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PHYLOGENY OF THE GRASSES (POACEAE) REVISITED

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ABSTRACT

The most robust previously published phylogeny for the overall structure of the grass family (Poaceae) shows three early diverging lineages and two major derived clades, the BEP clade and the PACCAD clade (Grass Phylogeny Working Group 2001). A few key taxa were incompletely sampled, however, and support for the BEP clade was moderate at best and relationships among the major lineages within the PACCAD clade remained unresolved. In addition, recent studies indicated that the sister group to Poaceae may be Joinvilleaceae and/or Ectociaceae, the latter of which were not previously sampled. In this study, missing structural data were determined and analyzed as well as sequence data for *ndhF* and *rbcL*, the two most complete plastid sequence data sets. Sampling was increased with a particular focus on key taxa such as *Danthoniopsis*, *Eriachne*, *Micraira*, and *Streptogyna* and a representative of the outgroup, Ectociaceae. A total of 61 ingroup and two outgroup taxa were analyzed using maximum parsimony for total data, and maximum parsimony, Bayesian inference, and neighbor joining for the molecular data. A strongly supported clade of ((*Eriachne*, *Isachne*) *Micraira*) was recovered as a sister subfamily to Arundinoideae and excluded from Panicoideae. *Arundinaria* was strongly united with Bambusoideae. The position of *Streptogyna* was weakly supported among Ehrhartoideae, and is still unresolved. An outgroup effect on ingroup topology was observed demonstrating that highly divergent outgroups may unpredictably alter ingroup relationships.

Key words: Bayesian inference, Grass Phylogeny Working Group, MrBayes, *ndhF*, phylogeny, Poaceae, *rbcL*.

INTRODUCTION

During the past decade our knowledge of plant evolution has been substantially advanced by the professional cooperation of scientists in the botanical community. The Grass Phylogeny Working Group (GPWG) was founded in 1996 to advance knowledge in the field of grass systematics and evolution. A major goal of the GPWG was to infer a broad phylogeny for Poaceae by assembly and analysis of structural data and DNA sequences from multiple genes. A data matrix comprising six molecular sequence data sets including both plastid and nuclear loci, plastid restriction sites data and structural data, were assembled and posted at a web-accessible site together with the results of total-evidence phylogenetic analyses (now posted at www.umsl.edu/services/kellogg/gpwg/default.htm). Further collaborative work identified a growing consensus phylogeny (GPWG 2000).

Due to the strong support for portions of this phylogeny and the clear need for a revised classification of the family, a subfamilial classification of the grasses was devised based on the comprehensive phylogenetic analyses (GPWG 2001). Eleven previously published subfamilies and one new subfamily were recognized in this scheme. Support was assessed for two informal groups, the PACCAD clade (Panicoideae,

Arundinoideae s.s., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae, *Eriachne* R. Br., *Gynerium* Willd. ex P. Beauv. and *Micraira* F. Muell.) and the BEP clade (Bambusoideae, Ehrhartoideae, and Pooideae). This classification has been influential in other treatments (e.g., Judziewicz et al. 2000; Peterson et al. 2001; Barkworth et al. 2003; Soreng et al. 2003; Zuloaga et al. 2003).

Another goal of this collaboration was to help focus the further development of existing data sets in terms of taxon sampling and methods of analysis. This study continues that goal with special emphasis on acquisition of plastid sequence data missing from the GPWG (2001) study. In that study certain taxa (i.e., *Eriachne*, *Gynerium*, *Micraira*, and *Streptogyna* P. Beauv.) were treated as incertae sedis because of poorly supported or incompletely resolved phylogenetic placements in separate and combined analyses of the data sets. An issue for these problematic taxa was that data were incomplete. Here we address that issue by determination and analysis of sequences missing in the original matrix for these taxa as well as for *Danthoniopsis* Stapf (Danthonioideae). Moreover, data for species of two additional genera, *Isachne* R. Br. (classified in Panicoideae by GPWG 2001) and *Arundinaria* Michx. (Bambusoideae), were added to explore the effect of increased sampling of these critical taxa on selected points in the phylogeny.

New information on the phylogenetic position of Ectociaceae has been published based on a study of structural and molecular data (Michelangeli et al. 2003). Ectociaceae share with Poaceae several characteristics of pollen and fruit type (see also Rudall et al. 2005). Like Joinville-

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Table 1. Additional taxa for which structural characters were added to those analyzed previously by GPWG (2001) following the same character order and coding.

Taxon	Character
	00000000011111111122222222233333333334444444445555 12345678901234567890123456789012345678901234567890123
<i>Arundinaria</i>	1100111101001101013011001101321-0111010301101120----1
<i>Bambusa</i>	110011110-000--101301111110-321-0111010301101120----1
<i>Ecdeiocolea</i>	000--0-0-----0-----1011002220-0----??-0---?0-?100
<i>Isachne</i>	0101010101000--0112000001100221-1101110301001000----1
<i>Sorghum</i>	0A0A010A1A11111001200?0011002?1?1101110311100051-1??1

aceae, Ecdeiocoleaceae have a 6-kilobase inversion that occurs elsewhere only in grasses, and lack the *trnT* inversion that is restricted to the grasses. A total-evidence analysis resolves *Ecdeiocolea* F. Muell. as sister to Poaceae, with *Joinvillea* Gaudich. ex Brogn. & Gris the subsequent sister of this group (Michelangeli et al. 2003). Ecdeiocoleaceae were not previously included in GPWG (2000, 2001) studies. Thus, one representative, *E. monostachya*, was included here to ascertain the effect of this outgroup on the phylogenetic positions of Anomochloideae, Pharoideae, Puelioideae, and other early diverging Poaceae.

Finally, the relatively recent software implementation of Bayesian inference (BI) of phylogeny allows the opportunity to estimate a nonparametric ML bootstrap analysis without the same degree of computer burden that is associated with analyses using existing maximum likelihood (ML) software (Huelsenbeck and Ronquist 2001). Here, we compare the results of maximum parsimony (MP; Swofford 2002), neighbor-joining (NJ; Saitou and Nei 1987), and BI analyses (Huelsenbeck and Ronquist 2001). We follow a growing number of studies that contrast BI with other methods of phylogenetic inference (Suzuki et al. 2002; Wilcox et al. 2002; Alfaro et al. 2003; Douady et al. 2003; Taylor and Piel 2004), with explicit comparisons among methods.

MATERIALS AND METHODS

A data matrix for 63 taxa was assembled comprising the structural characters previously analyzed by GPWG (2001) and sequences of the two plastid loci, *rbcL* and *ndhF*. Representatives of all 12 of the previously recognized subfamilies were sampled including species of the problematic genera *Eriachne*, *Micraira*, and *Streptogyna*. The previous GPWG analysis (2001) included several “conglomerate” taxa. These taxa are presumptive monophyletic groups that were scored and analyzed as solitary terminals in the data matrix, though they included data representing multiple species or genera. In the present analysis, some of the conglomerate taxa were eliminated by completing the character sampling for one or two of the constituent taxa, and by removing data representing closely related species. These changes resulted in the replacement of the conglomerate taxon “*Miscanthus*” with *Sorghum* Moench, and the replacement of “*Pseudosasa*” with *Arundinaria* and *Bambusa* Schreb. The generation of new sequence data also resulted in the inclusion of two previously unsampled taxa (*Ecdeiocolea* and *Isachne*). Three relatively distantly related outgroup taxa (*Balaskion* Raf., *Elegia* L., and *Flagellaria* L.) were removed

from the taxon set, as were three grass genera (*Austrodanthonia* H. P. Linder, *Pappophorum* Schreb., and *Sporobolus* R. Br.) for which sequences of one or both of the two genes were unavailable. The structural portion of the data matrix included the same 53 characters that were analyzed by GPWG (2001), with the same encoding of states. Scores for these characters for the five new taxa specified above are provided in Table 1.

