

Int. J. Plant Sci. 170(1):117–131. 2009.
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1058-5893/2009/17001-0011\$15.00 DOI: 10.1086/593043

A PHYLOGENY OF *SETARIA* (POACEAE, PANICOIDEAE, PANICEAE) AND RELATED GENERA BASED ON THE CHLOROPLAST GENE *ndhF*

Elizabeth A. Kellogg,^{1,*} Sandra S. Aliscioni,[†] Osvaldo Morrone,[‡] José Pensiero,[§] and Fernando Zuloaga[‡]

*Department of Biology, University of Missouri–St. Louis, One University Boulevard, St. Louis, Missouri 63121, U.S.A.; [†]Cátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE, Buenos Aires, Argentina; [‡]Instituto de Botánica Darwinion, Labarden 200, San Isidro, Buenos Aires, Argentina; and [§]Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, 3080, Esperanza, Santa Fe, Argentina

The genus *Setaria* is the largest genus in the so-called bristle clade, a monophyletic group of panicoid grasses distinguished by the presence of sterile branches, or bristles, in their inflorescences. The clade includes both foxtail millet and pearl millet, the latter an important cereal crop in dry parts of the world. Other members of the clade are weeds that are widespread agricultural pests. Previous molecular phylogenetic studies have suggested that *Setaria* might not be monophyletic but did not have a large enough sample of species to test this rigorously. In addition, taxonomic studies have suggested a close relationship between *Setaria* and *Paspalidium*, with some authors combining them into a single genus, but molecular studies included too few *Paspalidium* accessions for a meaningful conclusion. Accordingly, we have produced 77 new sequences of the chloroplast gene *ndhF* for 52 species not in previous analyses. These were added to available sequences for 35 species in 10 genera of the bristle clade and four outgroup taxa. We find that *Setaria* species fall into several moderately to strongly supported clades that correlate with geography but not with the existing subgeneric classification. Relationships among these clades and among other genera within the bristle clade are unclear. Constraint experiments using the approximately unbiased test reject the monophyly of *Pennisetum*, *Setaria*, and *Setaria* plus *Paspalidium*, as well as several other groupings, although the test may be overly sensitive and prone to Type I error. The more conservative Shimodaira-Hasegawa test fails to reject monophyly of any of the tested clades.

Keywords: phylogeny, *ndhF*, *Setaria*, *Pennisetum*, *Cenchrus*.

Online enhancement: appendix table.

Introduction

Setaria P. Beauv. is a cosmopolitan genus of grasses with 114 species (Webster 1993) mostly from tropical and subtropical regions but including several that occur in cold regions of both hemispheres. The plants grow in open environments or in woodlands (Rominger 1962; Prasada Rao et al. 1987; Pensiero 1999), and many species are weedy. The Old World species are concentrated largely in tropical regions of Africa, including several species endemic to Madagascar (Stapf and Hubbard 1930), whereas in the New World the center of diversity is Brazil (Rominger 1962). The genus is of agricultural importance because some species are cultivated for grains or perennial forage grass and others are noxious weeds (Rominger 1962; Prasada Rao et al. 1987). Several species, such as *Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb. ex M.B. Moss, are extensively collected as wild cereals in African savannas in times of scarcity (Dalziel 1937); in addition, *Setaria italica* (L.) P. Beauv., foxtail millet, is cultivated in Asia and other regions as a cereal for humans (Naciri et al. 1992).

Within the grass family (Poaceae), *Setaria* belongs to subfamily Panicoideae, tribe Paniceae (Clayton and Renvoize

1986). A molecular phylogenetic analysis demonstrated that this tribe, as traditionally circumscribed, is paraphyletic and consists of two clades that differ in chromosome base number (Giussani et al. 2001). *Setaria* falls in the $x=9$ clade, which also contains the type genus of the tribe (*Panicum*); thus, the genus is contained within Paniceae whether the tribe is considered sensu stricto or sensu lato. Within the $x=9$ Paniceae, one strongly supported and easily recognized group is known informally as the “bristle clade” (Doust and Kellogg 2002) because of the presence of sterile inflorescence branches, or bristles, which are unique among the grasses. The group was first demonstrated to be monophyletic by Zuloaga et al. (2000) in a morphological cladistic analysis, a result supported by all subsequent molecular studies (Gómez-Martínez and Culham 2000; Duvall et al. 2001; Giussani et al. 2001; Aliscioni et al. 2003; Doust et al. 2007). The bristle clade includes 24 genera and ~310 species (Doust and Kellogg 2002).

Setaria, as conventionally circumscribed, is the most species-rich genus of the bristle clade. The genus includes annual or perennial plants with leaf blades flat, folded, or plicate; inflorescences are open or spiciform panicles with spikelets placed along primary branches, more or less contracted and occasionally reduced to racemes; spikelets are usually dorsally compressed and planoconvex, all or some subtended by one or more bristles that persist on the axis when the spikelets fall

¹ Author for correspondence; e-mail: tkellogg@umsl.edu.

at maturity; the glumes are unequal, the lower usually much smaller than the upper; the lower antherium is male or sterile, with its lemma membranous, and rarely coriaceous, and the palea varies from well developed to absent. The upper antherium is hermaphrodite, crustaceous or coriaceous, and typically ellipsoid, with a surface that is usually corrugate, granular, or rugose, although it is smooth in some species.

Stapf (1920) recognized *Paspalidium* as an independent genus segregated from *Setaria* and characterized it as having bristles present only at the ends of the primary branches; however, some species of the genus have bristles accompanying spikelets aside from the distal one. Webster (1987, 1995) recognized ~28 species in *Paspalidium*, 23 concentrated in Australia and five native to Asia and Africa. When the full range of variation in the inflorescences of *Setaria* and *Paspalidium* is considered, morphological intermediates are present and the two genera intergrade. As a consequence, Veldkamp (1994) and Webster (1993, 1995) transferred all species of *Paspalidium* to *Setaria*, a decision followed by O. Morrone, S. S. Aliscioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg (unpublished manuscript).

Previous authors have had differing opinions regarding the infrageneric classification of *Setaria*. Clayton and Renvoise (1986) recognized four sections within the genus based on the characters of the panicle, the presence of plicate leaf blades, and the rounded or keeled upper lemma: *Panicatrix* Stapf & C.E. Hubb., *Cymbosetaria* Stapf & C.E. Hubb., *Ptychophyllum* (A. Braun) Stapf, and *Setaria*. Rominger (1962) published a revision of the North American species and recognized subgenera *Ptychophyllum* (A. Braun) Hitchc., with six species; *Paurochaetium* (Hitchc. & Chase) Rominger, with 10 species; and *Setaria*, with 27 species. Pensiero (1999), in a revision of South American species of the genus, recognized subgenus *Ptychophyllum*, with 3 species; *Setaria*, with 39 species; and the newly established monotypic subgenus *Cernatum*, including *Setaria cernua* endemic to Ecuador. In a complete revision of the Old World species now in progress (O. Morrone, S. S. Aliscioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg, unpublished manuscript), three major groups are recognized on the basis of the type of inflorescence: 19 species with open panicles, 21 species with spiciform panicles, and 18 species with unilateral racemes along the panicle. Species of the last group have been placed by other authors within the genus *Paspalidium*.

A diagnostic attribute often used in identifying *Setaria* species is the number of bristles subtending the spikelet. However, from developmental analysis, this character is in fact a composite of at least three characters: the number of orders of branching, the number of primordia per order of branching, and the number of spikelets whose development is suppressed (Doust and Kellogg 2002).

Polyploidy is common in *Setaria*, at both inter- and intra-specific levels (Khosla and Sharma 1973); chromosome numbers vary from $2n=18$ (diploid) to $2n=108$ (dodecaploid; Hacker 1966). More than 82% of species of the genus may be polyploids (Caponio and Pensiero 2002). If these are allopolyploids, then the phylogenetic history of the genus and/or the bristle clade may be complex and at least partially reticulate.

In this study, we evaluate the phylogeny of the genus *Setaria* on the basis of the chloroplast gene *ndbF*. We analyze relationships among Old and New World species of *Setaria*

and allied genera in the tribe Paniceae, we test whether previous infrageneric categories established in the New World (Rominger 1962; Pensiero 1999) and Old World species (O. Morrone, S. S. Aliscioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg, unpublished manuscript) represent monophyletic groups, and we discuss the generic boundaries between *Paspalidium* and *Setaria*.

