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Phylogeny and Subfamilial Classification of the Grasses (Poaceae)

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PHYLOGENY AND  
SUBFAMILIAL  
CLASSIFICATION OF THE  
GRASSES (POACEAE)<sup>1</sup>

*Grass Phylogeny Working Group*<sup>2,3</sup>

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ABSTRACT

A large collaborative effort has yielded a comprehensive study of the phylogeny and a new subfamilial classification of the grass family (Poaceae/Gramineae). The study was conducted on an integrated and representative set of 62 grasses (0.6% of the species and ca. 8% of the genera) plus four outgroup taxa using six molecular sequence data sets (*ndhF*, *rbcl*, *rpoC2*, *phyB*, ITS2, and GBSSI or *waxy*), chloroplast restriction site data, and morphological data. A parsimony analysis using 2143 informative characters (the combined analysis) resulted in a single most parsimonious tree of 8752 steps with an RI of 0.556 and bootstrap support of > 90% for more than half of the internal nodes. Significant relationships that appear consistently in all analyses of all data sets and are strongly supported by the combined analysis include the following: Joinvilleaceae are sister to a monophyletic Poaceae; the earliest diverging lineages of the Poaceae are Anomochlooideae, Pharoideae, and Puelioideae, respectively; and all remaining grasses form a clade. Multiple monophyletic clades were recovered, including Bambusoideae s. str., Ehrhartoideae, Pooideae s.l., Aristidoideae, Danthoioideae, Chloridoideae s. str., Chloridoideae s.l., Panicoideae, Parianeae, Olyreae s. str., Oryzeae, Stipeae, Meliceae, *Lygeum* + *Nardus*, and *Molinia* + *Phragmites*. The PACCAD Clade is monophyletic, containing Aristidoideae, Danthoioideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Panicoideae, *Eriachne*, *Micraira*, and *Gynerium*. Based on the phylogeny, a classification of 11 previously published subfamilies (Anomochlooideae, Pharoideae, Puelioideae, Bambusoideae, Ehrhartoideae, Pooideae, Aristidoideae, Arundinoideae, Chloridoideae, Centothecoideae, and Panicoideae) and 1 new subfamily (Danthoioideae) is proposed. Several changes in the circumscription of traditionally recognized subfamilies are included. Previous phylogenetic work and classifications are reviewed in relation to this classification and circumscription, and major characteristics of each subfamily are discussed and described. The matrix, trees, and updated data matrix are available at (<http://www.virtualherbarium.org/grass/gpwg/default.htm>).

*Key words:* cereals, classification, DNA sequence data, evolution, grass, phylogeny, Poaceae.

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The economic and ecological significance of the grasses (Poaceae) has led to widespread interest in their evolution and classification. The cereals, sugarcane, bamboos, and forage and weedy grasses are of pre-eminent importance in human economy. Grasses, which occur in virtually every terrestrial habitat, cover as much as one-fifth of the Earth's land surface (Shantz, 1954). Long recognized as a "natural" group, the grass family includes approximately 10,000 species in over 700 genera (Dahlgren et al., 1985; Tzvelev, 1989; Watson & Dallwitz, 1992; Renvoize & Clayton, 1992). Efforts to produce a comprehensive, formal taxonomic structure of the family began over 200 years ago, while serious study of grass evolution began late in the 19th century.

The Grass Phylogeny Working Group (GPWG) was established in 1996 to (1) combine a series of existing data sets to produce a comprehensive phylogeny for the grass family; (2) focus taxon sampling in the development of existing and future data sets; and (3) reevaluate the subfamilial classification of the grass family based on the results of the phylogenetic analyses. We combined and performed cladistic analyses on eight data sets (one structural, four plastome, and three nuclear) from 62 grasses and 4 outgroup taxa. The phylogenetic results and a revised subfamilial classification of the grass family are presented in this paper.

#### REVIEW OF GRASS PHYLOGENY AND CLASSIFICATION

Historically, the Poaceae were thought to be related to Cyperaceae (Engler, 1892; Cronquist, 1981) based on floral reduction and chemical characters, but evidence accumulated during the past 15 years unequivocally shows that the similarities are convergent. Phylogenetic studies based on morphological and molecular characters show that the grasses are most closely related to Joinvilleaceae, Restionaceae, Anarthriaceae, and Ecdiocolaceae,

and are somewhat more distantly related to Flagelariaceae (Dahlgren et al., 1985; Campbell & Kellogg, 1987; Linder, 1987; Doyle et al., 1992; Kellogg & Linder, 1995; Briggs et al., 2000); this group forms the graminoid clade, a subset of the order Poales (APG, 1998). A sister-group relationship between Poaceae and Joinvilleaceae consistently has been supported (Campbell & Kellogg, 1987; Doyle et al., 1992; Clark et al., 1995; Soreng & Davis, 1998), although *rbcL* sequence data suggest that Joinvilleaceae + Ecdiocolaceae is the sister clade to Poaceae (Briggs et al., 2000).

The grass family was recognized as distinctive and coherent long before the term monophyly was ever applied. The fruit (caryopsis) is unique to the family, with the outer integument developmentally fused to the inner wall of the ovary. The embryo is lateral and, unlike most monocot embryos, is highly differentiated, with clear shoot and root meristems, leaves and vascular system. The pollen, as is typical for the whole order Poales, has only one aperture, but in grasses the pollen wall lacks scrobiculi. In all but the earliest-diverging lineage, the grass spikelet consists of a set of distichous bracts, the basal two empty (glumes) with a series of one to many bracts (lemmas) above, each lemma subtending a contracted floral axis on which is borne a presumed prophyll (palea), two or three reduced perianth parts (lodicules), the androecium, and the gynoecium (see discussion under Spikelet).

Although subdivision of the grasses into groups we today call tribes began in the 18th century (see reviews in Calderón & Soderstrom, 1980; Gould & Shaw, 1983; Pohl, 1987), works by Brown (1810, 1814) represent the earliest attempt to define groups of tribes, or what we now call subfamilies. Brown (1814) divided the grasses into the tribe Paniceae (roughly equivalent to the modern Panicoideae) and the tribe Poaceae (roughly equivalent to the Festucoideae of Hitchcock & Chase, 1950) based on spikelet compression, articulation, and floret number. Brown is credited with describing grass spikelets in detail and recognizing them as branched structures, as well as noting the tendency for the panicoids to grow in warm climates and the poods in cooler climates (Gould & Shaw, 1983; Pohl, 1987). Brown's division of the family into two major groups was formalized by Bentham (1878), was retained by Bentham and Hooker (1883) and Hackel (1887), and persisted well into the 20th century (e.g., Hitchcock, 1935; Hitchcock & Chase, 1950).

Several classifications for the grasses based on spikelet and inflorescence morphology were pro-

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posed in the 19th century (see reviews in Calderón & Soderstrom, 1980; Gould & Shaw, 1983; Campbell, 1985; Pohl, 1987), with usually nine or ten tribes recognized. Some tribes, for example Paniceae, Andropogoneae, and Bambuseae, contain largely the same genera now as nearly 200 years ago. Others, such as the various "poid" tribes, included disparate elements and are now seen as artificial.

Whether explicit or not, a different perspective on the evolution of grasses and relationships within the family began to emerge by the end of the 19th century. Workers such as Čelakovský (1889), Goebel (1895), and Schuster (1910) carefully analyzed spikelet structure and proposed that *Streptochaeta*, or something very much like it, was representative of the most primitive grasses. With the development of leaf anatomical (Duval-Jouve, 1875; Prat, 1932), embryological (van Tieghem, 1897), and cytological (Avdulov, 1931) data, a profound reassessment of evolutionary relationships among grasses began. Additional data on embryo anatomy (Reeder, 1957, 1961, 1962), starch grains (Tateoka, 1962), lodicules (Jirásek & Jozifová, 1968; Guédès & Dupuy, 1976), and leaf anatomy (Brown, 1958; Metcalfe, 1960) accumulated and were also incorporated into evolutionary and classification schemes. Several classification systems were published in the 20th century (e.g., Roshevits, 1937, 1946; Tateoka, 1957; Prat, 1960; Stebbins & Crampton, 1961; Jacques-Félix, 1962; Caro, 1982; Clayton & Renvoize, 1986; Tzvelev, 1989; Renvoize & Clayton, 1992; Watson & Dallwitz, 1992); major ones that are global in scope are compared in Table 1. The number of subfamilies recognized ranged from 2 (Tzvelev, 1989) to 13 (Caro, 1982). All but the Watson and Dallwitz (1992) classification, which is avowedly phenetic, were based on presumed evolutionary relationships. The major change was the subdivision of the old Festucoideae (or Pooideae) into several subfamilies; Panicoideae were retained almost without modification. Other differences among the major classification systems primarily relate to the treatment of Arundinoideae and Bambusoideae. Clayton and Renvoize (1986) in particular published a number of diagrams depicting relationships based on their synthesis of knowledge at that time. These diagrams have served as a starting point for much subsequent work.

Phenetic analyses of the grass family generally found groups consistent with the five or six subfamilies commonly recognized by the mid 1980s. Hilu and Wright (1982), in a cluster analysis of morphological and anatomical data, found eight major groups with strong support. Except for the cluster

of *Diarrhena*, *Nardus*, and *Lygeum*, the remaining seven clusters corresponded to the subfamilies Festucoideae, Oryzoideae, Arundinoideae, Centothecoideae, Panicoideae, Eragrostoideae, and Bambusoideae. Watson et al. (1985) used the DELTA system to conduct comprehensive phenetic analyses of the family, and their character list continues to be developed. Watson and Dallwitz (1992) initially recognized five subfamilies and subsequently updated their classification to include seven (Watson & Dallwitz, 1999; <http://biodiversity.uno.edu/delta/grass/www/class.htm>); these are Stipoideae, Pooideae, Bambusoideae, Centothecoideae, Arundinoideae, Chloridoideae, and Panicoideae. Subsequent phenetic analyses of immunological data (Esen & Hilu, 1989) and plastid DNA reassociation (Hilu & Johnson, 1991) were limited in sampling but in each case produced four major groups.

Only within the past 15 years have cladistic methods been applied to questions of grass phylogeny and evolution. The first attempt to produce an explicit hypothesis of relationships was the morphological phylogeny of Kellogg and Campbell (1987), who analyzed 33 characters scored for virtually all grass genera. The poidids (including Stipeae), Panicoideae, Chloridoideae, and Bambusoideae were consistently monophyletic in their analyses, but Arundinoideae were polyphyletic, and the poidid clade formed the basal lineage in the family. Bambusoideae s.l. (including herbaceous tribes such as Anomochloaeae, Phareaeae, Streptochaeteae, and Streptogyneae) were interpreted as monophyletic based on the presence of arm and fusoid cells; several tribes often included in the traditional Bambusoideae were placed in other clades (e.g., Brachyelytreae, Diarrheneae, and Phaenospermatidae in the poidid clade).

Hamby and Zimmer (1988) and Doebley et al. (1990) published the first molecular phylogenies for the family, based respectively on ribosomal RNA and plastid gene *rbcL* (ribulose 1,5-bisphosphate carboxylase/oxygenase, large subunit) sequence data. Relatively few taxa were sampled in both studies, but both supported the core Pooideae as well as the group that came to be known as the PACC clade (Davis & Soreng, 1993), containing subfamilies Panicoideae, Arundinoideae, Centothecoideae, and Chloridoideae.

The first extensive application of molecular data to grass phylogeny was undertaken by Davis and Soreng (1993), using plastid DNA restriction site variation for 31 taxa representing the six subfamilies of Clayton and Renvoize (1986). This study marked the beginning of wider sampling in the traditional Bambusoideae (= Bambusoideae s.l.), long



Table 1. Continued.

Phenospermeae	See Arundoideae Phyllorachideae	?	Phaenosperrmatae Phyllorachideae	Phaenosperrmatae Phyllorachideae	See Pooideae Phyllorachideae
In Oryzeae	See Arundoideae	<b>Ehrhartoideae</b> Ehrharteae	Ehrharteae	Ehrharteae	Ehrharteae
Centothecoaceae	See Arundoideae Microaireae	<b>Centhostecoideae</b> Centhosteaceae	<b>Centothecoideae</b> Centosteaceae	Centosteaceae	<b>Centothecoideae</b> Centothecoaceae Thysanolaeneae
Hordeae		<b>Festucoideae</b> Hordeae	<b>Pooideae</b> Triticeae	<b>Pooideae</b> Triticeae	<b>Pooideae</b> Triticeae
Brachypodiaceae					Brachypodiaceae
Bromeae					Bromeae
Festuceae		Festuceae			Poeae
Aveneae		Aveneae			"
Agrostideae		Agrostideae			"
Phalarideae		Phalarideae			"
Meliceae		In Festuceae	In Poeae	In Phleaeae Ampelodesmeae	" " Ampelodesmeae In Poeae
Stipeae	Monermeae	Monermeae	Meliceae	Phleaeae	In Poeae Sesleriteae
Nardeae	See Arundoideae	Stipeae	Brylkinieae	Meliceae	Meliceae
Lygeae	See Arundoideae	Incertae Sedis Incertae Sedis	Brylkinieae Hainardiaceae	"	Brylkinieae In Poeae
			Stipeae	In Poeae	Stipeae
			Nardeae	See Arundoideae	Nardeae
			Lygeae	See Arundoideae	Lygeae
		<i>Beckmannia</i>			Brachyelytreae Diarrheneae Phaenosperrmatidae
	<b>Arundoideae</b>	<b>Phragmitiformes</b>	<b>Phragmitoideae</b>	<b>Arundoideae</b>	<b>Arundoideae</b>
	Stipeae			Stipeae	See Pooideae
	Nardeae			Steyermarkochloaeae	See Panicoidae
	Lygeae			Nardeae	See Pooideae
				Lygeae	See Pooideae



Table 1. Continued.

Sporoboleae	In Chloriidae	Sporoboleae	Leptureae	"	"	"
—	<b>Panicoidae</b>	<b>Panicoidae</b>	<b>Panicoidae</b>	<b>Panicoidae</b>	<b>Panicoidae</b>	<b>Panicoidae</b>
In Eragrosteae	—	—	Steyermarkochloae	Steyermarkochloae	See Arundinoideae	Steyermarkochloae
—	? ?	? ?	Eriachneae	In Arundineae	See Arundinoideae	Incertae Sedis
In Festuceae	Isachneae	Isachneae	Hubbardieae	?	In Isachneae	Hubbardieae
Panicaceae	Panicaceae	Panicaceae	Isachneae	Isachneae	Isachneae	Isachneae
Boivinelleae	Boivinelleae	Boivinelleae	Panicaceae	Panicaceae	Panicaceae	Panicaceae
Meliniidae	Meliniidae	Meliniidae	"	"	"	"
In Paniceae	Anthephoreae	?	"	"	"	"
In Paniceae	Lecomtelleae	?	"	"	"	"
In Zoysieae	Trachyeae	?	"	"	"	"
In Zoysieae	?	?	"	"	"	"
In Meliniidae	?	Arthropogoneae	"	"	Neurachneae	"
Arundinelleae	See Phragmitiformes	See Phragmitiformes	Arundinelleae	In Paniceae	In Paniceae	Arundinelleae
Andropogoneae	Andropogoneae	Andropogoneae	Andropogoneae	Arundinelleae	Arundinelleae	Andropogoneae
Maydeae	Maydeae	Maydeae	"	"	Maydeae	"
		various isolated genera including <i>Phaenosperma</i>				
		<b>Incertae Sedis</b>				<b>Incertae Sedis</b>
		<i>Anomochloa</i>				Eriachneae
		<i>Centotheca</i>				Micraireae
		<i>Ehrharta</i>				Streptogyneae
		<i>Lygeum, Nardus</i>				<i>Cyperochloa</i>
		<i>Micraira</i>				<i>Gynurium</i>
		<i>Streptogyna</i>				
		<i>Thysanolaena</i> and other genera				



presumed to include the most ancestral elements of the grass family. Davis and Soreng's (1993) results supported an expanded pooid clade, the PACC (now PACCAD) clade, and suggested that the traditional Bambusoideae were not monophyletic.

Nadot et al. (1994) analyzed sequences of the plastid gene *rps4* (ribosomal plastid small subunit, protein 4) for 26 genera of grasses. Their sampling was heavily weighted toward the pooid grasses, but they did include three genera of woody bamboos and *Zizania* and *Oryza* of the ehrhartoids (oryzoids). They recovered both a monophyletic pooid clade (including *Stipa*) and the PACC clade. The bambusoid/oryzoid taxa were paraphyletic and formed a polychotomy with the PACC clade. Cummings et al. (1994), using sequence data from the plastid *rpoC2* (RNA polymerase II,  $\beta'$  subunit) gene, sampled only 13 genera, but did derive a monophyletic PACC clade and a monophyletic pooid clade. The *rbcl* sequence analysis of Barker et al. (1995) focused on the subfamily Arundinoideae. Both the PACC and pooid clades were shown to be monophyletic, although the traditional Arundinoideae appeared as polyphyletic. Bambusoideae, represented by a woody bamboo (*Bambusa*) and Ehrhartoideae (*Zizania* and *Oryza*), were paraphyletic to the rest of the family.

Clark et al. (1995) were the first to include a broad sample of bambusoid and ehrhartoid taxa. Using *ndhF* (NADH dehydrogenase, subunit F) sequence data, they confirmed the polyphyly of the traditional Bambusoideae and demonstrated that *Anomochloa* and *Streptochaeta*, two broad-leaved Neotropical forest genera, formed the earliest diverging branch of the family, with *Pharus*, another broad-leaved tropical forest genus, constituting the next most basal branch. Their results also confirmed strong support for monophyly of the PACC clade, an expanded pooid clade (including Stipeae, Phaenospermatideae, Brachyelytreae, and Diarrheneae), a derived, monophyletic core bambusoid clade (Olyreae + Bambuseae), and the polyphyly of the traditional Arundinoideae. They also recovered a weakly supported clade including the core bambusoids, ehrhartoids, and pooids, which they named the BOP clade (here updated to the BEP Clade based on nomenclatural priority of Ehrhartoideae over Oryzoideae). They concluded that many features previously used to define the traditional Bambusoideae, including the presence of arm and fusoid cells and pseudopetiolate leaf blades among others, were probable synapomorphies for the family.

The *rbcl* study of Duvall and Morton (1996) confirmed the basal placement of *Anomochloa*, as well

as the monophyly of the core Bambusoideae, in addition to supporting the PACC and pooid clades. The topology recovered by Liang and Hilu (1996) from analysis of *matK* (maturase K) sequence data was similar to the *rbcl* topologies, with the PACC and pooid clades sister to each other, and *Oryza* sister to that clade and a woody bamboo sister to the whole family. By this time, reassessment of subfamilial classification was necessary; Clark and Judziewicz (1996) resurrected Anomochlooideae and Pharoideae to accommodate the basal lineages of the family, which could not be retained in a monophyletic Bambusoideae.

Soreng and Davis (1998) combined a structural data set (including morphological, anatomical, chromosomal, and biochemical characters as well as structural features of the chloroplast genome) and an expanded chloroplast restriction site data set to analyze phylogenetic relationships within the grass family. They confirmed the basal positions of Anomochlooideae and Pharoideae, monophyly of an expanded Pooideae, monophyly of Panicoideae, Centothecoideae, and Chloridoideae, and polyphyly of the traditional Arundinoideae. The core Bambusoideae, supported as monophyletic in other analyses along with the ehrhartoid grasses, appeared as a set of clades paraphyletic to the [*Brachyleytrum* + (Pooideae + PACC)] clade. Soreng and Davis (1998) also identified structural synapomorphies for major clades, including, for example, loss of the epiblast and gain of an elongated mesocotyl internode in the PACC clade.

Barker et al. (1999) used sequences of the grass-specific insert in the chloroplast gene *rpoC2* (hereafter referred to only as *rpoC2*) to study relationships among a broad sample of "arundinoid" taxa. They were the first to include molecular data on such traditionally arundinoid genera as *Centropodia*, *Merxmuellera*, *Notochloe*, *Tribolium*, *Monachather*, *Pentaschistis*, *Prionanthium*, *Cortaderia*, and *Spartochloa*. Because Arundinoideae were known to be polyphyletic, previous classifications were not helpful in placing these genera. *rpoC2* sequences of *Anomochloa* and *Streptochaeta* could not be aligned with those of other grasses, so their basal position could not be tested. Relationships among the Bambusoideae, Pooideae s.l., and the PACC clade varied depending on the analytical method and inclusion of phylogenetically informative insertion/deletion characters. Consistent with previous studies, they identified Panicoideae and Chloridoideae as monophyletic. They showed clearly that a large clade corresponding to Danthoioideae is monophyletic and that this corresponds at least in part to the clade with haustorial synergids identi-

fied by Verboom et al. (1994). They also showed that the genus *Merxmuellera* is polyphyletic, with one species, *M. rangei*, most closely related to *Cenitropodia* and the chloridoids.

Hilu et al. (1999) sequenced the chloroplast gene *matK* for 62 species of Poaceae and produced a tree that was quite similar to those found in previous studies. *Streptochaeta* and *Anomochloa* were the earliest diverging lineages, although paraphyletic instead of monophyletic. The *matK* data supported a PACC clade and a clade including Pooideae and Bambusoideae. Oryzoideae (= Ehrhartoideae) was sister to the PACC clade, rather than the pooid/bambusoid clade, but this was not strongly supported.

Zhang (2000) used the intron in the chloroplast gene *rpl16* (ribosomal protein 16) to construct a phylogeny of the grasses and confirmed (again) the early divergence of *Anomochloa* and *Streptochaeta*, although his data did not support the monophyly of the pair. The next branch was *Pharus*. The *rpl16* data supported a PACC clade and a BEP Clade; *Puelia olyrififormis* was sister to the BEP Clade, with modest bootstrap support.

Analyses of nuclear sequence data have provided results complementary to those obtained for molecular plastid data sets. Mathews and Sharrock (1996) and Mathews et al. (2000) sequenced loci in the phytochrome gene family and resolved a topology similar to that derived from *ndhF* sequence data, although the phytochrome data provided significantly stronger support for the BEP Clade than did the *ndhF* data. Additionally, the basal positions of *Anomochloa*, *Pharus*, and *Puelia* (as noted in Zhang, 1996) were confirmed. Hsiao et al. (1999) inferred phylogenetic relationships within the grasses based on sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. As in the other studies, *Streptochaeta* and *Pharus* were resolved as the basal lineages, and monophyly of the PACC clade and monophyly of the Pooideae were strongly supported. Unlike previous studies, however, some of Hsiao et al.'s (1999) analyses found that the traditional Arundinoideae were monophyletic.

Combined analysis of sequence data from two chloroplast genes (*ndhF*, *rbcL*) and one nuclear gene (phytochrome B) provided strong support for the placement of *Puelia* + *Guaduella* as the next most basal lineage of the family after Anomochloideae and Pharioideae (Clark et al., 2000). These results necessitated the description of a new subfamily, the Puelioideae.

Mapping studies of the nuclear genome are in their infancy, but genome rearrangements are con-

sistent with certain broad phylogenetic groups (Kellogg, 1998). Unique combinations of linkage groups are synapomorphic for subfamilies Pooideae (Moore et al., 1995; Gale & Devos, 1998), Panicoideae (Moore et al., 1995; Gale & Devos, 1998; Wilson et al., 1999), and Ehrhartoideae (Kennard et al., 1999). In addition, unique linkages support monophyly of Triticeae (Devos et al., 1993) and Andropogoneae (Wilson et al., 1999).

Phylogenetic analyses of individual molecular data sets within the last decade have converged on a set of well-supported relationships within Poaceae. Changes in the circumscriptions of subfamilies, and in the number of subfamilies recognized, clearly are necessary. The GPWG analyses presented here provide robust support for the major clades within the grass family, and provide the basis for the first family-wide subfamilial classification based on an explicit phylogenetic hypothesis.

## MATERIALS AND METHODS

### ORGANIZATION OF THE GPWG

The Grass Phylogeny Working Group was formed explicitly to combine available data on the phylogeny of the grass family and to use these data to propose a new classification. Most contributors had already published papers on grass phylogeny and were invited to contribute their data, both published and unpublished. Each contributor retained control over his or her data and was free to publish at any time, but the group agreed that the entire data set would be published as a single paper. Most of the collaboration has been conducted via e-mail, and the entire group has never met in a single place. This may serve as a model for future collaborations in plant systematics.

A list of taxa was drawn up in 1995 by LGC, JID, and EAK to improve parallel sampling for all data sets (Table 2; Appendix I). This list was chosen to include as many of the major lineages in the family as possible, based on our knowledge from previous studies. Although sampling of taxa is still not perfectly parallel, many sequences were generated for this particular set of taxa. DNA was exchanged as necessary among members of the group. The list was expanded slightly based on results acquired during the study.

All sequences available at the end of 1997 were assembled by EAK into a single large data set in NEXUS format. The data set was then distributed via e-mail to all participants, who had the opportunity to comment on it. A "final" version of the data set was then distributed. Based on the results of the first round of analyses (GPWG, 2000), the

Table 2. Summary of genes and taxa included in the combined analysis. Taxa are listed approximately in the order in which they appear in Figure 1. cp rs = chloroplast restriction sites; GBS = GBSSI; struc. = structural data; \* = composite taxon, represented by sequences from several genera; # = composite taxon, represented by sequences of different species within the same genus (as in Appendix I). For details of species, authorities, original publications, and GenBank accession numbers, see Appendix I. *Merxmuellera m.* = *Merxmuellera macowanii*, *Merxmuellera r.* = *Merxmuellera rangei*.

Genus	cp rs	<i>ndhF</i>	<i>phyB</i>	<i>rbcL</i>	<i>rpoC</i>	GBS	ITS	struc.
<i>Flagellaria</i>	X	X	X	X				X
<i>Elegia</i> #		X		X				X
<i>Baloskion</i>	X	X		X				X
<i>Joinvillea</i> #	X	X	X	X	X		X	X
<i>Anomochloa</i>	X	X	X	X		X		X
<i>Streptochaeta</i> #	X	X	X				X	X
<i>Pharus</i> #	X	X	X			X	X	X
<i>Guaduelia</i>		X		X				X
<i>Puelia</i>		X	X	X				X
<i>Eremitis</i>	X	X	X			X		X
<i>Pariana</i>		X	X			X		X
<i>Lithachne</i> #	X	X	X	X			X	X
<i>Olyra</i> #	X	X	X		X			X
<i>Buergersiochloa</i>		X	X					X
<i>Pseudosasa</i> *	X	X	X	X	X			X
<i>Chusquea</i> #	X	X	X	X		X	X	X
<i>Streptogyna</i>		X	X					X
<i>Ehrharta</i> #	X	X	X		X		X	X
<i>Oryza</i>	X	X	X	X	X	X	X	X
<i>Leersia</i> #	X	X		X			X	X
<i>Phaenosperma</i>		X						X
<i>Brachyelytrum</i>	X	X					X	X
<i>Lygeum</i>	X	X	X		X	X	X	X
<i>Nardus</i>	X	X	X		X		X	X
<i>Anisopogon</i>	X	X	X		X		X	X
<i>Ampelodesmos</i>	X	X					X	X
<i>Stipa</i> #	X	X		X	X		X	X
<i>Nassella</i> #	X	X	X				X	X
<i>Piptatherum</i> #	X	X					X	X
<i>Brachypodium</i> #	X	X	X				X	X
<i>Melica</i> #	X	X	X			X	X	X
<i>Glyceria</i> #	X	X	X			X	X	X
<i>Diarrhena</i> #	X	X	X				X	X
<i>Avena</i> *	X	X	X	X	X		X	X
<i>Bromus</i> #	X	X	X	X	X		X	X
<i>Triticum</i> *	X	X	X	X		X	X	X
<i>Aristida</i> #	X	X	X	X	X		X	X
<i>Stipagrostis</i>		X		X	X		X	X
<i>Amphipogon</i> #	X	X		X	X		X	X
<i>Arundo</i>	X	X		X	X		X	X
<i>Molinia</i> *	X	X	X	X	X		X	X
<i>Phragmites</i>	X	X	X	X	X		X	X
<i>Merxmuellera m.</i>		X		X	X	X	X	X
<i>Karroochloa</i>		X		X	X	X	X	X
<i>Danthonia</i> #	X	X	X	X	X		X	X
<i>Austrodanthonia</i>		X		X	X	X	X	X
<i>Merxmuellera r.</i>		X		X	X	X	X	X
<i>Centropodia</i>		X		X	X	X	X	X
<i>Eragrostis</i> #	X	X	X	X	X		X	X
<i>Uniola</i>	X	X						X
<i>Zoysia</i> #	X	X						X

Table 2. Continued.

Genus	cp rs	ndhF	phyB	rbcL	rpoC	GBS	ITS	struc.
<i>Distichlis</i>	X	X						X
<i>Pappophorum</i> *		X		X	X			X
<i>Spartina</i> #	X	X			X		X	X
<i>Sporobolus</i> #	X	X	X				X	X
<i>Eriachne</i> #				X			X	X
<i>Micraira</i> #		X			X		X	X
<i>Thysanolaena</i>		X	X	X	X		X	X
<i>Gynerium</i>		X		X	X		X	X
<i>Chasmanthium</i> #	X	X	X	X	X		X	X
<i>Zeugites</i>		X						X
<i>Danthoniopsis</i> #		X	X			X		X
<i>Panicum</i> #	X	X	X		X		X	X
<i>Pennisetum</i> #	X	X	X	X	X	X	X	X
<i>Miscanthus</i> *	X	X	X	X	X	X	X	X
<i>Zea</i>		X	X	X	X	X	X	X

taxon list was expanded to include several more danthonioid taxa, and the matrix was compiled by JID. Although all participants in the GPWG were invited to undertake data analyses and comment on the final version of matrices, abstracts, and text, this was not required. Thus the analyses and text of this paper reflect largely the work of LGC, JID, and EAK, with input from several members of the group. The GPWG website was created and is maintained by GFG.

TAXA

The taxa used in this analysis include four genera representing the families Flagellariaceae, Restionaceae (two genera), and Joinvilleaceae as out-

groups. This choice was based on previous work (summarized in Kellogg & Linder, 1995) indicating that these represent the closest relatives of the grasses. The results of Briggs et al. (2000) suggest that the Ectodiaceae should be included in outgroup comparisons for the grasses in the future. Within the grass family, 62 exemplar species were chosen to represent the commonly recognized subfamilies Anomochlooideae, Arundinoideae, Bambusoideae, Centothecoideae, Chloridoideae, Ehrhartoideae (=Oryzoideae), Panicoideae, Pharoideae, and Pooideae, as well as species from several genera whose placement was uncertain (*Amphipogon*, *Anisopogon*, *Brachyelytrum*, *Buergeriochloa*, *Danthonia*, *Danthoniopsis*, *Eriachne*,

Table 3. Tree statistics for subsets of the data matrix. The percent missing data is the number of cells that are missing for the particular block when included in the total data matrix, and thus is equivalent to the number of missing taxa times the total number of informative characters plus missing data within sequences for scored taxa. Chloroplast r.s. = Chloroplast restriction sites.

Data set	# Taxa	Total # characters	# Inf. characters	% Missing data	Length	CI	RI	# Trees
Morphological	66	53	50	16.0	227	0.300	0.690	38,000+
Chloroplast r.s.	45	364	293	42.2	939	0.312	0.569	7
ndhF	65	2210	680	4.7	2894	0.379	0.582	16
phytochrome B	40	1182	417	45.5	1997	0.369	0.522	1
rbcL	37	1344	213	44.8	651	0.448	0.660	1
rpoC2	34	777	150	49.9	374	0.503	0.648	33
GBSSI	19	773	213	71.2	720	0.479	0.504	1
ITS	47	322	127	28.8	745	0.349	0.541	24
cp sequence data	66	4331	1043	25.2	3952	0.399	0.597	8
All cp data	66	4695	1336	26.5	4903	0.381	0.589	3
Nuclear	57	2277	757	51.9	3513	0.382	0.512	8
All molecular	66	6972	2093	33.6	8488	0.378	0.554	6
Combined data	66	7025	2143	33.2	8752	0.375	0.557	1

Table 4. List of structural characters and states. The first number in parentheses indicates the number of the same character in Soreng and Davis (1998), and the second number indicates the number of the same character in Kellogg and Campbell (1987); a “—” indicates that the character was not used in one or both of those analyses.

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**Culm**

- 1 (1;—). Perennating woody culms: 0 = absent; 1 = present.  
2 (2;—). Culm internodes: 0 = solid; 1 = hollow.

**Leaf**

- 3 (3;—). Leaf sheath margins: 0 = free; 1 = fused for at least 1/4 of length.  
4 (4;—). Adaxial ligule type: 0 = membrane (with or without fringe of hairs); 1 = fringe of hairs only.  
5 (5;—). Abaxial (contra-) ligule: 0 = absent; 1 = present.  
6 (—;—). Leaf blade: 0 = absent; 1 = present.  
7 (6;—). Pseudopetiole: 0 = absent; 1 = present.

**Spikelet**

- 8 (—;—). Floret with a structure identifiable as a palea, this recognized as present when a flower arises on a contracted axis above an enshrouding prophyll (or something like it), in the axil of a lemma: 0 = absent; 1 = present.  
9 (—;—). Spikelet pairs: 0 = absent; 1 = present.  
10 (7;—). Pedicel of spikelet: 0 = absent; 1 = present.  
11 (8; 3, 4). Proximal female-sterile florets in female-fertile spikelets: 0 = absent; 1 = present.  
12 (9; 6). Number of female-fertile florets per female-fertile spikelet: 0 = two or more; 1 = one.  
13 (10;—). Awn or mucro on fertile or sterile lemma: 0 = absent; 1 = present.  
14 (—;—). Number of awns: 1 = 1 awn; 2 = 13–23 awns (unique to *Pappophorum*); 3 = 3 awns.  
15 (11;—). Awn attachment: 0 = terminal / subterminal; 1 = from a sinus; 2 = dorsal.  
16 (12; 1). Disarticulation above glumes: 0 = absent; 1 = present.  
17 (13;—). Germination flap in lemma: 0 = absent; 1 = present.

**Flower**

- 18 (14;—). Lodicules: 0 = absent; 1 = present.  
19 (15; 7). Lodicule number: 2 = two; 3 = three.  
20 (16;—). Fusion of anterior pair of lodicules: 0 = free; 1 = fused.  
21 (17; 8). Distally membranous portion of lodicule: 0 = absent; 1 = present.  
22 (18; 9). Lodicule vascularization: 0 = very faint to absent; 1 = prominent.  
23 (19; 10). Inner whorl, posterior stamen: 0 = absent; 1 = present.  
24 (19; 10). Inner whorl, anterior stamen pair: 0 = absent; 1 = present.  
25 (19; 10). Outer whorl, anterior stamen: 0 = absent; 1 = present.  
26 (19; 10). Outer whorl, posterior stamen pair: 0 = absent; 1 = present.  
27 (—;—). Anthers tetrasporangiate, dithecal = 0; anthers bisporangiate, monotheical = 1.  
28 (20;—). Styles fused at least at base: 0 = absent; 1 = present.  
29 (21;—). Number of stigmas: 1 = one; 2 = two; 3 = three; 4 = four.  
30 (22;—). Highest order of stigmatic branching present: 1 = simple (unbranched, or with branches composed of single elongate papillate receptive cells, or with very short branches composed of a few papillate receptive cells, but in the latter case the stigmas linear in outline); 2 = primary (branches well developed, composed of series of dispersed papillate receptive cells, with secondary branches absent or minimally developed, stigmas lanceolate or broader); 3 = secondary (secondary to tertiary branches well developed, branches composed of series of dispersed papillate receptive cells).  
31 (23;—). Number of locules and ovules per pistil (all three families have one ovule per locule): 1 = one; 2 = two; 3 = three.

**Embryogeny**

- 32 (—;—). Haustorial synergids: 0 = absent; 1 = present.

**Fruit and Embryo**

- 33 (24; 11). Hilum: 0 = long-linear, > 1/3 length of grain; 1 = nonlinear, < 1/3 length of grain, elliptical or broader to punctiform.  
34 (25;—). Embryo position and structure: 0 = embedded, simple; 1 = lateral, grass-type.  
35 (26; 15). Embryo epiblast: 0 = absent; 1 = present.  
36 (27; 16). Embryo scutellar tail: 0 = absent; 1 = present.  
37 (28; 17). Embryo mesocotyl internode: 0 = negligible; 1 = elongate.  
38 (29; 18). Embryonic leaf margins: 0 = meeting; 1 = overlapping.  
39 (30;—). Endosperm lipid: 0 = absent; 1 = present.  
40 (31;—). Endosperm starch grain syndromes: 0 = *Triticum*-type (simple grains only, dimorphic in size, round or lenticular, free); 1 = *Festuca*-type (highly compound grains present, with or without simple grains also present); 2 = *Andropogon*-type (simple and compound grains both present, the latter consisting of few granules); 3 = *Panicum*-type (simple grains only, uniform in size, small to medium, angular or sometimes smooth walled, densely packed); 4 = *Brachelytrum*-type (simple only, large).

**Seedling**

- 41 (32; 20). Lamina of first seedling leaf: 0 = absent; 1 = present.
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Table 4. Continued.

**Vegetative Anatomy**

42 (—;—). Differentiation of leaf epidermal cells into long and short (cork) cells: 0 = absent (i.e., cells  $\pm$  undifferentiated); 1 = present (Campbell & Kellogg, 1987).

43 (34; 21). Multicellular microhairs: 0 = absent; 1 = present.

44 (35; 22). Occurrence in multicellular microhairs of a broad, short terminal cell, often with a longer basal cell, the walls of the terminal and basal cells similar in thickness: 0 = absent; 1 = present.

45 (36; 31). Arm cells: 0 = absent; 1 = present.

46 (37;—). Fusoid cells: 0 = absent; 1 = present.

**Chromosomes**

47 (—;—). Base chromosome number is same as state number except that 0 = 10; 1 = 11; 2 = 12; 3 = 13; 4 = 18; 5 = 19.

**Biochemistry**

48 (38; 30). Carbon fixation pathway: 0 =  $C_3$ ; 1 =  $C_4$  NADP-ME classical-type; 2 =  $C_4$  NADP-ME *Aristida*-type; 3 =  $C_4$  NAD-ME; 4 =  $C_4$  NADP-ME *Arundinelleae*-type; 5 =  $C_4$  NADP-ME *Eriachne*-type.

49 (39; 30). Carbon fixation PCK: 0 = absent; 1 = present.

**Deletion in Phytochrome B**

50 (—;—). 3-bp DNA deletion in phytochrome B: 0 = 3-bp DNA present (i.e., non-deleted state); 1 = DNA absent (i.e., deleted state; the deleted codon is at position 402 in the alignment of Mathews et al., 1995).

**Chloroplast Genome Structure**

51 (40;—). 6.4 kb inversion in the large single-copy region of the chloroplast genome, relative to the gene arrangement in *Nicotiana*: 0 = absent; 1 = present.

52 (41;—). *trnT* inversion in the large single-copy region of the chloroplast genome, relative to the gene arrangement in *Nicotiana*: 0 = absent; 1 = present.

53 (42;—). 15 bp insertion in *ndhF* at position 101951 of the chloroplast genome of *Oryza sativa*: 0 = absent; 1 = present.

*Lygeum, Micraira, Nardus, Pariana, Phaenosperma, Puelia, Streptogyna, Thysanolaena*).

For 31 of the terminal taxa in the matrix, all molecular data were taken from a single species; for an additional 27, data were from two or more species of the same genus (noted by # in Table 2; Appendix I). In eight cases, however, data from several genera were combined to create a “conglomerate” taxon (asterisks in Table 2). For example, although one listed representative of the Andropogoneae is *Miscanthus*, there is no *rbcL* sequence available for that genus. There is, however, a sequence for *Sorghum*. Thus the *Sorghum* sequence for *rbcL* was combined with the *Miscanthus* sequences for *ndhF*, creating a fictive taxon, an approach used previously by Kellogg and Linder (1995). This assumes that both genera are part of a monophyletic higher-level group (in this case, Andropogoneae, which are certainly monophyletic; Spangler et al., 1999). The results of such combinations are potentially misleading, in that they assume certain combinations of characters that may not ever actually occur in a single plant. We feel that the number of characters involved, however, is small, and the addition of phylogenetically informative characters by including the line of data outweighs the risk of misleading results. Any subsequent studies, particularly those for which there are more than two representatives of taxa combined

here, should break up each conglomerate taxon into real species (i.e., exemplar taxa).

The number of taxa was dictated by the numbers of available sequences in the largest of the original data sets (*ndhF* and chloroplast restriction sites). Recent work on large phylogenies suggests that phylogenetic accuracy is improved by a very dense sample of taxa (e.g., Hillis, 1996, 1998; Graybeal, 1998). Producing a large data set with perfectly parallel sampling, however, would have required either a centralized effort in a single lab, or a formal, coordinated, and separately funded effort among multiple labs, rather than the decentralized approach used here.

CHARACTERS

The data matrix included 7025 characters assembled from the following sources:

1. NADH dehydrogenase, subunit F (*ndhF*)—Clark et al. (1995, 2000); Davis et al. (this paper); Spangler et al. (1999).
2. Ribulose 1,5-bisphosphate carboxylase/oxygenase, large subunit (*rbcL*)—Barker et al. (1995); Barker (1997); Doebley et al. (1990); Duvall and Morton (1996).
3. RNA polymerase II,  $\beta''$  subunit (*rpoC2*)—Cumings et al. (1994); Barker et al. (1999).

4. Chloroplast restriction sites—Davis and Soreng (1993); Soreng and Davis (1998).
5. Phytochrome B (*phyB*)—Mathews and Sharrock (1996); Mathews et al. (2000).
6. Internal transcribed spacer of the nuclear ribosomal RNA (ITS)—Hsiao et al. (1998, 1999).
7. Granule bound starch synthase I (GBSSI, or *waxy*)—Mason-Gamer et al. (1998).
8. Morphology—Soreng and Davis (1998, and additional members of the GPWG, this paper).

Information on numbers of characters and taxa for each matrix is in Table 3, and the structural character list is in Table 4. The morphological (structural) matrix is in Appendix II. The first four data sets represent the chloroplast genome, and the next three the nuclear genome. Five data sets, *ndhF*, *rbcL*, *rpoC2*, *phyB*, and GBSSI, are all protein coding sequences; introns of GBSSI were not included in the alignments. The full data matrix included 66 taxa and 7025 characters, for a total of 463,650 cells. The amount of missing data for the total data set is 33.2% and varies among genes and taxa (Table 3). The full data matrix can be obtained from LGC, JID, EAK, or HPL, or from the GPWG website, or at TreeBASE (<http://herbaria.harvard.edu/treebase/index.html>).

Alignments were provided by the contributors except for the ITS data, for which the alignment was constructed by EAK, beginning with an initial alignment in ClustalW (Thompson et al., 1994) and then continuing by eye. It became apparent that ITS1 could not be aligned reliably across the family, so it was omitted from the data set. Later after extensive data exploration, several regions of ambiguous alignment were also omitted from ITS2. Gaps were treated as missing data. A few indels, identified as phylogenetically informative in analyses of individual data sets, were coded as binary characters and included in the structural data matrix (Appendix II).

#### DATA ANALYSIS

Data were analyzed by parsimony algorithms, as implemented in PAUP\*4.0 d64 (Swofford, 1998) on a Power Macintosh G3, and Nona (Goloboff, 1993) on an Intel-chip-based workstation running Windows NT. Data sets were analyzed individually by JID, LGC, EAK, and HPL to be sure that e-mail transmittal of such a large file had not introduced any errors (for which we suggest the term “network-induced homoplasy”). Numbers of informative characters and tree lengths were the same for the two programs, although in some cases the number of equally parsimonious trees differed because of

different ways the two programs count resolutions of polytomies.

