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A classification of the Chloridoideae (Poaceae) based on multi-gene phylogenetic trees

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ABSTRACT

We conducted a molecular phylogenetic study of the subfamily Chloridoideae using six plastid DNA sequences (*ndhA* intron, *ndhF, rps16-trnK, rps16* intron, *rps3*, and *rpl32-trnL*) and a single nuclear ITS DNA sequence. Our large original data set includes 246 species (17.3%) representing 95 genera (66%) of the grasses currently placed in the Chloridoideae. The maximum likelihood and Bayesian analysis of DNA sequences provides strong support for the monophyly of the Chloridoideae; followed by, in order of divergence: a Triraphideae clade with *Neyraudia* sister to *Triraphis*; an Eragrostideae clade with the Cotteinae (includes *Cottea and Enneapogon*) sister to the Uniolinae (includes *Entoplocamia, Tetrachne,* and *Uniola*), and a terminal Eragrostidinae clade of *Ectrosia, Harpachne*, and *Psammagrostis* embedded in a polyphyletic *Eragrostis*; a Zoysieae clade with *Urochondra* sister to a Zoysiinae (*Zoysia*) clade, and a terminal Sporobolinae clade that includes *Spartina, Calamovilfa, Pogoneura,* and *Crypsis* embedded in a polyphyletic *Sporobolus;* and a very large terminal Cynodonteae clade that includes 13 monophyletic subtribes. The Cynodonteae includes, in alphabetical order: Aeluropodinae (*Aeluropus*); Boutelouinae (*Bouteloua*); Eleusininae (includes *Apochiton, Astrebla* with *Schoenefeldia* embedded, *Austrochloris*, *Brachyachne, Chloris, Cynodon* with *Brachyachne* embedded in part, *Eleusine, Enteropogon* with *Eustachys* embedded in part, *Eustachys, Chrysochloa, Coelachyrum, Leptochloa* with *Dinebra* embedded, *Lepturus, Lintonia, Microchloa, Saugetia, Schoenefeldia, Sclerodactylon, Tetrapogon*, and *Trichloris*); Hilariinae (*Hilaria*); Monanthochloinae (includes *Distichlis, Monanthochloe,* and *Reederochloa*); Muhlenbergiinae (*Muhlenbergia* with *Aegopogon, Bealia, Blepharoneuron, Chaboissaea, Lycurus, Pereilema, Redfieldia, Schaffnerella,* and *Schedonnardus* all embedded); Orcuttiinae (includes *Orcuttia* and *Tuctoria*); Pappophorinae (includes *Neesiochloa* and *Pappophorum*); Scleropogoninae (includes *Blepharidachne, Dasyochloa, Erioneuron, Munroa, Scleropogon*, and *Swallenia*); Traginae (*Tragus* with *Monelytrum, Polevansia*, and *Willkommia* all embedded); Tridentinae (includes *Gouinia, Tridens, Triplasis*, and *Vaseyochloa*); Triodiinae (*Triodia*); and the Tripogoninae (*Melanocenchris* and *Tripogon* with *Eragrostiella* embedded). In our study the Cynodonteae still include 19 genera and the Zoysieae include a single genus that are not yet placed in a subtribe. The tribe **Triraphideae** and the subtribe **Aeluropodinae** are newly treated at that rank. We propose a new tribal and subtribal classification for all known genera in the Chloridoideae. The subfamily might have originated in Africa and/or Asia since the basal lineage, the Triraphideae, includes species with African and Asian distribution.

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1. Introduction

The grass (Poaceae) subfamily Chloridoideae was first validly published by Beilschmied (1833), a German botanist and pharmacist, who used an earlier description of sect. Chlorideae by Kunth (1815). That same year, Kunth (1833) published his *Agrostographia Synoptica* in which he recognized the following genera in the group (Chlorideae): *Chloris*, *Ctenium*, *Cynodon*, *Dactyloctenium*, *Eleusine*, *Eustachys*, *Gymnopogon*, *Harpochloa* Kunth, *Leptochloa*, *Microchloa*, *Pleuraphis* Torr., *Schoenefeldia*, *Spartina*, *Triplasis*, and 8 genera now treated as synonyms of *Bouteloua*. Clearly our modern understanding of Chloridoideae is much greater, and there now appear to be more than 1420 species in approximately 140 genera in the subfamily worldwide (Clayton et al., 2008; Watson and Dallwitz, 1992b).

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The core species in the subfamily share two structural synapomorphies: all exhibit Kranz or C_4 leaf anatomy (except *Eragrostis walteri* Pilg. from South Africa; Ellis, 1984) and most have chloridoid bicellular microhairs (broad, short terminal cell the same thickness as the basal cell) present on leaf surfaces. Two main subtypes of C_4 photosynthesis, NAD-ME (nicotinamide adenine dinucleotide co-factor malic enzyme) and PCK (phosphoenolpyruvate carboxykinase), have been found and verified by biochemical assay to occur in Chloridoideae (Gutierrez et al., 1974; Brown, 1977; Hattersley and Watson, 1992). Ecologically, there appears to be some separation in habitat preference according to C_4 subtype (Ellis et al., 1980; Hattersley, 1992). The PCK subtype is thought to represent an apomorphy in grasses because this C_4 cycle appears to be a modification of the NAD-ME subtype (Hattersley and Watson, 1992). In addition, the PCK subtype is known only to occur in grasses, whereas the NAD-ME subtype is also found in other monocot and dicot families (Hattersley and Watson, 1992; Peterson and Herrera Arrieta, 2001).

Other character trends in Chloridoideae include a base chromosome number of $x = 10$, fruits (caryopses) with nonlinear hila that are usually punctiform or small, embryos with elongated mesocotyl internodes, and two non-membranous (fleshy) lodicules (Soreng and Davis, 1998; GPWG, 2001). However, most of these character trends are seen in the closely related subfamilies Aristidoideae, Arundinoideae, Centothecoideae, Danthonioideae, and Panicoideae of the PACCAD clade. A recent morphological and ecological description of the subfamily is given in GPWG (2001). Some salient features include: plants herbaceous, rarely woody, occurring in dry climates, sheaths usually non-auriculate, inflorescence paniculate, racemose, or spicate, spikelets bisexual or unisexual (plants monoecious or dioecious) with one to many fertile florets, usually laterally compressed, usually disarticulating above the glumes, palea well developed, lodicules usually two, fleshy, ovary glabrous, styles and stigmas two, caryopsis with pericarp often free or loose, hilum short, endosperm hard without lipid, embryo with an epiblast (usually), scutellar cleft, and elongated mesocotyl internode.

The Chloridoideae have appeared monophyletic in all previous molecular analyses, however the classification within the subfamily, until recently, has been controversial (Van den Borre and Watson, 1997; Soreng and Davis, 1998; Hilu et al., 1999; Hsiao et al., 1999; Hilu and Alice, 2001; Roodt-Wilding and Spies, 2006; Columbus et al., 2007; Peterson et al., 2007; Soreng et al., 2009). Earlier studies based entirely on morphological characters, while not entirely misleading in depicting closely related genera, were often erroneous in elucidating evolutionary alignment of the tribes (Hilu and Wright, 1982; Van den Borre and Watson, 1997). Clayton and Renvoize (1986) recognized a large Eragrostideae tribe that included 77 genera whereas Columbus et al. (2007) and Peterson et al. (2007), based on parsimony analyses of DNA sequences, have circumscribed a much smaller Eragrostideae (±8 genera) and a very large Cynodonteae (±60 genera) that included the following 10 subtribes: Boutelouinae, Chloridinae, Eleusininae, Gouiniinae, Hilariinae, Monanthochloinae, Munroinae, Muhlenbergiinae, Orcuttiinae, and Traginae. Roodt-Wilding and Spies (2006) investigated phylogenetic relationships among 38 southern African chloridoid species using *trnL-F* and ITS sequences. The largest molecular phylogenetic survey of chloridoid grasses included 80 species in 66 genera (Columbus et al., 2007). A recent plastid multi-gene (three) phylogeny of the grasses incorporating 78 chloridoids provides a good estimate of the tribal relationships among this subfamily (Bouchenak-Khelladi et al., 2008). A major problem with this work is that it contains a large amount of missing data, since 61 of the 78 chloridoid species are based on single gene sequence, 10 species are based on two genes, and only seven species are based on three genes. Consequently, misleading results are reported such as *Schedonorus*, sister to *Lolium* in the Poeae, and *Anisopogon* in the Phaenospermateae are included in their Chloridoideae assemblage (Davis and Soreng, 2007). Placement of these two genera in the Chloridoideae was based on each containing a single *rbcL* sequence. Obviously, *Schedonorus* and *Anisopogon* should have been omitted from their study.

In our study, we provide the latest estimates of the phylogeny within the Chloridoideae by analyzing six sequences from the plastid genome — *rps3* (coding), *rps16* intron, *rps16-trnK* (spacer), *ndhF* (coding), *ndhA* intron, and *rpl32-trnL* (spacer); and one from the nuclear genome — ITS. To do this we assembled a large data set including 254 species in 99 genera. We compare phylogenetic trees based on ITS and plastid datasets, combine the data set in a total evidence tree, discuss previous molecular and morphological studies where appropriate, interpret biogeographical relationships, and present a new classification for the subfamily. Based on our phylogenetic evidence we propose a change in rank for two taxa, the subtribe Triraphidinae and the tribe Aeluropodeae.

2. Materials and methods

2.1. Taxon sampling

The following 8 taxa were chosen as outgroups from the PACCAD clade: one species of *Danthonia* (Danthonioideae), two species of *Rytidosperma* (Danthonioideae), two species of *Aristida* (Aristidoideae), and three species of *Chasmanthium* (Centothecoideae or Centotheceae) (GPWG, 2001; Davis and Soreng, 2007). The Chloridoideae subset of data is partitioned as following: six species of tribe Triraphideae, 34 species (two multiple accessions) of tribe Eragrostideae, 18 species of Zoysieae (one multiple accession) and 188 species (11 multiple accessions) of Cynodonteae. The dataset of Cynodonteae includes three species of subtribe Aeluropodinae, 10 species of Triodiinae, five species of Orcuttiinae, four species of Tridentinae, six species of Tripogoninae, 51 species of (five multiple accessions) of Eleusininae, 11 species of Traginae, one species (one multiple accession) of Hilariinae, three species (one multiple accession) of Monanthochloinae, 13 species of Boutelouinae, 10 species of Scleropogoninae, and 33 species (one multiple accession) of Muhlenbergiinae. In addition, the Cynodonteae includes 31 species (three multiple accessions) with uncertain taxonomic or phylogenetic position.

Voucher information and GenBank numbers for 268 original accessions representing 254 species are given in Appendix A. All vouchers are deposited in the Smithsonian Institution, United States National Herbarium (US). The majority of samples used in this study were collected by PMP during the period from 1984 to 2008. In addition, we sampled older herbarium specimens to maximize the number of genera in the Chloridoideae. Collections from areas we have not visited, i.e., India and Africa, were included.

2.2. DNA extraction, primers design, amplification, and sequencing

All procedures were performed in the Laboratory of Analytical Biology (LAB) at the Smithsonian Institution. DNA was isolated using the BioSprint 96 DNA Plant Kit (Qiagen, Valencia, California, USA) following the protocol of the manufacturer. PCR amplifications were performed in a MJ Research or PE 9700 thermal cycler. Genomic DNA was combined with 1× reaction buffer (200 mM Tris–HCl, 500 mM NH_4) (Bioline Biolase Taunton, Madison, USA) without Mg^{++}, 2 mM $MgCl_2$, 200 mM dNTP's, 1.5 μl of Taq polymerase (Bioline Biolase Taunton, Madison, USA), 40 pmol/μl each of forward and reverse primers. We targeted seven regions for sequencing: three from chloroplast genome large single-copy re-

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gions (LSC) – *rps3* (coding region), *rps16* intron and *3′rps16–5′trnK* (spacer); three from small single-copy regions (SSC) – *ndhF* (coding region), *ndhA* intron, and *rpl32-trnL* (spacer); and nrDNA ITS region. Intergenic spacers *rpl32-trnL* (SSC) and *rps16-trnK* (LSC) are two of the top ranked, most variable non-coding regions for phylogenetic studies in the Angiosperms (Shaw et al., 2007). To our knowledge, *rpl32-trnL* intergenic spacer and *ndhA* intron (SSC) have not been previously used for phylogenetic inference within the Poaceae. We have chosen the widely used *ndhF* gene (SSC) to recover phylogenetic relationships since it proved useful in other groups of grasses (Giussani et al., 2001; Soreng et al., 2007). Another commonly used gene in phylogenetic studies of Poaceae, *matK* (LSC) was initially considered as a comparative region to *ndhF* in our dataset (Hilu et al., 1999; Hilu and Alice, 1999;2001; Soreng et al., 2007; Schneider et al., 2009). However, difficulties with amplification of *matK* over the entire range of species and especially for old samples encouraged us to explore other coding regions of the chloroplast genome that could fit the conditions of easy amplification, of reasonable size with a sufficient amount of parsimony informative characters (PIC's). The *rps3* (LSC) gene was chosen as a substitute for *matK* in our analysis. To our knowledge, the *rps3* region has not been used for phylogenetic inference before. The combined datasets include 1574 sequences of the nuclear and chloroplast genome regions of chloridoid grasses and 49 sequences of the species from the adjacent phylogenetic groups of the PACCAD clade.

