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Phylogenetics of Muhlenbergiinae (Poaceae: Chloridoideae, Cynodonteae) Based on ITS and *trnL-F* DNA Sequences

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Abstract—Muhlenbergiinae are a subtribe in the grass (Poaceae) subfamily Chloridoideae, tribe Cynodonteae. The morphologically diverse group includes 10 genera and ca. 174 species and is restricted almost entirely to the New World, with a center of diversity in Mexico (125 species). With ca. 153 species, *Muhlenbergia* is by far the largest genus, and is divided into two subgenera, *Muhlenbergia* and *Trichochloa*, the latter with two sections. The other, much smaller genera are *Aegopogon* (4 species), *Bealia* (1), *Blepharoneuron* (2), *Chaboissaea* (4), *Lycurus* (3), *Pereilema* (4), *Redfieldia* (1), *Schaffnerella* (1), and *Schedonnardus* (1). We conducted a phylogenetic study of Muhlenbergiinae based on parsimony analysis of DNA sequences of the nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2) and chloroplast *trnL* intron, *trnL* 3' exon, and *trnL-trnF* intergenic spacer. All genera were sampled, including 52 species of *Muhlenbergia* representing both subgenera and sections. *Muhlenbergia* and *Pereilema* are not monophyletic in the resulting trees. The species of *Pereilema* and the other small genera are nested within *Muhlenbergia* in three main lineages. One of the lineages includes a monophyletic *Muhlenbergia* subgen. *Trichochloa*. Another lineage comprises species having leaf anatomy predictive of the PCK subtype of C_4 photosynthesis. Based on the results of this study, we favor expanding the circumscription of *Muhlenbergia* to include the other nine genera of the subtribe.

Keywords—classification, grasses, *Muhlenbergia*, PCK photosynthesis, phylogeny.

The grass subtribe Muhlenbergiinae (Poaceae, Chloridoideae, Cynodonteae) was first circumscribed by Pilger (1956) to include a single genus, *Muhlenbergia* Schreb., with eight sections. He also recognized the genus *Epicampes* J. Presl, which was placed in a separate subtribe, Sporobolinae, but this name is now treated as a synonym of *Muhlenbergia* (e.g., Soreng et al. 2008). Subsequent authors have agreed, with respect to *Epicampes* and *Muhlenbergia*, that Pilger's classification was not particularly reflective of phylogenetic relationships (e.g., Soderstrom 1967; Pohl 1969).

Muhlenbergiinae are highly variable morphologically, although the group can be generally characterized as follows: ligule a membrane (rarely a line of hairs); inflorescence a panicle, rebranched or composed only of primary branches; spikelets solitary, sometimes in pairs or triads, cleistogenes occasionally present in the leaf sheaths; floret 1 (rarely more), perfect, staminate, or sterile; glumes awned or unawned; lemmas 3-nerved, awned or unawned; base chromosome number x = 8–10 (Peterson et al. 1995, 1997, 2007a, b; Peterson 2000; Columbus et al. 2007). Based on leaf anatomy, two subtypes of C_4 photosynthesis, NAD-ME and PCK, are thought to occur in Muhlenbergiinae (Hattersley and Watson 1992; Columbus 1996, unpubl. data; Peterson 2000; Peterson and Herrera-Arrieta 2001), although only one species has been biochemically typed (PCK; Gutierrez et al. 1974).

The subtribe includes 10 genera and ca. 174 species, of which 96% (167) are native to the western hemisphere and >85% are found in North America (Peterson et al. 2007a). Several of the New World taxa are amphitropical disjuncts; those studied appear to have North American origins (Peterson and Herrera 1995; Sykes et al. 1997; Peterson and Morrone 1998; Peterson and Ortíz-Diaz 1998).

By far the largest genus in the subtribe is *Muhlenbergia*, which has ca. 153 species including seven species native to southeast Asia, five New World species with amphitropical distributions, and the important North American range grass *M. montana* (Peterson and Ortíz-Diaz 1998; Peterson 2003; Wu and Peterson 2006; Herrera-Arrieta and Peterson 2007; Peterson et al. 2007a, b). Seventy percent (107) of the species occur in Mexico, where 56 are endemic (Peterson and Herrera-Arrieta 2005; Peterson et al. 2007a). On the basis of morphology and leaf anatomy, Soderstrom (1967) recognized two subgenera within *Muhlenbergia* and divided subgen. *Podosemum* (Desv.) Soderstr. (= subgen. *Trichochloa* A. Gray, an older name) into two sections, *Epicampes* (J. Presl) Soderstrom and *Podosemum* (Desv.) Pilg. These infrageneric groups were also recognized by Peterson (2000) and Peterson and Herrera-Arrieta (2001) based on variation in leaf anatomy in a large sample of species. Other significant contributions to our understanding of *Muhlenbergia* include the following. Pohl (1969) revised a group of 12 species he believed to represent the entire subgen. *Muhlenbergia* in North America. Based on morphology, anatomy, cytology, and flavonoid chemistry, 29 annual species have been studied and placed into tentative natural groups (Peterson

and Rieseberg 1987; Peterson 1988a, b, 1989a; Peterson et al. 1989; Peterson and Annable 1991). Morden (1985) and Morden and Hatch (1987, 1996) investigated the morphology and anatomy of six species they referred to as the *M. repens* (J. Presl) Hitchc. complex. A series of studies of the *M. montana* complex, consisting of ca. 15 species, have been carried out using morphological, anatomical, and flavonoid data (Herrera-Arrieta and Bain 1991; Herrera-Arrieta and Grant 1993, 1994; Reeder and Reeder 1995; Herrera-Arrieta 1998). More recently, variation in inter-simple sequence repeats (ISSRs) has been evaluated for *M. capillaris* (Lam.) Trin., *M. expansa*, and *M. sericea* (Michx.) P.M. Peterson (Gustafson and Peterson 2007).

