Vulnerable" in New South Wales (Schedule 2, Threatened Species Conservation Act) and in Queensland (Schedule 3, Nature Conservation (Wildlife) Regulation 2006 SL No. 206 [Nature Conservation Act 1992]). Traditionally, the monotypic genus *Maundia* was classified as a member of the family Juncaginaceae (Mueller, 1858; Hutchinson, 1959; Eckardt, 1964; Cronquist, 1981; Dahlgren, Clifford & Yeo, 1985; Haynes, Les & Holm-Nielsen, 1998; Takhtajan, 1966, 2009; Seberg, 2007). Earlier classifications treated the family in a wide sense to include *Scheuchzeria* L. using the names Juncaginaceae s.l. (Buchenau & Hieronymus, 1889; Shipunov, 2003) or Scheuchzeriaceae (Buchenau, 1903), respectively. Nakai (1943) proposed a monogeneric family Maundiaceae. Subsequently, this family was accepted by Takhtajan (1987, 1997). Molecular data supported the idea that *Maundia* should be excluded from Juncaginaceae (von Mering & Kadereit, 2010). According to Iles, Smith & Graham (2013), *Aponogeton* L.f. (Aponogetonaceae), *Scheuchzeria* (Scheuchzeriaceae), *Triglochin* L. (Juncaginaceae) and *Maundia* form successive branches in a grade leading to a group of more specialized aquatic Alismatales such as Zosteraceae, Potamogetonaceae, Posidoniaceae, Cymodoceaceae and Ruppiaceae.

To date, there is no consensus regarding the family placement of *Maundia*. Aston (2011) and Reveal & Chase (2011) continued to use the traditional concept of Juncaginaceae, while Reveal (2011), Stevens (2001+) and Les & Tippery (2013) accepted the monogeneric Maundiaceae. Furthermore, APG III (2009) suggested that more study is needed before Maundiaceae is recognized as another monogeneric family in Alismatales. According to APG III (2009) and Stevens (2001+), it might be better in this case to create a larger single family for the larger clade. As reviewed by von Mering & Kadereit (2010), several morphological characters of *Maundia* flowers are controversially interpreted in the literature. These include the presence or absence of a perianth and bracts and interpretation of stamens as bisporangiate and monothecal or tetrasporangiate and dithecal, respectively. We use our new anatomical evidence to discuss these issues. In addition, we improve existing descriptions of carpel arrangement in *Maundia*. Ovule type (orthotropous vs. anatropous) was used as the main morphological character distinguishing Maundiaceae from Juncaginaceae (Nakai, 1943; Takhtajan, 1987, 1997). Although the orthotropous ovule is nearly always indicated in descriptions of *Maundia*, detailed descriptions of ovule anatomy are not available. Existing descriptions of fruit morphology in *Maundia* are controversial (e.g., Bentham, 1878; Cronquist, 1981; Aston, 2011). As pointed out by Thieret (1988), gynoecia of *Maundia* and *Tetroncium* Willd. apparently differ from those of other Juncaginaceae, and a developmental study of fruits of both genera is called for. The present study improves knowledge on fruits and seeds/ovules of *Maundia*. Finally, we provide observations on the vegetative anatomy and pollen morphology of *Maundia*.

Material and methods

The following collection was studied: *Maundia triglochinoides*: Australia, New South Wales, Porters Creek Wetland, Wyong, entry point into swamp, 33º15′36.7′′S, 151º26′11.4′′E, elev. 14 m, 3 Dec 2008, *L. Stanberg & G. Sainty LS 80* (NSW-810429, duplicates in C, K, MJG). The plant occurred in a depression (20% shaded) in *Melaleuca linearifolia* (Link) Craven woodland with occasional scattered *Eucalyptus robusta* Sm., with associated species such as *Typha orientalis* C.Presl., *Villarsia exaltata* (Sims) G.Don, *Persicaria* sp., *Baumea rubiginosa* Boeckeler, *B. articulata* (R.Br.) S.T.Blake, *Alternanthera denticulata* R.Br., *Juncus polyanthemus* Buchenau, *Lachnagrostis filiformis* Trin., *Sagittaria platyphylla* (Engelm.) J.G.Sm., *Carex polyantha* F.Muell., *C. appressa* R.Br., *Cyperus eragrostis* Lam., *Ranunculus inundatus* R.Br. ex DC., *Hypolepis* sp. In this locality, *Maundia* was locally abundant, forming an almost pure continuous stand in a depression covering c. 1 acre, in water 20–30 cm deep.

Inflorescences and fruits were fixed in FAA and stored in 70% ethanol. For light microscope observations, material was sectioned using standard methods of paraplast embedding and serial sectioning at 15 mm thickness (e.g. Barykina *et al*., 2004). Sections were stained with picroindigocarmine and carbolic fuchsine (Axenov, 1967) or alcian blue and safranin and mounted in Biomount. Both cross sections and longitudinal serial sections were made, of flowers, fruits and inflorescence axes. In addition, free-hand sections of peduncles and fruits were treated with phloroglucinol and hydrochloric acid (to reveal lignification of cell walls) or I/KI (to reveal starch) and subsequently observed in glycerol. Sections were examined and images of them were taken using a Zeiss Axioplan microscope. Three-dimensional models of floral vasculature were constructed using 3D-Doctor. For scanning electron microscopy (SEM), the material was dissected in 96% ethanol and dehydrated through absolute acetone and critical-point dried using a Hitachi HCP-2 critical point dryer, then coated with gold and palladium using an Eiko IB-3 ion-coater (Tokyo, Japan) and observed using CamScan 4 DV (CamScan, UK) at Moscow University. In addition to fixed material, herbarium specimens from several collections were studied. These are listed in the Appendix. Terminology used in the Results section reflects our preferred morphological interpretation; other interpretations are reviewed and critically evaluated in the Discussion.

Results

GENERAL MORPHOLOGY, PEDUNCLE ANATOMY AND INFLORESCENCE STRUCTURE

Maundia triglochinoides (Fig. 1A–E) is a perennial aquatic herb with all leaves restricted to a creeping rhizome. Erect ±flat linear, eligulate foliage leaves emerge from the water. Inflorescence peduncles are terete, long, exposing flowers above the water level, and lacking any foliage leaves or scales.

The peduncle (Figs 1F–I, 2) is covered by a one-layered epidermis. Epidermal cells are elongated along the peduncle. Stomata are present at least in the distal part of the peduncle (Fig. 2D). Guard cells are elongated along the length of the peduncle. Cortex is either absent (in this case, the outermost vascular bundles are adjacent to the epidermis) or represented by one to eight layers of thin-walled cells (Figs 1G–I, 2A). The stele contains numerous vascular bundles arranged without a clear pattern in cross-sections of peduncle. Central bundles are larger than peripheral ones (Fig. 1A).

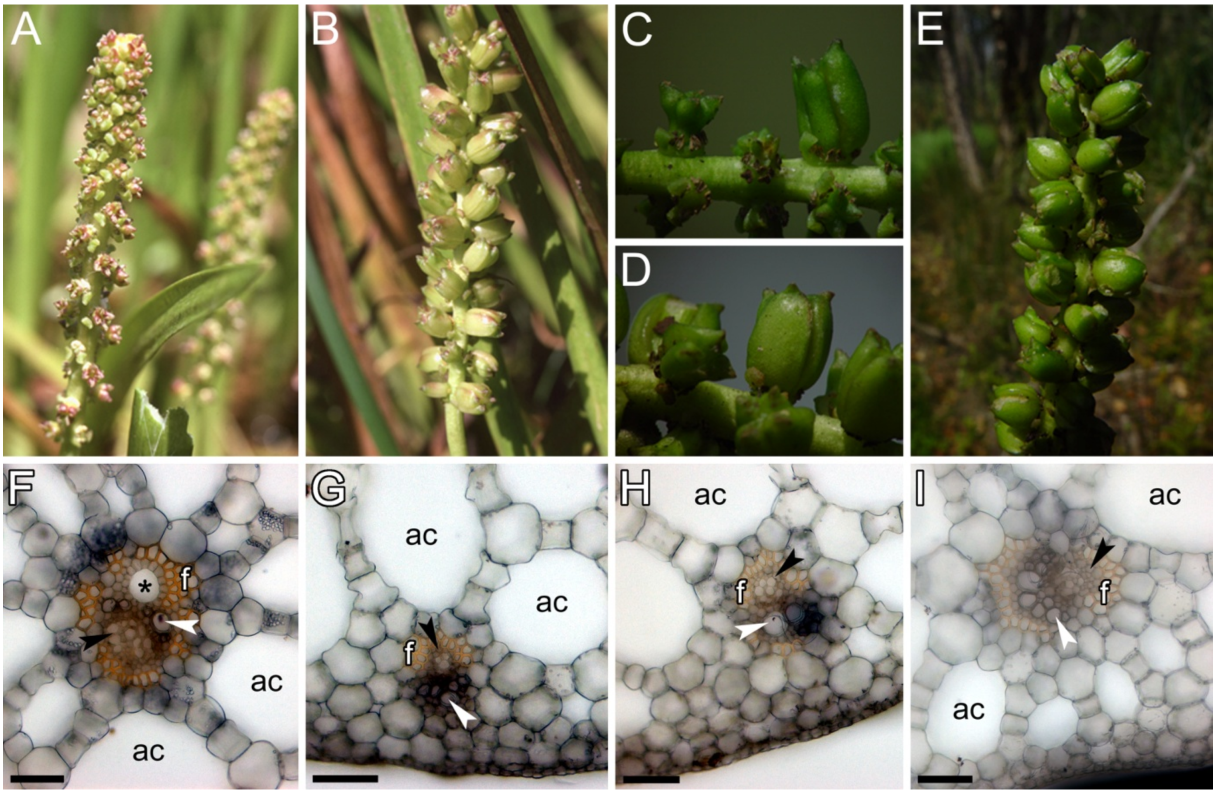

Fig. 1. *Maundia triglochinoides*. A–E, living plants (photographs taken in nature by S. Jacobs). A, inflorescence (bractless spike). B, postanthetic inflorescence. C,D, details of immature fruits. E, inflorescence axis with fruits. F–I, details of free-hand transverse section of peduncle, treated with phloroglucinol and hydrochloric acid (lignified cell walls orange). F, one of central vascular bundles. G–I, peripheral vascular bundles, epidermis of peduncle bottom. G,H, inverted peripheral bundles (typical condition). I, obliquely oriented bundle (rare condition). Scale bars, 100 µm (F–I). ac, air canals; f, fibres associated with vascular bundle; black arrowhead, phloem; white arrowhead, xylem tracheid; asterisk, protoxylem lacuna.

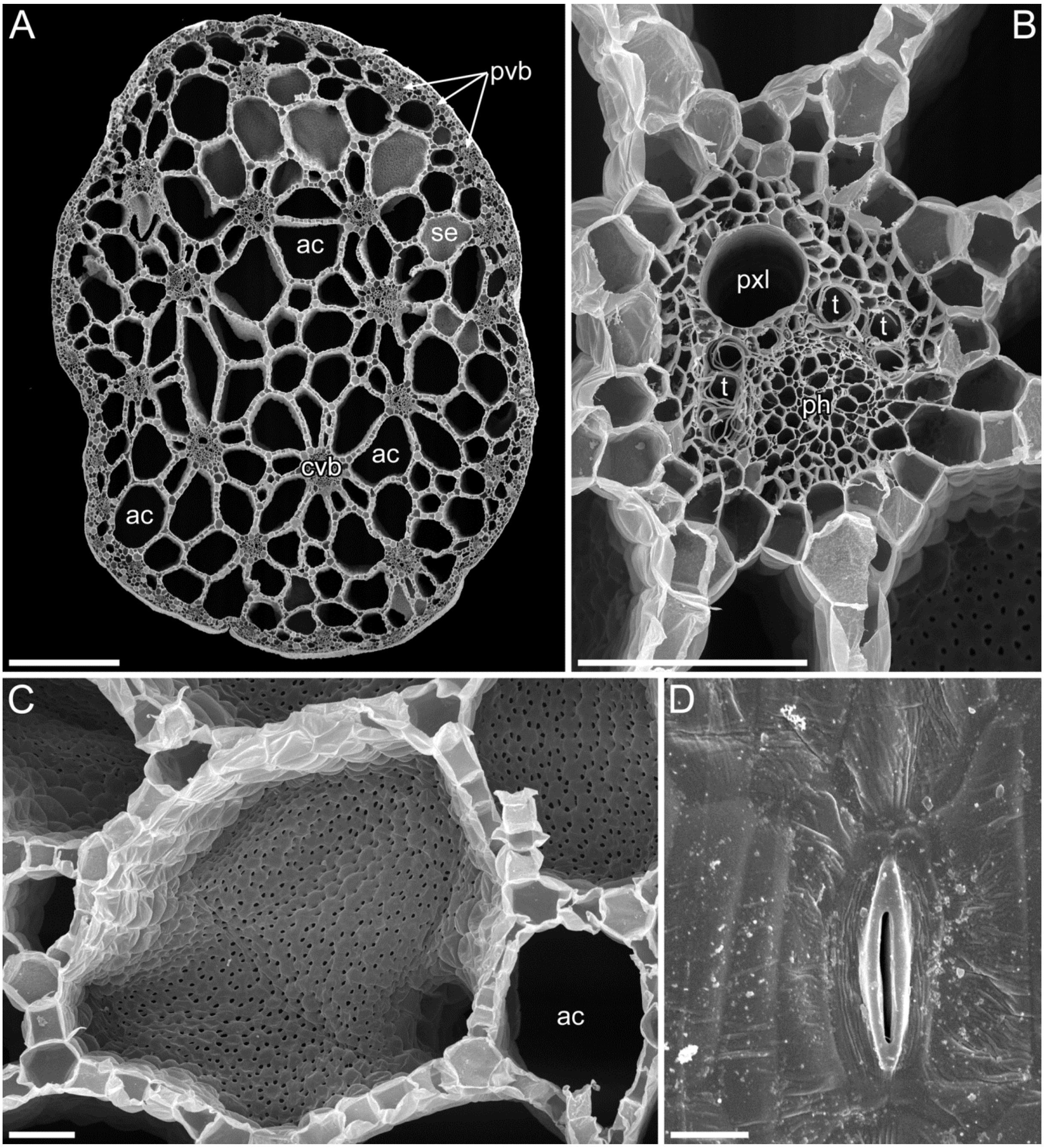

Fig. 2. *Maundia triglochinoides*. Peduncle anatomy and stoma on peduncle (scanning electron microscopy, SEM). A, Peduncle in transverse section. B, Detail of vascular bundle. C, Septum on air canal. D, Stoma on peduncle. Scale bars: 1 mm (A); 200 µm (B); 100 µm (C); 10 µm (D). ac, air canal; cvb, large central vascular bundle; ph, phloem; pvb, small peripheral vascular bundle; pxl, protoxylem lacuna; se, septum in air canal; t, tracheid with spiral thickenings.

The vascular bundles are collateral (Figs 1F–I; 1B). In large bundles, the protoxylem is represented by a lacuna (Figs 1F, 2B). The tracheids of the metaxylem form a horseshoe-shaped row in cross-sections, adjacent to the phloem (Fig. 1B). Tracheids possess spiral thickenings (up to five parallel spirals per cell). Vessels were not observed. Most peripheral bundles are inverted, i.e., with xylem oriented towards the epidermis and phloem towards the centre of the peduncle (Fig. 1G, H). One of the observed peripheral bundles was obliquely oriented (Fig. 1I). Each large bundle is surrounded by an almost complete sheath of thin-walled lignified fibres (Fig. 1F). Smallest bundles possess fibres

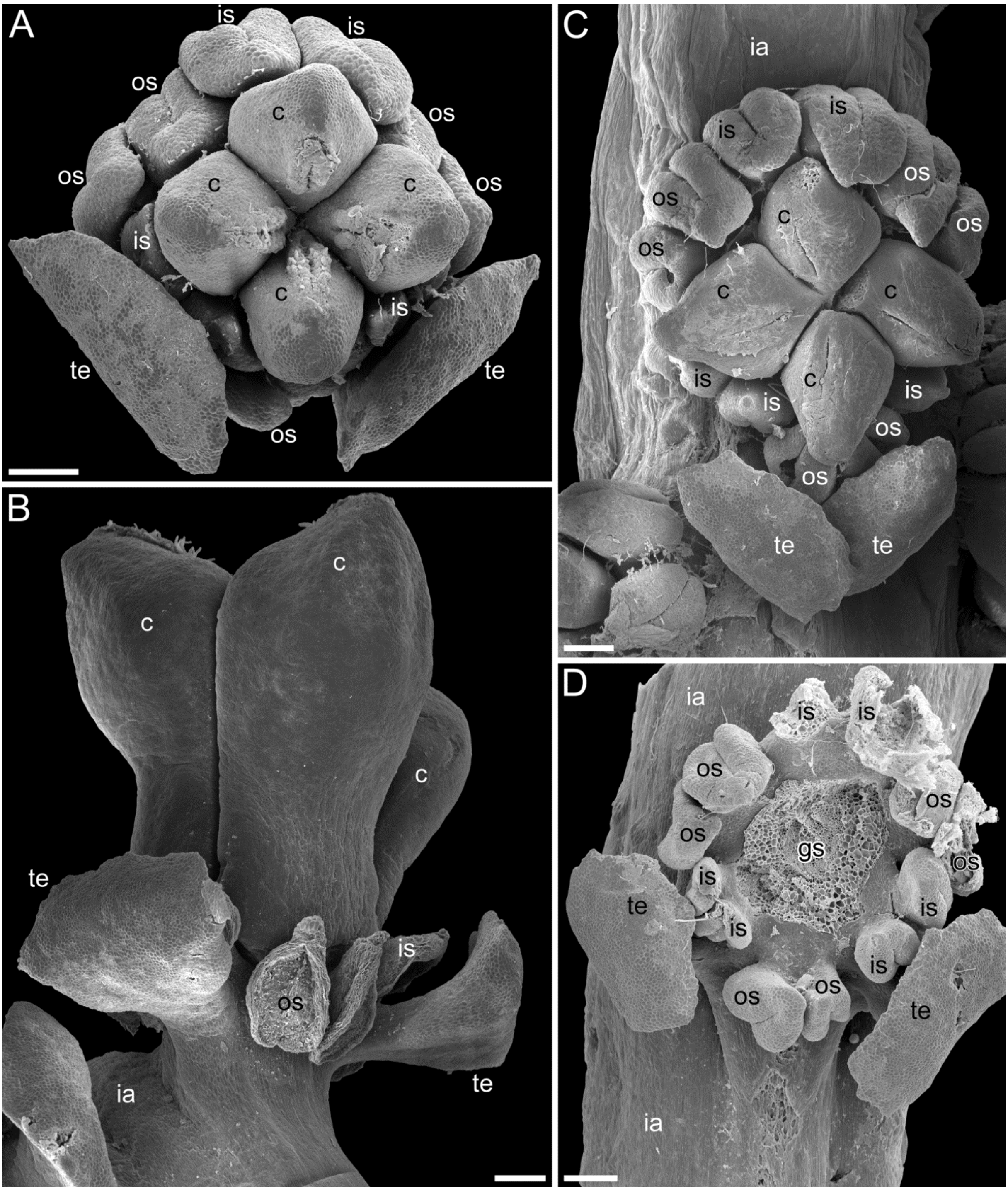

Fig. 3. *Maundia triglochinoides*. Flower groundplan, normal flowers (scanning electron microscopy, SEM). A, Flower from the central part of the inflorescence, top view. B, Post-anthetic flower from the abaxial side. C, Flower from the inflorescence base, top view. D, Flower with gynoecium removed. Scale bars: 500 µm (A–D). c, carpel; gs, stalk of removed gynoecium; ia, inflorescence axis; is, inner-whorl stamen; os, outer-whorl stamen; te, tepal.

along the phloem side only (Fig. 1G). Medium-sized bundles possess two groups of fibres, i.e., along the xylem and the phloem side (Fig. 1I). All space between the bundles is filled by an aerenchyma with large air canals separated by uniseriate files of thin-walled cells (Fig. 1A); these cells contain starch grains (there are fewer grains in the cells at the periphery of the peduncle). Along the length

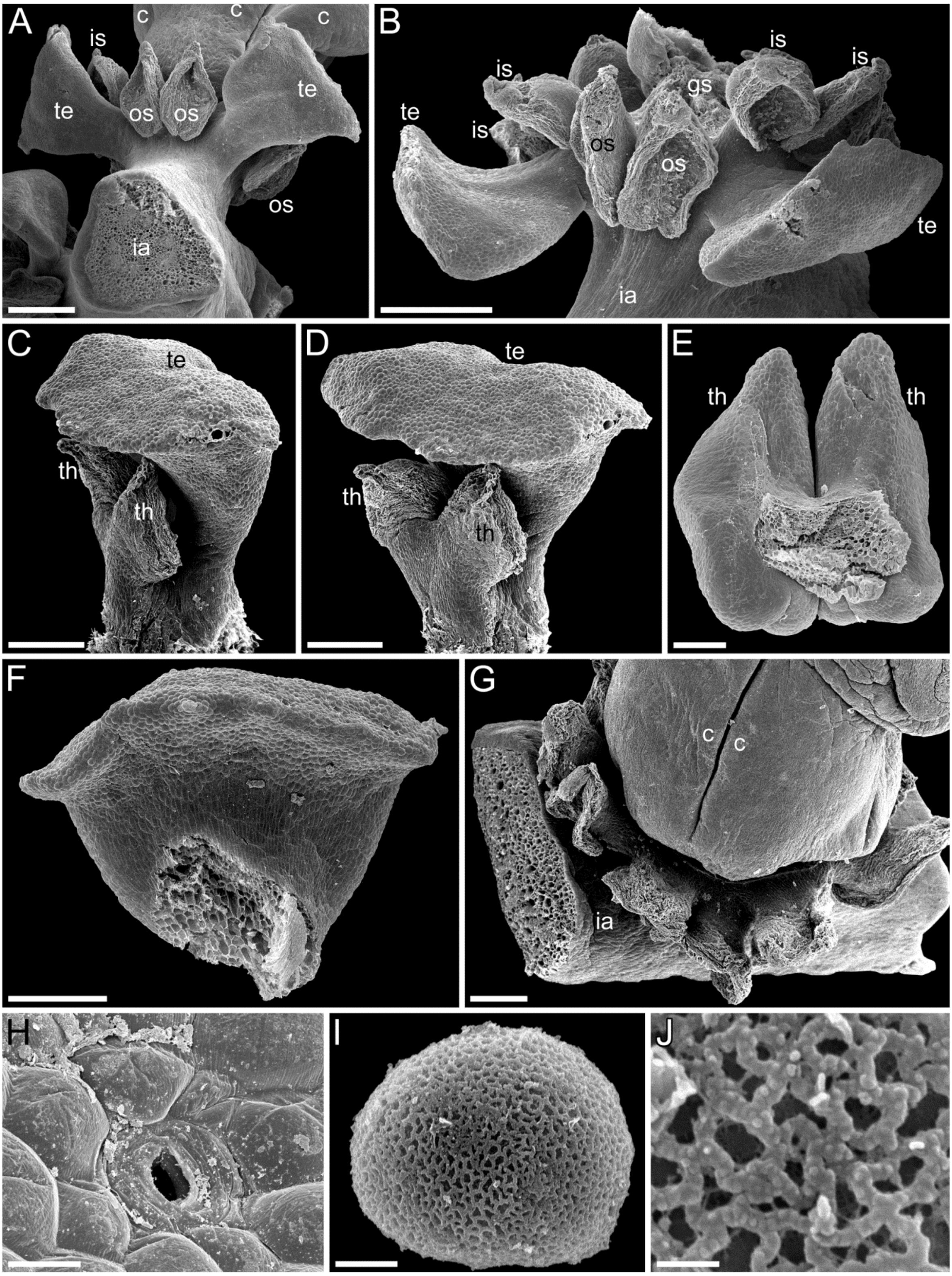

Fig. 4. *Maundia triglochinoides*. Stamens, tepals, pollen grains, stoma on tepal (scanning electron microscopy, SEM). A, Median abaxial outer-whorl stamen not associated with a tepal and tepals on radii of transversal-abaxial inner-whorl stamens. B, Flower with gynoecium removed showing median abaxial outer-whorl stamen, two tepals and inner-whorl stamens situated on radii of the tepals. C, D, Tepal–stamen pairs in different views. E, Removed stamen, adaxial view. F, Removed tepal, adaxial view. G, Basal part of young fruit to show non-abscised stamens. H, Stoma on tepal. I, Pollen grain. J, Exine sculpture. Scale bars: 1 mm (A, B); 500 µm (C, D, F, G); 300 µm (E); 20 µm (H); 5 µm (I); 1 µm (J). c, carpel; gs, stalk of removed gynoecium; ia, inflorescence axis; is, inner-whorl stamen; os, outer-whorl stamen; te, tepal; th, theca.

of the peduncle, the air canals are divided into chambers by transverse septa (Fig. 1A, C). Narrow perforations connecting adjacent air lacunae are present between cells forming a septum (Fig. 1C). Cells forming septa lack starch grains. No specialized mechanical elements are present in peduncles except the fibres associated with vascular bundles.

