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PHYLOGENETICS OF CHLORIDOIDEAE (GRAMINEAE): A PRELIMINARY STUDY BASED ON NUCLEAR RIBOSOMAL INTERNAL TRANSCRIBED SPACER AND CHLOROPLAST *trn*L–F SEQUENCES

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ABSTRACT

The phylogeny of Chloridoideae (Gramineae) was inferred from parsimony analyses of DNA sequences from two genomes—the chloroplast *trn*L intron, *trn*L 3′ exon, and *trn*L–F intergenic spacer, and the nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2). Eighty species representing 66 chloridoid genera were sampled, including all but four of the native New World genera. Analyses of the individual and combined data sets were performed. The phylogenies were found to be highly congruent. Of the four tribes and seven subtribes of Chloridoideae sensu Clayton and Renvoize (1986) whose phylogenetic status could be tested with our taxon sample, only Orcuttieae and Uniolinae were monophyletic. The phylogenies suggested significant homoplasy in morphological traits, including inflorescence type, number of florets per spikelet, and number of lemma nerves. We propose a new classification based on the three main clades in the phylogenies—tribes Cynodonteae, Eragrostideae, and Zoysieae. The Eragrostideae clade is well resolved and supported and is further divided into three subtribes, Cotteinae, Eragrostidinae, and Uniolinae. Cynodonteae include most of the genera in our study, but the clade is poorly resolved. However, a clade formed of *Muhlenbergia* and nine other genera is present in both phylogenies and is well resolved and supported. A number of interesting, well-supported relationships are evident in the phylogenies, including *Pappophorum–Tridens flavus*, *Tragus–Willkommia*, and *Gouinia–Tridens muticus–Triplasis–Vaseyochloa.* Except for *Bouteloua,* no genus represented by multiple species proved to be monophyletic in the phylogenies.

Key words: Chloridoideae, classification, Gramineae, homoplasy, ITS, phylogeny, Poaceae, *trn*L–*trn*F.

INTRODUCTION

The grass subfamily Chloridoideae is remarkable in its variation. Inflorescences range from diffuse and rebranched to a solitary spicate branch. Spikelets vary greatly in the number of florets (1–100+), lemma nerves (1–15), and awns (0–19), and in fertility (hermaphrodite, unisexual, or sterile florets) and disarticulation. Two types of C_4 photosynthesis (NAD-ME and PCK) are known, and one species of *Eragrostis* Wolf is C_3 (Ellis 1984; Hattersley and Watson 1992). Distributed worldwide, mostly in the tropics and subtropics, Chloridoideae are also diverse in numbers, with as many as 166 genera and some 1500 species (Van den Borre and Watson 1997).

Chloridoideae are monophyletic in virtually all phylogenetic analyses in spite of elusive non-molecular synapomorphies (Grass Phylogeny Working Group [GPWG] 2001 and refs. therein). They are one of the subfamilies in the PACCAD clade, along with Panicoideae, Aristidoideae, Centothecoideae, Arundinoideae, and Danthonioideae. Classification within the subfamily, however, has been controversial (see Jacobs 1987; Van den Borre and Watson 1997). The central issue has been whether to recognize the traditional tribes Cynodonteae (Chlorideae) and Eragrostideae as distinct. The much smaller tribes Orcuttieae and Pappophoreae have been widely accepted. In recent classifications in Clayton and Renvoize (1986) and Watson and Dallwitz (1994), Orcuttieae and Pappophoreae were recognized, but the latter authors merged Cynodonteae and Eragrostideae. Tests of these circumscriptions came with important contributions by Van den Borre and Watson (1997), who analyzed a large morphological and anatomical data set, and Hilu and Alice (2001), who analyzed sequences from the chloroplast gene *mat*K. Both of these studies rejected the traditional circumscriptions of Cynodonteae and Eragrostideae and revealed new groups that may better reflect evolutionary history.

In this study, we provide additional estimates of the phylogeny of Chloridoideae by analyzing sequences from two genomes—the chloroplast *trn*L intron, *trn*L 3′ exon, and *trn*L–*trn*F intergenic spacer (hereafter referred to as *trn*L–F), and the nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2; hereafter ITS). We compare these phylogenies to one another and to previous studies and classifications, in particular the detailed and widely followed classification in Clayton and Renvoize (1986). We also assess levels of homoplasy in morphological traits, seek characters supporting relationships in the molecular phylogenies, and propose changes to the classification.

MATERIALS AND METHODS

Taxa and Collections

We sampled 80 species representing 66 genera of Chloridoideae, including multiple species for some of the larger genera (Table 1). The sample emphasizes the New World and includes 36 and 60 endemic genera and species, respectively. *Lepturidium* Hitchc. & Ekman, *Rheochloa* Filg., P. M. Peterson & Y. Herrera, *Saugetia* Hitchc. & Chase, and *Steir-*

Table 1. Taxa and collections sampled, and GenBank accession numbers for *trn*L–F and ITS sequences. Collection/voucher numbers are those of the lead author unless indicated otherwise. Most determinations were made or verified by the lead author. Vouchers are deposited at RSA unless indicated otherwise.

Taxon	Collection/voucher	Source	GenBank accession	
			*trn*L–F	ITS
Aegopogon cenchroides Humb. & Bonpl. ex Willd.	*4380*	Venezuela: Mérida	EF156669	EF153020
Allolepis texana (Vasey) Soderstr. & H. F. Decker	*Bell 240*	USA: Texas	EF156670	EF153021
Bealia mexicana Scribn.	*3666*	Mexico: Chihuahua	EF156671	EF153022
Blepharidachne kingii (S. Watson) Hack.	*3855*	USA: California	EF156672	EF153023
Blepharoneuron tricholepis (Torr.) Nash	*3652*	Mexico: Chihuahua	EF156673	EF153024
Bouteloua aristidoides (Kunth) Griseb. var. *aristidoides*	*2444*	USA: Arizona	EF156674	EF153025
B. dactyloides (Nutt.) Columbus (syn. *Buchloë dactyloides* (Nutt.) Engelm.)	*2329*	Mexico: San Luis Potosí	EF156675	EF153026
B. trifida Thurb.	*2126*	USA: Texas	EF156676	EF153027
Calamovilfa longifolia (Hook.) Hack. ex Scribn. & Southw. var. *longifolia*	*3917*	USA: Kansas	EF156677	EF153028
Chaboissaea decumbens (Swallen) Reeder & C. Reeder	*3653*	Mexico: Chihuahua	EF156678	EF153029
Chloris cucullata Bisch.	*2903*	USA: Texas	EF156679	EF153030
C. elata Desv. (syn. *C. dandyana* C. D. Adams)	*3068*	Argentina: Corrientes	EF156680	EF153031
C. truncata R. Br.	*3203*	USA: California	EF156681	EF153032
Cottea pappophoroides Kunth	*3183*	Argentina: Salta	EF156682	EF153033
Crypsis vaginiflora (Forssk.) Opiz	*3831*	USA: California	EF156683	EF153034
Ctenium aromaticum (Walter) Alph. Wood	*3348*	USA: Louisiana	EF156684	EF153035
Cynodon dactylon (L.) Pers.	*2691*	USA: California	EF156685	EF153036
Dactyloctenium aegyptium (L.) Willd.	*2873*	Mexico: Tamaulipas	EF156686	EF153037
Dasyochloa pulchella (Kunth) Willd. ex Rydb. (syn. *Erioneuron pulchellum* (Kunth) Tateoka)	*2577*	Mexico: Querétaro	EF156687	EF153038
Dinebra retroflexa (Vahl) Panz. var. *retroflexa*	*Clarke s.n.* (23 Aug 2004)	USA: California	EF156688	EF153039
Distichlis spicata (L.) Greene	*Bell 231*	USA: California	EF156689	EF153040
Ectrosia leporina R. Br. var. *leporina*	*Bell 171*	Australia: Northern Territory	EF156690	EF153041
Eleusine indica (L.) Gaertn.	*2875*	Mexico: Tamaulipas	EF156691	EF153042
Enneapogon desvauxii P. Beauv.	*3133*	Argentina: San Juan	EF156692	EF153043
Enteropogon chlorideus (J. Presl) Clayton	*2939*	Mexico: Sonora	EF156693	EF153044
E. mollis (Nees) Clayton	*3438*	Peru: Lambayeque	EF156694	EF153045
Eragrostis amabilis (L.) Wight & Arn. ex Nees	*4317*	Venezuela: Distrito Capital	EF156695	EF153046
E. pectinacea (Michx.) Nees var. *pectinacea*	*2704*	Mexico: Sonora	EF156696	EF153047
E. sessilispica Buckley (syn. *Acamptoclados sessilispicus* (Buckley) Nash)	*3328*	USA: Texas	EF156698	EF153049
Erioneuron avenaceum (Kunth) Tateoka var. *avenaceum*	*2553*	Mexico: México	EF156699	EF153050
Eustachys distichophylla (Lag.) Nees	*3090*	Argentina: Córdoba	EF156700	EF153051
Fingerhuthia africana Nees ex Lehm.	*Snow & Burgoyne 7207* (MO)	Namibia: Erongo	EF156701	EF153052
Gouinia latifolia (Griseb.) Vasey var. *latifolia*	*3568*	Peru: Cusco	EF156702	EF153053
Gymnopogon ambiguus (Michx.) Britton, Sterns & Poggenb.	*3352*	USA: Texas	EF156703	EF153054
Hilaria cenchroides Kunth	*3758*	Mexico: Oaxaca	EF156704	EF153055
Jouvea pilosa (J. Presl) Scribn.	*Bell 247*	Mexico: Jalisco	EF156706	EF153057
Leptochloa dubia (Kunth) Nees (syn. *Diplachne dubia* (Kunth) Scribn.)	*3155*	Argentina: Catamarca	EF156707	EF153058
L. fusca (L.) Kunth subsp. *uninervia* (J. Presl) N. W. Snow (syn. *Diplachne uninervia* (J. Presl) Parodi)	*3111*	Argentina: Mendoza	EF156708	EF153059
L. panicea (Retz.) Ohwi subsp. *brachiata* (Steud.) N. W. Snow	*2700*	Mexico: Sonora	EF156709	EF153060
Leptothrium rigidum Kunth	*3429*	Peru: Piura	EF156710	EF153061
Lycurus setosus (Nutt.) C. Reeder	*3286*	USA: New Mexico	EF156711	EF153062
Melanocenchris abyssinica (R. Br. ex Fresen.) Hochst.	*4304.5*	India: Maharashtra	EF156712	EF153063
Microchloa indica (L. f.) P. Beauv.	*2979*	Mexico: Nayarit	EF156713	EF153064
Monanthochloë littoralis Engelm.	*Bell 236*	USA: Texas	EF156714	EF153065
Muhlenbergia emersleyi Vasey	*3275*	USA: New Mexico	EF156715	EF153066
M. montana (Nutt.) Hitchc.	*3375*	USA: Arizona	EF156716	EF153067
M. ramulosa (Kunth) Swallen	*3616*	Mexico: Sonora	EF156717	EF153068
Munroa squarrosa (Nutt.) Torr.	*3894*	USA: New Mexico	EF156718	EF153069

