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PHYLOGENY OF ABILDGAARDIEAE (CYPERACEAE) INFERRED FROM ITS AND *trnL*-F DATA

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ABSTRACT

Within the tribe Abildgaardiaceae, the relationships between *Fimbristylis* and its relatives have not been certain, and the limits of *Fimbristylis* have been unclear, with *Bulbostylis* and *Abildgaardia* variously combined with it and each other. The relationships and limits of tribes Abildgaardiaceae and Arthrostylideae and their genera were evaluated across 49 representative species using parsimony and maximum likelihood analyses of ITS (nuclear ribosomal) and *trnL*-F (plastid) DNA sequence data separately and combined. The evolutionary reconstructions derived from sequences of cpDNA and nrDNA disagree about the position of tribe Arthrostylideae relative to Abildgaardiaceae; *Arthrostylis* and *Actinoschoenus* are either nested within Abildgaardiaceae (*trnL*-F data) or very closely related to this tribe (ITS data). The reconstructions also disagree about the monophyly of genus *Abildgaardia* (excluding *A. vaginata*). *Crosslandia* and *A. vaginata* form a clade that is nested within *Fimbristylis*. *Bulbostylis* is monophyletic and clearly separated from *Fimbristylis*. Further sampling of taxa and characters is needed to resolve and/or strengthen support for some of these “deep” and fine-scale relationships.

Key words: *Abildgaardia*, Abildgaardiaceae, Arthrostylideae, *Bulbostylis*, *Crosslandia*, Cyperaceae, *Fimbristylis*, ITS, phylogeny, *trnL*-F.

INTRODUCTION

Botanists have consistently recognized Abildgaardiaceae, the focus of this study, as a tribe in family Cyperaceae subfamily Cyperoideae (Bruhl 1995; Goetghebeur 1998). A tribe equivalent to Abildgaardiaceae was first recognized informally as Fimbristylideae (Reichenbach 1828) and that name was published formally by Raynal (1978), but in the meantime Lye (1973) had published the name Abildgaardiaceae for the tribe. Abildgaardiaceae are composed of six genera (*Abildgaardia*, *Bulbostylis*, *Crosslandia*, *Fimbristylis*, *Nelmesia*, *Nemum*) or seven if *Tylocarya* is segregated from *Fimbristylis*, and about 480 species, mostly distributed in tropical or subtropical regions with a few cosmopolitan species. The group ranges from tiny annuals a few centimetres tall to tall rhizomatous perennials. The combination of a thickened style base (except in *Nemum*) and related embryo types (fimbristylidoid, bulbostylidoid and abildgaardioid; Goetghebeur 1986, 1998) differentiate Abildgaardiaceae from other tribes of the family Cyperaceae (Raynal 1973; Bruhl 1995; Goetghebeur 1998). As currently delimited, the biggest genus is *Fimbristylis* (about 300 spp.), found in all tropical to warm-temperate regions of the world with a heavy concentration in SE Asia, Malesia, and NE Australia (Goetghebeur 1998). *Bulbostylis* (150 spp.) is widely distributed in tropical and subtropical regions, especially in tropical Africa and South America (Goetghebeur and Coudijzer 1984, 1985; Lopez 1996; Prata et al. 2001). *Abildgaardia* (17 spp.) is also distributed widely in the tropics and sub-

tropics, but is concentrated in Australia and Africa. *Crosslandia* (up to 4 spp.; K. L. Clarke pers. comm.) is endemic to northern Australia. *Nemum* (10 spp.; Lye 1989) is found in tropical Africa, as is the monotypic *Nelmesia* (Goetghebeur 1998). Four of these genera, *Abildgaardia* (ca. 9 spp.), *Bulbostylis* (ca. 7 spp.), *Crosslandia*, and *Fimbristylis* (ca. 85 spp.) are native in Australia and were the focus of this study.

The position of Arthrostylideae (Goetghebeur 1986) in relation to other tribes within Cyperaceae has been unclear. Goetghebeur (1986) provisionally distinguished this tribe, which includes *Actinoschoenus*, *Arthrostylis*, *Trachystylis* S. T. Blake, and *Trichoschoenus* Raynal, from Schoeneae (with which it shares a *Schoenus*-type embryo) as having deciduous floral bracts in each female-fertile spikelet and lacking a perianth, but later included it in Schoeneae (Goetghebeur 1998). Bruhl (1995) recognized that this tribe had close connections to Abildgaardiaceae, and this was supported by the study of Muasya et al. (2000a). *Actinoschoenus* is the most widespread of the genera, with seven or eight species of scattered occurrence: from Gabon, SE Zaire, and Zambia, to Madagascar, Sri Lanka, Southeast Asia, Philippines, New Caledonia, and Australia. The monotypic *Arthrostylis* (*A. aphylla* R. Br.) occurs in northern Australia. The monotypic *Trichoschoenus* (*T. bosseri* J. Raynal) is endemic to Madagascar (Raynal 1973). *Trachystylis* is also monotypic; *T. stradbokensis* (Domin) Kük. is endemic to northeastern Australia.

Abildgaardiaceae were sparsely sampled along with other tribes of Cyperaceae using *rbcL* by Muasya et al. (1998) and Simpson et al. (2007). These limited data did not support Abildgaardiaceae as a monophyletic tribe, in contrast to previous non-molecular (Goetghebeur 1986; Bruhl 1995) and

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Table 1. Comparison of the intratribal classifications of Abildgaardiaceae. A = *Fimbristylis*, B = *Abildgaardia*, C = *Bulbostylis*, as circumscribed by Goetghebeur and Coudijzer (1985).

Genus	Goetghebeur & Coudijzer 1985; Goetghebeur 1986, 1998; Bruhl 1995	Lye 1973; Haines & Lye 1983	Kern 1974	Lye 1971	Koyama 1961	Clarke 1902	Kunth 1837	Vahl 1805
<i>Fimbristylis</i>	A	A	A, B	A	A, B, C	A, B	A	A, C
<i>Abildgaardia</i>	B	B, C		B		C	B	B
<i>Bulbostylis</i>	C		C	C			C	C: <i>Scirpus</i>

later combined molecular and non-molecular analyses (Muasya et al. 2000a).

Phylogenetic analyses of Cyperaceae, based on non-molecular data only, do not agree on the relationships of Abildgaardiaceae (Goetghebeur 1986; Bruhl 1995). Goetghebeur (1986) regarded Abildgaardiaceae as being closely related to his Eleocharideae and Fuireneae (see also Goetghebeur 1985). Eleocharideae did not often group with Abildgaardiaceae in Bruhl's (1995) analyses, and he included the former in a broad Scirpeae. The Arthrostyleae–Abildgaardiaceae complex and the tribe Scirpeae s.l. were sister groups in non-molecular (Bruhl 1995, analyses 3, 5, and 24) and two molecular studies (Muasya et al. 2000a, b). Bruhl (1995) also noted some characteristics of C_3 species of *Abildgaardia* and *Fimbristylis* (Abildgaardiaceae) as being similar to those in some members of Arthrostyleae. Additional study is needed to clarify these morphological and molecular results and to establish clear intertribal relationships.

Vahl (1805) set up the genus *Fimbristylis* for those species previously included in *Scirpus* s.l. that have spiral glumes, a biconvex or trigonous nut, and a basally expanded, usually ciliate, 2–3-branched style. He also set up the genus *Abildgaardia* for the species with laterally compressed spikelets, subdistichous basal glumes, and a trigonous style base that is persistent on the fruit.

Kunth (1837) proposed the genus *Bulbostylis* for three American species that had previously been included in *Isolepis* but which were, in his opinion, intermediate between that genus and *Fimbristylis*. The genus is conserved for nomenclatural purposes as being published by Kunth, but it was not fully recognized until much later (Clarke 1893: 651), by which time numerous species had been described by various authors.

