

## Chapter 1

### General introduction and aims

#### 1.1 Historical background and taxonomic problem

##### 1.1.1 Introduction

Cyperaceae are the third largest monocotyledon family composed of about 5500 species and about 110 genera (Govaerts *et al.* 2007), 14 tribes and two subfamilies (Simpson *et al.* 2007; Muasya *et al.* 2009). The family is common in temperate and cold temperate regions of the world, but very diverse in tropical regions occurring frequently in wetlands at almost all altitudes (Bruhl 1995; Goetghebeur 1998; Muasya *et al.* 1998; Musili 2007). Cyperaceae differ from other monocotyledonous families in the structural form and diversity of their spikelets (Cyperoideae) and spikelet-like units (Mapanioideae). Flowers in Cyperaceae have a solitary ovule, indehiscent fruit, and pollen mostly (Cyperoideae) in the form of pseudomonads (tetrads in which three microspores degenerate and only one develops) (Dahlgren *et al.* 1985; Haines and Lye 1993; Kubitzki 1998). Recent studies by Simpson *et al.* (2003) have, however, showed that pseudomonads are not found in all members of subfamily Mapanioideae in Cyperaceae where at least *Mapania* and *Diplasia* have ‘*Mapania*-type’ pollen which appears to be more like typical monoporate monocot pollen (dispersal as monads).

Cyperaceae was placed in the order Cyperales together with Poaceae by Cronquist (1988) on the basis of the reduced commelinid floral morphology, while Juncaceae and Thurniacaceae were placed in the order Juncales. Other workers (Takhtajan 1980; Dahlgren *et al.* 1985) have suggested that Cyperaceae is more closely related to Juncaceae by putting the families Cyperaceae, Juncaceae and Thurniacaceae in the order Cyperales. Cladistic analysis of morphological (Simpson 1995) and molecular data (Chase *et al.* 1993; Plunkett 1995) supports this classification. Bremer (2002) placed Cyperaceae and Thurniacaceae together with Poaceae in the order Poales based on the cladistic analysis of chloroplast DNA *rbcL* and *atpB* sequences, where graminoid and cyperoid clades were well supported by 80% and 100% jackknife values, respectively. This classification is congruent with results from Angiosperm Phylogeny Group (2003) and Bremer and Janssen (2006).

Cyperaceae have small flowers and highly condensed inflorescences that obscure the morphology of their floral parts (Kern 1974; Muasya *et al.* 1998). This has led to different

interpretations of floral morphology (e.g. see Bruhl 1991; Vrijdaghs *et al.* 2004; Vrijdaghs *et al.* 2005; Vrijdaghs 2009) and consequently has hampered classification (Table 1) and early estimates of relationship (cf. Goetghebeur 1986; Bruhl 1995).

Circumscription of subfamilies and tribes has thus been controversial (Clarke 1908; Koyama 1961; Goetghebeur 1986; Bruhl 1995; Goetghebeur 1998; Simpson *et al.* 2003; Simpson *et al.* 2007; Muasya *et al.* 2009). Most classifications of the family, and all those presented here (Table 1), recognise both subfamilies and tribes. Membership of the subfamilies has varied considerably. Use of mutigene region phylogenetic analysis, with (Simpson *et al.* 2003) or without (Muasya *et al.* 2009) morphological data, has firmed up the recognition of two subfamilies; Cyperoideae and Mapanioideae (Table 1).