PAUP\* analyses used 10 random addition sequences, MULPARS on, TBR branch swapping, and MAXTREES set to automatically increase by 100. Bootstrap analyses (bts) used the full heuristic option, 500 or 1000 replicates. Bremer support (abbreviated here as brs; Bremer, 1988; Källersjö et al., 1992; also called decay index, cf. Donoghue et al., 1992) was also calculated. For tree lengths up to 11 steps longer than the shortest tree (up to 8763 steps), all trees were saved and the strict consensus computed. Because of memory limitations the method of negative constraints (Baum et al., 1994) was used to compute higher Bremer support values. The search for optimal trees was found to be quite inefficient with this method and often led to inflated support values. To minimize this problem, each search was done with 10,000 random addition sequences. Even so, the search frequently found trees shorter than the negative constraint tree, indicating that the previous searches had missed some trees. Computing Bremer support thus took almost two weeks of computer time on a G3. For the tree presented here, we arbitrarily chose a cut-off of 34 steps, so brs values above that are simply reported as “> 34.”

To assess robustness of the results to choice of markers, each data set was analyzed by itself. The morphological data set was omitted from one analysis, and the chloroplast data were analyzed separately, as were the nuclear data. For analyses of individual data sets, PAUP\* was set to perform heuristic searches using maximum parsimony, gaps were coded as missing data, multistate taxa were coded as uncertain, and starting trees were obtained by ten random addition sequences, holding one tree at each step; branch swapping used tree bisection and regrafting (TBR), steepest descent was not in effect, and MULPARS was in effect. Bootstrap analyses of individual data sets were done to facilitate comparisons with combined analyses. All bootstraps of individual data sets included 500 bootstrap replicates; MAXTREES was set to 500 to minimize times for searches.

Analyses conducted with Nona ver. 1.6 (Goloboff, 1993) used the default settings *amb-* (clades resolved only if they have unambiguous support) and *poly=* (polytomies allowed). Tree searches involved 1000 Wagner tree initiations using random taxon entry sequences, followed by tree bisection reconnection (*tbr*) swapping with up to 20 most-parsimonious trees retained in each search (*hold/20, mult\*1000*); shortest trees retained from the subsearches were then *tbr*-swapped to completion,

with up to 10,000 trees held in memory (ho10000, max\*). Structural character autapomorphies of terminals, and synapomorphies of clades, were determined by optimizing the morphological data on most-parsimonious trees obtained by the various analyses, using Winclada ver. 0.9.99m6.1 (Nixon, 2000). Strict-consensus bootstrap frequencies for just the total evidence analysis (see Soreng & Davis, 1998) were computed with Clados ver. 1.9.95 (Nixon, 1993) running Nona (Goloboff, 1993) as a daughter process for the tree searches, using a copy of the data set from which uninformative characters had been removed (with the "mop" function of Winclada). One thousand bootstrap replicates were conducted, using the same ambiguity and polytomy settings as in the basic analyses. Each replicate consisted of 10 random taxon entry sequences followed by tbr swapping with up to 10 trees retained from each subsearch (ho/10, mult\*10), and with further tbr swapping then conducted on the resulting trees from the 10 subsearches, with 101 trees held (ho 101, max\*).

Uninformative characters were excluded for all analyses, so all tree statistics reported in this paper (consistency index [CI] and retention index [RI]) reflect only potentially phylogenetically informative characters.

Chloroplast and nuclear trees were compared using the incongruence length difference test (random partition test of Farris et al., 1994), as implemented in PAUP\*. They were also compared using simple inspection, as recommended by Mason-Gamer and Kellogg (1996). To compare tree topologies, constraint trees were constructed as necessary in MacClade (Maddison & Maddison, 1993); these were then loaded, the constraint enforced, and a heuristic search undertaken using the same parameters as in unconstrained searches.

The combined data were constrained to fit topologies suggested in previous studies by loading a constraint tree in PAUP\* and then searching for the most parsimonious tree compatible with that constraint tree. Constrained and unconstrained trees were compared using the Wilcoxon signed ranks test (WSR) as suggested by Templeton (1983) and implemented by Mason-Gamer and Kellogg (1996). Significance values were determined using a two-tailed test.

The entire data set could not be analyzed with neighbor-joining or maximum likelihood algorithms. The inclusion of morphological and restriction site data with sequences made it nonsensical to specify a single model of evolution. While a model could in principle be hypothesized for morphological or restriction site data, it would have to be

different from the model specified for sequence data. Calculation of base frequencies and transition/transversion ratios would be meaningless. Several neighbor-joining analyses were done with morphological and restriction site data omitted, but this also required omitting several taxa for which distances were then undefined because of missing data. By the time data sets and taxa were omitted, the results were difficult to compare to those of parsimony algorithms. Several maximum likelihood analyses were also undertaken on sequence data alone. These did not reach completion even after three to five days of analysis time. As with the neighbor-joining analyses, missing data and different models of evolution for the different genes made the results of questionable validity.

## RESULTS

Consensus trees for analyses of the individual data sets are presented in Appendix III-A to H and tree statistics in Tables 3 and 5. Note that the taxa included are generally selected from more comprehensive analyses that have been published elsewhere, as described in Materials and Methods. Many of the trees differed in topology, but in no case was a strongly supported group in one tree contradicted by a strongly supported group in another tree. We interpreted this as lack of significant conflict. Nonetheless, the ILD test indicated significant differences between the nuclear and chloroplast data sets, between nuclear protein-coding and chloroplast, between *ndhF* and *phyB*, and between *ndhF* and *rbcL*. These differences persisted in most cases even when taxa with conflicting placements were removed. In the only exception to this observation, *ndhF* and *phyB* were not significantly different if the PACCAD Clade was reduced to Panicoideae, Chloridoideae, and the clade of *Molinia* plus *Phragmites*. This provides weak evidence that differences in resolution of the PACCAD Clade (Panicoideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae) are partly responsible for the significant differences. Differences between *ndhF* and *rbcL*, however, are puzzling because both are part of the same linkage group. Because of the ambiguity of the results, we did not attempt to do all possible pairwise comparisons of trees. Despite the differences in the data sets, we chose to combine the data in a single analysis. Different histories for the various genes remain a formal possibility. However, in other investigations we have seen that the ILD test may return significant differences if there is extensive missing data (as we have in some data sets here)



Table 5. Bootstrap support values for subsets of the total data matrix. Numbers of nodes at particular support values are given as fractions of the total number of nodes, and also as decimals. Poly = polyphyletic; para = paraphyletic. Anom. = Anomochloidoideae; Phar. = Pharioideae; PueL. = Puelioideae. \*Panicoidae here include *Danthoniopsis*; if it is excluded then support values are much higher.

	Total data	Molec. data	Chloro-plast	Nuclear	Structural	<i>ndhF</i>	<i>cprs</i>	<i>rbcl</i>	<i>rpoC2</i>	<i>phyB</i>	GBSSI	ITS
# Nodes 100	27/64 (0.42)	25/64 (0.39)	22/64 (0.34)	2/55 (0.04)	0	23/63 (0.36)	2/43 (0.05)	8/35 (0.23)	2/32 (0.06)	4/38 (0.11)	3/17 (0.18)	1/45 (0.02)
# Nodes 90–99	9/64 (0.14)	14/64 (0.22)	14/64 (0.22)	12/55 (0.22)	1/64 (0.02)	11/63 (0.17)	8/43 (0.19)	7/35 (0.20)	6/32 (0.19)	13/38 (0.34)	4/17 (0.24)	5/45 (0.11)
# Nodes 70–89	11/64 (0.17)	8/64 (0.13)	7/64 (0.11)	5/55 (0.09)	4/64 (0.06)	4/63 (0.06)	9/43 (0.21)	6/35 (0.17)	4/32 (0.12)	10/38 (0.26)	1/17 (0.06)	4/45 (0.09)
Fraction nodes > 70	0.73	0.73	0.67	0.35	0.08	0.59	0.45	0.60	0.37	0.71	0.48	0.22
Poaceae	100	100	100	97	Para	100	98	99	Not tested	96	Not tested	Not tested
Spikelet Clade	100	99	98	87	Para	94	81	98	Not tested	54	Not tested	83
Bisigmatic Clade	100	100	100	97	<50	100	<50	Not tested	Not tested	79	100	98
BEP + PACCAD Clade	100	100	99	81	Poly	100	Not tested	Para	Not tested	75	Not tested	Not tested
Bambusoideae	97	98	97	Para	Poly	100	Para	Para	Para	92	Poly	75
Ehrhartoideae	100	100	100	92	Para	98	72	100	51	99	Not tested	54
Pooideae	100	100	93	95	Para	88	<50	70	88	94	77	19
BEP	71	90	62	50	Para	53	Para	Para	56	89	Poly	Para
Aristidoideae	100	100	100	84	84	100	Not tested	98	67	Not tested	Not tested	76
Chloridoideae	86	83	86	51	Para	<50	98	54	63	93	95	Poly
Panicoidae*	65	Poly	Para	63	Para	Poly	Para	95	93	99	61	Poly
Danthonioidae	98	97	98	Poly	Para	99	Not tested	83	70	Not tested	Poly	Poly
PACC	100	100	99	77	<50	100	95	73	73	90	Poly	<50
Arundinoideae s. str.	77	78	<50	Poly	Para	<50	Not resolved	23	Poly	52	Not tested	Poly
Centothecoideae	Para	Poly	Para	Para	Poly	Para	Not tested	64	Para	98	Not tested	Para

or if a single terminal taxon differs in its placement (Z. Magombo, pers. comm.). Because of the lack of obvious points of conflict between the data sets, and because of the clear congruence at the deep nodes with which we are concerned, we interpret the significant ILD tests as misleading.

Analyses of the complete data set were faster than analyses of many of the individual data sets, as has been found in studies of other large data sets (Soltis et al., 1998). For example, a heuristic search of the complete data set in PAUP\* on a Macintosh G3 with 10 random addition sequences took 19.6 seconds.

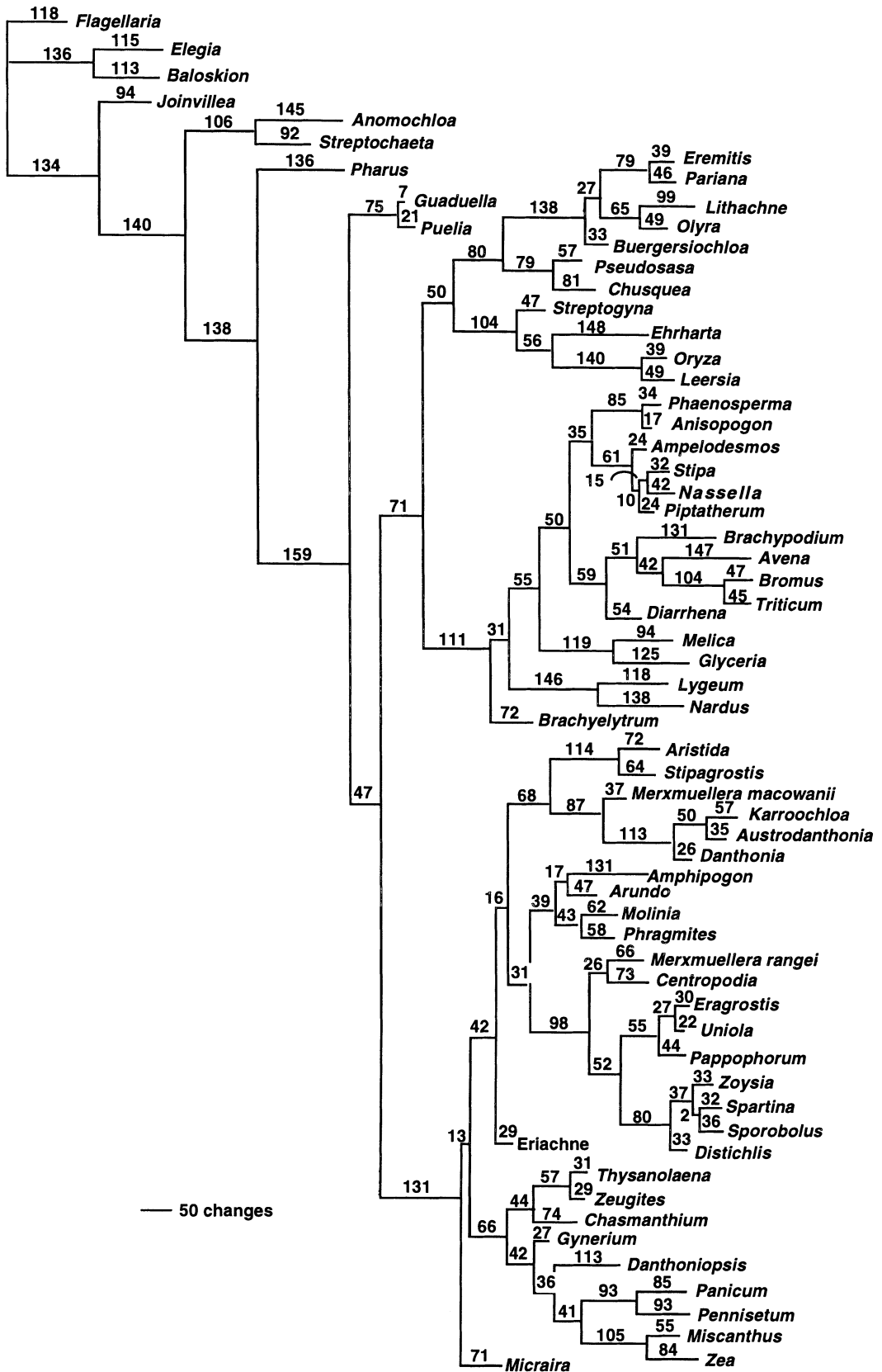
With all data combined, there were 2143 parsimony informative characters, which produced a single tree of 8752 steps, consistency index (CI) of 0.375, and retention index (RI) of 0.557 (Figs. 1 and 2). Bootstrap analyses (1000 replicates) indicated that 27 branches were supported in 100% of the bootstrap replicates, 9 branches in 90–99%, and 11 branches in 70–89% (Table 5). Put another way, of 64 internal nodes, slightly more than half (36) have bootstrap values over 90% and a clear majority (47) have values over 70%. Bootstrap values were virtually identical whether done using strict consensus bootstrap in Nona (Goloboff, 1993) or the frequency-within-replicates bootstrap in PAUP\*4.0 (Swofford, 1998); for comparison with individual analyses, we report the values from PAUP\*4.0.

The analysis of the combined data confirms many results of previous studies and clarifies some relationships that were previously ambiguous. The two species of Restionaceae form a clade. *Joinvillea* is sister to a monophyletic Poaceae. The three earliest diverging lineages are the Anomochlooideae, Pharioideae, and Puelioideae, in that order, together accounting for 30 species of grasses. The vast majority of extant grasses fall into two distinct lineages. One of these is the PACC clade (Davis & Soreng, 1993), here called the PACCAD Clade (Panicoidae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae) to reflect the inclusion of two additional subfamilies within the clade. Within this clade, Panicoidae s. str. (excluding *Danthoniopsis*) are monophyletic (bts 94; brs 10), as are the core Paniceae sampled here (bts 100; brs 25) and Andropogoneae (bts 100; brs 32). Other strongly supported groups in the PACCAD Clade correspond to Aristidoideae (bts 100; brs 25) and Danthonioideae (bts 98; brs 15). The traditional Chloridoideae are supported at bts 99 (brs 16), and the clade including the chloridoids plus *Centropodia glauca* and *Merxmuellera rangei* (Chloridoideae s.l.) is also reasonably well sup-

ported at bts 86 (brs 8). A clade corresponding to Arundinoideae s. str.—*Arundo*, *Amphipogon*, *Molinia*, and *Phragmites*—receives modest support from this analysis (bts 77, brs 6), but the sister relationship of *Molinia* and *Phragmites* is well supported (bts 100; brs 16). The other major clade (the BEP Clade) is less well supported (bts 71; brs 8) and includes Bambusoideae s. str., Ehrhartoideae (= Oryzoideae), and Pooideae. Bambusoideae are monophyletic (bts 97; brs 15), as is the clade including the herbaceous bamboos (bts 100; brs 18). Likewise Ehrhartoideae are monophyletic (bts 100; brs 24), as are Oryzeae (bts 100; brs > 34). Pooideae include *Brachyelytrum* (bts 100; brs 15), and most nodes within the pooid clade are strongly supported.

Despite the strong phylogenetic pattern shown by the combined analysis, placement of some taxa remains ambiguous. The major uncertainty remains the monophyly of the BEP Clade. As noted earlier (GPWG, 2000), it is almost equally parsimonious to place Pooideae as sister to the PACCAD Clade, and this makes evolution of particular morphological characters more parsimonious. The Pooideae plus PACCAD group appears in analyses of *rbcL* (Appendix III-C), chloroplast restriction sites (Appendix III-A), morphology (Appendix III-H), and ITS (Appendix III-F), whereas the BEP Clade is retrieved by analyses of *ndhF* (Appendix III-B), *rpoC2* (Appendix III-D), and *phyB* (Appendix III-E). GBSSI (Appendix III-G) forms a novel topology, in which neither the PACCAD nor the BEP Clades is monophyletic. An analysis combining *rbcL*, chloroplast restriction sites, ITS, and morphology retrieves, not surprisingly, a clade that links the Pooideae with the PACCAD Clade. Bootstrap analysis, however, finds that the Pooideae + PACCAD clade occurs in only 23% of the replicates, although it appears in 40% if *Streptogyna* is considered part of the clade. The BEP Clade was not found in any of the bootstrap partitions.

Constraining the entire data set to place Pooideae sister to the PACCAD Clade resulted in a single tree eight steps longer than the most parsimonious tree. The net change of eight steps, however, was produced by changes of one or two steps in 107 characters from throughout the data set. A Wilcoxon signed rank test (Templeton, 1983; Mason-Gamer & Kellogg, 1996) resulted in a test statistic of 2654; for  $n = 107$ , this corresponds to  $p < 0.406$  (two-tailed test). This means that we cannot rule out the possibility that Pooideae are indeed sister to the PACCAD Clade. This is true even if the morphological characters are excluded ( $z = 1.146$ ;  $P < 0.254$ ).



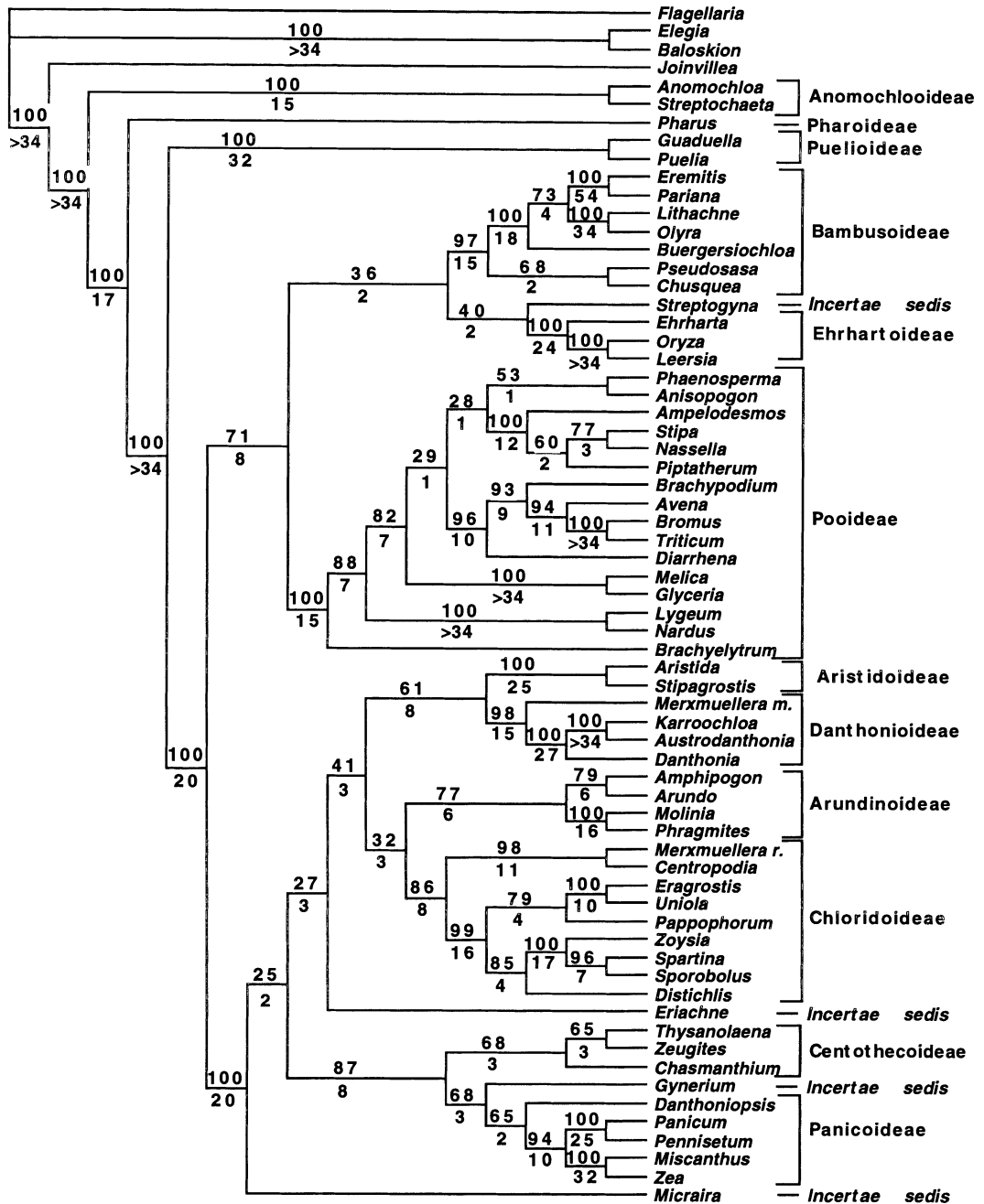


Figure 2. Same tree as Figure 1, showing percent of bootstrap replicates above lines and Bremer support below. Brackets indicate the revised classification for the Poaceae.

Figure 1. Single most parsimonious tree for the grasses and relatives, based on eight sets of data. Length = 8752 steps, CI = 0.375, RI = 0.557. Numbers above branches are numbers of unambiguous changes. Branches are drawn proportional to length.

The combined analysis places *Streptogyna* as sister to Ehrhartoideae, but this result is not strongly supported (bts 40; brs 2). This partly reflects missing data, in that only *ndhF* and *phyB* sequences are available for *Streptogyna*, in addition to morphological data. *ndhF* places *S. americana* sister to Ehrhartoideae, whereas *phyB* places it as sister to the entire BEP Clade, and morphological data fail to resolve its position.

The combined data place the woody bamboos, *Pseudosasa* and *Chusquea*, in a clade (bts 68; brs 2), as would be expected from previous studies (Zhang, 2000; Zhang & Clark, 2000). The pair appears monophyletic in chloroplast restriction site, morphological, and *phyB* trees. The two are paraphyletic, however, in trees using *rbcL* and *ndhF*, although this result is not well supported in these trees. "*Pseudosasa*" is a composite taxon, made up of data from several different genera, and this may also affect its placement in the combined tree.

*Phaenosperma* and *Anisopogon* are clearly members of the expanded pooid clade, where they are placed by all data sets, either singly or in combination. Their position within the clade, however, remains uncertain. They are sister taxa when all data are combined, but this result is not strongly supported (bts 53; brs 1); together they are sister to the Stipeae, also a poorly supported result (bts 28; brs 1). In *ndhF*, chloroplast restriction site, and *phyB* trees, *Anisopogon* is placed on a branch that diverges after the *Lygeum* + *Nardus* clade, but before the rest of the Pooideae (i.e., Stipeae, Meliceae, *Diarrhena*, *Brachypodium*, Aveneae, and Poae). ITS places it sister to Aveneae/Poae, and *rpoC2* places it sister to *Stipa*. In no case is the placement strongly supported. The position of *Phaenosperma* is based only on *ndhF* and morphological data, and the latter are largely uninformative about its position.

Meliceae are monophyletic in all gene trees and in the combined tree. Their position, however, varies among the individual gene trees. The combined tree provides good evidence that Meliceae diverged after *Lygeum* + *Nardus* (bts 82; brs 7), but evidence is weak that it was the next diverging branch (bts 29; brs 1). Other possible placements include sister to Stipeae (*ndhF*, *phyB*), sister to *Diarrhena* + *Brachypodium* + Aveneae/Poae (cp restriction sites), sister to a clade of *Brachypodium* + *Brachelytrum* + (*Lygeum* + *Nardus*) (ITS), or paraphyletic at the base of the Pooideae (GBSSI). The ambiguity cannot be ascribed to missing data, although additional sampling among early-diverging Pooideae might be warranted.

The positions of *Eriachne* and *Micraira* are not

firmly resolved by the combined data set, although both are clearly members of the PACCAD Clade. This almost certainly reflects missing data. In addition to morphological data, *Eriachne* is represented only by *rbcL* and ITS sequences, and *Micraira* by *ndhF*, *rpoC2*, and ITS. They are both isolated taxa, and in individual analyses fall at the base of other well-supported clades. The position of *Micraira* as sister to the entire PACCAD Clade appears only in the combined analysis, and likewise the position of *Eriachne* as sister to the Arundinoideae s. str. + Chloridoideae + Aristidoideae + Danthonioideae clade is both novel and poorly supported. Bootstrap analysis of the combined data set placed *Eriachne* and *Micraira* as sisters in 51% of the 1000 replicates, a position not supported by the most parsimonious tree.

Aristidoideae and Danthonioideae are both clearly monophyletic, and each is strongly supported by both bootstrap and decay analyses (bts 100 and 98; brs 25 and 15, respectively). In the combined tree they appear as sister taxa. The aristidoid/danthonioid clade is not strongly supported, however (bts 61; brs 8), and is reflected only in the *phyB* tree. *rbcL* places Aristidoideae sister to Chloridoideae, whereas *ndhF* and chloroplast restriction sites put Aristidoideae sister to the rest of the PACCAD Clade, and ITS places the subfamily sister to *Amphipogon* + Chloridoideae. *rpoC2* indicates that Aristidoideae is derived from within Arundinoideae. *ndhF* places Danthonioideae sister to Panicoideae + Centothecoideae, whereas chloroplast restriction sites do not resolve the position of *Danthonia*. GBSSI retrieves a novel arrangement in which Danthonioideae are polyphyletic, but this result is not strongly supported and is likely affected by skewed taxon sampling in the GBSSI data set. *rpoC2* suggests that Danthonioideae are sister to *Amphipogon*.

The relationships of *Zeugites*, *Thysanolaena*, *Chasmanthium*, *Danthoniopsis*, and *Gynerium* to each other and to the Panicoideae are not resolved by this analysis. The entire group is well supported as monophyletic (bts 87; brs 8), but other relationships are less clear. Only morphological and *ndhF* data are available for *Zeugites*, so its placement may be affected by missing data.

The morphological data have little effect on the analysis. When they are omitted, 6 trees are found in two islands (length 8488, CI = 0.378, RI = 0.554). The topology of the strict consensus (Appendix III-K) is similar to that of the combined tree except for the position of *Zeugites*, which is sister to *Danthoniopsis*, and *Gynerium*, which is sister to Panicoideae. In the consensus of the six trees, the

relationship of *Pseudosasa* and *Chusquea* is unresolved, as is the relationship of *Phaenosperma* and *Anisopogon*, and the position of Meliceae in the pooid clade. These are areas that were poorly supported even in the combined tree, and thus already known to be ambiguous. The most notable difference is the increased support for the BEP Clade, which is supported at a bootstrap value of 90%. The number of nodes with support greater than 90% (Table 5) is somewhat greater without the structural data, but the overall consistency index is not changed appreciably (Table 3).

The results for the entire data set largely reflect the results for the chloroplast data alone. The chloroplast data contribute 1336 potentially phylogenetically informative characters, or about 62% of the total. Analysis of these data alone produces 2 trees (length = 4903, CI = 0.381, RI = 0.589) that differ only in the relative positions of *Phaenosperma* and *Anisopogon* (Appendix III-I). The numbers of strongly supported nodes are about the same as for the entire data set (Table 5), and differences between the chloroplast tree and the entire data set are all in poorly supported areas of the tree (see below).

The chloroplast tree is only slightly affected by mixing sequence data with restriction site data. If the restriction site data are excluded so that the data set consists only of *ndhF*, *rbcL*, and *rpoC2* data, the tree is virtually identical to the chloroplast tree except that *Pseudosasa* plus *Chusquea*, and *Phaenosperma* plus *Anisopogon* form monophyletic pairs rather than being paraphyletic (not shown). *Piptatherum* and *Nassella* are paraphyletic rather than sisters, and the Meliceae are sister to the core Pooideae rather than to the Stipeae.

Analysis of only the three nuclear genes (*phyB*, *GBSSI*, and *ITS*) required elimination of nine taxa for which nuclear data were not available. The analysis thus included 57 taxa and 757 characters and found eight trees (length = 3513, CI = 0.382, RI = 0.512) on one island (Appendix III-J). The nuclear trees were not as well supported as the chloroplast tree or the tree for the entire data set, which presumably reflects extensive missing data for *GBSSI*, and a generally smaller number of informative characters. Only two nodes were supported in 100% of the 1000 bootstrap replicates, and 11 had values between 90 and 99%.

Analysis of chloroplast data plus the data from the two protein-coding nuclear genes (that is, excluding morphological and *ITS* data) has little effect on either topology or support for the tree, perhaps because omitting morphology and *ITS* only eliminates 178 characters, or about 8% of the total.

Differences appear only in the placement of the Meliceae, *Eriachne*, *Micraira*, *Zeugites*, and *Danthoniopsis*, all poorly supported areas of the trees.

## DISCUSSION

### WELL-SUPPORTED CLADES

Some relationships appear consistently in all analyses of all data sets and are strongly supported by the combined analysis. Among these are the following (in order from the bottom of the tree):

1. Joinvilleaceae are sister to Poaceae.
2. Poaceae are monophyletic.
3. The earliest diverging lineage of Poaceae is Anomochlooideae (even if *Anomochloa* and *Streptochaeta* prove to be two separate lineages, they would still be the two earliest-diverging lineages in the family).
4. The next diverging lineage is Pharoideae.
5. The next diverging lineage is Puelioideae.
6. All remaining grasses form a clade, which appears to have diversified well after the origin of the family.
7. Bambusoideae s. str., Ehrhartoideae, Pooideae s.l., Aristidoideae, Danthonioideae, Arundinoideae s. str., Chloridoideae s. str., Chloridoideae s.l., and Panicoideae are all monophyletic.
8. Bambuseae, Parianeae, Olyreae s. str., Oryzaeae, Stipeae, Meliceae, and *Lygeum* + *Nardus*, and *Molinia* + *Phragmites* are all monophyletic.
9. The PACCAD Clade—now including Panicoideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae, *Eriachne*, *Micraira*, and *Gynerium*—is monophyletic.

As noted in the introduction, all of these relationships have been supported by previous studies and none is unique to the combined analysis. Previous studies, however, were limited because they were based on a single gene, a modest number of morphological characters, and/or a restricted sample of taxa. Because of the strong support for the relationships found in the present study, we propose a revised subfamilial classification (see Taxonomic Treatment). The revisions primarily reflect changes in circumscriptions of the Bambusoideae and Arundinoideae and involve only a small fraction of the species in the family. Over three quarters of the species are included in the subfamilies Pooideae, Chloridoideae, and Panicoideae, the circumscriptions of which are changed only slightly by the revisions.

### MOLECULAR CHARACTERS

Virtually all of the phylogenetic signal in this analysis comes from the molecular data (Appendix

III-K), as expected. The combinable component consensus (Bremer, 1990) of the molecular trees (Kellogg, 1998) is remarkably well resolved; all nodes found in this consensus are strongly supported in the combined analysis presented here. When the molecular data are analyzed alone, all strongly supported nodes from the combined analysis are recovered, and support for the BEP Clade is increased.

Previous theoretical (Graybeal, 1998) and empirical (Soltis et al., 1998) studies have indicated that large numbers of characters may be necessary to resolve phylogenetic patterns, a conclusion only partially supported by this study. The molecular data alone and the entire data matrix included 2093 and 2143 phylogenetically informative characters, respectively (Table 3). These data sets found the largest percentage of nodes with bootstrap values above 70% (0.73 in both cases) but did not have the highest consistency or retention indices. The highest CI was produced by the *rpoC2* data alone (150 informative characters), although this could be in part an artifact of alignment (see below), and the highest RI by the *rbcL* data alone (213 informative characters). The fraction of nodes with bootstrap values over 70% was almost as high for the phytochrome B data alone (with 417 informative characters) as for all data combined. We conclude that, while large numbers of informative characters may provide increased reliability, small numbers are not necessarily misleading or inaccurate samples of the whole.

Other studies have shown that the number of taxa included may affect phylogenetic accuracy (e.g., Hillis, 1996, 1998; Graybeal, 1998), although this is not necessarily the case (Poe & Swofford, 1999). Certainly future studies should include more taxa than just the set of exemplars used here. However, the results numbered 1 to 9 above have been found in analyses of virtually every individual data set, as well as in the combined tree, and we would be surprised if they were overturned by inclusion of more taxa.

Most of the molecular data come from chloroplast genes, so it is not surprising that the tree from the chloroplast alone closely matches the tree for the entire data set. The *ndhF* data set is missing the least data (Table 3) and has the most informative characters of the molecular data sets, presumably because it is the longest molecule. Our results confirm the utility of this molecule for resolving relationships among grass genera (Clark et al., 1995; Giussani et al., in press).

Alignment is a particular problem for the *rpoC2* and ITS data used here. Alignment of the ITS data

was difficult and confirmed our suspicion that it may not be useful at this level of divergence. ITS1 and parts of ITS2 had to be eliminated because of difficulty in assessment of sequence similarity. The *rpoC2* sequences used here code for repeated amino acid motifs inserted into the protein. The insertion appears only in the grasses and thus constitutes a synapomorphy for the family (Cummings et al., 1994), although we did not code it as such in this analysis. The repeats are similar but not identical to each other in sequence, making alignment problematical, a point discussed at length by Barker (1995) and Barker et al. (1999). Efforts to improve alignments necessarily reduce apparent homoplasy; this may result in the high CI mentioned above. Although phylogenetic results from these molecules are similar to those from the other genes, by themselves the two sets of sequences do not permit confident assessments of relationships among subfamilies.

Trees from *ndhF* and *phyB*, individually, are particularly well resolved and well supported. Their congruence in early-diverging branches contributes to the strength of the overall topology of the combined data. In particular, *phyB* provides considerable support for the BEP Clade, a topology that is only weakly supported by *ndhF*, and not at all by several other data sets. The two data sets do appear to conflict in relationships among members of the PACCAD Clade, and this may be an area for future investigations.

As noted in Methods, sequences for a given genus were in some cases taken from different, congeneric species. This procedure assumes that the genus is monophyletic, an assumption that is almost certainly correct in many cases (e.g., *Joinvillea*, *Streptochaeta*), and perhaps not as likely in others. For example, the three species of *Stipa* sampled here have been placed in the genera *Achnatherum*, *Stipa*, and *Jarava* (Barkworth & Everett, 1987; Barkworth, 1993; Jacobs & Everett, 1997), which are distinct and possibly not a monophyletic group within Stipeae (Jacobs et al., 2000). While this problem is not likely to compromise our conclusions regarding subfamily relationships, it means that relationships among species of the Stipeae (or other tribes or genera where composite terminal taxa were used) cannot be addressed by this analysis.

#### STRUCTURAL CHARACTERS

The structural characters, comprising the morphological data set (Table 4), were optimized on the phylogeny (Fig. 3). Our results suggest that some

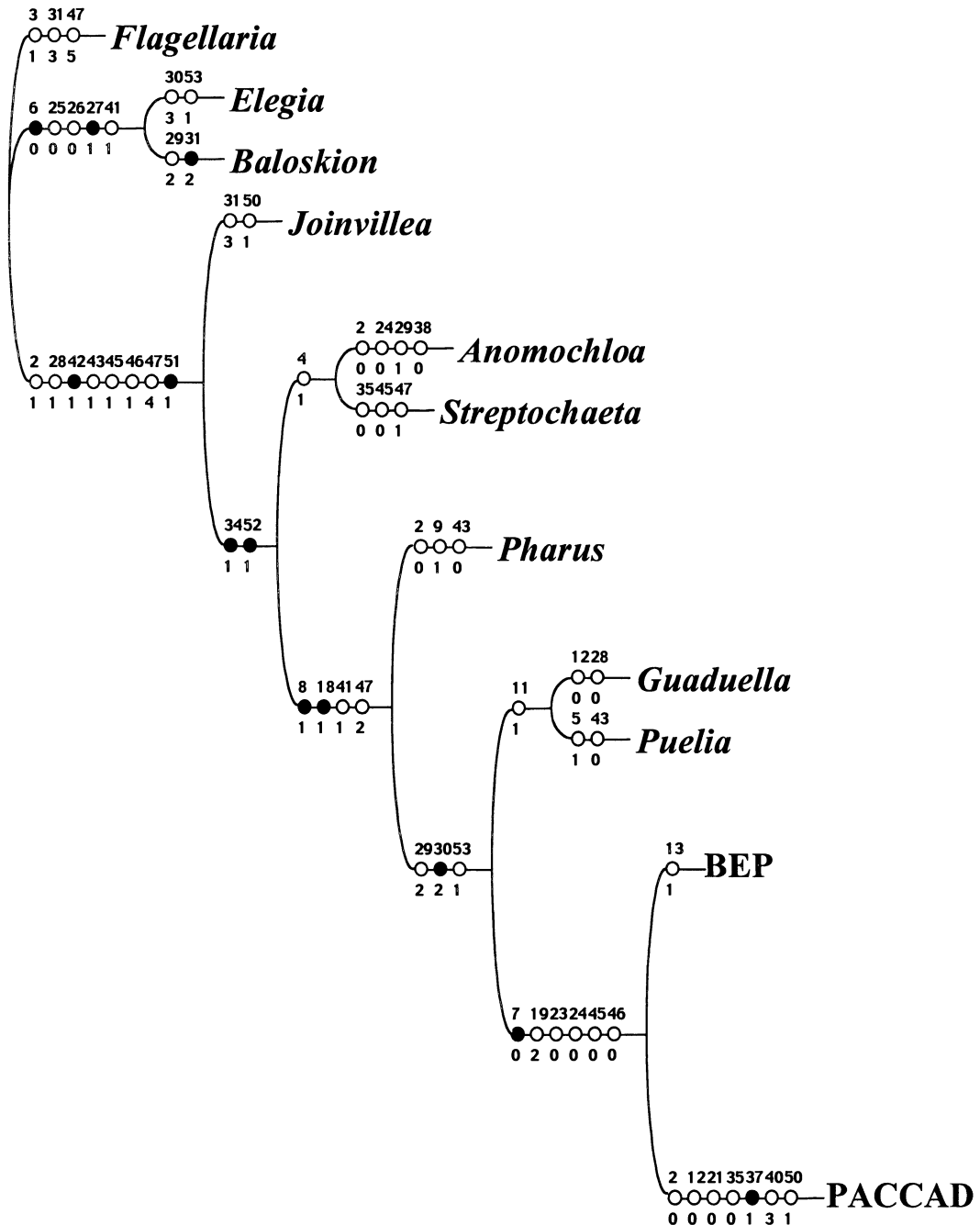


Figure 3 (pp. 395–397). Same tree as Figure 1, with structural characters mapped on using ACCTRAN optimization. Character number is above the branch, and the state to which the character changes is below. Filled circles represent unique occurrences of character states; open circles represent homoplasies.

of the morphological characters may be useful for delimiting groups within tribes or subfamilies, but are too variable to be useful in delimiting subfamilies; these include 2 (culms hollow or not), 3 (leaf sheath margins fused or free), 10 (pedicel present

or not), 11 (presence or absence of proximal reduced flowers), 12 (number of flowers per spikelet), 13 (presence or absence of awns), 15 (attachment of awns), 16 (disarticulation above or below glumes), 20 (lodicule fusion), 28 (style fusion), 29



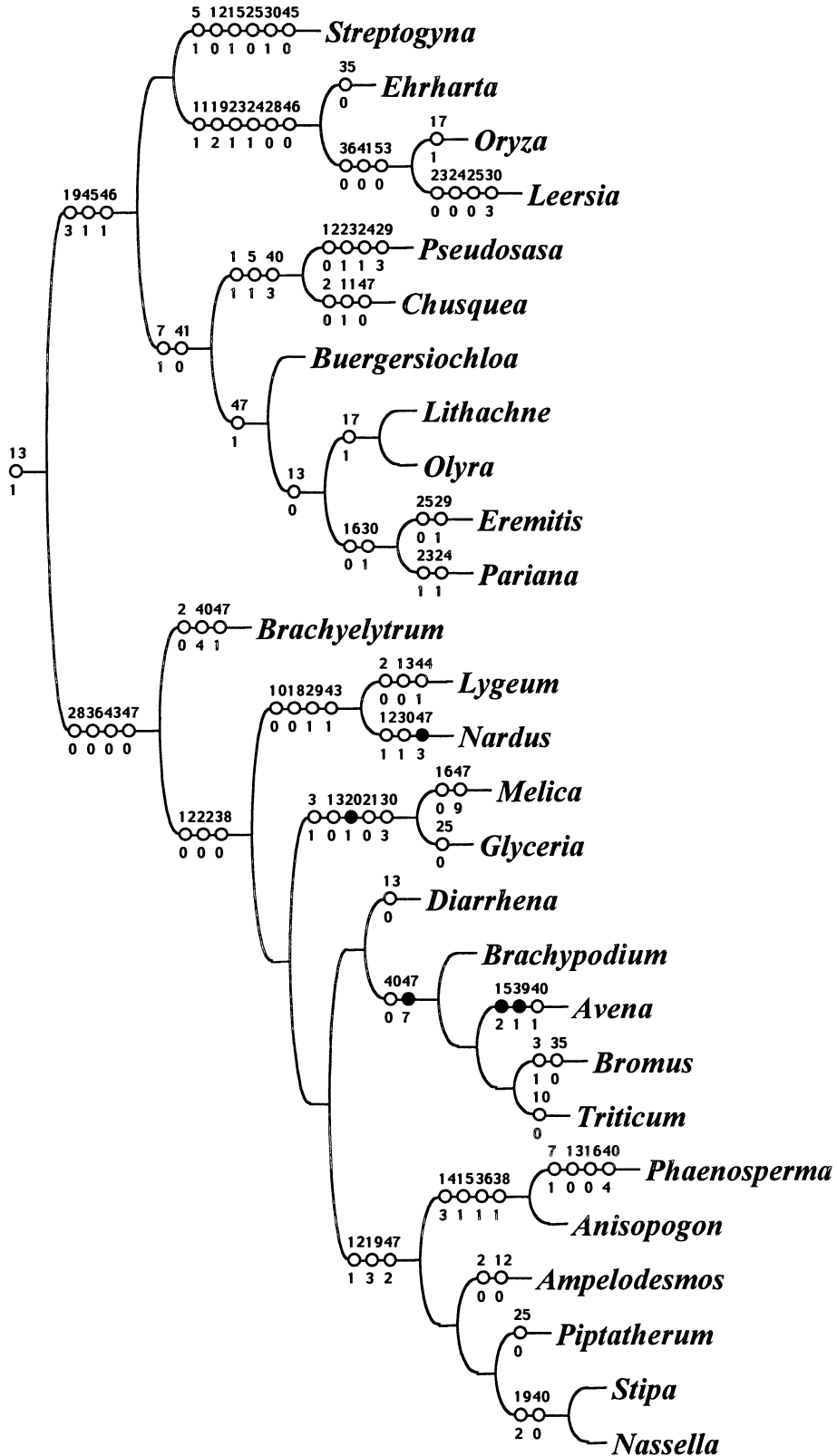


Figure 3. Continued.

