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A preliminary molecular phylogeny of *Pennisetum* and *Cenchrus* (Poaceae-Paniceae) based on the *trnL-F*, *rpl16* chloroplast markers

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Pennisetum (80–140 species) and *Cenchrus* (16–22 species) are closely related genera of Paniceae, both distributed in tropical and sub-tropical regions. The distinction between *Pennisetum* and *Cenchrus* is not clearly defined, and currently no comprehensive taxonomic treatment is available for either genus. Chloroplast DNA sequence data (*rpl16*, *trnL-F* region) were analyzed to test their monophyly, species relationships, and infrageneric classifications. Phylogenetic analyses of individual genomic regions, and a combined dataset, including indel-coded information, were performed under parsimony and Bayesian inference. Selected geographical, chromosomal and morphological characters were mapped onto the phylogeny to investigate evolutionary trends. Our results support a monophyletic bristle clade and the close relationship between *Pennisetum* and *Cenchrus*. Excluding *Pennisetum lanatum*, *Pennisetum* is paraphyletic because *Cenchrus* is nested within it. Sections *Pennisetum* and *Gymnotrix* are polyphyletic. Our molecular phylogenetic results show a close relationship among the domesticated species *P. glaucum*, *P. purpureum*, *P. squamulatum*, *P. nervosum*, and *P. sieberianum*, suggesting the potential use of these species in crop improvement. The *Pennisetum-Cenchrus* clade shows an independent reduction ($x = 5, 7, 8$) or duplication ($x = 17$) of the basic chromosome number from the ancestral $x = 9$. American *Pennisetum* and *Cenchrus* species appear to have originated from Old World species more than once. A core group of American species of *Cenchrus* with a basic chromosome number of $x = 17$ and retrorsely barbed bristles could be restricted to *Cenchrus* s.str.

KEYWORDS: bristle clade, *Cenchrus*, molecular phylogeny, *Pennisetum*, *rpl16*, *trnL-F*

INTRODUCTION

Pennisetum Rich. and *Cenchrus* L. are grasses belonging to the tribe Paniceae of the subfamily Panicoideae (Clayton & Renvoize, 1986). *Pennisetum* includes 80–140 species from the tropics and subtropics of both hemispheres (Brunken, 1977; Clayton & Renvoize, 1986) and occupies a wide range of habitats: savannas, woodlands and woody places in warmer regions of America, Africa and Asia; the genus is particularly diverse in Africa (Türpe, 1983; Clayton & Renvoize, 1986).

The genus is economically important since many of its species are cultivated or used for different purposes. *Pennisetum glaucum*, commonly known as "pearl millet", is the sixth-most important cereal crop in the world (Clayton & Renvoize, 1986; Crins, 1991). *Pennisetum purpureum* ("elephant grass"), and *Pennisetum clandestinum* ("kikuyu grass") are grown for pasture (Türpe, 1983; Clayton & Renvoize, 1986) and widely used for soil stabilization and pasture improvement (Webster, 1988). *Pennisetum setaceum* ("tender fountain grass"), *Pennisetum villosum* ("feathertop") and *Pennisetum alopecuroides* ("foxtail fountain grass"), among others, are cultivated as ornamentals for their striking, often purplish inflorescences (Crins, 1991; Rúgolo de Agrasar & Puglia, 2004).

No comprehensive taxonomic treatment is available for *Pennisetum* other than partial or regional floristic studies, which mostly differ in the circumscription of infrageneric taxa. The most recent treatment by Clayton & Renvoize (1986) divides the genus into five sections: *Dactylophora*, *Brevivalvula*, *Heterostachya*, *Pennisetum* and *Gymnotrix* (subsections: *Acrostigma*, *Pleurostigma*, *Beckeropsis*). However, there are some discrepancies in the infrageneric classification among different authors (Table 1). For example, *P.* sect. *Beckeropsis* has been considered as such (Roshevits, 1937), or as a subsection of *Gymnotrix* (Pilger, 1940; Clayton & Renvoize, 1986) or, treated as a different genus (Stapf & Hubbard, 1934; Chippindall, 1955), restricted to *P. unisetum*. *Pennisetum* sect. *Beckeropsis* is characterized by the presence of a single bristle subtending each spikelet and branched inflorescences.

Harlan & de Wet (1971) proposed a gene pool classification for cultivated plants, based on the ease with which

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a crop could be introgressed with itself and with wild crop relatives. Martel & al. (2004) applied this gene pool classification to *Pennisetum*, based on 16 species and results from a molecular phylogeny using the Internal Transcribed Spacer (ITS) of the ribosomal RNA locus. They placed the domesticated *Pennisetum glaucum* (2*x*, *x* = 7) and its wild relatives (*P. glaucum* subsp. *monodii* f. *mollissimum*, and f. *violaceum*) in the primary gene pool (GP-1); the secondary gene pool (GP-2) is represented by *P. purpureum* (4*x*, *x* = 7), and the tertiary gene pool (GP-3) includes the remaining species of *Pennisetum*.

Pennisetum shows differences in genome size (Martel & al., 1997), ploidy level (from diploid to octoploid), and basic chromosome numbers (*x* = 5, 7, 8, 9) (Jauhar, 1981; Wipff, 2003). Martel & al. (2004) pointed out that chromosome numbers based on *x* = 5, 7, or 8 could have occurred via structural mutations and loss of centromeres from an ancestral basic number *x* = 9 during evolution.

The genus *Cenchrus* consists of 16–22 species that inhabit bushlands, open grasslands, disturbed areas, and sandy soils in warmer regions of both hemispheres (DeLisle, 1963; Clayton & Renvoize, 1986; Webster, 1988; Crins, 1991; Stieber & Wipff, 2003). Most of the species are considered undesirable and noxious weeds such as *C. echinatus* L., but some, such as *C. ciliaris*, are important forage grasses (Correll & Johnston, 1970; Clayton & Renvoize, 1986; Stieber & Wipff, 2003). Species of *Cenchrus* mainly distributed in Africa and Asia are *x* = 9, while species with *x* = 17 are exclusively from the New World (DeLisle, 1963; Crins, 1991).

Pennisetum and *Cenchrus* are closely related genera (Türpe, 1983; Clayton & Renvoize, 1986; Crins, 1991), characterized by the presence of a highly modified inflorescence, with contracted and spiciform panicles, whose spikelets are subtended by sterile bristles that fall together with the spikelets at maturity (Clayton & Renvoize, 1986). Additional characters of inflorescence development support the relationship between the genera: the reduction of the internode on the secondary axis and on other axes, differential elongation of the bristles at maturity, and a change from equal numbers to more bristles than spikelets being initiated in early development (Doust & Kellogg, 2002). The two genera differ in the morphology of the involucral bristles; bristles in *Cenchrus* always show some degree of fusion at the base while in *Pennisetum* they are usually free (Hitchcock, 1951; DeLisle, 1963; Gould, 1975; Clayton & Renvoize, 1986; Crins, 1991). Moreover, the involucral bristles in *Cenchrus* are generally flat, stiff, spiny and retrorsely barbed while *Pennisetum* has filiform, and antrorsely scabrous bristles (DeLisle, 1963; Clayton & Renvoize, 1986; Crins, 1991; Wipff, 2001, 2003). Nevertheless *Pennisetum* and *Cenchrus* are not clearly defined (Türpe, 1983; Crins 1991; Wipff, 2001, 2003) due to variability in the basal fusion of the bristles (Hitchcock, 1951; DeLisle, 1963; Gould, 1975; Clayton & Renvoize, 1986), in the shape and stiffness of the bristles (Chippindall, 1955), in the basic chromosome number, and in the spiny nature and type of scabrosity of bristles (Wipff, 2003). As a result, some authors (e.g., Correll & Johnston, 1970) have treated *Pennisetum* as part of *Cenchrus*.

Previous molecular phylogenetic studies (Gómez-Martínez & Culham, 2000; Giussani & al., 2001; Doust & Kellogg, 2002; Doust & al., 2007) showed *Cenchrus* and *Pennisetum* in a well-supported monophyletic group within a monophyletic 'bristle clade'. The latter

Table 1. Summary of different infrageneric taxonomic treatments of *Pennisetum*.

