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PHYLOGENY OF ANDROPOGONEAE INFERRED FROM PHYTOCHROME B, GBSSI, AND *NDHF*

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Andropogoneae is a monophyletic tribe of 85 genera that includes *Zea* and *Sorghum*. All members exhibit C_4 photosynthesis and have inflorescences of paired spikelets. Previous studies of the chloroplast gene *ndhF* and the nuclear gene GBSSI identified numerous mutations that distinguish genera of the tribe but do not indicate relationships among them; the deep branches of the trees are quite short. Here we add newly collected data from phytochrome B to the data from the other two genes. The same pattern holds, with very short branches along the backbone of the tree indicating that the tribe resulted from rapid radiation. The phylogeny shows a single origin of a disarticulating rachis, which is a synapomorphy for the tribe. We find strong support for a core Andropogoneae that includes *Andropogon*, *Bothriochloa*, *Capillipedium*, *Cymbopogon*, *Dichanthium*, *Heteropogon*, *Hyparrhenia*, and *Schizachyrium* and support for its relationship with an expanded Saccharinae that includes *Microstegium*. The combined data reject the monophyly of subtribes Andropogoninae and Anthistiriinae and provide evidence that subtribes Sorghinae, Saccharinae, and Rottboelliinae are para- or polyphyletic, as is the traditional Maydeae. A relationship with *Zea* and *Tripsacum* is indicated for *Elionurus*, while *Chionachne* and *Phacelurus* are shown to diverge early in the history of the tribe. *Arundinella hirta* and *Arundinella nepalensis* can be included in an expanded Andropogoneae.

Keywords: Andropogoneae phylogeny, Poaceae, *Zea*, phytochrome B, GBSSI, *ndhF*, Bayesian inference.

Introduction

The grass tribe Andropogoneae includes both maize (*Zea mays* ssp. *mays*) and sorghum (*Sorghum bicolor*), two of the world's most important crops. In addition, it contains many of the dominant species of the American plains (e.g., *Sorghastrum nutans*, *Andropogon gerardii*, *Schizachyrium scoparium*) and the great tropical grasslands of Africa (e.g., *Hyparrhenia* spp., *Cymbopogon* spp., *Bothriochloa* spp.). Altogether there are 85 genera and ca. 1000 species in the tribe (Clayton and Renvoize 1986). Andropogoneae constitutes one of the two major tribes in the subfamily Panicoideae, which includes about one-third of all grass species and is itself strongly supported as monophyletic (GPWG 2000, 2001; Giussani et al. 2001).

Andropogoneae is often described as "natural" because of a large set of shared morphological characters (Clayton and Renvoize 1986; Clayton 1987). All members use the C_4 photosynthetic pathway, with NADP-ME as the primary decarboxylating enzyme (Hattersley and Watson 1992). They have a single sheath of cells around the vascular bundle, within which the plastids are agranal (Carolin et al. 1973); this correlates with a reduction in expression of the enzymes associated with photosystem II (Sheen and Bogorad 1986; Sinha and Kellogg 1996). Most species have pairs of spikelets in the inflorescence, one sessile and one on a pedicel, although in some species one or the other of these spikelets appears to be suppressed. Developmental studies have shown that the spikelet pair originates from a common primordium that subsequently forms two separate primordia (Kellogg 2000; Orr et al. 2001). Inflorescence form is highly variable. Some taxa have inflorescences that can be described as panicles, but many others have complex inflorescences with multiple orders of branching and repeated production of bracts and prophylls.

None of the foregoing characters is synapomorphic (Kellogg 2000). Other clades in Panicoideae have independently derived the C_4 pathway and use NADP-malic enzyme for decarboxylation; these exhibit the same internal anatomy as Andropogoneae. Paired spikelets occur in many panicoids and may in fact be plesiomorphic in the subfamily. As in Andropogoneae, paired spikelets develop from a common primordium. Many panicoids have paniculate inflorescences, similar to some Andropogoneae. Morphological characters by themselves thus do not support the monophyly of the tribe.

Clayton (1972, 1973) divided Andropogoneae into "awned" and "awnless" taxa. Later, Clayton and Renvoize (1986) divided the tribe into 11 subtribes, based largely on characters of the inflorescence. They suggested that the monoecious taxa—traditionally placed in Maydeae—did not form a natural group and were better separated into subtribes Tripsacinae, Coicinae, and Chionachninae. This contrasted with the phenetic study of Watson and Dallwitz (1992) that supported the Maydeae as a single cluster.

Kellogg and Watson (1993) undertook a phylogenetic analysis of morphological characters for all genera of Andropo-

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goneae. This study had a number of limitations, the largest being imposed by the lack of computer programs available for parsimony analysis of large data sets so that it was impossible to assess support for the tree. Additionally, all genera were assumed to be monophyletic. While this was a reasonable assumption for the genera with only one or two species (ca. 50%), it is not supported by more recent molecular data. They found that the distinction between awned and awnless taxa was largely supported but that most of the subtribes defined by Clayton and Renvoize (1986) were polyphyletic. They also found a monophyletic Maydeae. The latter conclusion is perhaps not surprising, as the data were largely the same as those used by Watson and Dallwitz (1992) in their phenetic study.

A recent analysis of plastid *ndhF* sequences from Andropogoneae and their outgroups showed that, for the set of taxa included in that study, the tribe is monophyletic and that *Arundinella* is the sister group (Spangler et al. 1999), agreeing with other analyses of molecular data (Mason-Gamer et al. 1998; Mathews et al. 2000; Lukens and Doebley 2001). Within Andropogoneae, neither *Sorghum* nor the subtribe Sorghinae was monophyletic, nor was the former Maydeae *sensu* Watson and Dallwitz (1992). All awnless taxa except for *Coix* formed a paraphyletic grade at the base of the tree. In the most parsimonious trees, the awned taxa formed a clade that included awnless *Coix*, but this group had no bootstrap support. Among the awned taxa, a poorly supported clade was identified and informally named the "core Andropogoneae." This clade included *Andropogon*, *Bothriochloa*, *Capillipedium*, *Cymbopogon*, *Dichanthium*, *Heteropogon*, *Hyparrhenia*, *Schizachyrium*, and *Sorghastrum*. Surprisingly, *Coix* also fell in this group although it is morphologically quite different. *Cleistachne* was nested within the *Sorghum* clade, which was sister to the "core Andropogoneae."

