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J. Gabriel Sánchez-Ken  
*Iowa State University, Ames*

Lynn G. Clark  
*Iowa State University, Ames*

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PHYLOGENETIC RELATIONSHIPS WITHIN THE CENTOTHECOIDEAE + PANICOIDEAE CLADE (POACEAE)  
BASED ON *ndhF* AND *rpl16* INTRON SEQUENCES AND STRUCTURAL DATA

J. GABRIEL SÁNCHEZ-KEN<sup>1,2,3</sup> AND LYNN G. CLARK<sup>1</sup>

<sup>1</sup>*Department of Ecology, Evolution and Organismal Biology, Iowa State University, 253 Bessey Hall,  
Ames, Iowa 50011-1020, USA*

<sup>2</sup>*Corresponding author (gabriels@hotmail.co.uk)*

ABSTRACT

The PACCAD clade (Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, Danthonioidae) is well supported in all recent studies of Poaceae. However, phylogenetic relationships within the clade are still unresolved, although several branches are strongly supported. In this study, we focus on the phylogeny of the Centothecoideae + Panicoideae clade, one of the major lineages within the PACCAD clade, and one of the most diverse in the family. Two previously unsampled tribes, Isachneae and Steyermarkochloae, are included, as are additional taxa of Centothecoideae. Phylogenetic analyses of plastid genome sequences (*ndhF* gene and *rpl16* intron) and structural data show increased support of the centothecoid–panicoid clade, but phylogenetic relationships between the two putative subfamilies remain unresolved. Centothecoideae may be sister to Panicoideae or they may be paraphyletic with respect to Panicoideae, as monophyly of Centothecoideae is weakly supported at best. Polyphyly of Panicoideae is demonstrated as Isachneae and Steyermarkochloae (only with *ndhF*) form well-supported clades with *Micraira–Eriachne* and *Chasmanthium*, respectively. Polyphyly of Arundinelleae is also confirmed as *Danthoniopsis* and related genera resolve elsewhere. Centothecoideae, as recently circumscribed, resolve as three strongly supported clades including *Danthoniopsis* and related genera. *Cyperochloa* (previously incertae sedis) and arundinoid *Spartochloa* are sister and fall within the centothecoids with strong support. Centothecoideae are polyphyletic and segregate into three major groups. One centothecoid genus, *Zeugites*, is demonstrably paraphyletic.

Key words: Arundinelleae, *Arundoclaytonia*, Centothecoideae, *Cyperochloa*, *Eriachne*, *Isachne*, *Micraira*, Panicoideae, *Spartochloa*.

INTRODUCTION

In the past 15 years, phylogenetic inference in the grasses has experienced great advances. The grass family (Poaceae), with at least 10,000 species (Watson and Dallwitz 1992), has received much attention due to its economic and ecological importance, as well as its great diversity and wide distribution, and has been analyzed rigorously from both morphological and molecular standpoints. Molecular systematic analyses include nuclear and chloroplast markers, such as plastid restriction fragments (Davis and Soreng 1993; Soreng and Davis 1998), sequences of plastid loci (*ndhF*: Clark et al. 1995, Catalán et al. 1997; *rbcL*: Doebley et al. 1990, Barker et al. 1995, Duvall and Morton 1996, Barker 1997; *rpl16*: Zhang 2000; *rpoC2*: Cummings et al. 1994, Barker et al. 1998; *rps4*: Nadot et al. 1994; *matK*: Liang and Hilu 1996, Hilu et al. 1999), and sequences of nuclear loci (ribosomal DNA: Hamby and Zimmer 1988; *adh*: Morton et al. 1996; ITS: Hsiao et al. 1999; *phyB*: Mathews and Sharrock 1996; *waxy*: Mason-Gamer et al. 1998). Most of these studies are consistent in recovering two major lineages, the BEP clade (Bambusoideae, Ehrhartoideae, Pooideae) and the PACCAD clade (Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, Danthonioidae) (Grass Phylogeny Working Group [GPWG] 2000, 2001).

The BEP clade receives moderate support at best, except for strong support from the *phyB* data (GPWG 2001), or it is not recovered at all, but the PACCAD clade is strongly supported in all analyses. Despite the robust nature of the PACCAD clade itself, relationships among its major lineages have remained ambiguous (GPWG 2001).

The GPWG (2001) compiled eight data sets (structural and molecular) to establish a comprehensive phylogenetic hypothesis for the family. The most-parsimonious cladogram from the combined analysis is topologically similar to those found in previous studies, with the PACCAD clade and the early diverging lineages well supported, and the BEP clade moderately supported. As in prior studies, Centothecoideae + Panicoideae are well supported as one of the major lineages within the PACCAD clade, but neither subfamily receives strong support.

Panicoideae account for about one-third of the species diversity of grasses, whereas Centothecoideae s.s. account for approximately 32 species (Watson and Dallwitz 1992). Panicoideae include six tribes recognized by the GPWG (2001) of which only three, Andropogoneae, Arundinelleae, and Paniceae, have been sampled for any molecular systematic analysis to date. Andropogoneae are clearly monophyletic with *Arundinella* Raddi as sister (Spangler et al. 1999). Arundinelleae as traditionally recognized are polyphyletic because *Danthoniopsis* Stapf (and presumably allied genera when sampled) resolve elsewhere within the Centothecoideae + Panicoideae clade (Clark et al. 1995). Important in terms of species diversity are Paniceae, but monophyly of

<sup>3</sup> Present address: National Herbarium of Mexico, Departamento de Botánica, Instituto de Biología, U.N.A.M., Tercer Circuito s/n, Ciudad Universitaria, Delegación Coyoacán, Apartado Postal 70-233, 04510 México, D.F., Mexico.

Table 1. Selected classifications of the grasses highlighting the placement of the centothecoids.

Prat (1932)	Tateoka (1957)	Jacques-Félix (1962)	Clayton and Renvoize (1986)	Watson and Dallwitz (1992)	GPWG (2001)
Festucoideae	Festucoideae	Olyroidées	Bambusoideae	Bambusoideae	Anomochloideae
Panicoideae	Panicoideae	Bambusoidées	Oryzoideae	<u>Centothecae</u>	Pharoideae
<u>Centothecoids</u>	Bambusoideae	Streptogynoidées	Pooideae	Pooideae	Puelioideae
Bambusoideae	Arundinoideae	Stipoidées	Arundinoideae	Arundinoideae	Bambusoideae
	<u>Centothecae</u>	Oryzoidées	Chloridoideae	Chloridoideae	Ehrhartoideae
		Ehrhartoidées	<u>Centothecoideae</u>	Panicoideae	Pooideae
		Zizanoïdées	Panicoideae		Panicoideae
		<u>Centothecoidées</u>			Arundinoideae
		Arundinoïdées			<u>Centothecoideae</u>
		Chloridoïdées			Chloridoideae
		Festucoïdées			Aristidoideae
		Panicoïdées			Danthonioideae
					Incertae Sedis

the tribe remains unresolved (Aliscioni et al. 2003). The remaining tribes (Hubbardieae, Isachneae, and Steyermarkochloaeae, the last originally placed in Arundinoideae) have not been sampled for molecular studies, and their placement in the subfamily relies solely on morphological characters.

The centothecoids were originally circumscribed by Benham (1881), who classified them within his tribe Festuceae. Early in the twentieth century, Centothecoideae were treated as a tribe or subtribe within Pooideae (e.g., Roshevits 1946) or Arundinoideae (e.g., Tateoka 1957), or as incertae sedis (e.g., Prat 1960), until Jacques-Félix (1962) recognized the group as a series (corresponding to a subfamily). Subsequently, its rank and position in the family have continued to be unstable (Table 1). Based on morphological characters, the centothecoids were considered related to the herbaceous bamboos by Soderstrom (1981), Watson et al. (1985), and Watson and Dallwitz (1992), although the last authors reinstated the centothecoids as a subfamily in an updated version of their classification (Watson and Dallwitz 1998). Other authors, based on morphology and anatomy, and more recently molecular data, related the centothecoids to Arundinoideae (Renvoize 1981; Hilu and Wright 1982; Davis and Soreng 1993; Hsiao et al. 1998; Watson and Dallwitz 1998). However, Cummings et al. (1994), Barker et al. (1995, 1998), Clark et al. (1995), Soreng and Davis (1998), and Gómez-Martínez and Culham (2000), based primarily on molecular data from one or two taxa, suggested that the centothecoids are more closely related to Panicoideae than to any other group, consistent with the GPWG (2000, 2001). Some of these studies also associated additional taxa (e.g., *Danthoniopsis* and *Thysanolaena* Nees) with the original centothecoid taxa (e.g., Clark et al. 1995). The relationship between panicoids and centothecoids was suggested many years ago by Prat (1932), even though he treated the centothecoids as incertae sedis in his later classification of the family (Prat 1960).

Centothecoideae are tropical and subtropical in distribution, usually growing in humid and shaded forest understories, although some taxa reach drier and more open habitats. Morphologically, centothecoids are characterized by a herbaceous habit similar to some early diverging grasses and bamboos, although *Thysanolaena* is reedlike (Jacques-Félix 1962; Decker 1964; Tenório 1978; Soderstrom 1981; Watson

and Dallwitz 1992; GPWG 2001). Most are small plants, with leaf blades widely lanceolate bearing a pseudopetiole and numerous cross veins producing obvious tessellation. A few members have linear blades, however, without the pseudopetiole or tessellation. The spikelets are extremely variable, as they may be bisexual (bearing bisexual or unisexual florets) or unisexual, with two to many florets; the proximal floret may be pistillate, sterile, or bisexual, whereas the distal florets may be staminate, sterile, or bisexual. The florets typically have two lodicules (but these may be fused or absent), one to three stamens, and one or two styles with feathery stigmas. The caryopsis has a punctate, basal hilum. The embryo is small, usually one-fourth to one-fifth as long as the endosperm, with an embryo formula of P + PP (defined as the centothecoid embryo) characteristic of all genera except *Zeugites* P. Browne, which has an F + PP or bambusoid-type embryo (Reeder 1962; Decker 1964).