Subsequent authors have varied from recognizing all three of these genera to treating them as either two genera or even one genus (Table 1). Bentham (1878), for example, included both *Bulbostylis*, as section *Oncostylis*, and *Abildgaardia*, as section *Abildgaardia*, in his *Fimbristylis* s.l. Koyama (1961) also included *Abildgaardia* and *Bulbostylis* in *Fimbristylis* on the basis of morphological similarities. He placed *Fimbristylis* in tribe Scirpeae s.l., but it is considered to be a member of Abildgaardiaceae by more recent authors, as discussed above.

The subdistichous arrangement of basal glumes in *Abildgaardia* is a clear difference from the spiral arrangement in most species of *Fimbristylis*, but other morphological differences are not clearcut. Van der Veken (1965) found that the embryos are of different types in *Abildgaardia*, *Bulbostylis*, and *Fimbristylis*, and support the separation of these genera. The *Abildgaardia* and *Bulbostylis* embryo types are

more similar to each other than to the *Fimbristylis* embryo type, as discussed by Goetghebeur and Coudijzer (1984). Van der Veken (1965: 327) found that, in a few species then placed in *Fimbristylis* (*F. cioniana* Savi, *F. hensii* C. B. Clarke, and *F. hispidula* (Vahl) Kunth), the embryos are not of the *Fimbristylis*-type but rather variants of the *Bulbostylis*-type, and these species were subsequently transferred to *Bulbostylis* (Haines in Appendix 3 of Haines and Lye 1983; Goetghebeur and Coudijzer 1985). Lye (1973) and Haines and Lye (1983) included *Bulbostylis* in *Abildgaardia* while keeping *Fimbristylis* separate. Kern (1974) treated *Abildgaardia* as a section of *Fimbristylis*, but he considered *Bulbostylis* to be morphologically clearly circumscribed. Kern (1974: 542–543) also included *Actinoschoenus* (subsequently regarded as belonging to tribe Arthrostyleae) in his *Fimbristylis* s.l., but he was clearly aware of the many differences, including embryo type: “it may be better to reinstate the genus *Actinoschoenus*.” In this, he was following Raynal (1967), who argued cogently for recognizing *Actinoschoenus*. Goetghebeur and Coudijzer (1984, 1985) recognized *Abildgaardia*, *Bulbostylis*, and *Fimbristylis* in their treatment of the Central African species of these genera. They regarded the first two genera as “independent specialized offsprings from a more primitive fimbristylidoid stock” (1985: 209).

The monotypic *Tylocarya*, from Southeast Asia, was sunk in *Fimbristylis* by Kern (1958) as *Fimbristylis nelmesii* J. Kern, and this has been followed by recent authors, e.g., Goetghebeur (1998) and Simpson and Koyama (1998).

There has been no serious debate until now about the boundaries of the rest of the genera of Abildgaardiaceae, which are distinct morphologically. *Crosslandia* has unisexual flowers and is amphicarpous (Wilson 1980; see also Bruhl 1994), whereas all of the other genera of Abildgaardiaceae have bisexual flowers and mostly only aerial spikelets. *Nelmesia* is characterized among the members of Abildgaardiaceae by having a single-terminal spikelet without an obvious inflorescence bract and each flower subtended by a single hypogynous scale (Goetghebeur 1986, 1998; but see Bruhl et al. 1992 and Bruhl 1995 for an alternative interpretation of its inflorescence). In *Nelmesia* and most species of *Bulbostylis*, the style base is persistent. *Nemum* differs from all other genera in lacking a distinct, thickened or slightly thickened style base. Embryologically, *Crosslandia* has a *Fimbristylis* embryo type (Goetghebeur 1986), while *Nelmesia* and *Nemum* have embryos that are variants of the *Bulbostylis* type (Van der Veken 1965).

The sister group relationships within Abildgaardiaceae were unresolved in Bruhl's (1995) and Goetghebeur's (1986) investigations. Bruhl (1995), in one of his analyses, found that

Crosslandia and *Tylocarya* made a clade and their sister group was *Fimbristylis*; these three genera with *Abildgaardia* made a clade that, with *Bulbostylis*, formed a monophyletic group. In a molecular study by Muasya et al. (1998) using the chloroplast genome *rbcL* data only, *Nemum* and *Bulbostylis* were grouped together but did not form a monophyletic group with *Fimbristylis* and *Abildgaardia*, i.e., they were paraphyletic. This result was contradicted later by Muasya et al. (2000a), combining *rbcL* and morphological data. Although later phylogenetic studies of Cyperaceae based on *rbcL* sequences provided significant resolution in relation to evolutionary relationships at family level as well as phylogeny of non-molecular features, some clades (including Abildgaardieae and Arthrostylideae) remain unconvincingly resolved and weakly corroborated (Muasya et al. 2000a).

Cladistic analyses of molecular data for one gene may provide valuable insights into the taxonomy and phylogeny of plants (Chase et al. 1993; Baldwin et al. 1995; Smith and Carroll 1997); however, analyses of single genes are gene trees and might not represent species trees (Avice et al. 1983; Doyle 1992). The *trnL*-F region has a few advantages over the rest of the chloroplast genes/regions, notably that it is easy to amplify across a wide taxonomic range using the universal primers designed by Taberlet et al. (1991). The primers used to amplify the region can also be used to sequence it entirely in some species, and the numerous large indels (insertions and deletions) provide additional phylogenetic information (Bayer and Starr 1998). Within Cyperaceae, this region has already proved to be informative, being used successfully to deal with systematic uncertainties in tribe Cariceae (Yen and Olmstead 2000a, b) and Cypereae (Muasya et al. 2001, 2002). Therefore, we chose the *trnL*-F region as our chloroplast DNA source in this study.

In Cyperaceae, sequence diversity in the ITS region of nrDNA has proven fruitful in constructing phylogenetic hypotheses at lower taxonomic levels (Roalson and Friar 2000; Roalson et al. 2001). Hence, we chose this region not only for the primary purpose of exploring the relationship between Abildgaardieae and Arthrostylideae, but also to investigate relationships within *Fimbristylis*.

Previous molecular investigations have sampled only a few species from each genus within Abildgaardieae. Additional study with a much larger sample, including more controversial and transitional species, is necessary to establish intertribal relationships.

No cladistic test of the monophyly of *Fimbristylis* has been carried out until this present study, nor has there been any section-level cladistic study of the genus. Kern (1974) recognized 18 sections within *Fimbristylis* s.l., but the monophyly and relationships of these subgroups of *Fimbristylis* have not been assessed, and clear morphological synapomorphies for these groups have not been readily apparent.

In this paper, a phylogenetic reconstruction of Abildgaardieae is presented, using nrDNA internal transcribed spacer (ITS) and *trnL*-F sequences separately, to seek resolution within the tribes Abildgaardieae and Arthrostylideae. After that, both *trnL*-F and ITS data are used to see if polytomies can be resolved with combined data.

MATERIALS AND METHODS

Plant Samples

The 63 accessions of 44 species are listed with voucher information and NCBI accessions in Table 2. Material was silica dried (Chase and Hillis 1991) or conserved in CTAB (saturated brine containing cetyl trimethylammonium bromide/disinfectant; Rogstad 1992, modified by Thomson 2002) and mostly collected specifically for this project by Kerri Clarke, Karen Wilson, and Jeremy Bruhl as cited in Table 2.

