MOLECULAR SYSTEMATIC STUDIES IN COMMELINID MONOCOTS

by

Jeffery Michael Saarela

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ABSTRACT

To clarify phylogenetic relationships among the major commelinid monocot lineages, among families in the orders Commelinales and Poales, and among grass subfamilies (Poaceae), I surveyed multiple plastid protein-coding regions and associated noncoding regions from exemplar taxa sampled broadly across these lineages. I also characterized phylogenetic relationships in the grass genus *Bromus*, using two plastid regions and a nuclear region. In commelinids, phylogenetic inferences using parsimony and likelihood are generally congruent, and my analyses provide strong support for many aspects of commelinid relationships. Commelinales and Zingiberales are sister taxa, but the positions of Arecales and Dasypogonaceae are not clear. Commelinales includes a Commelinaceae–Hanguanaceae clade and a (Philydraceae, (Haemodoraceae–Pontederiaceae)) clade. *Philydrella* is the sister group of the rest of Philydraceae. Poales includes a cyperid clade (Thurniaceae, (Cyperaceae–Juncaceae)), and a 'core Poales' clade consisting of a graminid clade (Flagellariaceae, (Joinvilleaceae, (Ecdeiocoleaceae–Poaceae))) and a restiid clade (Anarthriaceae, (Centrolepidaceae–Restionaceae)). Ecdeiocoleaceae are moderately supported as the sister group of Poaceae in analyses in which taxon sampling is most dense. The position of the aquatic Mayacaceae differs between parsimony and likelihood analyses, perhaps reflecting a long-branch artifact. Rapateaceae are part of a clade that includes all Poales except Bromeliaceae and Typhaceae, and the positions of these latter two families at the base of the Poales subtree are unresolved. Within Poaceae, Anomochlooideae and the BEP clade are supported strongly, Anomochlooideae, Pharoideae, and Puelioideae are the

successive sister groups (respectively) of the remaining grasses, and Bambusoideae and Pooideae are sister taxa. A generally accelerated substitution rate in Poaceae plastid genomes is shared with most, but not all, Poales lineages. Independent analyses of plastid and nuclear ribosomal data in *Bromus* indicate that several traditionally recognized sections are monophyletic, but sect. *Bromopsis* is not. There is some evidence of incongruence between plastid and nuclear linkage groups in *Bromus*, particularly with respect to relationships among major lineages. Based on plastid data, the dwarf aquatic family Hydatellaceae are not commelinids or (more broadly) monocots, but the sister group of water lilies (Nymphaeales), a result corroborated by nuclear data and morphology.

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PREFACE

"If organisms have reached their present state by a process of evolution, it follows that they have a built-in classification, and man's problem is to find it."

R. Holttum, 1967

"Systematics is . . . a synthetic interdisciplinary science that crowns the whole edifice of biology."

Armen Takhtajan, 1997

"Most major civilizations are based on the triploid endosperm of grasses."

David Mabberley, 1997

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DEDICATION

For my

Mom and Dad

CO-AUTHORSHIP STATEMENT

Chapter 2 is based on a manuscript that has been accepted for publication: Saarela, J. M., Peterson, P. M., Keane, R. M., Cayouette, J., and Graham, S. W. In Press. Molecular phylogenetics of *Bromus* (Poaceae: Pooideae) based on chloroplast and nuclear DNA sequence data. In: Columbus, J. T., Friar, E. A., Porter, J. M., Prince, L. M., and Simpson, M. G. [Eds.]. Monocots: Comparative Biology and Evolution, Poales. Rancho Santa Ana Botanic Garden, Claremont, California, U. S. A. The project was suggested by P. M. Peterson. I conducted all laboratory work and data analyses, and wrote the manuscript. Most of the plant samples were contributed by P. M. Peterson, R. M. Keane, and J. Cayouette. Sean W. Graham provided insights into data analyses and contributed to the writing.

Chapter 3 is a draft manuscript that will be submitted for publication: Saarela, J. M., and Graham, S. W. Phylogenetic relationships among the subfamilies of grasses (Poaceae) and related families inferred from a large plastid data set. I designed the project in consultation with S. W. Graham, and I conducted all laboratory work and data analyses, and wrote the manuscript. Sean W. Graham provided insights into data alignment and analyses, and he contributed to the writing.

Chapter 4 is a draft manuscript that will be submitted for publication: Saarela, J.M., Rai, H. S., Chang, Y., Prentis, P., Marchant, A., Briggs, B. G., and Graham, S. W. Higher-order phylogenetic relationships in the commelinid monocots, with a focus on the orders Commelinales and Poales. Sean W. Graham and I designed the project, and I carried out the

laboratory work (H. S. Rai contributed sequence data for three taxa included in this study, and Y. Chang contributed partial sequence data for one taxon). Critical DNA samples were provided by P. Prentis, A. Marchant, and B. G. Briggs. Sean W. Graham and I compiled alignments, and I conducted all analyses and wrote the manuscript, with contributions from S. W. Graham.

Chapter 5 is based on a manuscript that is in review: Saarela, J. M., H. S. Rai, J. A. Doyle, P. K. Endress, S. Mathews, A. D. Marchant, B. G. Briggs, and S. W. Graham. Hydatellaceae are not monocots but a lineage near the base of angiosperm phlogeny. I generated all of the plastid molecular data, except the plastid *ndh*F gene and short *rbc*L gene (generated by H. S. Rai) and the plastid *trn*L–*trn*F region (generated by A. Marchant and B. G. Briggs). The nuclear *PHYC* data was contributed by S. Mathews, and morphological characters were scored by J. A. Doyle and P. K. Endress. All authors contributed to analyses and preparation of the final manuscript.

CHAPTER 1

Introduction

1.1 Overview of Monocot Systematics

The monocots (Monocotyledoneae) are a large and diverse clade of flowering plants with ~60,000 species in ~92 families (APG II 2003), representing ~25% of total angiosperm diversity. They include many familiar ornamental and horticultural taxa, such as bananas, bromeliads, cattails, grasses, lilies, onions, orchids, and palms. They are readily distinguished from other angiosperms by their (usually) parallel-veined leaves, sheathing leaf bases, stems with scattered bundles, no vascular cambium, sieve cell plastids with cuneate proteinaceous crystalloids (i.e., P2-type), and embryos with a single cotyledon (Judd et al. 2002; Chase 2004). Among flowering plants, monocots are arguably the best-studied major lineage (Chase 2004); nonetheless, substantial questions remain about several aspects of their evolutionary history.

Molecular phylogenetic studies (e.g., Chase et al. 1995, 2000, 2006; Givnish et al. 1999, 2006; Fay et al. 2000; Bremer 2002; Caddick et al 2002; Davis et al. 2004; Tamura et al. 2004; Graham et al. 2006) have identified 11 major monocot lineages as orders (Acorales, Alismatales, Arecales, Asparagales, Commelinales, Dioscoreales, Liliales, Petrosaviales, Poales, and Zingiberales; APG II 2003, Chase 2004). The small family, Dasypogonaceae, remains unplaced to order (APG II 2003). Most studies agree that *Acorus* (Acorales) is the sister-group of the rest of the monocots, and that Alismatales and Petrosaviales respectively are the next successive sister groups of a large clade that includes Asparagales and the commelinid monocots (e.g., Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006). The

relative arrangements of Dioscoreales, Liliales, and Pandanales along the monocot backbone are still in question (e.g., Graham et al. 2006). The commelinid monocots – a large and heterogeneous group that includes about one third of monocot diversity – consist of the remaining four orders and Dasypogonaceae (Chase et al. 1995; APG II 2003). A roughly similar group of taxa was recognized by Dahlgren and Rasmussen (1983) based on morphology, and referred to subsequently as the Bromelianae–Commelinanae–Zingiberanae (BCZ) or Arecanae–Bromelianae–Commelinanae–Zingiberanae (ABCZ) clade (e.g., Clark et al. 1993). Although support for the commelinid clade is strong, several aspects of relationship among and within its major lineages are not clear, such as the relative arrangement of most of its five major clades, and relationships among families within Commelinales and Poales, the order that includes the grasses (Poaceae).

The grasses are a large and taxonomically difficult commelinid family. Because of their economic and ecological importance, Poaceae have received substantial systematic study, and multiple major lineages (subfamilies) in the family have been identified. Relationships among several of these, however, are not clear (GPWG 2001; Duvall et al. 2006). Moreover, with 600 to 800 genera and approximately 11,000 species, only a fraction of the species has been included in molecular phylogenetic studies. One major genus is *Bromus*, a large (~160 species) and widespread taxon that includes important forage grasses (such as *B. inermis* Leyss.) and weeds of major economic concern [such as cheatgrass (*B. tectorum* L.) and ripgut grass (*B. diandrus* Roth.)], in addition to a number of species that have narrow distributions. *Bromus* is also the sister group of a commercially important clade (tribe Triticeae) that includes barley, rye, and wheat.

Reconstructions of monocot phylogeny at multiple hierarchical levels have provided evolutionary frameworks for identifying major monocot lineages and their inter-relationships, and for reconstructing the origins of several floral and other morphological characters (e.g., Furness and Rudall 1998, 2003; Smith and Harris 1999; Zona 2001; Prychid et al. 2003; Rudall and Bateman 2004; Linder and Rudall 2005; Gunawardena and Dengler 2006). Within Poaceae, phylogenies have permitted researchers to develop (and test) hypotheses about the origin and diversification of the grass spikelet, the organ in which cereal grains are produced (e.g., Rudall et al. 2005; Preston and Kellogg 2006). Better sampled and more robust inferences of monocot evolution should provide an even firmer footing for performing these types of studies.

1.2 Objectives of the Thesis

The objectives of this thesis are to characterize phylogenetic relationships at a variety of hierarchical levels in commelinid monocots: among species in the grass genus, *Bromus* (Chapter 2); among subfamilies in the grass family, Poaceae (Chapter 3); and among and within major commelinid monocot lineages (Chapter 4). I also present a surprising discovery involving the reduced aquatic family Hydatellaceae, thought to belong to the commelinid monocots, but shown here to belong to the basal angiosperm grade that includes Amborellaceae and water lilies (Nymphaeales) (Chapter 5).

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CHAPTER 2[1]

Molecular Phylogenetics of *Bromus* (Poaceae: Pooideae) Based on Chloroplast and Nuclear DNA Sequence Data

2.1 INTRODUCTION

Bromus L. is a large genus that is widely distributed in temperate and mountainous regions of the Northern and Southern hemispheres. Several species are important forage grasses (e.g., Ferdinandez and Coulman 2000; Ferdinandez et al. 2001; Puecher et al. 2001); some were important cereal crops in the past (Scholz and Mos 1994); and many are invasive weeds (e.g., Ainouche et al. 1999; Novak and Mack 2001; Keane 2002; Ogle et al. 2003). *Bromus* is distinguished from other grass genera by the combination of several morphological characters, including: leaf sheath margins that are connate for most of their length; awns that are almost always subapically inserted; hairy apical bilabiate appendages of the ovary; and simple starch grains (Wagnon 1952; Smith 1970).

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2.1.1. Phylogenetic Position

The eastern Asian genus *Littledalea* Hemsl., with three species, was believed by Stebbins (1981) to be the closest living relative of *Bromus*; tribe Bromeae currently comprises these two genera (Smith 1970; Clayton and Renvoize 1986; Tsvelev 1989; Grass Phylogeny Working Group [GPWG] 2001). However, preliminary plastid sequence data indicate that *Littledalea* and *Bromus* do not form a clade, thus Bromeae may not be monophyletic (J. M. Saarela, unpubl. data). Other genera believed previously to be closely related to *Bromus*, based on morphological similarities, include *Megalachne* Steud., *Metcalfia* Conert, *Pseudodanthonia* Bor & C. E. Hubb., and *Sinochasea* Keng (Smith 1970; Stebbins 1981), but these genera are now considered distantly related (Clayton and Renvoize 1986; Soreng et al. 2003). Phylogenetic analyses of chloroplast DNA restriction site variation and DNA sequence data indicate that Bromeae are the sister group of Triticeae (e.g., Davis and Soreng 1993; Catalán et al. 1997; Hilu et al. 1999; Hsiao et al. 1999; Soreng and Davis 1998, 2000; GPWG 2001).

2.1.2. Taxonomy and Classification

Bromus is a taxonomically difficult genus with a complex nomenclatural history [see Wagnon (1952), Smith (1970), and Acedo and Llamas (1999) for comprehensive reviews], and many species are difficult to distinguish due to their high degree of morphological similarity. As with many other genera of grasses, many species are polyploids, and hybridization is believed to have played an important role in the evolution of many species in the genus (Stebbins 1981). The complexity of *Bromus* is exemplified in the more than 1200 taxa that have been described, according to the International Plant Names Index (2004). The

most recent estimates of the number of species in the genus are 160 (Acedo and Llamas 2001) and 142 (Clayton et al. 2002 onwards), although estimates have ranged from around 100 (Gould and Shaw 1983) to 400 (Soderstrom and Beaman 1968). Several species complexes have been the subject of recent taxonomic investigations (e.g., Scholz 1981; Naranjo et al. 1990; Sales and Smith 1990; Sales 1993, 1994a; Smith and Sales 1993; Zajac 1996a, b; Allison et al. 2001; Bacic and Jogan 2001; Peterson et al. 2002; Spalton 2002a; J. M. Saarela and P. M. Peterson, unpubl. data), and new taxa continue to be collected and described (e.g., Smith 1985a; Veldkamp et al. 1991; Peterson and Planchuelo 1998; Scholz 1997, 1998; Acedo and Llamas 1997; Bomble and Scholz 1999; Holmström and Scholz 2000; Spalton 2001; J. M. Saarela and P. M. Peterson, unpubl. data). Because of its large size, taxonomical complexity, and wide geographic range, no comprehensive worldwide treatment of all the species in *Bromus* exists, but many floristic treatments and keys of *Bromus* have been published for various geographic regions in the New World (e.g., Shear 1900; Wagnon 1952; Mitchell 1967; Soderstrom and Beaman 1968; Pinto-Escobar 1981, 1986; Matthei 1986; Allred 1993; Pavlick 1995; Gutiérrez and Pensiero 1998; Planchuelo and Peterson 2000) and the Old World (e.g., Veldkamp et al. 1991; Forde and Edgar 1995; Chen and Kuoh 2000; Spalton 2002b, 2004). Genetic variation within and among many species has been studied using data from isozymes (Kahler et al. 1981; Ainouche et al. 1995, 1999; Oja 1998, 1999, 2002a, b, 2005; Bartlett et al. 2002), as well as an array of DNA-based molecular techniques, including RAPDs and AFLPs (Ferdinandez et al. 2001; Massa et al. 2001; Puecher et al. 2001; Ferdinandez and Coulman 2002) and microsatellites (Green et al. 2001; Ramakrishnan et al. 2002). A physical map of the chloroplast genome has been constructed for one species, *B. inermis* (Pillay 1993).

The infrageneric classification of *Bromus* has received considerable study. The genus has been variously split into several groups that have been recognized as sections, subgenera, or generic segregates (Table 2.1). Smith (1970) reviewed the morphological characteristics and nomenclature of the commonly recognized groups in the genus, and accepted five distinct sections, characterized by minor differences in the structures of the spikelets. Using data from crossing experiments, Stebbins (1981) recognized seven subgenera (although one, subgenus *Boissiera*, is not validly published at this rank) based on their morphological distinctiveness and the apparent high degree of genetic divergence among them. He argued that the subgenera of *Bromus* are too distinct to be treated as sections, since they seemed more distantly related to one another than are several other genera of grasses. Other authors believe that each of these groups is sufficiently distinct to be regarded as genera (e.g., Tsvelev 1976). No taxonomic consensus exists, and infrageneric taxa in *Bromus* are recognized currently as distinct genera (e.g., Catalán et al. 1997; Green et al. 2001; Spalton 2002b, 2004), subgenera (e.g., Acedo and Llamas 1999), or sections (e.g., Smith 1985b; Pavlick 1995; Planchuelo and Peterson 2000). The sectional classification of Smith (1970) has been followed by most recent North American authors, and is employed here, incorporating the recent modifications of Smith (1985a) and Scholz (1998); all species mentioned below are species of *Bromus*.

Each section of *Bromus* can be identified using a combination of several morphological characters, including the number of nerves on the first and second glumes, spikelet shape and compression, and lemma and awn morphology (Table 2.2). Additional data from embryo morphology (Kosina 1996), floral microstructures (Kosina 1999), micromorphology of the lemmas and paleas (Acedo and Llamas 2001), and anatomy (Acedo

and Llamas 1999) have recently been collected to aid in the infrageneric classification. Insights obtained from these studies generally agree with the classification schemes based on macromorphological evidence.

Section *Bromopsis* is the largest section, comprising approximately 60 species that occur naturally in Eurasia, Africa, and North and South America, and thus occurs in all regions where brome grasses are native (Stebbins 1981; Armstrong 1991). The section includes diploids, tetraploids, hexaploids, octoploids, and decaploids (Stebbins 1981). Section *Bromopsis* comprises at least two geographically, morphologically, and cytologically distinct groups. North American taxa, and the *B. ramosus* complex from the Old World, are loosely tufted (non-rhizomatous), short-lived perennials or biennials [except *B. texensis* (Shear) A. S. Hitch., an annual] with small anthers and large chromosomes, and the majority are diploids (Wagnon 1952; Armstrong 1981, 1983, 1991; Stebbins 1981). Old World taxa and *B. pumpellianus*, which occurs in North America and the Old World, are densely tufted or rhizomatous long-lived perennials with large anthers and smaller chromosomes, and the majority are polyploids (tetra-, hexa-, octo-, and decaploids) (Wagnon 1952; Armstrong 1981, 1983, 1991; Stebbins 1981). Armstrong (1983, 1991) suggested that these two groups might have separate evolutionary histories, based on difficulties in crossing North American and Eurasian taxa, and noted that valid names are available at sectional rank for each of these groups if such recognition becomes appropriate. Cytology and evolutionary relationships of the South American species are poorly known (Stebbins 1981).

Section *Bromus* comprises 30–40 diploid and tetraploid annual species native to Europe and Asia. One species, *B. arenarius* Labill., is thought by some authors to be the only native *Bromus* species in Australia; others believe the species is introduced there (Stebbins

1981). Many species are invasive and widely distributed in other regions of the world (Keane 2002). For example, the 11 species of sect. *Bromus* that occur in North America are all introduced (Pavlick 1995). Species in the section are morphologically similar (e.g., Smith and Sales 1993; Oja et al. 2003), and several subsectional classifications of sect. *Bromus* have been proposed (Smith 1972). The tetraploid species in sect. *Bromus* are believed to be allopolyploids (Stebbins 1981), and their putative intrasectional origins have been elucidated using data from serology (Smith 1972), allozymes (Ainouche et al. 1995; Oja 1998) and DNA sequence data (Ainouche and Bayer 1997; Ainouche et al. 1999). One group of tetraploid species, the *B. pectinatus* complex, is believed to be of hybrid origin between sects. *Bromus* and *Genea* (Scholz 1981; Stebbins 1956, 1981).

Section *Ceratochloa* comprises 10–16 perennial species native to North and South America. All taxa in this section are polyploids (octo-, hexa-, and 12-ploid) (Stebbins 1981; Pavlick 1995). Species boundaries in sect. *Ceratochloa* are uncertain due to presumed hybridization and morphological intergradation among taxa, which have resulted in various taxonomic treatments (e.g., Soderstrom and Beaman 1968; Stebbins 1981; Pavlick 1995; Planchuelo and Peterson 2000). Some species complexes in sect. *Ceratochloa* have recently been revised. Based on genetical and morphological studies of six hexaploid and octoploid species from Patagonia, Massa et al. (2001, 2004) distinguished only two morphologically and genetically distinct taxa, which they treated as two species. Similar revisionary work is necessary to characterize morphological and molecular variation among North American taxa of sect. *Ceratochloa.*

Section *Genea* comprises diploid, tetraploid, hexaploid, and octoploid annual species native to the Mediterranean, southwestern Asia, northern Europe, and northern Africa.

Several species are invasive [e.g., cheatgrass (*B. tectorum* L.), ripgut grass (*B. diandrus* Roth.), and red brome (*B. madritensis* subsp. *rubens* (L.) Husnot)] and have become widely distributed far beyond their native range (e.g., Pavlick 1995). Species in sect. *Genea* are highly variable morphologically, and many taxa have been proposed. Recent revisionary work has reduced the number of species to five, including several intraspecific taxa (Sales 1993, 1994a). Section *Genea* is thus the only geographically widespread section of *Bromus* that has received monographic-level taxonomic attention. Based on this taxonomic framework, Sales (1994b) proposed hypotheses for the origins of taxa and patterns of adaptive radiation that have occurred within the section. Isozyme data have indicated that the three diploid species of sect. *Genea* are putative donors of genomes in the origins of the polyploid species in the section (e.g., Oja 1998, 2002b,c).

The remaining sections in *Bromus* (*Boissiera*, *Neobromus*, *Nevskiella*, and *Triniusia*) are individually small, but contribute substantially to morphological variation in the genus as a whole (Table 2.2). Section *Neobromus* comprises two annual hexaploid species native to the Pacific coasts of North and South America (Pavlick 1995; Matthei 1986). Sections *Nevskiella* (diploid; Armstrong 1991) and *Boissiera* (diploid [Smith 1972] or tetraploid [Oja and Jaaska 1998]) are both monotypic, while section *Triniusia* comprises two diploid species (Scholz 1998); species in these three sections are all annuals native to Asia and the eastern Mediterranean. Sections *Boissiera* and *Triniusia* were included within sect. *Bromus* by Smith (1970), but they were treated as distinct subgenera by Stebbins (1981; Table 2.1). They have both recently been recognized as distinct sections by Smith (1985a) and Scholz (1998), respectively (Table 2.1).

2.1.3. Phylogenetic Relationships

Past attempts to understand phylogenetic relationships in *Bromus* among species and infrageneric taxa have been based largely on data from morphology (e.g., Shear 1900; Wagnon 1952), karyology (including chromosome number, satellite type, chromosome size, and DNA quantities) and hybridization experiments (e.g., Stebbins and Togby 1944; Stebbins 1947, 1956, 1981; Schulz-Schaeffer 1960; Wilton 1965; Armstrong 1975, 1981, 1983; Kozuharov et al. 1981; Naganowska 1993a,b), serology (Smith 1969, 1972), and allozymes (e.g., Oja 1998; Oja and Jaaska 1998; Oja 2005). Chromosome numbers, polyploidy, genome size, karyotypes, c-banding, cross-compatibility, and genome homology within *Bromus* have been summarized by Armstrong (1991).

Five systematic studies have been conducted in *Bromus* using data from DNA, although the number of species included in each study was relatively limited. Pillay and Hilu (1990, 1995) studied cpDNA restriction site variation among 32 *Bromus* species, and identified two major clades: one comprising sects. *Ceratochloa* and *Neobromus*, the other comprising sects. *Bromopsis*, *Bromus*, and *Genea*. Species of sect. *Bromopsis* occurred in three different lineages, indicating that this taxon is not monophyletic, but the data did not support the New World/ Old World split hypothesized by Armstrong (1983) on the basis of morphology and chromosome pairing data. Sections *Bromus* and *Genea* were not monophyletic; species from both sections were intermixed in a single clade. Joachimiak et al. (2001) used RAPD data to portray relationships among nine species representing four infrageneric taxa in *Bromus* from the New and Old Worlds. Based on a phenetic analysis, they identified two distinct clusters: one comprising sect. *Ceratochloa*, and the other comprising sects. *Bromopsis, Bromus*, and *Genea*. However, because of their small sample

size, and the low level of molecular divergence detected, they were unable to make definitive statements regarding relationships in *Bromus*. Ainouche and Bayer (1997) and Ainouche et al. (1999) used sequence data from the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA to study the phylogeny of sect. *Bromus*. Based on an analysis of 22 species from sect. *Bromus* (including sect. *Triniusia*) and three species from other infrageneric groups, they found sect. *Bromus* to be monophyletic. They also studied the origin of some tetraploid species in the section. Little sequence heterogeneity was detected within tetraploid species, and they found that the inclusion of allotetraploid taxa with diploid taxa did not change the underlying topology of the trees obtained, compared to trees obtained from analyses of the diploid taxa alone.

2.1.4. Objectives of the Study

To further characterize phylogenetic relationships in *Bromus* s. l., I obtained new sequence data from the chloroplast *trn*L(UAA) intron, the rapidly evolving 3' end of the chloroplast *ndh*F gene, and the nuclear ribosomal internal transcribed spacer regions, from 46 exemplar *Bromus* species that represent a large proportion of the geographical and morphological diversity in the genus. The specific objectives of this study were to use DNA sequence data to: (1) test the monophyly of the currently recognized infrageneric groups in *Bromus*; and (2) determine phylogenetic relationships among infrageneric groups and species.

2.2 MATERIALS AND METHODS

2.2.1. Taxon Sampling

Exemplars from each of the currently recognized sections in *Bromus* were included in this study, except for the two monotypic sections, *Boissiera* and *Nevskiella*. Attempts to extract DNA from a herbarium specimen of *B. gracillimus* Bunge (sect. *Nevskiella*) were unsuccessful, and material of *B. pumilio* (Trin.) P. M. Sm. (sect. *Boissiera*) was not available. Table 2.3 lists the species sampled [following the classification schemes of Smith (1970) and Scholz (1998)], their geographic origins, vouchers, and GenBank accession numbers for the sequences. One individual of each species was examined, except for *B. madritensis* subsp. *rubens,* for which three individuals were sampled, and *B. anomalus*, for which two individuals were sampled. Samples were obtained from silica-gel dried leaf material from field collections, from plants grown in the greenhouse from seed obtained from the Western Regional Plant Introduction Station (United States Department of Agriculture, Pullman, Washington, USA) and Plant Gene Resources of Canada (Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada), and from herbarium specimens. All taxonomic identifications were confirmed using the available world taxonomic literature of *Bromus*. Outgroup taxa from tribes Triticeae and Poeae were chosen based on previous molecular investigations of the grasses (see Catalán et al. 1997; GPWG 2001). The *Bromus* and *Festuca breviglumis* sequences used in this study are new. Sequence data for *Hordeum vulgare* and *Triticum aestivum* were obtained from GenBank.

2.2.2. DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987) with 2% ß-mercaptoethanol added to each extraction. DNA extracts and PCR amplifications were purified using a Qiagen PCR Purification kit (Qiagen, Santa Clarita, California, USA) following manufacturer instructions.

The *trn*L (UAA) intron was amplified and sequenced with primers developed by Taberlet et al. (1991). The region I refer to as ITS, which includes two spacer regions, ITS 1 and ITS 2, and the 5.8S rDNA locus, was amplified and sequenced using primers published by White et al. (1990), Hsiao et al. (1994), and Blattner (1999). The 3' end of *ndh*F was amplified and sequenced using primers designed by Olmstead and Sweere (1994) and Graham et al. (1998). Amplification reactions consisted of 26.5 µl sterile water, 5µl 10x buffer, 4 µl 10 mM dNTPs, 3 µL 25 mM $MgCl_2$, 5 µL of each 5 pmol/µl primer, 1 µl of template DNA, and 0.5 µl of *Taq* DNA polymerase (1 unit). The thermal profile was: 1 cycle of 3 min at 94°C; 35 cycles of: 30 sec at 94°C, 1 min at 42.5°C, 2 min at 72°C; and 1 cycle of 5 min at 72°C.

Sequencing products were generated using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) with 50 ng of template DNA and the following thermal profile: 25 cycles of 10 sec at 96°C, 5 sec at 45°C, and 4 min at 60°C. For each sample, one or several duplicate sequencing reactions were included using a second DNA extract from the same source material. Sequencing reactions were analyzed using an Applied Biosystems Prism 377 automated DNA sequencer.

Sequence data were assembled and edited using Sequencher 4.1 (Genes Code Corporation, Ann Arbor, Michigan, USA). Consensus sequences were exported for each taxon and aligned manually using Se-Al ver. 1.0 alpha 1 (Rambaut 1998) according to guidelines outlined in Graham et al. (2000). Gaps in the final matrix were coded as missing data. Several inferred indels in the *trn*L intron were scored as binary characters. Alignments were imported into PAUP* version 4.0b10 (Swofford 2002). All sequence data have been submitted to Genbank (Table 2.3).

2.2.3. Phylogenetic Analyses

For the ITS data set, a heuristic search was conducted with 100 random starting trees, tree-bisection-reconnection (TBR) branch swapping, and all character and character-state changes equally weighted. A two-tier approach was taken for the heuristic searches of the combined plastid data because an upper limit on the number of most-parsimonious trees was unattainable with the available computational resources and time: (1) 100 independent heuristic searches each with a random starting tree, saving 100 trees each (MaxTrees set to 100), were performed with the parameters noted above; and (2) another heuristic search, with the same parameters as above, was conducted, except that the shortest of the 10,000 trees from step 1 were used as starting trees, and MaxTrees was set to 50,000. I also implemented the parsimony ratchet (Nixon 1999) using PAUPRat (Sikes and Lewis 2001) to search for shorter trees with the combined plastid data set. The incongruence length difference (ILD) test (Farris et al. 1994, 1995) was used to test for conflict among plastid and nuclear data partitions, with MaxTrees set to 500. In addition, trees and bootstrap analyses derived from plastid and nuclear data were compared visually to assess the robustness of topological

incongruence (e.g., Graham et al. 1998). I computed strict consensus trees from all of the most-parsimonious trees for each of the data partitions. I present phylograms of one randomly chosen tree from each of these analyses, and indicate which clades on the phylograms collapse in the strict consensus trees. Branch support was assessed using maximum parsimony bootstrap analysis (Felsenstein 1985) from 500 replicates using the heuristic search option, with one random starting tree, TBR branch swapping, and MaxTrees set to 500 per replicate. I use the terms 'weak', 'moderate', and 'well' to refer to bootstrap values that range from <50-70, 71-90, and 91-100, respectively.

2.3 RESULTS

2.3.1. Analyses of ITS Sequences

The boundaries of ITS 1, 5.8S, and ITS 2 follow Eckenrode et al. (1985), Yokota et al. (1989), and Kolosha and Fodor (1990). Lengths of ITS 1 and ITS 2 ranged from 216–219 and 213–216 base pairs (bp), respectively. The 5.8S rRNA gene was 163 bp in length. A small region of 10 bp (positions 108–117) in ITS 1 was difficult to align across taxa, and was excluded from all analyses. The ITS data matrix, without excluded sites, was 606 aligned nucleotides in length. Of these characters, 382 were constant, 224 were variable, and 125 (20.6%) were parsimony informative. Among the ingroup taxa, 437 characters were constant, 169 were variable, and 104 (17.2%) were parsimony informative. The heuristic searches of the ITS data set recovered 449 most-parsimonious trees (tree length = 380 steps, consistency index [CI] = 0.713, retention index [RI] = 0.826).

Several clades receive good bootstrap support (Fig. 2.2.1). The monophyly of the genus is moderately supported (bootstrap proportion [BP] = 75%). Section *Bromopsis* is not

monophyletic. A well-supported clade (BP = 99%) consisting of two North American *Bromopsis* species, *B. attenuatus* and *B. dolichocarpus*, is the sister group of the rest of the genus, the latter clade with BP = 100%. The next major split in *Bromus* is between a well-supported clade (BP = 100%) of four South American species of sect. *Bromopsis* (*B. lanatus*, *B. modestus*, *B. pellitus*, and *B. pflanzii*) and all remaining species of *Bromus*. The latter clade is weakly supported (BP = 59%).

A large and well-supported clade (BP = 96%) includes five species of sect. *Bromopsis* of Eurasian origin [*B. erectus*, *B. inermis*, *B. korotkoyi*, *B. pumpellianus* (which is also native in the New World), and *B. riparius*], one species of sect. *Bromopsis* from South American (*B. brachyanthera*), and the monophyletic sects. *Ceratochloa*, *Genea*, and *Neobromus* (BP = 92%, 100%, and 94% respectively). The Eurasian representatives of sect. *Bromopsis* are not united in a single clade.

A second large, weakly-supported clade (BP < 50%) contains the remaining North American species of sect. *Bromopsis*, *B. ramosus* (a species classified in sect. *Bromopsis* from the Old World), and sects. *Bromus* and *Triniusia* (Fig. 2.1). Several well-supported relationships are evident among some species of sect. *Bromopsis* from North America. Section *Triniusia* is monophyletic (BP = 94%), and is part of a well-supported clade that otherwise only includes representatives of sect. *Bromus* (BP = 100%).

2.3.2. Analyses of Plastid Sequences

The sequence data obtained for the 3' end of *ndh*F corresponds to positions 1441 to 2076 of *ndh*F in *Oryza sativa* (NC_00132). The sequenced portion of the 3' end of *ndh*F was 662 bp in length in all taxa, except for *B. grandis*, which had a six bp insertion. I was not

able to obtain data for *ndh*F from six taxa (Table 2.3). The unambiguously aligned *ndh*F matrix was 668 bp long; 576 nucleotides were constant, 92 were variable, and 44 (6.5%) were parsimony informative. Among the ingroup taxa, 616 characters were constant, 52 were variable, and 31 (4.6%) were parsimony informative. The *trn*L intron ranged in length from 568–586 bp. Several indels were present in the final data matrix; three of these were phylogenetically informative and were coded as binary characters in the analysis. In the *trn*L intron, two regions of 18 bp (positions 1397-1414) and 11 bp (positions 1755-1765) were homopolymer repeats of variable length that were difficult to align; these regions were excluded from all analyses. The aligned *trn*L intron matrix (including binary characters but without excluded sites) consisted of 646 aligned nucleotides; 578 were constant, 68 were variable, and 28 (4.9%) were parsimony informative. Among the ingroup taxa, 610 characters were constant, 36 were variable, and 23 (3.5%) were parsimony informative. No sequence data were obtained from either plastid locus for *B. modestus* and *B. nottowayanus*, and four species are represented solely by data from the *trn*L intron (Table 2.3). The heuristic search of the combined plastid data recovered 50,000 most parsimonious trees (tree length = 218 steps, CI =0.817, RI = 0.882).

In the analyses of combined plastid data there is moderate phylogenetic structure that is supported by bootstrap analysis (Fig. 2.2). The monophyly of the genus *Bromus* is well supported (BP = 99%). Taxa classified in sects. *Bromus*, *Genea*, and *Triniusia* form a well-supported monophyletic group (BP = 91%), but none of the three sections is monophyletic. *Bromus pectinatus* (sect. *Bromus*) and *B. diandrus* (sect. *Genea*) comprise a well-supported clade (BP = 100%) that is weakly supported (BP = 56%) as the sister group of *B. madritensis* subsp. *rubens* (sect. *Genea*). Other species of sect. *Bromus* and species of sect. *Triniusia* are

mixed in a clade (BP = 86%). Species of sects. *Ceratochloa*, *Neobromus*, and *B. brachyanthera* (sect. *Bromopsis*) comprise a weakly-supported clade (BP = 62%). Section *Neobromus* is not monophyletic, and the monophyly of sect. *Ceratochloa* is weakly supported (BP = 63%). A large clade of 23 New and Old World species of sect. *Bromopsis* is weakly supported (BP = 68%).

2.3.3. Incongruence Among Data Partitions

The ILD test indicated significant incongruence between the nuclear ribosomal and plastid data partitions ($p < 0.01$). Overall, the trees derived from the nuclear ribosomal data were more resolved than trees derived from the plastid data. There were some well-supported clades whose positions differed substantially among trees, although not always with strong support. Topologically, the greatest differences between the plastid and nuclear ribosomal trees were the positions and monophyly of sects. *Bromus*, *Trinusia*, and *Genea*. In the nuclear trees, species from sects. *Bromus* and *Triniusia* formed a clade, and sect. *Genea* was well supported as monophyletic; a close relationship between these two clades was not inferred (Fig. 2.1). In the plastid trees, species from these three sections were intermixed in a well-supported clade (Fig. 2.2); for example, *Bromus pectinatus* (sect. *Bromus*) and *B. diandrus* (sect. *Genea*) comprise a well-supported clade. Other incongruencies involve relationships among species of sect. *Bromopsis* (Fig. 2.2). The plastid trees include species of sect. *Bromopsis* from the Old World in a weakly-supported clade with some North American species of sect. *Bromopsis*, while the nuclear ribosomal trees indicate a more distant relationship between these Old World (with the exception of *B. ramosus*) and North

American species. Because of these possible instances of intergenomic conflict, I did not conduct analyses of the combined nuclear ribosomal and plastid data.

2.4 DISCUSSION

2.4.1. Phylogenetic Utility of the Regions Examined

Of the three regions examined, the nuclear ribosomal region was the most variable and accounted for 65.8% of the total number of parsimony-informative characters among all three data sets (among ingroup taxa). Resolution (number of bifurcated nodes in the strict consensus) was greater in the nuclear ribosomal phylogeny compared with the plastid phylogeny, probably because of the greater amount of variation in the former data set. The least parsimony-informative variation (among ingroup taxa) was observed in the *trn*L intron, which accounted for 14.5% of the total number of informative characters in all three data sets. Although this intron is commonly used for lower-level phylogenetic studies, several investigators have reported a paucity of phylogenetically informative characters in it to sufficiently resolve relationships among closely related grass genera and species (e.g., Hodkinson et al. 2002), and a wide variety of other plant taxa (e.g., Bruneau et al. 2001; Klak et al. 2003; Shaw et al. 2005). The 3' end of *ndh*F provided 19.6% of the total parsimony-informative variation among all three data sets (among the ingroup taxa), 1.35 times as many parsimony informative characters as the *trn*L intron for approximately the same length. The complete *ndh*F region has been used in several phylogenetic studies of grasses at the familial, subfamilial, tribal and generic levels (e.g., Clark et al. 1995; Catalán et al. 1997; Spangler et al. 1999; Giussani et al. 2001; Aliscioni et al. 2003). The more rapidly evolving 3' end of *ndh*F has been used at the genus level in grasses (e.g., Catalán and Olmstead 2000) and other

plants (e.g., Graham et al. 1998; Davis et al. 2002; Winkworth et al. 2002; Graham and Barrett 2004). The greater level of sequence variation detected in the 3' end of *ndh*F compared with the variation detected in the more commonly used *trn*L intron indicates that the former warrants consideration for use in the resolution of relationships at similar taxonomic levels in other groups.

2.4.2. Incongruence Between Nuclear Ribosomal and Plastid Data Partitions

Significant incongruence was detected between the nuclear ribosomal and plastid data partitions using the ILD test. The ILD test is commonly employed by systematists to examine congruence among data partitions, but there is growing evidence (e.g., Yoder et al. 2001; Barker and Lutzoni 2002) that the test can be misleading and should not be used to determine data combinability. Thus, I also visually compared trees derived independently from the plastid and nuclear data partitions for regions of incongruence, and found that each contained some moderately- to well-supported clades whose composition and position differed among trees. Because of this possible intergenomic conflict, I did not conduct analyses of the combined plastid and nuclear ribosomal data.

Incongruence among trees is not uncommon in phylogenetic studies that employ multiple gene regions, particularly when the data are from different genomes (e.g., Hardig et al. 2000; Les et al. 2002). Although often viewed as a hindrance to reliable phylogenetic estimation, incongruence can potentially provide insight into past evolutionary events, such as hybridization, introgression, and lineage sorting (Wendel and Doyle 1998). The current data suggest that some of these phenomena may have been involved in the evolutionary history of *Bromus*. However, it is difficult to infer the exact evolutionary processes that have

led to the differing gene trees, as reticulate patterns of evolution are difficult to study in a cladistic framework, and ideally requires gene trees inferred from more than two linkage groups. Nonetheless, previous studies have indicated that hybridization, alloploidy, and introgression may have been prominent in the evolution of many *Bromus* species and sections [reviewed by Stebbins (1981) and Armstrong (1991)]. The implications of the different gene histories detected here in understanding the evolutionary history of infrageneric groups in *Bromus* are discussed below. Clarification of the contribution of these processes to the evolutionary history of *Bromus* will require better-supported phylogenetic trees from multiple genetic linkage groups.

2.4.3. Phylogeny and Classification

In all analyses there is moderate to strong support for the monophyly of the genus *Bromus* s. l., based on current outgroup and ingroup taxon sampling. These findings agree with Ainouche and Bayer's (1997) study of sect. *Bromus*, and broader studies of grass phylogeny that have included several species of *Bromus* s. l. (e.g., Catalán et al. 1997; Hsiao et al. 1999), which all identified *Bromus* s. l. as a monophyletic taxon.

Sections *Bromus*, *Triniusia*, and *Genea* — The molecular evidence indicates that species of sect. *Triniusia* are nested within a clade that includes species of sect. *Bromus* (Figs. 2.1, 2.2). Section *Triniusia* was originally circumscribed as a monotypic section that included one species, *B. danthoniae*, characterized by three awns on each of the uppermost lemmas of the spikelets (Scholz 1998), but most authors have included this species in sect. *Bromus* (e.g., Smith 1970, 1972; Ainouche and Bayer 1997). A close relationship between *B. danthoniae* and *B. pseudodanthoniae* was not hypothesized until Scholz (1998) observed

that *B. pseudodanthoniae* sometimes has three awns on the uppermost lemmas of its spikelets, and that in portions of their ranges in the Middle East these two taxa intergrade. As a result, Scholz (1998) treated *B. pseudodanthoniae* as a subspecies of a polymorphic *B. danthoniae*, and re-circumscribed sect. *Triniusia* to include two morphologically similar species and several intraspecific taxa (*B. danthoniae* Trin. subsp. *danthoniae*, *B. danthoniae* subsp. *pseudodanthoniae* [Drobov] H. Scholz, *B. danthoniae* subsp. *rogersii* C. E. Hubb. ex H. Scholz, and *B. turcomanicus* H. Scholz). Scholz's (1998) recognition of sect. *Triniusia* is supported by isozyme data, which found *B. danthoniae* to be distinct from diploid members of sect. *Bromus* (Oja and Jaaska 1998), although serological evidence found *B. danthoniae* to be closely allied to species of sect. *Bromus*, including *B. pumilio* (classified currently in sect. *Boissiera* but often included in sect. *Bromus*), a species that also has multiple awns on the uppermost lemmas of its spikelets (Smith 1972). I did not sample *B. turcomanicus*, thus I was unable to fully test the monophyly of sect. *Triniusia* sensu Scholz (1998). However, my data confirm the close relationship hypothesized between *B. danthoniae* and *B. pseudodanthoniae*, and indicate that these species are nested phylogenetically within sect. *Bromus* (Fig. 2.1) or perhaps a somewhat broader clade (Fig. 2.2). These data are in accordance with the findings of Ainouche and Bayer (1997), who included *B. danthoniae* in their study of sect. *Bromus*. Recognition of sect. *Triniusia* renders sect. *Bromus* paraphyletic; it should therefore continue to be treated as a synonym of sect. *Bromus*, as past authors have done (e.g., Smith 1970, 1972; Ainouche and Bayer 1997). The distinct morphological characters that separate *B. danthoniae* and its close relatives from other species in sect. *Bromus* arose from within sect. *Bromus*.

The two sources of molecular evidence are in conflict in regards to the monophyly of sects. *Bromus* and *Genea*, due to the position of *B. pectinatus*. Sections *Bromus* (including sect. *Triniusia;* see above) and *Genea* (based on sampling only two of the approximately five species in the section) are each robustly supported as monophyletic in the nuclear ribosomal trees (Fig. 2.1). However, the plastid trees indicate that *B. pectinatus* (sect. *Bromus*) is the sister group of *B. diandrus* (sect. *Genea;* Fig. 2.2). Species of the *B. pectinatus* complex (only one species sampled here), a group of five tetraploid species that range from southern Africa to Tibet, classified in sect. *Bromus*, are morphologically similar to species of sect. *Genea*, with lemmas that taper toward the apex and paleas whose morphology is intermediate between the two sections (Smith 1972; Scholz 1981; Stebbins 1981; Sales 1993). A close relationship between *B. pectinatus* and sect. *Genea* is also supported by data from isozymes (Oja 2005) and embryo structure (Kosina 1996). Based on its morphological intermediacy, Stebbins (1956, 1981) suggested that the *B. pectinatus* complex (represented by *B. arenarius* in his studies) may be an intersectional amphidiploid that originated via a hybridization event between species of sects. *Genea* and *Bromus*. The conflicting positions of *B. pectinatus* in my plastid and nuclear ribosomal trees lend support to this hypothesis, indicating that the genome donors in the origin(s) of the complex were likely from sects. *Genea* and *Bromus*. Sampling of additional species of the *B. pectinatus* complex, and additional linkage groups, would be valuable, and may provide further insight into their origin(s). If *B. pectinatus* is a species of hybrid origin that arose after sects. *Bromus* and *Genea* initially diversified and it is excluded from consideration, then sects. *Bromus* and *Genea* are monophyletic. The morphological characteristics outlined in Table 2.2, widely employed in taxonomic keys to separate these two lineages (e.g., Pavlick 1995), constitute possible synapomorphies for these

clades; however, validation of these hypotheses will require rigorous reconstructions of character evolution on robustly supported and fully resolved gene trees.