Anatomically, Centothecoideae are considered unique within the grass family with regard to certain features of the leaf blade and epidermis (Renvoize 1986). Centothecoideae are typical C<sub>3</sub> plants, but in most members the mesophyll is unusual because it is differentiated into tissues resembling palisade parenchyma and spongy mesophyll. This is rare in the grasses, although there are many bamboos that possess a similar, but probably not homologous, condition. The chlorenchyma cells of centothecoids are lobed and resemble the arm (or rachymorph) cells of the bamboos and some other taxa. The base chromosome number,  $x = 12$ , is known for some genera, with polyploidy present in some species (Soderstrom and Decker 1973; Soderstrom 1981).

In a previous study (Tenório 1978), Centothecoideae were classified into two tribes, Centothecae and Zeugiteae, but this work was never published and suffered from ambiguities and lack of information. The current classification of the subfamily by the GPWG (2001) includes two tribes, Thysanolaenaeae (one genus) and Centothecaeae (11 genera), distributed in Africa, America, Asia, and Australia (Fig. 1). Monophyly of the centothecoids exclusive of *Thysanolaena* has never been rigorously tested, and to date there is no known morphological synapomorphy that defines it as a natural group, with the possible exception of the palisade mesophyll, which could be interpreted as a synapomorphy that was lost in some taxa.

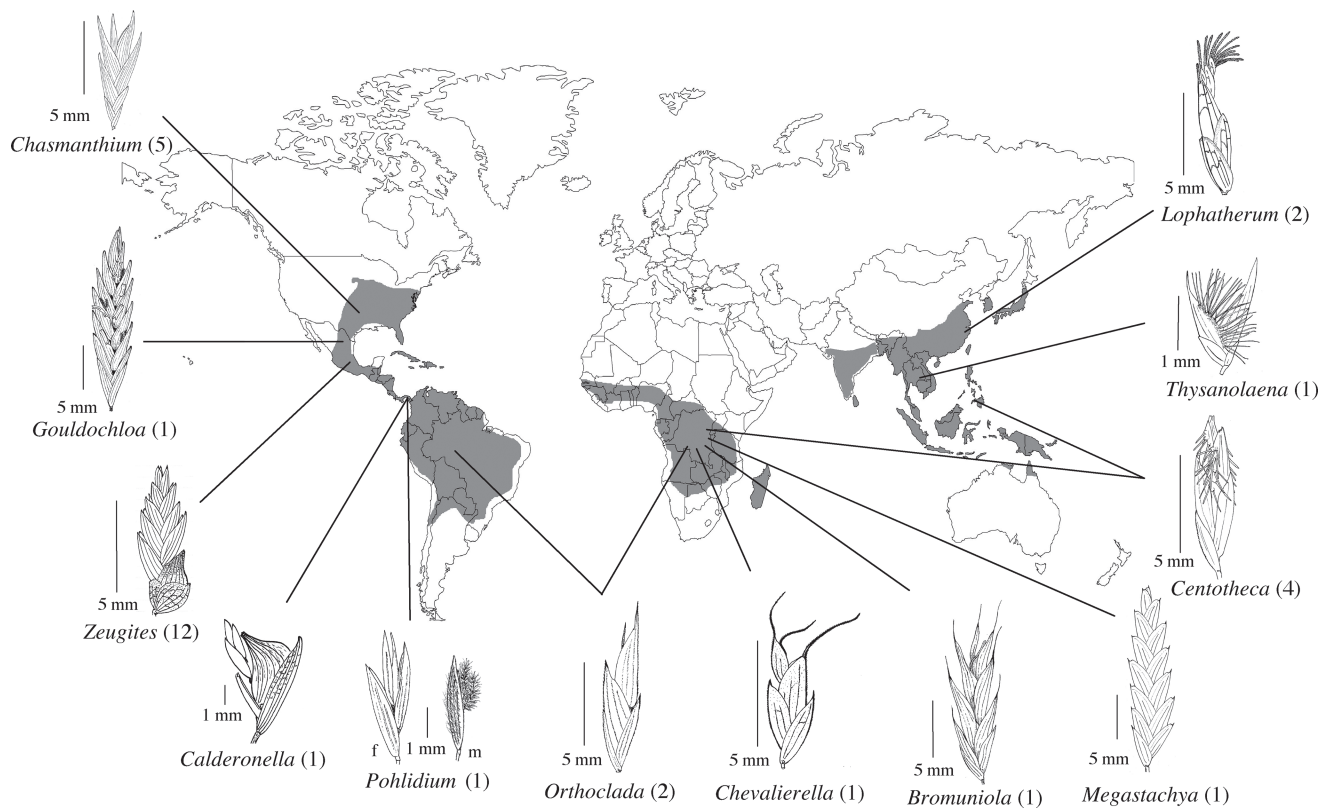


Fig. 1.—Geographical distributions and spikelets of the genera of Centothecoideae s.s. Number of species in parentheses. m = male; f = female.

Although the rank and position of Centothecoideae have been unstable, the circumscription remained stable, with 11 genera, until the recent inclusion of *Thysanolaena* (GPWG 2001). Genera such as *Cyperochloa* Lazarides & L. Watson, *Danthoniopsis*, *Gynerium* P. Beauv., and *Spartochloa* C. E. Hubb. have shown various relationships with *Thysanolaena* based on molecular data (Clark et al. 1995; Barker 1997; Barker et al. 1998; Hsiao et al. 1998; Bess et al. 2005). Since *Thysanolaena* is now considered a centothecoid, these other genera might belong to the group as well.

Lazarides and Watson (1986), relying on numerical analysis of morphological characters, suggested that *Cyperochloa* is an isolated genus of Danthoniaceae, within Arundinoideae. Watson and Dallwitz (1992) later created the tribe *Cyperochloae* nom. nud., positioned in the same subfamily. Barker (1997), based on *rbcL* sequence data, demonstrated that the genus is more closely related to *Thysanolaena* than to Arundinoideae. Another poorly known genus, the arundinoid *Spartochloa*, appears related to *Cyperochloa* and *Thysanolaena* based on ITS sequences (Hsiao et al. 1999) and to *Thysanolaena* based on *rpoC2* sequences (Barker et al. 1998).

*Gynerium*, a reed previously classified within Arundinoideae (Clayton and Renvoize 1986), appeared as sister to *Thysanolaena* based on *rbcL* sequences (Barker et al. 1995; Barker 1997; GPWG 2001), although it resolved as sister to Panicoideae with *ndhF* sequences (GPWG 2001). It is also surprising that *Danthoniopsis*, a panicoid genus in tribe Arundinelleae (Clayton and Renvoize 1986), is embedded in or sister to Centothecoideae in analyses of *ndhF* sequence

data (Clark et al. 1995), or is sister to the panicoids (Spanler et al. 1999; Mathews et al. 2000; Giussani et al. 2001; GPWG 2001). In a more complex scenario, *Tristachya* Nees, another putative member of Arundinelleae and morphologically close to *Danthoniopsis*, is embedded within Andropogoneae based on *rbcL* data (Linder et al. 1997) and within Paniceae based on *phyB* (Mathews et al. 2002). *Danthoniopsis* and *Tristachya* differ greatly from the centothecoids in morphology as well as by their  $C_4$  photosynthetic pathway.

One explanation for the lack of phylogenetic resolution within the Centothecoideae + Panicoideae clade might be poor or incomplete sampling. In almost all phylogenetic analyses to date, Centothecoideae have been represented by only one or two members, and Panicoideae by only three tribes. Some of the unsampled taxa may represent early diverging lineages that would be critical in establishing a robust phylogeny for the entire clade. Here we conduct a phylogenetic analysis of the Centothecoideae + Panicoideae clade, with particular emphasis on the centothecoids, based on sequences of two markers from the plastid genome (*ndhF* and the *rpl16* intron) and a morphological data set, in order to (1) test the monophyly of Centothecoideae and explore its internal phylogenetic structure, and (2) elucidate phylogenetic relationships of Centothecoideae to Panicoideae. We sample five tribes of Panicoideae, almost all centothecoid genera, and a number of genera of previously uncertain placement. Our results (1) confirm strong support for both the PACCAD and Centothecoideae + Panicoideae clades, (2) provide strong support for alternate placements of two

Table 2. Taxa and genic regions included in the study, and GenBank accession numbers and vouchers/sources. *Aristida* and *Chusquea* are composite taxa that include two species each. Vouchers without the herbarium indicated are deposited at ISC. When two vouchers are listed, the first is for *rpl16* and the second for *ndhF*. Sources of the GenBank numbers are as follows: <sup>a</sup>Catalán et al. (1997), <sup>b</sup>Clark et al. (1995), <sup>c</sup>Giussani et al. (2001), <sup>d</sup>GPWG (2001), <sup>e</sup>Kelchner and Clark (1997), <sup>f</sup>Kelchner and Wendel (1996), <sup>g</sup>Mathews et al. (2002), <sup>h</sup>Ogihara et al. (2002), <sup>i</sup>Shimada and Sugiura (1991), <sup>j</sup>Spangler et al. (1999), <sup>k</sup>Zhang (2000), <sup>l</sup>Zhang and Clark (2000), <sup>m</sup>M. R. Duvall, Northern Illinois University, USA, <sup>n</sup>this study. PI = USDA Plant Introduction Station (Pullman, Washington, USA) as source of seed.

