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## PHYLOGENETICS OF PANICEAE (POACEAE)<sup>1</sup>

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Paniceae demonstrate unique variability of photosynthetic physiology and anatomy, including both non-Kranz and Kranz species and all subtypes of the latter. This variability suggests hypotheses of independent origin or reversals (e.g., from C<sub>4</sub> to C<sub>3</sub>). These hypotheses can be tested by phylogenetic analysis of independent molecular characters. The molecular phylogeny of 57 species of Paniceae was explored using sequences from the grass-specific insert found in the plastid locus *rpoC2*. Phylogenetic analyses confirmed some long-recognized alliances in Paniceae, some recent molecular phylogenetic results, and suggested new relationships. Broadly, Paniceae were found to be paraphyletic with Andropogoneae, *Panicum* was found to be polyphyletic, and *Oplismenus hirtellus* was resolved as the sister group to the remaining ingroup species. A particularly well-supported clade in the *rpoC2* tree included four genera with non-Kranz species and three with distinctively keeled paleas. As previously suggested, the PCK (phosphoenol pyruvate carboxykinase) C<sub>4</sub> subtype arose once within Paniceae. All clades with non-Kranz species had Kranz ancestors or sister taxa suggesting repeated loss of the Kranz syndrome.

**Key words:** Kranz; molecular phylogenetics; non-Kranz; Paniceae; Poaceae; *rpoC2* insert.

The Paniceae are a highly diverse and species-rich assemblage of >2000 species comprising fully one-fifth of the total species of grasses. Diversity is expressed in variable floral development, inflorescence architecture, leaf anatomy, and photosynthetic physiology among species of the 100-odd genera in the tribe. Photosynthetic physiology and corresponding leaf anatomy (i.e., non-Kranz C<sub>3</sub> or Kranz C<sub>4</sub>) are unusually variable in Paniceae (Brown, 1977; Hattersley and Watson, 1992). The more prevalent C<sub>3</sub> photosynthesis is correlated with three characters: (1) irregular chlorenchyma; (2) starch formation confined to the mesophyll; and (3) four or more chlorenchyma cells between adjacent bundle sheaths. In C<sub>4</sub> species, the starch-forming chlorenchyma is restricted to a bundle sheath, and there are fewer cells between adjacent bundle sheaths (two to four) than in C<sub>3</sub> grasses. The C<sub>3</sub> state is generally considered to be plesiomorphic. However, with regard to Paniceae, Clayton and Renvoize (1986, p. 260) urge “some caution . . . in regarding the non-Kranz genera as primitive in any absolute sense. . . .”

There are also distinguishable C<sub>4</sub> subtypes. Decarboxylation of four-carbon compounds in C<sub>4</sub> species is catalyzed either by NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), or PEP-carboxykinase (PCK) (see Hattersley and Watson [1992] and Sinha and Kellogg [1996] for physiological differences between subtypes). The most common condition in Paniceae is NADP-ME, although PCK species are not rare.

Photosynthetic physiology is nearly invariant within entire subfamilies of Poaceae with only three exceptions. Nine of the 12 subfamilies (following Grass Phylogeny Working Group [GPWG], 2000, in press) are each wholly non-Kranz. All Kranz grasses are found in a large and diverse lineage known as the PACCAD clade (GPWG, 2000, in press). Aristidoideae

are Kranz except for *Sartidia* (four species). All chloridoid grasses are Kranz except for two species (*Eragrostis walteri* and *Merxmuellera rangei*). Panicoideae as a whole, and Paniceae in particular, are uniquely variable, comprising many taxa which are C<sub>3</sub> as well as taxa of all C<sub>4</sub> subtypes. This variation affords a unique opportunity for phylogenetic study. Hypotheses of independent origin and putative ancestral states for each subtype, which are generally unknown, can be tested in a phylogenetic framework.

The Poaceae has a generally well-studied phylogeny (e.g., GPWG, 2000, in press), however, the most intensively sampled phylogenetic work in Paniceae published to date is limited to two studies. The first, a cladistic study of 67 exomorphological and anatomical characters taken largely from herbarium specimens of 110 taxa, was conducted by Zuloaga, Morrone, and Giussani (2000). The authors considered this substantial study to be “preliminary,” undoubtedly because of the size of Paniceae, and support statistics for the cladogram were not reported. Of note was the fact that *Panicum*, as expected, was polyphyletic. For example, *Panicum* subgenus *Megathyrus* clustered with a *Urochloa/Eriochloa* clade, whereas *Panicum* section *Laxa* was in a somewhat more basal clade with *Arthropogon* and other taxa with fusoid-like cells.

