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Proposal for an Expanded *Distichlis* (Poaceae, Chloridoideae): Support from Molecular, Morphological, and Anatomical Characters

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Abstract*—**The *Distichlis* clade comprises *Distichlis* (7 species), *Monanthochloë* (2), and *Reederochloa* (1). All species except *D. distichophylla* (endemic to Australia) and *D. spicata* (widespread in the New World) are restricted either to North or South America. We investigated phylogenetic relationships within the clade using chloroplast (*trnL–F* and *ndhF*) and nuclear ribosomal (internal transcribed spacers and 5.8S) DNA sequences. We also studied lemma micromorphology, leaf blade anatomy, macromorphology, and biogeography in a phylogenetic context. The *Distichlis* clade is strongly supported in the molecular analyses. A morphological synapomorphy for the clade is the presence of a single papilla on the center of each subsidiary cell of lemma stomata. Other diagnostic features include dioecy, rhizomes or stolons, conspicuously distichous leaves, 5–13 lemma nerves, dumbbell- or flask-shaped bicellular microhairs with sunken basal cells, and growth in alkaline or saline soils. The nuclear and chloroplast phylogenies indicate that *Monanthochloë* and *Reederochloa* are nested within a paraphyletic *Distichlis*, and a number of structural characters, including leaf blade length, number of spikelets per inflorescence, and number of florets per spikelet, also fall within the range of variation in *Distichlis*. Therefore, we propose expanding the circumscription of *Distichlis* to include *Monanthochloë* and *Reederochloa*, and make the following new combinations: ***Distichlis acerosa, ***D. eludens***, and ***D. littoralis***. Biogeographical analysis revealed that the group likely originated in North America followed by a number of long-distance dispersal events, including back dispersals.

***Keywords*—**Biogeography, generic delimitation, *Monanthochloë*, phylogeny, *Reederochloa*, taxonomy.

Clayton and Renvoize (1986) treated seven genera in the grass subtribe Monanthochloinae (Poaceae, Chloridoideae): *Distichlis* Raf., *Monanthochloë* Engelm., *Reederochloa* Soderstr. & H. F. Decker, *Aeluropus* Trin., *Allolepis* Soderstr. & H. F. Decker, *Jouvea* E. Fourn., and *Swallenia* Soderstr. & H. F. Decker. With the exception of *Aeluropus* and one species of *Distichlis*, the subtribe is limited to the New World. Its members are characterized by conspicuously distichous leaves, vigorous rhizomes or stolons, 5–13 lemma nerves, and occurrence in xeric and/or saline/alkaline habitats (Clayton and Renvoize 1986; Watson and Dallwitz 1994; Peterson et al. 1995, 1997). Most of the genera are dioecious, but *Aeluropus* and *Swallenia* have hermaphroditic flowers. The association of these genera can be traced to Stebbins and Crampton (1961) who included them (with *Vaseyochloa* Hitchc.) in tribe Aeluropodeae.

A phylogeny of 84 genera of Chloridoideae, based on nuclear (internal transcribed spacer region [ITS]) and chloroplast (*trnL–F* region, *ndhF*) sequences (Bell 2007) demonstrated that *Aeluropus* is closely related to the Old World genera *Odyssea* Stapf and *Triodia* R. Br. and that *Swallenia* is in a clade with the New World genera *Blepharidachne* Hack., *Dasyochloa* Willd. ex Rydb., *Erioneuron* Nash, *Munroa* Torr., and *Scleropogon* Phil. Therefore, Monanthochloinae, sensu Clayton and Renvoize (1986), are not monophyletic. The same conclusion was reached by Hilu and Alice (2001), who sampled three genera of Monanthochloinae in their phylogenetic study of Chloridoideae based on chloroplast *matK* sequences. However, in Bell (2007) and a study of Chloridoideae by Columbus et al. (2007) based on *trnL–F* and ITS sequences, there is strong support for a clade comprising *Distichlis*, *Monanthochloë*, and *Reederochloa*, hereafter called the *Distichlis* clade. The sister of the *Distichlis* clade remains uncertain, although *Allolepis* (1 species), *Bouteloua* Lag. (57 species), *Jouvea* (2 species), and *Eragrostis obtusiflora* are among the candidates (Bell 2007).

The *Distichlis* clade has an amphitropical distribution in alkaline and saline soils, including coastal marshes, of temperate and subtropical North and South America (with one species found in similar habitats of southern Australia; Fig. 1). The largest genus of the clade, *Distichlis*, is usually treated as having seven species (McVaugh 1983; Pohl 1994; Zuloaga et al. 1994; Espejo Serna et al. 2000; Felger 2000; Peterson et al. 2001; Barkworth 2003; Nightingale and Weiller 2005). Six species have fairly narrow distributions and are relatively uniform in morphology although collectively they range from the diminutive mat-forming *D. australis* with leaf blades less than 1 cm long to *D. palmeri* that grows to 60 cm tall and has blades up to 12 cm long. The seventh species, *D. spicata*, is widely distributed in North and South America. It is also the most variable in features such as plant height, leaf blade length and divergence from culm, inflorescence size, pedicel length, spikelet size, and presence and density of hairs on leaf sheaths and blades. Several infraspecific taxa and distinct species have been recognized (Fassett 1925; Beetle 1943, 1945, 1955; Hitchcock 1950; Beetle et al. 1987; Zuloaga et al. 1994; ; Espejo Serna et al. 2000; Peterson et al. 2001; Negritto et al. 2003). There is, however, considerable overlap in the characters used to circumscribe these taxa, which often have not been accepted (Reeder 1943; McVaugh 1983; Pohl 1994; Felger 2000; Barkworth 2003).

Possessing long- and short-shoots, the growth form of *Monanthochloë* is distinctive. The long shoots are prostrate (stolons) to erect with long (to 15 cm) internodes. The short shoots arise from long shoots and bear closely spaced leaves having rigid, subulate blades shorter than 2 cm. The inflorescence is reduced to a single inconspicuous spikelet at the end of a short shoot. The spikelets lack glumes and have one or more reduced florets above the 1–3 fertile florets. Another diagnostic characteristic of *Monanthochloë* is the two aristae on the vegetative prophylls (Villamil 1969). *Monanthochloë* includes two species. *Monanthochloë littoralis* grows in coastal salt marshes of subtropical North America and, rarely, inland in the Chihuahuan and Sonoran deserts; *M. acerosa* is restricted to the salinas of central Argentina. In overall morphology, *M. acerosa* is more robust (thicker stems and wider leaf blades) than *M. littoralis*.

Limited to central Mexico, *Reederochloa* is a monotypic ge-

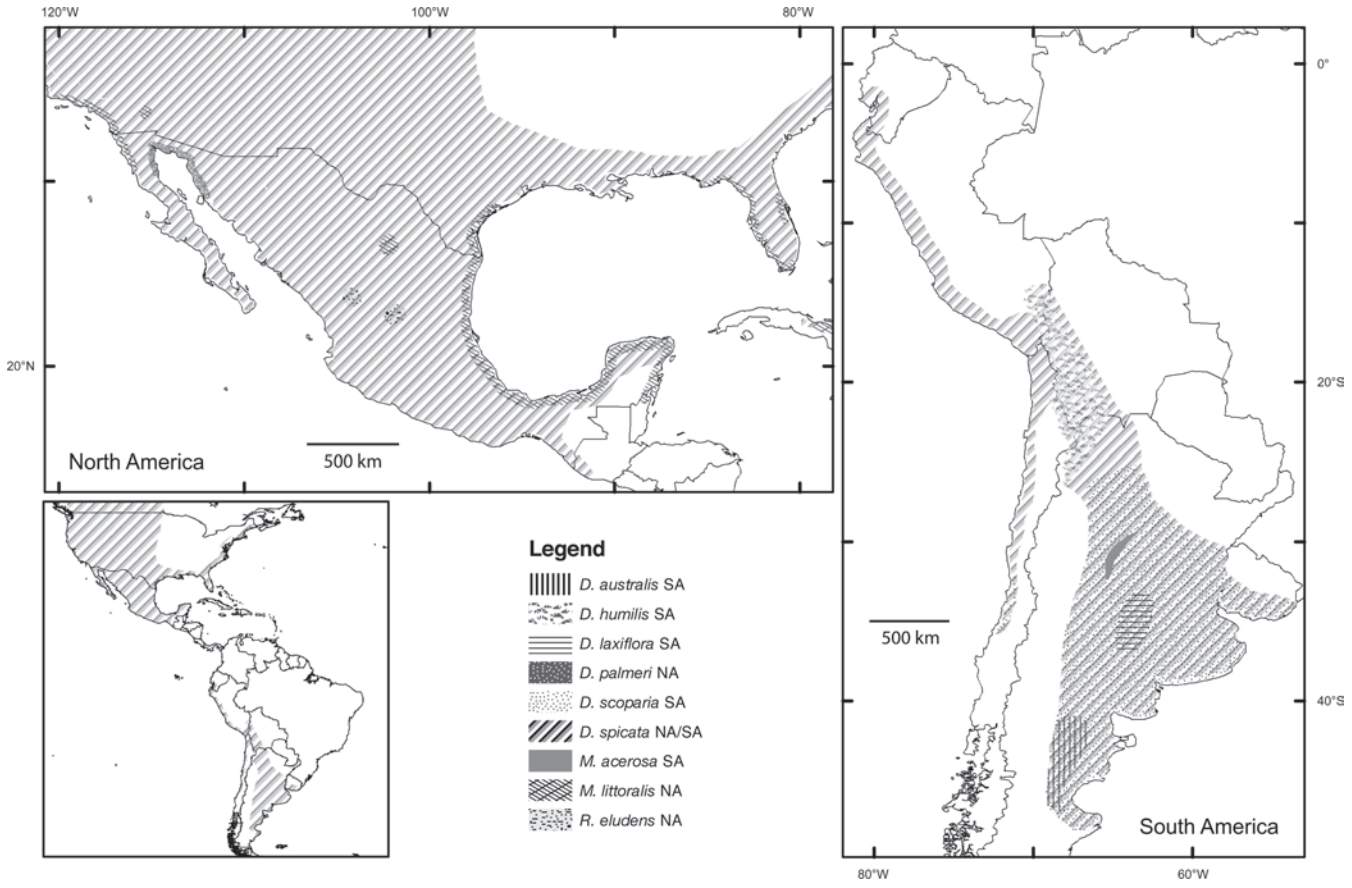


FIG. 1. Approximate distributions of the New World members of the *Distichlis* clade. *Distichlis distichophylla* is restricted to Australia (not shown). At lower left is the overall distribution of *D. spicata*. Note that the ranges of many species overlap. *D.* = *Distichlis*; *M.* = *Monanthochloë*; *R.* = *Reederochloa*; NA = North America; SA = South America.

nus that mainly differs from *Distichlis* and *Monanthochloë* in having dimorphic inflorescences, the female inflorescence positioned among the leaves, the male exserted. In describing *Reederochloa*, Soderstrom and Decker (1964) noted that it shares many features with *Distichlis* and *Monanthochloë*, including bulbous bicellular microhairs in which the basal cell is sunken into the epidermis. Table 1 summarizes the characters used to distinguish the three genera.

As no detailed phylogenetic studies of the *Distichlis* clade have been carried out, we gathered and analyzed DNA sequences from the chloroplast and nuclear genomes representing all species in the three genera. We also examined lemma micromorphology and leaf blade anatomy. Our goals were to evaluate morphology, anatomy, biogeography, and the classification in a phylogenetic context.

MATERIALS AND METHODS

***Taxon Sampling*—**Each recognized species in the *Distichlis* clade was sampled across its geographic range using two or more specimens. Outgroup taxa selected, based on Bell (2007), were *Allolepis*, *Bouteloua*, *Eragrostis obtusiflora*, and *Jouvea*. *Bouteloua dactyloides* was chosen to represent *Bouteloua* in the molecular data set. Appendix 1 presents source and voucher information for all specimens. Vouchers were prepared from plants collected in the field or greenhouse and are deposited at RSA unless otherwise noted.