Taxon	GenBank accession no.		Voucher/source
	<i>rpl16</i> intron	<i>ndhF</i>	
<b>Subfamily Bambusoideae</b>			
<i>Arundinaria gigantea</i> (Walter) Muhl.	AF133465 <sup>k</sup>	U21846 <sup>b</sup>	Zhang 8400703
<i>Chusquea circinata</i> Soderstr. & C. E. Calderón	–	U21991 <sup>b</sup>	Quail Botanic Garden, California, USA (no voucher)
<i>C. oxylepis</i> (Hack.) Ekman	U62786 <sup>e</sup>	–	Clark 1069
<i>Pariaria radiciiflora</i> Sagot ex Döll	U54740 <sup>f</sup>	AF182354 <sup>l</sup>	Clark & Zhang 1344
<b>Subfamily Ehrhartoideae</b>			
<i>Oryza sativa</i> L.	NC001320 <sup>i</sup>	NC001320 <sup>i</sup>	No voucher
<b>Subfamily Pooideae</b>			
<i>Glyceria striata</i> (Lam.) Hitchc.	AY847071 <sup>x</sup>	AF251453 <sup>d</sup>	Davis & Soreng s. n. (BH)
<i>Melica altissima</i> L.	AY847072 <sup>x</sup>	AF251452 <sup>d</sup>	PI 325418 (BH)
<i>Triticum aestivum</i> L.	AB042240 <sup>h</sup>	U71025 <sup>a</sup>	No voucher
<b>Subfamily Danthonioideae</b>			
<i>Danthonia californica</i> Bol.	–	AF251459 <sup>d</sup>	PI 232247 (BH)
<i>Merxmuellera macowanii</i> (Stapf) Conert	AY847074 <sup>x</sup>	AF251457 <sup>d</sup>	Barker 1008 (BOL)
<i>Karroochloa purpurea</i> (L. f.) Conert & Türpe	AY847075 <sup>x</sup>	AF251458 <sup>d</sup>	Linder 5360 (BOL)
<b>Subfamily Aristidoideae</b>			
<i>Aristida purpurea</i> Nutt. var. <i>curvifolia</i> (E. Fourn.) K. W. Allred	AY847076 <sup>x</sup>	–	Sánchez-Ken & Calzada s. n. (24 Oct 2000)
<i>A. purpurea</i> Nutt. var. <i>longiseta</i> (Steud.) Vasey ex Rothr.	–	U21966 <sup>b</sup>	Gabel 270
<i>Stipagrostis zeyheri</i> (Nees) De Winter	AY847077 <sup>x</sup>	AF251455 <sup>d</sup>	Barker 1133 (BOL)
<b>Subfamily Chloridoideae</b>			
<i>Eragrostis curvula</i> (Schrad.) Nees	AY847078 <sup>x</sup>	U21989 <sup>b</sup>	Clark 1303
<i>Merxmuellera rangei</i> (Pilg.) Conert	AY847079 <sup>x</sup>	AF251461 <sup>d</sup>	Barker 960 (GRA)

Table 2. Continued.

Taxon	GenBank accession no.		Voucher/source
	<i>rpl16</i> intron	<i>ndhF</i>	
<i>Sporobolus indicus</i> (L.) R. Br.	AY847080 <sup>x</sup>	U219983 <sup>b</sup>	Clark 1293
<b>Subfamily Arundinoideae</b>			
<i>Arundo donax</i> L.	AF133485 <sup>k</sup>	U21998 <sup>b</sup>	No voucher
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	AY847081 <sup>x</sup>	U21997 <sup>b</sup>	Sánchez-Ken & Calzada 646; Clark 1294
<i>Spartochloa scirpoides</i> (Steud.) C. E. Hubb.	AY847114 <sup>x</sup>	AY847140 <sup>x</sup>	Lepschi & Craven 4519 (CANB)
<b>Subfamily Panicoideae</b>			
<b>Tribe Andropogoneae</b>			
<i>Andropogon gerardii</i> Vitman	AY847082 <sup>x</sup>	AF117391 <sup>g</sup>	PI 477973 (no voucher)
<i>Sorghum bicolor</i> (L.) Moench	AF133478 <sup>k</sup>	U21981 <sup>b</sup>	No voucher
<i>Zea mays</i> L.	AF133477 <sup>k</sup>	U21985 <sup>b</sup>	No voucher
<b>Tribe Arundinelleae</b>			
<i>Arundinella hirta</i> (Thunb.) Tanaka	AY847085 <sup>x</sup>	AF117393 <sup>i</sup>	PI 263693 (no voucher)
<i>Loudetia simplex</i> (Nees) C. E. Hubb.	AY847088 <sup>x</sup>	AY847117 <sup>x</sup>	Malcomber 3082 (MO)
<i>Tristachya biseriata</i> Stapf	AY847089 <sup>x</sup>	AY847118 <sup>x</sup>	Malcomber 3101 (MO)
<b>Tribe Paniceae</b>			
<i>Paspalum conjugatum</i> P. J. Bergius	AY847083 <sup>x</sup>	AY029669 <sup>c</sup>	Sánchez-Ken s. n. (Dec 2003); Morrone 3339 (SI)
<i>Pennisetum setaceum</i> (Forssk.) Chiov.	AY847084 <sup>x</sup>	AY029673 <sup>c</sup>	Morden 1101
<b>Tribe Isachneae</b>			
<i>Isachne arundinacea</i> (Sw.) Griseb.	AY847090 <sup>x</sup>	AY847119 <sup>x</sup>	Sánchez-Ken & Ponce S. 680
<b>Tribe Steyermarkochloaeae</b>			
<i>Arundoclaytonia dissimilis</i> Davidse & R. P. Ellis	AY847092 <sup>x</sup>	AY847121 <sup>x</sup>	Calderón et al. 2706
<b>Subfamily Centothecoideae</b>			
<b>Tribe Thysanolaeneae</b>			
<i>Thysanolaena latifolia</i> (Roxb. ex Hornem.) Honda	AY847093 <sup>x</sup>	U21984 <sup>b</sup>	Sánchez-Ken s. n. (17 Apr 2003)
<b>Tribe Centothecaeae</b>			
<i>Bromuniola gossweileri</i> Stapf & C. E. Hubb.	AY847096 <sup>x</sup>	AY847124 <sup>x</sup>	Malaisse 12258 (BR)

Table 2. Continued.

Taxon	GenBank accession no.		Voucher/source
	<i>rpl16</i> intron	<i>ndhF</i>	
<i>Calderonella sylvatica</i> Soderstr. & H. F. Decker	AY847104 <sup>x</sup>	AY847131 <sup>x</sup>	<i>Sánchez-Ken &amp; Ponce S. 661</i>
<i>Centotheca lappa-cea</i> (L.) Desv.	AY847094 <sup>x</sup>	AY847122 <sup>x</sup>	<i>Carvalho 4086 (BM)</i>
<i>Chasmanthium latifolium</i> (Michx.) H. O. Yates	AY847098 <sup>x</sup>	AY029694 <sup>c</sup>	<i>Sánchez-Ken s. n. (17 Apr 2003); no voucher</i>
<i>C. laxum</i> (L.) H. O. Yates	AY847099 <sup>x</sup>	AY847126 <sup>x</sup>	<i>Sánchez-Ken s. n. (17 Apr 2003)</i>
<i>C. sessiliflorum</i> (Poir.) H. O. Yates	AY847100 <sup>x</sup>	AY847127 <sup>x</sup>	<i>Sánchez-Ken s. n. (17 Apr 2003)</i>
<i>Gouldochloa curvifolia</i> Valdés-Reyna, Morden & S. L. Hatch	AY847097 <sup>x</sup>	AY847125 <sup>x</sup>	<i>Sánchez-Ken &amp; Calzada 652</i>
<i>Lophatherum gracile</i> Brongn.	AY847102 <sup>x</sup>	AY847129 <sup>x</sup>	<i>Riedel s. n. (7 Jul 2002)</i>
<i>Megastachya mucronata</i> (Poir.) P. Beauv.	AY847095 <sup>x</sup>	AY847123 <sup>x</sup>	<i>Poulsen 777 (C)</i>
<i>Orthoclada laxa</i> (L. C. Rich.) P. Beauv.	AY847101 <sup>x</sup>	AY847128 <sup>x</sup>	<i>Sánchez-Ken 659</i>
<i>Pohlidium petiolatum</i> Davidse, Soderstr. & R. P. Ellis	AY847103 <sup>x</sup>	AY847130 <sup>x</sup>	<i>Sánchez-Ken et al. 685</i>
<i>Zeugites americana</i> Willd.	AY847105 <sup>x</sup>	AY847132 <sup>x</sup>	<i>Shaw 160</i>
<i>Z. capillaris</i> (Hitche.) Swallen	AY847106 <sup>x</sup>	AY847133 <sup>x</sup>	<i>Sánchez-Ken et al. 626</i>
<i>Z. hackelii</i> Swallen	AY847107 <sup>x</sup>	AY847134 <sup>x</sup>	<i>Sánchez-Ken et al. 614</i>
<i>Z. mexicana</i> (Kunth) Trin. ex Steud.	AY847108 <sup>x</sup>	AY847135 <sup>x</sup>	<i>Sánchez-Ken et al. 621</i>
<i>Z. munroana</i> Hemsl.	AY847109 <sup>x</sup>	AY847136 <sup>x</sup>	<i>Sánchez-Ken &amp; Calzada 636</i>
<i>Z. pittieri</i> (Hack.) Swallen	AF133483 <sup>k</sup>	U21987 <sup>a</sup>	<i>Clark 1171</i>
<i>Z. pringlei</i> Scribn.	AY847110 <sup>x</sup>	AY847137 <sup>x</sup>	<i>Sánchez-Ken et al. 623</i>
<i>Z. sagittata</i> W. Hartley	AY847111 <sup>x</sup>	AY847138 <sup>x</sup>	<i>Medina L. et al. 1269 (MEXU)</i>
Unstable taxa within the Centothecoideae + Panicoideae clade			
<i>Danthoniopsis dinteri</i> (Pilg.) C. E. Hubb	AY847086 <sup>x</sup>	AY847116 <sup>x</sup>	<i>Sánchez-Ken s. n. (17 Apr 2003)</i>
<i>D. petiolata</i> (J. B. Phipps) W. D. Clayton	AY847087 <sup>x</sup>	U22008 <sup>b</sup>	<i>Clark 1173</i>

Table 2. Continued.