The second, a molecular study (sequences of the plastid *trnL-F* region) of 32 species plus outgroups, was conducted by Gómez-Martínez and Culham (2000). Some of their main conclusions and discoveries about Paniceae were the following: (a) Setariinae and Digitariinae were both found to be paraphyletic with other genera of Paniceae; (b) the identification of a “setae-bearing clade” (*Cenchrus*, *Pennisetum*, and *Setaria*); (c) a monophyletic clade of species that are exclusively C<sub>4</sub> PCK, including *Panicum maximum* and *Brachiaria*; (d) a polyphyletic *Panicum* that supports Zuloaga, Morrone, and Giussani’s conclusions (2000); and (e) moderately supported monophyly of each of *Paspalum* and *Brachiaria*. We present here a complementary molecular analysis of Paniceae utilizing a different plastid locus, the grass-specific insert found in *rpoC2*, which encodes subunit C2 of a plastid-encoded RNA polymerase.

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## MATERIALS AND METHODS

DNA extracts were obtained from the Native Hawaiian Plant DNA Library (C. Morden, University of Hawaii, Honolulu, Hawaii, USA) and from the Kellogg DNA Library (J. H. Cota-Sánchez, University of Saskatchewan, Saskatoon, Canada, and E. Kellogg, University of Missouri, St. Louis, Missouri, USA). Sampling maximized representation of the variation in Paniceae (28 genera) with constraints imposed by the large number of species in the tribe and the availability of material. Multiple species were included for some of the larger genera and especially for *Panicum* (12 species). A region containing the grass-specific insert in the *rpoC2* locus was amplified with one of two pairs of primers, depending on successful amplification, either “rpoC2-1” and “rpoC2-2” or “rpoC2-U” and “rpoC2-D” (sequences in Barker, Linder, and Harley, 1999). Amplified products were purified and concentrated using Microcon-YM100 concentrators (Millipore, Bedford, Massachusetts, USA) following the manufacturer's instructions. Automated sequencing of these templates was performed (Core Laboratory Facility, Biological Sciences, Northern Illinois University, DeKalb, Illinois, USA) with the same primers that were used for amplification. Both strands were sequenced.

Molecular phylogenetic studies conducted broadly across Poaceae have suggested that *Danthoniopsis* is either the immediate sister group to the bulk of the other panicoid grasses or a member of the sister clade (Clark, Zhang, and Wendel, 1995; Spangler et al., 1999; Mathews, Tsai, and Kellogg, 2000). The *rpoC2* insert sequence of *Danthoniopsis dinterii* was thus also sequenced and was specified as the outgroup for phylogenetic analyses.

Nucleotide sequences were translated with Gene Inspector (version 1.5, Textco, West Lebanon, New Hampshire, USA) prior to alignment. A rule-governed alignment of the amino acid sequences was performed following Barker, Linder, and Harley (1999). These rules were devised to preserve heptameric amino acid repeats commonly found in the *rpoC2* insert, which are most likely the result of slipped-strand mispairing mutations (Cummings and Kellogg, 1994). We further followed a conservative approach to alignment, recognizing new heptameric insertions only if they could not otherwise be derived by one or two single nucleotide substitutions from existing adjacent sequences. Also aligned were *rpoC2* insert sequences of *Brachiaria serrata*, *Hyparrhenia hirta*, *Panicum maximum*, *Rhynchelytrum repens*, and *Saccharum officinarum* previously determined by Barker, Linder, and Harley (1999); and one of *Pennisetum* sp. determined by Cummings, King, and Kellogg (1994). Representatives of three tribes, Paniceae (57 spp.), Andropogoneae (2 spp.), and Arundinelleae (1 outgroup sp.) were thus included. After the amino acid sequences had been aligned, a corresponding alignment of nucleotide sequences was generated.