Material and Methods

Taxon Sampling

Data were available in GenBank for 31 species in 10 genera of the ingroup as well as four outgroup taxa. To assemble as large a set of taxa as possible for *Setaria* and its relatives, we undertook collecting trips to various parts of South America and South Africa. Leaves were collected in silica gel, and voucher specimens were collected in sets of two to four for deposit in local herbaria, as well as at SI and MO. Additional species were sampled from herbarium specimens. In total, this analysis presents 77 new sequences for 52 species not in previous analyses, plus an additional six sequences for six species added provisionally (see "Data Analysis"). Information on species, voucher specimens, and GenBank numbers is in the appendix in the online edition of the *International Journal of Plant Sciences*.

DNA Extraction and Sequencing

DNA was extracted from field-collected plants dried in silica gel for all American and some Old World species; DNA for the remainder of the Old World species came from herbarium material. DNA extractions were conducted with a modified CTAB protocol similar to that used by Giussani et al. (2001). Some DNA extractions, especially for herbarium material, were conducted using DNeasy Plant Mini Kit (Qiagen, Valencia, CA). DNA was amplified by using the polymerase chain reaction (PCR) with a battery of primers in different combinations. Amplification for most taxa was done in two overlapping fragments with the primers 5F/1318R and 972F/2110R (Olmstead and Sweere 1994; Clark et al. 1995), but for difficult taxa we amplified the gene in smaller fragments, using 5F/972R, 536F/1318R, 972F/1821R, and 1318F/2110R. Additional primers were designed by S. S. Aliscioni for some herbarium material that was more difficult to amplify: 445F (5'-TTTGGGAACCTGTCCGAATG-3'), 787F (5'-CCTCTTTCATATCCCTACC-3'), 1170F (5'-CCTCTTGCTTGCTTCTGG-3'), 1535F (5'-GTCTATCCTCATGAAACRGG-3'), and 1630R (5'-CCAATGAACAAAGTAAAAAG-3'), which was used by Doust et al. (2007).

Some PCR products were cleaned and sequenced on an ABI 377 sequencer at University of Missouri–St. Louis with procedures described by Giussani et al. (2001). Other PCR products were cleaned and sequenced by Macrogen (Seoul, South Korea). Forward and reverse strands were sequenced for all fragments, with a minimum overlap of 80%. Sequences were assembled and edited using Chromas Pro, version 1.22 (Technelysium, Tewantin, Australia). *Phred* values (Ewing and Green 1998; Ewing et al. 1998) were assessed in SeqMan (DNASTAR, Madison, WI).

Data Analysis

Sequences were aligned by eye in MacClade 4.0 (Maddison and Maddison 2005) and were trimmed to remove part of the 3' end, for which many sequences were incomplete. To check sequence accuracy, all sequences were translated to amino acids. Point substitutions that caused stop codons or nonconservative changes in amino acid were checked against the original sequencing trace files. In some cases, the sequence was eliminated from further analysis at this stage. It was not necessary to introduce gaps to align the sequences. Several sequences generated from DNA extracted from herbarium specimens were found to have relatively high error rates. In cases in which the species was of particular interest, we retained these as provisional sequences and added them to a second round of less rigorous analyses (see below). The data matrix was assembled in NEXUS format and submitted to TREEBASE (<http://www.treebase.org>), accession number SN3906.

Parsimony analyses were conducted with the parsimony ratchet, as implemented in PAUPRat (Goloboff 1999; Nixon 1999; Sikes and Lewis 2001), running on the Beowulf cluster at University of Missouri–St. Louis. The analysis used 20 runs, each with 200 iterations, with 15% of the characters reweighted at each iteration (default settings). The resulting trees were filtered to retain only the shortest ones, and a strict consensus tree was constructed. Support for the parsimony tree was assessed with a bootstrap analysis implemented in PAUP* 4.0 (Swofford 2003), with tree bisection reconnection (TBR) branch swapping, MULPARS on, and MAXTREES set to 500. One hundred bootstrap replicates were analyzed, and a 50% majority-rule consensus tree was constructed.

To determine the best model for maximum likelihood (ML) analyses, data were submitted to Modeltest (Posada and Crandall 1998). Both the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC) selected TVM+I+G as the best-fit model, although the delta value between TVM+I+G and GTR+I+G was less than 2, indicating that both models provide a good fit for the data. We applied TVM+I+G with the settings Base = (0.2855 0.1546 0.1737), Nst = 6, Rmat = (2.2698 4.0502 0.5576 1.5325 4.0502), Rates = gamma Shape = 1.0535, and Pinvar = 0.5677. An ML analysis was then run in PAUP* 4.0 (Swofford 2003), with 10 random addition sequences and TBR branch swapping. Support for the ML tree was assessed by bootstrapping. One hundred bootstrapped data sets were constructed, and each was analyzed with the same likelihood parameters as the original analysis. The resulting trees were assembled into a 50% majority-rule consensus tree. For comparison with the results from PAUP*, we also ran an ML analysis in PHYML (Guindon and Gascuel 2003) on a Macintosh using OS X 10.4.11.

To determine the best model for Bayesian analyses, data were submitted to MrModeltest (Nylander 2004). The hLRT and AIC both selected GTR+I+G as the best-fit model. With this model and empirical base frequencies, a Bayesian analysis was then run in MrBayes (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), implemented in parallel (Altekar et al. 2004) on the Beowulf cluster, using flat priors (the default). We did three replicate analyses, each with four chains, running for 10 million generations. Trees were sampled every 500 generations. Plots of likelihood values versus

generation number were viewed in Excel; for the three runs, the likelihood values had stabilized by 26,000, 30,000, and 25,000 generations. Accordingly, the appropriate trees of each run were removed as burn-in. All remaining trees were assembled into a majority-rule consensus tree.

We tested whether the data were able to reject various hypotheses of relationships, using the Shimodaira-Hasegawa (S-H) test (Shimodaira and Hasegawa 1999) and the approximately unbiased (AU) test as implemented in CONSEL (Shimodaira and Hasegawa 2001). In these tests, constraint trees are constructed for each hypothesis to be tested. The data are then analyzed by ML to find the optimal tree that satisfies each constraint. Finally, the likelihood of the constrained tree is compared with that of the unconstrained tree, and the significance of the difference in likelihoods is assessed. We tested whether the maximum parsimony (MP) and Bayesian trees were significantly different from the ML tree. We also tested monophyly of various groups of taxa, as described below.

To determine an approximate placement for several incomplete or modest-quality sequences, we ran a second ML analysis and parsimony bootstrap with these sequences included. These placements are discussed as provisional.

Results

The aligned data matrix consists of 2043 nucleotide positions. The sequences are A-T rich (66%), as expected of a chloroplast gene. There are no indels in the alignment. In all, 385 characters are variable and 169 potentially parsimony informative, although many of these characters distinguish the outgroups from each other and from the ingroup. When only the ingroup is considered, 331 characters are variable and 147 potentially parsimony informative.

The parsimony ratchet found 3342 trees of 409 steps (uninformative characters excluded), with a consistency index (CI) of 0.504 and a retention index (RI) of 0.836. The ML analysis in PAUP* found a single tree with log likelihood of -7345.59754 , and the PHYML analysis had a log likelihood of -7354.72896 . The best topology visited by the Markov chain in the Bayesian analysis had a log likelihood of -7913.702 .

All methods of analysis produced similar results (fig. 1). The ingroup is strongly supported as monophyletic (ML bootstrap support, MP bootstrap support, and Bayesian posterior probability of 99, 100, and 100, respectively; 99/100/100 in the notation used hereafter). Twelve distinct clades (roman numerals in fig. 1) are consistently retrieved in all analyses and receive moderate to strong support. The ML tree from PHYML is similar to that produced by the PAUP* ML analysis, with the only differences in regions of the tree that are poorly supported; accordingly, the results presented here refer to the PAUP* tree unless otherwise noted. Only seven taxa (*Paspalidium rarum*, *Paspalidium geminatum*, *Plagiosetum refractum*, *Pseudoraphis paradoxa*, *Setaria magna*, and *S. cernua*) are not consistently assigned to any of these clades.

