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MOLECULAR PHYLOGENETICS OF *BROMUS* (POACEAE: POOIDEAE) BASED ON CHLOROPLAST AND NUCLEAR DNA SEQUENCE DATA

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ABSTRACT

We conducted a phylogenetic analysis to characterize relationships among *Bromus* and test the monophyly of five of the seven morphologically distinct groups within *Bromus* (Poaceae: Pooideae) that have been treated as sections, subgenera, or genera. We sequenced the chloroplast *trn*L (UAA) intron, the 3′-end of the chloroplast *ndh*F gene, and the internal transcribed spacers (ITS) of the nuclear ribosomal DNA region for 46 species that represent a large proportion of the morphological and geographical diversity in the genus. Independent analyses of plastid and nuclear ribosomal data identified several lineages in *Bromus,* but there is some evidence of incongruence between these linkage groups. Nuclear ribosomal trees indicate that two clades comprising some North and South American species of sect. *Bromopsis* are the successive sister groups of the rest of the genus, and that Old World species of sect. *Bromopsis* are more closely related to sects. *Ceratochloa* and *Neobromus* than they are to the remaining North American species of sect. *Bromopsis.* In contrast, plastid trees indicate a close relationship between Old World and some North American species of sect. *Bromopsis.* In the nuclear ribosomal trees, sects. *Genea* and *Bromus* (if sect. *Triniusia* is included within it, as treated by most authors) are monophyletic and not closely related. In the plastid trees, species of these two sections are intermixed, supporting a hybrid origin for *B. pectinatus.* The monophyly of sect. *Ceratochloa* is supported in the plastid and nuclear ribosomal trees, and the monophyly of sect. *Neobromus* is robustly supported in the nuclear ribosomal trees. Current classification schemes do not reflect phylogenetic relationships in *Bromus.* Tentative evidence of conflict among nuclear and plastid data partitions needs clarification with more robustly supported plastid and nuclear ribosomal gene trees.

Key words: Bromeae, *Bromus,* ITS, *ndh*F, phylogenetics, Poaceae, Pooideae, *trn*L intron.

INTRODUCTION

Bromus is a large genus that is widely distributed in temperate and mountainous regions of the Northern and Southern hemispheres. Several species are important forage grasses (e.g., Ferdinandez and Coulman 2000; Ferdinandez et al. 2001; Puecher et al. 2001); some were important cereal crops in the past (Scholz and Mos 1994); and many are invasive weeds (e.g., Ainouche et al. 1999; Novak and Mack 2001; Ogle et al. 2003). *Bromus* is distinguished from other grass genera by the combination of several morphological characters, including: leaf sheath margins that are connate for most of their length; awns that are almost always subapically inserted; hairy apical bilabiate appendages of the ovary; and simple starch grains (Wagnon 1952; Smith 1970).

Phylogenetic Position

The eastern Asian genus *Littledalea* Hemsl., with three species, was believed by Stebbins (1981) to be the closest living relative of *Bromus*; tribe Bromeae currently comprises these two genera (Smith 1970; Clayton and Renvoize 1986; Tsvelev 1989; Grass Phylogeny Working Group [GPWG] 2001). However, preliminary plastid sequence data indicate that *Littledalea* and *Bromus* do not form a clade, thus Bromeae may not be monophyletic (J. M. Saarela unpubl. data). Other genera believed previously to be closely related to *Bromus,* based on morphological similarities, include *Megalachne* Steud., *Metcalfia* Conert, *Pseudodanthonia* Bor & C. E. Hubb., and *Sinochasea* Keng (Smith 1970; Stebbins 1981), but these genera are now considered distantly related (Clayton and Renvoize 1986; Soreng et al. 2003). Phylogenetic analyses of chloroplast DNA restriction site variation and DNA sequence data indicate that Bromeae are the sister group of Triticeae (e.g., Davis and Soreng 1993; Catalán et al. 1997; Hilu et al. 1999; Hsiao et al. 1999; Soreng and Davis 1998, 2000; GPWG 2001).

Taxonomy and Classification

Bromus is a taxonomically difficult genus with a complex nomenclatural history (see Wagnon 1952, Smith 1970, and Acedo and Llamas 1999 for comprehensive reviews), and many species are difficult to distinguish due to their high degree of morphological similarity. As with many other genera of grasses, many species are polyploids, and hybridization is believed to have played an important role in the evolution of many species in the genus (Stebbins 1981). The

complexity of *Bromus* is exemplified in the more than 1200 taxa that have been described, according to the International Plant Names Index (2004). The most recent estimates of the number of species in the genus are 160 (Acedo and Llamas 2001) and 142 (Clayton et al. 2002 onwards), although estimates have ranged from around 100 (Gould and Shaw 1983) to 400 (Soderstrom and Beaman 1968). Several species complexes have been the subject of recent taxonomic investigations (e.g., Scholz 1981; Naranjo et al. 1990; Sales and Smith 1990; Sales 1993, 1994*a*; Smith and Sales 1993; Zajac 1996*a, b*; Allison et al. 2001; Bacic and Jogan 2001; Peterson et al. 2002; Spalton 2002*a*; J. M. Saarela and P. M. Peterson unpubl. data), and new taxa continue to be collected and described (e.g., Smith 1985*a*; Veldkamp et al. 1991; Acedo and Llamas 1997; Scholz 1997, 1998; Peterson and Planchuelo 1998; Bomble and Scholz 1999; Holmström and Scholz 2000; Spalton 2001; J. M. Saarela and P. M. Peterson unpubl. data). Because of its large size, taxonomic complexity, and wide geographic range, no comprehensive worldwide treatment of all species in *Bromus* exists, but many floristic treatments and keys of *Bromus* have been published for various geographic regions in the New World (e.g., Shear 1900; Wagnon 1952; Mitchell 1967; Soderstrom and Beaman 1968; Pinto-Escobar 1981, 1986; Matthei 1986; Allred 1993; Pavlick 1995; Gutiérrez and Pensiero 1998; Planchuelo and Peterson 2000) and the Old World (e.g., Veldkamp et al. 1991; Forde and Edgar 1995; Chen and Kuoh 2000; Spalton 2002*b,* 2004). Genetic variation within and among many species has been studied using data from isozymes (Kahler et al. 1981; Ainouche et al. 1995, 1999; Oja 1998, 1999, 2002*a, b,* 2007; Bartlett et al. 2002), as well as an array of DNA-based molecular techniques, including RAPDs and AFLPs (Ferdinandez et al. 2001; Massa et al. 2001; Puecher et al. 2001; Ferdinandez and Coulman 2002) and microsatellites (Green et al. 2001; Ramakrishnan et al. 2002). A physical map of the chloroplast genome has been constructed for one species, *B. inermis* (Pillay 1993).

The infrageneric classification of *Bromus* has received considerable study. The genus has been variously split into several groups that have been recognized as sections, subgenera, or generic segregates (Table 1). Smith (1970) reviewed the morphological characteristics and nomenclature of the commonly recognized groups in the genus, and accepted five distinct sections, characterized by minor differences in the spikelets. Using data from crossing experiments, Stebbins (1981) recognized seven subgenera (although one, *Boissiera,* is not validly published at this rank) based on their morphological distinctiveness and the apparent high degree of genetic divergence among them. He argued that the subgenera of *Bromus* are too distinct to be treated as sections, since they seemed more distantly related to one another than are several other genera of grasses. Other authors believe that these groups are sufficiently distinct to be regarded as genera (e.g., Tsvelev 1976). No taxonomic consensus exists, and infrageneric taxa in *Bromus* are recognized currently as distinct genera (e.g., Catalán et al. 1997; Green et al. 2001; Spalton 2002*b,* 2004), subgenera (e.g., Acedo and Llamas 1999), or sections (e.g., Smith 1985*b*; Pavlick 1995; Planchuelo and Peterson 2000). The sectional classification of Smith (1970) has been followed by most recent North American authors, and is employed here, incorporating later modifications by Smith (1985*a*) and Scholz (1998); all species mentioned below are treated as species of *Bromus.*

Each section of *Bromus* can be identified using a combination of several morphological characters, including the number of nerves on the first and second glumes, spikelet shape and compression, and lemma and awn morphology (Table 2). Additional data from embryo morphology (Kosina 1996), floral microstructures (Kosina 1999), micromorphology of the lemmas and paleas (Acedo and Llamas 2001), and anatomy (Acedo and Llamas 1999) have recently been collected to aid in the infrageneric classification. Insights obtained from these studies generally agree with the classification schemes based on macromorphological evidence.

Section *Bromopsis* is the largest section, comprising approximately 60 species that occur naturally in Eurasia, Africa, and North and South America, and thus is represented in all regions where brome grasses are native (Stebbins 1981; Armstrong 1991). The section includes diploids, tetraploids, hexaploids, octoploids, and pentaploids (Stebbins 1981). Section *Bromopsis* comprises at least two geographically, morphologically, and cytologically distinct groups. North American taxa, and the *B. ramosus* complex from the Old World, are loosely tufted (nonrhizomatous), short-lived perennials or biennials (except *B. texensis* (Shear) Hitchc., an annual) with small anthers and large chromosomes, and the majority are diploids (Wagnon 1952; Armstrong 1981, 1983, 1991; Stebbins 1981). Old World taxa and *B. pumpellianus,* which occurs in North America and the Old World, are densely tufted or rhizomatous, long-lived perennials with large anthers and smaller chromosomes, and the majority are polyploids (tetra-, hexa-, octo-, and decaploids) (Wagnon 1952; Armstrong 1981, 1983, 1991; Stebbins 1981). Armstrong (1983, 1991) suggested that these two groups might have separate evolutionary histories, based on difficulties in crossing North American and Eurasian taxa, and noted that valid names are available at sectional rank for each of these groups if such recognition becomes appropriate. Cytology and evolutionary relationships of the South American species are poorly known (Stebbins 1981).