PAUP\* 4.0b4a (Swofford, 2000) analyses were performed on the aligned nucleotide sequences. Gaps were scored as “missing.” Gapped positions found in half or more of the species were excluded from the phylogenetic analysis, an approach that minimized the detrimental impact of excessive amounts of missing values on phylogenetic analysis while still preserving character data found for the majority of the taxa. Gaps were encoded as binary characters, appended to the aligned nucleotide sequences, and included in the phylogenetic analysis. Parsimony analysis of these data was a computer-intensive activity that consumed 400 h of computing time (plus >600 h of preliminary analyses) on a cluster of four desktop computers. To facilitate the analysis, tasks were subdivided into smaller parts that were run separately, each with specified limits on MAXTREES. Eighty-three such random addition sequence replicates, each with a different specified starting seed and tree-bisection-reconnection (TBR) swapping, were run with MAXTREES limited to 10 000. The results of 28 of these 83 subanalyses found sets of less parsimonious trees and were discarded. One additional replicate was also run with MAXTREES set to 500 000. The separate tree files were then combined with concurrent elimination of duplicate trees. A bootstrap analysis was run with 5000 subsamples, TBR swapping, and MAXTREES set at 1000.

## RESULTS

We determined *rpoC2* insert sequences for 54 species (Table 1) in this study (GenBank accession numbers GBAN-

AF372160–AF372213). All of the sequences included the expected heptameric-length amino acid repeat structure earlier observed by Cummings, King, and Kellogg (1994).

Thirty-four insertions/deletions were scored in the combined *rpoC2* insert data set for 60 species, ranging in size from 3 to 138 base pairs (bp) in length. The longest of these by far was a 138-bp autapomorphic insertion in the sequence for *Leptocoryphium lanatum*. Excluding this largest gap, the final aligned sequence length was 762 bp with 41% scored as missing, largely because of inferred insertions/deletions (alignment is available on request to the first author).

Exclusion of the ten gaps found in >50% of the taxa resulted in analysis of 450 nucleotide sites together with 34 binary-coded gaps, giving 484 total characters. Of these, 143 were parsimony informative, including 114 of the nucleotide sites and 29 of the encoded gaps. Once duplicate trees were eliminated from the trees combined from the 56 separate analyses,  $1 \times 10^6$  equally parsimonious trees of length 496 remained (see consensus tree, Fig. 1). Retention index (RI) was 0.7156 and consistency index (CI; excluding uninformative characters) was 0.4571.

## DISCUSSION

Phylogenetic analysis of *rpoC2* insert sequences confirmed some long recognized alliances in Paniceae as well as the recent molecular phylogenetic conclusions of Gómez-Martínez and Culham (2000) and those of our companion paper in this issue, the *ndhF* study of Giussani et al. (2001). Some new relationships were also suggested. Of particular interest is the distribution of  $C_3$  and  $C_4$  taxa across our *rpoC2* phylogeny (Fig. 1).

A clade of four taxa with non-Kranz species (*Steinchisma* [which includes anatomically intermediate  $C_3$ - $C_4$  species], *Plagiantha*, *Otachyrium*, and *Panicum laxum*) clustered with a fifth Kranz species, *Leptocoryphium lanatum*, and was strongly supported (bootstrap value = 94%). A similar association was noted by Giussani et al. (2001). The phylogenetic association between *Plagiantha* and *Otachyrium* has been previously noted (Zuloaga, Morrone, and Giussani, 2000). Further, a unique morphological synapomorphy has been previously recognized in the three most derived genera in this clade, *Steinchisma*, *Plagiantha*, and *Otachyrium*. Species in these genera are characterized by a distinct modification of the palea of the lower floret to produce enlarged keels or flanks at maturity (Clayton and Renvoize, 1986). These compelling morphological and molecular synapomorphies strongly support the monophyly of this “keeled clade.”

The keeled clade is in a polytomy with two other groups toward the base of the tree, a strongly supported *Arthropogon lanceolatus* and *Panicum prionitis* (bootstrap value = 90%), and a weakly supported clade of *Paspalum* (three species) that is paraphyletic with *Thrasya* (two species). Clayton and Renvoize (1986, p. 289) observed that the distinction between these latter two genera is “rather arbitrary,” which is consistent with the topology of the tree of Fig. 1.

Non-Kranz species were also found in seven other clades. One of these, *Oplismenus hirtellus*, was resolved as the sister taxon to all other ingroup species, *contra* Giussani et al. (2001). Clayton and Renvoize (1986) previously recognized the plesiomorphic nature of *Oplismenus*. If *Oplismenus hirtellus* is, in fact, the sister group to the Paniceae, then the non-Kranz  $C_3$  condition could be interpreted as plesiomorphic for

TABLE 1. Species sequenced for this study. Taxonomic arrangement follows Clayton and Renvoize (1986). Tribal, subtribal, or subgeneric designations are given parenthetically. Tribe is Paniceae and subtribe is Setariinae unless otherwise indicated.

