CHAPTER 5

Micromorphological pollen data join molecular data

5.1 Introduction

5.1.1 A review of palynological studies

5.1.1.1 Early studies (1682–1969)

The study of pollen has been connected closely to the progress of microscopic techniques. Grew (1682) and Malpighi (1687) were perhaps the pioneers in pollen morphology. The development of improved optics was fairly slow during the next century so that there are few significant findings till the remarkable drawings undertaken by Francis Bauer in the early 1800s (Fergusson 2000). With considerable improvements in optical technologies in the early 19th Century new interest in the study of pollen morphology developed and workers such as Hassal (1842a, b) demonstrated the potential of pollen as a possible taxonomic data source. Hassal's work was interesting due to the assessment of the usefulness of pollen morphology as a means of classification. He studied a wide range of pollen grains across some 60 families and illustrated over 150 pollen grains, distinguishing *Convolvulus*, with three-furrowed pollen, from *Calystegia*, with panto-porate pollen, from *Ipomoea*, with spiny pollen. Wodehouse (1935) gives an extensive review of the history of palynology up to 1935. However, he overlooked Hassal.

There then followed the era of a great school of plant systematics in Germany at the end of the 19th Century and the beginning of the 20th Century. Gilg (1895) investigated Gentianaceae and acknowledged pollen data as a valuable part in his systematic work. Wodehouse in the U.S.A. published several important publications on pollen morphology. including a series on the identification and classification of plants and the evolutionary value of pollen grain characters (e.g. Wodehouse 1928). Many of these works were collected in a classic textbook 'Pollen Grains' (Wodehouse 1935).

In the early 1940s there was considerable interest in pollen in relation to systematics and, without doubt, Erdtman (1960) made the most significant contribution. He refined core techniques applied in palaeobotany; this provided detailed inspection as well as careful assessment of the cleared, resistant pollen exine. He also described the pollen morphology of many angiosperm families (Erdtman 1952).

The complexity of the terms used to describe pollen grains and disagreements between specialists had a major deterrent effect and brought pollen studies into some disrepute among taxonomists of the period. The remarkable collection of definitions and illustration of pollen published by Kremp (1965) called attention to this problem. However, the first-class, clear and simplified glossary of Punt *et al.* (1994) dispelled most of these problems.

The arrival of a new technique (transmission electron microscopy or TEM) in the late 1950s saw the development of a parallel field of pollen study relevant to systematics and evolution. The beginning of understanding of the fine structure of the exine and the development of the pollen grain are documented in such classic papers as those of Rowley (1959), Larson *et al.* (1962), Heslop-Harrison (1963, 1968), Horvat (1966), and Roland (1968). The arrival of the earliest scanning electron microscopes (SEMs) in the mid 1960s together with the publication of Erdtman's (1969) book set the scene for the beginning of a new era in the study of pollen grains.

5.1.1.2 More recent studies (1970 to present)

The last 30 years have seen considerable change in the ways in which pollen data are distinguished and incorporated into analyses. Pollen data from the light microscope and the scanning electron microscope and also transmission electron microscope with an effective well-documented taxonomic input have resulted in high-quality comparative pollen-morphological studies that have made very important contributions to our understanding of systematic and evolutionary relationships. Among the earliest to benefit from these novel techniques and undertake comprehensive systematic investigations were Ferguson and Webb (1970) on the genus *Saxifraga* and Cerceau-Larrival (1971) on the Apiaceae, and many other works followed rapidly. Harley and Ferguson (1990) reviewed the contribution of SEM to pollen morphology and systematics. The classic publication by Heslop-Harrison (1976) about the adaptive significance of the exine, together with Chaloner's (1976) work on the adaptive features in fossil exines and Van Campo's (1976) successiform hypothesis were major contributions that focused pollen-morphological studies on understanding the evolution of pollen characters.

A few other significant papers among many hundreds in using SEM for pollen morphology include Punt (1975), Skvarla *et al.* (1977), Nowicke and Skvarla (1977, 1981),

Le Thomas (1980, 1981), Gadek *et al.* (1989), Guinet and Ferguson (1989), Harley (1991), and Ferguson and Harley (1993).

5.1.1.3 Pollen studies in monocotyledons

Pollen studies in the monocotyledons have not received as much attention as in the dicotyledons. Pollen morphology was one of the several suites of characters investigated in the comparative studies of Dahlgren and Clifford (1982) and Dahlgren *et al.* (1985), to reach a better phylogenetic classification of the monocotyledons. Almost concurrently, Zavada (1983) summed up the knowledge involving pollen openings and exine structure. He also discussed phylogenetic 'trends' of these data (Zavada 1983, p. 334). Since then an ongoing increase in the use of pollen morphological data in phylogenetic analyses has been seen (Harley and Zavada 2000).

The inconsistency with which pollen data are described in previous works makes those characters hard to collect from the literature. All authors excluded size for good reason, as even in small studies it is difficult to define the limits of character states of that character (Harley and Zavada 2000), though it might be worth exploring in the case of fine scale species limits questions if suitable grains are sampled.

In some projects, particularly broad investigations on the monocotyledons (Stevenson and Loconte 1995; Chase *et al.* 1995), many pollen characters have been extracted from earlier studies. In some of those there might be a danger, as pollen was not for the most part critically assessed or examined from an evolutionary perspective (Harley and Zavada 2000).

Furness and Rudall (2003) pointed out the significance of the operculate apertures on the pollen grains of monocots. They stated that within monocots, monosulcate-operculate pollen is absent from basal monocots and relatively infrequent among commelinids, except for some Dasypogonaceae, Arecaceae, and Poales (in which the aperture is reduced to an ulcus). They claimed that as the operculum has evolved several times independently in monocots, it may have a variety of related functions, including protection of the delicate apertural area. Pollen grains in Cyperaceae lack the operculum.

5.1.2 Combined datasets

In my study, I had to address the suitability of combining two molecular datasets and one morphological dataset. The question is that if results of individual datasets differ, how best to reach a general conclusion?

Donoghue and Sanderson (1992) discussed the issue of whether molecular or morphological data should be preferred to each other or be combined to reach more resolution. Their final recommendation was "both morphological and molecular data should be used in reconstructing phylogeny".

The total homoplasy might not have the important role that the distribution of homoplasy does (Jansen *et al.* 1990). Thus, it should not be awkward to assemble highly homoplasious with highly confident datasets (Sanderson and Donoghue 1989). This is defensible, at least, when confidence has been estimated through resampling methods such as bootstrap (Felsenstein 1985; Sanderson 1989). Conversely, because consistency is the function of the power of various, independent synapomorphies (Hennig 1979; Sanderson 1989), confidence can be low, even when homoplasy is low or does not exist; this is applicable even in the cases that only one synapomorphy exists for supporting each clade (Donoghue and Sanderson 1992).

Moreover, the selective significance of characters may have no particular link with the level of homoplasy that they exhibit; neutrality does not always result in low level of homoplasy. Numerous mutations can happen at one nucleotide site throughout the evolution of a lineage and all being equal, the probability of such multiple hits increases with time (Donoghue and Sanderson 1992). In these cases, the chance of high homoplasy is elevated (Mishler *et al.* 1988).

In the phylogenetic context, gathering multiple data sets and undertaking data partitions are the first steps to combine datasets. Whether a researcher should treat the evidence from these partitioned data differently is the main challenge.

Separate analyses of different datasets appear to present an appropriate situation for using consensus approaches. When the most parsimonious cladogram relative to non-molecular characters differs from the one constructed for the molecular characters, it is reasonable to look for the consensus. This will mostly provide an unresolved cladogram in which the suggestions about how those separate cladograms agree will be captured.

Barrett *et al.* (1991) argued against the use of consensus trees and instead suggested pooling the data and searching for the most parsimonious tree for all different data. However, as Kluge (1983) mentioned, when many molecular and few non-molecular characters go together, the result of pooling might be a "swamp" for non-molecular data.

Hillis (1987) proposed a consensus of all these ideas. He stated that classifications must be based on consensus cladograms, but that the best estimation of the phylogeny and character evolution is understood by the most parsimonious tree obtained from the pooled data. Moreover, the explanations of Barrett *et al.* (1991) about the advantages of pooled data over the consensus of separate trees according to the 'principle of total evidence' are good motivations for combining the data and construct the trees in light of all data. In recent years, many researchers have adopted this method and found high resolutions in their study groups (e.g. Olson 2002; Lundberg and Bremer 2003; both of these studies investigated three molecular and one morphological dataset).

5.1.3 Use of pollen data in phylogenetic studies

More recently macromolecular systematics and a good understanding of the composition of DNA have led to attempts to use this new source of data together with the older data sources to elucidate systematic and evolutionary relationships. Advances in pollen morphology have largely come from a multidisciplinary and collaborative team approach, which Ferguson (1984) predicted as the way forward for comparative studies. Workers such as Doyle and Le Thomas (1994) on Annonaceae, Persson *et al.* (1994) on Solanaceae and Giveyrel *et al.* (1998) on Asclepiadaceae applied combined datasets in their analyses. They not only could make sound assessment of evolutionary relationships but also evaluate characters including pollen and throw more light on the evolution of individual pollen structural features. This approach might help to answer a fundamental question in comparative pollen morphology, and in fact in morphological studies as a whole, as to which features show structural homology and which show functional homology.

Tomlinson (2000) mentioned that exine structure may be less important phylogenetically but that also, like many characters, it can show up weaknesses in purely molecular gene sequencing data. Stefanovic *et al.* (1998), using 28 S rRNA sequences, suggested that the conifers form a monophyletic group. These results were at variance with pollen data

(Kurmann 1994). Such results raise the issue of why pollen morphology may vary so much without apparent phylogenetic importance and returns to the question of adaptive significance, although the alternative hypothesis could be that the phylogeny the pollen data were being tested against wasn't very good.

Pollen type is a pollen morphological character of pollen grains, which can be distinguished either as one distinct character or as a unique combination of characters. While the pollen type has been supported for comparative applications, it is inappropriate to be considered as a separate character for phylogenetic analyses. It is possible that species that have very similar pollen might be, in fact, closely related taxa in a phylogenetic analysis. However, a pollen type itself should not be considered as an informative source of information for phylogenetic analyses otherwise it will be just a repetition of scoring the individual characters because its distinctiveness relies on a combination of characters. Each pollen character should be separated and treated objectively as an individual feature in a data matrix. Pollen type simply sounds like another of those bucket-characters (e.g. embryo type in Bruhl 1995) that needs to be reduced to its component characters to reduce homoplasy and increase homology. An understanding of pollen characters in order to estimate whether similarity or dissimilarity exists among comparable components is fundamentally crucial (Harley and Zavada 2000).

The pollen features included in phylogenetic analyses seem to vary depending on the preparation method sin addition to the exploratory methods used (LM, SEM and/or TEM) (Harley and Zavada 2000).

There is much evidence of homoplasy in palynological characters, although perhaps no more so than for any other characters (Ferguson 2000). Several workers distinguished the repeated patterns of character conversion and Van Campo's (1976, p. 127) key evolutionary "tendencies" are the prime case. Characters might be taxonomically informative at particular hierarchical ranks within each particular set of taxa under study. The real advance in taxonomy is that it is now possible to separate homoplasy and homology through cladistic analysis. High levels of homoplasy in features such as pollen ornamentation are an inherent component of the diversity of life. The level of homoplasy may restrict the extent and taxonomic level at which a particular character proves informative but that does not reduce the utility of exploring this aspect of morphological diversity. Nor has it been confirmed that pollen data are more homoplasious than other sources of data. Due to the strong selective

pressure on non-vegetative characters (Goldstein *et al.* 2001), notions of the undoubted strength of these characters may be misleading (Fergusson 2000).

5.1.3.1 Pollen studies in Cyperaceae

Cyperaceae and Juncaceae have monad-like tetrads with no dividers between their four nuclei. Three of these nuclei move towards the constricted end of each pollen and deteriorate. The fourth nucleus migrates towards the other end of the pollen, the large end (Kirpes *et al.* 1996).

A comprehensive review of the previous works on Cyperaceae has been provided in Chapter 1, section 1.2.1.1. The latest key specifically on Cyperaceae pollen is that of Faegri and Iverson (1975, p. 253). In this key the features of the specific apertures, as being different from the pore at the larger end of the pear-shaped pollen grains of Cyperaceae, have been suggested to have a great impact on the identification of these pollen grains.

Microscopic characters, including micromorphology and leaf anatomy, have been shown to be valuable in resolving incongruences between estimated phylogeny based on macromorphology and molecular data in the graminoids (e.g. Columbus 1999). These features are being studied in the Abildgaardieae and Arthrostylideae by K. Clarke in her Ph.D. project (pers. comm.). Like other non-molecular data sources, pollen has the potential to contribute characters to phylogenetic studies to give better resolution. Few published works have applied the potential of this very informative sort of data in cladistic studies (e.g Blackmore and Cannon 1983; Blackmore *et al.* 1988; Abu-Asab and Cantino 1993; Abu-Asab *et al.* 1993; Bremer 1994; Persson *et al.* 1994; Knapp *et al.* 1997; Martin *et al.* 2001; Simpson *et al.* 2003).

The assessment of primary homology (Kitching *et al.* 1998) has a vital role in scoring the pollen data for phylogenetic studies. Without doubt particular features, like ornamentation, although seemingly similar among some taxa, can mask considerable differences related to ultrastructure. Hence, similar features recognised by light microscopy may be, in fact, different features when investigated thoroughly by SEM studies (Harley and Ferguson 1990; El-Ghazaly 2000) and this is exactly the case in this study.

Padhye and Makde (1980) showed that 25 species belonging to 10 genera possess the *Cyperus*-type pollen, which is where the pollen grains have only one 'colpus occupying practically the entire distal end of the grain'. In an alternative terminology (Hoen 1999) this type of pollen is said to have an ulcus occupying roughly the whole distal face of a grain. Sharma (1967) studied 21 taxa of Cyperaceae and most of them exhibit the *Cyperus*-type. Tiwari (1970) reported similar findings. Thus genera such as *Bulbostylis*, *Fimbristylis*, *Eleocharis*, *Fuirena*, and *Rhynchospora* were reported to demonstrate this pollen type (Padhye and Makde 1980). This was reported to be also a common aperture type in many other monocot families by Padhye and Makde (1980). Walker and Doyle (1975) and Harley and Zavada (2000) agree with Padhye and Makde in monoaperturate pollen grains being the dominant type in monocots although they refer to this type of pollen as monosulcate. Erdtman (1952) who reported such a pollen type in *Mapania* and allied taxa, considered it somewhat difficult to describe for Cyperaceae. Dahlgren and Clifford (1982, p. 164) described pollen in Cyperaceae as mostly having a distal ulcus, 'some with up to three additional lateral poroid or elongate tenuitates'. However, Bruhl (1995) followed Koyama (1956) in recognition of two states for pollen apertures in Cyperaceae: with few (1–6) apertures and with many (>6) apertures. He reported that only pollen grains of *Baumea*, *Machaerina* and *Tricostularia* possess many apertures and that pollen grains in most of the other genera are few-aperturate. Nevertheless, he suggested that based on the investigations by other researchers, uniaperturate pollen grains could be recognized, too. Simpson *et al.* (2003) examined *Mapania tenuscapa* using the SEM and reported a single, large, and distinct ulcus with 'an annulus and a finely reticulate aperture membrane'. They described the ulcus in *Diplasia* (Mapanioideae) as forming a cavity filled with material probably derived from the tapetum.

5.1.4 Different approaches in combining data

Several approaches have been suggested by researchers to deal with different datasets. The most popular ones are:

Hillis (1987) believed that the best estimate of phylogeny is derived from combined analysis, but congruence among individual analyses is convincing evidence that phylogeny is correctly estimated.

Kluge (1989) proposed the "total evidence" approach. He argued that data sets should be always combined, and that combined analysis makes individual results irrelevant. Combined analysis maximises explanatory power of data, regardless of whether individual results are consistent with combined results. His philosophical position was: let the characters decide among themselves. This position is appealing, but runs the risk of confounding characters of genes with characters of organisms (see Chapter 2, section 2.2.3.1).

Miyamoto & Fitch (1995) suggested a consensus approach, taking the opposite position from Kluge. According to their approach, individual datasets or process partitions must be always analysed separately. Confidence will be judged in points of consensus from resulting trees. In this approach, separate analyses give insight into individual data. Different results could indicate violation of underlying assumptions of analysis. There are arguments for separate analyses such as:

- Different datasets may be reconstructing different underlying trees (genes reconstruct gene trees: non-orthology problems)

Method of analysis may be inappropriate for one (or more) of datasets -- method may be inconsistent (converge on wrong answer as more data added) or biased for data or have greatly different rates of substitution.

Arguments against separate (rather than combining) data analysis are based on the fewer number of characters in individual analyses that cause more sampling variation

Conditional combination (Bull *et al.* 1993; de Queiroz *et al.* 1993; Rodrigo *et al.* 1993) bases the decision on whether to combine on degree of incongruence. Weak incongruence may be from inadequate sample size. Strong incongruence may indicate different histories or violation of assumptions.This method suggests statistical tests to get the confidence level. There are several statistical tests for incongruence (see Chapter 4, section 4.2.5.1) such as:

Parsimony-based tests:

Templeton test (Templeton 1983): or goodness of fit. This test asks the question of whether alternative topologies differ significantly in how well data partition fits

ILD (incongruence-length difference) test (Farris *et al.*, 1994): this test compares the difference between the number of steps required by individual and combined analyses

Topological incongruence test (Rodrigo *et al.* 1993)

Maximum-likelihood based tests:

Likelihood heterogeneity test (Huelsenbeck & Bull 1996): compares likelihood obtained under the constraint that the same phylogeny underlies all datasets versus the likelihood obtained when the constraint is relaxed.

Miyamoto and Fitch (1995) advocated separate analysis of multiple datasets, because the loss of independence of characters resulting from the combined analysis of multiple datasets weakens statistical support for phylogenetic inference. Instead, these authors favoured multiple independent confirmation of phylogeny based upon the analysis of data partitions separately as a means to enhance accuracy and lend statistical support to the inferred pattern of descent. Several authors (e.g. de Queiroz 1993; Huelsenbeck *et al.* 1996) have proposed a middle-ground compromise to these conflicting viewpoints, offering that independent datasets may be analysed in combination when the data partitions can be shown to be congruent. This approach of "Conditional Combination" mandates the prior demonstration of homogeneity of partitions. One favoured method to test sequence congruence has been the Partition Homogeneity test (PHT) (see PAUP* software package by Swofford) also known as the Incongruence Length Difference (ILD) test (see Farris *et al.* 1995), whereby separate datasets are randomly resampled across partitions without replacement, and these resampled data are used to generate trees by parsimony analysis. The sum of the branch lengths of the "best" tree obtained without resampling is then compared to the distribution of the sums of branch lengths taken from a set of trees inferred from randomly resampled data. These sums should be similar where datasets are congruent (the null hypothesis), and differ when incongruent (see Farris *et al.* 1995). This test is used widely to test congruence of partitioned datasets and has shown superior performance relative to other statistical tests used for this

purpose (Cunningham 1997). Barker and Lutzoni (2000), however, noted that the ILD test was susceptible to rejecting the null hypothesis of congruence erroneously when data partitions showed differing levels of homoplasy. This problem was similarly noted by Carbone *et al.* (1999)

5.1.5 Aspects of pollen morphology

5.1.5.1 Pollen terminology

The applied use of pollen data from an assortment of fields and the use of different techniques such as light microscopy, scanning electron microscopy and transmission electron microscopy by different researchers have contributed to a large number of terms associated with these data. Since the mean size of a pollen grain is about 30 μm in diameter, it is impossible to see the fine structures on the surface of pollen by light microscopy. This restricted early studies of pollen, and is partly responsible for the different terminologies that have developed.