Stapf & Hubbard (1934)	**Roshevits (1937)**	**Pilger (1940)**	**Chippindall (1955)**	**Brunken (1977)**	**Clayton & Renvoize (1986)**
Pennisetum	***Pennisetum***	***Pennisetum***	***Pennisetum***	***Pennisetum***	***Pennisetum***
		Subg. *Dactylophora*			Sect. *Dactylophora*
Sect. *Brevivalvula*	Sect. *Brevivalvula*	Subg. *Eriochaeta*		Sect. *Brevivalvula*	Sect. *Brevivalvula*
Sect. *Heterostachya*	Sect. *Heterostachya*			Sect. *Heterostachya*	Sect. *Heterostachya*
Sect. *Pennisetum*	Sect. *Pennisetum*	Subg. *Pennisetum*		Sect. *Pennisetum*	Sect. *Pennisetum*
Sect. *Penicillaria*	Sect. *Penicillaria*	Sect. *Penicillaria*			
Sect. *Gymnotrix*	Sect. *Gymnotrix*	Sect. *Gymnotrix*		Sect. *Gymnotrix*	Sect. *Gymnotrix*
Subsect. *Acrostigma*		Subsect. *Acrostigma*			Subsect. *Acrostigma*
Subsect. *Pleurostigma*		Subsect. *Pleurostigma*			Subsect. *Pleurostigma*
Beckeropsis	Sect. *Beckeropsis*	Subsect. *Beckeropsis*	***Beckeropsis***		Subsect. *Beckeropsis*

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includes all Paniceae with a chromosome base number of $x = 9$, having the NADP-me subtype of C_4 photosynthesis, and bristles in their inflorescences. Other genera in the clade include *Setaria* P. Beauv., *Ixophorus* Schltdl., *Stenotaphrum* Trin., *Paspalidium* Stapf, and *Pseudoraphis* Griff. (Gómez-Martínez & Culham, 2000; Zuloaga & al., 2000; Duvall & al.; 2001; Giussani & al., 2001; Doust & Kellogg, 2002; Doust & al., 2007). These molecular studies (Gómez-Martínez & Culham, 2000; Giussani & al., 2001), although based on a small number of species, showed a paraphyletic *Pennisetum* forming a monophyletic clade with *Cenchrus*. Subsequent studies, using chloroplast markers (*ndhF*, *trnL-trnF*; Doust & Kellogg, 2002), or chloroplast plus nuclear markers (*ndhF*, *Knotted1*; Doust & al., 2007), also found *Cenchrus* to be monophyletic and embedded within *Pennisetum*. These authors suggested the need to add more taxa and additional molecular markers to confirm their findings.

Our aim here is to clarify the phylogenetic relationships of *Pennisetum* and *Cenchrus* based on two non-coding chloroplast DNA markers (*rpl16*, *trnL-trnF* intergenic spacer), using a larger taxonomic sampling, and considering cytological and morphological variability of both genera. We test the monophyly of *Pennisetum* and *Cenchrus* and discuss diagnostic morphological characters, basic chromosome numbers, and geographic origin of major clades based on the most parsimonious hypotheses.

MATERIALS AND METHODS

Taxonomic sampling. — Seven species of *Cenchrus* and twenty species of *Pennisetum* were included as ingroup taxa. Sampling represents 31.8% and 25% of the total number of species considered by Clayton & Renvoize (1986) respectively and includes the variation in basic chromosome number, the genetic classification (Harlan & de Wet, 1971), and taxonomic sections of *Pennisetum* according to the most recent treatment (Clayton & Renvoize, 1986) (Table 1). Eleven representatives of the subfamily Panicoideae were selected as outgroup taxa following the results from previous phylogenetic studies (Gómez-Martínez & Culham, 2000; Zuloaga & al., 2000; Giussani & al., 2001; Doust & Kellogg, 2002; Doust & al., 2007) (Appendix 1). Of these, six species correspond to the bristle clade (*Ixophorus unisetus*, *Paspalidium geminatum*, *Pseudoraphis paradoxa*, *Setaria lachnea*, *Setaria sulcata*, *Stenotaphrum secundatum*); three species to the Paniceae $x = 9$ clade (*Panicum repens*, *Urochloa panicoides*, and *Sacciolepis vilvoides*); one species to the Andropogoneae $x = 10$ clade (*Zea mays*), and one species to the subfamily Centothecoideae (*Chasmanthium latifolium*). In order to check the phylogenetic position of the species, we sequenced, when available, two accessions per species, e.g., *Pennisetum montanum*, *P. purpureum* and *P. villosum; P. setaceum* (*Giussani s.n.*) was only amplified for the *trnL-F* region. All species and voucher specimens are listed in Appendix 1.

Plant material, DNA extraction, PCR amplification, and sequencing. — DNA extractions were performed mainly from plants collected in the field and dried in silica gel. A few species were amplified from herbarium specimens, or from seedlings (seeds were provided by several genetic resources centers: National Seed Laboratory, Plant Gene Resources of Canada, and U.S. Department of Agriculture). Total DNA was extracted from leaf tissue using a CTAB protocol modified for small amounts of plant material (Doyle & Doyle, 1987; Murray & Thompson, 1980; Saghai-Maroof & al., 1984). DNeasy Plant Mini Kits (Qiagen, Hilden, Germany) were used to extract DNA from herbarium material.

Two non-coding chloroplast DNA markers were amplified via the polymerase chain reaction (PCR): the intron and partial exon 2 of the gene encoding *rpl16*, and the *trnL-F* region, including *trnL* (UAA) intron, *trnL*(UAA) partial 3′exon, and the intergenic spacer between the *trnL*(UAA) 3′exon and the *trnF*(GAA) gene (*trnL-F* region). The *rpl16* intron was amplified using primers F71, 5′-GCTATGCT TAGTGTGTGTCTC-3′ and R1661, 5′-CCAKATTTTTC CACCACGAC-3′ as in Jordan & al. (1996) and Kelchner & Clark (1997), respectively. For difficult taxa the marker was amplified in smaller fragments using the following combination of primers: F71/R584 (R584: 5′-TTCCGC CATCCCACCCAATGAA-3′) and F584/R1661 (F584: 5′-TTCATTGGGTGGGATGGCGAA-3′). For sequencing reactions, two additional internal primers were used: F80, 5′-C/TTATTGCTTCGTATTGTCG-3′, and R270, 5′-TCATCCCTCCTTTTATCC-3′ as in Giussani & al. (2009) and Zhang (2000), respectively. The *trnL-F* region was amplified by using primers C, D, E and F as described in Taberlet & al. (1991); for difficult taxa in which primers C and F failed to amplify, two other primers were used Cii, 5′-TAGACGCTACGGACTTGATTG-3′ and Fdw, 5′-CAGTCCTCTGCTCTACCAGC-3′ (Giussani & al., 2009).

PCR amplifications were performed in a 25 ml reaction volume containing 20–40 ng, a final concentration of 1× PCR Buffer minus Mg, 5 mM $MgCl_2$, 0.025 mM dNTP each, 0.2 μM each primer, 0.2% BSA as a PCR additive, and 1.25–3 units *Taq* Polymerase (Invitrogen Life Technologies, São Paulo, Brazil). PCR was carried out in a thermal cycler (Eppendorf Mastercycler personal) using the following parameters: *trnL-F*: 1 cycle of 94°C for 5 min, 34 cycles of 94°C for 30 s, 48°C for 1 min, and 72°C for 1 min 30 s, and a final extension cycle of 72°C for 7 min; *rpl16*: 1 cycle of 94°C for 4 min, 34 cycles of

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94°C for 1 min, 55°C for 1 min, and 72°C for 2 min 30 s, and a final extension cycle of 72°C for 7 min. The PCR products were quantified by comparison to a low mass (20–30 ng/ml) DNA ladder on 1% agarose electrophoresis gel using 1× TBE buffer and stained with ethidium bromide.