Species	Voucher: collector, number, institution	DNA accession no.
<i>Arthropogon lanceolatus</i> Filg. (Arthropogoninae)	Filgueiras, 3588, SI	LG-179
<i>Digitaria ciliaris</i> (Retz.) Koeler (Digitariinae)	Morden, 1126, HAW	203 <sup>a</sup>
<i>Digitaria insularis</i> (L.) Fedde (Digitariinae)	Morden, 1091, HAW	110 <sup>a</sup>
<i>Digitaria radicata</i> (J. Presl) Miq. (Digitariinae)	Morden, 1169, HAW	194 <sup>a</sup>
<i>Digitaria setigera</i> Roth ex Roem. & Schult. (Digitariinae)	Morden, 1187, HAW	263 <sup>a</sup>
<i>Digitaria violascens</i> Link (Digitariinae)	Morden, 1183, HAW	259 <sup>a</sup>
<i>Altoparadisium chapadense</i> Filg., Davidse, Zuloaga and Morrone	Filgueiras and Fonseca 2987 (136E), SI	LG-70
<i>Brachiaria mutica</i> (Forssk.) Stapf.	Morden, 1166, HAW	265 <sup>a</sup>
<i>Chaetium bromoides</i> (J.Prensl) Benth. Ex Hemsl.	Clark, s.n., ISC	HC 99-15
<i>Echinochloa colona</i> (L.) Link	Morden, 1172, HAW	197 <sup>a</sup>
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Morden, 1171, HAW	196 <sup>a</sup>
<i>Echinochloa inflexa</i> (Poir.) Chase	Zuloaga, 6943, SI	LG-74
<i>Eriochloa punctata</i> (L.) Desv.	Morden, 1273, HAW	336 <sup>a</sup>
<i>Leptocoryphium lanatum</i> (Kunth) Nees	Zuloaga, 6813, SI	LG-10
<i>Mesosetum</i> sp.	Zuloaga, 6945, SI	LG-119
<i>Oplismenus hirtellus</i> (L.) P. Beauv.	Clark, s.n., ISC	HC 99-2
<i>Otachyrium versicolor</i> (Döll) Henrard	Zuloaga, 7027, SI	LG-126
<i>Panicum elephantipes</i> Nees ex Trin (Subg. <i>Panicum</i> )	Zuloaga, s.n., SI	LG-111
<i>Panicum fauriei</i> Hitch. (Subg. <i>Panicum</i> )	Morden, 1275, HAW	126 <sup>a</sup>
<i>Panicum mystasipum</i> Zuloaga and Morrone (Subg. <i>Panicum</i> )	Filgueiras, 2111, SI	LG-4
<i>Panicum nephelophilum</i> Gaudich. (Subg. <i>Panicum</i> )	Morden, 1544, HAW	1683 <sup>a</sup>
<i>Panicum pedersenii</i> Zuloaga (Subg. <i>Panicum</i> )	Zuloaga, 6926, SI	LG-106
<i>Panicum prionitis</i> Nees (Subg. <i>Agrostoides</i> )	Zuloaga, 6794, SI	LG-138
<i>Panicum repens</i> L. (Subg. <i>Panicum</i> )	PI 356816, Turkey	HC 99-1A
<i>Panicum tenuifolium</i> Hook. and Arn (Subg. <i>Panicum</i> )	Morden, 1333, HAW	567 <sup>a</sup>