Section *Bromus* comprises 30–40 diploid and tetraploid annual species native to Europe and Asia. One species, *B. arenarius* Labill., is thought by some authors to be the only native *Bromus* species in Australia; others believe the species is introduced there (e.g., Stebbins 1981). Many species are invasive and widely distributed in other regions of the world. For example, the 11 species of sect. *Bromus* that occur in North America are all introduced (Pavlick 1995). Species in the section are morphologically similar (e.g., Smith and Sales 1993; Oja et al. 2003), and several subsectional classifications have been proposed (Smith 1972). The tetraploid species in sect. *Bromus* are believed to be allopolyploids (Stebbins 1981), and their putative intrasectional origins have been elucidated using serology (Smith 1972), allozymes (Ainouche et al. 1995; Oja 1998), and DNA sequence data (Ainouche and Bayer 1997; Ainouche et al. 1999). One group of tetraploid species, the *B. pectinatus* complex, is believed to be of hybrid origin between sects. *Bromus* and *Genea* (Stebbins 1956, 1981; Scholz 1981).

Section *Ceratochloa* comprises 10–16 perennial species native to North and South America. All taxa in this section are polyploids (octo-, hexa-, and 12-ploid) (Stebbins 1981;

Pavlick 1995). Species boundaries in sect. *Ceratochloa* are uncertain due to hybridization and morphological intergradation, which have resulted in various taxonomic treatments (e.g., Soderstrom and Beaman 1968; Stebbins 1981; Pavlick 1995; Planchuelo and Peterson 2000). Some species complexes in the section have recently been revised. Based on genetic and morphological studies of six hexaploid and octoploid species from Patagonia, Massa et al. (2001, 2004) distinguished only two morphologically and genetically distinct taxa, which they treated as two species. Similar revisionary work is necessary to characterize morphological and molecular variation among North American taxa of sect. *Ceratochloa.*

Section *Genea* comprises diploid, tetraploid, hexaploid, and octoploid annual species native to the Mediterranean, southwestern Asia, and northern Europe. Several species are invasive (e.g., cheatgrass [*B. tectorum* L.], ripgut grass [*B. diandrus*], and red brome [*B. madritensis* subsp. *rubens*]) and have become widely distributed far beyond their native ranges (e.g., Pavlick 1995). Species in the section are highly variable morphologically, and many taxa have been proposed. Recent revisionary work has reduced the number of species to five, including several infraspecific taxa (Sales 1993, 1994*a*). Section *Genea* is thus the only geographically widespread section of *Bromus* that has received monographic-level taxonomic attention. Based on this taxonomic framework, Sales (1994*b*) proposed hypotheses for the origins of taxa and patterns of adaptive radiation that have occurred within the section. Isozyme data have indicated that the three diploid species of sect. *Genea* are putative donors of genomes in the origins of the polyploid species in the section (e.g., Oja 1998, 2002*b, c*).

The remaining sections in *Bromus* (*Boissiera, Neobromus, Nevskiella,* and *Triniusia*) are individually small, but contribute substantially to morphological variation in the genus as a whole (Table 2). Section *Neobromus* comprises two annual hexaploid species native to the Pacific coasts of North and South America (Matthei 1986; Pavlick 1995). Sections *Nevskiella* (diploid; Armstrong 1991) and *Boissiera* (diploid [Smith 1972] or tetraploid [Oja and Jaaska 1998]) are both monotypic, while sect. *Triniusia* comprises two diploid species (Scholz 1998); species in these three sections are all annuals native to Asia and the eastern Mediterranean. Sections *Boissiera* and *Triniusia* were included within sect. *Bromus* by Smith (1970). *Boissiera* was treated as a subgenus by Stebbins (1981; Table 1), but he included *Triniusia* in subgen. *Bromus.* Both taxa were recognized as sections by Smith (1985*a*) and Scholz (1998), respectively (Table 1).

Phylogenetic Relationships

Past attempts to understand phylogenetic relationships in *Bromus* among species and infrageneric taxa have been based largely on data from morphology (e.g., Shear 1900; Wagnon 1952), karyology (including chromosome number, satellite type, chromosome size, and DNA quantities) and hybridization experiments (e.g., Stebbins and Togby 1944; Stebbins 1947, 1956, 1981; Schulz-Schaeffer 1960; Wilton 1965; Armstrong 1975, 1981, 1983; Kozuharov et al. 1981; Naganowska 1993*a, b*), serology (Smith 1969, 1972), and allozymes (e.g., Oja 1998, 2007; Oja and Jaaska 1998). Chromosome numbers, polyploidy, genome size, karyotypes, c-banding, cross-compatibility, and genome homology within *Bromus* have been summarized by Armstrong (1991).

Five systematic studies have been conducted in *Bromus* using data from DNA, although the number of species included in each study was relatively limited. Pillay and Hilu (1990, 1995) studied chloroplast DNA restriction site variation among 32 *Bromus* species, and identified two major clades: one comprising sects. *Ceratochloa* and *Neobromus,* the other comprising sects. *Bromopsis, Bromus,* and *Genea.* Species of sect. *Bromopsis* occurred in three different lineages, indicating that this taxon is not monophyletic, but the data did not support the New World/Old World split hypothesized by Armstrong (1983) on the basis of morphology and chromosome pairing data. Sections *Bromus* and *Genea* were not monophyletic; species from both sections were intermixed in a single clade. Joachimiak et al. (2001) used RAPD data to portray relationships among nine species representing four infrageneric taxa in *Bromus* from the New and Old World. Based on a phenetic analysis, they identified two distinct clusters: one comprising sect. *Ceratochloa* and the other comprising sects. *Bromopsis, Bromus,* and *Genea.* However, because of the small sample size, and the low level of molecular divergence detected, they were unable to make definitive statements regarding relationships in *Bromus.* Ainouche and Bayer (1997) and Ainouche et al. (1999) used sequence data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA to study the phylogeny of sect. *Bromus.* Based on an analysis of 22 species from sect. *Bromus* (including sect. *Triniusia*) and three species from other infrageneric groups, they found sect. *Bromus* to be monophyletic. They also studied the origin of some tetraploid species in the section. Little sequence heterogeneity was detected within tetraploid species, and they found that the inclusion of allotetraploid taxa with diploid taxa did not change the underlying topology of the trees obtained, compared to trees obtained from analyses of the diploid taxa alone.

To further characterize phylogenetic relationships in *Bromus* s.l., we obtained new sequence data from the chloroplast *trn*L (UAA) intron, the rapidly evolving 3′-end of the chloroplast *ndh*F gene, and the nuclear ribosomal ITS region, from 46 exemplar *Bromus* species that represent a large proportion of the geographical and morphological diversity in the genus. The specific objectives of this study were to use DNA sequence data to (1) test the monophyly of the currently recognized infrageneric groups in *Bromus,* and (2) determine phylogenetic relationships among infrageneric groups and species.

MATERIALS AND METHODS

Taxon Sampling

Exemplars from each of the currently recognized sections in *Bromus* were included in this study, except for the two monotypic sections, *Boissiera* and *Nevskiella.* Attempts to extract DNA from a herbarium specimen of *B. gracillimus* Bunge (sect. *Nevskiella*) were unsuccessful, and material of *B. pumilio* (Trin.) P. M. Sm. (sect. *Boissiera*) was not available. Table 3 lists the species sampled (following the classification schemes of Smith 1970 and Scholz 1998), sources

Table 1. Summary of infrageneric classifications and generic segregations of *Bromus* following Smith (1970), Tsvelev (1976), and Stebbins (1981). Equivalent circumscriptions are aligned horizontally. Indented names were treated by the author as synonyms of the taxon above.

Sections (Smith 1970)	Subgenera (Stebbins 1981)	Genera (Tsvelev 1976)
Bromopsis Dumort. (as sect. *Pnigma* Dumort.)	*Festucaria* Gren. & Godr.	*Bromopsis* (Dumort.) Fourr.
Bromus	*Bromus*	*Bromus* L.
Triniusia (Steud.) Nevski[a]	*Triniusia* (Steud.) Pénzes	*Triniusia* Steud.
Boissiera (Hochst. ex Steud.) P. M. Sm.[b]	*Boissiera* nom. inval.	*Boissiera* Hochst. ex Steud.
Ceratochloa (P. Beauv.) Griseb.	*Ceratochloa* (P. Beauv.) Hack.	*Ceratochloa* P. Beauv.
Genea Dumort.		*Anisantha* C. Koch
	Stenobromus Hack.	
Neobromus (Shear) Hitchc.	*Neobromus* Shear	*Trisetobromus* Nevski[c]
Nevskiella (Krecz. & Vved.) Tournay	*Nevskiella* (Krecz. & Vved.) Krecz. & Vved.	*Nevskiella* Krecz. & Vved.