Table 1. Continued.

Taxon	Collection/voucher	Source	GenBank accession	
			*trn*L–F	ITS
Neeragrostis reptans (Michx.) Nicora (= *Eragrostis reptans* (Michx.) Nees)	*Hill 22450*	USA: Texas	EF156697	EF153048
Neobouteloua lophostachya (Griseb.) Gould	*3144*	Argentina: La Rioja	EF156719	EF153070
Neostapfia colusana (Burtt Davy) Burtt Davy	*Reeder & Reeder 6198*	USA: California	EF156720	EF153071
Orcuttia californica Vasey	*2687*	USA: California	EF156721	EF153072
Pappophorum vaginatum Buckley	*2540*	USA: Arizona	EF156722	EF153073
Pereilema crinitum J. Presl	*3621*	Mexico: Sonora	EF156723	EF153074
Pleuraphis jamesii Torr. (= *Hilaria jamesii* (Torr.) Benth.)	*3221*	USA: Wyoming	EF156705	EF153056
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	*Snow et al. 7023* (MO)	South Africa: Mpumalanga	EF156724	EF153075
Redfieldia flexuosa (Thurb. ex A. Gray) Vasey	*3910*	USA: Colorado	EF156725	EF153076
Reederochloa eludens Soderstr. & H. F. Decker	*Bell 250*	Mexico: San Luis Potosí	EF156726	EF153077
Schaffnerella gracilis (Benth.) Nash	*4040*	Mexico: San Luis Potosí	EF156727	EF153078
Schedonnardus paniculatus (Nutt.) Branner & Coville	*Reeder & Reeder 9431*	USA: Arizona	EF156728	EF153079
Scleropogon brevifolius Phil.	*4129*	Mexico: San Luis Potosí	EF156729	EF153080
Sohnsia filifolia (E. Fourn.) Airy Shaw	*4038*	Mexico: San Luis Potosí	EF156730	EF153081
Spartina pectinata Link	*3210*	USA: Missouri	EF156731	EF153082
Sporobolus indicus (L.) R. Br.	*2737*	Mexico: Sonora	EF156732	EF153083
S. pyramidatus (Lam.) Hitchc.	*4264*	USA: Florida	EF156733	EF153084
S. wrightii Munro ex Scribn.	*2507*	USA: Arizona	EF156734	EF153085
Swallenia alexandrae (Swallen) Soderstr. & H. F. Decker	*Bell 228*	USA: California	EF156735	EF153086
Tragus racemosus (L.) All.	*2228*	USA: Arizona	EF156736	EF153087
Trichloris crinita (Lag.) Parodi	*3109*	Argentina: Mendoza	EF156737	EF153088
Trichoneura elegans Swallen	*4299*	USA: Texas	EF156738	EF153089
Tridens flavus (L.) Hitchc. var. *flavus*	*3212*	USA: Missouri	EF156739	EF153090
T. muticus (Torr.) Nash var. *muticus*	*3254*	USA: Arizona	EF156740	EF153091
Triodia desertorum (C. E. Hubb.) Lazarides	*Bell 114*	Australia: Western Australia	EF156741	EF153092
Triplasis americana P. Beauv.	*4251*	USA: Florida	EF156742	EF153093
Tripogon spicatus (Nees) Ekman	*3108*	Argentina: San Luis	EF156743	EF153094
Tuctoria mucronata (Crampton) Reeder	*4682.5*	USA: California	EF156744	EF153095
Uniola paniculata L.	*4206*	USA: North Carolina	EF156745	EF153096
Vaseyochloa multinervosa (Vasey) Hitchc.	*4300*	USA: Texas	EF156746	EF153097
Willkommia texana Hitchc. var. *texana*	*4143*	USA: Texas	EF156747	EF153098
Zoysia matrella (L.) Merr. s.l.	*3985*	USA: Hawaii	EF156748	EF153099
	Outgroup			
Aristida adscensionis L.	*2991*	Mexico: Jalisco	DQ172196	DQ171972
Arundo donax L.	*3201*	USA: California	DQ172302	DQ172077
Chasmanthium latifolium (Michx.) H. O. Yates	*3211*	USA: Missouri	DQ172304	DQ172079
Hackelochloa granularis (L.) Kuntze	*2624*	Mexico: Michoacán	DQ172306	DQ172081
Panicum hirticaule J. Presl var. *hirticaule*	*2536*	USA: Arizona	DQ172307	DQ172082

achne Ekman, each with one or two species, are the only genera endemic to the New World that were not sampled. Despite the New World emphasis, of Clayton and Renvoize's (1986) five chloridoid tribes and nine subtribes, only Leptureae and Pommereullinae are not represented. Also unavailable at the time of the study was DNA of *Centropodia* Rchb. and *Merxmuellera rangei* (Pilg.) Conert, recently positioned in Chloridoideae (GPWG 2001). Five species representing four of the other PACCAD subfamilies were employed as the outgroup (Table 1).

Collection/voucher information is provided in Table 1. Most samples were from live, field-collected plants or plants grown from caryopses or transplants at Rancho Santa Ana Botanic Garden. One gram or more of healthy, living leaf material was removed from an individual plant and placed directly in liquid nitrogen, silica gel (Liston et al. 1990; Chase and Hills 1991), or a −80°C freezer for later DNA extraction, or the sample was processed immediately. In a few cases 20 mg samples were removed from dried herbarium specimens.

Table 2. DNA amplification and sequencing primers designed for this study. See Fig. 1 for locations of primers.

Name	5′ Sequence 3′	Comments
*trn*L5′ BR	GATATGGCGAAATCGGTAGA	Complement of Taberlet et al. (1991) primer ''b''
*trn*L INT1F	CTCAATGGAAGCTGTTCTAACG	
*trn*L INT1R	CGTTAGAACAGCTTCCATTGAG	
*trn*L INT2R	GCTATGTCAGTATCTATACGTG	
*trn*L INT3F	GAGAGAGTCCCATTCTACATGTC	
*trn*L3′ D2	TGGGGATAGAGGGACTTGAACCC	Modification of Taberlet et al. (1991) primer ''d''
*trn*F F2	CAGTCCTCTGCTCTACCAAC	

DNA Sequences

ITS sequences of *Bouteloua aristidoides*, *Cynodon dactylon*, and *Tragus racemosus* are from Columbus et al. (1998).

Three procedures were used to extract total cellular DNA: the CTAB protocol of Doyle and Doyle (1987) as modified in Columbus et al. (1998), the Cullings (1992) CTAB protocol, or the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA).

For amplification of *trn*L–F and ITS, *Taq* polymerase from Invitrogen (Carlsbad, California, USA) or Promega (Madison, Wisconsin, USA) was used, as well as PCR Master Mix (Promega) and PuReTaq Ready-To-Go PCR Beads (Amersham Biosciences, Piscataway, New Jersey, USA). Employing annealing temperatures of 52–55°C, primers ''c'' and ''f'' (Taberlet et al. 1991) were used to amplify *trn*L–F or, more frequently, primer ''*trn*L5′ BR'' (Table 2) was used instead of ''c'' (Fig. 1). Reactions sometimes included 5 or 10% dimethyl sulfoxide (DMSO) to facilitate amplification (Winship 1989; Varadaraj and Skinner 1994). Amplification of ITS generally followed Columbus et al. (1998), with an annealing temperature of 48°C, except that primer ''ITS-5m'' (Sang et al. 1995) was sometimes used in place of ''ITS5'' (White et al. 1990), and the reactions sometimes included 10% DMSO. PCR products were purified using the Morgan and Soltis (1993) PEG protocol or the QIAquick PCR Purification Kit (QIAGEN).