The starlike nature of the *ndhF* phylogeny indicated either convergent nucleotide evolution or rapid radiation of the lineages that comprise the tribe. If short internal branches in the *ndhF* phylogeny result from convergent evolution of the gene, we would not necessarily expect a similar pattern of short internal branches and relatively long terminal branches in phylogenies of other genes from Andropogoneae. Thus, it is notable that Mason-Gamer et al. (1998) reported a similar lack of substitutions along internal branches of the Andropogoneae clade in their nuclear phylogeny of GBSSI from Poaceae, as did Lukens and Doebley (2001) in their phylogeny of the nuclear gene *teosinte branched*.

We were interested to see if additional data from a nuclear gene would help resolve the phylogeny of the Andropogoneae. We have therefore sampled the nuclear gene phytochrome B (*PHYB*) from representative genera in Andropogoneae and added new sequences from *ndhF* and GBSSI. Here we report results from analysis of these data and from analyses of *PHYB*, GBSSI, and *ndhF* combined.

Material and Methods

Taxonomic Sampling, DNA Cloning, and Sequencing

Twenty-four species of Andropogoneae, five species of Paniceae, and two species of Arundinelleae were included in the *PHYB* survey; data from GBSSI, *ndhF*, or both were available from all but one of the taxa sampled (table 1). A 1.2-kilobase (kb) region of exon 1 of *PHYB* was amplified from total DNA of 20 newly sampled species of Andropogoneae (sources of DNA given in table 1) according to methods previously described (Mathews et al. 2000). Newly reported GBSSI and *ndhF* sequences were obtained according to methods reported in Mason-Gamer et al. (1998) and Spangler et al. (1999), respectively. We did not include the *PHYB*, the GBSSI (Mason-Gamer et al. 1998), or the *ndhF* (Spangler et al. 1999) sequences from *Sorghastrum* because they were obtained from different DNAs, some of which were from sources of uncertain identity.

Phylogenetic Analyses

A *PHYB* matrix of 1128 nucleotides from 31 species was assembled using Sequencher (Gene Codes). A nine-nucleotide gap was inserted in the sequence from *Paspalum* in a region previously noted for a high frequency of insertions and deletions (Mathews et al. 1995; Mathews and Sharrock 1996). A GBSSI matrix of 778 nucleotides from 19 species, a subset of the alignment that was analyzed by Mason-Gamer et al. (1998), was provided by R. J. Mason-Gamer, and GBSSI sequences from an additional seven species were provided by R. E. Spangler and R. J. Mason-Gamer. An *ndhF* matrix of 2124 nucleotides from 30 species, a subset of the alignment that was analyzed by Spangler et al. (1999), was provided by R. E. Spangler, and sequences from an additional two species were provided by R. E. Spangler and E. A. Kellogg. The three matrices were combined to produce a data set of 4030 nucleotides from 33 species. In the combined data set, *Andropogon gerardii* (*ndhF* and GBSSI sampled) and *Andropogon ternarius* (*PHYB* sampled) were combined in a single terminal. Likewise, *Ischaemum afrum* (*ndhF* and *PHYB* sampled) and *Ischaemum santapaui* (GBSSI sampled), *Paspalum simplex* (*PHYB* sampled) and *Paspalum paniculatum* (*ndhF* sampled), and *Panicum capillare* (*PHYB* sampled) and *Panicum virgatum* (*ndhF* sampled) were combined in single terminals. Combining congeneric species assumes that these terminals are monophyletic relative to the set of taxa included in the analysis (GPWG 2001).

Parsimony analyses of the single and combined matrices were conducted using PAUP* (Swofford 2000). Each heuristic search comprised 100 replicates of random taxon addition and TBR branch swapping. Characters were weighted equally, and gaps were treated as missing data. Branch lengths were determined under ACCTRAN (accelerated transformation) optimization in PAUP*, and unambiguous changes were identified using the Trace All Changes option in MacClade (Maddison and Maddison 1992). Bootstrap values (Felsenstein 1985) for branches in the *PHYB*, GBSSI, and combined trees were estimated in 100 bootstrap replicates in which the same search settings were implemented. Bootstrap values for branches in the *ndhF* tree were estimated in 100,000 bootstrap replicates of simple taxon addition without branch swapping because searches using random taxon addition aborted in the first replicate from lack of sufficient memory to store all possible trees. Phylogenies were rooted along the branch to *Danthoniopsis dinteri*. *Danthoniopsis* is strongly supported as a member of the Centothecoideae, which is itself clearly the sister group of

Table 1

Species Sampled for the Three-Gene Analysis of Andropogoneae, with Vouchers and GenBank Accession Numbers

Tribe and species	Voucher	*PHYB*[a]	*ndhF*[b]	GBSSI[c]
Andropogoneae:				
Andropogon gerardii Vitman	PI 477973	nd		
Andropogon ternarius Michx.	PI 301216	AF443798	nd	nd
Apluda mutica L.	PI 271556	AF443799		nd
Bothriochloa bladhii (Retz.) S.T. Blake	PI 384059	AF443800		
Capillipedium parviflorum (R. Br.) Stapf	PI 301782			
Chionachne koenigii Thw.	RS 97-18	AF443801		nd
Chrysopogon fulvus (Spreng.) Chiov.	PI 185144	AF443802		
Chrysopogon gryllus (L.) Trin.	PI 250984	AF443803		
Cleistachne sorghoides Benth.	IS 14346 (ISC)	AF443804		AF446081
Coelorachis selloana (Hack.) A. Camus	PI 404763	AF443805		AY062271
Coix aquatica Roxb.	456995 (MIN)	AF443806		
Cymbopogon flexuosus (Nees ex Steud.) Watson	PI 209700	AF443807		
Cymbopogon jwarancusa Schult.	PI 211159	nd		
Dichanthium aristatum (Poir.) C.E. Hubbard	PI 301994	AF443808		
Elionurus muticus Kuntze	JS 5865 (BLFU)	AF443809		nd
Heteropogon contortus (L.) Beauv.	PI 364892	AF443810		
Hyparrhenia hirta (L.) Stapf	PI 196827	AF443811		
Ischaemum afrum (J.F. Gmel.) Dandy	PI 364923	AF443812		nd
Ischaemum santapaui Bor	PI 213265	nd	Excluded	
Microstegium nudum (Trin.) A. Camus	SJ 7965	AF443813		AF446082
Miscanthus japonicus Anderss.	AA 301-80c			AF446083
Phacelurus digitatus (Sm.) Griseb.	PI 206746	AF443815		AF318769
Saccharum officinarum L.	RS s.n.	AF443816	AF443824	AF446084
Schizachyrium scoparium Nash	EAK V48	AF443817		
Sorghum bicolor (L.) Moench		AF182394		
Tripsacum dactyloides L.	EAK V49	AF443818		nd
Zea mays L.				
Arundinelleae:				
Arundinella hirta (Thunberg) C. Tanaka	PI 263693			
Arundinella nepalensis Trin.	PI 384059	nd		
Danthoniopsis dinteri (Pilger) C.E. Hubbard	PI 207548			
Paniceae:				
Panicum capillare L.			nd	nd
Panicum virgatum L.		nd	Clark et al. 1995	nd
Paspalum paniculatum L.	*Morrone 3554*		AY029667	
Paspalum simplex Morong	PI 271572	AF443814	nd	AF318770
Pennisetum alopecuroides (L.) Spreng	EAK s.n.		AY029672	
Tristachya superba (DeNot.) Schweinf. & Asch.	RS 97-26	AF443819	nd	nd
Urochloa mutica (Forsk.) Nguyen[d]	RS 97-25	AF443820		nd