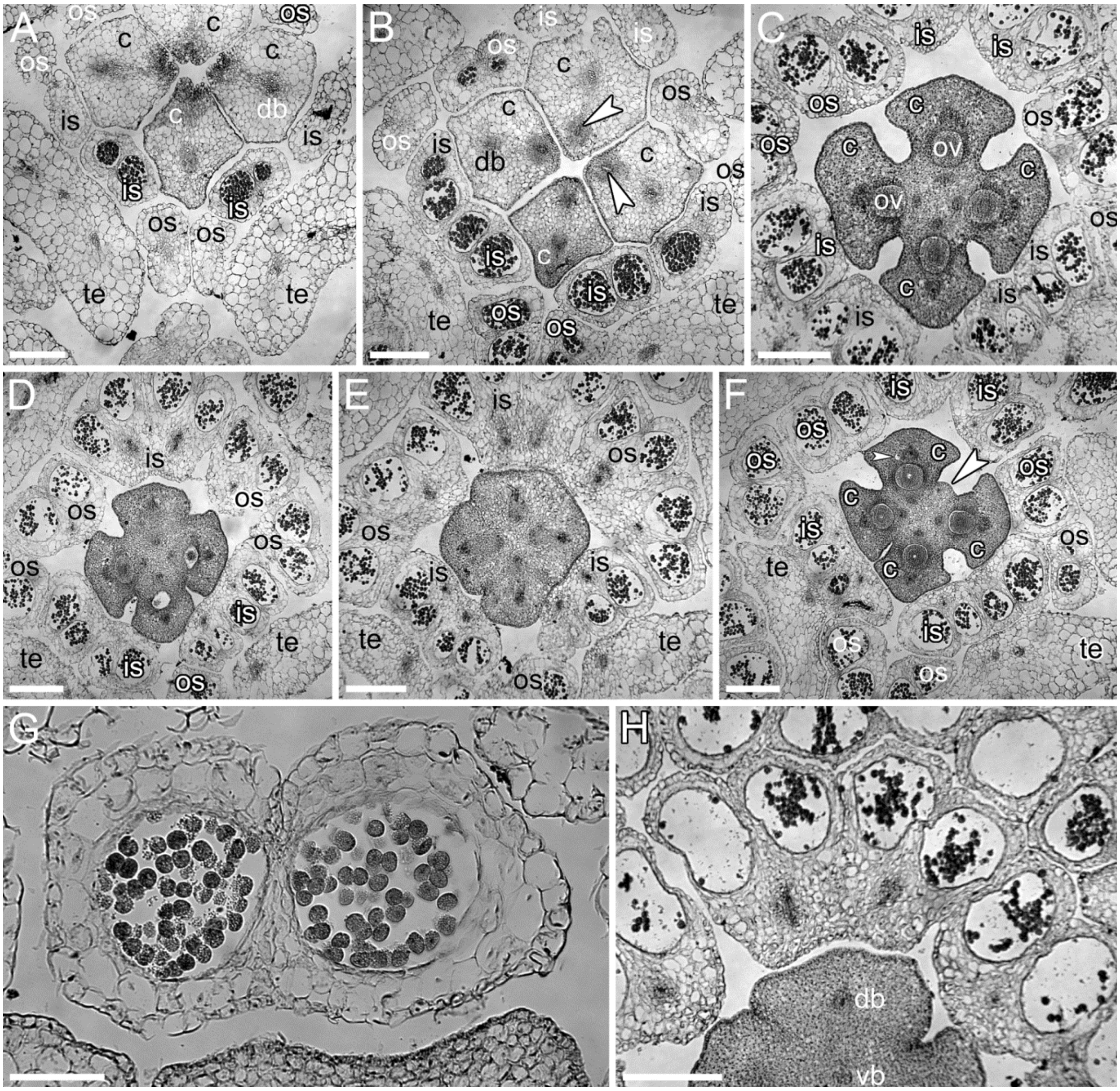

Fig. 5. *Maundia triglochinoides*. Transverse sections of anthetic flowers (light microscopy, LM). A–E, A descending series of sections of a flower. A, Level of oblique carpel mouths. B, Level of free ascidiate carpels with carpel canals (arrowheads) displaced towards ventral sides of carpels. C, Carpels are united via floral centre, just below the level of ovule attachment; note four ventral carpel bundles in the central part of the gynoecium. D, About the level of the micropyles of ovules (below the micropyle in the upper carpel, above the micropyle in the left-hand carpel); at this level, thecae of the inner-whorl stamens are united, whereas thecae of the outer-whorl stamens are free from each other. E, Below the ovary locules, each carpel with a dorsal and a ventral bundle; thecae of outer-whorl stamens are united. F, Another flower with asymmetric carpel arrangement and an unvascularized bulge (large arrowhead) that could be interpreted as an incipient fifth carpel. Small arrowhead indicates an enlarged cell flanking the dorsal bundle; these cells will be conspicuous in fruits. G, Stamen theca at the level above separation of free thecae. H, Anther at the level of united thecae; note the occurrence of two vascular bundles. Scale bars: 300 µm (A–F, H); 100 µm (G). c, carpel; db, dorsal carpel bundle; is, inner-whorl stamen; os, outer-whorl stamen; ov, ovule; te, tepal; vb, ventral bundle.

Inflorescences are unbranched spikes (Fig. 1A). Flowers are arranged along the inflorescence axis in regularly alternating trimerous whorls. In preanthetic inflorescences, the internodes of the inflorescence axis are short, the flowers are densely spaced, and the inflorescence axis is usually not visible without removing flowers. The most proximal internode can be slightly longer than the other internodes (this is also typical for several species of *Triglochin* – S.v.M., unpubl. data). Six orthostichies of flowers can be easily recognized. In postanthetic inflorescences, internodes of the inflorescence axis are longer and visible between the flowers or young fruits. At these stages, different flowers of the same whorl may be inserted at slightly different levels of the inflorescence axis. No flower-subtending bracts (or any rudiments) were observed (Figs 3B, D, 4A, B). Flowers are completely sessile, sometimes with the exception of the uppermost flowers in a spike. As no flower stalks are developed after anthesis, fruits remain sessile and perpendicular to the inflorescence axis (Fig. 1C–E).

MORPHOLOGY AND VASCULAR ANATOMY OF FLOWERS

Almost all flowers, except the uppermost ones, exhibit a stable groundplan. There are two tepals in transversal-abaxial positions, six stamens in two alternating trimerous whorls (an outer whorl with a median abaxial and two transversal-adaxial stamens and an inner whorl with a median adaxial and two transversal-abaxial stamens) and four carpels, of which two are in median and two in transversal positions (Figs 3, 4A, B, 5A–E). In one flower, a small, unvascularised outgrowth was found in the transversal-adaxial position; this could be interpreted as an incipient fifth carpel (Fig. 5F, arrowhead). In this flower, two carpels situated on the opposite radius were more closely spaced than in typical flowers (Fig. 5F).

Tepals are green and ca. 1.5 times as long as the stamens. They have narrow bases and are attached to the receptacle at the radii of the transversal-abaxial inner whorl stamens (Figs 3, 4A, B, 5A–F). Stamen and tepal bases can unite for a very short distance (Fig. 4C, D). The tepals are inserted at approximately the same distance from the flower centre as the outer-whorl stamens (Figs 3B, 4A, B). They have a short claw gradually extended into an almost orbicular blade, which is curved inwards (Fig. 4C, D, F). The tepal blade is conspicuously thick, consisting of several cell layers in cross sections of the middle part (Fig. 5A). Abundant stomata are present on the abaxial surface of the tepal blade. The guard cells are bean-shaped (Fig. 4H). Distinct cuticular ridges are present along the outer orifice of the aperture. Epidermal cells in the immediate vicinity of the guard cells are smaller than the rest of the epidermal cells; their number and arrangement relative to the guard cells do not appear to be precisely fixed.

Stamens are yellow, tetrasporangiate and dithecal (Fig. 5H). Stamen filaments are absent, and anther connectives are wide (wider than long) and very short, ca. one third as long as the thecae (Fig. 4C–E). Free thecae are strongly extended above the connective and slightly extended below the connective (Figs 4E, 5). Anther dehiscence is extrorse (Figs 3B, C, 4A, B). The line of dehiscence terminates a short distance from the acute distal end of a theca. At the obtuse proximal end of a theca, the dehiscence line curves from the outer to the inner side of the theca (Fig. 4E). The stamen epidermis lacks stomata. The endothecium cells show fibrous thickenings. Pollen grains are spherical, inaperturate, and the pollen surface is reticulate (Fig. 4I, J).

Carpels are pronouncedly ascidiate. In mature pre-anthetic flowers (the youngest stage available in this study), the carpels are congenitally united at the base via the floral centre, and the united part is about as long as the free parts of the carpels (Fig. 6B). The growth of the ventral sides of free parts of the carpels is apparently delayed with respect to their dorsal sides on early stages of

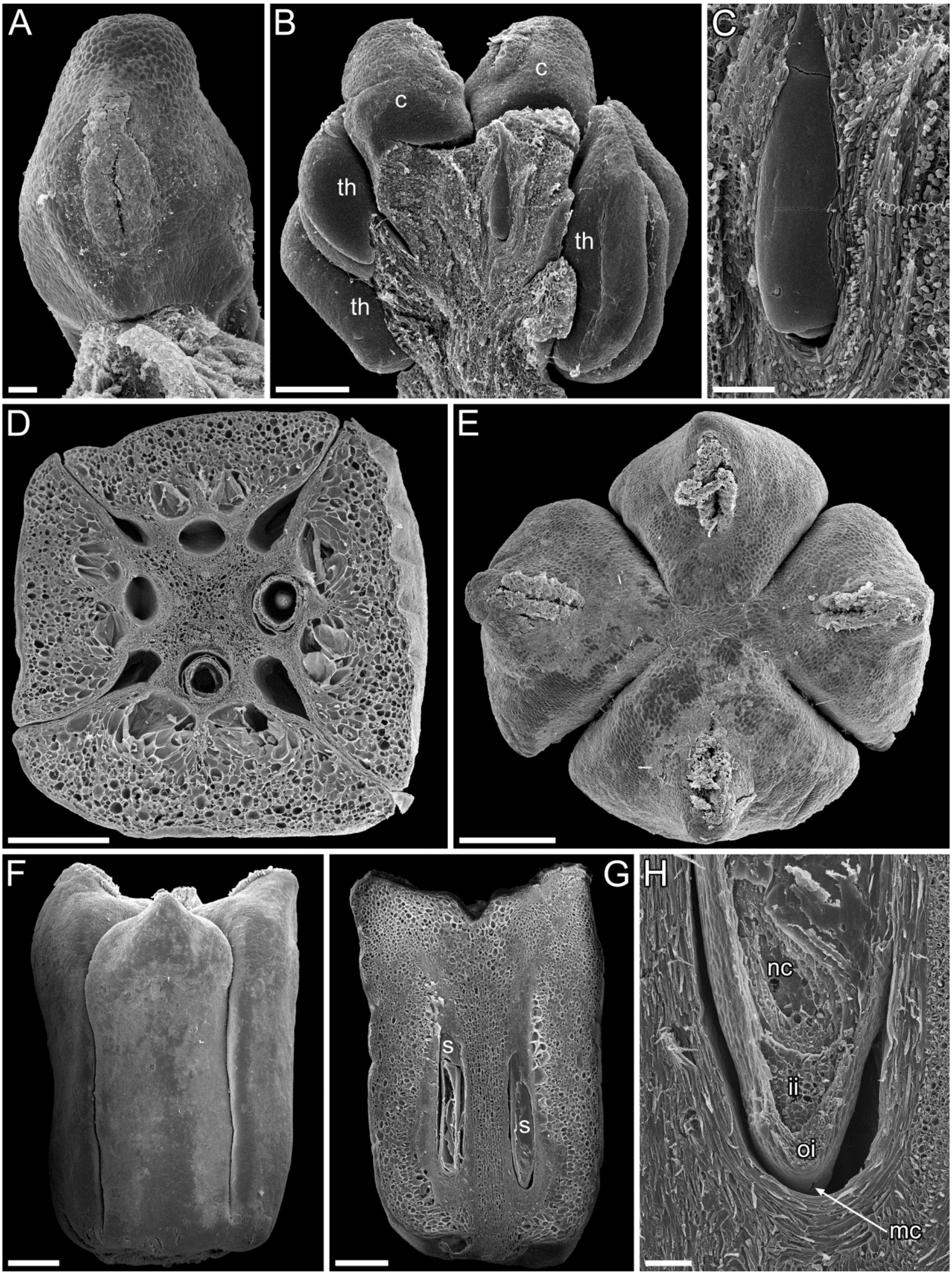

Fig. 6. *Maundia triglochinoides*. Gynoecium at anthesis and fruits (scanning electron microscopy, SEM). A, Free part of carpel at anthesis, ventral side. B, Longitudinally dissected anthetic flower showing relative length of united and free parts of carpels. C, Detail of (B) showing ovule. D, Cross-section of young fruit. E, Top view of young fruit. F, Side view of young fruit. G, Longitudinal section of young fruit. H, Detail of (G). Scale bars: 100 µm (A, C, H); 500 µm (B); 1 mm (D, E, F, G). c, carpel; ii, inner integument; mc, micropyle; nc, nucellus; oi, outer integument; s, developing seed; th, stamen theca.

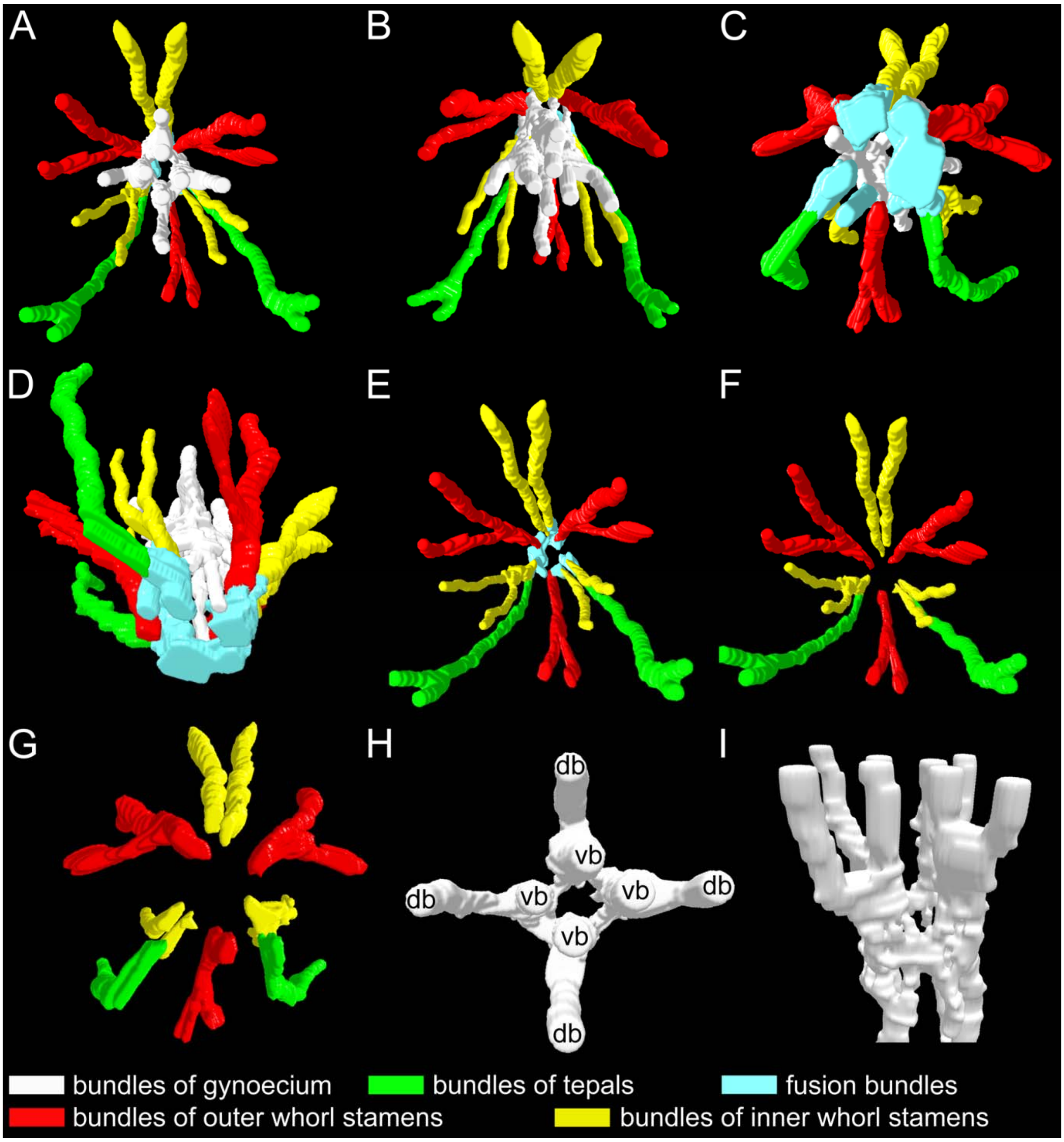

Fig. 7. *Maundia triglochinoides*. Three-dimensional reconstructions of floral vasculature. Fusion bundles = bundles formed by fusion of traces from different organ types. A–D, Different views of the entire floral vasculature. A, Top view. B, Oblique top view. C, Bottom view. D, Side view. E, Top view of vasculature with gynoecium bundles removed. F, Top view of vasculature with gynoecium and fusion bundles removed. G, The same as (F), bottom view. H, I, Top (H) and side (I) view of gynoecium vasculature. db, dorsal bundle; vb, ventral bundle.

development. In the earliest available stage, the carpel mouth (which is strongly oblique) is located on its inner side but does not extend along the entire length of the free part of a carpel (Fig. 6A, B). On the inner side of the free part of a carpel, there is a distinct congenitally closed portion below the mouth, which is the morphologically ventral area, and a portion above the mouth, which is part of the morphologically dorsal surface (Fig. 6A). Short stigmatic papillae are present around the carpel mouth (Fig. 6E). In cross sections below the carpel mouth, a canal elongated in a radial plane is

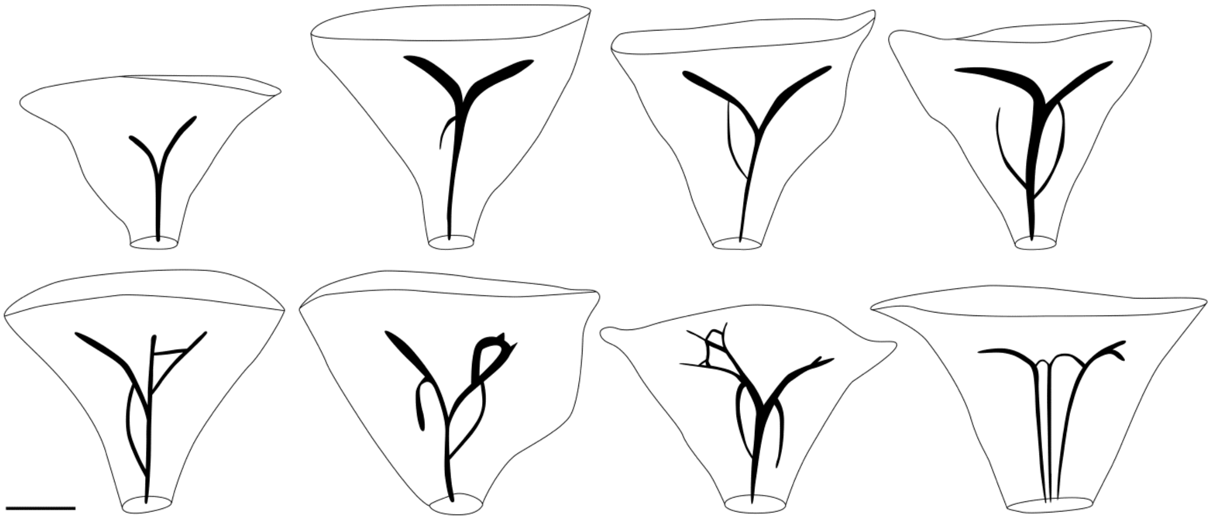

Fig. 8. *Maundia triglochinoides*. Sketches of cleared tepals viewed from the adaxial side showing variations in vasculature. Scale bar (common to all images): 500 µm.

present (Fig. 5B). The canal is very narrow compared to the width of the entire carpel. It is located on the ventral side of the carpel very close to its surface (Fig. 5B).

Each carpel has a single ovule inserted ventrally just below the level of carpel separation from the floral centre (Fig. 6B, C). The ovule is pendent, bitegmic and orthotropous. The micropyle is formed by the inner integument. The ovary locule is narrow compared to the carpel width, and circular in crosssection (Fig. 5C, F). The locule is closer to the floral centre than to the dorsal side of a carpel (Fig. 5C, F). The ovule fills the locule (Figs 5C, F, 6B, C). It is in close contact with the locule wall except in the micropylar region (Fig. 5D).

Each flower is supplied by a single strand of conductive tissues, which could also be viewed as a group of closely spaced bundles. Conductive tissues soon form a complete or incomplete ring (with xylem on the inner and phloem on the outer side) that is subdivided upwards into distinct individual bundles. Each tepal usually receives a single vascular bundle from the receptacle, which further branches in the tepal blade (Figs 5A, B, F, 7A–G, 8). Bundles are located closer to the adaxial than to the abaxial tepal surface. Examination of total removed tepals allowed assessing the variation in tepal vascularisation (Fig. 8). Some of the examined tepals were apparently three-traced. Anastomoses between tepal bundles are usually but not always present. Vein endings remain free. The endings in the tepal blade are usually thicker than the bundle(s) in the tepal claw. Some free endings are directed towards the tepal base (Fig. 8). Each stamen theca has a single vascular bundle extending into the very proximal portion of its free part (Fig. 5D); the rest of the theca is non-vascularised (Fig. 5G). Usually, these two bundles remain distinct in the anther connective (Fig. 5H) and fuse to form a common stamen trace in the flower receptacle (Fig. 7A–G). Inner whorl stamens are variable with respect to the presence or absence of this common stamen trace. In the latter case, the bundle from each theca downwards separately reaches the ring of conductive tissues at the base of the receptacle (Fig. 7E–G). The tepal trace joins the common trace of an inner whorl stamen of the same radius or enters the stele of the receptacle in between the free traces of the two thecae of such an inner whorl stamen (Fig. 7A–G). Each carpel has a dorsal and a ventral bundle (Figs 5B–F, H, 7H, I, 9D–F). The ventral bundle supplies the ovule, then continues for a certain distance along the

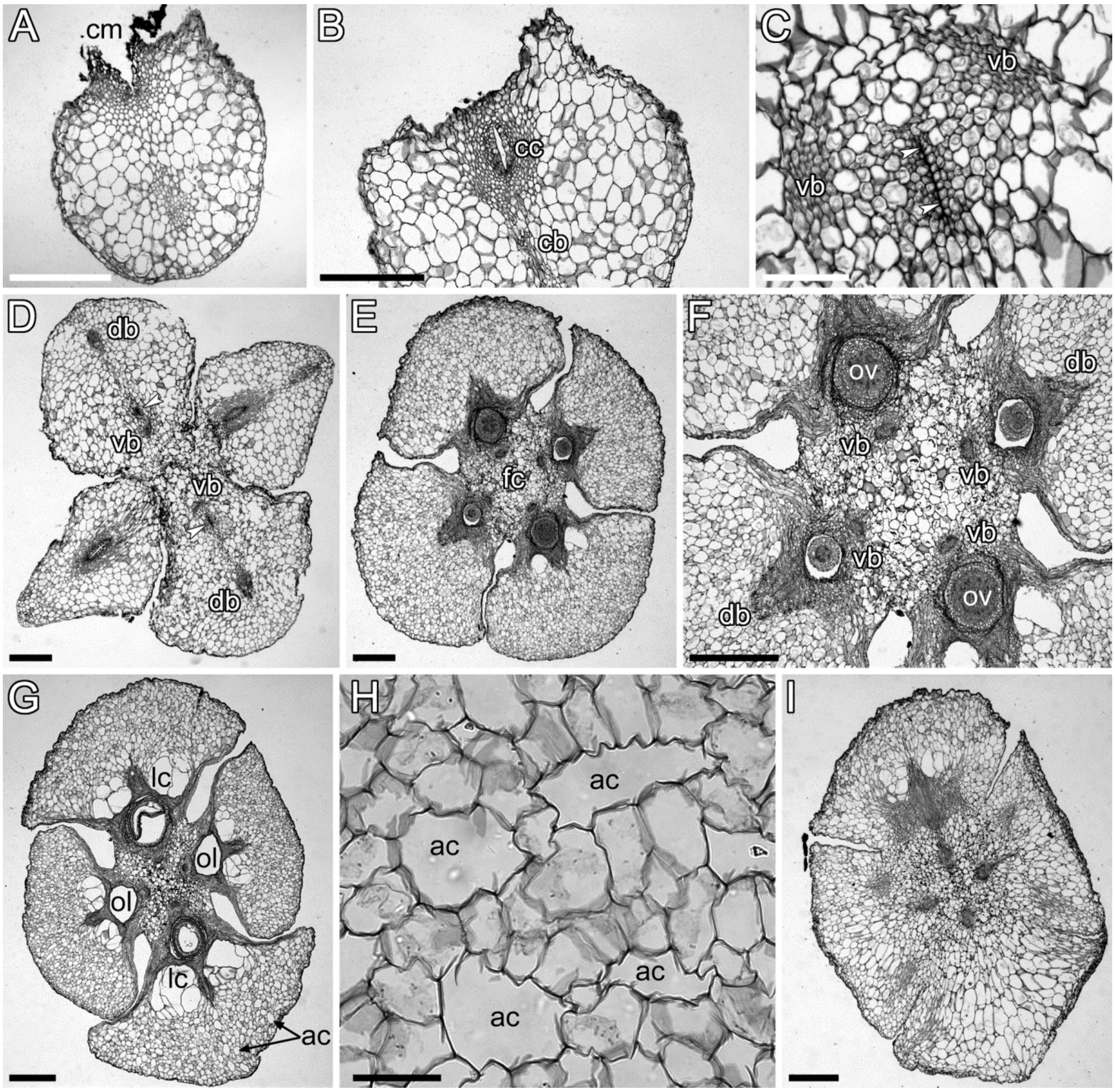

Fig. 9. *Maundia triglochinoides*. Descending series of transverse sections of young fruit (light microscopy, LM). A–C, Upper portion of the fruit with free carpel tips. A, Carpel tip with oblique carpel mouth. B, Carpel clearly ascidiate, with an open canal displaced towards its ventral side. C, Detail of section below (B) showing post-genitally closed carpel canal and two ventral bundles on either side of it. D–I, Levels at which carpels are united via floral centre. D, Just below the level of carpel separation. At this level, the upper left-hand and the lower right-hand carpels have post-genitally closed carpel canals, whereas two other carpels still have open canals. E, Upper part of ovary locules; two carpels with large ovules (sectioned at the level of the funiculus) completely filling the locules and two carpels with smaller, apparently (still?) unfertilized ovules. F, Detail of (E). G, Section at the middle part of the young fruit, below the level of micropyles of the two smaller ovules. H, Detail of pericarp tissue in dorsal part of a carpel with large air canals. I, Below ovary locules, each carpel supplied by a single vascular bundle. Scale bars: 500 μm (A, B, D–G, I); 100 μm (C); 50 μm (H). ac, air canal; cb, commissural vascular bundle connecting dorsal and ventral budles; cc, carpel canal; cm, carpel mouth; db, dorsal bundle; fc, floral centre; lc, large cells in pericarp; ol, ovary locule; ov, ovule; vb, ventral bundle; arrowheads, post-genitally closed carpel canal.

postgenitally closed carpel canal (Fig. 9D) and ultimately splits tangentially into two bundles above the level of its insertion (Fig. 9C). These two branches curve along either side of the carpel canal, approach each other and form a joint commissural bundle that unites with the dorsal bundle (Fig.

