Aliso: A Journal of Systematic and Evolutionary Botany

Volume 23 | Issue 1 Article 40

2007

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Elizabeth M. Skendzic
University of Wisconsin–Parkside, Kenosha

J. Travis Columbus
Rancho Santa Ana Botanic Garden, Claremont, California

Rosa Cerros-Tlatilpa
Rancho Santa Ana Botanic Garden, Claremont, California

Aliso 23, pp. 530–544

PHYLOGENETICS OF ANDROPOGONEAE (POACEAE: PANICOIDEAE) BASED ON NUCLEAR RIBOSOMAL INTERNAL TRANSCRIBED SPACER AND CHLOROPLAST *trn*L–F SEQUENCES

ELIZABETH M. SKENDZIC,[1,3,4] J. TRAVIS COLUMBUS,[2] AND ROSA CERROS-TLATILPA[2]

[1]*University of Wisconsin–Parkside, 900 Wood Road, Kenosha, Wisconsin 53141-2000, USA;*
[2]*Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California 91711-3157, USA*
[3]*Corresponding author (emskendzic@lcsc.edu)*

ABSTRACT

Phylogenetic relationships among 85 species representing 35 genera in the grass tribe Andropogoneae were estimated from maximum parsimony and Bayesian analyses of nuclear ITS and chloroplast *trn*L–F DNA sequences. Ten of the 11 subtribes recognized by Clayton and Renvoize (1986) were sampled. Independent analyses of ITS and *trn*L–F yielded mostly congruent, though not well resolved, topologies. *Arundinella* is sister to Andropogoneae in the *trn*L–F phylogeny and is nested within the tribe in the ITS and combined data trees. *Tristachya* is sister to Andropogoneae + *Arundinella* in the ITS phylogeny. Four clades are common to the ITS and *trn*L–F phylogenies and the trees from the combined data set. Clade A consists of *Andropogon*, *Diectomis*, *Hyparrhenia*, *Hyperthelia*, and *Schizachyrium.* Within this clade, *Andropogon distachyos*, *Hyparrhenia*, and *Hyperthelia* form clade C. Clade B consists of *Bothriochloa*, *Capillipedium*, and *Dichanthium*, and clade D includes *Chrysopogon* and *Vetiveria.* Analysis of the combined data resulted in an unsupported larger clade comprising clades A and B plus *Cymbopogon*, and a sister clade of *Heteropogon*, *Iseilema*, and *Themeda.* This larger clade is similar to the core Andropogoneae clade previously reported (Spangler et al. 1999; Mathews et al. 2002). Based on our sample, which represents 41% of the tribe's genera, most of Clayton and Renvoize's (1986) subtribes are not monophyletic.

Key words: Andropogoneae, Bayesian inference, cladistics, internal transcribed spacer (ITS), Panicoideae, parsimony, phylogeny, Poaceae, *trn*L–F.

INTRODUCTION

The grass tribe Andropogoneae (Panicoideae) boasts many species of economic and ecological importance worldwide. As a human staple, maize (*Zea mays* subsp. *mays*) evolved and sustained civilizations in North America in the same way that sorghum (*Sorghum bicolor*) did in Africa. Sudan grass (*Sorghum* ×*drummondii* (Nees ex Steud.) Millsp. & Chase), a hybrid of *S. arundinaceum* and *S. bicolor,* is cultivated for animal feed, and thus represents an indirect food crop. Sugar (*Saccharum officinarum* and related species), essential oils (*Cymbopogon* spp., *Vetiveria zizanioides*), and ornamental beads (*Coix lacryma-jobi*) are among the additional, varied products derived from species of Andropogoneae. Members of the tribe are often conspicuous, if not dominant, elements of grasslands and savannas, such as species of *Hyparrhenia*, *Imperata*, and *Themeda* (among numerous others) in Africa, and *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and *Sorghastrum nutans* (Indian grass) in North America. On the downside, *Imperata cylindrica*, *Ischaemum rugosum* Salisb., *Rottboellia cochinchinensis* (Lour.) Clayton, and *Sorghum halepense* are major worldwide weeds (Chapman 1996). Andropogoneae include ca. 1000 species, representing approximately one-tenth of the world's grass species (Clayton and Renvoize 1986; Watson and Dallwitz 1992), and are most diverse in the Old World. In the New World, Zuloaga et al. (2007) reported 230 species in 35 genera for Andropogoneae. All species undergo C_4 photosynthesis; correspondingly, although extending into warm temperate regions, the tribe is most prevalent in the tropics and subtropics.

Spikelets in Andropogoneae are usually arranged in pairs on spicate racemes (Fig. 1). The number and arrangement of spicate racemes on the flowering culm varies widely in the tribe. A single raceme may terminate the culm, whereas, at the opposite extreme, the racemes may form repeating units in large, much-branched, compound panicles. For most species, the spikelets of a pair are dissimilar, notably in that one is sessile and bisexual, and the other is pedicellate and staminate or neuter (Fig. 1). A triad of two pedicellate spikelets and one sessile spikelet usually terminates the spicate raceme (Fig. 1). Rarely, the entire inflorescence is reduced to a triad (e.g., *Apluda*). Some other variants include spikelets unpaired and solitary (e.g., *Dimeria* R. Br.), both spikelets of the pair bisexual (e.g., *Eulalia*, *Miscanthus*, *Saccharum*), spikelet pairs of one staminate or neuter subsessile spikelet and one bisexual pedicellate spikelet (*Trachypogon*), pedicellate spikelet reduced to just the pedicel (e.g., *Sorghastrum*), proximal and distal spikelets of the raceme dissimilar (e.g., *Euclasta*, *Heteropogon*, *Tripsacum*), and racemes unisexual and dimorphic (e.g., *Coix*, *Zea*). Each pair of spikelets generally disarticulates as a unit along with a segment of the raceme rachis and the pedicel (Fig. 2), a feature that seems to confer great seed dispersal ability.

The first thorough study of Andropogoneae was published by E. Hackel in 1889. He divided the tribe into five subtribes and 30 genera, some further divided into series, subgenera, and sections (Table 1). An example is the 13 subgenera rec-

[4]Present address: Lewis-Clark State College, Natural Sciences Division, Lewiston, Idaho 83501, USA.

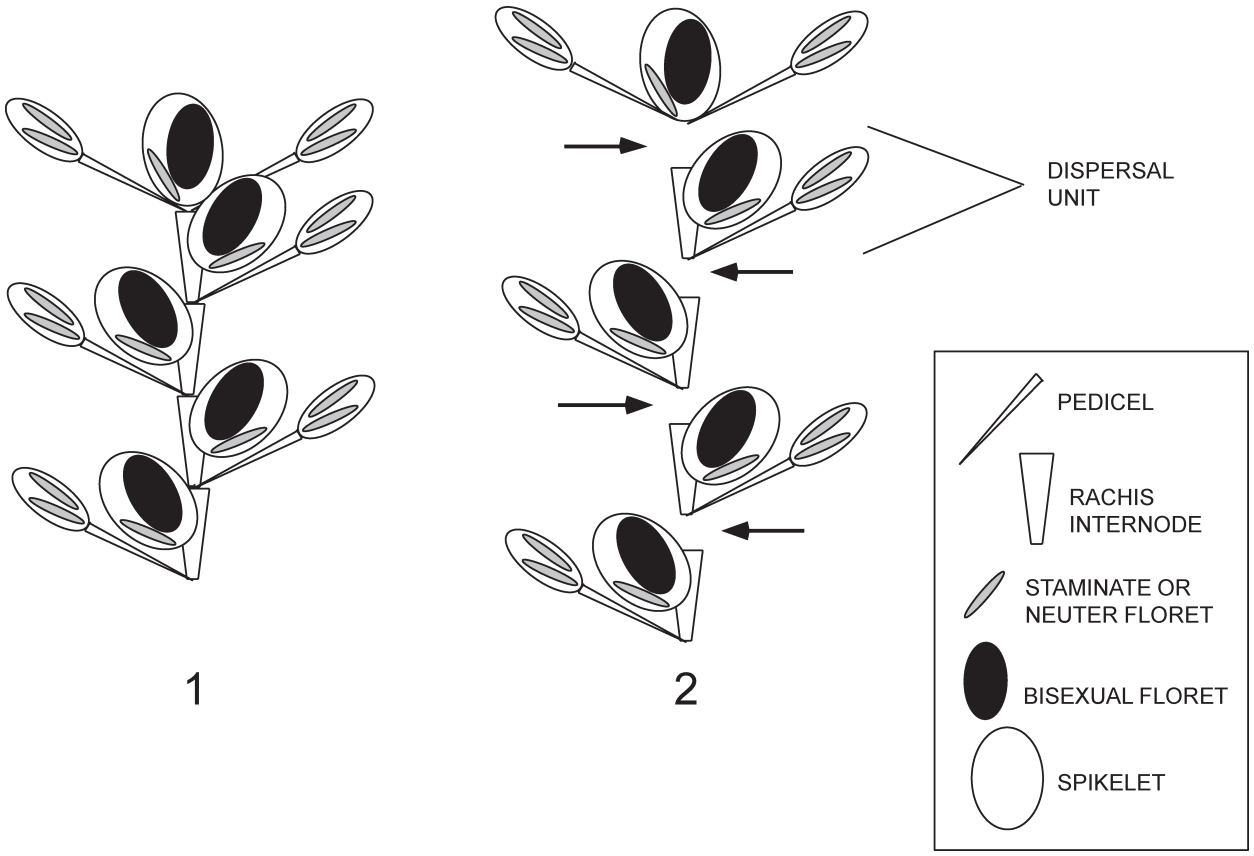

Fig. 1–2.—Spicate racemes of Andropogoneae showing typical spikelet arrangement and disarticulation.—1. Raceme with four pairs of spikelets (each pair with one sessile and one pedicellate spikelet) and a spikelet triad (one sessile and two pedicellate spikelets) at the apex.—2. Raceme with rachis disarticulation indicated by arrows, the dispersal unit consisting of a sessile spikelet, a pedicellate spikelet, and a rachis internode (two pedicellate spikelets and a sessile spikelet in the terminal triad).