Sequence determination followed standard methods (Clark et al. 1995; Duvall and Morton 1996). GenBank accession numbers for the complete set of sequences analyzed, together with voucher specimen information for newly determined sequences, are provided in Table 2. These include 32 *rbcL* and nine *ndhF* sequences newly determined for this study. Twelve of the 63 taxa are conglomerates with respect to the molecular data, where the two plastid sequences were determined for different congeners.

For the various phylogenetic analyses, *Joinvillea* and *Ecdeiocolea* were included individually and combined as alternative outgroups. Localized hotspots in *ndhF* sequences prone to insertions/deletions (indels) were excluded from the analyses, and sequence termini were trimmed to reduce missing data.

Maximum parsimony, NJ, and BI methods of phylogenetic inference were used; the first two as implemented in PAUP* vers. 4.0b10 (Swofford 2002) and the third as implemented in MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001).

Heuristic MP analyses for 1000 random-addition sequences and tree-bisection-reconnection (TBR) swapping were performed for the entire data matrix as well as for the sequence data only. Nonparametric parsimony bootstrap analysis (Felsenstein 1985) was conducted with 10 random-addition sequences for each of 1000 pseudoreplicates with TBR swapping.

The best-fit likelihood model for the sequence data was determined by using Modeltest vers. 3.06 (Posada and Crandall 1998) that selects the optimal model from 56 possibilities based on hierarchical likelihood ratio tests. The optimal model identified by Modeltest analysis was the transversional nucleotide substitution model, in which there are four different transversion rates and one transition rate, with gamma-distributed among-site rate variation, heterogeneous rates across sites and a proportion of invariable sites (i.e., TVM + Γ + I). Neighbor-joining analysis (Saitou and Nei 1987) was conducted under this model, again with 1000 bootstrap replicates.

Bayesian inference analyses were also performed on the sequence data alone. The TVM model, with five substitution types, is not implemented in MrBayes 3.0b4 so the more general GTR model with six substitution types ($nst = 6$) was used with a proportion of invariable sites and among-site rate variation for the remaining sites drawn from a Γ distribution ($rates = invgamma$). No prior probability distribution was assumed so that all trees were given equal weight a priori. Sequences were partitioned by gene and site-specific rates were allowed to vary across partitions ($ratepr = variable$). Ten random trees, generated in PAUP*, were specified as user-defined starting trees in ten replicate BI analyses to increase the coverage of tree space. All BI analyses were executed for 1,000,000 generations with trees sampled every 80 generations. The first 2501 trees were discarded ($burnin = 2501$), leaving 10,000 trees recovered from each replicate.

Maximum parsimony, NJ bootstrap, and BI analyses were repeated with the conditions specified above excluding, by turn, each of the two outgroups.

RESULTS AND DISCUSSION

Structural data comprised 53 characters. Conserved regions of sequence from which indels were excluded were unambiguously aligned to 3443 base pairs (bp) of which there were 1344 bp from *rbcL* and 2099 bp from *ndhF*. The total number of characters analyzed was 3496 of which 820 nucleotide and 48 structural characters were parsimony informative.

General Comparisons

Maximum parsimony analysis of the entire 3496 character by 63 taxon matrix (including both outgroups) produced 27 most-parsimonious trees of 4414 steps (consistency index [CI] excluding uninformative characters = 0.35; retention index [RI] = 0.59; see Fig. 1 for MP bootstrap results). The structures of the majority-rule bootstrap consensus (Fig. 1) and strict consensus of the MP trees were identical except for three nodes. In the strict-consensus tree, the *Anisopogon* R. Br./*Phaenosperma* Munro ex Benth. clade was united with selected Stipeae; the *Bambusa/Chusquea* Kunth clade diverged immediately after *Arundinaria*; and *Streptogyna* was united with Bambusoideae. All three of these nodes were unresolved in the MP bootstrap tree (Fig. 1).

Maximum parsimony analysis of the 3443 bp nucleotide partition produced a set of 45 trees of length 4032 (CI excluding uninformative characters = 0.36; RI = 0.60). The majority-rule parsimony bootstrap consensus tree is shown in Fig. 3 (right). The strict consensus trees of the two MP analyses were topologically similar. One notable difference between the two was that the nucleotide partition tree (Fig. 3, right) resolved a weakly supported BEP clade (bootstrap value [BV] = 65%) whereas the nucleotide + structural tree (Fig. 1) did not resolve this clade, but rather weakly united the pooid and PACCAD subclades (BV = 51%). Some other differences will be discussed below.

Neighbor-joining analysis of the nucleotide data partition produced a tree with a minimum-evolution score of 1.208. The bootstrap consensus NJ tree also failed to resolve the BEP clade (Fig. 2). The NJ analyses failed to resolve the branches of intermediate depth, i.e., the relationships among

the pooid, bambusoid–ehrhartoid, and PACCAD clades, unlike either the MP or the BI analyses. The remainder of this paper will include results from all three methods, but will emphasize results from only the latter two.

The ten replicate BI analyses for all 63 taxa, each starting with a different randomly assembled tree, recovered virtually identical trees in which posterior probability (PP) values varied slightly. The similarity of these trees is a positive indication that the global tree space was adequately sampled in our BI analyses to obtain an optimal result. The only topological difference was in the placement of *Anisopogon* and *Phaenosperma*. Three of the analyses recovered a clade of *Anisopogon* plus *Phaenosperma* (PP = 0.54–0.56) while the other seven left these taxa in an unresolved trichotomy with other pooids. We note that the *rbcL* sequence for *P. globosa* was the shortest sequence of this locus in the data matrix, only 1143 bp in length, and was truncated especially severely at the phylogenetically informative 3'-end, possibly accounting for this ambiguity in the BI analyses. One consensus BI tree is given, reflecting the more commonly recovered of the two topologies, and is shown in Fig. 3 (left) contrasted with the majority-rule MP bootstrap consensus tree from an analysis of the same data partition.

Here we also compare analyses from the plastid-only data against MP analyses of the original eight-data-set matrix of GPWG (2001). For some parts of the trees we find greater resolution and/or support over more characters than the previous analyses, especially in the BI trees. This may be due to fewer opportunities for conflict between data sets, a smaller proportion of missing data, a greater sensitivity to detect phylogenetic signal in an explicitly model-based approach, or some combination of these factors.