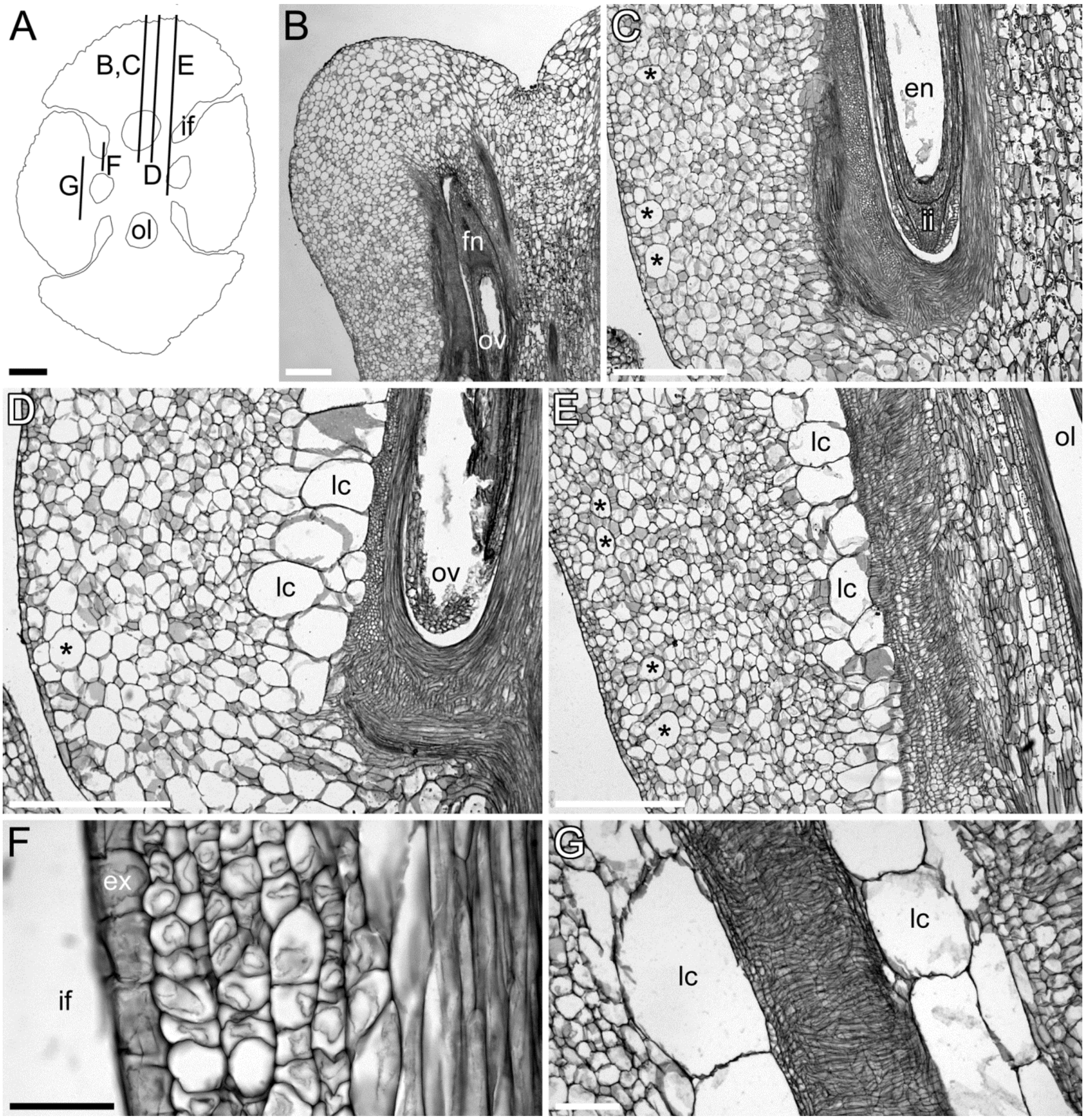

Fig. 10. *Maundia triglochinoides*. Pericarp histology on longitudinal sections of young fruits (light microscopy, LM). A, Outline of a transverse section based on Figure 9G showing orientation of sections illustrated in (B–G). B–G, Longitudinal sections. B, Upper part of a fruit. C, D, Lower part of a fruit. E–G, Middle part of a fruit. Scale bars: 500 µm (A–E); 40 µm (F); 200 µm (G). en, endosperm; ex, exocarp; fn, funiculus; if, intercarpellary furrow; ii, inner integument; lc, large cells in pericarp; ol, ovary locule; ov, ovule; asterisks, air canals.

9B). The dorsal and the ventral bundle of each carpel proximally unite into a single carpel trace (Figs 7H, I, 9I). Proximally in the receptacle, the vascular traces of the four carpels are connected by anastomoses forming an almost complete ring of conductive tissues (Fig. 7H, I). More proximally this ring again splits into separate strands that irregularly join stamen and tepal traces to form the proximal ring of conductive tissue in the flower receptacle (Fig. 7D).

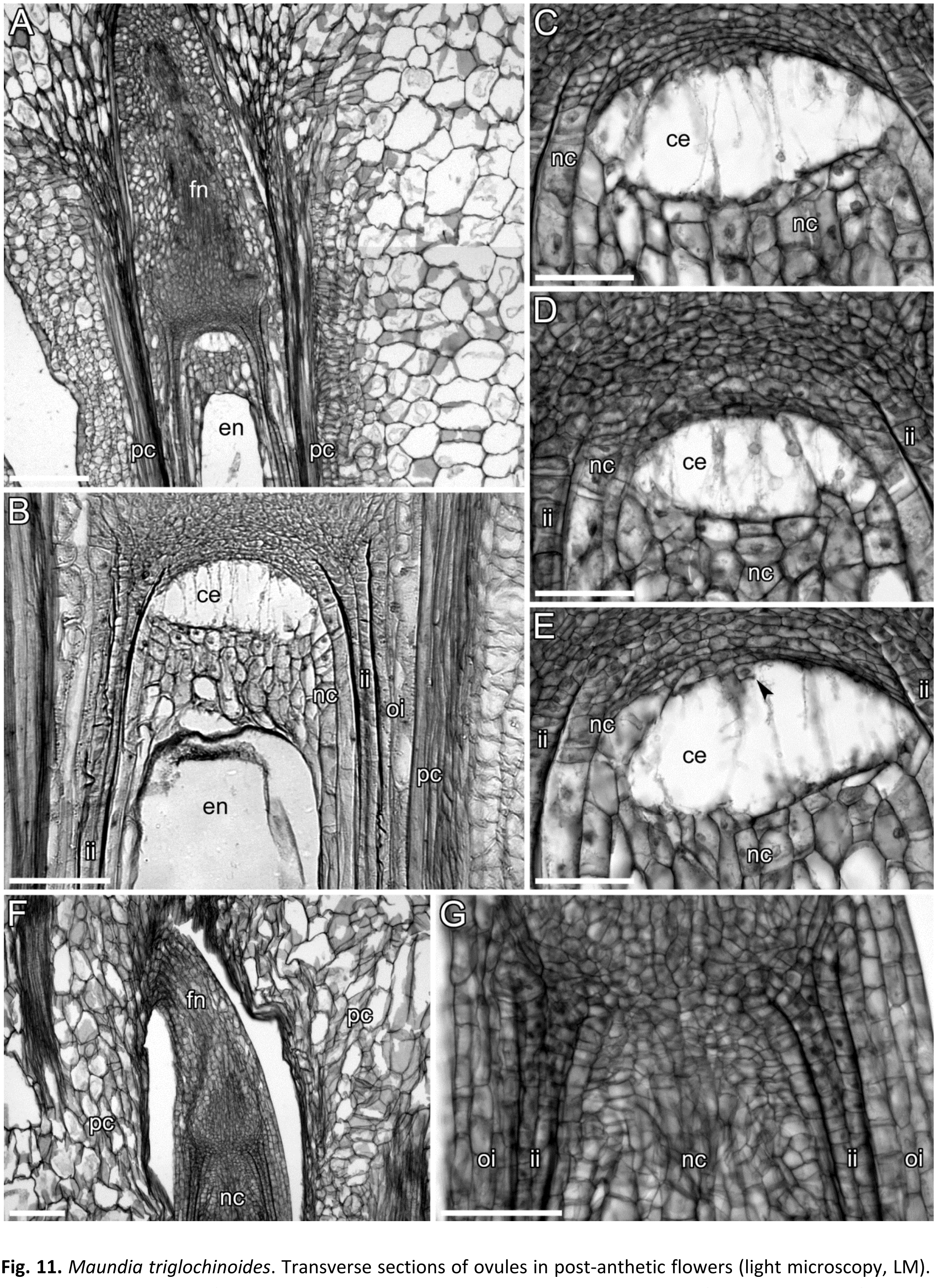

Fig. 11. *Maundia triglochinoides*. Transverse sections of ovules in post-anthetic flowers (light microscopy, LM). A, Funiculus with a ring of vascular tissue. B, Section above endosperm. C, Section of an apparently unfertilized ovule. D–F, Sections at the level of endosperm above embryo. G, Section through globular proembryo surrounded by endosperm. H, Section through gigantic basal cell of suspensor. I, Section through micropyle. Scale bars: 50 µm (A–I). db, dorsal bundle; en, endosperm; gc, gigantic basal cell of suspensor; ii, inner integument (tegmen); lc, large cells in pericarp; nc, nucellus; oi, outer integument (testa); pc, pericarp; pe, globular proembryo; vb, ventral bundle; asterisks, air canals in outer integument.

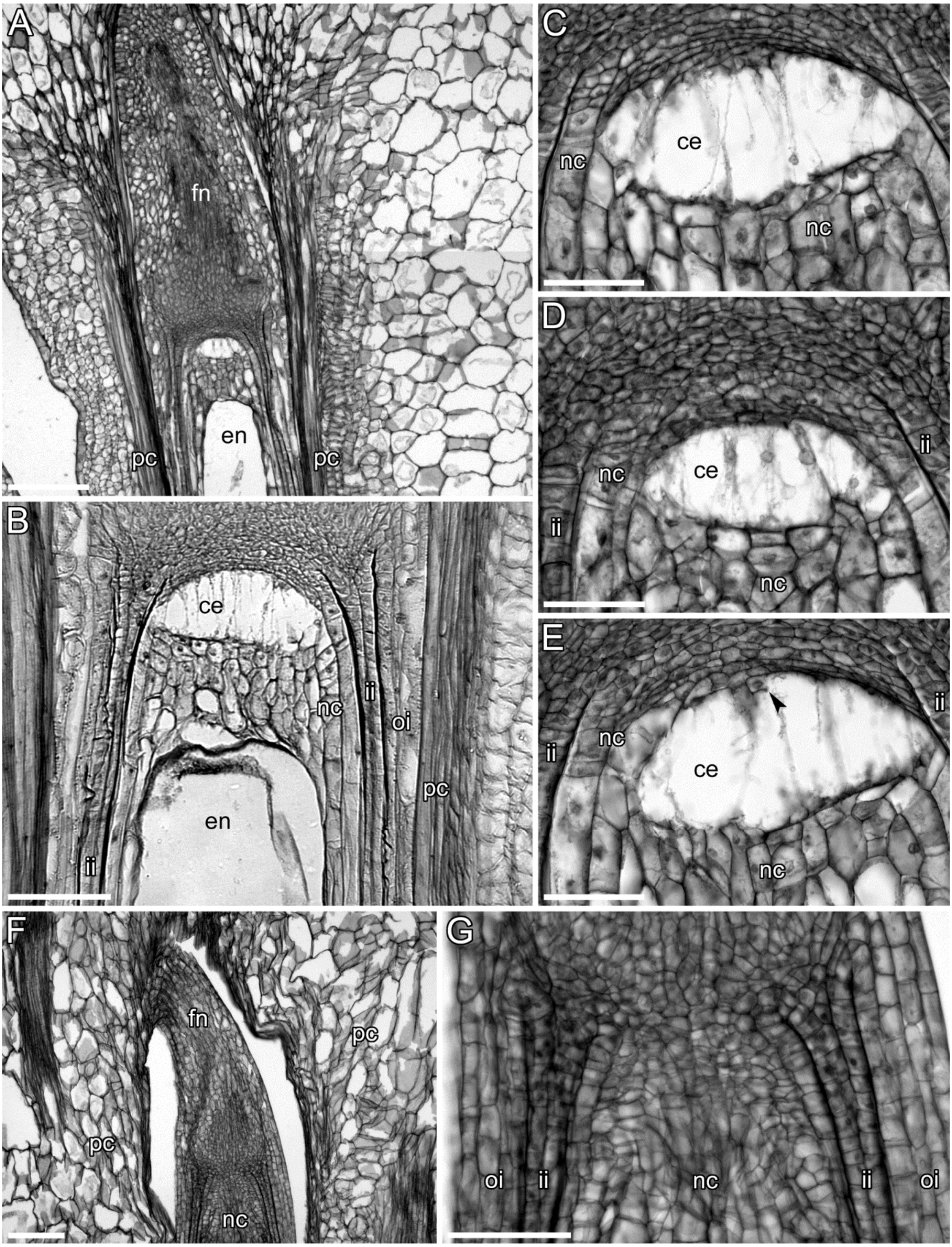

Fig. 12. *Maundia triglochinoides*. Chalazal parts of ovules of post-anthetic flowers on longitudinal sections (light microscopy, LM). A, Funiculus, chalaza and adjacent part of nucellus. B, Chalazal part of nucellus with nucellar coenocytic structure. C–E, Successive serial sections through a nucellar coenocyte. F, Funiculus, chalaza and adjacent part of nucellus of an apparently unfertilized ovule. G, Detail of (F). Scale bars: 200 µm (A); 100 µm (B, F); 50 µm (C–E, G). ce, nucellar coenocyte; en, endosperm, fn, funiculus; ii, inner integument (tegmen); nc, nucellus; oi, outer integument (testa); pc, pericarp; arrowhead, free end of degenerating wall between nucellar cells forming coenocyte.

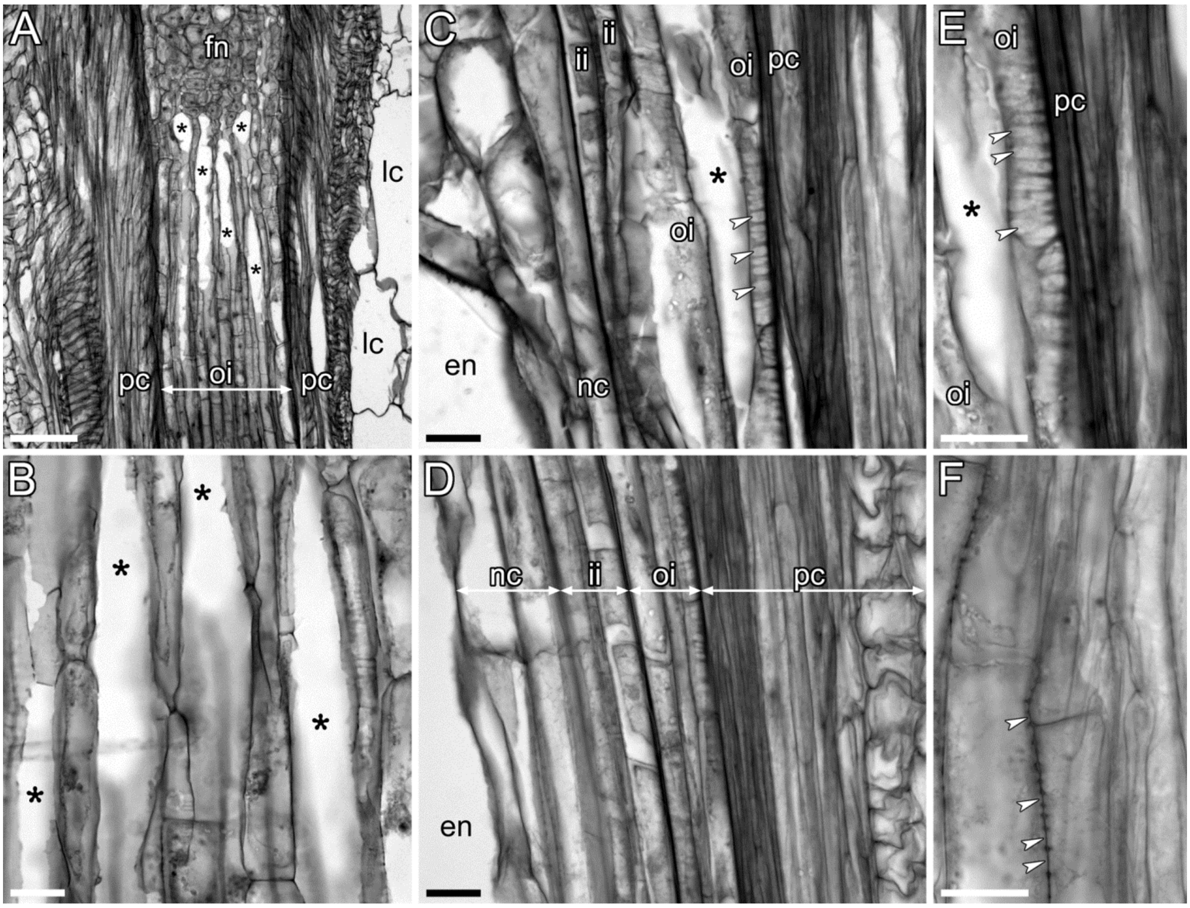

Fig. 13. *Maundia triglochinoides*. Outer integument (testa) anatomy on longitudinal sections of fertilized ovules (light microscopy, LM). A, Tangential section of an ovule through middle layer of outer integument. B, Detail of (A). C–E, Radial sections of ovules. C, Level of upper part of endosperm. D, Level of middle part of endosperm. E, Detail of outer epidermis of outer integument showing wide pores in anticlinal walls. F, Tangential section through outer epidermis of outer integument showing anticlinal walls in cross-section. Scale bars: 100 µm (A); 20 µm (B–F). en, endosperm; fn, funiculus; ii, inner integument; lc, large cells in pericarp; nc, nucellus; oi, outer integument (testa); pc, pericarp; asterisks, air canals in outer integument; arrowheads, pores in anticlinal walls of outer epidermis of outer integument (exotesta).

POST-ANTHETIC CHANGES IN GYNOECIA AND OVULES OF TYPICAL FLOWERS

Most, but not all flowers in an inflorescence develop fruits. In addition, a fruit may develop fewer than four seeds. This may be due to failure of cross-pollination if the plants are self-incompatible.

After pollination, the part of the gynoecium, where the carpels are united via the floral centre (Figs 6D, 9E–G), elongates considerably and becomes much longer than the free parts of the carpels (Fig. 6F, G). In addition, growth is more extensive in the floral centre and in ventral parts of the carpels. As a result, the carpel mouth surrounded by remains of stigmatic papillae is only slightly oblique at these late developmental stages (Fig. 6E) and carpels appear united via the floral centre along their entire length. In a very short basal-most portion of the fruits, the carpel flanks are united, and the furrows between the carpels are absent (Figs 4G, 6F).

Fruits are sessile on the inflorescence axis (Figs 1C–E, 4G). Stamens and often also tepals remain attached at the fruiting stage (Figs 1C–E, 4G). They are much shorter than the fruits, which are green, at least at immature stages. We have no evidence of fruit dehiscence, mericarp separation

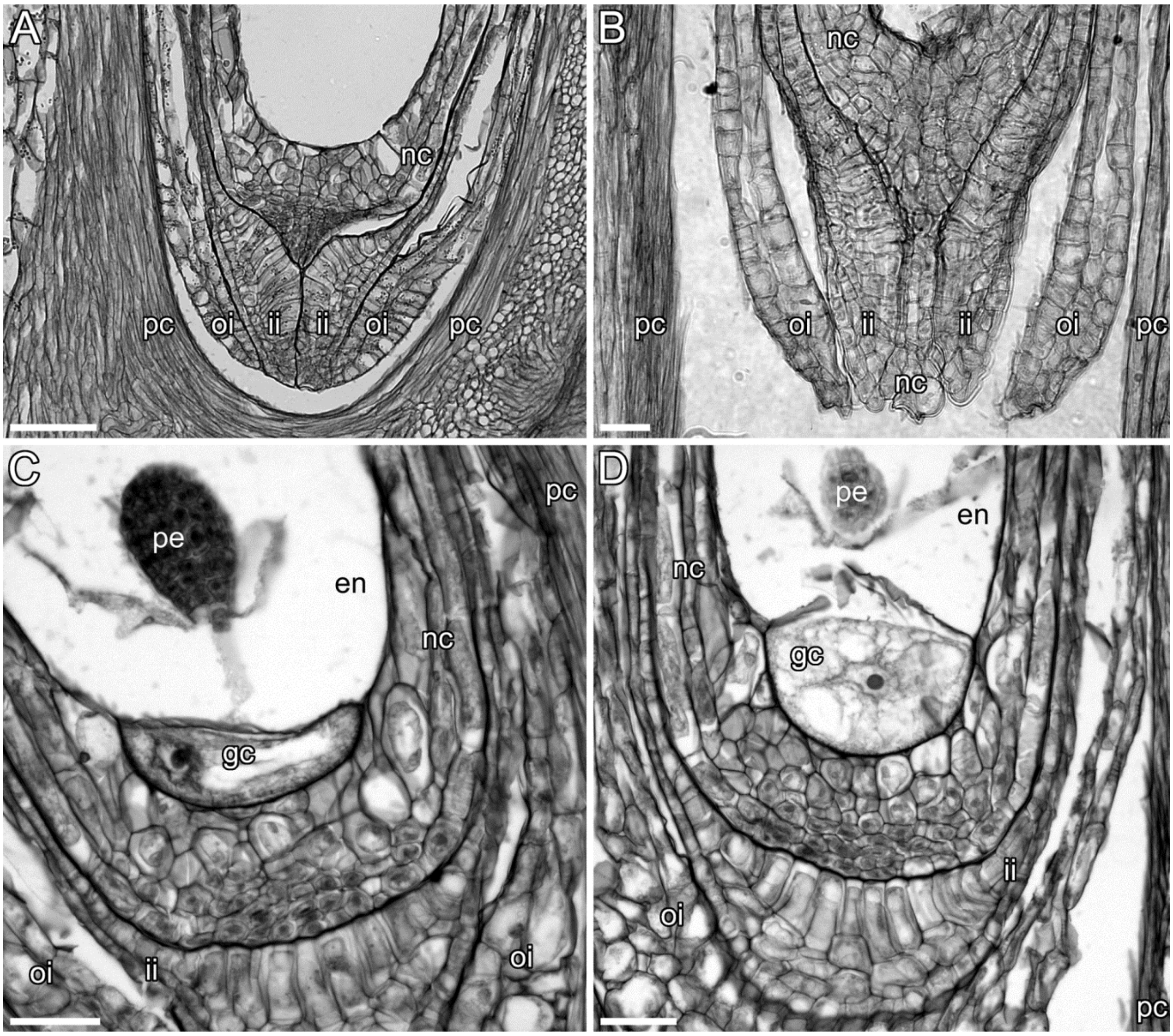

Fig. 14. *Maundia triglochinoides*. Micropylar parts of ovules of post-anthetic flowers in longitudinal sections (light microscopy, LM). A, Section through micropyle. B, Section through micropyle of an apparently unfertilized ovule. C, D, Non-median longitudinal sections showing proembryo at globular stage. Scale bars: 100 µm (A); 50 µm (B–D). en, endosperm; gc, gigantic basal cell of suspensor; ii, inner integument (tegmen); nc, nucellus; oi, outer integument (testa); pc, pericarp; pe, globular proembryo.

or specialized fruit abscission in our fixed material. Observations made on herbarium collections strongly support the indehiscent nature of the fruits of *Maundia*.