Note. Abbreviations for plant or sequence voucher numbers: AA = Arnold Arboretum, living collection; EAK = E. A. Kellogg; IS = International Crop Research Institute for the Semi-arid Tropics; JS = J. Spies; PI = USDA Plant Introduction numbers; RS = Russ Spangler; SJ = S. Jacobs. All vouchers are deposited at GH unless identified by a different acronym in parentheses (Holmgren et al. 1990). nd = not determined.

[a] 1128 base pairs from exon 1; from Mathews et al. (2000) unless otherwise noted.

[b] From Spangler et al. (1999) unless otherwise noted.

[c] From Mason-Gamer et al. (1998) unless otherwise noted.

[d] Cited as *Rattraya petiolata* in Spangler et al. (1999).

the Panicoideae (Spangler et al. 1999; Mathews et al. 2000; Giussani et al. 2001; GPWG 2001). Templeton tests (Templeton 1983; Larson 1994), performed using PAUP* (Swofford 2000), were used to evaluate conflicting topologies that resulted from separate analyses of single-gene matrices.

Bayesian analysis of the combined matrix was conducted using MrBayes version 2.01 (Huelsenbeck and Ronquist 2001). We assumed the GTR + Γ model of nucleotide substitution (Tavaré 1986; Yang 1994) and used the program's default uniform prior probabilities. Four Markov chain Monte Carlo chains were run for 1 million generations, each starting at a random tree; one chain was cold and three were heated (temp. = 0.2). The first 25,000 chains were discarded, after which every hundredth tree was sampled. Thus, inference is based on a sample of 9750 trees.

Results

We detected variation among multiple *PHYB* sequences in *Zea mays*, consistent with the presence of two loci in the maize

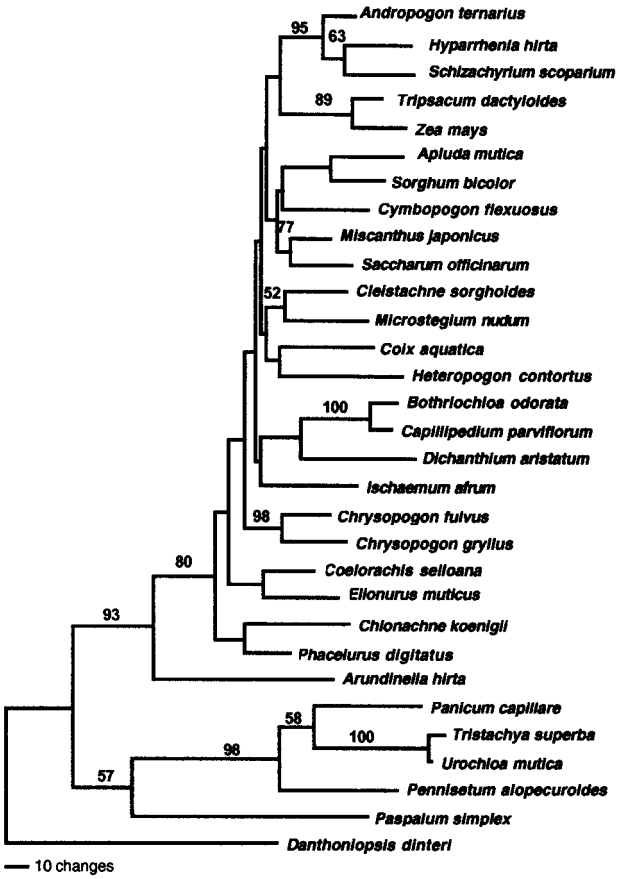

Fig. 1 One of 742 equally parsimonious trees based on sequences of *PHYB*. 187 sites were potentially phylogenetically informative. Length = 742 steps, CI (consistency index) = 0.47, RI (retention index) = 0.54.

genome, but we did not detect variation in multiple *PHYB* clones of the other species sampled. The two *PHYB* from maize were sister sequences in cladograms (not shown), and we arbitrarily included one in subsequent analyses. The final *PHYB* matrix comprised 1128 nucleotides, 187 of which were phylogenetically informative. Two percent of the cells in the *PHYB* matrix were coded as missing.

Parsimony analysis of the 31 *PHYB* sequences from Andropogoneae yielded 742 equally parsimonious trees of 790 steps with consistency indices (CI; excluding uninformative characters) and retention indices (RI) of 0.47 and 0.54, respectively. One of these trees is shown in figure 1. The *PHYB* data support the monophyly of Andropogoneae (bootstrap value of 80%) and the relationship of *Arundinella* + Andropogoneae (bootstrap of 93%), while the monophyly of the Paniceae is only weakly supported (bootstrap of 57%). Within Andropogoneae, *Tripsacum* is united with *Zea* (bootstrap of 89%), *Miscanthus* with *Saccharum* (bootstrap of 77%), *Microstegium* with *Cleistachne* (bootstrap of 52%), *Bothriochloa* with *Capillipedium* (bootstrap of 100%), and *Andropogon* with *Hyparrhenia* + *Schizachyrium* (bootstrap of 95%). The monophyly of *Chrysopogon* is supported by a bootstrap value of 98%. Within Paniceae, *Paspalum* is sister to the rest, *Pennisetum* diverges next (bootstrap of 98%), and *Panicum* is sister to *Tristachya* + *Urochloa*; the latter relationship is supported by a bootstrap value of 100%.