For several taxa, we were able to include multiple accessions. In many cases, multiple accessions of the same species had identical or nearly identical *ndhF* sequences, giving confidence that the sequences are highly reproducible. Examples of this include *S. barbata*, *Zuloagaea bulbosa*, *Paspalidium*

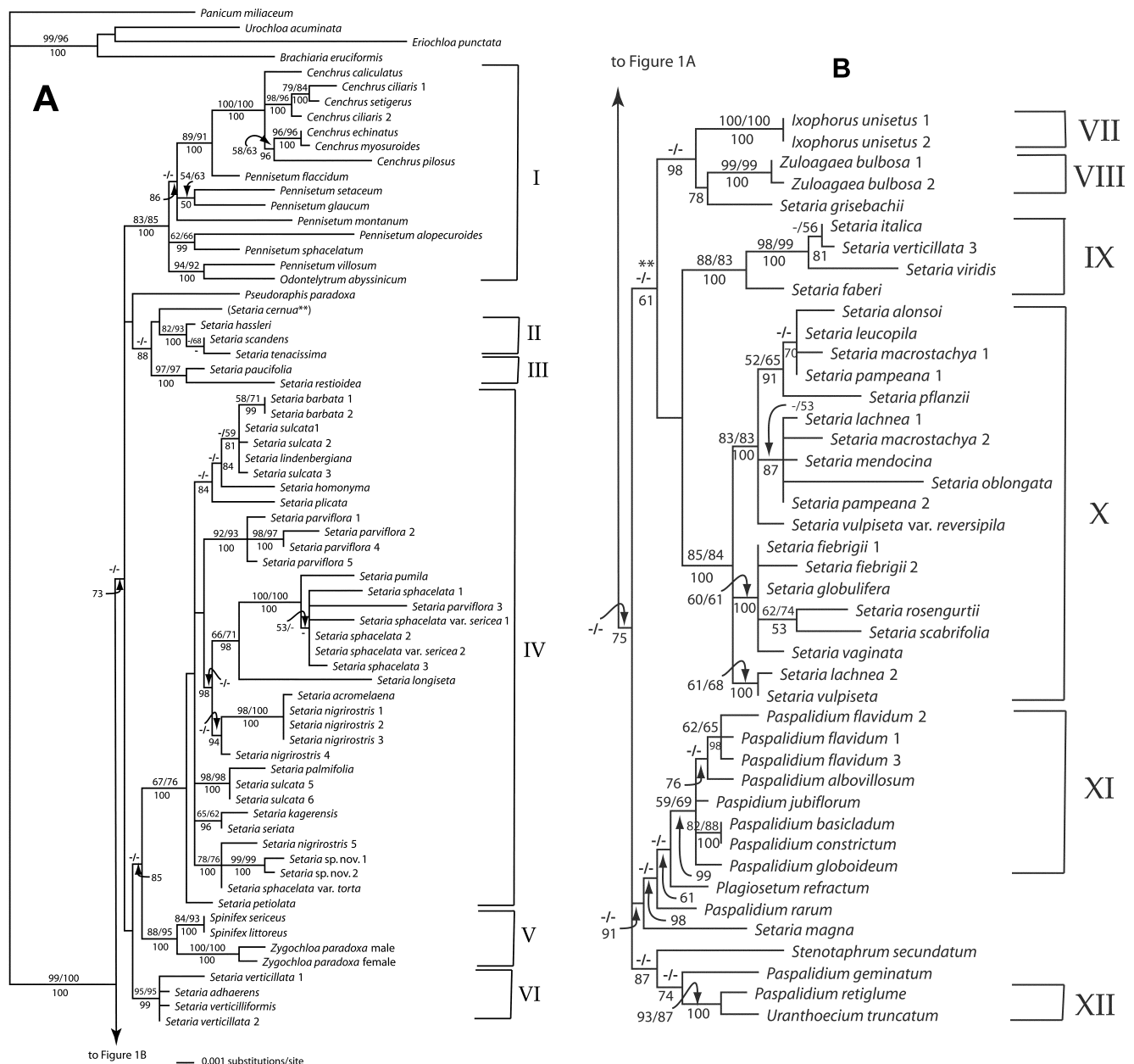


Fig. 1 Maximum likelihood phylogeny of the bristle clade. Maximum likelihood and parsimony bootstrap values are above the branches, and Bayesian posterior probability values are below the branches.

flavidum, and several accessions of *S. sulcata* and *S. nigrirostris*. In other cases, we found variation within a species, but the accessions still formed a clade (e.g., *Zygochloa paradoxa*, *S. sphacelata*, and *Ixophorus unisetus*). Finally, for a few taxa (e.g., *S. lachnea*, *S. macrostachya*, *S. pampeana*, *S. parviflora*, and *S. verticillata*), sequences from different accessions fell in different parts of the tree. These are discussed further below.

Relationships among the clades are poorly resolved, although all analyses placed clades I–VI in a single large clade (fig. 1A) and clades VII–XII in another large clade (fig. 1B). Only *S. cernua* (indicated by parentheses and double asterisks in fig. 1A) varied in its placement, falling as sister to

clade II in likelihood analyses, as sister to clade X in parsimony analyses, and in a polytomy with clades VII, VIII, and X (double asterisk next to 61) in Bayesian analysis. The node uniting clades I–VI is supported by a single mutation, a C-to-T transition at position 1707, which corresponds to a silent mutation in the third position of a phenylalanine codon; this character has a CI of 0.5. The T reverses to a C in *Pennisetum alopecuroides* and *S. plicata*. Along the internode leading to the clade of *S. alonsoi*, *S. leucopila*, *S. macrostachya*, *S. pampeana*, and *S. planzii* (a subgroup in clade X), the C changes to a G. The node uniting clades VII–XII is also supported by a single change, an A-to-G transition at position

354, which corresponds to a silent mutation in the third position of a glycine codon; this character has a CI of 1.0. As might be expected for clades supported by single mutations, the I–VI clade and the VII–XII clade receive <50% bootstrap support and are also unsupported by Bayesian analysis.

Clade I (76/84/100) includes *Cenchrus*, *Pennisetum*, and *Odontelytrum*. *Cenchrus* is strongly supported as monophyletic (100/100/100) and is derived from within a paraphyletic *Pennisetum*. *Odontelytrum* is also derived within *Pennisetum* and is strongly supported (94/92/100) as sister to *Pennisetum villosum*. Several strongly supported groups of species are evident within *Cenchrus*. *Cenchrus ciliaris* 2 is a sequence added in this analysis to previously published sequences; it is sister to the clade of *C. ciliaris* and *Cenchrus setigerus* and differs from the published *C. ciliaris* sequence by six mutations. Whether the difference between the two sequences of *C. ciliaris* represents real biological variation or simply errors in sequencing is unclear, but in either case a close relationship between *C. ciliaris* and *C. setigerus* is supported (98/96/100). The two species are distinct but morphologically similar, and a few workers have placed *C. setigerus* as a variety of *C. ciliaris* (see Wipff 2001). This analysis includes one more species of *Pennisetum* than previous analyses (*Pennisetum sphacelatum*), and relationships among the species of *Pennisetum* species remain largely unresolved.

Clades II and III plus *Pseudoraphis paradoxa* form a peculiarly heterogeneous group in the analyses presented here. Clade II is strongly supported (82/93/100) and consists of three South American species of *Setaria*; clade III includes *S. paucifolia* (of South America) as sister to *S. restioidea* (African; 97/97/100). Clades II and III are sisters, although without support, and in the PHYML analysis the two together are sister to clade IV (not shown). In our MP analysis, clades II and II are linked by two mutations, a T-to-C transition at position 144 (CI = 0.5) and a C-to-A transversion at position 1468 (CI = 0.11). While position 144 changes only once elsewhere on the tree (a parallelism along an internode linking clades VII, VIII, and X, which is present only in some parsimony trees), position 1468 changes nine times on the tree. In the ML analyses presented here, *S. cernua* (South American) is also a member of this group, although without support. Likewise, the linkage of clades II and III with the Australian *Pseudoraphis paradoxa* is unsupported.

Clade IV is a moderately supported clade (67/76/100) consisting entirely of African species, although several species in this clade are also reported from Asia; in parsimony trees it is supported by five mutations, all with relatively high CIs. Because of polyploidy and the morphological complexity of several of the African species, we included multiple accessions for many of them. Within the African clade are all the species placed by Rominger in section *Ptychophyllum*, a group with distinctive open panicles and plicate leaves. All members of the unsupported clade (-/84) comprising *S. barbata*, *S. sulcata* accessions 1–4, *S. lindenbergiana*, *S. homonyma*, and *S. plicata* share this same morphology. The two accessions of *S. barbata* are sisters and fall in a clade with four of the *S. sulcata* accessions plus *S. lindenbergiana*, *S. homonyma*, and *S. plicata*. The *S. sulcata* sequences in this clade are virtually identical to each other and to that of *S. lindenbergiana*. Sequences from other plants with plicate leaves form separate clades. In particular,

two additional accessions of *S. sulcata* form a separate, strongly supported clade (98/98/100) with the morphologically similar *S. palmifolia*, and *S. petiolata* is sister to all other African species. Thus, sect. *Ptychophyllum* is not monophyletic. *Setaria sulcata* is polyploid (Oliveira Freitas-Sacchet et al. 1984; table 1), so the separate lineages of this chloroplast gene could indicate multiple origins.