In cross section (Figs 6D, 9E–G), the carpel locules, which are rounded in outline, are close to the flower centre. Seeds are almost as wide as the locules, thus filling them completely. Only immature fruits were available, but further widening of locules is highly unlikely because of histological differentiation of endocarp and mesocarp cells in the latest available stages. Free peripheral (dorsal) parts of carpels are thick in a radial and especially in a tangential plane (Figs 6D, 9E–G). Lateral peripheral sides of adjacent carpels are in close proximity to each other, whereas adjacent carpels are separated by a considerable space near the floral centre (Figs 9F, 10A).

The exocarp is one-layered (Fig. 10F) and composed of short thin-walled epidermal cells. Stomata are present on the fruit surface, at least in the distal part. Guard cells are bean-shaped. The number and arrangement of epidermal cells surrounding the guard cells do not appear to be precisely fixed. Most of the multilayered mesocarp is composed of medium-sized thin-walled cells

(Figs 6D, 9G, 10). In the middle and outer part of the mesocarp, large spherical or radially elongated intercellular spaces are present (Figs 9G, H, 10C–E). The dorsal vascular bundles are massive and located close to the carpel locules (Figs 9F, 11I). On the left and right sides of the dorsal bundle, groups of very large (almost isodiametric) thin-walled cells are present (Figs 9G, 10G, 11I). These cells are recognizable already in anthetic flowers (Fig. 5F). They have a large nucleus and a large vacuole. The large cells do not degenerate at least until the stages illustrated in Figs 6D, 9 and 10. In the middle part of the fruit, each carpel locule is surrounded by several layers of fibres with relatively thin lignified cell walls. Fibres closest to the locules are elongated along the fruit length (Figs 6H, 10D, 11D).

We believe that only the innermost cell layer can be identified as an endocarp (i.e., a derivative of the inner epidermis of carpels), because the deeper cell layers are not aligned to the innermost cell layer in a way that could be interpreted as a result of cell divisions in periclinal planes. The rest of the cells elongated along the fruit length therefore belong to the mesocarp. Some other mesocarp fibres are oriented transversally (Figs 6H, 10D, G, 11D, I). These are situated (1) between the longitudinal fibres and dorsal bundles, extending along the inner margins of the groups of large cells and (2) in the peripheral part of the mesocarp adjacent to the furrows dividing the carpels. The ventral bundles, which are much smaller than the dorsal bundles, are situated at the periphery of the floral centre adjacent to the sheath of fibres surrounding the locules. The floral centre is parenchymatous (Fig. 9E, F). The sheath of fibres does not completely encircle the locules along their full length. Gaps are present in the apical part of the locules (Fig. 10B) and on the left and right side in the proximal part of the locules (Fig. 11I).

Crystals, tanniferous cells, oil cells or other cell types with conspicuous content are absent from the ovary wall or pericarp. Starch grains are abundant in parenchyma cells of the floral centre (these cells occupy a cross-shaped area on transverse sections of young fruits, with edges of the ‘cross’ alternating with ovary locules, Fig. 9G). Scattered starch grains are present in mesocarp cells situated in the peripheral parts of the carpels.

The ovules elongate considerably after fertilization, along with the elongation of the entire portion of the gynoecium consisting of united carpels (Fig. 6G). The funiculus remains much shorter than and as wide as the ovule (Figs 10B, 12A, 13A). It has a circular ring of vascular bundles that does not extend into integuments or the nucellus (Fig. 11A). The integuments are free from each other and from the nucellus throughout their entire length (Fig. 12), circular in cross section (Fig. 11). The outer integument consists of three to four cell layers and possesses a continuous outer and inner epidermis and a tissue with abundant lacunae in between (Figs 11, 13). In cross section, large lacunae are separated by smaller cells (or radial cell pairs) linking the outer and the inner epidermis. The lacunae (as well as the cells of the integument) are elongated along the length of the ovule. Cells of the outer epidermis of the outer integument possess characteristic thickenings on their radial walls (Fig. 13C, E, F). These can be interpreted as secondary thickenings with numerous very large and densely spaced pores, each extending along the whole depth of the radial wall. The inner integument is two-layered with almost equal isodiametric cells. Lacunae are absent in the inner integument. The two integuments are tightly appressed to each other (with cuticle in between), and the inner integument is appressed to the nucellus, also with cuticle in between. The micropyle is formed by the inner integument, which is more than two layered in this region (Fig. 14A). In the micropylar region, cells of the inner epidermis of the inner integument are elongated in a radial direction and their walls are thickened (Fig. 14A).

In cross sections through central parts of fertilized ovules with developing endosperm and embryo, the nucellus forms two to four cell layers surrounding the endosperm (Fig. 11D–G). Nucellar

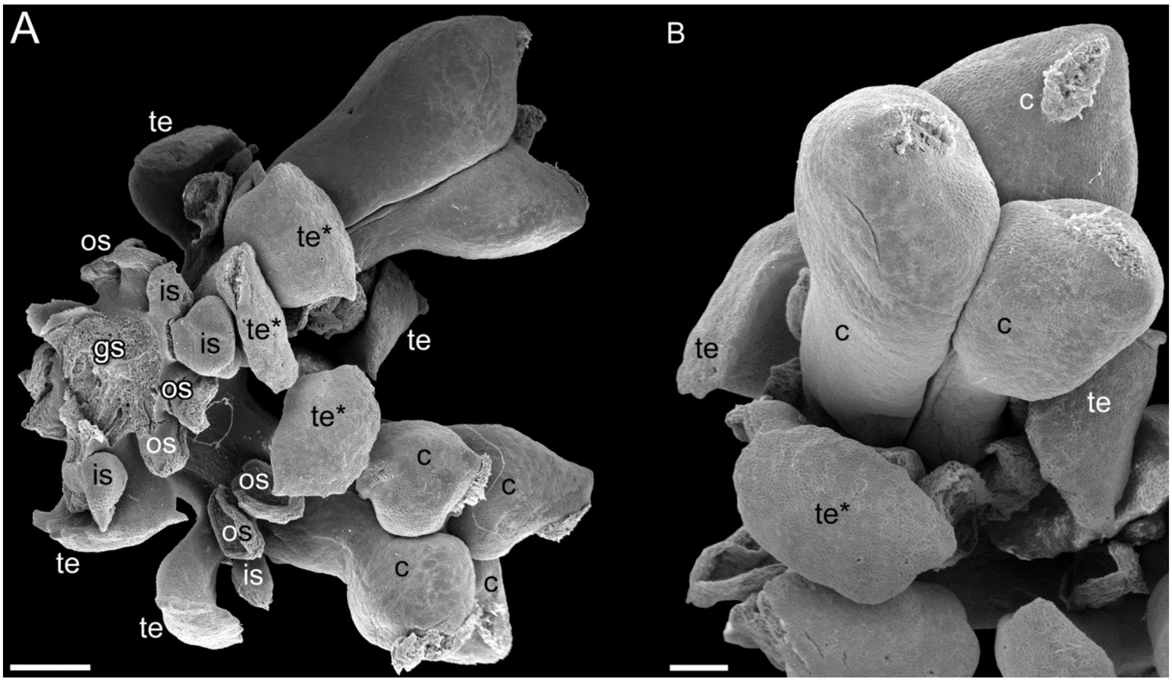

Fig. 15. *Maundia triglochinoides*. Flowers with three tepals at inflorescence tip (scanning electron microscopy, SEM). A, Top view of three flowers forming a whorl at inflorescence tip. Upper right flower (also illustrated in B) has three carpels, lower right flower has four carpels (adaxial one is smaller), left flower had damaged gynoecium, which was therefore removed. B, Another view of the flower from (A) with tricarpellate gynoecium. Scale bars: 1 mm (A); 500 µm (B). c, carpel; gs, stalk of removed gynoecium; is, inner-whorl stamen; os, outer-whorl stamen; te, transversal-abaxial tepal;
te*, adaxial tepal.

cells are thin-walled and elongated along the length of the ovule. Cells of the outer epidermis are larger than the rest of the nucellar cells. The innermost cell layers appear to degenerate during the course of endosperm development (hence the difference in the number of cell layers observed in different ovules). In the micropylar part of the ovule, the number of cell layers in the nucellus increases, and the cells are isodiametric. A narrow conical nucellar beak is present, extending towards the micropyle (Fig. 14A). In most ovules observed, the nucellar beak was short. In one unfertilized ovule (in a developing fruit with other ovules fertilized), the nucellar beak was very long and protruding through the micropyle and widening at its distal side (Fig. 14B). Cells of the chalazal part of the nucellus are almost isodiametric, except for nucellar epidermis cells. These cells are uniform, thin-walled in young ovules as well as in unfertilized ovules in developing fruits (Fig. 12F, G). However, in fertilized ovules, the chalazal part of the nucellus undergoes a radical transformation (Fig. 12A–E). The cell walls separating the nucellar cells closest to the chalaza disappear, thus resulting in the formation of a very large multinucleate coenocyte. The coenocyte contains a large vacuole with numerous cytoplasmic strands extending in chalazal-micropylar direction (Fig. 12B–E). Nuclei of the coenocyte appear to be functional. At least their nucleoli are stained in the same way as in nuclei of normal nucellar cells. Stages of degeneration of cell walls between adjacent nucellar cells were documented (Fig. 12E, arrowhead). Several layers of unfused uninucleate nucellar cells are present between the nucellar coenocyte and the endosperm. These cells are much larger than those in contact with the chalazal side of the coenocyte.

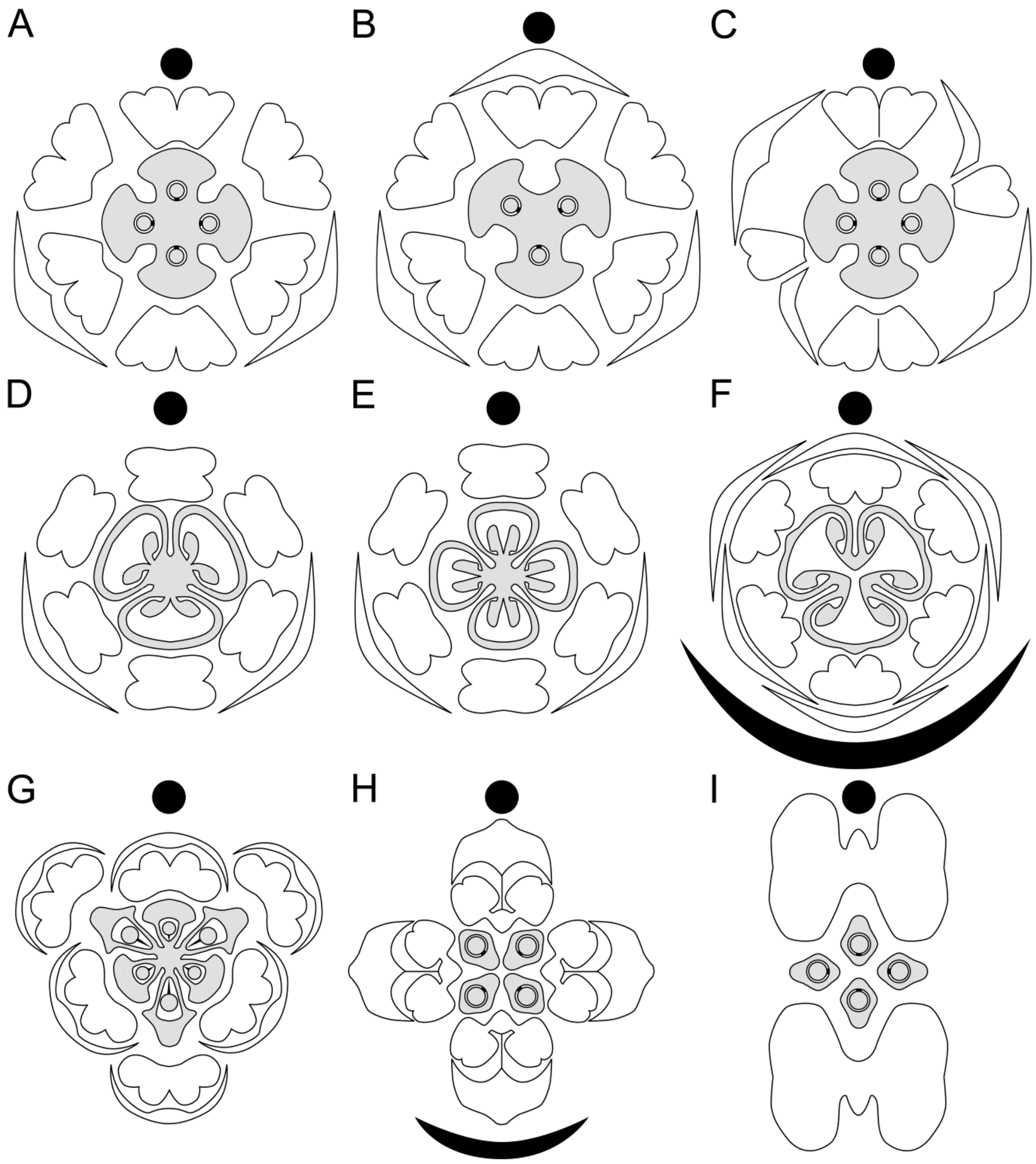

Fig. 16. Floral diagrams. A–C, *Maundia triglochinoides*. A, Normal flower. B, C, Flowers from inflorescence tips. D, E, *Aponogeton subconjugatus*. F, *Scheuchzeria palustris* L. G, *Triglochin maritima* L. H, *Potamogeton* sp. I, *Ruppia* sp. (four-carpellate flower). Black arches in (F, H), flower-subtending bracts; black dots, main inflorescence axis. Organ arrangement (but not stamen and carpel shape) in (D, E) is based on Tomlinson (1982). Gynoecium outline in (G) is based on Igersheim et al. (2001). Stamen and tepal shape in (H) is inspired by Singh (1965, *Potamogeton indicus*).

The globular stage of embryo development has a long and narrow suspensor ending in a gigantic basal cell with a large nucleus (Fig. 14C, D).

FLOWER VARIATION AT THE INFLORESCENCE TIP

Two inflorescence tips were available for detailed investigations. In one of them (Fig. 15), all flowers up to the inflorescence tip were arranged in clear trimerous whorls. In this specimen, flowers of the uppermost whorl differed from typical flowers of *Maundia* (Fig. 16A) in the occurrence of three tepals. Each of these three tepals was inserted on a radius of one of the inner whorl stamens, so that two were transversal-abaxial and the third was median-adaxial. Furthermore, one of the three flowers of the uppermost whorl possessed three carpels (inserted on the radii of the outer-whorl stamens, Figs 15B, 16B), another had four carpels, and the gynoecium of the other was damaged, making it unsuitable for investigation. In the four-carpellate gynoecium, the adaxial carpel was smaller than other carpels (Fig. 15A). Note that the adaxial carpel was missing from the tricarpellate gynoecium of another flower on the same inflorescence.

In the other inflorescence that was investigated in detail, two distal lateral flowers were attached at different levels of the inflorescence axis (below them, flowers were arranged in trimerous whorls typical of *Maundia*). The inflorescence axis was not extended above the level of the uppermost flower. In the absence of flower-subtending bracts in *Maundia*, a morphologically terminal position for the uppermost flower cannot be completely ruled out. However, as the flower was turned towards one side of the inflorescence and not developmentally accelerated, we prefer to interpret it as morphologically lateral. In this inflorescence, the distalmost flower (Fig. 16C) has four carpels in positions typical of flowers of *Maundia*, four stamens and four tepals. Two stamens are tetrasporangiate and dithecal (i.e., of a normal structure); these are in the median-adaxial and median-abaxial positions. Two other stamens are bisporangiate monothecal; these are located in the left and right transversal-adaxial positions. The four tepals are in diagonal positions. Two of them are asymmetric and associated with each of the monothecal stamens (vascular traces of a stamen and a tepal in both cases unite in the flower receptacle). Two other tepals are symmetrical and not associated with any stamens.

Discussion

PEDUNCLE ANATOMY AND OCCURRENCE OF STOMATA

Maundia shares many features of peduncle anatomy with other aquatic and semi-aquatic members of Alismatales, including the presence of aerenchyma with air canals divided into chambers by transverse, minutely perforate septa, and tracheids rather than vessels in the metaxylem (Tomlinson, 1982). A protoxylem lacuna is present in the peduncle bundles of many Alismatales, but according to Tomlinson (1982) it is not conspicuous in most Juncaginaceae (except *Cycnogeton* Endl.). Sclerenchyma forming a peripheral ring is present in peduncles of Juncaginaceae and Scheuchzeriaceae but absent from Potamogetonaceae and apparently Aponogetonaceae (Tomlinson, 1982). According to our data, the ring of sclerenchyma is absent from peduncles of *Maundia*. The occurrence of peripheral bundles is recorded in stems and peduncles of many members of the tepaloid clade of core Alismatales. An apparently unusual feature of *Maundia* is the inverted orientation of peripheral bundles, with xylem oriented towards the periphery of peduncle. Tomlinson (1982) did not record inverted peripheral bundles in peduncles of close phylogenetic relatives of *Maundia*. We were able to re-investigate the anatomical sections of *Cycnogeton procerum* (R. Br.) Buchenau (Juncaginaceae) used by Tomlinson and deposited in the Kew microscope slide collection.

Although Tomlinson did not describe explicitly the orientation of peripheral bundles in peduncles of *Cycnogeton*, we confirm that they are not inverted. We also re-investigated the material used by Tomlinson to describe the vegetative anatomy of *Maundia*. This is a set of sections of different organs, including a root tuber, which has a peculiar type of clustered root hair arrangement. Since *Maundia* lacks root tubers, it is possible that the material is misidentified. Due to the lack of voucher specimens, it was impossible to verify the correct identity of the material. Therefore, the present study probably provides the first detailed information on the vegetative anatomy of *Maundia*. Buchenau (1903) presented only a schematic illustration of a leaf cross-section. Schneider & Carlquist (1997) described the vessel structure in roots of *Maundia*, but no other details of root anatomy were given.

Stomata are tetracytic in *Scheuchzeria*, paracytic or tetracytic in Juncaginaceae and paracytic (when present) in Potamogetonaceae (Tomlinson, 1982). In *Aponogeton*, stomata are described as each having a pair of indistinct lateral subsidiary cells; one or both subsidiary cells are commonly segmented by a median anticlinal division, the subsidiary cells becoming obscure (Tomlinson, 1982). We found no data on the stomata of *Maundia* in the literature. The stomata documented in this study on tepals and fruits of *Maundia* possess several small cells surrounding the guard cells. Although developmental data are clearly needed for both taxa, the arrangement of surrounding cells is similar in *Maundia* and *Aponogeton* and cannot be readily described as either paracytic or tetracytic. Stomata on peduncles of *Maundia* are more similar to those described for leaves of Juncaginaceae (Tomlinson, 1982).

In Alismatales, stomata are present on carpels of Araceae, some Alismataceae, Aponogetonaceae, Butomaceae, Juncaginaceae, Limnocharitaceae and Scheuchzeriaceae (Igersheim, Buzgo & Endress, 2001). The present study revealed stomata on carpels of *Maundia*. In contrast, stomata are absent from carpels in a clade that is sister to *Maundia* (Potamogetonaceae, Zosteraceae, Ruppiaceae, Posidoniaceae, Cymodoceaceae). This difference is perhaps not surprising, as most of these taxa flower under water. However, flowers of some species of *Potamogeton* L. are exposed above the water, while submerged flowers are found in other species of the genus (Philbrick 1988).

MORPHOLOGICAL INTERPRETATION OF FLORAL PARTS

Our data confirm earlier observations that the flowers of *Maundia*, except the uppermost ones, uniformly possess two scale-like structures interpreted here as tepals. These phyllomes are inserted in the transversal-abaxial position. Three different interpretations of these scale-like structures have been proposed in the literature, namely as bracts (Uhl, 1947; Aston, 2011), perianth members (tepals: Bentham, 1878; Buchenau, 1903; Nakai, 1943; Haynes, Les & Holm-Nielsen, 1998; Buzgo *et al.*, 2006; Takhtajan, 2009 or sepals: Mueller, 1858) and connective appendages (Markgraf, 1936; Eckardt, 1964; Dahlgren, Clifford & Yeo, 1985). The same range of interpretations has been proposed for scale-like structures in reproductive structures of other members of core Alismatales (Helobiae), such as *Triglochin* s.l. and *Potamogeton* (see Kunth, 1841; Ascherson, 1889; Buchenau & Hieronymus, 1889; Markgraf, 1936; Miki, 1937; Uhl, 1947; Eames, 1961; Eckardt, 1964; Sattler, 1965; Singh, 1965; Posluszny & Sattler, 1973, 1974; Burger, 1977; Lieu, 1979; Dahlgren, Clifford & Yeo, 1985; Posluszny & Charlton, 1993; Endress, 1995; Mavrodiev & Sokoloff, 1998; Posluszny, Charlton & Les, 2000; Rudall, 2003; Buzgo *et al.*, 2006). The bract interpretation is typically used in the framework of the hypothesis that reproductive structures commonly termed flowers in this group in fact represent compact inflorescences (pseudanthia) composed of naked unisexual flowers. This hypothesis implies that each scale-like structure represents a flower-subtending bract of a male flower, and what is traditionally termed a stamen actually represents an entire male flower (e.g., Kunth, 1841; Miki, 1937; Uhl, 1947; Eames, 1961). A pseudanthial interpretation of the conventional flower of core Alismatales has been criticised on the grounds that the features used in support of this theory can also be found in some monocots belonging to other monocot orders (summarized in Endress, 1995; see also Lieu, 1979 and Buzgo *et al.*, 2006, for case studies in Juncaginaceae). We accept this criticism and follow the euanthial interpretation for flowers of most Alismatales (probably excluding Zannichelliaceae and Cymodoceaceae: Sokoloff, Rudall & Remizowa, 2006; Remizowa *et al.*, 2012b).

The euanthial interpretation implies that structures traditionally called flowers in taxa such as *Potamogeton* (Fig. 16H), *Triglochin* (Fig. 16G), and *Scheuchzeria* (Fig. 16F) are homologous with the uniaxial flowers of other angiosperms. In the framework of this view, it is clear that a euanthial interpretation can also be adopted for *Maundia*. If the flower of *Maundia* does not represent a pseudanthium, it is highly unlikely that the two scale-like structures are bracts. Indeed, their position excludes the possibility that these are flower-subtending bracts. For the reasons outlined below, we reject the interpretation of the two scale-like structures as prophylls (bracteoles) or connective appendages, and instead interpret them as tepals.

One could argue that the two scale-like structures represent prophylls (bracteoles) on the pedicel. However, bracteoles are unknown in any other member of the large clade to which *Maundia* belongs (Aponogetonaceae plus its sister clade, a group called ‘tepaloid alismatids’ by Posluszny & Charlton, 1993). Moreover, the hypothesis implies that bracteoles are present when a flower-subtending bract is absent. As pointed out by Remizowa *et al.* (2013), such a combination of characters is not observed in other early-divergent monocots.

Like the pseudanthial concept, a connective appendage interpretation for the scale-like structures of flowers of Juncaginaceae s.l. and/or Potamogetonaceae s.l., is based on various kinds of association between a scale and a stamen occurring on the same radius in a flower (Ascherson, 1889; Markgraf, 1936; Eckardt, 1964). This association can be manifested in a common vascular supply, and/or basal fusion of a stamen and a scale. Furthermore, as in *Triglochin*, the scales associated with inner-whorl stamens can be inserted above the outer-whorl stamens in the two-whorled androecium (e.g., Goebel, 1928; Uhl, 1947; Rudall, 2003; Remizowa, Sokoloff & Rudall, 2010). The connective appendage concept has been much criticised based on evidence from flower development and comparative morphology. In particular, the scale-like structures and stamens appear separately during flower development in both *Potamogeton* (Hegelmaier, 1870; Sattler, 1965; Posluszny & Sattler, 1973, 1974; Posluszny, 1981; Sun, Zhang & Chen, 2000; Nunes *et al.*, 2012) and *Triglochin* (Lieu, 1979; Bugzo *et al.*, 2006; Remizowa, Sokoloff & Rudall, 2013). The significance of developmental data should not be overestimated, because different thecae of the same anther occasionally appear separately on the floral apex (Posluszny & Sattler, 1973). Note that Eichler (1875) did not abandon the connective appendage interpretation for *Potamogeton* despite the excellent developmental study of Hegelmaier (1870). It is much more important that the intimate relationships between tepals and stamens occurring on the same radii can be found in a wide range of monocots belonging to different orders and these could merely reflect the pronounced sectorial differentiation in the flowers (Endress, 1995; Remizowa, Sokoloff & Rudall, 2010; Remizowa *et al.*, 2012a). As pointed out by Endress (1995), the pronounced association between tepals and stamens inserted on the same radii is more likely to appear in trimerous than in pentamerous flowers.