Taxon	GenBank accession no.		Voucher/source
	<i>rpl16</i> intron	<i>ndhF</i>	
Incertae sedis			
<i>Cyperochloa hirsuta</i> Lazarides & L. Watson	AY847112 <sup>x</sup>	AY847139 <sup>x</sup>	<i>Keighery 12559 (PERTH)</i>
<i>Eriachne pulchella</i> Domin	–	AY618659 <sup>m</sup>	<i>Jacobs 8720 (NSW)</i>
<i>Gynerium sagittatum</i> (Aubl.) P. Beauv.	AY847091 <sup>x</sup>	AY847120 <sup>x</sup>	<i>Sánchez-Ken &amp; Calzada 640</i>
<i>Micraira lazareidii</i> L. G. Clark, Wendel & Craven	AY847113 <sup>x</sup>	U21972 <sup>b</sup>	<i>Clark 1157</i>
<i>Streptogyna americana</i> C. E. Hubb.	AY847115 <sup>x</sup>	U21965 <sup>b</sup>	<i>Sánchez-Ken 657; Pohl &amp; Davidse 12310</i>

tribes currently classified within Panicoideae, and (3) resolve three major lineages within Centothecoideae but do not provide convincing evidence for the monophyly of the subfamily.

#### MATERIALS AND METHODS

##### Taxon Sampling

Plant materials were obtained from various sources, including field collections, herbarium specimens, purchased seeds, previous DNA extracts used by Clark et al. (1995) and Barker et al. (1998), and as gifts. A total of 56 species in 44 genera was included in this analysis (Table 2). Sampling included eight representatives of the BEP clade plus *Streptogyna* P. Beauv. as outgroups, and 47 species (plus one variety) of the PACCAD clade, which was considered to be the ingroup due to the inclusion of several genera of uncertain placement and uncertainty regarding the best outgroup for the Centothecoideae + Panicoideae clade. Five of the six tribes currently recognized in Panicoideae were sampled: Andropogoneae, Arundinelleae, Isachneae, Paniceae, and Steyermarkochloae. Both tribes of Centothecoideae were sampled, including all 11 currently recognized genera of Centothecoideae, except *Chevalierella* A. Camus. The five taxa listed as incertae sedis by the GPWG (2001) were sampled, as were *Danthoniopsis*, *Loudetia* Hochst., *Spartochloa*, and *Tristachya*.

##### Structural Characters

Seventy-one structural characters were employed including vegetative, reproductive, anatomical, and embryological characters, as well as chromosome numbers, photosynthetic pathways, and DNA insertions/deletions (indels) treated as binary characters (Table 3). Data for these characters were taken from the GPWG (2001) and supplemented by observations of herbarium and living material, as well as scoring the indels from sequences generated in this study. Anatomical characters were determined following standard procedures using paraffin embedding (Berlyn and Miksche 1976).

Table 3. List of the structural characters and states. Most of the characters and states were taken from the GPWG (2001), the remainder generated in this study. Provided are the lengths in base pairs (bp) and positions of insertions/deletions (indels) in the aligned DNA sequence matrix.

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*Culms*

1. Perennating woody culms: 0 = absent; 1 = present
2. Habit: 0 = reedlike; 1 = tufted; 2 = sprawling; 3 = stoloniferous
3. Internodes: 0 = solid; 1 = hollow
4. Rhizomes: 0 = leptomorph; 1 = pachymorph; 2 = absent
5. Internode length: 0 = short; 1 = elongate

*Leaf*

6. Adaxial ligule: 0 = membranous (fringed or not fringed); 1 = pilose
7. Abaxial ligule: 0 = absent; 1 = present
8. Leaf blade greatly reduced: 0 = absent; 1 = present
9. Leaf blade shape: 0 = linear; 1 = lanceolate; 2 = ovate
10. Leaf blade evidently tessellate: 0 = absent; 1 = present
11. Leaf blade base: 0 = symmetrical; 1 = asymmetrical
12. Pseudopetiole: 0 = absent; 1 = present
13. Pseudopetiole base and apex: 0 = epulvinate; 1 = unipulvinate; 2 = bipulvinate

*Spikelet*

14. Spikelet pairs: 0 = absent; 1 = present
15. Pedicel: 0 = absent; 1 = present
16. Floret sexuality in the same spikelet: 0 = all bisexual; 1 = unisexual, both male and female; 2 = unisexual, male or female only; 3 = male/sterile and bisexual
17. Bracts associated with spikelets: 0 = absent; 1 = present
18. Lower glume nerves: 0 = 1–3; 1 = only 3; 2 = 5; 3 = more than 5; 4 = 0
19. Proximal female-sterile florets in female spikelet: 0 = absent; 1 = present
20. Female-fertile florets per female spikelet: 0 = 2 or more; 1 = only 1
21. Female-fertile lemma gibbous at base: 0 = absent; 1 = present
22. Female-fertile lemma becoming indurate at maturity: 0 = absent; 1 = present
23. Awn or mucro on fertile or sterile lemma: 0 = absent; 1 = present
24. Number of awns = state
25. Awn attachment: 0 = terminal/subterminal; 1 = from a sinus; 2 = dorsal
26. Disarticulation above the glumes: 0 = absent; 1 = present
27. Germination flap: 0 = absent; 1 = present

*Flower*

28. Lodicules in female-fertile floret: 0 = absent; 1 = present
29. Lodicules in the male-fertile floret: 0 = absent; 1 = present
30. Lodicules fused: 0 = absent; 1 = present
31. Distally membranous portion of lodicule: 0 = absent; 1 = present
32. Lodicule vascularization: 0 = faintly or not vascularized; 1 = vascularized
33. Anterior stamen of outer whorl: 0 = absent; 1 = present
34. Posterior stamen pair of outer whorl: 0 = absent; 1 = present
35. Styles fused at least at base: 0 = absent; 1 = present
36. Highest order of stigmatic branching: 1 = simple; 2 = primary; 3 = secondary/tertiary
37. Rachilla: 0 = not extended beyond the female-fertile floret; 1 = extended beyond the female-fertile floret

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Table 3. Continued.

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*Fruit and Embryo*

38. Hilum shape: 0 = linear; 1 = nonlinear (punctate)
39. Embryo epiblast: 0 = absent; 1 = present
40. Embryo mesocotyl internode: 0 = negligible; 1 = elongated
41. Embryonic leaf margins: 0 = meeting; 1 = overlapping
42. Starch grain syndrome: 0 = *Triticum*-type (simple grains only, dimorphic in size, round or lenticular, free); 1 = Festucoid-type (highly compound grains present, with or without simple grains also present); 2 = *Andropogon*-type (simple and compound grains both present, the latter consisting of a few granules); 3 = Panicoid-type (simple grains only, small or medium in size, uniform in size, angular or sometimes rounded); 4 = *Brachyelytrum*-type (simple large grains)

*Vegetative Anatomy*

43. Microhairs: 0 = absent; 1 = present
44. Chloroid microhairs: 0 = absent; 1 = present
45. Balaniform microhairs: 0 = absent; 1 = present
46. Uncinate macrohairs: 0 = absent; 1 = present
47. Tissues resembling palisade parenchyma and spongy mesophyll: 0 = absent; 1 = present
48. Rosette cells: 0 = absent; 1 = present
49. Longitudinal-elongate-lobed (LEL) cells: 0 = absent; 1 = present
50. Haustorial synergids: 0 = absent; 1 = present

*Chromosomes*

51. Base chromosome number is same as state except 0 = 10; 1 = 11; 2 = 12; 3 = 13; 4 = 18

*Photosynthetic Pathway*

52. Carbon fixation pathway: 0 = C<sub>3</sub>; 1 = C<sub>4</sub> NADP-ME classical-type; 2 = C<sub>4</sub> NADP-ME *Aristida*-type; 3 = C<sub>4</sub> NADP-ME; 4 = C<sub>4</sub> NADP-ME *Arundinelleae*-type; 5 = C<sub>4</sub> NADP-ME *Eriachne*-type
53. PCK-type carbon fixation: 0 = absent; 1 = present

*ndhF Indels (a–c in Fig. 4)*

54. Deletion a, 1470–1475, 6 bp: 0 = absent; 1 = present
55. Deletion b, 1504–1512, 9 bp: 0 = absent; 1 = present
56. Insertion c, 1882–1887, 6 bp: 0 = absent; 1 = present

*rpl16 Intron Indels (d–r in Fig. 4)*

57. Deletion d, 258–262, 5 bp: 0 = absent; 1 = present
58. Insertion e, 365–367, 3 bp: 0 = absent; 1 = present
59. Deletion f, 395–402, 8 bp: 0 = absent; 1 = present
60. Deletion g, 395–399, 5 bp: 0 = absent; 1 = present
61. Deletion h, 427–433, 6 bp: 0 = absent; 1 = present
62. Deletion i, 443–447, 5 bp: 0 = absent; 1 = present
63. Deletion j, 513–518, 6 bp: 0 = absent; 1 = present
64. Insertion k, 600–603, 4 bp: 0 = absent; 1 = present
65. Deletion l, 746–754, 9 bp: 0 = absent; 1 = present
66. Deletion m, 768–773, 6 bp: 0 = absent; 1 = present
67. Insertion n, 831–852, 22 bp: 0 = absent; 1 = present
68. Deletion o, 798–916, 129 bp: 0 = absent; 1 = present
69. Deletion p, 923–925, 3 bp: 0 = absent; 1 = present
70. Insertion q, 989–991, 3 bp: 0 = absent; 1 = present
71. Insertion r, 1012–1015, 4 bp: 0 = absent; 1 = present

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#### DNA Sequencing

Total genomic DNA was extracted from 36 species, following the standardized CTAB-isopropanol precipitation protocols (Paterson et al. 1993) and the kits Nucleon® Phy-

topure® (Tepnel Life Sciences, Manchester, UK) and DNeasy® Plant (QIAGEN®, Valencia, California, USA). Plastid markers *ndhF* and the *rpl16* intron were chosen as they have provided good resolution among genera and subfamilies in previous studies and there were sequences available in GenBank. Amplification reactions for *ndhF* (ca. 2 kb) and the *rpl16* intron (ca. 1.2 kb) were conducted following known PCR protocols (Clark et al. 1995; Kelchner and Clark 1997; Zhang 2000). Amplified fragments were visualized and then cleaned with the QIAEX II Gel Extraction Kit (QIAGEN®). Sequencing reactions for both markers were carried out using specific primers designed in prior studies (Clark et al. 1995; Kelchner and Clark 1997). Sequencing of both strands was performed on the Automated DNA Sequencer ABI 377 (Perkin-Elmer, Applied Biosystems Division, Foster City, California, USA) at the Iowa State University DNA Sequencing and Synthesis Facility.