Within clade IV (African *Setaria*), several accessions of *S. parviflora* form a strongly supported clade (92/93/100). *Setaria sphacelata* is a morphologically intricate species complex with several varieties, three of which are included here. All accessions of the typical variety (indicated simply as *S. sphacelata* in fig. 1A) fall in a strongly supported (100/100/100) clade, along with the two accessions of *S. sphacelata* var. *sericea*. One accession of *S. parviflora* is a member of this clade, as is the weedy *S. pumila*. The one accession of *S. sphacelata* var. *torta* apparently has a chloroplast more similar to that of a specimen of *S. nigrirostris* and that of *Setaria* sp. nov. *Setaria longiseta* may be sister to the main *S. sphacelata* clade, although support for this is not especially strong (66/71/98). Sequences for three of the accessions of *S. nigrirostris* are nearly identical and form a strongly supported clade (98/100/100) with the morphologically similar annual species *S. acromelaena*. A fourth accession of *S. nigrirostris* is sister to this group, albeit with lower support (-/194). *Setaria kagerensis* and *S. seriata* are moderately supported as sisters (65/62/96).

Clade V consists of members of the dioecious Australian genera *Spinifex* and *Zygochloa*. The two are strongly supported as sisters (88/95/100), and the two accessions of each form strongly supported clades. The Bayesian analysis found weak support for a sister relationship between the African clade IV and the Australian dioecious clade V, although neither bootstrap analysis retrieved this grouping. The character linking clades IV and V is a C-to-G transversion at position 1708; this is a first-position mutation that changes a glutamine to glutamic acid. The same mutation also occurs in *Stenotaphrum secundatum*, and it reverses in *Setaria plicata*.

Clade VI is strongly supported (95/95/99) and includes two of the four accessions of the tetraploid species *S. verticillata*, plus *S. adhaerens* and *S. verticilliformis*. *Setaria verticillata* and *S. adhaerens* are characterized by retrorse barbs on the bristles, which are unique in the genus. The multiple placements of *S. verticillata* may indicate multiple origins of the polyploid and/or homoplasmy in the presence of retrorse barbs. In the PHYML analysis, clades V and VI are sisters (not shown).

Clades VII and VIII (fig. 1B) consist of the Central American polyploids *Ixophorus* and *Zuloagaea*. Both genera are monotypic, and multiple accessions of each confirm their monophyly. The two are morphologically quite distinct, and *Zuloagaea* is the only member of the entire ingroup that lacks bristles throughout development (Bess et al. 2006). Despite their morphological differences, the two genera are placed in a clade with *S. grisebachii* in all analyses, although only the Bayesian analysis provides any support for this relationship.

The type species of *Setaria*, *S. viridis*, is a member of clade IX, a well-supported group (88/83/100). *Setaria italica*, foxtail millet, was domesticated in western China from the weedy *S. viridis* (Le Thierry d'Ennequin et al. 2000; Benabdelmouna et al. 2001a), so their close relationship in this analysis is not surprising. Also in this clade is *S. faberi*, a species of China,

Table 1

Chromosome Numbers and Geographic Origin of the Species of *Setaria* and *Paspalidium* Included in the Analysis

Species	Origin	n		2n	
		Number	References	Number	References
<i>Paspalidium</i> :					
<i>P. albobillosum</i>	Australia	?		?	
<i>P. basicladum</i>	Australia	?		?	
<i>P. constrictum</i>	Australia	?		?	
<i>P. flavidum</i>	Asia	18	Bir and Chauhan 1990	~44	Sharma and Sharma 1979
<i>P. flavidum</i>	Asia	27	Mehra 1982; Bir and Sahni 1983; Nadeem Ahsan et al. 1994	54	Sinha et al. 1990
<i>P. flavidum</i>	Asia	56	Bir and Chauhan 1990		
<i>P. geminatum</i>	Africa	9	Rao and Mwasumbi 1981; Nadeem Ahsan et al. 1994	?	
<i>P. globoideum</i>	Australia	?		?	
<i>P. jubiflorum</i>	Australia	?		?	
<i>P. rarum</i>	Australia	?		?	
<i>P. retiglume</i>	Australia	?		?	
<i>Setaria</i> :					
<i>S. acromelaena</i>	Africa	?		?	
<i>S. adhaerans</i>	Asia	9	Gupta and Singh 1977	?	
<i>S. alonsoi</i>	America	?		?	
<i>S. barbata</i>	Africa	18	Olorode 1975	54	Gadella 1977
<i>S. barbata</i>	Africa	27	Christopher and Abraham 1976; Dujardin 1978	56	Sarkar et al. 1976
<i>S. barbata</i>	Africa	28	Sarkar et al. 1976		
<i>S. cernua</i>	America	?		?	
<i>S. faberi</i>	Asia	?		36	Probatova and Sokolovskaya 1983; Warwick et al. 1987, 1997
<i>S. fiebrigii</i>	America	18	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	36	Pensiero 1999
<i>S. globulifera</i>	America	?		?	
<i>S. grisebachii</i>	America	?		18	Reeder 1971
<i>S. hassleri</i>	America	?		?	
<i>S. homonyma</i>	Asia	18	Mehra and Sharma 1975	?	
<i>S. italica</i>	Asia	9	Khosla and Sharma 1973; Gupta and Singh 1977; Mehra 1982; Sinha et al. 1990	18	Christopher and Abraham 1976; Chikara and Gupta 1979; Frey et al. 1981; Li and Chen 1985; Zhou et al. 1989; Sinha et al. 1990; Kozuharov and Petrova 1991; Li et al. 1996; Wu and Bai 2000
<i>S. italica</i>	Asia			36	Li and Chen 1985
<i>S. kagerensis</i>	Africa	?		18 (9 II)	Lakshmi and Yacob 1978
<i>S. lachnea</i>	America	18	Gupta and Singh 1977	36	Bowden and Seen 1962; Manero de Zamelzú and Ochoa de Suárez 1991; Pensiero 1999
<i>S. leucopila</i>	America	?		54, 68, 72	Emery 1957a
<i>S. lindenbergiana</i>	Africa	?		?	
<i>S. longiseta</i>	Africa	18	Olorode 1975	36	Kammacher et al. 1973
<i>S. macrostachya</i>	America	27	Gupta and Singh 1977	54	Emery 1957b; Pensiero 1999
<i>S. magna</i>	America	?		36	Brown 1948
<i>S. mendocina</i>	America	?		?	
<i>S. nigrirostris</i>	Africa	9	Gupta and Singh 1977	36, 54 (18 II, 27 II)	Spies and du Plessis 1986
<i>S. nigrirostris</i>	Africa	18	Spies and du Plessis 1986		
<i>S. nigrirostris</i>	Africa	27	Spies and du Plessis 1986		
<i>S. oblongata</i>	America	18	Tiranti and Genghini 2000	?	
<i>S. palmifolia</i>	Asia	27	Mehra and Sharma 1975; Christopher and Abraham 1976; Mehra 1982	54	Christopher and Abraham 1976
<i>S. pampeana</i>	America	?		~50	Pensiero 1999
<i>S. parviflora</i>	Africa	18	Gupta and Singh 1977; Oliveira Freitas-Sacchet 1980; Mehra 1982	36	Gould and Soderstrom 1967; Pohl and Davidse 1971; Norrmann et al. 1994
<i>S. parviflora</i>	Africa			72	Gould and Soderstrom 1967; Fernández and Queiróz 1969
<i>S. paucifolia</i>	America	?		?	
<i>S. petiolata</i>	Africa	?		?	
<i>S. pflanzii</i>	America	?		36	Caponio and Pensiero 2002
<i>S. plicata</i>	?	36	Mehra 1982	?	