***DNA Extraction, Amplification, and Sequencing*—**Chloroplast (*trnL–F* [*trnL* intron, *trnL* 3′ exon, and *trnL–trnF* intergenic spacer] and *ndhF* [protein coding]) and nuclear ribosomal (ITS, comprising ITS1, the 5.8S gene, and ITS2) DNA sequences were selected for this study in order to provide phylogenetic estimates from two genomes (White et al. 1990; Taberlet et al. 1991; Olmstead and Sweere 1994; Baldwin et al. 1995; Clark et al. 1995; Álvarez and Wendel 2003). The ITS and *trnL–F* regions have provided informative characters in previous studies of Chloridoideae

TABLE 1. Characters used to distinguish *Distichlis*, *Monanthochloë*, and *Reederochloa*, including those in Soderstrom and Decker (1964) and Villamil (1969).

Character	*Distichlis*	*Monanthochloë*	*Reederochloa*
Rhizomes	Present	Infrequent	Absent
Stolons	Rare	Present	Present
Vegetative prophylls	Not aristate	Aristate	Not aristate
Ligule (membrane)	Ciliate	Ciliate	Eciliate
Inflorescence	Contained in foliage or exserted	Contained in foliage	Male exserted, female contained
Glumes	Present	Absent	Present
Lemma nerves	5–13 (both sexes)	9–11 (both sexes)	6–10 (male), 10–13 (female)
Lemma surface	Glabrous	Glabrous	Male glabrous, female pilose at base

(Columbus et al. 1998, 2000, 2007; Mant et al. 2000; Ortiz-Diaz and Culham 2000; Baumel et al. 2002; Shrestha et al. 2003; Neves et al. 2005; Roodt-Wilding and Spies 2006). The *ndhF* gene has also proven to be highly informative in phylogenetic studies of grasses, particularly of the family as a whole (Clark et al. 1995; Grass Phylogeny Working Group 2001). Sequences of *ndhF* can be aligned with a high degree of confidence which is often not the case for noncoding regions.

DNA was extracted from frozen or silica-gel dried samples collected from live plants (field or greenhouse) and from herbarium specimens. Two methods were used to extract total genomic DNA. The CTAB protocol of Doyle and Doyle (1987) as modified by Columbus et al. (1998) was used for frozen material. The DNeasy® Plant Mini Kit (QIAGEN, Valencia, California) was used for silica-gel desiccated material and herbarium samples.

Amplification was performed in 25 μl reactions using Promega *Taq* polymerase (Madison, Wisconsin) according to the manufacturer's directions. Reactions included 10% and 5% dimethyl sulfoxide (DMSO) for amplification of the ITS and chloroplast markers, respectively (Gouvea et al. 1990). Annealing temperatures were 48°C for ITS and 54°C for *trnL–F*. *ndhF* was amplified in a series of overlapping segments using annealing temperatures of 48°C and 51°C. All primers used in this study are listed in Appendix 2.

Amplification products were purified using the polyethylene glycol precipitation protocol of Morgan and Soltis (1993), resuspended in dH_2O, and sequenced using Applied Biosystems (ABI; Foster City, California) PRISM® Big Dye® vers. 3.1 according to the manufacturer's directions. An ABI 3100 Genetic Analyzer was used to separate and visualize the products at Rancho Santa Ana Botanic Garden.

Sequence Editing and Alignment—Sequences were assembled and edited using Sequencher™ vers. 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were aligned manually using Se-Al vers. 2.0a11 (Rambaut 2002) with gaps introduced to adjust for variations in sequence length. Unambiguous insertions and deletions (indels) shared by two or more sequences were scored as presence/absence characters at the end of the matrices by the simple indel coding procedure of Simmons and Ochoterena (2000) and Graham et al. (2000). Sequences are deposited in GenBank (see Appendix 1 for accession numbers). The data matrices along with the strict consensus tree from each analysis are deposited in TreeBASE (study number S1986).

Phylogenetic Analyses—Maximum parsimony (MP) analyses were implemented in PAUP* vers. 4.0b10 (Swofford 2002). Characters were treated as equally weighted and unordered, and were optimized via accelerated transformation. Gaps were treated as missing data. Characters within an ITS sequence having multiple nucleotides were interpreted as polymorphic. Heuristic searches were performed with random addition of sequences with 10 replicates (rearrangements limited to 1,000,000 per replicate), one tree held at each step, and TBR branch swapping. Collapse and MulTrees options were in effect. Steepest descent was not used. A strict consensus tree was calculated. The two chloroplast data sets (*trnL–F* and *ndhF*) were combined and analyzed simultaneously. As discussed below, significant conflict between the ITS and chloroplast trees precluded a combined analysis of these data sets. Exploratory analyses were performed using individual and multiple outgroup taxa, but outgroup choice did not affect ingroup topology or clade support. Bootstrap support was calculated using the same settings as above except 1000 replicates and simple addition of sequences were employed. Bremer decay values (Bremer 1988; Donoghue et al. 1992) were calculated in PAUP* 4.0b10 (Swofford 2002) as facilitated by MacClade 4.0 (Maddison and Maddison 2001).

Bayesian posterior probabilities were calculated using MrBayes vers. 3.0b4 (Huelsenbeck and Ronquist 2001). The general time reversible model was used (nst = 6, rates = invgamma) based upon the results of previous analyses (Bell 2007). Analyses were run for 2,000,000 generations and trees saved every 500 generations. The analysis was judged to have reached stationarity when the standard deviation between the split frequencies stabilized below 0.009. A majority-rule consensus tree was calculated from the pool of trees in the region of stationarity.

Maximum likelihood (ML) analyses were also carried out. Modeltest vers. 3.7 (Posada and Crandall 1998) using PAUP* 4.0b10 (Swofford 2002) was applied to each data set with model selection based upon the Akaike Information Criterion (Akaike 1974). The same model (SYM + G; Zharkikh 1994) was selected for ITS with or without the 5.8S region included. The model selected for the combined chloroplast data set was TVM + I + G (transversional model). For each data set a ML search was performed using TBR branch swapping and, as a starting tree, the tree with the highest log-likelihood score from the Bayesian analysis.

For the biogeographical analysis, each terminal was coded as to origin (North America, South America, or Australia). This information was optimized on trees having the highest log-likelihood scores from the Bayesian analyses of the ITS and chloroplast data sets. Character reconstruction employed Mesquite vers. 1.12 (Maddison and Maddison 2006) using parsimony (all possible reconstructions) and likelihood in order to estimate probabilities associated with equivocal nodes (Mk1 model; Lewis 2001).

Leaf Blade Transectional Anatomy—For those species with short, narrow leaf blades, a segment of a shoot with several leaves attached was fixed whole in FPA (1:1:18 37% formaldehyde: propionic acid: 70% ethanol). For all others, leaves were selected from the middle of a shoot and 5 mm long segments were removed from the middle third of the blade and fixed in FPA. All samples were collected from live plants. Processing of the samples, including transverse sectioning, staining, and preparation of permanent microscope slides, followed Columbus (1999). Slides are deposited at RSA. Descriptive terminology follows Ellis (1976). No preserved leaf material was available for *D. distichophylla*; observations of the leaf anatomy of *Bouteloua* were taken from Columbus (1996).

Lemma Micromorphology—The abaxial surface of mature lemmas from the first floret of spikelets removed from herbarium specimens was examined. Except for *D. laxiflora* and the perfect-flowered *Eragrostis obtusiflora*, both male and female lemmas were studied. Observations of the lemma micromorphology of *Bouteloua* were taken from Columbus (1996, 1999). Lemmas were sonicated in xylene for 30 or 45 min to remove surface waxes, mounted on aluminum stubs with carbon conductive adhesive tabs, desiccated in a chamber using silica gel, and sputter coated with gold using a PELCO SC-7 system (Ted Pella, Redding, California). Samples were examined at 10 kV with an International Scientific Instruments WB-6 scanning electron microscope (SEM) and photographed using Polaroid 55 positive-negative film. Descriptive terminology follows Ellis (1979) and Columbus (1996).

Results

Molecular Data—Details of the sequence and alignment lengths are presented in Table 2. There were no missing characters in the ITS data set. For the chloroplast data sets, there was 3.2% missing from *ndhF* (no sequence for *Peterson 12833*, *D. humilis*) and 0.1% missing from *trnL–F* (43 base pairs [bp] missing from the 3′ end of *Bell 392*, *M. acerosa*). A total of 17 sites were scored as polymorphic in the ITS data set. Most were autapomorphies; however, *Bell 231* and *237* (*D. spicata*) shared four polymorphic sites. Incongruously, one sample of *D. distichophylla* (*Walsh s. n.*) had five polymorphic sites in *trnL–F*.

There were five informative indels in ITS. Three insertions of 2 or 3 bp are shared by samples of *D. palmeri*. A 1 bp deletion is shared by samples of *Reederochloa*. A 3 bp repeat is shared by samples of *D. australis*. Four indels were scored for *trnL–F*. A 1 bp insertion is shared by *D. australis*, *M. littoralis*, and *Reederochloa*. Both species of *Monanthochloë* share a 6 bp deletion. A 19 bp repeat is shared by *D. spicata* from inland California (USA) and *D. distichophylla* from South Australia. A 24 bp repeat is shared by *D. spicata* from Coahuila (Mexico), British Columbia (Canada), Virginia (USA), and Peru. No indels were scored for *ndhF*, although there were two length variations, a 6 bp duplication in *Bell 330* (*D. australis*) and a 9 bp deletion in *Bell 240* (*Allolepis texana*).

Table 2. Summary information for the molecular data sets and descriptive statistics from the parsimony analyses. PIC = parsimony informative characters; CI = consistency index; RI = retention index.

Data set	Length (base pairs)	Aligned length	Trees	Steps	PIC	CI	RI
ITS	592–604	633	13	436	135	0.79	0.86
trnL–F	908–971	1044					
ndhF	2106–2112	2112					
trnL–F + ndhF		3156	360	257	79	0.82	0.87

Descriptive statistics from the parsimony analyses are presented in Table 2. Compared to the chloroplast data set, the smaller yet significantly more variable ITS data set yielded greater tree resolution. Trees from ML analyses of the ITS and chloroplast data sets are presented in Figs. 2, 3. With respect to the ingroup, the MP trees (not shown) from each analysis were congruent with the corresponding ML trees. The ITS trees from the analyses differed only in relative branch lengths and outgroup topology. The chloroplast ML tree had the same topology as many of the MP trees.