The plastid and nuclear ribosomal data sets infer different relationships between sects. *Bromus* and *Genea*. The nuclear ribosomal data do not infer a close relationship between sects. *Bromus* and *Genea* (Fig. 2.1), while the plastid data (Fig. 2.2) strongly support a clade containing all taxa from both sections. The placement of species from sects. *Genea* and *Bromus* together in a clade in the plastid trees corrobates the study of Pillay and Hilu (1995), although they did not detect sufficient chloroplast DNA variation to distinguish sects. *Bromus* and *Genea* as distinct monophyletic groups. Pillay and Hilu (1995) suggested that the similarity in chloroplast genomes among sects. *Genea* and *Bromus* may be the result of chloroplast transfer by hybridization and phylogenetic sorting. A close relationship between sects. *Genea* and *Bromus* is further supported by data from floral microstructural variation (Kosina 1999), and by their life histories. Both include only annual species (most other sections of *Bromus* comprise mostly perennial species), and both include many weedy species (Stebbins 1981). Stebbins (1981) also hypothesized a close relationship between sects. *Genea* and *Bromus*, and suggested that their origins probably involved different species of sect. *Bromopsis* as genome donors. My nuclear ribosomal data are potentially consistent with this hypothesis, as sect. *Bromus* is nested within a clade that includes species of sect. *Bromopsis* from North America and *B. ramosus* from the Old World, while sect. *Genea* is closely related to species of sect. *Bromopsis* from the Old World (excluding *B. ramosus*). These species groups of sect. *Bromopsis*, respectively, are potential candidates for genome donors in the origins of sects. *Bromus* and *Genea*.

Within sect. *Genea*, my data indicate a fairly substantial amount of genetic variability among individuals of *B. madritensis* subsp. *rubens*, in line with the results of a previous isozyme study (Kahler et al. 1981). The high genetic variation observed here seems to correspond with morphological variation that was high enough to result in the gross misidentification of one seed bank accession (see Table 2.3). The genetic variation observed in *B. madritensis* subsp. *rubens* raises the possibility that similar high levels of variation may be present in at least some other *Bromus* species.

Section *Bromopsis* — Section *Bromopsis*, the largest section currently recognized in *Bromus*, comprises several independent lineages and is not monophyletic in any of my analyses. These results are congruent with Pillay and Hilu (1995), who found members of sect. *Bromopsis* to occur in three distinct lineages (based on the plastid genome but with less taxon sampling). Based on my nuclear ribosomal data, *B. attenuatus* and *B. dolichocarpus*, two North American species of sect. *Bromopsis* native to northeastern Mexico and southern Mexico and Guatemala, respectively (Wagnon 1952; Soderstrom and Beaman 1968), are the sister group of the rest of *Bromus*. Four South American species of sect. *Bromopsis* (*B. lanatus*, *B. modestus*, *B. pellitus*, *B. pflanzii*) comprise a well-supported clade that is resolved as part of the second-deepest split in the genus. The plastid data alone do not support these phylogenetic placements, possibly because of insufficient variation; however, the plastid trees do indicate that these species are not part of the clade that includes other New and Old World species of sect. *Bromopsis* (Fig. 2.2), and they do not strongly rule out the relationships seen for the nuclear ribosomal data. Although they define deep splits on the molecular trees, the morphological characteristics of these species are not sufficiently distinct compared with other New World species of sect. *Bromopsis* for previous workers to have

considered them as major evolutionary lineages. However, Wagnon (1952) suspected that *B. attenuatus* and *B. dolichocarpus* are closely related to each other, and that they are distantly related to other North American species of sect. *Bromopsis*. The molecular data agree with this hypothesis. Further study is necessary to identify possible morphological synapomorphies for a *B. attenuatus/ B. dolichocarpus* clade as well as a putative clade of South American species that may be part of the second deepest split in *Bromus*. The deep phylogenetic positions of these two clades in the nuclear ribosomal trees suggest that the crown clade of *Bromus* originated in the New World. In contrast, Stebbins (1981) suggested that *Bromus* originated in Eurasia, with sects. *Neobromus*, *Ceratochloa*, and *Bromopsis* being the first to differentiate and subsequently spread to North and South America, followed by the evolution of sects. *Bromus*, *Genea*, and *Boissiera*.

The molecular evidence suggests that the remaining South American species of sect. *Bromopsis* sampled, *B. brachyanthera* (a hexaploid; Schifino and Winge 1983), is closely related to Old World species of sect. *Bromopsis* and sects. *Ceratochloa* and *Neobromus*. In the plastid trees, *B. brachyanthera* is the sister group of a clade corresponding to sect. *Ceratochloa* (Fig. 2.2), whereas in the nuclear ribosomal trees *B. brachyanthera* is the sister group of a clade comprised of sects. *Neobromus* and *Ceratochloa* (Fig. 2.1). Despite the close molecular relationship, *Bromus brachyanthera* is morphologically distinct from species in sect. *Ceratochloa* and *Neobromus*, having dorsiventrally flattened spikelets typical of other species in sect. *Bromopsis*, and straight awns. Stebbins (1981) suggested that some members of sect. *Bromopsis* may have donated genomes during the origin of sect. *Ceratochloa*. In line with this hypothesis, the phylogenetic affinities of *B. brachyanthera* and the Old World species of sect. *Bromopsis* with sect. *Ceratochloa* suggest that they, their

close relatives, or their immediate ancestors, are among the most likely candidates as possible genome donors. Inclusion of the six unsampled native species of sect. *Bromopsis* from South America (Planchuelo and Peterson 2000) in future studies should provide further insight into the evolution and relationships of this group of poorly understood species.

Armstrong (1983) hypothesized that the North American and Old World members of sect. *Bromopsis* may represent distinct evolutionary lineages. Species from North America are generally diploids (a few are tetraploids), and all have large chromosomes with pinhead satellites, whereas Old World species are generally polyploids with smaller chromosomes lacking pinhead satellites (Armstrong 1983). Exceptions are *B. pumpellianus*, which is native in North America and Eurasia and morphologically and cytologically similar to Old World taxa, and the *B. ramosus* complex of the Old World (represented here by *B. ramosus*), which is morphologically and cytologically similar to North American species of sect. *Bromopsis* (Armstrong 1983). Differences in floral microstructural variation further support the distinctiveness of these morphologically and cytologically differentiated groups (Kosina 1999). My nuclear ribosomal data may partly support these hypotheses, as species of sect. *Bromopsis* from North America (excluding *B. attenuatus* and *B. dolichocarpus*) and *B. ramosus* from the Old World form a clade that does not include the other Old World species (Fig. 2.1). Old World species of sect. *Bromopsis* (including *B. pumpellianus*) comprise several independent but closely related lineages that are part of a weakly-supported clade that also includes *B. brachyanthera* (sect. *Bromopsis*) and sects. *Ceratochloa* and *Neobromus* (Fig. 2.1). These relationships are consistent with the findings of Kosina (1996), who observed similarity in the embryo structure of species of sects. *Ceratochloa* and Old World species of sect. *Bromopsis*. In contrast, the plastid data include Old World species of sect.

Bromopsis in a large, weakly-supported clade with many North American species of sect. *Bromopsis* (Fig. 2.2). The gene trees thus indicate that most Old World and North American lineages of sect. *Bromopsis* share a similar plastid genome, but have conflicting nuclear ribosomal histories. The Old World species (most of which are polyploids) may have originated via a hybridization event, with a diploid member of sect. *Bromopsis* contributing the plastid genome. Additional representatives of sect. *Bromopsis* as traditionally circumscribed from the Old World, and improved genomic samplings, will be required to provide further insight into the evolution and relationships of these species. If it becomes desirable to formally recognize these Old World lineages, the sectional name *Pnigma* Dumort. is available for the clade that contains *B. inermis* Leyss. (Armstrong 1983).

Within the clade of North American species of sect. *Bromopsis* and *B. ramosus*, several weakly- to well-supported clades of two to five species are evident (Figs. 1, 2). Wagnon (1952) suggested several groupings of North American species of sect. *Bromopsis*, based on geographical distribution: (1) an Arctic group, (2) a Rocky Mountain Mexican Highland group, (3) a Pacific Slope group, and (4) an East-Midwest group. My trees are largely congruent with the East-Midwest group that Wagnon (1952) defined to include *B. ciliatus*, *B. kalmii*, *B. nottowayanus, B. pubescens, B. purgans* (here treated as *B. latiglumis*), and *B. texensis*. All of these species, except *B. texensis*, comprise a moderately-supported clade in the nuclear ribosomal trees; there is insufficient variation in the plastid data alone to support or reject such close species relationships. Wagnon (1952) noted that the placement of *B. texensis* might seem out of place in this group, since its geographic range is intermediate between other members of the East-Midwest group and members of the Rocky Mountain Mexican Highland group, but he included it because the morphology of its ligule is

similar to other members of the group. My data neither support nor reject Wagnon's (1952) other groups, as the phylogenetic relationships of many of these species are unresolved. The short branch lengths and lack of resolution among many of the North American species of sect. *Bromopsis* indicate that the species in this group likely diversified during a recent rapid radiation.

There has been much confusion about the species status of *B. richardsonii* and *B. ciliatus* (North American species of sect. *Bromopsis*). *Bromus richardsonii* is often treated as a synonym of *B. ciliatus* (e.g., Hitchcock and Chase 1951; Soderstrom and Beaman 1958; Allred 1993), although recent taxonomic study has indicated that these taxa are sufficiently distinct morphologically, cytologically, and genetically to warrant specific recognition (Peterson et al. 2002). My nuclear ribosomal data confirm that *B. richardsonii* is a distinct species, closely related to *B. mucroglumis* (although the species status of *B. mucroglumis* is also controversial; Wagnon 1952, Peterson et al. 2002); these two taxa do not share an immediate common ancestor with *B. ciliatus* (Figs. 2.1, 2.2).

It is clear from both plastid and nuclear ribosomal data that sect. *Bromopsis* is an artificial assemblage of species. The morphological characteristics traditionally used to circumscribe the section (Table 2.2) may therefore be a mixture of characters that are homoplasious or that represent symplesiomorphies of larger clades. The recognition of *Bromopsis* as a distinct section, subgenus, or genus (Table 2.1) is clearly not appropriate.

Sections *Ceratochloa* and *Neobromus* — Section *Ceratochloa* is weakly supported as monophyletic in the plastid trees (Fig. 2.2), and robustly supported as monophyletic in the nuclear ribosomal trees (Fig. 2.1). None of the sequence data is sufficiently variable to resolve relationships among species in the section, and several species are genetically

identical at the loci examined. Similarly, Pillay and Hilu (1990, 1995) found no chloroplast restriction-site variation among species of sect. *Ceratochloa*.

The plastid and nuclear ribosomal data are in conflict regarding the monophly of sect. *Neobromus*. In the plastid trees (Fig. 2.2), the two species of sect. *Neobromus* comprise a grade, in which *B. berteroanus* is the sister group of a clade comprising *B. gunckelli*, sect. *Ceratochloa* and *B. brachyanthera* (sect. *Bromopsis*). However, sect. *Neobromus* is a well-supported monophyletic group in the nuclear ribosomal trees (Fig. 2.1), a relationship not strongly rejected by the plastid data, and clearly both taxa are closely related. Both species are morphologically similar, sharing strongly twisted and divaricate awns (Table 2.2), hence their classification as a section. *Bromus berteroanus* (syn = *B. trinii* Desvaux) is morphologically similar to other grass genera because of its large glumes and a lemma that is deeply bilobed apically (Stebbins 1981), and in the past the species has been confused as a species of the genus *Trisetum* Pers. (Louis-Marie 1928), although this classification has not been followed by recent authors. My data confirm that *B. berteroanus* is a species of *Bromus*.

The weakly supported relationship between sects. *Ceratochloa* and *Neobromus* (Figs. 2.1, 2.2) agrees with previous hypotheses that these taxa share common ancestry. Stebbins (1981) reported a weak affinity between one genome of sects. *Neobromus* and *Ceratochloa*, and Pillay and Hilu (1995) found that these taxa shared eight synapomorphies based on chloroplast DNA restriction site variation. Unfortunately, neither of these studies included representatives of South American species of sect. *Bromopsis*, one of which appears here to be closely related to sects. *Ceratochloa* and *Neobromus* (Figs. 2.1, 2.2). Stebbins (1981) hypothesized that sects. *Neobromus* and *Ceratochloa* evolved early within *Bromus* because

of their small chromosome size and spikelets that resemble those in genera that he thought were derived from the ancestral complex from which *Bromus* originated (including *Littledalea, Megalachne, Metcalfia*, and *Pseudodanthonia*). The plastid data neither reject or support this hypothesis (Fig. 2.2), but the nuclear ribosomal data indicate that *B. attenuatus* and *B. dolichocarpus* (sect. *Bromopsis*), species that are morphologically distinct from taxa in sects. *Ceratochloa* and *Neobromus*, are part of a deep lineage that diverged early in the history of the genus (see above; Fig. 2.1). Sections *Ceratochloa* and *Neobromus* are nested deep within the nuclear ribosomal trees. Current knowledge, favouring distant phylogenetic positions of the morphologically similar genera thought previously to be closely related to sects. *Ceratochloa* and *Neobromus* (e.g., Soreng et al. 2003), further discounts Stebbins' (1981) hypotheses.

Sections *Boissiera* and *Nevskiella* — Material of *B. pumilio* (sect. *Boissiera*) was not available, and available material of *B. gracillimus* (sect. *Nevskiella*) was recalcitrant to molecular study, thus the phylogenetic positions of these taxa remain uncertain. *Bromus pumilio* was originally classified in its own genus, *Boissiera*, but was transferred to *Bromus* based on serological and morphological similarities to other *Bromus* species (Smith 1969). It has since been treated either in sect. *Bromus* (e.g., Smith 1970) or within its own section, *Boissiera* (Smith 1985a; Table 2.1), because of its unique morphology, having five to nine awns on each lemma (Table 2.2). Based on allozyme evidence, Oja and Jaaska (1998) found *B. pumilio* to be distinct from members of sect. *Bromus*, supporting its placement in its own section. The phylogenetic position of *B. gracillimus* (sect. *Nevskiella*), characterized by awns that are four to six times the length of the lemma (Table 2.2), remains unknown. It would be valuable to include both species in future molecular studies.

2.4.4. Conclusions and Future Directions

My study provides genus-wide phylogenetic hypotheses of relationships in *Bromus* s. l., based on DNA sequence data from the plastid genome and the nuclear ribosomal internal transcribed spacer regions, and provides a foundation for further phylogenetic study of the genus. Based on the nuclear ribosomal data, sects. *Bromus* (including sect. *Triniusia*), *Genea*, *Neobromus*, and *Ceratochloa* are monophyletic, and sect. *Bromopsis* comprises several distinct lineages. Plastid trees indicate that sects. *Genea* and *Bromus* are closely related, and the incongruence detected between the plastid and nuclear ribosomal data support a hybrid origin for the *B. pectinatus* complex (here represented by a single exemplar) between sects. *Bromus* and *Genea*. Plastid trees indicate a close relationship between Old World and some North American species of sect. *Bromopsis*, and the plastid and nuclear ribosomal data indicate that one South American species of sect. *Bromopsis* is not closely related to North American and Eurasian species traditionally classified in the same section. Most species of *Bromus* sampled had levels of sequence variation too low to allow complete resolution of relationships among close relatives at the species level (e.g., among North American members of sect. *Bromopsis* and within sect. *Ceratochloa*). Sequence data from additional nuclear loci, such as the granule-bound starch synthase gene (*waxy*; e.g., Mason-Gamer 2001), AFLPs (e.g., Beardsley et al. 2003; Després et al. 2003), or microsatellites (e.g., Alvarez et al. 2001) may provide further insight into species-level relationships in *Bromus*. Adding data from the plastid genome (e.g., Shaw et al. 2005) and the nuclear ribosomal region [the external transcribed spacer (ETS) of nuclear rDNA (e.g., Baldwin and Markos 1998; Markos and Baldwin 2002; Starr et al. 2003)] would also be valuable to improve resolution and support of trees inferred from these two linkage groups.

Recognition of the brome grasses as one distinct genus, *Bromus*, is in agreement with the molecular data, but current classification schemes do not satisfactorily reflect phylogenetic relationships within the genus, particularly with respect to the circumscription of sect. *Bromopsis*. However, before a revised infrageneric classification of *Bromus* is proposed, I advocate that substantially better sampling should be conducted of (1) DNA sequence regions, to obtain better support for phylogenetic relationships among taxa, and to further clarify incongruence among nuclear and plastid data partitions; and (2) taxa, to more adequately sample the molecular, morphological, and geographical variability in the genus. Although this is the largest study of *Bromus* phylogeny conducted thus far, all conclusions are based on a sample of less than one-third of the recognized species, mostly with one individual per taxon, and it is plausible that addition of other species will further contribute to and change understanding of evolution and phylogeny in this genus.

Table 2.1. Summary of infrageneric classificatons and generic segregations of *Bromus* following Smith (1970), Tsvelev (1976), and Stebbins (1981). Equivalent circumscriptions are aligned horizontally. Indented names were treated by the author as synonyms of the taxon above.

Sections (Smith 1970)	Subgenera (Stebbins 1981)	Genera (Tsvelev 1976)
Bromopsis Dumort. (as sect. *Pnigma* Dumort.)	*Festucaria* Gren. & Godr.	*Bromopsis* (Dumort) Fourr.
Bromus	*Bromus*	*Bromus* L.
Triniusia (Steud.) Nevski[a]	*Triniusia* (Steud.) Pénzes.	*Triniusia* Steud.
Boissiera (Hochst. ex Steud.) P. M. Smith[b]	*Boissiera* nom. inval..	*Boissiera* Hochst. ex Steud.
Ceratochloa (P. Beauv.) Griseb.	*Ceratochloa* (P. Beauv.) Hack.	*Ceratochloa* P. Beauv.
Genea Dumort.	*Stenobromus* Hack.	*Anisantha* C. Koch
Nevskiella (Krecz. & Vved.) Tournay	*Nevskiella* (Krecz. & Vved.) Krecz. & Vved.	*Nevskiella* Krecz. & Vved.
Neobromus (Shear) Hitchc.	*Neobromus* Shear	*Trisetobromus* Nevski

[a] Given sectional status by Scholz (1998).

[b] Given sectional status by Smith (1985a).

Table 2.2. Number of species, morphological characteristics, and native geographic distribution of sections in *Bromus* (after Smith 1970). The classification follows Smith (1970, 1985a) and Scholz (1998).

Section	No. Species	1st Glume Nerves	2nd Glume Nerves	Spikelet Shape	Lemmas	Native Geographic Distribution
Boissiera	1	3	5-9	Linear-lanceolate to oblong; terete	Oblong; awns five-nine	Asia, E Mediterranean
Bromopsis	~60	1(3)	3(5)	Narrow, lanceolate; terete	Rounded or slightly keeled; awn single, usually shorter than length of lemma, rarely absent	Eurasia, Africa, N and S America
Bromus	30--40	3-5	5-9	Ovate to ovate-lanceolate; terete to slightly compressed	Rounded; awn single, equalling or slightly exceeding length of lemma, rarely absent	Europe, Asia
Ceratochloa	10--16	3-5	5-7	Ovate or ovate-lanceolate; strongly compressed	Strongly keeled; awn single, short, often absent	N and S America
Genea	6	1	3	Cuneate, wider at top	Narrow and elongate; awns single, less than three times length of lemma	Mediterranean, SW Asia, N Europe

Table 2.2 continued

Section	No. Species	1st Glume Nerves	2nd Glume Nerves	Spikelet Shape	Lemmas	Native Geographic Distribution
Neobromus	2	1	3-5	Narrowly elliptic	Deep apical sinus and two long, narrow teeth; awn single, longer than length of lemma, geniculate	Pacific coast of N and S America
Nevskiella	1	1	3	Ovate-lanceolate to cuneiform, wider above; terete to slightly compressed	Rounded; awn single, 4-6 times length of lemma	Central Asia, Iran, Afghanistan
Triniusia	2	3-5	5-9	Ovate to lanceolate; compressed	Rounded; upper lemma with three awns; irregular apical notches	E Mediterranean, SW Asia

Table 2.3. Sections [following Smith (1970) and Scholz (1998)] and species sampled, sources of material, vouchers, and GenBank accession numbers for DNA sequences. WRPIS = Western Regional Plant Introduction Station (United States Department of Agriculture, Pullman, Washington, USA); PGRC = Plant Gene Resources of Canada. All numbers preceded by PI or CN are seed accession numbers. Missing GenBank accession numbers indicate that no sequence was obtained. Sequence data for two outgroup taxa (*Hordeum vulgare L.* L. and *Triticum aestivum* L.) were obtained from GenBank (accessions Z1159, X757505, U2003, AF521903, X75709, and NC_0027). Accessions of*B. madritensis subsp. rubens* are numbered 1--3.

Taxon	Geographic origin/ source	Voucher	GenBank accession number		
			ITS	*trn*L intron	*ndh*F
Section *Bromopsis*					
B. anomalus Rupr. ex Fourn. (acc. 1)	USA; PI 232199 (WRPIS)	*Keane 49* (ALTA)	AY367905	AY367955	AY368004
B. anomalus Rupr. ex Fourn. (acc. 2)	Mexico: Tamaulipas	*Peterson 15918 & Valdes-Reyna* (US)	AY367906	AY367956	AY368005
B. attenuatus Swallen	Mexico: Tamaulipas/ Nuevo León border	*Peterson 15926 & Valdes-Reyna* (US)	AY367910	AY367960	AY368009
B. brachyanthera Döll.	Bolivia: La Paz	*de Ros 9497* (US)	AY367908	AY367958	AY368007
B. ciliatus L.	Canada: Quebec	*Cayouette C8272 & Lavoie* (DAO)	AY367909	AY367959	AY368008
B. dolichocarpus Wagnon	Mexico: Michoacán	*Peterson 16128* (US)	AY367911	AY367961	
B. erectus Huds.	Turkey; PI 337652 (WRPIS), received as *B. benekenii* (Lange) Trimen	*Keane 8* (ALTA)	AY367907	AY367957	AY368006
B. exaltatus Bernh.	Mexico: Jalisco	*Peterson 16087 & Rosales* (US)	AY367912	AY367962	AY368010
B. frondosus (Shear) Woot. et Standl.	Mexico: Durango	*Peterson 15418 et al.* (US)	AY367913	AY367963	AY368011

Table 2.3 continued

Taxon	Geographic origin/ source	Voucher	ITS	*trn*L intron	*ndh*F
B. ramosus Huds.	UK	*Keane 101* (ALTA)	AY367929	AY367978	AY368023
B. richardsonii Link	USA: Arizona	*Peterson 15282 & Cayouette* (US)	AY367930	AY367979	AY368024
B. riparius Rehmann	Czech Republic/ Slovakia; PI 598590 (WRPIS)	*Keane 26* (ALTA)	AY367931	AY367980	AY368025
B. suksdorfii Vasey	USA: Washington	*Soreng 6352 & Soreng* (US)	AY367934	AY367983	AY368028
B. texensis (Shear) A. S. Hitchc.	USA: Texas	*Cayouette 668135* (DAO)	AY367935	AY367984	AY368029
Section *Bromus*					
B. japonicus Thunb.	Russia; PI 283198 CPI 24193 (WRPIS), received as *B. popovii* Drob.	*Keane 24* (ALTA)	AY367940	AY367989	AY368034
B. pectinatus Thunb.	Belgium; PI 442453 (WRPIS)	*Keane 23* (ALTA)	AY367939	AY367988	AY368033
B. scoparius L.	Turkey; PI 204425 (WRPIS)	*Keane 28* (ALTA)	AY367932	AY367981	AY368026
Section *Ceratochloa*					
B. carinatus Hook. et Arn.	Mexico: Durango	*Peterson 15421 et al.* (US)	AY367948	AY367997	AY368042
B. catharticus Vahl	Argentina; PI 578719 RGI 441 (WRPIS), received as *B. araucanus* Phil.	*Keane 5* (ALTA)	AY367954	AY368003	AY368048
B. cebadilla Steud.	Chile; PI 202696 (WRPIS), received as *B. coloratus* Steud.	*Keane 13* (ALTA)	AY367944	AY367993	AY368038
B. coloratus Steud.	Chile: Region I	*Peterson 15746 & Soreng* (US)	AY367943	AY367992	AY368037

Table 2.3 continued

Taxon	Geographic origin/ source	Voucher	ITS	*trn*L intron	*ndh*F
B. grandis (Shear) A. S. Hitchc.	USA: California	*Cayouette 7947a* (DAO)	AY367914	AY367964	AY368012
B. inermis Leyss.	USA: Arizona[a]	*Peterson 15295 & Cayouette* (US)	AY367915	AY367965	AY368013
B. kalmii A. Gray	Canada: Ontario; CN 51222 C7099 (PGRC)	*Keane 55* (ALTA)	AY367916	AY367966	AY368014
B. korotkoyi Drob.	China: Inner Mongolia	*Soreng 5160 et al.* (US)	AY367998	AY367988	AY368043
B. laevipes Shear	USA: California	*Peterson 14840 et al.* (US)	AY367917	AY367967	AY368015
B. lanatipes (Shear) Rydb.	USA: Arizona	*Peterson 15270 & Cayouette* (US)	AY367918	AY367968	
B. lanatus Kunth	Chile: Region I	*Peterson 15747 & Soreng* (US)	AY367919	AY367969	AY368016
B. latiglumis (Shear) A. S. Hitchc.	Canada: Ontario	*Cayouette 4336-1* (DAO)	AY367920	AY367970	AY368017
B. modestus Renvoize	Bolivia: La Paz	*Peterson 12639 et al.* (US)	AY367921		
B. mucroglumis Wagnon	USA: Arizona	*Peterson 15273 & Cayouette* (US)	AY367922	AY367972	AY368019
B. nottowayanus Fern.	USA: Illinois	*Chase 13512* (US)	AY367923		
B. pellitus Hack.	Argentina: Santa Cruz	*Peterson 17267 et al.* (US)	AY367951	AY368000	AY368045
B. pflanzii Pilg.	Bolivia: La Paz	*Luteyn & Dorr 13828* (US)	AY367924	AY367973	
B. porteri (Coult.) Nash	USA: Arizona	*Peterson 15245 & Cayouette* (US)	AY367925	AY367974	AY368020
B. pseudolaevipes Wagnon	USA: California	*Cayouette C7987* (DAO)	AY367926	AY367975	AY368021
B. pubescens Muhl. ex Willd.	USA: Virginia	*Peterson 15776 & Saarela* (US)	AY367927	AY367976	
B. pumpellianus Scribn.	Mongolia; PI 610833 (WRPIS)	*Keane 17* (ALTA)	AY367928	AY367977	AY368022

Table 2.3 continued

Taxon	Geographic origin/ source	Voucher	ITS	*trn*L intron	*ndh*F
B. marginatus Nees ex Steud.	USA: Oregon	*Soreng 6360 & Soreng* (US)	AY367921	AY367971	AY368018
B. striatus Hitchc.	France; PI 477988 974 (WRPIS)	*Keane 6* (ALTA)	AY367945	AY367994	AY368039
B. subvelutinus Shear.	Uzbekistan; PI 392355 (WRPIS)	*Keane 35* (ALTA)	AY367953	AY368002	AY368047
Section *Genea*					
B. diandrus Roth.	Germany; CN 31600 PGR 2848 (PGRC)	*Keane 25* (ALTA)	AY367936	AY367985	AY368030
B. madritensis L.	Iraq; PI 253735 (WRPIS),	*Keane 16* (ALTA)	AY367937	AY367986	AY368031
subsp. *rubens* (L.) Husnot (acc. 3)	received as *B. fasciculatus* C. Presl.				
B. madritensis L.	Iran; PI 239722 (WRPIS),	*Keane 20* (ALTA)	AY367938	AY367986	AY368032
subsp. *rubens* (L.) Husnot (acc. 2)	received as *B. madritensis* L.				
B. madritensis L.	Australia: Western Australia	*Peterson 14534 et al.* (US)	AY367950	AY367987	AY368044
subsp. *rubens* (L.) Husnot (acc. 1)					
Section *Neobromus*					
B. gunckelli Matthei	Chile: Region I	*Peterson 15697 & Soreng* (US)	AY367947	AY367996	AY368041
B. berteroanus Colla	Chile; PI 224789 (WRPIS)	*Keane 37* (ALTA)	AY367946	AY367994	AY368040
Section *Triniusia*					
B. danthoniae Trin.	Turkey; PI 598455 TU85-028-01 (WRPIS)	*Keane 15* (ALTA)	AY367941	AY367990	AY368035
B. pseudodanthoniae Drobov	Turkey; PI 204424 (WRPIS)	*Keane 21* (ALTA)	AY367942	AY367991	AY368036

Table 2.3 continued

Taxon	Geographic origin/ source	Voucher	ITS	*trn*L intron	*ndh*F
Outgroup					
Festuca breviglumis Swallen	Mexico: Durango	*Peterson et al. 16924* (US)	AY367952	AY368001	AY368046

[a] Collected outside native range.

Figure 2.1. Phylogram of one of 449 most-parsimonious (MP) trees found using data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. MP trees are each 380 steps, with a CI of 0.713 and RI of 0.826. Bootstrap support values greater than 50% are indicated. Nodes that collapse in the strict consensus tree are indicated with an arrow. Sections in *Bromus* [following Smith (1970) and Scholz (1998)] are indicated to the right of the tree. Native geographic distributions of species in sect. *Bromopsis* are indicated: light-shading = New World, dark shading = Old World, boxed species (*B. pumpellianus*) = New and Old World.

Fig. 2.1

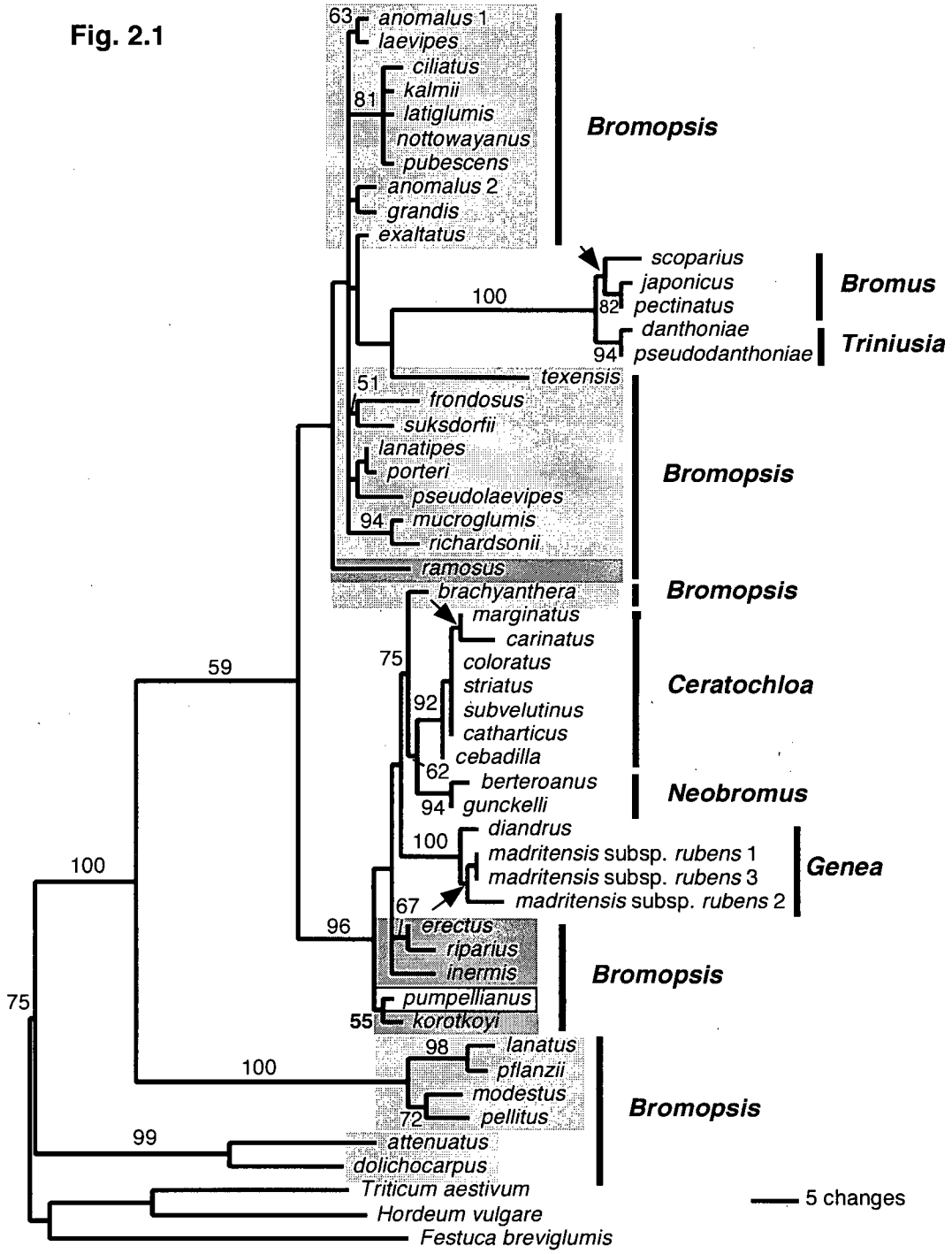
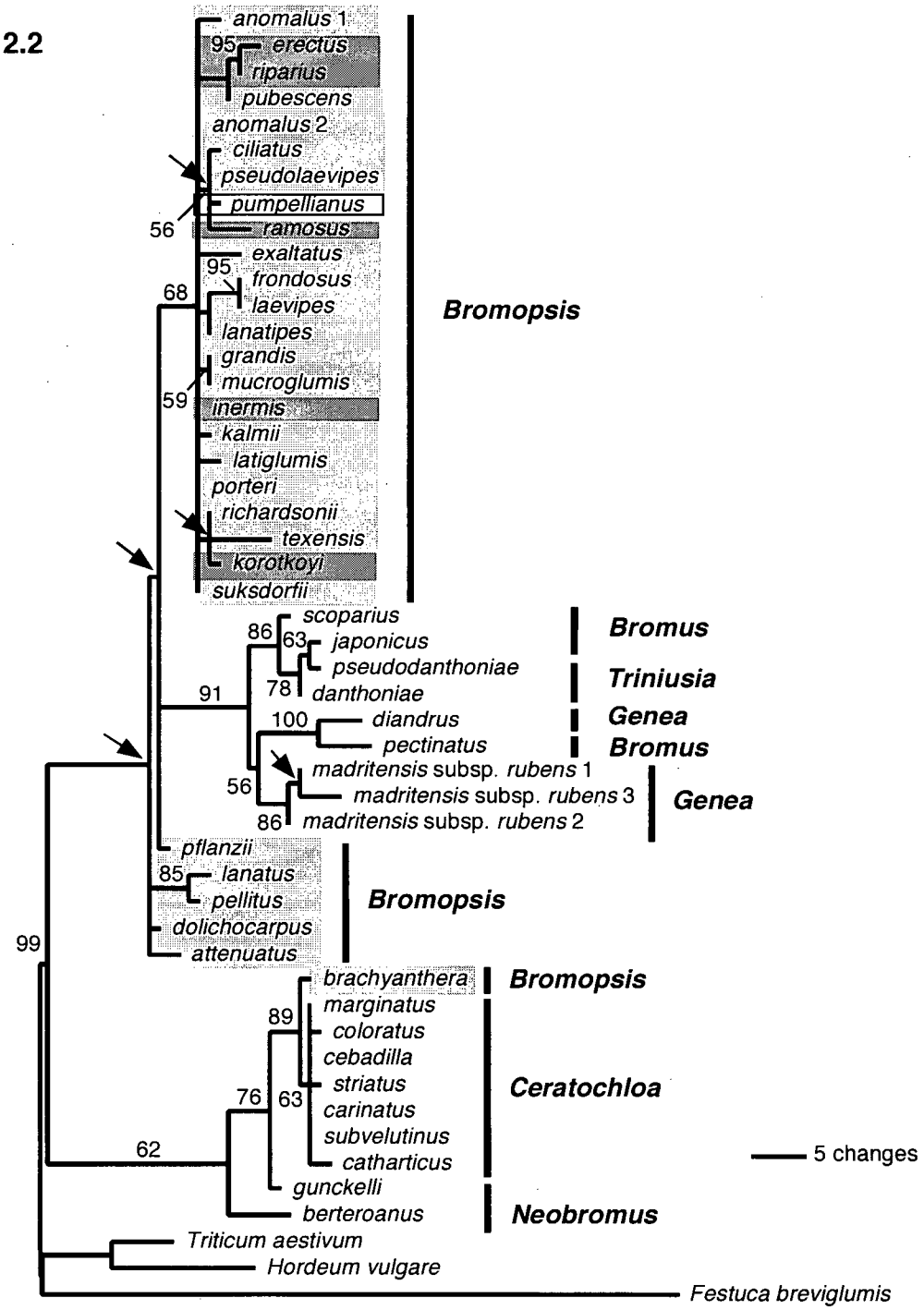

anomalus 1
laevipes
ciliatus
kalmii
latiglumis
nottowayanus
pubescens
anomalus 2
grandis
exaltatus
Bromopsis
scoparius
japonicus
pectinatus
Bromus
danthoniae
pseudodanthoniae
Triniusia
texensis
frondosus
suksdorfii
lanatipes
porteri
pseudolaevipes
mucroglumis
richardsonii
Bromopsis
ramosus
brachyanthera
marginatus
carinatus
coloratus
striatus
subvelutinus
catharticus
cebadilla
Ceratochloa
berteroanus
gunckelli
Neobromus
diandrus
madritensis subsp. rubens 1
madritensis subsp. rubens 3
madritensis subsp. rubens 2
Genea
erectus
riparius
inermis
pumpellianus
korotkoyi
Bromopsis
lanatus
pflanzii
modestus
pellitus
Bromopsis
attenuatus
dolichocarpus
Triticum aestivum
Hordeum vulgare
Festuca breviglumis
5 changes

Figure 2.2. Phylogram of one of 50,000 most-parsimonious (MP) trees found using combined plastid data from the *trn*L intron and the 3' end of *ndh*F. MP trees are each 218 steps, with a CI of 0.817 and RI of 0.882. Bootstrap support values greater than 50% are indicated. Nodes that collapse in the strict consensus tree are indicated with an arrow. Sections in *Bromus* [following Smith (1970) and Scholz (1998)] are indicated to the right of the tree. Native geographic distributions of species in sect. *Bromopsis* are indicated: light shading = New World, dark shading = Old World, boxed species (*B. pumpellianus*) = New and Old World.

Fig. 2.2


anomalus 1
95
erectus
riparius
pubescens
anomalus 2
ciliatus
pseudolaevipes
pumpellianus
56
ramosus
exaltatus
95
frondosus
68
laevipes
lanatipes
grandis
mucroglumis
59
inermis
kalmii
latiglumis
porteri
richardsonii
texensis
korotkoyi
suksdorfii
Bromopsis
scoparius
86
63
japonicus
pseudodanthoniae
78
danthoniae
Bromus
Triniusia
91
100
diandrus
pectinatus
Genea
Bromus
madritensis subsp. rubens 1
56
madritensis subsp. rubens 3
86
madritensis subsp. rubens 2
Genea
pflanzii
85
lanatus
pellitus
dolichocarpus
attenuatus
Bromopsis
99
brachyanthera
Bromopsis
marginatus
89
coloratus
cebadilla
striatus
76
63
carinatus
subvelutinus
catharticus
Ceratochloa
62
5 changes
gunckelli
berteroanus
Neobromus
Triticum aestivum
Hordeum vulgare
Festuca breviglumis

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CHAPTER 3[1]

Phylogenetic Relationships Among the Subfamilies of Grasses (Poaceae) and Related Families Inferred from a Large, Multigene Plastid Data Set

3.1. INTRODUCTION

3.1.1. The Grass Family (Poaceae)

The grasses (Poaceae) are the fourth largest family of angiosperms, with some 11,000 species classified in 600 to 900 genera (Campbell 1985; Clayton and Renvoize 1986; Tzvelev 1989). Grasses provide many of the world's major crops, and they are important components of most global ecosystems. As a result, the family has received substantial systematic study. Over the last two centuries, numerous classifications of Poaceae have been proposed [e.g., Brown 1810; Hitchcock and Chase 1951; Beetle 1955; Stebbins 1957; Hilu and Wright 1982; Clayton and Renvoize 1986; Tsvelev 1989; Watson and Dallwitz 1992; see Grass Phylogeny Working Group (GPWG; 2001) for a comprehensive review], based principally on data from morphology (e.g., Brown 1810), anatomy (e.g., Reeder 1957; Brown 1958; Tateoka et al. 1959), and cytology (e.g., Avdulov 1931; Brown and Emery 1957). Recent phylogenetic studies of morphological (e.g., Kellogg and Campbell 1987; Kellogg

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and Watson 1993) and molecular data (e.g., Barker et al. 1995; Columbus et al. 2000; Soreng and Davis 2000; Giussani et al. 2001; Ge et al. 2002; Mathews et al. 2002; Aliscioni et al. 2003; Chapter 2; see additional refs. below) have clarified taxonomic circumscriptions and hypotheses of evolutionary relationships throughout the family.

Several family-level phylogenetic investigations of Poaceae have been conducted with plastid and nuclear data. Plastid studies have been based on restriction-site variation (Davis and Soreng 1993; Soreng and Davis 1998, 2000) and DNA sequence data from single protein-coding genes (Duvall and Morton 1996; Cummings et al. 1994; Nadot et al. 1994; Clark et al. 1995, Catalán et al. 1997; Liang and Hilu 1996; Hilu and Alice 1999; Hilu et al. 1999), a noncoding region (Zhang 2000), and complete plastid genomes (Matsuoka et al. 2002). Nuclear studies have been conducted with various coding and noncoding regions (Hamby and Zimmer 1988; Hsiao et al. 1994, 1999; Mason-Gamer et al. 1998; Mathews and Sharrock 1996; Mathews et al. 2000). These, as well as additional studies with narrower taxonomic foci (e.g., Catalán et al. 1997; Barker et al. 2000; Gómez-Martinez and Culham 2000; Soreng and Davis 2000; Zhang and Clark 2000; Hilu and Alice 2000, 2001; Ge et al. 2002), have identified several major lineages in Poaceae with moderate to robust support. As a result, the broad phylogenetic structure of Poaceae is fairly well established. The GPWG (2000, 2001) based a new subfamilial classification on a fairly robust phylogeny generated using combined data from seven molecular datasets and morphology, although a few taxa (*Eriachne* R. Br., *Gynerium* Willd. ex P. Beauv., *Micraira* F. Muell, and *Streptogyna* P. Beauv.) were treated as *incertae sedis* because of unclear and poorly supported placements. Recent work, however, has placed these latter taxa in Poaceae with moderate to strong support (Duvall et al. 2006). Poaceae include three small subfamilies (Anomochlooideae,

Pharoideae, and Puelioideae) that are the successive sister groups (respectively) of a large clade that is divided into two major lineages. One lineage includes subfamilies Panicoideae [including Centothecoideae (Soreng et al. 2003; Duvall et al. 2006)], Aristidoideae, Chloridoideae, Arundinoideae, Danthonioideae, and the recently recognized Micrairoideae [the "PACMAD" clade (Duvall et al. 2006), called the "PACC" or "PACCAD" clade in earlier studies], and the other includes subfamilies Bambusoideae, Ehrhartoideae, and Pooideae (the "BEP" clade, also called the "BOP" clade in some earlier studies; GPWG 2001). The BEP clade, however, has not been uniformly recovered; several analyses indicate weakly that Pooideae may instead be the sister group of the PACMAD clade (e.g., Soreng and Davis 1998; GPWG 2001; Caetono-Anolles 2005a; Duvall et al. 2006). Relationships among subfamilies within these major lineages generally have been resolved inconsistently and are supported poorly (e.g., Duvall and Morton 1996; Hilu et al. 1999; Hsiao et al. 1999; Zhang 2000; GPWG 2001; Duvall et al. 2006). This is unfortunate, as understanding evolutionary patterns among the major lineages in Poaceae is critical for providing a robust framework for macro-evolutionary and comparative genomic investigations of extant grasses (including cereals) and their relatives (e.g., Kellogg 1998; Gaut 2000; Caetano-Anolles 2005b; Rudall et al. 2005), for the reconstruction of the timing and pattern of diversification of major clades in the family (e.g., Strömberg 2005; Prasad et al. 2005), and for improved understanding of the grass fossil record (e.g., Crepet and Feldman 1991; Prasad et al. 2005).

3.1.2. The Sister Group of Poaceae

Correct inference of the identity of the sister group of the grasses has also been problematic. Phylogenetic studies of morphological and molecular data indicate that the

closest living relatives of Poaeae are Joinvilleaceae (e.g., Campbell and Kellogg 1987; Doyle et al. 1992; GPWG 2001), Ecdeiocoleaceae (Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004), or a lineage that includes both families (Bremer 2000); relationships among these three families were unresolved in a recent analysis of the *ndh*F gene (Givnish et al. 2006). Additional molecular studies have indicated that either of these families is the sister-group of Poaceae, but none sampled both families (e.g., Davis and Soreng 1993; Davis 1995; Katayama and Ogihara 1996; Soreng and Davis 1998; Linder et al. 2000; Briggs et al. 2000; Hilu et al. 2003; Graham et al. 2006). In contrast to the large size and cosmopolitan distribution of Poaceae, Joinvilleaceae are monogeneric, with two species distributed throughout Malaysia and the south Pacific (Newell 1969), and Ecdeiocoleaceae are bigeneric, with two species distributed in southwestern Australia (Cutler and Shaw 1965; Briggs and Johnson 1998). A third undescribed species from western Australia, morphologically similar to *Ecdeiocolea monostachya*, has recently been discovered (B. G. Briggs, pers. comm.). Resolution of the sister group of Poaceae is important for making inferences about the origins and evolution of reproductive characteristics in Poaceae (e.g., Rudall and Bateman 2004; Rudall et al. 2005) and to clarify broader patterns of diversification within Poales.