<i>Panicum koolauense</i> H. St. John & Hosaka (Subg. <i>Dichantherium</i> )	Morden, 1244, HAW	351 <sup>a</sup>
<i>Panicum ovuliferum</i> Trin. (Subg. <i>Dichantherium</i> )	Zuloaga, 6803, SI	LG-77
<i>Panicum laxum</i> Sw. (Subg. <i>Phanopyrum</i> )	Zuloaga, 6756, SI	LG-29
<i>Panicum millegrana</i> Poir. (Subg. <i>Phanopyrum</i> )	Zuloaga, 6766, SI	LG-148
<i>Paspalidium geminatum</i> (Forssk) Stapf	Zuloaga, 6929, SI	LG-53
<i>Paspalum fimbriatum</i> Kunth	Morden, 1191, HAW	267 <sup>a</sup>
<i>Paspalum quadrifarium</i> Lam.	Morrone, S.N., SI	LG-136
<i>Paspalum scrobiculatum</i> L.	Morden, 1175, HAW	199 <sup>a</sup>
<i>Plagiantha tenella</i> Renvoize	Zuloaga, 6953, SI	LG-22
<i>Pseudechinochloa polystachya</i> (Kunth) Stapf	Zuloaga, 6798, SI	LG-52
<i>Sacciolepis indica</i> (L.) Chase	Morden, 1208, HAW	295 <sup>a</sup>
<i>Setaria gracilis</i> Kunth	Morden, 1156, HAW	181 <sup>a</sup>
<i>Setaria palmifolia</i> (J. König) Stapf	MO, living collection	HC 99-24
<i>Steinchisma hians</i> (Elliott) Nash	Zuloaga, 6773, SI	LG-89
<i>Stenotaphrum secundatum</i> (Walter) Kuntze	Morden, 1193, HAW	271 <sup>a</sup>
<i>Streptostachys ramosa</i> Zuloaga & Soderstr.	Zuloaga, 6960, SI	LG-101
<i>Tataniyx arnaces</i> (Trin.) Zuloaga & Soderstr.	Zuloaga, 6965, SI	LG-152
<i>Thrasya glaziovii</i> A.G. Burm	Filgueiras, 3482, SI	LG-145
<i>Thrasya petrosa</i> (Trin) Chase	Filgueiras, 3496, SI	LG-63
<i>Urochloa acuminata</i> (Renvoize) Morrone & Zuloaga	Zuloaga, s.n., SI	LG-60
<i>Urochloa reptans</i> (L.) Stapf	Morden, 1221, HAW	317 <sup>a</sup>
<i>Melinis minutiflora</i> Beauv. (Melinidinae)	Morden, 1109, HAW	128 <sup>a</sup>
<i>Cenchrus agrimonioides</i> Trin. (Cenchrinae)	Morden, 1554, HAW	1732 <sup>a</sup>
<i>Cenchrus ciliaris</i> L. (Cenchrinae)	Morden, 1179, HAW	203 <sup>a</sup>
<i>Cenchrus echinatus</i> L. (Cenchrinae)	Morden, 1168, HAW	193 <sup>a</sup>
<i>Pennisetum clandestinum</i> Hochst. ex Chiov. (Cenchrinae)	Morden, 1178, HAW	202 <sup>a</sup>
<i>Pennisetum purpureum</i> Schumach. (Cenchrinae)	Morden, 1173, HAW	206 <sup>a</sup>
<i>Pennisetum setaceum</i> (Forssk.) Chiov. (Cenchrinae)	Morden, 1101, HAW	120 <sup>a</sup>
<i>Danthoniopsis dinterii</i> (Pilg.) C. E. Hubb. (Arundinelleae)	PI 207548, Turkey	HC 99-13