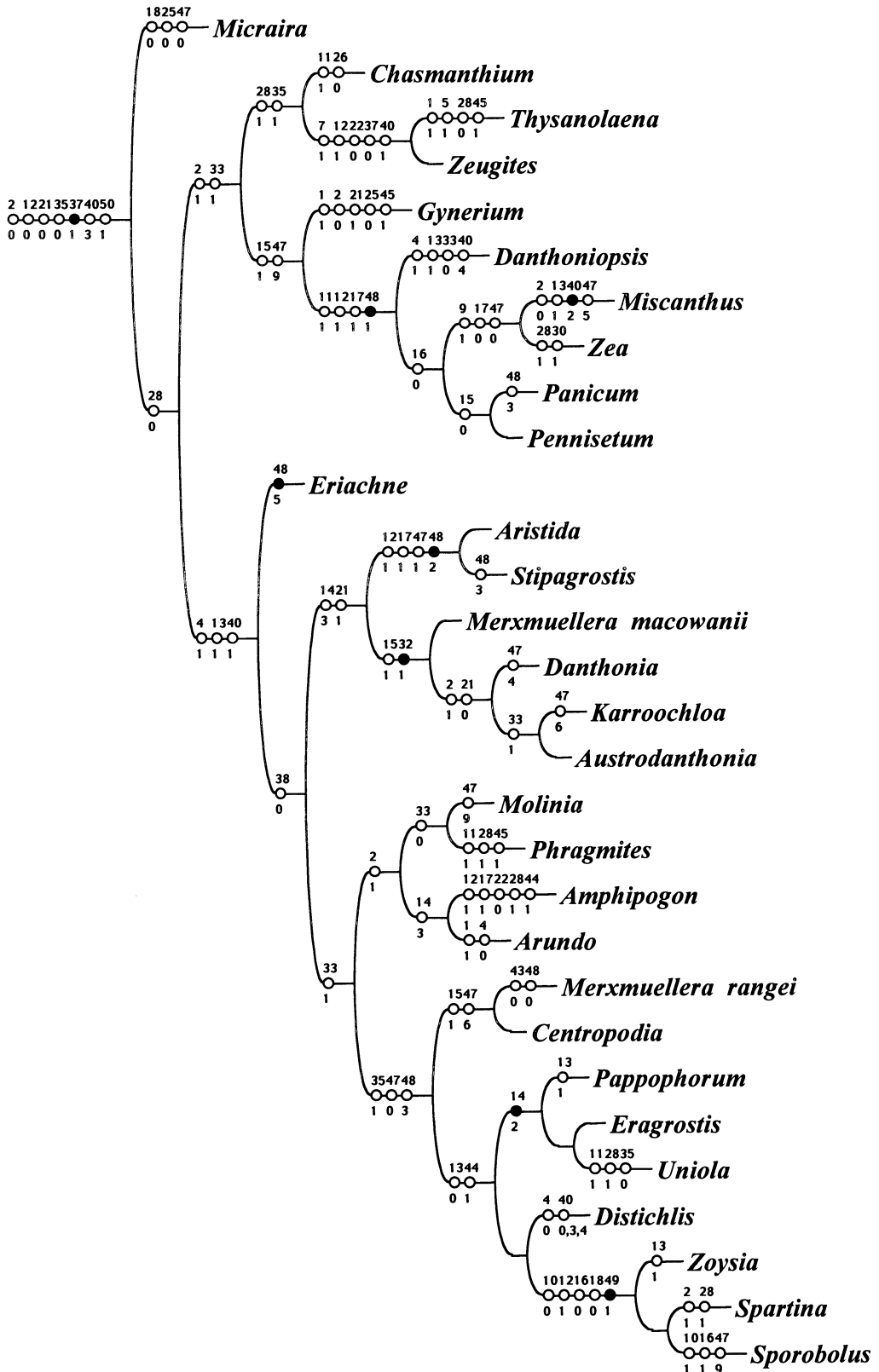


Figure 3. Continued.

(number of stigmas), 30 (highest order of stigmatic branching), 39 (lipid in the endosperm), 40 (endosperm starch grain syndrome), and 47 (base chromosome number).

For other structural characters, our assessment of character states is inadequate for use in phylogenetic analysis. For example, the woodiness of bamboo culms (char. 1) is due to numerous, closely spaced vascular bundles around the periphery of the culm, each bundle with massive sclerenchyma fiber caps on both sides, combined with heavily sclerified ground tissue, but isolated fiber strands may also occur (Soderstrom, 1981; Liese, 1998). It is not clear if the hardness of culms in *Arundo*, *Thysanolaena*, and *Gynerium* and a number of other "woody" taxa of the PACCAD Clade (Watson & Dallwitz, 1992) is derived in the same way; these taxa need more careful anatomical study. Similarly, the fringed membranous ligule of some Pooideae (char. 4) may not be the same as the ciliate membrane found in many members of the PACCAD Clade and requires developmental investigation. Spikelet pairing (char. 9) appears in various panicoide grasses and also in *Pharus*, but the pattern of development of the pairs in *Pharus* is unknown. Membranous lodicules (char. 21) are scored as being the same wherever they occur, but development of these structures has never been compared. Haustorial synergids are apparently uniquely derived within the Danthonioideae, but many taxa have not been investigated for this character. Embryo characters (35, 36, 37, 38) are often phylogenetically informative, but lack of observation of critical taxa makes their use difficult in some cases. Starch grains (char. 40) are classified according to their apparent structure when viewed with the light microscope. With much recent work done on the biochemistry and molecular genetics of starch granule formation (e.g., Whistler et al., 1984; Frazier et al., 1997), this character could and should be recircumscribed, although then much scoring will need to be redone. Arm cells and fusoid cells (chars. 45 and 46) are now seen to be ancestral in the family, yet their development and ultrastructure are poorly studied, and their physiological function is unknown. Comparisons of chromosome base numbers (char. 47) will almost certainly become more precise because of recent studies of nuclear genome arrangement (e.g., Gale & Devos, 1998).

Finally, some of the structural characters are genuine morphological puzzles, ones for which strict comparison is difficult. These include the floral bracts (glumes, lemmas, paleas, lodicules), which occur only in the grasses, and may not even occur in Anomochloideae. Homology of the grass

flower and spikelet affects characters 8, 18, and 19.  $C_4$  photosynthesis (chars. 48 and 49) is known to be a set of characters that do not co-vary; its origin is still poorly understood. These characters are discussed in more detail below.

#### SPIKELETS AND FLOWERS

*Spikelet.* The flowers of most grasses are arranged in bracteate units known as spikelets. These usually consist of two bract-like appendages (glumes) at the base of a central axis (rachilla) on which are borne one or more florets, all in a distichous pattern. According to Clayton (1965: viii), the "grass spikelet never ceases to fascinate, for the simplicity of its theme is matched by the elegance of its variations." The apparent simplicity of the grass spikelet notwithstanding, its origin and homologies, as well as those of the grass flower, have been much debated (see reviews in Clifford, 1987, and Soreng & Davis, 1998).

The phylogeny highlights the difficulty of discussing the evolution of the grass spikelet. The standard spikelet (or some modification of it) is present in all members of the Pooideae and the PACCAD Clade (Figs. 4 and 5). The pattern of bracts and flowers, however, is variable among Pharoideae, Puelioideae, Bambusoideae, and Ehrhartoideae (Fig. 4). Pharoideae bear single flowers, each with a lemma, a palea, and a pair of glumes, but no rachilla extension (Fig. 4B; Judziewicz, 1987; Soderstrom et al., 1987). Puelioideae have multi-flowered spikelets, in which each flower has a lemma and palea, and the whole unit has a pair of glumes (Fig. 4A). Proximal incomplete florets occur, but distal reduction is seen only in *Guadua*. In Bambusoideae, the unisexual, one-flowered spikelets of Olyreae are standard, but the bracteate, rebranching spikelets (pseudospikelets) of many Bambuseae are difficult to interpret (Fig. 4F; Judziewicz et al., 1999). The multiflowered spikelet of *Streptogyna* presents no difficulties of interpretation, but in the Ehrhartoideae, extra proximal, sterile bracts (usually called sterile lemmas) are common (Fig. 4G), and extreme reduction of glumes is known (Oryzeae). It is not clear whether the proximal sterile bracts of Ehrhartoideae are truly homologous to the proximal incomplete florets that occur in Puelioideae and some bambusoids, or if they are phylogenetically (and possibly developmentally and genetically) distinct. The uncertain position of *Streptogyna* makes the homology assessment even more ambiguous.

Most characters of the spikelet and the floret (chars. 9–12 and 14–17) are treated as inapplicable

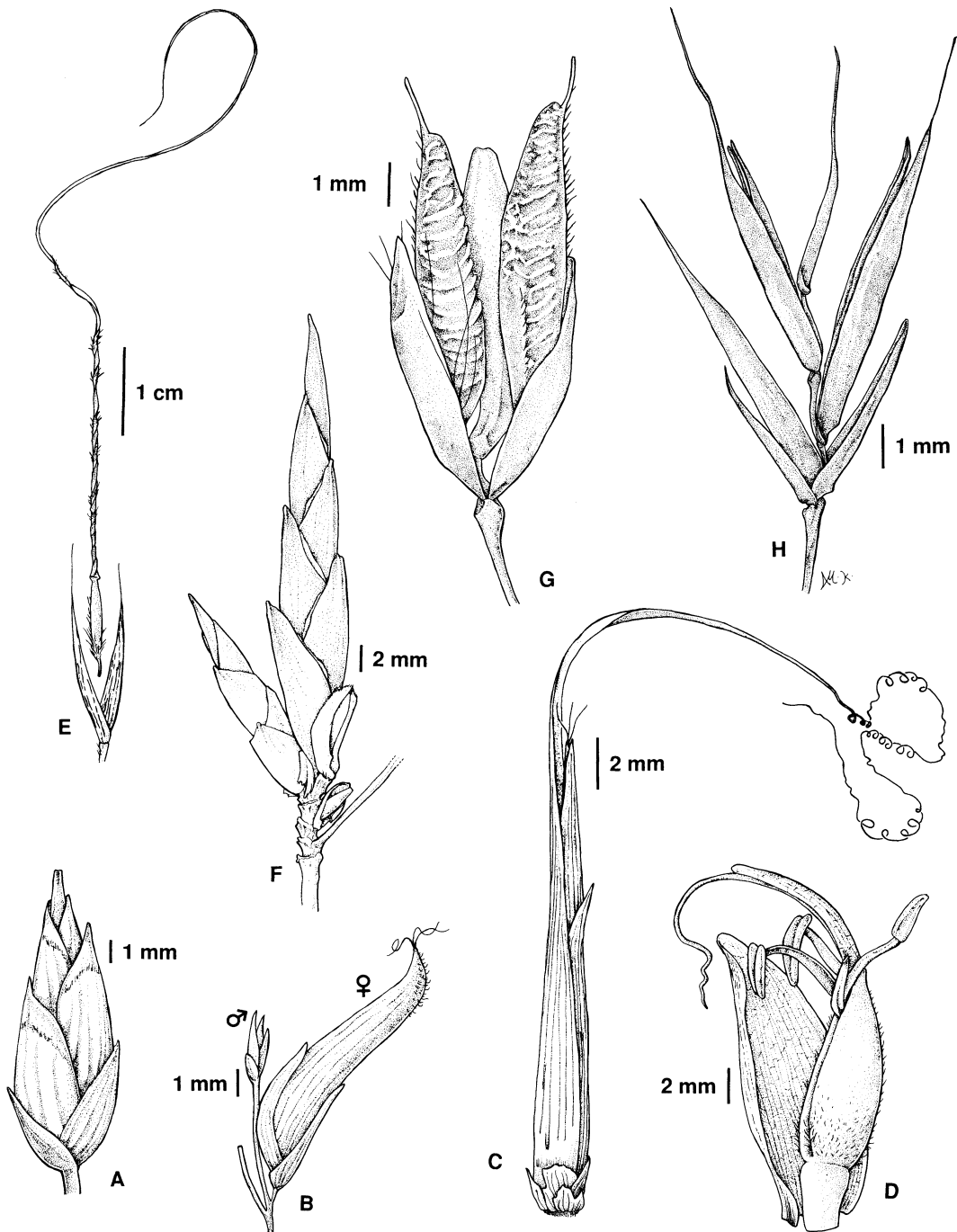


Figure 4. Spikelets and spikelet equivalents of early-diverging lineages and the BEP clade. —A. *Puelia schumanniana*, Puelioideae (*Letouzey 12930*, US). —B. *Pharus mezii*, Pharioideae (*Hinton 16059*, US; redrawn from Judziewicz, 1987). —C. *Streptochaeta spicata*, Anomochloideae (*Bailey & Bailey 723*, US; redrawn from Judziewicz & Soderstrom, 1989, from originals by A. Tangerini at US). —D. *Anomochloa marantoidea*, Anomochloideae (*Calderón 2046*, US; redrawn from Judziewicz & Soderstrom, 1989, from originals by A. Tangerini at US). —E. *Stipa comata*, Pooideae (*Pearson s.n.*, ISC). —F. *Guadua chacoensis*, Bambusoideae (*Nee 35467*, ISC). —G. *Ehrharta bulbosa*, Ehrhartoideae (*Barker 1119*, ISC). —H. *Festuca idahoensis*, Pooideae (*Pohl 15642*, ISC).

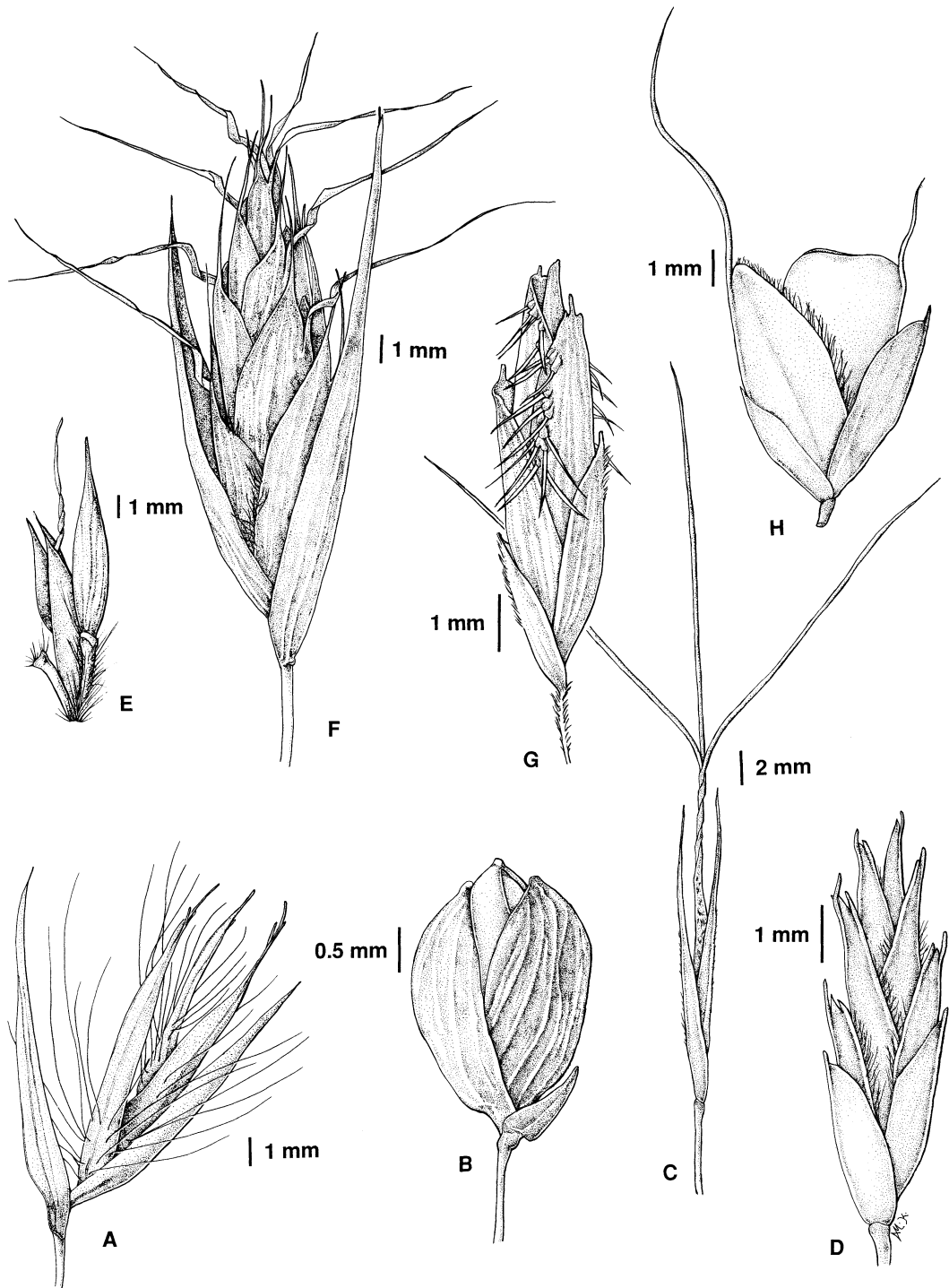


Figure 5. Spikelets of the PACCAD clade. —A. *Arundo donax*, Arundinoideae (Bradley & Sears 3558, ISC). —B. *Dichanthelium oligosanthes*, Panicoideae (Lelong 2063, ISC). —C. *Aristida arizonica*, Aristidoideae (Griffiths 7373, ISC). —D. *Tridens flavus*, Chloridoideae (Thorne 18302, ISC). —E. *Andropogon gerardii*, Panicoideae (Clark s.n., teaching collection, ISC). —F. *Danthonia californica*, Danthoioideae (Pohl 9459, ISC). —G. *Centotheca lappacea*, Centothecoideae (Liang 66250, ISC). —H. *Chloris cucullata*, Chloridoideae (Malacara & Gutierrez 33, ISC).

when grass-type spikelets and florets are absent, as in the non-grass outgroups (see char. 8). Although pseudospikelets occur in some genera of Bambuseae, the two in the present study are regarded as having true spikelets and florets, and thus scorable for features of these structures.

Neither *Anomochloa* nor *Streptochaeta* (Fig. 4C and D) has structures clearly homologous with glumes, lemmas, or paleas, and thus neither can be described as having grass-type spikelets or florets. We therefore follow Clark and Judziewicz (1996) in using the term "spikelet equivalent" to refer to the flowering units of the inflorescences in the Anomochlooideae to emphasize this lack of recognizable homology. Characters of the spikelet and the floret are scored as inapplicable or ambiguous in these two genera except for char. 13 (see Appendix IV). The grass spikelet may have originated either before or after the divergence of Anomochlooideae. If before, then the spikelet was extensively modified in the long history of Anomochlooideae. The origin certainly must have occurred before divergence of Pharoideae. We refer to the clade of all grasses except Anomochlooideae as the Spikelet Clade.

Bracts outside of the spikelets subtend inflorescence axes and often have a blade. Short to elongate prophylls are usually present on the branches subtended by these bracts. Such bracts occur primarily in Bambuseae and Andropogoneae, but they are not necessarily homologous between the two groups (see Renvoize & Clayton, 1992). Well-developed subtending bracts are usually absent in other members of the Spikelet Clade, although there may be a ridge or scar which presumably represents the subtending bract at the base of the inflorescence branch.

**Glumes.** We have assumed here that glumes are homologous across the Spikelet Clade. Glumes are typically defined as the two sterile bracts at the base of the spikelet, but additional sterile bracts (usually called sterile lemmas or sterile florets even if there is no evidence of any, even vestigial, floral axis) may occur between the glumes and the flower-bearing lemmas (e.g., Ehrhartoideae, *Chusquea*, many Centothecoideae). In general, the first (lower) glume is abaxial and the second (upper) glume is adaxial (E. A. Kellogg, pers. obs.; Clifford, 1987), but the first glume may be adaxial in position, as in a number of Paniceae (Clifford, 1987). Grassl (1956) and Stapleton (1997) argued that the first glume is actually a prophyll for Andropogoneae and Bambuseae, respectively, and that the prophyll was displaced upward to assume the position and function of a glume. Even if the first glume is abaxial,

the structure still could be derived as a prophyll if the axis rotated through 180° (Clifford, 1987). The gemmiparous bracts of many Bambuseae are essentially glumes with a bud in the axil. If the bud develops it becomes a second- or higher order pseudospikelet (Judziewicz et al., 1999). Glumes may be highly reduced or lost, as in Oryzaeae. A glume-like prophyll at the base of the pseudospikelet is observed in many Bambuseae (Fig. 4F), although the axis bearing the prophyll is not elongated (McClure, 1966).

**Numbers of florets.** The rachilla may or may not extend beyond the most distal floret, and reduced or modified florets may be present below or above (or both below and above) the fertile ones. For this analysis, we have assumed that the grass flower is terminal to the axis on which it is borne. In the Anomochlooideae, there is no identifiable palea and thus the flower appears to be truly terminal to the main sympodial axes of the inflorescence in *Anomochloa*, as discussed in Soreng and Davis (1998); the same is true under Soderstrom's (1981) interpretation of the spikelet equivalent of *Streptochaeta*. Within the Spikelet Clade, however, the flowers are borne on lateral branches, as indicated by the presence of a prophyll (i.e., the palea) in the proximal, adaxial position on the branch. This pattern is clear in those taxa with multiflowered spikelets or spikelets with one floret and a rachilla extension. There are a number of taxa with a single floret and no rachilla extension, including the Pharoideae, in which a well-developed palea is found in the floret. It is simple enough to imagine the reduction of the branch apex to the point where no evidence of a rachilla extension can be observed, but, as Soreng and Davis (1998) noted, the presence of a single-flowered floret (or equivalent) appears to be plesiomorphic for the family. This implies that either the rachilla extension and additional fertile florets evolved subsequently, or that multiflowered spikelets evolved before the divergence of the Pharoideae, and reduction to a single fertile floret occurred in that lineage (Soreng & Davis, 1998). Clearly, single-flowered spikelets evolved a number of times in various lineages in the BEP + PACCAD Clade.

**Lemma.** Each floral axis is subtended by a lemma (Fig. 6A and B), a structure that appears to be universally present across the Spikelet Clade. The lemma apparently is formed wholly by the spikelet meristem, and thus is a bract on the rachilla (Clifford, 1987). Lemma morphology is extremely variable, but the number of nerves is consistently odd, varying from 1 to 15 (Clifford, 1987). Some taxa

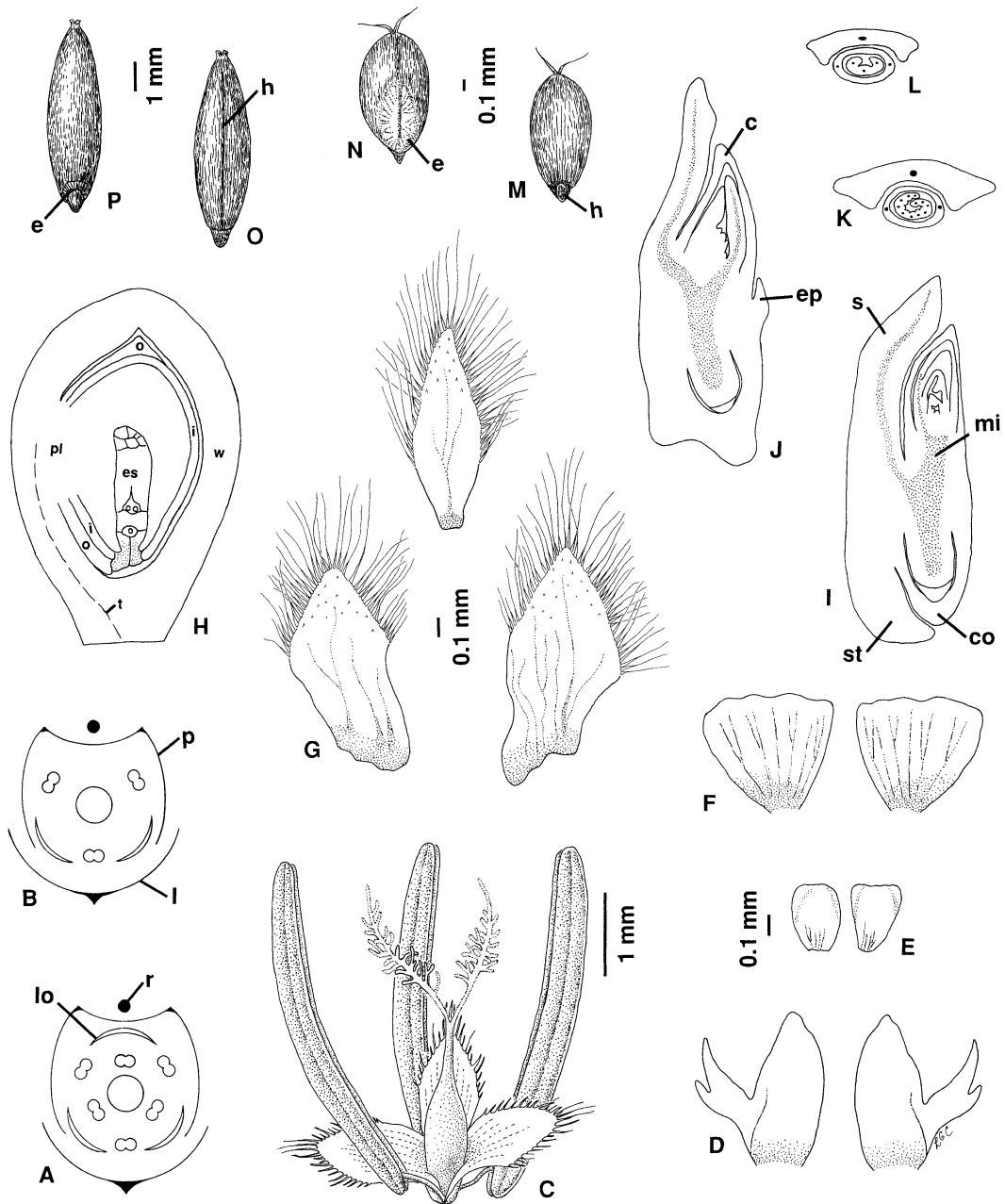


Figure 6. Grass flowers, fruits, and embryos. —A. Floral diagram of a grass with three lodicules and six stamens. —B. Floral diagram of a grass with two lodicules and three stamens. —C. Flower of *Yushania* (Bambuseae, original by D. Friedrich). —D. Lodicules of Pooideae (*Poa*, redrawn from Jirásek, 1968). —E. Lodicules of Chloridoideae (*Muhlenbergia*, redrawn from Soderstrom, 1967). —F. Lodicules of Panicoideae (*Setaria*, redrawn from Jirásek, 1968). —G. Lodicules of Bambusoideae (*Chusquea*), showing the anterior pair (lower two) and the posterior one (upper). —H. Generalized ovule section (Danthoioideae) showing haustorial synergids (stippled) (redrawn from Verboom et al., 1994). —I. Longitudinal section of a panicoid embryo showing presence of a scutellar tail (st) and the elongated mesocotyl internode (mi). —J. Longitudinal section of a poooid embryo showing presence of an epiblast (ep). —K. Cross section of a panicoid embryo apex showing overlapping embryonic leaf margins. —L. Cross section of a poooid embryo apex showing embryonic leaf margins that meet. M, N. Caryopsis of *Eustachys* (Chloridoideae). —M. Hilum side, showing a punctiform hilum (h). —N. Embryo side, showing the large embryo (e). O, P. Caryopsis of *Chusquea* (Bambusoideae) (redrawn from McClure, 1973). —O. Hilum side, showing a linear hilum (h). —P. Embryo side, showing the small embryo (e). c—coleoptile; co—coleorhiza; e—embryo; ep—epiblast; es—embryo sac; h—hilum; i—inner integument; l—lemma; lo—lodicule; mi—mesocotyl internode; o—outer integument; p—palea; pl—placenta; r—rachilla; s—scutellum; st—scutellar tail; t—vascular trace to placenta; w—ovary wall.

have more than two glumes at the base of the spikelet (see Glumes), but the sterile lemma of the panicoid spikelet does appear to represent an evolutionary reduction from a fertile floret.

*Palea.* The palea (Fig. 6A and B) is widely interpreted as a prophyll (Linder, 1987; Stapleton, 1997; Clayton, 1990; Soreng & Davis, 1998; Judziewicz et al., 1999), and prophylls do occur in the inflorescences of many Bambuseae and Andropogoneae (Judziewicz et al., 1999; E. A. Kellogg, unpublished obs.) relative to subtending bracts in precisely the same way a prophyll is related to a subtending leaf. Although the "palea as prophyll" explanation may be more parsimonious, the derivation of the palea from the fusion of two sepals also has been supported (Schuster, 1910; Stebbins, 1974; Irish, 1998; Schmidt & Ambrose, 1998). Nuñez (1968) and Clifford (1987) have suggested that the odd-nerved, often 1-keeled paleas in the Oryzae represent a separate origin of a palea-like structure, but this interpretation is inconsistent with the phylogeny. There is no reason to suppose paleas have been replaced with something different in Oryzae. Single-flowered spikelets lacking a palea (e.g., *Alopecurus*) probably represent loss of the rachilla extension and the palea on the floral axis.

Vegetative branching in the grasses and other monocots typically involves a leaf with a bud or branch in its axil, although bud displacement is observed in some palms and grasses (Dahlgren et al., 1985; Serebryakova, 1971; Fisher & Dransfield, 1977). The first appendage of the branching axis is an adaxial two-keeled bract (= prophyll). This prophyll encloses the bud, but often persists once the branch develops. The origin of the vegetative prophyll is not clear, but whether it is a single structure or the result of fusion of two bracts, ultimately it is foliar in nature (Stebbins, 1974). The relationship between the subtending leaf and the prophyll is generally constant, and therefore a prophyll marks the presence of a lateral branch. If the vegetative branching pattern is reiterated in the inflorescence, then the palea ought to be homologous to a prophyll, and prophylls should occur in fully bracteate inflorescences as the adaxial first appendage of branches in the axils of subtending bracts.

*Flower.* Grass flowers are made up of a gynoeceium, androecium, and two or three flap-like structures (lodicules) that force the floret open at maturity (Fig. 6A–G). The literature on the anatomy and development of the grass flower should probably be reinterpreted in the light of the present phylogeny. The origin and homology of the lod-

icules continue to be controversial. The grass flower has been interpreted variously as dichlamydeous (with two perianth whorls, the palea representing two fused sepals and the lodicules the petals), monochlamydeous (with only one perianth whorl, the lodicules, present, and the palea homologous to a prophyll), achlamydeous (with no perianth, the lodicules representing modified bracts or stipules), or pseudanthial (with the flower representing a highly reduced branch system, and the lodicules derived from leaves or branches) (Clifford, 1987). The monochlamydeous interpretation of the grass flower is the most widely accepted. Recent molecular genetic studies provide support for a petaloid homology of the lodicules (Irish, 1998; Schmidt & Ambrose, 1998; Ambrose et al., 2000), thus rejecting the achlamydeous and pseudanthial hypotheses. Ambrose et al. (2000) also provided genetic evidence that the palea and possibly also the lemma have characteristics in common with an outer perianth whorl, thus suggesting that the dichlamydeous interpretation might be revived. Bossinger (1990) and Pozzi et al. (2000) described mutations in barley in which the lemma is converted to a leaf. This may suggest that the lemma is more leaf-like than sepal-like. Kellogg (2000a) suggested that these two interpretations are not mutually exclusive, and that the complex structure of the grass spikelet may reflect simultaneous expression of both leaf and floral developmental "programs." If the latter interpretation is correct, then it may be meaningless to discuss whether the lemma is "really" a leaf or a sepal.

*Androecium.* All six stamens (arranged in two alternating whorls of three stamens each, Fig. 6A; see Clifford, 1987) are plesiomorphically present within the study group and at the point of origin of the grass family, but the entire outer whorl is lost in the Restionaceae. Within the grass family, all six stamens (both whorls) are maintained in the three earliest-diverging subfamilies (except for the loss of the inner anterior pair in *Anomochloa*, as noted above). The outer whorl is maintained throughout most of the grass family, except for the various autapomorphic losses and polymorphisms noted above. Loss of the entire inner whorl (e.g., Fig. 6B) is interpreted as a synapomorphy of the BEP + PACCAD Clade, but in one subclade within this large group (the bambusoid/ehrhartoid alliance) there are three or more independent reversions to presence of this whorl, and possibly a secondary loss in *Leersia*. An alternative interpretation of the outer stamen whorl, in the context of the present reconstruction of phylogenetic relationships, and involving no secondary origins, would have the in-



ner whorl lost independently in Pooideae and the PACCAD Clade, as well as in a series of small lineages within the bambusoid/ehrhartoid alliance. Alternatively, if a PACCAD + Pooideae lineage is considered, loss of the inner stamen whorl could be interpreted as a synapomorphy of that clade; the presence of this whorl in some genera of Ehrhartoideae and Bambusoideae might then be interpreted as retention of a plesiomorphy, while the absence in others would be interpreted as having arisen independently of the loss in the PACCAD + Pooideae.

#### BIOCHEMISTRY

The phylogeny suggests that the  $C_4$  photosynthetic pathway has evolved multiple times within the PACCAD Clade. In the  $C_4$  pathway, the Calvin-Benson cycle, and hence Rubisco, is relegated to the bundle sheath cells surrounding the veins (Hattersley & Watson, 1992; Sinha & Kellogg, 1996). Phosphoenolpyruvate carboxylase then catalyzes  $CO_2$  reduction in the mesophyll to produce the four-carbon compound oxaloacetate. This compound is then reduced to malate or aspartate and transported to the bundle sheath, where the newly fixed  $CO_2$  is immediately removed and taken up by Rubisco. This keeps  $CO_2$  concentration high at the active site of Rubisco, preventing competition by  $O_2$ . Consistent with the constant flow of materials between the bundle sheath and mesophyll,  $C_4$  species (Fig. 7C) have closer vein spacing than  $C_3$  species (Fig. 7B). Other anatomical manifestations of  $C_4$  photosynthesis include enlarged bundle sheath cells (Kranz anatomy), closely packed chlorenchyma cells, and in some, radiate chlorenchyma (Fig. 7C).

Despite the foregoing generalizations,  $C_4$  photosynthesis actually represents a suite of characters, rather than a single genetic and phylogenetic change. Only down-regulation of Rubisco in the mesophyll, up-regulation of PEP carboxylase, and closer vein spacing are common to all  $C_4$  grass lineages. The four-carbon compound that transports the carbon to the bundle sheath may be malate or aspartate, the decarboxylating enzyme may be a malic enzyme using NAD as a co-factor (NAD-ME), or using NADP (NADP-ME). If the former, additional decarboxylation activity may be provided by PEP carboxykinase, so PCK activity is dependent upon the presence of NAD-ME (Kanai & Edwards, 1999). Some  $C_4$  grasses have only one bundle sheath, whereas others have two. In those with two bundle sheaths the inner sheath may be made up of thick-walled cells, forming a conventional mesotome sheath, or it may be parenchymatous. There

are additional differences in expression of the enzymes involved in photosynthesis (Sinha & Kellogg, 1996). For example, all Chloridoideae except for the  $C_3$  *Eragrostis walteri* and *Merxmuellera rangei* form aspartate, use the NAD-malic enzyme, and have double bundle sheaths, with the inner one of thick-walled cells. (*Centropodia* has not been biochemically typed, but is anatomically the same as other NAD-ME taxa.) *Stipagrostis*, in Aristidoideae, is similar to the chloridoids in having two bundle sheaths, the outer of which appears to be carbon-reducing (Sinha & Kellogg, 1996). *Aristida* itself forms malate, using the NADP-malic enzyme; unlike other NADP taxa, it has two bundle sheaths. The ultrastructure of the outer sheath is similar to that of NADP species, but the inner sheath is more like an NAD or PCK plant; the extent to which these sheaths are developed varies throughout the genus (Brown, 1977; Carolin et al., 1973). *Eriachne* also uses NADP-ME and has a double bundle sheath, but unlike *Aristida* the inner sheath has thick-walled cells. The  $C_4$  Panicoideae also vary biochemically and histologically, although large groups are uniform. For example, the entire tribe Andropogoneae (ca. 100 genera and 1000 species) uses NADP-ME and has a single bundle sheath.

Both the phylogeny and structural/biochemical data indicate that the  $C_4$  pathway is not homologous wherever it occurs. The close relationship of the  $C_4$  lineages, however, suggests that there were a set of changes at the base of the entire PACCAD Clade that made the pathway easier to evolve. If this were true, then those changes, whatever they were, would be homologous, even though the final manifestations of the pathway are not.

#### IMPLICATIONS FOR MORPHOLOGICAL EVOLUTION

The phylogeny provides a unique and powerful tool for description of evolutionary pattern (Kellogg, 2000a). Major clades and evolutionary transitions are summarized in Figure 8. Additional detail can be found at <http://www.virtualherbarium.org/grass/gpwg/default.htm>.

*Sister relationship of Poaceae and Joinvilleaceae.* The presence of long and short cells in the leaf epidermis (char. 42), with at least some of the short cells containing silica bodies, unambiguously supports the sister relationship of Joinvilleaceae and Poaceae. This arrangement is apparently unique among angiosperms (Campbell & Kellogg, 1987; Kellogg & Linder, 1995). The presence of the 6.4 kb inversion in the chloroplast genome (char. 51) is also an unambiguous synapomorphy supporting this sister relationship, although it is not

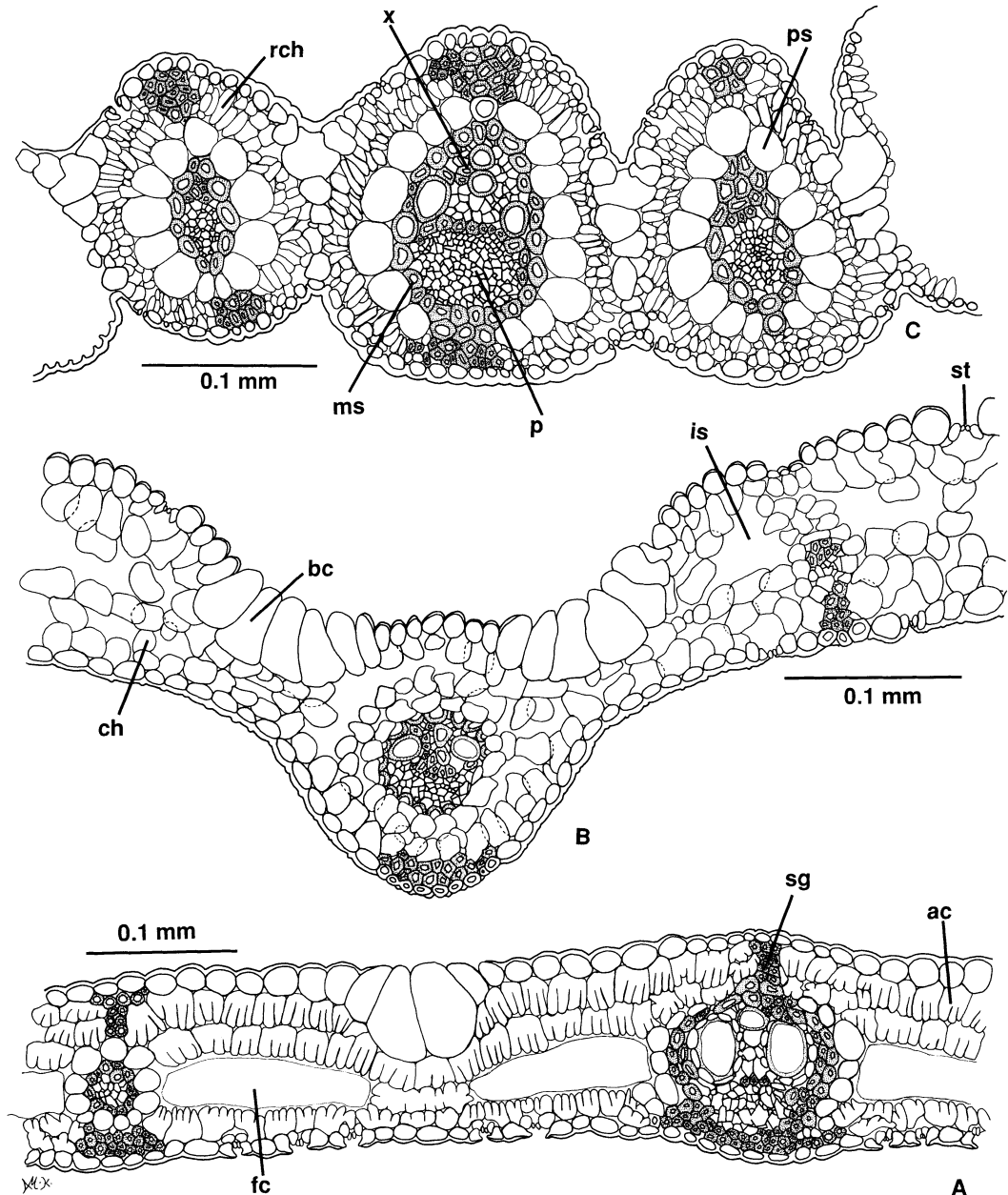
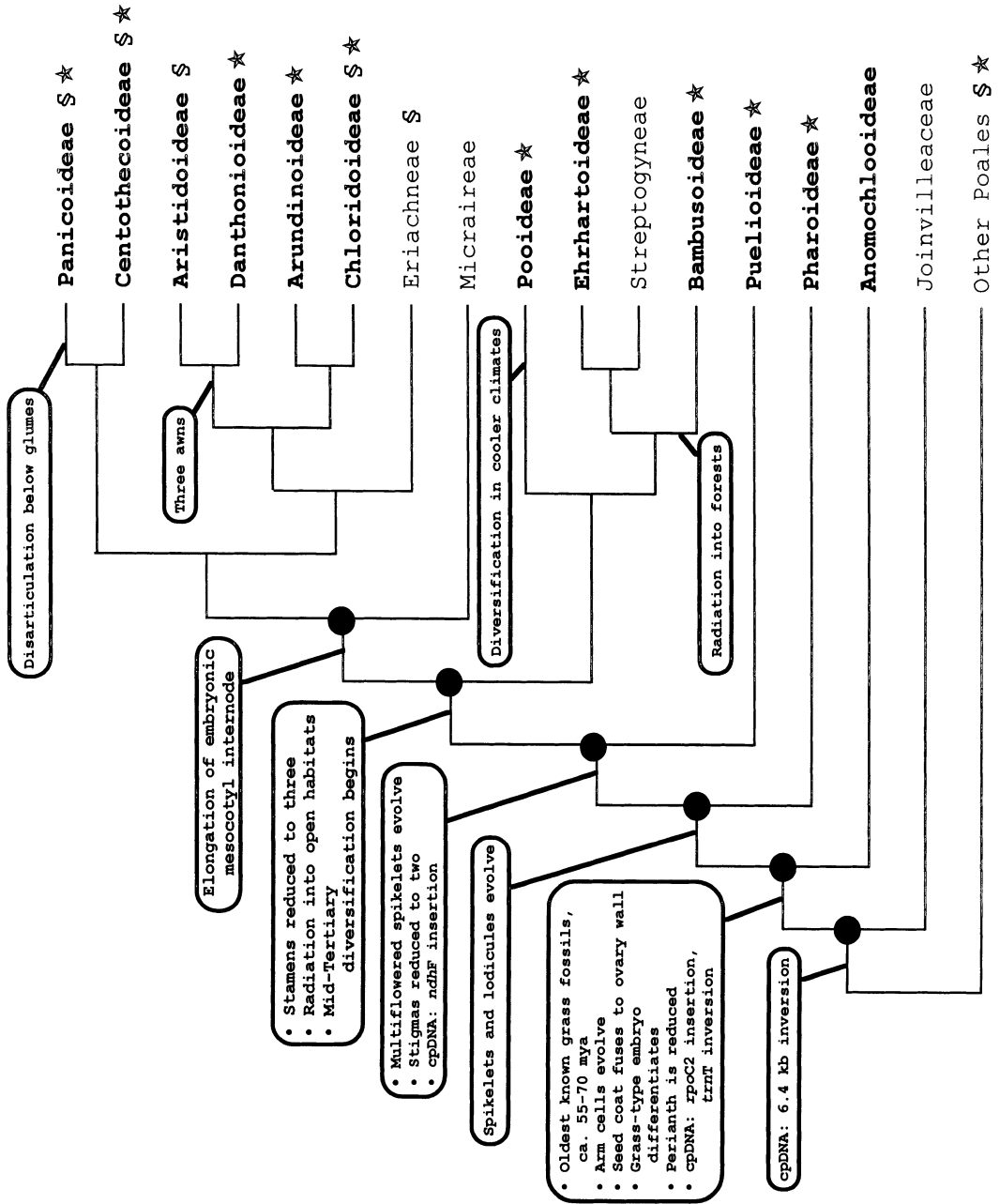


Figure 7. Leaf anatomy. —A. *Dinochloa maclelandii* (Soderstrom 2607). —B. *Poa* sp. (Carolina Biological Supply Co.). —C. *Bouteloua* sp. (Carolina Biological Supply Co.). ac—arm cell; bc—bulliform cell; ch—chlorenchyma; fc—fusoid cell; is—intercellular space; ms—mestome sheath; p—phloem; ps—outer parenchyma sheath; rch—radiate chlorenchyma; sg—sclerenchyma girder; st—stomatal apparatus; x—xylem.

known whether *Ectodiocoleaceae* possess this inversion (R. J. Soreng, pers. comm.).