3.1.3. Objectives of the Study

One strategy to obtain more fully resolved and better supported phylogenetic trees is to examine more characters per taxon (e.g., Hillis 1998; Poe and Swofford 1999; Graham and Olmstead 2000a; Rokas et al. 2003; Wortley et al. 2005; Graham et al. 2006). I take this approach here to clarify relationships among subfamilies within Poaceae, and to address the

identity of their sister group. I perform phylogenetic analyses of a large, plastid data set from representatives of most of the grass subfamilies. A similar complement of plastid regions has been used to resolve deep phylogenetic relationships among basal angiosperms (Graham and Olmstead 2000a,b; Graham et al. 2000), cycads (Rai et al. 2003; Zgurski 2004), and monocots as a whole (McPherson 2003; Zgurski 2004; Graham et al. 2006), but this combination of genes and intergenic spacers has not previously been sampled intensively within a single family. Within-family phylogenetic studies generally focus on an examination of a few rapidly evolving (often noncoding) regions, but larger genomic samples are becoming increasingly feasible and affordable to examine (Graham et al. 2006), and the slower rates in some of the regions examined are likely to be compensated for by the large number of regions examined, and by the generally elevated substitution rates found in Poaceae (e.g., Bousquet et al. 1992; Gaut et al. 1992, 1997).

3.2 MATERIALS AND METHODS

3.2.1. Taxonomic Sampling

I included at least one exemplar taxon from ten of the twelve Poaceae subfamilies according to GPWG (2001) and Duvall et al. (2006) (Table 3.1). Material representing subfamilies Micrairoideae and Puelioideae was not available for this study. It is arguably appropriate (e.g., Chase et al. 2006) to use one or a few exemplar species to represent each subfamily in this way, as the monophyly of each subfamily has been recovered repeatedly in most previous analyses (see earlier references). I obtained new sequence data for ten grass species, and used published sequence data from GenBank for *Oryza sativa* (Hiratsuka et al. 1989), *Triticum aestivum* (Ogihara et al. 2000) and *Zea mays* (Maier et al. 1995) (Table 3.1).

I obtained the *rbc*L sequence for *Pharus* from GenBank for a congeneric species (*P. latifolius* L.). Outside of Poaceae, I included new sequence data from one species of Joinvilleaceae (Table 3.1), and previously published data from exemplar taxa representing 59 other monocot families (Graham and Olmstead 2000a; Graham et al. 2006). This broad taxon sampling represents all orders of monocots, and includes Dasypogonaceae, a family currently unplaced to order (APG II 2003; Chase 2004).

3.2.2. Genomic Sampling

For most taxa, I extracted DNA from silica gel-dried leaf material using the CTAB protocol of Doyle and Doyle (1987), with the addition of 2% β-mercaptoethanol to each extraction. I amplified and sequenced multiple plastid genes involved in photosynthesis (i.e., *atp*B, *psb*B, *psb*C, *psb*D, *psb*E, *psb*F, *psb*H, *psb*J, *psb*L, *psb*N, *psb*T, *rbc*L), chlororespiration (*ndh*F, *ndh*B), and plastid translation (*rpl*2, 3'-*rps*12, *rps*7), and their associated noncoding regions [i.e., six intergenic regions in two of the photosystem II gene clusters, one intron each in *rpl*2, 3'-*rps*12 and *ndh*B, and intergenic spacer regions between 3'-*rps*12, *rps*7, *ndh*B, and *trn*L(CAA); see Graham and Olmstead (2000a) and Graham et al. (2006) for further details]. Most of the regions examined are in the single copy (SC) regions of the plastid genome, but four (*rpl*2, 3'-*rps*12, *rps*7, and *ndh*B) are in the large inverted repeat (IR) region in most land plants, including grasses examined thus far. I amplified and sequenced DNA using the methods and primers described in Graham and Olmstead (2000a), McPherson (2003), and Graham et al. (2006). I sequenced all regions in the forward and reverse directions, and obtained sequencing products from duplicate amplification products obtained

from independent DNA extractions to detect pipetting errors or cross-contamination (I observed none).

3.2.3. Data Assembly

I performed basecalling and contig assembly using Sequencher 4.1 (Genes Code Corporation, Ann Arbor, MI), determining gene boundaries by comparison to sequences of *Nicotiana tobacum* L. (Shinozaki et al. 1986) and *Ginkgo biloba* L. These sequences were added to previously published alignments that include representatives from across the seed plants (Graham and Olmstead 2000a; Rai et al. 2003; Graham et al. 2006) using Se-Al version 1.0 alpha (Rambaut 1998), according to guidelines outlined in Graham et al. (2000). With few exceptions, all taxa are represented nearly completely for all regions (Table 3.1). The length of the aligned data matrix is 28,062 bp. The unaligned sequence lengths of exemplars from the commelinid monocots examined range from ~13.9 kb in *Strelitzia* Ait. (see Graham et al. 2006) to ~15.8 kb in *Oryza* L., with a mean unaligned length of ~15.3 kb. The aligned data matrix is substantially larger than the unaligned length for any single taxon because of substantial gaps and/ or unalignable regions, including some from non-angiosperm taxa that are part of the matrix but not included in this study. Those noncoding regions that were too difficult to align were set aside as staggered gapped regions primarily consisting of unique sequences. The unique regions are effectively ignored for parsimony-based tree searches and scores (Graham et al., 2006), and should have only minimal effect for model-based methods (e.g., on estimation of base frequency parameter values). I coded gap cells in the data as "missing data" and did not attempt to score inferred insertion/deletion (indel) events.

3.2.4. Parsimony, Likelihood, and Bayesian Phylogenetic Analyses

I examined two different taxon sets, a large 79-taxon (58 family) set using maximum parsimony alone, and a smaller 28-taxon set (consisting of the 13 grasses, 10 exemplar Poales and several representatives of Commelinales and Zingiberales as outgroup taxa) with three different phylogenetic inference methods: (1) maximum parsimony (MP); (2) maximum likelihood (ML) analysis using PHYML version 2.4.4 (Guindon and Gascuel 2003), and; (3) Bayesian inference using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). The smaller taxon set was used for model-based approaches. For the monocot-wide taxon set I accepted *Acorus* as the probable sister group of the rest of the monocots (e.g., Duvall et al. 1993b; Chase et al. 2000; Graham et al. 2006); exemplar species from Commelinales and Zingiberales were used as outgroups for the reduced taxon set.

The MP searches were performed using PAUP* version 4.0b10 (Swofford 2002), with all characters and character-state changes equally weighted, using tree bisection-reconnection (TBR) branch-swapping, and with 100 random addition replicates performed for each search, but otherwise using default conditions. To examine whether the results are affected by data partitions that have different DNA substitutional dynamics, I analyzed four data subpartitions (defined as CHARSETs in the Nexus file) for the reduced taxon set using MP: (i) codon positions 1 and 2; (ii) codon position 3; (iii) all protein-coding regions, and; (iv) all noncoding regions.

For the model-based phylogenetic inference methods I chose optimal DNA substitution models using the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC), as implemented in ModelTest ver. 3.7 (Posada and Crandall 1998). The optimal model was GTR + Γ + I [general time reversible rate (GTR) model with

a proportion of invariant sites (I) considered and a gamma (Γ) distribution used to account for among-site rate variation)] for the combined data set, the protein-coding partition, and the noncoding partition; the TVM + Γ + I model is optimal for the partitions consisting of codon positions 1 and 2, and codon position 3 (for model details see ModelTest documentation; Posada and Crandall 1998). These models were used for the ML analyses of the respective data partitions, with model parameters estimated from the data, and otherwise using default settings in PHYML.

For Bayesian analyses, I used the GTR + Γ + I model of evolution in each analysis. For all analyses, I ran two parallel sets of four chains (three heated and one cold) simultaneously, starting each Markov chain from a random tree. I stopped runs after the average standard deviation of split frequencies (a convergence diagnostic) was less than 0.01. I sampled trees every 1,000 generations, discarding the first 25% of the trees as burn-in. I conducted Bayesian analyses for the data partitions analyzed using MP and ML, but also conducted analyses of the full data set with substitution-model parameters estimated independently ('unlinked') for each of several data subpartitions (Ronquist and Huelsenbeck 2003). Unlinking may be desirable when partitions of the data have different substitutional dynamics, as is clearly the case for different codon positions and between the IR (inverted repeat) and SC (single copy) regions in monocot plastid genomes (e.g., Graham et al. 2006). In total, I conducted four unlinked analyses (unlinked partitions are separated by a comma): (i) codon position 1, codon position 2, and codon position 3; (ii) SC codon positions 1 and 2, SC region codon position 3, IR codon positions 1 and 2, IR codon position 3; (iii) SC and IR protein-coding regions, IR noncoding regions, SC noncoding regions; and (iv) SC codon positions 1 and 2, SC codon position 3, SC noncoding regions, IR codon positions 1 and 2,

IR codon position 3, and IR noncoding regions. These variants exclude (i, ii) or include (iii, iv) noncoding data, and consider SC vs. IR variants of different codon positions as separate partitions (i.e., variants ii and iv), or lump these together (i, iii). To estimate posterior probabilities of individual clades for each analysis, I computed a majority-rule consensus tree in PAUP4.0b10.

I estimated branch support for MP and ML using nonparametric bootstrap analysis (Felsenstein 1985). For MP bootstrap analysis, I used 100 (79-taxon data set) and 1,000 (28-taxon data sets) bootstrap replicates, with the same search parameters as the initial search, but using ten random addition replicates per bootstrap replicate. For ML bootstrap analysis I used 500 bootstrap replicates. Clade posterior probabilies, estimated using MrBayes, are expressed as percentages here. With recognition of the arbitrariness in placing levels of bootstrap support into defined classes, I consider "strongly" supported or "robust" branches to have bootstrap support of 90% or more, "moderately" supported branches to have bootstrap support 70-89%, and "weakly" or "poorly" supported branches to have bootstrap support < 70%.

To compare support values among the various maximum parsimony, maximum likelihood, and Bayesian analyses conducted with the 28-taxon data set, I determined and graphed support values for all clades within Poaceae and among Ecdeiocoleaceae-Joinvilleaceae-Poaceae that were recovered with bootstrap or posterior probability support greater than 50% in at least one of the 19 analyses (see Fig. 4 in Graham et al. 1998). I recovered support values <50% from the bootstrap log for each analysis, ignoring values <5%. I identified each clade with a unique letter [clades "a" to "q" refer to branches recovered in Figs. 3.2, 3.3, "r" to "aa" to other branches, noted in legend to Fig. 3.4].

To assess heterogeneity in the rates of evolution among four monocot lineages, I conducted a series of likelihood ratio tests (LRT) at various hierarchical levels: 1) all monocots (n = 79); 2) commelinid monocots (excluding Dasypogonaceae and Arecaceae; n = 28); 3) Poales (n = 23); and 4) Poaceae (n = 13). I conducted the LRT test using the GTR + Γ + I model with and without a molecular clock enforced.

3.3 RESULTS

3.3.1. Sequence Characteristics

In the 28-taxon commelinid data set, there are 2,407 potentially parsimony informative characters (PPIC; Table 3.2). Among commelinid taxa, ~85% of the PPIC are from the single copy regions, whereas ~92% of the PPIC in Poaceae are from the single copy regions. Within the single copy regions, combined data from *atp*B, *ndh*F, and *rbc*L provide ~1.4 times as many PPIC in the commelinids and Poaceae compared with the combined photosystem II regions, for a similar length of sequence examined [a consequence of a lower subsitution rate, also reflected in fewer PPIC per unaligned nucleotide, and consistent with substitution rates noted for some of these genes in Olmstead and Palmer (1994)]. In single-copy regions, codon position 3 provides the greatest percentage of PPIC, and more PPIC per unaligned character than the noncoding regions. Noncoding nucleotides in the IR evolve at the same rate (Poaceae), or are more slowly evolving (commelinids) than IR codon position 3 (i.e., fewer PPIC per unaligned nucleotide in the latter case), but nonetheless provide more PPIC in total, because of the greater length (unaligned) of sequence examined (Table 3.2). The latter estimates may be more prone to sampling error, given the small amount of variation encountered within the inverted regions in Poaceae.

3.3.2. Phylogenetic Analyses

Maximum parsimony (MP) analysis of the large taxon set recovers six most-parsimonious trees. In the strict consensus tree, all nodes outside of Poaceae are resolved (branches collapsing in the strict consensus are noted in Fig. 3.1). Visual inspection of the phylogram indicates that members of the clade that includes Poaceae, Cyperaceae, Ecdeiocoleaceae, Mayacaceae, Restionaceae, and Xyridaceae generally have very long terminal branches compared with most other monocots, including other Poales, although there also appears to be considerable variation within this clade (cf. the relative lengths of the terminal branches ending in *Cyperus* and *Ecdeiocolea* to those ending in *Flagellaria* and *Joinvillea*; Fig. 3.1). Likelihood ratio tests with the GTR + Γ + I model of evolution with and without enforcement of a molecular clock confirm these observations. There is significant heterogeneity in the rate of evolution in the regions examined across the monocots, across the commelinid monocots, across Poales, and across Poaceae ($P < 0.05$; Table 3.3).

MP analysis of the complete 28-taxon data set recovers two most-parsimonious trees (one is shown in Figure 3.2). Likelihood analysis of this data set recovers a tree that is identical in topology to the parsimony tree, with the exception of relationships among Ecdeiocoleaceae–Joinvilleaceae–Poaceae (Fig. 3.2). A tree inferred from Bayesian analyses of the complete, unpartitioned 28-taxon data set is shown in Figure 3.3. In general, clades that receive moderate to high bootstrap support in maximum parsimony and maximum likelihood analyses receive posterior probabilities of greater value in Bayesian analyses, in line with recent findings of empirical and simulation studies (e.g., Rannala and Yang 1996; Leache and Reeder 2002; Suzuki et al. 2002; Whittingham 2002; Wilcox et al. 2002; Cummings et al. 2003; Erixon et al. 2003; Simmons et al. 2004). Clades that receive low

bootstrap support in parsimony analyses are not usually recovered by likelihood and Bayesian analyses, and vice versa. Within Poaceae, there is general congruence among trees inferred from most data partitions in parsimony, likelihood, and Bayesian analyses (Fig. 3.4). Analysis of the noncoding data partition, however, provides instances where there is moderate to strong support for several clades not recovered or well supported by analyses of other data partitions (Fig. 3.4: clades "r," "t," "u," "v," and "x"), and which conflict with moderately to strongly supported clades inferred from those other partitions. Topologies and support values do not vary substantially between the various partitioned and corresponding unpartitioned Bayesian analyses, except in parts of the tree that are generally weakly supported by the complete data set (Fig. 3.4c).

Relationships inferred along most of the backbone of Poaceae are supported robustly in all analyses, but support for relationships among several of the subfamilies varies (Figs. 3.2–3.4). In analyses involving all data sets, partitions and partitionings, and all phylogenetic methods, I recover strong support for the monophyly of Poaceae [maximum parsimony bootstrap proportion (BPMP) = 100%, maximum likelihood BP (BPML); Bayesian posterior probability, PP = 100%; clade "a"] and moderate to strong support for the monophyly of Anomochlooideae (*Anomochloa* and *Streptochaeta;* clade "c": BPMP = 78-100%, BPML = 90-100%, PP > 95%; Figs. 3.2–3.4. In parsimony, Bayesian and most likelihood analyes, I generally infer Anomochlooideae to be the sister group of the rest of the grasses (clade "b": BPMP = 89-100%, BPML = 83-100%, PP = 100%; Figs. 3.2-3.4), and Pharoideae to be the next successive sister group of the rest of the family (clade "d": BPMP = 100%, PP = 100%; Figs. 3.2–3.4). Likelihood anlayses of codon position 3, however, place Anomochlooideae and Pharoideae in a weakly supported clade (clade "aa": BPML = 69%; Fig. 3.4b) that is the

sister group of the rest of the grasses (i.e., the clade that includes the BEP and PACMAD subclades). The latter clade is robustly supported (clade "d": BPMP = 100%, BPML = 100%, PP = 100%; Figs. 3.2-3.4). The BEP (Bambusoideae, Ehrhartoideae, Pooideae) clade is supported to different degrees by different data partitions (clade "e": BPMP = 39-97%, BPML = 32-94%, PP = 88-100%; Figs. 3.2–3.4); but the PACMAD (Panicoideae, Arundinoideae, Chloridoideae, Aristidoideae, Danthonioideae) clade consistently has strong support (clade "h": BPMP = 99-100%, BPML = 99-100%, PP = 100%; Figs. 3.2–3.4). As might be expected, where support for individual clades is weaker across partitions, it is generally from partitions that have the least number of informative characters (Table 3.2)

Within the BEP clade, the two sampled taxa of Pooideae constitute a robustly supported clade in most analyses (clade "g", Fig. 3.4). When all data are examined, Bambusoideae are consistently inferred to be the sister group of Pooideae with moderate support (clade "f", Figs. 3.2, 3.3) and Ehrhartoideae are inferred to be the sister group of these two subfamilies (clade "e", Figs. 3.2, 3.3). Support for these relationships is again generally lower when data partitions with fewer PPIC per unaligned nucleotide are examined for parsimony and likelihood analyses, but not for most Bayesian analyses (for example, compare the different support values for clade "f" in Fig 3.4). Within the PACMAD clade, the monophyly of Panicoideae receives weak to strong support with various data partitions (clade "k", Fig. 3.4). In most cases, phylogenetic relationships among subfamilies in the PACMAD clade are not consistently resolved or supported. Internal branches are generally relatively short within this clade (Fig. 3.1). A clade consisting of Aristidoideae and Chloridoideae (clade "l", Fig. 3.4) is recovered with moderate to strong support in most

analyses, with less support from the smaller and less variable data partitions (differences in support values for this clade among partitions are less pronounced for Bayesian analyses).

Several hypotheses of relationship from analyses of the noncoding data partition conflict with analyses of most other data partitions or subpartitions. These include a sister-group relationship between *Triticum* (Pooideae) and Ehrhartoideae (clade "v": BPMP = 71%, BPML = 74%, PP = 100%; Fig. 3.4), a Chloridoideae–Danthonioideae–Panicoideae clade (clade "t": BPMP = 55%, BPML = 65%, PP = 98%; Fig. 3.4), and an Aristidoideae–Arundinoideae clade (clade "u": BPMP = 57%, BPML = 69%, PP = 99%; Fig. 3.4).

All analyses indicate that Ecdeiocoleaceae, Joinvilleaceae, and Poaceae are a strongly supported clade (clade "n": BPMP = 100%, BPML = 100%; PP = 100%; Figs. 3.2–3.4). All three possible arrangements among the three families are inferred in different analyses, often with low support, even considering all of the data combined. For example, in the monocot-wide parsimony analyses, Joinvilleaceae and Ecdeiocoleaceae are a weakly supported clade (BPMP = 43%, Fig. 3.1) that is the sister group of Poaceae, whereas in parsimony analyses restricted to commelinid taxa, Joinvilleaceae are weakly inferred to be the sister group of Poaceae (clade "m": BPMP = 31%; Fig. 3.2). In contrast, most likelihood and Bayesian analyses infer a sister-group relationship between Ecdeiocoleaceae and Joinvilleaceae, with weak to strong support (clade "o": BPML = 64-100%, PP = 53-100%; Fig. 3.4), and this two-family clade is robustly supported as the sister group of Poaceae (clade "n": Figs. 3.2–3.4). When the noncoding data are considered separately, Ecdeiocoleaceae are inferred to be the sister group of the grasses with moderate to robust support (clade "r": BPMP = 73%, BPML= 80%, PP = 86%, 96%; Fig. 3.4). In general, all three arrangements among Poaceae and relatives (clades "r", "o" and "m") have equally poor support in most MP analyses.

However, with the exception of analyses involving the noncoding data partition (and one of the data partitionings used in the Bayesian analysis) clade "o" (Ecdeiocoleaceae–Joinvilleaceae) is the only arrangement with reasonably strong support in the model-based analyses. This support is strongest for analyses that focus on coding regions (Fig. 3.4).

3.4. DISCUSSION

3.4.1. Molecular Evolution and Phylogenetic Utility of the Plastid DNA Regions in Poaceae

Visual inspection of branch variation in the phylogram here (Fig. 3.1) corroborates the findings of earlier studies on the accelerated substitution rate of grass plastid genomes compared with other monocots (e.g., Wilson et al. 1990; Bousquet et al. 1992; Gaut et al. 1992, 1996; Graham and Olmstead 2000a), and demonstrates that this increased substitution rate is not unique to Poaceae (Graham et al. 2006). The plastid rate elevation occurs in some, but not all, families within Poales [I have examined exemplar taxa here for only three fifths of Poales families, sensu APG II (2003), so this rate heterogeneity will require further characterization]. Comparison of likelihood models with and without a molecular clock enforced confirms that there is significant heterogeneity at various hierarchical levels among the monocots (Table 3.3). Most earlier studies did not characterize or comment on broader rate-heterogeneity within Poales, but published phylograms show generally increased branch lengths in the order compared with other monocots (e.g., Duvall et al. 1993a; Bremer 2002; Givnish et al. 2006), and grass long branches have been blamed on anomalous results in angiosperm-wide phylogenetic inference involving limited taxon sampling of whole plastid genomes (Stefanovic et al. 2004; Leebens-Mack et al. 2005). Various explanations have

been proposed for such rate heterogeneity, including a relaxation of purifying selection, demographic factors, and variable mutation rates among lineages (e.g., due to changes in DNA repair efficiency that may affect whole genomes or parts of them), or a combination of these factors (e.g., Cho et al. 2004; Parkinson et al. 2005; Young and dePamphilis 2005). Givnish et al. (1999) also observed this accelerated plastid substitution rate in Poales, and suggested that such genetic divergence may have arisen due to the limited seed dispersal abilities of many of the rate-accelerated lineages (i.e., wind or gravity dispersed), which might increase isolation of populations by distance, thereby promoting possible speciation and substantial genetic divergence. Two of the three closest relatives of Poaceae, Joinvilleaceae and Flagellariaceae (Figs. 3.1-3.3), are both fleshy-fruited, and both terminate branches here that appear to be less divergent than the predominantly non-animal dispersed Poaceae and Ecdeiocoleaceae (Fig. 3.1).

The regions sampled represent most of the diversity of evolutionary rates in the plastid genome, with the exception of the most rapidly evolving plastid regions (e.g., Small et al. 1998; Shaw et al. 2005). The combined plastid data contain sufficient phylogenetically informative variation to resolve several relationships in Poaceae with strong support, including some that were problematic previously, but they do not provide enough characters to robustly resolve some relationships that have consistently proven recalcitrant. The genes *atp*B, *ndh*F, and *rbc*L have been used commonly for phylogenetic reconstruction at higher taxonomic levels (e.g., Savolainen et al. 2000; Givnish et al. 2006). In Poaceae, *rbc*L and *ndh*F alone yield insufficient variation to provide strong support for deeper nodes in the family (Clark et al. 1995; Duvall and Morton 1996). In my analyses, *rbc*L, *ndh*F, and *atp*B

combined contribute approximately half of the parsimony informative variation in the data set (Table 3.2).

Inclusion of data from the more slowly evolving photosystem and inverted repeat regions of the plastid genome provides conservative characters. It has been suggested that they may be useful in reconstructing higher-level phylogenies in instances where long-branch attraction might otherwise complicate phylogenetic inference (e.g., Graham and Olmstead 2000a). These might also have better-than-expected phylogenetic utility in "lower-level" (within-family) phylogenetic analysis in Poaceae and relatives, because of the relatively accelerated plastid substitution rate in many Poales (Fig. 3.1). The photosystem II regions contribute approximately a third of the useful variation in the data set here (Table 3.2). Some of these have been used in recent phylogenetic studies (e.g., Sanderson et al. 2000; He-Nygrén and Sinikka 2003; Schütze et al. 2003; Cameron and Molina 2006), but most have been largely underutilized and underexplored in studies below the family level. The plastid inverted repeat regions (IR; 3'-*rps*12–*rps*7–*ndh*B, and *rpl*2) are by far the most conservative examined here, consistent with earlier studies (e.g., Wolfe et al. 1987; Goremykin et al. 1996; Graham and Olmstead 2000a; Graham et al. 2000; Masood et al. 2004). Most informative characters in the examined regions of the IR come from the noncoding portions, a function of the total amount of nucleotides examined for these portions (Table 3.2).

3.4.2. Phylogenetic Relationships in Poaceae

There is general consensus on most aspects of branching order at the base of the Poaceae subtree, here and elsewhere. All analyses here involving larger data partitions corroborate most earlier molecular studies that include *Anomochloa* and *Streptochaeta* in a

clade (Anomochlooideae; Clark and Judziewicz 1996) that is the sister group of the rest of Poaceae. It should be noted that I have not yet sampled Puelioideae [a small subfamily variously resolved as the sister group of all of Poaceae except Anomochlooideae and Pharoideae (Clark et al. 2000; GPWG 2001), or as the sister group of the BEP clade (Zhang 2000)]. Some previous analyses indicate that Anomochlooideae are not a natural group (e.g., Hilu et al. 1999; Mathews et al. 2000; Zhang 2000), but my data strongly support its monophyly. GPWG (2001) suggested that *Anomochloa* and *Streptochaeta* might be spuriously attracted in phylogenetic analyses due to long-branch attraction. This hypothesis could be tested in future work using a simulation approach (e.g., Sanderson et al. 2000).

In agreement with previous studies (e.g., Clark et al. 1995; Soreng and Davis 1995; Mathews et al. 2000; Zhang 2000; GPWG 2001), two branches ("b" and "d", Figs. 3.2–3.4) that collectively support a sister group relationship between *Pharus* (Pharoideae; Clark and Judziewicz 1996) and the BEP–PACMAD clade, are strongly supported in all analyses here. One possible moderate conflict to this finding from my data is that likelihood analysis of the third codon position data identifies an Anomochlooideae–Pharoideae clade ("aa" in Fig. 3.4), but this clade has very poor support from all other partitions and analytical methods, and may represent a spurious conflict. The "deep" positions of Anomochlooideae and Pharoideae in Poaceae in this and previous molecular analyses are consistent with substantial morphological differences that characterize these taxa compared with most grasses (see GPWG 2001).

The rest of the grasses form a very strongly supported clade (clade "d", the "core grasses", or BEP-PACMAD clade) in all of my analyses. This crown clade contains most grass diversity, and is subtended by a relatively long branch. Multiple putative

morphological synapomorphies have been identified for this major lineage (loss of pseudopetiole, reduction to two lodicules, loss of the inner whorl of stamens, and loss of arm cells and fusoid cells in the mesophyll), although several of these have undergone subsequent reversals in the clade (see GPWG 2001).

Phylogenetic relationships inferred among the nine subfamilies in the core grasses have varied among previous studies. Several analyses unite Bambusoideae, Ehrhartoideae, and Pooideae in a clade (the BEP clade) with poor (e.g., Clark et al. 1995; Cummings et al. 1994; Mason-Gamer et al. 1998; Zhang 2000) or moderate support (e.g., Mathews et al. 2000; GPWG 2001); other studies have found alternate topologies, including a weakly-supported sister-group relationship between Pooideae and the PACMAD clade (e.g., Duvall and Morton 1996; Soreng and Davis 1998; Hsiao et al. 1999; Caetano-Anolles 2005a; Duvall et al. 2006). No morphological synapomorphies have been identified for a BEP clade (GPWG 2001); six have been proposed for a Pooideae–PACMAD clade (Soreng and Davis 1998). My data recover the BEP clade with strong support in most analyses (clade "e", Figs. 3.2–3.4), an improvement for plastid data compared with the earlier multigene study [e.g., BP_{MP} = 95% here for the combined data, vs. 62% for plastid data in GPWG 2001); in contrast, I find only poor support for a Pooideae–PACMAD clade; the highest support for this clade (clade "s") is very weak (BP_{MP} = 52% here for codon positions 1 and 2 combined; Fig. 3.4).

The remainder of the sampled grasses here form the PACMAD clade (clade "h"), in agreement with all previous plastid analyses (e.g., Soreng and Davis 1998; Cummings et al. 1994; Clark et al. 1995; Duvall and Morton 1996; Zhang 2000), although I have not sampled the recently recognized subfamily Micrairoideae (Duvall et al. 2006). A substantial amount

of molecular evolution has clearly occurred in the plastid genome along the branch that subtends the PACMAD crown clade (e.g., Fig. 3.1), which has undoubtedly contributed to the consistent recovery of this clade in previous analyses (even using limited amounts of data), and the consistently strong support found here across all analyses and data partitions (Fig. 3.4). However, only two putative morphological synapomorphies (presence of an elongated mesocotyl internode, and loss of the epiblast) have been identified for the PACMAD grasses (GPWG 2001).

All possible topologies have been inferred among the three subfamilies that form the BEP clade. I find moderate to strong support for a sister-group relationship between Bambusoideae and Pooideae for several of my analyses (clade "f", Figs. 3.2–3.4). This relationship was also recovered in the analyses of Mathews and Sharrock (1996) and Zhang (2000), but with substantially lower support than is found here. In the previous multigene studies (GPWG 2001; Duvall et al. 2006), a sister-group relationship between Bambusoideae and Ehrhartoideae was recovered. In most cases here, support for a Bambusoideae–Ehrhartoideae clade is < 50%, but I find this topology with moderate support in one analysis (clade "z": BP_{ML} = 72% for analysis of codon positions 1 and 2 combined, Fig. 3.4), representing a moderate conflict with the other data partitions and analyses. A stronger conflict is also evident, as analysis of noncoding data places Ehrhartoideae and *Triticum* as a clade with moderate to strong support (clade "v": BP_{MP} = 71%; BP_{ML} = 74%; PP = 100%, Fig. 3.4). This arrangement was also weakly supported in an analysis of nuclear data from phytochrome B (Mathews et al. 2000). The cause of this incongruence is unknown.

Despite sampling an expanded data set, clear inference of phylogenetic structure within the PACMAD clade has not emerged from this study, although several aspects of

relationship are moderately to strongly supported. *Chasmanthium* and *Zea* are a well supported clade, consistent with the recent inclusion of the rather ill-defined Centothecoideae (GPWG 2001) in Panicoideae (e.g., Soreng et al. 2003; Duvall et al. 2006). In most instances, I infer a sister-group relationship between Aristidoideae and Chloridoideae, with moderate to robust support (clade "l": Figs. 3.2–3.4). This clade was inferred previously in an *rbc*L analysis (Duvall and Morton 1996). It is not in conflict with most of those inferred for the PACMAD clade in previous studies, as internal relationships in this clade generally have very weak support (GPWG 2001; Duvall et al. 2006). Analysis of the noncoding data partition here instead indicates a conflicting sister-group relationship between Arundinoideae and Aristidoideae, with moderate to strong support (clade "u"; Fig. 3.4), a topology that has not been seen in other studies (e.g., GPWG 2001). Bayesian analysis identifies a strongly supported clade that includes Arundinoideae, Aristidoideae, Chloridoideae, and Danthonioideae (clade "p", Figs. 3.3, 3.4). This clade was recovered in a previous *rbc*L study (Duvall and Morton 1996), but it does not receive bootstrap support higher than 50% in parsimony or likelihood analyses here (Fig. 3.4).

Most of the moderate to strong conflict observed here among data partitions involves noncoding regions. These may reflect substantially different "process partitions" for these regions (e.g., Gielly and Taberlet 1994; Shaw et al. 2005), or may be a simple function of more poorly aligned regions. The regions that are most difficult to align in the current data set all belong to the single-copy noncoding regions (i.e., the intergenic spacer regions between the various photosystem II genes). It may therefore be reasonable to exclude these regions from consideration, and thus ascribe the conflicting clades (i.e., clades "v", "u", "t", etc.) to alignment difficulties or inflated Bayesian support values. If so, greater weight

should be placed on the relationships inferred using only protein-coding regions (and perhaps the very conservative noncoding regions in the inverted repeat, not reported on separately here) using MP and ML. Aside from these conflicts, several aspects of relationship, particularly with the PACMAD clade, resist satisfactory resolution. These continuing difficulties may reflect the relatively short branches observed at deeper levels in the clade (Fig. 3.1), possibly reflecting a rapid initial radiation of each of the major lineages (GPWG 2001). These relationships are possibly difficult to resolve because too little information has been recovered to do so satisfactorily. Strong (high support values) and clear (congruent) inference of relationships in the PACMAD clade may require substantially more data per taxon (from the plastid or other genomes).

3.4.3. The Sister Group of Poaceae

Previous phylogenetic analyses of morphological and molecular data agree that Joinvilleaceae, Ecdeiocoleaceae, and Poaceae are a clade, but the identity of the sister group of the grasses has been variously resolved and remains unclear (e.g., Campbell and Kellogg 1987; Bremer 2000, 2002; Michelangeli et al. 2003; Davis et al. 2004). In my MP analyses, different taxon samplings yield different resolutions of relationships within this clade [(Poaceae, (Joinvilleaceae–Ecdeiocoleaceae)), Fig. 3.2; (Ecdeiocoleaceae, (Joinvilleaceae–Poaceae), Fig. 3.1], but this is not surprising, as branch support in the various parsimony analyses is consistently low and relatively evenly split among the different possibilities (clades "r", "o" and "m", Fig. 3.4a). In most likelihood and Bayesian analyses, however, Joinvilleaceae and Ecdeiocoleaceae are a clade (clade "o"; Figs. 3.2–3.4) with relatively strong support, particularly when protein-coding data are considered alone. By contrast,

when the noncoding data are considered independently (or in one case when they are considered as a separate partition in unlinked Bayesian analysis), Ecdeiocoleaceae are placed as the sister group of Poaceae, with moderate support in parsimony and likelihood analyses, and strong support in Bayesian analyses (clade "r"; Fig. 3.4). For the reasons discussed above, it may be reasonable to place more weight on the results from analyses that exclude some or all of the noncoding data (i.e., the finding of a sister-group relationship between Ecdeiocoleaceae and Joinvilleaceae). Indeed, for the noncoding data partition the terminal branch subtending Ecdeiocoleaceae is substantially longer than that for Joinvilleaceae and all of the grasses (data not shown), suggesting that there might be unrecognized alignment problems in the noncoding data for *Ecdeiocolea* (a subsequent review of the matrix could not identify substantial problems), or an unusual rate of molecular evolution for this data partition and taxon.

The Ecdeiocoleaceae–Joinvilleaceae clade seen in most model-based analyses of the larger data partitions here (that exclude noncoding data) has the highest support levels obtained to date for putative relationships among these three families. Before this hypothesis can be accepted, however, it clearly requires corroboration by additional lines of molecular evidence (for example, additional taxa and substantially more data may be necessary to recover an Ecdeiocoleaceae-Joinvilleaceae clade in parsimony analyses, if it is correct). It would also be valuable to identify possible synapomorphies (e.g., morphological, developmental, anatomical, chemical, molecular) that might define an Ecdeiocoleaceae–Joinvilleaceae clade.

3.4.4. Congruence and Discordance in the Data

Most relationships inferred here are either well supported (particularly among analyses performed on the larger data partitions; Fig. 3.4) and congruent among the various types of analyses performed, or are simply poorly supported across all analyses (e.g., several branches in the PACMAD clade). Broad-scale congruence is one of the best sources of evidence that phylogenetic inferences have correctly predicted historical relationships (e.g., Penny et al. 1982; Graham et al. 1998). In the face of this reassuring picture, instances of incongruence may suggest underlying problems with the data (e.g., that there may be alignment problems) or with particular analytical methods (e.g., the tendency for Bayesian analyses to produce inflated support values compared to bootstrap analysis, a result that seems to be generally supported here: Fig. 3.4). Because all the data considered here come from the same genetic linkage group (the plastid genome) it would be desirable to collect data from other linkage groups to detect sources of incongruence between gene and organismal phylogenies, such as lineage sorting of ancestral polymorphisms and ancient hybridization events (e.g., Maddison 1997; Maddison and Knowles 2006). However, the latter effects might be expected to have little consequence for most of the inferences made here, because of the long time periods separating most of the evolutionary splits examined.

In the current study I focussed on adding more data per exemplar taxon for making inferences of relationship with Poaceae and relatives, as this is expected to be an effective strategy for performing accurate phylogenetic inference (e.g., Swofford and Poe 1999; see Graham et al. 2006). Studies have also demonstrated that accurate inference of phylogenetic relationships can be enhanced by increasing the density of taxon sampling (e.g., Hillis 1998; Pollock et al. 2002; Zwickl and Hillis 2002). To estimate relationships among the major

clades of Poaceae for the current (or improved) genomic samplings, improving the taxon density may therefore also prove effective. One simple improvement would be to sample at least a few taxa per subfamily, chosen to straddle the root node of each of these major clades. However, with few exceptions [e.g., Puelioideae (see Chapter 4), and Micrairoideae within the PACMAD clade (Duvall et al. 2006)], it is doubtful that further additions can be made along the deepest branches of Poaceae phylogeny, or the branch between Poaceae and its closest relatives (Ecdeiocoleaceae and Joinvilleaceae).

Table 3.1. GenBank accession numbers and vouchers for sequence data from exemplar species in Poaceae and Joinvilleaceae. The *rbc*L sequence for *Pharus* (underlined) was obtained from GenBank; this sequence is from a closely related species. Data for three grasses (Ehrhartoideae: *Oryza sativa* L.; Pooideae: *Triticum aestivum* L.; and Panicoideae: *Zea mays* L. are from whole plastid genomes deposited on GenBank (accessions NC_001320, NC_002762 nad NC_001666.2, respectively).

Taxon	Voucher	Gene/ Region							Length (unaligned)	
		*atp*B	*ndh*F	*psb*B-T- N-H	*psb*D-C	*psb*E-F-L-J	*rbc*L	*rpl*2	3'*rps*12, *rps*7, and *ndh*B	All nucleotides	Protein-coding nucleotides
POACEAE											
Anomochlooideae											
Anomochloa marantoidea Brongn.	*Clark 1299* (ISC)									15.6 kb	12.2 kb
Streptochaeta angustifolia Soderstrom	*Clark 1304* (ISC)									15.6 kb	12.2 kb
Pharoideae											
Pharus lappulaceus Aubl.	*Clark 1329* (ISC)						AY357724			15.4 kb	12.1 kb
Bambusoideae											
Pseudosasa japonica (Siebold & Zucc. ex Steud.) Makino ex Nakai	*Saarela 265* (ALTA)									15.7 kb	12.3 kb
Pooideae											
Brachyelytrum aristosum (Michx.) P. Beauv. ex Trel.	*Saarela 50* (ALTA)									15.4 kb	12.0 kb
Aristidoideae										15.4 kb	12.1 kb
Aristida adscensionis L.	*Peterson et al. 16679* (US)										

Table 3.1 continued

Taxon	Voucher	Gene/ Region							Length (unaligned)	
		*atp*B	*ndh*F	*psb*B-T- N-H	*psb*D-C	*psb*E-F-L-J	*rbc*L	*rpl*2	3'*rps*12, *rps*7, and *ndh*B	All nucleotides	Protein-coding nucleotides
Arundinoideae										15.6 kb	12.2 kb
Phragmites australis (Cav.) Trin. ex Steud.	*Saarela 264 & Ferreira* (ALTA)										
Danthonioideae											
Danthonia californica Bol.	*Saarela 267, Greif, & Hernandez-Castillo* (ALTA)									15.4 kb	12.0 kb
Panicoideae											
Chasmanthium latifolium (Michx.) H. O. H. O. Yates	*Peterson 17532* (US)									15.4 kb	12.1 kb
Chloridoideae											
Bouteloua curtipendula (Michx.) Torr.	*Saarela 20* (ALTA)									15.2 kb	11.8 kb
JOINVILLEACEAE											
Joinvillea plicata (Hook. f.) Newell & Stone	*Thien 84* (NO)									14.8 kb	12.0 kb

[a]Partly sequenced genes by taxon, relative to sequences considered in Graham and Olmstead (2000a): (1) *rbc*L: *Brachyelytrum*, 195 bp missing at 3'-end; (2) *atp*B: *Chasmanthium and Aristida*, 193 bp missing at 5'-end, *Bouteloua*, missing 194 bp at 5'-end and 311 bp at 3'-end, *Danthonia*, missing 255 bp at 5'-end, *Phragmites*, missing 75 bp at 5'-end, *Anomochloa*, missing 57 bp at 5'-end, *Streptochaeta*, missing 52 bp at 5'-end, and *Brachyelytrum*, missing 71 bp at the 3'-end; (3) *ndh*F: *Pharus*, missing 176 bp at 3'-end; (4) *psb*E-F-L-J: *Aristida*, missing 58 bp at 3'-end.

Table 3.2. Number of characters (aligned and unaligned), potentially parsimony informative characters (PPIC), percentage of total PPIC, and number of PPIC per unaligned nucleotide in various partitions of the data.

Data partition	Number of characters (aligned)	Number of characters (unaligned)[a]	PPIC	% of total PPIC	Number of PPIC per unaligned nucleotide examined
Combined IR[b]					
Commelinids	11427	~5.7 kb	366	15.2	0.064
Poaceae	11427	~5.7 kb	61	8	0.011
*atpB, ndh*F, and *rbc*L					
Commelinids	5375	~5.0 kb	1193	49.6	0.239
Poaceae	5375	~5.0 kb	414	54.5	0.083
Combined photosytem II genes[c]					
Commelinids	11260	~5.0 kb	848	35.2	0.17
Poaceae	11260	~5.0 kb	285	37.5	0.057
All data					
Commelinids	**28062**		**2407[d]**		
Poaceae	**28062**		**760[d]**		
IR codon positions 1 and 2					
Commelinids	2456	~1.9 kb	99	4.1	0.052
Poaceae	2456	~1.9 kb	10	1.3	0.005
IR codon position 3					
Commelinids	1230	~0.9 kb	87	3.6	0.097
Poaceae	1230	~0.9 kb	12	1.6	0.013
IR noncoding					
Commelinids	7741	~3.0 kb	180	7.5	0.06
Poaceae	7741	~3.0 kb	39	5.1	0.013
SC codon positions 1 and 2					
Commelinids	6714	~6.4 kb	567	23.5	0.089
Poaceae	6714	~6.4 kb	177	23.3	0.028
SC codon position 3					
Commelinids	3386	~3.2 kb	1331	55.3	0.415
Poaceae	3386	~3.2 kb	483	63.4	0.015
SC noncoding					
Commelinids	6588	~0.5 kb	144	6	0.288
Poaceae	6588	~0.5 kb	40	5.3	0.08
All data					
Commelinids	**28062**		**2408[d]**	100	
Poaceae	**28062**		**761[d]**	100	

[a] Unaligned lengths were determined for a reference taxon, *Oryza sativa* L., for which complete data are available.

[b] Including genes, introns, and intergenic spacers.

[c] Including genes and intergenic spacers.

[d] Total PPIC vary between different combined analyses because the 53 bp overlap between *psb*D and *psb*C is considered in analyses of both SC codon positions 1 and 2 and SC codon position 3. This overlap was not considered twice when determining total number of characters.

Table 3.3. Likelihood ratio test (LRT) assessing heterogeneity among hierarchical lineages of monocots using the GTR + Γ + I[a] model of evolution with and without enforcement of a molecular clock. Likelihood scores were estimated based on a randomly chosen most-parsimonious tree.

Taxon set	Number of taxa (df)[c]	- ln likelihood: GTR + Γ + I	- ln likelihood: GTR + Γ + I + molecular clock	- 2 ln Λ[d]	P
Monocots	79 (77)	158371.77	162234.24	7724.94	< 0.05
Commelinid monocots[b]	28 (25)	76899.36	78885.92	3973.12	< 0.05
Poales	23 (21)	67608.54	69115.64	3014.2	< 0.05
Poaceae	13 (11)	39216.3	39541.78	650.96	< 0.05

[a] *Abbreviations*: GTR, general time-reversible; Γ, Gamma; I, proportion of invariable sites.

[b] Excluding Dasypogonaceae and Arecaceae.

[c] df = number of taxa - 2.

[d] Likelihood ratio test statistic.

Figure 3.1. Phylogram of one of six most parsimonius trees inferred for Poaceae and other monocots, based on a large plastid data set (*atp*B, *ndh*B, *ndh*F, ten photosystem genes, *rbc*L, *rps7*, 3'-*rps*12, *ndh*B and various introns and noncoding regions; see text). Tree length = 26,761 steps, consistency index = 0.370, retention index = 0.553. The clades marked with an arrow within Poaceae collapse in the strict consensus tree.