There is hard (i.e. statistically supported) conflict between the ITS and chloroplast phylogenies (shaded areas in Figs. 2, 3). In the ITS phylogeny, *D. humilis*, *D. laxiflora*, and *D. scoparia* resolve in a clade with *D. australis*, *Monanthochloë*, and *Reederochloa*, whereas in the chloroplast phylogeny the three species form a clade with *D. distichophylla*, *D. palmeri*, and *D. spicata*. The phylogenies agree with respect to the monophyly of *Monanthochloë* and the paraphyly of *Distichlis*. At the species level, all species are monophyletic in the ITS phylogeny except for the widespread and morphologically variable *D.*

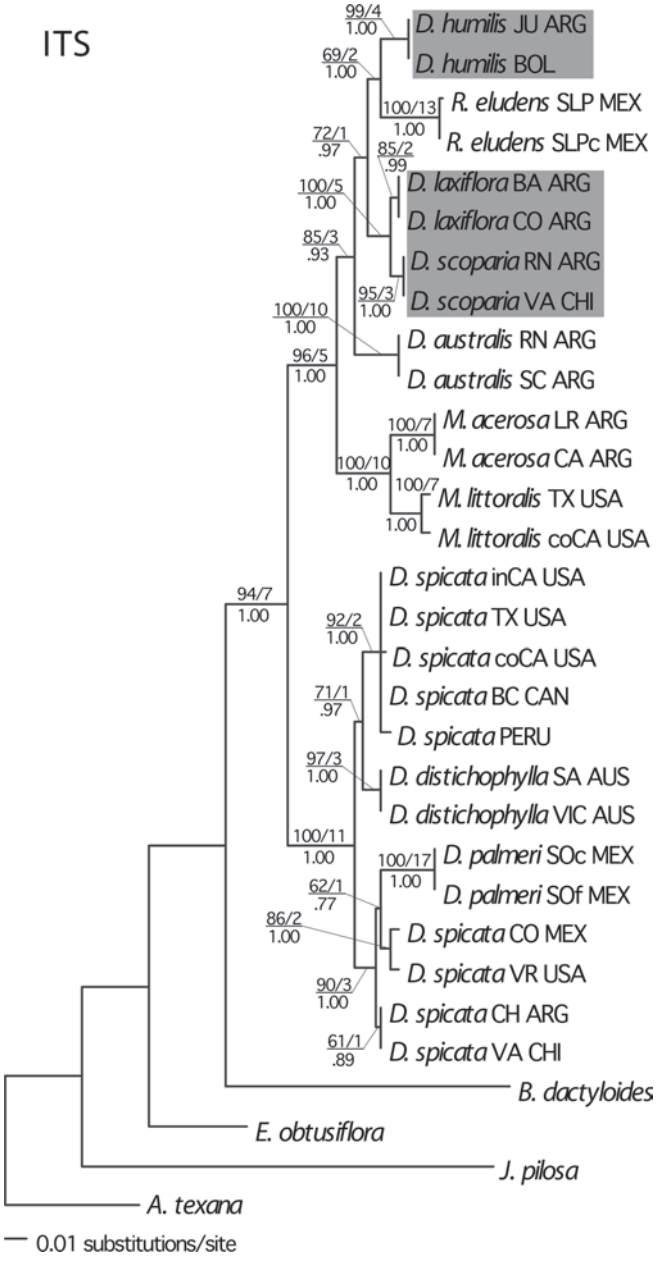


FIG. 2. Single tree derived from a maximum likelihood analysis of the ITS data set using the SYM + G model of evolution. Numbers above branches are bootstrap percentages and, following the slash, Bremer decay values. Numbers below branches are posterior probabilities from Bayesian analysis. Ingroup topology is the same as in all 13 equally parsimonious trees obtained from maximum parsimony analysis. Three species in positions conflicting with the chloroplast phylogeny (Fig. 3) are shaded. The geographic codes following the species names are explained in Appendix 1. *A.* = *Allolepis*; *B.* = *Bouteloua*; *D.* = *Distichlis*; *E.* = *Eragrostis*; *J.* = *Jouvea*; *M.* = *Monanthochloë*; *R.* = *Reederochloa*.

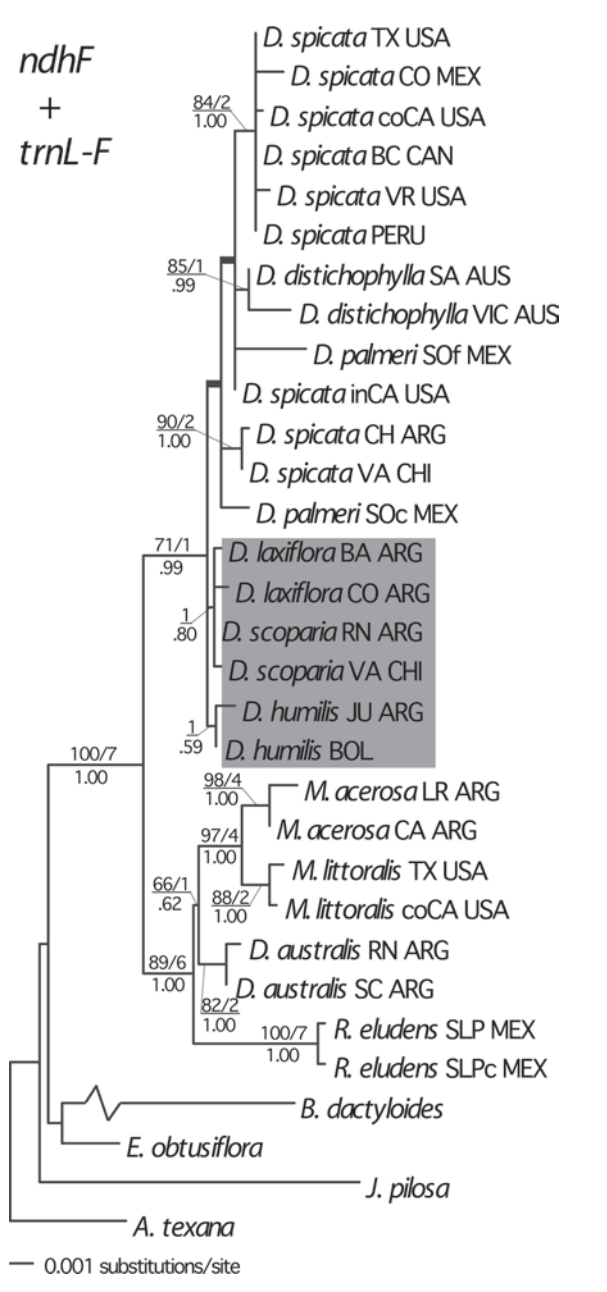
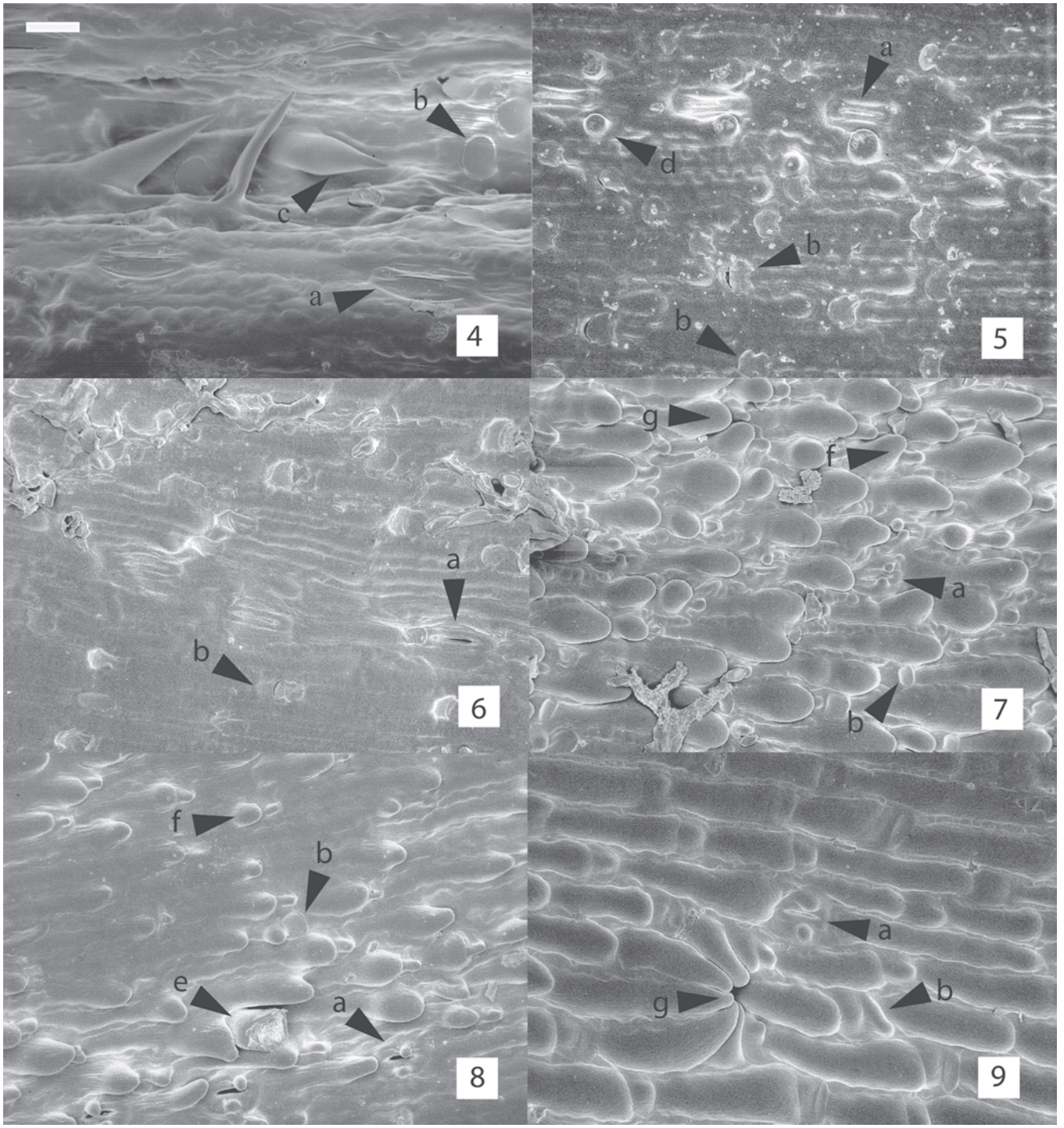


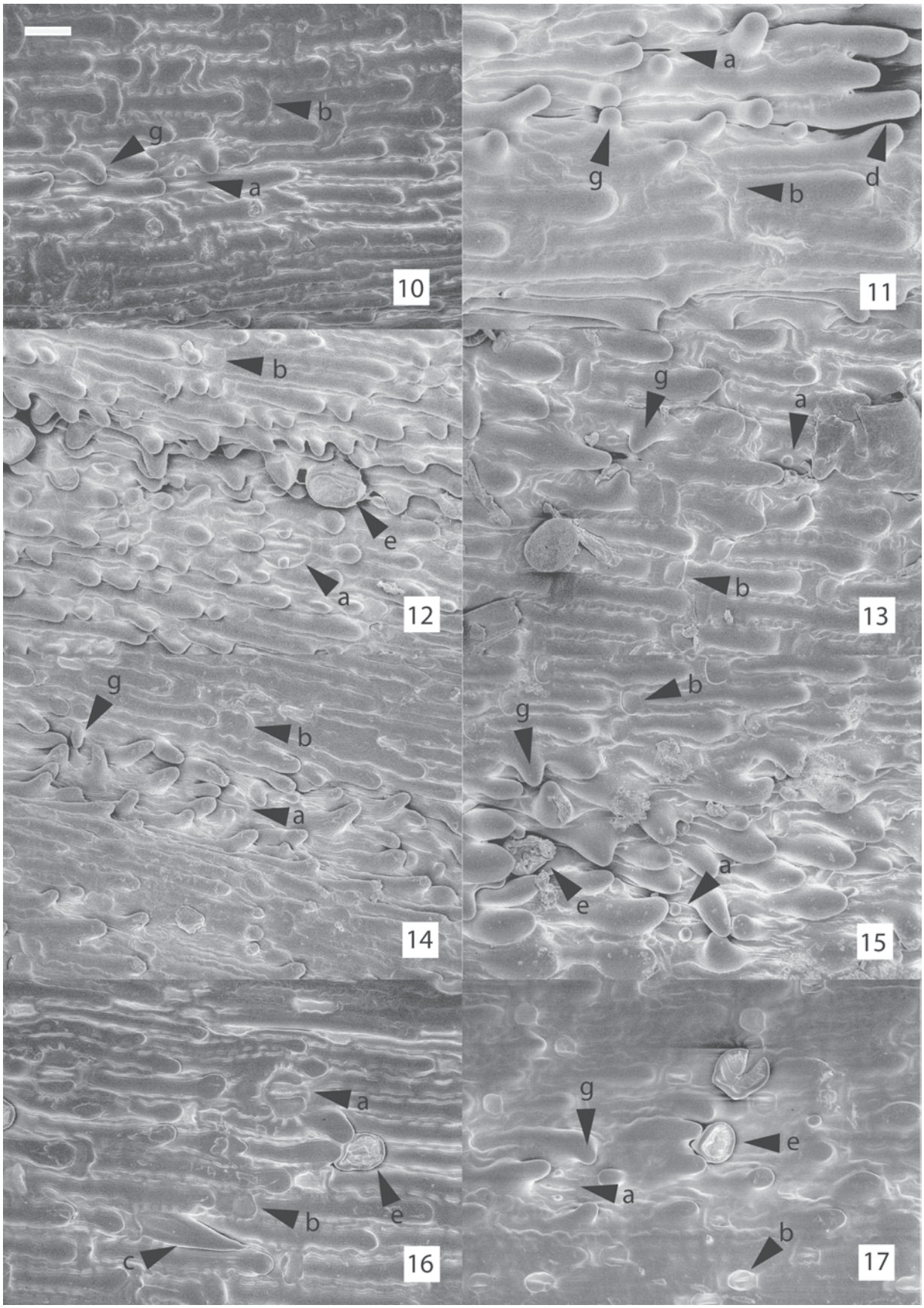
FIG. 3. Single tree derived from a maximum likelihood analysis of the combined *ndhF* and *trnL–F* data set using the TVM + I + G model of evolution. Numbers above branches are bootstrap percentages above 50 and, following the slash, Bremer decay values. Numbers below branches are posterior probabilities from Bayesian analysis. Thicker branches collapse in the strict consensus tree from the maximum parsimony analysis. Three species in positions conflicting with the ITS phylogeny (Fig. 2) are shaded. The geographic codes following the species names are explained in Appendix 1. Abbreviations are as in Fig. 2 caption.

spicata, which also possesses the greatest infraspecific sequence variation. *Distichlis distichophylla* and *D. palmeri* are nested within *D. spicata*. In the chloroplast phylogeny, low resolution and clade support render equivocal the monophyly of *D. spicata*, *D. laxiflora*, *D. palmeri*, and *D. scoparia*.