The remaining genera in Muhlenbergiinae have four or fewer species, and four are monotypic. All are limited to the New World except for an occurrence of *Aegopogon cenchroides* in Papua New Guinea (Veldkamp 1985). Five are endemic to North America. Apart from its presence in Papua New Guinea, *Aegopogon* Humb. & Bonpl. ex Willd. (four species) is distributed in North and South America. *Bealia* Scribn. (one species) is restricted to northern Mexico (Peterson 1989b). *Blepharoneuron* Nash (two species) is found in North America and includes *B. tricholepis*, an important range grass in the southwestern U.S.A. and northern Mexico (Peterson and Annable 1990, 2003). *Chaboissaea* E. Fourn. (four species) has three species in Mexico and *C. atacamensis* (Parodi) P.M. Peterson & Annable in Argentina and Bolivia (Peterson and Annable 1992; Peterson and Herrera 1995; Sykes et al. 1997). *Lycurus* Kunth (three species) has one species limited to North America and two amphitropical disjuncts, including *L. setosus* (Reeder 1985; Sánchez and Rúgolo de Agrasar 1986; Peterson and Morrone 1998). *Pereilema* J. Presl (four species) is distributed in North and South America. *Redfieldia* Vasey (one species) is endemic to the U.S.A. (Reeder 1976; Hatch 2003). *Schaffnerella* Nash (one species) is known only from San Luis Potosí, Mexico (Columbus et al. 2002). *Schedonnardus* Steud. (one species) is yet another genus with an amphitropical distribution (Peterson et al. 2007a).

Duvall et al. (1994) carried out the first molecular phylogenetic study involving members of Muhlenbergiinae. Analysis of chloroplast restriction site variation in 17 New World genera indicated that *Muhlenbergia*, which did not resolve as monophyletic (two species sampled), is closely related to *Bealia*, *Blepharoneuron*, *Chaboissaea*, *Lycurus*, *Pereilema*, and *Redfieldia*. In a study of Chloridoideae by Hilu and Alice (2001) based on chloroplast *matK* sequences, *Muhlenbergia*, which likewise was not monophyletic (two species sampled), formed a well-supported clade with *Aegopogon* and *Schedonnardus* (none of the other genera above was included in their study). Columbus et al. (2007) sampled all nine of these genera in a study of Chloridoideae based on sequences of the nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2; hereafter referred to as ITS) and the chloroplast *trnL* intron, *trnL* 3' exon, and *trnL-trnF* intergenic spacer (hereafter *trnL-F*). In both phylogenies, the nine genera along with *Schaffnerella* formed a well-supported clade (the *Muhlenbergia* clade), and *Muhlenbergia* (three

species sampled) again did not resolve as monophyletic. These 10 genera constitute subtribe Muhlenbergiinae (Peterson et al. 2001, 2007a; Columbus et al. 2007; Soreng et al. 2008).

Although Columbus et al. (2007) included all 10 genera of Muhlenbergiinae in their molecular phylogenetic study of Chloridoideae, only 12 species were sampled. In the present study we greatly expand sampling of the clade, particularly of the large genus *Muhlenbergia*. From this larger sample we analyze sequences of ITS and *trnL-F*, representing different genomes, and evaluate the existing classification in light of the molecular phylogenies.

Materials and Methods

Taxa and Collections—We sampled all 10 genera of Muhlenbergiinae, including both species of *Blepharoneuron* and 52 species of *Muhlenbergia* representing both of the subgenera and sections (Appendix 1). Because the sister of *Muhlenbergia* remains unknown, a species from each of five New World genera was selected for the outgroup based on current knowledge of the phylogeny of Chloridoideae (Bell 2007; Columbus et al. 2007; Bell and Columbus, unpubl. data).

Collection/voucher information is provided in Appendix 1. Leaf samples were removed from live, field-collected plants or in fewer cases from herbarium specimens. In the field, at least one gram of leaf material was removed for each sample and placed in liquid nitrogen or silica gel (Liston et al. 1990; Chase and Hills 1991).

DNA Sequences—ITS and *trnL-F* sequences of *Aegopogon cenchroides*, *Bealia mexicana*, *Blepharoneuron tricholepis*, *Chaboissaea decumbens*, *Lycurus setosus*, *Muhlenbergia emersleyi*, *M. montana*, *M. ramulosa*, *Pereilema crinitum*, *Redfieldia flexuosa*, *Schaffnerella gracilis*, *Schedonnardus paniculatus*, and the outgroup species were from Columbus et al. (2007).

Total cellular DNA was extracted using one of the following procedures: 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).

For amplification of ITS and *trnL-F*, *Taq* polymerase from Invitrogen (Carlsbad, California) or Promega (Madison, Wisconsin) was used, as well as PCR Master Mix (Promega) and PuReTaq Ready-To-Go PCR Beads (Amersham Biosciences, Piscataway, New Jersey). Employing an annealing temperature of 48°C, the procedure for amplifying ITS generally followed Columbus et al. (1998) except primer 'ITS-5m' (Sang et al. 1995) was sometimes used in place of 'ITS5' (White et al. 1990), and the reactions sometimes included 10% dimethyl sulfoxide (DMSO) to facilitate amplification (Winship 1989; Varadaraj and Skinner 1994). Primers 'c' and 'f' or '*trnL5*' BR' and 'f' (Taberlet et al. 1991; Columbus et al. 2007) and annealing temperatures of 52-55°C were used to amplify *trnL-F*; reactions sometimes included 5 or 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) 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 ITS, primers 'ITS5' and 'ITS4' were usually used for sequencing; 'ITS-5m', 'ITS5i', 'ITS4i', 'ITS2', and 'ITS3' were sometimes employed (White et al. 1990; Sang et al. 1995; Porter 1997). For *trnL-F*, primers 'c', 'd', 'e', and 'f' (Taberlet et al. 1991) were most often utilized for sequencing, although primers developed by Columbus et al. (2007) were commonly used to achieve reliable sequence determination of the entire region. Sequence fragments were assembled, edited, and a consensus sequence constructed using Sequencher vers. 3 or 4 (Gene Codes Corporation, Ann Arbor, Michigan). The bounds of ITS1, 5.8S, and ITS2 follow Columbus et al. (1998). The bounds of the *trnL* exons and intron and *trnL-trnF* intergenic spacer follow Columbus et al. (2007).