Table 1
(Continued)

Species	Origin	n		2n	
		Number	References	Number	References
<i>S. pumila</i>	Europe	9, 18+0–2B, 27	Sahni 1989	36	Baltisberger 1988; Sahni 1989; Devesa et al. 1991; Kozuharov and Petrova 1991
<i>S. pumila</i>	Europe	36	Sahni 1989; Nadeem Ahsan et al. 1994		
<i>S. vestioidea</i>	Africa	18	Dujardin 1978	?	
<i>S. rosenfurtii</i>	America	54	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	?	
<i>S. scabrifolia</i>	America	18	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	?	
<i>S. scandens</i>	?	?		?	
<i>S. seriata</i>	Africa	?		?	
<i>S. sphacelata</i>	Africa	9	Gupta and Singh 1977; Dujardin 1979; Rao and Mwasumbi 1981	36	de Wet 1954
<i>S. sphacelata</i>	Africa	18	Gupta and Singh 1977; Bir and Sahni 1986, 1987; Sahni 1989	54	Gupta and Singh 1977
<i>S. sphacelata</i>	Africa	27	Gupta and Singh 1977		
<i>S. sulcata</i>	Africa	16	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	32	Oliveira Freitas-Sacchet et al. 1980
<i>S. sulcata</i>	Africa	18	Olorode 1975; Dujardin 1978	36	Quarín 1977
<i>S. tenacissima</i>	America	?		36	S. Sede, A. Escobar, O. Morrone, and F. O. Zuloaga, unpublished manuscript
<i>S. vaginata</i>	America	18	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	?	
<i>S. verticillata</i>	Europe	9	Christopher and Abraham 1976	18	de Wet 1954; Wu and Bai 2000
<i>S. verticillata</i>	Europe	18	Gupta and Singh 1977; Bir and Sahni 1986; Bala and Sachdeva 1989, 1990; Sahni 1989	36	Váchová and Feráková 1980
<i>S. verticillata</i>	Europe	27	Christopher and Abraham 1976; Gupta and Singh 1977; Mehra 1982; Bir and Sahni 1983, 1985; Bala and Sachdeva 1989, 1990; Sahni 1989; Sinha et al. 1990	54	Khosla and Sharma 1973; Gupta and Singh 1977; Sinha et al. 1990
<i>S. verticillata</i>	Europe	36, 54	Bir and Sahni 1986; Sahni 1989		
<i>S. verticilliformis</i>	?	?		?	
<i>S. viridis</i>	Europe	9	Christopher and Abraham 1976; Gupta and Singh 1977; Koul and Gohil 1988, 1991	18	Khosla and Sharma 1973; Chopanov and Yurtsev 1976; Magulaev 1976; Váchová 1978; Kliphuis and Wieffering 1979; Belaeva and Siplivinsky 1981; Löve and Löve 1981; Guzik 1984; Li and Chen 1985; Kozuharov and Petrova 1991; Xu et al. 1992
<i>S. viridis</i>	Europe	18	Saxena and Gupta 1969; Löve and Löve 1981; Mulligan 1984		
<i>S. vulpiseta</i>	America	9	S. Sede, A. Escobar, O. Morrone, and F. O. Zuloaga, unpublished manuscript	36	Pensiero 1999
<i>S. vulpiseta</i>	America	18	Oliveira Freitas-Sacchet 1980; Oliveira Freitas-Sacchet et al. 1984	54	Norrmann et al. 1994
<i>S. vulpiseta</i> var. <i>reversipila</i>	America	?		?	

Note. Question mark indicates “unknown.”

previously suggested to be related to *S. viridis* (Fairbrothers 1959), along with another accession of the weedy polyploid *S. verticillata*.

Clade X is a large South American clade that appears in all analyses (85/84/100). This analysis is the first to sample this group extensively and thus to document its monophyly. Three subclades appear among the South American species. The first includes *S. alonsoi*, *S. leucopila*, *S. macrostachya* (two accessions), *S. pampeana* (two accessions), *S. pflanzii*, *S. lachnea*,

S. mendocina, *S. oblongata*, and *S. vulpiseta* var. *reversipila*. A second subclade includes *S. fiebrigii* (two accessions), *S. globulifera*, *S. rosenfurtii*, *S. scabrifolia*, and *S. vaginata*. The third clade includes a second accession of *S. lachnea* and a second of *S. vulpiseta*.

Clade XI consists of many, but not all, of the species of *Paspalidium* included in the analysis. These are all Australian species, and they are morphologically quite dissimilar from most of the species classified as *Setaria* (but see below). As the

generic name implies, species of *Paspalidium* look quite similar to the unrelated genus *Paspalum*. Most *Paspalidium* species have a relatively small number of primary inflorescence branches, each of which bears a relatively large number of spikelets; each primary branch terminates in a short bristle, but there are no bristles elsewhere in the inflorescence. The three accessions of *P. flavidum* form a moderately well supported clade, as do *Paspalidium basicladum* and *Paspalidium constrictum*. *Plagiosetum refractum* (= *Paraetaenum*) is an Australian species placed as sister to clade XI but without support. *Paspalidium rarum* is sister to clade XI plus *Plagiosetum* Benth., and it is supported in this position in the Bayesian analysis but not in the two bootstrap analyses. The node is supported by a single mutation, a nonhomoplasious, nonsynonymous G-to-T transversion at position 2015. This is a second position change that causes the amino acid to change from glycine to valine.

Setaria magna consistently appears with the *Paspalidium* clade, with moderate support from the Bayesian analysis but none from the two bootstrap analyses. This result is surprising because *S. magna* is morphologically quite different from *Paspalidium* and is a North American, rather than Australian, taxon.

Clade XII is a strongly supported (93/87/100) pairing of *Paspalidium retiglume* with *Uranthoecium truncatum*. The two species are morphologically distinct, with *P. retiglume* restricted to Western Australia. *Paspalidium geminatum* is sister to clade XII but without support, and *Stenotaphrum secundatum* is sister to *P. geminatum* plus clade XII, also without statistical support. As in species of *Paspalidium*, the secondary branches of *Stenotaphrum* end in a bristle; however, the main inflorescence axis of *Stenotaphrum* is broad and flattened, with the secondary branches embedded in it, a character not found in species of *Paspalidium*. Our data hint at the possibility that *Stenotaphrum* may be derived from a *Paspalidium*-like ancestor.

The *ndhF* sequences used in this analysis do not vary greatly among the species of the ingroup, leading to short internal branches in the tree. The short internal branches and lack of variation, in turn, mean that the data have little power to reject alternative phylogenetic hypotheses. We used S-H tests to determine whether we could reject alternative topologies but found that none could be statistically rejected by the data (table 2). The MP and Bayesian topologies were not significantly worse than the ML topology, as expected, given the considerable similarity among the trees. We tested the possible monophyly of *Pennisetum* and also the monophyly of *Pennisetum* plus *Cenchrus*. The latter case was a test of whether we can reject the possibility that *Odontelytrum* falls outside the *Pennisetum/Cenchrus* clade. Because we are interested in determining the limits of the genus *Setaria* for an upcoming monograph (O. Morrone, S. S. Aliscioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg, unpublished manuscript), we tested whether we could reject monophyly of *Setaria* or *Setaria* plus *Paspalidium*, but we could not reject either. Other tests of the monophyly of smaller clades are listed in table 2, which shows nonsignificant *P* values for all constraints.

The S-H test is known to be biased toward nonsignificance, particularly when the number of comparisons is large. We therefore also did an AU test as implemented in CONSEL (Shimodaira and Hasegawa 2001). This test is more sensitive but is also biased toward finding significant differences where there are none (Type I errors) and thus should be interpreted

with caution. The AU test firmly rejects monophyly of *Pennisetum* ($P < 0.007$), whereas monophyly of *Pennisetum* plus *Cenchrus* cannot be rejected, with or without inclusion of *Odontelytrum*. The AU test also rejects monophyly of African species of *Setaria* but not that of African *Setaria* except *S. restioidea*. This is evidence that the latter is indeed unrelated to other African species of the genus. Monophyly is also rejected for African taxa excluding *Ptychophyllum* and for all Australian taxa. Likewise, this test rejects monophyly of *Paspalidium* and of Australian *Paspalidium*. Monophyly of South American *Setaria* is rejected, probably reflecting the disparate placements of *S. magna* and members of clade II. Monophyly of *Setaria* alone is also rejected ($P < 0.036$), as is monophyly of *Setaria* plus *Paspalidium* ($P < 0.007$), but the data do not permit rejection of a broad concept of *Setaria* that would include *Ixophorus* and *Zuloagaea*.