***Lemma Micromorphology*—**Lemmas of all species in the *Distichlis* clade were found to have a single small papilla located on the center of stomatal subsidiary cells (Figs. 7–15, 17). In addition, we observed in many specimens multiple large papillae, arising from surrounding cells, that overarch and obscure the stomate (Figs. 10, 11, 13–15, 17). A few specimens of *D. laxiflora* and *D. scoparia* lack papillae on some subsidiary cells but, instead, the border between the subsidiary cells and adjoining cells is crenulate (Fig. 16). Large papillae were also associated with microhairs (Figs. 8, 12, 15–17); these were observed in all species in the clade. In *D. palmeri*, we observed complexes of papillae with the tips pinched together (Fig. 9). In general, papillae are abundant in



FIGS. 4–9. Lemma micromorphology. Lemma apex is to the right. Scale bar is 10 μm and applies to all figures. 4. *Jouvea pilosa* (*Bell 249* ♀). 5. *Eragrostis obtusiflora* (*Bell 295*). 6. *Allolepis texana* (*Bell 307* ♂). 7. *Distichlis spicata* (*Bell 375* ♂). 8. *Distichlis distichophylla* (*Everett 221* ♂). 9. *Distichlis palmeri* (*Felger 91–39* ♂). a = stomate; b = short cell; c = prickle hair; d = papilla(e); e = microhair; f = paired papillae; g = papillae complexes.



FIGS. 10–17. Lemma micromorphology, continued. Lemma apex is to the right. Scale bar is 10 μm and applies to all figures. 10. *Monanthochloë littoralis* (*Bell 260* ♂). 11. *Monanthochloë acerosa* (*Bell 389* ♂). 12. *Reederochloa eludens* (*Bell 312* ♂). 13. *Distichlis humilis* (*Bell 408* ♀). 14. *Distichlis australis* (*Bell 334* ♂). 15. *Distichlis scoparia* (*Bell 374*). 16 & 17. *Distichlis laxiflora* (*Daguerre 231* ♂). See Figs. 4–9 caption for explanation of letters.

all species in the clade, particularly in intercostal zones. Pairs of papillae, one large and one small, are sometimes found on the distal end of the long cell and on the adjacent short cell, respectively (Figs. 7, 8); these were observed in all species except *D. palmeri*, *M. acerosa*, and *Reederochloa*.

Costal-intercostal zonation is evident within the *Distichlis* clade and was observed in all members except *D. palmeri*, *M. acerosa*, and *Reederochloa*. Prickle hairs were observed in *D. laxiflora* (Fig. 16), *D. palmeri*, *D. scoparia*, *D. spicata*, and *M. acerosa*, and macrohairs were seen in *D. australis*, *D. palmeri*, *M. acerosa*, and *Reederochloa*.

We observed few differences between male and female lemmas of the same species. Consistent with Soderstrom and Decker's (1964) observations of *Reederochloa*, macrohairs are found at the base of the female lemmas but are absent from male lemmas. In *D. australis* there is a tendency for female lemmas to have more papillae than males.

Regarding the outgroup taxa, no papillae were observed in *Jouvea* (Fig. 4), and few were found in *Eragrostis obtusiflora* (Fig. 5) and *Allolepis* (Fig. 6). Stomata and prickle hairs were observed in all three taxa, but no papillae were seen on subsidiary cells. In *E. obtusiflora*, as in the *Distichlis* clade, papillae are sometimes in groups (frequently of four) associated with microhairs, their tips converging (not shown).

Leaf Blade Anatomy—All taxa in this study have Kranz anatomy with a double bundle sheath (XyMS+, Hattersley and Watson 1992; Figs. 18–31). The outer sheath is even in outline (Prendergast et al. 1987), and, when not entirely filling cells, the outer sheath chloroplasts are elongate and centripetally arranged. Mesophyll chlorenchyma is radiate. Sclerenchyma is generally associated with vascular bundles and blade margins. Uni- to multiseriate girders of colorless cells are present between vascular bundles of most species. Stomates are present on both surfaces. Bicellular microhairs were observed in all samples.

Members of the *Distichlis* clade share several additional anatomical features. *Distichlis*, *Monanthochloë*, and *Reederochloa* have dumbbell- or flask-shaped microhairs that stain dark, and the basal cell is "sunken" into the epidermis (Figs. 36–38). In addition, metaxylem vessels are relatively narrow in diameter ranging from 3–15 μm in median vascular bundles, with the largest vessels only observed in a few specimens of *D. spicata* (Figs. 22–31). In contrast, metaxylem vessels in the outgroup taxa are larger, 15–24 μm in diameter (Figs. 18–21). All species in the *Distichlis* clade also have papillae, hooks, and/or prickles on both blade surfaces.

In *D. palmeri* (Fig. 24) and *D. spicata* (Figs. 22, 23), blades have a wide V- or U-shape in transverse section. Unlike other species in the *Distichlis* clade, the two species have more vascular bundles (18–24 total, including 7–9 and 5–6 primary bundles in *D. spicata* and *D. palmeri*, respectively) and markedly sclerosed phloem (especially *D. palmeri*, Fig. 24). In addition to the difference between the two species in the number of primary bundles, most sclerenchyma in *D. spicata* is in the form of girders, these often interrupting the outer bundle sheath. However, in *D. palmeri* the girders, which do not interrupt the outer sheath, are limited to the abaxial side of the bundles; all adaxial sclerenchyma is in the form of strands.

Distichlis laxiflora and *D. scoparia* are almost indistinguishable anatomically; the blade is circular or U-shaped in outline with 7–15 total and 3–5 primary vascular bundles (Figs. 26, 27). *Distichlis scoparia* has slightly sclerosed phloem that was not observed in *D. laxiflora*. *Distichlis humilis* (Fig. 25) is quite similar to *D. laxiflora* and *D. scoparia* but has less sclerenchyma, mostly in the form of minute strands. *Distichlis australis* (Fig. 28) is like *D. humilis*, *D. laxiflora*, and *D. scoparia* but differs in having definite abaxial furrows, sclerenchyma only in strands except for a girder abaxial to the median vascular bundle, and few or no colorless cells between bundles (most cells contain some chloroplasts).

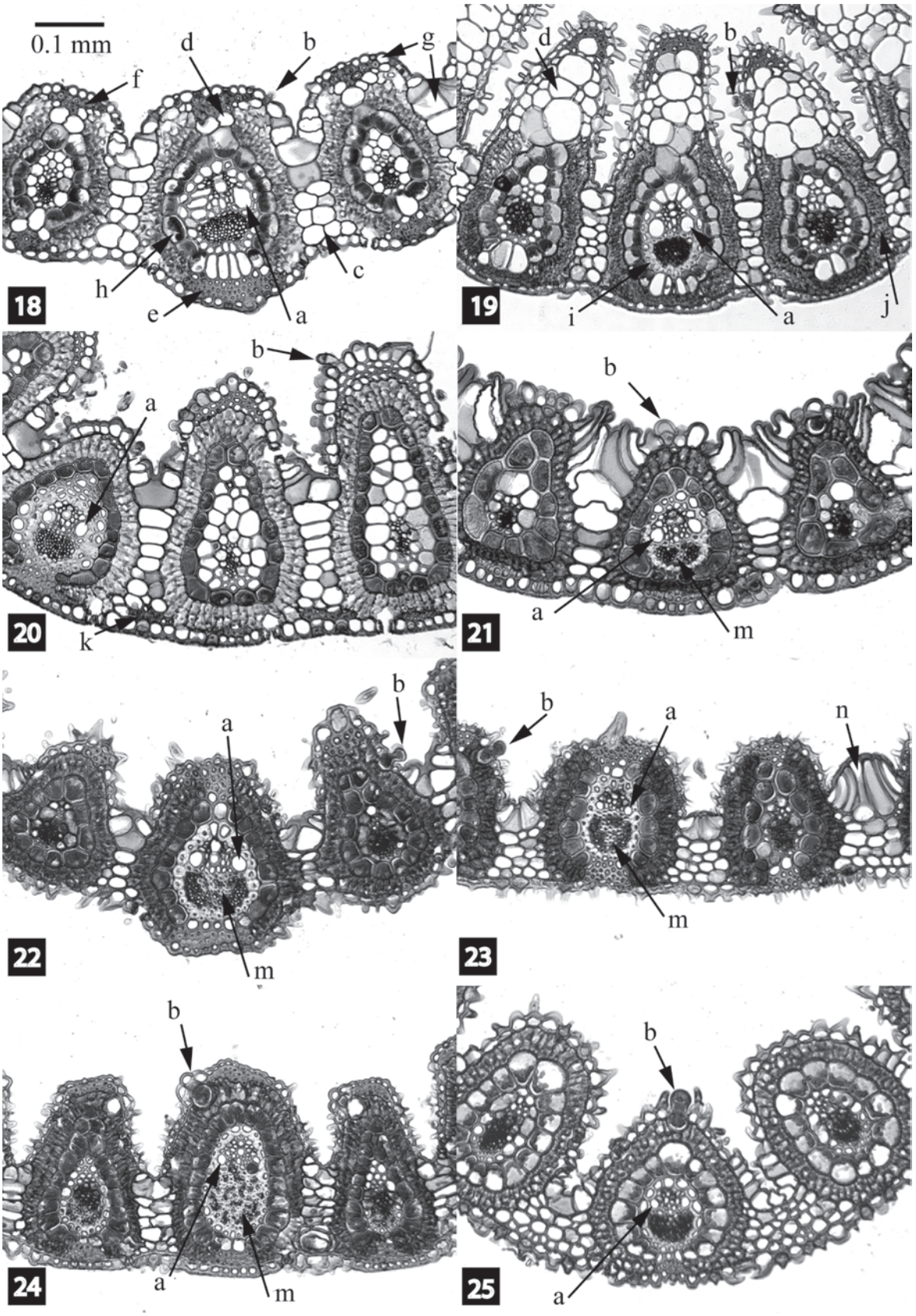
Both species of *Monanthochloë* have only three primary vascular bundles (Figs. 29, 30). There are few or no colorless cells between the bundles; those that approach being colorless cells contain some chloroplasts. Sclerenchyma is present only as strands adaxial and abaxial to all bundles and is absent adaxial to the median bundle. The two species differ in several aspects. The blade outline of *M. acerosa* (Fig. 29) is broadly V-shaped with 14 total vascular bundles while that of *M. littoralis* (Fig. 30) is more narrowly V- or U-shaped with 9 total bundles. *Monanthochloë acerosa* has distinct abaxial furrows. In *M. littoralis*, the outer wall and/or cuticle of epidermal cells is noticeably thicker especially on the abaxial surface between the bundles.