Parsimony analysis of the 32 *ndhF* sequences yielded 24 equally parsimonious trees of 501 steps with consistency (excluding uninformative characters) and retention indices of 0.60 and 0.70, respectively. One of these trees is presented in figure 2. Relationships supported by this *ndhF* data set are similar to those resolved in the larger analysis of Spangler et al. (1999). A core Andropogoneae that includes *Coix* was retained (without support) in the strict consensus of the 24 trees, and *Zea* and *Tripsacum* were united successively with *Elionurus* and then *Chionachne*. Both *Chrysopogon* and *Cymbopogon* are monophyletic (bootstraps of 83% and 82%, respectively). *Andropogoneae* is monophyletic (bootstrap of 59%) and is united with *Arundinella* (bootstrap of 100%). The newly sampled *Saccharum* is united with *Microstegium* and *Miscanthus* in the strict consensus tree, but the three taxa are unresolved in the bootstrap 50% majority rule consensus tree (not shown).

Parsimony analysis of the 26 GBSSI sequences yielded a single most parsimonious tree of 475 steps with consistency (excluding uninformative characters) and retention indices of 0.42 and 0.46, respectively. The GBSSI data support the separation of *Pennisetum* from *Arundinella* and Andropogoneae

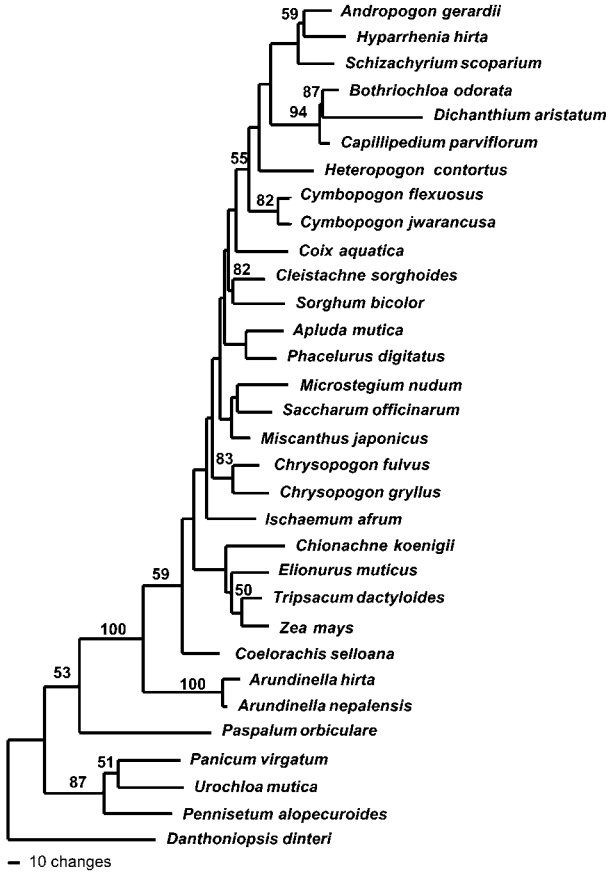

Fig. 2 One of 24 equally parsimonious trees based on sequences of *ndhF*. 126 sites were potentially phylogenetically informative. Length = 501 steps, CI = 0.6, RI = 0.7.

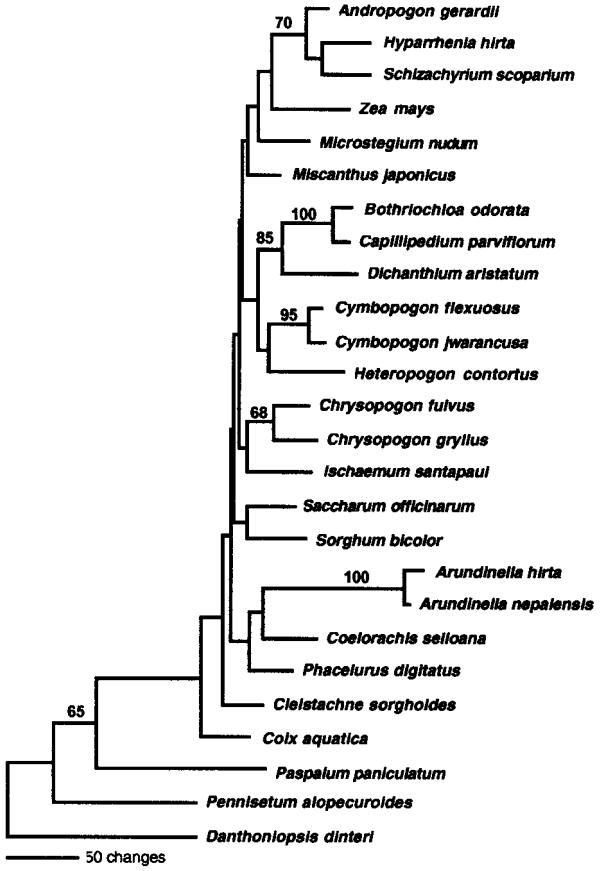

Fig. 3 Single most parsimonious tree based on sequences of GBSSI. 102 sites were potentially phylogenetically informative. Length = 475 steps, CI = 0.42, RI = 0.46.

(bootstrap of 65%), but Andropogoneae is not monophyletic in the most parsimonious tree (fig. 3). Within Andropogoneae, *Dichanthium* is united with *Bothriochloa* + *Capillipedium* (bootstrap of 85%), and *Andropogon* is united with *Hyparrhenia* + *Schizachyrium* (bootstrap of 70%). Both *Chrysopogon* and *Cymbopogon* are monophyletic (bootstraps of 68% and 95%, respectively).