[a] Given sectional status by Scholz (1998).
[b] Given sectional status by Smith (1985*a*).
[c] Outside geographic range of Tsvelev (1976).

of materials, vouchers, and GenBank accession numbers for the DNA sequences. One individual of each species was examined, except for *B. madritensis* subsp. *rubens,* for which three individuals were sampled, and *B. anomalus,* for which two individuals were sampled. Samples were obtained from silica-gel-dried leaf material from field collections, from plants grown in the greenhouse from seed obtained from the Western Regional Plant Introduction Station (United States Department of Agriculture, Pullman, Washington, USA) and Plant Gene Resources of Canada (Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan), and from herbarium specimens. All taxonomic identifications were confirmed using the available world taxonomic literature of *Bromus.* Outgroup taxa from tribes Triticeae and Poeae were chosen based on previous molecular investigations of the grasses (see Catalán et al. 1997; GPWG 2001). The *Bromus* and *Festuca breviglumis* sequences used in this study are new. Sequence data for *Hordeum vulgare* and *Triticum aestivum* were obtained from GenBank.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and

Table 2. Number of species, morphological characteristics, and native geographic distribution of sections of *Bromus.* The classification follows Smith (1970, 1985*a*) and Scholz (1998).

Section	No. species	1st glume nerves	2nd glume nerves	Spikelet shape	Lemmas	Native geographic distribution
Boissiera	1	3	5–9	Linear-lanceolate to oblong; terete	Oblong; awns 5–9	Asia, E Mediterranean
Bromopsis	ca. 60	1 (3)	3 (5)	Narrow, lanceolate; terete	Rounded or slightly keeled; awn single, usually shorter than length of lemma, rarely absent	Eurasia, Africa, N and S America
Bromus	30–40	3–5	5–9	Ovate to ovate-lanceolate; terete to slightly compressed	Rounded; awn single, equaling or slightly exceeding length of lemma, rarely absent	Europe, Asia
Ceratochloa	10–16	3–5	5–7	Ovate or ovate-lanceolate; strongly compressed	Strongly keeled; awn single, short, often absent	N and S America
Genea	6	1	3	Cuneate, wider at top	Narrow and elongate; awns single, less than 3 times length of lemma	Mediterranean, SW Asia, N Europe
Neobromus	2	1	3–5	Narrowly elliptic	Deep apical sinus and 2 long, narrow teeth; awn single, longer than length of lemma, geniculate	Pacific coasts of N and S America
Nevskiella	1	1	3	Ovate-lanceolate to cuneiform, wider above; terete to slightly compressed	Rounded; awn single, 4–6 times length of lemma	Central Asia, Iran, Afghanistan
Triniusia	2	3–5	5–9	Ovate to lanceolate; compressed	Rounded: upper lemma with 3 awns; irregular apical notches	E Mediterranean, SW Asia

Table 3. *Bromus* sections (following Smith 1970 and Scholz 1998) and species sampled, sources of material, vouchers, and GenBank accession numbers for DNA sequences. All numbers preceded by PI or CN are seed accession numbers. Missing GenBank accession numbers indicate that no sequence was obtained. WRPIS = Western Regional Plant Introduction Station (United States Department of Agriculture, Pullman, Washington, USA); PGRC = Plant Gene Resources of Canada (Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan).

Taxon	Geographic origin/source	Voucher	GenBank accession number		
			ITS	*trn*L intron	*ndh*F
Section *Bromopsis*					
B. anomalus Rupr. ex Fourn. (1)	USA: PI 232199 (WRPIS)	*Keane 49* (ALTA)	AY367905	AY367955	AY368004
(2)	Mexico: Tamaulipas	*Peterson 15918 & Valdes-Reyna* (US)	AY367906	AY367956	AY368005
B. attenuatus Swallen	Mexico: Tamaulipas/Nuevo León border	*Peterson 15926 & Valdes-Reyna* (US)	AY367910	AY367960	AY368009
B. brachyanthera Döll.	Bolivia: La Paz	*de Ros 9497* (US)	AY367908	AY367958	AY368007
B. ciliatus L.	Canada: Quebec	*Cayouette C8272 & Lavoie* (DAO)	AY367909	AY367959	AY368008
B. dolichocarpus Wagnon	Mexico: Michoacán	*Peterson 16128* (US)	AY367911	AY367961	—
B. erectus Huds.	Turkey; PI 337652 (WRPIS), received as *B. benekenii* (Lange) Trimen	*Keane 8* (ALTA)	AY367907	AY367957	AY368006
B. exaltatus Bernh.	Mexico: Jalisco	*Peterson 16087 & Rosales* (US)	AY367912	AY367962	AY368010
B. frondosus (Shear) Woot. & Standl.	Mexico: Durango	*Peterson 15418 et al.* (US)	AY367913	AY367963	AY368011
B. grandis (Shear) Hitchc.	USA: California	*Cayouette 7947a* (DAO)	AY367914	AY367964	AY368012
B. inermis Leyss.	USA: Arizona[a]	*Peterson 15295 & Cayouette* (US)	AY367915	AY367965	AY368013
B. kalmii A. Gray	Canada: Ontario; CN 51222 C7099 (PGRC)	*Keane 55* (ALTA)	AY367916	AY367966	AY368014
B. korotkoyi Drob.	China: Inner Mongolia	*Soreng 5160 et al.* (US)	AY367998	AY367988	AY368043
B. laevipes Shear	USA: California	*Peterson 14840 et al.* (US)	AY367917	AY367967	AY368015
B. lanatipes (Shear) Rydb.	USA: Arizona	*Peterson 15270 & Cayouette* (US)	AY367918	AY367968	—
B. lanatus Kunth	Chile: Region I	*Peterson 15747 & Soreng* (US)	AY367919	AY367969	AY368016
B. latiglumis (Shear) Hitchc.	Canada: Ontario	*Cayouette 4336-1* (DAO)	AY367920	AY367970	AY368017
B. modestus Renvoize	Bolivia: La Paz	*Peterson 12639 et al.* (US)	AY367921	—	—
B. mucroglumis Wagnon	USA: Arizona	*Peterson 15273 & Cayouette* (US)	AY367922	AY367972	AY368019
B. nottowayanus Fern.	USA: Illinois	*Chase 13512* (US)	AY367923	—	—
B. pellitus Hack.	Argentina: Santa Cruz	*Peterson 17267 et al.* (US)	AY367951	AY368000	AY368045
B. pflanzii Pilg.	Bolivia: La Paz	*Luteyn & Dorr 13828* (US)	AY367924	AY367973	—
B. porteri (Coult.) Nash	USA: Arizona	*Peterson 15245 & Cayouette* (US)	AY367925	AY367974	AY368020
B. pseudolaevipes Wagnon	USA: California	*Cayouette C7987* (DAO)	AY367926	AY367975	AY368021
B. pubescens Muhl. ex Willd.	USA: Virginia	*Peterson 15776 & Saarela* (US)	AY367927	AY367976	—
B. pumpellianus Scribn.	Mongolia; PI 610833 (WRPIS)	*Keane 17* (ALTA)	AY367928	AY367977	AY368022
B. ramosus Huds.	UK	*Keane 101* (ALTA)	AY367929	AY367978	AY368023
B. richardsonii Link	USA: Arizona	*Peterson 15282 & Cayouette* (US)	AY367930	AY367979	AY368024
B. riparius Rehmann	Czech Republic/Slovakia; PI 598590 (WRPIS)	*Keane 26* (ALTA)	AY367931	AY367980	AY368025
B. suksdorfii Vasey	USA: Washington	*Soreng 6352 & Soreng* (US)	AY367934	AY367983	AY368028
B. texensis (Shear) Hitchc.	USA: Texas	*Cayouette 668135* (DAO)	AY367935	AY367984	AY368029

[a] Collected outside native range.

Table 3. Continued.