Fig. 3.1

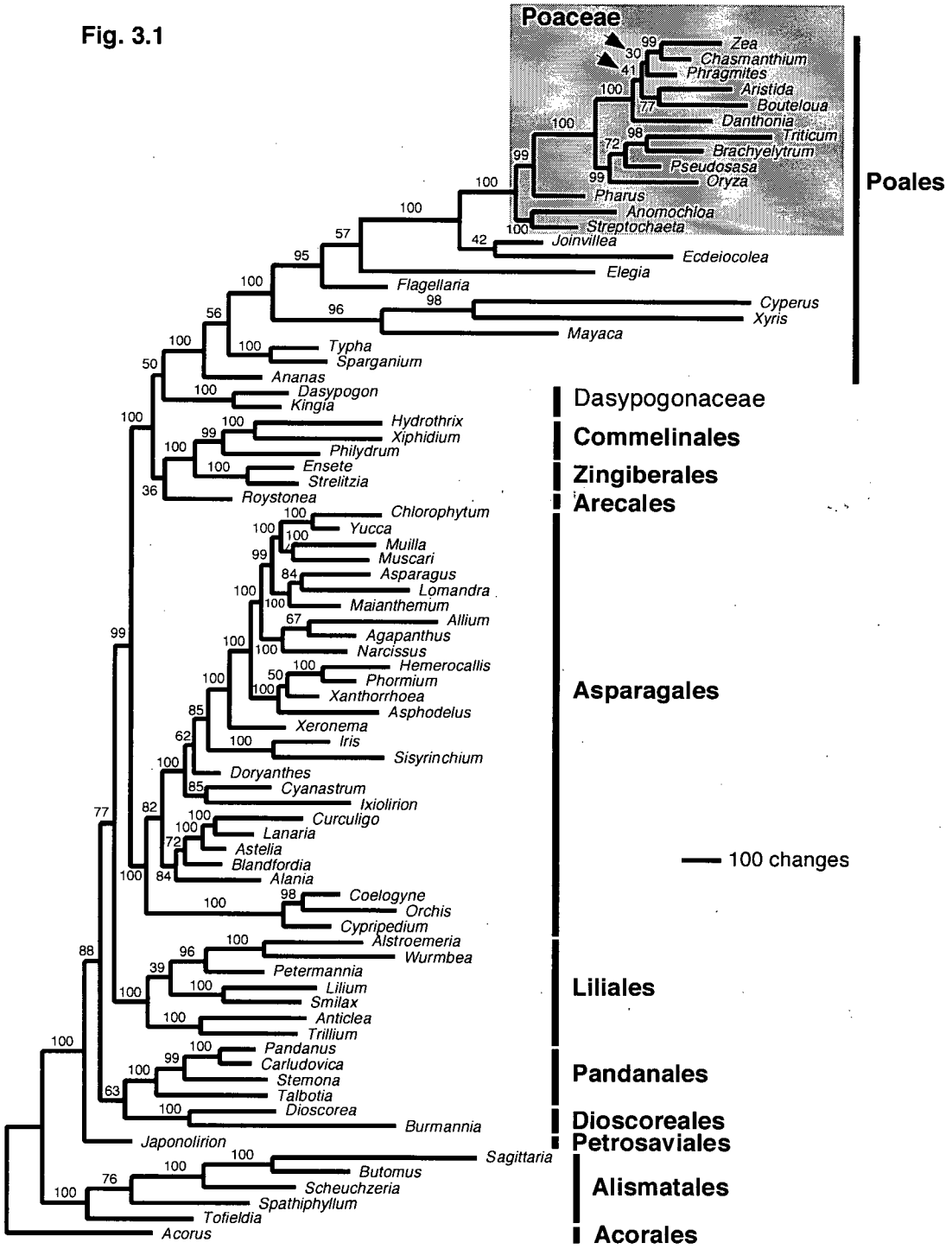

Poaceae
Zea
Chasmanthium
Phragmites
Aristida
Bouteloua
Danthonia
Triticum
Brachyelytrum
Pseudosasa
Oryza
Pharus
Anomochloa
Streptochaeta
Joinvillea
Ecdeiocolea
Elegia
Flagellaria
Cyperus
Xyris
Mayaca
Typha
Sparganium
Ananas
Dasypogon
Kingia
Hydrothrix
Xiphidium
Philydrum
Ensete
Strelitzia
Roystonea
Chlorophytum
Yucca
Muilla
Muscari
Asparagus
Lomandra
Maianthemum
Allium
Agapanthus
Narcissus
Hemerocallis
Phormium
Xanthorrhoea
Asphodelus
Xeronema
Iris
Sisyrinchium
Doryanthes
Cyanastrum
Ixiolirion
Curculigo
Lanaria
Astelia
Blandfordia
Alania
Coelogyne
Orchis
Cypripedium
Alstroemeria
Wurmbea
Petermannia
Lilium
Smilax
Anticlea
Trillium
Pandanus
Carludovica
Stemona
Talbotia
Dioscorea
Burmannia
Japonolirion
Sagittaria
Butomus
Scheuchzeria
Spathiphyllum
Tofieldia
Acorus
Poales
Dasypogonaceae
Commelinales
Zingiberales
Arecales
Asparagales
Liliales
Pandanales
Dioscoreales
Petrosaviales
Alismatales
Acorales
100 changes

Figure 3.2. One of two most parsimonious trees inferred from combined data from 17 chloroplast genes for Poaceae and related families in the commelinid monocots. Subfamilies within Poaceae, the BEP and PACMAD clades, and names of closely related families are indicated. Tree length = 10,318 steps, consistency index = 0.585, retention index = 0.611. Bootstrap values from parsimony and likelihood analyses are indicated above the branches (parsimony to the left, likelihood to the right). Clades marked with arrows collapse in the strict consensus tree. An Ecdeiocoleaceae–Joinvilleaceae clade inferred in the ML analysis (clade "o"; BP = 64% in likelihood analysis, 38% in parsimony analysis) is not shown here. In the likelihood tree, –ln = -77085.77. Letters "a" to "n" identify branches scored for analyses from partitions of the data.

Fig. 3.2

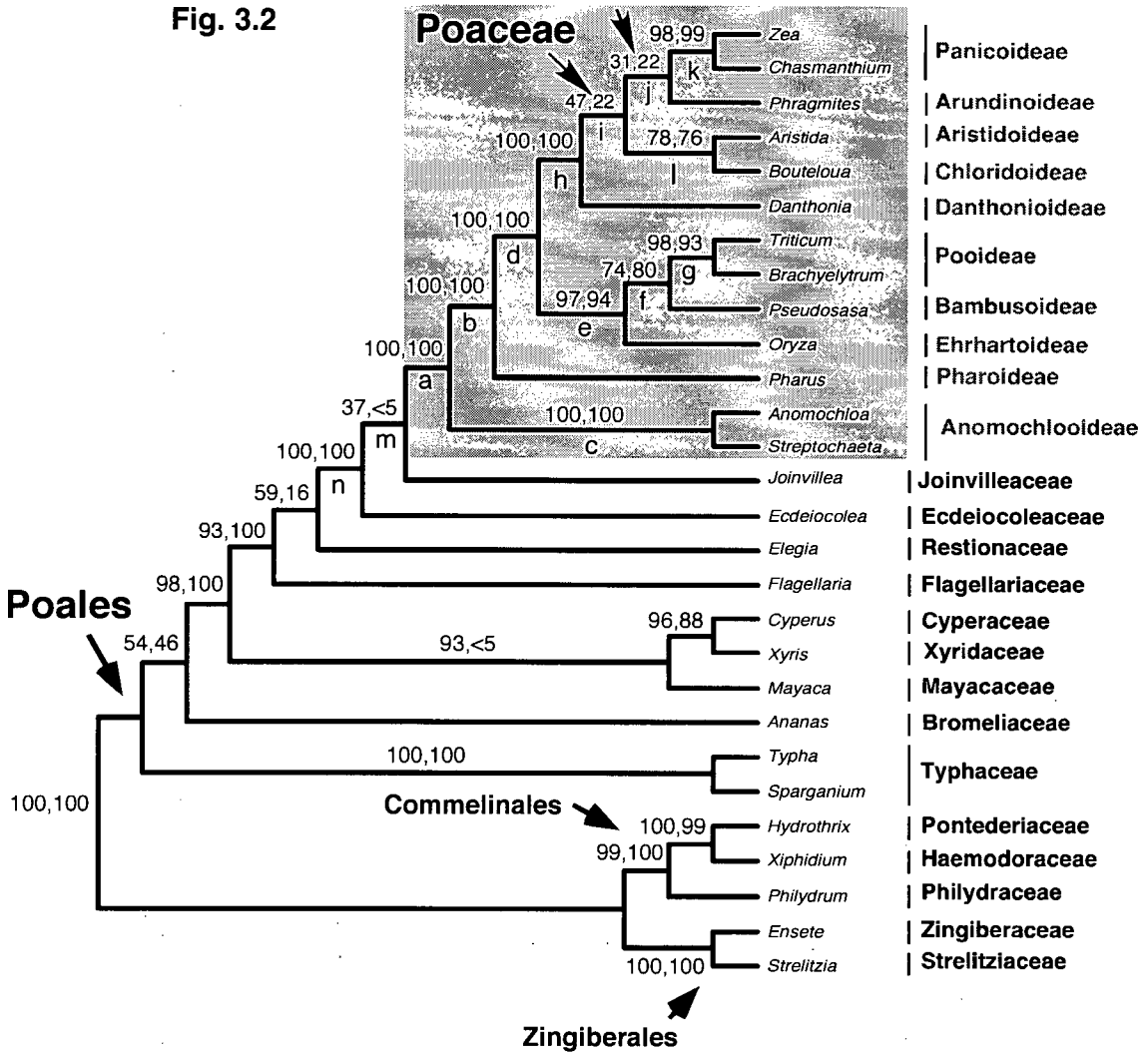

Poaceae
Poales
Commelinales
Zingiberales
98,99
31,22
47,22
100,100
78,76
100,100
98,93
74,80
100,100
97,94
100,100
37,<5
100,100
59,16
93,100
98,100
54,46
93,<5
96,88
100,100
100,100
100,99
99,100
100,100
a
b
c
d
e
f
g
h
i
j
k
l
m
n
Zea
Chasmanthium
Phragmites
Aristida
Bouteloua
Danthonia
Triticum
Brachyelytrum
Pseudosasa
Oryza
Pharus
Anomochloa
Streptochaeta
Joinvillea
Ecdeiocolea
Elegia
Flagellaria
Cyperus
Xyris
Mayaca
Ananas
Typha
Sparganium
Hydrothrix
Xiphidium
Philydrum
Ensete
Strelitzia
Panicoideae
Arundinoideae
Aristidoideae
Chloridoideae
Danthonioideae
Pooideae
Bambusoideae
Ehrhartoideae
Pharoideae
Anomochlooideae
Joinvilleaceae
Ecdeiocoleaceae
Restionaceae
Flagellariaceae
Cyperaceae
Xyridaceae
Mayacaceae
Bromeliaceae
Typhaceae
Pontederiaceae
Haemodoraceae
Philydraceae
Zingiberaceae
Strelitziaceae
PACMAD
BEP

Figure 3.3. Phylogenetic tree obtained from Bayesian analysis of combined data from 17 chloroplast genes for Poaceae and related families in the commelinid monocots. The analysis was conducted with the GTR + G + I model of evolution, with all data considered and an unpartitioned analysis. Numbers above branches are posterior probabilities. Subfamilies within Poaceae, the BEP and PACMAD clades, and names of closely related families are indicated. Letters ("a" to "q") identify branches scored for analyses from partitions of the data.

Fig. 3.3

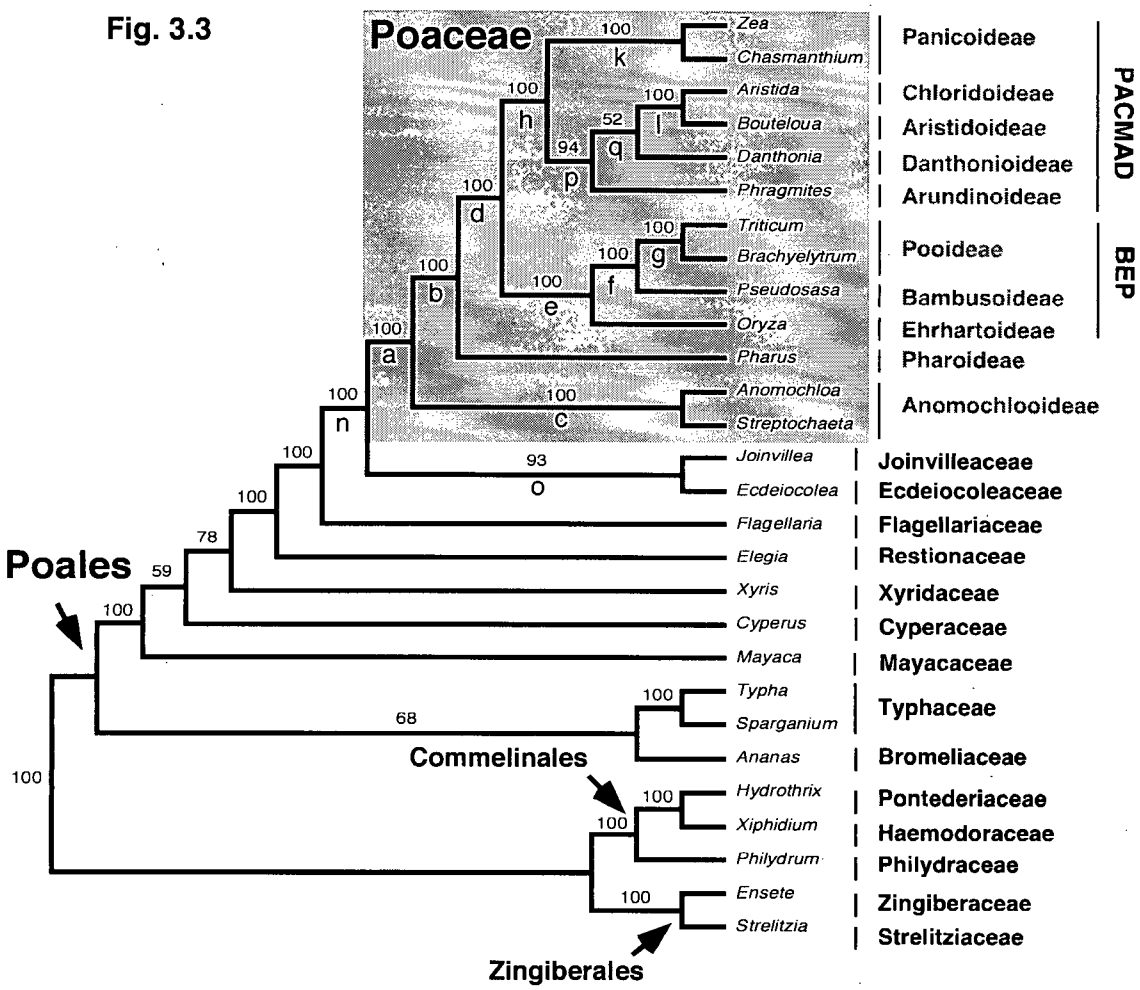

Poaceae
Zea
Chasmanthium
Aristida
Bouteloua
Danthonia
Phragmites
Triticum
Brachyelytrum
Pseudosasa
Oryza
Pharus
Anomochloa
Streptochaeta
Panicoideae
Chloridoideae
Aristidoideae
Danthonioideae
Arundinoideae
Pooideae
Bambusoideae
Ehrhartoideae
Pharoideae
Anomochlooideae
PACMAD
BEP
Joinvillea
Ecdeiocolea
Flagellaria
Elegia
Xyris
Cyperus
Mayaca
Typha
Sparganium
Ananas
Hydrothrix
Xiphidium
Philydrum
Ensete
Strelitzia
Joinvilleaceae
Ecdeiocoleaceae
Flagellariaceae
Restionaceae
Xyridaceae
Cyperaceae
Mayacaceae
Typhaceae
Bromeliaceae
Pontederiaceae
Haemodoraceae
Philydraceae
Zingiberaceae
Strelitziaceae
Poales
Commelinales
Zingiberales

Figure 3.4. Ranked profiles of clade support from analyses of various data partitions, as measured by (A) bootstrap proportions from parsimony analyses; (B) bootstrap proportions from likelihood analyses; and (C) posterior probabilities from Bayesian analyses. Letters "a" to "q" correspond to clades in Figs. 3.2, 3.3; "r" = (Ecdeiocoleaceae–Poaceae); "s" = (PACMAD–Pooideae); "t" = (Chloridoideae–Danthonioideae–Panicoideae); "u" = (Aristidoideae– Arundinoideae); "v" = (*Triticum*–Ehrhartoideae); "w" = Aristidoideae–Arundinoideae –Chloridoideae–*Zea*); "x" = (Chloridoideae–Danthoniodeae); "y" = (Aristidoideae– Chloridoideae–Danthoniodeae–*Zea*); "z" = (Bambusoideae–Ehrhartoideae); and "aa" = (Anomochlooideae–Pharoideae)]. Data are from 17 chloroplast genes from 28 taxa representing Poaceae and several closely related commelinid monocot families. Data partitions examined are indicated in each figure. † indicates analyses where substitution model parameters were estimated independently for each partition (partitions separated by semicolons). Clades with bootstrap proportions or posterior probabilities <5% are not indicated here.

Fig. 3.4A Parsimony

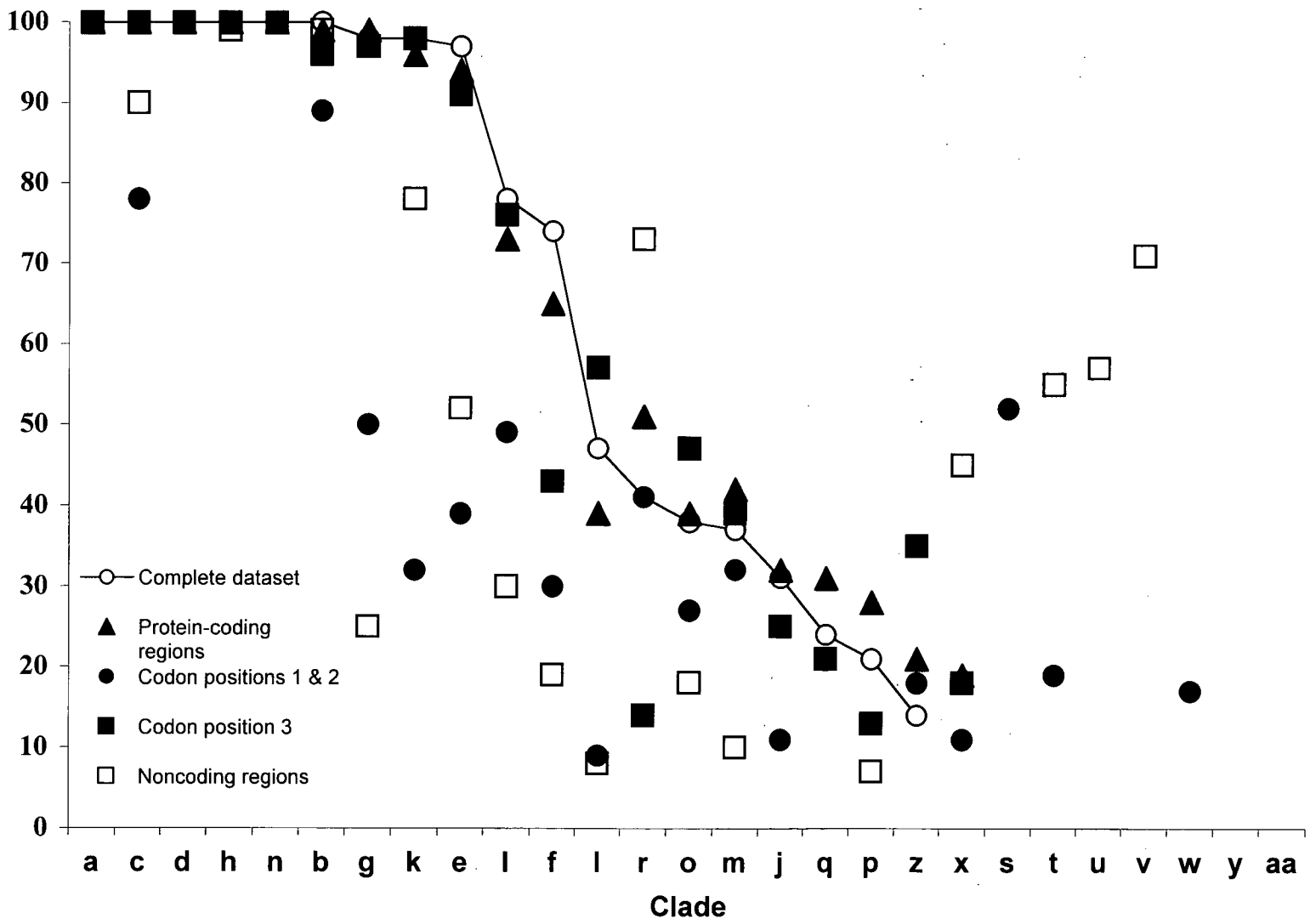


Fig. 3.4B Likelihood

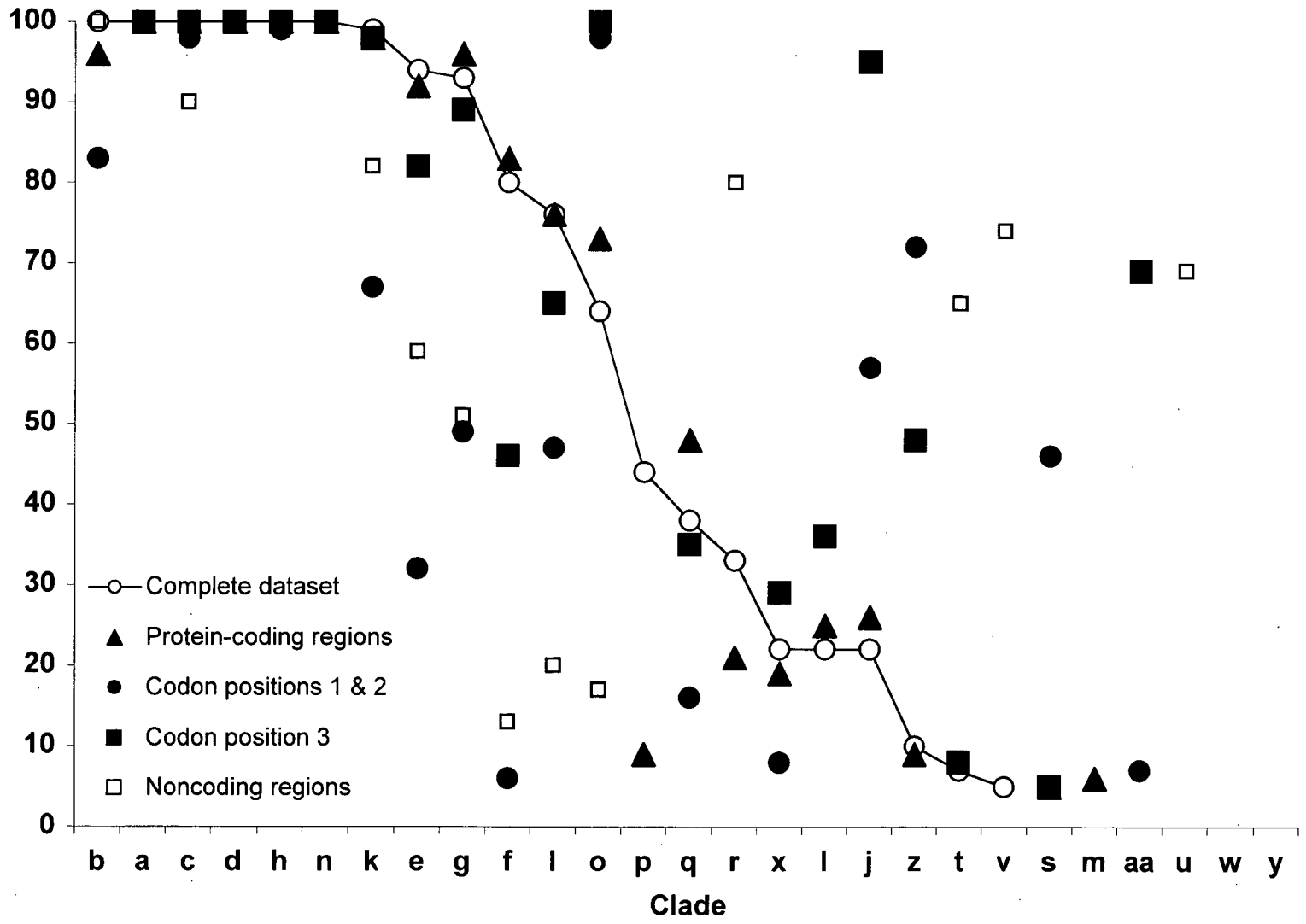
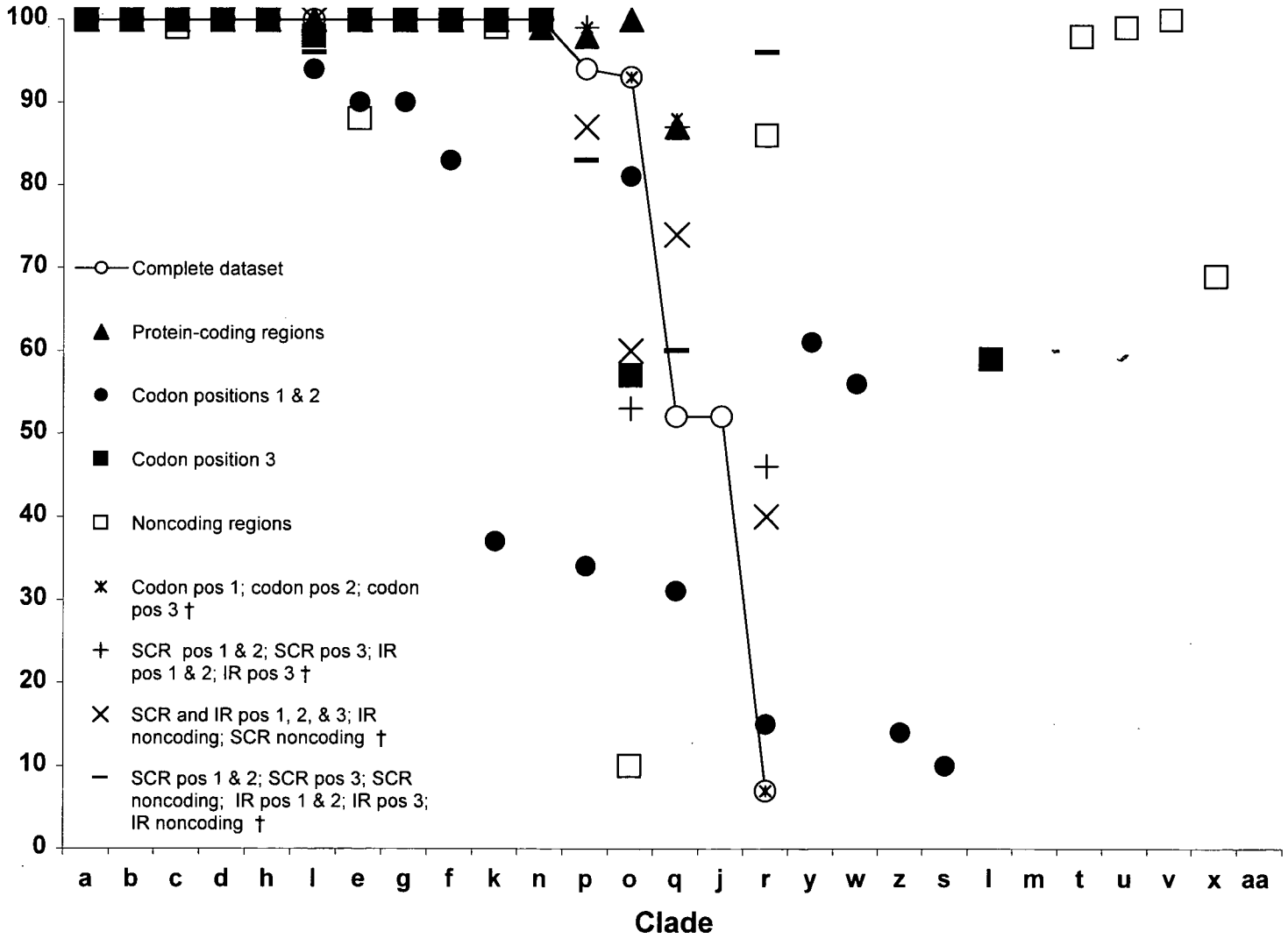


Fig. 3.4C Bayesian



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CHAPTER 4[1]

Higher-Order Phylogenetic Relationships in the Commelinid Monocots, with a Focus on the Orders Commelinales and Poales.

4.1. INTRODUCTION

4.1.1. The Commelinid Monocots

The commelinid monocots (Commelinidae) are a strongly supported clade (e.g., Givnish et al. 1999, 2006; Chase et al. 2000, 2006; Hilu et al. 2003; Davis et al. 2004; Graham et al. 2006) that includes approximately 32,000 species and 28 families – about half of all extant monocot species and one third of all monocot families. A similar group of taxa was identified in the first cladistic monocot study based on morphological characters (Dahlgren and Rasmussen 1983). Commelinids are an ancient angiosperm lineage; a recent estimate places the age of their stem node in the mid-Cretaceous at 122 mya (Janssen and Bremer 2004). They are morphologically and ecologically diverse, and include representatives of most major plant life forms, such as aquatics, submergents, epiphytes, xeromorphs, annuals, perennials, herbs, shrubs, and even trees. Commelinid monocots include several species of major and minor economic concern, such as the cereal grasses (e.g., *Hordeum* L., *Orzya* L., *Saccharum* L., *Sorghum* Moench, *Triticum* L., *Zea* L.), bananas

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[1] Chapter 4 represents a draft of a manuscript that will be submitted for publication:

Saarela, J. M., Rai, H. S., Prentis, P., Briggs, B. G, Marchant, A. D., and Graham, S. W. Phylogenetic relationships among the commelinid monocots, with a focus on the orders Commelinales and Poales.

(*Musa* L. spp.), pineapples (*Ananas* spp.), ginger (*Zingiber* spp.), water chestnut (*Eleocharis dulcis* Hensch.), the horticultural bird-of-paradise (*Strelitzia* Banks ex Dryander spp.), and the aquarium plant *Mayaca* Aublet.

The monophyly of the commelinid monocots is supported by several putative non-DNA synapomorphies, including the presence of ultraviolet fluorescing compounds (e.g., ester-linked ferulic acid and coamuric acid) in unlignified cell walls (Harris and Hartley 1976, 1980; Rudall and Caddick 1994; Rudall and Chase 1996), *Strelitzia*-like epicuticular waxes (long, often curly wax rodlets) (Barthlott and Frölich 1983; Frölich and Barthlott 1988), copiously starchy endosperm and possibly starchy pollen (Dahlgren and Rasmussen 1983; Dahlgren et al. 1985; Zona 2001), and the presence of silica bodies in leaves (Linder and Kellogg 1995). Several of these characters, however, are not present in all taxa, and some also occur outside the commelinid lineage. An additional cell-wall characteristic has recently been identified that may also be synapomorphic for all or most commelinids. Among nine commelinid families examined, the predominant noncellulosic polysaccharides in cell walls in all but Arecaceae are arabinose, xylose, and guluronic acids (highly substituted heteroxylans collectively referred to as glucuronoarabinoxylans [GAXs]; Harris et al. 1997).

Multiple molecular analyses have identified five major lineages with moderate to strong support within the commelinid monocots. In the most recent angiosperm classification, four of these are treated as orders (Arecales, Commelinales, Poales, and Zingiberales), and one family, Dasypogonaceae, is unplaced at the ordinal level (APG II 2003). Close relationships among several of the families in Commelinales, Poales, and Zingiberales have been hypothesized in the past (e.g., Dahlgren et al. 1985; Cronquist 1981,

1988). Affinities of the palms (Arecaceae, the one family in Arecales) — a morphologically distinct and easily recognizable group of trees, shrubs or lianas, with usually unbranched trunks and trimerous flowers — have also been hypothesized previously to be with other commelinid monocots, but also with the screw pines (Pandanaceae), the Panama-hat palm family (Cyclanthaceae), and aroids (Araceae) (Uhle et al. 1995). Placement of Dasypogonaceae, a small and morphologically diverse family of xeromorphic herbs, shrubs, and trees, with usually small and inconspicuous flowers (Dahlgren et al. 1985), among the commelinid monocots (Chase et al. 1995) was largely unexpected.

Dasypogonaceae Dumort. includes four Australian genera (*Baxteria* R. Br. ex Hook., *Calectasia* R. Br., *Dasypogon* R. Br., and *Kingia* R. Br.) that were previously included in the superficially similar family Xanthorreaceae (Asparagales sensu APG II 2003), but morphological and anatomical data indicate clearly that they are distinct (Dahlgren et al. 1985; Rudall and Caddick 1994; Rudall and Chase 1996). *Baxteria* and the tinsel lily genus, *Calectasia*, have sometimes been included in their own families, Baxteriaceae Takht. and Calectasiaceae Endlicher, respectively (e.g., Dahlgren et al. 1985; Takhtajan 1997). Nonetheless, molecular data indicate that the four dasypogonoid genera are a monophyletic group (Chase et al. 1995; Davis et al. 2004), and place them among other commelinids, a position supported by the presence of UV-flourescent compounds in their cell walls (e.g., Chase et al. 1993, 1995, 2006; Rudall and Caddick 1994; Rudall and Chase 1996; Givnish et al. 2006; Graham et al. 2006). Dasypogonaceae do not, however, have *Strelitzia*-like epicuticular wax and possibly starchy pollen, as do most other commelinid lineages (Barthlott and Frölich 1983; Frölich and Barthlott 1988; Rudall and Chase 1996; Zona 2001).

Phylogenetic relationships among the five major commelinid lineages have varied in previous studies (e.g., Chase et al. 1993, 1995, 2000, 2006; Givnish et al. 1999, 2006; Davis et al. 2004; Tamura et al. 2004; Graham et al. 2006). Most analyses infer a sister group relationship between Commelinales (see below), and Zingiberales. The latter order are a large clade that includes eight families of large, tropical, herbaceous monocots: the 'banana-families' (Lowiaceae, Heliconiaceae, Musaceae, and Strelitziaceae) with large, banana-like leaves and generally five fertile stamens, and the 'ginger-families' (a clade that includes Cannaceae, Costaceae, Marantaceae, and Zingiberaceae) generally with one fertile stamen (Kress et al. 2001). Arecales and Dasypogonaceae have been variously and weakly [i.e., bootstrap proportion (BP) < 70%] placed with respect to Commelinales–Zingiberales and Poales (e.g., Chase et al. 2000, 2006; Hilu et al. 2003; Davis et al. 2004; Tamura et al. 2004; Givnish et al. 1999, 2006; Graham et al. 2006). Some studies indicate that Arecales and Dasypogonaceae are the first two successive lineages among the five lineages; each has been identified as the sister-group of the rest of the commelinids (Chase et al. 2000; Davis et al. 2004). Two recent multigene studies suggest different sets of relationships, although support for these is consistently weak. A seven-gene study placed Dasypogonaceae as the sister group of Poales, and Arecales as the sister group of Commelinales–Zingiberales (Chase et al. 2006). In contrast, a 17-gene study placed Arecales as the sister group of Poales, and Dasypogonaceae as the sister group of Commelinales–Zingiberales (Graham et al. 2006).

4.1.2. Phylogenetic Relationships in the Order Commelinales

Most molecular studies of monocots have identified a clade that includes the families Commelinaceae, Haemodoraceae, Hanguanaceae, Philydraceae, and Pontederiaceae (e.g.,

Chase et al. 1993, 1995, 2000, 2006; Savolainen et al. 2000; Soltis et al. 2000; Givinish et al. 1999, 2006; Graham et al. 2002; Davis et al. 2004). In the most recent angiosperm classification, this clade is recognized as the order Commelinales (APG II 2003). In previous morphology-based classifications, these five families were not thought to be closely related, and were variously allied with or placed in taxa that are now included in most orders of monocots, sensu APG II 2003 (e.g., Cronquist 1981, 1988; Dahlgren and Rasmussen 1982; Dahlgren et al. 1985; Thorne 1992a,b; Takhtajan 1997). For example, Dahlgren et al. (1985) included Haemodoraceae, Philydraceae, and Pontederiaceae in their own monofamilial orders, Hanguanaceae in Asparagales, and Commelinaceae in an order (Commelinales) with the distantly related families Eriocaulaceae, Mayacaceae, Rapateaceae, and Xyridaceae.

Commelinales are relatively small in terms of numbers of genera and species (Table 4.1), but the order is geographically and ecologically diverse. Commelinaceae and Haemodoraceae are largely terrestrial families, Hanguanaceae generally occur in marshy habitats, Philydraceae occur in wet areas, and Pontederiaceae are freshwater aquatics. Within-family phylogenetic relationships have been characterized recently in Commelinaceae (Evans et al. 2000, 2003), Haemodoraceae (Simpson 1990; Hopper et al. 1999), and Pontederiaceae (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Graham et al. 1998, 2002), but no study has sampled the four genera of Philydraceae.

Inferred relationships among families in Commelinales have also varied. Most studies have included only one or a few Commelinales representatives (e.g., Chase et al. 1993; Davis 1995; Soltis et al. 2000; Tamura et al. 2004; Davis et al. 2004; Graham et al. 2006), but four studies have attained complete family-level sampling for the order. Based on the plastid

*rbc*L gene and the mitochondrial *atp*A gene, Davis et al. (2004) inferred a sister-group relationship between Philydraceae and Haemodoraceae, and found a clade that identified Hanguanaceae as the sister-group of Pontederiaceae–Commelinaceae. In contrast, analyses based on the plastid genes *ndh*F and *rbc*L inferred a sister group relationship between Philydraceae and Pontederiaceae, and found a clade that identified Haemodoraceae as the sister-group of Commelinaceae–Hanguanaceae (Graham et al. 2002). All relationships in these two-gene studies received bootstrap support less than 50%. Based on the plastid gene *ndh*F but with much denser sampling across monocots compared with Graham et al. (2002), Givnish et al. (2006) identified two well supported clades (Haemodoraceae–Pontederiaceae and Commelinaceae–Hanguanaceae), with Philydraceae inferred weakly to be the sister group of the rest of the order. Finally, in a recent study based on multiple mitochondrial, nuclear, and plastid regions, Chase et al. (2006) inferred a Commelinaceae–Hanguanaceae clade that is the sister group of a (Philydraceae (Haemodoraceae–Pontederiaceae)) clade. Both two-family clades received strong support (i.e., BP $\geq$ 90%) in this study, but the position of Philydraceae received only moderate support (i.e., BP = 70-89%). No study has robustly inferred relationships among all Commelinales families.

4.1.3. Phylogenetic Relationships in the Order Poales

The clade currently classified as the order Poales (APG II 2003) was identified in the first angiosperm- and monocot-wide molecular phylogenetic studies (e.g. Chase et al. 1993; Duvall et al. 1993), and it has since been recovered with moderate to strong support in several studies (e.g., Givnish et al. 1999, 2006; Tamura et al. 2004; Chase et al. 2006; Graham et al. 2006). According to the most recent monocot classification, Poales includes

17 families (Chase 2004; Table 4.1). In earlier classifications, several of these families were variously included in multiple orders (e.g., Cronquist 1981, 1988; Dahlgren et al. 1985), often with families with which they are now known to be related to only distantly. Close relationships among other families currently included in the order, however, have been hypothesized previously. For example, with the exception of Commelinaceae (see above), all families that Cronquist (1988) included in his subclass Commelinidae are currently part of Poales. Hydatellaceae, a family that has traditionally been difficult to place among any of the orders of monocots (e.g., Dahlgren et al. 1985; Hamann 1998) was included in Poales by APG II (2003), but recent work has demonstrated that it is not part of the clade, or even monocots as a whole (see Chapter 5).

Poales includes several taxa that are among the most readily-identifiable to family – and most taxonomically difficult – monocot groups, such as the grasses (Poaceae), sedges (Cyperaceae), and pineapples (Bromeliaceae). Many of the monocot families with highly modified, wind-pollinated flowers are part of Poales, although the order also includes several families with showy perianths or bracts, and insect pollination. Poales includes several small families with only one or a few genera and species, (e.g., Ecdeiocoleaceae, Thurniaceae), and others that are among the largest angiosperm families, with hundreds of genera, thousands of species, and cosmopolitan distributions (e.g., Poaceae, Cyperaceae) (Table 4.1). In addition to the 16 families currently included in the order (excluding Hydatellaceae; Table 4.1), several additional small, segregate families have been described and recognized recently (e.g., Hopkinsiaceae, Lyginiaceae, Mapaniaceae, Prioniaceae) (APG 1998; Briggs and Johnson 1998; Munro and Linder 1998; Shipunov 2003). These are included in more inclusive taxa (Table 4.1) following APG II (2003).

Molecular studies have clarified several aspects of relationship among Poales families (e.g., Givnish et al. 1999, 2006; Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004; Chase et al. 2006; Graham et al. 2006). Most studies reveal a basal grade of families that includes Bromeliaceae, Rapateaceae, and Typhaceae [the 'basal Poales' sensu Linder and Rudall (2005)], but the exact divergence pattern of these families is not well established. Most previous analyses indicate variously that Bromeliaceae and Typhaceae are a clade (Bremer et al. 2002; Chase et al. 2006; Givnish et al. 2006) or that they are respectiively the successive sister groups of the rest of Poales (Michelangeli et al. 2003; Graham et al. 2006). Rapateaceae have been variously inferred to be the sister group of Bromeliaceae (Clark et al. 1993; Chase et al. 1995; Linder and Kellogg 1995; Givnish et al. 1999), the sister group all Poales (Chase et al. 2000; Bremer et al. 2002), the sister group of the xyrid clade (Davis et al. 2004), or the sister group of all Poales except Bromeliaceae and Typhaceae (Chase et al. 2006; Givnish et al. 2006).

The remainder of Poales includes 13 families and four major clades [the 'cyperid', 'graminid', 'restiid', and 'xyrid' clades, sensu Linder and Rudall (2005)], although a few families have not been unequivocally placed in one of these groups. Most of these families have a substantially accelerated rate of plastid evolution compared with other monocots, but the reason for this is not yet clear (Givnish et al. 1999, 2006; Graham et al. 2006; Chapter 3). A close relationship has been inferred between the graminid and restiid clades (e.g., Michelangeli et al. 2003; Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006;), and this large clade has been referred to as the 'core Poales' (Linder and Rudall 2005; I use this designation here) or the 'graminoid' clade (Bremer 2002). Relationships among the core

Poales and the cyperid and xyrid clades, however, are not yet fully established (e.g., Bremer 2002; Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006).

The restiid clade includes the 'southern rush' families Anarthriaceae, Centrolepidaceae, and Restionaceae (Linder and Rudall 2005; Table 4.1). Analyses of different plastid genes variously resolve Centrolepidaceae as the sister group of Restionaceae (Briggs et al. 2000; Michelangeli et al. 2003), or include it within Restionaceae (Linder and Rudall 1993; Bremer 2002), the latter circumscription supported by morphological data (e.g., Hamann 1975; Linder and Kellogg 1995; Linder et al. 2000). It is not clear if Centrolepidaceae are a distinct lineage, or if they are part of a paraphyletic Restionaceae. The western Australian genera, *Hopkinsia* and *Lyginia*, were recently segregated from Restionaceae on the basis of several morphological and anatomical differences, and placed in their own monogeneric families, Hopkinsiaceae and Lyginiaceae (Briggs and Johnson 2000; Linder et al. 2000). The few molecular studies that have sampled these genera place them with the monogeneric Anarthriaceae in a clade that is the sister group of Centrolepidaceae/ Restionaceae (e.g., Briggs et al. 2000; Bremer 2002). Following recommendations by APG II (2003) not to recognize small, monogeneric families that are clearly sister taxa, *Hopkinisia* and *Lyginia* are now included with *Anarthria* in Anarthriaceae (APG II 2003).

The graminid clade includes Ecdeiocoleaceae, Joinvilleaceae, Poaceae, and sometimes Flagellariaceae (e.g., Michelangeli et al. 2003; Tamura et al. 2004; Davis et al. 2004; Linder and Rudall 2005; Chase et al. 2006). An Ecdeiocoleaceae–Joinvilleaceae–Poaceae clade has been consistently recovered with strong support, but relationships among these families remain largely equivocal (e.g., Campbell and Kellogg 1987; Doyle et al. 1992; Linder and Rudall 1993; Bremer 2000, 2002; Michelangeli et al. 2003; Davis et al. 2004;

Chapter 3). The identity of the sister-group of the grasses – the world's most economically important family of plants – is unfortunately not clear (Chapter 3). Resolution of this phylogenetically critical trichotomy has important implications for characterizing homologies of grass reproductive structures among monocots (e.g., Rudall et al. 2005). Flagellariaceae have been variously inferred to be the sister group of Ecdeiocoleaceae–Joinvilleaceae–Poaceae, a larger restiid–graminid clade, or the xyrid clade (e.g., Bremer 2002; Davis et al. 2004; Chase et al. 2006; Graham et al. 2006; Givnish et al. 2006).