Based on sequences of the complete chloroplast genome of *Oryza, Zea, Hordeum, Lolium, Triticum,* and *Agrostis* available from GenBank we designed primers for *rps3* and *ndhA* intron regions and modified or designed Poaceae specific primers for *3′rps16–5′trnK* and *rps16* intron regions. The programme FastPCR 4.0.27 was employed to adjust the temperature and the quality of the newly designed or modified primers in order to increase the PCR efficiency. The sequences, melting temperature, quality, and references for the primers used are given in Table 1. The *rps3* is a ribosomal protein S3 coding gene anchored at the 79300 position, according to the *Oryza* complete genome sequence, and flanked by the rpl22 (L22 – core protein of the large ribosomal subunit encoding region) from 5′ end and intergenic spacer with *rpl16* (L16 essential protein of the large subunit encoding region) from the 3′ end. Unlike its mitochondrial analogue that was previously used in phylogenetic studies (Jian et al., 2008), the plastid *rps3* gene lacks an intron and has reasonable size (~720 bp in *Oryza* and 678–684 bp in Chloridoideae) and is suitable for routine amplification and sequencing with one pair of primers. The fraction value of PIC's to the total sequence length for this region is comparable to the value of such fast-evolving plastid protein-coding genes as *ndhF* (Table 2). Two newly designed primers, *rps3*C28F and *rps3*C697R are anchored in conservative zones close to the 5′ and 3′ ends of the gene. The labelling numbers indicate positions of the primers from the first nucleotide of the start codon according to the *Oryza* sequence. Using this set of primers the portion of the *rps3* gene of approximately 580 bp (excluding primers area) was easily amplified and sequenced for the majority of the samples.

Of roughly 2230 bp of entire *ndhF* gene, ~740 bp fragment of the most variable 3′ end of the region was amplified and sequenced using one set of forward and reverse primers, *ndhF*1311F and *ndhF*2091R (Romaschenko et al., in press). We redesigned primers for amplification and sequencing of the *ndhA* intron making them less degenerate and more suitable for Poaceae than those designed by Small et al. (1998) or Shaw et al. (2005). In addition, the amplification with newly designed primers, *ndhA* × 4 and *ndhA* × 3 was steadier when working with older herbarium material. Both primers are anchored in flanking exons of the *ndhA* gene.

The amplification parameters for all plastid regions were: 95 °C for 3 min; followed by 35 cycles of 94 °C for 40 s, 51–56 °C for 40 s and 72 °C for 1 min 40 s; the temperature of the final extension was set for 72 °C for 10 min. Most of the plastid regions chosen for this study have a sequence length between 579 and 745 bp, which is suitable for routine amplification using standard PCR parameters and one set of primers for each region. The nuclear ribosomal ITS region was amplified using primers ITS4 and ITS5A using the following thermocycler settings: initial denaturation step of 4 min at 95 °C, followed 35 cycles at 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min 30 s, and a final extension of 10 min at 72 °C (Table 1).

All PCR products were cleaned with ExoSAP-IT (USB, Cleveland, Ohio, USA). DNA sequencing was performed with BigDye Terminator Cycle Sequencing v.3.1 (PE Applied Biosystems, Foster City, CA, USA) according to the following parameters: 80 °C, 5 min; 25 or 30 cycles of 95 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Sequenced products were analyzed on an ABI PRISM 3730 DNA Analyzer 7900HT (ABI). The regions *rpl32-trnL*, *rps3*, *rps16* intron, *3′rps16–5′trnK*, *ndhF* (coding region) and ITS were sequenced in one direction. Relatively short regions (500–750 bp) covered by our primers were easily interpreted allowing us to accumulate sequences from different parts of the genome for phylogenetic inference (Shaw et al., 2005;2007). Only *ndhA* intron (~933 bp) was sequenced in both directions and the program Sequencer 4.8 (Gene Code Corporation, 1991–2007) was employed to produce the contig sequence for the entire region.

2.3. Phylogenetic analyses

Sequence alignment was done manually using BioEdit v.7.0.5.3 (Hall, 1999). The indels and ambiguously aligned regions were

Table 1
Regions studied, sequences, melting temperature (°C), and quality of primers used for PCR and sequencing.

Region	Primers	Sequence (5′–3′)	Tm	Quality	Reference
ndhF	ndhF2091R	GACCCACTCCATTGGTAATTC	57.8	70	Romaschenko et al. (in press)
	ndhF1311F	ACTGCAGGATTAACTGCGTT	56.8	113	Romaschenko et al. (in press)
rpl32-trnL	trnL$^{(UAG)}$	CTGCTTCCTAAGAGCAGCGT	60.0	120	As Shaw et al. (2007)
	rpL32-F	CAGTTCCAAAAAAACGTACTTC	53.7	103	As Shaw et al. (2007)
rps16-trnK	rpS16–900F	TATCGAATCGTTGCAATTGATG	53.9	108	Modified rpS16R of Shaw et al. (2005)
	3914PR	CATTGAGTTAGCAACCCAGATA	55.3	105	Modified trnK3914F of Johnson and Soltis (1995)
rps3	rps3C697R	TCTTCGTCTACGAATATCCA	57.8	105	This study
	rps3C29F	TCAGACTTGGTACAACCCAA	53.4	64	This study
rps16 intron	rpS16F	AAACGATGTGGTAGAAAGCAAC	56.3	80	Modified as Shaw et al. (2005)
	rpS16R	ACATCAATTGCAACGATTCGATA	55.0	100	Modified as Shaw et al. (2005)
ndhA	ndhA × 4	CTAGCAATATCTCTACGTGYGATTCG	53.9	55	This study
	ndhA × 3	GACTGTGCTTCAACTATATCAACTG	53.7	69	This study
ITS	ITS5a	CCTTATCATTTAGAGGAAGGAG	53.7	82	Stanford et al. (2000)
	ITS4	TCCTCCGCTTATTGATATGC	55.0	38	White et al., 1990

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Table 2
Summary of six plastid regions and nrDNA ITS used in this study.

	ndhF	*rpl32-trnL*	*rps16-trnK*	*rps3*	*rps16* intron	*ndhA* intron	Plastid	ITS	Combined plastid + ITS
Aligned sequence length (alSL)	796	1389	1222	590	1368	1424	6789	814	7603
Average sequence length (SL)	734	695	723	579	745	933	4409	669	5078
No. of taxa	211	246	244	250	225	219	268	234	268
No. of excluded characters	2	364	213	0	0	0	579	89	668
Proportion of excluded characters (%)	0.3	26.2	17.4	0.0	0.0	0.0	8.5	10.9	8.8
No. of parsimony informative characters (PIC)	286	385	411	152	283	431	1948	428	2377
PIC/SL	0.39	0.554	0.568	0.263	0.380	0.462	0.442	0.640	0.468
PIC/TL	0.237	0.259	0.309	0.263	0.319	0.293	0.259	0.093	0.19.3
Tree length (TL)	1205	1487	1328	577	886	1470	7534	4627	12332
Consistency index (CI)	0.3627	0.4385	0.4819	0.3588	0.5011	0.4687	0.4153	0.209	0.3323
Homoplasy index (HI)	0.6373	0.5615	0.5181	0.6412	0.4989	0.5313	0.5847	0.791	0.6677
Retention index (RI)	0.7899	0.7997	0.8304	0.7876	0.8235	0.8243	0.7932	0.7346	0.7658
Rescaled consistency index (RC)	0.2865	0.3506	0.4002	0.2826	0.4127	0.3864	0.3294	0.1535	0.2545
AIC	GTR + G	GTR + G	HKY + G	TVM + G	TIM + G	GTR + G	GTR + G	GTR + G	GTR + G

excluded from analyses. The length of sequences and amount of excluded data for each region is presented in Table 2. No data was excluded from *rps3*, *rps16* intron, and *ndhA* intron. All gaps were treated as missing data. We used maximum likelihood and Bayesian analysis to infer phylogeny. The maximum likelihood analysis was conducted with the programme GARLI 0.951 (Zwickl, 2006). All separate and combined maximum likelihood analyses were run under single model GTR + I + G. The maximum likelihood bootstrap analyses were performed with the default parameters with "bootstrapreps" option set for 1000 replicates. The majority-rule consensus tree of resulting best trees found for each bootstrap reweighted dataset was constructed in PAUP* 4.0b10 (Swofford, 2000). The output file containing best trees found for each bootstrap reweighted dataset was then read into PAUP* 4.0b10 (Swofford, 2000) where the majority-rule consensus tree was constructed and bootstrap support values were calculated. Bootstrap (BS) values of 90–100 were interpreted as strong support; 70–89 as moderate, and 50–69 as weak.

Bayesian posterior probabilities were estimated using MrBayes 3.01 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2005) and the appropriate evolutionary models were selected using MrModeltest 1.1b (Nylander, 2002). MrModeltest selected models with gamma-distributed rate variation across sites with number of substitution types Nst = 6 for almost all datasets with exception of the *3′rps16–5′trnK* region where the HKY + G (Nst = 2) was selected as best-fitted model by Akaike information criterion (AIC) (Table 2). The plastid dataset and combined plastid + ITS dataset for Bayesian analysis were then partitioned into two subsets that were processed implementing different parameters suggested by MrModeltest concerning the model for among site rate variation, number of substitution types, substitution rates and gamma shape parameter. All other parameters were left at default settings. Each Bayesian analysis was initiated with random starting trees and was initially run for two million generations with sampling frequency of the chains set at the 100th iteration. The analysis was continued until the value of standard deviation of split sequences dropped below 0.01 as convergence diagnostic value (Huelsenbeck and Ronquist, 2001). The fraction of the sampled values discarded as burn in was set at 0.25. Posterior probabilities (PP) of 0.95–1.00 were considered statistically significant.

3. Results

3.1. Analysis of ITS sequences

The number of taxa included in the ITS analysis was 234 (including five outgroup); average sequence length was 669; number of PIC's was 428; and the tree length was 4627 with a consistency index (CI) of 0.209, homoplasy index (HI) of 0.791, retention index (RI) of 0.7346, and a rescaled consistency index (RC) of 0.1535 (Table 2). The best maximum likelihood tree with bootstrap (BS) values shown above the branches and posterior probabilities (PP) shown below the branches is illustrated in Fig. 1. The major tribes within a monophyletic Chloridoideae (BS = 95, PP = 1.00) are well resolved. The tribes Cynodonteae (BS = 69, PP = 1.00) and Zoysieae (BS = 63, PP = 0.92) are sister, sister to this clade (BS = 96, PP = 1.00) is the Eragrostideae (BS = 74, PP = 0.98), and sister to this clade (BS = 100, PP = 1.00) is the Triraphideae (BS = 97, PP = 1.00).

The Cynodonteae is composed of the following 13 well resolved subtribes: Aeluropodinae (BS = 100, PP = 1.00), Boutelouinae (BS = 94, PP = 1.00), Eleusininae (BS = 78, PP = 0.50), Tridentinae (BS = 88, PP = 0.99), Hilariinae (BS = 100, PP = 1.00), Monanthochloinae (BS = 81, PP = 1.00), Muhlenbergiinae (BS = 75, PP = 0.52), Scleropogoninae (BS = 53, PP = 0.87), Orcuttiinae (BS = 100. PP = 1.00), Pappophorinae (BS = 78, PP = 0.96), Traginae (BS = 100. PP = 1.00), Triodiinae (BS = 91, PP = 1.00), and the Tripogoninae (BS = 53). There is very little backbone support in the tree and relationships among subtribes are not well resolved. The Aeluropodinae, Boutelouinae, Orcuttiinae, Triodiinae, and Tripogoninae each contain multiple species in a single genus, *Aeluropus* (3 spp.), *Bouteloua* (12 spp.), *Tuctoria* (2 spp.), *Triodia* (10 spp.), and *Tripogon* (2 spp.), respectively. The Tridentinae includes four genera, *Gouinia, Tridens, Triplasis*, and *Vaseyochloa,* each represented by a single species. The Hilariinae includes two accessions of *Hilaria cenchroides*. The Traginae includes four genera: a polyphyletic *Tragus* that includes *Monelytrum luederitzianum-Willkommia sarmentosa-Willkommia texana* (BS = 69, PP = 1.00) that are sister to four species of *Tragus* (BS = 100, PP = 1.00), sister to all these species is *Polevansia rigida* (BS = 100, PP = 1.00). The Tripogoninae includes *Eragrostiella leioptera, Tripogon yunnanensis*, and *Tripogon spicatus* as a clade (BS = 100, PP = 1.00) and sister to this is *Melanocenchris royleana* (BS = 53). The Monanthochloinae includes three genera: a polyphyletic *Distichlis*, imbedded is a clade with *Monanthochloe littoralis* and *Reederochloa eludens* (BS = 66, PP = 1.00). The Scleropogoninae includes six genera: two species of *Munroa* are sister (BS = 100, PP = 1.00), sister to this is *Dasyochloa pulchella* (BS = 100, PP = 1.00), sister to this clade (BS = 100, PP = 1.00) are two species of *Erioneuron* (BS = 100, PP = 1.00); two species of *Blepharidachne* are sister (BS = 77, PP = 0.99), sister to this is an unsupported clade of *Scleropogon brevifolius*. *Blepharidachne, Dasyochloa, Erioneuron, Munroa*, and *Scleropogon* form a clade (BS = 78, PP = 1.00) and sister to this is *Swallenia alexandrae* (BS = 53, PP = 0.87). The Muhlenbergiinae includes nine genera (10 genera included in the plastid and combined tree): a poly-