The separate analyses of single-gene matrices resolved congruent relationships, with two exceptions. In each of the individual gene trees, a clade of *Bothriochloa*, *Capillipedium*, and *Dichanthium* is resolved. However, in the *PHYB* and GBSSI trees, *Bothriochloa* is united with *Capillipedium* (bootstraps of 100% in both trees), while in the *ndhF* tree, *Bothriochloa* is united with *Dichanthium* (bootstrap of 87%). Conversely, the relationship of *Cleistachne* and *Sorghum* is supported (bootstrap of 82%) by the *ndhF* data, while the *PHYB* data unite *Cleistachne* with *Microstegium* (bootstrap of 52%). Templeton tests indicate that the *ndhF* data cannot reject the placement of *Cleistachne* with *Microstegium* ($P < 0.08$) and that the *PHYB* data cannot reject the placement of *Cleistachne* with *Sorghum* ($P < 0.47$).

Parsimony analysis of data from the three genes combined (33 taxa) yielded a single most parsimonious tree of 1792 steps with consistency (excluding uninformative characters) and retention indices of 0.47 and 0.54, respectively (fig. 4). In the three-gene phylogeny, Andropogoneae is monophyletic (bootstrap of 95%, 16 unambiguous nucleotide changes) and is united with *Arundinella* (bootstrap of 100%, 31 unambiguous nucleotide changes). Paniceae is paraphyletic; *Paspalum* is united with *Arundinella* + Andropogoneae (bootstrap of 52%), as it is in both the *ndhF* and GBSSI trees. Within Andropogoneae, the awnless genera form a basal assemblage that is paraphyletic to the remaining genera. Among the awnless genera, just the relationship of *Tripsacum* and *Zea* is well supported (bootstrap of 95%). The awned genera form a clade supported by only four nucleotide substitutions, three of which change unambiguously on this branch. Within them, the core Andropogoneae (without *Coix*) is monophyletic (bootstrap of 64%; six unambiguous nucleotide substitutions). Within the core Andropogoneae, *Andropogon* is united with *Hyparrhenia* + *Schizachyrium* (bootstrap of 100%), *Dichanthium* is united with *Bothriochloa* + *Capillipedium* (bootstrap of 98%), and *Cymbopogon* is monophyletic (bootstrap of 99%). Among the remaining awned genera, only the relationship of *Miscan-*

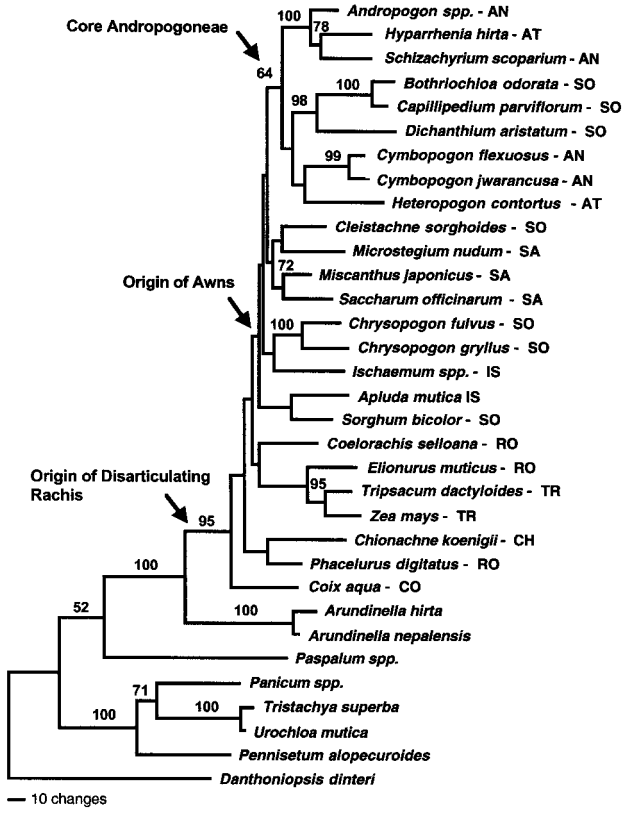

Fig. 4 Single most parsimonious tree based on combined sequences of *PHYB*, *ndhF*, and GBSSI. 415 sites were potentially phylogenetically informative. Length = 1792 steps, CI = 0.47, RI = 0.54. Abbreviations following taxon names indicate subtribal assignment according to Clayton and Renvoize (1986). *AN* = Andropogoninae; *AT* = Anthistiriinae; *SO* = Sorghinae; *SA* = Saccharinae; *IS* = Ischaeminae; *RO* = Rottboelliinae; *TR* = Tripsacinae; *CH* = Chionachninae; *CO* = Coicinae. The origin of awns and origin of the disarticulation of penultimate inflorescence branches ("rachis") are indicated by arrows. The latter marks the origin of the tribe Andropogoneae.

thus + *Saccharum* is supported (bootstrap of 72%). Despite support in the *ndhF* data for the placement of *Cleistachne* with *Sorghum* (bootstrap of 82%), the combined data place *Cleistachne* with *Microstegium* but without bootstrap support (fig. 4). This position is poorly supported in the *PHYB* tree and is not supported in the GBSSI tree (bootstrap of <5%).

We note the same starlike quality in the three-gene phylogeny (fig. 4) that we observe in the single-gene phylogenies (figs. 1–3). It is characterized by a backbone of very short internal branches leading to small groups of genera in contrast to long external branches leading to individual genera.

Bayesian inference of phylogeny (fig. 5) provides additional evidence of many relationships noted in the parsimony tree (fig. 4). The core Andropogoneae are monophyletic with a posterior probability (*P*) of 1.0, and they are united with a clade of Saccharinae + *Cleistachne* ($P = 0.73$). *Cleistachne* is united with *Microstegium* ($P = 0.87$), and they are sister to *Miscanthus* + *Saccharum* ($P = 0.88$). *Elionurus* is united with *Tripsacum* + *Zea* ($P = 0.97$). Moreover, the Bayesian tree suggests that *Chionachne* and *Phacelurus* are early-diverging lineages in Andropogoneae ($P = 0.99$).

Discussion

Addition of data from two nuclear genes, *PHYB* and GBSSI, to the analysis of relationships within Andropogoneae markedly strengthens the major conclusions resulting from analysis of the plastid data from *ndhF* (Spangler et al. 1999). The monophyly of the tribe and its sister-group relationship with *Arundinella* is strongly supported, as is the "core Andropogoneae," which excludes *Coix*.