*Monophyly of Poaceae.* Two characters in this analysis, the highly differentiated grass embryo (Fig. 6I and J) and its lateral position (char. 34) and the *trnT* inversion in the large single-copy re-

gion of the chloroplast genome (char. 52), unambiguously support the monophyly of Poaceae. Two other features unique to and characteristic of Poaceae but not included in this analysis are the caryopsis and the presence of intraaxinous channels in the pollen wall (Linder & Ferguson, 1985; Campbell & Kellogg, 1987; Kellogg & Linder, 1995).



The caryopsis is a single-seeded, usually dry, indehiscent fruit with the pericarp fused to the seed coat in the hilar region and otherwise closely adnate (Sendulsky et al., 1987). The caryopsis develops from a unilocular ovary containing a single ovule (Fig. 6H). Within Poaceae, the basic caryopsis has been modified to the fleshy (baccoid) or the achene-like (nuoid) caryopses of some woody bamboos (Sendulsky et al., 1987) or the follicoid or cistoid caryopses of some chloridoideae in which the seed is free from the pericarp or separates from it when moistened.

The highly differentiated grass embryo and its lateral position at the base of the caryopsis (char. 34; Fig. 6I and J) are synapomorphies for the family (Campbell & Kellogg, 1987; Kellogg & Linder, 1995). In the grasses, the embryo has leaves, vascular tissue, and clearly localized shoot and root meristems before the fruit is dispersed, and thus looks much more like a seedling than the embryos of non-grass relatives (Fig. 6I and J; Reeder, 1957; Sendulsky et al., 1987). Constant features of the grass embryo are the scutellum, coleoptile, and coleorrhiza. A scutellar cleft (Fig. 6I) may or may not separate the scutellum from the coleorrhiza, and the epiblast, an extra flap of tissue opposite the scutellum, may be present (Fig. 6J) or absent (Fig. 6I). Whether or not the embryonic leaf margins meet or overlap varies throughout the family (Fig. 6K and L).

Changes in the inflorescence occurred between the time that *Joinvillea* diverged and the time of divergence of Anomochlooideae. Relative to most of their poalean sister families (excluding Restionaceae, Ecteiocoleaceae, and Centrolepidaceae), the grasses, including Anomochlooideae, have well-developed bracts (what are normally called subtending bracts, prophylls, glumes, lemmas, and paleas) subtending and enclosing contracted inflorescence branches and flowers (Figs. 4 and 5). This appears to be a derived character marking the origin of the family, although this was not used explicitly as a character in this analysis. Under this interpretation, the characteristic grass spikelet (found in all grass-

es except Anomochlooideae) is then likely the result of a cumulative series of changes that occurred during the early history of the family. Clayton (1990), however, pointed out that there are well-developed bracts in the spikelets of Restionaceae, and what could be interpreted as subtending bracts are present, although not necessarily well developed, in Joinvilleaceae (Dahlgren et al., 1985). Clayton (1990) also noted that if the palea in grasses is interpreted as a prophyll, then there is no homolog for it among other Poales; however, prophylls are occasionally found at the base of spikelets in Restionaceae (H. P. Linder, pers. obs.).

The results of this analysis and prior studies (Clark et al., 1995; Soreng & Davis, 1998) support the following as plesiomorphic within the grass family: an herbaceous, perennial, rhizomatous habit; pseudopetiolate and relatively broad leaf blades bearing multicellular microhairs and anatomically with commissural veins, fusoid cells (Fig. 7A), and alternating long and short cells on the epidermis with at least some of the short cells including silica bodies; leaves with an adaxial ligule and open sheaths; a highly bracteate inflorescence; one-flowered spikelets or spikelet equivalents; six stamens in two whorls with tetrasporangiate, dithecal anthers; monoporate pollen with intraaxinous channels in the wall; a uniloculate, uniovulate gynoecium with three stigmas and one order of stigmatic branching; a basic caryopsis with a linear hilum; a highly differentiated, laterally positioned embryo with a scutellum, coleoptile, coleorrhiza, and a negligible mesocotyl internode; *Festuca*-type starch grains in the endosperm; and the  $C_3$  photosynthetic pathway. The grass spikelet and lodicules may have evolved before the divergence of the Anomochlooideae and the rest of the family, and were lost in the Anomochlooideae (cf. Soreng & Davis, 1998), but these features are not plesiomorphic under the present optimization. Bisexual flowers are probably also plesiomorphic, but unisexuality evolved early and in a number of different lineages within the family.

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Figure 8. Summary phylogeny of the grasses indicating significant morphological, ecological, and molecular (cpDNA = chloroplast DNA) events in the evolution of the family. Infrequent losses, parallel gains, and reversals are not shown for these characters. The 12 subfamilies recognized by the GPWG appear in boldface. Poales sensu APG include Cyperaceae. Marked taxa: (star) At least some included species have unisexual flowers/florets; (§) At least some included species have a  $C_4$  carbon fixation pathway, Kranz anatomy, or both. Dark circles indicate nodes strongly supported by all data combined (bootstrap > 99; Bremer support > 16). Subfamilies with common names: Aristidoideae (wiregrasses, etc.), Arundinoideae (reeds, etc.), Bambusoideae (bamboos), Chloridoideae (lovegrasses, tef, etc.), Danthonioideae (oat-grasses, pampas grass, etc.), Ehrhartoideae (rice, wild-rice, etc.), Panicoideae (maize, panic grasses, millets, sorghum, sugar cane, etc.), and Pooideae (barley, brome grasses, oats, rye, wheat, etc.).

*Anomochlooideae*. The basal divergence between Anomochlooideae and the rest of the Poaceae (the Spikelet Clade) is well supported based on molecular evidence. Monophyly of Anomochlooideae, however, is supported morphologically only by the unreversed presence of the adaxial ligule as a fringe of hairs, a character that appears elsewhere in the family. The pulvinus at the summit of the pseudopetiole is a possible synapomorphy for this clade but requires further study to determine similarities with the structure in other grasses. Molecular support for this clade may be due at least in part to long-branch attraction (see Unresolved questions).

Anomochlooideae have been recognized as a separate family (Nakai, 1943), a point of view that is completely consistent with the phylogeny. We have chosen here to retain Anomochlooideae within Poaceae because of the strong synapomorphies linking them (notably the caryopsis and the highly differentiated embryo, see Fig. 8). Retention of Anomochlooideae in Poaceae is also taxonomically conservative, in line with all previous studies of the family, and consistent with the efforts of the APG to limit monotypic or small families (Chase et al., 2000a, b).

Anomochlooideae are variable with respect to embryonic leaf margins and the epiblast. Embryonic leaf margins meet and the epiblast is present in *Anomochloa*, whereas the embryonic leaf margins overlap and the epiblast is absent in *Streptochaeta* (Judziewicz & Soderstrom, 1989). Both genera have an inconspicuous scutellar cleft. The coleoptile is usually represented as a more or less conical "cap" protecting the embryonic leaves and shoot apex, but the coleoptile margins are entirely free and overlapping in *Streptochaeta*, whereas in *Anomochloa* the margins at the base of the coleoptile are fused but free toward the apex, as is also seen in Pharoideae (Reeder, 1953; Judziewicz & Soderstrom, 1989).

The inflorescences of both *Anomochloa* and *Streptochaeta* are bracteate, but the lack of clear homology of these bracts with those of the standard grass spikelet has been noted. The spikelet equivalents in both *Anomochloa* and *Streptochaeta* are one-flowered and bisexual. The upper bract in *Anomochloa* exhibits a laminar anatomical structure, with the transversely elongated cell layer subjacent to the abaxial epidermis of the bract (Judziewicz & Soderstrom, 1989). This laminar structure is not found in bracts of *Streptochaeta*. (Similar but not identical laminar anatomy characterizes female lemmas in Pharoideae.) As Soreng and Davis (1998) pointed out, if the upper bract of the spike-

let equivalent of *Anomochloa* is indeed homologous to a standard grass lemma, a palea is lacking and the flower is terminal, whereas in a true grass floret the flower is borne on a lateral axis as indicated by the presence of the palea if it is interpreted as a prophyll. Some authors interpret the distal three bracts of the spikelet equivalent of *Streptochaeta* as lodicules and the next proximal two bracts as a bifid palea (e.g., Clayton, 1990), but there is no compelling evidence for this. The spikelet equivalent of *Streptochaeta* might represent a condensed branching system (Soderstrom, 1981), but it is not a pseudospikelet as found in the Bambuseae. In any case, lack of a palea in *Streptochaeta* also implies that the flower is terminal.

*The Spikelet Clade (Pharoideae + [Puelioideae + {BEP + PACCAD}])*. This clade, which includes all of the grasses except for Anomochlooideae, is defined by the unambiguous presence of true grass spikelets, florets (char. 8), and lodicules (char. 18). The plesiomorphic condition of the spikelet is clearly the presence of a pedicel (char. 10), two glumes, and a well-developed lemma and palea in the floret. The single-flowered spikelet may be synapomorphic for the family above the point of divergence of Anomochlooideae, with a transformation to multiflowered spikelets above Pharoideae and then numerous reversals, but the first true spikelets in grasses may have been multiflowered (see discussion under Spikelet). The plesiomorphic condition for lodicules is clearly three (char. 19), unfused (char. 20), and with a distally membranous portion (char. 21). Presence of unisexual flowers may be synapomorphic for this clade, with numerous reversals to bisexual florets, but it may be more likely that unisexuality arose multiple times. A base chromosome number of  $x = 12$  (char. 47) was established before the divergence of Pharoideae.

*Pharoideae*. Monophyly of this clade is strongly supported by the presence of resperate leaf blades, oblique lateral veins in the leaf blades, and uncinat hairs wholly or partially covering the female lemmas (Judziewicz, 1987). The female lemmas exhibit a laminar anatomical structure similar to that of Anomochlooideae, but the transversely elongated cell layer is subjacent to the adaxial epidermis. In Pharoideae, Anomochlooideae, and some Bambusoideae (Ghosal & Ram, 1985), the coleoptile margins are free for at least a portion of their length, but the distribution of this feature in the rest of the BEP + PACCAD Clade is not well documented. Pharoideae embryos have an epiblast and a small scutellar cleft, and embryonic leaf margins meet (Judziewicz, 1987). The Pharoideae uni-

formly exhibit one-flowered, unisexual, paired (char. 9) spikelets (Fig. 4B). When present in the male spikelets, the three lodicules are small, which may be plesiomorphic or may represent a reduction. Disarticulation is variable, in that the whole inflorescence may disarticulate as in *Scrotochloa*, or the whole inflorescence or branches usually disarticulate in *Pharus*, or female spikelets disarticulate above the glumes in *Leptaspis*, and perhaps also in the other genera (Soderstrom et al., 1987). These, along with the uncinat hairs, appear to be adaptations to epizoochorous dispersal. Multicellular microhairs (char. 43) are lost in Pharioideae.

*The Bistigmatic Clade (Puelioideae + [BEP + PACCAD]).* This clade is marked by three morphological synapomorphies: transformation from three to two stigmas (char. 29), transformation from one to two orders of stigmatic branching (char. 30), and presence of the 15 bp *ndhF* insertion (char. 53). Multiple florets per spikelet (char. 12; Fig. 5) may have arisen within Puelioideae as shown in Figure 3, or in the common ancestor of the Puelioideae + (BEP + PACCAD) clade. Regardless of which scenario is correct, reversals to one floret occurred numerous times in the BEP + PACCAD Clade. Disarticulation above the glumes (char. 16) clearly is established before the divergence of Puelioideae.

*Puelioideae.* The forest habitat and broad, pseudopetiolate leaf blades of the Anomochlooideae and Pharioideae are retained in this subfamily, but no unique morphological synapomorphies for Puelioideae have been identified. The culms apparently do not produce aerial branches, nor basal tillering as in Anomochlooideae and Pharioideae. The presence of proximal female-sterile florets in the spikelet (char. 11) is an unreversed synapomorphy for Puelioideae in this analysis, but is also a synapomorphy for Paniceae and autapomorphic for multiple other taxa on the tree. In Puelioideae, the pattern of sexuality in the spikelets is somewhat more complex than in many other subfamilies, but within a spikelet, at least some proximal florets are male. In *Guaduella*, the 1 to 3 proximal florets are male, and additional florets are bisexual with the distalmost one or few reduced, but in *Puelia* the proximal 3 to 6 florets are male or neuter, with the single apical floret unisexual and female. Multicellular microhairs (char. 43) are lost in *Puelia*, and a reversion to three stigmas (char. 29) occurs in some species of *Puelia*.

*The BEP + PACCAD Clade.* This clade includes the vast majority of grass species. Six mor-

phological synapomorphies support its monophyly: loss of the pseudopetiole (char. 7), reduction to two lodicules (char. 19), loss of the inner whorl of stamens (chars. 23 and 24), and loss of arm and fusoid cells (chars. 45 and 46). The pseudopetiole is regained in Bambusoideae, as well as in a few members of the PACCAD Clade. Arm and fusoid cells are also regained in Bambusoideae. The inner whorl of stamens is interpreted as having been regained three or four times within the bambusoid/ehrhartoid clade. Within the BEP + PACCAD Clade, the lamina on the first seedling leaf is lost only in Bambusoideae and Oryzae. Unisexual florets have evolved in most lineages of this clade, e.g., Olyreae (Bambusoideae), *Zizania* (Ehrhartioideae), *Lamarckia* (Pooideae), several genera of Chloridoideae and Centothecoideae, and very commonly in Panicoideae. Most lineages include taxa with one floret per spikelet and taxa with multiple florets per spikelet. The presence or absence of an epiblast is variable, as is the presence or absence of the scutellar cleft, although in the PACCAD Clade the scutellar cleft is generally present (Reeder, 1957).

The large number of reversals hypothesized in this part of the tree raises a number of intriguing questions regarding morphological evolution. We do not know anything about the development or underlying genetics of the characters, so we are forced into the agnostic assumptions that gains and losses are equally likely, and that pseudopetioles, arm cells, fusoid cells, epiblasts, and unisexual flowers are all developmentally and genetically the same wherever they occur. The changes that we interpret as reversals could actually represent retained primitive characters if loss of these characters is more likely than their regain. Equally possible, the changes interpreted as reversals could represent the origin of novel characters that look superficially similar to ancient ones. We have some evidence for the latter (see below) in that arm cells in the bambusoids are actually morphologically different from those in Anomochlooideae and Pharioideae (Zhang & Clark, 2000). Finally, the character optimizations reflect the hypothesis that the BEP Clade is monophyletic. If, as we outline below, the Pooideae are actually sister to the PACCAD Clade—a hypothesis that is neither favored nor excluded by the data—then the pattern of morphological evolution is different.

*The BEP Clade (= BOP clade of Clark et al., 1995).* This clade is supported by molecular sequence data, particularly from *ndhF*, *rpoC2*, and *phyB* (see Results), but other data sets support a

Pooideae + PACCAD clade (Soreng & Davis, 1998). In this analysis, constraining Pooideae + PACCAD as monophyletic was only slightly less parsimonious than BEP + PACCAD (see Results). In addition, no morphological synapomorphies supporting the BEP Clade have been identified. Loss of the lemma awn is optimized to this node, but awns are regained in many taxa within the BEP Clade. The lack of sequence data for *Streptogyna* contributes to the uncertainty of its position within the BEP Clade and may also affect assessment of the monophyly of the clade. *Streptogyna* appears as sister to Ehrhartoideae (Fig. 1), but in other analyses of these data it appears as sister to the rest of the BEP Clade.

**Bambusoideae.** Monophyly of the true bamboos (i.e., olyroid + woody bamboos) is supported by molecular data in this and other analyses (Clark et al., 1995; Zhang, 1996; Zhang & Clark, 2000). Morphologically, secondary gain of the pseudopetiole (char. 7) and secondary loss of the lamina of the first seedling leaf (char. 41) are synapomorphies. Although only presence or absence of arm cells was scored in this analysis, Zhang and Clark (2000) found that the presence of strongly asymmetrically invaginated arm cells (Fig. 7A) is a potential synapomorphy for this clade. Fusoid cells are characteristic of the Bambusoideae (Fig. 7A), but it is not known whether their presence represents retention of the plesiomorphic condition or reversal after loss of fusoid cells at the base of the BEP Clade. Bambuseae are here supported by the presence of perennating woody culms (char. 1), abaxial ligules (char. 5), and *Panicum*-type starch grains (char. 40). A secondary gain of the inner stamen whorl (chars. 23 and 24) occurred at least once but possibly several times. Olyreae have a synapomorphic base chromosome number of  $x = 11$  (char. 47), but the tribe is also characterized by unisexual spikelets.

**Ehrhartoideae.** This lineage is strongly supported by molecular data, and is characterized by the presence of one female-fertile floret per spikelet, often with one or two proximal female-sterile florets (char. 11). This character is coded as ambiguous in *Oryza* and *Leersia*, but if the vestigial structures at the base of the spikelets in these genera are interpreted as highly reduced glumes, then the presence of proximal female-sterile florets is an unambiguous synapomorphy. Two lodicules (char. 19) are found in this clade; in addition, the inner whorl of stamens (chars. 23 and 24) is regained, styles are not fused (char. 28), and fusoid cells (char. 46) are lost.

**Pooideae.** Monophyly of the pooid clade is strongly supported by molecular data including cpDNA restriction site data (Soreng et al., 1990; Davis & Soreng, 1993; Nadot et al., 1994; Soreng & Davis, 1998, 2000). Parallel-sided subsidiary cells, lack of microhairs, nonvascularized lodicules (Fig. 6D), and the presence of an epiblast and lack of a scutellar cleft in the embryo (Fig. 6J) are characteristic of a majority of the subfamily but do not constitute unequivocal synapomorphies. In this analysis, the loss of stylar fusion (char. 28) is an unreversed synapomorphy for the Pooideae. Loss of the scutellar tail (char. 36) is widespread in the clade, but polymorphisms prevent its unambiguous optimization. An unreversed transformation to faint or absent vascularization of the lodicules (char. 22) occurs within the Pooideae after the divergence of *Brachyelytrum*. A transformation to the embryonic leaf margins meeting (as opposed to overlapping; Fig. 6L) also occurs after the divergence of *Brachyelytrum* but is reversed in *Phaenosperma* (or *Phaenosperma* + *Anisopogon*). Multicellular microhairs (char. 43) are known only in *Lygeum* + *Nardus*; although this character is scored only for the abaxial leaf surface, it appears that Pooideae, at least above this divergence, are the only group of grasses to lose completely the ability to make multicellular microhairs anywhere on the plant (except possibly the lodicules). Chromosomal evolution in Pooideae is complex (see char. 47), but  $x = 12$  is apparently plesiomorphic in the BEP Clade, so numbers such as  $x = 10$  and  $x = 11$  in the earlier-diverging lineages of Pooideae may well be derived from this condition. The presence of  $x = 12$  in *Phaenosperma*, *Ampelodesmos*, and some Stipeae may be a retention;  $x = 7$  is clearly a synapomorphy of the core Pooideae (here represented by *Brachypodium*, *Avena*, *Bromus*, and *Triticum*). Two lodicules (char. 19) are found at the base of Pooideae, but a reversal to three occurs in Stipeae (in which another transformation, to two, occurs in *Nassella*); this is undoubtedly an oversimplification of the pattern in the Stipeae in which lodicule number varies considerably (Vickery et al., 1986). Loss of the distally membranous portion of the lodicule (char. 21) is a synapomorphy for Meliceae. The earliest-diverging lineages of the pooid clade have one floret per spikelet (char. 12) (although a rachilla extension is present in *Brachyelytrum*), multiple florets appear in Meliceae, single florets characterize the (*Phaenosperma* + *Anisopogon*) + Stipeae clade, multiple florets are found at the base of the core pooids, and many taxa within the core pooids have one floret per spikelet. Multiple independent origins of multiple florets per spikelet can be hypoth-

esized, but subsequent reduction to one floret per spikelet has clearly occurred in several groups. Patterns of divergence within this clade are complex and still are being evaluated, so some inferences regarding character evolution are likely to change.

*The PACCAD Clade.* Over half the species of the grass family are included in this clade. Even as early as the 1930s (Avdulov, 1931; Prat, 1932, 1936; Roshevits, 1937), taxa of this clade have been grouped together. Hilu and Wright (1982) were the first to retrieve this clade in a formal analysis, and subsequently support for the monophyly of the clade is found in all molecular analyses to date with sufficient sampling (Hilu & Esen, 1988; Hilu & Johnson, 1991; Davis & Soreng, 1993; Nardot et al., 1994; Barker et al., 1995; Clark et al., 1995; Duvall & Morton, 1996; Liang & Hilu, 1996; Mathews & Sharrock, 1996; Soreng & Davis, 1998; Hsiao et al., 1999; Mathews et al., 2000) except for Cummings et al. (1994), in which an oryzoid clade nested within the PACC clade. Davis and Soreng (1993) named this the PACC clade based on the four subfamilies that were then recognized as comprising the clade, but we here modify the name to reflect the recognition of two additional subfamilies, the Aristidoideae and the Danthoniodeae.

The PACCAD Clade is robustly supported based on molecular data and additionally is supported by the presence of an elongated mesocotyl internode (char. 37) and the loss of the epiblast (char. 35; Fig. 6I). The latter character reverses in the clade, so that secondary gain of the epiblast is an apparent synapomorphy for Centothecoideae. Two characters (chars. 21 and 50) are possible synapomorphies for the PACCAD Clade, but because of a lack of data or lack of a structure in *Micraira*, placement of these transitions is ambiguous. The lack of lodicules in *Micraira* prevents unambiguous placement of the loss of the distally membranous portion of the lodicule (char. 21), and *Micraira* remains unsampled for the presence or absence of the 3 bp deletion in phytochrome B (char. 50). Solid culm internodes (char. 2) are shown here as synapomorphic, although hollow ones reappear in other members of the clade. Non-linear hila (char. 33; Fig. 6M) are widespread in the PACCAD Clade, but the point of origin is ambiguous. The *Panicum*-type starch grain syndrome (char. 40) may be a synapomorphy for the PACCAD Clade, with a reversal to the *Festuca*-type in the clade containing *Eriachne*, Aristidoideae, Danthoniodeae, Arundinoideae, and Chloridoideae (the Ligule of Hairs Clade, as defined below), but other optimizations are possible. Two lodicules (char. 19) are estab-

lished for the BEP + PACCAD Clade, but no transformations to any other PACC number occur in the PACCAD Clade.

The positions of *Micraira* and *Eriachne* in the phylogeny are not well resolved, presumably due to a lack of sequence data for both genera (see Results and also Unresolved Questions). The two species of *Eriachne* are from quite distinct parts of the genus, based on the informal classification of Lazarides (1995). This undoubtedly affects the interpretation of character state transformations within the entire clade.

Early in the evolution of the PACCAD Clade, some lineages developed the capacity for  $C_4$  photosynthesis, apparently as an adaptation to high light/high temperature conditions and perhaps also to falling levels of atmospheric  $CO_2$  (Sage & Monson, 1999). Most members of the Panicoideae, all but two Chloridoideae, the Aristidoideae (except for *Sartidia*), and the Eriachneae are  $C_4$ . The poor resolution of the phylogeny at the base of the PACCAD Clade makes it impossible to determine precisely how many origins of  $C_4$  photosynthesis there were, but certainly there were at least two, and possibly more. The data are also consistent with a polymorphism at the base of the PACCAD Clade.

*The Panicoideae + Centothecoideae Clade.*

This clade was recovered in virtually all subanalyses, and had reasonable support (bts 85, brs 8) in the combined analysis. The presence of non-linear hila (char. 33; Fig. 6M) is a potential synapomorphy for this clade. Although support for the monophyly of Panicoideae (excluding *Gynerium* and *Danthoniopsis*) was strong (see Results), relationships of the centothecoid taxa, *Gynerium*, and *Danthoniopsis* to the Panicoideae and to each other remain unresolved, but the placement of *Gynerium* as sister to traditional Panicoideae is a novel result.

*Panicoideae.* The presence of proximal female-sterile florets (char. 11) and the transformation to the classical NADP-ME  $C_4$  subtype (char. 48) are unambiguous synapomorphies for *Danthoniopsis* + Panicoideae. Some reversions to the  $C_3$  pathway occur within the Paniceae among unsampled taxa, and at least one secondary transformation to the NAD-ME  $C_4$  subtype occurs in *Panicum*. This clade is also supported by the presence of one female-fertile floret (char. 12) as a reversal and the gain of a germination flap (char. 17), but the placement of this latter transformation is ambiguous. The loss of disarticulation above the glumes (char. 16) is a synapomorphy for Panicoideae excluding *Danthoniopsis*. The presence of paired spikelets (char. 9) is a synapomorphy of Andropogoneae in this



analysis, but paired spikelets do occur within Paniceae (e.g., *Brachiaria*, *Digitaria*, *Paspalum*).

*Centothecoideae.* Monophyly of this subfamily as currently constituted is not strongly supported in this analysis. The secondary gain of an epiblast (char. 35) is a possible synapomorphy (but is unknown for *Thysanolaena*), as is fusion of the styles (char. 28).

*The Ligule of Hairs Clade* (*Eriachne* + [*Aristidoideae* + *Danthonioideae*] + [*Arundinoideae* + *Chloridoideae*])). The adaxial ligule as a fringe of hairs (char. 4), awned lemmas (char. 13), and compound starch grains (char. 40) are synapomorphies of this clade, but characters 4 and 13 reverse multiple times, and character 40 once, within this clade, as well as elsewhere on the tree (Fig. 3). The recovery of this clade is a novel finding, but further investigation is warranted, given the lack of sequence data for *Eriachne*. The transformation to embryonic leaf margins meeting (char. 38; Fig. 6L) is an unreversed synapomorphy of the four subfamilies above *Eriachne*.

*The Aristidoideae + Danthonioideae Clade.*

Although each of these subfamilies is well supported as monophyletic, their sister relationship is another novel result, and one that is only moderately supported. Nonetheless, the presence of a basic pattern of three awns (char. 14; Fig. 5C and F) is an unreversed synapomorphy for this clade. Reappearance of the distal membranous portion of the lodicules (char. 21) also may be a synapomorphy, although this reverses within the *Danthonioideae*.

*Aristidoideae.* Gain of a germination flap (char. 17) and transformation to a base chromosome number of  $x = 11$  (char. 47) are unambiguous synapomorphies for the clade.

*Danthonioideae.* The presence of haustorial synergids (char. 32) is interpreted as an unreversed synapomorphy, but wider sampling within the clade is needed.

*Arundinoideae.* No unambiguous morphological support for the monophyly of this subfamily was found, although a reversal to hollow culms (char. 2) occurs in this clade.

*Chloridoideae.* Chloridoideae, including *Centropodia* and *Merxmuellera rangei*, are supported based on molecular data, although no clearcut morphological synapomorphies have been identified. Monophyly of *Centropodia* + *M. rangei* is well supported as is that of the traditional Chloridoideae (i.e., Chloridoideae s. str.), but support for the sister

relationship of these two clades is relatively modest. The gain of the NAD-ME C<sub>4</sub> subtype (char. 48) is a possible synapomorphy for the entire clade, however, and if so it would then revert to C<sub>3</sub> in *M. rangei*. The gain of chloridoid-type microhairs (char. 44) is a synapomorphy for the traditional Chloridoideae, although the character does occur elsewhere in the PACCAD Clade, and many genera of chloridoids also include species with panicoid-type microhairs (Jacobs, 1987; Van den Borre, 1994; Van den Borre & Watson, 1994). The pericarp is often free or loose, but this feature is not uniform and is also found in non-chloridoid grasses.

UNRESOLVED QUESTIONS

*Monophyly of Anomochlooideae.* *Anomochloa* and *Streptochaeta* are each distinctive genera but appear to have little in common. We have not yet found a uniquely derived morphological character that unites them as members of a single clade. Although the analyses presented here indicate that the two form a monophyletic group, analyses of single data sets sometimes show them to be paraphyletic or unresolved (Mathews et al., 2000; Hilu et al., 1999; Zhang, 2000). Because both genera occupy long branches in gene trees, they may form a clade only because of long-branch attraction (Felsenstein, 1978). Molecular studies of other species of *Streptochaeta* would help break up the long branch to *S. angustifolia* and might affect the monophyly of the clade. Resolution of *Anomochloa* and *Streptochaeta* as two separate basal lineages obviously would affect interpretations of character evolution within the family.

*Position of Streptogyna.* As noted in Results, the position of *Streptogyna* is ambiguous, apparently caused by lack of data. There are two species in the genus, one in the New World tropics and the other in Africa. Neither has been collected frequently, and we do not know of any plants in cultivation. Morphologically, the genus would fit comfortably within the Bambusoideae, but molecular data suggest that it is an early-diverging member of the BEP Clade or the Ehrhartoideae. The characters it shares with Bambusoideae are thus presumably ancestral, not indicative of relationship. Accurate placement of *Streptogyna* is necessary for interpretation of character evolution in the early-diverging members of Bambusoideae, Ehrhartoideae, and Pooideae.

*Early-diverging Pooideae.* The combined analysis confirms the position of *Brachyelytrum* as sister

to the rest of the Pooideae, and *Lygeum* + *Nardus* as the next-diverging lineage; both these results are well supported. The next diverging lineages include *Phaenosperryma*, *Anisopogon*, Stipeae, *Ampelodesmos*, Meliceae, and Diarrheneae, but the order of divergence is not resolved by any data collected to date. In the case of *Phaenosperryma*, *Anisopogon*, *Diarrhena*, and *Ampelodesmos*, the problem may be ascribed to insufficient sequence data in this analysis. For our sample of Stipeae and Meliceae, however, appreciable sequence data are available, yet the relative positions of the two lineages remain unclear. If the phylogenetic problem is indeed soluble with molecular data, the sample of genera and species in each tribe may have to be increased substantially. A combined analysis of cpDNA restriction sites and morphology (Soreng & Davis, 2000) represents the broadest taxon sample for Pooideae among studies to date. The order of divergence of these lineages affects interpretation of the evolution of such characters as parallel-sided subsidiary cells, loss of microhairs, and trends in reduction of chromosome number (Kellogg, 1998). The latter may correlate with a marked increase in genome size (Bennetzen & Kellogg, 1997) and may suggest possible mechanisms of genome evolution.

*The PACCAD Clade.* Relationships among the major lineages in the PACCAD Clade are not resolved by this or any other phylogenetic analysis to date. In the combined analysis, the branches at the base of the clade are short, marked by relatively few mutations each (11, 41, and 16 steps; Fig. 1). This suggests that the PACCAD radiation may have occurred relatively rapidly. If this is true, then relationships may remain difficult to resolve with certainty. The clade also contains a number of taxa of uncertain placement, many of which have received little or no attention in phylogenetic studies. The tribe Eriachneae, which includes the Australian genera *Eriachne* and *Pheidochloa*, is represented here only by an *rbcL* sequence of *Eriachne triodioides* and an ITS sequence of *E. trisetata*. The genus *Micraira*, the only member of the Australian tribe Micraireae, is represented only by an *ndhF* sequence of *M. lazaridis* and by an ITS sequence of *M. subulifolia*. Such genera as *Cyperochloa*, *Steyermarkochloa*, and the *Crinipes* group were not included in this combined analysis. An *rbcL* sequence of *Cyperochloa* places it with the centothecoids, whereas a sequence of the crinipoid genus *Styppeiochloa* places it sister to Arundineae s. str. (Barker, 1997; Linder et al., 1997).

The subfamilies recognized within the PACCAD Clade are, except for Centothecoideae, strongly

supported as monophyletic by our data. The list of genera included in and excluded from each subfamily, however, is based on a rather limited sample of species and genera, combined with inferences from classical morphological studies. In particular, the exact circumscriptions of Danthonioideae, Arundinoideae, and Centothecoideae are not precisely determined by this study. A comprehensive effort by multiple systematists is needed to improve understanding of the many poorly known species and genera.

*Centothecoideae.* Of the groups recognized here as subfamilies, Centothecoideae are the only one not strongly supported as monophyletic by the combined analysis. We have retained the subfamilial name and expanded the circumscription to include *Thysanolaena*, formerly a member of the Arundinoideae. As with the remainder of the PACCAD Clade, a clear picture of the limits of the centothecoid clade depends on much more data, particularly on the remaining centothecoid genera, but a study is under way (J. G. Sánchez-Ken, pers. comm.).

*Circumscription of tribes.* This paper does not address tribal circumscription. This will require far more extensive sampling, particularly in Pooideae, Panicoideae, Chloridoideae, and Bambusoideae, which constitute the four largest subfamilies. Choice of outgroups for such studies is now clear, however.

*Biogeography.* Present-day distributions do not indicate much about where the grasses originated. Restionaceae are clearly a Gondwanan group, with representatives in Africa and Australia. Joinvilleaceae, however, are insular, occurring on Borneo, New Caledonia, and Pacific Islands. The basal lineages of the grasses are found in the tropical regions of South America, Africa, and Asia; the Anomochlooideae are restricted to South and Central America (Judziewicz & Soderstrom, 1989), the Pharoideae are pantropical (Soderstrom et al., 1987), and the Puelioideae are restricted to tropical Africa (Soderstrom & Ellis, 1987; Clark et al., 2000). Due to the absence of an early fossil record, it is not clear how this distribution was established, whether by long-distance dispersal across the Atlantic and Indian Oceans, or whether across a continuous Gondwanan equatorial forest. Either way, the continent of origination cannot be determined with current data.

*Timing and causes of diversification.* The earliest unequivocal grass fossils are pollen grains from the Paleocene of South America and Africa,

deposited approximately 60 to 55 million years (my) ago (Jacobs et al., 1999), although some grains of *Monoporites* from the upper Maastrichtian (Cretaceous) may also represent remains of grasses (Linder, 1987). The earliest known grass macrofossil appears in an early Eocene formation (ca. 55 mya) in North America (Crepet & Feldman, 1991). Based on the fossil record therefore, the family is at least 55 my and possibly as much as 70 my old. Establishment of all major lineages had occurred by the mid-Miocene (Jacobs et al., 1999), which is about the time that grass-dominated ecosystems appeared.

Attempts to date particular nodes on the cladogram using molecular clocks are confounded by non-clocklike behavior of several of the genes (Gaut et al., 1996, 1997; Mathews et al., 2000; Kellogg & Russo, unpublished obs.). Using sequences of GBSSI, which has been shown to exhibit clocklike mutation, Gaut and Doebley (1997) placed the divergence of maize and *Pennisetum* at 25 mya, whereas Kellogg and Russo (unpublished) place the divergence of *Danthoniopsis dinteri* from the rest of the panicoids at ca. 16 mya. The two dates conflict with each other, but do suggest that the PACCAD Clade originated in the early Miocene or late Oligocene.

All  $C_4$  lineages are included in the PACCAD Clade, so paleontological evidence for  $C_4$  photosynthesis can establish a minimum age for the common ancestor of the clade. The earliest known  $C_4$  grass macrofossil is dated at 12.5 mya (Nambudiri et al., 1978), and the earliest isotopic evidence for  $C_4$  is ca. 15 mya (Kingston et al., 1994; Latorre et al., 1997). This suggests that the origin of the PACCAD Clade occurred no later than 15 mya and possibly as early as 25 mya.

Both fossil data and molecular clock estimates seem at odds with the apparent Gondwanan distribution of many grass taxa (see for example Simon & Jacobs, 1990). The Gondwanan distribution of such derived groups as the subfamily Danthonioidae might suggest that the PACCAD Clade originated sometime before the breakup of Gondwana, which would then place the origin of the family long before the earliest known fossils were deposited. This cannot be ruled out, of course, because it is an assumption based on negative evidence. If, however, we assume that groups within the PACCAD Clade originated before the breakup of Gondwana (a process hard to date precisely but perhaps 100–70 mya), then we would have to assume that the family originated more than 200–140 mya, before the time of the first appearance of angiosperms in the fossil record. It seems more likely, and more

consistent with available data, that the grasses achieved their Gondwanan distribution by dispersal (Soreng & Davis, 1998), as has been suggested for other taxa with an apparent Gondwanan distribution (e.g., *Adansonia*, Baum et al., 1998; Atherospermataceae, Renner et al., 2000).

The combination of fossil data and molecular clock evidence suggests that the major diversification of the grasses occurred between 15 and 25 mya, long after the origin of the family at 55 to 70 mya. This is consistent with the observed branch lengths on the phylogeny in Figure 1. There may have been many more representatives of the Anomochloideae, Pharoideae, and Puelioideae (or even additional lineages) that are now extinct, but grasses are generally rare in the fossil record until the Miocene (Jacobs et al., 1999). The simplest explanation is that the family diversified long after its origin. The novel characters that arose after the divergence of *Joinvillea*—the caryopsis, differentiated embryo, reduction in perianth—therefore did not lead immediately or directly to the current dominance of the family. Other characteristics acquired later in the evolution of the family may have been more important in its diversification and ecological success. Possibilities include such characters as formation of intercalary meristems or the acquisition of mechanisms for drought tolerance. We do not know the phylogenetic distribution of intercalary meristems, however, and it is possible that intercalary meristems of the leaves evolved after such meristems in the stems. This character needs to be investigated further. Acquisition of drought and heat tolerance would also be worth investigating, but would require a precise definition of what is meant by each term. The cellular components of such physiological responses are being identified and could perhaps be studied across a range of taxa.

#### CONCLUSIONS

We present here a resolved and strongly supported phylogeny of the grass family. It can be used to understand the diversification of morphology, genes, and genomes, to interpret comparative studies of cereal crops and forage grasses, and to develop hypotheses of adaptation to past and future environments. Some phylogenetic questions remain unresolved, and these affect inferences about such important characters as  $C_4$  photosynthesis. Nonetheless, this phylogeny is one of the most comprehensive and robust available for any family of plants, making the grasses an excellent clade for studies of evolutionary pattern and process.

## TAXONOMIC TREATMENT

Twelve subfamilies are recognized formally in this classification system (Table 1). A description is provided for each subfamily, and where appropriate, synonymy is indicated. To permit easy comparison with previous work, we have listed for each subfamily which of the tribes recognized by Clayton and Renvoize (1986) are to be included. In some cases (e.g., Pharoideae or Danthonioideae), the new circumscription of subfamilies makes tribal recognition largely unnecessary. For example, the subfamily Pharoideae includes three genera in a single tribe; the tribe is effectively redundant and serves no useful function in the subfamilial classification. Nonetheless we list the names for comparison.

Our sample of taxa was explicitly designed to explore relationships among major clades that can be recognized at the subfamilial level, but it is not dense enough to evaluate tribal limits. We have in many cases combined molecular data from several species to represent a genus (as is also commonly done for morphological analyses), and in a few cases have combined data from several genera that represent a putatively monophyletic group. Such combinations assume, rather than test, monophyly. We therefore refrain from formal discussion of tribal limits, which cannot be addressed by our data; these limits will have to be re-evaluated by future studies. Three tribes and two genera are placed as *Incertae Sedis* at the end of the classification, although the genera may be provisionally placed as noted below.

This classification reflects our attempt to use the phylogeny as the basis for recognizing subfamilies while remaining nomenclaturally conservative. Except for Centothecoideae, all subfamilies recognized are well supported as monophyletic in our analyses. While we could create an unranked classification for the grasses using our phylogeny, we feel that the practical interests of the potential users of this classification currently are best served by retaining the Linnaean hierarchy. Nonetheless we have applied informal names to several of the well-supported clades (see above).

The most significant changes in our proposed subfamily classification are the breakup of the traditional Bambusoideae and Arundinoideae and the expansion of Pooideae. The diversity encompassed by the traditional Bambusoideae (or Bambusoideae s.l.) is now recognized as Anomochlooideae, Pharoideae, Puelioideae, Bambusoideae s. str., and Ehrhartoideae. Elements of the traditional Arundinoideae are now recognized as Aristidoideae, Danthonioideae, and Arundinoideae s. str., with

Thysanolaeneae placed in the Centothecoideae and *Gynerium* as *Incertae Sedis*. *Centropodia* and *Merxmüllera rangei* are placed in Chloridoideae. Pooideae have grown by inclusion of Brachyelytreae, Lygeae, Nardeae, Phaenospematideae, Diarrheneae, Stipeae, and Ampelodesmeae, all formerly classified within either Bambusoideae or Arundinoideae by some authors; note, however, that Clayton and Renvoize (1986) placed Lygeae, Nardeae, and Stipeae in Pooideae in agreement with the classification proposed here. A detailed comparison of the GPWG classification with the major grass classification systems of the 20th century is presented in Table 1.

Primary sources for suprageneric names were the STAR Database (<http://matrix.nal.usda.gov:8080/star/supragenericname.html>), the Catalog of New World Grasses (<http://mobot.mobot.org/W3T/Search/nwgc.html>), and Clayton and Renvoize (1986). Diagnoses of the subfamilies were extracted from various sources including Clayton and Renvoize (1986) and Watson and Dallwitz (1992). Tribes in Chloridoideae and Panicoideae (except for the exclusion of Eriachneae) follow the treatment of Clayton and Renvoize (1986); tribes listed for the other subfamilies generally are treated according to more recent studies and/or consultation with specialists in those groups.

**Poaceae** (R. Br.) Barnh., Bull. Torrey Bot. Club 22: 7. 1895. (Nom. alt. Gramineae Juss., Gen. Pl.: 28. 1789.)

A monophyletic family, recognizable by the following synapomorphic morphological characters: Inflorescence highly bracteate. Perianth reduced or lacking. Pollen lacking scrobiculi, but with intraaxinous channels. Seed coat fused to inner ovary wall at maturity, forming a caryopsis. Embryo highly differentiated with obvious leaves, shoot and root meristems, and lateral in position.

**I. Anomochlooideae** Pilg. ex Potztl, in Willdenowia 1: 772. 1957. TYPE: *Anomochloa* Brongn. Figure 4C and D.

Syn.: Streptochaetoideae (Nakai) Butzin, Neue Unters. Blüte Gram.: 148. 1965.

Plants perennial, rhizomatous, herbaceous, of shaded tropical forest understories. Culms hollow or solid. Leaves with phyllotaxis either distichous or spiral; abaxial ligule absent; adaxial ligule a short fringe of cilia or absent, not membranous; blades usually relatively broad, venation parallel, with pseudopetioles short to very long, these with dark, turgid swellings (pulvini) at both ends (*An-*

*omochloa*) or only at the summit (*Streptochoeta*); sheaths non-auriculate. Inflorescences spicate, with complicated branching patterns, bracts outside of the spikelet equivalents present, large and with a blade or small and bladeless, or absent. Ultimate structures of the inflorescence (spikelet equivalents) of uncertain homology with typical grass spikelets but one-flowered and bisexual; bracts within the spikelet equivalents with phyllotaxis distichous or spiral, lacking uncinete macrohairs, sometimes awned but if so, the awns single; lodicules absent, or, in *Anomochloa*, their position occupied by a ring of short brownish cilia borne on a low membranous ring; stamens 4 or 6; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, style 1, stigma(s) 1 or 3. Caryopsis with the hilum linear, shallow and inconspicuous; endosperm hard, containing compound starch grains; embryo large, epiblast present or not, scutellar cleft present but shallow, mesocotyl internode absent, embryonic leaf margins overlapping or not. Basic chromosome numbers:  $x = 11$  or 18 (note: Clark & Judziewicz, 1996, erroneously cited these as 12 or 18).

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer absent, with fusoid cells very large and well developed, arm cells only weakly developed; Kranz anatomy absent; midrib complex; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with low dome-shaped and triangular subsidiary cells; bicellular microhairs very large (0.075–0.15 mm), the pointed apical cell usually one and a half times as long as the basally constricted basal cell; papillae absent.

*Photosynthetic pathway.* Presumed  $C_3$ .

INCLUDED TRIBES:

Anomochloae C. E. Hubb., in Hutchinson, Fam. Fl. Pl. 2: 219. 1934. TYPE: *Anomochloa* Brong.

Streptochoetaeae C. E. Hubb., in Hutchinson, Fam. Fl. Pl. 2: 205. 1934. TYPE: *Streptochoeta* Schrad. ex Nees.