Taxon	Geographic origin/source	Voucher	GenBank accession number		
			ITS	*trn*L intron	*ndh*F
Section *Bromus*					
B. japonicus Thunb.	Russia; PI 283198 CPI 24193 (WRPIS), received as *B. popovii* Drob.	*Keane 24* (ALTA)	AY367940	AY367989	AY368034
B. pectinatus Thunb.	Belgium; PI 442453 (WRPIS)	*Keane 23* (ALTA)	AY367939	AY367988	AY368033
B. scoparius L.	Turkey; PI 204425 (WRPIS)	*Keane 28* (ALTA)	AY367932	AY367981	AY368026
Section *Ceratochloa*					
B. carinatus Hook. & Arn.	Mexico: Durango	*Peterson 15421 et al.* (US)	AY367948	AY367997	AY368042
B. catharticus Vahl	Argentina; PI 578719 RGI 441 (WRPIS), received as *B. araucanus* Phil.	*Keane 5* (ALTA)	AY367954	AY368003	AY368048
B. cebadilla Steud.	Chile: PI 202696 (WRPIS), received as *B. coloratus*	*Keane 13* (ALTA)	AY367944	AY367993	AY368038
B. coloratus Steud.	Chile: Region I	*Peterson 15746 & Soreng* (US)	AY367943	AY367992	AY368037
B. marginatus Nees ex Steud.	USA: Oregon	*Soreng 6360 & Soreng* (US)	AY367921	AY367971	AY368018
B. striatus Hitchc.	France; PI 477988 974 (WRPIS)	*Keane 6* (ALTA)	AY367945	AY367994	AY368039
B. subvelutinus Shear.	Uzbekistan; PI 392355 (WRPIS)	*Keane 35* (ALTA)	AY367953	AY368002	AY368047
Section *Genea*					
B. diandrus Roth.	Germany; CN 31600 PGR 2848 (PGRC)	*Keane 25* (ALTA)	AY367936	AY367985	AY368030
B. madritensis L. subsp. *rubens* (L.) Husn.					
(1)	Australia: Western Australia[a]	*Peterson 14534 et al.* (US)	AY367950	AY367987	AY368044
(2)	Iran; PI 239722 (WRPIS), received as *B. madritensis*	*Keane 20* (ALTA)	AY367938	AY367986	AY368032
(3)	Iraq; PI 253735 (WRPIS), received as *B. fasciculatus* C. Presl	*Keane 16* (ALTA)	AY367937	AY367986	AY368031
Section *Neobromus*					
B. berteroanus Colla	Chile; PI 224789 (WRPIS)	*Keane 37* (ALTA)	AY367946	AY367994	AY368040
B. gunckelli Matthei	Chile: Region I	*Peterson 15697 & Soreng* (US)	AY367947	AY367996	AY368041
Section *Triniusia*					
B. danthoniae Trin.	Turkey; PI 598455 TU85-028-01 (WRPIS)	*Keane 15* (ALTA)	AY367941	AY367990	AY368035
B. pseudodanthoniae Drobov	Turkey; PI 204424 (WRPIS)	*Keane 21* (ALTA)	AY367942	AY367991	AY368036
Outgroup					
Festuca breviglumis Swallen	Mexico: Durango	*Peterson et al. 16924* (US)	AY367952	AY368001	AY368046
Hordeum vulgare L.			Z1159	X757505	U2003
Triticum avestivum L.			AF521903	X75709	NC0027

[a] Collected outside native range.

Doyle 1987) with 2% β-mercaptoethanol added to each extraction. DNA extracts and PCR amplifications were purified using a QIAGEN PCR Purification Kit (QIAGEN, Santa Clarita, California, USA) following manufacturer's instructions.

The *trn*L (UAA) intron was amplified and sequenced with primers developed by Taberlet et al. (1991). The region we refer to as ITS, which includes two spacer regions, ITS1 and ITS2, and the 5.8S rDNA locus, was amplified and sequenced using primers published by White et al. (1990), Hsiao et al. (1994), and Blattner (1999). The 3′-end of *ndh*F was amplified and sequenced using primers designed by Olmstead and Sweere (1994) and Graham et al. (1998). Amplification reactions consisted of 26.5 μl sterile water, 5 μl 10× buffer, 4 μl 10 mM dNTPs, 3 μL 25 mM $MgCl_2$, 5 μL of each 5 pmol/μl primer, 1 μl template DNA, and 0.5 μl *Taq* DNA polymerase (1 unit). The thermal profile was: 1 cycle of 3 min at 94°C; 35 cycles of 30 sec at 94°C, 1 min at 42.5°C, and 2 min at 72°C; and 1 cycle of 5 min at 72°C.

Sequencing products were generated using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) with 50 ng of template DNA and the following thermal profile: 25 cycles of 10 sec at 96°C, 5 sec at 45°C, and 4 min at 60°C. For each sample, one or several duplicate sequencing reactions were included using a second DNA extract from the same source material. Sequencing reactions were analyzed using an Applied Biosystems Prism 377 automated DNA sequencer.

Sequence data were assembled and edited using Sequencher vers. 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Consensus sequences were exported for each sample and aligned manually using Se-Al vers. 1.0 alpha 1 (Rambaut 1998) according to guidelines outlined in Graham et al. (2000). Gaps in the final matrix were coded as missing data. Several inferred insertions/deletions (indels) in the *trn*L intron were scored as binary characters. Alignments were imported into PAUP* vers. 4.0b10 (Swofford 2002) for analysis. All sequence data have been submitted to GenBank (Table 3).

Phylogenetic Analyses

For the ITS data set, a heuristic search was conducted with 100 random starting trees, tree-bisection-reconnection (TBR) branch swapping, and all character and character-state changes equally weighted. A two-tiered approach was taken for the heuristic searches of the combined plastid data because an upper limit on the number of most-parsimonious trees was unattainable with the available computational resources and time: (1) 100 independent heuristic searches each with a random starting tree, saving 100 trees each (MaxTrees set to 100), were performed with the parameters noted above, and (2) another heuristic search, with the same parameters as above, was conducted, except that the shortest of the 10,000 trees from step 1 were used as starting trees, and MaxTrees was set to 50,000. We also implemented the parsimony ratchet (Nixon 1999) using PAUPRat (Sikes and Lewis 2001) to search for shorter trees with the combined plastid data set. The incongruence length difference (ILD) test (Farris et al. 1994, 1995) was used to test for conflict between the plastid and nuclear data partitions, with MaxTrees set to 500. In addition, trees and bootstrap values derived from analyses of the plastid and nuclear data were compared visually to assess the robustness of topological incongruence (e.g., Graham et al. 1998). We computed strict consensus trees from all of the most-parsimonious trees for both of the data partitions. We present phylograms of one randomly chosen tree from each of these analyses, and indicate which clades on the phylograms collapse in the strict consensus trees. Branch support was assessed using maximum parsimony bootstrap analysis (Felsenstein 1985) from 500 replicates using the heuristic search option, with one random starting tree, TBR branch swapping, and MaxTrees set to 500 per replicate. We use weak, moderate, and well supported in reference to clades having bootstrap values of <71, 71–90, and 91–100, respectively.

RESULTS

Analyses of ITS Sequences

The boundaries of ITS1, 5.8S, and ITS2 follow Eckenrode et al. (1985), Yokota et al. (1989), and Kolosha and Fodor (1990). Lengths of ITS1 and ITS2 were 216–219 and 213–216 base pairs (bp), respectively. The 5.8S rRNA gene was 163 bp in length. A small region of 10 bp (positions 108–117) in ITS1 was difficult to align across taxa, and was excluded from all analyses. The ITS data matrix, without excluded sites, was 606 aligned nucleotides in length. Of these characters, 382 were constant, 224 were variable, and 125 (20.6%) were parsimony informative. Among the ingroup taxa, 437 characters were constant, 169 were variable, and 104 (17.2%) were parsimony informative. The heuristic searches of the ITS data set recovered 449 most-parsimonious trees (tree length = 380 steps; consistency index [CI] = 0.713; retention index [RI] = 0.826).

Several clades receive good bootstrap support (Fig. 1). The monophyly of the genus *Bromus* is moderately supported (bootstrap support value [BV] = 75%). Section *Bromopsis* is not monophyletic. A well-supported clade (BV = 99%) consisting of two North American *Bromopsis* species, *B. attenuatus* and *B. dolichocarpus,* is the sister group of the rest of the genus, the latter clade with BV = 100%. The next major split in *Bromus* is between a well-supported clade (BV = 100%) of four South American species of sect. *Bromopsis* (*B. lanatus, B. modestus, B. pellitus,* and *B. pflanzii*) and all remaining species of *Bromus.* The latter clade is weakly supported (BV = 59%).

A large and well-supported clade (BV = 96%) includes five species of sect. *Bromopsis* of Eurasian origin (*B. erectus, B. inermis, B. korotkoyi, B. pumpellianus* [which is also native in the New World], and *B. riparius*), one species of sect. *Bromopsis* from South American (*B. brachyanthera*), and the monophyletic sects. *Ceratochloa, Genea,* and *Neobromus* (BV = 92%, 100%, and 94%, respectively). The Eurasian representatives of sect. *Bromopsis* are not united in a single clade.

A second large, weakly supported clade (BV <50%) contains the remaining North American species of sect. *Bromopsis, B. ramosus* (a species classified in sect. *Bromopsis* from the Old World), and sects. *Bromus* and *Triniusia* (Fig. 1). Several well-supported relationships are evident among