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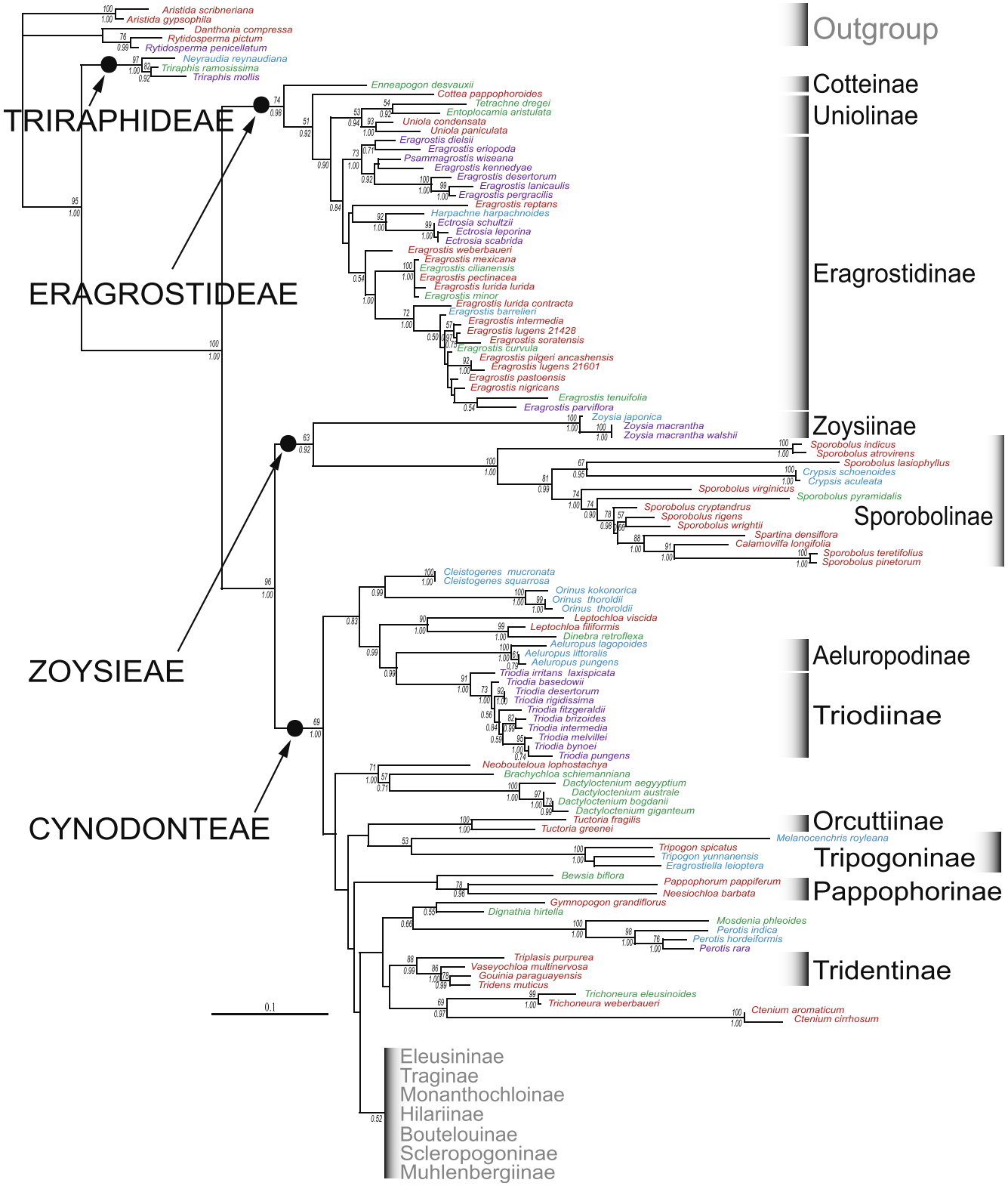

Fig. 1. Phylogram of best maximum likelihood tree from analysis of nuclear ITS data. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values; taxon colour indicates native distribution as follows: green = African, blue = Asian, purple = Australian, red = American (includes North and South America). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

phyletic *Muhlenbergia*, embedded are clades of *Bealia mexicana, Blepharoneuron shepherdii,* and *B. tricholepis* (BS = 86, PP = 0.99); three species of *Pereilema* (BS = 54, PP = 0.63); two species of *Aegopogon* (BS = 100, PP = 1.00); *Redfieldia flexuosa, Schedonnardus paniculatus*; two accessions of *Lycurus setosus* (BS = 100, PP = 1.00); and three species of *Chaboissaea* (BS = 99, PP = 1.00). The Eleusininae includes 20 genera: a polyphyletic *Cynodon* clade (BS = 100, PP = 1.00) that includes *Brachyachne convergens* and *B. tenella*, sister to this *is Chrysochloa hindsii* (BS = 52, PP = 0.65), sister to these is a clade (BS = 84, PP = 0.97) of three species of *Brachyachne* (BS = 100,

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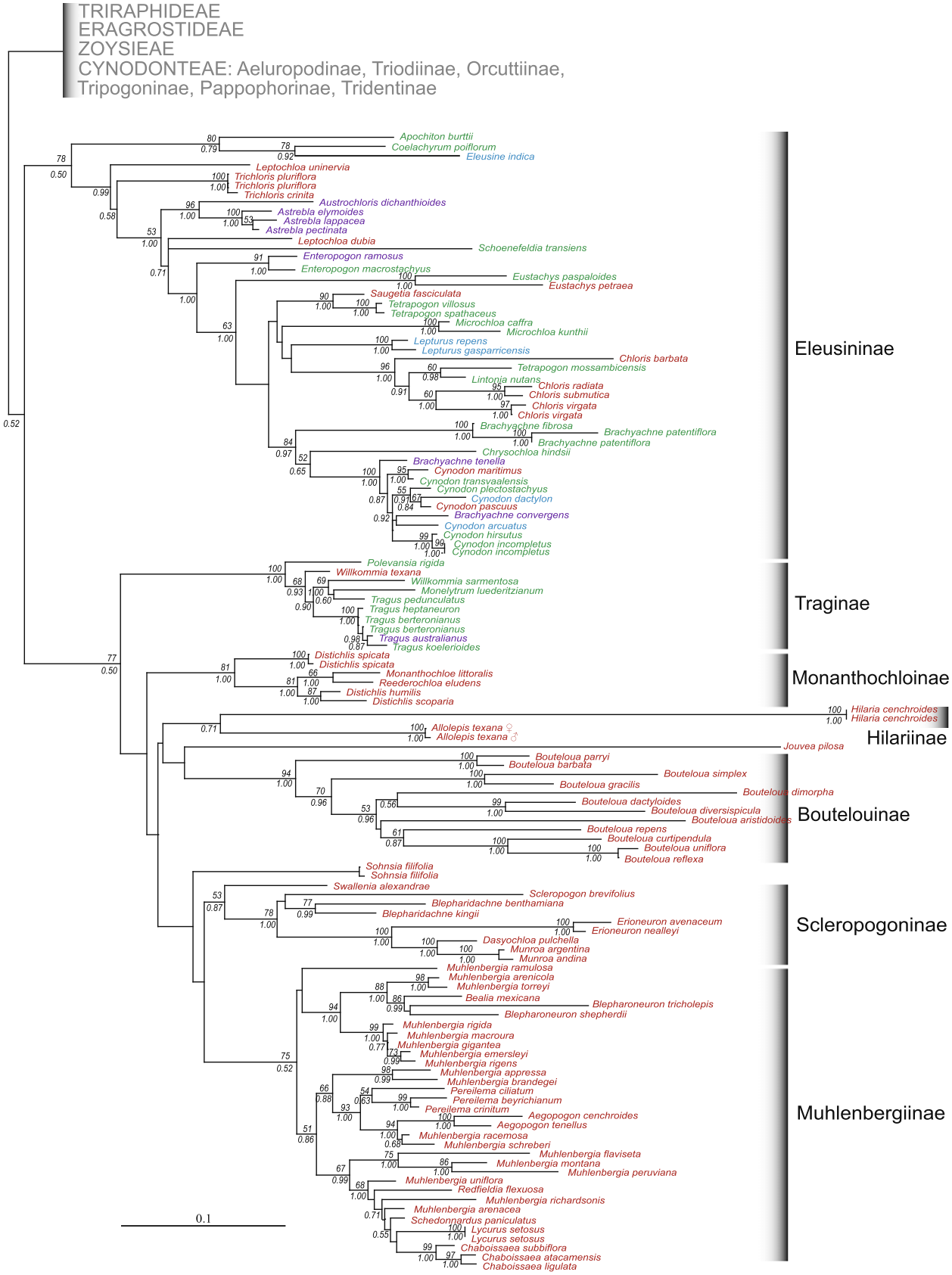

Fig. 1 (*continued*)

PP = 1.00); a polyphyletic *Chloris* clade (BS = 96, PP = 1.00) that includes a clade of *Tetrapogon mossambicensis* and *Lintonia nutans* (BS = 60, PP = 0.98); two species of *Lepturus* (BS = 100, PP = 1.00); two species of *Microchloa* (BS = 100, PP = 1.00); *Tetrapogon spathaceus* and *T. villosus* are sister (BS = 100, PP = 1.00), sister to this is *Saugetia fasciculata* (BS = 90, PP = 1.00); two species of *Eustachys*

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(BS = 100, PP = 1.00); two species of *Enteropogon* (BS = 91, PP = 1.00); three species of *Astrebla* (BS = 100, PP = 1.00) that is sister to *Austrochloris dichanthioides* (BS = 96, PP = 1.00); two species of *Trichloris* that includes two accessions of *T. pluriflora* (BS = 100, PP = 1.00); *Ceolachyrum poiflorum* and *Eleusine indica* are sister (BS = 78, PP = 0.92), sister to this is *Apochiton burtii* (BS = 80, PP = 0.79); and unsupported clades of *Leptochloa dubia, L. uninervia*, and *Schoenefeldia transiens*. The following genera do not align within these 13 subtribes: two species of *Cleistogenes* (BS = 100, PP = 1.00) form a clade (PP = 0.99) with three species of *Orinus* (BS = 100, PP = 1.00); *Leptochloa filiformis* and *Dinebra retroflexa* (BS = 99, PP = 1.00) are sister, sister to this is *Leptochloa viscida* (BS = 90, PP = 1.00); four species of *Dactyloctenium* form a clade (BS = 100, PP = 1.00), sister to this is *Brachychloa schiemanniana* (BS = 57, PP = 0.71), and sister to these is *Neobouteloua lophostachya* (BS = 71, PP = 1.00); *Bewsia biflora* is an unsupported sister to the Pappophorinae; *Dignathia hirtella* and *Gymnopogon grandiflorus* are sister (PP = 0.55); three species of *Perotis* form a clade (BS = 98, PP = 1.00), sister to this is *Mosdenia phleoides* (BS = 100. PP = 1.00); two species of *Ctenium* (BS = 100, PP = 1.00) form a clade, sister to this is a clade (BS = 69, PP = 0.97) with two species of *Trichoneura* (BS = 99, PP = 1.00); male and female accessions of *Allolepis texana* form a clade (BS = 100, PP = 1.00) that is sister (PP = 0.71) with the Hilariinae; *Jouvea pilosa* is an unsupported sister to the Boutelouinae; and two accessions of *Sohnsia filifolia* (BS = 100, PP = 1.00) are sister to an unsupported clade containing the Muhlenbergiinae and the Scleropogoninae.

Sister to the Cynodonteae is the Zoysieae that consists of two well resolved subtribes, the Sporobolinae (BS = 100, PP = 1.00) and the Zoysiinae (BS = 100, PP = 1.00). The Sporobolinae includes a polyphyletic *Sporobolus* with *Calamovilfa longifolia*, *Spartina densiflora*, and a clade of two species of *Crypsis* (BS = 100, PP = 1.00) embedded within. The Zoysiinae is represented by a single genus, *Zoysia* with three taxa in two species. Sister to the Cynodonteae and Zoysieae clade (BS = 96, PP = 1.00) is the Eragrostideae. The Eragrostideae is composed of three subtribes, the Eragrostidinae (PP = 0.90) is sister to the Uniolinae (BS = 53, PP = 0.94), this clade is sister to *Cottea pappophoroides* (PP = 0.90), and sister to all is *Enneapogon desvauxii* (BS = 51, PP = 0.92) (*Cottea* and *Enneapogon* form a well resolved Cotteinae in the plastid and combined tree, see Figs. 2 and 3). The Uniolinae contains a clade of two species of *Uniola* (BS = 93, PP = 1.00) that is sister to a clade with *Entoplocamia aristulata* and *Tetrachne dregei* (BS = 54, PP = 0.92). Within the Eragrostidinae, *Eragrostis* is polyphyletic and embedded within is a clade of three species of *Ectrosia* (BS = 99, PP = 1.00) that is sister to *Harpachne harpachnoides* (BS = 92, PP = 1.00); *Psammagrostis wiseana* is an unsupported member of an Australian clade (BS = 73, PP = 1.00) of *Eragrostis* that includes *E. desertorum, E. dielsii, E. eriopoda, E. kennedyae, E. lanicaulis*, and *E. pergracilis*. The Triraphideae is sister to the all remaining members of the Chloridoideae and this tribe consists of two genera: two species of *Triraphis* (BS = 82, PP = 0.92) form a clade that is sister to *Neyraudia reynaudiana* (BS = 97, PP = 1.00).