The blade outline of *Reederochloa* is U-shaped with 8–9 total and 3 primary vascular bundles (Fig. 31). Most sclerenchyma is present on the abaxial side, including strands between the outermost two or three vascular bundles, and there is none on the adaxial side of the inner bundles.

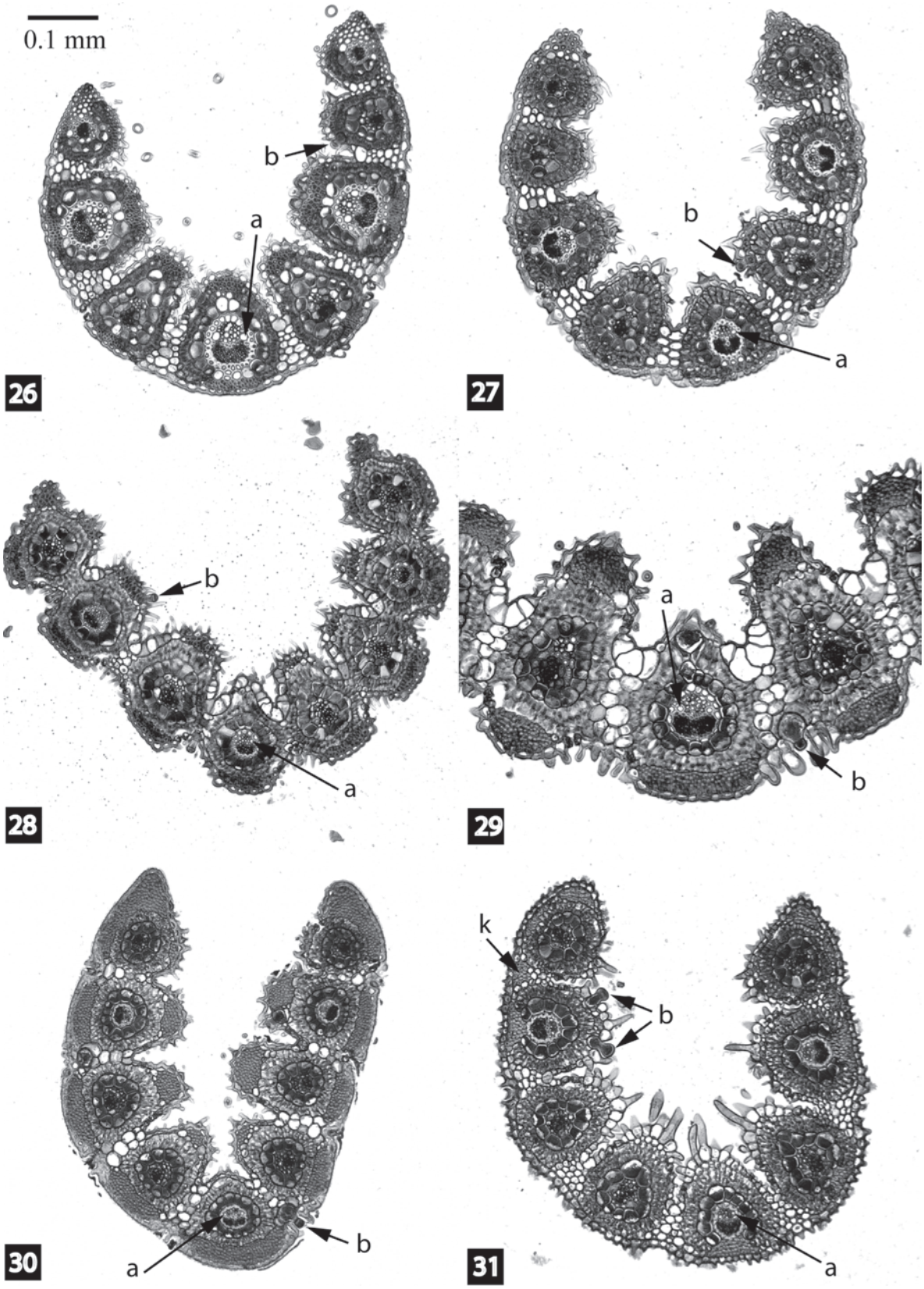
With respect to the outgroup, the blade of *Allolepis* (Fig. 18) is broadly V-shaped in outline. The two samples examined have 22 and 30 total and 7 and 10 primary vascular bundles, respectively. Abaxial and adaxial cells of the outer bundle sheath sometimes lack chloroplasts and are oval or oblong. One to a few colorless cells are present adaxial, and sometimes abaxial, to most vascular bundles. Both surfaces are relatively smooth with scant papillae, hooks, and/or prickles. The basal cell of microhairs is partially sunken into the epidermis; the sunken portion is generally rectangular, smaller than adjacent epidermal cells, and has dark-staining cytoplasm (Fig. 32). Because we were unable to obtain a section through an entire microhair (only a portion of the basal cell is shown in Fig. 32), we examined the abaxial and adaxial surfaces of the blade with SEM. We found that the microhairs are quite similar in shape to those of *Jouvea pilosa* (Fig. 34) with a turbinate basal cell and hemispheric distal cell. The microhairs are appressed and oriented toward the blade apex.

The blade of *Eragrostis obtusiflora* (Fig. 19) is U-shaped in outline. The two specimens examined have 20 and 21 total and 5 primary vascular bundles. As observed in *Allolepis*, abaxial outer bundle sheath cells sometimes lack chloroplasts and are oval or oblong. A prominent feature of *E. obtusiflora* is numerous colorless cells located adaxial to the vascular bundles; one to a few colorless cells were observed on the abaxial side of some bundles. Many papillae, hooks, and/or prickles are present on the adaxial epidermis; on the abaxial side they are present between vascular bundles. Microhairs of *E. obtusiflora* (Fig. 33) are similar to those of the *Distichlis* clade.

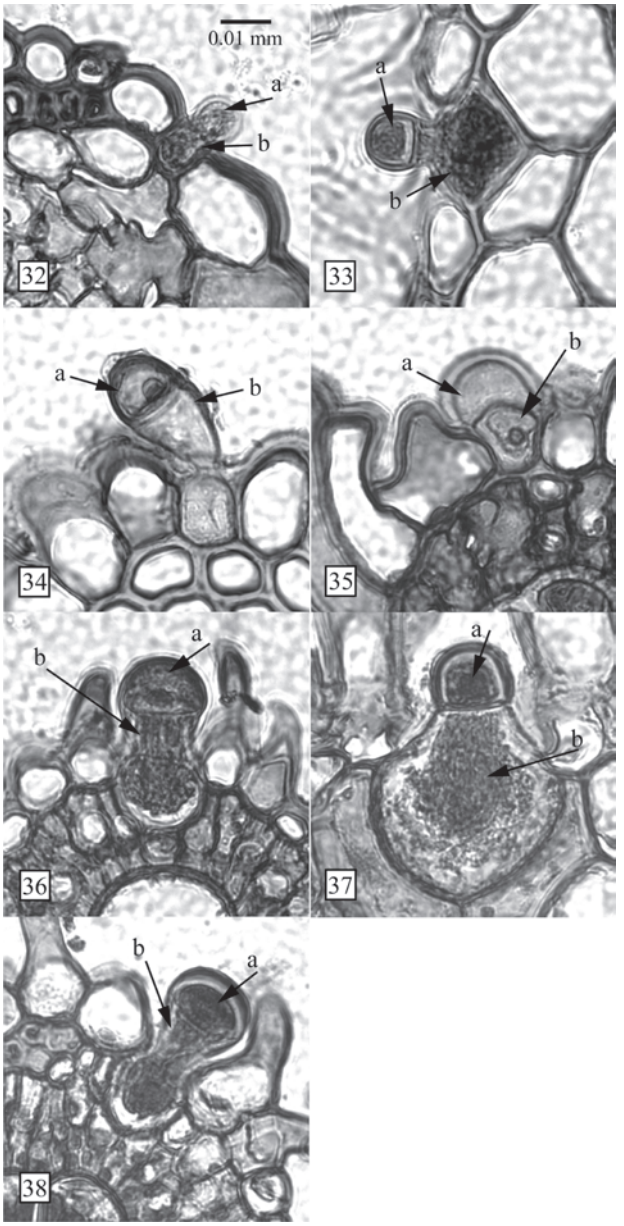
The blade of *Jouvea* is broadly V-shaped in outline. Two specimens of *J. pilosa* (each with 21 total and 7 primary vascular bundles; Fig. 20) and one specimen of *J. straminea* (19 total and 5 primary bundles; Fig. 21) were examined. In contrast to *Allolepis* and *E. obtusiflora*, all outer bundle sheath cells contain chloroplasts and some sclerenchyma was ob-



FIGS. 18–25. Leaf blade transectional anatomy. Adaxial surface is up. Scale is the same for all figures (and Figs. 26–31). 18. *Allolepis texana* (*Bell 240*). 19. *Eragrostis obtusiflora* (*Bell 305*). 20. *Jouvea pilosa* (*Columbus 3738*). 21. *Jouvea straminea* (*Bell 248*). 22. *Distichlis spicata* (*Bell 231*). 23. *Distichlis spicata* (*Bell 340*). 24. *Distichlis palmeri* (*Columbus 3586*). 25. *Distichlis humilis* (*Refulio 192*). a = metaxylem vessel in median vascular bundle (primary); b = bicellular microhair; c = girder of colorless cells; d = colorless cell; e = sclerenchyma girder; f = sclerenchyma strand; g = bulliform cell; h = outer bundle sheath; i = inner bundle sheath; j = mesophyll chlorenchyma; k = sclerenchyma between vascular bundles; m = sclerosed phloem; n = cushion base of macrohair.



FIGS. 26–31. Leaf blade transectional anatomy, continued. Adaxial surface is up. Scale is the same for all figures (and Figs. 18–25). 26. *Distichlis laxiflora* (*Bell 381*). 27. *Distichlis scoparia* (*Bell 343*). 28. *Distichlis australis* (*Bell 330*). 29. *Monanthochloë acerosa* (*Bell 389*). 30. *Monanthochloë littoralis* (*Bell 236*). 31. *Reederochloa eludens* (*Bell 252*). See Figs. 18–25 caption for explanation of letters.



FIGS. 32–38. Bicellular microhairs. Scale is the same for all figures. 32. *Allolepis texana* (*Bell 240*). 33. *Eragrostis obtusiflora* (*Bell 305*). 34. *Jouvea pilosa* (*Columbus 3738*). 35. *Jouvea straminea* (*Bell 248*). 36. *Distichlis humilis* (*Refulio 192*). 37. *Monanthochloë acerosa* (*Bell 389*). 38. *Reederochloa eludens* (*Bell 252*). a = distal cell; b = basal cell.

served between vascular bundles (Fig. 20). Some other differences were observed between *J. pilosa* and *J. straminea* and between *Jouvea* and other species in the study. Unlike *J. pilosa*, *J. straminea* has sclerosed phloem and lacks adaxial furrows and ribs except for some shallow furrows near the margin. Sclerenchyma is present on the abaxial side between many vascular bundles in *J. pilosa* but, in *J. straminea*, is found on the adaxial side only between the outermost pair of bundles. Adaxial bulliform cells between vascular bundles are organized into fan-shaped groups in *J. straminea* but not in *J. pilosa*. Present in furrows of *J. pilosa*, no papillae, hooks, and/or prickles were observed in *J. straminea*. In *J. pilosa*, microhairs are club-shaped with the turbinate basal cell ca. 2–3 × longer than the hemispheric distal cell (Fig. 34). The basal cell is possibly sunken and the cytoplasm does not stain dark. In *J. straminea*, the microhairs approach spherical in shape with cells of equal length, and the basal cell is sunken (Fig. 35); the cytoplasm of the basal cell is not as dark staining as in *Allolepis* and *E. obtusiflora* but has an evident nucleus.