Outgroup Effects

Molecular phylogenetic relationships within Poaceae have been shown to be influenced by outgroup selection in a smaller study of plastid DNA sequences (Duvall and Morton 1996). Here we explored the effect of outgroup membership on ingroup resolution and support in analyses of a larger molecular matrix. Twelve analyses were performed that differed in the choice of outgroup—*Joinvillea*, *Ecdeiocolea*, or both—across three phylogenetic methods and two data partitions (Table 3). The total-evidence analyses of GPWG (2001) identified three early diverging monophyletic groups, Anomochlooideae, Pharoideae, and Puelioideae. Among these taxa, only the earliest-diverging subfamily, Anomochlooideae (represented by *Anomochloa marantoidea* and *Streptochaeta sodiroana*), was influenced by outgroup selection. The subfamily was more strongly supported as monophyletic in analyses in which *Joinvillea* was the sole outgroup although it was unresolved in the NJ analysis. Anomochlooideae were retrieved in MP analyses with BV = 71–75% only when *Ecdeiocolea* was excluded. All BI analyses retrieved a monophyletic Anomochlooideae, identical to the findings of GPWG (2001) over the eight-data-set matrix. However, the PP associated with the subfamily decreased from a minimum value of 0.98 to 0.68 when *Joinvillea* was removed from the analysis. In MP and NJ analyses, when *Ecdeiocolea* was the only outgroup, an alternate topology resulted in which *Anomochloa* Brongn. diverged

Table 2. Total included taxa with GenBank accession numbers for *ndhF* and *rbcL* sequences. Congeners are listed separately on adjacent lines when different for *ndhF* and *rbcL*. Vouchers are indicated only for newly included taxa. Voucher information for other taxa can be found in Soreng and Davis (1998), Clark et al. (2000), or GPWG (2001). Standard herbarium codes are listed parenthetically. BBG = Berlin Botanic Garden, PI = USDA Plant Introduction Station (Pullman, Washington, USA), USNHG = U.S. National Herbarium Greenhouse.

Taxon	<i>ndhF</i>	<i>rbcL</i>	Voucher
<i>Ampelodesmos mauritanica</i> (Poir.) T. Durand & Schinz	AF251448	AY622885 ^a	BBG: <i>Royle & Schiers s. n.</i> (B)
<i>Amphipogon strictus</i> R. Br.	AF251456	U88403	
<i>Anisopogon avenaceus</i> R. Br.	AF251447	AY622886 ^a	<i>Linder 5590</i> (BOL)
<i>Anomochloa marantoidea</i> Brongn.	U21992	AF021875	
<i>Aristida congesta</i> Roem & Schult.		U31359	
<i>A. purpurea</i> Nutt. var. <i>longiseta</i> (Steud.) Vasey	U21966		
<i>Arundinaria gigantea</i> (Walter) Muhl.	U21846	AY618660 ^a	<i>Zhang 703</i> (ISC)
<i>Arundo donax</i> L.	U21998	U31360	
<i>Avena sativa</i> L.	U22000	L15300	
<i>Bambusa</i> aff. <i>bambos</i> (L.) Voss	U22001		
<i>B. multiplex</i> (Lour.) Raeusch. ex Schult. & Schult. f.		M91626	
<i>Brachyelytrum erectum</i> (Schreb.) P. Beauv.	U22005	AY622888 ^a	<i>Soreng 3427a</i> (BH)
<i>Brachypodium pinnatum</i> (L.) P. Beauv.	AY622312 ^a	AY632361 ^a	<i>PI-440170</i> (BH)
<i>Bromus inermis</i> Leyss.	AF251454	AY395531	
<i>Buergersiochloa bambusoides</i> Pilg.	AF182341	AY632362 ^a	<i>Dransfield 1382</i> (K)
<i>Centropodia glauca</i> (Nees) Cope	AF251462	U31100	
<i>Chasmanthium latifolium</i> (Michx.) H. O. Yates		U31101	
<i>C. laxum</i> (L.) H. O. Yates subsp. <i>sessiliflorum</i> (Poir.) L. G. Clark	U27296		
<i>Chusquea circinata</i> Soderstr. & C. E. Calderón		U13227	
<i>C. latifolia</i> L. G. Clark	U21990		
<i>Danthonia californica</i> Bol.	AF251459		
<i>D. spicata</i> (L.) P. Beauv. ex Roem. & Schult.		U31102	
<i>Danthoniopsis dinteri</i> (Pilg.) C. E. Hubb.		AY618661 ^a	<i>PI-207548</i> (A)
<i>D. petiolata</i> (J. B. Phipps) Clayton	U21988		
<i>Diarrhena obovata</i> (Gleason) Brandenburg	U21999	AY622890 ^a	<i>Tiedye 5186</i> (DAO)
<i>Distichlis spicata</i> (L.) Greene	AF251464	AY632363 ^a	No voucher
<i>Ecdiocola monostachya</i> F. Muell.	AY622313 ^a	AY123235	<i>Conran et al. 943</i> (ADU)
<i>Ehrharta calycina</i> Sm.	U21996	AY622891 ^a	<i>PI-208983</i> (BH)
<i>Eragrostis capensis</i> (Thunb.) Trin.		U31104	
<i>E. curvula</i> (Schrad.) Nees	U21989		
<i>Eremitis</i> Döll sp. nov.	AF182353	AY622892 ^a	<i>Soderstrom 2182</i> (US) or <i>USNHG-286</i> (US) ^b
<i>Eriachne pulchella</i> Domin	AY618659 ^a		<i>Jacobs 8720</i> (NSW)
<i>E. trisetata</i> Nees ex Steud.		AY626351 ^a	<i>Jacobs 8795</i> (NSW)
<i>Glyceria grandis</i> S. Watson	AY622314 ^a	AY632364 ^a	No voucher
<i>Guaduella marantifolia</i> Franch.	AF164777	AF164778	
<i>Gynerium sagittatum</i> (Aubl.) P. Beauv.	AF251466	U31105	
<i>Isachne arundinacea</i> (Sw.) Griseb.	AY847119 ^a	AY618662 ^a	<i>Sánchez-Ken 680</i> (ISC)
<i>Joinvillea ascendens</i> Gaudich. ex Brongn. & Gris.	U21973		
<i>J. plicata</i> (Hook. f.) Newell & B. C. Stone		L01471	
<i>Karroochloa purpurea</i> (L. f.) Conert & Türpe	AF251458	U31437	
<i>Leersia oryzoides</i> (L.) Sw.		U13228	
<i>L. virginica</i> Willd.	U21974		
<i>Lithachne humilis</i> Soderstr.	U21977	U13231	
<i>Lygeum spartum</i> L.	AF251445	AY622894 ^a	<i>Soreng 3698</i> (BH)
<i>Melica cupani</i> Guss.	AY622315 ^a	AY632365 ^a	<i>PI-383702</i> (BH)
<i>Merxmuellera macowanii</i> (Stapf) Conert	AF251457	U31438	
<i>M. rangei</i> (Pilg.) Conert	AF251461	AY640153 ^a	<i>Barker 960</i> (GRA)
<i>Micraira subulifolia</i> F. Muell.	AY622316 ^a	AY632366 ^a	<i>Jacobs 8671</i> (NSW)
<i>Molinia caerulea</i> (L.) Moench	U21995	AY632367 ^a	<i>Soreng 3305</i> (BH)
<i>Nardus stricta</i> L.	AF251446	AY622895 ^a	BBG: <i>Royle & Schiers s. n.</i> (B)
<i>Nassella viridula</i> (Trin.) Barkworth	AF251450	AY622896 ^a	<i>PI-387938</i> (BH)
<i>Olyra latifolia</i> L.	U21971	AY622897 ^a	<i>Peterson 7311</i> (US)
<i>Oryza sativa</i> L.	X15901	AC092750	
<i>Panicum virgatum</i> L.	U21986	AY632368 ^a	<i>PI-421520</i> (BH)
<i>Pariana radiceflora</i> Sagot ex Döll	AF182354	AY632369 ^a	<i>Clark & Zhang 1344</i> (ISC)

Table 2. Continued.