ognized in the large genus *Andropogon.* Many of the subgenera were later treated at the generic level, a trend still manifest in current classifications. Hackel (1887–1888) placed maize and relatives in a separate tribe, Maydeae. Recent classifications include Clayton and Renvoize (1986), Watson and Dallwitz (1992), Soreng et al. (2006), and Zuloaga et al. (2007) (Table 1). Clayton and Renvoize (1986) arranged the species into a single tribe, 11 subtribes, and 85 genera. Like Hackel, Watson and Dallwitz (1992) recognized two tribes (forming supertribe Andropogonodae), Andropogoneae and Maydeae, the former consisting of two large subtribes, Andropogoninae and Rottboelliinae. Overall, Andropogoneae (including Maydeae) are easily recognized as a group. They have paired spikelets, a C_4 photosynthetic pathway, and a single sheath of cells around the vascular bundle. These features, however, are present in other Panicoideae. A disarticulating rachis internode is a synapomorphy for the tribe (Mathews et al. 2002), but delimitation of genera and subtribes is problematic due to a lack of identifiable diagnostic characters (Clayton 1987; Spangler 2000; Mathews et al. 2002).

Clayton and Renvoize (1986) used morphological characters of the Andropogoneae inflorescence, rachis internode, and spikelet pair to suggest intratribal relationships along axes of increasing complexity. Kellogg and Watson (1993) performed a cladistic parsimony analysis on a large data set comprising 72 Andropogoneae/Maydeae genera and 220 characters (mostly morphological and anatomical). They found three groups roughly corresponding to Watson and Dallwitz's (1992) classification. The phylogenetic tree, however, did not support the subtribal classification of Clayton and Renvoize (1986).

DNA sequence data (chloroplast gene NADH dehydrogenase [*ndh*F]; nuclear genes granule-bound starch synthase [GBSSI or *waxy*], phytochrome B [*PHY*B], and teosinte branched 1 [*tb*1]) have also been used to investigate the phylogeny of Andropogoneae (Mason-Gamer et al. 1998; Spangler et al. 1999; Spangler 2000; Lukens and Doebley 2001; Giussani et al. 2001; Mathews et al. 2002). The largest number of genera and species of Andropogoneae in these studies is 23 and 41, respectively. The monophyly of the tribe and a sister relationship to *Arundinella* (tribe Arundinelleae sensu Clayton and Renvoize 1986) are supported. Kellogg (2000) recommended *Arundinella* to be included in Andropogoneae. Likewise, phylogenetic trees from all four markers show a similar pattern—internal branches are short and poorly supported, suggesting that the tribe resulted from a rapid radiation or that molecular evolution was relatively slow as these lineages arose (Mason-Gamer et al. 1998; Spangler et al. 1999). Despite much phylogenetic uncertainty, these studies cast doubt on the monophyly of Maydeae (Watson and Dallwitz 1992) and most of Clayton and Ren-

Table 1. Four classification systems of Andropogoneae and Maydeae compared for genera included in the study. All subtribes recognized in these classifications are shown. * = described after Hackel (1889).

Hackel 1887–1888, 1889 Tribe Subtribe Genus Series Subgenus Section	Clayton and Renvoize 1986 Tribe Subtribe Genus	Watson and Dallwitz 1992 Tribe Subtribe Genus	Zuloaga et al. 2003; Soreng et al. 2006 Tribe Subtribe Genus
Andropogoneae (Hackel 1889)	Andropogoneae	Andropogoneae	Andropogoneae
Dimerieae	Dimeriinae	Andropogoninae	Andropogoninae
Euandropogoneae	Andropogoninae		
Andropogon	*Andropogon*	*Andropogon*	*Andropogon*
Heterozygi			
Cymbopogon			
	Andropogoninae		Andropogoninae
Gymnanthelia	*Cymbopogon*	*Cymbopogon*	*Cymbopogon*
	Anthistiriinae		Anthistiriinae
Hyparrhenia	*Hyparrhenia*	*Hyparrhenia*	*Hyparrhenia*
	*Hyperthelia**	*Hyperthelia*	*Hyperthelia*
	Sorghinae		Sorghinae
Dichanthium	*Dichanthium*	*Dichanthium*	*Dichanthium*
	Anthistiriinae		Anthistiriinae
Heteropogon	*Heteropogon*	*Heteropogon*	*Heteropogon*
Isozygi			
	Sorghinae		Sorghinae
Amphilophis	*Bothriochloa*	*Bothriochloa*	*Bothriochloa*
	*Capillipedium**	*Capillipedium*	
Chrysopogon	*Chrysopogon*	*Chrysopogon*	*Chrysopogon*
	*Euclasta**	*Euclasta*	*Euclasta*
	Andropogoninae		Andropogoninae
Diectomis	*Andropogon*	*Diectomis*	
Schizachyrium	*Schizachyrium*	*Schizachyrium*	*Schizachyrium*
	Sorghinae		Sorghinae
	*Sorghastrum**	*Sorghastrum*	*Sorghastrum*
Sorghum	*Sorghum*	*Sorghum*	*Sorghum*
Vetiveria	*Vetiveria*	*Vetiveria*	
	Andropogoninae		Andropogoninae
Arthraxon	*Arthraxon*	*Arthraxon*	*Arthraxon*
	Rottboelliinae	Rottboelliinae	Rottboelliinae
Elionurus	*Elionurus*	*Elionurus*	*Elionurus*
	Anthistiriinae	Andropogoninae	Anthistiriinae
Iseilema	*Iseilema*	*Iseilema*	
Themeda	*Themeda*	*Themeda*	*Themeda*
	Germainiinae		Germainiinae
Trachypogon	*Trachypogon*	*Trachypogon*	*Trachypogon*
Ischaemeae	Ischaeminae		Ischaeminae
Apluda	*Apluda*	*Apluda*	*Apluda*
Rottboellieae	Rottboelliinae	Rottboelliinae	Rottboelliinae
Rottboellia			
Coelorachis	*Coelorachis*	*Coelorachis*	
	*Hackelochloa**	*Hackelochloa*	
	Rottboellia	*Rottboellia*	*Rottboellia*
Hemarthria	*Hemarthria*	*Hemarthria*	*Hemarthria*
Saccharieae	Saccharinae	Andropogoninae	Saccharinae
Erianthus	*Saccharum*	*Erianthus*	
Imperata	*Imperata*	*Imperata*	*Imperata*
Miscanthus	*Miscanthus*	*Miscanthus*	*Miscanthus*
Pollinia			
Eulalia	*Eulalia*	*Eulalia*	
Saccharum			
Eusaccharum	*Saccharum*	*Saccharum*	*Saccharum*
	Saccharum	*Narenga**	

Table 1. Continued.

Maydeae (Hackel 1887–1888)		Maydeae	
	Chionachninae		
Chionachne	*Chionachne*	*Chionachne*	
	Coicinae		Coicinae
Coix	*Coix*	*Coix*	*Coix*
	Tripsacinae		Tripsacinae
Euchlaena	*Zea*	*Euchlaena*	
Tripsacum	*Tripsacum*	*Tripsacum*	*Tripsacum*
Zea	*Zea*	*Zea*	*Zea*

voize's (1986) subtribes (some are explicitly rejected), although Tripsacinae (*Tripsacum* and *Zea*), of the nine nonmonotypic subtribes, are supported as monophyletic.

In the present study we sample more genera and species of Andropogoneae and employ two additional molecular markers—the internal transcribed spacers and intervening 5.8S gene of nuclear ribosomal DNA (ITS), and the *trn*L intron, *trn*L 3′ exon, and *trn*L–*trn*F intergenic spacer (hereafter simply *trn*L–F) of chloroplast DNA.

MATERIALS AND METHODS

Taxa and Collections

Eighty-five species representing 35 genera of Andropogoneae, including 11 of the largest, were sampled. The taxon names, sources of collections, and voucher information are provided in Table 2. Seeds obtained from the USDA National Germplasm Resources Laboratory were sown and plants grown to anthesis to obtain ample material and confirm identification. DNA sequences for 26 species (24 Andropogoneae) were obtained from GenBank (Table 2). With exception of the five Sánchez-Ken collections and sequence data obtained from GenBank, the first author identified all field- and greenhouse-grown source collections. The sampling represents 41% of the 85 genera in the tribe (Clayton and Renvoize 1986).