Taxa within Abildgaardieae (mainly from Australia) were selected to represent known morphological variation in the genera, particularly within *Fimbristylis*. These included 37 specimens of 27 species of *Fimbristylis*, 16 specimens of 12 species of three other genera of Abildgaardieae considered by various authors to be close to *Fimbristylis*: *Abildgaardia*, *Bulbostylis*, and *Crosslandia* (Table 2). It was not possible to obtain samples for some of the sections of *Fimbristylis* recognized by Kern (1974), nor for the African genera *Nelmesia* and *Nemum*. Species of *Fimbristylis* not placed in sections by Kern (1974) were placed by K LW, based on their morphology.

Six species from genera within tribes Scirpeae s.l. (Bruhl 1995) and Arthrostylideae (Goetghebeur 1986; Bruhl 1995) were also included. Within Scirpeae (sensu Bruhl 1995), *Eleocharis cylindrostachys* was selected to represent the Eleocharideae sensu Goetghebeur (1985, 1986, 1998). Also, one species each of *Bolboschoenus*, *Schoenoplectus*, and *Fuirena* were included in this study to cover Goetghebeur's (1998) Fuireneae.

Molecular Techniques

DNA was extracted using standard methods (see Wilkie 1997) with modifications, including purification using diatomaceous earth, as described by Gilmore et al. (1993). Two regions of the genome were amplified by PCR: (1) the part of the chloroplast DNA including the intron of the tRNA gene for leucine (UAA) (*trnL*), and the intergenic spacer (IGS) between *trnL* (5' exon) and the tRNA gene for phenylalanine (*trnF*) (Taberlet et al. 1991) (see Fig. 1); and (2) the internal transcribed spacer part of the nuclear ribosomal RNA coding region, including ITS1 and ITS2 (see Fig. 2). Primers used for PCR are shown in Table 3.

Taq polymerase from Bioline Co. (Alexandria, New South Wales, Australia) was used for PCR following the manufacturer's recommended protocols. For the ITS region, it was found that the addition of 10% DMSO (dimethylsulfoxide) was necessary for successful amplification. The reaction outline for the *trnL* intron and *trnL*-*trnF* IGS amplification included: a primary 94°C for 5 min, followed by 30 cycles of 94°C for 30 s; 60°C for 30 s; 72°C for 1 min. Double-stranded amplified products of the polymerase chain reaction (PCR) were purified by the "Concert Rapid PCR purification system" (Life Technologies, Inc., Burlington, Ontario, Canada) following the manufacturer's specifications. For each purified amplification product, both strands were cycle sequenced.

PCR amplifications for ITS involved a primary 95°C for 5 min, followed by 35 cycles of 95°C for 40 s; 63°C for 40 s; 72°C for 80 s and then another 72°C for 5 min. For the

Table 2. Taxa included in the phylogenetic study of Abildgaardieae using the sequences of the *trnL*-F and ITS regions. Tribal classification of Cyperaceae according to Goetghebeur (1998; small caps) and Bruhl (1995; in parentheses). Species were collected in Australia and deposited in NE, unless indicated otherwise. Provenance: NSW = New South Wales; NT = Northern Territory; Qld = Queensland; WA = Western Australia.

Species	Voucher, herbarium	Provenance	GenBank accession number <i>trnL</i> -F (ITS)
ABILDGAARDIEAE (Abildgaardieae)			
<i>Abildgaardia</i> Vahl			
<i>A. ovata</i> (Burm. f.) Kral	<i>Klaphake 1410</i> , NSW	NSW	AY506708 (AY506758)
“ <i>A. oxystachya</i> ” (<i>Fimbristylis oxystachya</i> F. Muell.) ^a	<i>Clarke 165</i>	WA	AY506704 (AY506762)
“ <i>A. pachyptera</i> ” (<i>Fimbristylis pachyptera</i> S. T. Blake) ^a	<i>Clarke 181</i>	NT	AY506707 (AY506760)
<i>A. schoenoides</i> R. Br.	<i>Clarke 70</i>	Qld	AY506706 (AY506761)
<i>A. vaginata</i> R. Br.	<i>Bruhl 2057</i>	NSW	AY506705 (AY506759)
<i>Bulbostylis</i> Kunth			
<i>B. barbata</i> (Rottb.) C. B. Clarke	<i>Clarke 113</i>	WA	AY506709 (AY506764)
<i>B. densa</i> (Wall.) Hand.-Mazz.	<i>Klaphake 1411</i>	NSW	AY506710 (AY506763)
<i>B. striatella</i> C. B. Clarke	<i>Bruhl 2084</i>	NSW	AY506711 (AY506765)
<i>B. sp. nov.</i> (NT1) ^b	<i>Clarke 184</i>	NT	AY506713 (AY506766)
(NT2) ^b	<i>Clarke 184a</i>	NT	AY506714
(NT3) ^b	<i>Clarke 184b</i>	NT	AY506712
<i>Crosslandia</i> W. V. Fitzg.			
<i>C. setifolia</i> W. V. Fitzg. (NT1)	<i>Clarke 246</i>	NT	AY506718 (AY506768)
(WA1)	<i>Clarke 115</i>	WA	(AY506770)
(NT2)	<i>Clarke 185</i>	NT	AY506716 (AY506769)
(NT3)	<i>Clarke 252</i>	NT	AY506717 (AY506767)
(WA2)	<i>Clarke 117</i>	WA	(AY506771)
<i>Fimbristylis</i> Vahl			
<i>F. acuminata</i> Vahl	<i>Wilson 10059</i> , NSW	WA	(AY506774)
<i>F. arnhemensis</i> Latz	<i>Clarke 177</i>	NT	AY506722 (AY506776)
<i>F. bisumbellata</i> (Forssk.) Bubani	<i>Clarke 107</i>	WA	AY506724 (AY506778)
<i>F. cephalophora</i> F. Muell.	<i>Wilson 10070</i> , NSW	WA	AY506720 (AY506777)
<i>F. cinnamometorum</i> (Vahl) Kunth (NSW)	<i>Bruhl 2058</i>	NSW	AY506721 (AY506772)
(Qld)	<i>Clarke 61</i>	Qld	AY506725 (AY506773)
<i>F. compacta</i> Turrill	<i>Wilson 10040</i> , NSW	WA	AY506723 (AY506775)
<i>F. cymosa</i> R. Br.	<i>Wilson 10041</i> , NSW	WA	AY506750 (AY506798)
<i>F. densa</i> S. T. Blake (WA)	<i>Clarke 119</i>	WA	AY506726 (AY506781)
(NT)	<i>Clarke 180</i>	NT	AY506747 (AY506779)
<i>F. dichotoma</i> (L.) Vahl	<i>Dalby 01/24</i> , NSW	NSW	AY506727 (AY506782)
<i>F. ferruginea</i> (L.) Vahl	<i>Hodgon 445</i> , NSW	NSW	AY506744 (AY506797)
<i>F. lanceolata</i> C. B. Clarke	<i>Wilson 10113</i> , NSW	WA	AY506730 (AY506786)
<i>F. laxiglumis</i> Latz	<i>Clarke 106</i>	WA	AY506736 (AY506785)
<i>F. littoralis</i> Gaud.	<i>Orel 10</i> , NSW	Dong Nai (Vietnam)	AY506715 (AY506790)
<i>F. microcarya</i> F. Muell. (WA1)	<i>Clarke 104</i>	WA	AY506731 (AY506787)
(WA2)	<i>Clarke 131</i>	WA	AY506735 (AY506788)
<i>F. neilsonii</i> F. Muell. (WA form)	<i>Wilson 10051</i> , NSW	WA	AY506719 (AY506784)
<i>F. nutans</i> (Retz.) Vahl	<i>Hodgon 541</i>	NSW	AY506733 (AY506789)
<i>F. pauciflora</i> R. Br.	<i>Clarke 50</i>	Qld	(AY506783)
<i>F. polytrichoides</i> (Retz.) R. Br. (Qld1)	<i>Clarke 91</i>	Qld	(AY506796)
(Qld2)	<i>Jacobs 8688</i> , NSW	Qld	AY506737 (AY506795)
<i>F. pterigosperma</i> R. Br.			
(WA1)	<i>Clarke 118</i>	WA	AY506729 (AY506794)
(WA2)	<i>Clarke 164</i>	WA	AY506732
(NT)	<i>Clarke 179</i>	NT	AY506734 (AY506793)

Table 2. Continued.