<sup>a</sup> DNA extract from the Hawaiian Plant DNA Library, University of Hawaii, Honolulu, Hawaii, USA. All other extracts are from the E. A. Kellogg DNA Library, University of Missouri, St. Louis, Missouri, USA.

the entire tribe. Each of the seven non-Kranz clades appears to have had Kranz ancestors at some point, suggesting multiple losses of the Kranz condition in Paniceae (though of the seven, only the Kranz ancestry of the keeled clade was strongly supported). Such a finding is consistent with the observation by Clayton and Renvoize (1986) that Paniceae have tended to radiate into mesic habitats where selection pressures favoring C<sub>4</sub> metabolism are minimized.

Paradoxically, the non-Kranz *Panicum koolauense* (*Panicum* subgen. *Dichantherium*) was associated with the two representative Andropogoneae (a wholly Kranz taxon) in our analysis, and this clade was embedded in moderately derived Paniceae. Spangler et al. (1999), Gómez-Martínez and Culham (2000), and Giussani et al. (2001) all found a strongly supported sister group relationship between Andropogoneae and the Kranz *Arundinella*, the latter of which was not included in

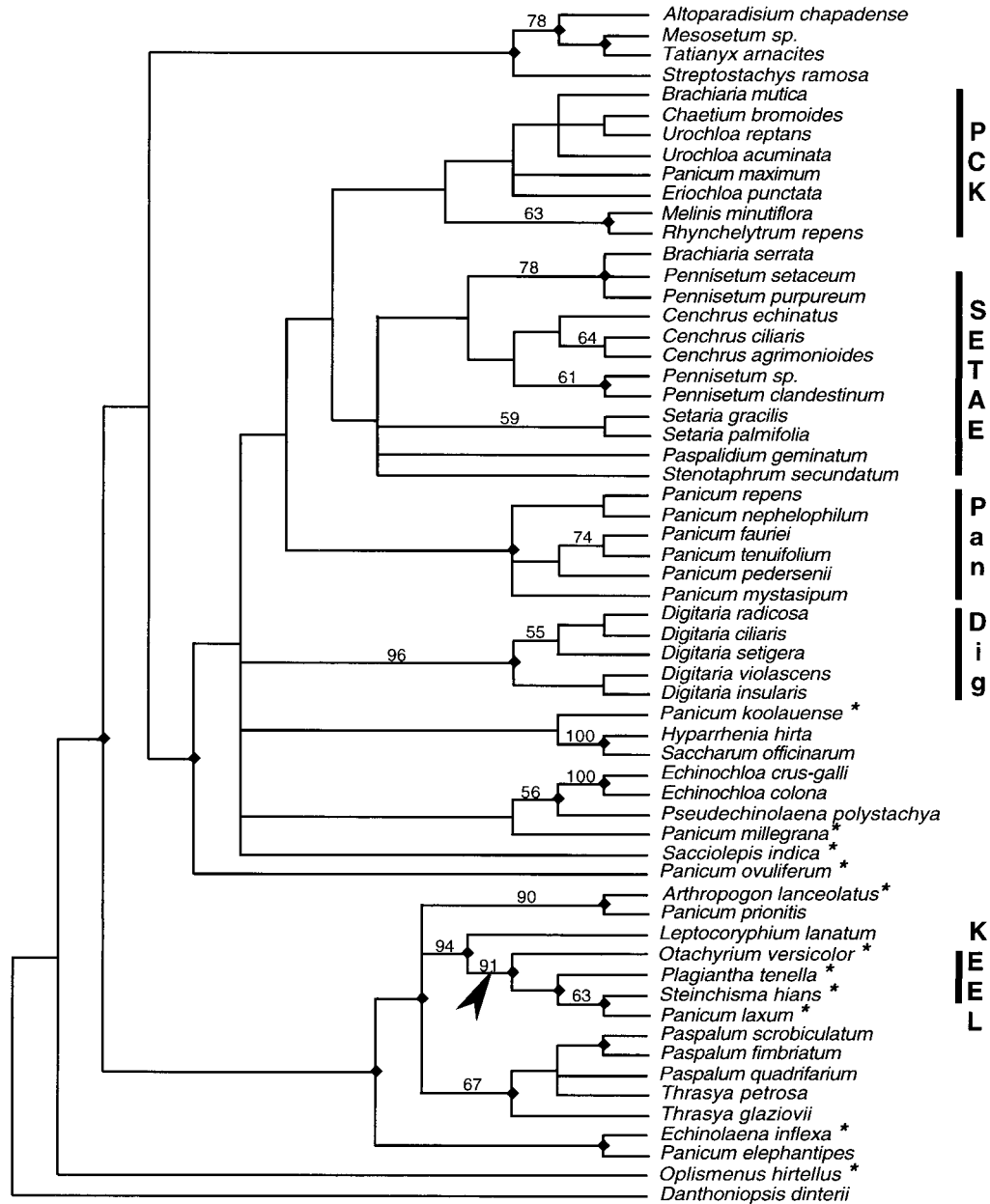


Fig. 1. Majority-rule consensus tree of  $1 \times 10^6$  trees of length 496 steps. Numbers are bootstrap values  $>50\%$ . Black diamonds ( $\blacklozenge$ ) indicate nodes that were also found in the strict consensus of these trees. Asterisks mark non-Kranz species (or in the case of *Steinchisma hians*, a  $C_3$ - $C_4$  intermediate). Arrowhead indicates the strongly supported clade containing species with modified lower paleas and/or that are non-Kranz. Some of the major clades are labelled with the following identifiers. "PCK" = PCK clade; "SETAE" = setae-bearing clade; "Pan" = core *Panicum* clade; "Dig" = *Digitaria* clade; "KEEL" = keeled clade

our study. Giussani et al. (2001) found a strongly supported relationship between *P. koolauense* and *P. sabulorum*, the latter of which species was not included here; however the position of this clade in their analysis was not strongly supported. Our results do suggest that Andropogoneae and Paniceae are paraphyletic; however, the weakly supported relationship between *Panicum koolauense* and Andropogoneae requires further study as it may be an artifact of the sampling in this study.