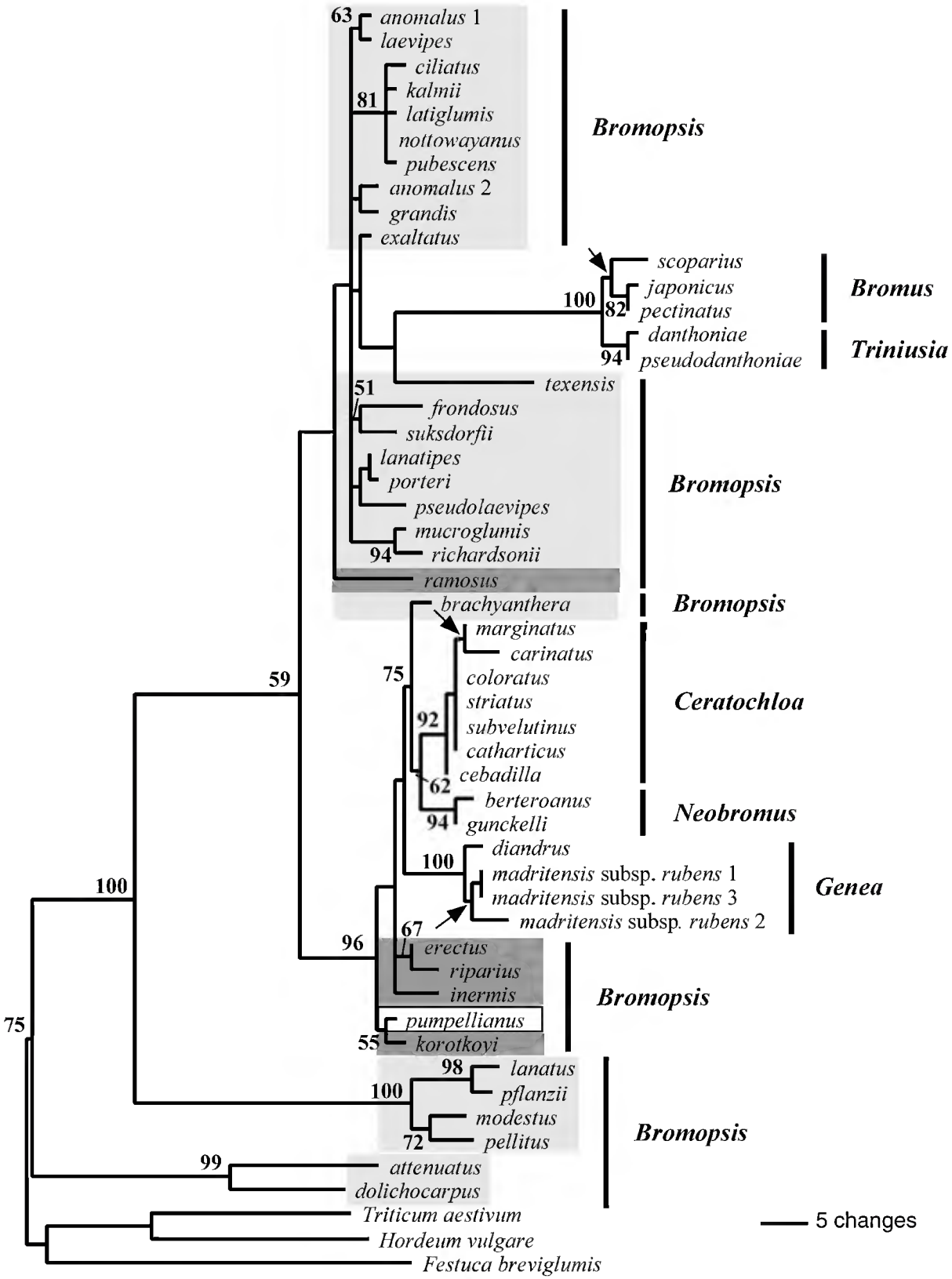

Fig. 1.—Phylogram of one of 449 most-parsimonious (MP) trees found using data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. MP trees are each 380 steps, with a CI of 0.713 and RI of 0.826. Bootstrap support values greater than 50% are indicated. Nodes that collapse in the strict consensus tree are indicated with an arrow. Sections in *Bromus* (following Smith 1970 and Scholz 1998) are indicated to the right of the tree. Native geographic distributions of species in sect. *Bromopsis* are indicated: light shading = New World; dark shading = Old World; boxed species (*B. pumpellianus*) = New and Old World.

some species of sect. *Bromopsis* from North America. Section *Triniusia* is monophyletic (BV = 94%), and is part of a well-supported clade (BV = 100%) that otherwise only includes representatives of sect. *Bromus.*

Analyses of Plastid Sequences

The sequence data obtained for the 3′-end of *ndh*F correspond to positions 1441–2076 of *ndh*F in *Oryza sativa* L. (GenBank accession no. NC00132). The sequenced portion was 662 bp in length in all taxa, except for *B. grandis,* which had a six bp insertion. The unambiguously aligned *ndh*F matrix was 668 bp long; 576 nucleotides were constant, 92 were variable, and 44 (6.5%) were parsimony informative. Among the ingroup taxa, 616 characters were constant, 52 were variable, and 31 (4.6%) were parsimony informative.

The *trn*L intron ranged in length from 568 to 586 bp. Several indels were present in the final data matrix; three of these were phylogenetically informative and were coded as binary characters in the analysis. Two regions of 18 bp (positions 1397–1414) and 11 bp (positions 1755–1765) were homopolymer repeats of variable length that were difficult to align; these regions were excluded from all analyses. The aligned *trn*L intron matrix (including binary characters but without excluded sites) consisted of 646 characters; 578 were constant, 68 were variable, and 28 (4.9%) were parsimony informative. Among the ingroup taxa, 610 characters were constant, 36 were variable, and 23 (3.5%) were parsimony informative.

No sequence data were obtained from either plastid locus for *B. modestus* and *B. nottowayanus,* and four species are represented solely by data from the *trn*L intron (Table 3). The heuristic search of the combined plastid data recovered 50,000 most-parsimonious trees (tree length = 218 steps; CI = 0.817; RI = 0.882).

In the most-parsimonious trees there is moderate phylogenetic structure that is supported by bootstrap analysis (Fig. 2). The monophyly of the genus *Bromus* is well supported (BV = 99%). Taxa classified in sects. *Bromus, Genea,* and *Triniusia* form a well-supported monophyletic group (BV = 91%), but none of the three sections is monophyletic. *Bromus pectinatus* (sect. *Bromus*) and *B. diandrus* (sect. *Genea*) form a well-supported clade (BV = 100%) that is weakly supported (BV = 56%) as the sister group of *B. madritensis* subsp. *rubens* (sect. *Genea*). The other species of sect. *Bromus* and species of sect. *Triniusia* are mixed in a clade (BV = 86%). Section *Ceratochloa,* sect. *Neobromus,* and *B. brachyanthera* (sect. *Bromopsis*) form a weakly supported clade (BV = 62%). Section *Neobromus* is not monophyletic, and the monophyly of sect. *Ceratochloa* is weakly supported (BV = 63%). A large clade of 23 New and Old World species of sect. *Bromopsis* is weakly supported (BV = 68%).

Incongruence Among Data Partitions

The ILD test indicated significant incongruence between the nuclear ribosomal and plastid data partitions ($P < 0.01$). Overall, the trees derived from the nuclear ribosomal data are more resolved than trees derived from the plastid data (Fig. 1, 2). There are some well-supported clades whose positions differ substantially among trees, although not always with strong support. Topologically, the greatest differences between the plastid and nuclear ribosomal trees are the positions and monophyly of sects. *Bromus, Genea,* and *Triniusia.* In the nuclear trees, species from sects. *Bromus* and *Triniusia* form a clade, and sect. *Genea* is well supported as monophyletic; a close relationship between the two clades is not inferred (Fig. 1). In the plastid trees, species from these three sections are intermixed in a well-supported clade (Fig. 2); for example, *B. pectinatus* (sect. *Bromus*) and *B. diandrus* (sect. *Genea*) form a well-supported clade. Other incongruencies involve relationships among species of sect. *Bromopsis* (Fig. 2). The plastid trees include species from the Old World in a weakly supported clade with some North American species, while the nuclear ribosomal trees indicate a more distant relationship between the Old World (with the exception of *B. ramosus*) and North American species. Because of these possible instances of intergenomic conflict, we did not conduct analyses of the combined nuclear ribosomal and plastid data.

DISCUSSION

Phylogenetic Utility of the Regions Examined

Of the three regions examined, the nuclear ribosomal region was the most variable and accounted for 65.8% of the total number of parsimony-informative characters (ingroup taxa only) among all three data sets. Resolution (number of bifurcated nodes in the strict consensus tree) was greater in the nuclear ribosomal phylogeny compared with the plastid phylogeny, probably because of the greater amount of variation in the former data set. The least parsimony-informative variation (among ingroup taxa) was observed in the *trn*L intron, which accounted for 14.5% of the total number of informative characters in all three data sets. Although this intron is commonly used for lower-level phylogenetic studies, several investigators have reported a paucity of phylogenetically-informative characters in it to sufficiently resolve relationships among closely related grass genera and species (e.g., Hodkinson et al. 2002), and a wide variety of other plant taxa (e.g., Bruneau et al. 2001; Klak et al. 2003; Shaw et al. 2005). The 3′-end of *ndh*F provided 19.6% of the total parsimony-informative variation (ingroup taxa only) among all three data sets, 1.35 times as many parsimony-informative characters as the *trn*L intron for approximately the same length. The complete *ndh*F region has been used in several phylogenetic studies of grasses at the familial, subfamilial, tribal, and generic levels (e.g., Clark et al. 1995; Catalán et al. 1997; Spangler et al. 1999; Giussani et al. 2001; Aliscioni et al. 2003). The more rapidly evolving 3′-end of *ndh*F has been used at the genus level in grasses (e.g., Catalán and Olmstead 2000) and other plants (e.g., Graham et al. 1998; Davis et al. 2002; Winkworth et al. 2002; Graham and Barrett 2004). The greater level of sequence variation detected in the 3′-end of *ndh*F compared with the variation detected in the more commonly used *trn*L intron indicates that the former warrants consideration for use in the resolution of relationships at similar taxonomic levels in other groups.

Incongruence Between Nuclear Ribosomal and Plastid Data Partitions

Significant incongruence was detected between the nuclear ribosomal and plastid data partitions using the ILD test.