*Notes.* There is no unique morphological synapomorphy for this subfamily, but both tribes lack lodicules and they apparently also lack grass-type spikelets. As noted above (Unresolved Questions), this lineage may not be monophyletic, in which case two subfamilies would need to be recognized. The subfamily includes 4 species.

**II. Pharoideae** (Stapf) L. G. Clark & Judz., Taxon 45: 643. 1996. TYPE: *Pharus* P. Browne. Figure 4B.

Syn.: Leptaspidoideae (Tzvelev) C. O. Morales, Sendtnera 5: 244. 1998. Nom. superfl.

Plants perennial, rhizomatous, monoecious, herbaceous, of shaded tropical to warm temperate forest understories. Culms hollow or solid. Leaves distichous; abaxial ligule absent; adaxial ligule a fringed membrane; blades resupinate, relatively broad, with pseudopetioles prominent and twisted, with lateral nerves diverging obliquely from midnerve and running straight to margins; sheaths non-auriculate. Inflorescences paniculate, the main axis and branches disarticulating or not, covered with uncinete macrohairs, bracts outside of the spikelets absent. Spikelets unisexual, one-flowered, mostly in male-female pairs on short branchlets, or some female spikelets solitary. Female spikelets large, short-stalked; glumes 2, shorter than the floret; lemma tubular or inflated, covered wholly or in part by uncinete macrohairs, awnless; palea well developed; lodicules absent; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, style 1, stigmas 3. Caryopsis with the hilum linear, extending the full length; endosperm hard, without lipid; embryo small, epiblast present, scutellar cleft present but shallow, mesocotyl internode absent, embryonic leaf margins overlapping. Male spikelets small, short- to long-stalked, membranous; glumes 2, shorter than the floret; lodicules 3 or 0, if present then minute, elliptic, glabrous, and nerveless; stamens 6. Basic chromosome number:  $x = 12$ .

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells large and well developed, arm cells weakly to moderately well developed; Kranz anatomy absent; midrib complex; inflated adaxial interstomatal cells present, bulliform cells poorly developed or absent.

*Foliar micromorphology.* Stomata with parallel-sided to dome-shaped subsidiary cells; bicellular microhairs and papillae absent.

*Photosynthetic pathway.* Presumed  $C_3$ .

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Phareae Stapf, in Thiselton-Dyer, Fl. Cap. 7: 319. 1898. TYPE: *Pharus* P. Browne.

*Notes.* In his original description of the tribe, Stapf specifically included *Olyra* (based on its uni-

sexual spikelets), but did not explicitly list *Pharus* or *Leptaspis*, although his choice of the name Phareae implicitly recognized the membership of *Pharus* in the tribe and automatically placed *Pharus* as its type, according to Article 10.6 of the *Code* (Greuter et al., 2000). As long as *Olyra* was retained in the same tribe as *Pharus*, Phareae was a superfluous name for the Olyreae. When *Pharus* and *Leptaspis* are segregated into their own tribe, and *Olyra* is excluded, then Phareae becomes the valid, correct name for the tribe. Clark and Judziewicz (1996) based the name of the subfamily on this tribal name. Tzvelev (1989) argued that the name Phareae was illegitimate because the type of the previously described tribe Olyreae was included in it, and provided the name Leptaspideae for this tribe. Morales (1998) agreed with Tzvelev and rejected the name Pharoideae for this subfamily, according to Article 52.1 of the *Code* (Greuter et al., 2000), replacing it with Leptaspidoideae. Under Article 52.3, however, "A name that was nomenclaturally superfluous when published is not illegitimate . . . if it is based on the stem of a legitimate generic name." We therefore accept the name Pharoideae for this subfamily, as *Pharus* is a legitimate generic name. The subfamily includes 12 species.

**III. Puelioideae** L. G. Clark, M. Kobay., S. Matthews, Spangler & E. A. Kellogg, Syst. Bot. 25: 181–187. 2000. TYPE: *Puelia* Franch. Figure 4A.

Plants perennial, rhizomatous, herbaceous, of shaded rainforest understories. Culms hollow. Leaves distichous; abaxial ligule absent (*Guaduelia*) or present (*Puelia*); adaxial ligule a fringed membrane; blades relatively broad, pseudopetiolate, venation parallel; sheaths non-auriculate. Inflorescences racemose or paniculate, bracts outside of the spikelets sometimes present. Spikelets with two glumes and several florets, the 1 to 3 proximal florets male, the next several florets female-fertile, with distal incomplete florets (*Guaduelia*), or the proximal 3 to 6 florets male or neuter with the single distal floret female (*Puelia*), disarticulating above the glumes and between the florets (*Guaduelia*) or not (*Puelia*); lemmas lacking uncinata macrohairs, awnless; palea well developed, sometimes tubular; lodicules 3, membranous, ciliate; stamens 6; ovary glabrous or hairy, an apical appendage present or not, haustorial synergids presumed absent, styles 2 or 3, the bases close, stigmas 2 or 3. Caryopsis with a long-linear hilum; embryo small. Basic chromosome number:  $x = 12$ .

*Foliar anatomy.* Mesophyll nonradiate, an ad-

axial palisade layer absent, fusoid cells well developed, arm cells only weakly developed; Kranz anatomy absent; midrib complex or less commonly simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with dome-shaped to triangular subsidiary cells; microhairs absent (*Puelia*) or multicellular, uniseriate microhairs present (*Guaduelia*); papillae present or more commonly absent.

*Photosynthetic pathway.* Presumed  $C_3$ .

INCLUDED TRIBES:

Guaduelleae Soderstr. & R. P. Ellis, in Soderstrom et al. (editors), Grass Syst. Evol.: 238. 1987. TYPE: *Guaduelia* Franch.

Puelieae Soderstr. & R. P. Ellis, in Soderstrom et al. (editors), Grass Syst. Evol.: 238. 1987. TYPE: *Puelia* Franch.

*Notes.* This subfamily, which comprises approximately 14 species, is poorly known, and morphological, anatomical, cytological, and ecological studies are needed.

**IV. Bambusoideae** Luerss., Grundz. Bot., ed. 5: 451. 1893. TYPE: *Bambusa* Schreb. Figures 4F, 6C, G, O, P, 7A.

Syn.: Olyroideae Pilger, Nat. Pfl.-Fam. ed. 2, 14d: 168. 1956.

Parianoideae (Nakai) Butzin, Neue Unters. Blüte Gram.: 148. 1965.

Plants perennial (rarely annual), rhizomatous, herbaceous or woody, of temperate and tropical forests, tropical high montane grasslands, riverbanks, and sometimes savannas. Culms hollow or solid. Leaves distichous; abaxial ligule absent (Olyreae) or present (Bambuseae); adaxial ligule membranous or chartaceous, fringed or unfringed; blades usually relatively broad, pseudopetiolate, venation parallel; sheaths often auriculate. Inflorescences spicate, racemose or paniculate, completing development of all spikelets in one period of growth and subtending bracts and prophylls usually absent, or pseudospikelets with basal bud-bearing bracts producing two or more orders of spikelets with different phases of maturity and subtending bracts and prophylls usually present. Spikelets (or spikelets proper of the pseudospikelets) bisexual (Bambuseae) or unisexual (Olyreae), consisting of 0, 1, 2 or several glumes, 1 to many florets; lemma lacking uncinata macrohairs, if awned, the awns single; palea well developed; lodicules usually 3 (rarely 0 to 6 or many), membranous, vascularized, often ciliate; stamens usually 2, 3, or 6 (10 to 40 in *Pariana*, 6

to 120 in *Ochlandra*); ovary glabrous or hairy, sometimes with an apical appendage, haustorial synergids absent, styles 2 or 3, sometimes very short but close, stigmas 2 or 3. Caryopsis with hilum linear (or rarely punctate), extending its full length (or rarely less than full length); endosperm hard, without lipid, containing compound starch grains; embryo small, epiblast present, scutellar cleft present, mesocotyl internode absent, embryonic leaf margins overlapping. Basic chromosome numbers:  $x = 7, 9, 10, 11$ , and 12.

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells large and well developed, arm cells usually well developed and strongly invaginated; Kranz anatomy absent; midrib complex or simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with dome-shaped, triangular, or parallel-sided subsidiary cells; bicellular microhairs present, panicoid-type; papillae common and abundant.

*Photosynthetic pathway.*  $C_3$ .

INCLUDED TRIBES:

Bambuseae Dumort., Anal. Fam. Pl.: 63. 1829.  
TYPE: *Bambusa* Schreb.

Olyreae Kunth ex Spenn., Fl. Friburg. 1: 172. 1825. TYPE: *Olyra* L. (Including *Buergersiochloae* Blake, *Blumea*, Suppl. 3: 62. 1946; *Parianeae* C. E. Hubbard, in Hutch., Fam. Fl. Pl. 2: 219. 1934.)

*Notes.* The current circumscription of this subfamily is much narrower than the traditional view. In their recent analysis, Zhang and Clark (2000) recovered two robustly supported clades, the olyroid bamboos and the woody bamboos, which they recognized as tribes Olyreae and Bambuseae, respectively. Following Zhang and Clark (2000), *Buergersiochloae* and *Parianeae* are included in Olyreae. This subfamily includes approximately 1200 species.

**V. Ehrhartoideae** Link, Hort. Berol. 1: 233. 1827. TYPE: *Ehrharta* Thunb. Figure 4G.

Syn.: Oryzoideae Kunth ex Beilschm., Flora 16(2): 52. 109. 1833.

Plants annual or perennial (rhizomatous or stoloniferous), herbaceous to suffrutescent, of forests, open hillsides, or aquatic habitats. Culms hollow or solid. Leaves distichous; abaxial ligule absent; adaxial ligule a fringed or unfringed membrane, or a fringe of hairs; blades rarely basally cordate or sag-

ittate (Phyllorachideae), somewhat broad to usually narrow, sometimes pseudopetiolate, venation parallel; sheaths sometimes bearing auricles. Inflorescences paniculate or racemose, bracts outside of the spikelets rarely present (*Humbertochloa*). Spikelets bisexual or unisexual, with glumes 2 (absent in some Oryzeae), sterile florets 0 to 2, and female-fertile floret 1, disarticulating above the glumes or infrequently primary branches disarticulating as units; lemma lacking uncinately macrohairs, if awned, the awn single; palea well developed; lodicules 2, membranous or rarely fleshy, heavily vascularized; stamens usually 3 or 6 (sometimes 1, 2, or 4); ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, fused basally or for their full length (*Zizaniopsis*), close, stigmas 2. Caryopsis with the hilum longitudinal; endosperm hard, without lipid, containing compound starch grains (rarely simple); embryo small, epiblast usually present (absent in *Ehrharta*), scutellar cleft usually present (absent in *Leersia* and *Potamophila*), mesocotyl internode absent (present but short in *Microlaena*), embryonic leaf usually with overlapping margins (meeting in *Potamophila*). Basic chromosome numbers:  $x = 12$  (10 in *Microlaena*; 15 in *Zizania*).

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer usually absent, fusoid cells absent or sometimes present (*Zizania* and *Zizaniopsis*), arm cells absent or present; Kranz anatomy absent; midrib simple or complex; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with dome-shaped or triangular subsidiary cells; bicellular microhairs present, panicoid-type; papillae often present in Oryzeae, otherwise absent.

*Photosynthetic pathway.*  $C_3$ .

INCLUDED TRIBES:

Ehrharteae Nevski, Trudy Bot. Inst. Akad. Nauk SSSR 4: 227. 1937. TYPE: *Ehrharta* Thunb.

Oryzeae Dumort., Observ. Gramin. Belg.: 83. 1824.  
TYPE: *Oryza* L.

Phyllorachideae C. E. Hubb., in Hook. Ic. Pl. 34: t. 3386, p. 5. 1939. TYPE: *Phyllorachis* Trimen.

*Notes.* Although we did not sample Phyllorachideae, we place it here based on morphological similarity. Nonetheless, any future studies of this clade should include this tribe to test its relationship to Ehrharteae and Oryzeae. Under the present circumscription, this subfamily includes approximately 120 species.

**VI. Pooideae** Benth., Fl. Hongk. 407. 1861.

TYPE: *Poa* L. Figures 4E, H, 6D, J, L, 7B.

Syn.: Avenoideae Link, Hort. Berol. 1: 108. 1827.

Festucoideae Link, Hort. Berol. 1: 137. 1827.

Glycerioideae Link, Hort. Berol. 1: 160. 1827.

Echinarioideae Link, Hort. Berol. 1: 197. 1827.

Cynosuroidae Link, Hort. Berol. 1: 198. 1827.

Anthoxanthoideae Link, Hort. Berol. 1: 232, 271. 1827.

Agrostidoideae Kunth ex Beilschm., Flora (Beib.) 16(2): 52, 104. 1833.

Stipoideae Burmeist., Handb. Naturgesch. 199. 1837.

Hordeoideae Burmeist., Handb. Naturgesch. 202. 1837.

Phalaroideae Burmeist., Handb. Naturgesch. 208. 1837.

Secaloideae Rouy, Fl. France 14: 2, 298. 1913.

Plants annual or perennial (rhizomatous, stoloniferous, or neither), herbaceous, of cool temperate and boreal regions, extending across the tropics in the high mountains. Culms hollow (rarely solid). Leaves distichous; abaxial ligule absent; adaxial ligule scarious or membranous, the margin not or infrequently short ciliate fringed (rarely long ciliate, *Anisopogon*); blades somewhat broad to usually narrow, rarely pseudopetiolate (*Phaenosperma*), venation parallel; sheaths sometimes auriculate. Inflorescences spicate, racemose, or paniculate, bracts outside of the spikelets absent or rarely present (e.g., *Sesleria*, *Echinaria*, *Ammochloa*). Spikelets bisexual, infrequently unisexual or mixed, usually with two glumes (rarely without glumes, *Lygeum*, or the first absent, *Hainardia*, *Lolium*, *Nardus*, except on terminal spikelets), 1 to many female-fertile florets with apical or infrequently basal reductions, compressed laterally, infrequently not or dorsally compressed, disarticulating above the glumes (infrequently below the glumes, some Poeae, or at the nodes of the inflorescence, various genera); lemma lacking uncinatate macrohairs, if awned, the awn single; palea usually present and well developed, but variable and sometimes very reduced or absent; lodicules 2 (rarely 3, *Anisopogon*, *Ampelodesmeae*, many *Stipeae* and few Poeae; fused, *Meliceae*; rarely absent, *Lygeum*, *Nardus*, and few Poeae), usually lanceolate, broadly membranous apically (fleshy, truncate, *Meliceae*), often lobed (*Triticeae*, *Poeae*), obscurely few-nerved, or infrequently  $\pm$  distinctly few-nerved, not or conspicuously ciliate on the margins; stamens usually 3 (infrequently 1 or 2); ovary glabrous or pubescent, rarely with an apical appendage (*Bromus*, *Diarrhena*) or rostellum (e.g., *Brachyelytrum*, *Rostraria*), haustorial synergids absent, styles usually 2, close, stigmas 2 (rarely 1, *Lygeum*, *Nardus*, and a few others, or 3, scattered genera). Caryopsis with the hilum linear and up to as long as the fruit, or subbasal and punctiform, linear, ellipsoidal, ovate,

or circular and less than  $\frac{1}{3}$  the length of the fruit; endosperm hard or sometimes soft or liquid (some Poeae), with or without lipids (some Poeae), containing compound starch grains, or simple starch grains (*Brachyelytreae*, *Bromeae*, *Triticeae*, some *Stipeae*); embryo small, epiblast present (rarely absent), scutellar cleft absent (rarely present, but not deeply incised), mesocotyl internode absent (rarely short, *Brachyelytrum*), embryonic leaf margins meeting (infrequently margins overlapping). Basic chromosome numbers:  $x = 7$  (*Bromeae*, *Triticeae*, *Poeae* generally, few *Brachypodieae*), 2, 4, 5, 6, 8, 9, 10, 11, 12, 13 represented in a few Poeae and the other tribes, generally medium or large.

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells absent, arm cells absent; Kranz anatomy absent; midrib simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with parallel-sided subsidiary cells; bicellular microhairs absent (rarely present, *Lygeum*, where chloridoid, *Nardus*, where panicoid), unicellular microhairs absent (rarely present, few *Stipeae*); papillae usually absent, when present rarely more than one per long cell.

*Photosynthetic pathway.* C<sub>3</sub>.

INCLUDED TRIBES:

*Ampelodesmeae* (Conert) Tutin, Bot. J. Linn. Soc.

76: 369. 1978. TYPE: *Ampelodesmos* Link.

*Brachyelytreae* Ohwi, Bot. Mag. Tokyo 55: 361.

1941. TYPE: *Brachyelytrum* P. Beauv.

*Brachypodieae* (Hack.) Hayek, Oesterr. Bot. Z.

74(10): 253. 1925. TYPE: *Brachypodium* P. Beauv.

*Bromeae* Dumort., Observ. Gramin. Belg.: 83.

1824. TYPE: *Bromus* L.

*Brylkinieae* Tateoka, Canad. J. Bot. 38: 962. 1960.

TYPE: *Brylkinia* F. Schmidt.

*Diarrheneae* (Ohwi) C. S. Campb., J. Arnold Arbor.

66: 188. 1985. TYPE: *Diarrhena* P. Beauv.

*Lygeae* J. Presl, Wsobecný Rostl. 2: 1708, 1753.

1846. TYPE: *Lygeum* Loeffl. ex L.

*Meliceae* Link ex Endl., Fl. Poson.: 116. 1830. [as

"Melicaceae"] TYPE: *Melica* L.

*Nardeae* W. D. J. Koch, Syn. Fl. Germ. Helv.: 830.

1837. TYPE: *Nardus* L.

*Phaenospermatideae* Renvoize & Clayton, Kew

Bull. 40: 478. 1985. TYPE: *Phaenosperma* Munro ex Benth.

*Poeae* R. Br., Voy. Terra Austral. 2: 582. 1814.

TYPE: *Poa* L. (Including *Aveneae* Dumort.,



Observ. Gramin. Belg.: 82. 1824; Agrostideae Dumort., Observ. Gramin. Belg.: 83. 1824.)  
Stipeae Dumort., Observ. Gramin. Belg.: 83. 1824 [as "Stipaceae"]. TYPE: *Stipa* L.  
Triticeae Dumort., Observ. Gramin. Belg.: 82, 84, 91. 1824. TYPE: *Triticum* L.

*Notes.* Relationships among some of the major lineages of the core Pooideae clade remain unresolved, and conflicts between molecular data and morphologically based tribal classifications exist (e.g., Poeae vs. Aveneae; see Soreng & Davis, 2000). This is one of several reasons that we do not offer a formal classification of tribes at this point. Relationships among the earlier diverging lineages of the whole pooid clade are only weakly supported, and also require further investigation. The tribal classification presented here is almost certain to change as additional data accumulate, and thus should be taken only as an indication of the taxa included within the subfamily. The subfamily includes approximately 3300 species.

**VII. Aristidoideae** Caro, Dominguezia 4: 16. 1982. TYPE: *Aristida* L. Figure 5C.

Plants annual or perennial, caespitose, herbaceous, xerophytic or less commonly mesophytic, of temperate, subtropical and tropical zones, often in open habitats. Culms solid or hollow. Leaves distichous; abaxial ligule absent or present as a line of hairs; adaxial ligule a fringed membrane or a fringe of hairs; blades relatively narrow, without pseudopetioles, venation parallel; sheaths non-auriculate. Inflorescences paniculate, bracts outside of the spikelets absent. Spikelets with bisexual florets, glumes 2, female-fertile floret 1, and no rachilla extension, cylindrical or laterally compressed, disarticulating above the glumes; lemma with three awns, the awns separate from each other, or fused below into a twisted column; palea short, less than half the lemma length; lodicules present or rarely absent, when present 2, free, membranous, glabrous, heavily vascularized; stamens 1 to 3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, close, stigmas 2. Caryopsis with the hilum short or long-linear; endosperm hard, without lipid, containing compound starch grains; embryo small (*Sartidia*) or large (*Aristida*, *Stipagrostis*), epiblast absent, scutellar cleft present or absent (*Sartidia*), mesocotyl internode elongated, embryonic leaf margins meeting. Basic chromosome numbers:  $x = 11, 12$ .

*Foliar anatomy.* Mesophyll radiate or nonradiate (*Sartidia*), an adaxial palisade layer absent,

fusoid cells absent, arm cells absent; Kranz anatomy absent (*Sartidia*) or present (*Stipagrostis*, *Aristida*), when present with one (*Stipagrostis*) or two (*Aristida*) parenchyma sheaths, although both not equally well developed throughout the genus; midrib simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata dome-shaped or triangular; bicellular microhairs present, panicoid-type; papillae absent.

*Photosynthetic pathway.* C<sub>3</sub> (*Sartidia*); C<sub>4</sub> (*Aristida*, NADP-ME; *Stipagrostis*, not biochemically typed, but anatomically NAD-ME; Hattersley & Watson, 1992).

INCLUDED TRIBE (NOW IDENTICAL TO THE SUBFAMILY AND THUS REDUNDANT):

Aristideae C. E. Hubbard, in Bor, Grasses Burma, Ceylon, India & Pakistan: 685. 1960. TYPE: *Aristida* L.

*Notes.* The presence of a basal column of the awn is a potential morphological synapomorphy for this clade. *Sartidia* diverges from *Stipagrostis* and *Aristida* in other respects, and should be sampled in future analyses. The subfamily includes approximately 350 species.

**VIII. Arundinoideae** Burmeist., Handb. Naturgesch.: 204. 1837. TYPE: *Arundo* L. Figure 5A.

Syn.: Phragmitoideae Parodi ex Caro, Dominguezia 4: 13. 1982.

Plants perennial (rarely annual), rhizomatous, stoloniferous, or caespitose, herbaceous to somewhat woody, of temperate and tropical areas, mesophytic or xerophytic, the reeds found in marshy habitats. Culms hollow or less commonly solid. Leaves distichous; abaxial ligule absent or rarely present as a line of hairs (*Hakonechloa*); adaxial ligule a fringed or unfringed membrane or a fringe of hairs; blades relatively broad to narrow, without pseudopetioles, venation parallel; sheaths usually non-auriculate. Inflorescences usually paniculate, rarely spicate or racemose, bracts outside of the spikelets absent. Spikelets with bisexual florets, glumes 2, a sterile lemma sometimes present, female-fertile florets 1 to several, apical reduction usually present, usually laterally compressed, disarticulating above the glumes; lemma lacking uncinat macrohairs, if awned, awn usually single, sometimes awns three, but then lacking a basal column; palea usually well developed; lodicules 2, free (rarely joined at the base), fleshy, glabrous or

infrequently ciliate, not or scarcely vascularized to heavily vascularized; stamens (1 to)3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, usually free, close, stigmas 2. Caryopsis with the hilum short or long-linear (*Molinia*); endosperm hard, without lipid, containing compound starch grains; embryo large or small (*Amphipogon*), epiblast absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting or overlapping (*Hakonechloa*). Basic chromosome numbers:  $x = 6, 9, 12$ .

*Foliar anatomy.* Mesophyll nonradiate or rarely radiate (*Arundo*, *Amphipogon*), without an adaxial palisade layer, without fusoid cells, arm cells absent or present (*Phragmites*); Kranz anatomy absent; midrib simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with low dome-shaped or triangular subsidiary cells; bicellular microhairs present or less commonly absent, when present of panicoid-type except in *Amphipogon*, which has unique microhair morphology; papillae absent except in *Amphipogon*.

*Photosynthetic pathway.* C<sub>3</sub>.

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Arundineae Dumort., Obs. Gram. Belg.: 82. 1824.  
TYPE: *Arundo* L.

*Notes.* The traditional Arundinoideae were well known as a dustbin group (e.g., Clayton & Renvoize, 1986; Kellogg & Campbell, 1987). A number of studies indicated that this subfamily as traditionally circumscribed was polyphyletic (e.g., Barker et al., 1995; Clark et al., 1995), although some support for a monophyletic Arundinoideae (including Arundinoideae s. str., Danthonioideae, Aristidoideae, *Micraira*, and *Eriachne*) was found by Hsiao et al. (1999). The results of the combined analysis presented here suggest that a monophyletic core arundinoid group does exist, even though individual data sets do not strongly support the group. The exact generic membership of the subfamily remains to be determined; however, we include the following genera: *Amphipogon*, *Arundo*, *Dregeochloa*, *Hakonechloa*, *Molinia* (and *Molinopsis* if recognized), and *Phragmites*. We provisionally place the crinipoid group (*Crinipes*, *Dichaetaria*, *Elytrophorus*, *Leptagrostis*, *Nematopoa*, *Piptophyllum*, *Stypeiuchloa*, and *Zenkeria*) here as well, based on molecular evidence from Linder et al. (1997) and Barker (1997). No morphological synapomorphies have been identified to support the

monophyletic arundinoid clade, although Linder et al. (1997) linked *Arundo*, *Phragmites*, and *Molinia* by the presence of hollow culm internodes, a punctiform hilum, and convex adaxial rib sides in the leaf blade. This subfamily clearly requires further study. The subfamily includes 33 to 38 species, counting the crinipoids.

**IX. Danthonioideae** Barker & H. P. Linder, subfam. nov. TYPE: *Danthonia* DC. Fl. Franc. 3: 32. 1805. Figure 5F.

Haec subfamilia ab aliis subfamiliis Poacearum synergidis haustorialibus, ligula ciliata, embryo mesocotyledone praedito, spicula pluriflora vel si uni- vel biflora nunc rachilla in extensionem desinente, stylorum basis plerumque distantibus atque anatomia "Kranz" et micropilis chloridoideis carentibus bene distincta.

Distinct from the other subfamilies of the grasses by the haustorial synergids, and by the conjunction of a ciliate ligule, the presence of an embryo mesocotyl, a several-flowered spikelet, which, if 1- or 2-flowered, has a rachilla extension, usually distinctly separated style bases, the absence of Kranz anatomy, and the absence of chloroid microhairs.

Plants perennial (caespitose, rhizomatous or stoloniferous) or less commonly annual, herbaceous or rarely suffrutescent, of mesic to xeric open habitats in grasslands, heathlands, and open woodlands. Culms solid or very rarely hollow. Leaves distichous; abaxial ligule usually absent (sometimes present in *Cortaderia*, *Karoochloa*, and *Pentstemon*); adaxial ligule a fringe of hairs or a fringed membrane; blades relatively narrow, without a pseudopetiole, venation parallel; sheaths not auriculate except in *Pentameris thuarii*. Inflorescences paniculate or less commonly racemose or spicate, bracts outside of the spikelets absent (but the subtending leaf  $\pm$  spatulate and disarticulating with the inflorescence in *Tribolium pusillum*). Spikelets bisexual (but sometimes without bisexual florets in *Cortaderia*) or unisexual (*Cortaderia*, *Lamprothyrus*), glumes 2 and usually equal, female-fertile florets 1 to 6 (to 20), with apical reduction and a rachilla extension usually present, laterally compressed, disarticulating above the glumes and between the florets, less commonly below the glumes; lemma lacking uncinata macrohairs, awn single and from a sinus; palea well developed, sometimes relatively short; lodicules 2, free (rarely joined), fleshy or rarely with an apical membranous flap, glabrous or ciliate, often with microhairs, sometimes heavily vascularized; stamens 3; ovary glabrous or rarely with apical hairs (*Pentameris*), apical appendage absent, haustorial synergids present, only weakly developed in a few taxa, styles 2, the bases usually widely separated, stigmas 2. Caryopsis with the hi-

lum short or long-linear; endosperm hard, containing compound starch grains (simple in *Prionanthium*); embryo large or small, epiblast absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting (overlapping in *Danthonia decumbens*). Basic chromosome numbers:  $x = 6, 7, 9$ .

*Foliar anatomy.* Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells absent, arm cells absent; Kranz anatomy absent; midrib simple, usually with one bundle, an arc of bundles in *Cortaderia*; adaxial bulliform cells present or absent.

*Foliar micromorphology.* Stomata with dome-shaped or parallel-sided subsidiary cells (rarely high dome-shaped or slightly triangular); bicellular microhairs present, panicoid-type, sometimes absent; papillae usually absent but often present in *Chionochloa* and *Merxmuellera*.

*Photosynthetic pathway.*  $C_3$ .

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Danthonieae Zotov, New Zealand J. Bot. 1 (1): 86. 1963. (Including Cortaderieae Zotov, New Zealand J. Bot. 1 (1): 83. 1963.) TYPE: *Danthonia* DC.

*Notes.* The presence of haustorial synergids in the ovule and distant styles support the monophyly of this clade (Verboom et al., 1994). Bilobed prophylls also may be a synapomorphy, but this feature has not been investigated sufficiently in the rest of the family. The results of this study indicate robust molecular support for the monophyly of this clade (excluding *Centropodia* and *Merxmuellera rangei*), but its placement within the larger PACCAD Clade is equivocal. Pending further studies of the diversity of the danthonioid grasses, we recognize only one tribe, which includes the following genera (sensu Barker et al., 2000): *Austroanthonia*, *Chaetobromus*, *Chionochloa*, *Cortaderia*, *Danthonia*, *Joycea*, *Karoochloa*, *Lamprothyrsus*, *Merxmuellera* (minus *M. rangei*), *Notochloe*, *Notodanthonia*, *Pentameris*, *Pentaschistis*, *Plinthanthesis*, *Prionanthium*, *Pseudopentameris*, *Rytidosperma*, *Schismus*, and *Tribolium*. The subfamily includes approximately 250 species.

**X. Centothecoideae** Soderstr. [as "Centostecoideae"], Taxon 30: 615. 1981. TYPE: *Centotheca* Desv. Figure 5G.

Plants annual or perennial (rhizomatous or stoloniferous), herbaceous or reedlike, of warm tem-

perate woodlands and tropical forests. Culms solid or hollow. Leaves distichous; abaxial ligule absent or present as a line of hairs (*Calderonella*, *Thysanolaena*); adaxial ligule membranous or ciliate, or membranous with ciliate margins; blades relatively broad to narrow, often pseudopetiolate, venation parallel; sheaths sometimes auriculate. Inflorescences racemose or paniculate, bracts outside of the spikelets absent. Spikelets bisexual or unisexual, (1 to)2- to many-flowered with reduction either above or below the fertile florets, often compressed laterally; lemma lacking uncinatate macrohairs, if awned, the awn single; palea usually well developed, sometimes relatively short; lodicules 2 or absent,  $\pm$  cuneate, many-nerved or less commonly not or scarcely vascularized; stamens (1 to)2 or 3; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, styles 2, free or fused, close, stigmas 2. Caryopsis with the hilum basal, punctiform; endosperm hard, without lipid, containing simple or compound starch grains; embryo small or large, the epiblast present, scutellar cleft present, mesocotyl internode present, embryonic leaf margins overlapping. Basic chromosome number:  $x = 12$  ( $x = 11$  or  $12?$  in *Thysanolaena*).

*Foliar anatomy.* Mesophyll nonradiate, often with an adaxial palisade layer, fusoid-like cells frequently present as extensions of the outer parenchyma bundle sheath, arm cells absent; Kranz anatomy absent; midrib simple; adaxial bulliform cells large.

*Foliar micromorphology.* Stomata with dome-shaped and/or triangular subsidiary cells; bicellular microhairs present, panicoid-type; papillae absent.

*Photosynthetic pathway.*  $C_3$ .

INCLUDED TRIBES:

Centothecoace Ridl., Mat. Fl. Malay Pen. 3: 122. 1907. TYPE: *Centotheca* P. Beauv.  
Thysanolaeneae C. E. Hubb., in Hutch., Fam. Fl. Pl. 2: 222. 1934. TYPE: *Thysanolaena* Nees.

*Notes.* Support for the monophyly of this subfamily as recognized here is moderate, and no morphological synapomorphies have been identified. The sister relationship between the centothecoid and panicoid clades, however, is relatively robust. The positions of *Gynerium* and *Danthoniopsis* are unstable. A majority of the Centothecoace are characterized by unusual leaf anatomy, including the presence of palisade mesophyll and laterally extended bundle sheath cells. Additional study of this clade is under way (J. G. Sánchez-Ken, pers.

comm.). The subfamily includes approximately 45 species.

**XI. Panicoideae** Link, Hort. Berol. 1: 202. 1827.

TYPE: *Panicum* L. Figures 5B, E, 6F, I, K.  
Syn.: Andropogonoideae Burmeist., Handb. Naturgesch.: 201. 1837.

Rottboellioideae Burmeist., Handb. Naturgesch.: 202. 1837.

Saccharoideae (Rchb.) Horan., Char. Ess. Fam.: 34. 1847.

Plants annual or perennial (rhizomatous, stoloniferous, caespitose or decumbent), primarily herbaceous, of the tropics and subtropics, but also diverse in the temperate zone. Culms solid or less commonly hollow. Leaves distichous; abaxial ligule usually absent, occasionally present as a line of hairs; adaxial ligule a fringed or unfringed membrane, or a fringe of hairs, or sometimes absent; blades relatively broad to narrow, sometimes pseudopetiolate, venation parallel; sheaths usually nonauriculate. Inflorescences panicles, racemes, or spikes, or complex combinations of these, bracts outside of the spikelets present (Andropogoneae) or absent (Paniceae). Spikelets bisexual or unisexual (if the latter plants dioecious or monoecious), frequently paired in combinations with long and short pedicels, usually with glumes 2, sterile lemma 1, and female-fertile floret 1, dorsally compressed or less commonly not compressed or laterally compressed, disarticulating below the glumes (above the glumes in Arundinelleae) or the inflorescence axes breaking apart; lemma lacking uncinata macrohairs, if awned, the awn single; palea well developed (Paniceae) or reduced to absent (Andropogoneae); lodicules 2 or sometimes absent, cuneate, free, fleshy, usually glabrous; stamens 3; ovary usually glabrous, apical appendage absent, haustorial synergids absent, styles 2, free or fused, close, stigmas 2 (rarely 1 or 3). Caryopsis with the hilum usually short; endosperm hard, without lipid, containing simple or less commonly compound starch grains; embryo usually large, epiblast absent or rarely present, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins overlapping or rarely meeting. Basic chromosome numbers:  $x = 5, (7), 9, 10, (12), (14)$ .

*Foliar anatomy.* Mesophyll radiate or nonradiate, an adaxial palisade layer absent, fusoid cells absent except in *Homolepis* and *Streptostachys*, arm cells usually absent; Kranz anatomy present or absent; midrib simple or rarely complex; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with triangu-

lar or dome-shaped subsidiary cells; bicellular microhairs present, panicoid-type, rarely absent; papillae absent or present (mostly in the Andropogoneae).

*Photosynthetic pathway.* C<sub>3</sub>, C<sub>4</sub> (PCK, NAD-ME and NADP-ME), and some C<sub>3</sub>/C<sub>4</sub> intermediates.

INCLUDED TRIBES:

Andropogoneae Dumort. [as "Andropogineae"], Observ. Gramin. Belg.: 84. 1824. TYPE: *Andropogon* L.

Arundinelleae Stapf, Fl. Cap. 7: 314. 1898. TYPE: *Arundinella* Raddi.

Hubbardieae C. E. Hubb., in Bor, Grasses India Burma Ceylon Pakistan: 685. 1960. TYPE: *Hubbardia* Bor.

Isachneae Benth., J. Linn. Soc. Bot. 19: 30. 1881. TYPE: *Isachne* R. Br.

Paniceae R. Br., Voy. Terra Austr. 2: 582. 1814. TYPE: *Panicum* L.

Steyermarkochloae Davidse & R. P. Ellis, Ann. Missouri Bot. Gard. 71: 994. 1984. TYPE: *Steyermarkochloa* Davidse & R. P. Ellis.

*Notes.* While support for the panicoid/centothecoid clade is high, relationships within the clade remain unclear. No robust phylogeny for the Panicoideae is yet available, although work is in progress (Giussani et al., in press; Duvall et al., in press). Preliminary results indicate that the Paniceae as currently circumscribed may not be monophyletic, and that the large genus *Panicum* is polyphyletic (Zuloaga et al., 2000; Gómez-Martínez & Culham, 2000). Andropogoneae + *Arundinella* appear to be monophyletic (Spangler et al., 1999); other genera of the Arundinelleae are likely to be distributed among the Andropogoneae, Paniceae, and perhaps even the Centothecoideae (Kellogg, 2000b). This subfamily includes approximately 3270 species.

**XII. Chloridoideae** Kunth ex Beilschm., Flora 16(2): 52, 105. 1833. TYPE: *Chloris* Sw. Figures 5D, H, 6E, M, N, 7C.

Syn.: Pappophoroideae Burmeist., Handb. Naturgesch. 205. 1837.

Eragrostoideae Pilger, Nat. Pfl.-Fam. ed. 2, 14d: 167. 1956.

Plants annual or perennial (rhizomatous, stoloniferous, caespitose or decumbent), herbaceous (rarely woody), of dry climates, especially in the tropics and subtropics, also found in the temperate zone. Culms solid or hollow. Leaves distichous; abaxial ligule usually absent, rarely present as a line of hairs; adaxial ligule a fringed or less commonly

unfringed membrane; blades relatively narrow, without pseudopetioles, venation parallel; sheaths usually non-auriculate. Inflorescences paniculate, paniculate with spicate branches, racemose, or spicate, bracts outside of the spikelets absent. Spikelets bisexual or sometimes unisexual (if so the plants dioecious or monoecious), with glumes 2, rarely a sterile lemma, and female-fertile florets 1 to many, apical reduction usually present, usually laterally compressed, sometimes dorsally compressed, usually disarticulating above the glumes (below in a few *Eragrostis* species); lemma lacking uncinata macrohairs, if awned, the awns single or if multiple, lacking a basal column; palea well developed; lodicules 2 or absent, fleshy, glabrous; stamens 1 to 3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, close, stigmas 2. Caryopsis with the pericarp often free or loose; hilum short; endosperm hard, without lipid, containing simple or compound starch grains; embryo large or rarely small, epiblast present or rarely absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting or rarely overlapping. Basic chromosome numbers:  $x = (7), (8), 9, 10$ .

*Foliar anatomy.* Mesophyll usually radiate, without an adaxial palisade layer, fusoid cells absent, arm cells absent; Kranz anatomy present; midrib simple; adaxial bulliform cells present.

*Foliar micromorphology.* Stomata with dome-shaped or triangular subsidiary cells; bicellular microhairs present, usually chloridoid-type; papillae absent or present.

*Photosynthetic pathway.*  $C_3$  (*Eragrostis walteri*, *Merxmuellera rangei*), otherwise  $C_4$  (PCK, NAD-ME, but reported as NADP-ME in *Pappophorum*, by Hattersley & Watson, 1992; the latter may be an error).

#### INCLUDED TRIBES:

- Cynodonteae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Cynodon* Rich.  
 Eragrostideae Stapf, Fl. Cap. 7: 316. 1898. TYPE: *Eragrostis* Wolf.  
 Leptureae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Lepturus* R. Br.  
 Orcuttiae Reeder, Madroño 18: 20. 1965. TYPE: *Orcuttia* Vasey.  
 Pappophoreae Kunth, Rév. Gramin. 1: 82. 1829. TYPE: *Pappophorum* Schreb.

#### INCERTAE SEDIS:

*Centropodia* Reichenb., *Merxmuellera rangei* (Pilg.) Conert

*Notes.* Reduction in the number of veins in the lemma is a general trend within the subfamily but is clearly not a synapomorphy. Except for the  $C_3$  *Eragrostis walteri* and *Merxmuellera rangei*, the Chloridoideae are uniformly  $C_4$  with both the NAD-ME and PCK subtypes. The current tribal classification for this subfamily conflicts with molecular data and is likely to be modified (Hilu et al., 1999). This subfamily includes approximately 1400 species.

#### XIII. Incertae Sedis

- Eriachneae (Ohwi) Eck-Borsb., Blumea 26: 128. 1980.  
 Micraireae Pilger, Nat. Pfl.-Fam. Ed. 2, 14d: 167. 1956.  
 Streptogyneae C. Calderón & Soderstr., Smithsonian Contr. Bot. 44: 18. 1980.  
*Cyperochloa* Lazarides & L. Watson, Brunonia 9: 216. 1987.  
*Gynerium* Willd. ex P. Beauv., Ess. Agrostogr. 138, 153, t. 24. 1812.

*Notes.* These five taxa are left Incertae Sedis because the data presented here do not firmly support their inclusion in any of the 12 subfamilies. This approach has also been taken by the APG (1998) for taxa of uncertain placement. Some possible placements of the five taxa above will require publication of new names, and we feel strongly that nomenclatural changes should not be made until appreciable data support the conclusion. That said, recent unpublished data (J. G. Sánchez-Ken, pers. comm.) suggest that *Gynerium* can be placed as its own tribe in Panicoideae, and the tribal name may be available by the time this paper is published (Sánchez-Ken & Clark, 2001). It is likely that *Cyperochloa* will be placed in Centothecoideae, but this is based on its morphological similarities to *Spartochloa* and not on any data on *Cyperochloa* itself. Streptogyneae will probably fall within Ehrhartoideae, but limitations of our data and lack of support in our trees make us cautious about placing it there unequivocally; there may be an argument for recognition of the tribe as its own subfamily. The name Micrairoideae has been published (Pilger, 1956). Our data are too limited and the placement of the group too uncertain to add it as a thirteenth subfamily, although flora writers may choose to do so. Our data on *Eriachne* are weak, and show only that the genus does not fall within the Pani-

coideae, where it has been placed traditionally. Its placement near the base of the PACCAD Clade is based on a single-stranded *rbcL* sequence from one species, and an ITS sequence from a second. The two species represent two sections of the genus, one of which has actually been recognized as its own genus. We therefore feel that *Incertae Sedis* best reflects what we know of the position of the tribe—its position is uncertain.

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Appendix I. Taxa included. For each data set, species name, voucher, and reference are listed, as well as GenBank accession numbers for gene sequences. EAK = Elizabeth Kellogg; HPL = Peter Linder; JID = Jerrold Davis; LGC = Lynn Clark; NPB = Nigel Barker; PMP = Paul Peterson; RJS = Robert Soreng; SJ = Surrey Jacobs; WZ = Weiping Zhang; XL = Ximena Londoño; BBG = Berlin Botanic Garden; BHC = L. H. Bailey Hortorium Conservatory; FTG = Fairchild Tropical Garden; NTBG = National Tropical Botanical Garden (Hawaii); PI = USDA Plant Introduction Station (Pullman, Washington) as source of seed.

