Chapter 1

General introduction

Abildgaardieae Lye

The tribe Abildgaardieae Lye is one of the tribes within the subfamily Cyperoideae (Table 1.1), in the family Cyperaceae and, depending on the classification used (Goetghebeur 1986; Bruhl 1995; Goetghebeur 1998; Simpson et al. 2005, in press), is composed of five, six or seven genera: *Abildgaardia* Vahl (TYPE genus), or as *Fimbristylis* Vahl section *Abildgaardia* Benth., *Fimbristylis* Vahl, *Bulbostylis* Kunth, *Crosslandia* W.Fitzg., *Nemum* Desv.ex Ham., *Nelmesia* Van der Veken and *Tylocarya* Nelmes, or as *Fimbristylis nelmesii* (Kern 1958, 1974; Simpson 1993; Goetghebeur 1998; World Checklist of Monocotyledons 2004).

History of the tribe Abildgaardieae

The genera *Abildgaardia* (expanded to include species of *Bulbostylis*) and *Nemum* were separated from *Fimbristylis* (Lye 1973) and *Scirpus* (Raynal 1973; Lye 1973) respectively, who placed them into his new tribe Abildgaardieae Lye. *Nelmesia* was also considered for possible inclusion. Although Lye had formed the new tribe, the publication 'Sedges and Rushes of East Africa' (Haines and Lye 1983) retained the three genera in the tribe Scirpeae, simply commenting that several smaller tribes (including Abildgaardieae) would be a better option. In his systematic study of the family Cyperaceae, Goetghebeur (1986) included *Crosslandia*, *Fimbristylis*, *Tylocarya*, and *Nelmesia* in Lye's tribe Abildgaardieae. Bruhl (1995) supported the

Table 1.1 Tribes of the Cyperaceae. Bruhl's classification shows the tribal and generic concepts used for this study. Alternative classifications provide comparative placement of genera studied within the confines of the present project. Some taxa from *Schoenoplectus** (includes *Schoenoplectiella* Lye) were used as additional outgroup taxa for cladistic analyses. The number of genera and species, respectively, are shown in brackets after each tribe in the most recent studies.

Bruhl (1995)	**Goetghebeur (1998)**	**Goetghebeur(1986)**	**Kern (1974) (for Malesia)**	**Bentham and Hooker (1880)**
Subfamily: Cyperoideae **Tribes:** Cyperaeae (17/878) Scirpeae (28/518) **Genus:** *Schoenoplectus** Abildgaardieae (7/430) **Genera:** *Abildgaardia* *Bulbostylis* *Crosslandia* *Fimbristylis* *Nemum* *Nelmesia* *Tylocarya* Arthrostylideae (4/6) (name invalid) **Genera:** *Actinoschoenus* *Arthrostylis* *Trachystylis* *Trichoschoenus* **Subfamily:** Caricoideae **Tribes:** Rhynchosporeae (4/273) Schoeneae (27/379) Cryptangieae (5/92) Trilepideae (4/15) Cariceae (6/2089) Sclerieae (2/201) Bisboeckelereae (4/22) Hypolytreae (14/159)	**Subfamily:** Mapanioideae **Tribes:** Hypolytreae (9/130) Chrysitricheae (4/13) **Subfamily:** Cyperoideae **Tribes:** Scirpeae (6/60) Fuireneae (5/90) **Genus:** *Schoenoplectus** Eleocharideae (3/200) Abildgaardieae (6/420) **Genera:** *Fimbristylis* (including *Tylocarya*) *Crosslandia* *Bulbostylis* *Abildgaardia* *Nemum* *Nelmesia* **Tribes:** Cypereae (19/900) Dulichieae (3/10) Schoeneae (29/700) **Genera:** *Arthrostylis* *Actinoschoenus* *Trichoschoenus* *Trachystylis* **Subfamily:** Scleriodeae **Tribes:** Cryptangieae (4/50) Trilepideae (4/15) Sclerieae (1/250) Bisboeckelereae (4/25) # **Subfamily:** Caricoideae **Tribe:** Cariceae (5/2150)	**Subfamily:** Mapanioideae **Tribes:** Hypolytreae Chrysitricheae **Subfamily:** Cyperoideae **Tribes:** Scirpeae Fuireneae **Genus:** *Schoenoplectus** Eleocharideae Abildgaardieae **Genera:** *Fimbristylis* *Tylocarya* *Crosslandia* *Bulbostylis* *Abildgaardia* *Nemum* *Nelmesia* **Tribes:** Ficineae Cypereae Dulichieae Arthrostylideae (provisional name) **Genera:** *Arthrostylis* *Actinoschoenus* *Trichoschoenus* *Trachystylis* Rhynchosporeae Schoeneae **Subfamily:** Scleriodeae **Tribes:** Cryptangieae Trilepideae Sclerieae Bisboeckelereae **Subfamily:** Caricoideae **Tribe:** Cariceae	**Subfamily:** Cyperoideae **Tribes:** Hypolytreae Cyperaeae **Genera:** *Fimbristylis* (includes *Abildgaardia* and *Actinoschoenus* as sections) *Bulbostylis* *Scirpus* *Fuirena* *Lipocarpha* *Eleocharis* *Cyperus* Rhynchosporeae **Subfamily:** Caricoideae **Tribes:** Sclerieae Cariceae **Genus:** *Schoenoplectus**	**Monoclines:** **Tribe:** Scirpeae **Genera:** *Fimbristylis* (included *Abildgaardia* and *Bulbostylis* [= Oncostylis] as sections) *Scirpus* sect. Schoenoplectus (*Schoenoplectus**) **Tribes:** Hypolytreae Rhynchosporeae **Genera:** *Arthrostylis* *Actinoschoenus* **Diclines:** **Tribes:** Cryptangieae Sclerieae Cariceae

N.B. # Goetghebeur (1995) has monotypic genera *Exochogyne* and *Koyamaea* as *incertae sedis*

placement of the genera within the tribe in his systematic study where vegetative anatomy was a focus.

The tribe Fimbristylideae Cherm. ex Raynal linked the related *Fimbristylis* and *Bulbostylis*, but was not widely used. Kukkonen (1991) did not recognise either tribe Fimbristylideae or Abildgaardieae, but retained the tribe Scirpeae.

There has been little disagreement with the general boundaries of the tribe, however, the limits of some of the main genera within the tribe have been disputed and are still unresolved (Greuter et al. 1993; Simpson 1993; World Checklist of Monocotyledons 2004).

General history of genera of the Abildgaardieae

Fimbristylis, *Abildgaardia*, and *Bulbostylis*

Vahl (1805) separated 21 species from *Scirpus*, placing 19 species plus four new species into his genus *Fimbristylis*, and two species, *Abildgaardia monostachya* (L.) Vahl (= *A. ovata* (Burm.f.) Kral or *Fimbristylis ovata* (Burm.f.) J.Kern) and *A. tristachya* Vahl (= *A. triflora* (L.) Abeywickr. or *Fimbristylis triflora* (L.) K.Schum. ex Engl.) into *Abildgaardia*. The generic status of *Fimbristylis* was widely accepted by the botanists of the day, but not *Abildgaardia*. Robert Brown (1810) collected extensively in Australia and described two new species of *Abildgaardia*, *A. vaginata* R.Br. and *A. schoenoides* R.Br. Botanists were tempted by the generic character of distichous-subdistichous glumes used to define *Abildgaardia*, with many new species assigned to *Abildgaardia*. Kunth (1837) certainly accepted the new genus, acknowledging 14 species in his circumscription. Mueller, however, preferred

Hasskarl's placement of *Abildgaardia monostachya* into *Fimbristylis* as *F. monostachya* (L.) Hassk., naming his new species *F. oxystachya* F.Muell. Initially Bentham (1861) used *Abildgaardia* as a genus and included *A. monostachya*, *A. eragrostis* and *A. fusca* as species for the Hong Kong Flora. He commented on the habit being similar to *Fimbristylis* and that only the distichous glumes in *Abildgaardia* were different. It was in Bentham's later classifications (Bentham 1878; Bentham and Hooker 1880) that *Abildgaardia* was demoted to a section within *Fimbristylis*, where he included *F. oxystachya*, *F. macrantha* Boeck., *F. squarrulosa* (syn. *A. schoenoides*), *F. brownii* Benth. (= *A. vaginata*) and *F. dallachyi* (= *F. fimbristyloides*) with *F. monostachya*. It was at this time that Bentham (1878) also demoted *Bulbostylis* to *Fimbristylis* section *Oncostylis* (*F. barbata* (Rottb.) Benth. and *F. capillaris* A.Gray), so that the expanded genus *Fimbristylis* then comprised five sections: *Heleocharoides*, *Dichelostylis*, and *Trichelostylis*, *Abildgaardia*, and *Oncostylis*.

Bulbostylis was reinstated to generic status when Clarke (1900, 1908) recognised four sections of *Fimbristylis*: *Eleocharoides*, *Dichelostylis*, *Trichelostylis*, and *Abildgaardia*. Clarke (1908) then assigned the species of *Bulbostylis* (some moved from *Isolepis* R.Br.) into five sections based on morphological features, narrowing groups within sections according to their broad geographical distribution (Table 1.2).

The genera remained stable for some time, until Koyama (1961) incorporated *Bulbostylis* once again into *Fimbristylis*, although he chose the rank of subgenus. The move was generally not accepted and Van der Veken (1965) showed that species of *Bulbostylis* shared an embryo type that was different to that in species of *Fimbristylis*. Kern (1974) maintained *Bulbostylis* as a genus and *Abildgaardia* as a section of *Fimbristylis*.

Table 1.2 Classification of *Bulbostylis* by Clarke (1908). Species sampled in the tribal study are indicated by an *.

Section I. Stylus 2-fidus	Section II. Stylus 3-fidus	Section III. Stylus 3-fidus. Culmus 1-cephalus	Section IV. Stylus 3-fidus. Capitula umbellatae	Section V. Stylus 3-fidus. Spiculae umbellatae
*B. humilis** (as *B. striatella*)	**Africanae**	**Gerontogeae** (old world)	**Gerontogae**	**Cosmopolitan**
	B. festucoides	*B. barbata**	*B. zeyheri*	*B. capillaris**
	B. renschii	*B. rarissima*	*B. kirkii*	**Gerontogeae**
	B. zambesica	*B. lichtensteiniana*	*B. trabeculata*	*B. puberula**
	*B. breviculmis** (as *B. striatella*)	*B. schoenoides*	*B. japonica*	*B. taylori*
	B. sphaerocarpus	*B. scleropus*	**Neogeae**	*B. burchellii*
	B. schlechteri	*B. parvinux*	*B. vestita*	*B. filiformis** (as *B. hispidula*)
	B. funckii	*B. cinnamomea*	*B. graminifolia*	*B. abortive*
	Americanae	*B. collina*	*B. consanguinea*	*B. biovini*
	B. leucostachya	*B. filamentosa*	*B. junciformis*	*B. coleotricha*
	B. conifera	*B. laniceps*	*B. nesiotis*	*B. johnstoni*
	B. lanata	*B. cardiocarpa*		*B. parva*
	B. paradoxa	*B. erratica*		*B. mucronata*
	B. schaffneri	*B. burkei*		*B. andongensis*
	B. pauciflora	*B. astrosanguinea*		*B. transiens*
	B. floccosa	*B. comorensis*		*B. melanocephala*
	B. aturensis	*B. schimperiana*		*B. macra*
		B. fimbristyloides		*B. megastachys*
		B. aphyllanthoides		*B. oritrephes*
		B. buchanani		*B. trichobasis*
		B. subspinescens		*B. cylindrical*
		Neogeae (new world)		**Neogeae**
		B. stenophylla		*B. fendleri*
		B. warei		*B. langsdorfiana*
		B. subaphylla		*B. scabra*
		B. glaziovii		*B. arenaria*
		B. fimbriata		*B. tenella*
		B. sphaerocephala		*B. circinata*
				B. paraensis
				B. jacobinae
				B. laeta
				B. asperula
				B. micans

In more recent times, Lye (1973; Haines and Lye 1983) placed *Bulbostylis* as a subgenus of *Abildgaardia* (*Abildgaardia* had nomenclatural priority), based on a similar embryo type that was distinct from that found in species of *Fimbristylis*. Goetghebeur and Coudijzer (1984, 1985) disagreed with Lye's change and stated that *Bulbostylis* and *Abildgaardia* had evolved separately from *Fimbristylis*. Differences between the embryo types, in conjunction with nut epidermal (fruit wall) ornamentation and the presence of long white hairs at the mouth of the leaf sheath, factored in keeping *Bulbostylis*, *Abildgaardia* and *Fimbristylis* as distinct genera. Robyns and Tournay (1955), and Kral (1971) had already adopted the equal generic rank in their regional studies. In later studies Lye (1995, 1996, 2000) accepted the generic rankings of *Bulbostylis*, *Abildgaardia* and *Fimbristylis*.

Workers at Kew Herbarium (Hooper 1973; Kern 1974; Simpson 1993; Simpson and Koyama 1998) have been steadfast in retaining *Abildgaardia* as a section of the genus *Fimbristylis*, while maintaining *Bulbostylis* as a separate genus. Kral (1971, 2002), Goetghebeur and Coudijzer (1984, 1985); Goetghebeur (1986, 1998), Greuter et al. (1993), Gordon-Gray (1995), and Bruhl (1995) all accepted *Abildgaardia* as a genus, equal in rank with *Bulbostylis* and *Fimbristylis*. The widespread *Abildgaardia ovata*, plus the African species *A. triflora*, and *A. hygrophila* (Goetghebeur and Coudijzer 1984), and the American species *A. mexicana*, *A. baeothryon* and *A. papillosa* (Kral and Strong 1999) are currently assigned to the genus *Abildgaardia*. The Australian species *Fimbristylis macrantha*, *F. oxystachya*, and *F. pachyptera*, in section *Abildgaardia* of *Fimbristylis*, were provisionally placed in the reinstated genus *Abildgaardia* (Goetghebeur 1986), however, the new combinations were never validly published. *Fimbristylis squarrulosa* (section *Abildgaardia*) was not included in Goetghebeur's study, but is accepted in the

Australian Plant Name Index (http://www.anbg.gov.au/cpbr/databases/apni.html) as *Abildgaardia schoenoides* R.Br.

Crosslandia W.Fitzg. was described as a monotypic genus (Fitzgerald 1918) from the Kimberley region in Western Australia, however, Goetghebeur (1986) proposed a second species be described, primarily on the variation of the inflorescence. The provisional name *C. anthelata* was never validly published.

Nemum Desv. ex Ham. is another relatively old genus raised from *Scirpus* and has had varied acceptance. Kunth (1837) and Steudal (1855) included *Nemum* in their classifications, however, Clarke (1908) retained the species in *Scirpus* section *Nemum* (*S. spadiceus* Boeck. and *S angolensis* C.B.Clarke). Raynal (07-1973) reinstated *Nemum* to generic status as did Lye (11-1973), when he included the genus in the tribe Abildgaardieae. *Nemum* is currently generally accepted as a genus (Greuter et al. 1993, Simpson 1993; World Checklist of Monocotyledons 2004).

Nelmesia Van der Veken is a monotypic genus (Van der Veken 1955), with *N. melanostachya* known from a single collection in Africa (Goetghebeur 1986).

Tylocarya Nelmes, described from Thailand, is also a monotypic genus, *Tylocarya cylindrostachya* Nelmes (Nelmes 1949), but was recombined as a species of *Fimbristylis* by Kern as *F. nelmesii* J.Kern (Kern 1958). *Tylocarya* has had mixed acceptance as a genus (Bruhl 1995), however, the most supported current view is as a species of *Fimbristylis* (Simpson 1993; Goetghebeur 1998).

Embryo morphology and Anatomy

In Cyperaceae, the application of characters from embryo morphology and the type of photosynthetic pathway in systematic studies has been well documented, especially when the use of plant morphology has its limitations.

Van der Veken (1965) sampled embryos from 342 species across 16 genera, and found six different embryo types within *Scirpus* s.l., three of those types correlate specifically with *Fimbristylis*, *Bulbostylis* and *Abildgaardia*. Investigating the embryo type has proved useful in the placement of difficult taxa. For example, species of the now accepted *Bulbostylis hispidula* group were controversially placed in *Fimbristylis*, until it was found that sampled material had a Bulbostylis-type embryo. This embryo type determined the placement of members of the *B. hispidula* group in *Bulbostylis*.

Metcalfe (1971) sampled the vegetative anatomy of the family extensively and related variation in leaf, culm and root anatomy to the classification popular at the time. The variation in anatomy, specifically the arrangement of tissues in vascular bundles, has since been associated with the photosynthetic pathway, and has evolved many times in the family (Bruhl et al. 1987; Bruhl and Perry 1995; Soros and Bruhl 2000; Soros and Dengler 2001). In addition to the C_3 photosynthetic type of anatomy there are four C_4 anatomical types that are clearly associated with specific generic types (Figure 1.4). These types are the: rhynchosporoid, chlorocyperoid, fimbristyloid and eleocharoid types (Bruhl et al. 1987; Soros and Dengler 2001). Some studies have related the type of anatomy to the classification of taxa at the broad tribal level (Bruhl 1995; Soros and Bruhl 2000), or at lower ranks e.g. the chlorocyperoid photosynthetic pathway in *Cyperus* sect. *Pinnati* (Wilson 1991).

General aim

The general aim of this thesis is to test monophyly of and within the tribe Abildgaardieae, using characters derived from plant morphology, embryo morphology and vegetative anatomy.

To address the main aim of the thesis it has been necessary to define the limits and assess monophyly of genera (specifically *Abildgaardia*, *Crosslandia* and to a lesser extent, Australian *Bulbostylis* and *Fimbristylis*) within the tribe Abildgaardieae prior to the full tribal analysis that tests monophyly. The main aim is divided into minor objectives that are addressed as separate chapters to incorporate species level work where necessary (i.e. *Crosslandia*, *Abildgaardia* and *Bulbostylis*). The minor objectives are given in the introductions of the individual chapters.

Thesis outline

The history of the tribe and taxonomic problem areas are outlined above (Chapter 1), followed by general materials and methods (Chapter 2). In Chapter 3, the limits of the genus *Crosslandia* are defined with the number of species within *Crosslandia* assessed. A cladistic analysis was used to determine if the species form a monophyletic group. The limits of the disputed genus or section *Abildgaardia* are defined in Chapter 4, by determining the number of species that combine to form the group, and the limits assessed across their global distribution. Cladistic analysis was used to test monophyly of all the species of *Abildgaardia* defined in the phenetic study and of selected samples of *Fimbristylis*. The number of species assigned to *Bulbostylis* is large and in need of revision globally. As only a small number of species occur in Australia, the limits of the Australian species are assessed and

compared globally in Chapter 5. The final analysis in Chapter 6 brings together data from Chapters 3–5, and data for the remaining genera *Nemum*, *Nelmesia* and *Tylocarya* (= *Fimbristylis nelmesii*), to complete the analysis of the tribal group. Finally, the general conclusion attempts to synthesise the findings of all the data chapters and suggest areas where further research is needed.

Chapter 2

General materials and methods

Plant material

Data for phenetic and cladistic studies were obtained primarily from dried herbarium material. Field collections that supplemented loan material were focused in the regions of the East Kimberley in Western Australia, Kakadu National Park in the Northern Territory, far north Queensland, and to a lesser extent southeast Queensland and northeast New South Wales. Herbarium vouchers for all material collected are lodged with the N.C.W. Beadle Herbarium (NE) at the University of New England. Duplicate specimens will be distributed to other herbaria after manuscripts have been prepared for publication.

Many field collections included fixed material for my anatomical studies and silica-gel samples for other projects (e.g. Ghamkhar et al. 2005, in press). Fixed specimens were placed in Formalin–Propiono–Alcohol (FPA), constituting proportions of Formalin, Propionic acid, and 70% ethanol (5:5:90), immediately upon collection. Specimens were held in fixative for at least one week to ensure adequate penetration of the tissues. In the laboratory, fixative was replaced with 70% ethanol (with 1% glycerol added) to facilitate long-term storage and safe handling.

Specimen loans were provided by Australian herbaria BRI, CANB, DNA, MBA, MEL, NSW, PERTH, QRS, and overseas herbaria EA, K, L, MO, NU, P, PRE (Holmgren et al. 1990).

Taxa Studied

Genera assigned to the tribe Abildgaardieae Lye (see Lye 1973; Goetghebeur 1986, 1998; Bruhl 1995) were sampled to test monophyly of the tribe. Representatives from *Nemum*, *Nelmesia* and *Tylocarya* (= *Fimbristylis nelmesii*), all non-Australian genera, were only included in the tribal cladistic analysis. Generic limits (*Abildgaardia–Fimbristylis*; *Crosslandia*) and contentious species limits (i.e. variation in *Crosslandia setifolia*, *Bulbostylis pyriformis–B. hispidula* complex, and the *B. densa* complex) required selection of particular taxa with the focus primarily on Australian material. In addition, putative new species (i.e. *Bulbostylis* sp. aff. *barbata*, *Fimbristylis* sp. aff. *odontocarpa*, *Abildgaardia* sp. aff. *schoenoides*, *A.* sp. aff. *pachyptera*), possible new combinations (i.e. *Abildgaardia vaginata*, *Fimbristylis spiralis*, *F. macrantha*, *F. oxystachya*, *F. pachyptera*), or taxa to be reinstated (*A. schoenoides*) were included in analyses to test species limits.

The large number of species that are assigned to *Bulbostylis* and *Fimbristylis* prevented a comprehensive assessment of either genera. The study of species for *Bulbostylis* was restricted to those species that occur in Australia, including unnamed Australian collections, and some representative overseas species (i.e. 15 taxa, 10 occur in Australia). Nine representative species of *Fimbristylis* were selected from five of Kern's (1974) sections (excluding section *Abildgaardia*); sections *Fimbristylis*, *Fuscae*, *Leptocladae*, *Tenerae*, and *Trichelostylis*. *Fimbristylis depauperata* R.Br. (= *F. dichotoma* (L.) Vahl subsp. *depauperata* (R.Br.) J.Kern) was selected to represent the TYPE section of *Fimbristylis*, *Fimbristylis* section *Fimbristylis* for cladistic analysis. Members from *Fimbristylis* section *Fuscae* were selected due to the history where species from both *Fimbristylis* sections *Abildgaardia* and *Fuscae* were sometimes combined in the one section due to the

distichous arrangement of the glumes. The remainder of the species (*F. schultzii*, *F. blakei*, *F.* sp. L, *F. furva*, and *F. microcarya*) were included in an attempt to capture some of the sectional variation not yet sampled.

Specimens were identified using regional floras (Rye 1992; Wilson 1993) and keys (Sharpe 1986; Latz 1990). Regional keys have obvious shortcomings when species occur outside the regional range. To offset this problem and to ensure correct assignment, i.e. when species limits were being assessed, Australian material was compared to TYPE specimens where possible.

An extensive and all-inclusive global assessment of the tribe Abildgaardieae was not possible due to the large number of species that fall within the current circumscription (Chapter 1). In an attempt to accommodate the global shortcomings of a restrictive national study, overseas specimens were included in analyses when the range for a species was extended (i.e. *Bulbostylis densa*), cosmopolitan (i.e. *Abildgaardia ovata*, *Bulbostylis barbata*,) or previously unreported in Australia (i.e. *Bulbostylis humilis*). Keys developed for specific countries or regions provided a basis for specimen identification of taxa on loan from international herbaria (Kral 1971; Kern 1974; Haines and Lye 1983; Adams 1994; Gordon-Gray 1995).

Not all taxa used in the cladistic analyses were included in the phenetic studies. Lists for specific taxa used in phenetic and cladistic analyses can be found in the relevant sections of the chapters dealing with generic groups, i.e. *Crosslandia* (Chapter 3), *Abildgaardia* (Chapter 4), *Bulbostylis* (Chapter 5), *Nemum*, *Nelmesia*, and *Tylocarya* (Chapter 6). The complete list of sampled taxa and specimens can be found in Appendix 1. A list of the more than 4400 loan specimens plus specimens

personally inspected at herbaria (BRI, CANB, DNA, MEL, MBA, NSW, NE, QRS, and PERTH) during the course of the study may be obtained upon request.

Sampling

Dried herbarium samples provided the basis for scoring morphological characters. To assess variation in plant morphology, ten specimens (where available) were selected to cover the geographical range of species when species had well-defined boundaries. Taxa with uncertain limits required a larger sample size to encompass the greater variation and thus define the entities (e.g. *Crosslandia*). Duplicate sheets were examined where possible to ensure a more thorough sampling, especially for species that exhibit polymorphism. Whenever possible, the collections chosen were whole plants that were in good general condition, with mature fruit, plus multiple culms and leaf blades (if present). If an individual specimen had all the necessary material to score all states, only that individual was sampled (i.e. single collected specimen on a herbarium sheet). Sometimes scoring from other individuals collected from the same site was needed – either from the same sheet or from duplicate specimens. When multiple individuals were scored, care was taken to ensure that all were the same taxon, as some sheets contained mixed taxa. If a duplicate sheet was required for sampling, the herbarium code and sheet information were included in the table of taxa (Appendix 1).

Phenetic studies

Exploratory pattern analyses were undertaken using the program PATN v 3.6 (Belbin 1993). The more recent PATN (for Windows) v 3.03 (2004) is limiting in that only a single similarity coefficient is allowed, preventing the use of merged data

sets and character weighting. In addition, Principal Components Analysis is not available, further limiting pattern exploration.

Taxa for phenetic analyses

Phenetic analyses were used to determine species boundaries. During assessment of species for a given genus, Operative Taxonomic Units (OTUs) forming well-defined species were selected as reference taxa. For example, in the analyses of species for the *Crosslandia* group, specimens from *Abildgaardia* or *Bulbostylis* that could not be assigned to a currently accepted species were excluded from the *Crosslandia* analyses. Species lists for sampled specimens used in phenetic analyses are found in the relevant data chapters 3, 4, and 5 (see also Appendix 1 for specimen information).

Characters for phenetic analyses

Phenetic studies were based on morphological data. Both qualitative and quantitative characters were used in phenetic analyses. Qualitative characters consisted of both binary and multistate forms. Binary characters were scored as either present or absent (0/1) for the two states (attributes) of the character. Multistate characters, where more than two states were observed, were converted into individual presence/absence type data (i.e. binary form). Preparing multistate characters in this manner prevented loss of information when a character had more than four states (Crisp and Weston 1993). Binary characters were given the weight value of 1, while multistate attributes were weighted according to the number of states (columns) per character, i.e. each character was given a weight value of 1. For example, a character with four states would have each attribute (column) assigned a

weight of 0.25. Attribute (column) weight values were updated with each phenetic analysis following the addition or exclusion of OTUs. Removing OTUs to reanalyse subsets usually created invariant columns that were removed from the data set, altering the number of states per character and therefore the weight for each attribute column.

Polymorphic characters were restricted in the phenetic analyses; however, when included these states were presented in separate columns of binary form (0/1) and given a weight of 0.5 for each of the two attribute columns formed. Two examples of polymorphic characters were variation in stamen number (e.g. in some species of *Bulbostylis*) and the presence or absence of prophyllar buds within an inflorescence–synflorescence (i.e. secondary floral growth from the axil of the prophyll as seen in some species of *Bulbostylis*). Most of the species under study exhibit regularity in the number of stamens per floret and the presence/absence of prophyllar bud growth; however, the observed variation required a realistic assessment in these otherwise stable characters.

Quantitative characters consisted of measurement data that, where available, were the mean of at least five values. This sampling strategy was applied to prevent destruction of specimens, especially in species with few mature spikelets or ripe fruits. Loose material from herbarium sheets was scored when available in preference to removing nuts and glumes *in situ*. TYPE specimens were rarely included in analyses and when it was necessary (e.g. *Fimbristylis odontocarpa* S.T.Blake), only loose nuts and glumes were scored from the sheets. All measurements were recorded in millimetres for consistency. Some data were converted into ratio coefficients or proportions, i.e. glume (floral bract) 1/(width/length), 1/(nut stipe length/ nut length) to provide additional information to

individual length or width measurements. Converted values were kept to a minimum, and so do not dominate the analyses. Lists of the attributes used in phenetic analyses can be found in the relevant chapters.

Analysis preparation

Data were stored within Microsoft Office Excel and files saved as comma delimited files (*.csv) for direct use within PATN.

To accommodate the nature of mixed data sets, various types of data manipulation and association measures were tested for robustness of the results. Non-weighted characters were analysed and compared to separate analyses where characters were weighted. After setting parameters and prior to producing association matrices, characters were weighted using the data transformation and standardisation module (TRND option 10, under 'Manipulation').

The application of various coefficients to different types of data matrices produced association matrices. Gower's General Similarity coefficient, known to be useful for binary, multistate and quantitative data (Stuessy 1990), was used here for data matrices combining quantitative and qualitative data, and quantitative matrices used in 'merged' analyses. Qualitative matrices used for 'merged' analyses were subjected to the Kulcynski coefficient which, although not generally used in phenetic analyses, is good for presence/absence data where polarity is expected (Crisp and Weston 1993); 0/0 matches are not considered important (Belbin 1993).

Data matrices analysed were grouped as:

1. whole data set, columns not weighted (cols = 1); ASO = Gower Similarity Coefficient;

2. whole data set, columns weighted (chars = 1); ASO = Gower Similarity Coefficient;

3. quantitative data set, columns not weighted (cols = 1); ASO = Gower Similarity Coefficient;

4. qualitative data set, columns not weighted (cols = 1); ASO = Kulcysnki Coefficient; and

5. qualitative data set, columns weighted (chars = 1); ASO = Kulcynski Coefficient.

Data sets 3, 4 and 3, 5 were merged within PATN (under 'Manipulation', Left-right & up-down merging). Merging data sets containing different types of data and using different association measures is more reliable than applying different association measures to one data set (Belbin 1993). The two association matrices were then standardised and added together in the association transforming and standardising (TRNA) module, located under 'Manipulation' option 9.

The lowest stress values (in the ordination module) were obtained from merged data sets, however, weighted quantitative and qualitative data (option 2 using the Gower similarity coefficient) resulted in the same group formation of OTUs. Although it was necessary initially to explore differences between treatment of data and association coefficients, there was no great advantage in the time taken. Therefore, option 2 (whole data set, columns weighted i.e. characters =1, using the Gower metric similarity coefficient) was applied to all the final phenetic analyses found in chapters 3, 4, and 5.

Association matrices were then subjected to ordination, cluster and network analyses within PATN.

Analyses

Phenograms from cluster analyses are generally useful as rough guides to taxonomic structure based on similarity/dissimilarity measures, with more accurate and useful results obtained from ordination approaches based on multidimensional scaling (Stuessy 1990). Phenograms portray close 'relationships' rather than the distant 'relationships' of ordination diagrams (Stuessy 1990) based purely on the similarity of taxa and are best interpreted with no evolutionary foundation.

Ordination, cluster, and network analyses were applied to association matrices. Combining these three techniques provides a thorough assessment for similarity/dissimilarity of taxa (Belbin 1993).

Ordination

Ordination reveals 'real' groups based on the underlying pattern in the data, whereas in cluster analysis taxa are forced to form groups. Ordination plots have been used as the basis for group formation (usually species), as emphasis is on the greater the distance between clusters the greater their dissimilarity. Cluster and network analyses were compared to ordinations to assess robustness of the data.

Both Multidimensional Scaling (MDS) and Principal Components Analysis (PCA) were applied to association matrices. Gower's coefficient is an interval measure and not appropriate for Semi Strong Hybrid (SSH) multidimensional scaling as SSH techniques apply to ratio data only. Therefore, care must be taken to select the 'interval' and not 'ratio' option when applying the ordination procedure. Crisp and

Weston (1993) performed ordinations using both interval and ratio strategies to avoid any loss of information, a practice followed here. However, only scatter plots using the 'interval' strategies were presented in the results.

Gower's coefficient requires that association values be unimodal if the coefficient is to be a valid measure for the data (Belbin 1993). In addition, an appropriate ratio-ordinal cut value within the SSH module must be determined by the type of data analysed. For example, ordinal data should have a cut value less than the minimum value within the association matrix, and interval data must have a cut value greater than the highest association value (Belbin 1993). Selecting 'HIST' from the analyses module (option 12) enables assessment of modality and maximum/minimum association values. When combined quantitative and qualitative data showed bimodal histograms of association values, the data sets were rerun as separate matrices and merged. The resultant histograms of merged association values were usually unimodal. Principal Components Analysis (PCA) using Gower's similarity coefficient was compared with MDS of combined data, and merged quantitative and qualitative matrices that were suitable only for use with MDS type ordinations.

Ordinations were run in two and three dimensions to achieve the lowest stress values, an indication of the level of best fit of the data to the number of axes used. Those that were 3-dimensional usually achieved acceptable recommended stress values (around 0.1), while higher stress values (0.15-0.17) were frequent in 2-dimensions. Often the groups formed were similar (robust) for the various dimensions (2 or 3) and whether interval or ratio method was applied to the ordination. All 3-dimensional ordinations were observed using Statistica version 5.1B (Statsoft 1996); this program allows for rotation of the points around the axes and visual conformation of the groups formed. For ease of presentation, scatter plots

from 2-dimensional ordinations (often with borderline stress values) were presented in the results sections when grouping was similar to the more robust 3-dimensional ordinations. When 2-dimensional groups did not clearly represent the points in 3-dimensional space, group borders were drawn onto the xy scatter plot to reflect the groups observed in the xyz plot. The stress values for both 2-dimensional and 3-dimensional ordinations are given for comparison in the results section for each data chapter. Two-dimensional ordinations were plotted using Microsoft® Excel 2000 (Microsoft 1999).

Table 2.1 Ordination stress values. Kruskal's goodness of fit associates stress levels to how well the data fit the number of axes used in any given ordination. From Belbin (1993 p:133 of the 'Technical Reference').

Stress value	Goodness of fit
> 0.2	poor
0.15 > 0.2	be cautious
0.1 > 0.15	fair (wish it were better)
0.05 > 0.1	satisfactory
< 0.05	impressive
0	perfect!

Classification

Clustering strategies may provide differing results depending on both the data and strategy selected. Various strategies were performed and compared with each other and the ordination scatter plot for each data set.

Flexible unweighted pair group arithmetic averaging (UPGMA) or flexible weighted pair group arithmetic averaging (WPGMA) provided phenograms that most closely resembled the groups found in ordination space. Equal weight is given to objects (not groups) in the UPGMA strategy so that during the fusion process groups are weighted proportionally to the number of objects contained within each group. In

contrast, groups are weighted equally regardless of the number of objects in the flexible WPGMA fusion strategy (Belbin 1993). Assigning beta values allows for attribute space distortion, either contraction or dilation depending on the assigned sign. A beta value of –0.1 was used for UPGMA or WPGMA strategies, as negative β is reported to aid known partition recovery (Belbin 1993), although changing the β value did not alter OTU partitioning in these analyses. Both strategies frequently produced similar groups for OTUs with minor variations, however, the strategy closest to the groups formed in the ordination scatter plot was selected for presentation.

Network analysis

Minimum spanning trees (MST) from the network module accurately represent close objects/neighbours, with uncertainty proportional to the increase in object separation. Minimum spanning trees compliment the ordination where the greater the distance between objects the lower the affinity of those objects. When used in conjunction with the ordination, MST connections can confirm or refute close 'relationships' (Belbin 1993), i.e. of similar/dissimilar groups. Minimum spanning trees were not presented for the larger data sets due to their complexity, although they were examined and compared to the ordination scatter plot and phenogram for general OTU group robustness.

Evaluation

Attributes with the greatest influence for a given ordination were evaluated using the principal axis correlation (PCC) module by selecting 'SCAT' from the ordination menu, under 'evaluation'. Attributes are fitted to an ordination space using multiple-

linear regression (Belbin 1993). Those character states (attributes) with 70-80% (depending on the number of attributes) or greater influence on the ordination were plotted to link with the matching scatter plot.

Character states influencing cluster analysis grouping in the phenogram were firstly extrapolated using group definition (GDEF) and then box and whisper (GSTA) module applied (neither presented). The latter uses the non-parametric Kruskal-Wallis statistic for quantitative (continuous) data (option 1) and constancy percentages for nominal (presence/absence) data (option 2). Operative Taxonomic Units could be forced into larger groups using GDEF to observe major group boundaries and the attributes forcing the OTUs into the groups investigated. The attributes found in the evaluation module for classification were the same as those associated with the ordination. Therefore, only results from the PCC are presented.

For any given data set, all taxa and characters were included in the initial analyses. Subsets of the first analysis were then rerun for unresolved groups, or finer analysis of the larger groups (i.e. generic groups formed) to assess species grouping. Invariant columns (character states) were removed prior to reanalysing. Characters that may have had undue influence in an analysis (i.e. presence of basal spikelets or amphicarpy) were removed and rerun to test the robustness of the analyses.

Ordination scatter plots and cluster analysis phenograms provided groups used as 'terminal taxa' within cladistic analyses.

Cladistic studies

Analysis preparation

For cladistic analyses, data were collated from the multiple OTUs scored in phenetic analyses so that each defined group of OTUs was converted into data for each terminal taxon.

Ingroup

Ingroup taxa were based on the tribe Abildgaardieae as outlined by Goetghebeur (1986) and Bruhl (1995). All genera were included in the tribal analysis (Chapter 6). When species level assessment was required, subsets of genera and species were analysed initially as smaller groups (Chapters 3, 4 and 5).

Outgroup

Data were polarised using the outgroup method (Maddison and Maddison 1992). Some members from the provisional tribe 'Arthrostylideae' (Goetghebeur 1986), i.e. *Arthrostylis* (Queensland), *Actinoschoenus* (Northern Territory), and *Trachystylis* (Queensland), plus species from *Schoenoplectus* (Reich.) Palla and *Schoenoplectiella* Lye (tribe Scirpeae or Fuireneae) (Appendix 1) were selected as outgroup taxa based on the sister relationships with the Abildgaardieae (Bruhl 1995). As taxa from the 'Arthrostylideae' have been placed variously within genera of the ingroup, (Clarke 1908; Kern 1974; Latz 1990) species of *Schoenoplectus* provided some distinct character differences to polarise the data.

Characters

Character states used in the phenetic analyses were converted into data suitable for cladistic study within the DELTA Editor v 1.04 (Dallwitz et al. 1999). Quantitave characters were included to provide additional information for phylogenetic assessment (Poe and Wiens 2000), especially at the species level (González-Elizondo et al. 1997). Quantitative characters were gap coded using a line graph to find gaps in the OTU mean data from the phenetic analysis to define the number of states, which were scored as ordered multistate characters within the DELTA data set. All other characters were scored as unordered. Spikelet width measurements were omitted from cladistic analysis and used for descriptive purposes only (not included), as gaps within the measurement data were not clear-cut. Although quantitative characters may be polymorphic, the potential for added information has been documented (Poe and Wiens 2000; Wiens 2000) and were therefore included in the present study.