Placement of *Paspalum* ($P = 0.99$) as sister to Andropogoneae s.l. is consistent with comprehensive studies of Panicoideae, which place Paniceae species with $x = 10$ (such as *Paspalum*) in a clade separate from the $x = 9$ species (Gómez-Martinez and Culham 2000; Giussani et al. 2001). The studies of Panicoideae weakly support a link between Andropogoneae and $x = 10$ Paniceae, a conclusion that our data also support.

The combined data resolve some clades not seen in the single-gene phylogenies and provide greater support for several others. *Zea* is united with *Tripsacum* (bootstrap 95%; $P = 1.0$), and they form a clade with *Elionurus* ($P = 0.97$). *Cleistachne* is united with *Microstegium* ($P = 0.87$), and they form a clade with *Miscanthus* and *Saccharum* ($P = 0.88$); these four genera are sister to the "core Andropogoneae" ($P = 0.73$).

Much evidence links *Zea* and *Tripsacum*. The species are interfertile (Mangelsdorf and Reeves 1931; Li et al. 1997), and they are placed together in both morphological and molecular phylogenies (Hamby and Zimmer 1988; Kellogg and Watson 1993; Buckler and Holtsford 1996; Spangler et al. 1999; Lukens and Doebley 2001). Thus, all current evidence, including that presented here, places *Tripsacum* as the sister genus of *Zea* and does not support its direct involvement in the origin and domestication of maize (Bennetzen et al. 2001).

The phylogeny presented here is similar to that of Lukens and Doebley (2001), which used many of the same plant and DNA accessions and investigated 18 genera. They found, as we did, that *Zea* and *Tripsacum* are sister taxa, and they identified a "core Andropogoneae" that included a subset of the

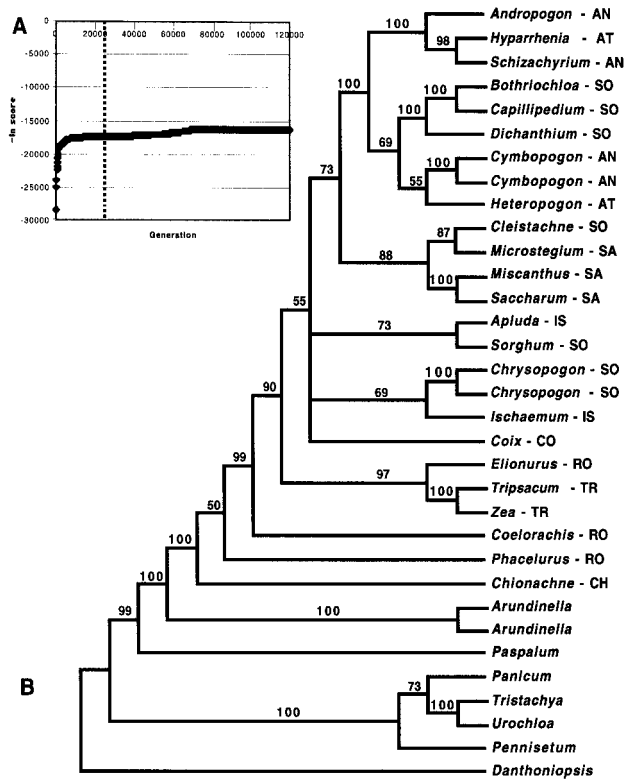

Fig. 5 *A*, The −ln likelihood scores plotted against generations in the Markov chain; stationarity was reached at about generation 70,000; the first 25,000 generations were discarded. *B*, 50% majority rule consensus of 9750 trees sampled in Bayesian analysis of the combined sequences of *PHYB*, *ndhF*, and GBSSI. Posterior probabilities are indicated above the branches. Abbreviations following taxon names indicate subtribal assignment according to Clayton and Renvoize (1986). *AN* = Andropogoninae; *AT* = Anthistiriinae; *SO* = Sorghinae; *SA* = Saccharinae; *IS* = Ischaeminae; *CO* = Coicinae; *RO* = Rottboelliinae; *TR* = Tripsacinae; *CH* = Chionachninae.

taxa that we sampled. The material identified by the USDA as *Heteropogon contortus* (PI 216429) is the same seed lot that produced plants of *Hyparrhenia hirta* in our greenhouse. The material is in the same place in the *tb1* tree and in the combined tree presented here, in a strongly supported clade with *Andropogon*. We hypothesize that the USDA identification is erroneous, or the seed lot became contaminated after its accession into the plant introduction system. A similar problem appears with PI 356816, which we have vouchered and identified as *Panicum repens*, rather than *Panicum virgatum* as indicated by the USDA and the *tb1* tree. The material was collected in Papua New Guinea, where *P. virgatum* does not occur, indicating that *P. repens* is the correct identity of the accession.

We observe the same lack of molecular divergence among clades when the data are combined as was observed in the single-gene phylogenies, including the *tb1* phylogeny (Lukens and Doebley 2001). The concentration of nucleotide changes on terminal branches and the relative lack of change along the backbone suggest rapid diversification. We cannot test this hypothesis without better sampling of lineages within the tribe,

but other explanations, such as nucleotide convergence, seem unlikely given that the pattern is characteristic of three independent gene phylogenies from two different genomes.

Implications for Morphological Evolution

Although Andropogoneae is generally described as a "natural" group (Clayton 1987), most of the presumed synapomorphies actually characterize deeper nodes in the tree and/or are homoplasious among panicoid grasses (Kellogg 2000). However, disarticulation of the penultimate inflorescence axes (the branches that bear the spikelet pairs) originates at the point indicated in figure 4 and thus helps define the tribe. This disarticulation pattern permits the spikelet pair to be the dispersal unit rather than the individual spikelet, floret, or caryopsis, and it may have played a role in the rapid appearance of distinct lineages in the tribe. For example, the disarticulating rachis might have facilitated innovation in modes of dispersal, thus allowing more frequent establishment of geographically isolated populations (Doyle and Donoghue 1986).

The parsimony tree (fig. 4) is congruent with Clayton's (1972, 1973) division of the tribe into awned and awnless genera and, with respect to this character, is also largely consistent with the morphological phylogeny presented by Kellogg and Watson (1993). However, while this tree suggests a single unreversed gain of awns, support for a monophyletic awned clade is lacking (figs. 4, 5), and the awned clade does not appear in the Bayesian tree because of the position of *Coix*.