Once we accepted the presence of a perianth in *Triglochin* and *Potamogeton*, we see no argument against accepting the scale-like structures in *Maundia* as tepals. Like tepals of related taxa, those of *Maundia* are inserted on the radii of stamens. Our data on floral vasculature do not show a substantial difference between vascular supply of tepals in *Maundia* and some *Potamogeton* species (Uhl, 1947).

For the purposes of evolutionary morphology and taxonomy, the most important conclusion is that scale-like structures of *Triglochin*, *Scheuchzeria*, *Potamogeton* and *Maundia* are homologous to each other. When flowers of *Triglochin* are interpreted as lacking bracts and having tepals but flowers of *Maundia* as having bracts but lacking tepals (Aston, 2011), such an interpretation artificially increases the degree of morphological difference between the two taxa. These problems should be considered in character scoring for morphological cladistic analyses.

There are two contrasting interpretations of stamen morphology in *Maundia*. In one interpretation, the typical flower has up to 12 monothecal bisporangiate stamens (Mueller, 1858). In another interpretation, the flower has tetrasporangiate dithecal stamens, with each stamen being split to its very base (most authors: e.g., Bentham, 1878; Buchenau 1903; Markgraf, 1936; Aston, 2011). We follow the second interpretation, because it creates a much smaller gap between *Maundia* and related taxa. With the exception of obviously reduced and highly transformed taxa such as Zannichelliaceae and Cymodoceaceae (where we accept a pseudanthial interpretation of the reproductive structures, see above), all the close relatives of *Maundia* possess tetrasporangiate and dithecal stamens. Although the two thecae of *Maundia* stamens are largely free, they remain basally united. The occurrence of individual vascular bundles supplying each theca in *Maundia* by no means supports the assignment of each theca to an individual monothecal stamen. Although one-traced stamens with a single unbranched vascular bundle are most common among angiosperms, taxa with multi-bundled and even multi-traced stamens are known from all major phylogenetic lineages, including monocots (e.g., Remizowa *et al.*, 2011), eudicots (e.g., Nuraliev, Sokoloff & Oskolski, 2011) and magnoliids (e.g., Canright, 1952). Patterns of variation in stamen supply described here for *Maundia* are identical to those described by Uhl (1947) in *Potamogeton*.

RELATIVE POSITIONS OF FLORAL PARTS IN *MAUNDIA*: AN INTRIGUING SIMILARITY TO *APONOGETON*

Detailed observations on the flower groundplan in *Maundia* were published by Markgraf (1936). He found that most flowers (except at the very tip of the inflorescence) possess six stamens and four carpels. He interpreted the flower as tetramerous, with two inner-whorl stamens lost. The outer-whorl stamens are in median and transversal positions. These stamens are not associated with scale-like structures. The two stamens of the inner whorl are in positions intermediate between abaxial and transversal. Markgraf implied that the inner whorl of the androecium alternates with the outer whorl, and the inner-whorl stamens are therefore in diagonal positions, and the two stamens of the adaxial part of the flower are absent. The two inner-whorl stamens that are present are associated with scale-like structures, which Markgraf interpreted as connective appendages. However, according to our interpretation, these scale-like structures are tepals, and this term will be used below. In the uppermost part of the inflorescence, Markgraf found flowers with four tepals. Two additional tepals were inserted in positions intermediate between adaxial and transversal. This observation was used by Markgraf in support of the tetramerous interpretation of the entire flower. The four carpels, according to Markgraf, are inserted in one whorl in diagonal positions. This orientation of carpels created a problem with alternation of whorls in the flower. Indeed, the carpels and the inner-whorl stamens are inserted on the same radii. Markgraf postulated that the gynoecium of *Maundia* was originally two-whorled (as in other Juncaginaceae), and the outer-whorl carpels were lost during evolution, but their positional information is retained. This interpretation was supported by the sterility of the outer-whorl carpels in most species of *Triglochin*. In some species of *Triglochin*, the outer-whorl carpels are much reduced.

Diagrams of Markgraf (1936) were reproduced (with reference) by Uhl (1947) and Eckardt (1964), who apparently did not study material of *Maundia* in detail themselves. However, both reproduced diagrams differ from the original, and in both cases no comments regarding these differences are provided. Uhl (1947) changed the carpel position from diagonal to median and transversal (i.e., on the radii of the outer-whorl stamens). Eckardt (1964) illustrated eight stamens in two whorls and four tepals associated with inner-whorl stamens, in what he called a normal flower.

Our data show an arrangement of organs that differs from all diagrams discussed above. In our material, carpel orientation was never diagonal (Fig. 16A, C). The four carpels were always inserted in median and transverse positions, as illustrated by Uhl (1947). This arrangement was also the case of the uppermost flowers in the inflorescence, if these are four-carpellate. In our interpretation, the androecium is two-whorled and trimerous. The tepals are associated with the anterior inner-whorl stamens. We lacked sufficient material to investigate the variation in inflorescence tip morphology in *Maundia*, because of the rare nature of the plant and different preservation of inflorescence tips in our material. However, none of the flowers from the upper part of inflorescence showed clear evidence of tetramery, except in the gynoecium. Moreover, in the inflorescence with three distal-most flowers forming a whorl, each flower possessed a trimerous whorl of tepals and two trimerous whorls of stamens. At least in one case, a completely trimerous flower was observed at the inflorescence tip, where the gynoecium was also trimerous (Fig. 16B).

Several authors, starting with Mueller (1858), indicate the occurrence of two to four perianth members in *Maundia* (Thompson, 1961; Haynes, Les & Holm-Nielsen, 1998). However, none of them discuss the position of flowers with different perianth morphology within inflorescences. According to our data, variation in tepal number occurs only in the final whorl of flowers. Except at the inflorescence tip, all observed flowers consistently possessed two tepals (see also Aston, 2011). Apparently, the records of variation between two and four tepals, along with the common presence of four carpels, were the source of interpretation of *Maundia* flowers as dimerous (Dahlgren, Clifford & Yeo, 1985; Haynes, Les & Holm-Nielsen, 1998). Our data do not support a dimerous interpretation.

Data on carpel number in *Maundia* also differ in various publications. While Mueller (1858) indicated three to four carpels and Bentham (1878) noted “carpels usually 2 or 3, sometimes 4”, Aston (2011) stated that there are usually four, rarely two or three carpels. Unfortunately, these authors did not consider flower position in the inflorescence. We did not find a bicarpellate gynoecium. Our analysis of fixed material and herbarium collections showed that the four-carpellate condition is the typical one in *Maundia* (see also Thompson, 1961).

The floral diagram of *Maundia* is almost identical to that of most species of *Aponogeton* (see Buzgo *et al.*, 2006). In *Aponogeton*, as in *Maundia*, flowers are arranged in spikes and lack any signs of flower-subtending bracts. With some exceptions (including *A. distachyus* L.f. and an early-divergent species *A. hexatepalus* H.Bruggen – Les, Moody & Jacobs, 2005), flowers of *Aponogeton* typically possess two tepals in transversal-abaxial positions, six stamens in two whorls (outer median stamen abaxial) and three, sometimes four carpels (Singh & Sattler, 1977; Tomlinson, 1982; Remizowa, Sokoloff & Rudall, 2010). When four carpels are present in *Aponogeton*, their position is the same as in *Maundia* (Tomlinson, 1982). When three carpels were present in our material of *Maundia*, their position is the same as in *Aponogeton*. In both *Maundia* and *Aponogeton*, there is a tendency to develop the third (adaxial) tepal in the uppermost flowers of an inflorescence.

The similarity in flower groundplan between *Maundia* and *Aponogeton* is intriguing because (1) this is a highly unusual flower organization apparently not found in other monocots and (2) the two genera are *not* sister taxa (Iles, Smith & Graham, 2013; Les & Tippery, 2013). There are other examples in biological evolution when similar and unusual novelties evolved independently in closely related but not sister lineages. For example, the order Alismatales is the only group of seed plants that includes marine taxa, the so-called seagrasses. In three families of seagrasses, the pollen grains are filiform, a condition unique among angiosperms. Analysis of this character based on a molecular phylogenetic tree topology (Iles, Smith & Graham, 2013) and additional structural evidence strongly suggest three independent origins of filiform pollen in Alismatales and more than one shift from continental aquatic to marine habitats (Remizowa *et al*., 2012). In the case of parallel evolution of filiform pollen, as in some other examples, independent appearance of an unusual character (which could be described as a homoplastic tendency: Sanderson, 1991) has an obvious adaptive signifycance.

In contrast, there is no obvious adaptive significance for the occurrence of two abaxial-transversal tepals in *Maundia* and *Aponogeton*. The two genera differ in pollination ecology. *Aponogeton* is insect-pollinated, with tepals being white, pink(ish)/purple, yellow to green and attractive in many species, while *Maundia* is apparently wind-pollinated (though detailed observations are needed), with tepals being green and non-attractive. We suggest that developmental and spatial constraints rather than functional significance are responsible for the homoplastic appearance of similar floral types in *Maundia* and *Aponogeton*. Many members of core Alismatales have spikes or racemes with whorled arrangement of flowers. Whorled flower arrangement is otherwise extremely rare in monocots (e.g., Sokoloff *et al.*, 2009; Remizowa *et al.*, 2013). Flowers are closely spaced in developing racemose inflorescences of Alismatales. In the absence of flower-subtending bracts (another feature common in Alismatales but otherwise rare in monocots – Remizowa, Sokoloff & Rudall, 2013; Remizowa *et al.*, 2013), physiological interactions between adjacent sites of flower initiation must be important for pre-patterning of floral organs. We argue that only a limited number of flower organizations are available that allow the most compact spacing of developing flowers and the most complete use of space on the surface of the inflorescence axis. The condition found in *Maundia* and *Aponogeton* is one of them. In this respect, it is tempting to consider the well-known similarity between flowers of taxonomically unrelated *Potamogeton* and some Cyclanthaceae and Pandanaceae (e.g., Miki, 1937) as reflecting similar spatial constraints. Apart from Alismatales, Cyclanthaceae is another aberrant monocot group with whorled flower arrangement.

POLLEN

Our data confirm information on *Maundia* pollen morphology provided by the Australasian Pollen and Spore Atlas (APSA Members, 2007). Pollen morphology of *Maundia* is very similar to that of related members of Alismatales (Grayum, 1992; Furness & Banks, 2010). As stated by Grayum (1992) the "genera [*Triglochin*, *Lilaea*, *Tetroncium*] are quite uniform palynologically, and hardly to be distinguished on this basis from *Potamogeton*". The same applies to *Maundia* pollen and further studies, e.g. on pollen wall ultrastructure might reveal informative characters. Monosulcate pollen of *Aponogeton* is different from that of taxa listed above (Grayum, 1992; Furness & Banks, 2010). *Aponogeton* also differs from other core Alismatales (no data on *Maundia*) in possessing simultaneous rather than successive type of microsporogenesis (Furness & Banks, 2010).

OVULES AND SEEDS

Our study fully supports the occurrence of pendent, orthotropous ovules in *Maundia*. To our knowledge, this feature was questioned only by Bentham (1878) who thought that Mueller's (1858) original description was incorrect, which is not the case. We provide the first detailed observations on the histology of the integuments and the nucellus in *Maundia*. According to our data, the outer integument consists of three to four cell layers and contains conspicuous intercellular canals (apparently air canals) aligned along the ovule length. These canals are conspicuous in fertilized ovules with developing embryo. Shaffer-Fehre (1987) studied the mature seeds of *Maundia* and found no evidence of a mesotesta and no air canals. She also noticed an obliteration of exotesta cells. We hypothesize that the mesotesta obliterates along with the exotesta, making the air canals inconspicuous in mature seeds.

We assume that the presence of air canals in the testa is of phylogenetic significance in Alismatales. According to literature, conspicuous air spaces are present in the testa of *Butomus* L., *Enhalus acoroides* (L.f.) Royle (Hydrocharitaceae) and *Aponogeton* (Melikian, 1985; Plisko, 1985; Teryokhin, 1985). We found no published evidence for the occurrence of similar intercellular spaces in other Alismatales, though more detailed observations in wide range of taxa in various developmental stages are certainly necessary.

According to available publications, the formation of a coenocytic structure in the nucellus of fertilized ovules of *Maundia* has no exact parallels among other angiosperms. In particular, it has nothing in common with various kinds of specialized structures described in the chalazal part of ovules of various angiosperms, such as a hypostase, podium, postament etc. (reviewed by Shamrov, 2008, see also Rudall, 1997). In many angiosperms, nucellar cells degenerate during endosperm and embryo development, but cell degeneration typically takes place in areas of the nucellus that are in a direct contact with endosperm and embryo. In *Maundia*, reorganization of the nucellus takes place in its chalazal-most part, and normal tissue of cells with cell walls remain between the nucellar coenocyte and the endosperm. In addition, formation of the coenocyte does not cause cell death in *Maundia*, at least during the developmental stages available for the present study. The nuclei of the coenocyte do not appear to be degenerating.

Among angiosperms, loss of cell walls between cells in tissue of the nucellus resulting in the formation of a coenocytic structure is known in the eudicot family Podostemaceae (Went, 1908; Razi, 1949; Jäger-Zürn, 1967; 1997; Nagendran, Arekal & Subramanyam, 1977; Nagendran, Anand & Arekal, 1980). Here, the nucellar coenocyte (also known as nucellar plasmodium) serves as a structure that substitutes an endosperm, which is missing in Podostemaceae. So the functional significance of this feature in Podostemaceae is obvious. We have no plausible interpretation of the functional role of the nucellar coenocyte in *Maundia*. It may play a role in transferring nutrients from the funiculus to the nucellus. *Maundia* appears to have an unusually well-developed vasculature in the funiculus. Instead of a single bundle, a ring of vascular bundles is present. Rudall (1997) highlighted the similarity between the formation of the nucellar plasmodium in Podostemaceae and the formation of the coenocytic structure in the monocot *Pandanus* (Pandanales), in which diploid nuclei of nucellar cells penetrate into the embryo sac where they further divide (Cheah & Stone, 1975). The formation of multinucleate structures in both *Pandanus* (Chubirko, 1990; Kamelina, 2011) and *Maundia* requires further investigation. Kamelina (2011) suggested that the coenocytic structure in *Pandanus* containing up to 200 diploid nuclei could form due to apomictic endosperm development.

FRUITS

Cronquist (1981) stated that, as in *Triglochin* and *Tetroncium*, the mature carpels of *Maundia* separate from the persistent central axis and open ventrally. Our data do not support this observation. Although we did not observe fully mature fixed fruits, their anatomical structure excludes the possibility of regular carpel separation from a persistent central column (as in many Juncaginaceae) and ventral carpel dehiscence. The floral centre of *Maundia* has no mechanical tissue that should be expected in a persistent column. The sclerenchymatous layer of each carpel is continuous on the ventral side, making ventral dehiscence impossible. Our observations of fully mature fruits in herbarium material also did not reveal carpel separation and dehiscence. According to Bobrov *et al*. (2009), fruits of *Maundia* are schizocarps with indehiscent mericarps (regma syncarpia), but we did not record separation of mericarps in studied fixed and herbarium material. Our data are congruent with the earlier description of Bentham (1878), noting: “the carpels almost drupaceous, each with a tiny cartilaginous endocarp with an acute dorsal rib”. One of specimens of *Maundia* (*Briggs 10003*) includes the note "Individual carpels tending to separate in fallen fruits". However, in almost all fruits that dropped off during the drying process all carpels remained united. Those carpels that have separated each possess a segment of the floral centre adhering to the ventral surface (B.G. Briggs, Royal Botanic Gardens, Sydney, pers. comm.). This observation agrees with Aston's (2011) description of fruiting carpels remaining united and falling together or tardily separating at maturity, and indehiscent.

To summarize, in the absence of regular carpel separation before fruit detachment from the maternal plant, fruits of *Maundia* do not belong to the carpological type that is characteristic of *Triglochin*. We would like to note in this context that fruit diversity in Juncaginaceae requires further investigation. In *Triglochin maritima* L., as in many other species, one-seeded parts (fruitlets or mericarps, depending on the interpretation of the gynoecium) separate from a persistent stalk-like structure (called carpophore or column) along their entire length. In *T. plaustris* L., the one-seeded parts remain united with the stalk-like structure only at their distal-most parts. According to observations in NW Russia (M.V. Remizowa, unpubl. data), the fruits of *T. palustris* do not disintegrate completely in the year of their formation. They remain attached to persistent upright inflorescence axes until the next season when individual one-seeded parts ultimately separate. Finally, in the recently described species *T. buchenaui* Köcke, Mering & Kadereit (Köcke *et al.*, 2010), a carpophore (column) is absent, and one-seeded parts separate each possessing a segment of the floral centre, as in occasional instances of carpel separation in *Maundia*.

The absence of a detailed survey of pericarp histology in Juncaginaceae does not allow conducting a comprehensive comparison with *Maundia*. An illustrated description of fruit anatomy is available for *Triglochin palustris* (Petrova, 1985). In addition, preliminary data on some African *Triglochin* species are reported by Lock *et al.* (2011). A shared feature of *Maundia* and *T. palustris* is the occurrence of mechanical tissue in the inner part of the pericarp, surrounding the fruit locules (however, mechanical tissue is also present in the inner part of the pericarp in many related groups such as Potamogetonaceae and Ruppiaceae – Teryokhin, 1985). It is not clear from the description in Petrova (1985) whether these cells are isodiametric or elongated along the length of the fruit and whether all layers of the mechanical tissue belong to the endocarp (as interpreted in Bobrov *et al.*, 2009). According to Lock *et al.* (2011), as in *Maundia*, mesocarp contributes to the mechanical tissue surrounding the locules in African species. Differences include (1) the presence of air spaces between the seed and the pericarp in *Triglochin*, (2) the presence of transversally elongated fibres in *Maundia*,

(3) the presence of large cells flanking the dorsal bundle in *Maundia*, and (4) much greater width of the pericarp in *Maundia*.

Of special interest is the presence of intercarpellary fusion in a very short proximal portion of the fruits in *Maundia*. This fusion is not detectable at anthesis, apparently because the basal-most part of the gynoecium forms after fertilization. This phenomenon is significant for understanding gynoecium evolution in Alismatales, if the fusion of carpel flanks is accepted (other interpretations would be accepting the occurrence of a widened fruit stalk or united carpel stipes, as in *Harperocallis* McDaniel in Tofieldiaceae – Remizowa *et al.*, 2011). Most members of the tepaloid clade of Alismatales possess either free carpels or carpels united via the floral centre (Igersheim, Buzgo & Endress, 2001; Remizowa, Sokoloff & Rudall, 2010). The only well-known exception is *Scheuchzeria*, where carpels form a conspicuous unilocular symplicate zone (see Eber, 1934; Igersheim, Buzgo & Endress, 2001). Carpel fusion via the floral centre is in many respects related to apocarpy, and both conditions are currently considered derived character states in monocots (Endress & Doyle, 2009; Remizowa, Sokoloff & Rudall, 2010). The condition found in *Scheuchzeria* is likely plesiomorphic. The short fusion between carpel flanks in the basal-most fruit region of *Maundia* could be considered as a rudiment of an ancestrally syncarpous gynoecium construction. In this respect, we emphasize that a re-investigation of gynoecium and fruit structure in *Tetroncium*, an early-divergent member of Juncaginaceae, is urgently needed (see also Thieret, 1988). As long as *Tetroncium* is placed in Juncaginaceae and as its flowers (though dioecious and dimerous) are generally similar to those of *Triglochin* and *Cycnogeton*, it might be logical to suppose that carpels are united via the floral centre in *Tetroncium* as in other Juncaginaceae. Surprisingly, as illustrated by Hooker (1847), *Tetroncium* has carpels united to form a unilocular ovary with incomplete septa. If Hooker's data are correct, then in terms of the gynoecium morphology *Tetroncium* is closer to *Scheuchzeria* than to *Triglochin, Cycnogeton* (and *Maundia*).

TAXONOMIC AND EVOLUTIONARY IMPLICATIONS

Our results show that morphology does not contradict the molecular phylogenetic placement of *Maundia* as sister to a group comprising members of Potamogetonaceae, Zosteraceae, Ruppiaceae, Cymodoceaceae and Posidoniaceae. As pointed out by von Mering and Kadereit (2010), the presence of pendent orthotropous ovules is an obvious synapomorphy of this lineage, including *Maundia*. Most members of the large clade that is sister to *Maundia* have underwater pollination and thus their flowers are quite reduced compared with those in *Maundia* and other Alismatales with emergent flowers. Less reduced flowers are characteristic of *Potamogeton*, where, at least in most species, pollination takes place above the water level. Therefore, it is most appropriate to compare the floral morphology of *Maundia* and *Potamogeton*. Tepal morphology is similar; in both genera, tepals are clawed, green, with abundant stomata, single-traced (probably rarely three-traced in *Maundia*), but the tepal bundle branches in the tepal blade (Hegelmaier, 1870; Uhl, 1947; Singh, 1965; Sattler, 1965). The latter character is of particular importance, because branching tepal bundles are not universally present in Alismatales (e.g., unbranched in *Triglochin* – Uhl, 1947). Some patterns of infraspecific variation in tepal vasculature in *Maundia* (this study) resemble those present in *Potamogeton* (Hegelmaier, 1870; Sattler, 1965), such as the presence vs. absence of anastomoses between bundles. Details of stamen vascular supply (and relationships between stamen and tepal traces) are similar in *Maundia* and at least some species of *Potamogeton* (Uhl, 1947). The occurrence of four carpels, however, cannot be viewed as a similarity between *Maundia* and *Potamogeton*, because those of *Maundia* are in median and transversal positions while those of *Potamogeton* are in diagonal positions. In terms of carpel position, *Maundia* could be compared with *Tetroncium* and the four-carpellate flowers of *Ruppia* L. In *Ruppia* (Fig. 16I) and apparently in *Tetroncium*, carpels are arranged in dimerous whorls. Developmental data are required to understand whether the gynoecium of *Maundia* is formed by one tetramerous or two dimerous whorls. Stamens of *Maundia* with almost free thecae are similar to those of *Ruppia*.

Although there are important similarities between *Maundia* and members of its sister group, some morphological characters highlight resemblances with other tepaloid Alismatales. In general habit as well as in the occurrence of strong carpel fusion via the floral centre, *Maundia* still resembles Juncaginaceae, the family where the genus has been traditionally placed. In flower groundplan, *Maundia* is similar to *Aponogeton*, a similarity that should be taken into account because of the rarity and unusual nature of this flower organisation. The occurrence of air canals in the testa is another shared feature of *Maundia* and *Aponogeton* (though it is also present in some more distantly related Alismatales, see above). Similarities between *Maundia* and *Aponogeton* have been noted as far back as by Mueller (1858). In a handwritten annotation to one of his herbarium specimens (*F. Mueller* s.n., K000098531), Mueller stated: “Stigma and sepals like *Aponogeton* – Fruit and anthers like *Triglochin*”.

Furthermore, *Maundia* possesses several features that appear to be unique or rare in Alismatales, including the overall fruit structure (carpels united via flower centre, but specialized fruit fragmentation absent; spongy outer and stony inner pericarp layers, large cells in pericarp), inverted peripheral bundles in peduncle and the enigmatic formation of the nucellar coenocyte during embryo development.

As *Maundia* exhibits a mosaic of features characteristic of other families of tepaloid core Alismatales (Table 1), and taking into account its isolated phylogenetic placement, we prefer to segregate it in a family of its own, Maundiaceae. As pointed out by APG III (2009) and discussed by Iles, Smith & Graham (2013), an alternative to the recognition of another monogeneric family in Alismatales would be to create an expanded (but highly heterogeneous) family for the larger clade. If this alternative is followed, we suggest accepting only four families in the order Alismatales: (1) Tofieldiaceae, (2) Araceae, (3) a family comprising members of Alismataceae (incl. Limnocharitaceae), Butomaceae and Hydrocharitaceae, i.e., the petaloid clade of core Alismatales (Fig. 17A) and (4) a family comprising members of Aponogetonaceae, Scheuchzeriaceae, Juncaginaceae, Maundiaceae, Potamogetonaceae (incl. Zannichelliaceae), Zosteraceae, Cymodoceaceae, Ruppiaceae and Posidoniaceae, i.e., the tepaloid clade of core Alismatales (Fig. 17A). This broad concept would accommodate similarities between Aponogetonaceae and Maundiaceae as well as those between Scheuchzeriaceae and Juncaginaceae. Figure 17A shows some potential morphological synapomorphies (and autapomorphies) of various clades in a molecular phylogenetic tree of core Alismatales. Analyzing data in Fig. 17A reveals one of the most significant problems of the broad family concept. Namely, the petaloid clade is marked by several potential synapomorphies, while assessing clear and unambiguous synapomorphies for the tepaloid clade is more problematic (but see Stevens, 2001+).