Polymorphism

Taxa, mostly species, which exhibit a variable range across character states for a particular character, occur frequently within the study group, particularly in *Bulbostylis* and *Fimbristylis*. These polymorphic characters present problems at a cladistic level where discrete states of homologous characters are required as a basic assumption in a phylogeny for any given group of taxa (Hennig 1979). There are numerous ways to code for polymorphism (Wiens 2000), all producing different tree topologies, however, excluding polymorphic characters and employing only fixed states provided the poorest tree topology of all methods when tested by Wiens. Breaking down the units of the species into separate samples is the best way to show polymorphism within a species, as polymorphism represented by one collated species

representative (n=1) cannot be detected in an analysis (Wiens 2000). In PAUP* (Swofford 2001), only one state of a polymorphic character is specifically assigned in an analysis, thus affecting parsimony, and the resultant tree topologies. Data for species in this study were presented as a single line within the DELTA data set rather than multiple individual samples due to the large number of species being analysed and time constraints. Polymorphic characters were included in this study despite loss of some information during analyses, as many taxa were observed to be constant for some of these characters (e.g. inflorescence–synflorescence type; presence or absence of hairs on culms, leaf blades, or glumes; style indumentum; etc.).

Characters were given equal weight, as is the default option in PAUP* (Swofford 2001). Although a larger number of character states may give undue weight to a character, it was not possible to scale character states to provide differential weights, as the many odd numbers of states prevented real scale values (whole numbers) to be produced for use within PAUP*.

Characters from embryo morphology plus leaf blade and culm anatomy were added to the data set at the terminal taxon stage (see Appendix 2).

Leaf blade and culm anatomy

Anatomical characters were included in cladistic analyses as Metcalfe (1969, 1971) showed that the anatomical pattern found within the Cyperaceae on the whole might reflect generic relationships and provide an indication of tribal grouping. More recently, vascular bundle types, specifically from leaf blades, have been described and associated with specific photosynthetic pathways (generally C_3 and C_4) (Bruhl et al. 1987; Bruhl and Perry 1995; Soros and Bruhl 2000; Soros and Dengler 2001) and related to genera. In addition to the ancestral C_3-type of anatomy, the Fimbristylis-

type was the only one of the four types of Kranz anatomy (C_4) described across Cyperaceae that applied to taxa within this study (Figure 2.1). Photosynthetic pathways were scored from leaf blades, or culms when plants were leafless. Although there are differences between leaf blade and culm anatomy, the type of photosynthetic pathway could still be assigned easily.

Anatomical structure may vary with environmental influences, or be useful only for diagnostic purposes (Metcalfe 1971). However, general leaf blade and culm shape, vascular bundle arrangement, and photosynthetic pathway are usually constant. Following Metcalfe's work, other anatomical features, such as shape of sclerenchyma and mesophyll chlorenchyma, number of vascular bundles, and the arrangement of vascular bundles and sclerenchyma within the organs, were included as potentially useful traits.

Culm and leaf blade anatomy were sampled from the mid-third area for three specimens from each species (where possible). Fixed material was used when available, or rehydrated 'green' herbarium material provided satisfactory sections in most cases. Selected organ segments from dried herbarium sheets were placed in a beaker of cold water with a few drops of detergent, heated until boiling point was reached, then removed from the heat and allowed to cool. These segments were then ready to cut by hand using a double-sided razor blade, or were stored in 70% ethanol until needed.

Hand cut sections were stained initially with Bismarck Brown, however, better tissue definition was obtained using the Astra Blue–Basic Fuchsin double-staining technique of Kraus et al. (1998), omitting bleaching, acetic acid pre-stain rinse and picric acid differentiation (picric acid has explosive properties when dry). Good

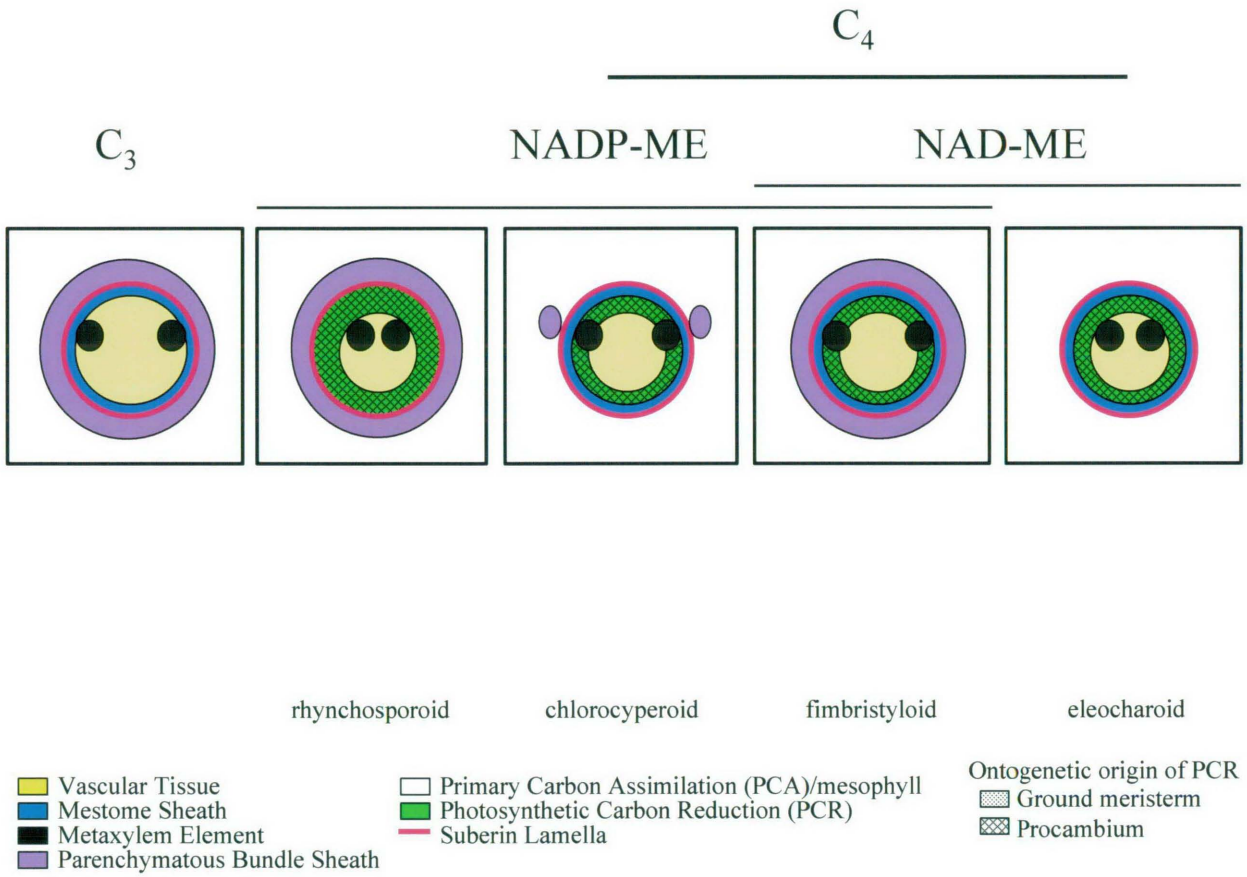


Figure 2.1 Schematic representation of the 'type' of variation in the photosynthetic pathway that correlates with the arrangement of tissues within vascular bundles in Cyperaceae (from Soros & Bruhl 2000; Soros & Dengler 2001). Photosynthetic pathways that apply to this study are C_3 and C_4 fimbristyloid.

tissue differentiation of sections was obtained without these extra steps. Sections were then mounted for microscopic examination as semi-permanent slides using clear glycerine jelly (see Appendix 3 for recipe). Once familiar with the tissues, observations could be made without the use of stains and hand cut sections were mounted directly into 50% glycerol for speedier microscopic examination. These sections could be made permanent at a later date.

Root anatomy was initially examined for useful characters, and although there appeared to be potential for some characters such as the endodermis shape and tissue layers, time constraints prevented a comprehensive assessment. These root characters were therefore eliminated from the analyses.

Characters taken from anatomical studies were used in cladistic and not phenetic analyses.

Embryo morphology

Micromorphology of the mature embryo has been widely used since Van der Veken's work in 1965 as an aid to assigning taxa to genera, or at least, to exclude taxa that do not fit within a given embryo–type group (Gordon-Gray 1971; Kern 1974; Goetghebeur 1986). Van der Veken (1965) sampled embryos across the Cyperaceae, describing six main embryo types. Although the same embryo type can be be found among different genera, Van der Veken noted that different embryo types do not occur within a given genus.

Embryological features, such as development, general size and shape, plus position of the primordial root and shoot, are known to be relatively stable and therefore taxonomically useful (Maheshwari 1964; Davis 1966; Johri 1992).

Sampling one or two embryos from a specimen can reflect the embryo morphology of the whole plant, especially when compared with specimens of the same species. Selecting three specimens (where possible) across the range of each defined species group increased the reliability of the limited sample size (when compared to morphological characters). For consistency, embryo morphology was sampled for the same taxa used in the anatomical and scanning electron microscopy studies. Characters from embryo morphology were included in the cladistic studies only.

Embryos were selected from wet (fixed material) or dried material (rehydrated as in anatomical work) if the former was not available. Fruits were carefully dissected to release the embryo from the proximal portion of the ovary.

To observe primordial root and shoot orientation for scoring, embryos were cleared. Chlorolactophenol was used initially as the clearing medium. Phenol is included as a preservative; however, this was later omitted, as it has known carcinogenic properties. In addition to increasing the refractive index of the medium, chloral hydrate also has preservative properties; the need for phenol as a preservative was redundant. The Chlorolactoglycerol (glycerol 10 mL, distilled water 10 mL, lactic acid 10 mL, chloral hydrate 1.6 g) was used to clear the remainder of the embryos.

Whole embryos were placed directly into clearing fluid in a single cavity slide or double cavity slide, if small enough, and a no.1 coverslip placed over the cavity. It was necessary to make deep well slides to accommodate the larger embryos for species from genera such as *Abildgaardia* and thus allow manipulation of the embryo during microscopy. Deeper well slides were made by affixing five 22x22 mm (no. 1) coverslips atop each other with Eukitt®, so that the coverslip piles on each side

partly covered the cavity of a single cavity slide. This created a smaller, deeper area in which to place the larger-sized embryo. Prepared slides were left to dry for two days in a 40°C oven prior to adding the clearing medium, embryo, and final coverslip. It was often necessary to add additional clearing fluid from the front or back gaps to ensure that bubbles were removed from the cavity area before microscopy.

Frequently, embryos would not clear and remained clouded, obscuring the inner areas of the embryo (i.e. primordial leaf blades and vascular tissue). Neither bleaching the embryos prior to clearing, or lengthening the time in the clearing medium was helpful. For this reason, some embryos were embedded using the paraffin wax method and sectioned using a rotary microtome. Wax embedded serial sections, which were prepared according to Johansen (1940), allowed me to become familiar with the embryo structure prior to assessing cleared material. These sections were stained with Safranin-O and Fast Green and made permanent by mounting in Eukitt®.

Cleared embryos were best observed soon after the clearing medium was added. The longer the embryos were kept in the clearing fluid, the less visible were the inner areas as starch bodies burst and released their 'oily' contents, obscuring the young organs. Extra care was taken if embryos were kept in the clearing fluid for extended periods as the embryo tissues became very soft and were easily damaged when moved under the coverslip. It was difficult to assess the presence of second or third primordial leaves, especially in the smallest embryos where the tissues contain dense cytoplasm. There was inconsistency for scoring second and/or third leaf data, therefore these data were omitted. Experimenting further with various pre-treatments

and clearing media is necessary to obtain greater definition and detail in embryos across the range of taxa.

Embryos were scored according to the general embryo types outlined in Haines and Lye (1983) (Figure 2.2). Once familiar with the embryo structure, embryos were scored using a compound microscope with the stage diaphram shut down to increase contrast.

Scanning electron microscopy

Nuts may exhibit surface patterning with: pits, tubercules, ridges and undulations; trichomes of various complexity; spines; or sometimes secondary or tertiary sculpturing (Lye 2000). Variation of the epidermal sculpturing has been a useful taxonomic character at the species level (Haines and Lye 1983; Gordon-Gray 1995), and conformity of cellular shape and arrangement has had some use at the generic level (Goetghebeur and Coudijzer 1984).

Nuts selected from dried herbarium specimens for examination of anatomy and embryo morphology were prepared for scanning electron microscopy (SEM) to provide comparable detail of the nut epidermis (see Appendix 1 for sampled specimens). When fruit availability was limited (i.e. from international loans, TYPE specimens – with prior permission, or when material was very scarce), some nuts used for SEM were rehydrated and the embryo dissected for embryo morphology. The success of rehydration depended on the condition of the nut prior to gold sputter-coating for SEM.

One to three nuts (depending on size and availability) were affixed to stubs using double-sided tape and sputter-coated with gold for four minutes in the Polaron gold

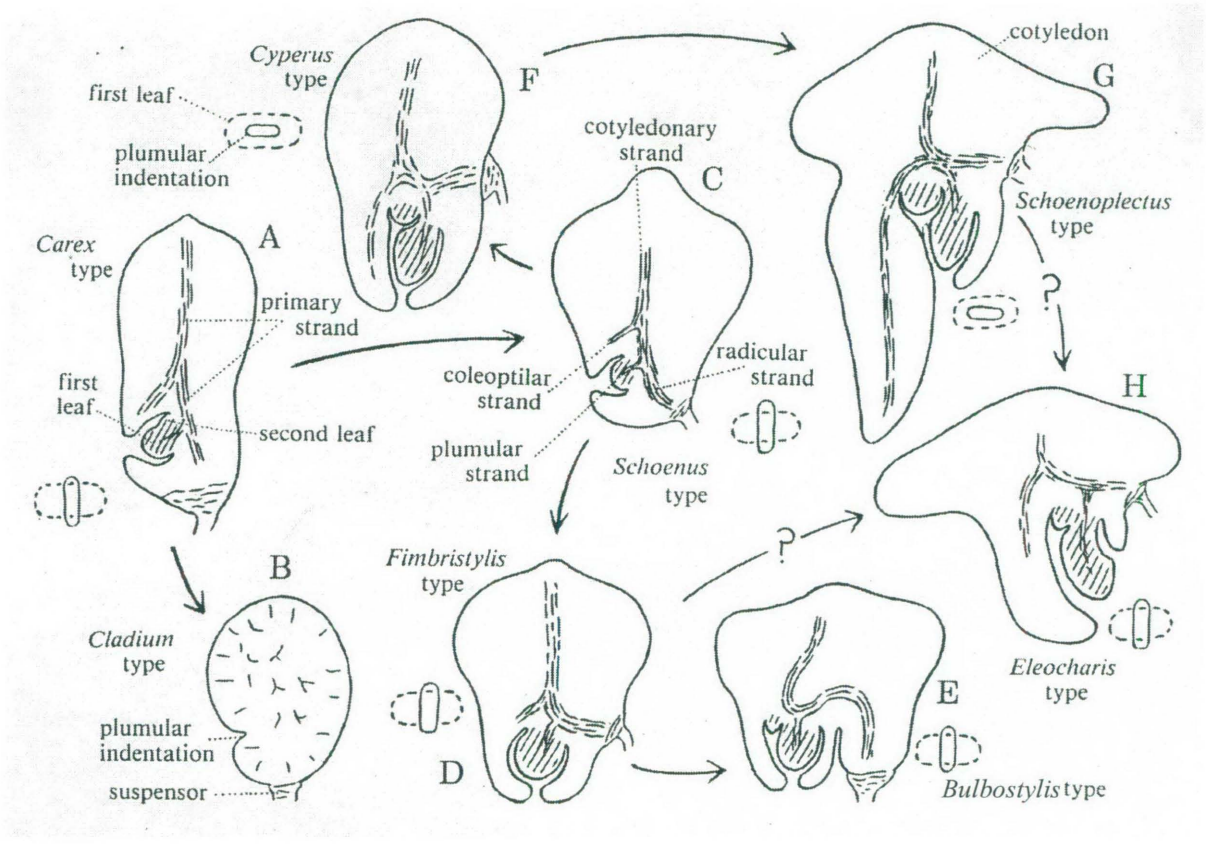


Figure 2.2 General embryo types of the Cyperaceae (adapted from Haines and Lye 1983 after Van der Veken 1965). The Fimbristylis-, Schoenus-, Bulbostylis-, Carex- and Schoenoplectus-types as pictured were found in this study, plus the Abildgaardia-type (not pictured), that shares the same primordial shoot and root orientation as Bulbostylis-type, but is distinctly larger, has a broader cotyledon, and a well-developed second primordial leaf.

sputter-coater E5100. Scanning electron microscopy was performed using a JEOL JSM-5800LV Scanning Microscope at 20 kV. Images were saved to disc in Tagged Image Format files (*.tif).

Some rachillas, styles and pollen were also assessed using SEM. Problems obtaining resolution at higher magnification prevented detail of style papillae and pollen features to be captured. Scoring for these characters was therefore abandoned.

Inflorescence–synflorescence homology

It is necessary to ensure that the characters scored are homologous if a given tree topology is to be accepted as the best fit for a given data set. Penet et al. (2005) reported that the monosulcate pollen grain found throughout the Asparagales was not homologous; they observed different developmental pathways during cytokinesis in the pollen tetrad of different genera. Scoring pollen simply as monosulcate would be misleading in a cladistic analysis. A similar situation occurs within the Cyperaceae synflorescence, where the commonly termed 'head' of sessile spikelets is not homologous across the species sampled. Differences in branching and arrangement of sessile spikelets that form a terminal 'head' was explored to ensure homology for inflorescence–synflorescence structure between scored taxa.

Fixed or rehydrated synflorescences were examined for all species that exhibit 'heads' of sessile spikelets. Lateral branches were determined by the position of the prophyll that was always present within the synflorescence in all but solitary spikelets where only the terminal florescence was present. Inflorescence–synflorescence terminology follows that of Weberling (1989) and more specifically,

the anthelodium and paniculodium synflorescence structures outlined by Vegetti (2003).

Analyses

A nexus file was generated from the DELTA data set from within DELTA to perform maximum parsimony analysis within PAUP* 4b10 (Swofford 2001). The large number of taxa to be analysed prevented exhaustive searches (for less than 11 taxa), or branch-and-bound analysis (up to 22 taxa) that guarantee to find all the shortest trees for a data set (Swofford 2001). Smaller data sets were analysed initially to explore speciation of some groups prior to assessing all members of the tribe for monophyly. However, the data set with the smallest number of taxa analysed was still too large for exhaustive or branch-and-bound methods.

Heuristic searches for optimal trees for simple and random addition-sequences of 10–2000 replicates using tree bisection-reconnection (TBR) branch-swapping, holding 1, 5, 10 or 100 trees at each replication were evaluated. Taxon order within DELTA data sets was also manually randomised (working with a newly saved file to avoid program bugs corrupting the original data set) and rerun using simple and random addition-sequences as outlined above to explore further optimal trees. Trees of the same tree length were recovered in all test analyses, the difference between analyses was that number of most parsimonious trees increased as the addition-sequence replication was increased. Also, manually randomising taxa within DELTA prior to generating the nexus file, increased the number of trees found within an analysis, compared to data sets where the taxa were not manually randomised. Heuristic searches were performed using 1000 random addition-sequence replicates, holding five trees at each step, on data sets where the taxa were manually

randomised, thus saving computational time while optimising retrieval of the number of shortest trees.

Evaluation

Bremer support values and bootstrap frequencies are included with the relevant cladogram of each analysis, as different aspects of group support are obtained by the two methods (Ramirez 2005).

Bootstrap analysis (Felsenstein 1985), where 'taxa are held constant and characters sampled with replacement to build a series of new data sets the same size as the original' (Swofford 1991 p: 62 PAUP 3.1 Users Manual), was used to provide statistical confidence to the relationship hypothesis provided by the heuristic search. Bootstrapping (100–1000 bootstrap replicates) was performed based on 10, 100 or 1000 random addition-sequence replicates to assess the variation of results. Bootstrap analysis using 10 addition-sequence replicates and 1000 bootstrap replicates provided comparable values of support against analysis using 1000 random addition-sequence replicates, but with much less computational time; Bootstrap values presented in Chapters 3, 4, 5, and 6 are based on 10 random addition-sequence replicates. Branch support was evaluated as >50<70% was weak support, >70<85% indicated moderate support, and >85% was strong support. Values that were <50% indicate no support and the values omitted. The majority-rule consensus constructed from the bootstrap replicates must be considered against the assumptions that the characters are independent and are representative of all the characters. On this basis, the non-statistical Bremer decay values were also used to assess the level of branch support.

Bremer support (Bremer 1994) or decay analysis, was obtained by expanding the tree length of the shortest trees(s) obtained from parsimony analysis by 1 step (s +1, s+2, s+3, s+4, etc.), while maintaining the settings for the original heuristic search. A tree from strict consensus determined the level of collapse or stability of branches, adding a value of 1 for each stable branch that remained after each analysis. Increasing the tree length by an extra step for each subsequent run and examining the strict consensus, provided the branch support values that were used in conjunction with bootstrap analysis. Usually five runs, increasing the tree length up to five steps, were sufficient to collapse most if not all of the main internal branches. As the number of taxa grew in subsequent analyses, the heuristic searches required too much computational time and were abandoned after the second or third extra step. Bremer support was not presented in Chapters 5 and 6 due to computational difficulties; Bootstrap support alone was presented on the cladograms.

MacClade v 3.08 (Maddison and Maddison 1992) was used to trace characters on trees and explore branching. TREEVIEW v 1.6.6 (Page 1996) allowed trees to be viewed and saved into a format appropriate for presentation within this thesis.

Photomicroscopy

Images from anatomical and embryo morphological studies were initially captured using a Nikon Coolpix 990 digital camera attached to a Leitz Larborlux S compound microscope. More recent image capture, including phase contrast microscopy of embryos, was performed using an Olympus BH-2 compound microscope with the Nikon Digital Sight camera control unit (DS-L1), DS-5M camera head attached.

Lower magnification images were obtained with the same Nikon Digital Sight system attached to the WILD Photomakroskop M400.

Chapter 3

Crosslandia W.Fitzg.: a phenetic and cladistic study

Introduction

This chapter focuses on the specific delimitation of *Crosslandia setifolia* W.Fitzg., the provisional *C. anthelata* Goetgh., *Fimbristylis spiralis* R.Br., and *Abildgaardia vaginata* R.Br. and their generic placement.

Fitzgerald's (1918) description of the monotypic genus *Crosslandia*, collected from Goody Goody Western Australia, is based on the capitate inflorescence structure, male aerial floret, and the presence of female spikelets at the base of the plant. *Crosslandia*, therefore, is defined by male aerial florets and female basal spikelets. Hutchinson (1959), using Engler's system of classification, placed *Crosslandia* in the tribe Sclerieae, based on the male spikelets in capitate inflorescences on long slender peduncles, and numerous female basal spikelets among the leaves. While Fitzgerald (1918: p123) had noted in his protologue that 'the plant bears a close resemblance to some of the capitate *Schoeni*', he demonstrated the affinity *Crosslandia setifolia* has with members of *Fimbristylis* through nut and style features, and highlighted the differences for generic separation. Anatomical studies undertaken by Metcalfe (1969, 1971) revealed that the leaf and culm anatomy of *Crosslandia setifolia* (*H. S. McKee 8432*; Blain, Northern Territory) were similar to species of *Fimbristylis*, especially in the arrangement of vascular bundle sheaths. *Crosslandia* was subsequently placed nearer *Fimbristylis* within the tribe Scirpeae (Hooper 1973). In Raynal's (1973) reconstruction of

phylogeny for the genera of the Cyperoideae *Crosslandia* was placed on the same lineage as *Eleocharis*, *Fimbristylis* (including *Abildgaardia*), *Nelmesia*, *Nemum* and *Bulbostylis*.

Goetghebeur (1986) observed that some *Crosslandia* material similar to the TYPE, i.e. having spikelets forming dense heads, had female florets distally within the spikelet. He also noted that the inflorescence structure in some specimens was 'anthelate' (having one spikelet per ray), with some of the 'anthelate' material bearing bisexual aerial florets rather than male florets within the spikelets. Specimens with the 'anthelate' inflorescence, as distinct from the 'capitate' sort, were given the provisional name *Crosslandia anthelata*. Goetghebeur, working on a broad study of Cyperaceae from Europe, had limited material of *Crosslandia* and was, therefore, not able to capture the full variation of the species at that time. The provisional name was never validated and a later treatment of Cyperaceae by Goetghebeur (1998) in 'The Families and Genera of Vascular Plants' did not mention the variation he had found previously.

Fimbristylis spiralis is the only member of the genus *Fimbristylis* bearing female basal spikelets (Latz 1990), and apart from the spirally arranged glumes, seems to share with *Crosslandia setifolia* similar morphology for general habit (although smaller), basal spikelet shape and nut characters. The original collection by Brown was made from the remote Arnhem Bay (north-east Arnhem Land). No mention of the basal spikelets was made in Brown's (1810) protologue. Bentham (1878) too did not refer to basal spikelets in the description of *F. spiralis* in 'Flora Australiensis'. All collections, including the TYPE specimen, were made from the north-east to east coastal edge of Arnhem Land, Northern Territory. The only other known collections are from Groote Eylandt, Northern Territory, *R.L. Specht 235* (MEL 2048472,

CANB); Rose River, Gulf of Carpentaria (as '?*Crosslandia setifolia*'), Northern Territory, *C.R. Dunlop 2957* (DNA 36442); and Cape Shield, Blue Mud Bay, NT, *G.J. Leach 3601 & I.D. Cowie* (NSW 422184, CANB 478018).

During this study, I observed female basal spikelets, at various stages of development, in herbarium specimens of *Abildgaardia vaginata*. These basal spikelets have a similar morphology to those found in both *Crosslandia* and *Fimbristylis spiralis*. Basal spikelets are not consistent with the generic delimitation of *Abildgaardia* and have not been noted previously in the literature. In addition, the nut of *A. vaginata* differs from *A. ovata* and *A. oxystachya*, *A. macrantha*, *A. pachyptera*, and *A. schoenoides* provisionally placed within *Abildgaardia* by Goetghebeur (1986); the nut resembles those from *Crosslandia* and *Fimbristylis spiralis*. Goetghebeur did not list *A. vaginata* with the species in his treatment of *Abildgaardia*.

Abildgaardia vaginata R.Br. has a chequered nomenclatural past. Blake (1947) noted that Bentham transferred *A. vaginata* to *Fimbristylis* Section II *Abildgaardia*, with the name change to *Fimbristylis brownii* Benth., a move that seems illegitimate. The name *F. vaginata* (Boiv. ex C.B.Clarke) was not occupied until 1895; in 1915 Domin made the combination *F. vaginata* from *A. vaginata*, however, the prior use of the name by Clarke in 1895 made the combination illegitimate. Bentham's *F. leptoclada* (based on two collections: Dallachy, Rockingham Bay in far north Queensland and O'Shanessy, from Rockhampton) in 'Flora Australiensis' (Bentham 1878), non *F. leptoclada* Benth. in Flora Hong Kong (Bentham 1861), was assigned to Section IV *Trichelostylis*, Series I *Oligostachyae* along with *F. spiralis*. *Fimbrisitylis leptoclada* is now a synonym of *Abildgaardia vaginata* or *Fimbristylis brownii*, depending on the classification system used. Clarke (1908) placed

F. spiralis and *F. brownii* in Section *Trichelotylis* Series A: *Oligostachyae* based on the 3-fid style and solitary spikelets, while the multi-spikeleted *F. lepotoclada* (with few spikelets according to Bentham) was in the same section, assigned to Series B.

The studies by Kral (1971), and Goetghebeur and Coudijzer (1985) indicated that *Abildgaardia* should have equal rank to that of *Fimbristylis* and *Bulbostylis*. This view has been accepted by cyperologists in Australia and the U.S.A., and some cyperologists in Europe. Simpson (1993) chose to retain the broader generic concept and, therefore, accepted *Fimbristylis brownii* as the correct name.

New information has led to questions whether *Fimbristylis spiralis* and *Abildgaardia vaginata* would be better placed within the genus *Crosslandia*. Also, is the variation within *Crosslandia setifolia* consistent with Goetghebeur's (1986) proposed recognition of a new species, *Crosslandia anthelata*?

Materials and methods

Taxa

To assess species limits of *Abildgaardia vaginata*, *Crosslandia setifolia* and *Fimbristylis spiralis*, and to see if they form a monphyletic group, specimens from *Abildgaardia*, *Crosslandia* and *Fimbristylis* were sampled (Table 3.1). Species limits were set using phenetic analysis. To define the taxa within *Crosslandia*, the relationships of the species were tested to assess monophyly using cladistic analysis.

Abildgaardia oxystachya, *A. pachyptera*, and *A. macrantha*, provisionally named in the genus *Abildgaardia* (Goetghebeur 1986), were included with *A. schoenoides* and *A. ovata* in this assessment.

Twenty-three specimens of *Crosslandia setifolia* s.l. (Table 3.1) were sampled across the geographic range (Northern Territory and Western Australia) to encompass the variable inflorescence morphology and floret sex within the species.

Sampling for *Fimbristylis spiralis* was restricted to three known collections (see Table 3.1), excluding the TYPE specimen housed at BM. Fragments of the ISOTYPE from Arnhem Bay, Northern Territory, *R. Brown* (KEW) were available from Queensland Herbarium (BRI 340661), although they were not suitable for sampling. The remoteness of the habitat (far north-east Northern Territory) prohibited field collections during the course of this study.

Representative taxa from *Fimbristylis* were included to compare *Fimbristylis spiralis* species limits with other members of the genus. Species of *Bulbostylis* were included due to movement of taxa between *Abildgaardia*, *Fimbristylis,* and *Bulbostylis*.

Herbarium material on loan from herbaria BRI, CANB, DNA, MBA, MEL, NSW, and PERTH supplemented NE collections (Holmgren et al. 1990).

Phenetic study

Quantitative (21) and qualitative (54) morphological attributes were scored from 165 samples (Operative Taxonomic Units, OTUs), from *Crosslandia*, *Abildgaardia*, *Bulbostylis* and *Fimbristylis* for the initial analyses.

Characters

Character state definitions are mostly self-explanatory (Table 3.2), with the more complex characters detailed in the text when necessary. Inflorescence–

Table 3.1 Specimens sampled as the focus group in the assessment of the genus *Crosslandia*. The label corresponds to phenetic analyses. N.T. = Northern Territory, W.A. = Western Australia, Qld = Queensland, N.S.W. = New South Wales. See Appendix 1 for specimen details.

Species	Label	State	Collector
Crosslandia	C1	N.T.	Chippendale G. 1268
setifolia	C2	W.A.	Poulton G. 5
	C3	W.A.	Wilson K.L. 4885
	C4	N.T.	Craven L.A. 7928, Whitbread G.
	C5	N.T.	Blake S.T. 17420
	C6	N.T.	Cowie I.D. 4639
	C7	W.A.	Pullen R.
	C8	N.T.	Wilson K.L. 5260
	C9	W.A.	Wilson K.L. 4859
	C10	W.A.	Wilson K.L. 4803
	C11	N.T.	Lazarides M. 8, Adams L.
	C12	W.A.	Burbidge N. 5703
	C13	N.T.	Dunlop C.R. 6789
	C14	W.A.	Clarke K.L. 166, Bruhl J.J., Wilson K.L.
	C15	N.T.	Blake S.T. 16585
	C16	W.A.	Van Rijn P.J. 19
	C17	N.T.	Clarke K.L. 155, Bruhl J.J., Wilson K.L., Cowie I.D.
Crosslandia	C18	N.T.	Dunlop C.R. 6854, Wightman G.
anthelata	C19	N.T.	Dunlop C.R. 3446
	C20	N.T.	Bruhl J.J., Hunter J.T., Egan J. 1268
	C21	N.T.	Dunlop C.R. 3408
	C22	N.T.	Wilson K.L. 5150, Dunlop C.R.
	C23	N.T.	Thompson H.S. 403
Fimbristylis	F1	N.T.	Specht R.L. 235
spiralis	F2	N.T.	Dunlop C.R. 2957
	F3	N.T.	Leach G. 3601, Cowie I.D.
Abildgaardia	Av1	Qld	Blake S.T. 15540, Webb L.J.
vaginata	Av2	N.S.W.	Floyd A.G.F. AGF2205
	Av3	Qld	Brass L.J. 18362
	Av4	N.S.W.	O'Hara J. 3472 and Coveny R.
	Av5	Qld	Blake S.T. 8598
	Av6	N.T.	Cowie I.D. 6801
	Av7	N.T.	Brennan K. 2588
	Av8	N.S.W.	Bell D.M.
	Av9	Qld	Forster P.I. PIF9732
	Av10	Qld	Forster P.I. PIF16257
	Av11	Qld	Blake S.T. 8222
	Av12	Qld	Blake S.T. 22499
	Av13	Qld	Brass L.J. 1924
	Av14	Qld	Sharpe P.R. 5299 and Bird L.

Table 3.2 Attribute codes and definitions used for the main phenetic analyses for *Crosslandia*, including corresponding initial weight values. Weight values changed in subset analyses.

Attribute	Description	Weight
char1	Mean aerial spikelet width in mm (spikelets with mature fruit) at the widest point	1
char2	Mean aerial nut length in mm from base of stipe to nut apex (excluding persistent style base)	1
char3	Mean aerial nut width in mm at the widest point	1
char4	Aerial nut length:width (ratio 1:W/L=x; convert to decimal 1/x)	1
char5	Mean aerial nut 'stipe' length in mm	1
char6	Stipe length/nut length (proportion)	1
char7	Mean aerial anther length in mm (including appendages)	1
char8	Mean aerial style length in mm (including style base to base of style arm junction)	1
char9	Mean aerial style width in mm (at mid third)	1
char10	Style length:width (1:W/L=x; convert to decimal 1/x)	1
char11	Mean aerial stylebase length in mm (from base to constriction at style junction)	1
char12	Mean aerial stylebase width in mm (at widest point)	1
char13	Style base length:width (1:W/L=x; convert to decimal 1/x)	1
char14	Mean aerial glume length in mm (from base of nerve to apical point)	1
char15	Mean aerial glume width in mm (at widest point)	1
char16	Aerial glume length:width (1:W/L=x; convert to decimal 1/x)	1
char17	Mean leaf width in mm (at mid third)	1
char18	Mean culm width in mm (at mid third)	1
char19	Mean root width in mm (one cm below plant base)	1
char20	Mean inflorescence–synflorescence length in mm (from base of main bract to furthermost point of spikelets)	1
char24	Stamen number (actual)	1
char25	Style base persists on nut even if temporarily, as style always separates from style base	0.5
char26	Style base falls in tact with style	0.5
char27	Style glabrous (processes absent)	0.33
char28	Style with fimbriolia 40-60μm (somewhat flattened processes)	0.33
char29	Style fimbria 100-140μm (distinctly flattened processes)	0.33
char33	Basal spikelets 0–absent: always only aerial; 1–present: basal spikelets (morphologically distinct) as well as aerial spikelets	1
char35	Plant habit 0–annual 1–perennial	1
char36	Floret sex 0–always bisexual 1–mixed aerial floret sex: functionally male, female or some bisexual	1
char37	Nut outline elliptic	0.167
char38	Nut outline obovate (2:1 or 3:2) to widely obovate	0.167
char39	Nut outline turbinate (top-like)	0.167
char40	Nut outline pyriform (pear-shaped)	0.167
char41	Nut outline obcordate	0.167
char42	Nut outline capitate or club shaped (with a prominent stipe)	0.167
char43	Nut epidermis without protuberances	0.1
char44	Nut epidermal cell walls raised while lumen appears sunken or flat	0.1
char45	Nut epidermis is sparse and irregularly puncticulate (from a central raised silica body in some cells)	0.1
char46	Nut epidermis puncticulate, as all cells with a central silica body	0.1

Table 3.2 cont'd

char47	Nut epidermal individual cells raised indiscriminately (not multiple as seen in large warts)	0.1
char48	Nut epidermal individual cells raised evenly over nut	0.1
char49	Nut epidermis with warts (cluster of multiple raised cells) arranged in vertical rows along the face	0.1
char50	Nut epidermis with warts sparse and unevely distributed	0.1
char51	Nut epidermis with pronounced warts formed by clusters of raised cells that have dense distribution	0.1
char52	Nut epidermis is rugose (cells saised in horizontal waves)	0.1
char53	Nut 0- not winged 1- winged (flattened extensions from the nut sides, including any extended notching on nut 'margins')	1
char55	Pilose hairs at leaf /sheath junction 0-absent 1-present (at least in young plants)	1
char56	Inflorescence: solitary (1 spikelet only-1st order primary main florescence (HF) only)	0.2
char57	Inflorescence–synflorescence: main florescence (HF) and one to multiple primary coflorescences (Cof) that are 'rayed' spikelets (on lengthed epipodia) or sometimes sessile	0.2
char61	Multiple order 'rayed' spikelets (ie 2nd order or greater ramification)	0.2
char62	'Head' of sessile spikelets (primary reduced anthelodium with epipodia highly reduced) terminal on the culm	0.2
char63	'Head' of sessile spikelets on lateral branch additional to the main terminal 'head'	0.2
char64	Glumes arranged distichously (spikelet distinctly compressed as glumes arise opposite the previous glume in the same plane)	0.25
char65	Glumes sub-distichous (spikelet somewhat compressed as not all opposite pairs sit in the same plane, rachilla may twist distally)	0.25
char66	Glumes distichously spiral (spiro-distichous – glumes are opposite each other but ascending pairs are arranged spirally)	0.25
char67	Glumes tristichously spiral, arranged in an ascending spiral	0.25
char68	Aerial glume margins entire (all glumes)	0.2
char69	Aerial glume margins ciliolate (thin hair-like process)	0.2
char70	Aerial glume margins fimbriolate (somewhat flattened)	0.2
char71	Aerial glume margins fimbriate (distinctly flattened)	0.2
char73	Aerial glume margins ciliate (fine hairs 0.5 mm long)	0.2
char74	Stigma number (actual)	1
char79	Ligule 0–absent 1–present	1
char80	Leaf blades always present on an individual	0.33
char81	Some leaf blades present, some as subulate points in an individual	0.33
char82	Leaf blades always absent in an individual	0.33
char83	Inflorescence–synflorescence bracts absent (usually in solitary spikelets)	0.5
char84	Inflorescence–synflorescence bracts present and distinct	0.5
char85	Inflorescence–synflorescence bracts glume-like	0.5
char86	Inflorescence–synflorescence bracts leaf-like	0.5
char87	Main inflorescence–synflorescence bracts shorter than inflorescence–synflorescence length	0.33
char88	Main inflorescence–synflorescence bracts equals inflorescence–synflorescence length	0.33
char89	Main inflorescence–synflorescence bracts longer than inflorescence–synflorescence length	0.33
char90	Prophyllar buds present within the inflorescence–synflorescence (polymorphic)	0.5
char91	Prophyllar buds absent within the inflorescence–synflorescence (polymorphic)	0.5

synflorescence structure and floret sex variability are two cases that required extra attention. Schematic representations for inflorescence–synflorescence structure and floret sex variation found within the *Crosslandia* study group are presented in the results section for this chapter.

Pattern Analyses

Patterns within the data were explored using the program PATN v 3.6 (Belbin 1993), by subjecting the data to ordination, cluster and network analyses (see Chapter 2 for details). Gower's similarity coefficient applied to weighted data sets proved suitable for these data and was used here.