Palynologists, up until now, do not have a uniformly agreed-upon terminology to describe pollen. They continue to apply terms linked with a variety of nomenclatural approaches, often merging them, as well as imparting new meaning to terms. This has resulted in the continuing development of palynological glossaries (e.g. Kremp 1965; Punt *et al.* 1994; Hoen 1999). In this study, I have used the terminology given in Hoen (1999) (http://www.bio.uu.nl/~palaeo/glossary/glos-int.htm), which is the online version of the second edition of Punt *et al.* (1994).

The outer coating of a spore wall or sporoderm (from Latin *sporodermis*, seed-skin) is named the exine (from Latin *exterius*, outer). It is normally ornamented by processes, reticulately branched and anastomosing ornaments, etc., that can help to identify the genus or even species of a particular specimen. It is even sometimes informative at levels lower than species. The varied look of this outer layer and its resistance to decay and chemicals are of great use in contemporary palynology. Morphological features of the mature pollen grains are generally categorised into five groups namely apertures, exine ornamentation (sculpturing), exine strata, dimensions and form in order of giving descriptive information about pollen grains of each taxon (Ravikumar 1984).

5.1.5.2 The character states

Harley and Zavada (2000) discussed at length the pollen features and character states that are potentially useful in cladistic analysis of monocot pollen grains. The major groups of features that I have selected for this study include:

Characters related to the exine openings (apertures);

Characters associated with the exine sculpturing;

Characters related to pollen appearance, symmetry and polarity;

Characters related only to the size.

The reason for this particular selection among the characters that have been used in this study is the ease of technical methods for observing these features and their character states in my study group, as well as high variation of these features among genera in Abildgaardieae. The ease of comparison to light microscopic data in future is another reason for this selection.

5.1.5.2.1 Apertures and their phylogenetically significant aspects

Punt *et al.* (1994) defined an aperture mainly based on the definition provided by Erdtman (1952) as "a specialized region of the sporoderm that is thinner than the remainder of the sporoderm and generally differs in ornamentation and/or in structure". The two basic kinds of pollen grain are the porate and colpate. Porate pollen has round apertures (pores) in its exine, whereas colpate pollen has elongated furrows named colpae (also referred to as sulci by some authors, e.g. Harley and Zavada 2000). Phylogenetically, furrows are considered to be a primitive type; pores probably developed later through the tightening of furrows (Iwanami *et al.* 1988). The occurrence of furrows in more primitive plants (ferns, mosses, and primitive angiosperms) and pores in more evolved families supports this (Erdtman 1969).

Shah (1962) asserted that pollen grains of Cyperaceae have a distal ulceroid aperture. Wodehouse (1965) found a solitary, poorly defined, irregularly formed germ aperture at the bigger end of the pollen grain. Erdtman (1966) explained that an aperture form with an ulceroid opening at the large end together with three peripheral, more or less

indistinctly marked minute openings or prolonged apertures appears to be the commonest type in Cyperaceae.

When the pollen germinates, a pollen tube emerges through one of the apertures. The apertures might also allow volume modifications caused by change in moisture. Pollen tubes germinate between the ridges in polyplicate (colpate) pollen (Hesse and Halbritter 1993; Weber *et al.* 1999).

5.1.5.2.2 Apertures and the effect of acetolysis

One of the methods that have been widely used in pollen studies to fix the pollen grains is called acetolysis. The acetolysis mixture is made up of one volume of concentrated sulphuric acid and nine volume of acetic anhydride. This mixture is added to the pollen grains and then goes through a process of boiling and washing (Erdtman 1969). This technique usually works in a wide range of plants but causes considerable damages in fragile pollen grains such as those of Cyperaceae (Raynal and Raynal 1971; Van Wichelen *et al.* 1999). There are also reports in other families about acetolysis producing artifacts in structure and therefore in dimensions. As a result, apertures collapse along their longer axis and this aperture rupture causes unnatural shapes of pollen (Nowicke and Miller 1990).

5.1.5.2.3 Exine outer ornamentation

The outer parts (exine) of pollen grains have different kinds of sculpturing (also called 'ornamentation'). There is an astounding diversity in pollen structure including exine ornamentation in plants. Yet there appears to be uniformity within each species (Ravikumar 1984; Iwanami *et al.* 1988).

Anyone who works on monocotyledonous pollen (particularly Cyperaceae pollen) knows that a major problem examining the sculpturing of these pollen grains by SEM is the collapse of the grains even after critical point drying (e.g. Van Wichelen *et al.* 1999: Harley and Zavada 2000). This may be due to the relatively thin walls of monocotyledons compared to dicotyledons (Zavada 1983), or the collapse might be due to structural variation of the partition layers as well as aperture position, or a combination

of both. Both features give rigidity to a pollen wall, which permits the wall to keep its integrity in water pressure (Harley and Zavada 2000). However, it should be pointed out that the collapse of pollen grains does not always happen. Even different intrafamilial groups may show different behaviours (e.g. Zavada 1983). Tribe Schoeneae of Cyperaceae is a good example of such differences in response to the treatments (Wheeler and Bruhl 2000).

The outmost layer of the exine is named the "tectum". This layer partially or fully covers the residual inner layers (Erdtman 1969, p. 42). A tectum may be "smooth" ("psilate") or carry various kinds of carving and decoration (Erdtman 1969, p. 42).

A tectum may be one-pieced, with or without holes or perforations, or divided into smaller parts by empty spaces called "lumina". Projections rising from any part of the general exine surface are called processes (Fig. 5.1). They may be pointed, and then are called "spinules" if their length does not exceed 3 μm and "spines" if their length is more than 3μm. Blunt, drumstick-shaped swellings with an inflated top plus a small shaft are called "pila" (sing. "pilum"). Blunt processes, wider than tall and irregularly roughened are named warts ("verrucae") (Fig. 8E in Walker and Doyle 1975) if they are not constricted at the base or "gemmae" (sing. "gemma") if they have constrictions. Processes known as "clavae" or clubs are longer than wide and pointed towards the base (Fig. 5.1). Very small sphere-shaped processes having a diameter up to 0.5 μm are often described as "granules" (Erdtman 1969).

Transitions from a particular type of process to another are common. Various, often intergrading, process types (spinose, spinulose, baculate, granulate, scabrate, etc.) may be observed on different parts of a pollen grain's surface (Erdtman 1969).

Fig. 5.1. Examples of exine ornamentation (topography). A: spine, B: spinule, C: bacule, D: wart (verruca), E: gemma, F: pilum, G: clava (modified from Erdtman 1969).

5.1.5.2.4 Shape

In "peroblate" pollen grains (Latin *per*, very, much; *oblatus*, flat) the equatorial breadth is more than twice the length of the polar axis. In "perprolate" pollen it is less than the length of the polar axis (Erdtman 1969).

Between these extremes a broad range is distinguishable: in "oblate" pollen grains the ratio of P (polar axis) to E (equatorial diameter) is defined as 4:8 to 6:8, with a transition through "suboblate" (6:8–7:8), "spheroidal" (7:8–8:7), and "subprolate" (8:7–8:6), to "prolate" grains (8:6–8:4) (Erdtman 1969)

5.1.5.2.5 Size

Because of the compound structure of the outmost layer of pollen grains, it is necessary to specify the exine elements between which the measurements are taken. Sometimes the dimensions of the space surrounded by the exine are also given (Erdtman 1969).

Size of pollen grains varies widely: from 10 to 200 µm in diameter (Iwanami *et al.* 1988). The following classes of dimension of pollen grains have been distinguished using the length of the longest axis (Erdtman 1969):

Very small: less than 10 µm; examples: *Lycoperdon*, *Myosotis palustris*.

Small: 10–25 µm; example: *Daphne mezereum*.

Medium-sized: 25–50 µm; example: *Centaurea cyanus*.

Large: 50–100 µm; examples: *Pinus* spp. (the longest axis 80 µm).

Very large: 100 – 200 µm (size can be rounded to the nearest 10 µm); example: spruce (longest axis ca. 125 µm).

Gigantic: bigger than 200 µm.

The size can be affected by the chemica solution used and/or the embedding medium (see Faegri and Iversen 1966). The pollen grains on a slide may also vary in size depending on their stage of maturity or the inwards collapse of the grains (Erdtman 1969).

5.1.6 Palynological characterisation of taxa above the rank of order

Modification of a basic monosulcate aperture type results in the aperture variation seen in monocotyledons. It seems that aperture position and number result from different developmental process compared to the dicotyledons (Harley and Zavada 2000).

5.1.7 Preparation method for scanning electron microscopy

As Van Wichelen *et al.* (1999) stated, for a clear view of the strata in a pollen wall, removal of cytoplasm from the pollen grain is important. The standard procedure for this is acetolysis, as described by Erdtman (1960, 1969). However, the pollen grains of some plants are so severely damaged by acetolysis that other methods must be applied in these plants to avoid those problems (Erdtman 1969). Recent workers (Raynal and Raynal 1971; Sultan *et al.* 1994) have agreed that acetolysis results in the breakdown of pollen in Cyperaceae. Raynal and Raynal (1971) developed a modified acetolysis mixture in which lactic acid was a constituent; this gave a much better result with light microscopy. Padhye and Makde (1980) used Erdtman's acetolysis technique with some modifications recommended by Nair (1960). Van Wichelen *et al.* (1999) mentioned that due to the existence of a thin exine in the pollen grains of most Cyperaceae, acetolysis is an extremely drastic approach, which causes the pollen grains to become completely dissolved or collapsed. Another technique is treatment by potassium hydroxide (KOH) (Fernandez 1987; Van Wichelen *et al.* 1999). KOH deflocculates the pollen grains and lets them separate from each other while helping them to retain their three dimensional shape. KOH may cause swelling of pollen beyond their normal size, but does not affect the form of the porus and colpus, as can acetolysis (Moore *et al.* 1991). Therefore KOH treatment is preferable to acetolysis in the study of colpus and porus characters. In contrast, as Rudall *et al.* (2003) observed in Araceae, acetolysis can help to dissolve calcareous artefacts, which might be misleading and scored as sculptures, but no such artefacts are known in Cyperaceae.

Van Wichelen *et al.* (1999) treated dry pollen grains of various species of Cyperaceae by five different methods prior to SEM examination. These methods were:

Unprocessed pollen: the dry pollen grains were directly put on a stub,

Treatment with Agepon (a wetting agent used as a final bath after the last wash in the process of film development): to bring back the original shape of the rehydrated and shrunk pollen grains.

Acetolysis according to Reitsma (1969) by expansion of dried pollen grains before acetolysis through boiling with %10 potassium hydroxide, water, glacial acetic acid or lactic acid: with two different treatments, with heating (strong reaction) and without heating (only by using a wetting agent).

KOH method: with four different conditions using only 10% KOH or Agepon prior to 10% KOH and different durations for each treatment.

Critical point drying: the pollen grains were put through a series of treatments before critical point drying. These treatments included treating in Agepon and washing with distilled water, then four different treatments as listed below:

no treatment.

fixation in formaldehyde 3.5% overnight, then washing with distilled water.

fixation in glutaraldehyde 2% overnight, then washing with distilled water.

treatment with KOH 10%, then washing with distilled water.

then passing the pollen grains through an alcohol series (25-50-75-94-100%).

Van Wichelen *et al.* concluded that for SEM studies, critical point drying using Agepon and KOH gives the best results.

5.1.8 Pollen grains in this study

An extended evolutionary study of the tribe Abildgaardieae, using representatives that have not been sampled before and two sources of DNA data, has been presented (Chapters 3 and 4). The aims of the current study are to:

1. Investigate the pollen of 45 species of Abildgaardieae and its allies in Australia and to assess whether pollen characters can contribute taxonomic evidence; and
2. Identify monophyletic taxa at tribe as well as genus level, using cladistic analyses of combined DNA sequences and pollen features.

5.2 Materials and methods

Morphological features of the pollen grains of 45 of 427 species in six of 10 genera belonging to two tribes Abildgaardieae, and Schoeneae and three species in the outgroups in Eleocharideae and Fuireneae, (as recognised by Goetghebeur 1998) have been studied using SEM. The sample included representatives of 15 sections out of Kern's 18 sections of *Fimbristylis* (sections *Signatae*, *Dipsaceae*, *Fuscae*, and *Rigidulae* were not sampled). One to three specimens (Appendix 1) were scored for each species; 30 anthers per specimen and 20 pollen grains per anther were examined and the observations checked against the literature (Erdtman 1969; Huang 1972; Padhye and Makde 1980; Van Wichelen *et al.* 1999; Punt *et al.*

1993; Sultan *et al.* 1994). Fertile anthers were selected using a binocular microscope and were dissected by very fine forceps. Conflicts about pollen features in the previous works conducted by other authors were resolved by comparison with their descriptions based on my observations of herbarium specimens. Pollen grains of *Bulbostylis* sp. nov. and three *Fimbristylis* species were double checked under the light microscope at the University of New South Wales and Royal Botanic Gardens Sydney to confirm the existence or absence of apertures. As these observations indicated consistent scoring of these characters, and the process was time consuming, the LM was not used for all samples. Specimens have been lodged with the N.C.W. Beadle Herbarium (NE, University of New England) and National Herbarium of New South Wales (NSW, RBG Sydney) (Appendix 1).

For the present survey, I experimented with three techniques:

1. Unprocessed pollen: the dry pollen grains were put directly on double-sided tape fixed on a stub using one third of the specimens,
2. Pollen treated by Agepon ™ (Agfa) and KOH (10%) (see section 5.2.1) followed by dehydration using an alcohol series (see section 5.2.3),
3. Pollen treated simply with boiling water (see section 5.2.2) and then alcohol series, prior to SEM examination (see section 5.2.3).

The first two methods were based on Van Wichelen *et al.* (1999) although the second one was modified in duration of treatments. The third method was a modification of the alcohol series of Van Wichelen *et al.* (1999) for rehydration and dehydration of pollen grains.

5.2.1. Treatment with Agepon

Rehydration of pollen grains re-established the fresh shape of the dried and shrunk grains. This was done by soaking dried flowers in an Agepon ™ solution in water (1:200) in a small container. The duration of the Agepon treatment has to be determined empirically. I used an average time of 15-20 min for rehydration followed by 10 min in 10% KOH solution.

5.2.2 Treatment with boiling water

Rehydration was done simply using boiling water (for 5 minutes). Flowers were first boiled for about 5 min and left to cool before removing the anthers. I did not use Agepon in this treatment as boiling water could do the same job as Agepon does. In addition, boiling water

made separation of anthers and pollen grains from flowers much easier. Then the anthers were treated by 10% KOH for 10 min.

5.2.3 Fixation with alcohol

Intact anthers were placed in permeable cylindrical containers (1.2 cm height, 1 cm diameter), with microscopic pores fitted to avoid losing any pollen grains. For rehydration of pollen, in some cases I put these tubes into Agepon for 15 minutes, and then washed them with distilled water, after that I left them in 10% KOH for 10 minutes, and then dipped them in distilled water again. In other cases, I simply boiled the tubes in water for 5 minutes. Next, I passed the tubes through a series of ethanol dilutions (25, 50, 75, 95, 100%) used for dehydration. Each step took no less than 2 hours (following Van Wichelen *et al.* 1999). The best outcomes in this study were found to be:

25% alcohol for 3 h,

50% alcohol overnight,

75% alcohol for 3 h,

95% alcohol for 5 h,

100% alcohol overnight.

5.2.4 Scanning electron microscopy (SEM)

Prior to observing the pollen grains in the scanning electron microscope, it is necessary to dry and vacuum-coat them with gold at 30 nm. Most of the samples were critical point dried to avoid shrunken pollen grains. After drying, the samples were stuck to stubs by double-sided sticky tape. The samples were observed and photographed (Fig. 5.3) in a Cambridge Stereoscan 360 SEM operated at 12 kV and features of the pollen grains recorded (Table 5.3).

5.2.5 Light microscopy (LM)

For light microscopy the washed pollen grains (still in a little alcohol) were viewed under a Wild Photomakroskop M400 light microscope with 40X objective using normal brightfield optics to observe pollen of *Bulbostylis* sp. nov., *Fimbristylis tristachya*, *F. velata*, *and F. xyridis*., as they were mounted in the 50% glycerin jelly (Zander 1997, 2003). A cover slip

was lowered carefully on to the slide to prevent the development of bubbles. The slides were put on a hot plate at 60°C to spread the glycerine jelly. The cover slip was sealed with clear nail varnish.

In the SEM study of pollen, I observed two flowers per specimen, five anthers per flower, and five pollen grains per anther making the total of 50 pollen grains per specimen. Each grain was observed from four directions, which are compared to the directions of the globe, i.e. the polar north and south and equatorial views as well as a side view at a 90-degree angle from its equatorial view. Calculation of means of the polar and equatorial axes was based on the sizes of 50 (rarely fewer) pollen grains. Measurements of sculpture were based on 50 observations for each specimen without any exception. Most micrographs were taken of the equatorial view because it reveals morphological characters of the exine sculpture as well as the placement of apertures.

5.2.6 Pollen terminology in this study

In general this study follows the terminology recommended in Hoen (1999) (http://www.bio.uu.nl/~palaeo/glossary/glos-int.htm), which is the online version of the second edition of Punt *et al.* (1994). My morphological data matrix includes 10 pollen characters not previously discussed in Cyperaceae (characters 5, 7-11, and 13-16 in table 5.1 and Appendix 2).

5.2.6.1 Apertures

There are a few polymorphic characters related to apertures. The terms applied here include "aperture", an area of the pollen wall that is thinner than other areas of the pollen wall, "pore", a more or less circular aperture, "colpus" is an elongated, aperture with a length/breadth ratio greater than 2, "colpoid", a more or less lengthened aperture-like structure; and "ulcus", a very large circular aperture situated at the distal pole of the pollen grain, which extends roughly to the visible outer limits of the pollen.

The difference between furrows and pores is morphological: furrows are boat-shaped with acute ends. Pores are, in most cases, isodiametric or, if these apertures are lengthened, the sides are curved.

5.2.6.1.1 Existence of aperture

Two character states were used:

1. Present: if any kind of aperture (pore, colpus, colpoid or ulcus) is observable;
2. Absent: without any aperture, i.e. inaperturate.

5.2.6.1.2 Aperture type

Aperture type has been defined as: 'a modification of the exine from which the pollen tube can potentially exit the pollen grain' (Harley and Zavada 2000, p. 196). Four main character states have been described (Hoer 1999), based on the presence of only colpoids and ulci in the study group:

Colpoid present or absent: a colpus means an ellipsoid opening with its long axis parallel to the elongated axis of the pollen grain. An almost equivalent term for colpate is sulcate, although sulcus, as a rule, is longer than colpus (Kremp 1965), and as Erdtman (1952, p. 12) has stated, is a colpus that touches or passes through the distal pole. Colpoid apertures are more or less similar to colpi (Erdtman 1952, p. 461) but they differ in the depth and distinctness of their furrow, i.e. colpoids are not as deep and distinct as colpi;

Ulcus present or absent: an ulcus is an extremely large (relative to the size of the pollen grain), roughly circular opening with a normally ill-defined margin that, when visible, extends approximately to the outer limits of pollen. An ulcus is usually situated on the distal side (aperture position is treated as a separate character).