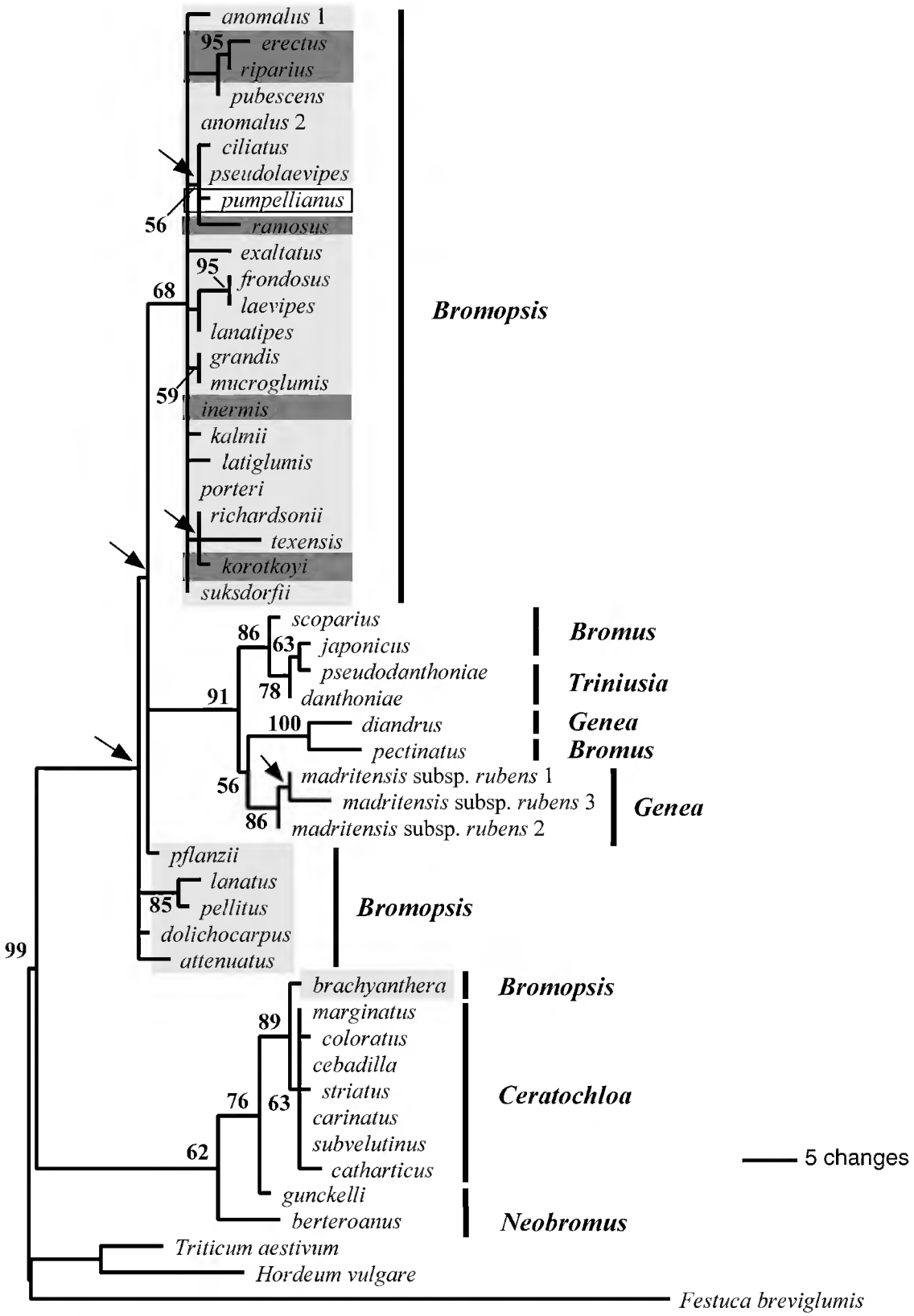

Fig. 2.—Phylogram of one of 50,000 most-parsimonious (MP) trees found using combined plastid data from the *trn*L intron and the 3′-end of *ndh*F. MP trees are each 218 steps, with a CI of 0.817 and RI of 0.882. Bootstrap support values greater than 50% are indicated. Nodes that collapse in the strict consensus tree are indicated with an arrow. Sections in *Bromus* (following Smith 1970 and Scholz 1998) are indicated to the right of the tree. Native geographic distributions of species in sect. *Bromopsis* are indicated: light shading = New World; dark shading = Old World; boxed species (*B. pumpellianus*) = New and Old World.

The ILD test is commonly employed by systematists to examine congruence among data partitions, but there is growing evidence (e.g., Yoder et al. 2001; Barker and Lutzoni 2002) that the test can be misleading and should not be used to determine data combinability. Thus, we also visually compared trees derived independently from the plastid and nuclear data partitions for regions of incongruence, and found that each contained some moderately to well-supported clades whose composition and position differed. Because of this possible intergenomic conflict, we did not conduct analyses of the combined plastid and nuclear ribosomal data.

Incongruence among trees is not uncommon in phylogenetic studies that employ multiple gene regions, particularly when the data are from different genomes (e.g., Hardig et al. 2000; Les et al. 2002). Although often viewed as a hindrance to reliable phylogenetic estimation, incongruence can potentially provide insight into past evolutionary events, such as hybridization, introgression, and lineage sorting (Wendel and Doyle 1998). Our data suggest that some of these phenomena may have been involved in the evolutionary history of *Bromus*. However, it is difficult to infer the exact evolutionary processes that have led to the differing gene trees, as reticulate patterns of evolution are difficult to study in a cladistic framework, and gene trees inferred from more than two linkage groups are ideally required. Nonetheless, previous studies have indicated that hybridization, alloploidy, and introgression may have been prominent in the evolution of many *Bromus* species and sections (reviewed by Stebbins 1981 and Armstrong 1991). The implications of the different gene histories detected here in understanding the evolutionary history of infrageneric groups in *Bromus* are discussed below. Clarification of the contribution of these processes to the evolutionary history of *Bromus* will require better-supported phylogenetic trees from multiple genetic linkage groups.

Phylogeny and Classification

In all analyses there is moderate to strong support for the monophyly of the genus *Bromus* s.l., based on current outgroup and ingroup taxon sampling. These findings agree with Ainouche and Bayer's (1997) study of sect. *Bromus,* and broader studies of grass phylogeny that included several species of *Bromus* s.l. (e.g., Catalán et al. 1997; Hsiao et al. 1999), which all identified *Bromus* s.l. as a monophyletic taxon.

Sections Bromus, Genea, *and* Triniusia.—The molecular evidence indicates that species of sect. *Triniusia* are nested within a clade that includes species of sect. *Bromus* (Fig. 1, 2). Section *Triniusia* was originally circumscribed as a monotypic section that included *B. danthoniae,* characterized by three awns on each of the uppermost lemmas of the spikelets (Scholz 1998), but most authors have included this species in sect. *Bromus* (e.g., Smith 1970, 1972; Ainouche and Bayer 1997). A close relationship between *B. danthoniae* and *B. pseudodanthoniae* was not hypothesized until Scholz (1998) observed that the latter species sometimes has three awns on the uppermost lemmas of its spikelets, and that in portions of their ranges in the Middle East the two taxa intergrade. As a result, Scholz (1998) treated *B. pseudodanthoniae* as a subspecies of a polymorphic *B. danthoniae,* and recircumscribed sect. *Triniusia* to include two morphologically similar species and several subspecies (*B. danthoniae* subsp. *danthoniae, B. danthoniae* subsp. *pseudodanthoniae* (Drobow) H. Scholz, *B. danthoniae* subsp. *rogersii* C. E. Hubb. ex H. Scholz, and *B. turcomanicus* H. Scholz). Scholz's (1998) recognition of sect. *Triniusia* is supported by isozyme data, which show *B. danthoniae* to be distinct from diploid members of sect. *Bromus* (Oja and Jaaska 1998), although serological evidence show *B. danthoniae* to be closely allied to species of sect. *Bromus,* including *B. pumilio* (classified currently in sect. *Boissiera* but often included in sect. *Bromus*), a species that also has multiple awns on the uppermost lemmas of its spikelets (Smith 1972). We did not sample *B. turcomanicus,* thus we were unable to fully test the monophyly of sect. *Triniusia* sensu Scholz (1998). However, our data confirm the close relationship hypothesized between *B. danthoniae* and *B. pseudodanthoniae,* and indicate that these species are nested phylogenetically within sect. *Bromus* (Fig. 1) or perhaps a somewhat broader clade (Fig. 2). These data are in accordance with the findings of Ainouche and Bayer (1997), who included *B. danthoniae* in their study of sect. *Bromus.* Recognition of sect. *Triniusia* renders sect. *Bromus* paraphyletic; it therefore should continue to be treated as a synonym of sect. *Bromus,* as has been done previously (e.g., Smith 1970, 1972; Ainouche and Bayer 1997). The distinct morphological characters that separate *B. danthoniae* and its close relatives from other species in sect. *Bromus* arose from within sect. *Bromus.*