**Table 1. Comparison of suprageneric classifications in Cyperaceae**

Goetghebeur 1986	Bruhl 1995	Goetghebeur 1998	Simpson <i>et al.</i> (2007)	Muasya <i>et al.</i> (2009)
<b>Cyperoideae</b>	<b>Cyperoideae</b>	<b>Cyperoideae</b>	<b>Cyperoideae</b>	<b>Cyperoideae</b>
Abildgaardieae	Abildgaardieae	Abildgaardieae	Abildgaardieae	Abildgaardieae
Arthrostylideae	Arthrostylideae		Schoeneae 3 Chrysitricheae 2	
Cypereae	Cypereae	Cypereae	Cypereae 1, 2 & 3	Cypereae
Ficinieae				
Dulichieae		Dulichieae	Dulichieae	Dulichieae
Eleocharideae	Scirpeae	Eleocharideae	Eleocharideae	Eleocharideae
Fuireneae		Fuireneae	Fuireneae1&2	Fuireneae 1, 2, 3 & 4
Scirpeae		Scirpeae	Scirpeae1&2	Scirpeae 1 & 2
-	<b>Caricoideae</b>	-	-	-
Schoeneae	Schoeneae	Schoeneae	Schoeneae1&2	Schoeneae 1, 2 & 3
Rhynchosporeae	Rhynchosporeae			Rhynchosporeae
<b>Sclerioideae</b>	-	<b>Sclerioideae</b>	<b>Sclerioideae</b>	-
Bisboeckelereae	Bisboeckelereae	Bisboeckelereae	Bisboeckelereae	Bisboeckelereae
Cryptangieae	Cryptangieae	Cryptangieae	Cryptangieae	Cryptangieae
Sclerieae	Sclerieae	Sclerieae	Sclerieae	Sclerieae
Trilepideae	Trilepideae	Trilepideae	Trilepideae	Trilepideae
<b>Caricoideae</b>	-	<b>Caricoideae</b>	<b>Caricoideae</b>	-
Cariceae	Cariceae	Cariceae	Cariceae	Cariceae
<b>Mapanioideae</b>	-	<b>Mapanioideae</b>	<b>Mapanioideae</b>	<b>Mapanioideae</b>
Chrysitricheae		Chrysitricheae	Chrysitricheae 1	Chrysitricheae
Hypolytreae	Hypolytreae	Hypolytreae	Hypolytreae	Hypolytreae

### 1.1.2 History of generic circumscription in Schoeneae

Schoeneae in its current circumscription is a large tribe of 29 genera comprising about 700 species with most generic and species diversity in the southern hemisphere, concentrated in Australia and South Africa (Goetghebeur 1998); this appears to have involved extensive

dispersal (Verboom 2006). Various older classifications treat members of the Schoeneae under the Rhynchosporae. More recently the Schoeneae has been recognised as being non-monophyletic (Table 1).

Generic composition of Schoeneae has varied since the first publication of the name by Dumortier (1827). This is due to disagreement about the inclusion or exclusion of genera including those in the tribes Arthrostylideae and Rhynchosporae. Most classifications however, have recognised a core group of Schoenoid genera (Table 2).

An early, explicit test of the monophyly of the tribe based on analysis of morphology yielded ambiguous results (Bruhl 1995). Of the 25 cladistic analyses, only five clearly supported the monophyly of tribe Schoeneae. It was discovered that the constitution of paraphyletic groups in the tribe was due to the segregation of *Cladium* and *Rhynchocladium*. A set of phenetic analyses in Bruhl's study mostly resulted in a highly distinctive Schoenoid cluster but it was paraphenetic with the inclusion of Arthrostylideae and/or Rhynchosporae. Bruhl included 27 genera in the tribe.

Muasya *et al.* (1998) used *rbcL* sequences and to estimate phylogeny of Cyperaceae. This analysis recovered a monophyletic Schoeneae *sensu stricto* (i.e. without *Cladium* and Rhynchosporae) with no Bootstrap support (BS <50%). Only six genera and nine species were sampled in Schoeneae making the assessment inadequate for resolving the monophyly of the tribe. Muasya *et al.* (2000a) combined *rbcL* data with Bruhl's (1995) morphological data without change or modification of the characters, which yielded marginally better support (BS=65%).

Zhang *et al.* (2004a) and Verboom (2006) used plastid *trnL-F* sequences to examine Schoenoid relationships. The results showed *Cladium* as a sister clade to Schoeneae, Rhynchosporae, Scirpeae and Cypereae. This is congruent with earlier cladistic analyses of Cyperaceae based on morphology (Bruhl 1995; Goetghebeur 1986; Simpson 1995) and DNA sequence data (Muasya *et al.* 1998) which collectively provide substantial information for understanding generic relationship patterns within Schoeneae. In all these analyses, there was limited sampling of genera within the tribe Schoeneae, For example, Zhang *et al.* (2004a) sampled only 46 species in 16 genera as ingroups and limited outgroups (only two species of *Rhynchospora* (six specimens) were included i.e. *Rhynchospora brownii* Roem. & Schult and *R. corymbosa* (L.) Britton. Verboom (2006) sampled 44 species, 11 of which were outgroups,

and 33 ingroup representing 13 genera in Schoeneae. Therefore, their work is inadequate to evaluate Schoenoid monophyly.