Fragments were assembled and edited with Autoassembler (Perkin-Elmer, Applied Biosystems Division). A total of 44 *rpl16* and 25 *ndhF* sequences was generated. Published sequences, nine *rpl16* and 29 *ndhF*, were obtained from GenBank (Table 2). Sequences were aligned manually with Se-Al vers. 2.09a (Rambaut 1996). Alignment introduced gaps that were treated as binary, presence/absence characters in the structural data set when they appeared to be parsimony informative (Giribert and Wheeler 1999).

#### Phylogenetic Analysis

A 55-taxon by 3394-character matrix was constructed with three character partitions, *ndhF* (2067 chars.), *rpl16* intron (1256 chars.), and structural data (71 chars.). The matrix included two composite genera, *Aristida* L. and *Chusquea* Kunth (Table 2). The data matrix has been submitted to TreeBASE ([www.treebase.org](http://www.treebase.org)). Parsimony analyses were conducted using PAUP\* (Swofford 1999). Each heuristic search comprised 10,000 replicates of random taxon addition and tree-bisection-reconnection (TBR) branch swapping. Characters were weighted equally and gaps were treated as missing data. Branch lengths were determined under ACC-TRAN (accelerated transformation) optimization in PAUP\*, and unambiguous changes were identified using the Trace All Changes option in MacClade (Maddison and Maddison 1992). To assess the relative support for clades, bootstrap analyses were performed with 1000 replicates in a heuristic search using random entry followed by TBR branch swapping (MULTREES) (Felsenstein 1985). In addition, Bremer support (“decay”) values were obtained using PAUP\* by generating strict consensus trees for all topologies found up to six or more steps longer than the most-parsimonious trees (Bremer 1988; Donoghue et al. 1992).

To ascertain potential conflicts among topologies, separate phylogenetic analyses of each partition were performed (Miyamoto and Fitch 1995; Huelsenbeck et al. 1996; Seelanan et al. 1997; Page and Holmes 1998). Then Incongruence Length Differences (ILD) tests (implemented as the Partition Homogeneity test in PAUP\*; Farris et al. 1994) were conducted to evaluate possible conflicts among the topologies that resulted from separate analyses of partitions. Finally, an analysis of combined data sets was performed.

#### RESULTS AND DISCUSSION

##### *ndhF* and *rpl16* Intron Sequences

The assembled matrix contained 55 taxa and 3394 characters, or 186,670 cells, of which approximately 3% were scored as missing. Most of the sequences generated were complete except for partial *rpl16* intron sequences obtained from *Bromuniola* Stapf & C. E. Hubb. (557 base pairs [bp]), *Centotheca* Desv. (815 bp), *Cyperochloa* (931), *Gouldochloa* Valdés-Reyna, Morden & S. L. Hatch (982 bp), *Micraira* F. Muell. (757 bp), *Zeugites americana* (944 bp), and *Z. munroana* (952 bp). Sequences of the *rpl16* intron for *Danthonia* DC. and *Eriachne* R. Br. were missing due to lack of plant materials. It is possible that the specific primers could not bind due to mutations or structural properties at the binding sites. Likewise, for *ndhF* only partial sequences for *Arundoclaytonia dissimilis* (1111 bp) and *Z. americana* (1788 bp) were obtained. Amplification of several different segments was attempted without success.

Alignment of the *ndhF* sequences was straightforward, whereas for the *rpl16* intron there were some sections that were impossible to align unambiguously and therefore were excluded from the analyses. Alignment produced several gaps that were treated as parsimony informative and coded as presence/absence characters in the structural data set (chars. 54–71, Table 3). The 15 coded indels in the *rpl16* intron varied in length from 3 to 10 bp, except for an insertion of 22 bp and a large deletion of 129 bp. For *ndhF*, the three parsimony-informative indels varied from 6 to 9 bp in length.

The *rpl16* intron and *ndhF* sequences were both AT rich, as expected, with the A content higher in the intron than in *ndhF*. The *rpl16* intron had approximately 41% A, 14% C, 18% G, and 27% T, whereas the *ndhF* gene had 28% A, 17% C, 18% G, and 37% T. The total number of parsimony-informative nucleotide bases was 365 for the *rpl16* intron and 482 for *ndhF*.

##### Phylogenetic Analysis

The three partitions—*rpl16* intron, *ndhF*, and structural data—were compared, and nonsignificant values from the ILD test were obtained, suggesting that a combined analysis could be done. There were a few conflicts between the morphological and molecular data that probably were due to convergent evolution of some characters, as has been shown in other studies (e.g., several origins of the C<sub>4</sub> photosynthetic pathway [Sinha and Kellogg 1996; Giussani et al. 2001], plant habit). The positions of some taxa, some represented by partial sequences, were unstable and lacked strong support.

Phylogenetic analysis of the combined data sets yielded four equally most-parsimonious trees of 2881 steps, a consistency index (CI) of 0.438, and a retention index (RI) of 0.638. Figure 2 shows the strict consensus cladogram with bootstrap (bts) and Bremer support (brs) values. Figure 3 shows the strict consensus tree with bootstrap and Bremer support from the analysis of the *ndhF* partition alone (192 trees, 1417 steps, CI = 0.451, RI = 0.650). Recovery of a PACCAD clade in both analyses did not require constraints as in the GPWG (2001) analyses. Because of the nature of the combined data, it could not be analyzed with maximum



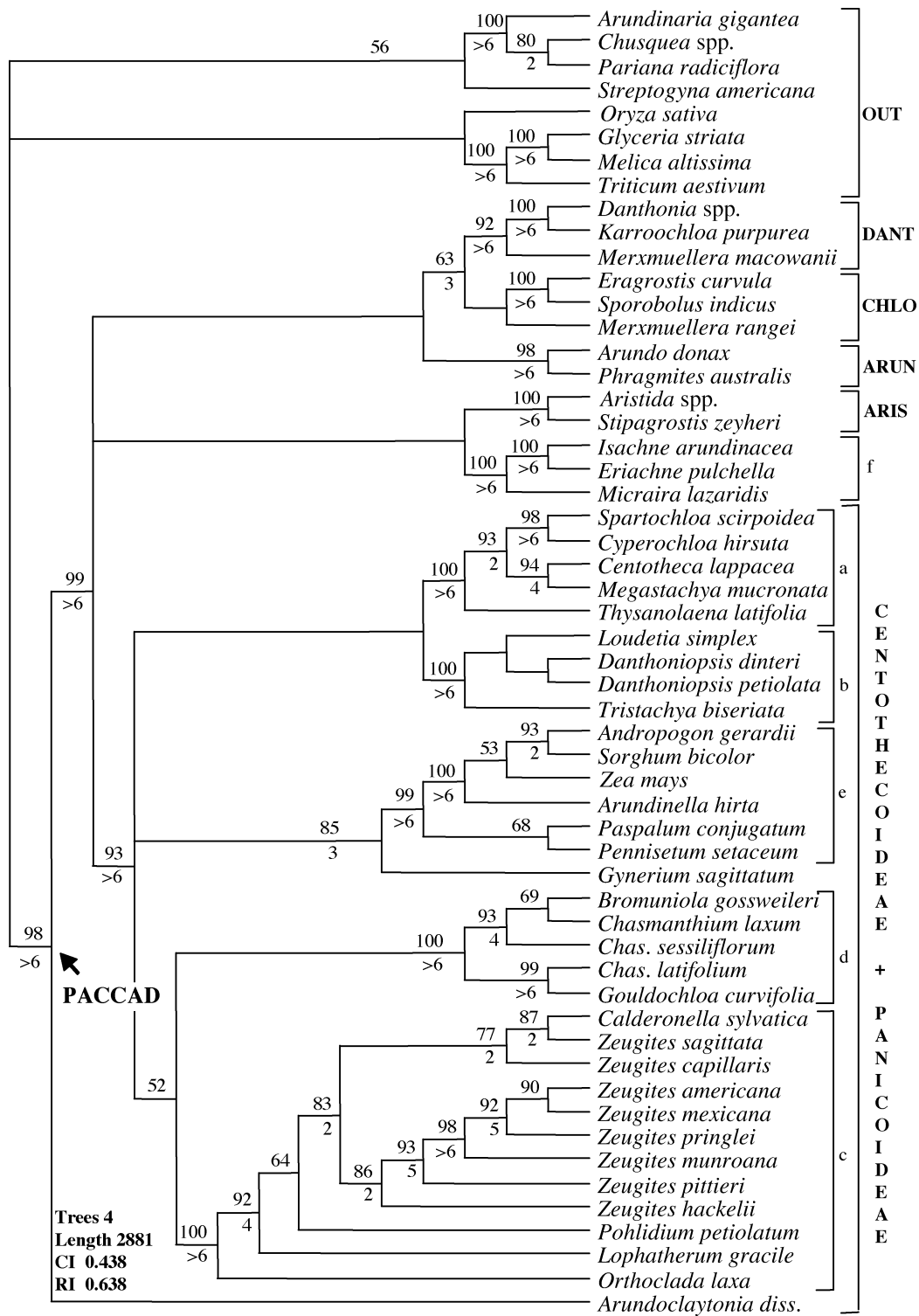


Fig. 2.—Strict consensus tree based on combined *ndhF*, *rp16* intron, and structural data, with bootstrap (>50%) and Bremer support values above and below the lines, respectively. PACCAD = Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, and Danthonioideae; ARIS = Aristidoideae; ARUN = Arundinoideae; CHLO = Chloridoideae; DANT = Danthonioideae; OUT = out-group; a = *Thysanolaena*–*Centotheca*–*Cyperochloa* clade; b = *Danthoniopsis* clade; c = *Zeugites* clade; d = *Chasmanthium* clade; e = Panicoideae tribes Andropogoneae, Arundinelleae s.s., and Paniceae; f = *Micraira* clade.