Species	Voucher, herbarium	Provenance	GenBank accession number <i>trnL</i> -F (ITS)
<i>F. punctata</i> R. Br.			
(WA1)	Clarke 109	WA	AY506741
(WA2)	Clarke 114	WA	AY506739
(WA3)	Clarke 133	WA	AY506740
<i>F. rara</i> R. Br.	Clarke 105	WA	AY506728 (AY506780)
<i>F. schultzei</i> Boeck.	Clarke 108	WA	AY506748 (AY506791)
<i>F. sericea</i> R. Br.	Wilson 10042, NSW	WA	AY506743 (AY506803)
<i>F. sieberiana</i> Kunth	Jacobs 8659, NSW	Qld	AY506742 (AY506801)
<i>F. tetragona</i> R. Br.			
(Qld)	Clarke 11	Qld	AY506749 (AY506800)
(WA)	Clarke 173	WA	AY506746 (AY506799)
<i>F. tristachya</i> R. Br.	Clarke 3	Qld	AY506745 (AY506802)
<i>F. velata</i> R. Br.	Wilson 10028, NSW	WA	AY506738 (AY506792)
<i>Nelmesia</i> Van der Veken	Not sampled		
<i>Nemum</i> Desv. ex Ham.	Not sampled		
<i>Tylocarya</i> Nelmes	Not sampled		
ELEOCHARIDEAE (Scirpeae)			
<i>Eleocharis</i> R. Br.			
<i>E. cylindrostachys</i> Boeck.	Wilson 9844, NSW	NSW	AY506696 (AY506751)
FUIRENEAE (Scirpeae)			
<i>Bolboschoenus</i> Palla			
<i>B. caldwelii</i> (V. J. Cook) Soják	Ghamkhar A1, NSW	NSW	AY506698 (AY506752)
<i>Schoenoplectus</i> Palla			
<i>S. litoralis</i> (Schrader) Palla	Clarke 26	Qld	AY506697 (AY506753)
<i>Fuirena</i> Rottb.			
<i>F. ciliaris</i> (L.) Roxb. ^c	Clarke 7	Qld	DQ075251 (DQ075252)
SCHOENEAE (Arthrostylidae)			
<i>Actinoschoenus</i> Benth.			
" <i>A. composita</i> " ^a (<i>Fimbristylis composita</i> Latz)			
(NT1)	Clarke 214, NE, NSW	NT	AY506701 (AY506754)
(NT2)	Clarke 213	NT	AY506702 (AY506755)
(NT3)	Clarke 186, NE, NSW	NT	AY506703
<i>Arthrostylis</i> R. Br.			
<i>A. aphylla</i> R. Br.			
(NT2)	Clarke 183	NT	AY506700 (AY506757)
(NT3)	Clarke 194, NSW	NT	AY506699 (AY506756)

^a Combinations are not yet published; ^b Three populations of the same species; ^c This specimen was sequenced but was not used in the analyses because of sequence mismatches with other specimens.

first 10 cycles, the annealing temperature was reduced by 1°C for each cycle, while for the rest (25 cycles) it remained 53°C.

Multiple alignments of the sequences were made by "manual" adjustment of alignments calculated by either CLUSTALX (Thompson et al. 1997) (using a gap cost: gap extension cost of 10:5) or Dialign (Morgenstern 1999) (using

a threshold $T = 7$). Aligned data matrices for the ITS and *trnL*-F data sets have been submitted to Tree BASE (<http://www.treebase.org/treebase/index.html>).

Insertions and deletions can have considerable value for phylogenetic inference (e.g., Golenberg et al. 1993; Bayer and Starr 1998; Downie et al. 1998), so one must consider

Fig. 1.—The primers used in this study to amplify the *trnL*-F region.

Fig. 2.—The primers used in this study to amplify the ITS region. Depicted in black are the large (26S) subunit and the 18S and 5.8S portions of the small subunit of the nuclear ribosomal (nr) DNA.

Table 3. Sequences of the 13 primers used for the amplification of two noncoding regions of cpDNA and nrDNA. The code prefix of A or B refers to forward and reverse strands of cpDNA, respectively.

DNA region	Code	Sequence 5'–3'
<i>trnL</i> –F (cpDNA)	B49317 ^a	GGAAATCGGTAGACGCTACG
	A49855 ^a	GGGGATAGAGGGACTTGAAC
	AdTabB1 ^b	AAGTGGTAAC TTCCAAATC
	AdTabA2#2 ^b	ATTGACATGTAGAATGGGACTC
	AbilB2 ^c	ACAGAGTCCATCTCGCATGTC
	AdTabA3 ^b	TTCCGTTGAGTCTCTGCACCTATC
	B49873 ^a	GGTTCAAGTCCCTCTATCCC
ITS (nrDNA)	A50272 ^a	ATTTGAACTGGTGACACGAG
	ITS1 ^d	TCCGTAGGTGAACCTGCGG
	ITS2 ^d	CCTGCGTCTTCATCGATGC
	ITS3 ^d	GCATCGATGAAGAACGAGC
	ITSZ ^c	GGAAGTAAAAAGGCGTAACAA
	ITS8 ^c	CGCCTGACCTGGGGTAT

^a Taberlet et al. (1991); ^b Briggs et al. (2000); ^c Designed or modified in this study; ^d White et al. (1990).

counting gaps as having the status of coded characters added in the sequence matrix. The exclusion of gaps and elimination of coded gap characters from a non-coding sequence has been recommended to assess the impact of point substitutions alone (e.g., Kelchner and Clark 1997). We conducted a similar analysis considering coded gaps only and excluding all other characters in the matrix as Kelchner (2000) recommended. Finally, a combined analysis of the sequences plus the gaps was conducted.

Phylogenetic Analyses

Separate analyses.—The aligned *trnL*–*trnF* and ITS sequences were analyzed separately using PAUP* vers. 4.0b10 (Swofford 2001). The phylogenetic relationships within the Abildgaardieae–Arthrostylidae group based on their *trnL* intron and *trnL*–*trnF* IGS and also ITS data were analyzed using two methods: maximum parsimony (MP) and maximum likelihood (ML).

Cladistic analysis of sequences, as well as tests of clade support (bootstrap analyses), used PAUP*. Sequence divergence among taxa was calculated by typing the SHOWDIST command for the *trnL*–F and ITS regions.

To evaluate the phylogenetic utility of all insertions/deletions, two distinct tests were performed (1) using base substitutions alone, and (2) both substitutions and insertions/deletions. In the second test, both mutational outcomes (replacements and insertions/deletions) were positioned so as to maximize the number of matching nucleotides in a given sequence position and overlapping gaps of different lengths were considered as different mutational events.