Cycle sequencing was carried out with the Applied Biosystems (ABI; Foster City, California, USA) DyeDeoxy or BigDye (vers. 3.1) Terminator Cycle Sequencing Kit, and sequencing products were visualized on an ABI PRISM 373A DNA Sequencer or 3100 Genetic Analyzer, respectively. For *trn*L–F, primers ''c'', ''d'', ''e'', and ''f'' (Taberlet et al. 1991) were most often employed for sequencing, but ''*trn*L INT3F'' (Table 2) was commonly used in place of ''e'' to enable reliable sequence determination of the *trn*L 3′ exon and flanking regions (Fig. 1). New primers were designed (Table 2; Fig. 1) primarily to improve sequence quality downstream from poly-n strings (predominately adenine and thymine). For ITS, primers ''ITS5'' and ''ITS4'' were usually used for sequencing, but ''ITS-5m'', ''ITS5i'', ''ITS4i'', ''ITS2'', and ''ITS3'' were sometimes employed (White et al. 1990; Sang et al. 1995; Porter 1997). Sequence fragments were assembled, edited, and a consensus sequence constructed using Sequencher vers. 3 or 4 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The bounds of the *trn*L exons and intron and *trn*L–*trn*F intergenic spacer were determined by comparison with the annotated sequence of *Zea mays* L. (GenBank accession X86563). The bounds of ITS1, 5.8S, and ITS2 follow Columbus et al. (1998).

Analyses

Sequences were aligned manually using Se-Al vers. 2.0 (Rambaut 2001). Unambiguous nucleotide insertions or deletions (indels) shared by two or more species were scored as presence/absence characters at the end of the data matrix following the simple indel coding method of Simmons and Ochoterena (2000).

Parsimony analyses of the *trn*L–F, ITS, and combined *trn*L–F/ITS data sets were performed using PAUP* vers. 4.0b10 (Swofford 2002). Characters (nucleotide sites and coded indels) were treated as unordered and weighted equally, and were optimized via accelerated transformation. For a given ITS sequence, a site possessing multiple nucleotides was treated as a polymorphism. Gaps were treated as missing data. For each heuristic search, 1000 random stepwise-addition replicates were executed, holding one tree per step, using tree bisection-reconnection branch swapping, collapsing branches with a maximum length of zero, and saving all shortest trees (MulTrees). Because exploratory analyses of the *trn*L–F matrix yielded many thousands of trees and could not be run to completion, for the final analysis of this data set we limited each replicate to one million rearrangements.

To determine statistical support for clades, bootstrap analyses (Felsenstein 1985) were performed in PAUP*. The same settings as above were employed except for the exclusion of uninformative characters and random stepwise-addition replicates was set to one. One thousand bootstrap replicates were performed on each data set. In addition, Bremer values (decay indices; Bremer 1988; Donoghue et al. 1992)

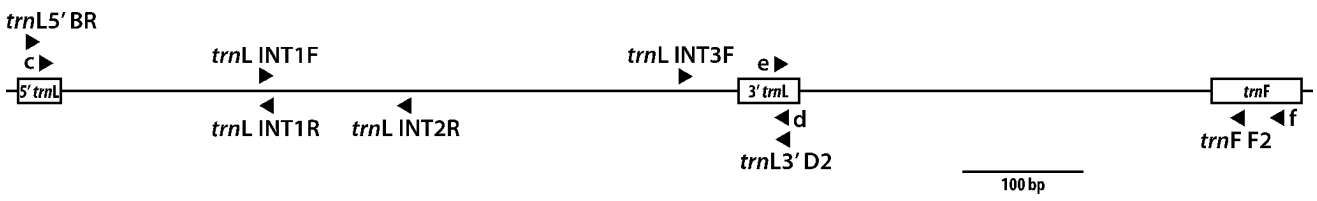

Fig. 1.—Locations of amplification and sequencing primers used in this study.

Table 3. Summary information for the data sets and results of the analyses.

	*trn*L–F	ITS	*trn*L–F + ITS
Average guanine/cytosine content (%)[a]	*trn*L intron: 33.0	ITS1: 56.3	—
	*trn*L 3′ exon: 46.0	5.8S: 54.4	—
	*trn*L–*trn*F spacer: 27.9	ITS2: 57.6	—
Sequence length (base pairs)[a]	860–1025	565–612	—
Aligned sequence length	1712	812	2524
Insertions/deletions coded	38	0	38
Total characters	1750	812	2562
Parsimony informative characters	243 (13.9%)	382 (47.0%)	626 (24.4%)
Most parsimonious trees	360,636	17	114
Tree length	906	3664	4602
Consistency index[b]	0.51	0.27	0.30
Retention index	0.69	0.50	0.53

[a] Ingroup only. [b] Excluding parsimony uninformative characters.

Table 4. Nucleotide insertions and deletions (indels) in *trn*L–F scored as presence/absence characters for the analyses (characters 1713–1750 in the *trn*L–F data matrix). Indels 1–19 are in the *trn*L intron and 20–38 are in the *trn*L–*trn*F intergenic spacer. Indels 9, 26, and 30 involve a subset of the outgroup and cannot be readily classed as insertions or deletions based on this data set.

Number	Kind	Length (base pairs)	Position in *trn*L–F data matrix
1	Insertion	2	219–220
2	Insertion	5	246–250
3	Insertion	23	269–291
4	Insertion	5	309–313
5	Insertion	8	324–342
6	Insertion	1	346
7	Insertion	5	360–364
8	Insertion	5	473–477
9	?	1	493
10	Insertion	2	494–495
11	Insertion	4	539–542
12	Insertion	12	568–579
13	Insertion	8	639–646
14	Insertion	1	675
15	Insertion	6	693–698
16	Deletion	1	784
17	Insertion	5	801–805
18	Deletion	5	808–822
19	Insertion	5	810–814
20	Insertion	6	987–992
21	Deletion	29	1013–1061
22	Insertion	5	1032–1036
23	Insertion	1	1068
24	Insertion	5	1078–1082
25	Insertion	23	1111–1133
26	?	3	1258–1260
27	Insertion	5	1267–1271
28	Insertion	6	1310–1315
29	Insertion	29	1329–1357
30	?	5	1359–1363
31	Deletion	9	1368–1377
32	Deletion	2	1369–1370
33	Deletion	6	1370–1376
34	Insertion	10	1402–1411
35	Insertion	6	1527–1532
36	Insertion	12	1557–1568
37	Insertion	5	1578–1582
38	Insertion	1	1705

were calculated using MacClade vers. 4.05 (Maddison and Maddison 2002) and PAUP*.

We relied heavily on the descriptions in Clayton and Renvoize (1986) and Watson and Dallwitz (1994) in making comparisons among taxa.

RESULTS

For each sample, complete sequences were obtained of the *trn*L intron, *trn*L 3′ exon, *trn*L–*trn*F intergenic spacer, ITS1, 5.8S, and ITS2. Sequences are available from GenBank with accession numbers as in Table 1. Summary information for the data sets and results of the analyses are given in Table 3. The data matrices along with the strict consensus tree from each analysis are available from TreeBASE (study accession S189, matrix accessions M3471–M3473).

Aligning the *trn*L–F sequences required the creation of many gaps equivalent to one or more base pairs. We found that most of the nucleotide insertions are duplications. Thirty-eight indels were coded for analysis (Table 4). Length variation associated with strings of the same nucleotide (mostly adenine and thymine) usually were not coded due to uncertainties about homology. Based on the phylogenetic trees presented below, several of the coded indels proved to be homoplastic. Of the 1750 total characters in the *trn*L–F data set, 243 (13.9%) are parsimony informative. The analysis yielded over 360,000 most parsimonious trees 906 steps long and with a consistency index of 0.51. Figure 2 is one of the shortest trees, showing branches (dotted) not present in the strict consensus tree.

In contrast to *trn*L–F, aligning the shorter but more divergent ITS sequences was challenging and not confidently achieved for ITS1 and ITS2. However, exploratory parsimony analyses based on different alignments always yielded the same strongly supported clades. Due to uncertainties about homology, we elected not to code gaps. Although the ITS data set has fewer total characters (812) than the *trn*L–F data set, a greater number of the ITS characters (382, 47.0%) are parsimony informative. The ITS data also yielded fewer trees (17) of far greater length (3664 steps) and with more homoplasy (consistency index = 0.27). Figure 3 is one of the shortest trees, showing branches (dotted) not present in the strict consensus tree.