Taxon	<i>ndhF</i>	<i>rbcL</i>	Voucher
<i>Pennisetum glaucum</i> (L.) R. Br.	AF499149	L14623	
<i>Phaenosperma globosa</i> Munro ex Benth.	U22006	AY632370 ^a	Clark 1292 (ISC)
<i>Pharus latifolius</i> L.	U21993	AY357724	
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	U21997	U29900	
<i>Piptatherum miliaceum</i> (L.) Coss.	AY622317 ^a	AY622898 ^a	PI-284145 (BH)
<i>Puelia schumanniana</i> Pilg. ^c	AF164779	AF164780	
<i>Sorghum bicolor</i> (L.) Moench	U21981	1515164A	
<i>Spartina pectinata</i> Link	AF251465	AY632371 ^a	No voucher
<i>Stipa barbata</i> Desf.	AF251449		
<i>S. dregeana</i> Steud.		U31442	
<i>Stipagrostis zeyheri</i> (Nees) DeWinter	AF251455	U31378	
<i>Streptochaeta sodiroana</i> Hack.	AY622318 ^a	AY632372 ^a	Peterson 9525 (US)
<i>Streptogyna americana</i> C. E. Hubb.	U21965	AY618663 ^a	Pohl & Davidse 12310 (ISC)
<i>Thysanolaena maxima</i> (Roxb.) Kuntze	U21984	U31380	
<i>Triticum aestivum</i> L.	AB042240	D00206	
<i>Uniola paniculata</i> L.	AF251463	AY632373 ^a	No voucher
<i>Zea mays</i> L.	U21985	NC001666	
<i>Zeugites pittieri</i> Hack.	U21987	AY632374 ^a	Clark 1171 (ISC)
<i>Zoysia</i> Willd. sp.		AY632375 ^a	No voucher
<i>Z. matrella</i> (L.) Merr.	U21975		

^a Sequences newly determined in this paper.

^b Specific voucher unknown due to a clerical error.

^c Listed as *P. ciliata* Franch. in Clark et al. (2000) and GPWG (2001).

immediately after the outgroup, followed by the separate divergence of *Streptochaeta*.

Unexpectedly, the BEP clade, which is situated at an intermediate position in the phylogeny, showed a similar outgroup effect. The support for this clade decreased in both BI and MP analyses of the nucleotide partition when *Joinvillea* was excluded (Table 3). The BEP clade was unresolved in all NJ analyses and paraphyletic in all MP analyses that included both data partitions in which Pooideae were weakly united with the PACCAD clade (BV = 51–52%).

We interpret these results to be the consequence of the greater divergence of the *Ecdeiocolea* sequences from the root of our trees than those of *Joinvillea*. *Ecdeiocolea* is borne on the longest branch in our trees (not shown, but branch length data available on request). Possibly this results in long-branch attraction between *Ecdeiocolea* and other long-branch taxa such as *Anomochloa*, *Ehrharta* Thunb. and/or *Lithachne* P. Beauv., although we did not specifically test for this kind of artifact. Our results resemble those obtained in analysis of *matK* sequences by Hilu et al. (1999). In that study, the choice of *Restio tetraphyllum* Labill. (= *Baloskion tetraphyllum* (Labill.) B. G. Briggs & L. A. S. Johnson), also exceptionally divergent, plus *Joinvillea ascendens* as the outgroup similarly failed to retrieve either Anomochlooideae or the BEP clade while still preserving much of the remaining phylogenetic structure of the family.

The outgroup effect is least pronounced in our BI analyses, which resolve both Anomochlooideae and the BEP clade as monophyletic irrespective of outgroup membership. This observation is consistent with a hypothesis of long-branch attraction, an effect for which BI is less biased than MP.

PACMAD Clade

GPWG (2000, 2001) identified a PACCAD clade. Here, we recommend that some of the subfamilies constituting this

clade be altered to reflect extensive research in progress. Namely, Micraioideae are added to the group (Sánchez-Ken et al. in press) and Centothecoideae are submerged into Panicoideae (Zuloaga et al. 2003; Sánchez-Ken et al. in prep.). We thus recommend the use of the alternate acronym PACMAD to reflect these modifications. We resolved the PACMAD clade with the strongest support (BV = 100, PP = 1.00) in all analyses (Fig. 1–3). Relationships within the group were largely unresolved in MP (Fig. 1, 3) and NJ (Fig. 2) analyses. Bayesian inference analyses united Micraioideae with Arundinoideae (PP = 1.00; Fig. 3). Consistent with the work in progress cited above, Centothecoideae were paraphyletic with Panicoideae, so that *Chasmanthium* Link was in a sister position to the remaining taxa from these two subfamilies plus *Gynerium* (Gynerieae) (PP = 1.00 for this entire clade; Fig. 3). *Danthoniopsis* and *Thysanolaena* Nees (the latter a centothecoid genus) were sister taxa (BV = 56–61%, PP = 0.92–0.97; Fig. 1, 3), in contrast to the weakly supported position of *Danthoniopsis* as an early diverging element of Panicoideae in prior analyses (GPWG 2001). Analyses of the additional plastid sequence data here suggest a somewhat different circumscription for Panicoideae and we recommend continued re-examination of the relationship between centothecoid grasses and Panicoideae. Other relationships among PACMAD subfamilies were not as strongly supported in BI trees and will not be discussed.