PCR products were sent to Macrogen, Inc. (Seoul, Korea), where they were cleaned using a Montage PCR purification kit from Millipore following the manufacturer's protocol, and sequenced using the ABI PRISM BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using selected primers to complete a bi-directional contig of the full sequence. Assembly and editing of DNA sequences was conducted using the program ChromasPro version 1.34 (Technelysium Pty, Ltd, Tewantin, Australia). Sequences were aligned using ClustalW (Thompson & al., 1994) followed by manual editing using BioEdit version 7.0.5.3 (Hall, 1999).

All sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov); and GenBank accession numbers are provided in Appendix 1. Alignments and phylogenetic trees were submitted to TreeBASE: study accession number: S2140).

Phylogenetic analyses. — Individual genomic regions (*trnL-F*, *rpl16*), and a combined dataset using both markers, were analyzed under parsimony using TNT version 1.1 (Goloboff & al., 2000), and under Bayesian inference (BI) using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001).

For parsimony analyses, uninformative characters were removed prior to all analyses, and informative characters were considered unordered and equally weighted. Gaps were treated as missing data. Indels were coded as binary (present/absent) characters following the "simple indel coding" method (Simmons & Ochoterena, 2000), and added to the matrix. Analyses were run using sequence aligned matrices (SAM), and sequence + indel coded matrices (ICM). Parsimony analyses of each dataset consisted of a series of heuristic searches using 1,000 random addition sequences (RAS), swapping the initial tree with bisection-reconnection (TBR), and saving five trees per replicate. Then, all optimal trees were branch-swapped by TBR until completion. Branch length, consistency and retention indexes (CI/RI), and the strict consensus of all most parsimonious trees were calculated. Jackknife support (Farris & al., 1996) and Bremer support (Bremer, 1994) were used to estimate support for individual nodes. Jackknife (JK) analyses were conducted using 1,000 replicates starting each replica with a single Wagner tree, and TBR swapped holding a single optimal tree; resampling probability was set to 36. Bremer support (BS) was calculated to find sub-optimal trees of 1, 2, 3 up to 10 steps longer than the shortest trees, saving a maximum of 65,000 suboptimal trees per step.

Modeltest version 3.7 (Posada & Crandall, 1998) was used to determine models of DNA sequence evolution used in BI analyses for each partition separately: *rpl16* intron, *trnL* intron and *trnL-trnF* intergenic spacer. The nucleotide substitution model was selected using the Akaike information criterion (AIC; Akaike, 1974). The TVM + I + G model (Rodriguez & al., 1990) was selected for the *rpl16* intron, the K81uf + G model (Kimura, 1981) for the *trnL* intron, and the K81uf + I model (Kimura, 1981) for the *trnL-F* intergenic spacer. The analyses were performed for the *rpl16* and *trnL-F* regions separately and combined, considering the selected evolutionary model for each partition as previously stated. Bayesian phylogenetic analyses were conducted by Markov chain Monte Carlo (MCMC) with four chains over one million generations, sampling every 100 generations. All trees obtained before stationary generations were discarded as burn-in trees and BI posterior probabilities (PP) of clades were calculated.

Optimization of non-molecular characters. — Fifteen non-molecular characters were coded and unambiguously optimized onto the molecular phylogeny obtained under parsimony. These characters were mapped onto the molecular phylogeny to infer evolutionary trends and to determine the taxonomic value of diagnostic features. Exomorphological data were obtained from the examination of herbarium material (Appendix 3 in the online versionof this article), and some anatomical and morphological data (Tsvelev, 1983), geographical origin (Türpe, 1983; Stieber & Wipff, 2003; Wipff, 2003), and basic chromosome numbers (DeLisle, 1963; Martel & al., 1997; Yukio & al., 2006; Indexes to Plant Chromosome Numbers [IPCN], http://mobot.mobot.org/W3T/Search/ipcn.html), were also compiled. Character list and codification is shown in Appendix 2 while the non-molecular data matrix is shown in Table 2.

RESULTS

Sequences. — *trnL-F region.* – Forty-one sequences were obtained for the *trnL-F* region, including three sequences from GenBank (Appendix 1). Sequences ranged from 803 (*Pennisetum montanum*, *Morrone 4931*) to 955 bp (*Chasmanthium latifolium*). *Pennisetum villosum* shows the longest sequence (883 bp) among the ingroup taxa.

The aligned data matrix consists of 1,028 bp, of which 51 (4.96%) sites are parsimony informative. The *trnL* intron has an aligned length of 576 bp, while the *trnL-trnF* intergenic spacer has 402 bp. In addition, 40 gaps were

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Table 2. Data matrix of non-molecular characters used to optimize onto the molecular trees.

	Non-molecular characters[a]														
Taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Cenchrus brownii	2	6	1	1	0	1	2	0	1	1	1	0	0	1	0
Cenchrus ciliaris	01	2	1	1	0	1	1	0	1	1	0	0	0	1	0
Cenchrus echinatus	2	6	1	1	0	1	2	0	1	1	1	0	0	1	0
Cenchrus incertus	2	6	1	1	0	1	2	0	1	1	1	0	0	1	1
Cenchrus myosuroides	2	2	1	1	1	1	1	0	0	1	1	0	0	1	1
Cenchrus pilosus	2	6	1	1	0	1	2	0	1	1	0	0	0	1	0
Cenchrus setigerus	01	2	1	1	0	1	1	0	1	1	0	0	0	1	0
Chasmanthium latifolium	2	0	0	0	-	0	-	-	-	-	-	0	-	1	-
Ixophorus unisetus	2	2	1	0	1	1	0	0	-	-	-	0	0	1	-
Panicum repens	0	2	0	0	-	0	-	-	-	-	-	0	1	1	-
Paspalidium geminatum	2	2	1	0	1	1	0	0	-	-	0	0	0	1	-
Pennisetum alopecuroides	1	2	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum chilense	2	-	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum clandestinum	0	2	1	1	01	1	0	0	0	0	0	0	0	1	0
Pennisetum flaccidum	1	2	1	1	0	1	0	0	0	0	0	0	0	1	0
Pennisetum frutescens	2	-	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum glaucum	1	4	1	1	0	1	0	0	0	0	0	0	0	0	0
Pennisetum lanatum	1	2	1	1	0	1	0	1	-	-	0	0	1	1	-
Pennisetum latifolium	2	2	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum macrourum	0	2	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum montanum	2	3	1	1	1	1	0	0	-	-	0	0	0	1	-
Pennisetum nervosum	2	2	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum polystachion	0	2	1	1	0	1	0	0	0	0	0	0	1	1	0
Pennisetum purpureum	0	4	1	1	0	1	0	0	0	0	0	0	0	0	0
Pennisetum ramosum	0	5	1	1	1	1	1	0	1	1	0	0	0	1	0
Pennisetum setaceum	0	2	1	1	0	1	0	0	0	0	0	0	0	1	0
Pennisetum sieberianum	1	-	1	1	0	1	0	0	0	0	0	0	0	0	0
Pennisetum squamulatum	0	4	1	1	0	1	0	0	0	0	0	1	0	1	0
Pennisetum tristachyon	2	-	1	1	1	1	0	0	0	0	0	0	0	1	0
Pennisetum unisetum	0	-	1	1	1	1	0	0	-	-	0	0	0	1	-
Pennisetum villosum	0	2	1	1	0	1	0	0	0	0	0	0	0	1	0
Pseudoraphis paradoxa	1	2	1	1	1	1	0	0	-	-	0	0	1	1	-
Sacciolepis vilvoides	2	2	0	0	-	0	-	-	-	-	-	0	1	1	-
Setaria lachnea	2	2	1	0	1	1	0	0	-	-	0	0	0	1	-
Setaria sulcata	0	2	1	0	1	1	0	0	-	-	0	0	0	1	-
Stenotaphrum secundatum	2	2	1	0	1	1	0	0	-	-	0	0	0	1	-
Urochloa panicoides	0	2	0	0	-	0	-	-	-	-	-	0	0	1	-
Zea mays	2	1	0	0	-	0	-	-	-	-	-	0	0	1	-

[a]Characters are described in Appendix 2.
More than one number in a cell indicates a polymorphic character state; - indicates inapplicable character state.

coded, of which 19 correspond to the intron and 21 to the intergenic spacer. Fifteen gaps (37.5%) out of forty were phylogenetically informative (Table 3; Table 4 in the online version of this article).

rpl16 intron. – A total of 40 sequences were obtained for the *rpl16*, including one sequence from GenBank; only *Cenchrus setigerus* failed to amplify (Appendix 1). The *rpl16* sequence length varied from 1,028 bp in *Pennisetum unisetum* to 1,228 bp in *Sacciolepis vilvoides*, with *P. latifolium* having the longest sequence within the ingroup (1,106 bp). The *rpl16* aligned data matrix consists of 1,367 bp, and 56 bp (4.1%) are phylogenetically informative. A total of 55 gaps were coded, of which 16 (29.09%) are informative (Table 3; Table 5 in the online version of this article).