Following the initial run, groups that were distinct and separated (i.e. *Bulbostylis*, *Abildgaardia*, *Fimbristylis*) were removed from the analyses and the subsets were rerun to explore the remaining *Abildgaardia vaginata–Crosslandia–Fimbristylis spiralis* group more closely. Patterns were also explored after removing basal spikelet data to ensure that these data did not unduly influence the groups formed in other analyses. Floret sex variation was expanded for the *Crosslandia–F. spiralis* data set (after removing *Abildgaardia vaginata*) to capture the full variation within the analysis.

Cladistic study

Ingroup

For the cladistic study, data were collated from the multiple OTUs scored in phenetic analyses so that each species group was converted into data for a single taxon. Species from *Abildgaardia* and *Bulbostylis* that had well-defined species limits in the phenetic study were used to represent genera in the cladistic analyses.

Representative species across the sections of *Fimbristylis* (Table 3.3; see also Appendix 1) complete the ingroup taxa included to test monophyly of *Crosslandia setifolia*, *Fimbristylis spiralis* and *Abildgaardia vaginata* at the generic level and assess their relationships.

Embryo morphology and anatomy

Goetghebeur (1986) reported that *Crosslandia* has a variant of the Fimbristylis-type embryo. Embryos of *Fimbristylis spiralis* and *Abildgaardia vaginata* were dissected from the fruit and compared to embryos of *Crosslandia* and other taxa included in the study.

Leaf blade and culm anatomy were examined for specific anatomical features (see cladistic character list Appendix 2) to compare *Crosslandia*, *Fimbristylis spiralis* and *Abildgaardia vaginata* with the other study taxa that provided generic anatomical contrasts.

Inflorescence–synflorescence structure

The inflorescence–synflorescence structures are complex across some of the taxa sampled. The variable inflorescence–synflorescence structure within the *Crosslandia* group required a detailed investigation, as the provisional *C. anthelata* was based primarily on a different inflorescence type to that of *C. setifolia.*

Table 3.3 Taxa included in the cladistic analyses to assess the relationships of *Crosslandia setifolia*, provisional *C. anthelata*, *Fimbristylis spiralis* and *Abildgaardia vaginata*. See Table 3.1 for *Crosslandia* specimen list and Appendix 1 for specimen details.

Taxa	No. of specimens sampled
Ingroup	
Abildgaardia macrantha (provisional)	10
Abildgaardia ovata	11
Abildgaardia oxystachya (provisional)	10
Abildgaardia pachyptera (provisional)	11
Abildgaardia schoenoides	11
Abildgaardia vaginata	14
Bulbostylis barbata	12
Bulbostylis densa	10
Crosslandia anthelata (provisional)	5
Crosslandia setifolia	18
Fimbristylis blakei	2
Fimbristylis cinnamometorum	5
Fimbristylis depauperata	2
Fimbristylis fimbristyloides	4
Fimbristylis furva	2
Fimbristylis microcarya	2
Fimbristylis schultzii	2
Fimbristylis sp *L.* (Flora of the Kimberley)	2
Fimbristylis spiralis	3
Outgroup	
Actinoschoenus compositus (provisional)	4
Arthrostylis aphylla	4
Schoenoplectiella laevis	5
Schoenoplectiella lateriflora	5
Schoenoplectus tabernaemontani	3

PAUP* Analyses

Data from 24 species and 156 characters for ingroup and outgroup taxa were subjected to parsimony analysis within PAUP* 4b10 (Swofford 2001) using heuristic techniques (hsearch swap=TBR addseq=random nreps=1000 hold=5 multrees=yes). When multiple parsimonious trees were retrieved, a strict consensus was compared to each tree. A single tree that most closely resembled the tree from the strict consensus was presented in the results section.

Decay and Bootstrap analyses were applied to the data set to assess relative branch support (see Chapter 2 for details). Characters with the strongest branch association were plotted onto the cladogram, using MacClade v 3.08 (Maddison and Maddison 1992) to trace characters.

Results

Phenetic study

Crosslandia, Abildgaardia, Fimbristylis, *and* Bulbostylis

The OTUs from *Bulbostylis*, *Fimbristylis*, *Abildgaardia*, and *Crosslandia* revealed distinct groups in both 3-dimensional (stress value=1.0) and 2-dimensional (stress value=0.17) ordinations (Figure 3.1). *Fimbristylis spiralis* OTUs formed a small group on the edge of the *Crosslandia anthelata* cloud that separated from the *Crosslandia setifolia* OTUs. A clear-cut group was formed by OTUs of *Abildgaardia vaginata*, separated from all other OTUs of *Abildgaardia*. In 3-dimensional space, species formed by the OTUs of *Bulbostylis* were separate from the remaining OTUs.

Similarly, OTUs of *Fimbristylis* (excluding *F. spiralis*) and *Abildgaardia* (excluding *A. vaginata*) formed separate clusters.

Characters having the greatest correlation (>80%) with the ordination pattern were: style glabrous, style base persistent on nut, and pilose hairs present at leaf/sheath junction – features distinctly associated with species from *Bulbostylis*. The remaining characters: style length and width, aerial glume length and width, plus nut length and width separated taxa within the *Abildgaardia–Fimbristylis–Crosslandia* group (Figure 3.2).

Cluster analysis (Figure 3.3) produced groupings similar to the ordination pattern, with five definite groups being formed and numbered to correspond with the ordination groups (Figure 3.1). The OTUs of *Fimbristylis spiralis* were associated with the OTUs of *Crosslandia* s.l. forming a broad group (G3), as no grouping for the *C. anthelata* OTUs (C18-23) was apparent. The three OTUs of *Fimbristylis spiralis* held together as a group within G3. The OTUs of *Abildgaardia vaginata* (G2) grouped next to OTUs of *Crosslandia* s.l. and *F.spiralis*, but remained distinct from G3. The remaining OTUs clustered into generic groups of *Fimbristylis* (G4), *Abildgaardia* (G1), and *Bulbostylis* (G5). The presence or absence of basal spikelets did not seem to influence the pattern of ordination and cluster analyses. The ordination and phenogram that resulted following removal of basal spikelet data, and re-analysis, confirmed that any association between *Crosslandia setifolia*, *Fimbristylis spiralis* and *Abildgaardia vaginata* was not due solely, or in particular, to the presence of basal spikelets.

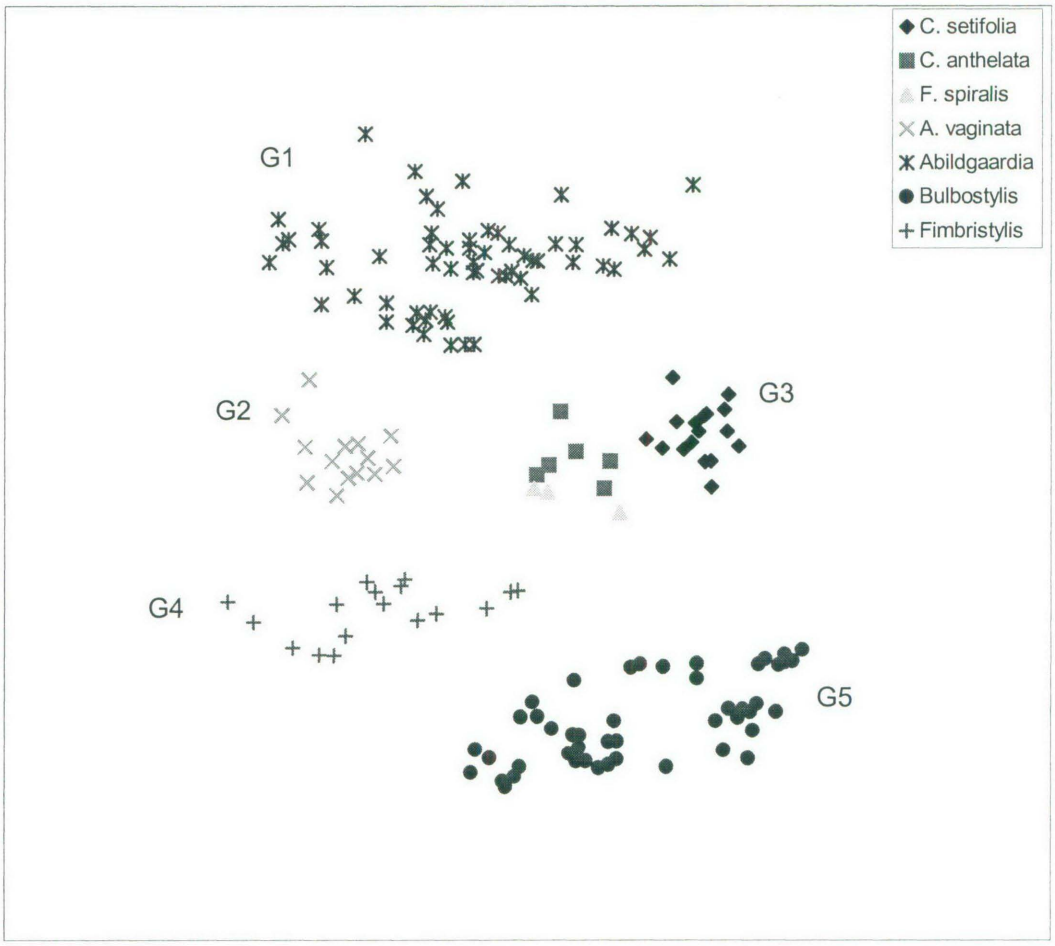


Figure 3.1 MDS ordination in 2-dimensions (stress = 0.17). OTU groups for *Crosslandia setifolia*, *C. anthelata* and *Fimbristylis spiralis* (G3), *Abildgaardia vaginata* (G2), *Abildigaardia* spp. (G1), *Fimbristylis* spp. (G4) and *Bulbostylis* spp. (G5).

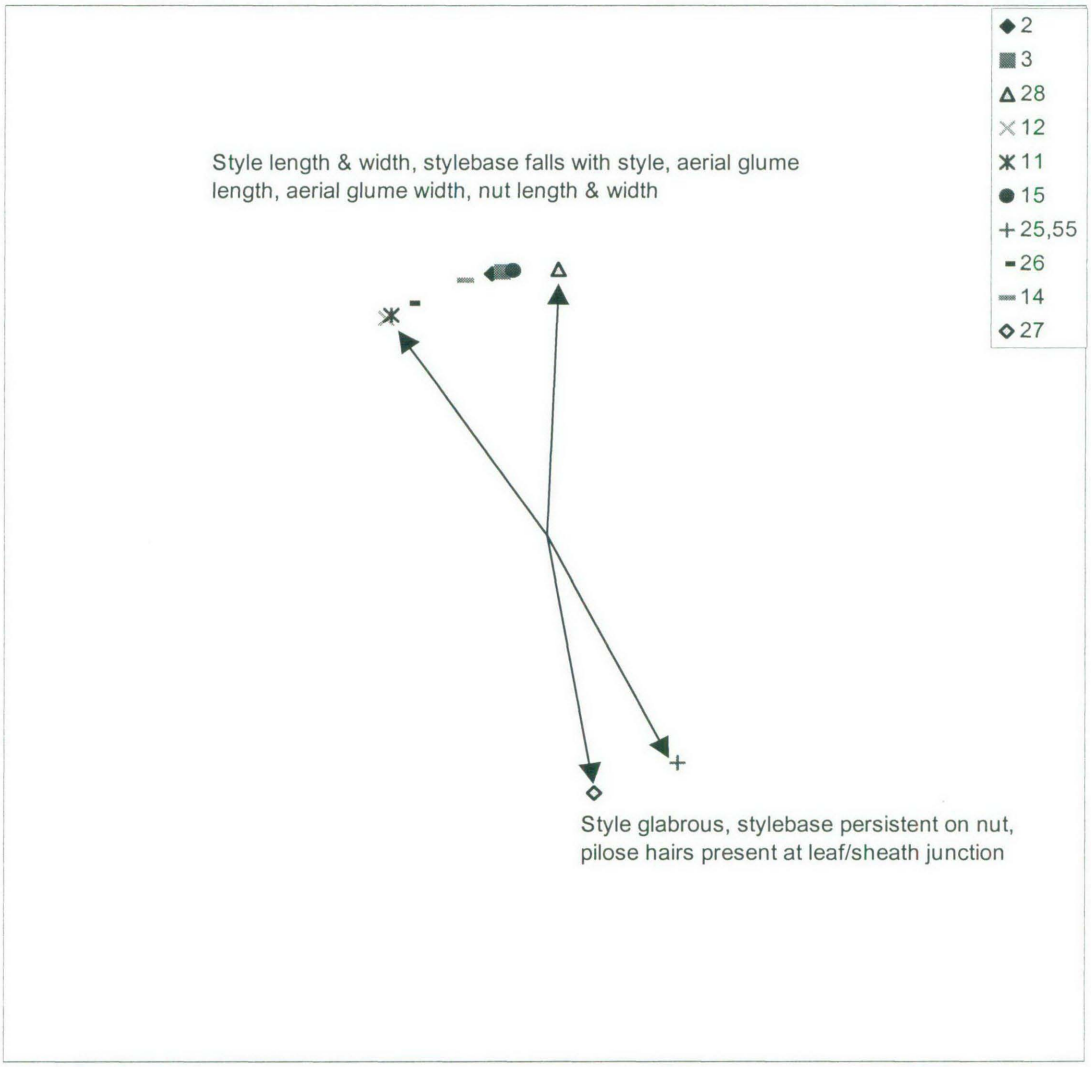


Figure 3.2 Correlation of attributes with ordination space in figure 3.1. Attributes with > 80% are shown; 27 (glabrous style), 25 (persistent style base on nut) and 55 (pillose hairs at leaf/sheath junction) separate OTUs of *Bulbostylis* from other taxa. See Table 3.2 for attribute definitions.

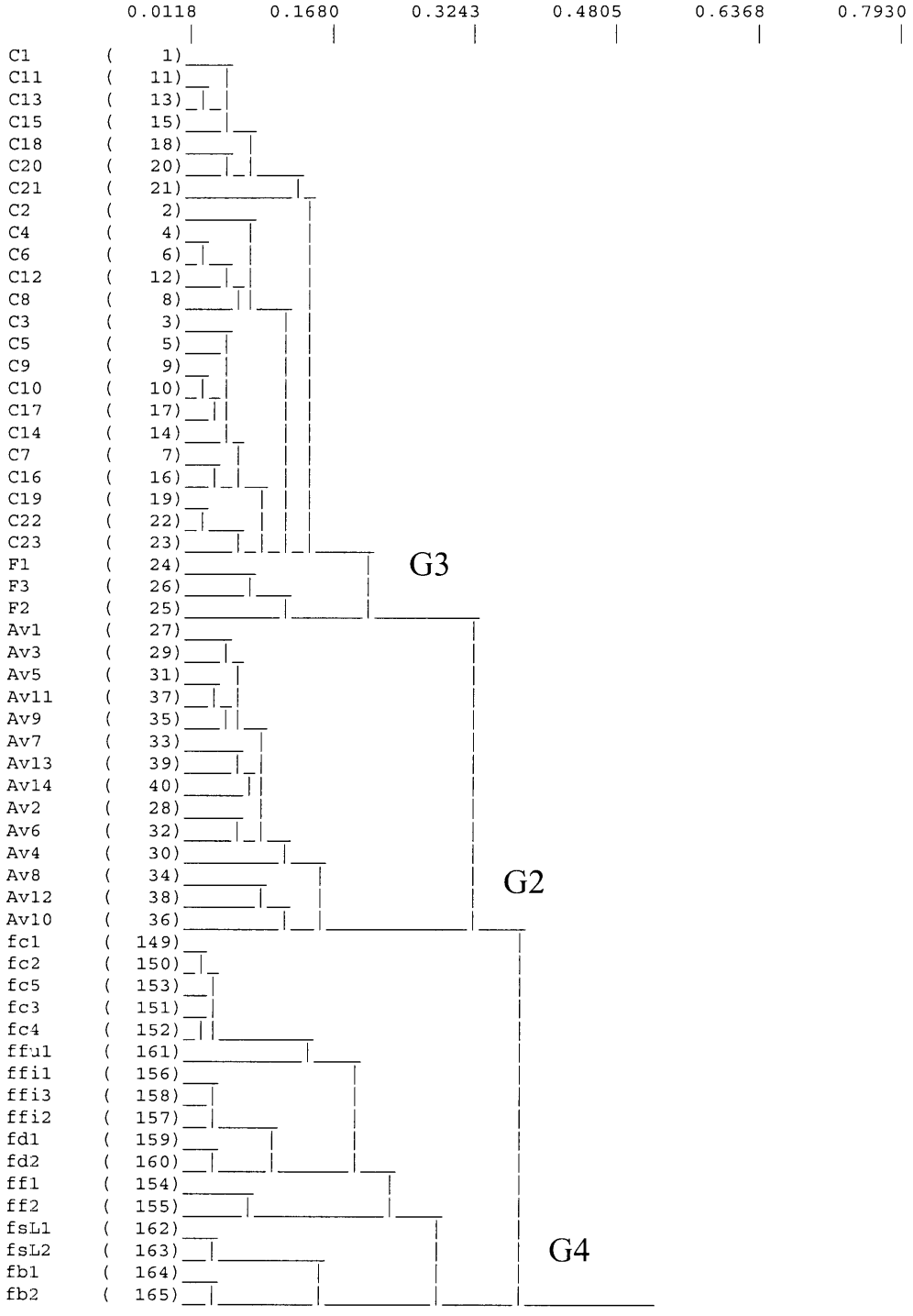

0.0118
0.1680
0.3243
0.4805
0.6368
0.7930
C1 (1)
C11 (11)
C13 (13)
C15 (15)
C18 (18)
C20 (20)
C21 (21)
C2 (2)
C4 (4)
C6 (6)
C12 (12)
C8 (8)
C3 (3)
C5 (5)
C9 (9)
C10 (10)
C17 (17)
C14 (14)
C7 (7)
C16 (16)
C19 (19)
C22 (22)
C23 (23)
F1 (24)
F3 (26)
F2 (25)
Av1 (27)
Av3 (29)
Av5 (31)
Av11 (37)
Av9 (35)
Av7 (33)
Av13 (39)
Av14 (40)
Av2 (28)
Av6 (32)
Av4 (30)
Av8 (34)
Av12 (38)
Av10 (36)
fc1 (149)
fc2 (150)
fc5 (153)
fc3 (151)
fc4 (152)
ffu1 (161)
ffi1 (156)
ffi3 (158)
ffi2 (157)
fd1 (159)
fd2 (160)
ff1 (154)
ff2 (155)
fsL1 (162)
fsL2 (163)
fb1 (164)
fb2 (165)
G3
G2
G4

Figure 3.3

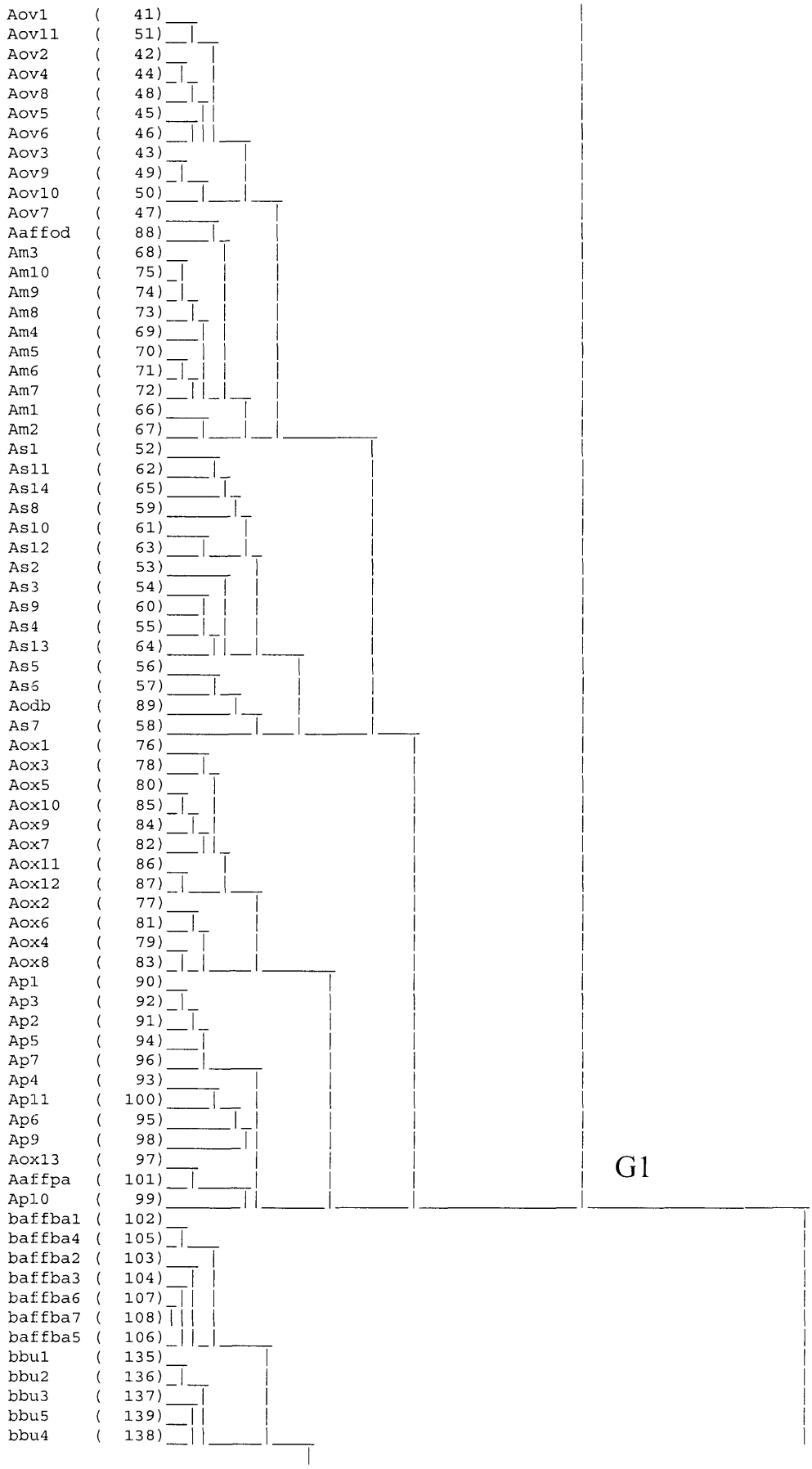
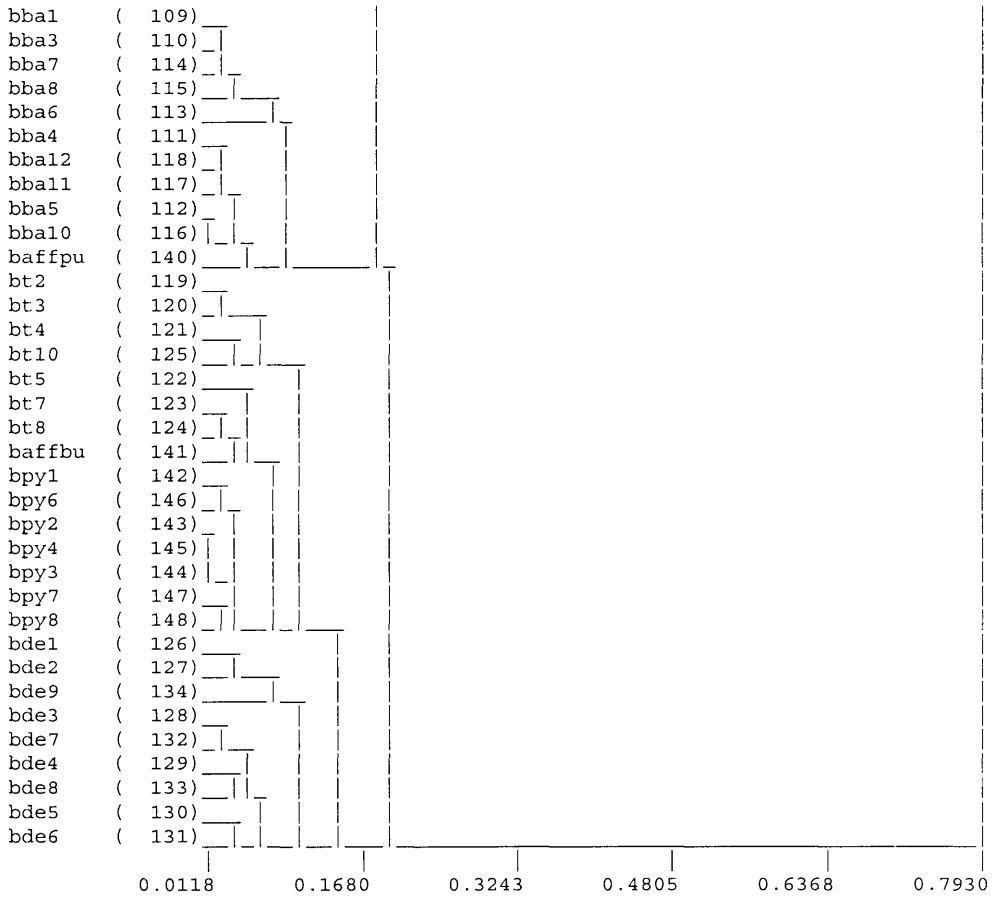

Aov1 (41)
Aov11 (51)
Aov2 (42)
Aov4 (44)
Aov8 (48)
Aov5 (45)
Aov6 (46)
Aov3 (43)
Aov9 (49)
Aov10 (50)
Aov7 (47)
Aaffod (88)
Am3 (68)
Am10 (75)
Am9 (74)
Am8 (73)
Am4 (69)
Am5 (70)
Am6 (71)
Am7 (72)
Am1 (66)
Am2 (67)
As1 (52)
As11 (62)
As14 (65)
As8 (59)
As10 (61)
As12 (63)
As2 (53)
As3 (54)
As9 (60)
As4 (55)
As13 (64)
As5 (56)
As6 (57)
Aodb (89)
As7 (58)
Aox1 (76)
Aox3 (78)
Aox5 (80)
Aox10 (85)
Aox9 (84)
Aox7 (82)
Aox11 (86)
Aox12 (87)
Aox2 (77)
Aox6 (81)
Aox4 (79)
Aox8 (83)
Ap1 (90)
Ap3 (92)
Ap2 (91)
Ap5 (94)
Ap7 (96)
Ap4 (93)
Ap11 (100)
Ap6 (95)
Ap9 (98)
Aox13 (97)
Aaffpa (101)
Ap10 (99)
baffba1 (102)
baffba4 (105)
baffba2 (103)
baffba3 (104)
baffba6 (107)
baffba7 (108)
baffba5 (106)
bbu1 (135)
bbu2 (136)
bbu3 (137)
bbu5 (139)
bbu4 (138)
G1

Figure 3.3



G5

Figure 3.3 WPGMA phenogram (β=-0.1) using the Gower metric similarity coefficient showing groups that correspond with the ordination (Figure 3.1). Group numbers correspond with the groups in the ordination: OTU groups *Crosslandia setifolia* s.l. and *Fimbristylis spiralis* (G3), *Abildgaardia vaginata* (G2), *Abildgaardia* spp. (G1), *Fimbristylis* spp. (G4) and *Bulbostylis* spp. (G5). See Table 3.1 and Appendix 1 for OTU and specimen details.

Network analyses (not presented) supported the general generic/species groupings found using ordination and cluster techniques.

Crosslandia, Fimbristylis spiralis *and* Abildgaardia vaginata

Analyses following the removal of OTUs of *Bulbostylis*, *Abildgaardia* (not *A. vaginata*) and *Fimbristylis* (not *F. spiralis*) resulted in the formation of four groups in the 3-dimensional ordination (stress values 3D=0.08, 2D=0.11). The OTUs of *Crosslandia anthelata* (G3), *C. setifolia* (G2) and *Fimbristylis spiralis* (G4) grouped more closely to each other than to OTUs of *Abildgaardia vaginata* (G1) (Figure 3.4).

Characters having greater than 80% correlation in separating OTUs within the ordination were generally associated with the two major groups formed (Figure 3.5).

Annual habit and the characters: leaf blades always present, and inflorescence bracts leaf-like, were correlated with *Crosslandia* and *Fimbristylis spiralis*; perennial habit and the characters: inflorescence bracts glume-like and the low prevalence of basal spikelets, were correlated with *Abildgaardia vaginata*.

Similar groups were revealed in both the phenogram and ordination, with one major difference in pattern between the two analyses. In the phenogram, OTUs of *Fimbristylis spiralis* (G4) were nested within the OTUs of *Crosslandia anthelata* (G3) (Figure 3.6).

Crosslandia *complex*

Operative taxonomic units of *Abildgaardia vaginata* were omitted from subsequent analyses, resulting in the formation of three groups (stress values 3D=

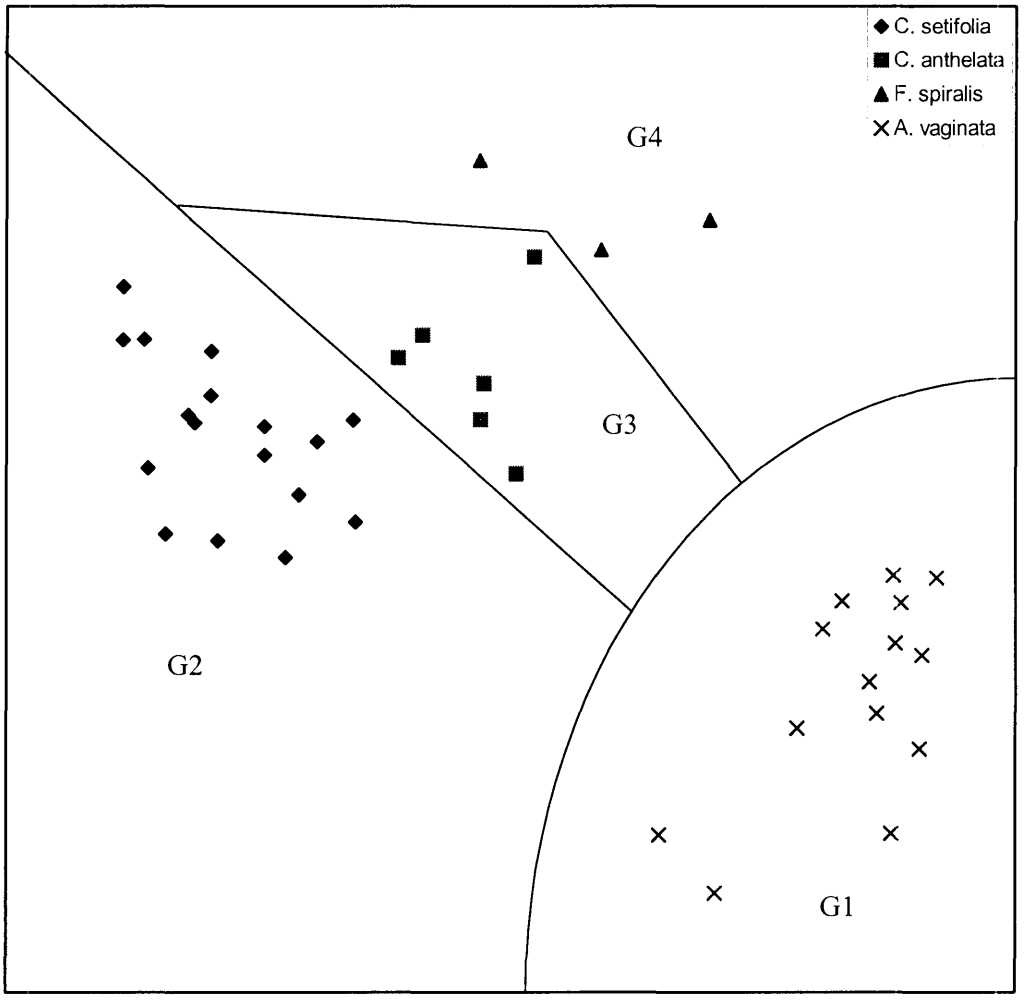


Figure 3.4 MDS ordination in 2-dimensions (stress = 0.11) showing groups formed when *Abildgaardia vaginata* is included within the *Crosslandia* complex. Group separation for *C. anthelata*, *C. setifolia* and *Fimbristylis spiralis* was distinctly resolved in three dimensions (stress = 0.08) as indicated by the group borders.

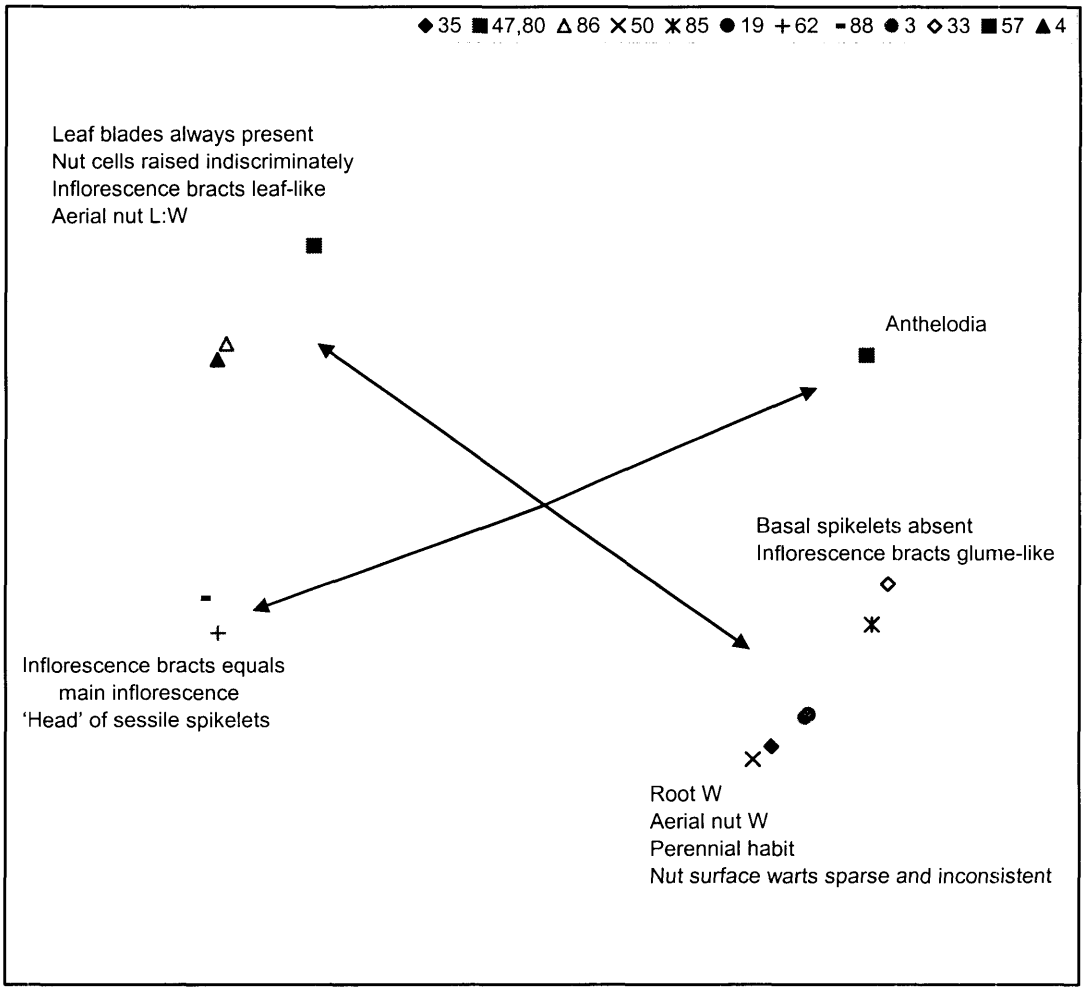


Figure 3.5 Correlation of characters that fit the ordination space in Figure 3.4. Characters with greater than 80% influence on the ordination are shown for the *Crosslandia s.l.* L=length, W=width. See Table 3.2 for attribute definitions.

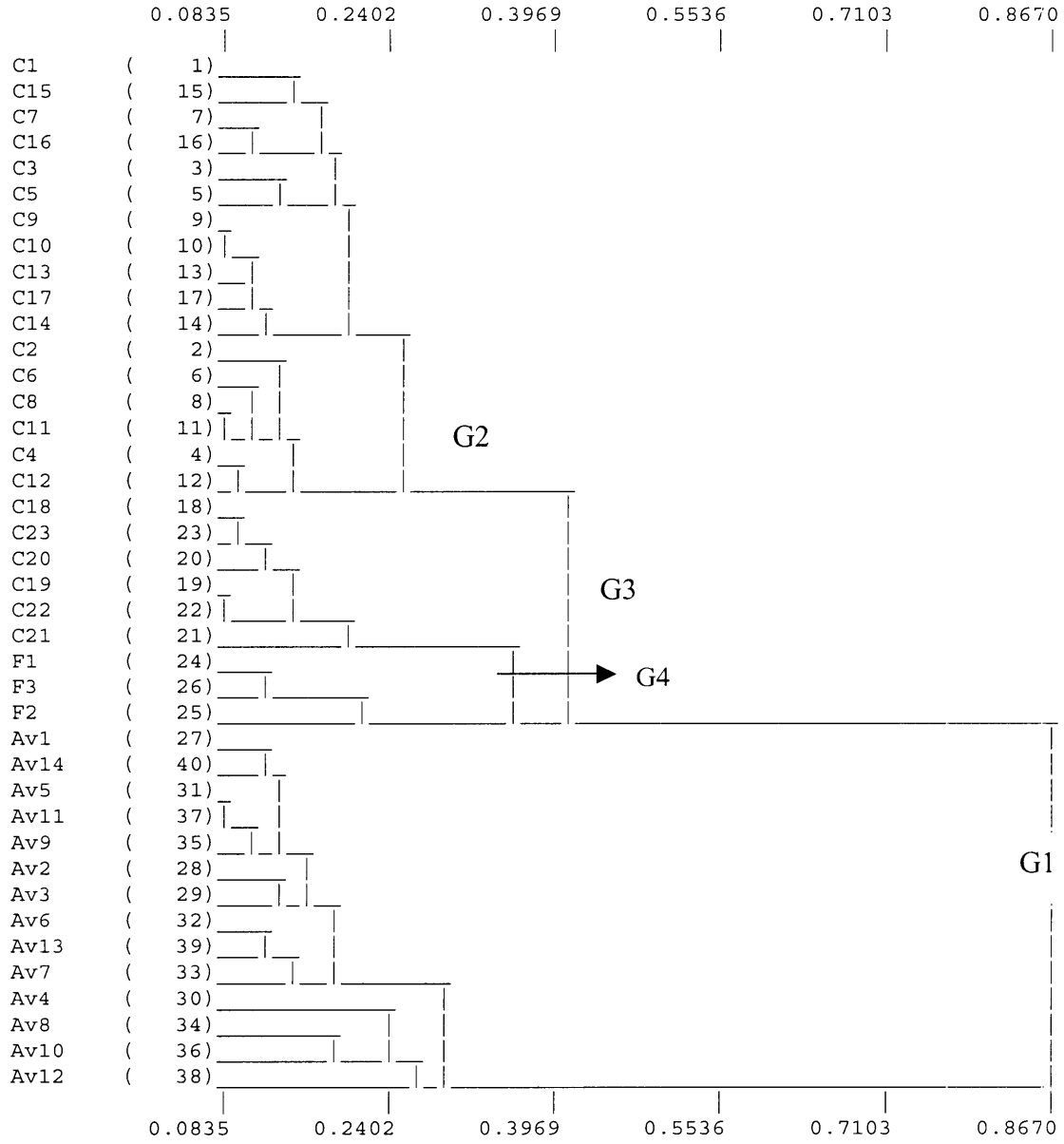


Figure 3.6 UPGMA phenogram (β=-0.1) using the Gower metric similarity measure showing four groups. Group numbering corresponds to the ordination in Figure 3.4 after the removal of *Bulbostylis*, *Fimbristylis* and *Abildgaardia* (excluding *A. vaginata*). See Table 3.1 and Appendix 1 for OTU and specimen details.