The cyperid clade – equivalent in circumscription to the former Cyperales sensu Dahlgren et al. (1985) – includes the large and cosmopolitan sedge (Cyperaceae) and rush (Juncaceae) families, and Thurniaceae (e.g., Givnish et al. 1999, 2006; Chase et al. 2000, 2006; Bremer 2002; Michelangeli et al. 2004; Linder and Rudall 2005). A sister-group relationship between Cyperaceae and Juncaceae is supported by morphological and molecular data, with Thurniaceae then resolved as their sister group. Cyperaceae are morphologically diverse, and recent molecular work on the family has identified two major sedge clades (e.g., Muasya et al. 1998, 2000; Simpson et al. 2003, 2006) which are recognized as subfamilies Mapaniodeae C. B. Clark and Cyperoideae in the most recent Cyperaceae classification (Simpson 2006). Shipunov (2003) recognized Mapanioideae at family rank (Mapaniaceae Shipunov), but recognition of two sedge families has not been accepted by cyperologists. The monotypic South African genus, *Prionium*, was traditionally included in Juncaceae on the basis of morphological characteristics (Cutler 1969; Dahlgren and Clifford 1982; Dahlgren et al. 1985; Simpson 1995), but early molecular analyses indicated that it is not part of Juncaceae and it was therefore placed in its own family, Prioniaceae (Munro and Linder 1998; APG 1998). Subsequent molecular studies have

placed *Prionium* in a clade with *Thurnia* (Thurniaceae) (e.g., Chase et al. 1993; Munro and Linder 1998; Bremer 2002; Davis et al. 2004), and it is now included in Thurniaceae (APG II 2003; see rationale for combining monogeneric families discussed above). Several molecular studies have indicated that Juncaceae may not be monophyletic, because the South American cushion-forming genus *Oxychloe* (traditionally included in Juncaceae) was placed as the sister group of, or within, Cyperaceae (e.g., Chase et al. 1993; Plunkett et al. 1995; Muasya et al. 1998, 2000). However, these earlier studies were based on misidentified material; subsequent analyses have found that *Oxychloe* is part of Juncaceae, indicating that the family as traditionally circumscribed is natural (Kristiansen et al. 2005; Roalson 2005).

Family composition of the xyrid clade has varied among studies, and support for the clade and relationships within it have generally been low (e.g., Chase et al. 2000, 2006; Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004; Givnish et al. 2006). Linder and Rudall (2005) suggested that the xyrid clade probably contains the families Eriocaulaceae, Hydatellaceae, Mayacaceae, and Xyridaceae. Several studies have inferred a close relationship between Eriocaulaceae and Xyridaceae (Stevenson and Loconte 1995; Chase et al. 2000, 2006; Bremer 2002; Givnish et al. 2006). Some studies indicate that Xyridaceae may not be monophyletic (e.g., Davis et al. 2004), a finding that is possibly consistent with the sometimes-recognized segregate family, Abolbodaceae Nakai (e.g., Steyermark 1984; APG 1998). Mayacaceae have been variously inferred to be the sister group of Xyridaceae (Bremer 2002; Davis et al. 2004), the cyperid clade (Chase et al. 2006), or the cyperid clade and Eriocaulaceae–Xyridaceae (Givnish et al. 2006). Hydatellaceae have been placed among other xyrid families in some previous studies, but this was based on a contaminant *rbc*L sequence that is a chimera of a grass and a moss (Chapter 5). I have demonstrated that

Hydatellaceae are not part of Poales (Chapter 5), and I therefore do not discuss this family further here.

4.1.4. Objectives of the Current Study

Major outstanding questions in higher commelinid monocot phylogenetics include the relative branching orders of Arecales, Dasypogonaceae, Commelinales–Zingiberales, and Poales; relationships among the five families in Commelinales, particularly the position of Philydraceae; and relationships among families in Poales, particularly the relative branching orders of Bromeliaceae, Rapateaceae, and Typhaceae, the positions of Flagellariaceae and Mayacaceae, the compositions and interrelationships of the xyrid, cyperid, restiid, and graminid clades, and the identity of the sister group of the grasses. Strategies to obtain more resolved and better supported phylogenetic trees include examining more characters per taxon (e.g., Hillis 1998; Poe and Swofford 1999; Graham and Olmstead 2000a; Rokas et al. 2003; Graham et al. 2006), and sampling major lineages more densely (e.g., Hillis 1998; Swofford et al. 2001). The former approach has helped resolve several aspects of deep relationship among basal angiosperms (Graham and Olmstead 2000a, b; Graham et al. 2000), cycads (Rai et al. 2003), Liliales (Zgurski 2004), Asparagales (McPherson 2003), Poaceae (see Chapter 3), and monocots as a whole (Graham et al. 2006). To clarify relationships in the commelinid monocots, I take this approach here, supplemented with denser taxon sampling than attempted before. My approach is to sample multiple plastid genes and associated noncoding regions (Graham and Olmstead 2000a) from multiple exemplar taxa (in most cases) for each of the commelinid families, with a focus on the orders Commelinales and Poales.

4.2 MATERIALS AND METHODS

4.2.1. Taxonomic Sampling

The current taxon sampling includes representatives from each of the five major commelinid lineages, including Dasypogonaceae, Arecales, and all families in Commelinales and Poales. I sampled multiple representatives from most families in Commelinales and Poales, and single exemplars from Eriocaulaceae, Restionaceae, and the monogeneric Flagellariaceae, Hanguanaceae, and Mayacaceae (Table 4.2). This taxon sampling also includes exemplars from each of the segregate families that have been recognized recently (i.e., Abolbodaceae, Hopkinsiaceae, Lyginiaceae, Mapaniaceae, Prioniaceae; see Introduction). To maximize the morphological, molecular, and taxonomic diversity sampled in large families, I attempted to choose exemplar taxa from different clades (often classified as subfamilies or tribes in rank-based classifications) that span the root node of each family, based on earlier phylogenetic studies (e.g., Simpson 1990; Bruhl 1995; Terry et al. 1997; Graham et al. 1998, 2002; Hopper et al. 1999; Horres et al. 2000; Givnish et al. 2000, 2006; GPWG 2001; Evans et al. 2003; Drábková et al. 2003; Linder et al. 2003; Simpson et al. 2003; Campbell 2004; Roalson 2005). In the current study, 28 taxa are new, compared to the eleven new taxa examined in Chapter 3.

In Commelinales, taxon sampling is greatest in Philydraceae, where I sampled a representative from each of its three to four genera. I attempted to sample each of the six species in Philydraceae, but DNA for two species was not available. Material from three *Helmholtzia novoguineensis* (K. Krausse) Skotts. herbarium specimens [*Brass 13431* (GH), *Brass 12859* (GH), and *Hoogland & Craven 11068* (GH)] and a collection of *Philydrella pygmaea* (R. Br.) Caruel [*Morrison s.n.* (BRI)] was recalcitrant to molecular study. Within

Poales, taxon sampling is most dense within Poaceae, where I include 13 taxa examined previously (Chapter 3), recently published sequence data from three Poaceae plastid genomes (Table 4.3) and new sequence data for *Puelia oylriformis* (Puelioideae), one of two grass subfamilies not included in my earlier study (Chapter 3).

4.2.2. Genomic Sampling

I obtained DNA from various sources, or extracted DNA from field-collected, silica gel-dried leaf material, using the CTAB protocol of Doyle and Doyle (1987), with the addition of 2% β-mercaptoethanol to each extraction. For a subset of taxa, two different species (*Anigozanthos*, *Mapania*) or two different individuals (*Anarthria*) were used to obtain the plastid data (Table 4.2). I amplified and sequenced multiple plastid genes involved in a range of functions, including photosynthesis (*atp*B, *psb*B, *psb*C, *psb*D, *psb*E, *psb*F, *psb*H, *psb*J, *psb*L, *psb*N, *psb*T, *rbc*L), chlororespiration (*ndh*B and *ndh*F), and plastid translation (*rpl*2, *rps*7, 3'-*rps*12), and associated noncoding regions (intergenic spacers that span *psb*B–T–N–H, *psb*E–F–L–J, and 3'-*rps*12–*rps*7 gene clusters, and introns in *rpl*2, 3'-*rps*12, and *ndh*B), using the methods and primers described in Graham and Olmstead (2000a), McPherson (2003), and Graham et al. (2006). I used additional primers from Zurawski et al. (1984), Olmstead and Sweere (1994), Hoot et al. (1995), Kim and Jansen (1995); Neyland and Urbatsch (1996a,b), and Graham et al. (1998), and the following two previously unpublished primers designed by H. S. Rai (UBC): C91F: 5'-TTGTGAGGTACARCAATTATTAGG-3' (*atp*B), and F45R: 5'-CATTAAAGAGCG TTTCCAC-3' (*psb*D). I sequenced all regions in forward and reverse directions, and in most cases I obtained sequencing products from duplicate PCR products amplified from independent DNA extractions to detect pipetting errors or cross-contamination (I observed

none). A subset of sequences for regions that I was unable to amplify and sequence satisfactorily are from GenBank (Table 4.2).

4.2.3. Data Assembly

I performed basecalling and contig assembly using Sequencher 4.1 (Genes Code Corporation, Ann Arbor, MI), and determined gene boundaries by direct comparisons with sequences of *Nicotiana tabacum* L. (Shinozaki et al. 1986) and *Ginkgo biloba* L. I added sequences to a previously published alignment that includes representatives from across extant seed plants, including monilophytes, conifers, cycads, gnetophytes, basal angiosperms, magnoliids, eudicots and monocots (Graham and Olmstead 2000a; Rai et al. 2003; McPherson 2003; Zgurski 2004; Graham et al. 2006). The matrix includes two exemplar taxa from each of Dasypogonaceae and Zingiberales (Musaceae and Strelitziaceae), and a single exemplar from Arecaceae. I added data for portions of the *psb*L-*psb*J intergenic spacer and *psb*J missing from a previous version of the matrix for *Acorus calamus* L. (positions 63750-63950; NC_007407; Goremykin et al. 2005), 195 bp from the 3'-end of *rbc*L that was not included in previous versions of the matrix for *Triticum aestivum* (NC_002762; Ogihara et al. 2000) and *Zea mays* (NC_001666; Maier et al. 1995), and data for the current plastid genome sampling for 20 taxa representing 17 families and 14 orders of eudicots (APG II 2003), and four monocot taxa (three grasses and an orchid), most from recently published plastid genomes (Table 4.3). The eudicot exemplars included here are an expansion from the seven orders represented in Graham and Olmstead (2000a). The *ndh*F gene is absent from the genome of *Phalaenopsis afrodite* (Chang et al. 2006), and *ndh*F data are not available for *Ranunculus macranthus*. The final matrix includes 221 taxa.

I aligned sequences manually using Se-Al version 1.0 alpha (Rambaut 1998) according to guidelines outlined in Graham et al. (2000). The total length of the aligned data matrix is 31, 285 base pairs (bp). The unaligned total sequence length of exemplar taxa added here range from ~10.4 kb in *Anarthria* to ~15.1 kb in *Aphelia*; one major exception is *Lyginia*, for which I was only able to obtain ~6.4 kb of sequence data. For multiple taxa I was unable to amplify and sequence ~200 bp at the 3'-end of *rbc*L and the second exon of 3'-*rps*12. Aside from these instances, all taxa are nearly completely represented for all regions. The aligned data matrix is substantially larger than the unaligned length for any single taxon because of substantial gaps and/ or unalignable regions, including some from non-angiosperm taxa that are part of the matrix but not included in this study. I set aside noncoding regions that were too difficult to align as staggered unique gapped regions. The unique regions are effectively ignored for parsimony-based tree searches and scores (Graham et al. 2006), and should have only minimal effect for model-based methods (e.g., on estimation of base frequency parameter values). I did not include insertion/ deletion (indel) events in the analysis, and coded gaps in the data as 'missing data'. Further details on the alignment and the data matrix are outlined in Graham and Olmstead (2000a), Rai et al. (2003), McPherson (2003) and Graham et al. (2006).

4.2.4. Parsimony and Likelihood Phylogenetic Analyses

In a previous study, I observed several instances of moderate to strong conflict between protein coding and noncoding partitions of the data examined here for fewer monocots (Chapter 3). Until the source of this conflict can be resolved, it seems reasonable to exclude most of these noncoding regions from consideration. In my analyses here, I

therefore include only protein-coding regions and some very conservative noncoding regions in the inverted repeat, including three introns in *rpl*2, 3'-*rps*12, and *ndh*B, and intergenic spacers between 3'-*rps*12-*rps*7, *rps*7-*ndh*B, and *ndh*B-*trn*L(CAA) (i.e., intergenic spacers between the various photosystem II genes are excluded). In Chapter 3, I also observed substantial inflation of Bayesian posterior probabilities compared with bootstrap support from parsimony and likelihood analyses, consistent with the findings of several studies (e.g., Suzuki et al. 2002; Cummings et al. 2003; Erixon et al. 2003); I therefore do not include Bayesian analyses here.

I examined two different taxon sets with maximum parsimony: a large angiosperm-wide 159-taxon set, and a smaller 113-taxon set representing all major lineages of monocots, with dense sampling in commelinids. For maximum likelihood analysis, I examined a 69-taxon subset of this smaller taxon set that included all commelinid taxa and five outgroup taxa from Asparagales and Liliales. For the 159-taxon set, I accepted *Amborella* as the probable sister group of the rest of the angiosperms (e.g., Graham and Olmstead 2000a; Hilu et al. 2003; Chapter 5) and used it to root the trees. For the 113-taxon set I accepted *Acorus* as the probable sister group of the rest of the monocots (e.g., Duvall et al. 1993b; Chase et al. 2000; Graham et al. 2006), and for the 69-taxon set I rooted the trees with the exemplars from Asparagales and Liliales.

I performed MP searches using PAUP* version 4.0b10 (Swofford 2002), with all characters and character-state changes equally weighted, using tree bisection-reconnection (TBR) branch-swapping, and with 10 random addition replicates performed for each search, but otherwise using default conditions. I performed the maximum likelihood searches using PHYML version 2.4.4 (Guindon and Gascuel 2003). I chose an optimal DNA substitution

model using the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC), as implemented in ModelTest ver. 3.7 (Posada and Crandall 1998). The optimal model was GTR + Γ + I [general time reversible rate (GTR) model with a proportion of invariant sites (I) considered and a gamma (Γ) distribution used to account for among-site rate variation]. I estimated all model parameters from the data, and otherwise used default settings in PHYML.

I estimated branch support for MP and ML using nonparametric bootstrap analysis (Felsenstein 1985). For MP bootstrap analysis, I used 100 bootstrap replicates, with the same search parameters as the initial search, but using a single random addition replicate per bootstrap replicate. For ML bootstrap analysis I used 100 bootstrap replicates. I consider "strongly" supported or "robust" branches to have bootstrap support of 90% or more, "moderately" supported branches to have bootstrap support 70-89%, and "weakly" or "poorly" supported branches to have bootstrap support < 70%. I obtained bootstrap support values <50% from the bootstrap log in PAUP*.

4.3 RESULTS

4.3.1. Outgroup Relationships

Outgroup relationships for basal angiosperms, monocots as a whole, Asparagales, and Liliales are discussed elsewehere (Graham and Olmstead 2000a; Graham et al. 2000, 2006; McPherson 2003; Zgurski 2004), therefore I do not consider these further here. My angiosperm-wide analysis (Fig. 4.1) included 20 eudicot exemplars not included in these previous analyses. Within the eudicots, I generally identify the same higher order lineages (i.e., rosids and asterids), Saxifragales, and a grade of 'basal' lineages (Ranunculales,

Proteales, Trochodendrales, Gunnerales) that have been inferred in previous angiosperm-wide studies (Fig. 4.2; reviewed in Soltis et al. 2005). However, relationships among the rosid lineages differ in several aspects compared with a recent analysis that considered data for complete plastid genomes for a smaller complement of taxa and with no Rosales exemplars (Cai et al. 2006). Interrelationships among Vitales, asterids, and rosids are not clear here (Fig. 4.2). I infer monocots to be the sister group of eudicots and *Ceratophyllum*, but this relationship receives very weak support here [maximum parsimony bootstrap proportion (BPMP) < 50%; Fig. 4.2). Eudicots are strongly supported as monophyletic (BPMP = 100%; Fig. 4.2). The monophyly of the monocots is only weakly supported (BPMP = 56%, Fig. 4.2; but see Graham et al. (2006) for details on how exclusion of *Ceratophyllum* from analyis substantially increases bootstrap support for monocots as a whole).

4.3.2. Relationships in the Commelinid Monocots

Within the monocots, commelinid monocots are strongly supported as a clade [BPMP = 100% and maximum likelihood bootstrap proportion (BPML) = 100%; Figs. 4.3–4.5] that is the sister-group of Asparagales (BPMP = 99%; Figs. 4.3, 4.4). Commelinales, Poales, and Zingiberales sensu APG II (2003) are each strongly supported as monophyletic in parsimony and likelihood analyses [BPMP = 100% (for each order) and BPML = 100% (for Poales and Zingiberales) and 94% for Commelinales; Figs. 4.4, 4.5]. Commelinales and Zingiberales are strongly supported as a clade (BPMP = 100% and BPML = 100%; Figs. 4.3–4.5), but the relative positions of Arecales, Dasypogonaceae, Commelinales–Zingiberales, and Poales are not clear here. In parsimony and likelihood analyses, Arecales are variously placed as the sister group of Commelinales–Zingiberales [BPMP = 46% (Fig. 4.4) and BPML = 30 % (data

not shown) or the sister group of all commelinids except Dasypogonaceae [BPMP = 37% (data not shown) and BPML = 37%, Fig. 4.5], and Dasypogonaceae are variously placed as the sister-group of all remaining commelinids [BPMP = 13% (data not shown) and BPML= 34%, Fig. 4.5)], the sister group of all commelinids except Arecales (BPMP = 32% and BPML = 34%, data not shown), or the sister group of Poales [BPMP = 53% (Fig. 4.4) and BPML = 13% (data not shown)]. Maximum likelihood analysis provides weak support for a sister group relationship between Commelinales–Zinbigerales and Poales (BPML = 64%; Fig 4.5); this relationship receives very weak support from parsimony analysis (BPMP = 37%; data not shown).

4.3.3. Phylogenetic Relationships in Commelinales

Commelinaceae and Hanguanaceae are strongly supported as sister taxa (BPMP and BPML = 100%; Figs 4.4, 4.5). I find moderate support for the branch supporting a sister-group relationship between Philydraceae and Haemodoraceae–Pontederiaceae (BPMP = 80% and BPML = 83%, Figs. 4.4, 4.5), and moderate to strong support for the branch within Philydraceae that identifies a sister group relationship between *Philydrella* and *Philydrum–Helmholtzia* (BPMP = 94% and BPML = 84%; Figs. 4.4, 4.5); Philydraceae are monophyletic (BPMP and BPML = 100%; Figs. 4.4, 4.5).

4.3.4. Phylogenetic Relationships in Poales

All families in Poales sampled here with more than one exemplar per family are monophyletic for the current taxon sampling and receive 100% bootstrap support from parsimony and likelihood analyses (Figs. 4.4, 4.5). Within Poales, I find a moderately to

strongly supported clade that includes all Poales families except Bromeliaceae and Typhaceae (BPMP = 78% and BPML = 98%; Figs. 4.4, 4.5); the relative positions of these latter two families at the base of Poales are not clear here. Rapateaceae are weakly identified as the sister group of the rest of this large clade (BPMP = 59% and BPML = 35%; Figs. 4.4, 4.5). I also find 99-100% bootstrap support from parsimony and likelihood analyses for several groups in Poales, including a cyperid clade (Thurniaceae, (Juncaceae–Cyperaceae)), a restiid clade (Anarthriaceae (Centrolepidaceae–Restionaceae)), and an Ecdeiocoleaceae–Joinvilleaceae–Poaceae clade (Figs. 4.4, 4.5). Flagellariaceae and these latter three families are a clade with moderate support (BPMP = 87%; Fig. 4.4) to strong (BPML = 98%; Fig. 4.5), and these four families (the graminid clade) are strongly supported as the sister group of the restiid clade (Figs. 4.4, 4.5). The graminid and restiid clades together represent the core Poales. Within the graminid clade, Ecdeiocoleaceae are weakly inferred to be the sister group of Poaceae (BPMP = 62% and BPML = 62%; Figs. 4.4, 4.5). Relationships among subfamilies within Poaceae are generally consistent with those inferred in my earlier study (Chapter 3), and Puelioideae are inferred here with strong support (BPMP and BPML = 100%; Figs. 4.4, 4.5) to be the third of three successive lineages (following Anomochlooideae and Pharoideae, respectively) that are the sister groups of the remaining grasses.

Relationships among Eriocaulaceae, Mayacaceae, and Xyridaceae differ between maximum parsimony and maximum likelihood analyses. In parsimony analysis, a clade that includes these three families receives very weak support (BPMP = 56%; Fig. 4.4), and Eriocaulaceae and Mayaceae are moderately supported as sister taxa (BPMP = 78%; Fig. 4.4). The position of this xyrid clade with respect to the cyperid clade or the core Poales is not clear here (Fig. 4.4). By contrast, likelihood analyses identify a moderately supported

Eriocaulaceae–Xyridaceae clade (BPML = 89%; Fig. 4.5) that is strongly supported as the sister group of the core Poales (BPML = 93%; Fig. 4.5). Mayacaceae are very weakly inferred to be the sister group of this large clade (BPML = 12%; Fig. 4.5).

4.4 DISCUSSION

4.4.1. Phylogenetic Relationships Among the Major Lineages of Commelinid Monocots

My phylogenetic analysis of multiple plastid genes and conservative noncoding regions sampled broadly across multiple monocot taxa provides strong support for the commelinid monocot clade (Figs 4.4, 4.5). Several putative non-DNA synapomorphies have been identified for this clade (see Introduction). Within the commelinid monocots, I identify a strongly supported sister-group relationship between Commelinales and Zingiberales (Figs. 4.4, 4.5), consistent with the findings of several recent studies (e.g., Chase et al. 2000, 2006; Soltis et al. 2000; Hilu et al. 2003; Givnish et al. 2006; Graham et al. 2006). This large clade includes 13 families [five in Commelinales (see below), and eight in Zingiberales (see Kress et al. 2001)]. The Zingiberales are well supported by morphological and molecular data (e.g., Kress 1995; Kress et al. 2001; Givnish et al. 2006). A few possible non-DNA synapomorphies for a Commelinales–Zingiberales clade have been identified, including the presence of phenylphenalenones (known in Haemodoraceae, Musaceae, Pontederiaceae, and Strelitziaceae; see Otálvaro et al. 2002), inflorescences with many-flowered cincinnal branches, and invasive or plasmodial tapetum (Stevens 2006).

Higher-order relationships among Arecales, Dasypogonaceae, Commelinales–Zingiberales, and Poales are not consistently resolved here, and no particular relationships receive strong support. In parsimony and likelihood analyses, Arecales and Dasypogonaceae

are variously placed among Commelinales–Zingiberales and Poales. In some of the shortest trees from parsimony, Dasypogonaceae are weakly placed in a clade with Poales (BP_{MP} = 53%; Fig. 4.4); this topology receives only 13% bootstrap support from maximum likelihood analysis (data not shown). Chase et al. (2006) found this same placement for Dasypogonaceae, with a similar level of support from parsimony. Consistent difficulty in placing Arecales and Dasypogonaceae among commelinid monocots may reflect a rapid initial radiation of the major commelinid lineages (i.e., divergence of major lineages within a relatively short time). Indeed, Jansenn and Bremer (2004) dated the crown nodes of Arecaceae and Dasypogonaceae to the mid-Cretaceous at 100–110 Mya, a period in which angiosperms were undergoing major radiation according to the fossil record (e.g., Herendeen and Crane 1995). Typical of rapid radiations, the lengths of these deep internal commelinid branches are relatively short (Figs. 4.4, 4.5). Inability to clearly (i.e., no collapsed branches in a strict consensus tree and no conflicts among analyses) and strongly (i.e., high support) infer deep commelinid relationships is therefore likely a function of sampling error; not enough characters have been examined to characterize the divergence pattern of these ancient lines for the regions examined (Graham et al. 2006).

Additional sampling from the plastid or other genomes will likely be necessary to place Arecales and Dasypogonaceae with strong support, in combination with denser taxon sampling, particularly in these two lineages. My current sampling includes two (*Dasypogon* and *Kingia*) of the four Dasypogonaceae genera, representing each of two putative clades in the family (e.g., Davis et al. 2004), but *Baxteria* and *Calectasia* should also be included in future work. I have also only included a single exemplar from the large family Arecaceae. Recent work has identified two major clades in Arecaceae that differ in their rates of plastid

evolution. One clade (subfamily Arecoideae), which includes *Roystonea* (examined here), has a relatively slow rate of plastid evolution, and the other clade (subfamily Calamoideae) has a more rapid rate of plastid evolution (Asmussen 2006). Inclusion of exemplars from Calamoideae in future studies may help place Arecaceae among commelinids.

4.4.2. Phylogenetic Relationships in Commelinales

Until recently, Commelinaceae, Hanguanaceae, Haemodoraceae, Philydraceae, and Pontederiaceae (Commelinales) were not all considered to be closely related, although close affinities among some of these families had been suggested previously (e.g., Cronquist 1981, 1988; Dahlgren et al. 1985; Thorne 1992a, b). Previous molecular data support their inclusion in a single clade (e.g., Chase et al. 1995, 2000, 2006; Graham et al. 2002; Davis et al. 2004; Givnish et al. 1999, 2006), although only a subset of these studies included all five families. I also find strong support for this five-family lineage here (Figs. 4.4, 4.5). Only a few potential non-DNA synapomorphies have been identified for Commelinales in its current circumscription, including abundant helobial endosperm, tannin cells in the perianth, and the presence of sclereids in the placenta (e.g., Givnish et al. 1999; Judd et al. 2002; Soltis et al. 2005).

Haemodoraceae–Philydraceae–Pontederiaceae — Several workers have suggested close relationships among Haemodoraceae, Philydraceae, and Pontederiaceae (e.g., Simpson 1990, 1993; Kress 1995; Linder and Kellogg 1995). Only a single molecular study places these three families in a clade, and this topology receives only moderate support (Chase et al. 2006). Here I also find a moderately supported Haemodoraceae–Philydraceae–Pontederiaceae clade (Figs. 4.4. 4.5). Several putative non-DNA synapomorphies have been

identified for this three-family lineage, including the presence of styloids, tanniniferous tepals that are generally persistent in fruit (e.g., Soltis et al. 2005), and tepalar perianths. The aquatic or semi-aquatic habit shared by Philydraceae and Pontederiaceae (Haemodoracaee are terrestrial) might have arisen only once in the lineage with a reversal in Haemodoraceae, or it may have arisen independently in both lineages (Barrett and Graham 1997). The three families also include species with enantiostyly, a unique floral asymmetry polymorphism in which the style is variously deflected to the left or right side of a flower that is relatively rare in angiosperms (Graham and Barrett 1995; Jesson and Barrett 2003). This character has been suggested previously as a possible synapomorphy for this three-family clade (Graham and Barrett 1995; Givnish et al. 1999). Among monocots, enantiostyly occurs in multiple genera in Haemodoraceae, Philydraceae, and Pontederiaceae, but also in the closely related Commelinaceae and the distantly related Tecophilaeaceae (Asparagales) (see Graham and Barrett 1995). Dimorphic enantiostyly, one of two enantiostyly subtypes in which left- and right-styled flowers occur on different individuals as a genetic polymorphism (Barrett et al. 2000), has been positively confirmed in monocots only in Haemodoraceae and Pontederiaceae (Jesson and Barrett 2002a, b; Jesson and Barrett 2003). Given the limited occurrence of dimorphic enantiostyly in both families (Jesson and Barrett 2003), it is possible that it originated in parallel in these lineages.

Within the Haemodoraceae–Philydraceae–Pontederiaceae clade, I infer Philydraceae to be the sister-group of Haemodoraceae–Pontederiaceae (Figs. 4.4, 4.5), with moderate support, similar to that found by Chase et al. (2006). All three families have an inner and outer tepaloid whorl, but in Philydraceae, the outer petaloid whorl is substantially smaller than the inner petaloid whorl, whereas in Haemodoraceae and Pontederiaceae, the outer and

innter petaloid whorls are similar in size (Soltis et al. 2005). The latter character (equal tepal size) may therefore constitute a synapomorphy for a Haemodoraceae–Pontederiaceae clade. A sister group relationship between Pontederiaceae and Haemodoraceae is further consistent with aspects of their pollen morphology (Simpson 1987).

Commelinaceae-Hanguanaceae — In the current analyses I find a strongly supported sister-group relationship between Commelinaceae and Hanguanaceae (Figs. 4.4, 4.5), consistent with the findings of most monocot tree of life work (Givnish et al. 1999, 2006; Chase et al. 2006; but see Tamura 2004). The spiderwort or wandering jew family, Commelinaceae, consists of herbs with cymose inflorescences, short-lived flowers with differentiated perianths (i.e., petals and sepals) and moniliform anthers, and the fruit is a capsule or berry (Dahlgren et al. 1985; Faden 1998; Soltis et al. 2005). Hanguanaceae are herbs with (sometimes broad) petiolate leaves, paniculate inflorescences with sessile, small flowers, and the fruit is a berry (Bayer et al. 1998; Stevens 2006). Commelinaceae were previously included in an order (Commelinales) with Eriocaulaceae, Mayacaceae, Rapateaceae, and Xyridaceae based on their shared petaloid and sepaloid perianths (Dahlgren et al. 1985), but Commelinaceae are not closely related to these families and this character is therefore homoplasious or symplesiomorphic. *Hanguana* was originally included in Flagellariaceae (Backer 1951), but removed to its own monogeneric family on the basis of differences in vegetative morphology and anatomy (Airy Shaw 1965). It has been variously allied with or placed in taxa that are now included in five orders of monocots, including Asparagales (Dahlgren et al. 1985), Liliales (Cronquist 1981), Poales (Backer 1951), and Zingiberales (Rudall et al. 1999). From a biodiversity perspective, Hanguanaceae is probably the most poorly known family in Commelinales and even commelinid monocots;

ongoing research indicates that there are multiple undescribed *Hanguana* species in Borneo and western Malaysia that need to be evaluated (Bayer et al. 1998; P. M. Boyce, pers. comm.).

A sister-group relationship between Commelinaceae and Hanguanaceae was largely unexpected. Only one putative non-DNA synapomorphy (largely non-photosynthetic cotyledons; Tillich 1996; Tillich and Sill 1999) has been identified for a Commelinaceae–Hanguanaceae clade (Stevens 2006). A 5-bp insertion in *mat*K shared by *Monochoria* (Pontederiaceae) and *Hanguana*, which Tamura et al. (2004) suggested as a possible synapomorphy for a Hanguanaceae–Pontederiaceae clade, likely is homoplasy. A recent morphological analysis found that *Hanguana* shares many morphological characteristics with Zingiberales (Rudall et al. 1999); these characters may be symplesiomorphies. Other similarities between Commelinaceae and Hanguanaceae have been observed recently, but it is not clear if these are symplesiomorphies, synapomorphies, or parallelisms. Tillich (1996) noted that an undescribed species of *Hanguana* (subsequently named *H. bogneri*; Tillich and Sill 1999) with broad, petiolate leaves, is similar in habit to some Commelinaceae genera, such as *Palisota*. In both Hanguanaceae and Commelinaceae the seed coats are testal in origin, although this character state occurs in other commelinid families (Tillich 1996). Also, Hanguanaceae and *Cartonema* – the putative sister-group of the rest of Commelinaceae (e.g., Evans et al. 2000, 2003; Figs. 4.4, 4.5) that has sometimes been recognized in its own family, Cartonemataceae Pichon (e.g., Tomlinson 1969) – have inconspicuous root collars (Tillich 1996). Rudall and Bateman (2004) observed that *Hanguana* and several species of *Cartonema* have actinomorphic flowers, and suggested that actinomorphic flowers may have

arisen only once in Hanguanaceae and Commelinaceae, and undergone a subsequent reversal in the remainder of Commelinaceae.

Philydraceae — Philydraceae are a small monocot family characterized by a perennial, rhizomatous or cormose habit, with basal and distichous ensiform leaves, bracteate inflorescences, indeterminate racemose inflorescences with flowers subtended by spathelike bracts, and zygomorphic flowers with four perianth parts and a single stamen (Simpson 1985; Hamann 1998). Three or four genera are currently recognized in Philydraceae. Generic circumscriptions of *Philydrum* (one species) and *Philydrella* (two species) are without controversy [but see Adams (1987) for nomenclatural issues with the name *Philydrella* Caruel]. Nonetheless, in this study I could not test the monophyly of *Philydrella* with molecular data because I was not able to sample *P. pygmaea*, the other species in the genus. The generic circumscription of *Helmoltzia* has varied previously. The genus *Helmholtzia* F. Muell. was established in 1865 to include a single species, *H. acorifolia* F. Muell. A second species, *H. glaberrima* (Hook f.) Caruel, was originally described as a species of *Philydrum* (*P. glaberrimum* Hook. f., 1873) but later transferred to *Helmholtzia*. Skottsberg (1932), however, placed this taxon in a monotypic genus, *Orthothylax* (Hook. f.) Skottsb. [*O. glaberrimus* (Hook. f.) Skottsb.], on the basis of several morphological differences with *H. acorifolia* [free perianth segments (vs. a conspicuous perianth tube in *H. acorifolia*); zygomorphic pistil (vs. actinomorphic pistil); partly unilocular ovary (vs. trilocular ovary); and dry, loculicidally dehiscent capsule (vs. an ovary that does not split)] (Skottsberg 1934), and he described a second species, *H. novoguineensis* (K. Krause) Skottsb., from Papua New Guinea. Subsequent authors have variously recognized *Orthothylax* as a distinct genus (e.g., Cronquist 1981; Hamann 1966, 1998; Simpson 1985) or included it within *Helmholtzia* (e.g.,

Adams 1987; Prentis et al. 2006). The molecular data presented here indicate that *H. acorifolia* and *H. glaberrima* are sister taxa, consistent with their possible inclusion in a single genus. The current data are not, however, inconsistent with the recognition of two genera, with the caveat that I am not able to test the monophyly of *Helmholtzia* s.s., because I have not sampled *H. novoguineensis*, the only remaining taxon in this species complex.

My complete genus-level sampling for Philydraceae indicates that the family is a well-supported natural taxon (Figs. 4.4, 4.5), consistent with the conclusions of a previous morphological investigation (Hamann 1966). Hamann (1966, 1998) recognized two ecologically distinct groups in Philydraceae: one including *Helmholtzia* and *Orthothylax*, distributed in montane rainforests; the other including *Philydrum* and *Philydrella*, distributed in lowland areas. My data support a close relationship between *Helmholtzia* and *Orthothylax*. *Philydrella* is the sister group of a clade that includes *Philydrum* and *Helmholtzia–Orthothylax* (Figs. 4.4, 4.5); the latter relationship receives moderate to strong support. A sister-group relationship of *Philydrella* with the rest of the family is possibly consistent with several morphological, biogeographical, and ecological differences among *Philydrella* and *Helmholtzia*, *Orthothylax*, and *Philydrum*. Hamann (1966) observed that *Philydrella* has stomata with two lateral subsidiary cells that are smaller than other epidermal cells, *Philydrum* has two or sometimes four subsidiary cells arranged like those in *Philydrella*, whereas *Helmholtzia* and *Orthothylax* have four or more subsidiary cells arranged polarly or laterally, that are generally the same size as other epidermal cells. Additionally, *Helmholtzia*, *Orthothylax*, and *Philydrum* occur in wet habitats mostly in eastern Australasia, and they are each characterized by distichous, isobifacial-ensiform leaves, rhizomes, long woolly hairs on the flowering stem and bracts, and inflorescences of a terminal spike with

many flowers and lateral spikes (Hamann 1966, 1998). In contrast, *Philydrella* occurs in the drier climate in western Australia, and is characterized by a single terete leaf with an acuminate blade, corms, glabrous flowering stems and bracts, and a terminal spike with few flowers and no lateral spikes (Hamann 1966, 1998). Simpson (1985) noted that pollen grains of *Helmholtzia*, *Orthothylax* and *Philydrum* have a reticulate nonapertural wall sculpting, whereas *Philydrella* differs in having a regulate nonapertural sculpturing. This minor character may constitue an additional synapomorphy for a *Helmholtzia–Orthothylax–Philydrum* clade. The complete genus-level phylogeny for the family determined here should provide a framework to test hypotheses of evolution in Philydraceae (P. Prentis, pers. comm.).

4.4.3. Higher-Order Relationships in Poales

Poales sensu APG II (2003) is strongly supported as monophyletic in my analyses (Figs. 4.4, 4.5). I find moderate to strong support here for a major lineage that includes all Poales families except Bromeliaceae and Typhaceae (Figs. 4.4, 4.5); the positions of these latter two families at the base of the Poales subtree are not resolved here. Rapateaceae are weakly inferred to be the sister group of the rest of this large clade. These findings are generally consistent with three recent studies (Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006), although the latter study did not include Rapateaceae. Most previous studies did not recover this 14-family clade at all, or recovered it with only weak support (e.g., Chase et al. 2000; Michelangeli et al. 2003; Davis et al. 2004). Despite the increasingly strong molecular evidence for this clade, no non-DNA synapomorphies for it are known at present (Soltis et al. 2005). Within this major Poales lineage, I infer a xyrid clade with differing

compositions in parsimony vs. likelihood analyses, a cyperid clade, and the core Poales which consists of the graminid and restiid clades. Relationships among the core Poales, the xyrids, and the cyperids are not resolved in all analyses here, and it is not clear if Mayacaceae belongs to the xyrid clade.

4.4.3.1. The Core Poales

The core Poales (Anarthriaceae, Centrolepidaceae, Ecdeiocoleaceae, Flagellariaceae, Joinvilleaceae, Poaceae, and Restionaceae) have long been considered to be closely related. These families constituted Poales in Dahlgren et al's (1985) classification, and they form a clade in most recent analyses (e.g., Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004; Givnish et al. 2006; Chase et al. 2006). I find strong support here (Figs. 4.4, 4.5) for the core Poales, an improvement on the moderately strong support found for the clade by Chase et al. (2006). Several putative synapomorphies for a core Poales clade have been identified, including distichous leaves with a (usually) open sheath, stomata with dumbbell-shaped guard cells (although this is not the case in *Flagellaria*; Sack 1994), monoporate pollen with an annular ring, apical placentation, and nuclear endosperm development (e.g., Kellogg and Linder 1995; Soltis et al. 2005).

4.4.3.2. The Graminid Clade

Within the core Poales, I identify a strongly supported graminid clade that includes Ecdeiocoleaceae, Flagellariaceae, Joinvilleaceae, and Poaceae (Figs. 4.4, 4.5). This Ecdeiocoleaceae–Joinvilleaceae–Poaceae clade has been recovered with strong support in several molecular analyses (e.g., Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004;

Chapter 3), and the monophyly of this three-family group is further supported by a shared 6-kb plastid inversion that is not found in other Poales lineages (e.g., Doyle et al. 1992; Katayama and Ogihara 1996; Michelangeli et al. 2003). Despite strong support for this clade, relationships among these three families have been difficult to resolve unambiguously. Identification of the sister-group of the grasses is critical to homologize Poaceae reproductive structures with those of their closest relatives (Rudall et al. 2005; see Chapter 3), and to facilitate reconconstruction of the sequence of events that lead to the origin of the highly modified grass spikelet.

Several recent studies have inferred Ecdeiocoleaceae to be the sister-group of the grasses, although this topology has generally received only weak support (e.g., Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004). Here, I also identify Ecdeiocoleaceae as the sister-group of the grasses. Although I find only weak support for an Ecdeiocoleaceae-Poaceae clade (Figs. 4.4, 4.5), this level of support is greater than that inferred for this clade in most previous studies (e.g., Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004). In a previous study I identified an Ecdeiocoleaceae–Joinvilleaceae clade with weak support from parsimony analyses and moderate support from model based analyses, particularly when protein-coding data were considered alone (Chapter 3). An Ecdeiocoleaceae–Joinvilleaceae clade here receives only 11 and 27% bootstrap support from parsimony and likelihood analyses, respectively (data not shown). Increased support for an Ecdeiocoleaceae–Poaceae clade here compared with my earlier study and other previous work is likely based on a combination of more data and increased taxon sampling in the former family (I have sampled both genera), which has helped break up the relatively long terminal *Ecdeiocolea* branch (see Chapter 3). It is possible that with even more data per

taxon, it will be possible to achieve strong support for this clade (if it is true), and unambiguously identify the sister-group of the grasses. Possible non-DNA synapormorphies for an Ecdeiocoleaceae–Poaceae clade include scrobiculate pollen (Campbell and Kellogg 1987; Michelangeli et al. 2003) and operculate pollen pores (Michelangeli et al. 2003). The presences of six anthers may also constitute a synapomorphy for this clade (Rudall et al. 2005). Some 'early-diverging' grasses have six anthers (e.g., Anomochlooideae; GPWG 2001), and one genus (*Georgeantha*) of Ecdeiocoleaceae has this character (*Ecdeiocolea* has four anthers) (Rudall et al. 2005).

Flagellariaceae — Flagellariaceae are a small, paleotropical family of rattan-like climbers with dichotomous branching, leaves terminated in tendrils, and large, paniculate inflorescences with perfect flowers (Stevens 2006). In its original circumscription, Flagellariaceae included the superficially similar genera *Flagellaria*, *Hanguana*, and *Joinvillea* (e.g., Backer 1951), but the latter two genera have since been segregated to their own families based on differences in vegetative morphology and anatomy (Airy Shaw 1965, Tomlinson and Smith 1970). Although Flagellariaceae have most characters typical of core Poales (see above), they have been difficult to place in core Poales (e.g., Takhtajan 1997) because they are unique in several aspects. Autopomorphies for Flagellariaceae include embryo-sac development of the *Allium* type, binucleate pollen, crassinucellate ovules, multicellular stigmatic papillae, and a unique guard-cell morphology (e.g., Campbell and Kellogg 1987; Sack 1994; Appel and Bayer 1998). In molecular analyses, Flagellariaceae have been variously placed as the sister group of Ecdeiocoleaceae–Joinvilleaceae–Poaceae (e.g., Chase et al. 2000, 2006; Givnish et al. 2006), the sister group of the rest of core Poales

(e.g., Michelangeli et al. 2003; Graham et al. 2006), or the sister group of the xyrid clade (Davis et al. 2004).

Here I infer *Flagellaria* to be part of the graminid clade; they are the sister-group of Ecdeiocoleaceae-Joinvilleaceae-Poaceae (Figs. 4.4, 4.5), with moderate to strong support, similar to that found by Chase et al. (2006) for the same position. Placement of Flagellariaceae as the sister-group of a Restionaceae-Ecdeiocoleaceae-Poaceae clade by Graham et al. (2006), who considered a subset of the taxa data that I examine here, may be an artifact of incomplete taxon sampling; they included only four of the seven core Poales families, mostly with single exemplars. A sister-group relationship between Flagellariaceae and Ecdeiocoleaceae-Joinvilleaceae-Poaceae is consistent with the presence of a 28-kb plastid inversion found in these four families (although this inversion also occurs in some Restionaceae) (Doyle et al. 1992; Michelangeli et al. 2003). Flagellariaceae lacks the 6-kb inversion shared by Ecdeiocoleaceae-Joinvilleaceae-Poaceae (Doyle et al. 1992; Michelangeli et al. 2003). Inclusion of Flagellariaceae in the graminid clade might also be consistent with a microstructural cell-wall characteristic. Poaceae and Flagellariaceae have a similar cell-wall-labelling pattern of (1→3), (1→4)-ß-D-glucans that is distinct from the pattern observed in other major Poales lineage, although only a few Poales families have been examined for this character (Trethewey et al. 2005).

Ecdeiocoleaceae — Ecdeiocoleaceae are strongly xeromorphic herbs with leaves reduced to sheaths, spicate inflorescences with male and female flowers, and fruits that are achenes or nutlets (Briggs 2005). The tussocky cord rush, *Ecdeiocolea monostachys*, was previously included in Restionaceae, but subsequently segregated to its own family on the basis of multiple vegetative and reproductive characteristics (Cutler and Shaw 1965).

Ecdeiocoleaceae were thought previously to be closely related to Restionaceae (e.g., Dahlgren et al. 1985; Manning and Linder 1990; Kellogg and Linder 1995; Stevenson and Loconte 1995) largely based on their superficially similar habit, but it is now clear that they are related more distantly (see above). Similarity between these two families may be a convergence in response to their xeromorphic habitat.

A second genus and species of Ecdeiocoleaceae, *Georgeantha hexandra*, was described only recently, differing from *E. monostachya* in its rhizomatous habit, culms with multiple internodes, branched inflorescences with up to three spikelets (each with five to eleven flowers), tricarpellate ovules, six stamens, and loculicidally dehiscent fruits (Briggs and Johnson 1998; Briggs 2005; Rudall et al. 2005). By contrast, *E. monostachya* has a caespitose habit, no culm internodes, unbranched inflorescences (with up to 50 flowers in a single spikelet), unicarpellate ovules, four stamens, and indehiscent fruits (Briggs and Johnson 1998; Rudall et al. 2005). The two genera are further distinguished by unique flavonoid patterns (Williams et al. 1997). Both species have been included in two molecular analyses (Briggs et al. 2000; Bremer 2002) which robustly supported the monophyly of Ecdeiocoleaceae. In the current study, I also identify an *Ecdeiocolea–Georgeantha* clade, with strong support (Figs. 4.4, 4.5). I observe substantial plastid variation between these two taxa that is possibly correlated with the strong morphological differences between the genera. A second undescribed species of *Ecdeiocolea* from western Australia has recently been discovered (B. G. Briggs, personal communication). This unnamed taxon differs from *E. monostachya* in having a more elongated rhizome, more spaced-out culms, and a generally stouter form (B. G. Briggs, unpublished data). The new taxon appears to be much less widespread than *E. monostachya*, but over most of its range the two species are extremely

common and grow together with no apparent ecological distinction (B. G. Briggs, unpublished data). There is currently no molecular data for this new species, and it should be a priority for inclusion in future studies to test the monophyly of *Ecdeiocolea* and *Georgeantha*, and to further characterize levels of plastid variation among these taxa. Inclusion of data for this species may also provide additional insight into relationships among Ecdeiocoleaceae–Joinvilleaceae–Poaceae.