The bootstrap option (Felsenstein 1985) of the program and decay analysis (Bremer 1988) were used to measure relative support in the unweighted analysis. For the parsimony analyses, we conducted heuristic tree searches using the Fitch criterion (unordered, equal weights; Fitch 1971) with 1000 replicates of tree-bisection-reconnection (TBR) swapping, but permitting only 10 trees to be held each time, saving time in tree swapping. Successive approximation

weighting (Farris 1969) was performed using the rescaled consistency index until an equal tree length was obtained in two (for *trnL*–F region) and three (for ITS region) successive rounds. Internal support was estimated through 1000 bootstrap replicates (Felsenstein 1985). Trees were reconstructed using simple taxon addition and TBR swapping, retaining groups with more than 50% support in the final bootstrap consensus tree. We categorized bootstrap values as: weak, 50–70%; moderate, 71–80%; and strong, 81–100%. Weighted parsimony analysis was also performed as indicated by the norm of Albert and Mishler (1992) following estimation of a transition/transversion proportion (using a few commands within PAUP*: DSET DISTANCE = ABS; SUBST = TRATIO; SHOWDIST, SAVEDIST FORMAT = TAB-TEXT). Decay indices for individual clades were obtained by comparing the strict consensus of all equal-length trees, using SIMPLE addition sequence and TBR in PAUP*.

The Hasegawa-Kishino-Yano model with rate heterogeneity was used for a maximum likelihood analysis with a few taxa selected from different clades of the parsimony analysis to see if the results agreed with those obtained from the other analyses. This model was optimized to the data set (transition/transversion ratio estimated; base frequency estimated; percentage of constant sites estimated; variable sites set as independent approximation to the gamma distribution with the shape parameter estimated). The ML analysis was based on a smaller sample of species to reduce the analysis run time.

Combined analyses.—A partition homogeneity test (PHT; an approach that uses a resampling method to estimate the degree to which two data sets or their subsets are in agreement; also known as incongruence length difference test, or ILDT test) (Mickeyevich and Farris 1981; Farris et al. 1994; see also Swofford 1991) refuted significant conflict between our *trnL*–F data set and the ITS data set, so a combined analysis was then performed. The seven accessions that were not sequenced in rDNA studies and the five accessions that were not sequenced in cpDNA experiments (see Table 2) were treated as unknown in the other data set, then included in our combined analyses.

For parsimony testing, heuristic searches were conducted on the combined data set (addition sequence random, 100 replicates, TBR branch swapping, “MulTrees” on, “steepest descent” off). After running the analyses in the parsimony criterion using unweighted characters, successive approximation weighting was implemented to get more details about the infrageneric groupings. Strict, majority-rule, and semi-strict consensus trees were obtained for our combined data analysis.

RESULTS

Combining the indels with the sequences resulted in the highest level of resolution in all three data sets (*trnL*–F, ITS, and combined *trnL*–F and ITS). Therefore, only these analyses will be discussed.

trnL–F Structure, Size, and Composition

Originally we intended to analyze the *trnL* intron and *trnL*–F spacer regions separately, but because the intron was

Table 4. Sequence characteristics of the *trnL* intron, *trnL*-F IGS, and the ITS region.

	<i>trnL</i> -F IGS	<i>trnL</i> intron	ITS1	ITS2	5.8S
Length range ^a	333–1600	576–772	197–223	237–279	162–165
Aligned length ^a	870 ^b	1082	315	343	166
No. of indels	63	23	22	36	—
GC content (%)	40	32	50.9	51.6	59.6

^a Base pairs; ^b excluding *Fimbristylis polytrichoides* with its extraordinary additional >1000 base pairs insertion (see text for discussion).

so highly conserved, and its tree was largely unresolved, we decided to combine these two regions to maximize the information available. The length of the *trnL*-F IGS section and the *trnL* intron sequences obtained in this study (Table 4) varied from 909 base pairs (bp) (*F. sericea*) to 2372 bp (*F. polytrichoides*). The aligned sequences after deletion of the unmatched sequence region of *F. polytrichoides* (see below) were 1952 bp long (Table 4). In the IGS region, the nucleotide sites varied (333–486 bp) due to a large number of indels (Table 4). *Abildgaardia vaginata* and *Crosslandia setifolia* (Clarke 115) had a variant dinucleotide repeat [d(A–T)]n/[d(T–A)]n for that region. The nucleotide sequences of the *trnL* intron include a very variable section at 410–490. Of the 1952 bp of the aligned *trnL*-F region, 1292 positions (66.2%) were invariant; of the remaining 640 variable positions (Table 5) 328 positions (16.8%) were potentially parsimony informative.

The mean nucleotide makeups (A, T, C, G) of our *trnL* intron and *trnL*-F IGS fragments were 0.36, 0.31, 0.16, and 0.17, corresponding to 0.27, 0.41, 0.15, and 0.17 in the *trnL*-F IGS and 0.35, 0.31, 0.15, and 0.19 in the *trnL* region. While the overall transition: transversion ratio was 1.74, it was 1.88 for the spacer region and 1.70 for *trnL*. The total G/C content was 36%.

Figure 3 alignment of our *trnL* intron sequences of ten species of the Abildgaardieae–Arthrostylideae group showing indels as homologies among the members of these two close tribes and the role of indels in revealing relationships among these taxa. This figure shows, in a very short sequence, indels of one to few bases and reveals the relationships among the species of different genera and differences between the species of one genus in the *trnL* intron.

Fimbristylis polytrichoides has the largest *trnL*-F IGS ever recorded, with 1600 base pairs. Examination showed that this was due to an insertion of approximately 1000 bp. Part of this insertion appeared to have been derived from

elsewhere in the cpDNA—a GenBank search found a match with some parts of another noncoding region of rice (*Oryza sativa*) cpDNA. The sequence of the other part did not match anything in GenBank. This oddity will be investigated further and reported on separately.

ITS Structure, Size, and Composition

The unaligned ITS sequence varied between 596 bp in *Abildgaardia ovata* and 667 bp in *Fimbristylis neilsonii*. After aligning 59 ITS sequences, the aligned data set was 824 bp long, comprising 435 variable characters (Table 5) of which 359 were informative. Among these, 312 were informative concerning *Fimbristylis*. Uncorrected pairwise distances of taxa ranged from 0.2% (*Fimbristylis lanceolata* to *F. compacta*) to 32.1% (*Bolboschoenus caldwellii* to *Crosslandia setifolia*).

In this study, the frequency of base substitutions that might be informative is lower in ITS1 (11.1%) than in ITS2 (15.1%), and the frequency of potentially informative indels is markedly different (22 and 36, respectively, Table 4). This indicates that ITS1 is under more structural constraint than ITS2 in Abildgaardieae.

An examination of the allocation of the 58 potentially informative indels (Table 4) on the successive weighting consensus cladogram (not shown) indicates that 26 are consistent with a single origin. The remaining 32 indels require reversals. Indel 26 is four bases long and occurs at the base of the *F. compacta*-*F. lanceolata* clade and also in *F. arnhemensis* (all appear in clade B; see Fig. 4) and *Bulbostylis* sp. nov. Indel 58 displays a two-base addition at the base of clade X, but is absent within the *Fimbristylis rara*-*F. ferruginea* clade (Fig. 4). Indel 23 is two bases long and requires four origins: within the Arthrostylideae clade and the Abildgaardieae clade in outer clades, and in the clade made up of the Northern Territory specimens of *Crosslandia* and

Table 5. Comparison of genetic evolution in the plastid and nuclear regions. This comparison is based on the Fitch analysis for taxa having a complete set of sequences, showing tree statistics and average number of changes per variable site (tree length/variable characters).