Species of *Digitaria* and *Panicum,* two panicoid genera in Paniceae, were chosen as the outgroup. *Arundinella* and another genus in Arundinelleae, *Tristachya*, were also sampled (Table 2). *Tristachya biseriata* Stapf resolved within Andropogoneae in cladistic parsimony and neighbor-joining analyses of chloroplast *rbc*L (ribulose-bisphosphate carboxylase) sequences emphasizing Arundinoideae (Barker et al. 1995). *Tristachya superba* (De Not.) Schweinf., however, resolved outside Andropogoneae in a three-gene phylogeny of Andropogoneae (Mathews et al. 2002).

For most samples, ca. 1 g of healthy leaf tissue was either frozen in liquid nitrogen for later processing or used immediately for DNA isolation. A few samples were obtained from herbarium specimens by removing 0.1 g of leaf tissue.

DNA Extraction, Amplification, and Sequencing

For frozen and fresh tissue, total genomic DNA was isolated using the CTAB protocol of Doyle and Doyle (1987) as modified in Columbus et al. (1998). For tissue from herbarium specimens, the DNeasy® Plant Mini Kit from QIAGEN (Valencia, California, USA) was used following the manufacturer's protocol. Primers ''ITS4'' and ''ITS5'' (White et al. 1990) were employed to PCR-amplify ITS, and primers ''BR'' (Columbus et al. 2007) and ''f'' (Taberlet et al. 1991) were used to amplify *trn*L–F. For ITS an initial denaturing step of 1 min at 97°C was followed by 40 cycles of 1 min at 97°C, 1 min at 48°C, and 2 min at 72°C, and concluded with a final extension step of 7 min at 72°C. For *trn*L–F an initial denaturing step of 1 min at 97°C was followed by 40 cycles of 1 min at 97°C, 1 min at 54°C, and 2 min at 72°C, and concluded with a final extension step of 7 min at 72°C. Purification was carried out using the polyethylene glycol precipitation protocol (Morgan and Soltis 1993) or with the QIAquick® PCR purification kit (QIAGEN) following the manufacturer's protocol. Templates were sequenced on an ABI 373 or GA 3100 automated DNA sequencer (Perkin Elmer-Applied Biosystems, Foster City, California, USA). Primers employed for sequencing the ITS region were the same as those used for amplification plus ''ITS2'' and ''ITS3'' (Porter 1997). For *trn*L–F, the internal primers ''LL1R'' and ''LL3F'' (Columbus et al. 2007) were employed along with those used for amplification.

Sequences were assembled, edited, and consensus sequences constructed using Sequencher vers. 4.0.5 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The bounds of ITS were determined by comparison with sequences in Hsiao et al. (1994). The bounds of *trn*L–F were determined by comparison with the *Zea mays* chloroplast genome sequence (Maier et al. 1995).

Sequences were visually aligned utilizing Se-Al vers. 2.0 (Rambaut 2001). Indels were not coded.

Phylogenetic Analyses

Parsimony.—Maximum parsimony (MP) analysis of each matrix was conducted using PAUP* vers. 4.0b10 (Swofford 2002). The parsimony ratchet (Nixon 1999) was employed, as implemented in PAUPRat (Sikes and Lewis 2001). Each analysis consisted of 20 independent runs of 200 iterations each. Characters (nucleotide sites) were treated as unordered and weighted equally. Gaps were treated as missing data, and if a sequence exhibited multiple nucleotides at a site it was treated as a polymorphism. Employing tree-bisection-reconnection (TBR) branch swapping, a heuristic search was performed using as starting trees those from the parsimony ratchet analysis. The ''collapse branches if maximum length is zero'' option was selected, and ten trees per replicate were saved. The ''steepest descent'' option was not in effect. All minimum-length trees were saved and a strict consensus tree was generated. Bootstrap (BS) support for nodes was deter-

Table 2. Taxa, collections, and GenBank accession numbers of DNA sequences used in the study. Literature references are provided for sequences not reported herein. Vouchers are deposited at RSA unless otherwise specified. USDA National Plant Germplasm System accession numbers begin with ''PI''. * = Collected from the living collections at the Royal Botanic Gardens, Kew, Surrey, UK, or Agriculture Development and Advisory Service, Arthur Rickwood Research Station, Cambridge, UK.

Taxon	Voucher/Accession/Origin	GenBank accession no.		Reference
		ITS	*trn*L–F	
Andropogon L.				
bicornis L.	*Columbus 4076*, Mexico	DQ005013		
distachyos L.	*Skendzic 5075*, PI 300696, Hungary	DQ005014	DQ004951	
gerardii Vitman	*Skendzic 5073*, commercial seed	DQ005015	DQ004952	
glomeratus (Walter) Britton, Sterns & Poggenb.	*Columbus 4238*, USA		DQ004953	
ternarius Michx.	*Columbus 4022*, Mexico		DQ004954	
Apluda L.				
mutica L.	*Skendzic 5077*, PI 219568, Pakistan	DQ005016	DQ004955	
Arthraxon P. Beauv.				
hispidus (Thunb.) Makino (1)	*Columbus 4373*, Venezuela	DQ005017		
(2)	*Sánchez-Ken 635* (ISC), Mexico	DQ005018	DQ004956	
Arundinella Raddi				
hirta (Thunb.) Tanaka	*Skendzic 5082*, PI 263693, South Korea	DQ005019	DQ004957	
nepalensis Trin. (1)	*Skendzic 5101*, PI 384059, India	DQ005020	DQ004958	
(2)	*Jacobs 7181* (NSW?)	AF019816		Hsiao et al. 1999
Bothriochloa Kuntze				
alta (Hitchc.) Henrard	*Columbus 4049*, Mexico	DQ005021	DQ004959	
barbinodis (Lag.) Herter	*Skendzic 5099*, PI 404743, Uruguay	DQ005022		
bladhii (Retz.) S. T. Blake	*Columbus 4615*, Mexico		DQ004960	
compressa (Hook. f.) Henrard	*Skendzic 5085*, PI 301282, India	DQ005023	DQ004961	
ewartiana (Domin) C. E. Hubb.	*Skendzic 5083*, PI 300724, Australia		DQ004962	
grahamii (Haines) Bor	*Skendzic 5084*, PI 301384, Singapore	DQ005024	DQ004963	
ischaemum (L.) Keng (1)	*Skendzic 5100*, PI 477958, USA	DQ005025	DQ004964	
(2)	*Manrique 1820* (COCA), Mexico	DQ141239	DQ141238	
laguroides (DC.) Herter	*Skendzic 5095*, PI 404289, Brazil	DQ005026	DQ004965	
macra (Steud.) S. T. Blake	*Skendzic 5084*, PI 301274, Australia	DQ005027	DQ005097	
pertusa (L.) A. Camus	*Skendzic 5090*, PI 301743, India	DQ005028	DQ004966	
springfieldii (Gould) Parodi	*Skendzic 5096*, PI 337509, Argentina	DQ005029	DQ005098	
sp.	No voucher, Colorado, USA (37°09′37″N, 104°06′08″W)	DQ005051		
Capillipedium Stapf				
spicigerum S. T. Blake	*Skendzic 5089*, PI 301773, Australia	DQ005030	DQ004967	
venustum (Thwaites) Bor	*Skendzic 5086*, PI 301731, Guyana	DQ005031	DQ004968	
Chionachne R. Br.				
cyathopoda (F. Muell.) F. Muell. ex Benth.	*Jacobs 7180* (NSW?)	AF019819		Hsiao et al. 1999
Chrysopogon Trin.				
serrulatus Trin.	*Skendzic 5076*, PI 219579, Pakistan	DQ005032	DQ005099	
Coelorachis Brongn.				
rugosa (Nutt.) Nash	*Columbus 4286*, USA	DQ005033	DQ004969	
Coix L.				
lacryma-jobi L.	*Skendzic 5074*, commercial seed	DQ005034	DQ004970	
Cymbopogon Spreng.				
citratus (DC.) Stapf. (1)	Royal Botanic Gardens, Kew 081-940.1171	AF019823		Hsiao et al. 1999
(2)	*Hodkinson 129* (K),*		AY116258	Hodkinson et al. 2002*a*
flexuosus (Nees ex Steud.) Will. Watson (1)	*Skendzic 5078*, PI 209700, India	DQ005036	DQ005100	
(2)	*Skendzic 5079*, PI 210336, India	DQ005035	DQ004971	
martinii (Roxb.) Will. Watson	*Skendzic 5070*, commercial plant	DQ005037		
Dichanthium Willemet				
annulatum (Forssk.) Stapf (1)	*Skendzic 5102*, PI 469251, USA	DQ005038	DQ004974	
(2)	*Sánchez-Ken 630* (ISC), Mexico	DQ005039	DQ004975	
(3)	*Skendzic 5080*, PI 219976, Afghanistan	DQ005040	DQ004973	
sericeum (R. Br.) A. Camus (1)	*Skendzic 5087*, PI 302033, USA	DQ005042	DQ005093	
(2)	*Skendzic 5091*, PI 302062, Australia	DQ005041	DQ004972	

Table 2. Continued.