3.2. Analysis of plastid sequences

The number of species included in the plastid analysis was 254 (268 total taxa, 14 species with two samples; 8 outgroup); average sequence length was 4409; number of PIC's was 1948; and the tree length was 7534 with a consistency index (CI) of 0.4153, homoplasy index (HI) of 0.5847, retention index (RI) of 0.7932, and a rescaled consistency index (RC) of 0.3294 (Table 2). The overall topology of the plastid-derived phylogram is very similar to the ITS tree, although BS and PP values are usually higher for most clades (Fig. 2). The Eragrostideae (BS = 96, PP = 1.00) and Zoysieae (BS = 97, PP = 1.00) are now strongly supported and the Cynodonteae (BS = 85, PP = 1.00) is moderately supported. The Triraphideae (BS = 91, PP = 1.00) includes a monophyletic *Triraphis* clade (BS = 52, PP = 0.80) of five species that is sister to *Neyraudia reynaudiana*. Within the Eragrostideae, *Cottea pappophoroides* and *Enneapogon desvauxii* form a strongly supported Cotteinae clade (BS = 100, PP = 1.00) that is sister to remaining members (BS = 91, PP = 1.00). The Uniolinae (BS = 80, PP = 1.00) consists of *Entoplocamia aristulata* and *Tetrachne dregei* clade (BS = 74, PP = 1.00) sister to a monophyletic *Uniola* with *U. condensata* and *U. paniculata* (BS = 96, PP = 1.00). The Eragrostidinae still contains a polyphyletic *Eragrostis* (BS = 91, PP = 1.00) with most nodes supported by high BS and PP values. A major clade of *Eragrostis* species primarily from the Americas and Africa (BS = 65, PP = 0.84) is sister to a strongly supported clade of *Harpachne harpachnoides* plus three species of *Ectrosia* (BS = 100, PP = 1.00), together these are sister to a strongly supported Australian clade (BS = 100, PP = 1.00) of *Psammagrostis wiseana* plus five species of *Eragrostis*. In the Zoysieae, *Pogoneura biflora* is embedded within a polyphyletic *Sporobolus* and is aligned in a clade with two species of *Crypsis* (BS = 100, PP = 1.00). The bootstrap and posterior probability values (BS = 64, PP = 1.00) for the Sporobolinae are lower than in the ITS tree. *Urochondra setulosa* is sister to all remaining members of the Zoysieae (BS = 81, PP = 1.00).

In the Cynodonteae, the 13 subtribes are well resolved although the Eleusininae has no bootstrap value (PP = 1.00), Tridentinae (BS = 61, PP = 1.00), Hilariinae (BS = 95, PP = 1.00), Orcuttiinae (BS = 91, PP = 1.00), and Traginae (BS = 92, PP = 1.00) have somewhat lower support values and the Boutelouinae (BS = 100, PP = 1.00), Monanthochloinae (BS = 97, PP = 1.00), Muhlenbergiinae (BS = 95, PP = 1.00), Scleropogoninae (BS = 99, PP = 1.00), and Pappophorinae (BS = 89, PP = 1.00) have higher support values. *Lepturidium insulare*, not in the ITS data set, is the closest sister to the Muhlenbergiinae, followed by *Sohnsia* with weak support (BS = 53, PP = 0.81). Alignment of the taxa within the Muhlenbergiinae is very similar to the ITS tree, although some support values are higher in the plastid phylogeny. *Schaffnerella gracilis* (not in ITS data) is sister (BS = 88, PP = 0.99) to *Lycurus* and together these are sister (BS = 78, PP = 0.99) to the *Chaboissaea* clade (BS = 100, PP = 1.00). The Scleropogoninae is not the immediate sister to the Muhlenbergiinae as in the ITS tree but is sister to the *Sohnsia–Lepturidium*–Muhlenbergiinae clade. Within the Scleropogoninae, *Scleropogon* is the basal member and *Swallenia* is sister to *Blepharidachne*. Monanthochloinae is sister to the Boutelouinae (BS = 77, PP = 1.00). In between the Pappophorinae and the Eleusininae is the Tripogoninae (PP = 1.00) that includes *Melanocenchris* sister to *Tripogon* plus *Eragrostiella* embedded within. This same relationship was recovered in the ITS phylogeny but the Tripogoninae was placed as sister to the Orcuttiinae. The Pappophorinae falls between the Tripogoninae and the Traginae. Within the Eleusininae, *Leptochloa uninervia* is basal followed by *Austrochloris dichanthioides* as sister to four species of *Astrebla* (BS = 63, PP = 0.91) with *Schoenefeldia gracilis* embedded within. *Lintonia nutans* is embedded in a clade with four other species of *Chloris* (BS = 90, PP = 1.00). Five species of *Lepturus* form a monophyletic lineage (BS = 70, PP = 1.00) as does *Microchloa* (BS = 100, PP = 1.00). *Brachyachne chrysolepis, B. fibrosa*, and B. *pateniflora* again form a strongly supported clade (BS = 100, PP = 1.00), and nine species of *Cynodon* form a strongly supported clade (BS = 90, PP = 1.00) with *Brachyachne convergens* and *B. tenella* embedded within (also found in the ITS tree). *Saugetia* forms a strongly supported clade (BS = 99, PP = 1.00) with two species of *Tetrapogon*. In the incertae sedis genera, the *Orinus* clade (BS = 100, PP = 1.00) forms a clade (BS = 70, PP = 1.00) with the Triodiinae (BS = 90, PP = 1.00) and this clade is sister to the Aeluropodinae. *Bewsia biflora* and *Gymnopogon grandiflorus* are sister (BS = 78, PP = 1.00) and they form a clade (BS = 61, PP = 1.00) with two species of *Dignathia* (BS = 98, PP = 1.00).

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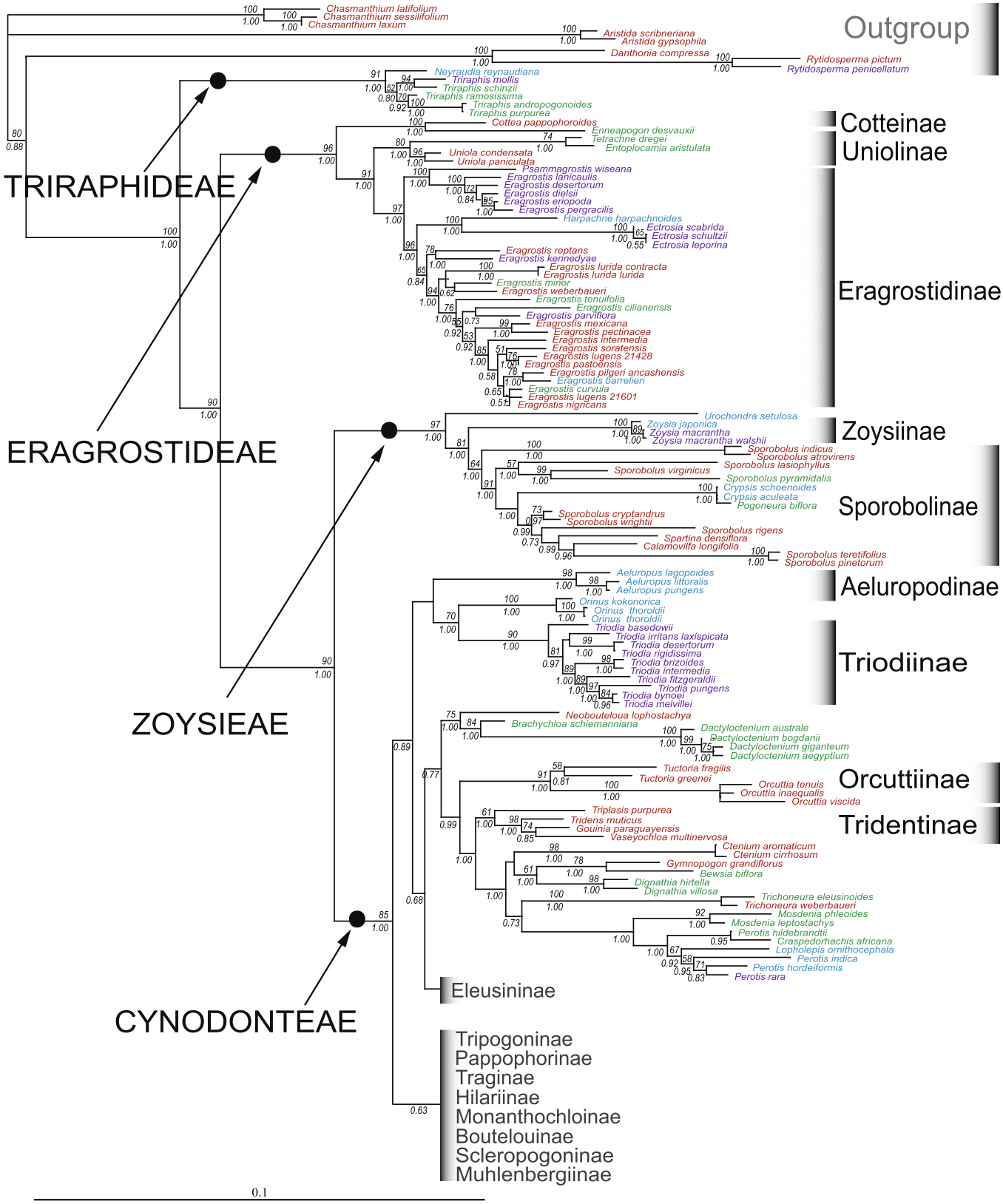

Fig. 2. Phylogram of best maximum likelihood tree from analysis of plastid data. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values; taxon colour indicates native distribution as follows: green = African, blue = Asian, purple = Australian, red = American (includes North and South America). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

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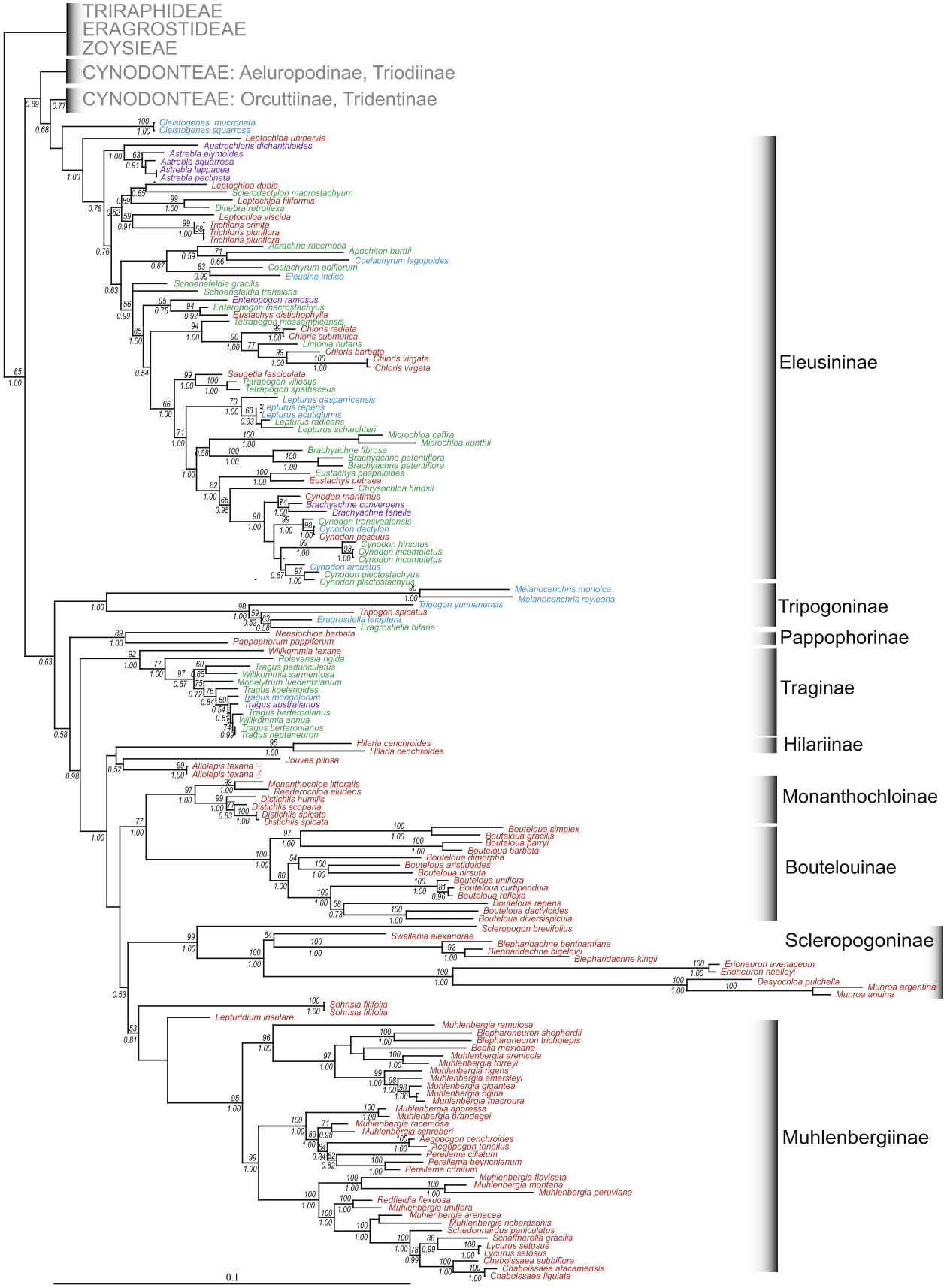