Micraioideae

Eriachne and *Isachne* were strongly supported as sister taxa in all analyses (BV = 92–99%, PP = 1.00; Fig. 1–3). Further, in all analyses this clade was united with *Micraira subulifolia* with the strongest support (BV = 100%, PP = 1.00). In a recent phylogenetic study of another plastid locus, the *rpoC2* insert, Micraioideae, represented by *Eriachne*

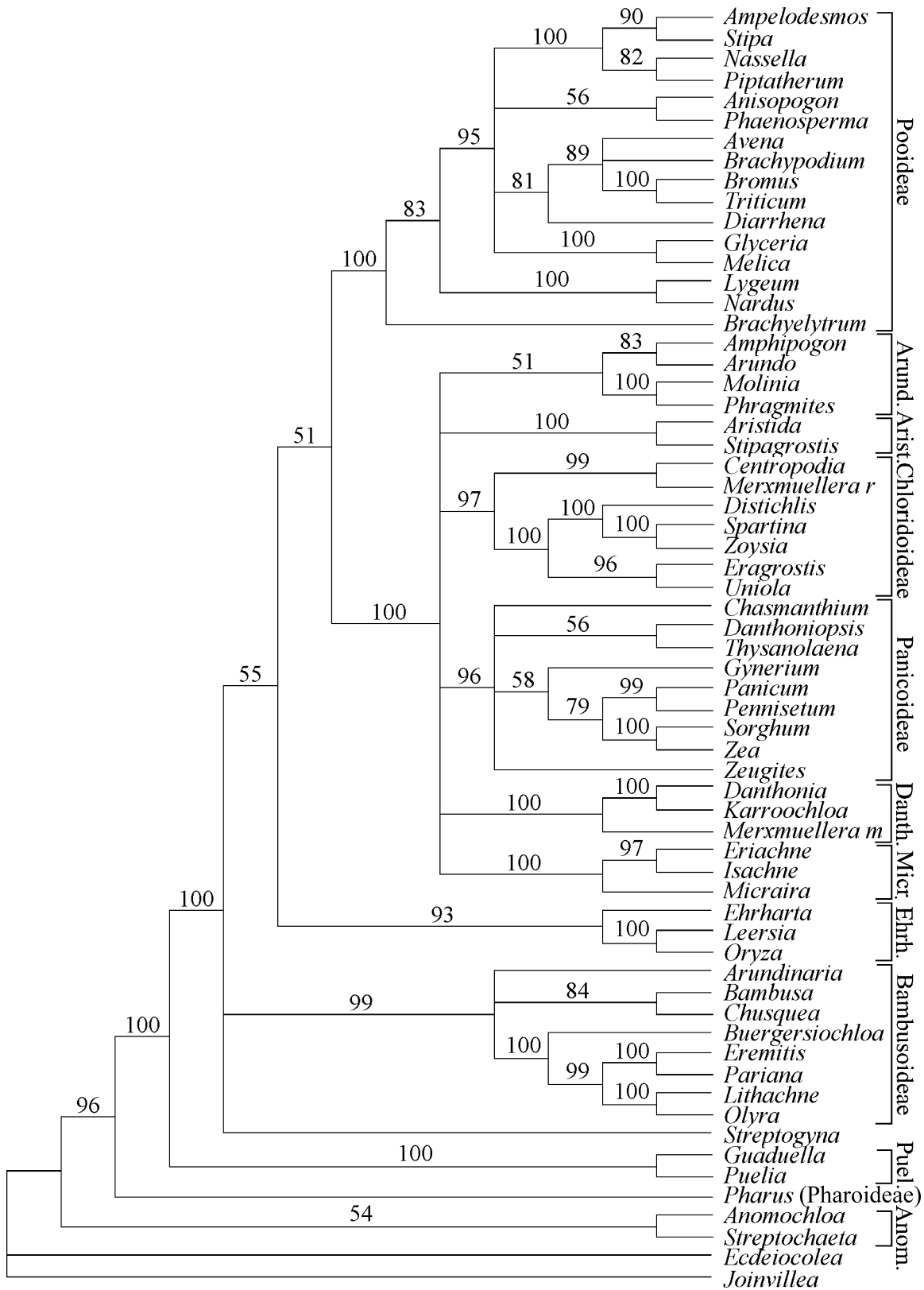


Fig. 1.—Majority-rule maximum parsimony bootstrap consensus tree inferred from combined analysis of data from two plastid genes (*rbcL* and *ndhF*) and 53 structural characters. Bootstrap values appear along the branches. Subfamily membership is indicated following GPWG (2001) with two amendments: (1) Centothecoideae are submerged within Panicoideae and (2) Micrairoideae are recognized. Abbreviations: Anom. = Anomochloideae, Arist. = Aristidoideae, Arund. = Arundinoideae, Danth. = Danthonioideae, Ehrh. = Ehrhartioideae, Micr. = Micrairoideae, and Puel. = Puelioideae.

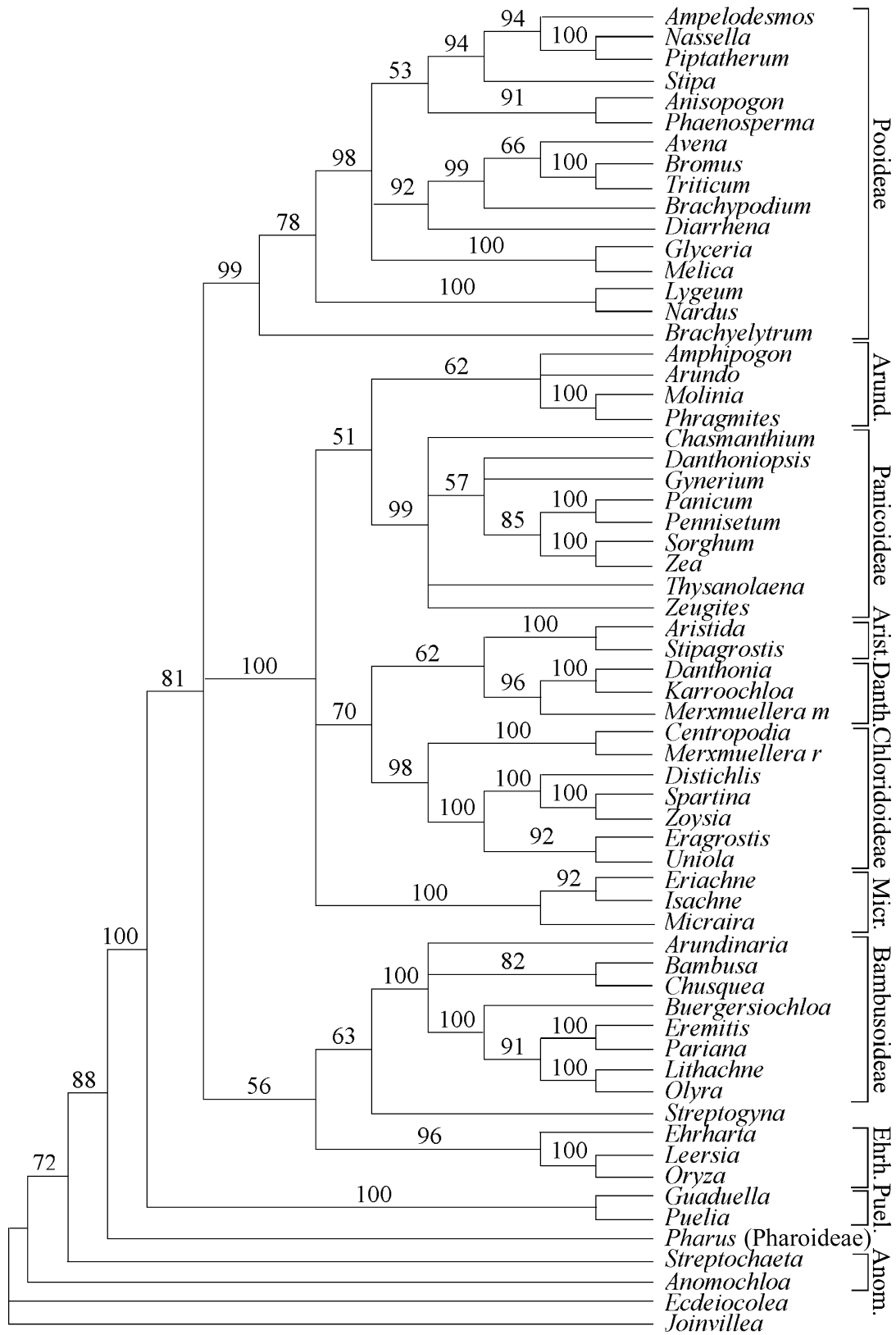


Fig. 2.—Majority-rule neighbor-joining bootstrap consensus tree inferred from analysis of the nucleotide partition only. Bootstrap values appear along the branches. See Fig. 1 caption for explanation of subfamilies.