Sequence attributes for the combined datasets, excluding and including gaps, is presented in Table 3.

Phylogenetic analyses. — All datasets were analyzed separately and combined, and both parsimony and Bayesian Inference yielded almost the same results. Hence, results from parsimony will be reported together with support values and posterior probabilities of major clades.

***trnL-F* analyses. —** The parsimony analysis of the *trnL-F* region yielded 56 most parsimonious trees (L = 89 steps, CI = 0.66, RI = 0.86; uninformative characters not included). The consensus tree (not shown) supported monophyly of the Paniceae $x = 9$ clade (JK = 70; BS = 2; PP = 0.66), and the bristle clade (JK = 98; BS = 5; PP = 1.00). All species of *Pennisetum* except *P. lanatum* fell within a strongly supported *Pennisetum-Cenchrus* clade (JK = 94; BS = 5; PP = 1.00). *Pennisetum* is paraphyletic because all species of *Cenchrus* are nested within the genus. In the strict consensus tree, *Pennisetum-Cenchrus* showed a basal polytomy formed by seven species of *Pennisetum* plus two major clades: clade A (BS = 1; PP = 0.86) composed by *P. flaccidum*, *P. frutescens*, *P. polystachion*, *P. purpureum* (*Morrone 4473*), *P. ramosum*, *P. setaceum*, and all species of *Cenchrus*; and clade B (JK = 71; BS = 1; PP = 1.00) which includes *P. glaucum*, *P. nervosum*, *P. purpureum* (*Morrone 4220*), *P. sieberianum*, and *P. squamulatum*. Within clade A, all *Cenchrus* species are closely related in clade A2 (JK = 52; BS = 1; PP = 0.98) but failed to form a monophyletic group since this clade also includes *P. purpureum* and *P. setaceum*. However, a sequence of *Pennisetum setaceum* (*Giussani s.n.*) and a sequence of *P. purpureum* (*Morrone 4220*) were related to the basal polytomy and to clade B, respectively (not shown); hence the relationship of these two species with *Cenchrus* is not confirmed.

When including indel information, analysis of the *trnL-F* dataset resulted in eight most parsimonious trees (L = 111, CI = 0.66, RI = 0.86), with a similar consensus topology to that from sequence data alone (not shown). However, support values increased for most clades, e.g.,

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Table 3. Attributes of both partitions and combined molecular data matrices.

			SAM			ICM			SAM + ICM			
Genomic regions	**Seq**	**Length (bp)**	**TC**	**PIC**	**%**	**TC**	**PIC**	**%**	**TC**	**PIC**	**%SAM**	**%ICM**
trnL-F	41	803–955	1,028	51	4.96	40	15	37.50	1,068	66	77.27	22.73
rpl16	40	1,028–1,228	1,367	56	4.10	55	16	29.09	1,422	72	77.80	22.20
trnL-F + rpl16	41	–	2,395	107	4.47	95	31	32.63	2,490	138	77.54	22.46

The data include the number of sequences analyzed (Seq), and the length of non-aligned sequences (Length). For the sequence aligned matrix (SAM), and the indels coded matrix (ICM), the total number of characters (TC), and the number and percentage of parsimony informative characters (PIC and %, respectively) are presented. %SAM and %ICM refer to the percentage of informative characters obtained from the SAM and ICM, respectively.

clade A (JK = 78; BS = 3) and clade A2 (JK = 59; BS = 1). Also a new clade A3 (JK = 62; BS = 1), including *P. purpureum* (*Morrone 4473*), *C. ciliaris*, and *C. setigerus* was recovered; these species share a 2-bp gap in the *trnL* intron (character 18; Table 4 in the online version of this article).

***rpl16* analyses.** — Independent parsimony analysis of the *rpl16* dataset yielded 75 most parsimonious trees (L = 101, CI = 0.65, RI = 0.80). The consensus tree shows a well-supported Paniceae *x* = 9 (JK = 98; BS = 7; PP = 1.00) but low support for the bristle and *Pennisetum-Cenchrus* clades (BS = 1; PP = 0.78) (not shown). However, this partition is useful for resolving some infrageneric relationships within *Pennisetum* and *Cenchrus*. The strict consensus tree shows two similar clades to the ones previously found by the *trnL-F* dataset: clade A (excluding *C. setigerus* because it failed to be amplified for this marker) (BS = 1; PP = 0.73), and clade B (JK = 86; BS = 3; PP = 1.00) sister to *P. alopecuroides* (BS = 1; PP = 0.84). Additionally, two other well-supported clades are found in *Pennisetum*: clade C with *P. chilense*, *P. latifolium*, *P. tristachyon*, and both specimens of *P. montanum* (JK = 52; BS = 1; PP = 1.00), and clade D that includes *P. clandestinum* and *P. macrourum* (JK = 83; BS = 3; PP = 1.00). When including indels, the analysis resulted in 2,535 most parsimonious trees (L = 129, CI = 0.63, RI = 0.78). The consensus tree was poorly resolved, not recovering the bristle clade, *Pennisetum-Cenchrus*, clade A, and the sister relationship between *P. alopecuroides* and clade B. However clades B, C and D and all other minor clades (like A1 with all taxa defined for clade A except for *P. flaccidum*) were recovered with slightly improved support (not shown).

Combined *trnL-F* + *rpl16* analyses. — Some major and minor clades are common between both partition consensus trees (*trnL-F,rpl16*) when analyzed separately, although support for most clades is low to moderate. Analyses of both markers, whether including or excluding indels, offered no contradictory information, so we assumed partitions to be congruent and hence combined both datasets.

The parsimony search yielded 15 most parsimonious trees (L = 192, CI = 0.65, RI = 0.83; not shown). In this analysis, we found all clades previously defined in either the *trnL-F* or the *rpl16* partitions, but support values and posterior probabilities were considerably increased in almost all cases.

The addition of indel information resulted in 93 trees (L = 241, CI = 0.64, RI = 0.82). These were congruent with those produced by all individual analyses, but the strict consensus was better resolved and more strongly supported (Fig. 1). As a consequence the remainder of this paper will rely on results of the combined datasets with indels included. Posterior probabilities values reported from now on, and in Fig. 1, correspond to the BI analysis of the combined dataset although excluding the indel coded information.

The highly supported bristle clade (JK = 99; BS = 9; PP = 1.00) is correlated with a Kranz MS leaf anatomy and the presence of sterile bristles in their inflorescences (characters 3 and 6; Fig. 2). The monophyletic *Pennisetum-Cenchrus* clade (JK = 97, BS = 7; PP = 1.00) is supported by seven molecular synapomorphies, five of them corresponding to the *trnL-F* dataset while the other two synapomorphies are from the *rpl16* dataset. This clade is characterized by the same type of inflorescence articulation, shared with *P. lanatum* and *Pseudoraphis paradoxa* (character 4; Fig. 2). As previously shown by the *trnL-F* partitioned analysis, *Pennisetum lanatum* is the only ingroup species that lies phylogenetically outside the *Pennisetum-Cenchrus* clade. This species is differentiated by branched bristles (character 8; Fig. 2). When *P. lanatum* is not considered as part of *Pennisetum*, *Pennisetum* is paraphyletic because it includes all species of *Cenchrus*. Basal phylogenetic relationships in the *Pennisetum-Cenchrus* clade are poorly supported, with only weak evidence that clade D+*P. villosum* (JK = 53; BS = 1; PP < 0.5) is the sister clade to all other species of *Pennisetum* and *Cenchrus*. In addition, the combined dataset recovers highly supported clades such as A, A1, A2, A5, A6, B, C and D (Fig. 1), previously found in the partitioned analyses.