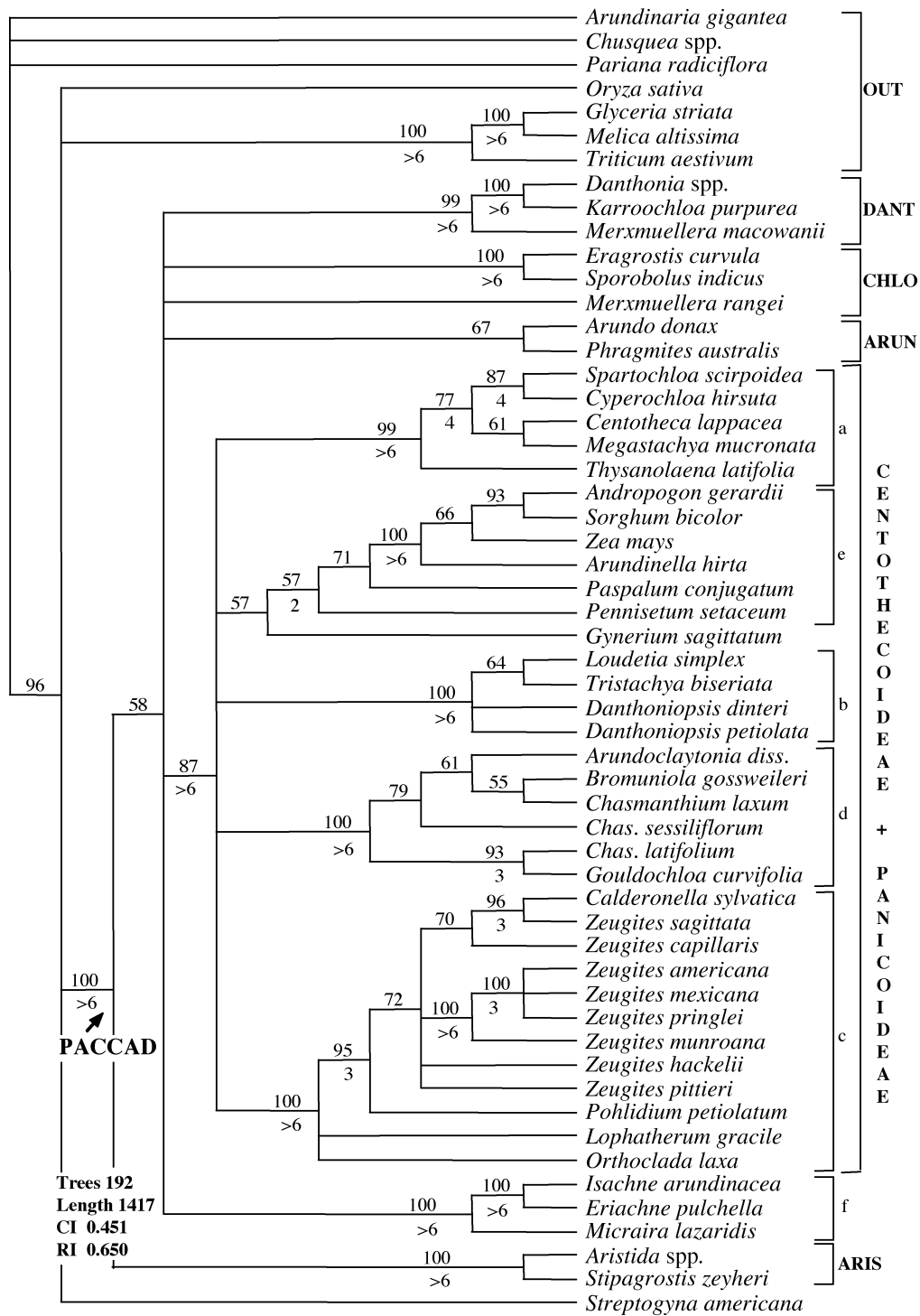


Fig. 3.—Strict consensus tree based on *ndhF* sequences only, with bootstrap values (>50%) above lines. See Fig. 2 caption for explanation of abbreviations.

likelihood (Page and Holmes 1998) or Bayesian analysis (Huelsenbeck and Ronquist 2001). The inclusion of coding and noncoding sequences and structural data made the search for a single model of nucleotide evolution impractical. However, after this analysis was carried out, Nylander et al. (2004) suggested that different models of evolution can be incorporated into such analyses, including structural data.

*PACCAD Clade*

The strict consensus cladogram based on the combined data (Fig. 2) shows 99% bts and >6 brs values supporting the PACCAD clade, but the bootstrap support slightly decreases with the inclusion of *Arundoclaytonia* Davidse & R. P. Ellis (98% bts, >6 brs). Confirming the findings of the

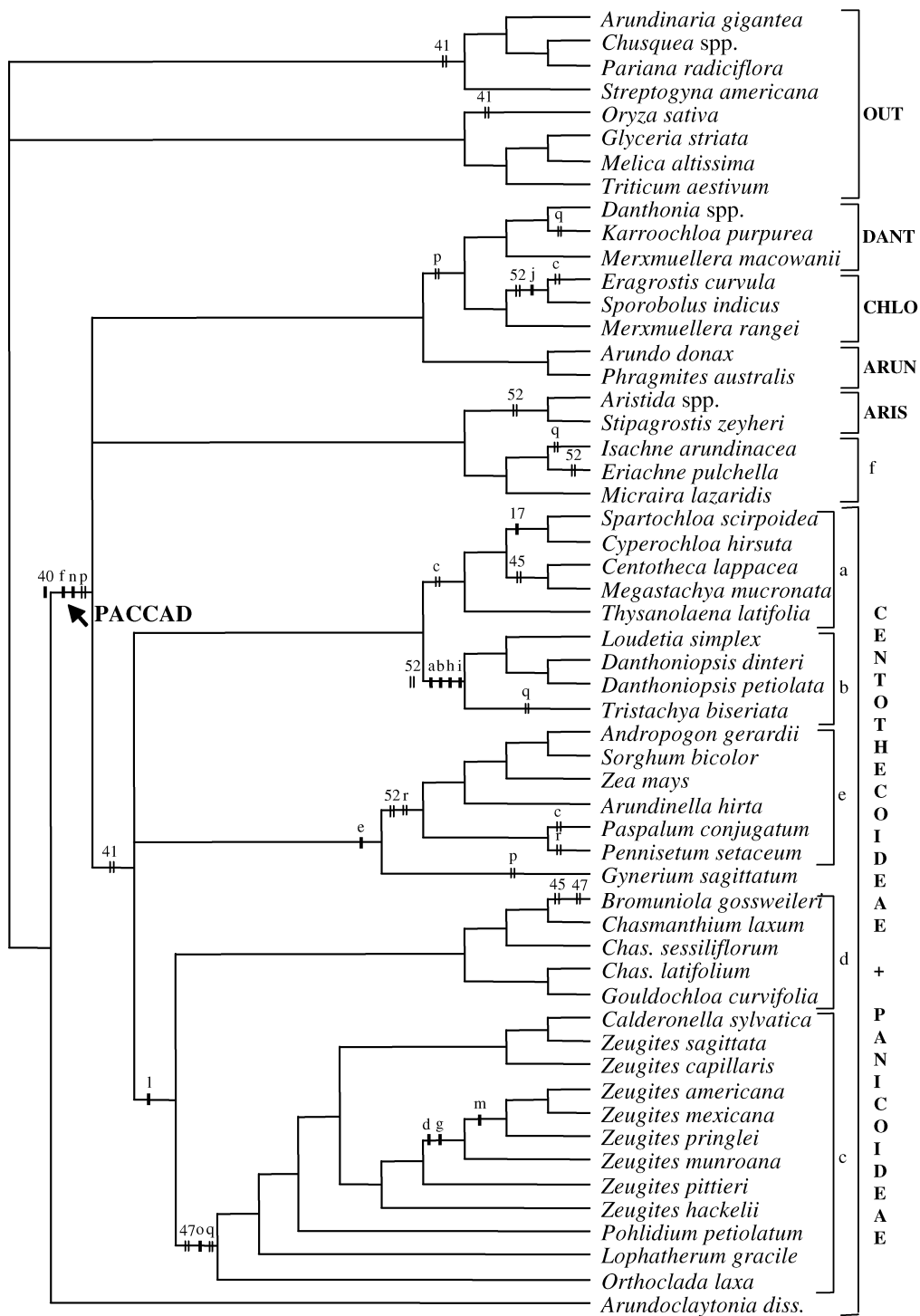


Fig. 4.—Same tree as in Fig. 2, showing mapped *ndhF* and *rpl16* intron insertions/deletions (letters a–r, representing chars. 54–71 in Table 3) and important non-molecular synapomorphies (chars. 17, 40, 41, 45, 47, and 52; Table 3) for the PACCAD, Centothecoideae + Panicoideae, and *Zeugites* (c) clades. Solid bars = unambiguous synapomorphies; double bars = homoplasies or character transformations. See Fig. 2 caption for explanation of other abbreviations.