More recently, Simpson *et al.* (2007) have showed that the tribe Schoeneae sensu Goetghebeur (1998) is not monophyletic, finding four major clades: *Cladium*, *Carpha* + *Lagenocarpus*, *Rhynchospora* + *Pleurostachys*, and the fourth comprising all other genera in the tribe. Their recognition of *Cladium* as a separate clade supports findings of Goetghebeur (1986), Bruhl (1995), and Muasya *et al.* (1998). Zhang *et al.* (2004a) had previously showed that *Carpha* formed a sister clade to the rest of Schoeneae. The placement of the sole *Lagenocarpus* sample was unusual since the genus has usually been placed in tribe Cryptangieae. The placement of *Rhynchospora* + *Pleurostachys* within Schoeneae was in accord with Goetghebeur (1998), whereas they had been put in tribe Rhynchosporeae by some workers (e.g. Goetghebeur 1986; Bruhl 1995).

Bruhl *et al.* (2008) indicated that Schoeneae is monophyletic without *Cladium*. A molecular study on Cyperaceae tribal classification by Muasya *et al.* (2009), using more samples, resolved four clades within an expanded Schoeneae: (1) *Cladium*, (2) *Gymnoschoenus*, (3) the bulk of genera (*Caustis* to *Didymiandrum*), and (4) *Rhynchospora* + *Pleurostachys*. This differs from the Simpson *et al.* (2007) analysis in that tribes Sclerieae and Bisboeckelereae were unequivocally included in the same major clade (3) as part of an expanded Schoeneae. Another difference was that tribe Cryptangieae (represented by three species in this study) formed a subclade within (3), i.e. separate from *Gymnoschoenus* (2). *Carpha* formed a subclade within clade (3) (with weak support).

Different interpretations of the classification of Schoeneae are summarised in Table 2. The situation is now that these studies need to be extended to clarify relationships more satisfactorily, by sampling more species and more genera and by using different sequences, allied to morphological and anatomical data.

**Table 2. Genera included in Schoeneae by different authors.** (x) indicates inclusion in tribe, (-) indicates the genus is either not recognized by the author and is treated as part of another genus, or it is not included in this tribe by the authors.

Genera	Clarke (1908)	Küikenthal (1940)	Goetghebeur (1986)	Bruhl (1995)	Goetghebeur (1998)	Simpson <i>et al.</i> (2007)	Muasya <i>et al.</i> (2009)
<i>Actinoschoenus</i>	-	-	-	-	×	-	-
<i>Arthrostylis</i>	×	-	-	-	×	-	-
<i>Baumea</i>	-	-	×	×	-	×	×
<i>Capeobolus</i>	-	-	-	-	-	-	×
<i>Carpha</i>	×	×	×	×	×	×	×
<i>Caustis</i>	×	-	×	×	×	×	×
<i>Cladium</i>	×	-	×	×	×	×	×
<i>Costularia</i>	×	×	×	×	×		×
<i>Cyathochaeta</i>	-	-	×	×	×	-	×
<i>Cyathocoma</i> ( <i>Macrochaetium</i> )	×	-	×	×	×	-	×
<i>Ecklonia</i>	×	-	-	-	-	-	-
<i>Epischoenus</i>	×	-	×	×	×	-	×
<i>Evandra</i>	×	-	×	×	×	×	×
<i>Gahnia</i>	×	-	×	×	×	×	×
<i>Gymnoschoenus</i>	×	×	×	×	×	×	×
<i>Lepidosperma</i>	×	-	×	×	×	×	×
<i>Lophoschoenus</i>	-	-	-	×	-	-	-
<i>Machaerina</i>	-	-	×	×	×	-	×
<i>Mesomelaena</i>	×	×	×	×	×	×	×
<i>Microschoenus</i>	×	-	-	-	-	-	-
<i>Morelotia</i>	-	-	×	×	×	-	×
<i>Neesenbeckia</i>	-	-	×	×	×	×	×
<i>Oreobolus</i>	×	×	×	×	×	×	×
<i>Phylloscirpus</i>	×	-	-	-	-	-	-
<i>Pleurostachys</i>	-	-	-	-	×	×	-
<i>Ptilothrix</i> ( <i>Ptilanthelium</i> <i>auct.</i> )	×	×	×	×	×	-	×
<i>Reedia</i>	×	-	×	×	×	-	-
<i>Rhynchocladium</i>	-	-	×	×	×	-	-
<i>Rhynchospora</i>	-	-	-	-	×	×	-
<i>Schoenoides</i>	-	-	-	×	-	-	-
<i>Schoenus</i>	×	×	×	×	×	×	×
<i>Tetaria</i>	×	×	×	×	×	-	×
<i>Trachystylis</i>	-	-	-	-	×	-	-
<i>Trianoptiles</i> ( <i>Ecklonea</i> )	×		×	×	×		×
<i>Tetrariopsis</i>	-	-	-	×	-	-	-
<i>Trichoschoenus</i>	-	-	-	-	×	-	-
<i>Tricostularia</i>	×	-	×	×	×	×	×