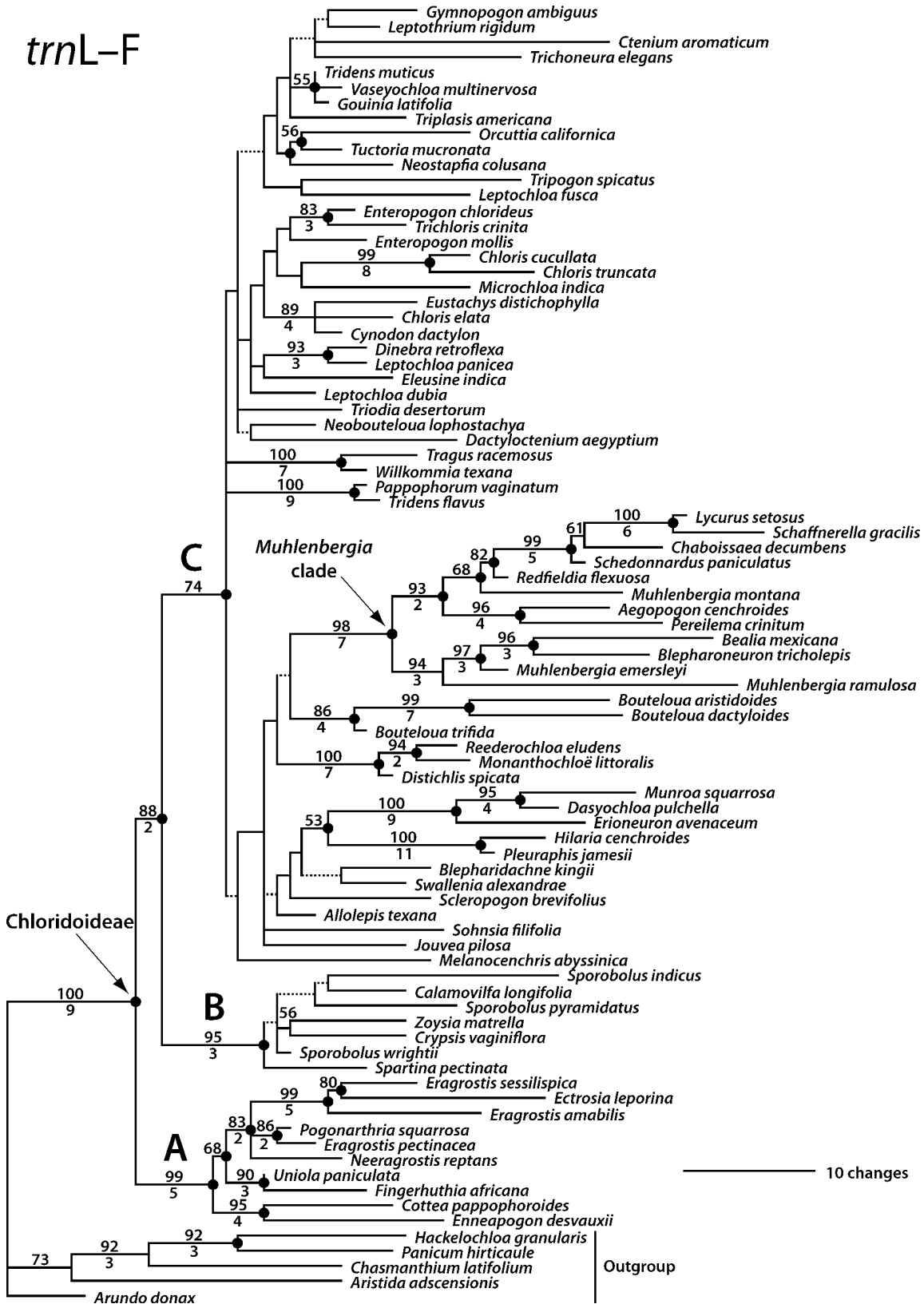

Fig. 2.—One of 360,636 most parsimonious trees, arbitrarily selected and drawn as a phylogram, resulting from analysis of *trn*L–F sequences. Dotted branches are not present in the strict consensus tree. Numbers above and below branches are bootstrap percentages (≥50%) and Bremer values (≥2), respectively. Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of *trn*L–F and ITS.

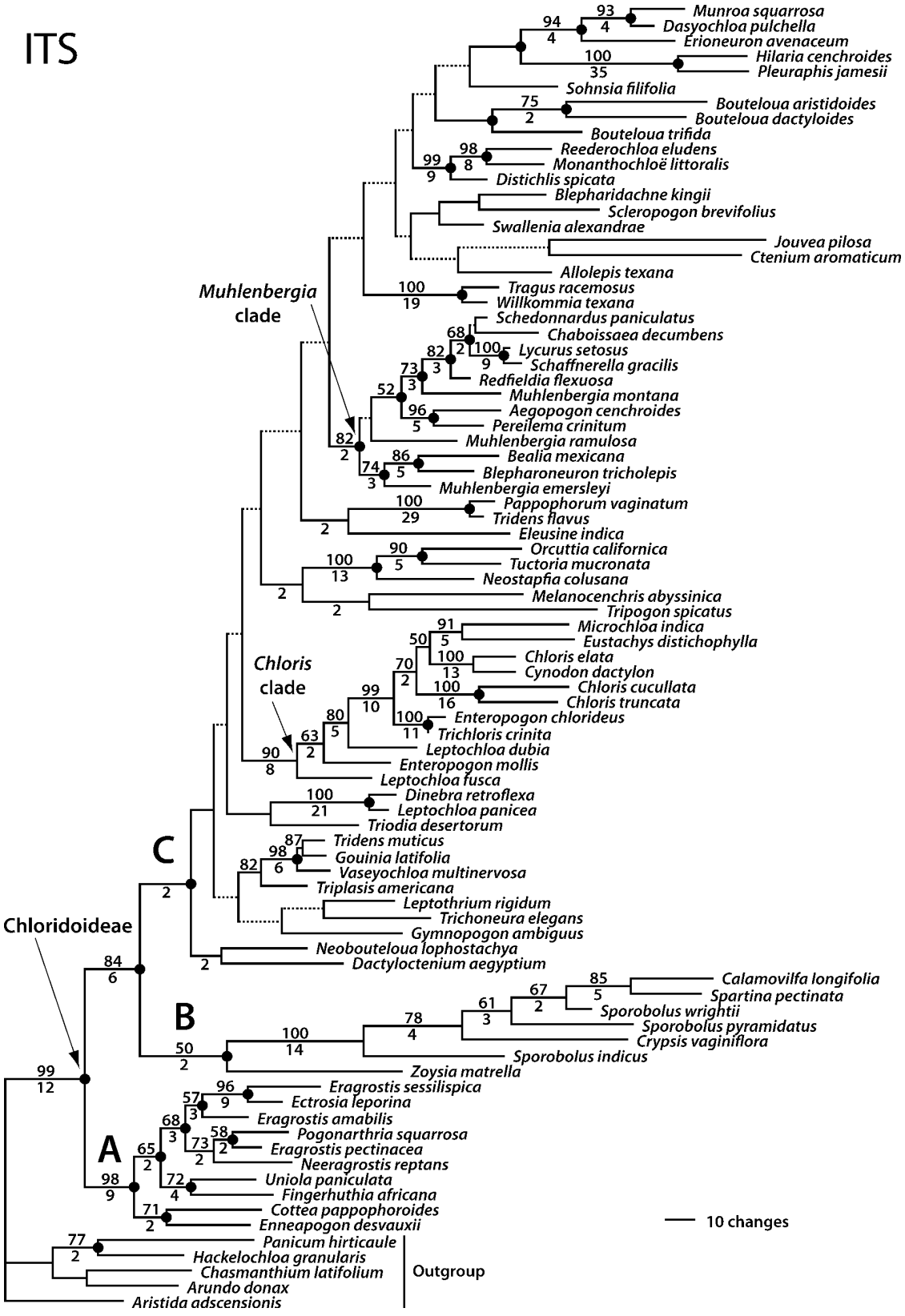

Fig. 3.—One of 17 most parsimonious trees, arbitrarily selected and drawn as a phylogram, resulting from analysis of ITS sequences. Dotted branches are not present in the strict consensus tree. Numbers above and below branches are bootstrap percentages (≥50%) and Bremer values (≥2), respectively. Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of *trn*L–F and ITS.

Analysis of the combined *trn*L–F/ITS data set resulted in 114 most parsimonious trees. Figure 4 is the strict consensus tree.

Thirty-seven clades are common to all trees resulting from the three analyses (bulleted nodes in Fig. 2–4). One of these clades corresponds to Chloridoideae and four are early diverging clades within the subfamily (A, B, C, and B + C in Fig. 2–4; clade designations follow Hilu and Alice 2001). Relationships within clade A are completely resolved in the ITS phylogeny (Fig. 3) and *trn*L–F + ITS trees (Fig. 4) and are congruent with the *trn*L–F phylogeny (Fig. 2), which has one polytomy. Likewise, relationships within clade B are completely resolved and congruent in the ITS and *trn*L–F + ITS trees; however, relationships are virtually unresolved in the *trn*L–F phylogeny. Clade C contains most of the genera and species sampled in the study. Unfortunately, as a whole, relationships are poorly resolved in clade C. However, common to all trees from all analyses is a clade comprising *Muhlenbergia* Schreb. and nine other genera (the *Muhlenbergia* clade), and common to the ITS and *trn*L–F + ITS trees, but not the *trn*L–F trees, is a clade containing *Chloris* Sw., five other genera, and two of the three sampled species of *Leptochloa* P. Beauv. (the *Chloris* clade). The only supported topological conflict between the *trn*L–F and ITS phylogenies involves *Chloris* and relatives: *Eustachys* Desv. forms a clade with *Cynodon* Rich. and *Chloris elata* (bootstrap [BS] 89%, Bremer value [BV] 4) in the *trn*L–F phylogeny yet forms a clade with *Microchloa* R. Br. (BS 91%, BV 5) in the ITS phylogeny.