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The position of *P. montanum* and *P. villosum* was confirmed by two different vouchers (Appendix 1). However, one specimen of *P. purpureum* falls in clade A2 and the other in clade B; therefore, its phylogenetic placement is contradictory.

Some of the characters used to differentiate *Pennisetum* and *Cenchrus* are shown as homoplasious. Fusion of bristles is present in all species of *Cenchrus* as well as in *P. ramosum*. Characters such as the shape and stiffness of the internal bristles (characters 9 and 10; Fig. 2) showed more than one independent origin. On the other hand, the presence of retrorsely barbed bristles (character 11; Fig. 2) is a derived state of clades A5 (*C. incertus*, *C. myosuroides*) and A6 (*C. brownii*, *C. echinatus*, *C. pilosus*), with the exception of *C. pilosus*. Clade A5 is also characterized by the presence of stiff external bristles (character 15; Fig. 2).

Geographic origin of species. — According to this phylogeny, groups of species with the same geographical distribution seem to have originated independently. Three geographic groups were observed: clade D + *P. villosum*

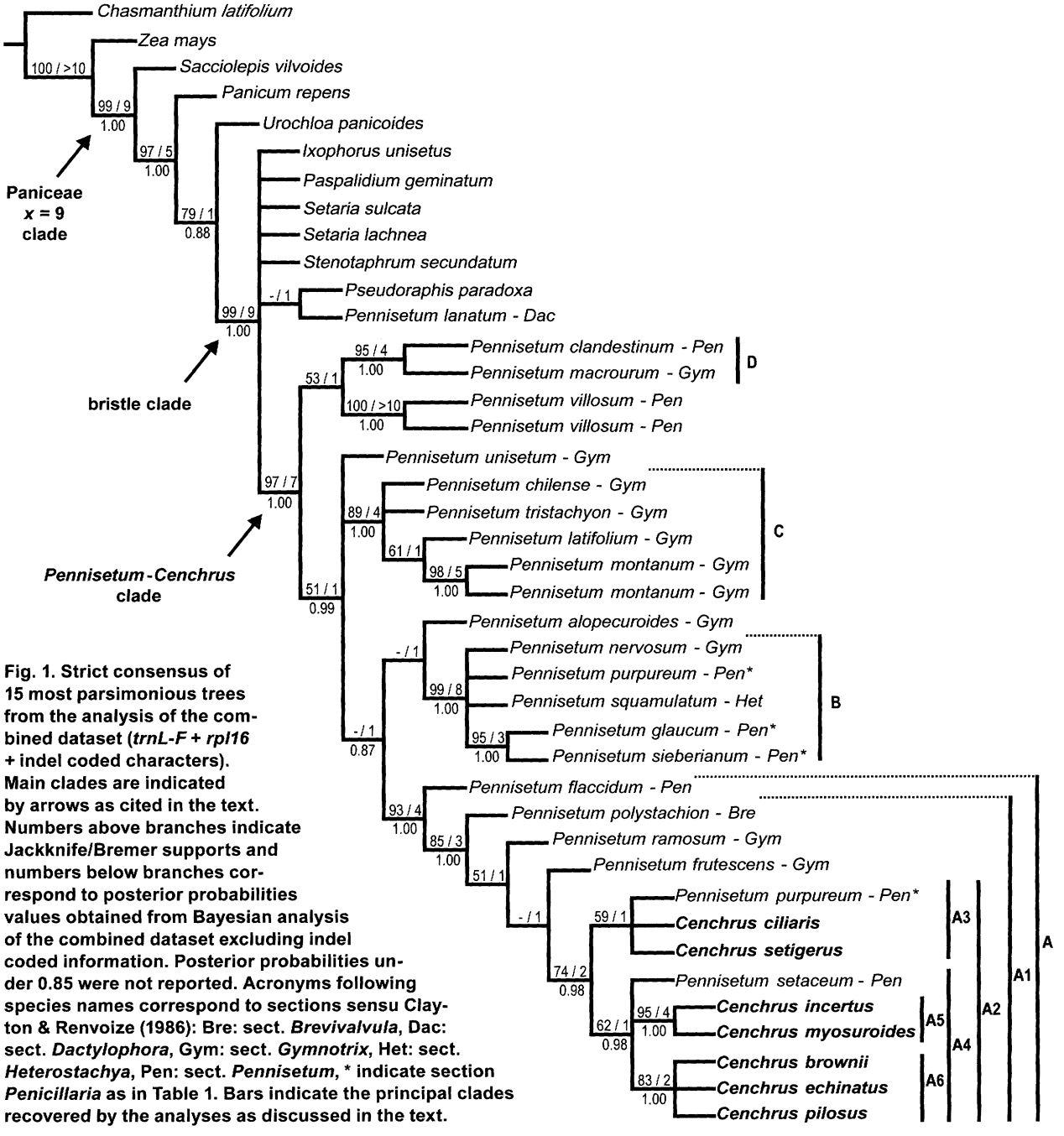


Fig. 1. Strict consensus of 15 most parsimonious trees from the analysis of the combined dataset (*trnL-F* + *rpl16* + indel coded characters). Main clades are indicated by arrows as cited in the text. Numbers above branches indicate Jackknife/Bremer supports and numbers below branches correspond to posterior probabilities values obtained from Bayesian analysis of the combined dataset excluding indel coded information. Posterior probabilities under 0.85 were not reported. Acronyms following species names correspond to sections sensu Clayton & Renvoize (1986): Bre: sect. *Brevivalvula*, Dac: sect. *Dactylophora*, Gym: sect. *Gymnotrix*, Het: sect. *Heterostachya*, Pen: sect. *Pennisetum*, * indicate section *Penicillaria* as in Table 1. Bars indicate the principal clades recovered by the analyses as discussed in the text.

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includes species native to Africa; clade C is formed only by South-American species, while clades A5 + A6 (*Cenchrus* s.str.) share an American origin, being distributed from southern U.S. to South America (character 1; Fig. 2).

Basic chromosome numbers. — The ancestral condition for the bristle clade is a basic chromosome number of $x = 9$. Several species of *Pennisetum* with $x = 5$ and 8 could have originated independently as derivative states, only once during the evolution of the *Pennisetum-Cenchrus* clade (character 2; Fig. 2); while there could be up to two changes to $x = 7$, assuming the position of both specimens of *P. purpureum*. The basic chromosome number is ambiguous for clade A4 because the basal relationships of the species having $x = 9$ or 17 are not resolved.