Genus	Species	Voucher	Reference	GenBank #
<i>ndhF</i>				
<i>Flagellaria</i>	<i>indica</i> L.	LGC & WZ 1305 (ISC)	Clark et al. (1995)	U22007
<i>Elegia</i>	<i>stipularis</i> Mast.	<i>Eldenas</i> 2 (BOL)	This paper	AF251443
<i>Baloskion</i>	<i>tetraphyllum</i> (Labill.) B. G. Briggs & L. A. S. Johnson	Kew-6565-1977 (BH)	This paper	AF251444
<i>Joinvillea</i>	<i>ascendens</i> Gaudich. ex Brongn. & Gris.	NTBG-800379 (living)	Clark et al. (1995)	U21973
<i>Anomochloa</i>	<i>marantioidea</i> Brongn.	LGC 1299 (ISC)	Clark et al. (1995)	U21991
<i>Streptochaeta</i>	<i>angustifolia</i> Soderstr.	LGC 1304 (ISC)	Clark et al. (1995)	U21982
<i>Pharus</i>	<i>latifolius</i> L.	LGC 1302 (ISC)	Clark et al. (1995)	U21992
<i>Guaduelia</i>	<i>marantifolia</i> Franch.	<i>Kobayashi et al.</i> 1539 (ISC)	Clark et al. (2000)	AF164777
<i>Puelia</i>	<i>ciliata</i> Franch.	<i>Kobayashi et al.</i> 1541 (ISC)	Clark et al. (2000)	AF164779
<i>Eremitis</i>	sp. nov.	LGC & WZ 1343 (ISC)	Zhang & Clark (2000)	AF182353
<i>Pariana</i>	<i>radiciflora</i> Sagot ex Döll	LGC & WZ 1344 (ISC)	Zhang & Clark (2000)	AF182354
<i>Lithachne</i>	<i>humilis</i> Soderstr.	LGC 1298 (ISC)	Clark et al. (1995)	U21977
<i>Olyra</i>	<i>latifolia</i> L.	XL & LGC 911 (ISC)	Clark et al. 1995	U21971
<i>Buergersiochloa</i>	<i>bambusoides</i> Pilg.	<i>Dransfield</i> 1382 (K)	Zhang & Clark (2000)	AF182341
<i>Arundinaria</i>	<i>gigantea</i> (Walter) Muhl.	WZ 8400703 (ISC)	Clark et al. (1995)	U21846
<i>Chusquea</i>	<i>latifolia</i> L. G. Clark	LGC & XL 417 (ISC)	Clark et al. (1995)	U21989
<i>Streptogyna</i>	<i>americana</i> C. E. Hubb.	<i>Pohl &amp; Davidse</i> 12310 (ISC)	Clark et al. (1995)	U21965
<i>Ehrharta</i>	<i>calycina</i> Sm.	NPB <i>s.n.</i> (BOL)	Clark et al. (1995)	U21995
<i>Oryza</i>	<i>sativa</i> L.	Sugiura (1989)	Clark et al. (1995)	X15901
<i>Leersia</i>	<i>virginica</i> Willd.	LGC 1316 (ISC)	Clark et al. (1995)	U21974
<i>Phaenosperma</i>	<i>globosum</i> Munro ex Benth.	LGC 1292 (ISC)	Clark et al. (1995)	U22005
<i>Brachyelytrum</i>	<i>erectum</i> (Schreb.) P. Beauv.	LGC 1330 (ISC)	Clark et al. (1995)	U22004
<i>Lygeum</i>	<i>spartum</i> L.	RJS 3698 (BH)	This paper	AF251445
<i>Nardus</i>	<i>stricta</i> L.	BBG: <i>Royl &amp; Schiers s.n.</i> 1988 (B)	This paper	AF251446
<i>Anisopogon</i>	<i>avenaceus</i> R. Br.	HPL 5590 (BOL)	This paper	AF251447
<i>Ampelodesmos</i>	<i>mauritanica</i> (Poir.) T. Durand & Schinz	BBG: <i>Royl &amp; Schiers s.n.</i> 1988 (B)	This paper	AF251448
<i>Stipa</i>	<i>barbata</i> Desf.	PI-229468 (BH)	This paper	AF251449
<i>Nassella</i>	<i>viridula</i> (Trin.) Barkworth	PI-387938 (BH)	This paper	AF251450
<i>Oryzopsis</i> (=Piptatherum)	<i>racemosa</i> (Sm.) Ricker ex Hitchc.	LGC & WZ 1288 (ISC)	Clark et al. (1995)	U21924
<i>Brachypodium</i>	<i>distachyon</i> (L.) P. Beauv.	PI-422452 (BH)	This paper	AF251451
<i>Melica</i>	<i>altissima</i> L.	PI-325418 (BH)	This paper	AF251452
<i>Glyceria</i>	<i>striata</i> (Lam.) Hitchc.	JID & RJS <i>s.n.</i> (BH)	This paper	AF251453
<i>Diarrhena</i>	<i>obovata</i> (Gleason) Brandenburg	LGC & WZ 1216 (ISC)	Clark et al. (1995)	U21998
<i>Avena</i>	<i>sativa</i> L.	material from R. Wise (ISU)	Clark et al. (1995)	U22000
<i>Bromus</i>	<i>inermis</i> Leyss.	PI-314071 (BH)	This paper	AF251454

## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Hordeum</i>	<i>vulgare</i> L.	material from R. Wise (ISU)	Clark et al. (1995)	U22003
<i>Aristida</i>	<i>purpurea</i> Nutt. var. <i>longi- seta</i> (Steud.) Vasey ex Rothr.	<i>Gabel 2700</i> (ISC)	Clark et al. (1995)	U21966
<i>Stipagrostis</i>	<i>zeyheri</i> (Nees) DeWinter	<i>NPB 1133</i> (BOL)	This paper	AF251455
<i>Amphipogon</i>	<i>strictus</i> R. Br.	<i>HPL 5634</i> (BOL)	This paper	AF251456
<i>Arundo</i>	<i>donax</i> L.	<i>LGC s.n.</i> (ISC)	Clark et al. (1995)	U21997
<i>Molinia</i>	<i>caerulea</i> (L.) Moench	<i>LGC 1165</i> (ISC)	Clark et al. (1995)	U21994
<i>Phragmites</i>	<i>australis</i> (Cav.) Trin. ex Steud.	<i>LGC 1294</i> (ISC)	Clark et al. (1995)	U21996
<i>Merxmuellera</i>	<i>macowanii</i> (Stapf) Conert	<i>NPB 1008</i> (BOL)	This paper	AF251457
<i>Karroochloa</i>	<i>purpurea</i> (L.f.) Conert & Türpe	<i>HPL 5360</i> (BOL)	This paper	AF251458
<i>Danthonia</i>	<i>californica</i> Bolander	PI-232247 (BH)	This paper	AF251459
<i>Austrodanthonia</i>	<i>laevis</i> (Vickery) H. P. Linder	<i>HPL 5633</i> (BOL)	This paper	AF251460
<i>Merxmuellera</i>	<i>rangei</i> (Pilg.) Conert	<i>NPB 960</i> (GRA)	This paper	AF251461
<i>Centropodia</i>	<i>glauca</i> (Nees) Copt	<i>NPB 967</i> (BOL)	This paper	AF251462
<i>Eragrostis</i>	<i>curvula</i> (Schrad.) Nees	<i>LGC 1303</i> (ISC)	Clark et al. (1995)	U21988
<i>Uniola</i>	<i>paniculata</i> L.	<i>JID s.n.</i> (BH)	This paper	AF251463
<i>Zoysia</i>	<i>matrella</i> (L.) Merr.	<i>LGC 1174</i> (ISC)	Clark et al. (1995)	U21975
<i>Distichlis</i>	<i>spicata</i> (L.) E. Green subsp. <i>stricta</i> (Torr.) R. F. Thorne	<i>Allred s.n.</i> (BH)	This paper	AF251464
<i>Pappophorum</i>	<i>bicolor</i> E. Fourn.	<i>Pohl 12464</i> (ISC)	This paper	AF352581
<i>Spartina</i>	<i>pectinata</i> Link	<i>LaDuke s.n.</i> (BH)	This paper	AF251465
<i>Sporobolus</i>	<i>indicus</i> (L.) R. Br.	<i>LGC 1293</i> (ISC)	Clark et al. (1995)	U21983
<i>Micraira</i>	<i>lazaridis</i> L. G. Clark, Wendel & Craven	<i>LGC 1157</i> (ISC)	Clark et al. (1995)	U21972
<i>Thysanolaena</i>	<i>maxima</i> (Roxb.) Kuntze	FTG (living)	Clark et al. (1995)	U21984
<i>Gynerium</i>	<i>sagittatum</i> (Aubl.) P. Beauv.	<i>LGC &amp; P. Asimbaya 1472</i> (ISC)	This paper	AF251466
<i>Chasmanthium</i>	<i>laxum</i> (L.) H. O. Yates	<i>D. Lewis s.n.</i> (ISC)	Clark et al. (1995)	U27296
<i>Zeugites</i>	<i>pittieri</i> Hack.	<i>LGC 1171</i> (ISC)	Clark et al. (1995)	U21987
<i>Danthoniopsis</i>	<i>petiolata</i> (J. B. Phipps) Clayton	<i>LGC 1173</i> (ISC)	Clark et al. (1995)	U22008
<i>Panicum</i>	<i>virgatum</i> L.	<i>LGC 1164</i> (ISC)	Clark et al. (1995)	U21986
<i>Pennisetum</i>	<i>alopecuroides</i> (L.) Spreng.	<i>RJS s.n.</i> (BH)	This paper	AF251467
<i>Miscanthus</i>	<i>japonicus</i> Andersson	Arnold Arboretum 301– 80c (living)	Spangler et al. (1999)	AF117417
<i>Zea</i>	<i>mays</i> L. cv. 'B73'	Material from M. Lee (ISU)	Clark et al. (1995)	U21985
rbcL				
<i>Flagellaria</i>	<i>indica</i>	<i>Chase 206</i> (NCU)	Chase et al. (1993)	L12678
<i>Elegia</i>	<i>capensis</i> (Burm. f.) Schel- pe	<i>Chase 209</i> (NCU)	Duvall & Morton (1996)	L12675
<i>Baloskion</i>	<i>tetraphyllum</i>	No voucher	Katayama & Ogihara (1996)	D38296
<i>Joinvillea</i>	<i>plicata</i> (Hook. f.) Newell & B. C. Stone	<i>Thren 84</i> (NO)	Duvall & Morton (1996)	L01471
<i>Anomochloa</i>	<i>marantoidea</i>	<i>LGC 1299</i> (ISC)	Duvall & Morton (1996)	AF021875
<i>Guaduella</i>	<i>marantifolia</i>	<i>Kobayashi et al. 1539</i> (ISC)	Clark et al. (2000)	AF164778

## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Puelia</i>	<i>ciliata</i>	<i>Kobayashi et al. 1541</i> (ISC)	Clark et al. (2000)	AF164780
<i>Lithachne</i>	<i>humilis</i>	<i>LGC s.n.</i> (ISC)	Duvall & Morton (1996)	U13231
<i>Bambusa</i>	<i>multiplex</i> (Lour.) Raeusch. ex Schult. & Schult. f.	<i>Sanders 62–616</i> (UCR)	Duvall & Morton (1996)	M91626
<i>Chusquea</i>	<i>circinata</i> Soderstr. & C. Calderón	Quail Botanic Garden (living)	Duvall & Morton (1996)	U13227
<i>Oryza</i>	<i>sativa</i>	No voucher	Nishizawa & Hirai (1987)	D00207
<i>Leersia</i>	<i>oryzoides</i> (L.) Sw.	<i>LGC s.n.</i> (ISC)	Duvall & Morton (1996)	U13228
<i>Stipa</i>	<i>dregeana</i> Steud. var. <i>dregeana</i>	<i>McDowell s.n.</i> (BOL)	Barker et al. (1995)	
<i>Avena</i>	<i>sativa</i>	No voucher	Duvall et al. (1993)	L15300
<i>Bromus</i>	<i>inermis</i> Leyss.	No voucher	Seberg & Linde-Laursen (1996)	Z49836
<i>Hordeum</i>	<i>vulgare</i>	No voucher	Zurawski et al. (1984)	X00630
<i>Aristida</i>	<i>congesta</i> Roem. & Schult.	<i>NPB 1130</i> (BOL)	Barker et al. (1995)	U31359
<i>Stipagrostis</i>	<i>zeyheri</i>	<i>NPB 1133</i> (BOL)	Barker et al. (1995)	U31378
<i>Amphipogon</i>	<i>strictus</i>	<i>HPL 5634</i> (BOL)	Barker (1997)	U88403
<i>Arundo</i>	<i>donax</i>	<i>NPB 1131</i> (BOL)	Barker et al. (1995)	U31360
<i>Moliniopsis</i>	<i>japonica</i> (Hack.) Hayata	<i>Kobayashi 1253</i>	Barker et al. (1995)	U31439
<i>Phragmites</i>	<i>australis</i>	<i>NPB 1132</i> (BOL)	Barker et al. (1995)	U29900
<i>Merxmuellera</i>	<i>macowanii</i>	<i>NPB 1008</i> (BOL)	Barker et al. (1995)	U31438
<i>Karroochloa</i>	<i>purpurea</i>	<i>HPL 5360</i> (BOL)	Barker et al. (1995)	U31437
<i>Danthonia</i>	<i>spicata</i> (L.) P. Beauv. ex Roem. & Schult.	<i>EAK V10</i> (GH)	Barker et al. (1995)	U31102
<i>Centropodia</i>	<i>glauca</i>	<i>HPL 5410</i> (BOL)	Barker et al. (1995)	U31100
<i>Eragrostis</i>	<i>capensis</i> (Thunb.) Trin.	<i>NPB 1135</i> (BOL)	Barker et al. (1995)	U31104
<i>Enneapogon</i>	<i>scaber</i> Lehm.	<i>NPB 1023</i> (BOL)	Barker et al. (1995)	U31103
<i>Eriachne</i>	<i>triodioides</i> Domin	<i>EAK s.n.</i> (GH)	This paper	AF352580
<i>Thysanolaena</i>	<i>maxima</i>	Kew 1979–3225 Warr (living)	Barker et al. (1995)	U31380
<i>Gynerium</i>	<i>sagittatum</i>	Kew 1991–1276 Kall (living)	Barker et al. (1995)	U31105
<i>Chasmanthium</i>	<i>latifolium</i> (Michx.) H. O. Yates	<i>Snow 5944</i>	Barker et al. (1995)	U31101
<i>Pennisetum</i>	<i>glaucum</i> (L.) R. Br.	No voucher	Doebley et al. (1990)	L14623
<i>Sorghum</i>	<i>bicolor</i> (L.) Moench	No voucher	Lou et al. (1989)	1515164A
<i>Zea</i>	<i>mays</i>	No voucher	Gaut et al. (1992)	Z11973
<i>rpoC2</i>				
<i>Joinvillea</i>	<i>plicata</i>	No voucher	Barker et al. (1999)	AF001864
<i>Olyra</i>	<i>latifolia</i>	<i>HPL 5742</i> (BOL)	Barker et al. (1999)	U90825
<i>Bambusa</i>	<i>vulgaris</i> Schrad. ex J. C. Wendl.	Durban Botanic Garden (living)	Barker et al. (1999)	U90824
<i>Ehrharta</i>	<i>dura</i> Nees ex Trin.	<i>NPB 1118</i> (BOL)	Barker et al. (1999)	AF064761
<i>Oryza</i>	<i>sativa</i>	No voucher	Hiratsuka et al. (1989)	X15901
<i>Lygeum</i>	<i>spartum</i>	Kew (living)	Cummings et al. (1994)	L25381
<i>Nardus</i>	<i>stricta</i>	Kew (living)	Cummings et al. (1994)	L25382
<i>Anisopogon</i>	<i>avenaceus</i>	<i>HPL 5590</i> (BOL)	Barker et al. (1999)	U92263
<i>Stipa</i>	<i>dregeana</i>	<i>McDowell s.n.</i> (BOL)	Barker et al. (1999)	U90826
<i>Briza</i>	<i>maxima</i> L.	<i>EAK s.n.</i> (GH)	Cummings et al. (1994)	L25376
<i>Bromus</i>	<i>tectorum</i> L.	<i>EAK s.n.</i> (GH)	Cummings et al. (1994)	L25377

## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Aristida</i>	<i>congesta</i> Roem. & Schult. subsp. <i>barbicollis</i> (Trin. & Rupr.) DeWinter	<i>NPB 1130</i> (BOL)	Barker et al. (1999)	U90827
<i>Stipagrostis</i>	<i>zeyheri</i> subsp. <i>zeyheri</i>	<i>NPB 1133</i> (BOL)	Barker et al. (1999)	U90828
<i>Amphipogon</i>	<i>strictus</i>	<i>HPL 5634</i> (BOL)	Barker et al. (1999)	U94335
<i>Arundo</i>	<i>donax</i>	<i>NPB 1131</i> (BOL)	Barker et al. (1999)	U92264
<i>Moliniopsis</i>	<i>japonica</i>	<i>Kobayashi 1253</i>	Barker et al. (1999)	U95081
<i>Phragmites</i>	<i>australis</i>	<i>NPB 1132</i> (BOL)	Barker et al. (1999)	U95130
<i>Merxmuellera</i>	<i>macowanii</i>	<i>NPB 1008</i> (BOL)	Barker et al. (1999)	U95076
<i>Karroochloa</i>	<i>purpurea</i>	<i>HPL 5360</i> (BOL)	Barker et al. (1999)	U94824
<i>Danthonia</i>	<i>spicata</i>	<i>EAK V10</i> (GH)	Barker et al. (1999)	U93362
<i>Austrodanthonia</i>	<i>laevis</i>	<i>HPL 5633</i> (BOL)	Barker et al. (1999)	U96313
<i>Merxmuellera</i>	<i>rangei</i>	<i>NPB 960</i> (GRA)	Barker et al. (1999)	U95077
<i>Centropodia</i>	<i>glauca</i>	<i>HPL 5410</i> (BOL)	Barker et al. (1999)	U92265
<i>Eragrostis</i>	<i>capensis</i>	<i>NPB 1135</i> (BOL)	Barker et al. (1999)	U96317
<i>Enneapogon</i>	<i>scaber</i>	<i>NPB 1023</i> (BOL)	Barker et al. (1999)	U96319
<i>Spartina</i>	<i>alterniflora</i>	<i>EAK s.n.</i> (GH)	Cummings et al. (1994)	L25386
<i>Micraira</i>	<i>lazaridis</i>	<i>LGC 1157</i> (ISC)	Barker et al. (1999)	U96318
<i>Thysanolaena</i>	<i>maxima</i>	Kew, 1979–3225 Warr (living)	Barker et al. (1999)	U96315
<i>Gynerium</i>	<i>sagittatum</i>	Kew, 1991–1276 Kall (living)	Barker et al. (1999)	U94392
<i>Chasmanthium</i>	<i>latifolium</i>	<i>Snow 5944</i>	Barker et al. (1999)	U94334
<i>Panicum</i>	<i>maximum</i> Jacq.	<i>NPB 1125</i> (BOL)	Barker et al. (1999)	AF000021
<i>Pennisetum</i>	sp.	No voucher	Cummings et al. (1994)	L25383
<i>Sorghum</i>	<i>bicolor</i>	No voucher	Chen et al. (1993)	Z14983
<i>Zea</i>	<i>mays</i>	No voucher	Igloi et al. (1990)	X86563
Phytochrome B				
<i>Flagellaria</i>	<i>indica</i>	<i>RJS 77 394</i> (BH)	Mathews & Sharrock (1996)	U61203
<i>Joinvillea</i>	<i>ascendens</i>	<i>Moore 10438</i> (NY)	Mathews & Sharrock (1996)	U61205
<i>Anomochloa</i>	<i>marantoidea</i>	<i>LGC 1299</i> (ISC)	Mathews et al. (2000)	AF137291
<i>Streptochaeta</i>	<i>angustifolia</i>	<i>LGC 1304</i> (ISC)	Mathews et al. (2000)	AF137328
<i>Pharus</i>	<i>lappulaceus</i> Aubl.	<i>LGC 1329</i> (ISC)	Mathews et al. (2000)	AF137321
<i>Puelia</i>	<i>ciliata</i>	<i>Kobayashi et al. 1541</i> (ISC)	Mathews et al. (2000)	AF137324
<i>Eremitis</i>	sp. nov.	<i>LGC &amp; WZ 1343</i> (ISC)	Mathews et al. (2000)	AF137304
<i>Pariana</i>	<i>radiciflora</i>	<i>LGC &amp; WZ 1344</i> (ISC)	Mathews et al. (2000)	AF137317
<i>Lithachne</i>	<i>pauciflora</i> (Sw.) P. Beauv.	<i>LGC 1297</i> (ISC)	Mathews et al. (2000)	AF137307
<i>Olyra</i>	<i>latifolia</i>	<i>XL &amp; LGC 911</i> (ISC)	Mathews et al. (2000)	AF137315
<i>Buergersiochloa</i>	<i>bambusoides</i>	<i>Dransfield 1382</i> (K)	Mathews et al. (2000)	AF137295
<i>Pseudosasa</i>	<i>japonica</i> (Sieb. & Zucc. ex Steud.) Makino ex Nakai	<i>EAK V6</i> (A)	Mathews et al. (2000)	AF137323
<i>Chusquea</i>	<i>oxylepis</i> (Hack.) Ekman	<i>LGC 1069</i> (ISC)	Mathews et al. (2000)	AF137298
<i>Streptogyna</i>	<i>americana</i>	<i>Johnston 433</i>	Mathews et al. (2000)	AF137329
<i>Ehrharta</i>	<i>erecta</i> Lam.	<i>EAK V44</i> (GH)	Mathews et al. (2000)	AF137302
<i>Oryza</i>	<i>sativa</i>	no voucher	Dehesh et al. (1991)	X57563
<i>Lygeum</i>	<i>spartum</i>	<i>RJS 3698</i> (BH)	Mathews et al. (2000)	AF137309
<i>Nardus</i>	<i>stricta</i>	BBG; <i>Royle &amp; Schiers s.n.</i>	Mathews et al. (2000)	AF137313
<i>Anisopogon</i>	<i>avenaceus</i>	<i>HPL 5590</i> (BOL)	Mathews et al. (2000)	AF137290
<i>Nassella</i>	<i>viridula</i>	<i>Lavin s.n.</i> (MONT)	Mathews et al. (2000)	AF137314
<i>Brachypodium</i>	<i>pinnatum</i> (L.) P. Beauv.	<i>PI-440176</i> (GH)	Mathews et al. (2000)	AF137294
<i>Melica</i>	<i>cupanii</i> Guss.	<i>PI-383702</i> (BH)	Mathews et al. (2000)	AF137310

## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Glyceria</i>	<i>grandis</i> S. Watson	JID & RJS s.n. (BH)	Mathews et al. (2000)	AF137305
<i>Diarrhena</i>	<i>obovata</i>	LGC & WZ 1216 (ISC)	Mathews et al. (2000)	AF137301
<i>Phalaris</i>	<i>arundinacea</i> L.	RJS 3427 (BH)	Mathews et al. (2000)	AF137320
<i>Bromus</i>	<i>inermis</i>	Lavin s.n. (MONT)	Mathews et al. (2000)	U61193
<i>Triticum</i>	<i>aestivum</i> L.	Mason-Gamer s.n. (GH)	Mathews et al. (2000)	AF137331
<i>Aristida</i>	<i>purpurea</i> subsp. <i>longiseta</i>	Lavin s.n. (MONT)	Mathews et al. (2000)	AF137292
<i>Molinia</i>	<i>caerulea</i>	RJS 3305 (BH)	Mathews et al. (2000)	AF137312
<i>Phragmites</i>	<i>australis</i>	Keller s.n. (GH)	Mathews et al. (2000)	AF137322
<i>Danthonia</i>	<i>spicata</i>	EAK V10 (GH)	Mathews et al. (2000)	AF137299
<i>Eragrostis</i>	<i>cilianensis</i> (All.) Vignolo ex Janch.	Lavin s.n. (MONT)	Mathews et al. (2000)	U61200
<i>Sporobolus</i>	<i>giganteus</i> Nash	PMP 10008 (US)	Mathews et al. (2000)	AF137327
<i>Thysanolaena</i>	<i>maxima</i>	Farnsworth s.n. (GH)	Mathews et al. (2000)	AF137330
<i>Chasmanthium</i>	<i>latifolium</i>	EAK V13 (A)	Mathews et al. (2000)	AF137297
<i>Danthoniopsis</i>	<i>dinteri</i> (Pilg.) C. E. Hubb.	PI-207548 (GH)	Mathews et al. (2000)	AF137300
<i>Panicum</i>	<i>capillare</i> L.	Lavin s.n. (MONT)	Mathews et al. (2000)	AF137316
<i>Pennisetum</i>	<i>alopeuroroides</i>	EAK s.n. (A)	Mathews et al. (2000)	AF137318
<i>Miscanthus</i>	<i>japonicus</i>	Arnold Arboretum 301– 80C (living)	Mathews et al. (2000)	AF137311
<i>Zea</i>	<i>mays</i>	Lavin s.n. (MONT)	Mathews et al. (2000)	AF137332
Chloroplast restriction site polymorphisms				
<i>Flagellaria</i>	<i>indica</i>	BHC-77394	Soreng & Davis (1998)	
<i>Baloskion</i>	<i>tetraphyllum</i>	Kew-6565–1977 (BH)	Soreng & Davis (1998)	
<i>Joinvillea</i>	<i>ascendens</i>	NTBG-800379 ( <i>H. Moore 10438</i> )	Davis & Soreng (1993)	
<i>Anomochloa</i>	<i>marantoidea</i>	LGC 1299 (ISC)	Soreng & Davis (1998)	
<i>Streptochaeta</i>	<i>sodiroides</i> Hack.	PMP 9525 (US)	Soreng & Davis (1998)	
<i>Pharus</i>	<i>latifolius</i>	BHC from USZ	Davis & Soreng (1993)	
<i>Eremittis</i>	sp.	USNHG-153, <i>Soderstrom</i> 2182 (US) or USNHG- 286 (US)	Soreng & Davis (1998)	
<i>Lithachne</i>	<i>humilis</i>	BHC from U. S. National Zoological Gardens	Davis & Soreng (1993)	
<i>Olyra</i>	<i>latifolia</i>	PMP 7311 (US)	Soreng & Davis (1998)	
<i>Pseudosasa</i>	<i>japonica</i>	BHC-71467	Davis & Soreng (1993)	
<i>Chusquea</i>	aff. <i>subulata</i> L. G. Clark	PMP 9499 (US)	Soreng & Davis (1998)	
<i>Ehrharta</i>	<i>calycina</i>	PI-208983 (BH)	Soreng & Davis (1998)	
<i>Oryza</i>	<i>sativa</i>	no voucher	Hiratsuka et al. (1989)	
<i>Leersia</i>	<i>virginica</i>	RJS 3399 (BH)	Davis & Soreng (1993)	
<i>Brachyelytrum</i>	<i>erectum</i>	RJS 3427 (BH)	Davis & Soreng (1993)	
<i>Lygeum</i>	<i>spartum</i>	RJS 3698 (BH)	Soreng & Davis (1998)	
<i>Nardus</i>	<i>stricta</i>	BBG: seed from <i>Royle</i> & <i>Schiers</i> s.n. 1988, <i>Hempel</i> s.n. 1987 (B)	Davis & Soreng (1993)	
<i>Anisopogon</i>	<i>avenaceus</i>	HPL 5590 (BOL)	Soreng & Davis (1998)	
<i>Ampelodesmos</i>	<i>mauritanica</i>	BBG: <i>Royle</i> & <i>Schiers</i> s.n. 1988 (B)	Soreng & Davis (1998)	
<i>Stipa</i>	<i>barbata</i>	PI-229468 (BH)	Davis & Soreng (1993)	
<i>Nassella</i>	<i>viridula</i>	PI-387938 (BH)	Soreng & Davis (1998)	
<i>Piptatherum</i>	<i>miliaceum</i> (L.) Coss.	PI-284145 (BH)	Davis & Soreng (1993)	
<i>Brachypodium</i>	<i>pinnatum</i>	PI-440170 (BH)	Davis & Soreng (1993)	
<i>Melica</i>	<i>altissima</i>	PI-325418 (BH)	Davis & Soreng (1993)	
<i>Glyceria</i>	<i>striata</i>	JID & RJS s.n. (BH)	Davis & Soreng (1993)	
<i>Diarrhena</i>	<i>obovata</i>	Seed from <i>Tiedye 5186</i> (DAO)	Davis & Soreng (1993)	



## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Avena</i>	<i>barbata</i> Pott ex Link	No voucher	Soreng & Davis (1998)	
<i>Bromus</i>	<i>inermis</i>	<i>RJS 3428</i> (BH), PI-314071 (BH)	Davis & Soreng (1993)	
<i>Triticum</i>	<i>aestivum</i> L. cv. 'Susquehanna'	<i>RJS s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Aristida</i>	<i>purpurea</i>	<i>Allred s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Amphipogon</i>	<i>strictus</i>	<i>HPL 5634</i> (BOL)	Soreng & Davis (1998)	
<i>Arundo</i>	<i>donax</i>	FTG-83-130 (BH)	Soreng & Davis (1998)	
<i>Molinia</i>	<i>caerulea</i>	<i>RJS 3305</i> (BH)	Soreng & Davis (1998)	
<i>Phragmites</i>	<i>australis</i>	<i>RJS 3884</i> (BH)	Davis & Soreng (1993)	
<i>Danthonia</i>	<i>californica</i>	<i>PI-232247</i> (BH)	Davis & Soreng (1993)	
<i>Eragrostis</i>	<i>curvula</i>	<i>PI-365034</i> (BH)	Davis & Soreng (1993)	
<i>Uniola</i>	<i>paniculata</i>	<i>JID s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Zoysia</i>	sp.	<i>JID s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Distichlis</i>	<i>spicata</i> subsp. <i>stricta</i>	<i>Allred s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Spartina</i>	<i>pectinata</i>	<i>LaDuke s.n.</i> (BH)	Soreng & Davis (1998)	
<i>Sporobolus</i>	<i>giganteus</i>	<i>PMP 10008</i> (US)	Soreng & Davis (1998)	
<i>Chasmanthium</i>	<i>latifolium</i>	Cornell University gardens (living)	Davis & Soreng (1993)	
<i>Panicum</i>	<i>virgatum</i>	USDA 421520 (BH)	Soreng & Davis (1998)	
<i>Pennisetum</i>	<i>alopecuroides</i>	<i>RJS s.n.</i> (BH)	Davis & Soreng (1993)	
<i>Miscanthus</i>	<i>sinensis</i> Andersson var. <i>gracillimus</i> Hitchc.	<i>RJS s.n.</i> (BH)	Davis & Soreng (1993)	
ITS				
<i>Joinvillea</i>	<i>plicata</i>	<i>Wilson 7126</i>	Hsiao et al. (1999)	AF019784
<i>Streptochaeta</i>	<i>sodiroana</i>	<i>PMP &amp; Annable 9525</i> (US)	Hsiao et al. (1999)	AF019785
<i>Pharus</i>	<i>latifolius</i>	<i>PMP &amp; Annable 6944</i> (US)	Hsiao et al. (1999)	AF019786
<i>Lithachne</i>	<i>humilis</i>	Utah State University s.n. (living)	Hsiao et al. (1999)	AF019787
<i>Chusquea</i>	<i>latifolia</i>	<i>LGC &amp; XL 417</i> (ISC)	Hsiao et al. (1999)	AF019788
<i>Microlaena</i>	<i>stipoides</i> (Labill.) R. Br.	Kew 1973-15875 (living)	Hsiao et al. (1999)	AF019791
<i>Oryza</i>	<i>sativa</i>	No voucher	Takaiwa et al. (1985)	
<i>Leersia</i>	<i>hexandra</i> Sw.	<i>Jacobs 7782</i>	Hsiao et al. (1999)	AF019793
<i>Brachyelytrum</i>	<i>erectum</i>	Intermountain Herbarium 1669	Hsiao et al. (1999)	AF019794
<i>Lygeum</i>	<i>spartum</i>	<i>Catalan 1593</i>	Hsiao et al. (1999)	AF019797
<i>Nardus</i>	<i>stricta</i>	Intermountain Herbarium 203443	Hsiao et al. (1999)	AF019796
<i>Anisopogon</i>	<i>avenaceus</i>	<i>Dalby 94/01</i>	Hsiao et al. (1999)	AF019800
<i>Ampelodesmos</i>	<i>mauritanica</i>	Kew 150-90.00982 (living)	Hsiao et al. (1999)	AF019799
<i>Stipa</i>	<i>ichu</i> (Ruiz & Pavón) Kunth	<i>Renwoize &amp; Flores 5301</i> (K)	Hsiao et al. (1999)	AF019803
<i>Nassella</i>	<i>leucotricha</i> (Trin. & Rupr.) R. W. Pohl	<i>Houck s.n.</i>	Hsiao et al. (1999)	L36520
<i>Piptatherum</i>	<i>songaricum</i> (Trin. & Rupr.) Roshev. ex Nikitina	<i>Hsiao 199</i>	Hsiao et al. (1999)	AF019802
<i>Brachypodium</i>	<i>mexicanum</i> (Roem. & Schult.) Link	University of Leicester Botanic Gardens 347	Hsiao et al. (1999)	AF019805
<i>Melica</i>	<i>californica</i> Scribn.	<i>Curto 719</i>	Hsiao et al. (1999)	L36518
<i>Glyceria</i>	<i>striata</i>	<i>Curto 826</i>	Hsiao et al. (1999)	L36516
<i>Diarrhena</i>	<i>americana</i> P. Beauv.	Intermountain Herbarium 218465	Hsiao et al. (1999)	AF019798

## Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Avena</i>	<i>longiglumis</i> Durieu	Fritz, CN	Hsiao et al. (1999)	Z11758
<i>Bromus</i>	<i>inermis</i>	Hsiao 103	Hsiao et al., (1994)	L11579
<i>Hordeum</i>	<i>vulgare</i>	Hsiao 200	Chatterton et al. (1992)	Z11759
<i>Aristida</i>	<i>purpurea</i>	Intermountain Herbarium 209381	Hsiao et al., 1999	AF019807
<i>Stipagrostis</i>	<i>zeyheri</i> subsp. <i>zeyheri</i>	NPB 1133	Hsiao et al. (1999)	AF019845
<i>Amphipogon</i>	<i>caricinus</i> F. Muell.	Macfarlane 2155	Hsiao et al. (1998)	AF019849
<i>Arundo</i>	<i>donax</i>	Hsiao 196, Evans s.n.	Hsiao et al. (1999)	AF019809
<i>Molinia</i>	<i>caerulea</i>	Kew1973-10386	Hsiao et al. (1999)	AF109857
<i>Phragmites</i>	<i>australis</i>	Chatterton s.n.	Hsiao et al. (1999)	AF019810
<i>Merxmuellera</i>	<i>macowanii</i>	Kew 142-83.01715	Hsiao et al. (1998)	AF019863
<i>Karroochloa</i>	<i>purpurea</i>	HPL 5360	Hsiao et al. (1998)	AF019874
<i>Danthonia</i>	<i>californica</i>	Curto 974	Hsiao et al. (1999)	AF019813
<i>Rytidosperma</i>	<i>pumilum</i> (Kirk) H. P. Linder	HPL 5747	Hsiao et al. (1998)	AF019878
<i>Merxmuellera</i>	<i>rangei</i>	NPB 960 (GRA)	Hsiao et al. (1998)	AF019862
<i>Centropodia</i>	<i>glauca</i>	NPB 967	Hsiao et al. (1998)	AF019861
<i>Eragrostis</i>	<i>dielsii</i> Pilg. ex Diels & Pritz.	Jacobs 7195	Hsiao et al. (1999)	AF019834
<i>Spartina</i>	<i>gracilis</i> Trin.	Intermountain Herbarium 194828	Hsiao et al. (1999)	AF019844
<i>Sporobolus</i>	<i>airoides</i> (Torr.) Torr.	Curto s.n.	Hsiao et al. (1999)	AF019842
<i>Eriachne</i>	<i>triseta</i> Nees ex Steud.	Jacobs 7184	Hsiao et al. (1999)	AF019818
<i>Micraira</i>	<i>subulifolia</i> F. Muell.	Clarkson 10300	Hsiao et al. (1999)	AF019859
<i>Thysanolaena</i>	<i>maxima</i>	Kew1979-3225	Hsiao et al. (1999)	AF019854
<i>Gynerium</i>	<i>sagittatum</i>	Kew1991-1276Kall	Hsiao et al. (1999)	AF019853
<i>Chasmanthium</i>	<i>latifolium</i>	Intermountain Herbarium 216008	Hsiao et al. (1999)	AF019815
<i>Panicum</i>	<i>bisulcatum</i> Thunb.	Hsiao 160, PI-19486	Hsiao et al. (1999)	AF019829
<i>Pennisetum</i>	<i>setaceum</i> (Forssk.) Chiov.	Curto 976	Hsiao et al. (1999)	AF019833
<i>Miscanthus</i>	<i>sinensis</i>	Evans s.n.	Hsiao et al. (1999)	AF019822
<i>Zea</i>	<i>mays</i> L. subsp. <i>mexicana</i> (Schrad.) Iltis	Hsiao 197	Hsiao et al. (1999)	AF019817
GBSSI				
<i>Anomochloa</i>	<i>marantoidea</i>	LGC 1299 (ISC)	Mason-Gamer et al. (1998)	AF079290
<i>Pharus</i>	<i>lappulaceus</i>	LGC 1329 (ISC)	Mason-Gamer et al. (1998)	AF079298
<i>Eremitis</i>	sp. nov.	LGC & WZ 1343 (ISC)	Mason-Gamer et al. (1998)	AF079295
<i>Pariana</i>	<i>radiciflora</i>	LGC & WZ 1344 (ISC)	Mason-Gamer et al. (1998)	AF079297
<i>Chusquea</i>	<i>exasperata</i> L. G. Clark	LGC et al. 1093 (ISC)	Mason-Gamer et al. (1998)	AF079293
<i>Oryza</i>	<i>sativa</i>	No voucher	Wang et al. (1994)	X65183
<i>Lygeum</i>	<i>spartum</i>	RJS 3698	Mason-Gamer et al. (1998)	AF079289
<i>Melica</i>	<i>cupanii</i>	PI-383702 (A)	Mason-Gamer et al. (1998)	AF079296
<i>Glyceria</i>	<i>grandis</i>	JID & RJS s.n.	Mason-Gamer et al. (1998)	AF079291
<i>Hordeum</i>	<i>vulgare</i>	No voucher	Rohde et al. (1988)	X07932
<i>Merxmuellera</i>	<i>macowanii</i>	NPB 1008 (BOL)	This paper	AF353520
<i>Karroochloa</i>	<i>purpurea</i>	HPL 5360 (BOL)	This paper	AF353519
<i>Austrodanthonia</i>	<i>laevis</i>	HPL 5633 (BOL)	This paper	AF353517

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
<i>Merxmuellera</i>	<i>rangei</i>	NPB 960 (GRA)	This paper	AF353521
<i>Centropodia</i>	<i>glauca</i>	NPB 967 (BOL)	This paper	AF353518
<i>Danthoniopsis</i>	<i>dinteri</i>	PI-207548 (A)	Mason-Gamer et al. (1998)	AF079251
<i>Pennisetum</i>	<i>alopecuroides</i>	Park Seed 3650 (A)	Mason-Gamer et al. (1998)	AF079288
<i>Sorghum</i>	<i>bicolor</i>	PI-156549 (A)	Mason-Gamer et al. (1998)	AF079258
<i>Zea</i>	<i>mays</i>	No voucher	Klöggen et al. (1986)	X03935

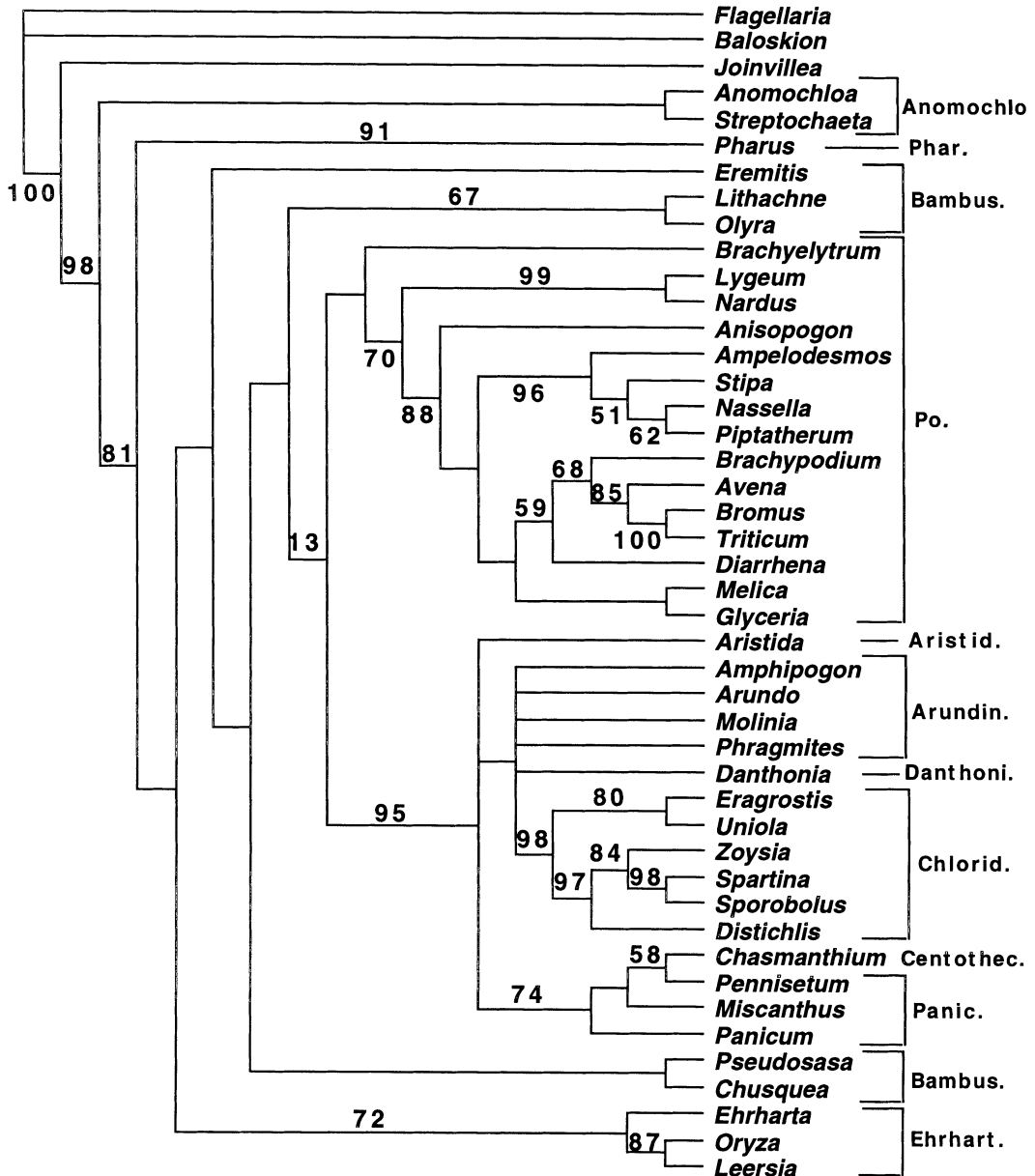
Appendix II. Matrix of structural characters, as assembled for analysis in NONA (Goloboff, 1993). Taxa in the matrix appear in groupings according to what was known about the phylogeny at the time the taxon sampling list was prepared. Thus, the four outgroups come first, followed by the early-diverging taxa, then bambusoids, rices, pooids, etc. Abbreviations of taxon names and associated underlines are required by the program. Characters and character states are described in Table 4, and are optimized on the cladogram in Figure 3. Codes used for polymorphisms (presence of two or more states) and subset ambiguities (when one or more states are not present, but the observed attribute cannot be assigned to any of the states not eliminated) are as follows: ? = unobserved; - = inapplicable; \ = intermediate/uncertain homology/unassignable to defined states; A = [01]; C = [03]; D = [12]; E = [13]; F = [23]; H = [034]; J = [234]; K = [0134]; L = [14]; N = [29]; Q = [07]; R = [012]; S = [57]; T = [127].