0.13, 2D=0.19). The OTUs of *Crosslandia anthelata* (with lengthened epipodia within the anthelodium, i.e. rayed) formed a group (G2=C18, C19, C20, C21, C22, C23), which separated more distinctly than in previous analyses from OTUs of *Crosslandia setifolia* (with capitate synflorescences, i.e. sessile spikelets) (G1). *Fimbristylis spiralis* formed a group (G3) that was distinct from the *Crosslandia* clusters (Figure 3.7).

Characters with the highest correlation with the ordination pattern that separated OTUs of *Fimbristylis spiralis*, from the 'rayed anthelodium' type of *Crosslandia*, and the typical 'capitate' type of *Crosslandia* were synflorescence related (chars 56, 57 and 62, respectively – see Figure 3.8). In the network analysis, all OTUs that were collected from the Northern Territory were connected, with OTUs of *Abildgaardia vaginata* and *Fimbristylis spiralis* diverging from the OTUs of *Crosslandia anthelata* (Figure 3.9).

Cluster analysis (Figure 3.10) revealed groups consistent with those formed in the ordination in Figure 3.7 and MST (Figure 3.9). The clusters recovered from the phenetic analyses correspond to *Crosslandia setifolia*, *C. anthelata*, *Fimbristylis spiralis*, *and Abildgaardia vaginata*. To assess their relationships, these entities were used as terminal taxa in cladistic analyses.

Cladistic analysis

Four most parsimonious trees were retrieved from a heuristic search (tree length=725, CI=0.5862, HI=0.4138, RI=0.5757, RC=0.3375). Differences for terminal taxa within the *Crosslandia* clade were displayed in two of the four trees. Trees 1 and 3 showed *Abildgaardia vaginata* and *Crosslandia setifolia* nested within

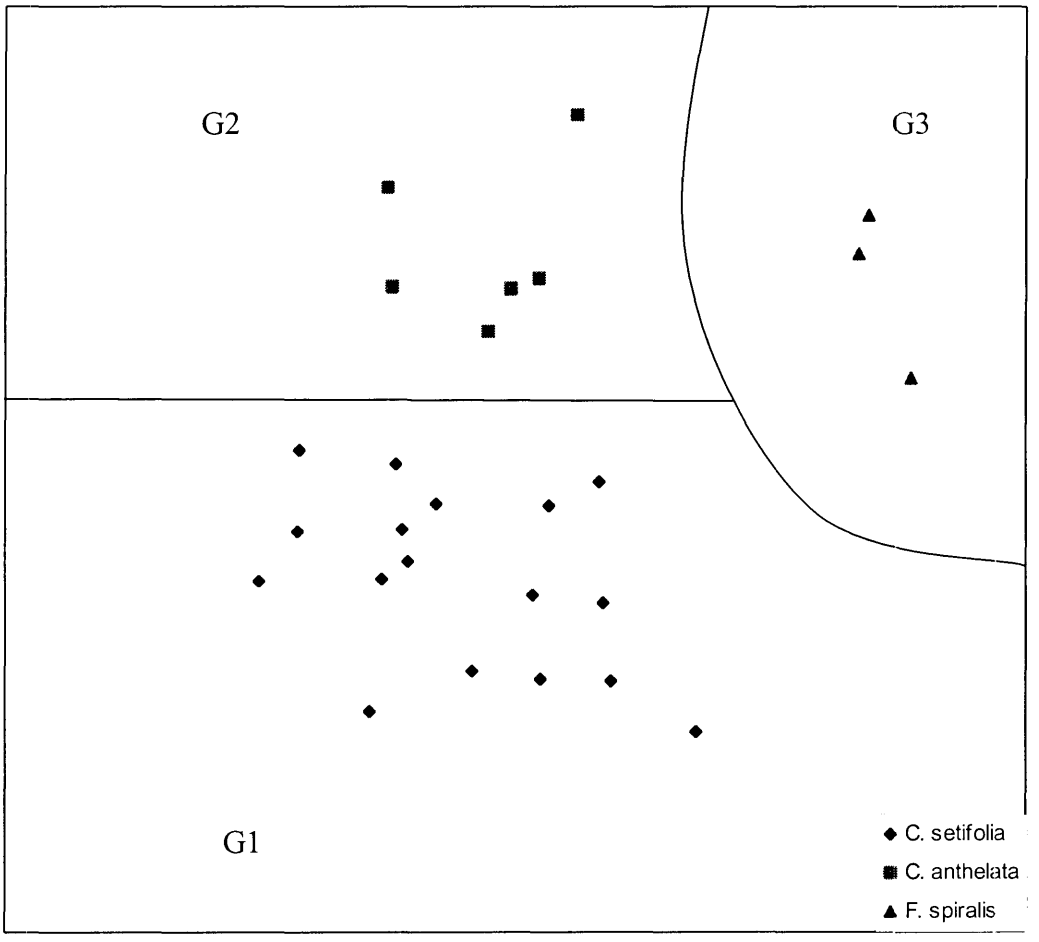


Figure 3.7 MDS ordination in 2-dimensions (stress = 0.19) showing three groups: *Crosslandia setifolia* (G1), *Crosslandia anthelata* (G2), and *Fimbristylis spiralis* (G3). The boundaries indicate the tighter grouping that was observed in the 3-dimensional scatter plot (stress = (0.13).

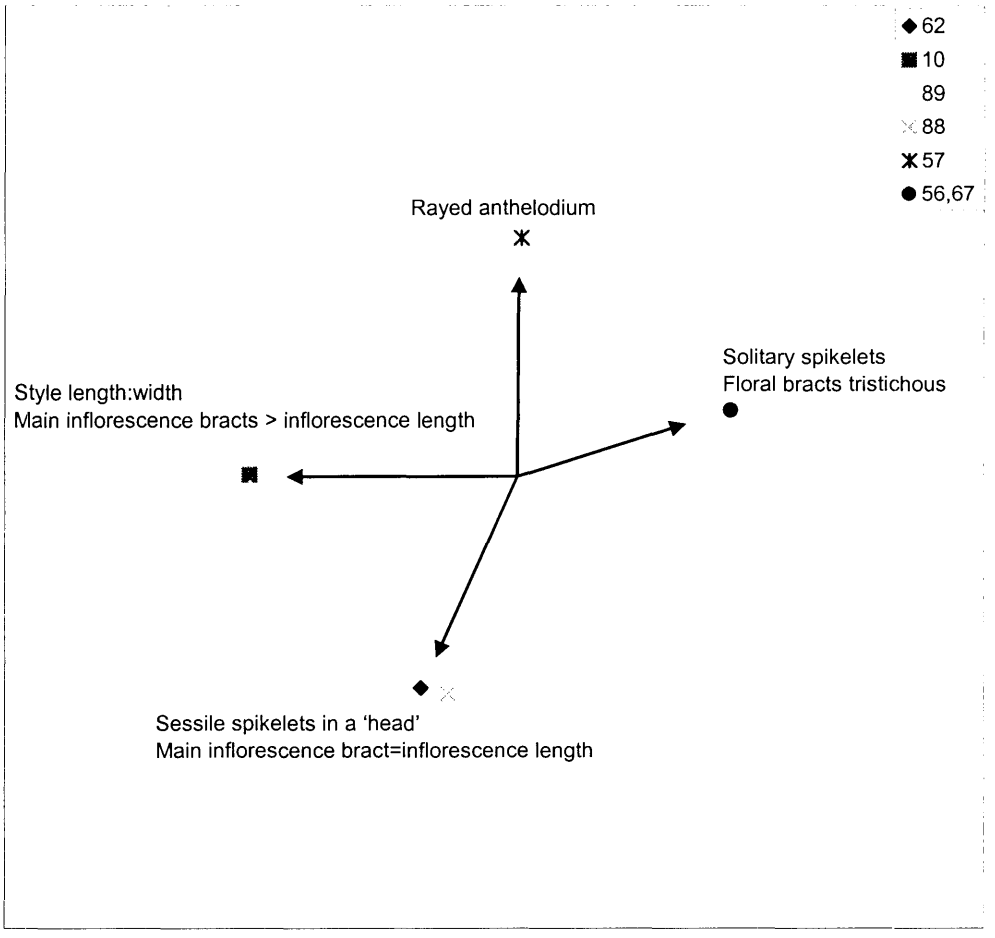


Figure 3.8 Correlation of attributes with the ordination in Figure 3.7. Attributes having greater than 80% influence on separating OTUs into three groups are presented. G2 was separated from the other groups due to the rayed anthelodium (char 57). *Fimbristylis spir*alis characters were mainly solitary spikelets (char 56) and glumes spirally arranged (char 67). Capitate synflorescence (char 62) pulled the typical *Crosslandia setifolia* into G1. See Table 3.2 for attribute definitions

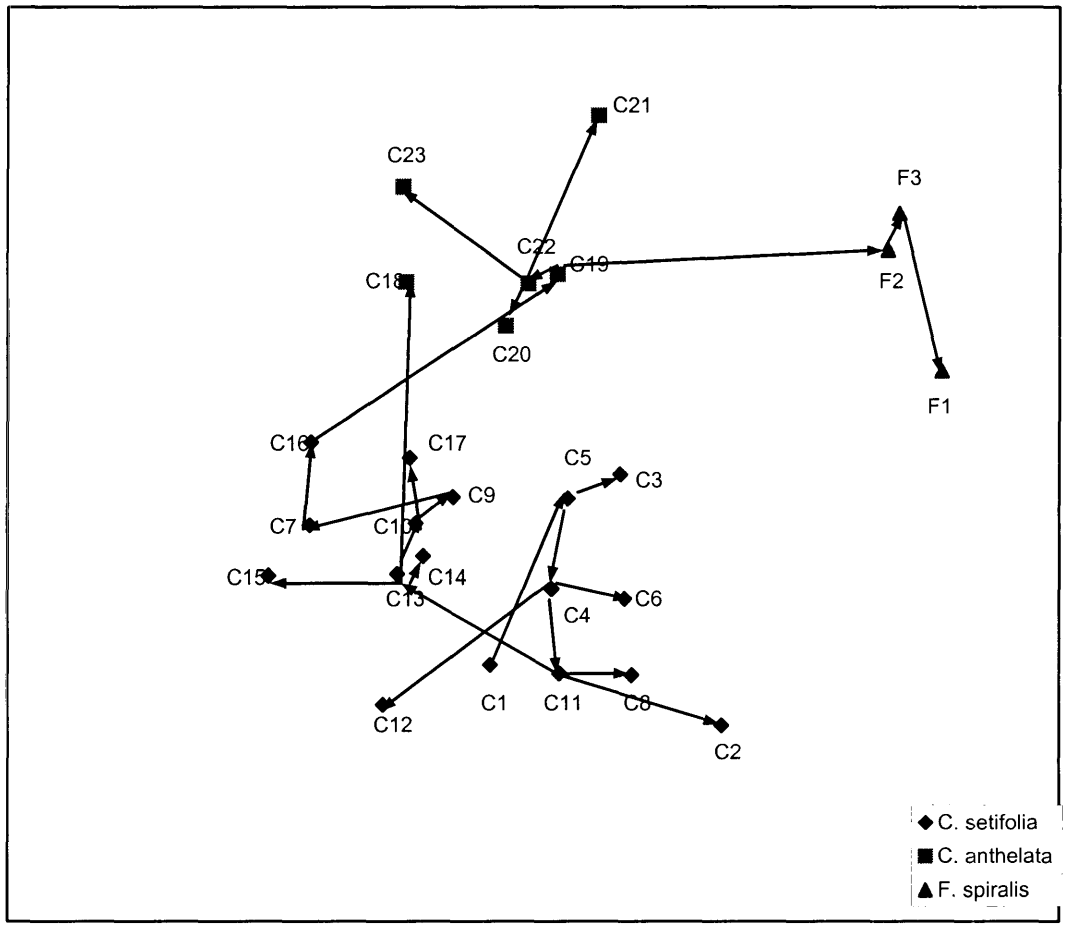


Figure 3.9 Minimum spanning tree (MST) for OTU linkages of *Crosslandia setifolia*, *C. anthelata* and *Fimbristylis spiralis* that correspond to the ordination in Figure 3.4. See Table 3.1 and Appendix 1 for specimen details.

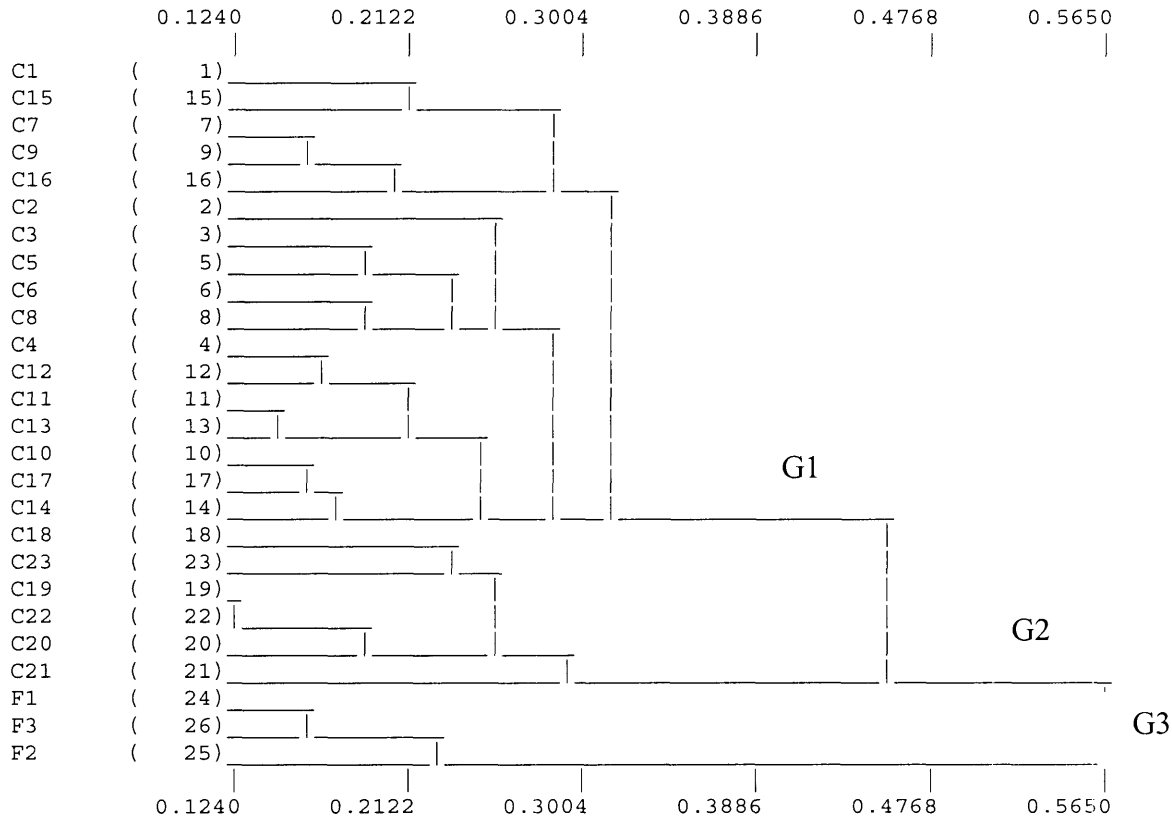


Figure 3.10 UPGMA phenogram (β=-0.1) using the Gower metric similarity measure that corresponds to the ordination in Figure 3.7. The separation of OTUs of *Crosslandia setifolia* as G1 (same synflorescence structure as the TYPE specimen), *Crosslandia anthelata* with solitary or small groups of sessile spikelets on lengthened epipodia (rays) as G2 and *Fimbristylis spiralis* as a distinct group G3 are supported. See Table 3.1 and Appendix 1 for specimen details.

Fimbristylis spiralis, which in turn was nested within *Crosslandia anthelata*. Trees 2 and 4 both placed *Crosslandia setifolia* and *C. anthelata* as sister to *Abildgaardia vaginata* and *Fimbristylis spiralis*. Strict consensus of the four trees did not indicate branch stability for the terminal branches of the *Crosslandia* clade, however, the position of *Crosslandia setifolia* and *C. anthelata* as sister terminal taxa is most likely due to their close similarity. Therefore, tree 2 is presented in the results (Figure 3.11). *Crosslandia setifolia*, *C. anthelata*, *Fimbristylis spiralis*, and *Abildgaardia vaginata* formed a monophyletic group, sister to the remaining species of *Abildgaardia*. The *Abildgaardia–Crosslandia* clade was sister to the *Fimbristylis–Bulbostylis* clade (containing *F. depauperata*, the representative of *Fimbristylis* section *Fimbristylis* that contains the TYPE species for the genus). *Fimbristylis* was rendered non-monophyletic by the exclusion of two members (not counting *F. spiralis*) of *Fimbristylis*: *F* sp. L and *F. blakei*.

Bremer support and Botstrap analyses indicated that branches containing clades *Abildgaardia* spp. (Decay=4 Bootstrap=98%), *Bulbostylis* spp. (Decay=4 Bootstrap=94%), *Actinoschoenus compositus* and *Arthrostylis aphylla* (Decay=3 Bootstrap=93%), plus *Schoenoplectiella laevis* and *S. lateriflora* (Decay=2 Bootstrap=88%) have strong support (Figure 3.11). Support for the *Crosslandia* clade was weaker (Decay=1 Bootstrap=58%), while the internal branch to the *Abildgaardia–Crosslandia* clade was moderately supported (Decay=2 Bootstrap=67%).

Despite weak support, the *Crosslandia* group was consistently retrieved in the most parsimonious trees of subsequent reruns using different RSEED values, nreps, nchuck and chucksore values.

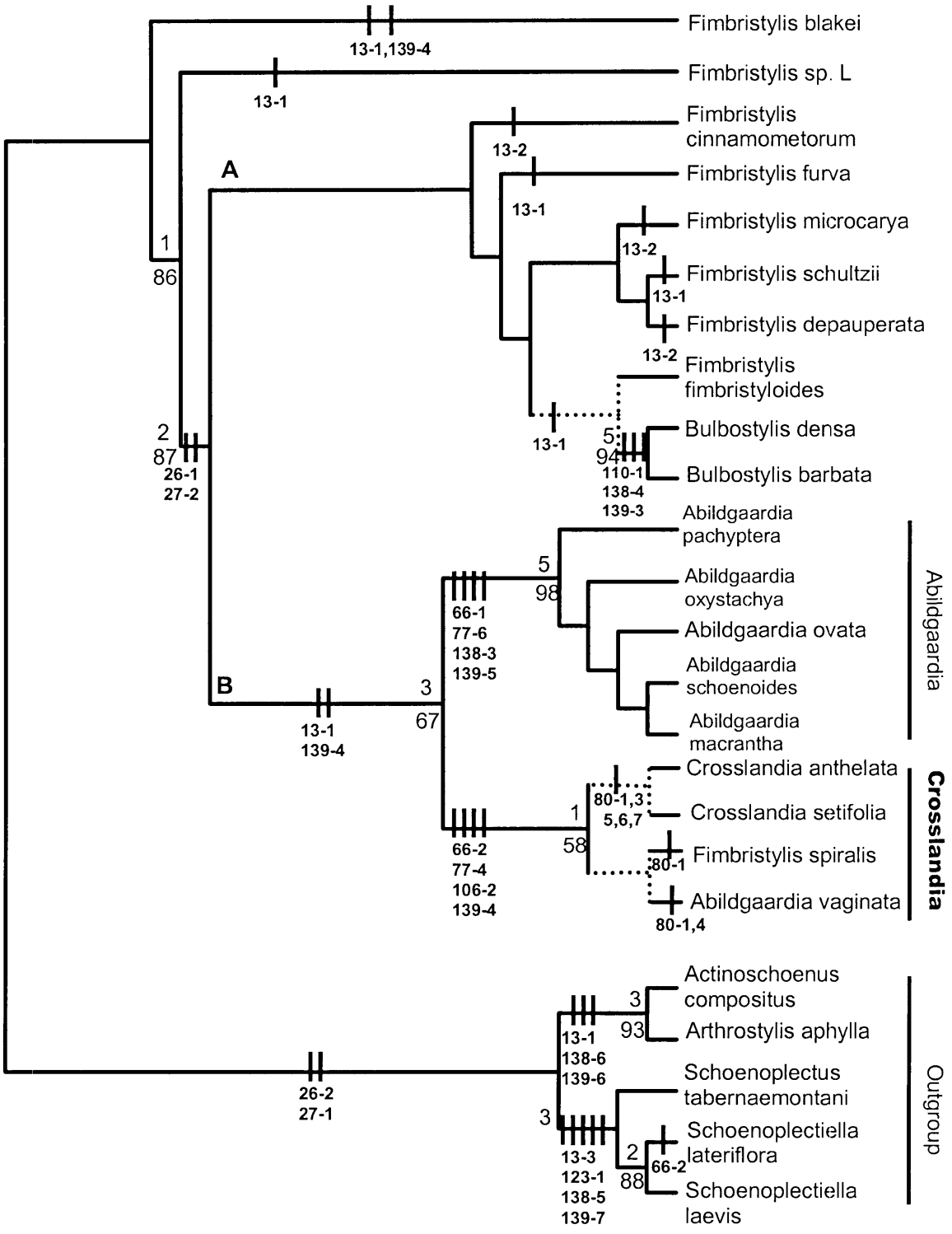


Figure 3.11 Cladogram for tree 2 of 4 shortest trees (tree length=725) to assess monophyly for *Crosslandia*. *Crosslandia setifolia*, *C. anthelata*, *Fimbristylis spiralis* and *Abildgaardia vaginata* form a monophyletic group sister to species of *Abildgaardia*. Bootstrap support values are given below the branch and decay indices above. The dashed lines indicate collapsed branches obtained from the strict consensus. See Appendix 1 for specimen details and Appendix 2 for characters used in analysis.

Support for *Actinoschoenus* and *Arthrostylis* as outgroup taxa was not strong; with the internal branch connecting the *Actinoschoenus–Arthrostylis* and *Schoenoplectus–Schoenoplectiella* as sister groups collapsing early in Bremer support analysis.

Noteworthy characters

Vascular bundle anatomy (including photosynthetic pathway) and embryo characters had significant association with group formation as indicated by branch support for the internal branching of *Abildgaardia*, *Crosslandia*, *Actinoschoenus–Arthrostylis*, and *Schoenoplectus–Schoenoplectiella* (Figure 3.11). Outgroup and ingroup were largely separated on characters relating to differences in photosynthetic pathway. The observed differences in embryo morphology between the generic groups, which linked *Abildgaardia vaginata*, *Fimbristylis spiralis* and *Crosslandia* proved to be important within the cladistic analysis (see Figure 3.11).

Observations

Species that form the *Crosslandia* complex display considerable morphological variability – specifically in *Crosslandia setifolia* and *C. anthelata* inflorescence–synflorescence structure and floret sex distribution within the spikelets.

Inflorescence–synflorescence structure

Inflorescence–synflorescence structure within the *Crosslandia* group was highly variable (Figures 3.12–17). A simple solitary spikelet or one to two primary lateral rayed spikelets are common in *Fimbristylis spiralis* (Figure 3.12). Solitary spikelets were also observed in *Abildgaardia vaginata*, along with primary lateral rays that may be substituted by one or two primary sessile spikelets. Occasional secondary

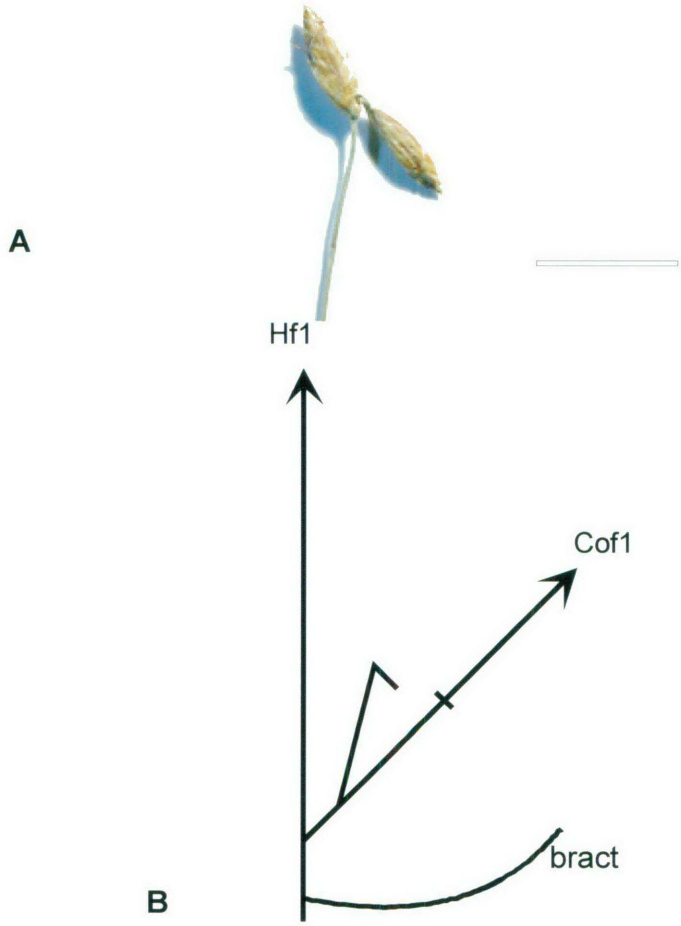


Figure 3.12 Highly reduced anthelodium A. *Fimbristylis spiralis* (ISOTYPE fragment BRI AQ341194) with bisexual florets that are spirally arranged (scale bar=10 mm). B. Diagramatic representation of image A. HF1=main florescence; Cof1=primary coflorescence which bears a prophyll indicating the lateral growth. The lowest bract subtends the coflorescence. Bar across the coflorescence indicates that the spikelet is 'rayed' as the epipodia is lengthened.

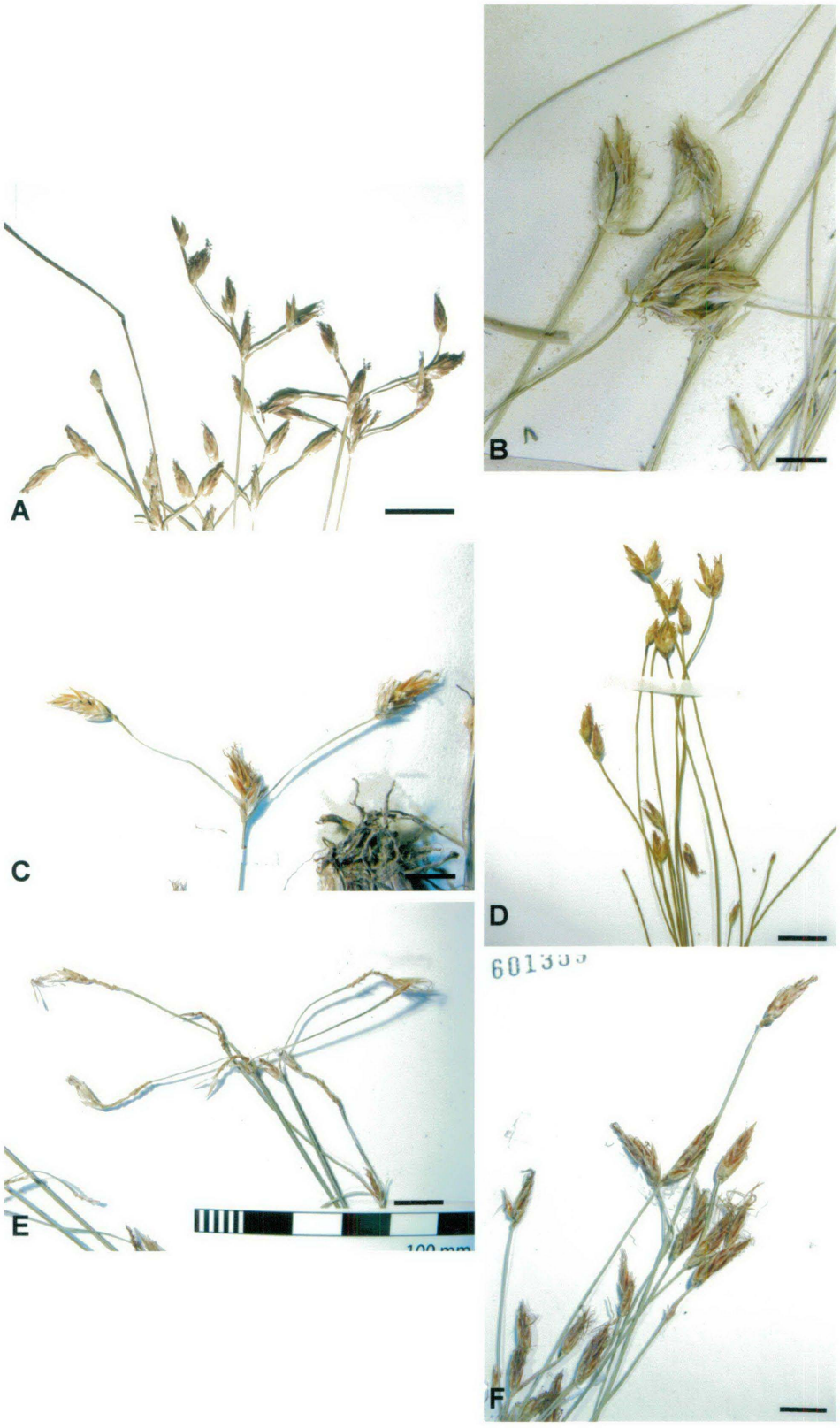


Figure 3.13. Inflorescence–synflorescence variation observed within *Abildgaardia vaginata*. A. Sample Av7 collected from the Northern Territory with open primary anthelodia and depauperate secondary florescences. B. Multiple spikelets that may be sessile or on lengthened epipodia (rays) produce a congested inflorescence (Av13). C. Open reduced anthelodia are seen in Queensland material (Av11) with primary florescences. D. Geminate spikelets (primary coflorescence is sessile) or a single rayed primary coflorescence in Av5 fits the TYPE description by Brown (1810). E. Extremely lengthened epidodia are unique and seen in Av6 from the Northern Territory F. The simplest inflorescence type, solitary spikelets (Av10), are common in material collected from SE Queensland and NSW. Drawn scale bars=10 mm

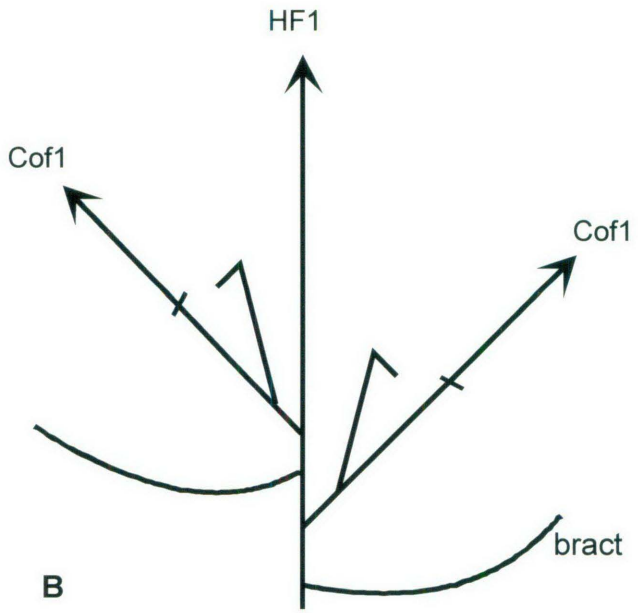


Figure 3.14 The reduced anthelodium. A. *Crosslandia anthelata* (C20) showing the simple structure of the inflorescence formed from the main florescence (HF1) and two primary rayed (indicated by the bar) coflorescences (Cof1) as represented in B (scale bar=5 mm).

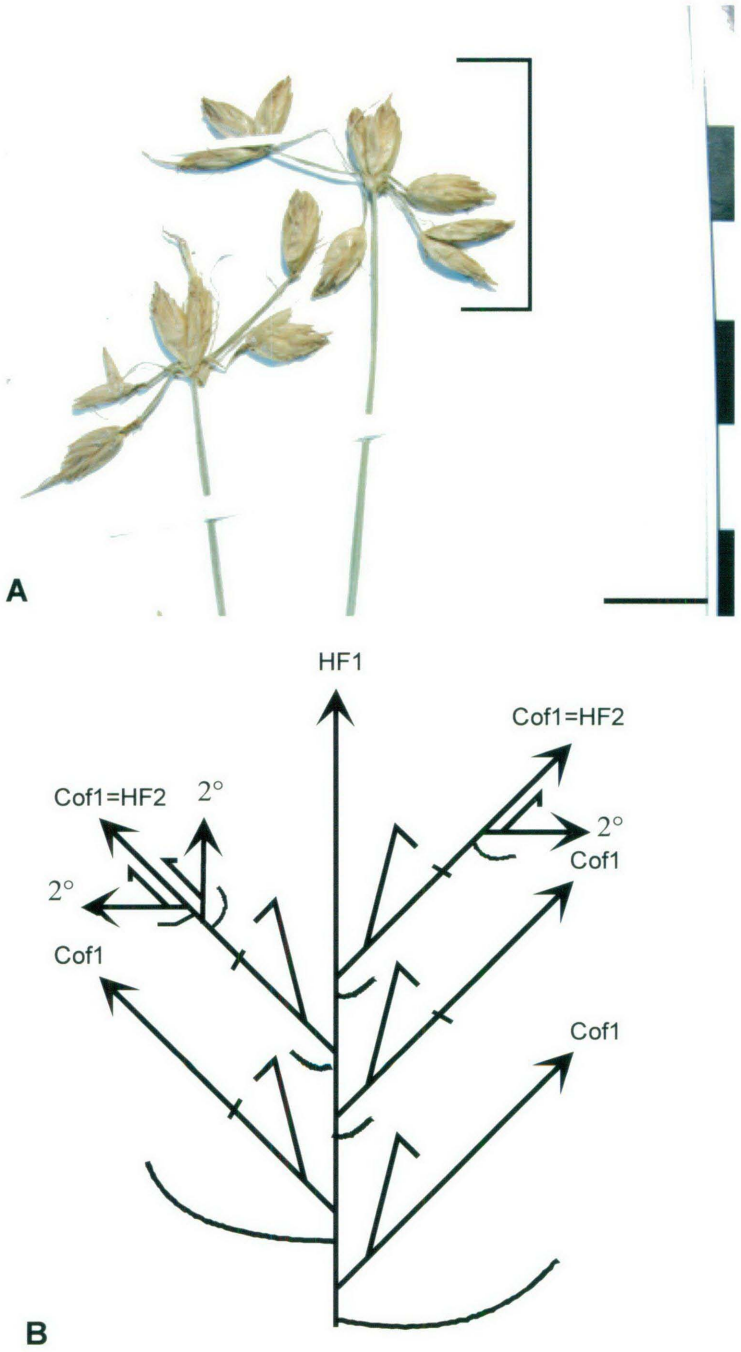


Figure 3.15 Highly reduced secondary anthelodium. A. *Crosslandia anthelata* (C19) inflorescence with multiple rayed coflorescences (Cof1), indicated by the bar in the schematic diagram B. Some sessile secondary paracladia (2°) are present on two of the rayed coflorescences that now represent the secondary main florescence (HF2) (scale bar=8 mm).

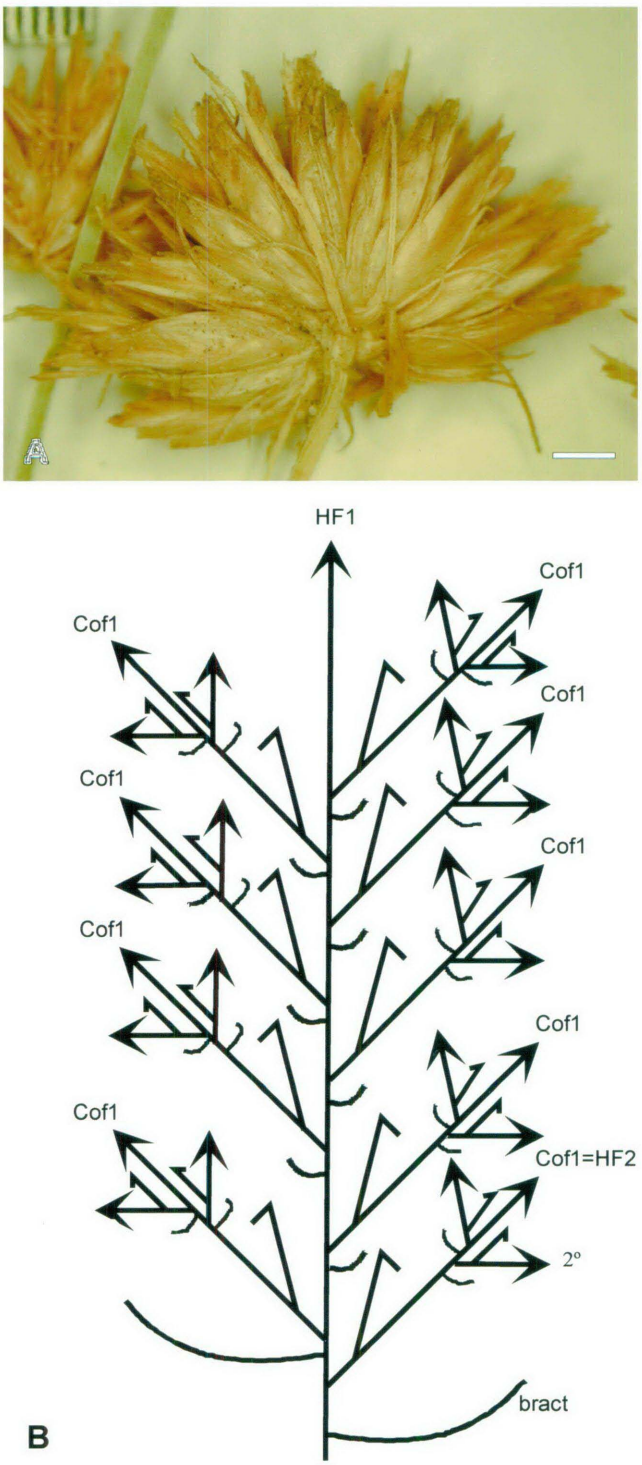


Figure 3.16 A terminal head of spikelets as sessile ramified reduced anthelodia.
A. *Crosslandia setifolia* (C14) has the synflorescence structure that is typical for the TYPE collection from Goody Goody, Western Australia (scale=2mm). B. All primary (HF1 and Cof1) and secondary (2°) spikelets are sessile in the schematic for the synflorescence of A. See Appendix 1 for specimen details.