Fig. 2 (*continued*)

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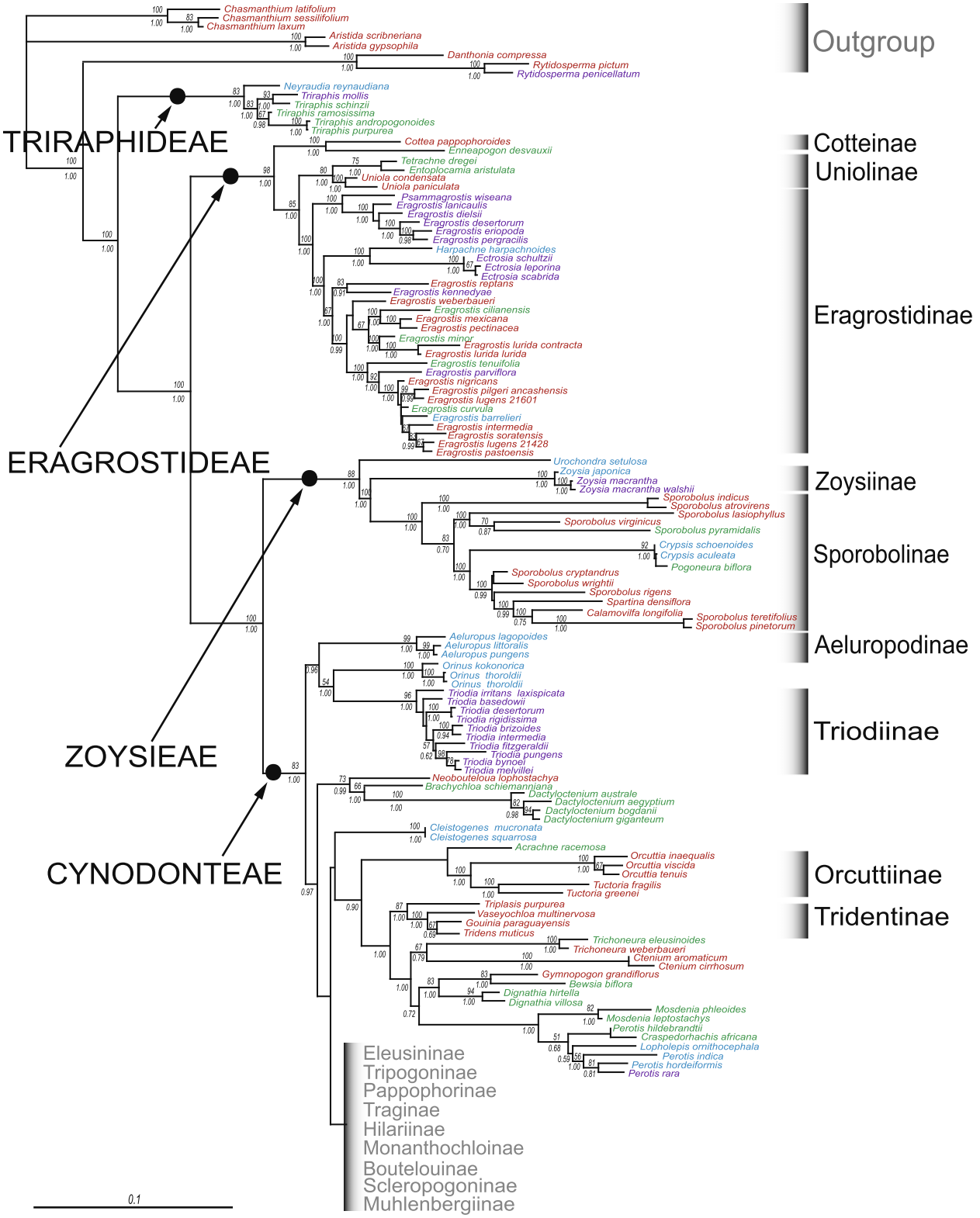

Fig. 3. Phylogram of best maximum likelihood tree from analysis of combined plastid and nuclear ITS data. Numbers above branches represent bootstrap values; numbers below branches are posterior probability values; taxon colour indicates native distribution as follows: green = African, blue = Asian, purple = Australian, red = American (includes North and South America). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

3.3. Combined plastid and ITS sequences

The number of taxa included in the combined plastid and ITS analysis was 268 (8 outgroup); average sequence length was 5078; number of PIC's was 2377; and the tree length was 12332 with a consistency index (CI) of 0.3323, homoplasy index (HI) of 0.6677, retention index (RI) of 0.7658, and a rescaled consistency index (RC) of 0.2545 (Table 2). The overall topology of the com-

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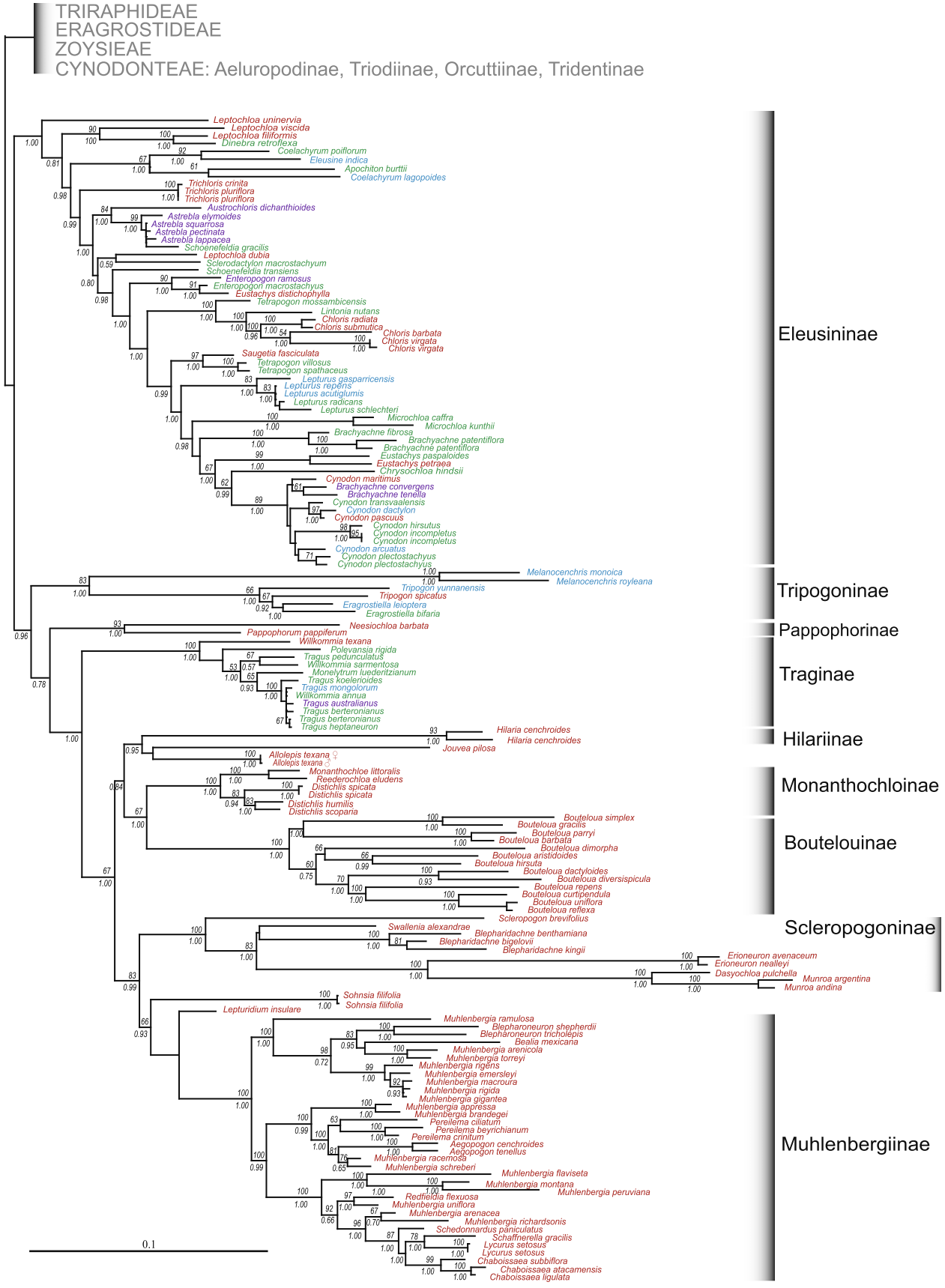

Fig. 3 (*continued*)

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bined plastid and ITS-derived phylogram is similar to the plastid tree. The major tribes within a monophyletic Chloridoideae (BS = 100, PP = 1.00) are well resolved. The Cynodonteae (BS = 83, PP = 1.00) and Zoysieae (BS = 88, PP = 1.00) form a clade (BS = 100, PP = 1.00) and they are sister to the Eragrostideae (BS = 98, PP = 1.00), and sister to all of these tribes is the Triraphideae (BS = 83, PP = 1.00).

Within the Cynodonteae, the Aeluropodinae, Boutelouinae, Hilariinae, Monanthochloinae, Muhlenbergiinae, Scleropogoninae, Orcuttiinae, Pappophorinae, Traginae, and the Triodiinae are strongly supported clades (BS = 93–100, PP = 1.00). The Eleusininae has only posterior probability support (PP = 1.00) and the Tridentinae (BS = 87, PP = 1.00) and Tripogoninae (BS = 83, PP = 1.00) are moderately supported. There is support for relationships among these subtribes since there is some structure within the deep nodes of this phylogram. The Muhlenbergiinae is the most derived subtribe, there is weak support for the *Sohnsia–Lepturidium*–Muhlenbergiinae clade (BS = 66, P = 0.93), and the Scleropogoninae is moderately supported as the sister to *Sohnsia–Lepturidium*–Muhlenbergiinae clade (BS = 83, PP = 0.99). The Boutelouinae and the Monanthochloinae are weakly supported (BS = 67, PP = 1.00) as sister and the Hilariinae–Boutelouinae–Monanathochloinae–Scleropogoninae–*Sohnsia–Lepturidium*–Muhlenbergiinae is weakly supported (BS = 67, PP = 1.00). The Traginae has posterior probability support (PP = 1.00) as sister to the Hilariinae–Boutelouinae–Monanathochloinae–Scleropogoninae–*Sohnsia–Lepturidium*–Muhlenbergiinae clade. Alignment within the Eleusininae is very similar to that found in the plastid tree, again with strong support (BS = 99–100, PP = 1.00) for the following: three species of *Brachyachne*, three species of *Trichloris*, a polyphyletic *Astrebla* with *Schoenefeldia gracilis* embedded, four species of *Chloris*, and two species of *Microchloa*. A polyphyletic *Cynodon* that includes *Brachyachne convergens* and *B. tenella* and five species of *Lepturus* are moderately supported (BS = 89, PP = 1.00).

3.4. Nomenclature novelties

Based on our results we propose a new classification for the Chloridoideae (Table 3) and the following names are necessary to realign the genera now recognized in the subfamily. *Merxmuellera papposa* (Nees) Conert, *M. rangei* (Pilg.) Conert, and the four species now currently placed in *Centropodia* Rchb. are not included in the proposed new classification since their placement as sister to the Chloridoideae is equivocal in all molecular trees to date (Barker et al., 1999, 2000; GPWG, 2001; Roodt-Wilding and Spies, 2006; Bouchenak-Khelladi et al., 2008). One or both genera may possibly align within the Chloridoideae but this has not been thoroughly investigated.

Tribe Cynodonteae, subtribe **Aeluropodinae** P. M. Peterson, stat. nov. — TYPE: *Aeluropus* Trin., Fund. Agrost. 143, pl. 12. 1820. Basionym: Aeluropodeae trib. Nevski ex Bor, Oesterr. Bot. Z. 112: 184. 1965. Included genera: *Aeluropus*.

Tribe **Triraphideae** P. M. Peterson, stat. nov. — TYPE: *Triraphis* R. Br., Prodr. 185. 1810. Basionym: Triraphidinae subtrib. Stapf, Fl. Trop. Afr. 9: 22. 1917. Included genera: *Neyraudia, Triraphis.*

4. Discussion

4.1. Phylogenetic relationships

4.1.1. Tribes and subtribes

In our overall tribal relationships, Cynodonteae and Zoysieae are sisters, and sister to this are Eragrostideae, and sister to all are Triraphideae. This corroborates results by Hilu et al. (1999) using only a few taxa, by Columbus et al. (2007) in their ITS and *trnL-F* study, and by Bouchenak-Khelladi et al. (2008) in their three plastid gene (*rbcL, matK*, and *trnL-F*) survey of the grasses. Only the latter study included members of the Triraphideae, and they found it to be sister to the Eragrostideae, and these in turn sister to a clade containing the Zoysieae and the Cynodonteae. Bell and Columbus (2008a) reported in an oral paper and abstract that their combined data set of ITS, *trnL-F*, and *ndhF* sequences yielded a tree with the following order of divergence: "*Triraphis*, an *Eragrostis* clade, and a *Sporobolus* clade that is sister to a large clade with two sub lineages, one comprised of primarily Old World and cosmopolitan taxa and the other primarily New World." Based on *matK* sequences and a full sampling of genera and species, Hilu and Alice (2001) found support for the recognition of the Zoysieae, Eragrostideae, Cynodonteae, and *Triraphis* but their order of derivation was equivocal. Our hypothesized phylogeny is the first to verify with medium to strong support values the stepwise derivation of the Triraphideae, Eragrostideae, Zoysieae, to the Cynodonteae.