GPWG (2001), the elongated mesocotyl in the embryo represents the morphological synapomorphy for this clade (Fig. 4, char. 40). In this analysis, an insertion of 22 bp in the *rpl16* intron (Fig. 4, indel n) was found to be another potential synapomorphy for the clade. The major lineages of the clade are also well supported, with *A. dissimilis* (Pani-

coideae: Steyermarkochloae) sister to the remainder of the PACCAD clade (Fig. 2). Trees resulting from analysis of the *rpl16* intron alone (not shown) show the same major groups as in other analyses, but with less resolution, and also place *A. dissimilis* as sister to the remainder of the PACCAD clade. Trees resulting from analysis of *ndhF* alone (Fig. 3) are sim-

ilar to the combined analysis but with lower resolution and, interestingly, show *A. dissimilis* embedded in the *Chasmanthium* Link (Centothechoideae: Centotheceae) clade with strong support (100% bts, >6 brs). It should be pointed out, however, that the *ndhF* sequence from this species was incomplete. Within the remainder of the PACCAD clade, little or no support was found for relationships among most of the major internal clades (Fig. 2, 3).

Danthonioideae and Chloridoideae were moderately and not supported, respectively, in the combined analyses, with 92% bts and >6 brs for the former (Fig. 2). These subfamilies are weakly supported as sister groups with 63% bts and 3 brs. Also, strongly supported in this analysis were Aristidoideae (100% bts, >6 brs), Arundinoideae (98% bts, >6 brs), and a clade (100% bts, >6 brs) formed by *Eriachne* (Eriachneae) and *Micraira* (Micraireae) (both incertae sedis, GPWG 2001) plus *Isachne* R. Br. (Panicoideae: Isachneae). These results are largely consistent with the phylogenetic analyses of the GPWG (2000, 2001). The only difference found here is the position of the incertae sedis genera with *Isachne*, which is panicoid in virtually all classifications (e.g., Clayton and Renvoize 1986; Watson and Dallwitz 1992, 1998; GPWG 2001). This analysis rejects the hypothesis that the tribes Isachneae and potentially Steyermarkochloae are panicoid, and demonstrates the polyphyly of the subfamily as currently circumscribed.

In previous analyses, *Eriachne* is an isolated genus based on *rbcL* sequence data (GPWG 2001) and it forms a clade with *Micraira* based on ITS sequence data (Hsiao et al. 1999). *Micraira*, on the other hand, is an unstable taxon in the *ndhF* and *rpoC2* phylogenies (Barker et al. 1995; Clark et al. 1995), and in a combined analysis of all sequences available, *Micraira* is sister to the other members of the PACCAD clade (GPWG 2001). The clade in our study (also Duvall et al. 2007) comprising *Eriachne*, *Isachne*, and *Micraira*, here called the *Micraira* clade, is strongly supported (Fig. 2, 3, clade f) and contains both C<sub>3</sub> (*Isachne* and *Micraira*) and C<sub>4</sub> (*Eriachne*) plants, suggesting another probable independent origin of the C<sub>4</sub> photosynthetic pathway (Fig. 4, char. 52) outside Aristidoideae, Chloridoideae, and Panicoideae (Giussani et al. 2001; GPWG 2001). Vegetatively, the three genera of the *Micraira* clade are divergent from one another, but they share two fertile florets per spikelet. The gross morphology of the florets varies, in part because *Eriachne* and *Micraira* have membranous bracts vs. the panicoid-like indurate bracts in the fertile florets of *Isachne*. This feature of *Isachne* appears to represent convergent evolution with members of Panicoideae. Geographically, *Micraira* is restricted to Australia; *Eriachne* occurs in Australia, China, and the Indo-Malayan region; and *Isachne* occurs in the tropical and subtropical regions of the world.

#### Centothechoideae + Panicoideae Clade

The Centothechoideae + Panicoideae clade (excluding *Arundoclaytonia*) is also well supported based on the combined data (Fig. 2, 93% bts, >6 brs). The overlapping embryonic leaf margins (Fig. 4, char. 41) are a synapomorphy, although this character state is also present in Bambusoideae and *Oryza* L. Panicoideae as currently circumscribed are demonstrated to be polyphyletic because *Isachne* is embed-

ded in another clade (Fig. 2, 3). *Arundoclaytonia* is sister to the other PACCAD clade representatives in trees derived from all data (Fig. 2), or, as mentioned above, it is part of a centothechoid clade in the *ndhF* trees (Fig. 3). However, Panicoideae s.s., including only Andropogoneae, Arundinelleae s.s. (*Arundinella*), and Paniceae, are well supported as monophyletic (Fig. 2, clade e, 99% bts, >6 brs). *Gynerium* is resolved as sister to Panicoideae s.s. with moderate bootstrap support (85%) and Bremer support of 3 (Fig. 2, 3). Centothechoideae do not have support from the combined data (Fig. 2), although there are several internal clades that are highly supported. The lack of resolution within the Centothechoideae + Panicoideae clade might be the result of potentially critical taxa of both groups not sampled in this study (*Chevalierella* and *Orthoclada africana* C. E. Hubb. in Centothechoideae, and *Hubbardia* Bor and *Steyermarkochloa* Davidse & R. P. Ellis in Panicoideae), insufficient sequence data, or because there was a rapid radiation as Spangler et al. (1999) suggested for Andropogoneae. One strongly supported clade (100% bts, >6 brs), the *Danthoniopsis* clade (Fig. 2, clade b), includes *Danthoniopsis*, *Loudetia*, and *Tristachya*; however, internal support for relationships is lacking. A second clade, the *Thysanolaena*–*Centothecha*–*Cyperochloa* clade (Fig. 2, clade a), is also strongly supported (100% bts, >6 brs) and includes *Thysanolaena* as sister to two clades that also have strong support, the *Centothecha* clade (*Centothecha* and *Megastachya* P. Beauv.; 94% bts, 4 brs) and the *Cyperochloa* clade (*Cyperochloa* and *Spartochloa*; 98% bts, >6 brs). A third strongly supported clade, the *Chasmanthium* clade (Fig. 2, clade d, 100% bts, >6 brs), includes *Bromuniola*, *Chasmanthium*, and *Gouldochloa* (plus *Arundoclaytonia*, represented by a partial sequence in the *ndhF* analysis; Fig. 4). A fourth clade, the *Zeugites* clade (Fig. 2, clade c, 100% bts, >6 brs), is also strongly supported and includes a basal *Orthoclada* C. E. Hubb., followed by *Lophatherum* Brongn., *Pohlidium* Davidse, Soderstr. & R. P. Ellis, and *Zeugites*, with *Calderonella* Soderstr. & H. F. Decker embedded in *Zeugites*.

#### Arundinelleae

The position of the *Danthoniopsis* clade (Fig. 2, 3, clade b) again confirms the polyphyly of Arundinelleae as shown in other studies (Mason-Gamer et al. 1998; Spangler et al. 1999; Mathews et al. 2000; Giussani et al. 2001; GPWG 2001). The position of the clade remains uncertain due to the lack of support, although nuclear sequence data suggest that it may be sister to the other panicoids (J. G. Sánchez-Ken unpubl. data). Nevertheless, our results are in conflict with the topology based on *rbcL* sequence data in which *Tristachya biseriata* is embedded within Andropogoneae (Linder et al. 1997). To complicate matters, *T. superba* (De Not) Schweinf. & Arch. is nested within Paniceae based on *phyB* sequence data (Mathews et al. 2002). *Danthoniopsis petiolata* (as *Rattraya petiolata* J. B. Phipps) is sister to Paniceae and separate from *D. dinteri* based on *ndhF* sequence data (Spangler et al. 1999; Spangler 2000). However, this outcome may be the result of a misidentification that cannot be corroborated because there is no voucher specimen. In the present analysis both species of *Danthoniopsis*, plus *Loudetia* and *Tristachya*, form a strongly supported clade in-

cluding two pairs of synapomorphic indels each in *ndhF* and *rpl16* (Fig. 4, indels a, b, h, and i). It is interesting to note that the *rpl16* intron sequence of *Loudetia* has a 123 bp gap, missing in the other members of the *Danthoniopsis* clade but similar to one found in the *Zeugites* clade, and most probably not homologous. The genera of the *Danthoniopsis* clade are morphologically and phytogeographically very similar (Phipps 1967a, b); although they need detailed study, it is clear that they must be excluded from Arundinelleae. *Arundinella* and probably *Garnotia* Brongn. (Gould 1972; Clayton and Renvoize 1986) are the two genera that may end up forming an emended Arundinelleae.

#### Thysanolaena–Centotheca–Cyperochloa Clade

*Centotheca* and *Megastachya*, forming a strongly supported clade (Fig. 2), are closely related to each other based on morphology, anatomy, cytology, and molecular data. They occupy similar humid and sometimes shaded habitats, and exhibit a similar geographic distribution, as both are sympatric in Africa, with *Centotheca* reaching Asia, the Pacific Islands, and Australia. Although *Centotheca* is the type genus of the subfamily, this clade is clearly distinct from the other major lineages of Centothecoideae. The *Centotheca* clade is sister to the *Cyperochloa* clade (Fig. 2, 3), consisting of two little-known, rare Australian genera, *Cyperochloa* and *Spartochloa*. *Cyperochloa* was previously considered a member of Arundinoideae (Lazarides and Watson 1986; Watson and Dallwitz 1992, 1998) and recently was treated as incertae sedis by the GPWG (2001). Barker (1997), based on *rbcL* sequence data, first suggested an alliance between *Cyperochloa* and *Thysanolaena*. Later, Barker et al. (1998), based on *rpoC2*, showed the relationship between *Spartochloa* and *Thysanolaena*. Hsiao et al. (1999), based on ITS (nuclear) sequences, suggested that *Cyperochloa* was closely related to *Spartochloa* and *Thysanolaena*. It is probable that this relationship was not even considered by the GPWG (2001) because of the striking differences in morphology between *Thysanolaena* and the other genera. *Cyperochloa* and *Spartochloa* are morphologically similar, sharing reduced leaf blades, numerous florets per spikelet, sterile bracts below the spikelet, and a  $C_3$  photosynthetic pathway; they are also restricted to a semi-arid region of southwest Australia.