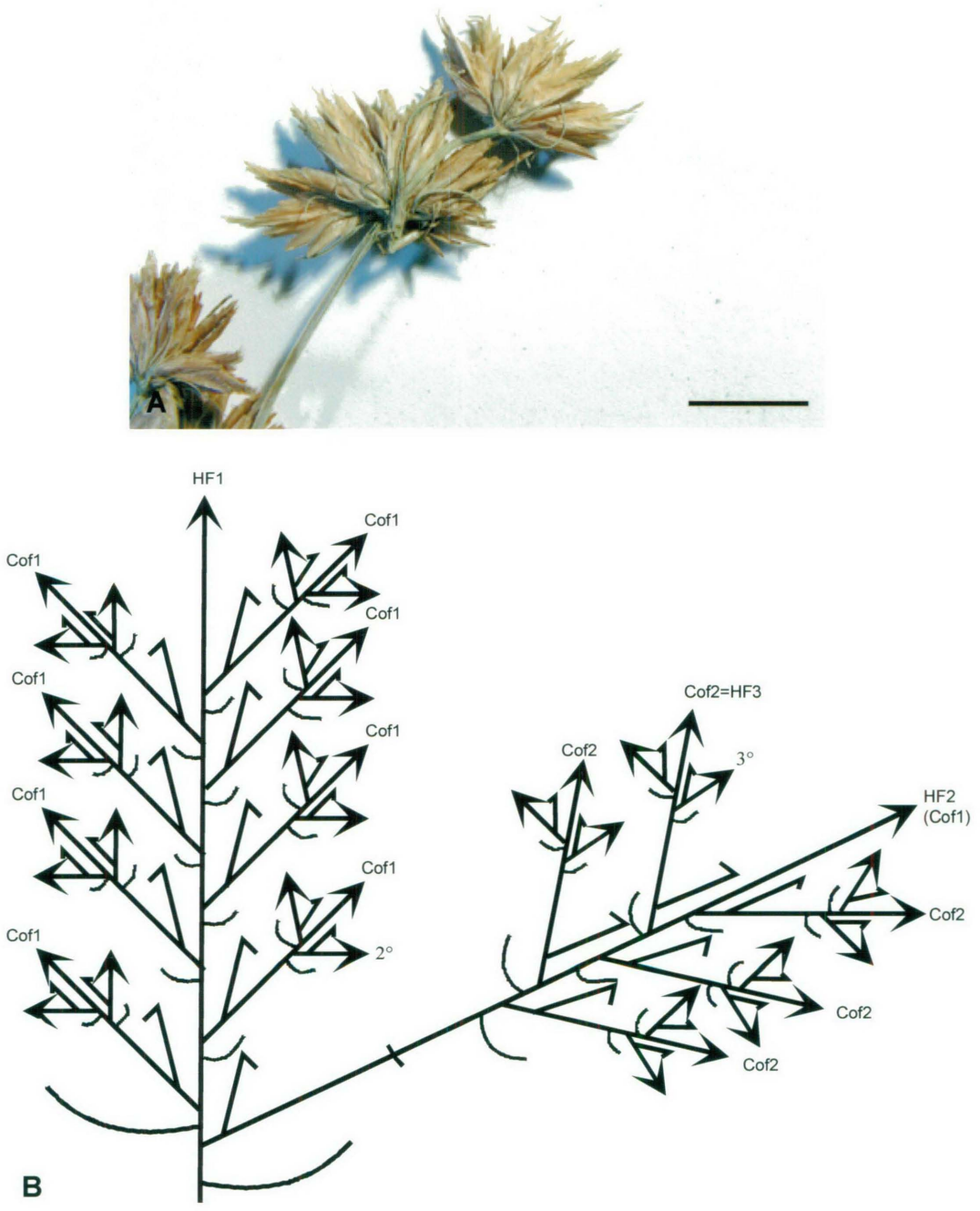


Figure 3.17 Lateral head of sessile spikelets. A. In *Crosslandia setifolia* lateral heads of spikelets may be formed in addition to the terminal head (e.g. in C14 scale=8 mm). The extended growth of one (rarely two) epipodium or 'ray' (marked with a bar) produces sessile secondary coflorescences (Cof2) with sessile tertiary paracladia (3°) as represented in the schematic diagram B. See Appendix 1 for specimen details.

lateral branches (secondary paracladia) arising from the primary coflorescence may also be present (Figure 3.13).

The reduced anthelodia of primary rayed spikelets (primary coflorescences) seemed to separate specimens of *Crosslandia* collected in Kakadu National Park, Northern Territory (Figure 3.14) easily from material producing 'heads' of sessile primary coflorescences collected in Western Australia (the origin of the TYPE) (Figure 3.16). Sampling across the range, however, revealed synflorescences with:

1. usually simple rayed spikelets as 2–4 coflorescences (Figure 3.14);

2. a mixture of 4–6 coflorescences, sessile or rayed, bearing solitary, or 2–3 sessile spikelets as primary coflorescence paracladia (Figure 3.15);

3. heads of sessile spikelets formed from primary florescences, i.e. main florescence plus primary coflorescences and their paracladia (Figure 3.16); and

4. characters as for 3, with occasional lengthened primary ray or rays, bearing a lateral 'head' of spikelets (Figure 3.17).

Although no specimens from Western Australia were observed to have the simplest inflorescence–synflorescence structure (see type 1 above), samples from the Northern Territory displayed all four types.

Floret sex

Of the 23 specimens of *Crosslandia setifolia* s.l. sampled, 19 have female florets distal within some aerial spikelets (Table 3.4 and Figure 3.18). When present, female florets always occur in the distal position of aerial spikelets (Figure 3.18 B–E) and

Table 3.4 Floret sex distribution seen in aerial spikelets for sampled specimens in *Crosslandia setifolia* and the provisional *C. anthelata*. W.A. = Western Australia, N.T. = Northern Territory. See Table 3.1 and Appendix 1 for OTU label and specimen details.

Species	OTU label	State	Floret sex bisexual	Floret sex male	Floret sex female
C. setifolia	C2	W.A.	1(distal)	1(proximal or all)	1(distal)
C. setifolia	C3	W.A.	1(mid only with distal female)	1(proximal or all)	1distal
C. setifolia	C7	W.A.	1(proximal or all)	0	1(distal)
C. setifolia	C9	W.A.	1(proximal)	1(proximal) or all florets	1(distal)
C. setifolia	C10	W.A.	1(proximal, distal or all)	1(proximal) or all florets	1(distal)
C. setifolia	C12	W.A.	1(mid)	1(proximal or all)	1(distal)
C. setifolia	C14	W.A.	0	1(proximal or all)	1(distal)
C. setifolia	C16	W.A.	1(proximal or all)	0	1(distal)
C. setifolia	C17	W.A.	0	1(proximal) or all florets	1(distal)
C. setifolia	C1	N.T.	1(proximal)	1(proximal or all)	1(distal)
C. setifolia	C4	N.T.	1distal)	1(proximal or all)	1(distal)
C. setifolia	C5	N.T.	1(mid)	1(proximal) or all florets	1(distal)
C. setifolia	C6	N.T.	0	1(proximal or all)	1(distal)
C. setifolia	C8	N.T.	0	1(proximal or all)	1(distal)
C. setifolia	C11	N.T.	0	1(all)	0
C. setifolia	C13	N.T.	0	1(all)	0
C. setifolia	C15	N.T.	1(proximal)	1(proximal or all)	1(distal)
C. anthelata	C18	N.T.	0	1	0
C. anthelata	C19	N.T.	1(proximal or all)	0	1(distal)
C. anthelata	C20	N.T.	0	1(proximal or all)	1(distal)
C. anthelata	C21	N.T.	1(mid)	1(proximal or all)	0
C. anthelata	C22	N.T.	0	1(proximal or all)	1(distal)
C. anthelata	C23	N.T.	0	1(proximal or all)	1(distal)

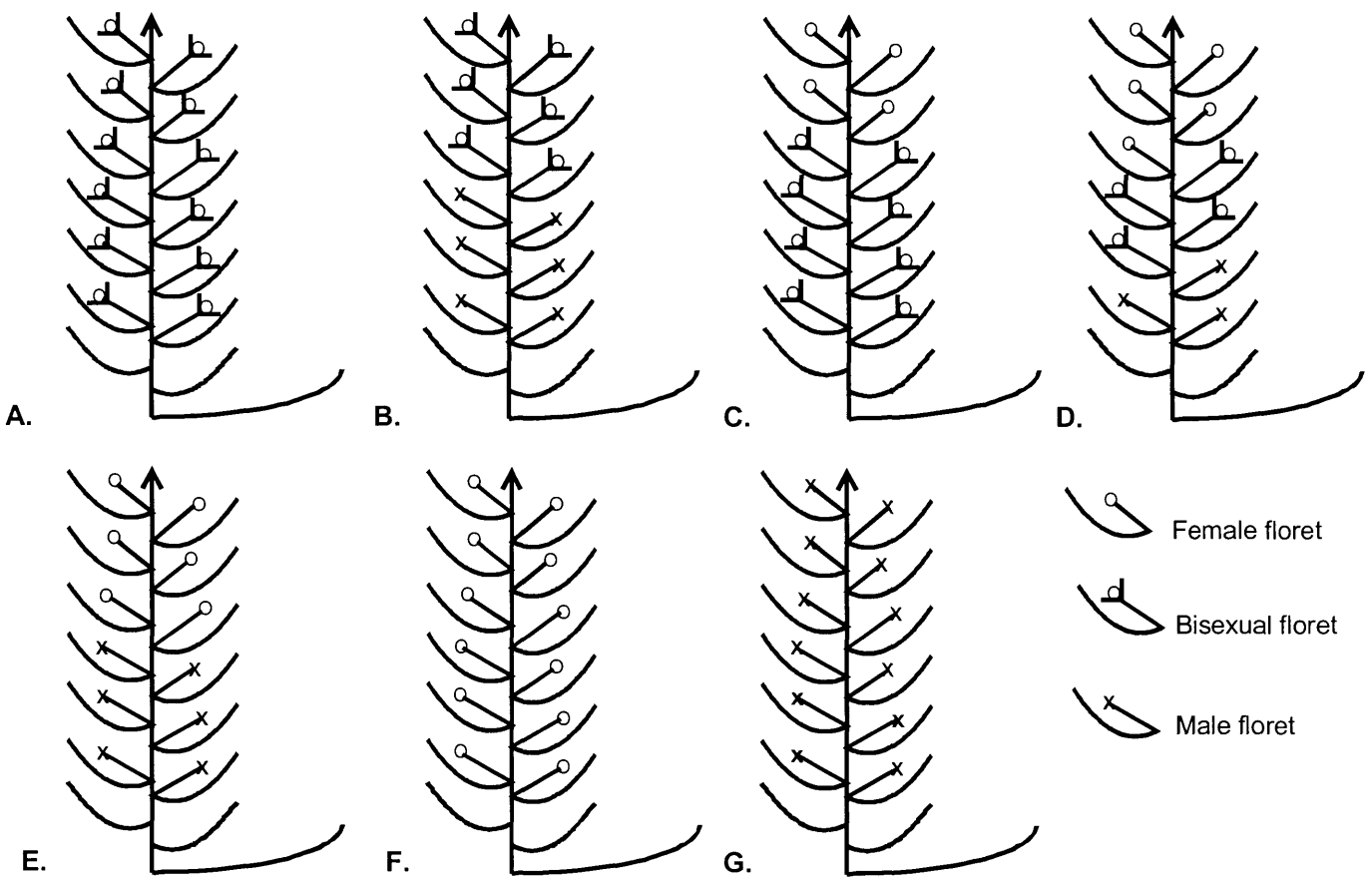


Figure 3.18 Floret sex variation exhibited in aerial or basal spikelets within *Crosslandia* as defined here. *Crosslandia setifolia, C. anthelata* (A-G see Table 3.3 for sample list and floret sex distribution) *Fimbristylis spiralis* (A), and *Abildgaardia vaginata* (A and rarely C). The all female florets in spikelet F. occur only in basal spikelets.

are easily recognised by the change in shape of the spikelet due to the narrowly elongated glumes. These distal female aerial portions usually resemble the all female basal spikelets that also have narrowly elongated glumes, and are conspicuously clustered at the base of the plant. Female florets (aerial or basal) appear to have never possessed rudimentary androecia, as no male parts were visible in mature female florets and filament scars were not observed.

Bisexual florets within aerial spikelets occurred in 13 of the 23 specimens sampled. Distribution of the bisexual florets varied considerably, from being positioned proximally (following the non-reproductive glumes with female florets distal) (Figure 3.18 C), at mid-spikelet (following male functional florets with female florets distal) (Figure 3.18 D), or distally (following male functional florets) (Figure 3.18 B). In four of the 13 samples, some spikelets were observed to have only bisexual florets (excluding the lowest empty glumes). When spikelets of *Crosslandia setifolia* and *C. anthelata* consisted of all bisexual florets, it appears that the female proximal florets were undeveloped, as other spikelets in the same samples had female distal florets present. The bisexual floreted spikelet in *Crosslandia setifolia* and *C. anthelata* may easily revert to unisexual female florets distally and therefore does not consistently produce bisexual only spikelets as seen in *Abildgaardia*, *Fimbristylis* and *Bulbostylis*.

The pattern of sexuality seen in the TYPE specimen of *Crosslandia setifolia* with aerial spikelets consisting of all functionally male florets (Figure 3.18 G) was represented by only three of the 23 specimens sampled. Those three specimens, however, were not restricted to Western Australian material. In the other material sampled, male only spikelets regularly occurred amongst plants with female distal or

bisexual mix types. Rudimentary female organs were always present in the male florets of all specimens.

It was possible then for a single plant to possess aerial spikelets with floret sex arranged as: all male, male proximal and bisexual distal, male proximal and female distal, bisexual proximal and female distal, or all bisexual. In contrast, bisexual florets were constant in aerial spikelets in specimens of *Abildgaardia vaginata* and *Fimbristylis spiralis* (Figure 3.18 A), except for one of the sampled specimens of *A. vaginata* (Av14), where female florets were present in some spikelets.

Floret sex in basal spikelets was more constant – usually bearing all-female florets in a spikelet (Figure 3.18 F). Some basal spikelets in *Crosslandia setifolia* (not observed in *C. anthelata*), *F. spiralis* and *A. vaginata* contained minor variations in floret sex. Basal spikelets with all bisexual florets (C15, F1and Av12), or bisexual proximal and female distal florets (C17) were concealed amongst the usual all-female spikelets. No functionally male-only florets were seen in basal spikelets in any of the material examined.

No correlation was apparent between the distribution of floret sex within aerial or basal spikelets and inflorescence pattern within *Crosslandia setifolia* or *C. anthelata*.

Basal spikelets

The presence of basal spikelets forms one of the main consistent features for the group (Figure 3.19). Basal spikelets were observed in 10 of the 103 specimens of *A. vaginata* examined for this study, and were usually poorly developed and easily overlooked. Three of these 10 specimens were included in analyses; only specimen Av8, collected from New South Wales, produced a mature nut in a well-developed

basal spikelet (Figure 3.20). The other two sampled specimens where basal spikelets were observed (Av10 and Av12) were collected from Queensland.

The morphology of basal spikelets in all species that form the *Crosslandia* group was distinct from aerial counterparts in the shape of spikelets, and floret features such as glume shape and length, style length, and nut size (Figures 3.20–21). With the exception of *Abildgaardia vaginata*, the nuts in the plants examined were produced mainly from the conspicuous basal spikelets (Figure 3.22).

Embryo morphology

Embryo morphology for *Abildgaardia vaginata*, *Crosslandia* and *Fimbristylis spiralis* was the Crosslandia-variant of the Fimbristylis-type (Figure 3.23 A–E). The maturity of the embryo is crucial when defining the type; immature embryos show the Schoenus-type embryo formation (Figure 23 F).

Vegetative anatomy

Abildgaardia vaginata occasionally produces leaf blades and these were compared with species of *Crosslandia* and *Abildgaardia*. Leaf blade and culm anatomy for *Fimbristylis spiralis* and *Abildgaardia vaginata* were of the general C_4 fimbristyloid type, as was *Crosslandia*.

Transverse sections revealed some differences among the other species assessed for the *Crosslandia* complex. There were three to four hypodermal layers in the leaf blade of *Abildgaardia vaginata*, while these cells may be absent from *Crosslandia* and *Fimbristylis spiralis* or present as one or two rows (the second often an incomplete row) (Figure 3.24). *Abildgaardia ovata* also had hypodermal tissue formed from one complete row and some cells in an incomplete second row, but the

Figure 3.19 Basal spikelets that show different morphology to aerial spikelets observed in *Crosslandia* s.l. Some of the variation observed in basal spikelets of A. *Crosslandia setifolia* (C14), B. *C. anthelata* (C21), C. *Abildgaardia vaginata* (Av8) and D. *Fimbristylis spiralis* ISOTYPE fragment (BRI AQ341194) (scale=5 mm). See Appendix 1 for OTU and specimen details.

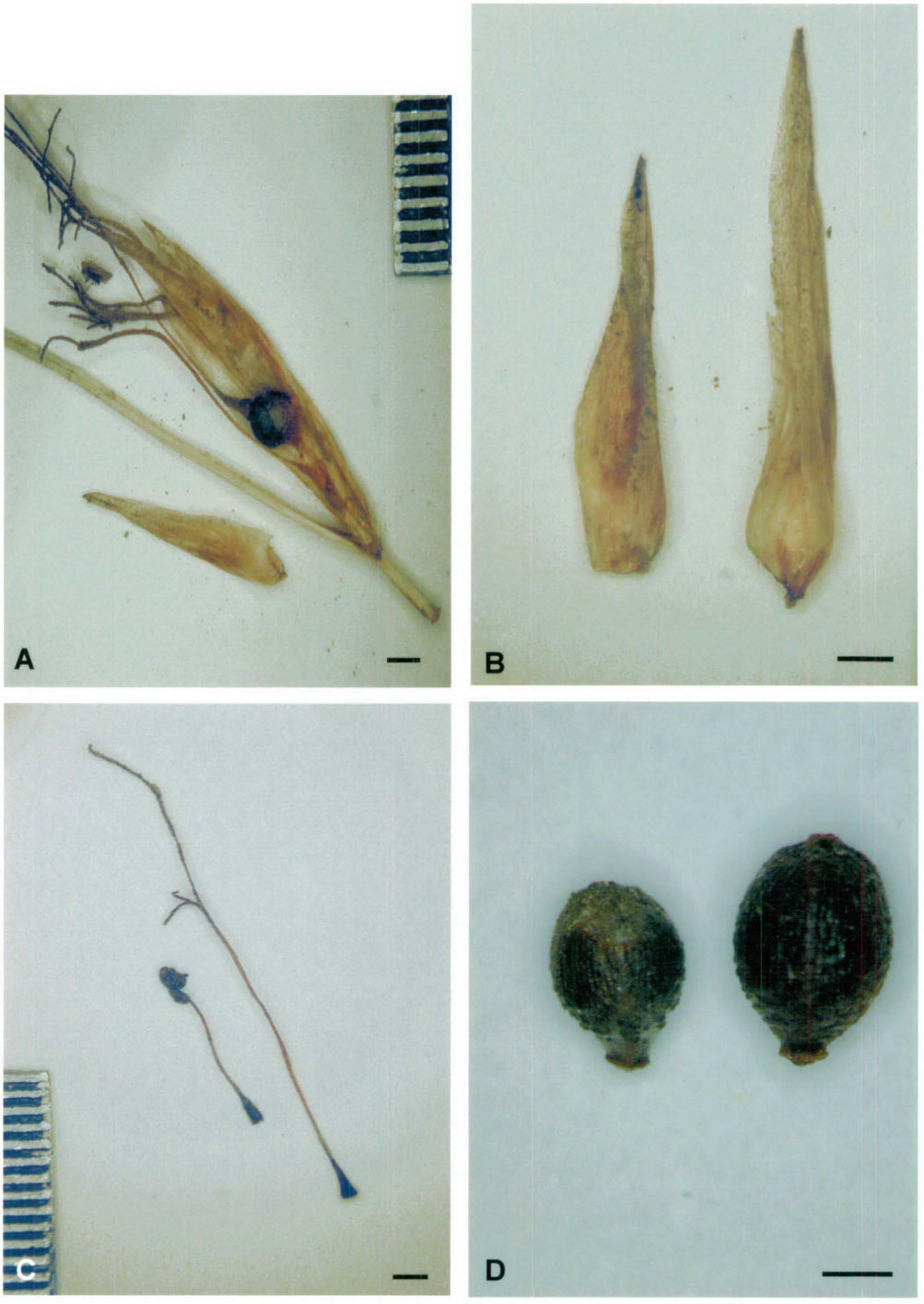


Figure 3.20 Comparative floral parts from aerial and basal spikelets of *Abildgaardia vaginata* (Av8). A. Basal spikelet with mature nut. B. Aerial glume (left) and basal glume (right) showing size difference. C. Aerial style is distinctly shorter than the basal style. D. Aerial nut (left) is distinctly smaller than the basal nut (right). Scale=1 mm.

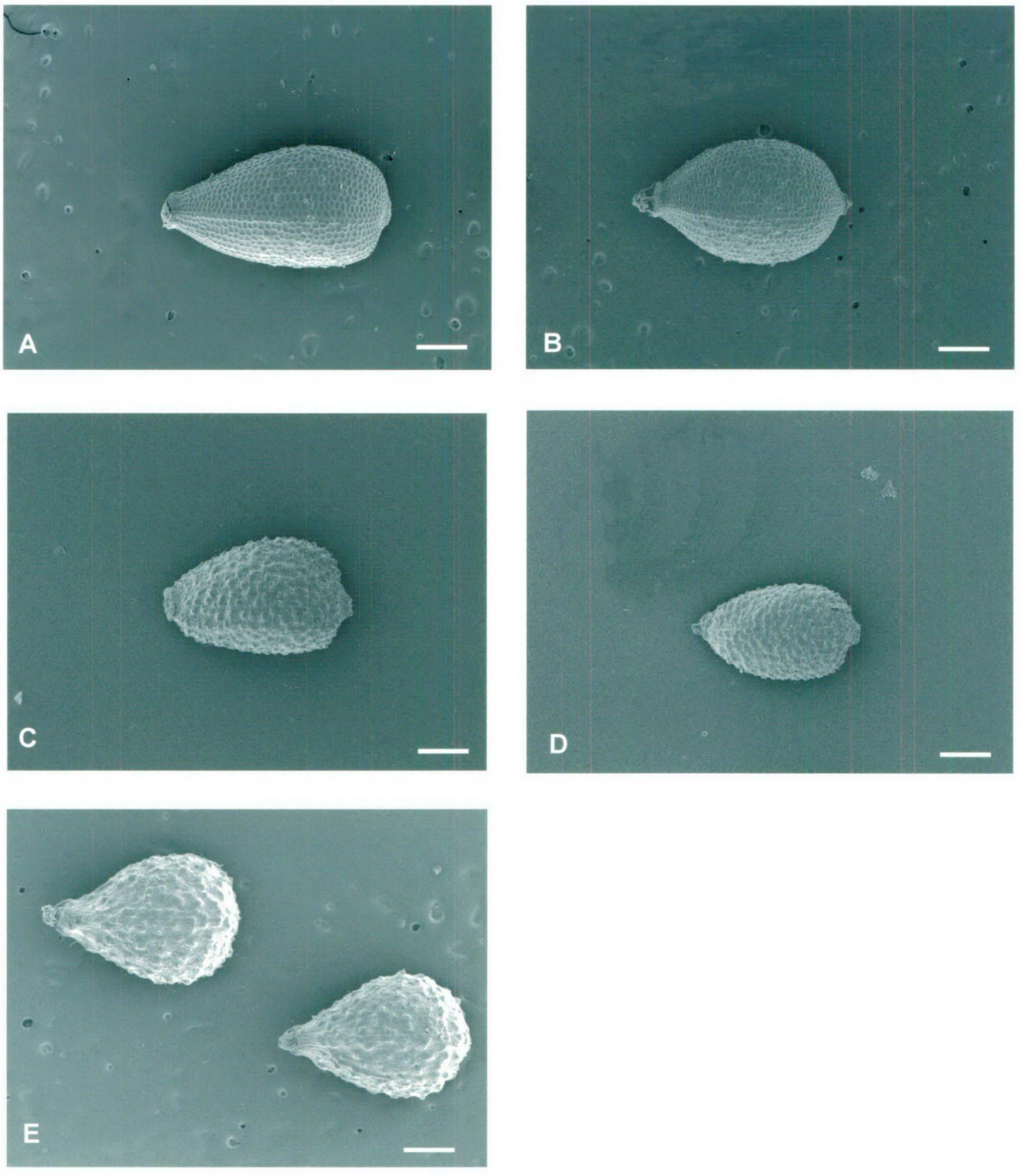


Figure 3.21 Scanning electron micrographs of nuts from the provisionally defined *Crosslandia* s.l. Similarities in the nut features for provisional *Crosslandia anthelata* (C19) A. basal nut and B. aerial nut; *Fimbristylis spiralis* (F1) C. basal nut and D. aerial nut; and E. aerial nuts from *Abildgaardia vaginata* (Av12). Scale=500 μm. See Appendix 1 for specimen details.

Figure 3.22 Basal spikelets at the plant base of *Crosslandia setifolia* (C12 collected from Western Australia). Many aggregated sessile spikelets are distinct at the plant base and exhibit different morphology to the aerial spikelets. Most of the nuts produced by the plant are from these basal spikelets. Scale bar=125 mm. See Appendix 1 for specimen details.

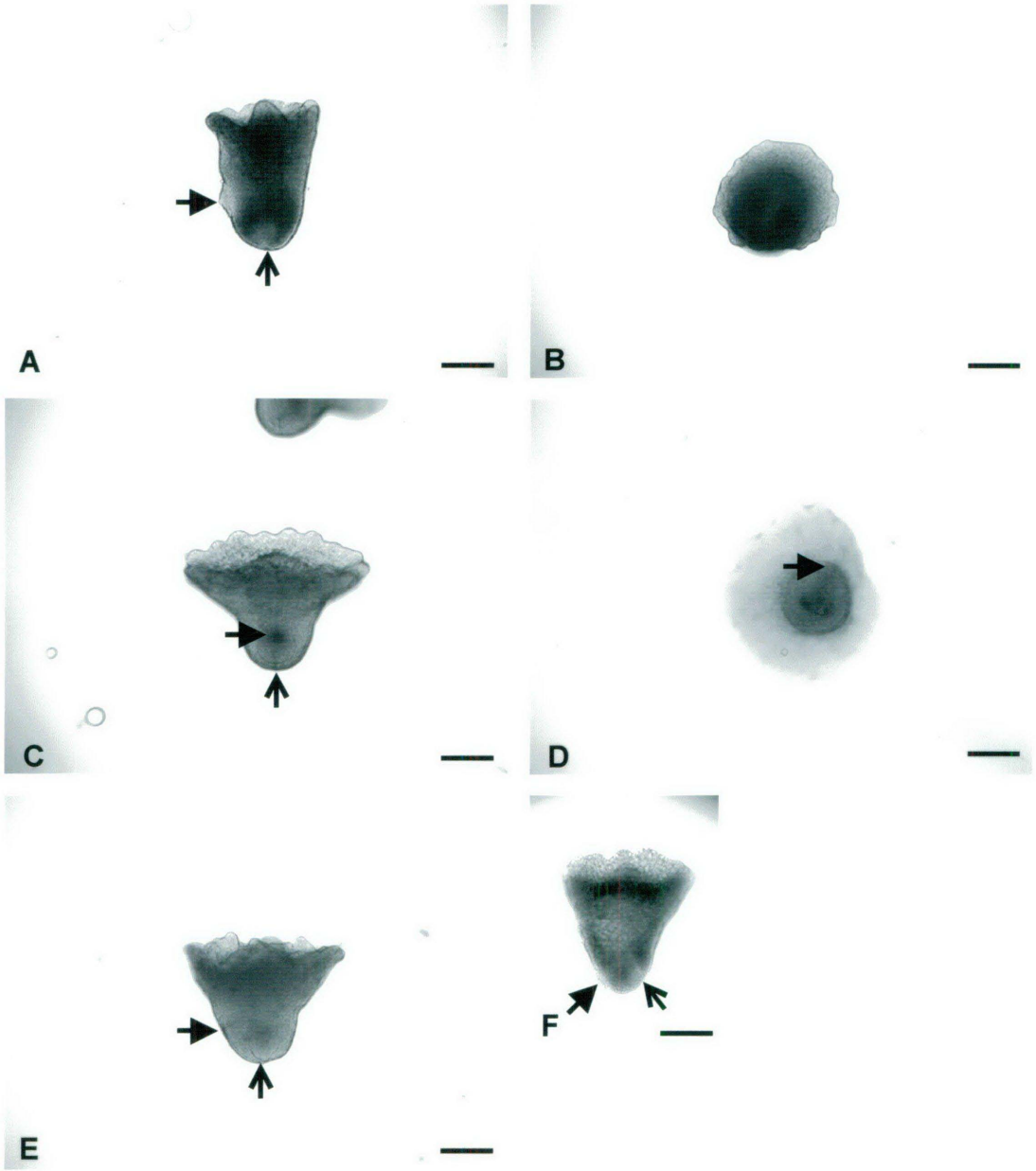


Figure 3.23 Variation in embryos of *Crosslandia setifolia* and *Abildgaardia vaginata*. A. and B. *Crosslandia setifolia*, C17 collected from Western Australia, C. and D. *Abildgaardia vaginata* Av2 collected from New South Wales, and E. Av3 (collected from Queensland), share the Fimbristylis-type embryo. F. *Abildgaardia vaginata* Av6 (embryo not fully developed – collected from the Northern Territory) has the Schoenus-type embryo, where the primordial shoot and root are situated sub-basally (see text p 80). Scale bar=200 μm. See Table 3.1 and Appendix 1 for specimen details.

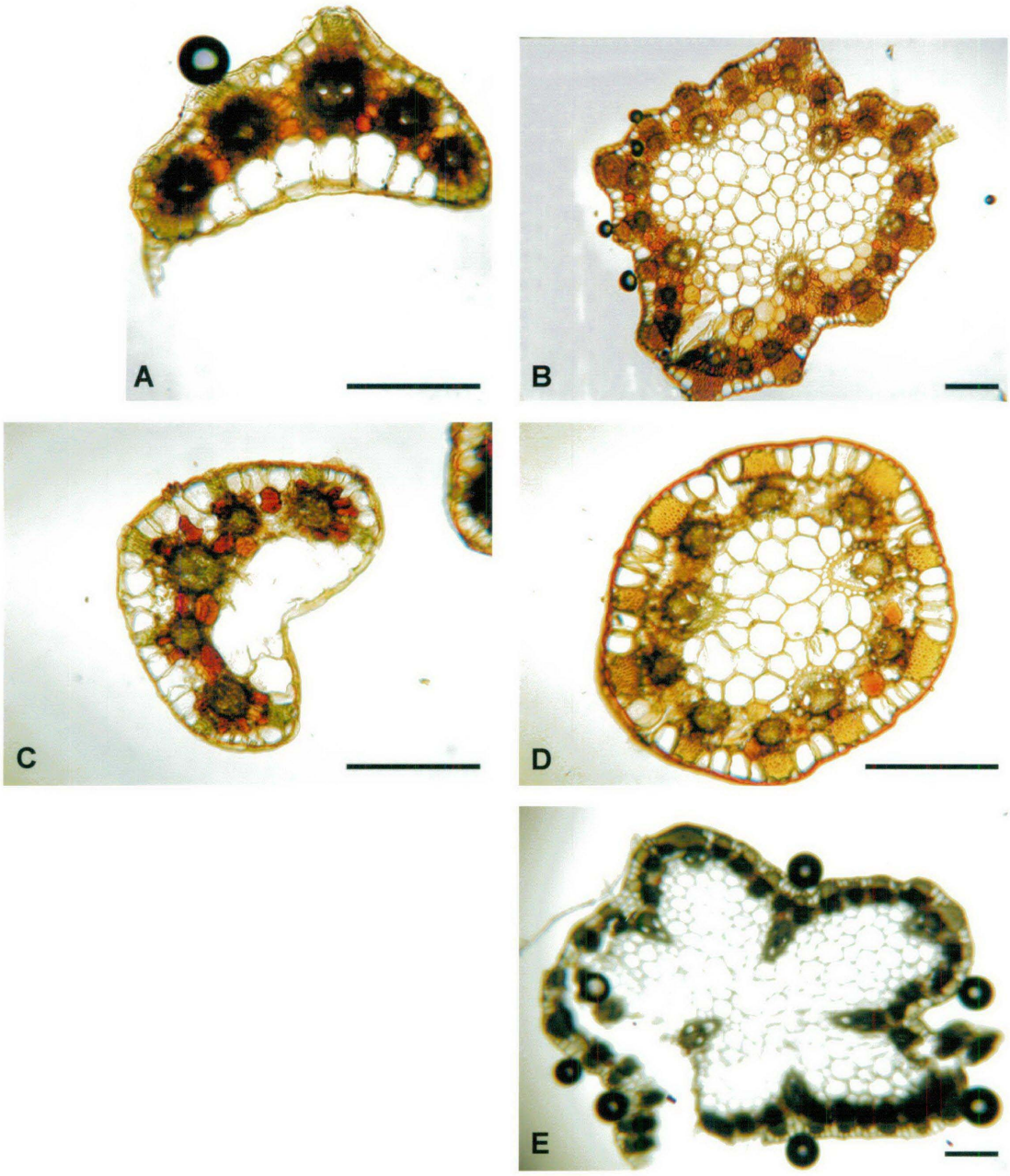


Figure 3.24 Leaf and/or culm transverse sections showing C_4 fimbristyloid anatomy. A. *Crosslandia setifolia* (C17) leaf and B. culm; C. *Fimbristylis spiralis* (F3) leaf and D. culm; E. *Abildgaardia vaginata* (Av6) culm. Scale bar=200 μm. See Table 3.1 and Appendix 1 for specimen details.

hypodermis is not a constant feature for species of *Abildgaardia* (see Chapter 4 for details).

Culm shape for the *Crosslandia* complex was either elliptic to wavy (sometimes deeply) elliptic with the number of sclerenchyma strands constantly less than the number of vascular bundles. In contrast, sclerenchyma strands in species of *Abildgaardia* equalled the number of vascular bundles within the culm.

Discussion

The four separate species (*Crosslandia anthelata*, *C. setifolia*, *Fimbristylis spiralis* and *Abildgaardia vaginata*) within *Crosslandia* as found in phenetic analyses, formed a monophyletic group in the cladistic analysis. Evidence from phenetic and cladistic studies indicates that all four taxa should be recognised as species in the genus *Crosslandia*.

Cladistic analysis clearly finds that *Fimbristylis* is non-monophyletic, with the combined *Crosslandia–Abildgaardia* and *Fimbristylis* s.s.–*Bulbostylis* clade nested within two species currently accepted in the genus *Fimbristylis*. The nested species of *Bulbostylis* also renders the *Fimbristylis* s.s. clade non-monophyletic. The position of *Bulbostylis* is possibly an artifact of the small sample size of *Bulbostylis* used in this analysis (see Chapters 5 for greater detail on *Bulbostylis*).

The genus *Fimbristylis* is large and morphologically heterogeneous and its taxonomy is unresolved. Although *Crosslandia* is nested within taxa currently accepted as *Fimbristylis*, sinking *Crosslandia* into *Fimbristylis* is not recommended while the generic limits for *Fimbristylis* are uncertain. It will take some time to

resolve taxonomic issues due to the large number of species assigned to *Fimbristylis*, and the great amount of variation present across those species. Meanwhile, defining the species and generic limits for *Crosslandia* could only benefit future work on *Fimbristylis*. The presence of basal spikelets across the *Crosslandia* group, plus the floret sex variation in *Crosslandia setifolia* and *C. anthelata* do not fall into the current generic limits for *Fimbristylis*; this adds to the justification of maintaining *Crosslandia*.

New combinations can now be made for the species of *Crosslandia,* based on the phenetic and cladistic data. This study supports Goetghebeur (1986) in that the *Crosslandia* 'rayed' and 'capitate spikeleted' forms of inflorescence–synflorescence, although highly variable, can be separated into two entities. The TYPE specimen for *Crosslandia setifolia* from Western Australia represents the densely spikeleted form, which is consistent for the State. Inflorescence variation is greatest in the Northern Territory for the current *Crosslandia setifolia* as recognised here.

The occurrence of all four species in the Northern Territory indicates that this area is the centre of diversity for the *Crosslandia* group. Although there were limited samples of *Fimbristylis spiralis,* this species appears to be morphologically uniform and apparently geographically isolated in coastal north-east Arnhem Land. *Abildgaardia vaginata* shows a broader range of distribution, occurring in the Northern Territory, Queensland, and coastal New South Wales. The diminished capacity to produce basal spikelets, the presence of bisexual florets and the extended distribution reflects the adaptability of this species compared to other species of *Crosslandia*. The synapomorphy of functionally unisexual florets in the aerial spikelets of the mixed floret sex seen in *Crosslandia setifolia and C. anthelata* indicates evolution from the bisexual condition, and distinguishes the two species

from *Abildgaardia vaginata* and *Fimbristylis spiralis*, both of which regularly have bisexual aerial florets (see Figure 3.18 and Table 3.3). A concurrent study tracing the molecular phylogeny of some members of the tribe Abildgaardieae (Ghamkhar et al. 2005, in press) showed strong support for the genetic closeness of *Abildgaardia vaginata* and *Crosslandia setifolia*. However, the restricted sample size used in the study (one specimen of *Abildgaardia vaginata* – from New South Wales; five *Crosslandia* s.l. specimens – three from Northern Territory, two from Western Australia; and no *Fimbristylis spiralis* samples) provided no additional insight into the relationships of the *Crosslandia* group to that already obtained from plant morphology, anatomy and embryo morphology of this study. More detailed gene analyses on populations for all four species would be necessary to assess fully the genetic diversity and thereby the genetic relationships within the *Crosslandia* group. Additional sampling, generally between the Western Australian and Northern Territory gradient, is also recommended, including sampling closer to the *Fimbristylis spiralis* sites.

The current description of inflorescence variation for *Crosslandia setifolia* is clearly inadequate. Also, the observed variation of mixed floret sex within spikelets in *C. setifolia* and *C. anthelata* (see also Goetghebeur 1986) has not been formally published.

Unisexual flowers are common in some groups of the Cyperaceae and have independent origins (Bruhl 1995; Goetghebeur 1998). The mixed floret sex seen in *Crosslandia* is exceptional within the tribe Abildgaardieae, and the spikelet sex arrangement is similar to the expressed floret sex in species of *Scleria*; explaining the placement of *Crosslandia* into Sclerieae by Hutchinson 1934, 1959). Terminal female spikelets with no male rudiments occur in several species of *Scleria* (includes

Acriulus), and intermediates occur between the bisexual spikelet, male spikelet and the strictly female spikelet (Kern 1963). *Scleria* differs from *Crosslandia* in having spikelets that consist of a single fertile floret (spikelets being female, male or bisexual), rather than the multi-floreted spikelet with mixed floret sex in *Crosslandia*. There are no structural discrepancies within the *Crosslandia* spikelet to indicate that the variable sex expression results from a composite floral structure such as a 'spike' e.g. as seen in the Cariceae (Kern 1958; Timonen 1998; Starr et al. 2004), or 'synanthium' e.g. as in *Mapania* (Simpson 1992); on the contrary, all florets conform to the typical base plan of the scirpoid floral arrangement (Vrijdaghs et al. 2005).