DISCUSSION

Comparison with Previous Molecular Phylogenetic Studies

Although taxon sampling differs between our study and Hilu and Alice's (2001) phylogenetic study of 56 genera of Chloridoideae based on chloroplast *mat*K sequences, in common are 37 genera, so comparisons can be made with some confidence. The results of the two studies are in fact quite similar, including the presence of clades A, B, and C in the *mat*K, *trn*L–F (Fig. 2), ITS (Fig. 3), and *trn*L–F + ITS (Fig. 4) trees, and the level of resolution within each clade. The only apparent inconsistency involves *Pappophorum* Schreb., which is situated among *Eragrostis* species in clade A of Hilu and Alice (2000, 2001), but is in clade C in our study. Ingram and Doyle (2004, 2007) explained that the *mat*K sequence of *Pappophorum* appears to be a sequence from a species of *Eragrostis*, and *trn*L–F and ITS sequences from additional species of *Pappophorum* confirm the position of the genus in clade C (J. T. Columbus and R. Cerros unpubl. data). If the *Pappophorum* sequence indeed represents a species of *Eragrostis* in Hilu and Alice (2001), then the *mat*K phylogeny is congruent with the *trn*L–F and ITS phylogenies. It also should be pointed out that we did not sample the two species of *Eragrostis* that resolved in clade B of the *mat*K phylogeny apart from the other species in clade A, nor did we sample other species of *Eragrostis* morphologically close to *Sporobolus* R. Br. (Clayton and Renvoize 1986). The similarity among the three phylogenies extends to the level of resolution: high in clade A and low in clade C. With respect to clade B, the chloroplast *mat*K and *trn*L–F phylogenies are similar in their low resolution, whereas relationships within the clade are completely resolved in the ITS phylogeny, although a couple of the clades are not well supported.

Although the *mat*K phylogeny is congruent with the *trn*L–F and ITS phylogenies, the relationships among clades A, B, and C were not resolved by parsimony analysis of equally weighted characters in Hilu and Alice (2001). The three clades, each well supported, form a polytomy along with *Triraphis* R. Br., which we did not sample. In our study, clades B and C are sister, and A is sister to B + C. These clades and relationships are well supported in all analyses except with respect to clade B in the ITS phylogeny (BS 50%, BV 2) and clade C in separate analyses of the *trn*L–F (BS 74%, BV 1) and ITS (BS <50%, BV 2) data sets (Fig. 2, 3). The combined *trn*L–F/ITS data yielded better support for clade C (BS 78%, BV 8; Fig. 4). In their analyses of the *mat*K data set, Hilu and Alice (2001) also performed parsimony analyses using differential character weighting and a neighbor-joining analysis. These analyses yielded the same relationships among clades A, B, and C, although without support, as we obtained from parsimony analyses of our data. As well, *Triraphis* resolved as sister to the remaining Chloridoideae (BS 72–74%). A year earlier, Hilu and Alice (2000) published the results of a parsimony analysis of *mat*K sequences representing a smaller number (26) of chloridoid genera. In addition to the unlikely position of *Pappophorum* among species of *Eragrostis* (discussed above), *Muhlenbergia* is in a clade with *Sporobolus*, which is in conflict with our study and Hilu and Alice (2001), who indicated that the *Muhlenbergia* sample was actually a species of *Sporobolus.* These errors notwithstanding, clades A, B, and C are resolved in Hilu and Alice (2000), though clade C is not well supported, and the sequence of divergence of these clades is the same as in our study and in the Hilu and Alice (2001) analyses described above.

Two other phylogenetic studies based at least in part on molecular data do not agree well with our results. Hilu and Esen (1993) examined relationships within Chloridoideae based on the size and immunological similarities of prolamins, a class of seed storage proteins. Trees resulting from analyses of these data for 11 genera bear little resemblance to the *mat*K, *trn*L–F, and ITS phylogenies, the only exception being the consistent grouping of *Chloris* with *Cynodon.* Many chloridoid genera were sampled in Hodkinson et al.'s (2007) supertree analysis of the grass family that included over 400 genera and combined 62 source trees based on molecular and non-molecular data. With respect to Chloridoideae, the *mat*K, *trn*L–F, and ITS phylogenies are not congruent with the supertree, wherein members of clades A, B, and C are intermixed, and *Microchloa*, along with *Austrochloa* Lazarides and *Kengia* Packer, fall outside the subfamily in a clade labeled as incertae sedis.

Other molecular phylogenetic studies of grasses have not focused on Chloridoideae as a whole. In most cases a limited number of chloridoids have been included in family-wide studies, or studies have focused on groups within the subfamily. In a study focused on *Eragrostis* based on chloroplast *rps*16 and nuclear *waxy* (granule-bound starch synthase I; GBSSI) sequences, Ingram and Doyle (2004, 2007) sampled 21 chloridoid genera. Rooted with the chloridoid genus *Coelachyrum* Hochst. & Nees, which was not sampled in

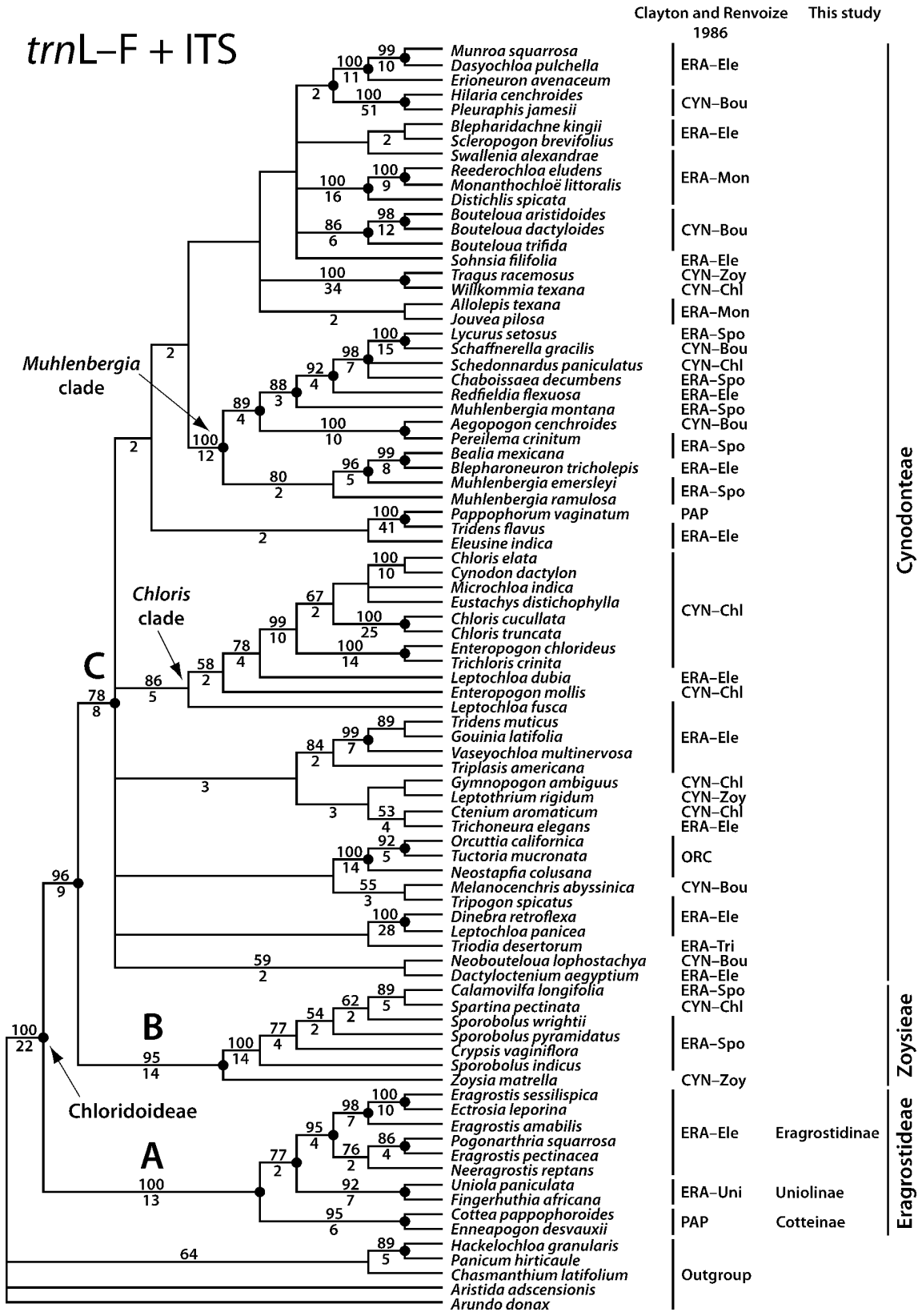

Fig. 4.—Strict consensus of 114 most parsimonious trees resulting from analysis of combined *trn*L–F and ITS sequences. Numbers above and below branches are bootstrap percentages (≥50%) and Bremer values (≥2), respectively. Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of *trn*L–F and ITS. Abbreviations of tribes and subtribes recognized in Clayton and Renvoize (1986) are as follows: CYN = Cynodonteae, ERA = Eragrostideae, ORC = Orcuttieae, PAP = Pappophoreae, Bou = Boutelouinae, Chl = Chloridinae, Ele = Eleusininae, Mon = Monanthochloinae, Spo = Sporobolinae, Tri = Triodiinae, Uni = Uniolinae, Zoy = Zoysiinae.