5.2.6.1.3 Aperture number

Three character states have been used here out of six suggested by Harley and Zavada (2000) as two of their states were not observed in this study and another one (inaperturate) was scored in section 5.2.6. .1:

1. One: the single distinct aperture is a common ulcerate or porate aperture;
2. Two;
3. Three;
4. Four or more.

5.2.6.1.4 Aperture position

Four character states have been used here based on comparisons between Erdtman (1958, 1969) and Hoen (1999):

1 Distal (Ana-aperturate): usually a distinct aperture centred at the distal face;

2 Equatorial (Zono-aperturate): aperture(s) centred at or just about the equator;

3 Random: both distal and equatorial apertures present.

4 None: no distal or equatorial apertures.

5.2.6.1.5 Aperture margin

This character is from Hoen (1999). Erdtman (1969, p. 240) has provided a good definition of this character:a distinct zone surrounding the aperture. He explains further that the delimitation from the rest of the exine is usually due to differences in thickness of the sexine or nexine (sexine or nexine thicker or thiner than in surrounding parts of the exine). Although I was not able to see and compare the thickness of sexine and nexine, I was clearly able to distinguish whether these distinct surrounding areas with a different texture from the rest of the exine exist or not. Hence I considered two character states:

Distinct margin present;

Distinct margin absent.

5.2.6.2 Shape and polarity of grains

There are a few polymorphic characters associated with form and polarity of pollen grains in the study group. The character states have been selected based on comparison of the character states suggested by Erdtman (1969), Moore *et al.* (1991), Hoen (1999), and Harley and Zavada (2000).

5.2.6.2.1 Pollen shape

Six character states (following Erdtman 1952; Hoen 1999) have been used:

Oblate, pyriform (pear-shaped) (P/E = 4:8 to 6:8);

Suboblate (P/E = 6:8 to 7:8);

5 Spheroidal (polar axis/equatorial diameter = 7:8 to 8:7): most common state in my study group;

6 Subprolate (P/E = 8:7 to 8:6);

7 Prolate, pyriform (pear-shaped) (P/E = 8:6 to 8:4):

8 Perprolate (polar axis/equatorial view > 2.

5.2.6.2.2 Polarity of pollen

Three character states apply here:

1. Heteropolar: describes pollen in which the distal and proximal faces of exine are different, either in shape, ornamentation or apertural system. This is the most common condition in my study group, where many pollen grains appear to have a single opening positioned at the distal side;
2. Isopolar: the distal and proximal faces look alike;
3. Paraisopolar: almost isopolar but the two faces are of unequal size.

5.2.6.3 Sculpturing

5.2.6.3.1 Distinctness of exine sculpturing

The surface of pollen may have ornamentations (called apiculate elements by Hoen (1999)) as mentioned in section 5.1.5.2.3. If present, they may be easy to observe or hardly visible except at very high magnification, giving two character states to this character:

Distinct: processes easily visible even at lower magnifications;

Indistinct: Only visible in magnifications more than 10,000.

5.2.6.3.2 Sculpturing type

The sculpturing or ornamentation of exine (if present) may be of different kinds. Comparing suggestions from Erdtman (1969), Moore *et al.* (1991), and Hoen (1999) four character states are recognised here:

1. Verrucate: processes are round and wider than high, and are then called warts (verrucae) if they are not constricted at the base;
2. Gemmate: processes round and wider than high are called gemmae if they have a basal constriction;
3. Granulate: very small spherical bodies with a diameter up to 0.5 µm are described as granules;

4. Scabrate: a surface faintly uneven because of processes projecting not more than 0.5 µm is scabrous.

5.2.6.3.3 Sculpturing ordination

The arrangement of sculpturing on the tectum (the outmost layer of exine) shows two different states:

Inordinate (without any obvious order);

Ordinate (obviously ordered in straight or curved lines).

This character and its states have been introduced here for the first time in the family Cyperaceae. It has been used by Iversen and Troels-Smith (1950, p. 46) in the past.

5.2.6.3.4 Sculptural density

The estimated number of sculptural elements in an area of 100 μm^2 of the surface of the exine is called sculptural density (Hoen 1999). Three different categories were distinguished at first:

Low density: 400–900;

Medium density: 900–1500;

High density: 1500–3500.

These character states were found to be too variable among the species and even within a species and therefore were not valuable at any level from species to tribe.

5.2.6.4 Size of pollen

As a rule, I did not include projections higher than 0.5 µm (such as spinules and verrucae; Fig. 5.1) in measuring the length of pollen grains. The following pollen size classes are distinguished in the study group (size expressed as length of the longest axis) based on Erdtman (1969, p. 36):

Small: 10–25 µm;

Medium: 25–50 µm.

Mature pollen grains were measured. Samples were compared with different preparation techniques (sections 5.2.1, 5.2.2, and 5.2.3), but no major difference due to preparation technique was observed.

5.2.6.5 AMB = ambitus (outline)

Erdtman (1969) defined AMB as the outline of a pollen grain observed with a pole uppermost (with the polar axis directed towards the observer). Two character states were distinguished in my study group:

1. Pleurotreme: angular pollen grains with the central part of each aperture situated from about halfway between to adjacent to the angles;
2. Goniotreme: pollen grains are again angular but the centres of the apertures (or some of them) are at the angles.

5.2.6.6 Areolae

Erdtman (1952, p. 460) defined areolae as grooves separating tiny sexinous, mostly circular or otherwise polygonal fragments more or less making a 'negative reticulum'. Based on the presence or absence of this feature two character states were recognised:

Areolae present;

Areolae absent.

5.2.6.7 Perforation of tectum

Little holes in the tectum are called perforations (Jackson 1949; Erdtman 1969). There are two states for this character:

Present: tectum with perforations;

Absent: without perforations.

5.2.6.8 Contact area

The area on the proximal face of a pollen grain that is interpreted as having been in contact with the other members of the tetrad is called the contact area (Potonie 1934). Two character states were considered in this study:

1. Visible: with distinct contact area;
2. Undetectable: without distinct contact area.

Although it was very difficult to distinguish between the absence and presence of this area, I only scored pollen grains with highly distinct contact areas as visible.

5.2.6.9 Informative characters

Among the characters discussed above, some were found to be either valuable or of very little value (e.g. pollen shape, sculptural density, and contact area) in my study group after a preliminary cladistic analysis of pollen and combined pollen-molecular data. Hence, only 13 unordered characters were finally assessed on herbarium or fresh material (Table 5.1).

Variation within micromorphological characters may be overlooked if care is not taken and if the sample is small. Given the limited availability of pollen grains due to the stage at which most specimens had been collected (i.e. collected at very young or old stages relative to flowering), I did not have access to a wide range of pollen grains at different stages of pollen development in most cases.However, for the majority of pollen characters I have described here, little variation was found within specimens or within species, although I have only looked at 1–3 specimens per species.

5.2.7 Choosing terminal taxa

All the morphological features in this data set are visible on herbarium specimens using the SEM. The morphological data include 47 taxa with 13 attributes (Table 5.1) making a sum of 658 cells in the matrix. Data are missing for only 16 cells, which are almost exclusively from species of *Fimbristylis* (Appendix 2).

5.2.8 Phylogenetic analyses

All pollen morphological data were entered and defined in DELTA version 1.04 (Dallwitz 1980; Dallwitz, *et al.* 1993, 1999) and then processed and prepared as a nexus file for the phylogenetic analyses. As the micromorphological data set differed from the molecular data in size and structure, it was necessary to analyse it somewhat differently (Bradford and Barnes 2001). The following options in PAUP* version 4.0b10 were used for pollen data: characters unweighted and unordered, searches heuristic, starting trees obtained via random stepwise addition with 1000 replicates, TBR branch swapping, COLLAPSE option on, STEEPEST DESCENT option off, MULTREES on 500 trees retained in each replicate.

The pollen matrix was explored in several ways. The first was a limitless parsimony method using all 16 initially extracted characters and no constraints. Because this did not resolve any relationships, a subset of 13 characters was selected based on three options:

excluding autapomorphic characters, and including characters exhibiting variability among genera and higher consistency indices in the preliminary analyses. Again the parsimony method was used. This analysis still failed to resolve many relationships, so the next analysis was constrained by a tree built in MacClade (Maddison and Maddison 2001) based on strongly or moderately well supported clades found by the molecular analyses. This analysis of my pollen data accepted only the most parsimonious trees congruent with the topology of the constraint tree. Multiple heuristic searches were conducted used TBR branch swapping. Due to the weak support for the paraphyly of *Fimbristylis* in my molecular data (Fig. 4.5), that topology was constrained to treat species of *Fimbristylis* as paraphyletic in the third analysis to see if the paraphyly was highly supported by pollen data or not. That was also done due to the minimal character variety in my pollen dataset.

A fourth non-molecular analysis was undertaken to resolve relationships among tribes and unplaced genera by using a subset of characters that were chosen as follows:

1. The support provided to certain relationships by *trn*L–*trn*F (*trn*L–F) and ITS characters gave me an idea about the homoplasy in some pollen characters amongst tribes even though others appeared to be relatively conserved.
2. Given the supposition that the conservative characters could resolve intertribal relationships more reliably than the more homoplasious ones, a subset of less homoplasious characters was chosen.
3. This subset of 13 characters (out of 16 original characters) was chosen with each character varying primarily among the tribes or unplaced genera and with an RI of 0.5 or greater from the third analysis. The 13 chosen characters have been set in bold in Table 5.1.

5.2.8.1 Combined molecular-morphological analysis

In my study, I had to address the suitability of combining two molecular datasets and one morphological dataset. A further question is that if results of individual datasets differ, how best to reach a general conclusion? As discussed in section 5.1.2, combination of such datasets is recommended (e.g. by Donoghue and Sanderson 1992).

In the phylogenetic context, gathering multiple data sets and undertaking data partitions are the first steps to combine datasets. Whether a researcher should treat the evidence from these partitioned data differently is the main challenge.

Separate analyses of different datasets appear to present an appropriate situation for using consensus approaches. When the most parsimonious cladogram relative to non-molecular characters differs from the one constructed for the molecular characters, it is reasonable to look for the consensus. This will mostly provide an unresolved cladogram in which indications of how those separate cladograms agree will be captured.

Barrett *et al.* (1991) argued against the use of consensus trees and instead suggested pooling the data and searching for the most parsimonious tree for all different data. However, as Kluge (1983) mentioned, when many molecular and few non-molecular characters go together, the result of pooling might be a "swamp" for non-molecular data.

Therefore, I implemented a consensus of all these ideas, which is the method that Hillis (1987) proposed. He stated that classifications must be based on consensus cladograms, but that the best estimation of the phylogeny and character evolution is understood by the most parsimonious tree obtained from the pooled data. Another explanation for using this method in this research would be that the overall trees of the pollen morphological and the combined ITS–*trn*L–F look much the same and the serious conflict is between the two molecular datasets rather than between pollen and combined molecular data. This justifies the use of combining all non-molecular and molecular datasets to find out which molecular dataset is more supported by non-molecular data. Moreover, the explanations of Barrett *et al.* (1991) about the advantages of pooled data over the consensus of separate trees, according to the 'principle of total evidence', are good motivations for combining the data and constructing the trees in light of all data. In recent years, many researchers have adopted this method and found high resolutions in their study groups (e.g. Olson 2002; Lundberg and Bremer 2003, both studies investigated three molecular and one morphological dataset).

The partition of homogeneity test (PHT) has been employed in the present study as a test of congruence of sequence data prior to the combined analysis, although I recognise that spurious rejection of partition homogeneity may be an issue (Yoder *et al.* 2001).

The PHT has been subject to recent criticism for its performance in determining sequence congruence (Barker and Lutzoni 2000). Certain datasets, which have been demonstrated to be congruent, have not been reported so using this test. As such, the PHT should only be used as a guideline and not an absolute determinant of congruence; however, it remains widely used for this purpose. Also, several sources question the very close relation between the rejection of homogeneity by the partition homogeneity test and preclusion of data set combination (see Yoder *et el.* 2001 and references therein). However, I found this test less strict compared with other data homogeneity tests such as the Templeton test for my data sets. The *trn*L–F, ITS, and pollen morphological datasets of the study group were combinable according to the partition of homogeneity tests (PHT) achieved between these three data sets. The significance level of congruence was marginal; PHT yielded a p-value of 0.058, and therefore I combined all information in a large matrix for further analysis. I combined the three data sets to investigate how the combined analysis compared to the topologies of three separate data sets, and where they agreed and disagreed. In particular, clades that get lower support in the combined analysis might indicate data conflict.

The morphological character states of the studied species were used together with ITS and *trn*L–F sequences only in sequenced species in the combined molecular-morphological analysis. These pollen data were also mapped onto my molecular tree (Fig. 4.6) using MacClade in order to examine direction of evolution in the characters. Constraint analyses considering the monophyly of different sculpturing types were conducted putting species of *Fimbristylis*, *Abildgaardia* and *Crosslandia* and *Actinoschoenus* with mostly verrucate and scabrate sculptures together and keeping *Bulbostylis*, *Arthrostylis* and the outgroup with granulate sculptures separate from the first four genera.

Parsimony analyses of the molecular-morphological data sets were subsequently carried out using heuristic search, unordered and unweighted characters, MULTREES option on, TBR branch swapping, and 10000 random addition sequences to search for multiple islands of most-parsimonious trees saving 500 trees at each stage (Maddison 1991). In all analyses potential phylogenetically informative indels were scored as binary characters (0 and 1). Bootstrap values using heuristic searches, with the MULTREES option off, TBR branch swapping, five random additions, and 10000 replicates were performed to assess relative

support for the identified clades. *Eleocharis*, *Bolboschoenus* and *Schoenoplectus* were selected as outgroups to polarise the characters.

5.2.9. Producing a pollen key for the study group

Pollen morphological data entered in DELTA were exported using the TOKEY directive and a key was produced (section 5.4.2) automatically using the associated program KEY using 'key5' (Dallwitz *et al.* 1993 onwards, 1999 onwards).

5.3 Results

5.3.1 Pollen morphological data

According to previous studies in Cyperaceae (Chapter 1), the lateral apertures, if present, are not easy to distinguish in unprocessed pollen, due to the presence of cytoplasm (Van Wichelen *et al.* 1999). Various treatments applied here (sections 5.2.1, 5.2.3, and 5.2.4) gave different results but rehydration by boiling water and dehydration by alcohol series (section 5.2.3) reliably allowed determination of number and nature of the apertures and dimensions of pollen grains. In particular, the dimensions of treated pollen grains were easily determined. The photomicrographs of pollen are shown in Figures 5.2–5.33. The morphological observations have been summarised in Table 5.3. In the Abildgaardieae different pollen types are found. Characters such as pollen grain shape, size, and exine sculpture, varied between species (Table 5.3).

In all samples exine is ornamented showing different but related states (Figs 5.4 and 5.5). Verrucate pollen was found in *Abildgaardia pachyptera* (Fig. 5.20), only one sample of three *Crosslandia setifolia* specimens from Northern Territory (Fig. 5.21), *Fimbristylis lanceolata* (Fig. 5.22), *F. pauciflora*, *F. sericea*, *F. sphaerocephala* (Fig. 5.23), *F. tristachya*, and *Actinoschoenus* sp. (all 3 specimens) (Fig. 5.24).

The pollen surface of certain species is perforated (Figs 5.26 and 5.28). Different surface patterns were due largely to variation in the perforation of the tectum. For species such as *Bulbostylis barbata*, the sculpture varied from tiny tectal holes (= perforations) to tiny projections (= granules) of two different sizes depending on the locality on the exine, though all specimens of this species could be described as granulate.

The shape of Abildgaardieae pollen varied from oblate spheroidal to prolate spheroidal (these mixtures of character states mean that plants have pollen grains with both characters states), subprolate and prolate (Figs. 5.2–5.16; Appendices 2 and 3). Since there was variation in pollen shape within a species and variation between species was not distinct for my sample, pollen shape is therefore not a good character for distinguishing the study group species (Figs 5.2–5.16, 5.29–5.31).

Table 5.1. Palynological characters used in the analyses and their Consistency Indices (CI).

Characters in bold were found to be most informative and reliable in this study.

Character	Character state	CI
1. Aperture	present (0) absent (1)	0.88
2. Aperture type	colpoid (0) ulcerate (1) both colpoid and ulcerate (2) None of the two types of apertures (3)	1.00
3. Number of apertures	1 (0) 2(1) 3 (2) 4 and higher (3)	1.00
4. Position of aperture	Distal (0) Equatorial (1) Random (2) None (3)	0.64
5. Aperture margin	distinct margin present (0) without margin (1)	0.68
6. Pollen shape	oblate (0) suboblate (1) spheroidal (2) subprolate (3) prolate (4) perprolate (5)	0.33

Table 5.1. (*continued*)

Character	Character state	CI
7. Polarity of pollen	heteropolar (0) isopolar (1) parasiopolar (2)	0.64
8. Apiculate elements	distinct (0) hardly visible (1)	0.54
9. Sculpturing type	gemmate (0) granulate (1) scabrate (2) verrucate(3)	1.00
10. Sculpturing ordination	inordinate (0) ordinate (1)	0.64
11. Sculptural density	400 –900 (0) 900 –1500 (1) 1500 –3500 (2)	0.33
12. Pollen size	small (0) medium (1)	1.00
13. AMB	pleurotreme (0) goniotreme (1)	0.78
14. Areolae	present (0) absent (1)	1.00
15. Perforation of tectum	present (0) absent (1)	1.00
16. Contact area	visible (0) undetectable (1)	0.33

Most pollen grains of the individuals in this study had a number of lateral colpoids and most species had a distal ulcus. Only four species (*Abildgaardia ovata*, *Fimbristylis littoralis*, *F. tetragona*, and *F. rara*) have pollen grains with no aperture. The prevailing outline of the pollen grains of the study group in equatorial view is more or less pyriform. There is little

variation in size of pollen across the species sampled (Tables 5.1 and 5.2. Size varies (13.9–42.1 μm) for the polar diameter (P) and (13.2–27.8 μm) for the equatorial diameter (E). Most species of *Fimbristylis* have small pollen except for *F. acuminata*, which has both small and medium-sized pollen, and *F. laxiglumis* and *F. nutans*, which have medium-sized pollen (Table 5.2). In Padhye and Makde (1980), pollen of *F. complanata* and *F. argentea* fell within the small group, *F. falcata* and *F. miliacea* in both groups, and *F. cymosa* in the medium group. Van Wichelen *et al.* (1999) obtained a different result for *F. complanata* namely that its pollen was medium-sized.

During this study, the only types of aperture found were the ulcus (Figs 5.2, 5.3, 5.4, 5.12, 5.17, 5.27, and 5.30), colpoids (Figs 5.2, 5.4, 5.8, 5.9, 5.15, 5.16, 5.17, 5.29, 5.30, and 5.31), and pores (Fig. 5.11). The pollen grains of most (55.3%) species studied are identical in terms of aperture appearance. This type of pollen has been characterised as having an ulcus occupying roughly the whole distal face of a grain. This was found in a number of species of *Fimbristylis* (Fig. 5.17), two species of *Abildgaardia* (Fig. 5.12), all four *Bulbostylis* species (Figs. 5.2, 5.3), *Crosslandia setifolia* (Fig. 5.4), *Arthrostylis aphylla*, *Actinoschoenus* sp., and all the outgroup members (Fig. 5.19).