The two sources of molecular evidence are in conflict with regard to the monophyly of sects. *Bromus* and *Genea,* due to the position of *B. pectinatus.* Sections *Bromus* (including sect. *Triniusia*; see above) and *Genea* (based on sampling only two of the approximately five species in the section) are each robustly supported as monophyletic in the nuclear ribosomal trees (Fig. 1). However, the plastid trees indicate that *B. pectinatus* (sect. *Bromus*) is the sister group of *B. diandrus* (sect. *Genea*; Fig. 2). Species of the *B. pectinatus* complex (only one species sampled here), a group of five tetraploid species that range from southern Africa to Tibet and classified in sect. *Bromus,* are morphologically similar to species of sect. *Genea,* with lemmas that taper toward the apex and paleas whose morphology is intermediate between the two sections (Smith 1972; Scholz 1981; Stebbins 1981; Sales 1993). A close relationship between *B. pectinatus* and sect. *Genea* is also supported by data from isozymes (Oja 2007) and embryo structure (Kosina 1996). Based on its morphological intermediacy, Stebbins (1956, 1981) suggested that the *B. pectinatus* complex (represented by *B. arenarius* in his studies) may be an intersectional amphidiploid that originated via a hybridization event between species of sects. *Bromus* and *Genea.* The conflicting positions of *B. pectinatus* in our plastid and nuclear ribosomal trees lend support to this hypothesis, indicating that the genome donors in the origin(s) of the complex were likely from sects. *Bromus* and *Genea.* Sampling of additional species of the *B. pectinatus* complex, and additional genetic linkage groups, would be valuable, and may provide further insight into their origin(s). If *B. pectinatus* is a species of hybrid origin that arose after sects. *Bromus* and *Genea* initially diversified, and it is excluded from consideration, then sects. *Bromus* and *Genea* are monophyletic. The morphological characteristics

outlined in Table 2, widely employed in taxonomic keys to separate these two lineages (e.g., Pavlick 1995), constitute possible synapomorphies for these clades; however, validation of these hypotheses will require rigorous reconstructions of character evolution on robustly supported and fully resolved gene trees.

The plastid and nuclear ribosomal data sets infer different relationships between sects. *Bromus* and *Genea.* The nuclear ribosomal data do not infer a close relationship between the sections (Fig. 1), while the plastid data strongly support a clade containing all taxa from both sections (Fig. 2). The placement of species from sects. *Bromus* and *Genea* together in a clade in the plastid trees corroborates Pillay and Hilu (1995), although they did not detect sufficient chloroplast DNA variation to separate the sections into distinct monophyletic groups. Pillay and Hilu (1995) suggested that the similarity in chloroplast genomes between sects. *Bromus* and *Genea* may be the result of chloroplast transfer by hybridization and phylogenetic sorting. A close relationship between the sections is further supported by data from floral microstructural variation (Kosina 1999), and by their life histories. Both include only annual species (most other sections of *Bromus* comprise mostly perennial species), and both include many weedy species (Stebbins 1981). Stebbins (1981) also hypothesized a close relationship between sects. *Bromus* and *Genea,* and suggested that their origins probably involved different species of sect. *Bromopsis* as genome donors. Our nuclear ribosomal data are potentially consistent with this hypothesis, as sect. *Bromus* is nested within a clade that includes species of sect. *Bromopsis* from North America and *B. ramosus* from the Old World, while sect. *Genea* is closely related to species of sect. *Bromopsis* from the Old World (excluding *B. ramosus*). These species groups of sect. *Bromopsis,* respectively, are potential candidates for genome donors in the origins of sects. *Bromus* and *Genea.*

Within sect. *Genea,* our data indicate a fairly substantial amount of genetic variability among individuals of *B. madritensis* subsp. *rubens,* in line with the results of a previous isozyme study (Kahler et al. 1981). The high genetic variation observed here seems to correspond with morphological variation that was high enough to result in the gross misidentification of one seed bank accession (see Table 3). The genetic variation observed in *B. madritensis* subsp. *rubens* raises the possibility that similar high levels of variation may be present in at least some other *Bromus* species.

Section Bromopsis.—Section *Bromopsis,* the largest section currently recognized in *Bromus,* comprises several independent lineages and is not monophyletic in any of our analyses. These results are congruent with Pillay and Hilu (1995), who found members of the section to occur in three distinct lineages (based on the plastid genome but with less taxon sampling). Based on our nuclear ribosomal data, *B. attenuatus* and *B. dolichocarpus,* two North American species of sect. *Bromopsis* native to northeastern Mexico and southern Mexico and Guatemala, respectively (Wagnon 1952; Soderstrom and Beaman 1968), are the sister group of the rest of *Bromus* (Fig. 1). Four South American species (*B. lanatus, B. modestus, B. pellitus,* and *B. pflanzii*) form a well-supported clade that is resolved as part of the second deepest split in the genus. The plastid data alone do not support these phylogenetic placements, possibly because of insufficient variation; however, the plastid trees do indicate that these species are not part of the clade that includes other New and Old World species of sect. *Bromopsis* (Fig. 2), and they do not rule out the relationships inferred from the nuclear ribosomal data. The morphological characteristics of the species are not sufficiently distinct compared with other New World species of sect. *Bromopsis* for previous workers to have considered them major evolutionary lineages. However, Wagnon (1952) suspected that *B. attenuatus* and *B. dolichocarpus* are closely related to each other, and that they are distantly related to other North American species of sect. *Bromopsis.* The molecular data agree with this hypothesis. Further study is necessary to identify possible morphological and/or anatomical synapomorphies for a *B. attenuatus–B. dolichocarpus* clade as well as a putative clade of South American species that may be part of the second deepest split in *Bromus.* The deep positions of these two clades in the nuclear ribosomal trees suggest that the crown clade of *Bromus* originated in the New World. In contrast, Stebbins (1981) suggested that *Bromus* originated in Eurasia, with sects. *Bromopsis, Ceratochloa,* and *Neobromus* being the first to differentiate and subsequently spreading to North and South America, followed by the evolution of sects. *Boissiera, Bromus,* and *Genea.*

The molecular evidence suggests that the remaining South American species of sect. *Bromopsis* sampled, *B. brachyanthera* (a hexaploid; Schifino and Winge 1983), is closely related to Old World species of sect. *Bromopsis* and sects. *Ceratochloa* and *Neobromus.* In the plastid trees, *B. brachyanthera* is the sister group of a clade corresponding to sect. *Ceratochloa* (Fig. 2), whereas in the nuclear ribosomal trees the species is the sister group of a clade comprising sects. *Ceratochloa* and *Neobromus* (Fig. 1). Despite the close molecular relationship, *B. brachyanthera* is morphologically distinct from species in sects. *Ceratochloa* and *Neobromus,* having dorsiventrally flattened spikelets typical of other species in sect. *Bromopsis,* and straight awns. Stebbins (1981) suggested that some members of sect. *Bromopsis* may have donated genomes during the origin of sect. *Ceratochloa.* In line with this hypothesis, the phylogenetic affinities of *B. brachyanthera* and the Old World species of sect. *Bromopsis* with sect. *Ceratochloa* suggest that these species of sect. *Bromopsis,* their close relatives, or their immediate ancestors are among the most likely candidates as possible genome donors. In future studies, inclusion of the six unsampled native species of sect. *Bromopsis* from South America (Planchuelo and Peterson 2000) should provide further insight into the evolution and relationships of this group of poorly understood species.

Armstrong (1983) hypothesized that the North American and Old World members of sect. *Bromopsis* may represent distinct evolutionary lineages. Species from North America are generally diploids (a few are tetraploids), and all have large chromosomes with pinhead satellites, whereas Old World species are generally polyploids with smaller chromosomes lacking pinhead satellites (Armstrong 1983). Exceptions are *B. pumpellianus,* which is native in North America and Eurasia and morphologically and cytologically similar to Old World taxa, and the *B. ramosus* complex of the Old World (represented here by *B. ramosus*), which is

morphologically and cytologically similar to North American species of sect. *Bromopsis* (Armstrong 1983). Differences in floral microstructural variation further support the distinctiveness of these morphologically and cytologically differentiated groups (Kosina 1999). Our nuclear ribosomal data may partly support these hypotheses, as species of sect. *Bromopsis* from North America (excluding *B. attenuatus* and *B. dolichocarpus*) and *B. ramosus* from the Old World form a clade that does not include the other Old World species in the section (Fig. 1). Old World species of sect. *Bromopsis* (including *B. pumpellianus*) occur in several independent lineages that are part of a well-supported clade that also includes *B. brachyanthera* (sect. *Bromopsis*) and sects. *Ceratochloa, Genea,* and *Neobromus* (Fig. 1). These relationships are consistent with the findings of Kosina (1996), who observed similarity in the embryo structure of species of sect. *Ceratochloa* and Old World species of sect. *Bromopsis.* In contrast, the plastid data include Old World species of sect. *Bromopsis* in a large, weakly supported clade with many North American species of the section (Fig. 2). The gene trees thus indicate that most Old World and North American lineages of sect. *Bromopsis* share a similar plastid genome, but have conflicting nuclear ribosomal histories. The Old World species (mostly polyploids) may have originated via a hybridization event, with a diploid member of sect. *Bromopsis* contributing the plastid genome. Additional Old World representatives of sect. *Bromopsis* as traditionally circumscribed, and improved genomic sampling, will be required to provide further insight into the evolution and relationships of these species. If it becomes desirable to formally recognize these Old World lineages, the sectional name *Pnigma* Dumort. is available for the clade that contains *B. inermis* (Armstrong 1983).