Character numbers	Taxon
	0000000001111111112222222222333333333344444444445555
	123456789012345678901234567890123456789012345678901234567890123
Flagellaria	\01-01A0-----0\\ \111003130-0----?00?-0050-\000
Elegia	00--0-0-----0\\ \1100103310-0----?1-0---?0-??1
Baloskion	0?0--0-0-----0\\ \1100102D2?-0----011-0---T0-?0?0
Joinvillea	01000110-----0\\ \1110A3130-0----??110\140-1100
Anomochloa	0001011\-\ \0---\0\\ \10110\111?011100?101101140-0110
Streptochaeta	01010110-\ \0---\0\\ \11101311?010101?10?100110-0110
Pharus	0000011111010--A0130??111101311?01110101110-1120-0110
Guaduella	0100011101100--101301111100221?01?????110A1?0-??1
Puelia	0100111101110--101301111101F21?01?????10-1120-0??1
Eremitis	0100011101010--001301100010\111?011101?1011011\0-01?1
Pariana	010001110A010--0\1301111110A211?011101?011011D0-0??1
Lithachne	01000111A010--11130110011012F1?0111010?01101110-0111
Olyra	0100011101010--11130110011012F1?0111010101101110-01?1
Buergersiochloa	010001110101110101301100A1012?1?01?????11011?0-0??1
Pseudosasa	110011110100A101013011111013210011101?301101120-01?1
Chusquea	100011110111A--1013011001101221?0111010?01101100-01?1
Streptogyna	010011A101001111013011000101F11?011101?K11100120-0??1
Ehrharta	0A00010101111101012011111002210010101011A0A020-01?1
Oryza	010001A01\1A10\11201111110A221?0110010101101020-0110
Leersia	0100010101\1A10\0120A1000100231?011001010110A020-?1?0
Phaenosperma	0A00011101010--001301\0011002D1?01110104?10-0020-??1
Brachyelytrum	0000010101011101012011001100221?011A01?4110-0010-?111
Lygeum	000001010\000--1?0----00110\121?0110000111110000-0111
Nardus	0100010100011101?0---00110\111?0110000111100030-01??
Anisopogon	0100010101011311013010001100F21001?????1?10-00-0-0111
Ampelodesmos	00000101010011A1013010001100221?0110000?110-0020-?1?1
Stipa	01000101010111A101F010001100J2100110000K110-00R0-?1?1
Nassella	01000101010111A1012010001100221?0110000C110-00\0-01?1
Piptatherum	010001010101A10101301000100221?01????01110-0020-?1?1
Brachypodium <sub>d</sub>	0100010101001101?1201?001100221?01A00000110-00S0-01?1
Melica <sub>a</sub>	01100101010A0--0012100001100231?011000011A0-0090-01?1

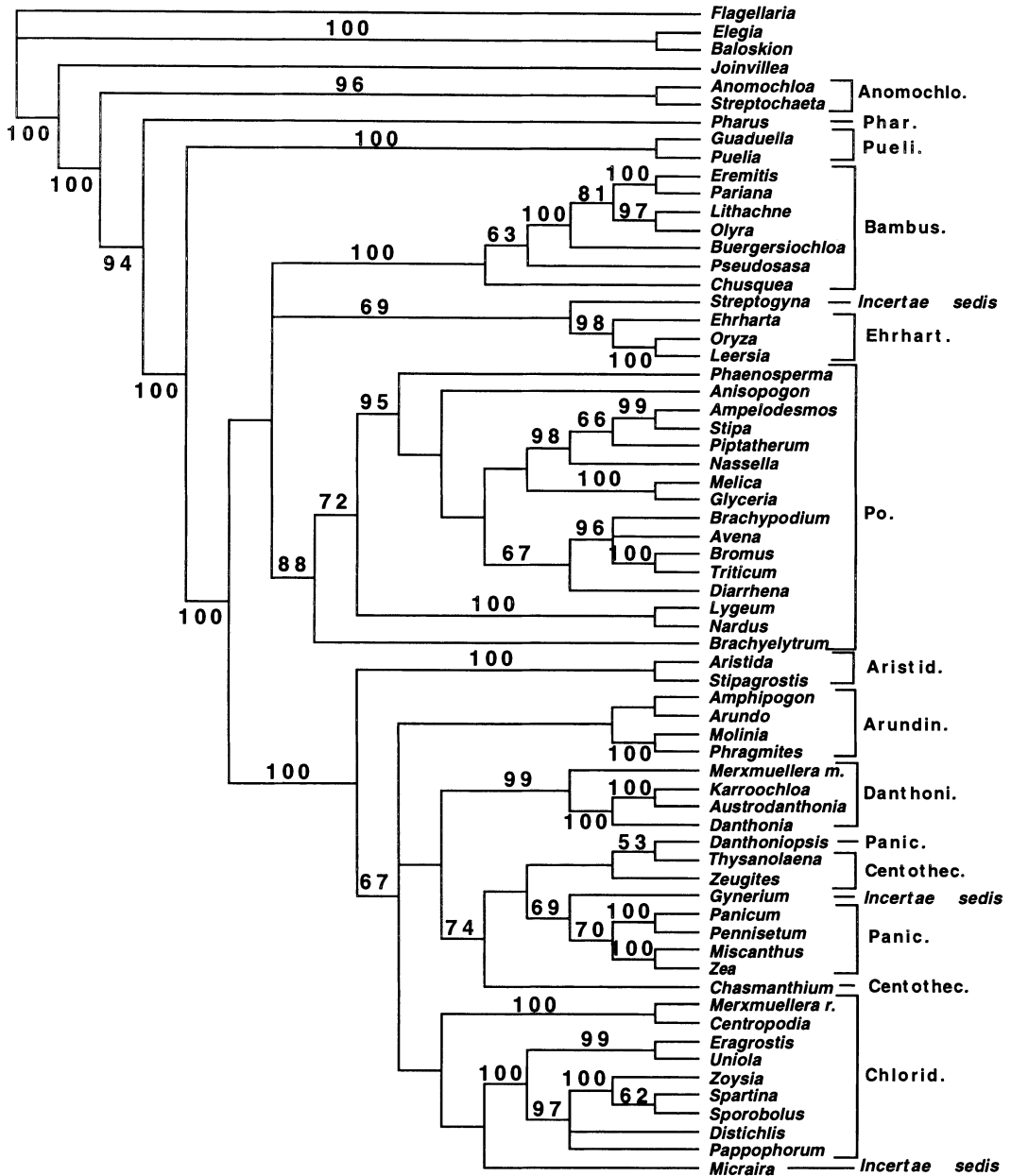
Appendix II. Continued.

Character numbers	0000000001111111112222222222333333333344444444445555
Taxon	12345678901234567890123456789012345678901234567890123
Glyceria_s	0110010101000--1012A00000100231?011000011A0-0000-01?1
Diarrhena	01000101010A0--101201000A100221?011A0A01110-0000-01?1
Avena	0100010101001E21012010001100221?011A0\111A0-0070-01?1
Bromus	011001010100A1R1012010001100221?010000001A0-0070-01?1
Triticum	010001010000A101012010001100221?01100\001A0-0070-01?1
Aristida	000A010101011E011120110011002210010110011A100012-11?1
Stipagrostis	0A010101010113011A2011001100221?010110?D111000130???1
Amphipogon	01010101010113A1112000001101221?110110?1?11100-0-?1?1
Arundo	110001010100AEA1012001001100221?110110?1?A100020-?111
Molinia	010101010100A101?12A01001100221?0101100111100090-1111
Phragmites	\10101010110A1010120010011012210\101100111101020-1111
M_merxmullera_m_	0001010101001E11012011001100221101???????11000?0-???1
Karoochloa	0101010101001E110120010011002211110110???1100060-???1
Danthonia	010101010100131101200100110022110101100D11100040-11?1
Austrodanthonia	01010101010013110120010011002211110110???1100020-???1
R_merxmullera_r_	0?01010101001E11012001001100221?????????10-0060-???1
Centropodia	0001010101001111012001001100221011?????L?11000630???1
Eragrostis	0\01010101000--1012001001100221?11111001111A000301111
Uniola	0\01010101100--1012001001101221?110110???11110003\?1?1
Zoysia	000A010A0001110000---00A10A221?1111100D111100031?1?1
Distichlis	0000010101000--1012001001100221?1111100H?11100030?1?1
Pappophorum	0?010101010A120101200\001100221?\111100???111000E-???1
Spartina	0101010100010--000---001101221?1111100111A100Q31?1?1
Sporobolus	000A010101010--10A200100AA002D1?1111100E11110093A11?1
Eriachne	0A01A101010AA1A101200100A100221?010111?1111000?5-???1
Micraira	000A01A101AA0--100---000101221?0101???H11100000-???1
Thysanolaena	1100111101010--101200000A100221?11???????11010D0-1111
Gynerium	100AA1\101000--101201\000100221?11???????11010\0-???1
Chasmanthium_l	0100010101100--1012001001001221?1111110311100020-1111
Zeugites	010001110101A10A01200\001101221?111101?1?11000\0-???1
Danthoniopsis	0A01A1A1A1111E11112001001100221?01?????4111000NL-1?1?1
Panicum	0100010101110--01120A100110022101101110E111000\3011?1
Pennisetum	010A01010111A1001A200100110A221?1101110311100091-11?1
Miscanthus	000001011111110012001001100221?110111?211100051-11?1
Zea	010001011A110--0?12001001101211?\101110311100001-11?1

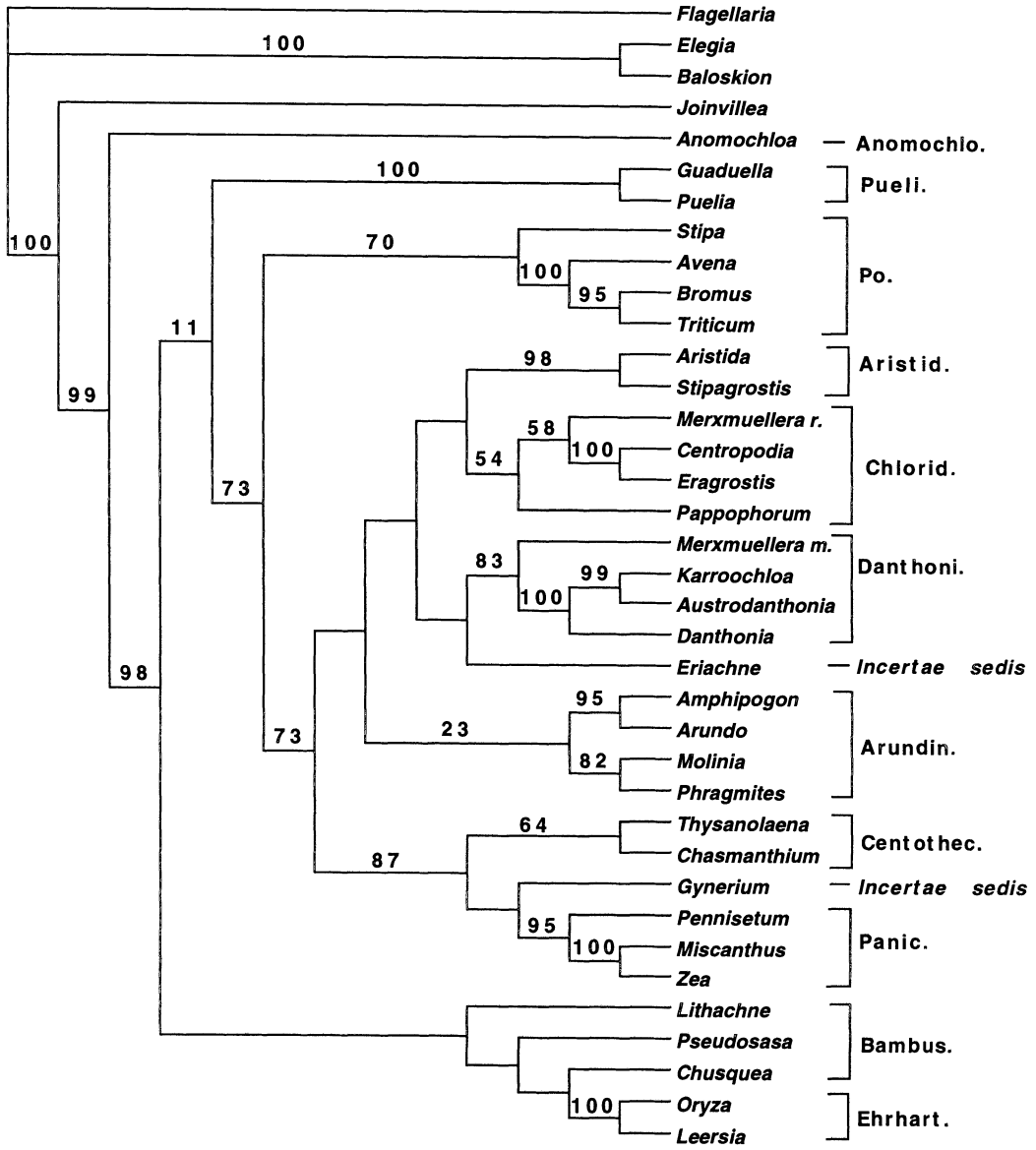
Appendix III. Consensus trees for individual data sets and combinations of data sets. Numbers above branches indicate percent of 500 bootstrap replicates, except for K (all molecular data), for which 1000 replicates were done. Tree statistics are listed in Table 3. The GPWG classification is overlain on each tree for comparison with Figure 2. —A. Chloroplast restriction sites; strict consensus of seven trees. —B. *ndhF*; strict consensus of 16 trees. —C. *rbcL*; single most parsimonious tree. —D. *rpoC2*; strict consensus of 33 trees. —E. Phytochrome B; single most parsimonious tree. —F. ITS; strict consensus of 24 trees. —G. GBSSI, single most parsimonious tree. —H. Structural data; strict consensus of 38,000 trees. —I. Chloroplast data; strict consensus of two trees. —J. Nuclear data; strict consensus of eight trees. —K. All molecular data; strict consensus of six trees.



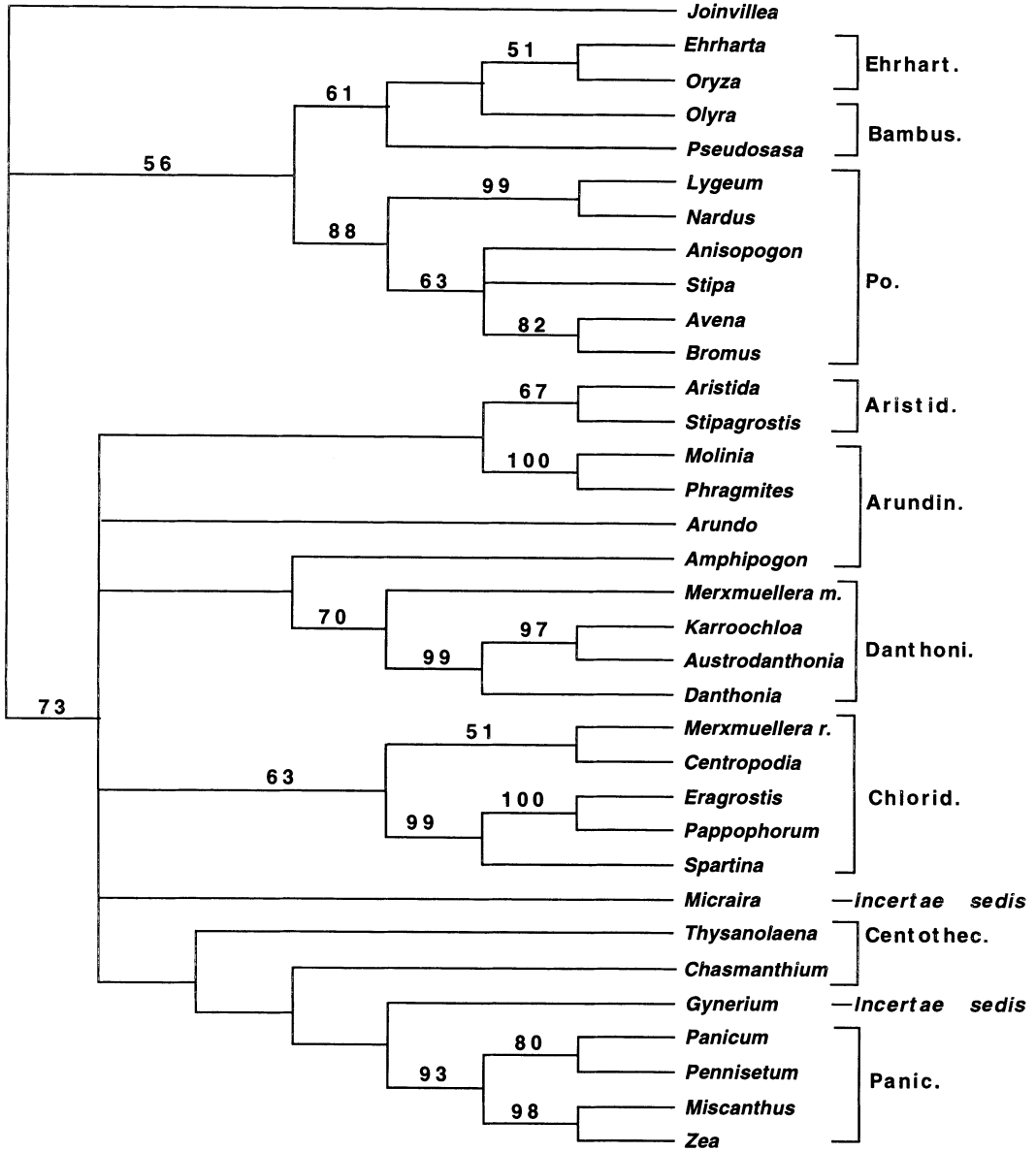
Appendix III—Figure A, cp restriction sites



Appendix III—Figure B, *ndhF*

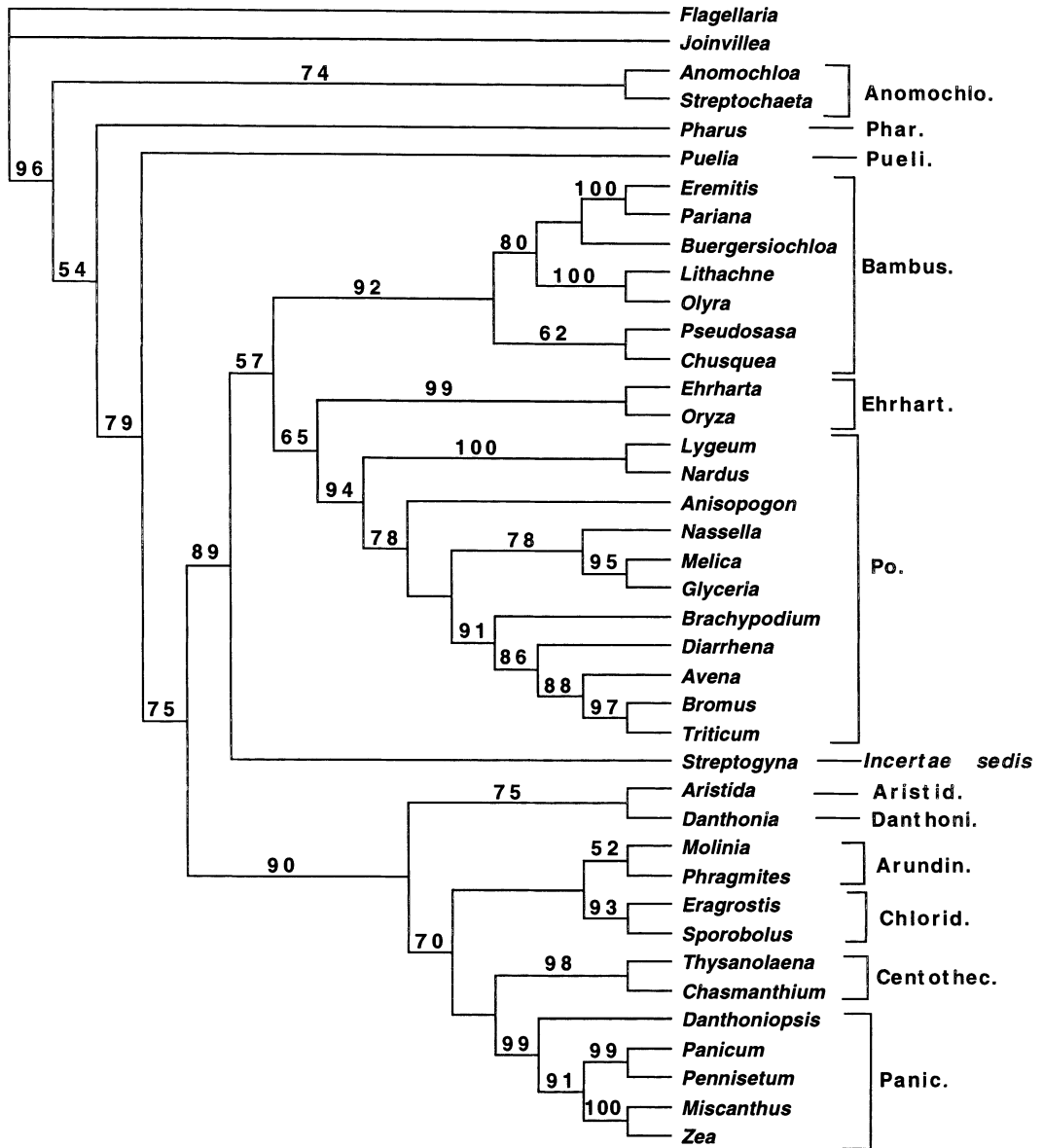


Appendix III—Figure C, *rbcL*.

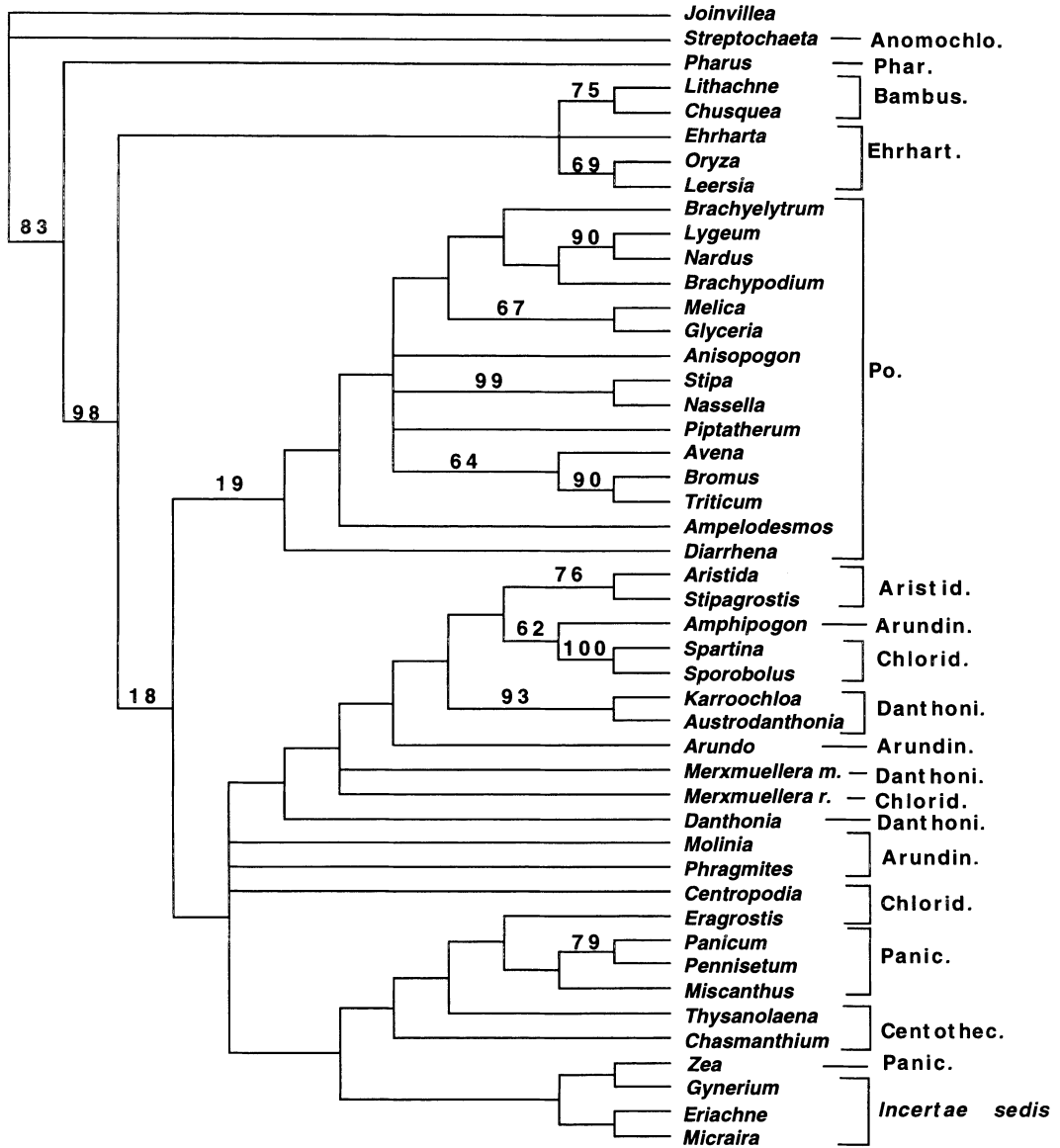


Appendix III—Figure D, *rpoC2*

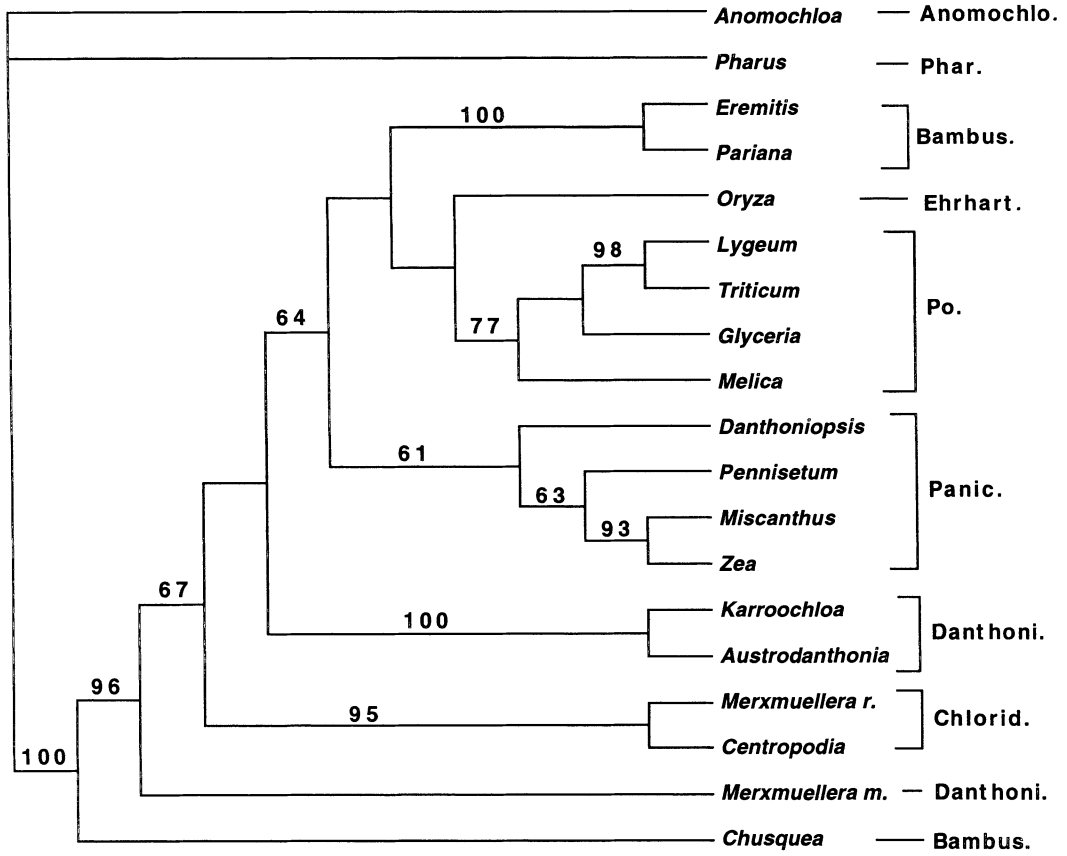




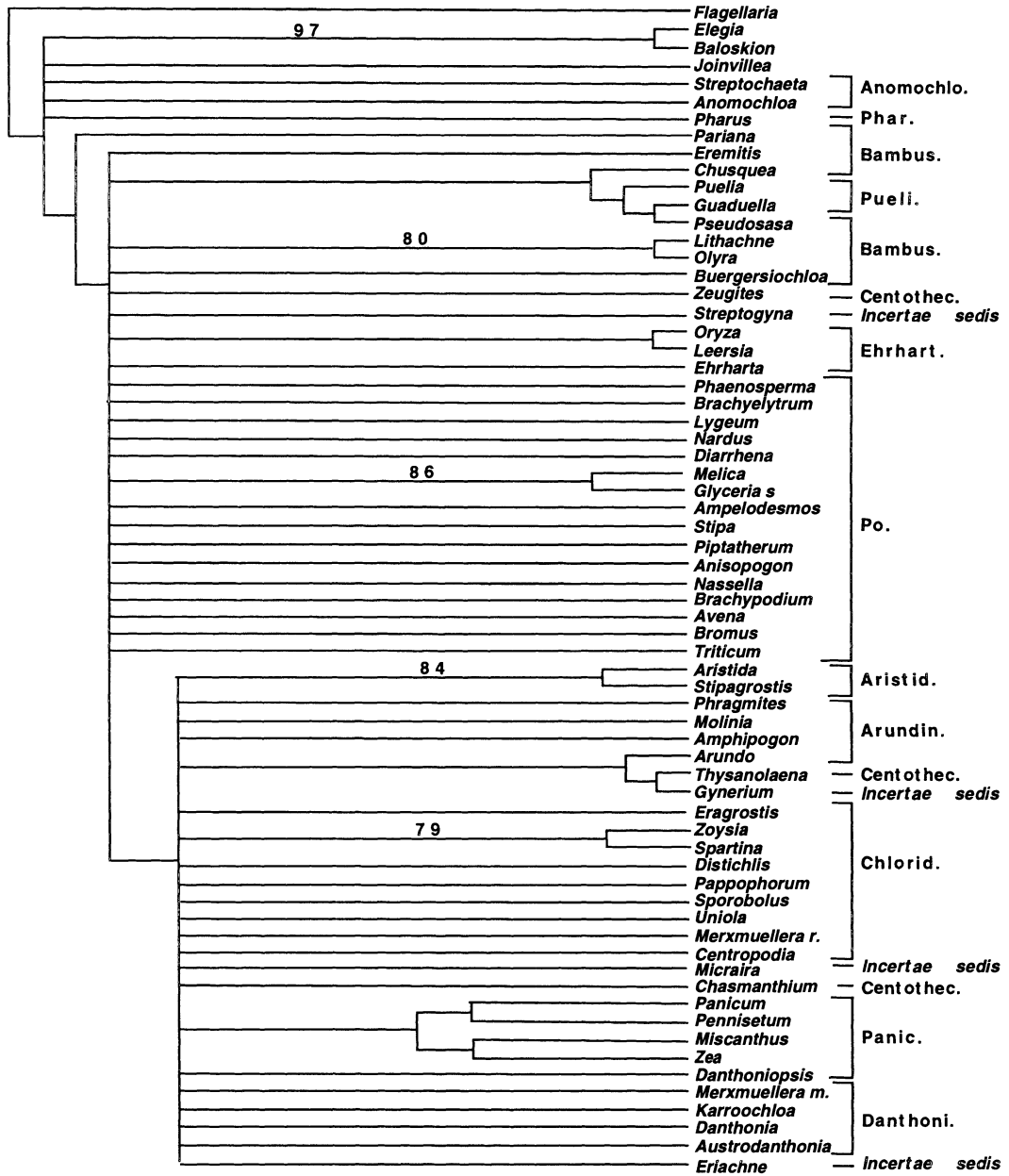
Appendix III—Figure E, phy B



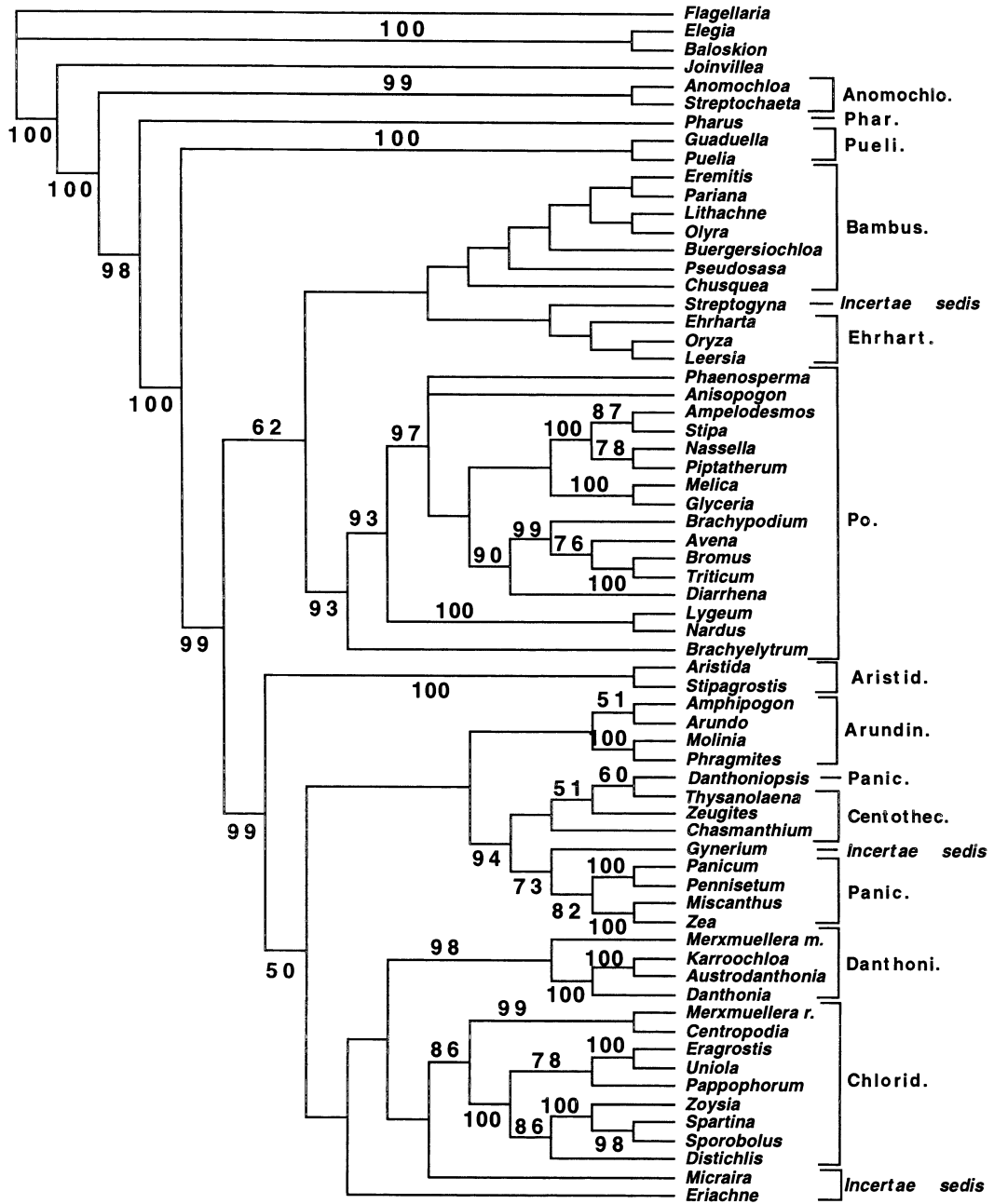
Appendix III—Figure F, ITS



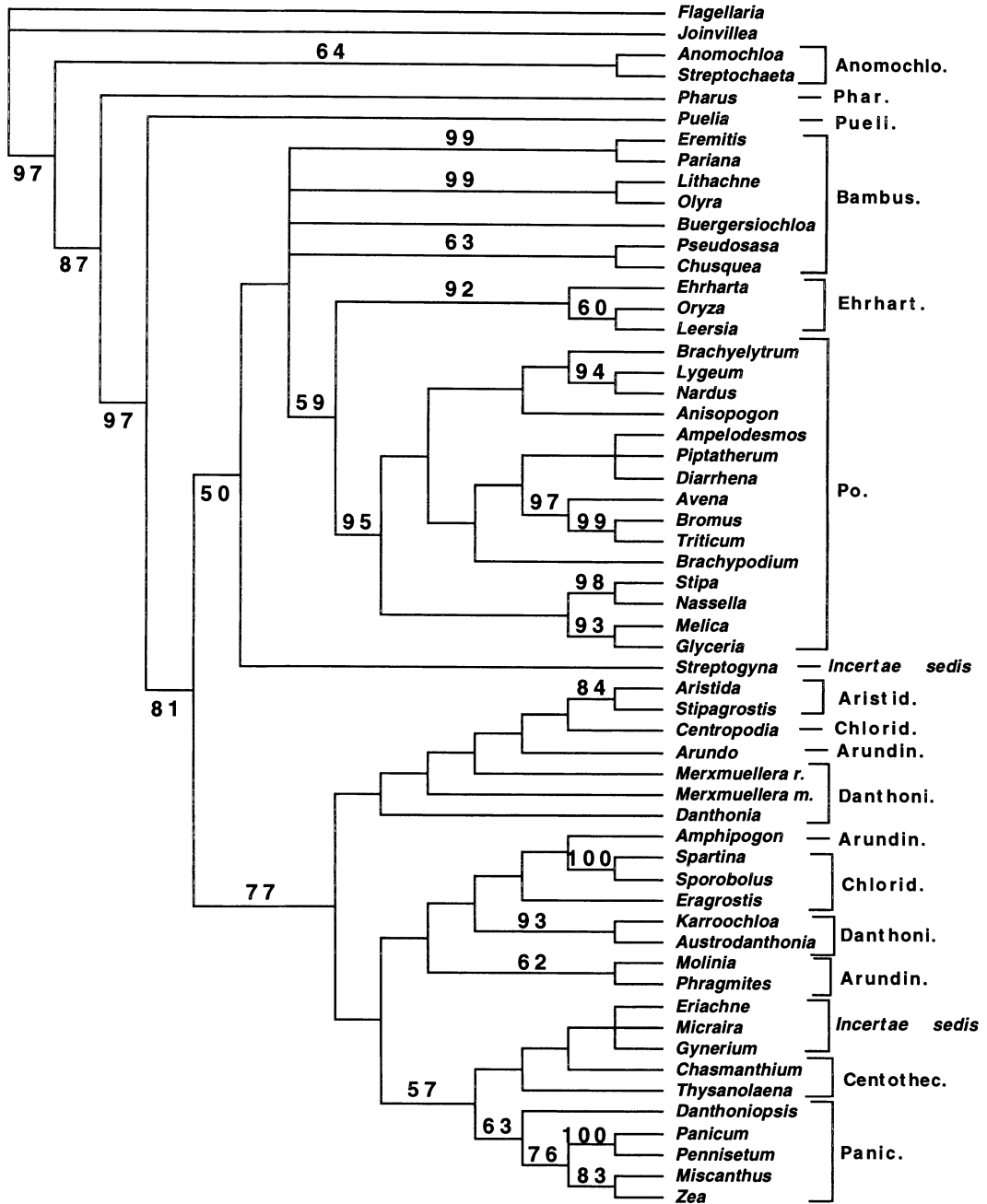
Appendix III—Figure G, GBSSI



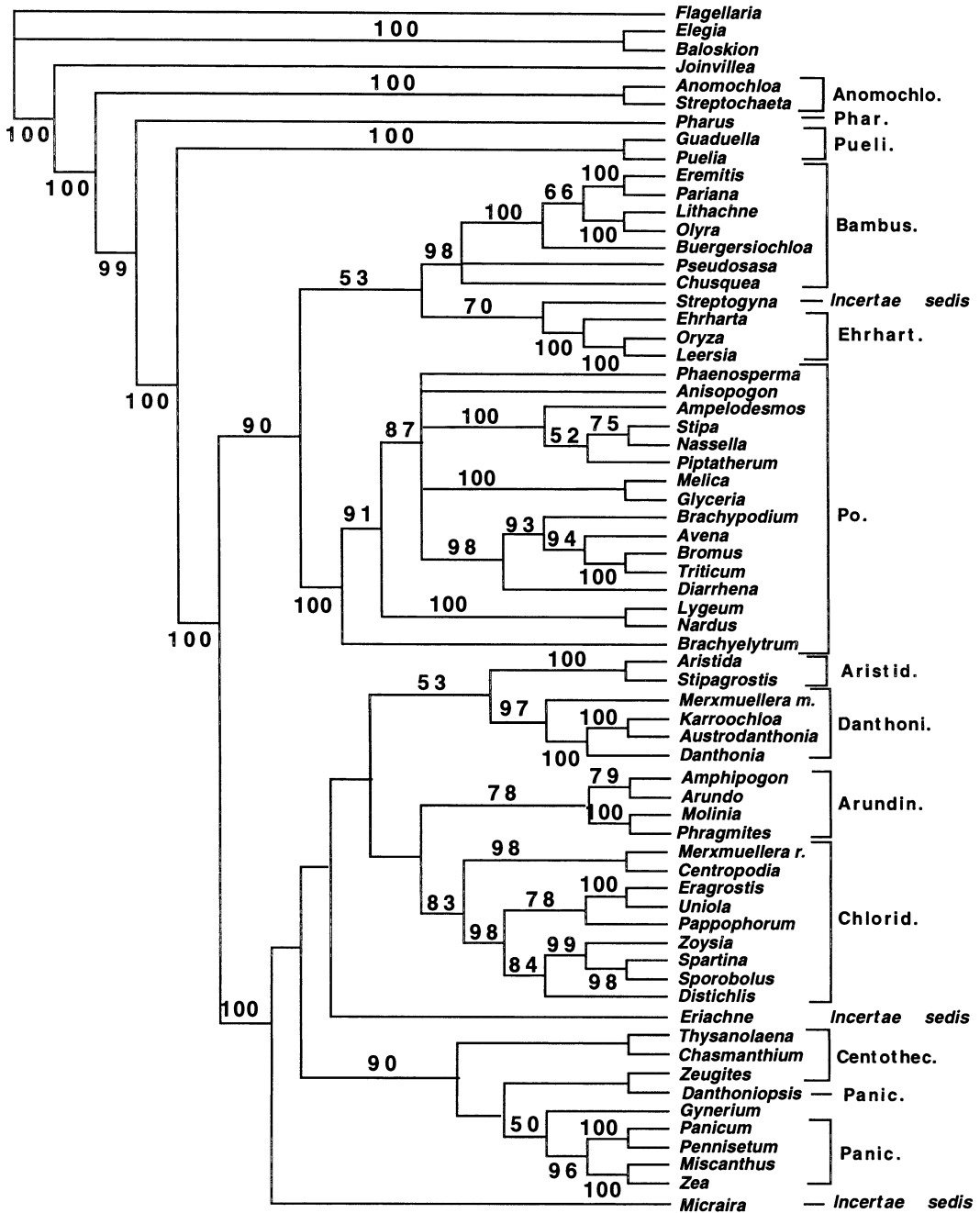
Appendix III—Figure H, structural



Appendix III—Figure I, all chloroplast



Appendix III—Figure J, all nuclear



Appendix III—Figure K, all molecular

## Appendix IV. Notes on morphological characters.

In this section the structural characters are defined and/or discussed, numbered as in Table 4, and their distribution on the most parsimonious tree is outlined. The behavior of each character on the most parsimonious tree is signified by a series of three numbers (number of steps, CI, and RI); p/a refers to presence/absence of a character.

## CULM

1 (4 steps, CI = 0.25, RI = 0.25). Perennating woody culms p/a: Highly lignified, perennial culms are absent among outgroups (except for the score of "uncertain" in *Flagellaria*) and in most grasses. While most, if not all, grasses produce some lignin in their culms, the distinction between "woody" and "herbaceous" is usually easy to draw, and our scoring was based on this qualitative criterion. Of the taxa in this analysis, presence is an unambiguous and unreversed synapomorphy of Bambuseae, and an autapomorphy of *Arundo*, *Thysanolaena*, and *Gynerium*; *Phragmites* is scored as intermediate.

2 (14 steps, CI = 0.07, RI = 0.18). Hollow culms p/a: This character is variable in the grasses (14 steps), and polymorphic in at least five of the sampled genera, and many additional ones. Occurrence of a small pore was scored as intermediate. Solid culms are uncommon and scattered in occurrence in the early-diverging lineages and the BEP Clade, but are frequent in the PACCAD Clade, where they are often associated with  $C_4$  photosynthesis. There are no unambiguous synapomorphies in this analysis and the character is highly homoplasious globally, but, as is well known among grass systematists, for many small groups of genera and species one state or the other of this character likely is a synapomorphy.

## LEAF

If the leaf blade is absent, characters 45 and 46 are considered inapplicable, but characters 3, 4, and 5 are scored.

3 (3 steps, CI = 0.33, RI = 0.33). Leaf sheath margins free/fused: Fused margins are an unreversed synapomorphy of Meliceae, and autapomorphies of *Flagellaria* and *Bromus*. Fused sheaths are frequent in Poeae and Aveneae and may provide a tribal or more local synapomorphy, or may be plesiomorphic. Sampling outside the grasses would help establish the point of origin of the free leaf sheath.

4 (5 steps, CI = 0.20, RI = 0.76). Adaxial ligule type: The membranous ligule is the most common state in the sample. Transformation to a fringe of hairs is an unreversed synapomorphy of Anomochlooideae, and a synapomorphy of the clade of *Eriachne* plus its sister group (a set of four subfamilies, Aristidoideae, Danthonioideae, Arundinoideae, and Chloridoideae), although the character reverses multiple times within this group. The ligule as a fringe of hairs also is an autapomorphy of *Danthoniopsis*.