Joinvilleaceae — The reed-like joinvillea family, Joinvilleaceae, which includes robust, grass-like plants with strongly plicate leaves and terminal panicles of small, perfect, flowers (Stevens 2006), were thought previously to be the sister group of Poaceae. In the current study, this relationship receives only 25 and 11% bootstrap support from parsimony and likelihood analysis, respectively (data not shown), and is clearly not favoured. One possible synapomorphy for a Joinvilleaceae–Poaceae clade that has been hypothesized is the presence of long and short cells in the leaf epidermal cells of both families (e.g., Campbell and Kellogg 1987). Long and short epidermal cells, however, have also been noted in a few palm (Arecaceae) and sedge (Cyperaceae) genera (Tomlinson 1969, Metcalfe 1971), indicating that this character may be a symplesiomorphy, or that it may have originated multiple times across Poales. Michelangeli et al. (2003) also recently reported the presence of long and short epidermal cells in Ecdeiocoleaceae. It may therefore unite Ecdeiocoleaceae, Joinvilleaceae, and Poaceae.

Within Joinvilleaceae, I have sampled *Joinvillea plicata* and *J. ascendens*, the two currently recognized species in the monogeneric family (Newell 1969). I detect minimal plastid variation between these two species (Figs. 4.4, 4.5). The low level of variation is

possibly consistent with the limited morphological variation that distinguishes them; they differ only in tepal shape and the frequency of bristles on the laminar surface (Newell 1969).

Poaceae — The grass family – the largest Poales family, with ~11,000 species (Table 4.1) – has received substantial systematic attention, and its general phylogenetic structure is fairly well established (see GPWG 2001; Chapter 3). The most recent classification of the family includes twelve subfamilies (GPWG 2001; Soreng et al. 2006; Duvall et al. 2006). In my previous study, I examined phylogenetic relationships among ten of these major lineages with the same complement of plastid data examined here. Here I infer the same relationships found in the previous study, generally with similar levels of support. In this study I have added data for a species of *Puelia* (subfamily Puelioideae), one of two major lineages not included in my previous study, and three additional exemplar taxa from available plastid genomes [*Oryza nivara* (Ehrhartoideae); *Saccharum officinarum* and *Sorghum bicolor* (Panicoideae)]. I have not sampled the recently recognized subfamily Micrairoideae (Duvall et al. 2006). The previously unsampled subfamily, Puelioidoeae, is inferred here with strong support (Figs. 4.4, 4.5) to be the third of three successive lineages (i.e., including Anomochlooideae and Pharoideae) that are the respective sister groups of the rest of the grasses). This placement is consistent with most earlier studies that have sampled Puelioideae (e.g., Clark et al. 2000; GPWG 2001), although one study placed *Puelia* with moderate support as the sister group of the BEP clade (Zhang 2000). A Puelioideae–BEP clade here receives < 5% bootstrap support from parsimony and likelihood analyses (data not shown).

Concerning the other taxa added in comparison to Chapter 3 (i.e., *Oryza nivara*, *Saccharum*, and *Sorghum*), I detect only minimal plastid variation among wild and cultivated

rice, the two sampled *Oryza* species, consistent with previous plastid genome comparisons of these taxa (Masood et al. 2004). In the PACMAD clade, my within-subfamily taxon sampling is most dense in Panicoideae, where I sampled four exemplar taxa. I find *Chasmathium* [currently included in tribe *Centotheceae* Ridl. (Soreng et al. 2006), but formerly included in a separate subfamily, Centothecoideae (GPWG 2001)] to be the sister group of a clade that includes *Zea*, *Saccharum*, and *Sorghum* (these latter genera are currently included in various subtribes in tribe Andropogoneae Dumort.; Soreng et al. 2006). This strongly supported set of relationships is consistent with other recent studies of the subfamily (e.g., Spangler et al. 1999; GPWG 2001; Mathews et al. 2002). Other higher-level relationships within the PACMAD clade receive very weak support here (Figs. 4.4, 4.5), consistent with most of my previous parsimony and likelihood analyses (Chapter 3).

4.4.3.3. The Restiid Clade

The remaining families in the core Poales (Anarthriaceae, Centrolepidaceae, and Restionaceae) are strongly supported here as the restiid clade (sensu Linder and Rudall 2005), as previous studies have found (e.g., Bremer 2002; Chase et al. 2006; Michelangeli et al. 2003; Davis et al. 2004; Givnish et al. 2006). Putative non-DNA morphological synapomorphies for this clade include dioecy (although this is also found in other Poales lineages, such as Cyperaceae and Poaceae), chlorenchyma with peg cells, and dorsifixed anthers (Soltis et al. 2005).

Restionaceae and Anarthriaceae — The cape-reed or restio family, Restionaceae, are xeromorphic herbs with reduced leaves, and small, imperfect flowers aggregated into spikelets (Stevens 2006). Restionaceae are the largest family in the restiid clade, and second

largest family among core Poales (Table 4.1). Family-level circumscriptions of Restionaceae have been controversial. The genus *Anarthria* was originally included in Restionaceae, but placed in its own family, Anarthriaceae, on the basis of multiple anatomical and reproductive differences (Cutler and Airy Shaw 1965; Linder and Rudall 1993). Previous molecular studies have placed *Anarthria* as the sister-group of Restionaceae (e.g., Briggs et al. 2000; Bremer 2002; Michelangeli et al. 2003; Chase et al. 2006), consistent with its placement in its own family. Two additional genera, *Hopkinsia* and *Lyginia*, were traditionally included in Restionaceae, but have long been recognized as abberant in that family based on multiple morphological and embryological differences (Briggs and Johnson 2000). *Lyginia* and *Hopkinsia* also have unique flavonoid patterns with respect to each other, *Anarthria*, and other Restionaceae (Williams et al. 1997). Briggs and Johnson (2000) proposed the new families, Hopkinsiaceae and Lyginiaceae, for these genera. Molecular studies indicate that *Hopkinsia* and *Lyginia* form a clade with *Anarthria* (Briggs et al. 2000; Bremer 2002), and the three genera are now included in Anarthriaceae (APG II 2003). Here I also strongly infer a sister group relationship between *Hopkinsia* and *Lyginia*, and these genera are the sister group of *Anarthria* (Fig. 4.4, 4.5). A possible non-DNA synapomorphy for Anarthriaceae s. l. is tetrathecate anthers (Linder and Rudall 1993).

Centrolepidaceae — The family status of Centrolepidaceae, a small Australasian group of dwarf, aquatic annuals or cushion-forming perennials with minute, spike-like bi- or unisexual inflorescences comprised of sterile bracts that surround highly reduced unisexual flowers (Cooke 1998), has also been controversial. Centrolepidaceae are morphologically similar to Restionaceae in several respects, and the two have been considered closely related (e.g., Dahlgren and Clifford 1982). The superficially similar genera *Hydatella* and *Trithuria*

were previously included in Centrolepidaceae, but are now included in Hydatellaceae (Hamann 1976; see Chapter 5). Workers have variously suggested that Centrolepidaceae should be included in Restionaceae (e.g., Hamann 1975) or that these families should not be considered closely related (e.g., Cutler 1969; Linder and Ferguson 1985). In previous molecular analyses, Centrolepidaceae have been included in Restionaceae (e.g., Briggs 2000; Bremer 2002), or inferred to be the sister group of Restionaceae (e.g., Michelangeli et al. 2003; Chase et al. 2006). Here, I have sampled only a single exemplar from Restionaceae [*Elegia*, representing an African clade, one of two major clades in the family; Linder et al. 2003], thus I am unable to test the monophyly of Restionaceae with respect to Centrolepidaceae. Nonetheless, I infer the two Centrolepidaceae taxa that I have sampled to be a clade that is the sister group of this single exemplar (Figs. 4.4, 4.5). Broader sampling in Restionaceae, including representatives from the Australian clade, is a priority for future work. Both Centrolepidaceae exemplars sampled here appear to have a substantially elevated rate of plastid evolution compared with Anarthriaceae and *Elegia* Restionaceae.

4.4.3.4. The Cyperid Clade

My analyses identify a well-supported cyperid clade that includes Cyperaceae, Juncaceae, and Thurniaceae, consistent with the findings of earlier studies (e.g., Chase et al. 2000, 2006; Michelangeli et al. 2003; Davis et al. 2004; Givnish et al. 2006). Several synapomorphies have been identified for this three-family lineage, including solid stems with tristichous leaves, pollen shed in tetrads, and chromosomes with diffuse centromeres (Plunkett et al. 1995; Munro and Linder 1998; Soltis et al. 2005). Within the cyperid clade, I find Thurniaceae to be the sister group of a Cyperaceae–Juncaceae clade. Close affinities

between Cyperaceae and Juncaceae have been hypothesized previously (e.g., Dahlgren et al. 1985; Thorne 1992a, b), and several potential synapomorphies for this clade have been identified (e.g., absence of calcium oxalate raphides, simultaneous microsporogenesis, usually paracytic stomata; Simpson 1995).

In parsimony and likelihood analyses, the cyperid clade is very weakly supported as the sister group of a large clade that includes the core Poales and Eriocaulaceae, Mayacaceae, and Xyridaceae (Figs. 4.4, 4.5). This relationship contrasts with several recent studies (Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006) that found moderate support for a clade that includes the cyperid and xyrid families. Precise placement of the cyperid clade among Poales will likely require additional data.

Thurniaceae — The South African genus *Prionium*, which occurs on stream and river margins (Munro and Linder 1998), was previously included in Juncaceae, although it has long been recognized as aberrant in that family because of its woody, aerial rhizomes and unique leaf anatomy (e.g., Dahlgren et al. 1985; Balslev 1998). Cutler (1969) suggested that *Prionium* should not be included in Juncaceae, but he did not formally remove it from the family. The first molecular evidence for *Prionium* inferred the genus to be the sister group of Cyperaceae–Juncaceae (e.g., Chase et al. 1993; Plunkett et al. 1995; Munro and Linder 1998), and it was therefore recently segregated to its own monogeneric family, Prioniaceae (Munro and Linder 1998). The small South American genus, *Thurnia*, is a rhizomatous, sedge-like herb with small, tepaloid flowers arranged in a dense inflorescence, and subulate-pointed seeds (Dahlgren et al. 1985). *Thurnia* has been variously included in Rapateaceae or Juncaceae (see Cutler 1965), but it was placed in its own family on the basis of distinct leaf anatomy, in addition to seed and embryo morphology (e.g., Cutler 1965; Dahlgren et al.

1985; Kubitzki 1998), with suggested affinities to Cyperaceae and Juncaceae (e.g., Cutler 1969; Cronquist 1981) and possibly Rapateaceae or Xyridaceae (Dahlgren et al. 1985). In some morphological analyses, *Thurnia* has been placed as the sister group of Juncaceae and *Prionium* (e.g., Stevenson and Loconte 1995; Rudall et al. 1999), and in others, *Prionium* and *Thurnia* are inferred to be sister taxa nested within Juncaceae (Simpson 1995). Recent molecular studies place *Thurnia* and *Prionium* in a clade that is the sister group of Cyperaceae–Juncaceae (e.g., Michelangeli et al. 2003; Davis et al. 2004; Givnish et al. 1999, 2006). Because these genera are closely related and morphologically similar (i.e., both genera have serrate leaf margins, erect stems and pentacyclic inflorescences; Stevens 2006) and because they are monogeneric sister taxa, Prioniaceae was included in Thurniaceae in the most recent angiosperm classification (APG II 2003). Consistent with this recent work, I also find a strongly supported sister group relationship between *Prionium–Thurnia*, and find Thurniaceae to be the sister-group of Cyperaceae–Juncaceae (Figs. 4.4, 4.5).

Cyperaceae — The sedge family, Cyperaceae, is a large and morphologically diverse group characterized by usually three-angled stems, reduced flowers surrounded by sterile bracts, and achene fruits (Stevens 2006). The most recent classification of the family recognizes two subfamilies (Mapanioideae and Cyperoideae) and twelve tribes (Simpson et al. 2006). Here, I have sampled representatives from each subfamily and four tribes in Cyperoideae. The relationships that I infer among these (Figs. 4.4, 4.5) are consistent with relationships found in previous studies (e.g., Muasya et al. 1998, 2000; Givnish et al. 2006; Simpson et al. 2006), although my sampling is currently much sparser.

4.4.3.5. The Xyrid Clade

Support levels and family composition for a xyrid clade are different in maximum parsimony and maximum likelihood analyses here, because of conflicting placements for Mayacaceae. In my parsimony analysis, I find a weakly supported xyrid clade (Fig 4.4) that includes Eriocaulaceae, Mayacaceae, and Xyridaceae; the relationships of this lineage with other Poales is not clear (Fig. 4.4). In contrast, in my likelihood analysis, I find a moderately supported xyrid clade that includes only Eriocaulaceae and Xyridaceae (Fig. 4.5), and this lineage is strongly supported as the sister group of the core Poales (Fig. 4.5); Mayacaceae are not part of this clade (Fig. 4.5). The aquatic bogmoss family, Mayacaceae, has a *Lycopodium*-like habit, and is characterized by spirally arranged leaves, solitary actinomorphic flowers, a showy perianth, and tricarpellate ovaries (Faden 1985; Venturelli and Bouman 1986). By contrast, the pipewort family, Eriocaulaceae, are characterized by a tufted basal rosette of leaves with capitula arranged in racemes (Unwin 2004), and Xyridaceae are petaloid monocots with variously arranged equitant leaves, a bracteate conelike-spike or capitulum-like inflorescence situated on a scape, and a two-whorled, tripartite perianth (Kral 1998). Mayacaceae have been variously included in a clade with Eriocaulaceae–Xyridaceae (e.g., Michelangeli et al. 2003), Bromeliaceae–Rapateaceae (Givnish et al. 1999), or placed as the sister group of Xyridaceae (e.g., Bremer 2002), a cyperid clade (e.g., Chase et al. 2006), or a xyrid–cyperid clade (e.g., Givnish et al. 2006). In parsimony analysis, I find Mayacaceae and Eriocaulaceae to be moderately supported as sister taxa (Fig. 4.4), and Xyridaceae to be their sister group. No previous study has inferred the Eriocaulaceae–Mayacaceae clade that I find here. Most studies place Eriocaulaceae and Xyridaceae in a robustly supported clade [e.g., Chase et al. 2006; Givnish et al. 2006; but see

Michelangeli et al. (2003) and Davis et al. (2004)]. In contrast to my parsimony analysis, I find this latter relationship from maximum likelihood analysis with moderate support (Fig. 4.5). Several morphological synapomorphies have been identified for an Eriocaulaceae–Xyridaceae clade, including a differentiated perianth, a basal rosette growth habit, paracytic stomata, dimerous flowers, ovules with thin-walled megasporangia, and spinulate/ echinate pollen (Dahlgren et al. 1985; Soltis et al. 2005). However, if Eriocaulaceae and Mayacacae are in fact sister taxa, and Xyridaceae are their sister group, then these putative Eriocaulaceae–Xyridaceae synapomorphies might actually be deeper symplesiomorphies. Alternatively, they may represent synapomorphies for a broader Eriocaulaceae–Mayacaceae–Xyridaceae clade, but this hypothesis necessitates substantial subsequent morphological change in the *Mayaca* lineage.

Difficulty inferring relationships clearly and strongly among the xyrid families is likely a function of their substantially accelerated plastid substitution rates, particularly in Mayacaceae. In the current study, branches for Eriocaulaceae, Mayacaceae, and Xyridaceae are very long; three of the four exemplars (*Eriocaulon*, *Mayaca*, and *Xyris*) are the longest among Poales taxa examined here (Figs 4.4, 4.5). It is well known that bootstrap analysis might be misleading for taxa with long branches (e.g., Felsenstein 1978; Hendy and Penny 1989), thus long branch attraction might be distorting inference of relationships among Eriocaulaceae, Mayacaceae, and Xyridaceae. The possibility of a long-branch artifact here is further substantiated by the moderately conflicting topologies inferred from parsimony and likelihood analyses. Likelihood analyses are known to be generally less prone to long- branch artifacts compared with parsimony analyses (e.g., Swofford et al. 2001; Sanderson and Shaffer 2002). Given previous difficulties placing Mayacaceae among Poales (e.g., Bremer

2002), Givnish et al. (2006) suggested that this taxon might act like a 'wild-card' in phylogenetic analyses; the current analyses uphold this hypothesis. Now that data are available for Eriocaulaceae, Mayacaceae, and Xyridaceae, as well as related families in Poales, future work could use a simulation approach (e.g., Huelsenbeck 1997; Sanderson et al. 2000) to determine if difficulty in placing Mayacace is a function of systematic bias (i.e., long branch attraction).

Increased taxon sampling in each family may also address this issue, by dividing long branches. For Mayacaceae, most phylogenetic studies have included sequence data only for *Mayaca fluviatilis*, the widest ranging species in the genus (Lourteig 1952); no studies have included more than one *Mayaca* species, and levels of plastid variation among the four or so species in the family are unknown. Recent morphological and molecular studies of Eriocaulaceae have identified two major clades in the family, recognized as subfamilies Eriocauloideae (two genera) and Paepalanthoideae (multiple genera) (Giulietti et al. 1995; Unwin 2004). I have included only a single representative for this family here, from the generally aquatic subfamily Eriocauloideae (Unwin 2004). Future work should sample additional *Mayaca* and Eriocauloideae species, and also sample from from the morphologically diverse and generally terrestrial Paepalanthoideae.

Xyridaceae — The monophly of Xyridaceae s. l. has been questioned in several studies. Most species in Xyridaceae are in the widespread genus *Xyris*, and one or a few species are in *Abolboda* Humb. & Bonpl., *Achlyphila* Maguira & Wurdack, *Aratitiyopea* Steyerm. & Berry, and *Orectanthe* Maguire (see Kral 1988, 1992, 1998; Campbell and Stevenson 2005). Xyridaceae are morphologically diverse, and some authors have placed *Abolboda*, *Aratitiyopea*, and *Orectanthe* in a segregate family, Abolbodaceae (e.g., APG

1998). *Achlyphila,* however, is morphologically intermediate between *Xyris* and Abolbodaceae; thus, most authors recognize only a single family (e.g., Dahlgren et al. 1985; Kral 1992, 1998). Nonetheless, some phylogenetic analyses indicate that the family is not monophyletic (e.g., Michelangeli et al. 2003; Davis et al. 2004), and others support Xyridaceae s. l. as a natural unit (e.g., Bremer 2002; Givnish et al. 1999, 2006; Chase et al. 2006), though sometimes with only weak support. In a preliminary study of morphological characters, Rudall and Sajo (1999) were unable to resolve relationships among Xyridaceae genera. They noted substantial variation in several embryological characters, and suggested that recognition of two families may be justified. In a more comprehenseive morphological study, Campbell (2004) inferred two major clades in Xyridaceae: one incuding *Xyris* (subfamily Xyridoideae s. s.), the other including *Achlyphila* (traditionally included in subf. Xyridoideae) and subfamily Abolboideae (*Aratitiyopea—Orectanthe* and *Abolboda*). In the current study I have sampled a single exemplar from each of these two major groups (i.e., *Aratitiyopea* and *Xyris*), and I recover strong support for a monophyletic Xyridaceae (Figs. 4.4, 4.5). Nonetheless, given the lack of monophyly for Xyridaceae in some studies and limited taxon sampling in the family in most studies (including the current one), extensive sampling of all five Xyridaceae genera, potentially including multiple species from *Xyris* and *Abolboda* (the two largest genera in the family; Kral 1988, 1992), should be carried out in future work, to further test the monophyly of the family and the phylogenetic hypotheses presented by Campbell (2004). At present, Xyridaceae are the largest family in Poales for which no family-wide phylogenetic hypotheses based on molecular data exist.

4.4.3.6. The 'Basal' Poales

Here I find Rapateaceae to be moderately supported as part of a clade that includes all of Poales except Bromeliaceae and Typhaceae (Figs. 4.4, 4.5), consistent with the recent analyses of Chase et al. (2006) and Givnish et al. (2006). Rapateaceae are a family of terrestrial, epiphytic, or amphibious herbs characterized by aerial stems with unifacial or bifacial leaves, and pedunculate inflorescences with petaloid flowers (Stevenson et al. 1998). Their superficial similarities to Eriocaulaceae and Xyridaceae are likely parallelisms (Soltis et al. 2005). No non-DNA synapomorphies are known for the large clade that includes Rapateaceae and the rest of Poales except Bromeliaceae and Typhaceae.

Bromeliaceae and Typhaceae have been consistently placed near the base of the Poales subtree, but their relationship to each other and the rest of Poales is not clear (e.g., Bremer 2002; Michelangeli et al. 2003; Chase et al. 2000, 2006; Graham et al. 2006; Givnish et al. 1999, 2006). The cattail family, Typhaceae, includes temperately-distributed rhizomatous herbs with erect leaves, and densely cylindrical inflorescences with reduced staminate and pistillate flowers. Typhaceae have been variously circumscribed to include or exclude the bur-reed genus, *Sparganium* (Sparganiaceae) (e.g., Cronquist 1981; Thorne 1992a, b; APG 1998; APG II 2003; Chase 2004). *Typha* and *Sparganium* are ecologically, cytologically, embryologically, and morphologically similar (e.g, Thieret and Luken 1996), and in most molecular studies (including the current study; Figs. 4.4, 4.5) they are a clade (e.g., Givnish et al. 1999, 2006; Graham et al. 2006; Chase et al. 2006), consistent with their inclusion in a single family. By contrast, the bromeliad family, Bromeliaceae, is a morphologically, physiologically, and ecologically variable neotropical group characterized

by a (usually) basal rosette of leaves, often coloured inflorescence bracts, and a petaloid perianth.

Here, Bromeliaceae and Typhaceae are a grade at the base of Poales, with either Bromeliaceae or Typhaceae very weakly inferred to be the sister group of the rest of Poales (Figs. 4.4, 4.5). By contrast, a Bromeliaceae–Typhaceae clade has been inferred with weak to moderate support in several studies (e.g., Bremer 2002; Chase et al. 2006; Givnish et al. 2006); here, a Bromeliaceae–Typhaceae clade receives only 16 and 37% bootstrap support from parsimony and likelihood analyses (data not shown). Regardless of their exact branching orders, 'deep' positions for Bromeliaceae and Typhaceae in Poales are consistent with cell-wall structural data. All sampled Poales families except Bromeliaceae and Typhaceae have (1→3), (1→4)-ß-D-glucans in their cell walls (Trethewey et al. 2005).

Given the difficulties observed here and elsewhere for inferring the exact positions of Bromeliaceae and Typhaceae at the base of Poales, additional sampling of plastid regions and possibly taxa may help clarify their positions. In this study I have sampled two exemplars that likely span the root of Bromeliaceae (e.g., Terry et al. 1997; Givnish et al. 2006), but given the low levels of plastid variation observed across Bromeliaceae (e.g., Givnish et al. 2006), it seems unlikely that further taxon samplings from the family will substantially affect inference of its position in Poales. Overall levels of plastid variation in Typhaceae are not known, as few species have been included in molecular studies. However, relatively high morphological variation in *Typha* (i.e., *viz. T. latifolia* L. vs. *T. minima* Hoppe.) and *Sparganium* (see Cook and Nichols 1986, 1987) suggests the possibility of additional molecular variation that might be useful in placing the family.

Few non-DNA characters are known that support the possible branching orders for Bromeliaceae and Typhaceae at the based of Poales. A close affinity between these families was hypothesized by Dahlgren et al. (1985), based on the presence of helobial endosperm in both families, but this character also occurs in Commelinales, Juncaceae, and Zingiberales (Linder and Kellogg 1995). Both Bromeliaceae and Typhaceae lack the mitochondrial gene *sdh*4 (Adams et al. 2002), and some authors have suggested that this loss might represent a synapomorphy for a Bromeliaceae–Typhaceae clade (Soltis et al. 2005; Stevens 2006). However, this should be viewed with caution, as *sdh* and ribosomal protein genes in the mitochondrial genome have been lost across the angiosperms multiple times (Adams et al. 2002; Ong and Palmer 2006).

4.4.4. Molecular Evolution in the Commelinid Monocots

Poaceae have been known to have an accelerated plastid substitution rate among monocots since some of the earliest plant molecular phylogenetic studies (Gaut et al. 1992), but more recent work has demonstrated that this accelerated substitution also occurs in several closely related Poales lineages (e.g., Bremer 2002; Givnish et al. 2006; Graham et al. 2006; Chapter 3). Here I have sampled representatives from all Poales families. Examination of branch variation in the order suggests that all families except Bromeliaceae, Rapateaceae, and Typhaceae (the basal Poales), and possibly Flagellariaceae, share this rate acceleration to some extent (Figs. 4.4, 4.5). Branch lengths in most Poales are substantially longer than those observed here in most other monocots, most eudicots, and basal angiosperms (Fig. 4.1). Within this 'rate-accelerated' Poales clade, there is also evidence of substantial variation in branch lengths among examined taxa (Chapter 3). Flagellariaceae

and Joinvilleaceae have shorter branches, whereas those for Centrolepidaceae, Eriocaulaceae, Mayacaceae, and Xyridaceae are substantially longer. The implications of these long branches for phylogenetic analyses are not evident. Although strong concordance for most Poales branches in parsimony and likelihood analyses here suggests that long branches are not causing misinference of relationships, discordance in the placement of Mayacaceae between parsimony and likelihood analyses (see above) suggest that they may do so, at least with respect to this taxon. Various explanations have been proposed for plastid rate heterogeneity among plants, including one, or a combination of, a relaxation of purifying selection, demographic factors, and variable mutation rates among lineages (e.g., due to changes in DNA repair efficiency that may affect whole genomes or parts of them) (e.g., Cho et al. 2004; Parkinson et al. 2005; Young and dePamphilis 2005).

Large-scale sequencing projects involving multiple taxa and genomic regions have often provided insight into molecular evolution of particular loci. For example, here I have detected an intronless version of the inverted repeat 3'-*rps*12 locus in *Luzula* (Juncaceae). The 3'-*rps*12 intron is present in most land plant taxa that have been examined so far (e.g., Graham and Olmstead 2000a, b, 2006; McPerson 2003; Rai et al. 2003; Zgurski 2004), but multiple, putatively independent losses for this locus have been documented in several taxa in Fabaceae (Graham et al. 2000; Lin et al. unpub. data, GenBank number: NC_003119.6), monilophytes (H. S. Rai et al. unpub. data), *Cuscuta europaea* L. (Convolvulaceae; Freyer et al. 1995), *Anomene* L. (Ranunculaceae; Hoot and Palmer 1994), and the Asparagales families Asphodelaceae and Hemerocallidaceae (McPherson et al. 2004). An intronless 3'-*rps*12 in *Luzula* is the first report for this loss in monocots outside Asparagales (McPherson et al. 2004), but it is possible that further monocot sampling will reveal additional instances for it.

The 3'-*rps*12 intron is present in *Juncus*, the other Juncaceae exemplar examined here, indicating that the intron loss is not characteristic of all of Juncaceae, although it might be a synapomorphy for the putatively monophyletic *Luzula* or broader clades that also include members of the non-monophyletic *Juncus* (Drábková et al. 2003; Roalson et al. 2005). Future work should more thoroughly explore and characterize the distribution of the 3'-*rps*12 intron in Juncaceae.

Table 4.1. Families recognized currently in Commelinales and Poales (sensu APG II 2003), their generic and species level diversity, and distribution.

Indented families have been recognized recently in some treatments, but are included in the previous taxon by APG II (2003).

Order and Family	No. Genera/ Species	Distribution	Reference(s)
Commelinales			
Commelinaceae R. Br.	41 / ~ 6,500	Africa, Asia, Australia, North America, South America	Cronquist 1981; Faden 1998
Haemodoraceae R. Br.	14 / 107	Australia,W and S North America, South America, S Africa	Simpson 1990; Hopper et al. 1999
Hanguanaceae Airy Shaw	1 / 3-15	Indo-Malaysia, N Australia	Bayer et al. 1998; P. M. Boyce, pers. comm.
Philydraceae Link	3-4 / 6	SE Asia, Australia	Hamann 1966, 1998; Adams 1987
Pontederiaceae Kunth. in Humb., Bonpl. & Kunth	3-4 / 30-35	Africa, Australia, Eurasia, North America, South America	Eckenwalder and Barrett 1986; Graham and Barrett 1995
Poales			
Anarthriaceae Cutler and Airy Shaw	1 / 5	W Australia	Cutler and Shaw 1965
Hopkinsiaceae B. G. Briggs & L.A.S. Johnson	1 / 2	W Australia	Briggs and Johnson 2000
Lyginiaceae B. G. Briggs & L.A.S. Johnson	1 / 3	W Australia	Briggs and Johnson 2000
Bromeliaceae Juss.	56 / 2,600	S North America, S America, W Africa	Smith and Till 1998
Centrolepidaceae Endl.	3 / 35	SE Asia, Australia, S South America	Cooke 1998; Stevens 2006

Table 4.1 continued

Order and Family	No. Genera/ Species	Distribution	Reference(s)
Cyperaceae Juss.	~120 / ~ 5,000	Worldwide	Bruhl 1995
Ecdeiocoleaceae Cutler and Airy Shaw	2 / 3	W Australia	Cutler and Shaw 1965; Briggs and Johnson 1998; Briggs, pers. comm.
Eriocaulaceae Desv.	11 / ~ 1,110	SE Asia, Africa, N America, S America, Australia	Stutzel 1998; Sano 2004; Unwin 2004
Flagellariaceae Dum.	1 / 4	Africa, SE Asia, NE Australia	Backer 1951
Joinvilleaceae Tomlinson and A.C. Sm.	1 / 2	SE Asia	Newell 1969; Tomlinson and Smith 1970
Juncaceae Juss.	7 / 442	Worldwide	Kirschener 2002a-c
Mayacaceae Kunth	1 / 4	W Africa, SE North America, South America	Lourteig 1952
Poaceae Barnhart	600-800 / ~ 11,000	Worldwide	Clayton et al. 2002 onwards; P. M. Peterson, pers. comm.
Rapateaceae Dum.	16 / ~ 100	W Africa, South America	Givnish et al. 2000
Restionaceae R. Br.	55 / ~ 490	S Africa, SE Asia, Australia, S South America	Linder et al. 1998
Thurniaceae Engl.	1 / 3	N South America	Cutler 1965; Dahlgren et al. 1985
Prioniaceae S. L. Munro & H. P. Linder	1 / 1	S Africa	Munro and Linder 1998

Table 4.1 continued

Order and Family	No. Genera/ Species	Distribution	Reference(s)
Typhaceae Juss.	1 / 8-13	Worldwide	Thieret and Luken 1996; Mavrodiev 2002
Sparganiaceae Schultz-Schultzenst.	1 / 14	N Africa, Australia, Eurasia, North America	Cook and Nichols 1986, 1987
Xyridaceae C. A. Agardh.	5 / ~385	SE Asia, Africa, N America, Australia, S America	Kral 1988, 1992, 1998; Campbell 2004a
Albolbodaceae Nakai	3 / 23	South America	Kral 1998

Table 4.2. Voucher information and GenBank accession numbers for plastid regions for exemplar commelinid monocot taxa in Poales and Commelinales. Source details and GenBank information for previously published taxa used in this study (including several commelinid taxa) can be found in Graham et al. (2006), Saarela et al. (in press), and Chapter 3. Alternative source details (e.g., living collection accession numbers) are given in place of voucher information in two cases. GenBank numbers for sequences from other studies are underlined; taxon identity and references for these sequences are included as footnotes. Regions for which no sequence was obtained are indicated by a hyphen.

Taxon	Voucher Information	Gene or Region							
		*atp*B	*ndh*F	*psb*B, T, N, and *psb*H	*psb*D, *psb*C	*psb*E, F, L, and *psb*J	*rbc*L	*rpl*2	3′*rps*12, *rps*7, and *ndh*B
POALES									
Anarthriaceae									
*Anarthria prolifera*R. Br.	*Brummitt 213376*(K)						AF148760.1[4]		
*Anarthria scabra*R. Br.	*Briggs 9581* (NSW)								
Hopkinsia anoectocolea(F. Muell.) D. F. Cutler	*Briggs 9376* (NSW)	—					AF148777[5]		
Hopkinsia anoectocolea(F. Muell.) D. F. Cutler	*Briggs 9475* (NSW)	—							
*Lyginia imberbis*R. Br.	*Briggs 9477* (NSW)	AJ419130[1]			—		AF148787[6]		
Bromeliaceae									
Brocchinia micrantha(Baker) Mez	No voucher; U.Wisconsin Botany Greenhouse living collection								
Centrolepidaceae									
*Aphelia brizula*F. Muell.	*Hopper 8532* (KPBG)								
Centrolepis monogyna(Hook. f.) Benth.	*Linder* 5689 (K)								

Table 4.2 continued

Taxon	Voucher Information	Gene or Region							
		*atp*B	*ndh*F	*psb*B, T, N, and *psb*H	*psb*D, *psb*C	*psb*E, F, L, and *psb*J	*rbc*L	*rpl*2	3'*rps*12, *rps*7, and *ndh*B
Cyperaceae									
Carex cordillerana Saarela & B. A. Ford	*Saarela 196* (ALTA)						L12672[7]		
Eleocharis palustris (L.) Roemer & JA Schultes	*Saarela 258* (UBC)								
Gahnia baniensis Benl.	*Simpson s.n.* (K)								
Mapania meditensis D. A. Simpson	*Simpson et al. 2515* (K)						DQ058337[8]		
Mapania cf. *pubisquama* Cherm	*Walters et al. 563* (MO)								
Ecdeiocoleaceae									
Georgeantha hexandra B.G. Briggs & L.A.S. Johnson	*Briggs 9530* (NSW)	—					AF148772.1[9]		
Eriocaulaceae									
Eriocaulon compressum Lam.	*Unwin 241* (MU)								
Joinvilleaceae									
Joinvillea ascendens Gaudich. ex. Brongn. & Gris.	*Weston 2501* (NSW)								
Juncaceae									
Juncus effusus L.	*Rai 1004* (ALTA)								
Luzula sp.	*Peterson, Saarela, and Smith 18634* (US)		—						

Table 4.2 continued

Taxon	Voucher Information	Gene or Region							
		*atp*B	*ndh*F	*psb*B, T, N, and *psb*H	*psb*D, *psb*C	*psb*E, F, L, and *psb*J	*rbc*L	*rpl*2	3'*rps*12, *rps*7, and *ndh*B
Poaceae									
Puelia olyriformis (Franch.) Clayton	*Bradley et al. 1060* (MO)						AF164870[10]		
Rapateaceae									
Rapatea sp.	*Chase 195* (K)								
Stegolepis sp.	*Kubitzki et al. 97-30* (HBG)						AY123242[11]		
Thurniaceae									
Prionium serratum (L. f.) Dr,ge	No voucher; National Botanic Garden of Belgium living collection (Acc. 19880003)								
Thurnia sphaerocephala (Rudge) Hook. f.	*Kelloff et al. 1335* (US)		AY208986[2]				AY123239[12]		
Xyridaceae									
Aratitiyopea lopezii (L.B. Sm.) Steyerm. & P.E. Berry	*van der Werff, Vasquez, and Gray 16131* (MO)								
Xyris jupicai Rich.	*Goldman 1766* (BH)	AY465541	AF547017	AY465566	AY465670	AY465594	AY465698	AY465722	AY465622
COMMELINALES									
Commelinaceae									
Cartonema philydroides F. Muell.	Hort. Munich Bot. Gard. s.n.	AY147602	AY147767	AY147508	AY147647	AY147555	AY149352	AY147694	AY147459
Palisota bogneri Brenan	*Stockey and Rothwell s. n.* (ALTA)	AY147606	AY147771	AY147513	AY147652	AY147560	AY149356	AY147699	AY147464

Table 4.2 continued

Taxon	Voucher Information	Gene or Region							
		*atp*B	*ndh*F	*psb*B, T, N, and *psb*H	*psb*D, *psb*C	*psb*E, F, L, and *psb*J	*rbc*L	*rpl*2	3'*rps*12, *rps*7, and *ndh*B
Tradescantia ohiensis Raf.	*Saarela 321* (UBC)						AF31228[13]		
Haemodoraceae									
Anigozanthos flavidus DC	*Neyland 1884* (MCN)								
Anigozanthos flavidus DC	*Chase 159* (NCU)								
Hanguanaceae									
Hanguana malayana (Jack) Merr.	*Stockey and Rothwell 1* (ALTA)						AM110254[14]		
Philydraceae									
Helmholtzia acorifiolia F. Muell.	*Prentis s. n.* (BRI acc. AQ 741268)								
Helmholtzia glaberrima (Hook. f.) Caruel	*Prentis s. n.* (BRI)								
Philydrella drummondii L. G. Adams	*Davis 10650* (PERTH)								
Pontederiaceae									
Eichhornia crassipes (Mart.) Solms	*Barrett 814* (TRT)		U41599.2[3]						

Previously published sequence data: [1] *Lyginia barbata*, Bremer (2002); [2] *Thurnia sphaerocephala*, Givnish et al. (2006); [3] *Eichhornia crassipes*, Graham et al. (1998), [4] *Anarthria polyphylla*, Briggs et al. (2000); [5] *Hopkinsia adscendens*, Briggs et al. (2000); [6] *Lyginia barbat*a, Briggs et al. (2000); [7] *Carex hostiana*, Chase et al. (1993); [8] *Mapania cuspidata*, Verboom et al. (2006); [9] *Georgeantha hexandra*, Briggs et al. (2000); [10] *Puelia ciliata*, Clark et al. (2000); [11] *Stegolepis parvipetala*, Michelangeli et al. (2003); [12] *Thurnia polycephala*, Michelangeli et al. (2003), [13] *Hanguana malayana*, Chase et al. (2000); [14] *Tradescantia soconuscana*, Evans et al. (2003).

Table 4.3. Additional eudicot and monocot taxa included in phylogenetic analyses with GenBank accession numbers and references. All data are from complete plastid genomes, except for *Ranunculus macranthus*.

Taxon	GenBank Accession	Reference
Eudicots		
Apiales		
Apiaceae, *Daucus carota* L.	NC_008325	Ruhlman et al. (2006)
Araliaceae, *Panax schinseng* Nees.	NC_006290	Kim and Lee (2004)
Asterales		
Asteraceae, *Helianthus annuus* L.	NC_007977	Timme et al. (unpublished)
Asteraceae, *Lactuca sativa* L.	NC_007578	Kanamoto et al. (unpublished)
Caryophyllales		
Chenopodiaceae, *Spinacia oleracea* L.	NC_002202	Schmitz-Linneweber et al. (2001)
Cucurbitales		
Cucurbitaceae, *Cucumis sativus* L.	NC_007144	Plader et al (unpublished); Kim et al. (2006)[1]
Fabales		
Fabaceae, *Glycine max* (L.) Merr.	NC_007942	Saski et al. (2005)
Fabaceae, *Lotus japonicus* L.	NC_002694	Kato et al. (2005)
Malphigiales		
Salicaceae, *Populus alba* L.	NC_008235	Okumura et al. (unpublished)
Malvales	NC_002694	Kato et al. (2006)
Malvaceae, *Gossypium hirsutum* L.	DQ345959	Lee et al. (2006)
Myrtales		
Myrtaceae, *Eucalyptus globulus* Labill.	AY780259	Steane (2005)
Onagraceae, *Oenothera elata* Kunth	NC_002693	Hupfer et al. (2000)
Proteales		
Platanaceae, *Platanus occidentalis* L.	DQ923116	Moore et al. (2006)
Rosales		
Moraceae, *Morus indica* L.	NC_008359	Ravi et al. (unpublished)
Ranunculales		
Berberidaceae, *Nandina domestica* Thunb.	DQ923117	Moore et al. (2006)

Table 4.3 continued

Taxon	GenBank Accession	Reference
Ranunculaceae, *Ranunculus macranthus* Scheele	DQ069337-DQ0069702	Leebens-Mack et al. (2005)
Sapindales		
Rutaceae, *Citrus sinensis* (L.) Osbeck	DQ864733	Bausher et al. (2006)
Solanales		
Solanaceae, *Solanum lycopersicum* L.	DQ347959	Daniell et al. (2006)
Solanaceae, *Atropa belladonna* L.	NC_004561	Schmitz-Linneweber et al. (2001)
Vitales		
Vitaceae, *Vitis vinifera* L.	NC_007957	Jansen et al. (2006)
Monocots		
Asparagales		
Orchidaceae, *Phalaenopsis aphrodite* Rchb. f.	NC_007499	Chang et al. (2005)
Poales		
Poaceae, *Saccharum officinarum* L.	NC_006084	Asano et al. (2004)
Poaceae, *Oryza nivara* S. D. Sharma & Shastry	NC_005973	Masood et al. (2004)
Poaceae, *Sorghum bicolor* L.	AC144549	Wiley et al. (unpublished)

[1] *psb*DC obtained from this genome.

Figure 4.1. One of 28 most-parsimonious trees inferred from a large plastid data set (*atp*B, *ndh*B, *ndh*F, *rbc*L, ten photosystem II genes, *rpl*2, *rps*7, and 3'-*rps*12, and several noncoding regions in the inverted repeat; see text) showing relationships among 159 angiosperm taxa. The tree is presented as a phylogram (ACCTRAN optimization).

Fig 4.1
Angiosperms

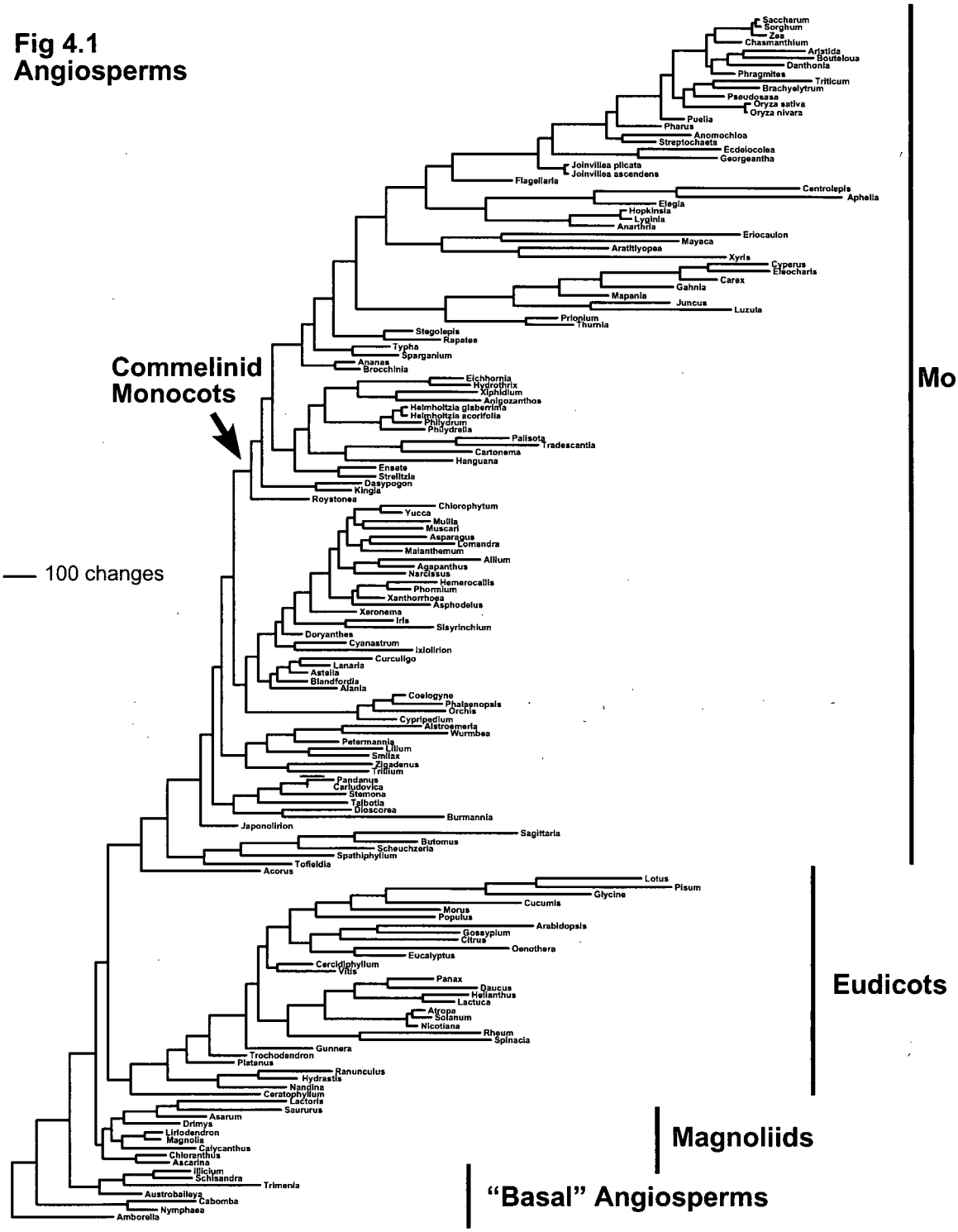

Figure 4.2. One of 28 most-parsimonious trees inferred from a large plastid data set (*atp*B, *ndh*B, *ndh*F, *rbc*L, ten photosystem II genes, *rpl*2, *rps*7, and 3'-*rps*12, and several noncoding regions in the inverted repeat; see text) showing relationships among the major angiosperm lineages (eudicots, monocots, magnoliids, and basal angiosperms). The analysis considered 159 taxa. Monocots are collapsed into a single branch for clarity. Small arrows indicate branches that collapse in a strict consensus tree. Numbers above branchs are results of bootstrap analysis.