Analyses—Sequences were aligned manually using Se-Al vers. 2.0 (Rambaut 2002). For *trnL-F*, 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).

Maximum parsimony analyses of the ITS, *trnL-F*, and combined ITS/*trnL-F* data sets were performed using PAUP* vers. 4.0b10 (Swofford 2002). The program defaults and the settings used in Columbus et al. (2007) were employed except 500 random stepwise-addition replicates were executed for each heuristic search (aside from *trnL-F*, discussed below), holding two trees per step, and each replicate was limited to 10 million rearrangements.

Bayesian, bootstrap, and Bremer/decay analyses were performed on the three data sets to determine support for clades. Bayesian posterior probabilities were estimated using MrBayes vers. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2005). The DNA substitution models GTR+I+G and K81uf+G were selected for ITS and *trnL-F*, respectively, using the Akaike (1974) information criterion in Modeltest vers. 3.7 (Posada and Crandall 1998). Because the latter model is not implemented in MrBayes, the more complex (parameter-rich) GTR+G model was used (Ronquist et al. 2005). For analysis of the combined ITS/*trnL-F* data set, the matrix was partitioned by locus and the respective model was applied to each partition. For each analysis, two parallel runs with four chains (Markov Chain Monte Carlo) were carried out for 5,000,000 generations to accumulate a minimum sample of 25,000 trees (one from every 100th generation) from each run after the standard deviation of split frequencies stabilized below 0.01; trees collected up to this point were discarded as 'burnin'. The posterior probability estimates were calculated by constructing a majority rule consensus tree in PAUP* from the >25,000 trees sampled in each analysis. Nonparametric boot-

strap analyses (Felsenstein 1985) were carried out in PAUP* and employed the same settings as in the parsimony analyses except uninformative characters were excluded and random stepwise-addition replicates was set to one. Five hundred bootstrap replicates were executed. Bremer values (decay indices; Bremer 1988; Donoghue et al. 1992) were calculated using MacClade vers. 4.05 (Maddison and Maddison 2002) and PAUP* and the same settings as in the parsimony analyses except the number of random stepwise-addition replicates was 10.

The incongruence length difference (ILD) test (Farris et al. 1995), implemented in PAUP* as the partition homogeneity test, was performed to assess incongruence between the ITS and *trnL-F* data sets. The search settings used are as described above for the bootstrap analyses. One thousand ILD replicates were executed.

Results

For each sample, complete sequences were obtained of ITS1, 5.8S, ITS2, and the *trnL* intron, *trnL* 3' exon, and *trnL-trnF* intergenic spacer. Sequences are available from GenBank; accession numbers are provided in Appendix 1. Summary information for the data sets and results of the analyses are given in Table 1. The data matrices along with the strict consensus tree from each analysis are available from TreeBASE (study accession S2438, matrix accessions M4632-M4634).

Aligning the ITS sequences was relatively straightforward but equivocal for some regions of ITS1 and ITS2. However, exploratory parsimony analyses using different alignments yielded the same well-supported clades. Because of ample variation in the data set (34.3% of the characters are parsimony informative) and, in part, uncertainties about homology, gaps were not coded for the analysis. However, several gaps turned out to be synapomorphies for clades that were

	ITS	*trnL-F*	ITS + *trnL-F*
Sequence length (base pairs)[a]	588-605	886-1026	–
Aligned sequence length	668	1319	1987
Insertions/deletions coded	0	15	15
Total characters	668	1334	2002
Parsimony informative characters	229	124	353
Most parsimonious trees	17,057	600,696	7518
Tree length	1393	379	1802
Consistency index[b]	0.40	0.64	0.43
Retention index	0.66	0.89	0.71

Table 1. Summary information for the data sets and results of the analyses. [a]Ingroup only. [b]Excluding parsimony uninformative characters.

Number	Kind	Length (base pairs)	Position in *trnL-F* data matrix
1	Deletion	6	152-157
2	Insertion	5	220-224
3	Insertion	12	298-309
4	Insertion	5	311-315
5	Insertion	1	506
6	Insertion	20	534-553
7	Insertion	6	595-600
8	Insertion	5	615-619
9	Insertion	23	892-914
10	Insertion	18	969-986
11	Insertion	5	1013-1018
12	Insertion	29	1028-1056
13	Deletion	10	1066-1075
14	Deletion	5	1089-1138
15	Insertion	3	1189-1191

Table 2. Nucleotide insertions and deletions (indels) in *trnL-F* scored as presence/absence characters for the analyses (characters 1320-1334 in the *trnL-F* data matrix). Indels 1-8 are in the *trnL* intron and 9-15 are in the *trnL-trnF* intergenic spacer.

already supported by Bayesian posterior probabilities of 0.99 or 1.0. Parsimony analysis of the 668 character data matrix yielded 17,057 most parsimonious trees 1393 steps long and with a consistency index of 0.40. Figure 1 is the strict consensus tree.

Aligning the *trnL-F* sequences was achieved with greater confidence but required the creation of many gaps equivalent to one or more base pairs. Most of the nucleotide insertions are clear-cut duplications. Fifteen indels were coded for the analysis (Table 2), all of which proved to have phylogenetic signal. Although the *trnL-F* data set has about twice as many total characters (1334) as the ITS data set, it has about half the number of parsimony informative characters (124, or 9.3% of the total characters). However, homoplasy is appreciably lower in *trnL-F* (consistency index = 0.64). Parsimony analysis of *trnL-F* also yielded many more trees, over 600,000. In fact, only 157 replicates could be completed due to the memory capacity of the computer. Shown in Fig. 2 is the strict consensus tree.