***Biogeography*—**When the geographic distributions are optimized on the molecular phylogenies, six steps (long-distance dispersal events) are required in the ITS phylogeny versus seven steps in the chloroplast phylogeny (Fig. 40). There are two equivocal nodes in the ITS tree and eight in the chloroplast tree.

DISCUSSION

***Analyses of Molecular Data*—**The conflict between the ITS and chloroplast phylogenies involves the positions of *D. humilis* and the *D. laxiflora*/*D. scoparia* clade (Figs. 2, 3). As discussed below, overall morphology and leaf anatomy are more congruent with the ITS phylogeny than with the chloroplast phylogeny.

Lineage sorting is one possible reason for the conflict. The chloroplast haplotypes in *D. humilis*, *D. laxiflora*, and *D. scoparia* are more closely related to those in the *D. spicata* clade than those in the *D. australis*/*Monanthochloë*/*Reederochloa* clade. Lineage sorting involving the chloroplast genome has been demonstrated in a number of studies (e.g. Comes and Abbott 2001; Jakob and Blattner 2006).

Introgression, in particular chloroplast capture, is another possibility. Stephenson (1972) reported possible hybridization between *D. spicata* and *M. littoralis*, species that often grow sympatrically, at a site in Baja California, Mexico. As well, the three species in the clade for which chromosome numbers are known are polyploids that could have originated via hybridization (2*n* = 38, 40, 42, 72; Reeder 1977, 1984). Additional evidence for hybridization involves highly polymorphic ITS sequences found in individuals of *D. humilis* growing sympatrically with *D. spicata* in Chile (H. Bell, unpubl. data). Cloning of ITS revealed that the copies are consistent with derivation from both species. A number of species in the clade have sympatric populations. The lead author is expanding infraspecific sampling in part to examine the frequency and role of hybridization.

***Lemma Micromorphology*—**The presence of papillae on stomatal subsidiary cells is a synapomorphy for the *Distichlis* clade. Stomatal papillae were not observed in the outgroup species we sampled and were not reported by Columbus (1996, 1999) for *Bouteloua* and other genera. In addition, stomatal papillae have not been reported in other studies of the

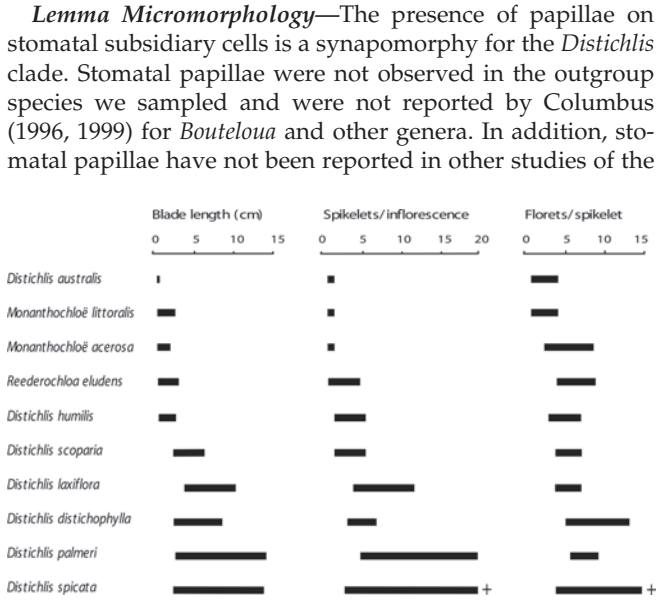


FIG. 39. Variation in blade length, number of spikelets per inflorescence, and number of florets per spikelet for species in the *Distichlis* clade.

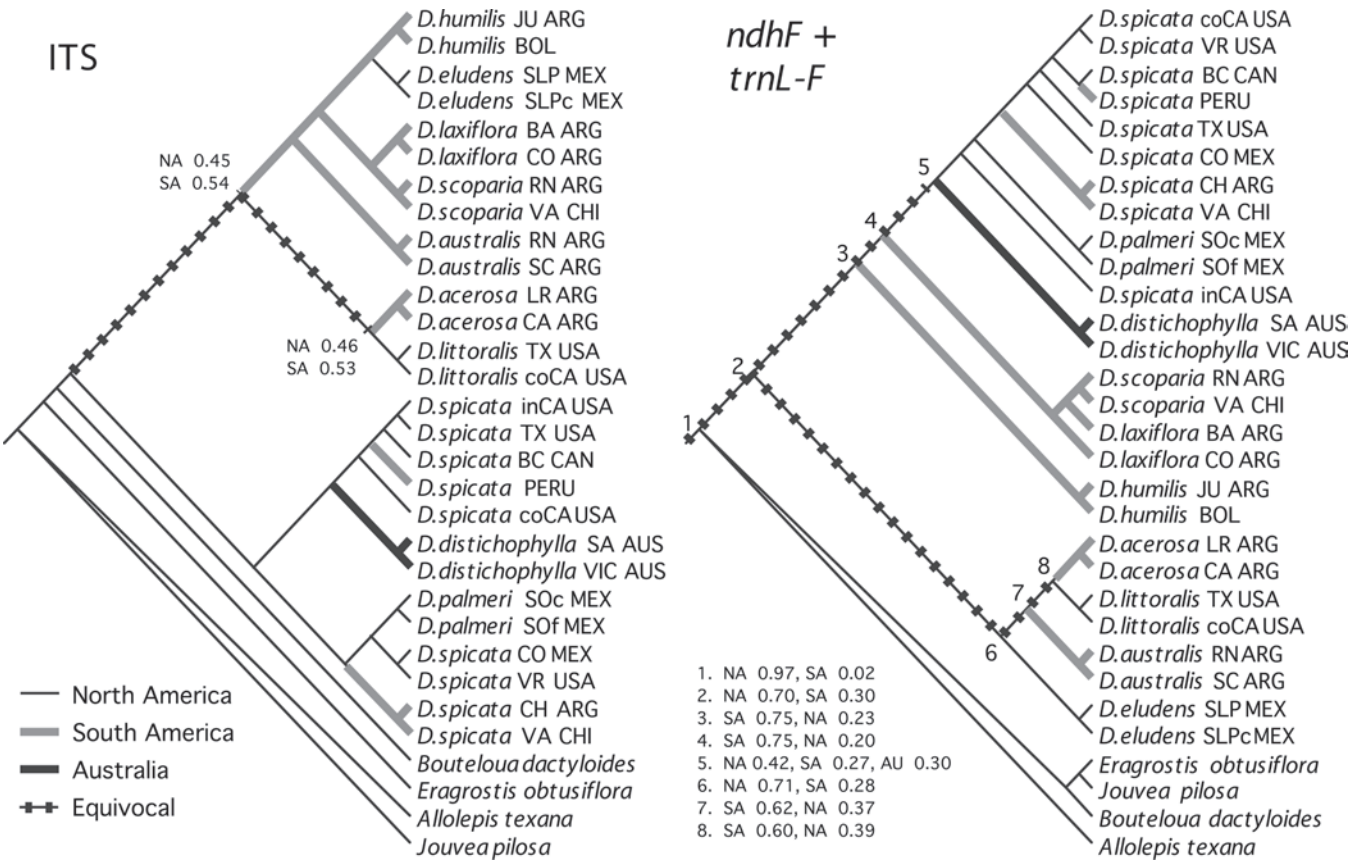


FIG. 40. Geographic distributions optimized on the ITS and chloroplast phylogenies using parsimony. Trees shown are those with the highest log-likelihood values from Bayesian analyses. Probabilities derived from likelihood analysis are provided for the equivocal nodes. AU = Australia; NA = North America; SA = South America. Geographic codes following species names are explained in Appendix 1.

lemma epidermis in chloridoids (Vignal 1984; Peterson 1989; Valdés-Reyna and Hatch 1991; Snow 1996; Rúgolo de Agrasar and Vega 2004). The presence of stomata themselves on lemmas may not be common in Chloridoideae. We observed stomata in all species studied, including the outgroup species, and Columbus (1996) reported stomates in *Bouteloua* and other genera. However, except for Vignal (1984), who observed stomates in a minority of species he surveyed, the presence of stomates was either not mentioned in other studies or described as "abnormal" (Snow 1996).

The groups of large papillae that surround and sometimes overarch stomata and microhairs are a feature of the lemma in the *Distichlis* clade that is uniquely shared by one of the outgroup species, *Eragrostis obtusiflora*. The groups of papillae were not observed in all samples of the *Distichlis* clade, and in *E. obtusiflora* they were observed only in association with microhairs. Nevertheless, the feature could be a synapomorphy for the *Distichlis* clade and *E. obtusiflora*. It should be mentioned that stomatal papillae and overarching papillae have been observed on the leaf blade of *D. spicata* (Hansen et al. 1976) features that were not reported in the Kenyan fossil species D. africana Dugas & Retallack (Dugas and Retallack 1993), which casts doubt on the taxonomic position of the species. Detailed comparative studies of the leaf epidermis of the study group could very well reveal informative variation.

In many chloridoids, the lemma surface is characterized by a regular pattern of long and short cells, frequently with the outer wall of the short cells partially or completely collapsed (Vignal 1984; Valdés-Reyna and Hatch 1991; Columbus 1996, 1999; Snow 1996; Rúgolo de Agrasar and Vega 2004). In contrast, the surface pattern in the study group appears more irregular, including variable numbers of short cells between long cells and few short cells having collapsed outer walls, suggesting the walls are thicker than those in other chloridoids.

Leaf Blade Anatomy—All species examined have Kranz anatomy that is predictive of the NAD-ME type of C_4 photosynthesis (Prendergast et al. 1987; Prendergast and Hattersley 1987; Hattersley and Watson 1992); however, none of the species has been biochemically typed. *Distichlis distichophylla*, the only species we lacked liquid-preserved samples of, is also reported to have Kranz anatomy with two bundle sheaths, the outer sheath even in outline (Metcalfe 1960; Hattersley and Watson 1976; Watson and Dallwitz 1994), which are features consistent with the NAD-ME C_4 type.

The leaf anatomy of *D. humilis*, *D. laxiflora*, *D. palmeri*, and *D. scoparia* is presented here for the first time. All other species have been studied previously (Holm 1891, 1901; Grob 1896; Ogden 1897; Harshberger 1909; Prat 1936; Brown 1958; Metcalfe 1960; Decker 1964; Soderstrom and Decker 1964, 1965; Caceres 1969; Villamil 1969; Anderson 1974; Hansen et al. 1976; Renvoize 1983; Hattersley and Watson 1992; Watson and Dallwitz 1994; Gómez-Sánchez and Koch 1998; Gómez-Sánchez et al. 2001). Our findings are in general agreement with these studies, although, as discussed below, there are issues regarding characterization of microhairs.

A potential synapomorphy for the *Distichlis* clade, based on the outgroup employed, is a reduction in the diameter of metaxylem vessels. However, some samples of *D. spicata* had vessels as large as the smallest vessels in the outgroup, so the reduction may have occurred during diversification of the *Distichlis* clade, or the larger vessels in *D. spicata* may represent a reversal.