For several species, we were able to obtain only partial sequences because of limited and/or poor-quality material, but we wished to include them on a provisional basis as a hypothesis for further testing. We were able to obtain a good sequence for *S. atrata* only for positions 1–1535, whereas for *S. orthosticha* we had data only from bp 962 to the 3' end, and for *S. nicorae* we were confident only of the 3' end. The sequence for *Paspalidium udum* was not fully double-stranded, and the sequences were of poor quality. The position of *S. grandis* was unstable in preliminary analyses, as was that of *S. appendiculata*, so these were removed from the main analyses on the grounds that their placement was uninformative and obscured other relationships.

Four ML trees were recovered with the provisional sequences included; these were largely congruent with trees from the other analyses (fig. 2). The likelihood score was 7507.69467. Analysis of the modified data set with the parsimony ratchet retrieved 3809 trees of 434 steps, with CI = 0.4988 and RI = 0.8306.

Setaria atrata fell in a weakly supported position sister to clade III, whereas *S. appendiculata* and *S. grandis* were in a larger group including clades II and III plus *S. cernua* and *Pseudoraphis paradoxa* in some of the trees; this group collapses in the consensus of the four trees (asterisk in fig. 2). *Setaria orthosticha* fell in clade IV with the other African taxa, as expected, and *S. nicorae* fell with the South American species of clade X. *Paspalidium udum* was sister to clade XII, but without bootstrap support, within the larger unsupported clade that included *P. geminatum* plus *Stenotaphrum*.

Discussion

Major Results and Comparison with Other Studies

The monophyly of the bristle clade was demonstrated by previous molecular phylogenetic studies, but this conclusion was based on only a handful of species sampled (Gómez-Martínez and Culham 2000; Duvall et al. 2001; Giussani et al. 2001; Aliscioni et al. 2003). Data presented here greatly increased the sample of *Setaria* species and of other genera with inflorescence bristles (e.g., *Pseudoraphis* Griff., *Spinifex* L., *Zygochloa* S.T. Blake, *Uranthoecium* Stapf, and *Plagiosetum* Benth.). Monophyly of the bristle clade is confirmed.

The analyses presented here build on those presented previously by Doust and Kellogg (2002), Kellogg et al. (2004),

Table 2
Results of Shimodaira-Hasegawa (S-H) and Approximately Unbiased (AU) Tests

Constraint ^a	–ln L ^b	Diff –ln L ^c	<i>P</i> _{S-H} ^d	<i>P</i> _{AU} ^e
Unconstrained	7345.59754	Best		
Parsimony strict consensus	7350.60514	5.00760	.901	.445
Bayesian 50% majority rule	7362.03801	16.44046	.649	.097
<u>African <i>Setaria</i></u>	7373.16556	27.56802	.397	.031*
<u>African <i>Setaria</i> except <i>S. restioidea</i></u>	7348.30893	2.71139	.975	.574
All Australian taxa	7378.82214	33.22459	.278	.001**
<u>Australian <i>Paspalidium</i></u>	7373.24268	27.64513	.401	.044*
<u>African taxa other than <i>Ptychophyllum</i></u>	7383.55432	37.95678	.215	<.0001**
<u><i>Paspalidium</i></u>	7380.96048	35.36294	.267	.013*
<u><i>Pennisetum</i></u>	7375.91471	30.31716	.343	.007**
<i>Pennisetum</i> + <i>Cenchrus</i>	7345.59754	.0000	.998	.784
<i>Pennisetum</i> + <i>Cenchrus</i> + <i>Odontelytrum</i>	7345.59754	.0000	.998	.784
<i>Ptychophyllum</i>	7347.09661	1.49907	.951	.602
South American <i>Setaria</i>	7385.91153	40.31399	.196	.003**
<u><i>Setaria</i></u>	7381.01154	35.41400	.254	.036*
<u><i>Setaria</i> + <i>Paspalidium</i></u>	7395.94014	50.34260	.116	.007**
<i>Setaria</i> + <i>Ixophorus</i> + <i>Zuloagaea</i>	7366.89720	21.29966	.541	.179
<i>Paspalidium</i> + <i>Stenotaphrum</i>	7348.93628	3.33874	.983	.357
<i>Paspalidium</i> + <i>Uranthoecium</i> + <i>Plagiosetum</i>	7352.44773	6.85019	.955	.429

Note. Underlining indicates monophyletic groups significantly rejected by the data under the AU test.

^a “Unconstrained” refers to the best maximum likelihood (ML) tree; parsimony strict consensus and Bayesian 50% majority rule are the best trees from the maximum parsimony and Bayesian analyses, respectively. All other constraints indicate the group constrained to be monophyletic.

^b Log likelihood of the tree when the same model as for the ML tree is used.

^c Difference between the log likelihood of the constrained tree and the best tree.

^d *P* value for the difference under the S-H test

^e *P* value for the difference under the AU test.

* *P* ≤ 0.05.

** *P* ≤ 0.01.

Bess et al. (2006), and Doust et al. (2007), although the number of species is more than double that in the Doust et al. study. In particular, this study adds considerably to our knowledge of relationships among African and South American species of *Setaria*. Major results include the discovery of large, geographically coherent clades corresponding to African *Setaria*, South American *Setaria*, and Australian *Setaria/Paspalidium* (see “Implications for Morphological Evolution”). As in previous studies, the *Pennisetum/Cenchrus* clade is monophyletic, but in this analysis support for the relationship is stronger than that retrieved previously.

In the course of this study, we discovered an error in our previously published work (Bess et al. 2006; Doust et al. 2007). Those analyses had placed *S. macrostachya* (a New World species) in a clade otherwise consisting of African species. On rechecking the voucher specimens, we discovered that the original plants that we had grown (from seeds from the USDA) were in fact *S. macrostachya*; however, voucher specimens taken several years later from the same pot indicated that the pot had become contaminated with *S. parviflora*. Thus, the published sequence of “*S. macrostachya*” (GenBank AY029678) was generated from *S. parviflora*. The name is corrected in the present tree and in GenBank.

The number of potentially informative characters in the ingroup (147) in this analysis is small relative to the number of

ingroup taxa (108); this alone provides a partial explanation for the lack of resolution of the backbone of the tree. Nevertheless, the high RI suggests that the characters included have generally low homoplasy and are quite informative. This is reflected in the high MP and ML bootstrap values for a number of the clades. The fact that many accessions have nearly identical sequences also indicates that the variation, though low, is an accurate reflection of the patterns of mutation in the sequences.

The use of DNA from herbarium specimens for several taxa increases the possibility of error. For these specimens, the gene had to be amplified in four fragments rather than the two overlapping fragments used for fresh or silica-dried material. The inability to amplify large fragments presumably points to degradation of the DNA. The small number of informative characters, combined with the possibility of error, means that the placement of some taxa, particularly those in boldface in figure 2, should be considered provisional until confirmed by additional genes and accessions.

The lack of resolution of the tree also led to the nonsignificant S-H tests. The AU test was able to reject a number of possible groups, indicating that the data do have some power. However, the AU test is biased in the opposite direction from the S-H test (in favor of rather than against finding significant differences), so these results must be verified with another data set.

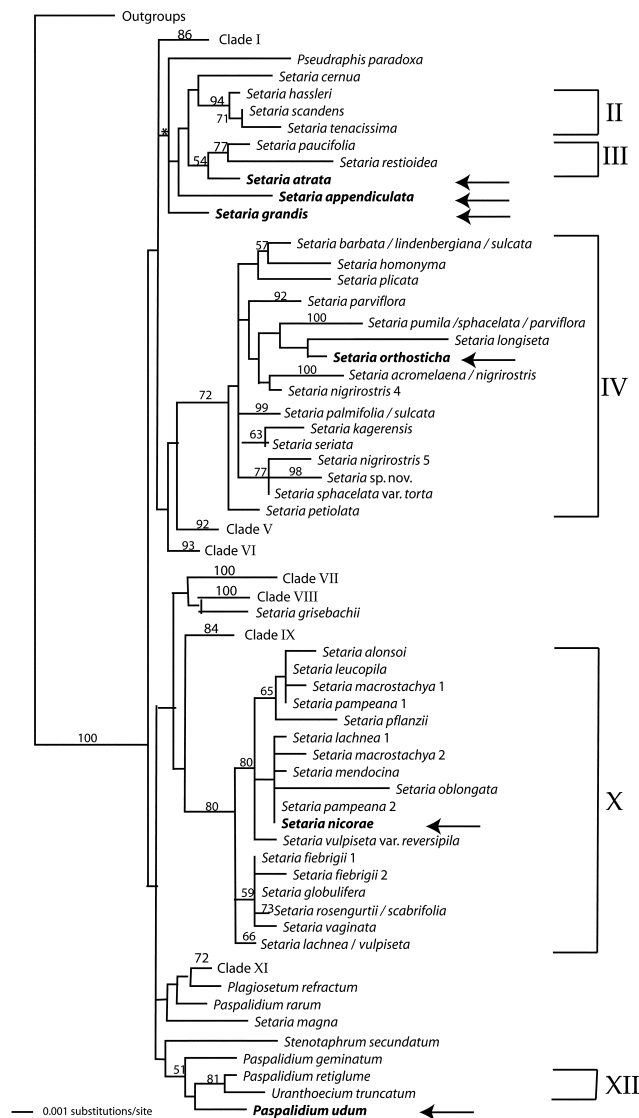


Fig. 2 Maximum likelihood phylogeny of the bristle clade, showing provisional placement of six partial sequences, indicated by arrows and boldface. Numbers above branches are parsimony bootstrap values. Clade numbers as in figure 1.