Taxon	Voucher/Accession/Origin	GenBank accession no. ITS	GenBank accession no. *trn*L–F	Reference
Diectomis Kunth				
fastigiata (Sw.) P. Beauv. (1)	*Columbus 4057*, Mexico	DQ005043		
(2)	*Columbus 3728*, Mexico		DQ004977	
(3)	*Ramos 770* (COCA), Mexico	DQ005044	DQ004976	
Elionurus Humb. & Bonpl. ex Willd.				
tripsacoides Humb. & Bonpl. ex. Willd. (1)	*Manrique 1904* (COCA), Mexico	DQ005047		
(2)	*Manrique 1895* (COCA), Mexico	DQ005046	DQ004978	
Erianthus Michx.				
arundinaceus (Retz.) Jeswiet		AF345212		Chen et al. 2003
fulvus (R. Br.) Kunth		AF345220		Chen et al. 2003
giganteus (Walter) P. Beauv.	*Columbus 4263*, USA	DQ005048	DQ004979	
ravennae (L.) P. Beauv.	*C. Evans s.n.* (herbarium not indicated)	AF019824		Hsiao et al. 1999
rockii Keng		AF345217		Chen et al. 2003
strictus Baldwin	*Columbus 4181*, USA	DQ005049	DQ004980	
Euclasta Franch.				
condylotricha (Hochst. ex Steud.) Stapf	*Columbus 4107*, Mexico	DQ005050		
Eulalia Kunth				
aurea (Bory) Kunth (1)	*Skendzic 5098*, PI 371930, Australia	DQ005052	DQ004982	
(2)	*Skendzic 5081*, PI 249139, Australia	DQ005053	DQ005101	
Hackelochloa Kuntze				
granularis (L.) Kuntze	*Columbus 2624*, Mexico	DQ172081	DQ172306	
Hemarthria R. Br.				
uncinata R. Br.	*Jacobs 7770* (NSW?)	AF019821		Hsiao et al. 1999
Heteropogon Pers.				
contortus (L.) P. Beauv. ex Roem. & Schult.	*Skendzic 5097*, PI 364892, South Africa	DQ005055	DQ004984	
Hyparrhenia Andersson ex E. Fourn.				
hirta (L.) Stapf	*Skendzic 5103*, PI 516599, Morocco	DQ005056	DQ004986	
Hyperthelia Clayton				
dissoluta (Nees ex Steud.) C. E. Hubb.	*Columbus 4063*, Mexico	DQ005057	DQ004985	
Imperata Cirillo				
cylindrica (L.) Raeusch. (1)		AF345653		Chen et al. 2003
(2)	*Marsden 3* (K),*		AY116262	Hodkinson et al. 2002*a*
Iseilema Andersson				
membranaceum (Lindl.) Domin	*Skendzic 5092*, PI 240840, Australia	DQ005058		
prostratum (L.) Andersson	*Skendzic 5093*, PI 213524, India	DQ005059	DQ004987	
Miscanthus Andersson				
floridulus (Labill.) Warb. ex K. Schum. & Lauterb.		AF345215		Chen et al. 2003
×*giganteus* J. M. Greef. & Deuter ex Hodk. & Renvoize	*Cult. Kew 1780* (K),*	AJ426563		Hodkinson et al. 2002*b*
sacchariflorus (Maxim.) Hack.	*Hodkinson s.n. 1987–2727* (K),*	AJ426564		Hodkinson et al. 2002*a*
sinensis Andersson (1)	*C. Evans s.n.* (herbarium not indicated)	AF019822		Hsiao et al. 1999
(2)	*Skendzic 5069*, commercial plant	DQ005060	DQ005095	
Narenga Bor				
porphyrocoma (Hance ex Trimen) Bor		AF345236		Chen et al. 2003
Rottboellia L. f.				
aurita Steud.	*Skendzic 5104*, PI 404628, Paraguay	DQ005063	DQ004989	
Saccharum L.				
barberi Jeswiet		AF345200		Chen et al. 2003
officinarum L.	*Skendzic 5068*, commercial plant	DQ005064	DQ005096	
robustum E. W. Brandes & Jeswiet ex Grassl		AF345239		Chen et al. 2003
sinense Roxb.		AF345243		Chen et al. 2003
spontaneum L.		AF345245		Chen et al. 2003
Schizachyrium Nees				
brevifolium (Sw.) Nees ex Büse (1)	*Columbus 3618*, Mexico	DQ005065		
(2)	*Columbus 4055*, Mexico	DQ005066		
cirratum (Hack.) Wooton & Standl.	*Tah 12* (COCA), Mexico	DQ005067	DQ004990	
gaumeri Nash	*Santana-Michel 1156* (COCA), Mexico	DQ005068		

Table 2. Continued.

Taxon	Voucher/Accession/Origin	GenBank accession no. ITS	GenBank accession no. *trn*L–F	Reference
malacostachyum (J. Presl) Nash	*Columbus 3727*, Mexico	DQ005069	DQ004991	
neomexicanum (Nash) Nash	*Columbus 4006*, USA		DQ004992	
sanguineum (Retz.) Alston	*Columbus 4045*, Mexico	DQ005070	DQ004993	
scoparium (Michx.) Nash (1)	*Skendzic 5105*, PI 476298, USA	DQ005071	DQ004994	
(2)	*Skendzic 5071*, commercial plant	DQ005072	DQ004995	
semitectum (Swallen) Reeder	*Garcia s.n.* (Nov 1991, COCA), Mexico	DQ005073	DQ004996	
tenerum Nees (1)	*Columbus 4054*, Mexico	DQ005074	DQ004998	
(2)	*Columbus 3729*, Mexico	DQ005075	DQ004997	
Sorghastrum Nash				
incompletum (J. Presl) Nash (1)	*Columbus 4056*, Mexico	DQ005076	DQ004999	
(2)	*Columbus 2623*, Mexico	DQ005077	DQ005000	
nutans (L.) Nash (1)	*Skendzic 5072*, commercial plant	DQ005079	DQ005001	
(2)	*Skendzic 5065*, USA	DQ005080	DQ005102	
secundum (Elliot) Nash	*Columbus 4243*, USA	DQ005078	DQ005002	
Sorghum Moench				
arundinaceum (Desv.) Stapf	*Skendzic 5106*, PI 524718, Sudan	DQ005081	DQ005003	
bicolor (L.) Moench subsp. *bicolor*	China	U04789		Sun et al. 1994
halepense (L.) Pers.	*Sánchez-Ken 602* (ISC), Mexico	DQ005082	DQ005004	
Themeda Forssk.				
triandra Forssk.	*Skendzic 5094*, PI 300141, South Africa	DQ005083	DQ005005	
Trachypogon Nees				
plumosus (Humb. et Bonpl. ex Willd.) Nees	*Columbus 4306*, Venezuela	DQ005085	DQ005006	
secundus (J. Presl) Scribn.	*Columbus 4115*, Mexico	DQ005084		
Tripsacum L.				
australe H. C. Cutler & E. S. Anderson	Timothy 68-67-1, Brazil	U46655		Buckler and Holtsford 1996
dactyloides (L.) L.	*Sánchez-Ken 607* (ISC), Mexico	DQ005086	DQ005007	
laxum Nash	de Wet 3766, Mexico	U46659		Buckler and Holtsford 1996
maizar Hern.-Xol. & Randolph	de Wet 3721, Mexico	U46657		Buckler and Holtsford 1996
Tristachya Nees				
avenacea (J. Presl) Scribn. & Merr.	*Columbus 4077*, Mexico	DQ005087		
leucothrix Trin. ex Nees	*Beck 5018* (DEK), South Africa	DQ005088		
Vetiveria Bory				
zizanioides (L.) Nash	*Skendzic 5107*, PI 538754, India	DQ005089	DQ005009	
Zea L.				
diploperennis H. H. Iltis, Doebley & R. Guzmán (1)	M001, Mexico	U46593		Buckler and Holtsford 1996
(2)	*Iltis & Guzmán 29115* (WIS), Mexico	DQ005091	DQ005011	
(3)	*Sánchez-Ken 624* (ISC), Mexico	DQ005090	DQ005010	
luxurians (Durieu & Asch.) R. M. Bird	Iltis G-5, Guatemala	DQ005092	DQ005012	
mays L. subsp. *mays*			X86563	Maier et al. 1995
subsp. *mexicana* (Schrad.) H. H. Iltis	*Hsiao 197* (UTC?)	AF019817		Hsiao et al. 1999
perennis (Hitchc.) Reeves & Mangelsd.	Ames 21881, Mexico	U46588		Buckler and Holtsford 1996
Outgroup				
Digitaria Haller				
ciliaris (Retz.) Koeler	*Jacobs 7230* (NSW?)	AF019826		Hsiao et al. 1999
sanguinalis (L.) Scop.	*Skendzic 5067*, USA	DQ005045		
Panicum L.				
hirticaule J. Presl var. *hirticaule*	*Columbus 2536*, USA	DQ172082	DQ172307	
virgatum L.	*Skendzic 5066*, USA	DQ005062		

mined from 10,000 replicates of TBR branch swapping using the ''fast stepwise-addition'' option.