our study but is a member of clade C in Hilu and Alice (2000, 2001), clades corresponding to our clades A and B are present and well supported in the *rps*16 and *waxy* trees, although not all of the genera that we sampled were sampled by Ingram and Doyle (2004, 2007) and vice versa. Within clade A, *Acamptoclados* Nash (as *Eragrostis sessilispica* in our study), *Neeragrostis* Bush, and *Pogonarthria* Stapf are part of a well-supported clade (the *Eragrostis* clade) along with other species of *Eragrostis*, which is consistent with our trees (Fig. 2–4). Clades consistent with our *Cottea* Kunth–*Enneapogon* Desv. ex P. Beauv. clade and *Fingerhuthia* Nees ex Lehm.–*Uniola* L. clade are present in the *rps*16 phylogeny, but not in the *waxy* phylogeny, which is not well resolved with respect to the genera in question. Relationships among these two clades and the *Eragrostis* clade are not resolved in the *rps*16 phylogeny, in contrast to the *mat*K (Hilu and Alice 2001), *trn*L–F (Fig. 2), and ITS (Fig. 3) phylogenies, wherein the *Fingerhuthia–Uniola* clade is sister to the *Eragrostis* clade, and sister to this clade is the *Cottea–Enneapogon* clade. Within clade B, a well-supported sister relationship between *Calamovilfa* (A. Gray) Hack. ex Scribn. & Southw. and *Spartina* Schreb. is common to the *rps*16, *waxy*, ITS, and *trn*L–F + ITS trees. As well, consistent between Hilu and Alice (2000, 2001) and Ingram and Doyle (2004, 2007) is the presence of *Eragrostis advena* (Stapf) S. M. Phillips (as *Thellungia advena* Stapf in Ingram and Doyle 2004, 2007), not sampled in our study, in clade B. Ingram and Doyle (2004, 2007) also sampled two species of *Pappophorum*, which form a well-supported clade apart from clades A and B.

Based on chloroplast restriction site variation, Duvall et al. (1994) conducted a phylogenetic study of 17 genera in Eragrostideae. *Aegopogon* Humb. & Bonpl. ex Willd., *Schaffnerella* Nash, and *Schedonnardus* Steud. (all Cynodonteae; Clayton and Renvoize 1986) were not sampled in their study, but *Muhlenbergia* and six other genera form a clade (BS 90%) consistent in composition with our *Muhlenbergia* clade (Fig. 2–4), including a sister relationship between *Bealia* Scribn. and *Blepharoneuron* Nash (BS 81%), and the non-monophyly of *Muhlenbergia.* Intergeneric relationships outside the *Muhlenbergia* clade are less certain. However, a clade comprising *Dasyochloa* Willd. ex Rydb., *Erioneuron* Nash, and *Munroa* Torr., which is well supported in our trees, is also present in the Duvall et al. (1994) trees from one of their analyses.

Ortiz-Diaz and Culham (2000) studied the phylogeny of *Sporobolus* and relatives using ITS sequences. Three chloridoid species were employed as the outgroup, including one species of *Eragrostis. Spartina* and *Zoysia* Willd. were not sampled. Their analyses yielded a well-supported clade (jackknife 100%) consistent with clade B (Fig. 2–4; Hilu and Alice 2001; Ingram and Doyle 2004, 2007). As in our study, *Sporobolus* is not monophyletic in the analyses of Ortiz-Diaz and Culham (2000), wherein *Calamovilfa*, *Crypsis* Aiton, and two species of *Eragrostis*, including *E. advena*, are nested within *Sporobolus* with support.

Although sampling of Chloridoideae has been limited in family-wide molecular phylogenetic studies of grasses, several provide support for relationships in our study. Hilu et al.'s (1999) *mat*K phylogeny of grasses (13 chloridoid genera sampled) is consistent with our study, including support for the relationships of clades A, B, and C, except *Pappophorum* resolved in clade A, not C (discussed above). The phylogenies in Soreng and Davis (1998) and GPWG (2001) based on chloroplast restriction site variation are fully resolved with respect to Chloridoideae (*Distichlis* Raf., *Eragrostis*, *Spartina*, *Sporobolus*, *Uniola*, and *Zoysia* sampled) and are likewise consistent with our study. However, in other analyses of molecular data sets in GPWG (2001) that include *Pappophorum* in addition to the six genera above, there is conflict with respect to the position of *Pappophorum.* In analyses of all chloroplast data (i.e., chloroplast restriction sites + *ndh*F + *rbc*L + *rpo*C2) and all molecular data (i.e., chloroplast data + nuclear GBSSI + ITS + *phy*B), *Pappophorum* is sister to the *Eragrostis–Uniola* clade (BS 78% in both trees), whereas in the *ndh*F phylogeny it forms a clade with the four other genera (BS 97%), which is consistent with our study. *Pappophorum* also appears in trees from individual analyses of the *rbc*L and *rpo*C2 data sets, but sampling of chloridoids therein is insufficient to determine which of the above conflicting topologies these phylogenies support, and, unlike the *ndh*F data set, the source(s) of the material used for sequencing *rbc*L and *rpo*C2 is not provided (GPWG 2001).

Analyses of ITS data in studies of Gramineae as a whole have yielded results inconsistent with ours. Hsiao et al. (1999) sampled nine chloridoid genera. *Eragrostis* is well supported as sister to the remaining chloridoids, and *Spartina* and *Sporobolus* form a well-supported clade. However, the *Spartina–Sporobolus* clade (= clade B) is nested within genera that are in our clade C, a relationship that received statistical support in some but not all of their analyses. The GPWG (2001) sampled five chloridoid genera in their ITS analysis, but the subfamily did not resolve as monophyletic, although *Spartina* and *Sporobolus* form a well-supported clade.

Other family-wide studies that provide some insights into relationships within Chloridoideae based on molecular data include Clark et al.'s (1995) *ndh*F phylogeny and Duvall et al.'s (2007) chloroplast phylogeny based on *ndh*F and *rbc*L sequences. Only four chloridoid genera (*Eragrostis*, *Eustachys*, *Sporobolus*, and *Zoysia*) were sampled in the *ndh*F analyses, but *Sporobolus* and *Zoysia* form a well-supported clade. Seven chloridoids (including *Distichlis*, *Eragrostis*, *Spartina*, *Uniola*, and *Zoysia,* but not *Pappophorum*) were sampled in analyses of the *ndh*F + *rbc*L data set and their relationships are fully resolved, well supported, and congruent with our study.

Comparisons with Recent Classifications and Studies Based on Non-Molecular Data

Because Clayton and Renvoize's (1986) classification of Chloridoideae is one of the most recent, detailed, and widely followed worldwide treatments of the subfamily, we show in Fig. 4 (the strict consensus tree from the *trn*L–F + ITS analysis) the tribes and, as applicable, the subtribes associated with the genera we sampled. We did not sample Leptureae and Pommereullinae, and Triodiinae are represented by a single species in our study. Except for Orcuttieae (all three genera sampled) and Uniolinae (two of the four genera sampled), the remaining three tribes and five subtribes are

not monophyletic in the *trn*L–F and ITS phylogenies, although low resolution in clade C leaves open the possibility that Monanthochloinae are monophyletic. These results are consistent with the *mat*K phylogeny (Hilu and Alice 2001), wherein Triodiinae (three of the four genera sampled) are also monophyletic. In fact, all classifications of Chloridoideae correspond poorly to the molecular phylogenies.

Quantitative analyses of non-molecular data involving a significant number of chloridoid genera (Hilu and Wright 1982; Phillips 1982; Van den Borre and Watson 1997; Peterson 2000) likewise demonstrate conflict with the molecular phylogenies. In a detailed study, Van den Borre and Watson (1997) conducted phenetic and cladistic analyses of Chloridoideae based on 120 morphological and leaf anatomical characters scored for all 166 recognized genera and two subgenera of *Eragrostis.* An outcome was an informal classification of the subfamily consisting of three tribes, two subtribes, four groups (at tribal level), and four subgroups (at subtribal level). Of these, Boutelouinae (= *Bouteloua* Lag.; Columbus 1999) and Orcuttieae are monophyletic in the *mat*K (Hilu and Alice 2001), *trn*L–F, and ITS phylogenies, and Triodieae (= Triodiinae in Clayton and Renvoize 1986) and the *Monanthochloë* subtribal group (represented by *Monanthochloë* Engelm. and *Reederochloa* Soderstr. & H. F. Decker in Hilu and Alice 2001) are monophyletic in the *mat*K phylogeny. However, although Pappophoreae group together in all of the Van den Borre and Watson (1997) analyses (in contrast to the molecular phylogenies), genera in Cynodonteae and Eragrostideae (Clayton and Renvoize 1986) are intermixed as in the molecular phylogenies (Fig. 4). A few of these intertribal groupings are reflected in the molecular phylogenies, revealing that some morphological and/or anatomical characters track the molecular phylogenies more closely than others. In two cases, genera in Cynodonteae having only primary inflorescence branches, *Schedonnardus* and *Spartina*, group with genera in Eragrostideae mostly having rebranched inflorescences. *Schedonnardus* groups with *Bealia*, *Blepharoneuron*, *Chaboissaea* E. Fourn., and *Muhlenbergia* (along with other genera in the *Muhlenbergia* clade in our study) in both studies, and *Spartina* groups with *Calamovilfa*, *Crypsis*, and *Sporobolus* (= clade B in our study excluding *Zoysia*; *Sporobolus* is not part of the group in some of Van den Borre and Watson's 1997 analyses) (Fig. 2–4). Morphological and anatomical characters common to the members of each group are detailed in Van den Borre and Watson (1997) and are summarized below. Therefore, with respect to *Schedonnardus* and *Spartina*, an inflorescence composed only of primary branches has been an unreliable character for classification, as this inflorescence type is inferred from analyses of molecular and non-molecular data to have evolved independently in these two lineages apart from other origins elsewhere in the subfamily. Another case where there is support for a close relationship between members of Cynodonteae and Eragrostideae in the molecular phylogenies and the Van den Borre and Watson (1997) analyses involves *Chloris* (Cynodonteae), *Leptochloa* (Eragrostideae), and relatives. Two species of *Leptochloa*, sometimes treated in *Diplachne* P. Beauv. (Table 1), are members of the well-supported *Chloris* clade in the ITS (Fig. 3) and ITS + *trn*L–F (Fig. 4) trees. A number of other genera in Eragrostideae group with *Chloris* and relatives in Van den Borre and Watson (1997), including *Eleusine* Gaertn. *Eleusine* and *Leptochloa* also form a clade (C_1) with *Chloris* and relatives in the *mat*K phylogeny (Hilu and Alice 2001). *Eleusine* and *Leptochloa* both have an inflorescence of primary branches only, as do *Chloris* and relatives, but they have been classified apart from *Chloris* in Eragrostideae because most species have two or more fertile florets per spikelet (Clayton and Renvoize 1986). In this case, the inflorescence type is more indicative of relationship than the number of fertile florets per spikelet.