### 1.1.3 Circumscription of *Schoenus* L. and its species

*Schoenus* L. has about 120 species, in its current circumscription, most of which are endemic to Australia (Kern 1974; Wilson 1993). Species of *Schoenus* are also found in New Guinea and South-East and eastern Asia, New Zealand, New Caledonia, Micronesia and other Pacific islands, the Indian sub-continent, Europe, the Middle East, and some parts of South America, South and East Africa and North America (Kern 1974; Kükenthal 1938, 1940; Wilson 1993).

Nine species were assigned to this genus in *Species Plantarum* (Linnaeus 1753). The work of later authors has seen only two of these species remain: *Schoenus nigricans* L. and *S. ferrugineus* L. (Kern 1974). The treatment of *Schoenus* by Kükenthal (1938, 1940) is the most comprehensive to date, on the basis of its world coverage and the wide range of the morphological characters used in defining subgenera and sections. Kern (1974) recognised five sections and 12 species in the Flora Malesiana area. Wilson (2003) modified Kükenthal's (1938, 1940) classification, and recognised two subgenera, 11 sections and about 110 species. In the absence of compelling morphological evidence and without an estimate of phylogeny, four of the new species were not assigned by her to any of the sections (Table 3).

The group has received little phylogenetic study to date. Verboom (2006) represented the genus with a single species (*Schoenus nigricans*) in his broad study of Schoenoid phylogeny, while Zhang *et al.* (2007) included only two species—*S. paludosus* (R.Br.) Poir. and *S. turbinatus* (R.Br.) Roem. & Schult.—in a phylogenetic study of *Carpha* and related genera combining morphological and molecular data. They found *Schoenus* to be non-monophyletic, which is corroborated by Bruhl *et al.* (2008).

**Table 3. Classification of *Schoenus* sensu (Kükenthal 1938, 1940) modified by Wilson (2003)**

Subgenus	Section <sup>1</sup>	No. of spp. per section	
		Kükenthal 1938, 1940	Wilson 2003 <sup>2</sup>
<i>Schoenus</i> ( <i>Eu-Schoenus</i> of Kük.)	<i>Longisetes</i> Kük.	5	5
	<i>Paniculati</i> (Benth.) C.B.Clarke ( <i>Scrobiculati</i> Kük.)	3	3 (3)
	<i>Nudicaules</i> Kük.	14	14(3)
	<i>Calostachyi</i> (Benth.) C.B.Clarke	7	13(1)
	<i>Laxi</i> (Benth.) Kük.	6	5
	<i>Stricti</i> (Benth.)	6	7
	<i>Helothrix</i> (Nees) Kük.	14	30 (4)
	<i>Humiles</i> C.B.Clarke ( <i>Oligostachyi</i> (Benth.) Kük.)	8	9
	<i>Repentes</i> Kük.	5	5(1)
	<i>Schoenus</i> Kük. ( <i>Foliati</i> Kük)	7	10
	<i>Subaphylli</i> Kük.	5	11
	[unassigned new species]		4
	<i>Pseudomesomelaena</i> Kük.		2

<sup>1</sup>Names in parentheses used by Kükenthal but not recognised by Wilson (2003). <sup>2</sup>Number of species in Malesia in parentheses (Kern 1974).