The grains in *Schoenoplectus*, *Arthrostylis*, and *Actinoschoenus* look different, however. *Schoenoplectus litoralis* (Fig. 5.13) is noteworthy because of its irregularly distributed apertures (not obvious at this magnification) as well as its different shape, which is elliptic.. It has an ulcus at the distal end and four equatorial pores. In contrast, pollen grains in *Arthrostylis aphylla* (Fig. 5.18) are 5-aperturate with a very regular distribution. In *Actinoschoenus* accessions (Fig. 5.14) examined in this study, there is only one aperture: a distal ulcus. *Abildgaardia pachyptera*, *A. schoenoides*, the Western Australian accession of *Crosslandia setifolia*, *Fimbristylis cymosa*, *F. densa*, *F. laxiglumis*, *F. nutans*, *F. pterygosperma*, *F. schultzii*, *F. sericea*, *F. sieberiana*, *F. sphaerocephala*, *F. tristachya*, *F. velata*, and *F. xyridis* only have colpoids. The type of aperture is not recognisable in *F. polytrichoides*.

The aperture position is more complex and variable in the species of *Abildgaardia* (Figs 5.15 and 5.31) and much more so in *Fimbristylis* (Figs 5.7, 5.8, 5.9, 5.10, 5.16, and 5.17) than in *Bulbostylis* (Figs 5.2, 5.3, 5.12, 5.25, 5.28, 5.29, 5.30) (Appendix 2). While the most

common aperture positions in *Fimbristylis* are zono-aperturate and both zono- and ana-aperturate, some species have distal apertures (ana-aperturate), only.

Table 5.2. Pollen morphological apomorphies of genera (in Abildgaardieae) and tribes in Arthrostylideae, Fuireneae, and Scirpeae.

Tribe Abildgaardieae

Abildgaardia Vahl: Areola all around the pollen grains. Aperture without margin. Pollen shape spheroidal, prolate, or subprolate.

Bulbostylis Kunth: Aperture present. Four or more apertures. Position of aperture both distal and equatorial. Both ulcus and colpoid apertures. Sculpture granulate. Centre of the apertures lies midway between the angles. Heteropolar. Areola only in apertures. Distinct aperture margin. Apiculate elements hardly visible. Pollen shape spheroidal, prolate, or oblate.

Crosslandia W. V. Fitzgerald: Aperture present. Four or more apertures. Small size. Centre of the apertures lie midway between the angles. Heteropolar. Areola all around the pollen grains. Aperture without margin. Apiculate elements distinct.

Fimbristylis Vahl: Apomorphies unknown.

Tribe Arthrostylideae

Aperture present. Four or more apertures. Heteropolar. Distal ulcus present. Distinct aperture margin. Apiculate elements distinct. Pollen shape spheroidal, prolate, or subprolate.

Tribe Fuireneae (*Bolboschoenus* and *Schoenoplectus*)

Aperture present. Four or more apertures. Position of aperture both distal and equatorial. Both ulcus and colpoid apertures. Sculpture granulate. Medium size. Centre of the apertures lies midway between the angles. Heteropolar. Areola only in apertures. Distinct aperture margin. Perforation of tectum present. Apiculate elements hardly visible. Pollen shape prolate, or subprolate.

Tribe Scirpeae (*Bolboschoenus*, *Schoenoplectus*, and *Eleocharis*)

Aperture present. Four or more apertures. Position of aperture both distal and equatorial. Both ulcus and colpoid apertures. Sculptures granulate. Medium size. Centre of the apertures lies midway between the angles. Heteropolar. Areola only in apertures. Distinct aperture margin. Perforation of tectum present. Apiculate elements hardly visible. Pollen shape spheroidal, prolate, or subprolate.

In *F. sphaerocephala* (Fig. 5.22) the sculptural density is more (2535 in 100 μm^2) than other species sampled of this genus. In *Eleocharis* (Fig. 5.18) the sculptural density is 1356 in 100 μm^2. The remarkable diversity of the sculptural density in *Crosslandia setifolia* (Figs 5.21) is unique (840–3275 in 100 μm^2) and was not found in any other taxa examined in this

study. This character was frequently associated with sculpturing type but it was not considered in the second, third, and fourth analyses, as it is too variable within species.

5.3.2. Morphological analyses

The first unconstrained pollen analysis comprised 47 specimens and 16 characters (Table 5.3). A total of 989 equally parsimonious trees were located in two large islands, each tree with 107 steps (CI = 0.29, RI = 0.62). Because the islands were so large, all highly parsimonious topologies may not have been found, because searches were stopped before completion (the computer memory was exhausted and the analysis did not stop automatically) (see also Chapter 4, section 4.2.5 for information about the memory of the computer used in this study).

The same 47 specimens together with the 13 selected characters (Table 5.1) were used in the second unconstrained analysis. Equally parsimonious trees were found in two outsized islands again, each tree with 55 steps (CI = 0.36, RI = 0.76). Due to the very large size of the islands, there is no certainty that the most highly parsimonious topologies were obtained. Again, the search was aborted before completion due to the exhaustive memory use of the computer. Two clades resolved in the strict consensus of all searches (Fig. 5.34) are: *Abildgaardia ovata*, *A. pachyptera*, *A. schoenoides*, *Fimbristylis littoralis*, and *Crosslandia setifolia* (WA accession); and (*Fimbristylis tetragona* + *F. velata*), *F. nutans*, *F. laxiglumis*, *F. cymosa*. None of these clades is completely compatible with my molecular outcomes (Chapter 4) and are not strongly supported. *Abildgaardia* is polyphyletic as *A. macrantha* and *A. oxystachya* are not grouped with *A. ovata*, *A. pachyptera*, and *A. schoenoides* (Fig. 5.34). The accessions of *Crosslandis setifolia* show a complex relationship. The Northern Territory accessions of this species display a paraphyletic relationship. However, the Western Australian accession groups with *Abildgaardia ovata*, *A. pachyptera*, *A. schoenoides*, and *Fimbristylis littoralis*, suggesting polyphyly for *Crosslandia setifolia*.

Table 5.3. Summary of a few important morphological features for pollen grains of the study group, chosen based on their consistency indices.

N/A = not available. See text for discussion and Table 5.1 for pollen characters.

Species	Polarity (character 7)	Size (character 12)	Sculpturing (character 9)	Aperture number and type (characters 1 – 4)	Figure
Abildgaardieae					
Abildgaardia macrantha	Heteropolar	Small	Scabrate	1 distal ulcus + 2 lateral colpoids	–
Abildgaardia ovata	Paraisopolar	Medium	Scabrate	Clear apertures not observed	–
Abildgaardia oxystachya	Heteropolar	Small	Scabrate	1 distal ulcus + 3 lateral colpoids	–
Abildgaardia pachyptera	Paraisopolar	Small	Verrucate	4 lateral colpoids	5.15, 20
Abildgaardia schoenoides	Paraisopolar	Medium	Scabrate	6 lateral colpoids	5, 31
Bulbostylis barbata	Heteropolar	Medium	Granulate	1 distal ulcus + 6 lateral colpoids	5.2, 28
Bulbostylis densa	Heteropolar	Small	Granulate	1 distal ulcus + 4 lateral colpoids	–
Bulbostylis sp. nov.	Heteropolar	Medium	Granulate	1 distal ulcus + 6 lateral colpoids	5.3, 24, 25, 30
Bulbostylis striatella	Heteropolar	Medium	Granulate	1 distal ulcus + 4 lateral colpoids	5.12
Crosslandia setifolia NT1	Heteropolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	5.4
Crosslandia setifolia NT2	Heteropolar	Small	Verrucate	1 distal ulcus + 4 lateral colpoids	5.21
Crosslandia setifolia WA1	Heteropolar	Small	Scabrate	4 lateral colpoids	–

Table 5.3. (*continued*)

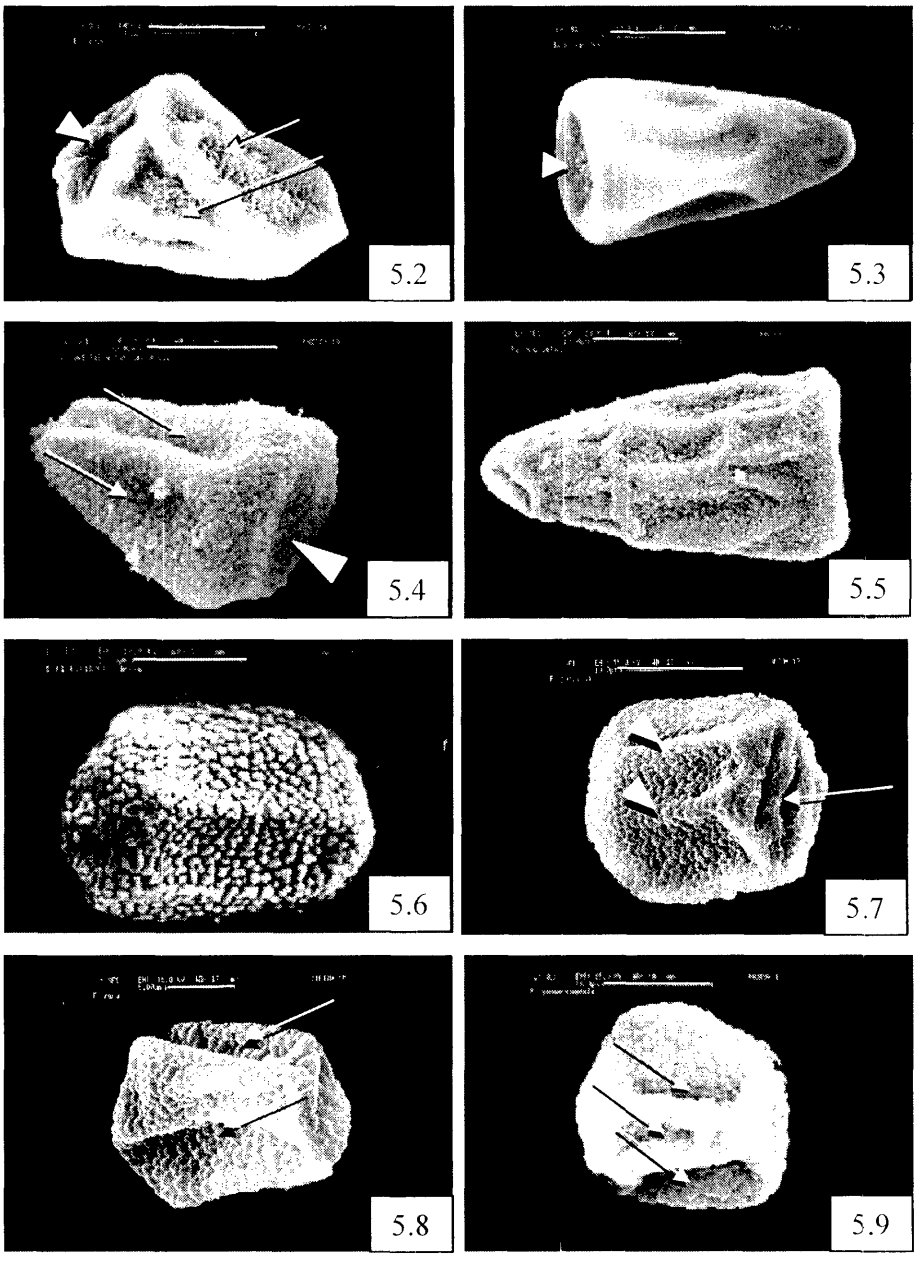
Species	Polarity (character 7)	Size (character 12)	Sculpturing (character 9)	Aperture number and type (characters 1 – 4)	Figure
Fimbristylis acuminata	Heteropolar	Small/Medium	Scabrate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis cephalophora	Heteropolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	5.23
Fimbristylis compacta	Heteropolar	Small	Granulate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis complanata	Heteropolar	Small	Scabrate	1 distal ulcus	–
Fimbristylis cymosa	Paraisopolar	Small	Scabrate	4 lateral colpoids	5.16
Fimbristylis densa	Heteropolar	Small	Scabrate	3 lateral colpoids	5.6
Fimbristylis depauperata	Heteropolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis dichotoma	Heteropolar	Small	Scabrate	N/A	–
Fimbristylis ferruginea	Heteropolar	Small	Gemmate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis furva	Isopolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis lanceolata	Heteropolar	Small	Verrucate	1 distal ulcus	5.10
Fimbristylis laxiglumis	Paraisopolar	Medium	Scabrate	4 lateral colpoids	–
Fimbristylis littoralis	Paraisopolar	Small	Scabrate	Clear apertures not observed	–
Fimbristylis microcarya	Heteropolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	5.33

Table 5.3. (*continued*)

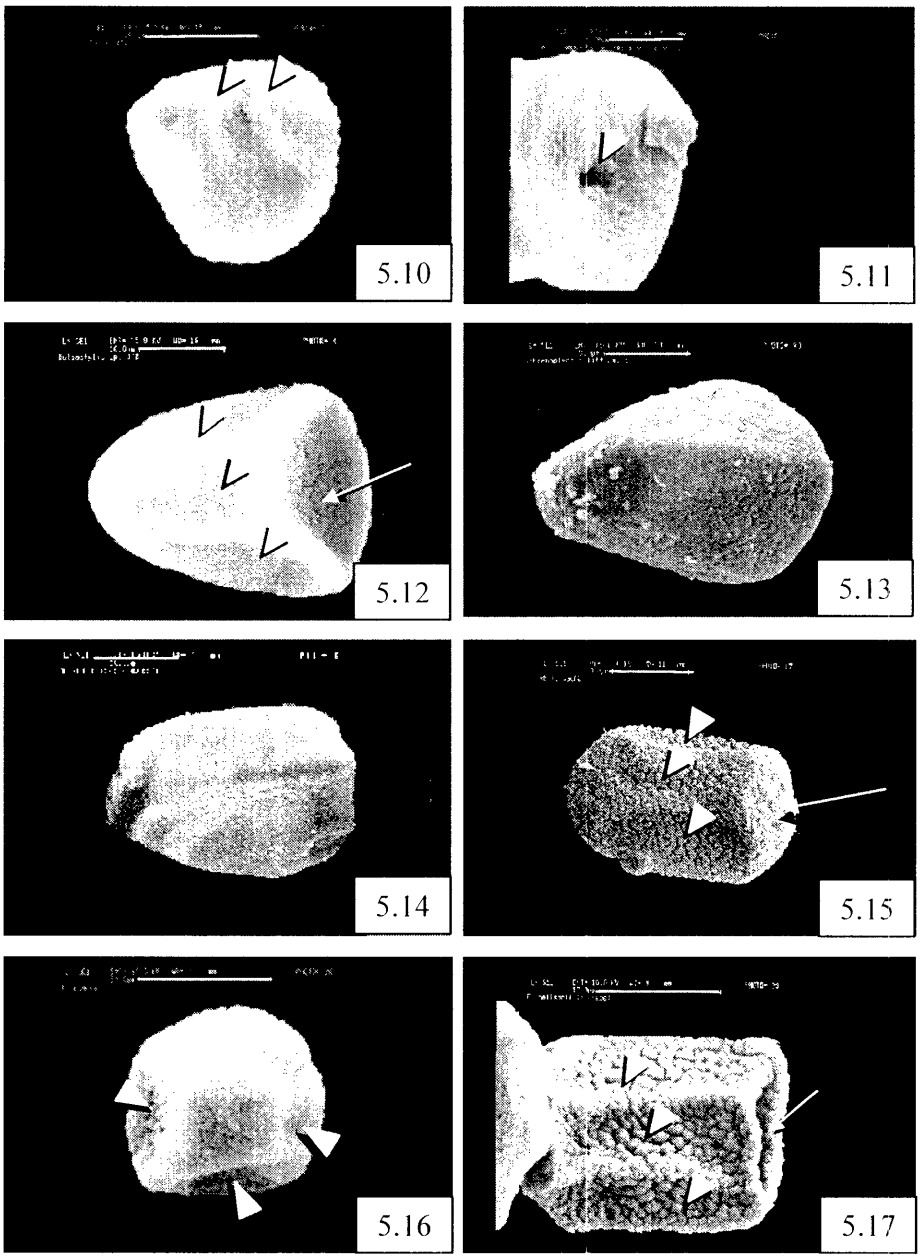
Species	Polarity (character 7)	Size (character 12)	Sculpturing (character 9)	Aperture number and type (characters 1 – 4)	Figure
Fimbristylis neilsonii (WA form)		Heteropolar	Small Scabrate	1 distal ulcus + 4 lateral colpoids	5.17
Fimbristylis nutans	Isoploar	Medium	Scabrate	3 lateral colpoids	–
Fimbristylis pauciflora	Paraisopolar	Small	Verrucate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis phaeoleuca	Heteropolar	Small	Scabrate	1 distal ulcus + 4 lateral colpoids	–
Fimbristylis polytrichoides	N/A	N/A	Scabrate	N/A	
Fimbristylis pterygosperma	Heteropolar	Small	Scabrate	3 lateral colpoids	–
Fimbristylis punctata	Heteropolar	Small	Scabrate	1 distal ulcus + 3 lateral colpoids	5.7
Fimbristylis rara	Heteropolar	Small	Scabrate	Clear apertures not observed	5.8
Fimbristylis schultzii	Isopolar	Small	Scabrate	4 lateral colpoids	–
Fimbristylis sericea	Isopolar	Small	Verrucate	4 lateral colpoids	–
Fimbristylis sieberiana	Heteropolar	Small	Scabrate	4 lateral colpoids	–
Fimbristylis sphaerocephala	Heteropolar	Small	Verrucate	3 lateral colpoids	5.9
Fimbristylis tetragona	N/A	Small	Granulate	Clear apertures not observed	–
Fimbristylis tristachya	Heteropolar	Small	Verrucate	4 lateral colpoids	–

Table 5.3. (*continued*)