Bayesian.—Bayesian analyses were carried out primarily to assess support for clades. The nucleotide substitution model employed for each data set was selected using Modeltest 3.06 (Posada and Crandall 1998; Posada 2001). Using MrBayes vers. 3.0b4 (Huelsenbeck and Ronquist 2001), we ran four chains (Markov Chain Monte Carlo), one cold and three heated. To explore the tree space, five million generations were performed with trees sampled every 100 generations. A majority-rule consensus tree was calculated using PAUP* (Swofford 2002). Trees from the first 268,000 generations were discarded. Clades with posterior probability (PP) values above 94% were considered well supported.

Partition homogeneity test.—To assess data combinability, congruence analyses (Farris et al. 1995) of the *trn*L–F and ITS data sets were conducted. This was implemented in PAUP* (Swofford 2002) as the partition homogeneity test using 1000 replicates and TBR branch swapping (simple addition sequence, Multrees, and steepest descent options selected) with the maximum number of trees retained for each replicate limited to 100.

RESULTS

Sequencing of ITS and *trn*L–F for all samples was attempted, but in some instances one of the markers could not be sequenced due to technical problems. GenBank accession numbers for all sequences are provided in Table 2.

ITS

Aligned sequences of the entire region consisted of 637 characters including gaps. Of these, 340 were variable and 255 were potentially phylogenetically informative. The MP analysis yielded 2746 most parsimonious trees of 1453 steps, with a consistency index (CI) of 0.37 and a retention index (RI) of 0.70. The strict consensus of tree is shown in Fig. 3, including BS percentages and PP values greater than 70% and 94%, respectively. For the Bayesian analysis we employed the GTR + I + G (nst = 6, rates = gamma) model (Posada and Crandall 1998; Posada 2001). Burn-in (or the time for each parameter to reach a stationary phase) was visually determined to be at 502,100 generations at a −ln likelihood score of 9513.49; these generations were discarded from the analysis. The MP and Bayesian strict consensus trees were congruent, and the PP values are shown in Fig. 3.

trn*L–F*

Some regions in the *trn*L–F intergenic spacer were difficult to align, so 227 characters were excluded from the analyses. The data matrix consisted of 1140 included characters with gaps. Of these characters, 160 were variable and 69 were potentially phylogenetically informative. The MP analysis yielded 3993 most parsimonious trees of 204 steps, with CI = 0.87 and RI = 0.91. Less variable than ITS, the *trn*L–F data set also exhibited less homoplasy. The strict consensus of the *trn*L–F trees is shown in Fig. 4, including BS percentages and PP values greater than 70% and 94%, respectively. For the Bayesian analysis we used the F81 + G (nst = 1, rates = equal) model (Posada and Crandall 1998; Posada 2001). Burn-in was visually determined to be at 634,000 generations at a −ln likelihood score of 2528.384; these generations were discarded from the analysis. The MP and Bayesian strict consensus trees were congruent, and the PP values are shown in Fig 4.

ITS + trn*L–F*

Results from the partition homogeneity test ($P = 0.55$) indicated that ITS and *trn*L–F data sets were congruent. Thus, the data sets were combined for analysis. The majority-rule consensus tree from the Bayesian analysis is shown in Fig. 5. No supported topological conflicts were found among the trees from all of the analyses.

Four clades (A–D) are common to all trees (Fig. 3–5). Clade A consists of *Andropogon*, *Diectomis*, *Hyparrhenia*, *Hyperthelia*, and *Schizachyrium.* Within this clade, *Andropogon distachyos*, *Hyparrhenia*, and *Hyperthelia* form clade C. Clade B consists of *Bothriochloa*, *Capillipedium*, and *Dichanthium*, and clade D includes *Chrysopogon* and *Vetiveria.* Clade A is not supported in the independent analyses of the ITS and *trn*L–F data sets, but is supported by a PP of 1.0 in the analysis of combined data. Clade B is supported in the ITS (PP 1.0), *trn*L–F (BS 82%, PP 1.0), and combined data (BS 77%, PP 1.0) trees. As a whole, relationships are not well resolved in clades A and B. The smaller clade C lacks BS support in all trees but is supported by a PP of 0.97 and 1.0 in the *trn*L–F and combined data trees, respectively. Clade D is supported in all analyses by a PP of 1.0 but lacks BS support. A large clade composed of clade A, clade B, *Cymbopogon*, *Heteropogon*, *Iseilema*, and *Themeda* resolves in the combined data tree but lacks support. The same clade plus *Elionurus* is resolved in the *trn*L–F phylogeny and is supported by a PP of 1.0. This larger clade does not resolve in the ITS tree. Common to the *trn*L–F (BS 99%, PP 1.0) and combined data (BS 100%, PP 1.0) trees is a clade of *Sorghum arundinaceum* and *S. halepense.* This clade plus *S. bicolor* is supported in the ITS analyses (BS 99%, PP 1.0). A *Tripsacum* + *Zea* clade is supported in the ITS and combined data analyses (BS 100%, PP 1.0 for both). This clade does not resolve in the analyses of *trn*L–F.

DISCUSSION

The low resolution and support for clades in the ITS, *trn*L–F, and combined trees are consistent with previous studies and may reflect a rapid radiation or slowdown in molecular evolution as these lineages arose (Mason-Gamer et al. 1998; Spangler et al. 1999). Low rates of nucleotide substitution among members of Andropogoneae have been reported by Mason-Gamer et al. (1998) for GBSSI, Spangler et al. (1999) for *ndh*F, Lukens and Doebley (2001) for *tb*1, and Mathews et al. (2002) for *PHY*B.

Andropogoneae share a common ancestor with Arundinelleae (Clayton and Renvoize 1986), and three genera of Arundinelleae have been included in molecular phylogenetic studies of Andropogoneae: *Arundinella*, *Danthoniopsis*, and *Tristachya. Arundinella* is strongly supported as sister to Andropogoneae in analyses of GBSSI (Mason-Gamer et al. 1998), *ndh*F (Spangler et al. 1999; Giussani et al. 2001; Mathews et al. 2002), *tb*1 (Lukens and Doebley 2001), ITS