Polyploidy

For several taxa, multiple accessions are placed differently in the different analyses. For most such taxa, chromosome numbers indicate polyploidy (table 1). Multiple origins of allopolyploids are not uncommon, and our data suggest that this may have occurred in *Setaria*. Allotetraploids have been reported in *S. verticillata* and *S. faberi* (Benabdelmouna et al. 2001b). Our data also suggest multiple possible allopolyploid origins of *Setaria sulcata* and *S. parviflora*, with different maternal parents leading to distinct placements of *ndbF* sequences in this phylogeny. This will have to be verified by studies of nuclear genes, such as those presented by Doust et al. (2007). We do not have chromosome numbers for the individual accessions used in this study and so are unable to assess whether their disparate placements reflect different ploidal levels, although this

is certainly possible. Members of other widespread polyploid complexes, such as *S. sphacelata* and *S. nigrirostris*, appear to have similar *ndbF* sequences and to form groups that are monophyletic or nearly so.

Biogeography

Major clades in the phylogeny correspond to geographic groups. However, because the relationships among the major clades are unclear, the biogeographic history of the group is also obscure. Some authors have suggested that tropical Africa might be the center of origin and diversification of the genus (Stapf and Hubbard 1930; Lakshmi and Ranjekar 1984). However, other authors have questioned how the species might have arrived in Eurasia after migration out of Africa (Rominger 1962; Prasada Rao et al. 1987; Simpson 1990), and even more problematic is the route by which *Setaria* might have dispersed to the New World (Dekker 2003).

Implications for Morphological Evolution

The phylogeny shows several morphologically distinctive clades—for example, *Pennisetum/Cenchrus/Odontelytrum*, *Spinifex/Zygochloa*, and *Paspalidium*—intermingled with other clades with no apparent morphological synapomorphies. Many of the clades in the latter category make up a paraphyletic *Setaria*. Despite the lack of morphological synapomorphies, however, most of the clades supported in the analyses are geographically coherent.

The morphological characters used traditionally to circumscribe the infrageneric categories as sections, subgenera, or informal groups in *Setaria* (Rominger 1962; Clayton and Renvoize 1986; Pensiero 1999; O. Morrone, S. S. Aliscioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg, unpublished) appeared as homoplasies in the phylogeny presented here. Thus, the previously recognized infrageneric groups are not monophyletic and must be taxonomically recircumscribed. The current tree is not strongly supported enough to warrant taxonomic revisions, but several preliminary observations are possible.

South American species. Clade II is composed of *S. hassleri*, *S. scandens*, and *S. tenacissima*. These three species are distributed from southern Mexico, Central America, and the Antilles to South America but are not related to the rest of the South American species. Like other typical species of *Setaria* with “bottle-brush inflorescences,” members of clade II have cylindrical, dense, and continuous spiciform panicles. Unlike other *Setaria* species, however, they have both antrorse and retrorse prickles on the same bristle. Therefore, this character represents a synapomorphy of this group. In addition, these species are annual, differing from the South American species in clade X, which are perennial.

Clade X is one of the mostly strongly supported groups in this analysis and includes the majority of the species sampled from South America (*S. alonsoi*, *S. fiebrigii*, *S. globulifera*, *S. lachnea*, *S. leucopila*, *S. macrostachya*, *S. mendocina*, *S. nicorae*, *S. oblongata*, *S. pampeana*, *S. pflanzii*, *S. rosengurtii*, *S. scabrifolia*, *S. vaginata*, and *S. vulpiseta*). A few of these extend their range as far as North America. These species are perennials and have panicles that are usually contracted and spiciform or occasionally pyramidal, with spikelets associated with one bristle

(rarely two); the bristles always bear antrorse prickles. No obvious morphological characters correlate with the species groups identified within this clade; however, the weak support for the various species groups suggests that additional analyses should be undertaken with more variable markers.

The last South American species included in this analysis is *S. paucifolia*, a caespitose perennial species of wet habitats characterized by linear, rigid, and sparse leaves. The upper lemmas have long hairs at the margins, the upper paleas have short cilia, and the bristles also bear long hairs (Pensiero 1988), all characters that are unique among South American species, although the African species *S. atrata* also may bear hairs on the bristles. While *S. paucifolia* is quite distinct from other South American species, it is morphologically similar to the African species *S. restioidea*, although the latter lacks hairs on the bristles. The two are sisters in clade III. Similarities have also been noted among *S. paucifolia*, *S. restioidea*, and *S. atrata* (= *S. rigida* Stapf, fide O. Morrone, S. S. Alicioni, F. O. Zuloaga, J. F. Pensiero, and E. A. Kellogg, unpublished manuscript; Clayton and Renvoize 1982, 520–541; Pensiero 1999). Our partial sequence of *S. atrata* is sister to *S. paucifolia* and *S. restioidea*, as expected from its morphology. Thus, morphological and phylogenetic data suggest that *S. paucifolia* is closely related not to other South American species but to the African species *S. restioidea* and *S. atrata*. The possible Africa–South America disjunction will require additional sampling and a focused study of this group of species.

Setaria cernua is a South American species sister to clade II in likelihood analyses, sister to clade X in parsimony analyses, and in a polytomy with clades VII, VIII, and X in Bayesian analysis. It is morphologically distinct from all other South American species, being characterized by the presence of conspicuous superficial rhizomes up to 1 cm in diameter, tillers with many strongly keeled leaves that superficially resemble those of some members of Iridaceae, a single bristle associated with each distal spikelet, and a lower antherium male with three developed anthers, the upper antherium being shorter than the spikelet. Its isolated phylogenetic position thus correlates with its morphological distinctiveness.

Old World species. A robust group recovered in this analysis is clade IX, which includes *S. faberi*, *S. italica*, *S. viridis* (the type species of the genus), and *S. verticillata*. These species are all aggressive annual colonizers in their native Old World and have become established as weeds in the New World (Prasada Rao et al. 1987). They are characterized by contracted spiciform panicles and spikelets associated with one bristle (rarely two to five bristles), with all prickles antrorse, except in *S. verticillata*, which has retrorse prickles. Similarities between *S. faberi* and *S. viridis* were noted by Fairbrothers (1959). *Setaria italica* is a domesticated species derived from wild populations of *S. viridis* (Le Thierry d'Ennequin et al. 2000; Benabdelmouna et al. 2001a).

Clade VI includes *S. verticillata* and *S. adhaerens*, two species with retrorse prickles on the bristles, a distinctive character in the genus that is easily assessed in the field simply by touching the bristles. On the other hand, *S. verticilliformis*, with antrorse prickles, is also included in this clade. *Setaria verticilliformis* has been interpreted as a hybrid between *S. verticillata* and *S. viridis* (Husnot 1896–1899; Léveillé 1917; des Abbayes et al. 1971; Stace 1975; Clayton 1980).

Clade IV represents a large and strongly supported monophyletic group of African and Asian species; these exhibit two quite different sorts of morphology. The first includes annual or perennial plants with leaves usually pseudopetiolate and plicate, panicles usually loose and open, slender spikelets, a single bristle subtending the terminal spikelet of each branch, and an occasional bristle below some of the lower spikelets (*S. barbata*, *S. homonyma*, *S. kagerensis*, *S. longiseta*, *S. lindenbergiana*, *S. petiolata*, *S. plicata*, *S. seriata*, and *S. sulcata*). Plicate leaves are distinctive and unique in *Setaria*, and open panicles are unique in clade IV, but plants with these features do not form a monophyletic group. If plicate leaves and open panicles are synapomorphic, the characters must have reversed several times. In the PHYML analysis, *S. plicata* is sister to all of clade IV, which would support this possible optimization. However, conclusions on the evolution of this character would require a more detailed analysis of the African clade.