Analysis of the combined ITS/*trnL-F* data set resulted in 7518 most parsimonious trees, fewer than in the independent analyses. Figure 3 is the strict consensus tree.

Comparing the strict consensus trees from each analysis, the ITS (Fig. 1) and combined data (Fig. 3) trees are better resolved than the *trnL-F* tree (Fig. 2), and have more clades supported by posterior probabilities ≥0.95. However, 16

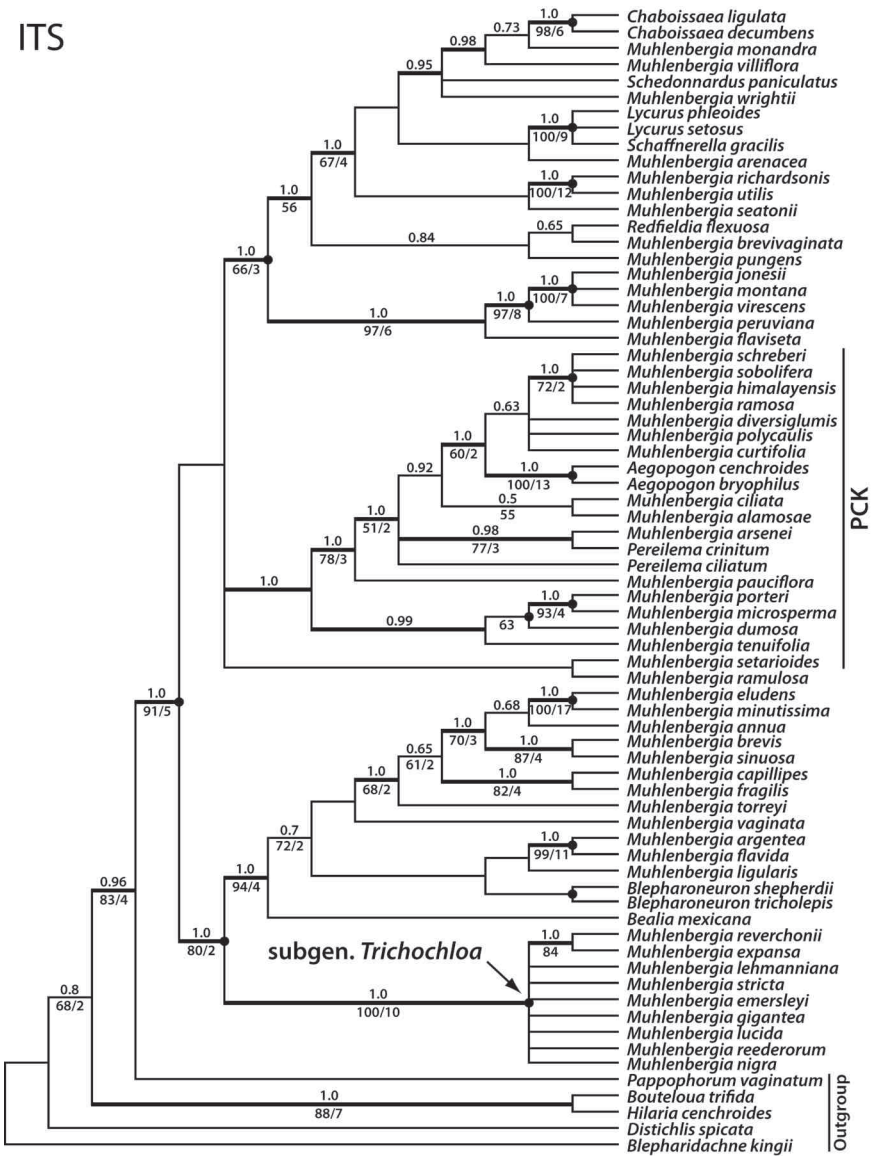

Fig. 1. Strict consensus of 17,057 most parsimonious trees resulting from analysis of ITS sequences. Numbers above branches are Bayesian posterior probabilities (≥0.5). Thick branches reflect posterior probabilities ≥95%. Numbers below branches are bootstrap percentages (≥50%) and Bremer values (≥2). Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of ITS and *trnL-F*.