Functional male florets possessing sterile vestigial female organs are 'type I' unisexual flowers according to Mitchell and Diggle (2005); 'type II' unisexual flowers are unisexual from inception (the floral meristem initiates only the male or female organs, omitting the hermaphroditic stage). Female florets in aerial or basal spikelets of *Crosslandia* appear to be type II flowers. Mitchell and Diggle (2005) mapped unisexual flower types onto a composite angiosperm phylogeny for dioecious taxa and then analysed the information to determine the number of evolutionary origins (Mitchell and Diggle 2005). A similar exercise could be applied to taxa with unisexual florets within the Cyperaceae, to see where *Crosslandia* and the type I and type II florets fit into the evolution of unisexual florets within the family. In addition, examining the developmental stages of both the functionally staminate and pistillate florets within *Crosslandia* could determine whether androecia in pistillate flowers become sterile at a very early stage and, therefore, these pistillate flowers could actually be type I unisexual flowers.

The attenuated glumes of female florets in *Crosslandia* have different morphology to glumes in male and bisexual florets. Some grass species (e.g. maize and *Tripsacum*), although grass spikelets are not homologous with those in Cyperaceae, may show glume morphology that correlates with unisexual floret sex expression; grass florets in the Andropogoneae always having type I unisexual florets, i.e. with vestigial male or female organs (Le Roux and Kellogg 1999). In Cyperaceae, type II female florets occur singly or in small clusters at the plant base in *Trianoptiles*, with these amphicarpous plants producing aerial spikelets with bisexual florets (Levyns 1943). The female florets are enclosed by a glumaceous 'utricle' where modified glumes tightly hold the nut (Haines and Lye 1977). In *Crosslandia*, multiple female florets occur in basal (usually solely female) or aerial spikelets (as mixed sex), where the glume (one per nut) also tightly encloses the nut; the attenuated apex of the glume wraps around and supports the long, thin, narrow style.

Delimiting *Crosslandia setifolia* s.l. into two species is consistent with the phenetic analyses and reflects the inflorescence–synflorescence variability across the sampled geographical range. All observed specimens with fewer spikelets on rays (lengthened epipodia) occur within the Northern Territory (apparently concentrated in the Kakadu National Park area), and specimens with many sessile spikelets forming distinct 'heads' occur in Western Australia; this provides support for the separation.

Despite the unavoidably small sample size, combining *Fimbristylis spiralis* into the genus *Crosslandia* and retaining species level distinction seems justified based on: differences in nut size (smaller overall), only bisexual aerial florets present, and glumes always spirally arranged (Table 3.5). Plants of *Fimbristylis spiralis* were found to be consistently smaller in habit than those of *Crosslandia setifolia* or the

provisional *C. anthelata*; however, the extreme conditions of the coastal environment where *Fimbristylis spiralis* grows may affect the plant size. Further sampling from the remote locations in the Northern Territory is needed to test this hypothesised classification.

Abildgaardia vaginata should be placed in *Crosslandia*. This move is supported by *Abildgaardia vaginata* having an embryo type similar to that of *Crosslandia*, not the *Abildgaardia*-type embryo observed for other members of *Abildgaardia* in this study. Leaf blade and culm anatomy in *Abildgaardia vaginata* are also similar to *Crosslandia*, as are basal spikelets, when present.

New combinations are now provisionally put forward prior to valid publication.

Nomenclature of *Crosslandia*

Genus: *Crosslandia* W.Fitzg.

TYPE: ***Crosslandia setifolia*** W.Fitzg.

1. *Crosslandia anthelata* Goetgh. ex K.L.Clarke J.J.Bruhl & K.L.Wilson sp. nov. ined.

2. *Crosslandia setifolia* W.Fitzg.

3. *Crosslandia spiralis* (R.Br.) K.L.Clarke & K.L.Wilson comb. nov. ined.

Fimbristylis spiralis R.Br. syn. nov. (photograph and fragments of the TYPE held at Queensland Herbarium, BRI 340661 were seen)
Iria spiralis (R.Br.) Kuntze
Scirpus spiralis (R.Br.) Poir

4. *Crosslandia vaginata* (R.Br.) K.L.Clarke comb. nov. ined.

Abildgaardia vaginata R.Br.
Fimbristylis brownii Benth.
F. leptoclada Benth (non. Fl. Hongk.)
F. vaginata (R.Br.) Domin (non Boiv. ex C.B.Clarke in Dur. & Schinz)
F. vaginata f. *leptoclada* (Benth.) Domin
F. vaginata (R.Br.) Domin f. *vaginata*

Table 3.5 Comparison of species to be assigned to *Crosslandia*. Characters taken from phenetic and cladistic analyses. *Crosslandia setifolia* is the TYPE species of the genus. W.A. = Western Australia, N.T. = Northern Territory, Qld = Queensland, N.S.W. = New South Wales.

	Crosslandia setifolia	***Crosslandia anthelata***	***Abildgaardia vaginata***	***Fimbristylis spiralis***
Inflorescence–synflorescence	distinct 'heads' of sessile spikelets, sometimes with lateral 'heads'	rayed solitary spikelets or rayed solitary plus some sessile spikelets	solitary spikelet or rayed solitary spikelets sometimes with sessile spikelets	solitary spikelet or rayed solitary spikelets
Primary coflorescence number	5-8	2-4	0-4	0-4
Floret sex within a spikelet	mixed	mixed	bisexual	bisexual
Aerial nut range: length/width	1.25/0.65 to 1.9/1.0	1.35/0.8 to 1.75/1.25	1.2/0.9 to 1.9/1.4	1.2/0.7 to 1.35/0.75
Glume arrangement	subdistichous	subdistichous to spiral	subdistichous	tristichously spiral
Basal spikelets (amphicarpy)	always present	always present	sometimes present	always present
Plant habit	annual	annual	perennial	annual
Distribution	W.A. and N.T.	N.T.	N.T., Qld, N.S.W.	N.T.

Chapter 4

Abildgaardia Vahl: a phenetic and cladistic study

Introduction

In this chapter I focus on the limits and relationships of taxa within *Abildgaardia* Vahl to determine the most appropriate taxonomic rank of *Abildgaardia* – either as a separate genus or as a section of *Fimbristylis*.

Species currently circumscribed under *Abildgaardia* (as a genus or as *Fimbristylis* section *Abildgaardia*), are concentrated in Australia (Blake 1969; Goetghebeur 1998). Australian species occur mostly in northern regions of Australia. *Abildgaardia ovata* (= *Fimbristylis ovata* (Burm.f.) J.Kern) is the only species found in temperate coastal regions of eastern Australia (extending south into New South Wales).

Kral (1971) reinstated *Abildgaardia* as a genus, elevated from *Fimbristylis* section *Abildgaardia*. *Abildgaardia ovata* (Burm.f.) Kral and *A. mexicana* (Palla) Kral were recombined for the move. Goetghebeur and Coudijzer (1984, 1985) followed the generic elevation, recognising *A. hygrophila* (Gordon-Gray) Lye (1971) and *A. triflora* (L.) Abeywickr. (syn. *A. tristachya* Vahl), as species in addition to *A. ovata*. Species that formed *Abildgaardia* were defined by the following characteristics: perennial habit, leaf sheath orifice glabrous, one to few large spikelets, spikelets laterally compressed, glumes distichous, fruits stipitate-capitate, and 3-fid style (Goetghebeur and Coudijzer 1984). Australian endemics under

Fimbristylis section *Abildgaardia*, *F. macrantha*, *F. oxystachya* (Bentham 1878) and *F. pachyptera* (Blake 1940), were provisionally named as species of the genus *Abildgaardia* (*A. macrantha*, *A. oxystachya*, *A. pachyptera* respectively) by Goetghebeur (1986) in his doctoral dissertation. The provisional names were never formalised. *Abildgaardia schoenoides* R.Br (syn. *Fimbristylis squarrulosa*) and *Fimbristylis odontocarpa* S.T.Blake were not included in Goetghebeur's studies, but fit the general characteristics of species of *Abildgaardia*.

Bulbostylis parvinux C.B.Clarke and *Fimbristylis variegata* Gordon-Gray were transferred by Lye (1971) to *Abildgaardia*. Gordon-Gray (1995) did not accept the transfer while embryographic studies were lacking. Goetghebeur and Coudijzer (1985) decided that *B. parvinux* truly belonged within *Bulbostylis* and retained *Fimbristylis variegata*, rejecting a transfer to *Abildgaardia*. Bruhl (1995) found that *Abildgaardia variegata* and *A. hygrophila* possessed C_3 anatomy, and referred to them as C_3 *Abildgaardia*. More recently Bruhl and Wilson (2005, in press) have included the former under *Fimbristylis* and the latter under *Abildgaardia.*

Kral and Strong (1999) reinstated *Fimbristylis bahiensis* Steud. into *Abildgaardia* as *A. baeothryon* St Hil. and described a new species *A. papillosa* Kral & M.Strong, which they regarded as being close to *A. baeothryon*. The limits of the genus *Abildgaardia* were extended to incorporate the two species.

I find the three species *Abildgaardia baeothryon*, *A. papillosa*, and *A. hygrophila*, more closely resemble taxa from *Fimbristylis* in their nut size, colour, and epidermal sculpturing; and glume characteristics, although superficially similar to species of *Abildgaardia*, also resemble glumes in some species of *Fimbristylis* and *Crosslandia*.

Some Australian collections with intermediate morphology did not fit into current species descriptions or keys (Sharpe 1986; Latz 1990; Rye 1992). Intermediates were observed for *A. oxystachya*, *A. pachyptera*, and *A. schoenoides* (*A.* sp. aff. *schoenoides* 1: As5 and As6, *A.* sp. aff. *schoenoides* 2: As7 and required assessment as to their placement. The putative new species *A.* sp. aff. *pachyptera* and *F.* sp. aff. *odontocarpa* also required attention.

Prior to testing the monophyly of *Abildgaardia*, it was necessary first to test and set species limits within *Abildgaardia*.

Materials and Methods

Taxa

As the previous main phenetic analysis in chapter 3 recovered *Abildgaardia* as a distinct group, only the species from *Abildgaardia* under study were included in the phenetic analyses for this chapter. All Australian taxa provisionally placed in the genus *Abildgaardia* (*A. oxystachya*, *A. pachyptera*, *A. macrantha*) by Goetghebeur (1986) plus *A. ovata, A. schoenoides* and *Fimbristylis odontocarpa,* were sampled to allow species level assessment. Specimens of *Fimbristylis* sp. aff. *odontocarpa*, and *A.* sp. aff *pachyptera* were included in the group requiring phenetic analysis.

A total of 62 specimens for *Abildgaardia* formed the basis for the phenetic study (Table 4.1). Specimens sampled were selected to encompass morphological variability over the geographic range of available material. *Abildgaardia ovata* is the only cosmopolitan species of the genus occurring in Australia. *Abildgaardia vaginata* was not included in this study; this species was removed from *Abildgaardia*

Table 4.1 Specimens sampled as the focus group in the assessment of Australian *Abildgaardia*. The 'OTU' corresponds to the sample used in phenetic analyses. States are given for Australian collections. N.T. = Northern Territory, W.A. = Western Australia, Qld = Queensland, N.S.W. = New South Wales. See Appendix 1 for specimen details.

Species	OTU	State	Collector
Abildgaardia	Aov1	Qld	Specht R.L. 408, Reeves R.D.
ovata	Aov2	Qld	Batianoff G.N. 11056
	Aov3	Qld	O'Shanessy P.A. 1656
	Aov4	N.S.W.	Wilson K.L. 5818
	Aov5	N.S.W.	Johnson L.A.S.
	Aov6	N.S.W.	Rodd A.N. 2277
	Aov7	N.S.W.	Mueller F.
	Aov8	N.S.W.	Rodd A.N. 2434
	Aov9	Qld	Stanley T. 8019
	Aov10	Qld	Neldner V.J. 3905
	Aov11	Qld	Clarke K.L. 99, Bruhl J.J.
Abildgaardia	As1	W.A.	Wilson K.L. 4888
schoenoides	As2	W.A.	Dunlop C.R. 7838
	As3	W.A.	Mitchell A.A. 2129
	As4	N.T.	Dunlop C.R. 8651, White
	As7	Qld	Bruhl J.J. 487
	As8	Qld	Jacobs S.W.L. 5903
	As9	Qld	Clarke K.L. 70, Bruhl J.J.
	As10	WA	Clarke K.L. 157, Bruhl J.J, Wilson K.L.
	As11	N.T.	Clarke K.L. 216, Bruhl J.J, Wilson K.L., Cowie I.D.
	As12	W.A.	Clarke K.L. 120, Bruhl J.J, Wilson K.L
	As13	N.T.	Clarke K.L. 230, Bruhl J.J, Wilson K.L., Cowie I.D.
	As14	N.T.	Perry R. 222
A. sp. aff. *schoenoides* 1	As5	N.T.	Bruhl J.J. 1261, Hunter J.T., Egan J.
	As6	N.T.	Dunlop C.R.5863, Craven L.A.
Abildgaardia	Am1	W.A.	Hartley T.G. 14405
macrantha	Am2	N.T.	Cowie I.D. 6202, Booth R.
	Am3	N.T.	Wilson K.L. 4971
	Am4	N.T.	Dunlop C.R. 4102
	Am5	Qld	Clarkson J. 8324
	Am6	Qld	Wilson K.L. 8073, Clarkson J., Jacobs S.W.L.
	Am7	Qld	Clarkson J. 6624
	Am8	N.T.	Cowie ID 5260, Taylor S.
	Am9	N.T.	Dunlop C.R. 3453
	Am10	N.T.	Clarke K.L. 249, Bruhl J.J, Wilson K.L., Cowie I.D.
Abildgaardia	Aox1	W.A.	Latz P.K. 4038
oxystachya	Aox2	Qld	Blake S.T. 15725, Webb
	Aox3	N.T.	Bruhl J.J. 1252
	Aox4	N.T.	Latz P.K. 8667
	Aox5	N.T.	Wilson K.L. 5369
	Aox6	W.A.	Clarke K.L. 124, Bruhl J.J, Wilson K.L.
	aox7	Qld	Blake S.T. 19620
	aox8	W.A.	Cane S. 53
	aox9	W.A.	Hartley T.G. 14357

Table 4.1 cont'd

	Aox10	W.A.	Carr G.W. 4377, Beauglehole A.C.
	Aox11	N.T.	Booth R. 618
	Aox12	Qld	Blake S.T. 13611
	Aox13	N.T.	Wightman G. 424 and Dunlop C.R.
Fimbristylis sp. aff. *odontocarpa*	Aaffod	W.A.	Carey J.
Fimbristylis odontocarpa	Aod1	Qld	Blake S.T. 13582
	Aod2	Qld	Turpin G.P., Thompson E.J.
Abildgaardia pachyptera	Ap1	N.T.	Hunter J.T. 1547, Bruhl J.J.
	Ap2	N.T.	Dunlop C.R. 9041
	Ap3	N.T.	Jones M., Booth R. 24
	Ap4	W.A.	Dunlop C.R. 5339
	Ap5	N.T.	Wilson K.L. 5109, Taylor S.
	Ap6	N.T.	Wilson K.L. 5207
	Ap7	N.T.	Chippendale G
	Ap9	N.T.	Clarke K.L. 253, Bruhl J.J, Wilson K.L., Cowie I.D.
	Ap10	N.T.	Clarke K.L. 181, Bruhl J.J, Wilson K.L., Cowie I.D.
	Ap11		Adams L.A. 1715
Abildgaardia sp. aff. *pachyptera*	Aaffpa	N.T.	Clarke K.L. 201, Bruhl J.J, Wilson K.L., Cowie I.D.

and placed in *Crosslandia* (Chapter 3). *Abildgaardia vaginata*, is provisionally called *Crosslandia vaginata*.

Representative specimens of *Abildgaardia triflora*, *A. mexicana*, *A. hygrophila*, *A. baeothryon,* and *Fimbrisytlis variegata* were sampled only for cladistic analysis.

Phenetic Study

Pattern Analyses

Invariant character states were removed from the initial PATN data set used to assess the *Crosslandia* group, and the revised data subjected to multivariate analyses, where characters (not states) were given equal weight (see Chapter 2 for full details). Twenty-one quantitative and 16 qualitative morphological characters (Table 4.2) were scored from 62 samples (Operative Taxonomic Units – OTUs) of Australian *Abildgaardia*.

Data were subjected to ordination, cluster and network analyses as detailed in Chapter 2, and the combined data set analysed using the Gower metric similarity-coefficient is presented in the results section.

Groups that were defined in the first run of analyses for all OTUs of *Abildgaardia* in this chapter were removed and data re-analysed to explore grouping of the remaining taxa. As with the previous study, 2-dimensional scatter plots adequately displayed the separation of taxa and were preferred for presentation purposes.

Groups defined in the phenetic study formed the terminal taxa used in cladistic analysis.

Table 4.2 Attribute codes and definitions used in the main phenetic analyses for the Australian *Abildgaardia*, including corresponding initial weight values. Weight values changed in subset analyses.

Attribute	Description	Weight
char1	Mean aerial spikelet width in mm (spikelets with mature fruit) at the widest point	1
char2	Mean aerial nut length in mm from base of stipe to nut apex (excluding persistent style base)	1
char3	Mean aerial nut width in mm at widest point	1
char4	Aerial nut length:width (ratio 1:W/L(x) (to decimal 1/x)	1
char5	Mean aerial nut 'stipe' length in mm	1
char6	Stipe length/nut length (proportion)	1
char7	Mean aerial anther length in mm (including appendages)	1
char8	Mean aerial style length in mm (including style base to base of style arm junction)	1
char9	Mean aerial style width in mm (at mid third)	1
char10	Style length:width (ratio 1:W/L(x) to decimal 1/x)	1
char11	Mean aerial stylebase length in mm (from base to constriction at style junction)	1
char12	Mean aerial stylebase width in mm (at widest point)	1
char13	Style base length:width (ratio 1:W/L(x) to decimal 1/x)	1
char14	Mean aerial glume length in mm (from base of nerve to apical point)	1
char15	Mean aerial glume width in mm (at widest point)	1
char16	Glume length:width (ratio 1:W/L(x) to decimal 1/x)	1
char17	Mean leaf width in mm (at mid third)	1
char18	Mean culm width in mm (at mid third)	1
char19	Mean root width in mm (one cm below plant base)	1
char20	Mean inflorescence–synflorescence length in mm (from base of main bract to furthermost point of spikelets)	1
char24	Stamen number (actual)	1
char35	Plant habit 0–annual 1–perennial	1
char41	Nut outline obcordate	0.5
char42	Nut outline capitate or club shaped (with a prominent stipe)	0.5
char43	Nut epidermis without protuberances	0.33
char50	Nut epidermis with some warts that are sparse and unevenly distributed	0.33
char51	Nut epidermis with pronounced warts formed by raised multiple cell clusters that are dense and evenly distributed	0.33
char53	Nut 0- not winged 1- winged (flattened extensions from the nut sides. Includes any extended notching on nut 'margins')	1
char65	Glumes 1-distichous to sub-distichous (spikelet somewhat compressed, but rachilla may twist distally); 0-glumes tristichously spiral	1
char80	Leaf blades always present in an individual	0.5
char81	Some leaf blades present, some as subulate points in an individual	0.5
char83	Inflorescence–synflorescence bracts absent (usually in solitary spikelets)	0.5
char84	Inflorescence–synflorescence bracts present and distinct	0.5
char85	Inflorescence–synflorescence bracts glume-like	0.5
char86	Inflorescence–synflorescence bracts leaf-like	0.5
char87	Main inflorescence–synflorescence bracts 1-shorter than the inflorescence–synflorescence; 0-equal or longer than main inflorescence–synflorescence	1

Cladistic study

Ingroup

Members of *Fimbristylis*, *Crosslandia* and *Bulbostylis* used in Chapter 3 were also included in the cladistic component of this chapter. Overseas specimens for the cosmopolitan *A. ovata* were compared to the *A. ovata* group defined in the phenetic analyses and five overseas samples added to the cladistic data set to cover the extended geographical range. Additional species assigned to *Abildgaardia* occurring outside of Australia: *A. triflora*, *A. mexicana*, *A. baeothryon*, including the C_3 species *Abildgaardia hygrophila* and *Fimbristylis variegata* (syn. *Abildgaardia variegata*) (Bruhl 1995), were added to the ingroup to assess monophyly of the genus (Table 4.3). No specimens of *A. papillosa* were available.

Embryo morphology

The Abildgaardia-type embryo was first observed by Van der Veken (1955) based on material of *A. ovata* and *A. oxystachya* (at the time assigned under *Fimbristylis* section *Abildgaardia*). In his study, Van der Veken commented on the possibility of reinstating genera, including *Abildgaardia*, based on the embryo types observed. Goetghebeur (1986) expanded on Van der Veken's work and found that embryos from *A. macrantha* (= *F. macrantha*) and *A. pachyptera* (= *F. pachyptera*) were also of the Abildgaardia-type. Embryo morphology was sampled for all species included in the cladistic analysis.

Table 4.3 Taxa included in the cladistic analyses to assess the relationships of species in *Abildgaardia*. Species from *Crosslandia* included here were defined in Chapter 3. See Appendix 1 for specimen details.

Taxa	No. of specimens sampled
Ingroup	
Abildgaardia baeothryon	4
Abildgaardia hygrophila	2
Abildgaardia macrantha (provisional)	10
Abildgaardia mexicana	5
Fimbristylis odontocarpa (section *Abildgaardia*)	2
Fimbrisytlis sp. aff. *odontocarpa*	1
Abildgaardia ovata	16
Abildgaardia oxystachya (provisional)	13
Abildgaardia pachyptera (provisional)	11
Abildgaardia schoenoides	11
Abildgaardia triflora	4
Abildgaardia sp. aff. *pachyptera*	1
Abildgaardia sp. aff. *schoenoides* 1 (As5, As6)	2
Abildgaardia sp. aff. *schoenoides* 2 (As7)	1
Bulbostylis barbata	20
Bulbostylis densa	15
Crosslandia anthelata (provisional)	5
Crosslandia setifolia	18
Crosslandia spiralis (provisional)	3
Crosslandia vaginata (provisional)	14
Fimbristylis blakei	2
Fimbristylis cinnamometorum	5
Fimbristylis depauperata	2
Fimbristylis fimbristyloides	4
Fimbristylis furva	2
Fimbristylis microcarya	2
Fimbristylis schultzii	2
Fimbristylis sp L. (Kimberley Flora)	2
Fimbristylis variegata	1
Outgroup	
Actinoschoenus compositus (provisional)	4
Arthrostylis aphylla	4
Schoenoplectiella laevis	5
Schoenoplectiella lateriflora	5
Schoenoplectus tabernaemontani	3
Trachystylis stradbrokensis	7

Anatomy

Variation in vegetative anatomy Gordon-Gray (1971) and photosynthetic pathways (Gilliland and Gordon-Gray 1978; Bruhl 1995) for species of *Abildgaardia* (C_4 fimbristyloid and C_3) prompted further investigation here. Leaf blade and culm anatomy were, therefore, assessed for species being tested for placement in *Abildgaardia*. Anatomical data were included in the cladistic analysis.

PAUP Analyses*

Data for ingroup and outgroup taxa, comprising 34 terminal taxa and 149 characters, were subjected to parsimony analysis within PAUP* (Swofford 2001) using heuristic techniques (hsearch swap=TBR addseq=random nreps=1000 hold=5 multrees=yes) (see Chapter 2 for full details). Branch support was determined using Bremer support and Bootstrap analysis. Characters were traced using MacClade v 3.08 (Maddison and Maddison 1992) and the most relevant characters plotted onto the cladogram selected for presentation.

Results

Phenetic study

Operative Taxonomic Units formed disjunct groups that represent the genera *Abildgaardia*, *Crosslandia*, *Fimbristylis* and *Bulbostylis* (see also chapter 3), with some species groups apparent within *Abildgaardia* at this broad level (Figure 4.1). When OTUs from the *Abildgaardia* group were analysed separately, OTU group boundaries were slightly relaxed, revealing broad variation within some species groups (Figure 4.2). Group formation in figure 4.2 was correlated with nut outline,

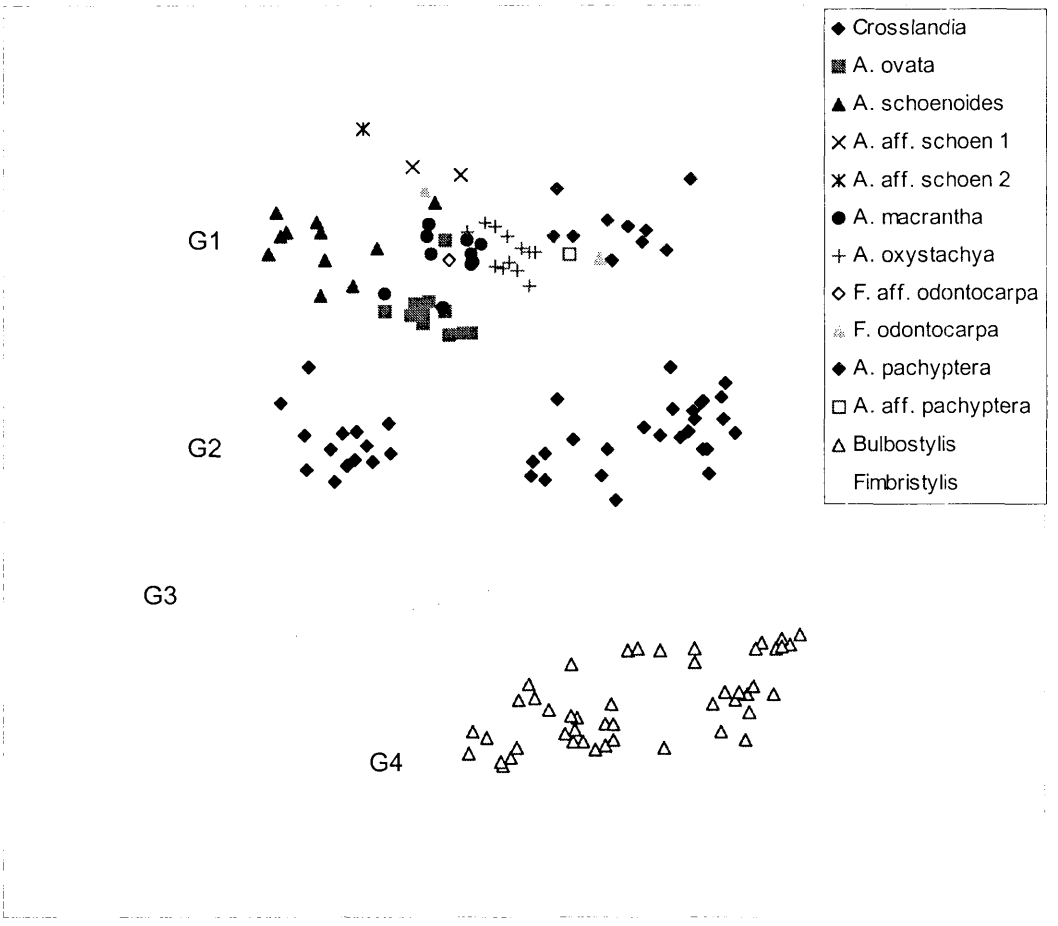


Figure 4.1 MDS ordination in 2-dimensions for OTUs of *Abildgaardia*, *Crosslandia*, *Fimbristylis* and *Bulbostylis*. OperativeTaxonomic Units that divide into generic groups *Abildgaardia* (G1) *Crosslandia* (G2), *Fimbristylis* (G3) and *Bulbostylis* (G4) are clearly shown within the ordination. Groups of OTUs that form species in *Abildgaardia* are discernable at this broad level. Stress value=0.17. See Chapter 3 for PCC and classification that correspond with this ordination.

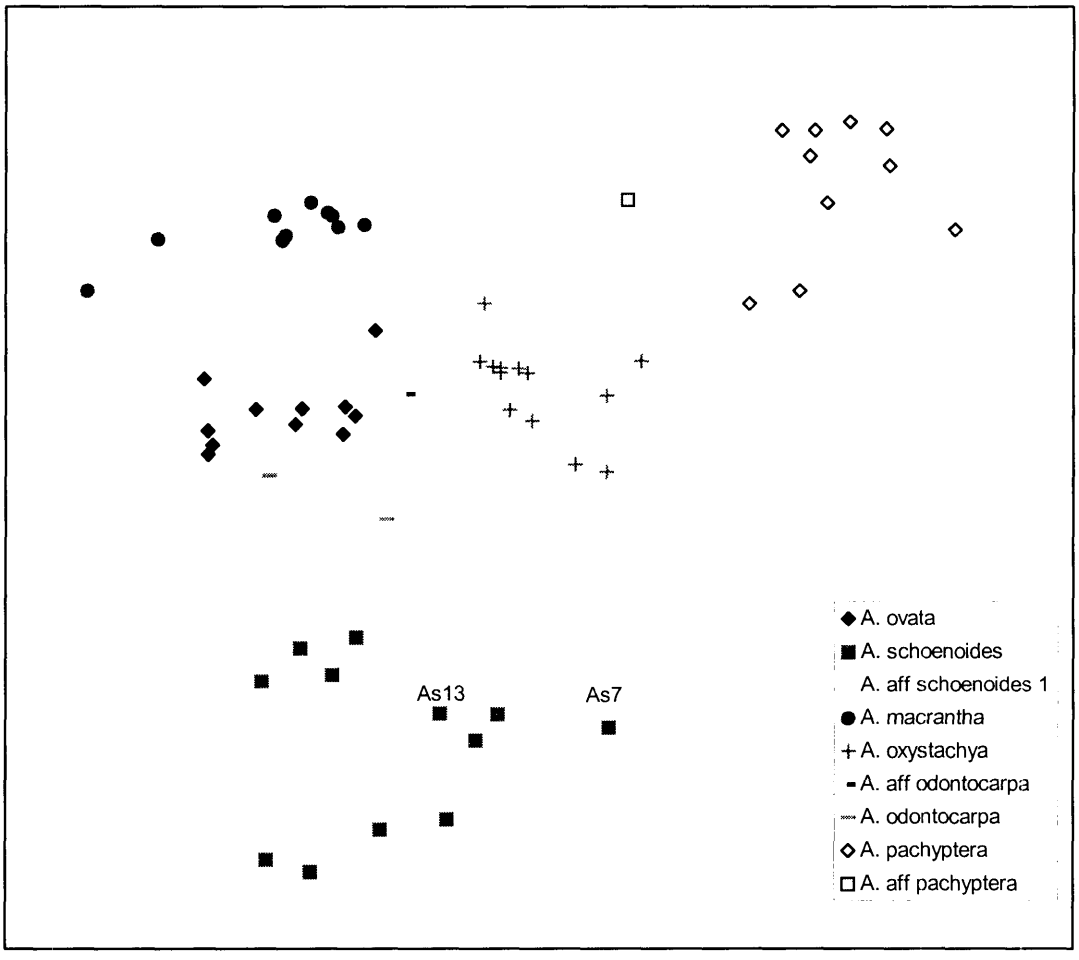


Figure 4.2 MDS ordination (stress = 0.13) showing OTUs forming broad species groups within *Abildgaardia*. As13 (*A. schoenoides*) with perianth nests within the main group, and As7 falls on the outer limits. OTUs for *A.* aff *schoenoides* 1 are separate from the remaining *A. schoenoides* OTUs. The OTU of *A.* sp. aff. *pachyptera* is distinctly separated from all other groups. See Table 4.1 and Appendix 1 for OTU and specimen details.

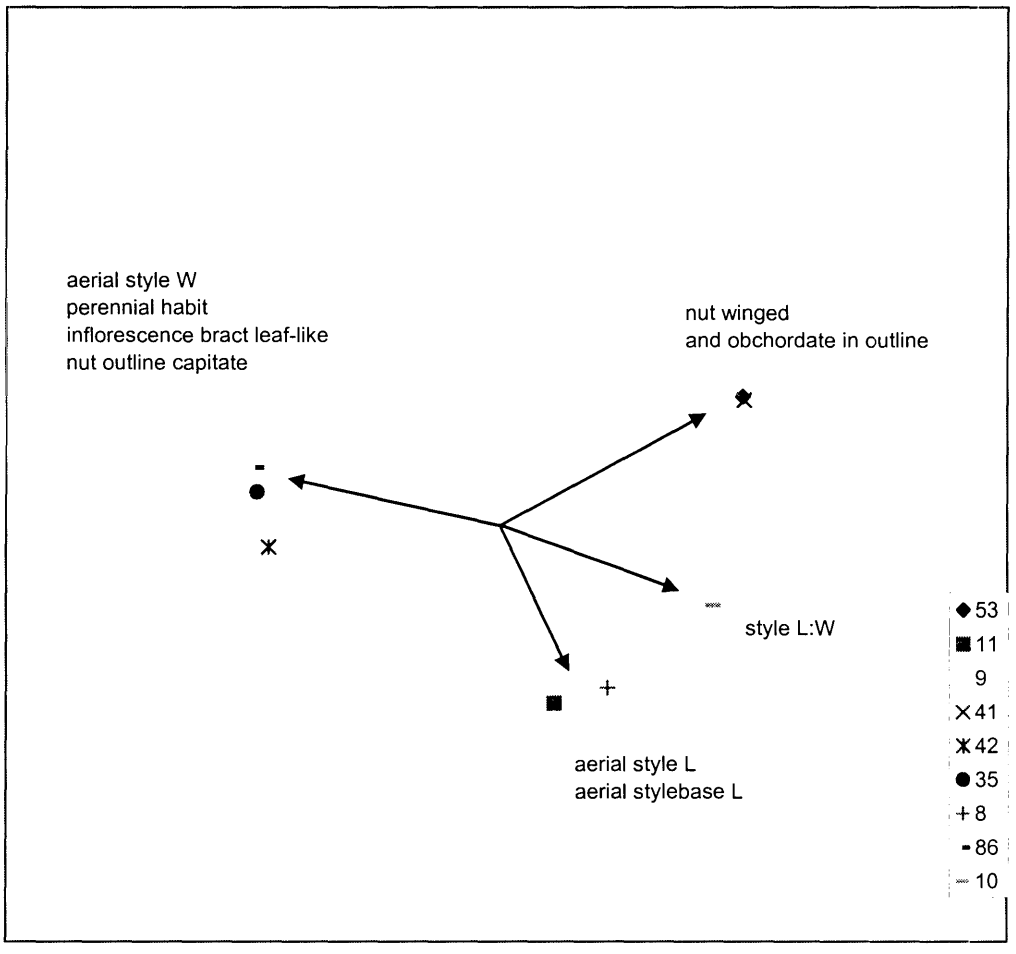


Figure 4.3. Correlation of attributes with ordination space in Figure 4.2. Attributes with >80% influence on OTU group formation are shown. See Table 4.2 for attribute definitions. L=length, W=width

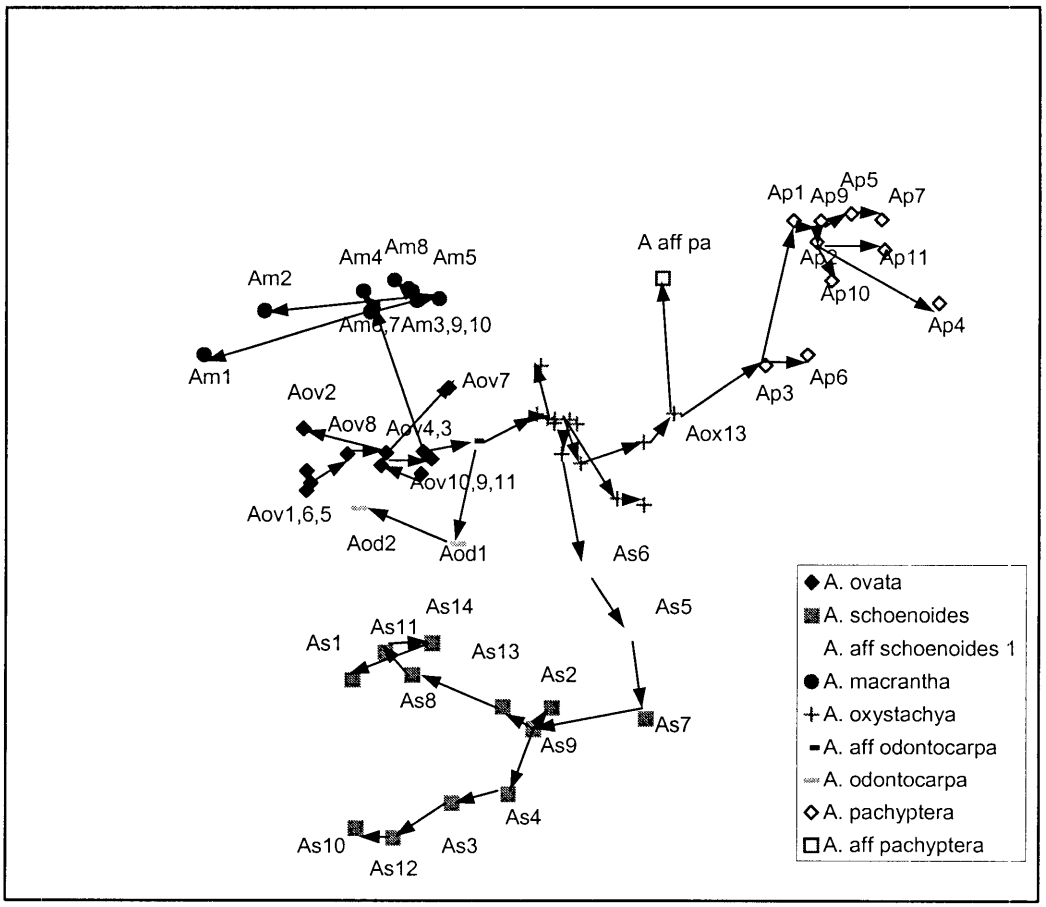


Figure 4.4 Minimum spanning tree (MST) from network analysis that corresponds to the ordination in Figure 4.2. See Table 4.1 and Appendix 1 for OTU and specimen details.