Table 1. Overview of selected characters in *Maundia* and some other members of the tepaloid clade of core Alismatales. The following major sources were used: Uhl (1947); Gibbs (1974); Goldblatt & Johnson (1979+); Tomlinson (1982); Plisko (1985); Petrova (1985); Teryokhin (1985); Shaffer-Fehre (1987); Takhtajan (2009); Igersheim *et al.* (2001); Les *et al.* (2005); von Mering & Kadereit (2010); Furness & Banks (2010); Hellquist & Jacobs (2011); Kamelina (2011).

Character	*Aponogeton* (Aponogetonaceae)	*Scheuchzeria* (Scheuchzeriaceae)	*Cycnogeton* (Juncaginaceae)	*Triglochin* (Juncaginaceae)	*Tetroncium* (Juncaginaceae)	*Maundia* (Maundiaceae)	*Potamogeton* (Potamogetonaceae)	*Ruppia* (Ruppiaceae)
Foliage leaves on elongate shoots emerging above substrate present (P) or absent (A)	A	P	A	A	A	A	P	P
Leaf intravaginal squamules transformed into hairs (Yes/No)	N	Y	?	N	?	?	N	N
Foliage leaves ligulate (L) or eligulate (E)	E	L	E	L	E	E	L	E
Leaves differentiated into petiole and lamina (Yes/No)	Y or N	N	N	N	N	N	Y or N	N
Leaf apical pore absent (A) or present (P)	A or P	P	?	A	?	?	P	A
Sclerenchymatous cylinder in inflorescence peduncles (flowering stems) absent (A) or present (P)	?A	P	P	P	?	A	A	A
Inverted bundles in inflorescence peduncles (flowering stems) present (P) or absent (A)	A	A	A	A	?	P	A	A
*Crystals in vegetative organs present (P) or absent (A)	P	P	A	A	A	A	A	A
**Laticifers (P) or absent (A)	P	A	?	P	?	A(?)	A	A
Spathe enclosing young inflores-cence present (P) or absent (A)	P	A	A	A	A	A	A	A
Flower-subtending bracts absent (A), present and vascularised (V), present and unvascularized (U)	A	V	A	A	A	A	A or U	A

Tepals present (P) or absent (A)	P	P	P	P	P	P	P	A
Perianth normally consisting of two transversal-abaxial tepals (Yes/No)	Y(N)	N	N	N	N	Y	N	-
Vascular bundle branching in tepal blade present (P) or absent (A)	A(P)	A	A	A	?	P	P	-
Plants monoecious, flowers usually bisexual (M) or plants dioecious (D)	M(D)	M	M	M	D	M	M	M
Typical stamen number	(4-)6(-18)	6	6	(1-)6	4	6	4	2
Stamen filaments present (Yes/No)	Y	Y	N	N	N	N	N	N
Microsporogenesis successive (SU) or simultaneous (SI)	SI	SU	?	SU	?	?	SU	SU
Pollen: monads (M), dyads (D)	M	D	M	M	M	M	M	M
Pollen: monosulcate (M), inaperturate (I), triaperturate (T)	M	I	I	I	I	I	I	T
Pollination: biotic (B), abiotic (A)	B	A	A	A	A	A	A	A
Carpels in two trimerous whorls (Yes/No)	N (Y)	N	Y	Y	N	N	N	N
When four carpels are present, these are in diagonal (D) or median and transversal (MT) positions	MT	?	-	***	MT	MT	D	MT
Carpels free (F) or united (U)	U(F)	U	U	U	U	U	F(U)	F
Symplicate zone of gynoecium present (P) or absent (A)	A	P	A	A	?	A	A	A
Stomata on carpel surface present (P) or absent (A)	P	P	?	P	?	P	A	A
Ovules per carpel	>1	>1	1	1	1	1	1	1
Ovules anatropous (A) or orthotropous (O)	A	A	A	A	A	O	O	O
Nucellar coenocyte absent (A) or present (P)	A	A	?	A	?	P	A	A

Carpophore/column in fruits absent (A) or present (P)	A	A	A	P(A)	A	A	A	A
Postanthetic elongation of carpel stalks absent (A) or present (P)	A	A	A	A	A	A	A	P
Stamens and tepals abscise after anthesis (A) or normally remain unshed, at least stamen connectives (U)	A or U	U	A	A	U	U	U	U
Seeds with persistent, many-layered mesotesta of thick-walled cells (Yes/No)	N	Y	N	N	N	N	N	N
Cyanogenic compounds present (P) or absent (A)	A	P	A	P	?	?	A	A
Chromosome number, 2n	16, 24, 32, 40, 56	22	16, 32, 64	12, 18, 24, 36, …, 156	?	?	14, …, 52, 104	20, 40
Floral diagrams, Fig. 16	D,E	F	=G	G		A,B	H	I

* Data from Tomlinson (1982) that need further verification (see Prychid & Rudall, 1999).

** This character clearly needs further investigation as published data are inconsistent.

*** Four-carpellate gynoecia only rarely occur in *Triglochin*, but both kinds of carpel arrangement (D and MT) are recorded (Goebel, 1928).

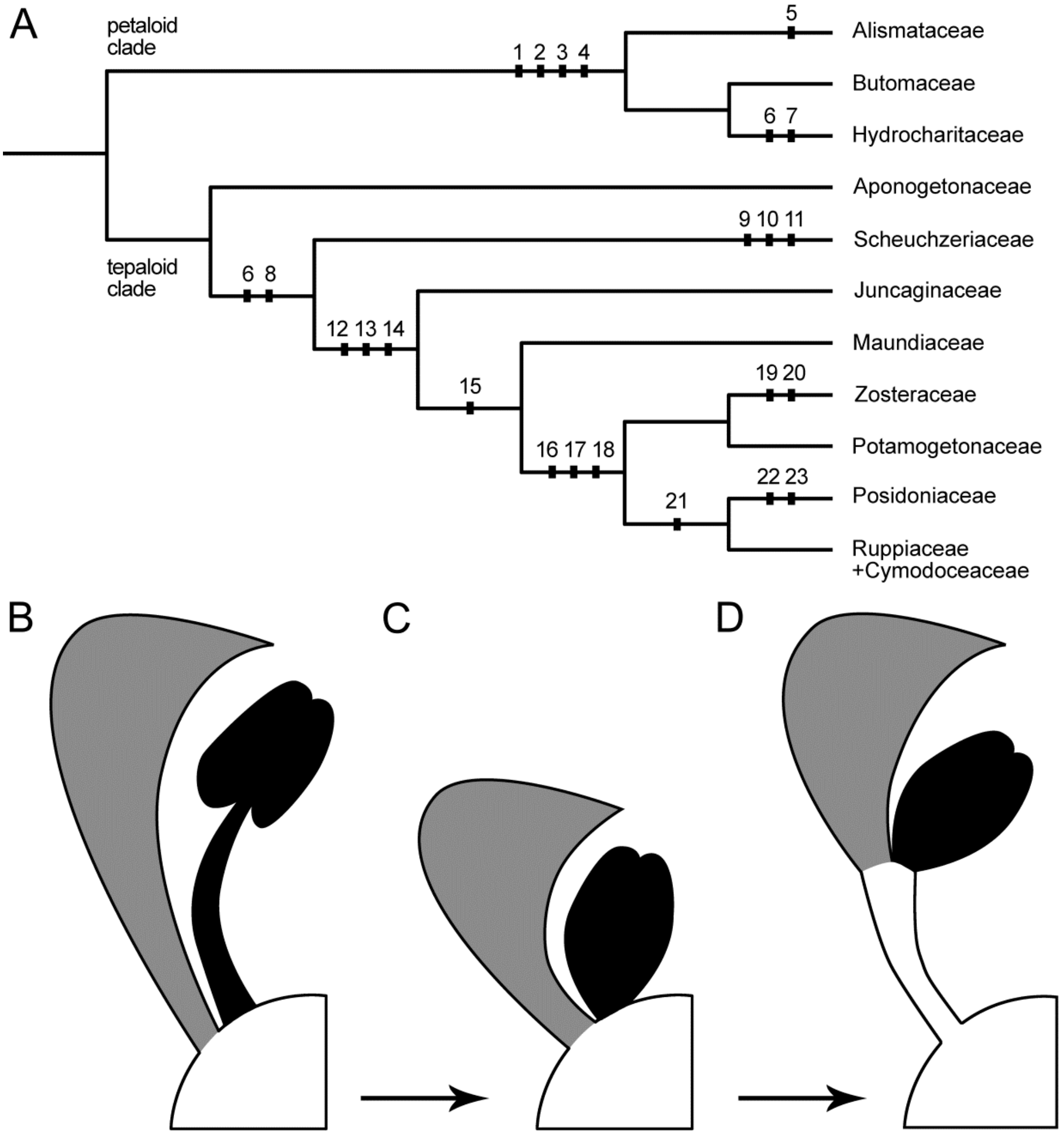

Fig. 17. A, Molecular-based phylogenetic tree of Alismatales (based on Iles et al., 2013) with potential morphological synapomorphies of different clades. B–D, Hypothetical series of morphological transformations of tepal/stamen complexes in the tepaloid clade of Alismatales. Schematic longitudinal sections, tepal grey, stamen black. B, Scheuchzeriaceae, Aponogetonaceae. C, Juncaginaceae, Maundiaceae. D, Potamogeton. Morphological synapomorphies: 1, lateral flowers with floral prophylls; 2, perianth differentiated into sepals and petals (reduced in specialized aquatic forms of Hydrocharitaceae); 3, stamen pairs in androecium present (reduced in specialized aquatic forms of Hydrocharitaceae); 4, placentation diffuse-laminar (except in forms with single ovule per carpel); 5, pollen pantoporate; 6, pollen inaperturate (in specialized submerged aquatic forms with reduced exine or, in Ruppia, triaperturate); 7, ovary inferior; 8, pollination abiotic (also in specialized Hydrocharitaceae); 9, leaf intravaginal squamules transformed into hairs; 10, flowersubtending bracts large, vascularized, basal ones similar to foliage leaves; 11, pollen in dyads; 12, stamen filament loss; 13, carpels pronouncedly ascidiate; 14, carpels with single ovule (also in some members of the petaloid clade); 15, ovule pendent and orthotropous; 16, leaves on elongate shoots emerging above substrate (also in some Hydrocharitaceae); 17, stomata on carpel surface absent (also in specialized submerged Hydrocharitaceae); 18, carpels free (as an exception, weakly united via flower centre: *Potamogeton crispus*); 19, inflorescence axis

flattened; 20, flowers monomerous (also in *Triglochin scilloides*); 21, loss of perianth (also in some other specialized aquatics); 22, ovule campylotropous, with integumental outgrowth; 23, stamens flattened, tepal-like after abscission of thecae.

Analysing morphological data on tepaloid core Alismatales in a phylogenetic context allows discussion of stamen evolution in this group (Fig. 17B–D). The possession of stamens with sessile anthers is a synapomorphy of the clade that includes Juncaginaceae and its sister lineage. The absence of stamen filaments is characteristic of all members of this clade. At first glance, most species of *Potamogeton* (Potamogetonaceae) represent an exception because the anthers are stalked. However, this stalk is common to the anther and the perianth member and appears late in flower development due to interprimordial and intercalary growth (Hegelmaier, 1870; Sattler, 1965; Posluszny and Sattler, 1974). In a phylogenetic context, the common stalk should be better interpreted as a novel structure rather than a product of congenital fusion of between the stamen and the tepal. As pointed out by Posluszny and Sattler (1974: 216), it is not quite correct to say that the tepal is inserted at the stamen connective in *Potamogeton*. With respect to the relative position of the anther and the adjacent tepal, *Potamogeton* does not differ from taxa such as Juncaginaceae s.str. and Maundiaceae.

Conclusions and outlook

Phylogenetic relationships within Juncaginaceae as proposed by this study (Chapter 1) have been confirmed by more recent studies. The placement of *Maundia* outside the family Juncaginaceae was recovered by two studies using other molecular markers (Iles et al. 2013, Les & Tippery 2013). However, the exact position of *Maundia* in relation to the members of the "*Potamogeton* clade" (Chapter 1) could not be clarified yet (Iles et al. 2013, Les & Tippery 2013). The proposed placement in a separate family Maundiaceae (Chapter 1) was discussed by APG III (2009) and Iles et al. (2013) and Maundiaceae has been accepted by some authors until further evidence becomes available (Stevens 2001+, Les & Tippery 2013). The study on the flower structure of *Maundia* (Chapter 5) adds new evidence to this discussion.

Important achievements presented in this thesis comprise the re-circumscription of the family (Chapter 1), investigations of the major lineages within *Triglochin* (Chapter 1 and 2), and the revision of the *Triglochin bulbosa* complex (Chapter 3). It could be demonstrated that the combination of molecular, morphological, ecological and distributional data (i.e. a total evidence approach) gives best results for a sound phylogenetic analysis and classification. However, this study has also shown the necessity for further taxonomic and revisionary work in the family.

Re-circumscription of Juncaginaceae is an important basis for future studies and allows new insight into the evolutionary history of the family and related Alismatales. For the first time, a species level phylogeny is available for *Triglochin* (Chapter 2) enabling further research on the evolutionary history of the genus. The revision of the *T. bulbosa* complex (Chapter 3) and the newly described taxa from South Africa expand our knowledge of the flora in the Cape Floristic region.

During the course of the project, further research questions were detected that could not be addressed due to difficulties in obtaining living plant material for study. Examples of such knowledge gaps include chromosome numbers as well as embryological data for *Tetroncium* and *Maundia*. Karyological studies of *Triglochin* could yield valuable information as chromosome numbers are unknown, for example, in the annual Australian *Triglochin* species and some taxa of the *T. bulbosa* complex.

Ongoing investigations include taxonomic studies, for example in the *Triglochin maritima* complex, including the search for type material and lectotypifications which are essential for future revisions. Collaborative research on the phylogeny and systematics of annual *Triglochin* species from Australia has recently been initiated. Furthermore, investigations of fruit morphology and development in the group are continuing in collaboration with colleagues (Chapter 5; Lock et al. 2011).

Online platforms or virtual research environments such as the *EDIT Platform for Cybertaxonomy* (Berendsohn 2010) or the Scratchpads (Smith et al. 2011) are increasingly used to build dynamic online information systems in order to facilitate access to and exchange of scientific data (Berendsohn et al. 2011, Smith et al. 2011). Information on Juncaginaceae has been very scattered. To improve access to varied information about the family an online platform has been established: *Juncaginceae of the World* (von Mering 2012+), a Scratchpad which is developed in close collaboration with the eMonocot project (http://e-monocot.org/). In addition to a continuously updated classification for Juncaginaceae ("taxonomic backbone"), it provides access to a bibliography and an increasing number of taxon pages with descriptions, images, and specimen information. New information, e.g. on type specimens or newly described taxa are continuously added to this online tool. Such an online resource is a basis for studies in the context of e-taxonomy, sometimes also called online-taxonomy or web-taxonomy (e.g., Godfray et al. 2007, Mayo et al. 2008) and might eventually result in an "online monography" of the family.

Identification keys have been prepared for the genera of the family (Chapter 1) and for the *Triglochin bulbosa* complex (Chapter 3). Identification tools including traditional dichotomous keys (single-access keys) and modern multi-access keys (generated with the programme Xper2, Ung et al. 2010) for all taxa of the family will be published and presented in online information systems. Two example keys for *Triglochin* in Europe and Central Europe have already been published on the open access platform *Offene Naturführer* (Hagedorn et al. 2010) and are available online (see Appendix 6, von Mering 2011).

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APPENDICES

Appendix 1: List of accessions (Chapter 1)

Appendix 2: Voucher information (Chapter 2)

Appendix 3: PCR information (Chapter 2)

Appendix 4: List of studied specimens of *Tetroncium magellancium* Willd. (Chapter 4)

Appendix 5: List of studied specimens of *Maundia triglochinoides* F.Muell. (Chapter 5)

Appendix 6: Identification keys for *Triglochin* in Europe

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., *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, —.

APPENDIX 2.

Voucher information and GenBank accession numbers for sequences analysed in this study.

Taxon	**Voucher** [Collector, collector no., herbarium code]	**Origin**	**I TS**	***psb*****A-*****trn*****H**	***mat*****K**
T. sp. A (Flora of Australia)	*Jacobs 4147a* (NSW)	Australia	x		
T. barrelieri Loisel.	*v. Mering s.n.* [2006] (MJG)	Italy, Sardinia	x	x	x
T. barrelieri Loisel.		Spain, Mallorca			
T. barrelieri Loisel.	*Uhink s.n.* [2005] (MJG)	Italy, Sicily	x		
T. barrelieri Loisel.	*Deil s.n.* [1990] (FB)	Spain	x		
T. barrelieri Loisel.	*Mateo et al. 4824/95* (RNG)	Morocco	x	x	
T. barrelieri Loisel.	*Sekegin & Sokoloff* [2004] (NW)	Cyprus	x		
T. barrelieri Loisel.	*Westberg 17/01/Gr* (MJG)	Greece	x		
T. barrelieri Loisel.	*Poelt & Poelt 207* (M)	Portugal		x	
T. buchenaui Köcke, Mering & Kadereit	*Köcke & Steffen* [2006] (MJG)	South Africa			
T. bulbosa L.	*Mucina & Jakubowsky 040806/02* (MJG)	South Africa	x		
T. bulbosa L.	*Köcke & Mucina 030406/ 22* (MJG)	South Africa	x		
T. bulbosa L.	*Köcke & Mucina 140406/01* (MJG)	South Africa	x		
T. bulbosa L.	*Mucina & Jakubowsky 040806/05* (MJG)	South Africa	x		
T. bulbosa L.	*Köcke & Mucina 140406/33* (MJG)	South Africa	x		
T. bulbosa L.	*Köcke & Steffen 190406/02* (MJG)	South Africa	x		
T. bulbosa L.	*Köcke & Steffen 190406/28* (MJG)	South Africa	x		
T. bulbosa L. ssp. *bulbosa*	*Linder 7076* (MJG)	South Africa	x	x	
T. bulbosa L. ssp. *calcicola* Mering, Köcke & Kadereit	*Mucina 251008/05* (MJG)	South Africa	x	x	
T. bulbosa L. ssp. *calcicola* Mering, Köcke & Kadereit	*Mucina & Merunkova 280908/04* (MJG)	South Africa	x	x	
T. bulbosa L. ssp. *calcicola* Mering, Köcke & Kadereit	*Merunkova KM 50/34* (MJG)	South Africa		x	
T. bulbosa L. ssp. *quarcicola* Mering, Köcke & Kadereit	*Schmiedel 124811* (MJG)	South Africa	x	x	
T. calcitrapa Hook.	*Wilson 1860a* (B)	Australia	x	x	
T. calcitrapa Hook.	*Greuter 18564* (B)	Australia	x		
T. calcitrapa Hook.	*Michell & Rister 1769* (B)	Australia	x		
T. centrocarpa Hook.	*Greuter 20973* (B)	Australia	x	x	
T. cf. *bulbosa*	*Wörz 04.10.06.01* (STU)	Australia	x	x	

T. compacta Adamson	*Naudé* s.n. (MJG)	South Africa	x		
T. compacta Adamson	*Goldblatt 1792* (C)	South Africa		x	
T. concinna Burtt Davy	*Rittenhouse 1937* (OSC)	USA, Oregon	x	x	
T. concinna Burtt Davy	*Ackermann 95* (B)	Chile		x	
T. concinna ssp. d*ebilis* (M.E.Jones) J.T.Howell	*Halse 2383* (C)	USA	x		
T. concinna ssp. d*ebilis* (M.E.Jones) J.T.Howell	*Tiehm 11558* (OSC)	USA, Nevada	x	x	
T. concinna ssp. d*ebilis* (M.E.Jones) J.T.Howell	*Wright 1807* (C)	USA, Oregon		x	
T. elongata Buchenau	*Vargas 537PV00* (MJG)	South Africa	x	x	x
T. gaspensis Lieth & D.Löve	*Teege s.n.* [2005] (MJG)	USA		x	
T. hexagona J.M.Black	*Latz 8458* (NSW)	Australia	x	x	
T. laxiflora Guss.	*v. Mering* s.n. (MJG) Sard5129 / 131_Sardinia	Italy, Sardinia	x	x	
T. laxiflora Guss.	*Mifsud* s.n. [2007] (MJG)	Malta	x	x	
T. laxiflora Guss.	*Rosello s.n.* (MJG)	Spain, Mallorca	x		
T. laxiflora Guss.	*Rosello s.n.* (MJG)	Spain, Mallorca	x		
T. maritima L.	*Lambracht s.n.* (MJG)	Germany	x		
T. maritima L.	*v. Mering s.n.* [2006] (MJG)	Germany		x	
T. maritima L.	*Höhn s.n.* [2005] (MJG)	Hungary	x	x	
T. maritima L.	seeds (BG Greifswald)	Mongolia	x		
T. maritima L.	*Nesbitt 1178* (RNG)	Turkey	x	x	
T. maritima L.	*Mehregan s.n.* [2002] (MJG)	Iran	x		
T. maritima L.	*Strauss s.n.* (GFW)	Mongolia	x	x	
T. maritima L.	*Ito & Tanaka 540-3* (MJG)	Japan	x	x	
T. maritima L.	*Masuda s.n.* [2006] (MJG)	Japan		x	
T. maritima L.	*Ito et al. 298-3* (MJG)	South Korea	x		
T. maritima L.	*Larson 11383* (OSC)	USA, South Dakota	x	x	
T. maritima L.	*Porsild & Weber 22933* (C)	USA, Colorado	x	x	
T. maritima L.	*Tiehm 10714* (OSC)	USA, Nevada	x	x	
T. maritima L.	*Coope 35* (OSC)	USA, Oregon	x		
T. maritima L.	*Pierce 1843* (C)	USA	x		
T. maritima L.	DQ786518			x	
T. maritima L.	*Garneaux & Tailleux s.n.* (O)	Canada, Quebec		x	
T. maritima ssp. *asiatica* Kitag.	*Ignatov 32* (MW)	Russia, Sakhalin	x	x	
T. milnei Horn	*Bidgood, Mbago & Vollesen 2603* (P)	Tanzania	x	x	
T. palustris L.	*Franklin 7426* (OSC)	USA, Utah	x		
T. palustris L.	*Strauss* [2002] (GFW)	Mongolia	x	x	
T. palustris L.	*Schönswetter & Tribsch T145* (WU)	Russia	x	x	
T. palustris L.	*de Lange* [1995] (AK)	New Zealand	x	x	
T. palustris L.	*Zündorf 21382* (JE)	Chile	x	x	
T. palustris L.	*Schmalz s.n.* [1997] (MJG)	Germany	x	x	
T. palustris L.	cult., BG Mainz	Germany	x		

T. scilloides (Poir.) Mering & Kadereit	*Moore s.n.* [2006] (MJG)	California	x	x	x
T. scilloides (Poir.) Mering & Kadereit	*Feuerer 9524a* (HBG)	Bolivia	x		
T. striata Ruiz & Pav.	*Glasson PD90/17* (OSC)	New Zealand	x	x	
T. striata Ruiz & Pav.	*Macmillan BH 98/32* (CHR)	New Zealand		x	
T. striata Ruiz & Pav.	*Gibbons 314* (C)	Australia	x	x	
T. striata Ruiz & Pav.	*Schmalz s.n.* (MJG)	Australia	x	x	x
T. striata Ruiz & Pav.	*Pullen 4322* (C)	Australia		x	
T. striata Ruiz & Pav.	*Myndel Pedersen s.n.* (C)	Brazil		x	
T. striata Ruiz & Pav.	SAfrcon0705064	South Africa	x		
T. striata Ruiz & Pav.	*Manitz RCH 71* (JE)	Chile	x	x	
T. striata Ruiz & Pav.	*Burnett 389* (OSC)	USA, Washington	x		
T. striata Ruiz & Pav.	*Leonard 2109* (C)	USA, North Carolina	x	x	
Cycnogeton multifructum (Aston) Mering & Kadereit	*Jacobs SJ9397* (NSW)	Australia	x	x	
Cycnogeton rheophilum (Aston) Mering & Kadereit	*Jacobs SJ9392* (NSW)	Australia	x	x	x

APPENDIX 3.

PCR information: primer sequences and origin, PCR ingredients and conditions. Notes: ITS, internal transcribed spacer; BSA, bovine serum albumine.

The complete ITS region (ITS1, the 5.8 gene, ITS2) was amplified as a single piece using the standard primers 18S and 28S (Muir and Schlötterer, 1999). For some accessions with low DNA quality, ITS was amplified in two overlapping fragments using the primer pairs ITS A, ITS C, and ITS B, ITS D (Blattner, 1999).