The *Thysanolaena–Centotheca–Cyperochloa* clade is defined by a synapomorphic insertion in *ndhF*, although it has arisen independently in *Eragrostis* Wolf and *Paspalum* L. (Fig. 4, indel c). The *Cyperochloa* clade is defined by the presence of sterile glume-like bracts below the spikelets (Table 3, char. 17). The *Centotheca* clade is defined by the presence of balaniform microhairs (Table 3, char. 45), which seem also to be present in *Bromuniola*. This tentative conclusion needs to be verified in *Bromuniola* because the quality of the dried material was not good in comparison to the living collections of *Centotheca* and *Megastachya* studied.

#### Chasmanthium Clade

The *Chasmanthium* clade is also an interesting group by the nature of its members (Fig. 2, 3, clade d). *Chasmanthium* is paraphyletic since it does not include *Bromuniola* and *Gouldochloa* in previous classifications (Clayton and Ren-

voize 1986; Watson and Dallwitz 1992, 1998). The three genera are morphologically very similar in that their spikelets usually have numerous florets and proximal sterile florets (Fig. 1). Hubbard (1926) previously pointed out the resemblance between *Bromuniola* and *Chasmanthium*, the latter of which was then a synonym of *Uniola* L. (Hitchcock 1951; Yates 1966) due to unrecognized convergent evolution in spikelet morphology. The three genera are widely disjunct, with *Bromuniola* restricted to tropical Africa, *Chasmanthium* to the central and southern USA, and *Gouldochloa* to northeastern Mexico (Fig. 1). As discussed above, based on a partial sequence, *Arundoclaytonia* is embedded within the *Chasmanthium* clade in the *ndhF* trees (Fig. 3), suggesting that tribe Steyermarkochloae in which *Arundoclaytonia* is classified is incorrectly placed within Panicoideae by the GPWG (2001).

Morphologically, the genus differs greatly from *Chasmanthium*, and its placement within that clade, if confirmed, is a striking result. The conflicting positions in the trees (Fig. 2, 3) could be due to the missing *ndhF* sequence data or potentially an erroneous *rpl16* intron sequence, which needs to be verified. It will be necessary to sample the remaining species of *Chasmanthium*, add the missing regions of the sequences (from *Arundoclaytonia*, *Bromuniola*, and *Gouldochloa*), and re-sequence the *rpl16* intron for *Arundoclaytonia* before better resolution of the phylogenetic relationships among these taxa can be obtained. Finally, *Bromuniola*, *Chasmanthium*, and *Gouldochloa* share a spikelet morphology, as described above, but the inclusion of *Arundoclaytonia* would render this group morphologically heterogeneous.

#### Zeugites Clade

The *Zeugites* clade (Fig. 2, 3, clade c) is a homogeneous group of five genera that is interesting morphologically and biogeographically, not unlike the *Chasmanthium* clade. The clade has two synapomorphies: tissues resembling palisade parenchyma and spongy mesophyll in the leaf chlorenchyma, and a deletion of 129 bp in the *rpl16* intron (Fig. 4, char. 47, indel o). The deletion in the *rpl16* intron is similar to one in *Loudetia*, and potentially also with those in *Sporobolus* R. Br. and *Merxmullera macowanii*, though the indel in the latter three species has a different length than the one in the *Zeugites* clade. Another indel (Fig. 4, indel l) supports the sister relationship between the *Chasmanthium* and *Zeugites* clades.

*Orthoclada* has two species, one in tropical Africa and the other in tropical America, that are strikingly similar and have spikelets with bisexual florets. *Lophatherum* is an Asian genus that has bisexual proximal florets and numerous sterile distal florets (Fig. 1). *Pohlidium*, endemic to Panama, is characterized by short culms and unisexual spikelets (Fig. 1). Lastly, *Zeugites*, with *Calderonella* nested within it, is moderately supported in trees from the combined analysis (Fig. 2, 83% bts, 2 brs) and is distributed from Mexico to northern South America. These two genera are vegetatively different, with *Calderonella* being caespitose vs. the reedlike or sprawling habit of *Zeugites*, but they share bisexual spikelets with unisexual florets. It is evident that *Zeugites* is par-

Table 4. Suggested redistribution of groups from the GPWG (2001). CP = Centothecoideae + Panicoideae.

GPWG (2001)	This study
<b>Centothecoideae</b>	<b>Centothecoideae</b>
Centothecaeae	<i>Centotheca</i> clade
Thysanolaeneae	<i>Cyperochloa</i> clade
<b>Panicoideae</b>	Thysanolaeneae
Andropogoneae	<i>Chasmanthium</i> clade
Arundinelleae ( <i>Loudetia</i> , <i>Tristachya</i> )	<i>Zeugites</i> clade
Hubbardieae	<b>Panicoideae</b>
Isachneae	Andropogoneae
Paniceae	Arundinelleae
Steyermarkochloaeae	Hubbardieae
<b>Unstable taxa within the CP clade</b>	Paniceae
<i>Danthoniopsis</i>	<b>Unstable taxa within the CP clade</b>
<b>Arundinoideae</b>	<i>Danthoniopsis</i> clade
<i>Spartochloa</i>	Gynerieae
<b>Uncertainae sedis</b>	Steyermarkochloaeae
<i>Cyperochloa</i>	<b><i>Micraira</i> clade</b>
Eriachneae	Eriachneae
<i>Gynerium</i>	Isachneae
Micraireae	Micraireae
Streptogyneae	<b>Uncertainae sedis</b>
	Streptogyneae

aphyletic and *Calderonella* must be placed within it in order to obtain a monophyletic *Zeugites*.

Evolution of spikelet sexuality within the *Zeugites* clade is interesting, in that bisexual spikelets and florets took two evolutionary pathways, one leading to *Pohlidium* with unisexual spikelets, and the other leading to *Zeugites* and *Calderonella*, each having bisexual spikelets and unisexual florets. It is unknown if the developmental pathway for unisexuality in this clade is similar to that found in Andropogoneae (Le Roux and Kellogg 1999), therefore this phenomenon merits more detailed study in this clade.

The *Zeugites* clade is among all centothecoids the most morphologically cohesive yet diverse in terms of number of species and genera. Anatomically, the tissues resembling palisade parenchyma and spongy mesophyll (Fig. 4, char. 47) represent a unique synapomorphy if this character is not considered homologous to the mesophyll of Bambusoideae, a likely conclusion given the distinctive structure of bambusoid arm cells. Scattered C<sub>3</sub> taxa in other subfamilies possess loose mesophyll, but it is never differentiated as in the *Zeugites* clade, thus we can assume that this character is a potential unique synapomorphy for the group.

This analysis shows that because Centothecoideae and Panicoideae, as currently circumscribed, cannot be recovered as monophyletic groups (Fig. 2, 3), it may be necessary to recognize an expanded subfamily Panicoideae. The expand-

ed subfamily could be characterized as a tremendous evolutionary experiment leading to the development of many morphologically divergent small groups. Such groups are well supported, as discussed above, and can be defined by several synapomorphies.

### Conclusions

Although the Centothecoideae + Panicoideae clade itself is well supported, resolution of relationships among the major internal branches is still lacking. This may be due to a lack of sampling of critical taxa, insufficient sequence data, or a putative rapid radiation as in Andropogoneae (Spangler et al. 1999).

Panicoideae as currently circumscribed (GPWG 2001) are polyphyletic. Isachneae must be excluded from either a narrow or broad circumscription of the subfamily, and Steyermarkochloaeae must be as well if a narrow concept of Panicoideae is adopted, which would include only Andropogoneae, Arundinelleae (comprising only *Arundinella* and probably *Garnotia*), and Paniceae. The positions of the *Danthoniopsis* clade, *Gynerium*, and Steyermarkochloaeae remain ambiguous, but preliminary analyses of nuclear data suggest that the *Danthoniopsis* clade may be sister to Panicoideae in the narrow sense above (J. G. Sánchez-Ken unpubl. data).

*Isachne* forms a monophyletic group with *Micraira* and *Eriachne*. This study supports its exclusion from Panicoideae, and further suggests that the *Micraira* clade should be recognized at the subfamily level (Duvall et al. 2007; Sánchez-Ken et al. 2007).

Centothecoideae sensu the GPWG (2001) are still not supported as monophyletic, even with the inclusion of various incertae sedis taxa. As noted above, there are various reasons why this might be the case, including two unsampled African taxa (*Chevalierella* and *Orthoclada africana*) that could affect the topology. The exploration of nuclear markers may bring resolution to the phylogenetic relationships within this clade and yield a monophyletic Centothecoideae, but, if not, a broad concept of Panicoideae, including Centothecoideae (sensu Zuloaga et al. 2003), would be warranted.

Regardless of subfamilial disposition, several clades within the centothecoids are well supported and need to be taxonomically and nomenclaturally defined. Tribe Centothecae as currently circumscribed is evidently paraphyletic and must be emended. The *Centotheca*, *Chasmanthium*, *Cyperochloa*, *Danthoniopsis*, and *Zeugites* clades need to be formally designated as tribes. Table 4 summarizes the new approach to classification of Centothecoideae + Panicoideae; these taxonomic changes are being prepared by the first author. *Calderonella* becomes synonymous with *Zeugites*, and it is likely that both *Bromuniola* and *Gouldochloa* should be synonymized under *Chasmanthium*. However, *Bromuniola*, *Chasmanthium*, and *Gouldochloa*, as well as *Arundoclaytonia*, have to be further analyzed before any taxonomic changes are undertaken.

Finally, in this study we establish the basis for further studies in elucidating phylogenetic relationships within the Centothecoideae + Panicoideae clade. Because the phylogenetic structure found in this study is based on maternally inherited DNA sequences and structural characters, the next step is the exploration of nuclear markers that may help to increase resolution within this clade and the entire PACCAD clade.

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