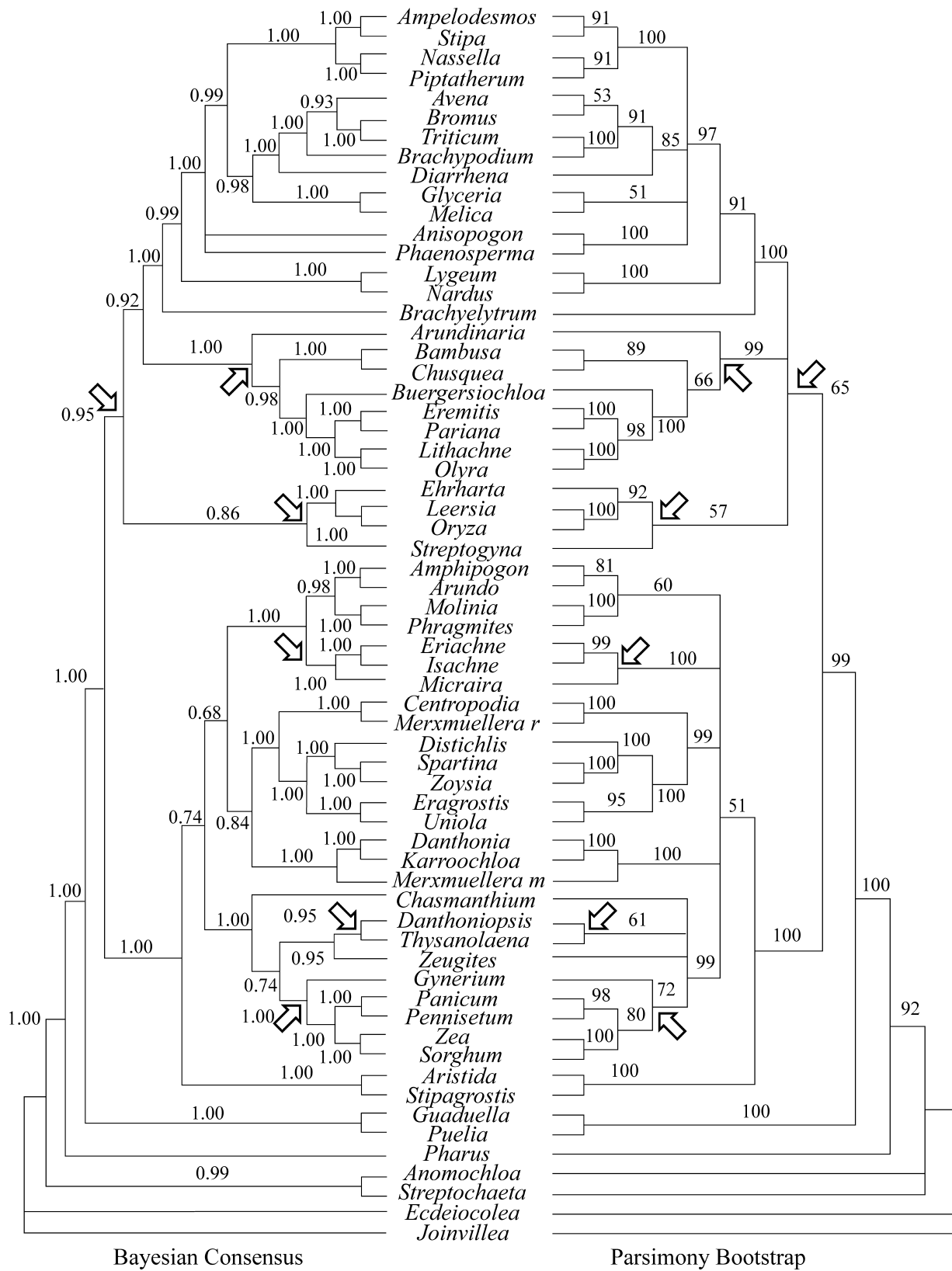


Fig. 3.—Majority-rule Bayesian inference consensus tree (left) and majority-rule maximum parsimony bootstrap consensus tree (right) inferred from analyses of the nucleotide partition only. Posterior probability and bootstrap values are indicated along the branches of the respective trees. Six points of congruence, associated with taxa newly added in this paper, are indicated with arrows.

Table 3. Effect of outgroup selection on the resolution and support of Anomochloideae and the BEP clade in 12 analyses that differ by method, data partition, and choice of outgroup. Values given are bootstrap (maximum parsimony [MP] and neighbor-joining [NJ]) or posterior probabilities (PP) when the group is monophyletic. For the full Bayesian inference (BI) analysis, the range is given for PP values across the ten replicate BI analyses.

Phylogenetic method: Data partition:	MP		NJ	BI
	Total evidence	Nucleotide	Nucleotide	Nucleotide
Outgroup effect—Anomochloideae				
<i>Joinvillea</i>	75	71	Unresolved	0.98
<i>Ecdeiocolea</i>	Paraphyletic	Paraphyletic	Paraphyletic	0.68
<i>Joinvillea</i> + <i>Ecdeiocolea</i>	54	Unresolved	Paraphyletic	0.98–0.99
Outgroup effect—BEP clade				
<i>Joinvillea</i>	Paraphyletic	66	Unresolved	0.94
<i>Ecdeiocolea</i>	Paraphyletic	63	Unresolved	0.83
<i>Joinvillea</i> + <i>Ecdeiocolea</i>	Paraphyletic	65	Unresolved	0.95–0.99

mucronata R. Br., *Isachne distichophylla* G. C. Munro ex Hillebr., and *Pheidochloa gracilis* S. T. Blake, were similarly resolved as monophyletic (Duvall et al. 2003). The position of Micrairoideae among the PACMAD subclades was unresolved in MP (Fig. 1, 3) and NJ (Fig. 2) analyses, but strongly supported as excluded from Panicoideae in a sister-group position with Arundinoideae in all BI analyses (PP = 0.99–1.00; Fig. 3).

In an early proposal by GPWG (1998), Micrairoideae were suggested as one of the subfamilies to be recognized based on preliminary evidence. However, the data accumulated by the time of the publication of GPWG (2001) were insufficient for confident recognition of this subfamily. Members of the subfamily share similar geographic distributions, and with the PACMAD clade exhibit an elongated mesocotyl internode in the embryo, although we are unaware of any unique structural synapomorphies for the subfamily. Nonetheless, the determination of missing plastid sequences in the GPWG (2001) data matrix has clearly resolved these taxa as members of a strongly supported subfamily with possible affinities to Arundinoideae. Sánchez-Ken et al. (in press) will provide an emended description for Micrairoideae.