Fig. 4.2
Angiosperms

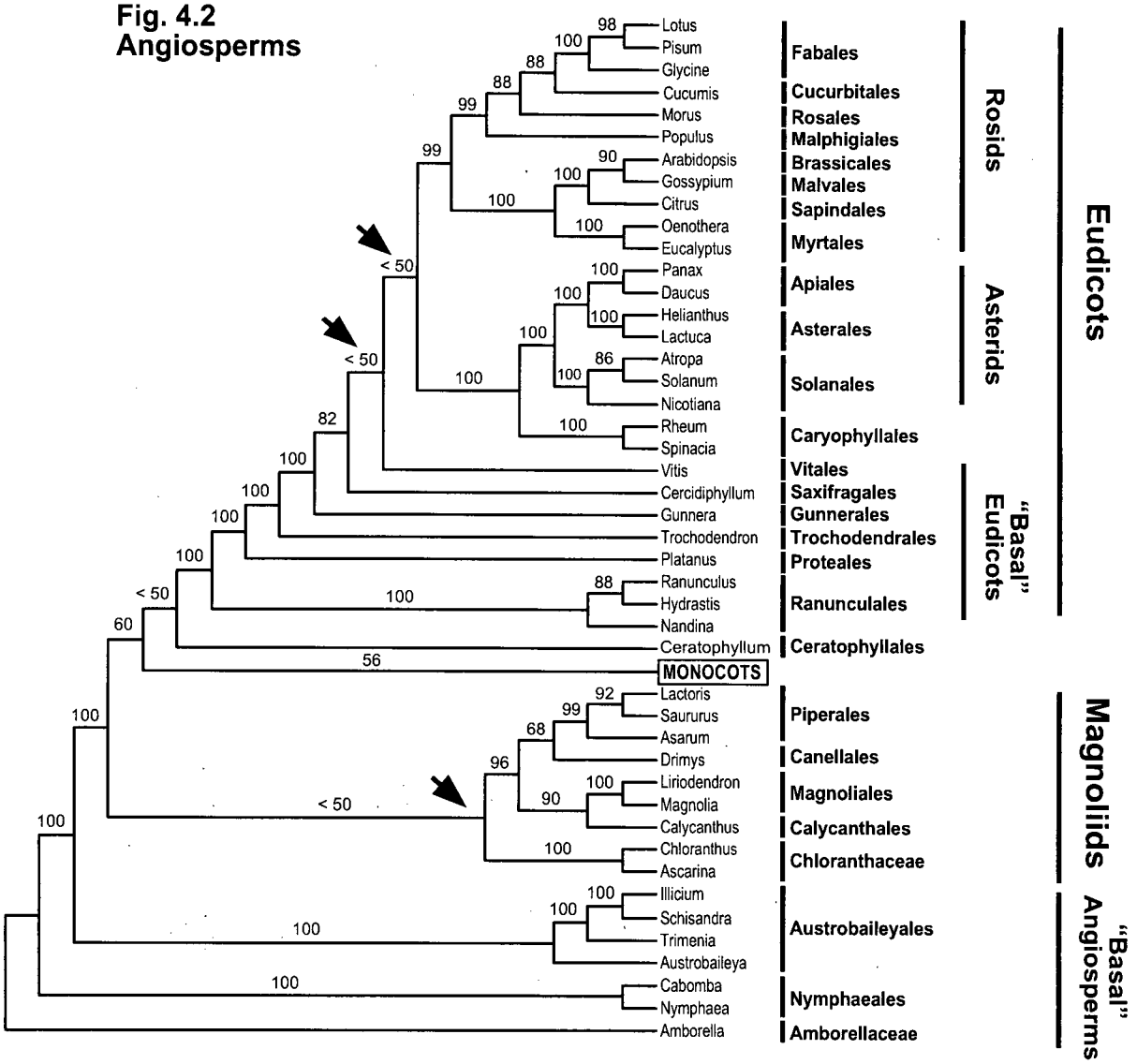

Figure 4.3. One of 12 most-parsimonious trees inferred from a large plastid data set (*atp*B, *ndh*B, *ndh*F, *rbc*L, ten photosystem II genes, *rpl*2, *rps*7, and 3'-*rps*12, and several noncoding regions in the inverted repeat; see text) showing relationships among all major monocot lineages (orders). The analysis considered 113 taxa. Commelinid monocots are collapsed into a single branch for clarity. Small arrows indicate branches that collapse in a strict consensus tree. Numbers above branchs are results of bootstrap analysis. Orders and families follow APG II (2003) using the optional "bracketed" system in Asparagales, except Petrosaviaceae are recognized as an order (see Chase 2004).

Fig. 4.3
Monocots

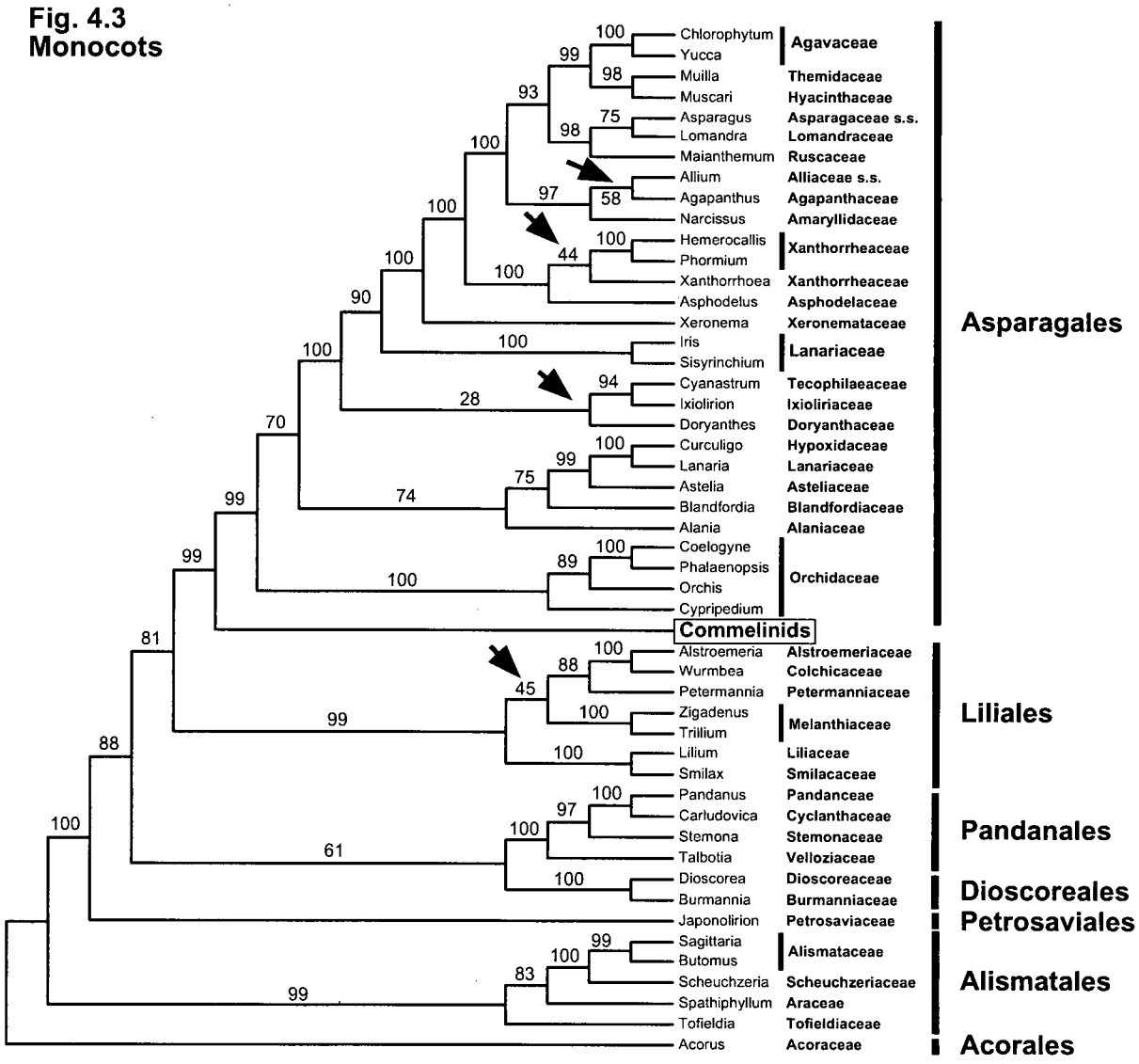

Figure 4.4. One of 12 most-parsimonious trees inferred from a large plastid data set (*atp*B, *ndh*B, *ndh*F, *rbc*L, ten photosystem II genes, *rpl*2, *rps*7, and 3'-*rps*12, and several noncoding regions in the inverted repeat; see text) showing relationships among the commelinid monocots. The analysis included 113 monocot taxa. All major lineages (orders) outside the commelinid monocots are collapsed into single branches for clarity. Small arrows indicate branches that collapse in a strict consensus tree. Numbers above or below branchs are results of bootstrap analysis. Orders and families follow APG II (2003), except Petrosaviaceae are recognized as an order (see Chase 2004). Subfamilies in Poaceae follow GPWG (2001) and Soreng et al. (2006).

Fig. 4.4
MP

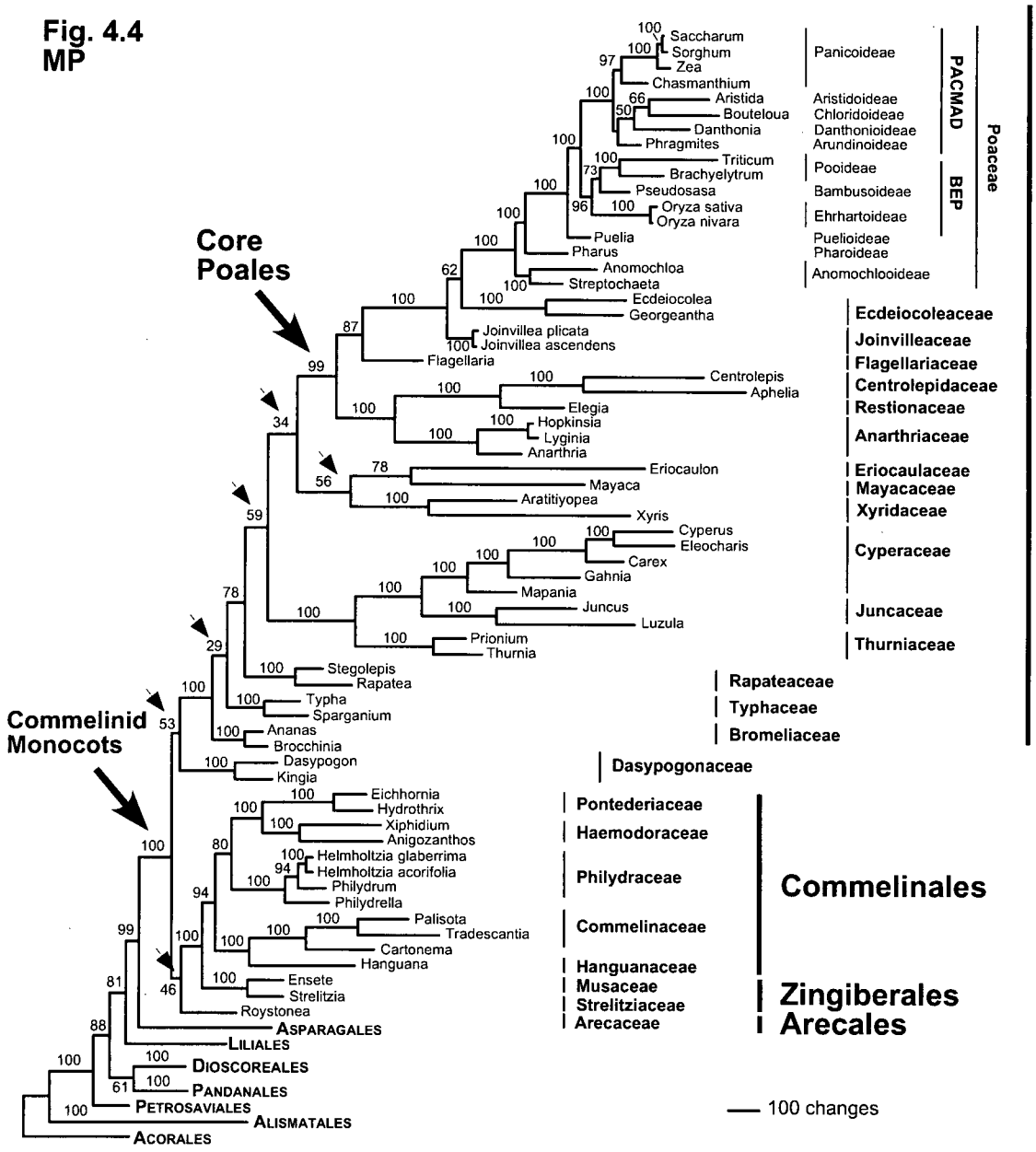
Core Poales
Commelinid Monocots
Saccharum
Sorghum
Zea
Chasmanthium
Aristida
Bouteloua
Danthonia
Phragmites
Triticum
Brachyelytrum
Pseudosasa
Oryza sativa
Oryza nivara
Puelia
Pharus
Anomochloa
Streptochaeta
Ecdeiocolea
Georgeantha
Joinvillea plicata
Joinvillea ascendens
Flagellaria
Centrolepis
Aphelia
Elegia
Hopkinsia
Lyginia
Anarthria
Eriocaulon
Mayaca
Aratitiyopea
Xyris
Cyperus
Eleocharis
Carex
Gahnia
Mapania
Juncus
Luzula
Prionium
Thurnia
Stegolepis
Rapatea
Typha
Sparganium
Ananas
Brocchinia
Dasypogon
Kingia
Eichhornia
Hydrothrix
Xiphidium
Anigozanthos
Helmholtzia glaberrima
Helmholtzia acorifolia
Philydrum
Philydrella
Palisota
Tradescantia
Cartonema
Hanguana
Ensete
Strelitzia
Roystonea
ASPARAGALES
LILIALES
DIOSCOREALES
PANDANALES
PETROSAVIALES
ALISMATALES
ACORALES
Panicoideae
Aristidoideae
Chloridoideae
Danthonioideae
Arundinoideae
Pooideae
Bambusoideae
Ehrhartoideae
Puelioideae
Pharoideae
Anomochlooideae
PACMAD
BEP
Poaceae
Ecdeiocoleaceae
Joinvilleaceae
Flagellariaceae
Centrolepidaceae
Restionaceae
Anarthriaceae
Eriocaulaceae
Mayacaceae
Xyridaceae
Cyperaceae
Juncaceae
Thurniaceae
Rapateaceae
Typhaceae
Bromeliaceae
Dasypogonaceae
Pontederiaceae
Haemodoraceae
Philydraceae
Commelinaceae
Hanguanaceae
Musaceae
Strelitziaceae
Arecaceae
Commelinales
Zingiberales
Arecales
Poales
100 changes

Figure 4.5. Optimal tree from maximum likelihood analysis inferred from a large plastid data set (*atp*B, *ndh*B, *ndh*F, *rbc*L, ten photosystem II genes, *rpl*2, *rps*7, and 3'-*rps*12, and several noncoding regions in the inverted repeat; see text) showing relationships among commelinid monocots. The analysis considered 69 taxa, including four exemplars from Asparagales and Liliales. The tree was inferred using the GTR + Γ + I model of evolution. Numbers above or below branchs are results of maximum likelihood bootstrap analysis. Orders and families follow APG II (2003), and subfamilies in Poaceae follow GPWG (2001) and Soreng et al. (2006).

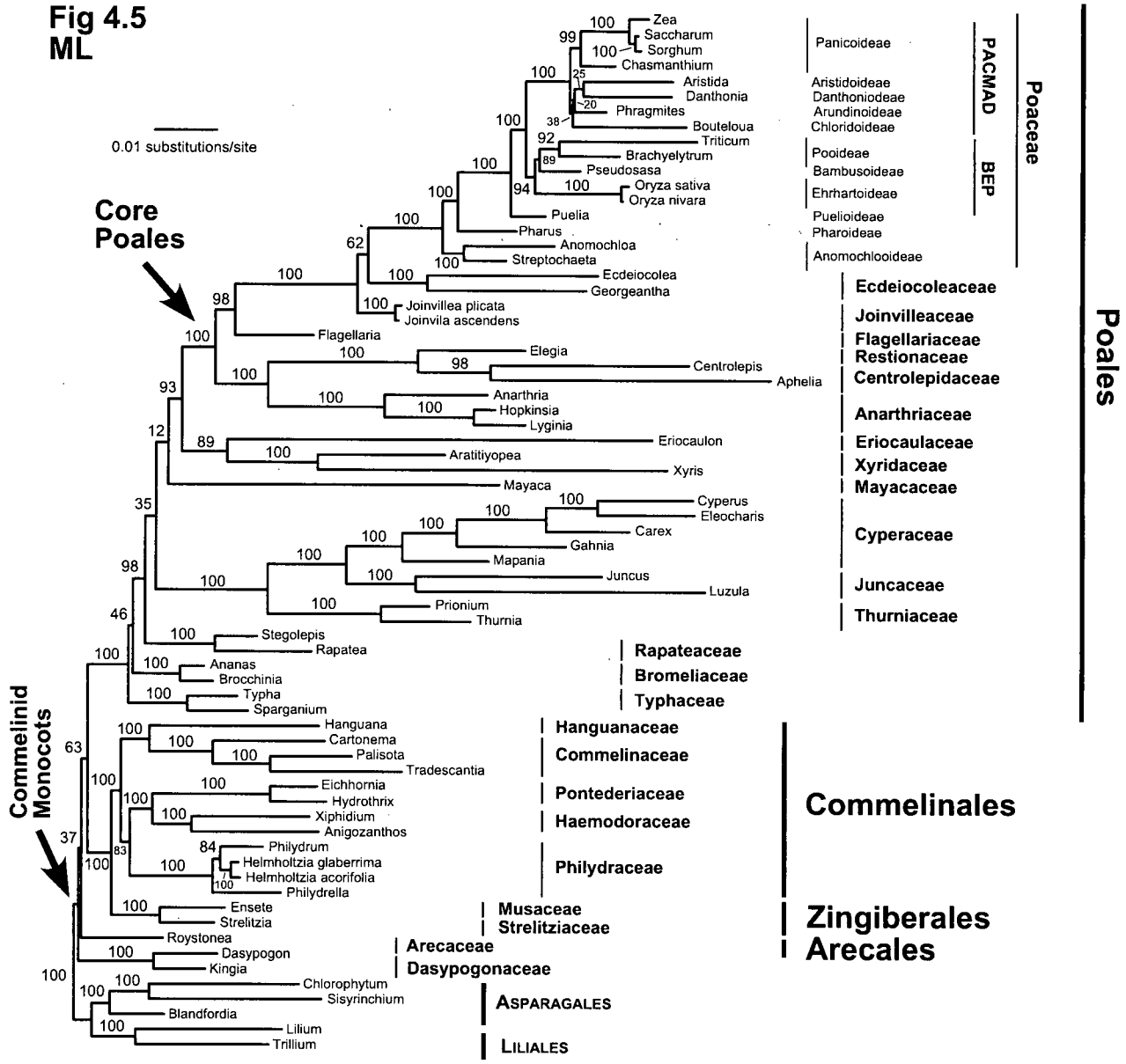
Fig 4.5
ML
0.01 substitutions/site
Core
Poales
Commelinid
Monocots
Zea
Saccharum
Sorghum
Chasmanthium
Aristida
Danthonia
Phragmites
Bouteloua
Triticum
Brachyelytrum
Pseudosasa
Oryza sativa
Oryza nivara
Puelia
Pharus
Anomochloa
Streptochaeta
Ecdeiocolea
Georgeantha
Joinvillea plicata
Joinvila ascendens
Flagellaria
Elegia
Centrolepis
Aphelia
Anarthria
Hopkinsia
Lyginia
Eriocaulon
Aratitiyopea
Xyris
Mayaca
Cyperus
Eleocharis
Carex
Gahnia
Mapania
Juncus
Luzula
Prionium
Thurnia
Stegolepis
Rapatea
Ananas
Brocchinia
Typha
Sparganium
Hanguana
Cartonema
Palisota
Tradescantia
Eichhornia
Hydrothrix
Xiphidium
Anigozanthos
Philydrum
Helmholtzia glaberrima
Helmholtzia acorifolia
Philydrella
Ensete
Strelitzia
Roystonea
Dasypogon
Kingia
Chlorophytum
Sisyrinchium
Blandfordia
Lilium
Trillium
Panicoideae
Aristidoideae
Danthoniodeae
Arundinoideae
Chloridoideae
Pooideae
Bambusoideae
Ehrhartoideae
Puelioideae
Pharoideae
Anomochlooideae
PACMAD
BEP
Poaceae
Ecdeiocoleaceae
Joinvilleaceae
Flagellariaceae
Restionaceae
Centrolepidaceae
Anarthriaceae
Eriocaulaceae
Xyridaceae
Mayacaceae
Cyperaceae
Juncaceae
Thurniaceae
Poales
Rapateaceae
Bromeliaceae
Typhaceae
Hanguanaceae
Commelinaceae
Pontederiaceae
Haemodoraceae
Philydraceae
Commelinales
Musaceae
Strelitziaceae
Zingiberales
Arecaceae
Dasypogonaceae
Arecales
ASPARAGALES
LILIALES

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CHAPTER 5[1]

Hydatellaceae are Not Monocots but a Lineage Near the Base of Angiosperm Phylogeny

5.1 INTRODUCTION

Although the relationship of angiosperms to other seed plants remains poorly resolved, great progress has been made in identifying the earliest extant splits in flowering-plant phylogeny with the discovery that the New Caledonian shrub *Amborella*, the water lilies (Nymphaeales), and the woody Austrobaileyales constitute a basal grade of lines that diverged before the main angiosperm radiation (e.g., Graham and Olmstead 2000; Mathews and Donoghue 2000; Zanis et al. 2002; Borsch et al. 2003; Qiu et al. 2005; Leebens-Mack et al. 2005). By focusing attention on these ancient lines, this finding has re-written our understanding of angiosperm structural and reproductive biology, physiology, ecology, and taxonomy (e.g., Doyle and Endress 2000; APG II 2003; Williams and Friedman 2004; Feild et al. 2004). The discovery of a new basal lineage would lead to further re-evaluation of the initial angiosperm radiation, but would also be unexpected, as nearly all of the ~460 flowering-plant families have been surveyed in molecular studies (APG II 2003).

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[1] A version of this chapter is in review:

Saarela, J. M., Rai, H. S., Doyle, J. A., Endress, P. K., Mathews, S., Marchant, A. D., Briggs, B. G., and Graham, S. W. Hydatellaceae are not monocots but a lineage near the base of angiosperm phylogeny.

One such group that has resisted placement is Hydatellaceae, a small, aquatic family of two genera (*Hydatella* Diels and *Trithuria* Hook. f.) restricted to Australasia and India. *Hydatella* and *Trithuria* were traditionally included in their own tribe *Diplanthereae* (=*Trithurieae*) (Hieronymous 1887; Gilg-Benedict 1930; Hamann 1964) in the superficially similar Centrolepidaceae, a family of highly reduced monocots. The minute reproductive structures of both groups are interpreted as multi-flowered inflorescences subtended by bracts; individual flowers are unisexual, consisting of a single stamen or carpel, with no associated perianth or bract. Despite these similarities, a number of structural differences warranted separation of Hydatellaceae from Centrolepidaceae (Hamann 1976). In fact, many features in Hydatellaceae are rare or unknown among 'core Poales' (a clade consisting of Centrolepidaceae, grasses and five other families; see Chapter 4), including monosulcate pollen, completely anatropous ovules, and typically starchy perisperm (seed storage tissue of nucellar origin). Other characteristics are virtually unknown in monocots (e.g., cellular endosperm development, restricted to the basal monocot genus *Acorus*). Based on morphological characteristics, a placement of Hydatellaceae within any of the major clades of monocots has therefore been viewed as problematic (e.g., Dahlgren et al. 1985; Hamann 1998). A single *rbc*L sequence for one species (*Trithuria submersa* Hook. f.) has been included in several studies, which generally places the family in Poales, seeming to confirm conventional views (e.g., Stevenson et. al. 2000; Michelangeli et al. 2003; Davis et al. 2004; Chase et al. 2006). Most molecular studies, however, have excluded the family due to unavailability of material and/ or poor quality DNA (e.g., Bremer 2002; Givnish et al. 2006). As part of a large-scale phylogenetic survey of grasses and relatives (Chapter 4), we re-examined patterns of variation along the published *rbc*L sequence from *T. submersa*, and

discovered that it is likely a PCR-based chimera of a grass and a moss sequence (confirmed by Dr. Jerrold Davis, Cornell University; pers. comm.).

The objectives of this study are to infer the position of Hydatellaceae among the flowering plants. Using morphology and molecular data for *Hydatella* and *Trithuria*, we show that Hydatellaceae have been misinterpreted as monocots, and are instead a highly modified and previously unrecognized ancient lineage of angiosperms.

5.2 MATERIALS AND METHODS

5.2.1. Taxonomic and Genomic Sampling

We obtained plastid sequence data from *Hydatella* (*H. inconspicua* Cheeseman) and *Trithuria* (*T. submersa*), and nuclear sequence data from *Trithuria*. Methods of DNA extraction, amplification, sequencing, and alignment follow Graham and Olmstead (2000), Borsch et al. (2003), and Graham et al. (2006) for plastid data, and Mathews and Donoghue (2000) for nuclear data. For *T. submersa*, we sequenced multiple plastid regions involved in photosynthesis (*atp*B, *psb*B, *psb*T, *psb*N, *psb*H, *rbc*L), chlororespiration (*ndh*F, *ndh*B), and translation (*rpl*2, 3'-*rps*12, *rps*7) (GenBank accession numbers: DQ915185–DQ915186, DQ915188-DQ915189) using DNA extracted by the Royal Botanic Gardens, Kew [*J. G. Conran 961 & P. J. Rudall* (ADU)]. Sequences for *ndh*F (AF547020) and the nuclear gene phytochrome C (*PHYC)* (DQ981794), as well as an additional *rbc*L sequence (DQ915187), were sequenced from an extraction prepared by K. Bremer, Uppsala University [*A. Doust 1123* (MELU)]. The latter extraction was also used to generate an *atp*B sequence for *T. submersa* in an earlier study (AJ419142; Bremer 2002). The two new *rbc*L sequences, one from each source of DNA, are identical for their 483 bp shared portion; we use the longer of

the two here (DQ915188; Conran and Rudall collection). The problematic published *rbc*L sequence for *T. submersa* [AF458076.1 (Stevenson et al. 2000; Michelangeli et al. 2003)] was obtained using a different DNA extraction generated from the Doust collection; that DNA extraction was not used here. We obtained sequence data from *H. inconspicua* for the plastid noncoding regions spanning *trn*L(UAA)-*trn*F(GAA) (DQ916291), from cultivated material sourced from Lake Rotokawau, New Zealand [*P. D Chapman s.n.* (NSW accession 428712)].

We added plastid data for *Trithuria submersa* (except for *trn*L-*trn*F; see below) to an existing alignment used in previous studies (Graham and Olmstead 2000; Rai et al. 2003; Graham et al. 2006) that includes basal angiosperms, eudicots, and monocots. Voucher information and GenBank numbers not previously published for taxa included here are: [*Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), *R.G. Olmstead 98-59* (WTU), GenBank numbers: AF238063, AY007488, AF239774, AY007473, AY007458, AF238049, except *A. thaliana atp*B and *rbc*L sequences are from the whole plastid genome (AP000423; Sato et al. 1999)], [*Ascarina lucida* Hook f. (Chloranthaceae), *Peter de Lange 3594* (AK), GenBank numbers: AF238064, AY116647, AF239776, AY007474, AY007459, AF239775, AF238050, AF238051], [*Chloranthus japonicus* Siebold (Chloranthaceae), *M.W. Chase 204* (NCU), GenBank numbers: AF238066, AY007490, AF239778, AY007476, AY007461, AF238053, except *C. japonicus atp*B (AJ235431.2; Savolainen et al. 2000) and *rbc*L (L12640; Qiu et al. 1993)], [*Gunnera chilensis* Lam. (Gunneraceae), no voucher, DNA U. Washington greenhouse (DNA = R.G. Olmstead #98-33), GenBank numbers: AF238068, AY007491, AF239781, AY007478, AY007463, AF238054, except *Gunnera atp*B (AF093374) and *rbc*L (AF093724) sequences are from *G. hamiltonii* Kirk ex W.S. Ham.

(Hoot et al. 1999)], and [*Schisandra chinensis* (Turcz.) Baill. (Schisandraceae), *Reznicek 10720* (MICH), GenBank numbers: AF238075, AY007498, AF239791, AY007485, AY007470, AF239790, AF238061, AF238062].

We added the *trn*L–*trn*F region from *H. inconspicua* to a larger published matrix for *trn*T-*trn*L-*trn*F (Borsch et al. 2003), and excluded several mutational hotspots (Borsch et al. 2003). We aligned the *T. submersa PHYC* sequence with previously published (Mathews and Donoghue 2000; Duvall et al 2006) and two unpublished sequences [(*Schisandra sphenathera* A. C. Smith (Schisandraceae), *Mathews 576* (A), GenBank number DQ981793), and (*Brasenia schreberi* J.F. Gmelin (Cabombaceae), no voucher, GenBank number DQ981792; see also Mathews and Donoghue (2000))].

5.2.2. Molecular Phylogenetic Analyses

For the plastid-based analyses (excluding *trn*L-*trn*F) involving *T. submersa*, we included all protein-coding genes sampled and several conservative noncoding regions [i.e., introns in *rpl*2, 3'-*rps*12 and *ndh*B; intergenic spacers between 3'-*rps*12–*rps*7 and *rps*7–*ndh*B (Graham and Olmstead 2000)]. We excluded intergenic spacer from two photosystem gene regions (*psb*B–*psb*H; *psb*E–*psb*J). The combined analyses also included several regions not yet obtained for *Trithuria* (i.e., *psb*D–*psb*C, *psb*E–*psb*J; intron and second exon of *ndh*B). For analyses of individual regions (subpartitions *atp*B, *ndh*F, *psb*B–*psb*H, *rbc*L, *rpl*2, and 3'-*rps*12–*ndh*B), we excluded outgroups (*Cycas* and *Ginkgo*) to minimize the effect of rooting uncertainty (Graham and Olmstead 2000) on inferred bootstrap support values, as we were interested primarily in the structure of the angiosperm subtree in these analyses. We excluded one variable region in the *PHYC* alignment from all analyses.

We performed heuristic maximum-parsimony (MP) and maximum-likelihood (ML) searches using PAUP* v. 4.0b10 (Swofford et al. 2002) and PHYML v. 2.4.4 (Guindon and Gascuel 2003), respectively, with default settings (e.g., TBR branch-swapping), except that MP searches used 100 random addition replicates. We determined optimal ML models using the likelihood ratio test and the Akaike information criterion, as implemented in ModelTest v. 3.7 (Posada and Crandall 1998); the two methods concurred in each case examined, except for *PHYC*. The optimal models favoured by the likelihood ratio test and the Akaike information criterion were GTR + Γ + I for *rbc*L, *atp*B, and combined data; TVM + Γ + I for *ndh*F, *psb*B-H, and 3'*rps*12-*ndh*B; and K81uF + Γ + I for *rpl*2 (see ModelTest documentation for details of individual DNA substitution models; Posada and Crandall 1998). The optimal models favoured by the likelihood ratio test and the Akaike information criterion for *PHYC* were TrN + Γ + I and GTR + Γ + I, respectively. The latter is a more general case of the former model, and it is the one that we used for the ML analysis of *PHYC*. We computed optimal model parameters with PAUP*, based on an MP tree from the corresponding analysis (e.g., Fig. 5.1), and used these values in the heuristic ML searches, except that we determined base frequencies empirically. We estimated branch support using bootstrap analysis, with 200 bootstrap replicates in each case (each with one random addition replicate in the MP analyses).

For the large plastid multigene data set that included *T. submersa*, we also assessed whether several suboptimal root positions (see arrows and asterisks in Fig. 5.1) could be distinguished from the optimal one. We used the Shimodaira-Hasegawa (Shimodaira and Hasegawa 1999) in PAUP* to (simultaneously) compare alternative root placements for the angiosperm subtree in Fig. 5.1. We performed this test using RELL (resampling estimated

log-likelihood) with 1,000 bootstrap replicates, and with all DNA substitution model parameters estimated from the data, except for base frequencies, which were calculated empirically. Results were evaluated as a one-tailed test, with an alpha-level of 0.05. We also analysed six individual plastid regions (*atp*B, *ndh*F, *psb*B–*psb*H, *rbc*L, *rpl*2, and 3'-*rps*12–*ndh*B) in separate unrooted MP and ML analyses to infer relationships in the absence of a root.

To demonstrate that the absence of several regions examined for taxa in the large multigene analysis, but not yet obtained for *Trithuria submersa* (i.e., *psb*D, *psb*C, *psb*E-*psb*F-*psb*L-*psb*J; the intron and second exon of *ndh*B), has little effect on basic tree inference, we re-ran the heuristic searches of the combined plastid data, considering only the regions obtained for *Trithuria*.

5.2.3. Morphological Phylogenetic Analyses

For morphological analyses, we added a consensus of *Hydatella* and *Trithuria* to a published data matrix (Doyle and Endress 2000) with modifications (Doyle 2005), and supplemented by data on four- vs. eight-nucleate female gametophytes (Williams and Friedman 2004). Scoring of most characters was based on Hamann (1975, 1976, 1998) and Dahlgren et al. (1985), with data from other sources on anatomy (Cutler 1969; Cheadle and Kosakai 1975), palynology (Ladd 1977), and seedling germination (Cooke 1983b). Characters and character scorings for Hydatellaceae are presented in Table 5.1.

Analysis of this data set without Hydatellaceae gives some weakly supported results that are strongly overruled by molecular data [e.g., Nymphaeales are linked with monocots and not located in the basal grade (Doyle and Endress 2000)]. We therefore analyzed this

data set using PAUP*, both without constraints and with relationships of other taxa constrained in heuristic MP searches using two backbone trees: one that corresponds to the MP tree from the previous combined morphological and molecular analysis (Doyle and Endress 2000), and a second (see Fig. 5.3) with modifications that take account increasingly robust molecular evidence on relationships in (eu)magnoliids (Zanis et al. 2002; Qiu et al. 2005) (Piperales, Canellales, Magnoliales, Laurales), eudicots (Kim et al. 2004), and monocots (Graham et al. 2006; see also Fig. 5.1). These constraints allow the position of Hydatellaceae to be determined by morphology. For the constrained analysis, we evaluated less than optimal arrangements of Hydatellaceae by searching for trees up to seven steps less parsimonious than the optimal tree length, and by moving taxa manually with MacClade v. 4.03 (Maddison and Maddison 2001). Apart from enforcing topological constraints and performing 100 random addition replicates per analysis, we used default settings for all heuristic searches. For the bootstrap analysis (performed without constraints), we used the settings described for the molecular analysis. We determined unambiguous synapomorphies using the "Trace all changes" tool in MacClade.

5.3 RESULTS

5.3.1. Molecular Analyses

Maximum parsimony (MP) and maximum likelihood (ML) analyses of multiple plastid genes and associated noncoding regions identify Hydatellaceae as the sister group of water lilies (Nymphaeales) (BP_{MP} = 80% and BP_{ML} = 100%; Fig 5.1). These analyses include several regions not yet obtained for *Trithuria*, but exclusion of these regions does not substantially affect tree inference or support values (data not shown). The topology that we

recovered for the full data set is also recovered for this reduced data set in the MP analysis (one of two MP trees) and the ML analysis. To corroborate this result using evidence from another genome, we sampled a portion of the nuclear gene phytochrome C (*PHYC*) from *T. submersa* and analyzed this sequence with orthologous sequences from other angiosperms that include most of the lineages in Fig. 5.1. In this analysis, we again observe a sister-group relationship between Hydatellaceae and water lilies, with moderate to strong bootstrap support (BP_{MP} = 70% and BP_{ML} = 90%; Fig. 5.2).

We also obtained plastid data from *Hydatella* for two noncoding regions that span the plastid tRNA genes *trn*L(UAA) and *trn*F(GAA) of *H. inconspicua*. Despite being based on a relatively limited amount of data, phylogenetic analyses again depict Hydatellaceae and Nymphaeales as sister taxa, with moderate bootstrap support (BP_{MP} = 75% and BP_{ML} = 69%; Fig. 5.3).

5.3.2. Morphological Analyses

Despite the extensive structural reduction of Hydatellaceae, we were able to score 59% of the morphological characters in the matrix (see Table 5.1). Analysis of the morphological data also showed that Hydatellaceae and Nymphaeales are sister taxa when relationships are not constrained, with 83% bootstrap support (Fig. 5.4); the clade consisting of Hydatellaceae and Nymphaeales is then sister to monocots, but with only 24% bootstrap support (Fig. 5.4). When backbone-relationships among other taxa are constrained to a tree according to recent molecular studies (see Methods), we also find that Hydatellaceae are the sister group of Nymphaeales (Fig. 5.5). We also find similar results when the original topology (Doyle and Endress 2000) is used.

A sister-group relationship between Hydatellaceae and Nymphaeales is supported by nine unequivocal synapomorphies (lack of a vascular cambium, anomocytic stomata, truncate anther connective, boat-shaped pollen, inner integument with two cell layers, sclerotic exotesta, seed operculum formed by cell enlargement in the inner integument, perisperm, hypogeal germination). Although most of these (except the seed operculum character state) occur in other taxa, they are not consistently associated. Hydatellaceae remain linked with Nymphaeales in all trees up to five steps less parsimonious than the shortest tree.

The next-best position for Hydatellaceae, six steps less parsimonious than the shortest tree, is nested within monocots, as the sister group of *Tofieldia* and *Butomus*. A sister-group relationship to all monocots is seven steps less parsimonious. In the latter case, the relationship of Hydatellaceae and monocots would be supported by only three unequivocal synapomorphies (no cambium, boat-shaped pollen, two-layered inner integument), all of which occur as convergences in Nymphaeales. Linear leaves and P2 sieve tube plastids would also support this relationship (they originate twice when Hydatellaceae are linked with Nymphaeales), but would be equivocal as synapomorphies, due to ambiguity concerning positions of character-state changes. Placement of Hydatellaceae in core Poales would require additional reversals (e.g., from orthotropous to anatropous ovules, porate to sulcate pollen, and nuclear to cellular endosperm development).

5.4 DISCUSSION

Molecular evidence has been particularly useful in clarifying the phylogenetic positions of groups with highly modified morphologies, such as holoparasites (e.g., Rafflesiaceae, Barkman et al. 2004), mycoheterotrophs (e.g., *Petrosavia* Becc., Cameron et al. 2003), and

certain aquatics (e.g., *Hydrostachys* Thou., Albach et al. 2001; Podostemaceae, Ueda et al. 1997). Hydatellaceae are also highly modified (i.e., extremely reduced) and have been difficult to place in morphology-based classifications. We find that combined analysis of multiple plastid regions from *Trithuria submersa* identify Hydatellaceae as the sister group of the water lilies (Nymphaeales; Fig. 5.1), with strong bootstrap support from maximum parsimony (MP) and maximum likelihood (ML) analyses. This surprising result is corroborated by data for *PHYC* from the nuclear genome (Fig. 5.2). Plastid data from *H. inconspicua*, representing the other genus in the family, for two noncoding regions that span the tRNA genes *trn*L(UAA) and *trn*F(GAA), also place Hydatellaceae as the sister group of Nymphaeales (Fig. 5.3). Based on these three independent lines of evidence, the placement of Hydatellaceae as the sister group of Nymphaeales indicates that water lilies are part of a larger lineage that evolved more extreme and diverse modifications for life in an aquatic habitat than previously recognized.

There is some uncertainty concerning the root of flowering-plant phylogeny (e.g., Soltis et al. 1999; Graham and Olmstead 2000; Mathews and Donoghue 2000; Zanis et al. 2002; Borsch et al. 2003; Qiu et al. 2005; Leebens-Mack et al. 2005), likely a function of the relatively long branch connecting angiosperms to other seed plants. As misrooting can lead to misinference of ingroup relationships (Graham et al. 2002), we determined plausible roots for the combined plastid tree using the Shimodaira-Hasegawa test (see Methods). A root on the *Trithuria* branch is among those rejected, but trees rooted at two positions not significantly worse than the optimal one (see arrows in Fig. 5.1) also indicate a sister-group relationship between Hydatellaceae and Nymphaeales. Additionally, when each of six distinct plastid regions (*atp*B, *ndh*F, *psb*B–*psb*H, *rbc*L, *rpl*2, and 3'-*rps*12–*ndh*B) is analysed

in separate unrooted MP and ML analyses (see Methods), we consistently observe a branch separating Hydatellaceae and Nymphaeales from all other angiosperms with strong bootstrap support (90-100%; data not shown).

Morphological data identify nine unequivocal synapomorpies supporting a sister-group relationship between Hydatellaceae and Nymphaeales (see Results; Fig. 5.5). Some of these features are among those originally used to segregate Hydatellaceae from Centrolepidaceae (Hamann 1976). Several other similarities support a link between Hydatellaceae and Nymphaeales, and a position among the most basal angiosperms. These include ascidiate carpels and a four-nucleate embryo sac, although the latter needs evaluation in the context of studies among related lineages (Williams and Friedman 2004). Both of these features are considered ancestral in angiosperms (Doyle and Endress 2000; Williams and Friedman 2004). Several character states found in Hydatellaceae are scattered across various monocot lineages, including perisperm (e.g., *Acorus* L., some Zingiberales; Rudall and Furness 1997), cellular endosperm (e.g., *Acorus*, but this requires further review; see Rudall and Furness 1997), and monosulcate pollen [e.g., *Acorus*, Petrosaviaceae (Cameron et al. 2003), some Asparagales (Penet et al. 2005)]. If Hydatellaceae were linked with monocots, the presence of these characters in Hydatellaceae would likely represent parallelisms or reversals. Better information on other characters (see Table 5.1) could affect support for the relationships inferred from morphology here. For example, cotyledon number is unknown in Hydatellaceae, which have a "minute, lens-shaped, incompletely developed embryo" (Hamann 1998), as in some Nymphaeales.

Our results have little effect on previous inferences of the growth habit and ecology of the common ancestor of extant angiosperms (Feild et al. 2004), because the closely related

Hydatellaceae and Nymphaeales are both aquatic herbs. A rooting of the flowering plants near *Amborella* and/or water lilies (Nymphaeales) has been robustly supported by numerous analyses of individual and combined sequences from the plastid and other genomes. One contrary result, however, is likely a function of long-branch attraction and low taxon sampling (see Leebens-Mack et al. 2005). If *Amborella* alone, or a clade consisting of *Amborella*, Nymphaeales and Hydatellaceae is sister to other angiosperms (see arrows in Fig. 5.1), parsimony optimization implies that the first angiosperms were woody and terrestrial, and that Hydatellaceae and Nymphaeales were an early line that invaded aquatic habitats. The third plausible rooting, in which Hydatellaceae and Nymphaeales are the sister group of all other angiosperms, would imply that the first angiosperms could be either woody and terrestrial or herbaceous and aquatic (Feild et al. 2004).

It would be misleading to view Hydatellaceae merely as reduced water lilies. First, Hydatellaceae occupy a different aquatic niche from Nymphaeales. No member of Nymphaeales has a phenotype that comes close to the minute, submergence-tolerant, moss-like habit of Hydatellaceae, evidently a convergence with the distantly related Centrolepidaceae. *Hydatella inconspicua* can be found growing and flowering at depths of a metre or more. In other species, plants are usually initially submerged, but may flower under water or on drying mud at the edges of seasonal pools or swamps. Second, Hydatellaceae have inflorescences rather than solitary flowers like Nymphaeales, indicating that their common ancestor with Nymphaeales could have had either condition. The Early Cretaceous aquatic genus, *Archaefructus* Sun, Dilcher, Zheng & Zhou, has been interpreted as the sister group of all extant angiosperms (Sun et al. 2002), or as a reduced aquatic nested within crown-group angiosperms (Friis et al. 2003). A link between *Archaefructus* and

Nymphaeales has been rejected (Crepet et al. 2004), but the possibility of a relationship of *Archaefructus* with Hydatellaceae should be investigated, since both taxa have inflorescences of naked, unisexual flowers. One recently described species of *Archaefructus* (*A. eoflora*) has a denser infloresence structure that is more comparable to that of Hydatellaceae than other species of *Archaefructus* (Ji et al. 2004)

Our current knowledge of Hydatellaceae is limited. The family has only recently been discovered in India (Yadav and Janarthanam 1995), and there is speculation that it has been overlooked elsewhere (Hamann 1998). Half of the ten or so species were described in the past 25 years (Cooke 1981, 1983; Yadav and Janarthanam 1995), and there appears to be substantial morphological variation among them; more species may therefore await discovery. It is clear that monographic work should be a high priority. Phylogenetic relationships need to be characterized within the family, in addition to studies of the developmental morphology, aquatic ecology, biogeography, and conservation status of these curious and neglected basal angiosperms.