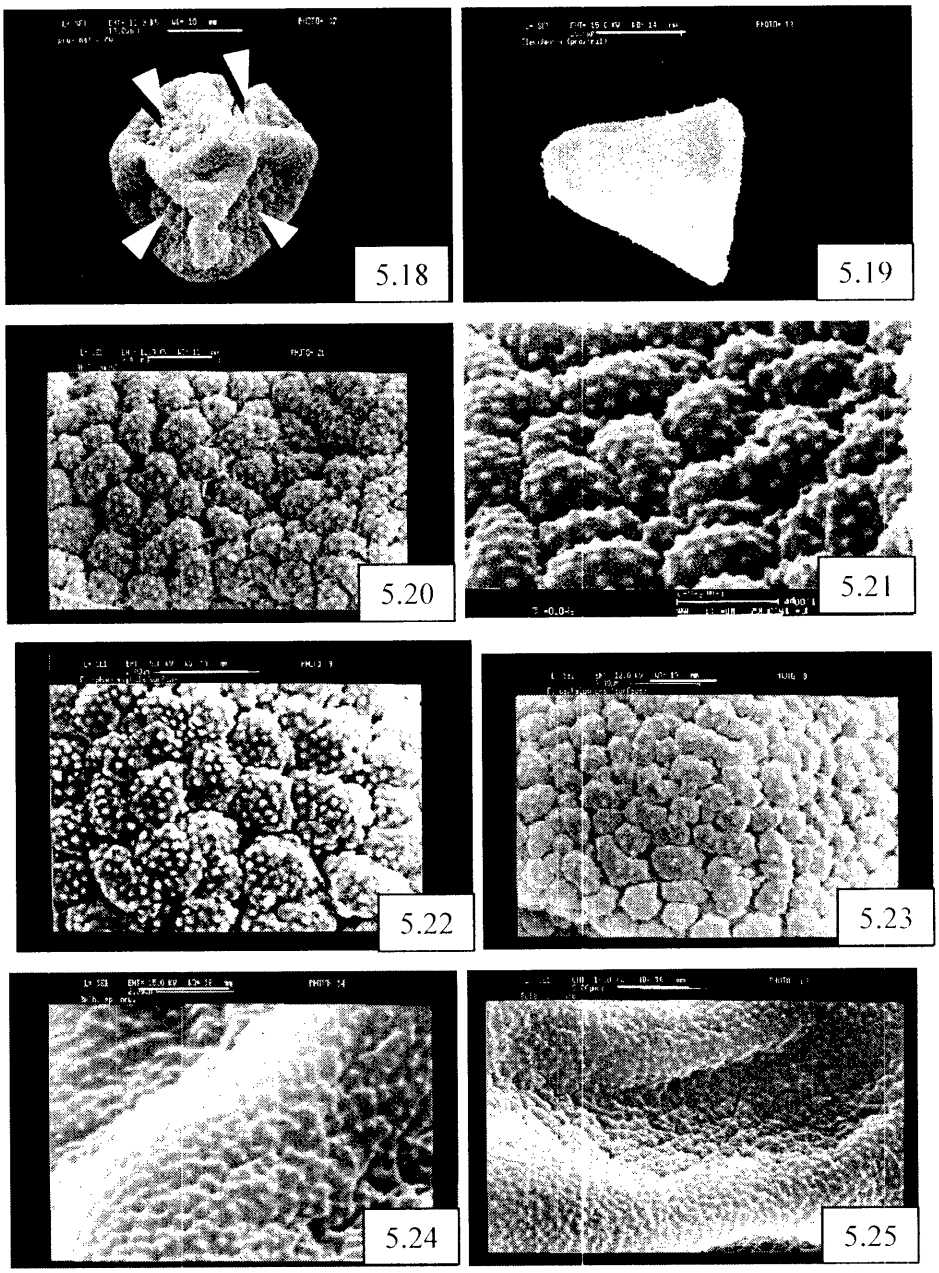
Species	Polarity (character 7)	Size (character 12)	Sculpturing (character 9)	Aperture number and type (characters 1 – 4)	Figure
Fimbristylis velata	Paraisopolar	Small	Granulate	3 lateral colpoids	–
Fimbristylis xyridis	Heteropolar	Small	Gemmate	4 lateral colpoids	–
Arthrostylideae					
Actinoschoenus sp. (*Fimbristylis composita*)	Heteropolar	Medium	Verrucate	1 distal ulcus	5.14, 11
Arthrostylis aphylla	Heteropolar	Small	Granulate	1 distal ulcus + 4 lateral colpoids	5.18
Outgroups					
Bolboschoenus caldwellii	Heteropolar	Medium	Granulate	1 distal ulcus + 5 lateral colpoids	5.5, 27
Eleocharis cylindrostachys	Heteropolar	Medium	Granulate	1 distal ulcus + 5 lateral colpoids	5.19, 26, 32
Schoenoplectus litoralis	Heteropolar	Medium	Granulate	1 distal ulcus + 3 lateral colpoids	5.13



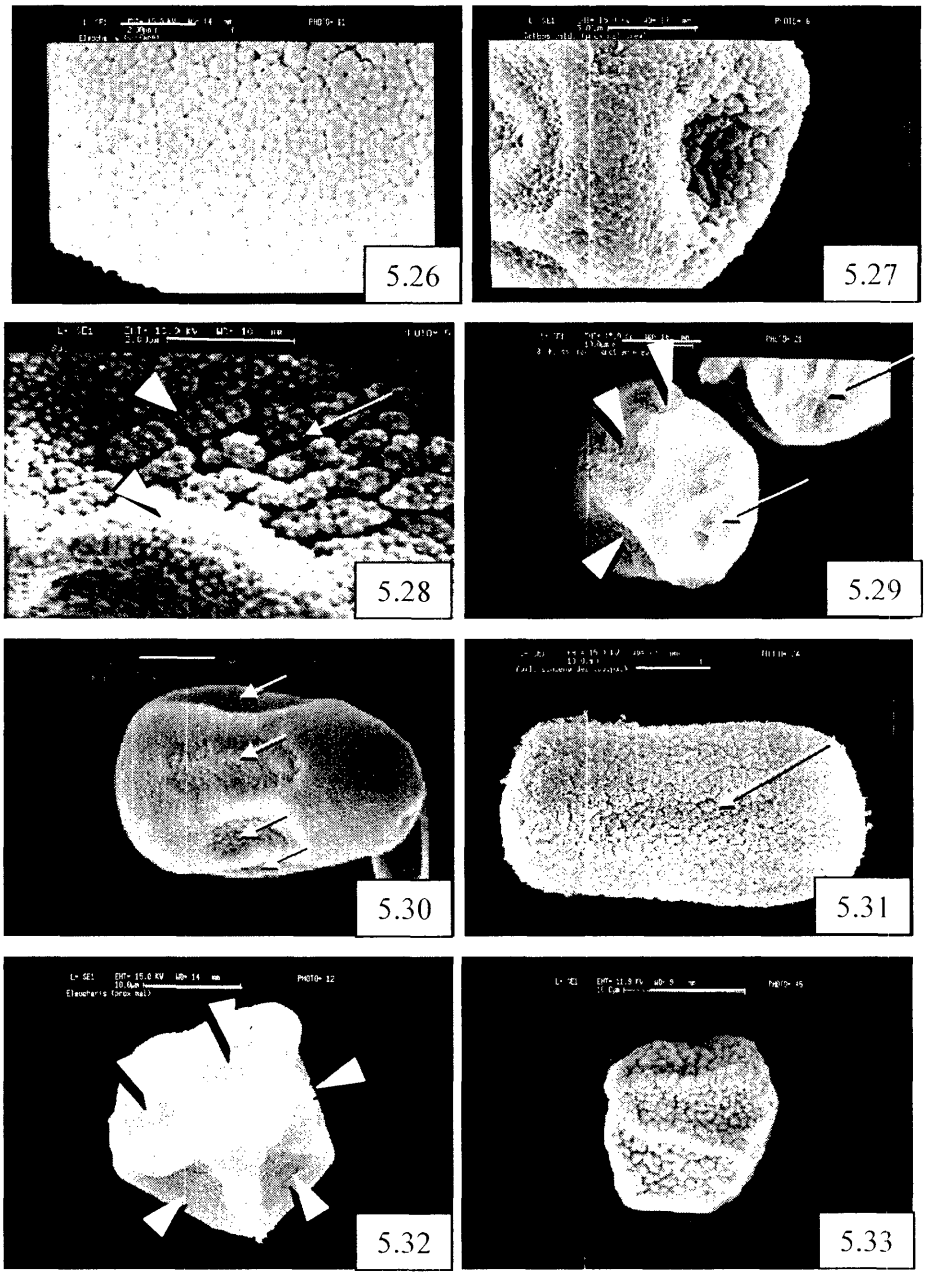
Figs 5.2–5.9. Scanning electron micrographs of pollen grains of study group. **Fig. 5.2.** *Bulbostylis barbata* with lateral colpoids (arrows) and distal ulcus (arrowhead). Scale = 10 µm. (See also Fig. 5.28). **Fig. 5.3.** *Bulbostylis* sp. nov. with distal ulcus (arrowhead). Scale = 20 µm. (Figs 5.2, 25, 30). **Fig. 5.4.** *Crosslandia setifolia* NT1 showing lateral colpoids (arrows) and distal ulcus (arrowhead). Scale = 10 µm. (See also Fig. 5.21). **Fig. 5.5.** *Bolboschoenus caldwellii*. Scale = 10 µm. (See also Fig. 5.27). **Fig. 5.6.** *Fimbristylis densa*. Scale = 10 µm. **Fig. 5.7.** *Fimbristylis punctata* showing proximal view (arrow) and ridges (arrowheads); also colpoids on each side of the ridges. Scale = 10 µm. **Fig. 5.8.** *Fimbristylis rara* showing lateral colpoids (arrows). Scale = 5 µm. **Fig. 5.9.** *Fimbristylis sphaerocephala* showing 3 lateral colpoids (arrows). Scale = 10 µm. (See also Fig. 5.22).



Figs 5.10–5.17. Scanning electron micrographs of pollen grains of study group. **Fig. 5.10.** *Fimbristylis lanceolata* showing ridges (arrowheads). Scale = 10 µm. **Fig. 5.11.** *Actinoschoenus* sp. with distal porus (arrowhead). Scale = 5 µm. (See also Fig. 5.14). **Fig. 5.12.** *Bulbostylis striatella* showing distal ulcus (arrow) and three colpoids (arrowheads). Scale = 10 µm. **Fig. 5.13.** *Schoenoplectus litoralis*, proximal view. Scale = 10 µm. **Fig. 5.14.** *Actinoschoenus* sp. without colpoids. Scale = 10 µm. (See also Fig. 5.11). **Fig. 5.15.** *Abildgaardia pachyptera* with colpoids (arrowheads) and lack of distal ulcus (arrow). Scale = 10 µm. (See also Fig. 5.20). **Fig. 5.16.** *Fimbristylis cymosa*: proximal view. Note the lateral colpoids (arrowheads). Scale = 10 µm. **Fig. 5.17.** *Fimbristylis neilsonii* (WA form) showing large distal ulcus (arrow) and colpoids with texture spaced further apart compared with neighbouring texture (arrowheads). Scale = 10 µm.



Figs 5.18–5.25 Scanning electron micrographs of pollen grains of study group. **Fig. 5.18.** *Arthrostylis aphylla*: proximal view. Note colpoids (arrowheads). **Fig. 5.19.** *Eleocharis cylindrostachys*. Scale = 10 µm. (See also Fig. 5.26). **Fig. 5.20.** *Abildgaardia pachyptera* showing the verrucate (verrucose) sculpturing. Scale = 2 µm. (See also Fig. 5.15). **Fig. 5.21.** *Crosslandia setifolia* NT2: verrucate (verrucose) sculpturing. Scale = 1 µm. (See also Fig. 5.4). **Fig. 5.22.** *Fimbristylia sphaerocephala*: verrucate (verrucose) sculpturing. Scale = 2 µm. (See also Fig. 5.9). **Fig. 5.23.** *Fimbristylis cephalophora*: scabrate sculpturing. Scale = 2 µm. **Fig. 5.24.** *Bulbostylis* sp. nov.: Granulate sculpturing. Scale = 2 µm. (See also Figs 5.25, 29, and 30). **Fig. 5.25.** *Bulbostylis* sp. nov.: Lateral colpoid and granulate sculpturing. Scale = 5 µm. (See also Figs 5.24, 29, and 30).



Figs 5.26–5.33 Scanning electron micrographs of pollen grains of study group. **Fig. 5.26.** *Eleocharis cylindrostachys*: Granulate sculpturing and perforation. Scale = 2 µm. **Fig. 5.27.** *Bolboschoenus caldwelii*: Distal ulcus. Scale = 3 µm. (See also Fig. 5.5). **Fig. 5.28.** *Bulbostylis barbata*: Perforation, ridges, and aperture. Scale = 2 µm. (See also Fig. 5.2). **Fig. 5.29.** *Bulbostylis* sp. nov. with colpoids (arrowheads) and contact area (arrows). Scale = 10 µm. (See also Figs 5.24 and 25). **Fig. 5.30.** *Bulbostylis* sp. nov. showing four of six lateral colpoids (arrows). Scale = 10 µm. (See also Figs 5.24, 25, and 29). **Fig. 5.31.** *Abildgaardia schoenoides* with very fine lateral colpoids (one arrowed). Scale = 10 µm. **Fig. 5.32.** *Eleocharis cylindrostachys* with five lateral colpoids (arrowheads). Scale = 10 µm. **Fig. 5.33.** *Fimbristylis microcarya*: small pollen, with three lateral colpoids visible. Scale = 10 µm.

Relationships within *Fimbristylis* are not resolved as the species of the genus make a large polytomy suggesting paraphyly (if *F. littoralis* is not considered). However, given the position of *Fimbristylis littoralis* within Abildgaardieae, grouping with three *Abildgaardia* species and the Western Australian accession of *Crosslandia setifolia*, *Fimbristylis* seems to be polyphyletic, too (Fig. 5.34). The point here, though, is the close relationship between *Abildgaardia* and *Fimbristylis littoralis* that supports the results of ITS and combined ITS/*trn*L–F analyses about the basal position of this species in the genus *Fimbristylis* and its intermediate position between the rest of *Fimbristylis* clades and *Abildgaardia* clade (Chapter 4, Figs 4.2 and 4.6). *Arthrostylis* and *Actinoschoenus* are again nested within Abildgaardieae and between *Bulbostylis* and the rest of the genera of this tribe, confirming the results from *trn*L–F and combined *trn*L–F/ITS analyses (Chapter 3, Fig. 3.7; Chapter 4, Fig. 4.6). Nonetheless, *Arthrostylis* and *Actinoschoenus* are paraphyletic here (Fig. 5.34).

The species of *Bulbostylis* studied here show paraphyly, although *B. densa* has more affinity to the rest of the tribe Abildgaardieae. Among outgroups, *Bolboschoenus caldwellii* is basal to the rest (Fig. 5.34). This is in contrast with the molecular results (Chapters 3 and 4, Figs 3.7, 4.2, and 4.6) in which *Bolboschoenus caldwelli* is grouped with *Schoenoplectus litoralis* leaving *Eleocharis cylindrostachys* outside the group.

Table 5.2 lists apomorphies of all specimens in my analysis. Only 0.15% of the cells (1 of 658) in the data matrix are scored as polymorphic.

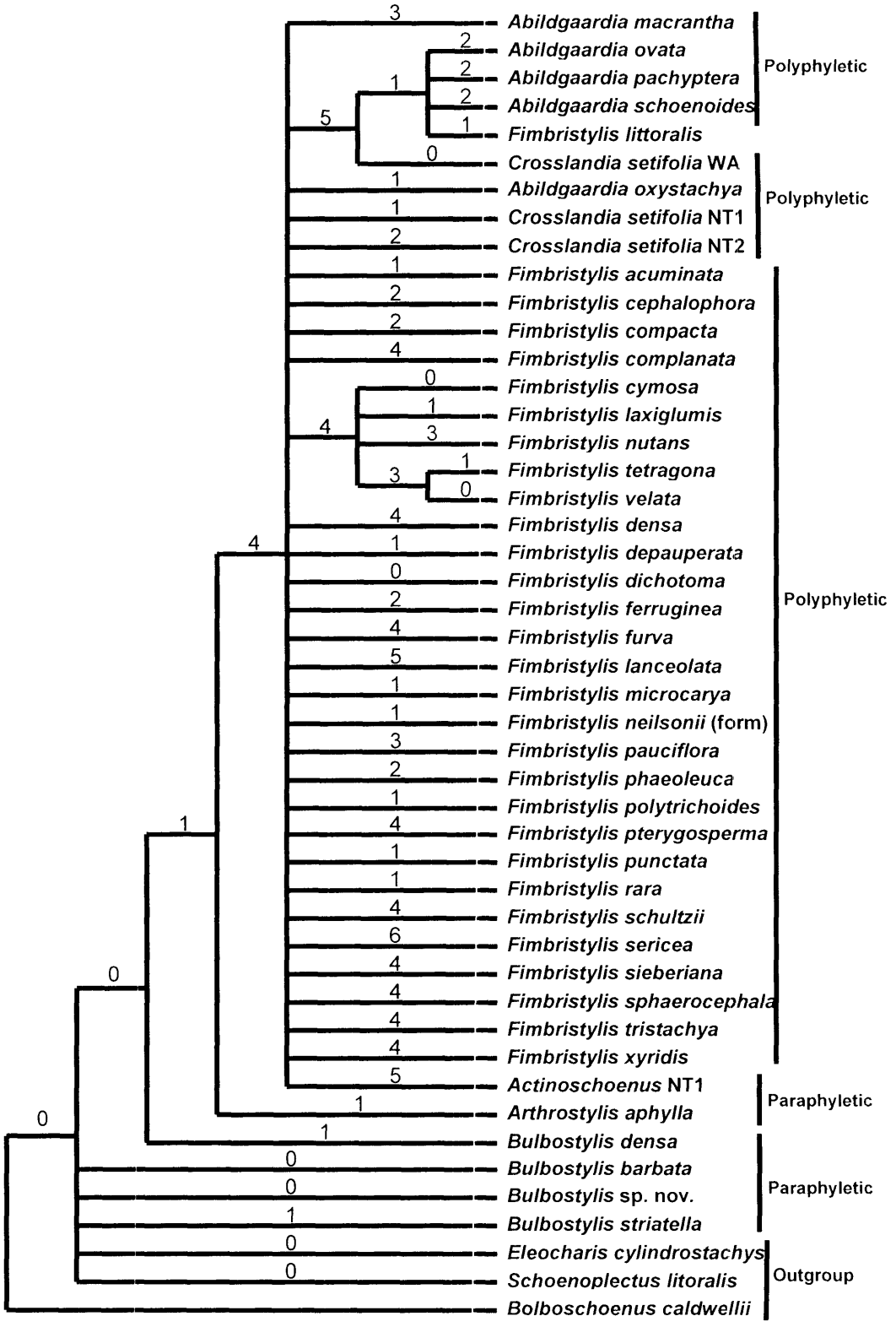

Abildgaardia macrantha
Abildgaardia ovata
Abildgaardia pachyptera
Abildgaardia schoenoides
Fimbristylis littoralis
Crosslandia setifolia WA
Abildgaardia oxystachya
Crosslandia setifolia NT1
Crosslandia setifolia NT2
Fimbristylis acuminata
Fimbristylis cephalophora
Fimbristylis compacta
Fimbristylis complanata
Fimbristylis cymosa
Fimbristylis laxiglumis
Fimbristylis nutans
Fimbristylis tetragona
Fimbristylis velata
Fimbristylis densa
Fimbristylis depauperata
Fimbristylis dichotoma
Fimbristylis ferruginea
Fimbristylis furva
Fimbristylis lanceolata
Fimbristylis microcarya
Fimbristylis neilsonii (form)
Fimbristylis pauciflora
Fimbristylis phaeoleuca
Fimbristylis polytrichoides
Fimbristylis pterygosperma
Fimbristylis punctata
Fimbristylis rara
Fimbristylis schultzii
Fimbristylis sericea
Fimbristylis sieberiana
Fimbristylis sphaerocephala
Fimbristylis tristachya
Fimbristylis xyridis
Actinoschoenus NT1
Arthrostylis aphylla
Bulbostylis densa
Bulbostylis barbata
Bulbostylis sp. nov.
Bulbostylis striatella
Eleocharis cylindrostachys
Schoenoplectus litoralis
Bolboschoenus caldwellii
Polyphyletic
Polyphyletic
Polyphyletic
Paraphyletic
Paraphyletic
Outgroup

Fig. 5.34. Strict consensus tree from a preliminary parsimony analysis of 13 pollen morphological characters. No constraint tree was used. Arthrostylideae is nested within Abildgaardieae. Numbers above the branches represent branch lengths.

The constrained analysis based on molecular results with 13 morphological characters found trees with 105 steps in two tree islands (CI = 0.29, RI = 0.68). Of the taxa without available DNA sequences, *Fimbristylis* species are placed within *Fimbristylis* sensu lato (including *Crosslandia setifolia*) as the sister taxon of clade X in Fig. 4.6 (Chapter 4), *Abildgaardia macrantha* is placed in *Abildgaardia* sensu stricto. *Bulbostylis* species are resolved as in the combined molecular analysis. The clade of five *Fimbristylis* species found in the unconstrained analysis is unresolved. Both islands allow the separation of the clades X and Y (Fig. 5.35) that is not shown in the unconstrained strict consensus trees (Fig. 5.34).