Within the clade that includes most of the North American species of sect. *Bromopsis,* several weakly to well-supported clades of two to five species are evident (Fig. 1, 2). Wagnon (1952) suggested several groupings of the North American species based on geographical distribution: (1) an Arctic group, (2) a Rocky Mountain–Mexican Highland group, (3) a Pacific Slope group, and (4) an East–Midwest group. Our nuclear ribosomal trees are largely congruent with the East–Midwest group that Wagnon (1952) defined to include *B. ciliatus, B. kalmii, B. nottowayanus, B. pubescens, B. purgans* L. (nom. rejic.; here treated as *B. latiglumis*), and *B. texensis.* All of these species, except *B. texensis,* make up a moderately supported clade in these trees; there is insufficient variation in the plastid data to support or reject such close species relationships. Wagnon (1952) noted that the placement of *B. texensis* might seem out of place in this group, since its geographic range is intermediate between other members of the East–Midwest group and members of the Rocky Mountain–Mexican Highland group, but he included it because the morphology of its ligule is similar to other members of the group. Our data neither support nor reject Wagnon's (1952) other groups, as the phylogenetic relationships of many of the species are unresolved. The short branch lengths and lack of resolution among many of the North American species of sect. *Bromopsis* indicate that the species in this group likely diversified during a recent rapid radiation.

There has been much confusion about the species status of *B. ciliatus* and *B. richardsonii* (North American species of sect. *Bromopsis*). *Bromus richardsonii* is often treated as a synonym of *B. ciliatus* (e.g., Hitchcock 1951; Soderstrom and Beaman 1968; Allred 1993), although recent taxonomic study has indicated that these taxa are sufficiently distinct morphologically, cytologically, and genetically to warrant specific recognition (Peterson et al. 2002). Our nuclear ribosomal data confirm that *B. richardsonii* is a distinct species, closely related to *B. mucroglumis* (although the species status of *B. mucroglumis* is also controversial; Wagnon 1952; Peterson et al. 2002); these taxa do not share an immediate common ancestor with *B. ciliatus* (Fig. 1, 2).

It is clear from both plastid and nuclear ribosomal data that sect. *Bromopsis* is an artificial assemblage of species. The morphological characteristics traditionally used to circumscribe the section (Table 2) may therefore be a mixture of characters that are homoplasious or that represent symplesiomorphies of larger clades. The recognition of *Bromopsis,* in its present circumscription, as a distinct section, subgenus, or genus (Table 1) is clearly not appropriate.

Sections Ceratochloa *and* Neobromus.—Section *Ceratochloa* is weakly supported as monophyletic in the plastid trees (Fig. 2), and robustly supported as monophyletic in the nuclear ribosomal trees (Fig. 1). None of the sequence data is sufficiently variable to resolve relationships among species in the section, and several species are genetically identical at the loci examined. Similarly, Pillay and Hilu (1990, 1995) found no chloroplast DNA restriction site variation among species of sect. *Ceratochloa.*

The plastid and nuclear ribosomal data are in conflict regarding the monophyly of sect. *Neobromus.* In the plastid trees (Fig. 2), the two species of sect. *Neobromus* comprise a grade, in which *B. berteroanus* is the sister group of a clade comprising *B. gunckelli,* sect. *Ceratochloa* and *B. brachyanthera* (sect. *Bromopsis*). However, sect. *Neobromus* is a well-supported monophyletic group in the nuclear ribosomal trees (Fig. 1), a relationship not strongly rejected by the plastid data, and clearly the species are closely related. Both species are morphologically similar, sharing geniculate awns (Table 2), hence their classification as a section. *Bromus berteroanus* (syn. *B. trinii* E. Desv.) is morphologically similar to other grass genera because of its large glumes and a lemma that is deeply bilobed apically (Stebbins 1981), and in the past the species has been confused as a species of the genus *Trisetum* Pers. (Louis-Marie 1928), although this classification has not been followed by recent authors. Our data confirm that *B. berteroanus* is a species of *Bromus.*

The weakly supported relationship between sects. *Ceratochloa* and *Neobromus* (Fig. 1, 2) agrees with previous hypotheses that these taxa share common ancestry. Stebbins (1981) reported a weak affinity between one genome of sects. *Ceratochloa* and *Neobromus,* and Pillay and Hilu (1995) found that these taxa shared eight synapomorphies based on chloroplast DNA restriction site variation. Unfortunately, neither of these studies included representatives of South American species of sect. *Bromopsis,* one of which appears here to be closely related to sects. *Ceratochloa* and *Neobromus* (Fig. 1, 2). Stebbins (1981) hypothesized that sects. *Ceratochloa* and *Neobromus* evolved early within

Bromus, based on their small chromosome size and spikelets that resemble those in genera he thought were derived from the ancestral complex that also produced *Bromus* (including *Littledalea, Megalachne, Metcalfia,* and *Pseudodanthonia*). The plastid data neither reject nor support this hypothesis (Fig. 2), but the nuclear ribosomal data indicate that *B. attenuatus* and *B. dolichocarpus* (sect. *Bromopsis*), species that are morphologically distinct from taxa in sects. *Ceratochloa* and *Neobromus,* are part of a deep lineage that diverged early in the history of the genus (see above; Fig. 1). Sections *Ceratochloa* and *Neobromus* are nested deep within the nuclear ribosomal trees. Current knowledge, favoring distant phylogenetic positions of the morphologically similar genera thought previously to be closely related to sects. *Ceratochloa* and *Neobromus* (e.g., Soreng et al. 2003), further discounts Stebbins's (1981) hypotheses.

Sections Boissiera *and* Nevskiella.—Material of *B. pumilio* (sect. *Boissiera*) was not available, and sampled material of *B. gracillimus* (sect. *Nevskiella*) was recalcitrant to molecular study, thus the phylogenetic positions of these taxa remain uncertain. *Bromus pumilio* was originally classified in its own genus, *Boissiera,* but was transferred to *Bromus* based on serological and morphological similarities to other *Bromus* species (Smith 1969). It has since been treated either in sect. *Bromus* (e.g., Smith 1970) or within its own section, *Boissiera* (Smith 1985*a*; Table 1), because of its unique morphology, having five to nine awns on each lemma (Table 2). Based on allozyme evidence, Oja and Jaaska (1998) found *B. pumilio* to be distinct from members of sect. *Bromus,* supporting its placement in its own section. The phylogenetic position of *B. gracillimus,* characterized by awns that are four to six times the length of the lemma (Table 2), remains unknown. It would be valuable to include both species in future molecular studies.

Conclusions and Future Directions

Our study provides genus-wide phylogenetic hypotheses of relationships in *Bromus* s.l., based on DNA sequence data from the plastid genome and the nuclear ribosomal internal transcribed spacer region, and provides a foundation for further phylogenetic study of the genus. Based on the nuclear ribosomal data, sects. *Bromus* (including sect. *Triniusia*), *Ceratochloa, Genea,* and *Neobromus* are monophyletic, and sect. *Bromopsis* comprises several distinct lineages. Plastid trees indicate that sects. *Bromus* and *Genea* are closely related, and the incongruence detected between the plastid and nuclear ribosomal data support a hybrid origin for the *B. pectinatus* complex (here represented by a single exemplar) between sects. *Bromus* and *Genea.* Plastid trees indicate a close relationship between Old World and some North American species of sect. *Bromopsis,* and the plastid and nuclear ribosomal data indicate that one South American species of sect. *Bromopsis* is not closely related to North American and Eurasian species traditionally classified in the same section. Most species of *Bromus* sampled had levels of sequence variation too low to allow complete resolution of relationships among close relatives at the species level (e.g., among North American members of sect. *Bromopsis* and within sect. *Ceratochloa*). Sequence data from additional nuclear loci, such as the granule-bound starch synthase gene (*waxy*; e.g., Mason-Gamer 2001), AFLPs (e.g., Beardsley et al. 2003; Després et al. 2003), or microsatellites (e.g., Alvarez et al. 2001) may provide further insight into species-level relationships in *Bromus.* Adding data from the plastid genome (e.g., Shaw et al. 2005) and the nuclear ribosomal region (the external transcribed spacer [ETS] of nuclear rDNA; e.g., Baldwin and Markos 1998; Markos and Baldwin 2002; Starr et al. 2003) would also be valuable to improve resolution and support of trees inferred from these two linkage groups.

Recognition of the brome grasses as one distinct genus, *Bromus,* is in agreement with the molecular data, but current classification schemes do not satisfactorily reflect phylogenetic relationships within the genus, particularly with respect to the circumscription of sect. *Bromopsis.* However, before a revised infrageneric classification of *Bromus* is proposed, we advocate that substantially better sampling should be conducted of (1) DNA sequence regions, to obtain better support for phylogenetic relationships among taxa, and to further clarify incongruence among nuclear and plastid data partitions, and (2) taxa, to more adequately sample the molecular, morphological, and geographical variability in the genus. Although this is the largest study of *Bromus* phylogeny conducted thus far, all conclusions are based on a sample of less than one-third of the recognized species, mostly with one individual per taxon, and it is plausible that addition of other species will further contribute to and change our understanding of evolution and phylogeny in this genus.

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