Table 5.1 Morphological characters and character states scored for Hydatellaceae. See Doyle and Endress (2000) and Doyle (2005) for more information on characters and character states.

Character: character states	Hydatellaceae
1 Habit: Tree or shrub, rhizomatous or scandent	rhizomatous or scandent
2 Stele: eustele, siphonostele, monocot type	?
3 Inverted vascular bundles: absent, present	?
4 Protoxylem lacunae: absent, present	siphonostele
5 Cambium: present, absent	absent
6 Storied structure: absent, present	?
7 Vessels: tracheids, porose pits, vessels	porose pits
8 Vessel grouping: solitary, multiples	?
9 Vessel perforations: scalariform, mixed, simple only	scalariform
10 Fiber pits: bordered, reduced	?
11 Ray width: narrow, wide	?
12 Paratracheal parenchyma: absent or scanty, well developed	?
13 Tangential parench bands: absent, present	?
14 Pith: uniform, septate	uniform
15 Secondary phloem: simple, stratified	?
16 Sieve tube plastids: starch, P1 type, P2 type	P2 type
17 Pericycle: separate fiber bundles, continuous fiber ring, composite with U sclereids, no sclerenchyma	?
18 Laticifers: absent, present	absent
19 Raphide idioblasts: absent, present	absent
20 Phyllotaxy: spiral, distichous, opposite	?
21 Nodes: multilacunar, one trace unilacunar, two trace unilacunar, trilacunar	?
22 Prophylls: paired lateral, single	?
23 Stipules: absent, adaxial axillary, interpetiolar	absent
24 Axillary squamules: absent, present	?
25 Leaf blade: bifacial, unifacial	?
26 Blade shape: obovate to elliptical, ovate, linear	linear
27 Major venation: pinnate, palmate or basally crowded	?
28 Base of blade: not peltate, peltate	?
29 Leaf dissection: simple, lobed, or dissected	simple
30 Marginal teeth: absent, chloranthoid, monimioid, platanoid	?
31 Stomata: paracytic, laterocytic, anomocytic, stephanocytic	anomocytic
32 Midrib vasculature: simple arc, arc with adaxial plate ring	?
33 Palisade parenchyma: absent, present	absent
34 Asterosclerids: absent, present	absent
35 Oil cells: absent, present	absent
36 Mucilage cells: absent, present	absent
37 Inflorescence: solitary, raceme or spike or botryoid, richly branched	raceme spike botryoid
38 Sex of flowers: bisexual, bisexual and unisexual, unisexual	unisexual

Table 5.1 (continued)

Character: character states	Hydatellaceae
39 Hypanthium: absent, present, inferior ovary	?
40 Perianth phyllotaxy: spiral, whorled	?
41 Perianth whorls: more than two, two, one, absent	absent
42 Perianth merosity: irregular, threes, twos fours or fives	?
43 Outer perianth cycle: undifferentiated, sepaloid	?
44 Calyx fusion: separate or basally fused, calyptrate	?
45 Nectar petals: absent, present	?
46 Stamen phyllotaxy: spiral, whorled, irregular	?
47 Stamen merosity: irregular, threes, twos fours or fives	?
48 Stamen fusion: free, connate	?
49 Stamen base: short, long and wide, long and narrow filament	long and narrow filament
50 Paired stamen glands: absent, present	absent
51 Connective apex: extended, truncate or rounded	truncate or rounded
52 Microsporangia: four, two	four
53 Pollen sacs: protruding, embedded	?
54 Orientation of dehiscence: introrse, latrorse to slightly introrse, extrorse	latrorse to slightly introrse
55 Dehiscence mode: longitudinal slit, H valvate, upward opening flaps	longitudinal slit
56 Connective hypodermis: unspecialized, endothecial	unspecialized
57 Tapetum: secretory, amoeboid	?
58 Microsporogenesis: simultaneous, successive	?
59 Pollen unit: monads, tetrads	monads
60 Pollen shape: boat shaped, globose	boat shaped
61 Apertures: monosulcate etc., inaperturate sulcuate, Garside tri or hexacolpate, tricolpate	monosulcate
62 Pollen size: large >50 micrometers, medium, small <20 micrometers	small
63 Infratectum: granular, intermediate, columellar	?
64 Tectum: continuous or microperforate, perforate to semitectate, reduced	continuous
65 Striate muri: absent, present	absent
66 Supratectal spinules: absent, present	present
67 Prominent spines: absent, present	absent
68 Aperture membrane: smooth, sculptured	?
69 Nexine stratification: homogeneous foot layer, footlayer and endexine, foliated nexine	?
70 Nexine thickness: absent or discontinuous, thin (less than 1:3), thick (1:3 or more)	?
71 Inner staminodes: absent, present	?
72 Carpel number: more than one, one	one
73 Carpel form: ascidiate, intermediate, plicate	ascidiate
74 Carpel sealing: by secretion, mixed partial PGF, mixed full PGF, postgenital fusion	?
75 Pollen tube transm. tissue: not differentiated, one layer diff., multilayered	?

Table 5.1 continued

	Character: character states	Hydatellaceae
76	Style: absent - sessile stigma, present	absent-sessile stigma
77	Stigma: extended, restricted	?
78	Stigma papillae: unicellular or smooth, uniseriate pluricellular, pluriseriate	uniseriate pluricellular
79	Compitum: absent, extragynoecial	?
80	Carpel fusion: apocarpous, paracarpous, eusyncarpous	apocarpous
81	Oil cells in carpels: not visible, intrusive	not visible
82	Septal nectaries: absent, present	absent
83	Ovule number: one, mostly two, more than two	one
84	Placentation: linear, laminar, diffuse	?
85	Ovule direction: pendent, horizontal, ascendent	pendent
86	Ovule curvature: anatropous, orthotropous, hemitropous	anatropous
87	Integuments: two, one	two
88	Outer integ shape: semiannular, annular	?
89	Outer integ lobation: unlobed, lobed	?
90	Outer integ thickness: 2 cells, 2 and 3 to 4, 4 and 5 or more	2 cells
91	Inner integ thickness: 2 cells, 2 and 3 or 3, 3 and more	2 cells
92	Chalaza: unextended, pachychalazal, perichalazal	unextended
93	Nucellus: crassinucellar, tenuinucellar, or pseudocrassi	crassinucellar
94	Fruit wall: fleshy, fleshy with endocarp, dry	dry
95	Fruit dehiscence: indehiscent, dehiscent	indehiscent/ dehiscent
96	Testa: nonmultiplicative, multiplicative	nonmultiplicative
97	Exotesta: normal, palisade or shorter sclerotic, tabular	palisade or shorter sclerotic
98	Mesotesta: unspecialized, sclerotic, fibrous, sarcotesta spongy	?
99	Endotesta: unspecialized single lignified layer, multiple lignified layer, tracheidal, palisade or shorter sclerotic	unspecialized single
100	Tegmen: unspecialized, both layers sclerotic, fibrous, exotegmen	absent
101	Ruminations: absent, testal, tegminal, or chalazal	absent
102	Operculum: absent, present	present
103	Aril: absent, present	absent
104	Endosperm development: cellular, nuclear, helobial	cellular
105	Endosperm in seed: present, absent	present
106	Perisperm: absent, present	present
107	Embryo: minute, large	minute
108	Cotyledons: two, one	?
109	Germination: epigeal, hypogeal	hypogeal
110	Embryo sac: 4 nucleate, 8 or 9 nucleate	4 nucleate

Figure 5.1. Phylogenetic placement of *Trithuria submersa* (Hydatellaceae) in angiosperms according to plastid data (multiple protein-coding genes and several noncoding regions; see text). MP and ML analyses yield the same topology (12,607 steps; -*ln*L = 88,820.974). Branch lengths are MP estimates (ACCTRAN optimization). Two outgroup taxa, *Cycas* and *Ginkgo*, have been trimmed for clarity (with the stem lineage shortened). Bootstrap support values are noted near branches (MP above or to the left, ML below or to the right). The optimal and two suboptimal roots (indicated with arrows) could not be distinguished from each other in a Shimodaira-Hasegawa test that simultaneously considered these and four additional candidate roots (indicated with asterisks; the latter four cases rejected at $P < 0.05$).

Fig. 5.1

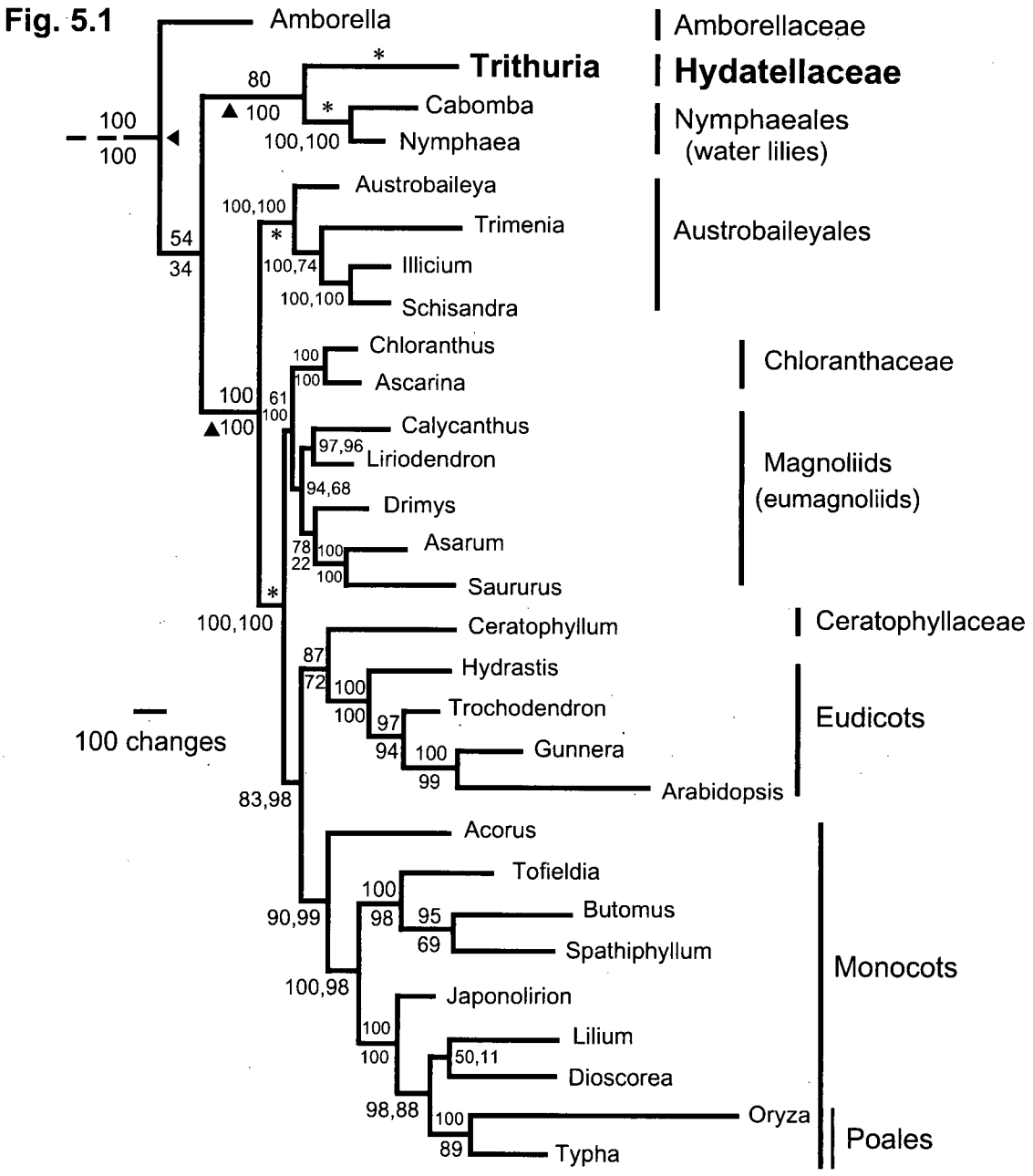

Amborella
Trithuria
Cabomba
Nymphaea
Austrobaileya
Trimenia
Illicium
Schisandra
Chloranthus
Ascarina
Calycanthus
Liriodendron
Drimys
Asarum
Saururus
Ceratophyllum
Hydrastis
Trochodendron
Gunnera
Arabidopsis
Acorus
Tofieldia
Butomus
Spathiphyllum
Japonolirion
Lilium
Dioscorea
Oryza
Typha
100 changes
Amborellaceae
Hydatellaceae
Nymphaeales
(water lilies)
Austrobaileyales
Chloranthaceae
Magnoliids
(eumagnoliids)
Ceratophyllaceae
Eudicots
Monocots
Poales

Figure 5.2. Phylogenetic placement of *Trithuria submersa* (Hydatellaceae) in angiosperms according to analyses of the nuclear phytochrome C locus (*PHYC*). The best MP tree (tree length: 4,074 steps) is shown; the ML tree (tree length: -*ln*L = 17,094.891) had the following general topology: (*Amborella*, ((Hydatellaceae, Nymphaeales), (Austrobaileyales, (Chloranthaceae, ((Canellales, (Laurales,Magnoliales)), (Monocots, (Piperales,Eudicots))))))), and with *Nelumbo* sister to (*Arabidopsis*, *Solanum*), but otherwise with the same structure within each clade as the MP tree. Branch lengths are MP estimates (ACCTRAN optimization). Bootstrap support values are noted near branches (MP above or to the left, ML below or to the right).

Fig 5.2

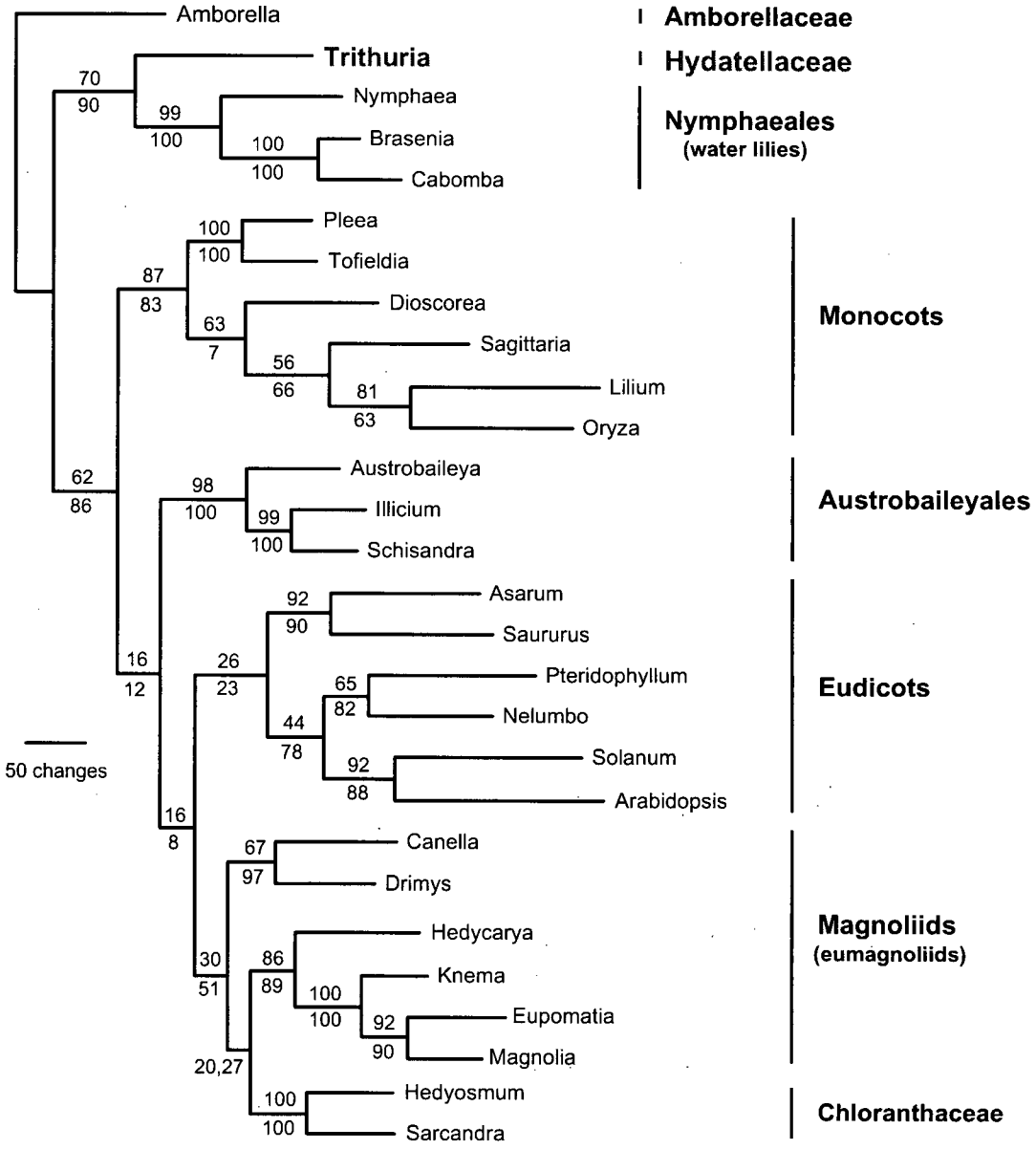

Amborella
Trithuria
Nymphaea
Brasenia
Cabomba
Pleea
Tofieldia
Dioscorea
Sagittaria
Lilium
Oryza
Austrobaileya
Illicium
Schisandra
Asarum
Saururus
Pteridophyllum
Nelumbo
Solanum
Arabidopsis
Canella
Drimys
Hedycarya
Knema
Eupomatia
Magnolia
Hedyosmum
Sarcandra
70
90
99
100
100
100
100
100
87
83
63
7
56
66
81
63
62
86
98
100
99
100
92
90
16
12
26
23
65
82
44
78
92
88
16
8
67
97
30
51
86
89
100
100
92
90
20,27
100
100
50 changes
Amborellaceae
Hydatellaceae
Nymphaeales
(water lilies)
Monocots
Austrobaileyales
Eudicots
Magnoliids
(eumagnoliids)
Chloranthaceae

Figure 5.3. Phylogenetic placement of *Hydatella inconspicua* (Hydatellaceae) for analyses of the *trn*T-*trn*L-*trn*F region, including the *trn*L-*trn*F region for *H. inconspicua*. One of two MP trees (tree length: 3,292 steps) is shown; the arrow indicates a branch that collapses in the strict consensus of the MP trees. The ML tree (tree length: -*ln*L = 19,968.977) has several additional differences in topology (i.e., *Nuphar* is depicted as sister to the remaining water lilies; Chloranthaceae sister to magnoliids). Branch lengths are MP estimates (ACCTRAN optimization). Three outgroup taxa (*Araucaria*, *Ginkgo* and *Pinus*) have been trimmed for clarity, with the stem lineage shortened. Bootstrap support values are noted near branches (MP above or to the left, ML below or to the right).

Fig. 5.3

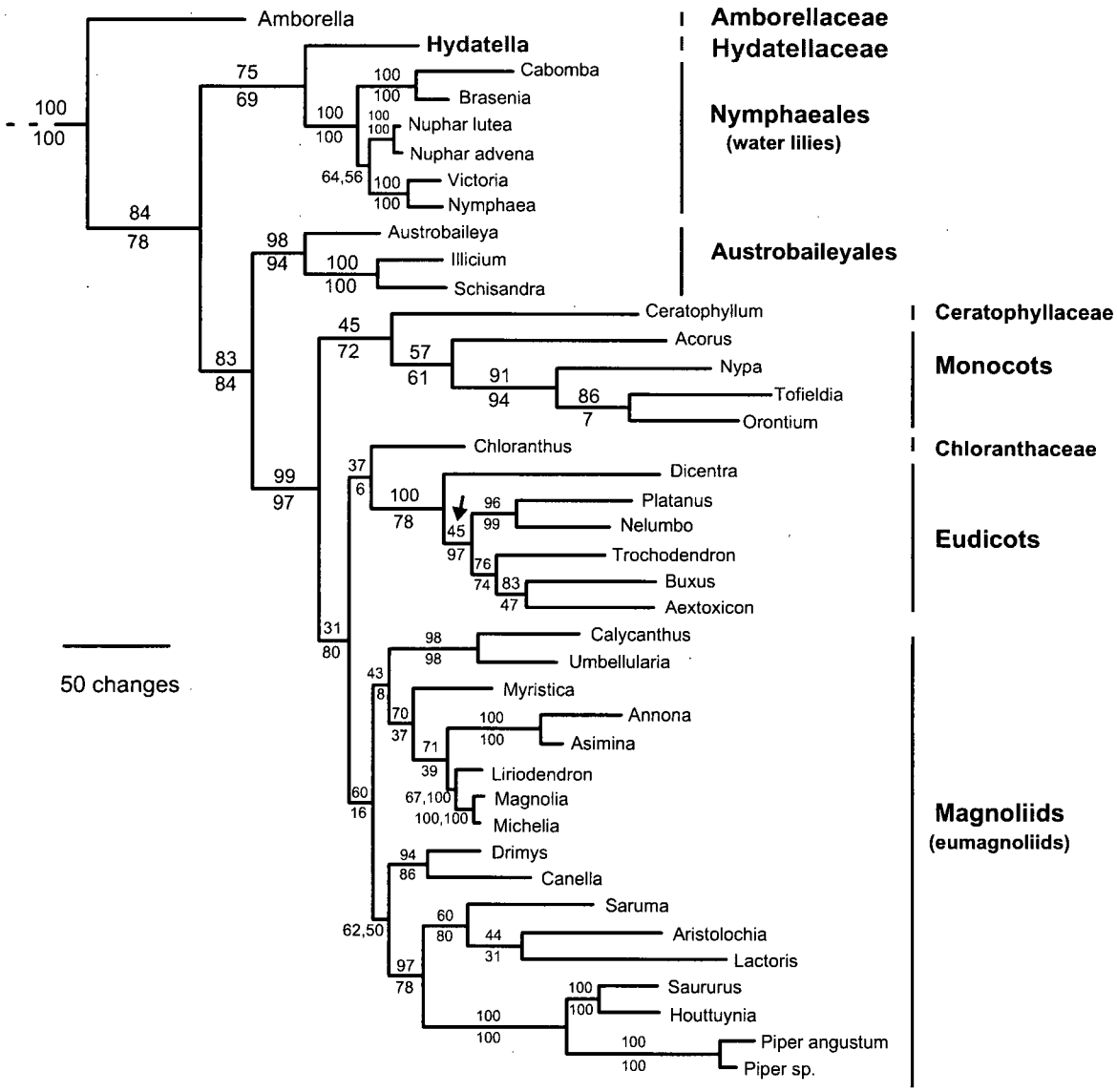

Amborella
Hydatella
Cabomba
Brasenia
Nuphar lutea
Nuphar advena
Victoria
Nymphaea
Austrobaileya
Illicium
Schisandra
Ceratophyllum
Acorus
Nypa
Tofieldia
Orontium
Chloranthus
Dicentra
Platanus
Nelumbo
Trochodendron
Buxus
Aextoxicon
Calycanthus
Umbellularia
Myristica
Annona
Asimina
Liriodendron
Magnolia
Michelia
Drimys
Canella
Saruma
Aristolochia
Lactoris
Saururus
Houttuynia
Piper angustum
Piper sp.
50 changes
Amborellaceae
Hydatellaceae
Nymphaeales
(water lilies)
Austrobaileyales
Ceratophyllaceae
Monocots
Chloranthaceae
Eudicots
Magnoliids
(eumagnoliids)

Figure 5.4. Most parsimonious position of Hydatellaceae based on morphology, with relationships of other taxa not constrained (see Methods). Bootstrap support values are noted near branches. Arrows indicate branches that collapse in a strict consensus tree.

Fig. 5.4

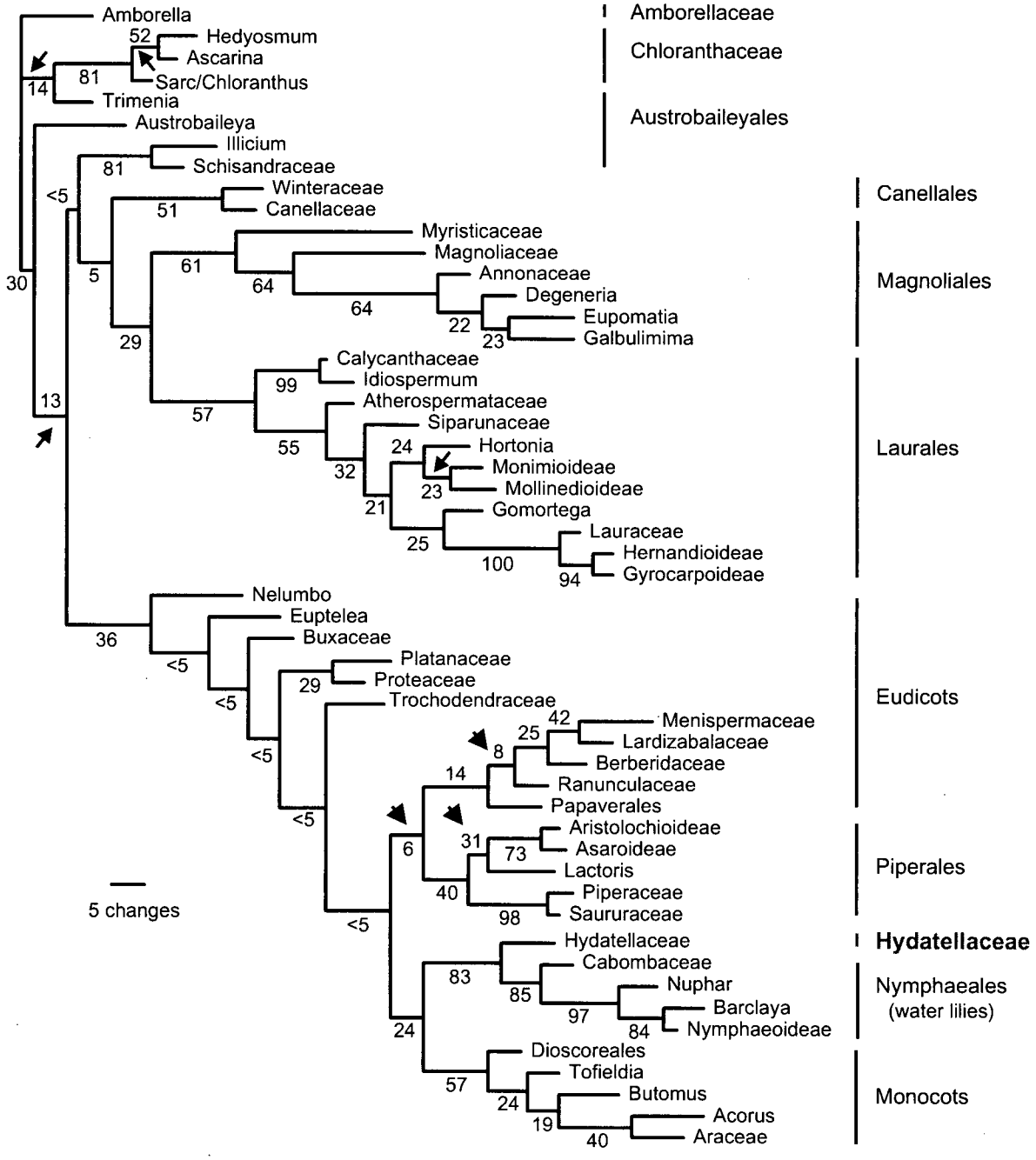
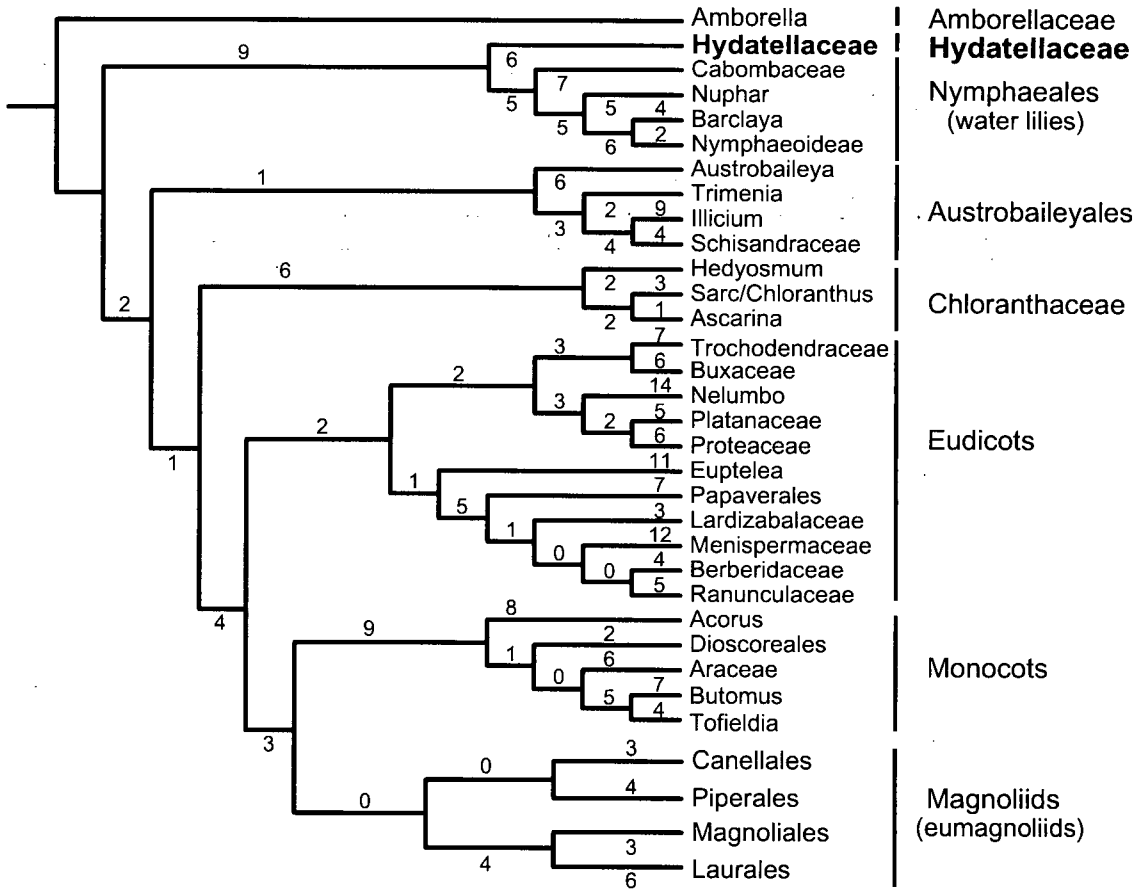

Amborella
Hedyosmum
Ascarina
Sarc/Chloranthus
Trimenia
Austrobaileya
Illicium
Schisandraceae
Winteraceae
Canellaceae
Myristicaceae
Magnoliaceae
Annonaceae
Degeneria
Eupomatia
Galbulimima
Calycanthaceae
Idiospermum
Atherospermataceae
Siparunaceae
Hortonia
Monimioideae
Mollinedioideae
Gomortega
Lauraceae
Hernandioideae
Gyrocarpoideae
Nelumbo
Euptelea
Buxaceae
Platanaceae
Proteaceae
Trochodendraceae
Menispermaceae
Lardizabalaceae
Berberidaceae
Ranunculaceae
Papaverales
Aristolochioideae
Asaroideae
Lactoris
Piperaceae
Saururaceae
Hydatellaceae
Cabombaceae
Nuphar
Barclaya
Nymphaeoideae
Dioscoreales
Tofieldia
Butomus
Acorus
Araceae
5 changes
Amborellaceae
Chloranthaceae
Austrobaileyales
Canellales
Magnoliales
Laurales
Eudicots
Piperales
Hydatellaceae
Nymphaeales
(water lilies)
Monocots

Figure 5.5. Most parsimonious position of Hydatellaceae based on morphology (774 steps), with relationships of other taxa constrained (see Methods). Numbers of unequivocal changes (with respect to the root indicated) are noted near each branch. For clarity, most phylogenetic structure within magnoliids (i.e., within Canellales, Piperales, Laurales and Magnoliales) has been excluded [see Doyle and Endress (2000) for details].

Fig. 5.5


Amborella
Hydatellaceae
Cabombaceae
Nuphar
Barclaya
Nymphaeoideae
Austrobaileya
Trimenia
Illicium
Schisandraceae
Hedyosmum
Sarc/Chloranthus
Ascarina
Trochodendraceae
Buxaceae
Nelumbo
Platanaceae
Proteaceae
Euptelea
Papaverales
Lardizabalaceae
Menispermaceae
Berberidaceae
Ranunculaceae
Acorus
Dioscoreales
Araceae
Butomus
Tofieldia
Canellales
Piperales
Magnoliales
Laurales
Amborellaceae
Hydatellaceae
Nymphaeales
(water lilies)
Austrobaileyales
Chloranthaceae
Eudicots
Monocots
Magnoliids
(eumagnoliids)

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CHAPTER 6

Conclusion

6.1 CONCLUSIONS AND FUTURE DIRECTIONS

Most previous phylogenetic studies of monocots have been based on only one or a few genes or regions, and multiple branches of the monocot tree of life have not yet been resolved clearly or supported strongly. In this thesis, I have used an expanded plastid data set to make phylogenetic inferences among major commelinid monocot lineages, among families within the commelinid orders Commelinales and Poales, and among major lineages in the grass family, Poaceae. I also conducted a phylogenetic study of the grass genus *Bromus*, using plastid and nuclear data. In line with several previous studies that used the same plastid regions examined here (Graham and Olmstead 2000; McPherson 2003; Zgurski 2004; Graham et al. 2006), my analyses of higher-order relationships in the commelinid monocots generally provide increased support for several relationships that have been inferred previously (e.g., Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006), and provide support for some relationships not inferred previously, including the position of Hydatellaceae outside of the monocots. My study of *Bromus* has provided new insights into the identities of major lineages in the genus, their interrelationships, and possible insight into past evolutionary processes acting in the genus, such as hybridization and polyploidy. Nonetheless, several higher-order commelinid relationships that have been difficult to infer in the past, and most aspects of recent relationships in *Bromus*, remain recalcitrant to clear and strong phylogenetic inference here.

In Chapter 2, I characterized phylogenetic relationships in the morphologically diverse and taxonomically complex grass genus *Bromus*, using sequence data from two plastid loci and a nuclear locus for 46 species (a third of known species-level diversity in the genus). Based on the nuclear ribosomal data, sects. *Bromus* (including sect. *Triniusia*), *Genea*, *Neobromus*, and *Ceratochloa* are monophyletic, and sect. *Bromopsis* comprises several distinct lineages. Plastid trees indicate that sects. *Genea* and *Bromus* are closely related, and the incongruence detected between the plastid and nuclear ribosomal data possibly supports a hybrid origin for the *B. pectinatus* complex between sects. *Bromus* and *Genea*. Plastid trees indicate a close relationship between Old World and some North American species of sect. *Bromopsis*, and the plastid and nuclear ribosomal data indicate that one South American species of sect. *Bromopsis* is not closely related to North American and Eurasian species traditionally classified in the same section. Most species of *Bromus* sampled had levels of sequence variation too low to allow complete resolution of relationships among close relatives at the species level. Recognition of the brome grasses as one distinct genus, *Bromus*, is in agreement with my molecular data, but current classification schemes do not satisfactorily reflect phylogenetic relationships within the genus, particularly with respect to the circumscription of sect. *Bromopsis*. However, before a revised infrageneric classification of *Bromus* is proposed, substantially better sampling should be conducted of different genomic regions, to obtain better support for phylogenetic relationships among taxa, and of taxa, to further clarify incongruence among different nuclear and plastid data partitions to more adequately sample the molecular, morphological, and geographical variability in the genus. Through this phylogenetic work on *Bromus*, I also discovered, described, and characterized the phylogenetic position of a new species from

Peru, *Bromus ayacuchensis* Saarela and P. M. Peterson *in ed.* (Saarela et al. in press), and I identified and clarified the taxonomic status of an endemic Californian species [*Bromus hallii* (Hitchc.) Saarela & P. M. Peterson; Saarela et al. 2005).

In Chapters 3 and 4, I characterized phylogenetic relationships among 10–11 of the twelve major lineages (subfamilies) of grasses. I observe general concordance in topology among most partitions of the data, and among different methods of phylogenetic analysis, but there are several instances of moderate to strong conflict (primarily between the protein coding and the noncoding data partitions) in various parts of the tree. My data provide strong support for relationships along much of the spine of Poaceae, congruent with the findings of a recent multigene study (GPWG 2001). Major findings of this study include strong support for the monophyly of Anomochlooideae, strong support for the position of Puelioideae as the third of three successive lineages that are the sister group of the rest of the grasses, substantially increased support from plastid data for the monophyly of the BEP clade, and moderate support for a sister-group relationship between Pooideae and Bambusoideae. Given this latter finding, I suggest that comparative genomic studies of the grasses (e.g., Xu et al. 2005) could benefit from analysis of a bamboo genome, which may provide additional insight into the evolution of Pooideae cereal crop genomes (e.g., *Avena* L., *Hordeum* L., *Triticum* L., *Secale* L.) compared with the more distantly related rice genome (Ehrhartoideae). As in previous studies, most relationships among subfamilies within the PACMAD clade are not inferred consistently or strongly here, perhaps a consequence of short internal branches. An Aristidoideae–Chloridoideae clade is recovered with moderate to strong support in most analyses, but the noncoding data do not recover this group. I have not sampled Micrairoideae, a recently recognized subfamily that is part of the PACMAD clade

(Duvall et al. 2006). Future work should sample this lineage for the regions examined here. Nonetheless, substantial work remains to unravel the phylogenetic history of the diverse PACMAD clade. A simple and potentially effective addition to the current analysis would be to sample more than one taxon per subfamily of Poaceae in future studies, and more data might be necessary in a subset of cases (e.g., within the PACMAD clade).

In Chapter 4, I broadly characterized phylogenetic relationships in the commelinid moncots, a large clade that includes about a third of monocot diversity. In line with recent studies (e.g., Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006), I inferred a strongly supported sister group relationship between Commelinales and Zingiberales. As in earlier studies, I was not able to infer the relative positions of Arecales, Dasypogonaceae, Commelinales–Zingiberales, and Poales with clear or strong support. Placements for these ancient lineages will likely require substantially more data per taxon, and possibly complete plastid genomes, which have helped clarify the positions of some taxa (e.g., Vitaceae; Jansen et al. 2006). Within Commelinales, I identified two major clades: one comprising Commelinaceae and Hanguanceae, the other Haemodoraceae, Philydraceae, and Pontederiaceae. In the latter clade, I inferred Philydraceae – a family whose exact position in Commelinales has been most controversial – to be the sister group of Haemodoraceae–Pontederiaceae, with moderate support, similar to that found in the only previous study to also infer this position (Chase et al. 2006). I also sampled each of the four genera in Philydraceae, and provide the first complete genus-level phylogeny for the family. I found *Philydrella* to be the sister group of a *Helmholtzia–Philydrum* clade with moderate support, a relationship that is seemingly consistent with several aspects of morphology in the family.

Within Poales, I identified a major clade that includes all Poales families except Bromeliaceae and Typhaceae, consistent with the findings of recent studies (e.g., Chase et al. 2006; Givnish et al. 2006; Graham et al. 2006). As in previous studies, the exact positions of Bromeliaceae and Typhaceae at the base of the Poales subtree are not resolved here. In line with previous studies, my analyses infer a strongly supported cyperid clade, in which Thurniaceae (*Thurnia* and *Prionium*) are the sister group of Cyperaceae–Juncaceae. However, the relationship of the cyperid clade to other Poales remains unresolved. The composition and relationships among taxa in the xyrid clade differ between my parsimony and likelihood analyses. With parsimony I find a weakly supported xyrid clade that includes Xyridaceae as the sister group of a more-strongly supported Eriocaulaceae–Mayacaceae clade, whereas with likelihood I find a strongly supported Eriocaulaceae–Xyridaceae clade that is the sister group of the core Poales, and the position of Mayacaceae is not resolved. These different relationships are likely a function of long branches in each of these families, particularly in the aquatic genus *Mayaca*. As likelihood methods are less prone to long-branch artifacts, it seems reasonable to have more confidence in the less certain placement for Mayacaceae inferred by this method. The close relationship inferred between Eriocaulaceae and Xyridaceae in likelihood analysis here has also been found in numerous other studies, and it is also supported by several putative morphological synapomorphies. Clearly, the position of Mayacaceae remains unestablished. A possible solution for this problem would be to sample additional species in each of these three families that potentially span the root node in each family, which might help break up long branches.

I identify a core Poales clade with strong support that includes a graminid clade and a restiid clade. Within the restiid clade, Anarthriaceae sensu APG II (2003) (i.e., including

Hopkinsia and *Lyginia*) are strongly supported as the sister group of Centrolepidaceae–Restionaceae. With only one exemplar sampled from Restionaceae, I am unable to determine if Centrolepidaceae are a distinct lineage, or if they are part of a broader Restionaceae, as has been suggested previously. Further sampling within Restionaceae will be necessary to address this unresolved question. I identify Flagellariaceae, a family whose position has been difficult to unambiguously resolve with both morphological and molecular data, to be strongly supported as the sister group of the rest of the graminid clade. The graminid clade also includes a strongly supported Ecdeiocoleaceae–Joinvilleaceae–Poaceae clade. In my first study (Chapter 3), I found relationships among Ecdeiocoleaceae, Joinvilleaceae, and Poaceae to be resolved variously, generally with low support. However, if the possibly conflicting noncoding data partition is excluded from consideration, a clade consisting of Ecdeiocoleaceae and Joinvilleaceae is inferred by model-based analyses to be the sister-group of Poaceae, with moderate to strong support. I expanded taxon sampling in and around this clade in Chapter 4, and found weak support for an Ecdeiocoleaceae–Poaceae clade, a topology that has also been inferred in several recent studies (e.g., Bremer 2002; Michelangeli et al. 2003; Davis et al. 2004; Chase et al. 2006). Despite exclusion of rapidly evolving noncoding regions, my parsimony and likelihood analyses in Chapter 4 did not infer the Ecdeiocoleaceae–Joinvilleaceae clade that I found in Chapter 3. This is likely a function of increased taxon sampling in Chapter 4, in which I sampled all Poales lineages, and also included *Georgeantha*, the other genus in Ecdeiocoleaceae (inclusion of *Georgeantha* appears to have divided the relatively long branch in *Ecdeiocolea* in Chapter 3). Nonetheless, support for an Ecdeicoloeaceae–Poaceae clade here remains unsatisfactorily weak. Increased support for this relationship (if it is true) will require more data, and may

benefit from inclusion of a second recently discovered species of *Ecdeiocolea* (B. G. Briggs, personal communication).

In Chapters 4 and 5, I demonstrated that several closely related families in Poales have a substantially accelerated plastid substitution rate compared with most other monocots. Now that several aspects of higher-order relationships in Poales are clear and well supported, the molecular evolution of the plastid regions examined here should be examined more closely. It would be worthwhile to characterize and compare rates of synonymous and non-synonymous substitutions in protein-coding regions within and across Poales and other monocot lineages, to distinguish among possible causes for this rate variation (i.e., relaxation of purifying selection, the role of genetic drift, changes in the underlying mutation rate, or a decrease in DNA repair efficiency; Young et al. 2005). I also found an intronless version of the 3'-*rps*12 locus in *Luzula* – the only report for this intron loss in monocots outside of Asparagales. Future work should characterize the distribution of this intron loss across Juncaceae.

Finally, in Chapter 5, I demonstrated that the putative monocot family Hydatellaceae, a small and highly reduced group of plants that has been difficult to place among monocots, is not a commelinid (Poales) at all, but the sister group of the water lilies (Nymphaeales). Previous placements for the family in Poales with molecular data were based on a single *rbc*L sequence, which I found to be a contaminant, chimeric sequence of a grass and a moss. New data for multiple plastid loci and the nuclear PHYC locus strongly place *Trithuria submersa* as the sister group of Nymphaeales. Plastid data for the *trn*L(UAA)–*trn*F(GAA) region from *Hydatella*, the other genus in the family, place Hydatellaceae in the same position. Morphological data are strongly congruent with these molecular results. Although

only 59% of 110 morphological characters used in previous studies of basal angiosperms could be scored for Hydatellaceae (e.g., Doyle 2005), analyses of morphological characters also identify a sister group relationship between Hydatellaceae and Nymphaeales, supported by nine unequivocal synapomorphies. Discovery of this new lineage of basal angiosperm has substantial implications for understanding early patterns of angiosperm diversification, and should provide a solid evolutionary framework for future work on the family.

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