Awns are not common in Panicoideae outside the Andropogoneae. The lemmas of the Paniceae are usually well developed and more or less robust. In the few Paniceae species with awns, the awns are either as robust as the lemmas or less robust. In contrast, the lemmas of the awned Andropogoneae frequently are much reduced and membranous, often occurring as a pair of fragile teeth at the base of the significantly more robust awn. Clayton (1987, p. 308) points out that this structure is "presumably the lemma of the upper floret," suggesting the distinctiveness of the structure. The awn is generally geniculate and often has a point of disarticulation at the top of the lemma or in the sinus between two lemma lobes. This general description applies to most awned Andropogoneae, although *Arthraxon*, with its tiny dorsally placed awn, may be an exception; no molecular data are available for this genus.

Awns occur sporadically in *Arundinella*. The awn of *Arundinella nepalensis* extends from the tip of the well-developed lemma. Three clear veins can be seen in the translucent lemma, the central one extending into the awn and the laterals converging toward the awn base. The base of the awn is flattened and brown, whereas the upper portion is more hairlike. The awn in *A. nepalensis* is thus only superficially similar to those in Andropogoneae, and the phylogeny indicates that awns in Andropogoneae and *Arundinella* are not homologous. A developmental study might indicate details of awn formation that distinguish the two groups.

On the abaxial leaf epidermis, many of the core Andropogoneae have a single oblique papilla on each of the intercostal cells, overarching the stomata (Watson and Dallwitz 1992). This character is unusual in Andropogoneae and among panicoids in general, although it is slightly homoplasious. Of the genera included in this study, *Andropogon* and *Schizachyrium* lack such papillae, whereas *Apluda* is reported to have them. Other awned genera, not sampled here, that have intercostal cells with one oblique papilla include *Agenium*, *Apocopis*, *Arthraxon*, *Diectomis*, *Dybowskia*, *Eremopogon*, *Euclasta*, *Homozeugos*, *Iseilema*, *Parahyparrhenia*, and *Spathia* (Watson and Dallwitz 1992). We predict, on the basis of their awns and their epidermal morphology, that they are also part of the core group of genera. *Urelytrum* is apparently the only awnless taxon with a single oblique papilla and may or may not be related to the taxa listed above. The apparent homoplasy in the character needs to be addressed by careful species-level studies.

The strong support for the clade containing *Andropogon*, *Hyparrhenia*, and *Schizachyrium* is one of the surprises produced by molecular data (figs. 1–5; Mason-Gamer et al. 1998; Spangler et al. 1999), as there are no obvious morphological characters that unite the three genera to the exclusion of other members of the tribe. *Schizachyrium* is a segregate of *Andropogon*, so the relationship of the two might have been predicted, but the inclusion of *Hyparrhenia* in this clade was unexpected. All three genera include a large number of species, and *Andropogon* is likely to be polyphyletic; future studies will thus have to investigate more species of each to test the connection demonstrated here.

Bothriochloa, *Capillipedium*, and *Dichanthium* are known to be interfertile at the tetraploid level, even though the diploids are intersterile (Harlan and DeWet 1963; DeWet and Harlan 1966; and references in both), and they form a clade in molecular phylogenies (Mason-Gamer et al. 1998; Spangler et al. 1999). *Bothriochloa bladhii* (=*B. intermedia*), the species investigated here, has been called a "compilospecies" in that it appears to have acquired characteristics of multiple other species via hybridization, polyploidy, and apomixis (DeWet and Harlan 1970). The differences found here between the nuclear and chloroplast trees are consistent with such a complex history. The combined tree reflects the two nuclear gene trees and is significantly different from trees in which *Bothriochloa* and *Dichanthium* are forced together ($P < 0.0001$). With only one species per genus, we cannot replicate the detailed sampling produced by DeWet and Harlan, but our preliminary data suggest that more molecular studies will support their earlier observations.

The monophyly of *Cymbopogon* was demonstrated clearly by data from GBSSI (Mason-Gamer et al. 1998) and is supported by the combined data presented here. The genus includes "lemongrass," *Cymbopogon citratus*, which is commonly used for flavoring in Asian cooking. Some species of the genus are strongly aromatic when crushed, although this characteristic occurs in other Andropogoneae as well. The relationship between *Cymbopogon* and *Heteropogon* is not strongly supported by these data, and no obvious morphological character unites them.

The relationship of *Cleistachne sorghoides* with *Sorghum* s.s. is supported by the overall resemblance of spikelets of the two genera and by data from *ndhF* (fig. 2; Spangler et al. 1999). However, our analyses of combined data suggest that *Cleistachne* is a member of Saccharinae and sister to *Microstegium* (figs. 4, 5; $P = 0.87$). This finding is consistent with the *tb1* tree (Lukens and Doebley 2001) and with an analysis that

included *Cleistachne* and all species currently placed in *Sorghum* (R. E. Spangler and E. A. Kellogg, unpublished data).

Miscanthus and *Saccharum* are morphologically quite similar and historically have been placed together (Bews 1929; Keng 1939; Pilger 1940, Celarier 1956; Clayton and Renvoize 1986). In *Miscanthus*, the rachis is tough and the "sessile" spikelets are actually short pedicellate, in contrast to the fragile rachis and truly sessile spikelets in *Saccharum*. The genera are among the few Andropogoneae (including, e.g., *Imperata* and *Eulalia*) in which sessile and pedicellate spikelets are similar in form and sex expression. This characteristic appears in our phylogeny to be a reversal rather than a plesiomorphy, as might be expected. Both genera, as with all others in the *Saccharum* group, share long hairs on the callus.

Chrysopogon is monophyletic. It is distinctive among the sampled genera in that its spikelets occur in threes. No obvious morphological character connects *Chrysopogon* with *Ischaemum*, despite the relationship indicated by the combined data.