5 (4 steps, CI = 0.25, RI = 0.25). Abaxial ligule p/a: Most grasses lack an abaxial ligule. Presence is an unreversed synapomorphy of Bambuseae, and an autapomorphy of *Puelia*, *Sireptogyna*, and *Thysanolaena*. Abaxial ligules occur sporadically in the PACCAD Clade, and are known in a few Pooideae.

6 (1 step, CI = 1.0, RI = 1.0). Leaf blade p/a: All sampled species have a leaf blade, except in Restionaceae, where loss of the blade is a synapomorphy. A few species in some grass genera, such as *Ehrharta*, lack a

developed blade, but these are clearly interpretable as losses.

7 (4 steps, CI = 0.25, RI = 0.80). Pseudopetiole p/a: The pseudopetiole is a constriction at the base of the leaf blade. Both states occur in the grasses and in the outgroups. Loss of the pseudopetiole is a synapomorphy of the clade that includes all grasses except Anomochlooideae, Pharoideae, and Puelioideae (i.e., the BEP + PACCAD clade). In the present cladogram, the pseudopetiole is interpreted as secondarily gained in Bambusoideae, *Thysanolaena* + *Zeugites*, and *Phaenosperma*.

## SPIKELET

8 (1 step, CI = 1.0, RI = 1.0). Floret p/a: The floret was defined for the morphological matrix as a unit of the grass inflorescence consisting of a subtending bract (= lemma) enclosing a short axillary axis bearing a flower, the first appendage of which is an adaxial, usually two-keeled bract (= palea). The floret is present only in grasses, but not in all grasses. Of the taxa included in this analysis, the floret is regarded as absent in *Streptochaeta* and of undetermined status in *Anomochloa*, based on the uncertain homologies of their floral bracts and the lack of an identifiable palea (Judziewicz & Soderstrom, 1989; Soreng & Davis, 1998). These two genera have flowers subtended by well-developed bracts, but not in any configuration that can be compared directly to the above definition. Gain of the floret is interpreted as a synapomorphy of the clade of all grasses except Anomochlooideae. Within the spikelet clade, the palea is absent in a number of taxa, including some species of *Agrostis*, Andropogoneae, and, in this analysis, *Zoysia*. All of these taxa, however, have an identifiable lemma and other congeners have paleas. Following a strict definition of the floret, *Zoysia* was scored as polymorphic for this character, although the phylogenetic context shows that a complete floret was present ancestrally.

9 (2 steps, CI = 0.50, RI = 0.50). Spikelet pairs: Spikelet pairs are infrequent in the grasses, and their origin is a synapomorphy of Andropogoneae in this analysis (but note that *Danthoniopsis* is regarded as polymorphic), although spikelet pairs are also found in some Paniceae. Their presence may also be an autapomorphy of *Pharus*, but since Anomochlooideae and non-grasses are not scored for this character, the placement of this transformation is ambiguous (i.e., paired spikelets could be interpreted as plesiomorphic among the floret-bearing grasses). Developmentally, spikelet pairing appears to occur in the same way wherever it appears in the Panicoideae (LeRoux & Kellogg, unpublished obs.), but developmental studies have not been done on *Pharus*, so spikelet pairing is an inference based on adult morphology alone.

10 (4 steps, CI = 0.25, RI = 0.0). Pedicel p/a: The pedicel is present in the earliest-diverging grass lineages that have spikelets. Multiple losses occur, but only the autapomorphic loss in *Triticum* can be placed unambiguously. Loss of the pedicel may be a synapomorphy of *Lygeum* + *Nardus*, but this is ambiguous because this character is scored as ambiguous for *Lygeum*.

11 (7 steps, CI = 0.14, RI = 0.45). Proximal female-sterile florets: Presence of proximal female-sterile florets is interpreted as an unreversed synapomorphy of Puelioideae and of Panicoideae (including *Danthoniopsis*, excluding *Gynerium*), and is a potential synapomorphy for Ehrhartoideae except that this is coded as ambiguous for *Leersia* and *Oryza*. Multiple origins occur elsewhere as



autapomorphies, e.g., in the traditional Aveneae (including Phalaridaeae), *Phragmites*, *Chasmanthium*, *Uniola*, and *Chusquea*, and there are no unambiguous losses once such flowers are gained. As noted in the discussion on spikelets, some proximal female-sterile florets may be homologous to glumes, as in Ehrhartoideae or some Bambuseae, whereas others, as in Panicoideae, are clearly derived from reduction of fertile florets.

12 (13 steps, CI = 0.07, RI = 0.47). Number of female-fertile florets per female-fertile spikelet: The plesiomorphic state among the grasses is one. Increase in the number of female-fertile florets has occurred multiple times, but the placement of these changes is ambiguous, in part because five genera are scored as polymorphic. The only unambiguous synapomorphic reduction from multiple female-fertile florets to one is for the clade of *Zoysia*, *Spartina*, and *Sporobolus*, although this may not hold up when sampling density is increased. Among unsampled taxa, there are numerous additional transformations to one floret.

13 (12 steps, CI = 0.08, RI = 0.52). Awn or mucro *p/a*: There are multiple origins of awns and mucros on the lemma, mostly of ambiguous placement. Approximately half the genera of the family have awns, so this is another example of a locally useful but globally highly homoplasious character. In *Streptochaeta*, eleven of the twelve bracts lack awns, whereas one (bract VI) has an awn (Judziewicz & Soderstrom, 1989). These bracts have been variously interpreted (Soderstrom, 1981), but the ones most closely associated with the flower lack awns, so interpretation of the single awned bract as a lemma is doubtful, and *Streptochaeta* consequently is scored as ambiguous for this character.

14 (4 steps, CI = 0.50, RI = 0.50). Number of awns present: This character is scored for taxa that are polymorphic for character 13, but it is inapplicable when character 13 is scored as state 0 or of questionable homology. *Danthonia* and *Austrodanthonia* are scored as state 3 to reflect the basic pattern in these taxa, in which the lemmas have nine veins, with the central vein forming a median, usually articulated or cork-screwed, awn, and the third vein out from the median on each side forms a hair-like awn or seta at the apex of its respective lateral lemma lobe. This seta varies from an acute lobe to a long hair-like extension. Occasionally the lateral lobes are fused with each other, and presumably with the base of the central awn. From the basic pattern all kinds of deviations occur, including fusion of the lateral lobes, loss of the setae, loss of the median awn, and fusion of the lateral lobes with the median awn still present as a stout mucro. In this analysis, presence of three awns is an unreversed synapomorphy of the clade of Aristidoideae + Danthoioideae, but this state also occurs in *Amphipogon* and *Anisopogon* and in other genera not sampled here such as *Plectrachne*, *Triodia*, and *Pentaschistis*, among others. Presence of numerous awns is an autapomorphy of *Pappophorum*.

15 (7 steps, CI = 0.28, RI = 0.44). Awn attachment: This character is scored for taxa that are polymorphic for character 13, but it is inapplicable when character 13 is scored as 0 or of questionable homology. Awn attachment at the apex of the lemma is the most common and widespread state of this character. Attachment in a sinus is an unreversed synapomorphy of Danthoioideae (although it appears to be attached at the apex of the lemma in some instances because of fusion of the lateral lobes with the base of the awn or loss of the lateral lobes; Linder &

Verboom, 1996; Linder & Davidse, 1997) and of *Merxmuellera rangei* + *Centropodia*. This state also occurs in *Streptogyna*, *Anisopogon*, *Bromus*, and some Panicoideae. Awn attachment on the back of the lemma is widespread in Aveneae; here it is an autapomorphy of *Avena*, the only taxon from the tribe in this analysis. Among unsampled grasses, dorsal attachment is known from one genus of Meliceae and a few genera of the PACCAD Clade including *Arthraxon*.

16 (6 steps, CI = 0.16, RI = 0.44). Disarticulation above glumes: Glumes are considered to be the two empty bracts subtending the spikelet, and glumes across the spikelet clade were assumed to be homologous even though there is disagreement on this point (see Discussion). This character is not scored in Anomochloioideae or in the non-grass outgroups, which lack spikelets and therefore glumes, but the presence of this type of disarticulation in at least some members of the Pharoioideae (e.g., *Leptaspis* and *Pharus*; Soderstrom et al., 1987), and in both genera of the Puelioideae, argue that this type of disarticulation is plesiomorphic in the Spikelet Clade. Synapomorphic loss of disarticulation above the glumes occurs in *Pariana* + *Eremitis* and in the clade within Panicoideae that consists of Paniceae + Andropogoneae. Additional losses, all autapomorphic or potentially so, occur in *Zoysia*, *Spartina*, *Phaenosperma*, and *Melica*.

17 (6 steps, CI = 0.16, RI = 0.37). Germination flap: The germination flap, a small flap of tissue at the base of the lemma through which the germinating embryo grows, is derived independently within the PACCAD and BEP Clades. There are unambiguous independent synapomorphic gains of the germination flap in Aristidoideae and Olyreae; germination flaps also are present in some Panicoideae (but placement of the transformation is ambiguous although no Andropogoneae have germination flaps), and the character is also autapomorphic in *Oryza* and in *Amphipogon*.

#### FLOWER

Characters 19–22 are scored as inapplicable for taxa that lack lodicules.

18 (4 steps, CI = 0.25, RI = 0.70). Lodicule *p/a*: Lodicules, as organs that become turgid at anthesis and force open the flower, occur in most grasses and are not present outside the grasses. The evidence that lodicules are modified tepals is not universally accepted. *Anomochloa*, *Streptochaeta*, and non-grasses were scored “uncertain homology” by Soreng and Davis (1998), but these two genera are scored as lacking lodicules in the present analysis. In the Pharoioideae, lodicules are present or absent in the male spikelets and lacking in female spikelets (Clark & Judziewicz, 1996). *Pharus* is scored here as having lodicules. Thus, the first unambiguous occurrence of lodicules is in the clade that consists of all grasses except Anomochloioideae. There is an unambiguous synapomorphic loss in *Lygeum* + *Nardus* and another in *Zoysia* + *Spartina* + *Sporobolus*, plus an autapomorphic loss in *Micraira*. Among unsampled grasses, 84 taxa lack lodicules; among these, 60 belong to the PACCAD Clade, and 13 to the Pooideae (Watson & Dallwitz, 1992). Lodicules (whatever their origin) might be plesiomorphic for the family, and then lost in the Anomochloioideae.

19 (5 steps, CI = 0.20, RI = 0.71). Number of lodicules: Three lodicules are present at the point of first unambiguous occurrence of lodicules (see char. 18), and this plesiomorphy is retained in Pharoioideae and Puelioideae.

Bambusoideae and *Streptogyna* also have three lodicules, while Ehrhartoideae have two. Transformation to two lodicules may be a synapomorphy of Ehrhartoideae, but cannot be inferred unambiguously because the earliest-diverging lineages of Pooideae also have two lodicules, as do all taxa of the PACCAD Clade that have been scored. Thus two lodicules may be a synapomorphy of the BEP + PACCAD group, with a reversal to three lodicules in the Bambusoideae/Ehrhartoideae group, and re-reversal to two lodicules in the Ehrhartoideae. Alternatively, two lodicules might have been retained in Ehrhartoideae (following the transformation from three to two in the origin of the BEP + PACCAD group) while *Streptogyna* and Bambusoideae independently experienced reversals to three; still other transformation sequences also are possible. Within Pooideae, an unambiguous transformation from two lodicules to three is a synapomorphy of the clade that includes *Anisopogon*, *Phaenosperma*, *Ampelodesmos*, and Stipeae, but within this group there is yet another unambiguous transformation back to two lodicules in *Nassella* (while *Stipa* is polymorphic).

20 (uninformative). Fusion of anterior pair of lodicules: Lodicules are unfused at the first point at which they are unambiguously present (see char. 18), and in almost all grasses. The anterior two are fused in *Melica*, but the character is polymorphic in *Glyceria*, so the transformation may be a synapomorphy of this pair of genera, but the precise placement is ambiguous. Elsewhere, *Molinia* also is polymorphic.

21 (5 steps, CI = 0.20, RI = 0.82). Distally membranous portion of lodicule: The earliest lodicules apparently had a distally membranous portion (see char. 18), and this state is retained in early-diverging lineages. Within the BEP Clade, loss of this membranous portion is an unreversed synapomorphy of Meliceae. Most elements of the PACCAD Clade lack a distally membranous portion of the lodicule, including various early-diverging lineages, but *Micraira*, because it lacks lodicules, is not scored for this character. Thus, transformation to this state may be a synapomorphy of the entire PACCAD Clade, or of the subset that includes all members except *Micraira*. Within the PACCAD Clade, a distally membranous lodicule in Aristidoideae, *Gynerium*, and *Merxmuellera macowanii* implies at least three additional steps in this character, including either three independent transformations to this lodicule type, or two independent gains including one in the common ancestry of Aristidoideae and Danthoniodeae, followed by a loss in the ancestor of *Danthonia*, *Karroochloa*, and *Austrodanthonia*. Polymorphism in *Panicum* and *Leersia* implies two additional transformations, a gain of the membranous portion of the lodicule within the former, and a loss within the latter.

22 (3 steps, CI = 0.33, RI = 0.83). Lodicule vascularization: Lodicules were originally vascularized (see char. 18). In the BEP Clade, a single unreversed loss of vascularization is inferred within Pooideae, after divergence of *Brachyelytrum* from the rest of the subfamily, but absence of lodicules in the *Lygeum* + *Nardus* group means that this transformation could have occurred before or after divergence of this group from the rest of the Pooideae. In the PACCAD Clade, an independent transformation to faint vascularization is an autapomorphy of *Amphipogon*. The state also occurs in *Thysanolaena*, but the association of the latter with *Zeugites*, which is not scored for this character, prevents an unambiguous placement of that transformation.

23 (5 steps, CI = 0.20, RI = 0.66). Posterior stamen

of the inner whorl: The posterior stamen of the inner whorl is present in all outgroups and among the earliest-diverging lineages in the grasses, so its presence is a plesiomorphy for the grasses. Loss of this stamen is a synapomorphy of the BEP + PACCAD clade, but it is regained at least three (possibly four) times in *Ehrharta*, *Oryza* (but not *Leersia*), *Pseudosasa*, and *Pariana*. The absence of this stamen in *Leersia*, coupled with its presence in *Oryza* and *Ehrharta*, is equally consistent with independent gains in the latter two, or a gain in the ancestor of Ehrhartoideae followed by a secondary loss in *Leersia*. This stamen is not gained elsewhere in the family.

24 (6 steps, CI = 0.16, RI = 0.54). Anterior stamen pair of the inner whorl: Except for the loss of this pair in *Anomochloa*, while the posterior stamen is retained, the distributions of states of these two characters (23 and 24) are identical. Thus, as with the posterior stamen of the inner whorl, this stamen pair is unambiguously interpreted as plesiomorphically present at the point of origin of the grasses, lost as a synapomorphy of the BEP + PACCAD clade, and regained three or four times in the Bambusoideae/Ehrhartoideae, possibly with a secondary loss in *Leersia*.

25 (8 steps, CI = 0.12, RI = 0.12). Anterior stamen of outer whorl: This stamen, though absent in Restionaceae, is present in *Flagellaria*, *Joinvillea*, and all early-diverging grass lineages, and thus is plesiomorphically present within the grasses, and lost independently in Restionaceae and various grass lineages. There are seven autapomorphic losses within the grasses (in *Streptogyna*, *Leersia*, *Eremitis*, *Glyceria*, *Piptatherum*, *Micraira*, and *Gynerium*), plus polymorphisms in *Buergersiochloa*, *Diarhena*, *Eriachne*, *Zoysia*, *Sporobolus*, and *Thysanolaena*. The loss in Restionaceae is the only unambiguous synapomorphic loss in the taxon sample.

26 (2 steps, CI = 0.50, RI = 0.50). Posterior stamen pair of outer whorl: This stamen pair, like the anterior stamen of the outer whorl, is present in all early-diverging grass lineages and in all outgroups except Restionaceae. Thus, like the anterior stamen and like all three stamens of the inner whorl, this pair is plesiomorphically present in the study sample as well as in grasses, and as with the anterior stamen of the outer whorl, all absences are interpreted as losses. Except for a few unscored taxa within the grasses, plus an autapomorphic loss in *Chasmanthium* and a polymorphism in *Sporobolus*, presence of this pair of stamens is constant within the grasses.

27 (1 step, CI = 1.0, RI = 1.0). Anthers tetrasporangiate, dithecal, vs. bisporangiate, monotheical: The presence of tetrasporangiate, dithecal anthers is interpreted as a plesiomorphy of the entire taxon sample, and of the grasses. The only transformation to bisporangiate, monotheical anthers is an unambiguous synapomorphy of Restionaceae.

28 (12 steps, CI = 0.08, RI = 0.31). Fusion of styles: The presence of one style (see char. 29) can be interpreted as either a fusion or reduction in number, so taxa scored with state 1 of character 29 are scored as ambiguous for character 28. Unfused styles in *Flagellaria* and both representatives of Restionaceae, and fused styles in *Streptochaeta*, *Pharus*, and *Puelia* together suggest that transformation to the fused state occurred near the origin of the grasses, but polymorphism in *Joinvillea* and the unknown state in *Anomochloa* prevent unambiguous placement of this transformation. Reversal to unfused styles is interpreted as an autapomorphy in *Guadua*, and as unreversed synapomorphies (i.e., lacking secondary reversion to

fused styles) of Pooideae and Ehrhartoideae (except for polymorphism in *Oryza*). Within the PACCAD Clade, fused styles appear to be plesiomorphic, with one or more transformations to the nonfused state, and multiple reversals to the fused state (e.g., in *Phragmites*, *Uniola*, and *Spartina*).

29 (6 steps, CI = 0.33, RI = 0.50). Number of stigmas: The plesiomorphic state for the grasses, as well as for the entire taxon set, is three, with autapomorphic transformations to two in *Baloskion* and, among earliest-diverging grass lineages, to one in *Anomochloa*. Transformation to two stigmas is a synapomorphy of the clade that includes all grasses except Anomochlooideae and Pharoideae (i.e., the Bistigmatic Clade), and there are additional transformations to one in *Eremitis* and in *Lygeum* + *Nardus*, and to three in *Pseudosasa*. Another transformation to one appears to occur in *Eremitis*, but this is the result of a miscoding, as there are actually two stigmas in *Eremitis* (V. Hollowell, pers. comm.). *Puelia* is actually polymorphic for this character, having two or three stigmas (the species in this analysis has two). Other polymorphisms (see data table) signify additional transformations. All members of the PACCAD Clade have two stigmas.

30 (8 steps, CI = 0.25, RI = 0.50). Highest order of stigmatic branching: Only one order of stigmatic branching occurs in *Flagellaria*, *Joinvillea*, Anomochlooideae, and Pharoideae, and this state therefore appears to be plesiomorphic for the grasses and for the entire taxon sample, with the various states in Restionaceae interpretable as apomorphic within that family. Transformation to two orders of branching appears to be a synapomorphy for the clade that includes all grasses except Anomochlooideae and Pharoideae (the Bistigmatic Clade), and this state, once established, is constant in Puelioideae and nearly so in the PACCAD Clade (exceptions being the presence of state one in *Zea*, and polymorphism in *Sporobolus*). Within the Bambusoideae/Ehrhartoideae, transformation to state one is an autapomorphy of *Streptogyna* and a synapomorphy of *Pariana* + *Eremitis*, while a transformation to state 3 is an autapomorphy of *Leersia*, and polymorphisms occur in unsampled Olyreae. Within the Pooideae, there is an autapomorphic transformation to state one in *Nardus*, a synapomorphic transformation to state three in Meliceae, and polymorphism in *Phaenosperma*.

31 (3 steps, CI = 0.66, RI = 0.0). Number of locules: All grasses have one locule with one ovule, while three locules, each with one ovule, are found in *Flagellaria* and *Joinvillea*, although there is a strong tendency to abort one or two of the ovules in both of these genera. A transformation from three locules to one may be a synapomorphy of the grasses. This transition has also occurred in Restionaceae, where the basal condition is three locules with numerous reductions to a single functioning locule, as in *Elegia* in our sample (Linder, 1992a, b). Although not sampled in this study, Anarthriaceae have three locules each with one ovule, but the fruit is 1-seeded, and Ecdiocoleaceae have two locules, each with one ovule, and the fruit is 1–2-seeded. Thus, reduction in both number of locules and number of ovules that develop in the fruit are common in the Poales; Centrolepidaceae, however, have uniloculate, uniovulate ovaries that are apparently monocarpellary (Dahlgren et al., 1985).

#### EMBRYOGENY

32 (1 step, CI = 1.0, RI = 1.0). Haustorial synergids p/a: Data are unavailable for many taxa, but haustorial

synergids are observed to be present only in the four sampled taxa of the Danthonioideae, and absent in all other taxa that have been examined (Verboom et al., 1994). Thus, presence of haustorial synergids is an unambiguous and unreversed synapomorphy of Danthonioideae, but continued investigation is warranted.

#### FRUIT AND EMBRYO

Characters 35–38 describe features of the typical grass-type embryo. They are inapplicable for non-grass genera, which lack the grass-type embryo (i.e., state 0 of char. 34). Data sources include those listed by Soreng and Davis (1998) plus Klak (unpublished). These characters can be difficult to score, and the literature contains conflicting reports for some taxa.

33 (5 steps, CI = 0.20, RI = 0.76). Hilum shape: This character is recognized as a feature of the caryopsis, and thus is inapplicable for non-grasses. Among grasses, taxa with a short hilum less than one-third the length of the grain are scored as ambiguous. All groups except the PACCAD Clade have a long hilum that is greater than one-third the length of the grain in our sample. This may thus be a synapomorphy of grasses (i.e., the plesiomorphic state for the caryopsis), but because the character is treated as inapplicable outside the grasses there is no observed transformation at the origin of the family. Nonlinear hila are reported from the Olyreae and the Poaeae/Aveneae in the BEP Clade. There are five character transformations within the PACCAD Clade, but the placements of two of these are ambiguous. All Centothecoideae and most Panicoideae have a nonlinear hilum that is less than ½ the length of the grain, but the hilum is long-linear and greater than ½ the length of the grain in *Danthoniopsis*, and this is interpreted as one origin of this state in the common ancestor of the clade, followed by a reversal in *Danthoniopsis*. Elsewhere in the PACCAD Clade, the only unambiguous transformation of this character is as a synapomorphy of *Karroochloa* + *Austrodanthonia*, a subset of Danthonioideae. This state also occurs in all taxa of Arundinoideae and Chloridoideae for which there are observations, except in *Molinia*. Thus, it is interpretable either as having arisen twice (once in *Amphipogon* + *Arundo*, and once in Chloridoideae), or as a synapomorphy of Arundinoideae + Chloridoideae, with reversion to a long-linear hilum in *Molinia* or in *Molinia* + *Phragmites* (not scored for *Phragmites*).

34 (1 step, CI = 1.0, RI = 1.0). Grass-type embryo p/a: The grass-type embryo is lateral, peripheral to the endosperm, and differentiated in fruit (Reeder, 1957; Cronquist, 1981; Sendulsky et al., 1987). This embryo type is absent outside the grass family and present in all grasses for which observations are available (unobserved in *Merxmüllera rangei*), and thus interpreted as an unambiguous and unreversed synapomorphy of the grasses.

35 (7 steps, CI = 0.14, RI = 0.66). Epiblast p/a: Because this character is inapplicable for non-grasses, its origin is not unambiguously fixed, but available data for the earliest-diverging lineages within the grasses (except *Streptochoeta*) indicate presence of an epiblast, and thus presence is interpretable as a plesiomorphy within the family. Loss of the epiblast is an unambiguous synapomorphy of the PACCAD Clade, but there are also three autapomorphic losses outside of the PACCAD Clade in *Streptochoeta*, *Ehrharta*, and *Bromus*, plus a polymorphism in *Brachypodium*. Within the PACCAD Clade the epiblast is secondarily gained in the Centothecoideae (but

there is no observation for *Thysanolaena*) and is a synapomorphy for either the Chloridoideae or a subset of that clade. The precise point of origin within the Chloridoideae is ambiguous because there is no observation for *Merxmuellera rangei* or *Centropodia*. Within the Chloridoideae the epiblast is lost in *Uniola*. This character is highly homoplasious within the family.

36 (3 steps, CI = 0.33, RI = 0.81). Embryo scutellar tail p/a: The scutellar tail is present in Anomochloideae and Pharoideae, throughout the PACCAD Clade (wherever observations are available) and in most taxa of the Bambusoid/Ehrhartoid clade, absent in most Pooideae, and unobserved in Puelioideae. Despite the variation just described, plus the inapplicability of this character outside the grasses and the absence of data for some critical taxa, the balance of evidence suggests that the scutellar tail is a plesiomorphy of the grasses. Within the Bambusoid/Ehrhartoid clade, loss of the scutellar tail is unambiguously interpreted as an unreversed synapomorphy of Oryzae. A more complex pattern is present in Pooideae, where the scutellar tail is usually absent, but is present in *Phaenosperryma*, and there are polymorphisms or conflicting reports for *Brachyelytrum*, *Diarrhena*, and *Avena*. These polymorphisms in the matrix prevent unambiguous optimization of the character in Pooideae. Loss of the scutellar tail may be a synapomorphy of Pooideae or of all Pooideae except *Brachyelytrum*; presence of the scutellar tail in *Phaenosperryma* may be either a unique reversal or a synapomorphy for *Phaenosperryma* and *Anisopogon* (which is not scored).

37 (2 steps, CI = 0.50, RI = 0.95). Embryo mesocotyl internode, negligible vs. elongated: The embryo mesocotyl internode is negligible in length in all early-diverging grass lineages for which scores are available (e.g., Anomochloideae, Pharoideae), so although this character is inapplicable for the non-grass outgroups the internode is likely to have been negligible at the origin of the grass-type embryo. All taxa that have been scored in the PACCAD Clade have an elongated internode. Transformation from a negligible to an elongated internode is either a synapomorphy of the entire PACCAD Clade or of the entire clade except *Micraira*, for which no observation is available.

38 (4 steps, CI = 0.25, RI = 0.85). Embryonic leaf margins meeting vs. overlapping. The margins of the embryonic leaf meet in *Anomochloa* but overlap in *Streptochaeta*, *Pharus* (unobserved in Puelioideae), and early-diverging lineages of both the BEP and PACCAD Clades. Thus, although the character is inapplicable in the non-grass outgroups, overlapping leaf margins are plesiomorphic at the origin of the grass-type embryo, with an autapomorphic transformation to margins meeting in *Anomochloa*, and with parallel synapomorphic transformations in both major lineages. Margins are overlapping in all observed taxa in the bambusoid/ehrhartoid alliance, as well as in *Brachyelytrum* and *Phaenosperryma* of the Pooideae (also, *Diarrhena* is polymorphic). Given this distribution, transformation to the margins meeting is interpreted as a synapomorphy of all Pooideae except *Brachyelytrum*, with a reversal either in *Phaenosperryma* or in the ancestor of *Phaenosperryma* and *Anisopogon* (there is no observation for the latter). All observations for Panicoidae and Centothecoideae, plus *Eriachne*, are of leaf margins overlapping, while all observations for the other four subfamilies of the PACCAD Clade are of leaf margins meeting. Thus, transformation to the latter state is an unambiguous and unreversed synapomorphy of the sister

group of *Eriachne*, the clade that includes Aristidoideae, Danthonioideae, Arundinoideae, and Chloridoideae.

39 (uninformative). Endosperm lipid p/a: Observations are unavailable for several taxa. Of those taxa that are scored, including *Baloskion*, only *Avena* has lipid in the endosperm. Thus, absence of lipid in the endosperm is plesiomorphic for the grass family and for the taxon set as a whole, with the presence of lipid in *Avena* an autapomorphy. Among unsampled grasses, all reports of endosperm lipid are from the Poaeae-Aveneae. Liquid and semi-liquid endosperm are indicative of the presence of lipid, but "semi-solid" and solid states do not imply absence of lipid (Terrell, 1971; Rosengurt et al., 1972).

40 (12 steps, CI = 0.33, RI = 0.20). Starch grain syndromes: Scoring here follows Tateoka's (1962) classification with one exception. Tateoka scored *Brachyelytrum* as having simple *Panicum*-type grains, but emphasized a major size difference, and we recognize *Brachyelytrum*-type as a separate state (see also Campbell et al., 1986). Watson and Dallwitz (1992) distinguished between starch grains "simple only" (coded here as [034]) or "compound" (coded here as [12]). Polymorphisms, ambiguity of state delimitation, and lack of observations together preclude unambiguous optimizations of many character-state transformations in this multistate character, but some patterns are evident. First, the *Festuca*-type grain (state 1) is present in *Baloskion* (the only non-grass that is scored) and is widespread in early-diverging grass lineages, among which other types are not observed. This pattern suggests that this starch grain syndrome is plesiomorphic for the grass family and for the taxon set as a whole. The *Triticum*-type syndrome (state 0) occurs in most "core" Pooideae (represented here by *Brachypodium*, *Avena*, *Bromus*, and *Triticum*) that are collectively the sister group of *Diarrhena*, and state 0 may be either a synapomorphy of this group (reversed, however, in *Avena*) or a parallelism that arises separately in *Brachypodium* and in the ancestor of *Bromus* + *Triticum*. The *Panicum*-type syndrome (state 3) may be a synapomorphy of Bambuseae, of *Stipa* + *Nassella*, and of all Panicoidae except *Danthoniopsis*, or of the entire PACCAD Clade. If the latter is true, then there is a reversal to the *Festuca*-type in the clade of *Eriachne* plus the set of four subfamilies that is its sister; the *Festuca*-type is also a potential synapomorphy of *Thysanolaena* + *Zeugites*, or an autapomorphy of *Zeugites*. The *Brachyelytrum*-type syndrome occurs in *Phaenosperryma* and *Brachyelytrum*. Available information suggests that *Stipa* may also have this state (see Soreng & Davis, 1998).

#### SEEDLING

41 (4 steps, CI = 0.25, RI = 0.70). Lamina of first seedling leaf p/a: The lamina of the first seedling leaf is absent in *Flagellaria* and Anomochloideae, and present in Restionaceae and Pharoideae, while the character is unobserved in other non-grass taxa and other early-diverging lineages within the grasses. Consequently, optimization of this character is ambiguous in this region of the tree. However, presence of the lamina is unambiguously established by the point of divergence of Pharoideae from the lineage that includes most other grasses; it is present in the PACCAD Clade and in Pooideae, but is lost twice within the BEP Clade, as a synapomorphy of Oryzae and as a synapomorphy of Bambusoideae.

#### VEGETATIVE ANATOMY

42 (uninformative). Differentiation of leaf epidermal cells into long and short cells: Differentiation is absent in

*Flagellaria*, inapplicable in Restionaceae (blades absent), and present in *Joinvillea* and all grasses (except for a few that are polymorphic). This differentiation is therefore established by the time of divergence of *Joinvillea* from the grasses, but the point of origin is ambiguous.

43 (6 steps, CI = 0.16, RI = 0.72). Multicellular microhairs p/a: Presence of multicellular microhairs on the abaxial surface of the leaf blades in *Joinvillea*, Anomochloideae, and *Guaduella*, and their absence in Restionaceae, Pharoideae, and *Puelia*, make it difficult to place the origin (or origins) of multicellular microhairs, but they appear to have been present in the common ancestor of *Joinvillea* and the grasses, and are inferred to have been lost independently (among the aforementioned taxa) in Pharoideae and *Puelia*. Multicellular microhairs are universally present within the PACCAD Clade in our sample, except for an autapomorphic loss in *Merxmuellera rangei* and a polymorphism in *Spartina*. The score for *M. macowanii* was inferred from reports for the rest of the genus; Ellis (1981) reported the absence of microhairs on the abaxial epidermis but did not investigate the adaxial epidermis, where they are most likely to occur. We note, however, that multicellular microhairs have not been detected on the abaxial leaf surface in some species of 40 PACCAD genera, but many of these genera are polymorphic for this character (Watson & Dallwitz, 1992). In contrast with their widespread occurrence throughout most of the family, multicellular microhairs occur in only two genera of the Pooideae, *Lygeum* and *Nardus*. Either microhairs were lost in the common ancestor of the subfamily, followed by a secondary gain in the common ancestor of *Lygeum* and *Nardus*, or were lost twice, once in *Brachyelytrum*, the other time in the ancestor of all pooids except *Brachyelytrum*, *Lygeum*, and *Nardus*. Some taxa reported to lack multicellular microhairs on the abaxial surface of the leaf blades may have such hairs on the adaxial leaf surface or elsewhere on the plant, particularly lemmas or lodicules, and more detailed examination should be undertaken to verify this. Only the clade within Pooideae that is the sister of *Lygeum* + *Nardus* lacks multicellular microhairs entirely, but even in this clade, unicellular microhairs have been reported in several genera of Stipeae (Watson & Dallwitz, 1992).

44 (3 steps, CI = 0.33, RI = 0.71). "Chloridoid-type" microhairs p/a: Tateoka et al. (1959) distinguished microhairs with short and wide apical cells as "Chloridoid-type," as they are mainly restricted to subfamily Chloridoideae. This type of hair is contrasted with "panicoid-type" microhairs, which have relatively longer and thin-walled terminal cells and are widespread among non-Chloridoid grasses. This distinction has been recognized in subsequent studies (Johnston & Watson, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992). Our scoring of presence vs. absence of chloridoid-type microhairs implies that the two types are clearly distinguishable, but in fact there is a continuum of variation between them (Van den Borre, 1994; Van den Borre & Watson, 1994; E. A. Kellogg, unpublished data). In future analyses this character should be considered very carefully, as many taxa may actually be intermediate or polymorphic. This character is inapplicable for taxa scored 0 for character 43. Absence of chloridoid microhairs is plesiomorphic for the family. This type of hair is gained in *Lygeum*, in *Amphipogon*, and as a synapomorphy in Chloridoideae as traditionally circumscribed (i.e., excluding *Centropodia* and *Merxmuellera rangei*). This gain in the Chloridoideae is unreversed, except for a polymor-

phism in *Eragrostis* (Van den Borre, 1994; Van den Borre & Watson, 1994).

45 (8 steps, CI = 0.12, RI = 0.46). Arm cells p/a: Arm cells are invaginated chlorenchyma cells that, when present, reach their maximum development in the layer of chlorenchyma beneath the upper surface of the leaf. Variation in arm cells in the Poaceae is known but has not been investigated, and only presence/absence was scored for this analysis. Presence in *Anomochloa*, Pharoideae, and Puelioideae (including a polymorphism in *Guaduella*), and absence in *Streptochoeta*, combined with absence outside the Poaceae, together suggest a first occurrence of arm cells near the origin of the family, but optimization of the transformation to arm cells at the point of origin of the family is ambiguous. Placement of additional gains and losses is complicated by a widespread occurrence in the bambusoid and ehrhartoid clades, polymorphism within *Ehrharta* and *Leersia*, total absence in Pooideae, and sporadic occurrence in the PACCAD Clade (in *Thysanolaena*, *Cynerium*, and *Phragmites*). This overall distribution is consistent with a variety of optimizations that imply multiple origins and losses. Some taxa with arm cells lack fusoid cells, and vice versa, but the occurrence of both cell types is correlated with broad leaf blades and the forest habitat.

46 (4 steps, CI = 0.25, RI = 0.76). Fusoid cells p/a: Fusoid cells are large, clear, cigar-shaped empty cells that flank each vascular bundle and can occupy up to 30% of the leaf blade volume. Although their function is still unknown, they appear to form gas spaces rather than liquid spaces and may play some role in photosynthesis (Clark, 1991). Fusoid cells are absent outside the Poales and, among the families sampled in this analysis, are also absent in Flagellariaceae. They are present in *Joinvillea* and all three of the early-diverging lineages within the grasses. Thus, presence of fusoid cells may be a synapomorphy of *Joinvillea* and Poaceae, but the precise point of origin of fusoid cells is ambiguous in the present analysis, in part because the character is scored as inapplicable in Restionaceae. Elsewhere in the grasses, fusoid cells occur only in Bambusoideae and *Streptogyna*. Fusoid cells are absent in Ehrhartoideae, Pooideae, and the PACCAD Clade, and this pattern is interpretable either as a synapomorphic loss in BEP + PACCAD (followed by secondary gain in *Streptogyna* and Bambusoideae), or parallel losses in Ehrhartoideae, Pooideae, and the PACCAD Clade. Fusoid-like cells are known in some Paniceae, but these appear to be derived from laterally extended bundle sheath cells and thus are not homologous to fusoid cells.

#### CHROMOSOMES

47 (23 steps, CI = 0.34, RI = 0.44). Base chromosome number: This multistate character varies among taxa, is scored as ambiguous in some, and is polymorphic in others, so the positions of most transformations cannot be reconstructed unambiguously. However, the base number  $x = 12$  in Pharoideae, Puelioideae, the BEP Clade, and the PACCAD Clade, suggests early establishment of this state. If the base numbers 11 (in *Streptochoeta*) and 18 (in *Joinvillea* and *Anomochloa*) are derived from 12, then  $x = 12$  may predate the origin of the grass family, but other reasonable interpretations also are possible. Nevertheless, our data support the interpretation that  $x = 12$  was established prior to divergence of Pharoideae and Puelioideae from the BEP + PACCAD lineage, and that this base number was maintained in some sublineages of both the BEP and PACCAD Clades. This number is main-

tained (or re-evolved) in *Streptogyna*, Ehrhartoideae, and some Bambusoideae, and transformations to other base numbers occur within the Bambusoideae (e.g.,  $x = 11$  in Olyreae). Within Pooideae, the occurrence of  $x = 10$  in *Lygeum*, *Diarrhena*, and some Meliceae suggests that this number was established early in the history of this subfamily (with  $x = 11$  in *Brachyelytrum* possibly derived from  $x = 10$  as well, but also possibly derived directly from  $x = 12$  in the common ancestor of Pooideae and the bambusoid/ehrhartoid lineage).  $x = 12$  in *Phaenosperma*, *Ampelodesmos*, and some Stipeae is interpreted here as a secondary transformation from  $x = 10$ , but placement of these lineages as early-diverging groups within Pooideae, and the occurrence of  $x = 12$  in other early-diverging lineages, suggests that this state may be a plesiomorphy retained from its original establishment near or before the point of origin of the family. Transformation to  $x = 7$  is a synapomorphy of the clade that includes *Brachypodium*, *Avena*, *Bromus*, and *Triticum*, which suggests that all other numbers within the tribes represented by this sample of genera (including  $x = 5$  in some *Brachypodium*, and various numbers in Aveneae and Poeae) are derived from  $x = 7$ . Poeae are not sampled in this study, but they would undoubtedly be placed within the  $x = 7$  clade. In the PACCAD Clade, the base number  $x = 12$  occurs in several disparate taxa and is interpreted as the plesiomorphic state of this clade. Among the many other base numbers in the PACCAD Clade, there are a few unambiguous autapomorphic transformations (e.g., to  $x = 9$  in *Molinia* and *Sporobolus*), but only one unambiguous synapomorphic transformation, to  $x = 11$ , in Aristidoideae.

#### BIOCHEMISTRY

48 (7 steps, CI = 0.57, RI = 0.75). Carbon fixation pathways: All of the non-grass outgroups and all grasses outside the PACCAD Clade share the  $C_3$  photosynthetic pathway. Taxa with all five  $C_4$  subtypes, as well as additional  $C_3$  taxa, occur within the PACCAD Clade. These are intermixed to such an extent that this character would be homoplasious even if all subtypes of  $C_4$  carbon fixation had been scored as a single state. One unambiguous point of origin of  $C_4$  carbon fixation is the transformation to NADP-ME decarboxylation (state 1) as a synapomorphy of Panicoideae (although recent studies on the phylogeny of Panicoideae show that even this is ambiguous (Giussani et al., in press)). The occurrence of NADP-ME decarboxylation (state 3) in *Panicum* is interpreted as a secondary transformation from NADP-ME. Outside of the Panicoideae and the Chloridoideae (discussed below), the occurrence of two different types of  $C_4$  decarboxylating enzymes in taxa with and without mesostome sheaths (states 2, 3, and 5, in *Aristida*, *Stipagrostis*, and *Eriachne*, respectively) requires three additional transformations, but various sequences of transformation among states can explain the variation observed in these three genera. Finally, the predominant occurrence of the NADP-ME type of decarboxylation in Chloridoideae, including *Centropodia* but not *Merxmuellera rangei* (which has  $C_3$  photosynthesis), suggests a synapomorphic gain of this syndrome in the ancestor of Chloridoideae, followed by a return to  $C_3$  photosynthesis in *Merxmuellera rangei*, but multiple gains of the NADP-ME subtype also are possible. Reversal from  $C_4$  to  $C_3$  also occurs in *Eragrostis walteri* (van den Borre, 1994). State 4, the NADP-ME *Arundinella*-type, occurs in

*Arundinella*, some species of *Microstegium*, *Arthraxon*, and in some species of *Danthoniopsis*, which is here scored as polymorphic.

49 (1 step, CI = 1.0, RI = 1.0). PCK-type carbon fixation, *p/a*: This syndrome arises once, as an unambiguous transformation from the "normal" NAD-ME  $C_4$  syndrome in a subclade of the Chloridoideae. *Zoysia* and *Spartina* exhibit PCK-type carbon fixation, and *Sporobolus* is polymorphic for presence/absence of this character.

#### INDEL IN PHYTOCHROME B

50 (2 steps, CI = 0.50, RI = 0.92). 3-bp deletion in phytochrome, *p/a*: The deleted genotype (state 1) occurs in all sampled taxa of the PACCAD Clade, while the undeleted genotype (state 0) occurs in all other sampled grasses. *Joinvillea* has a 3-bp deletion and *Flagellaria* a 12-bp deletion in this region. *Sesleria* (not included in the present taxon sample) also has a 3-bp deletion (Mathews et al., 1995). The deletion occurs in a region of exon I characterized by extensive length and nucleotide variability, and it seems likely that grasses outside the PACCAD Clade have a synapomorphic insertion and that the deletion in *Sesleria* is apomorphic. Under this reconstruction, the deleted genotype in the PACCAD Clade would be synapomorphic, but the absence of data for *Micraira* precludes unambiguous optimization of the transformation, which is either a synapomorphy of the entire PACCAD Clade (including *Micraira*) or of the subclade that consists of all taxa except *Micraira*.

#### CHLOROPLAST GENOME STRUCTURE

51 (1 step, CI = 1.0, RI = 1.0). 6.4 kb inversion in the chloroplast genome, *p/a*: Absence of the inversion in *Flagellaria* and *Baloskion* (no information for *Elegia*), coupled with presence in *Joinvillea* and all grasses that have been sampled, allows unambiguous optimization of this character as a synapomorphy of *Joinvillea* plus Poaceae.

52 (1 step, CI = 1.0, RI = 1.0). *trnT* inversion in the chloroplast genome, *p/a*: Absence of this inversion in *Flagellaria* and *Joinvillea* (no information for either genus of Restionaceae in the present study), coupled with presence in all grasses that have been sampled, allows unambiguous optimization of this character as a synapomorphy of Poaceae. Although this character has not been scored for *Elegia* or *Baloskion*, it has been scored in another genus of Restionaceae, *Chondropetalum* (Doyle et al., 1992), and absence of the inversion in that genus is consistent with the interpretation that this inversion is a synapomorphy of Poaceae.

53 (3 steps, CI = 0.33, RI = 0.71). 15 bp *ndhF* insertion, *p/a*: This insertion was previously reported as present in all sampled grasses except Anomochloideae, Pharoideae, and Oryzeae (though present in Ehrharteae), and absent outside the Poaceae (Clark et al., 1995). New sequences reported here confirm this distribution except for the presence of the insertion in *Elegia*. Thus, the insertion arises independently as an autapomorphy of *Elegia* and as a synapomorphy of the Bistigmatic Clade (all grasses except Anomochloideae and Pharoideae); it is secondarily lost (i.e., deleted) in the ancestor of (or within) the Oryzeae. Examination of this character in other Restionaceae and related families such as Anarthriaceae, Edeicolaaceae, and Centrolepidaceae is warranted.