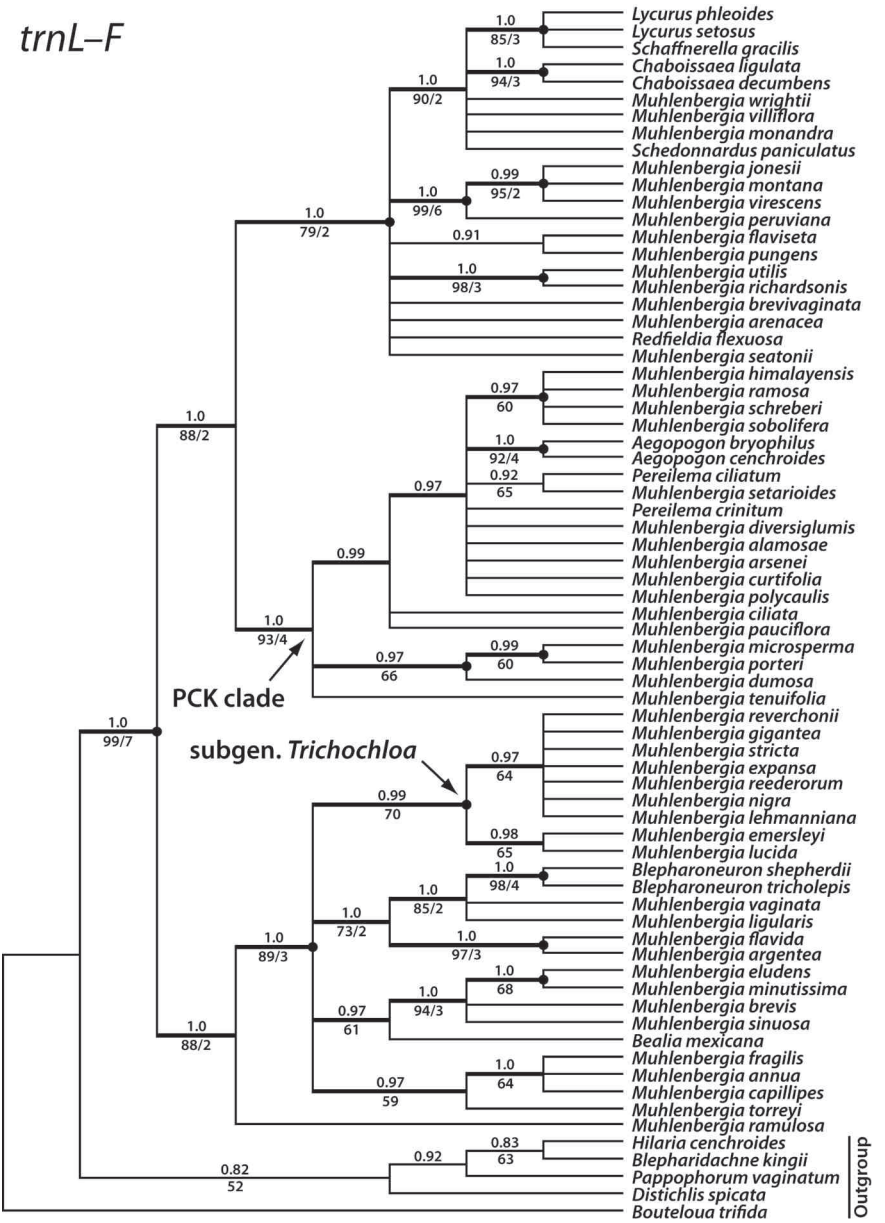

Fig. 2. Strict consensus of 600,696 most parsimonious trees resulting from analysis of *trnL-F* sequences. Numbers above branches are Bayesian posterior probabilities (≥0.5). Thick branches reflect posterior probabilities ≥95%. Numbers below branches are bootstrap percentages (≥50%) and Bremer values (≥2). Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of ITS and *trnL-F*.

clades are common to all trees from the three analyses (bulleted nodes). The only supported topological conflict between the ITS and *trnL-F* trees, based on posterior probabilities, involves *Muhlenbergia annua* and *M. setarioides* (discussed below).

The ILD test rejected the null hypothesis of congruence between the ITS and *trnL-F* data sets ($p = 0.001$). A second analysis was run after removing *Muhlenbergia annua* and *M. setarioides* from the data matrix. This resulted in a higher p value of 0.015 which fails to reject the null hypothesis at the 0.99 confidence level.

Discussion

Muhlenbergia is not monophyletic in either the ITS, *trnL-F*, or combined data trees because the other nine, smaller genera of Muhlenbergiinae are nested within it (Figs. 1-3). A possible outcome of the study was that the smaller genera might form one or a few clades. Instead, apart from *Lycurus* and *Schaffnerella*, which form a strongly supported clade, the other genera are scattered throughout the phylogeny in each of the major lineages. *Lycurus* (two of the three species sampled), however, resolved as monophyletic only in the ITS + *trnL-F* trees, and *Pereilema* (two of four species sampled) is not monophyletic in any of the trees from the three analyses. The other non-monotypic genera – *Aegopogon* (two of four species sampled), *Blepharoneuron* (both species sampled), and *Chaboissaea* (two of four species sampled) – are monophyletic in all trees from all three analyses. With regard to the subgenera and sections of *Muhlenbergia*, subgen. *Trichochloa* is well supported as monophyletic in all trees, but a lack of resolution within the clade due to low levels of variation precludes assessment of the two sections. Clearly, taxonomic changes are needed in order to better reflect phylogenetic relationships. Based on the results of this study, we favor expanding the circumscription of *Muhlenbergia* to include the other nine genera of the subtribe.

There are three major lineages within the ingroup. One includes *Chaboissaea*, *Lycurus*, *Redfieldia*, *Schaffnerella*, *Schedonnardus*, and species of *Muhlenbergia* subgen. *Muhlenbergia*. The clade is present in all trees from each analysis and is supported by posterior probabilities of 1.0. In the *Lycurus*/*Schaffnerella* subclade, the two genera differ in a number of aspects (e.g., lower glume development, lemma nerve number, disarticulation), but inflorescences of both are composed only of short primary branches, each bearing a small number of spikelets, and one of the glumes (the lower glume in *Lycurus*, upper in *Schaffnerella*) bears multiple awns. Species of *Chaboissaea* have one or two (occasionally three) florets, the lower floret perfect and the upper staminate or sterile (Peterson and Annable 1992; Peterson 2000). *Redfieldia* is also unusual in having two or more florets per spikelet, as well as a ligule of hairs. *Schedonnardus* stands out by having lengthy primary inflorescence branches that do not rebranch. The lineage includes two subclades of *Muhlenbergia* species representing groups that have been the focus

of several studies. *Muhlenbergia richardsonis* and *M. utilis* are part of the *M. repens* complex of six rhizomatous species with short, contracted inflorescences and awnless spikelets (Morden 1985; Morden and Hatch 1987, 1996). *Muhlenbergia flaviseta*, *M. jonesii*, *M. montana*, and *M. virescens* are perennial species in the *M. montana* complex, characterized in part by 3-nerved (often 3-toothed and/or 3-awned) upper glumes and lower leaf sheaths that often become flat, somewhat papery, and coiled in age (Reeder and Reeder 1995; Herrera-Arrieta 1998). However, one member of the complex sensu Herrera-Arrieta (1998), *M. argentea*, which has slightly compressed-keeled sheaths, 1-nerved upper glumes, and flattened caryopses (Reeder and Reeder 1995), forms a strongly supported clade with *M. flavida* in another of the three major lineages within the ingroup. Nested within the *M. montana* complex/clade in the ITS (Fig. 1) and ITS + *trnL-F* (Fig. 3) trees is *M. peruviana*, an annual species that usually has 2-3-nerved/toothed upper glumes (Peterson and Annable 1991).