There is significant variation in the number of vascular bundles per leaf blade. *Distichlis palmeri*, *D. spicata*, and all of the outgroup species we sampled have relatively broad, open blades usually possessing more than 17 bundles per blade, whereas the remainder of the ingroup species have narrower, more infolded blades usually having fewer bundles. Examination of leaf blades from herbarium specimens of *D. distichophylla* revealed at least 17 bundles per blade. Therefore, based on the selected outgroup and the molecular phylogenies, within the *Distichlis* clade there has been a reduction in vascular bundle number along with an increased infolding of the blade. These coupled traits map less parsimoniously on the chloroplast phylogeny (Fig. 3) than on the ITS phylogeny (Fig. 2), wherein the shift to narrow blades represents a unique synapomorphy for the clade comprising *D. australis*, *D. humilis*, *D. laxiflora*, *D. scoparia*, *Monanthochloë*, and *Reederochloa*. Markedly sclerosed phloem found in *D. distichophylla* (Metcalfe 1960), *D. palmeri*, and *D. spicata* is a synapomorphy for the clade comprising these species. Nearly identical anatomically are the blades of *D. laxiflora* (Fig. 26) and *D. scoparia* (Fig. 27), which is consistent with their similar morphologies and minimal sequence divergence (Figs. 2, 3); Beetle (1955) treated *D. laxiflora* as a form of *D. scoparia*. We found no anatomical synapomorphies for *Monanthochloë*. Both species have few or no colorless cells between the vascular bundles, but this is shared with *D. australis*.

Microhairs representing the three genera in the *Distichlis* clade are shown in Figs. 36–38. They are dumbbell- or flask-shaped, stain dark, and the basal cell is clearly sunken into the epidermis. Among the outgroup taxa, including *Bouteloua* (Columbus 1996, 1999), only *Eragrostis obtusiflora* (Fig. 33) has the same kind of microhair, which could be evidence of a sister relationship. Microhairs in *Allolepis* (Fig. 32, only the base shown), *Bouteloua* (Columbus 1996, 1999), and *Jouvea pilosa* (Fig. 34) are all of the club-shaped type predominant in Chloridoideae (Tateoka et al. 1959). *Jouvea straminea* (Fig. 35) differs in having short, nearly spherical microhairs scarcely exserted from the epidermis. A drawing in Renvoize (1983, his Fig. 2C) shows a detached, club-shaped microhair for *Reederochloa*, but this differs from Soderstrom and Decker (1964) and our findings (Fig. 38).

Microhairs resembling those in the *Distichlis* clade and *E. obtusiflora* have been reported for other chloridoid grasses (and some Panicoideae), including *Cynodon dactylon* (L.) Pers., *Dactyloctenium aegyptium* (L.) Willd., *Eleusine indica* (L.) Gaertn., and *Spartina foliosa* Trin. (Levering and Thomson 1971; Liphschitz and Waisel 1974; Oross and Thomson 1982; Amarasinghe and Watson 1988). In addition, Metcalfe (1960, his Fig. IIA-9, as macrohairs), Renvoize (1985), and Watson and Dallwitz (1994) reported "button mushroom" microhairs in tribe Orcuttieae (three genera), which are similar to the flask-shaped microhairs we observed in the *Distichlis* clade and *E. obtusiflora*. The positions of these taxa in the ITS, *trnL–F*, and *ndhF* phylogenies of Chloridoideae (Bell 2007; Columbus et al. 2007) point to independent origins of this microhair type within the subfamily.

Many authors refer to bicellular microhairs as salt glands (Levering and Thomson 1971; Anderson 1974; Liphschitz and Waisel 1974; Oross and Thomson 1982; Arriaga 1992; Ramadan 2001). Salt secretion from microhairs has been demonstrated in several chloridoid grasses (Amarasinghe and Watson 1988; Ramadan 2001; Bell and O'Leary 2003) and is associated with the presence of numerous mitochondria and partitioning membranes in the basal cell (both dark staining; Oross and Thomson 1982; Amarasinghe and Watson 1988). Microhairs of Orcuttieae are associated with aromatic secretions (Reeder 1965; Roalson and Columbus 1999). Interestingly, Barrow et al. (2007) present evidence that microhairs in Chloridoideae may be fungal in origin.

It is clear that morphological and anatomical studies are required in concert to characterize microhairs. Micromorphological studies of the surface alone will not reveal all features of the basal cell, including the degree to which it is contained within the epidermis.

Morphology—Species in the *Distichlis* clade share a number of characteristics including dioecy, vegetative reproduction via stolons or rhizomes, conspicuously distichous leaves, and many-nerved lemmas. However, these traits are also distributed among the outgroup taxa, so none is a clear synapomorphy for the clade. The only non-molecular synapomorphy we have uncovered is the presence of papillae on subsidiary cells of lemma stomata.

Mirroring the micromorphological, anatomical, and molecular variation we discuss above, there is considerable macromorphological variation in the *Distichlis* clade. Some of the variation is summarized in Table 1 with respect to the three constituent genera. In addition, Fig. 39 shows the intra and interspecific variation in three characters—leaf blade length, number of spikelets per inflorescence, and number of florets per spikelet—which can be viewed as proxies for plant size. It can be seen that the most widespread species, *D. spicata*, is also the most variable. Another species of *Distichlis*, *D. australis*, is the least variable and most diminutive species in the clade. Note also that *Monanthochloë* and *Reederochloa* fall within the range of variation found in the paraphyletic *Distichlis*.

The monophyletic *Monanthochloë* has two morphological synapomorphies—the absence of glumes and the presence of aristae on the prophylls. The two species of *Monanthochloë* along with *D. australis* each have only one spikelet in the inflorescence, which represents an unequivocal synapomorphy in the chloroplast phylogeny (Fig. 3) but not in the ITS phylogeny (Fig. 2), wherein the three species do not form a clade. *Distichlis australis* was first described as a species of *Monanthochloë* but was later positioned in *Distichlis* by Villamil (1969) based on the presence of glumes and the absence of stolons and aristate prophylls.

Distichlis humilis, *D. laxiflora*, and *D. scoparia*, which as a group occur in different positions in the ITS and chloroplast phylogenies (Figs. 2, 3), are morphologically intermediate in a number of traits between *D. australis*/*Monanthochloë*/*Reederochloa* and *D. distichophylla*/*D. palmeri*/*D. spicata* (Fig. 39). However, as pointed out above, all three species plus *D. australis*, *Monanthochloë*, and *Reederochloa* share narrow, infolded leaf blades, which represents a synapomorphy according to the ITS phylogeny (Fig. 2).

Taxonomy—In spite of the conflict between the ITS and chloroplast phylogenies, they both support the monophyly of *Monanthochloë* and paraphyly of *Distichlis* (Figs. 2, 3). Based

on these phylogenies, some species of *Distichlis* (*D. australis* for certain) are more closely related to *Monanthochloë* and *Reederochloa* than to the species in the *D. spicata* clade. *Distichlis australis* could be returned to *Monanthochloë*, but the expanded genus would not be monophyletic in the ITS phylogeny and would be based on only one morphological feature (one spikelet per inflorescence). Similarly unsatisfactory alternatives are further expanding the circumscription of *Monanthochloë* or establishing new genera.

We propose expanding *Distichlis* to include the species of *Monanthochloë* and *Reederochloa*. *Distichlis* is the oldest name. With this circumscription, *Distichlis* is strongly supported as monophyletic (Figs. 2, 3). The genus is also morphologically cohesive, characterized by dioecy, stolons or rhizomes, conspicuously distichous leaves, and many-nerved lemmas. In addition, all species grow in alkaline or saline soils. Below are the necessary nomenclatural combinations, an emended description of *Distichlis*, and a key to the species.

Distichlis acerosa (Griseb.) H. L. Bell & Columbus, comb. nov. *Halochloa acerosa* Griseb., Symb. Fl. Argent. 285–286. 1879.

Distichlis eludens (Soderstr. & H. F. Decker) H. L. Bell & Columbus, comb. nov. *Reederochloa eludens* Soderstr. & H. F. Decker, Brittonia 16: 335–336. 1964.

Distichlis littoralis (Engelm.) H. L. Bell & Columbus, comb. nov. *Monanthochloë littoralis* Engelm., Trans. Acad. Sci. St. Louis 1: 437–439, pl. 13–14. 1859.

Distichlis Raf., descr. emend

Dioecious (possibly rarely monoecious) perennials with well-developed creeping rhizomes or stolons; leaves mostly cauline, conspicuously distichous; sheath open; ligule a membrane, ciliate or eciliate; auricle absent but tufts of hairs sometimes present; blade expanded to permanently infolded; inflorescence unbranched or a contracted panicle of primary branches (sometimes rebranched), with 1–50+ spikelets, exserted (sometimes only males) or positioned within foliage; spikelet oblong to lanceolate, more or less laterally compressed; florets 2–15+, sterile florets above; glumes present or absent, shorter than adjacent lemmas, 1–9-nerved; lemma entire, awnless, 5–13-nerved, usually glabrous, female lemma usually firmer than the male; palea slightly shorter to equaling lemma, 2-nerved and -keeled; stamens 3, stigmas 2; fruit an ellipsoid caryopsis; disarticulation above glumes (females) or florets sometimes persistent (males).

1. Leaf blades < 2.5 cm long; plants stoloniferous or rhizomatous.
   2. Leaf blades subulate, usually < 1.5 cm long; inflorescence a single spikelet.
      3. Plants stoloniferous (rarely rhizomatous); glumes absent.
         4. Stolons to 1.5 mm in diameter, wire-like; stolon leaf sheaths to 1.5 cm long, deciduous; North America. ..... *D. littoralis*
         4. Stolons to 3 mm in diameter, cord-like; stolon leaf sheaths to 2.5 cm long, persistent; central Argentina. ..... *D. acerosa*
      3. Plants rhizomatous, forming low mats; glumes present; southern Argentina (Patagonia). ..... *D. australis*
   2. Leaf blades not subulate, usually > 1.5 cm long; inflorescence with up to 10 spikelets.
      5. Plants stoloniferous, forming low mats; female lemma pilose at base; central Mexico. ..... *D. eludens*
      5. Plants rhizomatous; female lemma glabrous; Andean salinas. ..... *D. humilis*
1. Leaf blades usually > 2.5 cm long; plants rhizomatous (rarely stoloniferous).
   6. Leaf blades < 2 mm wide.
      7. Leaf blades straight; inflorescence with ≤ 5 spikelets; Argentina, Chile, Uruguay. ..... *D. scoparia*
      7. Leaf blades wavy; inflorescence usually with > 5 spikelets; central Argentina. ..... *D. laxiflora*
   6. Leaf blades usually > 3.5 mm wide.
      8. Leaf blades usually < 10 cm long; Australia. ..... *D. distichophylla*
      8. Leaf blades up to 14 cm long; New World.
         9. Lemmas > 7 mm long; plants usually > 30 cm tall; salt marshes at N end of the Gulf of California (Mexico). ..... *D. palmeri*
         9. Lemmas < 6 mm; plants usually < 30 cm; widely distributed in North and South America. ..... *D. spicata*

***Biogeography*—**The biogeography of *Distichlis* is complex (Fig. 1). The genus has an amphitropical distribution in the New World (absent from northern South America and most of Central America), and one species, *D. distichophylla*, is endemic to Australia. *Distichlis spicata* is broadly distributed in North and South America. *Distichlis distichophylla*, *D. humilis*, *D. littoralis*, and *D. scoparia* are each confined to a single continent but are fairly widely distributed within it. The remaining species—*D. acerosa*, *D. australis*, *D. eludens*, *D. laxiflora*, and *D. palmeri*—are much more restricted.

As mentioned above, the sister of the *Distichlis* clade remains uncertain, although two potential synapomorphies (microhair structure and groups of large papillae on the lemma) are shared between the clade and *Eragrostis obtusiflora*. The remaining outgroup taxa are predominantly North American; *Jouvea straminea* and a few species of *Bouteloua* occur in South America, but only one species is endemic there. Therefore, a North American origin for the *Distichlis* clade is suggested by the distributions of the potential sisters, even though more species in the clade occur in South America.