Gynerieae

Gynerium was treated as incertae sedis by GPWG (2001) because of insufficient support for its phylogenetic placement. Gynerieae, composed of the monotypic *Gynerium*, were concurrently recognized by Sánchez-Ken and Clark (2001) and provisionally included in the circumscription of Panicoideae. Although none of the new taxa added to our analysis were of close phylogenetic proximity to Gynerieae, a sequence of *rbcL* from *Danthoniopsis*, missing from the GPWG (2001) matrix, was determined. *Gynerium* is here united in a sister-group relationship to the Andropogoneae (here represented by *Sorghum* and *Zea* L.)/Paniceae (*Panicum* L., *Pennisetum* Rich.) clade (BV = 58–72% in MP analyses, PP = 1.00 in all BI replicates; though unresolved in NJ analyses; Fig. 1–3), consistent with the MP results of Barker et al. (1995). Thus, the addition of the missing *Danthoniopsis* sequence, combined with the greater sensitivity of the BI approach, has produced unexpectedly strong support for the position of Gynerieae in Panicoideae.

Streptogyneae

Additional plastid sequences for *Streptogyna* did not produce a robust phylogenetic placement for Streptogyneae, a tribe of one genus and two widely distributed species. The position of *Streptogyna* is unresolved in MP bootstrap analyses of structural and molecular data (Fig. 1). Neighbor-joining analyses of the nucleotide partition place *Streptogyna* as the sister taxon to Bambusoideae (BV = 63–64%; Fig. 2). Maximum parsimony and BI analyses of the sequence data unite *Streptogyna* with Ehrhartoideae (BV = 57%, PP = 0.76–0.90; Fig. 3) as was previously suggested (GPWG 2001). There is a minor outgroup effect here as well, with the lowest PP value (0.76) obtained with *Ecdeiocolea* as the sole outgroup, and a higher range of values (0.82–0.90) obtained when both outgroup taxa were included.

Arundinaria and Bambuseae

Arundinaria gigantea is distinguished as a representative of the sole native genus of Bambuseae in the USA (Triplett et al. 2004). Previous molecular studies have been inconclusive as to its phylogenetic position, either because of under-sampling of Bambusoideae (e.g., Nadot et al. 1995) or insufficient phylogenetic information (Clark et al. 1995; GPWG 2001). More recent studies with broader sampling and additional morphological characters provide robust support for a North Temperate woody bamboo clade including *Arundinaria*, but the relationship of this clade to other lineages within the bamboos remains unresolved (Zhang and Clark 2000; Ní Chonghaile 2002; Clark et al. 2006). Our MP and BI analyses of the nucleotide partition support an early diverging position for *A. gigantea* in Bambusoideae (BV = 64–66%, PP = 0.96–0.98; Fig. 3), but we note the paucity of sampling of Bambuseae here. In our other analyses, the position of *A. gigantea* was unresolved (Fig. 1, 2).

Arundinaria has been classified in Bambuseae in recent and older treatments (Clayton and Renvoize 1986; Watson and Dallwitz 1992 onwards; Judziewicz et al. 2000). In our MP and BI analyses of nucleotide data, such a classification results in a Bambuseae (here represented by *Bambusa*, *Chusquea*), which is paraphyletic with Olyreae (*Buergersiochloa* Pilg., *Eremitis* Döll, *Lithachne*, *Olyra* L., *Pariana* Aubl.) (Fig. 3). This is consistent with other analyses in which mo-

lecular data alone produce a paraphyletic Bambuseae (Zhang and Clark 2000; Clark et al. 2006). The addition of certain morphological characters, particularly the differentiation of culm and foliage leaves, provides moderate to strong support for a monophyletic Bambuseae (Zhang and Clark 2000; Clark et al. 2006). This character and others relating to the complex branching of the woody bamboos were not included in the GPWG (2000, 2001) analyses or in our analyses here.

Conclusion

Here we have compared analyses of three of the original eight data sets assembled by GPWG (2000, 2001) in which missing sequences were determined and data were added for several ingroup and outgroup taxa. While this is a more limited character data matrix, our trees show considerable topological congruence with those of GPWG (2001). Comparison of analyses of the separate nucleotide partition with the combined data indicated that the former better resolve weakly supported nodes (e.g., of *Streptogyna*, *Arundinaria*, and the BEP clade) and show slightly higher CI and RI values as well as greater bootstrap support across the tree (Fig. 1, 3). This is consistent with the observation that the level of variation in structural characters is too high to be useful for resolving relationships between subfamilies (GPWG 2001).

The comparison between BI and nonparametric parsimony bootstrap analyses is informative. Previous studies that contrasted BI against other methods differed considerably in the choice of simulated and/or real data, number and source of loci, organisms and objectives. However, a consensus is emerging. Bayesian PP values are slightly less biased predictors of phylogenetic accuracy (e.g., Alfaro et al. 2003), consistent with our results. We found the ingroup phylogeny of Poaceae to be somewhat sensitive to outgroup membership, both at the earliest-diverging nodes and at weakly supported intermediate nodes (Table 3). We thus caution against the exclusive use of a highly diverged outgroup, such as *Ecdiocollea* or *Restio* Rottb., for MP analyses of Poaceae, although BI analyses are less affected by what may be a long-branch attraction bias.

Posterior probability values are also generally somewhat less conservative measures than nonparametric bootstrap values (Taylor and Piel 2004). Our results are largely consistent with this observation. To illustrate, mean support values from the same set of 47 resolved nodes in the BI and MP trees (Fig. 3) were calculated. The mean PP value (and range) for these nodes was 0.99 (0.74–1.00) while the mean BV over the same nodes was 90% (51–100%). Moreover, error rates associated with PP >0.99 have been found to be similar to those for bootstrap values >95% (Taylor and Piel 2004). Thus, the more liberal PP values require a somewhat different interpretation with regard to levels of support than nonparametric BVs.

The concern that missing data were responsible for some of the unstable results in the analyses of GPWG (2001) was well founded. Our analyses of a matrix with 41 additional plastid sequences and corresponding structural data offer support for the phylogenetic position of taxa previously treated as incertae sedis, even in the absence of the other

molecular data in the original GPWG (2000, 2001) studies, and contribute to continuing progress in grass phylogenetics.