	ITS	<i>trnL</i> intron	<i>trnL</i> -F spacer
Variable/total	435/824	265/1012	375/940
Tree length	1283	398	849
CI (after excluding autapomorphies)	0.49	0.66	0.69
RI	0.72	0.76	0.74
Changes per site	2.9	1.5	2.3

Arthr. aphylla ----AGCCTAA-CAAAAAATTGATTCAA-----AAAAAA
 Abil. pachyptera----AGCCTAA-CAAAAAATTGATTCAAAAAAGTCAAAAAA
 Bulb. barbata ATTGAGCCTAA-CAAAAAATTGATTCAA-----AAAAAA
 Cross. setifolia----AGCCTAA-CAAAAAATTGATTCAA-----AAAAAA
 Fimb. dichotoma -----AA-CAAAAAATTGATTCAA-----AAAAAA
 Fimb. polytri. -----AATCAAAAAATTGATTCA-----AAAAAA
 Fimb. velata -----AATCAAAAAATTGATTCAA-----AAAAAA
 Fimb. sieberiana----AG-----CAAAAAATTGATTCA-----AAAAAA
 Fimb. schultzei ----AGCCTAA-CAAAAAATTGATTCAAAAAAGTCAAAAAA
 Fimb. tetragona -----CAAAAAATTGATTCA-----AAAAAA

Fig. 3.—Part of the nucleotide sequence of the *trnL* intron in eight species of Abildgaardieae and Arthrostylideae showing indels as homologies among these taxa. An indel is shared between *Abildgaardia pachyptera* and *Fimbristylis schultzei*. Abbreviations: Arthr. = *Arthrostylis*; Abil. = *Abildgaardia*; Bulb. = *Bulbostylis*; Cross. = *Crosslandia*; Fimb. = *Fimbristylis*; polytri. = *polytrichoides*. A dash indicates the absence of a base.

Fig. 4.—Strict consensus tree from 24 most-parsimonious trees for the ITS data set unweighted Fitch parsimony. Bootstrap values from 1000 replicates are in percent above the branches; * = 100%. As there was no obvious difference among the sequences of different accessions of *Bulbostylis* sp. nov., they have all been covered by one accession name in this tree. *Abil.* = *Abildgaardia*; *Bulb.* = *Bulbostylis*; *Cross.* = *Crosslandia*; *Fimb.* = *Fimbristylis*. Abbreviations on the right denote tribes of Cyperaceae sensu Goetghebeur (1986): ABIL = Abildgaardieae, ARTH = Arthrostylideae, FUI = Fuireneae, and ELEO = Eleocharideae. NSW = New South Wales; NT = Northern Territory; WA = Western Australia. Clades I and II refer to the main clades; A–H, X, and Y refer to clades discussed in the text.

Abildgaardia vaginata as well as in *F. littoralis* in inner parts of the tree (clades as seen in Fig. 4).

Phylogenetic Analyses

The representatives of Scirpeae s.l. and Eleocharideae appear as outgroups to Abildgaardieae and Arthrostylideae, as

designed in Fig. 4–7. Specimens of the same species usually grouped together in *trnL*–*F*, ITS, and combined analyses. An exception was the position of *Abildgaardia vaginata* within a clade of *Crosslandia setifolia* individuals (Fig. 4–7).

trnL–*F* Phylogenetic analysis.—Using substitutions only for the *trnL*–*F* data, heuristic search calculation using PAUP*

Fig. 5.—One of 280 shortest maximum parsimony trees of the *trnL*-F intergenic spacer and intron (*trnL*) based on unweighted analysis of only the nucleotide substitution data (excluding indels). The number above each branch shows the branch length. *Eleocharis* was used to root this tree. Tree length is 1273. Consistency index is 0.667 (0.565 without uninformative data). Retention index is 0.767. *Abil.* = *Abildgaardia*; *Bulb.* = *Bulbostylis*; *Cross.* = *Crosslandia*; *Fimb.* = *Fimbristylis*. NSW = New South Wales; NT = Northern Territory; Qld = Queensland; WA = Western Australia. Clades I and II are the main clades of Abildgaardieae; III is Arthrostylideae.

resulted in 280 most-parsimonious trees. One of these trees is shown in Fig. 5 (consistency index 0.67; tree length 1273, and 1010 ignoring autapomorphies). Highest resolution of the ingroup for the *trnL*-F data was achieved with base substitutions plus the indel matrix. The strict consensus of these

trees (not shown) reveals Arthrostylideae nested between the species of *Bulbostylis* and the rest of Abildgaardieae. The last forms a polytomy of species currently assigned to *Fimbristylis*, *Abildgaardia*, and *Crosslandia*.

The MP unweighted (Fig. 5) and successive approxima-

Fig. 6.—One of nine maximum parsimony trees from successive approximation weighting (SAW) for the *trnL*–*F* data set. Arrows mark clades absent from the strict consensus tree of Fitch (open) or both Fitch and SAW (solid) analyses. Numbers above branches are SAW bootstrap percentages; Fitch decay values below branches; * = 100% bootstrap support. *Abil.* = *Abildgaardia*; *Bulb.* = *Bulbostylis*; *Cross.* = *Crosslandia*; *Fimb.* = *Fimbristylis*. Clades I and II are the main clades of *Abildgaardieae*; III is *Arthrostylideae*.

tion weighted (Fig. 6) trees differed noticeably in the placement of *Fimbristylis littoralis*. However, successive approximation weighting recovered the same main branches, particularly at generic level (Fig. 6). The *trnL*–*F* phylogeny for species of *Abildgaardieae* has two main clades (I and II). *Bulbostylis* never appeared in a clade with the rest of *Abildgaardieae* s.s. in any of the analyses. *Abildgaardia vaginata* is separate from the rest of *Abildgaardia*, forming a clade with samples of *Crosslandia setifolia*, nested or grouped with the species of *Fimbristylis* in all of the most-parsimo-

nious, strict consensus, and successive approximation-weighted trees (Fig. 4–7).

ITS phylogenetic analysis.—The initial search applying equal weights found 24 most-parsimonious trees of 1283 steps (Table 4). The consistency index was 0.49 (0.46 excluding uninformative characters) and the retention index (RI) 0.72 (Table 4). The strict consensus is shown in Fig. 4, with bootstrap values provided above the branches of each clade. In an attempt to test the tree space left behind, the

Fig. 7.—Strict consensus tree from 12 most-parsimonious trees for the combined data set from the *trnL-F* and the ITS regions produced by unweighted Fitch parsimony. Bootstrap values are indicated as percent (on top of branches) after 1000 replicates; * = 100%; decay values are indicated below branches. *Abil.* = *Abildgaardia*; *Bulb.* = *Bulbostylis*; *Cross.* = *Crosslandia*; *Fimb.* = *Fimbristylis*. Abbreviations on the right denote tribes of Cyperaceae sensu Goetghebeur (1986): ABIL = Abildgaardiaceae, ARTH = Arthrostylideae, FUI = Fuireneae, and ELEO = Eleocharideae. NSW = New South Wales; NT = Northern Territory; WA = Western Australia; A, D, F, G, I, J, X, and Y refer to clades also present in Fig. 4.

strict consensus tree was then employed as a reverse constraint in a heuristic search with 100 replicates with random taxon addition, saving 500 trees per replicate. No shorter trees were achieved in any of these searches, nor were any equally parsimonious trees inconsistent with Fig. 4.

Arthrostylideae (clade 1) are well separated from Abild-

gaardiaceae (clade 2). The representatives of Abildgaardiaceae have been divided into two main clades indicated as I and II in Fig. 4. There is strong bootstrap support for both clades (100 and 91%, respectively). All species of *Abildgaardia* group together except *A. vaginata*, which is grouped with *Crosslandia setifolia* accessions and three species of *Fim-*

bristylis in clade A (Fig. 4). Species assigned to *Fimbristylis* appeared only in the Abildgaardieae I clade, subclades X and Y (except for *F. littoralis*, but possible problems with that sample mean the placement of this species needs further study; Fig. 4). Relationships among lineages observed within clade X are, for the most part, uncertain. Within clade X, the species have been placed in four poorly resolved clades labelled A–D. *Fimbristylis acuminata* and *F. nutans* plus *F. pauciflora* are weakly grouped in clade A with *Crosslandia* and *A. vaginata*. The monophyly of *Crosslandia* is strongly supported (together with *A. vaginata*) within clade A. The interesting point here is the position of *A. vaginata*, which is always between the two sets of accessions of *C. setifolia* collected from two different ecogeographical regions (Western Australia and Northern Territory). The *Abildgaardia* clade, followed by *Fimbristylis littoralis*, is sister to main clade I. *Bulbostylis* is well supported in clade II, with *Bulbostylis* sp. nov. sister to all the other taxa.