A second major lineage consists of *Aegopogon*, *Pereilema*, and species of *Muhlenbergia* subgen. *Muhlenbergia*, including the type, *M. schreberi* (PCK clade in Figs. 2 and 3). *Aegopogon* is unique by having short, pendent primary inflorescence branches each bearing a triad of spikelets, one perfect and two staminate or sterile (one often not developed in *A. bryophilus*). *Pereilema*, not monophyletic, is distinguished by the presence of bristles in the inflorescence. All species in the lineage possess leaf anatomy predictive of the PCK subtype of C_4 photosynthesis (Columbus 1996, unpubl. data; Peterson 2000; Peterson and Herrera-Arrieta 2001). In contrast to the *trnL-F* (Fig. 2) and ITS + *trnL-F* (Fig. 3) trees, wherein the PCK species form a strongly supported clade, *M. setarioides* does not group with the other PCK species in the ITS phylogeny (Fig. 1). However, there is no support for its position outside the clade, and it is sister to the other PCK species in the Bayesian majority rule consensus tree (not shown). In the *trnL-F* phylogeny, *M. setarioides* is supported as nested within the PCK clade. Based on the *trnL-F* and combined data trees, therefore, the PCK subtype is inferred to have evolved a single time from an NAD-ME ancestor. Of additional note, the only non-American species in the study, the Asian *M. himalayensis* and *M. ramosa*, are well nested within the PCK clade, indicating one or more past migrations from the New World, most likely North America, to the Old World.

The third major lineage includes *Bealia*, *Blepharoneuron*, *Muhlenbergia* subgen. *Trichochloa*, and species of subgen. *Muhlenbergia*. *Muhlenbergia ramulosa*, a delicate annual, is supported as sister to the lineage in the *trnL-F* (Fig. 2) and combined data (Fig. 3) trees. However, in the ITS phylogeny (Fig. 1) its position within the ingroup lacks support and is hence uncertain, therefore a relationship as in the *trnL-F* phylogeny cannot be ruled out. Species of subgen. *Trichochloa* are tall, caespitose perennials with erect, stout to robust culms and nerveless or 1-nerved glumes (Soderstrom 1967). In contrast, like *M. ramulosa*, most of the other species in the lineage are small annuals. In leaf blade transectional anatomy, most species of subgen. *Trichochloa* have primary vascular bundles

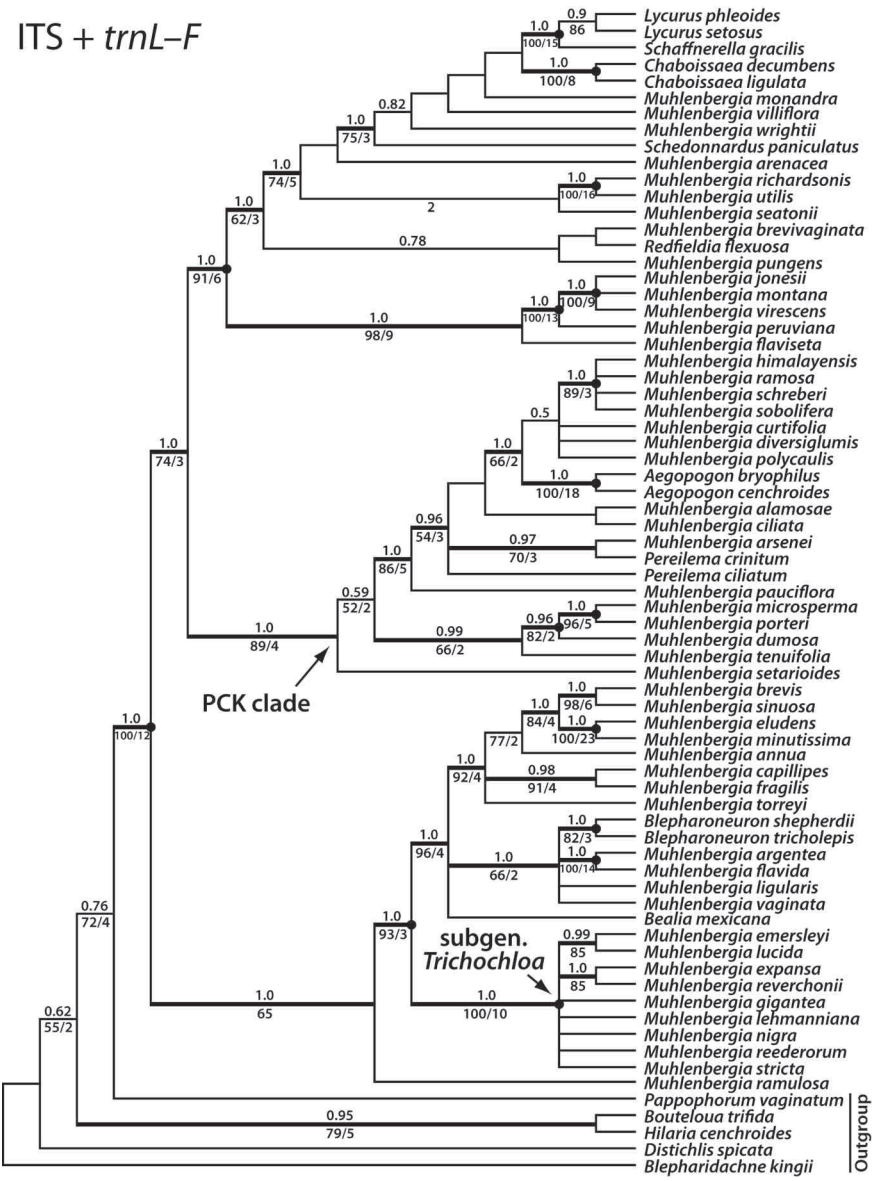

Fig. 3. Strict consensus of 7518 most parsimonious trees resulting from analysis of combined ITS and *trnL-F* sequences. Numbers above branches are Bayesian posterior probabilities (≥0.5). Thick branches reflect posterior probabilities ≥95%. Numbers below branches are bootstrap percentages (≥50%) and Bremer values (≥2). Bullets denote clades having the same composition of taxa in all most parsimonious trees from separate and combined analyses of ITS and *trnL-F*.