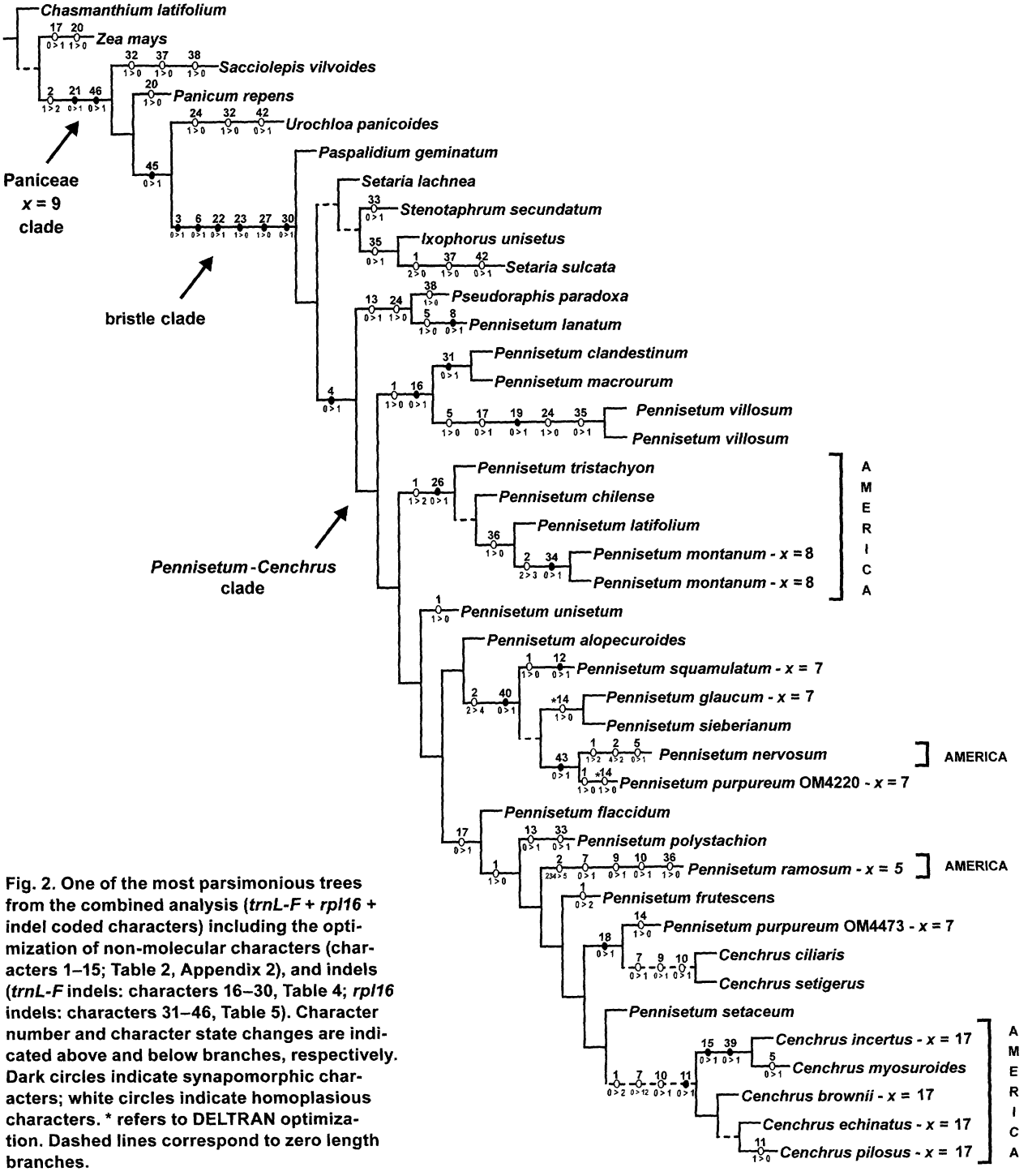


Fig. 2. One of the most parsimonious trees from the combined analysis (*trnL-F* + *rpl16* + indel coded characters) including the optimization of non-molecular characters (characters 1–15; Table 2, Appendix 2), and indels (*trnL-F* indels: characters 16–30, Table 4; *rpl16* indels: characters 31–46, Table 5). Character number and character state changes are indicated above and below branches, respectively. Dark circles indicate synapomorphic characters; white circles indicate homoplasious characters. * refers to DELTRAN optimization. Dashed lines correspond to zero length branches.

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DISCUSSION

Consistent with previous phylogenetic evidence, this study supports the monophyly of the Paniceae $x = 9$ clade, and that of the bristle clade (Clayton & Renvoize, 1986; Kellogg & Campbell, 1987; Gómez-Martínez & Culham, 2000; Zuloaga & al., 2000; Giussani & al., 2001; Doust & Kellogg, 2002; Aliscioni & al., 2003; Doust & al. 2007). The bristle clade is here clearly supported by eleven molecular changes, all of them unreversed synapomorphies (including four indels). Morphologically, the clade is defined by the presence of sterile bristles in their inflorescences and a Kranz-MS leaf anatomy (Gómez-Martínez & Culham, 2000; Zuloaga & al., 2000; Giussani & al., 2001; Doust & Kellogg, 2002; Doust & al., 2007). Our analyses also support previous studies in showing that species of *Pennisetum* and *Cenchrus* together form a monophyletic group (except for *P. lanatum*; see below).

Previous phylogenies incorporating *Pennisetum* and *Cenchrus* neither supported nor refuted the monophyly of *Pennisetum*, although *Cenchrus* was consistently shown to be monophyletic (Doust & Kellogg, 2002, Doust & al., 2007). However, our analyses suggest that neither genus is monophyletic.

Our study showed a dubious external position of *P. lanatum*. This species is characterized by its branched bristles, a character utilized by Clayton & Renvoize (1986) to place it in the monotypic sect. *Dactylophora*. Furthermore, these authors proposed a scheme of taxonomic relationships in which *P. lanatum* is considered as intermediate between *Pseudochaetochloa* Hitchc. and *Pennisetum*. Recent analyses from nuclear (*Knotted 1*) and chloroplast (*ndhF*) markers (Doust & al., 2007) have revealed different placements of *P. lanatum*: this species was included or excluded from the *Pennisetum-Cenchrus* clade respectively. Incongruence between both datasets could be explained by hybridization of *P. lanatum* with a related genus. However, because all available data are based on a single accession that is no longer available, possible contamination or sequencing errors cannot be ruled out. At any rate, the phylogenetic position of *P. lanatum* should be further confirmed with the addition of other specimens of this species and related genera.

Excluding *Pennisetum lanatum*, the genus *Pennisetum* is clearly paraphyletic with all species of *Cenchrus* nested within it, a result previously found by other authors (Gómez-Martínez & Culham, 2000; Giussani & al., 2001; Doust & Kellogg, 2002; Doust & al., 2007). Contrary to previous phylogenies (Doust & Kellogg, 2002; Doust & al., 2007), monophyly of *Cenchrus* is here contradicted by the inclusion of all species of *Cenchrus* together with two species of *Pennisetum* (*P. purpureum—Morrone 4473*; *P. setaceum—Morrone 5373*) in a moderately supported clade A2 (JK = 74; BS = 2; PP = 0.98; Fig. 1). This result is surprising since *P. setaceum* appeared related to *P. glaucum* in a previous report (Doust & Kellogg, 2002); while in our analyses another accession of *P. purpureum* (*Morrone 4220*) is nested in clade B, in agreement with the phylogenetic position of the species reported by Martel & al. (2004). *Pennisetum purpureum* is reported to include tetraploids (IPCN) as well as diploids. It is possible that the polyploids are formed from a cross between one parent from clade B and the other from clade A3, if the polyploidy were formed more than once, with different maternal parents, the species could appear to have two unrelated chloroplast sequences as shown here. Data from a nuclear gene would be required to test this hypothesis and a more exhaustive sampling of species of *Cenchrus* should be needed to further test the monophyly of the genus.

If *Cenchrus ciliaris* and *C. setigerus* are treated under *Pennisetum*, based on a common basic chromosome number $x = 9$, and the presence of antrorsely scabrous and not spiny bristles (Wipff, 2003), the name *Cenchrus* s.str. could be restricted to a core group of species (clades A5 + A6; Fig. 2). Monophyly of *Cenchrus* s.str. is neither confirmed nor rejected by our results, but optimization of non-molecular characters such as the geographic origin (America), basic number of chromosomes ($x = 17$, except in *C. myosuroides* with $x = 9$; Brown, 1959; Morrone & al. 2006), the degree of fusion of bristles (up to the middle of the bristles, except in *C. myosuroides*), the stiffness of internal bristles (stiff bristles) and the presence of retrorsely barbed bristles (except in *C. pilosus*) all could be used to support the delimitation of *Cenchrus* in a strict sense (Fig. 2). Additional molecular characters and a better sampling of the American species are required to further test the monophyly of this group.