The ITS and *trnL*–*F* ML analyses (not shown) yielded trees with (Arthrostyleae (*Bulbostylis* (*Schoenoplectus*, *Bolboschoenus*)) (the rest of Abildgaardieae))). Except for the anomalous position of *Schoenoplectus* and *Bolboschoenus*, on the *trnL*–*F* tree, the results are essentially the same results as those of the MP trees (Fig. 4, 5). Given the limited data set and the mostly similar results, only the MP analyses are presented for brevity. The unexpected position of *Bolboschoenus* and *Schoenoplectus* in the *trnL*–*F* ML tree does not concern us because it was not seen in any other analysis. However, a possible close relationship between *Schoenoplectus* and some species of *Abildgaardia* was suggested by Bruhl (1995: 206): “. . . my observations on the embryo of the *C*₃ species of *Abildgaardia* reveal *Fimbristylis*–*Schoenoplectus*-like embryo morphology.”

Phylogenetic analysis of combined ITS/*trnL*–*F* data.—The combined data set has 2776 characters, 1075 of which are potentially phylogenetically informative. Fitch analysis generated 12 cladograms, each 2709 steps long, CI = 0.55, RI = 0.71. Partition homogeneity test (PHT) indicated that the *trnL*–*F* and ITS data sets were congruent ($P = 0.85$) in their phylogeny estimations. Therefore, we combined the two data sets in a large matrix for further analysis to examine inconsistencies.

The results of our combined analysis (Fig. 7) show that tribe Abildgaardieae, excluding *Bulbostylis*, makes a strongly supported clade (bootstrap value = 100%), but support is poor for many major branches within it. *Bulbostylis* forms a highly supported clade with Arthrostyleae, nested between the *Bulbostylis* clade and the rest of Abildgaardieae. *Fimbristylis* is paraphyletic as currently circumscribed; the *Fimbristylis* s.s. clade (clade Z in Fig. 7) includes species in two other genera: *Abildgaardia* and *Crosslandia*.

DISCUSSION

The preponderance of substitutions over deletions in the ITS region, and the short length of the insertions (mostly 1–2 bp), are in line with the conclusion (Baldwin et al. 1995) that these spacers are under structural constraint due to their role in the maturation of nuclear RNAs. Most indels might be the results of slippage at some stage in DNA replication (Levinson and Gutman 1987; Stephan 1989). By contrast,

the large deletions in all sequenced species except *Abildgaardia ovata* and *Bulbostylis* sp. nov. (indel 3; 52 bp) are of the type consistent with unequal crossing over of rDNA repeat units (Smith 1976). Indels of the latter kind may bear useful apomorphies in intensive investigations of this tribe.

The distributions of indels are congruent with the estimate of phylogeny obtained primarily from the substitution data, and they can be seen to support many of the clades. For example, all accessions of *Crosslandia setifolia* and the only accession of *Abildgaardia vaginata* are characterized by four unique indels (1, 12, 20, and 31) plus another one (33) that also arises in other lineages in the ITS data set. All five informative indels in ITS required only a single origin when their distributions were mapped on the strict consensus tree (figure not shown), whereas 23 of the 97 informative indels in the *trnL*–*F* region required more than one origin. Multiple origins of such indels in intergenic spacers have been frequently observed (e.g., Golenberg et al. 1993; Lowrey et al. 2001). The outgroup taxa differ from the ingroup by four indels, two in the ITS region (10 and 24) and two in the *trnL*–*F* region (73 and 86). This shows that the indels are not highly differentiating between the outgroup taxa and the ingroup, suggesting more divergent taxa ought to be included in the outgroup in future studies. Further, the existence of two common indels among all species of *Bulbostylis*, *Arthrostyleae*, *Actinoschoenus*, and the outgroup suggests that these indels might be informative more broadly across Cyperaceae.

Relationship Between Tribes Abildgaardieae and Arthrostyleae

Our phylogenetic analyses recover the ingroup comprising members of tribes Abildgaardieae and Arthrostyleae as a monophyletic group relative to the outgroups (*Eleocharis* in tribe Eleocharideae and *Schoenoplectus* and *Bolboschoenus* in tribe Fuireneae). The genera sampled from Arthrostyleae (*Actinoschoenus* and *Arthrostyleae*) form a monophyletic group, but the results are inconclusive as to whether this clade is sister to Abildgaardieae (ITS: Fig. 4) or nested within Abildgaardieae (*trnL*–*F*: Fig. 5 and 6; combined data: Fig. 7).

Arthrostyleae were recognized provisionally by Goetghebeur (1986) and that hypothesis received some support from Bruhl (1995), most of whose analyses showed that the relationships of Arthrostyleae were with *Abildgaardia* or Abildgaardieae as a whole, and only in a few analyses with Schoeneae. However, more recently Goetghebeur (1998) in his summary treatment of the family, without further analysis, included the members of Arthrostyleae under Schoeneae. In analyses of combined molecular and morphological data (Muasya et al. 2000a), and in a recent expanded analysis of *rbcL* data for Cyperaceae (Simpson et al. 2007) that included *Arthrostyleae* (Arthrostyleae/Schoeneae) and *Abildgaardia*, *Bulbostylis*, *Fimbristylis*, and *Nemum* (six species of Abildgaardieae), *Arthrostyleae* was sister (but unsupported in the strict consensus) to Abildgaardieae. The results to date would argue for the inclusion of members of Arthrostyleae in Abildgaardieae, but some caution is required.

Sampling in *Nelmesia*, *Nemum*, *Trachystylis*, *Trichoschoenus*, and also morphologically different accessions of

Actinoschoenus for *trnL*-F and ITS, as well as analysis of another DNA region plus morphological and embryological data are needed to produce better resolution. It would also seem appropriate to expand and combine data sets for Abildgaardieae, Arthrostylidae, and Schoeneae to test more thoroughly their relationships.

All genera of Arthrostylidae have the C₃ photosynthetic pathway, as do the sampled outgroups, except for some C₄ species in *Eleocharis* (Bruhl et al. 1987; Ueno et al. 1989; Bruhl and Perry 1995; Bruhl and Wilson 2006). In contrast, all the genera of Abildgaardieae sampled are C₄ (Bruhl and Wilson 2007), except for one species each of *Abildgaardia* and *Fimbristylis* in Africa (not sampled by us) that are C₃. This distribution of photosynthetic pathways is interesting but, given the independent origin of the C₄ pathway in several lineages within Cyperaceae (Soros and Bruhl 2000), further sampling across taxa representing different photosynthetic pathways in Abildgaardieae and Eleocharidae is desirable to test its phylogenetic importance here, especially in the context of *Crosslandia* and *A. vaginata* (see below).

Resolution of the Genera of Abildgaardieae

Within Abildgaardieae, only *Bulbostylis* is monophyletic in all three analyses. Its position varies from being sister to the rest of Abildgaardieae in the ITS analysis to sister to these taxa plus Arthrostylidae (*trnL*-F and combined analyses). We consider these results, together with the morphological similarity of *Bulbostylis* to the other genera in tribe Abildgaardieae and Arthrostylidae (Bruhl et al. 1992; Bruhl 1995), to be further evidence that the two tribes should be joined (cf. Simpson et al. 2007).