Marker	Primer sequence 5'-3'	Source	PCR ingredients	PCR conditions
ITS	**18S:** CCT TMT CAT YTA GAG GAA GGA G	Muir et al. 2001	$MgCl_2$ 0.48mM Polymerase 0.04 U/µL dNTPs 0.2 mM	Pretreatment: 94°C, 60s Denaturation: 94°C, 18s Annealing: 52°C, 30s
	ITS 4: TCC TCC GCT TAT TGA TAT GC	White et al. 1990	Primer 0.8 mM BSA 0.1 mg/ml	Elongation: 72°C, 60s Post treatment: 52°C, 78s, 72°C, 8min Cycles: 35
*psb*A-*trn*H	**psbA-F:** GTT ATG CAT GAA CGT AAT GCT C	Sang et al. 1997	$MgCl_2$ 0.48mM Polymerase 0.04 U/µL dNTPs 0.2 mM	Pretreatment: 94°C, 60s Denaturation: 94°C, 20s Annealing: 52°C, 30s Elongation: 72°C, 60s Post treatment: 52°C, 80s, 72°C, 8min Cycles: 35
	trnH-R: CGC GCA TGG TGG ATT CAC AAA TC	Sang et al. 1997	Primer 0.8 mM BSA 0.8 mg/ml	
*mat*K	see von Mering & Kadereit 2010			

APPENDIX 4.

List of studied specimens of *Tetroncium magellancium* Willd.

ARGENTINA: PROVINCIA DE RÍO NEGRO: Departamento Bariloche: Parque Nacional Nahuel Huapi, Laguna Ortiz Basualdo, terreno pantanoso, 07.01.1952, *O. Boelcke 5366 & M. N. Correa* (SI image!). Parque Nacional Nahuel Huapi, Pampa Linda, mállin Amer, high raised bog above Lago Mascardi, alt. 1000 m, 08.12.1973, *V. Markgraf s.n.* (P). Parque Nacional Nahuel Huapi, between Puerto Blest and Lago Frias, alt. 750 m, 18-19.12.1978, *S. Laegaard 12533* (K). PROVINCIA SANTA CRUZ: Dpto. Lago Argentino: Brazo Norte, valle de la Cristina, ac de los Cipresas, al este lago Pearson, alt. 450 m, 17.2.1953, *F. B. Vervoorst 4496* (NY, P). Brazo Onelli, mallín al NW del puesto abandonado Neumann, alt. 350 m, 26.2.1953, *F. B. Vervoorst 4564* (NY). Mayo Glacier, Shipton Expedition to Patagonia (Lago Argentino) 1958-59, in small bog in clearing in *Nothofagus betuloides* wood, near the front of the glacier, 49° 80'-51° S, 72°-73° 30' W, alt. c. 400 ft., 30.01.1959, *P. W. James 1500* (BM, SI image!). PROVINCIA DE TIERRA DEL FUEGO, ANTÁRTIDA E ISLAS DEL ATLÁNTICO SUR: Departamento Río Grande: Source grande de Río Grande. Alt. 400-450 m, 06.03.1896, *N. Alboff s.n.* (SI image!). Nahe Lago Verde an Ruta B, 09.01.1989, *J. Poelt s.n.* (M). Departamento Ushuaia: St. Vincent's Bay [Buen Thetis or Thetis Bay], Captain Cook's first voyage, H.M.S. Endeavour, 1768-1771, in collibus subalpinus, 14.01.1769, *J. Banks & D. Solander s.n.* (BM). Ushuaia, in a bog in the *Nothofagus pumilio* forest, above the town, alt. c. 300 m, 01.02.1940, *R. Santesson 396* (K, P). Lago Victoria, turbera, 07.11.1965, *Luis Mendoza s.n.* (BAB image!). Estancia Harberton, Moat Bay, alt. c. 60-100 ft., hill behind lake, growing in *Astelia* bog, 01.01.1966, *R. N. P. Goodall 247* (RNG). Estancia Harberton, Harberton swamp, alt. c. 60-100 ft., in *Sphagnum*, deeply buried, only 2-3 ins. Showing, 01.02.1966, *R. N. P. Goodall 300* (RNG). Estancia Harberton, Harberton swamp, NW end near fence, elev. 0-60 m, plants growing in green and reddish *Sphagnum*, *Sphagnum* very wet and easily broken apart, showing above moss, 28.12.1966, *R. N. P. Goodall 443* (BAB image!, NY, RNG, SI image!). Estancia Harberton, Harberton swamp, elev. 0-60 m, growing in *Sphagnum* roots, have haustoria of *Nanodea mucosa*, 29.04.1967, *R. N. P. Goodall 795* (P). Bahía Aguirre, c. 5 km E of Puerto Espagnol, 54° 53' S 65° 54' W, 30-80 m, boggy ground with *Astelia*. 14.02.1968, *D. M. Moore 1831* (K, RNG). Río Lashifashaj valley, c. 1 km N of Laguna Victoria, 54° 48' S 67° 27' W, *Sphagnum* swamp, 01.03.1968, *D. M. Moore 2082* (BAB image!, RNG). Estancia Harberton, Cambaceres Bay, in swamp. 03.12.1968, *R. N. P. Goodall 1882* (RNG). Loma Larga Forte, 900 ft., in open burned woods, not grazed, 22.01.1968, *R. N. P. Goodall 1309* (B). Hill to the North of abandoned settlement at Bahía Thetis, top of hill is *Astelia* formation with numerous small pools, each edged with *Tetroncium*, sterile at this time, 20.11.1969, *R. N. P. Goodall 2311* (RNG, SI image!). Tierra del Fuego australis, Rancho Hambre, 54° 45' S, 67° 54' W, turbal sphagnoso, abierto, copiosamente, 140 m, 16.01.1970. *H. Roivainen s.n.* (RNG). Lapataia, growing in *Sphagnum* swamp surrounding Laguna Negra, to the West of Río Lapataia, 02.03.1970, *R. N. P. Goodall 2424* (BAB image!, SI image!). Lapataia, Laguna Negra, in wet *Sphagnum* swamp, 06.12.1970, *R. N. P. Goodall 2634* (RNG). Pink Mountain, edge of mountain torrent at SE edge of mountain and in *Sphagnum* swamp at base of mountain, 2000 to 1500 ft., 11.03.1971, *R. N. P. Goodall 3700* (SGO). Mitre Península of Isla Grande, Bahía Buen Suceso, 54° 48' S 65° 20' W, 14.10.1971, *T. R. Dudley, R. N. P. Goodall & G. Crow 272* (BAB image!). Tra Lapataia ed il lago Roca (ovest di Ushuaia), torbiera nella parte orientale della Laguna Negra, sui cuscini di *Sphagnum*, Spedizione Scientifica Italiana Mares AMF in Patagonia, Terra del Fuoco ed Antartide organizzata dal Gruppo Ricerche Scientifiche Tecniche Subacqueo di Firenze, 23.01.1974, *R. E. G. Pichi Sermolli & P. Bizzarri 7539* (K, P, SI image!). Ushuaia, Weg zum Nationalpark /camino al Parque Nacional, 27.12.1976, *P. Seibert 183 et al. / T.B.P.A. 2213* (BAB image!, M). At Passo Garibaldi, alt.
750-800 m, 26.-31.01.1979, *S. Laegaard 13296* (K). Cordon del Toro at Host. Alakush, W of Ushuaia, alt. 600 m, 01.-04.02.1979, *S. Laegaard 13338* (K). Isla de los Estados, Bahía Franklin, entra Caleta Croix y lago Gaona, 54°52'S 64°41'W, abundante localmente en charcos en turbera dominada por *Astelia*, 10.12.1999, *F. Biganzoli 699* (SI image!).

CHILE: IX REGIÓN DE LA ARAUCANÍA: Provincia de Malleco: Parque Nacional de Nahuelbuta, centro del parquet, 37°43'S 73°02'W, 1250 m, 28.12.1968, *M. Ricardi 5697 & C. Marticorena 1858* (CONC image!, SI image!). X REGIÓN DE LOS LAGOS: Provincia de Chiloé: Isla Grande de Chiloé, altiplanicie central (campaños), January 1902, *M. Espinosa s.n.* (SGO). Cordillera San Pedro, wet hollow in campaña, 15.11.1958, *E. J. Godley 474a* (BM). Cordillera San Pedro, wet hollows in open campaña, 18.11.1958, *E. J. Godley 503* (SGO). Isla Grande de Chiloé, Cordillera de Piuchén, 42°22'S 73°59'W, 640 m, 12.02.1983, *C. Villagrán 4907* (CONC image!). Llicaldad, en turbera esfagnosa, 42°29'S 73°50'38''W, 495 m, 18.02.2010, *S. Pfanzelt 455* (CONC image!). Provincia de Palena: 5.9 km N jct. rds. to Palena and Chaitén, Villa Sta. Lucia, bog in *Nothofagus* forest, 560 m, March 1985, *T. F. Stuessy et al. 7170* (CONC image!). XI Región Aysén del General Carlos Ibáñez del Campo: Provincia de Capitán Prat: [Messier Channel], Halt Bay [Bahía Halt], 21.03.1868, *R. O. Cunningham s. n.* (K). [Sarmiento Channel], Puerto Bueno, 08.12.1868, *R. O. Cunningham s. n.* (K). Glaciar "Hammick", 48°50'S 74°13'W, común en pantanos, ± 30 m, 28.12.1967, *S. F. Anliot 6033* (SGO). Villa O'Higgins, Cuenca del Río Mosco, turbera, subcuenca del Río Claro, 48°27'S 72°28'W, 785 m, 20.03.2003, *N. García 21* (CONC image!). XII Región de Magallanes y de la Antártica Chilena: Provincia de Última Esperanza: [Sarmiento Channel], Puerto Bueno (Puerto bono), Expédition de la Magicienne, 1876-1879, lieux humides et tourbeux, 15.02.1877, *L. Savatier s.n.* (P 2 sheets). [Wellington Island], Eden [Puerto Edén], Expédition de la Magicienne 1876-1879, 24.01.1879, *L. Savatier s.n.* (K, P 3 sheets). Canal Smith, February 1900, *R.* [s.c., s.n.] (SGO). Canal Smith [Canal Smyth, Smyth Channel], January 1924, *M. Gusinde s.n.* (M). Puerto Eden, Wellington Is., lowland bog, near sea, 06.12.1958, *E. J. Godley 631a*, *632b* (BM). Bahía del Indio, Lote San Isidro, Río Yumbel, interior, en turbales herbaceous, 25.02.1973, *E. Pisano V. 3968* (RNG). Seno Unión, N side, Ancón Sin Salida, 95 m, 52°9'S 73°21'W, Y201, bog with scattered trees, 10.01.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 876* (RNG). Seno Unión, 52°25'S 73°35'W, 13.01.1976, *Ulrich Eskuche / T.B.P.A. 695* (BAB image!). Isla Piazzi, Caleta Ocasión, Abra Leackey's Retreat, 150 m, 51°44'S 74°1'W, S231, rocky hill summit, wet seepage area, 16.01.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1003* (BAB image!, RNG). Isla Piazzi, Caleta Ocasión, Abra Leackey's Retreat, 80m, 51°44'S, 74°1'W, S231, coastal bog, *Donatia*/*Tetroncium* dominant, 18.01.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1069* (BAB image!, RNG). Isla Rennel Norte, Canal Smyth, 51°54'S 74°12'W, W231, bog, 24.01.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1152* (BAB image!, RNG). Isla Vidal Gormaz, Seno Nantuel, Bahía María Angélica, 30 m, 51°53'S 74°41'W, W251, *Donatia* bog, 01.02.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1237* (BAB image!, RNG). Isla Vidal Gormaz, Seno Nantuel, Estero Lobos, 51°53'S 74°41'W, W251, *Donatia* bog, 04.02.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1395* (RNG). Isla Virtudes, Canal Eliás, Puerto Virtudes, 51°33'S 74°54'W, P261, bog, pool with organic bottom material, 10.02.1976, *O. Dollenz, D. M. Moore, E. Pisano V. & A. A. Saenz / T.B.P.A. 1477* (BAB image!, RNG). Seno Última Esperanza, Lago Azul, E side, 51°27'S 73°18'W, N201, dryish bog, hummocks with some water between, 10.01.1977, *D. M. Moore & E. Pisano V. / T.B.P.A. 1541* (RNG). Seno Última Esperanza, Puerto Bella Vista, S shore, 51°31'S 73°16'W, P201, bog above coastal forest, higher ground at margin of bog, 11.01.1977, *D. M. Moore & E. Pisano V. / T.B.P.A. 1599* (RNG). Seno Última Esperanza, Puerto Toro, Río Serrano, penin. W side estuary, 51°25'S 73°5'W, N191, *Sphagnum* bog with scattered trees of *Pilgerodendron*, *N. antarctica* & *N. betuloides*, 16.01.1977, *D. M. Moore & E. Pisano V. / T.B.P.A. 1733* (RNG). Seno Última Esperanza, Lote Sanchez, costa E Río Serrano, 51°26'S 73°5'W, N192, turbal esfagnoso, en cojines de *Sphagnum magellanicum*, 26.01.1977, *E. Pisano V. / T.B.P.A. 2039* (RNG). Península Roca, Seno Resi, ca. 175 m, 51°51'S 73°2'W, W191, urbal interior, común, 22.01.1978, *E. Pisano V. / T.B.P.A. 2811* (BAB image!, RNG). Puerto Bella Vista, 51°30'S 73°15'W, 06.12.1979, *F. Roig, O. Dollenz & E. Méndez / T.B.P.A. 5139 Censo 38* (BAB image!). Puerto Bella Vista, 51°30'S 73°15'W, 09.12.1979, *F. Roig, O. Dollenz & E. Méndez / T.B.P.A. 5300 Censo 77* (BAB image!). W coast of Brunswick Peninsula, where Puerto Prat / Los Canellos road meets, 05.04.1985, *B. J. Wallace 089/85* (SGO). Fiordo Peel, Río Murtillar, 50°27'S 73°37'W, en turbales musgosos en afloramientos rocosos, 18.11.1985, *E. Pisano V. 5925* (RNG). Fiordo Peel, Río Murtillar, 50°27'S 73°37'W, forma tapices extensos en sitios muy húmedos en turbales esfagnosos, 19.11.1985, *E. Pisano V. 5948* (RNG). Fiordo Peel, Río al E del cerro Aguilera, 50°30'S 73°44'W, en turbales esfagnosos sobre afloramientos rocosos, sitios inundados, 15.12.1985, *E. Pisano V. 6070* (RNG). Provincia de Magallanes: Détroit de Magellan, s. loc., s. d., *Léman s.n.* (P). Port Famine [Puerto (del) Hambre], Capt. King's Voyage to South America in *H.M.S. Adventure* (King's Voyage) 1826-1830, *[J.] Anderson s.n.* (BM, K). Détroit de Magellan, Port Gallant (Ports Gallant, Famine, etc.), Voyage de l'Astrolabe et de la Zélée 1838-1840, *H. Jacquinot & [J.] Hombron s.n.* (P). Détroit de Magellan, Port Famine, Voyage de l'Astrolabe et de la Zélée, 1838-1840, [*E.*] *Le Guillou s.n.* (P). Détroit de Magellan, Baie St. Nicholas et Bougainville [St. Nicholas Bay], Voyage de l'Astrolabe et de la Zélée, 1838-1840, [*E.*] *Le Guillou s.n.* (P). Port Gallant [Puerto Gallant], Extra-Tropical South America Survey of H. M. S. Nassau 1866-1869, 19.04.1869, *R. O. Cunningham s. n.* (K, NY). Isla Dawson, Laguna Buen Pastor, 23.12.1910, *A. Benove s.n.* (SI image!). Isla Dawson, Bahía Lomas, 23.12.1910, *A. Benove s.n.* (SI image!). Punta Arenas, turberas, Jan.-March 1917, *G. Bonarelli 38* (SI image! 2 sheets, ZT). [Punta Arenas], Laguna Lynch, turbera, Jan.-March 1917, *[G.] Bonarelli 38a* (SI image!). S from Punta Arenas along W side of Straits, c. 4 km N of Fuerte Bulnes, wet boggy depression among *Nothofagus antarcticus* woodland, abundant on bare peat in *Sphagnum* bog, assoc. *Drosera uniflora*, 23.03.1964, *D. M. Moore 1132* (AAS image!, K, RNG, SGO). Fiordo Silva Palma, Angostura Titus, costa opuesta a ex aserradero, en turbales esfagnosos, 08.01.1973, *E. Pisano V. 3771* (RNG). Laguna Parrillar, costa E, en turbales esfagnosos, 01.02.1973, *E. Pisano V. 3934* (RNG). Estancia Skyring, Punkt 046, 52°25'S 71°52'W, 25.02.2001, *A. Vogel s.n.* (MJG). Provincia de Tierra del Fuego: Pantanos cerca del Rio Fontaine, Expeditio suecia 1907-1909, 01.03.1908, *C. Skottsberg s.n.* (SGO). Provincia de la Antártica Chilena: Tierra del Fuego, Orange Harbor, U.S. Exploring Expedition (Wilkes Expedition) 1838-1842, [January - April 1839], s. coll., s.n. (K, US image!). Hermite Island [Isla Hermite], Cape Horn, Antarctic Expedition 1839-1843, [20.09.-07.11.1842], *J. D. Hooker s.n.* (K, P 2 sheets). Terre de feu, Île Hoste [= Isla Hoste], Baie Orange, Mission du Cape Horn, 28(?).07.1883, *P. Hariot s.n.* (P). Terre de Feu, s. loc., 1890-1891, *Rousson & Willems* s.n. (P). Terre de Feu, s. loc., 1892, *Willems & Rousson* s.n. (LY image!). Canal Beagle [Beagle Channel], Rimolino-Sumpf, February 1922, *M. Gusinde s.n.* (BR, K, M). Puerto Williams, Navarino Is., lowland *Sphagnum* bog, 08.01.1959, *E. J. Godley 924* (SGO), *E. J. Godley 924a* (BM), *E. J. Godley 924b* (SGO). Puerto Toro, Isla Navarino, en turbales, 17.01.1972. *E. Pisano V. 3460* (RNG). Isla Wollaston, Caleta Lientur, 55°44'S 67°19'W, en turbal musgoso, escaso, 17.02.1980, *E. Pisano V. 5006* (RNG). Arch. Cabo de Hornos, Isla Grevy, Rada Norte, Ba. Gretton, en sitios pantanosos de turbales pulvinados, 16.01.1982, *E. Pisano V. 5385* (RNG). Isla Hoste, Caleta Awaiakirrh, en sitios inundados de turbales esfagnosos, 23.01.1982, *E. Pisano V. 5446* (RNG). Île Picton [Picton Island], Caleta Banner, 55°01'S 66°56'W, 500 m au S du hameau, à mi-chemin du lac, alt. 20 m, 30.12.1998, *L. Gautier 3414* (M).

FALKLAND ISLANDS (ISLAS MALVINAS): EAST FALKLAND: s. loc., s. d. [1842], *J. D. Hooker s. n.* (K). Canopus Hill, East of Stanley, 51° 41´S 57° 47´ W, assoc. *Juncus scheuchzerioides* and *Cortaderia pilosa*, male and female plants growing about 25 m apart, 14.12.2006, *R. W. Woods s.n. & M. Morrison* (MJG). Cape Pembroke, close to Stanley airport, acid grassland, associated with grass, *Gunnera*, *Carex fuscula*, small fern & christmas bush, 30.01.2009, *Millenium Seed Bank (T. Heller & L. Taylor) MSB-FI 25* (K image!). WEST FALKLAND: s. loc., s. d., *[W.] Wright s. n.* (K). Between Teal River and Goring House, Chartres, just N of Rocky inlet, 19(17?).12.1949, *W. J. L. Sladen* Fa125/49 (AAS image!, BM). Roy Cove, Sharp Peak, N side of flat ground by sea, wet patch of ground, assoc. *Unicinia brevicaulis*, *Cortaderia*, *Oreobolus*, 13.02.1964, *D. M. Moore 866* (K, RNG).

GOUGH ISLAND: Crest of ridge between Glen & 1st Guleh, frequent on wet places and in high level bogs from 1200 ft. upwards, alt. 1500 ft., 01.12.1955, *N. M. Wace 22* (BM). Base of Hag's Tooth, alt. 1500 ft., frequent especially in wet places, on shallow peat overlying rocks, 07.01.1956, *N. M. Wace 76* (BM, BOL). X St. Gonydale Raised Bog, alt. 1500 ft, 18.01.1956, *N. M. Wace 99* (BM). Tafelkop, in wet high altitude mire/grassland, 40° 20' S 9° 54' W, 500 m asl, 21.09.2006, *R. M. Wanless s.n.* (MJG).

Note: Specimens with the abbreviation "T.B.P.A." and a number were collected during the project *Transecta Botánica de Patagonia Austral* (Boelcke & al. 1985).

APPENDIX 5.

List of studied herbarium specimens of *Maundia triglochinoides* F.Muell.

AUSTRALIA. **Queensland:** South East Queensland [Moreton]: Moreton Bay, s. d., *F. Mueller s. n.* (K). Slacks Creek, Logan River, s. d., *N. Michael s. n.* (BRI). Bald Hills Road, in shallow water, 27°2'S 153°0'E, s. d., *S. T. Blake 20053* (K). Between Petrie and Redcliffe, in freshwater creek , 08 x 1959, *S. T. Blake 21028* (BRI). Woodford, in shallow shelving edge of One Mile Creek, 26°5'S 152°4'E, 16 iii 1960, *S. T. Blake 21205* (BRI, K). One Mile Creek at Woodford, N of George Street and opposite Nicklaus Street, growing in narrow, recently flooded creek bed, now without free water, 26°57'S 152°47'E, 28 v 1997, *H. I. Aston 2883 & T. Spokes* (BRI). **New South Wales:** Wyong district, Porters Creek Wetland, c. 1.5 km n of Watanobbi, 33°15'25''S 151°26'00''E, water channel in area of moist dense woodland of *Melaleuca ericifolia*, 13 ii 2009, *B.G. Briggs 10003* (NSW – image!). Kogarah Swamp, ca. 7 miles SW of Sydney, 17 i 1903*; J. H. Camfield s.n.* (BRI, K, NSW). Sans Souci, 34°00'S 151°07'E, 18 i 1903, *J. H. Camfield* (NSW). Approx. 1 km W of Pacific Highway, between Tuggerah and Wyong, 33°18'S 151°25'E, small creek, 12 xii 1978, *S. W. L. Jacobs 3461* (NSW, 2 sheets). Wyong, swamp in centre of race course, 33°17'S 151°26'E, 12 xii 1978, *S. W. L. Jacobs 3464*, *S. W. L. Jacobs 3465* (NSW). 7 km along Colletts Crossing Road S from Wooli-Pillar Valley road, 29°50'S 153°12'E, lagoon with *Melaleuca quinquenervia* in woodland of scribbly gum, bloodwood, stringybark*, Casuarina littoralis*, 20 xii 1978, *K. L. Wilson 4001* (NSW). Tucabia district, swamp near Upper Coldstream, 29°37'S 153°07'E, 25 xi 1979, *R. Pressey s. n.* (NSW). Moffats Swamp, 2 km E of Ringwood Road, Medowie, 32°45'S 151°53'E, in and beside drain in swamp below sewage treatment works, with *Eleocharis sphacelata*, 22 xii 1979, *K. L. Wilson 3088* (NSW). 1 km along Yellow Cutting Road from Newfoundland Road, Newfoundland State Forest, 29°55'S 153°09'E, small stream bordered by wet forest in Euc. forest, dominant in small pool, 20 xii 1981, *K. L. Wilson 3993* (NSW). Collombatti Creek, 10 km NNW of Kempsey, 30°59'10''S 152°49'50''E, 10 xii 1983, *R. Pressey 30* (NSW). Tuggerah, on Gadlock Rd., N of Johnson Rd., 33°19'S 151°25'E, pond in swampy area beside road, 30 xi 1990, *S. Papassotiriou 13 & S. W. L. Jacob*s (NSW). Porters Creek Wetland, Wyong, entry to wetland from unnamed short road running NE off Fishburn Rd and just NW of Augusta Close, Watanobbi. Entry point into swamp from Railway Rd (dirt track on W side of rail line). 33°15'36.7''S 151°26'11.4''E, elev. 14 m, 3 xii 2008, *L. C. Stanberg LS80 & G. Sainty* (MJG).