The best matching constrained analysis with 13 selected characters (analysis four) found 380 trees with 58 steps (CI = 0.34, RI = 0.67). The strict consensus of these trees groups *Abildgaardia*, *Crosslandia*, and *Fimbristylis littoralis* in a clade that is a sister group to ??a largely unresolved polytomy of the other *Fimbristylis* species and *Actinoschoenus* (Fig. 5.36). Intratribal relationships from this analysis are the same as shown in Fig. 5.34.

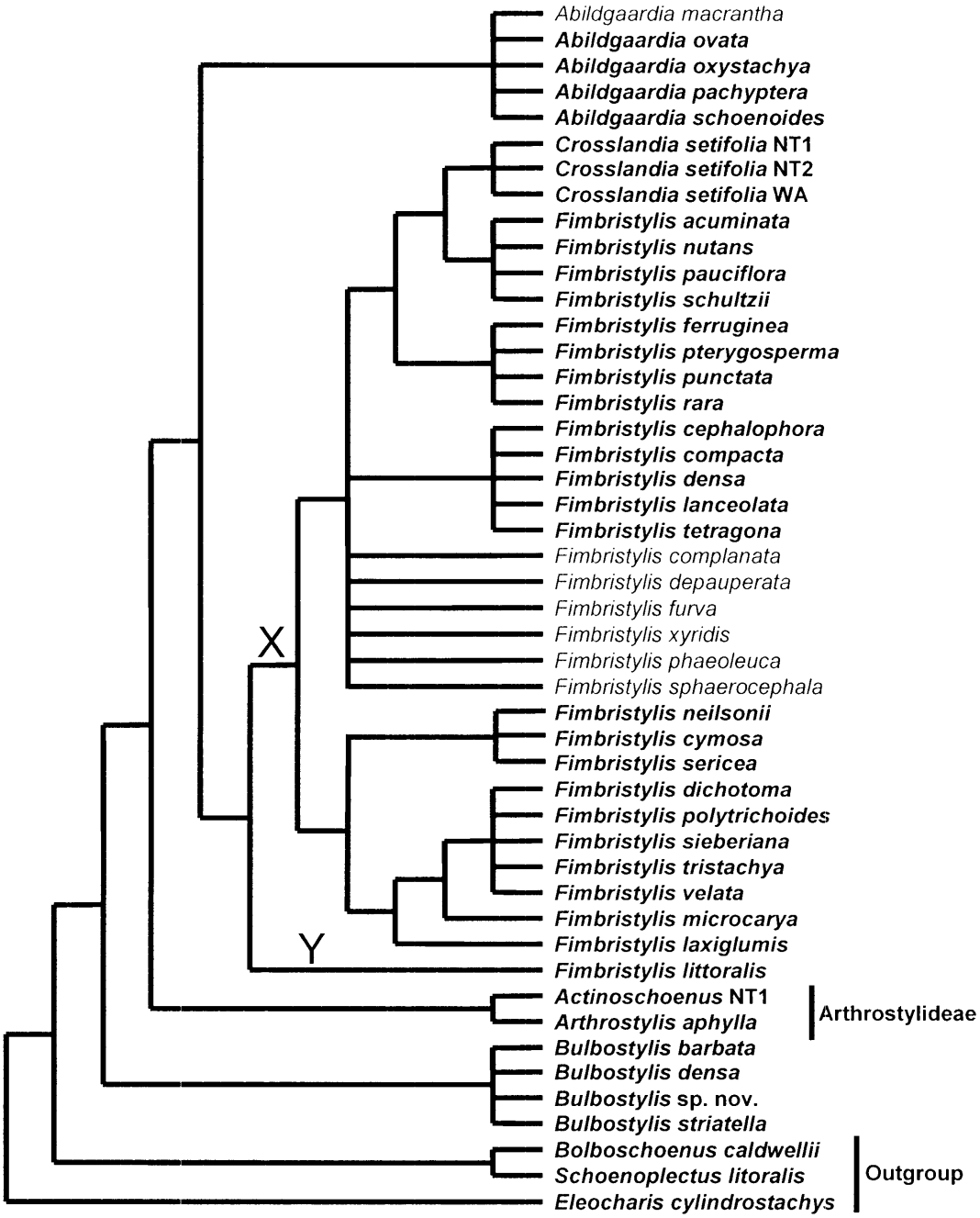


Fig. 5.35. Strict consensus tree from a parsimony analysis of pollen morphological characters using a constraint tree based substantially on clades found in molecular analyses. Strict consensus trees corroborate Arthrostylideae nested within Abildgaardieae in both unconstrained and constrained analyses. Unbolded species were not sampled in the molecular study (Chapters 3 and 4).

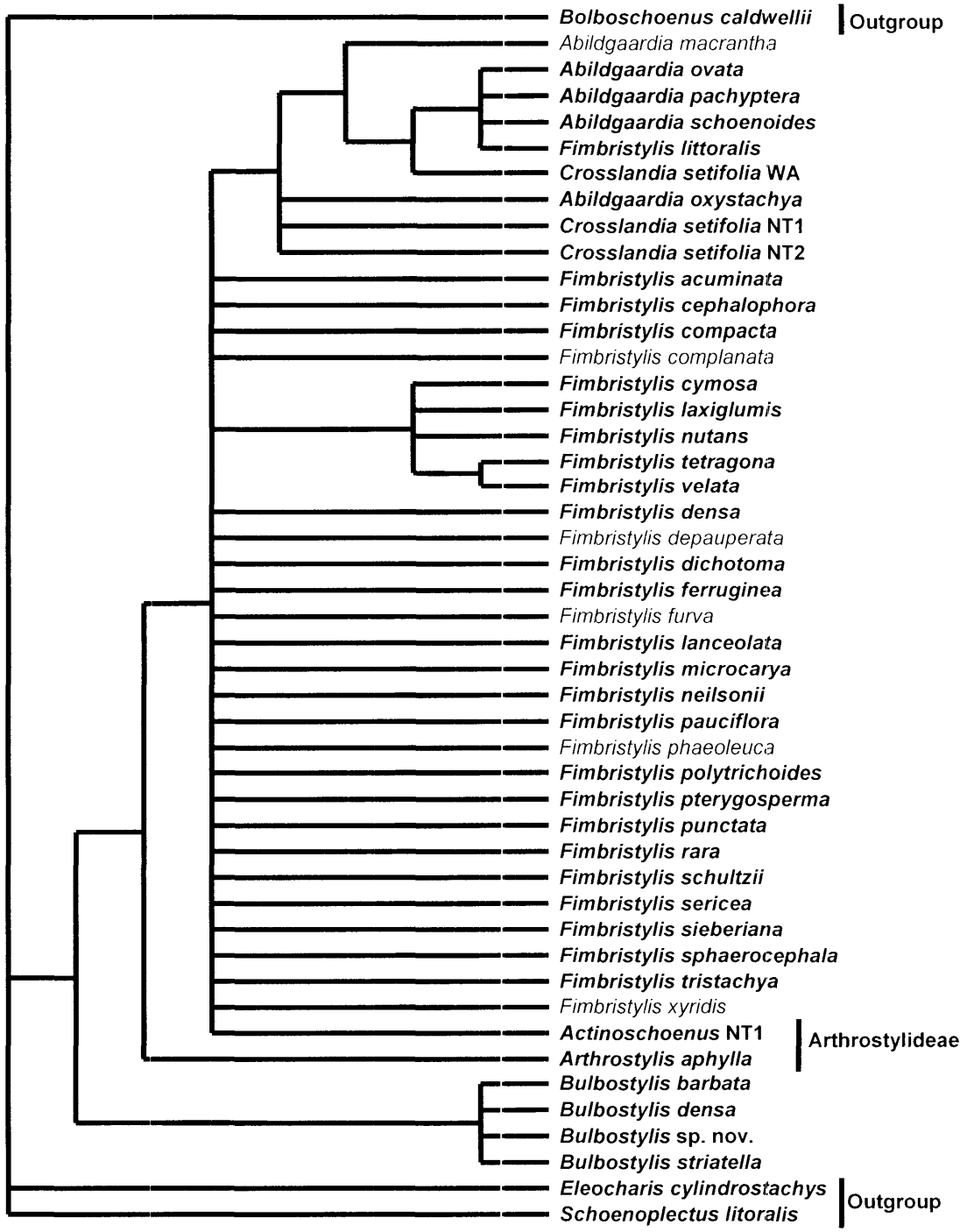


Fig. 5.36. Strict consensus tree from a parsimony analysis of pollen morphological characters using the best matching constraint tree (with the lowest difference in steps). Strict consensus tree corroborates Arthrostylideae nested within Abildgaardieae in both unconstrained and constrained analyses.

5.3.3. Combined analyses

Fusion of the three different molecular and morphological data sets of 60 taxa in one matrix yielded 2872 positions and 797 parsimony-informative characters (Table 5.4). A parsimony analysis generated two islands containing 36 most equally parsimonious trees each 2816 steps long, with CI of 0.539 and RI of 0.702. Both molecular and molecular–morphological combined topologies (Figs4.5 and 5.37) recover Abildgaardieae sensu stricto [?in whose sense? better to say 'as circumscribed by G 1998' or whoever] as a non-monophyletic tribe. Both combined trees diagnosed very similar monophyletic groups although the topology within clade X is not exactly the same in the two trees. Clade Y within *Fimbristylis* is a consistent subgroup of this genus although not strongly supported in any of the trees (Figs 4.5 and 5.37). Other monophyletic groups are genera that have been already recognised within Abildgaardieae (*Bulbostylis* and *Abildgaardia*?) except the *Crosslandia* clade, which includes *Abildgaardia vaginata* and falls within the limits of *Fimbristylis*. The members of tribe Arthrostylideae that are always nested within Abildgaardieae but separate from *Fimbristylis* and *Bulbostylis* are monophyletic, too. Within the Abildgaardieae sensu stricto the *Bulbostylis* clade has high support in both combined trees (BS = 100). The relationships amongst the four studied genera have been largely resolved except the relationships between *Crosslandia* and *Fimbristylis*; however, the position of *Abildgaardia vaginata* within the *Crossslandia* clade is highly resolved (Fig. 5.37).

Comparison between the trees from both combined analyses (Figs 4.6 and 5.37), reveals slight differences in resolution and support. In the combined molecular–morphological tree (Fig. 5.37), *Fimbristylis densa* was sister to the *Crosslandia* clade with no support (BS < 50 %), whereas in the combined molecular tree (Fig. 4.6), *F. densa* is located far from this clade with a strong support (BS = 100 %). While *F. rara* and *F. ferruginea* seem to be very close species in both trees, the position of the clade of two taxa is resolved differently in the two trees. In the combined molecular–morphological tree, these two taxa are grouped with *F. pauciflora* with no support (BS < 50%) while in the combined molecular tree these two taxa make a clade with *F. pterygosperma* and *F. punctata* (clade D in Fig. 4.6; BS < 50%) that would exist in the combined molecular–morphological tree if *F. pauciflora* was excluded (Fig. 5.37). Also, some differences were found relating to higher taxonomic levels: in the combined molecular tree (Fig. 4.5), the *Fimbristylis–Crosslandia–Abildgaardia vaginata*

clade was resolved with no support (BS < 50 %) but this clade was moderately resolved (BS 74 %) in the combined molecular–morphological tree. Despite low resolution of the clades X and Y as sister groups in both combined analyses, support for this sister relationship is higher in the combined molecular–morphological tree by comparison with the combined molecular tree (BS = 68% and BS < 50 %, respectively).

Table 5.4. Comparisons of the different data sets.

Subtracting the molecular from the molecular-pollen morphological figures gives a total of 13 pollen characters.

Datasets/Statistics	No. of taxa	No. of characters*	Informative characters
*trn*L–F	55	1980	424 (21.41%)
ITS	53	878	359 (40.89 %)
Combined molecular	60	2858	783 (27.39 %)
Molecular–morphological	60	2871	797 (27.75 %)

* Length of aligned sequences (including phylogenetically scored, informative indels)

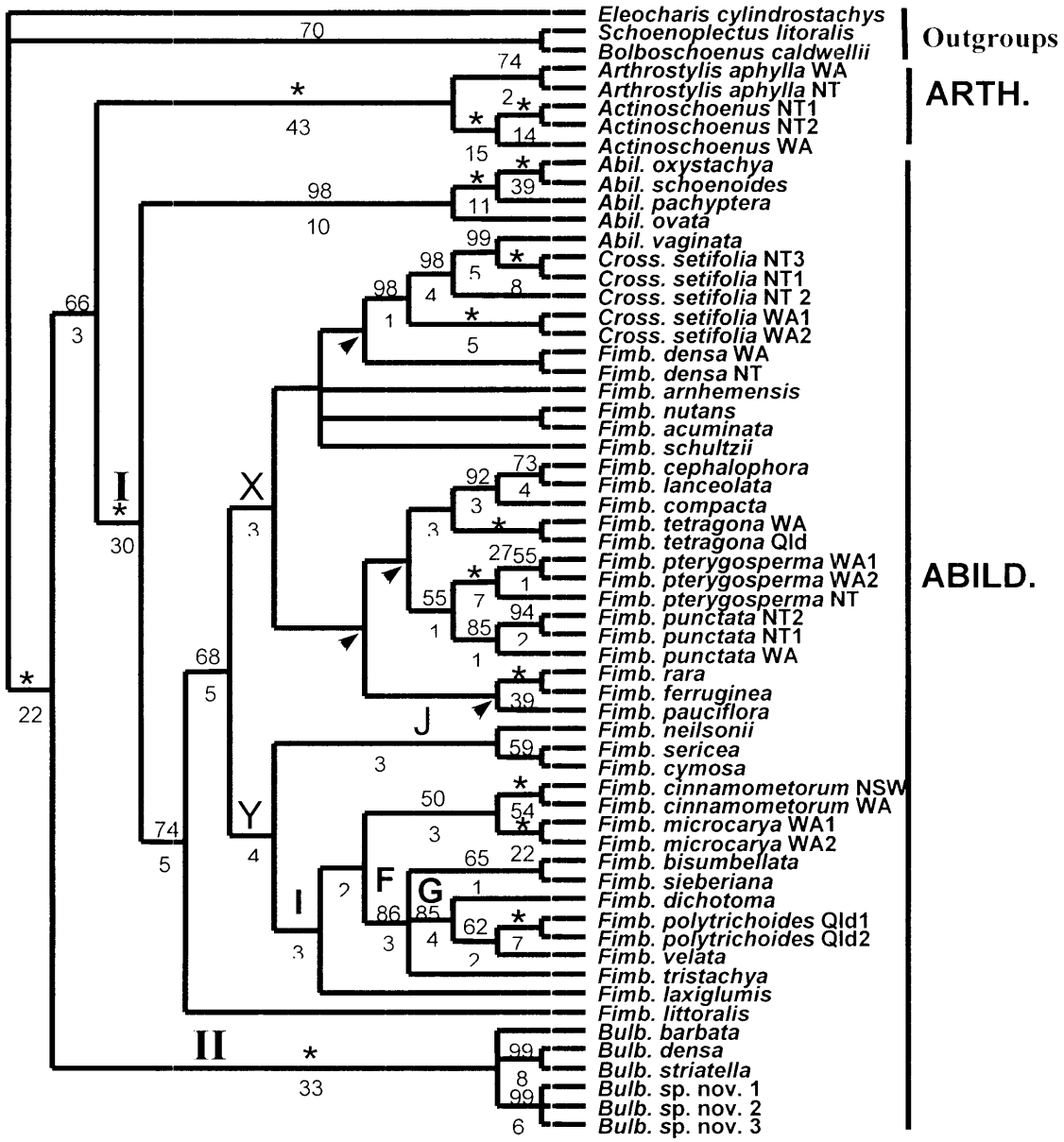


Fig. 5.37. Strict consensus tree from 36 most parsimonious trees for the combined molecular and pollen morphological datasets unweighted Fitch-parsimony. Percent bootstrap values are given above branches: star represents 100. Bremer support (below branches) is indicated too. Codes denote tribes of Cyperaceae sensu Bruhl (1995); ABILD. = Abildgaardieae and ARTH. = Arthrostylideae. WA = Western Australia; NT = Northern Territory; NSW = New South Wales; Qld = Queensland. Arrows mark clades absent in the strict consensus tree of the combined ITS/trnL–F using Fitch analysis (Fig. 4.5). **I** refers to clades that include the species of *Abildgaardia*, *Fimbristylis*, and *Crosslandia* and **II** refers to the Bulbostylis clade. X, and Y refer to clades present in Figs 4.1 and 4.5, too.

5.4. Discussion

5.4.1. Pollen studies

The difference between other classifications (Goetghebeur 1986, 1998; Bruhl 1995) and my molecular estimate of phylogeny (Figs. 4.2 and 4.6) raises the question of which relationships indicated by my molecular data are supported by morphological variation

Erdtman (1952) stated that the pollen grains with a distal ulcerate aperture and three faintly indicated poroid or elongate openings on their lateral faces is the most frequent aperture form in Cyperaceae. Walker and Doyle (1975) referred to the pollen grains of Cyperaceae as mostly one or four aperturate. Harley and Zevada (2000), however, without referring to Cyperaceae in particular, stated that uniaperturate pollen grains are more frequent in monocots than multiaperturate pollen grains. My results conform to those of Erdtman (1952) and Walker and Doyle (1975) with most of the species sampled possessing a distal ulceroid aperture and three indistinct poroid or elongate openings on the lateral faces of the pollen grains.

Four genera (*Abildgaardia*, *Actinoschoenus*, *Arthrostylis*, and *Crosslandia*) and 45 species have been examined by scanning electron microscope and reported for the first time. *Fimbristylis complanata* has been already exa nined by SEM (van Wichelen *et al.* 1999). However, as I studied only 43 of 491 taxa within the Abildgaardieae–Arthrostylideae group, I am cautious in drawing phylogenetic conclusions.

Pollen grains in the Abildgaardieae seem to include different forms. In most cases, pollen attributes seem to be species-specific (Table 5.3), and pollen of these species can readily be distinguished by a combination of characters. Although there appear to be differences in pollen morphological characters between species, intraspecific variation also occurs. For example, the different accessions of *Crosslandia setifolia* show different character states in a few characters such as aperture type, sculpturing type, and perforation of tectum, although this might be due to poorly defined species limits. In this assessment, the Abildgaardieae can be split into two types based upon their pollen grain morphology (Appendix 2, Tables 5.2 and 5.3, Figs 5.2–5.4, 5.6–5.12, 5.14–5.18, 5.20–5.25, 5.28–5.31). For instance, *Bulbostylis barbata* (Fig. 5.2) and *Bulbostylis* sp. nov. (Fig. 5.24) can be distinguished from the other members of this tribe, because the number of apertures (seven) in these two species is different from the other species of *Bulbostylis* and also the rest of the species sampled within

the tribe. Padhye and Makde (1980) mentioned *Bulbostylis barbata* as a species with monocolpate pollen grains but I found that all *Bulbostylis* species studied in my study including *B. barbata* have at least four lateral colpoids and one distal ulcus. My observations are consistent with those of Van Wichelen *et al.* (1999) for *B. megastachys*.

Data on the pollen grain form of *Eleocharis* have been contradictory in the literature. According to Shah (1972) and Huang and Chung (1971), pollen grains in *Eleocharis* have one aperture. Padhye and Makde (1980) also mentioned *Eleocharis capitata* as a species with monocolpate pollen grain, while Sultan *et al.* (1994) and Van Wichelen *et al.* (1999) found 4-aperturate pollen grains in this species. However, I found pollen grains of *Eleocharis cylindrostachys* with six apertures: five lateral colpoids and one distal ulcus (Fig.5.32). *Eleocharis* is a very big genus and somewhat variable, therefore variation in this genus is possible. However, my results on *Eleocharis cylindrostachys* mainly agree the findings of Sultan *et al.* (1994) and Van Wichelen *et al.* (1999) on other species of this genus. Most of my findings disagree with the information presented by Padhye and Makde (1980) and this is probably due to their use of light microscopy only.