***Pennisetum* infrageneric classifications.** — Not considering the monotypic section *Dactylophora* and sections *Brevivalvula* and *Heterostachya*, each of which is represented here by only one species, sects. *Gymnotrix* and *Pennisetum* are polyphyletic in the molecular phylogeny (Fig. 1). Only clade C is completely formed by a group of species of *P.* sect. *Gymnotrix*. The presence of glabrous involucres has been commonly used to differentiate this section; however, optimization of this character on the most parsimonious trees showed that glabrous involucres are plesiomorphic within the *Pennisetum-Cenchrus* clade, while plumose involucres have arisen several times (character 5; Fig. 2).

Pennisetum polystachion, representing sect. *Brevivalvula*, is included in clade A (Fig. 1) and the deciduous upper anthecium, commonly used in the diagnosis of *Brevivalvula* (character 13; Fig. 2), appeared only once within the *Pennisetum-Cenchrus* clade. However, this character state is also present in *P. lanatum*, *Pseudoraphis paradoxa*, *Panicum repens* and *Sacciolepis vilvoides*. Heteromorphous spikelet clusters (character 12; Fig. 2)

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were only found in *P. squamulatum*, the only species representing *P.* sect. *Heterostachya* (Stapf & Hubbard, 1934).

The presence of pilose anther-tips (character 14; Fig. 2) was used by Stapf & Hubbard (1934) to characterize *P.* sect. *Penicillaria*, here represented by *P. glaucum*, *P. purpureum* (*Morrone 4220*) and *P. sieberianum*. Sect. *Penicillaria* is a superfluous name since it includes the type species of *Pennisetum* (Chase, 1921). Nevertheless, we analyzed the relationships of the taxa considered in this section (Stapf & Hubbard, 1934; Roshevits, 1937; Pilger, 1940) with other species of *Pennisetum*. Our phylogeny showed *P. glaucum*, *P. purpureum* (*Morrone 4220*) and *P. sieberianum* closely related to each other in clade B together with *P. nervosum*, and *P. squamulatum* (Fig. 1).

Recognition of *Beckeropsis* as a monotypic genus based on *Pennisetum unisetum* (Stapf & Hubbard, 1934; Chippindall, 1955) is not sustained by this study, since the species is included within the core clade *Pennisetum-Cenchrus*. Similarly, all phylogenetic trees showed the inclusion of *P. clandestinum* in *Pennisetum-Cenchrus* in contrast with the recently published hypothesis by Scholz (2006), who segregated this species in monotypic genus *Kikuyuochloa*.

Although our sampling of sections is sparse, this molecular phylogeny shows considerable disagreement with earlier infrageneric treatments based on morphology. Therefore, it will be necessary to analyze a larger sampling of species and morphological and molecular data to further evaluate the circumscription of infrageneric categories within the genus *Pennisetum*.

Consistent with the genetic classification proposed by Harlan & de Wet (1971) for cultivated plants, *Pennisetum* species belonging to the primary (GP-1: *P. glaucum*) and secondary (GP-2: *P. purpureum*) gene pools (Martel & al., 2004) are found to be phylogenetically closely related in a highly supported clade B (JK = 99; BS = 8; PP = 1.00; Fig. 1). *Pennisetum squamulatum*, previously placed in the tertiary gene pool (GP-3), (Martel & al., 2004), was found to have the same basic chromosome number $x = 7$ (Yukio & al., 2006) as that of *P. glaucum* and *P. purpureum*. This new cytological evidence, in addition to the success in crossing *P. squamulatum* with *P. glaucum* (Dujardin & Hanna, 1983; Ozias-Akins & al., 1993) suggests the inclusion of *P. squamulatum* in the secondary gene pool. Our phylogenetic results also show a close relationship between the domesticated species *P. glaucum* (GP-1) with *P. purpureum*, and *P. squamulatum*, as well as with *P. nervosum* and *P. sieberianum* (clade B). All these species should be considered as putative crop wild relatives (CWR, following Maxted & al., 2006) to *P. glaucum* and considered within the secondary gene pool.

Basic chromosome number and geographic distribution. — Optimization of the basic chromosome number determined that $x = 9$ is the ancestral condition for the ingroup. Within *Pennisetum*, a tendency to reduction of the basic chromosome number (as proposed by Martel & al., 2004) may be hypothesized, because of derived position of species having $x = 8$, 7 and 5 (Fig. 2). On the other hand, the direction of chromosome evolution in *Cenchrus* is the opposite: New World species of *Cenchrus* have $x = 17$ and are placed in one of the most recently divergent clades of this phylogeny, deriving from an $x = 9$ ancestor. The basic chromosome number $x = 17$ could be derived from $x = 9$ through direct loss of one pair of chromosomes in the tetraploid form (Avdulov, 1931), or by fusion and loss of part of a chromosome containing a centromere (Darlington, 1956).

Our phylogenetic results failed to clarify the geographic origin of *Pennisetum-Cenchrus* because it is ambiguous for basal branches. However, it is possible that American *Pennisetum* and *Cenchrus* species could have multiple origins from Old World (African and Asian) species: New World *Pennisetum* species could have had at least three independent dispersal events while the American *Cenchrus* species have originated once or twice, depending on the resolution of clade A4.

Future evidence, including other species of *Pennisetum* and *Cenchrus*, and additional DNA markers, is necessary to realign the classification and propose natural taxonomic groups for these genera of Paniceae.

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Appendix 1. Voucher information and GenBank accession numbers for the species sequenced in this study. Specimens are deposited in the following herbaria: K, MO, SI. (*) indicates sequences from unpublished manuscripts; (**) indicates sequences obtained from GenBank; (–) not sequenced; NSL = National Seed Laboratory; PI = U.S. Department of Agriculture; CN = Plant Gene Resources of Canada.

Species: voucher, *trnL-F* acc. no, *rpl16* acc. no.