The second morphological group of species in clade IV, including *S. acromelaena*, have linear leaves that are not plicate and contracted panicles with numerous bristles associated with each spikelet. Like the plicate-leaved species, the linear-leaved species do not constitute a monophyletic group. *Setaria sulcata* and *S. parviflora* were interpreted by Rominger (1962) and Pensiero (1999, 2003) as American species, but this study suggests that both originated in the Old World and may have been introduced to the New World.

Clade XI includes many of the species of *Paspalidium*. In this group, only the uppermost spikelet on the primary branch is subtended by a bristle, a synapomorphy that may provide evidence of monophyly of all *Paspalidium* plus *Stenotaphrum*. *Paspalidium rarum* is also included in this clade, but the placement is supported only in the Bayesian analysis. This species differs in having inflorescences with the lateral branches reduced to one to three spikelets, a unique morphological feature that distinguishes it from the other species of the clade. The arrangement of the spikelets along the main axis of the inflorescence in *P. rarum* is similar to that in *Stenotaphrum* (Webster 1987), although the latter genus appears more closely related to *Paspalidium geminatum*, *Paspalidium retiglume*, and *Uranthoecium truncatum*. *Paspalidium geminatum* is semi-aquatic (Allen 2003), a habit shared with other African species, such as *Paspalidium obtusifolium* and *Paspalidium punctatum* (Gibbs-Russell et al. 1991); the latter two species were not available for this study but may also fall in this position in the phylogeny.

Clade XII is newly identified in this study and links the rare *P. retiglume* of Western Australia with *U. truncatum*, which has a more eastern distribution on the same continent. Although *Uranthoecium* has short lateral branches similar to those of some other species of *Paspalidium*, it has a disarticulating rachis and distinctive truncate glumes, which together make it look rather different from *Paspalidium*. A better understanding of the identity of *Paspalidium* and allied taxa requires including other morphologically similar American species, that is, *S. pradana* (León) León, *S. reverchonii* (Vasey) Pilg., and *S. utowananaea* (Scribn.) Pilg., among others.

Setaria magna is a distinctive, robust annual species with culms as much as 4 m tall and densely flowered panicles up to 40 cm long; it is found in wetlands, in saline marshes, or

on the shores of lakes. The position of this species as sister to the *Paspalidium* clade (supported only by Bayesian analysis) is a surprising result. *Setaria magna* and *Paspalidium* do not share geographical distributions or obvious macromorphological characters.

Implications for Classification

A major impetus for this study was the need for a comprehensive monograph of the Old World species of *Setaria* to complement the available revisions of the New World species (Rominger 1962; Pensiero 1999, 2003). The monograph, in turn, requires an assessment of generic limits.

In this study, monophyly of traditional *Setaria* is not supported, as evidenced by the significant AU test (table 2). This confirms phylogenetic studies using a nuclear locus (*Knotted1*) in addition to *ndbF* (Doust et al. 2007), which also found that *Setaria* is a collection of unrelated groups. The authors suggested that it is not possible to ally *Setaria* with any other genus to make a monophyletic group, unless the alliance is with all members of the bristle clade.

The most conservative course of action would be to refrain from making any nomenclatural changes and to continue to recognize *Setaria*, *Paspalidium*, and the other major genera, such as *Pennisetum* and *Cenchrus*, even though *Setaria* and *Pennisetum* are paraphyletic. At the other extreme would be the radical solution of placing each of the twelve major clades in its own genus or the even more radical solution of placing the entire bristle clade in a single genus.

New World *Setaria* species with plicate leaves were placed in subgenus *Ptychophyllum* (A. Braun) Hitchc. by Rominger (1962) in his taxonomic revision of North American species and by Pensiero (1999) in his revision of South American species. Rominger (1962) mentioned six species in North America: *S. palmifolia*, native to India, *S. barbata*, apparently native to Africa, *S. sulcata*, and three species from South America (*S. paniculifera*, *S. crus-ardeae*, and *S. speciosa*). Pensiero (1999) has since synonymized *S. paniculifera* with *S. palmifolia* and *S. crus-ardeae* and *S. speciosa* with *S. sulcata*. Our data cannot rule out monophyly of subg. *Ptychophyllum*, but they firmly reject monophyly of the remaining, non-*Ptychophyllum* species from Africa (table 2).

The uncertain position of *S. cernua* supports the decision by Pensiero (1999) to establish a new monotypic subgenus within *Setaria* for this species. If the various clades in this phylogeny were to be recognized as segregate genera, *S. cernua* would probably have to be considered incertae sedis for the time being.

The genus *Paspalidium* was segregated from *Setaria* by Stapf (1920). Later, several authors (Veldkamp 1980; Clayton and Renvoize 1986; Webster 1987; Davidse and Pohl 1992) pointed out intermediate species that prevent a clear distinction between *Paspalidium* and *Setaria*. None of the putatively intermediate species were available for this analysis. Veldkamp (1994) reduced *Paspalidium* to *Setaria*, transferring all Southeast Asian species to the latter genus, including the type *P. geminatum*; Webster (1995) subsequently transferred the Australian species. Our data reject the monophyly of *Setaria* plus *Paspalidium*. In addition, a monophyletic clade of the Australian species *Paspalidium globoideum*, *P. jubiflorum*, *P. basicladum*, *P. constrictum*, *P. flavidum*, and *P. albavillosum*

is recovered in all analyses. (Note that *P. flavidum* is widely distributed, extending from Réunion to southern China and through the Pacific.) *Setaria pradana* (León) León, *S. reverchonii* (Vasey) Pilg., and *S. utowanaea* (Scribn.) Pilg. may also be related to *Paspalidium*, and they must be included in future analyses. These species were placed by Rominger (1962) in subgenus *Paurochaetium* (Hitchc. & Chase) Rominger.

Whatever the fate of *Setaria* and *Paspalidium*, our data support transfer of the African genus *Odontelytrum* to *Pennisetum*. Each spikelet in *Odontelytrum* is subtended by a bractlike involucre with a single bristle; morphologically this appears similar to the involucral bristles common in *Pennisetum* and *Cenchrus* (Clayton and Renvoize 1986), although developmental study would be required to support this rigorously.

Many of the species with plicate leaves have been synonymized under the name *S. sulcata*. Our sample of plants included ones variously determined as *S. palmifolia*, and *S. poiretiana*; these all fell within the groups that also contained *S. sulcata* specimens, lending support to the decision of Pensiero (1999) to interpret these as members of a single widespread species.

Subtribe Cenchrinae Dumort and subtribe Setariinae Dumort are recognized in the Catalogue of New World Grasses (Soreng et al. 2000–), the former containing *Cenchrus*, *Pennisetum*, *Paratheria*, and *Antheophora* and the latter including *Ixophorus*, *Paspalidium*, *Setaria*, *Setariopsis*, and *Stenotaphrum*. In addition, subtribe Spinificinae Owhi is recognized from the Old World. Unpublished *ndbF* data exclude *Antheophora* from the bristle clade (E. A. Kellogg and A. Penly, unpublished data), and molecular data are not available for *Paratheria*. If *Antheophora* is excluded, however, Cenchrinae could be monophyletic.

We suspect that *Setaria*, as currently accepted, will ultimately be split into several genera. However, neither the number of genera nor the relationships among them are resolved by the data presented here, even though we have more than doubled the taxon sample relative to previous studies. Bess et al. (2006) mentioned that a fully resolved phylogeny of the bristle clade will not be available in the near future, and therefore some nomenclatural changes are needed within this group. Additional studies of development, morphology, and anatomy could discover additional synapomorphies that might help develop a more satisfactory taxonomic resolution.

Acknowledgments

We would like to thank Nigel Barker and Syd Rhamdani for assistance in field collecting, Lyn Fish for access to specimens and databases at the herbarium in Pretoria, Surrey Jacobs for material of *Plagiosetum* and *Uranthoecium*, Paulo Camara and Chris Gillispie for help with sequencing, and Scot Kelchner and an anonymous reviewer for comments that greatly strengthened the manuscript. We thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for research grant PIP 5453, Agencia Nacional de Promoción Científica y Técnica (ANPCyT) for grants 11739, 13374, and 32664, the U.S. National Science Foundation for grant DEB-0108501, and the National Geographic Society for research grant 7792-05; grants from Buenos Aires University (UBACYT-G003) and the Myn del Botanica Foundation supported collecting by S. S. Aliscioni.

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