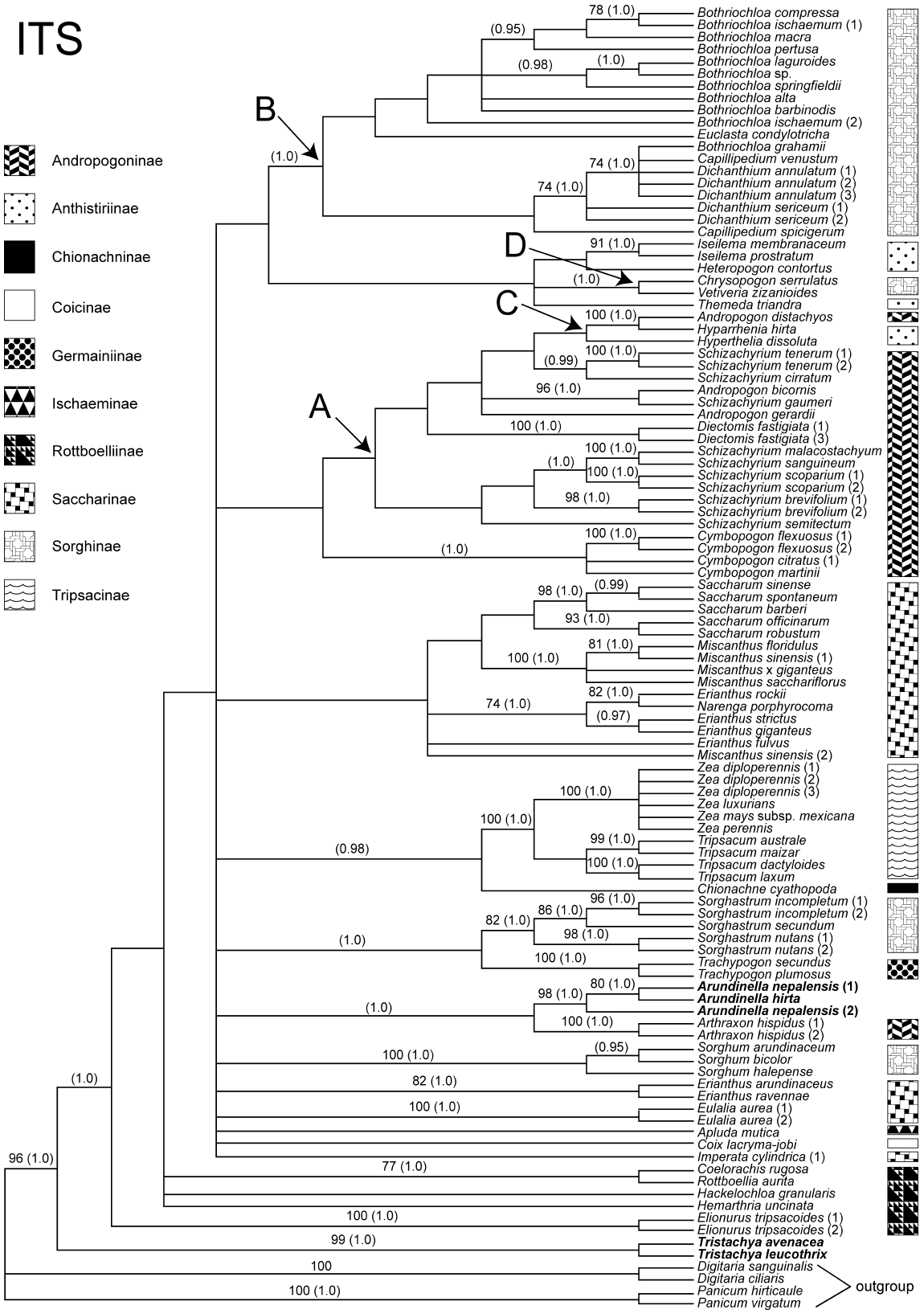

Fig. 3.—Strict consensus of 2746 most parsimonious trees (length = 1453, consistency index = 0.37, retention index = 0.70) from parsimony analysis of the ITS region. Bootstrap values ≥70% are shown above the branches and posterior probability values ≥95% are in parentheses. Taxon names in bold are in tribe Arundinelleae sensu Clayton and Renvoize (1986). The subtribes are from Clayton and Renvoize (1986). Clades A, B, C, and D are referred to in the text.

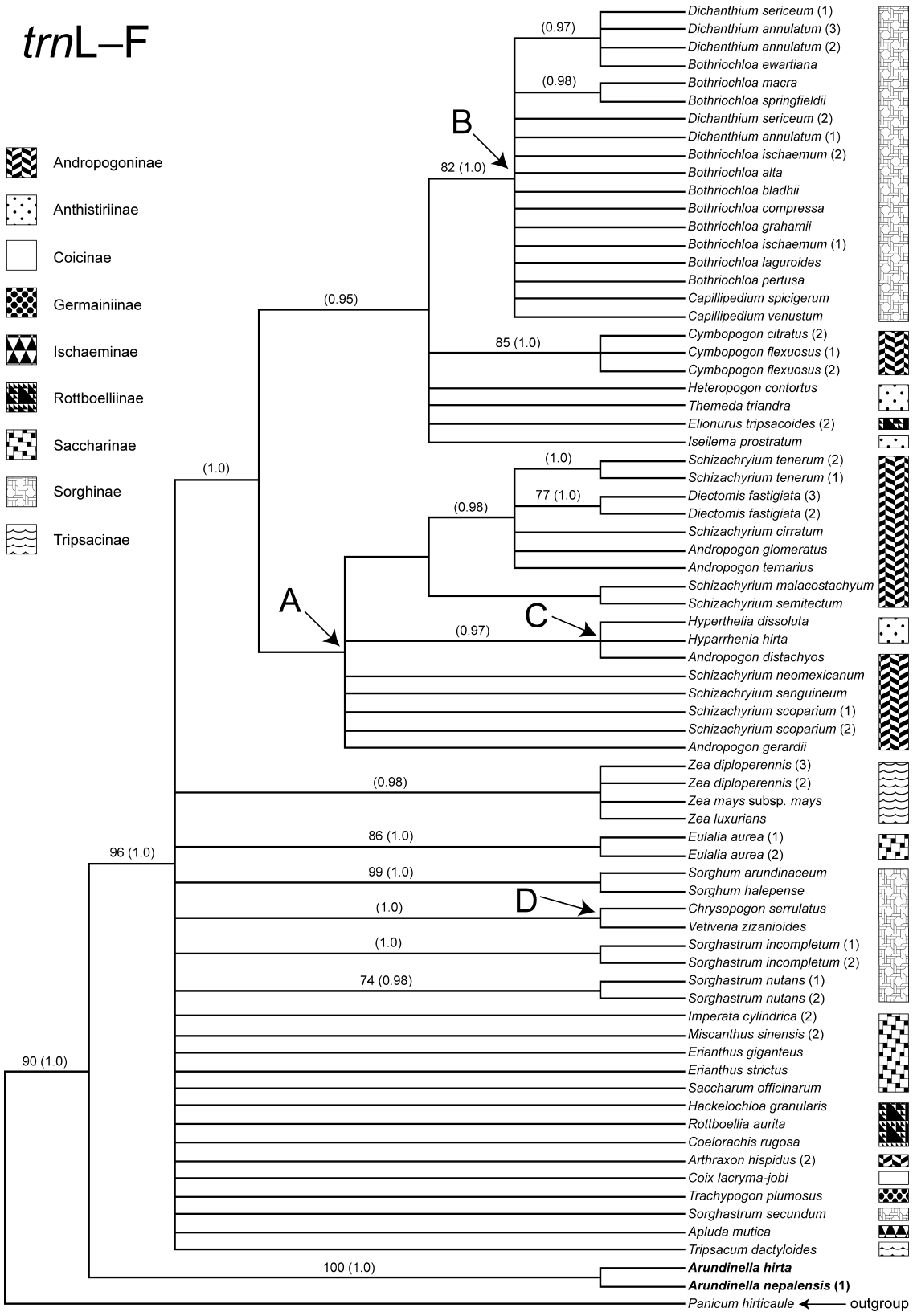

Fig. 4.—Strict consensus of 3993 most parsimonious trees (length = 204, consistency index = 0.87, retention index = 0.91) from parsimony analysis of the *trn*L–F region. Bootstrap values ≥70% are shown above the branches and posterior probability values ≥95% are in parentheses. Taxon names in bold are in tribe Arundinelleae sensu Clayton and Renvoize (1986). The subtribes are from Clayton and Renvoize (1986). Clades A, B, C, and D are referred to in the text.

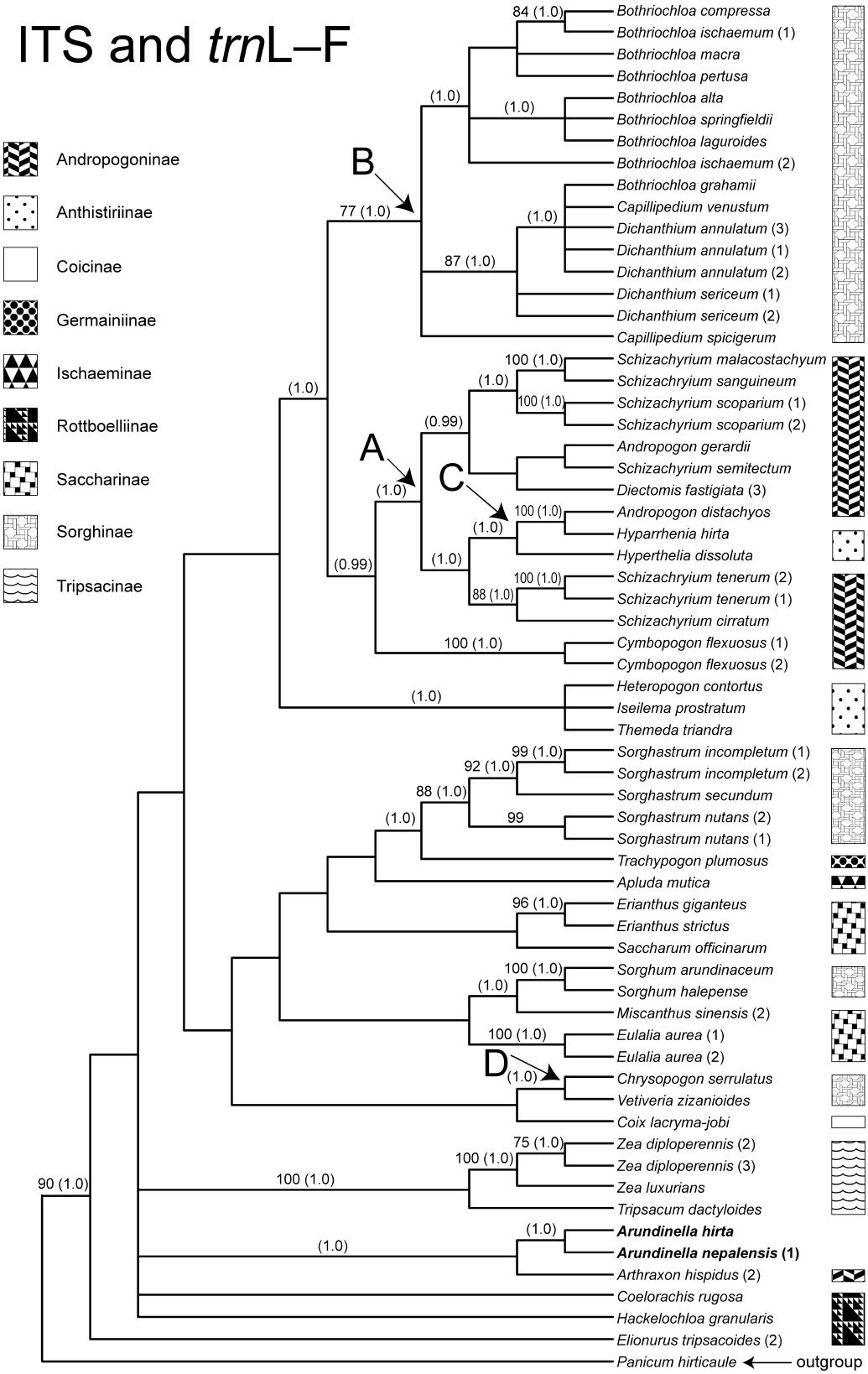

Fig. 5.—Majority-rule consensus tree from Bayesian analysis of the combined ITS and *trn*L–F data sets. Bootstrap values ≥70% are shown above the branches and posterior probability values ≥95% are in parentheses. Taxon names in bold are in tribe Arundinelleae sensu Clayton and Renvoize (1986). The subtribes are from Clayton and Renvoize (1986). Clades A, B, C, and D are referred to in the text.