Most of the potential synapomorphies at deep nodes in figure 5.37 are homoplasious in more terminal parts of the tree, implying that reconstruction of ancestral states is very uncertain (see Omland 1999 for a review of assumptions and potential problems of ancestral state reconstructions). However, characters that may mark early-divergent clades are granulate sculpturing, medium size, areolae only in apertures, and perforated tectum (Figs 5.34, 5.35, and 5.36, Tables 5.1 and 5.2).

The pollen in Abildgaardieae possesses fragile exine, which is barely ornamented. The sculpturing is a key feature in drawing up the boundaries of the tribes and some genera. The present assessment confirms the importance of the exine sculpture as an informative part of pollen grain and providing information through its characters and character states, being mostly gemmate, granulate, scabrate, and verrucate. The genus *Fimbristylis* differs in the distinctness and size of the sculpturing (section 5.3.1, Figs 5.22 and 5.23).

Species of *Fimbristylis* exhibit diverse aperture shapes and distribution (Figs 5.8, 5.9, 5.16, 5.17, Tables 5.2 and 5.3). The pollen grains demonstrate 0–4 apertures, which may be haphazardly distributed or may be 1 distal ulcus and 2 or 3 zonal colpoids. The pollen in *Bulbostylis* and some species of *Fimbristylis* have more than four apertures. Those of

Bulbostylis are slightly granulate whereas those of *Fimbristylis* are distinctly scabrate, verrucate, or gemmate. At least one character is shared only by species of *Bulbostylis*: areolae only in apertures, a character unique in the tribe.

This pollen study (Figs 5.4, 5.5, and 5.6) does not support the merge of *Fimbristylis* and *Bulbostylis* as was proposed in Koyama (1961). However, pollen morphology does not reject the merger of *Abildgaardia* with *Fimbristylis* as proposed by Clarke (1902) and Kern (1974).

The comparison of pollen size, aperture type, and exine sculpture showed that *Bulbostylis*, *Arthrostylis*, *Bolboschoenus* and *Eleocharis* are more similar than *Bulbostylis* and the rest of Abildgaardieae (Fig. 5.4), especially in exine sculpture (Figs 5.20–24, 5.26, 5.28). For Abildgaardieae a distinction can be made between taxa with numerous lateral colpoids and those with few or no lateral colpoids (Figs 5.2–9, 5.12–19; Table 5.3).

The size of pollen was used for cladistic analysis because it is not difficult to determine states and also it was not a homoplasious character according to the results of the preliminary analyses. Pollen size presents useful phylogenetic evidence in classifying certain taxa such as *Bulbostylis* in this study and in other studies of genera *Myosotis* (Boraginaceae), *Annona* and *Cymbopetalum* (Annonaceae), *Cucurbita* (Cucurbitaceae) (Walker and Doyle 1975), and subfamilies Atherospermatoideae and Gomortegaceae at infrafamilial level and Illiciaceae, Schisandraceae, Trimeniaceae, Austrobaileyaceae, Piperaceae, Lactoridaceae, Himantandraceae, Degeneriaceae, Eupomatiaceae at family level (Sampson 2000). Within the larger clade X (Fig. 5.37) is a basal clade in which pollen size is medium. The difference of size in the previous studies reporting the pollen size of some species of Abildgaardieae (e.g. Padhye and Makde 1980; Van Wichelen *et al.* 1999, section 5.3.1) might be due to the use of herbarium material by Van Wichelen *et al.* (1999) versus fresh material by Padhye and Makde (1980) or to different preparation techniques.

For some characters similar character states exist in different species (or even genera). There were palynological distinctions that occurred in only a few taxa. However, these characters are clearly homoplasious. Several losses and gains were needed to obtain these character states when the morphological data were compared with my molecular tree(s). The presence of an obvious contact area and the shape of pollen grains are examples of such characters.

While the outgroup species *Bolboschoenus caldwelli* and *Schoenoplectus litoralis* have an obvious contact area on their pollen grains, most of the ingroup taxa have no visible contact area. However, the occurrence of contact area in some members of the ingroup looks highly random. For example, *Arthrostylis aphylla* shows the feature whereas *Actinoschoenus*, which is supposedly the closest ally of *Arthrostylis* in the study group, does not. *Bulbostylis* sp. nov. is the only species of *Bulbostylis* among the species sampled that possesses this feature. Also, in pollen grains of *Fimbristylis sieberiana*, *F. microcarya*, *F. compacta*, *F. lanceolata*, and *F. punctata*, the contact area is visible (Figs 4.6 and 5.7). As all of these species (except *Fimbristylis compacta* and *F. lanceolata*) are located far from each other and in different clades (Fig. 4.6), the feature is probably either poorly defined and not useful for phylogenetic analyses or has occurred several times within the Arthrostylideae–Abildgaardieae group. The second assumption seems less likely according to the principles of parsimony. Furthermore, none of the *Fimbristylis* species that possess a contact area belong to the same sections suggested by Kern (1974).

Prolate pollen shape is an example of a randomly distributed character state. It is observable in *Bolboschoenus caldwelli* (one of the outgroups), *Bulbostylis* sp. nov., *Abildgaardia ovata*, *A. pachyptera*, *A. schoenoides*, one of the accesssions of *Crosslandia setifolia* from the Northern Territory, *Fimbristylis lanceolata*, *F. laxiglumis*, *F. nutans* and *F. xyridis*. This character is also too variable within species (even in an specimen) as two or more character states occur in different specimens of *Arthrostylis aphylla*, *F. accuminata*, *F. neilsonii*, and *F. velata* and in the other *Crosslandia setifolia* collected from the Northern Territory. This phenomenon as well as the existence of very diverse morphologies in closely related species raises the fundamental question of the origin and persistence of such characters. As a result, I did not add these characters to my final morphological phylogenetic analyses.

Other conspicuous pollen characters (pollen shape, and presence or absence of a distal ulcus) are homoplasious among and within clades at the tribal level. Van Wichelen *et al.* (1999) found obovate pollen (corresponds to subprolate in my study) in *Fimbristylis complanata*, while *Bulbostylis megastachys* was reported as pyriform. Padhye and Makde (1980) showed *Fimbristylis complanata* and *Bulbostylis barbata* had a similar pollen shape which was ellipsoid (corresponds to prolate in my study), but that pollen of *F. cymosa* and *F.*

falcata were ellipsoidal convexo-convex (corresponds to oblate in my study) and that of *F. miliacea* ellipsoidal (and circular in lateral view; this does not correspond to any character state in my study). Pollen shape was too variable within my study group (even within the same species). One possible interpretation of these results is that there are a limited number of structurally defined pollen shapes, each with potential variation that may or may not be manifested or another interpretation might be that shape is too influenced by preparation technique to be comparable.

More species need to be sampled, but it is clear from this work that pollen grain size, exine sculpture, areolae, and perforation of tectum (if used under identical conditions) have good potential for systematic and phylogenetic purposes at tribe and genus levels. Also, these characters can be used as supplementary characters in the identification of Abildgaardieae species. However, the relationships shown in palynology are not as clear as those shown by DNA sequence comparisons as reported in Chapters 3 and 4.

The constrained analysis founded on the combined molecular tree forces 50 more steps than the unconstrained analysis. Although this seems like many more steps, it is only about 8% more homoplasy overall (RI-unconstrained = 0.76 versus RI-constrained = 0.68). Furthermore, the general topology of the genus *Bulbostylis* and the genera of Arthrostylideae within the constrained and non-constrained trees are similar, suggesting that the position of these genera on the constrained tree is still believable although this is hard to say for the topology and relationships of *Abildgaardia*, *Fimbristylis* and *Crosslandia*.

Padhye and Makde (1980) examining five species of *Fimbristylis*, described this genus as having a granulate exine (except *F. miliacea* which had a reticulate/foveolate exine).Within my study group, the plesiomorphic form for sculpturing type is granulate. A large clade can be recognised as having states other than granulate (Figs. 5.34 and 5.36 and Table 5.3). Sculpture type within this clade has apparently reversed at least twice, once in *Fimbristylis compacta*, and at least once more if *F. velata* and *F. tetragona* are placed in the clade. This analysis suggests that the presence of areolae all around the pollen grain might have evolved in the possible *Abildgaardia–Fimbristylis–Crosslandia* clade, as well as in *Actinoschoenus* (Arthrostylideae). Similarities in the perforation of the tectum noted among Scirpeae, *Arthrostylis* and *Bulbostylis* are evidently plesiomorphic.

Aperture type appears not to be homologous among the study group, except that it is constant in *Bulbostylis* (Table 5.2). Studies of aperture development are needed to investigate aperture variation within and between more closely related species. The use of TEM might be needed to investigate structure in three dimensions, too.

Medium-sized pollen appears to be plesiomorphic in this group. The small type (Fig. 5.33) may not be homoplasious, evolving once in the *Fimbristylis–Abildgaardia–Crosslandia* clade (Figs. 5.34 and 5.36 and Table 5.3).

Presence of more than one aperture is plesiomorphic and one-aperturate is an apomorphic state, while the inaperturate state developed at least twice: in [1] *Fimbristylis littoralis* and *Abildgaardia ovata*, and [2] *F. rara*, (Fig. 5.36 and Table 5.3). The number of apertures is variable and homoplasious (Fig. 5.36 and Tabel 5.2). The plesiomorphic condition is tetratreme and higher, with a few reversals to this state in the species of *Abildgaardia* and *Fimbristylis*.

On the basis of pollen characters, especially aperture status and surface characters, taxa within Abildgaardieae–Arthrostylideae studied in this work can be distinguished (the key provided in section 5.4.2) (Figs 5.34–5.36).

5.4.1.1 Proposed pollen types

Two distinct pollen forms are recognised in Abildgaardieae and Arthrostylideae. They differ in the sculpture type, existence or absence of perforation of the tectum, position of the areolae, and grain size. Type I is characterised by granulate pollen with distinct perforations on the tectum and no areolae on non-apertural parts, and of medium size; this is seen in *Bulbostylis* and *Arthrostylis*. Type II pollen grains, seen in *Abildgaardia*, *Fimbristylis*, *Crosslandia*, and *Actinoschoenus*, have indistinct or absent perforations on the tectum, areolae all over the pollen grain, and are small in size.

5.4.2 A key to the pollen of the Abildgaardieae–Arthrostylideae group

A key was produced as described in section 5.2.9 based on the pollen morphological data to:

1. help in identifying the species within the Abildgaardieae–Arthrostylideae group.

2. highlight the ease and advantage of using DELTA to organise such non-molecular data for taxonomic studies in Cyperaceae.
3. show the taxonomic utility of pollen data in Cyperaceae, as suggested by Wheeler and Bruhl (2000).
4. point to issues in need of further study within this data source for this group.

The key was completed with 44 taxa included in the data set and the same number of taxa appeared in the key. This indicates the relatively efficient nature of this data source for identification and highlights its taxonomic power. *Fimbristylis polytrichoides* appears four times in the key. This variability is for two reasons: first there are a few missing data for this species and, second, pollen of *F. polytrichoides* exhibits polymorphisms for some characters such as presence of a distal ulcus.

1. Sculpturing type gemmate..... 2
   Sculpturing type granulate..... 3
   Sculpturing type scabrate..... 9
   Sculpturing type verrucate..... 27

2(1). Position of aperture zono (apertures arranged in an equatorial zone); aperture type colpoid (length/breadth>2); distal ulcus absent; areolae all around the pollen grain.....***Fimbristylis xyridis***
   Position of aperture random (both distal and equatorial); aperture type both ulcus and colpoid; distal ulcus present; areolae only in apertures.....
   ....***Fimbristylis ferruginea***

3(1). Size small (10-25 µm longest axis)..... 4
   Size medium (25-50 µm longest axis)..... 8

4(3). Polarity of pollen heteropolar (the two faces are different); distal ulcus present
   5
   Polarity of pollen paraisopolar (almost isopolar but the two faces are of unequal size); distal ulcus absent..... 7

5(4). Areolae all around the pollen grain; arrangement of the elements on the tectum ordinate; perforation of tectum absent.....***Fimbristylis compacta***
   Areolae only in apertures; arrangement of the elements on the tectum inordinate; perforation of tectum present..... 6

6(5). Apiculate elements distinct.....***Arthrostylis aphylla***
   Apiculate elements hardly visible..... ***Bulbostylis densa***

7(4). Apertures present (3 lateral colpoids) ..... ***Fimbristylis velata***
   Aperture absent..... ***Fimbristylis tetragona***

8(3). Perforation of tectum present..... ***Bulbostylis* sp. nov.**
. ***Bulbostylis barbata***
Perforation of tectum absent..... .....***Bulbostylis striatella***

9(1). Polarity of pollen heteropolar (the two faces are different)..... 10
Polarity of pollen isopolar (the distal and proximal faces look alike)..... 19
Polarity of pollen paraisopolar (almost isopolar but the two faces are of unequal size)..... 22

10(9). Arrangement of the elements on the tectum inordinate..... 11
Arrangement of the elements on the tectum ordinate..... 18

11(10). Areolaeall around the pollen grain..... 12
Areolae only in apertures..... 17

12(11). Perforation of tectum present..... 13
Perforation of tectum absent..... 14

13(12). Number of apertures tritreme; distal ulcus present; aperture type both ulcus and colpoid; position of apertures random (both distal and equatorial)
.....***Abildgaardia macrantha***
Number of apertures tetratreme and higher; distal ulcus absent; aperture type colpoid (length/breadth>2); position of apertures equatorial
.....***Crosslandia setifolia* WA**

14(12). Apertures present..... 15
Aperture absent..... ..... ***Fimbristylis rara***

15(14). Aperture type colpoid (length/breadth>2); position of apertures (3 colpoids) equatorial..... ***Fimbristylis pterygosperma***
.....***Fimbristylis densa***
Aperture type a distal ulcus (length/breadth< or = 2)....***Fimbristylis complanata***
Aperture type both distal ulcus and colpoids; position of apertures random 16

16(15). Distal ulcus present.....***Fimbristylis punctata***
.....***Fimbristylis dichotoma***
....***Crosslandia setifolia* NT**
....***Fimbristylis acuminata***
....***Abildgaardia oxystachya***
Distal ulcus absent.....***Fimbristylis cephalophora***

17(11). Aperture margin distinct.....***Fimbristylis depauperata***
Aperture without margin..... .....***Fimbristylis phaeoleuca***

18(10). Distal ulcus present.....***Fimbristylis neilsonii***
.....***Fimbristylis microcarya***
.....***Fimbristylis polytrichoides* (Qld 1)**
Distal ulcus absent.....***Fimbristylis sieberiana***
.....***Fimbristylis polytrichoides* (Qld 2)**

19(9). Arrangement of the elements on the tectum inordinate.....20
Arrangement of the elements on the tectum ordinate.....21

20(19). Size small (10-25 µm); distal ulcus present; aperture without margin; number of apertures 4 or more .....*Fimbristylis schultzii*
Size medium (25-50 µm); distal ulcus absent; aperture margin distinct; number of apertures 3 .....*Fimbristylis nutans*

21(19). Areola all around the pollen grain; aperture margin distinct.....*Fimbristylis polytrichoides* **(Qld 1 and 2)**
Areola only in apertures; aperture without margin...*Fimbristylis furva*

22(9). Perforation of tectum present.....23
Perforation of tectum absent.....25

23(22). Size small (10-25 u the longest axis).....*Fimbristylis littoralis*
Size medium (25-50 u the longest axis).....24

24(23). Apertures present; apiculate elements hardly visible..*Abildgaardia schoenoides*
Aperture absent; apiculate elements distinct.....*Abildgaardia ovata*

25(22). Arrangement of the elements on the tectum inordinate.....26
Arrangement of the elements on the tectum ordinate.....
Fimbristylis polytrichoides **(Qld 1 and 2)**

26(25). Size small (10-25 µm).....*Fimbristylis cymosa*
Size medium (25-50 µm).....*Fimbristylis laxiglumis*

27(1). Aperture type colpoid (length/breadth>2).....28
Aperture type distal ulcus (length/breadth ≤ 2).....30
Aperture type both distal ulcus and colpoid.....31

28(27). Polarity of pollen heteropolar (the two faces are different).....29
Polarity of pollen isopolar (the distal and proximal faces look alike).....*Fimbristylis sericea*
Polarity of pollen paraisopolar.....*Abildgaardia pachyptera*

29(28). Number of apertures 3; aperture without margin....*Fimbristylis sphaerocephala*
Number of apertures 4 or more; aperture margin distinct.....*Fimbristylis tristachya*

30(27). Size small (10-25 µm); arrangement of the elements on the tectum ordinate; AMB pleurotreme (centre of the apertures lies midway between the angles).....*Fimbristylis lanceolata*
Size medium (25-50 µm); arrangement of the elements on the tectum inordinate; AMB centre of the apertures lies on the angles.....*Actinoschoenus* **sp.**

31(27). Polarity of pollen heteropolar (the two faces are different); arrangement of the elements on the tectum inordinate; aperture without margin.....***Crosslandia setifolia* NT2**
Polarity of pollen paraisopolar (almost isopolar but the two faces are of unequal size); arrangement of the elements on the tectum ordinate; aperture margin distinct..... .....***Fimbristylis pauciflora***

Considerably more utility is gained by using INTKEY (Dallwitz *et al.* 1999 onwards), also available with the DELTA package.

5.4.3. Combined morphological-molecular studies

The most parsimonious trees corroborate two distinct groups based on the pollen type, placing the genera with type I pollen in the basal clades. Cladistic analysis of pollen morphology did not group *Arthrostylis* and *Actinoschoenus* together (Fig. 5.34). In contrast, my combined morphological–molecular analysis has *Actinoschoenus* and *Arthrostylis* as sisters (Fig. 5.37). Although bootstrap support for the latter arrangement is not strongly supported due to the conflict in the position of *Actinoschoenus* in molecular and morphological data, the bootstrap increased from 58% in the combined molecular analysis to 66% in the combined molecular–morphological analysis.

The position of *Bulbostylis* as a distinct clade appears to be unambiguous; it also occupies an isolated location in my ingroup. The four accessions of *Bulbostylis* are particularly closely allied and are only a little different in their palynological characters. Further, a basal location for *Bulbostylis* in the Abildgaardieae–Arthrostylideae complex was suggested by *trn*L–F (Chapter 3), combined molecular (Chapter 4), pollen, and combined morphological–molecular data, but not by ITS data although the constraints that support this position in ITS data are not much longer than ITS most parsimonious trees. The basal location of *Bulbostylis* is partly in agreement with the results obtained from morphological and physiological characters by Bruhl (1995). He did not mention the position of *Bulbostylis* in relation to Arthrostylideae but he did mention the close relationships between *Abildgaardia* C_3 species and *Arthrostylis*. My results on the basal position of *Bulbostylis* oppose Gordon-Gray's (1971, p. 565) view. She recognised *Bulbostylis* as "the more recent, derived" genus in Abildgaardieae. They also disagree with Goetghebeur's (1986) judgement about grouping *Abildgaardia* and *Bulbostylis* together far from *Fimbristylis* and *Crosslandia*, although my results support his view about the close relationship between *Fimbristylis* and *Crosslandia*.

Further, *Abildgaardia* seems to be much closer to the *Fimbristylis–Crosslandia* clade than to *Bulbostylis*, which is an isolated and belongs to an earlier derived lineage.