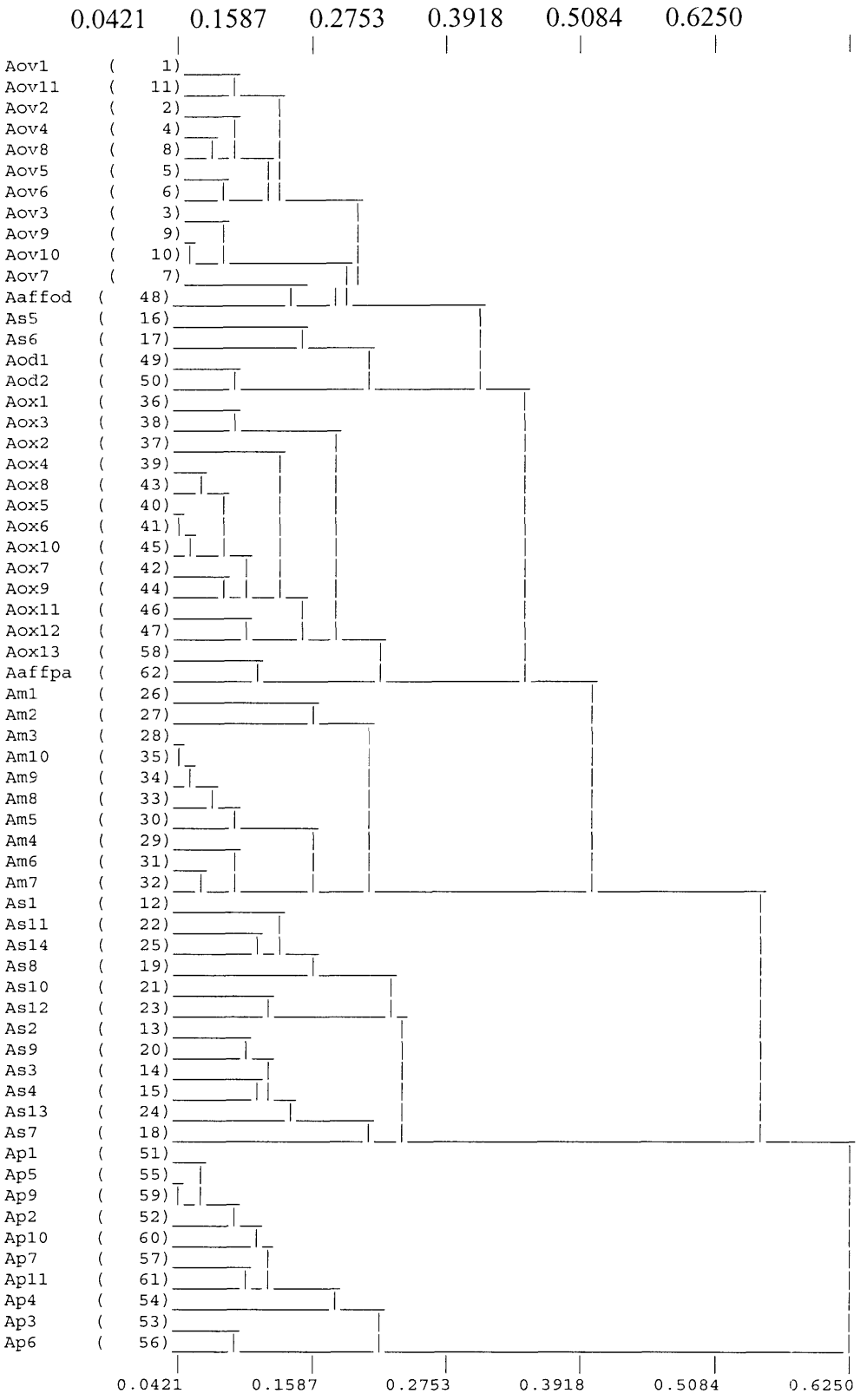


Figure 4.5. WPGMA (β=-0.1) phenogram for the full *Abildgaardia* analyses, using the Gower metric association measure, corresponds to the ordination in Figure 4.2. *Abildgaardia* sp. aff. *schoenoides* 1 OTUs are separated from *A. schoenoides* s.s. in the phenogram and As13, As7 nested within *A. schoenoides*. The OTU *A.* sp. aff. *pachyptera* is nested within *A. oxystachya* and not *A. pachyptera*. The two outlying *A. macrantha* OTUs (Am1, Am2) are nested with the main *A. macrantha* group. See Table 4.1 and Appendix 1 for OTU and specimen details.

nut wing presence/absence, style and style base length, and plant habit (Figure 4.3). The broad species groups observed in the ordination were consistent in network (Figure 4.4) and cluster analysis (Figure 4.5).

Abildgaardia schoenoides *group*

Minor internal groups of OTUs that broadly form the species *Abildgaardia schoenoides* were consistent in the ordination scatter plot (Figure 4.2), phenogram (Figure 4.5), and MST (Figure 4.4). The minor group of OTUs As1, As8, As11 and As14 are spread across the geographical range, and do not appear to be consistent with any other morphological, anatomical, or embryological feature that could justify separation of these OTUs from the remainder of the main group.

In the ordination scatter plot the OTU *A.* sp. aff. *schoenoides* 2 (As7) is placed at the extreme edge of the *A. schoenoides* limits (Figure 4.2), and links *A.* sp. aff. *schoenoides* 1 and other OTUs *A. schoenoides* in the MST (Figure 4.4); As7 is nested with OTUs of *A. schoenoides* in the phenogram (Figure 4.5). The placement of As7 is therefore considered to fall within the limits of *A. schoenoides*. The one OTU (As13) collected from the edge of the Arnhem Land Plateau with perianth bristles present (see observations section) was distinctly nested within the *A. schoenoides* cluster in all analyses.

Specimens of *A.* sp. aff. *schoenoides* 1 (As5, As6) remain separated from OTUs of *A. schoenoides*, consistent with the habit being annual and not obviously perennial as in the TYPE specimen of *Fimbristylis squarrulosa*.

Abildgaardia pachyptera – A. oxystachya *group*

Operative Taxonomic Units that grouped as the species *A. pachyptera* were distinct in the ordination due to their winged nuts and obcordate nut outline (Figure 4.3). Two of the specimens sampled Ap3 and Ap6, appear transitional between *A. pachyptera* and *A. oxystachya* (Figure 4.2, 4.4, 4.5), with nut attributes intermediate to the two species. Cluster analysis groups Ap3 and Ap6 together as a minor group nested within *A. pachyptera* (Figure 4.5), and the MST links *A. oxystachya* to *A. pachyptera* them within the other OTUs of the species (Figure 4.4).

The OTU of the collection labelled *A.* sp. aff. *pachyptera* is distinctly separated from the main group of *A. pachyptera* OTUs in the broad ordination of all OTUs for *Abildgaardia* (Figure 4.2 and 4.4), and in the subset analyses for ordination (2-D stress=0.1; 3-D stress=0.07) (Figure 4.6), but is grouped with *A. oxystachya* in the phenogram of the same analyses (Figure 4.7) as it was in the full *Abildgaardia* cluster analysis (Figure 4.5).

Operative taxonomic units of *Abildgaardia oxystachya* also exhibited a minor group (aox1, aox3, aox11) within the ordination (Figure 4.2) that was not substantiated in the phenogram (Figure4.5) or by MST linkages (Figure 4.4). Again an intermediate specimen (aox13) stretches the *A. oxystachya* limits and pushes towards the limits of *A. pachyptera* within the ordination scatter plot; any connection between the two species was not seen in subsequent analyses (Figures 4.6-7).

Analyses of the data subset containing OTUs for *A. oxystachya*, *A. pachyptera* and *A.* sp. aff. *pachyptera* confirmed the three species groups in the ordination (Figure

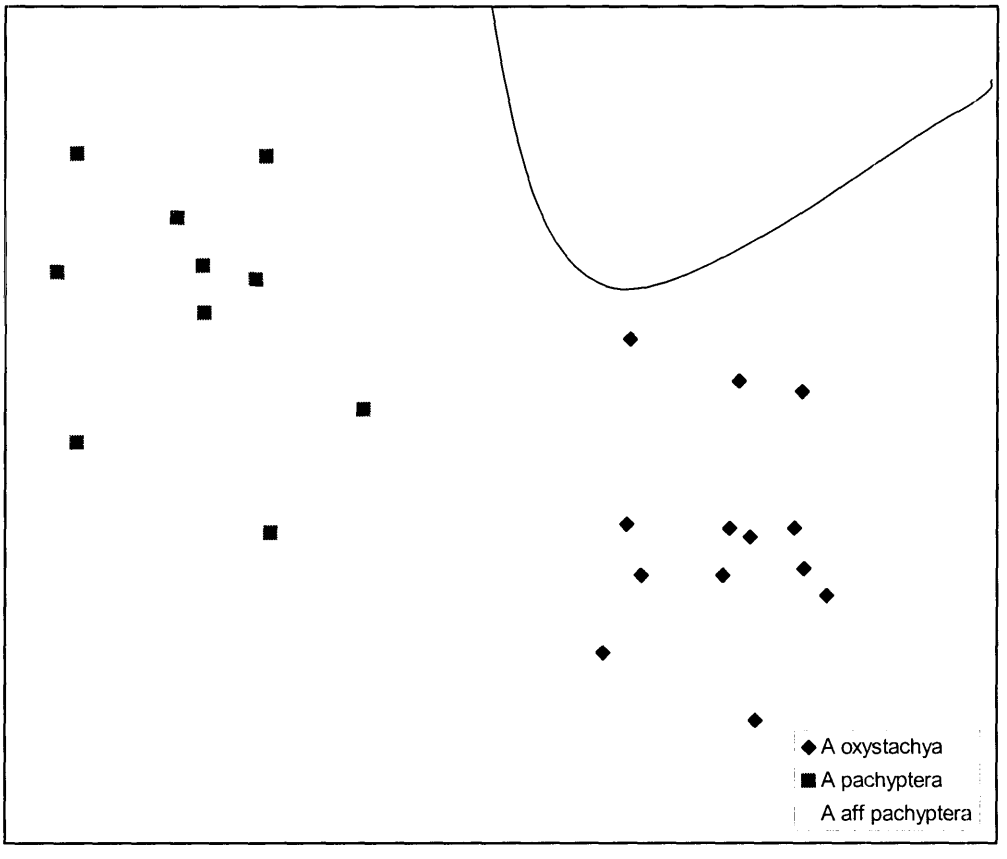


Figure 4.6 Two dimensional MDS ordination (stress = 0.1) showing OTUs grouped as *Abildgaardia oxystachya*, *A. pachyptera* and *A.* sp. aff. *pachyptera*. The drawn border separating *A.* sp. aff. *pachyptera* indicates the stronger separation of this OTU from the remainder of the OTUs in 3-dimensional analysis (stress = 0.07). See Appendix 1 for specimen details.

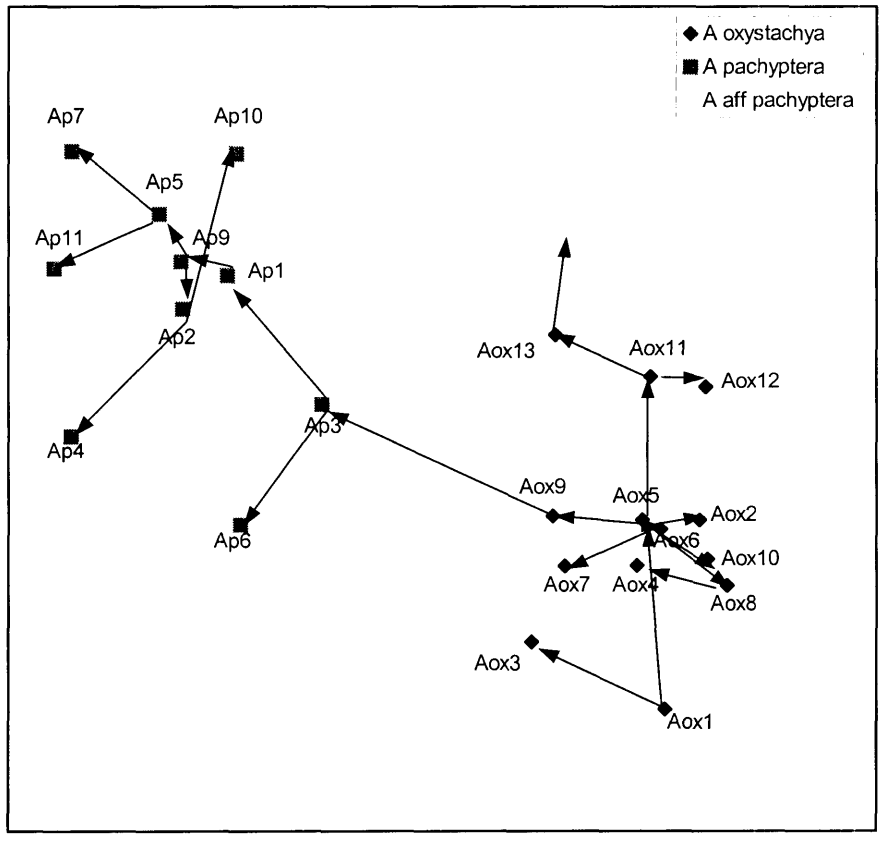


Figure 4.7 Minimum spanning tree from network analysis that corresponds to the ordination in Figure 4.6. See Table 4.1 and Appendix 1 for OTU and specimen details.

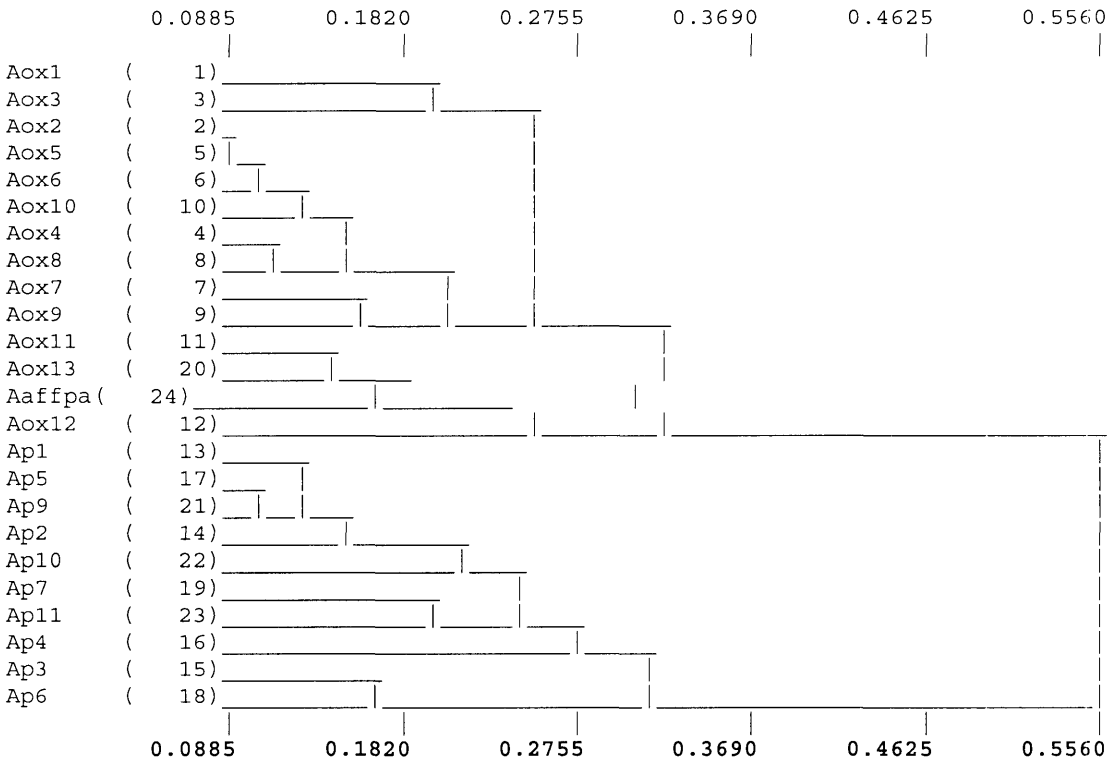


Figure 4.8 WPGMA (β=-0.1) phenogram, using the Gower metric association measure, that corresponds to the ordination in Figure 4.4. OTUs of *Abildgaardia oxystachya*, *A. pachyptera* and *A.* sp. aff. *pachyptera* form distinct groups separate to *A. oxystachya*, in which *A.* sp. aff. *pachyptera* is nested. See Table 4.1 for OTU specimen information and Appendix 1 for specimen details.

4.6), observed when all OTUs for *Abildgaardia* were analysed. Within the minimum spanning tree the OTU for *A.* sp. aff. *pachyptera* terminates the *A. oxystachya* chain of OTUs, and is not nested within the group (Figure 4.7), however, the single OTU for *A.* sp. aff. *pachyptera* was nested within OTUs of *A. oxystachya* in the phenogram (Figure 4.8).

Abildgaardia macrantha *group*

OTUs of *A. macrantha*: Am3-10 formed a tight cluster in all ordination analyses with Am1 and Am2 as distinct outliers from the main group (Figure 4.2). Phenograms show the outlier OTUs as a minor internal group in both the broad *Abildgaardia* group (Figure 4.5) and subset analyses (not presented). Network analysis was less consistent, with Am1 and Am2 linked to OTUs of *A. macrantha* at separate ends of minor chains in the full *Abildgaardia* analysis (Figure 4.4), while in a subset analysis these samples were together, linked at the end of the *A. macrantha* chain (not presented).

Abildgaardia ovata – F. odontocarpa *group*

Within ordination and cluster analyses for the full *Abildgaardia* data set the cluster of OTUs that combine as *A. ovata* included the single sample of *Fimbristylis* sp. aff. *odontocarpa* on the outer limits of the group and within the group respectively (Figures 4.2 and 4.5). Operative Taxonomic Units for *Fimbristylis odontocarpa* were placed near to the *Abildgaardia ovata* group in the phenogram, although distinctly separated within the ordination. Subset analyses showed *Fimbristylis* sp. aff. *odontocarpa* separate to, but near the perimeter of the *A. ovata* group in the ordination scatter plot (Figure 4.9), and in the phenogram was retained within the

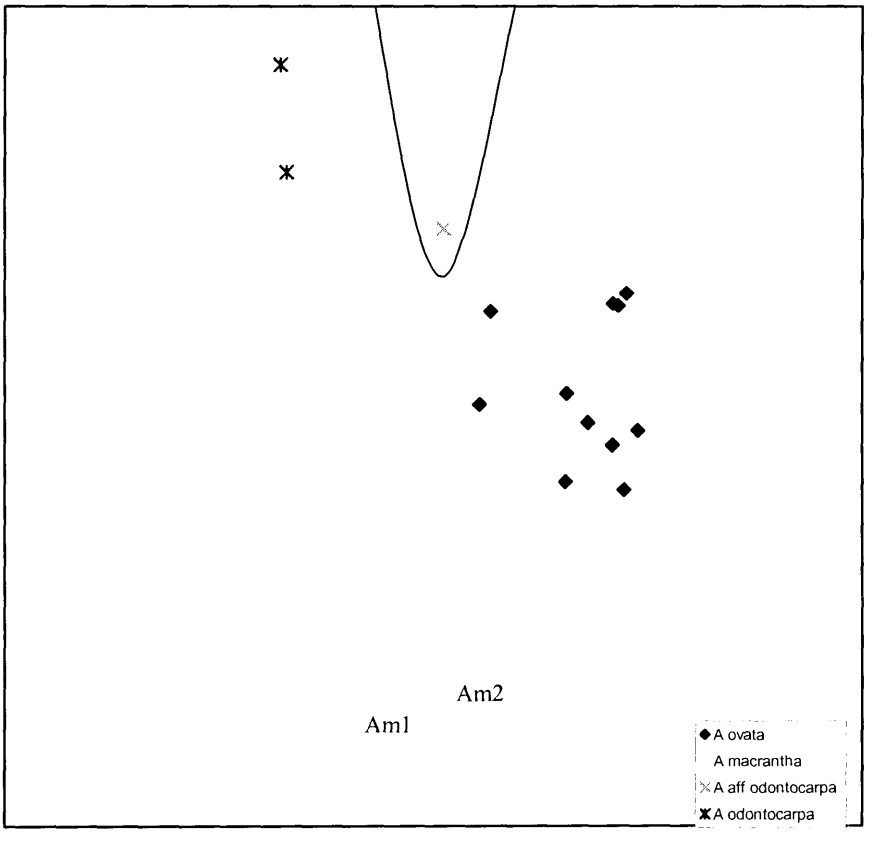


Figure 4.9 MDS ordination in 2-dimensions (stress = 0.17) showing OTUs grouped as *Abildgaardia ovata*, *A. macrantha, F. odontocarpa,* and *F.* sp. aff. *odontocarpa*.. The border drawn around *F.* sp. aff. *odontocarpa* indicates the stronger separation of this OTU from the remainder of the OTUs in 3-dimensions (stress=0.09). Am1 and Am2 were more broadly separated from each other in the 3-dimensional ordination. See Table 4.1 and Appendix 1 for OTU and specimen details.

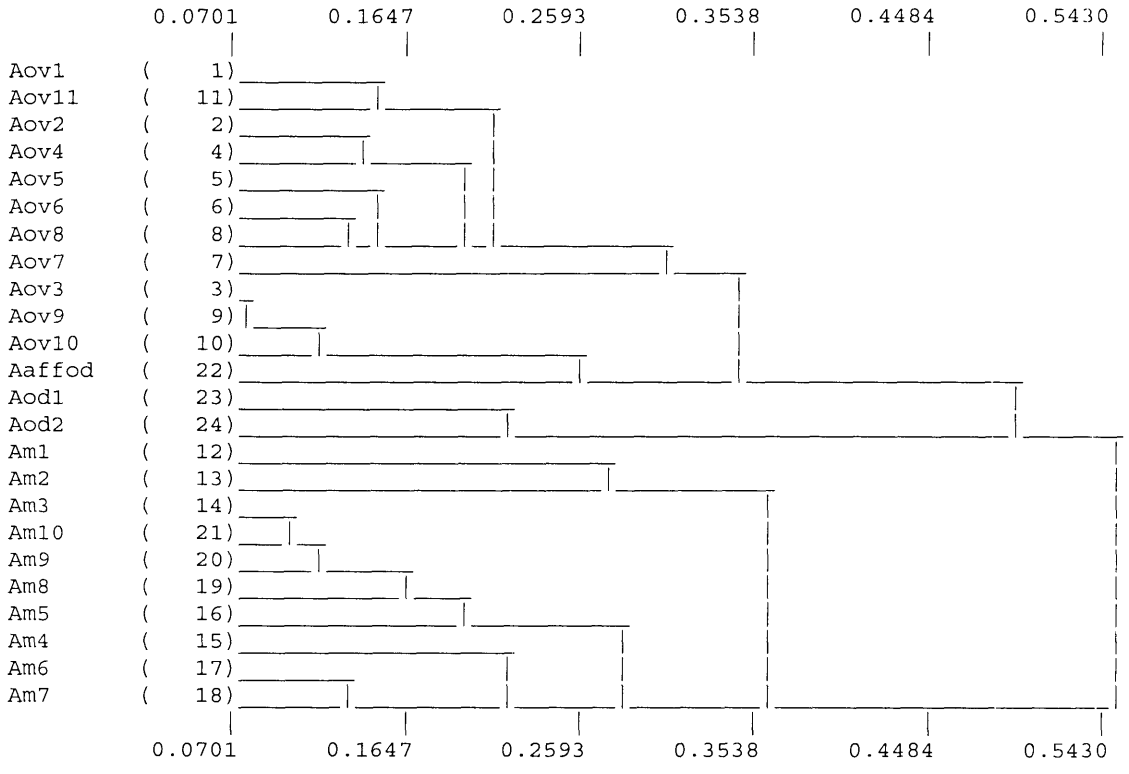


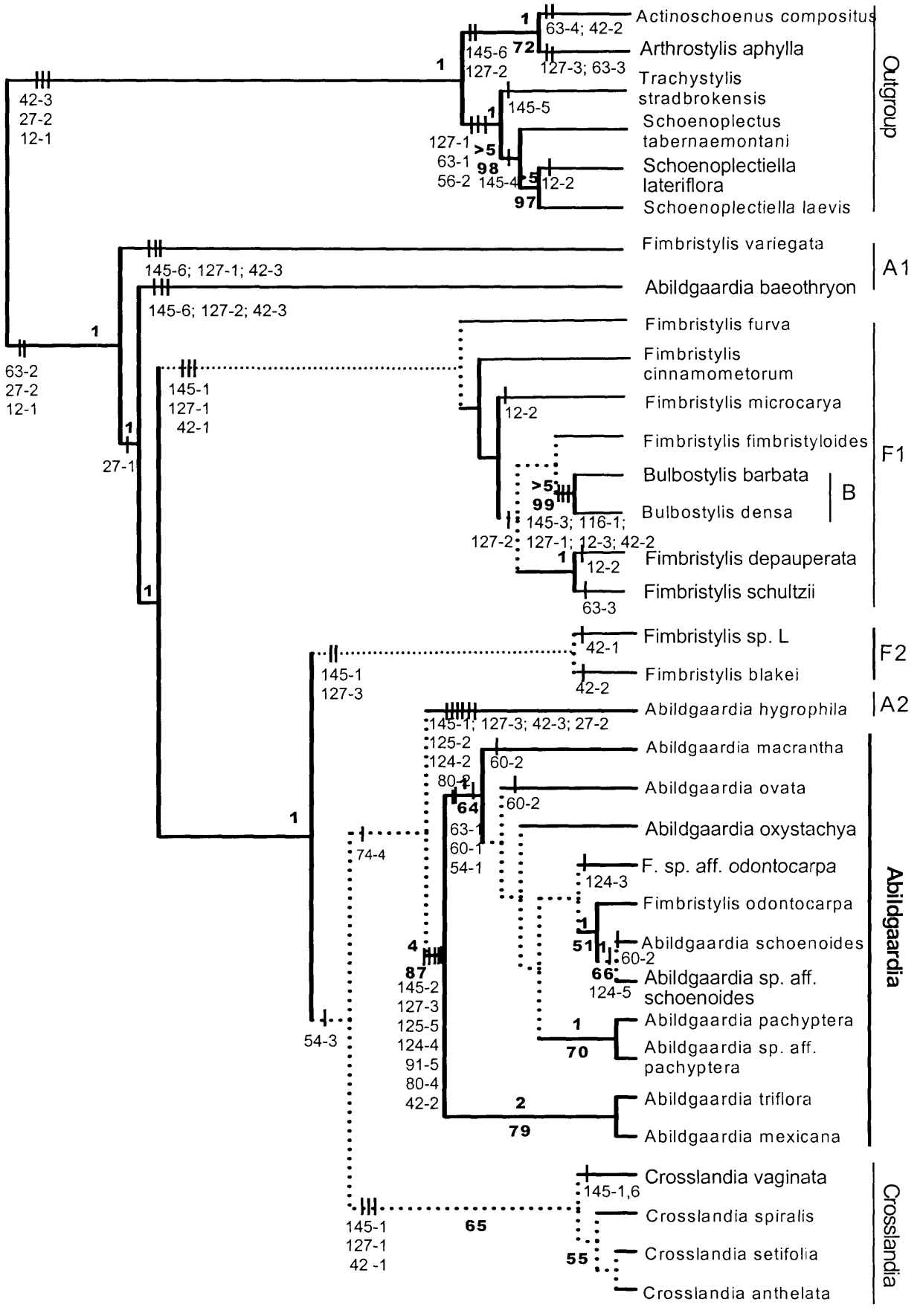
Figure 4.10 WPGMA (β=-0.1) phenogram, using the Gower metric association measure, that corresponds to the ordination in Figure 4.6. OTUs of *Abildgaardia ovata* (including *F.* sp. aff. *odontocarpa*), *F. odontocarpa* group separately to OTUs of *A. macrantha*. Am1 and Am2 form a minor internal group within OTUs of *A. macrantha*. OTUs for *F. odontocarpa* form a distinct group and *F.* sp. aff.. *odontocarpa* is nested within OTUs of *A. ovata*. See Table 4.1 and Appendix 1 for OTU and specimen details.

A. ovata group (Figure 4.10). The minimum spanning tree links *Fimbristylis* sp. aff. *odontocarpa* between *A. ovata* and *A. oxystachya*, and *Fimbristylis odontocarpa* connected as a terminal branch in the full *Abildgaardia* analysis (not presented). However the subset minimum spanning tree reveals *Fimbristylis* sp. aff. *odontocarpa* and *Fimbristylis odontocarpa* as chains linked to *Abildgaardia ovata* at opposite ends of the *A. ovata* chain (not presented).

Abildgaardia ovata, *A. macrantha*, *A. oxystachya*, *A. pachyptera*, *A.* aff. *pachyptera*, *A. schoenoides*, *A.* aff. *schoenoides* 1, *Fimbristylis odontocarpa*, and *F* .sp. aff. *odontocarpa*, as defined in the phenetic analyses, formed terminal taxa for the Australian species of *Abildgaardia*.

Cladistic Analysis

There were 126 most parsimonious trees retrieved (tree length=987, CI=0.4863, HI=0.5137, RI=0.5848, RC=0.2844) from the heuristic search. Tree number 23 was selected to show branch support and character placement, as it was one of the trees with essentially the same topology as the strict consensus. All species assessed as *Abildgaardia* did not form a monophyletic group (Figure 4.11). *Abildgaardia ovata*, *A. schoenoides*, *A.* sp. aff. *schoenoides* 1, *A. macrantha*, *A. oxystachya*, *A. pachyptera*, *A.* sp. aff. *pachyptera*, *A. triflora*, *A. mexicana*, *Fimbristylis odontocarpa* and *F. sp.* aff. *odontocarpa* formed a monophyletic group with strong branch support (Decay=3 Bootstrap=87%). The *Abildgaardia* s.s. group was sister to *A. hygrophila* which does not have the typical *Abildgaardia* embryo or nut characters of other taxa from *Abildgaardia* (see observations section) and therefore is not considered as a species of *Abildgaardia*. Synapomorphies for the *Abildgaardia* clade


Actinoschoenus compositus
Arthrostylis aphylla
Trachystylis stradbrokensis
Schoenoplectus tabernaemontani
Schoenoplectiella lateriflora
Schoenoplectiella laevis
Outgroup
Fimbristylis variegata
Abildgaardia baeothryon
A1
Fimbristylis furva
Fimbristylis cinnamometorum
Fimbristylis microcarya
Fimbristylis fimbristyloides
Bulbostylis barbata
Bulbostylis densa
B
Fimbristylis depauperata
Fimbristylis schultzii
F1
Fimbristylis sp. L
Fimbristylis blakei
F2
Abildgaardia hygrophila
A2
Abildgaardia macrantha
Abildgaardia ovata
Abildgaardia oxystachya
F. sp. aff. odontocarpa
Fimbristylis odontocarpa
Abildgaardia schoenoides
Abildgaardia sp. aff. schoenoides
Abildgaardia pachyptera
Abildgaardia sp. aff. pachyptera
Abildgaardia triflora
Abildgaardia mexicana
Abildgaardia
Crosslandia vaginata
Crosslandia spiralis
Crosslandia setifolia
Crosslandia anthelata
Crosslandia

that separate the *Abildgaardia* s.s. clade from *A. hygrophila* are: Abildgaardia-type embryo (145-2), nut length greater than 1.35 mm, most greater than 2mm (124-3,4,5), nut width greater than 1.5 mm (124-5), glume nerve area broad (91-5), glume margins indistinct from glume back (80-4), culm sclerenchyma strands equal the number of vascular bundles in the outer ring (42-2), and C_4 photosynthetic pathway (27-1). In addition, there is no branch support for the combined clade *Abildgaardia* s.stricto plus *Abildgaardia hygrophila* (Figure 4.11).

Within the *Abildgaardia* clade all Australian species including the cosmopolitan *A. ovata* formed a group sister to *A. triflora* and *A. mexicana*, with both internal branches receiving weak support (Bootstrap=64%; Decay=1) (Figure 4.11).

Species currently accepted in the genus *Fimbristylis* were polyphyletic, with the bulk of *Fimbristylis* species (including *F. depauperata* from *Fimbristylis* section *Fimbristylis* of the TYPE species), and nested species of *Bulbostylis*, grouped as sister to the broad group containing *Abildgaardia* s.s. and *Crosslandia*.

Abildgaardia baeothryon and *Fimbristylis variegata* occur basally in the tree, with *A. baeothryon* nested within *F. variegata* and sister to all terminal taxa of *Fimbristylis*, *Abildgaardia* and *Crosslandia*.

Observations

Inflorescence–synflorescence structure

Species of *Abildgaardia s.s.* possess the simplest inflorescence structure of all the genera within the tribe Abildgaardieae. All Australian species of *Abildgaardia*

consistently have the most reduced inflorescence type, seen as a solitary spikelet (Figure 4.12 A, B). Occasionally, *Abildgaardia ovata* may have a single primary coflorescence (rarely 2) present in addition to the main florescence, as seen in specimens across the global range of this cosmopolitan species (Figure 4.12 C).

The African *A. triflora* and American *A. mexicana* have the most complex inflorescence structure for *Abildgaardia* with one to four primary coflorescences (Figures 4.12 D, E). Spikelet coflorescences in *A. mexicana* are always sessile and are therefore the only species of *Abildgaardia* with depauperate 'heads'.

Perianth

Perianth bristles were observed in a specimen (As13) collected from the edge of the Arnhem Plateau in Kakadu National Park, that otherwise resembles the other OTUs of *Abildgaardia schoenoides*. The presence of perianth in this material is unique among taxa of the Abildgaardieae. The two bristles with antrorse prickle hairs (Figure 4.13) were obscure within the depauperate spikelets and were not observed in all florets.

Nut shape and pattern

Nuts within the *Abildgaardia* s.s. are large (> 2mm), frequently capitate or club shaped, and have a distinct narrowed stipe (Figure 4.14-15). The exception in nut shape and stipe presence is in *A. pachyptera*, where nuts may have winged extensions on the adaxial margins of the plano-convex nut (Figure 4.15). Intermediate forms between wide wings and shorter notched margins are seen between specimens of *A. pachyptera* (Figure 4.15) and and to some extent in samples

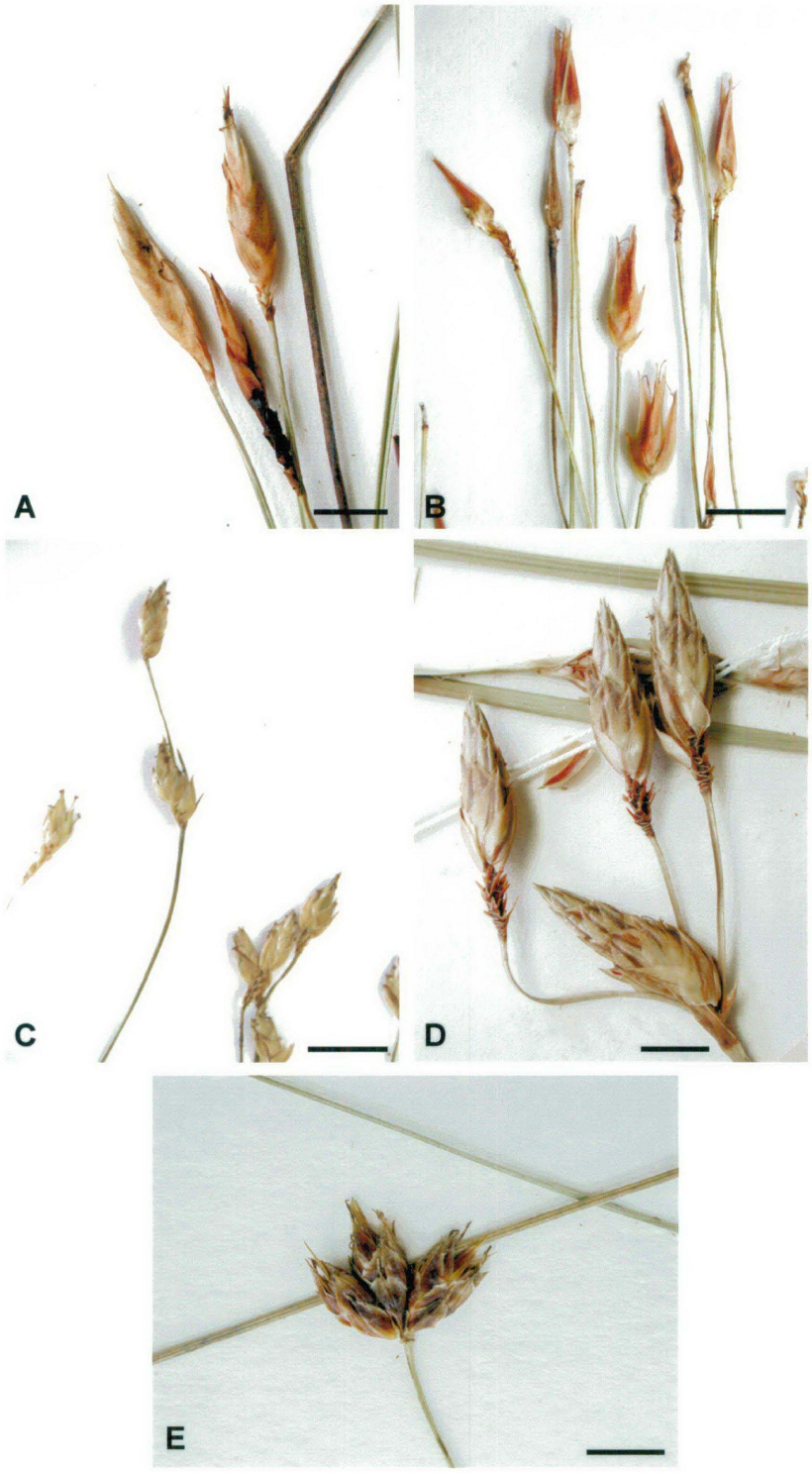


Figure 4.12 Variation of the inflorescence–synflorescence structure seen in species of *Abildgaardia*. Solitary spikelets are most common in *Abildgaardia*, especially in the Australian species as seen in A. *A. macrantha* (Am8) and B. *A.* sp. aff. *pachyptera* (Aaffpach). C. *A. ovata* (Aov9) occasionally produces one or two coflorescences (rays), while multiple coflorescences are common in D. *A. triflora* (*P.J. Greenway 1859*). E. In *A. mexicana* (*C.J. Pringle 3127*) one or two (rarely 3) coflorescence spikelets are sessile. See Table 4.1 and Appendix 1 for OTU and specimen details.

Figure 4.13 Perianth in *Abildgaardia*. Specimen of *Abildgaardia schoenoides* (As13) collected from Arnhem Land Plateau, showing A. and B. one of two perianth bristles. B. At higher magnification the antrorse barbs are evident. Scale bar A=500 μm and B=50 μm. See Table 4.1 and Appendix 1 for OTU and specimen details.