When the geographic distributions are optimized on the molecular phylogenies (Fig. 40), six steps are required in the ITS phylogeny versus seven in the chloroplast phylogeny. There are two equivocal nodes in the ITS tree and eight in the chloroplast tree. One of the equivocal nodes in the chloroplast tree represents the root of the *Distichlis* clade. The likelihood analysis yielded a 0.70 probability that the root is North American, which is the unequivocal result in the ITS phylogeny. Although the ITS phylogeny provides a more parsimonious explanation for the present distribution of *Distichlis*, both phylogenies indicate multiple long-distance dispersal events, including back dispersals. The ITS phylogeny suggests that *D. spicata* from Virginia (U.S.A.) and Coahuila (Mexico) are more closely related to *D. palmeri* (northwestern Mexico) and *D. spicata* from Argentina and Chile than they are to *D. distichophylla* (Australia) and *D. spicata* from California (U.S.A.), Texas (U.S.A.), British Columbia (Canada), and Peru (Fig. 40). As well, the North American endemics *D. eludens* and *D. littoralis* are more closely related to South American endemics than to other North American species. The number of long-distance dispersals inferred from the analyses is interesting considering all species of *Distichlis* are dioecious, which means both morphs are required to estab-

lish a sexually reproducing population. However, all species reproduce via rhizomes or stolons, so an introduced genotype could spread vegetatively and persist.

The Australian endemic *D. distichophylla*, described in 1805 (as *Uniola* L.), deserves special comment. Morphologically, it falls within the range of variation of *D. spicata* and has been considered conspecific (Beetle 1945; Peterson et al. 2001), and it along with *D. palmeri* render *D. spicata* paraphyletic in the molecular phylogenies (Figs. 2, 3). It is possible that its presence in Australia may be the result of a recent, inadvertent introduction of *D. spicata* from the New World, perhaps in ballast from transpacific shipping that began long before 1805. Caryopses, rhizomes, or entire plants could have been transported in this manner and led to its establishment. This could explain the occurrence of female dominated populations in Victoria and New South Wales (Connor and Jacobs 1991).

Future Directions—Using molecular, anatomical, and morphological data, this study advances our understanding of the *Distichlis* clade and improves the classification. We hypothesize *Eragrostis obtusiflora* to be the sister of *Distichlis*, but additional supporting data are needed. This hermaphroditic species is distantly related to the other species of *Eragrostis* (Van den Borre and Watson 1997; Gómez-Sánchez and Koch 1998; Bell 2007), and we are erecting a new genus to accommodate it (H. Bell et al. in prep.). As mentioned above, interspecific hybridization appears to be taking place in *Distichlis*. More sampling and data are needed to examine the level of gene flow occurring among species. As well, additional sampling of the *D. spicata* clade in particular is needed to assess species boundaries, including whether or not *D. distichophylla* is distinct.

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APPENDIX 1. Taxa and collections sampled, and GenBank accession numbers for the ITS, *trnL–F*, and *ndhF* sequences (in that order). Unless otherwise indicated, vouchers are deposited at RSA. [L] utilized for lemma micromorphology; [B] utilized for leaf blade anatomy; * in Bell's (2007) molecular phylogenetic study.

INGROUP. ***Distichlis australis*** (Speg.) Villamil, Río Negro, Argentina RN ARG, *Bell 330*[L,B,*], EF196875, EF196903, EF561650; Chubut, Argentina, *Bell 334*[L]; Santa Cruz, Argentina, *Bell 349*[L]; Santa Cruz, Argentina SC ARG, *Bell 357*[L,B], EF196876, EF196904, EF561651. ***Distichlis distichophylla*** (Labill.) Fassett, Victoria, Australia VIC AUS, *Cochrane 1198** (MEL), EF196877, EF196905, EF561652; South Australia, Australia SA AUS, *Walsh s. n.* (12 Oct 2003), EF196878, EF196906, EF561653; New South Wales, Australia, *Everett 221*[L] (NSW); Victoria, Australia, *Jacobs 5505*[L] (NSW); New South Wales, Australia, *Jacobs 6600*[L] (NSW). ***Distichlis humilis*** Phil., Jujuy, Argentina JU ARG, *Bell 405*[L,B,*], EF196879, EF196907, EF561654; Jujuy, Argentina, *Bell 406*[L]; Jujuy, Argentina, *Bell 408*[L,B]; Bolivia, *Columbus 4702*[B]; Bolivia BOL, *Peterson 12833* (US), EF196880, EF196908, —; Bolivia, *Refulio 192*[B]. ***Distichlis laxiflora*** Hack., Buenos Aires, Argentina BA ARG, *Bell 367*[B,*], EF196881, EF196909, EF561656; Córdoba, Argentina CO ARG, *Bell 381*[L,B], EF196882, EF196910, EF561657; Buenos Aires, Argentina, *Daguerre 231*[L] (BAA). ***Distichlis palmeri*** (Vasey) Fassett, Sonora, Mexico SOc MEX, *Columbus 3586*[L,B,*], EF196883, EF196911, EF561658; Sonora, Mexico SOf MEX, *Felger 91-39*[L], EF196884, EF196912, EF561659. ***Distichlis scoparia*** (Nees ex Kunth) Arechav., Río Negro, Argentina RN ARG, *Bell 328*[L,*], EF196885, EF196913, EF561660; Río Negro, Argentina, *Bell 329*[B]; Río Negro, Argentina, *Bell 337*[L]; Chubut, Argentina, *Bell 339*[L]; Santa Cruz, Argentina, *Bell 343*[B]; Santa Cruz, Argentina, *Bell 346*[B]; Valparaiso, Chile VA CHI, *Bell 374*[L,B], EF196886, EF196914, EF561661. ***Distichlis spicata*** (L.) Greene, inland California, USA inCA USA, *Bell 231*[L,B,*], EF153040, EF156689, EF561662; Texas, USA TX USA, *Bell 237*, EF196887, EF196915, EF561663; Coahuila, Mexico CO MEX, *Bell 245*[L,B], EF196888, EF196916, EF561664; Durango, Mexico, *Bell 253*[L]; coastal California, USA coCA USA, *Bell 259*, EF196890, EF196918, EF561665; British Columbia, Canada BC CAN, *Bell 277*[L,B], EF196891, EF196919, EF561666; Virginia, USA VR USA, *Bell 290*[L,B], EF196892, EF196920, EF561667; Chubut, Argentina CH ARG, *Bell 340*[L,B,*], EF196893, EF196921, EF561668; Valparaiso, Chile VA CHI, *Bell 375*[L,B], EF196895, EF196922, EF561669; Peru PERU, *Columbus 3432*, EF196896, EF196923, EF561670. ***Monanthochloë acerosa*** (Griseb.) Speg. (= *Distichlis acerosa* (Griseb.) H. L. Bell & Columbus), La Rioja, Argentina LR ARG, *Bell 389*[L,B,*], EF196897, EF196924, EF561671; Catamarca, Argentina CA ARG, *Bell 392*[L,B], EF196898, EF196925, EF561672. ***Monanthochloë littoralis*** Englem. (= *Distichlis littoralis* (Engelm.) H. L. Bell & Columbus), coastal Texas, USA TX USA, *Bell 236*[L,B,*], EF153065, EF156714, EF561673; coastal California, USA coCA USA, *Bell 260*[L,B], EF196900, EF196927, EF561674. ***Reederochloa eludens*** Soderstr. & H. F. Decker (= *Distichlis eludens* (Soderstr. & H. F. Decker) H. L. Bell & Columbus), San Luis Potosí, Mexico SLP MEX, *Bell 250*[L,B,*], EF153077, EF156726, EF561675; Durango, Mexico, *Bell 252*[B]; Durango, Mexico, *Bell 312*[L]; San Luis Potosí, Mexico SLPc MEX, *Columbus 4133*[L,B], EF196901, EF196928, EF561676. **OUTGROUP.** ***Allolepis texana*** (Vasey) Soderstr. & H. F. Decker, Texas, USA, *Bell 240*[L,B,*], EF153021, EF156670, EF561646; Coahuila, Mexico, *Bell 307*[L,B]. ***Bouteloua dactyloides*** (Nutt.) Columbus, Querétaro, Mexico, *Columbus 2329**, EF153026, EF156675, EF561647. ***Eragrostis obtusiflora*** (E. Fourn.) Scribn., Michoacán, Mexico, *Bell 314**, EF196874, EF196902, EF561648; Arizona, USA, *Bell 295*[L,B]; Michoacán, Mexico, *Bell 305*[B]. ***Jouvea pilosa*** (J. Presl) Scribn., Jalisco, Mexico, *Bell 247*[L,B,*], EF153057, EF156706, EF561649; Jalisco, Mexico, *Columbus 3738*[B]; Jalisco, Mexico, *Bell 249*[L]. ***Jouvea straminea*** E. Fourn., Jalisco, Mexico, *Bell 248*[L,B].

APPENDIX 2. Primers for amplification (A) and sequencing (S) used in the study. ITS primers are from White et al. (1990). *trnL–F* primers c, d, e, and f are from Taberlet et al. (1991), and primers *trnL*5′ BR, *trnL*3′ D2, and *trnF* F2 are from Columbus et al. (2007). *ndhF* primers 1F, 1318R, 1318F, and 2110R are from Olmstead and Sweere (1994). Additional *ndhF* primers were designed using Oligo 4.0-s (Molecular Biology Insights, Inc., Cascade, Colorado). Primer names are bolded.

ITS: 5 (A/S) (5′–GGAAGTAAAAGTCGTAACAAGG–3′); **4** (A/S) (5′–TCCTCCGCTTATTGATATGC–3′); **3** (S) (5′–GCATCGATGAAGAACG-CAGC–3′); **2** (S) (5′–GCTGCGTTCTTCATCGATGC–3′).

trnL–F: **c** (S) (5′–CGAAATCGGTAGACGCTACG–3′); **d** (S) (5′–GGGGATAGAGGGACTTGAAC–3′); **e** (S) (5′–GGTTCAAGTCCCTC-TATCCC–3′); **f** (A/S) (5′–ATTTGAACTGGTGACACGAG–3′); ***trnL5′* BR** (A/S) (5′–GATATGGCGAAATCGGTAGA–3′); ***trnL3′* D2** (S) (5′–TGGGGATAGAGGGACTTGAACCC–3′); ***trnF* F2** (S) (5′–CAGTCCTCTGCTCTACCAAC–3′).

ndhF: **1F** (A/S) (5′–ATGGAACA[GT]ACATAT[CG]AATATGC–3′); **250R** (S) (5′–TAACATAATAGAAGTAAGGG–3′); **250F** (S) (5′–CCCTTACTTCTATTATGTTA–3′); **580R** (S) (5′–ATCCCGAAACTC-TAAACTAC–3′); **580F** (A/S) (5′–GTAGTTTAGAGTTTCGGGAT–3′); **779R** (S) (5′–AAATCCCCGCAGCAACCATA–3′); **779F** (A/S) (5′–TATGGTTGCTGCGGGGATTT–3′); **957R** (A/S) (5′–CCTAGAGCTAA-CATCATATAACC–3′); **957F** (A/S) (5′–GGTTATATGATGTTAGCTC-TAGG–3′); **1197R** (A/S) (5′–ATTCCACCTCTTGCTTGCTT–3′); **1197F** (A/S) (5′–AAGCAAGCAAGAGGTGGAAT–3′); **1318R** (A/S) (5′–CGAAACATATAAAATGC[AG]GTTAATCC–3′); **1318F** (A/S) (5′–GGATTAAC[CT]GCATTTTATATGTTTCG–3′); **1333R** (A/S) (5′–ACGCAAATACCCATCAAAAG–3′); **1333F** (A/S) (5′–CTTTTGATGGGTATTTGCGT–3′); **1587R** (A/S) (5′–AGCATAGTATTTCC[AC]GTTTC–3′); **1587F** (S) (5′–GAAAC[GT]GGAAATACTATGCT–3′); **1788R** (A/S) (5′–CAGAAGAAATTGCATTAGT–3′); **1788F** (S) (5′–ACTAATGCAATTTCTTCTG–3′); **1956R** (S) (5′–AACC[AG]CGATTATATGACCA–3′); **1956F** (S) (5′–TGGTCATATAATCG[CT]GGTT–3′); **2110R** (A/S) (5′–CCCCCTA[CT]ATATTTGATACCTTCTCC–3′).