Cenchrus brownii Roem. & Schult.: VENEZUELA, alrededores de la universidad, *Ramia & Marrero 9349* (SI), EU940005, EU931888. *Cenchrus ciliaris* L.: ARGENTINA, Jujuy, Santa Bárbara, Camino de San Pedro a El Piquete, Ruta Prov. 1, 6 km de El Piquete, 24°15′S 64°42′W, 12 Oct 2000, *Zuloaga & Morrone 7088* (SI), EU940006, EU931889. *Cenchrus echinatus* L.: ARGENTINA, Jujuy, Santa Bárbara, Ruta Prov. 80, 12 km de Puesto Nuevo camino a La Estrella (límite con Salta), 23°49′S 64°13′W, 29 Apr 2003, *Morrone & al. 4474* (SI), EU940007, EU931890. *Cenchrus incertus* M.A. Curtis: ARGENTINA, Córdoba, San Marcos Sierras, 5 km de S. M. Sierras camino a San Esteban, Río Quilpo, Balneario Tres Piletas, 25 Jan 2005, *Morrone & Giussani 5166* (SI), EU940008, EU931891. *Cenchrus myosuroides* Kunth: ARGENTINA, Jujuy, Santa Bárbara, Camino de San Pedro a El Piquete, Ruta Prov. 1, 6 km de El Piquete, 24°15′S 64°42′W, 12 Oct 2000, *Zuloaga & Morrone 7090* (SI), EU940009, EU931892. *Cenchrus pilosus* Kunth: *K. Hiser 57* (MO), EU940010, EU931893. *Cenchrus setigerus* Vahl: PI 215637, EU940011,–. *Chasmanthium latifolium* (Michx.) H.O. Yates: ARGENTINA, Buenos Aires, Vicente López, Munro, cultivada, *Giussani 353* (SI), EU871113*, EU871064*. *Ixophorus unisetus* (J. Presl) Schltdl.: MEXICO, Michoacán, Ruta 15, km 371, entre Churitzio y La Barca, 7 Oct 2001, *Morrone & Giussani 3638* (SI), EU939980; MEXICO, Mexico, Carretera Tejupilco-Bejucos, desviación a Acamuchitlan, 1 km de la carretera, 18°50′08″N 100°15′59″W, 22 Sep 2001, *Zuloaga & al. 7360* (SI), EU931860. *Panicum repens* L.: AF509963**; URUGUAY, Colonia, Nueva Palmira, Río Uruguay, 33°51′14″S 58°24′36″W, 31 Mar 2005, *Morrone & al. 5229* (SI), EU920053*. *Paspalidium geminatum* (Forssk.) Stapf: ARGENTINA, Corrientes, Curuzú Cuatiá, Ruta Prov. 126, entre Cazadores Correntinos y Sauce, 27 Feb 1999, *Zuloaga & Morrone 6929* (SI), EU939981, EU931861. *Pennisetum alopecuroides* (L.) Spreng.: PI 269235, EU939986, EU931865. *Pennisetum chilense* (E. Desv.) B.D. Jacks. ex R.E. Fr.: ARGENTINA, Jujuy, Tumbaya, Ruta Nacional 9, 1 km pasando Tumbaya hacia Tilcara, 23°50′12″S 65°28′36″W, 09 Feb 2005, *Zuloaga & al. 8617* (SI), EU939987, EU931866. *Pennisetum clandestinum* Hochst. ex Chiov.: ARGENTINA, Buenos Aires, cultivada en jardines de Agronomía (Universidad de Buenos Aires), 18 Aug 2005, *Morrone s.n.* (SI), EU939988, EU931867. *Pennisetum flaccidum* Griseb.: PI 315868, EU939989, EU931868. *Pennisetum frutescens* Leeke: ARGENTINA, Buenos Aires, cultivada en jardines de Agronomía (Universidad de Buenos Aires), 18 Aug 2005, *Morrone s.n.* (SI), EU939990, EU931869. *Pennisetum glaucum* (L.) R. Br.: NSL 114290, EU939991, EU931870. *Pennisetum lanatum* Klotzsch: PI 269866, EU939992, EU931871. *Pennisetum latifolium* Spreng.: BOLIVIA, La Paz, Larecaja, 1 km de Sorata, vía el camino por "Altai Oasis", 15°46′09″ S 68°39′12″W, *Morrone & Belgrano 4940* (SI), EU939993, EU931872. *Pennisetum macrourum* Trin.: AY116266**; CN 87800, EU931873. *Pennisetum montanum* (Griseb.) Hack.: ARGENTINA, Jujuy, Tumbaya, Volcán, a 1–2 km de la ciudad, alrededores de la Laguna de Volcán, 23°55′S 65°28′W, 25 Apr 2003, *Morrone & al. 4368* (SI), EU939994, EU931874; BOLIVIA, La Paz, Larecaja, 2 km de Sorata vía el camino por "Altai Oasis", 15°46′12″S 68°39′35″W, 24 Mar 2004, *Morrone & Belgrano* 4931 (SI), EU939995, EU931875. *Pennisetum nervosum* (Nees) Trin.: BOLIVIA, Santa Cruz, Prov. Andrés Ibañez, 58 km de Santa Cruz camino a Samaipata, pasando 17 km Agostura, 18°09′S 63°34′W, 30 Oct 2002, *Morrone & Giussani 4267* (SI), EU939996, EU931876. *Pennisetum*

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Appendix 1. Continued.

polystachion (L.) Schult.: BOLIVIA, Santa Cruz, Velasco, camino de San Ignacio a Santa Ana, ca. 11 km al S de San Ignacio de Velasco, 16°27′12″S 60°55′34″W, 30 Mar 2004, *Morrone & Belgrano 5060* (SI), EU939997, EU931877. *Pennisetum purpureum* Schumach.: BOLIVIA, La Paz, Prov. Nor Yungas, a 47 km de Yolosa camino hacia Caranavi, pasando 24 km Chálla, 24 Oct 2002, *Morrone & al. 4220* (SI), EU939998, EU931878; ARGENTINA, Jujuy, Santa Bárbara, Ruta Prov. 80, 12 km de Puesto Nuevo camino a La Estrella (límite con Salta), 23°49′S 64°13′W, 29 Apr 2003, *Morrone & al. 4473* (SI), EU939999, EU931879. *Pennisetum ramosum* (Hochst.) Schweinf.: CN 84079, EU929056*, EU931880. *Pennisetum setaceum* (Forssk.) Chiov.: ARGENTINA, Buenos Aires, Vicente López, Munro, cultivada, 1 Aug 2005, *Morrone 5373* (SI), EU940000, EU931881. *Pennisetum sieberianum* (Schltdl.) Stapf & C.E. Hubb.: PI 532675, EU940001, EU931882. *Pennisetum squamulatum* Fresen.: PI 248534, EU929057*, EU931883. *Pennisetum tristachyon* (Kunth) Spreng.: BOLIVIA, La Paz, Prov. Nor Yungas, 38 km de Coroico camino a Puente Villa, pasando 6 km Coripata, 25 Oct 2002, *Morrone & al. 4234* (SI), EU940002, EU931884. *Pennisetum unisetum* (Nees) Benth.: SUDAN, Bushbuck Hill, E of Itibol, 4°01′N 32°52′E, 10 Nov 1980, *Friis & Vollesen 129* (K), EU929058*, EU931885. *Pennisetum villosum* R. Br. ex Fresen.: PI 304750, EU940003, EU931886; *Doust 1500* (MO), EU940004, EU931887. *Pseudoraphis paradoxa* (R. Br.) Pilg.: *Doust 1505* (MO), EU939982, EU931862. *Sacciolepis vilvoides* (Trin.) Chase: BRAZIL, *Longhi-Wagner & al. 9429* (SI), EU871142*, EU871093*. *Setaria lachnea* (Nees) Kunth: ARGENTINA, Misiones, Leandro N. Alem, Vieja ruta 14 de San José a Cerro Azul, 27°45′S 55°38′W, 21 Feb 1999, *Zuloaga & Morrone 6758* (SI), EU939984, EU931863. *Setaria sulcata* Raddi: ARGENTINA, Salta, Orán, Ruta Nac. 18, 8 km de la Ruta Nac. 50 camino a Isla de Cañas, 1 May 2003, *Morrone & al. 4548* (SI), EU939983, EU920054*. *Stenotaphrum secundatum* (Walter) Kuntze: ARGENTINA, Buenos Aires, Vicente López, Munro, cultivada, 6 Sep 2005, *Morrone 5374* (SI), EU939985, EU931864. *Urochloa panicoides* P. Beauv.: MEXICO, Mexico, Carretera Tejupilco-Bejucos, desviación a Acamuchitlan, 1 km de la carretera, 18°50′08″N 100°15′59″W, 22 Sep 2001, *Zuloaga & al. 7358* (SI), EU920056*, EU920055*. *Zea mays* L.: X86563**, AF133477**.

Appendix 2. List and codification of non-molecular, unordered and unpolarized characters mapped onto the molecular phylogeny.

1 Geographic origin (Africa = 0; Asia = 1; America = 2)
2 Basic chromosome number (x = 12 = 0; x = 10 = 1; x = 9 = 2; x = 8 = 3; x = 7 = 4; x = 5 = 5; x = 17 = 6)
3 Leaf anatomy (Kranz-PS = 0; Kranz-MS = 1)
4 Disarticulation in the base of the primary branches (absent = 0; present = 1)
5 Involucre (plumose = 0; glabrous =1)
6 Bristles (absent = 0; present = 1)
7 Fusion of bristles (not fused, bristles free = 0; basal = 1; up to the middle of the bristles = 2)
8 Bristles (unbranched = 0; branched = 1)
9 Shape of internal bristles (filiform = 0; flat = 1)
10 Texture of internal bristles (not rigid = 0; stiff = 1)
11 Scabrosity of bristles (antrorse = 0; retrorse = 1)
12 Spikelets (isomorphic in the same inflorescence = 0; dimorphic in the same inflorescence = 1)
13 Disarticulation at the base of the upper anthecium (absent = 0; present = 1)
14 Anther-tips (pilose = 0; glabrous = 1)
15 External bristles (not rigid = 0; stiff = 1)

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