(Hodkinson et al. 2002*a*), and *PHY*B (Mathews et al. 2002). However, the genus is nested within Andropogoneae in the Mathews et al. (2002) analysis of GBSSI. We found statistical support for the position of *Arundinella* as sister to Andropogoneae in the *trn*L–F phylogeny (BS 90%, PP 1.0; Fig. 4), in accordance with most previous studies, but it is nested within Andropogoneae in the ITS (Fig. 3) and the combined data (Fig. 5) trees.

Tristachya is nested within Andropogoneae in the Barker et al. (1995) *rbc*L analysis. We found statistical support for *Tristachya* as sister to Andropogoneae + *Arundinella* in the ITS tree (BS 96%, PP 1.0; Fig. 3), but limited outgroup sampling constrained our ability to explore the relationship of *Arundinella* and *Tristachya* to Andropogoneae. We were unable to obtain *trn*L–F sequences for *Tristachya.*

Arundinella is sister to *Arthraxon* in the ITS (Fig. 3) and combined data (Fig. 5) trees (PP 1.0 in both), but this association lacks BS support, and supporting non-molecular synapomorphies are not obvious. *Arthraxon* is a small genus that differs substantially from all other members of subtribe Andropogoninae and from *Arundinella* (Clayton and Renvoize 1986). In fact, both genera are unique within their tribes. *Arthraxon* has a geniculate dorsal awn on the lemma of the sessile spikelet, whereas species of *Arundinella* with awned lemmas, as well as most genera of Andropogoneae, have an apical awn originating from between the lobes of the lemma. The spikelets in *Arundinella* are atypical of Arundinelleae in that they are in pairs instead of triads. Spikelets in *Arthraxon* are single as a result of the reduction of the pedicellate spikelet. Based on micromorphological characters of the leaf epidermis, Mathews et al. (2002) speculated that *Arthraxon* would be a member of the core Andropogoneae clade.

The four clades common to the ITS, *trn*L–F, and ITS + *trn*L–F trees are discussed as follows.

Clade A

Andropogon, *Diectomis*, *Hyparrhenia*, *Hyperthelia*, and *Schizachyrium* form a clade in the ITS, *trn*L–F, and combined data trees (Fig. 3–5). The clade is also present in the GBSSI (Mason-Gamer et al. 1998; Mathews et al. 2002), *ndh*F (Spangler et al. 1999; Giussani et al. 2001; Mathews et al. 2002), and *PHY*B (Mathews et al. 2002) phylogenies, except *Diectomis* and *Hyperthelia* were not sampled in these studies.

We cannot identify a morphological synapomorphy for clade A, which mostly comprises genera in subtribe Andropogoninae. *Hyparrhenia* and *Hyperthelia* have paired racemes while *Diectomis* and *Schizachyrium* have single racemes. *Andropogon* is variable, with paired or digitate (rarely single) racemes. Taxonomically, *Diectomis* has been considered a synonym of *Andropogon* (Clayton and Renvoize 1986; Zuloaga et al. 2003, 2007; Soreng et al. 2006) or a distinct genus (Watson and Dallwitz 1992) while *Schizachyrium* has long been segregated from *Andropogon* (Bentham and Hooker 1883; Hackel 1889; Hitchcock 1950; Swallen 1955; Clayton and Renvoize 1986; Watson and Dallwitz 1992). Our results suggest that *Andropogon* and *Schizachyrium* are not monophyletic.

Clade C

Within clade A, clade C includes *Andropogon distachyos*, *Hyparrhenia hirta*, and *Hyperthelia dissoluta* (Fig. 3–5). *Hyparrhenia* and *Hyperthelia* are members of subtribe Anthistiriinae. They differ in their rounded versus grooved lower glume but otherwise are very similar, with paired racemes and one or two homogamous basal spikelet pairs. *Andropogon distachyos* is similar to *Hyparrhenia* and *Hyperthelia* in that it has paired racemes. *Hyparrhenia* is largely an African genus, with *H. hirta* common in tropical savannas. Likewise, most *Hyperthelia* species are restricted to Africa, with only *H. dissoluta* introduced in tropical America. *Andropogon distachyos* is distributed throughout the tropics and in the Mediterranean.

Clade B

A clade formed of *Bothriochloa*, *Capillipedium*, and *Dichanthium* is present in the ITS, *trn*L–F, and combined data trees (Fig. 3–5). *Euclasta,* for which we were unable to obtain a *trn*L–F sequence, is a member of the clade in the ITS phylogeny. The clade is also present in the GBSSI (Mason-Gamer et al. 1998; Mathews et al. 2002), *ndh*F (Spangler et al. 1999; Giussani et al. 2001; Mathews et al. 2002), and *PHY*B (Mathews et al. 2002) phylogenies, except *Euclasta* was not sampled and only one species of each genus was included in these studies. There is a *Bothriochloa* + *Capillipedium* clade in the *tb*1 phylogeny (Lukens and Doebley 2001); *Dichanthium* and *Euclasta* were not sampled. Mathews et al. (2002) found differences between their nuclear and chloroplast phylogenies wherein *Dichanthium* is sister to *Bothriochloa* + *Capillipedium* in the *PHY*B and GBSSI phylogenies, and *Capillipedium* is sister to *Bothriochloa* + *Dichanthium* in the *ndh*F phylogeny.

Morphologically, *Bothriochloa* and *Capillipedium* have pedicels and rachis internodes with a translucent median line and thickened margins, unlike *Dichanthium.* Conversely, one or more homogamous spikelet pairs are usually present in *Dichanthium* but absent in *Bothriochloa* and *Capillipedium.* A member of clade B in the ITS phylogeny, *Euclasta* (with two species) has homogamous pairs of spikelets and rachis internodes and pedicels with a translucent median line, strongly resembling *Bothriochloa* except for the pitted lower glume in the latter. The four genera have similar leaf blade micromorphology (Watson and Dallwitz 1992). These genera are characterized by complex patterns of hybridization, polyploidy, and apomixis, and it has been suggested that they be united (Harlan and De Wet 1963).

Bothriochloa, *Capillipedium*, and *Dichanthium* are each suggested not to be monophyletic in the ITS and combined data trees, and the clade is virtually unresolved in the *trn*L–F phylogeny. *Pseudosorghum* A. Camus, a genus not sampled in our study, has been described as a link between *Sorghum* and the *Bothriochloa* group (Clayton and Renvoize 1986) within subtribe Sorghinae. It would be interesting to assess the relationship of *Pseudosorghum* to the *Bothriochloa* + *Capillipedium* + *Dichanthium* clade.

Clade D

Vetiveria and *Chrysopogon*, both in subtribe Sorghinae, form a clade in the ITS, *trn*L–F and combined data trees

(Fig. 3–5). *Chrysopogon* is monophyletic in analyses of GBSSI, *ndh*F, and *PHY*B sequences (Spangler et al. 1999; Mathews et al. 2002). *Vetiveria* has not previously been sampled for a molecular phylogenetic study. It is not surprising that *Chrysopogon* and *Vetiveria* are closely related because these genera have many intermediate species, as noted by Hackel (1889) and more recently by Veldkamp (1999), who suggested reducing *Vetiveria* to *Chrysopogon*, as was carried out by Zuloaga et al. (2003, 2007) and Soreng et al. (2006). The genera have a compound panicle very similar to that of *Bothriochloa*, but they do not form a clade with *Bothriochloa* and relatives in our study nor in previous studies. Leaf blade micromorphology also sets them apart. *Chrysopogon* and *Vetiveria* have an abaxial leaf epidermis without papillae while *Dichanthium*, *Bothriochloa*, and *Capillipedium* have papillae (Watson and Dallwitz 1992).