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LITERATURE CITED

- ALFARO, M. E., S. ZOLLER, AND F. LUTZONI. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molec. Biol. Evol.* **20**: 255–266.
- BARKER, N., H. P. LINDER, AND E. HARLEY. 1995. Polyphyly of Arundinoideae (Poaceae): evidence from *rbcL* sequence data. *Syst. Bot.* **20**: 423–435.
- BARKWORTH, M. E., K. M. CAPELS, S. LONG, AND M. B. PIEP (editors) [FLORA OF NORTH AMERICA COMMITTEE]. 2003. Magnoliophyta: Commelinidae (in part): Poaceae, Part 2. Flora of North America North of Mexico, Vol. 25. Oxford University Press, New York, USA. 783 p.
- CLARK, L. G., S. DRANSFIELD, J. TRIPLETT, AND J. G. SÁNCHEZ-KEN. 2006. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae), pp. 315–332. In J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], *Monocots: comparative biology and evolution—Poales*. Rancho Santa Ana Botanic Garden, Claremont, California, USA.
- , M. KOBAYASHI, S. MATHEWS, R. SPANGLER, AND E. KELLOGG. 2000. The Puelioideae, a new subfamily of Poaceae. *Syst. Bot.* **25**: 181–187.
- , W. ZHANG, AND J. F. WENDEL. 1995. A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. *Syst. Bot.* **20**: 436–460.
- CLAYTON, W. D., AND S. A. RENVOIZE. 1986. *Genera graminum: grasses of the world*. Her Majesty's Stationery Office, London, UK. 389 p.
- DOUADY, C. J., F. DELSUC, Y. BOUCHER, W. F. DOOLITTLE, AND E. DOUZERY. 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molec. Biol. Evol.* **20**: 248–254.
- DUVALL, M., AND B. MORTON. 1996. Molecular phylogenetics of Poaceae: an expanded analysis of *rbcL* sequence data. *Molec. Phylogen. Evol.* **5**: 352–358.
- , D. SAAR, W. S. GRAYBURN, AND G. HOLBROOK. 2003. Complex transitions between C₃ and C₄ photosynthesis during the evolution of Paniceae: a phylogenetic case study emphasizing the position of *Steinchisma hians* (Poaceae), a C₃–C₄ intermediate. *Int. J. Pl. Sci.* **164**: 949–958.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- GRASS PHYLOGENY WORKING GROUP [GPWG; AUTHORS LISTED ALPHABETICALLY: N. P. BARKER, L. G. CLARK, J. I. DAVIS, M. R. DUVALL, G. F. GUALA, C. HSIAO, E. A. KELLOGG, H. P. LINDER, R. J. MASON-GAMER, S. Y. MATHEWS, M. P. SIMMONS, R. J. SORENG, AND R. E. SPANGLER]. 1998. Proposal for a subfamilial reclassification of the Poaceae. Third international symposium on grass systematics and evolution. University of New South Wales, Sydney, Australia, 27 Sep–2 Oct 1998. Abstract.

- . 2000. A phylogeny of the grass family (Poaceae) as inferred from eight character sets, pp. 3–7. In S. W. L. Jacobs and J. Everett [eds.], *Grasses: systematics and evolution*. CSIRO Publishing, Collingwood, Victoria, Australia.
- . 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). *Ann. Missouri Bot. Gard.* **88**: 373–457.
- HILU, K. W., L. A. ALICE, AND H. LIANG. 1999. Phylogeny of Poaceae inferred from *matK* sequences. *Ann. Missouri Bot. Gard.* **86**: 835–851.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- JUDZIEWICZ, E. J., R. J. SORENG, G. DAVIDSE, P. M. PETERSON, T. S. FILGUEIRAS, AND F. O. ZULOAGA. 2000. Catalogue of New World grasses (Poaceae): I. Subfamilies Anomochlooideae, Bambusoideae, Ehrhartoideae, and Pharoideae. *Contr. U.S. Natl. Herb.* **39**: 1–128.
- MICHELANGELI, F. A., J. I. DAVIS, AND D. W. STEVENSON. 2003. Phylogenetic relationships among Poaceae and related families as inferred from morphology, inversions in the plastid genome, and sequence data from the mitochondrial and plastid genomes. *Amer. J. Bot.* **90**: 93–106.
- NADOT, S., G. BITTAR, L. CARTER, R. LACROIX, AND B. LEJEUNE. 1995. A phylogenetic analysis of monocotyledons based on the chloroplast gene *rps4*, using parsimony and a new numerical phenetics method. *Molec. Phylogen. Evol.* **4**: 257–282.
- NÍ CHONGHAILE, G. 2002. Systematics of the woody bamboos (tribe Bambuseae). Ph.D. thesis, University of Dublin, Trinity College, Ireland.
- PETERSON, P. M., R. J. SORENG, G. DAVIDSE, T. S. FILGUEIRAS, F. O. ZULOAGA, AND E. J. JUDZIEWICZ. 2001. Catalogue of New World grasses (Poaceae): II. Subfamily Chloridoideae. *Contr. U.S. Natl. Herb.* **41**: 1–255.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- RUDALL, P. J., W. STUPPY, J. CUNNIFF, E. A. KELLOGG, AND B. G. BRIGGS. 2005. Evolution of reproductive structures in grasses (Poaceae) inferred by sister-group comparison with their putative closest living relatives, Ecdicoleaceae. *Amer. J. Bot.* **92**: 1432–1443.
- SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4**: 406–425.
- SÁNCHEZ-KEN, J. G., AND L. G. CLARK. 2001. Gynerieae, a new Neotropical tribe of grasses (Poaceae). *Novon* **11**: 350–352.
- , E. KELLOGG, AND E. KAY. In press. Reinstatement and emendation of the subfamily Micrairoideae (Poaceae). *Syst. Bot.*
- SORENG, R. J., AND J. I. DAVIS. 1998. Phylogenetics and character evolution in the grass family (Poaceae): simultaneous analysis of morphological and chloroplast DNA restriction site character sets. *Bot. Rev. (Lancaster)* **64**: 1–85.
- , P. M. PETERSON, G. DAVIDSE, E. J. JUDZIEWICZ, F. O. ZULOAGA, T. S. FILGUEIRAS, AND O. MORRONE. 2003. Catalogue of New World grasses (Poaceae): IV. Subfamily Pooideae. *Contr. U.S. Natl. Herb.* **48**: 1–730.
- SUZUKI, Y., G. GLAZKO, AND M. NEI. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 16138–16143.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4.0. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- TAYLOR, D., AND W. PIEL. 2004. An assessment of accuracy, error and conflict with support values from genome-scale phylogenetic data. *Molec. Biol. Evol.* **21**: 1534–1537.
- TRIPLETT, J. K., A. S. WEAKLEY, AND L. G. CLARK. 2004. Systematics of *Arundinaria* sensu stricto (Bambusoideae, Poaceae) in North America. Botany 2004. Snowbird Resort, Salt Lake City, Utah, USA, Jul 31–Aug 2004. Abstract.
- WATSON, L., AND M. J. DALLWITZ. 1992 onwards. Grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Vers. 10 Aug 2003. <http://delta-intkey.com> (Jun 2005).
- WILCOX, T., D. ZWICKL, T. HEATH, AND D. HILLIS. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molec. Phylogen. Evol.* **25**: 361–371.
- ZHANG, W., AND L. G. CLARK. 2000. Phylogeny and classification of the Bambusoideae (Poaceae), pp. 35–42. In S. W. L. Jacobs, and J. Everett [eds.], *Grasses: systematics and evolution*. CSIRO Publishing, Collingwood, Victoria, Australia.
- ZULOAGA, F., O. MORRONE, G. DAVIDSE, T. S. FILGUEIRAS, P. M. PETERSON, R. J. SORENG, AND E. JUDZIEWICZ. 2003. Catalogue of New World Grasses (Poaceae): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae. *Contr. U.S. Natl. Herb.* **46**: 1–662.