The combined analysis produces an additional supported node compared to the palynological analysis, grouping all *Abildgaardia* species (all but *A. pachyptera* with perforated pollen grains) except *A. vaginata* together. This grouping includes both small- and medium-sized pollen grains. *A. vaginata* consistently grouped with *Crosslandia*.

A few pollen characters confirm patterns of distribution corroborating the molecular cladogram. Very constant from beginning to end is the *Fimbristylis–Crosslandia* group, indicating an actual close affiliation between the two genera. The morphological analyses by Goetghebeur (1985) and Bruhl (1995) also suggested this affinity. Therefore I propose merging *Crosslandia* in *Fimbristylis*. An obvious morphological diversity was seen among different accessions of *Crosslandia setifolia*. I assume this has a very important message, as there is no such difference among accessions of any other species in my study group. In addition to the macromorphological differences (e.g. inflorescence) between accessions from Western Australia and accessions from the Northern Territory, there is diversity among the pollen grains of the accesssions from the same area (Northern Territory). This indicates that there are either more than one species of *Crosslandia* (see Goetghebeur 1985) or a few infraspecific taxa within *Crosslandia setifolia*. However, any change in taxonomic delimitation must wait until the morphological and anatomical studies of K. Clarke (pers. comm.) are completed. None of the sections within *Fimbristylis* suggested by Kern (1974) are supported by the analyses on pollen data. The only clade that groups five species of *Fimbristylis* together (Fig. 5.4) comprises *F. cymosa* (section *Cymosae*), *F. laxiglumis* (section *Leptocladae*), *F. nutans* (section *Nutantes*), *F. tetragona* (section *Mischospora*), and *F. velata* (section *Pogonostylis*). This amalgamation of five different sections in one clade indicates that the pollen data of the sampled species of *Fimbristylis* does not support the existence of sections suggested by Kern (1974). However, here, I recognise a monophyletic group Y within *Fimbristylis* (Fig. 5.37) as a subgenus of *Fimbristylis*. I will discuss the support for these sections by my molecular and combined pollen morphological–molecular analyses in Chapter 6.

My results argue against the monophyly of Abildgaardieae sensu stricto and, therefore, in favour of its recircumscription to include Arthrostylideae. Both combined molecular and

combined morphological–molecular analyses recognise Abildgaardieae (as currently circumscribed) as a non-monophyletic group. This is due to a 100% bootstrap and 22 Bremer support for either splitting Abildgaardieae to *Bulbostylis* and the rest of the tribe, or merging Abildgaardieae and Arthrostylideae (Fig. 5.37). Additionally, the ITS tree (Fig. 4.2) scores poorly when either the morphological data alone or the combined data are mapped onto it, while the scores for the combined morphological–molecular trees are only three steps longer when the ITS data are mapped onto them. This is a signal of the consistency of the combined morphological–molecular tree.

I conclude that pollen characters are useful taxonomic indicators for Abildgaardieae–Arthrostylideae complex, and despite the conflicts in some cases with DNA data, these two datasets are consistent in general and agree on the position of *Bulbostylis*, *Arthrostylis* and *Actinoschoenus* in particular (Figs 4.6, 5.34 and 5.37). From a palynological standpoint, *Arthrostylis* and *Actinoschoenus* should be included in the tribe Abildgaardieae and molecular phylogeny results strengthen this opinion (Fig. 5.37). *Bulbostylis* appears to be older than *Fimbristylis*. As Goetghebeur (1998) admitted in his survey of Cyperaceae, some of his proposed tribes need 'a further division' and I would add that his Schoeneae and Abildgaardieae need to be redefined. More palynological analyses in those genera of the tribes Abildgaardieae and Arthrostylideae that have not been included in this study, as well as mitochondrial DNA investigations, will be valuable to resolve a well-defined inter- and intratribal circumscription, within the Abildgaardieae–Arthrostylideae clade. Because the relationships of *Fimbristylis* and its allied genera have failed to reach a highly consistent agreement by the cladistic methods to date, and because more inclusive morphological studies of this group of genera are in progress (K. Clarke's ongoing Ph.D. research project), it would be premature to make any formal taxonomic changes at this time.

CHAPTER 6

General discussion

6.1 Towards a new resolution

6.1.1. Subfamilial and suprageneric delimitaion

This study represents the most comprehensive cladistic analysis to date undertaken on Abildgaardieae. Generic classification in Abildgaardieae into genera *Fimbristylis*, *Abildgaardia*, and *Bulbostylis* based on the length of hairs in leaf sheath orifice and the size and surface characters of the achene is corroborated by molecular (particularly ITS) data. However, the status of many of the sections in *Fimbristylis*, recognised through one to few non-molecular autapomorphies, was unconfirmed. *Fimbristylis s.str.*, as circumscribed now, is paraphyletic.

Taxa defined by *trn*L–*trn*F (*trn*L–F) data in *Fimbristylis* conflict with the traditional classification, infrageneric groups like sections were not identified, and some morphologically distantly related taxa (according to Kern 1974) may belong to the same clade. Data that are 100% harmonized are seldom, if ever, known, no matter how well the morphology is studied or the DNA sequencing is performed.

I refrain from presenting formal changes in classification. Cladograms, in general, have been revised over time thanks to new characters, reinterpreted assumptions of similarity and upgraded analytical approaches. I view this study as preliminary to a great deal of further study. Both sampling from a broader range of species and surveys of more genes and spacers are needed. In addition, non-molecular variation has to be re-evaluated in light of the relationships found with the molecular phylogenetic data.

My cpDNA analysis (Chapter 3) recovers a clade comprising *Fimbristylis*, *Abildgaardia*, and *Crosslandia* as sister to Arthrostylideae. This topology is not similar to that reported in earlier phylogenetic studies of intrafamilial groups within Cyperaceae (e.g. Muasya *et al.* 1998; Muasya *et al.* 2000a). More studies are needed for verifying whether this clade really is better treated as a tribe separate from Arthrostylideae in a narrow sense (not all genera of Arthrostylideae were included in this study).

In accordance with the results of Muasya *et al.* (2000a) using successive weighting of the cladograms inferred from *rbc*L data, *Abildgaardia* comes together with *Fimbristylis* in a clade (100% bs).The robust (100%) bootstrap value in this analysis

for the Arthrostylideae—Abildgaarddieae clade and also a definite relationship between *Arthrostylis* and C_3 species of *Abildgaardia* in another cladistic study based on non-molecular characters (Bruhl 1995), suggest the close relationship of *Arthrostylis* (Arthrostylideae) and Abildgaardieae.

6.1.2. Generic relationships in Abildgaardieae and Arthrostylideae

The ITS region (Chapter 4) and *trn*L–F IGS and *trn*L intron (Chapter 3) are adequately variable for resolving infratribal relationships in the Abildgaardieae. As the ITS region and also *trn*L–F region are unlikely to be functionally connected, both data sets were studied separately for checking the congruence. The ITS outcomes are incongruent with the previous classifications. Combining the two data sets reduced random error and generated a superior estimate of tree resolution (Chapter 4).

Insertions/deletions made up a critical part of the sequence divergence observed at the infra- and the intergeneric levels. They appeared no less valuable than nucleotide substitutions reported elsewhere (Curtis and Clegg 1984; Wolfe *et al.* 1987; Zurawski and Clegg 1987; Clegg and Zurawski 1991; Gielly and Taberlet 1994a).

The results obtained from this work allow me to make the following points about the evolution of *Fimbristylis*. This genus displays some specialisation in morphology, which leads to debate about its close allies. This controversy still remains, even after considering the molecular data used in this study.

The trees suggest a major split between two groups of Abildgaardieae with deciduous and persistent style-base. *Bulbostylis* is the root lineage for other genera of Abildgaardieae. The *Bulbostylis* clade is strongly consistent in both combined molecular and molecular–pollen morphological trees (100% bootstrap support in both trees), while *Fimbristylis* is not confirmed as monophyletic. *Crosslandia*, *Abildgaardia*, and *Fimbristylis* form a monophyletic clade in all analyses (Figs 3.7, 4. 6, and 5.37). However, relationships between these subgroups were not completely resolved. The two major clades of the members of Abildgaardieae in the *trn*L–F tree show dramatically different pollen characters as well (Figs 5.2–5.33). In the last clade *Crosslandia* is monophyletic if *Abildgardia vaginata* is included. The tree in Fig. 5.37 is similar to those suggested by either morphological or embryological data. Differences between this analysis and the previous studies are in the relations between *Abildgaardia* and *Crosslandia* and the placement of *Arthrostylis*.

Several studies suggest that the genus *Arthrostylis* is best placed in its own tribe Arthrostylideae (Goetghebeur 1986, Bruhl 1995) and may have its closest relatives among the genera *Eleocharis* (Goetghebeur 1986), *Abildgaarda* (Bruhl 1995), or *Schoenus* (Goetghebeur 1998). The close relationship of *Arthrostylis* with *Abildgaardia* (Bruhl 1995) is backed by my cpDNA, combined molecular, pollen morphological, and combined molecular–pollen morphological analyses (Chapters 3, 4, and 5 respectively), although I have not sampled any C_3 species of *Abildgaardia*.

Testing the proposed position for the members of Arthrostylideae by Goetghebeur (1998) within the tribe Schoeneae, the *trn*L–F data of a few genera of Schoeneae (sequenced by Zhang 2003) were far from easy to align with my data. Alignment of *Schoenus*, *Carpha*, *Gahnia*, and even *Rhynchospora* (Rhynchosporeae) with *Arthrostylis* and *Actinoschoenus* was unmanageable and needed the imposition of 43–65 long gaps. This does not rule out the possibility that a number of unsampled taxa of Schoeneae might share more contemporaneous common heritage with Arthrostylideae, particularly in view of different opinions in relation to the boundaries of Schoeneae as well as putatively allied genera.

Achieving better resolution will need further studies using nuclear genes or examining more phylogenetically informative morphological data. *Fimbristylis* and *Abildgaardia* are often considered as nearest relatives to each other based on resemblances in the morphological features (Bruhl 1995, Goetghebeur 1998). My analyses strongly support (100% bs) *Abildgaardia* as the closest lineage to *Fimbristylis*, among all genera involved in these analyses. Additional characters are needed to verify this. *Fimbristylis* was considered to lack a close affinity with *Bulbostylis* in the analysis of *rbc*L sequences by Muasya *et al.* (1998) .This study confirms low affinity between these two genera compared with other genera of Abildgaardieae sampled. However, the tree resulted from ITS data does not show this.

In the *trn*L–F tree (Fig. 3.7), *Abildgaardia s. str.* is not monophyletic seeing that other genera of Abildgaardieae are embedded in it. *Abildgaardia* is polyphyletic in this cladogram, with *A. schoenoides* and *A. oxystachya* grouped together but separate from *A. ovata* and *A. pachyptera*. However, the ITS tree differs, in grouping these four species together. Despite its quite short length, the usefulness of ITS at infrageneric level in Cyperaceae is high. Despite the firm conviction of numerous previous authors about the morphological blurriness of the delimitation of

Fimbristylis and *Abildgaardia*, a view supported by my *trn*L–F analysis, the phylogenetic trees produced from the ITS, combined molecular, and combined molecular-pollen morphological data strongly suggest that these genera should not be merged. This is with the exception of *Abildgaardia vaginata*, which should be included in *Crosslandia* if that is separated from *Fimbristylis*. Several monophyletic clades are found in these analyses, a few of them adequately supported.

Fimbristylis s. str. is paraphyletic according to the *trn*L–F tree as the species of *Abildgaardia* are nested within the genus (Fig. 3.7), but this is not supported by the other analyses. On the basis of these other analyses, *Fimbristylis s.l.* could be maintained as a monophyletic genus if *Crosslandia* and *Abildgaardia vaginata* were included. At the moment I refrain from formalising any revised classification and suggest a more intensive study of the tribe.

6.1.3. Sections within *Fimbristylis*

Fimbristylis s. str. was divided into five sections with one of them made up of four series by Bentham (1878) and later into eighteen sections by Kern (1974), who included *Actinoschoenus* and *Abildgaardia* in his somewhat broader concept of the genus. The DNA data present no additional support for recognising these sections except series *Glomeratae* of Bentham's section *Trichelostylis*. Within *Fimbristylis*, only three infrageneric groups (sections *Abildgaardia*, *Actinoschoenus*, and *Cymosae sensu* Kern) show bootstrap/jackknife confirmation of the sections suggested by Kern (1974) (Table 4.5) and, of course, *Abildgaardia and Actinoschoenus* are treated as genera in this study.

Gross morphological features used by Kern in his diagnostic key to sections are not unique to a particular section, thus reducing the systematic value of those features (Table 6.1). Dividing *Fimbristylis* to two subgenera on the basis of the current sampling might be appropriate given the clear consistency of these two subgeneric groups (X and Y, Figs 4.2, 4.6, and 5.37) in all combined and also ITS analyses. However, these two *Fimbristylis* subgroups have low internal support. Bentham (1878) had grouped four of the species in subgroup Y (*F. cymosa*, *F. neilsonii*, *F. sericea* and *F. tristachya* [as *F. macrostachya*]) in series *Glomeratae* of section *Trichelostylis* but many other previously suggested sections of *Fimbristylis* are para- or polyphyletic on the basis of the current sampling. Sections *Tenerae*, *Heleocharoides*, *Leptocladae*, *Dichelostylis*, *Nutantes*, and *Fimbristylis* are non-

monophyletic (Table 4.5, Fig. 5.37). Sections *Trichelostylis*, *Signatae*, *Fuscae*, *Neodichelostylis*, *Mischospora*, *Miliaceae* and *Pogonostylis* are not tested in relation to monophyly in this work since just one taxon of each of these sections has been included. Sections *Rigidulae* and *Dipsaceae* have not been sampled. Further work is needed to answer the questions about the genealogy of the sections and species.

The topologies found in this study, in addition to outgroup relationships, suggest that sectional characters are homoplastic. This appears to play the main role in the ambiguity surrounding the classification of the infrageneric units of the genus.

In summary, my data are insufficient for clarifying how the species of *Fimbristylis* are best grouped. Phylogenies based on molecular and pollen data included in this study should stimulate research on non-molecular characters, as incongruities of the old classifications at infrageneric (and even higher) level(s) become evident. Therefore, comprehensive studies of non-molecular characters are still desirable to test evolutionary hypotheses inferred from the molecular data. Taken together these data sets will provide reciprocity that will allow us to improve our understanding of structural evolutionary change.

The phylogenetic position of the basal species, *F. littoralis*, cannot be fully resolved by the present study because not all the members of the genus have been used. Interpretation of phylogenetic changes in morphological and molecular characters should be accomplished in another study using a wider sample of species of *Fimbristylis*.

6.1.4. Final message

I did not aim to do any classification in this study. The aim of this study was, rather, exploring the phylogenetic relationships among the genera of Abildgaardieae and among tribes Abildgaardieae and Arthrostylideae. Classifications will be reviewed after the completion of morphological and anatomical studies by Kerri Clarke (Ph.D. student, UNE).

This study has provided increased support for resolution within the Abildgaardieae, demonstrated the utility of non-coding DNA also at tribal and generic levels, achieving my first aim (to resolve [using pollen morphological characters and DNA sequences] weakly supported relationships that have been recovered in non-molecular analyses by previous researchers; see Chapters 1, 3, 4, and 5). I have been able to resolve with strong support the basal interrelationships among *Bulbostylis*,

Abildgaardia, and *Fimbristylis*. Resolution in tribes Arthrostylideae and Abildgaardieae, remains partly unclear. The utility of the palynological characters is also at tribal level. This information has achieved my second aim (to test the taxonomic value of pollen characters in the tribes Abildgaardieae, Arthrostylideae and their allies; and identification of the level at which these characters are informative; see Chapters 1, and 5). The two noncoding markers proved almost equally useful at the tribal level, even though *trn*L–F seems generally more informative at tribal and generic levels and ITS is generally more useful at generic and infrageneric levels. My third aim (to evaluate conflicts between previous molecular and non-molecular studies by studying molecular [DNA] sequence data of *trn*L–F IGS and *trn*L intron, and ITS; see Chapters 1, 2, 3, and 4) has been achieved by this evaluation. Finally, I was able to address my fourth aim (are Abildgaardieae monophyletic and what is their relationship with Arthrostylideae) by finding that the latter tribe is nested within the former.

The results obtained from this work allowed me to address two aspects of the phylogeny of Abildgaardieae. First, Abildgaardieae shows morphological specialisation, which has led to controversy about the genera included in the tribe or in neighboring tribes. The sequences I looked at proved the utility of molecular data in analysing the phylogeny of the Abildgaardieae but other sequences are needed to resolve relationships more fully, especially below the generic level. Second, it should be pointed out that corroboration of relations within Abildgaardieae depends on increasing the sample. Because of the size of Abildgaardieae (particularly genera *Fimbristylis* and *Bulbostylis*), wide and inclusive sampling is a necessity, and further sampling should improve or alter weakly supported branches. However, well-supported clades (80% or more) will probably remain even with increased sampling. A few genera, especially within Abildgaardieae, have not been sampled, and therefore their positions in the Abildgaardieae—Arthrostylideae group remain unclear and require further investigation. This does not discount the possibility of the group sharing more recent common ancestry with some unsampled species of *Eleocharis* rather than with *Schoenoplectus* or *Bolboschoenus* or Arthrostylideae, especially given the diverse views about the limits of *Eleocharis* and its closest relatives. Further careful selection of taxa and use of other phylogenetically informative genes, non-coding regions and morphological characters in future studies should result in a comprehensive and robust estimate of phylogeny of

Table 6.1. Some characters used by Kern (1974) and others for delimiting sections in *Fimbristylis s.s.* and related genera.

Genus/Section	Ligule	Inflorescence	Nut surface	Style
Crosslandia	absent	anthelate/capitate	tuberculate	triquetrous
Abildgaardia	absent/reduced to a sheath	anthelate/ capitate/single	tuberculate	triquetrous
Actinoschoenus	absent	capitate	smooth/verruculose	flat
Fimbristylis				
Sect. Trichelostylis	a fringe of short hairs	anthelate/capitate	smooth/verruculose	triquetrous
Sect. Miliaceae	absent	anthelate/capitate	smooth/verruculose	triquetrous
Sect. Cymosae	absent	anthelate/capitate	smooth/verruculose	triquetrous
Sect. Tenerae	absent	anthelate/capitate	smooth/verruculose	triquetrous
Sect. Leptocladae	absent	anthelate/capitate	smooth/verruculose	triquetrous
Sect. Heleocharoides	absent	single spikelet	smooth/verruculose	triquetrous
Sect. Signatae	absent	anthelate	tuberculate	triquetrous
Sect. Fuscae	absent	single/ numerous simple	smooth/verruculose	triquetrous
Sect. Dichelostylis	a fringe of short hairs	anthelate	smooth	flat
Sect. Fimbristylis	a fringe of short hairs	anthelate	tuberculate	flat
Sect. Rigidulae	absent	anthelate	smooth/verruculose	flat
Sect. Pogonostylis	absent	anthelate	smooth	flat
Sect. Neodichelostylis	a fringe of short hairs	single spikelet	smooth/verruculose	flat
Sect. Nutantes	absent	single spikelet	tuberculate	flat
Sect. Mischospora	absent	single spikelet	tuberculate	flat
Sect. Dipsaceae	absent	anthelate	with appendages	flat

Abildgaardieae. Further investigation of Abildgaardieae and its close tribes, should involve analysing new sequences and comparison with formerly published sequences. New molecular phylogenies should be compared with existing taxonomic concepts based on morphology. Still, as Doyle (1993) suggests, even obtaining a result that is robust by all available standards might not guarantee finding a true phylogeny.