## 1.2 Summary of taxonomic problem

Infra- and suprageneric classification of *Schoenus* is not yet resolved satisfactorily. Like other Cyperaceae, *Schoenus* and allied genera have reduced vegetative and reproductive structures which results in uncertain homologies and hence differences in opinion about their phylogenetic relationships. The study of spikelet morphology in the genus and close relatives by Zhang *et al.* (2004a, 2004b) gave results which undermined traditional and other interpretations of floral character homologies. For example, possession of elongated and prominently zigzag internodes above the fertile nodes of the ‘rachilla’ is the character state that has been used to distinguish *Schoenus* from other genera (Clarke 1908; Kern 1974; Wilson 1993; Goetghebeur 1998). This character state was found to be inconsistent in *Schoenus*, as three species in the morphological analyses—*S. rhynchosporoides* (Steud.) Kük. *S. paludosus* and *S. turbinatus*—do not have elongated and prominently zigzag upper internodes of the ‘rachilla’ (Zhang *et al.* 2004a, 2004b, 2007). A completely different interpretation of the rachillas has been given by Vrijdaghs *et al.* (2007), which highlights the need for estimation of phylogeny using data independent of spikelet morphology.

Preliminary molecular phylogenies indicate *Schoenus* is polyphyletic and there are some taxonomic complexes e.g. *S. melanostachys* R.Br. and *S. apogon* Roem. & Schult. (Zhang *et al.* 2007; Bruhl *et al.* 2008). To reliably resolve the relationships and determine the limits of *Schoenus* and its species, sampling across and beyond the genus together with phylogenetic and phenetic analyses are required respectively.

### 1.3 Aims of the study

1. To reliably resolve relationships and reconstruct phylogeny of *Schoenus* using ITS nrDNA sequence data to test the monophyly of *Schoenus* in tribe Schoeneae and evaluate relationships within *Schoenus* (and the status of its current classification).
2. To test and set species limits within five *Schoenus* sections sensu Kükenthal (1938, 1940; as amended by Wilson 2003) using phenetic analysis of morphological data.
3. To explore the taxonomic value of culm anatomy characters in *Schoenus* L.
4. To resolve the *S. melanostachys* R.Br. complex.

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## Chapter 6

### General Conclusions

#### 6.1 Introduction

This chapter reviews the aims of the study as stated in Chapter 1 and provides a synopsis for the results of analyses undertaken to address these aims. The overall contribution of this study to systematic knowledge is reviewed and the limitations of this study are discussed. Future directions for systematic studies in *Schoenus* are outlined.

#### 6.2 Overall contribution of this project to systematic knowledge

This study represents a significant contribution to the systematics of the family Cyperaceae, meeting the broad aim of improving systematic understanding of *Schoenus* through investigating each of the aims in Chapter 1. It demonstrates the fact that it is better to utilize as many sources of data as possible (Stuessy 1990) as follows: resolving relationships and reconstructing phylogeny of species using ITS data (Chapter 2); setting and testing species limits using morphological data and phenetic analysis (Chapter 3); exploring the taxonomic value of anatomical characters in the genus (Chapter 4); and resolving the *S. melanostachys* R.Br. complex (Chapter 5).

#### 6.3 Summary of findings for each project aim

##### 6.3.1 Phylogeny of *Schoenus*

The phylogeny of *Schoenus* has been reconstructed using ITS sequence data for 15 genera (3 outgroups) and 131 species (3 outgroups), including 75 species of *Schoenus*, which has also clarified some species relationships. *Schoenus* is found to be non-monophyletic, corroborating previous studies (Verboom 2006; Zhang *et al.* 2007; Bruhl *et al.* 2008; Muasya *et al.* 2009) that were based on much more limited sampling of *Schoenus* than here. The strongly supported clades based on ITS data can also be characterized by morphological characters. *Schoenus* subgen. *Pseudomesomelaena* and *Schoenus grandiflorus* are separate from core *Schoenus* and are embedded with other genera in Schoeneae (i.e. *Morelotia* and *Tricostularia*) and likely indicates the need for another genus to accommodate the subgenus. The place of *S. grandiflorus* needs further study. The phylogenetic analysis also reveals numerous well supported terminal groupings and taxa, but the ITS data did not fully resolve relationships in *Schoenus* at species level. Sections of *Schoenus sensu* Kükenthal (1938;

1940) as modified by Wilson (2003) are largely recovered, agreeing with the morphological basis for those classifications. Our complementary multi-gene region analysis of phylogeny (Gibbs et al. in prep.) will help to improve the resolution of sections, fine-scale species relationships and other species limits.