that are rectangular or obovate/elliptic, have sclerosed phloem and a crown of inflated cells on the adaxial side, and are >1/3 larger than the secondary and tertiary bundles (Soderstrom 1967; Peterson and Herrera-Arrieta 2001). Sclerosed phloem is also found in some members of the *M. montana* complex. Peterson and Annable (1991) pointed out that the annuals *M. brevis* and *M. depauperata* Scribn., the latter not sampled in our study, have a number of morphological features in common with *Lycurus*, including 2-nerved/awned lower glumes. However, *M. brevis* resolves well apart from *Lycurus* in the ITS and *trnL-F* phylogenies, suggesting these traits evolved in parallel.

In conclusion, this study represents a significant step forward in our understanding of the phylogeny of Muhlenbergiinae. Greater taxon sampling and improved resolution from additional data will add new insights. Detailed studies of morphology, anatomy, development, and cytology undoubtedly will uncover additional synapomorphies for clades that were revealed in the molecular study.

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Appendix 1

Taxa and collections (including sources) sampled, and GenBank accession numbers for ITS and *trnL-F* sequences (in that order). Columbus and Peterson vouchers are deposited at RSA and US, respectively. * *Muhlenbergia* subgen. *Trichochloa*.

* * *

Ingroup: *Aegopogon bryophilus* Döll, Peru (Cusco), *Columbus 3565*, GQ397862, GQ397916; *A. cenchroides* Humb. & Bonpl. ex Willd., Venezuela (Mérida), *Columbus 4380*, EF153020, EF156669; *Bealia mexicana* Scribn., Mexico (Chihuahua), *Columbus 3666*, EF153022, EF156671; *Blepharoneuron shepherdii* (Vasey) P.M. Peterson & Annable, Mexico (Chihuahua), *Peterson & R.M. King 8222*, GQ397863, GQ397917; *B. tricholepis* (Torr.) Nash, Mexico (Chihuahua), *Columbus 3652*, EF153024, EF156673; *Chaboissaea decumbens* (Swallen) Reeder & C. Reeder, Mexico (Chihuahua), *Columbus 3653*, EF153029, EF156678; *C. ligulata* E. Fourn., Mexico (Jalisco), *Columbus 3705*, GQ397864, GQ397918; *Lycurus phleoides* Kunth,

Mexico (Puebla), *Columbus 2638*, GQ397865, GQ397919; *L. setosus* (Nutt.) C. Reeder, U.S.A. (New Mexico), *Columbus 3286*, EF153062, EF156711; *Muhlenbergia alamosae* Vasey, Mexico (Chihuahua), *Peterson, M.B. Knowles, C.H. Dietrich & S.M. Braxton 13573*, GQ397866, GQ397920; *M. annua* (Vasey) Swallen, Mexico (Sonora), *Columbus 2724*, GQ397867, GQ397921; *M. arenacea* (Buckley) Hitchc., U.S.A. (New Mexico), *Columbus 3292*, GQ397868, GQ397922; *M. argentea* Vasey, Mexico (Chihuahua), *Peterson, C.R. Annable & Y. Herrera 8044*, GQ397869, GQ397923; *M. arsenei* Hitchc., Mexico (Baja California), *Peterson, C.R. Annable, R.F. Thorne & R.D. Noyes 5222*, GQ397870, GQ397924; *M. brevis* C.O. Goodd., U.S.A. (Texas), *Columbus 3308*, GQ397871, GQ397925; *M. brevivaginata* Swallen, Mexico (Durango), *Peterson, M.B. Knowles, C.H. Dietrich & S.M. Braxton 13682*, GQ397872, GQ397926; *M. capillipes* (M.E. Jones) P.M. Peterson & Annable, Mexico (Sonora), *Columbus 3622*, GQ397873, GQ397927; *M. ciliata* (Kunth) Trin., Mexico (Jalisco), *Columbus 4094*, GQ397874, GQ397928; *M. curtifolia* Scribn., U.S.A. (Arizona), *Peterson & C.R. Annable 5631*, GQ397875, GQ397929; *M. diversiglumis* Trin., Mexico (Sonora), *Columbus 3614*, GQ397876, GQ397930; *M. dumosa* Scribn. ex Vasey, Mexico (Sonora), *Columbus 3602*, GQ397877, GQ397931; *M. eludens* C. Reeder, Mexico (Chihuahua), *Peterson, C.R. Annable & Y. Herrera 7939*, GQ397878, GQ397932; *M. emersleyi* Vasey*, U.S.A. (New Mexico), *Columbus 3275*, EF153066, EF156715; *M. expansa* (Poir.) Trin.*, U.S.A. (Louisiana), *Columbus 3350*, GQ397879, GQ397933; *M. flavida* Vasey, Mexico (Sonora), *Columbus 3615*, GQ397880, GQ397934; *M. flaviseta* Scribn., Mexico (Durango), *Columbus 3683*, GQ397881, GQ397935; *M. fragilis* Swallen, Mexico (Sonora), *Columbus 3606*, GQ397882, GQ397936; *M. gigantea* (E. Fourn.) Hitchc.*, Mexico (Sinaloa), *Peterson, M.B. Knowles, C.H. Dietrich & S.M. Braxton 13414*, GQ397883, GQ397937; *M. himalayensis* Hack. ex Hook. f., China (Xizhang = Tibet), *R.J. Soreng, Peterson & Sun Hang 5666* (US), GQ397884, GQ397938; *M. jonesii* (Vasey) Hitchc., U.S.A. (California), *Peterson & C.R. Annable 4861*, GQ397885, GQ397939; *M. lehmanniana* Henrard*, Panama (Chiriqui), *Peterson & C. R. Annable 7372*, GQ397886, GQ397940; *M. ligularis* (Hack.) Hitchc., Venezuela (Barinas), *Peterson & R.M. King 11182*, GQ397887, GQ397941; *M. lucida* Swallen*, Mexico (Chihuahua), *Columbus 3656*, GQ397888, GQ397942; *M. microsperma* (DC.) Kunth, Mexico (Sonora), *Columbus 3601*, GQ397889, GQ397943; *M. minutissima* (Steud.) Swallen, Mexico (Sonora), *Columbus 3617*, GQ397890, GQ397944; *M. monandra* Alegría & Rúgolo, Peru (Lima), *Peterson & N.F. Refulio Rodriguez 17990*, GQ397891, GQ397945; *M. montana* (Nutt.) Hitchc., U.S.A. (Arizona), *Columbus 3375*, EF153067, EF156716; *M. nigra* Hitchc.*, Mexico (México), *Columbus 4603*, GQ397892, GQ397946; *M. pauciflora* Buckley, U.S.A. (New Mexico), *Columbus 3269*, GQ397893, GQ397947; *M. peruviana* (P. Beauv.) Steud., Mexico (Chihuahua), *Columbus 3641*, GQ397894, GQ397948; *M. polycaulis* Scribn., Mexico (Chihuahua), *Peterson, C.R. Annable & Y. Herrera 7938*, GQ397895, GQ397949; *M. porteri* Scribn. ex Beal, U.S.A. (Arizona), *Columbus 3240*, GQ397896, GQ397950; *M. pungens* Thurb. ex A. Gray, U.S.A. (Nebraska), *Columbus 3229*, GQ397897, GQ397951; *M. ramosa* (Hack. ex