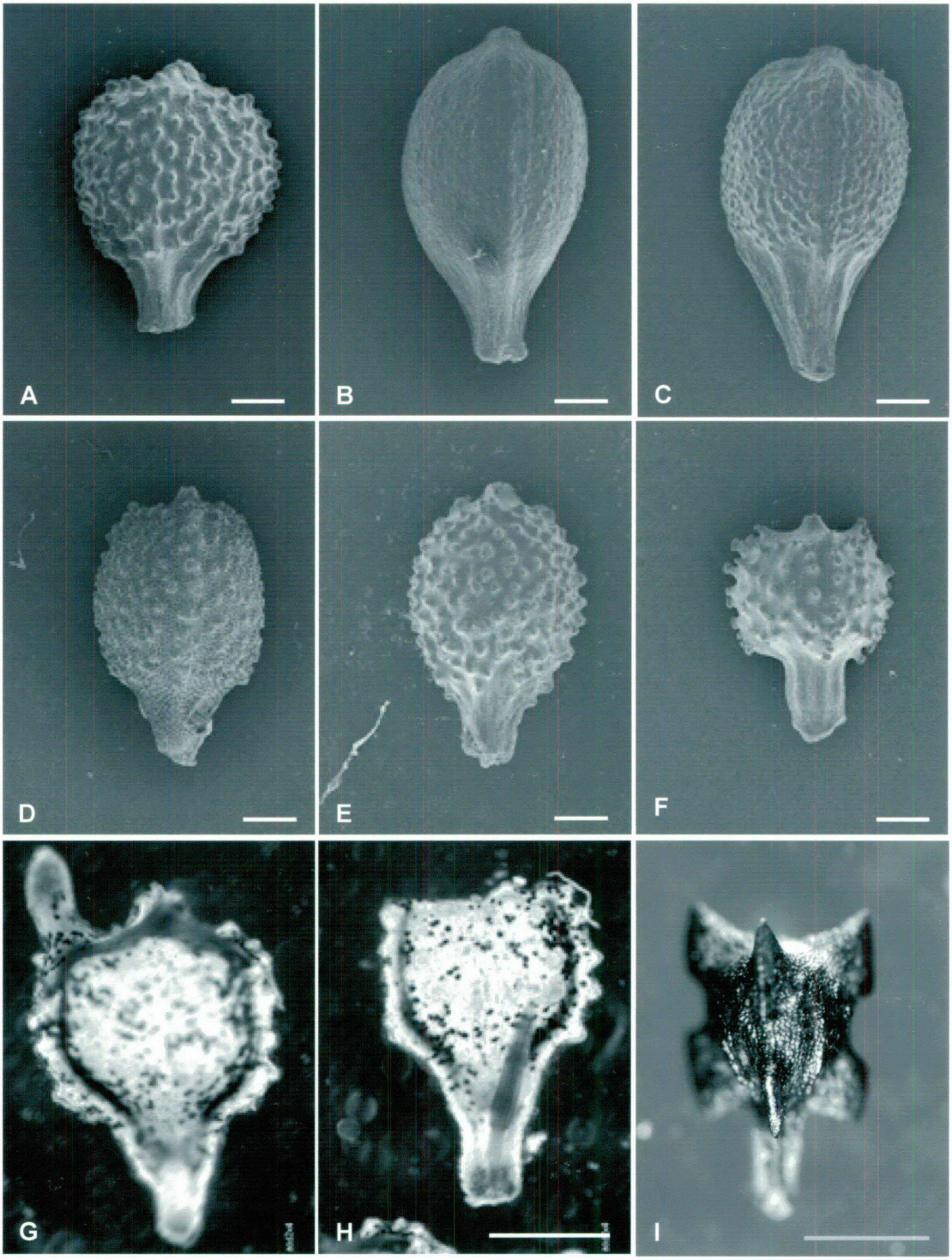


Figure 4.14 Scanning electron micrographs (SEM) and light micrographs (LM) of nuts in species of *Abildgaardia*. A. SEM of nut in *A. ovata* (Aov2), B. and C. *A. macrantha* (Am3 and Am7), D. and E. *A. oxystachya* (Aox5 and Aox1), F. *Fimbristylis* sp. aff. *odontocarpa* (Faffod), G. and H. LM of nut from *F. odontocarpa* (Fod2) with a horn-like apical protusion (G) and large tubercules in view (H). I. Nut from *A*. sp. aff. *pachyptera* (Aaffpach) showing the distinctive projections from the face margins. Scale bars A-F=500 μm, G-I=1000 μm. See Table 4.1 and Appendix 1 for OTU and specimen details.

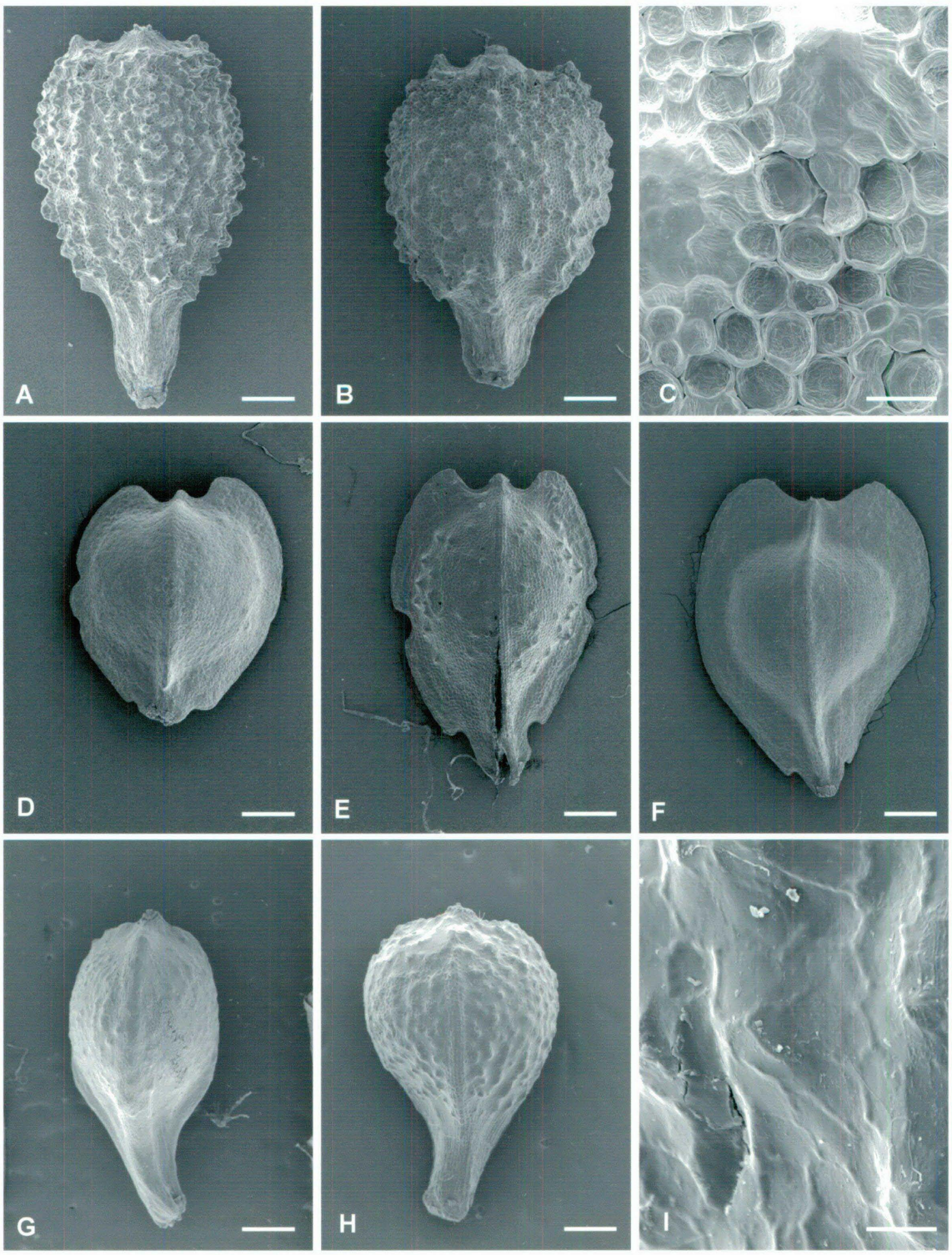


Figure 4.15 Scanning electron micrographs (SEM) of nuts in species of *Abildgaardia*. A. *A. schoenoides* nut (As9), B. *A.* sp. aff. *schoenoides* nut (As6) and C. epidermal cells of the nut in B at higher magnification. D., E. and F. The nut of *A. pachyptera* (Ap4, Ap3 and Ap2) is variable in the size of the nut 'wings', 'wing' notching and epidermal sculpturing. G. *A. triflora* (*F. Malaisse 400 & P. Goetghebeur*) nut that is almost smooth as large tubercules are absent. H. Nut of *A. mexicana (C.G. Pringle 3127*) and I. epidermal cells of the nut in H. at higher magnification. Scale bars A-B, D-H=500 μm; C-I=1000 μm. See Table 4.1 and Appendix 1 for OTU and specimen details.

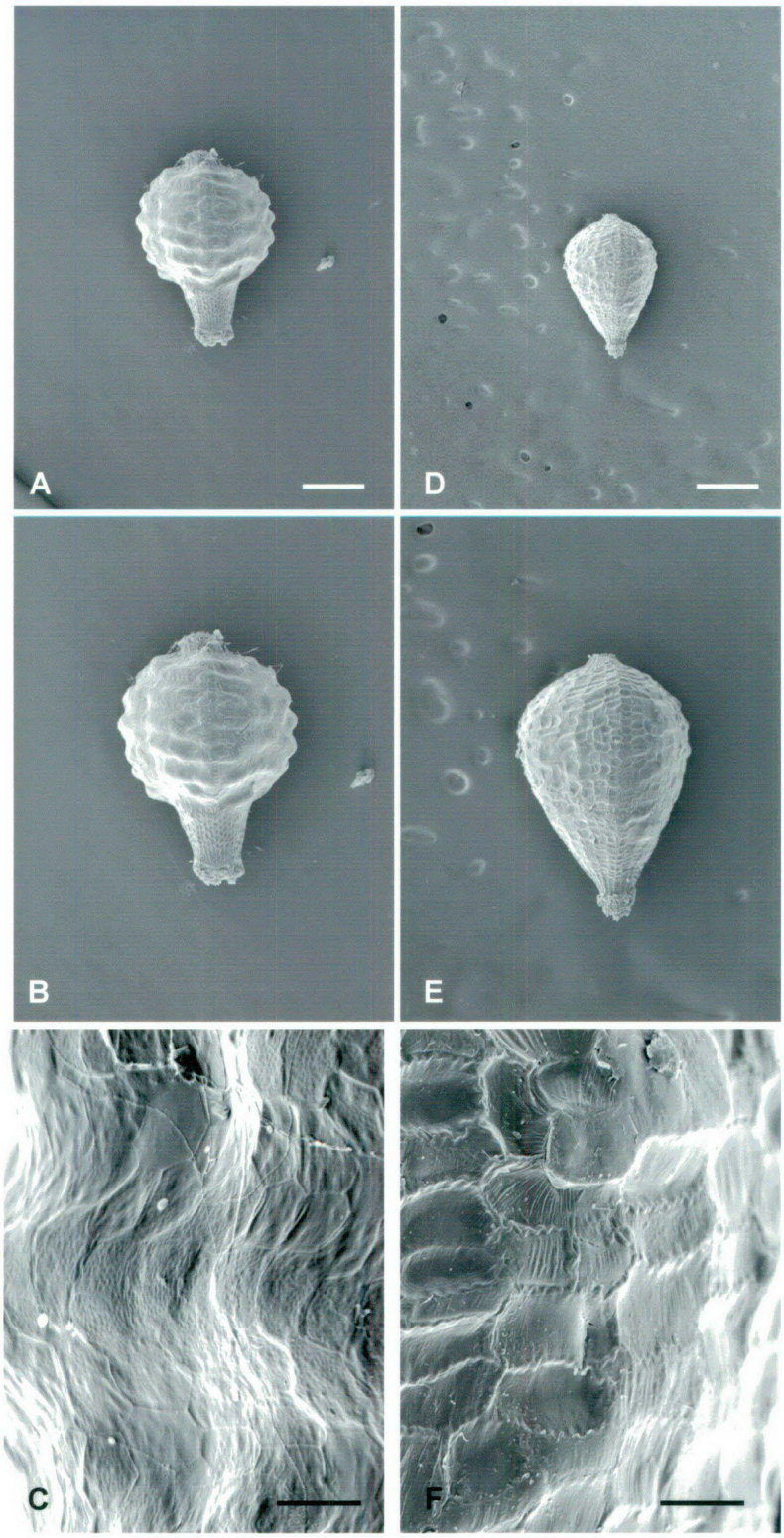


Figure 4.16 Scanning electron micrographs for *Abildgaardia hygrophila* and *Fimbristylis variegata*. A. *Abildgaardia hygrophila* (*C.J. Ward 2794*) nut at magnification (35x) comparable to other species of *Abildgaardia*, showing rugose sculpturing and B. at higher magnification (45x). C. Nut in A and B at higher magnification to view epidermal cell shape and cell walls. D. *Fimbristylis variegata* (*J. Browning 834*) at 35x magnification and E. at higher magnification (65x). F. Nut epidermal cells for *F. variegata* differ in shape, being almost rectangular, and have sinuose cell walls. Scale bar A, D=500 μm; C, D=50 μm. See Appendix 1 for specimen details.

of *A. oxystachya* (not shown). In *A.* sp. aff. *pachyptera* nut margins are deeply notched in all three planes (Figure 4.15), and in *F. odontocarpa* prominent horns at the apex of the nut may be present (Figure 4.14). Tubercules are common as multi-celled protuberances from the nut epidermis and are most prominent in *F.* sp aff. *odontocarpa* as large flat-topped protusions (Figure 4.14). Distinct tubercules are absent in some *A. pachyptera* (Figure4.15 F), and almost so in other samples of *A. pachyptera*, *A. macrantha,* and *A. triflora* (Figure 4.14-15).

The nut epidermis in *A. hygrophila* is rugosely sculptured, although the cell shape is hexagonal and the cell walls are straight (Figure 4.16). In *A. baeothryon* the small white nut has circular epidermal cells but the cells walls are sinuose. Ovate to circular nut epidermal cells are present in *Fimbristylis variegata* and the cell walls also are sinuose (Figure 4.16).

Embryo

The Abildgaardia–type embryo was present in all species that formed the monophyletic group *Abildgaardia*. Embryo size and shape was consistent within a species and varied between species (Figure 4.17-18). Embryos for *A. baeothryon* and *Fimbristylis variegata* were of the Schoenus–type, and the Fimbristylis–type in *Abildgaardia hygrophila* (Figure 4.18).

Anatomy

Leaf blade and culm anatomy confirmed that all species of the *Abildgaardia* s.s. group shared the C_4 photosynthetic pathway of the Fimbristyloid–type. Anatomical structure for *Abildgaardia* was consistent across the species (Figure 4.19-20). Leaf

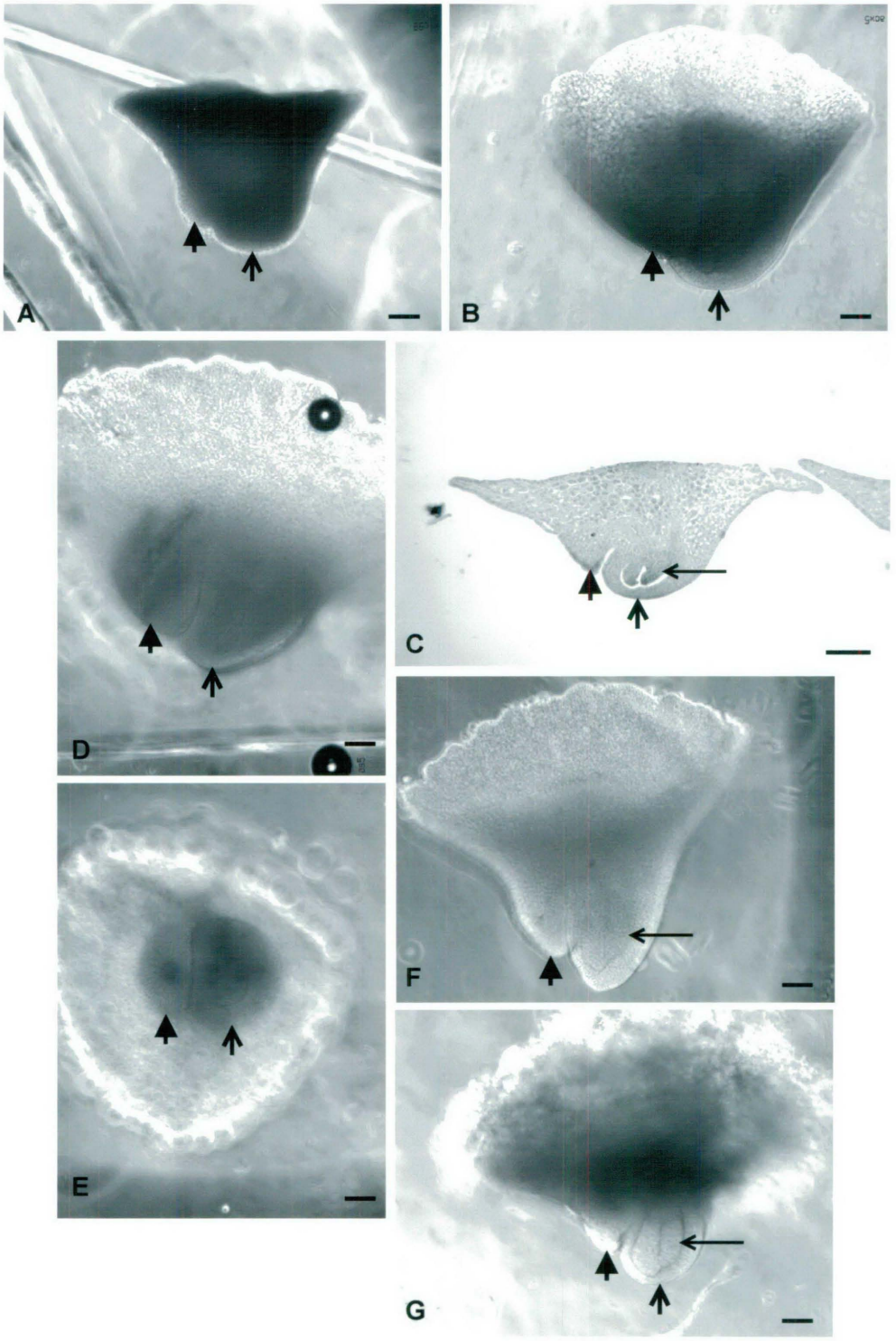


Figure 4.17 Light micrographs of mostly whole cleared embryos for Abildgaardia-type embryos in species of *Abildgaardia*. A. Embryo of *A. ovata* (Aov11), B. *A. oxystachya* (Aox5) and C. longitudinal section (Aox12) showing well developed first and second primordial leaves surrounding a small third leaf. D. *A.* sp. aff. *schoenoides* (As5), E. and *Abildgaardia schoenoides* (As7) are shown from a top view of the basal orientated root and shoot, F. side view of embryo in E. G. In *F.* sp. aff. *odontocarpa* (Faffod) the well-developed second leaf is clearly visible (thin arrow). Scale bars=100 µm. Solid arrow=root, open arrow=shoot, thin arrow =second primordial leaf. See Appendix 1 for specimen details.

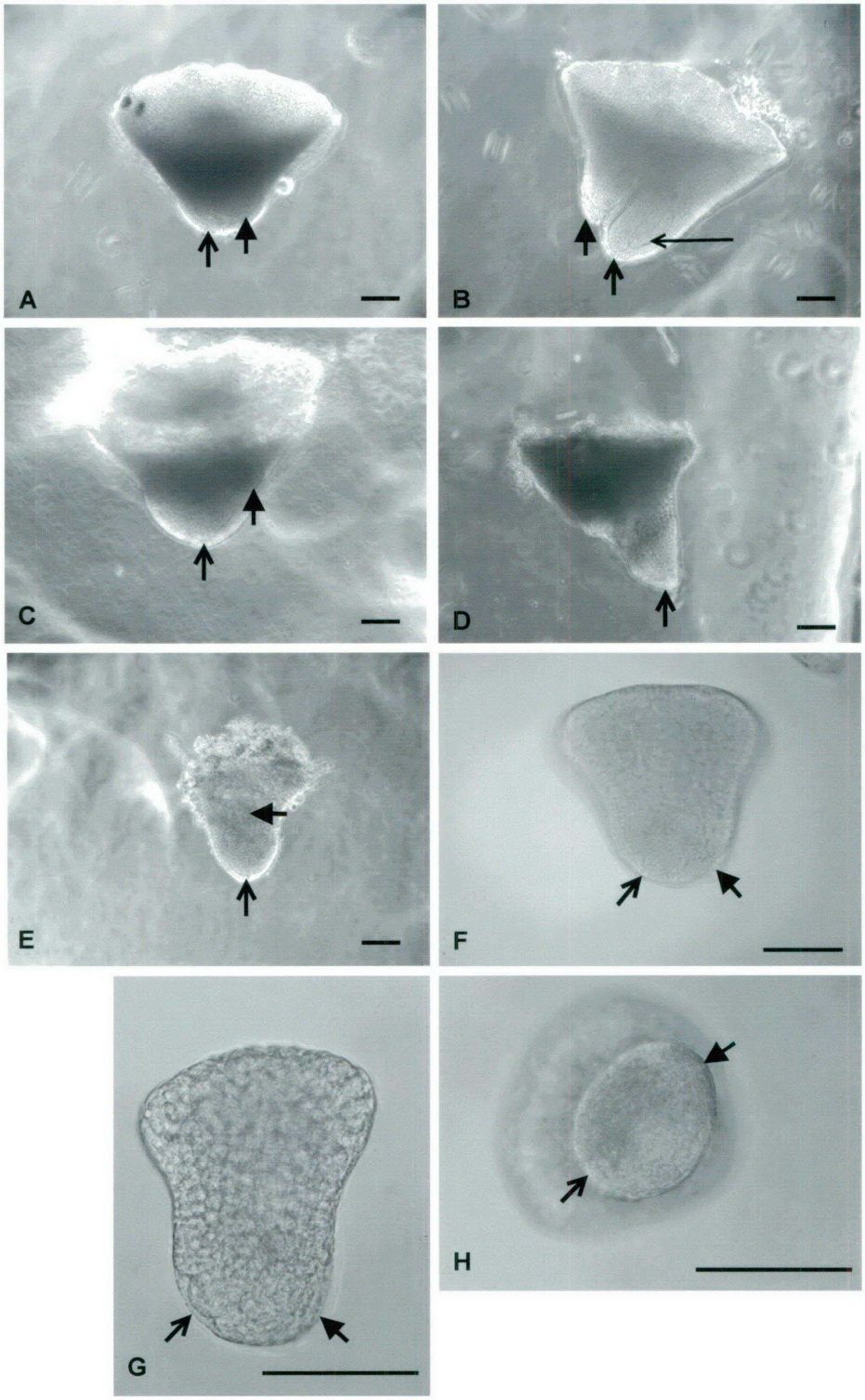


Figure 4.18 Light micrographs of whole cleared embryos for some species assigned to *Abildgaardia*. A. *Abildgaardia macrantha* (Am10) B. *A.* sp. aff. *pachyptera* (Aaffpach), C. *A. triflora (P.J. Greenway 1859)* D. *A. mexicana (C.G. Pringle 3127* – embryo damaged*)* all share the Abildgaardia-type embryo. E. *Abildgaardia hygrophila* (*K.L. Tinley 307*) has a Fimbristylis-type embryo (embryo partially damaged) and F. *Fimbristylis variegata* (*J. Browning 834*) (previously in *Abildgaardia*) and G. *Abildgaardia baeothryon* (*J. Almeida de Jesus 1466*) share the Schoenus-type embryo. The orientation of the sub-basal root and shoot (of G) are shown in top view in H. Scale bars=100 µm. Solid arrow=root, open arrow=shoot, thin arrow=second primordial leaf. See Appendix 1 for specimen details.

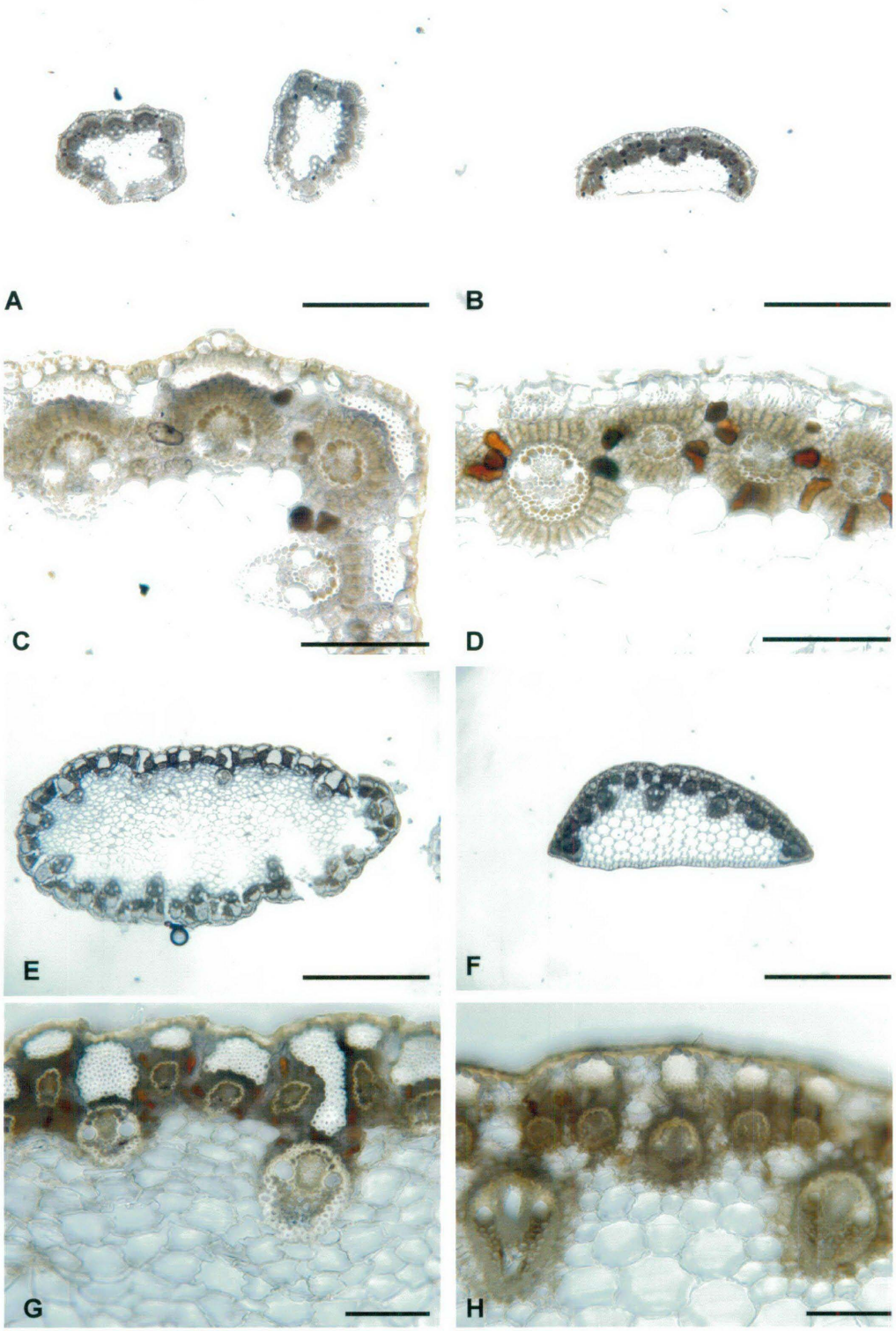


Figure 4.19 Culm and leaf blade transverse sections for two species of *Abildgaardia* showing the typical outlines, arrangement of sclerenchyma strands per vascular bundle, and C_4 fimbristyloid anatomy. A. *Abildgaardia ovata* (Aov11.) culm and B. leaf blade sections at low magnification, with C. culm and D. leaf blade at higher magnification. E. *Abildgaardia triflora* (*P.J. Greenway 1859*) culm and F. leaf blade sections at low magnification with G. culm and H. leaf at higher magnification, showing a second semi row of vascular bundles in the leaf section. Scale bars=100 μm. See Table 4.1 and Appendix 1 for OTU and specimen details.

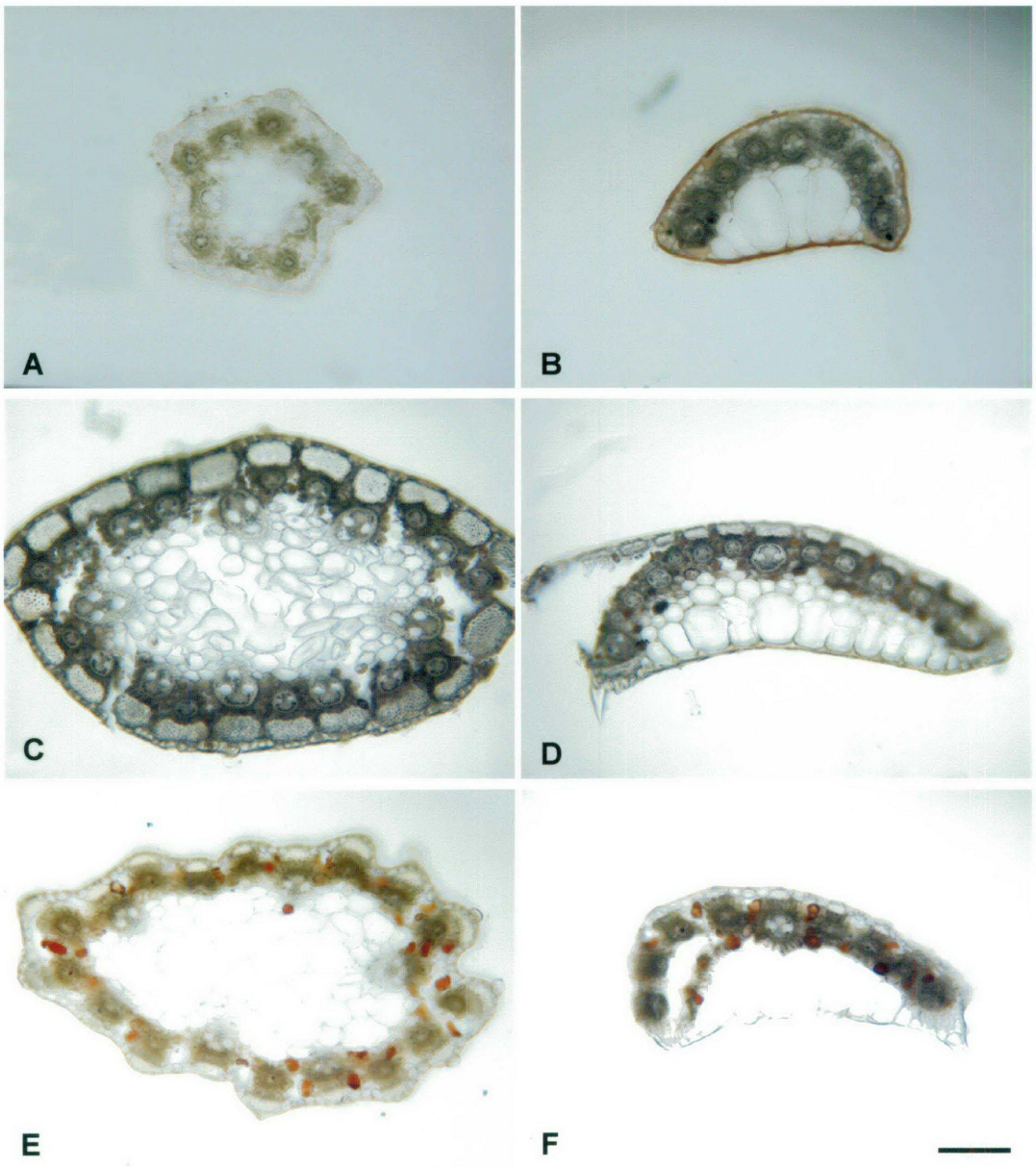


Figure 4.20 Culm and leaf blade transverse sections for species of *Abildgaardia* showing the typical outlines, arrangement of sclerenchyma strands per vascular bundle, and C_4 fimbristyloid anatomy. A. *A.* sp. aff. *pachyptera* (Aaffpach) culm and B. leaf blade sections. C. *Fimbristylis odontocarpa* (Fod2) culm and D. leaf blade sections. E. *Abildgaardia oxystachya* (Aox4) culm and F. leaf blades sections. Scale bars=200 µm. See Table 4.1 and Appendix 1 for OTU and specimen details.

blades and culms mostly have sclerenchyma equalling the number of vascular bundles. The exception is seen in the leaf of *A. triflora*, where a second layer of vascular bundles occurs; although the outer bundles are usually in line with a sclerenchyma strand (Figure 4.19). Leaf blade shape in transverse section is generally crescentiform and a hypodermis may be present as 2 or 3 cell layers; well developed in *A. triflora*. Culms are mostly elliptic or sometimes hexagonal, as in *A. pachyptera* and *A.* sp. aff. *pachyptera* (Figure 4.20).

In contrast, *Abildgaardia hygrophila* and *Fimbristylis variegata* both share C_3 photosynthetic pathway and vary in general vegetative anatomy, however the sections were not suitable for photography.

Discussion

Abildgaardia, as defined by the synapomorphies of C_4 fimbristyloid photosynthetic pathway, Abildgaardia-type embryo, and nut length, and is composed of 11 species. Nine species are found in Australia, eight of these are endemics.

The caution by Bruhl and Wilson (2005, in press) regarding the inconsistency of the photosynthetic pathway in *Abildgaardia* is refuted by this study, which shows that all species in the *Abildgaardia* clade share the C_4 fimbristyloid photosynthetic pathway.

This study finds that *Abildgaardia baeothryon* and *A. hygrophila* have been misplaced in *Abildgaardia* as indicated by the embryo type (Schoenus- and Fimbristylis-types respectively) and the C_3 anatomy of *A. hygrophila*. *Fimbristylis hygrophila* and *A. baeothryon* (= *Fimbristylis bahiensis*) nut characters are atypical

for the genus *Abildgaardia*, and along with *F. variegata*, resemble those of some species of *Fimbristylis*. Evidence from this study does not support the inclusion of *Abildgaardia hygrophila* or *A. baeothryon* in *Abildgaardia* and confirms the exclusion of *F. variegata* (Goetghebeur and Coudijzer 1984, 1985; Goetghebeur 1986; Gordon-Gray 1995; Bruhl and Wilson 2005, in press). Therefore, *Fimbristylis bahiensis*, *F. hygrophila*, *F. variegata* and should all be excluded from *Abildgaardia* Detailed studies are needed to ascertain the correct placement of these three species, which is not necessarily in *Fimbristylis*.

Kral and Strong (1999) described their new species *A. papillosa* as being close to *A. baeothryon* (= *Fimbristylis bahiensis* Steud.). Based on this similarity and the nut characters described in the protologue, it seems reasonable now that *A. papillosa* does not belong in the genus *Abildgaardia*. Assessing the embryo and anatomy of *A. papillosa* is a necessary step to confirm the correct placement of this species. *Abildgaardia papillosa* should therefore also be excluded from *Abildgaardia*.

Goetghebeur and Coudijzer (1985 p:209) regarded *Abildgaardia* and *Bulbostylis* as 'genera with offspring that were derived from a more primitive fimbristylidoid stock'. The perianth bristles found in some florets from a single collection of *Abildgaardia schoenoides* (As13 – KLC230) supports their statement, as perianth has been lost from all other members of the Abildgaardieae and appears to be a remnant feature retained in this sample. The uncontroversial placement of As13 within the *A. schoenoides* s.s. limits, indicate that other characters for this specimen are consistent with the species; the only difference between the sample As13 and the other OTUs is the presence of perianth bristles.

The two samples As5 and As6 that form *A.* sp. aff. *schoenoides* 1 and the main group of *A. schoenoides* need to be compared to the TYPE for *A. schoenoides* collected by Robert Brown. The TYPE specimen for *Fimbristylis squarrulosa* was assigned by Ferdinand von Mueller for a collection from Victoria River and was not based on the Robert Brown specimen collected from the Gulf of Carpentaria. Both TYPE collections do not necessarily represent the same species and need to be compared. Brown's (1810) protologue does not give an adequate description to ascertain the similarity between his specimen and that used by Mueller. The TYPE specimen for *Fimbristylis squarrulosa* is distinctly perennial and corresponds to the specimens currently included as *A. schoenoides* s.stricto. The correct application of the name *A. schoenoides* to one of the delimited groups (*A. schoenoides* or *A.* sp. aff. *schoenoides* 1) must be resolved before relegating *Fimbristylis squarrulosa* as synonym of *Abildgaardia schoenoides*, or a new species described (i.e. As5 and As6).

Although *Abildgaardia* sp. aff. *pachyptera* and *Fimbristylis* sp. aff. *odontocarpa* are from single collections, they form discrete entities and will be formally described subsequently in a valid publication.

This study indicates that *Abildgaardia* should have generic status given that *Fimbristylis* is non-monophyletic, and that *F. depauperata* (from the TYPE section *Fimbristylis* section *Fimbristylis*) falls within a clade that is sister to the clade that contains the species of *Abildgaardia*. Given that *Crosslandia* and *Abildgaardia* form monophyletic groups and that *Fimbristylis* is non-monophyletic, it seems best at present to accept all three as genera. The relationships of *Fimbristylis* need to be assessed in a separate study. This analysis therefore supports the trend in accepting *Abildgaardia* and *Fimbristylis* as equally ranked genera (Gordon-Gray 1971; Kral

1971; Lye 1973; Goetghebeur and Coudijzer 1984, 1985; Goetghebeur 1986; Gordon-Gray 1995; Lye 1995; Kral and Strong 1999).

Recent phylogenies based on DNA sequence data consistently retrieved *Abildgaardia* as sister to *Fimbristylis* (Muasya et al. 2000; Ghamkhar et al. 2005, in press; Simpson et al. 2005, in press). Sample size for *Abildgaardia* used in all studies was small with studies by Muasya et al. (2000) and Simpson et al. (2005, in press) incorporating only one representative, *A. ovata*. In Simpson et al. (Simpson et al. 2005, in press) *A. ovata* and *Arthrostylis aphylla* formed an unresolved group.

New combinations as proposed by Goetghebeur (1986) can now be formally published. Species that comprise the genus *Abildgaardia* in this study are presented here prior to formal publication.

Nomenclature of *Abildgaardia*

Abildgaardia Vahl, Enumeratio Plantarum 2. 296 (1806).

LECTOTYPE: *Abildgaardia monostachya* (L.) Vahl

1. ***Abildgaardia macrantha*** (Boeck.) Goetgh. ex K.L.Clarke, K.L.Wilson, J.J.Bruhl comb. nov. ined.
Fimbristylis macrantha Boeck.

2. ***Abildgaardia mexicana*** (Palla) Kral
Fimbristylis mexicana Palla
Fimbristylis crassipes Boeck. (non Flora 41: 602 1858)

3. ***Abildgaardia odontocarpa*** (S.T.Blake) K.L.Clarke comb. nov. ined.
Fimbristylis odontocarpa S.T.Blake

4. ***Abildgaardia oxystachya*** (F.Muell.) Goetgh. ex K.L.Clarke, K.L.Wilson, J.J.Bruhl comb. nov. ined.
Fimbristylis oxystachya F.Muell.

5. ***Abildgaardia ovata*** (Burm.f.) Kral
Cyperus monostachyos L.
Abildgaardia monostachya (L.) Vahl,
Fimbristylis monostachya (L.) Hassk.,
Iriha monostachya (L.) Kuntze,
Fimbristylis ovata (Burm.f.) J.Kern

6. ***Abildgaardia pachyptera*** (S.T.Blake) Goetgh. ex K.L.Clarke, K.L.Wilson, J.J.Bruhl comb. nov. ined.
Fimbristylis pachyptera S.T.Blake

7. ***Abildgaardia schoenoides*** R.Br.
Fimbristylis squarrulosa F.Muell. (although need to check the TYPE for *A. schoenoides* to confirm synonomy)

8. ***Abildgaardia triflora*** (L.) Abeywickr.
Cyperus triflorus L.
Abildgaardia tristachya Vahl
Schoenus cyperoides Retz.
Fimbristylis triflora (L.) K.Schum. ex Engl.
Abildgaardia triflora (L.) Lye (superfluous)

9. Naming *Abildgaardia* sp. aff. *schoenoides* 1 (As5, As6) is pending comparison with the *A. schoenoides* TYPE specimen.

10. *A.* sp. aff. *odontocarpa*, and **11.** *A.* sp. aff. *pachyptera* are yet to be named.