Goetghebeur and Coudijzer (1984: 209) suggested that *Bulbostylis* and *Abildgaardia* (without *A. vaginata*) represent “independent specialized offsprings from a more primitive fimbristylidoid stock.” Although we agree that *Bulbostylis* is an independent genus, our results place *Bulbostylis* as a basal lineage relative to *Abildgaardia*, *Crosslandia*, and *Fimbristylis* (Fig. 4–7).

Abildgaardia, as currently circumscribed, is not monophyletic in any of the analyses. There is poor recovery of the genus in the *trnL*-F analysis, with its species scattered across several subclades within main clade I (Fig. 5). However, there is better recovery in the ITS (Fig. 4) and combined (Fig. 7) analyses, with four species forming a well-defined clade that is sister to a *Fimbristylis*—*Crosslandia*—*A. vaginata* clade. This last clade is a constant feature of our analyses, but it is mostly nested within *Fimbristylis*, and indicates the need either to break up *Fimbristylis* further or to include *Crosslandia* and *A. vaginata* in *Fimbristylis*. Excluding *A. vaginata*, the ITS (Fig. 4) and combined analyses (Fig. 7) do present the first multispecies sample of *Abildgaardia* in a molecular phylogeny reconstruction that argues for the retention of *Abildgaardia* distinct from *Bulbostylis* (in contrast to Lye 1971, 1973, 1983) and *Fimbristylis* (in contrast to Simpson et al. 2007).

All species of *Abildgaardia* that we sampled have the C₄ photosynthetic pathway (Bruhl and Wilson 2006). The C₃ species of *Abildgaardia* and *Fimbristylis* (Bruhl and Wilson 2006) need to be analyzed to assess their relationship to the

Table 6. Assignment of species to sections in *Fimbristylis* s.l. according to Kern (1974), with interpolation of Australian endemics by K. L. Wilson (underlined) on the basis of morphology.

Section number	Section name	Species
1	<i>Trichelostylis</i>	<i>Fimbristylis microcarya</i>
2	<i>Miliaceae</i>	<i>F. littoralis</i> , <i>F. miliacea</i>
3	<i>Cymosae</i>	<i>F. cymosa</i> , <i>F. sericea</i>
4	<i>Tenerae</i>	<i>F. cephalophora</i> , <i>F. compacta</i> , <i>F. schultzei</i>
5	<i>Leptocladae</i>	<i>F. lanceolata</i> , <i>F. laxiglumis</i> , <i>F. neilsonii</i> , <i>F. rara</i>
6	<i>Heleocharoides</i>	<i>F. densa</i> , <i>F. pauciflora</i> , <i>F. pterigospema</i>
7	<i>Signatae</i>	<i>F. arnhemensis</i>
8	<i>Abildgaardia</i>	<i>Abildgaardia ovata</i> , <i>A. oxystachya</i> , <i>A. pachyptera</i> , <i>A. schoenoides</i> , <i>A. vaginata</i>
9	<i>Fuscae</i>	<i>Fimbristylis cinnamometorum</i>
10	<i>Dichelostylis</i>	<i>F. ferruginea</i> , <i>F. sieberiana</i> , <i>F. tristachya</i>
11	<i>Fimbristylis</i>	<i>F. bisumbellata</i> , <i>F. dichotoma</i>
13	<i>Pogonostylis</i>	<i>F. velata</i>
14	<i>Neodichelostylis</i>	<i>F. polytrichoides</i>
15	<i>Nutantes</i>	<i>F. acuminata</i> , <i>F. nutans</i> , <i>F. punctata</i>
16	<i>Mischospora</i>	<i>F. tetragona</i>
18	<i>Actinoschoenus</i>	<i>Actinoschoenus</i> sp.

C₄ species as well as sampling more broadly across the rest of *Abildgaardia*.

Crosslandia has been treated as a monotypic genus (Fitzgerald 1918), or as possibly having a second species (Goetghebeur 1986, 1998; Bruhl 1995), or even including four species (K. L. Clarke pers. comm.). The relationships are neither constant nor resolved among the samples of *C. setifolia*, indicating that our data do not resolve these relationships.

Given that the *Crosslandia* and *A. vaginata* group is nested within *Fimbristylis* in all our analyses, there is strong evidence that these species should be treated as part of *Fimbristylis* or alternatively that *Fimbristylis* should be broken up more finely. However, we did not have DNA samples of *F. spiralis*, which is morphologically similar to *C. setifolia*. It would seem prudent to extend the molecular sample more widely across this group before implementing changes to generic boundaries.

Fimbristylis is a large genus of about 300 species, and has been divided into a number of sections on morphological characters (Kern 1974). All of the species of *Fimbristylis* sampled fall within one major clade in all of our analyses, but *Crosslandia* and *A. vaginata* are nested within this clade. We sampled 1–5 species in 16 of the 18 sections of *Fimbristylis* recognized by Kern (including *Abildgaardia* and *Actinoschoenus*, which were both treated as sections by Kern; see Table 6). Australian species not assigned by Kern were placed in sections by K LW on the basis of their morphology (Table 6). Few of those sections (excluding *Abildgaardia* and *Actinoschoenus*) are recovered. Indeed, none is recovered in the *trnL*-F analysis (Fig. 5, 6). In the ITS analysis (Fig. 4), (1) *F. cymosa* and *F. sericea* of sect. *Cymosae*,

and (2) *F. acuminata* and *F. nutans* of sect. *Nutantes* group together; similarly (3) *F. compacta* and *F. cephalophora* of sect. *Tenerae* are together, but *F. schultzei* is distant. In the combined analysis (Fig. 7), only pairs (1) and (2) are recovered but *F. punctata*, traditionally in section *Nutantes*, is well removed from pair (2). Further sampling and analysis of species in molecular studies, coupled with analysis of morphological variation, should improve resolution. It may be that some of the morphological variation that has been used to characterize the sections (Kern 1974), e.g., inflorescence form, is plesiomorphic and therefore not indicative of phylogeny. Certainly such morphological characters contain considerable homoplasy (Bruhl 1995; Muasya et al. 2000a).

CONCLUSIONS

Our phylogenetic analyses of ITS and the *trnL-trnF* IGS and *trnL* intron data contribute to an understanding of relationships across tribes and genera of Arthrostylideae and Abildgaardieae and in particular support a close association between Arthrostylideae and Abildgaardieae. Analyses of combined data sets resulted in better clade support, as was the case for Muasya et al. (1998, 2000a). Support for the distinctness of *Bulbostylis* from the rest of Abildgaardieae is an important outcome of this study. Although the monophyly of *Abildgaardia* as currently delimited is not supported by our *trnL-F* data, there is the possibility of monophyly for this genus if *A. vaginata* is excluded, as shown by our ITS and combined analyses. Further, our results support inclusion of *Crosslandia* and *A. vaginata* in an expanded *Fimbristylis*, whose internal relationships, however, remain poorly resolved.

The ITS and the *trnL-trnF* IGS and *trnL* intron data have been of limited use for answering questions about close relationships and for the deeper branches in Abildgaardieae. The relatively poor resolution at the terminal and basal branches might be explained by the suggestion that Abildgaardieae have evolved recently, though this seems unlikely (Bremer and Janssen 2006). Another explanation for the low resolution of the current data sets might be peripheral segregation and disintegration of populations (Soltis and Soltis 1994). In any case, higher resolution as well as stronger corroboration of terminal branches can possibly be achieved by using faster evolving genes. One considerable issue in the current study could be undersampling. Even though we studied 40 species, this is less than 10% of the 480 species in this group. There is a need for collection and analysis of many more samples (more species of *Fimbristylis* and samples of the genera not included here, plus other critical species referred to above). Using combined molecular and non-molecular information along with more intense sampling should resolve relationships within Abildgaardieae and close tribes including Arthrostylideae.

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