The five species of *Digitaria* were strongly supported as monophyletic (bootstrap value = 96%) though the relatively distant and well-supported position of *Leptocoryphium* indi-

cated that *Digitariinae*, in which these two genera are traditionally classified, was polyphyletic. Clayton and Renvoize (1986) suggested that *Digitaria* was phylogenetically basal in Paniceae, whereas the genus is considerably more derived in our *rpoC2* phylogeny.

Thirteen species of *Panicum* were included in this analysis. Six of these, *P. fauriei*, *P. mystasipum*, *P. nephelophilum*, *P. pedersenii*, *P. repens*, and *P. tenuifolium*, all subg. *Panicum*, associated together into a very weakly supported clade (bootstrap  $<50\%$ ). The remaining seven species were variously allied with diverse elements of Panicoideae, though again with

weak support. The association of *P. maximum* with *Urochloa/Eriochloa* in the PCK clade and *P. laxum*'s position in the same general clade as *Arthropogon lanceolatus* are noteworthy; both positions were observed by Zuloaga, Morrone, and Giussani (2000) and both relationships were recovered by Giussani et al. (2001). Either *rpoC2* has insufficient phylogenetic information to resolve a monophyletic *Panicum* or any of the subgenera sensu Zuloaga (1986) therein, or *Panicum* is extraordinarily polyphyletic. The correlated morphophysiological synapomorphies of *P. maximum* (see below) and *P. laxum* with other members of their respective clades suggests that *Panicum* has at least some degree of polyphyly (Gómez-Martínez and Culham, 2000; Zuloaga, Morrone, and Giussani, 2000; Giussani et al., 2001).

The *rpoC2* data offered weak support for two other groups identified by Gómez-Martínez and Culham (2000) and Giussani et al. (2001). The "setae-bearing clade" (*Cenchrus*, *Pennisetum*, and *Setaria* together with *Paspalidium*, *Stenotaphrum*, and *Brachiaria serrata* in our analysis) was monophyletic in the majority rule consensus (Fig. 1). Significantly, *Cenchrus* and *Pennisetum*, which Clayton and Renvoize (1986, p. 304) described as "not sharply separated" were paraphyletic with each other within this clade. Also resolved was the PCK clade (*Brachiaria mutica*, *Chaetium bromoides*, *Eriochloa punctata*, *Melinis minutiflora*, *Panicum maximum*, *Rhynchelytrum repens*, and two species of *Urochloa*). Note that *Rhynchelytrum*, which was sister taxon to *Melinis*, is sometimes synonymized with the latter (Dallwitz, Paine, and Zurcher, 1999). The *rpoC2* insert sequence of *Brachiaria serrata*, which clustered neither with its congener (*B. mutica*) nor with other members of the PCK clade, was obtained from a previously published study (Barker, Linder, and Harley, 1999). The unexpected association of this species with *Pennisetum* in the setae-bearing clade needs further study.

A distinctive linear hilum has been observed in two of the genera included here, *Tatianyx* and *Mesosetum* (Sendulsky, Filgueiras, and Burman, 1986). Species of these two genera were clustered together in our analysis albeit with only weak support, suggesting that this character might be synapomorphic for these taxa. The moderately supported association of *Altoparadisium chapadense* to this clade (bootstrap value = 78%) is, to our knowledge, a new indication of relationship and was also recovered in the *ndhF* study of Giussani et al. (2001).

In spite of the somewhat limited phylogenetic information in the *rpoC2* insert sequences, correlations were nonetheless found between clades identified from the phylogenetic analysis of this region and morphology (e.g., the "linear hilum," "keeled," and "setae-bearing" clades), physiology (the PCK clade) as well as with other plastid phylogenies (Gómez-Martínez and Culham, 2000; Giussani et al., 2001). Increased sampling of molecular characters for this large tribe may rearrange or increase support for basal nodes. As previously reported, we found that the gaps in the *rpoC2* insert sequences are a

substantial component of the phylogenetic information that the region has to offer. Our molecular phylogenetic results offer further impetus for a taxonomic reorganization of Paniceae.

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