In sum, congruent, well-supported relationships in molecular phylogenies can lead us to those morphological and anatomical traits that are synapomorphies, even though these traits may be homoplastic in the larger context of the family or subfamily. In the following section we briefly explore morphological variation in light of the molecular phylogenies and, in concert with Peterson et al. (2007), propose changes to the classification based on what we know about relationships among Chloridoideae.

A Proposed Classification

The classification proposed in Peterson et al. (2007) is discussed here primarily with respect to tribes, which correspond to clades A, B, and C in the *mat*K (Hilu and Alice 2001), *trn*L–F (Fig. 2), and ITS (Fig. 3) phylogenies. We also discuss the subtribes in clade A. In the new classification, clades A, B, and C correspond to Eragrostideae, Zoysieae, and Cynodonteae, respectively (Fig. 4). Each clade is statistically supported in all analyses except for clades B and C in the analysis of the ITS data set.

Eragrostideae (clade A).—As can be gleaned from Fig. 4, the circumscription of Eragrostideae differs significantly from Clayton and Renvoize (1986). Based on current sampling, members of subtribes Monanthochloinae, Sporobolinae, and Triodiinae are excluded along with most genera in Eleusininae. Included are Uniolinae, some Eleusininae, and some Pappophoreae, each of these groups corresponding to well-supported (except in ITS) clades in the *mat*K (Hilu and Alice 2001), *rps*16 (Ingram and Doyle 2004, 2007), *trn*L–F (Fig. 2), ITS (Fig. 3), and *trn*L–F + ITS (Fig. 4) trees. These clades are classified as three subtribes: Uniolinae, Eragrostidinae, and Cotteinae, respectively (Peterson et al. 2007). Eragrostidinae and Uniolinae are sister, and sister to this clade are Cotteinae in each of the phylogenies above except *rps*16 (relationships unresolved); the *mat*K and ITS + *trn*L–F data sets provided statistical support for these relationships. Predominant features in the tribe include a ligule of hairs, multiple fertile florets per spikelet, and lemma nerves three or more. Lemmas in Cotteinae and Uniolinae have five or more nerves, in contrast to the typically three-nerved lemma in Eragrostideae, which indicates, based on their relationships, that five or more nerves is ancestral and there has been a reduction in nerve number in the Eragrostideae clade.

Cotteinae.—The long-recognized tribe Pappophoreae is polyphyletic in our molecular phylogenies. Of the three genera sampled (of five), *Cottea* and *Enneapogon* form a clade (Cotteinae) and *Pappophorum* forms a well-supported clade with *Tridens flavus* in clade C (Fig. 2–4). *Tridens* Roem. &

Schult. is not monophyletic in our phylogenies (discussed below), but analyses of *trn*L–F and ITS sequences from other species of *Pappophorum* and *Tridens* confirm a close relationship (J. T. Columbus and R. Cerros unpubl. data). The main characters that have been used to circumscribe Pappophoreae are lemmas with many nerves and awns and/or lobes, but the molecular phylogenies tell us that these traits evolved independently in *Pappophorum* and Cotteinae. Reeder (1965) provided evidence of a more distant relationship between *Pappophorum* and the other genera in the tribe than was previously thought. Unlike *Pappophorum*, Cotteinae possess many-nerved glumes and distinctive, elongate bicellular microhairs, among other differences. We have not yet carried out detailed morphological and anatomical studies comparing *Pappophorum* and *Tridens*, but examination of *Pappophorum* specimens revealed the presence of hairs along the central and marginal nerves of the lemma, which are also found in *Tridens.* Hilu and Alice (2001) and Ingram and Doyle (2004, 2007) sampled *Schmidtia* Steud. ex J. A. Schmidt, another genus in Pappophoreae, which resolved in the Cotteinae clade in the *mat*K and *rps*16 phylogenies.

Uniolinae.—Although *Entoplocamia* has yet to be sampled, Clayton and Renvoize's (1986) Uniolinae are monophyletic in the *mat*K (Hilu and Alice 2001), *trn*L–F (Fig. 2), and ITS (Fig. 3) phylogenies. Two of the four genera, *Fingerhuthia* and *Uniola*, were sampled in our study, and Hilu and Alice (2001) and Ingram and Doyle (2004, 2007) also sampled *Tetrachne* Nees. In the *rps*16 phylogeny, *Stiburus* Stapf is also in the clade (Ingram and Doyle 2004, 2007). The genus is sometimes included in *Eragrostis* and was not sampled in our study nor in Hilu and Alice (2001).

Eragrostidinae.—The Eragrostidinae clade in our study is represented by *Ectrosia* R. Br., *Neeragrostis*, *Pogonarthria*, and three species of *Eragrostis*, all classified in Clayton and Renvoize's (1986) subtribe Eleusininae (Fig. 2–4). Relationships are fully resolved in the ITS and *trn*L–F + ITS trees, and all clades in the latter analysis are well supported. *Eragrostis* is not monophyletic. In the *mat*K phylogeny (Hilu and Alice 2001), the clade comprises *Eragrostiella* Bor, *Heterachne* Benth., and several species of *Eragrostis*, but relationships are not well resolved or supported. In Ingram and Doyle's (2004, 2007) studies focused on *Eragrostis*, the Eragrostidinae clade includes *Acamptoclados* (as *E. sessilispica* in our study), *Diandrochloa* De Winter, *Neeragrostis*, *Pogonarthria*, and many species of *Eragrostis*, including the type species, *E. minor* Host. The *rps*16 phylogeny is virtually unresolved with respect to this clade, in contrast to the well-resolved *waxy* phylogeny, but *Eragrostis* is not monophyletic in either phylogeny. However, *Acamptoclados*, *Diandrochloa*, and *Neeragrostis* are often treated as synonyms of *Eragrostis* (e.g., Clayton and Renvoize 1986). Ingram and Doyle (2004, 2007) suggested that *Pogonarthria* also should be included in the genus. Our study shows that *Ectrosia* likewise is nested within *Eragrostis.* Unlike most species of *Eragrostis*, lemmas of *Ectrosia* and *Pogonarthria* are acuminate to one-awned. *Pogonarthria* also has an inflorescence of primary branches only (these tardily deciduous) in contrast to the rebranched inflorescence characteristic of most species of *Eragrostis.* A number of additional genera morphologically similar to *Eragrostis* need to be included in future molecular studies.

Zoysieae (clade B).—The five genera that form Zoysieae in our study are positioned in two tribes in Clayton and Renvoize (1986). *Calamovilfa*, *Crypsis*, and *Sporobolus* were placed in Eragrostideae subtribe Sporobolinae based on rebranched inflorescences and spikelets with a single floret. *Spartina* was placed in Cynodonteae subtribe Chloridinae based on spikelets having a single fertile floret and arranged along one side of nondeciduous, primary inflorescence branches, and *Zoysia* was positioned in subtribe Zoysiinae based on a spiciform inflorescence and spikelets having a single floret and falling as a single unit. The molecular phylogenies indicate that the single floret per spikelet is indicative of relationship among these genera exhibiting morphologically diverse inflorescences, although numerous other chloridoids have spikelets with a single floret. Other prevalent features in Zoysieae include a ligule of hairs, one-nerved, awnless lemmas, and a free pericarp. Many species in the tribe grow in sandy, saline, and/or wet soils.

As mentioned above, some species of *Eragrostis* that we did not sample, including *E. advena*, resolved in this clade in the *mat*K phylogeny (Hilu and Alice 2001), Ortiz-Diaz and Culham's (2000) ITS phylogeny, and (as *Thellungia* Stapf) in the *rps*16 and *waxy* phylogenies (Ingram and Doyle 2004, 2007). Clayton and Renvoize (1986) pointed out that a few species of *Eragrostis*, including *E. advena*, are morphologically close to *Sporobolus.* Morphological support for a close relationship of this species to *Sporobolus* and relatives are its one-nerved lemma and free pericarp. Clearly, more of these morphologically intermediate species need to be sampled in future studies.