Inflorescence form is homoplasious on the phylogeny. Many members of Andropogoneae have spikelets arranged in digitate racemes, and these can be solitary, paired, or numerous. For example, the inflorescences of *Cymbopogon* and *Hyparrhenia* are similar in being composed of paired racemes. The two genera also can be difficult to separate since the racemes in both may be reflexed. Nonetheless, the genera are apparently not closely related. *Cymbopogon* is instead sister to *Heteropogon*, which has single unbranched racemes, and *Hyparrhenia* is sister to *Schizachyrium*, which also has unbranched racemes. Other taxa such as *Sorghum*, *Capillipedium*, and *Chrysopogon*, which are unrelated, have more highly branched inflorescences that are generally described as panicles. The inference from the phylogeny is thus that inflorescence form is not a good predictor of relationship. Conversely, inflorescence form must be fairly easy to modify in evolutionary time and is worth more detailed study.

Implications for Classification

The sister taxon relationship between Andropogoneae and *Arundinella* supports the suggestion of Kellogg (2000) that the latter be included within the former tribe and that the tribe Arundinelleae be abandoned as a taxonomic group. The data presented here also support previous suggestions that *Danthoniopsis* (formerly placed in Arundinelleae) be excluded from Arundinelleae and Andropogoneae (Giussani et al. 2001; GPWG 2001). No rooting of the tree would place *Danthoniopsis* sister to or contained within Andropogoneae.

The combined data do not support the subtribal designations of Clayton and Renvoize (1986), a result already noted for the GBSSI (Mason-Gamer et al. 1998), *ndhF* (Spangler et al. 1999), and *tb1* (Lukens and Doebley 2001) trees. Moreover, hypothesis tests based on parsimony analyses explicitly reject monophyly of Andropogoninae and Anthistiriinae. Trees in which Andropogoninae were forced to be monophyletic were significantly longer than the most parsimonious trees ($P < 0.0039$), as were trees forcing monophyly of Anthistiriinae ($P < 0.0009$).

The monophyly of the remaining tribes we sampled is not always rejected in parsimony-based hypothesis tests, but their monophyly is challenged by the Bayesian tree (fig. 5). Sorghinae *sensu* Clayton and Renvoize (1986) is polyphyletic (figs. 4, 5). Most of the equally parsimonious trees in which Sorghinae is monophyletic are not significantly different from the tree in figure 4 ($P < 0.089$ to $P < 0.101$), but our data do reject the monophyly of Sorghinae in some trees ($P < 0.028$). Moreover, Sorghinae is clearly polyphyletic in the Bayesian tree; the segregation of some members is suggested with a posterior probability of 1.0 (fig. 5). Similarly, Saccharinae is paraphyletic (figs. 4, 5). And while its monophyly cannot always be rejected ($P < 0.025$ to $P < 0.353$), the Bayesian tree suggests that *Cleistachne* (Sorghinae) and *Microstegium* (Saccharinae) are sister taxa ($P = 0.87$). Rottboelliinae is polyphyletic, and monophyly is not rejected by parsimony analyses ($P < 0.500$), but *Elionurus* is clearly segregated from other Rottboelliinae in Bayesian analyses (fig. 5; $P = 0.97$). Ischaeminae (*Apluda*, *Ischaemum*) is polyphyletic, and monophyly cannot be rejected in parsimony analyses ($P < 0.746$), but the two are clearly not related in the Bayesian analysis. Only Tripsacinae is monophyletic; as defined by Clayton and Renvoize (1986), the subtribe includes the genera *Tripsacum* and *Zea*, both of which are sampled here and are strongly supported as sister taxa. Coicinae is monotypic, and we have sampled only the type genus of Chionachninae and so did not test its monophyly.

The combined tree also does not support recognition of "Maydeae" *sensu* Watson and Dallwitz (1992), which is here shown to be polyphyletic. Traditionally this tribe included *Zea*, *Tripsacum*, *Coix*, *Polytoca*, *Chionachne*, *Sclerachne*, and *Trilobachne*, all of which are monoecious, with staminate and pistillate spikelets clearly separated in the inflorescence (or, in *Zea*, in separate inflorescences). On the tree presented here, the monoecious genera do not form a monophyletic group, and monoecy has apparently arisen multiple times.

Heteropogon contortus is also monoecious (LeRoux and Kellogg 1999), with all spikelets in the proximal part of the inflorescence being staminate and those in the distal part being pistillate. Curiously, the species is not usually mentioned in discussions of Maydeae, perhaps because other species of *Heteropogon* are andromonoecious. The proximal spikelet pairs in a number of genera in Andropogoneae are wholly staminate, with the more distal spikelets being bisexual. Monoecy could thus be derived from andromonoecy by suppression of stamen development in the distal spikelets.

We thus confirm the conclusions of previous workers (Kellogg 2000 and citations therein) that the subtribal classification of Clayton and Renvoize (1986) should be abandoned and the tribe Maydeae should no longer be recognized. However, a number of informal groups are well supported by the molecular data. There is clear evidence for a lineage that we have called the "core Andropogoneae" and of a putative sister lineage comprising an expanded Saccharinae that includes *Microstegium*. Epidermal cells with a single oblique papilla may constitute a morphological synapomorphy for the "core Andropogoneae," although some homoplasy appears in the character. We also provide evidence that *Elionurus* is closely related to *Tripsacum* + *Zea*, a relationship noted in the *ndhF* but not in other molecular trees. Finally, our data suggest that *Chionachne* and *Phacelurus* are early-diverging members of the tribe, a placement that has not been suggested previously, to our knowledge.

These relationships remain to be evaluated in a more ex-

tensive study of the 85 genera of Andropogoneae recognized by Clayton and Renvoize (1986). Our sample of 25 species constitutes less than 3% of the ca. 1000 species of the tribe, although the 23 genera represent more than one-fourth of the total number of genera. However, the monophyly of most genera has not been tested by this or by any other phylogenetic study. The *ndhF* data of Spangler et al. (1999) show that *Microstegium* is polyphyletic and *Sorghum* may be as well (Spangler 2000; R. E. Spangler and E. A. Kellogg, unpublished data). *Andropogon* is large and variable and is the genus from which all the others have been removed; this suggests that it will prove to be paraphyletic, although this hypothesis is currently untested.

The strongly supported subgroups that have emerged from this study are likely to be clarified by inclusion of more species, including a broader range of morphological and molecular variation. We are optimistic that additional taxonomic sampling will result in better resolution of relationships (Hillis 1996; Soltis et al. 1998), and this will be a major focus of our future studies.

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