### **6.3.2 Testing and setting species limits in *Schoenus***

Species limits for 38 of 46 species currently assigned to five sections of *Schoenus* have been confirmed. Species complexes have been largely resolved, especially where there have been conflicting taxonomic opinions by previous authors. Taxonomic changes in the genus, including four new species, are proposed along with a partial taxonomic treatment.

### **6.3.3 Taxonomic value of culm anatomy characters in *Schoenus***

Culm anatomical features have been found here to have taxonomic value in *Schoenus* at and below species level. They help to clarify patterns of variation at inter- and infraspecific levels and to resolve species complexes. An automatically-generated printed sequential key for identification (using DELTA and KEY) is presented that efficiently covers the species of *Schoenus* sampled. The same data have been used to produce an INTKEY dataset for interactive identification and data retrieval, which we plan to make available.

### **6.3.4 New species distinguished from *S. melanostachys* R.Br. complex**

A new species has been distinguished and described (Chapter 5). *Schoenus rupicola* Musili & J.J.Bruhl was previously treated as part of *S. melanostachys* (Kükenthal 1938; Kern 1974; Wilson 1993).

## **6.4 Utility of data sources used**

### **6.4.1 Molecular data**

ITS rDNA sequence data proved useful in reconstructing phylogeny and testing monophyly in *Schoenus* (Chapter 2). The utility of molecular data extends through to sections and species in *Schoenus*. At species level, however, the ITS data did not fully resolve relationships and additional sequences will be needed to refine the classification of certain groups of species. Also needed is the combination of molecular and morphological data to robustly define the clades, explore character evolution, and produce an updated infrageneric classification.

## 6.4.2 Non-molecular data

### 6.4.2.1 Morphological data

Phenetic approaches used in this study to deal with numerically complex data have been effective in determining species limits, as found in many previous studies (e.g. Crisp and Weston 1993; Brown and Wiecek 1996; Duretto and Ladiges 1997; Plunkett et al. 2009). Morphological data proved extremely useful in setting and testing species limits in *Schoenus* (Chapters 3, 5). Phylogenetic analyses of combined morphological and molecular sequence data can yield markedly increased levels of resolution and support compared with analyses of either data type alone (Wortley and Scotland 2006). Combined morphological and molecular data should be useful in addressing taxonomic issues at higher levels in *Schoenus*.

### 6.4.2.2 Anatomical data

Data from culm anatomy were of taxonomic value in *Schoenus*. Anatomical characters pulled together OTUs sampled for each species in most cases, showing consistency in the important characters for species. Anatomical data were also adequate to generate a complete identification key using KEY for the species of *Schoenus* studied. The anatomy dataset however, had no power to resolve phylogenetic relationships for the species of *Schoenus* indicating high levels of homoplasy.

### 6.4.2.3 Other sources of data

Data from ecological preferences and distribution patterns were important in supplementing morphological and anatomical data in defining species (Chapters 3, 5).

## 6.5 Study limitations

The main limitation for this study was shortage or, in some cases, lack of study materials particularly from the tropics and South America. Despite this, the broad and necessary range of variation is considered to have been covered for nearly all species.

In culm anatomy, it was difficult to make good sections from dried herbarium specimens (especially those that are extremely hard and fibrous) even when rehydrated. In such cases, the sections were useful in gathering data, but were of mixed quality for photography and publication.

### 6.6 Future directions should include:

- Establishing fully the limits and relationships of *Schoenus* and its internal clades, using a combination of data from additional gene regions and other lines of evidence.
- Evaluation of species limits in the other sections of *Schoenus* and providing revised taxonomic treatments of them.
- A culm anatomical study in the rest of Schoeneae to look at the distribution of anatomical characters and their correlation with morphological and molecular characters at specific and higher levels.

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