In the Triraphideae, Bouchenak-Khelladi et al. (2008) were first to show strong support for *Neyraudia* and *Triraphis* as being sister. We offer moderate support for the monophyly of *Triraphis* having sampled five of the seven known species. Clayton and Renvoize (1986) pointed out that *Triraphis* was perhaps an ally of *Neyraudia* since both genera possess slender panicoid-like microhairs and the two have keeled lemmas that are villous on the lateral nerves (Watson and Dallwitz, 1992a). Hilu and Alice (2001) and Bouchenak-Khelladi et al. (2008) who both used the same *matK* sequence place this taxon in the Uniolinae. We have ITS and *rps16*-spacer sequences support (although only weakly) for *Tetrachne* as sister to *Uniola*. We have moderate to strong support (BS = 85, PP = 1.00) for order of divergence of the Cotteinae, Uniolinae, and the Eragrostidinae. Within the Eragrostidinae, our data indicate that *Eragrostis* is polyphyletic since three genera (*Ectrosia, Harpachne harpachnoides*, and *Psammagrostis wiseana*) are embedded within. We advocate subsuming the previous three genera within *Eragrostis* since it would be much easier to expand the circumscription than to begin splitting out small clades within this large genus (400 + spp., Simon et al., 2009). *Ectrosia* along with *Pogonarthria squarrosa* (Roem. & Schult.) Pilg. were found embedded within a polyphyletic *Eragrostis* (Columbus et al., 2007). Based on a survey of *rps16* and nuclear waxy gene sequences from a broad range of *Eragrostis* species, Ingram and Doyle (2003, 2004, 2007) have advocated that other small segregate genera such as *Acamptoclados* Nash, *Diandrochloa* De Winter, and *Neeragrostis* Bush be included with *Eragrostis*. Roodt-Wilding and Spies (2006) in their ITS and *trnL-F* strict consensus tree found *Catalepis, Cladoraphis,* and *Pogonarthria* all embedded in a polyphyletic *Eragrostis* clade. There is a geographic signal within our expanded *Eragrostis* since the clade containing *Psammagrostis wiseana, E. desertorum, E. dielsii, E. eriopoda, E. lanicaulis*, and *E. pergracilis*, all endemic to Australia, are sister to the remaining species in the genus. The thickened to coriaceous spikelets of *Psammagrostis* are very similar to those found in *E. dielsii* and *E. lanicaulis* (Clayton and Renvoize, 1986; Lazarides, 1997). *Harpachne* has a raceme inflorescence with reflexed spikelets that are very similar to other species of *Eragrostis* whereas species of *Ectrosia* have 1-nerved glumes and awned lemmas that are 1–3-nerved (Watson and Dallwitz, 1992a; Nightingale and Weiller, 2005a). Mucronate lemmas have been reported for a few species of *Eragrostis* but by addition of the 12 Australian–Malaysian species of *Ectrosia* the circumscription will need emendation.

Sister to the Eragrostidinae is the Uniolinae where we provide the first molecular evidence for *Entoplocamia* and *Tetrachne* (BS = 75, PP = 1.00 in Fig. 3) each monotypic genera from Africa, as being sister to two species of *Uniola*. Clayton (1982) included *Fingerhuthia* Staph and *Tetrachne* in this subtribe, and this has been corroborated with molecular studies (Hilu and Alice, 2001; Colum-

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Table 3
A proposed tribal and subtribal classification of genera in subfamily Chloridoideae (Poaceae). The proposed assignments are based on plastid and nuclear DNA analyses (*= not examined in this study) and/or morphology. See footnotes legend below for placement of taxon by Clayton and Renvoize (1986).

Subfamily Chloridoideae

Incertae sedis: **Afrotrichloris* Chiov., **Daknopholis* Clayton, **Decaryella* A. Camus, **Desmostachya* (Hook.f) Stapf, **Drake-Brockmania* Stapf, **Farrago* Clayton, **Habrochloa* C.E. Hubb., **Halopyrum* Stapf, **Hubbardochloa* Auquier, **Indopoa* Bor, **Kampochloa* Clayton, **Kaokochloa* De Winter, **Leptocarydion* Stapf, **Leptothrium* Kunth, **Lepturopetium* Morat, **Lophachme* Stapf, **Myriostachya* (Benth.) Hook.f., **Neostapfiella* A. Camus, **Ochthochloa* Edgew., **Odyssea* Stapf, **Oropetium* Trin.,**Pogonochloa* C.E. Hubb., **Pommereulla* L.f., **Pseudozoysia* Chiov., **Psilolemma* S.M. Phillips, **Silentvalleya* V.J. Nair, **Tetrachaete* Chiov., **Viguierella* A. Camus

Tribe Triraphideae P.M. Peterson: *Neyraudia* Hook. f.[a], *Triraphis* R. Br.[a]

Tribe Eragrostideae Stapf

Incertae sedis: **Cladoraphis* Franch.

Subtribe Cotteinae Reeder[b]: *Cottea* Kunth[b], *Enneapogon* P. Beauv.[b], **Schmidtia* Steud. ex J.A. Schmidt

Subtribe Eragrostidinae J. Presl[a]: **Catalepis* Stapf, *Ectrosia* R. Br.[a] [includes **Ectrosiopsis* (Ohwi) Ohwi ex Jansen, see Nightingale and Weiller, 2005], *Eragrostis* Wolf[a](includes **Acamptoclados* Nash, **Diandrochloa* De Winter, and *Neeragrostis* Bush), *Harpachne* A. Rich.[a], **Heterachne* Benth., **Pogonarthria* Stapf, *Psammagrostis* C.A. Gardner & C.E. Hubb.[a], **Richardsiella* Elffers & Kenn.-O'Byrne, **Steirachne* Ekman

Subtribe Uniolinae Clayton: *Entoplocamia* Stapf, **Fingerhuthia* Nees ex Lehm., **Stiburus* Stapf, *Tetrachne* Nees, *Uniola* L.

Tribe Zoysieae Benth.[c]

Incertae sedis: *Urochondra* C.E. Hubb.[d]

Subtribe Zoysiinae Benth.[c]: *Zoysia* Willd.

Subtribe Sporobolinae Benth[e]: *Calamovilfa* (A. Gray) Scribn.[d], *Crypsis* Aiton[d], *Pogoneura* Napper[a], *Spartina* Schreb.[f], *Sporobolus* R. Br.[d], **Thellungia* Stapf

Tribe Cynodonteae Dumort.

Incertae sedis: *Acrachne* Wight & Arn. ex Chiov.[a], *Allolepis* Soderstr. & H.F. Decker[g], *Bewsia* Goossens[a], *Brachychloa* S.M. Phillips[a], *Cleistogenes* Keng[a], *Craspedorhachis* Benth.[f], *Ctenium* Panz.[f], *Dactyloctenium* Willd.[a], *Dignathia* Stapf[m], *Gymnopogon* P. Beauv.[f], *Jouvea* E. Fourn.[g], *Lepturidium* Hitchc. & Ekman[f], *Lopholepis* Decne.[m], *Mosdenia* Stent[l], *Neobouteloua* Gould[l], *Orinus* Hitchc.[a], *Perotis* Aiton[l], *Sohnsia* Airy Shaw[a], *Trichoneura* Andersson[a]

Subtribe Aeluropodinae P.M. Peterson: *Aeluropus* Trin.[e]

Subtribe Triodiinae Benth.[g]: **Monodia* S.W.L. Jacobs, **Symplectrodia* Lazarides, *Triodia* R. Br. (includes **Plectrachne* Henrard see Lazarides et al., 2005)

Subtribe Orcuttiinae P.M. Peterson & Columbus[h]: **Neostapfia* Burtt Davy, *Orcuttia* Vasey, *Tuctoria* Reeder

Subtribe Tridentinae Keng & Keng f.[a]: *Gouinia* E. Fourn. ex Benth. & Hook[a] (includes *Schenckochloa* J.J. Ortiz), *Tridens* Roem. & Schult.[a], *Triplasis* P. Beauv.[a], *Vaseyochloa* Hitchc.[a]

Subtribe Eleusininae Dumort.[e]: *Apochiton* C.E. Hubb.[a], *Astrebla* F. Muell.[i], *Austrochloris* Lazarides[f], *Brachyachne* (Benth.) Stapf[f], *Chloris* Sw.[f], *Chrysochloa* Swallen[f], *Coelachyrum* Hochst. & Nees[a](includes *Coelachyropsis* Bor, *Cypholepis* Chiov.), *Cynodon* Rich.[f], *Dinebra* Jacq.[a], *Eleusine* Gaertn.[a], *Enteropogon* Nees[f], *Eustachys* Desv.[f], **Harpochloa* Kunth, *Leptochloa* P. Beauv.[a], *Lepturus* R. Br.[j], *Lintonia* Stapf[i], *Microchloa* R. Br.[f], **Oxychloris* Lazarides, **Rendlia* Chiov., **Rheochloa* Filg., P.M. Peterson & Y. Herrera, *Tetrapogon* Desf.[f], *Trichloris* Benth.[f], *Saugetia* Hitchc. & Chase[k], *Schoenefeldia* Kunth[f], *Sclerodactylon* Stapf[a]

Subtribe Tripogoninae Stapf[a]: *Eragrostiella* Bor[a], *Melanocenchris* Nees[l], *Tripogon* Roem. & Schult.[a]

Subtribe Pappophorinae Dumort.[b]: *Neesiochloa* Pilg.[a], *Pappophorum* Schreb.[b]

Subtribe Traginae P.M. Peterson & Columbus: *Monelytrum* Hack.[m], *Polevansia* De Winter[f], *Tragus* Haller[m], *Willkommia* Hack.[f]

Subtribe Hilariinae P.M. Peterson & Columbus: *Hilaria* Kunth[l], **Pleuraphis* Torr.[n]

Subtribe Monanthochloinae Pilg. ex Potztal[e]: *Distichlis* Raf. (includes *Monanthochloe* Engelm., *Reederochloa* Soderst. & H.F. Decker)

Subtribe Boutelouinae Stapf: *Bouteloua* Lag. (includes *Buchloe* Engelm., **Buchlomimus* Reeder, C. Reeder & Rzed., *Cathesticum* J. Presl, *Chondrosum* Desv., *Cyclostachya* Reeder & C. Reeder, **Griffithsochloa* G.J. Pierce, *Opizia* J. Presl, **Pentarraphis* Kunth, **Pringleochloa* Scribn., and **Soderstromia* C.V. Morton, see Columbus, 1999)

Subtribe Scleropogoninae Pilg.[a]: *Blepharidachne* Hack.[a], *Dasyochloa* Rydb.[o], *Erioneuron* Nash[a], *Munroa* Torr.[a], *Scleropogon* Phil.[a], *Swallenia* Soderstr. & H.F. Decker[g]

Subtribe Muhlenbergiinae Pilg.[d]: *Aegopogon* Humb. & Bonpl. ex Willd.[l], *Bealia* Scribn.[p], *Blepharoneuron* Nash[a], *Chaboissaea* E. Fourn[p], *Lycurus* Kunth[d], *Muhlenbergia* Schreb.[d], *Pereilema* J. Presl[d], *Redfieldia* Vasey[a], *Schaffnerella* Nash[l], *Schedonnardus* Steud.[f]

[a] Placed in tribe Eragrostideae, subtribe Eleusininae.
[b] Placed in tribe Pappophoreae Kunth.
[c] Placed in tribe Cynodonteae.
[d] Placed in tribe Eragrostideae, subtribe Sporobolinae.
[e] Placed in tribe Eragrostideae.
[f] Placed in tribe Cynodonteae, subtribe Chloridinae J. Presl.
[g] Placed in tribe Eragrostideae, subtribe Monanthochloinae.
[h] Placed in tribe Orcuttieae Reeder.
[i] Placed in tribe Cynodonteae, subtribe Pommereullinae Pilg. ex Potztal.
[j] Placed in tribe Leptureae Holmberg.
[k] Placed in *Enteropogon*.
[l] Placed in subtribe Boutelouinae.
[m] Placed in subtribe Zoysiinae.
[n] Placed in *Hilaria*.
[o] Placed in *Erioneuron*.
[p] Placed in *Muhlenbergia*.

bus et al., 2007; Bouchenak-Khelladi et al.,2008). Roodt-Wilding and Spies (2006) in their strict consensus tree of ITS sequence data found strong support for a clade containing *Entoplocamia* and *Fingerhuthia*. All three genera have a raceme inflorescence, a line of hairs for the ligule, and 5–11-nerved lemmas (Watson and Dallwitz, 1992a). The basal member of the Eragrostideae is the Cotteinae and this has been recovered along with the inclusion of *Schmidtia* Steud. ex J.A. Schmidt in other molecular studies (Hilu and Alice, 2001; Columbus et al., 2007; Bouchenak-Khelladi et al., 2008).