Matsum.) Makino, China (Yunnan), *R.J. Soreng, Peterson & Sun Hang 5302* (US), GQ397898, GQ397952; *M. ramulosa* (Kunth) Swallen, Mexico (Sonora), *Columbus 3616*, EF153068, EF156717; *M. reederorum* Soderstr.*, Mexico (Durango), *Columbus 3686*, GQ397899, GQ397953; *M. reverchonii* Vasey & Scribn.*, U.S.A. (Texas), *Columbus 3332*, GQ397900, GQ397954; *M. richardsonis* (Trin.) Rydb., U.S.A. (Colorado), *Peterson & C.R. Annable 7832*, GQ397901, GQ397955; *M. schreberi* J.F. Gmel., U.S.A. (Alabama), *Columbus 4190*, GQ397902, GQ397956; *M. seatonii* Scribn., Mexico (Puebla), *Peterson & C.R. Annable 9946*, GQ397903, GQ397957; *M. setarioides* E. Fourn., Mexico (Oaxaca), *Peterson & C.R. Annable 9897*, GQ397904, GQ397958; *M. sinuosa* Swallen, U.S.A. (Arizona), *Peterson & C.R. Annable 7920*, GQ397905, GQ397959; *M. sobolifera* (Muhl. ex Willd.) Trin., U.S.A. (Maryland), *Peterson & J.M. Saarela 15773*, GQ397906, GQ397960; *M. stricta* (J. Presl) Kunth*, Mexico (Tamaulipas), *Peterson & R.M. King 8324*, GQ397907, GQ397961; *M. tenuifolia* (Kunth) Kunth, Mexico (Chihuahua), *Columbus 3662*, GQ397908, GQ397962; *M. torreyi* (Kunth) Hitchc. ex Bush, U.S.A. (Colorado), *Peterson & C.R. Annable 12005*, GQ397909, GQ397963; *M. utilis* (Torr.) Hitchc., U.S.A. (Texas), *Columbus 3333*, GQ397910, GQ397964; *M. vaginata* Swallen, Mexico (Chihuahua), *Peterson & R.M. King 8220*, GQ397911, GQ397965; *M. villiflora* Hitchc. var. *villiflora*, Mexico (Coahuila), *Peterson, C.R. Annable & J. Valdes Reyna 10040*, GQ397912, GQ397966; *M. virescens* (Kunth) Trin., Mexico (Chihuahua), *Peterson, C.R. Annable & Y. Herrera 8006*, GQ397913, GQ397967; *M. wrightii* Vasey ex J.M. Coult., U.S.A (New Mexico), *Columbus 3278*, GQ397914, GQ397968; *Pereilema ciliatum* E. Fourn., Mexico (Oaxaca), *Columbus 3756*, GQ397915, GQ397969; *P. crinitum* J. Presl, Mexico (Sonora), *Columbus 3621*, EF153074, EF156723; *Redfieldia flexuosa* (Thurb. ex A. Gray) Vasey, U.S.A. (Colorado), *Columbus 3910*, EF153076, EF156725; *Schaffnerella gracilis* (Benth.) Nash, Mexico (San Luis Potosí), *Columbus 4040*, EF153078, EF156727; *Schedonnardus paniculatus* (Nutt.) Branner & Coville, U.S.A. (Arizona), *Reeder & Reeder 9431* (RSA), EF153079, EF156728.

Outgroup: *Blepharidachne kingii* (S. Watson) Hack., U.S.A. (California), *Columbus 3855*, EF153023, EF156672; *Bouteloua trifida* Thurb., U.S.A. (Texas), *Columbus 2126*, EF153027, EF156676; *Distichlis spicata* (L.) Greene, U.S.A. (California), *Bell 231* (RSA), EF153040, EF156689; *Hilaria cenchroides* Kunth, Mexico (Oaxaca), *Columbus 3758*, EF153055, EF156704; *Pappophorum vaginatum* Buckley, U.S.A. (Arizona), *Columbus 2540*, EF153073, EF156722.