Core Andropogoneae Clade

Spangler et al. (1999; *ndh*F) discussed a clade (not statistically supported) referred to as the ''core Andropogoneae'', comprising *Andropogon*, *Bothriochloa*, *Capillipedium*, *Coix*, *Cymbopogon*, *Dichanthium*, *Heteropogon*, *Hyparrhenia*, *Schizachyrium*, and *Sorghastrum.* Mathews et al. (2002; GBSSI, *ndh*F, *PHY*B), who did not sample *Sorghastrum*, also make reference to the core Andropogoneae clade. The genera do not form a clade in individual analyses of *PHY*B and GBSSI but do so in analyses of *ndh*F and GBSSI + *ndh*F + *PHY*B, except that the morphologically divergent *Coix* falls outside the clade in the analysis of combined data. Five of the core genera form a clade in the *tb*1 phylogeny in Lukens and Doebley (2001), who did not sample *Dichanthium*, *Hyparrhenia*, and *Schizachyrium*, whereas *Coix* and *Sorghastrum* fall outside the clade.

Analysis of ITS + *trn*L–F (Fig. 5) resulted in a clade lacking support that includes *Andropogon*, *Diectomis*, *Hyparrhenia*, *Hyperthelia*, and *Schizachyrium* (clade A), *Bothriochloa*, *Capillipedium*, and *Dichanthium* (clade B), and *Cymbopogon*, *Heteropogon*, *Iseilema*, and *Themeda.* This large clade is similar to the core Andropogoneae clade (Spangler et al. 1999; Mathews et al. 2002) in its assemblage of genera except that *Diectomis*, *Iseilema*, *Hyperthelia*, and *Themeda* were not sampled in the earlier studies. As in Lukens and Doebley's (2001) *tb*1 phylogeny, *Coix* and *Sorghastrum* appear elsewhere in the tree.

With the exception of *Arthraxon*, *Chrysopogon*, *Sorghastrum*, *Sorghum*, and *Vetiveria*, all other genera in subtribes Andropogoninae, Anthistiriinae, and Sorghinae that we sampled are included in the core Andropogoneae. None of the five genera above has the translucent median line and thickened margins of pedicels and rachis internodes characteristic of *Bothriochloa* and *Capillipedium.* With respect to *Sorghum* and *Sorghastrum*, our findings are in better agreement with Keng's (1939) subtribal circumscriptions than they are with Clayton and Renvoize's (1986) expanded Sorghinae. In Keng's study, Bothriochloeae included *Bothriochloa*, *Capillipedium*, *Dichanthium*, and *Euclasta*, while Sorgheae included *Astenochloa* Büse, *Cleistachne* Benth., *Pseudosorghum*, *Sorghastrum*, and *Sorghum.* Sorghinae sensu Clayton and Renvoize (1986) have not resolved as a monophyletic group in previous studies (Spangler et al. 1999).

The core Andropogoneae clade including *Elionurus* resolves in the *trn*L–F phylogeny (Fig. 4), but *Elionurus* falls outside the clade in the combined data tree (Fig. 5). This larger clade is not found in the ITS tree. The phylogenetic position of *Elionurus* remains unclear. The genus is a member of subtribe Rottboelliinae and has single racemes and thick pedicels and rachis internodes with no evident similarities to genera in the core Andropogoneae. In the ITS (Fig. 3) and combined data (Fig. 5) trees, *Elionurus* is sister to the other Andropogoneae + *Arundinella*, although this relationship lacks statistical support. *Elionurus* is sister to the *Tripsacum* + *Zea* clade in analyses of *ndh*F and GBSSI + *ndh*F + *PHY*B (Spangler 1999; Mathews et al. 2002), but this relationship also lacks statistical support. *Elionurus* is not sister to this clade in the *tb*1 phylogeny, nor is its relationship to other genera resolved (Lukens and Doebley 2001). Of the other five total genera of Rottboelliinae we sampled, only *Coelorachis* and *Rottboellia* form a clade in the ITS phylogeny (Fig. 3; BS 77%, PP 1.0).

Cymbopogon (subtribe Andropogoninae) is monophyletic in all trees from our analyses. It is also monophyletic in Mason-Gamer et al. (1998), Spangler et al. (1999), and Mathews et al. (2002). In the ITS (Fig. 3) and combined data (Fig. 5) trees, *Cymbopogon* is sister to clade A, which largely consists of genera in the same subtribe plus *Hyparrhenia* and *Hyperthelia* (subtribe Anthistiriinae). Morphologically, *Cymbopogon* shows similarities to *Andropogon* in its complex inflorescence and two-keeled glume of the sessile spikelet, and to *Hyparrhenia* in its paired, sometimes deflexed racemes and wide spatheoles. Hackel (1889) treated *Cymbopogon* as a subgenus of *Andropogon* under sect. *Gymnanthelia.* The genus is largely confined to the Old World tropics and is distinguished from related genera by its aromatic odor (Soenarko 1977).

Other Taxa and Relationships

Sorghum is monophyletic in our study (Fig. 3–5). However, our sample represents only one of the *Sorghum* clades in Spangler et al.'s (1999) *ndh*F study, wherein the genus is not monophyletic. The genus is also not monophyletic in the Hodkinson et al. (2002*a*) ITS study. It is monophyletic in the *tb*1 analysis of Lukens and Doebley (2001), which, like our study, had a limited sample of the genus. A large genus traditionally divided into five sections (Garber 1950), *Sorghum* includes species of agricultural importance as well as troublesome weeds. In studying the generic limits of *Sorghum*, Spangler et al. (1999) found at least three distinct lineages, but the need for additional molecular and morphological studies was suggested. The species included in our study correspond to the African and Mediterranean lineage within *Sorghum* subgen. *Eu-Sorghum* (Spangler 2000; Dillon et al. 2004). Studies based on ITS1 and *ndh*F sequences (Dillon et al. 2004) support a reduction in the number of subgenera from five to three.

A clade including *Sorghum* and *Miscanthus*, present only in the combined data tree (Fig. 5), is also present in the *ndh*F phylogeny (Spangler et al. 1999; Giussani et al. 2001) along with *Cleistachne* and *Microstegium.* Based on ITS and *trn*L–F data, *Miscanthus* s.l. and *Saccharum* s.l. are not monophyletic, but more studies are needed to understand subtribe

Saccharinae (Hodkinson et al. 2002*a*). At least six genera (including *Sorghum*) hybridize with *Saccharum*, and a marked colinearity of their genomes has been reported (Hodkinson et al. 2002*b*).

Species of *Sorghastrum* form a clade in ITS (Fig. 3) and combined data (Fig. 5) trees but do not resolve as a clade in the *trn*L–F phylogeny (Fig. 4). Only one species was studied by Spangler et al. (1999), which fell outside the *Sorghum* clades.

Subtribe Tripsacinae (Clayton and Renvoize 1986) is monophyletic in the ITS (Fig. 3) and combined data (Fig. 5) trees. It includes *Tripsacum* and *Zea* and is consistent with analyses of morphology (Kellogg and Watson 1993), ITS (Buckler and Holtsford 1996; Hodkinson et al. 2002*a*), GBSSI (Mathews et al. 2002), *ndh*F (Spangler et al. 1999; Giussani et al. 2001; Mathews et al. 2002), *PHY*B (Mathews et al. 2002), and *tb*1 (Lukens and Doebley 2001). *Zea* is also monophyletic in our study, as well as in previous studies (Buckler and Holtsford 1996; Mason-Gamer et al. 1998; Lukens and Doebley 2001; Hodkinson et al. 2002*a*).

Chionachne (subtribe Chionachneae) is sister to *Tripsacum* + *Zea* in the ITS tree (Fig. 3); a *trn*L–F sequence was not obtained for *Chionachne*. In the *ndh*F trees in Spangler et al. (1999) and Mathews et al. (2002), *Chionachne* is also part of a clade that includes *Tripsacum* and *Zea*, but *Elionurus* falls in the clade as well. *Chionachne*, *Tripsacum*, and *Zea*, along with *Coix* (subtribe Coicinae), form Hackel's (1887–1888) and Watson and Dallwitz's (1992) Maydeae, but *Coix* is not part of this clade in our study or previous studies (Lukens and Doebley 2001; Mathews et al. 2002), thus rejecting the monophyly of Maydeae. Maydeae (Watson and Dallwitz 1992) are circumscribed largely based on their unisexual fertile spikelets. Clayton and Renvoize (1986) instead recognized three subtribes with unisexual spikelets (Chionachninae, Coicinae, and Tripsacinae), which differ in the arrangement of male and female spikelets. Chionachninae have male and female spikelets in the same inflorescence; Coicinae have male and female spikelets in the same inflorescence but separated by a prophyll; and Tripsacinae have male and female spikelets in separate parts of the same inflorescence or in different inflorescences.

Concluding Remarks

Clearly, more studies are needed to better understand evolutionary relationships within Andropogoneae, including increased sampling and additional DNA sequence data. Collaboration with agrostologists in Africa and Asia will be necessary to broaden the sampling of Andropogoneae to include more taxa from parts of the world where it is most diverse. Further, additional morphological and developmental data will certainly contribute to a better understanding of this complex tribe.

ACKNOWLEDGMENTS

We thank the USDA Plant Genetic Resources Conservation Unit, Georgia, for providing seeds, and Melvin Duvall for providing DNA of *Tristachya leucothrix*. We also thank Toby Kellogg for her support during the initial stages of the project, and Lucinda McDade, Paul Peterson, and Victor Steinmann for reviews of the manuscript. This study was funded by National Science Foundation grant DEB-0074777 to EMS.

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