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The Sporobolinae in our study includes a polyphyletic *Sporobolus* with *Calamovilfa, Crypsis, Pogoneura*, and *Spartina* embedded within. These genera have been verified as being embedded with *Sporobolus* in other molecular studies with the exception of *Pogoneura* (reported here) (Ortiz-Diaz and Culham, 2000; Hilu and Alice, 2001; Columbus et al., 2007; Bouchenak-Khelladi et al., 2008). Even though *Sporobolus* includes 200 species worldwide (Simon et al., 2009) and sampling within the genus for molecular studies have been rather small (42 species in Ortiz-Diaz and Culham, 2000), we recommend expansion of *Sporobolus* to include these genera. The monotypic genus, *Pogoneura biflora* from east Africa, is morphologically quite distinct from others members of *Sporobolus* since it has 2 or 3-flowered spikelets with short awned lemmas (Clayton and Renvoize, 1986). Sister to the Sporobolinae and Zoysiinae in our plastid and combined trees (we lack ITS sequence) is *Urochondra setulosa*, a monotypic species from northeast Africa. Like *Zoysia, Urochondra* has 1-flowered spikelets with 1-nerved and awnless glumes, 1-nerved lemmas, and lacks lodicules (Watson and Dallwitz, 1992a).

The most derived tribe within the Chloridoideae, the Cynodonteae exhibits a wide range of morphological variation and we currently recognize 13 well supported subtribes. Relationships among these subtribes are fairly well elucidated since there are support indexes at many of the deep nodes in our combined plastid-ITS phylogram. Our clade uniting, in order of divergence, the Hilariinae with *Allolepis* and *Jouvea*, Monanthochloinae, Boutelouinae, Scleropogoninae, *Sohnsia, Lepturidium*, and Muhlenbergiinae is almost entirely New World (western hemisphere) in origin and current distribution.

The Muhlenbergiinae are here represented by 33 species and it is clear that *Muhlenbergia* is polyphyletic and that *Aegopogon, Bealia, Blepharoneuron, Chaboissaea, Lycurus, Pereilema, Redfieldia, Schaffnerella,* and *Schedonnardus* are nested within (Duvall et al., 1994; Columbus et al., 2007;in press; Peterson et al., in review). The subgeneric classification within the Muhlenbergiinae has recently been studied and there is strong support for a subgeneric classification to recognize five clades (Peterson, 2000; Peterson and Herrera Arrieta, 2001; Peterson et al., in review). In the combined plastid and ITS tree in Peterson et al. (in review) there is moderate support for *M.* sect. *Bealia* that includes *Bealia*, two species of *Blepharoneuron*, *M. arenicola,* and *M. torreyi* (BS = 83, PP = 0.95); strong support of *M.* subg. *Trichochloa* that includes *M. emersleyi, M. gigantea, M. macroura, M. rigens*, and *M. rigida* (BS = 99, PP = 1.00); strong support for *M.* subg. *Muhlenbergia* that includes two species of *Aegopogon*, three species of *Pereilema, M. appressa, M. brandegei, M. racemosa*, and *M. schreberi* (BS = 100, PP = 0.99); strong support for *M.* subg. *Clomena* that includes *M. flaviseta, M. montana,* and *M. peruviana*; and strong support for a *M.* unranked *Pseudosporobolus* clade that contains three species of *Chaboissaea*, two species of *Lycurus*, *Redfieldia, Schaffnerella, Schedonnardus, M. arenacea, M. richardsonis*, and *M. uniflora* (BS = 92, PP = 0.66).

Sister to the Muhlenbergiinae–*Lepturidium*–*Sohnsia* clade is the Scleropogoninae that consists of the following six genera: *Blepharidachne, Dasyochloa, Erioneuron, Munroa, Scleropogon*, and *Swallenia*. In a combined ITS and *trnL-F* tree, Columbus et al. (2007) obtained strong support for a clade of *Dasyochloa, Erioneuron*, and *Munroa*. In this same tree Columbus et al. (2007) recovered an unsupported clade containing *Blepharidachne, Scleropogon,* and *Swallenia*. Morphologically, these species share lemmas that are often chartaceous to coriaceous with ciliate margins and usually narrow and condensed inflorescences (Watson and Dallwitz, 1992a). Florets are perfect in *Dasyochloa, Erioneuron*, and *Swallenia*, can be unisexual in *Blepharidachne* and *Munroa,* while individual plants of *Scleropogon* are male or female (dioecious) or sometimes of both sexes (monoecious). We provide the first molecular support for including *Blepharidachne, Scleropogon*, and *Swallenia* in the Scleropogoninae, hence placing Munroinae in synonymy (Peterson et al., 1995).

In our study the Monanthochloinae and Boutelouinae are sister (BS = 67, PP = 1.00 in Fig. 3) and both subtribes have been the subject of molecular studies. Based on ITS and *trnL-F* sequences Columbus (1999) and Columbus et al. (1998, 2000) subsumed nine genera (many of these were unisexual) to accommodate a monophyletic *Bouteloua*. We also support this view since at least three (*Buchloe, Cathestecum*, and *Opizia*) were included in our analysis and all our trees depict a strongly supported Boutelouinae (excluding *Neobouteloua*). In a three gene study (ITS, *ndhF,* and *trnL-F*) of the Monanthochloinae, Bell and Columbus, 2008b proposed expanding *Distichlis* to include two species of *Monanthochloe* and *Reederochloa eludens* (=*Distichlis eludens* (Soderstr. & H. F. Decker) H. L. Bell & Columbus). Our data does not refute this decision and we agree with their interpretation even though our combined and plastid trees placed *M. littoralis* and *Reederochloa* in a clade as sister to the remaining three species of *Distichlis*. Our analysis did not include *M. acerosa* (Griseb.) Speg. (=*Distichlis acerosa* (Griseb.) H. L. Bell & Columbus) or *Distichlis australis* (Speg.) Villamil, both members of a clade with *M. littoralis* (=*Distichlis littoralis* (Engelm.) H. L. Bell & Columbus) and *Reederochloa* that was sister to all other species of *Distichlis* (Bell and Columbus, 2008b).

The Traginae in our study includes a polyphyletic *Tragus* and *Willkommia* with a single species of *Monelytrum* (ditypic) and *Polevansia* (monotypic) imbedded within. Clayton and Renvoize (1986) indicate that *Polevansia* is "like *Willkommia*" in that both have Inflorescences with several racemes on an elongated axis and dorsally compressed spikelets; while *Tragus* and *Monelytrum* have cylindrical false racemes with each branch short pedunculate, dorsally compressed spikelets, and lower glumes that are usually reduced to small scales. Columbus et al. (2007) was first to link *Willkommia* with *Tragus* where they were aligned as a pair with strong support. Our study is the first molecular evidence to link *Monelytrum* and *Polevansia* with *Tragus*–*Willkommia*. Six out of eight possible species of *Tragus* and three of the four species of *Willkommia* are included in our study (Clayton et al. 2008). This is very strong evidence for placing *Willkommia* within *Tragus* and it appears that *Monelytrum* and *Polevansia* should also be subsumed within *Tragus*. Species of this tribe are distributed in Africa and only *Willkommia texana* is endemic to the New World in USA and Argentina.

We provide the first molecular support for including *Neesiochloa* (monotypic) in the Pappophorinae. All nine species of *Pappophorum* and *Neesiochloa barbata* are from the New World. The alignment of this subtribe is interesting since it is not part of the New World clade of Hilariinae–*Jouvea*–*Allolepis*–Monanthochloinae–Boutelouinae–Scleropogoninae–*Sohnsia*–*Lepturidium*–Muhlenbergiinae clade but is found in a different position in each of the three trees.

We have moderate support (BS = 83, PP = 1.00 in the combined tree) for the Tripogoninae where *Tripogon* is polyphyletic with two species of *Eragrostiella* embedded within. Sister to this are two species of *Melanocenchris*. This is the first time that *Eragrostiella* and *Tripogon* have been linked and they are morphologically similar since both have winged paleas, strongly keeled and glabrous lemmas that are 1–3-nerved, and spike to racemose inflorescences (Watson and Dallwitz, 1992a). We recommend realignment of *Tripogon* to include *Eragrostiella*. Columbus et al. (2007) found *Melanocenchris* sister to *Tripogon* in their strict consensus tree based on *trnL-F* and ITS sequences. Most species in this subtribe are from the Old World tropics to Australia and a single species is found in tropical America (Clayton et al., 2008).

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We have low bootstrap values (BS = 78 in ITS tree) but high posterior probability (PP = 1.00 in combined tree) for the Eleusininae and we have pretty good structure for this large and enigmatic subtribe. The Eleusininae as here recognized is a combination of the Pommereullinae, Chloridinae, and Eleusininae sensu Clayton and Renvoize (1986) and earlier authors. Morphologically and geographically this is a very diverse group although most species have racemose inflorescences and many species are found in tropical environments in Africa, Southeast Asia, the Americas, and Australia. Our trees lend support for the monophyly of *Lepturus, Microchloa*, and *Trichloris*; and support the expansion of the following: *Astrebla* (*Schoenefeldia gracilis* embedded in overall tree only), *Chloris* (*Lintonia* and *Tetrapogon mossambicensis* as sister or embedded within), *Cynodon* (*Brachyachne convergens* and *B. tenella* embedded), and *Enteropogon* (*Eustachys distichophylla* embedded in plastid and combined trees). *Brachyachne, Eustachys, Leptochloa*, and *Tetrapogon* are all clearly polyphyletic, although there is strong support for three species of *Brachyachne* (BS = 100, PP = 1.00 in all trees), two species of *Eustachys* (BS = 100, PP = 1.00 in all trees), and two species of *Tetrapogon* (BS = 100, PP = 1.00 in all trees). Clearly there is more work to be done to sort out relationships among these genera and to determine monophyletic lineages. This subtribe recovered by Roodt-Wilding and Spies (2006) whose strict consensus trees obtained from ITS and the combined ITS–*trnL-F* sequences indicates moderate to strong support for a clade containing *Chloris, Cynodon, Eustachys, Harpochloa, Microchloa*, and *Rendlia*.

The identical four genera (*Gouinia, Tridens, Triplasis*, and *Vaseyochloa*) in the Tridentinae were found to have moderate support (BS = 84) by Columbus et al. (2007) in their *trnL-F* + ITS strict consensus tree. Our results are concordant with theirs and we used *Gouinia paraguayensis* and *Triplasis purpurea*, two different species in our study. Members of the Tridentinae usually have pubescent lemma nerves, a line of hairs for the ligule, and keeled florets, although the monotypic *Vaseyochloa* has dorsally rounded florets (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992a). Based on data reported in Columbus et al. (2007), Peterson et al. (2007) erected the subtribe Gouiniinae to include *Gouinia* and *Vaseyochloa*; this name is now placed in synonymy to accommodate the larger assemblage.

The Orcuttiinae is a small group of nine annuals known from California, Baja California, and Baja California Sur whose unusual features of glandular hairs, leaves without ligules, and mushroom-button bicellular microhairs were first noted by Crampton (1959) and later recognized as a tribe by Reeder (1965). We have not sampled *Neostapfia* in our study but we still have strong support for two species of *Tuctoria* being sister to three species of *Orcuttia* (BS = 100, PP = 1.00 in the combined tree). This subtribe was recovered with strong support (BS = 100) in Columbus et al. (2007) in their *trnL-F* + ITS strict consensus tree and by Bouchenak-Khelladi et al. (2008) in their Bayesian consensus tree. Roalson and Columbus (1999) presented a phylogeny based on morphological characteristics that depict *Tuctoria* as a grade. Even though we lack one species of *Tuctoria* and two species of *Orcuttia* it appears that these two genera warrant recognition.

We have strong support for the monophyly of *Aeluropus* (BS = 99, PP = 1.00 in combined tree) and *Triodia* (BS = 96, PP = 1.00 in combined tree), and for their treatment in separate subtribes, the Aeluropodinae and Triodiinae, respectively. We indicate that these two subtribes could be sister (PP = 0.99 in our ITS tree) to one another; and we have weak support for Aeluropodinae as sister to the *Orinus*–Triodiinae clade (BS = 54, PP = 1.00 in our combined tree). A molecular study of ITS, *trnL-F*, and *ndhF* sequences indicated that *Aeluropus* was related to the African *Odyssea* Stapf (not sampled in our study) and the Australian *Troidia* (Bell 2007).

4.1.2. Incertae sedis genera

In the ITS tree species of *Orinus* and *Cleistogenes* form a clade (PP = 0.99) but in the plastid and combined trees they are not aligned near one another. Morphologically, these species although at first appearing very similar, have quite a few distinguishing characteristics with the former having long scaly rhizomes, pungent leaf blade apices, membranous ligules, and rachilla extensions beyond the upper floret, whereas the latter genus is composed of tufted perennials with unarmed leaf blades, a line of hairs for a ligule, and the occurrence of cleistogamous spikelets. *Orinus* contains four species from the Himalayas to western China (three spp. endemic) while *Cleistogenes* contains 13 species with 10 species occurring in China (five spp. endemic) (Chen and Phillips, 2006a,b). The relationship between these two genera warrants further study since we report equivocal results.