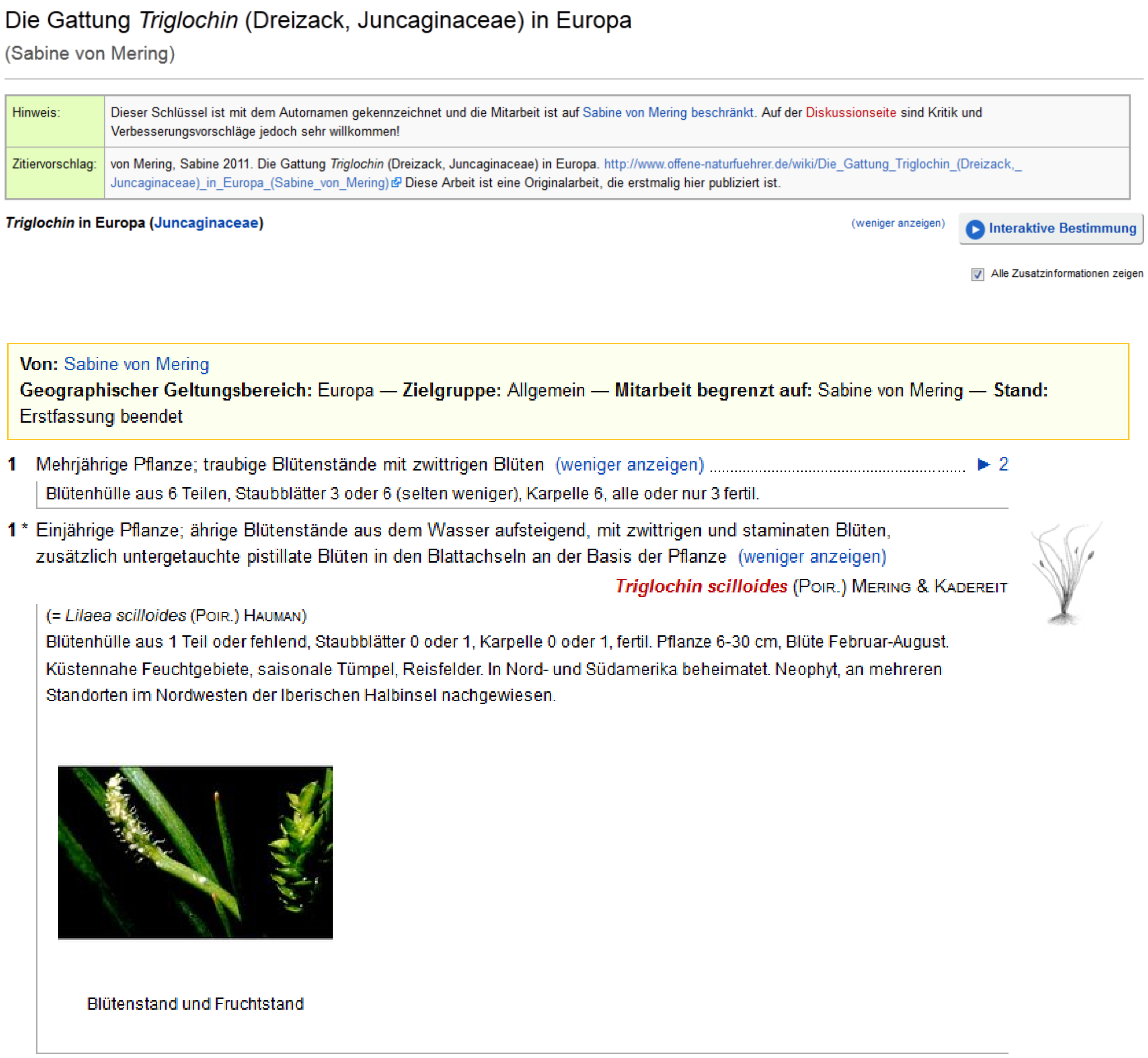
APPENDIX 6.

Interactive identification keys for *Triglochin* in Europe published on the internet platform *Offene Naturführer*. For technical details see Hagedorn et al. (2010).

Identification key for the genus ***Triglochin* in Europe** (in German), published online at http://www.offene-naturfuehrer.de/wiki/Die_Gattung_Triglochin_(Dreizack,_Juncaginaceae)_in_Europa_(Sabine_von_Mering)

von Mering S. 2011: Die Gattung *Triglochin* (Dreizack, Juncaginaceae) in Europa. – Published at http://www.offene-naturfuehrer.de/wiki/Die_Gattung_Triglochin_(Dreizack,_Juncaginaceae)_in_Europa_(Sabine_von_Mering). Diese Arbeit ist eine Originalarbeit, die erstmalig hier publiziert ist.

Another interactive identification key for ***Triglochin* in Central Europe** (also in German) is published online at http://offene-naturfuehrer.de/web/Triglochin_%28Mitteleuropa%29



Die Gattung *Triglochin* (Dreizack, Juncaginaceae) in Europa

(Sabine von Mering)

Hinweis:	Dieser Schlüssel ist mit dem Autornamen gekennzeichnet und die Mitarbeit ist auf Sabine von Mering beschränkt. Auf der Diskussionseite sind Kritik und Verbesserungsvorschläge jedoch sehr willkommen!
Zitiervorschlag:	von Mering, Sabine 2011. Die Gattung *Triglochin* (Dreizack, Juncaginaceae) in Europa. http://www.offene-naturfuehrer.de/wiki/Die_Gattung_Triglochin_(Dreizack,_Juncaginaceae)_in_Europa_(Sabine_von_Mering) Diese Arbeit ist eine Originalarbeit, die erstmalig hier publiziert ist.

***Triglochin* in Europa (Juncaginaceae)**

☑ Alle Zusatzinformationen zeigen

Von: Sabine von Mering
Geographischer Geltungsbereich: Europa — **Zielgruppe:** Allgemein — **Mitarbeit begrenzt auf:** Sabine von Mering — **Stand:** Erstfassung beendet

1 Mehrjährige Pflanze; traubige Blütenstände mit zwittrigen Blüten (weniger anzeigen) .... ► 2

Blütenhülle aus 6 Teilen, Staubblätter 3 oder 6 (selten weniger), Karpelle 6, alle oder nur 3 fertil.

1 * Einjährige Pflanze; ährige Blütenstände aus dem Wasser aufsteigend, mit zwittrigen und staminaten Blüten, zusätzlich untergetauchte pistillate Blüten in den Blattachseln an der Basis der Pflanze (weniger anzeigen)

Triglochin scilloides (POIR.) MERING & KADEREIT

(= *Lilaea scilloides* (POIR.) HAUMAN)

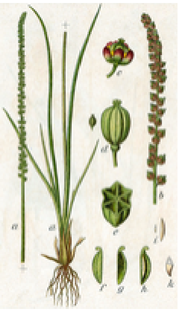
Blütenhülle aus 1 Teil oder fehlend, Staubblätter 0 oder 1, Karpelle 0 oder 1, fertil. Pflanze 6-30 cm, Blüte Februar-August. Küstennahe Feuchtgebiete, saisonale Tümpel, Reisfelder. In Nord- und Südamerika beheimatet. Neophyt, an mehreren Standorten im Nordwesten der Iberischen Halbinsel nachgewiesen.

Blütenstand und Fruchtstand

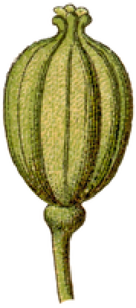
2 Fertile Karpelle 6; Früchte eiförmig, 4-6 mm lang, Teilfrüchte bei Reife vom Karpophor abfallend (weniger anzeigen)

Strand-Dreizack, Salz-Dreizack – *Triglochin maritima* L.

Mehrjährige Pflanze, häufig Horste bildend. Rhizom kräftig, schräg aufsteigend oder horizontal, ohne Ausläufer. Blätter derb, bis > 3 mm dick; mit langer, ungeteilter Ligula. Traubige Blütenstände dichtblütig; Blüten unscheinbar, sternförmig, grünlich, oft etwas rötlich; Narbe 6-fedrig, weiß bis hellviolett; alle 6 Karpelle fertil. Pflanze 10-75 cm hoch, Blüte (Mai-)Juni-August. In ganz Europa weit verbreitet. Salzstandorte, an den Küsten häufiger in Salzmarschen, selten an Binnensalzstellen. Salztolerant.

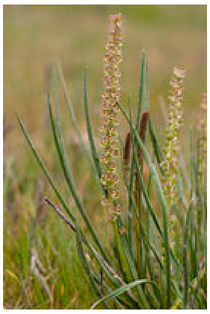


Triglochin maritima



Reife Frucht

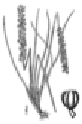
Blühende Pflanze



Junge Früchte

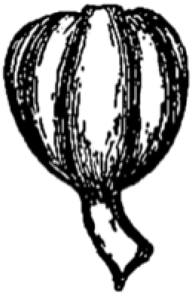
2* Fertile Karpelle 3 .... ► 3

3 Früchte ± kugelig, etwa so lang wie breit; Pflanze mit Rhizom, Ausläufer bildend; in Europa nur im Westen der Iberischen Halbinsel vorkommend (weniger anzeigen)



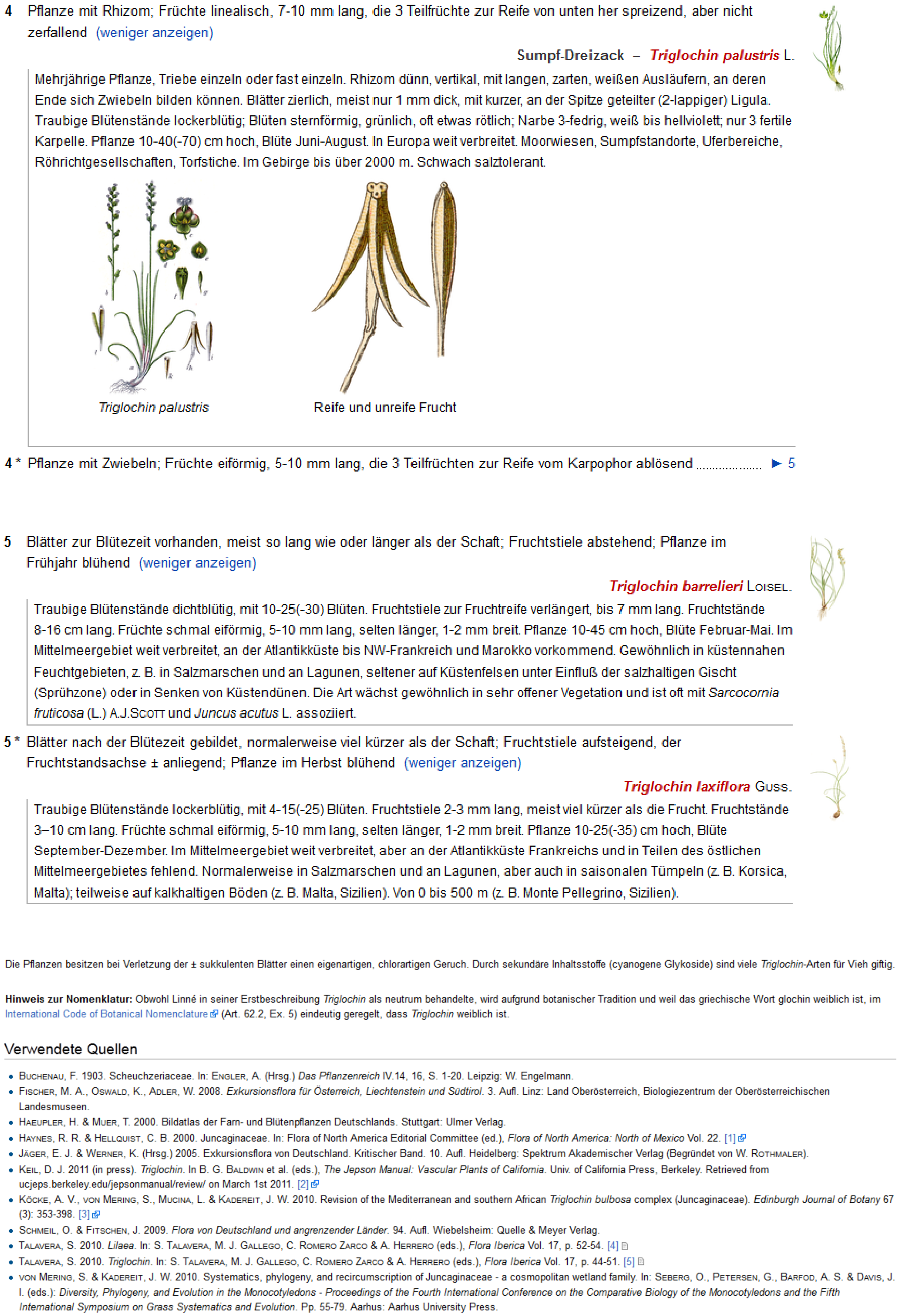
Triglochin striata Ruiz. & Pav.

Traubige Blütenstände dichtblütig, mit (15-)20-80(-100) Blüten, (1,5)3-8,5 cm lang. Früchte 1–2 mm lang; Teilfrüchte gerippt. Pflanze 10-40 cm hoch, Blüte Mai-Dezember. Permanente und temporäre Feuchtgebiete (Flussufer, Sümpfe, Tümpel etc.). Sehr weit, v. a. südhemispherisch verbreitet (Australien, Tasmanien, Neuseeland, südliches Afrika, Südamerika), aber auch in Teilen Nordamerikas. Neophyt, an mehreren Standorten im Westen der Iberischen Halbinsel nachgewiesen.



Frucht

3* Früchte ± länglich, mehrfach länger als breit; Pflanze mit Rhizom oder Zwiebeln .... ► 4



4 Pflanze mit Rhizom; Früchte linealisch, 7-10 mm lang, die 3 Teilfrüchte zur Reife von unten her spreizend, aber nicht zerfallend (weniger anzeigen)

Sumpf-Dreizack – ***Triglochin palustris*** L.

Mehrjährige Pflanze, Triebe einzeln oder fast einzeln. Rhizom dünn, vertikal, mit langen, zarten, weißen Ausläufern, an deren Ende sich Zwiebeln bilden können. Blätter zierlich, meist nur 1 mm dick, mit kurzer, an der Spitze geteilter (2-lappiger) Ligula. Traubige Blütenstände lockerblütig; Blüten sternförmig, grünlich, oft etwas rötlich; Narbe 3-fedrig, weiß bis hellviolett; nur 3 fertile Karpelle. Pflanze 10-40(-70) cm hoch, Blüte Juni-August. In Europa weit verbreitet. Moorwiesen, Sumpfstandorte, Uferbereiche, Röhrichtgesellschaften, Torfstiche. Im Gebirge bis über 2000 m. Schwach salztolerant.

Triglochin palustris

Reife und unreife Frucht

4* Pflanze mit Zwiebeln; Früchte eiförmig, 5-10 mm lang, die 3 Teilfrüchten zur Reife vom Karpophor ablösend .... ► 5

5 Blätter zur Blütezeit vorhanden, meist so lang wie oder länger als der Schaft; Fruchtstiele abstehend; Pflanze im Frühjahr blühend (weniger anzeigen)

Triglochin barrelieri Loisel.

Traubige Blütenstände dichtblütig, mit 10-25(-30) Blüten. Fruchtstiele zur Fruchtreife verlängert, bis 7 mm lang. Fruchtstände 8-16 cm lang. Früchte schmal eiförmig, 5-10 mm lang, selten länger, 1-2 mm breit. Pflanze 10-45 cm hoch, Blüte Februar-Mai. Im Mittelmeergebiet weit verbreitet, an der Atlantikküste bis NW-Frankreich und Marokko vorkommend. Gewöhnlich in küstennahen Feuchtgebieten, z. B. in Salzmarschen und an Lagunen, seltener auf Küstenfelsen unter Einfluß der salzhaltigen Gischt (Sprühzone) oder in Senken von Küstendünen. Die Art wächst gewöhnlich in sehr offener Vegetation und ist oft mit *Sarcocornia fruticosa* (L.) A.J.Scott und *Juncus acutus* L. assoziiert.

5* Blätter nach der Blütezeit gebildet, normalerweise viel kürzer als der Schaft; Fruchtstiele aufsteigend, der Fruchtstandsachse ± anliegend; Pflanze im Herbst blühend (weniger anzeigen)

Triglochin laxiflora Guss.

Traubige Blütenstände lockerblütig, mit 4-15(-25) Blüten. Fruchtstiele 2-3 mm lang, meist viel kürzer als die Frucht. Fruchtstände 3–10 cm lang. Früchte schmal eiförmig, 5-10 mm lang, selten länger, 1-2 mm breit. Pflanze 10-25(-35) cm hoch, Blüte September-Dezember. Im Mittelmeergebiet weit verbreitet, aber an der Atlantikküste Frankreichs und in Teilen des östlichen Mittelmeergebietes fehlend. Normalerweise in Salzmarschen und an Lagunen, aber auch in saisonalen Tümpeln (z. B. Korsica, Malta); teilweise auf kalkhaltigen Böden (z. B. Malta, Sizilien). Von 0 bis 500 m (z. B. Monte Pellegrino, Sizilien).

Die Pflanzen besitzen bei Verletzung der ± sukkulenten Blätter einen eigenartigen, chlorartigen Geruch. Durch sekundäre Inhaltsstoffe (cyanogene Glykoside) sind viele *Triglochin*-Arten für Vieh giftig.

Hinweis zur Nomenklatur: Obwohl Linné in seiner Erstbeschreibung *Triglochin* als neutrum behandelte, wird aufgrund botanischer Tradition und weil das griechische Wort glochin weiblich ist, im International Code of Botanical Nomenclature (Art. 62.2, Ex. 5) eindeutig geregelt, dass *Triglochin* weiblich ist.

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SUMMARY

I investigated the systematics, phylogeny and biogeographical history of Juncaginaceae, a small family of the early-diverging monocot order Alismatales which comprises about 30 species of annual and perennial herbs. A wide range of methods from classical taxonomy to molecular systematic and biogeographic approaches was used.

In **Chapter 1**, a phylogenetic analysis of the family and members of Alismatales was conducted to clarify the circumscription of Juncaginaceae and intrafamilial relationships. For the first time, all accepted genera and those associated with the family in the past were analysed together. Phylogenetic analysis of three molecular markers (*rbc*L, *mat*K, and *atp*A) showed that Juncaginaceae are not monophyletic. As a consequence the family is re-circumscribed to exclude *Maundia* which is proposed to belong to a separate family Maundiaceae, reducing Juncaginaceae to include *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.str. *Lilaea* is nested within *Triglochin* s. str. 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 given, and several new combinations are made.

In **Chapter 2**, phylogenetic relationships in *Triglochin* were investigated. A species-level phylogeny was constructed based on molecular data obtained from nuclear (ITS, internal transcribed spacer) and chloroplast sequence data (*psb*A-*trn*H, *mat*K). Based on the phylogeny of the group, divergence times were estimated and ancestral distribution areas reconstructed. The monophyly of *Triglochin* is confirmed and relationships between the major lineages of the genus were resolved. A clade comprising the Mediterranean/African *T. bulbosa* complex and the American *T. scilloides* (= *Lilaea s.*) is sister to the rest of the genus which contains two main clades. In the first, the widespread *T. striata* is sister to a clade comprising annual *Triglochin* species from Australia. The second clade comprises *T. palustris* as sister to the *T. maritima* complex, of which the latter is further divided into a Eurasian and an American subclade. Diversification in *Triglochin* began in the Miocene or Oligocene, and most disjunctions in *Triglochin* were dated to the Miocene. Taxonomic diversity in some clades is strongly linked to habitat shifts and can not be observed in old but ecologically invariable lineages such as the non-monophyletic *T. maritima*.

Chapter 3 is a collaborative revision of the *Triglochin bulbosa* complex, a monophyletic group from the Mediterranean region and Africa. One new species, *Triglochin buchenaui*, and two new subspecies, *T. bulbosa* subsp. *calcicola* and subsp. *quarcicola*, from South Africa were described. Furthermore, two taxa were elevated to species rank and two reinstated. Altogether, seven species and four subspecies are recognised. An identification key, detailed descriptions and accounts of the ecology and distribution of the taxa are provided. An IUCN conservation status is proposed for each taxon.

Chapter 4 deals with the monotypic *Tetroncium* from southern South America. *Tetroncium magellanicum* is the only dioecious species in the family. The taxonomic history of the species is described, type material is traced, and a lectotype for the name is designated. Based on an extensive study of herbarium specimens and literature, a detailed description of the species and notes on its ecology and conservation status are provided. A detailed map showing the known distribution area of *T. magellanicum* is presented.

In **Chapter 5**, the flower structure of the rare Australian endemic *Maundia triglochinoides* (Maundiaceae, see Chapter 1) was studied in a collaborative project. As the morphology of *Maundia* is poorly known and some characters were described differently in the literature, inflorescences, flowers and fruits were studied using serial mictrotome sections and scanning electron microscopy. The phylogenetic placement, affinities to other taxa, and the evolution of certain characters are discussed. As *Maundia* exhibits a mosaic of characters of other families of tepaloid core Alismatales, its segregation as a separate family seems plausible.

ZUSAMMENFASSUNG

Die Systematik, Phylogenie und Biogeographie der Juncaginaceae, einer kleinen Familie aus der Monokotylen-Ordnung Alismatales mit etwa 30 ein- oder mehrjährigen krautigen Arten wurde untersucht. Verschiedene Ansätze von der klassischen Revision bis hin zu molekularsystematischen und biogeographischen Methoden wurden genutzt.

Kapitel 1 beschreibt die Ergebnisse einer phylogenetischen Analyse der Familie und Vertreter der Alismatales, um die Abgrenzung der Juncaginaceae und Gattungsbeziehungen zu klären. Erstmals wurden alle akzeptierten Gattungen und in der Vergangenheit mit der Familie assoziierte Taxa gemeinsam analysiert. Die phylogenetische Analyse von drei molekularen Markern (*rbc*L, *mat*K, und *atp*A) zeigt, dass die Juncaginaceae nicht monophyletisch sind. Die Familie wurde neu umschrieben: nach Ausgliederung von *Maundia*, die zur separaten Familie Maundiaceae gestellt wurde, umfassen die Juncaginaceae *Tetroncium*, *Cycnogeton* und *Triglochin*. *Tetroncium* ist Schwester zum Rest der Familie, allerdings nur mit schwacher Unterstützung. *Cycnogeton* wurde wieder in Gattungsrang erhoben (vorher in *Triglochin* eingeschlossen) und ist mit großer Unterstützung Schwester zu *Triglochin* s.str. *Lilaea* fällt in *Triglochin* s. str. und ist gut unterstützt als Schwester des *T. bulbosa*-Komplexes. Die Ergebnisse der molekularen Analysen werden zusammen mit morphologischen Merkmalen diskutiert. Außerdem werden ein Schlüssel zu den Gattungen der Familie vorgelegt sowie einige neue Kombinationen gemacht. In **Kapitel 2** werden die phylogenetischen Beziehungen innerhalb von *Triglochin* untersucht. Eine Phylogenie auf Artniveau wurde mit nukleären (ITS, internal transcribed spacer) und plastidären Sequenzdaten (*psb*A-*trn*H, *mat*K) erstellt. Basierend auf der Phylogenie wurden eine Altersbestimmung der Gruppe durchgeführt und die Ursprungsgebiete rekonstruiert. Die Monophylie von *Triglochin* wurde bestätigt und die Beziehungen zwischen den Hauptlinien der Gattung aufgeklärt. Eine Gruppe bestehend aus dem *T. bulbosa*-Komplex (Mittelmeer, Afrika) und der in Amerika verbreiteten *T. scilloides* (= *Lilaea s.*) ist Schwester zum Rest der Gattung, der aus zwei Hauptlinien besteht. In der ersten ist die weitverbreitete *T. striata* Schwester zu einer Gruppe australischer einjähriger *Triglochin*-Arten. In der zweiten Hauptlinie besteht ein Schwestergruppenverhältnis zwischen *T. palustris* und dem *T. maritima*-Komplex, wobei letzterer in eine eurasische und eine amerikanische Linie zerfällt. Diversifikation in *Triglochin* begann im Miozän oder Oligozän, die meisten Disjunktionen wurden in das Miozän datiert. Die taxonomische Diversität in einigen Gruppen ist stark mit Habitatwechseln verknüpft. Viel geringere taxonomische Diversität wurde in alten aber ökologisch weniger variablen Linien wie der nicht monophyletischen *T. maritima* beobachtet. **Kapitel 3** ist eine gemeinschaftliche Revision des *Triglochin bulbosa*-Komplexes, einer monophyletischen Gruppe aus dem Mittelmeergebiet und Afrika. Aus Südafrika wurden eine neue Art, *Triglochin buchenaui*, und zwei neue Unterarten, *T. bulbosa* subspp. *calcicola* und *quarcicola*, beschrieben. Außerdem wurden zwei Taxa in den Artrang erhoben und zwei weitere wieder als Arten bestätigt. Insgesamt werden sieben Arten und vier Unterarten anerkannt. Ein Bestimmungsschlüssel, detaillierte Beschreibungen und Angaben zur Ökologie und Verbreitung der Taxa werden präsentiert. Ein Gefährdungsstatus nach IUCN-Kriterien wird für alle Taxa vorgeschlagen. In **Kapitel 4** wird die monotypische Gattung *Tetroncium* aus dem südlichen Südamerika untersucht. *Tetroncium magellanicum* ist die einzige diözische Art der Familie. Die taxonomische Geschichte der Art wird beschrieben, Typusmaterial ausfindig gemacht und ein Lectotypus für den Namen designiert. Basierend auf Bearbeitungen von Herbarmaterial sowie Literaturangaben werden die Art detailliert beschrieben und Angaben zur Ökologie und dem Gefährdungsstand gemacht. Eine Punktverbreitungskarte zeigt das bekannte Areal von *T. magellanicum*. In **Kapitel 5** wird die Blütenmorphologie von *Maundia triglochinoides* (Maundiaceae, siehe Kapitel 1) in einem Gemeinschaftsprojekt untersucht. Infloreszenzen, Blüten und Früchte wurden mittels Serienschnitten und Rasterelektronenmikroskopie analysiert, um widersprüchliche Literaturangaben zu klären. Die Beziehungen zu anderen Taxa und die Evolution ausgewählter Merkmale werden diskutiert. Da *Maundia* Merkmale verschiedener Familien der tepaloiden Kern-Alismatales vereint, erscheint eine Abgrenzung als separate Familie plausibel.