The *mat*K and *trn*L–F phylogenies are virtually unresolved with respect to relationships in the Zoysieae clade (Hilu and Alice 2001; Fig. 2). However, *Calamovilfa* and *Spartina* form a well-supported clade in the *rps*16, *waxy*, ITS, and *trn*L–F + ITS trees (Ingram and Doyle 2004, 2007; Fig. 3, 4). In addition, *Zoysia* is supported as sister to the other members of the clade in the ITS and *trn*L–F + ITS trees. Peterson et al. (2007) placed *Zoysia* in subtribe Zoysiinae apart from the other genera (Sporobolinae) based on, among other characters, a suppressed or highly reduced lower glume and fused pericarp. Our phylogenies also show that *Sporobolus* is not monophyletic. *Spartina* and *Zoysia* were not sampled in the Ortiz-Diaz and Culham (2000) study focused on *Sporobolus*, but their ITS phylogeny shows *Calamovilfa*, *Crypsis*, and two species of *Eragrostis* nested within *Sporobolus*, which was represented by many species.

Cynodonteae (clade C).—Cynodonteae, the most densely sampled tribe in our study, display nearly the full range of morphological variation seen in the entire subfamily. Relatively low resolution and support within the clade (Fig. 2–4), perhaps resulting from one or more rapid diversification events, severely hinder classification as well as studies of character evolution and biogeography. Nonetheless, some well-supported clades provide important insights into relationships, and these are discussed below. Peterson et al. (2007) recognized ten subtribes, but about half of the genera in the tribe are treated as incertae sedis with respect to sub-

tribe. Additional data are needed to further resolve relationships in this morphologically diverse clade.

One well-supported clade corresponds to Orcuttieae, the lone tribe in Clayton and Renvoize (1986) that is monophyletic in our study. Peterson et al. (2007) treated this clade as subtribe Orcuttiinae. All three genera were sampled in the *mat*K (Hilu and Alice 2001), *trn*L–F (Fig. 2), and ITS (Fig. 3) phylogenies. In each phylogeny *Neostapfia* Burtt Davy is sister to the *Orcuttia* Vasey–*Tuctoria* Reeder clade, although relationships lack statistical support in the *trn*L–F trees. This topology supports Roalson and Columbus's (1999) hypothesis of relationships based on non-molecular data.

The largest clade within Cynodonteae that resolved with statistical support in our study consists of *Muhlenbergia* and nine other genera (the *Muhlenbergia* clade, Fig. 2–4). Although there are some topological differences between the *trn*L–F and ITS trees, the conflict involves clades lacking statistical support in one or both phylogenies. Relationships among *Chaboissaea*, *Schedonnardus*, and *Lycurus* Kunth–*Schaffnerella* remain uncertain, but the position of *M. ramulosa* is well supported in the *trn*L–F and *trn*L–F + ITS trees. As testament to homoplasy in inflorescence form, genera in the *Muhlenbergia* clade (= subtribe Muhlenbergiinae, Peterson et al. 2007) were classified in two tribes and four subtribes by Clayton and Renvoize (1986; Fig. 4), although most species share membranous ligules, one floret per spikelet, and three-nerved lemmas. *Redfieldia* Vasey is intriguing in having a ligule of hairs and two or more florets per spikelet. The genus is also in the *Muhlenbergia* clade in Duvall et al.'s (1994) phylogenetic study based on chloroplast restriction site variation. Because of the anomalous morphological features, the authors suggested that the monotypic *Redfieldia* may be of hybrid origin, involving a species outside the clade, but there is no evidence for this based on our molecular phylogenies. As in Duvall et al. (1994), *Muhlenbergia* is not monophyletic in our study.

Another large clade that is well supported in the ITS and *trn*L–F + ITS trees but not in the *trn*L–F phylogeny is the *Chloris* clade (Fig. 2–4), including *Chloris*, *Cynodon*, *Enteropogon* Nees, *Eustachys*, *Microchloa*, *Trichloris* E. Fourn. ex Benth., and two of three sampled species of *Leptochloa.* Peterson et al. (2007) placed all of these genera in subtribe Chloridinae except for *Leptochloa* (incertae sedis). Inflorescences of all members of the *Chloris* clade bear only nondeciduous primary branches, the spikelets arranged along one side, and the lemmas are three nerved. Except for the two species of *Leptochloa* we sampled, which have multiple fertile florets per spikelet, the other genera in the clade share a single fertile floret per spikelet, usually accompanied by one or more sterile upper florets. This distinction led Clayton and Renvoize (1986) to place *Leptochloa* in Eragrostideae apart from the other genera in Cynodonteae. Each genus in the *Chloris* clade that is represented in our study by two or more species—*Chloris*, *Enteropogon*, and *Leptochloa*—is not monophyletic in the *mat*K (Hilu and Alice 2001), *trn*L–F (Fig. 2), and ITS (Fig. 3) phylogenies. The third species of *Leptochloa* we sampled, *L. panicea*, forms a well-supported clade with *Dinebra* Jacq. outside the *Chloris* clade. In the *mat*K phylogeny, *Dinebra*, *Eleusine*, and several other genera that we did not sample form a clade (C_1) with the genera represented in our *Chloris* clade (Hilu and Alice 2001). Phillips (1973), in a taxonomic revision of *Dinebra*, stated that the genus is closely related to *Leptochloa*, differing in part by its deciduous inflorescence branches. Additional data are required to evaluate relationships between *Chloris* and its near relatives.

Erioneuron is well supported as sister to the *Dasyochloa–Munroa* clade in our molecular phylogenies (Fig. 2–4). This topology differs from an analysis of chloroplast restriction site variation in Duvall et al. (1994), wherein *Dasyochloa* is sister to *Erioneuron–Munroa.* In 1961, Tateoka conducted a study of *Tridens,* at the time circumscribed to include *Dasyochloa* and *Erioneuron.* Based on morphological, anatomical, and cytological evidence, he resurrected *Erioneuron,* treated *Dasyochloa* as a synonym therein, and hypothesized a closer relationship of the genus to *Munroa* than to *Tridens.* Originating from a study by Sánchez (1983), *Dasyochloa* is now widely recognized. For a fuller discussion of these genera, including the characters they share, see Peterson et al. (1995, 1997, 2007). These authors placed the three genera in subtribe Munroinae.

Even with *Dasyochloa* and *Erioneuron* removed, *Tridens* is not monophyletic in the *trn*L–F and ITS phylogenies (Fig. 2, 3). As discussed above, *T. flavus* and *Pappophorum* form a well-supported clade in both phylogenies. *Tridens muticus*, on the other hand, forms a clade with *Gouinia* E. Fourn. ex Benth. & Hook. f. and *Vaseyochloa* Hitchc. The clade is well supported in all but the *trn*L–F analysis, wherein the relationships among the three taxa are also unresolved. In the ITS and *trn*L–F + ITS trees (Fig. 3, 4), *Vaseyochloa* is sister to *Gouinia–T. muticus*, a relationship that receives bootstrap support. Furthermore, in the same trees, *Triplasis* P. Beauv. is sister to the *Gouinia–Tridens muticus–Vaseyochloa* clade, a relationship also receiving support. Peterson et al. (2007) placed *Gouinia* and *Vaseyochloa* in subtribe Gouiniinae (*Tridens* and *Triplasis* were treated as incertae sedis). These four taxa have inflorescences of primary branches only, these persistent and rarely rebranched, pedicellate spikelets with multiple fertile florets, and hairs along the central and marginal nerves of the lemma.

Another interesting result of our analyses is the well-supported *Tragus* Haller–*Willkommia* Hack. clade (Fig. 2–4). Clayton and Renvoize (1986) treated these genera in separate subtribes of Cynodonteae—Zoysiinae and Chloridinae, respectively. Peterson et al. (2007) placed *Tragus* and *Willkommia* in subtribe Traginae. The genera differ in a number aspects, most notably in the five to seven rows of long, usually hooked projections on the upper glume of *Tragus.* However, traits in common include inflorescences with primary branches only, dorsally compressed spikelets, a single floret per spikelet, and three-nerved lemmas.

The remaining well-supported clades in the *trn*L–F and ITS phylogenies are the *Bouteloua* and *Distichlis* clades (Fig. 2–4). Because *Aegopogon* and *Schaffnerella* are in the well-supported *Muhlenbergia* clade, Clayton and Renvoize's (1986) Boutelouinae are rejected as monophyletic. Columbus et al. (1998, 2000) carried out molecular phylogenetic studies of the *Bouteloua* clade based on *trn*L–F and ITS sequences. In the *Distichlis* clade, *Reederochloa* and *Monanthochloë* are well supported as sister. Low resolution in the Cynodonteae clade leaves open the possibility that Monanthochloinae sensu Clayton and Renvoize (1986) are mono-

phyletic. Peterson et al. (2007) placed the members of these two clades in Boutelouinae (= *Bouteloua*) and Monanthochloinae, respectively.

Concluding Remarks

The results of this study, in concert with previous research, point to significant homoplasy in morphological characters which hinders efforts to produce a classification of Chloridoideae based on common ancestry. The problem is by no means restricted to the subfamily, yet the molecular phylogenies indicate homoplasy in all of the principal characters that have been employed in classification of the chloridoids, notably inflorescence type, number of florets per spikelet, and number of lemma nerves. Although far from exhaustive, a great deal is known about the morphology, anatomy, and cytology of chloridoid grasses. Where we are most deficient, however, is in our understanding of phylogenetic relationships. Large molecular studies are needed not only to improve the classification of this diverse, widespread group, but also to evaluate existing morphological, anatomical, and other data in a phylogenetic context to gain new insights into character evolution and biogeography.

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