In our study *Dactyloctenium* forms a strongly supported monophyletic genus with four species represented, including the Australian, *D. australe*. The genus contains about 13 species mainly from Africa to India and is characterized by digitate inflorescences composed of several linear to narrowly oblong secund spikes (Phillips, 1974). It has been linked to *Eleusine* but differs from the latter by having each raceme terminate in a bare rachis extension rather than a fertile floret (Clayton and Renvoize, 1986). We are the first to report *Brachychloa* as sister with weak support (BS = 66 in combined tree) and sister to these is *Neobouteloua lophostachya* with moderate support (BS = 73 in combined tree). Columbus et al. (2007) found a weakly supported clade (BS = 59) containing *D. aegytium* and *N. lophostachya* in their strict consensus tree based on *trnL-F* and ITS sequences.

We are first to provide molecular evidence for the polyphyletic origin of *Perotis* (primarily African in distribution with 13 species) since *Lopholepis ornithocephala* (monotypic) and *Craspedorhachis africana* are embedded within a weakly supported clade (BS = 51, PP = 0.68 in the combined tree) (Clayton et al., 2008). We have only a single sequence (*rps3*) for *Craspedorhachis* and therefore its placement within *Perotis* may be due to a lack of variation within this single marker. *Lopholepis* is morphologically similar to *Perotis* as both share narrow racemes with spikelets borne on a short pedicel and falling with it, flat leaf blades that are cordate near base, laterally compressed spikelets, glumes that are longer than the floret (in *Lopholepis* this is developed into an obliquely constricted structure resembling a birds head), and awnless lemmas (Bor, 1960; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992a). Sister to the *Perotis–Lopholepis* clade are *Mosdenia leptostachys* (PP = 1.00 in the combined tree) and *M. phleoides* (placed as a synonym of *M. leptostachys* in Clayton et al., 2008). Morphologically, *Mosdenia* is similar to *Perotis* but it has sessile spikelets and awnless glumes (Clayton and Renvoize, 1986). *Dignathia* (east African to India) appears monophyletic (BS = 94, PP = 1.00 in the combined tree) and these two species form a clade (BS = 83, PP = 1.00) with *Gymnopogon grandiflorus* (primarily New World in distribution; one species India to Thailand) and *Bewsia biflora* (monotypic from Africa) (Clayton and Renvoize, 1986). These species then form a clade with *Mosdenia–Perotis–Lopholepis* (PP = 0.72 in the combined tree). A monophyletic *Trichoneura* (distributed in Africa, Asia, and America) with two species forms a strongly supported clade (BS = 100, PP = 1.00 in the combined tree) and they are sister (BS = 67, PP = 0.79) to a monophyletic *Ctenium* (distributed in Africa, Madagascar, and America) with two species. Together, all these species form a clade (PP = 1.00) in the combined tree. Perhaps with greater sampling among species of *Ctenium*, *Gymnopogon, Trichoneura,* and all other genera not placed in a subtribe, we will be able to better resolve relationships and circumscribe other lineages within the Cynodonteae.

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4.2. Biogeography

All three phylograms indicate that the Chloridoideae might have originated in Africa and/or Asia since the basal lineage, the tribe Triraphideae, includes sister genera, *Neyraudia* and *Triraphis,* both with African and Asian distribution. *Neyraudia* contains four species all native to Asia with *N. arundinacea* (L.) Henrard (not in our study) also occurring in tropical Africa (Chen and Phillips, 2006c; Clayton et al., 2008). *Triraphis* consists of 8 species with six of these native to Africa; *T. mollis* R. Br. native to Australia and *T. devia* Filg. & Zuloaga (not in our study) native to South America (Filgueiras and Zuloaga, 1999; Nightingale and Weiller, 2005b). The latter species is more than likely derived and recently dispersed to South America. Because more than half of the genera of Chloridoideae reside in Africa and the larger tribes, such as, the Eragrostideae, Zoysieae, and Cynodonteae, excluding the Muhlenbergiinae, have centers of diversity there, Hartley and Slater (1960) concluded that the subfamily probably originated on the African continent (during the Oligocene) and spread to other parts of the world. Our data is equivocal and we cannot satisfactorily choose Africa or Asia as the likely area for the origin of the subfamily.

Within the tribe Eragrostideae, the Cotteinae is sister to the Unioolinae–Eragrostidinae clade (Figs. 2 and 3) and includes three genera: *Cottea* with a single species distributed in the Americas; *Schmidtia* (not in our study) with two species centered in Africa; and *Enneapogon* with 16 species native to Australia (15 of these endemic), 8 species native to Africa, and one species, *E. desvauxii*, distributed worldwide (Weiller and Lazarides, 2005; Clayton et al., 2008). Therefore, it seems likely that the tribe Eragrostideae might have originated in Australia and/or Africa and then radiated to all parts of the world. *Eragrostis,* the largest chloridoid genus estimated to have 423 species in the derived subtribe Eragrostidinae, has 212 species in Africa, 153 species in the Americas, 74 species in Australasia, 56 in tropical Asia, and 51 in temperate Asia (Clayton et al., 2008; Simon et al., 2009).

Zoysia, the basal lineage within the tribe Zoysieae includes 11 species, six species in Asia, four in Australasia, one in Africa, one in the Pacific, and three introduced in the Americas (Peterson et al., 2001; Clayton et al., 2008). The Zoysieae might have originated in Asia where it is most speciose today and subsequently radiated. *Sporobolus* with 200 species in the derived subtribe Sporobolinae, includes 86 species in the Americas, 83 species in Africa, 59 species in Asia, and 23 species in Australasia (18 native to Australia) (Simon, 2005; Clayton et al., 2008; Simon et al., 2009).

Even though we have rather poor backbone support for the derivation of subtribes within the tribe Cynodonteae, the total evidence phylogram (Fig. 3) suggests that Asia and/or Africa might have been the area of origin. The basal lineage, subtribe Aeluropodinae, consists of 5–10 species, five distributed in Asia (two endemic to China), two in Africa, and two in Europe (Chen and Phillips, 2006d; Clayton et al., 2008). Sister to the Aeluropodinae is *Orinus* with all four species from Asia and subtribe Triodiinae with 68 species in three genera (*Monodia* S.W.L. Jacobs and *Symplectrodia* Lazarides (not in our study) and *Triodia*) all endemic to Australia (Lazarides et al., 2005; Nightingale and Weiller, 2005c; Nightingale et al., 2005).

As mentioned earlier, the derived clade containing the Hilariinae with *Allolepis* and *Jouvea*, Monanthochloinae, Boutelouinae, Scleropogoninae, *Sohnsia, Lepturidium*, and Muhlenbergiinae is almost entirely distributed in the Americas. Only six species of *Muhlenbergia* and one species of *Distichlis* are known to be disjunct in southeastern Asia and Australia, respectively. Earlier population studies of *Chaboissaea*, *Lycurus*, *Scleropogon*, and *Muhlenbergia torreyi* indicate that the subtribe Muhlenbergiinae probably originated in North America and has since radiated to South America multiple times (Peterson and Herrera Arrieta, 1995; Peterson and Columbus, 1997; Sykes et al., 1997; Peterson and Morrone, 1998; Peterson and Ortiz-Diaz, 1998; Peterson et al. in review). Based on four Asian species studied within *Muhlenbergia,* there is evidence for a single colonization event from the Americas to southeastern Asia (Peterson et al., in review).

Three other subtribes, the Tridentinae, Orcuttiinae, and Pappophorinae are also entirely New World in distribution but these are usually aligned near Old World-African subtribes and incertae sedis genera in our tree where there is little deep support among the nodes (see Fig. 3). The subtribe Tripogoninae contains three genera: *Eragrostiella* with six species, five distributed in tropical Asia, one in Australasia, and one in Africa; *Melanocenchris* with three species, two known in Africa and two from Asia; and *Tripogon* with approximately 30 species, 22 of these species from tropical Asia, nine from Africa, and three from the Americas. The subtribe Traginae is composed of four genera: *Monelytrum* and *Polevansia* each with a single species from Africa; *Tragus* with nine species, six in Africa, five in Asia, one endemic to Australia, and four introduced in the Americas (Peterson et al., 2001; Nightingale and Weiller, 2005d; Clayton et al., 2008). The subtribe Eleusininae is a diverse assemblage of at least 25 genera (as treated here) and is widely distributed.

4.3. Cytology

Within the subfamily Chloridoideae there is a high frequency of polyploids ranging from diploid to 20-ploid (*Pleuraphis mutica* Buckley, not sampled in our study) and many of these are thought to be alloploids suggesting extensive hybridization (Roodt and Spies, 2003). The common base chromosome number for all chloridoid tribes treated here is $x = 10$ and this is the predominant number found in the Triraphideae, Eragrostideae, Zoysieae, and Cynodonteae. Lower base numbers are common in the Cotteinae ($x = 9$, 10), Sporobolinae ($x = 7$, 8, 9, 10), Eleusininae ($x = 9$, 10), Hilariinae ($x = 9$), Scleropogoninae ($x = 7$, 8, 10), and Muhlenbergiinae ($x = 8$, 9, 10) (Watson and Dallwitz, 1992a).

4.4. C_4 evolution

According to molecular dating C_4 photosynthesis in the subfamily Chloridoideae originated between 32 and 25 mya in the early Miocene–late Oligocene (Christin et al., 2008; Vicentini et al., 2008; Bouchenak-Khelladi et al., 2009). The genetic changes responsible for the evolution of C_4 PCK subtype are still unidentified (Christin et al., 2009) and the development of this subtype is probably not identical, i.e., not analogous, in all lineages (Kellogg, 1999). In the Chloridoideae approximately 68% of the species are NAD-ME and as many as 31% have been estimated to be PCK (Taub, 2000). Based on a list of genera containing C_4 grasses (Sage et al., 1999), within the Chloridoideae the PCK subtype has arisen many times and is found in the Triraphideae (*Neyraudia*), Eragrostideae, Zoysieae, and Cynodonteae. However, most of the PCK-like species were identified solely on their anatomical descriptions and very few species have actually been investigated biochemically to determine the predominant decarboxylating enzyme (Sage et al., 1999). In addition to being polyphyletic, the three largest genera: *Eragrostis* (Eragrostideae), *Muhlenbergia* (Cynodonteae), and *Sporobolus* (Zoysieae) apparently contain both PCK and NAD-ME species. With the exception of the Aeluropodinae, Triodiinae, and Orcuttiinae, all subtribes treated have at least one taxon that has been identified as having the PCK subtype. The Orcuttiinae with nine species in three genera (*Neostapfia, Orcuttia*, and *Tuctoria*), are the only members of the Chloridoideae that have been identified to be NADP-ME (nicotinamide adenine dinucleotide phosphate cofactor malic enzyme) (Keeley, 1998). The NADP-ME is the primary

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decarboxylating enzyme in the Panicoideae and is found in over 90% of the species in this subfamily (Taub, 2000).

5. Conclusion

In this study we have performed a multi-gene phylogenetic analysis of the Chloridoideae with the largest sample size published to date at the species, generic, subtribal, and tribal levels. We have produced a robust classification of the tribes (Triraphideae, Eragrostideae, Zoysieae, and Cynodonteae) and subtribes (Cotteinae, Uniolinae and Eragrostidinae in the Eragrostideae; Zoysiinae and Sporobolinae in the Zoysieae; Aeluropodinae, Triodiinae, Orcuttiinae, Tridentinae, Eleusininae, Tripogoninae, Pappophorinae, Traginae, Hilariinae, Monanthochloinae, Boutelouinae, Scleropogoninae, and Muhlenbergiinae in the Cynodonteae) based on our phylogenetic inferences from six plastid and one nuclear DNA sequences (see Fig. 3, Table 3). We have moderate to strong support for all clades representing the tribes and subtribes (except subtribe Eleusininae where we have only posterior probability support, PP = 1.00) and all were resolved as monophyletic. The Chloridoideae might have originated in Africa and/or Asia since the basal lineage, the tribe Triraphideae, includes species with African and Asian distribution. In our study we have 20 incertae sedis genera (not placed within a subtribe) primarily within the Cynodonteae (19) and a single genus (*Urochondra*) in the Zoysieae. Based on our phylogenetic treatment the following 15 genera are polyphyletic: *Astrebla, Brachyachne, Chloris, Cynodon, Distichlis* (ITS tree only), *Enteropogon, Eragrostis, Eustachys, Leptochloa, Muhlenbergia, Perotis, Sporobolus, Tetrapogon, Tragus,* and *Tripogon*; and the following 22 genera with two or more species were always portrayed as monophyletic: *Aeluropus, Blepharidachne, Bouteloua, Cleistogenes, Ctenium, Dactyloctenium, Dignathia, Erioneuron, Lepturus, Melanocenchris, Microchloa, Mosdenia, Munroa, Orcuttia, Orinus, Trichloris, Trichoneura, Trioidia, Triraphis, Tuctoria, Uniola,* and *Zoysia*. Other genera depicted as monophyletic but found embedded within other genera were: *Aegopogon, Blepharoneuron, Chaboissaea, Lycurus* (all in *Muhlenbergia*), *Ectrosia* (in *Eragrostis*), and *Eragrostiella* (in *Tripogon*). Even though we have tried to sample as many chloridoid genera as possible (95) there are still approximately 46 (of these 30 are monotypic and 11 are ditypic) remaining that we have not yet included in our study. The majority of these unsampled genera are primarily distributed in Africa and we hope to gather these in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.01.018.

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